The Lichenologist **47**(6): 369–378 (2015) doi:10.1017/S0024282915000353

Epiphloea belongs to *Collemataceae* (Lecanoromycetes, lichenized Ascomycota)

Matthias SCHULTZ, Mats WEDIN, Henrike DIEL and Maria PRIETO

Abstract: The cyanolichen genus *Epiphloea* is currently included within the *Heppiaceae* (Lichinomycetes) based on ascus characteristics. The presumed presence of a prototunicate ascus has been used as support for this classification, despite the incongruence with other characters (e.g. spores). Here, we use a molecular phylogeny of the two markers mtSSU rDNA and *Mcm7* to investigate the position of *Epiphloea*. In addition, we have re-investigated the ascus characteristics. Our results place the two species, *Epiphloea byssina* and *E. terrena*, within the *Collemataceae*, nested in *Leptogium* s. str. The ascus type in both species is shown to be Lecanoralean and similar to the ascus in other *Collemataceae*, with a strongly amyloid tube-like structure. This observation supports the placement within Lecanoromycetes and refutes the earlier suggested affinities with *Heppiaceae* and Lichinomycetes. The correct names for these species are *Leptogium byssinum* and *Leptogium terrenum*.

Key words: cyanolichens, *Leptogium*, lichens, Lichinomycetes, phylogeny, systematics, taxonomy

Accepted for publication 13 *August* 2015

Introduction

Current progress in our understanding of the relationships among Ascomycota is substantial, and the recent advances in the phylogeny and evolution of lichen-forming Ascomycota associating with cyanobacteria is no exception (Schoch *et al*. 2009; Wedin *et al.* 2009, 2011; Otálora & Wedin 2013; Otálora *et al.* 2013, 2014; Spribille & Muggia 2013; Ekman *et al.* 2014; Magain & Sérusiaux 2014; Miądlikowska *et al.* 2014). Identifying and interpreting characters relevant for the classification of higher taxonomic ranks is a fundamental question in the phylogenetic-systematic study of the Ascomycota. Fungal classification is full of examples of groups where the current taxonomy is based on erroneous or dubious observations or interpretations of morphological structures. This is a problem which is particularly relevant for small and inconspicuous groups of cyanobacterial lichens.

During our ongoing studies of the phylogeny and character evolution of the Lichinomycetes, one of the largest remaining poorly understood groups of cyanobacterial lichens, a highly deviant genus was found to be *Epiphloea*, where the sequences of all molecular markers obtained were very different from other Lichinomycetes. Preliminary BLAST searches showed similarities with the *Collemataceae*, another group of cyanobacterial lichens. *Epiphloea* has for a long time been considered closely related to the Lichinomycete genus *Heppia* in *Heppiaceae* (Zahlbruckner 1924–1925; Eriksson 1999; Lumbsch & Huhndorf 2007), although they differ in spore characteristics and photobiont (i.e. *Nostoc* and muriform spores in *Epiphloea*, *Scytonema* and simple spores in *Heppia*). In the latest major treatment of *Epiphloea*, Jørgensen (2007) reported the asci to be prototunicate (thin-walled throughout and opening by apical rupturing), a common trait in Lichinomycetes. Jørgensen's statement, however, differs from earlier observations by Zahlbruckner (1919), who

M. Schultz: Herbarium Hamburgense, Biocenter Klein Flottbek, University of Hamburg, Ohnhorststraße 18, D-22609 Hamburg, Germany. Email: matthias.schultz@uni-hamburg.de
M. Wedin and M. Prieto: Swedish Museum of Natural History, Department of Botany, P.O. Box 50007, SE-10405 Stockholm, Sweden.
H. Diel: University Medical Center Hamburg-Eppendorf, Campus Forschung (N27), Martinistraße 52, D-20246 Hamburg, Germany.

described the asci as having rounded and, at first, thickened tips that suggests a non-prototunicate ascus type.

The aim of this study is to clarify the phylogenetic placement of *Epiphloea*. To achieve this, we performed a phylogenetic analysis which included putative relatives based on DNA sequences of the mitochondrial SSU rDNA and the nuclear protein coding gene *Mcm7*. In order to re-evaluate and assess relevant morphological attributes, we studied the thallus and ascoma characteristics of both species in the genus, including type material, with particular focus on the ascus apex characteristics.

Material and Methods

Molecular study

For the phylogenetic study, we included three samples of *Epiphloea* and a wide selection of *Collemataceae* members (Table 1), representing all groups identified by Otálora *et al.* (2013, 2014). As in these previous studies, we used two members of *Pannariaceae*, *Pannaria rubiginosa* (Thunb. ex Ach.) Delise and *Staurolemma omphalarioides* (Anzi) P. M. Jørg. & Henssen, as outgroups rooting the tree with the latter. DNA was extracted using DNeasy Plant Mini Kit (Qiagen) and innuPREP Plant DNA Kit (Analytik Jena), according to the manufacturers' instructions. Amplifications were performed using 1:10 diluted DNA and with mtSSU1 and mtSSU3R (Zoller *et al.* 1999) primers for the mtSSU region. For amplification of the *Mcm7* region, we used the primers *Mcm7*-709for and *Mcm7*-1348rev (Schmitt *et al.* 2009). When no bands were obtained in the PCR, we carried out a nested PCR using 1 µl of the PCR product and the internal primers *Mcm7*-CalicF and *Mcm7*-CalicR (Prieto *et al.* 2013).

PCR amplifications were performed using Illustra™ Hot Start Mix RTG PCR beads (GE Healthcare, UK) in a 25 µl volume, containing 3 µl of diluted genomic DNA, 10 µM of each primer and distilled water. Amplifications were performed using the following procedure: initial denaturation at 95 °C for 15 min, followed by 35 cycles of 95 °C for 45 s, 56 °C for 50 s, 72 °C for 1 min, followed by a final extension at 72 °C for 5 min. PCR products were subsequently purified using the enzymatic method Exo-sap-IT (USB Corporation, Santa Clara, California, USA) or spin columns (Geneaid Gel/PCR DNAFragments Extraction Kit). The purified PCR products were sequenced using the same amplification primers.

Sequences were assembled and edited using Sequencher v. 4.10.1. (Genes Codes Corporation, Ann Arbor) and deposited in GenBank (Table 1). These sequences were added to the *Collemataceae* alignment provided by Otálora *et al.* (2013), using MacClade 4.01 (Maddison & Maddison 2001) and adjusted and reduced manually. Ambiguous regions (*sensu* Lutzoni *et al.* 2000) and introns were delimited manually and excluded from the phylogenetic analyses.

Independent analyses were carried out in both data sets (i.e. mtSSU and *Mcm7* alignments) using maximum likelihood-based inference (ML) in RAxML ver. 8.1.11 (Stamatakis 2014) and a GTRGAMMA model for tree inference. Bootstrapping was performed with a GTRCAT model and 1000 replicates. In order to check incongruence between the two analyses, we compared ML-BS individual gene trees, considering a conflict when a supported clade (bootstrap support >70%) for one marker was contradicted with significant support by another. Because no supported nodes were in conflict, the data were combined into a single data matrix. The combined maximum likelihood (ML) analyses were run with three distinct partitions (mtSSU, 1st and 2nd codon position for *Mcm7* and the 3rd codon position for *Mcm7*), using the same settings as in the individual analysis.

We selected the best-fit models of nucleotide substitutions based on the Akaike Information Criterion (AIC) using jModeltest 0.1.1 (Posada 2008). The GTR model (Rodríguez *et al.* 1990) was selected for the three partitions, with a gamma distributed rate variation across sites with four categories and an estimated proportion of invariable sites. All parameters were unlinked, with rates allowed to vary across partitions under a flat Dirichlet prior.

The Bayesian inference was performed using MrBayes 3.2.3 (Ronquist *et al.* 2012). Two runs of 10 million generations, starting from an initial random tree and employing four simultaneous chains, were executed. A tree was saved every 100th generation. To ensure that stationarity and convergence were reached, to verify if mixing was appropriate, and choose a suitable burn-in, we plotted the log-likelihood values against the time generation with Tracer v.1.5.0 (Rambaut & Drummond 2007). A burn-in sample of 25 000 trees was discarded for each run. The remaining 150 000 trees (pooled from both independent runs) were used to estimate branch lengths and posterior probabilities (PPs). The analyses were run on the CIPRES Science Gateway v. 3.3 (Miller *et al.* 2010).

Selected specimens examined. Epiphloea byssina (Hoffm.) Henssen & P. M. Jørg. **Germany:** *Baden-Württemberg*: Heidelberg, *W. v. Zwackh-Holzhausen* (Lich. exs. 174) (UPS 111320 (L-62566)—neotype!; M-0154536, M-0154537—isoneotypes!). *Bavaria*: ad terram nudam prope Eichstadt [Eichstätt] in Bavaria, *F. Arnold* (Körber, Lich. Sel. Germ. 60; as *Collema cheileum* var. *byssinum*) (B).—**Poland:** *Wojew. Dolnośląskie*: Silesia, Hirschberg, auf Mauern, *J. v. Flotow* (Deutsch. Lich. 143A; as *Collema cheileum* var. *byssaceum*) (HBG).—**Sweden:** *Uppland*: Bondkyrko par., Norby, auf lehmigen Äckern, 7 vii 1920, *G. Du Rietz* (as f. *obscurius* Du Rietz) (B).—**Norway:** *Oppland*: Vågå municip., Nordherad, E of Svarthåmårbekken, by the parking lot just S of the road Fv454, on soil, 61·8674°N, 8·9886°E, 680 m, 30 vi 2013, *M. Westberg* (S F264803).—**Russia:** *Sverdlovsk*: Distr. Pervouralsk, Sloboda village, limestone outcrops on Chusovaya River, on soil, 2002, *A. Paukov* AGP20020804-02 (UFU) (dupl. hb. Schultz).

TABLE 1. *GenBank accession numbers for specimens used in this study. New sequences are indicated by accession numbers in bold.*

Species	Voucher specimen	GenBank Acc. number	
		mtSSU	*Mcm7*
Blennothallia crispa (Huds.) Otálora *et al.* 1		JX992918	JX992976
B. crispa (Huds.) Otálora *et al.* 2		JX992919	JX992977
B. crispa (Huds.) Otálora *et al.* 3		JX992920	JX992978
Collema furfuraceum (Arnold) Du Rietz		EU982567	JX992982
C. leptaleum Tuck. 1		JX992928	JX992986
C. leptaleum Tuck. 2		JX992929	JX992987
C. nigrescens (Huds.) DC.		EU982563	JX992989
C. rugosum Kremp.		JX992936	JX992995
C. subconveniens Nyl. 1		JX992937	JX992996
C. subconveniens Nyl. 2		JX992938	JX992997
Enchylium bachmanianum (Fink) Otálora *et al.*		JX992914	JX992974
E. polycarpon (Hoffm.) Otálora *et al.* 1		JX992935	JX992994
E. polycarpon (Hoffm.) Otálora *et al.* 2		JX992935	JX992994
E. tenax (Sw.) Gray		EU982556	JX992998
Lathagrium auriforme (With.) Otálora *et al.*		EU982568	-
L. cristatum (L.) Otálora *et al.*		DQ917409	JX992979
L. fuscovirens (With.) Otálora *et al.*		JX992923	JX992983
L. undulatum (Flot.) Otálora *et al.*		EU982554	JX993000
Leptogium azureum (Sw.) Mont.		JX992939	JX993002
L. biloculare F. Wilson		JX992942	JX993004
L. brebissonii Mont.		EU982583	JX993005
L. britannicum P. M. Jørg. & P. James		JX992944	JX993037
L. byssinum (Hoffm.) Zwackh ex Nyl.	Norway, *Westberg* (S F264803)	**KT240180**	**KT240183**
L. corticola (Taylor) Tuck.		EU982585	JX993008
L. crispatellum Nyl.		JX992945	JX993009
L. cyanescens (Rabenh.) Körb.		EU982561	JX993010
L. dactylinum Tuck.		JX992946	JX993011
L. denticulatum Nyl. 1		JX992947	JX993012
L. denticulatum Nyl. 2		JX992948	JX993013
L. digitatum (A. Massal.) Zahlbr.		JX992950	JX993016
L. furfuraceum (Harm.) Sierk		EU982553	JX993017
L. isidiosellum (Riddle) Sierk		JX992953	JX993018
L. juressianum Tav.		JX992954	JX993019
L. laceroides B. de Lesd.		JX992955	JX993020
L. malmei P. M. Jørg.		JX992956	JX993023
L. marginellum (Sw.) Gray		JX992957	JX993024
L. papillosum (B. de Lesd.) C. W. Dodge		JX992961	JX993027
L. phyllocarpum (Pers.) Mont. 1		EU982589	JX993028
L. phyllocarpum (Pers.) Mont. 2		EU982588	JX993029
L. pseudofurfuraceum P. M. Jørg. & A. K. Wallace		EU982562	JX993031
L. resupinans Nyl.		JX992963	JX993033
L. reticulatum Mont.		JX992964	JX993038
L. rivulare (Ach.) Mont. 1		JX992965	-
L. rivulare (Ach.) Mont. 2		JX992966	-
L. saturninum (Dicks.) Nyl. 1		EU982569	JX993034
L. saturninum (Dicks.) Nyl. 2		AY340499	JX993035
L. sessile Vain.		JX992967	JX993007
L. terrenum Nyl. 1	Portugal, *van den Boom* 41781 (hb. van den Boom)	**KT240181**	**KT240184**
L. terrenum Nyl. 2	Portugal, Amieiro, *Marques* (PO)	**KT240182**	**KT240185**
L. velutinum P.M. Jørg.		JX992972	JX993041
Pannaria rubiginosa (Thunb.) Delise		AY360513	JX993042
Paracollema italicum (B. de Lesd.) Otálora *et al.* 1		JX992925	JX992984

https://doi.org/10.1017/S0024282915000353 Published online by Cambridge University Press

TABLE 1. *Continued*

Species	Voucher specimen	GenBank Acc. number mtSSU	*Mcm7*
P. italicum (B. de Lesd.) Otálora *et al.* 2		JX992926	JX992985
Pseudoleptogium diffractum (Körb.) Müll. Arg.		JX992949	JX993015
Rostania multipunctata (Degel.) Otálora *et al.*		JX992930	JX992988
R. occultata (Bagl.) Otálora *et al.*		JX992931	JX992990
Scytinium biatorinum (Nyl.) Otálora *et al.*		JX992940	JX993003
S. fragrans (Sm.) Otálora *et al.*		JX992922	JX992981
S. lichenoides (L.) Otálora *et al.*		DQ923120	JX993021
S. magnussonii (Degel. & P. M. Jørg.) Otálora *et al.*		EU982565	JX993022
S. palmatum (Huds.) Gray 1		JX992959	JX993025
S. palmatum (Huds.) Gray 2		JX992960	JX993026
S. parvum (Degel.) Otálora *et al.*		JX992933	JX992992
S. plicatile (Ach.) Otálora *et al.*		GQ259033	JX993030
S. pulvinatum (Hoffm.) Otálora *et al.*		EU982590	JX993032
S. schraderi (Bernh.) Otálora *et al.*		EU982559	JX993036
S. turgidum (Ach.) Otálora *et al.*		EU982592	JX993040
Staurolemma omphalarioides (Anzi) P. M. Jørg. & Henssen		EU982560	JX993043

Epiphloea terrena (Nyl.) Trevis. **France:** *Languedoc-Roussillon, Dept. Pyrénées Orientales*: Colliour, Pla de las Fourques, in fossa arcis Fortrand, 5 vii 1872, *W. Nylander* (H-NYL 42806—lectotype!). *Provence-Alpes-Côte d'Azur, Dept. Vaucluse*: Vaucluse, Morières, Plateau de Gadagne, ad terram argillaceo-sabulosum secus viam im vicinitate Querceti ilicis, 12 ii 1971, *G. Clauzade & C. Roux* (Vězda, Lich. sel. exs. 987) (W 1975-261).—**Portugal:** *Norte, Distr. Vila Real*: Amieiro, terricolous on side of dust road, UTM 29TPF 3471, 7 iii 2012, *J. Marques* (PO) (HBG DNA no. 3731); Vale do Moinho, terricolous on side of dirt road, 7 iii 2012, *J. Marques* (PO) (dupl. hb. M. Schultz 17149). *Centro, Distr. Coimbra:* Ribeira de Relvas, 2011, *J. Marques* 686 (PO) (dupl. hb. M. Schultz 17150). *Algarve, Distr. Faro*: NE of Albufeira, *c.* 7 km ENE of Paderne, along new road from Espargal to the south, W slope of small hill, low calcareous outcrops and a few *Ceratonia siliqua* trees, terricolous, 37·214°N, 8·1202°W, 235 m, 2009, *P. van den Boom* 41781 (hb. P. van den Boom) (HBG DNA 3707).—**Spain:** *Canary Islands*: Tenerife, NW, near Buenavista del Norte, road to La Costa and Punta de la Laja, coastal scrub with *Euphorbia* spp., ± exposed soil crust, 28·375°N, 16·833°W, 50 m, 2001, *M. Schultz* 17083e (hb. M. Schultz).

Heppia despreauxii (Mont.) Tuck. **USA:** *Arizona*: Cochise Co., *c.* 2 km S of Tombstone, soil over calcareous rock, 1999, *M. Schultz* 16097a (hb. M. Schultz).

Results

A total of six sequences were generated for this study (Table 1). The combined data set consisted of 69 taxa and 1227 unambiguously aligned sites, 663 for the mtSSU and 564 for the *Mcm7*.

Maximum likelihood analyses resulted in a single most likely tree with an ln-likelihood of −13452·12. The harmonic mean ln-likelihood from the Bayesian analysis was −13968·6. The tree topologies obtained by the maximum likelihood and the Bayesian approaches did not show any significant conflict (Fig. 1).

Discussion

Here we show that *Epiphloea* clearly belongs within the *Collemataceae*. This is not surprising as the *Epiphloea* species were classified in *Collema* or *Leptogium* when they were originally described and are very similar to other *Collemataceae* (*sensu* Wedin *et al.* 2009

FIG. 1. Phylogenetic position of *Epiphloea* within the family *Collemataceae*. Best tree from RAxML with bootstrap support (ML-BS) and posterior probabilities (PP) obtained in the Bayesian analysis. * Indicates a support value of 100% for ML-BS and 1 for PP. Supported clades by both analyses (ML-BS > 70, PP > 0·95) are marked with thicker black branches and with thicker grey branches when the node is only supported by one of the two analyses.

https://doi.org/10.1017/S0024282915000353 Published online by Cambridge University Press

and Otálora *et al.* 2013) in morphology (i.e. thallus structure, photobionts and ascospores; Fig. 2). Pycnidia have not been reported previously in the two *Epiphloea* species nor have we succeeded in seeing them. Contrary to some recent suggestions, our observations of type and other material showing that the asci in *Epiphloea* are Lecanoralean, with well-developed apical domes and distinct tube-like amyloid apical structures similar to other *Collemataceae* (Fig. 3A–C). This observation is clearly supported by the phylogenetic relationship found here. In contrast, the asci are always thin-walled in *Heppiaceae* (Fig. 3D), where *Epiphloea* used to be classified. It should be noted, however, that juvenile asci of *Epiphloea* are sometimes still thin-walled due to incomplete development, whereas the mature asci observed by us always had amyloid apical structures. Thus the phylogenetic placement is also supported by the ascus apex characters.

Both *Epiphloea* species are part of *Leptogium* s. str. (*sensu* Otálora *et al.* 2014), and both already have names coined in this genus. *Leptogium* s. str. chiefly contains corticolous species with a few exceptions such as *Leptogium cyanescens* (corticolous to saxicolous) or *L. britannicum* (growing on coastal soils). *Leptogium byssinum* (Hoffm.) Zwackh ex Nyl. and *L. terrenum* Nyl. are two further examples of non-corticolous, soil dwelling species in the newly circumscribed genus. Furthermore, the thallus anatomy, especially in *L. terrenum*, adds to the variability of the genus by including species with a ± paraplechtencymatous thallus (otherwise found in some species of *Scytinium*). On the other hand, both species share the typical eucortex of *Leptogium*: a single layer of isodiametric cells in *L. byssinum* (Fig. 3F) and two to three rows of isodiametric cortex cells in *L. terrenum* (Fig. 3E).

Leptogium byssinum and *L. terrenum* form the sister clade to *Leptogium rivulare*, *L. crispatellum* and *L. biloculare*, a relationship which is not so easy to explain. Difficulties in the interpretation may be due to a still incomplete taxon sampling. *Leptogium rivulare* has an unique ecology, growing on seasonally submerged exposed roots, soil or occasionally on rock along the margins of sluggish rivers and ponds. *Leptogium crispatellum* is an epiphyte known only from New Zealand. *Leptogium biloculare* is an epiphytic species occurring in moist, montane to subalpine regions in Australia. The three species seem to form a natural group within *Leptogium* s. str., but their molecular relationship with *Epiphloea* is not obviously corroborated by morphological and anatomical evidence. It rather seems that *Leptogium byssinum* and *L. terrenum* form a group within *Leptogium* s. str., with preference for disturbed and dry soil habitats combined with an otherwise somewhat unusual thallus anatomy. The general similarity in overall macro-morphology of *L. byssinum* and *L. terrenum* to species of *Heppia* (Lichinomycetes) is likely to be the result of parallel evolution, as these distinctly unrelated taxa have adapted to similar environmental conditions in dry soils where they form part of biological soil crusts.

Nomenclatural Summary

Leptogium (Ach.) Gray nom. cons. prop. (Jørgensen *et al.* 2013)

= *Epiphloea* Trevis., *Rendiconti Reale Ist. Lombardo Sci., ser. 2* **13**(3)**:** 73 (1880) syn. nov.; type: *E. terrena* (Nyl.) Trevis.
= *Amphidium* Nyl., *Lich. Pyren. Orient.*: 72 (1891) nom. illeg., non Schimp. 1856 (bryophytes, nom. cons.); type: *Amphidium terrenum* (Nyl.) Nyl.

Leptogium byssinum (Hoffm.) Zwackh ex Nyl.

Actes Soc. Linn. Bordeaux **21:** 270 (1857); *Epiphloea byssina* (Hoffm.) Henssen & P. M. Jørg., *Nordic Lich. Fl.* **3:** 144 (2007) syn. nov.

The following description largely follows that given by Jørgensen (1994), but according to our observations the asci are Lecanoralean with a distinct apical dome and a strongly amyloid tube-like structure (Fig. 3C).

Thallus forming a thin crust over bare soil breaking up into irregularly shaped areoles up to 3 mm in size, consisting of brownish granules that sometimes become increasingly dissolved into bluish, leprose granules resembling soralia (Fig. 2D), or it remains ± crustose and corticate (Fig. 2C), attached to the substratum by pale rhizohyphae. *Photobiont* layer with short chains of *Nostoc* and

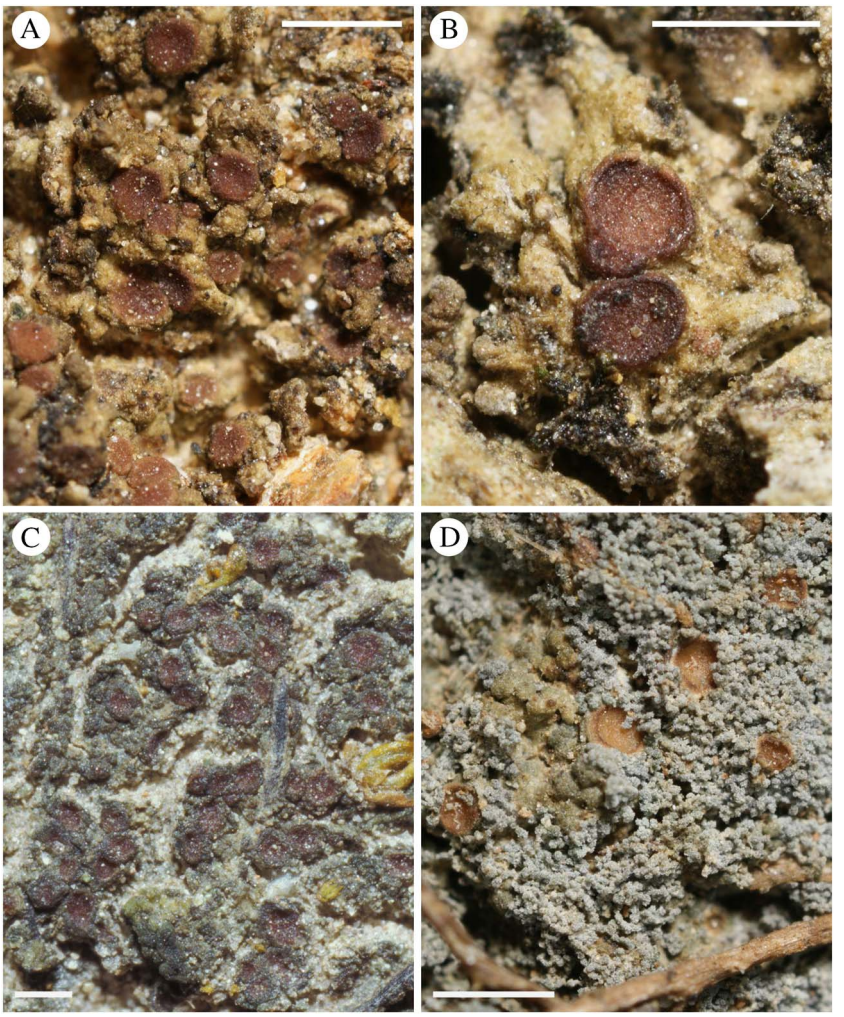

FIG. 2. A & B, *Leptogium terrenum*; A, olivaceous, small-squamulose thallus, apothecia adnate, with dark red discs (*Marque*s s. n., dupl. hb. M. Schultz 17149); B, apothecia with distinct proper exciple (*Marques* 686, dupl. hb. M. Schultz 17150). C & D, *Leptogium byssinum*; C, dark olivaceous, crustose thallus, with semi-immersed apothecia (Flotow, Deutsch. Lich. 143A, HBG); D, thallus almost entirely dissolved into leprose, bluish (olive) granules, apothecia with brownish discs (Paukov AGP20020804-02, dupl. hb. M. Schultz). Scales = 1 mm. In colour online

densely reticulate to paraplectenchymatous hyphae. *Medulla* absent, upper and lower cortex composed of a single row of isodiametric cells, 4·5–8·0 × 4–6 µm in size.

Apothecia circular, immersed to semi-immersed, rarely adnate, up to 2 mm diam.; *disc* dark reddish to brownish, initially concave, later plane, surrounded by thin thalline margin which becomes obscured in sorediate thallus parts (Fig. 2C & D). *Hymenium* 100–150 µm high, KOH/IKI + blue; *proper exciple* laterally thin, up to 10 µm thick, composed of ellipsoid to elongated cells, 5–8 × 3·0–3·5 µm, exciple apically thickened, cells roundish, 8·0–11·5 × 7–9 µm, pale reddish brown-coloured, sub-hymenial layer 30–40 µm high; *paraphyses*

https://doi.org/10.1017/S0024282915000353 Published online by Cambridge University Press

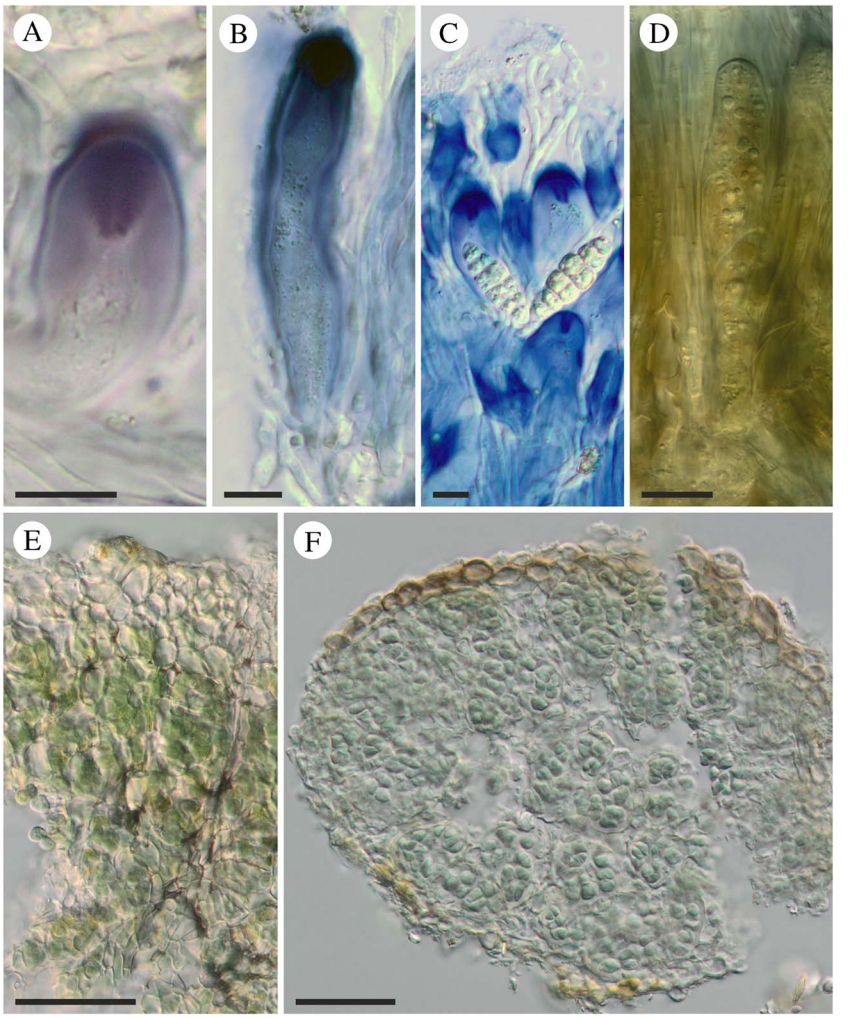

FIG. 3. A–D, asci stained with Lugol's solution after pretreatment with KOH. A & B, *Leptogium terrenum*, ascus with amyloid, tube-like apical structure (Vězda, Lich. sel. 987, W 1975-261); C, *Leptogium byssinum*, like A & B, but with muriform ascospores (Körber, Lich. sel. 60, B); D, *Heppia despreauxii*, thin-walled, prototunicate ascus not staining with iodine, ascospores simple (*Schultz* 16097a, hb. Schultz); E, *Leptogium terrenum*, thallus cross-section showing upper cortex of isodiametric cells, photobiont layer with densely reticulate, vertically elongated cells (*Marques* s. n., dupl. hb. M. Schultz 17149); F, *Leptogium byssinum*, thallus granule surrounded by single-row cortex, photobiont layer with densely reticulate hyphae (Du Rietz, B). Scales = 10 µm. In colour online.

simple, straight, 1–2 µm thick, terminal cells slightly enlarged. *Asci* narrowly clavate, 60–100 × 10–15 µm in size, 8-spored, Lecanoralean, with a distinct amyloid tube-like apical structure. *Ascospores* hyaline, ellipsoid, muriform, 16–28 × 7–15 µm.

Pycnidia unknown.

Leptogium terrenum Nyl.

Flora **56:** 195 (1873); *Epiphloea terrena* (Nyl.) Trevis., *Rendiconti Reale Ist. Lombardo Sci., ser. 2* **13**(3): 73 (1880)

https://doi.org/10.1017/S0024282915000353 Published online by Cambridge University Press

syn. nov.; *Amphidium terrenum* (Nyl.) Nyl., *Lich. Pyren. Orient.*: 72 (1891) syn. nov.

Thallus resembling *Heppia*, with smaller irregularly shaped squamules; thallus subcrustose, (yellowish) olive, 0·4–1·2 mm wide, tightly to loosely adpressed (Fig. 2A), often divided into small lobules 0·4–0·8 mm in size, up to 150 µm thick, pale below, attached to the substratum by robust, pale rhizohyphae that are indistinctly separated from the photobiont layer and composed of 1–2 rows of relatively large, isodiametric cells 10–15 µm in size. *Photobiont* layer with short chains of *Nostoc* and mostly vertically arranged, reticulate hyphae composed of elongated cells (Fig. 3E). *Medulla* absent, lower cortex usually inconspicuous (obscured by rhizohyphae), with 1–2 rows of small, ± isodiametric cells, 5·0–7·5 µm in size.

Apothecia circular, at first semi-immersed, soon adnate, 0·6–0·9 mm diam.; *disc* reddish to reddish brown, widely exposed (Fig. 2B). *Hymenium* 100–125 µm high, KOH/IKI + blue; *proper exciple* distinct, hyaline, composed of small cells of 5–10 × 3–5 µm, subhymenial layer up to 100 µm thick (medial sections!); *paraphyses* simple, straight, 1·5–2·0 µm thick, terminal cells slightly enlarged. *Asci* narrowly clavate, 80–90 × 11–22 µm, 8-spored, Lecanoralean with a distinct amyloid tube-like apical structure (Fig. 3A & B). *Ascospores* hyaline, muriform, 15–26 × 9·5–12·0 µm.

Pycnidia unknown.

This project was funded by two grants from the Swedish Taxonomy Initiative (Svenska Artprojektet) to MP in collaboration with MW and MS. We would like to thank Joana Marques (Kew), Martin Westberg (NRM) and Pieter van den Boom (Son) for putting fresh material at our disposal. Alexander Paukov (Yekaterinburg) is thanked for additional study material. The curators of the herbaria in B, GZU, M, STU, UPS and W are thanked for handling loan requests and we are grateful to M. G. Otálora for sharing the alignment and for her useful comments.

References

Ekman, S., Wedin, M., Lindblom, L. & Jørgensen, P. M. (2014) Extended phylogeny and a revised generic classification of the *Pannariaceae* (*Peltigerales*, Ascomycota). *Lichenologist* **46:** 627–656.

Eriksson, O. E. (1999) Outline of Ascomycota – 1999. *Myconet* **3:** 1–88.

Jørgensen, P. M. (1994) Further notes on European taxa of the lichen genus *Leptogium*, with emphasis on the small species. *Lichenologist* **26:** 1–29.

Jørgensen, P. M. (2007) *Heppiaceae*. In *Nordic Lichen Flora. Volume 3. Cyanolichens* (T. Ahti, P. M. Jørgensen, H. Kristinsson, R. Moberg, U. Søchting & G. Thor, eds): 43–45. Uppsala: Nordic Lichen Society.

Jørgensen, P. M., Otálora, M. A. G. & Wedin, M. (2013) Proposal to conserve the name *Leptogium* (lichenized Ascomycota) with a conserved type. *Taxon* **62:** 1333–1334.

Lumbsch, H. T. & Huhndorf, S. M. (eds) (2007) Outline of Ascomycota – 2007. *Myconet* **13:** 1–58.

Lutzoni, F., Wagner, P., Reeb, V. & Zoller, S. (2000) Integrating ambiguously aligned regions of DNA sequences in phylogenetic analyses without violating positional homology. *Systematic Biology* **49:** 628–651.

Maddison, W. P. & Maddison, D. R. (2001) *MacClade: Analysis of Phylogeny and Character Evolution*. Version 4.01. Sunderland, Massachusetts: Sinauer Associates.

Magain, N. & Sérusiaux, E. (2014) Do photobiont switch and cephalodia emancipation act as evolutionary drivers in the lichen symbiosis? A case study in the *Pannariaceae* (*Peltigerales*). *PLoS ONE* **9:** e89876. doi:10.1371/journal.pone.0089876

Miądlikowska, J., Richardson, D., Magain, N., Ball, B., Anderson, F., Cameron, R., Lendemer, J., Truong, C. & Lutzoni, F. (2014) Phylogenetic placement, species delimitation, and cyanobiont identity of endangered aquatic *Peltigera* species (lichen-forming Ascomycota, Lecanoromycetes). *American Journal of Botany* **101:** 1141–1156.

Miller, M. A., Pfeiffer, W. & Schwartz, T. (2010) Creating the CIPRES Science Gateway for inference of large phylogenetic trees. In *Proceedings of the Gateway Computing Environments Workshop (GCE), 14 November 2010, New Orleans, Louisiana*, pp. 1–8.

Otálora, M. A. G. & Wedin, M. (2013) *Collema fasciculare* belongs in *Arctomiaceae*. *Lichenologist* **45:** 295–304.

Otálora, M. A. G., Aragón, G., Martínez, I. & Wedin, M. (2013) Cardinal characters on a slippery slope – a re-evaluation of phylogeny, character evolution, and evolutionary rates in the jelly lichens (*Collemataceae* s. str). *Molecular Phylogenetics and Evolution* **68:** 185–198.

Otálora, M. A. G., Jørgensen, P. M. & Wedin, M. (2014) A revised generic classification of the jelly lichens. *Collemataceae. Fungal Diversity* **64:** 275–293.

Posada, D. (2008) JModelTest: phylogenetic model averaging. *Molecular Biology and Evolution* **25:** 1253–1256.

Prieto, M., Baloch, E., Tehler, A. & Wedin, M. (2013) Mazaedium evolution in the Ascomycota (Fungi) and the classification of mazaediate groups of unclear relationship. *Cladistics* **29:** 296–308.

Rambaut, A. & Drummond, A. J. (2007) *Tracer v.1.4*. http://beast.bio.ed.ac.uk/Tracer.

Rodríguez, F., Oliver, J. F., Marin, A. & Medina, J. R. (1990) The general stochastic model of nucleotide substitution. *Journal of Theoretical Biology* **142:** 485–501.

Ronquist, F., Teslenko, M., van der Mark, P., Ayres, D., Darling, A., Höhna, S., Larget, B., Liu, L., Suchard, M. A. & Huelsenbeck, J. P. (2012) MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology* **61:** 539–542.

Schmitt, I., Crespo, A., Divakar, P. K., Frankhauser, J. D., Herman-Sackett, E., Kalb, K., Nelsen, M. P., Rivas Plata, E., Shimp, A. D., Widhelm, T. *et al.* (2009) New primers for promising single-copy genes in fungal phylogenetics and systematics. *Persoonia* **23:** 35–40.

Schoch, C. L., Sung, G.-H., López-Giráldez, F., Townsend, J. P., Miądlikowska, J., Hofstetter, V., Robbertse, B., Matheny, P. B., Kauff, F., Wang, Z. *et al.* (2009) The Ascomycota tree of life: a phylum wide phylogeny clarifies the origin and evolution of fundamental reproductive and ecological traits. *Systematic Biology* **58:** 224–239.

Spribille, T. & Muggia, L. (2013) Expanded taxon sampling disentangles evolutionary relationships and reveals a new family in *Peltigerales* (Lecanoromycetidae, Ascomycota). *Fungal Diversity* **58:** 171–184.

Stamatakis, A. (2014) RAxML Version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* **30:** 1312–1313.

Wedin, M., Wiklund, E., Jørgensen, P. M. & Ekman, S. (2009) Slippery when wet: phylogeny and character evolution in the gelatinous cyanobacterial lichens (*Peltigerales*, Ascomycetes). *Molecular Phylogenetics and Evolution* **53:** 862–871.

Wedin, M., Jørgensen, P. M. & Ekman, S. (2011) *Vahliellaceae*, a new family of cyanobacterial lichens (*Peltigerales*, Ascomycetes). *Lichenologist* **43:** 67–72.

Zahlbruckner, A. (1919) Vorarbeiten zu einer Flechtenflora Dalmatiens. *Oesterreichische Botanische Zeitschrift* **68:** 60–77, 148–165, 237–253, 297–326.

Zahlbruckner, A. (1924–1925) *Catalogus Lichenum Universalis. Band III*. Leipzig: Borntraeger.

Zoller, S., Scheidegger, C. & Sperisen, C. (1999) PCR primers for the amplification of mitochondrial small subunit ribosomal DNA of lichen-forming ascomycetes. *Lichenologist* **31:** 511–516.