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Droppings of Svalbard reindeer (*Rangifer tarandus platyrhynchus*) as a reservoir of cultivable micromycetes on Spitsbergen

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Abstract: Fungi are highly diverse, yet only a minor part of the total estimated species has been cultured and characterized. This might be especially true for Arctic, where studies on the fungal diversity are still scarce. For that reason, our aim was to analyze fungal diversity in the droppings of *Rangifer tarandus platyrhynchus*. The samples of feces from 32 adult individuals were collected in the southern or central parts of the Wedel Jarlsberg Land (Spitsbergen, Svalbard Archipelago) and assessed for micromycetes diversity using a combination of classical and molecular identification approaches. We found 16 fungal species, out of which three were described as mesophilic, two as psychrotolerant and eleven as psychrophilic. The identified Arctic fungi belonged to eleven genera out of which representatives of *Naganishia* genus (formerly belonging to *Cryptococcus albidus* clade) were the most abundant fungal species isolated. Additionally, to our knowledge, we firstly recorded *Botrytis cinerea* in polar areas. We conclude that droppings of *R. tarandus platyrhynchus* are a source of different fungal taxa, including fungi potentially pathogenic towards humans, plants and insects.

Keywords: Arctic, Wedel Jarlsberg Land, microscopic fungi, excrement.

Introduction

The Svalbard Archipelago, located in the Arctic Ocean between Norway and the North Pole, is one of the best studied Arctic locations in terms of biology and ecology. Due to the permission of the Norwegian authorities for conducting research on Svalbard by any nation, the archipelago is also often visited by Arctic microbiologists. Previous studies on Arctic microbial communities have focused on Bacteria (Larose *et al.* 2010; Singh *et al.* 2016), Archaea (Zarsky *et al.* 2013), viruses (Anesio *et al.* 2007; Bellas *et al.* 2013) or fungi (Butinar *et al.* 2007; Edwards *et al.* 2013; Singh *et al.* 2014). The latter has been estimated to include *ca*. 2.3% of the world's fungal species richness (Singh *et al.* 2012). Considering that Arctic fungi have been isolated from various substrates and habitats, Singh *et al*. (2012) indicated that fungal diversity in the Arctic soil had been investigated only to a limited extent. Similar conclusions were made in late 1990s by Elvebakk *et al*. (1996) who enlisted almost 400 Svalbard fungal species (including fungi-like organisms) which belonged to Ascomycota, Basidiomycota, Chytridiomycota, Zygomycota, Myxomycota, and Oomycota.

Some environments are considered particularly difficult in terms of sampling due to their physicochemical properties which lead to an uneven distribution of the microorganisms (Ranjard and Richaume 2001; Grundmann 2004). According to Lombard *et al.* (2011) some highly condensed samples (such as soil) are challenging for extraction and their subsequent analyses (even in case of metagenomics analyses). The environmental extremes distinctive for polar regions (such as low temperature, dryness, exposure to UV-radiation, and organic matter deficiency) additionally result in low microbial diversity present in samples (Ali *et al.* 2013). Concluding, some natural enrichment must be present in the studied samples prior the sampling. Bradshaw *et al.* (2022) stated that one approach to improve sampling efficiency for fungi is to use mammalian feces source that can function as a so-called "proxy".

Identification of dormant fungal spores in mammalian feces is considered a reliable indicator of potentially metabolically active mycelium presence at a given location at a given moment (Langer 2002; Padmanabhan *et al.* 2013). Such timing is crucial in case of High Arctic environments, which are characterized by nitrogen and phosphorus cycles driven by the presence of vegetation and litter (animal feces or cadavers) in the soil during Arctic winter or summer (Zmudczyńska *et al.* 2012). Such uneven distribution in nutrient availability also affects higher organisms like Svalbard reindeer [*Rangifer tarandus platyrhynchus* (Vrolik, 1829)], which due to incapability of migration to different feeding areas adapted its digestive system to such cycles. It was proven that Svalbard reindeer's droppings originating from the winter diet vastly differ in phosphorus, carbon and nitrogen content rather than those originating from the summer diet (Hayashi *et al.* 2014).

For those reasons, our aim was to investigate the micromycetes diversity inhabiting Arctic environments in Wedel Jarlsberg Land (Spitsbergen, Svalbard Archipelago) during Arctic summer by treating *R. tarandus platyrhynchus* droppings as a fungal reservoir.

Materials and Methods

Sample collection and the studied area. — Fresh fecal samples (I–XV) were collected from 32 adult *R. tarandus platyrhynchus* individuals from fifteen sampling points in the southwest part of the Wedel Jarlsberg Land (Fig. 1). The sampling points spread from 77°00'06"N, 15°22'34"E to 77°04'09"N, 15°10'13"E (Table 1). Selected *R. tarandus platyrhynchus* individuals were followed until they defecated. Fresh portions of feces were aseptically collected, placed in plastic, sterile tubes (Biologix), and frozen at −20°C within max. 5 h. Samples were collected in the late Arctic summer of 2016, between July 15th and July 20th, which according to Mathiesen *et al*. (2005) corresponds to the peak point of nutrient availability and reindeer digestibility.

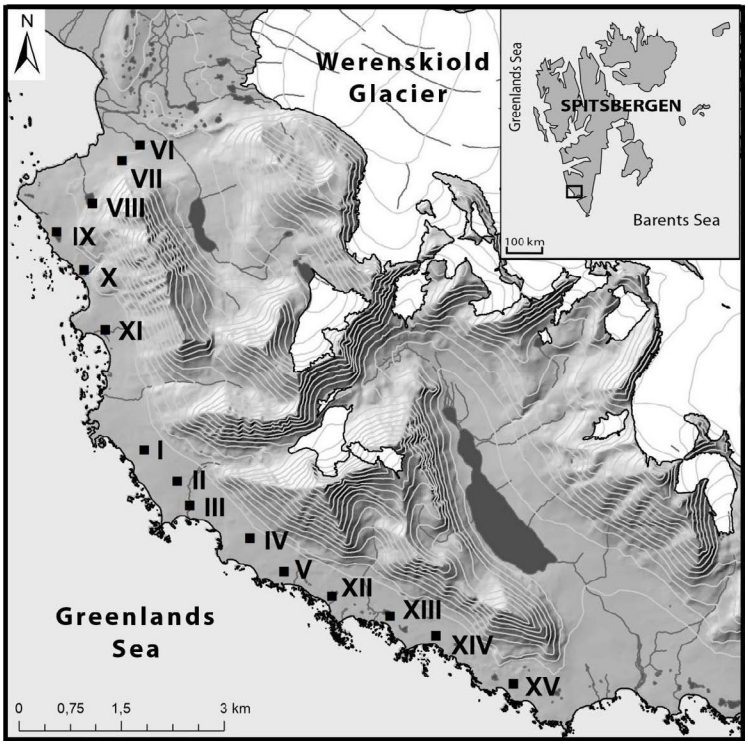

Fig. 1. Study sites in Wedel Jarlsberg Land, Spitsbergen, Svalbard Archipelago. The exact geographic coordinates of the sites are given in Table 1.

Table 1

Location of the study sites.

Study sites	Latitude (N)	Longitude (E)	Specific location
I	77°01'52"	15°10'20"	flush
II	77°01'38"	15°11'25"	shore of the small lake
III	77°01'27"	15°11'50"	shore of the brook/flush
IV	77°01'12"	15°13'51"	flush
V	77°00'57"	15°14'58"	flush
VI	77°04'09"	15°10'13"	flush
VII	77°04'02"	15°09'37"	flush
VIII	77°03'43"	15°08'38"	shore of the lake
IX	77°03'30"	15°07'25"	shore of the small lake
X	77°03'13"	15°08'21"	shore of the brook/flush
XI	77°02'46"	15°09'03"	shore of the brook/flush
XII	77°00'46"	15°16'33"	flush
XIII	77°00'37"	15°18'30"	shore of the small lake
XIV	77°00'28"	15°20'01"	flush
XV	77°00'06"	15°22'34"	flush

The studied area is under influence of an Arctic climate with long periods of darkness during the polar night, long and cold winters, as well as short and cool summers. Along the coast, on the border between sea and land, tundra with lichens, mosses, fungi and small flowering plants develop in valleys and on mountain slopes. These are mainly places where large colonies of seabirds exist and provide fertile guano and thus attract herbivores or predators.

The annual mean air temperature in 2016 was 0.3°C with maximum 11.3°C in July and minimum −18.5°C in March, and annual mean −3.6°C with maximum 16.5°C in July 2020 and minimum −35.9°C in January 1981 in period of 1979−2020 respectively. Mean air temperature in July 2016 was 6.3°C with maximum on July 8th (11.3°C) and minimum air temperature was noted on July 29th (3.8°C). In 2016, 156 days have been reported with rainfall and 152 days with snowfall along with annual precipitation amount 805.8 mm. Annual precipitation relation the in period of 1979−2020 was 466.6 mm. Accordingly to precipitation data, July was very wet with the sum of precipitation stood at 54.5 mm (the average sum precipitation in July in period of 1979−2020 was 42.6 mm).

Isolation of fungi from samples. — One gram (1 g) of each sample was homogenized, suspended in 9 mL sterile H_2O_{dd}, incubated at room temperature (20 min; 25 ± 1°C; n = 3), and serially diluted up to 10^{-3}. Volume of 100 µL of each dilution (from 10^{-0} to 10^{-3}) was transferred into potato dextrose agar (PDA, Biomaxima, Poland) in three repetitions and incubated for 10 days at either 5°C or 23°C. The grown colonies of fungi at each dilution were separated by the single

spore sub-culturing on PDA slants (Ogórek *et al.* 2019). At this point, fungal colony forming units (CFUs) per 1 g of droppings were calculated. Simultaneously, the cultures were inoculated on PDA plates and used for morphological/molecular identification.

Fungal identification. — The initial identification was performed by direct observation of macro- and micromorphology of the micromycetes grown on PDA medium. During interpretation of the results available monographs were used (Peterson 2004; Houbraken *et al.* 2011; Ogórek *et al.* 2018; Dyląg *et al.* 2019). To further confirm the species affiliation, fungal internal transcribed spacer regions (ITS) were sequenced. Briefly, DNA was extracted from a 21-day-old culture on PDA using Bead-Beat Micro AX Gravity kit (A&A Biotechnology, Gdańsk, Poland) according to the manufacturer's instructions. ITS regions were amplified using the ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') primer set (White *et al.* 1990). Polymerase chain reaction (PCR) was performed in a T100 Thermal Cycler (Bio-Rad, Berkeley, CA, USA), according to Ogórek *et al.* (2016). The PCR products were verified by electrophoretic separation on a 1.2% agarose gel, purified using Clean-UP kit (A&A Biotechnology, Gdańsk, Poland), and sequenced by the service of Macrogen Europe (Amsterdam, Netherlands, http://dna.macrogen.com/eng/).

Data analyses. — Raw sequence readings were analyzed using the BioEdit Sequence Alignment Editor (http://www.mbio.ncsu.edu/bioedit/bioedit.html). Then, the sequences were compared with those deposited in the GenBank of the National Center for Biotechnology Information (NCBI, Bethesda, Rockville, MD, USA) using the BLAST algorithm (http://www.ncbi.nlm.nih.gov/), and submitted into this database. The obtained data (the differences in colony forming units, CFUs, at different sites) were analyzed using Statistica 13.0 software (StatSoft Polska Sp. z o.o., Kraków, Poland). For this purpose, one-way analysis of variance (ANOVA) was applied, and means were compared using the Tukey HSD (Honest Significant Difference) test at $\alpha \leq 0.05$. To determine the diversity of fungal communities at specific research sites, the Shannon Diversity Index (H) was used and calculated from the following equation: $H = -å\ P_i(\ln P_i)$, where Pi stands for the proportion of each community in the sample (Shannon and Wiener 1963; Spellerberg *et al.* 2003).

Results

A total number of 681 fungal isolates were extracted from the samples of *R. tarandus platyrhynchus* droppings collected at the 15 sampling points, marked from I to XV. Basing on macro- and micromorphology, the isolates were clustered into 16 major groups. Next, the ITS rDNA sequencing was performed, which resulted in affiliating the fungi into 16 different species (Table 2). The lengths of

Table 2

BLAST analysis of rDNA ITS sequences of micromycetes cultured from droppings of Svalbard reindeer. All E values were equal to zero.

Identified fungi	Phylum	Morpho-logical forms	Isolate number	Isolation temperature		GenBank accession no.	The sequence length (bp)	Identity with sequence from GenBank		
				5°C	23°C			Query cover (%)	Identity (%)	Accession
Aspergillus fumigatus Fresenius, 1863	Ascomycota	filamentous	UWR_298		×	OM347927	429	100.00	100.00	MK623263.1
Botrytis cinerea Persoon, 1794	Ascomycota	filamentous	UWR_299	×		OM347928	435	100.00	100.00	KX025175.1
Cystobasidium laryngis (Reiersöl) Yurkov *et al.*, 2014	Basidiomycota	yeast	UWR_300	×	×	OM347929	461	100.00	99.78	KU145526.1
Goffeauzyma gilvescens (Chernov *et* Babeva) Liu *et al.*, 2015	Basidiomycota	yeast	UWR_302	×		OM347931	523	100.00	100.00	LC203691.1
Hypocreales sp. Lindau, 1897	Ascomycota	filamentous	UWR_303	×		OM347932	368	99.00	98.91	KP691493.1
Isaria farinosa (Holmskjold) Fries, 1832	Ascomycota	filamentous	UWR_304	×		OM347933	468	100.00	100.00	KY646427.1
Lecanicillium sp. Gams *et* Zare, 2001	Ascomycota	filamentous	UWR_305	×		OM347934	427	100.00	100.00	MF682448.2

Identified fungi	Phylum	Morpho-logical forms	Isolate number	Isolation temperature		GenBank accession no.	The sequence length (bp)	Identity with sequence from GenBank		
				5°C	23°C			Query cover (%)	Identity (%)	Accession
Mucor hiemalis Wehmer, 1903	Mucoromycota	filamentous	UWR_306	×		OM347935	408	100.00	99.75	MT514377.1
Naganishia albidosimilis (Vishniac *et* Kurtzman) Liu et al., 2015	Basidiomycota	yeast	UWR_307	×	×	OM347936	537	100.00	99.81	MN371855.1
Naganishia diffluens (Zach) Liu *et al.*, 2015	Basidiomycota	yeast	UWR_308	×		OM347937	367	100.00	100.00	MK782253.1
Naganishia liquefaciens (Saito *et* M. Ota) Liu *et al.*, 2015	Basidiomycota	yeast	UWR_309		×	OM347938	524	100.00	99.81	MN244413.1
Parengyodontium album (Limber) Tsang *et al.*, 2016	Ascomycota	filamentous	UWR_301	×		OM347930	458	100.00	100.00	KJ767115.1
Penicillium corylophilum Dierckx, 1901	Ascomycota	filamentous	UWR_310		×	OM347939	467	100.00	100.00	MN636222.1
Thelebolus globosus Brumm. *et* de Hoog, 2005	Ascomycota	filamentous	UWR_311	×		OM347940	362	100.00	100.00	MN445140.1

Identified fungi	Phylum	Morpho-logical forms	Isolate number	Isolation temperature		GenBank accession no.	The sequence length (bp)	Identity with sequence from GenBank		
				5°C	23°C			Query cover (%)	Identity (%)	Accession
Thelebolus microsporus (Berkeley *et* Broome) Kimbrough, 1967	Ascomycota	filamentous	UWR_312	×		OM347941	427	100.00	100.00	MG586989.1
Thelebolus stercoreus Tode, 1790	Ascomycota	filamentous	UWR_313	×		OM347942	372	100.00	99.73	MH860784.1

PCR products of the sequences from isolated fungal species ranged from 362 to 537 bps. All sequences were submitted to GenBank under the accession numbers from OM347927 to OM347942. Basing on BLAST analysis, the E values were zero, the percentages of the query cover amounted to 100%, except for 99% in case of isolate no. UWR_303 (*Hypocreales* sp.). The lowest acquired identity was 98.91% in case of isolate no. UWR_303 (*Hypocreales* sp.), whereas in case of the remaining fungi, the identity ranged from 99.73 to 100% (Table 2).

The identified Arctic fungi belong to either Ascomycota (ten isolates), Basidiomycota (five isolates) or Mucoromycota (one isolate), include seven families (Aspergillaceae, Cordycipitaceae, Cystobasidiaceae, Filobasidiaceae, Mucoraceae, Sclerotiniaceae, Thelebolaceae), and eleven genera (*Aspergillus*, *Botrytis*, *Cystobasidium*, *Goffeauzyma*, *Isaria*, *Lecanicillium*, *Mucor*, *Naganishia*, *Parengyodontium*, *Penicillium* and *Thelebolus*). Thus, the most species belonged to Filobasidiaceae family (four species), and *Naganishia* or *Thelebolus* genera (included three species each) (Table 2).

In the study were the only representatives of mesophilic fungi, which were reported in four sampling sites – *A. fumigatus* in XII, *N. liquefaciens* in XI and XV, and *P. corylophilum* in XIII (Table 3). In sampling site XV only *N. liquefaciens* was isolated. *Cystobasidium laryngis* and *Naganishia albidosimilis* displayed psychrotolerance and were present in eight and 14 different sampling sites, respectively. The remaining fungi were identified as psychrophiles, out of which each species was isolated from one up to ten different sampling sites.

The highest diversity of fungal communities was detected in study site X (0.647) for species cultured at 5°C, and in the study site XI (0.298) for species cultured at 23°C, as shown by the values of the Shannon Diversity Index. The highest overall value of the index was 0.625 at the study site X (Table 3).

The overall distribution of the number of fungal colonies capable of growing at either 5°C or 23°C is shown in Fig. 2. Despite higher species diversity of psychrophiles and psychrotolerants (Table 3), the abundance of fungal colonies growing at 23°C was generally higher than at 5°C. This was especially visible in the sample sites II, V, VI, VII, IX, XI and XIV, where CFU values differed significantly between incubation temperatures. The values of CFU of fungi capable of growing at 5°C were not statistically different in different locations, although the highest isolate density was found in location I (7.55×10^3), while in location XV no fungal isolates were detected. On the other hand, the highest values of CFU of fungi capable of growing at 23°C were detected at sampling sites II, and VI, whereas the lowest in I-IV, V, VI, VIII, X–XIII, and XV (Fig. 2).

Overall, the most frequently detected fungal species was *N. albidosimilis*, accounting for 70% of species isolated from all study locations and incubation temperatures. In turn, the most abundant fungal species capable of growing at 5°C were *N. diffluens* (30%), *B. cinerea* (22%), followed by *N. albidosimilis* and *G. gilvescens* (13% and 12%, respectively). The dominant species isolated at 23°C was also *N. albidosimilis* (97%) (Fig. 3).

Table 3

List of micromycetes isolated from the droppings of Svalbard reindeer) at 5°C ([1]) and at 23°C ([2]).

Fungi		Study sites														
		I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV	XV
Aspergillus fumigatus													$\times^{2}$			
Botrytis cinerea		$\times^{1}$														
Cystobasidium laryngis		$\times^{1}$		$\times^{1}$		$\times^{2}$	$\times^{1}$			$\times^{1}$	$\times^{1}$	$\times^{1}$			$\times^{1}$	
Goffeauzyma gilvescens		$\times^{1}$		$\times^{1}$	$\times^{1}$		$\times^{1}$	$\times^{1}$	$\times^{1}$	$\times^{1}$	$\times^{1}$	$\times^{1}$	$\times^{1}$		$\times^{1}$	
Hypocreales sp.									$\times^{1}$					$\times^{1}$		
Isaria farinosa										$\times^{1}$						
Lecanicillium sp.		$\times^{1}$														
Mucor hiemalis											$\times^{1}$					
Naganishia albidosimilis		$\times^{2}$	$\times^{1, 2}$	$\times^{2}$	$\times^{1, 2}$	$\times^{2}$	$\times^{1, 2}$	$\times^{1, 2}$	$\times^{2}$	$\times^{1, 2}$	$\times^{2}$	$\times^{2}$	$\times^{2}$	$\times^{2}$	$\times^{2}$	
Naganishia diffluens			$\times^{1}$	$\times^{1}$	$\times^{1}$		$\times^{1}$	$\times^{1}$	$\times^{1}$		$\times^{1}$		$\times^{1}$	$\times^{1}$	$\times^{1}$	
Naganishia liquefaciens												$\times^{2}$				$\times^{2}$
Parengyodontium album													$\times^{1}$			
Penicillium corylophilum														$\times^{2}$		
Thelebolus globosus			$\times^{1}$	$\times^{1}$	$\times^{1}$	$\times^{1}$		$\times^{1}$		$\times^{1}$	$\times^{1}$		$\times^{1}$			
Thelebolus microsporus		$\times^{1}$					$\times^{1}$			$\times^{1}$						
Thelebolus stercoreus					$\times^{1}$	$\times^{1}$									$\times^{1}$	
Shannon Index	for fungal communities at 5°C	0.132	0.505	0.327	0.246	0.168	0.419	0.515	0.374	0.588	0.647	0.292	0.295	0.301	0.287	0.000
	for fungal communities at 23°C	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.298	0.050	0.083	0.000	0.000
	overall	0.342	0.056	0.476	0.397	0.418	0.500	0.267	0.517	0.249	0.625	0.259	0.478	0.490	0.252	0.000

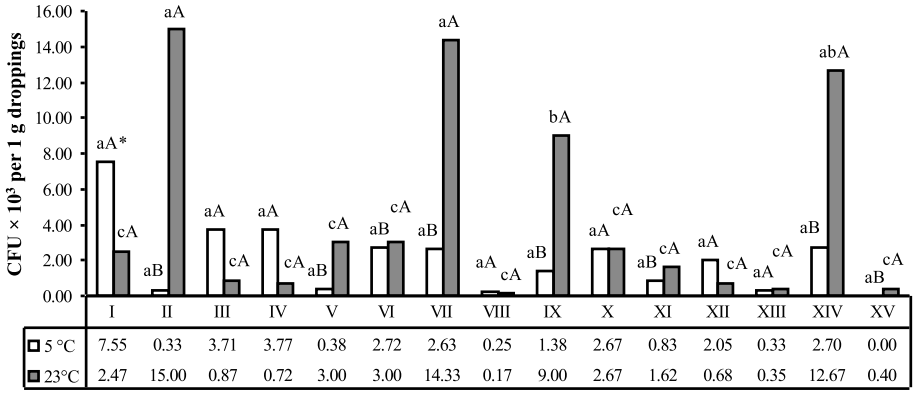

Fig. 2. Number (CFU × 10^3 per 1 g droppings) of fungi isolated from droppings of Svalbard reindeer (*R. tarandus platyrhynchus*) and incubated at 5°C or 23°C.

*number of fungal growth followed by the same letter are not statistically different (Tukey HSD test, $\alpha \leq 0.05$). Small letters indicate the differences between study sites in number of fungi cultured in a given incubated temperature. Capital letters indicate the differences between incubated temperature in number of fungi cultured in a given study site.

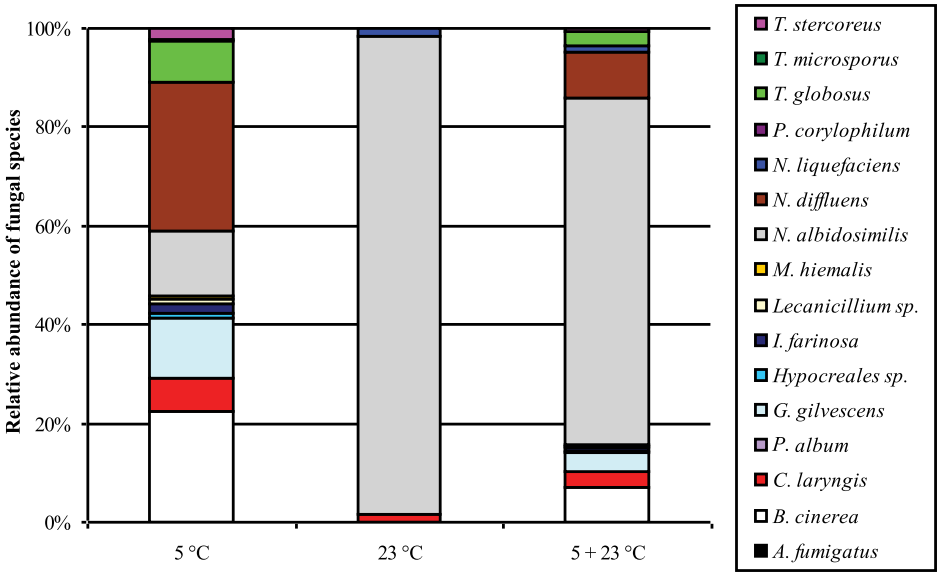

Fig. 3. Relative abundance of fungal species isolated from the droppings of Svalbard reindeer.

The distribution of fungal species among different sampling sites is shown in Figs 4 and 5. In case of fungi isolated at 5°C there was none one dominant species for all samples (Fig. 4). *B. cinerea* was the dominant species in the sampling site I (92.7%); *N. diffluens* in the sampling sites II, III, VI, VII and XIV (50%, 76.4%, 61.1%, 64.3%, and 80.2%, respectively); *Hypocreales* sp. in the sampling sites XIII and VIII (50% and 66.6%, respectively); *N. albidosimilis* in

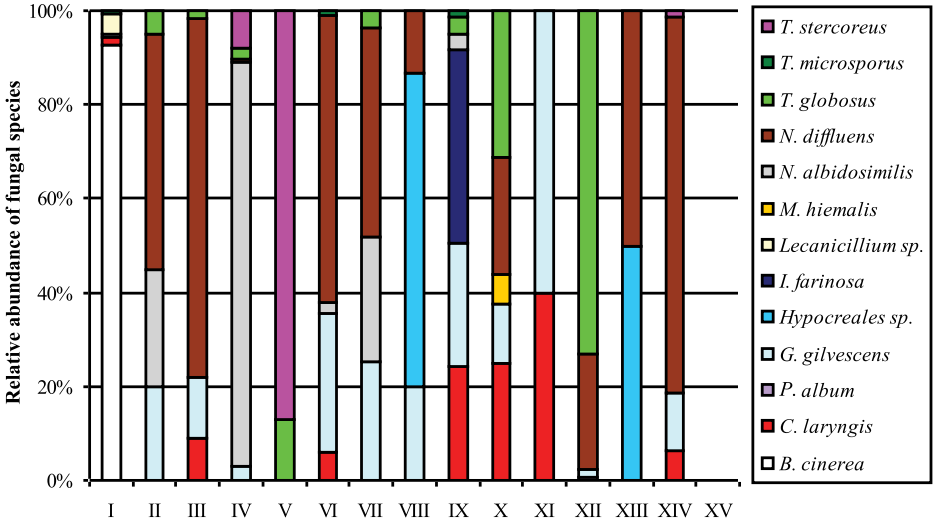

Fig. 4. Relative abundance of fungal species isolated from the droppings of Svalbard reindeer at 5°C in particular study sites. In location XV, no fungi were isolated at this temperature.

the sampling site IV (85.8%); *T. stercoreus* in the sampling site V (86.9%); *I. farinosa* in the sampling site IX (40.9%); *T. globosus* in the sampling sites X and XII (31.2%, and 73.1%, respectively); *G. gilvescens* in the sampling site XI (60%). In sampling site XV non-fungal colonies capable of growing at 5°C were detected (Fig. 4).

On the other hand, at 23°C lower fungal diversity was noted (Fig. 5). In each sampling site (excluding sample site XV) *N. albidosimilis* was the only or the dominant species (from 55.6% to 100%). In sampling sites V and XIII *N. albidosimilis* was accompanied by *P. corylophilum* (66.6%, and 95.2%, respectively); in sampling site XI by *N. liquefaciens* (55.6%); in sampling site XII by *A. fumigatus* (97.5%).

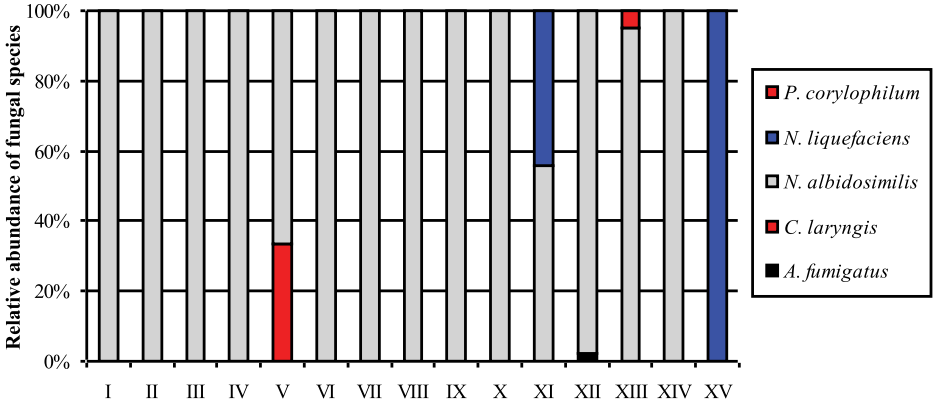

Fig. 5. Relative abundance of fungal species isolated from the droppings of Svalbard reindeer at 23°C in particular study sites.

Discussion

The study was carried out to explore the micromycetes diversity from Spitsbergen (Svalbard Archipelago) using droppings of Svalbard reindeer as a reservoir of cultivable fungi. We followed the broad definition of cold-adapted fungi, which divides them into psychrophiles and psychrotolerants (Tsuji 2018). Accordingly, psychrophilic fungi are capable of growing at or below 0°C, have maximum growth temperatures (MGT) of ≤ 20°C; while psychrotolerant fungi can grow close to 0°C, but have MGT > 20°C. We estimated whether the isolated fungi belong to psychrophiles, psychrotolerants or mesophiles, which cannot grow close to 0°C, and have MGT > 20°C. Most of the fecal fungi could grow at 5°C, indicating that psychrophilic fungi were predominantly isolated in the study sites. This observation is in contrast with the results of previous reports (Bergero *et al.* 1999; Singh *et al.* 2012), where mostly psychrotolerant fungi were described. One of the possible explanations might be the location of the studied areas in Spitsbergen. The present study was performed in Wedel Jarlsberg Land (southern part of Spitsbergen), whereas Bergero et al. (1999) were performing research within Arctic soils of Franz Joseph Land and Singh *et al.* (2012) within Ny-Ålesund (northern part of Spitsbergen). The differences might origin in the fact that the mentioned studies were performed either a few or a dozen years before the present study. Additionally, the samples described here were directly frozen during sampling as it was not possible to transfer the material and perform isolation immediately. Thus, we took the risk of avoiding some fungal species to preserve the samples. Nevertheless, either psychrotolerant or psychrophilic fungi are characterized by physical adaptations that allow overcoming low temperatures or dryness by chlamydospores formation and mycelial thickening (Robinson 2001). Similar features were also spotted during micro-morphological observations of the identified fungi.

During the present study, representatives of Filobasidiaceae family (*Naganishia* spp. and *Goffeauzyma gilvescens*) were the most abundant. Out of which, the most predominant species isolated from 14 out of 15 sampling sites, was psychrotolerant *N. albidosimilis*. It contributed to 70% of all species isolated from different locations at both incubation temperatures. *N. albidosimilis* was firstly described in 1992 as *Cryptococcus albidosimilis*, isolated from soil samples in Linnaeus Terrace (Antarctica) along with *Cryptococcus antarcticus* (Vishniac *et al.* 1992). Later, it was commonly reported in Arctic areas, including soil samples from East Ongul Island (East Antarctica) (Tsuji 2018), or was reported as *C. albidosimilis* in samples of ice cores of Midre Lovénbreen glacier (Svalbard) (Singh *et al.* 2013). *N. albidosimilis* is a promising producer of such enzymes as amylase, or other enzymes degrading starch, xylose or cellobiose (Vishniac *et al.* 1992). *Naganishia* spp. are predominantly isolated from such ecosystems as water, soil, or glaciers. Connell *et al.* (2008) described a few *Naganishia* spp. inhabiting South Victoria Land (Antarctica), which included

psychrophilic or psychrotolerant *N. albida*, *N. antarctica*, *N. adeliensis*, *N. albidosimilis*, *N. friedmannii* and *N. vishniacii*. A few years later *N. liquefaciens* was isolated from soil in Alaska (USA) (Fonseca *et al.* 2011). Interestingly, *N. liquefaciens* was the only species isolated from sampling site XV, the most eastern point of sampling in the present studies, characterized by the least humidity, where the wetlands are limited and turn into rocky terrain. *Naganishia liquefaciens* was one of three species of mesophilic fungi, isolated herein, whereas *N. albidosimilis* occurred to be psychrotolerant. On the other hand, *N. diffluens* was characterized by psychrophilicity. This is in agreement with Liu *et al.* (2015) who described *Naganishia* genus as diverse in case of basic physiology and even morphology. In case of *N. diffluens*, it was reported in snow samples at Amundsen–Scott South Pole Station (Hayward *et al.* 2021). Additionally, *N. diffluens* seems to be resistant towards different cellular stresses as it was isolated from hypersaline water (Mokhtarnejad *et al.* 2016) or anthropogenic polluted environments (Babič *et al.* 2017). The closely related to *Naganishia* spp., *G. gilvescens* (formerly *Cryptococcus gilvescens*) was reported as psychrophilic and dominant in the XI site (close to the shore, pebble beach and Trappers cottage). *G. gilvescens*, similarly to most *Cryptococcus* spp., produces polysaccharide capsules and is commonly described in Arctic environments (Carrasco *et al.* 2012; Białkowska *et al.* 2017).

Besides of *N. liquefaciens* both species of Aspergillaceae family, *A. fumigatus* and *P. corylophilum,* were representatives of mesophilic fungi unable to grow in 5°C. Either *A. fumigatus* or *P. corylophilum* have been already described in Arctic environments (Sonjak *et al.* 2006; Korfanty *et al.* 2022). *Aspergillus fumigatus* is a thermotolerant ascomycete mold with a ubiquitous presence around the world, even in arid region (Korfanty *et al.* 2022). It is worth mentioning that over the past 30 years, there have been rising incidences of triazole-resistant among *A. fumigatus* infections worldwide, including the identifications of triazole-resistant strains in triazole untreated patients (Sonjak *et al.* 2006).

Some notable examples are psychrophilic *Thelebolus* spp., most commonly isolated at 5°C in sampling sites V, X and XII. *T. globosus*, *T. microsporus* and *T. stercoreus* have already been described in Arctic environments (de Hoog *et al.* 2005; Godinho *et al.* 2019). *T. microsporus* was described as a dimorphic fungus, which produces hyphae at 2°C, chlamydospores at 12°C and yeast-like cells at 22°C (Sazanova *et al.* 2019). In our studies, *T. microsporus* was not able to propagate at 23°C (Tables 2 and 3), thus we assumed that in case of the dimorphism trait some differences might occur among different strains of *T. microsporus* isolates.

Hypocreales sp. is reported as a dominant species in sampling sites VIII and XIII (in this location along with *B. cinerea*) at 5°C. Despite the fact that some unassigned species of the order *Hypocreales* have already been reported in Arctic regions (Meyling *et al.* 2012; Zhang *et al.* 2020), it is not certain whether the

present isolates was already discovered in such regions. It is worth mentioning that the most close (98.91% ITS sequence identity, Table 1) *Hypocreales* sp. 24392-01-02-02 isolate (Bohuski *et al.* 2015) was obtained from captive snakes and wild snake populations in eastern North America (Bohuski *et al.* 2015). However, due to low identity and query cover we believe that the present *Hypocreales* sp. isolate might be a yet undiscovered species, assigning of which seems to be of a great importance in nearest future.

Similarly, sampling site I was predominantly inhabited by *Botrytis cinerea*, and the species was only found in that site (the coast, where migrating birds commonly arrive for the breeding period). Commonly known as a "grey mould", *B. cinerea* is a phytopathogen most notably known for infecting wine grapes. To best of our knowledge there are no reports on its occurrence in Arctic regions. *B. cinerea* was described to infect *Rubus arcticus*, a slow-growing bramble commonly found in Alaska. However, the studied cases of *B. cinerea*-infected *R. arcticus* were outside of polar regions (namely: South Savo Experimental Station in central Finland) (Ruokola 1981).

Another examples of fungi present only in one sampling sites include psychrophilic species such as: *Lecanicillium* sp. (sample site I), *Parengyodontium album* (sampling site XII), *Isaria farinosa* (sampling site IX) and *Mucor hiemalis* (sampling site X). Isolates of *Lecanicillium* sp. have been already described in Arctic regions (Pearce *et al.* 2009), however ITS rDNA sequence identity of the present isolate is similar at ≤ 95% to already known Arctic *Lecanicillium* spp., such as *L. lecanii*, *L. muscarium* and *L. longisporum*. It is worth mentioning that the present *Lecanicillium* sp. isolate display 100% sequence identity towards *Lecanicillium* sp. Kd1 strain, isolated from mineral building materials in Russian urban areas (Ponizovskaya *et al.* 2019). Thus, at this point it is not certain whether the present isolate was already described in Arctic areas without further analysis. On the other hand, isolates of entomopathogenic *I. farinosa*, facultative human parasite *M. hiemalis*, and extremophilic *P. album* have been detected in polar/Arctic areas (Meyling *et al.* 2012; Leplat *et al.* 2020). Interestingly, the taxonomic position of the latter species has been changed quite frequently and here initially it was identified by its former taxonomic name, which was *Engyodontium album* (Leplat *et al.* 2020). Psychrotolerant, entomopathogenic *Cystobasidium laryngis*, which consisted as 20–40 % of fungal colonies at 5°C in sampling sites IX–XII, has been already isolated as endophytic symbiont yeast associated with the Antarctic angiosperms *Deschampsia antarctica* E. Desv. and *Colobanthus quitensis* (Kunth) Bartl. (Santiago *et al.* 2017).

Conclusions

The study contributed to gaining new insight into micromycetes inhabiting droppings of *R. tarandus platyrhynchus* in Wedel Jarlsberg Land (Spitsbergen, Svalbard Archipelago). We concluded that droppings of *R. tarandus platyrhynchus* might be a source of different fungal taxa, including fungi potentially pathogenic towards humans, plants, and insects. We believe that our study has also proved that identification of fungal spores in mammalian feces might be a reliable indicator of a metabolically active micromycetes present at a given location. Thus, representatives of *Naganishia* genus (formerly belonging to *Cryptococcus albidus* clade) might be the most abundant fungal species in southwest part of the Wedel Jarlsberg Land (Spitsbergen, Svalbard Archipelago, Arctic). Additionally, to our knowledge, we firstly recorded *Botrytis cinerea* in polar areas. However, due to highly diverse nutrient requirements of the Fungi kingdom we believe that some uncultivable fungi might have also been present in the studied samples. Especially since our study was mostly culture-based. Thus, we believe that our study might lead to further research and better understanding of fungal diversity in Arctics using *R. tarandus platyrhynchus* droppings as a reservoir.

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