

## Molecular phylogeny of parmotremaoid lichens (Ascomycota, Parmeliaceae)

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**Abstract:** *Parmotrema* is one of the larger genera segregated from *Parmelia s. lat.* Additional genera recently have been segregated from this large genus based mainly on morphological and chemical features. We have employed molecular data from three genes to continue a revision of the generic concept within the parmelioid lichens. A Bayesian analysis of nuclear ITS, LSU rDNA and mitochondrial SSU rDNA sequences was performed. The genera *Canomaculina*, *Concamerella*, *Parmelaria* and *Rimelia* appear nested within *Parmotrema*. Alternative hypotheses to maintain the independence of *Canomaculina*, *Concamerella* and *Rimelia* are shown to be highly unlikely and are rejected. As a consequence these three genera are reduced to synonymy with *Parmotrema*. An alternative topology segregating *Parmelaria* from *Parmotrema s. lat.* cannot be rejected with the dataset at hand. However we have established that this genus is closely related to *Parmotrema* rather than to cetrarioid species as was considered previously. The revised genus *Parmotrema* includes species that have an upper cortex consisting of a palisade plectenchyma or rarely paraplectenchyma with vaults, have a pored or fenestrated epicortex, lack pseudocyphellae, have or lack cilia, have laminal, perforate or eperforate apothecia, usually have simple rhizines and filiform, cylindrical, bacilliform or sublageniform conidia. It is closely related to *Flavoparmelia* but the status of these genera requires further investigation. Nineteen new combinations are made.

**Key words:** Bayesian statistics, *Canomaculina*, combined analysis, *Concamerella*, *Parmelaria*, *Parmotrema*, *Rimelia*

### INTRODUCTION

The *Parmeliaceae* is one of the most common and well known ascomycete families comprising more than 2400 species in about 85 genera (Hawksworth et al 1995, Blanco et al 2004a, b). Within this large family, parmelioid lichens, which formerly were placed in the huge genus *Parmelia*, are a monophyletic group based on mitochondrial SSU sequences analysis (Crespo et al 2001). They also are defined morphologically in typically having rhizinate thalli with laminal lecanorine apothecia, a *Lecanora*-type ascus and simple hyaline ascospores. Parmelioid lichens comprise more than 1500 species and exhibit significant biodiversity, especially in oceanic-temperate, tropical and subtropical ecosystems.

In the past few decades the number of genera comprising the *Parmeliaceae* has increased significantly, particularly among the parmelioid lichens, due in part to a narrower generic concept (Hale 1984a). Hale initially proposed an infrageneric classification for the large and polyphyletic genus *Parmelia s. lat.* but several new genera subsequently were erected. These segregations were based mainly on morphological, anatomical and chemical characters (Culbertson and Culbertson 1981; Elix 1993; Elix and Hale 1987; Elix et al 1986; Hale 1974a, b, 1984b, 1986; Krog 1982; Kurokawa 1991; Sipman 1980). Many of these genera have not been fully accepted by a number of European lichenologists (e.g., Clauzade and Roux 1985; Eriksson and Hawksworth 1986, 1992, 1998; Poelt and Vezda 1981; Santesson 1984; Purvis et al 1992; Nimis 1993; Llimona and Hladun 2001) and some of the segregates recently have been combined on the basis of morphological and/or molecular evidence (e.g., *Rimeliella* within *Canomaculina*; *Chondropsis*, *Paraparmelia* and *Neofuscelia* within *Xanthoparmelia* [Elix 1997, 2003; Hawksworth and Crespo 2002, Blanco et al 2004a]; *Melanelixia* and *Melanohalea* segregated from *Melanelia* [Blanco et al 2004b]).

*Parmotrema* (Massalongo 1860) is one of the larger genera segregated from *Parmelia s. lat.* It includes

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more than 300 species with an apparent center of speciation in the Pacific Islands tropical and subtropical regions of South America. The species of the genus are characterized by a pored epicortex, large thalli with broad lobes, a broad, naked marginal zone on the lower surface, and large, thick-walled, ellipsoid ascospores, sublageniform or filiform conidia (Elix 1993) and (commonly) marginal cilia. Hale (1974a) resurrected the genus *Parmotrema* (Hale 1965) based on *Parmelia* subg. *Amphigymnia* (Vain.) Dodge. Four related genera more recently have been segregated based on morphological and chemical characters: *Rimelia* Hale & Fletcher (ca 20 species), *Rimeliella* Kurok. (12 species), *Canomaculina* Elix & Hale (13 species) and *Parmotremopsis* Elix & Hale (two species). Two further genera, *Concamerella* Culberson & Culberson and *Parmelaria* Awasthi, have been shown to be related to *Parmotrema* by molecular studies (Blanco et al 2004a).

Hale and Fletcher (1990) segregated *Rimelia* based on the presence of effigurate maculae on the upper surface, which fissure to form fine, reticulate cracks, as well as differences in rhizines, conidial, apothecial and chemical characters.

Although Elix (1993) regarded *Canomaculina* and *Parmotremopsis* as close to *Parmotrema*, the two genera comprised species formerly accommodated in the genus *Parmelina* (Elix and Hale 1987). They differed from other parmelioid segregates by the presence of effigurate maculae on the upper surface (*Canomaculina*), the nature and distribution of the cilia, the lobe and rhizine morphology, the size of the ascospores and conidia and the different centers of distribution. *Canomaculina* has its center of distribution in South America, whereas *Parmotremopsis* occurs mainly in Central America and the Caribbean. We unfortunately have not been able to obtain fresh material of *Parmotremopsis*.

*Concamerella* (Culberson and Culberson 1981), a genus comprising two species, has been included in the analysis because previous molecular studies indicated that it was closely related to *Canomaculina* (Blanco et al 2004a). To our knowledge its relationship with *Parmotrema* has not been recognized previously. Indeed the overt morphological features of *Concamerella* are similar to those of *Cetrariastrum* (Elix 1993), a genus that is not related phylogenetically (Crespo et al personal communication).

*Parmelaria* (Awasthi 1987), another genus of two species, is characterized by thick-walled ascospores, marginal to submarginal perforate apothecia, marginal verruciform pycnidia and broad lobes with marginal cilia. It occurs in temperate and subtemperate parts of the Himalaya. Its systematic position is uncertain. It originally was placed in *Parmelia* subg. *Am-*

*phigymnia* (Culberson 1962) because of obvious morphological similarities, such as presence of marginal cilia, large perforated apothecia and large, thick-walled ascospores. This placement subsequently was refuted by Hale (1965, 1974a) because he interpreted the presence of marginal pycnidia as excluding *Parmelia* subg. *Amphigymnia*. The genus currently is considered more closely related to cetrarioid lichens based on the presence of marginal apothecia and pycnidia (Randlane and Saag 1993, Randlane et al 1997).

Relatively few phylogenetic studies have been carried out on the genera that are close to *Parmotrema*, which also are called parmotremoid genera. Crespo et al (1999) achieved a statistic analysis, using morphological and chemical characters in parmelioid, that was not conclusive in demonstrating the relationship among parmotremoid genera. Louwhoff and Crisp (2000) performed a cladistic study also based on morphological and chemical characters of 43 species of *Parmotrema* and included representatives of some related genera such as *Rimelia*, *Canomaculina* and *Parmotremopsis*. They found *Canomaculina* and *Rimelia* clustered within *Parmotrema* and concluded that subgeneric rank within *Parmotrema* might be more appropriate for these two segregate genera.

Using morphological and molecular markers (including nuclear ITS and mitochondrial SSU), Crespo and Cubero (1998), Crespo et al (1999) and Crespo et al (2001) concluded that *Parmotrema* and *Rimelia* formed a strongly supported monophyletic group and that *Parmotrema* would become paraphyletic if *Rimelia* were kept segregated.

To revise the generic concept of the parmotremoid lichens we have included all the above genera in a molecular study using three sets of sequences: mt SSU rDNA, nu ITS and nu LSU rDNA. We conducted a Bayesian analysis of the combined dataset.

#### MATERIALS AND METHODS

*Taxon sampling.*—Sequences of the nu ITS rDNA, nu LSU rDNA and mt SSU rDNA were obtained from 25 taxa representing nine genera and including six type species. Sixty-six new sequences were obtained from 23 specimens (TABLE I). In addition 23 sequences were downloaded from GenBank (TABLE II). *Flavopunctelia flaventior* was used as outgroup because it previously has been shown to belong to a sister group of all the specimens included in the current study.

*Molecular methods.*—Total DNA was extracted according to a slightly modified DNeasy Plant Mini Kit (Qiagen) protocol (Crespo et al 2001). Dilutions  $10^{-1}$  of the total DNA were used for PCR amplifications of the genes coding for the nu

TABLE I. Species and specimens of *Parmeliaceae* from which new sequences were obtained for this study

Species	Country	Collector(s)	Herbarium acc. No.	GenBank acc. No.		
				nu LSU	nu ITS	mt SSU
<i>Canomaculina subcaperata</i> (Kremp.) Elix	Australia	Kantvilas	HO 324283	AY584829	AY586557	AY586581
<i>Canomaculina subtinctoria</i> (Zahlbr.) Elix	India	Divakar	GPGC 02- 000696	AY584830	AY586558	AY586582
<i>Canoparmelia crozalsiana</i> (de Lesd) Elix & Hale	Spain	Crespo et al	MAF 7658	AY584831	AY586571	AY586594
<i>Flavoparmelia baltimorensis</i> (Gyeln. & Föriß) Hale 1	USA	Molina	MAF 7660	AY584832	AY586559	AY586583
<i>Flavoparmelia baltimorensis</i> (Gyeln. & Föriß) Hale 2	USA	Molina	MAF 10174	AY584833	AY586560	AY586584
<i>Flavoparmelia caperata</i> (L.) Hale 2	China	Crespo et al	MAF 10175	AY584834	AY586561	AY586585
<i>Flavoparmelia soledians</i> (Nyl.) Hale	Spain	Crespo et al	MAF 10176	AY584835	AY586562	AY586586
<i>Flavopunctelia flaventior</i> (Stirt.) Hale 2	China	Hawksworth	DCH-19	—	AY586563	AY586587
<i>Parmelaria subthomsonii</i> D. D. Awasthi	India	Chatterjee and Divakar	LWG 20- 77151	AY584836	AY586564	AY586588
<i>Parmotrema crinitum</i> (Ach.) M. Choisy	Portugal	Crespo	MAF 6061	AY584837	AY586565	AY586589
<i>Parmotrema hypoleucinum</i> (J. Steiner) Hale	Spain	Crespo et al	MAF 7637	AY584839	AY586567	AY586590
<i>Parmotrema perforatum</i> (Jacq.) A. Massal.	USA	Marler	Cole 7983	AY584840	AY586568	AY586591
<i>Parmotrema perlatum</i> (Eschw.) M. Choisy	Portugal	Crespo et al	MAF 6965	AY584838	AY586566	AY586580
<i>Parmotrema pseudoreticulatum</i> (Tav.) Hale	Spain	Crespo	MAF 7650	AY584849	AY586578	AY586600
<i>Parmotrema robustum</i> (Degel.) Hale	Portugal	Crespo et al	MAF 10166	AY584841	AY586569	AY586592
<i>Parmotrema tinctorum</i> (Despr. ex Nyl.) Hale	Australia	Louwhof, Molina and Elix	MAF 10163	AY584842	AY586570	AY586593
<i>Punctelia pseudocoralloidea</i> (Gyelnik) Kalb. & Gotz	Australia	Louwhoff, Molina and Elix	MAF 6922	AY584843	AY586572	AY586595
<i>Punctelia rudecta</i> (Ach.) Krog 1	USA	Molina	MAF 7661	AY584844	AY586573	AY586596
<i>Punctelia rudecta</i> (Ach.) Krog 2	USA	Molina	MAF 10162	AY584845	AY586574	AY586597
<i>Punctelia subflava</i> (Taylor) Elix & J. Johnst.	Australia	Elix	42705-CNB	AY584846	AY586575	—
<i>Rimelia cetrata</i> Hale & A. Fletcher	Uruguay	Scarabino	H. S. Osorio 9424	AY584847	AY586576	AY586598
<i>Rimelia clavulifera</i> (Räsänen) Kurok.	China	Crespo et al	MAF 10164	AY584848	AY586577	AY586599
<i>Rimelia reticulata</i> (Taylor) Hale & A. Fletcher 2	Portugal	Crespo and Jones	MAF 6067	AY584850	AY586579	—

ITS, LSU rRNA and mt SSU rRNA sequences. Primers for amplification of fungal nu ITS rDNA were ITS1F (Gardes and Bruns 1993), ITS4 (White et al 1990), ITS1-LM (Myllys et al 1999) and ITS2-KL (Lohtander et al 1998); nu LSU rDNA was amplified using the primers LROR and LR5 (Vilgalys <http://www.biology.duke.edu/fungi/mycolab/primers.htm>), and mt SSU rDNA was amplified using the primers mr SSU1 and mr SSU3R (Zoller et al 1999), NMS1 and NMS2 (Li et al 1994), MSU1 and MSU7 (Zhou and Stanosz

2001). The PCR cocktail (total volume 50 µL) contained 10 µL diluted DNA, 5 µL of 10× DNA polymerase buffer (Biotools) (including MgCl<sub>2</sub> 2 mM, 10 mM Tris-HCl, pH 8.0, 50 mM KCl, 1 mM EDTA, 0.1% Triton X-100), 1 µL of deoxynucleotide triphosphate (dNTPs) with 10 mM of each base, 2.5 µL of each primer (10 µM), 1.25 µL of DNA polymerase (1U/µL) and 27.75 µL dH<sub>2</sub>O.

The amplifications for nu ITS and LSU rDNA were carried out in an automatic thermocycler, Techne Progene.

TABLE II. Species and specimens of *Parmeliaceae* downloaded from GenBank

Species	GenBank acc. No.		
	nu LSU	nu ITS	mt SSU
<i>Canomaculina haitiensis</i> (Hale) Elix	AY578918	AY581055	AY582295
<i>Canomaculina pilosa</i> (Stizenb.) Elix & Hale	AY578919	AY581056	AY582296
<i>Concamerella fistulata</i> (Taylor) W. L. Culb. & C. F. Culb.	AY578920	AY581057	AY582297
<i>Flavoparmelia caperata</i> (L.) Hale 1	AY578922	AY581059	AF 351163
<i>Flavopunctelia flaventior</i> (Stirt.) Hale 1	AY578923	AY581060	AF 351164
<i>Punctelia borrieri</i> (Sm.) Krog	AY578954	AY581088	AY582324
<i>Punctelia subflava</i> (Taylor) Elix & J. Johnst.	—	—	AF 351183
<i>Punctelia subrudecta</i> (Nyl.) Krog	AY578955	AY581089	AY582325
<i>Rimelia reticulata</i> (Taylor) Hale & A. Fletcher	—	—	AF351184

These PCR cycling parameters were used to amplify both these regions: initial denaturation at 94 C for 5 min, and 30 cycles of 94 C for 1 min, 54–60 C (ITS rDNA) and 60 C (LSU rDNA) for 1 min, 72 C for 1.5 min and a final extension at 72 C for 5 min. The PCR amplification for mitochondrial rDNA was carried out in a Hybaid OmniGene thermocycler and was performed with this program: initial denaturation at 94 C for 5 min and 35 cycles of 94 C for 1 min, 57–58 C for 1 min, 72 C for 1.5 min and a final extension at 72 C for 5 min.

Fragments were cleaned using Biotools Bioclean Columns kit or, in case of an impure product, the bands were cut with Biotools Bioclean kit, according to the manufacturer's instructions. Cleaned PCR products were sequenced. To obtain complete overlapping sequences in both directions these primers, in addition to those also used for PCR amplifications, were used: (i) for the ITS rDNA: ITS2 and ITS3 (White et al 1990) were used when long PCR products were obtained due to the presence of group I introns at the very end of nu SSU (Gargas et al 1995); (ii) for the nu LSU rDNA: LR3 and LR3R (Vilgalys <http://www.biology.duke.edu/fungi/mycolab/primers.htm>); and (iii) for the mt SSU rDNA, mr SSU2 and mr SSU2R (Zoller et al 1999). The ABI Prism™ Dye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems) was used and cycle sequencing was carried out with these settings: denaturation for 3 min at 94 C and 25 cycles at 96 C for 10 sec, 50 C for 5 sec and 60 C for 4 min. Sequencing reactions were electrophoresed on a 3730 DNA analyzer (Applied Biosystems). Sequence fragments were assembled with SeqMan 4.03 (DNASTar) and manually adjusted. Introns in the SSU and LSU as well as partial nu SSU and LSU rDNA sequences at either end of the ITS sequences were removed.

*Sequence alignments.*—Most phylogenetic reconstruction methods rely on multiple alignments. However multiple alignments can be ambiguous if sequences are highly divergent. Therefore we have used an alignment procedure employing a linear Hidden Markov Model (HMM), as implemented in the software SAM (Hughey and Krogh 1996). Sequences of 30 specimens (TABLES I, II) were aligned sep-

arately for the three genes. Regions that could not be aligned with statistical confidence were excluded from the phylogenetic analysis.

*Phylogenetic analysis.*—The alignment was analyzed with PAUP\* 4.0b10 (Swofford 2003) and MrBayes 3.0 (Huelsenbeck and Ronquist 2001). The data were analyzed using a Bayesian approach (Huelsenbeck et al 2000, Larget and Simon 1999). A Bayesian phylogenetic tree-sampling technique was used to estimate the posterior probability of phylogenetic trees using a Markov Chain Monte Carlo (MCMC) method (Larget and Simon 1999).

MrBayes was employed to sample the trees. The likelihood model was set to general time reversible model (Rodríguez et al 1990) including estimation of invariant sites and assuming a discrete gamma distribution with six rate categories (GTR+I+G) for the single-gene and the combined analyses. No molecular clock was assumed. A run with 2 000 000 generations starting with a random tree and employing 12 simultaneous chains was executed. Every 100th tree was saved into a file.

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>) and determined that stationarity was achieved when the log-likelihood values of the sample points reached a stable equilibrium value (Huelsenbeck and Ronquist 2001). The initial 1000 trees were discarded. The sumt command of MrBayes was used to produce a 50% majority-rule consensus trees with branch lengths. Posterior probabilities equal to and above 95% were considered significant supports. Phylogenetic trees were drawn using TreeView (Page 1996). In addition a maximum parsimony analysis was performed using PAUP\*. A heuristic search with 200 random taxon addition replicates was conducted with TBR branch swapping and MulTrees option in effect, equally weighted characters and gaps treated as missing data. Bootstrapping was performed based on 2000 replicates with random sequence additions.

We used a Bayesian approach to examine the heterogeneity in phylogenetic signals among the three data partitions (Buckley et al 2002). For the three genes and the



TABLE III. Probabilities of four phylogenetic null hypotheses being correct. Each test is based on a B/MCMC tree sample of 10 000 trees. Probabilities significant at  $<0.05$  are denoted “\*” and at  $<0.001$  denoted “\*\*\*”

Null hypothesis	Probability
<i>Canomaculina</i> forms an independent genus	0.000**
<i>Concamerella</i> forms an independent genus	0.043*
<i>Rimelia</i> forms an independent genus (including <i>P. pseudoreticulatum</i> )	0.000**
<i>Parmelaria</i> forms an independent genus	0.332

concatenated analyses, the set of topologies reaching 0.95 posterior probability was estimated. The combined analysis topology then was compared for conflict with the 0.95 posterior intervals of the single gene analyses. If no conflict was evident, it was assumed that the two datasets were congruent and could be combined.

**Hypothesis testing.**—Bayesian hypothesis testing was used to determine whether groupings corresponding to different views in lichen classifications appear in alternative topologies in suboptimal trees. Four hypothesized phylogenetic relationships were tested as null hypotheses using a MCMC tree sampling (TABLE III).

For hypothesis testing a new Bayesian analysis with MrBayes as described above was performed with the same settings as in the estimation of the phylogeny using the combined dataset. Ten thousand trees at the equilibrium state per null hypothesis were used for this analysis. The probability of the null hypothesis being correct is calculated by counting the presence of this topology in the MCMC sample (Lewis 2001). The frequency of trees in the MCMC sample agreeing with the null hypothesis was calculated using the filter command in PAUP\* with a constraint describing the null hypothesis.

## RESULTS

A total of 21 new mt SSU rDNA, 23 new nu ITS rDNA and 22 new nu LSU rDNA sequences were generated. In addition, 23 sequences were downloaded from GenBank and aligned with the new ones. We produced a matrix of 598 characters for mt SSU, 533 for nu ITS and 1038 for nu LSU. After SAM alignment 549 unambiguous nucleotide position characters were kept for the analysis in the mt SSU, 509 in the nu ITS and 834 in the nu LSU. Seventy-three characters were variable in mt SSU, 186 in nu ITS and 93 in nu LSU. The final alignment of the 30 taxa studied (TABLES I, II) was 1892 positions in length. The alignments are deposited in TreeBase (acc. SN1867).

The topology of trees obtained using maximum parsimony and Bayesian methods was identical and therefore only the results of the Bayesian analyses are discussed here in detail. The likelihood parameters in the sample had these average values ( $\pm$  one stan-

dard deviation): likelihood (LnL) =  $-7280.132$  ( $\pm 0.188$ ), base frequencies  $\pi(A) = 0.266$  ( $\pm 0.002$ ),  $\pi(C) = 0.212$  ( $\pm 0.002$ ),  $\pi(G) = 0.269$  ( $\pm 0.001$ ),  $\pi(T) = 0.253$  ( $\pm 0.001$ ), rate matrix  $r(AC) = 1.349$  ( $\pm 0.095$ ),  $r(AG) = 2.898$  ( $\pm 0.171$ ),  $r(AT) = 1.45$  ( $\pm 0.097$ ),  $r(CG) = 0.523$  ( $\pm 0.038$ ),  $r(CT) = 9.870$  ( $\pm 0.877$ ),  $r(GT) = 1.0$  ( $\pm 0.0$ ), and the gamma shape parameter  $\alpha = 0.664$  ( $\pm 0.027$ ).

In the majority rule consensus tree (FIG. 1) three main monophyletic groups are present. All *Punctelia* species form a well supported group (1.0 pp). *Punctelia* is the sister group of all species included in the analysis. The three species of *Flavoparmelia* also are placed in a supported monophyletic group (1.0 pp). *Canoparmelia crozalsiana* is basal to a well supported group (1.0 pp) including *Parmelaria subthomsonii*, *Rimelia*, *Canomaculina*, *Parmotrema* and *Concamerella* species. Within this group *Parmelaria subthomsonii* and *Parmotrema tinctorum* are sister taxa but their relationship lacks support. The two taxa are sister of a supported monophyletic group (0.96 pp) that includes the bulk of parmotremaid species. Within this clade *Rimelia reticulata* specimens and *Parmotrema pseudoreticulatum* together form a well supported group (1.0 pp). The type species of *Rimelia*, *R. cetrata*, is basal to this group but without support. Three species groups, including two *Canomaculina* and four *Parmotrema* species form supported groups each (1.0 pp). No additional clades are resolved within the parmotremaid taxa.

The topology of the 50% majority rule consensus tree is in disagreement with current classifications. Species currently placed in *Canomaculina*, *Concamerella*, *Parmelaria* and *Rimelia* are nested within *Parmotrema*. Therefore we tested whether topologies supporting these as segregate genera are present in suboptimal trees and whether the data are sufficient to reject independence of the genera. The results of the hypothesis testing are shown (TABLE III). *Canomaculina*, *Concamerella* and *Rimelia* are rejected as independent genera at a  $P = 0.05$  threshold. However, the independence of *Parmelaria* cannot be rejected with the data at hand.

## DISCUSSION

The current study based on three ribosomal gene regions yielded a phylogeny of *Parmotrema* and allied genera. As in previous studies (Crespo et al 1999, 2001), *Flavoparmelia* is a sister group of the parmotremaid lichens which are monophyletic. The single sequence of *Canoparmelia crozalsiana* is basal to the other parmotremaid lichens. This placement, however, lacks support. Additional *Canoparmelia* species (including the type species *C. texana*) need to be

sampled before any conclusions can be made regarding the relationships of this genus. However our data suggest that the closest relatives of the parmotrema lichens do have a pored epicortex.

The remaining parmotrema lichens form a strongly supported monophyletic group. Relationships within this group are resolved only partially, indicating that further studies to elucidate phylogenetic relationships among the parmotrema lichens should employ different molecular markers. This also applies to the phylogenetic position of the genus *Parmelaria*. Although our results suggest that this genus may be more appropriately treated as a synonym of *Parmotrema*, the data do not reject *Parmelaria* being an independent monophyletic lineage. Despite this we have confirmed Culberson's (1962) view that the species studied is parmelioid rather than cetrarioid lichen.

The combined analysis of the three datasets clarifies the relationships among species formerly included in *Canomaculina*, *Concamerella* and *Rimelia*. Analysis of the single datasets revealed the same basic topology as the combined dataset but was unable to reject alternative topologies in most cases (data not shown). The genera *Canomaculina* and *Rimelia* were regarded previously as being closely related to *Parmotrema* based on morphological evidence (Elix 1993), but this relationship was not supported in a statistic analysis of all genera included in *Parmeliaceae* (Crespo et al 1999). However Louwhoff and Crisp (2000) have suggested that these two genera should be included in *Parmotrema* based on a cladistic study, based also on morphological and chemical characters but precisely focused on this group. The distinction of the two genera was based on differences in lobe width, maculae and type of cilia and rhizines in the case of *Canomaculina* and on difference of rhizines, conidia, spore size and medullary chemistry in *Rimelia*. Our data indicate that these characters are inappropriate for the recognition of monophyletic groups in these lichens. The distinction of *Canomaculina* and *Parmotrema* is in part consequence of historic classifications because these two genera formerly were placed in different groups of *Parmelia s. lat.* A similar case occurred in *Canomaculina* and *Rimeliella*, genera that subsequently were synonymized by Elix (1997). While *Canomaculina* was segregated by *Parmelina* (Elix and Hale 1987), *Rimeliella* was segregated from *Parmotrema* (Kurokawa 1991). Subsequent morphological analyses established that they were synonymous (Elix 1997). Likewise our results suggest that *Canomaculina* and *Rimelia* are congeneric with *Parmotrema*. Our molecular analysis is in full agreement with the morphological analysis performed by Louwhoff and Crisp (2000).

The genus *Concamerella* previously was considered to be close to *Cetrariastrum* and *Everniastrum* (Culberson and Culberson 1981, Elix 1993), rather than the parmotrema genera. This was mainly due to their morphological similarities, in particular the linear-elongate, dichotomously divided lobes with a canaliculate lower surface. At first glance the relationship with *Parmotrema* is somewhat surprising. However the lobe morphology in *Parmotrema* is quite variable and includes taxa with narrow, elongated lobes such as *P. moreliense* (B. de Lesd.) W. Culb. & C. Culb., *P. paramoreliense* W. Culb. & C. Culb. (Culberson and Culberson 1981) and *P. blanchetianum* (Müll. Arg.) Kalb. Given these morphological similarities and the topology of the molecularly derived phylogenetic tree (FIG. 1), we propose to reduce the parmotrema genera *Rimelia*, *Canomaculina* and *Concamerella* into synonymy with *Parmotrema*.

The *Flavoparmelia* clade is supported strongly as a monophyletic group and also is sister group of the remaining parmotrema lichens. *Flavoparmelia* and *Canoparmelia* are similar morphologically to *Parmotrema* in having a pored epicortex and broad, naked, erhizinate regions at the margins of the lobes. However *Canoparmelia* and *Flavoparmelia* differ from *Parmotrema s. lat.* in lacking cilia and perforate apothecia and having fungal cell walls containing isolichenan rather than lichenan. A close relationship between *Flavoparmelia* and *Parmotrema* has been suggested previously by Crespo and Cubero (1998) who pointed out the similarity of lichenicolous *Abrothallus* species within these lichens. It is interesting to note, from an evolutionary point of view, that the whole ingroup ensemble in a single branch (1.00 pp), has a common lichenicolous species, *Abrothallus microspermus* (Cole and Hawksworth 2001). Sharing a common lichenicolous fungus has been used widely as a supporting character and for indication of phylogenetic relationships in lichenology (e.g., Rambold and Triebel 1992, Triebel et al 1995).

Our new classification of the majority of parmotrema lichens into one genus, based on the results of our molecular analysis, is corroborated by their common morphological characters (as outlined below) and similar distribution patterns. An alternative classification would be to further segregate small species groups as separate genera within *Parmotrema*. Such further distinction would be based on thalline morphological characters usually employed at species level. Such a procedure clearly would be unacceptable given the rather small genetic differences found among genera in *Parmeliaceae* in comparison with other groups of euascomycetes (Lumbsch 2002).

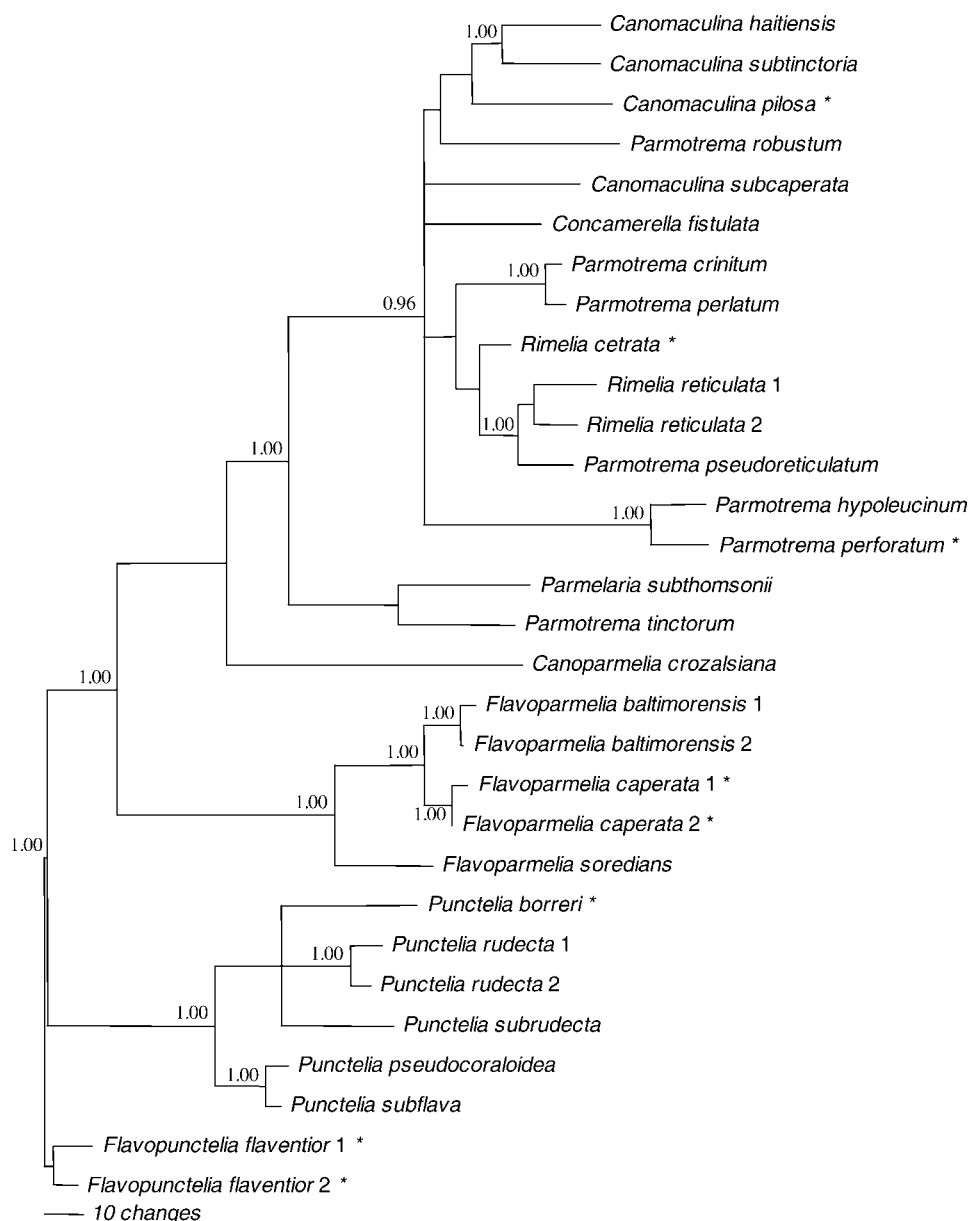


FIG 1. Ninety-five percent majority rule consensus tree of 19 000 trees visited during a B/MCMC tree sampling procedure. Numbers at nodes are posterior probabilities values above or equal 95%. Types species are indicated by\*.

#### AMENDED GENUS DESCRIPTION

**Parmotrema** Mass., Atti imp. Reg. Ist. Venet. Sci., III 5:248.1860. Type: *P. perforatum* (Jacq.) Mass.

*Parmelia* subgen. *Amphigymnia* Vain., Acta Soc Fauna Fl Fenn 7(7):28 1890. Type: *Parmotrema perforata* (Jacq.) Ach.

= *Canomaculina* Elix & Hale, Mycotaxon 29:239. 1987. Type: *C. pilosa* (Stirt.) Elix & Hale (*Parmelia pilosa* Stirt.).

= *Concamerella* W. Culb & C. Culb., Bryologist 84:307. 1981. Type: *C. pachyderma* (Hue) W. Culb. & C. Culb. (*Parmelia pachyderma* Hue).

= *Rimelia* Hale & Fletcher, Bryologist 93:23. 1990. Type:

*R. cetrata* (Ach.) Hale & Fletcher (*Parmelia cetrata* Ach.).

= *Rimeliella* Kurok., Ann. Tsukuba Bot. Gard. 10:1. 1991. Type: *R. subcaperata* (Kremp.) Kurok. (*Parmelia subcaperata* Kremp.).

*Thallus* foliose, loosely adnate to adnate. Lobes broad, apically rotund, or dichotomously/subdichotomously divided 1–30 mm wide, sometimes marginally lacinate; margins entire or variously incised or ornamented, with or without cilia; cilia simple or branched, not bulbate. Upper surface gray to gray-green, yellowish gray, dark gray or pale green (atranorin and chloroatranorin, rarely with additional

usnic acid), pale with conspicuous white stippled, flat or rarely convex, shiny or dull, smooth, undulate to rugose or foveolate, with or without maculae, soredia, isidia and dactyls, without pseudocyphellae; maculae may form an intricate network, ultimately fissuring into reticulate cracks. Upper cortex of palisade plectenchyma or vaulted paraplectenchyma with pored epicortex. *Cell walls* containing lichenan intermediate between *Cetraria*-type lichenan and *Xanthoparmelia*-type lichenan. *Medulla* white, wholly pigmented or pigmented adjacent to lower cortex. Lower surface brown, black or glossy black, rhizinate (more than 1 mm wide); rhizines central or grouped subapically, usually rather sparse, simple, rarely branched, or irregularly squarrosely branched, slender or coarse, brown or black. *Ascomata* apothecial, laminal, commonly pedicellate or subpedicellate; disk perforate or not; thalline exciple smooth or rugose, sometimes maculate, eciliate. *Asci* elongate, clavate, *Lecanora*-type, apically thickened, without an internal apical beak, discharge rostrate, 8-spored. Ascospores ellipsoidal or rarely reniform or subglobose, large and thick-walled,  $8\text{--}37 \times 5\text{--}18 \mu\text{m}$ . *Conidiomata* pycnidial, laminal, immersed, punctiform. *Conidiophores* of type V or VI sensu Vobis (1980). *Conidiogenous cells* terminal or intercalary. *Conidia* sublageniform ( $3\text{--}10 \times 1 \mu\text{m}$ ), filiform ( $8\text{--}20 \times 1\text{--}1.5 \mu\text{m}$ ), bacilliform ( $4\text{--}16 \times 1\text{--}1.5 \mu\text{m}$ ) or cylindrical ( $9\text{--}13 \times 1 \mu\text{m}$ ).

*Chemistry*: medulla containing orcinol depsides,  $\beta$ -orcinol depsides, orcinol depsidones,  $\beta$ -orcinol depsidones, aliphatic acids, pulvinic acid derivatives, anthraquinones and xanthenes.

The genus as it is now circumscribed includes ca 350 species that have their center of distribution in tropical regions of the world, especially in the Pacific Islands South America. It is characterized by having an upper cortex of palisade plectenchyma or paraplectenchyma with vaults, a pored epicortex, lack of pseudocyphellae, presence or absence of cilia, laminal perforate or eperforate apothecia, ellipsoid ascospores and filiform, cylindrical, bacilliform or sublageniform conidia.

#### NEW NAMES AND COMBINATIONS

As a consequence of a revised generic concept of *Parmotrema* several new combinations are necessary and are proposed below.

***Parmotrema albinatum*** (K.H. Moon, Kurok. & Kashiw.) O. Blanco, A. Crespo, Divakar, Elix & Lumbsch comb. nov.  $\equiv$  *Rimelia albinata* K.H. Moon, Kurok. & Kashiw., J Jpn Bot 76:322. 2001.

***Parmotrema bonplandii*** (Mata) O. Blanco, A. Crespo, Divakar, Elix & Lumbsch comb. nov.  $\equiv$  *Rimelia bonplandii* Mata, Bryologist 97:432. 1994.

***Parmotrema cristobalii*** (Ferraro & Elix) O. Blanco, A. Crespo, Divakar, Elix & Lumbsch comb. nov.  $\equiv$  *Rimeliella cristobalii* Ferraro & Elix, Mycotaxon 49: 406. 1993.  $\equiv$  *Canomaculina cristobalii* (Ferraro & Elix) Elix, Mycotaxon 65:477. 1997.

***Parmotrema fistulatum*** (Tayl.) O. Blanco, A. Crespo, Divakar, Elix & Lumbsch comb. nov.  $\equiv$  *Parmelia fistulata* Tayl., Lond Jour Bot 6:168. 1847.  $\equiv$  *Concamerella fistulata* (Tayl.) W Culb. & C. Culb., Bryologist 84:308. 1981.

***Parmotrema hawaiiense*** (H. Magn.) O. Blanco, A. Crespo, Divakar, Elix & Lumbsch comb. nov.  $\equiv$  *Parmelia hawaiiensis* H. Magn. in Magn. & Zahlbr., Ark Bot 31A (6): 106. 1944.  $\equiv$  *Rimelia hawaiiensis* (Magn.) Hale & Fletcher, Bryologist 93:28. 1990.

***Parmotrema laciniellum*** (Ferraro et Elix) O. Blanco, A. Crespo, Divakar, Elix & Lumbsch comb. nov.  $\equiv$  *Canomaculina laciniella* L.I. Ferraro & Elix, Mycotaxon 74:391. 2000.

***Parmotrema lobuliferum*** (Ribeiro & Marcelli) O. Blanco, A. Crespo, Divakar, Elix & Lumbsch comb. nov.  $\equiv$  *Canomaculina lobulifera* Ribeiro & Marcelli, Mitt. Inst. Allg. Bot. Hamburg 30–32:128. 2002.

***Parmotrema melanochaetum*** (Kurok.) O. Blanco, A. Crespo, Divakar, Elix & Lumbsch comb. nov.  $\equiv$  *Parmelia melanochaeta* Kurok. in Hale & Kurok., Contrib US Nat Herb 36:133. 1964.  $\equiv$  *Canomaculina melanochaeta* (Kurok.) Elix, Mycotaxon 47:127. 1993.

***Parmotrema muelleri*** (Vain.) O. Blanco, A. Crespo, Divakar, Elix & Lumbsch comb. nov.  $\equiv$  *Parmelia muelleri* Vain., Acta Soc Fauna Fl Fenn 7: 49. 1890.  $\equiv$  *Canomaculina muelleri* (Vain.) Elix & Hale, Mycotaxon 29:240. 1987.

***Parmotrema pachydermum*** (Hue) O. Blanco, A. Crespo, Divakar, Elix & Lumbsch comb. nov.  $\equiv$  *Parmelia pachyderma* Hue, Nouv Arch Mus Hist Nat, 4e ser 1:137. 1899.  $\equiv$  *Concamerella pachyderma* (Hue) W. Culb. & C. Culb., Bryologist 84:308. 1981.

***Parmotrema pontagrossense*** (Eliasaro & Adler) O. Blanco, A. Crespo, Divakar, Elix & Lumbsch comb. nov.  $\equiv$  *Rimelia pontagrossensis* Eliasaro & Adler, Mycotaxon 66:127. 1998.

***Parmotrema pustulatum*** (Elix & Bawingan) O. Blanco, A. Crespo, Divakar, Elix & Lumbsch comb. nov.  $\equiv$  *Rimelia pustulata* Elix & Bawingan, Mycotaxon 81:252. 2002.

***Parmotrema ramescens*** (Zahlbr.) O. Blanco, A. Crespo, Divakar, Elix & Lumbsch comb. nov.  $\equiv$  *Parmelia ramescens* Zahlbr. in H. Magn. & Zahlbr., Ark Bot 31A(6):96. 1944.  $\equiv$  *Rimelia ramescens* (Zahlbr.) K. H. Moon & Kurok., J Jpn Bot 76:326. 2001.

***Parmotrema reparatum*** (Stirt.) O. Blanco, A. Crespo, Divakar, Elix & Lumbsch comb. nov.  $\equiv$  *Parmelia reparata* Stirt., Scott. Naturalist 4:201. 1877–1878.



- = *Canomaculina reparata* (Stirt.) Kurok., Bull Natn Sci Mus, Tokyo, ser B. 27:2. 2001.
- Parmotrema ruminatum** (Zahlbr.) O. Blanco, A. Crespo, Divakar, Elix & Lumbsch comb. nov. = *Parmelia ruminata* Zahlbr. in H. Magn & Zahlbr., Ark. Bot. 31A(6):107. 1944. = *Rimelia ruminata* (Zahlbr.) Hale & Fletcher, Bryologist 93: 28. 1990. (1990)
- Parmotrema sticticum** (Louwhoff & Elix) O. Blanco, A. Crespo, Divakar, Elix & Lumbsch comb. nov. = *Rimelia stictica* Louwhoff & Elix, Mycotaxon 68: 452. 1998.
- Parmotrema succinreticulatum** (Eliasaro & Adler) O. Blanco, A. Crespo, Divakar, Elix & Lumbsch comb. nov. = *Rimelia succinreticulata* Eliasaro & Adler, Mycotaxon 63:50. 1997.
- Parmotrema tandilense** (Adler & Elix) O. Blanco, A. Crespo, Divakar, Elix & Lumbsch comb. nov. = *Canomaculina tandilensis* Adler & Elix, Mycotaxon 30:339. 1987.
- Parmotrema ventanicum** (Adler & Elix) O. Blanco, A. Crespo, Divakar, Elix & Lumbsch comb. nov. = *Canomaculina ventanica* Adler & Elix, Mycotaxon 30:340. 1987.
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