Unravelling the phylogenetic relationships of lichenised fungi in Dothideomyceta

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Abstract: We present a revised phylogeny of lichenised fungi (Arthoniomycetes and Dothideomycetes) based on a combined data set of nuclear large subunit (nLSU) 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: Chrysothrixaceae (Chrysotrix), Arthoniaceae (Arthonia s. I., Cryptothecia, Herpothallus), and Roccellaceae (Chiocordot, Combma, Dendrographa, Dichosporidium, Enterographa, Erythrodeecten, Lecanactic, Opegrapha, Roccella, Roccelligorgia, Schismatomma, Simyrella). The widespread and common Arthonia caesia is strongly supported as a non-pigmented member of Chrysotrichia. Monoblastiaceae, Sigulaceae, and Trypetheliaceae are recovered as unrelated, monophyletic clades within Dothideomycetes. Also, the genera Arthropneus (Arthropneusaceae) and Cystocoleus and Racodium (Capnodiales) are confirmed as Dothideomycetes but unrelated to each other. Mycomicrothelia is shown to be unrelated to Arthropneus s. I., but is supported as a monophyletic clade sister to Trypetheliaceae, which is supported by hamathecum characters. The generic concept in several groups is in need of revision, as indicated by non-monophyly of genera, such as Arthonia, Astrotelium, Cryptothecia, Cryptothelium, Enterographa, Opegrapha, and Trypethelium in our analyses.

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

INTRODUCTION

Mutualism is one of the three main modes of nutrition within Ascomycota, besides saprotrophy 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, Persch et al. 2004, Wedin et al. 2005, del Prado et al. 2006, Mладикосква 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, Gueldan 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, Persch et al. 2004, Wedin et al. 2005, Mладикосква 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 cladenised clade, Arthoniomycetes, with about 1 500 species (Tehler 1995, Myllys et al. 1998, Sundin 2000, Teher & Irestedt 2007, Ertz et al. 2008). This class was recently shown to include the mazaeidate 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 apothecoid ascomata with partially ascolocular development and bitunicate asci (Hennessen & 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 Chrysotrichaceae, a small family of two genera (Byssocaulon, Chrysotrix) and little over 20 species (Kirk et al. 2008). The third primarily lichenised class is Lichinomycetes (350 species).
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 Verrucariaceae and once at base of Pyrenulales), though, this is uncertain as the ancestral state of the common ancestor to Verrucariales, Verrucarioideae 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 Dothidiomyceta, a name introduced by Schoch et al. (2009a, b). The ancestral state of Dothideomycota 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 Dothideomycota, specifically Dothideomycetes.

The following families have been confirmed or are believed to belong in either Chaetothyriomycetidae or Dothideomycetes: Verrucariales (930 species), Pyrenulaceae (280 species), Celastraceae (eight species), Microthelopsisidae (three species), and Pyrenostrichidae (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 Verrucales (Poelt 1973, Henssen & Jahns 1974, Hofherr 1986, Kirk et al. 2001, Eriksson et al. 2004, Cannon & Kirk 2007), and much of the confusion regarding previous classifications of these pyrenomycous lichens stems from the fact that Pyrenulales were at some point considered synonymous with the ascochloric Melanommatales (currently regarded synonymous with Pleosporales; Barr 1980, Hirs 1984, 1990, 1991, 1995), whereas other workers considered Pyrenulales to be ascochymenial (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 Capnodioidae/Dothideomycetes (Muggia et al. 2007), as well as Julella, Mycoroporum, Collemopsis (Pyrenocollina), and others, of unconfirmed affinities (Hirs 1995). Yet other lineages, such as the recently discovered Eremithallus (Lücking et al. 2008) or the genera Thelocarpon and Veizdae (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 polybori (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 Dothideomycota. 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 Dothideomycota 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 Dothideomycota 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 REDExtract-N-Amp Plant PCR Kit (SL 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 mssU1, mRSU2, mRSU2r and mRSU3r 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, 2 µL of each dNTP, 2 µL of 100 ng/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 agarose gel under UV light and bands were gel extracted, heated
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 point, from which a large number of sequences were removed. Lecanoromycetes, Arthoniomycetes, Dothideomycetes (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 a final 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 (Leotiomycetes, Sordariomycetes, Eurotiomycetes, Lecanoromycetes, Arthoniomycetes, Dothideomycetes). Arthoniomycetes and Dothideomycetes 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 Arthoniomycetes (Arthoniales) largely confirmed previous analyses, with Chrysothricaceae forming an additional family within this clade (Fig. 1). Arthoniaceae s. l. and Roccellaceae s. 1. 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-Herpotrichiaceae), the Tylophoraceae, Roccellaceae s. str., Opegraphaceae s. str., and possibly Chiodoraceae (as Chiodoctraceae is closely related to Erythroderon and Dichosporidium whereas the sequenced C. natalense is apparently not a Chiodoctraceae s. str.). Surprisingly, Arthonia caesia clustered with Chrysortheciaceae and not Arthoniaceae. Herpotrichon rubrocinctum is nested within Cryptotheca s. l.

Six distinct, lichenised lineages were confirmed as belonging to Dothideomycetes (Fig. 1): the order Trypetheliaceae, the families Arthoniaceae, Monoblastiacaeae, and Strigulaceae, and the genera Cystoceleus and Racodium. The latter two (Cystoceleus and Racodium) are members of the order Capnodiales, whereas Arthoniaceae, represented by the species Arthonia salica, was confirmed as clustering within Pleosporales. However, Arthoniaceae as currently defined, including the genera Julelia (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 Kirschsteiniothelia aethiops, but without support. Monoblastiacaeae was strongly supported and included four genera with one species each in this analysis: Acrocladia subglabrosa, Anisomendium ubiquianum, Megalotremis verrucosa, and Trypetheliopsis (syn. Musaespora) kalbii. Initially we also included a GenBank sequence of Anisomendium polyponi in the data set, but the nuLSU sequence was recovered in Eurotiomycetes 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 perithelial and ascospore core genera Astrolithium, Laurera, and Trypetheliaceae, 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 Arthoniomycetes and Dothideomycetes. 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 Arthoniomycetes (not included in our sampling).

Arthoniomycetes 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 Arthoniomycetes in three families: Arthoniaceae, Chrysortheciaceae, and Roccellaceae (Tehler 1995, Grube 1998, Teherl & Tresstl 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
unclear. Along with previous data (Tehter 1995, Myllys et al. 1998, Tehter & 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-Enterographa complex: while the sequenced Chiodecton natalense appears to be unrelated to the morphologically and anatomically similar Dichosporidium and Erythrodecton (Thor 1990), Enterographa and the similar Schismatomma (Sparrius 2004) were found in three different clades related to either Chiodecton natalense (Schismatomma), Dichosporidium (Enterographa crassa), and Opegrapha (Enterographa anguinella), respectively. This is in agreement with
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 Chiodolecton-like species (Dichosporidium and Erythrodecton). Consequently, Ertz et al. (2009) transferred Enterographa anguinella 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 Roccellolographa cretacea.
Hemithallium rubrocininctum is now confirmed as an ascomycete in Arthoniaceae. 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 Hemithallium nested within Cryptothecia, supporting the previous hypothesis that byssoid-islidate 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-Hemithallium 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 Typhorhion, a genus that has passive spore dispersal and was previously assigned to Calicariaes, is here confirmed as a member of Arthoniaceae s. l., in agreement with Lambrecht et al. (2009a).

The strongly supported placement of Arthonia caesia within Chrysotrichiaceae is unexpected; however, fertile species of Chrysotrichiaceae are very similar to Arthonia in ascma morphology and anatomy, and particularly A. caesia and allies can be easily perceived as non-pigmented species of Chrysotrichiaceae 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 Chrysotrichiaceae. The latter genus was previously placed in its own family Chrysotrichaceae, 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 Chrysotrichaceae 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. rubrocinincta, two species with reddish pigments, form a weakly supported group. If future efforts confirm this grouping, the name Coniocarpus can be used for this clade (Cáceres 2007).

In contrast to Arthoniaceae, the overwhelming majority of Dothideomycetes species are non-lichenised. In addition to Arthotheliumaceae, Trypetheliaceae and Cystocteolus 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 (Schöch 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 (= Musaeospora) have developed unique pycnidia that in part are similar to campyliida and hypophyphores 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 Phyllocrateria 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 Phyllobatheliales 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 epiideral thallus with algae belonging to Trentepohliaceae; however, Anisomeridium is often found lichicolous and Apriotia grows on bryophytes. Detailed studies in Costa Rica suggest Trypetheliales 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). Trypetheliales 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 multisepate or muriform ascospores (Harris 1984, 1990, 1995, Aptroot 1991b, Aptroot et al. 2008). The secondary chemistry is equally simple, with lichenexanthone and pigments as most common substances, i.e. polyketide derived aromatic compounds produced through the acetyl-polyomalonyl 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
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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 Trypetheliaceae. 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.

Fig. 3. Select lichenised Dothideomycetes; A. Arthopyrenia cinchonae; B. Mycomicrothelia modesta; C. Anisomeridium subprostans; D. Anisomeridium spec. (pycnidia); E. A. foliicola (pycnidia); F. Capretta amazonensis (pycnidia); G. Megalotremis cauli flora (pycnidia); H. Trypetheliopsis (= Musaespora) coccinea (campylidia); I. Strigula viridasea; J. S. laurensformis (pycnidia); K. S. smaragdula; L. Flavobathelium epiphyllum; M. Phyllobathelium firmum; N. P. leguminosae (pycnidia); O. Pseudopyrenula subnudata; 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.
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 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

| Genus              | Zahlbruckner 1926 | Barr 1987  | Harris 1995 | current  |
|--------------------|-------------------|------------|-------------|----------|
| Celothelium        | Pyrenocarpaceae   | Loculoascomycetes | Loculoascomycetes | Eurotiomycetes |
|                    | (as Leptorhaphis) | Pleosporales | Melanommatales | Pyrenulales |
| Pyrenula           | Pyrenocarpaceae   | Loculoascomycetes | Loculoascomycetes | Eurotiomycetes |
|                    | Trypetheliaceae   | Melanommatales | Melanommatales | Pyrenulales |
| Lithothelium       | Pyrenocarpaceae   | Loculoascomycetes | Loculoascomycetes | Eurotiomycetes |
|                    | Pleosporales      | Theleniellaceae | Cylindriellaceae | Cylindriellaceae |
| Pyrenula           | Pyrenocarpaceae   | Pyrenulaceae | Pyrenulaceae | Pyrenulaceae |
|                    | Trypetheliaceae   | Pyrenulaceae | Pyrenulaceae | Pyrenulaceae |
| Arthopyrena        | Pyrenocarpaceae   | Loculoascomycetes | Loculoascomycetes | Dothideomycetes |
|                    | Pleosporales      | Pleosporales | Pleosporales | Pleosporales |
|                    | Arthopyreniaceae  | Pleosporales | Arthopyreniaceae | Arthopyreniaceae |
| Acrocodia          | Pyrenocarpaceae   | Loculoascomycetes | Loculoascomycetes | Dothideomycetes |
|                    | Pleosporales      | Pleosporales | Pleosporales | Pleosporales |
|                    | Arthopyreniaceae  | Pleosporales | Arthopyreniaceae | Arthopyreniaceae |
| Anisomeridium      | Pyrenocarpaceae   | Loculoascomycetes | Loculoascomycetes | Dothideomycetes |
| (as Arthopyrenia)  | Pleosporales      | Melanommatales | incertae sedis | Melanommatales |
| Phyllobathelium    | Pyrenocarpaceae   | Loculoascomycetes | Loculoascomycetes | Dothideomycetes |
|                    | Pleosporales      | Pleosporales | Pleosporales | Pleosporales |
|                    | Monoblastiaceae   | Monoblastiaceae | Monoblastiaceae | Monoblastiaceae |
| Strigula           | Strigulaceae      | Strigulaceae | Strigulaceae | Strigulaceae |
|                    | Chaetothyriales   | Strigulaceae | Strigulaceae | Strigulaceae |
| Astrophyrella      | Pyrenocarpaceae   | Loculoascomycetes | Loculoascomycetes | Dothideomycetes |
|                    | Pleosporales      | Pleosporales | Pleosporales | Pleosporales |
|                    | Acrocoriaceae     | Acrocoriaceae | Monoblastiaceae | Monoblastiaceae |
| Camphyrothelium    | Pyrenocarpaceae   | Loculoascomycetes | Loculoascomycetes | Dothideomycetes |
|                    | Pleosporales      | Pleosporales | Pleosporales | Pleosporales |
|                    | Monoblastiaceae   | Monoblastiaceae | Monoblastiaceae | Monoblastiaceae |
| Laurera            | Pyrenocarpaceae   | Trypetheliaceae | Trypetheliaceae | Trypetheliaceae |
|                    | Pleosporales      | Pleosporales | Pleosporales | Pleosporales |
| Pseudopyrenula     | Pyrenocarpaceae   | Trypetheliaceae | Trypetheliaceae | Trypetheliaceae |
|                    | Pleosporales      | Pleosporales | Pleosporales | Pleosporales |
| Trypethelium       | Pyrenocarpaceae   | Trypetheliaceae | Trypetheliaceae | Trypetheliaceae |
|                    | Pleosporales      | Pleosporales | Pleosporales | Pleosporales |
| Mycomicrothelia    | Pyrenocarpaceae   | Loculoascomycetes | Loculoascomycetes | Dothideomycetes |
| (as Microthelium)  | Pleosporales      | Pleosporales | Pleosporales | Pleosporales |
|                    | Arthopyreniaceae  | Arthopyreniaceae | Arthopyreniaceae | Arthopyreniaceae |
| Porina             | Pyrenocarpaceae   | Hymenoascomycetes | Lecanoromycetes | Lecanoromycetes |
|                    | Pleosporales      | Pleosporales | Pleosporales | Pleosporales |
|                    | Trichotheliales   | Ostropales | Ostropales | Ostropales |
| Trichothelium      | Pyrenocarpaceae   | —           | Trichotheliales | Porinaceae |
|                    | Strigulaceae      | —           | Strigulaceae | Strigulaceae |

Table 2. Systematic placement of selected pyrenocarpous lichens according to different concepts.
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, asciaceous 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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### 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         | nuLSU Accession | mtSSU Accession |
|------------------------|--------------------|-----------------|-----------------|
| Acrocordia subglobosa  | Palice s.n., Poland (F) | GU327681        |                 |
| Amphiphilia umbrina    |                    | FJ76863         | FJ713609        |
| Anisomeridium ubianum  | Lumbsch 19845, Fiji (F) | GU327709        | GU327682        |
| Aproclia terricola     |                    | DQ328995        |                 |
| Arthonia caesia        |                    | FJ469668        | FJ469671        |
| Arthonia didyma        |                    | EU704083        | EU704047        |
| Arthonia dispersa      |                    | AY571381        | AY571383        |
| Arthonia radiate       |                    | EU704048        |                 |
| Arthonia ruana (79B)   | Zimmerman 1117, Germany (F) | GU327683        |                 |
| Arthonia rubrocincta   | Nelsen 4010, U.S.A. (F) | GU327684        |                 |
| Arthopyrenia salicis  |                    | AY538339        | AY538345        |
| Ascolobus crenulatus   |                    | AY607730        | AY607742        |
| Asterothelium cinnamomeum |                  | AY584652        | AY584632        |
| Astrothelium confusum  | Nelsen 4004a, Peru (F) | GU327710        | GU327685        |
| Bacidia schweinfurthii |                    | DQ752911        | DQ752998        |
| Bathelium degenerans   |                    | DQ328987        | DQ328998        |
| Bimuria novae-zelandiae|                    |                 |                 |
| Bionectria ochroleuca  |                    | AY489716        | FJ713619        |
| Botryosphaeria dothidea|                    | DQ678051        | FJ713628        |
| Botryosphaeria stevensii|                  | DQ678064        |                 |
| Botryosphaeria tzaige  |                    | DQ767655        |                 |
| Botryotinia fuckeliana |                    | AY544651        | AY544732        |
| Calicopsis orientalis  |                    | DQ470987        | FJ7190654       |
| Calicopsis pinee       |                    | DQ678097        | FJ7190653       |
| Camarops ustulinaoides |                    | DQ470941        | FJ7190588       |
| Capnodium coffeae      |                    | DQ247800        | FJ7190609       |
| Capronia pilosella     |                    | DQ823099        | FJ725725        |
| Ceratotheca essertoria  |                    | DQ678091        | FJ7190647       |
| Chelidonia stercorea   |                    | AY544661        | AY544733        |
| Chiodecton natalense   |                    | EU704085        | EU704051        |
| Chlorociboria aeruginosa|                   | AY544669        | AY544734        |
| Chrysotrichum flavovirens (L466) | Perlmutter 786, U.S.A. (NCU) | GU327711        | GU327686        |
| Chrysotrichum xanthina  | Nelsen 4005, U.S.A. (F) | GU327712        | GU327687        |
| Cladosporum cladosporoides |               | DQ678057        | FJ7190628       |
| Cochliobolus heterostrophus |               | AY544645        | AY544737        |
| Cochliobolus sativus    |                    | DQ678045        | FJ7190589       |
| Columnosphaeria fagi   |                    | DQ470956        | FJ7190608       |
| Combea mollusca        |                    | AY571382        | AY571384        |
| Coniotrichum palmarum  |                    | DQ767653        | FJ7190638       |
| Cordyceps capitata     |                    | AY489721        | FJ7190628       |
| Cryptothecia assimilis  | Lumbsch 19815, Fiji (F) | GU327688        |                 |
Table 1. (Continued).

| Taxon                                      | Collection                  | Accession Number          |
|--------------------------------------------|-----------------------------|---------------------------|
| Cryptothecium candida                      | Nelsen 4000a, Peru (F)      | GU327714, GU327689        |
| Cryptothelium amazonum (47)                |                             | GU327714, GU327690        |
| Cryptothelium occidiogenum                 |                             | DQ328991                  |
| Cryptothelium sepultum (63C)               | Nelsen 4001a, Peru (F)      | GU327714, GU327690        |
| Cudoniella cf. clavus                      |                             | DQ470944, FJ13604        |
| Cystocoleus ebeeneus                       |                             | EU048578, EU048584        |
| Cryptothelium amazonum                     |                             | EU048579, EU048585        |
| Cryptothelium cecidiogenum                 |                             | EU048580, EU048586        |
| Cudoniella cf. clavus                      |                             | EU048587                  |
| Cryptothelium sepultum                     |                             | EU048587                  |
| Delitschia winteri                         |                             | EU048587                  |
| Denrographa alectoroides (100)             | Lumbsch 19914g, U.S.A. (F)  | GU327715, GU327691        |
| Denrographa leucophae f. minor             |                             | AF279382, AY548811        |
| Dendryphia arenaria                        |                             | DQ470971, FJ190617        |
| Dermatocarpon minutum                      |                             | AY584644, AY584616        |
| Diaporthe eres                             |                             | AF408350, FJ190607        |
| Dichosporidium boschianum (89B)            | Lumbsch 19815a, Fiji (F)    | GU327716, GU327692        |
| Dirina catalinariae                        |                             | EF081387                  |
| Dothidea insculpta                         |                             | DQ247802, FJ190602        |
| Dothidea sambuci                           |                             | AY544681, AY544739        |
| Dothiora cannabinae                        |                             | DQ470984, FJ190636        |
| Eleutherascus lectardi                     |                             | DQ470966, FJ190606        |
| Elatino centrolobi                         |                             | DQ678094, FJ190651        |
| Elsinoe phaseoli                           |                             | DQ678095, FJ190652        |
| Elsinoe veneta                             |                             | DQ767658, FJ190650        |
| Endocarpon pallidulum                      |                             | DQ823097, FJ225674        |
| Enterographa anguinella                    |                             | EU704086, EU704054        |
| Enterographa crassa                        |                             | EU704088, EU704056        |
| Erythrodecton granulatum                   |                             | EU704090, EU704058        |
| Eupenicillium javanicum                    |                             | EF413621, FJ225778        |
| Exophiliala salminus                       |                             | EF413609, FJ225745        |
| Flavobathelium epiphyllum (67)             | Lücking s.n. Panama (F)     | GU327717                  |
| Glomerella cingulata                       |                             | AF543786, FJ190626        |
| Glyphium elatum                            |                             | AF346420, AF346425        |
| Gnomonia gnomon                            |                             | AF408361, FJ190615        |
| Guignardia gaulteriae                      |                             | DQ678089, FJ190646        |
| Hachystothelella rubrocinctum (128)         | Nelsen 4006, U.S.A. (F)     | GU327693                  |
| Herpotrichia diffusa                       |                             | DQ678071, DQ384076        |
| Hypocrea lutea                             |                             | AF543791, FJ13620         |
| Hysteropatella f. elliptica                |                             | DQ767657, FJ190649        |
| Kirschsteiniothelia aethiopis              |                             | AY016361, FJ190604        |
| Lachnum virgineum                         |                             | DQ678046, FJ190590        |
| Lecanactis abietina                        |                             | AY544646, AY544745        |
| Lecanactis sp.                             |                             | FJ267702                  |
| Lecanora hybocarpa                         |                             | AY548812, AY548813        |
| Macrooomina phaseolina                     |                             | EU704091, EU704059        |
| Macrophomina phaseolina                    |                             | DQ782910, DQ912273        |
| Macrophomina phaseolina                    |                             | DQ678088, FJ190645        |
| Taxon                                      | Collection                  | Accession Number | nuLSU          | mtSSU          |
|-------------------------------------------|-----------------------------|------------------|---------------|---------------|
| Megalotremis verrucosa (104)              | Lücking 26316, Colombia (F) | GU327718         | GU327694      |
| Moniliá saxa                              |                             | AYS44670         | AYS44748      |
| Mycomicrothelia hemispherica (102)        | Lücking 28641, Nicaragua (F)| GU327719         | GU327695      |
| Mycomicrothelia miculiformis (101B)       | Lücking 28637, Nicaragua (F)| GU327720         | GU327696      |
| Mycomicrothelia obovata (96)              | Nielson 4007a, Peru (F)     | GU327721         | GU327697      |
| Mycosphaerella fijensis                  |                            | DQ678098         | FJ190566      |
| Mycosphaerella punctiformis               |                            | DQ470968         | FJ190611      |
| Myriangium duriaeí                        |                            |                  |               |
| Nectria cinnabarina                       |                            | U00748           | FJ13622       |
| Opegrapha celtidicola                     |                            | EU704094         | EU704066      |
| Opegrapha filicina                        |                            | EU704095         | EU704067      |
| Opegrapha lillygya                        |                            | EU704096         | EU704068      |
| Opegrapha varia                           |                            | EU704013         | EU704075      |
| Ophionectria trichospora                  |                            | AF543790         | FJ13626       |
| Pelitgera degenií                         |                            | AYS84657         | AYS84628      |
| Penicillium freii                         |                            | AYS64958         | AYS84712      |
| Pertusaria dacylvina                      |                            | DG782907         | DG792973      |
| Phaeotrichum benjaminii                   |                            | AY004340         | AYS38349      |
| Phoma herbarum                            |                            | DQ678066         | FJ190640      |
| Phyllothrophium anomalam (242)            | Lücking s.n., Panama (F)   | GU327722         | GU327698      |
| Phyllothrophium firmum (HTL3175)          | Lücking s.n., Panama (F)   | GU327723         |               |
| Pleospora herbarum var. herbarum          |                            | DQ247804         | FJ190610      |
| Preussia terricola                        |                            | AY544686         | AY544754      |
| Pseudopynenua subgregaria (106)           |                             | GU327724         | GU327699      |
| Pseudopynenua subnudata                   |                            | DQ328997         |               |
| Pyrenophora phaeocomes                     |                            | DQ499596         | FJ190591      |
| Pyrenophora trifici-repentis               |                            | AY544672         | FJ13605       |
| Pyrenula pseudobufonia                     |                            | AY640962         | AYS84720      |
| Pyrgillus javanicus                        |                            | DG823103         | FJ225774      |
| Pyxine subcinerea                          |                            | DG853802         | DG912292      |
| Racodium rupestre                         |                            | EU048583         | EU048588      |
| Ramichloridium anops                       |                            | EU048582         | EU048589      |
| Roccella canariensis                       |                            | DG823102         | FJ225752      |
| Roccella luciformalis                      |                            | AY773328         |               |
| Roccella montagnei (109)                   | Lumbsch 19700a, India (F)  | GU327725         | GU327700      |
| Roccella tuberculata                       |                            | AY779328         |               |
| Roccella reticulata                        |                            | DQ836966         | FJ772240      |
| Schismatommata decolorans                 |                            | AYS48815         | AYS48816      |
| Schismatommata periculum                   |                            | AF279408         | AYS71390      |
| Scorias spongiosa                         |                            | DG878075         | FJ190643      |
| Scutellinia scutellata                     |                            | DG247806         | FJ190587      |
| Simonyella variegata                       |                            | AYS84631         |               |
| Sphinctrina turbinata                      |                            | EF413362         | FJ713611      |
| Spiromastix warcupii                       |                            | DG782909         | FJ225794      |
| Sporormiella minima                        |                            | DG678056         | FJ190624      |
| Taxon                                | Collection                        | nuLSU     | mtSSU     |
|--------------------------------------|-----------------------------------|-----------|-----------|
| Staurophle frustulenta               | Lücking s.n., Costa Rica (F)      | DQ823098  | FJ225702  |
| Strigula nemathora (72)              | Lücking s.n., Costa Rica (F)      | DQ823098  | FJ225702  |
| Strigula schizospora (73)            | Lücking s.n., Costa Rica (F)      | DQ823098  | FJ225702  |
| Styphodon rhothites lucinioides      |                                   | AY004342  | AF346428  |
| Sydowia polyspora                    |                                   | DQ878058  | FJ190631  |
| Syncisia farinacea                   |                                   | EF081452  |           |
| Trematosphaeria heterospora          |                                   | AY016369  | AF346429  |
| Trematosphaeria pertusa              |                                   | DQ878072  | FJ190641  |
| Trimmatostroma abietis               |                                   | DQ878092  | FJ190648  |
| Trypetheliopsis kalbii (243)         | Lücking s.n., Panama (F)          |           | GU327703  |
| Trypetheliopsis eluteriic            |                                   | DQ328989  |           |
| Trypetheliopsis eluteriic (111)      | Lumbsch 19701a, India (F)         | GU327726  | GU327704  |
| Trypetheliopsis marsicum             |                                   | DQ329007  |           |
| Trypetheliopsis marsicum (132)       | Nelsen 4008, U.S.A. (F)           | GU327727  | GU327705  |
| Trypetheliopsis nitidiusculum (139)  | Nelsen 4002a, U.S.A. (F)          | GU327728  | GU327706  |
| Trypetheliopsis papulosum (97)       | Nelsen 4009a, Peru (F)            | GU327729  | GU327707  |
| Trypetheliopsis platystomum          |                                   | DQ329009  |           |
| Trypetheliopsis tropicum (25)        | Nelsen 4003, Thailand (F)         | GU327730  | GU327708  |
| Tubeufia cerea                       |                                   | DQ470982  | FJ190634  |
| Tylophorion crassiusculum            |                                   | EU670258  |           |
| Tylophorion moderatum                |                                   | EU670256  |           |
| Tyranosorus pinicola                 |                                   | DQ470974  | FJ190620  |
| Vibrissea truncorum                  |                                   | FJ176874  | FJ190635  |
| Westendyella cylindrical             |                                   | AY004343  | AF346430  |
| Xylaria hypoxylon                    |                                   | AY544648  | AY544760  |