

Unravelling the phylogenetic relationships of lichenised fungi in Dothideomyceta

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Abstract: We present a revised phylogeny of lichenised Dothideomyceta (*Arthoniomycetes* and *Dothideomycetes*) based on a combined data set of nuclear large subunit (nuLSU) and mitochondrial small subunit (mtSSU) rDNA data. Dothideomyceta is supported as monophyletic with monophyletic classes *Arthoniomycetes* and *Dothideomycetes*; the latter, however, lacking support in this study. The phylogeny of lichenised *Arthoniomycetes* supports the current division into three families: *Chrysothrichaceae* (*Chrysothrix*), *Arthoniaceae* (*Arthonia* s. l., *Cryptothecia*, *Herpothallon*), and *Roccellaceae* (*Chiodecton*, *Combea*, *Dendrographa*, *Dichosporidium*, *Enterographa*, *Erythrodictyon*, *Lecanactis*, *Opegrapha*, *Roccella*, *Roccellographa*, *Schismatomma*, *Simonyella*). The widespread and common *Arthonia caesia* is strongly supported as a (non-pigmented) member of *Chrysothrix*. *Monoblastiaceae*, *Strigulaceae*, and *Trypetheliaceae* are recovered as unrelated, monophyletic clades within *Dothideomycetes*. Also, the genera *Arthopyrenia* (*Arthopyreniaceae*) and *Cystocoleus* and *Racodium* (*Capnodiales*) are confirmed as *Dothideomycetes* but unrelated to each other. *Mycocomrothelia* is shown to be unrelated to *Arthopyrenia* s. str., but is supported as a monophyletic clade sister to *Trypetheliaceae*, which is supported by hamathecium characters. The generic concept in several groups is in need of revision, as indicated by non-monophyly of genera, such as *Arthonia*, *Astrothelium*, *Cryptothecia*, *Cryptothelium*, *Enterographa*, *Opegrapha*, and *Trypethelium* in our analyses.

Key words: *Arthoniomycetes*, Ascolocularous fungi, bitunicate fungi, *Dothideomycetes*, lichens, phylogeny, ribosomal DNA.

INTRODUCTION

Mutualism is one of the three main modes of nutrition within *Ascomycota*, besides saprotrophism and parasitism. A large number of mutualistic ascomycetes form symbiotic relationships with algae and/or cyanobacteria, so-called lichens. Of the 64 000 species currently accepted in *Ascomycota* (Kirk *et al.* 2008), about almost 30 % (17 600) are lichen-forming fungi (Feuerer & Hawksworth 2007, Kirk *et al.* 2008). Lichenised fungi differ from all other fungi in the formation of complex, persistent vegetative thalli, which makes them a prime subject for evolutionary studies.

It was long believed that lichens evolved several times independently within *Ascomycota* (and *Basidiomycota*), an idea supported by the first molecular study testing this hypothesis (Gargas *et al.* 1995). Lutzoni *et al.* (2001, 2004) were unable to conclusively determine whether there were multiple gains of lichenisation or whether an initial lichenisation event occurred deep within *Ascomycota*, however, Lutzoni *et al.* (2001) found some *Eurotiomycetes* to be secondarily de-lichenised. This is particularly intriguing as *Eurotiomycetes* includes economically important fungi in the genera *Aspergillus* and *Penicillium* that feature a complex secondary chemistry similar to that found in lichens produced by homologous polyketide synthase genes (Grube & Blaha 2003, Kroken *et al.* 2003, Schmitt *et al.* 2005, Schmitt & Lumbsch 2009).

Since then, the phylogeny and classification of *Ascomycota* has further advanced (Lindemuth *et al.* 2001, Lumbsch *et al.* 2001, 2002a, b, 2004, Grube *et al.* 2004, Lücking *et al.* 2004, Lutzoni *et al.* 2004, Persoh *et al.* 2004, Wedin *et al.* 2005, del Prado *et al.* 2006, Miadlikowska *et al.* 2006, Schmitt *et al.* 2006, Spatafora

et al. 2006, Hibbett *et al.* 2007, Hofstetter *et al.* 2007, Lumbsch & Huhndorf 2007a, Schoch *et al.* 2006, 2009a–c). Our current understanding suggests that there were several lichenisation events but also some major delichenisation events during the evolution of *Ascomycota* (Gargas *et al.* 1995, Lutzoni *et al.* 2001, Liu & Hall 2004, Gueidan *et al.* 2008, Schoch *et al.* 2009a). The largest clade of lichenised fungi, *Lecanoromycetes*, with 14 000 accepted species, appears to be the result of a single lichenisation event with at least one major delichenisation event in *Ostropales* and several delichenisation events throughout the class (Lumbsch *et al.* 2004, Persoh *et al.* 2004, Wedin *et al.* 2005, Miadlikowska *et al.* 2006, Hofstetter *et al.* 2007, Schoch *et al.* 2009a, Baloch *et al.* in prep.). A similar pattern is suggested within the second largest lichenised clade, *Arthoniomycetes*, with about 1 500 species (Tehler 1995, Myllys *et al.* 1998, Sundin 2000, Tehler & Irestedt 2007, Ertz *et al.* 2008). This class was recently shown to include the mazaediate genus *Tylophoron* (Lumbsch *et al.* 2009a), previously considered to be related to pyrenocarpous lichens (Aptroot *et al.* 2008). *Arthoniomycetes* is composed primarily of lichenised fungi producing apothecia or apothecioid ascomata with partially ascolocular development and bitunicate asci (Henssen & Jahns 1974, Eriksson & Winka 1997). The base of this clade was reconstructed as lichenised (Schoch *et al.* 2009a) and it is presumed that non-lichenised and lichenicolous species within the class represent reversions to the unlichenised state. One family that has not yet been confirmed within *Arthoniomycetes* using molecular data is *Chrysothrichaceae*, a small family of two genera (*Byssocaulon*, *Chrysothrix*) and little over 20 species (Kirk *et al.* 2008). The third primarily lichenised class is *Lichinomycetes* (350 species).

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The remaining lichenised fungi are primarily restricted to *Dothideomycetes* and *Eurotiomycetes* (subclass *Chaetothyriomycetidae*). Gueidan *et al.* (2008) demonstrated that lichenisation may have evolved at least twice within *Eurotiomycetes* (once at base of *Verrucariales* and once at base of *Pyrenulales*), though, this is uncertain as the ancestral state of the common ancestor to *Pyrenulales*, *Verrucariales* and *Chaetothyriales*, is not unambiguously resolved (Gueidan *et al.* 2008, Schoch *et al.* 2009a). Within both *Verrucariales* and *Pyrenulales*, there appears to be at least one loss of lichenisation each. *Dothideomycetes* and *Arthoniomycetes* together form the rankless clade Dothideomyceta, a name introduced by Schoch *et al.* (2009a, b). The ancestral state of Dothideomyceta and *Dothideomycetes* nodes are not resolved with confidence (Gueidan *et al.* 2008, Schoch *et al.* 2009a, b). In this paper we do not aim to resolve this issue but rather attempt to clarify, confirm or reject the placement of lichenised lineages within Dothideomyceta, specifically *Dothideomycetes*.

The following families have been confirmed or are believed to belong in either *Chaetothyriomycetidae* or *Dothideomycetes*: *Verrucariaceae* (930 species), *Pyrenulaceae* (280 species), *Celotheliaceae* (eight species), *Microtheliopsidaceae* (three species), and *Pyrenothrichaceae* (three species) in *Chaetothyriomycetidae* (Herrera-Campos *et al.* 2005, del Prado *et al.* 2006, Lücking 2008), and *Trypetheliaceae* (200 species), *Monoblastiaceae* (130 species), *Strigulaceae* (120 species), and *Arthopyreniaceae* (120 species) in *Dothideomycetes* (Lutzoni *et al.* 2004, del Prado *et al.* 2006, Lumbsch & Huhndorf 2007b). Most of these families have traditionally been placed within *Pyrenulales* (Poelt 1973, Henssen & Jahns 1974, Hafellner 1986, Kirk *et al.* 2001, Eriksson *et al.* 2004, Cannon & Kirk 2007), and much of the confusion regarding previous classifications of these pyrenocarpous lichens stems from the fact that *Pyrenulales* were at some point considered synonymous with the ascolocular *Melanommatales* (currently regarded synonymous with *Pleosporales*; Barr 1980, Harris 1984, 1990, 1991, 1995), whereas other workers considered *Pyrenulales* to be ascolohymenial (Henssen & Jahns 1974). The fact that *Trypetheliaceae* have no close relative within *Dothideomycetes* was reflected in the establishment of a separate order, *Trypetheliales* (Aptroot *et al.* 2008).

In addition to the aforementioned families, there are several genera of uncertain position, such as *Cystocoleus* and *Racodium*, both of which belong in *Capnodiales/Dothideomycetes* (Muggia *et al.* 2007), as well as *Julella*, *Mycoporum*, *Collemopsidium* (*Pyrenocollema*), and others, of unconfirmed affinities (Harris 1995). Yet other lineages, such as the recently discovered *Eremithallus* (Lücking *et al.* 2008) or the genera *Thelocarpon* and *Vezeadaea* (Reeb *et al.* 2004, Lumbsch *et al.* 2009b) appear to fall outside the currently accepted classes known to contain lichen-forming fungi. The current phylogeny of *Chaetothyriomycetidae* suggests that the two large lichen-forming families in this subclass may have emerged from distinct lichenisation events, however, this could not be resolved with confidence (see node 18 in fig. 1 and table 1 of Gueidan *et al.* 2008, Schoch *et al.* 2009a). It thus appears that *Dothideomycetes*, the largest class of *Ascomycota* with an estimated number of 19 000 species (Kirk *et al.* 2008), a class that has largely been neglected when assessing the phylogeny of lichenised fungi, might be the only class within *Ascomycota* containing several lineages that evolved through independent lichenisation. In addition to *Trypetheliaceae*, at least two other families, which exhibit substantial radiation accompanied with morphological variation at the generic and species level (*Monoblastiaceae* and *Strigulaceae*) have been suggested to

belong to *Dothideomycetes*. The only sequenced species of *Strigula* has been suggested to belong to *Eurotiomycetes* (Schmitt *et al.* 2005); however, re-examination of the specimen used in this study showed that it belonged in *Verrucariaceae*. Therefore the phylogenetic position of *Strigulaceae* remains unresolved. In addition, *Anisomeridium polypori* (*Monoblastiaceae*) was suggested to belong to *Dothideomycetes* (James *et al.* 2006).

In this paper, we are using nuclear large subunit (nuLSU) and mitochondrial small subunit (mtSSU) rDNA data, to construct a phylogeny of lichenised fungi with bitunicate asci, focusing on Dothideomyceta. We also present novel data that require adjustments in the systematic classification of taxa within both classes. A further objective was to begin to examine generic concepts within the family *Trypetheliaceae*, which is comprised of 11 genera (Lumbsch & Huhndorf 2007b) and approximately 200 species (Harris 1984, Aptroot 1991b, del Prado *et al.* 2006).

MATERIAL AND METHODS

Taxon sampling

Representatives of lichenised Dothideomyceta taxa were obtained through recent field work in the U.S.A., Central and South America, Europe, India, Thailand, and Fiji. Newly generated sequences were supplemented with other lichenised and non-lichenised Dothideomyceta from GenBank plus additional taxa in *Pezizomycetes*, *Leotiomycetes*, *Sordariomycetes*, *Eurotiomycetes*, and *Lecanoromycetes*, chiefly from a previous alignment published by Schoch *et al.* (2009a). In total, we analysed 162 operational taxonomic units (OTUs) representing 152 species and 111 genera. All OTUs included in the analyses, along with GenBank accession numbers and collection information for newly sequenced samples, are listed in Table 1 - see online Supplementary Information.

Molecular methods

The Sigma REExtract-N-Amp Plant PCR Kit (St. Louis, Missouri, U.S.A.) was used to isolate DNA, following the manufacturer's instructions, except only 10 µL of extraction buffer and 10 µL dilution buffer were used, following Avis *et al.* (2003). Dilutions of these extractions (rather than the stock DNA solution) were found to work best for PCR (C. Andrew, pers. comm. 2009), and a 20× DNA dilution was then used in subsequent PCR reactions.

Samples were PCR amplified and/or sequenced using the mrSSU1, mrSSU2, mrSSU2r and mrSSU3r primers (Zoller *et al.* 1999) for the mitochondrial small subunit (mtSSU) and the AL2R (Mangold *et al.* 2008), LR3R, LR3, LR5, LR6, LR7 (Vilgalys & Hester 1990) primers for the nuclear ribosomal large subunit rDNA (nuLSU). The 10 µL PCR reactions consisted of 5 µM of each PCR primer, 3 mM of each dNTP, 2 µL of 10 mg/mL 100x BSA (New England Biolabs, Ipswich, Massachusetts, U.S.A.), 1.5 µL 10× PCR buffer (Roche Applied Science, Indianapolis, Indiana, U.S.A.), 0.5 µL *Taq*, approximately 2 µL diluted DNA, and 2 µL water. The PCR cycling conditions were as follows: 95 °C for 5 min, followed by 35 cycles of 95 °C for 1 min, a locus-specific annealing temperature for 1 min, and 72 °C for 1 min, followed by a single 72 °C final extension for 7 min. An annealing temperature of 53 °C was used for mtSSU, while 57 °C was used for nuLSU.

Samples were visualised on a 1 % ethidium bromide-stained agarose gel under UV light and bands were gel extracted, heated at 70 °C for 5 min, cooled to 45 °C for 10 min, treated with 1 µL

GELase (Epicentre Biotechnologies, Madison, WI, U.S.A.) and incubated at 45 °C for at least 24 h. The 10 µL cycle sequencing reactions consisted of 1–1.5 µL of Big Dye v. 3.1 (Perkin-Elmer Applied Biosystems, Foster City, California, U.S.A.), 2.5–3 µL of Big Dye buffer, 6 µM primer, 0.75–2 µL Gelased PCR product and water. The cycle sequencing conditions were as follows: 96 °C for 1 min, followed by 25 cycles of 96 °C for 10 s, 50 °C for 5 s and 60 °C for 4 min. Samples were precipitated and sequenced in an Applied Biosystems 3730 DNA Analyser (Foster City, California, U.S.A.), and sequences assembled in Sequencher 4.9 (Gene Codes Corporation, Ann Arbor, Michigan, U.S.A.).

Phylogenetic analysis

The alignment of Schoch *et al.* (2009a) was used as a starting point, from which a large number of sequences were removed. Newly generated sequences were added and manually aligned (nuLSU), or were separately aligned, added to the Schoch *et al.* (2009a) alignment, and manually adjusted (mtSSU). In addition to a representative set of dothideomycetous fungi, members of several *Ascomycota* classes were retained and *Pezizomycetes* taxa were used as the outgroup. The entire set of sequences generated in the present study plus those from GenBank were aligned in Se-AL v. 2.0a11 (Rambaut 1996) and BioEdit 7.0.9 (Hall 1999). An iterative procedure was used for the nuLSU in which ambiguous regions were aligned with Muscle 3.6 (Edgar 2004) through Mesquite 2.71 (Maddison & Maddison 2009); the alignment was again manually refined and other portions realigned with Muscle. After a final manual refinement, ambiguous regions and introns were removed and the alignment was deposited in TreeBase.

Alignments for each gene were concatenated in Mesquite 2.71 (Maddison & Maddison 2009) and analysed under the maximum likelihood (ML) optimality criterion in RAxML 7.0.4 (Stamatakis 2006). The data set was partitioned by locus and the GTRMIXI model with twenty-five rate parameter categories (default) was used for each partition. In addition, support was estimated by performing 1000 bootstrap replicates, and clades with bootstrap support of 70 % or greater were considered strongly supported. Additionally, the data sets were analyzed in GARLI 0.96 (Zwickl 2006) using the GTR-gamma-invariant model which is similar to the model used in RAxML.

RESULTS

The final alignment consisted of 1 915 unambiguously aligned characters (1 199: nuLSU; 716: mtSSU). Both ML analyses recovered the major class-level ingroup nodes (Fig. 1) corresponding to other recent studies (*Leotiomyces*, *Sordariomyces*, *Eurotiomyces*, *Lecanoromyces*, *Arthoniomyces*, *Dothideomyces*). *Arthoniomyces* and *Dothideomyces* form a strongly supported sister-group relationship, corresponding to Dothideomyceta. Individual gene phylogenies suggested some incongruence between loci (unpubl. data), however, the topology in the combined analysis is in agreement with previously reported phylogenies and we did not exclude taxa.

The phylogeny of *Arthoniomyces* (*Arthoniales*) largely confirmed previous analyses, with *Chrysothrichaceae* forming an additional family within this clade (Fig. 1). *Arthoniaceae s. l.* and *Roccellaceae s. l.* are both monophyletic and well separated. However, several smaller lineages that eventually could be reinstated at the family level show strong support: *Arthoniaceae*

s. str., *Cryptotheciaceae* (*Cryptothecia-Herpothallon*), the *Tylophoron* clade, *Roccellaceae s. str.*, *Opegraphaceae s. str.*, and possibly *Chiodectonaceae* (as *Chiodecton sphaerale* is closely related to *Erythrodictyon* and *Dichosporidium* whereas the sequenced *C. natalense* is apparently not a *Chiodecton s. str.*). Surprisingly, *Arthonia caesia* clustered with *Chrysothrichaceae* and not *Arthoniaceae*. *Herpothallon rubrocinctum* is nested within *Cryptothecia s. l.*

Six distinct, lichenised lineages were confirmed as belonging to Dothideomycetes (Fig. 1): the order *Trypetheliales*, the families *Arthopyreniaceae*, *Monoblastiaceae*, and *Strigulaceae*, and the genera *Cystocoleus* and *Racodium*. The latter two (*Cystocoleus* and *Racodium*) are members of the order *Capnodiales*, whereas *Arthopyreniaceae*, represented by the species *Arthopyrenia salicis*, was confirmed as clustering within *Pleosporales*. However, *Arthopyreniaceae* as currently defined, including the genera *Julella* (not sequenced) and *Mycomicrothelia*, is not monophyletic, as the sequenced species of *Mycomicrothelia* appeared outside *Pleosporales* and form a sister-group to *Trypetheliaceae*.

Strigulaceae is represented by five samples of the three genera *Flavobathelium*, *Phyllobathelium*, and *Strigula*, which formed a supported monophyletic clade sister to *Kirschsteiniethelia aethiops*, but without support. *Monoblastiaceae* was strongly supported and included four genera with one species each in this analysis: *Acrocordia subglobosa*, *Anisomeridium ubianum*, *Megalotremis verrucosa*, and *Trypetheliopsis* (syn. *Musaespora*) *kalbii*. Initially we also included a GenBank sequence of *Anisomeridium polypori* in the data set, but the nuLSU sequence was recovered in *Eurotiomyces* and the taxon was excluded from the final analysis. It is possible that this sequence is derived from a contaminant or that it was confused with a similar species in an unrelated lineage.

Trypetheliaceae was strongly supported as monophyletic, being sister to the genus *Mycomicrothelia*. There was no support for the traditional separation into the perithecial and ascospore core genera *Astrothelium*, *Laurera*, and *Trypethelium*, as species of these genera were found scattered over the *Trypetheliaceae* clade.

DISCUSSION

This is the first molecular phylogenetic study that includes presumably all major lichenised lineages within Dothideomyceta. This rankless taxon was informally introduced by Schoch *et al.* (2009a, b) for the clade including *Arthoniomyces* and *Dothideomyces*. The sister group of Dothideomyceta is not yet resolved but Ruibal *et al.* (2009; this volume) demonstrated an unnamed lineage of melanised rock-inhabiting fungi to be basal to *Arthoniomyces* (not included in our sampling).

Arthoniomyces is the second largest class of primarily lichenised *Ascomycota* and exhibits considerable morpho-anatomical variation (Fig. 2). The molecular phylogeny presented here confirms the current classification of lichenised *Arthoniomyces* in three families: *Arthoniaceae*, *Chrysothrichaceae*, and *Roccellaceae* (Tehler 1995, Grube 1998, Tehler & Irestedt 2007). The morphological concept used to classify the single order included few large genera, with *Arthonia* and *Opegrapha* having the highest number of species (500 and 300, respectively). The infrageneric relationships of these species were repeatedly discussed and there was common agreement that these genera were not monophyletic and include morphologically distinct groups. Similarly the relationships of other genera with fewer species or of monospecific genera in the family *Roccellaceae* was

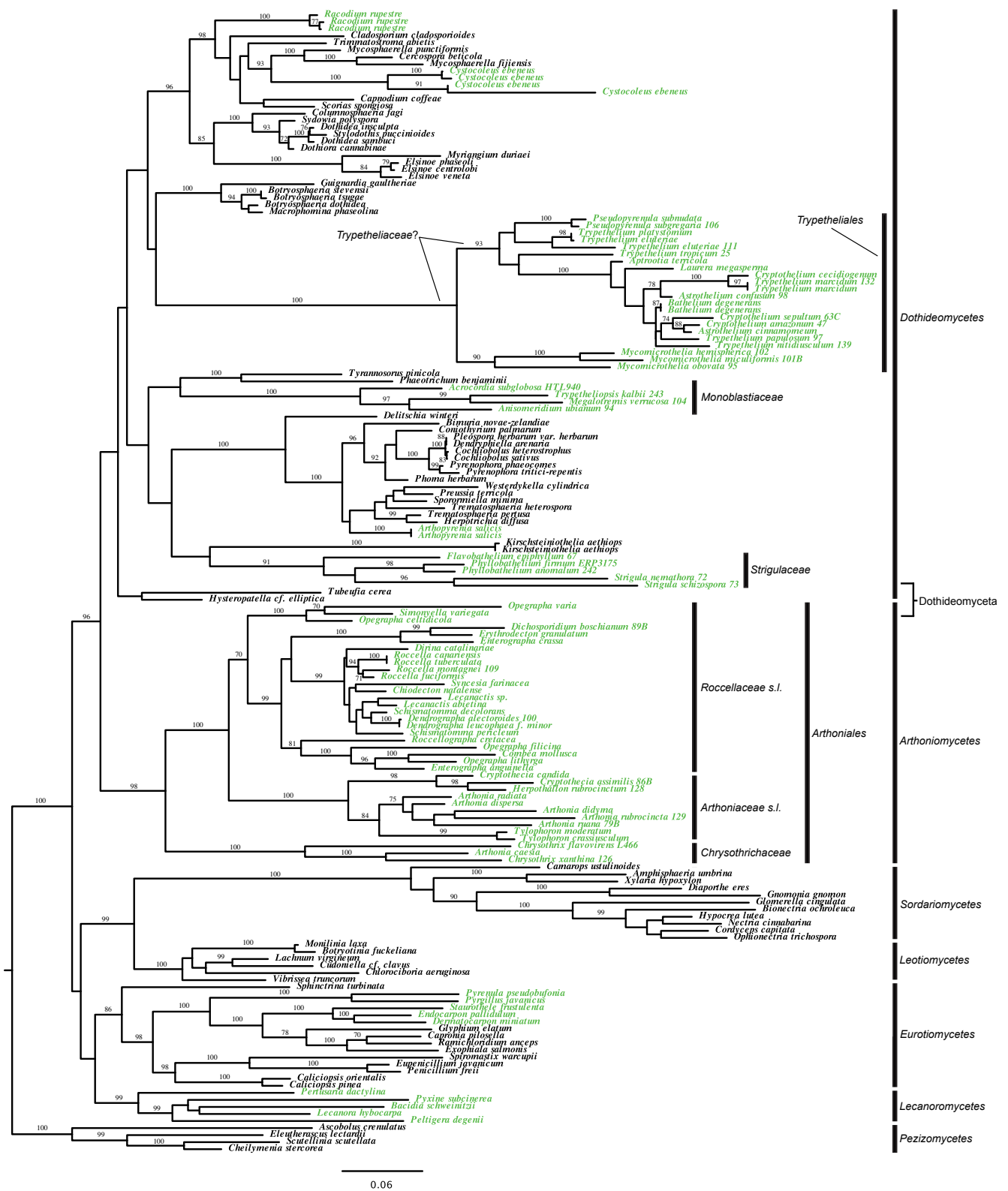


Fig. 1. The ML tree from RAxML maximum likelihood analysis with bootstrap percentages equal to or greater than 70 are plotted above or below branches. Lichenised taxa are in green, while non-lichenised taxa are in black.

unclear. Along with previous data (Tehler 1995, Myllys *et al.* 1998, Tehler & Irestedt 2007) and recent results by Ertz *et al.* (2009), the present tree is a further step to resolve these questions based on molecular data.

Little can be said regarding generic concepts of most genera, as the taxon sampling is still far too incomplete for this group, but it appears that some of the traditional concepts based on fruit body structure are not supported, which suggests some degree of parallel

evolution. An example is the *Chiodecton-Enterograpta* complex: while the sequenced *Chiodecton natalense* appears to be unrelated to the morphologically and anatomically similar *Dichosporidium* and *Erythrodictyon* (Thor 1990), *Enterograpta* and the similar *Schismatomma* (Sparrius 2004) were found in three different clades related to either *Chiodecton natalense* (*Schismatomma*), *Dichosporidium* (*Enterograpta crassa*), and *Opegrapha* (*Enterograpta anguinella*), respectively. This is in agreement with



Fig. 2. Select lichenised *Arthoniomycetes*. A. *Chrysothrix xanthina*; B. *C. septemseptata*; C. *Arthonia caesia*; D. *A. cyanea*; E. *A. pulcherrima*; F. *A. rubrocincta*; G. *Cryptothecia candida*; H. *Herpothallon rubrocinctum*; I. *Tylophoron crassiusculum* (teleomorph); J. *T. crassiusculum* (anamorph); K. *Opegrapha filicina*; L. *O. astraea*; M. *Enterographa anguineella*; N. *Syncesia glyphysoides*; O. *S. byssina*; P. *Lecanactis epileuca*; Q. *Chiodecton sphaerales*; R–S. *Erythrodection granulatum*; T. *Dichosporidium boschianum*; U. *D. nigrocinctum* (ascomata); V. *D. nigrocinctum* (isidia); W. *Mazosia rotula*; X. *Roccella* spec. Photo credits: R. Lücking.

Ertz *et al.* (2009), who showed that *Enterographa* is not monophyletic and groups either with the core *Opegrapha* clade (here represented by *O. lithyrgica*), or with *Chiodecton*-like species (*Dichosporidium* and *Erythrodection*). Consequently, Ertz *et al.* (2009) transferred *Enterographa anguineella* to *Opegrapha*. Not surprisingly, neither *Arthonia* nor *Opegrapha* are monophyletic. Ertz *et al.* (2009) showed convincingly that despite different ascomatal structure, *Opegrapha atra* and *O. calcarea* (with distinct excipulum) are closely related to *Arthonia radiata* (lacking an excipulum), which is confirmed by

similarities of ascus structure and pigment type. Subsequently, Ertz *et al.* (2009) suggested these two *Opegrapha* species be recognised as belonging to *Arthonia*. *Opegrapha varia* and *O. celtidicola* form another monophyletic lineage together with *Simonyella variegata*. Most likely this branch also includes other *Opegrapha* species, according to the results of Ertz *et al.* (2009). *Opegrapha s. str.* forms a further lineage including *O. lithyrgica*, which is closely related to the type species *O. vulgata* (Ertz *et al.* 2009), the foliicolous *O. filicina*, as well as *Combea mollusca* and *Roccellographa cretacea*.

Herpothallon rubrocinctum is now confirmed as an ascomycete in *Arthoniomycetes*. This seems trivial as the species also morphologically shows clear affinities with *Cryptothecia* (Aptroot *et al.* 2008), but the position of this taxon was questioned long ago and was even considered a basidiomycete (see discussion in Withrow & Ahmadjian 1983, Aptroot *et al.* 2008). Our analysis shows *Herpothallon* nested within *Cryptothecia*, supporting the previous hypothesis that byssoid-isidiate species within this complex are indeed members of *Cryptothecia* rather than forming a separate genus, as proposed by Aptroot *et al.* (2008). However, a larger taxon sampling is needed to resolve the *Cryptothecia*-*Herpothallon* complex, especially considering that there are other genera such as *Stirtonia* involved and even further new genera have been segregated recently (Aptroot *et al.* 2009, Frisch & Thor 2010). The fruticose *Roccella* species form a clearly monophyletic branch together with several crustose species representing various genera; this assemblage of core *Roccellaceae* has already been recognised previously (Tehler 1995, Myllys *et al.* 1998, Tehler & Irestedt 2007). The placement of *Tylophoron*, a genus that has passive spore dispersal and was previously assigned to *Caliciales*, is here confirmed as a member of *Arthoniaceae* s. l., in agreement with Lumbsch *et al.* (2009a).

The strongly supported placement of *Arthonia caesia* within *Chrysothrix* is unexpected; however, fertile species of *Chrysothrix* are very similar to *Arthonia* in ascoma morphology and anatomy, and particularly *A. caesia* and allies can be easily perceived as non-pigmented species of *Chrysothrix* in apothecial anatomy and morphology and thallus structure (including the chlorococcoid photobiont). Similar *Arthonia* species include *A. cupressina*, which is closely related to *A. caesia*. Further studies are needed to elucidate which additional *Arthonia* taxa need to be placed in *Chrysothrix*. The latter genus was variously placed in its own family *Chrysothrichaceae* mainly due to the presence of pulvinic acids as secondary metabolites but also in *Arthoniaceae* due to similarities in ascus characters (Grube 1998). The present data strongly support *Chrysothrichaceae* as a separate family, especially as it is sister to all remaining *Arthoniales* and not to *Arthoniaceae*. It is therefore necessary to transfer *Arthonia caesia* (which lacks pulvinic acids) and related species to this family. The other *Arthonia* species sampled group form a fairly well supported monophyletic group, which includes a species formerly assigned to *Arthothelium*, i.e. *Arthonia ruana*, because of its muriform ascospores; however, it has been known for some time that most species with muriform ascospores are more closely related to *Arthonia* than to the type of *Arthothelium*, *A. spectabile* (Tehler 1990, Sundin & Tehler 1998, Cáceres 2007, Grube 2007), which has not yet been sequenced. Notably, *Arthonia didyma* and *A. rubrocincta*, two species with reddish pigments, form a weakly supported group. If future efforts confirm this grouping, the name *Coniocarpon* could be used for this clade (Cáceres 2007).

In contrast to *Arthoniomycetes*, the overwhelming majority of *Dothideomycetes* species are non-lichenised. In addition to *Arthopyreniaceae*, *Trypetheliaceae* and *Cystocoleus* and *Racodium* (Muggia *et al.* 2007), this study confirms the placement of *Monoblastiaceae* and *Strigulaceae* within *Dothideomycetes*. Although our support for the *Dothideomycetes* node is weak, the included non-lichenised taxa are well supported within this class in other studies (Schoch *et al.* 2006, 2009a, b); in addition, placement within *Dothideomycetes* is strongly supported. Both, *Monoblastiaceae* and *Strigulaceae* are comparatively large with over 100 accepted species each and show substantial morphological and ecological radiation (Fig. 3); both are chiefly tropical. The mostly corticolous

Monoblastiaceae range from barely lichenised forms with exposed perithecia (many species of *Anisomeridium*) to taxa with well-developed, corticate thalli (*Anisomeridium* p.p., *Megalotremis*, *Trypetheliopsis*). Ascospores vary from small to large and thick-walled but are always simple or transversely septate only (Harris 1995). Substantial variation is found in the conidiomata, and many species, particularly in the genera *Caprettia*, *Megalotremis*, and *Trypetheliopsis* (= *Musaespora*) have developed unique pycnidia that in part are similar to campylidia or hyphophores found in certain *Lecanoromycetes* (Aptroot & Sipman 1993, Lücking *et al.* 1998, Aptroot *et al.* 2008, Lücking 2008). Secondary substances are few, including lichexanthone and anthraquinones. All species of *Monoblastiaceae* in which conidiomata are known share a particular synapomorphy: the conidia are always embedded in a strongly coherent, gelatinous matrix. Thus, besides the uniform hamathecium and ascus anatomy, there is substantial phenotypic evidence for monophyly of this family, now confirmed by molecular data.

Strigulaceae share many characteristics with *Monoblastiaceae*, specifically the ascus type and the mostly 1- or 3-septate ascospores, although some species have muriform ascospores (Harris 1995, Aptroot *et al.* 2008, Lücking 2008). Species in this family are found on a variety of substrata, including rocks, bark, and living leaves. Poorly developed thalli are found in corticolous species with barely lichenised thalli and exposed perithecia (*Strigula* p.p.), whereas the genera *Flavobathelium*, *Phyllobathelium*, and *Phyllocratera* include taxa with well-developed, corticate thalli. Also in this family, the most characteristic synapomorphy are the conidia, which feature terminal gelatinous appendices (Harris 1995, Lücking 2008). Unfortunately, our taxon sampling of this family is poor but sufficient to confirm its monophyly and its placement in *Dothideomycetes*. This is the first molecule-based support for the inclusion of *Phyllobatheliaceae* within *Strigulaceae*, a concept first presented by Harris (1995).

The largest lichenised family within *Dothideomycetes*, *Trypetheliaceae*, contains members that are typically lichen-forming and tropical to subtropical in distribution, with some taxa extending into temperate regions (Aptroot 1991, Harris 1995, Brodo *et al.* 2001, Aptroot *et al.* 2008). The species are almost exclusively corticolous, forming a crustose, endo- or epiperidermal thallus with algae belonging to *Trentepohliaceae*; however, *Anisomeridium* is often found lignicolous and *Aptrootia* grows on bryophytes. Detailed studies in Costa Rica suggest *Trypetheliaceae* to occur primarily on trunks and branches of trees in exposed habitats of lowland to lower montane (200–1000 m) rain and dry forests and savannas with rather distinct dry season (Aptroot *et al.* 2008, Rivas-Plata *et al.* 2008). *Trypetheliaceae* species are quite variable in perithecial morphology (Fig. 3) but have a rather uniform hamathecium composed of thin, anastomosing pseudoparaphyses embedded in a stiff gelatinous matrix. The most characteristic synapomorphy are the usually hyaline ascospores with internal wall thickenings that cause more or less diamond-shaped septa, but these wall thickenings are often reduced or absent in species with multiseptate or muriform ascospores (Harris 1984, 1990, 1995, Aptroot 1991b, Aptroot *et al.* 2008). The secondary chemistry is equally simple, with lichexanthone and pigments as most common substances, i.e. polyketide derived aromatic compounds produced through the acetyl-polymalonyl pathway (Elix & Stocker-Wörgötter 2008). However, the number of species with substances present is much higher in *Trypetheliaceae* than any other lineage within *Dothideomycetes*: more than 70 species are known to produce secondary substances in this family. The core genera *Astrothelium*, *Campylothelium*, *Cryptothelium*, *Laurera*, and *Trypethelium*, are

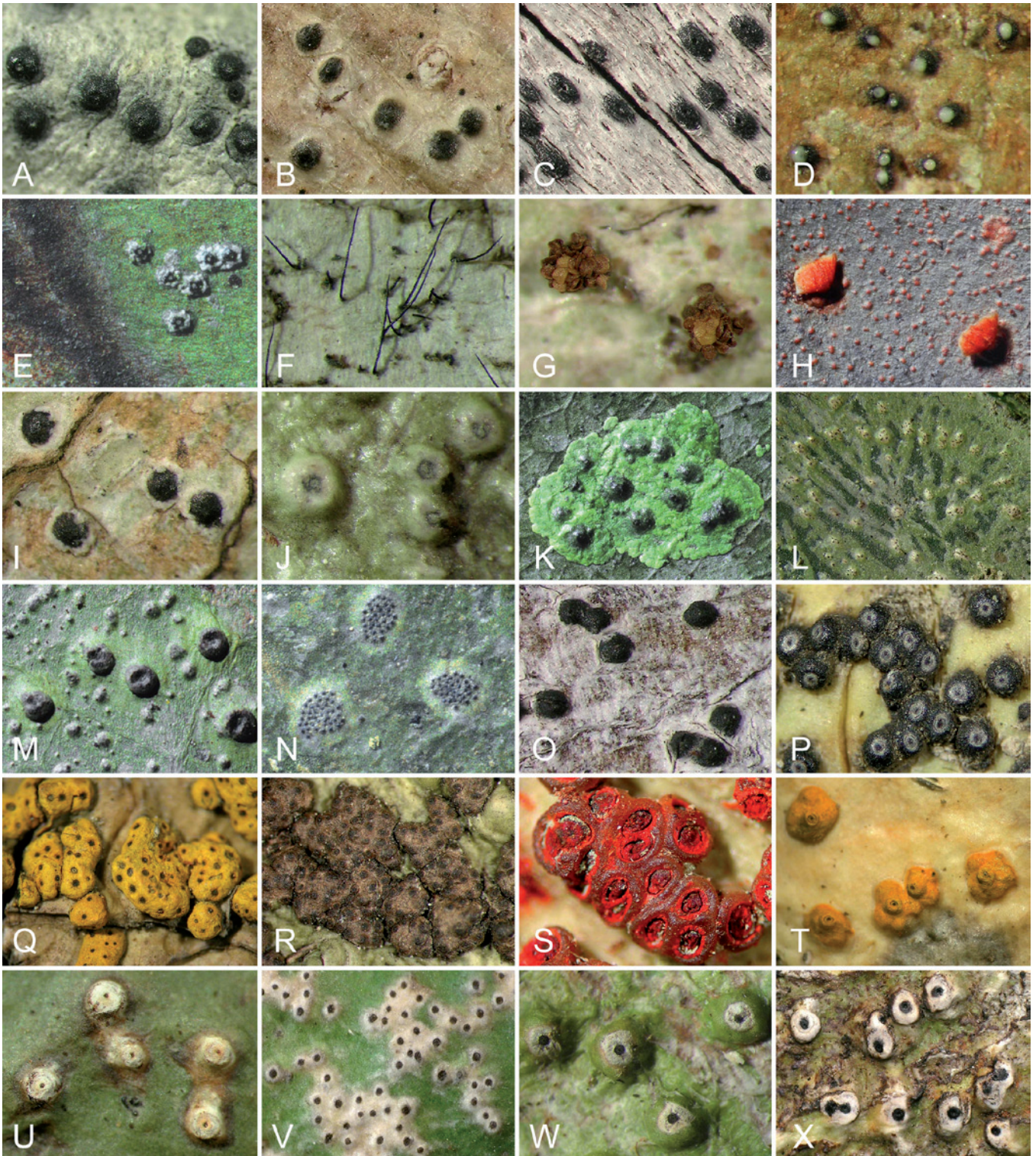


Fig. 3. Select lichenised *Dothideomycetes*; A. *Arthopyrenia cinchonae*; B. *Mycomicrothelia modesta*; C. *Anisomeridium subprostans*; D. *Anisomeridium spec.* (pycnidia); E. *A. foliicola* (pycnidia); F. *Capretzia amazonensis* (pycnidia); G. *Megalotremis cauliflora* (pycnidia); H. *Trypetheliopsis* (= *Musaespora*) *coccinea* (campylidia); I. *Strigula viridiseda*; J. *S. laureriformis* (pycnidia); K. *S. smaragdula*; L. *Flavobathelium epiphyllum*; M. *Phyllobathelium firmum*; N. *P. leguminosae* (pycnidia); O. *Pseudopyrenula subnuda*; P. *Trypethelium tropicum*; Q. *T. platystomum*; R. *Bathelium degenerans*; S. *Laurera purpurina*; T. *Astrothelium cinnamomeum*; U. *A. eustomum*; V. *Trypethelium nitidiusculum*; W. *Laurera megasperma*; X. *Campylothelium spec.* Photo credits: R. Lücking.

separated primarily on the basis of perithecial arrangement and ostiolar orientation (solitary vs. aggregate, apical vs. excentric) and ascospore septation (transverse vs. muriform; Harris 1990, 1995, del Prado *et al.* 2006). Because of the schematic classification, Harris (1995) suggested that these genera may be polyphyletic, and del Prado *et al.* (2006) subsequently illustrated the non-monophyly of *Trypethelium*. Aptroot *et al.* (2008) echoed Harris's (1995) sentiment and stated that generic concepts in *Trypetheliaceae* are in need of revision.

Surprisingly, *Mycomicrothelia* was recovered as sister to *Trypetheliaceae*. *Mycomicrothelia* has traditionally been considered a sister genus to *Arthopyrenia* with brown ascospores (Harris 1995). However, the hamathecium at least of the sequenced species is identical to that found in *Trypetheliaceae*, whereas *Arthopyrenia* has thicker and less branched and anastomosing pseudoparaphyses. Moreover, the ascospores are of a different type, often with internal wall thickenings. It remains to be tested whether *Arthopyrenia* and *Mycomicrothelia* in their current circumscriptions are monophyletic

Table 2. Systematic placement of selected pyrenocarpous lichens according to different concepts.

Genus	Zahlbruckner 1926	Barr 1987	Harris 1995	current
Celothelium	<i>Pyrenocarpeae</i>	<i>Loculoascomycetes</i>	<i>Loculoascomycetes</i>	<i>Eurotiomycetes</i>
	(as <i>Leptorhaphis</i>)	<i>Pleosporales</i>	<i>Melanommatales</i>	<i>Pyrenulales</i>
	<i>Pyrenulaceae</i>	<i>Pleosporaceae</i>	<i>Thelenellaceae</i>	<i>Celotheliaceae</i>
Lithothelium	<i>Pyrenocarpeae</i>	<i>Loculoascomycetes</i>	<i>Loculoascomycetes</i>	<i>Eurotiomycetes</i>
	<i>Astrotheliaceae</i>	<i>Melanommatales</i>	<i>Melanommatales</i>	<i>Pyrenulales</i>
Pyrenula	<i>Pyrenocarpeae</i>	<i>Pyrenulaceae</i>	<i>Pyrenulaceae</i>	<i>Pyrenulaceae</i>
	<i>Pyrenulaceae</i>			
Arthopyrenia	<i>Pyrenocarpeae</i>	<i>Loculoascomycetes</i>	<i>Loculoascomycetes</i>	<i>Dothideomycetes</i>
	<i>Pyrenulaceae</i>	<i>Pleosporales</i>	<i>Pleosporales</i>	<i>Pleosporales</i>
		<i>Arthopyreniaceae</i>	<i>Pleosporaceae</i>	<i>Arthopyreniaceae</i>
Acrocordia	<i>Pyrenocarpeae</i>	<i>Loculoascomycetes</i>	<i>Loculoascomycetes</i>	<i>Dothideomycetes</i>
Anisomeridium	(as <i>Arthopyrenia</i>)	<i>Melanommatales</i>	<i>Melanommatales</i>	<i>incertae sedis</i>
	<i>Pyrenulaceae</i>	<i>Acrocoriaceae</i>	<i>Monoblastiaceae</i>	<i>Monoblastiaceae</i>
Phyllobathelium	<i>Pyrenocarpeae</i>	<i>Loculoascomycetes</i>	<i>Loculoascomycetes</i>	<i>Dothideomycetes</i>
Strigula	<i>Strigulaceae</i>	<i>Chaetothyriales</i>	<i>Melanommatales</i>	<i>incertae sedis</i>
		<i>Strigulaceae</i>	<i>Strigulaceae</i>	<i>Strigulaceae</i>
Astrothelium	<i>Pyrenocarpeae</i>	<i>Loculoascomycetes</i>	<i>Loculoascomycetes</i>	<i>Dothideomycetes</i>
	<i>Astrotheliaceae</i>	<i>Melanommatales</i>	<i>Melanommatales</i>	<i>Trypetheliales</i>
Campylothelium	<i>Pyrenocarpeae</i>	<i>Trypetheliaceae</i>	<i>Trypetheliaceae</i>	<i>Trypetheliaceae</i>
	<i>Paratheliaceae</i>			
Laurera	<i>Pyrenocarpeae</i>			
	<i>Trypetheliaceae</i>			
Pseudopyrenula	<i>Pyrenocarpeae</i>			
	<i>Pyrenulaceae</i>			
Trypethelium	<i>Pyrenocarpeae</i>			
	<i>Trypetheliaceae</i>			
Mycomicrothelia	<i>Pyrenocarpeae</i>	<i>Loculoascomycetes</i>	<i>Loculoascomycetes</i>	<i>Dothideomycetes</i>
	(as <i>Microthelia</i>)	<i>Pleosporales</i>	<i>Pleosporales</i>	<i>Trypetheliales</i>
	<i>Strigulaceae</i>	<i>Arthopyreniaceae</i>	<i>Arthopyreniaceae</i>	<i>Trypetheliaceae?</i>
Porina	<i>Pyrenocarpeae</i>		<i>Hymenoascomycetes</i>	<i>Lecanoromycetes</i>
	<i>Pyrenulaceae</i>		<i>Trichotheliales</i>	<i>Ostropales</i>
Trichothelium	<i>Pyrenocarpeae</i>		<i>Trichotheliaceae</i>	<i>Porinaceae</i>
	<i>Strigulaceae</i>	—		

genera or whether at least some species currently assigned to these genera perhaps represent further lichenised lineages within *Dothideomycetes*. Whether *Mycomicrothelia* should be included within *Trypetheliaceae* or receive its own family rank is open to question. *Mycomicrothelia* has primarily thin-walled, dark brown ascospores, whereas in *Trypetheliaceae* they are primarily thick-walled with diamond-shaped lumina and hyaline (brown only in *Aptrootia* and *Architrypethelium*). Understanding the phylogenetic position of *Polymeridium*, which also has thin-walled ascospores, will hopefully help clarify this.

In spite of the many characters in parallel with *Monoblastiaceae* and *Strigulaceae*, also the *Trypetheliaceae* plus *Mycomicrothelia* (*Trypetheliales*) are quite unique genetically and there is no evidence that the three families would be related to each other or with *Arthopyreniaceae*. This supports the notion of several shifts in lichenisation within the *Dothideomycetes* (Aptroot 1991a,

1998). However, the often barely lichenised thalli in certain species of *Anisomeridium*, *Arthopyrenia*, *Julella*, *Mycomicrothelia*, *Mycoporum*, *Pseudopyrenula*, and *Strigula* (Aptroot 1991a, Aptroot 1998, Harris 1995) suggest that these species can possibly switch between being (almost) non-lichenised to distinctly lichenised, a situation also found in the unrelated genus *Stictis* within *Lecanoromycetes* (Wedin *et al.* 2004).

The present study clarifies the systematic position of further pyrenocarpous lichenised lineages within the *Ascomycota* and shows that previous concepts in part diverged widely from our present understanding but also came surprisingly close even without molecular evidence (Table 2). This study emphasises that pyrenocarpous lichens with bitunicate asci are not only not monophyletic, but belong to at least two different classes (*Dothideomycetes* and *Eurotiomycetes*) and several different orders and families; the data at hand also suggest that these

represent several independent lineages of lichenisation. Although we consider this study a contribution to clarify the systematic position of pyrenocarpous lichens and the evolution of lichenisation within *Dothideomycetes*, much remains to be done, considering that at present only a fraction of the presumably 600 species of lichens belonging in this class have been studied using DNA sequences. In particular, clarifying the generic and species concepts within *Monoblastiaceae*, *Strigulaceae*, and *Trypetheliaceae*, speciose families that are important elements of crustose lichen communities especially in the tropics, will be a major challenge in the near future.

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REFERENCES

- Aptroot A (1991a). Tropical pyrenocarpous lichens. A phylogenetic approach. In: *Tropical lichens: their systematics, conservation and ecology* (Galloway DJ, ed.). Clarendon Press, U.K.: 253–273.
- Aptroot A (1991b). A monograph of the *Pyrenulaceae* (excluding *Anthracotheceum* and *Pyrenula*) and the *Requienellaceae*, with notes on the *Pleomassariaceae*, the *Trypetheliaceae* and *Mycomicrothelia* (lichenized and non-lichenized Ascomycetes). *Bibliotheca Lichenologica* **44**: 1–178.
- Aptroot A (1998). Aspects of the integration of the taxonomy of lichenized and non-lichenized pyrenocarpous ascomycetes. *Lichenologist* **30**: 501–514.
- Aptroot A, Sipman H (1993). *Musaespora*, a genus of pyrenocarpous lichens with campyloidia, and other additions to the foliicolous lichen flora of New Guinea. *Lichenologist* **25**: 121–135.
- Aptroot A, Lücking R, Sipman HJM, Umaña L, Chaves JL (2008). Pyrenocarpous lichens with bitunicate asci: a first assessment of the lichen biodiversity inventory of Costa Rica. *Bibliotheca Lichenologica* **97**: 1–162.
- Aptroot A, Thor G, Lücking R, Elix JA, Chaves JL (2009). The lichen genus *Herpothallon* re-instated. *Bibliotheca Lichenologica* **99**: 19–66.
- Avis P, McLaughlin DJ, Dentinger BC, Reich PB (2003). Long-term increase in nitrogen supply alters above- and below-ground ectomycorrhizal communities and increases the dominance of *Russula* spp. in a temperate oak savanna. *New Phytologist* **160**: 239–253.
- Barr ME (1983). The ascomycete connection. *Mycologia* **75**: 1–13.
- Barr ME (1987). *Prodromus to class Loculoascomycetes*. Hamilton I. Newell, Inc., Amherst, Massachusetts, published by the author.
- Brodo IM, Sharnoff SD, Sharnoff S (2001). *Lichens of North America*. Yale University Press, U.S.A.
- Cannon PF, Kirk PM (2007). *Fungal families of the world*. CABI Publishing, U.K.
- del Prado R, Schmitt I, Kautz S, Palice Z, Lücking R, Lumbsch HT (2006). Molecular data place *Trypetheliaceae* in *Dothideomycetes*. *Mycological Research* **110**: 511–520.
- Edgar RC (2004). MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Research* **32**(5): 1792–1797.
- Elix JA, Stocker-Wörgötter E (2008). Biochemistry and secondary metabolites. In: *Lichen biology*, 2nd edn (Nash III TH, ed.). Cambridge University Press, U.K.: 104–133.
- Eriksson OE, Barah H-O, Curra RS, Hansen K, Kurtzman CP, et al. (2004). Outline of *Ascomycota*. *Myconet* **10**: 1–99.
- Eriksson OE, Winka K (1997). Supraordinal taxa of *Ascomycota*. *Myconet* **1**: 1–16.
- Ertz D, Miadlikowska J, Lutzoni F, Dessein S, Raspé O, et al. (2009). Towards a new classification of the *Arthoniales* (*Ascomycota*) based on a three-gene phylogeny focussing on the genus *Opegrapha*. *Mycological Research* **113**: 141–152.
- Feurerer T, Hawksworth DL (2007). Biodiversity of lichens, including a world-wide analysis of checklist data based on Takhtajan's floristic regions. *Biodiversity and Conservation* **16**: 85–98.
- Frisch A, Thor G (2010). *Crypthonia*, a new genus of byssoid *Arthoniaceae* (lichenised *Ascomycota*). *Mycological Progress*: In press.
- Gargas A, DePriest PT, Grube M, Tehler A (1995). Multiple origins of lichen symbioses in fungi suggested by SSU rDNA phylogeny. *Science* **268**: 1492–1495.
- Grube M (1998). Classification and phylogeny in the *Arthoniales* (lichenized Ascomycetes). *Bryologist* **101**: 377–391.
- Grube M (2007). *Arthonia*. In: *Lichen Flora of the Greater Sonoran Desert Region. Volume 3*. (TH Nash III, C Gries, F Bungartz, eds). Lichens Unlimited, Arizona State University, Tempe, U.S.A.: 39–61.
- Grube M, Blaha J (2003). On the phylogeny of some polyketide synthase genes in the lichenized genus *Lecanora*. *Mycological Research* **107**: 1419–1426.
- Grube M, Baloch E, Lumbsch HT (2004). The phylogeny of *Porinaceae* (*Ostropomycetidae*) suggests a neotenic origin of perithecia in *Lecanoromycetes*. *Mycological Research* **108**: 1111–1118.
- Gueidan C, Ruibal Villaseñor C, Hoog GS de, Gorbushina AA, et al. (2008). A rock-inhabiting ancestor for mutualistic and pathogen-rich fungal lineages. *Studies in Mycology* **61**: 111–119.
- Hafellner J (1986). Systematics of lichenized fungi. *Progress in Botany* **48**: 316–333.
- Hall TA (1999). BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acid Symposium Series* **41**: 95–98.
- Harris RC (1984). The family *Trypetheliaceae* (*Loculoascomycetes*: lichenized *Melanommatales*) in Amazonian Brazil. *Acta Amazonica* **14** (Supplement): 55–80.
- Harris RC (1990). *Some Florida lichens*. Published by the author, U.S.A.
- Harris RC (1991). A revision of *Polymeridium* (Muell. Arg.) R.C. Harris (*Trypetheliaceae*). *Boletim do Museu Paraense Emílio Goeldi. Série Botânica* **7**: 619–644.
- Harris RC (1995). *More Florida lichens*. Including the 10¢ tour of the pyrenolichens. Published by the author, U.S.A.
- Henssen A, Jahns HM (1974). *Lichenes*. Georg Thieme Verlag, Stuttgart, Germany.
- Herrera-Campos M, Huhndorf S, Lücking R (2005). The foliicolous lichen flora of Mexico IV: a new, foliicolous species of *Pyrenothrix* (*Chaetothyriales*: *Pyrenothrichaceae*). *Mycologia* **97**: 356–361.
- Hibbett DS, Binder M, Bischoff JF, Blackwell M, Cannon PF, et al. (2007). A higher-level phylogenetic classification of the Fungi. *Mycological Research* **111**: 509–547.
- Hofstetter V, Miadlikowska J, Kauff F, Lutzoni F (2007). Phylogenetic comparison of protein-coding versus ribosomal RNA-coding sequence data: a case study of the *Lecanoromycetes* (*Ascomycota*). *Molecular Phylogenetics and Evolution* **44**: 412–426.
- James TY, Kauff F, Schoch C, Matheny PB, Hofstetter V, et al. (2006). Reconstructing the early evolution of the fungi using a six-gene phylogeny. *Nature* **443**: 818–822.
- Kirk PM, Cannon PF, Mintner DW, Stalpers JA (2008). Ainsworth & Bisby's dictionary of the Fungi, 10th edn. CAB International, U.K.
- Kroken S, Glass NL, Taylor JW, Yoder OC, Turgeon BG (2003). Phylogenomic analysis of type I polyketide synthase genes in pathogenic and saprobic ascomycetes. *Proceedings of the National Academy of Sciences (U.S.A.)* **100**: 15670–15675.
- Lindemuth R, Wirtz N, Lumbsch HT (2001). Phylogenetic analysis of nuclear and mitochondrial rDNA sequences supports the view that *loculoascomycetes* (*Ascomycota*) are not monophyletic. *Mycological Research* **105**: 1176–1181.
- Liu YJ, Hall BD (2004). Body plan evolution of ascomycetes, as inferred from an RNA polymerase II phylogeny. *Proceedings of the National Academy of Sciences (U.S.A.)* **101**: 4507–4512.
- Lücking R (2008). Foliiicolous lichenized fungi. *Flora Neotropica Monograph* **103**: 1–873.
- Lücking R, Becker U, Follmann G (1998). Foliiikole Flechten aus dem Tai-Nationalpark, Elfenbeinküste (Tropisches Afrika). II. Ökologie und Biogeografie [Foliiicolous lichens from the Tai National Park, Ivory Coast (Tropical Africa). II. Ecology and biogeography]. *Herzogia* **13**: 207–228.
- Lücking R, Stuart BL, Lumbsch HT (2004). Phylogenetic relationships of *Gomphillaceae* and *Asterothyriaceae*: evidence from a combined Bayesian analysis of nuclear and mitochondrial sequences. *Mycologia* **96**: 283–294.
- Lücking R, Lumbsch HT, Di Stéfano JF, Lizano D, Carranza J, et al. (2008). *Eremithallus costaricensis* (*Ascomycota*: *Lichinomycetes*: *Eremithallales*), a new fungal lineage with a novel lichen symbiotic lifestyle discovered in an urban relict forest in Costa Rica. *Symbiosis* **46**: 161–170.
- Lumbsch HT, Lindemuth R (2001). Major lineages of *Dothideomycetes* (*Ascomycota*) inferred from SSU and LSU rDNA sequences. *Mycological Research* **105**: 901–908.
- Lumbsch HT, Schmitt I, Döring H, Wedin M (2001). Molecular systematics supports the recognition of an additional order of *Ascomycota*: the *Agyriales*. *Mycological Research* **105**: 16–23.
- Lumbsch HT, Schmitt I (2002). Molecular data shake the *Pertusariaceae* tree into order. *Lichenology* **1**: 37–43.
- Lumbsch HT, Wirtz N, Lindemuth R, Schmitt I (2002). Higher level phylogenetic relationships of *euscomycetes* (*Pezizomycotina*) inferred from a combined

- analysis of nuclear and mitochondrial sequence data. *Mycological Progress* **1**: 57–70.
- Lumbsch HT, Schmitt I, Palice Z, Wiklund E, Ekman S, Wedin M (2004). Supraordinal phylogenetic relationships of *Lecanoromycetes* based on a Bayesian analysis of combined nuclear and mitochondrial sequences. *Molecular Phylogenetics and Evolution* **31**: 822–832.
- Lumbsch HT, Huhndorf SM (2007a). Whatever happened to the pyrenomyces and loculoascomycetes? *Mycological Research* **111**: 1064–1074.
- Lumbsch HT, Huhndorf SM (2007b). Outline of *Ascomycota* – 2007. *Myconet* **13**: 1–58.
- Lumbsch HT, Lücking R, L. Tibbell (2009a). Molecular data place *Tylophoron* as an additional calicioid genus in the *Arthoniales* (*Ascomycota*). *Bibliotheca Lichenologica* **99**: 285–296.
- Lumbsch HT, Zimmermann DG, Schmitt I (2009b). Phylogenetic position of ephemeral lichens in *Thelocarpaceae* and *Veizdaeeaceae* (*Ascomycota*). *Bibliotheca Lichenologica* **100**: 389–398.
- Lutzoni F, Pagel M, Reeb V (2001). Major fungal lineages are derived from lichen symbiotic ancestors. *Nature* **411**: 937–940.
- Lutzoni F, Kauff F, Cox CJ, McLaughlin D, Celio G, Dentinger B, et al. (2004). Assembling the fungal tree of life: progress, classification, and evolution of subcellular traits. *American Journal of Botany* **91**: 1446–1480.
- Maddison WP, Maddison DR (2009). Mesquite: a modular system for evolutionary analysis. Version 2.71 <http://mesquiteproject.org>
- Mangold A, Martin MP, Lücking R, Lumbsch HT (2008). Molecular phylogeny suggests synonymy of *Thelotremataceae* within *Graphidaceae* (*Ascomycota: Ostropales*). *Taxon* **57**: 476–486.
- Miadlikowska J, Kauff F, Hofstetter V, Fraker E, Grube M, et al. (2006). New insights into classification and evolution of the *Lecanoromycetes* (*Pezizomycotina, Ascomycota*) from phylogenetic analyses of three ribosomal RNA- and two protein-coding genes. *Mycologia* **98**: 1090–1103.
- Myllys L, Källersjö M, Tehler A (1998). A comparison of SSU rDNA data and morphological data in *Arthoniales* (*Euascomycetes*) phylogeny. *The Bryologist* **101**: 70–85.
- Persoh D, Beck A, Rambold G (2004). The distribution of ascus types and photobiontal selection in *Lecanoromycetes* (*Ascomycota*) against the background of a revised SSU nrDNA phylogeny. *Mycological Progress* **3**: 103–121.
- Poelt J (1973). Classification. - In: *The Lichens*. (V. Ahmadjian & M.E. Hale, eds). Academic Press, New York and London: 599–632.
- Rambaut A (1996). Se-AL: Sequence Alignment Editor. Available at <http://evolve.zoo.ox.ac.uk/>
- Reeb V, Lutzoni F, Roux C (2004). Contribution of *RPB2* to multilocus phylogenetic studies of the *euascomycetes* (*Pezizomycotina, Fungi*) with special emphasis on the lichen-forming *Acarosporaceae* and evolution of polyspory. *Molecular Phylogenetics and Evolution* **32**: 1036–1060.
- Rivas-Plata E, Lücking R, Lumbsch HT (2008). When family matters: an analysis of *Thelotremataceae* (lichenized *Ascomycota: Ostropales*) as bioindicators of ecological continuity in tropical forests. *Biodiversity and Conservation* **17**: 1319–1351.
- Ruibal C, Gueidan C, Selbmann L, Gorbushina AA, Crous PW, et al. (2009). Phylogeny of rock-inhabiting fungi related to *Dothideomycetes*. *Studies in Mycology* **64**: 123–133.
- Schmitt I, Yamamoto Y, Lumbsch HT (2006). Phylogeny of *Pertusariales* (*Ascomycotina*): resurrection of *Ochrolechiaceae* and new circumscription of *Megasporaceae*. *Journal of the Hattori Botanical Laboratory* **100**: 753–764.
- Schmitt I, Lumbsch HT (2009). Ancient horizontal gene transfer from bacteria enhances biosynthetic capabilities of fungi. *PLoS ONE* **4**: e4437.
- Schmitt I, Martin MP, Kautz S, Lumbsch HT (2005). Diversity of non-reducing polyketide synthase genes in the *Pertusariales* (lichenized *Ascomycota*): A phylogenetic perspective. *Phytochemistry* **66**: 1241–1253.
- Schoch CL, Shoemaker RA, Seifert KA, Hambleton S, Spatafora JW, Crous PW (2006). A multigene phylogeny of the *Dothideomycetes* using four nuclear loci. *Mycologia* **98**: 1041–1052.
- Schoch CL, Sung G-H, López-Giráldez F, Townsend JP, Miadlikowska J, et al. (2009a). The *Ascomycota* tree of Life: a phylum wide phylogeny clarifies the origin and evolution of fundamental reproductive and ecological traits. *Systematic Biology* **58**: 224–239.
- Schoch CL, Wang Z, Townsend JP, Spatafora JW (2009b). *Geoglossomycetes* cl. nov., *Geoglossales* ord. nov. and taxa above class rank in the *Ascomycota* Tree of Life. *Persoonia* **22**: 129–138.
- Schoch CL, Crous PW, Groenewald JZ, Boehm EWA, Burgess TI, et al. (2009c). A class-wide phylogenetic assessment of *Dothideomycetes*. *Studies in Mycology* **64**: 1–15.
- Sparrus L (2004). A monograph of *Enterographa* and *Sclerophyton*. *Bibliotheca Lichenologica* **89**: 1–141.
- Spatafora JW, Johnson D, Sung GH, Hosaka K, O'Rourke B, et al. (2006). A five-gene phylogenetic analysis of the *Pezizomycotina*. *Mycologia* **98**: 1020–1030.
- Stamatakis A (2006). RAxML-VI-HPC: Maximum Likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics* **22**: 2688–2690.
- Strimmer K, Rambaut A (2002). Inferring confidence sets of possibly misspecified gene trees. *Proceedings of the Royal Society of London, Biological Sciences* **269**: 137–142.
- Sundin R (2000). Phylogeny and taxonomy within *Arthonia* Ach. In: *The Fourth IAL Symposium, Progress and Problems in Lichenology at the Turn of the Millennium*. Universitat de Barcelona, Barcelona, Spain.
- Sundin R, Tehler A (1998). Phylogenetic studies of the genus *Arthonia*. *Lichenologist* **30**: 381–413.
- Tehler A (1990). A new approach to the phylogeny of *euascomycetes* with a cladistic outline of *Arthoniales* focussing on *Roccellaceae*. *Canadian Journal of Botany* **68**: 2458–2492.
- Tehler A (1995). *Arthoniales* phylogeny as indicated by morphological and rDNA sequence data. *Cryptogamic Botany* **5**: 82–97.
- Tehler A, Irestedt M (2007). Parallel evolution of lichen growth forms in the family *Roccellaceae* (*Arthoniales, Ascomycota*). *Cladistics* **23**: 432–454.
- Vilgaly R, Hester M (1990). Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. *Journal of Bacteriology* **172**: 4238–4246.
- Wedin M, Döring H, Gilenstam G (2004). Saprotrophy and lichenization as options for the same fungal species on different substrata: environmental plasticity and fungal lifestyles in the *Stictis-Conotrema* complex. *New Phytologist* **164**: 459–465.
- Wedin M, Wiklund E, Crewe A, Döring H, Ekman S, et al. (2005). Phylogenetic relationships of *Lecanoromycetes* (*Ascomycota*) as revealed by analyses of mtSSU and nLSU rDNA sequence data. *Mycological Research* **109**: 159–172.
- Withrow K, Ahmadjian V (1983). The ultrastructure of lichens. VII. *Chiodecton sanguineum*. *Mycologia* **75**: 337–339.
- Zahlbruckner A (1926). *Catalogus Lichenum Universalis*. - Borntraeger/Leipzig, Germany.
- 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.
- Zwickl DJ (2006). *Genetic algorithm approaches for the phylogenetic analysis of large biological sequence datasets under the maximum likelihood criterion*. Ph.D. dissertation - The University of Texas at Austin, U.S.A.

SUPPLEMENTARY INFORMATION

Table 1. Taxa included in this study with GenBank accession numbers and collection information. Numbers following taxon names are DNA identification numbers used in this study.

Taxon	Collection	Accession Number	
		nuLSU	mtSSU
<i>Acrocordia subglobosa</i> (HTL940)	Palice s.n., Poland (F)		GU327681
<i>Amphisphaeria umbrina</i>		FJ176863	FJ713609
<i>Anisomeridium ubianum</i> (94)	Lumsch 19845j, Fiji (F)	GU327709	GU327682
<i>Aptrootia terricola</i>			DQ328995
<i>Arthonia caesia</i>		FJ469668	FJ469671
<i>Arthonia didyma</i>		EU704083	EU704047
<i>Arthonia dispersa</i>		AY571381	AY571383
<i>Arthonia radiate</i>			EU704048
<i>Arthonia ruana</i> (79B)	Zimmerman 1117, Germany (F)		GU327683
<i>Arthonia rubrocincta</i> (129)	Nelsen 4010, U.S.A. (F)		GU327684
<i>Arthopyrenia salicis</i>		AY538339	AY538345
		AY607730	AY607742
<i>Ascobolus crenulatus</i>		AY544678	FJ713607
<i>Astrothelium cinnamomeum</i>		AY584652	AY584632
<i>Astrothelium confusum</i> (98)	Nelsen 4004a, Peru (F)	GU327710	GU327685
<i>Bacidia schweinitzii</i>		DQ782911	DQ972998
<i>Bathelium degenerans</i>			DQ328987
			DQ328988
<i>Bimuria novae-zelandiae</i>		AY016356	FJ190605
<i>Bionectria ochroleuca</i>		AY489716	FJ713619
<i>Botryosphaeria dothidea</i>		DQ678051	FJ190612
<i>Botryosphaeria stevensii</i>		DQ678064	
<i>Botryosphaeria tsugae</i>		DQ767655	
<i>Botryotinia fuckeliana</i>		AY544651	AY544732
<i>Caliciopsis orientalis</i>		DQ470987	FJ190654
<i>Caliciopsis pinea</i>		DQ678097	FJ190653
<i>Camarops ustulinoides</i>		DQ470941	FJ190588
<i>Capnodium coffeae</i>		DQ247800	FJ190609
<i>Capronia pilosella</i>		DQ823099	FJ225725
<i>Cercospora beticola</i>		DQ678091	FJ190647
<i>Cheilymenia stercorea</i>		AY544661	AY544733
<i>Chiodecton natalense</i>		EU704085	EU704051
<i>Chlorociboria aeruginosa</i>		AY544669	AY544734
<i>Chrysothrix flavovirens</i> (L466)	Perlmutter 786, U.S.A. (NCU)	GU327711	GU327686
<i>Chrysothrix xanthina</i> (126)	Nelsen 4005, U.S.A. (F)	GU327712	GU327687
<i>Cladosporium cladosporioides</i>		DQ678057	FJ190628
<i>Cochliobolus heterostrophus</i>		AY544645	AY544737
<i>Cochliobolus sativus</i>		DQ678045	FJ190589
<i>Columnosphaeria fagi</i>		DQ470956	FJ713608
<i>Combea mollusca</i>		AY571382	AY571384
<i>Coniothyrium palmarum</i>		DQ767653	FJ190638
<i>Cordyceps capitata</i>		AY489721	FJ713628
<i>Cryptothecia assimilis</i> (86B)	Lumsch 19815l, Fiji (F)		GU327688

Table 1. (Continued).

Taxon	Collection	Accession Number	
		nuLSU	mtSSU
<i>Cryptothecia candida</i>			EU704052
<i>Cryptothelium amazonum</i> (47)	Nelsen 4000a, Peru (F)	GU327713	GU327689
<i>Cryptothelium cecidiogenum</i>			DQ328991
<i>Cryptothelium sepultum</i> (63C)	Nelsen 4001a, Peru (F)	GU327714	GU327690
<i>Cudoniella cf. clavus</i>		DQ470944	FJ713604
<i>Cystocoleus ebeneus</i>		EU048578	EU048584
		EU048579	EU048585
		EU048580	EU048586
			EU048587
<i>Delitschia winteri</i>		DQ678077	FJ190644
<i>Dendrographa alectoroides</i> (100)	Lumbsch 19914g, U.S.A. (F)	GU327715	GU327691
<i>Dendrographa leucophaea f. minor</i>		AF279382	AY548811
<i>Dendryphiella arenaria</i>		DQ470971	FJ190617
<i>Dermatocarpon minutum</i>		AY584644	AY584616
<i>Diaporthe eres</i>		AF408350	FJ190607
<i>Dichosporidium boschianum</i> (89B)	Lumbsch 19815a, Fiji (F)	GU327716	GU327692
<i>Dirina catalinariae</i>		EF081387	
<i>Dothidea insculpta</i>		DQ247802	FJ190602
<i>Dothidea sambuci</i>		AY544681	AY544739
<i>Dothiora cannabinae</i>		DQ470984	FJ190636
<i>Eleutherascus lectardii</i>		DQ470966	FJ190606
<i>Elsinoe centrolobi</i>		DQ678094	FJ190651
<i>Elsinoe phaseoli</i>		DQ678095	FJ190652
<i>Elsinoe veneta</i>		DQ767658	FJ190650
<i>Endocarpon pallidulum</i>		DQ823097	FJ225674
<i>Enterographa anguinella</i>		EU704086	EU704054
<i>Enterographa crassa</i>		EU704088	EU704056
<i>Erythrodictyon granulatum</i>		EU704090	EU704058
<i>Eupenicillium javanicum</i>		EF413621	FJ225778
<i>Exophiala salmonis</i>		EF413609	FJ225745
<i>Flavobathelium epiphyllum</i> (67)	Lücking s.n. Panama (F)	GU327717	
<i>Glomerella cingulata</i>		AF543786	FJ190626
<i>Glyphium elatum</i>		AF346420	AF346425
<i>Gnomonia gnomon</i>		AF408361	FJ190615
<i>Guignardia gaultheriae</i>		DQ678089	FJ190646
<i>Herpothallon rubrocinctum</i> (128)	Nelsen 4006, U.S.A. (F)		GU327693
<i>Herpotrichia diffusa</i>		DQ678071	DQ384076
<i>Hypocrea lutea</i>		AF543791	FJ713620
<i>Hysteropatella cf. elliptica</i>		DQ767657	FJ190649
<i>Kirschsteiniothelia aethiops</i>		AY016361	FJ190604
		DQ678046	FJ190590
<i>Lachnum virgineum</i>		AY544646	AY544745
<i>Laurera megasperma</i>		FJ267702	
<i>Lecanactis abietina</i>		AY548812	AY548813
<i>Lecanactis sp.</i>		EU704091	EU704059
<i>Lecanora hybocarpa</i>		DQ782910	DQ912273
<i>Macrophomina phaseolina</i>		DQ678088	FJ190645

Table 1. (Continued).

Taxon	Collection	Accession Number	
		nuLSU	mtSSU
<i>Megalotremis verrucosa</i> (104)	Lücking 26316, Colombia (F)	GU327718	GU327694
<i>Monilinia laxa</i>		AY544670	AY544748
<i>Mycocrothelia hemispherica</i> (102)	Lücking 28641, Nicaragua (F)	GU327719	GU327695
<i>Mycocrothelia miculiformis</i> (101B)	Lücking 28637, Nicaragua (F)	GU327720	GU327696
<i>Mycocrothelia obovata</i> (95)	Nelsen 4007a, Peru (F)	GU327721	GU327697
<i>Mycosphaerella fijiensis</i>		DQ678098	FJ190656
<i>Mycosphaerella punctiformis</i>		DQ470968	FJ190611
<i>Myriangium duriaei</i>		DQ678059	AY571389
<i>Nectria cinnabarina</i>		U00748	FJ713622
<i>Opegrapha celtidicola</i>		EU704094	EU704066
<i>Opegrapha filicina</i>		EU704095	EU704067
<i>Opegrapha lithyriga</i>		EU704096	EU704068
<i>Opegrapha varia</i>		EU704103	EU704075
<i>Ophionectria trichospora</i>		AF543790	FJ713626
<i>Peltigera degenii</i>		AY584657	AY584628
<i>Penicillium freii</i>		AY640958	AY584712
<i>Pertusaria dactylina</i>		DQ782907	DQ972973
<i>Phaeotrichum benjaminii</i>		AY004340	AY538349
<i>Phoma herbarum</i>		DQ678066	FJ190640
<i>Phyllobathelium anomalum</i> (242)	Lücking s.n., Panama (F)	GU327722	GU327698
<i>Phyllobathelium firmum</i> (HTL3175)	Lücking s.n., Panama (F)	GU327723	
<i>Pleospora herbarum</i> var. <i>herbarum</i>		DQ247804	FJ190610
<i>Preussia terricola</i>		AY544686	AY544754
<i>Pseudopyrenula subgregaria</i> (106)	Lücking 24079, Thailand (F)	GU327724	GU327699
<i>Pseudopyrenula subnudata</i>			DQ328997
<i>Pyrenophora phaeocomes</i>		DQ499596	FJ190591
<i>Pyrenophora tritici-repentis</i>		AY544672	FJ713605
<i>Pyrenula pseudobufonia</i>		AY640962	AY584720
<i>Pyrgillus javanicus</i>		DQ823103	FJ225774
<i>Pyxine subcinerea</i>		DQ883802	DQ912292
<i>Racodium rupestre</i>		EU048583	EU048588
		EU048581	
		EU048582	EU048589
<i>Ramichloridium anceps</i>		DQ823102	FJ225752
<i>Roccella canariensis</i>		AY779328	
<i>Roccella fuciformis</i>		AY584654	EU704082
<i>Roccella montagnei</i> (109)	Lumbsch 19700a, India (F)	GU327725	GU327700
<i>Roccella tuberculata</i>		AY779328	
<i>Roccellographa cretacea</i>		DQ883696	FJ772240
<i>Schismatomma decolorans</i>		AY548815	AY548816
<i>Schismatomma pericleum</i>		AF279408	AY571390
<i>Scorias spongiosa</i>		DQ678075	FJ190643
<i>Scutellinia scutellata</i>		DQ247806	FJ190587
<i>Simonyella variegata</i>			AY584631
<i>Sphinctrina turbinate</i>		EF413632	FJ713611
<i>Spiromastix warcupii</i>		DQ782909	FJ225794
<i>Sporormiella minima</i>		DQ678056	FJ190624

Table 1. (Continued).

Taxon	Collection	Accession Number	
		nuLSU	mtSSU
<i>Staurothele frustulenta</i>		DQ823098	FJ225702
<i>Strigula nemathora</i> (72)	Lücking s.n., Costa Rica (F)		GU327701
<i>Strigula schizospora</i> (73)	Lücking s.n., Costa Rica (F)		GU327702
<i>Stylodothis puccinioides</i>		AY004342	AF346428
<i>Sydowia polyspora</i>		DQ678058	FJ190631
<i>Synnesia farinacea</i>		EF081452	
<i>Trematosphaeria heterospora</i>		AY016369	AF346429
<i>Trematosphaeria pertusa</i>		DQ678072	FJ190641
<i>Trimmatostroma abietis</i>		DQ678092	FJ190648
<i>Trypetheliopsis kalbii</i> (243)	Lücking s.n., Panama (F)		GU327703
<i>Trypethelium eluteriae</i>			DQ328989
<i>Trypethelium eluteriae</i> (111)	Lumbsch 19701a, India (F)	GU327726	GU327704
<i>Trypethelium marcidum</i>			DQ329007
<i>Trypethelium marcidum</i> (132)	Nelsen 4008, U.S.A. (F)	GU327727	GU327705
<i>Trypethelium nitidiusculum</i> (139)	Nelsen 4002a, U.S.A. (F)	GU327728	GU327706
<i>Trypethelium papulosum</i> (97)	Nelsen 4009a, Peru (F)	GU327729	GU327707
<i>Trypethelium platystomum</i>			DQ329009
<i>Trypethelium tropicum</i> (25)	Nelsen 4003, Thailand (F)	GU327730	GU327708
<i>Tubeufia cerea</i>		DQ470982	FJ190634
<i>Tylophoron crassiusculum</i>		EU670258	
<i>Tylophoron moderatum</i>		EU670256	
<i>Tyrannosorus pinicola</i>		DQ470974	FJ190620
<i>Vibrissea truncorum</i>		FJ176874	FJ190635
<i>Westerdykella cylindrical</i>		AY004343	AF346430
<i>Xylaria hypoxylon</i>		AY544648	AY544760