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New records of lichenized fungi for Antarctica

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Abstract: Three lichenized fungal species collected from James Ross Island (eastern coast of Antarctic Peninsula): *Cladonia acuminata* (Ach.) Norrl., *Rhizocarpon pusillum* Runemark and *Rhizoplaca parilis* S.D. Leav., Fern.-Mend., Lumbsch, Sohrabi *et* St. Clair are reported from Antarctica for the first time. Detailed morphological and anatomical properties of these species along with photographes based on Antarctic specimens are provided here. In addition, the nrITS gene regions of the selected specimens are studied and the phylogenetic positions of the species are discussed. The nrITS data for *Rhizocarpon pusillum* is provided for the first time. According to our studies the lichen biodiversity of the Antarctic is still poorly known and molecular studies are very important in order to present the correct lichen biodiversity of Antarctica.

Keywords: Antarctic, biodiversity, James Ross Island, lichens.

Introduction

Antarctica is a continent dominated by lower plant groups in deglaciated areas. The flora of the Antarctic is composed predominantly of mosses and lichens with a few liverwort species and two native species of vascular plants (Øvstedal and Lewis Smith 2001).

In Antarctic lichens a diversity gradient exists along the Antarctic Peninsula with a strong decline in species richness from 62°S to around 70°S (Peat *et al.* 2007). These authors report that the distribution pattern of Antarctic lichens shows 3 clusters – (1) the South Orkney and South Shetland Islands and the northern and western sections of the Antarctic Peninsula; (2) the eastern and southern sections of the Antarctic Peninsula; and (3) Eastern Antarctica. James Ross Island belongs to the region fitting group 2 which, according to Terauds and Lee (2016), belongs to the the North-East Antarctic Peninsula region. The lichen flora of the James Ross Island has been investigated by Øvstedal and Lewis Smith (2001) and a comprehensive list of species known for the island is available in the herbarium of the British Antarctic Survey (BAS) and from their database (Antarctic Plant Database). Recently, over 100 lichen species are reported for James Ross Island and the Trinity Peninsula in the above-mentioned database. Within last decade, several ecological and ecophysiological studies have been published covering different aspects of lichen abundance at the James Ross Island, such as local microclimate effects on lichen abundance (Láska *et al.* 2011), and sucessional gradient (Bohuslavová *et al.* 2018). A recent study (Sancho *et al.* 2019) gave an overview of the lichen flora in the Antarctic, especially in the relation to lichen responses to environmental factors including global change. The authors suggest that lichen growth and diversity (1) might be used for biomonitoring of environmental changes, and (2) contribute to our understanding of drivers of climate change responses in the Antarctic. At James Ross Island some lichen species have been used for the study of lichen responses to long-term manipulated warming effects using the approach of open top chambers (Barták *et al.* 2019). Within the last decade, several new lichen species have been recorded from James Ross Island as the materials collected during an expedition in 2017, where we participated, are gradually analyzed and determined with molecular taxonomic tools (*e.g.*, Halici *et al*. 2017, 2018). In the present study, we bring records and supplementary description of three lichen species newly found at James Ross Island with DNA based identification methods.

Materials and methods

Samples of lichenized fungi were collected from James Ross Island which belongs to the North-East Antarctic Peninsula region (Terauds and Lee 2016). The specimens detailed below are deposited in Erciyes University Herbarium Kayseri, Turkey (ERCH). They were numbered starting with 'JR' and added to the database of the herbarium under those numbers. All the lichen specimens were examined by standard microscopic techniques. Hand-cut sections were studied in water, potassium hydroxide (KOH) and Lugol's solution (I). Measurements were made in water. Ascospores were measured from five different ascomata for each species. The measurements are given as minimum–maximum, from N measurements. TLC (Thin-Layer Chromotography) was carried out to determine some of the compounds, using solvent system C (Orange *et al.* 2001) when the results of spot tests were inconclusive. The descriptions summarized below for each species are based on the specimens collected from James Ross Island by the authors.

DNA isolation, PCR and sequencing. — Samples of freshly collected specimens were cleaned under a stereoscopic microscope and ground in 2 ml Eppendorf tubes with sterile plastic pestles. Total DNA was extracted from apothecia by using the DNeasy Plant Mini Kit (Qiagen) according to the manufacturer's instructions. PCR was carried out in 50 µL reaction volumes using 25 µl of Trans Bio Novo 2x Easy Taq© PCR Super Mix (Catalog No. AS111), 1 µl of each primer (ITS1F and ITS4), 4 µl of genomic DNA and 19 µl nuclease free water on a thermal cycler equipped with a heated lid. ITS4 (TCCTCCGCTTATTGATATGC) (White *et al.* 1990) and ITS1-F (CTTG GTCATTTAGAGGAAGTAA, Gardes and Bruns 1993) were used to amplify the ITS sequences. Polymerase chain reaction (PCR) amplification was performed under the following conditions: an initial denaturation for 5 min. at 95°C; 10 cycles at 30 sec. at 95°C, 30 sec. at 55°C, and 1 min. at 72°C; and 25 cycles with 30 sec. at 95°C, 30 sec. at 52°C, and 1 min. at 72°C. A final extension step of 8 min. at 72°C was added, after which the samples were kept at 4°C. The PCR products were visualized on 1.6% agarose gel as a band of approximately 500 or 700 bp.

Sequence alignment and phylogenetic analysis. — Sequence analyses of the lichen samples obtained from the PCR products were performed by the BM Labosis laboratory. Sequence results of the lichen samples were checked in GenBank (NCBI) by blast similarity search. Our ITS sequences plus sequences obtained from Genbank were aligned by the ClustalW plug-in in the BioEdit program (Hall 1999) and manually adjusted. The selection of sequences from Genbank was made by considering the morphological relationships as well as the molecular results of the studied samples (Table 1). For the reconstruction of phylogenetic trees, the MEGA 7 (Molecular Evolutionary Genetics Analysis) program was used (Tamura *et al.* 2013). Maksimum Likelihood was chosen, using the model Kimura 2-parameter. Pairwise deletion was applied to gaps in data and, for a control, the reliability of the inferred tree was tested by 1,000 bootstrap replications. The out-groups used in the phylogenetic trees were chosen to be phylogenetically related with the in-groups.

Table 1

List of species used in phylogenetic trees. The newly generated sequences are in bold.

GenBank Number	Species	Locality
MW938045	*Cladonia acuminata* (JR 0.029)	James Ross Island, Antarctica
MW938044	*Cladonia acuminata* (JR 0.201)	James Ross Island, Antarctica
MW938041	*Rhizocarpon pusillum* (JR 0.030)	James Ross Island, Antarctica
MW938040	*Rhizocarpon pusillum* (JR 0.031)	James Ross Island, Antarctica
MW938043	*Rhizocarpon pusillum* (JR 0.040)	James Ross Island, Antarctica
MW938042	*Rhizoplaca parilis* (JR 0.179)	James Ross Island, Antarctica

GenBank Number	Species	Locality
JN621928	*Cladonia acuminata*	Canada
JN621932	*Cladonia acuminata*	USA
JN621933	*Cladonia acuminata*	Canada
JN621911	*Cladonia cariosa*	Spain
FR695863	*Cladonia cariosa*	Spain
JN621906	*Cladonia cariosa*	Portugal
JN621907	*Cladonia cariosa*	Spain
JN621908	*Cladonia cariosa*	Spain
JN621909	*Cladonia cariosa*	Spain
JN621910	*Cladonia cariosa*	Spain
JN621912	*Cladonia cariosa*	USA
JN621913	*Cladonia cariosa*	Norway
JN621915	*Cladonia cariosa*	Finland
JN621916	*Cladonia cariosa*	Finland
JN621917	*Cladonia cariosa*	Russia
JN621937	*Cladonia latiloba*	Brazil
JN621935	*Cladonia subcariosa*	USA
JN621936	*Cladonia subcariosa*	USA
JN621914	*Cladonia symphycarpa*	Norway
JN621918	*Cladonia symphycarpa*	Bosnia and Herzegovina
JN621919	*Cladonia symphycarpa*	Spain
JN621921	*Cladonia symphycarpa*	Spain
JN621923	*Cladonia symphycarpa*	Sweden
JN621926	*Cladonia symphycarpa*	Germany
JN621924	*Cladonia symphycarpa*	USA
JN621930	*Cladonia symphycarpa*	Ukraine
JN621931	*Cladonia symphycarpa*	Bosnia and Herzegovina
MK625448	*Rhizocarpon atroflavescens*	China
MK629879	*Rhizocarpon atroflavescens*	China
MK629881	*Rhizocarpon atroflavescens*	China
MH979409	*Rhizocarpon effiguratum*	China
MH979410	*Rhizocarpon effiguratum*	China
AF250805	*Rhizocarpon geographicum*	–
AF483619	*Rhizocarpon geographicum*	Norway
DQ534482	*Rhizocarpon geographicum*	Antarctica
KX550103	*Rhizocarpon geographicum*	Turkey

GenBank Number	Species	Locality
DQ534483	*Rhizocarpon nidificum*	Antarctica
AF483618	*Rhizocarpon norvegicum*	Norway
KY680775	*Rhizocarpon norvegicum*	Russia
KY680776	*Rhizocarpon norvegicum*	Russia
KY680779	*Rhizocarpon smaragdulum*	Russia
NR152547	*Rhizocarpon smaragdulum*	Russia
MH979404	*Rhizocarpon superficiale*	China
MH979405	*Rhizocarpon superficiale*	China
MH979406	*Rhizocarpon superficiale*	China
KU934705	*Rhizoplaca* aff. *porterii “nevadensis”*	–
HM577242	*Rhizoplaca chrysoleuca*	USA
HM577243	*Rhizoplaca chrysoleuca*	USA
KU934617	*Rhizoplaca chrysoleuca*	Russia
KU934618	*Rhizoplaca chrysoleuca*	Russia
KU934619	*Rhizoplaca chrysoleuca*	Russia
HM577303	*Rhizoplaca haydenii* subsp. *arbuscula*	USA
HM577304	*Rhizoplaca haydenii* subsp. *arbuscula*	USA
AY530885	*Rhizoplaca huashanensis*	China
HM577295	*Rhizoplaca idahoensis*	USA
HM577296	*Rhizoplaca idahoensis*	USA
HM577297	*Rhizoplaca idahoensis*	USA
KU934640	*Rhizoplaca macleanii*	Antarctica
KU934641	*Rhizoplaca macleanii*	Antarctica
MK970668	*Rhizoplaca macleanii*	Victoria Land, Antarctica
MK970669	*Rhizoplaca macleanii*	Victoria Land, Antarctica
MK970670	*Rhizoplaca macleanii*	Victoria Land, Antarctica
JX948273	*Rhizoplaca melanophtalma*	Iran
JX948274	*Rhizoplaca melanophtalma*	Iran
JX948292	*Rhizoplaca melanophtalma*	Iran
KP314423	*Rhizoplaca melanophtalma*	Svalbard
MK811669	*Rhizoplaca melanophtalma*	Norway
MK812478	*Rhizoplaca melanophtalma*	Norway
NR120221	*Rhizoplaca melanophtalma*	Spain
KU934699	*Rhizoplaca novomexicana*	USA
KU934700	*Rhizoplaca novomexicana*	USA
KU934706	*Rhizoplaca novomexicana*	USA

GenBank Number	Species	Locality
HM577305	*Rhizoplaca occulta*	USA
HM577306	*Rhizoplaca occulta*	USA
HM577307	*Rhizoplaca occulta*	USA
NR119880	*Rhizoplaca occulta*	USA
JX948220	*Rhizoplaca parilis*	Chile
JX948223	*Rhizoplaca parilis*	Chile
JX948224	*Rhizoplaca parilis*	Chile
JX948226	*Rhizoplaca parilis*	Chile
JX948227	*Rhizoplaca parilis*	Chile
HM577317	*Rhizoplaca parilis*	USA
NR119881	*Rhizoplaca parilis*	USA
JX948225	*Rhizoplaca parilis*	Chile
HM577318	*Rhizoplaca parilis*	USA
NR119882	*Rhizoplaca polymorpha*	USA
KU934778	*Rhizoplaca polymorpha*	USA
KU934779	*Rhizoplaca polymorpha*	USA
HM577377	*Rhizoplaca porterii*	USA
HM577379	*Rhizoplaca porterii*	USA
JX948228	*Rhizoplaca porterii*	USA
KU934833	*Rhizoplaca porterii*	USA
KU934834	*Rhizoplaca porterii*	USA
NR119883	*Rhizoplaca porterii*	USA
HM577291	*Rhizoplaca shushanii*	USA
HM577292	*Rhizoplaca shushanii*	USA
HM577293	*Rhizoplaca shushanii*	USA
KU934859	*Rhizoplaca shushanii*	USA
KU934860	*Rhizoplaca shushanii*	USA
NR119879	*Rhizoplaca shushanii*	USA
AF163113	*Rhizoplaca subdiscrepans*	–
KP226212	*Rhizoplaca subdiscrepans*	–
KU934894	*Rhizoplaca subdiscrepans*	Russia
KU934900	*Rhizoplaca subdiscrepans*	Russia
HQ650649	*Catolechia wahlenbergii*	–
KM250247	*Pilophorus clavatus*	South Korea
MH481906	*Protoparmelia badia*	Japan

Species list

Cladonia acuminata (Ach.) Norll.

Detailed descriptions of this species were provided by Osyczka *et al.* (2011) and Pino-Bodas *et al.* (2013).

Primary thallus squamulose, persistent, light greenish to gray. Squamules rather large and conspicuous; up to 0.5 mm wide, and up to 1 mm long, entire or irregularly crenate-edged or wavy or sinuous edged, lobate, lobes ascending and nearly concave. Podetia rarely developed, when present grayish white, blunt at the tips, without scypi, up to 1.6 cm tall, 0.5 cm thick at base, mostly simple and not branched. Podetial surface squamulose at base and granulose at upper parts. Apothecia and pycnidia not seen (Fig. 1).

Fig. 1. *Cladonia acuminata*. Squamulose thallus and podetium with squamules at the base.

Chemistry. – Podetia and primary thallus K+ yellow to orange, KC-, Pd+ yellow. Atranorin and norstictic acid identified by TLC.

Remarks. – *Cladonia acuminata* belongs to the *Cladonia cariosa* group which includes *C. cariosa* (Ach.) Spreng., *C. symphycarpa* (Ach.) Fr. and *C. acuminata*. These three species constitute a monophyletic group according to Stenroos *et al.* (2002). Our specimen fell into that group, in a strongly supported clade with Genbank accessions JN621920.1, JN621922.1, and JN621932.1 of *C. acuminata*

(Fig. 2). From the other species of the group, *C. symphycarpa* differs in mostly lacking podetia, and *C. acuminata* differs from the two other species by having sorediate podetia which are mostly unbranched or rarely dichotomously branched near the tips (Ahti 2000). While *C. cariosa* mostly have apothecia on the podetia, *C. acuminata* rarely have; and our Antarctic specimens also lack apothecia. These three species in the *Cladonia cariosa* group have atranorin in common and they all grow on calcareous substratum (Stenroos *et al.* 2002).

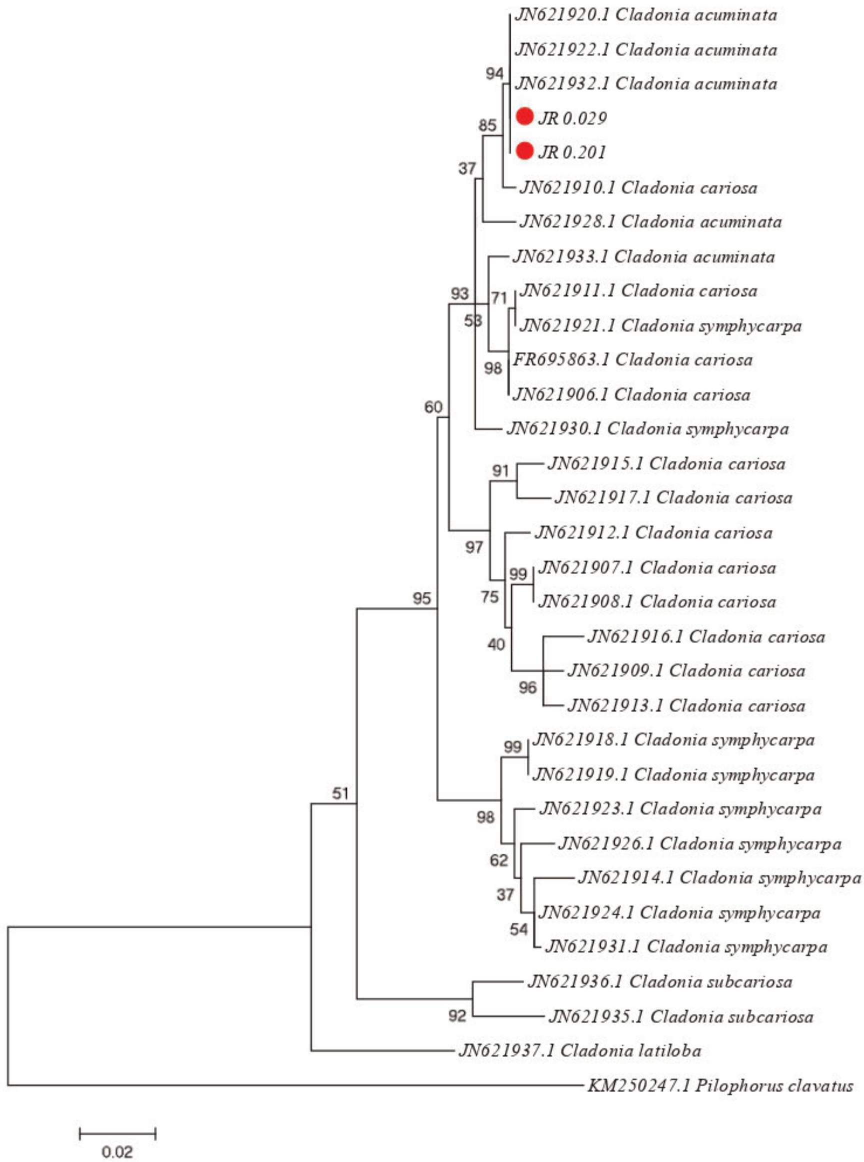

Fig. 2. Maximum Likelihood (ML) analysis inferred from ITS region sequences of the

Cladonia cariosa group

Cladonia acuminata has a bipolar distribution and mostly grows on calcareous soil which is rich in humus (Osyczka *et al.* 2011). In James Ross Island we found this species from two different localities on sandy soils, close to the sea shore growing with many terricolous lichens such as *Physconia muscigena* (Ach.) Poelt, *Peltigera antarctica* C.W.Dodge, *P. castanea* Goward, Goffinet *et* Miądl. and *Solorina spongiosa* (Ach.) Anzi. The geographically nearest record of this species is from the Navarino Island of Chile (Burgaz and Raggio 2007) at 400 m altitude. It is also known from the Arctic, e.g. Greenland (Hansen 2007; Alstrup *et al.* 2009) and Svalbard (Konoreva *et al.* 2019).

Specimens examined. – Antarctica, Antarctic Peninsula, James Ross Island, Solorina Valley (63° 52′ 39.0″ S, 57° 46′ 51.6″ W, alt. 2 m.), on soil. Leg. M.G. Halici and M. Bartak (JR 0.029); Lachman Bay, (63° 47′ 22.5″ S, 57° 48′ 12″ W, alt. 36 m.), on soil. Leg. M.G. Halici and M. Bartak (JR 0.201).

Rhizocarpon pusillum Runemark

Lichenicolous on *Sporastatia testidunea* in the early stages of development but later sometimes independent (Fig. 3).

Thallus continuous, forming areolate pacthes in the host thallus up to 5 cm. Areoles bright greenish yellow, angular, flat or weakly concave, up to 2 mm.

Fig. 3. Early stages of *Rhizocarpon pusillum* growing on *Sporastatia testudinea.* Dark brown color of the stone (lower left corner) indicates a proximity to ground level where more moisture is avaliable than on lichen-free tops of the stones (upper right corner).

Apothecia black, mostly angular, rarely rounded, flat or convex, slightly white pruniose, 0.15−0.8 mm, apothecial margin distinct, prominent, white-greyish, thicker at young ones. Epihymenium brown, 40−70 µm, N+ red, K+ weakly reddish. Hymenium brownish hyaline, N+ red, K+ red, 60-85 µm. Hypothecium dark brown, 90 µm. Asci 8-spored. Ascospores brown, one septate, widely elipsoid or almost subglobose, (12–)14,5–16–17,5(–19) × (6–)8,5–10–11,5(–13) µm (n=31) and spore length/width ratio (1,23–)1,37–1,65–1,92(–2,5) µm (n=31). Paraphyses simple, not branched, has oil droplets, strongly adglutinated, end cells enlarged to 3.5-4 µm. Pycnidium not observed (Fig. 4).

Chemistry. – Thallus and medulla K-, C-, I-, KI- and Pd+ yellow. Rhizocarpic acid and Psoromic acid identified by TLC.

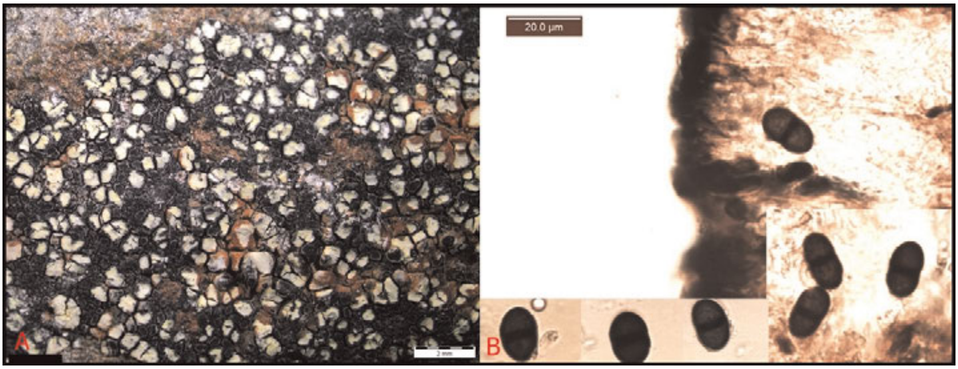

Fig. 4. *Rhizocarpon pusillum* **A.** Thallus, **B.** Ascospores.

Two other yellow *Rhizocarpon* species with 1-septate ascospores were previously reported from the Antarctic: *Rhizocarpon adarense* (Darb.) I.M. Lamb and *R. superficiale* (Schaer.) Malme. *Rhizocarpon pusillum* differs from these species by its lichenicolous habit on *Sporastatia testudinea* (Ach.) A. Massal. (Wang *et al.* 2015). Other morphological differences were summarized in Table 2. The other yellow *Rhizocarpon* species with one-septate ascospores which are not known from the Antarctic are: *R. alpicola* (Wahlenb.) Rabenh., *R. effigurata* (Anzi) Th. Fr., *R. eupetraeoides* (Nyl.) Blomb. *et* Forssell, *R. inarense* (Vain.) Vain., *R. norvegicum* Räsänen and *R. parvum* Runemark. Among these species, only *R. effiguratum, R. norvegicum* and *R. parvum* are known to be lichenicolous, but on different hosts (on *Pleopsidium flavum* (Trevis.) Körb., the Acrosporaceae, and *Tremolecia atrata* (Ach.) Hertel, respectively) (Table 2).

There were no sequences of *Rhizocarpon pusillum* in GenBank. According to our ITS phylogeny, *R. pusillum* is closely related to *R. superficiale* which also has one-septate ascospores (Fig. 5).

In James Ross Island, this species is very common on basaltic rocks. It starts its life cycle on *Sporastatia testudinea,* and usually damages the whole host thalli and becomes independent. Occurences of *R. pusillum* on the stones form an

Table 2

Comparision of yellow *Rhizocarpon* species with 1-septate ascospores.

	R. adarense	***R. alpicola***	***R. effiguratum***	***R. eupetraeoides***	***R. norvegicum***	***R. parvum***	***R. pusillum***	***R. inarense***	***R. superficiale***
Lichenicolous	No	No	Yes	No	No	Yes	Yes	No	No
Secondary Chemistry	rhizocarpic acid	rhizocarpic acid, psoromic acid, gyrophoric acid, and atranorin.	rhizocarpic acid, often psoromic acid, stictic acid (sometimes)	rhizocarpic acid, norstictic or psoromic acid or norstictic or bourgeanic acid	rhizocarpic acid with or without psoromic acid.	rhizocarpic acid	rhizocarpic acid, psoromic acid	rhizocarpic acid, norstictic acid, sometimes with traces of psoromic or gyrophoric acid	rhizocarpic acid, stictic acid complex
Spot Tests	Thallus and medulla K-, C-, I-, KI-	Medulla K-, Pd + yellow	Medulla K-, C-, KC-, P+ yellow, I+ violet	Medulla K+ red, P+ yellow and I+ violet	Medulla K-, P-, or P+ yellow, 1+ dark blue	Medulla K-, C-, P-, I+ violet	Thallus and medulla K-, C-, I-,KI- and Pd+ yellow	Medulla K+ yellow	Medulla K+ yellow or orange, K/I-
Ascospore sizes	11–18 × 5–10 µm	20–33 × 9–17 µm	9–14 x 4–8 µm	22–34 × 9–17 µm	9–15 x 6–7 µm	9–15 x 6–7 µm	12–19 × 6–13 µm	21–30 × 10–12 µm	11–12 × 8–9 µm
Literature	McCarthy and Elix (2014)	Smith *et al.* (2009)	Nash *et al.* (2004); Hawksworth *et al.* (2008)	Nash *et al.* (2004); Hawksworth *et al.* (2008)	McCarthy and Elix (2014)	Wang *et al.* (2015)	This article	Nimis (2016)	Fryday and Øvstedal (2012)

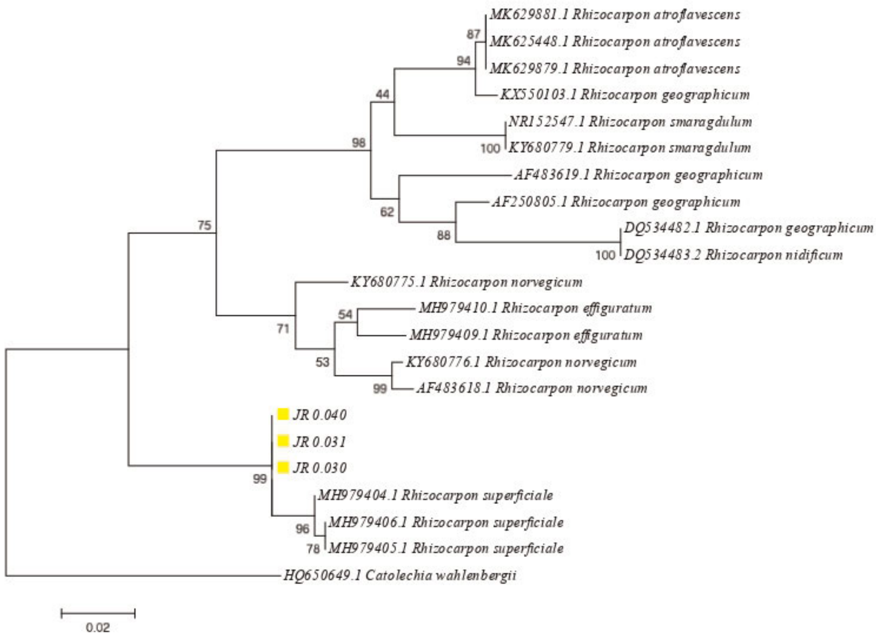

Fig. 5. Maximum Likelihood (ML) analysis inferred from ITS sequences of *Rhizocarpon pusillum* and related species.

irregular pattern on sedimentary rock plateau (Northern coast of James Ross Island, neighbourhood of *J.G. Mendel* station) and is restricted to the leeward side of a stone or boulder. This is due to the fact that snow depositions do not form a layer of constant thickness but rather accumulations on the leeward side while the winward side and the top of the stones remain snow-free. Because southern to western wind prevail (Bohuslavová *et al.* 2018; Kavan *et al.* 2018) at the localities, *S. testudinea* and *R. pusillum* are found on N to E sides of the stones/boulders where, close to ground surface (see Fig. 4) more moisture is available thanks to gradually melting snow accumulation. The locality of collection with the patterend distribution of volcanic stones belongs to glacial-sculpted erosional surface of sedimentary rock (Jennings *et al.* 2021).

Rhizocarpon superficiale was reported from James Ross Island by Øvstedal and Lewis Smith (2001), but we could not collect this species although we made field excursion almost in all deglaciated parts of the island for two months. Probably the records of *R. superficiale* belongs to *R. pusillum*. Another explanation could be that the collection site of *R. superficiale* reported by Øvstedal and Lewis Smith (2001)- top of hill located South of the Santa Marta Cove, has not yet been sampled systematically since the 1990-ies and, therefore, the occurence of the species can not be proven.

Rhizocarpon pusillum is a cosmopolite species with bipolar distribution and has been reported from Asia, Europe, North America, New Zealand (Thomson

1997; Feuerer and Timdal 2004; Matwiejuk 2008; Hafellner 2015; Wang *et al.* 2015), China (Wang *et al.* 2015), Turkey (Halici *et al.* 2005), and Greenland (Hansen 1982, 2002, 2012).

Specimens examined. – Antarctica, Antarctic Peninsula, James Ross Island, Dirty Valley, (63° 48′ 38.1″ S, 57° 51′ 36″ W, alt. 92 m.), the locality is a shallow small-area valley located 750 m NW from the Panorama Pass, on rock. Leg. M.G. Halici and M. Bartak (JR 0.030); neighbourhood of V-Shape Valley (63° 48′ 52.2″ S, 57° 54′ 52.8″ W, alt. 102 m.), on rock. Leg. M.G. Halici and M. Bartak (JR 0.031 and JR 0.040).

***Rhizoplaca parilis* S. Leavitt, F. Fernández-Mendoza, Lumbsch, Sohrabi *et* L. St. Clair**

Thallus crustose, yellow-green, attached to the substratum in one point, almost vagrant, lobate, edges of the lobes blue-blackish. Apothecia abundant, aggregated, lecanorine, immersed then sessile, convex or not, especially mature ones strongly convex. Apothecia disc black, white pruinose, especially mature ones heavily white pruinose, (0.4–)–0.45–0.5–0,55– (–0.6) mm (Fig. 6). Epihymenium black-green, 35–100 µm. *Hymenium hyaline*, 50–90 µm. Hypotechium hyaline, 120 µm. Asci 8-spored, 40 × 8 µm. Ascospores simple, hyaline, subglobose or eliptic, 9–10 × 4–5 µm. Paraphyses simple, not branched, tips somewhat enlarged, 3 µm.

Chemistry. – Thallus and medulla K- and C-.

Fig. 6. *Rhizoplaca parilis*. Habitus.

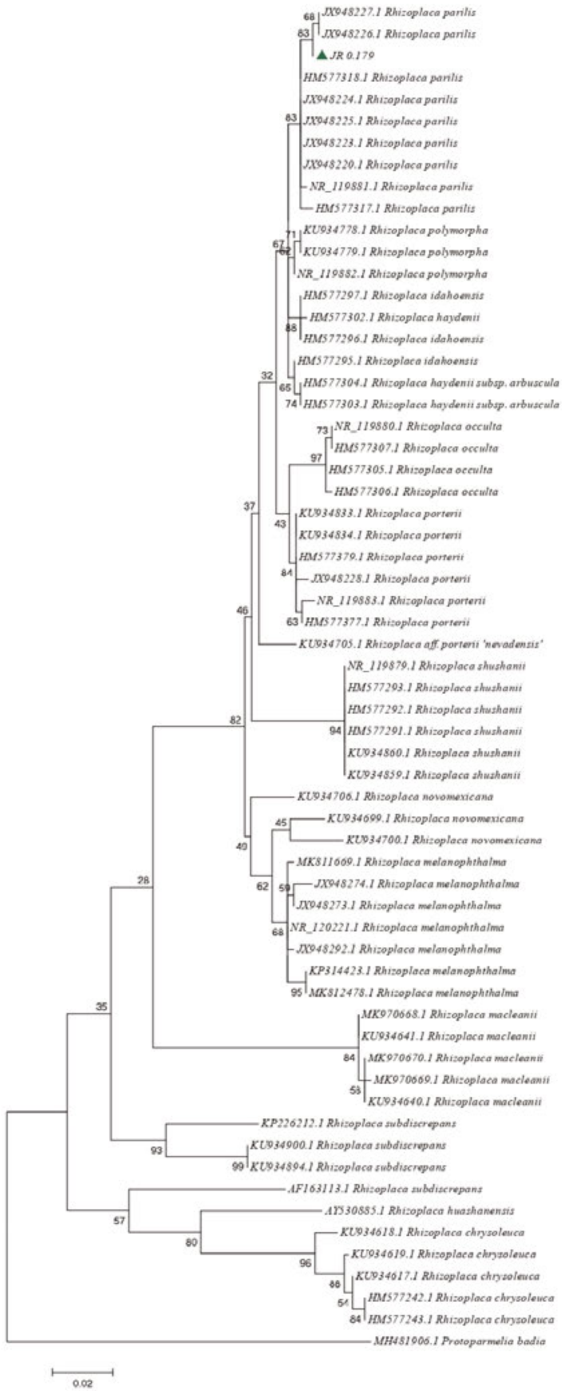

Fig. 7. Phylogenetic reconstruction based on the maximum likelihood (ML) criterion, inferred from ITS sequences of *Rhizoplaca parilis* and the other species of the genus.

Rhizoplaca parilis is a cryptic species recently described in the *Rhizoplaca melanophthalma* complex. Except for the genetics, the only differences between these two species are the occurence and amounts of orsellinic, lecanoric, and gyrophoric acids (Leavitt *et al.* 2013). Phylogenetically *R. parilis* and *R. melanophthalma* (DC.) Leuckert occurs at different clades within the genus. Our sequence was recovered within the *R. parilis* clade (Fig. 7). These two species also have similar ecological characteristics, both occur on calcium-poor rocks such as basalt, granite, schist (Leavitt *et al.* 2013). As far as we know, no samples reported as *Rhizoplaca melanophthalma* from Antarctica were DNA barcoded and the previous reports of this species may belong to *R. parilis*. Since the lichens of genus *Rhizoplaca* are found on only few small-area spots on James Ross Island, typically close to bird nesting sites enriched by nutrients from ornithoguano, future field ecophysiological studies should address the occurence of *R. melanophthalma* and *R. parilis* in particular spots.

Specimen examined. — Antarctica, Antarctic Peninsula, James Ross Island, Berry Hill Mesa, (63° 48′ 42.0″, 57° 50′ 5.4″ W, alt. 345 m.), on rock. Leg. M. G. Halici *et* M. Bartak (JR 0.179).

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