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Eurotiomycetes: Eurotiomycetidae and Chaetothyriomycetidae

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Abstract: The class Eurotiomycetes (Ascomycota, Pezizomycotina) is a monophyletic group comprising two major clades of very different ascomycetous fungi: (i) the subclass Eurotiomycetidae, a clade that contains most of the fungi previously recognized as Plectomycetes because of their mostly enclosed ascomata and prototunicate asci; and (ii) the subclass Chaetothyriomycetidae, a group of fungi that produce ascomata with an opening reminiscent of those produced by Dothideomycetes or Sordariomycetes. In this paper we use phylogenetic analyses based on data available from the Assembling the Fungal Tree of Life project (AFTOL), in addition to sequences in GenBank, to outline this important group of fungi. The Eurotiomycetidae include producers of toxic and useful secondary metabolites, fermentation agents used to make food products and enzymes, xerophiles and psychrophiles, and the important genetics model *Aspergillus nidulans*. The Chaetothyriomycetidae include the common black yeast fungi, some of which are pathogens of humans and animals, as well as some primarily lichenized groups newly found to be phylogenetically associated with this group. The recently proposed order Mycocaliciales shows a sister relationship with Eurotiomycetes. The great majority of human pathogenic Pezizomycotina are Eurotiomycetes, particularly in Eurotiales, Onygenales and Chaetothyriales. Due to their broad importance in basic research, industry and public health, several genome projects have focused on species in Onygenales and Eurotiales.

Key words: Cleistothecium, industrial fungi, medically important fungi

INTRODUCTION

Molecular data have been crucial in defining the class Eurotiomycetes, which is now known to comprise a morphologically and ecologically disparate set of fungi. Early phylogenetic analyses of the Ascomycota based on nuclear small ribosomal subunit sequences revealed a common ancestry between *Capronia pilosella* (Chaetothyriales), with bitunicate asci and ascolocular development, and the fungi then recognized as the monophyletic Plectomycetes (Geiser and LoBuglio 2001) and as Eurotiomycetes by Eriksson (1999) (Berbee 1996, Spatafora et al 1995), which generally produce prototunicate asci in enclosed ascomata. This link led Berbee (1996) to propose that the Eurotiomycetes evolved by loss of the bitunicate ascus and its corresponding mode of forcible discharge. The possibility of the cleistothecium evolving by means of paedomorphosis or ''arrested development'' was proposed by Eriksson (1982). Later phylogenetic analyses further supported a connection between the Chaetothyriales and the prototunicate Eurotiomycetes (Liu et al 1999, Lumbsch et al 2000, Silva-Hanlin and Hanlin 1999, Winka et al 1998). Based on the distinctiveness of Chaetothyriales, Chaetothyriomycetes (Eriksson and Winka 1997) and Chaetothyriomycetidae (Kirk et al 2001) were proposed as supraordinal taxa.

Despite the generally low node support in some previous studies of the Eurotiomycetes comprising both Eurotiomycetidae and Chaetothyriomycetidae (Liu and Hall 2004, Lutzoni et al 2001, Reeb et al 2004), the result appears to be supported by multiple datasets involving multiple genes and taxon sets (Lutzoni et al 2004). As shown in this volume, weighted parsimony analysis of sequences from five gene regions yielded 89% bootstrap support and Bayesian analysis yielded 100% posterior probability for the common ancestry of the subclasses (Spatafora

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et al 2006). Furthermore this analysis placed Coryneliales in a basal position within a strongly supported Eurotiomycetidae clade, indicating that members of this clade evolved from fissitunicate/ascolocular ancestors (Schoch et al 2006, Spatafora et al 2006).

Changing concepts of class Eurotiomycetes.—Nannfeldt (1932) played a key role in integrating ontogenetic features into ascomycete taxonomy, creating three major groups based on ascomal development. One of these groups, Plectascales, was defined based on the production of naked asci and antheridia. In this group enclosed cleistothecial ascomata form after ascogenous hyphae are enveloped in sterile mycelia. This group included all fungi with globose ascomata and irregularly disposed evanescent asci.

Eurotiomycetidae is a monophyletic group encompassing a wide variety of morphologies, which includes many traditionally defined plectomycete-like fungi. Based on phylogenetic analyses of the nuclear ribosomal small subunit RNA gene (SSU), Eriksson (1999) proposed the class Eurotiomycetes for the clade comprising most fungi known morphologically as ''Plectomycetes'' and outlined as ''the monophyletic Plectomycetes'' by Geiser and LoBuglio (2001). Although some concepts have included perithecial taxa (Benny and Kimbrough 1980, Luttrell 1951) ''Plectomycetes'' generally refers to fungi with these morphological characters: (i) thin-walled, globose to pyriform, prototunicate asci (i.e. asci with walls that break down at maturity to release the ascospores within the ascoma in a passive fashion, rather than forcible discharge through an apical pore); (ii) ascomata without a distinct hymenial layer and with asci forming scattered within the ascomatal cavity; (iii) unicellular ascospores; (iv) ascomata varying from gymnothecial to cleistothecial, lacking an ostiolar opening and with highly variable cleistothecial peridia, that may or may not be produced within a stromatal structure; and (v) highly variable phialoblastic and thallic hyphomycetous anamorphs (Alexopoulos et al 1996, Geiser and LoBuglio 2001). Both Eriksson (1999) and Geiser and LoBuglio (2001) recognized similar fungi as making up this clade. Eriksson organized them into two major orders. Eurotiales contained families Elaphomycetaceae, Monascaceae and Trichocomaceae and Onygenales comprised Arthrodermataceae, Ascosphaeraceae, Eremascaceae, Gymnoascaceae and Onygenaceae. Based on SSU sequences Gibas et al (2002) defined the new order Arachnomycetales to accommodate fungi in the genus *Arachnomyces*. This genus previously had been placed within Onygenales based on morphology, but DNA characters grouped it with Eurotiales. Previous analyses based on SSU sequences showed *Ascosphaera* and *Eremascus* to form a clade distinct from Onygenales (Berbee and Taylor 1992), leading Geiser and LoBuglio (2001) to infer Ascosphaerales as a distinct order. (Lutzoni et al 2004) added further evidence in support of Ascosphaerales based on SSU and nuclear ribosomal large subunit RNA gene (LSU) sequences, showing *Ascosphaera apis, Eremascus albus* and *Paracoccidioides brasiliensis* to form a strongly supported clade.

Newly included orders.—The subclass Chaetothyriomycetidae first was proposed to accommodate the order Chaetothyriales (Kirk et al 2001). The order Verrucariales subsequently was shown to be sister to Chaetothyriales (Lutzoni et al 2001) and was added to this subclass (Eriksson 2001). Recent molecular studies also placed the order Pyrenulales in this group (reviewed in Lutzoni et al 2004). Members of these three orders all are characterized by perithecial ascomata and bitunicate asci with a dehiscence ranging from fissitunicate to evanescent. Historically classifications of ascomycetes treated either lichenized or nonlichenized taxa, but rarely both, because they were recognized as two different groups (Acharius 1810, Lindau 1897, Zahlbruckner 1926). It was only in the 20th century that these classifications were merged (Luttrell 1951, 1955; Nannfeldt 1932), and indeed the Chaetothyriomycetidae stands as the prime example of the need for such a combined system. The family Pyrenulaceae, together with species from the Verrucariaceae, then were included in the order Xylariales based on their ascus type and centrum, identified as Xylaria-type (Luttrell 1951, 1955). In the same classification the family Herpotrichiellaceae was assigned to Pleosporales based on its centrum development (Luttrell 1951, 1955).

Eriksson's (1982) system divided what is now recognized as the Pezizomycotina into four groups according to ascus type; the new orders Pyrenulales and Verrucariales were placed in the bitunicate group. The existing families Chaetothyriaceae and Herpotrichiellaceae were included in the bitunicate order Dothideales (Eriksson 1982). Barr was one of the first authors to consider the three orders Verrucariales, Chaetothyriales and Pyrenulales (at that time included in the Melanommatales) as closely related, based on ascus type (bitunicate or secondarily prototunicate) and hamathecium structure (insertion of sterile filaments in the centrum, either pseudoparaphyses or periphysoids) (Barr 1983). Molecular studies confirmed the close phylogenetic relationships among these three orders as proposed by Barr (Lutzoni et al 2004 and references therein). The Verrucariales was added to the Chaetothyriomyceti-

dae (Eriksson 2001), followed by the Pyrenulales (Eriksson 2006).

Inclusion of Mycocalicales and Coryneliales.—Mycocaliciales was described to include nonlichenized members of the families Mycocaliciaceae and Sphinctrinaceae (Tibell and Wedin 2000). These fungi are parasites or commensals on lichenized or saprotrophic fungi and produce stalked or sessile apothecial ascomata, often referred to as mazaedia. Analysis of nuclear ribosomal small subunit data showed a weakly supported phylogenetic connection between *Mycocalicium* and plectomycetous Eurotiomycetes (Wedin et al 1998). A broader analysis of lichenized fungi, including Mycocaliciales, recovered a moderately supported clade that placed Mycocaliciales basal to a group that included Eurotiales, Lichinales (now recognized at the class level), Chaetothyriales and Verrucariales (Wedin et al 2005), leading to its placement as an order of uncertain position within Eurotiomycetes (Eriksson 2006).

Our aim in this paper is to combine ribosomal as well as protein-coding DNA sequences obtained by the AFTOL project and those available through GenBank and the various genome projects to infer a phylogeny for the class. The inferred phylogeny will be used as the basis for a discussion of character evolution in this diverse and important group of fungi.

MATERIALS AND METHODS

Sampling and alignments.—Sequence data were obtained from GenBank and the Assembling the Fungal Tree of Life Project (AFTOL, http://www.aftol.org). All strains and sequences used in this study are provided (SUPPLEMENTARY TABLE I). DNA alignments were assembled with Clustal X (Thompson et al 1997) and manually edited with the shareware package BioEdit (v7.0; Tom Hall, Carlsbad, California). Newly generated DNA sequences were deposited at GenBank (SUPPLEMENTARY TABLE I). Isolates obtained from the culture collections were verified where possible by comparison with DNA sequences obtained from previous studies and deposited at GenBank. Herbaria and culture collections where strains and specimens used in this study are deposited are listed (SUPPLEMENTARY TABLE I).

Phylogenetic analysis.—Maximum and weighted parsimony (MP and WP) analyses were performed in PAUP* v4.0b (Swofford 2002) on a combined dataset with a total of 81 taxa that included 49 taxa classified as Eurotiomycetes and representatives of all classes of Ascomycota except the Laboulbeniomycetes, Lichinomycetes and Orbiliomycetes. The sequence alignment was submitted in TreeBASE (www.treebase.org) under accession No. SN2996. Thirteen taxa used contained data only for the ribosomal loci but were included to maximize taxon sampling. Comparative analyses with only ribosomal data yielded congruent topologies (data not shown). We rooted the tree with four taxa from the class Pezizomycetes as outgroups (*Pyronema domesticum, Caloscypha fulgens, Aleuria aurantia* and *Gyromitra californica*) (not shown in figure).

For WP analyses unambiguously aligned regions were subjected to symmetric step matrices for 11 partitions (i.e. nuc SSU rDNA, nuc LSU rDNA, and codon positions of tef1, RPB1 and RPB2) to incorporate the differences in substitution rates and patterns as described in Lutzoni et al (2004). MP and WP analyses were performed with these settings: 100 replicates of random sequence addition, TBR branch swapping, and MULTREES in effect. Maximum likelihood was performed with RAxML-VI-HPC (Stamatakis et al 2005) using a GTRCAT model of evolution with 50 rate categories. In all preceding cases nodal support was verified by nonparametric bootstrapping under the conditions mentioned, with 500 replicates.

Initial incongruence in the single gene trees for the taxa used was tested by examining single gene analyses with WP under the conditions previously mentioned for a set of taxa containing data for all four loci (Lutzoni et al 2004). A 70% majority rule consensus tree was compared in each case.

A Bayesian analysis was performed with a parallelized version of MrBayes v3.1.2 across four processors (Huelsenbeck and Ronquist 2001, Ronquist and Huelsenbeck 2003). MrBayes was run with these parameters: a general time reversible (GTR) model of DNA substitution with gamma-distributed rate variation across sites (invariance, partitioning across genes and codons). A Markov chain Monte Carlo (MCMC) analysis with metropolis coupling was run starting from a random tree for 10×10^6 generations, sampling every 100th cycle. Four chains were run simultaneously with the initial 10 000 cycles discarded as burn-in. Two additional runs with 5×10^6 generations were compared to confirm that stationarity in likelihood values was reached and compared. The phylogenies obtained in all cases were congruent. A 50% majority rule tree from a total of 90 000 trees obtained from a single run is presented (FIG. 1).

RESULTS AND DISCUSSION

An inferred Eurotiomycete phylogeny (FIG. 1) indicates that the class is monophyletic and contains two strongly supported subclasses, Eurotiomycetidae and Chaetothyriomycetidae. A discussion of the major characters associated with the Eurotiomycetes and its resident taxa follows with attention given to their evolution.

Eurotiomycetidae.—Eurotiomycetidae (FIGS. 2–7) comprises most of the taxa known previously as ''the monophyletic Plectomycetes'' (Geiser and LoBuglio 2001) and ''Eurotiomycetes'' (Eriksson 1999), as well as the Coryneliales, which possess more dothideomycete-like characters.

Coryneliales.—The missing link? Members of Coryneliales (*Caliciopsis orientalis, Ca. pinea* and *Cornelia uberata*) form a strongly supported, basally positioned

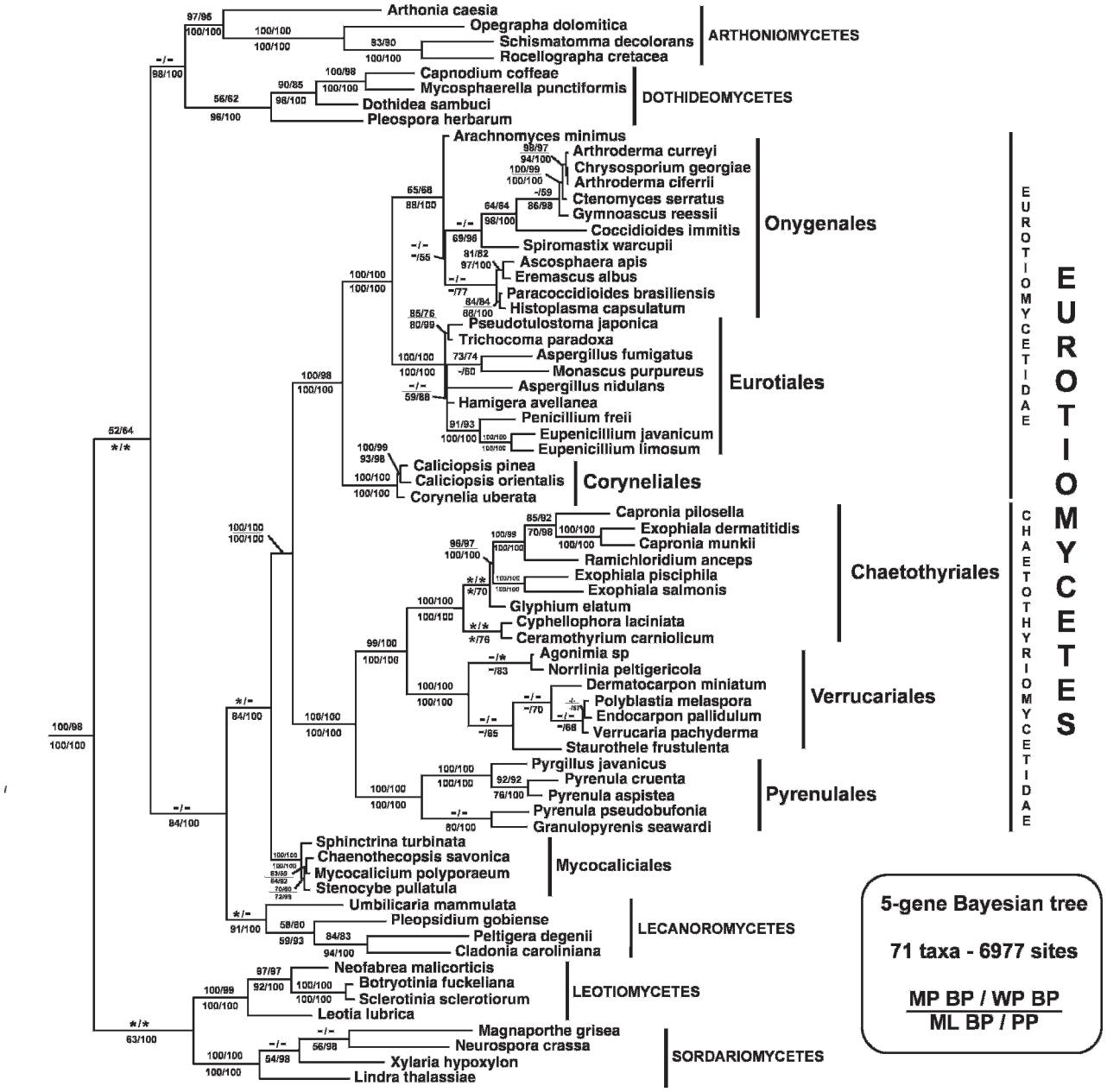


FIG. 1. Eurotiomycete phylogeny. Fifty percent Majority rule consensus tree of 90 000 trees obtained by Bayesian inference under GTR+I+Γ applied across 11 partitions. MP BP= maximum parsimony bootstrap, WP BP = weighted parsimony bootstrap, ML BP = maximum likelihood bootstrap, PP = Bayesian posterior probability. Dashes are shown for nodes with < 50% support and an asterisk indicates nodes that are differently resolved under the specific statistical sampling method used.

clade within the Eurotiomycetidae, consistent with the results based on a comprehensive phylogenetic analysis of five loci across the Pezizomycotina (Spatafora et al 2006). Coryneliales is associated with fissitunicate asci and ascolocular ascomata (FIGS. 1–2) and was considered an order of uncertain position within the Pezizomycotina (Eriksson 2006). Neither character is observed elsewhere in the Eurotiomycetidae, and their absence strongly indicates that they were lost in concert as the unitunicate Eurotiomycetidae evolved modes of passive spore discharge. The results presented in this paper are consistent with previous analyses showing that the SSU sequence of *Corynelia uberata* indicated its relationship to the Eurotiomycetes (Inderbitzin et al 2004, Winka 2000). *Caliciopsis* and other taxa in the Coryneliales possess morphological characters that bridge those found in taxa in the Chaetothyriomycetidae with characters that dominate elsewhere in the Eurotiomycetidae. Coryneliales tend to produce plectomycete-like, globose to

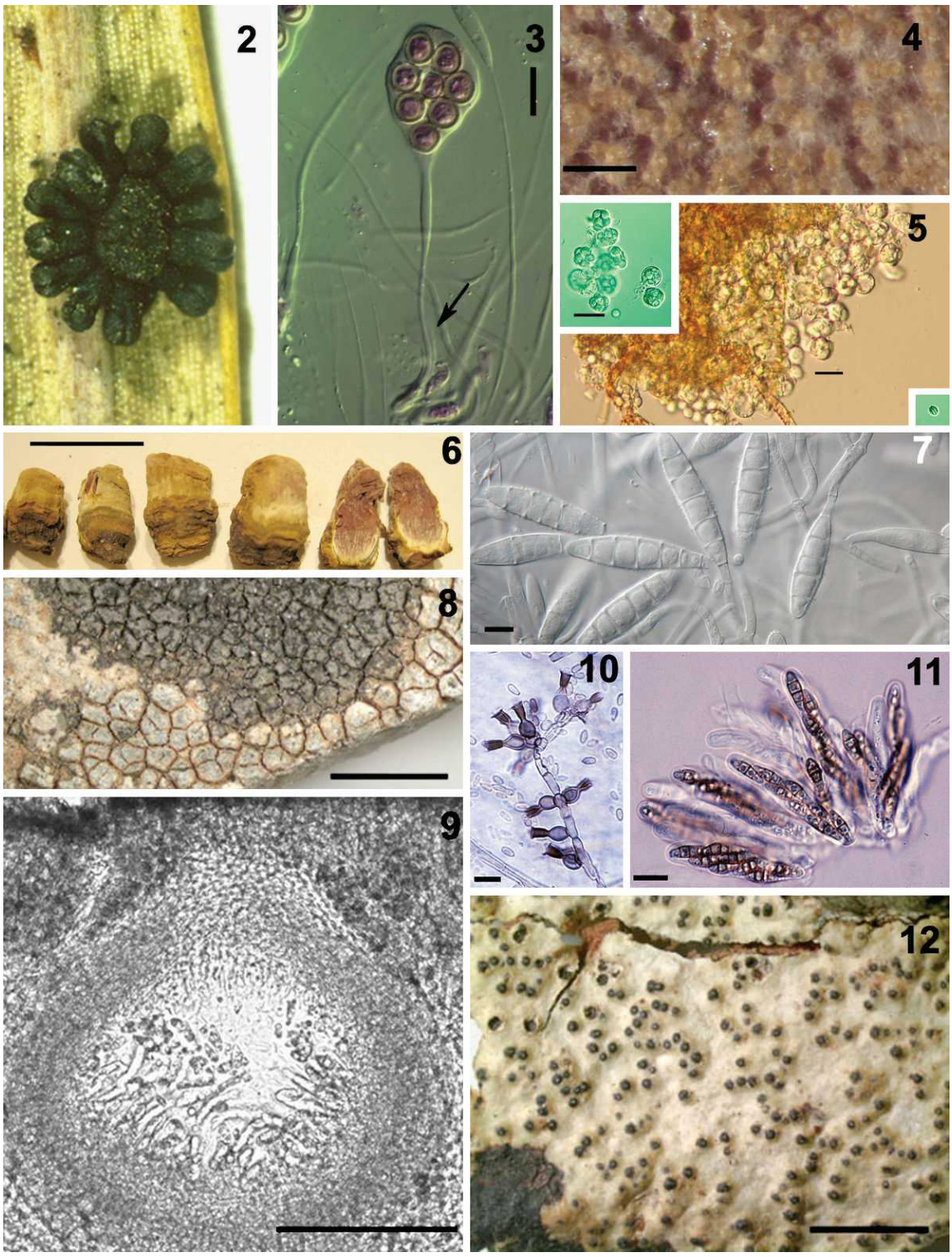
pyriform asci that have been described mostly as unitunicate or prototunicate, which have a thin wall that deliquesces at maturity to release ascospores within the ascoma (Barr and Huhndorf 2001). Ascospores usually are single-celled and spherical, and they are released through irregular openings in the ascomata, which lack sterile hyphal elements. In contrast to their clademates members of Coryneliales also show nonplectomycete-like characteristics: Ascomatal development is ascolocular and asci possess long tails that are retained from their ascohymenial origins.

Luttrell (1951, 1955, 1973) consistently recognized Coryneliales as a member of Pyrenomycetes, the unitunicate perithecial fungi that mostly occupy Sordariomycetes in the current system, and proposed that it gained its ascostromatic characteristics through a reduction of the perithecium. The asci of Coryneliales were considered unitunicate, and by definition fungi with unitunicate asci could not be considered Loculoascomycetes in Luttrell's system, even if they showed ascolocular centrum development. Luttrell (1951) clearly saw Coryneliales as problematic in his system of classification based on ascomal ontogeny, and it was with some qualification that he placed them in the Pyrenomycetes. However later studies of the corynelialean ascus revealed that it was indeed bitunicate, albeit in a distinctive way that appears to be a transition state between the bitunicate asci observed in the Chaetothyriomycetidae and the prototunicate asci characteristic of the classical Plectomycetes. Based on light microscopic studies, Johnston and Minter (1989) described asci in the Coryneliales as bitunicate with an outer wall that breaks away to release a thin-walled, young ascus (FIG. 3). However the two walls are present only in the earliest stages of development, before the delimitation of ascospores, so that their bitunicate nature is easily overlooked. In *Corynelia uberata* the outer wall breaks near the ascus base long before the formation of ascospores. The asci lengthen before and during ascospore formation, yielding the distinctive long-tailed asci harboring eight single-celled ascospores. At maturity the inner ascus walls break open in an irregular fashion, often on the side of the ascus, releasing free ascospores into the ascomatal cavity. The mature, unitunicate asci are thus essentially prototunicate, lacking an apical pore or fissure from which ascospores are released. Johnston's and Minter's (1989) observation of bitunicate asci in this group, along with the seemingly ascolocular mode of ascomatal development, seemed to seal its placement among the Loculoascomycetes. However these authors suggested that there were fundamental structural differences between the wall layers found in the Coryneliales and those found in other bitunicate ascomycetes and cast doubts on their homology.

Most members of the Coryneliales are biotrophic on woody hosts, especially on Podocarpaceae in the southern hemisphere and some northern temperate species that occur on conifers. The presence of spermogonia and spermatia acting as male gametangia also distinguish Coryneliales from Onygenales and Eurotiales, both of which produce hyphal gametangia that are difficult or impossible to distinguish (Benjamin 1955). Only one genus in Coryneliales, *Coryneliopsis*, has a known anamorph that is placed in the coelomycetous genus *Anthracoderma*.

Core Eurotiomycetidae.—Onygenales (including Ascosphaerales/Arachnomycetales) and Eurotiales. These orders form the core taxa referred to as ''monophyletic Plectomycetes'' by Geiser and LoBuglio (2001) and originally defined as Eurotiomycetes by Eriksson (1999). They produce a variety of ascomata that may include cleistothecia, some sort of ascostroma, and in some cases neither. Asci are always produced in a scattered fashion and do not form in a distinctive fertile layer. Asci tend to be pyriform to globose and evanescent, and bear eight, unordered ascospores. Cleistothecial and stromatic ascomata characteristic of this group are depicted (FIGS. 4–6).

Onygenales. A well-resolved Onygenales clade is supported with two subclades: the strongly supported Onygenales *sensu stricto* and a weakly supported clade that comprises fungi previously classified as Onygenales, Ascosphaerales and Arachnomycetales (FIG. 1). Members of Onygenales produce highly variable ascomata, ranging from macroscopic ascostromata to more reduced cleistothecial and gymnothecial forms (Currah 1985). Cleistothecial peridia are highly variable, often composed of distinctively shaped hyphae. Anamorphs in Onygenales are almost exclusively thallic, involving the production of simple terminal and intercalary arthroconidia as well as more elaborate forms (FIG. 7). An ability to degrade keratin is found frequently among members of this order, and this trait correlates with the common status of such species as pathogens of vertebrates. Onygenalean mammalian pathogens include agents of histoplasmosis and coccidioidomycosis, which cause respiratory infections in mammals that occasionally disseminate to other parts of the body. As the major group of dermatophytic fungi, they also cause superficial tinea infections. Despite this association with mammalian disease, onygenalean fungi are probably mostly saprophytic, and they are frequently isolated from soils. Currah's (1985) monograph recognized four families within Onygenales, Arthrodermataceae, Onygenaceae, Gymnoascaceae and Myxotrichaceae. A possible connec-



FIGS. 2–12. Representative sexual and asexual structures of Eurotiomycetes. 2–3. Coryneliales: *Corynelia uberata* (photographs courtesy Peter Johnston). 2. Ascostroma on *Podocarpus* leaf. 3. Ascus with basal remnants of broken outer wall visible (arrow). Bar = 10 µm. 4–6. Eurotiales. 4. *Eupenicillium* cleistothecia/ascostromata in culture. Bar = 1 mm. 5. Ruptured *Eurotium* cleistothecium, globose asci (upper left) and lenticular ascospore (lower right). Bars = 10 µm. 6. Noncleistothecial *Trichocoma paradoxa* ascostromata. Bar = 10 mm. 7. Onygenales: Thallic *Microsporum gypseum* macroconidial anamorph (photograph courtesy James A. Scott). Bar = 10 µm. 8–9. Verrucariales. 8. *Verrucaria aspiciliicola* thallus on rock. Bar = 100 µm. 10–11. 9. Cross-section of *Dermatocarpon miniatum* perithecium, with asci and hamathecial

tion between Myxotrichaceae and certain Leotiomycetes was noted, however based on ecology and morphology, and the connection was confirmed later based on nucleotide sequence analysis (Currah 1994, Sugiyama et al 1999, Wang et al 2006). Detailed microscopic studies of ascomal development in *Myxotrichum arcticum* also revealed strong evidence for a connection to the Leotiomycetes, specifically a distinct hymenial layer, paraphyses, and stipitate asci (Tsuneda and Currah 2004). These results strongly support removal of Myxotrichaceae from the Onygenales.

Ascosphaerales/Arachnomycetales. Ascosphaerales, as previously defined, comprise osmotolerant to osmophilic fungi with reduced ascomata and thallic anamorphs. Early workers placed these fungi in Hemiascomycetes because of their reduced hyphal systems (Bessey 1950). In light of its reduced gametangia, the genus *Ascosphaera* in Ascosphaeraceae was proposed as a member of Plectascales (Spiltoir and Olive 1955). Tightly aggregated asci and ascospores take the form of spore balls, which are released from a mature spore cyst. In mass the spore balls have a chalky appearance, the basis of the term ''chalkbrood'' applied to the disease these fungi cause in insects. Larvae infected with the fungus become filled with spore balls, giving them a chalky appearance. The genus *Eremascus* is morphologically distinct, and its connection to *Ascosphaera* went unnoticed until the advent of molecular phylogenetics (Berbee and Taylor 1992); the strongly supported relationship based on SSU sequences led Geiser and LoBuglio (2001) to recognize *Ascosphaera* and *Eremascus* together as a distinct order. *Eremascus* produces a thin mycelium and undifferentiated gametangia, with globose, eight-spored asci that are typical of the plectomycetous Eurotiomycetes. Eriksson et al (2004) recognized two families for these fungi, Ascosphaeraceae and Eremascaceae, placed in Onygenales, but Lutzoni et al (2004) found that *Ascosphaera* and *Eremascus* occurred in a strongly supported clade that included *Paracoccidioides brasiliensis*, distinct from the major clade of Onygenales. *Paracoccidioides brasiliensis* is a human pathogenic fungus previously considered aligned with Onygenales. This dimorphic fungus produces multiply budding cells in its yeast phase, and an aleurioconidial stage reminiscent of chrysosporium-like stages found across Onygenales as defined broadly. Based on a phylogenetic analysis of nuclear ribosomal small subunit data which showed it to be distinctive from other members of Onygenales, the order Arachnomycetales was erected to accommodate the genus *Arachnomyces* (Gibas et al 2002). However that analysis did not include sequences from *Ascosphaera, Eremascus* and *Paracoccidioides.* Maximum parsimony analysis of the dataset in our study yielded a weakly supported clade that includes *Arachnomyces, Ascosphaera, Eremascus, Paracoccidioides* and *Ajellomyces capsulata* (results not shown), opening up the possibility that the clades inferred as Arachnomycetales by Gibas et al (2002) and as Ascosphaerales by Lutzoni et al (2004) represent the same group. However these data are based mostly on SSU and LSU data alone, and neither Bayesian (FIG. 1) nor ML analysis (results not shown) support the inclusion of *Arachnomyces* in this clade, so we will cautiously follow Eriksson (1999) and recognize these fungi as members of Onygenales.

Eurotiales. Eurotiales, which receives consistently strong support in our analysis, contains a diverse range of ascomatal types similar to that found in Onygenales. Asci are globose, and ascospores tend to be lenticular, often with two equatorial rings. Ascomata include highly reduced forms with hyphal peridia, cleistothecia, cleistothecia borne within a surrounding stroma, and noncleistothecial stromata (Malloch 1981). Highly reduced forms include *Byssochlamys,* which produces asci in loosely organized hyphal structures, sometimes referred to as protothecia. Cleistothecial forms comprise a variety of peridial types, ranging from hyphal forms (often referred to as gymnothecia) found in genera such as *Talaromyces,* to more rigid, pseudoparenchymatous forms found in genera such as *Eurotium.* When a stroma is present, it may or may not surround a cleistothecium. Stromatic/cleistothecial ascomata are diverse. In *Emericella* cleistothecia have a distinct peridium, with an outer layer of stromatic cells of unknown function called Hülle cells (Malloch 1985). *Elaphomyces* produces the largest ascomata in the Eurotiomycetes, hypogeous, truffle-like fruiting bodies measuring up to ~3 cm across. Finally eurotialean ascomata sometimes take a stromatic form that is not enclosed and does not take the form of a cleistothecium. These include *Trichocoma,* which produces a brush-like ascostroma in which ascogenous hyphae and asci extend like bristles from a stromatic base, and the more recently discovered genus *Pseudotulostoma.* In this genus a tulostomoid (i.e. similar to a stalked puffball) inner structure enclosing asci and ascospores

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elements visible. Bar = 100 µm. Chaetothyriales: *Capronia pilosella.* 10. *Phialophora* anamorph, featuring phialides with distinctive collarettes. Bar = 10 µm. 11. Fissitunicate asci with eight septate ascospores. Bar = 10 µm. 12. Pyrenulales: *Pyrenula concatervans* thallus on bark, with perithecial ascomata visible. Bar = 5 µm.

extends from an *Elaphomyces*-like ascostroma, leaving behind a structure analogous to an agaricalean volva (Miller et al 2001).

Eurotiales includes many well known and common fungi, particularly those with phialidic *Aspergillus* and *Penicillium* asexual stages. Most eurotialean fungi are saprotrophic and represent some of the most catabolically and anabolically diverse microorganisms known. Some species are capable of growing at extremely low water activities (i.e. xerotolerant and/or osmotolerant), low temperatures (psychrotolerant) and high temperatures (thermotolerant). These properties, combined with the ability to produce diverse sets of toxic secondary metabolites such as aflatoxins, ochratoxins and patulins, make these fungi important agents of food spoilage. Other secondary metabolites produced by eurotialean fungi are useful as pharmaceuticals, including antibiotics such as penicillin and the anticholesterolemic agent lovastatin. The aggressively saprotrophic nature of some species also makes them ideal industrial fungi because they produce copious and diverse enzymes that degrade a wide variety of complex biomolecules, secrete them efficiently, and work well in fermentation systems. The broad importance of these fungi, coupled with their ease of manipulation in the laboratory, has led to a long standing interest in their genetics, and more recently, to complete-genome sequencing of several *Aspergillus* and *Penicillium* species. *Aspergillus nidulans*, with its homothallic *Emericella* sexual stage, has been used in classical and molecular genetic studies for nearly 70 y.

Chaetothyriomycetidae.—This (FIGS. 8–12) is the second major clade of Eurotiomycetes. This subclass and its three resident orders received consistently high support across all analyses (FIG. 1).

Verrucariales. The order Verrucariales includes mainly taxa living with autotrophic organisms to form lichen symbioses. The majority of their photosynthetic partners belong to the Chlorophyta, but heterokont (phaeophyte, xanthophyte) and rhodophyte partners also are known (Friedl and Büdel 1996, Kohlmeyer and Volkmann-Kohlmeyer 1998). This large phylogenetic diversity of photosynthetic symbionts in the Verrucariales differs from the largest group of lichens (Lecanoromycetes), probably because of the exceptionally broad habitat preference across members of this order, ranging from dry to aquatic conditions, including freshwater and marine habitats. The two partners of these symbioses usually share a mutualistic relationship, but some of these fungi depart from this ''reciprocal benefit'' principle and act as parasites on other lichens. Some of the parasites are still associated with a photobiont, but they complement their nutrient uptake by invading and parasitizing thalli of other lichens (lichenicolous lichens, FIG. 8). Others have lost their association with the photobiont and live as commensals or parasites on other lichens (lichenicolous fungi). Therefore, although most of the Verrucariales are mutualists, living strategies in this order are diverse, including commensalism and parasitism.

Verrucariales are cosmopolitan and include mostly saxicolous species, which colonize rocks ranging from small pebbles in rivers or glades to boulders and entire cliffs but also manufactured substrates such as concrete or stone walls (FIG. 8). They are particularly diverse on calcareous substrates, where they grow either as epiliths (over the surface of the rock) or endoliths (within a superficial layer of the rock). Although members of the Verrucariales are saxicolous, a significant number of taxa grow on other substrates, such as soil, bark or wood, mosses or other lichens. Verruclariales constitute the largest group of maritime lichens and are present on rocky shores worldwide.

Species of Verrucariales are diverse in thallus morphology, including foliose-umbilicate, squamulose, crustose and granulose forms. Consistent with their narrow range of thallus coloration, these fungi lack the secondary metabolite diversity so characteristic of Lecanoromycetes. However some species of Verrucariales are rich in a dark pigment probably related to melanin, possibly an ancestral character shared with Chaetothyriales. Similar to the situation among members of Pyrenulales, vegetative propagules such as isidia or soredia are rare in the Verrucariales, but asexual reproduction is still possible because squamulose and crustose-areolate thalli are prone to fragmentation. Sexual reproductive structures certainly play a major role in dispersal in this group. Verrucariales is characterized by perithecial ascomata ranging from superficial to entirely immersed in the thallus. The hamathecium is often absent or when present formed by an evanescent tissue of gelatinized pseudoparaphyses. The ostiole is distinctively covered by periphyses (FIG. 9). Asci are described as bitunicate with a mode of dehiscence that is fissitunicate to evanescent (Eriksson 1982, Janex-Favre 1971). Spores are highly variable, from colorless to brown, and simple to muriform.

Chaetothyriales. This order includes nonlichenized ascomycetes divided in two different families, the Chaetothyriaceae and the Herpotrichiellaceae. The members of Chaetothyriaceae are known as epiphytes (Batista and Ciferri 1962) but it is still unclear whether many of these species are saprophytic or biotrophic (Barr 1987). Species of the Herpotrichiellaceae are known either from their sexually reproducing state (teleomorph), their clonally reproducing state (ana-

morph) or both. Teleomorphs include small inconspicuous saprophytes, mainly growing on dead plants and wood (Barr 1987; Untereiner and Naveau 1999) whereas anamorphs also are found as animal and human pathogens. These parasites, often called black yeasts, can induce skin and nervous system infections in healthy or immunocompromised patients (de Hoog et al 2000 and references therein) and their addition to the Eurotiomycetes places them with most other animal pathogens in the Pezizomycotina. Most of these species are known only by their anamorphs, and it is only through recent molecular analyses that anamorph-teleomorph connections have been made (Untereiner 2000 and references therein).

Sexual stages in Chaetothyriaceae are found mainly in the tropics where they occur on the leaves and bark of plants (Batista and Ciferri 1962). The sexually reproducing Herpotrichiellaceae grow on dead plants or wood, but their anamorphs are cosmopolitan and can be isolated from a large variety of substrates including bathwater, plants and soil (de Hoog et al 2000 and references therein). Their ubiquitous presence in nature indicates that they are mainly opportunistic pathogens, although the recent diversification of some species and their narrow range of host specialization suggest that they can be systemic pathogens (de Hoog et al 2000, Haase et al 1999). Recent studies showed that some slow growing melanized fungi inhabiting rocks in harsh environments belong to the Chaetothyriales (Ruibal 2004, Sterflinger et al 1999).

The order Chaetothyriales is characterized by a dark mycelium, growing as a loose net of hyphae (mycelial pellicle) over the substrate in the Chaetothyriaceae (Batista and Ciferri 1962), or as inconspicuous immersed mycelium in the teleomorphs of Herpotrichiellaceae (Untereiner 2000). Anamorphic Chaetothyriales are characterized primarily by melanized torulose hyphae, but they also can exhibit yeast-like, meristematic and filamentous forms. Production of asexual spores is also pleiomorphic in this order, with annellidic (e.g. *Exophiala*), phialidic (e.g. *Phialophora*, FIG. 10), and blastic conidiogeneses (e.g. *Ramichloridium anceps*) (de Hoog et al 2000). The dark coloration of the mycelium is due to the production of a melanin pigment, which was shown to contribute to the resistance of these fungi to host immune responses (Schnitzler et al 1999). However the presence of melanin alone is not sufficient to explain pathogenicity, and additional factors must be involved to explain the virulence of these fungi (de Hoog et al 2000). The perithecial ascomata of Chaetothyriales are erumpent or superficial, sometimes setose, with or without a periphysate ostiole (Kirk et al 2001). The excipulum pigmentation is variable, and the wall is thin and pseudoparenchymatous. The hamathecium consists of short apical periphysoids. Asci are clavate, with a fissitunicate mode of dehiscence and a thickening of the apical region (FIG. 11). Spores are hyaline to pale gray and transversally septate to muriform.

Pyrenulales. Pyrenulales includes mostly lichenized taxa, the majority of them belonging to *Anisomeridium* and *Pyrenula*, both with more than 100 accepted species. These lichenized Pyrenulales are associated with green algae belonging exclusively to the Trentepohliaceae, a family characterized by its orange carotenoid pigments. This order also includes some saprophytic nonlichenized taxa mostly found in the family Requienellaceae (Aptroot 1991). Recent molecular studies showed that the family Trypetheliaceae did not cluster with the family Pyrenulaceae but is nested with the Dothideomycetes (Lutzoni et al 2004, del Prado et al 2006).

The great majority of the lichenized Pyrenulales colonize trees, where they occur exclusively on bark. One notable exception on leaf surfaces, however, are species of *Strigula*, associated with the orange-pigmented green alga, *Cephaleuros parasiticus*; the alga has been described mistakenly as a fungus on numerous occasions (Holcomb and Henk 1994, Reynolds and Dunn 1984). The nonlichenized taxa are found on bark, leaves or wood. Some Pyrenulales occur in temperate climates where they often are confined to ancient woodlands, but this group is predominantly tropical, where members are diverse as epiphytes in rainforests. Although many recent collecting efforts have been carried out in the tropics, many regions are still understudied, and the species diversity of this order is probably greatly underestimated (Aptroot 2001, Aptroot and Sipman 1997).

Lichenized Pyrenulales species are characterized by a thin thallus, either immersed in the substrate or superficial (FIG. 12). The structure of their thalli is never as complex as in some other lichens, and they never form foliose or fruticose thalli (Aptroot 1991). Vegetative propagules such as soredia and isidia, typically found in lichens from the Lecanoromycetes, are absent. Similarly, secondary metabolites, so informative for species identification within the Lecanoromycetes, are rare in this order. Pyrenulales are characterized by perithecial ascomata that are ostiolate, often papillate and sometimes aggregated. The hamathecium generally consists of narrow trabeculate pseudoparaphyses, subsequently replaced by unbranched paraphyses in the family Pyrenulaceae (Kirk et al 2001). In the Requiellenaceae, the hamathecium differs by having unbranched and sparsely septate paraphyses (Boise, 1986). Asci are functionally bitunicate and they have ascohymenial origins (Janex-

Favre 1971). Spores are colorless or brown and transversally septate to muriform.

Mycocaliciales. Mycocaliciales showed a sister relationship with the Eurotiomycetidae + Chaetothyriomycetidae clade that was strongly supported by ML, BP and Bayesian PP, but not by MP or WP (FIG. 1). This relationship suggests possible inclusion of Mycocaliciales in Eurotiomycetes as a third subclass, but we postpone including it because it is based only on SSU and LSU data from Mycocaliciales. A number of morphological characters, however, unite this order with taxa in both Eurotiomycetidae and Chaetothyriomycetidae, which might reflect common ancestry. The mazaedial ascomata in this group are reminiscent of the stalked ascomata produced by *Onygena* (Onygenales) and *Pseudotulostoma* (Eurotiales), and asci in some taxa within Sphinctrinaceae are evanescent, providing a general connection to Eurotiomycetidae. Their parasitic and/or commensal associations with other fungi, particularly lichens, provide an ecological connection with many taxa in Chaetothyriomycetidae. The spotty distribution of these characters in Mycocaliciales and Eurotiomycetes does not necessarily compel the hypothesis that they are ancestral but perhaps indicates an evolutionary plasticity toward them within these groups.

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