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Occurrence of *Psychrophilomyces antarcticus* in the Arctic

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ABSTRACT

The fungus *Psychrophilomyces antarcticus* M.M. Wang & Xing Z. Liu, previously known only from the Qinghai-Tibet Plateau and Antarctica, was isolated in the Arctic from sediments on the littoral of the Kara Sea (Shokalsky Island) and represented by strains VKM F-5025/CBS 151455 and VKM F-5026/CBS 151456. The macro- and micro-morphology of the isolate was studied together with partial sequences of the DNA regions ITS, LSU, TEF1, and β-TUB, as well as the optimal values for temperature, pH and salinity. Slight differences between our two isolates and previously known strains of *P. antarcticus* were found in morphology (colony color and shape, size of phialides and spores) and physiological traits (optimal growth temperature, range of acceptable temperature and pH values for growth, colony odor, and degree of mucosity under extreme conditions). However, the high similarity in the sequenced conservative DNA sequences from 100 to 97% indicates that these slight morphological and physiological variations of the studied strains of *P. antarcticus* are intraspecific variation. Our study demonstrates that *P. antarcticus* is a cold-adapted poly-extreme tolerant species that can occur and function in extremely cold areas, such as high alpine, Antarctic, and Arctic regions.

1. Introduction

Earth has environmentally harsh and extremely cold areas, such as high alpine and polar regions, that are nearly inhabitable for life. High mountain and polar regions share some comparable climate characteristics, including low temperature, intense ultraviolet radiation, and frequently limited nutrient availability in their substrates (Tsuji and Hoshino, 2019; Selbmann et al., 2021; Zhang et al., 2020). Nevertheless, some organisms manage to inhabit such unfriendly environments.[1] To survive or to even thrive, the organisms in these regions possess adaptations that protect them against the environment's stressful conditions. Examples of adaptations of microorganisms to cold environments include changes in the structure (fluidity) and functions of cellular membranes, unique genome structure, gene expression changes, production of cold-adapted and cold-active enzymes, antifreeze proteins that protect microorganisms from these adverse conditions (Dasila et al., 2022; Yusof et al., 2021). In the review by Yusof et al. (2021), the results of studies on the adaptive mechanisms of the Antarctic yeast fungus *Glaciozyma antarctica* are collected and described in detail. The researchers also highlight the significant potential role of cold-resistant bacteria and fungi in biotechnology and their important role in cold ecosystems (Dasila et al., 2022; Yusof et al., 2021).

Fungi are among the most abundant groups in high alpine and polar regions due to their adaptive flexibility (Coleine et al., 2022). The adaptability of fungi enables them to perform their functions as decomposers, symbionts, parasites, and predators even in such extreme ecosystems, allowing for their important contribution to carbon, nitrogen, and other nutrient cycles. In recent years, several studies have identified the uniqueness and similarity of fungal communities in geographically distant yet similar regions of the planet (Noffsinger et al., 2020). These investigations are important for understanding the roles of ecological selection and fungal dispersal in shaping fungal communities in such widely separated extreme habitats (Cox et al., 2016). However, despite these investigations no conclusions can be drawn about

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[1] VKM F-5025, VKM F-5026 – official strains numbers in the All-Russian Collection of Microorganisms; CBS 151455, CBS 151456 – official strains numbers in the Westerdijk Institute Collection (WI-KNAW Collections) of Microorganisms.

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similarity of fungal alpine and polar regions communities because of limited research and a lack of data (Cox et al., 2016). The situation is further complicated by the fact that permafrost zones are shrinking due to global warming and, as a result, the number of microorganisms adapted to cold conditions is decreasing (Tsuji et al., 2022; Wang et al., 2015). Additionally, data is severely limited on the stress tolerance of fungi found in extreme environments, which hinders understanding of the extent to which these fungi actively function and are involved in global biogeochemical processes, as well as how their activity will change in response to global climate change (Coleine et al., 2022; Harrison et al., 2013; Noffsinger et al., 2020).

Several recent studies show the presence of similar fungal species in the Arctic and Antarctic (Cox et al., 2016; Iliushin, 2020), as well as in high mountain regions and Antarctica (Wang et al., 2015). However, we have not seen any mention of fungal species found in a high mountain region as well as both polar regions. In 2011, three fungal species belonging to the genus *Psychrophila* were first isolated from samples taken from glaciers in the Qinghai-Tibet Plateau highlands and from Antarctic soil samples (Wang et al., 2015). However, the study used the invalid name *Psychrophila*, but it is validated here with a new generic name *Psychrophilomyces* (see the section "Taxonomy" below). The genus *Psychrophilomyces* was described with the type species *Psychrophilomyces antarcticus*. To date, *P. antarcticus* has only been described in the aforementioned study by Wang et al. (2015) and has not yet been found in any other region. *Psychrophilomyces* species are psychrotolerant[2] characterized as fungi that prefer cold environments and are well adapted to habitats with low temperatures. Wang et al. (2015) provided a comprehensive description of the morphological features of *P. antarcticus*, as well as gene sequences for ITS, LSU, β-TUB, and TEF1 genes.

In 2015, we discovered two strains that resembled *P. antarcticus* in samples of littoral sediments from the Arctic Shokalsky Island. Through a combination of morphological and molecular analysis, as well as a study of their physiological characteristics, we determined that these Arctic isolates shared similarities with the Tibetian and Antarctic *P. antarcticus* isolates. This marks the first time *P. antarcticus* has been detected in the Arctic, and we provide the details of the identification process in this study. Recognizing the lack of information regarding fungi with the ability to tolerate extreme conditions (Coleine et al., 2022; Harrison et al., 2013), we also examined the growth rate of these strains under different temperatures, salinity levels, and pH values. These factors were selected based on the isolation of the strains from the littoral zone of the Kara Sea, situated in the Arctic region. Our objective was to ascertain the temperature range and optimal growth conditions for the strains, as well as their preferred salinity, considering their origin from sea sediments. Additionally, we conducted tests to evaluate their growth rate under varying pH levels, as pH is recognized as one of the important limiting factors for fungal development (Ali et al., 2017; Jiang et al., 2022; Sharma et al., 2016).

2. Material and methods

2.1. Study area and sampling

Sediment samples of the littoral zone of the Shokalsky Island coast in the Arctic were collected with permission from the management of the state nature reserve "Gydanskiy". This flat area of the island measures approximately 20 × 30 km and is located in the Kara Sea. It is covered with tundra vegetation, as well as numerous swamps, lakes, and streams (Kalyakin et al., 2000). The average air temperature in July and August is around +5 °C, and the frost-free period does not exceed 70 days. The island is located at the exit of the Ob Bay, and its Western coast is significantly influenced by river discharge, resulting in the transport of large amounts of terrigenous materials and the desalination of marine waters (Galimov et al., 2006; Zatsepin et al., 2010).

In August 2015, 23 sediment samples were collected from the mid-littoral zone at four locations on Shokalsky Island, near the mouths of the streams. Two isolates of *Psychrophilomyces antarcticus* were obtained from one of the seven samples of sandy sediments collected on the middle littoral zone of the island's western coast, north of the mouth of the Pereprava River (72°55′37.5"N 74°17′32.3"E, Fig. 1). Initially, the top 2 mm of soil were removed with a sterile knife, and 2 cm^3 of sediments were collected by a sterile syringe inserting it vertically to the sediment. The material was then placed in a sterile craft paper bag, dried, and stored at 6 °C and further cultured on a nutrient medium for 2.5 months.

2.2. Detection of the Psychrophilomyces antarcticus strains

2.2.1. Cultivation of samples

For plating, 1 g of the sample was taken and evenly distributed among five Petri dishes containing Malt Extract Agar medium (distilled water, Malt Extract to a total sugar content of 0.2%, aquarium marine salt 35‰ (Red Sea Salt, moderate alkalinity), gentamicin (Microgen) - 160 mg/L, lincomycin (Microgen) - 600 mg/L). The seeded dishes were incubated at +4 °C for two months, after which all grown colonies were isolated into pure cultures for subsequent identification.

2.2.2. Identification of fungi

The sorting and initial identification of fungal isolates were conducted based on morphological and cultural characteristics. Molecular methods were then used to determine the taxonomic affiliation of the cultures, which was difficult to determine based on morphology alone.

2.2.3. Morphological observations

Primary identification of strains, later assigned to the species *P. antarcticus*, was conducted using an Olympus CX 23 microscope after the culture slides were prepared by transferring a portion of the colony from the medium onto a glass slide. For further accurate identification, slide cultures were prepared as follows: a drop of PDA medium was placed on a sterile glass slide, a fungal culture was inoculated onto it, covered with a cover slip, and then placed in a sterile Petri dish. Each fungus was inoculated in eight replicates. Petri dishes with cultures on slides were wrapped with Parafilm and incubated at 10 °C for 4 weeks. Subsequently, sporulation was studied and photographed by using Zeiss and Leica microscopes at magnifications of ×63 and ×100, respectively.

2.2.4. DNA extraction, PCR amplification and sequence analysis

Total genomic DNA (gDNA) was extracted from the mycelium of both strains using the chloroform/isopropanol method (Karakousis et al., 2006; Grum-Grzhimaylo et al., 2016). The primers ITS-1f and ITS-4r (White et al., 1990) were used to amplify a portion of the nuclear ribosomal gene (rDNA) including the two internal transcribed spacer regions (ITS1 and ITS2) and the 5.8S rDNA. The primers LR0R and LR7 (Vilgalys and Gonzalez, 1990) were used for amplification of part of the nuclear large subunit nrDNA gene (LSU); EF1-728F and EF1-986R (Carbone and Kohn, 1999) were used to amplify partial translation elongation factor 1-α gene (TEF1), and Bt-2a and Bt-2b (Glass and Donaldson, 1995) were used to amplify partial β-tubulin gene (β-TUB). PCR mixes (Promega Corp., Madison, Wisconsin) contained 0.5 μL 25 mM $MgCl_2$, 5 μL 5x PCR GoTaq buffer (Promega), 1 μL 10 mM dNTP, 1 μL 10 mM of each primer, 0.1 μL GoTaq G2 polymerase (Promega) and 2 μL gDNA, and filled with MQ-water to 25 μL. The amplification program included initial step of 94 C for 5 min, then 35 cycles of 94 C for 1 min, 52 C for 1 min and 72 C for 1 min followed by final extension at 72 C for 7 min. PCR products were purified using the PCR clean-up Gel extraction kit (Macherrey-Nagel) following the instruction manual. The purified

[2] Psychrotolerant species – fungi that can grow close to 0 °C, with optimum growth temperatures of >15 °C and maximum growth temperatures of >20 °C (Coleine et al., 2022).

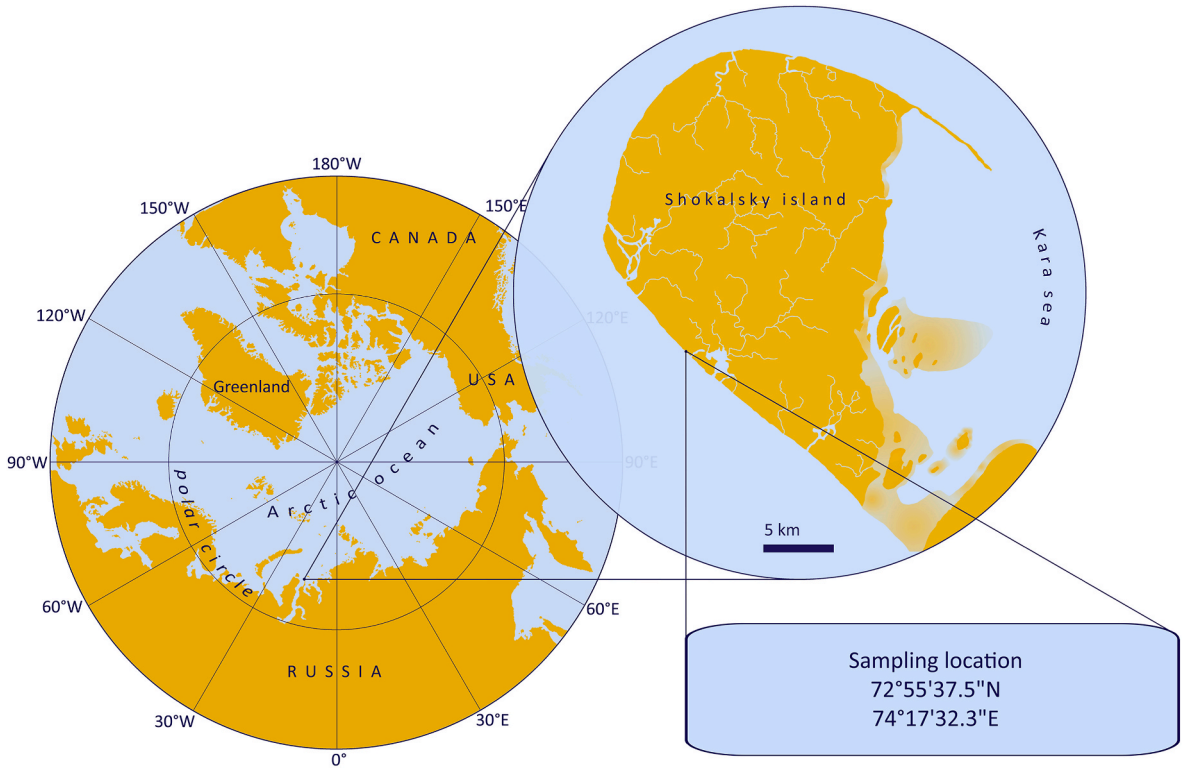

Fig. 1. Sampling point on Shokalsky Island from which two strains of *P. antarcticus* were isolated.

PCR products were sent for Sanger sequencing to Eurofins Genomics (EU). Sequences were compared with the data from GenBank using BLAST similarity searches (blast).

2.2.5. Phylogentic analysis

In total, we used 13 strains (11 of *Psychrophilomyces*) to reconstruct phylogeny and confirm identification of our two strains (Supplementary Material 1). We used four loci, LSU, ITS, TEF1, β-TUB) for each strain, except *P. lagodekhiensis* CBS122314 (this strain had only LSU and ITS available). Individual gene matrices were aligned with MAFFT (v7.505) with automatic alignment strategy, and trimmed with Trimal (v. 1.5. rev0) with -nogaps flag to generate gapless alignments. PhyKIT (v. 1.20.0) was used to concatenate the four alignments together, but retaining partition information for each gene. We then used IQ-TREE (v. 2.2.0.3) to test the DNA substitution model for each partition and generate maximum likelihood tree with SH-aLRT and ultrafast bootstrap supports (-m TEST –alrt 1000 -B 1000), with *T. globosum*_HAILUO215 as outgroup. Aesthetics were added with Adobe Illustrator CC 2018 (Adobe Inc., 2018).

2.2.6. Submitting to collections

Both strains were deposited in the All-Russian collection of microorganisms (VKM) and in the Westerdijk Institute Collection (WI-KNAW Collections) of Microorganisms. Newly generated sequences were deposited in GenBank (Table 1).

Table 1
GenBank Accession Numbers of our *Psychrophilomyces antarcticus* strains.

Strain No		Accession Numbers			
VKM	WI-KNAW	ITS	LSU	TEF1	β-TUB
VKM F-5025	CBS 151455	**PP390063**	**PP390068**	**PP935172**	**PP722763**
VKM F-5026	CBS 151456	**PP390064**	**PP390069**	**PP935173**	**PP722764**

2.3. Exploring Psychrophilomyces antarcticus tolerance to extreme environmental factors

2.3.1. Growth at different temperatures

Both fungal strains were inoculated by transferring a 1 × 1 mm piece into the beginning of a raised tube or the center of a Petri dish with PDA medium, with 3–6 repetitions for different temperature values (Supplementary Material 2A), and incubated for 35 days at the following temperature values: −2.0, −0.5, 0, +5, +10, +15, +16.5, +18, +20, +22.5, +25 °C.

Specimens were incubated in standard incubators within a temperature range of +5 to +25 °C. To guarantee a controlled temperature of 0 °C, we used a floating basket inside a styrofoam box containing ice. Placing the box in a +4 °C room allowed the ice to last for two weeks. To develop subzero incubator boxes, we prepared a sodium chloride (NaCl) solution to produce ice and water, based on desired temperatures (Supplementary Material 2B). This approach aimed to target the freezing point, density, specific heat, and dynamic viscosity of the sodium chloride and water coolant mixture (engineeringtoolbox). The incubator boxes containing ice and water were similarly prepared to the zero incubator and maintained at a temperature of +4 °C. To maintain the appropriate temperature in the hand made incubators, we replaced the ice every two days.

2.3.2. Growth at different salinities and temperatures

Both fungal strains were inoculated by transferring a 1 × 1 mm piece into the center of Petri dishes containing MEA medium (15 g malt extract [Millipore (Canada)], 15 g agar per liter of distilled water) with 0 %, 1.2 % and 3.4 % of NaCl, six repetitions for each salinity. Three replicates of each strain on different salinity media were incubated at 6, 15, 18 and 20 °C for 35 days.

2.3.3. Growth at different pH values

To conduct a growth experiment at different pH values, media based on phosphate buffer were prepared as follows (per 1 L): 0.2 M buffer –

500 mL, malt extract – 15 g, agar – 15 g, distilled water – 500 mL. Stock solutions were prepared for buffer preparation (Supplementary Material 2C), which were then combined in different proportions to obtain a buffer with the desired pH value (Supplementary Material 2D). Both fungal strains were inoculated by transferring a 1 × 1 mm piece onto the center of Petri dishes containing media with four repetitions for each pH values, and incubated for 35 days.

2.3.4. Colony measurement

The radius of each colony was measured in two directions from the inoculum, and their average length was calculated. Then, the average values of colony diameters or radii of all replicates were calculated and graphs with standard deviation were created in Excel.

3. Results

3.1. Phylogenetic and morphological observation

As part of our study of the littoral sediments in Shokalsky Island, we isolated strains VKM F-5025/CBS 151455 (further designated by No 1) and VKM F-5026/CBS 151456 (further designated by No 2). BLAST analysis of the partial ITS, LSU, TEF1 and β-TUB genes sequences showed high similarity between our two isolates, as well as between our isolates and all known *Psychrophilomyces antarcticus* strains (Suppplem. material 3). Phylogenetic analysis resolves all four species of *Psychrophilomyces* and places our two strains firmly (86.6/81 SH-aLRT/ultrafast bootstrap support) within the *P. antarcticus* species clade (Fig. 2).

3.1.1. Morphological description of strain 1

Colonies on PDA at 10 °C attained 14 mm diam after 5 wk, OGT 20 °C, eurypsychrophile; colonies rose-cream white, aerial mycelium were less abundant or sparse on the surface of the colony (Fig. 3). The reverse is white. Conidiophores were sometimes short, or much differentiated, conidiogenesis phialidic, phialides short, hyaline, flask-shaped, single or in groups, 5.1–8.0 × 2.5–4.5 µm, apically tapering into a broad funnel, bottleneck-like constriction; the collarette was 2.1–4 µm, wedge-shaped, widely flaring; vegetative hyphae hyaline, sometimes agglomerate to bundles or swollen to irregular shapes, 2–4 µm. Conidia were in the heads, hyaline, 1-celled, smooth, mostly globose, 2.0–2.2 µm diam.

3.1.2. Morphological description of strain 2

Colonies on PDA at 10 °C attained 14 mm diam after 5 wk, OGT 22.5 °C, eurypsychrophile; colonies were creamish, aerial mycelium was practically absent; if present, it was in the form of a small tuft in the center of the colony (Fig. 4). The reverse is yellowish only in the center

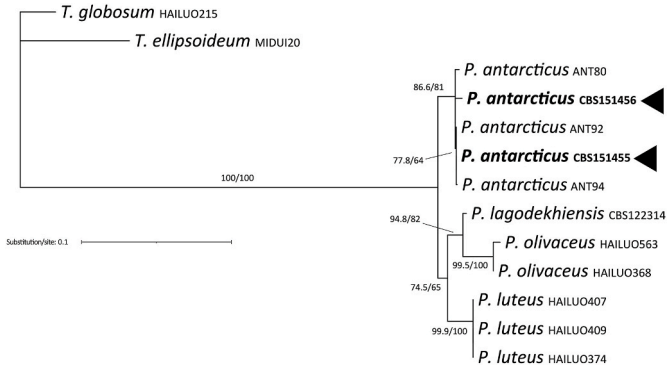

Fig. 2. Phylogenetic placement of *P. antarcticus* based on combined LSU + ITS + TEF1+ β-TUB dataset. Maximum likelihood phylogeny was reconstructed using IQ-TREE with automatic best substitution model selection for each partition. SH-aLRT and ultrafast bootstrap supports are indicated at corresponding nodes.

while closer to the edges it is lighter. Conidiophores were sometimes short, or much differentiated, conidiogenesis phialidic, phialides short, hyaline, flask-shaped, mostly single, 5.1–8.0 × 2.5–4.5 µm, apically tapering into a broad funnel, bottleneck-like constriction; the collarette was 2.1–4 µm, wedge-shaped, widely flaring; vegetative hyphae hyaline was sometimes agglomerate to bundles or swollen to irregular shapes, 2–4 µm. Conidia in the heads, 1-celled, smooth, mostly globose, 2.0–2.2 µm diam.

3.1.3. Growth at different temperature

As a result of measuring the linear growth rate of strains 1 and 2 at different temperatures (from −2.5 to 25 °C), optimal growth temperatures (OGT) were identified: 20 °C for strain 1 and 22.5 °C for strain 2 (Fig. 5; Supplementary Material 4). Both strains showed the ability to grow at subzero temperatures; at 25 °C, strain 1 practically stopped growing, and strain 2 grew slowly.

3.1.4. Growth at different salinities and temperatures

The linear growth rate of strains 1 and 2 slightly decreased with increasing salinity from 0 to 3.4% NaCl at temperatures of 15, 18, and 20 °C. At a temperature of 6 °C, the maximum growth rates of both strains were observed at a salinity of 1.2% NaCl (Fig. 6; Supplementary Material 5). It was also noted that at temperatures of 15, 18, and 20 °C and in the absence of NaCl in the medium, colonies grew dry, whereas with increasing salinity, colonies became slimy, aerial mycelium disappeared, and more hyphae agglomerated to bundles were detected upon microscopic examination. Sporulation was present at all salinity levels at temperatures of 15, 18, and 20 °C. At a temperature of 6 °C, colonies were slimy at all salinity levels, with abundant aerial hyphae, and sporulation was nearly absent. It was also observed that in the presence of NaCl in the medium, colonies remained small longer during the first 10–14 days, but by day 35, when measurements were taken, they nearly caught up in growth rate to colonies growing at zero salinity.

3.1.5. Growth at different pH values

The study of the linear growth rate of strains 1 and 2 at pH values of 5, 6, 7, 8, and 9 revealed a deceleration of growth rate from pH 5 to 7 and complete cessation of growth at pH 8 for strain 1. Strain 2 exhibited a similar trend, but retained the ability to grow even at high pH values (Fig. 7). Colonies of the strains became more compact, more submerged in the substrate, lost aerial mycelium, and exhibited changes in color as pH increased (Supplementary Material 6).

3.1.6. Taxonomy

Psychrophilomyces O.A. Grum-Grzhim. *nom. nov.* Mycobank MB 856658.

Replaced synonym: *Psychrophila* M.M. Wang & Xing Z. Liu, Persoonia 34: 105. 2014.

Non *Psychrophila* (DC.) Bercht. & J.Presl, Prir. Rostlin Aneb. Rostl. 1: 79. 1823.

Description and illustration: Wang et al. (2015).

Type species: *Psychrophilomyces antarcticus* (=*Psychrophila antarctica* M.M. Wang & Xing Z. Liu).

Psychrophilomyces antarcticus (M.M. Wang & Xing Z. Liu) O.A. Grum-Grzhim. *comb. nov.* Mycobank MB 856660.

Basionym: *Psychrophila antarctica* M.M. Wang & Xing Z. Liu, Persoonia 34: 106. 2014.

Type: Antarctic, Great Wall Station, S62°12′ W58°57′, from soil, Jan. 2011, T. Zhang (dried culture HMAS244374 holotype, living culture extype CGMCC315133 (ANT92)).

Psychrophilomyces lagodekhiensis (Unter., Réblová & Bills) O.A. Grum-Grzhim. *comb. nov.* Mycobank MB 856661.

Basionym: *Psychrophila lagodekhiensis* Unter., Réblová & Bills, Mycologia 111: 1020. 2019.

Type: Georgia, Lagodekhi, from the rhizosphere of *Hedera helix* at 645 m, Feb 4, 2007, G.F. Bills (dried culture on MLA UAMH 12042

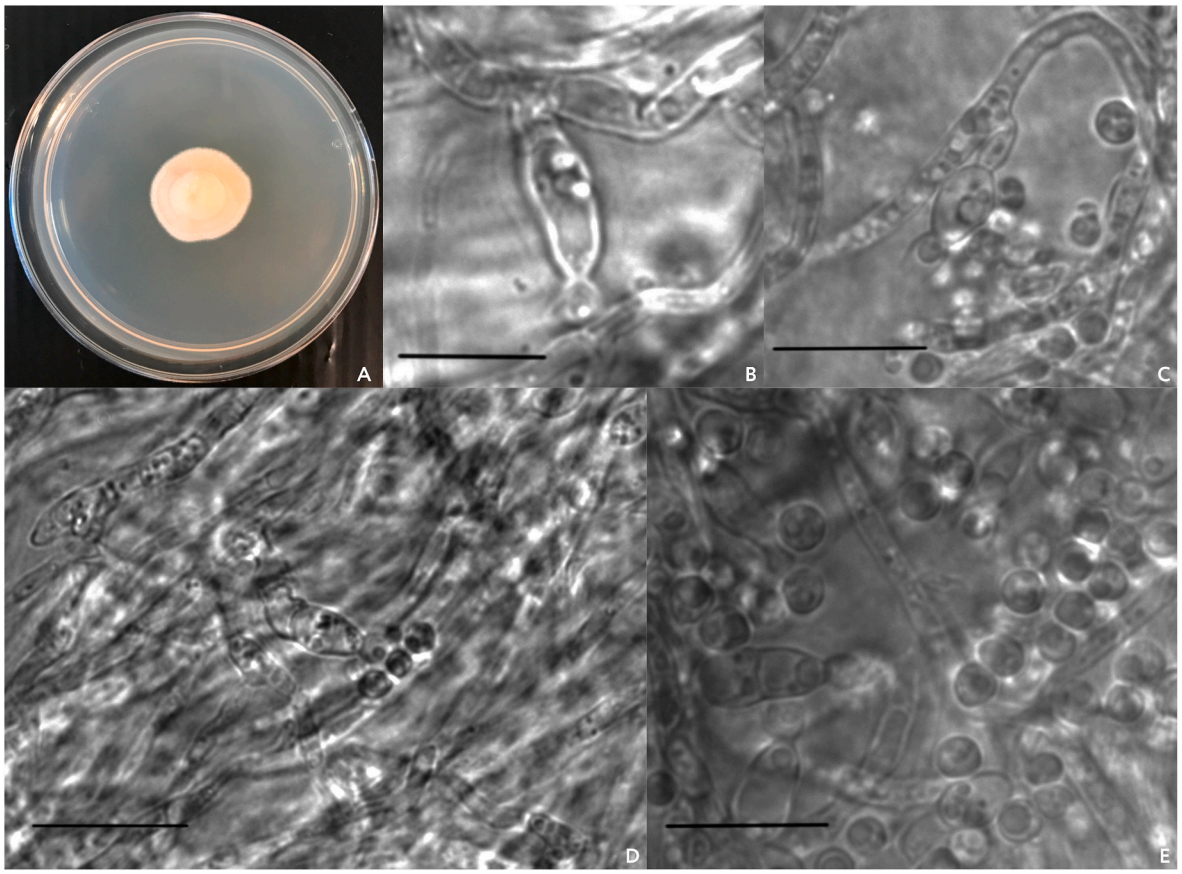

Fig. 3. *Psychrophilomyces antarcticus* (from strain 1) A. Colony morphology at 10 °C after 35 days; B – E. mycelium, conidiophores, conidiogenous cells and conidia. — Scale bars = 10 μm.

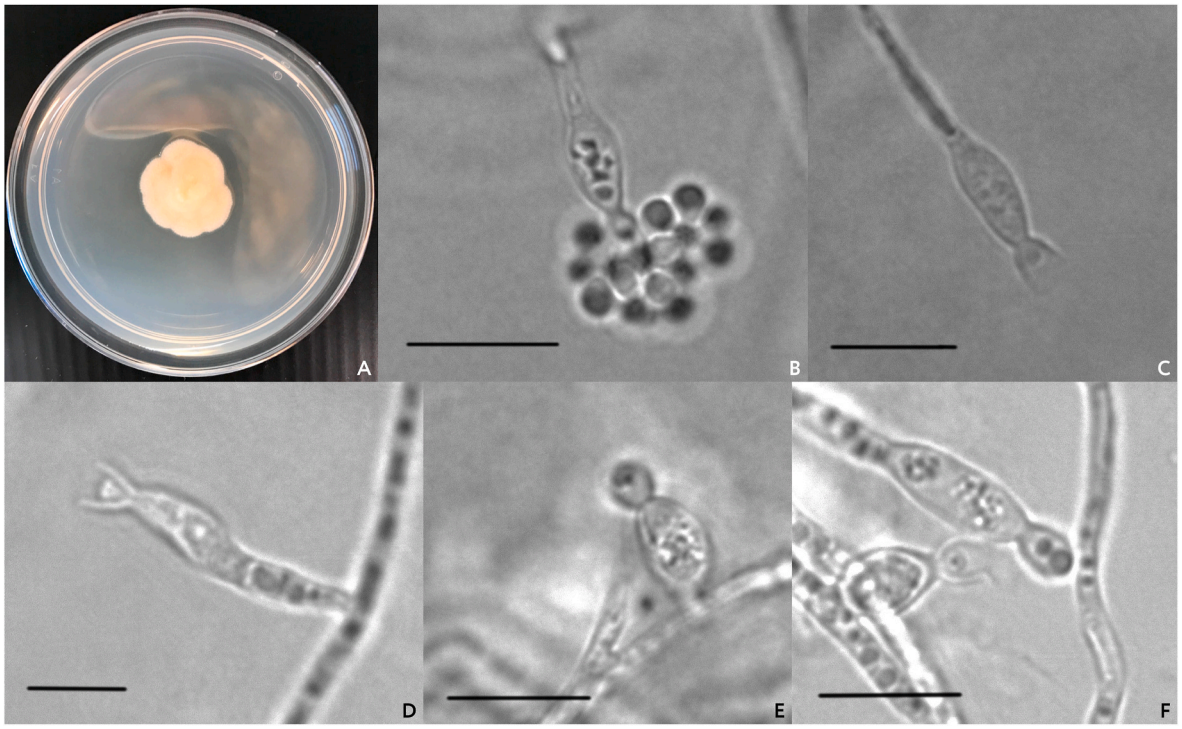

Fig. 4. *Psychrophilomyces antarcticus* (from strain 3) A. Colony morphology at 10 °C after 35 days; B – E. mycelium, conidiophores, conidiogenous cells and conidia. — Scale bars = 10 μm.

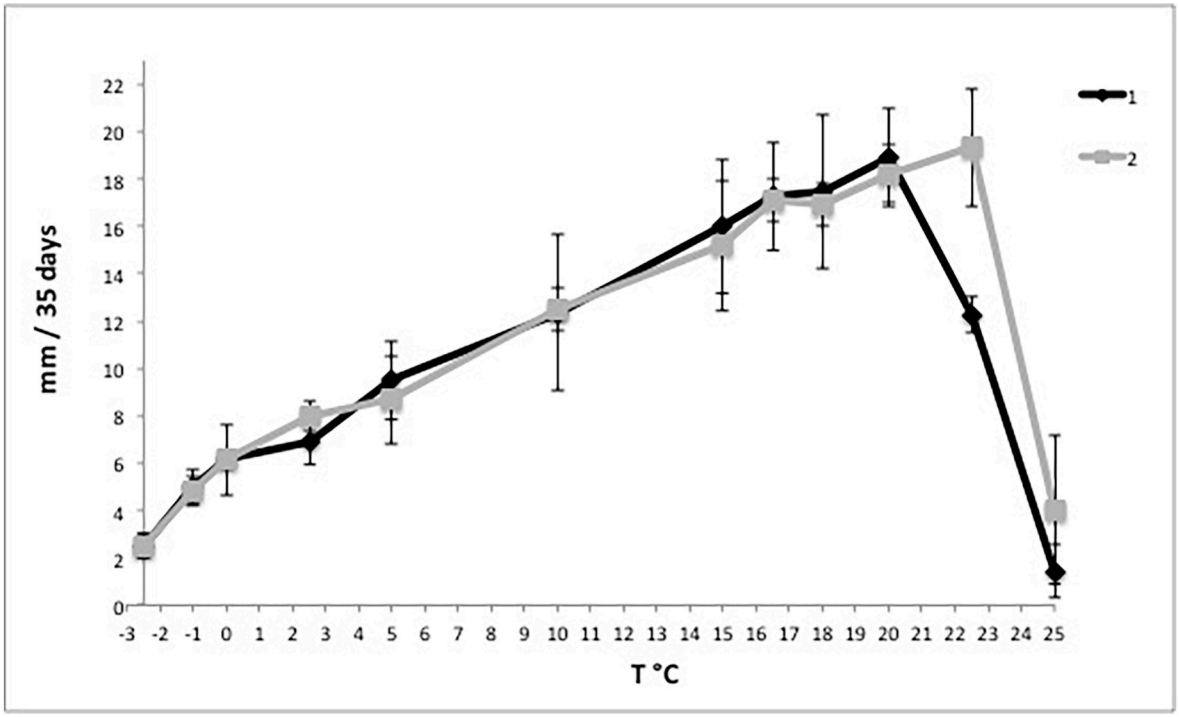

Fig. 5. Linear growth rate of strains 1 and 2 at different temperatures (diameter in mm/35 days).

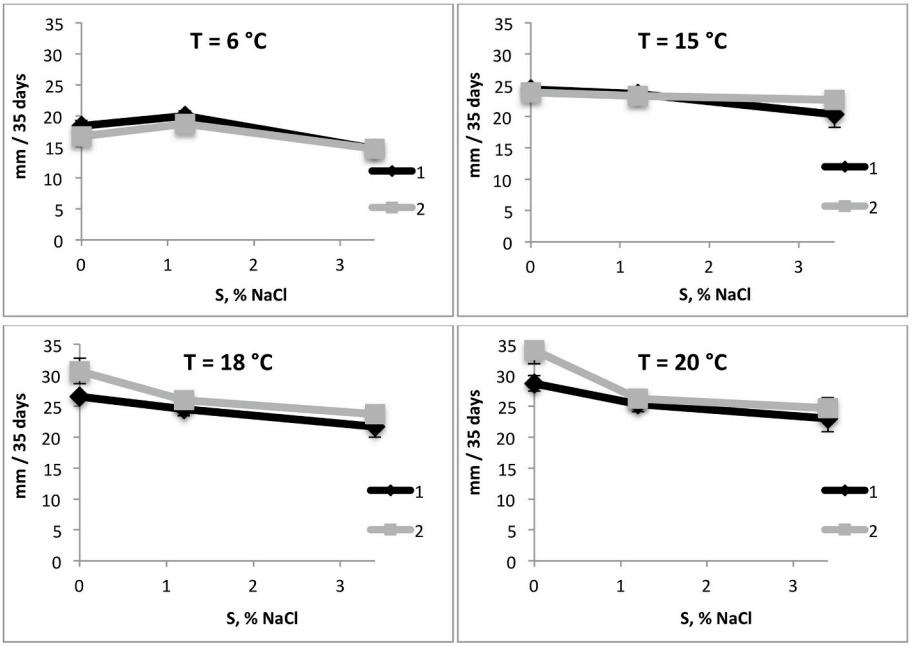

Fig. 6. Linear growth rate of strains 1 and 2 at different temperatures and salinities (diameter); T – temperature, S – salinity.

holotype, living culture ex-type CBS 122314).

Psychrophilomyces luteus (M.M. Wang & Xing Z. Liu) O.A. Grum-Grzhim. *comb. nov*. Mycobank MB 856663.

Basionym: *Psychrophila lutea* M.M. Wang & Xing Z. Liu, Persoonia 34: 106. 2014.

Type: China, Sichuan, Hailuogou Glacier, N29°33′ E101°58′, from soil, Apr. 20, 2011, M. Wang (dried culture HMAS244372 holotype, living culture ex-type CGMCC315134 = HAILUO409).

Psychrophilomyces olivaceus (M.M. Wang & Xing Z. Liu) O.A. Grum-Grzhim. *comb. nov*. Mycobank MB 856664.

Basionym: *Psychrophila olivacea* M.M. Wang & Xing Z. Liu, Persoonia 34: 107. 2014.

Type: China, Sichuan, Hailuogou Glacier, N29°33′ E101°58′, from soil, Apr. 20, 2011, M. Wang (dried culture HMAS244375 holotype, living culture ex-type CGMCC315135 = HAILUO368).

4. Discussion

Interest in studying and comparing fungal communities in extremely cold habitats, such as high altitude and polar regions, is constantly

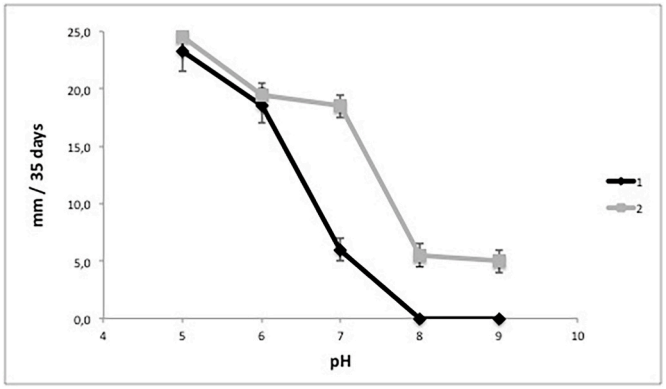

Fig. 7. Linear growth rate of strains 1 and 2 at different pH values (diameter).

increasing for several reasons. Firstly, the mycobiota of these territories is understudied due to their inaccessibility (Bridge and Spooner, 2012). Some studies show that fungi can play an important ecological role in cold regions as decomposers, symbionts, and parasites (Barbosa et al., 2017; da Silva et al., 2022; Makhalanyane et al., 2016; Robicheau et al., 2019; Tsuji and Hoshino, 2019; etc.). However, due to the limited amount of mycological research, it is difficult to assess the contribution of fungi in high altitude and polar regions to global biospheric processes (da Silva et al., 2019). Secondly, cold-adapted fungi may have unique biochemical and physiological characteristics and may be applied in biotechnology, for instance, as cold-active enzymes, materials for new drugs, biosurfactants, as well as in molecular biology, food industry, beverage production, detergents, etc. (Tsuji et al., 2022; Sarsan et al., 2024). Therefore, the importance of cold-adapted fungi for both the biosphere and human use are evident. Another reason for the increasing interest in these fungi is related to the discovery of identical species known only in such remote places as high altitude and polar regions of the Earth, which is interesting from the perspective of the evolution, adaptation, distribution, and dispersal of these fungi (Wang et al., 2015; Cox et al., 2016). And finally, with global warming, the activity of cold-adapted and mesophilic fungi is changing, affecting the intensity of greenhouse gas emissions and other global processes (Dunleavy and Mack, 2021; Semenova et al., 2015; Wild et al., 2014). Therefore, studying the diversity of fungi in extremely cold habitats is important for a better understanding of the effect of global warming, which appears to lead to a reduction in fungal species diversity and loss of psychrophilic species, as shown in a recent study by Tsuji et al. (2022).

The fungal diversity of Walker Glacier in the Canadian High Arctic was studied recently by Tsuji et al. (2022). They demonstrated the presence of unique ice-dependent mycobiota on this glacier, with strains that were unable to compete or survive in the glacial environment because of warming. The accelerating retreat of Arctic glaciers due to global warming is leading to habitat loss and may ultimately result in the extinction of these cold-adapted fungi. In other areas along the margins of the Little Ice Age in Canada and Greenland, the most high-latitude coastal region of the Arctic, there is recent evidence of complete loss of certain types of ecosystems and widespread ice depletion (Vincent and Mueller, 2019). Thus, psychrophilic fungal taxa of high-mountain and polar regions are gradually losing their habitat, underscoring the importance of local biodiversity research. Conservation efforts should include genomic research and cryopreservation of samples of Arctic ecological microbiomes, as well as the further isolation of fungal strains from these habitats for cultivation and analysis (Tsuji et al., 2022).

Cold-adapted enzymes have many unique characteristics due to their thermolabile nature and high activity at very low temperatures. Psychrophilic enzymes exhibit higher and faster activity than mesophilic or thermophilic enzymes, even at low enzyme concentrations under optimal conditions, making them more efficient and cost-effective (Sarsan et al., 2024). All of these reasons emphasize the importance of preserving and studying cold-adapted fungal species.

In 2015, Wang et al. described the genus *Psychrophilomyces* (Helotiales) containing three psychrotolerant species: *P. antarcticus* (the type species), *P. luteus*, and *P. olivaceus* while studying fungi from the Qinghai-Tibet Plateau. *P. antarcticus* was also found by these researchers in soil samples from Antarctica. Since these authors provided a detailed morphological description of the fungal species they discovered, sequences of four gene loci (ITS, LSU, β-TUB, and TEF1), and results of incubation at temperatures of 4, 10, and 20 °C, we were able to compare our Arctic strains with their data based on these parameters.

The high similarity at four gene loci, morphology, and optimal growth temperature of our Arctic fungal cultures with Antarctic and Tibetan cultures of *P. antarcticus* warrant them to be classified as this species. Since we could not find mentions of *P. antarcticus* in other regions, we provisionally consider this species to be known only in Tibet, Antarctica, and the Arctic. In terms of temperature, this fungus belongs to psychrotolerant species, so it is most active in cool conditions (10–23 °C), functions slowly at low temperatures (−2.5-5 °C), and quickly loses the ability to grow at temperatures above 25 °C. According to our growth study results at different salinity levels, *P. antarcticus* belongs to halotolerant[3] species and can grow in salty ecosystems up to at least 3.4% (w/v) NaCl. It is interesting that many well-known halotolerant species of fungi, originally discovered in saltworks, have later been found in Arctic glacier ice and other polar environments. This can be explained by the low water activity as a common critical parameter in both environments, and therefore the xerophilic nature of these species. For example, in polar glaciers, abundant marine and other halotolerant yeasts capable of growth at salinities of 5% and 10% NaCl in combination with temperatures of 4, 10, and 24 °C have been found (Butinar et al., 2007). Examples of halotolerant mycelial fungi may include cosmopolitan species of the genus *Cladosporium*. For instance, *Cladosporium halotolerance*, initially isolated from hypersaline habitats, has subsequently been repeatedly found in Antarctica (Zalar et al., 2007; Gunde-Cimerman and Zalar, 2014; Grum-Grzhimaylo et al., 2024). This species can tolerate salinities up to 20% NaCl, but thrives in non-saline environments as well (Zalar et al., 2007). More examples of halotolerant fungi, known in both hypersaline and cold habitats, can be found in publications by Coleine et al. (2022), Gunde-Cimerman and Zalar (2014). We have not tested the maximum salinity that *P. antarcticus* can tolerate, but it would be interesting to conduct such a study in the future. The observed sliming of colonies during an increase in salinity, as well as at temperatures close to zero, may represent an adaptation of this fungus to extreme conditions. With respect to pH, our cultures are considered acidotolerant.[4] Thus, *P. antarcticus* can be classified as a polyextremotolerant[5] species at least in relation to three factors - low temperature, high salinity, and high acidity.

The obtained data partially correlate with the conditions characteristic of the ecotope from which our cultures were isolated: a cold region and a salinity of approximately 1.2% NaCl. The results of testing our cultures at different pH values correlate with the results of our experiment on other fungi from the Shokalsky Island, most of which achieve maximum growth rates at pH 5 (unpublished data). We cannot correlate with the pH of the sampling areas from which our fungi were isolated, as we do not have data on their pH. However, Shokalsky Island is predominantly covered with oligotrophic tundra lakes, with a pH ranging from 5.2 to 6.8 (gdanskiynp.ru). Additionally, this island is located near the mouth of the Ob River into the Kara Sea, which significantly freshens

[3] Halotolerant fungi can grow in salt environments (1.2–2.9% NaCl) such as coastal dunes and saline deserts but that can still grow without salt (Coleine et al., 2022).

[4] Acidotolerant fungi can live in acidic habitats but also able to grow under neutral or even alkaline pH (Coleine et al., 2022).

[5] Polyextremotolerant species thrive under multiple extreme conditions (Coleine et al., 2022).

the seawater and potentially lowers its pH. These facts suggest that the sediments pH at the sampling site was likely reduced.

The two arctic strains show similarities of 99% (a difference of 2 nucleotides) for ITS and LSU, 100% for β-TUB, and 98% for TEF1 (a difference of 6 nucleotides; Supplem. material 3B), while both cultures are 98–100% similar to *P. antarcticus* strains from Genbank (Supplem. material 3A). In terms of morphology, cultures 1 and 2 differ slightly in colony color, texture, and odor. No significant differences in sporulation were found. In terms of physiology, there are differences in the optimal temperature value and the ability to grow at high pH values (Figs. 4 and 6). The phenotypic differences we described may be adaptive traits of these strains to environmental variation within the normal range of response.

It is difficult to explain how a particular species ended up in such geographically distant places from each other. Cox et al. (2016), who compared the mycoflora of Antarctica and the Arctic, suggest that microorganisms with well-developed dispersal abilities can inhabit the opposite poles of the Earth and then thrive there as a result of environmental selection. However, the organization of phialospores in slimy heads in *P. antarcticus* do not suggest effective dispersal, for example, through the wind. It is possible for fungi to be transported from one pole to another on floating materials or aquatic animals, but this dispersal mode is not possible to the high-altitude Qinghai-Tibet Plateau. Fungal spores may also be spread on the bodies of migratory birds. Such a mode of dispersion between the Qinghai-Tibet Plateau and both polar regions is hypothetically possible if there are bird species that make such migrations. Our results are consistent with Baas-Becking's statement (1934) on microbial biogeography "everything is everywhere, but the environment selects" (Baas-Becking, 1934). However, there has been debate over the correctness of this statement for many years (Cox et al., 2016). Thus, the question of the origin or dispersal of fungi known only in different poles of the Earth and high mountain regions remains unresolved.

Summarizing our research, *Psychrophilomyces antarcticus* a polyextremotolerant species, is found in high alpine, Antarctic, and Arctic regions. Arctic strains have been deposited in two established microbial collections; thus, they can be considered preserved for further research.

CRediT authorship contribution statement

Olga A. Grum-Grzhimaylo: Writing – original draft, Visualization, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Ekaterina N. Bubnova:** Writing – review & editing, Visualization, Investigation. **Alexey A. Grum-Grzhimaylo:** Data curation. **Alfons J.M. Debets:** Writing – review & editing, Supervision. **Duur K. Aanen:** Writing – review & editing, Supervision.

Disclosure statement

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Declaration of interest

None.

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Appendix A. Supplementary data

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