

## Two new species of basidiomycetous yeast *Mrakia* sp. isolated from Ward Hunt Lake in the Canadian High Arctic

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Ward Hunt Lake is located on Ward Hunt Island, off the northern coast of Ellesmere Island at the northern limit of North America (83°05.226'N; 74°08.721'W). The lake is 0.37 km<sup>2</sup>, of which the majority is shallow (i.e., <2 m), with a maximum depth of 9.7 m. Ward Hunt Lake is perennially ice-covered. However, the ice cover of Ward Hunt lake thinned from 2008 onward, and the lake became ice-free in 2011 (1,2).

In 2018, as part of a microbial survey in the lake, 3.3m depth of lake sediments were collected and transferred aseptically to sterile 5-mL sample tubes. Within one hour of sampling, the tubes were transferred to a -20°C freezer at the field laboratory and then stored at that temperature until subsequent analysis.

Subsamples (0.1 g) of the lake sediment were placed on potato dextrose agar (PDA; Difco, Becton Dickinson Japan, Tokyo, Japan) containing 50 µg/mL chloramphenicol and incubated at 10°C for a period of up to 3 weeks. We isolated fungi growing on the PDA based on colony morphology. Each colony with a different morphology was purified by repeated streaking on fresh PDA. DNA was extracted from fungal colonies using an ISOPLANT II kit (Wako Pure Chemical Industries, Osaka, Japan) according to the manufacturer's protocols. The extracted DNA was amplified by polymerase chain reaction (PCR) using KOD-plus DNA polymerase (Toyobo, Osaka, Japan). After that, the DNA was purified using Sephadryl S-400HR (Sigma-Aldrich Japan, Tokyo). Sequences were determined using an ABI Prism 3130xl Sequencer (Applied Biosystems, Life Technologies Japan, Tokyo).

A total of 102 fungal strains were isolated from the 2018 sediment samples. Based on the internal transcribed spacer (ITS) region and 26S rDNA D1/D2 domain sequence similarity, these strains were classified into 19 genera and 28 species. The dominant fungi belonged to the genera *Mrakia* (32.7%), *Vishniacozyma* (13.1%), and *Pseudogymnoascus* (9.3%).

Phylogenetic analysis of two strains showing low sequence homology with the ITS and D1/D2 regions. Result of the phylogenetic analysis, these two strains may be new species of the genus *Mrakia*. Further experiments will be conducted on these strains to propose them as new species, *Mrakia wardhuntensis* and *M. yamadae*.

### References

1. Paquette, M.D. Fortier, D. R. Mueller, D. Sarrazin, and W. F. Vincent, Rapid disappearance of perennial ice on Canada's most northern lake, *Geophys. Res. Lett.*, 42, 1433–1440, 2015.
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