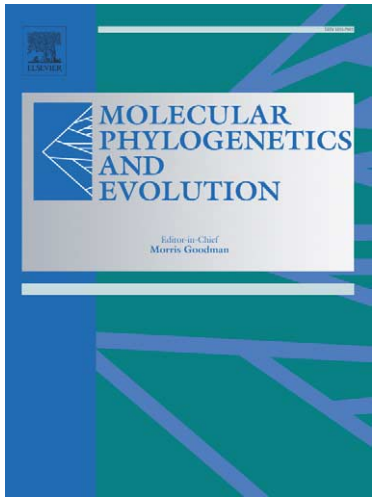

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PII: S1055-7903(06)00485-4
DOI: 10.1016/j.ympev.2006.11.029
Reference: YMPEV 2423

To appear in: *Molecular Phylogenetics and Evolution*

Received Date: 14 September 2006
Revised Date: 13 November 2006
Accepted Date: 25 November 2006

Please cite this article as: Crespo, A., Thorsten Lumbsch, H., Mattsson, J-E., Blanco, O., Divakar, P.K., Articus, K., Wiklund, E., Bawingan, P.A., Wedin, M., Testing morphology-based hypotheses of phylogenetic relationships in Parmeliaceae (Ascomycota) using three ribosomal markers and the nuclear *RPB1* gene, *Molecular Phylogenetics and Evolution* (2006), doi: 10.1016/j.ympev.2006.11.029

Testing morphology-based hypotheses of phylogenetic relationships in Parmeliaceae (Ascomycota) using three ribosomal markers and the nuclear *RPB1* gene

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Received xx September 2006; revised xxx

Running title: Parmeliaceae phylogeny

Abstract

Parmeliaceae is the largest family of lichen-forming fungi with more than 2000 species and includes taxa with different growth forms. Morphology was widely employed to distinguish groups within this large, cosmopolitan family. In this study we test these morphology-based groupings using DNA sequence data from three nuclear and one mitochondrial marker from 120 taxa that include 59 genera and represent the morphological and chemical diversity in this lineage. Parmeliaceae is strongly supported as monophyletic and six well-supported main clades can be distinguished within the family. The relationships among them remain unresolved. The clades largely agree with the morphology-based groupings and only the placement of four of the genera studied is rejected by molecular data, while four other genera belong to clades previously unrecognised. The classification of these previously misplaced genera, however, has already been questioned by some authors based on morphological evidence. These results support morphological characters as important for the identification of monophyletic clades within Parmeliaceae.

Keywords: Parmeliaceae; Lecanorales; Ascomycota; Lichens; Phylogeny; Growth forms; Morphological characters.

1. Introduction

Symbiotic associations with photosynthetic active partners, such as algae or cyanobacteria, are among the most widespread life strategies of fungi and are especially common in Ascomycota. Roughly 40% of all Ascomycota form such symbiotic relationships that are called lichens (Kirk et al., 2001). Unlike most non-lichenized fungi, lichens have a diverse vegetative morphology as a consequence of their dual nature. These morphologies primarily reflect the necessity of these symbiotic systems to expose sufficient area with algae or cyanobacteria to light for photosynthesis. Foliose lichens for example have a leaf-like appearance while fruticose lichens increase their surface by dividing the thalli to form branches (Ott and Lumbsch, 2001). The water regime plays another important role in the morphology of lichens. In foliose lichens a water film between the thallus and the contacting substrate can be gradually taken up by the lower surface (Jahns, 1984). Since water uptake occurs over the whole surface of the lichen thallus, deeply divided fruticose lichens have been shown to be extremely effective in use of fog, snow or dew (e.g., Lange et al., 1990); epicortical thin polysaccharide layers interrupted by several structures as pores, fenestrations (Blanco et al. 2004a) or pseudocyphellae also play an important role in water isolation and gas exchange (Hale, 1973). The different morphologies found in lichens have been widely used in taxonomy of these organisms. However, molecular studies demonstrated that growth forms are of no importance at the family and in some cases even generic level (e.g., Stenroos and DePriest, 1998; Wedin et al. 1999; Ekman, 2001; Schmitt et al., 2001; Blanco et al. 2004b). While these phylogenetic studies showed that morphology cannot be schematically applied to circumscribe higher taxa, there is no random pattern of morphological characters in the published phylogenies. Hence, the question remains to what extent growth form characters can be used for the circumscription of monophyletic lineages.

Parmeliaceae is an ideal model to study the question of the importance of growth forms in the taxonomy of these fungi. This family includes morphologically very diverse lichens,

including crustose (e.g., *Protoparmelia*; Henssen, 1995), peltate (e.g., *Omphalodiella*; Henssen, 1991), subcrustose (e.g., *Karoowia*; Hale, 1989), foliose (e.g., *Parmelia;* Elix, 1993), umbilicate (e.g., *Xanthomaculina*; Hale, 1985), fruticose (e.g., *Usnea*; Motyka, 1936) or subfruticose (e.g., *Almbornia*; Esslinger 1981) species and even lichenicolous fungi devoid of any own photosynthetic partner, such as *Phacopsis* and *Nesolechia* (Persoh and Rambold, 2002), were placed here. Parmeliaceae includes approximately 2000 species in some 90 genera and represents the largest family within Lecanorales. The family belongs to the core of the Lecanorales closely related to other large families like the Lecanoraceae and Cladoniaceae (Wedin et al., 2000; Ekman & Tønsberg, 2002; Tehler et al., 2003; Lutzoni et al., 2004). This order is the most speciose within the class Lecanoromycetes which itself includes the bulk of lichen-forming fungi (Eriksson, 2006).

Based on different morphological characters, several genera were segregated at family level from Parmeliaceae. This includes Alectoriaceae, Anziaceae, Cetrariaceae, Cornicul ariaceae, Everniaceae, Hypogymniaceae, and Usneaceae (Eriksson and Hawksworth, 1998）. While most of these segregates were not used recently, Anziaceae, Hypogymniaceae, and Usneaceae have been accepted by some authors (e.g., Poelt, 1973; Elix and James, 1992; Golubkova, 1994; Wirth and Büdel, 1994; Kärnefelt et al., 1998; Stevens, 1999; McCarthy, 2003), based chiefly on deviating thallus morphology. Further, Alectoriaceae was accepted in several publications (Brodo, 1986; Esslinger, 1989; Eriksson and Hawksworth, 1992; Kärnefelt and Thell, 1992), mainly including taxa with deviating ascospores (Table 1). Based on similarities in micromorphological characters, such as a shared type of ascoma ontogeny and a characteristic structure in the ascoma anatomy, called a cupulate exciple, Henssen and Jahns (1973) accepted the morphologically diverse Parmeliaceae in a wider sense. In phylogenetic analyses based on molecular markers, all the proposed segregated families were shown to be nested within

Parmeliaceae (Mattsson and Wedin, 1999; Wedin et al., 1999; Arup et al. 2006). Hence, currently a wider concept of Parmeliaceae is generally accepted (Eriksson, 2006).

Although molecular data supported a wider concept of Parmeliaceae, it is currently not clear if the different growth forms characterize natural groups within Parmeliaceae. Based on similarities in growth forms or micromorphological similarities (Table 1), different informal groupings have often been distinguished (Krog, 1982; Goward, 1985; Kärnefelt and Thell, 1992; Kärnefelt et al., 1992, 1998; Elix, 1993; Kärnefelt, 1998). These have usually been named after a characteristic genus e.g., parmelioid lichens, which share the typical foliose, dorsiventral growth form and laminal pycnidia and apothecia with the genus *Parmelia*, or cetrarioid lichens, which have erect foliose or subfruticose thalli with marginal apothecia and pycnidia like the genus *Cetraria.* Whether or not these groups represent monophyletic lineages remains to be investigated. In previous phylogenetic studies, a core group of parmelioid genera was found to be monophyletic (Crespo et al., 2001; Blanco et al., 2006), while Thell et al. (2004) failed to get support for parmelioid lichens as being monophyletic. In the latter study the parmelioid lichens fall into two separate groups, while a core group of cetrarioid lichens was supported as monophyletic. In the present study we addressed the question of phylogenetic patterns of the morphological variation observed in Parmeliaceae using a data set of 120 taxa using four loci, including nuclear and mitochondrial ribosomal DNA and one protein-coding gene *RPB1*. Our sampling includes 59 genera of Parmeliaceae that represents all growth forms found within the family.

2. Materials and methods

2.1. Taxon sampling

We sampled 116 species of Parmeliaceae, including the major genera and representatives of the morphological and chemical diversity within this group (Table 2). The sample includes 59 of

the 89 genera currently accepted in Parmeliaceae (Eriksson, 2006). Most of the genera not sampled in the present study have earlier been shown to belong to monophyletic groups well covered here (Thell et al., 2002; 2004; Blanco et al., 2006; Divakar et al., 2006). Sequences of three Lecanoraceae species were also included. *Cladonia rangiferina* was used as outgroup as a representative of the closely related Cladoniaceae. GenBank accession numbers and voucher information are given in Table 3.

2.2. Molecular methods

Samples prepared from freshly collected, frozen samples or herbarium specimens were ground with sterile glass pestles. Total genomic DNA was extracted using the DNeasy Plant Mini Kit (Qiagen) according to the manufacturer's instructions. Dilutions (10^{-1} up to 10^{-2}) or undiluted DNA was used for PCR amplifications of the internal transcribed spacer (ITS) and the genes coding for the nuclear LSU rRNA, mitochondrial SSU and the protein coding *RPB1* gene, respectively. Primers for amplification were: a) for the nuclear LSU rDNA: nu-LSU-0155-5' (Döring et al., 2000), nu-LSU-0042-5' (=LR0R) (Vilgalys unpublished, http://www.botany.duke.edu/fungi/mycolab), nu-LSU-1432-3' (=LR7), LR5 and nu-LSU-1125-3' (=LR6) (Vilgalys and Hester, 1990), b) for the nuclear ITS rDNA: ITS1F (Gardes and Bruns, 1993), ITS4 (White et al., 1990) and ITS1-LM (Myllys et al., 1999) and ITS2-KL (Lohtander et al., 1998), c) for the mitochondrial SSU rDNA: mr SSU1 and mrSSU3R (Zoller et al., 1999), and MSU 7 (Zhou et al., 2001), and d) for *RPB1* nu DNA: g*RPB1*-A (Stiller and Hall, 1997) and f*RPB1*-C (Matheny et al., 2002), and RPr2 (Wirtz et al., in prep.). The 25 µL PCR reactions contained 2.5 µL buffer, 2.5 µL dNTP mix, 1 µL of each primer (10 µM), 5 µL BSA, 2 µL Taq, 2 µL genomic DNA extract and 9 µL distilled water. Alternatively, amplifications were performed

in 50 µL volumes containing a reaction mixture of 10 µL genomic DNA, 5 µL of 10X DNA polymerase buffer (Biotools) (containing $MgCl_2$ 2mM, 10 mM Tris-HCl, pH 8.0, 50 mM KCl, 1 mM EDTA, 0.1% Triton X-100), 1µL dNTP mix, containing 10mM of each base, 2.5 µL of each primer (10 µM), 1.25 µL of DNA polymerase (1U/µL) and 27.5 µL distilled water. PCR on some samples was performed using Amersham Pharmacia Biotech Ready-To-Go Beads. Thermal cycling parameters were: initial denaturation for 3 min at 95°C, followed by 30 cycles of 1 min at 95°C, 1 min at 52°C, 1 min at 73°C, and a final elongation for 7 min at 73°C. Amplifications of some samples were carried out in a Techne Progene thermocycler and performed using the following programs: initial denaturation at 94°C for 5 min, and 30 cycles of: 94°C for 1 min, 54–60°C (ITS nrDNA), 60°C (LSU nrDNA), 57–58°C (SSU mtrDNA) and 52°C (*RPB1* nrDNA) for 1 min, 72°C for 1.5 min, and a final extension at 72°C for 5 min.

Amplification products were viewed on 1% agarose gels stained with ethidium bromide and subsequently purified using the QIAquick PCR Purification Kit (Qiagen) and DNA Purification Column kit (Biotools) according to the manufacturer's instructions. The cleaned PCR products were sequenced using the same primers used in the amplifications. The ABI Prism™ Dye Terminator Cycle Sequencing Ready reaction kit (Applied Biosystems) was used and the following settings were carried out: denaturation for 3 min at 94°C and 25 cycles at: 96°C for 10 sec, 50°C for 5 sec and 60° for 4 min. Sequencing reactions were electrophoresed on a 3730 DNA analyser (Applied Biosystems). Sequence fragments obtained were assembled with SeqMan 4.03 (DNAStar) and manually adjusted.

2.3. Sequence alignments

We employed an alignment procedure that uses a linear Hidden Markov Model (HMM) as implemented in the software SAM (Sequence Alignment and Modelling system; Karplus et al., 1998) for separate alignments of the nu ITS, nu LSU and mt SSU data sets. Regions that were not aligned with statistical confidence using SAM were excluded from the phylogenetic analysis. In the combined data sets missing sequence portions were coded as "?". The alignment of the *RPB1* sequences was performed using Clustal W (Thompson et al., 1994).

2.4. Phylogenetic analyses

The phylogenetic analyses of the alignments included a maximum parsimony (MP) and a Bayesian approach (B/MCMC) (Huelsenbeck et al., 2001; Larget and Simon, 1999). To test for potential conflict, parsimony bootstrap analyses were performed on each individual data set and ≥70% bootstrap consensus trees were examined (De Queiroz, 1993; Lutzoni et al., 2004).

Maximum parsimony analyses were performed using the program PAUP* (Swofford, 2003). Heuristic searches with 200 random taxon addition replicates were conducted with TBR branch swapping and MulTrees option in effect, equally weighted characters and gaps treated as missing data. Bootstrapping (Felsenstein, 1985) was performed based on 2000 replicates with random sequence additions. To assess homoplasy levels, consistency index (CI), and retention index (RI) were calculated from combined parsimony search.

The B/MCMC analysis of the combined data set was performed using the MrBayes 3.1.2 program (Huelsenbeck and Ronquist, 2001). We used the general time reversible model of nucleotide substitution (Rodríguez et al., 1990) including estimation of invariant sites, assuming a discrete gamma distribution with six rate categories and allowing site-specific rates

(GTR+I+G+SS) by using the covarion (Tuffley and Steel, 1998) option of MrBayes. The data set was portioned into six parts (nu ITS, nu LSU, mt SSU, 1st, 2nd, 3rd codon positions of *RPB1*). Each partition was allowed to have its own model parameters as proposed by Nylander et al. (2004). No molecular clock was assumed. A run with 4,000,000 generations starting with a random tree and employing 12 simultaneous chains was executed. Every 100th tree was saved into a file. The first 200,000 generations (i.e. the first 2000 trees) were deleted as the "burn in" of the chain. We plotted the log-likelihood scores of sample points against generation time using TRACER 1.0 (http://evolve.zoo.ox.ac.uk/software.html?id=tracer) to ensure that stationarity was achieved after the first 200,000 generations by checking whether the log-likelihood values of the sample points reached a stable equilibrium value (Huelsenbeck and Ronquist, 2001). Of the remaining 76,000 trees (38,000 from each of the parallel runs) a majority rule consensus tree with average branch lengths was calculated using the sumt option of MrBayes. Posterior probabilities were obtained for each clade. Only clades that received bootstrap support equal or above 70% under MP (Hillis and Bull, 1993) and posterior probabilities ≥ 0.95 were considered as strongly supported. Phylogenetic trees were drawn using the program Treeview (Page, 1996).

3. Results

For this study 242 new sequences were obtained, including 50 nu LSU, 46 mt SSU, 49 nu ITS, and 97 *RPB1* sequences. For four species no mt SSU and 20 species no *RPB1* sequence could be obtained, and in twelve species sequences of different specimens had to be used in the combined analyses. The sequences were aligned with sequences obtained from GenBank as listed in Table 3. The data matrix of 2731 unambiguously aligned nucleotide position characters in the combined

analysis, including 784 of the mt SSU, 845 of the nu LSU, 496 of the nu ITS rDNA, and 606 of the *RPB1* rDNA. 412 sites in the mt SSU, 503 in the nu LSU, 336 in the ITS rDNA, and 288 in the *RPB1* data set were constant. Parsimony informative sites were 313 in the mt SSU, 249 in the nu LSU, 109 in the ITS rDNA, and 263 in the *RPB1* data set. Ambiguously aligned regions and major insertions, representing spliceosomal and group I introns in the nuclear ribosomal DNA (Bhattacharya et al., 2000; Cubero et al., 2000; Gargas et al., 1995), were excluded from all analyses. 1192 characters were variable in the combined data set. The MP ≥70% bootstrap support method for testing data sets for incongruence indicated that phylogenetic signal between the four loci was high overall, with the majority of clades supported by one single-gene analysis not being contradicted in the others (data not shown) and hence a combined analysis was performed. Only very few internal nodes, in which strongly supported clades were contradicted in another analysis, were found: a) in the mt SSU and nu LSU bootstrapping tree *Parmelia discordans* and *P. serrana* clustered together, while in the nu ITS *P. saxatilis* is sister group of *P. serrana*, and in the nu *RPB1* tree *P. saxatilis* is sister group of *P. discordans*; b) *Melanohalea elegantula* in the mt SSU bootstrapping tree grouped with *M. exasperata*, whereas in the nu LSU and nu *RPB1 M. elegantula* is sister group of *M. aff. exasperata*. The combined alignment is available in TreeBASE (http://www.treebase.org/treebase).

Maximum parsimony analysis of the combined data set yielded a consensus tree that did not contradict the Bayesian tree topology. Nine most parsimonious trees were found 8031 steps long (CI=0.22, RI=0.55). 934 positions in the matrix were parsimony-informative.

In the B/MCMC analysis of the combined data set, the likelihood parameters in the sample had the following mean (Variance): LnL = –41918.956 (0.685), base frequencies $\pi(A)_{\{all\}} = 0.27$ (0.0003), $\pi(C)_{\{all\}} = 0.223$ (0.0003), $\pi(G)_{\{all\}} = 0.248$ (0.0004), $\pi(T)_{\{all\}} = 0.259$ (0.0003), rate

matrix $r(AC)_{\{all\}} = 0.084$ (0.0002), $r(AG)_{\{all\}} = 0.203$ (0.0006), $r(AT)_{\{all\}} = 0.087$ (0.0002), $r(CG)_{\{all\}} = 0.055$ (0.0002), $r(CT)_{\{all\}} = 0.512$ (0.0008), $r(GT)_{\{all\}} = 0.058$ (0.0002), the gamma shape parameter $alpha_{\{nu\ LSU\}} = 0.376$ (0.0005), $alpha_{\{nu\ ITS\}} = 0.825$ (0.0005), $alpha_{\{mt\ SSU\}} = 0.476$ (0.0006), $alpha_{\{1st\ codon\ \textit{RPB1}\}} = 0.289$ (0.0003), $alpha_{\{2nd\ codon\ \textit{R\ PB1}\}} = 0.135$ (0.0002), $alpha_{\{3rd\ codon\ \textit{RPB1}\}} = 6.45$ (0.01), the proportion of invariable sites $P(invar)_{mean/all} = 0.389$ (0.0004), and the proportions of $state(off->on)_{\{all\}} = 0.37$ (0.007), $state(on->off)_{\{all\}} = 0.371$ (0.006).

Since the topologies of the MP and B/MCMC analyses did not show any strongly supported conflicts, only the 50% majority-rule consensus tree of Bayesian tree sampling is shown with those nodes in bold that received strong support (i.e. PP ≥0.95 in B/MCMC analysis and MP bootstrap ≥70%) in both the MP and Bayesian analyses (Fig. 1).

In the majority-rule consensus tree of combined data set shown in Fig. 1, Parmeliaceae is strongly supported as monophyletic. The crustose *Protoparmelia badia* is sister to all remaining Parmeliaceae. The backbone of the phylogeny of the remaining Parmeliaceae lacks support. However, several clades are strongly supported that partially agree with previously distinguished morphological groupings. The relationships of these monophyletic lineages, however, are not resolved with confidence. The species of the genus *Usnea* are strongly supported as monophyletic. Other taxa like *Evernia* or *Protousnea,* previously classified as usneoid (Table 2), do not cluster with *Usnea* in our analysis. Further, several genera are strongly supported as monophyletic, but without a strongly supported suprageneric relationships; this includes *Bryoria, Menegazzia,* and *Platismatia*. Moreover, the phylogenetic position of eight genera remains unresolved: *Allantoparmelia, Anzia, Cornicularia, Evernia, Imshaugia, Oropogon, Pannoparmelia* and *Protousnea*.

Six strongly supported clades were found in our analysis, each of which includes species of different genera. A core group of parmelioid lichens is supported as monophyletic, which includes genera that were incorporated in Blanco et al. (2006), and in addition the lichenicolous *Nesolechia oxyspora* (=*Phacopsis oxyspora*), the peltate *Omphalodiella patagonica, Cetrelia,* a genus previously considered as cetrarioid, and three genera previously considered as parmelioid based on morphological characters: *Almbornia, Namakwa,* and *Xanthomaculina* (Table 2). Alectorioid genera, including *Alectoria, Pseudephebe* and *Sulcaria* are strongly supported as monophyletic. *Everniopsis trulla* and *Psiloparmelia* spp. form a strongly supported sister-group, here called psiloparmelioid. A core group of cetrarioid lichens form a strongly supported clade, including *Arctocetraria, Cetraria, Cetrariella, Cetreliopsis, Flavocetraria, Tuckermannopsis, Vulpicida*, and *Melanelia.* The genera *Arctoparmelia, Brodoa, Hypogymnia* and *Pseudevernia* form a monophyletic group that corresponds to parts of the hypogymnioid group as circumscribed by Krog (1982). However, *Menegazzia*, a genus previously considered as hypogymnioid based on morphology, forms an independent monophyletic group with unresolved relationship. *Letharia* and *Lethariella* form another well-supported sister-group (letharioid group).

4. Discussion

The four-region data set, including three nuclear (nu LSU, nu ITS, *RPB1*) and one mitochondrial (mt SSU) markers yielded in a more structured and better supported topology of Parmeliaceae than possible in previous studies including fewer markers or a more restricted taxon sampling (e.g., Mattsson and Wedin, 1998, 1999; Wedin et al., 1999; Thell et al., 2004). The nuclear and mitochondrial gene partitions supported the same overall topology. No supported

intragenomic and no substantial intergenomic conflict was found for the major clades. The combined four-region data set provided the most robust support of Parmeliaceae phylogeny overall, although several clades could not be resolved with confidence. This phenomenon suggests that several additional loci and extended taxon sampling will be necessary to further resolve the phylogenetic relationships in this fungal family.

Although the backbone of the Parmeliaceae phylogeny is not resolved with confidence, we can nevertheless draw several conclusions on phylogenetic relationships. The family itself is strongly supported and six well-supported clades can be distinguished, although the relationships of these clades remain unknown. The traditional morphological concept of the family was based on the foliose or fruticose growth form, trebouxioid photobiont, with two single-layered cortices, usually rhizines, lecanorine ascomata, branched paraphyses, hyaline, non-septate ascospores, and pycnidial conidiomata (Kirk et al., 2001). As indicated in Table 1, the present phylogenetic concept includes genera that are not consistent with these criteria (e. g. pigmented, septate or curved ascospores, complex cortex, etc.). One of the most notable results is the position of the crustose genus *Protoparmelia*, which was placed here based on ascomatal characters (Henssen, 1995; Eriksson, 2006). In spite of the deviating growth form, the present phylogeny supports a close relationship of *Protoparmelia* with other Parmeliaceae, as was found by Arup et al. (2006). In this study, where a number of potential relatives to Parmeliaceae were included in a combined mt SSU and nu LSU rDNA phylogeny, *Protoparmelia* or a group consisting of *Protoparmelia* and Gypsoplacaceae formed the sister-group to Parmeliaceae. Arup et al. (2006) concluded that it is difficult to include *Protoparmelia* in Parmeliaceae as has often been done recently, without also including the morphologically deviating Gypsoplacaceae. However, although the present knowledge support a very close relationship of *Protoparmelia* with Parmeliaceae, an increased

taxon sampling is necessary to evaluate what classification of this genus that is best and most informative.

In the following we concentrate on the discussion of the six well-supported clades.

Alectorioid group. This clade includes the genera *Alectoria, Pseudephebe* and *Sulcaria.* Alectorioid lichens in a strict sense (Alectoriaceae s. str.) include genera with fruticose growth form and pigmented, simple or septate to muriform ascospores (Eriksson and Hawksworth, 1985; Kärnefelt and Thell, 1992). However, in the present phylogeny genera with brown, septate ascospores (*Alectoria* and *Sulcaria*) are grouped with *Pseudephebe*, which has hyaline, simple ascospores. Therefore, ascospore pigmentation is not useful to circumscribe the alectorioid clade (Table 1). Krog (1982) and Esslinger (1989) had previously argued that using variation in thallus morphology better circumscribes these taxa than classifications that emphasize variation in ascospore pigmention (Eriksson and Hawksworth, 1985; Kärnefelt and Thell, 1992). The genera *Bryoria* and *Oropogon*, which are usually placed in the alectorioid group, do not cluster here, but their relationships are not resolved with confidence and hence our data are not sufficient to reject a placement of those taxa into the alectorioid clade. Alectorioid genera in the new concept are characterized by a fruticose, often beard-like pendent or caespitose thallus, *Cetraria*-type polysaccharides in cell walls (Common, 1991), and periclinally arranged hyphae in a, often multi-layered, cortex. Additional studies are necessary to understand the circumscription of this clade.

Cetrarioid group. This group includes *Arctocetraria, Cetraria, Cetrariella, Cetreliopsis, Flavocetraria, Tuckermannopsis,* and *Vulpicida*, genera that were earlier placed in *Cetraria* based on morphological features (Tables 1, 2), and also includes *Melanelia.* All genera include erect foliose to subfruticose species. *Melanelia* was shown to be polyphyletic previously and the

parmelioid genera *Melanohalea* and *Melanelixia* were segregated from *Melanelia* s. str (Blanco et al., 2004a). The close relationship of *Melanelia* with cetrarioid lichens was indicated by Thell et al. (2002, 2004) and Blanco et al., (2004a, 2006), but in these studies the relationships lacked support. This study strongly supports the placement of *Melanelia* s. str. as a cetrarioid genus, a relationship which is supported by morphology. The taxa in the cetrarioid clade typically have a characteristic combination of characters including marginal, more or less stalked, pycnidia, usually marginal apothecia, and *Cetraria*-type polysaccharides in their cell walls. Although the single characters (marginal pycnidia and apothecia / *Cetraria*-type lichenan) occur outside the clade, none of the taxa outside the cetrarioid clade show this combination of characters. Thell et al. (2002, 2004) have shown that *Tuckernaria, Nephromopsis, Kaernefeltia, Ahtiana, Masonhalea* and *Allocetraria* also belong to this group. *Cetrelia* and *Parmelaria* which previously were considered as cetrarioid based on several morphological traits (e.g., marginal pycnidia and apothecia, sparsely rhizinate lower surface) cluster in the parmelioid group, the latter in agreement with Blanco et al. (2005).

Hypogymnioid group. This clade corresponds to the genera *Arctoparmelia, Brodoa, Hypogymnia* and *Pseudevernia.* Together with *Menegazzia* most of these genera were traditionally considered as hypogymnioid based on foliose growth form, lack of rhizines, hollow and loose medulla. Moreover they have been separated at family level by some authors (Poelt, 1973; Elix and James, 1992; Kärnefelt et al., 1992). However, in the present study, the species of *Menegazzia* form a monophyletic group without a resolved relationship. Hence, a placement of this genus in the hypogymnioid clade cannot be rejected with the data at hand. *Arctoparmelia* has not previously been grouped with the hypogymnioid taxa, since it possesses rhizines, which are usually lacking in this group. However, *Arctoparmelia* has a loosely compact medulla (Divakar

and Upreti, 2005), as is the case in other hypogymioid taxa. In fact, the only morphological character that would characterize this clade is the loose or hollow medulla; additional studies are needed in this group.

Lethariod group. This clade consists of *Letharia* and *Lethariella* supporting Krog (1976, 1982) who suggested the two genera to be sister-groups based on morphological characters. A close relationship of the two genera to *Usnea* s. lat., that was suggested by Krog (1982) could not be supported, but since there is no well-supported sister-group relationship of *Usnea*, this cannot be rejected either. The lethariod clade includes species with fruticose thallus with a thin, soft and spongy cortex and atranorin as major cortical constituent (Krog, 1976).

Parmelioid group. This is the largest clade within Parmeliaceae, representing about 75% of the described species in the family and characterized by foliose thallus, rhizines on the lower surface, laminal apothecia, *Lecanora*- type asci and simple hyaline ascospores (Crespo et al., 2001). However, deviating growth forms are also included as the peltate *Omphalodiella,* subcrustose *Karoowia*, subfruticose *Almbornia* and umbilicate *Xanthomaculina.* It includes numerous genera encompassing species that were traditionally included in *Parmelia* (*Almbornia, Bulbothrix, Canoparmelia, Everniastrum, Flavoparmelia, Flavopunctelia, Hypotrachyna, Karoowia, Melanelixia, Melanohalea, Myelochroa, Namakwa, Parmelia, Parmelina, Parmelinella, Parmelinopsis, Parmotrema, Pleurosticta, Punctelia, Relicina, Xanthomaculina, Xanthoparmelia*), but also *Parmeliopsis,* the cetrarioid genera *Cetrelia* and *Parmelaria,* the peltate *Omphalodiella,* and the lichenicolous fungus *Nesolechia.* Basically the same seven major clades of parmelioid lichens were found here as shown by Blanco et al. (2006). Polyphyly of some genera, such as *Canoparmelia* and *Xanthoparmelia,* indicates that the current generic

concept is in need of revision. This issue needs to be addressed using extended taxon samplings of these genera.

The placement of several cetrarioid genera in the parmelioid clades demonstrates that the pycnidial or apothecial location cannot be used alone to morphologically distinguish the two clades. However, these two genera (*Cetrelia, Parmelaria*) belong to broadly lobed cetrarioid taxa. Furthermore, *Cetrelia* has isolichenan as cell wall polysaccharide (Elix, 1993), which is typical for several groups in parmelioid genera (Blanco et al., 2006), but not present in the cetrarioid clade as circumscribed here. Moreover, *Cetrelia* was regarded as a "parmelioid *Cetraria*" by Culberson and Culberson (1968) based on their morphological and anatomical similarities to parmelioid species. *Parmelaria*, which structural polysaccharide is not yet determined, is morphologically similar to the parmelioid genus *Parmotrema,* except for the pycnidial and apothecial location (Culberson, 1962). *Parmelaria* was nested in *Parmotrema* in previous molecular studies (Blanco et al., 2005). The placement of *Parmeliopsis* with deviating pycnidia-type and *Nesolechia,* which is a lichenicolous fungus having a cupulate exciple, among parmelioid lichens has been shown and discussed previously (Crespo et al., 2001; Blanco et al., 2006, Persoh and Rambold, 2002). The peltate *Omphalodiella* has not been studied using molecular data before. However, already Henssen (1991) noted the similarities in hymenial characters and cortical chemistry of *Xanthoparmelia* and *Neofuscelia*. The latter is now treated as a synonym of *Xanthoparmelia* (Blanco et al., 2004b).

Other genera earlier included in the parmelioid group based on morphology, such as *Allantoparmelia, Arctoparmelia, Melanelia* and *Psiloparmelia,* do not fall into this clade. This is in concordance with recent molecular studies (e.g., Blanco et al., 2004a, b, 2006; Thell et al., 2004). Based on morphological and chemical characters, these genera were already shown to be

aberrant within parmelioid genera. *Allantoparmelia* was placed near hypogymnioid lichens (Elix, 1993), *Arctoparmelia* was shown to have a deviating polysaccharide-type in the cell walls (Elix, 1993), and finally *Psiloparmelia*, which has a different type of epicortical layer (Lumbsch et al., 1992).

Psiloparmelioid group. This clade corresponds to the genera *Everniopsis* and *Psiloparmelia.* A close relationship of these two genera has not been proposed previously and morphological characters that would characterize this group are currently not known. However, both genera have a similar thallus surface, contain both usnic acid and atranorin in the cortex, and have bifusiform conidia (Elix, 1993); while *Psiloparmelia* has isolichenan, in *Everniopsis* the cell wall polysaccharide has not been determined. The two genera are widely distributed in higher elevations of the neotropics and also occur in South Africa. Additional morphological and chemical studies are necessary to clarify the relationships of the two genera.

The monophyletic, well-supported clades agree partially with groups with a circumscription based on morphology. 53 of the 59 genera included in this study were placed in one of the six morphological groups (Table 1) previously recognized. Of these, the position of 35 genera could be supported by molecular data while the placement of 12 genera remained unresolved. Only a small percentage of genera were misplaced based on morphology. This includes the genera *Arctoparmelia, Cetrelia, Melanelia, Parmelaria* and *Parmeliopsis.* All genera that were previously considered as being aberrant within their presumed group based on other morphological, chemical or structural (growth forms) characters, suggesting that the classifications previously used were based on oversimplifications. In the case of the genera *Everniopsis* and *Psiloparmelia,* the genera were found to belong to previously unrecognised clades. It should be noted that, although widely different growth forms occur in some clades (e.g.

the parmelioid group), most smaller clades are very well circumscribed morphologically by their growth form and further morphological characters, such as cortical structure (e.g., the alectorioid group). Cell wall polysaccharides have been revealed as important traits to characterize monophyletic groups (e. g. *Xanthoparmelia*–type lichenan within parmelioid genera, cf. Elix, 1993; Blanco et al., 2004b). They have not been studied in detail in most of the groups of Parmeliaceae, however, and much further research is needed to accomplish this.

Our results clearly indicate that morphological characters are useful when characterizing and identifying monophyletic groups within Parmeliaceae, but that the interpretation of the morphological diversity found within this, the most species-rich lineage of fungi, has been too superficial. A more detailed investigation of the distribution and development of important morphological characteristics is clearly needed simultaneously with additional molecular studies, to understand the phylogeny and evolution, and to facilitate the classification of this ecologically important group of lichen-forming fungi.

Acknowledgments

This project has been supported by the Spanish Ministry of Education and Science (CGL2004-1848/BOS) to AC and a Juan de la Cierva grant to PKD; a start up fund of the Field Museum to HTL, a start up fund to JEM from Södertörns University College, and grants to MW from the Kempe foundation (JCK-2026), the Royal Academy of Sciences, Magn. Bergvalls Stiftelse, and the Swedish Research Council (NFR B 5101-20005187, VR 620-2001-5756, VR 621-2002-349, VR 621-2003-303). Sequencing was carried out at the Unidad de Genómica (Parque Científico de Madrid) by M. Isabel García, the Pritzker Laboratory of Molecular Systematics at The Field Museum, Chicago, the Natural History Museum, London, and the Umeå

Plant Science Centre. We are indebted to various colleagues for sending fresh material of several species, notably to Johannes Bergsten, Jack Elix, Gintaras Kantvilas, Maria Ines Messuti, and Bengt Oldhammer. Nora Wirtz kindly provided the sequences of *Usnea antarctica* and *U. trachycarpa* for our study. Carin Olofsson, Emma Persbo, David Tingström, Heidi Döring, and Iyabo Osifeso assisted with laboratory work. JEM acknowledges the Museum of Evolution (Uppsala University) and the Natural History Museum in London for providing the possibility to work on this project. AC is indebted to Walter Obermayer (GZU) for providing material and facilities for studying the collection. Ralph Common is thanked for sharing unpublished information on polysaccharide types in Parmeliaceae.

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Fig. 1. Phylogenetic relationships of Parmeliaceae inferred from a combined analysis of nuclear ITS, LSU, mitochondrial SSU rDNA, and nuclear *RPB1* sequences. 50% majority-rule consensus tree of 38,000 trees sampled using a Bayesian MC/MCMC analysis. Branches with posterior probabilities above 0.94 and also bootstrap support under parsimony equal or above 70% are indicated in bold.

Table 1

Morphologically defined groups in Parmeliaceae

Group	References	Main characteristics
Alectorioid (Alectoriaceae) s. lat.	Poelt (1973), Krog (1982), Esslinger (1989)	Thallus fruticose, cortical layer composed of periclinally arranged hyphae, sometimes additional layers present
Alectorioid (Alectoriaceae) s. str.	Eriksson and Hawksworth (1985), Kärnefelt and Thell (1992)	Thallus fruticose, ascospores pigmented, simple or septate to muriform
Anzioid (Anziaceae)	Poelt (1973)	Thallus foliose, asci polyspored, with curved ascospores
Cetrarioid	Goward (1985), Kärnefelt et al. (1992)	Thallus foliose to subfruticose, pycnidia and apothecia marginal
Hypogymnioid (Hypogymniaceae)	Poelt (1973)	Thallus foliose, lacking rhizines, medulla loose, often hollow
Parmelioid	Goward (1985), DePriest (1999)	Thallus foliose, pycnidia and apothecia laminal
Usneoid (Usneaceae)	Hale (1983), Kärnefelt et al. (1998)	Thallus fruticose, cortex para- and/or prosoplectenhchymatous, never of periclinally arranged hyphae

Table 2

Genera of Parmeliaceae included in this study and their group placement according to morphological characters following Krog (1982), Goward (1985), Kärnefelt and Thell (1992), Kärnefelt et al. (1992, 1998), Elix (1993), and Kärnefelt (1998) and according to molecular markers; * alectorioid s. lat. group following Poelt (1973), Krog (1982), and Esslinger (1989); - genus not placed in group; -- placement in group not supported

Genus	Morphological group	Growth form	No of described species	Phylogenetic group placement according to this study
Alectoria	alectorioid	fruticose	8	alectorioid
Allantoparmelia	parmelioid	foliose	3	--
Almbornia	parmelioid	subfruticose	2	parmelioid
Anzia	anzioid	foliose	Ca. 45	-
Arctocetraria	cetrarioid	subfruticose	2	cetrarioid
Arctoparmelia	parmelioid	foliose	5	hypogymnioid
Brodoa	hypogymnioid	foliose	3	hypogymnioid
Bryoria	alectorioid*	fruticose, caespitose	Ca. 75	--
Bulbothrix	parmelioid	foliose	52	parmelioid
Canoparmelia	parmelioid	foliose	49	parmelioid
Cetraria	cetrarioid	subfruticose	Ca. 30	cetrarioid
Cetrariella	cetrarioid	erect foliose	2	cetrarioid
Cetrelia	cetrarioid	foliose	18	parmelioid

Cetreliopsis	cetrarioid	foliose	8	cetrarioid
Cornicularia	-	caespitose, erect	1	-
Evernia	usneoid	subfruticose	Ca. 5	-
Everniastrum	parmelioid	foliose	40	parmelioid
Everniopsis	usneoid	subfruticose	1	psiloparmelioid
Flavocetraria	cetrarioid	erect foliose	2	cetrarioid
Flavoparmelia	parmelioid	foliose	35	parmelioid
Flavopunctelia	parmelioid	foliose	7	parmelioid
Hypogymnia	hypogymnioid	foliose	Ca. 50	hypogymnioid
Hypotrachyna	parmelioid	foliose	Ca. 190	parmelioid
Imshaugia	-	foliose	7	-
Karoowia	parmelioid	subcrustose	19	parmelioid
Letharia	usneoid	fruticose	Ca. 5	letharioid
Lethariella	usneoid	fruticose	11	letharioid
Melanelia	parmelioid	foliose	8	cetrarioid
Melanelixia	parmelioid	foliose	11	parmelioid
Melanohalea	parmelioid	foliose	19	parmelioid
Menegazzia	hypogymnioid	foliose	Ca. 60	--
Myelochroa	parmelioid	foliose	28	parmelioid
Namakwa	parmelioid	foliose	2	parmelioid

Nesolechia	-	lichenicolous fungi	4	parmelioid
Omphalodiella	-	peltate	1	parmelioid
Oropogon	alectorioid	fruticose	Ca. 40	--
Pannoparmelia	parmelioid	foliose	5	--
Parmelaria	cetrarioid	foliose	2	parmelioid
Parmelia	parmelioid	foliose	45	parmelioid
Parmelina	parmelioid	foliose	15	parmelioid
Parmelinella	parmelioid	foliose	5	parmelioid
Parmelinopsis	parmelioid	foliose	25	parmelioid
Parmeliopsis	-	foliose	Ca. 6	parmelioid
Parmotrema	parmelioid	foliose	Ca. 350	parmelioid
Platismatia	cetrarioid	foliose	10	--
Pleurosticta	parmelioid	foliose	3	parmelioid
Protoparmelia	-	crustose	Ca. 20	-
Protousnea	usneoid	fruticose	8	-
Pseudephebe	alectorioid*	fruticose	3	alectorioid
Pseudevernia	hypogymnioid	foliose	4	hypogymnioid
Psiloparmelia	parmelioid	foliose	12	psiloparmelioid
Punctelia	parmelioid	foliose	34	parmelioid
Relicina	parmelioid	foliose	54	parmelioid

Sulcaria	alectorioid	fruticose	4	alectorioid
Tuckermannopsis	cetrarioid	erect foliose	11	cetrarioid
Usnea	usneoid	fruticose	500	-
Vulpicida	cetrarioid	erect foliose	6	cetrarioid
Xanthomaculina	parmelioid	umblicate	2	parmelioid
Xanthoparmelia	parmelioid	foliose	Ca. 800	parmelioid

Table 3
Species and specimens of Parmeliaceae analysed. New sequences not used in previous studies are in bold.

Species	Voucher	Locality	Collectors	mt SSU	Nu ITS	Nu LSU	*RPB1*
Alectoria nigricans	UPS	Sweden: Dalarna	Lundqvist 8377	**DQ923620**	**DQ979996**	**DQ923649**	--
A. nigricans	UPS	Norway: Troms	Wedin 7297	--	--	--	**DQ923676**
A. ochroleuca	BM	Austria: Styria	Wedin Aug. 1998	DQ899289	**DQ979997**	DQ899288	--
A. ochroleuca	UPS	Sweden: Härjedalen	Wedin 6542	--	--	--	**DQ923677**
A. sarmentosa	UPS	Sweden: Västerbotten	Wedin 6350	DQ899291	**DQ979998**	DQ899290	**DQ923678**
Allantoparmelia alpicola	UPS	Sweden, Lycksele Lappmark	Wedin 7159	**DQ923621**	**DQ979999**	**DQ923650**	**DQ923679**
Almbornia azaniensis	MAF-Lich 14269	South Africa: Matroosberg	Crespo et al. s.n.	**EF025478**	**EF042900**	**EF042910**	**EF092098**
A. cafferensis	MAF-Lich 14268	South Africa: Matroosberg	Crespo et al. s.n.	**EF025479**	**EF042901**	**EF042911**	--
Anzia colpodes	UPS	USA: Tennessee, Smoky Mtn. National Park	Lumbsch 4.VI.04	**DQ923622**	**DQ980000**	**DQ923651**	--
Arctocetraria andrejewii	UPS	USA: Alaska	Zhurbenko 1189	**DQ923623**	**DQ980001**	**DQ923652**	**DQ923680**
Arctoparmelia centrifuga	MAF-Lich 6879	Sweden: Umea	Eriksson s.n.	AF351156	AY581054	AY578917	**EF092099**
Brodoa atrofusca	MAF-Lich 6780	Spain: Zamora	Crespo et al. s.n.	AY643090	**EF042902**	AY607824	**EF092100**
B. intestiniformis	UPS	Sweden: Härjedalen	Wedin 6329	**DQ923624**	**DQ980002**	**DQ923653**	**DQ923681**
B. oroarctica	UPS	Norway: Troms	Wedin 7293	**DQ923625**	**DQ980003**	**DQ923654**	**DQ923682**
Bryoria capillaris	UPS	Sweden: Uppland	Mattsson 4009	**DQ923626**	AF058032	**DQ923655**	--
B. capillaris	UPS	Sweden: Västerbotten	Wedin 7624	--	--	--	**DQ923683**
B. fremontii	UPS	Sweden: Västerbotten	Wedin 6349	**DQ923627**	**DQ980004**	**DQ923656**	--
B. fremontii	UPS	Sweden: Västerbotten	Wedin s.n.	--	--	--	**DQ923684**
B. fuscescens	MAF-Lich 6923	Spain: Salamanca	Crespo et al. s.n.	AF351158	**EF042903**	**EF042912**	**EF092101**
Bulbothrix meizospora	GPGC 02-000786	India: Uttaranchal	Divakar s.n.	AY611127	AY611068	AY607780	**EF092102**
B. setschwanensis	MAF-Lich 10212	China: Chu Xiong County	Crespo et al. s.n.	--	AY611069	AY607781	**EF092103**
Canoparmelia carneopruinata	F	Costa Rica: Sarchi	Lücking 15171a	**EF025480**	**EF042904**	**EF042913**	--
C. crozalsiana	MAF-Lich 7658	Spain: Cádiz	Crespo et al. s.n.	AY586594	AY586571	AY584831	**EF092104**
C. pruinata	MAF-Lich 14270	Australia: Tutanning Natural Reserve	E. McCrum s.n.	**EF025481**	**EF042905**	**EF042914**	--
C. texana	MAF-Lich 14273	Australia: Bermagui	Elix 31550	**EF025482**	--	--	--
C. texana	GPGC 02-000637	India: Uttaranchal	Divakar s.n.	--	**EF042906**	**EF042915**	**EF092105**
Cetraria islandica	UPS	Sweden: Västerbotten	Wedin 15/5/05	AY340486	AF117995	AY340539	**DQ923685**

Cetrariella delisei	UPS	Sweden: Västerbotten	Wedin 6351	**DQ923628**	**DQ980005**	**DQ923657**	--
C. delisei	UPS	Sweden: Västerbotten	Wedin 7625	--	---	--	**DQ923686**
Cetrelia chicitae	--	Philippines: Mt. Data National Park, Mt. Province	Bawingan CL 0650	**DQ923629**	**DQ980006**	**DQ923658**	--
C. olivetorum	UPS	England: Devonshire	Wedin 6272	**DQ923630**	**DQ980007**	**DQ923659**	--
Cetreliopsis rhytidocarpa	--	Philippines: Mt. Ugo, Tinongdan, Benguet	Bawingan CL 0582	**DQ923631**	**DQ980008**	**DQ923660**	--
Cladonia rangiferina	UPS	Sweden: Jämtland	Wedin 6935	AY300881	AF458306	AY300832	**DQ915595**
Cornicularia normoerica	UPS	Norway: Sör-Tröndelag	Hatten et al. 9302	**DQ923632**	**DQ980009**	**DQ923661**	**DQ923687**
Evernia mesomorpha	UPS	Sweden: Dalarna	Oldhammer s.n.	**DQ923633**	**DQ980010**	**DQ923662**	--
E. prunastri	UPS	Sweden: Ångermanland	Wiklund 2000	**DQ923634**	AF058033	AF107562	--
E. prunastri	F	Germany: Hesse	Schmitt s.n.	--	--	--	**EF105428**
Everniastrum cirrhatum	**--**	Costa Rica: San José	Trest 149	AY611128	AY611070	AY607782	--
E. nepalense	GPGC 02-000924	India: Uttaranchal	Divakar s.n.	AY611129	AY611071	AY607783	**EF092106**
Everniopsis trulla	F	Perú: Ancash	Lumsbch et al. 19308c	**EF108289**	**EF105411**	**EF108290**	**EF105429**
Flavocetraria nivalis	BM	Sweden: Jämtland	Wedin 5052	**DQ923635**	**DQ980011**	**DQ923663**	--
F. nivalis	UPS	Sweden:Västerbotten	Wedin 15/9/03				**DQ923688**
Flavoparmelia caperata	MAF-Lich 6045	Spain: Teruel	Crespo et al. s.n.	AF351163	AY581059	AY578922	**EF092107**
F. soredians	MAF-Lich 10176	Spain: Cáceres	Crespo et al. s.n.	AY586586	AY586562	AY584835	**EF092108**
F. springtonensis	MAF-Lich 14271	Australia: Flinders Ranges	Elix 31200	**EF025483**	**EF042907**	**EF042916**	**EF092109**
Flavopunctelia flaventior	MAF-Lich 6046	Spain: Teruel	Crespo et al. s.n.	AF351164	AY581060	AY578923	**EF092110**
Hypogymnia physodes	--	--	--	AY756400	AF058036	AY756338	AY756407
H. vittata	UPS	Sweden: Jämtland	Wedin 15/7/00	DQ900629	**DQ980012**	DQ900637	--
H. vittata	UPS	Sweden: Västerbotten	Wedin 6814	--	--	--	**DQ923689**
Hypotrachyna ciliata	MAF-Lich 10185	China: Yunnan, Jianchian County	Crespo et al. s.n.	AY785280	AY785273	AY785266	**EF092111**
H. revoluta	MAF-Lich 6047	Spain: Vizcaya	Noya & Olea s.n.	AF351166	AY611075	AY607787	**EF092112**
H. sinuosa	MAF-Lich 10179	United Kingdom: Scotland	Coppins s.n.	AY611133	AY611076	AY607788	**EF092113**
Imshaugia aleurites	MAF-Lich 6877	Australia: Australian Capital Territory	Louwhoff et al.	AY351167	AY611126	AY607840	**EF092114**
Karoowia saxeti	EBL	Taiwan: Pigntung County	Aproot 53350	AY582299	AY581063	AY578926	**EF092115**
Lecanora hybocarpa	F	Spain: Guadalajara	Lumbsch s.n.	**EF105417**	**EF105412**	**EF105421**	**EF105430**
L. paramerae	F	Spain: Guadalajara	Lumbsch s.n.	**EF105418**	**EF105413**	**EF105422**	**EF105431**
L. sulphurea	F	Spain: Guadalajara	Lumbsch s.n.	**EF105419**	AF070030	**EF105423**	**EF105432**
Letharia columbiana	UPS	USA: California	Moberg 11301	**DQ923636**	**DQ980013**	**DQ923664**	--
Lethariella cashmeriana	UPS	Tibet: Sichuan	Obermayer 8335	**DQ923637**	**DQ980014**	**DQ923665**	**DQ923690**

'Melanelia' disjuncta	UPS	Sweden: Lycksele Lappmark	Wedin 7143	**DQ923638**	**DQ980015**	**DQ923666**	**DQ923691**
M. hepatizon	UPS	Sweden: Västerbotten	Wedin 6821	**DQ923639**	--	--	--
M. hepatizon	UPS	Sweden: Västerbotten	Wedin 6812	--	**DQ980016**	**DQ923667**	**DQ923692**
M. stygia	BM	Sweden: Hälsingland	Wedin 5080	**DQ923640**	AY611121	AY607835	--
M. stygia	UPS	Sweden: Västerbotten	Wedin 7626	--	--	--	**DQ923693**
Melanelixia fuliginosa 1	MAF-Lich 10223	Spain: La Rioja	Blanco s.n.	AY611146	AY611089	AY607801	**EF092116**
M. fuliginosa 2	MAF-Lich 10222	Spain: Burgos	Crespo s.n.	AY611142	AY611085	AY607797	**EF092117**
M. glabra	MAF-Lich 10228	Spain: Guadalajara	Crespo et al. s.n.	AY611144	AY611087	AY607799	**EF092118**
M. subargentifera	MAF-Lich 6049	Spain: Teruel	Crespo et al. s.n.	AY611155	AY611098	AY607810	**EF092119**
M. subaurifera	MAF-Lich 10215	United Kingdom: England, London	Crespo s.n.	AY611156	AY611095	AY607811	**EF092120**
Melanohalea aff. exasperata	MAF-Lich 10230	Spain: Asturias	Blanco s.n.	AY611153	AY611095	AY607808	**EF092121**
M. elegantula	MAF-Lich 10218	Spain: Madrid	Crespo & Divakar s.n.	AY611135	AY611078	AY607790	**EF092122**
M. exasperata	MAF-Lich 10214	Spain: Guadalajara	Blanco s.n.	AY611138	AY611081	AY607793	**EF092123**
M. exasperatula	MAF-Lich 10213	Spain: Madrid	Crespo et al. s.n.	AY611147	AY611090	AY607802	**EF092124**
M. olivacea	H	Finland: Puolanca	Vitikainen 16196	AY611148	AY611091	AY607811	**EF092125**
M. subelegantula	NDA	USA: Oregon	Esslinger 16132	AY611171	AY611115	AY607829	**EF092126**
Menegazzia confusa	UPS	Australia: Tasmania	Kantvilas 167/00	**DQ923641**	**DQ980017**	**DQ923668**	--
M. myriotrema	UPS	Australia: Tasmania	Kantvilas 169/00	DQ899303	**DQ980018**	DQ899302	--
M. terebrata	UPS	Sweden: Gästrikland	Wedin 4392	DQ899305	**DQ980019**	DQ899304	**DQ923694**
Myelochroa aurulenta	MAF-Lich 13992	India: North Sikkim	Divakar s.n.	**EF025484**	DQ279530	**EF042917**	**EF092127**
M. irrugans	MAF-Lich 10207	China: Yunnan, Jianchian County	Crespo et al. s.n.	AY611160	AY611103	AY607815	**EF092128**
M. metarevoluta	MAF-Lich 10208	China: Yunnan, Jianchian County	Crespo et al. s.n.	AY611159	AY611102	AY607814	**EF092129**
Namakwa exornata	MAF-Lich 14266	South Africa: Cape Region	Crespo et al. s.n.	**EF025485**	**EF042908**	**EF108318**	**EF092130**
Nesolechia oxyspora	UPS	Norway: Troms	Fröberg 10/08/03	**DQ923642**	**DQ980020**	**DQ923669**	--
Oropogon sperlingii	F	Perú: Ancash	Lumbsch et al. 19326a	--	**EF105414**	**EF105424**	**EF105433**
Omphalodiella patagonica	UPS	Argentina: Río Negro	Lumbsch et al. 11036a	**DQ923643**	**DQ980021**	**DQ923670**	--
Pannoparmelia angustata	MAF-Lich 7321	Australia: Molonglo Gorge Reserve	Elix 42640	AF351170	AY785272	AY785265	**EF092131**
Parmelaria subthmonsonii	LWG 20-77151	India: Sikkim	Chatterjee & Divakar s.n.	AY586588	AY586564	AY584836	--
Parmelia discordans	MAF-Lich 10232	United Kingdom: Scotland	Hawksworth s.n.	DQ287841	AY583212	**EF042918**	**EF092132**
P. saxatilis	UPS	Sweden: Västerbotten	Wedin 7091	AF351172	AF058037	AY300849	**DQ923695**

P. serrana	MAF-Lich 9756	Spain: Madrid	Crespo & Divakar s.n.	AY582319	AY295109	AY578948	**EF092133**
P. squarrosa	MAF-Lich 7288	USA: Virginia	Flenniken 4737	AY611162	AY036975	AY607815	**EF092134**
P. sulcata	MAF-Lich 6054	United Kingdom: England, Norfolk	Lambley s.n.	AY582320	AY581083	AY578949	**EF092135**
Parmelina quercina	MAF-Lich 6057	Spain: Madrid	Crespo s.n.	AY611164	AY611105	AY607818	**EF092136**
P. tiliacea	MAF-Lich 6056	Spain: Teruel	Crespo s.n.	AY351173	AY581084	AY578950	**EF092137**
Parmelinella wallichiana	LWG-2077171	India: Sikkim	Chatterjee & Divakar s.n.	AY611165	AY611106	AY607819	--
Parmelinopsis horrescens	MAF-Lich 9913	Spain: La Coruña	Carballal s.n.	AY582321	AY581085	AY578951	**EF092138**
P. minarum	MAF-Lich 7639	Spain: Cádiz	Crespo et al. s.n.	AY582322	AY581086	AY578952	**EF092139**
P. neodamaziana	MAF-Lich 10182	Australia: Motion National Park	Louwhoff et al. s.n.	AY611166	AY611107	AY607820	**EF092140**
P. subfatiscens	MAF-Lich 6878	Australia: Motion National Park	Louwhoff et al. s.n.	AF351174	AY611108	AY607821	**EF092141**
Parmeliopsis ambigua	--	--	--	AF351175	AF410829	AY607822	--
P. hyperopta	MAF-Lich 10181	Spain: Madrid	Blanco s.n.	AY611167	AY611109	AY607823	**EF092142**
Parmotrema cetratum	MVM	Uruguay: Maldonado	Osorio 9424	AY586598	AY586576	AY584847	**EF092143**
P. haitiense	MAF-Lich 7657	Australia: Australian Capital Territory	Lowhoff et al. s.n.	AY582295	AY581055	AY578918	**EF092144**
P. perforatum	**--**	USA: North Carolina	Cole 7983	AY586591	AY586568	AY584840	**EF092145**
P. perlatum	MAF-Lich 6965	Portugal: Sintra	Crespo et al. s.n.	AY586580	AY586566	AY584838	**EF092146**
Platismatia glauca	--	--	--	AY756404	AF058035	AY756342	AY756410
P. norvegica	UPS	Sweden: Jämtland	Tibell 22720	**DQ923644**	**DQ980022**	**DQ923671**	**DQ923696**
Pleurosticta acetabulum	MAF-Lich 9914	Spain: Guadalajara	Crespo et al. s.n.	AY582323	AY581087	AY578953	**EF092147**
Protoparmelia badia	F	Spain: Guadalajara	Lumbsch s.n.	**EF105420**	AF070023	**EF105425**	**EF105434**
Protousnea magellanica	UPS	Argentina: Nequen	Messuti 14.XI.01	**DQ985194**	**DQ985192**	**DQ985193**	**DQ985195**
Pseudephebe pubescens	MAF-Lich 6774	Spain: Zamora	Crespo s.n.	AF351180	AY611125	AY607839	**EF092148**
Pseudevernia furfuracea	F	Germany: Hesse	Schmitt s.n.	AY611169	AY611112	AY607826	**EF105435**
Psiloparmelia denotata	F	Perú: Ancash	Lumbsch et al. 19302g	--	**EF105415**	**EF105426**	**EF105436**
P. sp.	F	Perú: Ancash	Lumbsch et al. 19322h	--	**EF105416**	**EF105427**	**EF105437**
Punctelia borreri	MAF-Lich 9919	Portugal: Castello Vide	Crespo et al. s.n.	AY582324	AY581088	AY578954	**EF092149**
P. pseudocoralloidea	MAF-Lich 6922	Australia: New South Wales	Louwhoff et al. s.n.	AY586595	AY586572	AY584843	**EF092150**
P. rudecta	MAF-Lich 10162	USA: New York	Molina s.n.	AY586597	AY586574	AY584845	**EF092151**

Relicina subnigra	MAF-Lich 10184	Australia: Molonglo Gorge Reserve	Louwhoff et al.	AY785281	AY785274	AY785267	**EF092152**
Sulcaria sulcata	UPS	India: Uttar Pradesh	Tibell 22073	**DQ923645**	**DQ980023**	**DQ923672**	--
S. virens	UPS	India: Uttaranchal	Tibell 23383	**DQ923646**	**DQ980024**	**DQ923673**	--
Tuckermannopsis chlorophylla	UPS	Sweden: Västerbotten	Wedin 6995	**DQ923647**	**DQ980025**	**DQ923674**	**DQ923697**
Usnea antarctica	F	Antarctica: Livingston Island	Lumbsch 19029c	**New**	**New**	**New**	**New**
U. florida	UPS	Sweden: Uppland	Mattsson 4001 .	--	AJ457147	**New**	**New**
U. trachycarpa	F	Argentina, Tierra de Fuego	Lumbsch 19001a	**New**	--	**New**	--
U. trachycarpa	F	Argentina, Tierra de Fuego	Wirtz & Messuti PA-12b	--	**New**	--	**New**
Vulpicida juniperina	UPS	Sweden: Uppland	Mattsson 4013	AY340535	AF058038	AY340577	--
V. pinastri	UPS	Sweden: Uppland	Mattsson 4004	**DQ923648**	AF058039	**DQ923675**	--
V. pinastri	UPS	Sweden: Västerbotten	Wedin 7620	--	--	--	**DQ923698**
Xanthomaculina hottentota	MAF-Lich 14267	South Africa: Cape Region	Crespo et al. s.n.	**EF025486**	**EF042909**	**EF042919**	**EF092153**
Xanthoparmelia brachinaensis	MAF-Lich 10669	Australia: Flinders Ranges	Elix 30651	--	AY581062	AY578925	**EF092154**
X. conspersa	MAF-Lich 6793	Spain: Zamora	Blanco & Crespo s.n.	AF351186	AY581096	AY578962	**EF092155**
X. mougeotii	MAF-Lich 9916	Spain: La Rioja	Blanco & Crespo s.n.	AY582336	AY581100	AY578967	**EF092156**
X. semiviridis	MAF-Lich 6876	Australia: New South Wales	Elix 30294	AF351158	AY581058	AY578921	**EF092157**

Figure 1