

The sister group relation of Parmeliaceae (Lecanorales, Ascomycota)

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Abstract: The family Parmeliaceae (Lecanorales, Ascomycota) is possibly the largest, best known and most thoroughly studied lichen family within its order. Despite this fact the relationship between Parmeliaceae and other groups in Lecanorales is still poorly known. The aim of the present study is to contribute to finding the sister group of Parmeliaceae as an aid in future studies on the phylogeny and character evolution of the group. We do this by sampling all potential relatives to the Parmeliaceae that we have identified, i.e. *Gypsoplaca*, *Japewia*, *Mycoblastus*, *Protoparmelia*, and *Tephromela*, a good representation of the major groups within the Parmeliaceae s. lat. and a good representation of other taxa in the core Lecanorales. We use molecular data from two genes, the large subunit of the nuclear ribosomal RNA gene (nrLSU) and the small subunit of the mitochondrial ribosomal RNA gene (mrSSU), and a Bayesian analysis of the combined data. The results show that the closest relatives to Parmeliaceae are the two genera *Protoparmelia* and *Gypsoplaca*, which are crustose lichens. Parmeliaceae in our sense is a well supported group, including also the family segregates Alectoriaceae, Hypogymniaceae, Usneaceae and Anziaceae.

Key words: DNA, *Gypsoplaca*, Lecanorales, molecular, mrSSU, nrLSU, phylogeny, *Protoparmelia*

INTRODUCTION

The family Parmeliaceae is possibly the largest, best known and most thoroughly studied family in the Lecanorales (Ascomycota), comprising more than 2000 predominantly foliose and fruticose lichens in 89 genera (Eriksson 2006). Many of the groups included here belong to the earliest described lichen genera, e.g. *Parmelia*, *Cetraria* and *Usnea*, and many of the species are important components of diverse lichen communities in different vegetation types all around the world. Parmeliaceae currently is regarded as a uniform monophyletic group circumscribed by similarities in ascoma ontogeny and the anatomy of the characteristic cupular exciple (Henssen and Jahns 1973, Kärnefelt et al 1998). Although many family segregates have been suggested for groups obviously deviating in growth form (e.g. Alectoriaceae, Hypogymniaceae and Usneaceae; see Wedin et al 1999 for a list of suggested segregates and other family names considered synonyms to Parmeliaceae) a broad circumscription of Parmeliaceae is the most widely accepted. Several molecular phylogenies either have taken this concept as a starting assumption for analyses of the group (Mattsson and Wedin 1998; Thell et al 2002, 2004; Articus 2004) or support at least partly this view (Mattsson and Wedin 1999, Persoh and Rambold 2002). However to our surprise no one seems to have tested this concept by including representatives of all commonly accepted family segregates in a phylogenetic analysis. In the most recent Outline of the Ascomycota (Eriksson 2006) only the family segregate Anziaceae is accepted.

Numerous recent studies have focussed on the delimitation and reclassification of groups related to *Parmelia* s. lat. within the Parmeliaceae (e.g. Blanco et al 2004a, 2004b, 2005; Thell et al 2004). However, despite being such a widespread, well known and well studied lichen group, the relationships among Parmeliaceae and other groups in Lecanorales are poorly known. Although several molecular studies show that Lecanorales (sensu Wiklund and Wedin 2003, Wedin et al 2005) forms a monophyletic group, excluding among others the families Umbilicariaceae, Candelariaceae and Acarosporaceae (Lücking et al 2004, Lumbsch et al 2004, Reeb et al 2004, Persoh et al 2004, Wedin et al 2005), the detailed sister group relation of Parmeliaceae is not yet known. This is hampering the investigations of the phylogenetic relationships within Parmeliaceae

s. lat. and investigations of morphological evolution within the group because proper outgroup rooting with a known close relative currently is not possible.

A possible candidate for the sister group of Parmeliaceae is *Protoparmelia*. This genus has been classified usually in Lecanoraceae because it includes crustose lichens with single, one-celled hyaline ascospores and an ascus of the *Lecanora*-type (Hafellner 1984; Eriksson and Hawksworth 1989, 1995). Studies of the ascoma ontogeny (Miyawaki 1991, Henssen 1995) however showed that *Protoparmelia* possesses the cupular exciple (cup-shaped structure below the hymenium) so typical for Parmeliaceae, and in the latest version of the Outline of Ascomycota (Eriksson 2005) *Protoparmelia* is listed as a member of Parmeliaceae. Several recent studies of Parmeliaceae phylogeny consequently use *Protoparmelia* as outgroup, even though this is an untested assumption and *Protoparmelia* might just as well be a member of the ingroup.

There are further possible sister group candidates however. A number of crustose groups, based on anatomical characteristics, could be related to Parmeliaceae. These include Mycoblastaceae and Tephromelataceae, *Japewia* (currently in Ramalinaceae), and our previous, preliminary, analyses of lecanoralean lichens also placed the enigmatic genus *Gypsoplaca* close to Parmeliaceae. *Gypsoplaca* (Gypsoplacaceae) includes just one species, *G. macrophylla*.

The aim of the present study is to contribute to the circumscription of a natural, monophyletic group corresponding to the family Parmeliaceae. We also wish to suggest possible sister groups of Parmeliaceae, whereby further and more detailed studies of the phylogeny and character evolution within this group may be aided. We do this by sampling DNA sequence data from the type species of the family (*Parmelia saxatilis*), representatives of all recently accepted family segregates (Alectoriaceae, Anziaceae, Hypogymniaceae, Usneaceae) plus the deviant *Menegazzia* and all potential close relatives of the Parmeliaceae that we have been able to identify (*Gypsoplaca*, *Japewia*, *Mycoblastus*, *Protoparmelia*, *Tephromela* and *Calvitimela*). It should be pointed out that, although Parmeliaceae at present contains almost 90 genera, our selection represents a fair selection of the morphological and taxonomical diversity in this group. Finally we have included a good representation of other taxa in the Lecanorales. Lecanoraceae, a family of crustose lichens showing similarities with the Parmeliaceae in ascoma characteristics, was particularly well sampled.

MATERIALS AND METHODS

Sampling of taxa and specimens.—Taxa were sampled from 35 genera representing nine families within the order Lecanorales (TABLE I). The outgroup species *Caloplaca flavorubescens* and *Xanthoria parietina* were chosen because they belong to Lecanorales sensu Wiklund and Wedin (2003) but are not closely related to any of the main target families Lecanoraceae or Parmeliaceae (Andersen and Ekman 2004).

DNA-extraction, PCR-amplification and sequencing.—Total DNA extraction, PCR amplification, PCR product purification and sequencing were performed as in Lindemuth et al (2001), Döring et al 2000 and Wiklund and Wedin (2003) or the PCR cycling parameters included an initial hold at 94 C for 5 min, then denaturation at 94 C for 1 min, annealing at 56 C for 1 min, decreasing 1 C per cycle for the first six of the 39 cycles (touchdown), and extension at 72 C for 3 min. We amplified the first part of the large subunit of the nuclear ribosomal RNA gene (nrLSU) and the first part of the small subunit of the mitochondrial ribosomal RNA gene (mrSSU).

Sequence alignment and phylogenetic analyses.—A total of 54 ingroup taxa and two outgroup taxa were aligned by hand. The alignment, which included 838 sites of nrLSU and 763 sites of mrSSU, was submitted to TreeBASE (study accession number S1769, matrix accession number M3227). For *Calvitimela armeniaca* and *C. aglaea* only the LSU gene was successfully sequenced. Ambiguously aligned parts were excluded. Gaps were treated as missing data. Phylogenetic analyses were carried out with Bayesian inference. Congruence between data partitions was tested by inspecting posterior probability scores ≥ 0.95 resulting from separate Bayesian analyses including all taxa, also *C. armeniaca* and *C. aglaea* for which mrSSU sequence data is lacking (de Queiroz 1993, Miadlikowska and Lutzoni 2000). Conflicts between the two datasets were considered significant if two relationships (one monophyletic and the other nonmonophyletic) for the same set of taxa were supported by $pp \geq 0.95$.

Bayesian tree inference was carried out with MrBayes version 3.1.2 (Ronquist and Huelsenbeck 2003). A suitable likelihood model was selected with decision theory as implemented in the software DT-ModSel (Minin et al 2003). The TrN+I+G model was found to be optimal for the nrLSU dataset while the TVM+I+G model was optimal for the mrSSU dataset. These models include respectively three and five reversible substitution types. However MrBayes does not allow the implementation of models having between three and five substitution types. Therefore, following the recommendation by Huelsenbeck and Rannala (2004), we chose the next more complicated model allowed in MrBayes (viz. GTR). We consequently implemented separate GTR+I+G models for each of the two partitions. All likelihood parameters, except branch lengths (assumed to be proportional across partitions) and tree topology, were unlinked. The number of discrete categories to be used in the gamma model was estimated according to the method used by Andersen and Ekman (2004). This procedure

TABLE I. Sequences used in the phylogenetic analyses with GenBank accession numbers. Newly produced sequences are marked in bold.

Taxon	Vouchers for new sequences	Accession number	
		nrLSU	mtSSU
<i>Adelolecia pilati</i>		AY300826	AY567713
<i>Alectoria ochroleuca</i>	Austria, Wedin Aug 1998 (BM)	DQ899288	DQ899289
<i>Alectoria sarmentosa</i>	Sweden, Wedin 6350 (UPS)	DQ899290	DQ899291
<i>Amandinea punctata</i>		AY340536	AY143399
<i>Anzia colpodes</i>	USA, Moberg 11469 (UPS)	DQ899292	DQ899293
<i>Bacidia rosella</i>		AY300829	AY300877
<i>Biatora vernalis</i>	Norway, Tønsberg 23757 (BG)	DQ838752	DQ838753
<i>Buellia disciformis</i>		AY340537	AY143401
<i>Calicium viride</i>		AY340538	AY143402
<i>Caloplaca flavorubescens</i>		AY300831	AY143403
<i>Calvitimela aglaea</i>	Austria, Ekman 3371 (BG)	DQ838754	—
<i>Calvitimela armeniaca</i>	Italy, Arup L97797 (LD)	DQ787367	—
<i>Cetraria islandica</i>		AY340539	AY340486
<i>Cladonia rangiferina</i>		AY300832	AY300881
<i>Dolichousnea longissima</i>	Japan, Bergsten 24/8/00 (UPS)	DQ899294	DQ899295
<i>Gypsoplaca macrophylla</i>	USA, Bratt et al 7025 (UPS) CO28	DQ899296	DQ899297
<i>Gypsoplaca macrophylla</i>	Russia, Zhurbenko 92104 (UPS) CO29	DQ899298	DQ899299
<i>Hypogymnia physodes</i>		AY756338	AY756400
<i>Japewia tornoenensis</i>	Sweden, Wedin 10/5/03 (UPS)	DQ899300	DQ899301
<i>Lecania cyrtella</i>		AY300840	AY567720
<i>Lecanora intricata</i>	Austria, Arup L97031 (LD)	DQ787345	DQ787346
<i>Lecanora achariana</i>	Arup L03216 (LD)	DQ787341	DQ787342
<i>Lecanora aff. allophana</i>		AY853376	AY567710
<i>Lecanora argopholis</i>	Sweden, Arup L97568 (Arup), Austria, Arup L97504 (LD)	DQ787357	DQ787358
<i>Lecanora campestris</i>	Sweden, Arup L97370 (LD)	DQ787361	DQ787362
<i>Lecanora carpinea</i>	Austria, Arup L97007 (LD), Sweden, Arup L03192 (LD)	DQ787363	DQ787364
<i>Lecanora glabrata</i>	Sweden, Fritz (LD), Arup L011003 (LD)	DQ787359	DQ787360
<i>Lecanora muralis</i>	Austria, Wilfling 2536 (GZU), Sweden, Arup L03196 (LD)	DQ787339	DQ787340
<i>Lecanora perpruinosa</i>	Austria, Wilfling et al 1224 (GZU), Arup L97320 (LD)	DQ787343	DQ787344
<i>Lecanora polytropha</i>	Austria, Grube (GZU), Sweden, Arup L03568 (LD)	DQ787347	DQ787348
<i>Lecanora sulphurea</i>	Sweden, Arup L96006, L01823 (LD)	DQ787355	DQ787356
<i>Lecanora valesiaca</i>	Italy, Arup L97881 (LD)	DQ787349	DQ787350
<i>Lecidella meiococca</i>		AY300842	AY567714
<i>Menegazzia myriotrema</i>	Australia, Kantvilas 169/00 (UPS)	DQ899302	DQ899303
<i>Menegazzia terebrata</i>	Sweden, Wedin 4392 (UPS)	DQ899304	DQ899305
<i>Metus conglomeratus</i>		AY340555	AY340510
<i>Miriquidica garovaglii</i>		AY756357	AY567711
<i>Mycoblastus affinis</i>	Sweden, Nordin 3216 (UPS)	DQ899306	DQ899307
<i>Mycoblastus sanguinarius</i>	Sweden, Wedin 6932 (UPS)	AY756341	AY756403
<i>Neophyllis melacarpa</i>		AY340556	AY340511
<i>Neuropogon antarctica</i>	Antarctica, Schroeter 20/02/02 (UPS)	DQ899309	DQ899310
<i>Parmelia saxatilis</i>		AY300849	AY340514
<i>Physcia aiopolia</i>		AY300857	AY143406
<i>Pilophorus strumaticus</i>		AY340559	AY340517
<i>Protoparmelia badia</i>	Austria, Arup L97026 (LD), Norway, Arup L03569 (LD)	DQ787365	DQ899311
<i>Protoparmelia picea</i>	Sweden, Arup L97388, L01841 (LD)	DQ787366	DQ899312
<i>Pyrrhospora quereia</i>		AY300858	AY567712
<i>Ramalina fastigiata</i>		AY756360	AY756375
<i>Rhizoplaca chrysoleuca</i>	Kazakhstan, Moberg, Nordin 190 (GZU)	DQ787353	
	Norway, Arup L03454 (Arup)		DQ787354
<i>Rhizopl. melanophthalma</i>	Austria, Obermayer 2701 (GZU), Chile, Arup L01500 (LD)	DQ787351	DQ787352
<i>Sphaerophourus globosus</i>		AY256751	AY256769
<i>Stereocaulon pascale</i>		AY340568	AY340525

TABLE I. Continued

Taxon	Vouchers for new sequences	Accession number	
		nrLSU	mtSSU
<i>Tephromela atra</i>		AY300865	AY762097
<i>Toninia cinereovirens</i>		AY756365	AY567724
<i>Xanthoparmelia conspersa</i>	Sweden, Mattsson 4008 (UPS)	DQ899313	DQ899314
<i>Xanthoria parietina</i>		AY340578	AY143408

resulted in a discrete gamma model with eight categories for the nrLSU dataset and seven for the mrSSU data.

Prior distributions were set to uniform for tree topology, gamma shape parameter and the proportion of invariable sites, to a flat dirichlet for the rate matrix and state frequencies and to exponential for the branch lengths (with inverse scale parameter 10). The two partitions were allowed to evolve at different rates by setting the rate prior to variable. Three parallel runs of Markov chain Monte Carlo were performed, each with 12 chains, 11 of which were incrementally heated with a temperature of 0.15. The appropriate degree of heating was determined by observing swap rates between chains in preliminary runs. Analyses were diagnosed every 100 000 generations in the last 50% of the tree sample and automatically halted when convergence was reached. Convergence was defined as a standard deviation of splits (of frequency ≥ 0.1) between runs below 0.01. Every 100th tree was sampled.

A maximum likelihood (ML) analysis was carried out to obtain a second measure of branch support. We used the software RAXML-VI-HPC v2.0 (Stamatakis, Ludwig, Meier 2005; Stamatakis Website at <http://www.ics.forth.gr/~stamatak/>). RAXML implements only the GTR model, which is hard-coded into the software. Partitioning data with independent models is allowed (but interpartition rate heterogeneity is not allowed). We divided our data into the same two partitions as in the Bayesian analysis. Rate heterogeneity among sites was assumed to be gamma distributed with four rate categories (the only number of rate categories allowed by RAXML). We could not assume a proportion of sites to be invariable because this is not allowed by RAXML. Branch support was assessed with 1000 bootstrap replicates. Random addition sequence parsimony trees were used as starting trees for likelihood optimization. In the initial rearrangement pruned subtrees were allowed to be reinserted up to 10 nodes away from their original position. Model parameters were optimized up to a difference of 0.1 ln likelihood units. For classification purposes branch support was considered strong if its posterior probability was 0.95 or higher and its bootstrap proportion was 75% or higher.

RESULTS

Thirty new DNA sequences of nuclear DNA of the ribosomal LSU and 27 new sequences of mitochondrial SSU were produced for the study (TABLE I). No

significant incongruence between the two datasets was detected and they consequently were combined. A total of 1601 sites were included in the final combined analysis. The majority rule consensus tree of the Bayesian analysis, based on the final 50% of three parallel runs of length 6 600 000 generations, is presented (FIG. 1) together with posterior probabilities and likelihood bootstrap proportions of branches.

DISCUSSION

Parmeliaceae (including Anziaceae, Alectoriaceae, Hypogymniaceae, Usneaceae and *Menegazzia*) forms a monophyletic, well supported group. This confirms results from other studies (e.g. Mattsson and Wedin 1999, Persoh and Rambold 2002) but also provides evidence for the inclusion of Anziaceae and *Menegazzia* in Parmeliaceae. This family, together with Gypsoplacaceae and *Protoparmelia*, forms a group that received strong support (pp = 1.00, bootstrap = 76). In the majority rule consensus tree *Protoparmelia* forms the sister group of Parmeliaceae, but this has low support (pp = 0.48, FIG. 2). A closer look at the possible topologies of the three branches Parmeliaceae s. str., *Protoparmelia* and *Gypsoplaca* shows that another topology is almost as probable (FIG. 2). With a posterior probability of 0.45 *Protoparmelia* and *Gypsoplaca* together form a sister group of Parmeliaceae, while a topology where *Gypsoplaca* alone is sister of Parmeliaceae has low support (pp = 0.07). We at present cannot decide whether *Protoparmelia*, or a group consisting of *Gypsoplaca* and *Protoparmelia*, is the sister group of Parmeliaceae. We thus cannot support including *Protoparmelia* in Parmeliaceae, as suggested by some authors, without including also Gypsoplacaceae in the family. To accept *Protoparmelia* as a crustose Parmeliaceae is admittedly not difficult because it shares the cupular exciple widely accepted as a characteristic feature for Parmeliaceae. However this feature occurs, with some variations, also in *Caloplaca*, *Collema*, *Rinodina* and *Lecanora*, which are not closely related to Parmeliaceae. *Gypsoplaca*

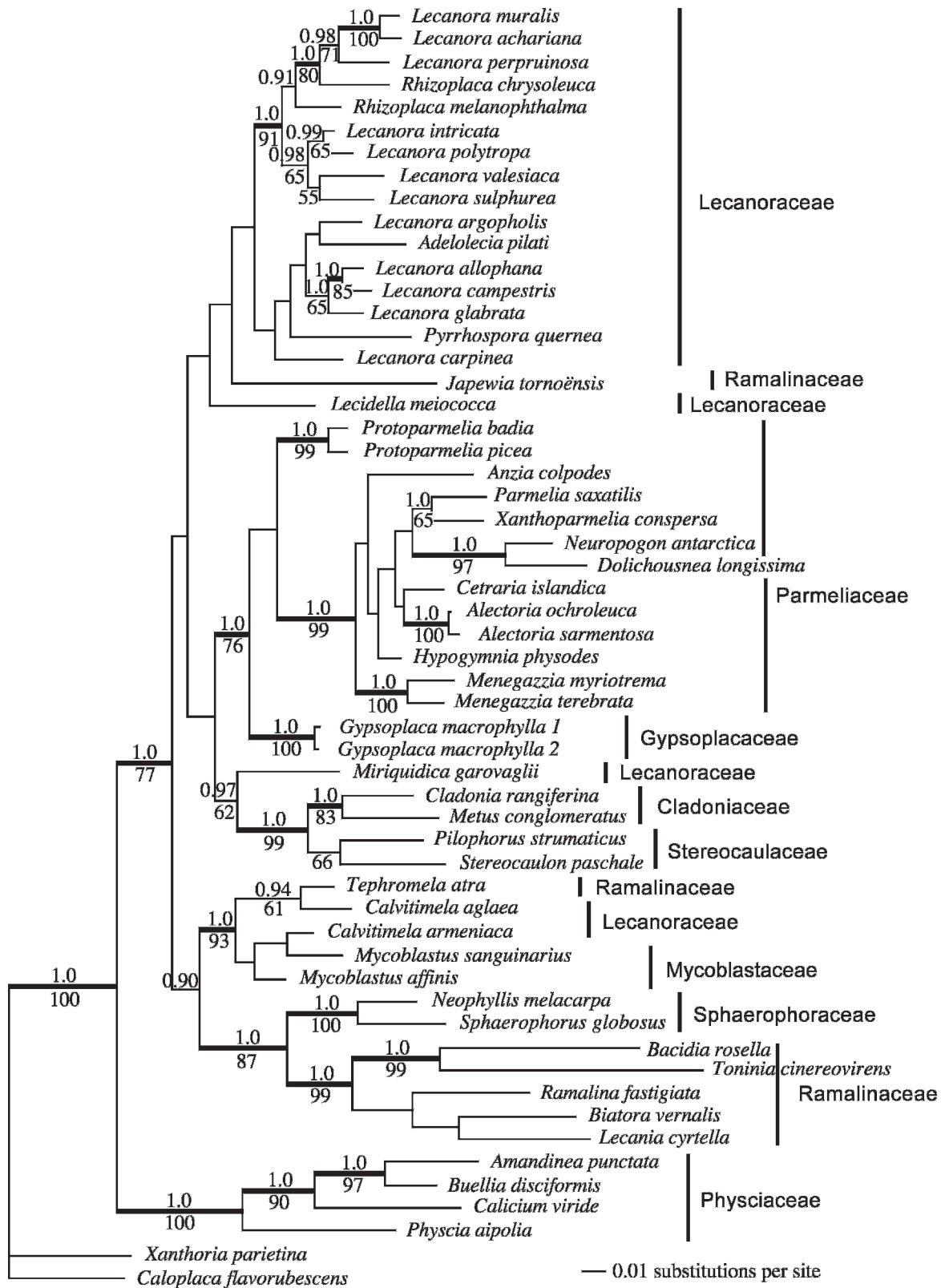


FIG. 1. Majority rule consensus tree with all compatible groups based on 99 000 trees visited during the stationary part of a B/MCMC tree sampling procedure. Posterior probabilities at or above 0.9 are presented above branches, likelihood bootstrap support above 50% below branches. Branches with posterior probabilities at or above 0.95 and bootstrap proportions above 75% are marked in bold. The classification to family follows Eriksson (2006) apart from Physciaceae, where the wide concept of Wedin et al (2002) is followed.

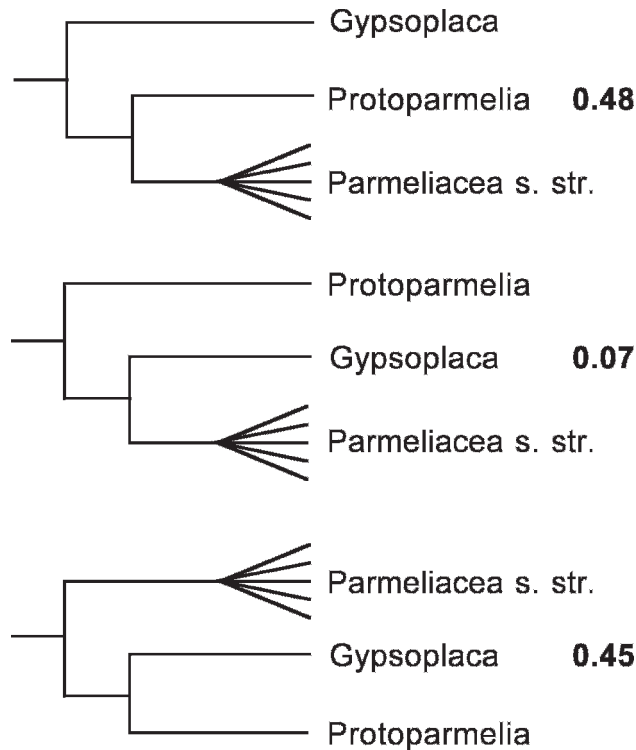


FIG. 2. Posterior probabilities for three different topologies in the Parmeliaceae and related taxa.

however lacks a cupular exciple and has a strongly aberrant apothecium ontogeny and anatomy. The matter of classification of these groups is obviously difficult and we refrain from suggesting any changes at this moment.

Protopermelia has been placed most often in Lecanoraceae, but this placement has been uncertain. Hafellner (1984) questioned the placement in Lecanoraceae, mainly because of the tholus shape, which differed slightly from that of *Lecanora*. Hertel (1984) added the presence of lobaric acid in *Protopermelia* to the list of characters not in concordance with a placement in Lecanoraceae. Later Miyawaki (1991) studied apothecial structures in *P. badia* and found a cupular exciple that is surrounded by a “saucer-shaped layer” of algal cells, which characterizes Parmeliaceae according to Henssen and Jahns (1973). Therefore they proposed a classification of *Protopermelia* within Parmeliaceae. According to Henssen (1995) most members of Parmeliaceae have a distinctly heteromerous thallus and subcrustaceous and foliose species have both an upper and a lower cortex. Typically this thallus is attached to the substrate either by rhizines, an umbilicus or by rhizoidal hyphae. In this respect *Protopermelia* differs from the general pattern, but this did not prevent

Henssen from accepting the genus as a member of Parmeliaceae s. lat.

The lichen genus *Gypsoplaca* includes just one species, *G. macrophylla*, which is brown, crustose or squamiform (Timdal 1990). Timdal erected this genus and the new family Gypsoplacaceae due to a remarkable ascoma ontogeny, which is characterized by an undelimited ascoma formed by paraphyses and ascogenous hyphae growing into the upper cortex and transforming it into a hymenium. The cortex is of “Kegelrinden” type (Poelt 1958, Timdal 1990). The asci are clavate, surrounded by an amyloid, gelatinous sheet and have a well developed, amyloid tholus with a deeper amyloid tube and usually an ocular chamber (Timdal 1990). This type of ascus is not rare and occurs for example among some of the *Cetraria*-like genera of Parmeliaceae (Thell et al 1995, Wedin et al 2000).

A few comments are needed concerning interesting phylogenetic relationships outside the focus of this study, the Parmeliaceae. Cladoniaceae, Stereocaulaceae and *Miriquidica garovaglii* together form a supported sister clade (pp = 0.97) of the group with Parmeliaceae, Gypsoplacaceae and *Protopermelia*. The relationship as sister clades however is not supported. The genus *Miriquidica* currently is classified in Lecanoraceae, but it should be noted that the species used in the analysis is not the type species. Another clade (without support) includes Lecanoraceae including *Japewia*. Although this has no support in the present analysis, a similar pattern where Lecanoraceae, Parmeliaceae, Cladoniaceae and Stereocaulaceae together form a monophyletic group has been found in other phylogenetic analyses (Wedin et al 2000, Persoh et al 2004). The support for Ramalinaceae is high (pp = 1.0, bootstrap value 99) if *Tephromela* and *Japewia*, both classified in the Ramalinaceae (Eriksson 2006), are excluded from that family. *Japewia* instead might belong in the Lecanoraceae, although branch support for this is low. *Tephromela*, *Calvitimela* and *Mycoblastus* together form a monophyletic group with high support (pp = 1.0, bootstrap value 93) but the two latter genera might not be monophyletic. Because of low branch support inside this group we cannot tell whether two families, Tephromelataceae and Mycoblastaceae, or just one family, Mycoblastaceae (including Tephromelataceae), should be recognized. However the results confirm that *Tephromela* does not belong to Ramalinaceae (Ekman and Wedin 2000) and that *Calvitimela*, contrary to the suggestion by Eriksson (2006), does not belong to Lecanoraceae. The close relationship among *Mycoblastus*, *Tephromela* and *Calvitimela* is perhaps somewhat surprising. Although they are morphologically similar there are consider-

able anatomic differences (e.g. in ascus anatomy, number and size of spores, and paraphyses) (Hafellner 1984). A more detailed molecular study is needed to investigate both the generic circumscription in the group and the taxonomy at family level.

We have found that Parmeliaceae appears to be a well supported group that includes also the sometimes recognized family segregates Alectoriaceae, Hypogymniaceae, Usneaceae and Anziaceae. Further studies are needed to identify well supported natural groups within this family and to identify morphological synapomorphies that can be used to characterize them. The closest relatives to the large macrolichens within Parmeliaceae are likely to be crustose or squamiform microlichens. This has potentially substantial implications for future investigations of phylogeny and character evolution in this group.

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