

***Bryobilimbia*, a new generic name for *Lecidea hypnorum* and closely related species**

Alan M. FRYDAY, Christian PRINTZEN and Stefan EKMAN

Abstract: The new generic name *Bryobilimbia* is proposed for *Lecidea hypnorum* and the closely related taxa *Lecidea ahlesii* var. *ahlesii*, *L. ahlesii* var. *nemoralis*, *L. diapsensiae*, *L. sanguineoatra* and *Mycobilimbia australis*. A phylogenetic analysis based on five genes shows that *Lecidea berengeriana* does not belong to this group but is more closely related to *Romjulularia*. Both groups of species have been included in *Mycobilimbia* by some authors but are instead shown to be most closely related to a group of genera (including *Clauzadea*, *Farnoldia*, *Lecidoma* and *Romjulularia*) that do not belong to *Lecideaceae* s. str. A neotype is selected for *Lichen sanguineoater* Wulfen and the new combinations *Bryobilimbia ahlesii* var. *ahlesii*, *B. ahlesii* var. *nemoralis*, *B. australis*, *B. diapsensiae*, *B. hypnorum* and *B. sanguineoatra* are proposed. A lectotype is also selected for *L. templetonii* Taylor.

Key words: Ascomycota, lichenized-fungi, phylogenetic analysis, systematics

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Introduction

It has long been recognized that *Lecidea hypnorum* Lib. and its close associates form a distinctive group unrelated to *Lecidea fuscoatra* Ach., the type species of the genus. It appears more closely related to *Clauzadea* Hafellner & Bellem. (Hawksworth & Coppins 1992), although Meyer (2002), in her study of *Clauzadea*, explicitly excluded *L. hypnorum* from that genus. The core species of the group were transferred to *Mycobilimbia* Rehm by Kalb & Hafellner in Wirth (1987) although they are clearly not congeneric with *M. obscurata* (Sommerf.) Rehm, the type species of that genus. Recent molecular studies (Arup 2004; Buschbom & Mueller 2004; Schmull *et al.* 2011) have confirmed that *Lecidea hypnorum* cannot be accommodated in either *Lecidea* or *Mycobilimbia*, and that it

is more closely related to *Clauzadea*, *Farnoldia* Hertel, *Lecidoma* Gotth. Schneid. & Hertel, and *Romjulularia* Timdal.

Currently, some authors prefer to describe additional species in, or transfer them to, *Mycobilimbia* (e.g., Hafellner 1989; Kalb & Hafellner 1992; Sarrión *et al.* 2003; Kantvilas *et al.* 2005), whereas others choose to retain the species in *Lecidea* (Hertel & Printzen 2004; Coppins & Fryday 2006; Fryday 2008; Aptroot *et al.* 2009). This situation, where two genera are available although the species are not congeneric with the type specimen of either, is clearly unsatisfactory and this study is an attempt to resolve the issue.

Materials & Methods

DNA extraction and PCR amplification

DNA was extracted from 2–3 apothecia per thallus using the QIAquick™ Plant Mini Kit (Qiagen) according to the manufacturer's instructions. Five gene loci were amplified with the following primers: ITS1F (Gardes & Bruns 1993) and ITS4 (White *et al.* 1990) for the internal transcribed spacer region of the ribosomal DNA (ITS), mrSSU1 (Zoller *et al.* 1999) and MSU7 (Zhou & Stanosz 2001) for part of the small subunit of the mitochondrial ribosomal DNA (mtSSU), LR0R and LR7 (Vilgalys

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Laboratory, Duke University: <http://www.biology.duke.edu/fungi/mycolab/primers.htm>) for the first part of the large subunit of the nuclear ribosomal DNA (nuLSU), RPB1-Af (Stiller & Hall 1997) and RPB1-Cr (Matheny *et al.* 2002) for part of the largest subunit of RNA polymerase II (RPB1), and RPB2-5f and RPB2-7Cr (Liu *et al.* 1999) for part of the second largest subunit of RNA polymerase II (RPB2). 25 µl PCR reactions were carried out using PCR-PuReTaq Ready-to-Go Beads™ (GE Healthcare). For ITS, mtSSU and nuLSU, reactions contained 5 µl of DNA extract, 1 µl of each forward- and reverse-primer (10 µM) and 18 µl of distilled water. Cycling conditions included initial denaturation at 94°C for 5 min; 5 cycles of 94°C for 30 s, 54°C for 30 s, 72°C for 1 min; 33 cycles of 94°C for 30 s, 48°C for 30 s, 72°C for 1 min; and a final extension step at 72°C for 10 min. For RPB1 and RPB2, PCR reactions contained 10 µl of DNA extract, 3–5 µl of each forward- and reverse-primer (10 µM) and 8 µl of distilled water. Cycling conditions were as follows: for RPB1 initial denaturation at 95°C for 5 min, 8 cycles of 95°C for 1 min, 58°C for 1 min, 72°C for 1 min 45 s, 34 cycles of 95°C for 1 min, 50°C for 1 min, 72°C for 1 min 45 s, and a final extension step at 72°C for 10 min; for RPB2 initial denaturation at 92°C for 2 min, 8 cycles of 94°C for 1 min, 59°C for 1 min, 72°C for 2 min, 33 cycles of 95°C for 30 s, 50°C for 30 s, 72°C for 2 min, and a final extension step at 72°C for 10 min. PCR products were run on agarose gels, bands cut out and purified using the QIAquick Gel Extraction Kit (Qiagen). Purified DNA was labelled with the BigDye™ Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems) and cycle sequenced at 94°C for 30 s, with 29 cycles of 95°C for 15 s, 45°C for 15 s, and 60°C for 4 min using the PCR primers. Sequences were determined on an ABI PRISM® 3730 DNA Analyzer (Applied Biosystems), and assembled and edited using Geneious Pro, version 5.6.5 (Biomatters Inc.).

BLAST searches in GenBank were performed to ascertain that all sequences used in the phylogenetic analyses originated from the lichens and not from contaminating organisms such as parasymbiotic fungi. Single gene datasets containing the sequences listed in Table 1 were compiled and aligned in Geneious Pro, version 5.6.5, using the Muscle algorithm with default settings. Regions of uncertain alignment were removed using GBLOCKS version 0.91b (Castresana 2000), applying default settings but allowing gap positions in half of the sequences. After excluding uncertain alignment, the RPB1 and RPB2 sequences of *Lecidea auriculata* consisted only of missing data. Accordingly, the species was removed from these datasets. Final alignments comprised 25 sequences, 373 bp (ITS), 26 seq., 620 bp (mtSSU), 27 seq., 902 bp (nuLSU), 12 seq., 663 bp (RPB1) and, 13 seq., 702 bp (RPB2).

Datasets were concatenated to yield a final alignment of 30 sequences and 3260 bp length. The optimal partitioning scheme and substitution models for each data partition, inferred with the help of PartitionFinder version 1.0.1 (Lanfear *et al.* 2012) using default settings and suggesting eleven data blocks (ITS1, 5.8S rDNA, ITS2, mtSSU, nuLSU, and three independent codon positions for RPB1 and RPB2), are listed in Table 2. We used the Markov Chain Monte Carlo (MCMC) ap-

proach implemented in MrBayes, version 3.2 (Ronquist *et al.* 2012) to infer phylogenetic trees for the single gene datasets, applying the substitution models and partitioning schemes inferred with PartitionFinder and default settings of MrBayes with three exceptions. We used a proportional model on partition-specific rates, gamma distributed rates across sites modelled as six discrete categories and with a mean 1 exponential prior, and an empirical Bayes approach to select the mean of the exponential branch length prior. The mean of the branch length prior was inferred by calculating ML trees for all single gene datasets and the concatenated dataset using raxmlGUI version 0.9 beta 2 (Stamatakis 2006; Silvestro & Michalak 2010) and applying either an unpartitioned GTRGAMMAI model (mtSSU, nuLSU) or a partitioned model with separate, proportional GTRGAMMAI models (the rest) and 20 runs. The mean branch lengths of the ML trees were then used as means of the exponential distributions.

The single gene MCMC trees were compared to identify conflicting phylogenetic signals between datasets. Three supported conflicts were detected (data not shown), which concerned the positions of *Lecidea lapicida* and *L. silacea*. In mtSSU, *L. lapicida* appeared as the sister taxon of *L. silacea*, in nuLSU its position was outside a well-supported clade of *L. silacea*, *L. confluens* and *Porpidia speirea*. In RPB2, it formed a well-supported clade with *L. fuscoatra*, *L. grisella* and *P. speirea* that excluded *L. silacea*. The different positions of *L. silacea* in nuLSU and RPB2, either inside or outside a clade with *P. speirea*, were also well supported. Because none of these conflicts concerned taxa connected with the *L. hypnorum* group, we decided to infer phylogenetic relationships based on a concatenated dataset containing all five gene regions. The inferred branch length prior for the MCMC analysis of this dataset followed an exponential distribution with mean 1/19. MrBayes was set to sample every 500th tree from three independent runs, each with four chains with the temperature increment parameter set to 0.15. The average standard deviation of bipartition frequencies among runs was calculated every 1 M generations to infer convergence of the Markov Chains, discarding the first 50% of the trees sampled as burn-in and including only those bipartitions with a frequency of at least 10%. The analysis was stopped after 2 M generations when the standard deviation had dropped below 0.01. Finally, we also calculated a ML bootstrap tree for the concatenated dataset using raxmlGUI and unlinked GTRGAMMAI models for the five partitions inferred by PartitionFinder.

Results

The results of the molecular analysis (Fig. 1) indicate that *L. hypnorum* belongs to a well-supported group of closely related species that also includes *Mycobilimbia australis* Kantvilas & Messuti, *Lecidea diapensiae* Th. Fr. and *L. sanguineoatra* auct. Because these species are also morphologically and anatomically

TABLE 1. Taxa and sequences used in this study. Collection data are given for samples that were used to generate new sequences.

Taxon	Origin	ITS	nuLSU	mtSSU	RPB1	RPB2
<i>Bryobilimbia australis</i> 1	Falkland Islands, East Falkland, by Argentine Cemetery, N Goose Green, 23 Jan. 2009, <i>S. Davey</i> s.n. (MSC)	n/a	KF683115	KF683099	n/a	n/a
<i>B. australis</i> 2	Chile, XII Region, Isla Grande de Tierra del Fuego, Seno de Almirantazgo, Bahía Blanca, 7 Dec. 2009, <i>S. Pérez-Ortega</i> 1419 (FR)	KF683092	KF683106	KF683100	KF683112	n/a
<i>B. diapensiae</i>	GenBank	HQ650660	HQ660539	HQ660564	n/a	n/a
<i>B. hypnorum</i>	Sweden, Jämtland, Åre par., Kvissle, by the shore of Indal river (Indalsälven) near the outflow into the lake Storsjön, 2006, <i>F. Jonsson & Z. Palice</i> 10747 (hb. Palice)	KF683093	n/a	n/a	n/a	n/a
<i>B. sanguineoatra</i> 1	GenBank	n/a	AY756354	AY762094	n/a	n/a
<i>B. sanguineoatra</i> 2 (= " <i>L. cf. hypnorum</i> ")	GenBank	HQ650664	AY533005	HQ660569	n/a	n/a
<i>B. sp.</i> (= " <i>Helocarpon crassipes</i> ")	GenBank	n/a	AY756322	n/a	AY756384	n/a
<i>Clauzadea monticola</i>	USA, Alaska, Hoonah-Angoon District, Glacier Bay National Park, Willoughby Island, 6 Sept. 2011, <i>A. M. Fryday</i> 9703 (MSC); <i>ibid.</i> , S. Marble Mountain, 7 Sept. 2011, <i>A. M. Fryday</i> 9728 (MSC) (used for RPB1 and RPB2)	KF683090	KF692710	KF683097	KF683113	KF683110
<i>Eremastrella crystallifera</i>	GenBank	n/a	n/a	EF524307	EF524327	n/a
<i>Farnoldia jurana</i>	GenBank	EU263920	AY532984	GU074511	n/a	n/a
<i>Lecidea atrobrunnea</i>	GenBank	HQ650657	HQ660535	HQ660560	n/a	HQ660524
<i>L. auriculata</i>	GenBank	HQ650658	HQ660536	HQ660561	HQ660552	HQ660525
<i>L. berengeriana</i>	GenBank	HQ650659	HQ660537	HQ660562	n/a	HQ660526
<i>L. confluens</i>	GenBank	EU263921	AY532994	GU074492	n/a	n/a
<i>L. fuscoatra</i>	GenBank	HQ650662	HQ660541	HQ660566	HQ660553	HQ660528
<i>L. grisella</i>	Great Britain, Scotland, V.C. 82, Mid-Lothian, Haddington, Garleton Hills, Yellow Craig, 7 Nov. 2010, <i>A. M. Fryday</i> 9437 & <i>B. J. Coppins</i> (MSC)	HQ605931	HQ660542	HQ660567	KF683115	HQ660529
<i>L. lapicida</i>	GenBank	HQ650665	HQ660544	HQ660570	n/a	HQ660530

TABLE 1. *Continued*

Taxon	Origin	ITS	nuLSU	mtSSU	RPB1	RPB2
<i>L. plana</i>	USA, Maine, Washington Co., Milbridge, McClelland Park, 15 June 2011, <i>A. M. Fryday</i> 9597 (MSC)	EU259903	n/a	KF683103	KF683116	KF683111
<i>L. silacea</i>	GenBank	HQ650629	AY756340	GU074496	DQ986820	DQ992431
<i>L. tessellata</i>	GenBank	HQ650671	HQ660548	GU074491	n/a	n/a
<i>L. cf. tessellata</i>	USA, California, San Benito Co., Clear Creek Management Area, San Carlos Creek, 21 Apr. 2011, <i>A. M. Fryday</i> 9481 (MSC)	KF683096	KF683108	KF683101	KF683114	n/a
<i>Lecidoma demissum</i>	GenBank	HQ650630	DQ986759	DQ986881	n/a	DQ992445
<i>Porpidia albocaerulescens</i> 1	GenBank	JQ301702	DQ986757	AY584718	DQ986828	DQ992443
<i>P. albocaerulescens</i> 2 (sorediate)	USA, Maine, Hancock Co., Black Mountain, mixed woodland and granitic rocks, 6 Aug. 2009, <i>A. M. Fryday</i> 9232 (MSC)	KF683095	KF683109	KF683102	n/a	n/a
<i>P. cinereoatra</i>	Canada, Newfoundland, Avalon Peninsula, Maritime Barrens Ecoregion, Hawke Hill Ecological Preserve, 0.1–2.0 km S of telecommunications towers along Trans-Canada Hwy, 8 Sept. 2007, <i>J. Guccion</i> 1187 (MSC)	KF683094	AY532941	KF683104	n/a	n/a
<i>P. flavicunda</i>	GenBank	n/a	DQ314903	n/a	n/a	DQ315026
<i>P. speirea</i>	GenBank	HQ650631	DQ986758	DQ986865	DQ986829	DQ992444
<i>Rhizocarpon disporum</i>	GenBank	HQ650708	AF356678	n/a	n/a	AY641069
<i>R. geographicum</i>	GenBank	DQ534482	AY853389	AF483187	n/a	n/a
<i>Romjularia lurida</i>	Spain, Aragón, Teruel, Villar del Cobo, Casas de Búcar, cruce Guadalaviar-Griegos, pista dirección Griegos, 6 Sept. 2010, <i>S. Pérez-Ortega</i> 1372 (FR)	KF683091	KF683107	KF683098	EF524328	n/a

TABLE 2. Optimal partitioning scheme and substitution models for each data partition inferred by PartitionFinder, version 1.0.1, and used in the phylogenetic analyses.

Subset	Best Model	Partitions	Subset Sites
1	GTR+ Γ	ITS1, ITS2	1–116, 268–373
2	GTR+I+ Γ	5.8S, nuLSU	117–267, 994–1895
3	HKY+I+ Γ	RPB1 codon 3, RPB2 codon 3, mtSSU	374–993, 1898–2558\3, 2561–260\3
4	SYM+ Γ	RPB1 codon 1, RPB2 codon 1	1896–2558\3, 2559–3260\3
5	GTR+ Γ	RPB1 codon 2, RPB2 codon 2	1897–2558\3, 2560–3260\3

similar, we describe below the new genus *Bryobilimbia* to accommodate them. *Lecidea berengeriana* (A. Massal.) Nyl., which is superficially similar to these species but can be distinguished by a number of characters (see below), is most closely related to *Romjularia lurida* (Ach.) Timdal. *Clauzadea monticola* (Ach.) Hafellner & Bellem. appears basal to this clade. These groupings receive high support from ML bootstrap values and posterior probabilities. The position of *Lecidoma demissum* (Rutstr.) Gotth. Schneid. & Hertel and *Farnoldia jurana* (Schaer.) Hertel is less clear. In the phylogenetic tree they form a sister group to the *Clauzadea*–*Romjularia* clade but the inter-relationships between these two clades and *Bryobilimbia* are not supported. The family *Lecideaceae* s. str., including *Lecidea* and *Porpidia* but also *Eremastrella*, appears monophyletic but the genera *Lecidea* and *Porpidia* are non-monophyletic.

Our phylogenetic analysis resulted in an anomalous position for two of the specimens for which sequences were obtained from GenBank; the sequences for *Helocarpon crassipes* Th. Fr. (AY756322, AY756384) placed this specimen firmly within our new genus, whereas the sequences for *Lecidea hypnorum* (HQ650664, AY533005, HQ660569) resulted in this species appearing paraphyletic with our newly obtained sequences of this species. However, examination of the specimens from which the GenBank sequences were obtained showed that they had been incorrectly identified: the *H. crassipes* specimen was a species of *Bryobilimbia*, although its identity is currently unclear, whereas the *L. hypnorum* collections were referable to *B. sanguineoatra*, which is consistent with their position in our analysis.

Taxonomic Innovations

Bryobilimbia Fryday, Printzen & S. Ekman gen. nov.

Mycobank No.: MB805035

Distinguished from *Lecidea* and *Mycobilimbia* by having a *Porpidia*-type ascus, from *Clauzadea* and *Porpidia* by the much thinner gelatinous coat in mature ascospores, and from *Lecidoma* and *Romjularia* by the inconspicuous thallus and darker hypothecium. Its distinctness is also supported by molecular phylogenetic analyses based on ITS, nuclear LSU, mitochondrial SSU, RPB1 and RPB2 sequences.

Type species: *Bryobilimbia hypnorum* (Lib.) Fryday, Printzen & S. Ekman.

Bryobilimbia ahlesii (Körb.) Fryday, Printzen & S. Ekman comb. nov.

Mycobank No.: MB805040

Biatora ahlesii Körb., *Parerga lichenol.* 161 (1865).—*Lecidea ahlesii* (Körb.) Nyl., *Flora* 55: 356 (1872); type: [Germany, Baden-Württemberg], in sylvis “montanus” [montanis] p[rope]. Heidelberg, 1852; [W.] *Zwackh* (H-Nyl. 20416—neotype, designated by Meyer 2002).

Lecidea delincta Nyl., *Flora* 55: 356 (1872); type: [Finland, Tavastia Australis], Kuhmois, 1866, *J. P. Norrlin* 362 (H-Nyl. 20423—lectotype, designated by Meyer 2002).

Lecidea valentior Nyl., *Flora* 60: 229 (1877); type: Hibernia [Ireland, Galway], Bois du Lough Inagh, 1876, *C. Lorbalestier* (H-Nyl. 20822—lectotype, designated by Meyer 2002).

Bryobilimbia ahlesii var. *nemoralis* (J. Lowe) Fryday, Printzen & S. Ekman comb. nov.

Mycobank No.: MB805047

Lecidea nemoralis J. Lowe, *Lloydia* 2: 264 (1939).—*Lecidea ahlesii* var. *nemoralis* (J. Lowe) Fryday & Coppins, *Bryologist* 109: 12 (2006); type: USA, New York, Adirondack Region, The Huntingdon Forest at Newcomb, on rock in brook bed, 1934, *J. L. Lowe* 5016 (MICH—holotype!).

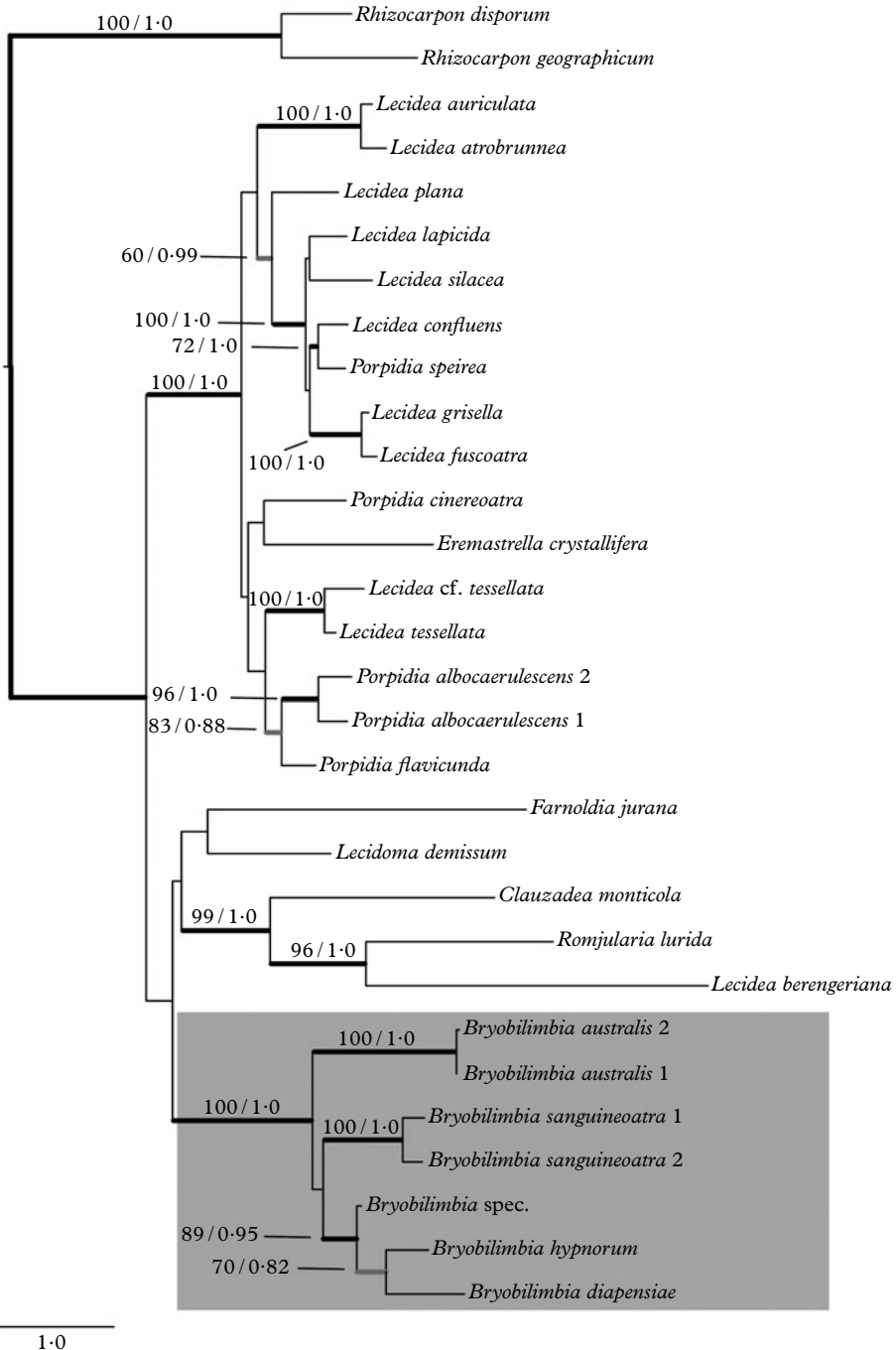


FIG. 1. Maximum likelihood phylogenetic tree of *Bryobilimbia* (shaded) and related genera and species. Two species of *Rhizocarpon* were used as the outgroup. Numbers on and beside branches denote ML bootstrap values and MCMC posterior probabilities. Bold branches have BP $\geq 70\%$ and PP ≥ 0.95 . Grey branches were only supported in one of the analyses. Total length was 3.076 for the ML tree and 2.319 for the MCMC tree.

Although we did not include *B. ahlesii* in our phylogenetic analysis, it has all the features characteristic of the genus and so we have no hesitation in including it in *Bryobilimbia*. It is unusual within the genus in that both varieties occur only on damp siliceous rock.

***Bryobilimbia australis* (Kantvilas & Messuti) Fryday, Printzen & S. Ekman comb. nov.**

Mycobank No.: MB805049

Mycobilimbia australis Kantvilas & Messuti, *Lichenologist* 37: 252 (2004); type: Australia, Tasmania, summit plateau of Projection Bluff, 41°43'S, 146°42'E, 1260 m, on peaty soil in alpine heathland, 1994, G. Kantvilas (3/94) & J. Jarman (HO—holotype; BCRU, BM, CHR—iso-types).

***Bryobilimbia diapensiae* (Th. Fr.) Fryday, Printzen & S. Ekman comb. nov.**

Mycobank No.: MB805052

Lecidea diapensiae Th. Fr., *Lichenes Arctoi*: 209 (1860).—*Biatora diapensiae* (Th. Fr.) Hellb., *Öfvers. kongl. Svenska Vetensk.-Akad. Förhandl.* 1875: 68 (1875); type: [Norway, Finnmark], Østfinnmark, Syd-Varanger, Elvenes, 15 July 1857, Th. M. Fries (UPS—lectotype, designated by Printzen in *Biblioth. Lich.* 60: 171, 1995).

The lectotype has an olivaceous subhymenium and this character is shared by many collections from *Diapensia*. However, other collections from *Diapensia* lack this pigment and are otherwise similar to *B. sanguineoatra*. The taxonomic status of these collections is currently unclear and warrants further study.

***Bryobilimbia hypnorum* (Lib.) Fryday, Printzen & S. Ekman comb. nov.**

Mycobank No.: MB805054

Lecidea hypnorum Lib., *Plantae cryptogamicae quas in Arduenna collegit M. A. Libert, fasc. 1, #12.* (1830).—*Mycobilimbia hypnorum* (Lib.) Kalb & Hafellner, in Wirth, *Die Flechten Baden-Württembergs. Verbreitungsatlas*: 511 (1987); type: [?Belgium, 'Ardennes'], ad rupes supra muscos, Libert [PRA—lectotype, designated by Vězda in *Lichenes Selecti Exs., Fasc. L (No. 1233)*, 1974, FR—isolectotype!].

Lecidea templetonii Taylor, in Mackay, *Flora Hibernica* 2: 123 (1836); type: Ireland, near Belfast, *Templeton* (FH—lectotype!, designated here; BM—probable isolectotype!).

Lecidea hypnorum was first described by Libert in 1830 (not 1853 as given by Zahlbruckner) and therefore has priority over *L. templetonii* Taylor.

Taylor (in Mackay 1836) mentions two collections of his new taxon: “on moss, near Belfast, Mr. Templeton; on turf, near Bantry, Miss Hutchins”. Both collections are present in Taylor’s herbarium in FH, with a further specimen of Templeton’s collection in BM (ex hb. Crombie). The Hutchins collection (near Bantry) is *Micarea inquinans* (Tul.) Coppins, but the Templeton collection (near Belfast) is clearly conspecific with *Lecidea hypnorum*. There are two specimens in the packet in FH and the upper one (Bar Code: 00377084) is designated here as the lectotype. There is a space on the sheet beside this specimen where another specimen has been removed and it is probable that this is the specimen that is now in BM.

***Bryobilimbia sanguineoatra* (Wulfen) Fryday, Printzen & S. Ekman comb. nov.**

Mycobank No.: MB805055

Lichen sanguineoater Wulfen, in Jacquin, *Coll. Botan.* 3: 117 (1789)—*Lecidea sanguineoatra* (Wulfen) Ach., *Method. Lich.*: 50 (1803).—*Mycobilimbia sanguineoatra* (Wulfen) Kalb & Hafellner *nom. inval.*, *Herzogia* 9: 75 (1992)); type: Ueber Mossen [sic, not “Moosen”], besonders *Hypnum cupressif.* auf Fichtenwurzeln im Walde hinter Schernfeld bei Eichstätt, Sommer 1862, [Arnold, *Lichenes exsiccati (Lichenes furae)* #229: UPS—neotype!; FR—isonotype!, designated here].

Based on Wulfen’s description, Arnold (1882) referred the taxon to *Bilimbia sabuletorum* (Schreb.) Arnold, the basionym of which (*Lichen sabuletorum* Schreb.) was described 18 years earlier than *Lichen sanguineoater*. Because no original material of Wulfen’s taxon is found in either M or W to confirm or reject Arnold’s determination, subsequent authors referred to the species as *L. sanguineoatra* auct. (e.g., Aptroot et al. 2009), or *L. sanguineoatra sensu* A. L. Smith (e.g., Index Fungorum 2012). In order to avoid further confusion, we here designate a neotype for Wulfen’s name that matches current usage.

Discussion

Systematic position

In the study by Buschbom & Mueller (2004), *Bryobilimbia sanguineoatra* [as *Mycobilimbia hypnorum* (Lib.) Kalb & Hafellner] grouped with *Clauzadea*, which in turn formed a sister group, although with little support, to a group containing *Farnoldia*, *Melanolectia* Hertel, *Notolecidea* Hertel and *Pachyphysis* R. C. Harris & Ladd, in a group outside the core *Lecideaceae*. However, for want of a better alternative, all of these genera were included in the *Lecideaceae* by Lumbsch & Huhndorf (2010).

Subsequent molecular work by Schmuil *et al.* (2011) has shown that the *L. hypnorum*-group is not congeneric with the '*Lecidea*' *berengeriana* group but is more closely related to *Lecidoma*, and also indicated an anomalous position of these two groups outside the major groups in Lecanoromycetidae. Our study confirms these relationships but does little to resolve them. It does, however, indicate that *Bryobilimbia* and *L. berengeriana* are more closely related to each other than suggested by Schmuil *et al.* (2011). Further work, including other species of *Bryobilimbia* and the *L. berengeriana* group (see excluded species), along with other taxa with a *Lecidea* or *Porpidia*-type ascus that were shown by Buschbom & Mueller (2004) to be related to *Farnoldia* (e.g., *Melanolectia*, *Pachyphysis*, *Poeltiaria*, *Poeltidea*, etc.), is required to clarify the higher systematic position of these two groups of species.

Conidiomata

Pycnidia are very rare in *Bryobilimbia*. To our knowledge, they have been reported only from *B. australis* by Kantvilas *et al.* (2005), who described them as "uncommon, black, rather glossy, superficial, 0.10–0.15 mm wide, resembling apothecial initials; ostiole gaping at maturity, becoming excavate; wall in section dark brown to purple-brown with blue-black, K⁺ aeruginose pigment. Conidia bacilliform, 4–5 × 1 µm". Unfortunately, Kantvilas *et al.* (2005) did not record details of the conidiophores or the attach-

ment of the conidia. However, inspection of a collection of *B. australis* housed in MSC (*Imshaug* 42125) confirmed the description given by Kantvilas *et al.* (2005) and revealed that the conidia were acrogenous, borne singly on conidiophores with an unbranched terminal cell measuring 12–15 × 3 µm.

Comparison of *Bryobilimbia* with similar genera

A number of other genera, all with *Porpidia*-type asci, have characters that are similar to those of our new genus. The differences between *Bryobilimbia* and similar genera are shown in detail in Table 3.

Clauzadea. The species of this genus differ in having branched, partly moniliform paraphyses, the ascospores having a poorly- to well-developed perispore and/or gelatinous coat, and in the extremely rare occurrence of blue-green granules in the hymenium, hypothecium, and excipulum. *Clauzadea* is also a genus of calcareous rocks, whereas the species of *Bryobilimbia* are primarily bryophilous (over various substrata). However, *L. ahlesii* and two undescribed species known to us occur on damp siliceous rocks and are also referable to *Bryobilimbia*.

Lecidea berengeriana. It is currently unclear which species formerly considered as belonging to the '*Lecidea hypnorum* group' (see below) are referable to *Bryobilimbia* and which are more closely related to *L. berengeriana*. However, it appears that *L. berengeriana* and closely related species are distinguished morphologically from *Bryobilimbia* by having a thick tartareous to subsquamulose thallus, capitate paraphyses to 6 µm wide, and ellipsoid conidia. This last character is also shared with *Romjularia*, supporting the relationship revealed by the molecular analysis. The mature ascospores also lack any perispore or gelatinous coat and there are rarely any blue-violet (K⁺ green) granules in apothecial sections, a feature that is characteristic of *Bryobilimbia*. There is also some evidence that apothecium development is gymnocarpous in *L. berengeriana* whereas it is hemiangiocarpous in *Bryobilimbia* (A. M. Fryday, unpublished data).

TABLE 3. Comparison of characters of *Bryobilimbia* and similar genera (significant characters in **bold**)

	<i>Bryobilimbia</i>	<i>Clauzadea</i>	<i>L. berengeriana</i>	<i>Lecidoma</i>	<i>Romjularia</i>
Thallus	effuse, thin, membranous, pale grey, sometimes with brownish tinge	generally immersed; when superficial grey or brown, granular or \pm areolate	mostly c. 0.2 mm thick, tartareous, white , obscuring the form of the underlying bryophytes or plant remains, surface of contiguous granular warts 0.1–0.3(–0.5) mm diam.	thick, coarsely areolate to subsquamulose , dark brown, rarely grey to grey-brown (shade morphs), attached directly to substratum by black hyphae	squamules to 5 mm, rounded at apices , at times minutely lobed, \pm concave, usually strongly imbricate, pale brown to dark brown, dull greenish when wet
Photobiont	Trebouxiod	Trebouxiod	Trebouxiod	chlorococcoid; dividing into 2–4 daughter cells	Trebouxiod
Apothecia	0.6–1.2 mm diam. (sometimes forming larger tuberculate clusters), dark brown to black, sessile; disc flat to convex	0.25–0.60–1.20 mm diam., red-brown to black, sessile or immersed in rock and sometimes leaving pits, naked or \pm pruinose	0.5–1.0(–1.5) mm diam., flat and \pm thinly marginate when young but soon convex and immarginate, medium to dark brown or blackish	0.5–2.0(–3.0) mm diam., discrete or becoming confluent into irregular shapes, immersed or adnate	to 1.0(–1.5) mm diam., mostly single, marginal or on surface, dark brown, at first flat, becoming weakly convex with an excluded exciple
Hymenium	often with scattered blue-violet (K+ green) granules	blue-violet (K+ green) granules absent	rarely with scattered blue-violet (K+ green) granules	blue-violet (K+ green) granules absent	blue-violet (K+ green) granules absent
Paraphyses	1.5–1.7 μ m wide, mostly simple, the apices gradually widening to 2.5 μ m	branched and occasionally anastomosing , scarcely or markedly swollen and pigmented at the apices	1.5–2.0(–2.5) μ m wide, becoming clavate to capitate and brown walled at apices and to 6 μm wide , mostly simple	straight, 2–3 μ m wide, thin walled, strongly conglutinate, only slightly branched at tips, apical cells swollen to 4 μ m with a thin, dark brown cap	straight, strongly conglutinate, sparingly branched and anastomosing, apical cells slightly swollen
Hypothecium	dark reddish brown	\pm colourless to red-brown or orange-brown	dark reddish brown	hyaline	pale brown
Ascospore septation	0(–1)	0	0	0	0
Perispore	with finely warted perispore and/or thin gelatinous coat	with a thin gelatinous coat when young	perispore and gelatinous coat absent	perispore and gelatinous coat absent	perispore and gelatinous coat absent
Conidia	bacilliform	bacilliform	ellipsoid	not seen	ellipsoid
Ecology	On bryophytes and plant debris over limestone, soil or trees or siliceous rock	On calcareous rocks	On bryophytes over \pm calcareous rocks or on exposed turf of mountain ridges or summits; localized	On peaty soils , decaying vegetation and acid gravels on exposed mountain sides and summits	On calcareous soils in crevices associated with limestone, sometimes directly on rock

Lecidoma. The single species, *L. demissum*, differs from *Bryobilimbia* most significantly in having a chlorococcoid photobiont that divides into 2–4 daughter cells, and a hyaline hypothecium (always red-brown in *Bryobilimbia*). The apothecia also lack the blue-violet (K+ green) granules in section that are characteristic of *Bryobilimbia*, and the mature ascospores lack any perispore or gelatinous coat. The species is restricted to acid soils in alpine regions.

Romjularia. The single species, *R. lurida*, differs from *Bryobilimbia* in having a thallus composed of squamules up to 5 mm across that are rounded at the apices, apothecia with a pale brown hypothecium, and ellipsoid conidia. The apothecia lack the blue-violet (K+ green) granules in section that are characteristic of *Bryobilimbia*, and the mature ascospores lack any perispore or gelatinous coat. The species is also restricted to calcareous soils associated with limestone.

Key to *Bryobilimbia* and related genera

This is a preliminary key to the genera of *Lecideaceae* s. lat. that appear closely related to *Bryobilimbia*. A full key to the genera of *Lecideaceae* s. lat. is in preparation and will be published elsewhere (Fryday & Hertel 2014).

- 1 Exciple thick and carbonaceous throughout, clearly separated from the paler hypothecium **Farnoldia**
Excipulum not carbonaceous throughout 2
- 2(1) Saxicolous. Thallus usually crustose, rarely squamulose. 3
Musci-colous, terricolous or on moribund *Diapensia*. Thallus crustose to squamulose; if directly on rock then thallus squamulose 6
- 3(2) Thallus distinctly squamulose. On limestone (rare occurrences for this usually terricolous species) **Romjularia**
Thallus crustose. On siliceous or calcareous rocks 4
- 4(3) Hypothecium and exciple usually dark brown (Arnoldiana-brown), if orange-brown (Superba-brown) then ascospores > 20 µm long; olivaceous pigments (Cinereorufagreen, K + HCl+ blue) usually present in epithecium and exciple cortex. Ascospores with well-developed gelatinous coat. Conidia borne apically . . . **Porpidia**
Hypothecium and exciple orange-brown (Superba-brown); usually lacking olivaceous pigments internally, if present then Baglietoana-green (K + HCl+ violaceous) in exciple, medulla and/or lower hymenium and hypothecium. Ascospores < 18 µm long with poorly- or well-developed gelatinous coat 5
- 5(4) On damp siliceous rocks; brown or olivaceous pigments present Conidia borne apically **Bryobilimbia**
On calcareous rocks; hypothecium paler than exciple, K–, only brown pigments present internally. Conidia borne laterally and apically **Clauzadea**
- 6(2) Terricolous. Thallus either distinctly squamulose or areolate-squamulose with distinct marginal lobes. 7
Musci-colous on rocks, trees or soil. Thallus crustose or minutely squamulose without distinct marginal lobes. 8
- 7(6) On acid alpine soils. Thallus areolate-squamulose with wide marginal lobes; hypothecium hyaline; paraphyses simple, thick (3–4 µm) distinctly capitate (5–7 µm) **Lecidoma**
On calcareous soils, often in limestone crevices. Thallus squamulose; hypothecium pale brown; paraphyses thinner **Romjularia**

- 8(6) Conidia bacilliform, *c.* 1 µm wide. Paraphyses only slightly swollen at apex. Thallus inconspicuous **Bryobilimbia**
 Conidia ellipsoid, >2 µm wide. Paraphyses distinctly swollen at apex (to 6 µm).
 Thallus granular to minutely squamulose **L. berengeriana-group**

Key to species of *Bryobilimbia* s. str.

In most cases the species currently placed in the new genus are easily separated and, where there is doubt, good descriptions are provided by Aptroot *et al.* (2009) and Kantvilas *et al.* (2005). The exception is *B. diapensiae*, for which no good, modern, English description exists, although descriptions of this species are provided by Foucard (2001; Swedish) and Fries (1874; Latin). *Bryobilimbia diapensiae* is distinguished by its habitat of moribund *Diapensia* and the presence of an olivaceous subhymenium.

- 1 On damp siliceous rocks 2
 Muscicolous on rocks, trees or soil 4
- 2(1) Ascospores 3.0–4.5(–6.0) µm wide (rare occurrences for this usually muscicolous species) **B. sanguineoatra**
 Ascospores (5–)6–7(–9) µm wide 3
- 3(2) Apothecia with brown pigments only **B. ahlesii var. ahlesii**
 Apothecia with brown and olivaceous green pigments **B. ahlesii var. nemoralis**
- 4(1) Mature apothecia forming large, blackberry-like clusters; exciple blue-black (K+ aeruginose). Terricolous in the southern cool temperate zone. **B. australis**
 Mature apothecia remaining single; exciple brown. Ecology and distribution various 5
- 5(4) Ascospores 4.5–6.0(–7.0) µm wide with a warted perispore, often 1-septate. Over bryophytes on calcareous rocks and trees **B. hypnorum**
 Ascospores narrow ellipsoid, <5 µm wide, smooth, simple. Over bryophytes on trees or directly on moribund *Diapensia* plants 6
- 6(5) Only brown apothecial pigments present. Growing over bryophytes, usually on trees but occasionally on siliceous rocks; rarely directly on bark or siliceous rock. Cool temperate to boreal **B. sanguineoatra**
 Subhymenium olivaceous-green. On moribund *Diapensia* in Arctic regions. **B. diapensiae**

Excluded Species

Mycobilimbia austrocalifornica (Zahlbr.) K. Knudsen

Opuscula Philolichenum 2: 36 (2005).—*Lecidea austrocalifornica* Zahlbr., *Cat. Lich. Univ.* 3: 738 (1925), nomen novum pro *Lecidea subplebeia* Nyl. in Hasse non *Lecidea subplebeia* Vain. (1890).

Lecidea subplebeia Nyl. in Hasse (non *Lecidea subplebeia* Vain.), *Bull. Torrey Bot. Club* 24: 447 (1897); type: USA, California, Los Angeles Co., Santa Monica Range, on earth, near Soldier's Home, November 1896, H. E. Hasse

s.n. (H-Nyl 12067—lectotype, designated by Knudsen 2005).

The type collection is *Carbonea latypizodes* (Nyl.) Knoph & Rambold and the other collections mentioned by Knudsen (2005) are *Placynthiella hyporhoda* (Th. Fr.) Coppins & P. James (C. Printzen, unpublished data).

In addition, the following species, which are traditionally included in the '*Lecidea hypnorum* group', are not here included in

Bryobilimbia. Our analysis shows that *Lecidea berengeriana* is not congeneric with *B. hypnorum*, and the other species are morphologically and anatomically closer to *Lecidea berengeriana*.

Lecidea berengeriana (A. Massal.) Nyl.

Lecidea berengeriana (A. Massal.) Nyl., *Not. Sällsk. Fauna Fl. Fenn. Förh.* **8**: 144 (1866).—*Biatora berengeriana* A. Massal., *Ric. auton. lich. crost.*: 128 (1852).—*Mycobilimbia berengeriana* (A. Massal.) Hafellner & V. Wirth, in Wirth, *Die Flechten Baden-Württembergs. Verbreitungsatlas*: 511 (1987).

Lecidea diplotypa Vain.

Lecidea diplotypa Vain., *Étud. class. lich. Brésil*, **11**: 30 (1890).—*Mycobilimbia diplotypa* (Vain.) Kalb, *Lichenes Neotropici*, Fascicle **IX** (nos 351–400) (Neumarkt): 11, no. 382 (1986).

Lecidea fissuriseda Poelt

Lecidea fissuriseda Poelt, *Mitt. Bot. Staatssamml. München* **4**: 181 (1961).—*Mycobilimbia fissuriseda* (Poelt) Poelt & Hafellner, in Hafellner, *Herzogia* **8**: 56 (1989).

Lecidea holopolia (Tuck.) Zahlbr.

Biatora holopolia Tuck, *Syn. N. Amer. Lich.* **2**: 26 (1888).—*Lecidea holopolia* (Tuck.) Zahlbr., *Cat. Lich. Univ.* **3**: 782 (1925).

Mycobilimbia olivacea Aragón, Sarrion & Hafellner, *Lichenologist* **35**: 3 (2003).

Lecidea strasseri Zahlbr.

Lecidea strasseri Zahlbr., *Verh. Zool.-Bot. Ges. Wien* **48**: 357 (1898).

Lecidea subfilamentosa (Zahlbr.) Fryday

Phyllopsora subfilamentosa Zahlbr., *Ann. Mycol.* **33**: 44 (1935).—*Fuscidea subfilamentosa* (Zahlbr.) Brako, in Egan, *Bryologist* **90**: 163 (1987).—*Lecidea subfilamentosa* (Zahlbr.) Fryday, *Lichenologist* **39**: 322 (2008).

Mycobilimbia meridionalis Kantvilas

Mycobilimbia meridionalis Kantvilas, *Lichenologist* **37**: 255 (2004).

Mycobilimbia parvilobulosa Aragón et al.

Mycobilimbia parvilobulosa Aragón, Sarrion & Hafellner, *Lichenologist* **35**: 6 (2003).

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REFERENCES

- Aptroot, A., Gilbert, O. L., Hawksworth, D. L. & Coppins, B. J. (2009) *Lecidea* Ach. (1803). In *The Lichens of Great Britain and Ireland* (C. W. Smith, A. Aptroot, B. J. Coppins, A. Fletcher, O. L. Gilbert, P. W. James & P. A. Wolseley, eds): 502–519. London: British Lichen Society.
- Arnold, F. (1882) Zur Erinnerung an F.X. Freiherr von Wulfen. *Verhandlungen des Zoologisch-Botanischen Vereins in Wien* **1882**: 143–166.
- Arup, U. (2004) Three overlooked *Lecidea* species in Sweden. *Symbolae Botanicae Upsalienses* **34**: 39–48.
- Buschbom, J. & Mueller, G. (2004) Resolving evolutionary relationships in the lichen-forming genus *Porpidia* and related allies (*Porpidiaceae*, Ascomycota). *Molecular Phylogenetics and Evolution* **32**: 66–82.
- Castresana, J. (2000) Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. *Molecular Biology and Evolution* **17**: 540–552.
- Coppins, B. J. & Fryday, A. M. (2006) Reassessment of some lichen species described by Josiah Lowe, and notes on some other North American lecidoid lichens. *Bryologist* **109**: 9–17.
- Foucard, T. (2001) *Svenska Skorplavaoch Svampar som Växer på Dem*. Stockholm: Interpublishing.
- Fries, T. M. (1874) *Lichenographia Scandinavica sive dispositio lichenum in Dania, Suecia, Norvegia, Fennia, Lapponia Rossica hactenus collectorum. Vol. I Archilichenes discocarpos continens. Pars II*. Upsaliae: Berling.
- Fryday, A. M. (2008) The genus *Fuscidea* (*Fuscideaceae*, lichenized Ascomycota) in North America. *Lichenologist* **40**: 295–328.
- Fryday, A. M. & Hertel, H. (2014) A contribution to the family *Lecideaceae* s. lat. (Lecanoromycetidae inc. sed., lichenized Ascomycota) in the southern subpolar region; including eight new species and some revised generic circumscriptions. *Lichenologist* **46**: in press.
- Gardes, M. & Bruns, T. D. (1993) ITS primers with enhanced specificity for basidiomycetes—application to the identification of mycorrhizae and rusts. *Molecular Ecology* **2**: 113–118.
- Hafellner, J. (1989) Die europäischen *Mycobilimbia*-Arten—eine erste Übersicht (lichenisierte Ascomycetes, Lecanorales). *Herzogia* **8**: 53–59.
- Hawksworth, D. L. & Coppins, B. J. (1992) *Lecidea* Ach. (1803). In *The Lichen Flora of Great Britain and Ireland* (O. W. Purvis, B. J. Coppins, D. L. Hawksworth, P. W. James & D. M. Moore, eds): 318–336. London: Natural History Museum Publications & British Lichen Society.

- Hertel, H. & Printzen, C. (2004) *Lecidea*. In *Lichen Flora of the Greater Sonoran Desert Region, Vol. 2* (T. H. Nash III, B. D. Ryan, P. Diederich, C. Gries & F. Bungartz, eds): 287–309. Tempe, Arizona: Lichens Unlimited, Arizona State University.
- Index Fungorum Partnership (2012) *Index Fungorum. A community resource*. Manaaki Whenua—Landcare Research, Lincoln, New Zealand & RBG Kew, Mycology, custodians. <http://www.indexfungorum.org>: accessed 23 January 2013.
- Kalb, K. & Hafellner, J. (1992) Bemerkenswerte Flechten und lichenicole Pilze von der Insel Madeira. *Herzogia* **9**: 45–102.
- Kantvilas, G., Messuti, M. I. & Lumbsch, H. T. (2005) Additions to the genus *Mycobilimbia* s. lat. from the Southern Hemisphere. *Lichenologist* **37**: 251–259.
- Knudsen, K. (2005) Lichens of the Santa Monica Mountains, Part One. *Opuscula Philolichenum* **2**: 27–36.
- Lanfear, R., Calcott, B., Ho, S. Y. W. & Guindon, S. (2012) PartitionFinder: combined selection of partitioning schemes and substitution models for phylogenetic analyses. *Molecular Biology and Evolution* **29**: 1695–1701.
- Liu, Y. L., Whelen, S. & Hall, B. D. (1999) Phylogenetic relationships among ascomycetes: evidence from an RNA polymerase II subunit. *Molecular Biology and Evolution* **16**: 1799–1808.
- Lumbsch, H. T. & Huhndorf, S. M. (2010) Outline of Ascomycota—2009. *Myconet* **14**: 1–42.
- Mackay, J. T. (1836) *Flora Hibernica, Comprising the Flowering Plants, Ferns, Characeae, Musci, Hepaticae, Lichenes and Algae of Ireland*. Dublin: W. Curry.
- Matheny, P. B., Liu, Y. J., Ammirati, J. F. & Hall, B. D. (2002) Using RPBI sequences to improve phylogenetic inference among mushrooms (*Inocybe*, *Agaricales*). *American Journal of Botany* **89**: 688–698.
- Meyer, B. (2002) Die Flechtengattung *Clauzadea*. *Sendtnera* **8**: 85–154.
- Meyer, B. & Printzen, C. (2000) Proposal for a standardized nomenclature and characterization of insoluble lichen pigments. *Lichenologist* **32**: 571–583.
- 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.
- Sarrión, F. J., Aragón, G., Hafellner, J., Rico, V. J. & Burgaz, A. R. (2003) Two new species of *Mycobilimbia* from Spain. *Lichenologist* **35**: 1–10.
- Schmull, M., Miądlikowska, J., Pelzer, M., Stocker-Wörgötter, E., Hofstetter, V., Fraker, E., Hodkinson, B. P., Reeb, V., Kukwa, M., Lumbsch, H. T., et al. (2011) Phylogenetic affiliations of members of the heterogeneous lichen-forming fungi of the genus *Lecidea sensu Zahlbruckner* (Lecanoromycetes, Ascomycota). *Mycologia* **103**: 983–1003.
- Silvestro, D. & Michalak, I. (2010) raxmlGUI: a graphical front-end for RAxML. *Organisms Diversity and Evolution* **12**: 335–337.
- Stamatakis, A. (2006) RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics* **22**: 2688–2690.
- Stiller, J. W. & Hall, B. D. (1997) The origin of red algae: implications for plastid evolution. *Proceedings of the National Academy of the United States of America* **94**: 4520–4525.
- White, T. J., Bruns, T., Lee, S. & Taylor, J. W. (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In *PCR Protocols: A Guide to Methods and Applications* (M. A. Innis, D. H. Gelfand, J. J. Sninsky & T. J. White, eds): 315–322. New York: Academic Press.
- Wirth, V. (1987) *Die Flechten Baden-Württembergs*. Stuttgart: Eugen Ulmer, GmbH & Co.
- Zhou, S. & Stanosz, G. R. (2001) Primers for amplification of mt SSU rDNA, and a phylogenetic study of *Botryosphaeria* and associated anamorphic fungi. *Mycological Research* **105**: 1033–1044.
- 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.