

# *Mrakia hoshinonis* sp. nov., a novel psychrophilic yeast isolated from a retreating glacier on Ellesmere Island in the Canadian High Arctic

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## Abstract

Four strains isolated from sediment sampled at the front of a retreating glacier on northern Ellesmere Island in the Canadian high Arctic, namely JCM 32575<sup>T</sup>, JCM 32576, JCM 32577 and JCM 32578, belong to a novel psychrophilic basidiomycetous yeast species in the genus *Mrakia*. Molecular phylogenetic analysis indicated that these strains are most closely related to the type strains of *Mrakia aquatica* and *Mrakianic combsii*, but with 8–9 and 7–12 nt substitutions in ITS and in the D1/D2 domain of the LSU rRNA gene, respectively. The strains grew at sub-zero temperatures and in vitamin-free media, with lipase and cellulase highly active even at –3 °C. These characteristics likely allow this yeast species to grow and survive in extremely cold, oligotrophic environments, such as the fronts of retreating glaciers in the high Arctic. The name *Mrakia hoshinonis* sp. nov. is proposed, with type strain JCM 32575<sup>T</sup> (UAMH 11969) and MycoBank number MB 825484.

## INTRODUCTION

The history of the genus *Mrakia* can be traced back to Komagata and Nakase [1], who identified *Candida curiosa* as a new species in frozen food. Subsequently, di Menna [2] classified three isolates from Scott Base on Ross Island in Antarctica as new species based on physiological characteristics: *Candida nivalis*, *Candida gelida* and *Candida frigida*. However, Fell *et al.* [3] observed that *Candida* species isolated from Antarctic soil exhibit a heterobasidiomycetous lifecycle, and hence reclassified *Candida stokesii*, *Candida nivalis*, *Candida gelida* and *Candida frigida* as *Leucosporidium stokeii*, *Leucosporidium nivalis*, *Leucosporidium gelidum* and *Leucosporidium frigidum*, respectively. Some two decades later, these taxa were again reclassified to a new genus, *Mrakia*, as *Mrakia stokesii*, *Mrakia nivalis*, *Mrakia frigida* and *Mrakia gelida*, since these isolates harbour a CoQ<sub>8</sub> system while other *Leucosporidium* species harbour CoQ<sub>9</sub> or CoQ<sub>10</sub> [4]. Finally, the genus *Mrakiella* was established to accommodate species of unknown sexual cycles in the *Mrakia* clade [5], but was eventually merged into *Mrakia* [6].

*Mrakia* strains have been isolated from a variety of extreme cold environments, including the Arctic, Siberia, the Alps,

Alaska, Patagonia and Antarctica [7–12]. It was estimated that *Mrakia* species account for approximately 24 % of culturable yeasts in soil from Ross Island, Antarctica [13]. Similarly, about 35 % of culturable fungi around Skarvsnes in East Antarctica are *Mrakia* [14]. These results imply that *Mrakia* is well adapted to the polar environment. To date, the genus *Mrakia* consists of nine species: *Mrakia aquatica*; *Mrakia arctica*; *Mrakia blollopis*; *Mrakia cryoconiti*; *Mrakia frigida*; *Mrakia gelida*; *Mrakia niccombsii*; *Mrakia psychrophila*; and *Mrakia robertii* [4, 6, 9, 15, 16].

We here describe four yeast colonies isolated from a retreating glacier in the Canadian high Arctic. Based on physiological testing and molecular analysis of ITS and the D1/D2 domain of the LSU rRNA gene, these strains were classified as a new basidiomycetous yeast species in the genus *Mrakia*, for which the name *Mrakia hoshinonis* sp. nov. is proposed.

## METHODS

### Yeast isolation

Sediment samples from the melting ice face and terminal deposits of Walker Glacier (unofficial name; 83° 00.601' N; 72° 12.387' W) on northern Ellesmere Island in the Canadian Arctic were collected on 16 July 2016 and aseptically

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**Keywords:** Arctic yeast; Basidiomycota; Cystofilobasidiales; rRNA gene D1/D2 domain; taxonomy.

**Abbreviation:** ITS, internal transcribed spacer.

The GenBank/EMBL/DDBJ accession numbers for the sequence of the ITS region and the D1/D2 domain of large subunit (LSU) rRNA gene are LC335798 for JCM 32575 (UAMH 11969), LC335799 for JCM 32576 (UAMH 11970), LC335800 for JCM 32577 (UAMH11971), and LC335801 for JCM 32578 (UAMH 11972). The MycoBank number for *Mrakia hoshinonis* sp. nov. is MB 825484

transferred into sterile 5 ml tubes; further site and sampling details are given in Tsuji *et al.* [17]. Within 1 h of sampling, samples were stored at  $-20^{\circ}\text{C}$  until analysis. Fungi were isolated from sediment samples taken at locations that were 40, 55, 70 and 132 m distance from the glacier terminus. The sediment samples (0.1 g) were directly plated on potato dextrose agar (Difco, Becton Dickinson) containing  $50\ \mu\text{g}\ \text{ml}^{-1}$  chloramphenicol, and incubated at  $10^{\circ}\text{C}$  for up to 3 weeks. Based on colony morphology, four yeast strains white to cream in colour were purified by repeated streaking on fresh potato dextrose agar. The resulting pure cultures, which we here propose as the new species, *Mrakia hoshinonis*, were deposited at the Japan Collection of Microorganisms, Riken, Japan, and at the UAMH Centre for Global Microfungal Biodiversity, University of Toronto, Canada.

### DNA sequencing and phylogenetic analysis

DNA was extracted using ISOPLANT II (Wako Pure Chemical Industries) according to the manufacturer's protocol. The sequences of the ITS region and the D1/D2 domain were amplified by PCR as described previously [14], using KOD-plus DNA polymerase (Toyobo) and the primers ITS1F (5'-GTAACAAGGTTTCCGT) and NL4 (5'-GGTCCGTGTTCAAGACGG), respectively. Amplicons were purified on Sephadryl S-400HR (Sigma-Aldrich), and sequenced on an ABI Prism 3130xl Sequencer (Applied Biosystems).

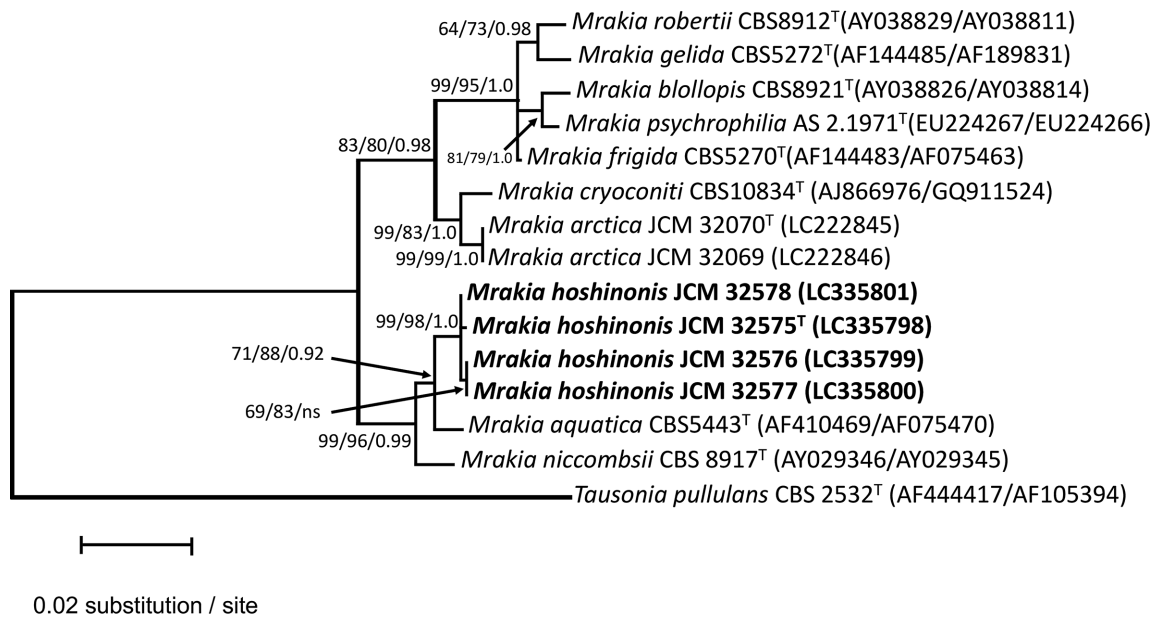
Concatenated ITS and D1/D2 sequences were aligned in MAFFT version 7.273 [18] using the L-INS-I algorithm and analysed in MEGA 7 [19] using a maximum-likelihood

HKY+G+I model and a maximum-parsimony TBR model. Bayesian inference trees were reconstructed in MrBayes 3.2.5 [20] using a GTR+I+G model, with 5 000 000 generations, two independent runs, four chains and default values for all other parameters. We discarded 25 % of the resulting trees, and a 50 % majority rule consensus tree was calculated from remaining trees to estimate posterior probabilities. Tree nodes were tested by bootstrap analysis with 1000 replicates, and a bootstrap percentage  $\geq 50\%$  or Bayesian posterior probability  $\geq 0.9$  was considered supportive.

Sequence similarity and nucleotide variations in ITS and D1/D2 sequences among isolates most closely related to *M. hoshinonis* were calculated using the EMBOSS water alignment tool ([www.ebi.ac.uk/Tools/psa/emboss\\_water/nucleotide.html](http://www.ebi.ac.uk/Tools/psa/emboss_water/nucleotide.html)).

### Morphological observation, physiological characteristics and mating tests

Growth was measured on potato dextrose agar from  $-3$  to  $30^{\circ}\text{C}$ . Carbon assimilation, nitrogen assimilation and other physiological characteristics were quantified according to standard methods [21] after 2 weeks at  $15^{\circ}\text{C}$  in glass vials containing appropriate media. Sexual state was examined after growth for up to 8 weeks at  $15^{\circ}\text{C}$  on yeast malt agar ( $3\ \text{g}\ \text{l}^{-1}$  yeast extract,  $3\ \text{g}\ \text{l}^{-1}$  malt extract,  $5\ \text{g}\ \text{l}^{-1}$  peptone,  $10\ \text{g}\ \text{l}^{-1}$  glucose and  $20\ \text{g}\ \text{l}^{-1}$  agar), 5 % malt extract agar (5 % MA,  $50\ \text{g}\ \text{l}^{-1}$  malt extract and  $30\ \text{g}\ \text{l}^{-1}$  agar) and corn meal agar (Difco). Data were collected from three independent vials or plates.



**Fig. 1.** Phylogenetic tree based on the concatenated sequences of the ITS region and the D1/D2 domain of the LSU rRNA gene in *Mrakia arctica* and closely related species. *Mrakia* strains described in this manuscript are highlighted in bold. *Tausonia pullulans* CBS 2532 was used as an outgroup. Numbers indicate bootstrap percentages over 50 % from maximum-likelihood and maximum-parsimony analyses with 1000 bootstrap replicates, as well as posterior probabilities above 0.9 from Bayesian-inference analysis. The tree backbone was reconstructed in MEGA 7.

**Table 1.** Nucleotide substitutions in the sequences of ITS region and the D1/D2 domain of the LSU rRNA gene in type strains of *Mrakia*

Values above the diagonal are number of nucleotide substitutions in the D1/D2 domain of the LSU rRNA gene. Values below the diagonal are number of nucleotide substitutions and sequence similarity (% in parentheses) in the sequences of the ITS region.

Species	<i>M. hoshinonis</i>	<i>M. aquatica</i>	<i>M. arctica</i>	<i>M. blollopis</i>	<i>M. cryoconiti</i>	<i>M. frigida</i>	<i>M. gelida</i>	<i>M. niccombsii</i>	<i>M. psychrophila</i>	<i>M. robertii</i>
<i>M. hoshinonis</i>	–	7	12	15	14	14	14	12	15	15
<i>M. aquatica</i>	8 (98.7)	–	16	19	16	18	18	10	18	19
<i>M. arctica</i>	47 (92.4)	52 (91.7)	–	10	7	7	7	13	10	8
<i>M. blollopis</i>	53 (91.4)	53 (91.8)	35 (94.4)	–	11	3	3	15	2	4
<i>M. cryoconiti</i>	50 (91.7)	49 (92.0)	16 (97.4)	34 (94.4)	–	10	10	14	11	11
<i>M. frigida</i>	50 (91.9)	50 (92.3)	30 (95.2)	11 (98.3)	31 (94.9)	–	0	14	3	1
<i>M. gelida</i>	53 (91.4)	53 (91.8)	38 (93.9)	15 (97.7)	37 (93.9)	16 (97.5)	–	14	3	1
<i>M. niccombsii</i>	9 (98.5)	8 (98.8)	52 (91.7)	54 (91.7)	50 (91.8)	52 (92.0)	52 (92.0)	–	13	18
<i>M. psychrophila</i>	42 (91.3)	53 (91.3)	30 (94.9)	8 (98.7)	31 (94.6)	11 (98.2)	8 (97.0)	55 (91.0)	–	4
<i>M. robertii</i>	53 (91.4)	52 (92.0)	40 (93.5)	17 (97.3)	37 (93.9)	17 (97.4)	18 (97.2)	53 (91.8)	17 (97.2)	–

### Secretion of extracellular enzymes

Extracellular cellulase, protease, and lipase activities were quantified on agar plates at –3, 4, 10, 15 and 20 °C for 3 weeks. Cellulose degradation was assessed as clear zones on yeast peptone dextrose agar (Difco) supplemented with 5.0 g l<sup>-1</sup> carboxymethylcellulose and stained with Congo red. Similarly, protease activity was assessed as clear zones on peptone dextrose agar containing 10 gl<sup>-1</sup> skim milk (Difco, Becton Dickinson), while degradation of long-chain esters was evaluated as opaque haloes on agar plates containing 10 gl<sup>-1</sup> Tween 80, 10 g l<sup>-1</sup> peptone, 5 g l<sup>-1</sup> NaCl, 0.10 g CaCl<sub>2</sub>·2H<sub>2</sub>O and 20 g l<sup>-1</sup> agar. Enzyme activities were calculated as (diameter of a clear or opaque zone – diameter of the colony)/diameter of the colony. Values >2.0, 1.0–2.0 and ≤1.0 were considered strongly positive, positive and weakly positive, respectively. Lack of clearing was considered to indicate an absence of extracellular enzymes. Data were collected from three individual experiments.

## RESULTS AND DISCUSSION

### DNA sequencing and phylogenetic analysis

A total of 325 fungal strains were isolated from nine sediment samples collected at the Walker Glacier site. Of these strains, 111 were classified as *Mrakia* (taxonomy: Basidiomycota, Agaricomycotina, Tremellomycetes, Cystofilobasidiales) by analysis of sequences of ITS and the D1/D2 domain of the LSU rRNA gene. Four strains (JCM 32575<sup>T</sup>, JCM 32576, JCM 32577 and JCM 32578) out of 111 *Mrakia* strains were classified as the new *Mrakia* species. JCM 32575<sup>T</sup> was isolated from a sample taken at 40 m, JCM 32576 was isolated from a sample taken at 55 m, JCM 32577 was isolated from a sample taken at 70 m and JCM 32578 was isolated from a sample taken at 132 m distant from the retreating glacier terminus. Phylogenetic analysis also indicated that these strains branched off *M. aquatica*, with 71 % bootstrap percentage, 88 % bootstrap percentage, and 0.92 Bayesian posterior probability in maximum-likelihood,

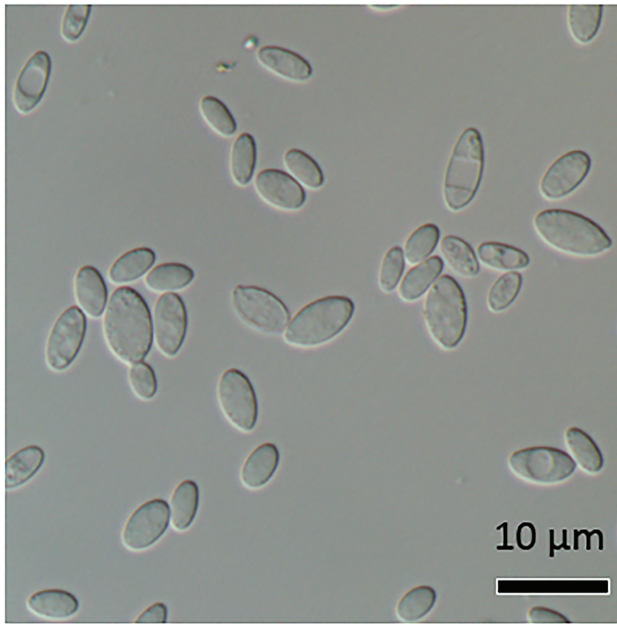
maximum-parsimony and Bayesian-inference trees, respectively (Fig. 1).

The strains were most closely related to *M. aquatica* and *M. niccombsii*, against which 7 and 12 nt substitutions were observed in the D1/D2 domain (Table 1). In addition, analysis of the same sequences in JCM 32575<sup>T</sup> showed 2 nt substitutions and one gap against JCM 32576, two gaps against JCM 32577 and one gap against JCM 32578. On the other hand, the sequence of the ITS region in JCM 32575 contained 8 and 9 nt substitutions in comparison to that of the ITS region in *M. aquatica* and *M. niccombsii*, respectively, with sequence identities of 98.7 and 98.5 %. Analysis

**Table 2.** Nutrient assimilation and growth characteristics that differentiate *Mrakia hoshinonis* from closely related species, *M. aquatica* and *M. niccombsii*

+, Positive; w, weak; s, slow; -, negative. Data are from Fell and Margesin [21], Thomas-Hall et al. [9] and this study.

Characteristics	<i>M. hoshinonis</i>	<i>M. aquatica</i>	<i>M. niccombsii</i>
Glucose fermentation	–/w	–/w	–
Assimilation of:			
Inulin	+	–	w
L-Rhamnose	+	–	+/w
D-Ribose	+	–	+/w
myo-Inositol	+	–	w
N-Acetyl-D-glucosamine	+	–	w
D-Glucosamine	–	–	+/w
Ribitol	+	+	–
Succinate	–	s	w
Growth:			
In vitamin-free media	+	–	–
On 50 % glucose	+	–	–
At 20 °C	+	+	+
At 25 °C	–	–	–



**Fig. 2.** Morphology of *Mrakia hoshinonis*. Photomicrograph of *M. hoshinonis* JCM 32575<sup>T</sup> grown on cornmeal agar for 10 days at 15 °C.

of the sequence of the ITS region of *M. psychrophila* indicated 8 nt differences and 98.7 % identity against *M. blollopis*, and 8 nt substitutions and 97.0 % sequence identity against *M. gelida* (Table 1). In contrast, 2 nt substitutions were noted between the sequences of the ITS region from JCM 32575<sup>T</sup> and JCM 32576, along with 1 nt substitution between JCM 32577 and JCM 32578. Based on these results, JCM 32575<sup>T</sup>, JCM 32576, JCM 32577 and JCM 32578 should be considered a new species, for which the name *Mrakia hoshinonis* sp. nov. is proposed. The type strain is JCM 32575<sup>T</sup> (UAMH 11969), with MycoBank number MB 825484.

*Mrakia hoshinonis* assimilated nitrate, produced starch-like compounds, grew maximally below 25 °C, and reacted with diazonium blue B and urease substrates, consistent with the characteristics of the genus *Mrakia* [21, 22]. In addition, the type strain JCM 32575<sup>T</sup> assimilated L-rhamnose, D-ribose, inulin, *myo*-inositol (weakly), *N*-acetyl-D-glucosamine and ribitol, and grew on vitamin-free 50 % w/v glucose agar but did not ferment glucose or consume D-glucosamine or citrate. In contrast, *M. aquatica* did not ferment or ferments glucose only weakly; did not grow in vitamin-free, 50 % w/v glucose agar; and did not assimilate L-rhamnose, D-ribose, inulin, *myo*-inositol or *N*-acetyl-D-glucosamine, but did assimilate succinate. Similarly, *M. niccombsii* did not ferment glucose; assimilated L-rhamnose, D-ribose, inulin (weakly), *myo*-inositol and *N*-acetyl-D-glucosamine, but not ribitol; and grew in vitamin-free, 50 % w/v glucose agar (Table 2). *M. hoshinonis* grew optimally at 15 °C but did not grow above 20 °C.

The optimum temperature for lipase secretion by *M. hoshinonis* was 10 °C, with evidence of strong lipase activity even at -3 °C (Table 3). In comparison, we previously reported that lipase activity in *Mrakia* from soils in Skarvsnes in the Antarctic was higher at 4 °C than at 15 °C [12, 23, 24]. *M. hoshinonis* also exhibits strong cellulase activity between -3 and 20 °C, although activity is maximal at 20 °C (Table 3). Protease activity was optimal at 10 °C. Of note, we previously reported that *Mrakia arctica* isolated from the same region of the Canadian Arctic has strong protease activity between 4 and 20 °C [16]. In contrast, Singh *et al.* [11] reported that *M. blollopis* isolated from the Norwegian Arctic has weak protease activity, while we found that an Antarctic *M. blollopis* strain lacks the protease K gene [14]. Similarly, *Mrakia* species in East Ongul Island, East Antarctica have weak protease activity even at 20 °C [16], perhaps implying that strong protease activity is unique to *Mrakia* species in the Canadian high Arctic.

## DESCRIPTION OF *MRAKIA HOSHINONIS* SP. NOV.

*Mrakia hoshinonis* (ho.shi.no'nis. N.L. gen. n. *hoshinonis* of Dr Tamotsu Hoshino at National Institute of Advanced Industrial Science and Technology, Japan, in recognition of his contributions to the study of fungi in polar environments).

Yeast cells after 10 days on yeast-malt agar are ovoid to elongated, 5–6 μm × 3–4 μm and proliferate by polar budding (Fig. 2). Formation of teliospores and basidiospores not observed. Pseudohyphae and true hyphae are not formed. Streak culture for 1 week at 15 °C on 5 % MA produces colonies that are white to yellowish cream, round, convex and smooth with an entire margin.

Glucose and sucrose are not fermented or weakly fermented. Glucose, D-galactose, sucrose, D-arabinose (weakly), L-arabinose, cellobiose, lactose, maltose, melibiose, melezitose, raffinose, D-ribose, L-rhamnose, L-sorbose, trehalose, D-xylose, inulin, galactitol, D-glucitol, *myo*-inositol (weakly), D-mannitol, ribitol (weakly in some cases, strongly in others), D-xylitol, glycerol, starch, salicin, succinate, D-gluconate, D-glucuronate, *N*-acetyl-D-glucosamine, ethanol (weakly), potassium nitrate and sodium nitrate are assimilated. Methanol, erythritol, DL-lactate, levucic acid, citrate and methyl α-D-glucoside are not assimilated. No growth on 5 % glucose medium with 10 % NaCl w/v and 0.01 % cycloheximide. Diazonium blue B test and urease activity are positive. Amino acids and vitamins are not required. The maximum temperature for growth is 20 °C, and optimal growth occurs at 15 °C. Grows on 50 % w/v glucose agar and at -3 °C on potato dextrose agar.

The holotype, JCM 32575, was isolated from sediment sampled in front of the retreating Walker Glacier on Ellesmere Island (83° 00' N, 72° 12' W), Canada, and is preserved in a metabolically inactive state at the Japan Collection of Microorganisms, Riken, Japan. The ex-type

culture was deposited at the UAMH Centre for Global Microfungal Biodiversity, University of Toronto, Canada as UAMH 11969. Strains JCM 32576 (UAMH 11970), JCM 32577 (UAMH 11971) and JCM 32578 (UAMH 11972) were also deposited at the Japan Collection of Microorganisms and the UAMH Centre for Global Microfungal Biodiversity as paratype cultures. The MycoBank number is MB 825484.

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#### Conflicts of interest

The authors declare that there are no conflicts of interest.

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