First record of a common endolithic lichenized fungus species *Catenarina desolata* Søchting, Søgaard & Elvebakk. from James Ross Island (Antarctic Peninsula)

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Abstract

Catenarina desolata, a species which was recently described from southernmost Chile is reported from the Antarctica for the first time. Although it was described as lichenicolous, in James Ross Island, many specimens belonging to this species have non lichenicolous habit. The dubious reports of *Caloplaca* aff. *anchon-phoenicon* from James Ross Island actually belongs to *Catenarina desolata*. Collections were evaluated using morphological, anatomical and molecular characteristics (nrITS). The morphological and ecological variations of this species are discussed in this paper.

Key words: Antarctica, first report, southern hemisphere, lichens, Teloschistaceae

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Introduction

As not many professional lichenologists visited Antarctica, the lichens of this continent, especially the crustose ones are unfortunately poorly known because of their cryptic habits. The lichen biodiversity of Antarctic continent corresponds to the fact that there are the harshest conditions for living organisms in the world. However, some review papers on the Antarctic lichen biodiversity exist. The biodiversity is best presented by Øvstedal et Smith (2001) and Olech (2004). One of the largest lichen family, Teloschistaceae includes more than 1000 species worldwide (Søchting et Lutzoni 2003, Gaya et al. 2012) and many novel genera in this familiy were recently described after molecular studies (Arup et al. 2013). The crustose Teloschistaceae species are one of the most common species in Antarctica. More than 30 species belonging to

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this family have been reported from this continent (*e.g.* Øvstedal et Smith 2001, Olech 2004, Søchting et al. 2004, Søchting et Castello 2012, Søchting et al. 2014). In last two decades, an increasing number of studies has appeared addressing either new lichen species of Teloschistaceae family from Antarctica (*see* e.g. Garrido-Benavent et al. 2016) or selected physiological issues such as *e.g.* intrathalline antioxidant contents (Bhattarai et al. 2008). For James Ross Island, there are several tens of lichen species reported (*see* BAS lichen database). Among them, however, only few belong to Teloschistaceae family and were studied occasionally in the field or in a laboratory (*e.g. Caloplaca* sp. – Láska et al. 2011, Nývlt et al. 2016).

In 2016-2017 austral summer period, the first and third authors of this study made extensive field excursions to determine the lichen biodiversity of James Ross Island, specifically in the Northern deglaciated part. The survey was done at several localities distant up to 13 km from the Czech Republic scientific base Johann Gregor Mendel Base. In the field excursions, we observed that *Catenarina desolata* was very common on volcanic, non-littoral rocks in the island. *C. desolata* is recently described by Søchting et al. (2014), As this species was only known from southernmost Chile and the Kerguelen Island (*see* Fig. 1), and there was a doubt that the Antarctic specimens published by Søchting et Øvstedal (1992) and Øvstedal et Smith (2001) as *Caloplaca* aff. *anchon-phoeniceon* actually belongs to *C. desolata* Søchting et al. (2014). In this study, we used molecular biology tools, fingerprinting in particular, to determine *C. desolata.* from the collections from James Ross Island, Antarctica and now it is sure that those records reported as *Caloplaca* aff. *anchon-phoeniceon* are *Catenarina desolata*.

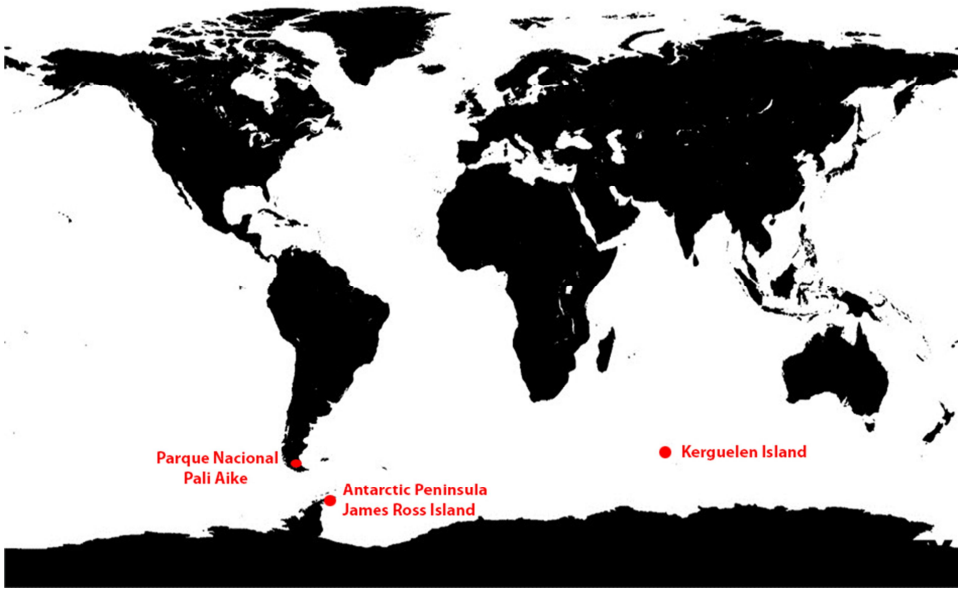

Fig. 1. Distribution map of *Catenarina desolata.*

Material and Methods

Lichen samples (JR 0.002 and JR 0.004 – the localities of collections are specified below) were collected from James Ross Island in Antarctica. Samples of freshly collected specimens were cleaned under a stereoscopic microscope and ground in 2 ml Eppendorf tubes with sterile plastic pestles.

JR 0.002: Antarctica, James Ross Island, Pukao boulder (local unofficial name), 63° 48' 22.5'' S, 57° 51' 00'' W, altitude 140 m, on the crevices of volcanic boulders, 27 January 2017, *M. G. Halici*.

JR 0.004: Antarctica, James Ross Island, Southeast of Johnson Mesa, 63° 49' 43.9'' S, 57° 51' 16'' W, altitude 285 m, on the crevices of volcanic boulders, 08 February 2017, *M. G. Halici*.

Species	Locality	nrITS
Catenarina desolata	Antarctica, James Ross Island (JR 0.002)	KY983102
Catenarina desolata	Antarctica, James Ross Island (JR 0.004)	KY983103
Catenarina desolata	Chile	KF657315
Catenarina desolata	Chile	KF657314
Catenarina desolata	Chile	KF657313
Catenarina desolata	Chile	KF657316
Catenarina desolata	Chile	KF657317
Catenarina vivasiana	Chile	KF657311
Catenarina vivasiana	Chile	KF657310
Catenarina vivasiana	Chile	KF657309
Catenarina vivasiana	Chile	KF657312
Wetmoreana sp.	Peru	KC179336
Wetmoreana decipioides	South Korea	KF264643
Wetmoreana decipioides	South Korea	KF264644
Wetmoreana appressa	Mexico	KC179332
Wetmoreana sp.	Peru	KC179335
Wetmoreana texana	USA, Texas	KF264658
Wetmoreana texana	USA, Texas	KF264657
Teloschistes flavicans	USA, Texas	KT291472

Table 1. Sequences used in the analyses; newly produced ones are in bold and the others were downloaded from the Genbank (gene bank numbers of the new sequences will be taken when the manuscript is accepted for publication).

DNA isolation, PCR and sequencing

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 3 µl of 10 x reaction buffer, 3 µl $MgCl_2$ (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 on a thermal cycler equipped with a heated lid.

ITS4 (TCCTCCGCTTATTGATATGC) (White et al. 1990) and ITS1-F (CTTG GTCATTTAGAGGAAGTAA, Gardes et Bruns 1993) were used to amplify the ITS sequence. Polymerase chain reaction (PCR) amplification was performed under the fol-

lowing conditions: an initial denaturation 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. 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% agarose gel as a band of approximately 500 or 800 bp.

Sequence alignment and phylogenetic analysis

Sequence analyzes 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 by blastn similarity search (Standard Nucleotide BLAST). ITS sequence results of lichen samples were analysed by using Clustal W option in the BioEdit program with the investigated samples and also with the samples obtained from Genbank and manually adjusted. Ambiguous regions were delimited and excluded from the alignment (Hall 1999). Thus identifications were confirmed. For phylogenetic tree, MEGA 6 (Molecular Evolutionary Genetics Analysis) program was used (Tamura et al. 2013). Maximum Likelihod was chosen to construct the phylogenetic tree, using the model Tamura 3-parameter. Pairwise deletion was applied to gaps in data, and for a control, the reliability of the inferred tree was tested by 1000 bootstrap replications. *Teloschistes flavicans* KT291472 was used as an outgroup.

Results and Discussion

Molecular results

The ITS sequences of the Antarctic *C. desolata* collected from James Ross Island were blasted against database of ITS sequences of two known species of *Catenarina* and the related genus *Wetmoreana* (Søchting et al. 2014). The resulting phylogenetic tree clearly shows that these Antarctic specimens which were probably reported as from Antarctica as *Caloplaca* aff. *anchon-phoeniceon* by Søchting et Øvstedal (1992) and Øvstedal et Smith (2001) are matched well with *C. desolata* (Fig. 2).

Morphology

A detailed description of *C. desolata* was provided by Søchting et al. (2014). The authors recognized this species with its lichenicolous habit. However, the specimens collected from James Ross Island (Antarctica) are not frequently lichenicolous and mostly endolithic (Fig. 3). According to our observations, it rarely grows on some crustose or foliose lichens and some of the specimens which are not lichenicolous have inconspicuous thalli. The Antarctic specimens reported by mistake as *Caloplaca* aff. *anchon-phoenicon* by Søchting et Øvstedal (1992) and Øvstedal et Smith (2001) have narrower ascospores (4.5─6 µm *vs.* (5.5─)6─6.5─7(─7.5) µm) as indicated by Søchting et al. (2014).

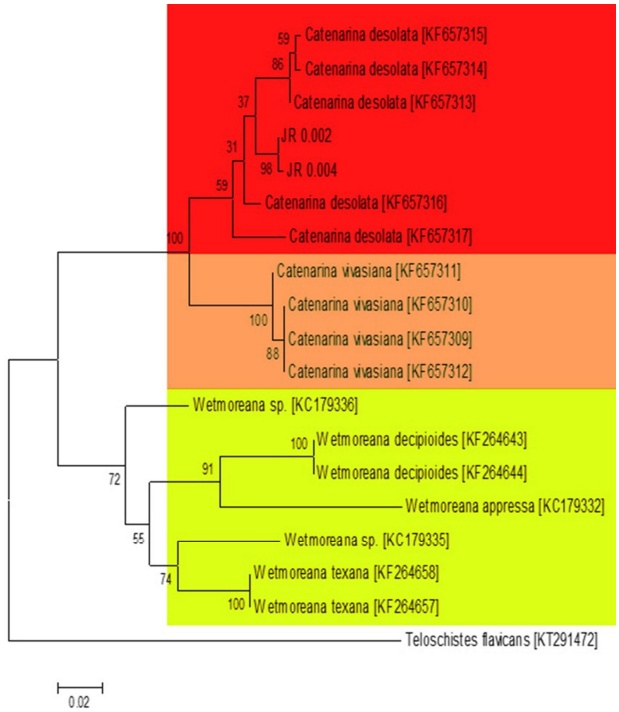

Fig. 2: Maximum Likelihood (ML) analysis inferred from ITS region sequences of *Catenarina desolata* and related species.

Fig. 3. *Catenaria desolata* from James Ross Island. Habitus.

Besides, *C. desolata* specimens collected from James Ross Island have ascospores with shorter septa (3.5 ± 1 *vs.* 5.5 ± 1, Søchting et al. 2014). *C. desolata* is easily recognised in the field with its deep red and slightly pinkish tinge minute apothecia of 0.5 ± 0.1 mm size.

Ecology and distribution

In James Ross Island, *C. desolata* is very common on volcanic non littoral rocks, but we did not observe any occurrence of this species on sedimentary rocks located along the northern coast of the Ulu peninsula at the altitudes below 100 m a.s.l. Rarely, it is lichenicolous found on crustose or foliose lichens such as *Leptogium* spp. but mostly it has an independent growth. It is quite common on the top and horizontal surfaces of big volcanic boulders but sometimes it is also seen in very humid conditions such as on small pepples present in the stream beds. This recently described species of *C. desolata* was only known from southernmost Chile and the Kerguelen Island but it is reported now for the first time from Antarctica. The distribution map of this species is given in Fig. 1.

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