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A revision of *Leptogium* (*Collemataceae*, lichenized Ascomycota) from Antarctica with a key to species

Marcos J. KITAURA, Mayara C. SCUR, Adriano A. SPIELMANN and Aline P. LORENZ-LEMKE

Abstract: With more than 180 known species, *Leptogium* has its greatest richness in tropical regions. Only three species have so far been reported from Antarctica but extensive surveys in the Antarctic Maritime Islands have shown that this is an underestimate. *Leptogium antarcticum* (non-isidiate, with medulla composed of columnar hyphae), *L. marcellii* (non-isidiate, with medulla composed of a sponge-like arrangement of hyphae) and *L. tectum* (isidiate, with medulla composed of columnar hyphae) are described here as new to science. The new species are compared with those already reported for the genus in Antarctica, namely *L. crispatellum*, *L. menziesii* and *L. puberulum*, and an identification key is provided. Sequences of ITS and mrSSU regions were obtained from recently collected *L. antarcticum*, *L. marcellii*, *L. puberulum* and *L. tectum* specimens. Morphological and anatomical data were compared along with available genetic data in order to delimit these species more accurately, using an integrative approach.

Key words: columnar hyphae, isidia, ITS, lichen anatomy, mrSSU, phylogenetic analysis

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Introduction

Leptogium (Ach.) Gray is a genus of large foliose, eucorticate and mainly epiphytic species distributed worldwide (Sierk 1964; Otálora *et al.* 2014), including polar regions such as Antarctica (Øvstedal & Lewis Smith 2001). *Leptogium* specimens are attached to substrata by hapters, rhizines or hairs (Kitaura 2012). A hairy species presents at least one surface covered by hairs (Sierk 1964; Awasthi & Akhtar 1977; Jørgensen 1997), either simple or agglutinated. In species with simple hairs, such as *L. puberulum* Hue, the lower side has a velvety appearance, whereas in species with agglutinated hairs, for example *L. menziesii* (Ach.) Mont., the lower side is characterized by a beard-like appearance (Jørgensen 1997). Species with hairs on one or both surfaces were traditionally included in the section *Mallotium* (Ach.) Gray (Jørgensen 1971, 1997); however, molecular analyses revealed that this section and the genus as a whole are polyphyletic (Otálora *et al.* 2013, 2014).

The Antarctic continent, together with its maritime islands south of latitude 60°S, represents one of the most extreme climates on Earth. Its terrestrial biota is composed almost exclusively of cryptogamic species. Lichens are the most diverse organisms with more than 500 cited species (Spielmann & Pereira 2012). *Leptogium* species are widespread and locally abundant in the Antarctic Maritime Islands and the Antarctic Peninsula, covering wet or moist stony ground/soil (Øvstedal & Lewis Smith 2001). However, only three *Leptogium* species have been previously reported from Antarctica, the hairy *L. puberulum* and *L. menziesii* and the non-hairy *L. crispatellum* Nyl. (Dodge 1973; Lindsay 1974, 1978; Øvstedal & Lewis Smith 2001, 2004).

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Leptogium puberulum is endemic to Antarctica and can be found on the Antarctic Peninsula as well as on the Maritime Islands (Hue 1915; Dodge 1965, 1968, 1973; Lewis Smith & Corner 1973; Lindsay 1974; Redon 1985; Jørgensen 1986; Lewis Smith 1993; Sancho *et al.* 1999; Øvstedal & Lewis Smith 2001; see also Olech 2001 for additional references) whereas *L. menziesii* is a subantarctic species, originally collected from the Strait of Magellan area (Acharius 1803) and also found on the Falkland Islands and South Georgia (Øvstedal & Lewis Smith 2001), in Australia (Verdon 1992; Øvstedal & Lewis Smith 2001), Chile (Galloway & Jørgensen 1995; Øvstedal & Lewis Smith 2001), Japan (Hue 1898), New Zealand (Galloway 1999; Øvstedal & Lewis Smith 2001) and Uruguay (Osorio 1972). *Leptogium crispatellum* was found on King George Island (Øvstedal & Lewis Smith 2004) and in New Zealand (Nylander 1888).

Through detailed morphological and anatomical descriptions, and using molecular analyses based mainly on recent collections, we propose three new species of *Leptogium* as new to science.

Materials and Methods

Taxon sampling and morphological studies

Specimens of *Leptogium antarcticum*, *L. marcellii* and *L. puberulum* were collected during the Brazilian Antarctic Expeditions (OPERANTAR XXVI, XXXIII, XXXIV) to the South Shetland Islands. *Leptogium tectum* and *L. puberulum* were collected during the Campaña Antártica de Verano (CAV 2015–2016) to James Ross Island (Table 1). All specimens were deposited in the herbarium of the Universidade Federal de Mato Grosso do Sul, Brazil (CGMS).

The type of *L. puberulum*, which is deposited in the herbarium of the Muséum National d'Historie Naturelle (PC), was not available for study. Specimens of *L. puberulum* were identified through published descriptions (Hue 1915; Dodge 1965; Lindsay 1974) and distinguished from other species by their typically thicker cortex with two or more layers of cells. The detailed species description provided here was based on fresh specimens collected from the Antarctic Maritime Islands. Additionally, we examined the holotype of *L. crispatellum* (H) and the neotype of *L. menziesii* (E).

Sections were hand-cut and all specimens were studied and described according to Kitaura & Marcelli (2013), using the same terminology but the sequence of characteristics was changed here. The term "lobule" was applied only to ornamentation with constricted bases, appearing to be vegetative propagules (Hale 1983). Crenulate, verruciform and denticular appendages without constricted bases were considered functionally vegetative and are mentioned in the description when present. Lobules originating from cracks in the thallus were not considered ornamentation and the term "corona" is used according to Kitaura & Marcelli (2013). The thalline exciple is the tissue or all the tissues outermost to the proper exciple of a lecanorine apothecium (Kirk *et al.* 2008), including the cortex of thalline exciple that can be composed of thick paraplectenchymatous tissue. The apical part of the thalline exciple cortex can differ from the base and mid-height, where structures such as denticules, isidia, lobules and circular ridges can arise, and we have highlighted this difference in the descriptions.

In *Leptogium*, the proper exciple is a thick paraplectenchymatous tissue between the hypothecium and thalline exciple and can be found in other species such as *L. chloromelum* (Sw.) Nyl., *L. sessile* Vain. and *L. phyllocarpum* (Pers.) Mont. (Swinscow & Krog 1988). The proper exciple is lacking in all known Antarctic species of *Leptogium*.

DNA extraction, amplification and sequencing

Total DNA was extracted from freshly collected specimens using the Wizard® Genomic DNA Purification Kit (Promega, Madison, WI, USA). The nuclear ribosomal internal transcribed spacer region (ITS) was chosen for the analyses based on its ability to accommodate variability for the delimitation of fungal species (Schoch *et al.* 2012). The small subunit of the mitochondrial ribosomal RNA gene (mrSSU) is a more conserved region and widely used for phylogenetic studies of lichen-forming ascomycetes (Zoller *et al.* 1999), including the *Collemataceae* revision of Otálora *et al.* (2014).

The ITS region was amplified using the primers ITS1F (Gardes & Bruns 1993) and ITS4 (White *et al.* 1990); the mrSSU region was amplified using mrSSU1 and mrSSU3R (Zoller *et al.* 1999). Amplifications for both regions were performed in 25 µl reactions containing 1 × PCR Buffer (Promega), 0·2 µM of each primer, 0·2 µM of dNTPs, 2 µM of $MgCl_2$, 1 unit DNA polymerase (Promega) and 5–20 ng genomic DNA. The PCR reactions were carried out in a Veriti Thermal Cycler (Applied Biosystems) using the following programs: (ITS) initial denaturation at 94 °C for 5 min, 30 cycles of 95 °C for 30 s, 54·8 °C for 30 s, 72 °C for 1 min and a final extension at 72 °C for 5 min; (mrSSU) initial denaturation at 5 min, 35 cycles of 95 °C for 1 min, 54 °C for 1 min, 72 °C for 1 min and a final extension at 72 °C for 10 min. The sequencing was performed with an ABI 3730 XL automatic sequencer (Applied Biosystems).

Phylogenetic analyses

The sequences obtained were assembled with Geneious 8.1.7 (Kearse *et al.* 2012) and submitted to GenBank (Table 1). Sequences from other *Leptogium* species (*sensu* Otálora *et al.* 2014) available in GenBank were used in the analyses (Table 1). The ITS and

TABLE 1. *Voucher information and GenBank Accession numbers for selected* Leptogium *species analyzed in this study. New sequences are indicated in bold. Specimen ID refers to those used in Figs 1 & 2. Unknown = voucher information unavailable. – = sequences not available.*

Species	Specimen ID	Voucher information	Reference	GenBank Acc. no. ITS	GenBank Acc. no. SSU
Leptogium antarcticum	1	Antarctica, King George Island, Keller Peninsula, *N. M. Koch* 5528 (CGMS)	Present study	**KY171869**	**KY171880**
L. azureum	1	Chile, *Cornejo* 26507 (MA)	Otálora *et al.* 2013	–	JX992939
	2	South Korea (unknown)	Jayalal *et al.* 2014	KJ409609	–
L. biloculare	1	New Zealand, *Wedin* 9093 (S)	Otálora *et al.* 2013	–	JX992942
L. brebissonii	1	Colombia, *Otálora* 4011 (S)	Otálora *et al.* 2013	–	JX992943
L. britannicum	1	Colombia, *Otálora* 080109 (S)	Otálora *et al.* 2013	–	JX992944
L. burnetiae	1	Spain, *Aragón* 141/97 (MA)	Otálora *et al.* 2013	–	EU982584
	2	South Korea (unknown)	Jayalal, *et al.* 2014	KJ409601	–
L. corticola	1	USA, *Nordin* 4084 (UPS)	Otálora *et al.* 2013	–	EU982585
L. crispatellum	1	New Zealand, *Wedin* 9206	Otálora *et al.* 2013	–	JX992945
L. cyanescens	1	Unknown	Rangsiruji *et al.* 2016	KT947075	KT947102
L. dactylinum	1	Mexico, *Nordin* 3916 (UPS)	Otálora *et al.* 2013	–	JX992946
L. denticulatum	1	Argentina, *Wedin* 8698 (S)	Otálora *et al.* 2013	–	JX992948
	2	South Korea (unknown)	Jayalal *et al.* 2014	KJ409597	–
L. digitatum	1	Colombia, *Otálora* 010109 (S)	Otálora *et al.* 2013	–	JX992950
L. furfuraceum	1	Spain, *Aragón* 16280 (MA)	Otálora *et al.* 2010, 2013	EU982634	EU982553
L. hibernicum	1	New Zealand, *Wedin* 8751 (S)	Otálora *et al.* 2013	–	JX992952
L. hildenbrandii	1	Rumania, Bayerova, *Halda & Palice* (UPS)	Otálora *et al.* 2013	–	JX992951
L. isidiosellum	1	Cuba, *Pérez-Ortega* 659 (S)	Otálora *et al.* 2013	–	JX992953
L. jurassianum	1	South Africa, *Nordin* 11812 (UPS)	Otálora *et al.* 2013	–	JX992954
L. laceroides	1	Colombia, *Otálora* 020109 (S)	Otálora *et al.* 2013	–	JX992955
L. malmei	1	Chile, *Santesson* 3960 (S)	Otálora *et al.* 2013	–	JX992956
L. marcellii	1	Antarctica, Dufayel Island, *N. M. Koch* 5552 (CGMS)	Present study	**KY171872**	**KY171883**
	2	Antarctica, King George Island, Admiralty Bay, *C. M.Bernardo* 487	Present study	**KY171871**	**KY171882**
L. marginellum	1	Cuba, *Pérez-Ortega* 664 (S)	Otálora *et al.* 2013	–	JX992958
L. papillosum	1	Argentina, *Wedin* 8750 (S)	Otálora *et al.* 2013	–	JX992961
L. pedicellatum	1	South Korea, *Thor* 17186 (UPS)	Otálora *et al.* 2013	–	JX992962
	2	South Korea, (unknown)	Jayalal *et al.* 2014	KJ409611	–
L. phyllocarpum	1	Costa Rica, *O'Brien* 03051203 (DUKE)	Otálora *et al.* 2013	–	EU982589
L. pseudofurfuraceum	1	Argentina, *Aragón* 16291 (MA)	Otálora *et al.* 2010, 2013	EU982645	EU982562
L. puberulum	1	Antarctica, King George Island, Hennequin Point, *C. M. Bernardo* 441 (CGMS)	Present study	**KY171876**	**KY171887**
	2	Antarctica, King George Island, Hennequin Point, *C. M. Bernardo* 486 (CGMS)	Present study	**KY171877**	**KY171888**
	3	Antarctica, King George Island, Hennequin Point, *A. P. Lorenz-Lemke* 345 (CGMS)	Present study	**KY171873**	**KY171884**
	4	Antarctica, King George Island, Hennequin Point, *A. P. Lorenz-Lemke* 348 (CGMS)	Present study	**KY171874**	**KY171885**

TABLE 1 (*continued*).

Species	Specimen ID	Voucher information	Reference	GenBank Acc. no. ITS	GenBank Acc. no. SSU
Leptogium puberulum	5	Antarctica, King George Island, Vauréal Peak, *A. P. Lorenz-Lemke* 409 (CGMS)	Present study	**KY171875**	**KY171886**
	6	Antarctica, James Ross Island, *M. J. Kitaura* 2802 (CGMS)	Present study	**KY171878**	**KY171889**
	7	Antarctica, James Ross Island, *M. J. Kitaura* 2804 (CGMS)	Present study	**KY171879**	**KY171890**
L. resupinans	1	Canary Islands, *Otálora* 010410 (S)	Otálora *et al.* 2013	–	JX992963
L. reticulatum	1	Colombia, *Otálora* 030810 (S)	Otálora *et al.* 2013	–	JX992964
L. rivulare	1	Sweden, *Delin* 20000416 (UPS)	Otálora *et al.* 2013	–	JX992965
L. saturninum	1	France, MA-Lichen 16024	Otálora *et al.* 2008, 2013	DQ466043	EU982569
L. sessile	1	Taiwan, *Aptroot* 52125 (DUKE)	Otálora *et al.* 2013	–	JX992968
L. tectum	1	Antarctica, James Ross Island, *M. J. Kitaura* 2948 (CGMS)	Present study	**KY171870**	**KY171881**
L. velutinum	1	Ecuador, *Prieto* 2010 (S)	Otálora *et al.* 2013	–	JX992972

mrSSU datasets were aligned using the MAFFT v.7.222 algorithm (Katoh *et al.* 2002) available in Geneious v.8.1.7, with scoring matrix 200PAM/k = 2, gap penalty = 1·53, offset value = 0·123 and automatic algorithm selection. Ambiguously aligned regions were removed using the Gblocks web server (Castresana 2000; Talavera & Castresana 2007) with the less stringent options.

The best substitution models used in the phylogenetic analyses were indicated by jModelTest v.2.1.7 (Guindon & Gascuel 2003; Darriba *et al.* 2012), using the Akaike Information Criterion (AIC) and the Bayesian Information Criterion (BIC), respectively: GTR + G and TRN + G for ITS, TPM2uf + I + G and HKY + I + G for mrSSU.

For the maximum likelihood (ML) inference, the 50% majority consensus rule tree was estimated using PhyML plug-in in Geneious 8.1.7 (Guindon & Gascuel 2003), with 5000 bootstrap replicates and the best substitution model for each alignment, taking into consideration the AIC results in jModelTest. For the Bayesian (BA) inference, the maximum clade credibility tree was generated using BEAST v.1.8.2 (Drummond *et al.* 2012) using the best substitution model resulting from each alignment, taking into consideration the BIC results in jModelTest. A Yule tree prior was assumed and for all other parameters the default priors were used. A run was performed for a chain length of 10 000 000, sampling every 1000 generations. The tree was generated from the combined sampled trees after discarding the first 20% as burn-in. Tracer 1.5 (Rambaut *et al.* 2014) was used to check MCMC convergence (effective sample sizes ≥200). Clades that received bootstrap support ≥75% in the ML analysis or posterior probabilities ≥0·95 were considered well supported. *Leptogium saturninum* (Dicks.) Nyl., *L. burnetiae* Dodge and *L. pedicellatum* P. M. Jørg. sequences were used as outgroups for both datasets (ITS and mrSSU) and *L. hildenbrandii* for the mrSSU dataset, bearing in mind their position in clade C and close relationship with the *Leptogium* clade B group (*sensu* Otálora *et al.* 2014) and the availability of sequences in GenBank. The trees were edited in FigTree v.1.3.1 (Rambaut 2009).

To provide additional support for species delimitation, the ITS sequences were used to calculate ML distances as the number of substitutions per site (s/s) using PAUP v.4.0b10 (Swofford 2003). Additionally, the ABGD web server (Puillandre *et al.* 2011) was used to estimate the barcode gap in the distribution of pairwise distances with Pmin = 0·001, Pmax = 0·1, different gap width values (0·5, 1·0 and 1·5), Nb bins = 20 as the settings, and the JC69 substitution model.

Results and Discussion

We obtained ITS and mrSSU sequences for one specimen each of *Leptogium antarcticum* and *L. tectum*, two specimens of *L. marcellii* and seven specimens of *L. puberulum*. ITS and mrSSU sequences from species positioned in *Leptogium* clade B (Otálora *et al.* 2014) were used in the phylogenetic analyses (Table 1). The sequence sizes varied between 484 and 587 base pairs (bp) for ITS, and 580 and 603 bp for mrSSU markers. After the removal of uncertain positions from the alignment, ITS and mrSSU datasets were composed of matrices of 491 and 596 bp, respectively.

For the different datasets and using ML and BA approaches, the trees showed similar topologies and the phylogenetic positions of the three new species were consistently

maintained. Thus, only maximum clade credibility (BA) trees are shown in Figs 1 (ITS) and 2 (mrSSU).

The ITS and mrSSU trees were in agreement regarding the highly supported close relationship between *L. puberulum* and *L. tectum* in a clade sister to *L. marcellii*. In addition, the ITS tree supports the relationship among all three species and *L. furfuraceum* (Harm.) Sierk and *L. pseudofurfuraceum* P. M. Jørg. & Wallace,

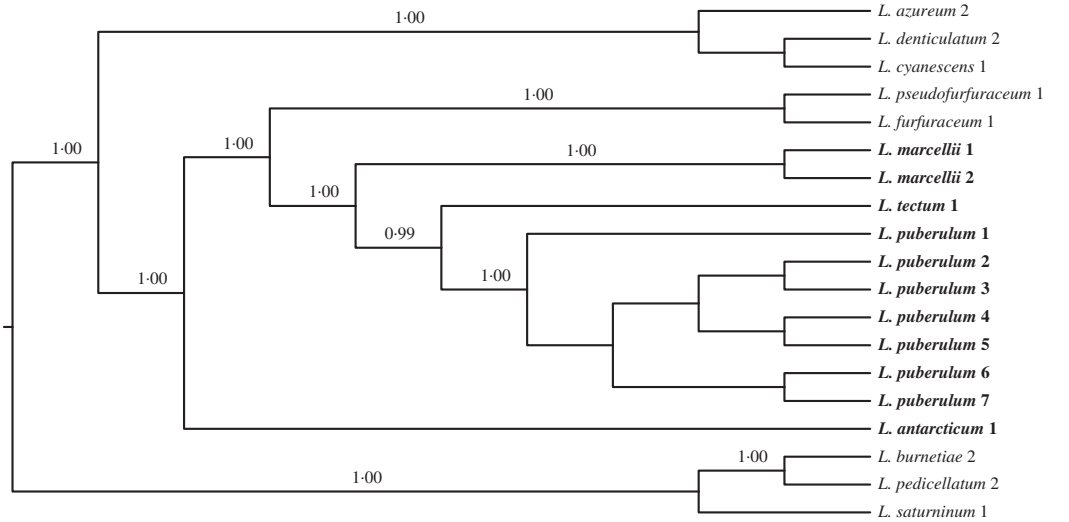

FIG. 1. Bayesian Maximum Clade Credibility tree based on the ITS dataset showing the phylogenetic relationships among *Leptogium antarcticum*, *L. marcellii*, *L. tectum* and other *Leptogium* species. Posterior probabilities (PP) >0·95 shown above branches. Details of specimens are given in Table 1. Sequences obtained in this study are in bold.

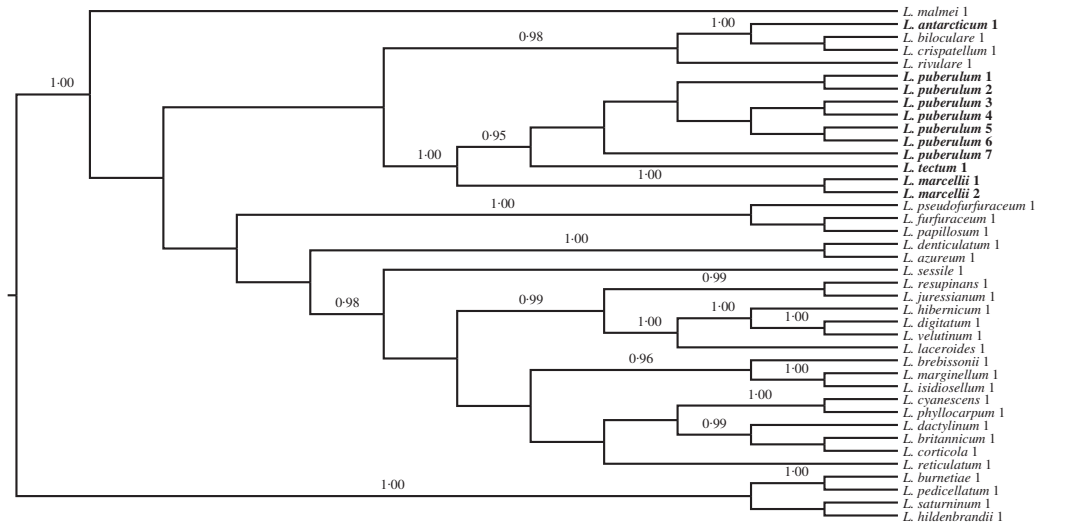

FIG. 2. Bayesian Maximum Clade Credibility tree based on the mrSSU dataset showing the phylogenetic relationships among *Leptogium antarcticum*, *L. marcellii*, *L. tectum* and other *Leptogium* species. Posterior probabilities (PP) >0·95 are above branches. Details of specimens are shown in Table 1. Sequences obtained in this study are marked in bold.

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but this is not apparent in the mrSSU tree (Fig. 2).

In the mrSSU analyses, *L. antarcticum* is related to *L. biloculare* F. Wilson, *L. crispatellum* and *L. rivulare* with high support values; however, with ITS the placement of these species inside the genus was unresolved because sequences for the latter three species were unavailable.

No morphological or geographical patterns were found in common between *L. antarcticum* and *L. biloculare*, *L. crispatellum* and *L. rivulare*. All trees showed the separation of clades B (*L. denticulatum* Nyl., *L. azureum* (Sw.) Mont., *L. cyanescens* (Rabenh.) Körber., *L. puberulum*, *L. rivulare* (Ach.) Mont., *L. biloculare*, *L. crispatellum* and others) and C (*L. saturninum*, *L. pedicellatum* and *L. burnetiae* and others) described by Otálora *et al.* (2014), the three new species belonging to clade B.

For the purpose of species delimitation and the first placement of the three new species proposed here, the data provided is adequate. However, more data from other markers is necessary, especially because the greatest availability of sequences was for the mrSSU marker and its conserved nature may not reflect the real position of the new species inside *Leptogium*.

The ITS pairwise distance comparisons among *L. antarcticum*, *L. tectum* and *L. marcellii*, and their respective most-related species, suggest interspecific diversity. Sequences of *L. tectum* and *L. puberulum* presented 0·0340 s/s of ML distance (*c.* 3·15% of divergence), *L. marcellii* and *L. puberulum* 0·060 s/s (6·12% of divergence) and *L. antarcticum* and *L. pseudofurfuraceum* 0·265 s/s (17·00% of divergence). Although the intra- and interspecific genetic distances of *Leptogium* are not well known, the frequently used 3% of divergence threshold corroborates the delimitation of the three new species (Nilsson *et al.* 2008; Blaalid *et al.* 2013). In all partitions of the ABGD analyses (between maximal distance $P = 0{\cdot}001$ and $P = 0{\cdot}01$, as well as with the three different gap width values) the three new species were retained in separate groups. Besides the ABGD analysis, even low ML distances (as the *c.* 3·15% of divergence between *L. tectum* and *L. puberulum*) were considered adequate to delimit *L. tectum* as a new species because of significant morphological differences. The integrative approach of this study allowed an accurate delimitation of three new *Leptogium* species from Antarctica. Expanding the geographical ranges of these species using detailed morphological and anatomical characters as well as genetic analyses will probably reveal more species and disclose the real diversity of the genus on this continent.

Species Descriptions

Leptogium antarcticum Scur, Lorenz-Lemke & Kitaura sp. nov.

MycoBank No.: MB 822500

Similar to *Leptogium puberulum* but with agglomerated, ascending and ornamented lobes; beard-like hairs on lower side; margin of apothecia with circular wrinkles.

Type: Antarctica, South Shetland Islands, King George Island, Keller Peninsula, near Comandante Ferraz Antarctic Station (EACF), on mosses, 62°05'05·2"S, 058°23′40'7"W, 24 m, 7 December 2015, *N. M. Koch* 5528 (CGMS—holotype).

(Fig. 3A–C)

Thallus c. 2·5 cm wide, 85–125 µm thick, dark brown to blackish under fluorescent light, opaque, matt or shiny, grey to dark brown when observed under stereomicroscope with white light; *maculae* absent; *lobes* 0·5–2·0 mm wide, agglomerated, slightly attached, ascending; *apices of lobes* rounded, slightly revolute, smooth to rarely with ornamentation; *lateral margins of lobes* smooth to irregular, ascending, thickened; *vegetative ornamentation* denticulate, verruciform, cylindrical to lobuloid, with rounded apices, simple, 0·05–0·15 × 0·05–0·15 mm, laminal and marginal, originating from ridges; *isidia* and *lobules* absent. Thallus attached by beard-like hairs, 3–7 cylindrical cells long, 0·05–0·65 mm long, usually cream-coloured; rhizines and hapters absent; *upper cortex* smooth to slightly rugulose when viewed at ×20, composed of 1–3 layers of paraplectenchymatous cells, up to 20 µm thick, isodiametric to rectangular cells of 4·0–7·0 µm diam., covered by thick amorphous layer (similar to cuticle of leaves); *lower cortex* tomentose, grey, smooth and beard-like hairy when viewed at

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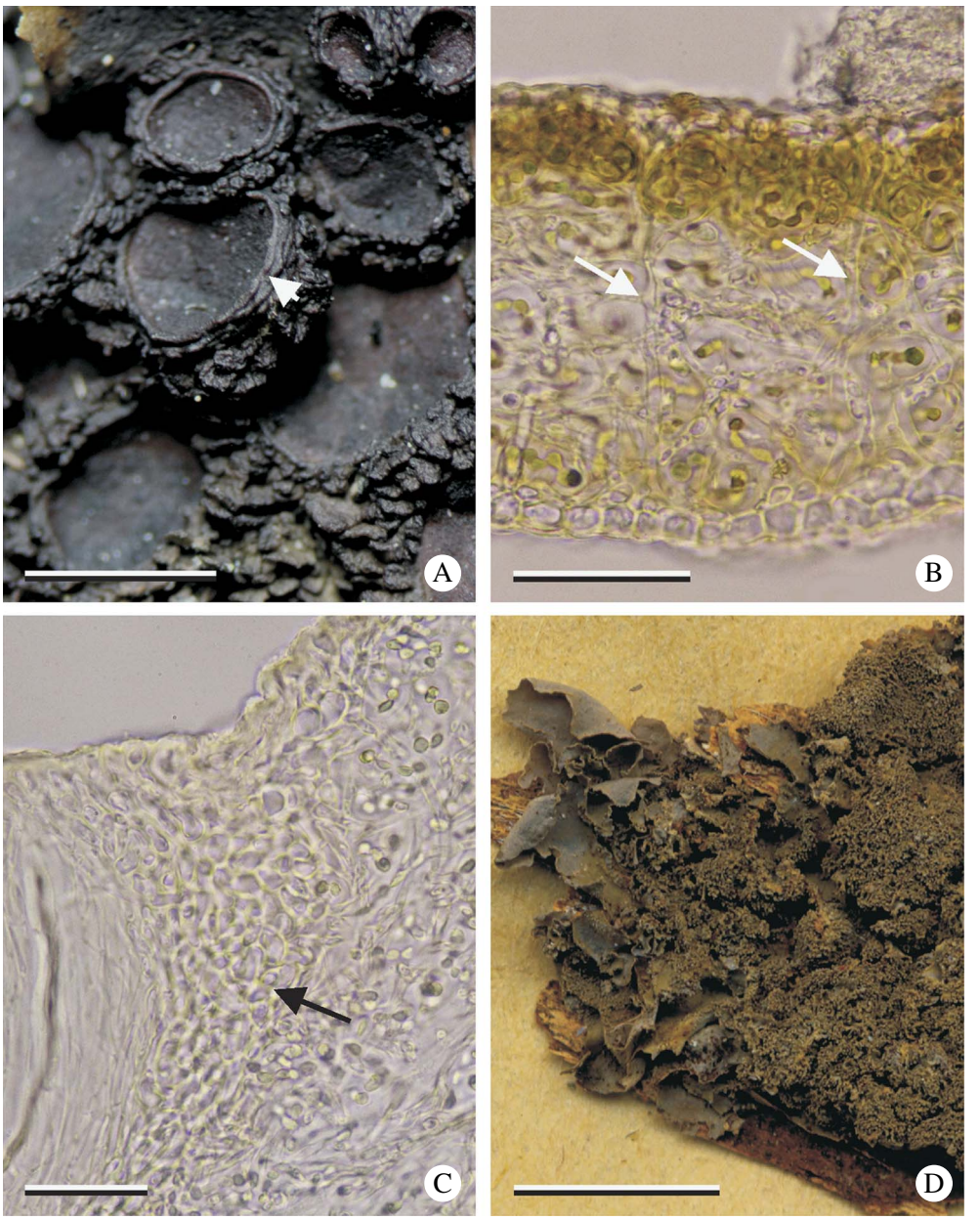

FIG. 3. A–C, *Leptogium antarcticum*; A, holotype, arrow = circular ridges; B, TS thallus, arrows = inclined columnar hyphae; C, TS apothecium, arrow = paraplectenchymatous parahymenial tissue. D, *Leptogium crispatellum*, thallus (holotype). Scales: A = 3 mm; B = 20 µm; C = 50 µm; D = 5 mm. In colour online.

×20, composed of one layer of isodiametric cells of 3·0–7·5 µm diam.; *medulla* with columnar hyphae *c.* 2·5 µm thick, inclined at *c.* 65° from the cortex or slightly sinuous, *c.* 4 cells long, frequent to scarce; *photobiont* cyanobacterium, yellow, frequent to abundant,

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spherical cells 2·5–5·0 µm diam., *c.* 8 cells per filament; *gelatinous matrix* frequent, hyaline.

Apothecia up to 3·0 mm diam., laminal, adnate; *disc* plane to concave; *basal paraplectenchymatous tissue* absent; *thalline exciple* cortex concolorous with the thallus, base and mid-height with verruciform ornamentation and lobules, with simple and minute hairs, margin smooth or with denticules and circular ridges (Fig. 3A) as in *L. sessile*; composed of paraplectenchymatous tissue, 25–30 µm (3–5 cells) thick at the base, 10–15 µm (*c.* 2 cells) thick at mid-height, 3·0–5·0 µm (one cell) thick at the apex; *corona* and *pedicel* absent; *parahymenial tissue* continuous with hypothecium, with paraplectenchymatous tissue (Fig. 3C), *c.* 100 µm (up to 10 cells) thick at the apex, as tall as hymenium; *proper exciple* absent; *hypothecium* 20·0–25·0 µm thick, colourless, cell tissue subparaplectenchymatous to elongated; *subhymenium* up to 40 µm thick, colourless; *hymenium c.* 140 µm thick; *asci* not observed; *ascospores* fusiform, 20–25 × 10–15 µm (3–4 × 1–2 cells), apices acute, submuriform to muriform.

Pycnidia rare, immersed in the thallus; *conidia* bacilliform to bifusiform, *c.* 2·5 × 1·0 µm.

Etymology. The epithet refers to the Antarctic region where the species was found.

Distribution. Currently known only from King George Island, South Shetland Islands, Antarctica.

Discussion. *Leptogium antarcticum* is characterized by the agglomerated lobes, verruciform ornamentation on the lamina and margin, beard-like hairs on the lower surface, amphithecia with ornamentation and simple hairs, and the smooth margin of apothecia with circular wrinkles similar to *L. sessile*, a non-hairy species without ornamentation. The medulla of *L. antarcticum* is composed of inclined (Fig. 3B) to slightly sinuous columnar hyphae (Table 2).

Leptogium crispatellum Nyl.

Lichenes Novae Zelandiae **1888:** 10 (1888); type: New Zealand, "corticola ad Greymouth", 1888, *R. Helms* 214 (H–NYL 41462!—holotype; see Galloway 1985).

(Figs 3D & 4A)

Thallus c. 2·5 cm wide, 55–70 µm thick, grey to bluish grey under fluorescent light, opaque, matt, bluish grey when observed under stereomicroscope with white light; *maculae* present; *lobes c.* 2 mm wide, agglomerated, slightly attached, revolute to ascending; *apices of lobes* rounded, revolute to ascending, smooth; *lateral margins of lobes* smooth, revolute, crispate, thin; *isidia* granular to flattened, 0·05–0·15 × 0·04–0·10 mm, simple to very branched, erect, firm, concolorous with the thallus or slightly blackish, usually marginal but sometimes laminal, grouped, abundant, granular when young and flattened with age; *lobules* round to oval, apices rounded to truncate, 0·05–0·10 × 0·10–0·20 mm, usually simple but occasionally branched in just one plane, erect, firm, concolorous with the thallus, usually marginal to laminal, densely aggregated, abundant; *thallus attached* by short hapters (Fig. 4A), hairs absent on the margin and cross-sections of the thallus; *upper cortex* smooth when viewed at ×20; consisting of one layer of isodiametric cells, *c.* 10 µm diam.; *lower cortex* bluish grey, smooth when viewed at ×20, composed of one layer of isodiametric cells, *c.* 7·5 µm diam.; *medulla* with columnar hyphae 2·5 µm thick, inclined at 80–70° from the cortex or slightly sinuous, 5–7 cells high; *photobiont* cyanobacterium, blue, scarce to frequent in medulla, cells elliptic, number of cells per filament not determined; *gelatinous matrix* scarce, varying from yellow next to the upper cortex to hyaline below.

Apothecia and *pycnidia* absent.

Distribution. Australia (Verdon 1992); New Zealand (Nylander 1888; Müller Argoviensis 1894; Galloway 1999); Tasmania (Verdon 1992); Subantarctic region (Marion and Heard Islands; Øvstedal & Lewis Smith 2001, 2004) and Antarctic region (South Shetland Islands; Øvstedal & Lewis Smith 2001, 2004).

Discussion. *Leptogium crispatellum* is characterized by agglomerated lobes, granular to flattened isidia and usually some rounded to oval lobules typically on the margin (Table 2). The thallus is thin (55–70 µm) with a smooth upper surface and the medulla is loosely packed with inclined or slightly sinuous columnar hyphae.

TABLE 2. *Characteristics distinguishing the* Leptogium *species found in Antarctica.*

	L. antarcticum	*L. crispatellum*	*L. marcellii*	*L. menziesii*	*L. puberulum*	*L. tectum*
Thallus width (cm)	2·5	2·5	4·0	7·0	9·0	1·5
Lobe structure	Agglomerated	Agglomerated	Agglomerated to overlapping distally	Overlapping	Agglomerated	Agglomerated
Vegetative ornamentation	Denticulate, verruciform to lobuloid, laminal and marginal	Absent	Absent	Absent	Crenulate, verruciform or denticulate, marginal	Verruciform, and usually marginal
Isidia	Absent	Granular to flattened, usually marginal	Absent	Absent	Absent	Granular to cylindrical, laminal
Lobules	Absent	Round to oval, usually marginal to laminal	Flattened, usually marginal	Absent	Absent	Absent
Thallus attachment	Tomentose, beard-like hairs	No hairs	Tomentose, simple to beard-like hairs	Tomentose, simple and beard-like hairs	Tomentose, simple hairs	Non-tomentose, interwoven hyphae
Number of cell layers in cortices	1–3	1	1–2(–3)	1	Upper:1–2; lower: 1–3 (–4)	Upper: 1; lower: 1–3
Medullar columnar hyphae	Inclined or slightly sinuous	Inclined to slightly sinuous	Absent, sponge-like arrangement of hyphae	Not observed	Erect to inclined	Erect to inclined
Parahymenial tissue	Paraplectenchymatous	Not observed	Elongated to colloplectenchymatous	Paraplectenchymatous	Colloplectenchymatous	Not observed

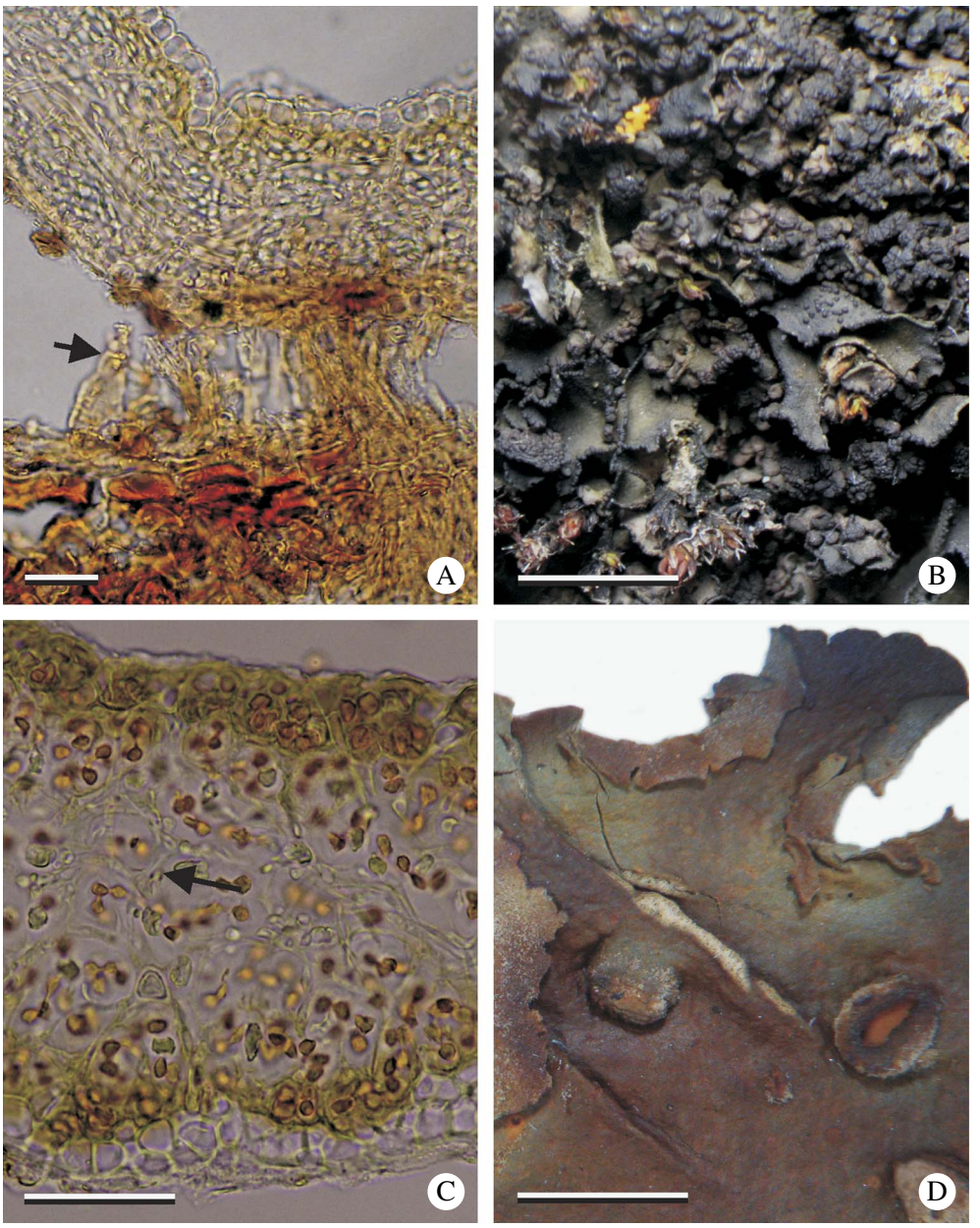

FIG. 4. A, *Leptogium crispatellum*; TS thallus, arrow = detail of hapter. B & C, *Leptogium marcellii*; B, holotype; C, TS thallus, arrow = sponge-like arrangement of the medullary hyphae. D, *L. menziesii* (neotype). Scales: A = 20 µm; B & D = 3 mm; C = 30 µm. In colour online.

The type of *L. crispatellum* is pasted on a card, therefore hairs were not observed on the margins of lobes and cross-section of the thallus. The thallus is attached to the substratum by short hapters (Fig. 4A). Neither Verdon (1992) nor Galloway (1999) mentioned the presence of hairs.

Leptogium crispatellum differs from *L. antarcticum* by the presence of hapters and granular to flattened isidia with *L. antarcticum* having beard-like hairs and irregular vegetative ornamentation, such as denticulate, verruciform, cylindrical to lobuloid structures.

The presence of *L. crispatellum* in Antarctica is based upon two specimens (*Ochyra* 2700/80 and 2708/80) that were cited in Øvstedal & Lewis Smith (2001, 2004) as *Leptogium* sp. A and *L. crispatellum*, respectively. However, both descriptions mentioned the presence of hairs on the lower side and the characteristics of these specimens from the South Shetland Islands and the Antarctic Peninsula did not match the holotype, which was collected in New Zealand. They could represent a new species or one of the species described in this work and more collections in other areas of Antarctica are required to confirm the presence of *L. crispatellum* on the continent.

Leptogium marcellii Kitaura, Lorenz-Lemke & A. A. Spielm. sp. nov.

MycoBank No.: MB 822501

Similar to *Leptogium puberulum* but with overlapping, ornamented lobes; beard-like hairs on lower surface; medullary hyphae loosely packed, with a sponge-like arrangement filled by the cyanobacterium and gelatinous matrix.

Type: Antarctica, South Shetland Islands, King George Island, Ezcurra Cove, Dufayel Island, on mosses, 62°10'06·5"S, 058°33'45·2"W, 16 m, 13 December 2015, *N. M. Koch* 5552 (CGMS—holotype).

(Fig. 4B & C)

Thallus c. 4 cm wide, 85–125 μm thick, dark brown to blackish under fluorescent light, opaque, matt, brownish to greenish grey when observed under the stereomicroscope with white light; *maculae* absent; *lobes* up to 4 mm wide, overlapped in the marginal part to agglomerated, slightly attached, ascending; *apices of lobes* rounded, slightly revolute and ascending, smooth or with ornamentation; *lateral margins of lobes* smooth to lobulate, ascending, crispate, thickened; *isidia* absent; *lobules* flattened, apices rounded, 0·2–1·0 μm diam., simple, erect, firm, concolorous with the thallus to blackish, usually scattered on the margin of the thallus, but also found in clusters on the lamina and on the margin of apothecia, frequent to rare; *thallus attached* by simple (non-agglutinated) hairs and beard-like hairs, more than 7 cylindrical cells long, 50 μm long or more when agglutinated, cream to white, abundant in the central region and scarce at the margin on the lower side; *rhizines* and *hapters* absent; *upper cortex* smooth to rugulose when viewed at ×20, composed of 1–2(–3) layers of paraplectenchymatous, isodiametric to rectangular cells, 5·0–10·0 × 5·0–7·5 μm, covered by thick amorphous layer (similar to the cuticle of leaves), *c.* 7·5 μm thick; *lower cortex* tomentose, light grey to whitish, smooth to rugulose when viewed at ×20, one layer of isodiametric cells, 5·0–7·5 μm diam.; *medulla* composed of loosely packed hyphae, in a sponge-like arrangement filled with the cyanobacterium; columnar hyphae absent; *photobiont* cyanobacterium, blue,, spherical cells, frequent within the medulla, *c.* 5·0 μm diam., number of cells per filament not determined; *gelatinous matrix* frequent, hyaline.

Apothecia (as determined only from additional material (*Spielmann* 4429) for which DNA sequences were not obtained) up to 2·5 mm diam., laminal, subpedicellate; *disc* concave; *basal paraplectenchymatous tissue* absent; *thalline exciple* cortex concolorous with the thallus to cream when pubescent, base and mid-height smooth to pubescent; *margin* ornamented with verruciform structures when young and with lobules when older, concolorous with the thallus; composed of paraplectenchymatous tissue, 25–30 μm (3–5 cells) thick at the base, 20–25 μm (2–3 cells) thick at mid-height, 12–17 μm (amorphous layer) thick at the apex; *corona* and *pedicel* absent; *parahymenial tissue* continuous with hypothecium, with cells elongated to colloplectenchymatous, *c.* 25 μm (3–4 cells) thick at the base, 25–75 μm (5–9 cells) thick at the apex, as tall as hymenium; *proper exciple* absent; *hypothecium* 12·5–15·0 μm, colourless, made of prosoplectenchymatous tissue of irregular cells; *subhymenium* 20–25 μm thick, colourless; *hymenium* 90–100 μm thick. *Asci* not observed; *ascospores* fusiform, 20·0–25·0 × 5·0–7·5 μm, apices acute, submuriform to muriform.

Pycnidia concolorous with the thallus, ostiole brown, sessile; *conidia* bifusiform to bacilliform, *c.* 5·00 × 1·25 μm.

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Distribution. Currently known only from King George and Livingston Islands, South Shetland Islands, Antarctica.

Discussion. *Leptogium marcellii* is characterized by the presence of overlapping lobes, with marginal lobules. The lamina of the thallus also has ornamentation but this is more frequent on the margin (Fig. 4B). The thallus is 85–125 µm thick, and the medullary hyphae are loosely packed in a sponge-like arrangement with the cyanobacterium. Columnar hyphae were not found in the specimens examined (Table 2); the pattern of medullary hyphae can be used to identify this species (Fig. 4C).

The only material with apothecia (*Spielmann* 4429) was collected in 2007 but DNA sequences were not obtained from this specimen. Nevertheless, the anatomical and morphological descriptions of this specimen were included in the description of *L. marcellii*. The apothecia have margins that are ornamented with verruciform structures when young and with lobules when older whereas those of *L. antarcticum* have smooth or denticulate margins. In addition, *L. antarcticum* has ornamented amphithecia whereas *L. marcellii* does not. *Leptogium marcellii* also has overlapping lobes and lobules on the margin, differing from *L. antarcticum* which has agglomerated lobes, verruciform ornamentation on the lamina and margin, and slightly sinuous columnar hyphae in the medulla.

Leptogium marcellii is named in honour of Dr Marcelo Pinto Marcelli, an outstanding Brazilian lichenologist who believed that columnar hyphae and medullary hyphae of sponge-like arrangement with the cyanobacterium are characteristics with taxonomic importance in *Leptogium*, a belief that has consistently inspired the first author.

Selected specimens examined. **Antarctica:** *South Shetland Islands*: King George Island, Admiralty Bay, Stenhouse Point, saxicolous, 62°04·307'S, 58°22·455'W, 2014, *C. M. Bernardo* [487] & *S. C. Feuerstein* (CGMS); Livingston Island, Byers Peninsula, on mosses in the soil with small pebbles, 62°39'18·1"S, 61°08'40·4"W, 2007, *A. A. Spielmann* [4429], *C. E. R. Schaefer* & *M. R. Francelino* (CGMS—fertile specimen).

Leptogium menziesii (Ach.) Mont.

Ann. Sci. Nat. Bot. Biol. **18**(3)**:** 313 (1852). —*Lichen menziesii* Sm., apud Ach., *Methodica Lichenum*: 221 (1803) *pro. syn.*—*Parmelia menziesii* Ach., *Methodica Lichenum*: 221 (1803).—*Collema menziesii* (Ach.) Ach., *Lich. Univer.*: 645 (1810); type: Argentina, Staten Island, near Cape Horn, February 1789, *Menzies* s. n. (E!—neotype designated by Jørgensen 1975).

(Figs 4D, 5A & B)

Thallus c. 7 cm wide, 50–75 µm thick, brownish grey under fluorescent light, opaque, matt, greenish grey with some brownish parts under stereomicroscope with white light; *maculae* absent; *lobes* up to 10 mm wide, overlapping, adnate; *apices of lobes* rounded, plane, smooth; *lateral margins of lobes* smooth, plane, straight, thin; *isidia* and *lobules* absent; *thallus attached* by simple and beard-like hairs, usually with 4 cylindrical cells, 100 µm long or more when agglutinated, cream on the lower cortex, abundant, *upper cortex* rugulose when viewed at ×20, composed of isodiametric cells, *c.* 10·0 × 5·0 µm, one layer of paraplectenchymatous cells; *lower cortex* tomentose, greenish grey, smooth and pubescent when viewed at ×20 magnification, isodiametric cells, *c.* 15·0 × 7·5 µm, one layer of paraplectenchymatous cells, covered by a thick amorphous layer (similar to the cuticle of leaves); *medulla* with hyphae and the cyanobacterium compactly woven in a closed pattern, columnar hyphae absent; *photobiont* cyanobacterium, blue, frequent within the medulla, cells elliptic, 5·0 × 7·5 µm, number of cells per filament not determined; *gelatinous matrix* scarce, hyaline.

Apothecia up to 1·5 mm diam., laminal, subpedicellate; *disc* concave; *basal paraplectenchymatous tissue* 35–40 µm (2–6 cells) thick, external cells covered by thicker amorphous layer; *thalline exciple* cortex yellow to beige, base and mid-height pubescent, *margin* smooth to slightly wrinkled, without ornamentation, with wrinkles visible to the naked eye, brown to blackish; composed of paraplectenchymatous tissue 65–70 µm (7–8 cells) thick at the base, 20–25 µm (*c.* 2 cells) thick at the mid-height, 20–25 µm (*c.* 2 cells) thick at the apex; *corona* absent; *pedicel* up to 0·5 mm long, smooth, pubescent, as tall as hymenium, with external cells of amphithecia covered by thicker

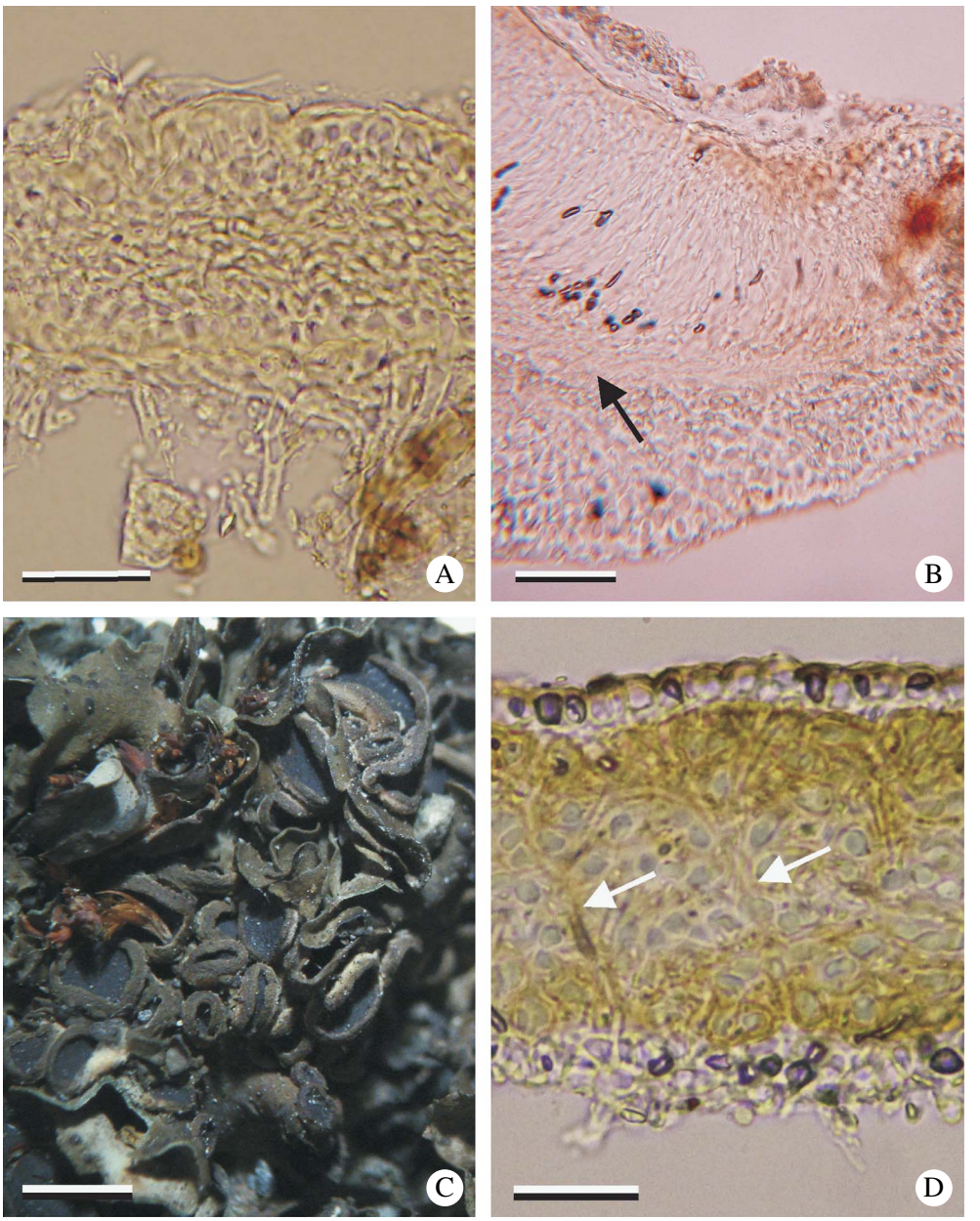

FIG. 5. A & B, *Leptogium menziesii*; A, TS thallus (poorly conserved specimen); B, TS apothecium, arrow = hypothecium. C & D, *Leptogium puberulum*; C, upper surface and apothecia; D, TS thallus, arrows = columnar hyphae. Scales: A = 30 µm; B & D = 50 µm; C = 3 mm. In colour online.

amorphous layer (similar to cuticle of leaves); *parahymenial tissue* continuous with hypothecium, with paraplectenchymatous tissue, 15–18 µm (3 cells) thick at the base, 15–20 µm (8 cells) thick at the apices; *proper exciple* absent; *hypothecium* 25–30 µm thick, yellow, of prosoplectenchymatous tissue of irregular cells (Fig. 6A – arrow); *subhymenium* 15–20 µm

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thick, yellow; *hymenium c.* 125 µm thick. *Asci* not observed; *ascospores* not found (22–27 × 8–10 µm; Jørgensen 1975).

Pycnidia laminal, usually close to the thallus margin, concolorous with the thallus or brown; *conidia* bacilliform, 3·75 × 1·25 µm.

Distribution. Argentina, Staten Island (Jørgensen 1975); Chile, Fuegia (Galloway & Jørgensen 1995); Falkland Islands; New Zealand; Peru; Antarctic region on South Georgia (Lindsay 1974; Jørgensen 1975; Øvstedal & Lewis Smith 2001).

Discussion. *Leptogium menziesii* is characterized by overlapping lobes, the base of the apothecia (Fig. 4D) and pycnidia being covered by hairs and the non-ornamented thallus and apothecia. When young, *Leptogium menziesii* has simple hairs that become beard-like with age. The apothecia have a prosoplectenchymatous hypothecium and paraplectenchymatous parahymenial tissue. The quick process used to dry the specimen probably modified the thallus colour as well as the medullary hyphae; thus, the absence of columnar hyphae in this specimen is questionable (Fig. 5A). *Leptogium menziesii* does not have any ornamentation, whereas all the new species described here are ornamented (Table 2).

Leptogium puberulum Hue

Lichenes. In *Deuxième Expédition Antarctique Française*: 14 (1915); type: South Shetland Islands, King George Island, Admiralty Bay, "sur les pierres des éboulis et récolté sur la terre recouvrant un nunatak à 300 mètres d'altitude, XXIII excursion", 25 December 1909, *Louis Gain*, 300 (PC—Hue's collection).

(Figs 5C & D, 6A)

Thallus up to 9 cm wide, 75–80 µm thick, usually brownish under fluorescent light, opaque, matt, whitish to beige or brown to blackish under stereomicroscope with white light; *maculae* not observed; *lobes* 2·0–5·5 mm wide, agglomerated, slightly attached, ascending; *apices of lobes* rounded, ascending, undulating, smooth to ornamented; *lateral margins of lobes* smooth, thickened, ascending, sinuous; *vegetative ornamentation* without constricted base, crenulate, verruciform or denticulate appendages, marginal, frequent to scarce; *isidia* and *lobules* absent; *thallus attached* by simple hairs, typically 4–6 cells long, spherical cells at the base (5–10 µm long) and cylindrical at the apex (10–15 µm long), cream to white; on the lower surface, frequent to abundant, the hairs agglutinated where the thallus is attached to the substratum; *upper cortex* smooth to slightly rugulose when viewed at ×20 magnification, 1–2 layers of isodiametric cells, 5·0 µm diam.; *lower cortex* tomentose, brown to whitish or yellowish, slightly rugulose when viewed at ×20 magnification, 1–3(–4) layers of isodiametric cells, 3·5–5·0 µm diam.; *medulla* with columnar hyphae 2·5 µm thick, erect to inclined, *c.* 70° from the cortex, 4–6 cells high, scattered between the medullary hyphae; *photobiont* cyanobacterium, green to blue, spherical cells 5·0 µm diam., frequent in medulla, number of cells per filament not determined; *gelatinous matrix* scarce, hyaline to brownish next to the cortices.

Apothecia 1·0–2·0(–5·5) mm diam., laminal, adnate to subpedicellate; *disc* concave to plane; *basal paraplectenchymatous tissue* absent; *thalline exciple* cortex brown when young to blackish yellow, base and mid-height smooth, glabrous or pubescent; *margin* usually smooth or with circular wrinkles similar to verruciform ornamentation in appearance, concolorous with the thallus, yellow to brown; composed of paraplectenchymatous tissue, 100 µm (6 cells) thick at the base, *c.* 40 µm (4–6 cells) thick at mid-height, 15–40 µm (3–6 cells) thick at the apex; *corona* interrupted, subtle, absent in young apothecia, generating verruciform ornamentation; *pedicel* originating from thallus, smooth or pubescent; *parahymenial tissue* continuous with the hypothecium, collo-plectenchymatous cells, 35–40 µm (6–8 cells) thick at the base, 35–40 µm (5–8 cells) thick at the apex, two thirds to the same height as the hymenium; *proper exciple* absent; *hypothecium* 35–40 µm thick, slightly yellow, composed of prosoplectenchymatous cells; *subhymenium* 10·0–12·5 µm thick, colourless; *hymenium* 100–125 µm thick. *Asci* not observed; *ascospores* fusiform, 25·0–30·0 × 7·5–12·5 µm, 4–5 × 2–3 cells, apices acute to obtuse, submuriform to muriform.

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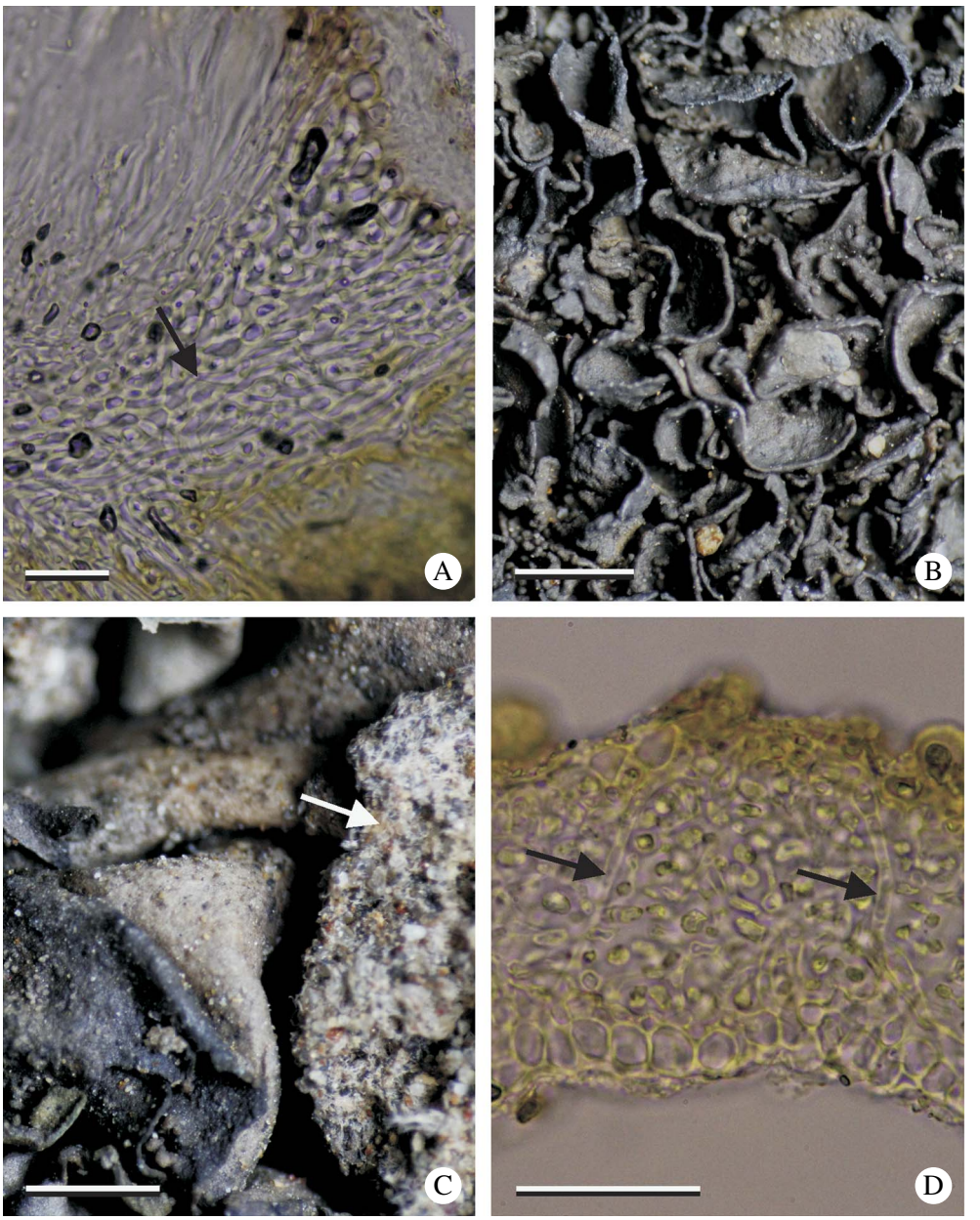

FIG. 6. A, *Leptogium puberulum*, TS apothecium, arrow = colloplectenchymatous cells. B–D, *Leptogium tectum*; B, upper surface; C, lower side showing absence of hairs, arrow = hyphae interwoven with sand grains; D, TS thallus with columnar hyphae (arrows). Scales: A = 20 µm; B & C = 3 mm; D = 50 µm. In colour online.

Pycnidia laminal to marginal, black; *conidia* bifusiform to bacilliform, 3·75 × 1·25 µm.

Distribution. Antarctic Peninsula, Alexander Islands, Cockburn Island, Bouvet Island, Melchior Islands, South Orkney

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Island, South Georgia, South Sandwich Island and South Shetland Islands (Hue 1915; Dodge 1965, 1968, 1973; Lewis Smith & Corner 1973; Lindsay 1974; Redon 1985; Jørgensen 1986; Lewis Smith 1993; Sancho *et al.* 1999; Øvstedal & Lewis Smith 2001).

Discussion. *Leptogium puberulum* is characterized by cortices composed of more than one layer of cells and hairs usually not agglutinated on the lower surface that are barely visible to the naked eye. The hairs are simple and short, usually 50 µm or 3–5 cells in length, giving the surface a velvety appearance. The hypothecium is prosoplectenchymatous and the parahymenial tissue colloplectenchymatous (Fig. 6A), differing from the apothecia of *L. menziesii* in which the hypothecium is prosoplectenchymatous and the parahymenial tissue is paraplectenchymatous. Jørgensen (1986) included *L. puberulum* in the *L. azureum* group (non-hairy group), based on a study of the species present on Bouvet Island. However, the same author considered *L. puberulum* as a hairy species and included it in the key to the section *Mallotium* (Jørgensen 1997). Dodge (1965) described the ascospores as ellipsoid, 30 × 15 µm, based on material from Deception Island (*Follmann* 13551D). This description is almost the same as that reported in the present study. However, Øvstedal & Lewis-Smith (2001) described the ascospores as broadly ovate with globose cells, measuring 17–20 × 14–16 µm; this material requires further examination.

Leptogium puberulum is similar to the non-ornamented species *L. menziesii* but differs by the presence of cortices with more than one layer of cells and the very different structure of the medulla (Table 2). Lobules may form on cracks of the thallus in *L. puberulum*, but in this case they probably are not a good taxonomic character because they are most likely simply adventitious.

Leptogium puberulum is locally abundant and widespread in the South Shetland Islands and James Ross Island (Øvstedal & Lewis Smith 2001). Specimens of *L. antarcticum* and *L. marcellii* were found together with *L. puberulum* but can be differentiated in the field by the presence of verruciform ornamentation and lobules, respectively.

Selected specimens examined. **Antarctica:** *South Shetland Islands*: King George Island, Hennequin Point, 2014, *C. Bernardo* [441, 486] *& S. C. Feuerstein* (CGMS); King George Island, Keller Peninsula, Point 35, 62°05'08·6"S, 058°23'56·6"W, saxicolous, 2016, *A. P. Lorenz-Lemke* [345, 348] & *H. C. Oliveira* (CGMS); King George Island, Vauréal Peak, Point 42, 62°10'59·6"S, 058° 17'23·6"W, on moss, 2016, *A. P. Lorenz-Lemke* [409] & *H. C. Oliveira* (CGMS). *James Ross Island*: Clearwater Plateau, 64°01'28·32"S, 057°41'51·09"W, 245 m, 2016, *M. J. Kitaura* [2802, 2804], *J. M. Lirio et al.*

Leptogium tectum Lorenz-Lemke, Kitaura & Scur sp. nov.

MycoBank No.: MB 822502

Similar to *Leptogium puberulum* but lobes agglomerated, ascending and usually with granular to cylindrical isidia that are usually on the margin; the thallus is attached to the substratum by interwoven hyphae.

Type: Antarctica, James Ross Island, Clearwater Mesa, near Soledad Lake, growing in a hollow caused by aeolic erosion in basaltic rock, saxicolous, 64°00'36·90"S, 57° 41'51·90"W, 213 m, 19 January 2016, *M. J. Kitaura* [2948], *S. Coria & J. M. Lirio* (CGMS—holotype).

(Fig. 6B–D)

Thallus c. 1·5(–2·0) cm wide, 75–150 µm thick, black under fluorescent light, opaque, usually matt, blackish grey to dark brown-grey when observed under stereomicroscope with white light; *maculae* absent; *lobes or squamules* 2–4 mm wide, agglomerated, ascending, slightly attached; *apices of lobes* rounded, ascending, smooth to irregularly ornamented; *lateral margins of lobes* smooth to irregularly ornamented, thickened, ascending, crispate; *vegetative ornamentation* verruciform, without constricted bases, usually marginal, apices rounded, simple; *isidia* granular to cylindrical, 0·10–0·15 × 0·075–0·1 mm, simple, erect, firm, concolorous with the thallus, laminal, grouped, rare to frequent, on the wider lobes and on both surfaces; *lobules* absent; *thallus attached* by interwoven hairs, originating at the base of lobes or squamules, hyphae mixed with sand grains or organic material, white to cream; *rhizines* and *hairs* not observed; *upper cortex*

smooth to slightly rugulose when viewed at ×20, composed of one layer of pseudoparaplectenchymatous cells, 2·5–5·0 µm diam.; *lower cortex* non-tomentose, light brown to yellowish, smooth to rugulose when viewed at ×20, 1–3 layers with irregular to paraplectenchymatous cells, 5·0–7·5 × 2·5–5·0 µm, *medulla* with erect to inclined columnar hyphae, 4(–6) cells long, scarce (Fig. 6D), between tightly packed cyanobacterial cells; *photobiont* cyanobacterium, green or yellow, frequent within the medulla, spherical cells, 5·0 µm diam., number of cells per filament not determined; *gelatinous matrix* scarce, hyaline.

Apothecia and *pycnidia* absent.

Etymology. The epithet is from the Latin '*tectus*, *-a*, *-um*', meaning 'covered, hidden'. Both specimens of *Leptogium tectum* were found in a hollow.

Distribution. Currently known only from James Ross Island, Antarctica.

Discussion. *Leptogium tectum* is characterized by agglomerated and ascending lobes or squamules, with verruciform ornamentation and isidia. The thallus is attached to the substratum by interwoven hyphae that originate in the centre of the thallus, covering the substratum (Fig. 6C).

The specimens of *Leptogium tectum* are small and were found inside a hollow created by aeolic erosion on basaltic rock. Neither specimen has hairs but both are characterized by interwoven hyphae growing between the sediments below the lower surface, attaching the thallus to the substratum and differ from those in *L. antarcticum* and *L. crispatellum* which are attached by hairs and short hapters, respectively. The interwoven hyphae are not agglutinated and differ from beard-like hairs and rhizines.

Isidia can be found on both upper and lower lobe surfaces, increasing the exposed surface to the environment. When young, they consist of bundles of a pigmented gelatinous matrix composed of some fungal hyphal cells, and a small number of cyanobacterial cells which originate in the upper layer of the medulla and were characterized as *saturninum*-type isidium described by Stone *et al.* (2016).

Material examined. **Antarctica:** *James Ross Island*: Clearwater Mesa, near Soledad Lagoon, growing in a hollow originating from aeolic erosion in basaltic rock, saxicolous, 64°00′36·90″S, 57°41′51·90″W, 213 m, 2016, *M. J. Kitaura* [2948 I], *S. Coria & J. M. Lirio*.

Key to *Leptogium* species in Antarctica

1 Thallus with hairs 2
Thallus without hairs . . . . . **L. crispatellum**

2(1) Lower cortex tomentose, composed of simple or beard-like hairs 3
Lower cortex non-tomentose but attached to the substratum by interwoven hyphae . . . . . **L. tectum**

3(2) Thallus with lobules or verruciform ornamentation 4
Thallus without lobules or verruciform ornamentation 5

4(3) Thallus with verruciform ornamentation on the lamina and margin; lobules absent; columnar hyphae present . . . . . **L. antarcticum**
Thallus without verruciform ornamentation; with lobules usually on the margin; columnar hyphae absent . . . . . **L. marcellii**

5(3) Cortices of the thallus with one layer of cells; hairs on the lower side, apothecia and pycnidia, with beard-like appearance . . . . . **L. menziesii**
Cortices of the thallus with more than one layer of cells; hairs usually on the lower cortex, with velvety appearance . . . . . **L. puberulum**

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