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PHYLOGENETIC STUDY OF *FULGENSIA* AND ALLIED *CALOPLACA* AND *XANTHORIA* SPECIES (TELOSCHISTACEAE, LICHEN-FORMING ASCOMYCOTA)[1]

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Fulgensia Massal. & De Not. is a widespread genus with considerable morphological and ecological heterogeneity across species. For this reason, the taxonomic delimitation of this genus has been controversial. Relationships among species of *Fulgensia*, *Caloplaca* Th. Fr., and *Xanthoria* (Fr.) Th. Fr. (*Lecanorales*) were investigated based on a comprehensive phylogenetic analysis of 62 DNA sequences from the nuclear ribosomal internal transcribed spacer (ITS) region using maximum parsimony (MP) and likelihood (ML). Ambiguously aligned (INAASE coded characters) and unambiguous regions were analyzed separately and combined when using MP as the optimization criterion. All our analyses confirm the polyphyly of this genus as three distinct lineages: *Fulgensia* sensu stricto, *F. australis*, and *F. schistidii*. We report here that *Caloplaca*, *Fulgensia*, and *Xanthoria* together form two main sister lineages. One lineage includes *Fulgensia schistidii* (part of the *C. saxicola* group), *Xanthoria*, and most of the lobed *Caloplaca* species belonging to the *Gasparrinia* group. A second main lineage comprises the remaining *Caloplaca* species, *Fulgensia* sensu stricto, and *F. australis*. Therefore, the traditional generic level classification schemes for the family Teloschistaceae appear to be highly artificial. All three genera were found to be nonmonophyletic. We demonstrate here that the ITS is appropriate to resolve relationships across the Teloschistaceae. However, a combination of an MP analysis, in which ambiguously aligned regions are accommodated using INAASE, with an ML analysis, in which phylogenetic confidence is estimated using a Bayesian approach, is needed.

Key words: ambiguous alignments; *Caloplaca*; *Fulgensia*; internal transcribed spacer; lichen-forming fungi; Markov chain Monte Carlo Bayesian inference; molecular phylogenetics; Teloschistaceae; *Xanthoria*.

The lichen-forming genus *Fulgensia* A. Massal. & De Not. includes terricolous, muscicolous, and saxicolous crustose species that are common and widespread on most continents. The species diversity and abundance is particularly important in the Mediterranean region. *Fulgensia* is classified within the family Teloschistaceae (Lecanorales Nannf.). Formerly, this family had been classified within the order Teloschistales, which was established as an independent order within the lichenized Ascomycota by Eriksson and Hawksworth (1986).

The genus was described by Massalongo and de Notaris (Massalongo, 1855) based on *Fulgensia fulgens* (Sw.) Elenkin, i.e., mainly comprising taxa with non-polarilocular, mostly simple ascospores, and with more or less pruinose and yellowish thalli containing anthraquinones. Zahlbruckner (1926, 1931) did not recognize *Fulgensia* as a distinct genus and subsumed it within *Caloplaca* Th. Fr. as a separate section. Dodge and Baker (1938), Räsänen (1943), and Rudolph (1955) revived the use of the name *Fulgensia* at the genus level.

Poelt (1965) redefined the previous delimitation of *Fulgensia* by including, in addition to taxa with simple ascospores, taxa with typical one-septate non-polarilocular ascospores. *Fulgensia* sensu Poelt contained 10 species distributed into two subgenera: *Fulgensia* subgen. *Fulgensia* Poelt and subgen. *Candelariopsis* (Sambo) Poelt. The former included six species: *F. delphinensis* Poelt, *F. desertorum* (Tomin) Poelt, *F. bracteata* (Hoffm.) Räsänen, *F. subbracteata* (Nyl.) Poelt, *F. fulgens* (Sw.) Elenkin, and *F. fulgida* (Nyl.) Szatala. This subgenus was characterized mainly by simple or one-septate ascospores, a thallus with a false upper cortical layer, and by their attachment to the substratum with a felt of hyphae (Poelt, 1965). The subgenus *Candelariopsis* included four taxa: *F. australis* (Arnold) Poelt, *F. chanousiae* (Sambo) Poelt, *F. schistidii* (Anzi) Poelt, and *F. pruinosa* (Körb.) Poelt. These species have one-septate ascospores, a thallus with a true upper cortical layer, and are attached to the substratum by hyphal strands or by their lower cortex (Poelt, 1965). Subsequently, three new *Fulgensia* species were described: *F. klementii* Kalb (Kalb, 1970), *F. poeltii* Llimona (Llimona, 1974), and *F. canariensis* Follmann (Follmann and Poelt, 1981).

Gilbert (1978) applied Poelt's concepts to *Fulgensia* populations found in the British Isles. He asserted the complexity of this genus by remarking that most of the species exhibit a large variability, implying the existence of several potential infraspecific taxa. Poelt's circumscription of *Fulgensia* has not always been followed. Some contemporary authors (Nowak and Tobolewski, 1975; Oksner, 1993) still recognize members of the *Candelariopsis* group as being *Caloplaca* species.

Apart from Poelt (1965), no further revision of the group had been made until the work of Westberg and Kärnefelt (1998), who recognized four groups within *Fulgensia*: group A (*F. australis* and *F. chanousiae*), group B (*F. canariensis*),

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group C (*F. schistidii*) and group D (*F. bracteata*, *F. delphinensis*, *F. desertorum*, *F. fulgens*, *F. fulgida*, *F. klementii*, *F. poeltii*, *F. pruinosa*, and *F. subbracteata*). In their study of *Fulgensia* and *Caloplaca*, Poelt (1965) and Kärnefelt (1989) came to the conclusion that species from the *Caloplaca aurea* group (not sampled here) were most similar to *Fulgensia* (*Candelariopsis*), the main differential feature being the type of ascospore septation. Based on the structure of the upper thallus cortex, Kärnefelt (1989) and Westberg and Kärnefelt (1998) proposed close affinities between species from the *C. aurea* group (e.g., *C. paulii* Poelt) and *F. canariensis* and *F. schistidii*. However, Navarro-Rosinés et al. (2000) suggested that *C. aurea* (Schaer.) Zahlbr. was more similar to subgenus *Fulgensia* than to subgenus *Candelariopsis*. Furthermore, the recently described *C. cancarixiticola* Nav.-Ros., Egea & Llimona shares several morphological features with *F. australis*, *F. canariensis*, and *F. schistidii* (Navarro-Rosinés et al., 2000).

In spite of all these morphological studies and the evident connections between *Fulgensia* and *Caloplaca*, only one molecular phylogenetic study of *Fulgensia* has been carried out (Kasalicky et al., 2000). Unfortunately, only two *Caloplaca* species (*C. flavorubescens* [Huds.] Laundon and *C. cerina* [Hedw.] Th. Fr.) and one *Xanthoria* (Fr.) Th. Fr. species (*X. parietina* [L.] Th. Fr.) were included to represent taxa outside *Fulgensia*. This sampling was sufficient to demonstrate the polyphyletic nature of *Fulgensia*, but was insufficient to reveal relationships of its separate lineages within the broad context of the Teloschistaceae.

During the molecular and morphological revision of the lobate *Caloplaca* (*Gasparrinia* sect.) by the first author, the need to clarify the delimitation between *Caloplaca* and *Fulgensia* became necessary. We report here the results of a phylogenetic study assessing relationships of *Fulgensia* species within the broader context of the Teloschistaceae. The conclusions presented here are based on sequences from the internal transcribed spacer (ITS) region for 58 specimens, representing 33 species (36 taxa) belonging to three genera (*Caloplaca*, *Fulgensia*, and *Xanthoria*) within the Teloschistaceae.

MATERIALS AND METHODS

Taxon sampling—A total of 24 specimens of *Fulgensia* (representing 10 taxa), 26 specimens of *Caloplaca* (representing 21 taxa) regarded as most closely related to *Fulgensia*, and eight specimens of *Xanthoria* (representing five species) were included in this study. Most of the sequences needed for this study were available in GenBank (Arup and Grube, 1999; Kasalicky et al., 2000; Lohtander et al., 2000; Martín and Winka, 2000; Dyer and Murtagh, 2001; Kroken and Taylor, 2001). Eight new ITS sequences were generated for this study. After comparing ITS sequences from a number of species belonging to different families with our alignment of ingroup sequences, four outgroup species were selected: *Protoparmelia badia* (Hoffm.) Haf. (Lecanoraceae), *Letharia vulpina* (L.) Hue, *L. columbiana* (Nutt.) J. W. Thomson, and *Usnea arizonica* Mot. (Parmeliaceae). See Supplemental Data accompanying the online version of this paper for voucher information and GenBank accession numbers for the 62 ITS sequences included in this study.

DNA isolation and sequencing—Genomic DNA was obtained from fresh samples and herbarium specimens (the oldest specimen was collected in 1995). DNA was isolated using the Puregene Kit (GENTRA Systems, Minneapolis, Minnesota, USA) following the manufacturer's protocol for filamentous fungi. DNA concentration was determined by visual comparison with positive control (λ 100 ladder, concentration 10, 20, 40 ng) on an ethidium-bromide-stained agarose gel. Symmetric polymerase chain reactions (PCR) were prepared for a 50.0 μL final volume containing 31.7 μL of sterile double-distilled water, 5.0 μL of 10× *Taq* polymerase reaction buffer (Boehringer-Mannheim, Indianapolis, Indiana, USA), 5.0 μL of 2.5 mmol/L dNTPs, 0.3 μL of *Taq* DNA polymerase (Boehringer-Mannheim), 2.5 μL for each of the 10 μmol/L primers ITS1F or ITS5 and ITS4 (Gardes and Bruns, 1993; White et al., 1990), 1.5 μL of 10 mg/mL bovine serum albumin (BSA; BioLabs, Beverly, Massachusetts, USA), 0.5 μL of 50 mmol/L $MgCl_2$, and 1 μL of template genomic DNA. The PCR was performed on Peltier Thermal Cyclers PTC-200 (MJ Research, Boston, Massachusetts, USA) under the following conditions: one cycle of 1 min at 95°C linked to 40 cycles of 1 min at 95°C, 45 s at 52°C, and 2 min at 72°C, with the last step increased by increments of 5 s for the last 15 cycles. A final extension step of 10 min at 72°C was added, after which the samples were kept at 4°C. The PCR products were purified using GELase Agarose Gel-Digesting Preparation (Epicentre Technologies, Madison, Wisconsin, USA) following the manufacturer's instructions or low-binding regenerated cellulose 30 000 nominal molecular weight limit (NMWL) filter units (Millipore, Bedford, Massachusetts, USA). Both strands of the purified PCR products were sequenced using PCR primers used for the symmetric amplification and primers 5.8S and 5.8SR (Vilgalys and Hester, 1990). Sequencing reactions were prepared in 10 μL final volume using BigDye Terminator (ABI PRISM, Perkin-Elmer Biosystems, Wellesley, Massachusetts, USA) and following the manufacturer's instructions. Sequenced products were precipitated with 10 μL of deionized sterile water, 2 μL of 3 mol/L sodium acetate, and 50 μL of 95% ethanol. Polyacrylamide gel electrophoresis was conducted using Long Ranger Singel packs (FMC BioProducts, Rockland, Maryland, USA) and an ABI 377 automated DNA sequencer (Perkin-Elmer, Applied Biosystems, Wellesley, Massachusetts, USA). Sequence fragments were subjected to BLAST searches to verify their identity and assembled using Sequencher version 3.0 (Gene Codes Corporation, Ann Arbor, Michigan, USA).

Sequence alignment—Sequences were aligned using Sequencher 3.0. The manual alignment was carefully inspected for the presence of ambiguously aligned regions caused by the insertion of gaps. The alignment was done separately in two blocks because of the difficulty of aligning two different groups of sequences. *Xanthoria fallax* (Hepp) Arnold was the only species alignable with both groups and was used to facilitate the final and global manual alignment. The delimitation of ambiguous regions, their unequivocal coding, and the elaboration of symmetric step matrices for each of these coded characters were generated using the program INAASE 2.3b (Lutzoni et al., 2000). The alignment is available in a Nexus format upon request to E.G. or F.L.

Phylogenetic analyses—All phylogenetic searches were performed with PAUP* 4.0b8 (Swofford, 2001) using maximum parsimony (MP) and maximum likelihood (ML) as optimization criteria. The same program was used for all bootstrap analyses. Constant sites and ambiguously aligned sites were removed from all analyses.

Maximum-parsimony (MP) analyses—Three different MP searches (MP1–MP3) were carried out.

A first MP analysis (MP1) was executed using exclusively unambiguously aligned sites. Changes among character states (including gaps as a fifth character state) were subjected to symmetric step matrices constructed as follows. The options Show character status/full details/hide excluded characters options from the Data menu in PAUP* were implemented. From the resulting table, the column States showing all nucleotide states found at each of the unambiguously aligned and nonconstant sites was saved as a separate text file. This file was used as an input file for the program STMatrix 2.1 (written by S. Zoller and available upon request from S.Z. or F.L.), which generates a step matrix (in Nexus format) by calculating frequencies of reciprocal changes from one state to another and converting them into costs of changes using the negative natural logarithm of the frequencies (Felsenstein, 1981; Wheeler, 1990).

The second MP search (MP2) was restricted to unequivocally coded (INAASE) characters replacing ambiguously aligned regions. Only coded characters with no more than 32 character states were subjected to the MP2

search. A total of eight coded characters out of 16 (corresponding to the 16 ambiguously aligned regions we delimited in this ITS alignment) fit this criterion, forming eight new (INAASE) characters. All pair-wise transformation costs were equal for one of these eight characters. The seven other coded characters (with consistent pair-wise sequence comparisons) were each subjected to a specific step matrix taking into account the optimal number of steps to transform one ambiguous sequence into another.

Before combining the unambiguously aligned sites with the eight coded (INAASE) characters for the MP3 analysis, global phylogenetic congruence was assessed for these two data partitions. This was done by inspecting internodes with bootstrap scores above 70% resulting from the separate MP1 and MP2 analyses (Mason-Gamer and Kellogg, 1996). Conflicts between the two data partitions would be considered significant only if the two bootstrap analyses provided support ≥70% for two different phylogenetic relationships for the same set of taxa (one being monophyletic and the other being nonmonophyletic).

All three MP searches were performed using heuristic searches with 1000 random-addition-sequences (RAS), tree bisection-reconnection (TBR) branch swapping, Multrees option in effect, and collapsing branches with maximum branch length equal to zero. The MP1 and MP2 searches were done in two steps. The goal of the first step was to find the length of the most parsimonious trees. This was done by saving only one tree for each RAS. The goal of the second step was to find all equally most parsimonious trees. This was done by saving all trees only when swapping on a tree equal or shorter than the shortest tree found in the first step. The MP3 was conducted in one step by saving all trees as soon as TBR swapping was initiated. Internode confidence was assessed by bootstrap analyses (Felsenstein, 1985) with full heuristic searches, 10 000 parsimony bootstrap replicates, using two RAS per bootstrap replicate and by saving no more than 10 trees per bootstrap replicate.

Maximum-likelihood (ML) analyses—Selection of the nucleotide substitution model and the estimation of most parameters were done with hierarchical likelihood ratio testing (HLRTs) using the program Modeltest 3.04 PPC (Posada and Crandall, 1998). The ML search was performed using the HKY substitution model (Hasegawa-Kishino-Yano, 1985) with base frequencies (A = 0.1539, C = 0.2704, G = 0.2263, T = 0.3494), ti/tv ratio = 3.2956, and unequal rates among sites. Rates for variable sites assumed to follow a gamma distribution with shape parameter = 1.9549 for an optimal number of rate categories equal to 3. The ML heuristic search was conducted on the same data set subjected to the MP1 search, with 1000 RAS, number of rearrangements limited to 20 000/RAS, TBR branch swapping, Multrees option in effect, and collapsing branches with maximum branch length equal to zero. The ML analysis was divided in three separate searches (300, 300, and 400 RAS, respectively).

We used a Bayesian Markov chain Monte Carlo (B/MCMC) method (Larget and Simon, 1999) to assess the level of confidence for relationships revealed by our ML search. The same model and number of rate categories as used in the ML search was used for the B/MCMC analyses. The tree sampling using this approach was done with MrBayes 1.11 (Huelsenbeck, 2000). The MCMC algorithm ran for 2 000 000 generations with four incrementally heated chains, starting from random trees and sampling one out of every 100th generation with DNA substitution parameters updated during the search. A majority-rule consensus tree was calculated with PAUP* from the last 15 000 out of the 20 000 trees sampled. The first 5000 trees (burnin) were excluded to avoid trees that might have been sampled prior to convergence of the Markov chains.

We repeated the B/MCMC tree sampling as described to confirm the result from the first Bayesian analysis. The final majority-rule consensus tree was obtained by pooling all trees selected from both runs (30 000 trees). The posterior probability of each topological bipartition was estimated by the frequency of these bipartitions across all 30 000 trees sampled. Internodes with posterior probabilities ≥95% were considered statistically significant.

RESULTS

The size of the ITS final data matrix for this study of the Teloschistaceae was 62 sequences by 674 sites. A total of 16 ambiguously aligned regions were delimited, resulting in the exclusion of 438 nucleotide sites. Constant sites (170) were excluded as well. Of the remaining 66 characters that were subjected to ML and B/MCMC analyses, 47 were parsimony-informative in MP1 analyses. The eight characters used in the MP2 analysis were derived from the eight ambiguous regions coded with INAASE. These eight characters were combined with the 66 characters of MP1 for a total of 74 nonconstant characters for MP3, 55 of which were parsimony-informative.

The unequally weighted MP1 search revealed 575 equally most parsimonious trees of 288.19 steps (Fig. 1). The unequally weighted MP2 search revealed 5712 equally most parsimonious trees in two islands. One island of 4032 trees was hit 101 times and the second island of 1680 trees was hit 49 times. The score of the best tree was 173.00 steps (Fig. 2).

The MP2 search, based on only the eight INAASE characters, was performed to determine the amount of phylogenetic signal that could be recovered from ambiguously aligned regions and to ensure that this signal was not incongruent with the unambiguously aligned portion of the data matrix. The phylogenetic signal recovered with INAASE provided a surprisingly high level of resolution and support for these eight characters. The phylogenetic signal retrieved from ambiguously aligned regions was most often complementary to the signal provided by nonambiguous sites. Of the 21 internodes with support ≥50% generated by the MP2 bootstrap analysis, 11 internodes had bootstrap values increased by an increment of at least 10% when compared to the MP1 bootstrap values, and six of these bootstrap values went from <70% to ≥70% (Fig. 2). Although topologies obtained from the separate analyses of unambiguous sites and INAASE characters were considerably different, no conflict was detected using the reciprocal 70% bootstrap criterion (see Materials and Methods), and, therefore, the two data sets were combined.

A total of 12 equally most parsimonious trees was found in one island that was hit 1000 times with the MP3 search based on combined unambiguously aligned sites and INAASE characters (Fig. 3). The score of the best tree was 473.70 steps. The inclusion of the eight ambiguously aligned regions as coded characters explains the increase in resolution from 575 to 12 equally most parsimonious trees and the faster computing time when the analysis was performed on the combined data set. The number of internodes with bootstrap support ≥70% went from 13, when the analysis was restricted to unambiguously aligned sites (MP1), to 25 when the eight INAASE characters were added to these unambiguous sites (Figs. 1 and 3).

Five equally most likely trees (−ln 874.90079), hit 183 times out of 1000 RAS, resulted from the ML search. The ML analysis generated a more resolved consensus tree with only five equally optimal trees compared to 12 for MP3. However, only 13 internodes have high support values (posterior probability [PP] ≥ 94%) with B/MCMC (Fig. 4) compared to 25 (bootstrap proportion [BP] ≥ 70%) for MP3 (Fig. 3). This extra level of support comes from the phylogenetic signal that was recovered with the eight INAASE characters from ambiguously aligned portions of the alignment (Fig. 2). When the MP bootstrap analysis was performed on the same data set that was used for the B/MCMC analysis, i.e., without the eight INAASE characters, internodes with high support were virtually the same (compare Fig. 1 with Fig. 4). The B/MCMC approach did provide significant support for two internodes that never received bootstrap support ≥70% (Fig. 4).

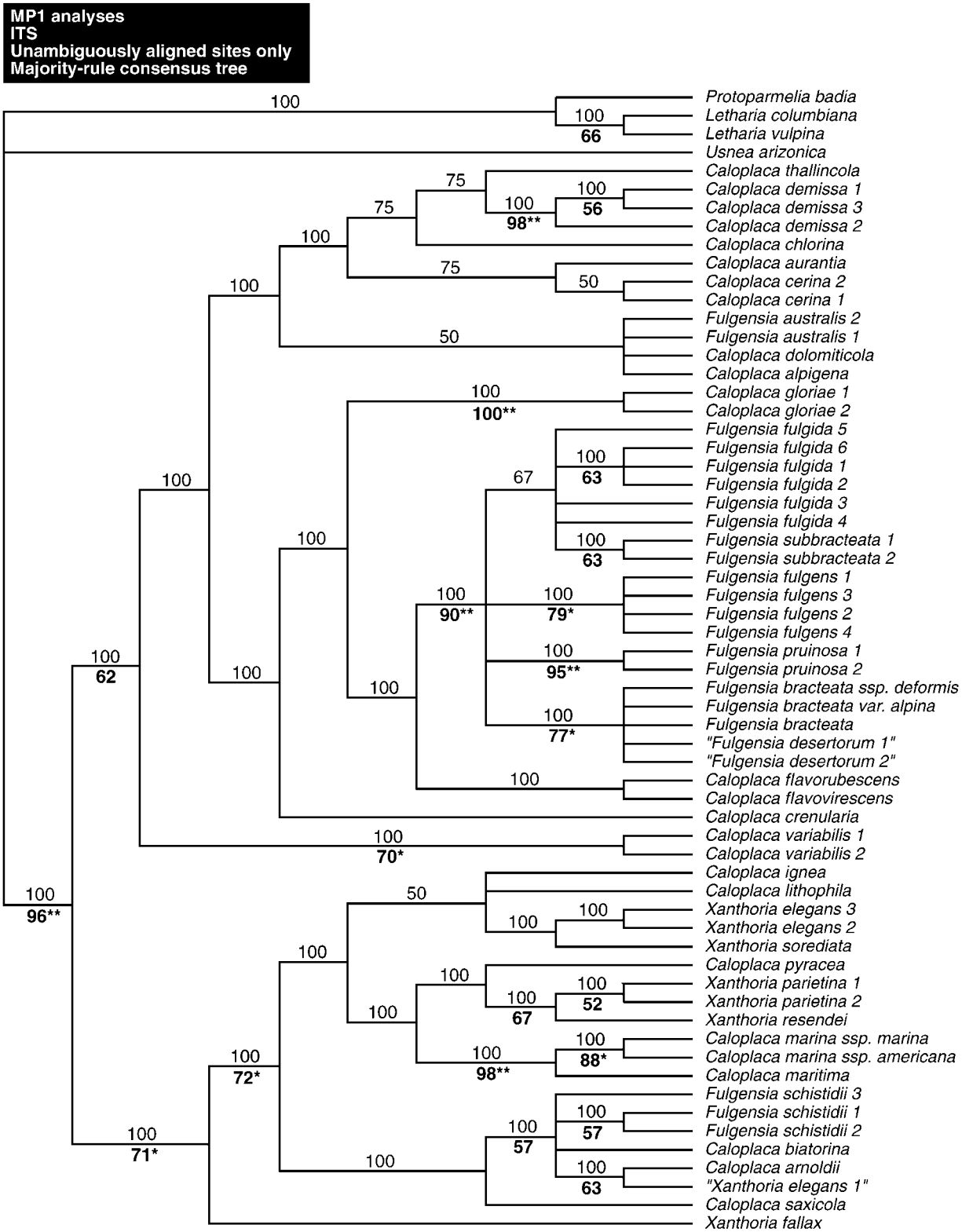

Fig. 1. Relationships among *Fulgensia* species and selected members of *Caloplaca* and *Xanthoria*, based on internal transcribed spacer (ITS) unambiguously aligned sites only. Majority-rule consensus tree of 575 equally most parsimonious trees generated by the maximum parsimony (MP1) analysis. Tree length = 288.19 steps. Numbers above each internode represent the percentage of these 575 trees that share these specific topological bipartitions. Numbers in boldface type below internodes are the percentages (≥50%) of 10 000 bootstrap replicates supporting specific topological bipartitions. Bootstrap values with one asterisk are ≥70% and bootstrap values with two asterisks are ≥90%.

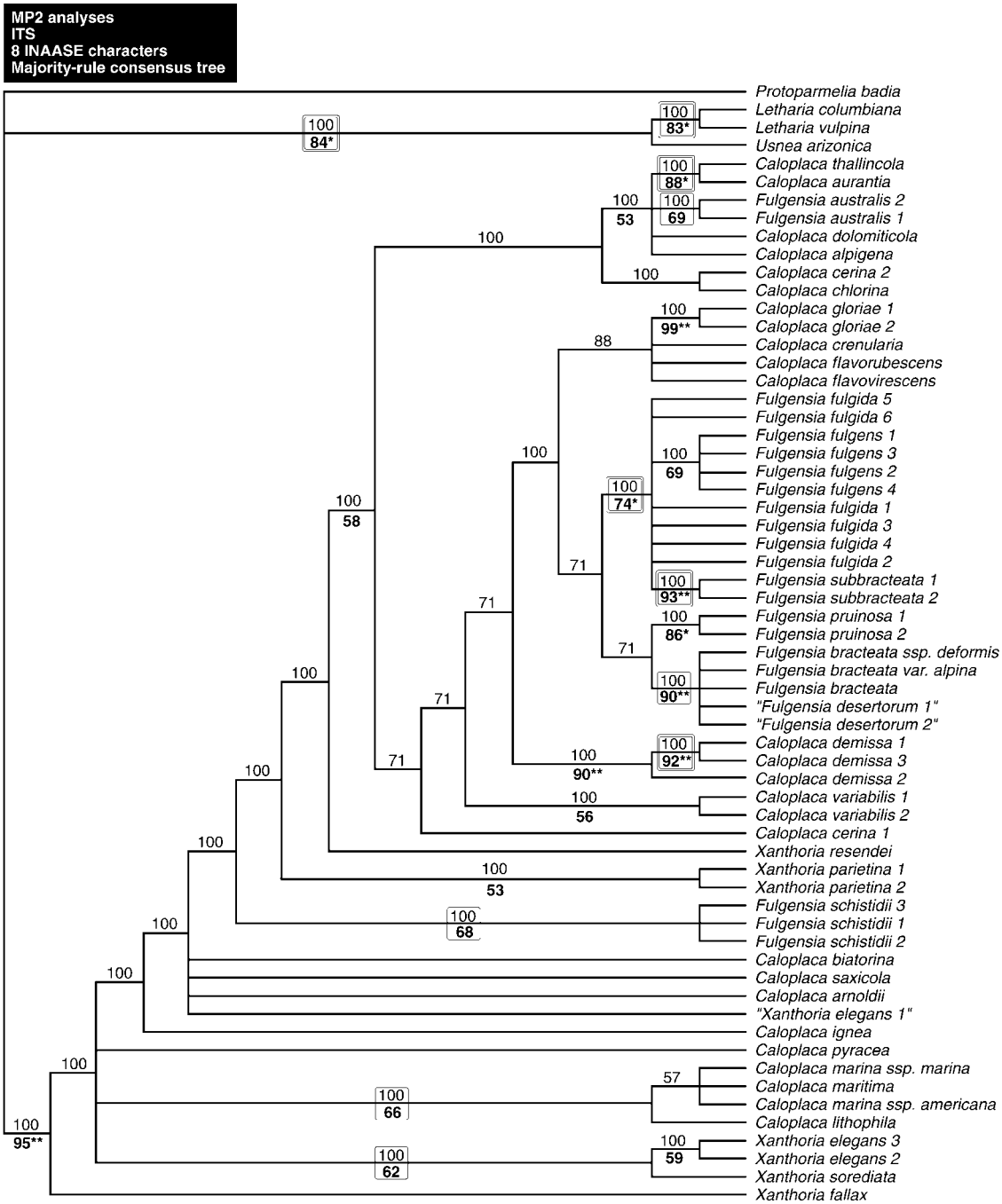

Fig. 2. Relationships among *Fulgensia* species and selected members of *Caloplaca* and *Xanthoria*, based only on eight INAASE characters derived from internal transcribed spacer (ITS) ambiguously aligned regions. Majority-rule consensus tree of 5712 equally most parsimonious trees generated by the maximum parsimony (MP2) analysis. Tree length = 173.00 steps. Numbers above each internode represent the percentage of these 5712 trees that share these specific topological bipartitions. Numbers in boldface type below internodes are the percentages (≥50%) of 10 000 bootstrap replicates supporting specific topological bipartitions. Bootstrap values with one asterisk are ≥70%, and bootstrap values with two asterisks are ≥90%. Boxes indicate 11 internodes with bootstrap values that increased by an increment of 10% or more compared to MP1 bootstrap values. Double boxes show six of these 11 internodes with bootstrap values ≥70% that were <70% in the MP1 analysis.

The outgroup rooted the ingroup always at the same internode, except for MP2 which was based strictly on the eight INAASE characters and lacked support. Even if our taxon sampling for the outgroup and ingroup was different from Arup and Grube (1999), our rooting of the ingroup is in total agreement with the result shown in their Fig. 4.

Phylogenetic relationships—Our results suggest that *Caloplaca*, *Xanthoria*, and *Fulgensia* are part of two main lineages within the Theloschistaceae: lineage 1 (PP = 99%, Fig. 4) and lineage 2 (BP = 89%, Fig. 3). Because *Xanthoria* and most lobate *Caloplaca* species are restricted to lineage 1, this lineage seems to include fewer species and to be more ho-

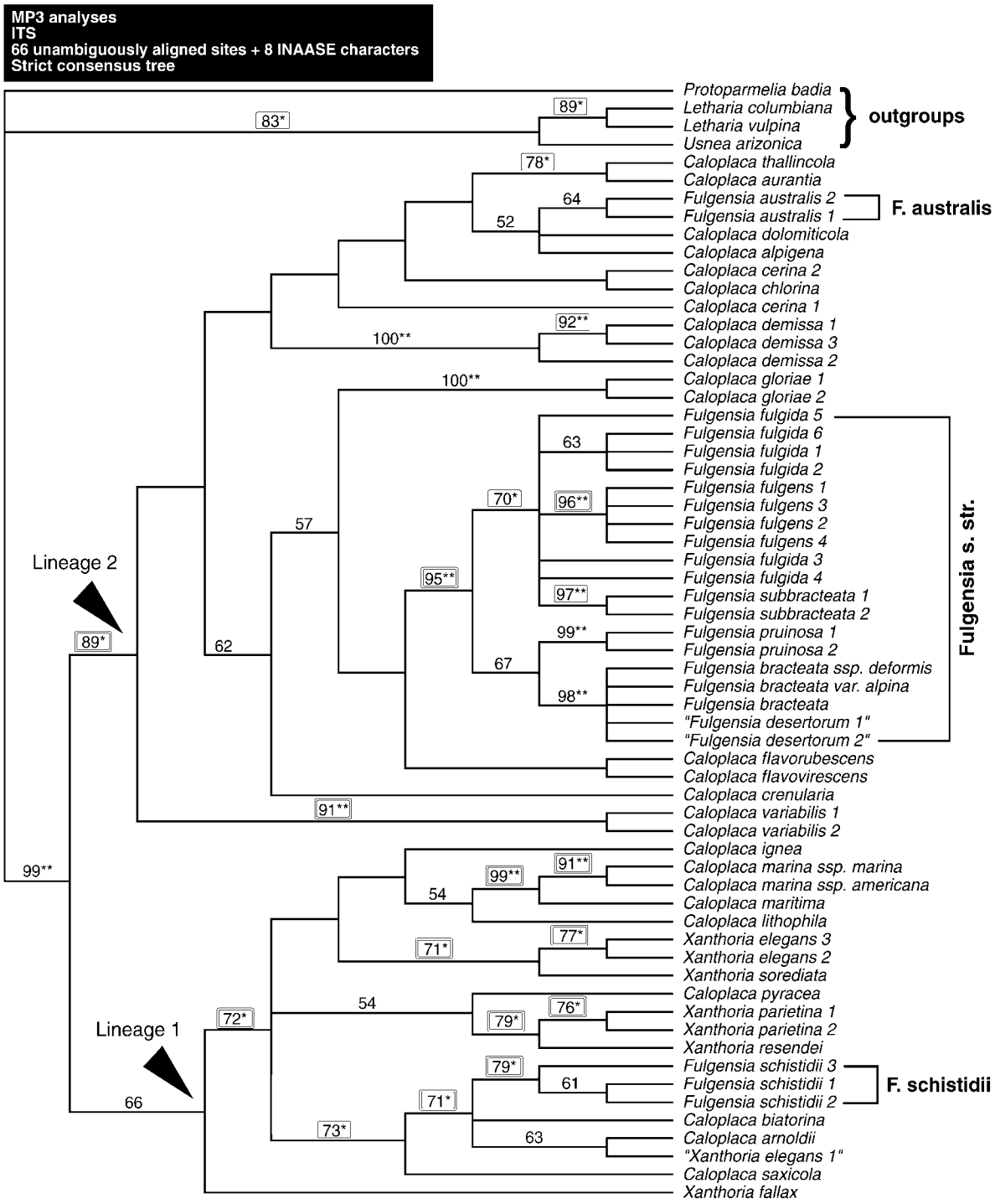

Fig. 3. Relationships among *Fulgensia* species and selected members of *Caloplaca* and *Xanthoria*, based on internal transcribed spacer (ITS) unambiguously aligned sites combined with eight INAASE characters. Strict consensus tree of 12 equally most parsimonious trees generated by the maximum parsimony (MP3) analysis. Tree length = 473.70 steps. Numbers above each internode represent the percentages (≥50%) of 10 000 bootstrap replicates supporting specific topological bipartitions. Bootstrap values with one asterisk are ≥70%, and bootstrap values with two asterisks are ≥90%. Boxes indicate all internodes with bootstrap support ≥70% that were <70% in the MP1 analysis, and double boxes show internodes with bootstrap support ≥70% that were <70% in the MP1 or MP2 analysis.

mogeneous than lineage 2. By including most members of *Fulgensia* and several representatives of at least five groups of *Caloplaca* (sensu Clauzade and Roux, 1985), lineage 2 is the most phenotypically diverse.

Fulgensia has three independent origins, confirming the polyphyly of this genus by Kasalicky et al. (2000). One of these origins gave rise to the largest group of *Fulgensia* species (BP = 95%, Fig. 3; PP = 98%, Fig. 4) and corresponds to the *Fulgensia* sensu stricto (s.s.) group proposed by Kasalicky et al. (2000). *Fulgensia* s.s. and *F. australis* are part of lineage

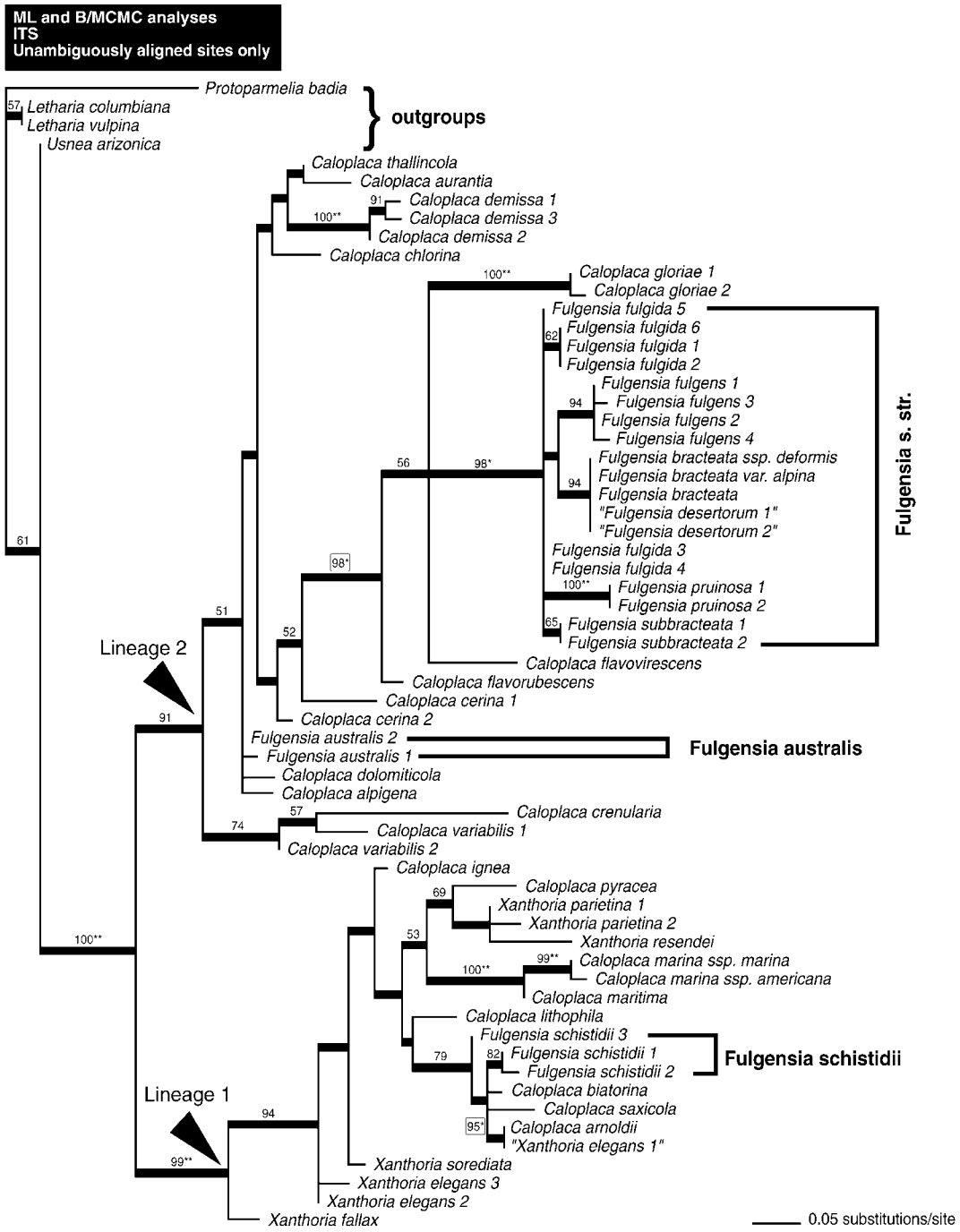

Fig. 4. Relationships among *Fulgensia* species and selected members of *Caloplaca* and *Xanthoria*, based on internal transcribed spacer (ITS) unambiguously aligned sites only. One of the five equally most likely trees generated by the maximum likelihood search (−ln likelihood = 874.90079). All internodes present in the strict consensus of these five trees are highlighted with thicker branches. Numbers above each internode represent posterior probabilities >50%. One asterisk indicates scores ≥95%, and two asterisks show scores ≥99%. Posterior probalities in boxes indicate two internodes with high support that never received bootstrap support ≥70% in MP1–MP3 analyses.

2 of the Teloschistaceae, whereas *F. schistidii* is part of lineage 1 of this family.

Our B/MCMC analysis shows that *Fulgensia* s.s. is nested within a subgroup of *Caloplaca* part of lineage 2. Exact relationships among the main species groups within lineage 2 remain uncertain with ITS. However, a closer (monophyletic) relationship of *Fulgensia* s.s. to *Caloplaca gloriae* Llimona & Werner, *C. flavovirescens* (Wulfen) DT. & Sarnth, and *C. flavorubescens* was found to be statistically significant (PP = 98%, Fig. 4).

The phylogenetic position of *Fulgensia australis* within lineage 2 is also uncertain. Both the MP and the ML analyses suggest a close relationship with *C. dolomiticola* (Hue) Zahlbr. and *C. alpigena* Poelt ined. (current name: *C. macrocarpa*

(Anzi) Zahlbr.), but virtually without any support (Figs. 3 and 4). *Caloplaca variabilis* (Pers.) Müll. Arg. was always at the base of lineage 2 in the combined analyses, but did not receive statistical support.

In general, the level of uncertainty is also high for relationships among main groups within lineage 1, except for the *Xanthoria fallax* group, which was constantly found to be part of the first divergence that took place within this lineage (PP = 94%, Fig. 4; BP = 72%, Fig. 3). This result is in agreement with the ITS tree from Arup and Grube (1999).

Fulgensia schistidii is most likely to form a monophyletic group with members of the *Caloplaca saxicola* group (BP = 73%, Fig. 3), rather than being nested within *Fulgensia* where it has been classified traditionally (Poelt, 1965) or being sister to *Xanthoria parietina* according to Kasalicky et al. (2000). In agreement with Arup and Grube (1999), *C. marina* (Wedd.) Zahlbr. and *C. maritima* B. de Lesd. are sister species with strong support. However, by adding *Fulgensia* species (Kasalicky et al., 2000) to the data set of Arup and Grube (1999), we lost the high support for the sister relationship of *C. lithophila* H. Magn. to the *C. marina* group.

Our study revealed a sister relationship between *X. sorediata* (Vain.) Poelt and *X. elegans* (Link) Th. Fr. (BP = 71%, Fig. 3), as well as between *X. parietina* and *X. resendei* Poelt & Tav. (BP = 79%, Fig. 3). However, one specimen of ''*X. elegans*'' (individual 1) was always found to be sister to *C. arnoldii* (BP = 63%, Fig. 3; PP = 95%, Fig. 4). This specimen of ''*X. elegans*'' with ''compact thalli'' was collected in eastern Antarctica and was part of a phylogenetic study by Dyer and Murtagh (2001). Based on their Figs. 2 and 4, this ''*Xanthoria elegans*'' with compact thalli is very likely a misidentified member of the *C. saxicola* group (GenBank AF278753). Even if we reassign ''*Xanthoria elegans* 1'' to *Caloplaca saxicola* sensu lato, our results clearly show that *Xanthoria* and *Caloplaca* do not form monophyletic groups (Figs. 3 and 4).

DISCUSSION

When comparing trees resulting from MP (with and without INAASE characters) and ML, we find few differences. The most obvious topological discrepancies were unsupported by BP or PP. We regard the analyses with MP3 (Fig. 3) and ML (Fig. 4) as being the most accurate reconstruction of the underlying phylogeny.

Adequacy of ITS in resolving relationships within the Teloschistaceae—One important finding to emerge from this study is that in spite of the small number (eight) of ambiguous regions that we were able to convert to characters with INAASE, these eight characters alone were able to generate a high level of resolution. However, the highest level of confidence with MP was obtained when these eight INAASE characters were added to the data matrix containing the nonambiguously aligned sites. Doing so, the number of internodes with bootstrap support ≥70% was more than doubled compared to the MP1 analysis.

It is clear that large ITS data sets within the Teloschistaceae will greatly benefit from methods like INAASE that are designed to recover phylogenetic signal from these ambiguously aligned regions. In this particular case, using B/MCMC, which seems to be a more statistically powerful method to assess the level of phylogenetic uncertainty (Alfaro et al., 2002; Kauff and Lutzoni, 2002), is not superior to using parsimony bootstrap with INAASE characters. This is likely to be true for alignments with high percentages of sites that are ambiguously aligned, until B/MCMC methods and ML searches can accommodate multiple models simultaneously, including models for characters with character states other than the usual four nucleotides (e.g., INAASE or morphological characters). It is possible that ITS alone could provide sufficient phylogenetic information to fully resolve relationships within the Teloschistaceae and provide high support values for most internodes, if a new method could capture phylogenetic signal from all ambiguously aligned regions instead of being restricted to eight of the 16 regions we delimited for this study.

Phylogenetic relationships of Fulgensia species within the Teloschistaceae—*Fulgensia pruinosa* had been included in subgenus *Candelariopsis* with *F. australis*, *F. chanousiae*, and *F. schistidii* by Poelt (1965). Westberg and Kärnefelt (1998), Kasalicky et al. (2000), and this study demonstrate that *F. pruinosa* belongs to the subgenus *Fulgensia*, corresponding to *Fulgensia* s.s. This group is characterized by a homogeneous cortical layer and abundant pruina.

As for Kasalicky et al. (2000), our analyses could not distinguish ''*F. desertorum*'' from *F. bracteata*. The high similarity of the ITS and LSU sequences suggest that more characters are needed to resolve this relationship. We believe that because of the overall phenotypic similarity of ''*F. desertorum*'' to *F. fulgens*, this odd result may also be explained by misidentification of the two specimens used by Kasalicky et al. to represent ''*F. desertorum*.'' Typical *F. desertorum* is xerophilous and of irano-turanian distribution (X. Llimona, personal communication), whereas specimens used by Kasalicky et al. (2000) have a very different ecology and were collected in Norway and northern Italy.

We confirm the conclusion by Westberg and Kärnefelt (1998) and Kasalicky et al. (2000) that *Fulgensia australis* does not belong to *Fulgensia* s.s. However, its phylogenetic placement is still unknown. This might be resolved by including more related taxa. For example, species from the *Caloplaca aurea* group share several morphological similarities with *F. australis*, i.e., lack of pruina, presence of septate spores, and saxicolous habit. Additional molecular characters might also be necessary to resolve the phylogenetic relationship of this problematic species.

Because of its placement in lineage 1, *F. schistidii* appears to be the most distantly related species to *Fulgensia* s.s. Westberg and Kärnefelt (1998) already pointed out the particularity of this species, creating a monospecific group for it. Our study revealed a tight relationship between *F. schistidii* and species of the *C. saxicola* group. The yellow-orange color, the septate spores, the compact cortex and medulla, as well as the presence of lobes (though sometimes highly reduced) corroborate in part this relationship.

Taxonomic conclusions—The type species of *Caloplaca* (*C. cerina*) is nested within lineage 2, together with *Fulgensia* s.s. and several morphologically diverse *Caloplaca* groups. Hence, lineage 2 forms a heterogenous group for which it is not yet possible to determine the taxonomic rank without a more extensive taxon sampling and more characters. However, we can conclude that this taxonomic unit contains both *Caloplaca* s.s. and *Fulgensia* s.s. We can also conclude that *Ful-*

gensia australis (Arnold) Poelt should revert to *Caloplaca australis* (Arnold) Zahlbr.

Lineage 1 is mostly formed by yellowish-orange lobed species that seem to be centered around subgenus *Gasparrinia* (Torn.) Th. Fr. (sensu Clauzade and Roux, 1985). Some of the most extreme forms in this lineage are represented by *Xanthoria* species, *Fulgensia schistidii*, and *Caloplaca pyracea* (Ach.) Th. Fr. Therefore, lineage 1 forms a fairly homogeneous group, especially when compared to lineage 2. In this context, the generic rank could be interpreted as being the most appropriate for lineage 1. Given that *Xanthoria parietina*, the type of *Xanthoria*, is part of this lineage, the name *Xanthoria* is available for lineage 1. This would mean that all *Caloplaca* species in this lineage and *Fulgensia schistidii* would have to be transferred to *Xanthoria*.

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