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Molecular phylogenetic study at the generic boundary between the lichen-forming fungi *Caloplaca* and *Xanthoria* (*Ascomycota*, *Teloschistaceae*)

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A molecular phylogenetic analysis of rDNA was performed for seven *Caloplaca*, seven *Xanthoria*, one *Fulgensia* and five outgroup species. Phylogenetic hypotheses are constructed based on nuclear small and large subunit rDNA, separately and in combination. Three strongly supported major monophyletic groups were revealed within the *Teloschistaceae*. One group represents the *Xanthoria fallax*-group. The second group includes three subgroups: (1) *X. parietina* and *X. elegans*; (2) basal placodioid *Caloplaca* species followed by speciations leading to *X. polycarpa* and *X. candelaria*; and (3) a mixture of placodioid and endolithic *Caloplaca* species. The third main monophyletic group represents a heterogeneous assemblage of *Caloplaca* and *Fulgensia* species with a drastically different metabolite content. We report here that the two genera *Caloplaca* and *Xanthoria*, as well as the subgenus *Gasparrinia*, are all polyphyletic. The taxonomic significance of thallus morphology in *Teloschistaceae* and the current delimitation of the genus *Xanthoria* is discussed in light of these results.

INTRODUCTION

The *Teloschistaceae* is a well-delimited family of lichenized fungi. Apart from a number of genera with one or two species, it consists of three small genera, *Teloschistes*, *Xanthoria* and *Fulgensia*, and one large genus, *Caloplaca* with more than 1000 published species names. Over time lichenologists have tried to subdivide the large genus *Caloplaca* into smaller homogenous taxonomic units, which have resulted in the proposal of a large number of genera or subgeneric entities (Kärnefelt 1989). These putative taxonomic circumscriptions have generally been based on only one character, such as ascospore septation, thallus morphology, or pigments. A lack of congruence with other characters and the lack of clear demarcations between the genera hampered these efforts. Lacking a better alternative, the current classification is very similar to that established more than a century ago. There seems, however, to be a consensus among taxonomists working on the *Teloschistaceae* that the delimitations of the classic neighbouring genera *Teloschistes*, *Xanthoria*, *Caloplaca* and *Fulgensia* are highly artificial and in need of revision (e.g. Kasalicky *et al.* 2000). In particular, the doubtful distinction between *Xanthoria* and *Caloplaca* subgenus *Gasparrinia* has been emphasized repeatedly (Poelt & Hafellner 1980, Kärnefelt 1989).

Taxonomy of Teloschistaceae *and its genera*

Hawksworth & Eriksson (1986) assigned the *Teloschistaceae* to the new order *Teloschistales*, and also proposed the inclusion of the *Fuscideaceae*. The *Teloschistales* was re-included in the *Lecanorales* and ranked at the suborder level by Rambold and co-workers (Rambold & Triebel 1992, Rambold, Schuhwerk & Triebel 1992, Hafellner *et al.* 1994). Tehler (1996) included the *Teloschistaceae*, *Letrouitiaceae* and *Fuscideaceae* in *Lecanorales* suborder *Teloschistineae*. The family *Teloschistaceae* has traditionally been characterized by having polarilocular ascospores and/or anthraquinones as secondary compounds. Honegger (1978) demonstrated the presence of a special ascus type, the *Teloschistes*-type, with an amyloid outer layer without visible apical structures, and with an irregular dehiscence. This ascus type was used by Bellemère, Hafellner & Letrouit-Galinou (1986) as a diagnostic character for the family *Teloschistaceae*.

Kärnefelt (1989) reviewed the older systems of classification of genera belonging to *Teloschistaceae* (incl. *Caloplacaceae*, *Teloschistaceae*, *Placodiaceae* and *Blasteniaceae*) and tentatively rejected a number of genera formerly recognized as members of the *Teloschistaceae*: *Blastenia*, *Follmannia*, *Gasparrinia*, *Huea*, *Kuttlingeria*, *Leproplaca*, *Mawsonia*, *Niorma*,

Polycauliona, *Pyrenodesmia* and *Xanthocarpia*. He accepted ten genera in the *Teloschistaceae*: *Apatoplaca*, *Caloplaca*, *Cephalophysis*, *Fulgensia*, *Ioplaca*, *Seirophora*, *Teloschistes*, *Xanthodactylon*, *Xanthopeltis*, and *Xanthoria*. *Apatoplaca* was later included in *Caloplaca* (Wetmore 1994) and *Seirophora* has proven to be a misinterpretation based on a mixture of a *Teloschistes* and a *Ramalina* (P. Frödén, pers. comm.). The genera *Josefpoeltia* and *Xanthomendoza* were later added to this list of accepted genera (Kondratyuk & Kärnefelt 1997).

Taxonomic status of Xanthoria *and* Caloplaca

The taxonomic circumscription of *Xanthoria*, which presently includes about 30 species, has remained rather stable since the genus was described by Fries (1860). It is distinguished from *Caloplaca* only by the presence of a lower cortex, which is more or less separated from the substratum. However, this diagnostic character is not always clear, and some species have been difficult to assign with certainty to one of the two genera. This applies to, for example, *Xanthoria elegans* and *Caloplaca lobulata*. Kondratyuk & Kärnefelt (1997) described two small genera, *Josefpoeltia* and *Xanthomendoza* based on morphological and anatomical features, and recently a number of *Xanthoria* species have been combined into *Xanthomendoza* (Søchting, Kärnefelt & Kondratyuk 2002).

Caloplaca is extremely heterogeneous in terms of thallus morphology, and ascoma anatomy. Ascospores are generally polarilocular, but in some species the septum is reduced to a central thickening thus presenting almost simple ascospores. Numerous anthraquinone syndromes are present in the genus, sometimes together with other lichen metabolites (Santesson 1970, Søchting 1997, 2001). The numerous genera proposed to accommodate parts of the genus *Caloplaca* are thoroughly discussed by Kärnefelt (1989). They are mostly based on single characters and are usually not accepted in recent publications and checklists.

In recent years several attempts have been made to divide *Caloplaca* into subgenera or sections based on secondary chemistry, ascospore morphology, cortical structure and thallus morphology. Wade (1965) listed four sections for *Caloplaca* species found in the British Isles: sects. *Caloplaca*, *Triophthalmidium*, *Gasparrinia* and *Leproplaca*. Clauzade & Roux (1985) listed the western European species under six subgenera, some of which were further subdivided (Table 1). Hansen, Poelt & Søchting (1987) tentatively subdivided the species from Greenland into the following species groups: *Sinapispermae*, *Citrinae*, *Nivales*, *Chalybaeae*, *Cerinae*, *Ferrugineae*, *Saxicolae*, *Trachyphyllae*, *Pauliae* and *Pyraceae*. Poelt & Hinteregger (1993) suggested 21 groups to accommodate about 60 species of the 104 Himalayan *Caloplaca*, but were not able to group about 40 species. They remarked that further studies would significantly change their grouping.

Table 1. Subgenera and their respective taxonomic subdivisions within the genus *Caloplaca* according to Clauzade & Roux (1985).

Genus	Subgenus	Group
Caloplaca	*Pyrenodesmia*	–
	Leproplaca	–
	Gasparrinia	*C. carphinea*
		C. aurea
		C. aurantia
		C. saxicola
		C. persica
	Xanthocarpia	–
	Gyalolechia	–
	Caloplaca	*C. citrina*
		C. cerina
		C. ferruginea

DNA sequences provide a new source of characters for constructing phylogenetic hypotheses within this family and allow an independent evaluation of characters that have been used so far for discriminating genera and subgeneric taxa. Arup & Grube (1999) published a phylogenetic analysis for 17 *Caloplaca* species and three *Xanthoria* species based on the ITS region to determine the taxonomic position of *Lecanora demissa*. Their study showed that *L. demissa* belongs in *Caloplaca s. str.*, and suggested that *Caloplaca* and *Xanthoria* are not monophyletic as currently circumscribed. In the same article, Arup & Grube recognized two large monophyletic groups of species, one with *Xanthoria* species mixed with lobate and crustose members of *Caloplaca*, and one with mainly crustose *Caloplaca*, including both species with orange and black apothecia.

Our study consists of a phylogenetic assessment of the genera *Xanthoria* and *Caloplaca*, particularly the species with lobate thalli (subgen. *Gasparrinia*), with the aim of evaluating thallus morphology as a diagnostic character at the generic level, and re-evaluating the taxonomic boundary between the two genera. To reach this goal, partial sequences from the nuclear small and large subunits of ribosomal RNA genes (SSU and LSU rDNA) were obtained for representative species of *Caloplaca*, *Fulgensia* and *Xanthoria*.

MATERIALS AND METHODS

Selected species

The species selected for the phylogenetic analyses are listed in Table 2 together with voucher information and GenBank accession nos for nuclear SSU and LSU rDNA sequences. Our taxon sampling consists of seven *Caloplaca* species (of which five belong to subgen. *Gasparrinia*), seven *Xanthoria* species, one *Fulgensia* species, and five outgroup species outside the *Teloschistaceae*. The choice of outgroup species was based on phylogenetic studies of the *Ascomycota* by Lutzoni, Pagel & Reeb (2001) and Bhattacharya *et al.* (2000), as

Table 2. Voucher/DNA source information, and GenBank accession numbers for small and large subunit nuclear ribosomal DNA sequences (SSU and LSU rDNA) used in this study. Sequences generated in this study are printed in bold.

Taxon	Locality, date, collector and herbarium/reference for sequences taken from GenBank	SSU GenBank accession no.	LSU GenBank accession no.
Teloschistaceae			
Caloplaca decipiens	**Denmark, 1 April 1995, *U. Søchting* (C)**	**AJ535280**	**AJ535267**
C. gomerana	Lutzoni *et al.* (2001)	AF356684	AF356685
C. holocarpa	**Denmark, *U. Søchting 7472* (C)**	**AJ535281**	**AJ535268**
C. saxicola	**USA: Alaska, *U. Søchting 7451* (C)**	**AJ535282**	**AJ535269**
C. scopularis	**Iceland, *U. Søchting 7521* (C)**	**AJ535283**	**AJ535270**
C. verruculifera	**Denmark, *Søchting 7157* (C)**	**AJ535284**	**AJ535271**
C. xanthostigmoidea	**USA: Alaska, *U. Søchting 7389* (C)**	**AJ535285 (5′ end)** **AJ535286 (3′ end)**	**AJ535272**
Fulgensia bracteata	**Sweden, *U. Søchting 7463* (C)**	**AJ535287 (5′ end)** **AJ535288 (3′ end)**	–
F. bracteata	**Austria, *F. Lutzoni 96.8.30-19* (C)**	–	**AJ535273**
Xanthoria andina	**Argentina, 19 Nov. 1992, *N. Scutari* (C)**	**AJ535289 (5′ end)** **AJ535290 (Internal fragment)** **AJ535291 (3′ end)**	**AJ535266**
X. borealis	**Russia: Taymyr Peninsula, *M. Zhurbenko 94411* (UPS)**	**AJ535292 (5′ end)** **AJ535293 (3′ end)**	**AJ535274**
X. candelaria	**USA: Alaska, 15 Aug. 1996, *U. Søchting* (C)**	**AJ353294**	**AJ535275**
X. elegans	**Denmark, 3 May 1995, *E. Fredtoft* (C)**	**AJ353295 (5′ end)** **AJ353296 (3′ end)**	**AJ535276**
X. parietina	Lutzoni *et al.* (2001)	AF356686	AF356687
X. poeltii	**Denmark, *U. Søchting 7473* (C)**	**AJ535297**	**AJ535277**
X. polycarpa	**Denmark, 3 May 1995, *E. Fredtoft* (C)**	**AJ535298**	**AJ535278**
Outgroup			
Heterodermia hypoleuca	**USA: North Carolina, *U. Søchting 7207*, det. R. Moberg (C)**	**AJ535299 (5′ end)** **AJ535300 (3′ end)**	**AJ535279**
Lobaria quercizans	Bhattacharya *et al.* (2000)	AF279396	AF279397
Rhizocarpon disporum	Lutzoni *et al.* (2001)	AF356677	AF356678
Sphaerophorus globosus	Gargas & Taylor (1995), Lutzoni *et al.* (2001)	L37532	AF356680
Stereocaulon paschale	Bhattacharya *et al.* (2000)	AF279412	AF279413

well as on the similarity of their LSU sequences with LSU sequences of the ingroup.

DNA isolation, amplification, sequencing and sequence alignment

Total DNA was isolated using the CTAB procedure of Armaleo & Clerc (1995). PCR-reactions were performed using the primer pair NS17 and NS24 or NSSU131 and NS24 for ≈1.0 kb fragment at the 5′ end of the nuclear SSU rRNA and the primer pair LR0R and LR7 for a 1.4 kb fragment at the 5′ end of the nuclear LSU rRNA (Table 3). The amplification was performed using an initial denaturation at 94 °C for 5 min, and subsequently a 40 cycle reaction with 94 ° for 1.3 min, 48 ° for 1.5 min, 72 ° for 2 min, and terminated with a 4 ° soak. The PCR products were purified with QIAquick PCR purification Kit (Qiagen) and eluted with sterile water.

Fragments were sequenced using Big Dye Terminator reaction kit (ABI PRISM, Perkin-Elmer). Primers used for cycle sequencing are listed in Table 3. Cycle sequencing was executed with the following programme: 25 cycles of 95 ° for 30 s, 48 ° for 15 s, 60 ° for 4 min. Sequenced products were precipitated with 10 µl of deionized sterile water, 2 µl of 3 M NaOAc, and 50 µl of 95% EtOH before they were loaded on an ABI 300 or ABI 377 (Perkin-Elmer, Applied Biosystems) automated sequencer.

Sequence fragments were edited and assembled using Sequencher software version 3.1.1 (GeneCodes, Ann Arbor). Each sequence fragment was subjected to BLAST searches to verify their identity. All sequences were aligned with Sequencher and subsequently adjusted manually. All DNA sequences have been deposited in GenBank (Table 2), and sequence alignments are available upon request from U.S.

Phylogenetic analyses

Alignments of the nuclear SSU and LSU rDNA were inspected for the presence of ambiguously aligned regions caused by the insertion of gaps. These regions were unequivocally coded to form a new set of characters replacing these regions in the phylogenetic analyses. Each of these characters, resulting from the coding of ambiguous regions, were subjected to a specific step matrix taking into account the optimal number of steps to transform one ambiguous sequence into another. Coding and the elaboration of symmetric step matrices for each of these coded regions were generated using the program INAASE 0.2c1 (Lutzoni *et al.* 2000). Unambiguous portions of the alignments were subjected to symmetric step matrices taking into consideration

Table 3. Primers used for symmetric PCR amplifications and cycle sequencing reactions.

Targeted region	Primer name	Sequence	Position and orientation[a]	Reference
SSU rDNA	NS17	5′-CATGTCTAAGTTTAAGCAA-3′	54–72	Gargas & Taylor (1992)
	NSSU131	5′-CAGTTATCGTTTATTTGATAGTACC-3′	107–131	Kauff & Lutzoni (2002)
	CNS26	5′-TCGAAAGTTGATAGGGCAG-3′	323–305	Gargas, DePriest & Taylor (1995)
	SR11R	5′-GGAGCCTGAGAAACGGCTAC-3′	389–408	Spatafora *et al*. (1995)
	NS2	5′-GGCTGCTGGCACCAGACTTGC-3′	573–553	White *et al*. (1990)
	SR7R	5′-TTAAAAAGCTCGTAGTTGAAC-3′	617–637	R. Vilgalys lab web site[b]
	NS21	5′-GAATAATAGAATAGGACG-3′	802–819	Gargas & Taylor (1992)
	NS20	5′-CGTCCCTATTAATCATTACG-3′	871–852	R. Vilgalys Lab web site[b]
	NS23	5′-GACTCAACACGGGGAAACTC-3′	1184–1203	Gargas & Taylor (1992)
	MB2	5′-GAGTTTCCCCGTGTTGAGTC-3′	1203–1184	Gargas *et al*. (1995)
	NS22	5′-AATTAAGCAGACAAATCACT-3′	1312–1297	Gargas & Taylor (1992)
	SR15	5′-CATCTAAGGGCATCACAG-3′	1445–1428	DePriest (1992)
	NS24	5′-AAACCTTGTTACGACTTTTA-3′	1769–1750	Gargas & Taylor (1992)
LSU rDNA	LR0R	5′-GTACCCGCTGAACTTAAG-3′	24–42	Rehner & Samuels (1994)
	TELSU9R	5′-CGAGTCGAGTTGTTTGGGAATGCAGC-3′	279–299	This study
	F377	5′-AGATGAAAAGAACTTTGAAAAGAGAA-3′	375–400	S. Rehner's lab
	P2R	5′-CTCTCTTTTCAAAGTTCTTTTCATCT-3′	400–375	Rehner & Samuels (1994)
	LR21	5′-ACTTCAAGCGTTTCCCTTT-3′	442–424	R. Vilgalys Lab web site[b]
	TELSU7R	5′-CAGCATCGGTTCGGGCGG-3′	508–526	This study
	TELSU7	5′-CCGCCCGAACCGATGCTG-3′	526–508	This study
	TELSU6R	5′-AGGATGCTGGCGTAATGG-3′	633–650	This study
	TELSU6	5′-CCATTACGCCAGCATCCT-3′	650–633	This study
	LR3R	5′-GTCTTGAAACACGGACC-3′	664–680	R. Vilgalys Lab web site[b]
	LR3	5′-GGTCCGTGTTTCAAGAC-3′	677–661	Vilgalys & Hester (1990)
	TELSU1R	5′-CGAGTGTTTGGGTGTCAA-3′	700–718	This study
	LR16	5′-TTCCACCCAAACACTCG-3′	716–700	Moncalvo *et al*. (1993)
	TELSU1	5′-TTGACACCCAAACACTCG-3′	718–700	This study
	TELSU5	5′-CTGGCTTCACCCTATTCAGGC-3′	874–854	This study
	TELSU4R	5′-CGTGCAAATCGATCGTCAAATT-3′	909–930	This study
	TELSU4	5′-AATTTGACGATCGATTTGCACG-3′	930–909	This study
	TELSU3R	5′-GTTTCCCTCAGGATAGC-3′	985–1001	This study
	LR5	5′-ATCCTGAGGGAAACTTC-3′	997–981	Vilgalys & Hester (1990)
	TELSU3	5′-GCTATCCTGAGGGAAAC-3′	1001–985	This study
	LR17R	5′-TAACCTATTCTCAAACTT-3′	1066–1083	R. Vilgalys Lab web site[b]
	TELSU2	5′-CGTTCGGTTCATCCCGCATCGCC-3′	1192–1170	This study
	TELSU12	5′-GGTAGCACGCTTGAGCGCC-3′	1359–1341	This study
	LR7	5′-TACTACCACCAAGATCT-3′	1483–1467	Vilgalys & Hester (1990)

[a] Position of the primers relative to *Saccharomyces cerevisiae* (SSU rDNA, Mankin *et al*. 1986) and *Schizosaccharomyces pombe* (LSU rDNA, Lapeyre *et al*. 1993). See also F. Lutzoni's lab web site: http://www.morag.com/lutzoni/primer.shtml.
[b] Department of Biology, Duke University; http://www.biology.duke.edu/fungi/mycolab.primers.htm.

the frequency of each class of possible changes as described in Fernández, Lutzoni & Huhndorf (1999). All analyses were conducted using PAUP* 4.0b4a on a Macintosh platform (Swofford 1998), and without constant sites.

The SSU and LSU rDNA data sets were analysed separately using maximum parsimony as the optimization criterion. The heuristic search for the best tree was performed with 1000 random addition sequence (RAS), tree-bisection-reconnection (TBR) swapping, MULTREES in effect, branches collapsed if maximum branch length is zero, and using gaps as a fifth character state for the unambiguously aligned part of the alignments. Bootstrap support (Felsenstein 1985) for these two partitions, when analysed separately, was estimated with 1000 replicates and 1 RAS per bootstrap replicate. Otherwise, the settings for heuristic search on each bootstrap data set was identical to heuristic searches described above on original data sets.

Conflicts among partitions were first detected by inspecting bootstrap scores above 70 % (Mason-Gamer & Kellogg 1996). If two bootstrap analyses, derived from two different partitions (A and B), provided support $\geqslant 70\,\%$ for two different phylogenetic relationships, this was interpreted as a potential incongruence between the two partitions. If no sign of incongruence was detected with this approach, no further tests were used to confirm this result and the two partitions were combined.

The combined SSU and LSU rDNA data sets were analysed using maximum parsimony (MP) and likelihood (ML) as optimization criteria. Three different searches were performed: (1) maximum parsimony search where every changes were equally weighted; (2) maximum parsimony search where changes were unequally weighted using step matrices as described above; and (3) maximum likelihood search. A hierarchical maximum likelihood ratio test with the

most-parsimonious tree derived from the unequally weighted combined analysis was used to select an evolutionary model and estimate all parameters needed for the ML search following a bottom-up approach (Huelsenbeck & Crandall 1997). The heuristic searches using MP were performed with 1000 RASs, tree-bisection-reconnection (TBR) swapping, MULTREES in effect, branches collapsed if maximum branch length is zero, and using gaps as a fifth character state for the unambiguously aligned part of the alignments. The same settings were used for the ML search except that gaps were not used as a fifth character state, INAASE characters were not included, and the search was restricted to 250 RAS. Bootstrap support (Felsenstein 1985) for the combined data set was estimated with 1000 replicates and 1 RAS per bootstrap replicate for equally weighted MP, 1000 replicates and 3 RAS per bootstrap replicate for unequally weighted MP, and 200 replicates and 1 RAS for ML.

RESULTS

The final length of the SSU rDNA alignment for 20 taxa was 1651 sites, of which 1456 were excluded because they were constant or were sites at both ends of the alignment with too many missing nucleotides. Of the 195 included sites, 82 were parsimony-informative. No regions of this alignment were recoded using INAASE (Lutzoni *et al*. 2000). The unequally weighted MP search using the SSU data set alone revealed 18 equally most parsimonious trees of 495.47 steps (Fig. 1a). These trees were found in one island that was hit for each of the 1000 RAS.

The final length of the LSU rDNA alignment for 20 taxa was 1378 characters, of which 1134 were excluded because they were constant, ambiguously aligned due to the presence of gaps, or contained too many missing nucleotides for sites at the ends of the alignment. Six ambiguously aligned regions were recoded and subjected to step matrices using INAASE, adding six for a total of 250 characters included in the unequally weighted MP search restricted to the LSU rDNA. Of these characters, 112 were parsimony-informative. The unequally weighted MP search using the LSU data set alone revealed a single most parsimonious tree of 817.5 steps (Fig. 1b). This tree was hit 912 times out of 1000 RAS. The two separate searches revealed the same two monophyletic groups (B and C) with high bootstrap support within the *Teloschistaceae* (Fig. 1a, b). An additional monophyletic group (A) with high bootstrap support (89 %) was found by the weighted MP search restricted to the LSU rDNA data.

No conflict was detected between the SSU and LSU partitions using the 70 % bootstrap criterion (see Materials and Methods). Most of the differences were due to the lower resolving power of the SSU compared to the LSU data. When the SSU and LSU data sets were combined, the equally weighted parsimony search found three equally most parsimonious trees of 748 steps. The three trees were found in one island that was hit for each of the 1000 RAS. The unequally weighted parsimony search of the combined data set revealed a single most parsimonious tree of 1331.12 steps, which was hit 334 times out of 1000 RAS. For the ML search using the combined data set, nucleotide frequencies were assumed to be unequal, all sites were assumed to evolve at the same rate, and a six parameter general-time-reversible model was implemented. One most likely tree was found ($-\ln = 3337.83407$) and was hit 250 times out of 250 RAS. The relationships within the *Teloschistaceae* were identical for these three analyses of the combined data set, except for the *Caloplaca holocarpa–C. saxicola–C. decipiens* monophyletic group that was unresolved (trichotomy) in the unweighted MP. For this reason we present here only the tree from the ML analysis (Fig. 1c).

The same three main lineages (A–C) found in the separate analyses were found when the data sets were combined. The bootstrap values for internodes supporting these groups increased or remained high when the data sets were combined. The number of internodes with bootstrap support $\geqslant 70$ % went from five when the SSU was analysed separately, to nine when LSU was analysed separately, to ten when the two data sets were combined (Fig. 1).

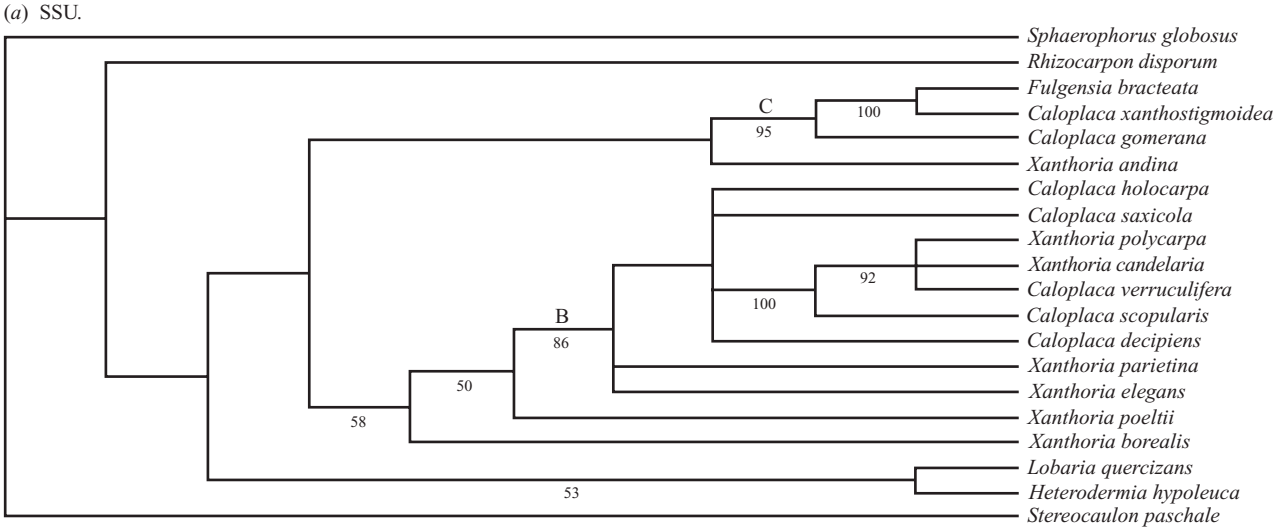
DISCUSSION

The most striking feature of the tree resulting from the phylogenetic analysis of the combined data set is the lack of monophyly for the genera *Xanthoria* and *Caloplaca* as currently circumscribed. The analyses of the combined data set (Fig. 1c) strongly support the existence of: (1) a minor lineage with two representative species belonging to the *Xanthoria fallax*-group (group A); (2) a major lineage where *Xanthoria* species are closely associated with species of *Caloplaca* subg. *Gasparrinia* (group B); and (3) another major lineage where two very different *Caloplaca* species are associated with a species of *Fulgensia* (group C).

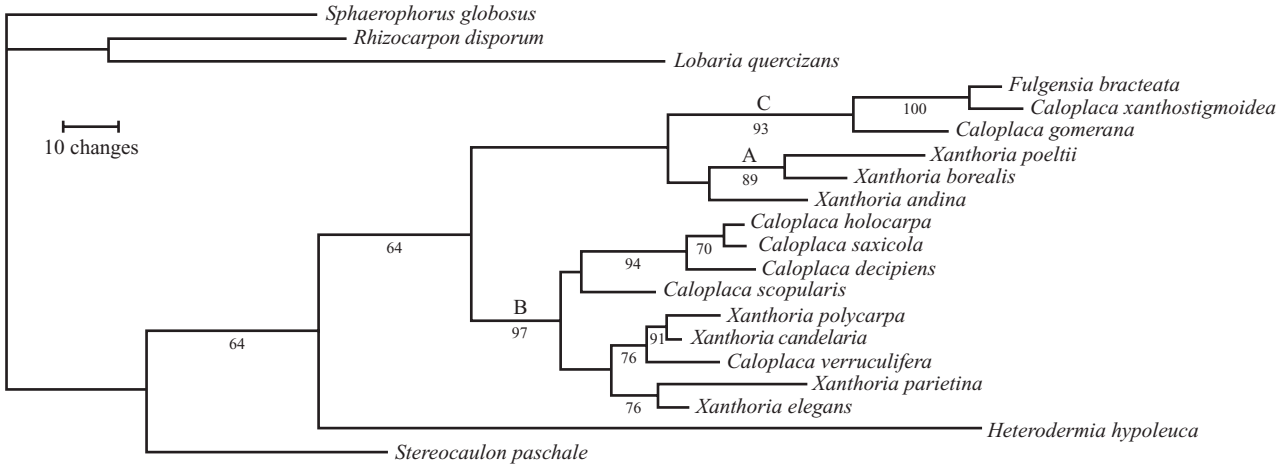
Lineage A contains the two foliose species *X. poeltii* (Fig. 2l) and *X. borealis* (Fig. 2k) that belong to a group of species within *Xanthoria* known as the *X. fallax*- or *X. ulophyllodes* group. This group, which was established by Poelt & Petutschnig (1992a, b) and later combined into *Xanthomendoza* by Søchting, Kärnefelt & Kondratyuk (2002), has true rhizines, a somewhat different structure of the thallus cortex, and narrow, oblong or bacilliform conidia (Lindblom 1997). Both species have chemosyndrome A, which is unlike most of the species in the group, which have A_3. Lindblom (1997) also reported this syndrome for *X. borealis*.

Lineage B includes several species belonging to *Xanthoria* and a number of *Caloplaca* species with more or less well-developed crustose thalli. Where a well-developed thallus exists, the upper cortex is

(*a*) SSU.



(*b*) LSU.



(*c*) SSU and LSU combined.

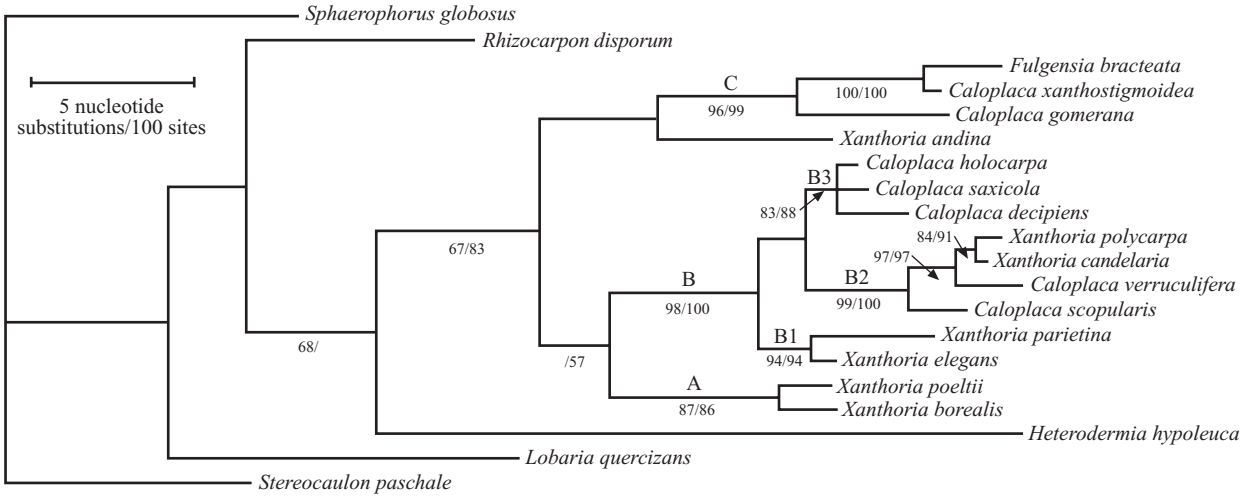


Fig. 1. Phylogenetic trees representing relationships within the *Teloschistaceae* with an emphasis on species at the generic boundary between *Caloplaca* and *Xanthoria*. Numbers below internodes are bootstrap values $\geqslant 50\,\%$. (*a*) Nuclear SSU rDNA, strict consensus of 18 most parsimonious trees; (*b*) Nuclear LSU rDNA, single most parsimonious tree; and (*c*) SSU and LSU combined, single most likely tree. Numbers before and after back slashes are bootstrap values using maximum likelihood and weighted maximum parsimony, respectively.

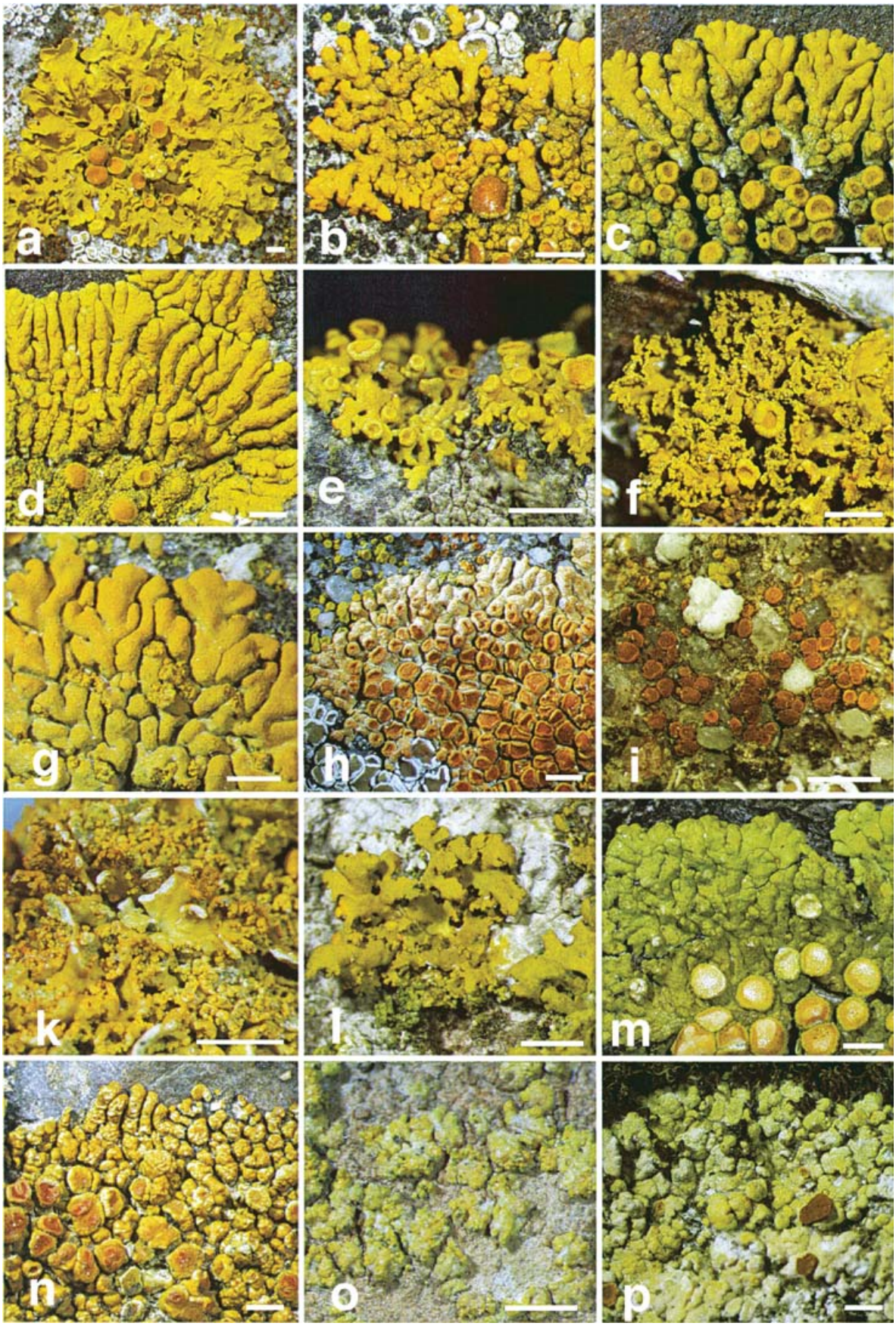


Fig. 2. (*a*) *Xanthoria parietina* (1998, US 9595b); (*b*) *X. elegans* (1995, US 7324); (*c*) *Caloplaca scopularis* (1997, US 7521); (*d*) *C. verruculifera* (1997, US 7522); (*e*) *X. polycarpa* (1996, US 7470); (*f*) *X. candelaria* (1997, US 7488); (*g*) *C. decipiens* (1995, US 7323); (*h*) *C. saxicola* (1998, US 9595c); (*i*) *C. holocarpa* (1998, US 9595); (*k*) *X. borealis* (1991, US 6321); (*l*) *X. poeltii* (1998, US 9226); (*m*) *X. andina* (1992, BAFC 37283, 37285); (*n*) *C. gomerana* (2000, US 9653); (*o*) *C. xanthostigmoidea* (1984, US 5126); and (*p*) *Fulgensia bracteata* (1996, US 7463). All specimens are deposited in C. Bars = 1 mm.

Table 4. Secondary metabolites analysed by HPLC for voucher specimens listed in Table 3. Relative composition calculated as percentage of total significant absorption peaks at 270 nm according to Søchting (1997, 2001). The chemosyndromes are named according to Søchting (*loc. cit.*) if published. Thallus samples are analysed in all species except for *Caloplaca holocarpa*, where apothecia were analysed. Dashes indicate that the compound was not found.

Taxon	Taxonomic group	Chemo-syndrome	parietin	teloschistin	fallacinal	parietinic acid	emodin	fragilin	7-clemodin	caloploicin	Other
Xanthoria borealis	A	A	94	1	2	2	1	–	–	–	–
X. poeltii	A	A	93	1	3	1	1	–	–	–	–
X. elegans	B1	A	93	1	2	1	2	–	–	–	–
X. parietina	B1	A	95	1	2	1	1	–	–	–	–
Caloplaca scopularis	B2	A	91	4	3	1	1	–	–	–	–
C. verruculifera	B2	A	96	–	2	1	1	–	–	–	–
X. candelaria	B2	A	94	1	2	1	1	–	–	–	–
X. polycarpa	B2	A	93	2	3	1	1	–	–	–	–
C. holocarpa	B3	A	94	1	3	1	1	–	–	–	–
C. saxicola	B3	A	91	1	3	2	1	–	–	–	–
C. decipiens	B3	A	97	1	1	1	–	–	–	–	–
C. gomerana	C		–	–	–	–	–	11	–	69	20
C. xanthostigmoidea	C	B_2	4	–	–	–	4	76	13	–	–
Fulgensia bracteata	C		75	1	2	1	1	–	–	–	20
X. andina		B_5	–	–	–	–	–	100	–	–	–

paraplectenchymatous or with irregular anticlinal hyphae (Arup 1995). The conidia in this clade are more or less ellipsoid. All species have parietin as the dominant anthraquinone together with smaller proportions of fallacinal, teloschistin, parietinic acid and emodin (Table 4; chemosyndrome A of Søchting 1997). This strongly supported clade is characterized by a signature sequence in the large subunit (Table 5).

Lineage B1 includes *X. elegans* and *X. parietina*, the latter being the type species of the genus. These species are all characterized by foliose thalli with paraplectenchymatous upper cortex (Fig. 2a–b), which are more or less closely attached to the substrate by their lower surface or by hapters (Kondratyuk & Poelt 1997). According to the analysis of ITS sequences by Arup & Grube (1999), *X. calcicola* is also part of our B1 group. Lineage B2 includes at its base two maritime, crustose, lobate species – *C. scopularis* and *C. verruculifera* (Fig. 2c–d). *X. polycarpa* and *X. candelaria* form a group sister to *C. verruculifera*. *X. polycarpa* has very short lobes and is invariably highly fertile (Fig. 2e). Its sister species *X. candelaria* is sorediate on the more or less upright lobes (Fig. 2f). Due to the presence of soredia, Poelt & Petutschnig (1992a) classified the latter species within the *Xanthoria fallax*-group (lineage A, Fig. 1c). However, *X. candelaria* differs from those species by its ellipsoid spermatia and the lack of rhizinae. Therefore, our molecular data support the taxonomic importance of conidium morphology. According to results derived from a more extensive SSU rDNA data, set we found that the subfruticose *C. coralloides* is a sister taxon to *X. polycarpa* (results not shown). *C. coralloides* initially produces elongated thallus lobes that are appressed to the substratum, but eventually develops a genuine subfruticose thallus. *C. coralloides*, *C. verruculifera* and *C. scopularis* all tend to have an irregular arrangement of the cortical hyphae and are able to produce pseudocyphellae on the upper cortex (Arup 1995). Our molecular phylogeny supports the suggestion made by Arup (*op. cit.*) that *C. coralloides* is related to a taxonomic group, that includes *C. alcarum*, *C. scopularis* and *C. verruculifera*.

The three *Caloplaca* species included in B3 are all saxicolous and often grow together on calcareous substrata, thus sharing similar ecological requirements. *C. decipiens* and *C. saxicola* both have effigurate marginal lobes and are accordingly typical members of the subgenus *Gasparrinia* (Fig. 2g, h). The position of *C. holocarpa* on this clade may be surprising because the thallus is almost inexistent, but its position here can be explained by a secondary reduction of the thallus into thin areoles without any trace of lobation (Fig. 2i). Wade (1965) used the name *C. holocarpa* in a broad sense to cover specimens growing on bark, wood and rock. Subsequently, the saxicolous specimens of this species complex have often been named *C. lithophila* (Hansen *et al.* 1987), and Clauzade & Roux (1985) used the name *C. tenuatula* for saxicolous specimens that form an inconspicuous orange or dark thallus between the apothecia on calcareous rocks. Claude Roux (pers. comm.) identified the voucher specimen of *C. holocarpa* included in this study as *C. tenuatula* subsp. *inconnexa*. The phylogenetic analysis of ITS sequences by Arup & Grube (1999) suggests that *C. lithophila*, *C. marina*, *C. maritima*, *C. pyracea*, *C. ignea*, *C. saxicola*, *C. arnoldii*, and *C. biatorina* are all part of group B3 (Fig. 1c).

The placodioid species in lineage B forms two separate groups: the *C. scopularis*-group (B2) and the *C. saxicola*-group (B3). In the *C. saxicola*-group there are several examples of thallus reduction (*C. holocarpa*, *C. lithophila* and *C. pyracea*) and it is likely that a large number of *Caloplaca* species with uneffigurate thalli with chemosyndrome A belong to this group. In the

Table 5. Part of the LSU alignment showing the signature sequence GACCAA for lineage B (see Fig. 1). Base numbering according to Lapeyre *et al*. (1993).

Taxon	LSU sequence. Bases 689–694 in italics	Lineage
Xanthoria elegans	AAGGAGTC***GACCAA***CTATGCGAGTGTTT	B
X. parietina	AAGGAGTC***GACCAA***CCGTGCGAGTGTTT	B
Caloplaca holocarpa	AAGGAGTC***GACCAA***CTATGCGAGTGTTT	B
C. saxicola	AAGGAGTC***GACCAA***CTATGCGAGTGTTT	B
C. decipiens	AAGGAGTC***GACCAA***CTATGCGAGTGTTT	B
X. polycarpa	AAGGAGTC***GACCAA***CTATGCGAGTGTTT	B
X. candelaria	AAGGAGTC***GACCAA***CTATGCGAGTGTTT	B
C. scopularis	AAGGAGTC***GACCAA***CTATGCGAGTGTTT	B
C. verruculifera	AAGGAGTC***GACCAA***CTATGCGAGTGTTT	B
X. poeltii	AAGGAGTC*TAACAT*CTATGCGAGTGTTT	A
X. borealis	AAGGAGTC*TAACAT*CTATGCGAGTGTTT	A
C. gomerana	AAGGAGTC*TAACAT*CTATGCGAGTGTTT	C
C. xanthostigmoidea	AAGGA:TC*TAACAT*CTATGCGAGTGTTT	C
Fulgensia bracteata	AAGGAGTC*TAACAT*CTATGCGAGTGTTT	C
X. andina	AAGGAGTC*TAACAT*CTATGCGAGTGTTT	
Stereocaulon paschale	AAGGAGTC*TAACAT*CTATGCGAGTGTTT	
Rhizocarpon disporum	AAGGAGTC*TAACAT*CTATGCGAGTGTTT	
Sphaerophorus globosus	AAGGAGTC*TAACAT*CTATGCGAGTGTTT	
Lobaria quercizans	AAGGAGTC*TAACAT*CTATGCGAGTGTTT	
Heterodermia hypoleuca	AAGGAGTC*GACCCT*GCGTGCGAGTGTTT	

C. scopularis-group, thallus reductions may also have occurred, but it is likely that placodioid thalli have also evolved into foliose thalli (*X. candelaria*, *X. polycarpa*), and in one case even into a subfruticose thallus (*C. coralloides*).

Lineage C includes three species belonging to *Caloplaca* and *Fulgensia*. *C. gomerana* and *F. bracteata* have crustose, more or less effigurate thalli giving them a superficial similarity to the *Gasparrinia*-group. However, they differ in ascospore characters and(or) secondary metabolite composition. *C. gomerana* (as *C. gloriae*) (Fig. 2n) was assumed by Llimona & Werner (1975) to be closely related to *C. saxicola* (*as C. murorum*), and Clauzade & Roux (1985) also assigned it to the *C. saxicola* group in subgen. *Gasparrinia* due to the effigurate lobation of its crustose thallus. However, our data show that *C. gomerana* belongs to a different lineage within the *Teloschistaceae*. Anatomically it also differs considerably from the *C. saxicola* group in having a prosoplectenchymatous upper cortex (Hansen *et al*. 1987) and by containing fragilin and caloploicin (Table 4), two compounds that also occur in *Fulgensia* (Søchting, unpubl.). The presence of fragilin is shared with *C. xanthostigmoidea* and *X. andina*, but it has not been found in the chemically homogenous lineages A and B.

C. xanthostigmoidea, studied by Søchting & Tønsberg (1997), has a crustose, yellowish thallus and resembles a *Fulgensia* except for the ascospores being polarilocular (Fig. 2o). *F. bracteata*, the sister species to *C. xanthostigmoidea*, has a crustose thallus consisting of areoles that are only rarely slightly radiate (Fig. 2p). *Fulgensia s. lat*. is characterized by simple or regular one-septate ascospores. Westberg & Kärnefelt (1998) concluded that *Fulgensia* is probably polyphyletic and the distinct lineages are probably related to different groups within the large genus *Caloplaca*. Based on a phylogenetic analysis of the ITS region and LSU rDNA data, Kasalicky *et al*. (2000) confirmed the polyphyletic nature of *Fulgensia* proposed by Westberg & Kärnefelt (1998) based on similarities and differences of anatomical and morphological features. Because the taxon sampling outside *Fulgensia* was minimal (only two *Caloplaca* and one *Xanthoria* species) in the Kasalicky *et al*. paper the affiliations of the three independent groups their study revealed remains uncertain. *F. bracteata* belongs to *Fulgensia s. str*., including *F. fulgens* and *F. fulgida*. Lineage C represents a highly heterogeneous assemblage of species belonging to *Fulgensia* and *Caloplaca*. Other genera within the *Teloschistaceae* might be part of this major lineage. Further studies within this lineage are likely to show relationships that will require changes to the current classification at the genus level.

The position of *X. andina* is not well supported in any of the phylogenetic analyses we conducted. This is a South American species with a thallus intermediate between foliose and crustose (Fig. 2m). It has a hardly recognizable lower cortex that is loosely attached to the substratum. Its secondary compound profile strongly suggests that it is part of lineage C, which is concordant but weakly supported by our SSU and LSU data when combined.

Most *Caloplaca* species included in this study have been assigned to subgen. *Gasparrinia*, a taxonomic entity defined by Clauzade & Roux (1985) and Poelt (1969) as *Caloplaca*'s with elongated marginal lobes and anthraquinones as metabolites. The difficulty of separating this subgenus from *Xanthoria* was discussed by Poelt (1983) and Kärnefelt (1989); later, Wetmore & Kärnefelt (1998) stated that the group of species covered by the above definition is extremely heterogeneous and cannot be recognized at any taxonomic level. It is also not clearly distinguished from the

remaining species of *Caloplaca*. The molecular data underline that the degree of attachment to the substratum and the degree of lobation of the thallus are versatile characters poorly suited for taxonomic distinction at the generic level within this family. Furthermore, species with similar placodioid thallus morphology are represented in several different lineages. Based on a phylogenetic analysis of ITS sequences, Søchting & Arup (2002) demonstrated that at least three more species, *C. aurantia*, *C. flavescens* and *C. thallincola*, formerly assigned to subgen. *Gasparrinia*, must be placed outside lineage B. These three species were placed in a distinct group within subgen. *Gasparrinia* (Table 1) by Clauzade & Roux (1985) due to their citriform ascospores, but Kärnefelt (1989) did not find strong support for a formal distinction of this group within or outside *Caloplaca*. The conidia are bacilliform (Søchting & Arup 2002) and the cortical structure of the group is diverse, but *C. thallincola* in particular has a prosoplectenchymatous structure. The chemosyndrome in *C. aurantia* belongs to type A_3, whereas that in *C. thallincola* and *C. flavescens* belongs to the common type A.

Our study clearly shows that the subgenus *Gasparrinia* is polyphyletic. The presence of foliose species at the base of lineage B (group B1, Fig. 1c) suggests that at least some placodioid species within this family are derived from a foliose ancestor. Further thallus reduction seems to have taken place in the *C. saxicola*-group resulting in crustose forms with endolithic thalli. Thallus habit (such as placodioid and foliose forms) is not an appropriate diagnostic character at the generic and subgeneric levels within the *Teloschistaceae*.

The results of our phylogenetic study necessitate a taxonomic reassessment of the genus *Xanthoria*. *X. andina* has been transferred to *Caloplaca* by Scutari, Rosato & Søchting (2002). All *Caloplaca* species that are part of lineage B (Fig. 1c) need to be transferred to *Xanthoria*, unless one or two new genera were erected to accommodate groups B2 and B3. However, the latter solution cannot be justified based on the present morphological, anatomical and chemical data, nor does the phylogenetic relationships shown here require the need to recognize more genera within the monophyletic lineage B. Moreover, contrary to the use of subgeneric divisions within lineage B, the establishment of new generic entities within lineage B would obscure the close relationships between *Xanthoria s. str.* and *Caloplaca* subgen. *Gasparrinia s. str.*

The sister relationship of the *X. fallax*-group (lineage A) to lineage B reported here based on SSU and LSU rDNA is strongly supported (BS = 91 %) by the phylogenetic analysis of ITS sequences by Arup & Grube (1999). Therefore, in the spirit of maintaining taxonomic stability, the *X. fallax*-group could be kept within a redefined genus *Xanthoria*, with multiple subgenera to accommodate the *X. fallax*-group and various other monophyletic lineages such as lineages B1–B3 (Fig. 1c). However, because of the extensive genetic divergence between lineages A and B, it could also be argued that the *X. fallax*-group should be recognized as a separate genus or transferred to *Xanthomendoza* based on the close similarity in ITS sequences (Søchting, Kärnefelt & Kondratyuk 2002).

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