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An acid deficient population of *Lambiella psephota* from Antarctica and a new combination in the genus from Campbell Island with a world-wide key to the genus

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Abstract

Only two species of the lichen genus *Lambiella* are known from Antarctica: *L. impavida* and *L. psephota*. Here we report a new chemotype of *L. psephota* collected from James Ross Island in the North-East Antarctic Peninsula region. nrITS, mtSSU and RPB1 gene regions of the norstictic acid deficient *L. psephota* were obtained, which showed the new chemotype phylogenetically belongs to *L. psephota.* We also transfer *Rimularia maculata* to *Lambiella*, and provide an identification key of the 17 known species of *Lambiella* world-wide.

Key words: Antarctica, biodiversity, lichenized fungi, *Xylographaceae.*

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Antarktika'dan *Lambiella psehota*'nın asit yoksunu popülasyonu, cinste Campbell Adası'ndan yeni bir kombinasyon ve tür tayin anahtarı

Özet

Antarktika'da likenleşmiş mantar cinslerinden biri olan *Lambiella*'nın *L. impavida* ve L. *psephota* olmak üzere iki adet türü bilinmektedir. Burada, Kuzey-Doğu Antarktika Yarımadası bölgesindeki James Ross Adası'ndan toplanan yeni bir *L. psephota* kemotipini rapor ediyoruz. Yeni kemotip filogenetik olarak *L. psephota*'ya aittir. Norstitik asit içermeyen *L. psephota*'nın nrITS, mtSSU ve RPB1 gen bölgeleri çalışılmıştır. Ayrıca bu yayınla birlikte *Rimularia maculata*'yı *Lambiella* cinsine aktarıyoruz ve dünya çapında bilinen 17 *Lambiella* türü için bir tayin anahtarı veriyoruz.

Anahtar kelimeler: Antarktika, biyoçeşitlilik, likenleşmiş mantarlar, *Xylographaceae.*

1. Introduction

The genus *Lambiella* was described by Hertel (1984) for the single species *L. psephota* (Tuck.) Hertel. He compared it with the genus *Rimularia*, from which it differed in having an amyloid (I+ violet) medulla, non gyrose apothecial disks lacking an umbo, and a lower hymenium with less reticulate paraphyses. Hertel & Rambold [1] decided that these differences were insufficient to justify a distinct genus and combined the species into *Rimularia* [1]. Several other similar species have subsequently either been transferred to, or described in that genus. However, molecular studies [2, 3] confirmed Hertel's original opinion that *L. psephota* did not belong in *Rimularia* and further showed that *Lambiella* belongs in *Xylographaceae* whereas *Rimularia* belongs in *Trapeliaceae*. Resl et al. [2] identified other phenotypic differences between the two genera (viz, the development of depsidones in the thallus of *Lambiella* [2]. and differences in the ascus apical apparatus – with all species of *Rimularia* developing a thin, vertical, nonamyloid tube that is absent in *Lambiella* species) and transferred 10 species of *Rimularia* to the genus *Lambiella* [2]. To date, 17 species are included in the genus *Lambiella* and others, currently still in *Rimularia*, almost certainly belong there. The species of *Lambiella*

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are generally reported from extreme latitudes or high altitudes. Although most 11 species of the genus are saxicolous, three species are muscicolous and three species are epiphytic on tree bark.

In the project aiming to determine the lichen biodiversity of James Ross Island (North-East Antarctic Peninsula region), which has a special location in the transition zone between maritime and continental climate types [4], we collected a *Lambiella* specimen that differed from the two species of the genus previously reported from Antarctica; *L. psephota* (Tuck.) Hertel and *L. impavida* (Th. Fr.) M. Westb. & Resl, in lacking norstictic acid. However, molecular data revealed that, phylogenetically, it belonged to *L. psephota* and so it is here reported as an acid-deficient chemotype of that species. We also take this opportunity to transfer an additional species of *Rimularia* described from Campbell Island, New Zealand to *Lambiella* and also provide an artificial identification key to the genus worldwide.

2. Materials and methods

2.1. Morphology and Thin-layer Chromatography

The lichen sample was collected from James Ross Island in Antarctica and is deposited in Erciyes University Herbarium Kayseri, Turkey (ERCH). The specimen was examined by standard microscopic techniques. Hand-cut sections were studied in water, potassium hydroxide (KOH) and Lugol's solution (I). Measurements were made in water. Ascospores were measured from five different ascomata for each taxon. The measurements are given as minimum–maximum, from n = 30 measurements. Thin-layer chromotography (TLC) was carried out to determine compounds, using solvent system C [5, 6].

2.2. Molecular Methods

2.2.1. DNA Isolation, PCR and Sequencing

Molecular works were conducted in the Molecular Biology Lab, University of Erciyes. Total DNA was extracted using the Dneasy Plant Mini Kit (Qiagen) following the manufacturer's protocol. DNA extractions were used as template in the PCR. The complete nrITS fragment (nrITS1-5.8S-nrITS2, ca. 500 bp) of the nuclear ribosomal DNA repeat was amplified by PCR using primers ITS1F and ITS4 [7, 8]. PCR was carried out in 50 µL reaction volumes using 4 µl of 10 x reaction buffer, 4 µl $MgCl_2$ (50 mM), 0.5 µl each primer, 2 µl dNTP (10 mM), 0.2 µl Taq DNA polymerase, 1 µl of genomic DNA and 37.8 µl dH_2O on a thermal cycler equipped with a heated lid. Primers used for PCR amplification of ITS regions were the fungi-specific primer ITS1-F (5′-CTTGGTCATTTAGAGGAAGTAA-3′) [8] and the universal primer ITS4 (5'- TCCTCCGCTTATTGATATGC-3') [6]. Primers used for PCR amplification of mtSSU and RPB1 regions were mrSSU1:F AGCAGTGAGGAATATTGGTC; mrSSU3R ATGTGGCACGTCTATAGCCC, [9] and RPB1-aFasc ADTGYCCYGGYCATTTYGGT [10] and RPB1-cR (CCCGCATNTCRTTRTCCATRTA [11]. The PCR was performed under the following conditions: An initial denaturation 4 min at 95 °C; 10 cycles with 1 min at 95 °C, 1.30 min at 56 °C, and 1 min at 72 °C; and 15 cycles with 1 min at 95 °C, 1.30 min at 51 °C, and 1 min at 72 °C; a final extension step of 8 min at 72 °C was added, after which the samples were kept at 4 °C. PCR products run on agarose gel and sequence analysis of nine lichen samples from which DNA bands were obtained. All amplified products were electrophoresed on a 1.6 % agarose gel and compared with a 1 Kb Plus DNA Ladder for size estimation.

2.2.2. Additional Sequences

20 ITS, 23 mtSSU and 12 RPB1 DNA sequences were downloaded from GenBank, representing a selection of 15 taxa in the genus *Lambiella* and the outgroup species *Xylographa trunciseda* (Table 1).

Table 1. mtSSU, ITS and RPB1 Sequences used in the analyses

Species	**mtSSU**	**Country**	**Year**	**ITS**	**Country**	**Year**	**RPB1**	**Country**	**Year**
Lambiella aliphatica	-			MN483114	USA	2020			
Lambiella arenosa	MF464550	USA	2017	MF464549	USA	2017			
Lambiella arenosa	MF464551	USA	2017	MF464548	USA	2017			
Lambiella caeca	KR017379	USA	2015	KR017065	USA	2015	KR017444	USA	2015
Lambiella caeca	KR017360	USA	2015	KR017131	USA	2015	KR017445	USA	2015
Lambiella caeca	KR017357	USA	2015	KR017090	USA	2015	KR017475	Canada	2015
Lambiella caeca	KR017391	Canada	2015						
Lambiella caeca	KR017329	USA	2015						
Lambiella caeca	KR017338	USA	2015						

Table 1. Continues

Lambiella furvella	KR017366	Sweden	2015	KR017118	Sweden	2015			
Lambiella fuscosora	KR017376	Russia	2015	KR017130	Russia	2015			
Lambiella globulosa	KR017369	Sweden	2015	KR017132	Sweden	2015	KR017442	Sweden	2015
Lambiella globulosa	KR017365	Sweden	2015	KR017105	Sweden	2015			
Lambiella gyrizans	KR017377	Sweden	2015	MH636003	USA	2018	KR017443	Sweden	2015
Lambiella hepaticola	-			MN483115	Chile	2020			
Lambiella impavida	KR017372	Sweden	2015	KR017115	Sweden	2015	KR017439	Sweden	2015
Lambiella impavida	KR017371	Sweden	2015	KR017114	Sweden	2015	KR017440	Sweden	2015
Lambiella impavida	KR017370	Sweden	2015	KR017127	Sweden	2015	KR017441	Sweden	2015
Lambiella impavida	KR017364	Sweden	2015	KR017104	Sweden	2015			
Lambiella insularis	KR017374	Sweden	2015	KR017100	Sweden	2015	KC222188	USA	2012
Lambiella insularis	KC222182	USA	2015				KR017477	USA	2015
Lambiella insularis	KR017375	Sweden	2015	KJ462268	USA	2014			
Lambiella psephota	DQ871019	Australia	2007				DQ870992	Australia	2006
Lambiella psephota	OK271049	Antarctica	2021	OK272466	Antarctica	2021	OK272501	Antarctica	2021
Lambiella sphacelata	KR017378	Sweden	2015	KR017113	Sweden	2015			
Xylographa trunciseda	KF360417	Norway	2013	KJ462454	Norway	2014	KR017430	Norway	2015

2.2.3. Sequence Alignment and Phylogenetic Analysis

Sequence editing was performed with the software Bioedit [12]. Each alignment was analysed by MEGA 7 software with using Tamura 3 parameter [13] and to infer a maximum likelihood tree with 1000 standard non-parametric bootstrap repetitions.

3. Results

3.1. Sequence Alignment and Phylogenetic Analysis

The mtSSU alignment comprised 21 accessions with a length of 816 bp. The ITS alignment consisted of 23 accessions with a length of 520 bp. The RPB1 alignment consisted of 12 accessions with a length of 668 bp (Table 1). *Xylographa trunciseda* was used for rooting all gene trees. In ITS (Figure 1), mtSSU (Figure 2) and RPB1 (Figure 3) trees the acid deficient *L. psephota* was resolved by forming a clade with *L. psephota*.

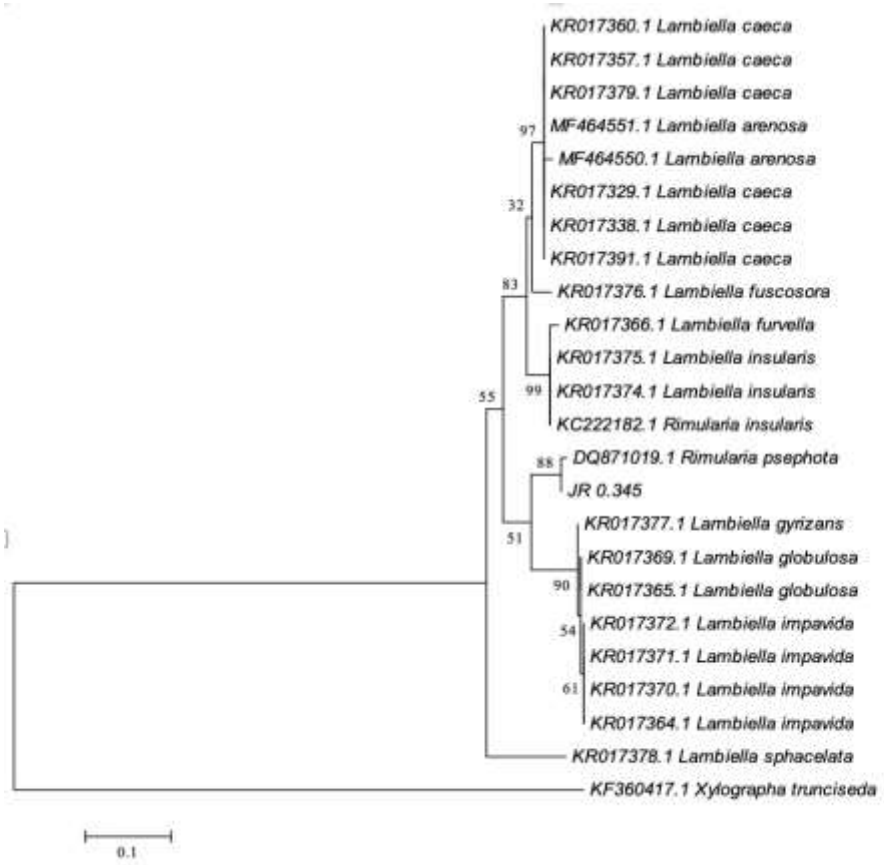

Figure 1. Maximum Likelihood (ML) analysis inferred from nrITS region sequences of the genus *Lambiella*.

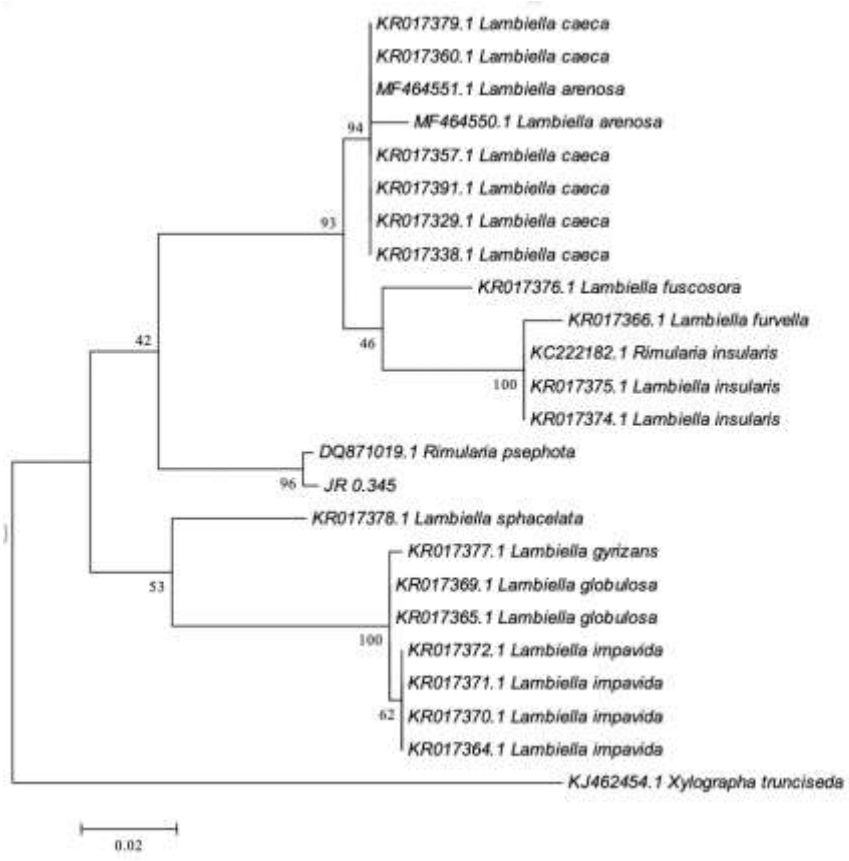

Figure 2. Maximum Likelihood (ML) analysis inferred from mtSSU region sequences of the genus *Lambiella*.

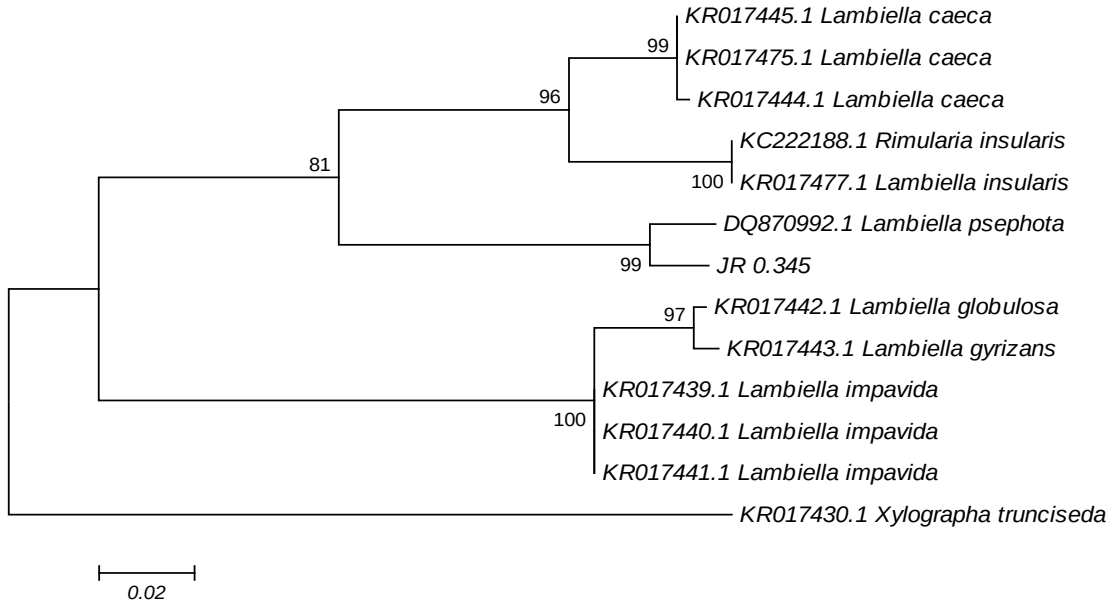

Figure 3. Maximum Likelihood (ML) analysis inferred from RPB1 region sequences of the genus *Lambiella*.

Acid deficient *Lambiella psephota*

Antarctic Peninsula, James Ross Island: Sharp Valley, 63°52'S, 58°4'W, alt. 200 m, 11 January 2017, *ERCH JR 0.345* (ERCH).

Description: Thallus violetish dark grey, composed of thick and convex more or less angular areoles (0.1–0.4 mm), in small patches to 1 cm diam. Cortex with a thin brownish layer above the photobiont layer. Medulla with numerous fine granules (not dissolving in K or N), I+ violet. Photobiont chlorococcoid, cells 12–15 × 10–15 µm. Apothecia black, lecideine, sessile, mostly in the margins of the areoles, rounded to angular, occasionally lirellate with slit-like disc, 0.1–0.3 mm diam., proper exciple thick, raised and persistent. Hymenium hyaline in most parts but violet or greenish tinges are present, I+ blue, 50–65 µm tall; epihymenium 10–25 µm thick, brownish grey (K+ purple; Sedifolia-grey). Hypothecium pale brown, 20–30 µm thick. Excipulum dark brown to almost black. Paraphysoids 1.5–2 µm thick, septate, branched and anastomosing, apices swollen to 4 µm. Asci 8-spored, broadly cylindrical to subclavate, *Lambiella*-type; 25–50 × 15–30 µm. Ascospores hyaline, simple, elliptic or subglobose, occasionally almost globose, (8–)9,5–12–14,5(–19) × (6–)7–8,5–10(–11) µm; length/width (1–)1,11–1,39–1,68(–2) µm (n=20) µm, perispore present, 0.5-1 µm thick. Conidiomata not observed (Figure 4,5).

Chemistry: K–, C–, KC–, Pd–. No lichen substances were detected by TLC.

Ecology: The specimen grows on basaltic non-maritime rocks at 200 m altitude without sea spray in James Ross Island (Antarctic Peninsula) with macrolichens such as *Umbilicaria decussata* and *Usnea antarctica*. In the valley where this specimen was collected, there are a lot of bird nests and nitrophilic lichens such as *Calogaya saxicola* and *Rusavskia elegans* are very common.

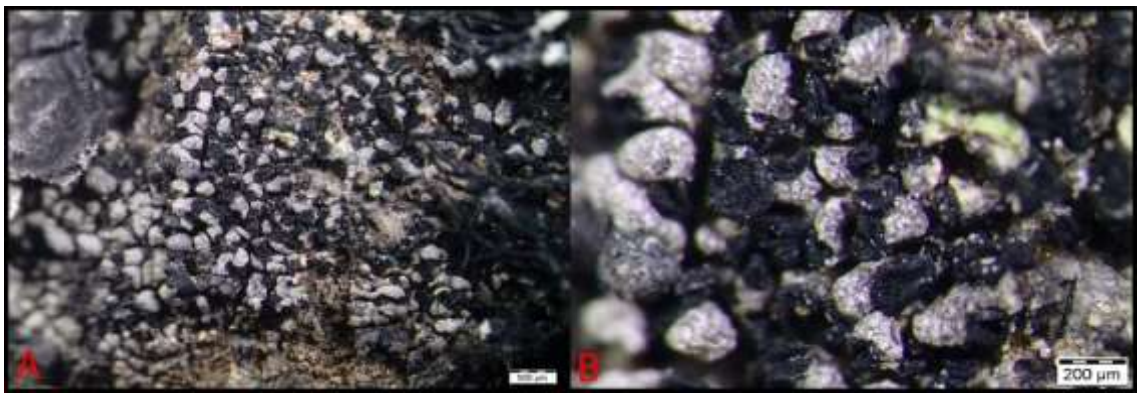

Figure 4. Acid deficient *Lambiella psephota*. **A.** Habitus. **B.** Apothecia and areoles.

Figure 5. Acid deficient *Lambiella psephota* **A.** Apothecial section. **B.** Ascus and ascospores. **C.** Medulla in K **D.** Epihymenium in K.

4. Conclusions and discussion

Lambiella subpsephota Fryday, described from the Falkland Islands (Islas Malvinas) [7], is similar to *L. psephota* in having a thallus with an amyloid medulla and an epihymenium containing sedifolia-grey (K+ purple). It resembles the acid deficient *L. psephota* in having a thallus lacking norstictic acid but differs in having a white to pale grey thallus, longer and larger ascospores ((8–)9,5–12–14,5(–19) × (6–)7–8,5–10(–11) µm vs. 13–15 × 9–10 µm, larger apothecia (0.4–0.6 mm vs 0.1–0.3 mm), a taller hymenia (80–100 µm vs 50–65 µm). It is also known only from maritime rocks, or at least rocks close to the sea [8].

Previously, only two species of *Lambiella*: *L. impavida* and *L. psephota* were reported from Antarctica [14]. In the field, *L. impavida* is easily distinguished from *L. psephota* by its dark brownish to blackish thallus [14] and microscopically by the K– epihymenium (K+ purple in *L. psephota*).

New Combination in *Lambiella*

The following new combination is required for *Rimularia maculata* Fryday, which was described from Campbell Island and is otherwise known only from the Auckland Islands [15]. It is probable that several other species of *Rimularia* should also be transferred to *Lambiella*, but as we have not seen material of them we refrain from doing so at this time.

Lambiella maculata (Fryday) Fryday *nov. comb.*

Mycobank: MB 842521

Rimularia maculata Fryday, Biblioth. Lichenol. **88**: 142 (2004). Type: New Zealand, Campbell Island, cliffs and shingle feldmarkat summit of Mt. Fizeau, 505 m., 10 Jam 1970, H.A. Imshaug (46761) & R.C. Harris (MSC—holotype, CHR, HO, M—isotypes).

An identification key to the species of Lambiella

1. On mosses or on bark.....**2**

1. Saxicolous..... **7**

2. On mosses.....**3**

2. On bark.....**5**

3. Thallus whitish, with lichen substances **4**

3. Thallus consisting of dispersed, brown bullate areoles, without lichen substances. Ascospores (7–)9–11.5(–13) × (4–)4.5–5(–6) µm. Falkland Islands, Tierra del Fuego, South America [15]…….....…***L. andreaeicola*** (Fryday) Fryday.

4. Thallus K+ faint yellowish, porphyrilic acid. Apothecia in clustures. Ascospores (7–)9–11.5(–13) × (4–)4.5–5(–6)µm. Tasmania, Australia (Coppins & Kantvilas, 2001), Campbell Island, New Zealand [16] Tierra del Fuego, Chile [17]…….....…….***L. hepaticicola*** (Kantvilas & Coppins) Resl & T. Sbrib

4. Thallus K+ red, norstictic acid. On boulders and rock outcrops in open arctic-alpine habitats. Scotland and North America [12] .....***L. sphacelata*** (Th. Fr.) M. Westb. & Resl

5. Non sorediate.....**6**

5. Sorediate, esorediate parts usually endosubstratal. Thallus with norstictic acid. Apothecia usually present, black. Ascospores (9.5–)11–16(–20) × (5–)7–11 µm. Scandinavia, Iceland, Scotland, Altai Mountains, Russia [18].....***L. fuscosora*** (Muhr & Tønsberg) M. Westb. & Resl

6. Thallus endosubstratal or forming a whitish stain. Ascospores on average over 11 µm long. Pacific Northwest US [19]…….....……..***L. arenosa*** McCune & Lumbsch

6. Thallus more conspicuous. Ascospores on average 8–11 µm long. Boreal and Great Lakes Regions of NE North America [20], SE Alaska [2] …….....… ***L. caeca*** (J. Lowe) Resl & T. Sprib.

7. Thallus with vegetative propagules **8**

7. Without vegetative propagules.....**9**

8. Thallus dull brownish grey, consisting of cylindrical to flattened or branched isidia. Apothecia usually in dense groups. Ascospores globose, 6–8 µm. Venezuela [21] …….....…… ***L. isidiata*** Aptroot

8. Thallus dark olive brown to nearly blackish, with minute granular isidia, usually sterile, C+ red, gyrophoric acid. Apothecia rarely seen. Ascospores 12–20 × 6.5–10 µm. Often lichenicolous on a wide range of crustose lichens on siliceous rocks. Europe, North America [22, 23]…….....……. ***L. furvella*** (Nyl. ex Mudd) M. Westb. & Resl

9. Not lichenicolous.....**10**

9. Lichenicolous on *Lecanora rupicola* group. Thallus brownish to olivaceous. Apothecia black, rounded to angular, between the areoles. Ascospores 8–14 × 4.5–7 µm. Europe, Asia, North America, suboceanic, almost cosmopolitian [22, 24] ……..….....…… ***L. insularis*** (Nyl.) T. Sprib.

10. Hymenium I+ blue; aliphatic acids absent **11**

10. Hymenium I+ wine red. Aliphatic acids by tlc. Thallus bicoloured, towards the margin dark grey, in the centre light grey, K–, C–. Apothecia rounded to angular, black, single or in groups of 2-3, with a distinct umbo. Ascospores (6.5–)8–9(–12) × (4.5–)5.5–6.5(–7) µm. Alaska, USA [17]. ..... ***L. aliphatica*** T. Sprib. & Resl

11. Thallus without norstictic acid or stictic acid.....**12**

11. Thallus with norstictic acid or stictic acid.....**14**

12. Thallus brown with numerous black carbonaceous ridges. Known only from the New Zealand subantarctic islands (Campbell Island and the Auckland Islands) ……..… ***L. maculata*** (Fryday) Fryday

12. Thallus grey, carbonaceous ridges absent…….....……..**13**

13. Thallus white to pale grey. Apothecia rounded to angular, occasionally lirellate, 0.4–0.6 mm diam. Hymenium 80–100 µm tall. Ascospores 13–15 × 9–10 µm. On maritime rocks. Falkland Islands, Tierra Del Fuego, South America [16] …….. ***L. subpsephota*** (Fryday) Fryday

13. Thallus violet grey. Apothecia rounded to angular, occasionally lirellate, 0.1–0.3 mm diam. Hymenium 50-60 µm tall. Ascospores 9–14 × 7–10 µm. On non-maritime rocks. Antarctic Peninsula Acid deficient ***L. psephota***

14. With norstictic acid.....**15**

14. With stictic acid.....**18**

15. Thallus pale to dark grey, sometimes brownish grey. Apothecia rounded to lirellate….....**16**

15. Thallus dark brownish to blackish. Ascospores 5.5–10 × 6–8 µm. North America, Scandinavia, Antarctic Peninsula [14]…….....…..…… ***L. impavida*** (Th. Fr.) M. Westb. & Resl

16. Epihymenium K+ purple (sedifolia-grey) ….....***L. psephota*** (Tuck.) Hertel subsp. **psephota**

16. Epihymenium K– …….....……..**17**

17. Thallus dark grey, formed of more convex areoles; apothecia slit-like. Known only from Scotland…….....…… ***L. mullensis*** (Stirt.) Fyday & Coppins

17. Thallus pale grey of flat areoles; apothecia angular but not slit-like. Northern Europe and North America …….....…… ***L. gyrizans*** (Nyl.) M. Westb. & Resl

18. Thallus dark grey. Ascospores (10–)12.5–14.5(–16) × (6–)7-8.5(–9.5) µm. Scotland [25].....***L. globulosa*** (Coppins) M. Westb. & Resl.

18. Thallus pale grey. Ascospores 7–11 × 4.5–7 µm. North America and North Europe [25].....***L. gyrizans*** (Nyl.) M. Westb. & Resl

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