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Melanelixia and *Melanohalea*, two new genera segregated from *Melanelia* (*Parmeliaceae*) based on molecular and morphological data

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This paper continues a revision of generic concepts in the parmelioid lichens using molecular data in order to reach a consensus among lichenologists over which segregates proposed over the last two decades should be accepted. Here we employ data from three gene portions to provide a basis for a revised generic concept of the brown parmelioid lichens hitherto classified in *Melanelia*. The phylogeny was studied using a Bayesian analysis of a combined data set of nuclear ITS, LSU rDNA and mitochondrial SSU rDNA sequences. 173 new sequences were obtained from 38 specimens of 15 *Melanelia* species, 37 related parmelioid species, and eight non-parmelioid species. The results indicate that *Melanelia* is not monophyletic but falls into four different clades. The genus *Melanelia* is restricted here to a small group of saxicolous lichens related to the type species *M. stygia*, and with bifusiform conidia, while the remaining species, most of which are primarily corticolous and have mainly cylindrical to filiform conidia, belong to two other clades recognised as two new genera: *Melanelixia* and *Melanohalea*, to accommodate the *M. exasperata* and *M. glabra* groups, respectively. 27 new combinations are made. The epicortex of *Melanelixia* species have pores or special structures termed here 'fenestrations', while most *Melanohalea* species are pseudocyphellate. *Pleurosticta* links to the *Melanohalea* clade but without strong support, and the phylogenetic position of *M. disjuncta* and its related species remains uncertain, linking with the *Xanthoparmelia* (syn. *Neofuscelia*) clade but also without strong support.

INTRODUCTION

The *Parmeliaceae* (*Lecanorales*) constitutes one of the largest families of lichen-forming ascomycetes. It is dominated by two large groups which had been placed into the huge genera *Cetraria* and *Parmelia* in traditional classifications. Generic concepts in lichenology started to change dramatically from the late 1960s, with the *Parmeliaceae* being a prominent example. Morphological and chemical as well as anatomical characters were used to segregate numerous groups as genera within the parmelioid lichens during this time (Hale 1984a, Elix 1993, Nimis 1998, DePriest 1999). While the segregated genera have been recognised by many lichenologists, acceptance has not been universal (e.g. Clauzade & Roux 1986, Eriksson & Hawksworth 1986, 1992, Poelt & Vězda 1981, Santesson 1984, Purvis *et al.* 1992, Nimis 1993, Llimona & Hladun 2001), primarily because correlations with ascomatal or pycnidial differences have often not been established (e.g. Hawksworth, James & Coppins 1980). Nevertheless, further investigations have shown that even some of the segregates were heterogeneous and consequently additional genera have continued to be proposed (e.g. Hale 1984b, 1986a, b, Elix, Johnston & Verdon 1986, Elix & Hale 1987).

Within the parmelioid genera, there is a group of taxa usually lacking atranorin or usnic acid in the cortex, but having a dark to medium brown thallus colour. These brown *Parmeliae* were monographed by Esslinger (1977), and are currently divided into five genera: *Allantoparmelia*, *Almbornia*, *Melanelia*, *Neofuscelia*, and *Pleurosticta*. Most species were placed in *Melanelia* and *Neofuscelia*, while *Allantoparmelia*, *Almbornia* and *Pleurosticta* contained only few taxa.

Allantoparmelia, a boreal and arctic-alpine genus, is believed to be related to *Brodoa* (Elix 1993); it is therefore not treated further here. We have not been

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able to get fresh material of *Almbornia*, fruticose lichens endemic to South Africa and perhaps related to *Neofuscelia* (Elix 1993). *Almbornia*, *Neofuscelia* and *Pleurosticta* are characterized by having a pored epicortex, while *Melanelia* spp. are usually considered to have pseudocyphellae (Elix 1993). The genus *Pleurosticta* was formerly placed in *Melanelia* (Esslinger 1978) as subgenus *Olivascentes*, but Lumbsch, Kothe & Elix (1988) resurrected the genus based partly on the deviating epicortex.

Neofuscelia species also have *Xanthoparmelia*-type lichenan, consistently bifusiform conidia, and occur exclusively on rocks. Molecular studies on this group show that the species belong to the same monophyletic clade as the yellow-green to yellow-grey usnic acid containing *Xanthoparmelia* species (Crespo, Blanco & Hawksworth 2001). The synonymy of the two genera has been proposed (Hawksworth & Crespo 2002), and after the study of sequences from many more species the necessary combinations of *Neofuscelia* species into *Xanthoparmelia* have been made (Blanco *et al*. 2004).

The remaining taxa referred to *Melanelia* were placed in two subgenera by Esslinger (1978), *Melanelia* and *Vainioellae*. The nominal subgenus included a few saxicolous species, such as *M. disjuncta*, *M. panniformis*, and the type species of *Melanelia*, *M. stygia*. Subgenus *Melanelia* is characterized by species with rather narrow lobes, which are often somewhat elongate, and flat to convex or concave. Thell (1995) transferred additional saxicolous species (the *Cetraria commixta* group) from *Cetraria* to *Melanelia*, placing them close to *M. stygia*. Subgenus *Vainioellae*, however, includes numerous primarily corticolous species that only rarely occur on siliceous rocks, and a few that are primarily saxicolous. The members of this subgenus include species with broad lobes that are round to rather elongate and more or less flat. The genus *Melanelia* is cosmopolitan and comprises *ca* 40 species most of which occur primarily or solely in the Northern Hemisphere.

The few molecular studies so far to have included *Melanelia* species (Crespo & Cubero 1998, Crespo *et al*. 1999, Thell 1999, Thell & Miao 1999) have been based on nuITS rDNA sequences alone, and were focused on other cetrarioid or parmelioid lichens, thus including few species of the genus. However, Crespo *et al*. (1999) noted that *M. glabra* and *M. exasperata* did not form a monophyletic group. Thell *et al*. (2002) examined a large number of cetrarioid and parmelioid lichens, including three species of *Melanelia*, using ITS and also β-tubulin gene sequences. Subsequently, Guzow-Krzeminska & Wegrzyn (2003) studied the phylogenetic relationships within *Melanelia* based on nuLSU rRNA gene sequences from ten *Melanelia* species.

The systematics of the brown parmelioid lichens is consequently in a somewhat confused state. In order to clarify the circumscription of the genus *Melanelia*, and to ascertain the relationships between the species referred to it and other parmelioid genera, we performed a multigene molecular study including 60 taxa belonging to the *Parmeliaceae*, of which 15 belonged to *Melanelia*, including the type species. We employed three data sets: the nuclear LSU (nuLSU), the nuclear ITS (nuITS) and the mitochondrial SSU (mtSSU) regions of the ribosomal DNA in a combined analysis. A Bayesian approach was used employing complex nucleotide substitution models in a parametric statistical framework (Larget & Simon 1999, Huelsenbeck *et al*. 2001).

MATERIALS AND METHODS

Taxon sampling

Sequence data of the nuITS rDNA, nuLSU rDNA and mtSSU rDNA were obtained from 53 parmelioid species. 175 new sequences were obtained from 85 specimens, as listed in Table 1. Moreover, 75 sequences were downloaded from GenBank (Table 2). *Pseudephebe pubescens* was used as outgroup since it has been considered as belonging to the alectorioid group of *Parmeliaceae* that is basal to the cetrarioid and parmelioid groups (Mattsson *et al*. 2004).

Molecular methods

Small samples prepared from freshly collected and frozen herbarium specimens were ground with sterile glass pestles. Total genomic DNA was extracted using the DNeasy Plant Mini Kit (Qiagen, Hilden) according to the manufacturer's instructions with slight modifications described in Crespo, Blanco & Hawksworth (2001). Dilutions of the total DNA were used for PCR amplifications of the genes coding for the nuITS and nuLSU rRNA, and the mtSSU rRNA. Fungal nuITS rDNA was amplified using the primers ITS1F (Gardes & Bruns 1993), ITS4 (White *et al*. 1990), ITS1-LM (Myllys *et al*. 1999) and ITS2-KL (Lohtander *et al*. 1998); nuLSU rDNA was amplified using the primers LROR and LR5 (R. J. Vilgalys, website; http://www.biology.duke.edu/fungi/mycolab/primers.htm), and mtSSU rDNA was amplified using the primers mrSSU1 and mrSSU3R (Zoller, Scheidegger & Sperisen 1999), NMS1 and NMS2 (Li, Rouse & German 1994), MSU1 and MSU7 (Zhou & Stanosz 2001). Amplifications were performed in 50 µl volumes containing a reaction mixture of 5 µl of 10× DNA polymerase buffer (Biotools, Madrid) (containing $MgCl_2$ 2 mM, 10 mM Tris-HCl, pH 8.0, 50 mM KCl, 1 mM EDTA, 0.1% Triton X-100), 1 µl of dinucleotide triphosphate (dNTPs), containing 10 mM of each base, 2.5 µl of each primer (10 µM), 1.25 µl of DNA polymerase (1 unit μl^{-1}) and 27.5 µl dH_2O. Finally, 40 µl of this mixture was added to 10 µl of DNA of each sample.

The amplifications for ITS and LSU rDNA were carried out in an automatic thermocycler Techne

Table 1. Species and specimens of *Parmeliaceae* from which new sequences were obtained for this study.

Species	Country	Collector (s)	Herbarium accession no.	GenBank accession no.		
				nuLSU	nuITS	mtSSU
Brodoa atrofusca	Spain	*Crespo*	MAF 6780	AY607824	–	AY643090
Bulbothrix meizospora	India	*Divakar*	GPGC 02-000786	AY607780	AY611068	AY611127
B. setschwanensis	China	*Crespo, Blanco & Argüello*	MAF 10212	AY607781	AY611069	–
Cetraria aculeata	Spain	*Crespo & Blanco*	MAF 6781	AY607825	AY611111	AY643091
Everniastrum cirrhatum	Costa Rica	*Trest*	Trest 149	AY607782	AY611070	AY611128
E. nepalense	India	*Divakar*	GPGC 02-000924	AY607783	AY611071	AY611129
Hypotrachyna endochlora	UK	*Coppins*	MAF 10178	AY607784	AY611072	AY611130
H. immaculata	Australia	*Louwhoff, Molina & Elix*	MAF 7462	AY607785	AY611073	AY611131
H. laevigata	UK	*Coppins*	MAF 10177	AY607786	AY611074	AY611132
H. revoluta	Spain	*Noya & Olea*	MAF 6047	AY607787	AY611075	–
H. sinuosa	UK	*Coppins*	MAF 10179	AY607788	AY611076	AY611133
Imshaugia aleurites	Australia	*Louwhoff, Molina & Elix*	MAF 6877	AY607840	AY611126	–
Melanelia disjuncta	Austria	*Mayrhofer & Arup*	Mayrhofer 13743	AY607789	AY611077	AY611134
M. elegantula 1	Spain	*Aragón, Herrero & Martínez*	MAF 5592	AY607836	AY611122	AY611177
M. elegantula 2	Spain	*Crespo et al.*	MAF 10226	AY607791	AY611079	AY611136
M. elegantula 3	Spain	*Crespo & Divakar*	MAF 10218	AY607790	AY611078	AY611135
M. elegantula 4	Spain	*Crespo*	MAF 10231	AY607806	AY611094	AY611151
M. elegantula 5	Spain	*Crespo & Divakar*	MAF 10224	AY607792	AY611080	AY611137
M. exasperata 1	Spain	*Crespo et al.*	MAF 7636	AY607795	AY611083	AY611140
M. exasperata 2	Spain	*Blanco*	MAF 10214	AY607793	AY611081	AY611138
M. exasperatula 1	Spain	*Crespo et al.*	MAF 10213	AY607802	AY611090	AY611147
M. exasperatula 2	USA	*Esslinger*	Esslinger 16554	AY607833	AY611119	AY611175
M. fuliginosa 1	Spain	*Crespo et al.*	MAF 7640	AY607796	AY611084	AY611141
M. fuliginosa 2	Spain	*Crespo*	MAF 10222	AY607797	AY611085	AY611142
M. fuliginosa 3	Spain	*Crespo et al.*	MAF 10219	AY607798	AY611086	AY611143
M. fuliginosa 4	Spain	*Blanco*	MAF 10223	AY607801	AY611089	AY611146
M. fuliginosa 5	Spain	*Crespo*	MAF 10229	AY607800	AY611088	AY611145
M. fuliginosa 6	USA	*Robertson*	Robertson 7140	AY607831	AY611117	AY611173
M. fuliginosa 7	USA	*Robertson*	Robertson 7139	AY607838	AY611124	AY611179
M. glabra 1	USA	*Robertson & Robertson*	Robertson 7137c	AY607828	AY611114	AY611170
M. glabra 3	Spain	*Crespo et al.*	MAF 10228	AY607799	AY611087	AY611144
M. olivacea	Finland	*Vitikainen*	Vitikainen 16196	AY607803	AY611091	AY611148
M. septentrionalis	Finland	*Ahti*	Ahti 60893	AY607805	AY611093	AY611150
M. stygia 1	Finland	*Haikonen*	Haikonen 20365	AY607809	AY611097	AY611154
M. stygia 2	Austria	*Hafellner & Hafellner*	Hafellner 51658	AY607835	AY611121	–
M. subargentifera 1	Spain	*Crespo et al.*	MAF 6049	AY607810	AY611098	AY611155
M. subaurifera 1	Spain	*Blanco*	MAF 10221	AY607807	AY611096	AY611152
M. subaurifera 2	Spain	*Blanco & Divakar*	MAF 10217	AY607812	AY611100	AY611157
M. subaurifera 3	Spain	*Blanco*	MAF 10216	AY607813	AY611101	AY611158
M. subaurifera 4	UK	*Crespo*	MAF 10215	AY607811	AY611099	AY611156
M. subaurifera 5	USA	*Robertson*	Robertson 7138	AY607832	AY611118	AY611174
M. subelegantula	USA	*Esslinger*	Esslinger 16132	AY607829	AY611115	AY611171
M. subolivacea	USA	*Esslinger*	Esslinger 16555	AY607837	AY611123	AY611178
M. aff. *elegantula* 1	USA	*Esslinger*	Esslinger 16362	AY607834	AY611120	AY611176
M. aff. *elegantula* 2	USA	*Esslinger*	Esslinger 16550	AY607830	AY611116	AY611172
M. aff. *exasperata* 1	Spain	*Blanco*	MAF 10227	AY607794	AY611082	AY611139
M. aff. *exasperata* 2	Spain	*Divakar*	MAF 10225	AY607804	AY611092	AY611149
M. aff. *exasperata* 3	Spain	*Blanco*	MAF 10230	AY607808	AY611095	AY611153
Myelochroa irrugans	China	*Crespo et al.*	MAF 10207	AY607815	AY611103	AY611160
M. metarevoluta	China	*Crespo et al.*	MAF 10208	AY607814	AY611102	AY611159
Parmelia pinnatifida	Rusia	*Schlensog*	MAF 7272	–	–	AY611161
P. squarrosa	Japan	*Harada*	MAF 7281	AY607816	–	AY611162
Parmelina pastillifera	Spain	*Crespo*	MAF 6058	AY607817	AY611104	AY611163
P. quercina	Spain	*Crespo*	MAF 6057	AY607818	AY611105	AY611164
Parmelinella wallichiana	India	*Chatterjee & Divakar*	LWG 20-77171	AY607819	AY611106	AY611165
Parmelinopsis minarum 2	China	*Crespo et al.*	MAF 10220	–	AY611110	AY611168
P. neodamaziana	Australia	*Louwhoff, Molina & Elix*	MAF 10182	AY607820	AY611107	AY611166
P. subfatiscens	Australia	*Louwhoff, Molina & Elix*	MAF 6878	AY607821	AY611108	–
Parmeliopsis ambigua	Spain	*Divakar*	MAF 10186	AY607822	–	–
P. hyperopta	Spain	*Blanco*	MAF 10181	AY607823	AY611109	AY611167
Pseudephebe pubescens	Spain	*Crespo*	MAF 6774	AY607839	AY611125	–
Pseudevernia furfuracea	Spain	*Crespo et al.*	MAF 6772	AY607826	AY611112	AY611169
Vulpicida pinastri	Spain	*Blanco*	MAF 6783	AY607827	AY611113	–

Table 2. Species and sequences of *Parmeliaceae* downloaded from GenBank.

Species	GenBank accession no.		
	nuLSU	nuITS	mtSSU
Arctoparmelia centrifuga	AY578917	AY581054	AF351156
Hypotrachyna revoluta	–	–	AF351166
H. taylorensis	AY578924	AY581061	AY582298
Imshaugia aleurites	–	–	AF351167
Melanelia glabra 2	AY578927	AY581064	AY582300
M. subargentifera 2	AY578928	AY581065	AY582301
Parmelia saxatilis	AY578947	AF350027	AF351172
P. serrana	AY578948	AY295109	AY582319
P. sulcata	AY578949	AY581083	AY582320
P. pinnatifida	–	AY036988	–
P. squarrosa	–	AY036975	–
Parmelina tiliacea	AY578950	AY581084	AF351173
Parmelinopsis horrescens	AY578951	AY581085	AY582321
P. minarum 1	AY578952	AY581086	AY582322
P. subfatiscens	–	–	AF351174
Parmeliopsis ambigua		AF410829	AF351175
Pleurosticta acetabulum	AY578953	AY581087	AY582323
Pseudephebe pubescens	–	–	AF351180
Vulpicida pinastri	–	–	AF351184
Xanthoparmelia aff. *glabrans*	AY578935	AY581072	AY582308
X. pulla	AJ 421433	AY581071	AF351169
X. delisei	AY578931	AY581068	AY582304
X. glabrans	AY578932	AY581069	AY582305
X. pokornyi 1	AY578934	AY037005	AY582307
X. pokornyi 2	AY578939	AY581075	AY582312
X. pulloides	AY578936	AY037004	AY582309
X. subincerta	AY578937	AY581073	AY582310
X. subprolixa	AY578938	AY581074	AY582311
X. loxodes 1	AY578949	AY581076	AY582313
X. loxodes 2	AY578933	AY581070	AY582306

Progene and performed using the following programs: initial denaturation at 94 °C for 5 min, and 30 cycles of: 94 ° for 1 min, 54–60 ° (ITS rDNA) and 60 ° (LSU rDNA) for 1 min, 72 ° for 1.5 min, and a final extension at 72 ° for 5 min. The PCR amplification for mitochondrial rDNA was carried out in a Hybaid OmniGene thermocycler and was performed using the following program: initial denaturation at 94 ° for 5 min and 35 cycles of: 94 ° for 1 min, 57–58 ° for 1 min, and 72 ° for 1.5 min, and a final extension at 72 ° for 5 min.

The PCR products were subsequently cleaned using the Bioclean Columms kit (Biotools) or, in case of an impure product, the Bioclean kit for purification of DNA bands from agarose gels (Biotools) according to the manufacturer's instructions. The cleaned PCR products were sequenced using the following primers, in addition to those also used for PCR amplifications: (1) 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 nuSSU (Gargas, DePriest & Taylor 1995); (2) for the LSU rDNA: LR3 and LR3R (Vilgalys website; see above); and (3) for the mtSSU rDNA: mrSSU2 and mrSSU2R (Zoller, Scheidegger & Sperisen 1999). The ABI Prism™ Dye Terminator Cycle Sequencing Ready reaction kit (Applied Biosystems, Foster City, CA) was used and the following settings were carried out: denaturation for 3 min at 94 ° and 25 cycles at 96 ° for 10 s, 50 ° for 5 s and 60 ° for 4 min. Sequencing reactions were electrophoresed on a 3730 DNA analyzer (Applied Biosystems). Partial nuSSU, sometimes including an intron at the end of the 3′, were removed before the aligment. Sequence fragments obtained were assembled with SeqMan 4.03 (DNAStar) and manually adjusted.

Sequence alignments

We used an alignment procedure employing a linear Hidden Markov Model (HMM) for the alignment, as implemented in the software SAM (Hughey & Krogh 1996). Sequences of 85 specimens (Tables 1–2) were aligned separately for the three genes. Regions that could not be aligned with statistical confidence were excluded from the phylogenetic analysis. Moreover, in the case of nuITS and mtSSU matrices, small regions clearly remained ambiguously aligned. These regions were consequently excluded and the remaining, were used in the subsequent analyses.

Phylogenetic analysis

The alignment was analysed using the programs PAUP* 4.0b10 (Swofford 2003) and MrBAYES 3.0 (Huelsenbeck & Ronquist 2001). The polarity of characters was assessed with outgroup comparison using *Pseudephebe pubescens* as outgroup. The data were analysed using a Bayesian approach (Larget & Simon 1999, Huelsenbeck, Rannala & Masly 2000). Posterior probabilities were approximated by sampling trees using a Markov Chain Monte Carlo (MCMC) method. The posterior probabilities of each branch were calculated by counting the frequency of trees that were visited during the course of the MCMC analysis.

The program MrBAYES was employed to sample the trees. The analysis was performed assuming the general time reversible model (Rodriguez *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. The nucleotide substitution model was selected using a likelihood ratio test (Huelsenbeck & Crandall 1997) with the program ModelTest (Posada & Crandall 1998). 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 & Ronquist 2001). The initial 1000 trees were discarded as burn-in before stationarity was reached. Using sumt command of

MrBAYES, a majority-rule consensus tree was calculated from 19 000 trees sampled after reaching likelihood convergence to calculate the posterior probabilities of the tree nodes. Unlike nonparametric bootstrap values (Felsenstein 1985), these are estimated probabilities of the clades under the assumed model (Rannala & Yang 1996) and hence posterior probabilities equal to and above 95% are considered significant supports. Phylogenetic trees were drawn using TREEVIEW (Page 1996).

We used a Bayesian approach to examine the heterogeneity in phylogenetic signal among the three data partitions (Buckley *et al*. 2002). For the three genes and the concatenated analyses, the set of topologies reaching 0.95 posterior probability was estimated. The combined analysis topology was then compared for conflict with the 0.95 posterior intervals of the single gene analyses. If no conflict was evident, it was assumed that the three data sets were congruent and could be combined. If conflict was evident, the three data sets were interpreted as incongruent and thus the combined analysis might be potentially misleading (Bull *et al*. 1993).

Hypothesis testing

One hypothesized phylogenetic relationship was tested as a null hypothesis using a MCMC tree sampling procedure to examine the possibility of presence of alternative topologies in suboptimal trees. The null hypothesis tested was: *Melanelia elegantula*, *M*. aff. *elegantula*, *M. exasperata*, *M*. aff. *exasperata*, *M. exasperatula*, *M. subelegantula*, *M. subolivacea*, *M. olivacea*, *M. septentrionalis*, *M. glabra*, *M. subargentifera*, *M. fuliginosa* and *M. subaurifera* form a monophyletic group.

For the hypothesis testing a run as described above was performed with the same settings as in the estimation of the phylogeny using the combined data set. 19 000 trees at the equilibrium state for the null hypothesis were used from 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, Lumbsch *et al*. 2004). The frequency of trees in the MCMC sample agreeing with the null hypothesis was calculated using the filter command in PAUP* (Swofford 2003), with a certain constraint describing the null hypothesis.

Scanning-electron microscopy

Small pieces of thalli *ca* 5 mm diam were cut from samples, air-dried, fixed to a metallic stub, and sputtered with gold-palladium in a vacuum. A Jeol (JSM 6400) scanning electron microscope was used for the analysis.

RESULTS

In all, 55 mtSSU rDNA, 59 new nuITS rDNA, and 61 new nuLSU rDNA, sequences were generated, and in addition 75 sequences were downloaded from GenBank and aligned with the newly obtained ones. We produced a matrix of 533 unambiguous nucleotide position characters in the mtSSU, 501 in the nuITS and 850 in the nuLSU. 572 characters were variable. The final aligment of the 85 taxa studied (Tables 1–2) was 1884 positions in length. The Bayesian approach to test heterogeneity in phylogenetic signal among the data partitions showed no significant incongruence and hence a combined analysis was performed.

The likelihood parameters in the sample had the following average values ($\pm$ one standard deviation): likelihood (LnL) = -1417.706 (± 1.486), base frequencies $\pi(A) = 0.256$ (± 0.002), $\pi(C) = 0.235$ (± 0.003), $\pi(G) = 0.27$ (± 0.003), $\pi(T) = 0.239$ (± 0.002), rate matrix $r(AC) = 1.017$ (± 0.082), $r(AG) = 2.488$ (± 0.171), $r(AT) = 1.713$ (± 0.121), $r(CG) = 0.614$ (± 0.056), $r(CT) = 9.689$ (± 0.676), $r(GT) = 1.0$ (± 0), the gamma shape parameter alpha = 0.594 (± 0.012), and the pinvar = 0.517 (± 0.004).

In the majority-rule consensus tree of 19 000 sampled trees (Fig. 1), *Melanelia* is not monophyletic and four different clades can be recognized (Groups I, II, III, and IV). *M. stygia*, the type species, is not placed within the parmelioid clade and its relationship with other taxa has not been established (Group I). The other taxa previously included in *Melanelia* appear in three different groups within the parmelioid lichens as outlined below. Among non-parmelioid species included, only *Vulpicida pinastri* and *Cetraria aculeata* form a supported monophyletic group.

The parmelioid species form a well-supported monophyletic group (pp 1.00) including the genera *Myelochroa*, *Parmelina*, *Xanthoparmelia* (syn. *Neofuscelia*), *Parmelinella*, *Bulbothrix*, *Hypotrachyna*, *Everniastrum*, *Parmelinopsis*, *Melanelia* (except the type species), *Parmeliopsis*, *Pleurosticta*, and *Parmelia s. str*. The parmelioid genera fall into two lineages, which, however, lack statistical support. One sister group includes again two unsupported sister groups. One of these includes species of *Parmelina*, *Myelochroa*, *Bulbothrix*, and *Parmelinella*. All these genera form well-supported monophyletic clades (pp 1.00) but their relationships lack support. Only *Parmelinella wallichiana*, the type species of that genus, is shown as the sister group of *Bulbothrix* (pp 1.00). The other sister group includes *Hypotrachyna*, *Everniastrum*, *Parmelinopsis*, and *Parmeliopsis* spp. The latter genus is basal and its relationships remain unsupported. The other taxa form a well supported monophyletic group (pp 1.00). The genus *Hypotrachyna* appears polyphyletic, while the taxa included from the genera *Everniastrum* and *Parmelinopsis* form monophyletic lineages. Further studies on the phylogeny of *Hypotrachyna* will be presented elsewhere.

The second major lineage of parmelioid lichens includes *Parmelia s. str*., *Pleurosticta*, brown *Xanthoparmelia* spp. (syn. *Neofuscelia*), and the bulk of species currently classified in *Melanelia* placed in three groups

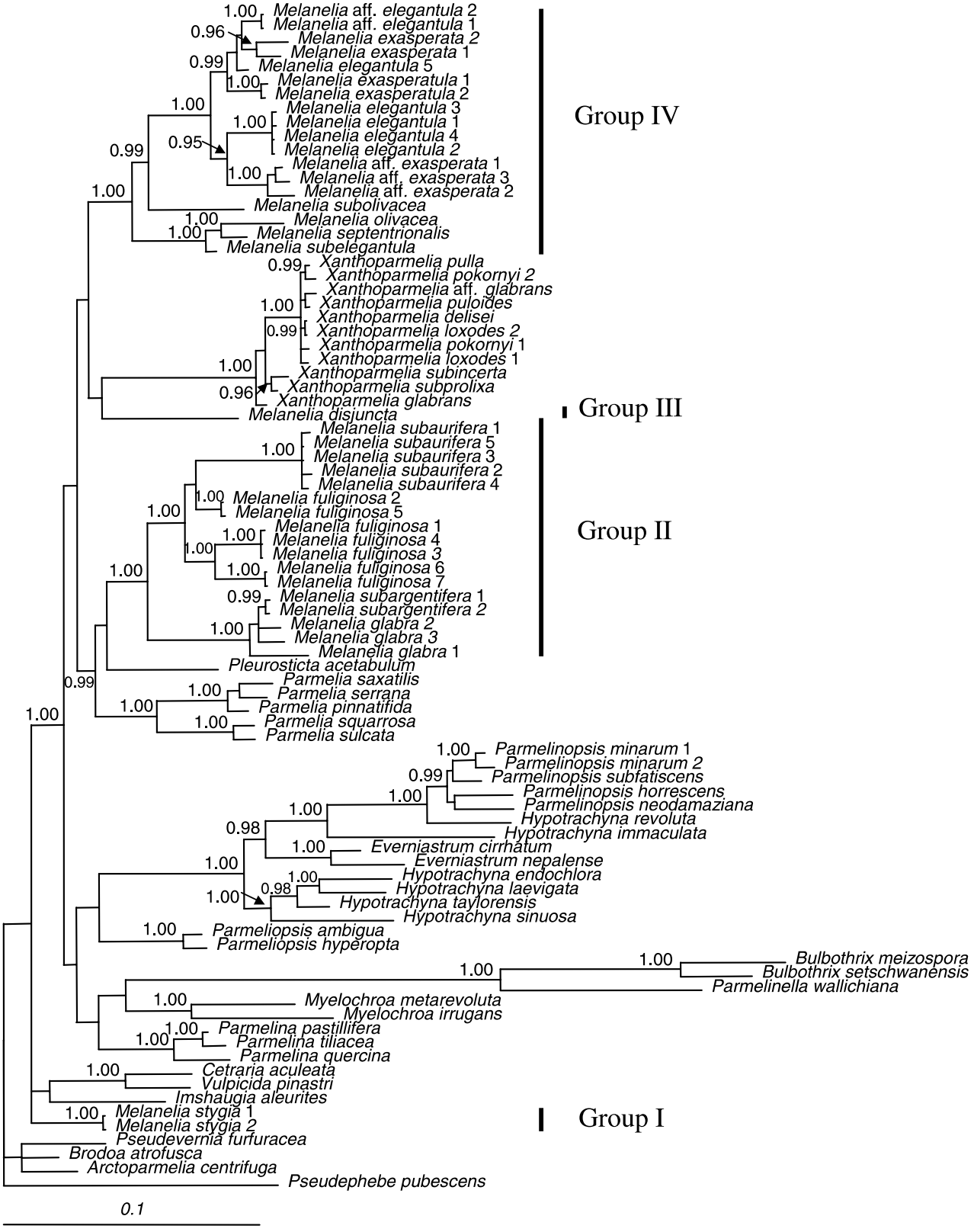


Fig. 1. 95% majority rule consensus tree of 19 000 trees visited during a B/MCMC tree sampling procedure. Numbers at nodes are posterior probabilities values above or equal 95%.

(II, III and IV). Group II includes: *M. glabra*, *M. subargentifera*, *M. fuliginosa* and *M. subaurifera*, which form a highly supported monophyletic group (pp 1.00). Group III contains *M. disjuncta* that appears as sister-group of the *Xanthoparmelia* species, but without significant support, and group IV, formed by *M. elegantula*, *M.* aff. *elegantula*, *M. exasperata*, *M.* aff. *exasperata*, *M. exasperatula*, *M. subelegantula*,

M. subolivacea, *M. olivacea*, and *M. septentrionalis*, forms another well-supported group (pp 1.00).

A Bayesian hypothesis test was performed testing the null hypothesis of groups II and IV forming a monophyletic lineage. This null hypothesis was rejected ($P<0.001$).

DISCUSSION

The results of our analysis including 15 *Melanelia* species and three molecular data sets, support previous studies that found *Melanelia* to be polyphyletic (e.g. Crespo *et al*. 1999, Thell *et al*. 2002, Mattsson *et al*. 2004). The genus as currently circumscribed falls into four groups: the type species *Melanelia stygia*, which does not belong to the main group of parmelioid lichens, and three groups that belong to a lineage of parmelioid lichens which also includes the genera *Xanthoparmelia* (syn. *Neofuscelia*), *Pleurosticta*, and *Parmelia s. str*.

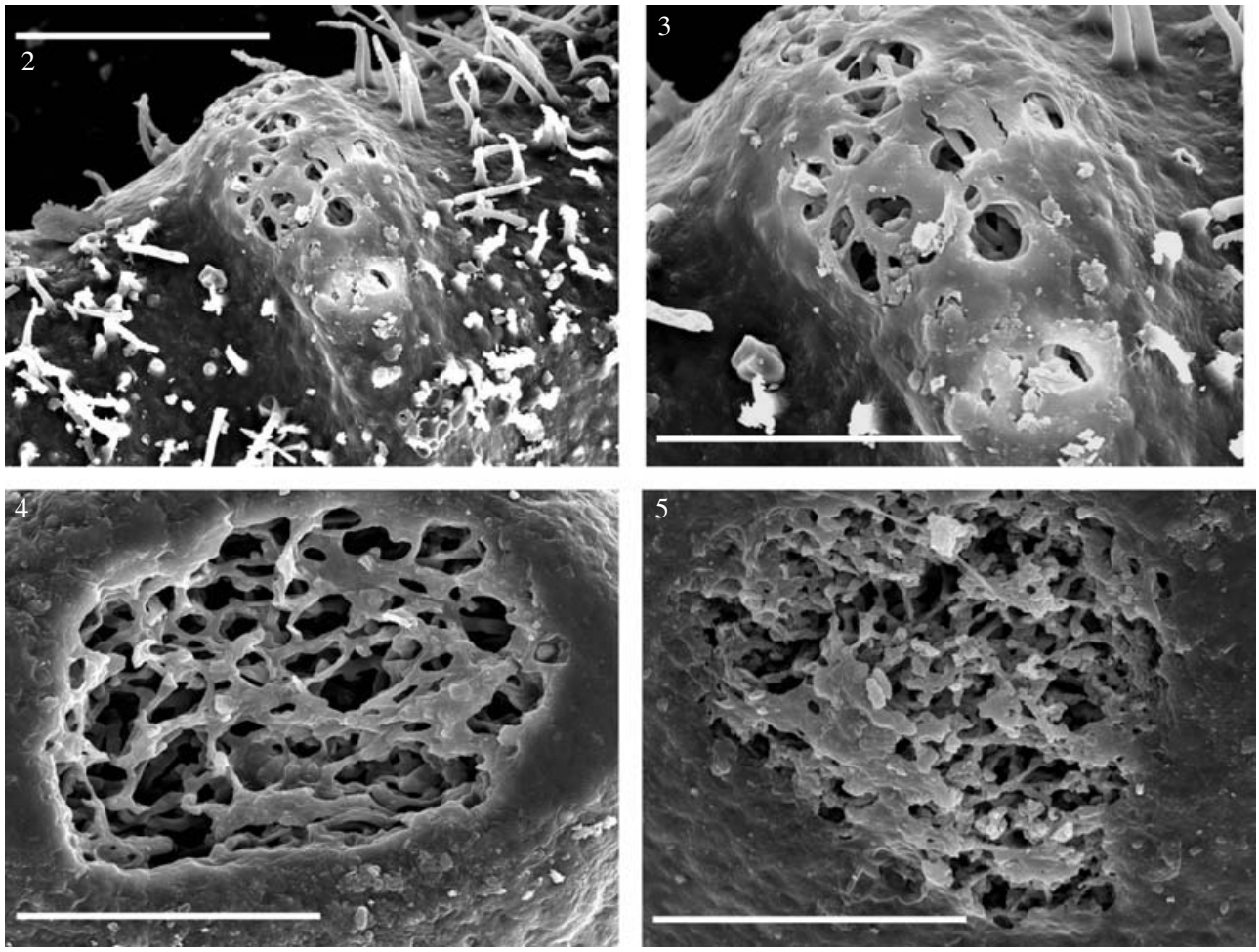
Although the placement of the type species of *Melanelia*, *M. stygia*, outside the parmelioid lichens is strongly supported (pp 1.00), its further relationships remain unclear. Additional studies employing a wider sampling of cetrarioid and other non-parmelioid taxa are necessary to elucidate the phylogenetic position of *Melanelia s. str*. *M. stygia* was included in the section *Melanoparmelia* of *Parmelia* (Esslinger 1977) and later in the nominal subgenus of *Melanelia* (Esslinger 1978) together with *M. disjuncta*, *M. panniformis*, *M. predisjuncta*, *M. sorediata*, and *M. tominii* (as *M. substygia*; Esslinger 1992). Chemically, *M. stygia* is unique in this group by containing the depsidone fumarprotocetraric acid, while four of the other species have long side-chain depsides, such as perlatolic and stenosporic acids. Moreover some morphological features, such as long, narrow, flat to convex inflated lobes, a thick upper cortex and effigurate pseudocyphellae, are common among these four taxa. Further, both *M. stygia* and *M. tominii* have cylindrical to bifusiform conidia. However, not all these taxa belong to *Melanelia s. str*. as *M. disjuncta*, is quite distantly clustered within the parmelioid genera, in a separate group (III) close to the species formerly placed in *Xanthoparmelia* (syn. *Neofuscelia*) (see below). Sequences were not available from *M. tominii*, which has the habit of *M. stygia* but contains the tridepside gyrophoric acid; its systematic position remains uncertain. However, a small group of cetrarioid lichens, the *Cetraria hepatizon* group, has been transferred to *Melanelia* by Thell (1995). This is supported by morphological and chemical characters (such as the presence of depsidones) and was corroborated by molecular data (Thell *et al*. 2002).

The taxa belonging to group II form a well supported monophyletic clade (pp 1.00) that contains four species (*M. glabra*, *M. subargentifera*, *M. fuliginosa*, and *M. subaurifera*). However, the species boundaries in this group are not well understood. *M. subaurifera* appears as a monophyletic taxon, but *M. fuliginosa* is paraphyletic, and *M. glabra* is also paraphyletic with *M. subargentifera* nested within. It is beyond the scope of this publication to elucidate the species concepts in this group, but our results indicate that additional research is needed. All these four species were classified in sect. *Vainioellae* by Esslinger (1977, 1978). The species of group II have broad, plain to concave and not inflated lobes, lack pseudocyphellae, contain lecanoric acid, and those in which we have found pycnidia have conidia which are cylindrical to fusiform and not clearly bifusiform. None of the species have pseudocyphellae, but either have scattered pores in the epicortex, or (in *M. glabra*) special structures which we will refer to as 'fenestrations' (Figs 2–3). These fenestrations comprise localized, generally ellipsoidal raised areas of the thallus with a mesh of rounded to somewhat irregularly shaped pores formed through the epicortical layer. In fenestrations, the pores tend to be larger than those seen in a pored epicortex, and are not dispersed over the whole surface as is the case in lichens with this feature (Hale 1973, 1981). Fenestrations differ from pseudocyphellae in that they are not clearly delimited, and further retain strands of broad epicortical tissue between the pores, so that the underlying medullary hyphae are not readily seen. The presence of pores in the epicortex in *M. acetabulum* was a major reason for resurrection of the genus *Pleurosticta*, but in that case the pores tend to be arranged in a characteristic reticulate pattern (Lumbsch, Kothe & Elix 1988) distinct from both the true pored epicortex and the fenestrated type. *M. glabra* was previously reported having 'obscure' pseudocyphellae in the amphithecium and extreme lobe edges (Esslinger 1977), but a SEM re-examination revealed that these were what we term fenestrations here.

The species of group II appear to be related to *Pleurosticta*, although this relationship lacks support. *Pleurosticta* can be distinguished by broader, marginally erhizinate lobes, the reticulated epicortical pores, and a different chemistry; a cortical pigment that turns violet in K and HNO_3, and depsidones in the medulla.

Group III includes the saxicolous *M. disjuncta* as the only species. As mentioned above, it has been considered as belonging to section *Melanoparmelia* of *Parmelia* (Esslinger 1977) or the nominal subgenus of *Melanelia* (Esslinger 1978). Based on morphological and chemical characters it appears to be related to *M. predisjuncta*, *M. panniformis*, and *M. sorediata*. Further molecular studies are needed to establish the phylogenetic relationships in this clade, though it is of interest that it appears as a sister group to the species now placed in *Xanthoparmelia*, although without support.

Group IV is a well-supported monophyletic clade (pp 1.00), which includes nine species which grow primarily on bark or wood (*M. subelegantula*, *M. septentrionalis*, *M. olivacea*, *M. subolivacea*, *M. elegantula*, *M.* aff. *elegantula*, *M. exasperatula*, *M. exasperata* and *M.* aff.



Figs 2–5. Scanning electron micrographs of cortical features. **Figs 2–3.** *Melanelixia glabra* (MAF 10228), showing fenestrations in the epicortex. **Fig. 4.** *Melanohalea exasperata* (MAF 7636) pseudocyphella. **Fig. 5.** *Melanelia stygia* (Hafellner 51658) pseudocyhella. Bar = 60 μm.

exasperata). All species in this clade in which we have found pycnidia have cylindrical to fusiform conidia. Again, the species delimitations in some taxa are not well understood. Two species, *M. elegantula* and *M. exasperata* appear polyphyletic and some morphological differences have been discovered for supporting new taxa; based on these differences the provisional names, *M.* aff. *elegantula*, and *M.* aff. *exasperata* have been used. Additional studies are currently under way to elucidate the species circumscriptions in these complexes. All species placed in group IV here were included in section *Vainioellae* by Esslinger (1977, 1978) together with those that are included in group II here. The taxa placed in group IV are characterized by broad, plane to concave non-inflated lobes, having a non-pored epicortex and usually with discrete pseudocyphellae (Figs 4–5), and either containing the depsidone fumarprotocetraric acid or lacking phenolic substances. The pseudocyphellae are often located on the tips of isidia or warts. Three boreal species (*M. septentrionalis*, *M. olivacea*, and *M. subelegantula*) form a strongly supported monophyletic group (pp 1.00), which is basal to the group, and *M. subolivacea* appears as the sister-group of the remaining species studied.

Our null hypothesis test rejected the monophyly of groups II and IV significantly. Given this molecular evidence and the morphological and chemical features that distinguish these groups, such as the pored or fenestrate epicortex in group II and non-pored epicortex and pseudocyphellae in group IV, depsides in group II and depsidones in group IV (Table 3), we recognize these two groups at generic level distinct from *Melanelia s. str*. The generic placement of group III requires additional studies, including taxa presumed to be related, such as *M. panniformis*, *M. predisjuncta* or *M. sorediata*.

In view of these results, we introduce two new generic names, *Melanelixia* and *Melanohalea*, for the species of groups II and IV respectively. In doing this, we recognize that our study included molecular data on only 15 of the 40 species of *Melanelia s. lat*., and that the inclusion of additional species could have had some impact on the results as we did not obtain strong support for the relationship between the major clades. Nevertheless, we are confident in the placement of the additional species transferred to one or the other of these genera here based on the morphological and chemical characters summarized in Table 3.

Table 3. Selected morphological and chemical features in the monophyletic groups of *Melanelia s. lat.*

Taxon	Epicortex	Pseudocyphellae	Upper surface	Lobes	Lobe periphery	Cortical hairs	Medullary chemistry	Substrate
Melanelia stygia	Non-pored	Flat, effigurate	Undulate	Long, narrow to terete	Plane to convex, inflated	Absent	Depsidones	Saxicolous
M. disjuncta	Non-pored	Flat, effigurate	Undulate	Short, narrow	Plane to convex, inflated	Absent	Long side-chain despides	Saxicolous
Melanohalea	Non-pored	On verrucae or isidial tips, circular to slightly elliptic	Undulate	Broad	Plane to concave, flat	Absent	Absent or depsidones	Corticolous or rarely saxicolous
saxicolous*Melanelixia*	Pored or fenestrate	Absent	Smooth	Broad	Plane to concave, flat	Present in some species	Depsides	Corticolous or sometimes saxicolous

Indeed, it would be contrary to the generic concept we would wish to promulgate to recognize as genera groups which could not be recognized without molecular data.

Our study of *Melanelia* supports the idea that medullary chemical characters, such as the presence of certain substance classes, are important for the circumscription of monophyletic groups at generic level in lichen-forming fungi. In other groups of lichen-forming fungi, such as *Lecanoraceae* or *Pertusariaceae* (Arup & Grube 1998, Schmitt & Lumbsch 2004), chemistry was also found to be an important indicator of phylogenetic relationships. On the other hand, cortical chemistry (presence or absence of atranorin *vs* usnic acid) has also been found to be of low taxonomic relevance in other parmelioid lichens (Elix 2003, Blanco *et al.* 2004). The study also demonstrates the importance of the cortical structure when studied at the SEM level, and apparently also correlations with conidium types, although the latter features and those of the excipular structures require more study.

TAXONOMY

Melanelixia O. Blanco, A. Crespo, Divakar, Essl., D. Hawksw. & Lumbsch, **gen. nov.**

Etym.: From *Melanelia*, and dedicated in honour of John A. Elix for his immense contributions to lichen systematics and chemistry, especially in *Parmeliaceae*.

Thallus foliosus, laxus vel modice adnatus, lobis 1–6 mm, plus minusque planis, acidum lecanorinum continens. Superne pseudocyphellis destitutus sed cum epicorticis fenestratis vel cum poris, HNO_3 non reagens.

Typus: *Melanelixia glabra* (Schaer.) O. Blanco, A. Crespo, Divakar, Essl., D. Hawksw. & Lumbsch 2004.

Thallus foliose, loosely to moderately adnate; lobes plane to concave, flat, short, apices rounded, 1–6 mm wide, eciliate; upper surface olive-green to dark brown, smooth to rugose, maculate or not, lacking pseudocyphellae, with or without soredia, isidia and cortical hairs; upper cortex paraplectenchymatous, 16–20 μm thick, covered by an epicortex (SEM) which has either dispersed pores or fenestrations; cell walls containing isolichenan; medulla white to pale yellow or occasionally orange in the lower parts; lower cortex flat, smooth, dark brown to black; rhizines simple with white tips. *Ascomata* apothecial, laminal, sessile to subpedicellate; disc imperforate, concave and becoming plane with age, pale to dark brown, amphithecium commonly maculate and with an abundantly fenestrated or pored epicortex. *Asci* elongate, clavate, *Lecanora*-type, apically thicked, without an internal apical beak, 8-spored. *Ascospores* ellipsoid to ovoid, colourless, thin-walled, simple, 9–15 × 5–11.5 μm. *Conidiomata* pycnidial, immersed, laminal. *Conidiophores* of type V or VI (Vobis 1980). *Conidia* cylindrical to fusiform, simple, hyaline, 5–8 × 1 μm.

Chemistry: Cortex with a brown coloured pigment but no other compounds; medulla containing depsides (lecanoric acid chemosyndrome), and (in two species) skyrin.

Observations: This new genus includes eight species that occur on bark and wood in the Northern Hemisphere. *Melanelixia* is characterized by having a pored or fenestrate epicortex, lacking pseudocyphellae and containing lecanoric acid as the primary medullary constituent. It is similar to *Pleurosticta*, which differs in having broader lobes, reticulated epicortical pores, a pigment that reacts violet in K and HNO_3, and the presence of depsidones in the medulla. Six rather similar species from the Southern Hemisphere which produce gyrophoric instead of lecanoric acid (Esslinger 1977), are not transferred to the genus here pending more detailed study.

Melanelixia albertana (Ahti) O. Blanco, A. Crespo, Divakar, Essl., D. Hawksw. & Lumbsch, **comb. nov.** Basionym: *Parmelia albertana* Ahti, *Bryologist* **72**: 236 (1969). Synonym: *Melanelia albertana* (Ahti) Essl., *Mycotaxon* **7**: 47 (1978).

Melanelixia fuliginosa (Fr. ex Duby) O. Blanco, A. Crespo, Divakar, Essl., D. Hawksw. & Lumbsch, **comb. nov.** Basionym: *Parmelia olivacea* var. *fuliginosa* Fr. ex Duby, *Bot. Gall.* **2**: 602 (1830). *Parmelia fuliginosa* ∗ *P. glabratula* Lamy, *Bull. Soc. Bot. France* **30**: 353 (1883); *Melanelia glabratula* (Lamy) Essl., *Mycotaxon* **7**: 48 (1978); *M. fuliginosa* (Fr. ex Duby) Essl., *Bryologist* **90**: 163 (1987).

Melanelixia glabra (Schaer.) O. Blanco, A. Crespo, Divakar, Essl., D. Hawksw. & Lumbsch, **comb. nov.** Basionym: *Parmelia olivacea* α. *corticicola a. glabra* Schaer., *Lich. Helv. Spicil.*: 466 (1840). Synonym: *Melanelia glabra* (Schaer.) Essl., *Mycotaxon* **7**: 47 (1978).

Melanelixia glabroides (Essl.) O. Blanco, A. Crespo, Divakar, Essl., D. Hawksw. & Lumbsch, **comb. nov.** Basionym: *Parmelia glabroides* Essl., *J. Hattori Bot. Lab.* **42**: 72 (1977). Synonym: *Melanelia glabroides* (Essl.) Essl., *Mycotaxon* **7**: 48 (1978).

Melanelixia huei (Asah.) O. Blanco, A. Crespo, Divakar, Essl., D. Hawksw. & Lumbsch, **comb. nov.** Basionym: *Parmelia huei* Asah., *J. Jap. Bot.* **26**: 194 (1951). Synonym: *Melanelia huei* (Asah) Essl., *Mycotaxon* **7**: 48 (1978).

Melanelixia subargentifera (Nyl.) O. Blanco, A. Crespo, Divakar, Essl., D. Hawksw. & Lumbsch, **comb. nov.** Basionym: *Parmelia subargentifera* Nyl., *Flora* **58**: 359 (1875). Synonym: *Melanelia subargentifera* (Nyl.) Essl., *Mycotaxon* **7**: 48 (1978).

Melanelixia subaurifera (Nyl.) O. Blanco, A. Crespo, Divakar, Essl., D. Hawksw. & Lumbsch, **comb. nov.** Basionym: *Parmelia subaurifera* Nyl., *Flora* **56**: 22 (1873). Synonym: *Melanelia subaurifera* (Nyl.) Essl., *Mycotaxon* **7**: 48 (1978).

Melanelixia villosella (Essl.) O. Blanco, A. Crespo, Divakar, Essl., D. Hawksw. & Lumbsch, **comb. nov.** Basionym: *Parmelia villosella* Essl., *J. Hattori Bot. Lab.* **42**: 95 (1977). Synonym: *Melanelia villosella* (Essl.) Essl., *Mycotaxon* **7**: 49 (1978).

Melanohalea O. Blanco, A. Crespo, Divakar, Essl., D. Hawksw. & Lumbsch, **gen. nov.**

Etym.: From *Melanelia*, and in honour of the father of modern studies on the *Parmeliaceae*, Mason E. Hale jr, who provided the foundations for subsequent contributions to our knowledge of this family.

Thallus foliosus, laxus vel modice adnatus, lobis 0.5–7 mm, plus minusque planis, acidum fumarprotocetraricum et norsticticum vel non secundarius metabolitus continens. Superne pseudocyphellatus, HNO_3 non reagens.

Typus: *Melanohalea exasperata* (De Not.) O. Blanco, A. Crespo, Divakar, Essl., D. Hawksw. & Lumbsch 2004.

Thallus foliose, loosely to moderately adnate; lobes plane to concave, flat, short, apices rounded, 0.5–7 mm wide, eciliate; upper surface olive-green to dark brown, smooth to rugose, emaculate, commonly pseudocyphellate on warts or on tips of isidia, with or without soredia and isidia; upper cortex paraplectenchymatous, 10–16 μm thick, epicortex not pored (SEM); cell walls containing isolichenan; medulla white; lower surface flat, smooth, pale brown to black; rhizines simple. *Ascomata* apothecial, laminal, sessile to subpedicillate; disc imperforate, concave and becoming convex with age, brown, amphithecium with pseudocyphellate papillae, without maculae. *Asci* elongate, clavate, *Lecanora*-type, apically thicked, without an internal apical beak, 8–32 spored. *Ascospores* globose to ovoid or ellipsoid, thin-walled, colourless, 5.5–20 × 4–12.5 μm. *Conidiomata* pycnidial, immersed, laminal. *Conidiophores* of type V or VI (Vobis 1980). *Conidia* cylindrical to fusiform, simple, colourless, 5–8.5 × 1 μm long.

Chemistry: Cortex with a brown coloured pigment but no other compounds; medulla containing depsidones (fumarprotocetraric acid, norstictic acid) or lacking secondary metabolites.

Observations: This new genus as circumscribed here includes 19 species, most of which have their primary distribution on bark and wood in the Northern Hemisphere, with three species occurring only in the Southern Hemisphere. The genus is characterized by pseudocyphellae, usually on warts or isidial tips, a non-pored epicortex, and a medulla containing depsidones or lacking secondary compounds.

Melanohalea elegantula (Zahlbr.) O. Blanco, A. Crespo, Divakar, Essl., D. Hawksw. & Lumbsch, **comb. nov.** Basionym: *Parmelia olivacea* * *P. aspidota* var. *elegantula* Zahlbr., *Verh. Vereins Natur- und Heilk. Preßburg* **8**: 39 (1894). Synonym: *Melanelia elegantula* (Zahlbr.) Essl., *Mycotaxon* **7**: 47 (1978).

Melanohalea exasperata (De Not.) O. Blanco, A. Crespo, Divakar, Essl., D. Hawksw. & Lumbsch, **comb. nov.** Basionym: *Parmelia exasperata* De Not., *Giorn. Bot. Ital.* **2**: 193 (1847). Synonym: *Melanelia exasperata* (De Not.) Essl., *Mycotaxon* **7**: 47 (1978).

Melanohalea exasperatula (Nyl.) O. Blanco, A. Crespo, Divakar, Essl., D. Hawksw. & Lumbsch, **comb. nov.** Basionym: *Parmelia exasperatula* Nyl., Flora 56: 299 (1873), syn. *Melanelia exasperatula* (Nyl.) Essl., *Mycotaxon* **7**: 47 (1978).

Melanohalea gomukhensis (Divakar, Upreti & Elix) O. Blanco, A. Crespo, Divakar, Essl., D. Hawksw. & Lumbsch, **comb. nov.** Basionym: *Melanelia gomukhensis* Divakar, Upreti & Elix, *Mycotaxon* **80**: 356 (2001).

Melanohalea halei (Ahti) O. Blanco, A. Crespo, Divakar, Essl., D. Hawksw. & Lumbsch, **comb. nov.** Basionym: *Parmelia halei* Ahti, *Acta Bot. Fenn.* **70**: 38 (1966). Synonym: *Melanelia halei* (Ahti) Essl., *Mycotaxon* **7**: 48 (1978).

Melanohalea inactiva (P. M. Jørg.) O. Blanco, A. Crespo, Divakar, Essl., D. Hawksw. & Lumbsch, **comb. nov.** Basionym: *Melanelia inactiva* P. M. Jørg., *N. Z. Jl Bot.* **28**: 10 (1990).

Melanohalea infumata (Nyl.) O. Blanco, A. Crespo, Divakar, Essl., D. Hawksw. & Lumbsch, **comb. nov.** Basionym: *Parmelia infumata* Nyl., *Flora* **58**: 359 (1875). Synonym: *Melanelia infumata* (Nyl.) Essl., *Mycotaxon* **7**: 48 (1978).

Melanohalea laciniatula (Flagey ex H. Olivier) O. Blanco, A. Crespo, Divakar, Essl., D. Hawksw. & Lumbsch, **comb. nov.** Basionym: *Parmelia exasperatula* var. *laciniatula* Flagey ex H. Olivier, *Rev. Bot. Bull. Mens.* **12**: 69 (1894). Synonym: *Melanelia laciniatula* (Flagey ex H. Olivier) Essl., *Mycotaxon* **7**: 48 (1978).

Melanohalea multispora (A. Schneid.) O. Blanco, A. Crespo, Divakar, Essl., D. Hawksw. & Lumbsch, **comb. nov.** Basionym: *Parmelia multispora* A. Schneid., *Guide Study Lich.*: 154 (1898). Synonym: *Melanelia multispora* (A. Schneid.) Essl., *Mycotaxon* **7**: 48 (1978).

Melanohalea olivacea (L.) O. Blanco, A. Crespo, Divakar, Essl., D. Hawksw. & Lumbsch, **comb. nov.** Basionym: *Lichen olivaceus* L., *Sp. Pl.* **2**: 1143 (1753). Synonym: *Melanelia olivacea* (L.) Essl., *Mycotaxon* **7**: 48 (1978).

Melanohalea olivaceoides (Krog) O. Blanco, A. Crespo, Divakar, Essl., D. Hawksw. & Lumbsch, **comb. nov.** Basionym: *Parmelia olivaceoides* Krog, *Norsk Polarinst. Skr.* **144**: 109 (1968). Synonym: *Melanelia olivaceoides* (Krog) Essl., *Mycotaxon* **7**: 48 (1978).

Melanohalea poeltii (Essl.) O. Blanco, A. Crespo, Divakar, Essl., D. Hawksw. & Lumbsch, **comb. nov.** Basionym: *Melanelia poeltii* Essl., *Mycotaxon* **28**: 215 (1987).

Melanohalea septentrionalis (Lynge) O. Blanco, A. Crespo, Divakar, Essl., D. Hawksw. & Lumbsch, **comb. nov.** Basionym: *Parmelia olivacea* var. *septentrionalis* Lynge, *Bergens Mus. Årbok* **1912** (10): 4 (1912). Synonym: *Melanelia septentrionalis* (Lynge) Essl., *Mycotaxon* **7**: 48 (1978).

Melanohalea subelegantula (Essl.) O. Blanco, A. Crespo, Divakar, Essl., D. Hawksw. & Lumbsch, **comb. nov.** Basionym: *Parmelia subelegantula* Essl., *J. Hattori Bot. Lab.* **42**: 89 (1977). Synonym: *Melanelia subelegantula* (Essl.) Essl., *Mycotaxon* **7**: 48 (1978).

Melanohalea subolivacea (Nyl.) O. Blanco, A. Crespo, Divakar, Essl., D. Hawksw. & Lumbsch, **comb. nov.** Basionym: *Parmelia subolivacea* Nyl., *in* Hasse, *Bull. Torrey Bot. Club* **24**: 445 (1897). Synonym: *Melanelia subolivacea* (Nyl.) Essl., *Mycotaxon* **7**: 49 (1978).

Melanohalea subverruculifera (J. C. Wei & Y. M. Jiang) O. Blanco, A. Crespo, Divakar, Essl., D. Hawksw. & Lumbsch, **comb. nov.** Basionym: *Parmelia subverruculifera* J. C. Wei & Y. M. Jiang, *Acta phytotax. sin.* **18**: 387 (1980). Synonym: *Melanelia subverruculifera* (J. C. Wei & Y. M. Jiang) J. C. Wei, *Enum. Lich. China*: 153 (1991).

Melanohalea trabeculata (Ahti) O. Blanco, A. Crespo, Divakar, Essl., D. Hawksw. & Lumbsch, **comb. nov.** Basionym: *Parmelia trabeculata* Ahti, *Acta Bot. Fenn.* **70**: 54 (1966). Synomym: *Melanelia trabeculata* (Ahti) Essl., *Mycotaxon* **7**: 49 (1978).

Melanohalea ushuaiensis (Zahlbr.) O. Blanco, A. Crespo, Divakar, Essl., D. Hawksw. & Lumbsch, **comb. nov.** Basionym: *Parmelia ushuaiensis* Zahlbr., *K. Svenska Vetensk. Handl.* **57**: 42 (1917). Synonym: *Melanelia ushuaiensis* (Zahlbr.) Essl., *Mycotaxon* **7**: 49 (1978).

Melanohalea zopheroa (Essl.) O. Blanco, A. Crespo, Divakar, Essl., D. Hawksw. & Lumbsch, **comb. nov.** Basionym: *Parmelia zopheroa* Essl., *J. Hattori Bot. Lab.* **42**: 96 (1977). Synonym: *Melanelia zopheroa* (Essl.) Essl., *Mycotaxon* **7**: 49 (1978).

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REFERENCES

Arup, U. & Grube, M. (1998) Molecular systematics of *Lecanora* subgenus *Placodium*. *Lichenologist* **30**: 415–425.

Blanco, O., Crespo, A., Elix, J. A., Hawksworth, D. L. & Lumbsch, H. T. (2004) A molecular phylogeny and new classification of parmelioid lichens containing *Xanthoparmelia*-type lichenan (*Ascomycota*: *Lecanorales*). *Taxon* **53**: in press.

Buckley, T. R., Arensburger, P., Simon, C. & Chambers, G. K. (2002) Combined data, Bayesian phylogenetics, and the origin of the New Zealand cicada genera. *Systematic Biology* **51**: 4–18.

Bull, J. J., Huelsenbeck, J. P., Cunningham, C. W., Swofford, D. L. & Waddell, P. J. (1993) Partitioning and combining data in phylogenetic analysis. *Systematic Biology* **42**: 84–397.

Clauzade, G. & Roux, C. (1986) ['1985'] Likenoj de okcidenta Europo. Ilustrita determinlibro. *Bulletin de la Société Botanique du Centre-Ouest, nouvelle série, numéro spécial* **7**: 1–893.

Crespo, A., Blanco, O. & Hawksworth, D. L. (2001) The potential of mitochondrial DNA for establishing phylogeny and stabilising generic concepts in the parmelioid lichens. *Taxon* **50**: 807–819.

Crespo, A. & Cubero, O. F. (1998) A molecular approach to the circumscription and evaluation of some genera segregated from *Parmelia s. lat*. *Lichenologist* **30**: 369–380.

Crespo, A., Blanco, O. & Hawksworth, D. L. (2001) The potential of mitochondrial DNA for establishing phylogeny and stablising generic concepts in the parmelioid lichens. *Taxon* **50**: 807–819.

Crespo, A., Gavilán, R., Elix, J. A. & Gutiérrez, G. (1999) A comparison of morphological, chemical and molecular characters in some parmelioid genera. *Lichenologist* **3**: 451–460.

DePriest, P. T. (1999) Development of Mason E. Hale's list of epithets in the parmelioid genera (lichen-forming *Ascomycotina*): a bibliographic review. *The Bryologist* **102**: 442–461.

Elix, J. A. (1993) Progress in the generic delimitation of *Parmelia* sensu lato lichens (*Ascomycotina*: *Parmeliaceae*) and a synoptic key to the *Parmeliaceae*. *The Bryologist* **96**: 359–383.

Elix, J. A. (2003) The lichen genus *Paraparmelia*, a synonym of *Xanthoparmelia* (*Ascomycota*, *Parmeliaceae*). *Mycotaxon* **87**: 395–403.

Elix, J. A. & Hale, M. E. (1987) *Canomaculina*, *Myelochroa*, *Parmelinella*, *Parmelinopsis* and *Parmotremopsis*, five new genera in the *Parmeliaceae* (lichenized *Ascomycotina*). *Mycotaxon* **29**: 233–244.

Elix, J. A., Johnston, J. & Verdon, D. (1986) *Canoparmelia*, *Paraparmelia* and *Relicinopsis*, three new genera in the *Parmeliaceae* (lichenized *Ascomycotina*). *Mycotaxon* **27**: 271–282.

Eriksson, O. & Hawksworth, D. L. (1986) An alphabetical list of the generic names of ascomycetes – 1986. *Systema Ascomycetum* **5**: 3–111.

Eriksson, O. E. & Hawksworth, D. L. (1992) Notes on ascomycete systematics – Nos 1294–1417. *Systema Ascomycetum* **11**: 49–82.

Esslinger, T. L. (1977) A chemosystematic revision of the brown *Parmeliae*. *Journal of the Hattori Botanical Laboratory* **42**: 1–211.

Esslinger, T. L. (1978) A new status for the brown *Parmeliae*. *Mycotaxon* **7**: 45–54.

Esslinger, T. L. (1992) The brown *Parmelia* type specimens of A. N. Oxner. *Lichenologist* **24**: 13–20.

Felsenstein, J. (1985) Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* **39**: 783–791.

Gardes, M. & Bruns, T. D. (1993) ITS primers with enhanced specificity for basidiomycetes-application to the identification of micorrhizae and rust. *Molecular Ecology* **2**: 113–118.

Gargas, A., DePriest, P. T. & Taylor, J. W. (1995) Positions of multiple insertions in SSU rDNA of lichen-forming fungi. *Molecular Biology and Evolution* **12**: 208–218.

Guzow-Krzeminska, B. & Wegrzyn, G. (2003) A preliminary study on the phylogeny of the genus *Melanelia* using nuclear large subunit ribosomal DNA sequences. *Lichenologist* **35**: 83–86.

Hale, M. E. (1973) Fine structure of the cortex in the lichen family *Parmeliaceae* viewed with the scanning-electron microscope. *Smithsonian Contributions to Botany* **10**: 1–92.

Hale, M. E. (1981) Pseudocyphellae and pored epicortex in the *Parmeliaceae*: their delimitation and evolutionay significance. *Lichenologist* **13**: 1–10.

Hale, M. E. (1984a) An historical review of the genus concept in lichenology. *Beiheft zur Nova Hedwigia* **79**: 11–23.

Hale, M. E. (1984b) *Flavopunctelia*, a new genus in the *Parmeliaceae* (*Ascomycotina*). *Mycotaxon* **20**: 681–682.

Hale, M. E. (1986a) *Arctoparmelia*, a new genus in the *Parmeliaceae* (*Ascomycotina*). *Mycotaxon* **25**: 251–254.

Hale, M. E. (1986b). *Flavoparmelia*, a new genus in the lichen family *Parmeliaceae* (*Ascomycotina*). *Mycotaxon* **25**: 603–605.

Hawksworth, D. L. & Crespo, A. (2002) Proposal to conserve the name *Xanthoparmelia* against *Chondropsis* nom. cons. (*Parmeliaceae*). *Taxon* **51**: 807.

Hawksworth, D. L., James, P. W. & Coppins, B. J. (1980) Checklist of British lichen-forming, lichenicolous and allied fungi. *Lichenologist* **12**: 1–115.

Huelsenbeck, J. P. & Crandall, K. A. (1997) Phylogeny estimation and hypothesis testing using maximum likelihood. *Annual Review of Ecology and Systematics* **28**: 437–466.

Huelsenbeck, J. P. & Ronquist, F. (2001) MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics* **17**: 754–755.

Huelsenbeck, J. P., Rannala, B. & Masly, J. P. (2000) Accomodating phylogenetic uncertainty in evolutionary studies. *Science* **288**: 2349–2350.

Huelsenbeck, J. P., Ronquist, F., Nielsen, R. & Bollback, J. P. (2001) Bayesian inference of phylogeny and its impact on evolutionary biology. *Science* **294**: 2310–2314.

Hughey, R. & Krogh, A. (1996) *SAM: sequence alignment and modeling software system*. [Technical Report UCSC-CRL-96–22.] University of California, Santa Cruz, CA.

Larget, B. & Simon, D. L. (1999) Markov chain Monte Carlo algorithms for the Bayesian analysis of phylogenetic trees. *Molecular Biology and Evolution* **16**: 750–759.

Lewis, P. O. (2001) Phylogenetic systematics turns over a new leaf. *Trends in Ecology and Evolution* **16**: 30–37.

Li, K.-N., Rouse, D. I. & German, T. L. (1994) PCR primers that allow intergeneric differentiation of ascomycetes and their application to *Verticillium* spp. *Applied Environmetal Microbiology* **60**: 4324–4331.

Llimona, X. & Hladun, N. L. (2001) Checklist of the lichens and lichenicolous fungi of the Iberian Peninsula and Balearic Islands. *Bocconea* **14**: 1–581.

Lohtander, K., Myllys, L., Sundin, R., Källersjö, M. & Tehler, A. (1998) The species pair concept in the lichen *Dendrographa leucophaea* (*Arthoniales*): analyses based on ITS sequences. *Bryologist* **101**: 404–411.

Lumbsch, H. T., Kothe, H. W. & Elix, J. A. (1988) Resurrection of the lichen genus *Pleurosticta* Petrak (*Parmeliaceae*: *Ascomycotina*). *Mycotaxon* **33**: 447–455.

Lumbsch, H. T., Schmitt, I., Palice, Z., Wiklund, E., Ekman, S. & Wedin, M. (2004) Supraordinal phylogenetic relationships of the *Lecanoromycetes* based on a Bayesian analyses of combined nuclear and mitochondrial sequences. *Molecular Phylogenetics and Evolution* **31**: 822–832.

Mattsson, J.-E., Articus, K., Wiklund, E. & Wedin, M. (2004) The monophyletic groups within the *Parmeliaceae*. In *Phylogenetic Studies in Usnea* (*Parmeliaceae*) *and allied genera* (K. Articus): *sine pagin*. [Comprehensive Summaries of Uppsala Dissertations from the Faculty of Science and Technology No. 931.] Acta Universitatis Upsaliensis, Uppsala.

Myllys, L., Lohtander, K., Källersjö, M. & Tehler, A. (1999) Sequence insertions and ITS data provide congruent information on *Roccella canariensis* and *R. tuberculata* (*Arthoniales*, *Euascomycetes*) phylogeny. *Molecular Phylogenetics and Evolution* **12**: 295–309.

Nimis, P. L. (1993) *The Lichens of Italy*. Museo Regionale di Scienze Naturali, Torino.

Nimis, P. L. (1998) A critical appraisal of modern generic concepts in lichenology. *Lichenologist* **30**: 427–438.

Page, R. D. M. (1996) Treeview: an application to display phylogenetic trees on personal computers. *Computer Applications in the Bioscience* **12**: 357–358.

Poelt, J. & Vězda, A. (1981) Bestimmungsschlüssel europäischer Flechten. Ergänzungsheft II. *Bibliotheca Lichenologica* **16**: 1–390.

Posada, D. & Crandall, K. A. (1998) Modeltest: testing the model of DNA substitution. *Bioinformatics* **14**: 817–818.

Purvis, O. W., Coppins, B. J., Hawksworth, D. L., James, P. W. & Moore, D. M. (1992) *The Lichen Flora of Great Britain and Ireland*. Natural History Museum Publications, London.

Rannala, B. & Yang, Z. (1996) Probability distribution of molecular evolutionary trees: a new method of phylogenetic inference. *Journal of Molecular and Evolution* **43**: 304–311.

Rodríguez, F., Oliver, J. F., Martín, A. & Medina, J. R. (1990) The general stochastic model of nucleotide substitution. *Journal Theoretical Biology* **142**: 485–501.

Santesson, R. (1984) *The Lichens of Sweden and Norway*. Swedish Museum of Natural History, Stockholm.

Schmitt, I. & Lumbsch, H. T. (2004) Molecular phylogeny of the *Pertusariaceae* supports secondary chemistry as an important systematic character set in lichen-forming ascomycetes. *Molecular Phylogenetics and Evolution*: in press.

Swofford, D. L. (2003) *PAUP*: Phylogenetic analysis using parsimony* (**and other methods*). Sinauer Associates, Sunderland, MA.

Thell, A. (1995) A new position of the *Cetraria commixta* group in *Melanelia* (*Ascomycotina*, *Parmeliaceae*). *Nova Hedwigia* **60**: 407–422.

Thell, A. (1999) Group I intron versus ITS sequences in phylogeny of cetrarioid lichens. *Lichenologist* **31**: 441–449.

Thell, A. & Miao, V. (1999) Phylogenetic analysis of ITS and group I intron sequences from European and North American samples of cetrarioid lichens. *Annales Botanici Fennici* **35**: 275–286.

Thell, A., Stenroos, S., Feuerer, T., Kärnefelt, I., Myllys, L. & Hyvönen, J. (2002) Phylogeny of cetrarioid lichens (*Parmeliaceae*) inferred from ITS and β-tubulin sequences, morphology, anatomy and secondary chemistry. *Mycological Progress* **1**: 335–354.

Vobis, G. (1980) Bau und Entwicklung der Flechten-Pycnidien und ihrer Conidien. *Bibliotheca Lichenologica* **14**: 1–141.

White, T. J., Bruns, T. D., Lee, S. & Taylor, J. (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In *PCR Protocols* (M. A. Innis, D. H. Gelfand, J. J. Sninsky & T. J. White, eds): 315–322. Academic Press, San Diego.

Zhou, S. & Stanosz, G. R. (2001) Primers for amplification of mtSSU rDNA, and a phylogenetic study of *Botryosphaeria* and associated anamorphic fungi. *Mycological Research* **105**: 1033–1044.

Zoller, S., Scheidegger, C. & Sperisen, C. (1999) PCR primers for the amplification of mitochondrial small subunit ribosomal DNA of lichen-forming ascomycetes. *Lichenologist* **31**: 511–516.

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