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RESEARCH ARTICLE

Leptogium Pirireisii, a new species of lichenized Ascomycota (Collemataceae) from James Ross Island in Antarctica

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ABSTRACT
The first author collected lichens in the austral summer of 2017 from James Ross Island, located in the North-East region of the Antarctic Peninsula, which is one of the lichen-rich islands in Antarctica, with around 60 species reported previously. In this project, the lichen biodiversity of the island is studied by using molecular techniques in addition to morphological characters. Our results show that the lichen biodiversity of Antarctica is not well known, as many undescribed or unreported species are still present. One of the undescribed species is *Leptogium pirireisii*, a cyanolichen with *Nostoc* photobiont, a nitrogen fixer. In the nuITS phylogenetic tree, *L. pirireisii* clustered, with high support, with the species *L. antarcticum*, *L. furfuraceum*, *L. marcellii*, *L. pseudofurfuraceum*, *L. puberulum* and *L. tectum*, which are all characterised by the presence of hairs, while these are absent in the new species. We also confirmed the occurrence of *L. antarcticum* on James Ross Island based on molecular data and further generated nuITS sequences of *L. puberulum*.

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Introduction

Leptogium is a genus of lichen-forming Ascomycota, which are associated with *Nostoc* as photobiont and have gelatinous appearance when wet (Otálora et al. 2014). The genus is distributed globally, including environments with severe climatic conditions such as Antarctica (Øvstedal and Smith 2001; Otálora et al. 2014). The diversity of *Leptogium* has been estimated as between 70 and 180 species (Otálora et al. 2014; Lücking et al. 2017), but the actual number of species is still unknown. During the last decade, *Leptogium* species have been studied in different parts of the world and, consequently, the number of species has increased (Jørgensen and Olley 2010; Liu and Guan 2012, 2015; Kitaura and Marcelli 2013; Stone et al. 2016; Jørgensen and Palice 2016; Harada 2017; Kitaura et al. 2019).

Molecular analyses of the genus have been accompanied by anatomical and morphological studies, and in many regions the diversity has been shown to be higher than previously thought. For example, nine species were previously known from three mountain regions in Kenya and Tanzania, but a phylogenetic revision revealed more than 60

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putative species using the nuITS region as tool (Kaasalainen et al. 2021). With respect to the Antarctica Islands, three species of *Leptogium*, *L. crispatellum* Nyl., *L. menziesii* (Ach.) Mont. and *L. puberulum* Hue, were previously reported, but an integrative analysis revealed the presence of other unrecognised species, as *L. antarcticum* Scur, Lorenz-Lemke & Kitaura on King George Island, *L. marcellii* Kitaura, Lorenz-Lemke & A. A. Spielm. on King George Island and Duffayel Island, and *L. tectum* Lorenz-Lemke, Kitaura & Scur on James Ross Island (Kitaura et al. 2018).

James Ross Island (64°15′ S, 57°45′ W) is a large, irregularly shaped island, 60 km long in N–S direction, and with a maximum elevation of 1630 m above sea level. It lies off the Trinity Peninsula and the N tip of the Antarctica Peninsula, separated from both by the Prince Gustav Channel (Stewart 2011). The island is one of the lichen-rich landmasses in Antarctica, with around 60 lichen species reported, with the dominance of *L. puberulum* and *Usnea* spp. (Bohuslavová et al. 2012; Halıcı et al. 2018, 2020).

This paper is part of the lichen diversity survey of James Ross Island, Antarctica, and aims to analyse the *Leptogium* species through integrative approaches. We propose *L. pirireisii* Halici, Kahraman & Kitaura, as a species new to science, and report *L. antarcticum* for the first time from James Ross Island, increasing the known diversity to 62 species.

Materials and methods

The collecting expedition to James Ross Island was realised in the austral summer of 2017 (January–March) (Figure 1). The *Leptogium* samples were collected from rocks by chisel and from soil by knife. They were wrapped in toilet paper and placed in paper bags. After

Figure 1. Location of James Ross Island.

arriving at the Mendel Polar Station, they were left to dry for three days in a room with air flow.

The morphology of the collected *Leptogium* samples was studied at the Erciyes University, following the protocol of Kitaura et al. (2018). For the newly described species, only one apothecium was found, therefore diametral sections were made with parsimony preserving part of the apothecium. Furthermore, we considered tufts or loosely aggregated hyphae as hapters, whereas the compacted strands of hyphae as rhizines (Hale 1979).

DNA isolation, PCR and sequencing

DNA isolation and PCR was performed in the molecular biology laboratory of Biology Department, Erciyes University. Thallus fragments were used for DNA isolation employing the DNeasy Plant Mini Kit (Qiagen, Cat. No. 69106), according to the manufacturer's protocol. The amplification of the Internal Transcribed Spacer (nuITS) region 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 each primer (ITS1F and ITS4, White et al. 1990; Gardes and Bruns 1993), 4 µl of genomic DNA and 19 µl nuclease free water on a thermal cycler equipped with a heated lid. Polymerase chain reaction (PCR) was performed under the following conditions: initial denaturation for 5 min. at 95°C; 10 cycles of: 30 s at 95°C, 30 s at 55°C and 1 min at 72°C; and 25 cycles with: 30 s at 95°C, 30 s at 52°C and 1 min at 72°C. A final extension step of 8 min at 72°C was added and the samples were kept at 4°C. The PCR products were visualised on 1.2% agarose gel as a band of approximately 500–700 base pairs. Sequencing was performed with an ABI 3730 XL automatic sequencer (Applied Biosystems).

Alignment and molecular analyses

All newly generated sequences were assembled in Geneious v.8.1.7 and submitted to Blast search to determine the most similar sequences (BLASTN 2.11.0+), with the maximum target sequences set to 100 (Zheng et al. 2000; Morguilis et al. 2008). The sequences resulting from the blast search were added to the sequences generated in the present study, constituting an initial dataset analyzed trough Bayesian Inference (see methods below), based on which a subset including the target taxa was selected as final dataset.

The sequences were aligned with MAFFT v.7.222 (Katoh et al. 2002), available in Geneious v.8.1.7, using the following settings: scoring matrix 200PAM/k = 2, gap penalty = 1.53, offset value = 0.123, and automatic algorithm selection. The alignments were manually checked and ambiguous regions were removed using the Gblocks web server (Castresana 2000; Talavera and Castresana 2007), selecting the less stringent options. The final dataset was composed of 22 sequences, originally 641 bases long and 452 bases long after the exclusion of unreliably aligned sites (Table 1). *Collema furfuraceum* (Schaer.) Du Rietz and *C. undulatum* Laurer ex Flot., with GenBank accession numbers GQ396263 and DQ466044, respectively, were used as outgroup, based on the Collemataceae phylogeny of Otálora et al. (2010).

Bayesian trees were generated in MrBayes v3.2.7 on XSEDE in the CIPRES Science Gateway (Miller et al. 2010; Ronquist et al. 2012). For the final dataset, the nucleotide

Table 1. Information of the final dataset selected for the genetic analysis of the present study.

Species ID	Collection locality	Genbank ID	Voucher Information	Reference
***Leptogium antarcticum* 1**	**Antarctica, James Ross Island**	**MZ156763**	**ERCH JR0.203**	**This study**
Leptogium antarcticum 2	Antarctica	KY171869	N. M. Koch 5528	Kitaura et al. (2018)
Leptogium azureum	South Korea	KJ409609	Unknown	Jayalal et al. (2014)
Leptogium cyanescens	South Korea	KJ409598	Unknown	Jayalal et al. (2014)
Leptogium denticulatum	South Korea	KJ409597	Unknown	Jayalal et al. (2014)
Leptogium furfuraceum	Spain	EU982635	MA-16282	Otálora et al. (2010)
Leptogium hibernicum	Azores	KX013722	Purvis & James s. n. (BM-000747636)	Bjelland et al. (2017)
Leptogium juressianum	Kenya	JX503813	Unknown	Kaasalainen et al. (2021)
Leptogium krogiae	Tanzania	KX013713	Krog s. n. (O-L-188660)	Bjelland et al. (2017)
Leptogium marcellii	Antarctica	KY171872	N. M. Koch 5552	Kitaura et al. (2018)
Leptogium pirireisii	**Antarctica, James Ross Island**	**MZ156771**	**ERCH JR0.326**	**This study**
***Leptogium puberulum* 1**	**Antarctica, James Ross Island**	**MZ156760**	**ERCH JR0.382**	**This study**
***Leptogium puberulum* 2**	**Antarctica, James Ross Island**	**MZ156760**	**ERCH JR0.394**	**This study**
***Leptogium puberulum* 3**	**Antarctica, James Ross Island**	**MZ156762**	**ERCH JR0.365**	**This study**
Leptogium puberulum 4	Antarctica, King George Island	KY171875	A. *P.* Lorenz-Lemke 409	Kitaura et al. (2018)
Leptogium pseudofurfuraceum	Argentina, Salta	EU982647	MA-16293	Otálora et al. (2010)
Leptogium rivulare	Canada	KU198880	Lewis 2406	Unpublished
Leptogium saturninum	Norway	KX013640	BG-L-14383	Bjelland et al. (2017)
Leptogium tectum	Antarctica, James Ross island	KY171870	M. J. Kitaura 2948	Kitaura et al. (2018)

substitution model used was TRN + Gamma and the substitution rates were set according to BIC results in jModelTest2 on XSEDE in the CIPRES Science Gateway (Miller et al. 2010; Darriba et al. 2012). Two independent runs were performed with a chain length of 10,000,000, sampling every 1,000 generations. The 50% majority-rule consensus tree was generated from the combined sampled trees after discarding the first 25% as burn-in. Clades with posterior probabilities ≥0·95 were considered well supported. The phylogenetic tree and the DNA sequence alignments are available from TreeBASE (http://purl.org/phylo/treebase/phylows/study/TB2:S27894).

Results

Phylogenetic analyses

Five new nuITS sequences of *Leptogium* spp. were generated in the present study, three of *Leptogium puberulum*, one of *L. antarcticum*, and one of the new species *Leptogium pirireisii*. The new species clustered, with high support, with other Antarctica species such as *L. marcellii*, *L. puberulum* and *L. tectum*, and further with *L. furfuraceum* (Harm.) Sierk from Spain and *L. pseudofurfuraceum* P.M. Jørg. & A.K. Wallace from Argentina (Figure 2). The sequences of *L. antarcticum* and *L. puberulum* grouped with the other sequences of their corresponding species, confirming previous identifications.

Leptogium pirireisii Halici, Kahraman & Kitaura, sp. nov. (Figure 3A–E)

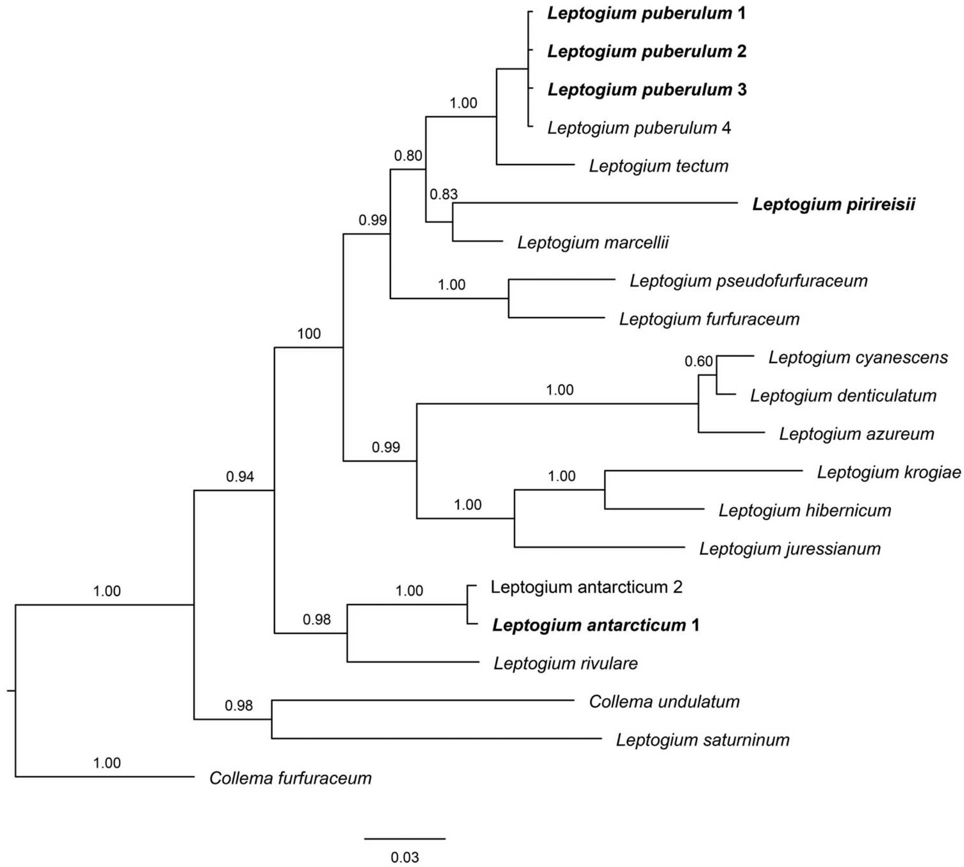

Figure 2. Inference of the phylogenetic relationships among *Leptogium* species resulting from Bayesian inference. Support values are above branches. Where more than one terminal per species is included, the numbers correspond to the specimen (see Table 1).

Mycobank No: 839689

Etymology: The epithet honors the Ottoman admiral Ahmed Muhiddin Piri (ca. 1465–1553), known as Piri Reis, who was a cartographer, geographer and navigator. His famous map published in 1513 included Antarctica and also some of the Subantarctic islands such as the Falkland Islands (Islas Malvinas).

***Diagnosis*:** Similar to *Leptogium rivulare* (Ach.) Mont., but differs by the thalli slightly attached by rhizines on the substrate, and ascospores 15–25 × 8–15 µm.

Type: Antarctica, James Ross Island, Panoramic Pass, 63° 48′ 56″ S, 57° 50′ 36″ W, alt. 220 m., on soil, 23.01.2017, Leg. M. G. Halıcı. JR 0.326 (ERCH Herbarium, holotype).

Description: *Thallus* 2.5–3.0 cm wide (Figure 3A), 100–300 µm thick, blackish under fluorescent light, opaque, matt, black (under a stereomicroscope with white light); maculae; lobes 0.3–0.7 mm wide, agglomerated, slightly attached, ascending; apices of lobes rounded, plane or involute, smooth; lateral margins of lobes smooth, plane or revolute; adventive ornamentation lobuliform, originated on the cracks, rare. Thallus attached by rhizines, as agglutinated strands of hyphae (Figure 3C), hapters and beard-like hairs absent; upper cortex smooth to slightly rugulose when viewed at 20×

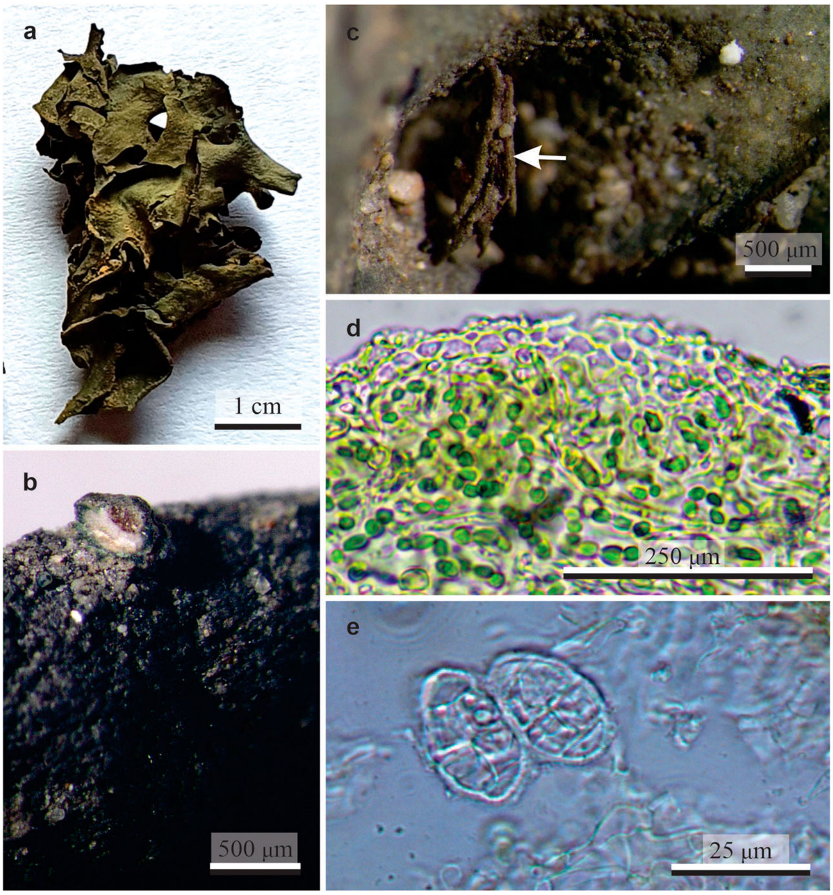

Figure 3. *Leptogium pirireisii* (holotype). **A,** Thallus. **B,** Details of the upper surface and apothecium. **C,** Detail of the rhizine, highlighting the compacted strands of hyphae (arrow). **D,** Transversal section of thallus, highlighting the paraplectenchymatous upper cortex. **E,** Ascospores.

magnification, composed of usually 2 layers of paraplectenchymatous cells (Figure 3D), up to 15 µm thick, isodiametric to rectangular cells of 8–13 × 5–10 µm diam.; lower cortex smooth when viewed at 20× magnification, blackish, composed of usually 2 layers of isodiametric cells of 10–12 × 5–8 µm diam.; medulla with columnar hyphae 3.0–6.0 µm thick, slightly sinuous, c. 4 cells long, frequent to scarce; photobiont cyanobacterium, yellowish green, frequent to abundant, spherical cells 5–6 × 3–4 µm diam., c. 5 cells per filament; gelatinous matrix frequent, hyaline.

Apothecium (only a single one found) 0.2 mm diam (Figure 3B), laminal, sessile; disc plane, brownish orange; epihymenium 20 µm tall; paraphyses unbranched, simple, septate, with oil droplets, tips clavate or slightly enlarged, 3–5 µm thick; hymenium hyaline, 80 µm tall; hypothecium hyaline, 60 µm tall. *Asci* 8-spored. *Ascospores* 15–25 × 8–15 µm, hyaline, ellipsoid, apices obtuse or almost orbicular, submuriform to muriform, with *ca*. 4 × 1–2 cells, with oil droplets when fresh (Figure 3E). *Pycnidia* not observed.

Ecology: *Leptogium pirireisii* is known from only one specimen, growing on soil in exposed cracks of large volcanic boulders, accomponied by other lichen species such as *Usnea antarctica* Du Rietz, *Parvoplaca athallina* (Darb.) Arup, Søchting & Frödén and *Megaspora verrucosa* (Ach.) Arcadia & A. Nordin.

Distribution: Thus far only known from James Ross Island, Antarctica.

Notes: *Leptogium pirireisii* differs from other species of the genus by the presence of glabrous thalli, with lobes covered by cortices usually with 2 layers of paraplectenchymatous cells, that are slightly attached to substrate by rhizines. Only one sessile and laminal apothecium was found with ascospores 15–25 × 8–15 µm.

Discussion

The results of the present study expand the known diversity of *Leptogium* in Antarctica, supporting the hypothesis that the diversity of the genus in the continent is higher than currently assumed (Kitaura et al. 2018). We also increase the known distribution of *L. antarcticum*, now including James Ross Island, based on both molecular and morphological data, highlighting the use of integrative approaches to study the diversity of *Leptogium* in this region (Bjelland et al. 2017; Kitaura et al. 2018; Kaasalainen et al. 2021). We provide additional sequence data for *L. puberulum*, an abundant species on James Ross Island, and present nuITS barcoding data, together with a morphological and anatomical descriptions, of the new species *L. pirireisii*.

Among the *Leptogium* species from Antarctica, *L. crispatellum* is another known species with glabrous thallus (Øvstedal and Smith 2004). However, *L. crispatellum* is characterised by the presence of isidia and lobules on the margins and lamina of the thallus, and presence of hapters (Kitaura et al. 2018), which differs of *L. pirireisii* that has only lobuliform structures originated along cracks, considered adventive lobules, i.e. responses to damage likely without taxonomic value, and attaches by rhizines. From phylogenetically related species, viz., *L. marcellii*, *L. puberulum* and *L. tectum*, they are hairy and differ from the new species that has glabrous thallus, corroborating with the earlier findings that the presence of hairs on the thallus surface in this genus is a homoplastic character, not denoting a natural group (Otálora et al. 2014).

Comparing with species that do not occur in Antarctica, *Leptogium pirireisii* is a small species as *Leptogium rivulare*, but the latter has thallus appressed to substrate and ascospores 15–20 × 7–8 µm (Jørgensen 2007), whereas the new species has thalli slightly attached to substrate and ascospores 15–25 × 8–15 µm. From phylogenetically close species, *L. furfuraceum* (Harm.) Sierk (France), *L. hibernicum* M.E. Mitch. ex P.M. Jørg. (Ireland), *L. juressianum* Tav. (Portugal), *L. krogiae* Bjelland, Frisch & Bendiksby (Tazmania) and *L. pseudofurfuraceum* P.M. Jørg. & Wallace (USA), they are isidiate and hairy (Sierk 1964; Kitaura and Marcelli 2012, 2013; Bjelland et al. 2017), and differ from *L. pirireisii* in which these characteristics are lacking. In addition, *L. denticulatum* Nyl. (Colombia) and *L. cyanescens* (Rabenh.) Körb. (Switzerland) has ornaments on the apothecia and thallus, respectively (Kitaura et al. 2015, 2019), whereas *L. pirireisii* has only adventive lobules on the cracks; and *L. azureum* (Sw.) Mont. (Jamaica) has thallus with 3–8 cm diam. and shortly pedicelate apothecia (Aragón et al. 2004), while *L. pirireisii* has thalli with 2.5–3.0 cm wide and sessile apothecium.

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