TAXONOMY

Phylogenetic generic classification of parmelioid lichens (Parmeliaceae, Ascomycota) based on molecular, morphological and chemical evidence

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Abstract Parmelioid lichens are a diverse and ubiquitous group of foliose lichens. Generic delimitation in parmelioid lichens has been in a state of flux since the late 1960s with the segregation of the large, heterogeneous genus *Parmelia* into numerous smaller genera. Recent molecular phylogenetic studies have demonstrated that some of these new genera were monophyletic, some were not, and others, previously believed to be unrelated, fell within single monophyletic groups, indicating the need for a revision of the generic delimitations. This study aims to give an overview of current knowledge of the major clades of all parmelioid lichens. For this, we assembled a dataset of 762 specimens, including 31 of 33 currently accepted parmelioid genera (and 63 of 84 accepted genera of Parmeliaceae). We performed maximum likelihood and Bayesian analyses of combined datasets including two, three and four loci. Based on these phylogenies and the correlation of morphological and chemical

characters that characterize monophyletic groups, we accept 27 genera within nine main clades. We re-circumscribe several genera and reduce *Parmelaria* to synonymy with *Parmotrema*. *Emodomelanelia* Divakar & A. Crespo is described as a new genus (type: *E. masonii*). *Nipponoparmelia* (Kurok.) K.H. Moon, Y. Ohmura & Kashiw. ex A. Crespo & al. is elevated to generic rank and 15 new combinations are proposed (in the genera *Flavoparmelia*, *Parmotrema*, *Myelochroa*, *Melanelixia* and *Nipponoparmelia*). A short discussion of the accepted genera is provided and remaining challenges and areas requiring additional taxon sampling are identified.

Keywords combined analysis; *Emodomelanelia*; generic concept; Lecanorales; large-scale phylogeny; lichens; lichenized fungi; *Nipponoparmelia*; Parmeliaceae; *Parmotrema*; taxonomy

Supplementary Material Figures S1–S2 and the Appendix are available in the free Electronic Supplement to the online version of this article (http://www.ingentaconnect.com/content/iapt/tax).

■ INTRODUCTION

The delimitation of genera in lichen-forming fungi has been in a state of flux since the late 1960s (Poelt, 1966; Hale, 1984b; Elix, 1993; Nimis, 1998; DePriest, 1999) with parmelioid lichens being a prominent example of this. No other group of lichenized ascomycetes was the subject of such vigorous and controversial debates about the recognition and circumscription of genera than these common foliose lichens. Parmelioid lichens have mostly foliose, dorsiventral thalli, usually with rhizines on the lower surface, laminal pycnidia and apothecia, *Lecanora*-type asci and simple hyaline ascospores (Crespo & al., 2001, 2007). With the exception of one study (Thell & al., 2004) that had parmelioid lichens fall into two separate groups, phylogenetic studies converged on one monophyletic core group of parmelioid genera (Crespo & al., 2001, 2007; Blanco & al., 2006). Parmelioid lichens represent the largest group within Parmeliaceae, including about 75% of the described species in this family, which is among the largest families of lichen-forming fungi with nearly 2500 species (Kirk & al., 2008). The parmelioid group comprises common and well-known species, such as *Parmelia sulcata*, *Flavoparmelia caperata*, *Parmotrema perlatum*, and *Punctelia subrudecta*, which are frequently used in biomonitoring studies of atmospheric pollution (Crespo & al., 1999b, 2004; Nimis & al., 2002). Besides the typical foliose growth forms, some genera with deviating morphologies, such as the peltate *Omphalodiella*, subcrustose *Karoowia*, subfruticose *Almbornia* and umbilicate *Xanthomaculina* have been shown to belong to the parmelioid lichens (Esslinger, 1981; Hale, 1985, 1989; Henssen, 1991; Thell & al., 2006). Recent molecular studies added *Parmeliopsis*, *Cetrelia* and *Parmelaria* (previously recognized as cetrarioid lichens), and even the lichenicolous fungus *Nesolechia*, to the parmelioid clade (Peršoh & Rambold, 2002; Blanco & al., 2004a, 2005; Crespo & al., 2007). As taxa were added to the parmelioid clade, taxa that were seen as typical parmelioid lichens, such as *Allantoparmelia*, *Arctoparmelia*, *Melanelia* s.str., and *Psiloparmelia*, have been found to belong to non-parmelioid groups (Crespo & al., 2007).

Traditional generic classification within parmelioid lichens has relied mostly on morphological and chemical characters of the thallus (Hale, 1974, 1984a, 1986a,b, 1988; Elix & Hale, 1987; Elix, 1993; Crespo & al., 1999a; Divakar & Upreti, 2005a). Acceptance of new genera, segregated from established genera of parmelioid lichens in the absence of ascomatal characters has not been uniform (Poelt & Vězda, 1981; Clauzade & Roux, 1986; Eriksson & Hawksworth, 1986; Purvis & al., 1992; Llimona & Hladun, 2001). Based on the late Mason Hale's 30-year study of Parmeliaceae, DePriest (1999) gave an overview of his generic delimitation of parmelioid lichens. Hale recognized 36 genera in this group. Subsequently, some of these genera were included within other genera based on morphological and/or molecular evidence. These include *Rimeliella*, which was placed into synonymy with *Canomaculina* (Elix, 1997); *Almbornia*, *Chondropsis*, *Namakwa*, *Paraparmelia*, *Neofuscelia*, and *Xanthomaculina*, which were included in *Xanthoparmelia* (Hawksworth & Crespo, 2002; Elix, 2003; Blanco & al., 2004b; Thell & al., 2006); and *Canomaculina*, *Concamerella*, and *Rimelia*, which were merged with *Parmotrema* (Blanco & al., 2005). Recently, two additional generic names (*Omphalodiella*, *Placoparmelia*) were added as synonyms of *Xanthoparmelia* (Amo & al., 2010a) and *Karoowia* was shown to be highly polyphyletic and nested within *Xanthoparmelia* as well (Amo & al., 2010b). Other genera were found to be polyphyletic, such as *Melanelia* (Blanco & al., 2004a), which fell into four distinct clades, two of which were recognized as new genera (*Melanelixia* and *Melanohalea*). *Hypotrachyna* provides another example, with *Cetrariastrum*, *Everniastrum* and *Parmelinopsis* clustered within a core group of *Hypotrachyna*, whereas a second group of *Hypotrachyna* formed a sister clade with *Bulbothrix* and *Parmelinella* (Divakar & al., 2006). The latter *Hypotrachyna* clade was subsequently segregated as *Remototrachyna* (Divakar & al., 2010). Similarly, an Australasian clade of *Parmelina* was described as a new genus—*Austroparmelina* (Crespo & al., 2010).

Although several studies have addressed generic delimitations of parmelioid lichens, the scope of these studies was limited to smaller groups of genera. With the exception of a checklist of European species (Hawksworth & al., 2008), a modern synthesis of the generic classification of parmelioid lichens, summarizing results from molecular phylogenetic studies is still lacking. To fill this gap, we initiated a collaborative research project (PARSYS, parmelioid systematics) to assemble the largest and most inclusive dataset of DNA sequences of parmelioid lichens to date to address the generic classification within this group.

Specifically, this study aimed at: (1) elucidating evolutionary relationships among parmelioid lichens with phylogenetic

analyses of a four-locus (the nuclear *RPB1* protein-coding gene, and the nuclear ITS, nuclear LSU, and mitochondrial SSU rDNA) dataset including 762 samples representing 470 species including almost all currently accepted genera; (2) providing a comprehensive and coherent classification at the generic level for parmelioid lichens reflecting current knowledge of phylogenetic relationships and giving a short morphological and chemical characterization of monophyletic genera; and (3) identifying generic groups that need additional molecular and morphological work before taxonomic changes can be proposed.

■ MATERIALS AND METHODS

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 and 1–25 ng genomic 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: (i) for the nuclear LSU rDNA: nu-LSU-0155–5′ (Döring & al., 2000), nu-LSU-0042–5′ (=LR0R) (Vilgalys, unpub., http://www.botany.duke.edu/fungi/mycolab), AL2R (Mangold & al., 2008), nu-LSU-1432–3′ (=LR7), LR5 and nu-LSU-1125–3′ (=LR6) (Vilgalys & Hester, 1990); (ii) for the nuclear ITS rDNA: ITS1F (Gardes & Bruns, 1993), ITS4 (White & al., 1990) and ITS1-LM (Myllys & al., 1999) and ITS2-KL (Lohtander & al., 1998); (iii) for the mitochondrial SSU rDNA: mrSSU1 and mrSSU3R (Zoller & al., 1999), and MSU 7 (Zhou & Stanosz, 2001), and (iv) for *RPB1* nuDNA: gRPB1-A (Stiller & Hall, 1997) and fRPB1-C (Matheny & al., 2002), and RPr2 (Wirtz & al., 2008). The 25 µL PCR reactions contained 1× buffer (containing 10 mM Tris pH 9.0, 2.5 mM $MgCl_2$, 50 mM KCl, 0.1% TritonX-100), 0.2 mM each dNTP, 0.5 µM each primer, 1.25 units *Taq* DNA polymerase (Applied Biosystems) and 1–10 ng genomic DNA extract. Alternatively, amplifications were performed in 50 µL volumes containing a reaction mixture of 5–25 ng genomic DNA, 1× DNA polymerase buffer (Biotools) (containing 2 mM $MgCl_2$, 10 mM Tris-HCl pH 8.0, 50 mM KCl, 1 mM EDTA, 0.1% Triton X-100), 0.2 mM each dNTP, 0.5 µM each primer and 1.25 units DNA polymerase (Biotools). PCRs on some samples were 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°C–60°C (ITS nrDNA), 60°C (LSU nrDNA), 57°C–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 with the following settings: denaturation for 3 min at 94°C and 25 cycles at: 96°C for 10 s, 50°C for 5 s and 60°C for 4 min. Sequencing reactions were electrophoresed on a 3730 DNA analyser (Applied Biosystems). Sequence fragments obtained were assembled with SeqMan v.4.03 (DNAStar) and manually adjusted.

Taxon sampling. — Data collection across all participants of the PARSYS project was facilitated using a web site (Zope 2.10.4) and an SQL database (Postgresql 8.2.4). Sequences which were newly generated for PARSYS were complemented with sequence data from GenBank (www.ncbi.nlm.nih.gov), using several scripts in the Python programming language (www.python.org) with modules provided from Biopython (Cock & al., 2009) to download and filter the available data. First, all available sequences belonging to Parmeliaceae and the genus *Phacopsis* (according to NCBI's taxonomy browser), plus sequences from the genus *Protoparmelia* as outgroup, were downloaded from GenBank. The choice of outgroup follows Crespo & al. (2007). Second, the downloaded data were filtered for sequences belonging to the nuclear ribosomal large subunit (nucLSU), the nuclear ribosomal intergenic spacers (nucITS, including the 5.8S rDNA), the mitochondrial ribosomal small subunit (mitSSU), and the largest subunit of the RNA polymerase II (*RPB1*). Other available gene loci were discarded due to insufficient data. Initial alignments were created with ClustalW (Thompson & al., 1994) and optimized manually using Seaview (Galtier & al., 1996). Mislabeled or otherwise unalignable sequences were removed during the process. Introns and ambiguously aligned regions were excluded prior to all analyses.

Dataset assembly. — The initial datasets for each locus were created by selecting from the initial alignments a maximum of two sequences per species. If more than two sequences were available for a given species, the longer sequences were given priority. As a consequence, the resulting single-locus datasets (1GENE, 762 OTUs) contained sequences from all species for which sequence information was available, but with no more than two OTUs per species. For a complete list of sequences see Appendix.

For the phylogenetic analysis of the combined data, three datasets were generated from the 1GENE single locus datasets by combining only those specimens for which sequence data from at least two (2GENE, 433 OTUs), at least three (3GENE, 323 OTUs), or all four (4GENE, 145 OTUs) loci per specimen was present, following the strategy of Miadlikowska & al. (2006).

Test for incongruence. — The 1GENE single locus datasets were used to test for topological incongruence among loci using the program compat3 (available at www.lutzonilab.net/downloads). For each locus individually, 500 bootstrap replicates (Felsenstein, 1985) were generated with RAxML v.7.0.4 (Stamatakis, 2006, Stamatakis & al., 2008) and all pairwise comparisons between the four loci were performed with compat3. A conflict between two loci was assumed when a clade was supported as monophyletic with a bootstrap frequency

≥75% in one tree, but supported as non-monophyletic in another (Mason-Gamer & Kellogg, 1996).

Phylogenetic analyses. — Phylogenetic searches were carried out by implementing maximum likelihood (ML) and Bayesian analyses on each of the three combined datasets (2GENE, 3GENE, and 4GENE). The analyses were run on the Duke Shared Cluster Resource (DSCR, Duke University, U.S.A.) and the computer cluster of the Nano+Bio Center (University of Kaiserslautern, Germany). If not stated otherwise, all three datasets were analyzed in the same way.

For one set of ML analyses the 2GENE, 3GENE and 4GENE combined datasets were partitioned into eight partitions: nucLSU, mitSSU, nucITS1, nuc5.8S, nucITS2, *RPB1* 1st position, *RPB1* 2nd position, and *RPB1* 3rd position. The ML analyses were carried out using RAxML v.7.0.4 (Stamatakis, 2006), implementing a GTR model of nucleotide substitution (Rodriguez & al., 1990) with a gamma shape distribution, and searching for the most likely tree with 500 heuristic replicates. Bootstrap frequencies (Felsenstein, 1985; Stamatakis & al., 2008) were estimated with 500 replicates.

The second set of ML analyses was implemented with GARLI v.0.96 (Zwickl, 2006). The 2GENE, 3GENE, and 4GENE datasets were analyzed unpartitioned, because GARLI currently does not support multiple partitions. Tree search was performed with 500 replicates using the standard settings of GARLI, implementing a GTR model of nucleotide substitution with a gamma shape distribution (approximated with four categories), and a proportion of invariable sites. Bootstrap frequencies were estimated with 500 replicates, limiting the parameter 'genthreshfortopoterm' to 10,000 generations, as suggested in the manual.

In the Bayesian analyses the 2GENE, 3GENE, and 4GENE combined datasets were partitioned as described above. Modeltest (Posada & Crandall, 1998) was used to estimate for each dataset and each of the above partitions individually the number of substitution types, to test for an implementation of a gamma shape distribution (approximated with four categories) and to test for a proportion of invariable sites. Three independent runs with 30,000,000 generations, and four independent chains each were started with MrBayes v.3.1.2 (Ronquist & Huelsenbeck, 2003) for each dataset, sampling every 500th tree. The temperature parameter for the $(MC)^3$ chains of MrBayes was lowered to 0.05 to ensure that a sufficient amount of state swapping occurred across the four chains. The burn-in fraction of sampled trees was estimated both by eye with ln-likelihood plots and using AWTY (Nylander & al., 2008).

Only nodes that received posterior probabilities equal and above 0.95 and ML-bootstrap support values equal or above 70% are interpreted as strongly supported.

■ RESULTS

DNA sequences and test for topological incongruence. — A total of 201 new sequences were generated for this study, including 43 nucITS, 49 nucLSU, 47 mitSSU, and 62 *RPB1* sequences. The 1GENE-nucLSU dataset contained 353 taxa with a total of 3242 characters, 1357 of which could be unambiguously aligned. The 1GENE-nucITS dataset had 731 taxa and 533 characters, with 366 alignable positions. The 1GENE-mitSSU dataset contained 374 taxa with 2488 characters, 641 of which were unambiguous. The 1GENE-*RPB1* dataset contained 205 taxa, and 615 of 760 characters could be unambiguously aligned. The test for topological incongruence (results not shown) displayed no supported conflicts at or above the generic level, and the single-gene datasets were thus combined for further analysis.

Phylogenetic analyses. — Analyses of three different datasets were performed: all OTUs with at least two loci (2GENE), at least three loci (3GENE), and a dataset only including OTUs with all four genes available (4GENE). There was no conflict (i.e., strongly supported conflicting topologies with ≥75% bootstrap support) between the phylogenetic trees obtained from these analyses and between ML and Bayesian analyses of each of the datasets. The trees obtained in the analyses on the 2GENE and 4GENE datasets are available as Figs. S1–S2, whereas the 3GENE dataset tree, being the best compromise between number of taxa and amount of missing loci, is shown in Fig. 1.

The combined 3GENE dataset contained 323 OTUs (311 nucLSU, 315 nucITS, 307 mitSSU, 181 *RPB1*) and a total of 2970 characters, allowing for a maximum of one missing locus per OTU. After excluding alignment positions with only undetermined character states (positions containing only Ns, gaps, or missing data), the nucLSU remained with 1351 unambiguous characters, and the mitSSU remained with 638 unambiguous characters. The total number of included characters for nucITS and *RPB1* remained unchanged with 366 and 615 characters, respectively.

The optimal ML tree estimated with RAxML (ln likelihood = –44,695.13) is shown in Fig. 1. Because inferences from RAxML, GARLI and the Bayesian analysis presented no conflict, the ML tree obtained using RAxML is shown with support from the other two methods of analysis included. The three independent runs of MrBayes plateaued at different likelihood levels, and after discarding the first 15,000,000 generations as burn-in (50%), the last 30,000 trees of the best run were used to calculate the posterior probabilities for internal branches.

Molecular phylogeny. — A cartoon phylogeny that summarises all generic relationships within Parmeliaceae that are supported in at least one of the single-locus analyses is presented in Fig. 2. The core group of parmelioid lichens as circumscribed by Crespo & al. (2007) is monophyletic and strongly supported in the 4GENE dataset, but does not receive significant support in the 2GENE and 3GENE datasets. The sister-group relationship of parmelioid lichens in Parmeliaceae is not resolved with confidence, since the sister-group relationship with *Usnea* lacks support in all datasets analyzed. Monophyly of Parmeliaceae is strongly supported. Within parmelioid lichens, several well-supported major clades are distinguished. The *Parmotrema* clade includes the following monophyletic genera: *Parmotrema* (with two individuals of *Canoparmelia norstictica* and one *Parmelaria* sp. nested within, and the *C. crozalsiana* group as sister to the remaining *Parmotrema*

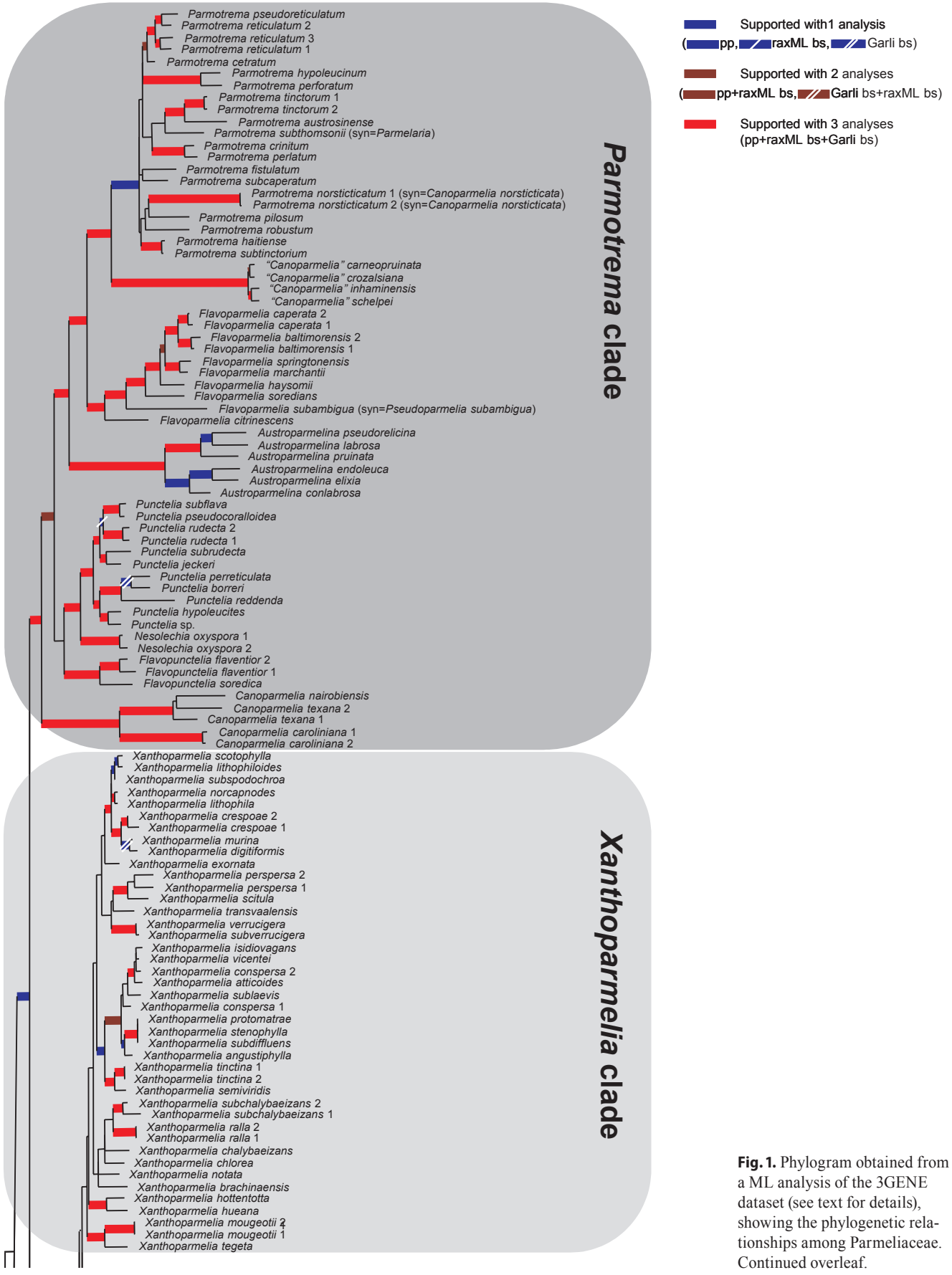
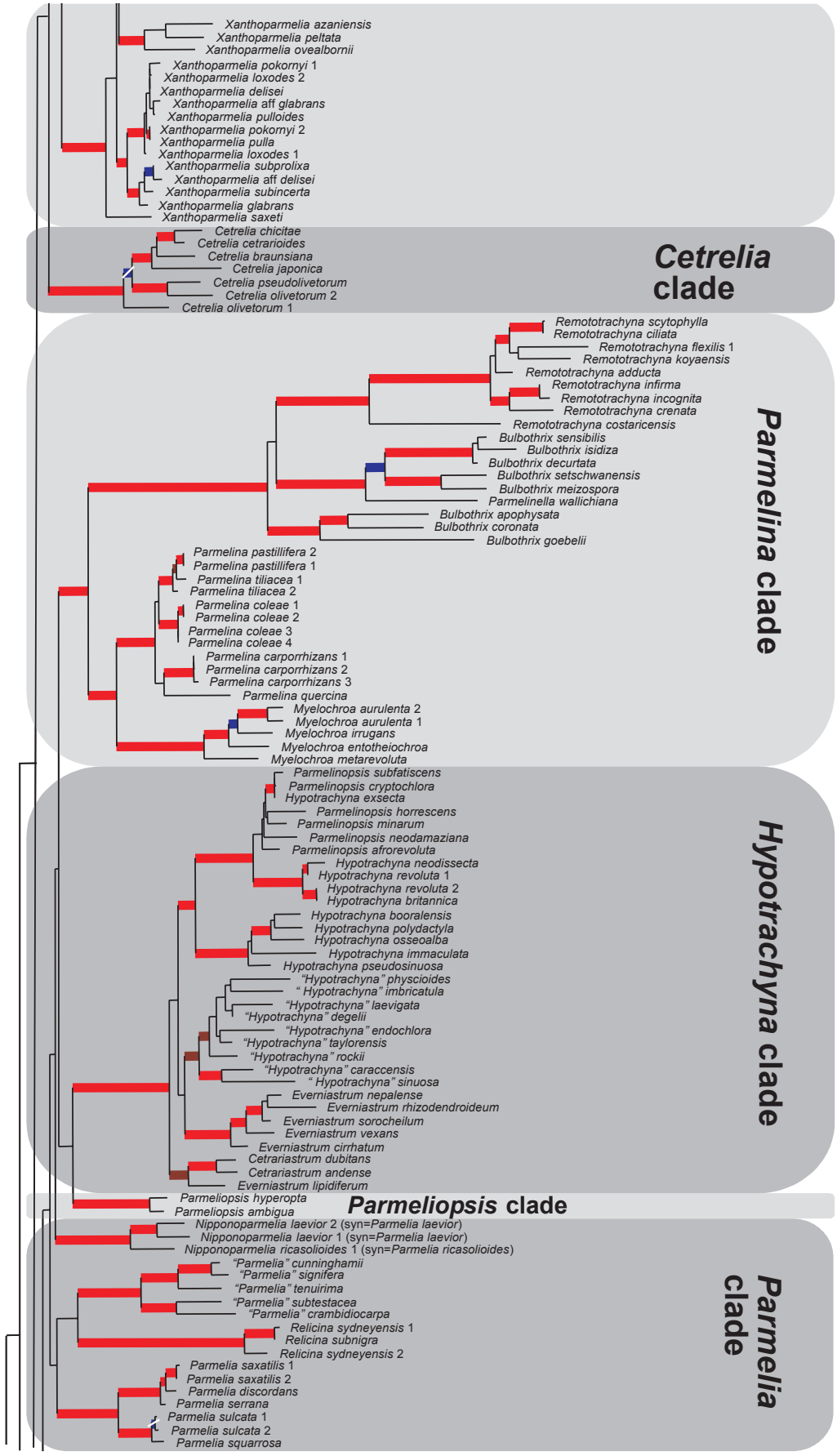


Fig. 1. Phylogram obtained from a ML analysis of the 3GENE dataset (see text for details), showing the phylogenetic relationships among Parmeliaceae. Continued overleaf.

Fig. 1. Continued.



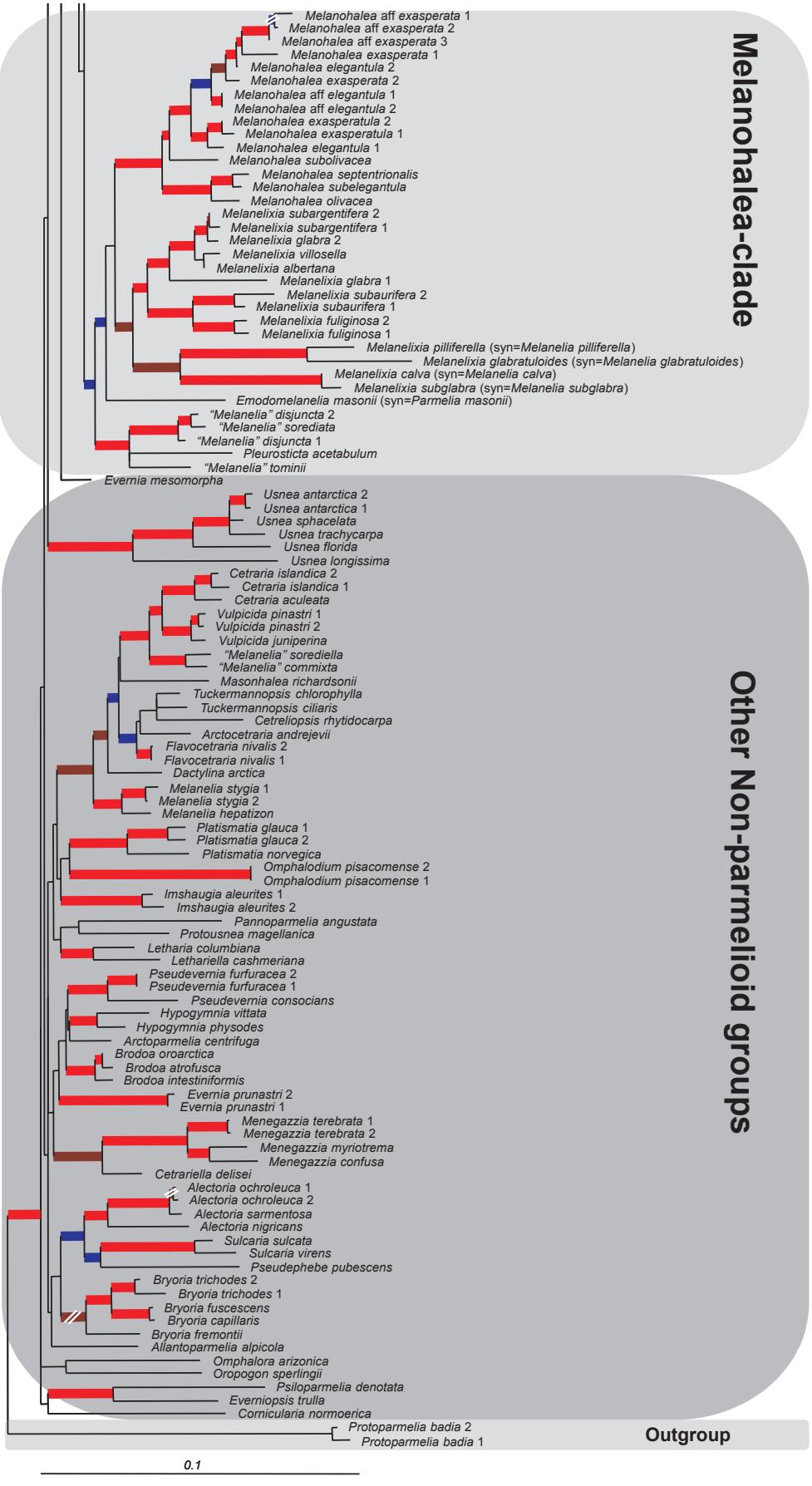


Fig. 1. Continued.

spp.), *Flavoparmelia* (with one *Pseudoparmelia* sp. nested within), *Austroparmelina*, *Punctelia*, *Nesolechia*, *Flavopunctelia*, and *Canoparmelia* s.str. The relationships among these lineages are strongly supported. The *Xanthoparmelia* clade is sister to the *Parmotrema* clade and this relationship is only supported in the Bayesian analysis. The former clade consists of a monophyletic genus *Xanthoparmelia*. The phylogenetic placement of the strongly supported, monophyletic genus *Cetrelia* is not recovered with confidence.

The *Parmelina* clade is strongly supported as monophyletic including the monophyletic genera *Myelochroa* and *Parmelina*, and in addition includes the monophyletic genus *Remototrachyna* and a paraphyletic *Bulbothrix*. In the 2GENE tree (Fig. S1), both specimens of *Hypotrachyna radiculata* compose a strongly supported lineage that is nested within *Myelochroa*. The strongly supported monophyletic *Hypotrachyna* clade includes the polyphyletic genera *Everniastrum*, *Hypotrachyna*, *Parmelinopsis*, and a monophyletic *Cetrariastrum*. In the 2GENE tree (Fig. S1), *Cetrariastrum* is nested within *Everniastrum*, but this relationship lacks support. The genera *Parmeliopsis* and the new genus *Nipponoparmelia* each form monophyletic groups with uncertain relationships. *Relicina* is strongly supported as monophyletic (including *Relicinopsis* in the 2GENE analysis, Fig. S1). The relationships of *Relicina* s.l., however, remain uncertain. *Parmelia* is polyphyletic, with one clade, segregated as *Nipponoparmelia* below with uncertain relationships, a clade of southern Hemisphere species (*Parmelia* spp. in Fig. 1) that is sister to *Relicina*, but without support, *Parmelia masonii*

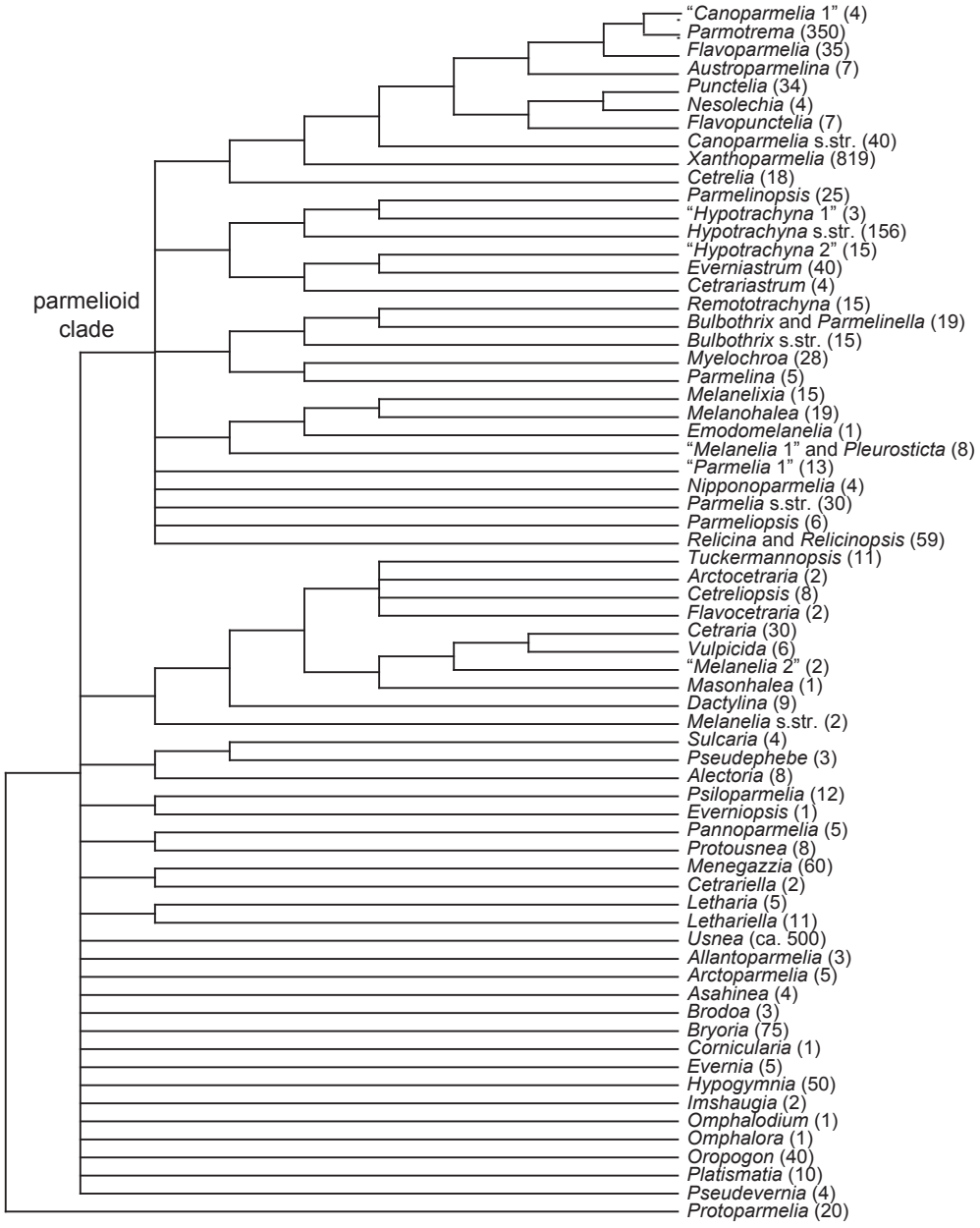


Fig. 2. Cartoon tree summarizing our current knowledge of phylogenetic relationships within Parmeliaceae. Only relationships that received significant support in at least one of the analyses part of this study are shown. Numbers of species in each clade are in parentheses.

as sister to *Melanelixia* + *Melanohalea*, and *Parmelia* s.str. (*Parmelia* spp. in Fig. 1); *Parmelia* s.str. is strongly supported as monophyletic, but lacking support regarding intergeneric relationships. The *Melanelixia* and *Melanohalea* clades form one well supported clade in the 4GENE analysis (Fig. S2), but do not receive significant support in the 2GENE or 3GENE analyses (Fig. S1 and Fig. 1, resp.). *Melanohalea* is monophyletic and *Melanelixia* as well, with a strongly supported sister-group relationship to four species from the southern Hemisphere (previously placed in *Melanelia*) that are transferred to *Melanelixia* below. Three other *Melanelia* spp. (*M. disjuncta*, *M. sorediata*, *M. tominii*) cluster in one monophyletic group with *Pleurosticta acetabulum*.

The phylogeny of non-parmelioid Parmeliaceae is beyond the focus of this paper and will not be discussed in detail here. The well-supported relationships agree mostly with those discussed in Crespo & al. (2007), including strongly supported monophyly of alectorioid (*Alectoria* incl. *Gowardia*, *Pseudephebe*, *Sulcaria*), cetrarioid (*Arctocetraria*, *Cetraria*, *Cetreliopsis*, *Dactylina*, *Flavocetraria*, *Melanelia*, *Tuckermannopsis*, *Vulpicida*), letharioid (*Letharia*, *Lethariella*), and psiloparmelioid lichens (*Everniopsis*, *Psiloparmelia*). Relationships among these groups and other monophyletic genera (e.g., *Bryoria*, *Platismatia*) remain largely unresolved. A more thorough taxon and gene sampling is needed to further assess phylogenetic relationships of these groups in Parmeliaceae.

A number of species in parmelioid lichens do not form monophyletic groups (e.g., *Canoparmelia texana*, *Hypotrachyna revoluta*, *Melanelia disjuncta*, *Melanohalea exasperata*, *Parmelina tiliacea*, *Parmotrema reticulatum*, *Punctelia rudecta*, *Xanthoparmelia pulla*; Fig. S1), indicating that additional studies are necessary to clarify their current delimitations, which are largely based on morphological and chemical characters.

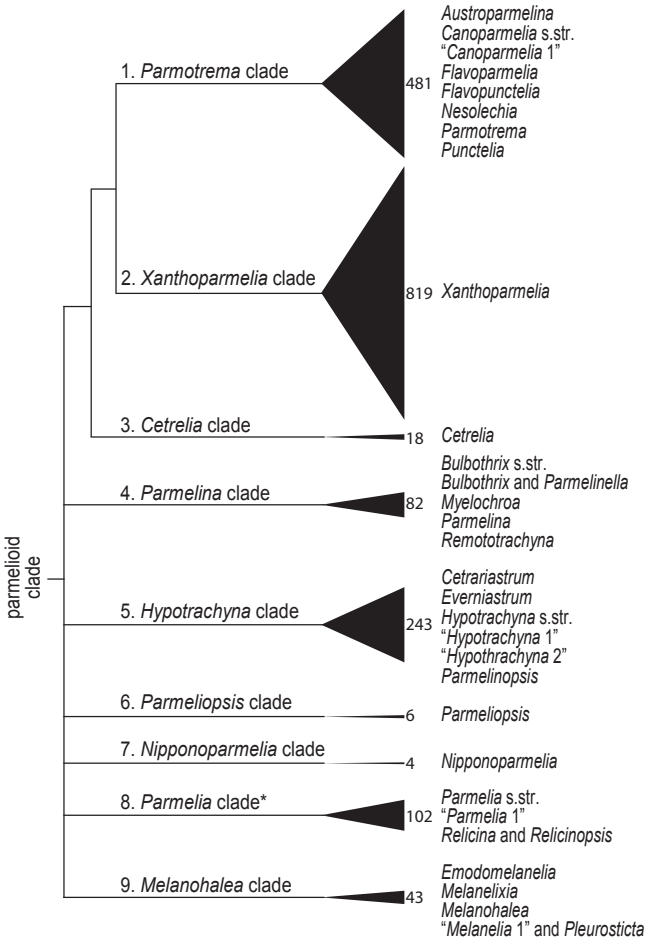


Fig. 3. Nine major groups within the parmelioid clade of Parmeliaceae sharing morphological and chemical characters. This is a cartoon tree summarizing our current knowledge of phylogenetic relationships based on nucITS, mitSSU, nucLSU, and *RPB1* single-locus and combined datasets discussed in the text. Naming and numbering of the clades follows descriptions in the text. The size of the triangles and number behind the triangles indicate the number of species currently included in each of the clades. Branch lengths in this tree are uninformative. *The *Parmelia* clade is not supported in molecular analyses, but due to shared morphological and chemical characteres we are treating the included taxa together in the text.

■ TAXONOMY

Although there is a growing body of studies focused on certain groups of parmelioid lichens, ours is the first study addressing generic classification of parmelioid lichens as a whole using molecular data. Our analyses confirm previous studies that the generic classification is in need of further revision, with several genera being para- or polyphyletic. Here we present a new generic classification based on our phylogenetic studies and morphological and chemical evidence, listed alphabetically within major clades as indicated in Figs. 1 and 3. Each of the clades and genera are briefly characterized morphologically (Fig. 4), anatomically, and chemically. Taxonomic changes are only proposed for well-supported clades. A new genus, *Emodomelanelia* is described here, *Nipponoparmelia* is elevated to generic rank, and an additional 15 new combinations are proposed. The genus *Parmelaria* is reduced to synonymy with *Parmotrema.* We identify several groups for which additional taxon sampling and/or generation of molecular data are necessary for unequivocal resolution of their phylogenetic placement. Data on conidia were studied for the included species.

1. *Parmotrema* clade

This clade includes species that contain a not well characterized but diagnostic cell wall polysaccharide (isolichenan). Most species have a pored epicortex, some have pseudocyphellae, and they contain either atranorin or usnic acid as a cortical pigment (Fig. 4A–D). The center of diversity is in the Southern Hemisphere, with numerous tropical and subtropical species, some of which extend into temperate regions (Blanco & al., 2005, 2006).

Austroparmelina A. Crespo, Divakar & Elix in Syst. Biodivers. 8: 216. 2010 – Type: *A. pseudorelicina* (Jatta) A. Crespo & al. in Syst. Biodivers. 8: 217. 2010 (*Parmelia pseudorelicina* Jatta in Boll. Soc. Bot. Ital., 1910: 254. 1911).

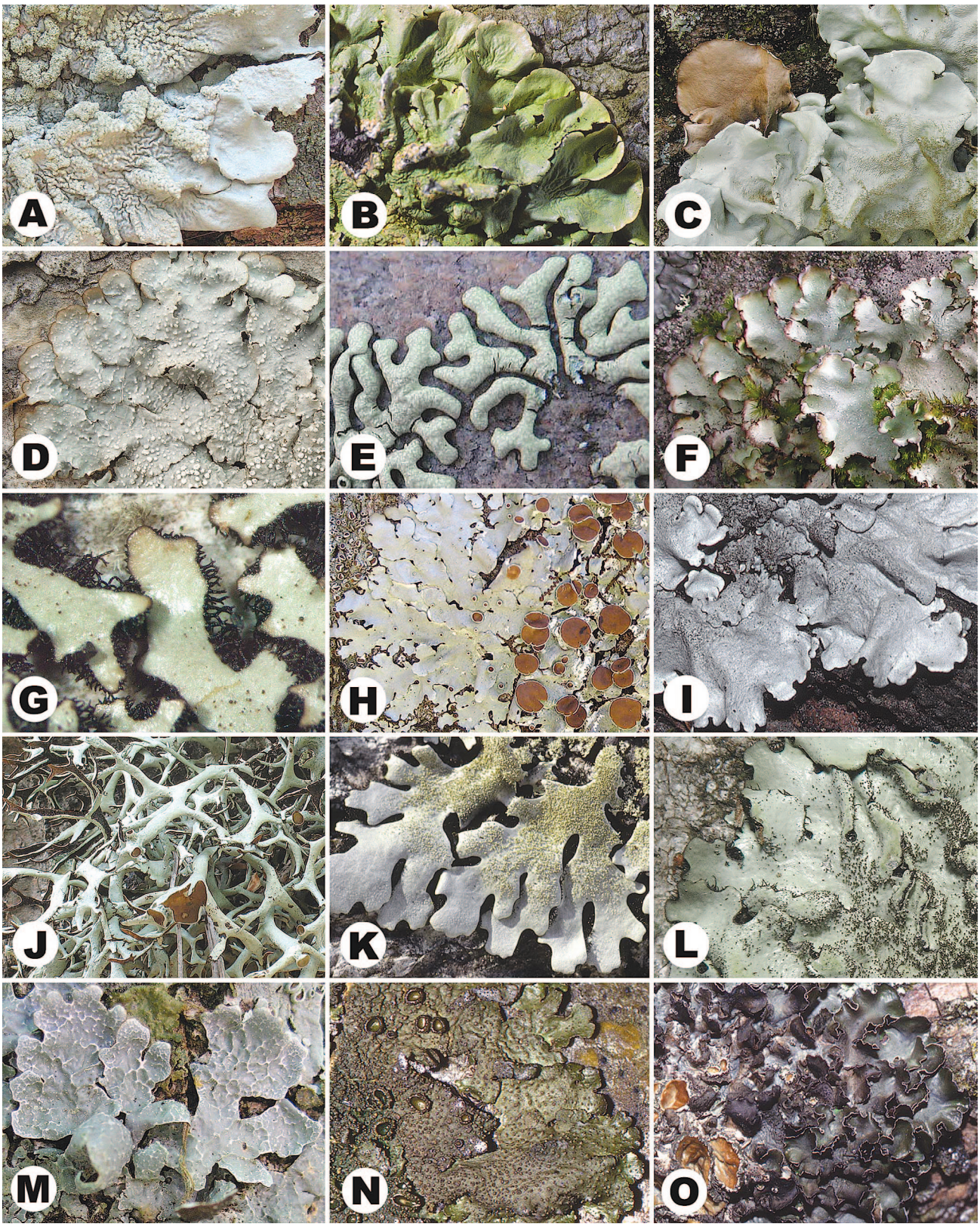


Fig. 4. Selected species representing clades and genera in the parmelioid Parmeliaceae. **A–D,** *Parmotrema* clade: **A,** *Canoparmelia texana*; **B,** *Flavoparmelia flaventior*; **C,** *Parmotrema tinctorum*; **D,** *Punctelia subrudecta*. **E,** *Xanthoparmelia* clade: *X. exornata*. **F,** *Cetrelia* clade: *C. braunsiana*. **G–I,** *Parmelina* clade: **G,** *Bulbothrix suffixa*; **H,** *Myelochroa irrugans*; **I,** *Parmelina tiliacea*. **J–L,** *Hypotrachyna* clade: **J,** *Everniastrum cirrhatum*; **K,** *Hypotrachyna imbricatula*; **L,** *Parmelinopsis horrescens*. **M,** *Parmelia* clade: *P. sulcata*. **N–O,** *Melanohalea* clade: **N,** *Melanohalea exasperata*; **O.** *Pleurosticta acetabulum*.

Diagnostic characters. – Lobes subirregular; pored epicortex present; isolichenan; conidia cylindrical.

Notes. – The genus *Austroparmelina* was recently described (Crespo & al., 2010) for species previously placed in the polyphyletic genera *Canoparmelia* and *Parmelina*. This genus is sister to a clade comprising *Flavoparmelia* + *Parmotrema*. It differs from *Parmotrema* in having adnate thalli, narrow lobes, rhizines on the lower surface extending to the margins and cylindrical conidia; and from *Flavoparmelia* in having a grey upper cortex (containing atranorin). The monophyly of this genus and its relationships are well-supported. Crespo & al. (2007) showed that "*Canoparmelia*" *pruinata* was not related to *Canoparmelia* s.str. Broadening taxon sampling to other species of *Canoparmelia* revealed (Fig. 1) that several species occurring in the Southern Hemisphere composed a novel clade in need of taxonomic recognition.

Canoparmelia Elix & Hale in Mycotaxon 27: 277. 1986 – Type: *C. texana* (Tuck.) Elix & Hale in Mycotaxon 27: 279. 1986 (*Parmelia texana* Tuck., in Amer. J. Sci. Arts, Ser. 2, 25: 424. 1858).

Diagnostic characters. – Lobes subirregular (Fig. 4A); pored epicortex present; isolichenan; conidia bifusiform.

Notes. – In its restricted circumscription, this genus includes species with broad and large ascospores, lacking depsidones and having a maculate upper surface. The genus in its restricted sense is a strongly supported monophyletic group whose common ancestry with the remaining genera within the *Parmotrema* clade is also strongly supported (Fig. 1). Molecular data suggest that *Canoparmelia* as originally circumscribed (Elix & al., 1986) is highly polyphyletic. Blanco and colleagues (Blanco & al., 2004b, 2005) showed that some *Canoparmelia* species clustered close to *Parmotrema*, and subsequently, Crespo & al. (2007) demonstrated that *other Canoparmelia* species were sister to *Flavoparmelia* and *Parmotrema*. These species were subsequently placed in the new genus *Austroparmelina* (Crespo & al., 2010). Here, we have found an additional species, *Canoparmelia norsticticata*, nested within *Parmotrema* (Fig. 1). This species is transferred to *Parmotrema* below, but the *C. crozalsiana* group that composes the sister lineage to *Parmotrema* requires additional study.

Flavoparmelia Hale in Mycotaxon 25: 604. 1986 – Type: *F. caperata* (L.) Hale in Mycotaxon 25: 604. 1986 (*Lichen caperatus* L., Sp. Pl. 2: 1147. 1753).

Diagnostic characters. – Broad lobes (Fig. 4B); pored epicortex present; isolichenan; conidia bifusiform; cortex with usnic acid.

Notes. – This genus was originally described as a segregate of *Pseudoparmelia* to accommodate species with broad lobes, containing usnic acid, isolichenan in cell walls, large ascospores and marginally erhizinate lobes (Hale, 1986b; Elix, 1993). The phylogeny of *Flavoparmelia* is currently under study (Blanco pers. comm.). The genus is sister to *Parmotrema* from which it differs in having bifusiform conidia and always containing usnic acid. Our analyses suggest the placement of *Pseudoparmelia subambigua* within *Flavoparmelia* and consequently it is here transferred to this genus.

Flavoparmelia citrinescens (Gyelnik) O. Blanco, A. Crespo & Elix, **comb. nov.** [MB 516787] ≡ *Parmelia citrinescens* Gyelnik in Ann. Mycol. 36: 271. 1938.

Flavoparmelia subambigua (Hale) O. Blanco, A. Crespo & Elix, **comb. nov.** [MB 516752] ≡ *Pseudoparmelia subambigua* Hale in Smithsonian Contr. Bot. 31: 50. 1976.

Flavopunctelia (Krog) Hale in Mycotaxon 20: 682. 1984 – Type: *F. flaventior* (Stirt.) Hale in Mycotaxon 20: 682. 1984 (*Parmelia flaventior* Stirt. in Trans. Glasgow Soc. Field- Naturalists 5: 212. 1877).

Diagnostic characters. – Lobes subirregular; non-pored epicortex, with punctiform pseudocyphellae; isolichenan; conidia bifusiform; containing usnic acid.

Notes. – This genus was originally described as a subgenus of *Punctelia* (Krog, 1982), but was later raised to generic level (Hale, 1984a) based on differences in conidial morphology. Molecular studies confirm the distinction of these two groups with roundish pseudocyphellae at generic level. This small genus (7 species) occurs in temperate and tropical regions on all continents except Australia.

Nesolechia A. Massal., Misc. Lichenol.: 43. 1856 – Type: *N. oxyspora* (Tul.) A. Massal., Misc.Lichenol.: 43. 1856 (*Abrothallus oxysporus* Tul. in Ann. Sci. Nat., Bot., sér. 3, 17: 116. 1852).

Diagnostic characters. – Lichenicolous fungus; gall forming; thallus endokapylic; apothecia with entire margin; conidia bacilliform.

Notes. – The genus *Nesolechia* was treated as a synonym of *Phacopsis* Tul. by Triebel & Rambold (1988) based on the absence of great anatomical differences between both genera. This was not accepted by some authors and Alstrup & Haswksworth (1990) offered several characters in which both genera differ. Subsequently, Diederich (2003) supported congenerity based on similarities of epihymenial and hypothecial pigments. The molecular study of species of *Phacopsis* and *Nesolechia* by Peršoh & Rambold (2002) revealed their placement in Parmeliaceae for the first time, later supported by Crespo & al. (2007). However results in Peršoh & Rambold (2002) revealed the polyphyly of the genus *Phacopsis*. Thus, until further research is made in this group we propose to keep the name *Nesolechia*. In our analysis specimens of *Nesolechia* represent the sister group to the genus *Punctelia*; this relationship is well-supported in all the trees generated.

Parmotrema A. Massal. in Atti Reale Veneto Sci. Lett. Arti, ser. 3, 5: 248. 1860 – Type: *P. perforatum* (Wulfen) A. Massal. in Atti Reale Veneto Sci. Lett. Arti, ser. 3, 5: 248. 1860 (*Lichen perforatus* Wulfen in Jacquin, Collectanea 1: 116. 1787 ['1786']).

= *Canomaculina* Elix & Hale in Mycotaxon 29: 239. 1987 – Type: *C. pilosa* (Stizenb.) Elix & Hale in Mycotaxon 29:

240. 1987 (*Parmelia pilosa* Stizenb. in Ber. Thätigk. St. Gallischen Naturwiss. Ges., 1888–89: 165. 1890).

= *Concamerella* W.L. Culb. & C.F. Culb. in Bryologist 84: 307. 1981 – Type: *C. pachyderma* (Hue) W.L. Culb. & C.F. Culb. in Bryologist 84: 308. 1981 (*Parmelia pachyderma* Hue in Nouv. Arch. Mus. Hist. Nat., ser. 4, 1: 137. 1899).

= *Parmelaria* D.D. Awasthi in J. Hattori Bot. Lab. 63: 368. 1987 – Type: *P. thomsonii* (Stirt.) D.D. Awasthi in J. Hattori Bot. Lab. 63: 368. 1987 (*Platysma thomsonii* Stirt. in Proc. Roy. Phil. Soc. Glasgow 11: 321. 1879), **syn. nov.**

= *Rimelia* Hale & A. Fletcher in Bryologist 93: 23. 1990 – Type: *R. cetrata* (Ach.) Hale & A. Fletcher in Bryologist 93: 26. 1990 (*Parmelia cetrata* Ach., Syn. Meth. Lich.: 198. 1814).

= *Rimeliella* Kurok. in Ann. Tsukuba Bot. Gard. 10: 1. 1991 – Type: *R. subcaperata* (Kremp.) Kurok. in Ann. Tsukuba Bot. Gard. 10: 7. 1991 (*Parmelia subcaperata* Kremp. in Vidensk. Meddel. Dansk Naturhist. Foren. Kjøbenhavn, ser. 3, 1873: 10. 1873).

Diagnostic characters. – Broad lobes (Fig. 4C); pored epicortex present; intermediate-type lichenan between *Cetraria*-type and *Xanthoparmelia*-type lichenan; conidia cylindrical.

Notes. – The genus as now circumscribed includes ca. 350 species that have their center of distribution in tropical regions of the world, especially in the Pacific Islands and South America (Blanco & al., 2005). The genus is sister to *Flavoparmelia*. Our analysis confirms the placement of *Parmelaria* within *Parmotrema* (the type of *Parmelaria*, *P. thomsonii*, clustered within *Parmotrema* in a single-gene analysis, data not shown) and consequently the two *Parmelaria* spp. and the species previously classified in *Canoparmelia* sensu lato, but which cluster in *Parmotrema* are transferred to *Parmotrema*.

Parmotrema norsticticatum (G.N. Stevens) A. Crespo, Divakar & Elix, **comb. nov.** [MB 516753] ≡ *Parmelia norsticticata* G.N. Stevens in Austral. J. Bot. 27: 881. 1980. ≡ *Canoparmelia norsticticata* (G.N. Stevens) Elix & Hale in Mycotaxon 27: 278. 1986.

Parmotrema subthomsonii (D.D. Awasthi) A. Crespo, Divakar & Elix, **comb. nov.** [MB 516754] ≡ *Parmelaria subthomsonii* D.D. Awasthi in J. Hattori Bot. Lab. 63: 370. 1987.

Parmotrema thomsonii (Stirt.) A. Crespo, Divakar & Elix, **comb. nov.** [MB 516755] ≡ *Platysma thomsonii* Stirt. in Proc. Roy. Phil. Soc. Glasgow 11: 321. 1879 ≡ *Parmelaria thomsonii* (Stirt.) D.D. Awasthi in J. Hattori Bot. Lab. 63: 368. 1987.

Punctelia Krog in Nord. J. Bot. 2: 290. 1982 – Type: *P. borreri* (Sm.) Krog in Nord. J. Bot. 2: 291. 1982 (*Lichen borreri* Sm. in Engl. Bot. 25: tab. 1780. 1807).

Diagnostic characters. – Lobes subirregular; non-pored epicortex, punctiform pseudocyphellae present (Fig. 4D); isolichenan; conidia unciform or cylindrical; containing atranorin, lacking usnic acid.

Notes. – This genus of ca. 45 species is cosmopolitan with highest diversity in the Neotropics and Africa. The most similar genus is *Flavopunctelia*, which differs in conidial morphology and the presence of usnic acid (Krog, 1982; Hale, 1984a). The genus is well-supported as monophyletic sister to *Nesolechia*.

2. *Xanthoparmelia* clade

This clade includes only the genus *Xanthoparmelia*, after *Karoowia* was recently included in *Xanthoparmelia* (Amo & al., 2010b). The clade includes species that have cell wall polysaccharides with *Xanthoparmelia*-type lichenan. Most species occur in the Southern Hemisphere in arid or semiarid subtropical areas, with some extending into temperate regions. The species in this clade lack true pseudocyphellae, have a pored epicortex (Fig. 4E), and show a considerable variation in cortical chemistry, including species containing usnic acid, atranorin or lacking cortical phenols (Blanco & al., 2004b; 2006).

Xanthoparmelia (Vain.) Hale in Phytologia 28: 485. 1974 – Type: *X. conspersa* (Ehrh. ex Ach.) Hale in Phytologia 28: 485. 1974 (*Lichen conspersus* Ehrh. ex Ach., Lichenogr. Suec. Prodr.: 118. 1798).

= *Almbornia* Essl. in Nord. J. Bot. 1: 125. 1981 – Type: *A. caferensis* Essl. in Nord. J. Bot. 1: 125. 1981.

= *Chondropsis* Nyl. ex Cromb. in J. Linn. Soc., Bot. 17: 397. 1879 – Type: *C. semiviridis* (F. Muell. ex Nyl.) Nyl. ex Cromb. in J. Linn. Soc., Bot. 17: 397. 1879 (*Parmeliopsis semiviridis* F. Muell. ex Nyl., Syn. Meth. Lich. 2: 57. 1869).

= *Karoowia* Hale in Mycotaxon 35: 182. 1989 – Type: *K. adhaerens* (Nyl.) Hale in Mycotaxon 35: 182. 1989 (*Parmelia adhaerens* Nyl. in J. Bot. 14: 19. 1876).

= *Namakwa* Hale in Mycotaxon 32: 169. 1988 – Type: *N. exornata* (Zahlbr.) Hale in Mycotaxon 32: 169. 1988 (*Parmelia conturbata* var. *exornata* Zahlbr. in Ann. Cryptog. Exot. 5: 251. 1932).

= *Neofuscelia* Essl. in Mycotaxon 7: 49. 1978 – Type: *N. pulla* (Ach.) Essl. in Mycotaxon 7: 52. 1978 (*Parmelia pulla* Ach., Syn. Meth. Lich.: 206. 1814).

= *Omphalodiella* Henssen in Lichenologist 23: 334. 1991 – Type: *O. patagonica* Henssen in Lichenologist 23: 335. 1991.

= *Paraparmelia* Elix & J. Johnst. in Mycotaxon 27: 279. 1986 – Type: *P. scotophylla* (Kurok.) Elix & J. Johnst. in Mycotaxon 27: 281. 1986 (*Parmelia scotophylla* Kurok. in Contr. U.S. Natl. Herb. 36: 185. 1964).

= *Placoparmelia* Henssen in Lichenologist 24: 134. 1992 – Type: *P. patagonica* Henssen in Lichenologist 24: 134. 1992.

= *Xanthomaculina* Hale in Lichenologist 17: 262. 1985 – Type: *X. hottentotta* (Ach.) Hale in Lichenologist 17: 264. 1985 (*Lichen hottentottus* Ach., Lichenogr. Suec. Prodr.: 155. 1798).

Diagnostic characters. – Pored epicortex present; *Xanthoparmelia*-type lichenan; conidia bifusiform or cylindrical; ascospores arachiform

Notes. – The genus *Xanthoparmelia* was the subject of several recent phylogenetic studies, resulting in a merging of a

number of previously recognized genera within it (Blanco & al., 2004b; Thell & al., 2006; Amo & al., 2010a,b); the most recent being *Karoowia*, *Omphalodiella* and *Placoparmelia* (Amo & al., 2010a,b). The clade is well-supported as monophyletic, but the absence of distinct morphological traits associated with well-supported monophyletic groups within the *Xanthoparmelia* clade prevents the recognition of smaller genera within this large genus. Morphological variation within this clade is likely to be driven by environmental factors and were overemphasized in previous classifications (Lumbsch & al., 2008).

3. *Cetrelia* clade

This clade consists only of the genus *Cetrelia*, which was traditionally regarded as cetrarioid based on the presence of marginal apothecia, but is now considered to belong to parmelioid lichens based on inferences from molecular data (Crespo & al., 2007). The genus has broadly lobed thalli and was previously regarded as a "parmelioid *Cetraria*" (Culberson & Culberson, 1968). Furthermore, *Cetrelia* has isolichenan as cell wall polysaccharide (Elix, 1993), which occurs in several groups of parmelioid genera, but is absent in the cetrarioid group (Crespo & al., 2007).

Cetrelia W.L. Culb. & C.F. Culb. in Contr. U.S. Natl. Herb. 34: 490. 1968 – Type: *C. cetrarioides* (Delise) W.L. Culb. & C.F. Culb. in Contr. U.S. Natl. Herb. 34: 498. 1968 (*Parmelia perlata* var. *cetrarioides* Delise in Duby, Bot. Gall. Pars Secunda: 601. 1830).

Diagnostic characters. – Broad lobes (Fig. 4F); non-pored epicortex, punctiform pseudocyphellae present; isolichenan; conidia bifusiform; apothecia marginal.

4. *Parmelina* clade

The *Parmelina* clade is enlarged from its previous concept (Blanco & al., 2006) to include *Bulbothrix*, *Parmelinella* and *Remototrachyna* in agreement with Divakar & al. (2010) as well as *Myelochroa* and *Parmelina*. The latter three genera were previously placed in the *Hypotrachyna* clade, but the relationships of these genera within the *Hypotrachyna* clade, in a restricted sense, lacked support. Species in the *Parmelina* clade have isolichenan in the cell walls, a pored epicortex, lack pseudocyphellae, and contain atranorin or usnic acid as cortical compounds (Fig. 4G–I).

Bulbothrix Hale in Phytologia 28: 479. 1974 – Type: *B. semilunata* (Lynge) Hale in Phytologia 28: 479. 1974 (*Parmelia semilunata* Lynge in Ark. Bot. 13: 23. 1914).

Diagnostic characters. – Lobes subirregular (Fig. 4G); conidia cylindrical or bifusiform; bulbate cilia present.

Notes. – The circumscription of this genus remains uncertain. In current phylogenies, the genus is paraphyletic. We have so far not been able to get sequences of the species that includes the type of the name and our taxon sampling is still poor. Additional data are required to address the issue of monophyly and circumscription of *Bulbothrix*.

Myelochroa (Asahina) Elix & Hale in Mycotaxon 29: 240. 1987 – Type: *M. aurulenta* (Tuck.) Elix & Hale in Mycotaxon 29: 240. 1987 (*Parmelia aurulenta* Tuck. in Amer. J. Sci. Arts, ser. 2, 25: 424. 1858).

Diagnostic characters. – Lobes subirregular (Fig. 4H); conidia cylindrical or bifusiform; yellow-orange medulla (secalonic acid derivatives).

Notes. – This relatively small genus (ca. 30 species) has its center of distribution in eastern Asia and is characterized by the presence of yellow-orange pigments (secalonic acid derivatives) in the medulla and simple to squarrosely branched rhizines. It is morphologically similar to *Parmelina*, which is also sister to it, but the latter differs in having a white medulla and lacking hopane triterpenes. Interestingly, these two clades have a largely vicariant distribution: while *Myelochroa* is chiefly distributed in eastern Asia, *Parmelina* is largely confined to winter-rain areas in the west of Europe and North America and adjacent regions. The status of the two genera merits further study. Results from Divakar & al. (2006) show the phylogenetic placement of *Hypotrachyna radiculata* within *Myelochroa*. Consequently, it is here transferred to this genus.

Myelochroa radiculata (Kurok.) Divakar & A. Crespo, **comb. nov.** [MB 516756] ≡ *Parmelia radiculata* Kurok., Studies Crypt. Papua New Guinea: 139. 1979 ≡ *Parmelina radiculata* (Kurok.) Streimann in Biblioth. Lichenol. 22: 92. 1986 ≡ *Parmelinopsis radiculata* (Kurok.) Elix & Hale in Mycotaxon 29: 243. 1987 ≡ *Hypotrachyna radiculata* (Kurok.) Elix in Austral. Lichenol. 48: 35. 2001.

Parmelina Hale in Phytologia 28: 481. 1974 – Type: *P. tiliacea* (Hoffm.) Hale in Phytologia 28: 481. 1974 (*Lichen tiliaceus* Hoffm., Enum. Lich.: 96. 1784).

Diagnostic characters. – Lobes subirregular (Fig. 4I); conidia cylindrical; medulla white; upper part of inner excipulum carbonized.

Notes. – When originally described, the genus *Parmelina* (Hale, 1974) included a number of unrelated elements that were subsequently placed elsewhere (Elix & Hale, 1987; Crespo & al., 2010). In its restricted sense, the genus is confined to the Northern Hemisphere with a center of distribution in western North America and Europe. In apothecial sections a thin carbonized layer is seen in the upper part of the inner excipulum, corresponding to an amphithecial ring seen in a superficial view of the ascomata. The closest relative, *Myelochroa*, is mainly distinguished from it based on chemical characters.

Parmelinella Elix & Hale in Mycotaxon 29: 241. 1987 – Type: *P. wallichiana* (Taylor) Elix & Hale in Mycotaxon 29: 242. 1987 (*Parmelia wallichiana* Taylor in London J. Bot. 6: 176. 1847).

Diagnostic characters. – Lobes subirregular; conidia cylindrical or bifusiform; bulbate cilia absent; yellow-grey upper cortex (secalonic acid derivatives).

Notes. – *Parmelinella* is nested within a clade of *Bulbothrix* and its generic status is in need of revision. A study addressing the generic concept in the *Bulbothrix/Parmelinella* group

requires more thorough taxon sampling. Under the current generic circumscription, this small genus of three species is distinguished from *Bulbothrix* mainly by the absence of bulbate cilia and the presence of secalonic acid derivatives, characters that are not supported as taxonomically important in molecular phylogenies of parmelioid lichens (Divakar & al., 2006).

Remototrachyna Divakar & A. Crespo in Amer. J. Bot. 97: 584. 2010 – Type: *R. flexilis* (Kurok.) Divakar & A. Crespo in Amer. J. Bot. 97: 586. 2010 (*Parmelia flexilis* Kurok. in Hara, Fl. Eastern Himalaya: 607. 1966).

Diagnostic characters. – Lobes broad; conidia bifusiform; outer exciple with very thick cell walls.

Notes. – This recently described genus (Divakar & al., 2010) was previously included in *Hypotrachyna*, but is not closely related to that genus, from which it differs in lobe morphology, rhizine length, hymenium height, exciple structure, and ascospore size. *Remototrachyna* is distinguished from *Bulbothrix* by having broader lobes, lacking bulbate cilia, a higher hymenium, and larger ascospores. *Remototrachyna* has its center of distribution in Southeast Asia.

5. *Hypotrachyna* clade

This clade includes the genera *Cetrariastrum*, *Everniastrum*, *Hypotrachyna*, and *Parmelinopsis*. All have isolichenan as cell wall polysaccharide. They are currently poorly known and have their center of species diversity in the tropical and subtropical regions of both Hemispheres. All taxa in this clade have a pored epicortex and lack pseudocyphellae (Fig. 4J–L). They may contain atranorin, usnic acid or lichexanthone as cortical substances. Some of the genera in this clade are not monophyletic. However, since the backbone of the phylogeny within the group is lacking support, we refrain from drawing nomenclatural conclusions before additional data become available.

Cetrariastrum Sipman in Proc. Kon. Ned. Akad. Wetensch., Ser. C, Biol. Med. Sci. 83: 335. 1980 – Type: *C. ecuadoriense* (R. Sant.) Sipman in Proc. Kon. Ned. Akad. Wetensch., Ser. C, Biol. Med. Sci. 83: 343. 1980 (*Parmelia ecuadoriensis* R. Sant. in Bot. Not. 1942: 328. 1942).

Diagnostic characters. – Lobes linearly elongate; conidia cylindrical; apothecia with solid stipe.

Notes. – This genus was separated from *Everniastrum*, which differs in having more regularly branched lobes, a hollow stipe, larger asci and a thinner hypothecium (Sipman, 1980, 1986). However, the distinction between the two genera is disputed (Culberson & Culberson, 1981). Additional data are necessary to clarify their taxonomic status.

Everniastrum Hale ex Sipman in Mycotaxon 26: 237. 1986 – Type: *E. cirrhatum* (Fr.) Hale ex Sipman in Mycotaxon 26: 237. 1986 (*Parmelia cirrhata* Fr., Syst. Orb. Veg. 1: 283. 1825).

Diagnostic characters. – Lobes linearly elongate (Fig. 4J); conidia bifusiform; apothecia with hollow stipe.

Notes. – The distinction of this genus from *Cetrariastrum* requires further studies, as discussed above. This is a pantropical genus with centers of distribution in the Neotropics and Asia.

Hypotrachyna (Vain.) Hale in Phytologia 28: 340. 1974 – Type: *H. brasiliana* (Nyl.) Hale in Phytologia 28: 340. 1974 (*Parmelia brasiliana* Nyl in Flora 68: 611. 1885).

Diagnostic characters. – Lobes dichotomously branched, with truncate apices (Fig. 4K); conidia bifusiform; rhizines richly dichotomously branched.

Notes. – This pantropical genus is paraphyletic in its current circumscription. Asian species with broad lobes were shown to be unrelated and consequently separated as *Remototrachyna* (Divakar & al., 2010). The classification of the remaining *Hypotrachyna* species needs to be addressed by a study including more taxa and molecular characters.

Parmelinopsis Elix & Hale in Mycotaxon 29: 242. 1987 – Type: *P. horrescens* (Taylor) Elix & Hale in Mycotaxon 29: 242. 1987 (*Parmelia horrescens* Taylor in Mackay, Fl. Hibern, Part 2: 144. 1836).

Diagnostic characters. – Lobes dichotomously branched, with truncate apices (Fig. 4L); conidia bifusiform or cylindrical; rhizines simple to sparsely dichotomously branched.

Notes. – This pantropical to temperate genus is paraphyletic and nested within *Hypotrachyna*. Traditionally it has been distinguished from *Hypotrachyna* based on the presence of cilia and less richly branched rhizines. It remains to be seen whether this genus can be kept separate from *Hypotrachyna* in a modified circumscription or needs to be synonymized with *Hypotrachyna*.

6. *Parmeliopsis* clade

This small clade includes only the genus *Parmeliopsis*, a genus of six species. The phylogenetic placement of this clade remains uncertain. It is unique among parmelioid lichens for having richly branched conidiophores (Vobis, 1980).

Parmeliopsis (Nyl.) Nyl. in Not. Sällsk. Fauna Fl. Fenn. Förh. 8: 121. 1866 – Type: *P. ambigua* (Wulfen) Nyl., Syn. Meth. Lich. 2: 54. 1869 (*Lichen ambiguus* Wulfen in Jacquin, Collectanea 4: 239. 1790).

Diagnostic characters. – Lobes subirregular; pored epicortex present; isolichenan; conidiophores branched; conidia falcate.

7. *Nipponoparmelia* clade

This newly discovered clade includes a group of East Asian species previously placed in *Parmelia* s.str. (Hale, 1987) that differs morphologically from other species in this genus by having lateral, punctate pseudocyphellae. It was later treated as a subgenus *Nipponoparmelia* within *Parmelia* (Kurokawa, 1994). The subgenus is here raised to the generic rank.

Nipponoparmelia (Kurok.) K.H. Moon, Y. Ohmura & Kashiw. ex A. Crespo & al., **stat. nov.** [MB 516758] ≡ *Parmelia* subg. *Nipponoparmelia* Kurok. in J. Jap. Bot. 69: 121. 1994 – Type: *N. laevior* (Nyl.) K.H. Moon, Y. Ohmura & Kashiw. ex A. Crespo & al.

Diagnostic characters. – Lobes subirregular; non-pored epicortex, punctiform pseudocyphellae present; conidia cylindrical.

Notes. – This small East Asian genus is characterized by marginal punctiform pseudocyphellae, grey to grey-brown thalli and simple to furcate rhizines. The following four species are included in the current circumscription of this genus.

Nipponoparmelia isidioclada (Vain.) K.H. Moon, Y. Ohmura & Kashiw. ex A. Crespo & al., **comb. nov.** [MB MB516759] ≡ *Parmelia isidioclada* Vain. in Bot. Mag. (Tokyo) 35: 48. 1921.

Nipponoparmelia laevior (Nyl.) K.H. Moon, Y. Ohmura & Kashiw. ex A. Crespo & al., **comb. nov.** [MB MB516760] ≡ *Parmelia laevior* Nyl., Lich. Jap.: 28. 1890.

Nipponoparmelia pseudolaevior (Asahina) K.H. Moon, Y. Ohmura & Kashiw. ex A. Crespo & al., **comb. nov.** [MB 516761] ≡ *Parmelia pseudolaevior* Asahina in J. Jap. Bot. 26: 331. 1951.

Nipponoparmelia ricasolioides (Nyl.) A. Crespo & Divakar, **comb. nov.** [MB 516762] ≡ *Parmelia ricasolioides* Nyl. in Flora 70: 135. 1887.

8. *Parmelia* clade

This group is not strongly supported as monophyletic but additional data are required to evaluate the relationships of the genera listed here. Morphologically these genera are quite diverse and also include biogeographically distant entities, such as the primarily northern hemispheric, temperate genus *Parmelia* and the tropical genera *Relicina* and *Relicinopsis.*

Parmelia Ach., Methodus: 153. 1803 – Type: *P. saxatilis* (L.) Ach., Methodus: 204. 1803 (*Lichen saxatilis* L., Sp. Pl. 2: 1142. 1753).

Diagnostic characters. – Lobes subirregular (Fig. 4M); non-pored epicortex, effigurate to elongate pseudocyphellae present; isolichenan; conidia cylindrical or bifusiform.

Notes. – Among the most surprising result of our analyses was the polyphyly of *Parmelia* s.str. A group of East Asian taxa with punctiform pseudocyphellae is segregated as *Nipponoparmelia* (see above), whereas a predominantly Australasian group of species related to *P. signifera* is tentatively kept in *Parmelia.* However, relationships among these well-supported groups are resolved only with short internodes, none of which well-spported. This predominantly Australasian clade includes species with usually broader lobes, but additional morphological studies are needed in addition to a more extensive taxon sampling and molecular characters, to better understand the phylogeny of this clade. *Parmelia* in its restricted sense is a small genus of temperate species, with a center of distribution in the Northern Hemisphere.

Relicina (Hale & Kurok.) Hale in Phytologia 28: 484. 1974 – Type: *Relicina eumorpha* (Hepp) Hale in Phytologia 28: 484. 1974 (*Parmelia eumorpha* Hepp in Zollinger, Syst. Verz.: 6, 9. 1854).

Diagnostic characters. – Lobes sublinear, subdichotomously to dichotomously branched; pored epicortex present; isolichenan; conidia bifusiform; cortex with usnic acid; bulbate cilia present.

Notes. – This tropical genus has its center of species diversity in eastern Asia and Australasia. It is similar to *Bulbothrix* in having bulbate cilia, but differs from that genus in cortical chemistry and conidia shape and is not closely related. Another similar genus is *Relicinopsis*, which primarily differs in lacking bulbate cilia and having fusiform conidia. Our studies indicate that the two genera are probably better regarded as synonymous, since *Relicinopsis* is nested within *Relicina* in the 1GENE analysis. However, this relationship lacks support and hence additional data are required before any nomenclatural conclusion is reached.

Relicinopsis Elix & Verdon in Mycotaxon 27: 281. 1986 – Type: *R. intertexta* (Mont. & Bosch) Elix & Verdon in Mycotaxon 27: 281. 1986 (*Parmelia intertexta* Mont. & Bosch. in Mont., Syll. Gen. Sp. Crypt.: 327. 1856).

Diagnostic characters. – Lobes sublinear, subdichotomously to dichotomously branched; pored epicortex present; isolichenan; conidia elongate fusiform or cylindrical; cortex with usnic acid; simple cilia present.

Notes. – The taxonomic status of this genus remains uncertain, see under *Relicina.*

9. *Melanohalea* clade

This clade is expanded in comparison with its previous circumscription (Blanco & al., 2006) to include also the *Melanelixia* clade. Genera in this clade have a cell wall polysaccharide that has not yet been identified and may or may not have a pored epicortex and/or pseudocyphellae. Neither atranorin nor usnic acid is present as cortical compounds, but species in this group contain melanoid substances that are responsible for their brown color (Fig. 4N–O). The *Melanelia disjuncta* group has not yet been assigned to a genus. The group certainly is not related to *M. stygia* the type of *Melanelia*, which is a cetrarioid genus. However, additional data are necessary to evaluate whether this group can be placed within one of the current genera in the *Melanohalea* clade or whether a new genus needs to be described to accommodate these taxa.

Emodomelanelia Divakar & A. Crespo, **gen. nov.** [MB 516763] – Type: ***Emodomelanelia masonii*** (Essl. & Poelt) Divakar & A. Crespo, **comb. nov.** [MB 516764] ≡ *Parmelia masonii* Essl. & Poelt in Bryologist 94: 203. 1991.

Thallus laxe adnatus, pallide ad obscure brunneus; cum pseudocyphellis marginalibus vel etiam laminalibus, albis; soralia isidiaque desunt; superficies inferior nigra; cum rhizinis simplicibus vel furcatis, nigris. *Apothecia* sessilia vel substipitata, cum marginibus pseudocyphellatis; ascosporae parietibus ca. 1–2 µm crassis. *Conidia* bifusiformia.

Diagnostic characters. – Thallus olive brown to brown; lobes narrow to moderately broad; non-pored epicortex, effigurate pseudocyphellae present; conidia bifusiform; cortex HNO_3+ green.

Etymology. – The epithet *emodo* refers to the Himalayas and *melanelia* to brown color of the thallus upper surface.

Notes. – This new monospecific genus includes a parmelioid species that combines characters typical for *Parmelia* s.str., such as laminal and marginal effigurate pseudocyphellae and large ascospores, with characters typical of the brown parmelioid genera, such as an olive-brown to brown upper surface and lack of atranorin. When describing the new species, Esslinger & Poelt (1991) pointed out that the placement in that genus was tentative. The species is an Asian endemic, known from mainland China, India, Nepal and Taiwan, where it is common on rocks in subalpine to alpine habitats (Esslinger & Poelt, 1991; Ahti & al., 1999; Kurokawa & Lai, 2001; Divakar & Upreti, 2005b).

Melanelixia O. Blanco & al. in Mycol. Res. 108: 881. 2004 – Type: *M. glabra* (Schaer.) O. Blanco & al. in Mycol. Res. 108: 882. 2004 (*Parmelia olivacea* α. *corticola* a. *glabra* Schaer., Lich. Helv. Spic. 10: 466. 1840).

Diagnostic characters. – Lobes subirregular, plane to concave; pored epicortex present; conidia cylindrical to fusiform; cortex HNO_3–.

Notes. – *Melanelixia* includes species occurring chiefly in temperate regions of the Northern and Southern Hemispheres that grow on bark and wood. It is characterized by having a pored (fenestrate) epicortex, lacking pseudocyphellae and containing lecanoric or gyrophoric acids as the primary medullary constituent. It is similar to *Pleurosticta*, which differs in having broader lobes, reticulated epicortical pores, a pigment reacting violet in K and HNO_3, and the presence of depsidones in the medulla. Four species from the Southern Hemisphere, which were previously placed here (Blanco & al., 2004a), but were not formally transferred since they contain gyrophoric acid rather than lecanoric acid like the Northern Hemisphere taxa (Esslinger, 1977), have been included in the phylogenetic analyses. They cluster with strong support as sister group to Northern Hemisphere *Melanelixia* spp. Consequently, they are here transferred into *Melanelixia*.

Melanelixia calva (Essl.) A. Crespo, Divakar & Elix, **comb. nov.** [MB 516765] ≡ *Parmelia calva* Essl. in J. Hattori Bot. Lab. 42: 60. 1977 ≡ *Melanelia calva* (Essl.) Essl. in Mycotaxon 7: 47. 1978.

Melanelixia glabratuloides (Essl.) A. Crespo, Divakar & Elix, **comb. nov.** [MB 516766] ≡ *Parmelia glabratuloides* Essl. in J. Hattori Bot. Lab. 42: 72. 1977 ≡ *Melanelia glabratuloides* (Essl.) Essl. in Mycotaxon 7: 48. 1978.

Melanelixia piliferella (Essl.) A. Crespo, Divakar & Elix, **comb. nov.** [MB MB516767] ≡ *Parmelia piliferella* Essl. in J. Hattori Bot. Lab. 42: 83. 1977 ≡ *Melanelia piliferella* (Essl.) Essl. in Mycotaxon 7: 48. 1978.

Melanelixia subglabra (Räsänen) A. Crespo, Divakar & Elix, **comb. nov.** [MB 516768] ≡ *Parmelia subaurifera* var. *subglabra* Räsänen in Ann. Bot. Soc. Zool.-Bot. Fenn. Vanamo 2(1): 19. 1932 ≡ *Parmelia subglabra* (Räsänen) Essl. in Bryologist 76: 307. 1973 ≡ *Melanelia subglabra* (Räsänen) Essl. in Mycotaxon 7: 48. 1978.

Melanohalea O. Blanco & al. in Mycol. Res. 108: 882. 2004 – Type: *M. exasperata* (De Not.) O. Blanco & al. in Mycol. Res. 108: 882. 2004 (*Parmelia exasperata* De Not. in Giorn. Bot. Ital. 2: 193. 1847).

Diagnostic characters. – Lobes subirregular, plane to concave (Fig. 4 N); pseudocyphellae present, on tuberculae; conidia elongate cylindrical to fusiform; cortex HNO_3–.

Notes. – This genus is most common in the Northern Hemisphere and includes species occurring on bark or wood. It is characterized by pseudocyphellae, often on warts or isidial tips, a nonpored epicortex, and a medulla containing depsidones or lacking secondary compounds.

Pleurosticta Petr. in Kryptog. Forsch. 2: 190. 1931 – Type: *P. lichenicola* Petr. in Kryptog. Forsch. 2: 190. 1931 (= pycnidia of *Pleurosticta acetabulum* (Neck.) Lumbsch & Elix in Lumbsch, Kothe & Elix in Mycotaxon 33: 453. 1988)

Diagnostic characters. – Broad lobes (Fig. 4O); pored epicortex present; isolichenan; conidia cylindrical to elongate fusiform; cortex HNO_3+ violet.

Notes. – This is a small genus of two species that is restricted to Eurasia and North Africa. Its relationships with other groups of brown parmelioid lichens requires further study.

Excluded genera and genera not studied

The following genera formerly placed in the parmelioid group based on morphology (Elix, 1993; DePriest, 1999) belong to other non-parmelioid groups: *Allantoparmelia* (Vain.) Essl., *Arctoparmelia* Hale, *Everniopsis* Nyl., *Imshaugia* S.L.F. Mey., *Melanelia* Essl., *Omphalodium* Meyen & Flot., *Omphalora* T.H. Nash & Hafellner, and *Psiloparmelia* Hale. *Everniopsis* and *Psiloparmelia* were previously shown to belong to a separate psiloparmelioid group within Parmeliaceae (Crespo & al., 2007), while numerous studies have shown that *Melanelia* s.str. belongs to the clade of cetrarioid lichens (Blanco & al., 2004a, 2006; Thell & al., 2004, 2009; Crespo & al., 2007). The placements of *Allantoparmelia* and *Imshaugia* within Parmeliaceae remain uncertain (Thell & al., 2004; Crespo & al., 2007), while *Arctoparmelia* has been shown to belong to the hypogymnioid group (Crespo & al., 2007). *Omphalodium* and *Omphalora* originated outside the parmelioid group (Thell & al., 2002, 2004).

The species that include the types of the following generic names have not yet been studied by molecular methods

and hence their placement and taxonomic status is unknown: *Bulborrhizina* Kurok., *Parmotremopsis* Elix & Hale, and *Pseudoparmelia* Lynge.

■ DISCUSSION

In this study we have gathered DNA sequence data available in GenBank and obtained 201 new sequences to address the generic circumscriptions in all clades of parmelioid genera. Our analyses identified eight well-supported major clades within parmelioid lichens, the majority agreeing with the clades found by Blanco & al. (2006). The exceptions include an enlarged circumscription of the *Parmelina* clade to include taxa previously placed in the *Hypotrachyna* clade and inclusion of the *Melanelixia* clade in the enlarged *Melanohalea* clade. The *Cetrelia*, *Nipponoparmelia*, *Parmeliopsis* clades are here newly recognized. We attempted to keep nomenclatural changes at a minimum. Although four clades were shown to be independent and merit recognition at some taxonomic level, we only proposed the acceptance of two new genera. One new genus (*Emodomelanelia*) is described and a new combination into *Nipponoparmelia* is made for species previously classified in the highly polyphyletic genus *Parmelia* sensu Hale (1987). Even after segregation of these two genera, *Parmelia* remains highly polyphyletic. The *Canoparmelia crozalsiana* and *Parmelia signifera* groups are shown to be unrelated to the species that include the types of their generic names. However, we refrain from describing new genera for those groups here, since our understanding of the morphological characters to circumscribe these clades is currently too poor. Additional taxonomic studies are necessary before nomenclatural changes are made.

We also identified a number of remaining problems in the generic classification of parmelioid lichens. These include the circumscription of *Parmotrema* (especially the relationships to the *Canoparmelia crozalsiana* group), the segregation of *Bulbothrix* into two clades with *Parmelinella* nested within one of these clades, the distinction of *Myelochroa* and *Parmelina*, the generic circumscription in the *Hypotrachyna* clade (i.e., the polyphyly of *Everniastrum*, *Hypotrachyna* and *Parmelinopsis*), polyphyly of *Parmelia* (with the Australasian *P. signifera* probably representing a distinct lineage), the relationships of *Relicina* and *Relicinopsis*, and the placement of the *Melanelia disjuncta* group. Further, no molecular data are available for the species that include the types of three generic names (*Bulborrhizina*, *Parmotremopsis*, *Pseudoparmelia*). Thus, our study has effectively focused forthcoming generic level phylogenetic studies in this lichen group on these problematic generic groups.

Although a number of problems remain, significant progress in our understanding of the phylogeny of parmelioid lichens has been made during the last decade. The classification of the family has now been put on a sound phylogenetic basis. A few new genera have been described, while many others have been synonymized. The taxonomic significance of morphological and chemical characters at the generic level was evaluated. Some vegetative characters, such as the presence of cilia and rhizine-types, are shown to be too homoplasious and hence of minor importance at the generic level for parmelioid lichens. More changes in the generic classification will ensue following additional studies directed at the problems identified here. Further, a major remaining problem is to resolve the backbone relationships among major clades of parmelioid lichens, which will require the sequencing of additional loci. However, a stable framework for the generic classification has been developed in this collaborative project. We hope that this framework will assist lichenologists in the near future to refine a generic classification of parmelioid lichens that reflects the phylogenetic relationships.

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