



## Short communication

## *Gelidatrema psychrophila* sp. nov., a novel yeast species isolated from an ice island in the Canadian High Arctic

Masaharu Tsuji <sup>a,\*</sup>, Yukiko Tanabe <sup>a,b</sup>, Warwick F. Vincent <sup>c</sup>, Masaki Uchida <sup>a,b</sup><sup>a</sup> National Institute of Polar Research (NIPR), 10-3 Midori-cho, Tachikawa, Tokyo, 190-8518, Japan<sup>b</sup> Department of Polar Science, SOKENDAI (The Graduate University for Advanced Studies), 10-3 Midori-cho, Tachikawa, Tokyo, 190-8518, Japan<sup>c</sup> Département de Biologie & Centre for Northern Studies (CEN), Université Laval, Québec, QC, G1V 0A6, Canada

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## ABSTRACT

A new cold-adapted yeast species, *Gelidatrema psychrophila* sp. nov., was isolated from a melt-pool microbial mat community, on an ice island located in Disraeli Fjord, Ellesmere Island, in the Canadian High Arctic. Molecular analysis of the D1/D2 domain sequence of the large subunit rDNA showed that this species is novel and could grow at sub-zero temperatures and in vitamin-free media. These characteristics were likely acquired by the yeast to survive in extreme, perennially cold oligotrophic environments.

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Basidiomycetous yeasts have been widely reported to represent the dominant fungi in polar regions (di Menna, 1966; Singh, Tsuji, Singh, Roy, & Hoshino, 2013; Tsuji, Uetake, & Tanabe, 2016; Tsuji, Fujii, et al., 2013; Tsuji, Yokota, Shimohara, Kudoh, & Hoshino, 2013). Many have been found only in their asexual stage, and are classified as the anamorphic genera *Cryptococcus* Vuillemin or *Rhodotorula* Harrison (Boekhout et al., 2011; Fonseca, Boekhout, & Fell, 2011; Sampaio, 2011). *Cryptococcus* species are distributed across four orders: Tremellales, Trichosporonales, Filobasidiales and Cystofilobasidiales (Fonseca et al., 2011). Species isolated from cold environments belong to all these orders (Buzzini, Branda, Goretti, & Turchetti, 2012). The genus *Gelidatrema* A.M. Yurkov, X.Z. Liu, F.Y. Bai, M. Groenew. & Boekhout is classified in the order Tremellales as a single-species lineage. *Gelidatrema spencermartinsiae* (C. García, Brizzio, Boekhout, Theelen, Libkind & van Broock) A.M. Yurkov, X.Z. Liu, F.Y. Bai, M. Groenew. & Boekhout, whose former name was *Cryptococcus spencermartinsiae* C. García, Brizzio, Boekhout, Theelen, Libkind & van Broock, was isolated from glacier meltwater in Patagonia, Argentina, and from apples in the Netherlands (Liu et al., 2015; de García et al., 2010).

In this study, two yeast colonies, cream in color, were isolated from an ice island habitat in the Canadian High Arctic. By molecular analysis of the large subunit 26S rDNA (LSU D1/D2 domain) sequences and physiological testing, we classified these strains into a new basidiomycetous yeast species in the genus *Gelidatrema* and propose the name *Gelidatrema psychrophila* sp. nov.

The ice island was in Disraeli Fjord, northern Ellesmere Island, in the Canadian High Arctic (lat. 82°50'N, long. 73°40'W), and was a remnant of the Ward Hunt Ice Shelf that collapsed in 2011–12; maps and further details about this site are given in Vincent et al. (2011). To investigate fungal diversity on the island, the ice island was accessed by helicopter on 18 Jul 2016, and microbial mat samples from the bottom of a shallow (0.3 m depth), freshwater melt pool were aseptically transferred to sterile 5-mL sample tubes by 5 points. The mats formed a loose, several mm-thick flocculent layer over the ice at the bottom of the pool, with a thin orange surface layer that overlaid olive colored organic 'matlets', as described in Mueller, VincentWF, and Laurion (2005). Within 1 h of sampling, the tubes were transferred to a –20 °C freezer, and were stored at that temperature until subsequent analysis. Each 0.1-g frozen iceberg sediment sample was directly placed on potato dextrose agar (PDA, Difco, Becton Dickinson Japan, Tokyo) containing 50 µg/mL chloramphenicol and incubated at 10 °C for a period of up to 3 wk. Yeast samples were chosen for isolation based

\* Corresponding author.

E-mail address: [spindletuber@gmail.com](mailto:spindletuber@gmail.com) (M. Tsuji).

**Table 1**  
The two *Gelidatrema psychrophila* strains examined in this study.

<i>Gelidatrema psychrophila</i> strain	Isolation source	Locality	Accession numbers (Nucleotide position in LSU D1/D2)
JCM 32067 <sup>T</sup> = HUT 7417 <sup>T</sup>	Melt-pool microbial mat on an ice island	Disraeli Fjords, Ellesmere Island, Canada	LC222847 (440–1074)
JCM 32068 = HUT 7418	Melt-pool microbial mat on an ice island	Disraeli Fjords, Ellesmere Island, Canada	LC222848 (439–1073)

on colony morphology and each colony with a different morphology was purified by repeated streaking on fresh PDA. The cultures of *Gelidatrema psychrophila* are deposited at the Japan Collection of Microorganisms (JCM), Riken, Japan, and at the HUT Culture Collection (HUT), Hiroshima University, Japan. Strain numbers are shown in Table 1.

DNA was extracted from yeast colonies using an ISOPLANT II kit (Wako Pure Chemical Industries, Osaka, Japan) according to the manufacturer's protocol. The extracted DNA was amplified by polymerase chain reaction (PCR), using KOD-plus DNA polymerase (Toyobo, Osaka, Japan). The internal transcribed spacer (ITS) region and LSU D1/D2 domain primers, and PCR conditions were as described previously (Tsuji, Kudoh, & Hoshino, 2016). The amplified DNA fragments were purified using Sephadryl S-400HR (Sigma–Aldrich Japan, Tokyo). Sequences were determined using an ABI prism 3130xl Sequencer (Applied Biosystems, Life Technologies Japan, Tokyo). GenBank accession numbers for all the sequences analyzed in this study are listed in Table 1.

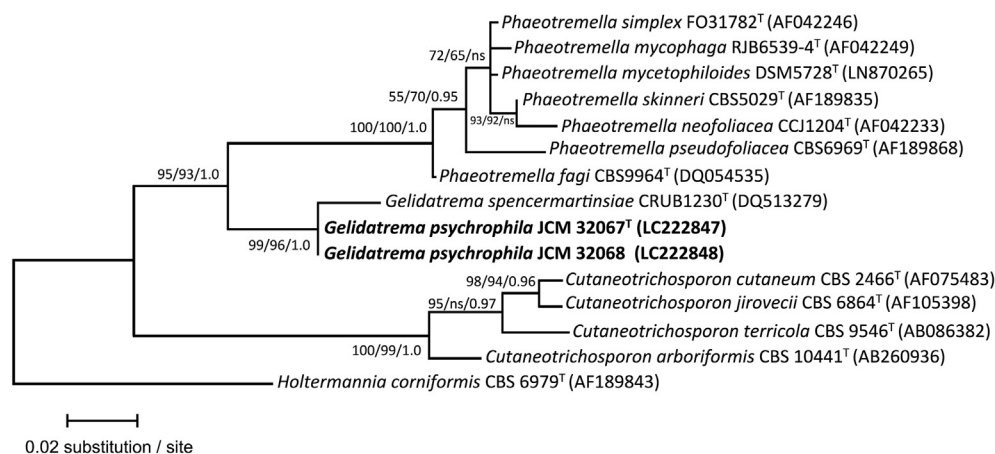
The LSU D1/D2 domain sequences were aligned with the MAFFT ver. 7.273 (Katoh & Standley, 2013) program using the L-INS-I algorithm and the alignments were deposited in TreeBASE (submission ID: S20821). Maximum likelihood (ML) with an HKY + G + I model and maximum parsimony (MP) analysis with a TBR model were performed using MEGA 7 (Kumar, Stecher, & Tamura, 2016). Bayesian inference (BI) was constructed using MrBayes 3.2.5 (Ronquist et al., 2012) with a GTR + I + G model and 5,000,000 generations, two independent runs, and four chains. The other parameters retained their default settings. We discarded 25% of these trees, with the remainder used to compute a 50% majority rule consensus tree to estimate posterior probabilities. A bootstrap analysis with 1000 replicates was performed to estimate the confidence of the tree nodes, and a bootstrap percentage (BP) of  $\geq 50\%$  or Bayesian posterior probability (BPP) of  $\geq 0.9$  was considered

supportive in all constructed trees in this study.

We also determined the sequence similarity and nucleotide variation in the LSU D1/D2 domain among the species most closely related to *G. psychrophila*, using the EMBOSS Water alignment tool ([http://www.ebi.ac.uk/Tools/psa/emboss\\_water/nucleotide.html](http://www.ebi.ac.uk/Tools/psa/emboss_water/nucleotide.html)).

The effect of temperature on the growth of fungi was determined on PDA plates in a range of  $-3\text{ }^{\circ}\text{C}$  to  $37\text{ }^{\circ}\text{C}$ . Carbon assimilation was assessed in glass vials with yeast nitrogen base liquid media according to standard methods (Kurtzman, Fell, Boekhout, & Robert, 2011), with an incubation period of 4 wk at  $17\text{ }^{\circ}\text{C}$ . Assimilation of nitrogen and other physiological tests were also carried out in glass vials containing liquid media with a yeast nitrogen base without amino acid (Kurtzman et al., 2011). Strains were examined for sexual state after growth and incubation  $17\text{ }^{\circ}\text{C}$  on the following media: yeast malt extract (YM) agar (3 g/L yeast extract, 3 g/L malt extract, 5 g/L peptone, 10 g/L glucose, and 20 g/L agar), 5% malt extract agar (50 g/L malt extract and 30 g agar/L), and corn meal agar (CMA, Difco). All experiments were carried out independently in three vials or on three plates.

A total of 85 fungal strains were isolated from the ice island mat samples that were collected in Disraeli Fjord, Ellesmere Island, Canada. Among these fungal strains, two strains isolated from different samples were classified as genus *Gelidatrema* according to phylogenetic analysis based on the LSU D1/D2 domain (Fig. 1). The strains cluster apart from other known strains, and were therefore considered a new species, which is here given name *G. psychrophila*. Based on the phylogenetic analysis of LSU D1/D2 domain sequences, *G. psychrophila* clearly branched from the *Phaeotremella* clade, supported by 95% BP, 93% BP, and 1.0 BPP using ML, MP and BI analysis, respectively (Fig. 1). Moreover, strains JCM 32067<sup>T</sup> and JCM 32068 were clearly branched from *G. spencermartinsiae* supported with 99% BP, 96% BP and 1.0 BI by ML, MP, and BI analyses, respectively (Fig. 1). By comparing their LSU D1/D2 domain sequences with those



**Fig. 1.** Phylogenetic tree based on the LSU D1/D2 domain. Maximum likelihood analysis of the LSU D1/D2 domain sequences of *Gelidatrema psychrophila* strains investigated in this study (in bold). *Holtermannia corniformis* CBS 6979 was designated as the outgroup. The tree backbone was constructed by maximum likelihood analysis with MEGA7. Bootstrap percentages of maximum likelihood and maximum parsimony analyses over 50% from 1000 bootstrap replicates and posterior probabilities of Bayesian inference above 0.9 are shown on the left on the branches. The scale bar represents 0.02 substitutions per nucleotide position. ns, not supported (bootstrap percentages <50% or Bayesian inference <0.9).

**Table 2**Number of nucleotide substitutions in the LSU D1/D2 region sequence among the species most closely related to *Gelidatrema psychrophila*.

Species	<i>G. psychrophila</i>	<i>G. spencermartinsiae</i>	<i>Phaeotremella fagi</i>	<i>P. mycetophiloides</i>	<i>P. mycophaga</i>	<i>P. simplex</i>
<i>G. psychrophila</i> JCM 32067 (LC 22847)	–	12	43	47	50	50
<i>G. spencermartinsiae</i> CRUB 1230 (DQ513279)		–	46	48	55	51
<i>Phaeotremella fagi</i> CBS 9964 (DQ05435)			–	10	16	15
<i>P. mycetophiloides</i> DSM 5728 (LN870265)				–	6	6
<i>P. mycophaga</i> RJB 6539-4 (AF042249)					–	11
<i>P. simplex</i> FO 31782 (AF042246)						–

Each value indicates the number of nucleotide substitutions (nt) between the indicated species pairs.

Column under species are indicated under species names.

Accession number of LSU D1/D2 domain is shown in parentheses for each species.

**Table 3**Comparison of physiological characteristics between *Gelidatrema psychrophila* and *G. spencermartinsiae*.

Species	Assimilation of			Growth on		
	Starch	Glycerol	<i>myo</i> -Inositol	Amino acid free	Vitamine-free	25 °C
<i>Gelidatrema psychrophila</i>	+	w	+	+	+	–
<i>G. spencermartinsiae</i>	–	–	w	+	+	w

Main physiological test results for the characteristics of *G. psychrophila* and *G. spencermartinsiae* are shown. Physiological data of related species were taken from de García et al. (2010) and this study. +, positive; w, weak; –, negative.

of related species, we showed that *G. psychrophila* differed 12 and 43 nucleotide substitutions with *G. spencermartinsiae* and *Phaeotremella fagi*, respectively (Table 2). Furthermore, *P. mycetophiloides* (Kobayasi) Millanes & Wedin was shown to differ by 6 nucleotides with *P. simplex* (H.S. Jacks. & G.W. Martin) Millanes & Wedin and *P. mycophaga* (G.W. Martin) Millanes & Wedin (Table 2). *Gelidatrema psychrophila* JCM 32067 differed by 3 nucleotide substitutions with JCM 32068 in ITS and LSU D1/D2 region.

The genus *Gelidatrema* is characterized by ovoid to ellipsoidal yeast cells. This genus can utilize D-glucuronate and *myo*-inositol and assimilate nitrate, but cannot produce starch-like compounds (de García et al., 2010). *Gelidatrema psychrophila* also showed these characteristics. Therefore, strains JCM 32067<sup>T</sup> and JCM 32068 were confirmed to belong to the genus *Gelidatrema* by both of phylogenetic analysis and physiological tests. The carbon assimilation patterns of *G. psychrophila* were highly similar to that of *G. spencermartinsiae*. However, glycerol was weakly assimilated, and starch was utilized by *G. psychrophila* (Table 3).

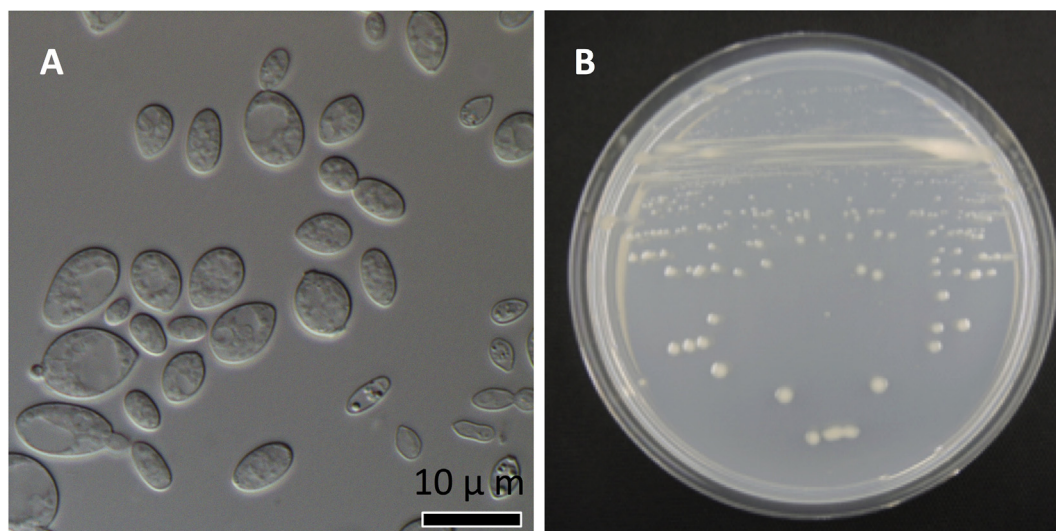
After incubation for 10 d on CMA at 17 °C, yeast cells of *G. psychrophila* exhibited an ovoid to elongated shape (6–10 μm × 5–8 μm) and reproduced by multilateral budding (Fig. 2). Furthermore, after 2 wk on YM agar, PDA, 5% malt extract agar and CMA, neither species exhibited produced ballistoconidia or pseudohyphae by agar plate and Dalmau plates test (Supplementary Figs. S1a–c). The optimum growth temperature of *G. psychrophila* was 17 °C. Besides, *G. psychrophila* did not grow at 25 °C, whereas *G. spencermartinsiae* weakly grew at 25 °C (Table 3). Additionally, *G. psychrophila* and *G. spencermartinsiae* can grow in cultures without amino-acid or in vitamins. These characteristics are likely to be suitable for surviving in oligotrophic glacial environments.

### Taxonomy

*Gelidatrema psychrophila* M. Tsuji, sp. nov.

Fig. 2.

Mycobank no.: MB 821524.



**Fig. 2.** Morphology of *Gelidatrema psychrophila*. *Gelidatrema psychrophila* grown on corn meal agar for 10 d at 17 °C. A. Photomicrograph. Bar = 10 μm. B. Growth on Petri dish.



Etymology: “*psychrophila*” refers to organisms that are capable of growth in cold temperatures, which is a characteristic of this species.

After 2 wk on CMA at 17 °C, colonies are shiny, mucoid, smooth, cream-colored and with an entire margin. Yeast cells after 10 d at 17 °C on CMA exhibit ovoid to ellipsoidal (6–10 µm × 5–8 µm), proliferating by multilateral budding. No positive mating reactions are observed. Ballistoconidia and pseudohyphae not produced.

Type: Canada, Ellesmere Island, 18 Jul 2016, isolated from an ice island microbial mat in Disraeli Fjord (holotype: strain JCM 32067<sup>T</sup> preserved in a metabolically inactive state at Japan Collection of Microorganisms, Riken, Japan; ex-type culture: HUT7417<sup>T</sup> deposited at the HUT Culture Collection, Hiroshima University, Japan; paratype: JCM 32068, HUT7418); ITS and LSU D1/D2 domain: LC222847 (JCM 32067<sup>T</sup>) and LC222848 (JCM 32068).

Glucose is not fermented. D-glucose, sucrose, galactose, trehalose, melezitose, starch, salicin, cellobiose, maltose, D-xylose, lactose, raffinose, melibiose, D-ribose, L-rhamnose, palatinose, glycerol (weak), ribitol, myo-inositol, L-arabinose, D-arabinose, galactitol, D-mannitol, sorbitol, succinic acid, D-glucuronate, N-acetyl-D-glucosamine, potassium nitrate, and sodium nitrate are assimilated. Inulin, methyl-α-D-glucoside, L-sorbose, ethanol, methanol, erythritol, D-glucosamine, DL-lactate, citrate and D-glucuronate, are not assimilated. Growth in vitamin-free medium is present. No growth occurs in 5% glucose medium with 10% NaCl (w/v), 50% (w/v) glucose medium and 0.01% cyclohexamide. Urease activity, acid production and diazonium blue B reaction are positive. Maximum growth temperature at 20 °C and optimal growth occurs at 17 °C. Growth on PDA is observed at –3 °C.

Habitat: Ice island melt-pool, Disraeli Fjords, northern Ellesmere Island, High Arctic Canada.

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## Appendix A. Supplementary data

Supplementary data related to this article can be found at <https://doi.org/10.1016/j.myc.2017.08.010>.

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