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PHYLOGENETIC CLASSIFICATION OF PELTIGERALEAN FUNGI (PELTIGERALES, ASCOMYCOTA) BASED ON RIBOSOMAL RNA SMALL AND LARGE SUBUNITS[1]

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To provide a comprehensive molecular phylogeny for peltigeralean fungi and to establish a classification based on monophyly, phylogenetic analyses were carried out on sequences from the nuclear ribosomal large (LSU) and small (SSU) subunits obtained from 113 individuals that represent virtually all main lineages of ascomycetes. Analyses were also conducted on a subset of 77 individuals in which the ingroup consisted of 59 individuals representing six families, 12 genera, and 54 species potentially part of the Peltigerineae/Peltigerales. Our study revealed that all six families together formed a strongly supported monophyletic group within the Lecanoromycetidae. We propose here a new classification for these lichens consisting of the order Peltigerales and two suborders—Collematineae subordo nov. (Collemataceae, Placynthiaceae, and Pannariaceae) and Peltigerineae (Lobariaceae, Nephromataceae, and Peltigeraceae). To accommodate these new monophyletic groups, we redefined the Lecanorineae, Pertusariales, and Lecanorales sensu Eriksson et al. (*Outline of Ascomycota*—2003, Myconet 9: 1–103, 2003). Our study confirms the monophyly of the Collemataceae, Lobariaceae, Nephromataceae, and Peltigeraceae, and the genera *Nephroma*, *Sticta*, and *Peltigera*. However, *Leptogium*, *Lobaria*, *Pseudocyphellaria*, and *Solorina* were found to be nonmonophyletic genera. Reconstruction of ancestral symbiotic states within the Peltigerales, using maximum likelihood (ML) and a Bayesian approach to account for phylogenetic uncertainty, revealed an evolutionary scenario in which bimembered associations with cyanobacteria were ancestral, followed by multiple independent acquisitions of green algae to form tripartite symbioses and rare subsequent losses of the cyanobiont to form bimembered symbioses with green algae.

Key words: ancestral state reconstruction; Collematineae; Lecanoromycetidae; lichen symbiosis; molecular phylogenetic classification of ascomycetes; nuclear ribosomal large (LSU nrDNA) and small (SSU nrDNA) subunits; Peltigerales; Peltigerineae.

Peltigeralean lichen-forming fungi (Peltigerineae/Peltigerales W. Watson, Ascomycota) are generally characterized by foliose, subfruticose, or granular-squamulose thalli; the presence of rhizines or tomentum on the lower side of the thalli; predominantly multiseptate, colorless or brown ascospores formed in asci with some lecanoralean features, i.e., reactive a-layer and thick c-layer; and mostly unbranched and free paraphyses. This group contains both bipartite (one mycobiont and one photobiont) and tripartite lichens (one mycobiont and two photobionts). Cyanobacteria (*Nostoc* Vauch., *Scytonema* Agardh, or less commonly, *Dichotrix* Zanardini) are the photobionts most frequently found in thalli of bipartite peltigeralean species or are restricted to internal or external cephalodia in tripartite species in which a green alga is the main photobiont (e.g., Tschermak-Woess, 1988; Rai, 1990; Schenk, 1992). Bipartite fungal symbioses with green algae (usually *Coccomyxa* Léger & Hesse, *Dictyochloropsis* Geitler, or *Myrmecia* Printz) are relatively rare in these lichens (e.g., Tschermak-Woess, 1988; Ahmadjian, 1993). The mycobiont of several tripartite peltigeralean lichens [*Nephroma* Ach., *Sticta* (Schreber) Ach., *Lobaria* (Schreber) Hoffm., *Psoroma* Michaux, and *Peltigera* Willd.] can also form a cyanomorph thallus, in which the same fungus is associated with only the cyanobacterial partner, forming a bipartite thallus that is attached or growing separately from the mother tripartite thallus (Tønsberg and Holtan-Hartwig, 1983; White and James, 1988; Armaleo and Clerc, 1991; Goffinet and Bayer, 1997; Paulsrud et al., 1998, 2000; Holien and Jørgensen, 2000). Peltigeralean lichens are worldwide in distribution; however, the Lobariaceae Chevall. are most abundant and diverse in the Southern Hemisphere, whereas other families (e.g., Peltigeraceae Dumort.) are more common and diverse in the Northern Hemisphere. These lichens grow on various substrates: mosses, rocks, soil, or bark, and mostly in moist to humid wooded habitats.

All lecanoralean taxa having cyanobacteria as their main photobiont are part of the suborder Peltigerineae (recognized also at the ordinal level Peltigerales; Kirk et al., 2001) when broadly delimited. This broad circumscription of the Peltigerineae/Peltigerales includes 7–8 families (Rambold and Triebel, 1992; Tehler, 1996; J. Hafellner, H. Hertel, G. Rambold, and E. Timdal, unpublished manuscript), whereas the narrow delimitation of this taxonomic group (Table 1) includes only 3–5 families (Henssen and Jahns, 1973; Poelt, 1974; Hafellner, 1988; Kirk et al., 2001; Eriksson et al., 2003). Differences in family numbers within the two major types of delimitations are due in part to the recognition of separate families for *Solorina* Ach. and *Nephroma* (Solorinaceae Bayrh. and Nephromataceae Wetm. ex J. C. David & D. Hawksw., respectively) instead of being classified within the Peltigeraceae sensu lato (Table 1). No matter how the Peltigeraceae and Lobariaceae (= Stictaceae sensu Henssen and Jahns, 1973) have been delimited, they have been always included within the Peltigeri-

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TABLE 1. Families included in the Peltigerineae/Peltigerales according to selected classifications/molecular phylogenetic studies compared to the classification proposed here.

Taxon	Henssen and Jahns, 1973	Poelt, 1974	Hafellner, 1988	Rambold and Triebel, 1992	Hafellner et al.[a]	Tehler, 1996	Kirk et al., 2001	Eriksson et al., 2003	Wiklund and Wedin, 2003	This study
Peltigerales	−	−	+	−	−	−	+	−	−	+
Peltigerineae	+	+	−	+	+	+	−	+	+	+
Collematineae	−	−	−	−	−	−	−	−	−	+
Peltigeraceae	+[b,c,d]	+[b]	+	+[b]	+	+[b,d]	+[b]	+[b]	+[b]	+[b]
Solorinaceae	−	−	+	−	+	−	−	−	−	−
Lobariaceae	+	+	+	+	+	+	+	+	+	+
Nephromataceae	−	+	+	+	+	+	+	+	+	+
Placynthiaceae	+	+[e]	+	+	+	+	+	+	+	+
Pannariaceae	−	−	−	+[f]	+[f]	+	−	−	+	+
Coccocarpiaceae	−	−	−	+	+	+	−	−	+	(+)[g]
Collemataceae	−	−	−	+	+	+	−	−	+	+
Massalongia	−	−	?	?	+	−	−	−	+	+

[a] J. Hafellner, H. Hertel, G. Rambold, and E. Timdal, unpublished manuscript.
[b] Solorinaceae included.
[c] Nephromataceae excluded.
[d] *Massalongia* included.
[e] *Massalongia* and *Psoroma* included.
[f] *Psoroma* excluded.
[g] Based on Wiklund and Wedin (2003).

neae/Peltigerales. All authors listed in Table 1 also consider the family Placynthiaceae A. E. Dahl as part of the Peltigerineae/Peltigerales; however, Hafellner (1988; = Lecotheciaceae Körber) suggested that this taxon may not belong in this group. Three additional families, Coccocarpiaceae (Mont. ex Müll. Stuttg.) Henssen, Collemataceae Zenker, and Pannariaceae Tuck., are sometimes considered as members of the Peltigerineae/Peltigerales (Rambold and Triebel, 1992; Tehler, 1996; J. Hafellner, H. Hertel, G. Rambold, and E. Timdal, unpublished manuscript). Otherwise, these three families have been placed within the heterogenous Lecanorales Nannf. (Poelt, 1974; Hafellner, 1988; Kirk et al., 2001), or more specifically in the suborder Lecanorineae (Eriksson et al., 2003).

Circumscriptions of families within the Peltigerineae/Peltigerales differ considerably among recent classifications. Only the family Lobariaceae, with *Lobaria*, *Pseudocyphellaria* Vainio, and *Sticta*, was consistently defined as such in all systems. The classification of several genera (e.g., *Degelia* Arv. & D. J. Galloway, *Leioderma* Nyl., *Leptochidium* Choisy, and *Massalongia* Körber) was sometimes considered unsettled within established families. For example, J. Hafellner, H. Hertel, G. Rambold, and E. Timdal (unpublished manuscript) placed 21 incertae sedis genera into three artificial groups. The establishment of these groups was based, in part, on differences of the ascus apical structure (tubelike vs. caplike). In his 1996 classification, Tehler listed *Fuscoderma* (D. J. Galloway & P. M. Jørg.) P. M. Jørg. & D. J. Galloway and *Siphulastrum* Müll. Arg. under ''unsettled family position'' within the Peltigerineae. Currently, these two genera are considered members of the Pannariaceae (Jørgensen and Galloway, 1992; Jørgensen, 1998; Kirk et al., 2001; Eriksson et al., 2003).

Since the early 1980s (Hafellner, 1984), ascus structure has been regarded as a highly relevant character for revealing relationships among lichen-forming ascomycetes at the genus and family levels. Asci with an amyloid (I+) tubelike structure in the tholus are present in members of the Peltigeraceae (*Peltigera*-type), Coccocarpiaceae, Collemataceae (*Collema*-type), and Placynthiaceae (*Placynthium*-type). In general, the tholus of the Lobariaceae and Pannariaceae has a more or less diffuse amyloid (I+) caplike structure. The Nephromataceae, with its nassasceous ascus, is the only family lacking an amyloid apical structure within the Peltigerineae/Peltigerales (Poelt, 1974; Bellemère and Letrouit-Galinou, 1981; Rambold and Triebel, 1992). The fissitunicate type of dehiscence detected in *Peltigera* using transmission electron microscopy (Honegger, 1978) is believed to occur also in the Nephromataceae (*Nephroma*), *Solorina saccata* (L.) Ach., and Lobariaceae (*Lobaria* and *Sticta*) based on evidence obtained with light microscopy (Richardson, 1970; Eriksson, 1981).

The distribution of secondary compounds across families within the broad selection of peltigeralean lichens sensu lato (s.l.) is heterogeneous. A great diversity of secondary compounds is found within the Peltigeraceae, Nephromataceae, and Lobariaceae (e.g., Culberson, 1969, 1970; Yoshimura, 1971; James and White, 1987; Galloway, 1988, 1991; White and James, 1988; Holtan-Hartwig, 1993; Kondratyuk and Galloway, 1995; Miadlikowska and Lutzoni, 2000). Galloway (1991) suggested that triterpenoids, which are widespread within *Peltigera*, *Pseudocyphellaria*, and *Lobaria*, could be especially useful in detecting evolutionary relationships within and among these genera. A distinctly smaller number of lichen substances (e.g., beta-orcinol depsidones, xanthones, anthraquinones, and triterpenoids) are found in selected genera from the Pannariaceae (e.g., Jørgensen and Galloway, 1992) and Coccocarpiaceae (e.g., Arvidsson, 1982). Taxonomically diagnostic compounds were not reported from members of the Collemataceae and Placynthiaceae (C. Culberson, personal communication).

Hawksworth (1982) proposed that the peltigeralean lichens were part of an ancient lineage within the Pezizomycotina O. E. Erikss. & Winka (Euascomycetes). This hypothesis was based mainly on the following observations: (1) the semifissitunicate ascus, which was thought to be ancestral to unitunicate and bitunicate asci; (2) the exceptionally high number of obligatory lichenicolous fungi growing on Peltigeraceae and Lobariaceae; (3) the wide distribution of many peltigeralean species; (4) the great diversity of secondary metabolites; and (5) the cyanobacterium being one of the earliest potential pho-

tobionts available for the origin of the lichens (Hawksworth, 1982, 1988a, b; Galloway, 1991). Broad molecular phylogenetic studies of the Ascomycota (Eriksson and Strand, 1995; Lutzoni et al., 2001; Kauff and Lutzoni, 2002) refuted this hypothesis.

The first molecular phylogenetic study to include peltigeralean species [*Peltigera neopolydactyla* (Gyelnik) Gyelnik, *Nephroma arcticum* (L.) Torss., and *Solorina crocea* (L.) Ach.] was carried out by Eriksson and Strand (1995) using 28 nucleotide sequences from the nuclear ribosomal subunit (SSU and nrDNA) and representing a broad spectrum of the Ascomycota. The reconstructed relationships allowed the authors to conclude that: (1) the Peltigerales are more closely related to Lecanorales than to, for instance, Helotiales Nannf. (≡ Leotiales Carpenter) or Pezizales C. Bessey; (2) the genus *Solorina* should be included in the family Peltigeraceae; and (3) the genus *Nephroma* deserves to be placed in its own family (Nephromataceae). Based on a combined analysis of the SSU and large subunit (LSU) nrDNA for a broad selection of species from the Ascomycota (52–60 species), Lutzoni et al. (2001) and Kauff and Lutzoni (2002) have demonstrated that the order Peltigerales is nested within the bitunicate ascohymenials (≡ Lecanoromycetidae; Taylor et al., in press). Moreover, the phylogenetic trees published by Kauff and Lutzoni (2002) show the Peltigeraceae (*Peltigera*), Lobariaceae (*Lobaria*), Collemataceae [*Leptogium* (Ach.) Gray], and Placynthiaceae [*Placynthium* (Ach.) Gray] forming a monophyletic and highly supported group.

The only molecular phylogenetic study to date specifically addressing relationships across the Peltigerineae was recently published by Wiklund and Wedin (2003). Based on a combined mitochondrial SSU and nuclear LSU rDNA data set for a broad sampling from the Lecanorales s.l., including all putative peltigeralean families, they concluded that the Peltigerineae (including the Coccocarpiaceae, Collemataceae, Lobariaceae, Nephromataceae, Pannariaceae, Peltigeraceae, and Placynthiaceae) is a monophyletic group. This well-supported suborder was sister to the rest of Lecanorales, suggesting that this was the first evolutionary split, taking place after the origin of the Lecanorales. The parsimony and jackknifing analyses resolved the Lobariaceae as a monophyletic group. Within this family, *Lobaria* and *Sticta* were monophyletic, whereas relationships among *Pseudocyphellaria* species were shown to be unresolved in their two majority-rule consensus trees resulting from parsimony jackknife analyses. They also concluded that the Placynthiaceae is a sister group to the Collemataceae and that *Leptogium* may be paraphyletic. Furthermore, they confirmed the nonmonophyly of the Pannariaceae that was first revealed by the ITS-based phylogenetic study of Ekman and Jørgensen (2002), who also showed that *Degelia* sect. *Amphiloma* (Fr.) P. M. Jørg. & P. James, *Fuscopannaria* P. M. Jørg. subg. *Micropannaria* P. M. Jørg., and *Moelleropsis humida* Gyelnik are not part of the Pannariaceae.

Phylogenetic relationships within the Lobariaceae inferred from ITS sequences were the focus of a recent study by Thomas et al. (2002) and by Stenroos et al. (2003). Based on selected taxa from New Zealand, Thomas et al. (2002) demonstrated that the Lobariaceae and genera *Nephroma*, *Peltigera*, and *Sticta* were monophyletic. These results confirm an earlier report that *Peltigera* is monophyletic (Miadlikowska and Lutzoni, 2000). They have also shown that the genus *Pseudocyphellaria* is a nonmonophyletic entity consisting of three independent lineages, including a monophyletic group of species with a white medulla and two groups of taxa with a yellow medulla. Contrary to Wiklund and Wedin (2003), Thomas et al. (2002) reported that the genus *Lobaria* is not monophyletic. The nonmonophyletic status of *Lobaria* and *Pseudocyphellaria*, as well as the monophyletic delimitation of *Sticta*, was also confirmed by Stenroos et al. (2003). Although molecular phylogenetic studies to date support the broad recognition of the Peltigerineae/Peltigerales including the Coccocarpiaceae, Collemataceae, Placynthiaceae, and Pannariaceae, none of these studies provided well-supported sister-group relationships of these taxa with other members of the Lecanorales s.l.

Because the delimitation of the Peltigerineae/Peltigerales varies remarkably among recent classifications, the main goal of our study was to provide a comprehensive molecular phylogeny for the peltigeralean fungi and establish a new classification if needed. By using a different selection of taxa, molecular markers, and phylogenetic methods that were used by Thomas et al. (2002), Stenroos et al. (2003), and Wiklund and Wedin (2003), we conducted an independent reassessment of the relationships within the Peltigerineae. Our specific objectives were to: (1) circumscribe the Peltigerineae/Peltigerales using monophyly as the grouping criterion; (2) provide a robust estimation of the phylogenetic placement of these lichen-forming fungi within the Ascomycota; (3) test the monophyly of selected genera and families of peltigeralean fungi; (4) evaluate the taxonomic rank (order to genus level) that should be applied to peltigeralean fung; and (5) reconstruct the evolution of symbiotic associations for peltigeralean fungi.

To achieve these goals, phylogenetic analyses of the nuclear LSU and SSU rDNA were conducted using maximum parsimony (MP) and maximum likelihood (ML) as optimality criteria for 113 specimens (59 within the Peltigerineae/Peltigerales), representing 108 species (54 within the Peltigerineae/Peltigerales), and 59 genera (12 within the Peltigerineae/Peltigerales). Representatives of virtually all major lineages of the Ascomycota were included in this study. Bootstrap proportions (BP; Felsenstein, 1985) and posterior probabilities (PP) obtained with Bayesian-Metropolis coupled Markov chain Monte Carlo (B-MCMCMC) tree sampling (Larget and Simon, 1999; Huelsenbeck, 2000) were used to estimate levels of confidence for topological bipartitions revealed by phylogenetic analyses. Symbiotic ancestral states for selected lineages within the Peltigerineae/Peltigerales were reconstructed using ML (Pagel, 1999) as implemented in Mesquite 0.995 (Maddison and Maddison, 2003a, b) on 20 000 trees sampled with B-MCMCMC.

MATERIALS AND METHODS

Taxon sampling—Fifty-three LSU (1.4 kb at the 5′ end) and SSU (1.0 kb at the 5′ end) sequences were selected from previous phylogenetic studies (Lutzoni et al., 2001; Kauff and Lutzoni, 2002) to provide a core phylogenetic framework of the Ascomycota for assessing phylogenetic placement(s) of the peltigeralean fungi. These 53 sequences represent 51 species from 13 of 15 ascomycete orders that include lichen-forming species, 11 of 31 orders incorporating only nonlichenized species (according to the classification of Hawksworth et al., 1995), and two basidiomycetes as outgroup. To these 53 core sequences, we added five LSU and SSU sequences from Schmitt et al. (2001) representing members of the Pertusariineae/Pertusariales M. Choisy ex D. Hawksw. & O. E. Erikss.: two *Ochrolechia* A. Massal. species (Pertusariaceae Körber ex Körber), and three *Coccotrema* Müll. Arg. species (Coccotremataceae Henssen ex J. C. David & D. Hawksw.). This core sampling of 58 specimens (56 species) included four genera from the Peltigerineae/Peltigerales: *Leptogium* (Collemataceae), *Placynthium* (Placynthiaceae), *Lobaria* (Lobariaceae), and *Peltigera* (Peltigeraceae).

We sequenced the same portion of the LSU and SSU nrDNA for an additional 55 specimens (51 species and 10 genera) representing six putative peltigeralean families (Collemataceae, Lobariaceae, Nephromataceae, Pannariaceae, Peltigeraceae, and Solorinaceae). All families included in the Peltigerineae/Peltigerales (except Coccocarpiaceae) under various classifications (Table 1) were represented in our overall sampling of 107 species. Of the 113 specimens included in our study, the 59 peltigeralean specimens (54 species and 12 genera) were considered part of the ingroup. Nine of the 12 genera were represented by at least two species. All putative genera (except *Siphulastrum* Müll. Arg. and *Dendriscocaulon* Nyl.) from Nephromataceae, Lobariaceae, Solorinaceae, and Peltigeraceae (sensu Eriksson et al., 2003) were part of our sampling. For the remaining three families, two genera were selected from the Pannariaceae and Collemataceae (2/14, *Psoroma* and *Pannaria* Del. ex Bory; 2/8, *Collema* F. H. Wigg. and *Leptogium*), and one of seven genera was sampled from the Placynthiaceae (*Placynthium*; following the classification of Eriksson et al., 2003). To test if phenotypic circumscriptions of widely recognized genera are monophyletic, several putative North American and European species from *Lobaria*, *Pseudocyphellaria*, *Sticta* (Lobariaceae), *Nephroma* (Nephromataceae), and *Peltigera* (Peltigeraceae) were selected. For the purpose of this study, we kept the generic concept of *Lobaria* s.l. including *Lobarina* (Vainio) Nyl. ex Cromb. [= *Lobaria scrobiculata* (Scop.) Nyl. ex Crombie group] from before the taxonomic changes introduced by Yoshimura (1998a). Because we wanted to evaluate the intrageneric classification of the genus *Peltigera* that we proposed in a previous paper (eight monophyletic sections based on morphological, chemical, and LSU data; Miadlikowska and Lutzoni, 2000), this genus was represented by the highest number of species (28). Voucher information and GenBank accession numbers for the 59 LSU and SSU nrDNA sequences from the Peltigerineae/Peltigerales included in this study have been archived as supplemental data accompanying the online version of this article.

To diminish the amount of ambiguity in the 113-specimen alignments and to improve phylogenetic resolution and support, we removed 36 sequences belonging to taxa that are clearly outside the Lecanoromycetidae for a second set of analyses. The two *Acarospora* A. Massal. species were used to root the ingroup of the trees derived from these 77-specimen data sets.

Molecular data—Genomic DNA was obtained from fresh samples and herbarium specimens. DNA isolation, symmetric PCR amplification, asymmetric PCR sequencing, PCR product purification, and automated sequencing were performed as presented in Miadlikowska and Lutzoni (2000) and Miadlikowska et al. (2003). The SSU symmetric PCR were done using primers nssu97a, nssu97b, or nssu131 (Kauff and Lutzoni, 2002) at the 5′ end in combination with NS22 (Gargas and Taylor, 1992) at the 3′ end. In addition to these primers, the following set of primers was used for cycle sequencing reactions: SR7, SR7R, SR11R, nssu634, nssu897R, nssu1088, and nssu1088R (Kauff and Lutzoni, 2002). The LSU and SSU sequences were subjected to BLAST searches for a first verification of their identities. They were assembled using Sequencher 4.1 (Gene Codes Corporation, Ann Arbor) and aligned manually with McClade 4.01 (Maddison and Maddison, 2001). The delimitation of ambiguous regions within alignments, their unequivocal coding, and the elaboration of symmetric step matrices for each of these coded characters were done using a method developed by Lutzoni et al. (INAASE 2.3b; 2000).

Phylogenetic analyses—Weighted maximum parsimony (MP) and maximum likelihood (ML) analyses were performed with PAUP* 4.0b.8a (Swofford, 2001). Analyses were done on separate and combined LSU and SSU data sets of 113 and 77 specimens. Parsimony ratchet search strategy (PAUPRat; Nixon, 1999; Sikes and Lewis, 2001) and its modified version for the likelihood search were implemented in PAUP* on selected MP and ML data sets.

Maximum parsimony analyses—Two MP searches were carried out, MP1 on the LSU + SSU 113 operational taxonomic unit (OTU) combined data set and MP2 on the LSU + SSU 77 OTU combined data set. Constant sites and ambiguously aligned sites were removed from the MP searches, and phylogenetic signal from ambiguous regions was recovered using INAASE 2.3b. Unambiguously aligned portions of the LSU and SSU alignments were subjected to symmetric step matrices using the computer program STMatrix 2.1 (written by S. Zoller and available upon request from S. Z. or F. L.) as outlined in Gaya et al. (2003). Gaps were treated as a fifth character state for the unambiguous portions of the alignments. The two MP analyses were performed using heuristic searches with 550 random-addition-sequences (RAS) for MP1 and 1000 RAS for MP2, TBR (tree bisection reconnection) branch swapping, Multrees option in effect, rearrangement limit = 8, and collapsing branches with maximum branch length equal to zero. PAUPRat was implemented with PAUP* on MP1 and MP2 data sets by performing 200 iterations with 15% of characters perturbed per iteration with the same parameters as for the original MP analyses. Branch support for MP1 and MP2 trees was estimated by bootstrap analyses (BP-MP; Felsenstein, 1985) by performing 200 bootstrap replicates with four RAS per bootstrap replicate with the same parameters as for the initial MP analyses.

Maximum likelihood analyses—Two ML searches were carried out, ML1 on the LSU + SSU 113 OTU combined data set and ML2 on the LSU + SSU 77 OTU combined data set. Constant sites were part of ML analyses. A hierarchical likelihood ratio test (Modeltest 3.04 PPC; Posada and Crandall, 1998) was used to select the nucleotide substitution models and parameters for ML searches. The ML1 search was performed using the TIMef+G+I substitution model with equal base frequencies, substitution rate matrix (A↔C = 1.0000, A⟨−⟩G = 2.8365, A⟨−⟩T = 0.6634, C⟨−⟩G = 0.6634, C↔T = 5.2300, and G⟨−⟩T = 1.0000), gamma distribution parameter $\alpha = 0.5165$, and proportion of invariable sites I = 0.5698. The ML2 search was performed using the TrNef+G+I substitution model with base frequencies (A = 0.2965, C = 0.2039, G = 0.2700, and T = 0.2296), substitution rate matrix (A⟨−⟩C = 1.0000, A⟨−⟩G = 2.7081, A⟨−⟩T = 1.0000, C⟨−⟩G = 1.0000, C⟨−⟩T = 6.0870, and G⟨−⟩T = 1.0000), gamma distribution parameter $\alpha = 0.5431$, and proportion of invariable sites I = 0.4159. The ML1 analysis was performed by TBR swapping on the MP1 tree with the Multrees option in effect, reconnection limit = 8, and all branches of effectively zero length collapsed. The ML2 analyses were done using heuristic searches with 50 RAS and the same settings as for the ML1 analysis. To confirm that the most likely tree was found, an additional TBR swapping on the MP2 tree was completed using the same search parameters as for the initial ML2 analysis. PAUPRat was implemented with PAUP* on the ML2 data set by performing 100 iterations with 15% of characters perturbed per iteration with the same parameters as for the initial ML2 analysis.

We used Bayesian posterior probabilities (PP) as implemented in ''MrBayes 2.01'' (Huelsenbeck, 2000) to assess confidence for relationships revealed by ML combined searches. All Bayesian analyses were initiated with random trees and flat priors. One of every 50 trees was sampled for 2 000 000 generations with four chains and substitution parameters updated during the search. To ensure that all chains converged at the same level, majority rule consensus trees were assembled with PAUP* using only the last 20 000 of the 40 000 trees sampled. Internodes with PP ≥95% were considered statistically significant. Throughout this paper, we refer to the Bayesian analysis of the ML1 data set and the resulting majority rule consensus tree as the B/ML1 analysis or tree. In addition to Bayesian searches, phylogenetic confidence for the ML2 data set was estimated with bootstrap resampling (BP-ML). We executed 100 bootstrap replicates with three RAS per bootstrap replicate with the same parameters as for the initial ML2 analysis.

Testing for phylogenetic congruence among data partitions—Before combining the LSU and SSU data sets for 113 and 77 specimens, phylogenetic congruence was assessed for each data partition. For MP analyses, this was done by inspecting internodes with bootstrap scores ≥70% resulting from the separate SSU and LSU bootstrap analyses (Mason-Gamer and Kellogg, 1996; outlined in Miadlikowska and Lutzoni, 2000). For ML analyses, congruence was tested by inspecting internodes with posterior probabilities ≥95% resulting from the separate Bayesian analyses (as outlined in Miadlikowska et al., 2002). Conflicts between the SSU and LSU data partitions were considered significant only if two different relationships (one monophyletic and the

other nonmonophyletic) for the same set of taxa were supported by BP ≥ 70% or PP ≥95%.

Reconstructing symbiotic ancestral states within the Peltigerineae/Peltigerales—Symbiotic ancestral states within the peltigeralean fungi were reconstructed based on the 77-specimen LSU + SSU data set phylogeny (ML2) for nine crucial and significantly supported nodes (PP ≥95%) within the Peltigerineae/Peltigerales. Three character states representing all possible symbiotic associations among peltigeralean fungi (0 = bimembered with a green alga, 1 = bimembered with a cyanobacterium, and 2 = trimembered with a cyanobacterium and a green alga) were considered putative ancestral symbiotic states. Ancestral states were reconstructed with maximum likelihood as the optimality criterion (Pagel, 1999) on the same 20 000 trees sampled with B-MCMCMC (as described earlier) using the Trace Character Over Trees option in Mesquite 0.995 (Maddison and Maddison, 2003a, b). Using a likelihood ratio test, the asymmetric two-parameter model was selected for this analysis. Only ancestral states reconstructed with raw likelihood scores greater than 2.0 (i.e., the default setting T = 2.0 in Mesquite), corresponding to a conservative approximation of proportional likelihood values >0.95 in our analysis, were considered to be significant.

RESULTS

Alignments—The final size of the combined alignment for the 113-specimen data set was 7483 sites (3204 LSU sites and 4279 SSU sites). A total of 5380 sites were excluded from the MP1, ML1, and B/ML1 analyses (2175 LSU sites representing 24 ambiguously aligned regions and introns at 14 splicing sites; 3205 SSU sites representing 17 ambiguous regions and introns at 15 splicing sites). Of the 2103 characters included in the ML1 and B/ML1 searches, 1163 were constant (515 LSU and 648 SSU sites) and 940 were variable (514 LSU and 426 SSU sites). For the MP1 analyses, the 1163 constant sites were excluded and 24 coded INAASE characters replaced the 10 and 14 ambiguously aligned regions from the LSU and SSU data sets, respectively, for a total of 964 variable characters. Of these, 618 were parsimony informative (363 LSU and 255 SSU characters).

The final size of the combined alignment for the 77-specimen data set was 5218 sites (2130 LSU and 3088 SSU sites). A total of 2968 sites were excluded from the MP2, ML2, and Bayesian analyses (1018 LSU sites representing 10 ambiguous regions and introns at six splicing sites; 1950 SSU sites representing four ambiguous regions and introns at 10 splicing sites). Of the 2250 characters included in the ML2 and Bayesian searches, 1635 were constant (774 LSU and 861 SSU sites) and 615 were variable (338 LSU and 277 SSU sites). For the MP2 analyses, the 1635 constant sites were excluded and seven coded INAASE characters replaced the three and four ambiguously aligned regions from the LSU and SSU data sets, respectively, for a total of 622 variable characters. Of these, 401 were parsimony informative (243 LSU and 158 SSU characters).

The LSU and SSU trees were congruent for both the 113- and 77-specimen data sets when using the reciprocal 70% BP and 95% PP criterion. Therefore, the LSU and SSU data sets were combined and analyzed simultaneously using maximum parsimony (MP1 and MP2, respectively) and maximum likelihood (ML1 and ML2, respectively) as optimization criteria.

Comparison of resolution and support among optimality criteria and taxon sampling—The MP1 search on the 113-specimen LSU + SSU combined data set with 24 INAASE characters revealed 60 equally most parsimonious trees (not shown) of 7039.72 steps, which were part of two islands. One island of one tree was hit once out of 550 RAS and the second island of 59 trees was hit 46 times (consistency index [CI, excluding uninformative characters] = 0.3679; retention index [RI] = 0.6751; tree not shown). The 30 most parsimonious trees found with PAUPRat (resulting from 30 of 200 iterations) were the same length as the optimal trees found with the MP1 search and were a subset of the 60 most parsimonious MP1 trees. The level of confidence provided by the bootstrap analysis was relatively low for the deep relationships among ascomycetes and for many internodes within the Peltigerineae/Peltigerales ingroup (Fig. 1).

The MP2 search on the 77-specimen LSU + SSU combined data set with seven INAASE characters revealed eight equally most parsimonious trees (not shown) of 3067.70 steps, which were part of a single island hit 228 times out of 1000 RAS (CI [excluding uninformative characters] = 0.4071; RI = 0.7956; result not shown). The same eight most parsimonious trees were found with PAUPRat, in 62 of 200 iterations. By restricting the outgroup to 18 taxa, the proportion of ambiguous sites decreased from 37%, in the 113-OTU data set (MP1) to 10% in MP2. However, the number of parsimony informative sites, including the INAASE characters, decreased by 217 characters. Despite this, the MP2 analysis resulted in higher phylogenetic resolution than was achieved with MP1 (eight vs. 60 MP1 trees), but the level of confidence provided by BP support was only moderately higher (mostly at the family level within the Peltigerineae/Peltigerales) (Figs. 1 and 2A).

A single most likely tree ($-\ln = 20\,413.471\,43$) was obtained from the ML1 search (not shown). The topology of this tree was almost identical with the B/ML1 tree (Fig. 1) and was similar to the MP1 majority-rule consensus tree. None of the discrepancies among bipartitions was significant when comparing PP support ≥95% in ML1 with BP values ≥70% in MP1. The level of confidence recovered from B/ML1 (Fig. 1) was higher than phylogenetic support obtained by bootstrapping the MP1 data set. Of the 59 nodes with PP ≥95% in B/ML1, 17 received bootstrap support <70% in the MP1 analysis (Fig. 1). Overall, none of the combined analyses on the 113 specimens provided well-resolved and supported deep relationships within the Pezizomycotina (Euascomycetes).

A single most likely tree ($-\ln = 12\,428.611\,70$; part of a single island hit 20 times out of 50 RAS) was revealed by the ML2 analysis (Fig. 2A). The same topology was obtained when swapping on the MP2 tree and when implementing PAUPRat, for which the most likely tree was hit 33 times out of 100 iterations. The same monophyletic groups were found by MP2 and ML2 analyses within the Peltigerineae/Peltigerales. However, 14 of 44 nodes with PP ≥95% in the ML2 received BP-MP support <70% in the MP2 analyses, whereas only eight (mostly short) internodes with significant posterior probabilities received BP-ML support below 70%. Therefore, a maximum likelihood based bootstrap analysis provided confidence values ≥70% for six additional topological bipartitions compared to a maximum parsimony based bootstrap analysis. Only one node with bootstrap value ≥70% in the MP2 was not significant (PP <95%) with the Bayesian inference part of ML2 (Fig. 2A). Phylogenetic relationships within the bitunicate ascohymenials (sensu Lutzoni et al., 2001; Kauff and Lutzoni, 2002) and the Peltigerineae/Peltigerales were better resolved and supported based on this 77-specimen ML2 analysis than on the 113-specimen B/ML1 analysis (Fig. 2A vs. Fig. 1). However, many of the topological bipartitions revealed ex-

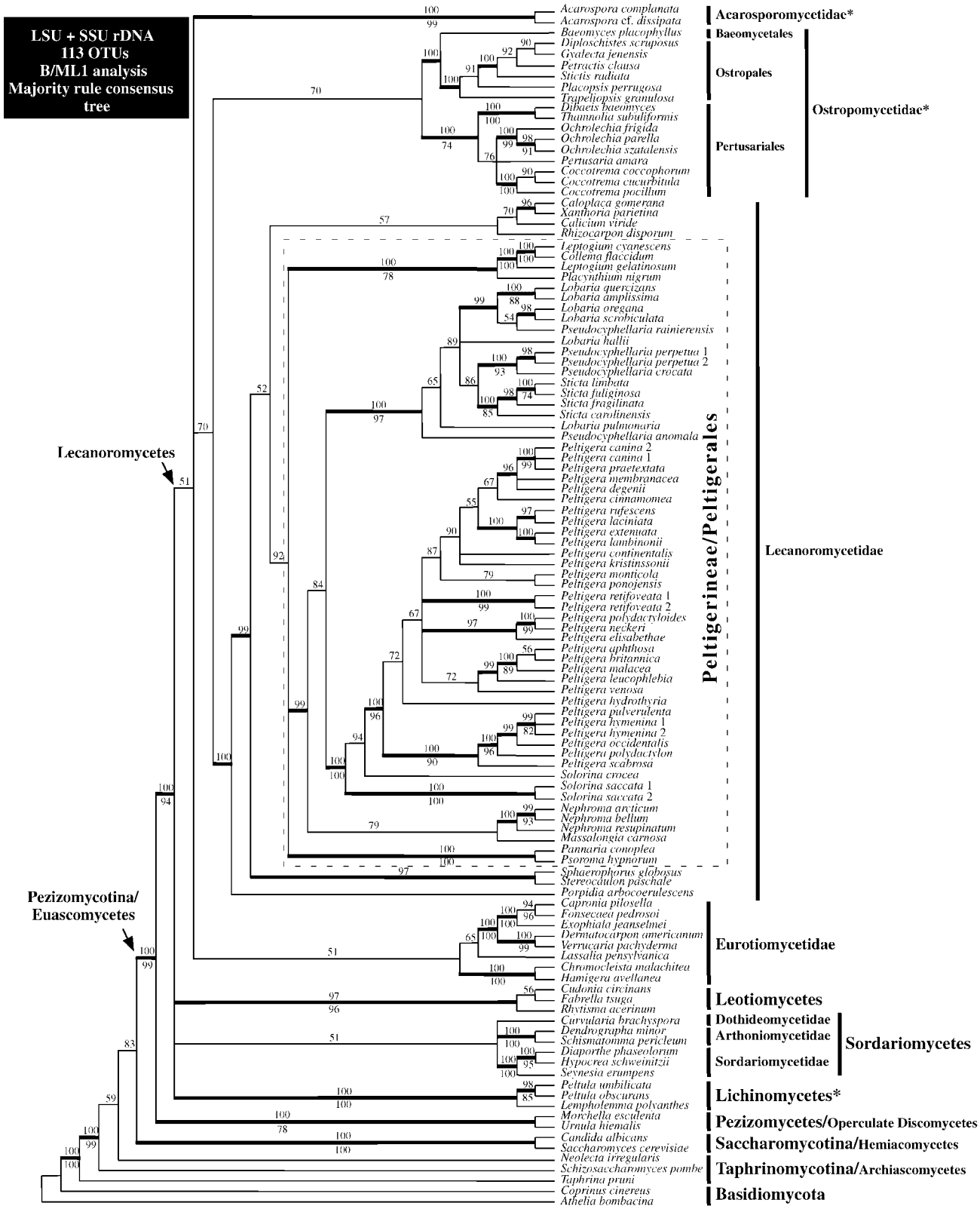

Fig. 1. Phylogenetic placement of putative members of the Peltigerineae/Peltigerales (Collemataceae, Lobariaceae, Nephromataceae, Pannariaceae, Placynthiaceae, Peltigeraceae, and *Massalongia*) within the Ascomycota—B/ML1 analysis. Majority rule consensus tree of 20 000 trees resulting from the Bayesian analysis of the combined nuclear LSU and SSU rDNA data set for 113 individuals that represent 108 species belonging to the Ascomycota and include 59 specimens that represent 54 species from the Peltigerineae/Peltigerales. Two basidiomycete species were used to root the ingroup of these trees. Posterior probabilities (PP) ≥50% are shown above internodes, which are thicker when PP ≥95%. Bootstrap proportions (BP) ≥70% from the MP1 analysis are shown below internodes. Dashed line delimits all peltigeralean lichens included in this study. Taxon names follow, in part, Eriksson et al. (2003), Kauff and Lutzoni (2002), and Taylor et al. (in press). Names followed by an asterisk are new names that will be formally introduced and described in more detail by V. Reeb, F. Lutzoni, and C. Roux (unpublished manuscript).

clusively by ML2 were not significantly supported. Five posterior probabilities became significant with ML2. Six of the internodes that were not found with B/ML1 were statistically significant (PP ≥95%) with the Bayesian inference restricted to 77 taxa. The reverse was also true for five internodes. None of the deep internodes within the Lecanoromycetidae was well supported based on this ML2 analysis.

Phylogenetic relationships within the Ascomycota—In general, the same main Ascomycota lineages as outlined by Lutzoni et al. (2001) and Kauff and Lutzoni (2002) are found in our B/ML1 tree (Fig. 1). Our results differ in the phylogenetic placement of the Pertusariales + Icmadophilaceae Triebel clade. We confirm here the monophyletic status of this group (PP = 100% and BP-ML = 96%; Figs. 1 and 2A). This group was sister to and classified within the bitunicate ascohymenials (node BA) by Lutzoni et al. (2001; but was not significant [PP = 91%]) and Kauff and Lutzoni (2002; PP = 96%, but BP <50%). In our study this group is sister to the Ostropales s.l. + Baeomycetales (Kauff and Lutzoni, 2002) group (but not significant [PP = 70%]; Fig. 1). Relationships within the Pertusariales (i.e., among *Coccotrema*, *Ochrolechia*, and *Pertusaria* DC.) are not well supported in our study. The family Coccotremataceae (represented by the genus *Coccotrema*) was consistently recovered as a highly significant monophyletic group in our study (PP = 100% and BS = 100%), but we could neither confirm nor reject the monophyly of the Pertusariaceae.

When comparing our results to Lutzoni et al. (2001) and Kauff and Lutzoni (2002), statistical significance (PP ≥95%) was maintained for the following major monophyletic groups within the Ascomycota: Pezizomycotina/Euascomycetes, the next internode after the divergence of the Pezizomycetes O. E. Erikss. & Winka (operculate discomycetes; node 2 in Lutzoni et al., 2001), Acarosporomycetidae (Acarosporaceae Zahlbr. in Lutzoni et al., 2001, and Kauff and Lutzoni, 2002; the recognition of this group at the subclass level will be done formally by V. Reeb, F. Lutzoni, and C. Roux, unpublished manuscript), unitunicate ascohymenials including Ostropales s.l. and Baeomycetales (the latter = Baeomycetaceae Dumort.; node 14 in Lutzoni et al., 2001), Leotiomycetes O. E. Erikss. & Winka (Helotiales-Rhytismatales M. E. Barr ex Minter in Lutzoni et al., 2001), Lichinomycetes (= Lichinales Henssen & Büdel in Lutzoni et al., 2001; the recognition of this group at the subclass level will be done formally by V. Reeb, F. Lutzoni, and C. Roux, unpublished manuscript), Pezizomycetes/operculate discomycetes and Saccharomycotina O. E. Erikss. & Winka (Hemiascomycetes). In our study, posterior probabilities dropped below the 95% level of significance for the Lecanoromycetes O. E. Erikss. & Winka (PP = 51%; node 6 ''Lecanoromycotina'' in Lutzoni et al., 2001), Eurotiomycetideae (PP = 51%), and Sordariomycetes O. E. Erikss. & Winka (PP = 51%; node 3 assigned for Arthoniales Henssen ex D. Hawksw. & O. E. Erikss. + Pyrenomycetes-Dothideales Lindau in Lutzoni et al., 2001, and Pyrenomycetideae + Loculoascomycetes in Kauff and Lutzoni, 2002). Within the latter group, Sordariomycetidae and Arthoniomycetidae are both well-supported monophyletic entities (PP = 100%, BP = 100%; Fig. 1). None of our analyses revealed the large order Lecanorales (sensu Eriksson et al., 2003, or Tehler, 1996) as monophyletic (Figs. 1 and 2A). Relationships among lecanoralean genera are not well established, except for the well-supported basal placement of *Porpidia albocoerulescens* (Wulfen) Hertel & Knoph (Cladoniineae sensu Tehler, 1996) in B/ML1 (PP = 99%; Fig. 1) and the close relationship of the ''Caliciales'' C. Bessey with members of the Teloschistales D. Hawksw. & O. E. Erikss. in ML2 (PP = 98%, Fig. 2A).

Phylogenetic delimitation of the peltigeralean fungi—No significant discrepancies were found between phylogenetic trees derived from the 113- and 77-specimen data sets in terms of relationships among peltigeralean fungi (Figs. 1 and 2A). All potential families (Table 1) belonging to the Peltigerineae/Peltigerales (Collemataceae, Placynthiaceae, Lobariaceae, Nephromataceae, Pannariaceae, Peltigeraceae, and Solorinaceae) are nested within the Lecanoromycetidae. They form a single monophyletic group with high support values (PP = 100% and BP-ML value = 81%; Fig. 2A). Based on the MP2 and ML2 analyses (Fig. 2A), two major robust monophyletic groups were reconstructed within the Peltigerineae/Peltigerales: the Pannariaceae + Collemataceae + Placynthiaceae group (PP = 100% and BP-ML = 77%) and the Nephromataceae + *Massalongia* + Lobariaceae + Peltigeraceae + Solorinaceae group (PP = 100% and BP-ML = 84%). The latter group was also revealed in the B/ML1 analysis (PP = 99%; Fig. 1). Based on our taxon sampling, the Collemataceae, Pannariaceae, Lobariaceae, Nephromataceae, and Peltigeraceae were each supported as monophyletic groups (all families PP = 100% and BP-ML ≥95%; Figs. 1 and 2A). Based on the ML2 data set, we found strong evidence for the Collemataceae and Placynthiaceae sharing a most recent common ancestor (PP = 100% and BP-ML = 91%) and for this group being sister to the family Pannariaceae (PP = 100% and BP-ML = 77%; Fig. 2A). The monogeneric family Solorinaceae is shown here as paraphyletic (but not significant), and together with the family Peltigeraceae, they form a monophyletic group (PP and BP = 100%; Figs. 1 and 2A). Phylogenetic placements of the Nephromataceae and *Massalongia carnosa* (Dickson) Körber within the Nephromataceae + *Massalongia* + Lobariaceae + Peltigeraceae + Solorinaceae group could not be resolved here with high confidence.

All analyses revealed *Lobaria*, *Pseudocyphellaria* (Lobariaceae), *Leptogium* (Collemataceae), and *Solorina* (Peltigeraceae) as being nonmonophyletic genera, whereas *Sticta* (Lobariaceae), *Nephroma* (Nephromataceae), and *Peltigera* (Peltigeraceae) were consistently monophyletic (PP = 100% and BP-ML ≥99%; Figs. 1 and 2A). All *Lobaria* species, except *L. pulmonaria* (L.) Hoffm. and *L. hallii* (Tuck.) Zahlbr. form a robust monophyletic entity together with *Pseudocyphellaria rainierensis* Imshaug (PP ≥98%; Fig. 2A). *Lobaria pulmonaria* (type species) belongs to a second distinct lineage of *Lobaria*, whereas the phylogenetic placement of *L. hallii* is unresolved. *Pseudocyphellaria* is partitioned into three separate lineages represented by *P. anomala* Brodo & Ahti (at the base of the Lobariaceae), *P. perpetua* McCune & Miadlikowska + *P. crocata* (L.) Vainio (nested within the Lobariaceae; PP = 100% and BP ≥93%), and *P. rainierensis* (part of the *Lobaria* group).

All *Peltigera* sections represented by more than one specimen were monophyletic and all, except sect. *Horizontales* Miadlikowska & Lutzoni, were highly supported (PP ≥95%; Fig. 2A). Phylogenetic relationships among sections are not well supported, except for the sister relationship of sect. *Peltigera* and *Retifoveatae* Miadlikowska & Lutzoni (PP >95%) and their sister relationship to sect. *Horizontales* (PP >95%; Fig. 2A).

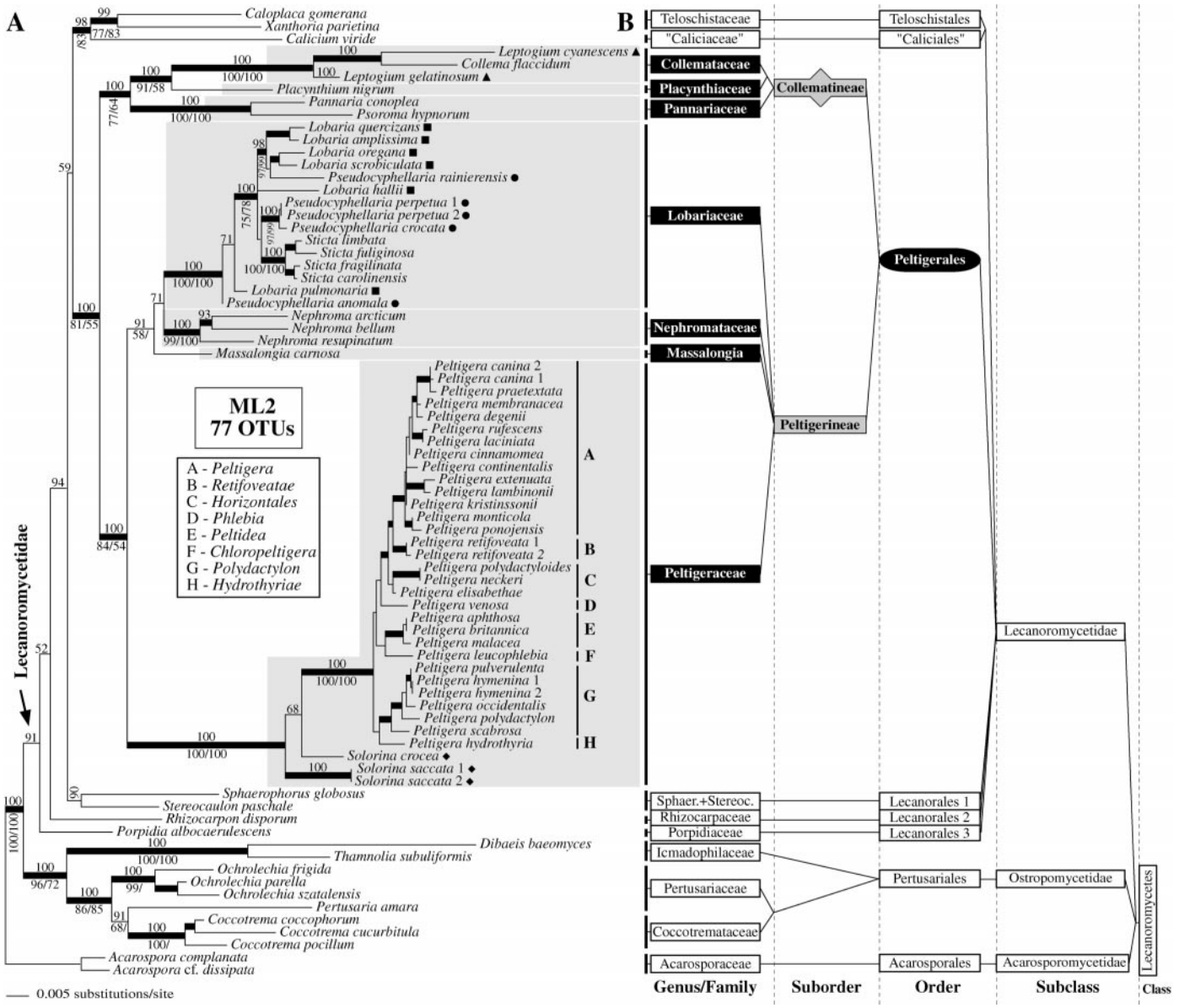

Fig. 2. Phylogenetic relationships among peltigeralean fungi and consequences of statistically significant monophyletic groups revealed by this study on alternative classifications. (A) Single most likely tree (ln likelihood = −12 428.611 70) revealed from the phylogenetic analysis (ML2) of the combined nuclear LSU and SSU rDNA for 77 individuals representing 72 species from the Lecanoromycetidae (including 59 specimens representing 54 species from the Peltigerales), Pertusariales, and Acarosporomycetidae. Except within genera, where posterior probabilities and bootstrap proportions are not included, PP ≥50% are shown above internodes and BP ≥50% are shown below internodes (BP-ML before and BP-MP after the slash). Internodes with PP ≥95% are represented by thicker lines. Shaded areas of the tree delimit families and one genus (*Massalongia*) within the Peltigerales. Symbols following names indicate species part of nonmonophyletic genera. Capital letters (A-H) within the genus *Peltigera* refer to the eight sections defined by Miadlikowska and Lutzoni (2000). (B) Schematic representation of the final classification proposed here for part of the Lecanoromycetes, with special emphasis on the Peltigerales, based on B/ML1 and ML2 trees (Figs. 1 and 2A). (C) Schematic representation of ranking inconsistencies and violation of monophyly when directly integrating phylogenetic relationships revealed by our study into the recent classification of the Ascomycota by Eriksson et al. (2003). Wavy lines highlight discrepancies between highly supported phylogenetic relationships revealed by this study and the classification proposed by Eriksson et al. Rank in quotation mark and question mark at the bottom of the figure indicate the lack of ranks in the classification by Eriksson et al. (2003) that are needed to accommodate the phylogenetic relationship reported here. Question marks in small vertical ovals indicate strongly supported relationships for taxa that were considered incertae sedis in Eriksson et al. (2003). Question marks in diamonds show the lack of a name for a monophyletic group at a given rank or the lack of a rank for a monophyletic group in the current classification by Eriksson et al. (2003). (D) Schematic representation of an alternative classification considered in this study in which the two major groups of peltigeralean fungi are recognized at the ordinal level. The question mark at the bottom of the figure indicates the lack of rank for the monophyletic peltigeralean fungi (black diamond with question mark) under this system. (B), (C), and (D). All taxonomic entities that are part of the Peltigerales are shown with a black background. Horizontal ovals represent taxonomic units that are part of the Peltigerales with the highest possible rank within various classification schemes. Gray boxes highlight suborders Collematineae and Peltigerineae. The oval and the box with diamonds indicate new taxon names (e.g., Collematineae).

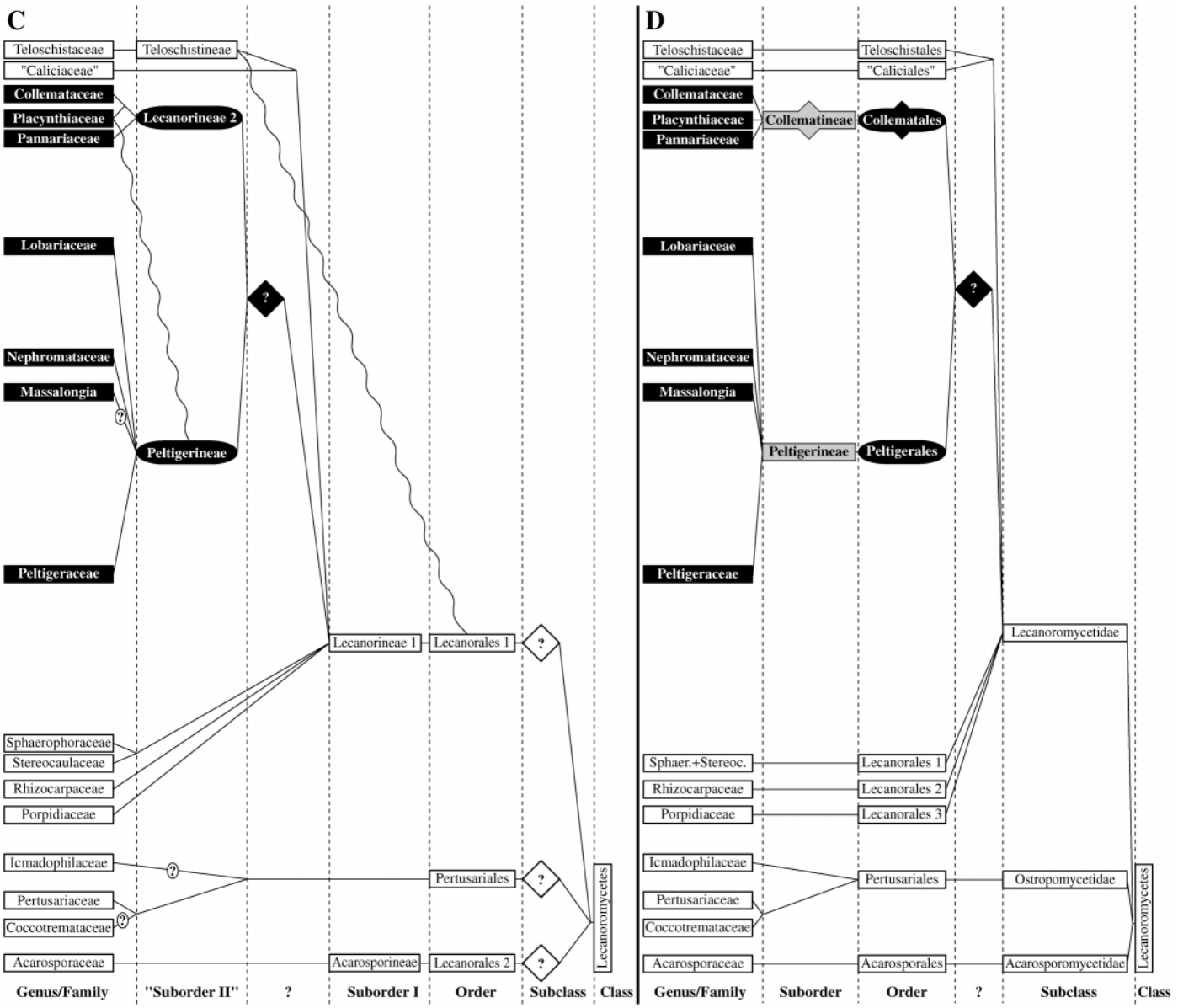

Fig. 2. Continued.

Reconstructing symbiotic ancestral states within the Peltigerineae/Peltigerales—We reconstructed ancestral symbiotic states for nine statistically significant nodes within the Peltigerineae/Peltigerales monophyletic group. For four nodes (2, 3, 5, and 6), the bimembered symbiotic state with cyanobacterium was significant (raw likelihood values >2.0) on 100% of the trees sampled from the posterior distribution (Fig. 3). This symbiotic state was also predominantly significant for nodes 1, 7, and 9, with frequencies ranging from 86 to 94%. Nodes 4 and 8 were mostly nonsignificant. The trimembered state was significant only for three nodes: 4, 8, and 9, but at a very low frequency of 1%. The bimembered symbiotic state with a green alga was never found to be highly probable for any of the ancestral nodes within the peltigeralean clade.

DISCUSSION

Phylogenetic relationships within the Ascomycota—Our results from the phylogenetic analysis of the combined 113-specimen data set (Fig. 1) are either in agreement with other global Ascomycota phylogenies (e.g., Lutzoni et al., 2001; Kauff and Lutzoni, 2002; Lumbsch et al., 2002) and the phylogenetic summary by Taylor et al. (in press) or the topological inconsistencies are not significant. For example, some of the highly supported groups recovered by Lutzoni et al. (2001) and Kauff and Lutzoni (2002) obtained posterior probabilities that were lower than the 95% PP threshold value in our study (e.g., monophyly of the Eurotiomycetidae and Sordariomycetes). This might be due to our more extensive taxon sampling designed to address phylogenetic relationships among peltigeralean fungi.

Our B/ML1 analysis (Fig. 1) indicates that the Acarosporomycetidae (= Acarosporineae sensu Eriksson et al., 2003) and the Pertusariales might be outside of the Lecanoromycetidae (= Lecanorales sensu Eriksson et al.). The close relationship shown here between the Ostropales (sensu Kauff and Lutzoni, 2002) and Pertusariales + Icmadophilaceae (= Os-

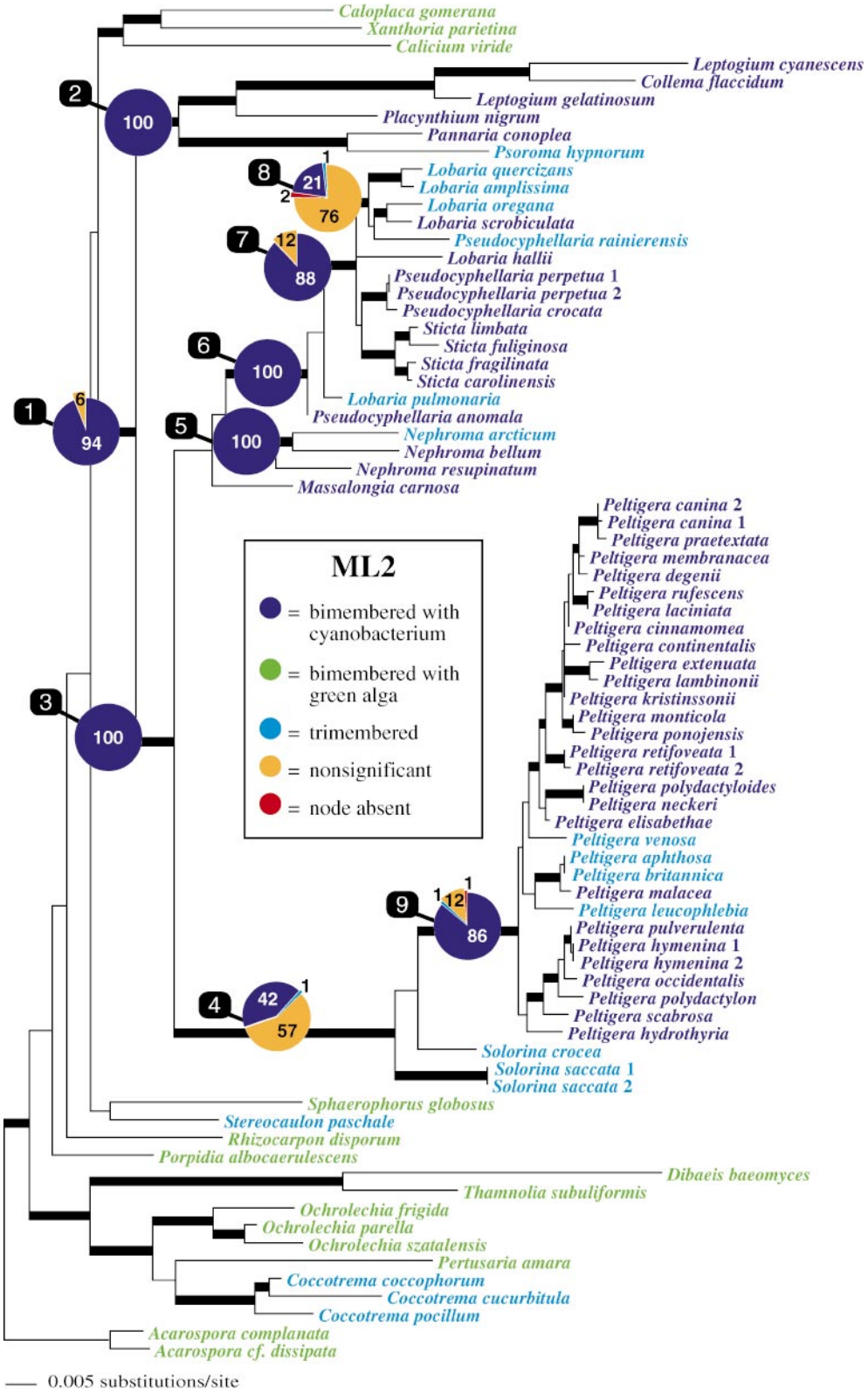

Fig. 3. Evolution of symbiotic associations within the Peltigerales shown on the ML2 tree (from Fig. 2A). Ancestral symbiotic states were reconstructed for nine nodes across 20 000 Bayesian trees using maximum likelihood (Pagel, 1999) as implemented in Mesquite 0.995 (Maddison and Maddison, 2003a, b). Percentages in pie charts refer to the percentage of trees for which the reconstructed ancestral state (see key) was significant at that node. Internodes with PP ≥95% are represented by thicker lines (see Fig. 2A).

tropomycetidae in Figs. 1 and 2), although not well supported (Fig. 1), differs from the results obtained by Kauff and Lutzoni (2002) in which the latter group was shown to be part of the bitunicate ascohymenials (= Lecanoromycetidae) with significant support. The recognition of the Ostropomycetidae will be done formally by V. Reeb, F. Lutzoni, and C. Roux (unpublished manuscript). This affiliation of the Pertusariales + Icmadophilaceae to the Ostropales + Baeomycetales group was also recovered, but with poor support, by Bhattacharya et al. (2000). Lumbsch et al. (2002) presented some evidence for the close relationship between members of the Pertusariales and Ostropales sensu Kauff and Lutzoni (2002), i.e., including Agyriales Clem. & Shear. Ekman and Tønsberg (2002) have also shown that *Coccotrema*, *Ochrolechia*, and *Pertusaria* are nested within the Ostropales sensu Kauff and Lutzoni (2002) with high posterior probability (PP = 96%).

The Pertusariaceae is the only family common to all previous classifications of the Pertusariineae/Pertusariales (e.g., Hafellner, 1988; Tehler, 1996; Eriksson et al., 2003). Lumbsch et al. (2001), based on a combined LSU and SSU nrDNA data set, found that the Coccotremataceae and Pertusariaceae form two monophyletic sister groups, which they refer to as the Pertusariales. Despite this result, Eriksson et al. (2003) maintains that the Coccotremataceae is a family with an uncertain position within the Ascomycota. Using a very different taxon sampling than Lumbsch et al. (2001), we confirm here the monophyly of the Pertusariaceae + Coccotremataceae group (PP = 100% and BP ≥85%, Fig. 2A). Therefore, the latter family is clearly part of the Pezizomycotina, outside the Pezizomycetes (PP = 100%; Fig. 1). However, compared to Lumbsch et al. (2001), we cannot decipher statistically between *Pertusaria* being sister to *Ochrolechia* vs. *Coccotrema* (Figs. 1 and 2A). Given that the Coccotremataceae is a monogeneric family (Schmitt et al., 2001) and sometime forms a strongly supported monophyletic group with members of the Pertusariaceae (Fig. 2A), subsuming Coccotremataceae within the Pertusariaceae should be considered in future studies.

We found with high statistical confidence that the Pertusariaceae + *Coccotrema* form a monophyletic entity with the Icmadophilaceae (PP = 100% and BP-ML = 96%; Figs. 1 and 2A). This robust sister relationship was revealed also by Lutzoni et al. (2001) and Kauff and Lutzoni (2002). Platt and Spatafora (2000), in their molecular phylogenetic study of the family Icmadophilaceae, rejected the inclusion of this family within the Lecanorales, but could not provide evidence favoring its affiliation with the Helotiales as was suggested earlier (Rambold et al., 1993; Tehler, 1996; Kirk et al., 2001). Eriksson et al. (2003) consider this family to be a familiae incertae sedis within the Lecanoromycetes. Based on former phylogenetic treatments that included this family, and based on the results from our study, we conclude that the Icmadophilaceae are part of the Pertusariales, an order that may belong to the Ostropomycetidae (Figs. 1 and 2A).

Phylogenetic relationships among peltigeralean fungi—Analyses of the 113- and 77-specimen LSU + SSU data sets showed that all putative peltigeralean families (Collemataceae, Placynthiaceae, Pannariaceae, Nephromataceae, Lobariaceae, Solorinaceae, and Peltigeraceae) and *Massalongia* together form a strongly supported monophyletic group within the Lecanoromycetidae (PP = 100% in the ML2 and BP-ML = 81%; Fig. 2A). This placement of the peltigeralean fungi among the derived lineages within the Ascomycota (Fig. 1) provides further evidence for the rejection of the hypothesis of the ancient origin of these lichen-forming fungi introduced by Hawksworth (1982, 1988a, b). Because of the phylogenetic relationships among peltigeralean fungi within the Lecanoromycetidae (Fig. 2A) and the current circumscription of the Lecanoromycetes according to Eriksson et al. (2003), we propose here that the monophyletic peltigeralean fungi be recognized at the order level (Peltigerales) as in previous classifications of the Ascomycota (e.g., Hafellner, 1988; Kirk et al., 2001; Table 1). With the exception of the Coccocarpiaceae (not part of our study), our revised circumscription of the Peltigerales is identical to the Peltigerineae sensu Wiklund and Wedin (2003), a study based on a combined mitochondrial SSU and nuclear LSU rDNA data set. Our phylogenetic delimitation of the peltigeralean fungi supports the broad taxonomic concept of these lichens by Rambold and Triebel (1992), Tehler (1996), and J. Hafellner, H. Hertel, G. Rambold, and E. Timdal (unpublished manuscript), except that they all recognized this taxonomic entity at the subordinal level (Peltigerineae) instead of the ordinal level (Peltigerales; Table 1).

Our study revealed two strongly supported monophyletic groups at the base of the Peltigerales (Fig. 2A). These two main peltigeralean groups (group 1 = Pannariaceae + Placynthiaceae + Collemataceae and group 2 = Nephromataceae + *Massalongia* + Lobariaceae + Peltigeraceae + Solorinaceae) differ from the two clades (group 1 = Pannariaceae and group 2 = the rest of the peltigeralean fungi) defined by the basal dichotomy of the study by Wiklund and Wedin (2003). However, because basal peltigeralean relationships received low support values or were unresolved by their parsimony jackknife analysis, this descrepancy between their study and our study does not appear to be significant.

We propose that the two well-supported monophyletic groups at the base of the Peltigerales, reported here, be recognized at the suborder level (Fig. 2B). One of these two groups corresponds to the Peltigerineae sensu stricto, which includes the Lobariaceae, Nephromataceae, Peltigeraceae, and *Massalongia* (Table 1). The second group is a new suborder, Collematineae, which includes the Collemataceae, Pannariaceae, and Placynthiaceae.

Suborder—Collematineae Miadlikowska & Lutzoni subordo nov.

Diagnosis—A subordine Peltigerineae differt in morphologia et anatomia thallorum. In contrario ad ea thalli crustosi et squamulosi aut gelatinosi homoeomericique frequenter occurrentes. Thalli foliosi si presentes numquam heteromerici. Substanciae chimicae utiles in taxonomia plerumque deficientes.

Description—Lichen-forming ascomycetes. Mostly bipartite with cyanobacteria, rarely tripartite. Thalli non- or gelatinous, squamulose, foliose, or subfruticose, rarely crustose; homoiomerous or heteromerous. Cortex present on both sides of the thallus or lower side ecorticated and sometimes with dark prothallus. Vegetative propagules (soredia, isidia, or phyllidia) common. Apothecia immersed or sessile; lecideine, biatorine, or lecanorine. Paraphyses simple or branched, sometimes strongly gelatinous. Ascospores aseptate, one- to multiseptate, or muriform, colorless. Asci with an amyloid external wall layer, I+ blue. Secondary substances uncommon or absent. On various substrates including stones, soil, bark, and mosses, often in moist habitats.

Type—Collemataceae Zenker.

Familiae alterae—Pannariaceae Tuck. and Placynthiaceae Å. E. Dahl.

Remarks—The suborder Collematineae includes peltigeralean fungi of varying morphological forms, but never species with conspicuous heteromerous thalli typical of Peltigerineae species. All species with gelatinous and homoiomerous thalli classified within the Peltigerales (Collemataceae), except the cyanomorph of *P. venosa* (L.) Hoffm. (Peltigeraceae), are part of the Collematineae. Members of this suborder are predominantly bipartite, forming associations mostly with the cyanobacteria *Nostoc* and *Scytonema*, but also with cyanobacteria of the *Dichotrix* type (e.g., in the Placynthiaceae). Unlike the Peltigerineae, tripartite symbioses are rarely found in this group (only in the Pannariaceae) and bipartite associations with green algae have never been reported. Contrary to the great diversity of secondary compounds found in the Peltigerineae, very few of these substances are detectable by TLC in the Collematineae. Asci with an amyloid tholus and colorless ascospores are two common characteristics of the Collematineae.

In his discussion of morphological and anatomical features of the genus *Coccocarpia* Pers., Arvidsson (1982) suggested that members of the Coccocarpiaceae where likely to be closely related to peltigeralean taxa such as Placynthiaceae, Peltigeraceae, and especially Pannariaceae. Based on the phylogenetic placement of *Steinera glaucella* (Tuck.) Dodge, the Coccocarpiaceae (at least in part) seems to be a member of the Peltigerales (Wiklund and Wedin, 2003). However, because its relationship within the Peltigerales is unresolved, the classification of this family at the subordinal level is unsettled at this time.

The Arctomiaceae Th. Fr., a small family of two genera (*Arctomia* Th. Fr. and *Wawea* Henssen & Kantvilas) associated with *Nostoc*, could be considered as another member of the Peltigerales. Henssen and Kantvilas (1985) demonstrated that there were structural similarities between *Wawea* and members of the Collemataceae and Coccocarpiaceae. Despite this phenotypic evidence, the Arctomiaceae have never been classified within the Peltigerineae or Peltigerales. Currently, this family is classified within the Lecanorales (Kirk et al., 2001) or in the Lecanorineae (Eriksson et al., 2003).

With the exception of the Placynthiaceae, our phylogenetically based circumscription of the suborder Peltigerineae is very similar to the narrow delimitation of the Peltigerales sensu Hafellner (1988) and Kirk et al. (2001), as well as of the Peltigerineae sensu Poelt (1974) and Eriksson et al. (2003; Table 1). As for the studies by Wiklund and Wedin (2003) and Thomas et al. (2002), the phylogenetic placement of the Nephromataceae sister to the Lobariaceae is not well supported at this time (Fig. 2A). We confirm here the monophyly of four of the six peltigeralean families (Fig. 2A): Collemataceae, Lobariaceae (shown earlier by Thomas et al., 2002 and Wiklund and Wedin, 2003), Nephromataceae, (shown earlier by Lohtander et al., 2002 and Thomas et al., 2002), and Peltigeraceae (shown earlier by Miadlikowska and Lutzoni, 2000). The monogeneric family Solorinaceae is reconstructed here as paraphyletic; however, its monophyly cannot be rejected due to the nonsignificant support obtained for its paraphyly (PP = 94% in B/ML and PP = 68% in ML2; Figs. 1 and 2). The highly supported monophyly of the *Solorina* + *Peltigera* group (PP and BP = 100%), which was also reported earlier by Miadlikowska and Lutzoni (2000), and the very likely paraphyly of *Solorina*, strongly support the recognition of the genus *Solorina* within the family Peltigeraceae. The latter circumscription is in agreement with all classifications of the lichen-forming ascomycetes proposed during the last 10 years (Table 1).

The family Pannariaceae is shown here forming a monophyletic group; however, the inclusion of *Degelia* in analyses by Wilkund and Wedin (2003; another putative member of the Pannariaceae according to Jørgensen and Galloway, 1992, and Eriksson et al., 2003) resulted in a nonmonophyletic delimitation of this family. Based on an extensive sampling, Ekman and Jørgensen (2002) demonstrated that the Pannariaceae (part of the Collematineae in this study; Fig. 2A and B) represents a polyphyletic assemblage and concluded that *Degelia*, *Fuscopannaria*, *Moelleropsis humida*, and perhaps *Parmeliella* Müll. Arg. do not belong to this family.

In addition to *Solorina* (Peltigeraceae), three genera were found to be nonmonophyletic: *Leptogium* (Collemataceae), *Lobaria*, and *Pseudocyphellaria* (Lobariaceae; Fig. 2A and B). In light of this new information, these four genera are in need of a comprehensive phylogenetic revision based on both phenotypic and genotypic evidence. Closer affiliation of *Leptogium cyanescens* (Rabenh.) Körber with *Collema* species than with other *Leptogium* species reported by Wiklund and Wedin is confirmed here with high support values. Based on ITS sequences, Thomas et al. (2002) and Stenroos et al. (2003) found that *Pseudocyphellaria* and *Lobaria* species are intercalated. In our study, *P. rainierensis* is nested within the main *Lobaria* clade, and *P. anomala* with *L. pulmonaria* forms a paraphyletic group at the base of the Lobariaceae (Fig. 2A). Contrary to the study by Thomas et al. (2002), restricted to *Pseudocyphellaria* species from New Zealand, our phylogenetic analysis of North American species shows the *Pseudocyphellaria* species with a white medulla (represented by *P. rainierensis*, *P. crocata*, and *P. anomala*) to be part of at least three separate lineages within the Lobariaceae. These results confirm the nonmonophyletic delimitation of this *Pseudocyphellaria* group revealed by Stenroos et al. (2003) based on *P. crocata* and *P. anomala* and the possible paraphyly of *P. aurata* (Ach.) Vainio, *P. crocata*, and *P. divulsa* (Taylor) Imshaug suggested by Wiklund and Wedin (2003).

As with *Pseudocyphellaria*, the genus *Lobaria* consists of more than one taxonomic unit, as was anticipated by Yoshimura (1998a) based on phenotypic characters and demonstrated phylogenetically by Thomas et al. (2002) and Stenroos et al. (2003). Our results confirm that *Lobaria pulmonaria* (part of the *L. pulmonaria* group; Yoshimura, 1971, 1998b) represents a lineage distinct from the remaining *Lobaria* species included in our data sets. Therefore, the name *Lobaria* should be restricted to species that share a most recent common ancestor with *L. pulmonaria*. Wiklund and Wedin (2003) obtained a different result, where *Lobaria* species [including *L. amplissima* (Scop.) Forss., *L. pulmonaria*, *L. retigera* (Bory) Trevisan, *L. scrobiculata* (Scop.) DC., and *L. virens* (With.) Laundon] form a well-supported monophyletic group. The use of mitochondrial rDNA sequences by Wiklund and Wedin (2003) might be the cause of this discrepancy with studies restricted to DNA sequences from the nuclear ribosomal tandem repeat unit. However, phylogenies derived from separate jackknife analyses of their nuclear and mitochondrial rDNA data sets were not statistically incongruent according to the authors.

The genus *Massalongia* is clearly a member of the suborder Peltigerineae (Fig. 2A and B). In Wiklund and Wedin (2003) and in our study (Fig. 1), this genus was shown to be sister to the genus *Nephroma*. However, the phylogenies from both studies are too uncertain to establish the precise affiliation of this genus within this suborder (see Figs. 1 and 2A).

Our study also confirms the monophyly of four of the eight sections [*Peltidea* (Ach.) Vainio, *Peltigera*, *Polydactylon* Miadlikowska & Lutzoni, and *Retifoveatae*] we delimited (Miadlikowska and Lutzoni, 2000) within *Peltigera* based on morphology, chemistry, and LSU nrDNA. With this new study, we did not obtain additional support for phylogenetic relationships among sections within *Peltigera*. *Peltigera hydrothyria* Miadlikowska & Lutzoni (2000; ≡ *Hydrothyria venosa* Russ.) is clearly a member of the monophyletic and highly supported genus *Peltigera* (Fig. 2A).

New classification for the peltigeralean and lecanoralean fungi—Based on our ML2 tree (Fig. 2A), we propose here a new classification for the peltigeralean fungi consisting of the order Peltigerales and two suborders—Collematineae and Peltigerineae (Table 1; Fig. 2B). Before proposing this new classification, we evaluated the most recent classification of the peltigeralean fungi (Eriksson et al., 2003) to determine if it could accommodate the new and statistically significant phylogenetic relationships revealed by our study. However, this was not possible because the taxonomic ranks in the classification by Eriksson et al. were inconsistent within the Lecanoromycetes (Fig. 2C).

These inconsistencies partly result from the recognition of the peltigeralean fungi at a rank that is too low because of their inclusion within the order Lecanorales under the classification system of Eriksson et al. (2003). Another consequence of this current classification is the lack of ranks below the ordinal level to classify taxa within the Lecanorales sensu Eriksson et al. (2003; see Fig. 2C). Therefore, the delimitation and ranking of the Lecanorales is at the core of this problem. Our solution to this problem is to recognize Lecanorales sensu Eriksson et al. at the subclass level (Lecanoromycetidae), which allows the recognition of the peltigeralean fungi at the ordinal level (Peltigerales; Fig. 2B). This adjustment to the classification by Eriksson et al. is derived from a bottom-up approach focusing on the Peltigerales (this study), Ostropales (Kauff and Lutzoni, 2002), and Acarosporaceae (V. Reeb, F. Lutzoni, and C. Roux, unpublished manuscript)—phylogenetic studies conducted within a broad and extensive sampling across the ascomycetes. These core alignments (SSU and LSU nrDNA) for representatives selected across the Ascomycota were generated from a top-down approach (Lutzoni et al., 2001). We believe that this concerted top-down and bottom-up approach will lead to a coherent and stable classification of the Ascomycota. A first summary of the overall classification of the Ascomycota resulting from this approach is presented in Taylor et al. (in press). The classification proposed here is in total agreement with this latter, summarized classification.

Our newly delimited Peltigerineae (Lobariaceae, Nephromataceae, and Peltigeraceae) includes all members of the suborder Peltigerineae sensu Eriksson et al. (2003), except for the exclusion of *Massalongia*, which was considered an incertae sedis within the Lecanorales by Eriksson et al., and the exclusion of the Placynthiaceae, which is nested within a different monophyletic group (Collematineae) according to our phylogenetic analyses (Fig. 2B and C). The two remaining families of peltigeralean fungi (Collemataceae and Pannariaceae), part of the Collematineae based on our study (Fig. 2B), were classified in the suborder Lecanorineae by Eriksson et al. (2003; Lecanorineae 2 in Fig. 2C). The placement of the Collemataceae and Pannariaceae in the Lecanorineae by Eriksson et al. was sufficient to render their circumscriptions of the Peltigerineae and Lecanorineae to be polyphyletic. The broad circumscription of the Lecanorineae by Eriksson et al. (2003) also resulted in suborders being nested within other suborders (e.g., Peltigerineae and Lecanorineae 2 nested within Lecanorineae 1) and the lack of a rank (Fig. 2C) for the monophyletic peltigeralean fungi (= Peltigerales in Fig. 2B). This broad circumscription of the Lecanorales by Eriksson et al. (2003) also caused the Lecanorineae (i.e., Lecanorineae 1 in Fig. 2C) to include one member (''Caliciaceae'') of a monophyletic group, but not the other (Teloschistaceae Zahlbr.). The phylogenetic relationships inferred from our study also demonstrate that with the exception of the Acarosporineae and Teloschistineae, the circumscription of the Lecanorineae sensu Eriksson et al. (2003; = Lecanorineae 1 in Fig. 2C) is redundant with their delimitation of the order Lecanorales (Lecanorales 1 in Fig. 2C).

To avoid violating monophyly as a grouping criterion and to have sufficient ranks to accommodate all major lineages of lecanoralean fungi, the classification of Eriksson et al. (2003) needs to be modified. Either the Placynthiaceae should be excluded from the Peltigerineae (sensu Eriksson et al.), or the Collemataceae and Pannariaceae should be classified within the Peltigerineae (sensu Tehler, 1996; Table 1) or within the Peltigerales (as proposed here; Fig. 2A). This latter change in classification also requires that two members of the sister group to the Peltigerineae (Collemataceae and Pannariaceae) be removed from the Lecanorineae (= Lecanorineae 2 in Fig. 2C) and that the sister group be recognized at the suborder level—Collematineae (Fig. 2B). Further changes are needed because no rank is available for the monophyletic group composed of the Peltigerineae and Collematineae (Fig. 2B). We propose here the use of the Peltigerales for this group (Fig. 2B), which simultaneously provides a basis to redelimit the Lecanorales by excluding the ''Caliciaceae'' and Teloschistineae from the Lecanorales sensu Eriksson et al. (2003). To our knowledge, this is a novel circumscription for the Peltigerales. In the past, the Peltigerales included only the Peltigeraceae, Lobariaceae, Nephromataceae, and Placynthiaceae, whereas the Peltigerineae was most often more comprehensive by also including the Pannariaceae, Coccocarpiaceae, and Collemataceae (Table 1). Our new classification of the peltigeralean fungi resolves the inconsistency between ranks and groups inherent to past classifications.

This consequential recircumscription of the Lecanorales requires a taxonomic reassessment of the ''Caliciales'' and Teloschistales (Fig. 2B). The family ''Caliciaceae'' was recently shown to be part of the *Buellia*-group within the Physciaceae Zahlbr. (Wedin et al., 2002; Wiklund and Wedin, 2003). As one of the consequences of this phylogenetic relationship, Wedin and Grube (2002) proposed to use the name Physciaceae to accommodate this newly delimitated group (Physciaceae + ''Caliciaceae''). If this nested phylogenetic placement of the ''Caliciaceae'' is confirmed, the ''Caliciales'' should be reinstated to refer to the Physciaceae + ''Caliciaceae'' group. Although the order Lecanorales sensu Kirk et al. (2001) is not monophyletic (due to the inclusion of the ''Caliciaceae'' and

Acarosporaceae), these authors recognize the Teloschistaceae/Teloschistineae at the ordinal level (Teloschistales).

If the suborder rank is used for all peltigeralean fungi (Peltigerineae ≡ Peltigerales in Fig. 2B), the two major lineages (Collematineae and Peltigerineae; Fig. 2B) would have to be recognized taxonomically at the family level (Collemataceae and Peltigeraceae). This change would have a major impact on the current family concept within the peltigeralean fungi by eliminating four widely accepted family names (Lobariaceae, Nephromataceae, Pannariaceae, and Placynthiaceae). Another potential solution would be to recognize the two major peltigeralean lineages at the order level—Peltigerales and Collematales (Fig. 2D). However, this taxonomic scenario would leave no rank for the Collematales + Peltigerales clade.

To accommodate all new and strongly supported phylogenetic relationships among and outside of the peltigeralean fungi reported here, Eriksson's classification (Eriksson et al., 2003) requires a re-delimitation of the Lecanorineae and Lecanorales. The use of subclasses Acarosporomycetidae, Eurotiomycetidae, Lecanoromycetidae (= Lecanoromycetes in Kirk et al., 2001), and Ostropomycetidae within the Lecanoromycetes (based on previous studies by F. L. [Lutzoni et al., 2001; Kauff and Lutzoni, 2002] and summarized in Taylor et al. [in press]), and the fragmentation of the Lecanorales into smaller monophyletic orders within this class are crucial to the establishment of a coherent phylogenetic classification of the Ascomycota.

Evolution of symbiotic associations within the Peltigerales—As circumscribed here, most members of the Peltigerales are associated with a cyanobacterium (= bimembered symbiosis with a cyanobacterium). A subgroup of these lichens have a green algal photobiont in addition to their cyanobacterial partner (= trimembered symbiosis). The few remaining species are associated strictly with a green alga (= bimembered symbiosis with a green alga). With the exception of the Arctomiaceae and Lichinales Henssen & Büdel, all bimembered Lecanoromycetes (sensu Eriksson et al., 2003) associated with a cyanobacterium are classified within the Peltigerales.

Ancestors to all peltigeralean fungi (Peltigerales; node 1; Fig. 3) and two main lineages within this group, Collematineae and Peltigerineae, were bipartite lichens with cyanobacteria as their photobionts (nodes 2 and 3; Fig. 3). Because taxa from the latter suborder are associated exclusively with *Nostoc*, whereas other cyanobacteria are also present in the Collematineae (e.g., *Dichotrix* in the Placynthiaceae), a switch from one type of cyanobacterium to another took place during the evolution of the Collematineae. Within the Peltigerineae, bimembered symbioses with *Nostoc* were found to be ancestral for members of the Nephromataceae and Lobariaceae (nodes 5 and 6; Fig. 3). Due to phylogenetic uncertainty within the Peltigerineae and the long branch subtending the Peltigeraceae (node 4), only a small fraction of the reconstructed ancestral symbiotic states were significant for nodes 4 and 8. Except for a few rare cases (nodes 4, 8, and 9), only ancestral bimembered associations with cyanobacteria were found to be statistically significant (Fig. 3).

Because the ancestor to all taxa that belong to the Peltigerales was reconstructed to be a lichen-forming fungus associated with a cyanobacterium (Fig. 3), this bipartite mutualistic assemblage was very likely the key trait (deQueiroz, 2002) that lead to the diversification of the Peltigerales. This was followed by multiple independent acquisitions of green algae to form tripartite symbioses (e.g., *Coccomyxa* in the Peltigeraceae and Nephromataceae, *Dictyochloropsis* in the Lobariaceae, *Myrmecia* in the Pannariaceae; Fig. 3) and presumably by subsequent losses of the cyanobacterium photobiont to form bimembered symbioses with green algae. This trend in the Peltigerales seems to be a clear case where ontogeny recapitulates phylogeny. Only later during the ontogenetic process is the green alga incorporated and becomes dominant, with the cyanobacteria usually restricted to cephalodia or absent from the thallus. Both in vivo and in vitro studies have demonstrated that the first developmental stage of tripartite lichens seems to consist always of an association between a fungus and a cyanobacterium (Tønsberg and Holtan-Hartwig, 1983; White and James, 1988; Stocker-Wörgötter and Türk, 1994; Yoshimura et al., 1994; Holtan-Hartwig, 1996; Stocker-Wörgötter, 2001). Additional evidence supporting bipartite symbiosis with green algae as the derived state from a trimembered symbiotic state is provided by species such as *Lobaria pulmonaria*, which are usually found in a trimembered state, but never form fruticose cyanomorphs. Further, the mycobiont of this species is sometimes associated with only green algae (Yoshimura, 1971). *Sticta canariensis* (Bory) Bory ex Delise provides a case where the same fungal species is associated with either a cyanobacterium or a green alga forming different thalli that can grow separately or together. This special photosymbiodeme also favors this trend where the loss of the cyanobacterium leads to the bipartite lichens with green algae classified within the Peltigerales. To detect how many times during the evolutionary history of the Peltigerineae each symbiotic state was gained and lost, a robust phylogeny is required, with at least one additional locus and with a more complete sampling having all symbiotic states within the Peltigerales proportionally represented.

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