

New records of *Oleoguttula mirabilis* and *Rachicladosporium antarcticum* from James Ross Island, Antarctica

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Abstract

A group of seven isolates of black meristematic fungi was collected from rocks within the framework of mycological research focused on the study of diversity of rock-inhabiting fungi on James Ross Island in Antarctica. Their identification was based on ITS rDNA sequence comparisons supported by morphological data. Obtained results assigned four analysed strains as *Oleoguttula mirabilis* and three isolates as *Rachicladosporium antarcticum*. To the best of our knowledge this is the second report of isolation of these two psychrophilic species from different parts of James Ross Island which imply that they probably represent a common part of its environmental microbial flora.

Key words: black yeast, ITS rDNA region, meristematic fungi, polar region, rock-inhabiting fungi

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Introduction

Rock-inhabiting fungi (RIF) represent a polyphyletic group of extremotolerant microorganisms which occur in different inhospitable environment such as cold or hot deserts (Ruibal et al. 2005, 2008; Selbmann et al. 2005, 2008, 2013, 2014; Egidi et al. 2014). Except natural habitats, a number of species have been isolated from marble monuments (Wollenzien et al. 1995, Sert et al. 2007, Isola et al. 2015) or

radiation-ravaged places (Zhdanova et al. 2000).

Antarctic rocks represent harsh habitat due to the presence of various stress factors, including nutrient deficiency, intense sunlight, high UV radiation, osmotic stress, temperature fluctuations or low water availability (Sterflinger 1998, 2006; Selbmann et al. 2008, 2011). Despite those adverse conditions, they are inhabited by various

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microbial communities including algae, bacteria, cyanobacteria, lichenised and non-lichenised fungi. Fungi colonise rocks on the surface (epilithic fungi) and/or inside the rock (endolithic fungi) (Friedmann 1982, Selbmann et al. 2005, 2013; Gorbushina 2007, Zucconi et al. 2014).

The ability of these fungi to survive in extreme conditions is given by their morpho-physiological properties. They are characterized by restricted growth leading to formation of small compact black colonies. The black coloration of cells is given by production of high amount of pigment melanin, which protects them against UV radiation (Selbmann et al. 2005, 2011). Meristematic growth characterised by isodiametrically dividing cells contributes to their resistance as well and enable them to survive desiccation and extreme temperatures (Urzi et al. 2000, Selbmann et al. 2005).

Many novel genera of RIF, such as *Cryomyces*, *Elasticomyces*, *Extremus*, *Oleoguttula*, *Recurvomyces*, *Vermiconidia*, and endemic genus *Friedmanniomyces* have been described from the cold environment of Antarctica in taxonomic papers of Selbmann et al. (2005, 2008) and Egidi et al. (2014). They are classified into the class *Dothideomycetes*, mainly within the order *Capnodiales* (Ruibal et al. 2009, Egidi et al. 2014, Quaedvlieg et al. 2014).

The genus *Oleoguttula* belongs to the family *Teratosphaeriaceae* within the order *Capnodiales* (Egidi et al. 2014, Quaedvlieg et al. 2014) The genus represented by a single psychrophilic species *Oleoguttula mirabilis* was proposed based on two isolates originated from rocks on James Ross Island by Selbmann and de Hoog (Egidi et al. 2014) and the name was validated by Crous et al. (2019). Recently, this species has also been recorded from ice with high ice algae abundance (called dark ice) from Greenland Ice Sheet (Perini et al. 2019). Phialidic conidiogenesis is the important morphological feature of this genus, which has not been observed in other RIF taxa

described from Antarctica and makes this species easy to distinguish from remaining RIF species (Egidi et al. 2014).

The genus *Rachicladosporium* belongs to the order *Capnodiales* and family *Cladosporiaceae* with phylogenetically closely related genera *Cladosporium*, *Dichocladosporium*, *Toxicocladosporium* and *Verrucocladosporium*. It was proposed by Crous et al. (2007) on a single isolate from leaf spots on *Luculia* sp. and assigned as *Rachicladosporium luculiae*. At present the genus comprise 14 species with validly published name that accommodate mainly epiphytic and rock-inhabiting fungi. A total, six new mostly psychrophilic species were described from rocks of which three species originated from the Italian Alps and two species from Antarctica, namely *Rachicladosporium mcmurdoi* and *Rachicladosporium antarcticum* (Egidi et al. 2014, Crous et al. 2019). *Rachicladosporium* is morphologically distinguishable from *Cladosporium* s. str. and related cladosporium-like genera by presence of apical rachis on conidiophores with inconspicuous to subconspicuous scars and unthickened, not darkened-refractive conidial hila (Crous et al. 2007). In contrast the rock-inhabiting species usually produce brown septate hyphae only, cladosporium-like cells are not observed (Egidi et al. 2014).

Morphological identification of many RIF species is difficult because some of them are highly pleomorphic or many species are morphologically scarcely differentiated and meristematic growth presents the only type of their reproduction (Urzi et al. 2000, Egidi et al. 2014). Therefore their identification is based primarily on phylogenetic analysis supported by morphological data.

In total, 115 isolates of black meristematic fungi were collected from rocks within the framework of mycological research focused on the study of diversity, taxonomy and phylogenetic relationships of rock-inhabiting fungi inhabiting James

Ross Island in Antarctica. For most isolates there were no phylogenetically close ITS rDNA sequences available in the GenBank nucleotide database enabling their reliable taxonomic identification (unpublished). Only seven RIF isolates de-

scribed in this study showed high similarity with reference sequences and could be identified to the species level. Taxonomic position of the remaining isolates was unclear and require further investigation.

Material and Methods

Sampling sites

The investigated area was located in the northern deglaciated part of the Ulu Peninsula, James Ross Island (64° 10' S, 57° 45' W), which is situated close to the northeastern extremity of the Antarctic Peninsula. The climate of the island is semiarid with the mean annual tempera-

ture around -7°C (Ambrožová and Láška 2016). The vegetation is represented by a great variety of terrestrial algae, cyanobacteria, lichens and mosses as the main components of communities forming Antarctic vegetation oases (Barták et al. 2015).

Sampling and isolation

Seven isolates of RIF analysed in this study were obtained from five samples of rock fragments collected in February and March during the Czech polar expedition in 2018. The rock samples were aseptically collected into sterile plastic tubes and preserved at 8 °C until isolation. RIF were isolated by the direct inoculation of rock fragments on Petri dishes with Dichloran rose bengal chloramphenicol agar (DRBC, Difco) and Malt yeast extract agar (MYEA, Selbmann et al. 2005). The plates were in-

cubated at 15°C under aerobic conditions and observed after four weeks. Axenic cultures of RIF were subcultured on agar plates with 2% Malt Extract Agar (MEA, Difco) and maintained at -70°C. Morphology of 12 weeks old isolates cultivated on MEA at 15°C was studied by microscopic observation in lactic acid using Olympus BX 50 light microscope. Microphotographs were done with a digital camera Artcam 500 MI.

DNA extraction and sequencing

Sequencing of the complete ITS rDNA region (ITS1-5.8S-ITS2) was used to delimit species of RIF's isolates and compare them with close relatives (de Hoog et al. 1999). Genomic DNA was isolated from 12 weeks old cultures grown on MEA plates at 15 °C. Mycelial fragments were disrupted using the FastPrep bead beating system (MP Biomedicals, Irvine, CA, USA) and purified with a High Pure PCR Tem-

plate Preparation Kit (Roche, Germany). The ITS rDNA region was amplified with the primers SR6R and LR1 (Vilgalys and Hester 1990). PCR conditions and sequencing method were performed according to Marvanová and Laichmanová (2014). Sequences obtained in this study were submitted to NCBI's GenBank nucleotide database (Table 1).

Species	Strain No.	Locality	ITS GenBank accession No.
<i>Aureobasidium subglaciale</i>	CBS 123387	Svalbard and Jan Mayen Island	NR_147323.1
<i>Cladosporium cladosporioides</i>	CBS 112388	Germany	NR_119839.1
<i>Cladosporium colombiae</i>	CBS 274.80B	Colombia	NR_119729.1
<i>Cladosporium verrucocladosporioides</i>	CBS 126363	South Korea	MH863939.1
<i>Oleoguttula mirabilis</i>	P10109	JRI, Antarctica (1)	MW222191
<i>Oleoguttula mirabilis</i>	P10406	JRI, Antarctica (2)	MW222211
<i>Oleoguttula mirabilis</i>	P10407	JRI, Antarctica (2)	MW222212
<i>Oleoguttula mirabilis</i>	P10444	JRI, Antarctica (1)	MW222225
<i>Oleoguttula mirabilis</i>	CCFEE 5522	JRI, Antarctica	KF309972.1
<i>Oleoguttula mirabilis</i>	CBS 136102	JRI, Antarctica	NR_144974.1
<i>Oleoguttula mirabilis</i>	EXF-13077	Greenland Ice Sheet	MK460370.1
<i>Oleoguttula mirabilis</i>	EXF-13078	Greenland Ice Sheet	MK460369.1
<i>Oleoguttula mirabilis</i>	EXF-13080	Greenland Ice Sheet	MK460371.1
<i>Rachicladosporium alpinum</i>	CBS 136040	Monte Rosa, Italy	NR_144965.1
<i>Rachicladosporium americanum</i>	CBS 124774	Virginia, USA	MH863412.1
<i>Rachicladosporium antarcticum</i>	P10108	JRI, Antarctica (3)	MW251711
<i>Rachicladosporium antarcticum</i>	P10377	JRI, Antarctica (5)	MW222195
<i>Rachicladosporium antarcticum</i>	P10413	JRI, Antarctica (4)	MW222216
<i>Rachicladosporium antarcticum</i>	CCFEE 5527	Antarctic Peninsula, Antarctica	NR_144970.1
<i>Rachicladosporium cboliae</i>	CBS 125424	Virginia, USA	NR_156538
<i>Rachicladosporium corymbiae</i>	CBS 145087	Ghana	NR_161143
<i>Rachicladosporium eucalypti</i>	CPC 23241	Ethiopia	NR_155718.1
<i>Rachicladosporium inconspicuum</i>	CBS 136043	Monte Rosa, Italy	NR_144966
<i>Rachicladosporium iridis</i>	CBS 282.49	Netherlands	NR_169891.1
<i>Rachicladosporium luculiae</i>	CBS 121620	Auckland, New Zealand	NR_160222
<i>Rachicladosporium mcmurdoi</i>	CBS 119432	McMurdo Dry Valleys, Antarctica	NR_144967.1
<i>Rachicladosporium monterosium</i>	CBS 137178	Monte Rosa, Italy	NR_144968.1
<i>Rachicladosporium paucitum</i>	CBS 136041	Trento, Italy	NR_144969
<i>Rachicladosporium pini</i>	CBS 129525	Netherlands	JF951145.1
<i>Toxicocladosporium banksiae</i>	CBS 128215	Australia	NR_152322.1
<i>Toxicocladosporium irritans</i>	CBS 185.58	Suriname	NR_152316.1
<i>Toxicocladosporium strelitziae</i>	CBS 132535	South Africa	NR_111765.1
<i>Pseudotaeniolina globosa</i>	CBS 109889	Italy	MH862844.1

Table 1. List of isolates used in the study. Isolates investigated in this work are highlighted in bold. Strains isolated from the rock are highlighted in yellow. *Notes:* JRI - James Ross Island, Antarctica. Numbers in parentheses shows sampling sites displayed in Fig. 1.

Phylogenetic analysis

The megablast search (Zhang et al. 2000) of ITS rDNA sequences was used to identify highly similar species to RIF isolates in the NCBI's GenBank nucleotide

database. The ITS rDNA sequences dataset for phylogenetic analysis involved 33 nucleotide sequences of which 26 sequences of close related taxa were retrieved from

the NCBI's GenBank (Table 1). They were aligned by the ClustalW program (Thompson et al. 1994) and manually checked and assembled. The phylogenetic analysis and dendrogram construction was conducted using MEGA X software (Kumar et al. 2018). The evolutionary history was

inferred by using the Maximum Likelihood method and Jukes-Cantor model and genetic distances were estimated using the Maximum Composite Likelihood (MCL) method (Jukes and Cantor 1969). Branch support was tested by 1000 replications in the bootstrap test (Felsenstein 1985).

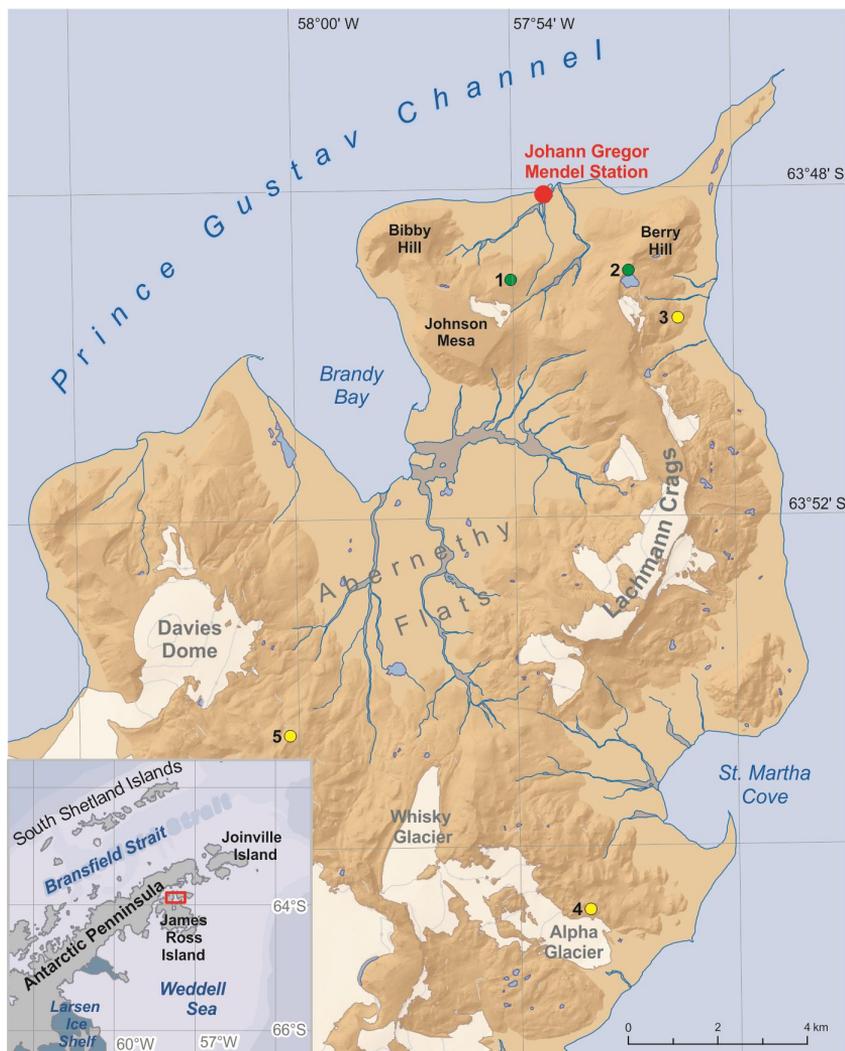


Fig. 1. Modified map of James Ross Island - Northern part ([1] - Czech Geological Survey 2009) showing the sampling sites of *Oleoguttula mirabilis* (green points) and *Rachicladosporium antarcticum* (yellow points) analysed strains. Notes: (1) – northeast side of Johnson Mesa, (2) – southeast side of Lachmann Crags, (3) – rock outcrop of Sekyra Peak, (4) – Panorama Pass, (5) – near Alpha Glacier.

Results and Discussion

Comparison of obtained ITS rDNA sequences within the nucleotide NCBI database showed *R. antarcticum* CCFEE 5527 (NR_144970.1) as 100% identical with RIF isolate P10108 and highly similar with RIF isolates P10377 and P10413 (99.59%), followed by *Rachicladosporium monterosium* CBS 137178 (NR_144961.1, 98.56%) and *R. mcmurdoi* CBS 119432 (NR_144967.1, 97.33%). The remaining four RIF isolates (P10109, P10406, P10407, P10444) showed 100% sequence similarity with *O. mirabilis* CCFEE 5522 (KF309973.1) and 99% sequence similarity with *O. mirabilis* CBS 136102 (NR_144974.1) and *O. mirabilis* strains EXF-13077, EXF-13078, EXF-13080. The genus *Oleoguttula* displayed very distinct ITS sequences from other sequences in the nucleotide NCBI database and *Pseudotaeniolina globosa* CBS 109889 (MH862844) isolated from stone of church in Italy was found as the phylogenetically closest relative (similarity 89.77%).

Phylogenetic analysis and Maximum Likelihood phylogenetic tree constructed using ITS rDNA sequences (Fig. 3) clearly showed the phylogenetic relation of three RIF isolates P10108, P10377 and P10413 from James Ross Island to *R. antarcticum* (*Rachicladosporium* clade) and P10109, P10406, P10407 and P10444 RIF isolates to *O. mirabilis* (*Oleoguttula* clade).

The three RIF isolates assigned in the *Rachicladosporium* clade clustered with the reference ITS sequences of 13 *Rachicladosporium* species. *Cladosporium* spp. and *Toxicocladosporium* spp. were included in the phylogenetic tree construction as closely related genera of the family *Cladosporiaceae*. Analysed RIF isolates P10108, P10377 and P10413 were clustered with *R. antarcticum* CCFEE 5527 (NR_144970.1) described from Antarctic Peninsula (Egidi et al. 2014) with 91% bootstrap support. The evolutionary divergences among strains placed in the sepa-

rate subbranch, including *R. antarcticum*, *R. monterosium* and *R. mcmurdoi* varied from 1.1 to 2.2%, while the evolutionary divergences among all *Rachicladosporium* species were in the range of 1.1 – 15.4% (data not showed). Pairwise distance calculation showed a minor percentage of nucleotide differences (0 – 0.5%) in the case of intraspecific variation in *R. antarcticum*.

Rachicladosporium antarcticum isolates were collected from three distant localities of James Ross Island (Fig. 1). The closest related species *R. monterosium* was isolated from Monte Rosa in the Italian Alps and *R. mcmurdoi* was collected from McMurdo Dry Valleys in Southern Victoria Land, Antarctica. These species are difficult to identify based on morphology because they are scarcely differentiated. They all develop brown, branched hyphae but *R. monterosium* often forms hyphae in strands with anastomosis and *R. antarcticum* produces torulose hyphae enteroblastically proliferating. *Rachicladosporium mcmurdoi* develops 1 – 2-celled conidia by arthric secession, while the formation of conidia or chlamydospor-like cells were not observed even after prolonged incubation of *R. monterosium* and *R. antarcticum* (Egidi et al. 2014). Studied isolates developed brown, branched, torulose hyphae (Fig. 2). Colonies on MEA attained 20 mm in diam. after 12 weeks at 15 °C. They were compact, elevated with folds, dark brown in observe and black in reverse (Fig. 2).

The *Oleoguttula* clade was composed of four analysed RIF isolates (P10109, P10406, P10407, P10444) collected from two different sampling sites on James Ross Island while strains of *O. mirabilis* (CBS 136102, CCFEE 5522) described by Selbmann and de Hoog and originated from James Ross Island (Egidi et al. 2014) were isolated from another locality than our isolates.

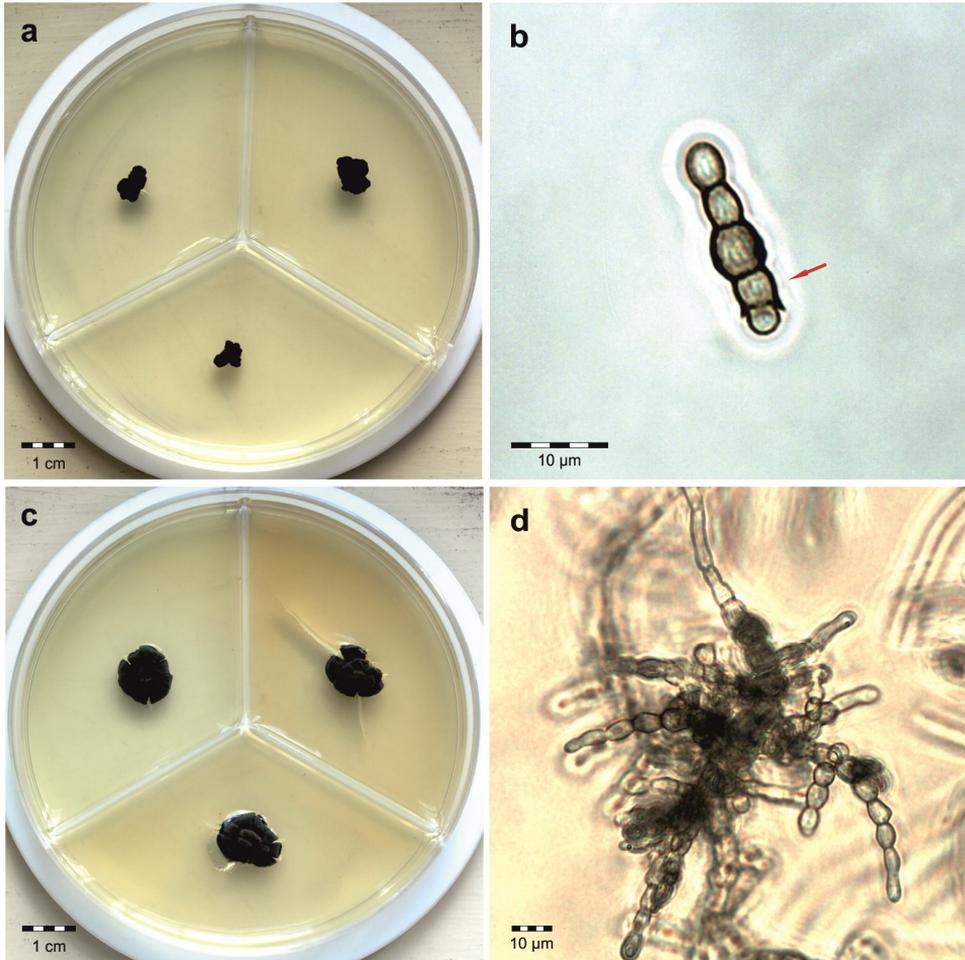


Fig. 2. **a-b** *Oleoguttula mirabilis* P10407; **a** – colony appearance on MEA after 12 weeks, **b** – wide opening, terminal phialide with conidia; **c-d** *Rachicladosporium antarcticum* P10108, **c** – colony appearance on MEA after 12 weeks, **d** – torulose, branched hyphae.

The remaining three strains (EXF-13077, EXF-13078, EXF-13080) of *Oleoguttula* clade were originated from the Greenland ice sheet (Perini et al. 2019). The intraspecific variability of ITS sequences in *O. mirabilis* varied from 0 to 0.5%. The morphology of studied isolates corresponded with the description of the

type culture *O. mirabilis* CBS 136102^T from Selbmann and de Hoog (Egidi et al. 2014). Colonies on MEA attained 9 mm in diam. after 12 weeks at 15°C. They were black in obverse and reverse, cerebriform with irregular margin. Unicellular, micro-nematous phialides with wide collarates and brown conidia were observed (Fig. 2).

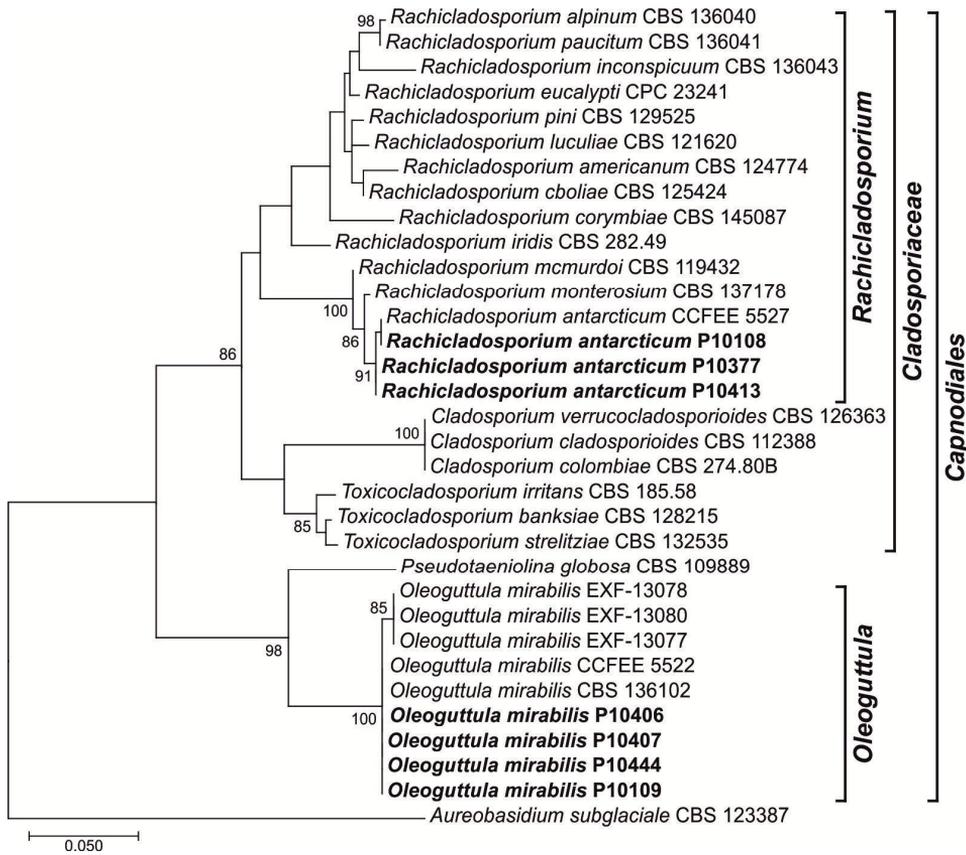


Fig. 3. Maximum Likelihood phylogenetic tree constructed using ITS rDNA sequences shows the phylogenetic position of studied *Oleoguttula mirabilis* and *Rachicladosporium antarcticum* isolates (in bold) among representative strains of the genera *Rachicladosporium* and *Oleoguttula*. All positions containing gaps and missing data were eliminated. There were a total of 391 positions in the final dataset. Bootstrap probability values (percentages of 1000 tree replications) greater than 85 % are indicated at branch points. *Aureobasidium subglaciale* CBS 123387 (NR 147323.1) was used as an outgroup. Bar, 0.05 substitutions per nucleotide position.

Conclusion

Oleoguttula mirabilis and *Rachicladosporium antarcticum*, two psychrophilic species inhabiting rocks in Antarctica were identified based on sequences similarity and phylogenetic analysis of ITS rDNA

region supported by morphological data. Both species were repeatedly collected from different parts of James Ross Island, which indicates their distribution across the deglaciated part of Ulu Peninsula.

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