A new record of lichenized fungus species for Antarctica: *Peltigera castanea* Goward, Goffinet & Miądl.

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Abstract

As a result of our studies aiming to determine the lichen mycota of the James Ross Island (Antarctic Peninsula), we report *Peltigera castanea*, a species in the *P. didactyla* complex from Antarctica and Southern Hemisphere for the first time. Collections were evaluated using morphological, anatomical and molecular characteristics (nrITS). *Peltigera castanea* has foliose, 4-6 cm lobate thallus; upper surface dark brown to chestnut brown, weakly tomentose (especially in the margins of the lobes) and sorediate. The morphological and ecological variations of this species are discussed in this paper.

Key words: Antarctica, first report, lichens, biodiversity, James Ross Island

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Introduction

Peltigera is one of the earliest generic lichen names proposed by Willdenow (1787). It is an extensively distributed genus of primarily terricolous and muscicolous macrolichens with more than 90 species recognized worldwide. Many early authors such as Acharius (1794), Duby (1830), Fries (1831) and Nylander (1863, 1866) used the name *Peltigera* as a synonym for *Peltidea* and they contributed the taxonomy of *Peltigera* and allied genera (Turk et al. 2015). Most of the *Peltigera* species are bipartite symbioses, with one cyanobacterial or chlorococcoid photobiont and one mycobiont. *Peltigera* species have thalli that range in colour from bluish grey to dark green and brown when moist (Manoharan-Basil et al. 2016). Usually the members of *Peltigera* have the largest thalli among lichens and this genus occupies a central position in the family *Peltigeraceae* (Miadlikowska and Lutzoni 2000).

In the austral summer of 2017; the first author collected lichens from James Ross Island, which is located in the North east part of Antarctic Peninsula. One of the *Peltigera* specimens was studied in detail and also its nrITS gene region. As a result, we concluded that this specimen belongs to *Peltigera castanea* which was previously known from British Columbia (Canada), Russia and Estonia (Goffinet et al. 2003, Degtjarenko et al. 2018, Magain et al. 2018). In the literature there are seven *Peltigera* species previously reported from Antarctica. These species are *Peltigera*

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didactyla, Peltigera rufescens, *Peltigera neckeri, Peltigera antarctica*, *Peltigera aubertii, Peltigera patagonica* and *Peltigera ponojensis* (Øvstedal and Lewis-Smith 2001, 2009; Halıcı et al. 2018). In this paper, we report *Peltigera castanea* as the eighth species of *Peltigera* in Antarctica, and provide detailed information about morphology, anatomy and molecular information of *P. castanea.*

Material and Methods

Lichen sample (JR 0.297; the locality of specimen is specified below) was collected from Solorina Valley, James Ross Island in Antarctica. Solorina valley is one of the longest valleys in the deglaciated area of James Ross Island and is a representative of the typical geomorphological landscape of this region. Samples of freshly collected specimens were cleaned under a stereoscopic microscope and deposited in ERCH Lichen Herbarium.

Morphology and anatomy of the specimens were studied using an Olympus SXZ 1000 stereomicroscope and a Leica DM 1000 Light microscope. For chemical constituents spot test reactions were applied under stereomicroscope. Thin layer chromotography (TLC) was carried out to determine some of the compounds in solvent system C (Orange et al. 2010) when the results of spot tests were inconclusive.

JR 0.297: Antarctica, Antarctic Peninsula, James Ross Island, Solorina Valley 63° 52′ 39.0″ S, 57° 46′ 51.6″ W, alt. 2 m, on moss, leg. M. G. Halıcı & M. Barták, 12.01.2017 (ERCH JR 0.279).

DNA isolation, PCR and sequencing

Lichen thalli without any fungal infection and visible damage were chosen for DNA isolation and little thallus fragments from tips of lobes were taken under a stereomicroscope. For DNA extraction, DNeasy Plant Mini Kit (Qiagen) were applied to sample according to manufacturer's instructions. PCR amplifications of ITS were performed using fungal-specific primers ITS4 (TCCTCCGCTTATTGATATGC) (White et al. 1990) and ITS1-F (CTTGGTCATT TAGAGGAAGTAA (Gardes and Bruns 1993). The 50 µL PCRs contained 3 µl of 10 x reaction buffer, 3 µl MgCl2 (50 mM), 0.5 µl each primer (ITS1F and ITS4), 1 µl dNTP (10 mM), 0.1 µl Taq DNA polymerase, 3 µl of genomic DNA and 38.9 µl dH_2O. PCR amplifications were carried out in a thermal cycler equipped with a heated lid, with the following conditions: an initial heating step for 5 min. at 95°C; 6 cycles with 1:30 min. at 94°C, 1:30 min. at 55°C, and 2 min. at 72°C; and 33 cycles with 1 min. at 94°C, 1 min. at 52°C, and 2 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. Amplification products were visualized on 1% agarose gel as a band of approximately 500 or 800 bp.

Sequence alignment and phylogenetic analysis

Sequence analyzes obtained from the PCR products were performed by the BM Labosis laboratory. Sequence identities were evaluated using by blast similarity search (Standard Nucleotide BLAST) function in GenBank® (NIH genetic sequence database). For sequence aligment, Clustal W option of Bioedit program was used and ITS sequence results of lichen samples and samples obtained from Genbank® were

manually adjusted in Bioedit program (Table 1).

Ambiguous regions were delimited and excluded from the alignment. Phylogenetic tree was created by using MEGA 6 (Molecular Evolutionary Genetics Analysis) program (Tamura et al. 2013). To construct the phylogenetic tree Maximum Likelihod was chosen and Tamura 3-parameter model was used. Pairwise deletion was applied to gaps in data and for a control, the reliability of the inferred tree was tested by 1000 bootstrap replications. *Solorina saccata* HQ650625 was used as an outgroup.

Species	Locality	nrITS
Peltigera castanea	Antarctica, Solorina Valley (JR 0.279)	MT632253
Peltigera castanea	Canada	MH758239
Peltigera castanea	Canada	AY266023
Peltigera castanea	Canada	AY266019
Peltigera castanea	Canada	AY266025
Peltigera antarctica	Chile	MH758274
Peltigera antarctica	Antarctica, South Orkney Islands	MH758275
Peltigera antarctica	Chile	MH758273
Peltigera canina	USA	MH758486
Peltigera canina	USA	MH758487
Peltigera didactyla	Norway	MH758244
Peltigera didactyla	USA	MH758245
Peltigera didactyla	Canada	MH758242
Peltigera extenuata	USA	MH758253
Peltigera extenuata	USA	MH758254
Peltigera lambinonii	Australia	AY257933
Peltigera lambinonii	Zaire	AY257934
Peltigera lambinonii	Zaire	AY266037
Peltigera monticola	USA	MH758313
Peltigera monticola	USA	MH758311
Peltigera neckeri	Norway	MK811778
Peltigera neckeri	Norway	MK811968
Peltigera neocanina	USA	MH758395
Peltigera neocanina	USA	MH758396
Peltigera neocanina	USA	MH758397
Peltigera neocanina	USA	MH758398
Peltigera rufescens	USA	MH758371
Peltigera rufescens	USA	MH758370
Peltigera sorediifera	Australia	MH758255
Peltigera sorediifera	Australia	MH758256
Peltigera ulcerata	Costa Rica	MH758264
Peltigera ulcerata	Peru	MH758265
Peltigera vainioi	Colombia	MH758268
Peltigera vainioi	Ecuador	MH758269
Solorina saccata	USA	HQ650625

Table 1. Sequences used in the analyses; newly produced one is in bold and the others were downloaded from the Genbank®.

Results and Discussion

Molecular results

The ITS sequence of Antarctic *P. castanea* collected from Solorina Valley was blasted against the database of ITS sequences of 13 known *Peltigera* species and the related genus *Solorina* for an outgroup.

The resulting phylogenetic tree clearly shows that our Antarctic *Peltigera* specimen (JR 0.279) is well matched with Canadian *P. castanea* specimens (Fig. 1).

Morphology

Peltigera castanea was described by Goffinet et al. (2003) with a detailed description. Below we provide a description of the Antarctic specimens of this species:

Thallus is foliose, small, 4-6 cm across, lobate; lobes are usually with upturned margins, 1-1.2 cm wide, 3-4.5 cm long, mostly strongly concave and sometimes weakly overlapping. Upper surface is bluish gray in the field but after waiting in the herbarium dark brown to chestnut brown, weakly tomentose (especially in the margins of the lobes), sorediate (Fig. 2).

Soredia are mostly clustered in a rounded soralia, brown. Lower surface is white to light cream with indistinct veins; rhizines present usually near the lobe tips and brownish or concolorous with veins and flocculent (Fig. 3). All spot tests are negative (thallus and medulla K-, C-, KC-, KI-, I-, Pd-). Photobiont is cyanobacterial, visible as dull blue-green layer. No apothecia or pycnidia were seen in the Antarctic specimens. TLC: No compounds were observed.

Peltigera castanea is a well-delimited species belonging to *P. didactyla* complex which comprises species of the section *Peltigera* with laminal or submarginal soredia (Goffinet et al. 2003). Goffinet et al. (2003) recognized 5 morphospecies in this complex by nrDNA sequences: *P. castanea*, *P. didactyla*, *P. extenuata*, *P. lambinonii,* and *P. ulcerata*. Unfortunately it may be very difficult to seperate these taxa in the field, and a detailed morphological and chemical works supported by molecular data is very important in the taxonomy of this complex. The most important morphological taxonomical character which seperates *P. castanea* from all these species is its characteristic chestnut brown upper surface. Moreover, *P. ulcerata* differs from *P. castanea* by having shiny upper surface which is not tomentose (even in the margins of the lobes) and more or less elongate soralia. The rhizines forms a dense mat in *P. lambinonii* whereas they are more sparse in *P. castanea*. *P. extenuata,* which is morphologically closest species to *P. castanea,* typically has gyrophoric acid derivates in the soralia (Goffinett et al. 2003).

P. didactyla, the only known species of the *P. didactyla* complex in Antarctica, has a cosmopolitian distribution in Antarctic Peninsula including James Ross Island (Lewis-Smith 1988, 2005a, b; Sancho et al. 1999, Egan 2006, Moudrá 2007, Zúñiga et al. 2015). This species often has apothecia in the margins of the lobes. *P. castanea* has rarely apothecia. Our samples of *P. castanea* which collected from James Ross Island have no apothecia. It can be hard to distinguish *P. didactyla* without apothecia from *P. castanea* but in this case rhizines should be observed more carefully as *P. castanea* has flocculent rhizines and *P. didactyla* has more discrete rhizines which are non- flocculent. In our opinion all the materials reported as *P. didactyla* from Antarctic Peninsula should be more carefully checked and also fingerprinted for a more precious classification.

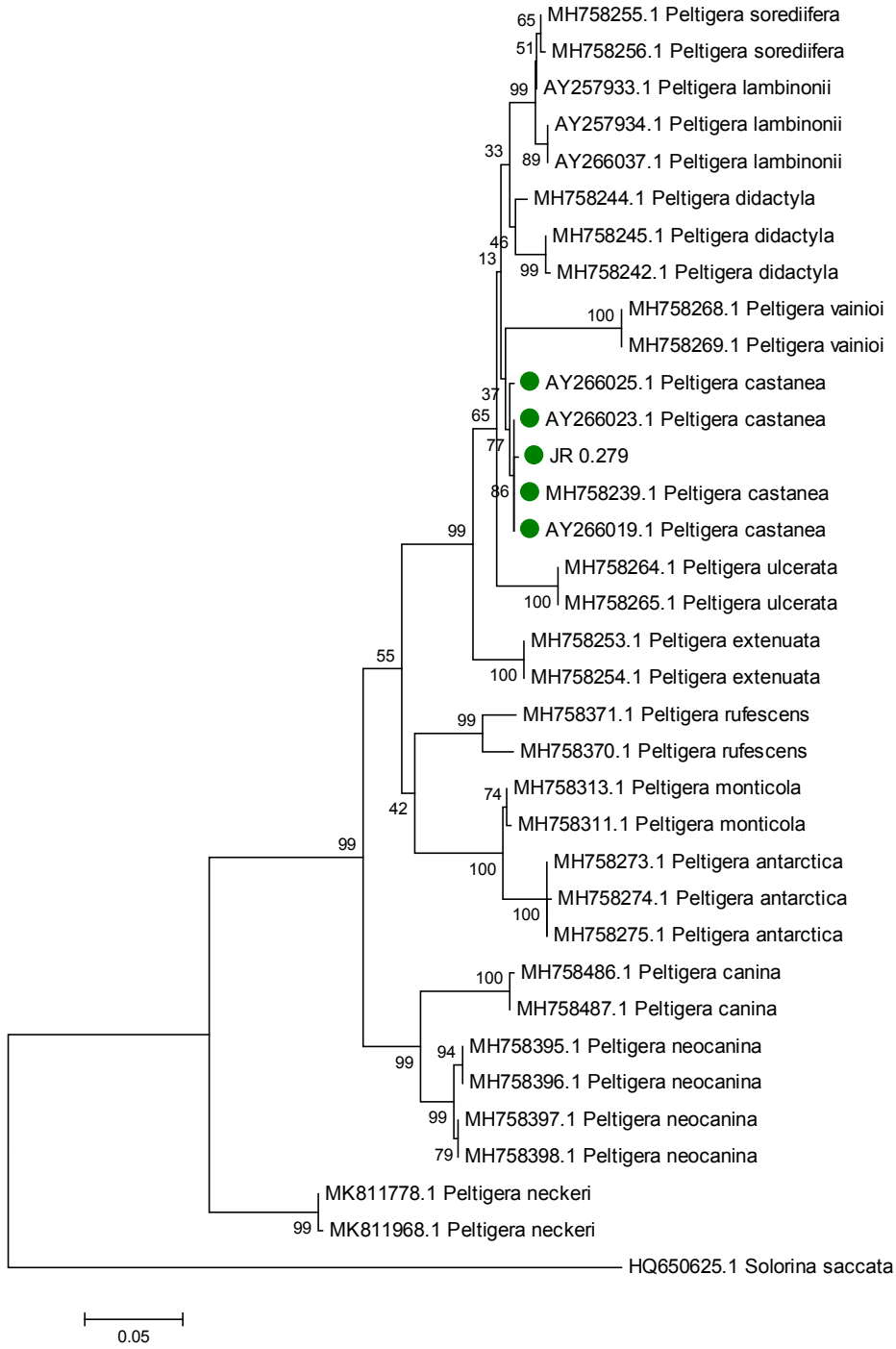

Fig. 1. Maximum Likelihood (ML) analysis inferred from ITS region sequences of *Peltigera castanea* and related species.

Fig. 2. Habitus of *Peltigera castanea.*

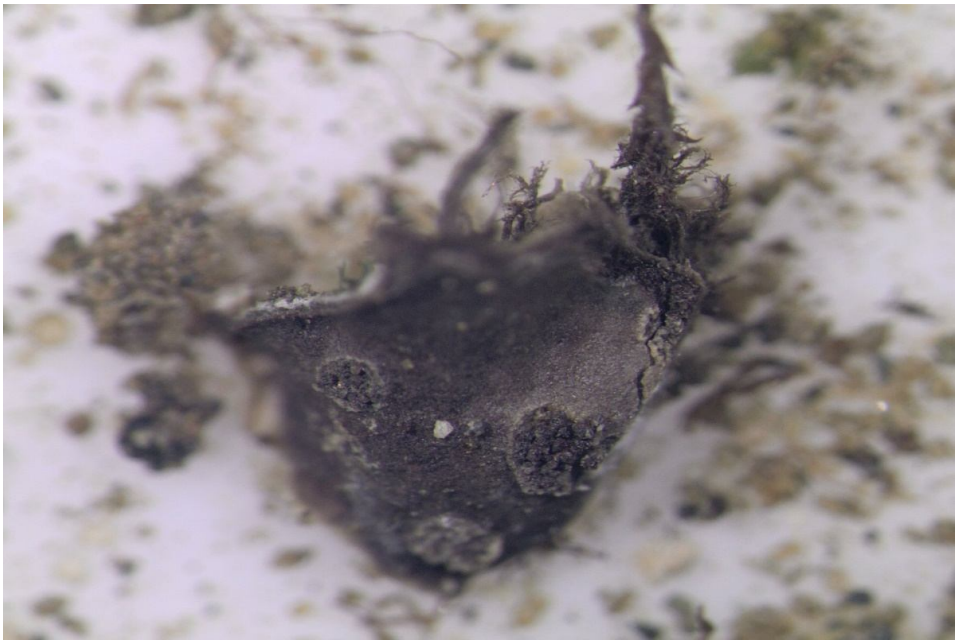

Fig. 3. *Peltigera castanea* from Solorina Valley, James Ross Island, Antarctica showing the rounded soralia and flocculent rhizines.

Ecology and Distribution

P. castanea was previously reported from North America (Canada), Russia and Estonia (Goffinet et al. 2003, Degtjarenko et al. 2018, Magain et al. 2018). This is the first record of *P. castanea* in Antarctica and in Southern Hemisphere (Fig. 4).

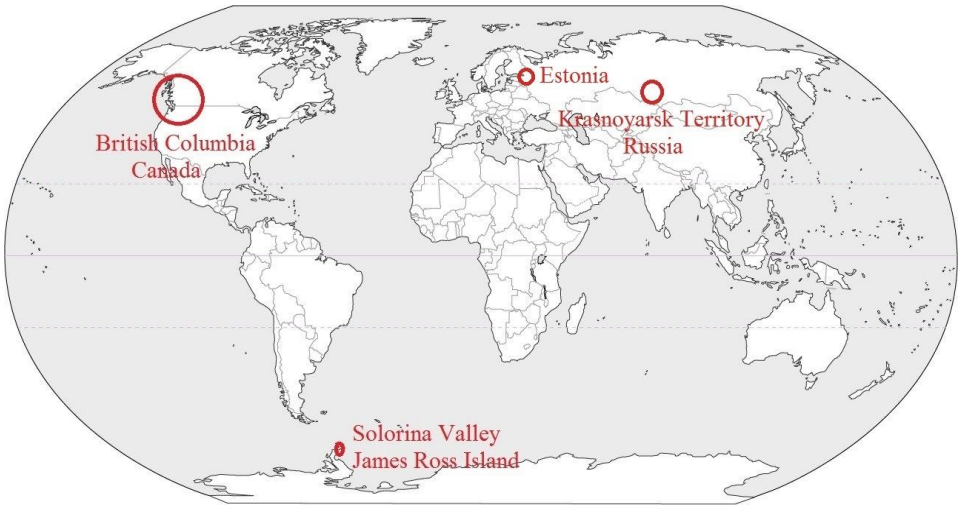

Fig. 4. Distribution map of *P. castanea*.

Fig. 5. *P. castanea* growing on moss in Solorina Valley, James Ross Island.

According to literature (Goffinet et al. 2003, Degtjarenko et al. 2018, Magain et al. 2018), this species prefers (oro)boreal forests and alpine heaths especially in open sites on xerophytic moss mats.

The specimens which were collected from Solorina Valley (James Ross Island) grew on soil or on mosses in the sandy terrace very close to seashore. At the sampling point, the terricolous lichens of the genera *Solorina*, *Cladonia* and *Psoroma* grew along with *P. castanea* (Fig. 5).

The area of the Solorina Valley outlet is rich in moss flora forming irregular patches along the seashore and margins of the Solorina stream. Several shallow depressions are located over the terrase. They are filled by meltwater at the begining of austral summer season and rich in microbiological mats formed by tens of algal and cyanobacterial species (Skácelová and Barták 2014). The regolith from Solorina Valley was analyzed by Coufalík et al. (2015), who revealed low mercury levels originated from weathering of bedrock. Consequently, Zvěřina et al. (2018) studied levels of cadmium, lead, and mercury in *Usnea antarctica* lichens.

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