

Collema fasciculare belongs in *Arctomiaceae*

Mónica A. G. OTÁLORA and Mats WEDIN

Abstract: The phylogenetic placement of *Collema fasciculare* (L.) F. H. Wigg, the most deviating species within *Collema* (*Collemataceae*, Lecanoromycetidae), was studied using maximum likelihood and Bayesian analysis of three molecular loci (mitochondrial SSU rDNA, and the protein-coding, nuclear RPB1 and MCM7 genes). *Collema fasciculare* belongs to *Arctomiaceae* (*Ostropomycetidae*) forming a strongly supported monophyletic group with members of *Arctomia*. The spores, paraphyses, asci and hymenial reactions in *Collema fasciculare* are similar to other *Arctomia*, but the ascoma ontogeny is somewhat different. Also *C. leptosporum* Malme, another species in the *Fasciculare* group, shows similarities with *Arctomia* regarding spores and asci. *Arctomia* is morphologically heterogeneous and the genus is in need of revision. Until then, the species of the *Fasciculare* group fit best in *Arctomia* s. lat. and the combinations *Arctomia fascicularis* (L.) Otálora & Wedin, *Arctomia leptospora* (Malme) Otálora & Wedin, *Arctomia papuanarum* (Degel.) Otálora & Wedin and *Arctomia wviformis* (Hue) Otálora & Wedin are proposed.

Key words: *Arctomia*, ascoma ontogeny, *Collemataceae*, cyanolichens, lichens, taxonomy

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Introduction

During recent decades, tremendous progress has been made in understanding relationships among Ascomycota by using DNA sequence data (Lutzoni *et al.* 2004; Miądlikowska *et al.* 2006; Hibbett *et al.* 2007; Ekman *et al.* 2008). As a consequence, the classification of Ascomycetes has undergone substantial changes (Hibbett *et al.* 2007; Kirk *et al.* 2008; Lumbsch & Huhndorf 2009). In addition, the constant increase in the number of taxa in phylogenetic studies has also revealed the phylogenetic placement of sterile or enigmatic taxa. In the lichen-forming Ascomycetes associating with cyanobacteria (cyanolichens), several recent major taxonomic changes have been proposed (Lumbsch *et al.* 2005; Wedin *et al.* 2009; Otálora *et al.* 2010; Muggia *et al.* 2011; Spribille & Muggia 2012). Molecular phylogenies suggest that cyanolichens form three

distinct and distantly related lineages: *Arctomiaceae* (Lumbsch *et al.* 2005; Wedin *et al.* 2005) and the *Peltigerales* (Wedin *et al.* 2005; Miądlikowska *et al.* 2006) in the Lecanoromycetes, and *Lichinales* in the Lichinomycetes (Reeb *et al.* 2004).

Although the *Peltigerales* is by far the most species-rich cyanolichen group, it contains lineages that have not yet been well studied. One of these is the *Collemataceae* s. str., a group of distinctly gelatinous cyanolichens which comprises two large genera; *Collema* F. H. Wigg. with *c.* 80 currently accepted species, and *Leptogium* (Ach.) Gray with *c.* 180 species (Kirk *et al.* 2008). The detailed phylogenetic relationships within the *Collemataceae* are still unclear, despite some recent studies (Otálora *et al.* 2008, 2010; Wedin *et al.* 2009). Although these studies have confirmed that *Collema* and *Leptogium* are non-monophyletic, they have also shown that the traditional family delimitation (Henssen 1965; Henssen & Jahns 1974; Henssen *et al.* 1981) did not reflect the phylogenetic relationships. Most genera previously classified in *Collemataceae* were shown to belong to

M. A. G. Otálora and M. Wedin: Cryptogamic Botany, Swedish Museum of Natural History, P.O. Box 50007, SE-104 05 Stockholm, Sweden.
Email: monica.garcia.otalora@gmail.com

Pannariaceae (Wedin *et al.* 2009; Otálora *et al.* 2010), and currently only *Collema* and *Leptogium* constitute the *Collemataceae*.

Another group of cyanolichens that was earlier considered to belong to *Peltigerales* is *Arctomiaceae*. *Arctomiaceae* was originally proposed by Fries (1860), but never gained wide acceptance, and *Arctomia* Th. Fr. was included in *Collemataceae* by Zahlbruckner (1925). Henssen (1969), however, resurrected *Arctomiaceae*, based on differences in the ascoma development between *Arctomia* and *Collemataceae*. Molecular sequence data confirmed that *Arctomiaceae* is distantly related to *Collemataceae* and suggested that it belongs to Ostropomycetidae (Lumbsch *et al.* 2005; Wedin *et al.* 2005).

When studying the phylogenetic relationships within *Collemataceae* s. str., we soon found that the most deviating species regarding spore and apothecial characteristics within *Collema* [*C. fasciculare* (L.) F. H. Wigg.] not only grouped outside *Collemataceae*, but also outside the *Peltigerales*. Preliminary BLAST searches suggested similarities to *Arctomiaceae*. *Collema fasciculare* is a widely distributed, epiphytic species, forming rounded cushions usually up to c. 2 cm diam. (rarely up to 7 cm) with a crustose to subfoliose, indistinctly lobed thallus, which becomes swollen and gelatinous when wet. Similar to *Collema*, it associates with *Nostoc*, has a homoiomerous thallus organization without a true cortex, lecanorine apothecia with a persistent thalline exciple, and lacks secondary metabolites. However, it differs from other *Collema* species in some distinct ascomatal traits. The paraphyses and ascospores of *C. fasciculare* are comparatively more flexuous and thinner than in other *Collema* species (Degelius 1954, 1974), and the proper exciple is poorly developed or sometimes absent. Based on these differences, Degelius proposed the informal subgeneric Fasciculare group in which he included the widely distributed species *C. fasciculare* (Degelius 1954) and the three rare or poorly collected tropical species *C. uviforme* Hue, *C. papuanorum* Degel., and *C. leptosporum* Malme (Degelius 1974). Although the morphological differences between

the members of the Fasciculare-group and other *Collema* species have largely been recognized and accepted by other lichenologists (Filson 1992; Jørgensen 2007), no molecular studies have investigated the phylogenetic position of any member of this group.

The aim of this study is to investigate the phylogenetic placement of *C. fasciculare*. To achieve this we performed phylogenetic analyses, including representatives from the two lineages of Lecanoromycetes where cyanolichens are classified (*Peltigerales* and *Arctomiaceae*), based on sequences of the mitochondrial SSU rDNA and the protein coding genes RPB1 (RNA polymerase II subunit 1) and MCM7. In order to identify morphological characters that explain the phylogenetic placement of *C. fasciculare*, we also studied the ascus structure and the ascoma ontogeny. In addition, we studied material of one other species of the Fasciculare group, *C. leptosporum*, and of *Arctomia* s. str., for comparison.

Materials and Methods

Specimens and markers

We used the mtSSU rDNA, RPB1 and MCM7 loci from a total of 35 species of Lecanoromycetes. *Collema fasciculare* was represented by four specimens originating from different parts of the world (New Zealand, Spain and Sweden). The other species of the Fasciculare-group were not represented in the sampling due to the lack of recent material. Only the sequences of *C. fasciculare* were generated for this study, the rest were obtained from GenBank (Table 1). A total of 39 mtSSU rDNA, 37 RPB1 and 23 MCM7 sequences were included in this study.

Additional material examined. *Arctomia delicatula* Th. Fr.—**Sweden:** Torne Lappmark: Karesuando par., Pältsan (Bealccan). alt. 1110 m, 2011, M. Westberg P108 (S).

Collema leptosporum Malme.—**Brazil:** Mato Grosso: Corumbá, in silva clararegionis calcariae, 14 viii 1894, Malme [Malme Lich. austr. amer. Exs. No 70] (S).

DNA sequencing

Total genomic DNA was extracted using DNeasy (Qiagen) according to the manufacturer's instructions. The mtSSU rDNA was amplified using the primers mtSSU1 and mtSSU3R (Zoller *et al.* 1999); the protein coding gene RPB1 using primers RPB1-Af (Stiller & Hall 1997) and RPB1-6R1asc (Hofstetter *et al.* 2007);

TABLE 1. List of taxa included in this study and GenBank accession numbers. New sequences are indicated in bold

| Species | | GenBank accession number | | |
|----------------------------------|----------------------------------|--------------------------|-----------------|-----------------|
| | | mtSSU | RPB1 | MCM7 |
| <i>Arctomia borbonica</i> 1 | | JX030032 | JX030034 | – |
| <i>A. borbonica</i> 2 | | JX030033 | JX030035 | – |
| <i>A. delicatula</i> | | AY853307 | DQ870929 | GQ272388 |
| <i>A. interfixa</i> | | DQ007348 | – | – |
| <i>A. teretiusscula</i> | | DQ007349 | DQ870930 | GQ272389 |
| <i>Aspicilia caesiocinerea</i> | | DQ780271 | DQ870931 | GQ272390 |
| <i>A. cinerea</i> | | DQ780272 | DQ870932 | GQ272391 |
| <i>Coccotrema cucurbitula</i> | | AF329161 | DQ870939 | GU980990 |
| <i>C. pocillarium</i> | | AF329166 | DQ870940 | GU980992 |
| <i>Collema fasciculare</i> 1 | Spain, Aragón & Martí. 3417 (MA) | KC118988 | KC118991 | KC118995 |
| <i>C. fasciculare</i> 2 | Sweden, Karström 562 (UPS) | KC118987 | KC118993 | KC118997 |
| <i>C. fasciculare</i> 3 | Spain, Aragón 1041/95 (MA) | KC118989 | KC118992 | KC118996 |
| <i>C. fasciculare</i> 4 | New Zealand, Wedin 8753 (S) | KC118990 | KC118994 | KC118998 |
| <i>C. fufuraceum</i> | | EU982567 | GQ259048 | JX992982 |
| <i>C. nigrescens</i> | | EU982563 | GQ259049 | JX992989 |
| <i>Diploschistes scruposus</i> | | AY584692 | DQ870943 | JX000163 |
| <i>Graphis scripta</i> | | AY853322 | DQ870947 | JX000164 |
| <i>Gregorella humida</i> | | AY853329 | DQ870946 | – |
| <i>Lecanora paramerae</i> | | EF105418 | DQ870950 | – |
| <i>Lepraria lobificans</i> | | DQ986887 | DQ986837 | – |
| <i>Leptogium lichenoides</i> | | DQ923120 | DQ917414 | JX993021 |
| <i>L. saturninum</i> | | EU982569 | GQ259064 | JX992985 |
| <i>Lobaria pulmonaria</i> | | AY340504 | DQ915591 | JX000169 |
| <i>Ochrolechia parella</i> | | GU980977 | DQ870959 | GQ272421 |
| <i>Pannaria rubiginosa</i> | | AY340513 | GQ259074 | JX993042 |
| <i>Peltigera aphthosa</i> | | AY340515 | DQ915598 | JX000176 |
| <i>Pertusaria amara</i> | | AY300900 | DQ870965 | GQ272423 |
| <i>P. corallina</i> | | AY300901 | DQ870967 | GU980997 |
| <i>Placynthium nigrum</i> | | EU982566 | GQ259079 | – |
| <i>Protopannaria pezizoides</i> | | AY340519 | GQ259081 | – |
| <i>Protothelenella corrosa</i> | | AY607746 | DQ870988 | – |
| <i>P. sphinctrinoidella</i> | | AY607747 | DQ870989 | – |
| <i>Ramalina complanata</i> | | DQ972986 | DQ973059 | – |
| <i>Rhizocarpon sphaerosporum</i> | | AY853340 | DQ870991 | – |
| <i>Schaereria corticola</i> | | AY300909 | DQ870993 | – |
| <i>Sphaerophorus globosus</i> | | DQ986866 | DQ986836 | JX000185 |
| <i>Thelenella antarctica</i> | | AY607749 | – | – |
| <i>T. muscorum</i> | | AY607743 | FJ941910 | – |
| <i>Warwea fruticulosa</i> | | DQ871023 | DQ871005 | – |

and MCM7 using the primers MCM7-709 and MCM7 1348 (Schmitt *et al.* 2009). PCR amplifications were performed using Illustra™ Hot Start PCR beads, according to the manufacturer's instructions. PCR-reactions were performed using the following cycling parameters: initial denaturation 95°C for 5 min, and 35 (mtSSU rDNA) or 40 (MCM7 and RPB1) cycles at

95°C for 1 min, 56°C for 50 s (mtSSU rDNA and RPB1) or 54°C for 1 min (MCM7), and 72°C for 1 min; this was followed by a final extension at 72°C for 8 min. PCR products were subsequently purified using the enzymatic method Exo-sap-IT provided by USB Corporation. Unidirectional dye-terminated sequencing for the forward and reverse reactions using the same

PCR primers were performed on an ABI 377 automated sequencer using Big Dye Terminator technology (Applied Biosystems, Warrington).

Phylogenetic methods

Alignments were constructed separately for each locus with the online version of MAFFT 6 using the auto option (Katoh & Toh 2008). In the case of mtSSU rDNA, ambiguous regions were delimited using GUIDANCE web service with the default cut-off of 0.95 (Penn *et al.* 2010). The alignments for the protein coding genes were adjusted in MacClade 4.01 (Maddison & Maddison 2001), utilizing the amino acid translations. Delimited ambiguous regions and introns were excluded from the phylogenetic analyses.

Phylogenetic relationships and confidence were inferred with a maximum likelihood (ML) and Bayesian approach (MB). The combinability of the single locus data sets was assessed by visual inspection of the individual bootstrap values (Mason-Gamer & Kellogg 1996; Wiens 1998). A conflict was considered significant when two data partitions supported conflicting monophyletic groups with ML bootstrap values $\geq 70\%$ in both trees. Because no significant conflicts were detected, it was assumed that the two data sets were congruent and could be combined. We constructed two different combined data sets, first a two-locus data set (mtSSU rDNA+RPB1) with two taxa having missing data for RPB1, and second a three-locus (mtSSU rDNA+RPB1+MCM7) dataset with 16 taxa having missing data for MCM7. Maximum likelihood analyses were performed on the two combined data sets using RAxML-HPC2 (Stamatakis 2006; Stamatakis *et al.* 2008) and implementing a GTRCAT model for the bootstrapping phase, and GTRGAMMA for the final tree inference using 4 and 7 partitions for the 2-locus data set and the 3-locus data set, respectively (three codon positions for each RPB1 and MCM7). In addition, a bootstrap analysis of 1000 pseudoreplicates was performed for each data set. The analyses were performed on the Cipres Web Portal (Miller *et al.* 2010).

The evolutionary models for Bayesian analyses were selected using the Bayesian Information Criteria (BIC), as implemented in JModeltest 3.06 (Posada 2008). The GTR+G model was used for mtSSU and RPB1 first position; SYM+G for RPB1 second position and MCM7 first and second position; and HKY+I+G for RPB1 third position and MCM7 third position. The Bayesian analyses were performed using MrBayes 3.2.0 (Ronquist *et al.* 2011). Two runs of 4 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 was reached and the runs converged at the same log-likelihood levels, we plotted the log-likelihood scores of sample points against generation time using Tracer 1.5 (Rambaut & Drummond 2007). The first 10 000 trees from each run were discarded as 'burn-in'. For the remaining trees in each analysis, a majority-rule consensus tree was assembled using the sumt option of MrBayes. The .con file created by MrBayes was visualized in FigTree V.1.3.1 (Rambaut 2009).

Morphological study

For the ontogenetic study, serial sections through thallus parts with different apothecial stages were prepared using a freezing microtome. The sections were mounted in lactophenol cotton blue and studied by light microscopy. Asci were studied on material mounted in water using hand-cut sections, before and after staining with Lugol's iodine solution, with and without pretreatment with 10% KOH.

Results

Twelve new sequences were generated in this study, four for each of the genes. These new sequences were aligned with 83 sequences obtained from GenBank (Table 1). After excluding 514 ambiguously aligned sites, the 3-locus data set contained 1807 sites, of which 984 were variable. Of the variable sites, 338 belonged to the mtSSU partition, 367 to RPB1 and 279 to the MCM7. In the 2-locus data set, the entire MCM7 partition was excluded. Maximum likelihood analyses resulted in a single most likely tree with ln-likelihood of -15922.3654 for the 2-locus and -21191.7847 for the 3-locus data sets. The majority rule consensus trees from the Bayesian analyses were based on 60 000 credible trees from two runs for each one of the data sets. The harmonic mean ln-likelihood was -15985.49 for the 2-locus data set and -21228.13 for the 3-locus data set. The tree topologies obtained from the 2-locus and 3-locus data sets did not show any significant conflict in either of the two methods of analysis, but the 3-locus data set resulted in lower branch support. Only the best tree from the maximum likelihood analysis of the 2-locus analysis is thus presented here, with both bootstrap support and posterior probabilities from both data sets (Fig. 1).

Morphological study

The earliest stages in the ascomatal development that we found were young primordia situated under the thallus surface in shallow thallus outgrowths (Fig. 2A). We did not see any stage corresponding to the outgrowth of generative tissue typical for the *Arctomiaceae* (Henssen 1969; Henssen & Kantvilas 1985;

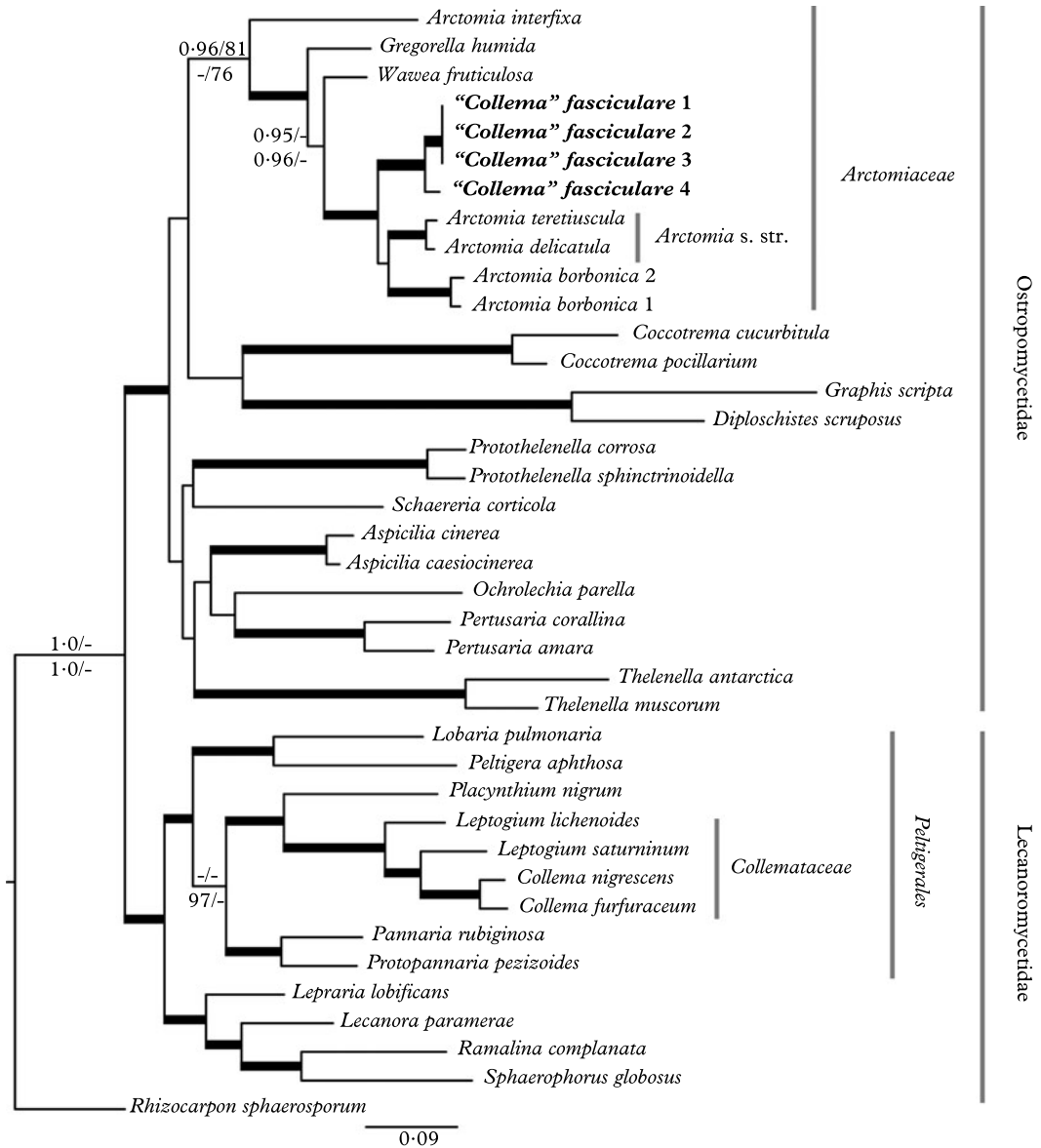


FIG. 1. Phylogenetic relationships of cyanobacterial lichens within the Lecanoromycetes. Support values above branches correspond to the values obtained from the 2-locus data set, and support values below branches correspond to the ones obtained from the 3-locus data set (mtSSU rDNA+RPB1+MCM7). Thicker internodes indicate significant support in the two data sets for the two phylogenetic methods (PP > 0.95, ML BS > 70). *Collema fasciculare* constitutes a monophyletic group sister to the rest of *Arctomia* s. str., within a monophyletic *Arctomiaceae* in the Ostropomycetidae.

Lumbsch *et al.* 2005), despite several attempts, although we cannot rule out that such a stage occurred but quickly became overgrown by thallus tissue. When the pri-

mordium grows and reaches the thallus surface (Fig. 2B & C), the upper surface disintegrates and a simple, very thin primordium wall is differentiated (Fig. 2C & D). This

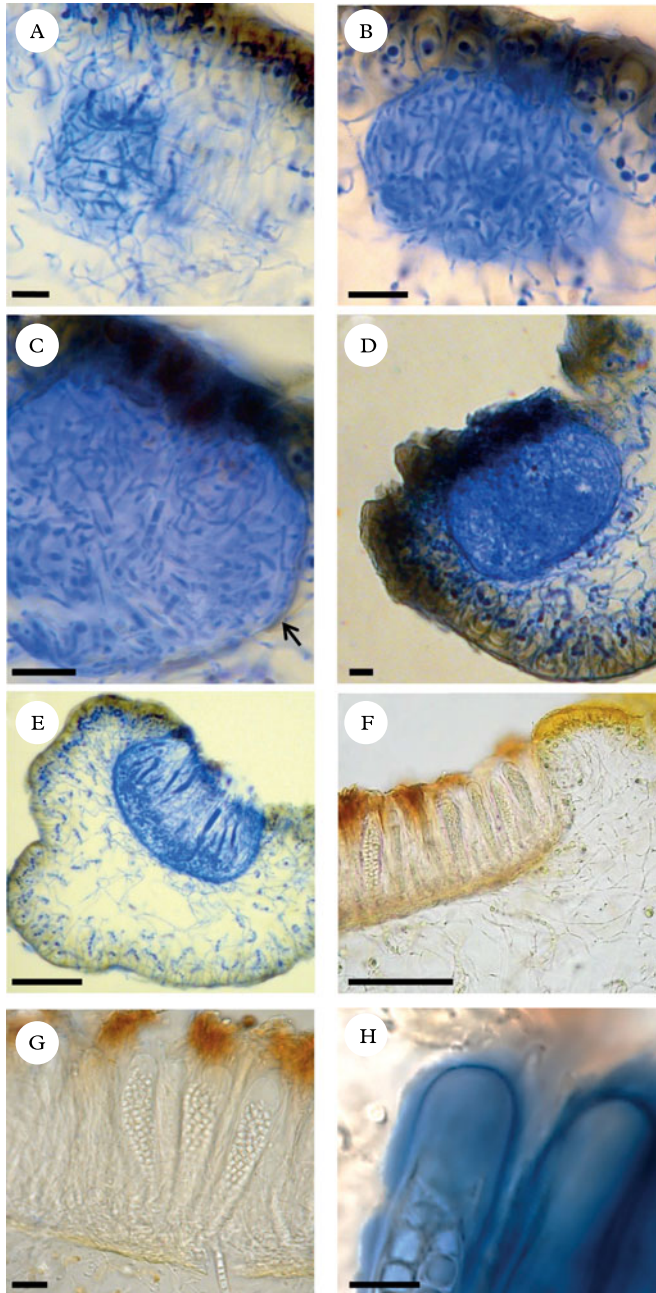


FIG. 2. Ascoma development in *Collema fasciculare* [Karström 562 (UPS)]. A, young primordium situated under the thallus surface; B, somewhat older primordium reaching the thallus surface; C, older primordium where a thin, slightly pigmented wall is differentiated (arrow); D, older primordium where the upper surface is disintegrating during the development; E, older stage showing young asci and young paraphyses; F, marginal part of mature apothecium with pigmented epihymenium and thalline exciple visible; G, detail showing the proper exciple, asci and ascospores; H, detail of asci in Lugol's iodine solution after pre-treatment with KOH. Scales: A–D, G & H = 10 μ m; E & F = 100 μ m.

wall is composed of thin-walled, slightly pigmented hyphae (Fig. 2C), which in the mature stage form the thin proper exciple (Fig. 2E–G). When the developing hymenium is exposed, asci and paraphyses are visible (Fig. 2E), and at the mature stage (Fig. 2F), an indistinct, thin thalline exciple is present. The paraphyses are thin (1.0–1.5 µm) and lax, branched, and distinctly brown-pigmented at the apices (Fig. 2G) which are slightly swollen. The ascus wall is I+ reddish to blue, KOH/I+ blue, and the tholus contains no amyloid structures (Fig. 2H). It contains eight long (*c.* 65–170 µm) and thin (*c.* 4.5–5.0 µm) spores formed by 10–17 cells (Fig. 2G & H).

We also studied sections of *C. leptosporum*, for comparison. In *C. leptosporum*, the proper exciple is distinct, euparaplectenchymatous and thick (70–105 µm); the paraphyses are 1.5–2.0 µm thick, simple and slightly swollen at the apices. *Collema leptosporum* has ascus characteristics similar to *C. fasciculare*, and the spores are also long (115–160 µm) and thin (2–4 µm), formed by 6–8 cells.

Discussion

The two cyanolichen lineages within Lecanoromycetes are both recovered here as monophyletic. *Arctomiaceae* formed a weakly-supported clade within Ostropomycetidae, and *Peltigerales* formed a well-supported clade within Lecanoromycetidae (Fig. 1). These results agree with previous Lecanoromycete studies (Lumbsch *et al.* 2005, 2007; Wedin *et al.* 2005). The monophyly of *Arctomiaceae* is well supported in the 2-locus analysis, but lacks Bayesian support in the 3-locus analysis. This is probably due to the large amount of missing data in the MCM7 data set. The backbone of the topology within Ostropomycetidae lacks support, which makes the detailed relationships of *Arctomiaceae* within this group uncertain. All analyses supported the monophyly of the four specimens of *C. fasciculare* with high confidence (1.0 PP, 100 ML) as a member of the monophyletic *Arctomiaceae*. *Arctomia delicatula*, *A. borbonica* Magain & Sérus., *A. teretiusscula*

P. M. Jørg. and *C. fasciculare* form a well-supported monophyletic group. *Collema fasciculare* is thus suggested here to belong to *Arctomia*. *Arctomia* was previously considered monophyletic (Lumbsch *et al.* 2005) but in our investigation it is paraphyletic, with *A. interfixa* (Nyl.) Vain. as sister to the rest of *Arctomiaceae*, including *Gregorella* Lumbsch and *Wawea* Henssen & Kantvilas. This is similar to the result of Magain & Sérusiaux (2012), who considered *A. interfixa* to be of uncertain position within *Arctomiaceae*, and who interpreted the paraphyly of *Arctomia* as the result of an incomplete data set. It should be noted that we too have no data of MCM7 and RPB1 for *A. interfixa*, and therefore this relationship is also uncertain in our investigation. The phylogenetic relationship between *Gregorella humida* and *Wawea fruticulosa* is also uncertain. Lumbsch *et al.* (2005) and Magain & Sérusiaux (2012) found them to form a well-supported monophyletic group, but neither our study nor Lumbsch *et al.* (2007) supported that relationship. The fact that *Arctomiaceae* is monophyletic including *Collema fasciculare* is, however, beyond doubt.

Degelius previously (1954: 103) suggested that, in view of the different type of ascospores and paraphyses, *C. fasciculare* should better be recognized as a separate genus. This recognition was never done, probably because of the many characteristics that this species shares with various species of *Collema*. For instance, *C. fasciculare* is similar to *C. leptaleum* Tuck. regarding habit. The anatomical characteristics of the thallus are similar to *C. multipartitum* Sm. and *C. multipartitatum* Degel., and the apothecial habit is similar to several other *Collema* species (Degelius 1954, 1974; Jørgensen 2007).

The ascoma ontogeny was the criterion used by Henssen (1969) to delimit the *Arctomiaceae*. The ontogeny had not been studied earlier in *C. fasciculare*, but our study suggested that there actually are distinct differences compared with other *Arctomiaceae*. Henssen (1969) excluded *Arctomia* from *Collemataceae* because of the distinct external outgrowth of generative tissue in which asconogonia develop, in *Arctomia*, and because the

whole ontogeny thus took place above the surface of the thallus proper. In *Collema*-*ceae* s. str., the development of the ascoma commences within the thallus (beneath the upper surface). Studies after Henssen (1969) have likewise found that the ontogeny in other *Arctomiaceae* starts with an outgrowth of generative tissue (Henssen & Kantvilas 1985; Lumbsch *et al.* 2005). We have no indications that such an outgrowth is produced in *C. fasciculare*, and as far as we have been able to find, the whole ontogeny in *C. fasciculare* takes place within the thallus beneath the upper surface (Fig. 2A), and the resulting apothecia have a distinct thalline excipulum (Fig. 2F).

Even if the ascoma ontogeny deviates somewhat from the typical *Arctomiaceae*, there are other important similarities. *Collema fasciculare* is similar to other *Arctomia* in the spores and paraphyses, the ascus structure, and in the hymenium type. The hymenial gel in *C. fasciculare* is hemi-amyloid, as in *Arctomia*. *Collema fasciculare* possesses asci with a non-amyloid tholus, similar to the *Trapelia*-type present in some *Arctomiaceae* (Fig. 2H). This ascus type lacks the typical internal amyloid tube structure present in *Collema*. The ascospores of *C. fasciculare* are further similar to the fusiform, multiseptate ascospores in *A. delicatula* and *A. teretiusscula*. Finally, the paraphyses in *C. fasciculare* are also similar to those in *Arctomia*.

Arctomia as circumscribed by Henssen (1969) and Jørgensen (2003), includes crustose-squamulose, semi-gelatinous cyanolichens with a single-layered cortex, biatorine apothecia and fusiform, multiseptate ascospores. It could be argued that the putative differences in ascoma ontogeny, and the fact that *C. fasciculare* is sister to *Arctomia* s. str., would be a reason to accept it as a distinct genus. Several rather deviating new species have been accepted in *Arctomia* lately, however, and the genus clearly needs a full revision, something that is beyond the scope of this study. Øvstedal & Gremmen (2001, 2006) described two *Arctomia* species which have relatively small, ellipsoid ascospores, with 3 transverse septa, which are different

to the ones in *Arctomia* s. str. and *C. fasciculare*. In fact, the authors claimed that these two species do not belong in *Arctomia* s. str., and that they should be studied further. Recently, Magain & Sérusiaux (2012) included the new species *A. borbonica*, which also deviates from the typical *Arctomia* species by producing goniocysts at the lobe margins and by having a thallus that, in general, is similar to the thallus in *Leptogium*. No apothecia are hitherto known from *A. borbonica*.

Even if including *C. fasciculare* in *Arctomia* results in a yet more morphologically heterogeneous genus, we believe that this is currently a better option than describing a new genus, as the family now is in desperate need of a thorough revision of the generic delimitations. *Collema leptosporum* also has asci and ascospores similar to *Arctomia* and to *C. fasciculare*. This suggests that *C. leptosporum* does not belong to *Collema* and it could be related to *Arctomiaceae*, although the proper exciple and paraphyses differ considerably from other *Arctomia* and from *C. fasciculare*. We feel, however, that *C. leptosporum* fits considerably better in *Arctomia* than in *Collema*. Finally, we suggest that the remaining two species of the Fasciculare-group (which we have not studied material of) should also be better placed in *Arctomia*, as it is clear from the literature (Degelius 1974) that they share important characteristics with *C. fasciculare* and *C. leptosporum*, particularly the *Arctomia*-type spores.

Nomenclature

***Arctomia fascicularis* (L.) Otálora & Wedin comb. nov.**

Mycobank No.: MB802158

Basionym: *Lichen fascicularis* L., *Mantissa Pl.* 1: 133 (1767).

***Arctomia leptospora* (Malme) Otálora & Wedin comb. nov.**

Mycobank No.: MB802161

Basionym: *Collema leptosporum* Malme, *Ark. f. Bot.* 19 (8): 6 (1924).

Arctomia papuanarum (Degel.) Otálora & Wedin comb. nov.

Mycobank No.: MB802165

Basionym: *Collema papuanorum* Degel., *Symb. Bot. Upsal.* 20 (2): 196 (1974).

Arctomia uviformis (Hue) Otálora & Wedin comb. nov.

Mycobank No.: MB802168

Basionym: *Collema uviforme* Hue, *Bull. Soc. Bot. France* 63 (28): 5 (1916).

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