

New insights into classification and evolution of the Lecanoromycetes (Pezizomycotina, Ascomycota) from phylogenetic analyses of three ribosomal RNA- and two protein-coding genes

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Abstract: The Lecanoromycetes includes most of the lichen-forming fungal species (>13 500) and is therefore one of the most diverse class of all Fungi in terms of phenotypic complexity. We report phylogenetic relationships within the Lecanoromycetes

resulting from Bayesian and maximum likelihood analyses with complementary posterior probabilities and bootstrap support values based on three combined multilocus datasets using a supermatrix approach. Nine of 10 orders and 43 of 64 families currently recognized in Eriksson's classification of the Lecanoromycetes (Outline of Ascomycota—2006 Myconet 12:1–82) were represented in this sampling. Our analyses strongly support the Acarosporomycetidae and Ostropomycetidae as monophyletic, whereas the delimitation of the largest subclass, the Lecanoromycetidae, remains uncertain. Independent of future delimitation of the Lecanoromycetidae, the Rhizocarpaceae and Umbilicariaceae should be elevated to the ordinal level. This study shows that recent classifications include several nonmonophyletic taxa at different ranks that need to be recircumscribed. Our phylogenies confirm that ascus morphology cannot be applied consistently to shape the classification of lichen-forming fungi. The increasing amount of missing data associated with the progressive addition of taxa resulted in some cases in the expected loss of support, but we also observed an improvement in statistical support for many internodes. We conclude that a phylogenetic synthesis for a chosen taxonomic group should include a comprehensive assessment of phylogenetic confidence based on multiple estimates using different methods and on a progressive taxon sampling with an increasing number of taxa, even if it involves an increasing amount of missing data.

Key words: Bayesian inference, Lecanoromycetes, lichen-forming ascomycetes, maximum likelihood, missing data, mitochondrial ribosomal small subunit (mitSSU), molecular phylogenetic classification, nuclear ribosomal large subunit (nuLSU), nuclear ribosomal small subunit (nuSSU), phenotypic characters, photobionts, phylogenetic confidence, phylogeny, RNA polymerase II largest subunit (*RPB1*), RNA polymerase II second largest subunit (*RPB2*)

INTRODUCTION

The Lecanoromycetes, as recognized in Eriksson's (2006) classification, is the largest class of Fungi. It includes the majority (about 90%) of all described lichen-forming Ascomycota (estimated to be > 13 500 species, Kirk et al 2001). A common character uniting members of this class is their ascohymental ascomatal ontogeny, with a predominance of apothecial fruiting bodies, although of diverse construction and shape. Perithecioid ascomata are known in only four of 64 families (Grube et al 2004, Schmitt et al 2005) of Eriksson's (2006) classification and in a few unclassified genera. In most lineages asci have a multilayered

ascal wall of which two layers are thick enough to be visible with light microscopy and display different types of dehiscence (predominantly rostrate but also semi-fissitunicate or bilabiate), however some members (calicioid lichen-forming fungi, such as *Calicium* and *Sphaerophorus*) produce asci with a single evanescent layer (Luttrell 1955; Eriksson 1981; Reynolds 1981, 1989; Tibell 1984; Hafellner 1988).

Members of the Lecanoromycetes form bipartite symbiotic associations with a broad range of photobionts, representing chlorococcalean algae (*Asterochloris*, *Coccomyxa s.l.*, *Dictyochloropsis s.l.* and *Trebouxia*, are the most frequent genera), filamentous algae (*Trentepohlia*, *Phycopeltis*) and cyanobacteria (*Calothrix*, *Nostoc*, *Scytonema* and *Stigonema*) (e.g. Tschermak-Woess 1988; Rikkinen 1995; Beck et al 1998, 2002; Rambold et al 1998; Peršoh et al 2004; Cordeiro et al 2005). Tripartite symbioses with cyanobacteria as the secondary photobiont (in terms of relative abundance in mature thalli) occur in several unrelated genera within the Lecanoromycetes, however they are particularly common in peltigeralean lichens (Peltigerales). Rambold et al (1998) suggested that photobionts associated with lichen-forming fungi could be used in lichen systematics. These authors detected a strong selectivity of mycobionts with respect to their photobionts at the rank of families and genera in the order Lecanorales.

Most members of the Lecanoromycetes are known to produce a wide variety of unique secondary compounds, especially polyketide derivatives (such as depsides and depsidones, anthraquinones and xanthenes) terpenes and pulvinic acid derivatives (e.g. Elix 1996). These chemical compounds are of biological and ecological importance (especially if present in the upper cortex of lichen thalli; e.g. Rundel 1978, Lawrey 1986, Solhaug and Gauslaa 1996, Pöykkö et al 2005) and of systematic (e.g. Elix 1993, Culberson and Culberson 1994, Schmitt and Lumbsch 2004) and evolutionary significance (e.g. Culberson 1986).

Circumscription and ranking of subgroups within the Lecanoromycetes varied in previous classifications, and the last major improvement before molecular phylogenetic studies was derived from the study of reproductive structures, in particular the apical structures of asci. These characters were used to delineate groups of lichen-forming fungi and resulted in a high number of families in the Lecanorales (Hafellner 1984). Because of controversy associated with the uniform implementation of these characters to circumscribe families across the Lecanorales (e.g. Timdal 1991) many families were redefined (e.g. Rambold and Triebel 1992, Hafellner 1993).

Molecular studies have substantially challenged

phenotypically based groupings applied to previous classifications, as well as resolved placement of many sterile taxa, and taxa with uncertain taxonomic affiliation. The Lecanoromycetes include a minimum of three subclasses, the Acarosporomycetidae, Ostropomycetidae and Lecanoromycetidae, according to Reeb et al (2004) and Lutzoni et al (2004). The Acarosporomycetidae encompasses a single family, the Acarosporaceae, defined in most cases by the presence of a crustose or squamulose thallus, a chlorococcoid photobiont (*Trebouxia*), apothecia of various structures, generally more than a hundred simple spores per ascus, and functionally unitunicate ascus with non- or slightly amyloid tholus and ocular chamber. The Ostropomycetidae includes lichenized and nonlichenized fungi (including lichenicolous taxa, Lücking et al 2005) with crustose, squamulose and filamentous thalli, trentepohlioid and chlorococcoid photobionts, ascomata of apothecial or perithecial type, eight or fewer spores per ascus and functionally unitunicate asci. Eriksson (2006) recognizes five orders in this subclass, Agyriales (two families), Gyalectales (two families), Ostropales (seven families), Pertusariales (three families) and Trichotheliales (two families). The subclass Lecanoromycetidae currently (Eriksson 2006) accommodates three recognized orders: Lecanorales (29 families), the most speciose group of the Lecanoromycetes; Peltigerales (seven families); and Teloschistales (three families). Six families (Brigantiaceae, Elixiaceae, Fuscideaceae, Phlyctidaceae, Umbilicariaceae and Vezdaeaceae) are of uncertain position within the Lecanoromycetidae and 30 genera could not be placed with certainty in any of the three existing subclasses of the Lecanoromycetes, according to Eriksson (2006). All members of this largest subclass within the Lecanoromycetes are discomycetes with apotheciate fruiting bodies and most species have chlorococcoid or cyanobacterial (in Peltigerales) primary photobionts. The lichenicolous living strategy (lichenized and nonlichenized fungi growing on lichens) is found in many groups of the Lecanoromycetidae, whose members also serve frequently as hosts for other such fungi (e.g. Clauzade et al 1989, Rambold and Triebel 1992, Kirk et al 2001, Lawrey and Diederich 2003).

Many recent phylogenetic studies have explored relationships within the Lecanoromycetes to evaluate delimitations of particular taxa and less frequently the validity of diagnostic features (especially ascomata and ascus characters) used to circumscribe taxa (e.g. Grube et al 2004, Schmitt et al 2005, Wedin et al 2005). Most of these studies were based on different combinations of two or three nuclear ribosomal genes (i.e. nucSSU, nucLSU and mitochondrial ribosomal

small subunit [mitSSU]) (e.g. Lumbsch et al 2001, 2004a; Lutzoni et al 2001; Ekman and Tønsberg 2002; Kauff and Lutzoni 2002; Lumbsch 2002; Lücking et al 2004; Wedin et al 2005) with only four phylogenetic studies using at least one protein coding gene (*RPB2*: Liu and Hall 2004, Lutzoni et al 2004 and Reeb et al 2004; *RPB1* and *RPB2*: Hofstetter et al 2007). Hofstetter et al (2007) evaluated the phylogenetic contribution (resolving power and statistical confidence) provided by protein-coding (*RPB1* and *RPB2*) and ribosomal RNA-coding (nucSSU, nucLSU and mitSSU) loci in a phylogenetic study of 82 members of the Lecanoromycetes. This study provided a robust phylogenetic framework and useful guidance for selecting loci in future multilocus studies on Lecanoromycetes and Pezizomycotina in general.

Two studies, Lumbsch et al (2004a) and Wedin et al (2005), were designed specifically to reconstruct phylogenetic relationships within the Lecanoromycetes at the family and higher levels as a framework for the evaluation of existing classifications. Although these studies, as well as Lutzoni et al (2004, 83 taxa using nucSSU+nucLSU), substantially increased taxon sampling compared to previously published two-gene phylogenies, many internodes including deep relationships among major groups in the Lecanoromycetes remained poorly supported when using ribosomal genes exclusively. Nevertheless they convincingly argued that ascus and ascoma characters should not be applied consistently to the same hierarchical levels across the Lecanoromycetes. Wedin et al (2005) also provided an overview of the recent major phylogenetic analyses of the Lecanoromycetes. A recent overview of coexisting classifications of the Lecanoromycetes at the order level also can be found in Lumbsch et al (2004a).

The main objectives of this study were to (i) increase significantly both taxon and character sampling to diminish phylogenetic uncertainty within the Lecanoromycetes, (ii) evaluate Eriksson's classification (2006) at the family and higher ranks, (iii) resolve the phylogenetic placement of taxa with unknown affinities and (iv) revisit the distribution and evolution of selected phenotypic characters (including photobionts and ascus structure) across the major groups within the Lecanoromycetes and their utility in lichen systematics.

Using a supermatrix approach we assembled three datasets with a progressively higher number of taxa and missing data. Internodal support estimated with maximum likelihood bootstrap (with RAxML and PHYML) and Bayesian posterior probabilities (with MrBayes) are compared and discussed in the context of missing data and phylogenetic reconstructions.

MATERIALS AND METHODS

Because of space limitation associated with this issue of *Mycologia*, this section is presented in SUPPLEMENT 1 (<http://www.mycologia.org>).

RESULTS AND DISCUSSION

Phylogenetic reconstructions and confidence.—Missing data in the 5+4-gene supermatrix (26%) and the 5+4+3-gene supermatrix (37%) datasets overall did not have a negative affect on phylogenetic resolution and support when using maximum likelihood (RAxML) and Bayesian methods (MrBayes) (see also Wiens 2006). However noticeably lower bootstrap values for several nodes were obtained from PHYML analyses on the 5+4-gene and 5+4+3-gene datasets (FIG. 1, second column vs. first and third columns of grid showing support for each internode). All phylogenies were concordant with the tree based on the most complete 5-gene dataset (the 5+4+3-gene tree is shown in FIG. 1). Only a few branches that were highly supported in the 5-gene phylogeny received no significant support (based on two or all three methods) in the 5+4-gene or 5+4+3-gene reconstructions (e.g. the monophyly of the group delimited by Parmeliaceae and Mycoblastaceae in the Lecanorales, FIG. 1). Adding taxa with missing data to the 5-gene and 5+4-gene datasets often improved phylogenetic confidence (e.g. the monophyly of the Collematineae and the Pertusariaceae). Comparing the three methods used to estimate phylogenetic confidence, we found that support provided by MrBayes generally was congruent with RAxML bootstrap values, whereas PHYML seems to require more data (less efficient) than the other two methods to provide significant support values and seems the least stable as the number of taxa and missing data increased.

Acarosporomycetidae/Acarosporales.—The phylogenetic distinctiveness of the Acarosporaceae was shown by Reeb et al (2004), who suggested recognizing this family at the subclass level (Acarosporomycetidae). This result was confirmed by Lutzoni et al (2004), Miadlikowska and Lutzoni (2004), Hofstetter et al (2007) and this study. In agreement with Reeb et al (2004) neither *Acarospora* nor *Sarcogyne* are monophyletic (FIG. 1). In our analyses *Polysporina* (*P. simplex*) diverged earlier than *Pleopsidium* and the remaining genera of the Acarosporales, a significant result based on all nine support values (but see Wedin et al 2005). *Pleopsidium* (FIG. 2), with *Lecanora*-type asci and ascospores that resemble those of *Lecanora*, was expected to be closely related to *Lecanora* (Hafellner 1993). The Acarosporales represent a strong case where taxa that appear phylogenetically

closely related have very different types of asci and differ considerably also in other characters such as hamathecium and secondary chemistry (including *Timdalia*, a member of the Acarosporaceae in Wedin et al 2005).

Candelariomycetidae/Candelariales.—One of the most surprising outcomes of all three dataset studies (although the strongest support came from the 5-gene analyses) is the placement of *Candelariella* (FIG. 3) and *Candelaria* (former Candelariaceae, Hakulinen 1954) outside the Lecanorales and Lecanoromycetidae (FIG. 1). Owing to the ascus type these genera often were considered close relatives of the Lecanoraceae and currently are classified in this family (Eriksson 2006). This unexpected placement of Candelariaceae also was found and discussed by Wedin et al (2005) and Hofstetter et al (2007), although in the latter study the Candelariaceae is strongly supported as the first phylogenetic split before the divergence of the Acarosporomycetidae. We confirm that this group should be recognized as a major independent lineage within the Lecanoromycetes by classifying it in its own subclass (Candelariomycetidae) the same way it was done to accommodate the unique phylogenetic placement of the Acarosporaceae. No morphological features are known to confirm the separation of these two genera from the Lecanoraceae and the Lecanoromycetidae. A revision of the genera *Candelaria* and *Candelariella* is needed, given that *Candelaria concolor* was found nested within *Candelariella* (FIG. 1).

Ostropomycetidae.—As revealed from analyses on 5+4-gene and 5+4+3-gene datasets, the subclass Ostropomycetidae is well supported as monophyletic (except by PHYML-BS). The phylogenetic tree presented here includes members of these four of five orders part of the current classification of the Ascomycota (Eriksson 2006): Agryariales, Gyalectales, Ostropales and Pertusariales (FIG. 1). The Baecomyetales and Loxosporaceae need to be recognized as members of the Ostropomycetidae, based on our results.

The Ostropales and Gyalectales are treated usually as Ostropales *s.l.* (Kauff and Lutzoni 2002, Lücking et al 2004) due in part to the poor taxon sampling and support these relationships received in past studies. In this study we show that the order Ostropales as circumscribed by Eriksson (2006) is nonmonophyletic due to the inclusion of the Gyalectales. For this reason the Gyalectales should be subsumed within the Ostropales *s.l.* as proposed by Kauff and Lutzoni (2002) and Lücking et al (2004). Because *Ostropa* is classified within the Stictidaceae, the Ostropales *s.str.* could be restricted to this family (well supported in FIG. 1, Ostropales 1), which would allow the recogni-

No. of genes/ data set	No. of taxa	% of missing data	RAxML BP	PHYML BP	MrBayes PP
5	111	17	1	2	3
5+4	188	26	4	5	6
5+4+3	274	37	7	8	9

not applicable due to missing taxa
 RAxML-BP/PHYML-BP $\geq 70\%$ / MrBayes-PP $\geq 95\%$
 RAxML-BP/PHYML-BP $< 70\%$ / MrBayes-PP $< 95\%$ / node does not exist

Primary photobiont:

- Trebouxia* or *Asterochloris*
- Trebouxia*
- Asterochloris*
- Myrmecia*
- Cystococcus*
- Trentepohlia*
- Dictyochloropsis*
- Coccomyxa*
- Other chlorococcoid algae
- Cyanobacteria
- Non-lichenized

Secondary photobiont:

- S/Sc* *Stigonema* / *Scytonema*
- Nostoc*
- Calothrix*

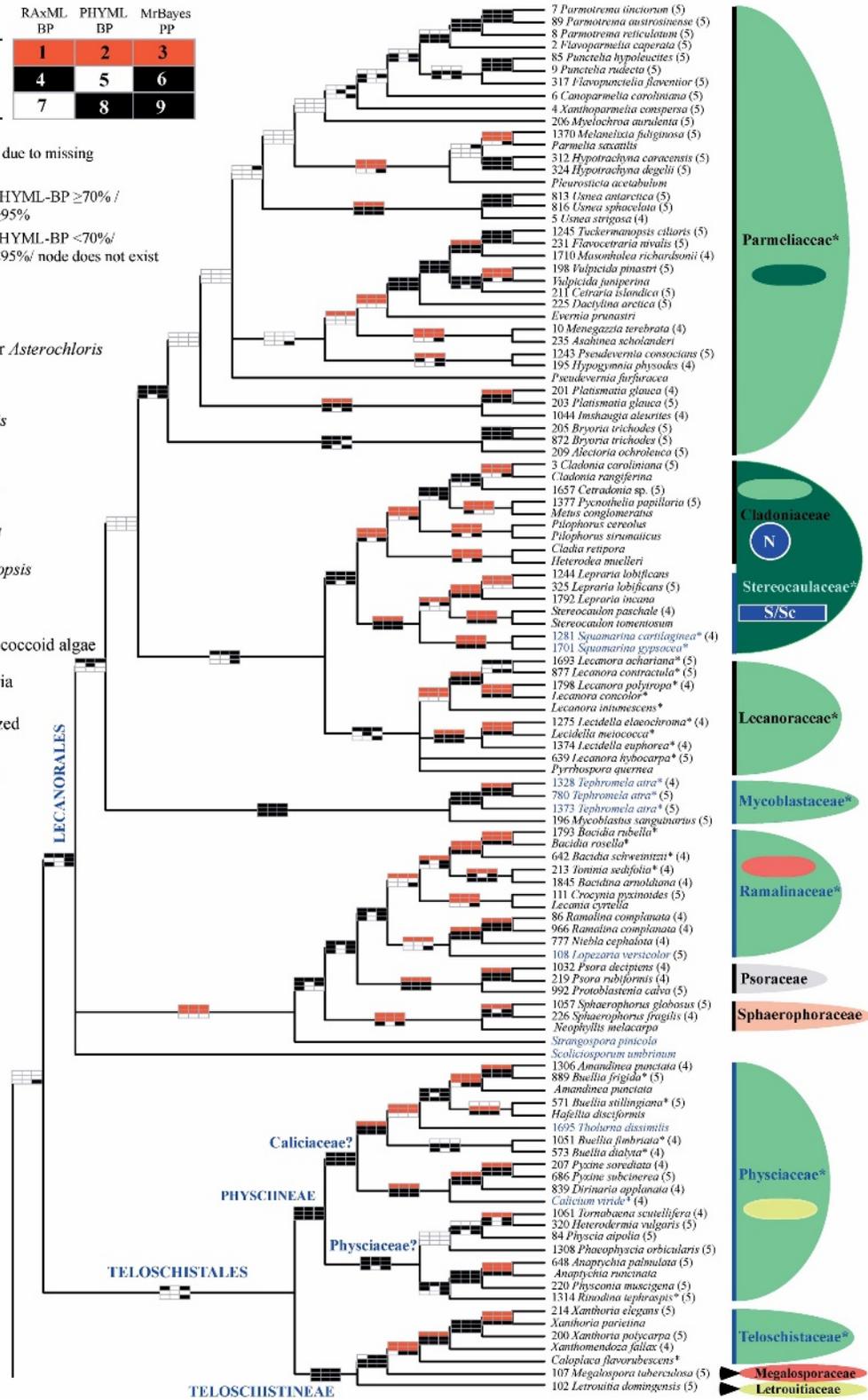


FIG. 1.

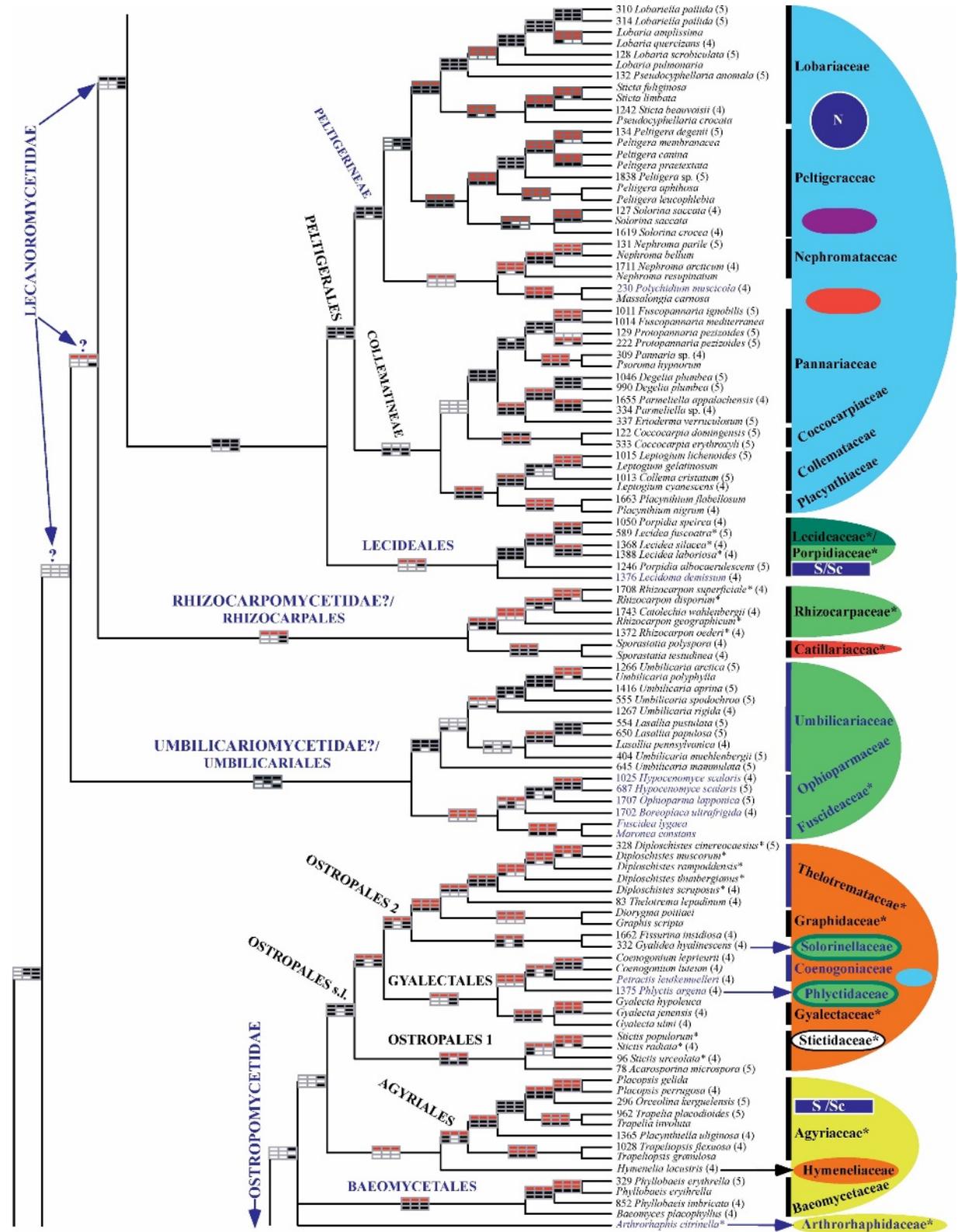


FIG. 1. Continued.

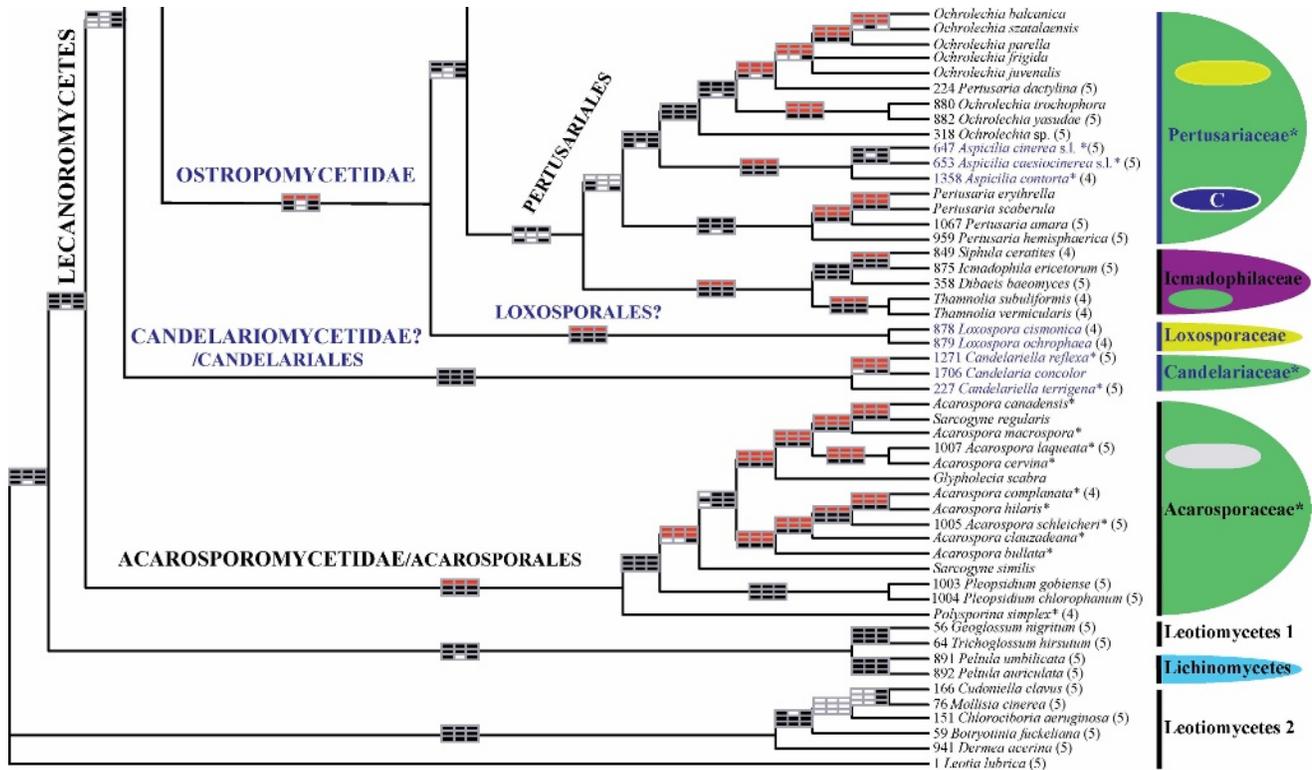


FIG. 1. Phylogenetic relationships among 264 putative members of the Lecanoromycetes based on Bayesian analyses of the combined nucSSU, nucLSU, mitSSU, *RPB1* and *RPB2* sequences (5+4+3-gene dataset) and 10 species used as outgroup (Geoglossaceae, Lichinomycetes and Leotiomycetes). This cladogram resulted from a 50% majority rule consensus of 30 000 trees sampled with Bayesian MCMCMC (SUPPLEMENT 1). Numbers in parentheses after taxon names indicate the dataset in which they were included: 5 refers to taxa present in the 5-, 5+4- and 5+4+3-gene datasets, and 4 refers to taxa present in the 5+4- and 5+4+3-gene datasets. When no numbers are found after names, taxa were included only in the 5+4+3-gene supermatrix. Stars indicate genera and families with lichenicolous members. Taxa at the tip of the tree shown in blue indicate phylogenetic placements that are newly revealed or significantly supported compared to previous studies. Taxa in blue at the family and higher levels indicate suggested changes in their circumscription and ranking that needs to be incorporated in future classifications of the Ascomycota. Names followed by a question mark indicate potential changes for future consideration. The nine-box grids on internodes indicate support with different phylogenetic methods (column 1 [boxes 1, 4, 7] = bootstrap values calculated with RAxML, column 2 [boxes 2, 5, 8] = bootstrap values calculated with PHYML, column 3 [boxes 3, 6, 9] = posterior probabilities calculated with MrBayes) based on different datasets (top row [boxes 1–3] being the smallest dataset [111 taxa] but with the least amount of missing data, and the bottom row [boxes 7–9] being the largest dataset [274 taxa] with the largest amount of missing data). Red boxes indicate cases where internodal support is not applicable due to at least one of the (usually two) immediately downstream branches being absent in the 188 or the 111 taxa datasets compared to the 274 taxa dataset. Black boxes indicate RAxML bootstrap values $\geq 70\%$ (column 1), PHYML bootstrap values $\geq 70\%$ (column 2) or MrBayes posterior probability values $\geq 95\%$ (column 3). White boxes indicate RAxML bootstrap values $< 70\%$, PHYML bootstrap values $< 70\%$ or MrBayes posterior probability values $< 95\%$. Colors on the right side of the figure indicate major types of primary photobionts associated with mycobionts within an order/family/monophyletic group based on available records for members classified in these taxa, even if not included in the tree. Presence of secondary photobionts (different genera of cyanobacteria) is indicated by a dark blue box (*Scytonema/Stigonema*), a circle (*Nostoc*) and an oval (*Calothrix*).

tion of the Graphidales (Ostropales 2; well supported monophyletic group including Graphidaceae, Asterothyriaceae [= Solorinellaceae; Henssen and Lücking 2002] and Thelotremaaceae in FIG. 1) and the Gyalectales (a poorly supported monophyletic group in FIG. 1, that would include the Coenogoniaceae, Gyalectaceae and Phlyctidaceae), thus partly reflecting the earlier classifications by Henssen and Jahns

(1974) and Sherwood (1977). However this three-order classification would remove the use of Ostropales *s.l.* for a well supported monophyletic group of lichen-forming fungi preferentially associated with *Trentepohlia*, (FIG. 1), which would leave this important internode and associated putative synapomorphy without a name and commonly used rank. An alternative solution to this problem would be the

use of suborders Graphidineae (Ostropales 2), Gyalectineae (Gyalectales) and Stictidineae (Ostropales 1) within the Ostropales *s.l.* as phylogenetically circumscribed here. Simultaneous inclusion of Odonotremataceae and Gomphillaceae in phylogenetic studies is necessary before any changes to the classification of the Ostropales *s.l.* are made.

The Ostropales *s.l.* includes morphologically and ecologically diverse lichens. Lücking et al (2004) demonstrated that the Gomphillaceae, with anastomosing paraphyses, are part of this group, and Grube et al (2004) have shown that the perithecial Porinaceae, with unitunicate asci and unbranched true paraphyses, also belongs to this clade. Lumbsch et al (2004b) confirmed that the mazaediate genus *Nad-vornikia* is a member of the Thelotremataceae and thus the Ostropales *s.l.* The genus *Phlyctis* was placed traditionally in the Lecanorales due to its amyloid hymenium and chlorococcoid photobiont; however its thallus and apothecial structure are more reminiscent of the Ostropales *s.l.* (RL unpublished) therefore supporting its placement in the latter group (Gyalectaceae).

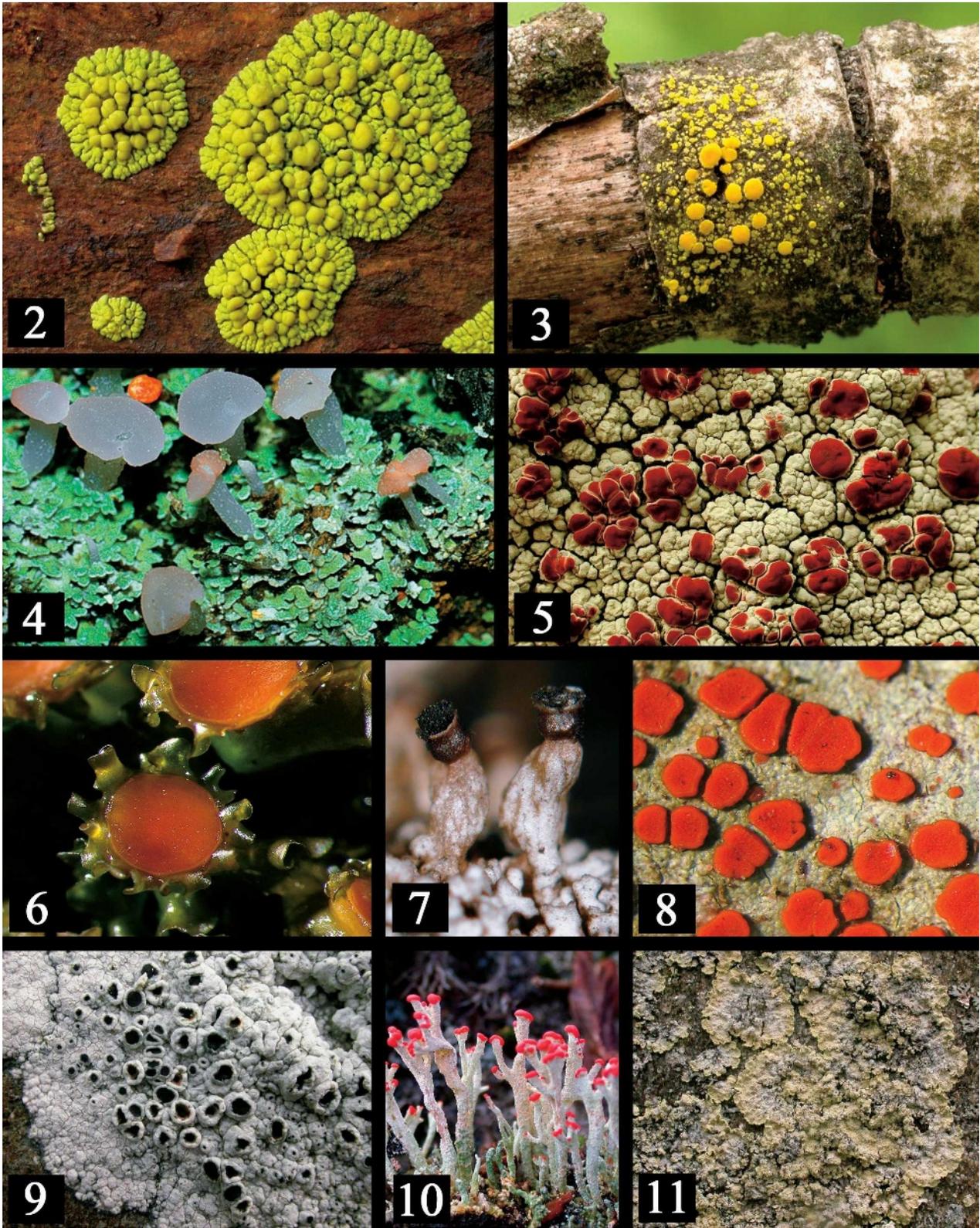
Our study shows a well supported sister clade relationship of Pertusariaceae (with nonmonophyletic *Ochrolechia* and *Pertusaria*) and Icmadophilaceae, to form the order Pertusariales (reconstructed as paraphyletic in Wedin et al [2005]) and the unexpected placement of *Aspicilia* (Hymeneliaceae) nested within the Pertusariaceae. Wedin et al (2005) and Hofstetter et al (2007) also suggested a close affinity among *Aspicilia* and members of the Pertusariaceae and Icmadophilaceae but without obtaining strong support for this relationship. *Aspicilia* was shown to be outside the family Hymeneliaceae more than 10 y ago based on morphological and isozyme data (Lutzoni and Brodo 1995). The current circumscription of the Hymeneliaceae and Pertusariaceae needs to be updated accordingly.

The Baeomycetaceae (with an uncertain placement in the Ascomycota according to Eriksson 2006) is delimited as monophyletic and a highly supported lineage (*Phyllobaeis* [FIG. 4] and *Baeomyces*) in our phylogeny (FIG. 1). *Arthrorhaphis* is also part of this subclass, although an accurate placement in the Ostropomycetidae remains unresolved. The latter is true for the Hymeneliaceae and Agryiales. Based on ribosomal genes Kauff and Lutzoni (2002) proposed an elevation of the Baeomycetaceae (represented in their tree by *Baeomyces placophyllus*) to the order level (Baeomycetales) in the Ostropomycetidae. This suggestion is confirmed by our study; however some putative close relatives of the Baeomycetaceae (*Ainoa* and *Anamylopsora*) were not included. The placement of *Arthrorhaphis citrinella* (a juvenile parasite of

Baeomyces) in the Ostropomycetidae, where two of its hosts belong, also was unexpected given the different ascomatal structures (see also Wedin et al 2005).

Our study also shows that *Loxospora* is part of the most basal divergence within the Ostropomycetidae (with significant support values, FIG. 1). Members of this genus have coccalean green algae and somewhat spirally arranged ascospores. This novel phylogenetic affinity revealed by this study is not surprising due to *Loxospora's* (Loxosporaceae) greater similarity (thallus structure) to Pertusariaceae than to Lecanorales, where this taxon is classified currently (Eriksson 2006). *Loxospora* was re-established as a genus by Hafellner (1984) and previously was classified in the Haematommataceae. Staiger and Kalb (1995) noticed anatomical characters that were not shared by other members of this family (e.g. the genus *Haematomma*) and created a separate family to accommodate *Loxospora*. Loose and thick paraphyses, predominance of elatinic acid and the presence of wide and grouped ascogenous hyphae in ascomatal primordia (cf. Brodo and Henssen 1995) are further characters that circumscribe this newly reconstructed lineage in the Ostropomycetidae. Because none of the members of the Haematommataceae has been included in phylogenetic analyses we cannot justify the exclusion of the Loxosporaceae from the Haematommataceae.

Lecanoromycetidae.—The delimitation of the Lecanoromycetidae is ambiguous due to a lack of support for the phylogenetic placement of the Catillariaceae (*Sporastatia*; Rambold and Triebel 1992, Eriksson 2006), Fuscideaceae, Ophioparmaceae, Rhizocarpaceae and Umbilicariaceae (FIG. 1). An early divergence of the Rhizocarpaceae as revealed here and in previous studies (Reeb et al 2004, Lutzoni et al 2004) was postulated by Honegger (1980) based on characters of the ascus tip. Our phylogeny confirms that the narrowest delimitation of the Lecanoromycetidae contains at least three main lineages (Miadlikowska and Lutzoni 2004, Hofstetter et al 2007): the Lecanorales, Peltigerales (and most closely related group, including the Lecideaceae and Porpidiaceae) and Teloschistales. If the current topology receives high support values in future studies, the Fuscideaceae-Ophioparmaceae-Umbilicariaceae and the Rhizocarpaceae-Catillariaceae monophyletic groups should be classified as separate orders (Umbilicariales and Rhizocarpales) within the Lecanoromycetidae. If future studies show that it is not possible to encompass these two new orders within a monophyletic Lecanoromycetidae it is likely that each group would have to be recognized at the subclass level



FIGS. 2–11. Lichen-forming members of the Lecanoromycetes. 2. *Pleopsidium chlorophanum*, Acarosporomycetidae, Acarosporales, Acarosporaceae (photo by E. Timdal). 3. *Candelariella lutella*, Candelariaceae (photo by E. Timdal). 4. *Phyllobaeis imbricata*. Close-up of fruiting bodies, Ostropomycetidae, Baeomycetaceae (photo by R. Lücking). 5. *Ophioparma ventosa*, Ophioparmaceae (photo by E. Timdal). 6. *Leptogium burgessii*. Close-up of fruiting bodies, Lecanoromycetidae,

(Umbilicariomycetidae and Rhizocarpomycetidae). Therefore no matter how these two monophyletic entities will be resolved in future studies, they both need to be recognized at least at the ordinal level, as proposed here.

Umbilicariomycetidae?/Umbilicariales.—Some novel and interesting relationships are found in the group containing the Umbilicariaceae. Placement of the Fuscideaceae outside Teloschistaceae was suggested by Lutzoni et al (2004), Reeb et al (2004) and Wedin et al (2005) and is confirmed in this study. We found it interesting that *Fuscidea* and *Maronea*, despite striking differences in ascocarp anatomy and spore number per ascus, share a unique ascus type and a distinctive type of epihymenial pigmentation, which led Hafellner (1984) to the description of the family Fuscideaceae. Therefore similarities in the ascus structure between Teloschistales and Fuscideaceae were misleading and turned out to be homoplastic.

Three other groups of lichen-forming fungi fall within the Fuscideaceae-Umbilicariaceae clade: Ophioparmaceae (represented by *Ophioparma* [FIG. 5], *Boreoplaca* [Lecanoromycetes genera *inc. sed.*] and *Hypocenomyce* [Lecideaceae] [FIG. 1]). Presence of an amyloid ascus with a tholus exhibiting a strongly amyloid dome in these otherwise morphologically and anatomically distinct genera was used by Wedin et al (2005) to support the monophyly (strong PP support) of *Boreoplaca*, *Hypocenomyce* and *Ophioparma* to form the extended family Ophioparmaceae. Lumbsch et al (2004a) found in their study based on nuLSU and mitSSU data that the family Elixiaaceae (with a single species *Elixia flexella*, not included in this study) formed a well supported monophyletic group within the family Umbilicariaceae and that the former family circumscription including species with foliose (umbilicate) thalli, possibly comprised a sister group of crustose species.

Rhizocarpomycetidae?/Rhizocarpales.—This order proposed here includes Rhizocarpaceae and *Sporastatia*. While excluding *Sporastatia* from the family Acarosporaceae, Hafellner (1995) already questioned its placement in the Catillariaceae, which could not be tested here. A close affiliation between *Sporastatia* and *Rhizocarpon* first was shown by Reeb et al (2004)

and corroborated by Buschbom and Mueller (2004) and Lutzoni et al (2004). Both taxa are strictly crustose and distinctly areolate. While members of the Rhizocarpaceae often form large, hyaline to brown, transversely septate to distinctly muriform ascospores, *Sporastatia* develops multiple, hyaline ascospores. The placement of *Catolechia* within *Rhizocarpon* (FIG. 1) is interesting because this genus, with thick squamulose thalli, reveals a strong pigmentation of the spore wall around the septum of mature ascospores (torus) but lacks the typical gelatinous perispore (“halo”). A closer relationship of *Catolechia* and *Rhizocarpon* rather than with *Buellia* was assumed by Hafellner (1978). Noteworthy in this group is also the tendency toward lichenicolous growth (e.g. in *Rhizocarpon* and *Epilichen*; Hafellner and Poelt 1976, Lawrey and Diederich 2003, Santesson et al 2004) in several parallel lineages however in none of them is the lichenization lost completely.

Peltigerales.—Strongly supported as monophyletic in all analyses, the order Peltigerales comprises two suborders, the Peltigerineae and Collematineae, as defined by Miadlikowska and Lutzoni (2004) based on ribosomal genes and confirmed by Hofstetter et al (2007) based on a five-locus analysis. Peltigerineae, which differs from the Collematineae by the development of conspicuous heteromerous thalli, the common occurrence of tripartite symbioses with *Nostoc* as a secondary photobiont (cephalodia), the presence of bipartite associations with green algae (*Coccomyxa*) and the production of diverse secondary compounds (mostly triterpenoids), includes Lobariceae, Peltigeraceae and Nephromataceae. For the first time the monophyly of *Solorina* (FIG. 1) is shown here to receive high bootstrap values. We found that *Polychidium*, a member of the Placynthiaceae (classified in the Collematineae), belongs to the Peltigerineae and we confirm that *Massalongia* is placed also in this suborder; however their sister relationship and phylogenetic placement within the Peltigerineae remains uncertain. The monophyly of the Collematineae, the second suborder within the Peltigerales, became significantly supported only when more members from each family were incorporated in the phylogenetic analyses (10 taxa in the 5-gene dataset

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Peltigerales, Collemataceae (photo by R. Lücking). 7. *Tholurna dissimilis*. Close-up of fruiting bodies, Lecanoromycetidae, Teloschistales, Caliciaceae (photo by E. Timdal). 8. *Pyrrhospora russula*, Lecanoromycetidae, Lecanorales, Lecanoraceae (photo by R. Lücking). 9. *Tephromela atra*, Lecanoromycetidae, Mycoblastaceae (photo by E. Timdal). 10. *Cladonia floerkeana*, Lecanoromycetidae, Lecanorales, Cladoniaceae (photo by E. Timdal). 11. *Lepraria membranacea*, Lecanoromycetidae, Lecanorales, Stereocaulaceae (photo by E. Timdal). All photographs by E. Timdal are available at http://www.toyen.uio.no/botanisk/lav/Photo_Gallery/PG_index.html.

vs. 19 in the 5+4+3-gene dataset). Collematineae as defined here includes the monophyletic Pannariaceae (*Degelia*, *Erioderma*, *Fuscopannaria*, *Parmeliella*, *Protopannaria* and *Psoroma*), Coccocarpiaceae (only *Coccocarpia* was sampled), Collemataceae (*Collema* and nonmonophyletic *Leptogium* [FIG. 6]) and Placynthiaceae (excluding *Polychidium*).

Lecideales.—Lecideaceae, intermixed with Porpidiaceae (*Porpidia*), appeared as a sister group of the Peltigerales (strongly supported in FIG. 1). This is surprising because the Lecideaceae seem to share no common features with members of the Peltigerales. Moreover the placement of *Porpidia* intermixed with *Lecidea* in the Lecideaceae, detected also by Buschbom and Mueller (2004), questions the recognition of the entire family Porpidiaceae based on ascus structures. A basal position of *Lecidoma* (former *Lecidea*) to Lecideaceae received only PP support in the 5+4-gene analysis. Both *Lecidoma* and *Porpidia* have asci with amyloid tube structures but of different features (Hafellner 1984), whereas in *Lecidea* this tube seems to be reduced to a minute structure in the tholus tip. These three genera have brown to dark ascospores, crustose to adpressed squamulose thalli with coccalean green algae, (*Asterochloris* and *Trebouxia*, Rambold et al 1998). In contrast to *Lecidea*, *Porpidia* species have prominent dark parathecial margins, halonate ascospores and intensely anastomosing paraphyses, while a carbonization of portions of the apothecia as in these two genera is not found in *Lecidoma*. Furthermore *Porpidia* and *Lecidea* are strictly saxicolous whereas *Lecidoma* grows on soil. Hafellner (1984) had introduced the monotypic family Lecidomataceae to accommodate the relatively unique genus *Lecidoma*.

Teloschistales.—The Letrouitiaceae, Megalosporaceae and Teloschistaceae forming a monophyletic group sister of the monophyletic Physciaceae is confirmed here for the first time (FIG. 1). These families differ considerably in the cortical pigmentation of their thallus. While the Physciaceae are diverse in their cortical pigmentation (mostly atranorin), predominance of anthraquinones is characteristic for the Teloschistaceae. Most representatives of the Teloschistaceae and Physciaceae are characterized by polar diblastic ascospores, which often display conspicuous endospore thickenings. *Letrouitia* (Letrouitiaceae) diverged earlier than *Megalospora* (Megalosporaceae) and Teloschistaceae. *Megalospora* is phenotypically different from the Teloschistales, especially in its peculiar ascus type (i.e. without any teloschistalean features, ascospores without internal thickenings, thallus chemistry and the lack of quinoid substances). *Caloplaca* is represented only by one

species here, and this phenotypically diverse genus and its relatives, *Fulgensia*, *Teloschistes* (both not included in this study) and *Xanthoria*, are all nonmonophyletic genera (Kasalicky et al 2000, Gaya et al 2003, Søchting and Lutzoni 2003, Gaya 2006).

Our results also confirm that the Caliciaceae, here represented by *Tholurna* (FIG. 7) and *Calicium*, are nested in the buellioid branch (*Buellia* and related genera) of the Physciaceae (Wedin et al 2002) and are not supported as a monophyletic group. Loss of active ascospore dispersal (i.e. evanescent asci) and evolution of mazaedial ascomata evidently occurred several times (e.g. Mycocaliciales shown to be sister of the Eurotiomycetes by Geiser et al [2006]), including in the buellioid clade. Placement of *Amandinea* within *Buellia* (see also Helms et al [2003]) raises doubts about the validity of the former genus, which is distinguished only by spermatial characters; however *Amandinea* species included in the analysis is not the generic type (*A. conioops*). Although the genus *Buellia* s.l. is extremely diverse (e.g. Marbach 2000) and pending a proper sampling in phylogenetic study, we propose to maintain the current classification. A close relationship between the genus *Pyxine* and *Dirinaria* was mentioned by Scheidegger et al 2001 and demonstrated in a phylogenetic study by Helms et al (2003). Both taxa have delayed ascospore septum formation, shared with the genus *Hafellia*, which is nested in the buellioid clade.

We propose here to establish two suborders within the Teloschistales, Physciineae and Teloschistineae. The phylogeny presented here (FIG. 1) shows two main lineages within the Physciineae, a predominantly buellioid clade (Caliciaceae) and a rinodinoid clade (*Rinodina* and related genera, Physciaceae).

Lecanorales.—The order Lecanorales includes eight families (FIG. 1), Cladoniaceae, Lecanoraceae, Mycoblastaceae, Parmeliaceae, Psoraceae, Ramalinaceae, Sphaerophoraceae and Stereocaulaceae. At least one further large family, the emended Pilocarpaceae (not sampled for this study), which also includes members of the Micareaceae and Ectolechiaceae (Eriksson 2006), was found to be closely related to Ramalinaceae by Andersen and Ekman (2004, 2005) and therefore is part of the Lecanorales.

The well supported Parmeliaceae is the most speciose family within the Lecanorales and comprises mostly foliose to fruticose lichens associated exclusively with coccalean green algae (predominantly *Trebouxia*, Rambold et al 1998). A diagnostic character for this family is the presence of a cupula in the ascospores, a well differentiated cup-shaped hyphal structure beneath the hypothecium (Henssen and Jahns 1974). All members also share a similar type of

ascus with a broadly shaped nonamyloid masse axiale. A series of papers recently reviewed the previously controversial classification within this family (e.g. Crespo et al 2001; Blanco et al 2004a, b, 2005, 2006; Thell et al 2004). Blanco et al (2006) recently demonstrated that the taxonomic value of key characters (presence of usnic acid and atranorin in the cortex of the thallus, occurrence of pseudocyphellae and pored epicortex) traditionally used to classify members of the Parmeliaceae at generic and suprageneric levels has been overemphasized in previous classifications. Most of the cetrarioid genera are grouped together, sister of *Dactylina* (FIG. 1). Another well supported monophyletic group includes *Alectoria* and *Bryoria*, both with fruticose, pendent to shrubby thalli and distinguished by cortical compounds (usnic acid vs. amorphous melanin-like substances) and hymenial characters including ascospore pigmentation (pigmented vs. hyaline). The lack of support for most deep internodes within the Parmeliaceae is due to the little divergence recorded within this strongly supported monophyletic group. ITS can be aligned across members of this family for example. Therefore the fastest evolving sites of the nucLSU, nucSSU and mitSSU, which would be most appropriate to increase phylogenetic confidence in this portion of the tree, had to be excluded from these analyses due to the presence of indels rendering positional homology too uncertain in these regions (a typical problem of broad selection of taxa that also include a large group of closely related taxa).

The Lecanoraceae comprise *Lecanora*, *Lecidella* and *Pyrrhospora* (FIG. 8) in our tree. This relationship is supported by tholus amyloidity in the ascus and the presence of a broad masse axiale, common features in all three genera. The sister relationship of *Mycoblastus* and *Tephromela* (FIG. 9), as also found by Wedin et al (2005), is unexpected. Both genera were classified in separate families by Hafellner (1984). They differ considerably in their ascospores (large and thick-walled in *Mycoblastus* vs. small and thin-walled in *Tephromela*) and secondary chemistry (*Mycoblastus* partly with chinoid substances vs. *Tephromela* partly with the rare α -collatolic acid) but share the tar-black pigmentation of the epihymenium, which can extend downward into the hymenium. The inclusion of further taxa will show whether two separate families, Tephromelataceae and Mycoblastaceae, are needed.

The monophyletic Psoraceae, Ramalinaceae and Sphaerophoraceae as well as their interfamilial relationships are all well supported. Our analyses support the Ramalinaceae to include the Bacidiaceae, as outlined by Ekman (2001, but see Andersen and Ekman 2005). *Lopezaria*, considered to be a genus of uncertain position within the Lecanoromycetes

(Eriksson 2006), is shown here to be a member of the Ramalinaceae. *Lopezaria* is similar in ascospore type and ecology to *Megalospora* but apparently not closely related to the latter, and its large, thick-walled ascospores thus have evolved independently. Apothecial features are otherwise similar to those of certain tropical *Bacidia* species and support its placement in the Ramalinaceae. Except for *Lopezaria*, asci in this group are of similar type, but diverse growth forms include crustose, squamulose and fruticose thalli. Hafellner (1988) regarded Lecaniaceae (as an available family name for crustose bacidioid lichens with lecanorine apothecia) and Ramalinaceae as members of the same evolutionary lineage in term of thallus evolution. *Scoliciosporum*, currently classified within the Lecanoraceae and *Strangospora*, currently with an uncertain placement in the Lecanoromycetes (Eriksson 2006), are shown here as members of the Lecanorales. Hafellner (1984) had introduced the family Scoliciosporaceae and later (Hafellner 1995) discussed a possible closer relationship of *Strangospora* and *Scoliciosporum*, both with similar ascomata and *Lecanora*-type asci, but the type species of these genera have different ascospores (polyspored one-celled vs. eight-spored phragmospore). The addition of more taxa from these genera is needed to resolve their affiliation within the Lecanorales.

Results from this study do not support previous subordinal circumscriptions within the Lecanorales (Hafellner et al 1993). Cladoniineae as shown here includes Cladoniaceae (*Cetradonia*, *Cladonia* [FIG. 10] and *Pycnothelia*) and Stereocaulaceae (*Lepraria* [FIG. 11], *Stereocaulon* and *Squamarina*). Both families share the same main photobiont type identified as *Asterochloris* (Rambold et al 1998) and, with the exception of *Squamarina*, asci with tholi provided with amyloid tube structures. Discovered for the first time by Ekman and Tønsberg (2002) the close relationship of *Lepraria* and *Stereocaulon* was supported only in the 5+4-gene phylogeny. For the first time *Squamarina* is well supported as being related to the Stereocaulaceae (shown by Stenroos and DePriest 1998 but not supported). However the inclusion of *Squamarina* in Stereocaulaceae is incongruent with morpho-anatomical characters, such as ascomatal and ecological attributes (e.g. all *Squamarina* grow on calcareous substrates). While the Cladoniaceae and Stereocaulaceae previously were placed in the informal *Micarea* group owing to ascus characters, Squamarinaceae was recognized as a separate group (Hafellner et al 1993). Ekman and Tønsberg (2002) demonstrated that the Lecanoraceae are more closely related to the Cladoniaceae and Stereocaulaceae than suggested by Hafellner et al (1993), who included the Lecanoraceae together with

the Parmeliaceae in the suborder Lecanorineae. Thus in our analysis the Cladoniineae are nested within the Lecanorineae using the previous subordinal concept (Poelt 1974). It is too early to propose a revised subordinal classification within the Lecanorales.

Photobiont selectivity as a taxonomic character.—Although photobiont relationships and life strategy characters were mainly disregarded in previous taxonomic treatments of the lichen-forming fungi, photobiont associations are highly structured across the Lecanoromycetes phylogeny (FIG. 1, similar to what was anticipated for the Lecanorales by Rambold et al 1998) suggesting that these symbiotic interactions are the result of a highly selective process and where shifts from one main type of photobiont to another were rare during the evolution of the lichen symbiosis. Large monophyletic groups of the Lecanoromycetes have preferences for certain types of photobionts (FIG. 1). For example members of the *Ostropales s.l.*, with mostly crustose thalli and high species diversity in wet tropical habitats, are predominantly associated with photobionts of the Trentepohliaceae, which do not occur as photobionts in the Acarosporomycetidae, Candelariomycetidae, Lecanoromycetidae, Rhizocarpaceae and Umbilicariales. Only a few lineages in this order, including Gomphillaceae, Asterothyriaceae (with *Gyalidea*), *Diploschistes*, *Phlyctis*, and *Stictis*, were able to switch from filamentous to chlorococcalean green algae or in rare cases (*Petractis*) to cyanobacteria (*Scytonema*) as their photosynthetic partner. Mycobionts of the Peltigerales (Lecanoromycetidae) have strong preferences for cyanobacteria (mostly *Nostoc*). In the Peltigerales bipartite associations with cyanobacteria seem to be the ancestral state, which either is maintained or switched repeatedly to coccalean green algae (*Coccomyxa* and *Dictyochloropsis* in *Peltigera*, *Lobaria* and *Sticta*), resulting either in phycosymbiodemes, tripartite symbioses or bipartite species that associate only with green algae in the later stage of development (Miadlikowska and Lutzoni 2004).

Photobiont-mycobiont patterns of associations can greatly contribute to our understanding of relationships and evolution of lichen-forming fungi, as already suggested by Rambold et al (1998). However this will require a re-examination of existing records of green algae and cyanobacteria reported to be associated with lichen-forming taxa based on recent phylogenetic treatments of these photobionts.

Ascomatal features as taxonomic characters.—As already reported by Reeb et al (2004), highly polyspor-

ous asci (i.e. > 100 spores/ascus) evolved independently in several lineages of the Lecanoromycetes (e.g. in *Acarosporaceae*, *Biatorella*, *Maronea*, *Sporastatia*, *Strangospora* and *Thelocarpon*). Less pronounced polyspory is found in many other groups throughout the Lecanoromycetes (e.g. in *Candelariella*, *Gyalidea* [species formerly assigned to *Solorinella*], *Gyalideopsis*, members of *Buellia*, *Caloplaca*, *Catillaria*, *Lecanora*, *Rinodina* and other genera). Unusually large ascospores (e.g. in *Asterothyrium*, *Megalospora*, *Mycoblastus*, *Pertusaria*, *Psorotheciopsis* and *Solorina*) or long-filiform ascospores (e.g. in *Bacidina*, *Bapalmuia*, *Stictis* and *Gomphillus*) also have evolved independently many times within the Lecanoromycetes.

Our study confirms that different types of asci can occur in a single lineage of closely related taxa or that the same ascus type can be found in distinct lineages (homoplasy). The widespread occurrence of the *Lecanora* type ascus (in Candelariaceae, *Lecanora*, Parmeliaceae, *Physcia*, *Pleopsidium*, *Scoliciosporum* and *Strangospora*) suggests that this type of ascus could be ancestral (Chadefaud et al 1968) as discussed by Wedin et al (2005) and therefore residual in many lineages of the Lecanoromycetes.

Characters of ascomatal architecture and pigmentation are also of varying significance for classification in the Lecanoromycetes. While a cupula structure in the ascomata of the Parmeliaceae is a characteristic feature of the whole group, such structures also occur intermittently in other Lecanoromycetes and can be found in species of *Caloplaca*, *Collema*, *Lecanora* and *Rinodina*. Although perithecioid ascomata characterize the large family Porinaceae, such ascomata also are found in smaller unrelated genera in the Ostropomycetidae (e.g. *Belonia*, *Protothelenella*, *Thelenella* or *Topelia*).

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