

An overview of the systematics of the Sordariomycetes based on a four-gene phylogeny

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Abstract: The Sordariomycetes is one of the largest classes in the Ascomycota, and the majority of its species are characterized by perithecial ascomata and inoperculate unitunicate asci. It includes more than 600 genera with over 3000 species and represents a wide range of ecologies including pathogens and endophytes of plants, animal pathogens and mycoparasites. To test and refine the classification of the Sordariomycetes *sensu* Eriksson (2006), the phylogenetic relationship among 106 taxa from 12 orders out

of 16 in the Sordariomycetes was investigated based on four nuclear loci (nSSU and nLSU rDNA, TEF and RPB2), using three species of the Leotiomycetes as outgroups. Three subclasses (i.e. Hypocreomycetidae, Sordariomycetidae and Xylariomycetidae) currently recognized in the classification are well supported with the placement of the Lulworthiales in either a basal group of the Sordariomycetes or a sister group of the Hypocreomycetidae. Except for the Microascales, our results recognize most of the orders as monophyletic groups. *Melanospora* species form a clade outside of the Hypocreales and are recognized as a distinct order in the Hypocreomycetidae. Glomerellaceae is excluded from the Phyllachorales and placed in Hypocreomycetidae incertae sedis. In the Sordariomycetidae, the Sordariales is a strongly supported clade and occurs within a well supported clade containing the Boliniales and Chaetosphaeriales. Aspects of morphology, ecology and evolution are discussed.

Key words: classification, ecology, evolution, Hypocreomycetidae, Sordariomycetidae, Xylariomycetidae

INTRODUCTION

The class Sordariomycetes is one of the largest monophyletic clades in the Ascomycota with more than 600 genera and 3000 known species (Kirk et al 2001). It includes most nonlichenized ascomycetes with perithecial (flask-shaped) or less frequently cleistothecial (nonostiolate) ascomata and inoperculate unitunicate or prototunicate asci (Alexopoulos et al 1996). The term “pyrenomycetes” was used to unite fungi with perithecial ascomata and unitunicate asci (Luttrell 1951). Its use was discontinued based on the placement of perithecial species outside the clade and the inclusion of species with prototunicate asci (e.g. *Corollospora* and *Ophiostoma*) to avoid confusion. In the current classification *sensu* Eriksson (2006) the Sordariomycetes comprises 16 orders in three subclasses (i.e. the Hypocreomycetidae, Sordariomycetidae and Xylariomycetidae) based on rDNA phylogenies.

Ecology and its importance.—Members of the Sordariomycetes are ubiquitous and cosmopolitan and function in almost all ecosystems as pathogens and

endophytes of plants, arthropod and mammals, mycoparasites and saprobes involved in decomposition and nutrient cycling. Most plant pathogens in the Sordariomycetes are distributed in the Diaporthales, Hypocreales, Microascales, Ophiostomatales, Phyllochorales and Xylariales. These include the best known plant pathogens (e.g. *Cryphonectria parasitica* [the causal agent of chestnut blight], *Magnaporthe grisea* [the cause of rice blast], *Ophiostoma ulmi* and *O. novo-ulmi* [Dutch elm disease causal agents], *Fusarium* species and *Rosellinia* species [Alexopoulos et al 1996, Samuels and Blackwell 2001]). In addition to pathogens of plants the Sordariomycetes includes endophytes that live inside the above-ground parts of apparently healthy plants (Alexopoulos et al 1996). The best studied endophytes belong to the Hypocreales (e.g. *Balansia* and *Epichloë*), Xylariales (e.g. *Nemania* and *Xylaria*) and *Colletotrichum*. Their infected host plants benefit from increased drought resistance, reduced feeding by insects and limited pathogen infections.

Members of the Hypocreales, Ophiostomatales and Microascales often are associated with opportunistic infections of humans and other animals (e.g. *Sporothrix schenckii*, *Fusarium solani* species complex and *Trichoderma* spp.) (Gugnani et al 1976, Summerbell 2003). As symbionts of arthropods the Sordariomycetes comprise a diverse assemblage of species that range from antagonistic to mutualistic (Samuels and Blackwell 2001). Members of the Microascales and Ophiostomatales (e.g. *Ceratocystis*, *Ophiostoma* and *Ambrosiella*) are associated with bark beetles in fungal spore dispersal. Species of the Hypocreales (e.g. *Cordyceps* and *Torrubiella*) directly parasitize a broad range of arthropods. The Hypocreales also is known to be rich in mycoparasites. Most species of *Hypomyces* are parasitic on fleshy fungal fruit bodies such as mushrooms and large apothecia (Rogerson and Samuels 1989). Some species of *Trichoderma*, used alone or combined with other biocontrol agents, have been applied in agriculture for plant disease management as an alternative to pesticides (Harman et al 2004).

Saprobic Sordariomycetes function in the decomposition and nutrient cycling of plant litter including wood, herbaceous stems and dung. Important taxa include *Neurospora crassa*, the model organism widely used in molecular and genetic studies, and *Chaetomium*, an important cellulolytic organism responsible for the destruction of paper and fabrics. The Sordariomycetes also contains species known as producers of some of the most important fungal secondary metabolites. These include the trichothecene mycotoxins produced by many members of the Nectriaceae and the *Stachybotrys* clade and the ergot

and other alkaloids produced by *Claviceps* and *Epichloë*. Marine fungi also are a good source of novel bioactive compounds (Bugni and Ireland 2004).

Morphology and classification.—The majority of Sordariomycetes produce perithecial ascomata (FIG. 1D–G). The shape, size, pigmentation, texture and position of ascomata were characters used in traditional taxonomy. For example the position of ascomata in relation to substrates was used in family delimitation of the Diaporthales by Barr (1978) but this classification was not supported by phylogenetic analyses using molecular characters (Castlebury et al 2002, Zhang and Blackwell 2001). A phylogenetic study by Miller and Huhndorf (2005) suggested that ascomal wall morphology is a better character than ascospore morphology in defining genera in the Sordariales. Nannfeldt (1932) and Luttrell (1951) first applied ontogenetic characters (FIG. 1F, G) in filamentous ascomycete classification. Although the ontogenetic characters do not always correspond well with molecular phylogenies, they are still informative characters in ordinal classification within the Sordariomycetes (Samuels and Blackwell 2001). The typical arrangement of asci in the Sordariomycetes is basal or peripheral in a hymenium (FIG. 1E). The presence or absence of an apical ring (amyloid or nonamyloid) is an important feature in Sordariomycetes classification (FIG. 1H–J). Asci of the Sordariomycetes typically are octosporous (FIG. 1K, M, N). Ascospore wall ornamentation has been used to delimit certain genera, but phylogenetic studies on the Melanosporales and Sordariales show that this character is prone to convergence and hence is phylogenetically uninformative. The Sordariomycetes is an anamorph-rich class, with significant diversity represented by hyphomycete and coelomycete species. Many species of the Hypocreales, Ophiostomatales and Chaetosphaerales have two or more distinguishable anamorphs (synanamorphs). Hyphomycetes occur throughout the class, but coelomycete anamorphs also occur, most notably in the Glomerellaceae and Diaporthales. In common with teleomorph characters many characteristics used to delimit anamorph genera (e.g. variations in conidiomata, pigmentation, conidiophore branching and conidial septation) are homoplastic in the Sordariomycetes. Despite this, recognizable patterns of anamorph morphological characters often allow recognition of phylogenetic groups (Seifert and Gams 2001).

Recent comprehensive taxonomic studies of families and higher taxa of the Sordariomycetes were published by Barr (1990), Samuels and Blackwell (2001) and Eriksson (2006). The classification

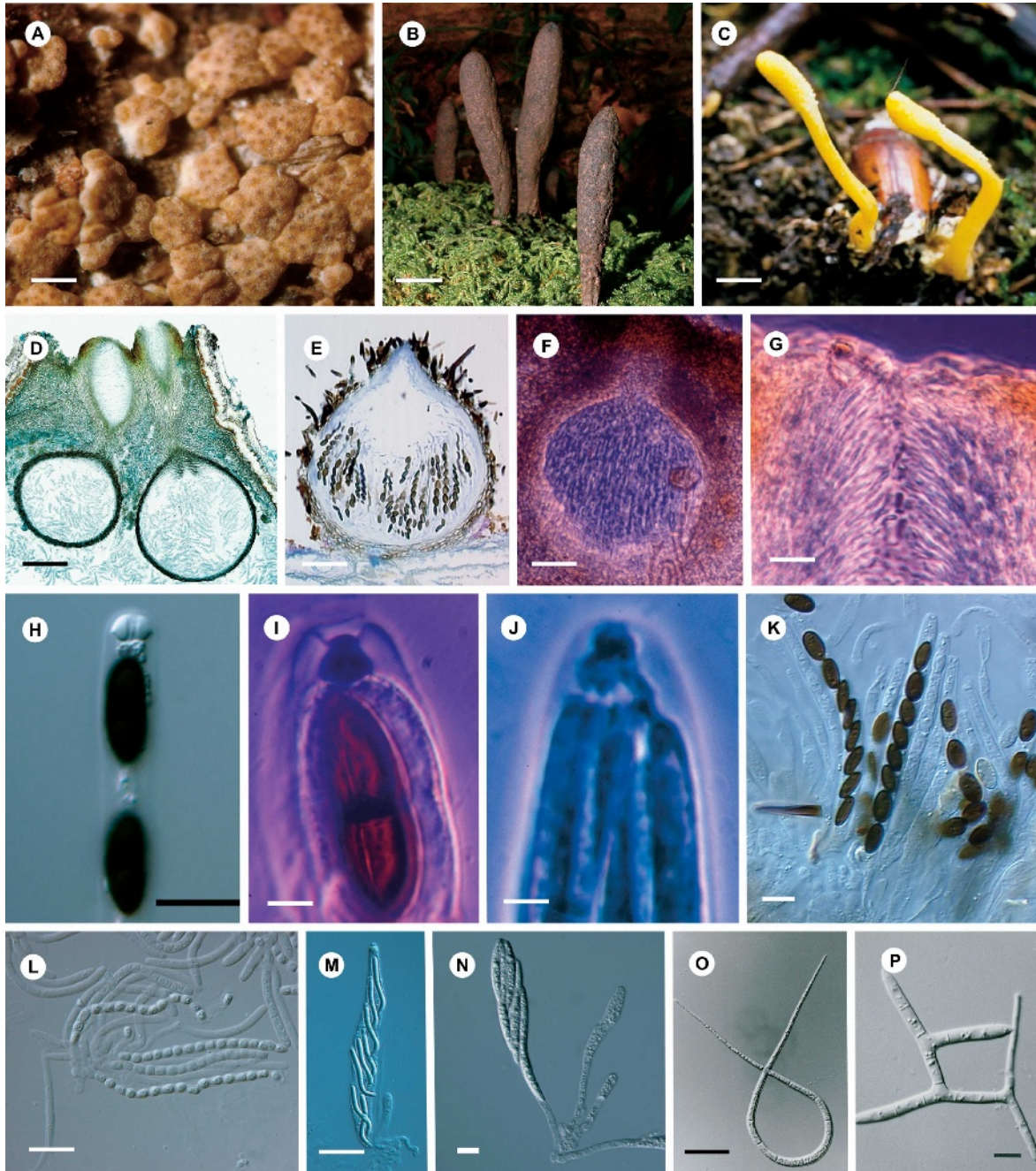


FIG. 1. Morphology of species in the Sordariomycetes. A. *Hypocrea lutea* BPI 744483, perithecia embedded in stromata, Hypocreales, bar = 1 mm. B. *Xylaria longipes*, mature stromata growing on a moss covered hardwood log, Xylariales, bar = 1 cm. C. *Cordyceps scarabaeicola*, mature stromata growing on a scarabaeid pupa, Hypocreales, bar = 5 mm. D. *Valsella salicis* BPI 748461, longitudinal section of perithecia, Diaporthales, bar = 100 μ m. E. *Coniochaeta leucoplaca* SMH 2578, longitudinal section of perithecium, Coniochaetales, bar = 40 μ m. F. *Hypomyces lactifluorum* DAOM 83684, apical paraphyses in perithecium, Hypocreales, bar = 90 μ m. G. *Hypomyces lactifluorum* DAOM 83684, paraphyses in perithecium, Hypocreales, bar = 11 μ m. H. *Jobellisia luteola* ANM 233, apical ring of ascus, Diaporthales, bar = 10 μ m. I. *Cainia desmazieri* DAOM 89953(a), apical ring of ascus, Xylariales, bar = 5 μ m. J. *Gaeumannomyces graminis* DAOM 19596, apical ring of ascus, Magnaporthaceae, bar = 9 μ m. K. *Coniochaeta leucoplaca* SMH 2578, asci with ascospores, Coniochaetales, bar = 10 μ m. L. *Hypocrea lutea* BPI 744503, asci and ascospores, Hypocreales, bar = 20 μ m. M. *Lasiochaeta ovina* SMH 1538, asci with globose subapical globules and ascospores, Sordariales, bar = 40 μ m. N. *Bertia moriformis* ANM 264, ascus, Coronophorales, bar = 10 μ m. O. *Lindra thalassiae* JK 2728 (IMS), septate ascospore without apical chambers, Lulworthiales, bar = 25 μ m. P. *Varicosporina ramulosa* JK 3907 (IMS), conidium, Halosphaeriales, bar = 5 μ m.

proposed by Barr (1990) was based on morphology while the other two were based on both morphological characters and sequences from the nuclear small subunit ribosomal RNA gene (nSSU rDNA). The Erysiphales and Coryneliales *sensu* Barr (1990) were excluded from the Sordariomycetes (Samuels and Blackwell 2001). Eriksson (2006) placed the Erysiphales in the Leotiomycetes, a sister taxon of the Sordariomycetes (see also Spatafora et al on Pezizomycotina, this issue). The extralimital pyrenomycete Laboulbeniales (Samuels and Blackwell 2001) has been recognized as a separate class (Eriksson 2006, Weir and Blackwell 2001). The main goals of this paper are twofold: to define monophyletic groups within the class and to infer the evolutionary relationships among major orders in the Sordariomycetes.

MATERIALS AND METHODS

Character and taxon sampling.—Genomic DNA extraction from specimens or cultures was performed according to standard methods as in Castlebury et al (2004). Amplifications and sequencing were performed for four nuclear gene regions (NS1-NS6 region of nSSU rDNA, LR0R-LR7 region of nLSU rDNA, 983F-2218r region of TEF, and 5–7 region of RPB2) under standard conditions using primers in the AFTOL Website (<http://aftol.biology.duke.edu/pub/primers/viewPrimers>). A total of 106 taxa were sampled to represent 12 orders of the Sordariomycetes following the classification of Eriksson (2006). Due to the lack of data from protein-coding genes, the members of other orders (i.e. the Calosphaerales, Meliolales, Phyllachorales and Trichosphaerales) are not represented in this study. (For voucher information and GenBank accession numbers see SUPPLEMENTARY TABLE I).

Phylogenetic analyses.—Sequences were edited with the Seqmerge program in GCG software (Accelrys, San Diego, California). Sequences of each gene were aligned manually and the concatenated alignment was deposited in TreeBASE (study No. SN2723). To detect incongruence among genes a 70% reciprocal MP bootstrap comparison (Mason-Gamer and Kellogg 1996) was used as a dataset of the 61 core taxa for which all four genes were available. Maximum and weighted parsimony (MP and WP) analyses were performed as described in Lutzoni et al (2004) on a combined dataset of the 106 taxa including taxa that were complete for at least two genes. Maximum likelihood (ML) analyses were conducted with Phylml (Guindon and Gascuel 2003) with the GTR+I+ Γ model. Bayesian analyses with Markov chain Monte Carlo (MCMC) algorithm were performed with the GTR+I+ Γ model for each of eight partitions using MrBayes 3.0b4 (Huelsenbeck and Ronquist 2001). Bayesian analysis with 10 000 000 generations was conducted in four chains with sampling every 100 generations. Ten independent analyses with 1 000 000 generations from different starting trees were performed to reconfirm the adequate log-likelihood convergence and mixing of chains. Nodal supports in MP, WP and ML analyses were

assessed by nonparametric bootstrapping (Felsenstein 1985). Nodal supports in the Bayesian analyses were examined by posterior probabilities from a 10 000 000 generation analysis. To examine potential impact of saturation in third codons of TEF and RPB2 on nodal supports in higher relationships, bootstrap proportions and posterior probabilities were reassessed after excluding the third codon positions of protein-coding genes.

RESULTS AND DISCUSSION

Data analyses.—The alignment for the phylogenetic analyses, after excluding ambiguously aligned regions, comprised 4514 base pairs, 1697 of which were parsimony informative. The reciprocal comparisons of 70% bootstrap trees did not reveal any incongruence (data not shown). The heuristic search in MP and WP analyses yielded four most parsimonious trees (MPTs) with 17 501 steps (CI = 0.1858, RI = 0.5361) and one MPT with 29 222.44 steps, respectively. ML heuristic search analysis resulted in a tree of $-81\,968.65$ log-likelihood. In all Bayesian analyses the log-likelihood was converged at approximately 30 000 generations and a 50% majority rule consensus tree was generated from the 10 000 000 generation analysis with the exclusion of the first 500 trees. The tree from WP analyses is provided (FIG. 2) with all bootstrap proportions (i.e. MP-BP, WP-BP and ML-BP) as well as the Bayesian posterior probabilities (PP) and the tree from the Bayesian analyses is presented (SUPPLEMENTARY FIG. 1). Internodes were considered strongly supported if they received all of bootstrap proportions (MP-BP, WP-BP and ML-BP) \geq 70% and posterior probabilities (PP) \geq 95% (Lutzoni et al 2004).

Systematics of the Sordariomycetes.—The four-gene phylogenetic analyses (FIG. 2) provide support for three major clades in the Sordariomycetes, corresponding to the three subclasses of Eriksson (2006). The Xylariomycetidae is placed as a basal group of the Hypocreomycetidae and the Sordariomycetidae, which are strongly supported after the exclusion of third codons in protein-coding genes. The Lulworthiales is basal to other Sordariomycetes according to the MP, WP and ML tree, but it is resolved as a sister taxon of the Hypocreomycetidae based on the Bayesian analyses (SUPPLEMENTARY FIG. 1). Because the four-gene phylogeny is largely consistent with the current classification, the ordinal relationships and characteristics of each clade are discussed below according to the classification of the Outline of Ascomycota (Eriksson 2006).

Xylariomycetidae.—This well supported monophyletic subclass contains a single order, the Xylariales, which

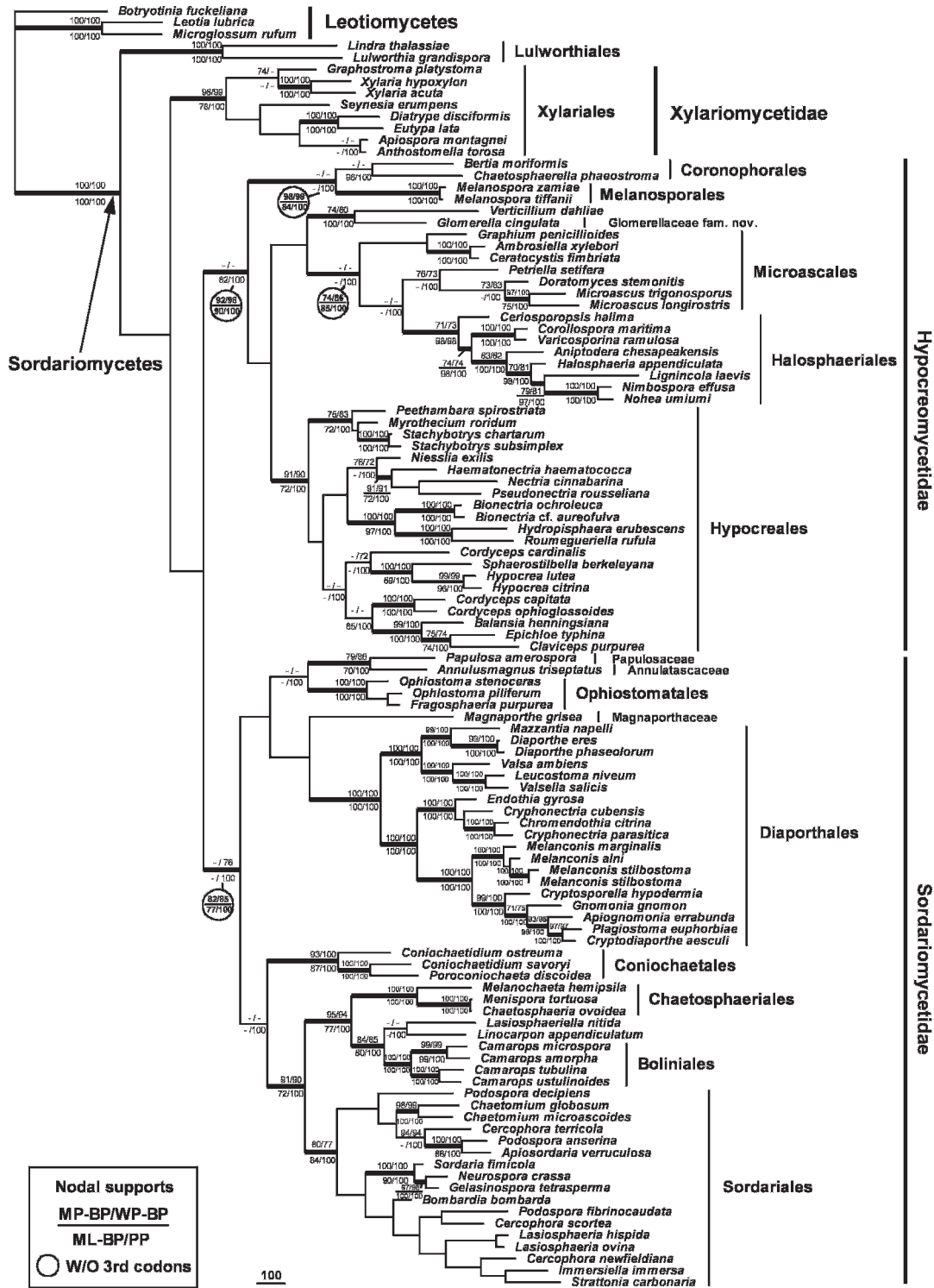


FIG. 2. Phylogenetic tree of the Sordariomycetes. The phylogeny is constructed from single most parsimonious tree from weighted parsimony analyses. Bootstrap proportions (MP-BP, WP-BP and ML-BP) of $\geq 70\%$ and posterior probabilities (PP) of $\geq 95\%$ are provided for the corresponding internodes. Bootstrap proportions and posterior probabilities in circles were calculated with the exclusion of the third codon positions in TEF and RPB2. The internodes that are supported by all bootstrap proportions and posterior probabilities are shown as a thicker line. The name of taxa (e.g. subclasses, orders, families) is provided to the right of species names.

is characterized by well developed stromata, dark colored perithecia, persistent asci often with amyloid apical rings and true paraphyses. Most species of the Xylariales are saprobes or plant parasites in terrestrial habitats. While most marine species are classified in the Halosphaeriales and Lulworthiales (Spatafora et al 1998) the sampled species, *Anthostomella torosa*, represents marine species in the Xylariales (Kohlmeyer and Volkmann-Kohlmeyer 2002). The Xylariales is one of the largest orders in the Sordariomycetes and includes more than 800 species in eight families according to the *Dictionary of the Fungi* (Kirk et al 2001). Some of its families are larger and more diverse than small orders in the class. Therefore we will discuss each family to not oversimplify the picture.

Family Xylariaceae is a large assemblage and the type family of the Xylariales. Perithecia are embedded in stromata, asci usually possess amyloid apical rings, and mature ascospores are usually unicellular with a prominent germ slit. A number of recent reports have dealt with phylogenetics within the family (e.g. Sánchez-Ballesteros et al 2000, Triebel et al 2005 and Hsieh et al 2005). Anamorphs of the Xylariaceae have sympodially proliferating conidiogenous cells with characteristic scars, and holoblastic, single-celled conidia with detachment scars corresponding with those on the conidiogenous cells. Variation in conidiophore branching and the nature of the conidiogenous cell proliferation (ampulliform, irregularly swollen, geniculate) allows the distinction of anamorph genera such as *Nodulisporium* and *Geniculosporium* and several others, which often delimit monophyletic groups (Ju and Rogers 1996). The Amphisphaeriaceae in the sense of Müller and von Arx (1973) included taxa that greatly resemble members of the Xylariaceae but with ascospores that (mostly) lack defined germination sites. Barr (1990) presented a much more restricted circumscription of the family, and Kang et al (2002) published a molecular-based phylogeny including the restricted group. The versicolored, multiappendaged conidia of the *Pestalotia* complex seem to be a synapomorphic character for these coelomycetous anamorphs of the Amphisphaeriaceae (Kang et al 2002).

Family Cainiaceae was established for a small assemblage of monocot inhabitants with two-celled ascospores ornamented with striations or multiple germination slits and asci with amyloid apical rings. The two-locus phylogeny of Lutzoni et al (2004) places *Cainia* among the Xylariales. Family Clypeosphaeriaceae *sensu* Barr (1990) includes genera with mostly iodine-negative ascical apices and germination sites, when present, that are pores. Important genera include *Clypeosphaeria* and *Endoxyla*. Family

Graphostromataceae contains *Graphostroma*. Although species have a stroma reminiscent of the Diatrypaceae and ascospores somewhat suggestive of that family, its affinities are clearly with the Xylariaceae (see FIG. 2). Family Diatrypaceae has long been recognized as a natural assemblage allied with the Xylariaceae. A cardinal feature is the pale brown allantoid ascospore. The anamorphs of the Diatrypaceae also have sympodially proliferating as well as percurrently proliferating conidiogenous cells (sometimes on the same conidiophore) but lack conspicuous scars and often have tiny conidiogenous cells and conidia densely packed into rudimentary coelomycetous conidiomata, sometimes little more than cavities in stroma (Glawe and Rogers 1982). Although circumscription of the family is clear and widely accepted (Barr 1990) the delimitation of genera is in part artificial (Acero et al 2004). Family Hyponectriaceae as circumscribed by Barr (1990) contains 13 genera. The assemblage is probably polyphyletic. The type genus *Hyponectria* falls within the Xylariales in the two-locus phylogeny of Lutzoni et al (2004). Family Myelostromataceae is monotypic. It generally is assigned to the Xylariales, but its true affinities remain uncertain.

Hypocreomycetidae.—This subclass is recognized here as a strongly supported monophyletic clade and includes its four orders (i.e. the Coronophorales, Halosphaeriales, Hypocreales and Microascales) in the classification *sensu* Eriksson (2006). The members of the subclass typically possess light colored perithecia. Asci are nonamyloid or amyloid, or lack apical rings, and true paraphyses are lacking in most members (FIG. 2). While the Coronophorales, Halosphaeriales and Hypocreales are recognized as monophyletic groups, the Microascales is rendered paraphyletic due to the placement of the Halosphaeriales. A new finding is that the sister group of the *Melanospora* clade is the Coronophorales and this long branch internode (SUPPLEMENTARY TABLE I) is strongly supported without third codon positions. Therefore *Melanospora* (type species: *M. zamiae*) is excluded here from the Hypocreales and recognized as a distinct order. The remaining clade in the subclass comprises *Glomerella cingulata* and an anamorphic species, *Verticillium dahliae*. The *Glomerellaceae* is validated with *G. cingulata* and placed within the Hypocreomycetidae.

Hypocreales.—The Hypocreales is a strongly supported monophyletic order that includes virulent plant and insect pathogens as well as useful mycoparasitic, endophytic and saprobic species (FIG. 2, Rossman et al 1999). This order is defined by species with soft textured, generally light to bright colored

perithecial fruiting bodies and unitunicate asci that develop among apical (and centripetal in some species) paraphyses that often dissolve at maturity. The colorless ascospores vary from nonseptate, globose to one- to multiseptate, ellipsoidal to filiform. The Hypocreales include more than 70 genera in four major families (Rossman et al 1999). The Bionectriaceae and Nectriaceae consist primarily of species previously placed in the large and artificial genus *Nectria* having nonstromatic, uniloculate perithecia. The genus *Nectria* (anamorph: *Tubercularia*) now is restricted to a related group of 23 species. The Nectriaceae (e.g. *Haematonectria*, *Nectria* and *Pseudonectria* in FIG. 2) is defined by the orange to red, KOH+ perithecia. The Bionectriaceae (e.g. *Bionectria*, *Hydropisphaera* and *Roumegueriella* in FIG. 2) consists primarily of pallid, nectria-like species such as *Bionectria* (anamorph: *Clonostachys*), some of which are used to control greenhouse pathogens. The Hypocreaceae includes *Hypocrea* (anamorph: *Trichoderma*), increasingly useful in the biological control of plant pathogens (Samuels 2006), and *Hypomyces* with diverse anamorphic states. The Clavicipitaceae produces a wide range of secondary metabolites and insect-associated genera including *Cordyceps*, *Hypocrella* and *Torrubiella*. The anamorphs of the Hypocreales are generally phialidic producing slimy conidia; chlamydospore-like anamorphs also are produced by many species. The anamorphs include the well known phialidic genera *Gliocladium*, *Trichoderma* (Hypocreaceae), *Clonostachys*, *Stilbella* (Bionectriaceae), *Cylindrocarpon*, *Cylindrocladium*, *Fusarium*, *Tubercularia* (Nectriaceae, Rossman et al 1999), and *Beauveria*, *Hirsutella*, *Metarhizium*, and *Tolypocladium* (Clavicipitaceae, Hodge 2003). The “toxic mold” fungus, *Stachybotrys chartarum*, represents part of a lineage separate from any of the defined families within the Hypocreales (Castlebury et al 2004).

Halosphaeriales.—This order is strongly supported (FIG. 2) as shown in Spatafora et al (1998). The order is characterized by usually submerged perithecial ascomata; an interascal tissue is absent, but the centrum is filled by a thin-walled pseudoparenchyma that dissolves or breaks up to form filamentous catenophyses. A small number of species in this order are found in freshwater, but the majority consists of marine species. The ascus wall in most species deliquesces releasing the ascospores, which are forced into the neck of the ascocarp by production of additional asci and ascospores. Within the order the distinctive ascospore appendages are important characters for delineating most genera. Several anamorphs have been attributed to the Halosphaeriales, including chlamydospore-like anamorphs and several

genera of so-called Ingoldian hyphomycetes. The hyphomycetous anamorph *Varicosporina ramulosa* (FIG. 1P) produces conidia and sclerocarps, sclerotium-like fruiting bodies, lacking sexual capacity. It was postulated that *V. ramulosa* is an ascomycete that has lost its ability to reproduce sexually (Kohlmeyer and Charles 1981). Sclerocarps are morphologically similar to ascomata of *Corollospora* species and well adapted to extreme conditions of sandy beaches, where they may be exposed to long periods of drying and extreme high or low temperatures.

Microascales.—This order is recognized here as a paraphyletic group with respect to the Halosphaeriales (FIG. 2). However taxonomic revision is premature due to poorly supported internal nodes and the need for greater taxon sampling. The Microscales is a small order of primarily saprobic fungi in soil, rotting vegetation and dung. A few species of this order cause plant diseases such as *Ceratocystis fimbriata*, transmitted by beetles to living trees and causing cacao wilt and many other economically important diseases. Other members such as species of *Pseudallescheria* cause incurable diseases of humans. The order is characterized by nonstromatic black perithecial ascomata with long necks or rarely with cleistothecial ascomata that lack paraphyses, and globose and evanescent asci, developing singly or in chains. Nonseptate, colorless ascospores often bear ornamenting ridges or wings. The anamorphs of the Microascaleae produce percurrently proliferating conidiogenous cells (annellides) and sometimes chlamydospore-like or aleurioconidial synanamorphs that are classified mostly in the hyphomycete genera *Scopulariopsis*, *Graphium* and *Scedosporium* (Abbott et al 1998, Okada et al 1998).

Coronophorales and Melanosporales.—The Coronophorales is composed of primarily wood-inhabiting taxa and defined by species with erumpent to superficial ascomata that often collapse upon drying and that sometimes possess an extensive hyphal subiculum or basal stroma. Many taxa contain Munk pores (small pores each of which is surrounded by a ring or thickening) in their ascomatal cell walls and a Quellkörper (a gelatinous mass of cells in the apical region of the ascoma, believed to function in rupturing the ascoma) in their centrum (Huhndorf et al 2004a). While Munk pores are found in a few taxa outside the order, the Quellkörper is a character unique to the Coronophorales. Filiform paraphyses are absent in the group, and in most species the asci are thin-walled, clavate, stipitate and lack an apical ring. Ascospores usually are hyaline, small and allantoid. Anamorphs are hyphomycetous when known. The typical characteristics of the Melanospor-

ales include translucent perithecial or cleistothecial ascomata, pseudoparenchymatous centra, absence of paraphyses in development, clavate evanescent asci and dark colored ascospores. Previous phylogenetic studies based on rDNA sequences suggested that *Melanospora* is within or near Hypocreales (Spatafora and Blackwell 1994, Zhang and Blackwell 2002). When analyzed with more taxa of the Sordariomycetes, *Melanospora* formed a distinct clade outside Hypocreales (Castlebury et al 2004). The four-gene phylogeny (FIG. 2) supports the exclusion of *Melanospora* from the Hypocreales. Furthermore a close relationship between the Coronophorales and the Melanosporales (FIG. 2) is recognized. Similar morphological and ecological features of the two orders include a pseudoparenchymatous ascomal wall, clavate, deliquescent asci, lack of paraphyses (with a few exceptions in the Coronophorales) and often a mycoparasitic habit.

Glomerellaceae.—The Glomerellaceae is a so far monotypic family characterized by black, nonstromatic perithecia, well developed periphysate ostioles, abundant thin-walled paraphyses, clavate asci with J-apical ring, and hyaline, smooth, often curved ascospores. Anamorphs known are *Colletotrichum* species. *Glomerella* had been placed in the Phyllachorales (see the orders of uncertain positions) but some of its features are clearly distinct from those of other Phyllachorales, such as its lack of stromatic tissue and its exclusively *Colletotrichum* anamorphs. Molecular phylogenetic analyses (Wanderlei-Silva et al 2003) confirmed these distinctions and they recognized *Glomerella* as a family in the Hypocreomycetidae. Further investigation is required to test whether *Verticillium dahliae*, a sister taxon of *G. cingulata* in the phylogeny presented here (FIG. 2), is actually a member of the Glomerellaceae. The anamorphic genus *Verticillium* apparently is related closely to the holomorphic genus *Plectosphaerella* (Zare et al 2004), which together might represent an undescribed family (Gams, pers comm). The family was invalidly published by Locquin (1984) and is validated here.

Glomerellaceae Locq. 1984 ex Seifert & W. Gams, fam. nov.

Glomerellaceae Locq., Mycol. Gén. Struct.: 175. 1984, nom. inval., Art. 36

Stromata absentia. Ascomata perithecia, nigra, saepe sclerotiformia, pariete crasso, ostiolum periphysatum. Textura interascalis paraphysibus veris, tenuitunicatis. Asci clavati, brevi-stipitati, tenuitunicati non fissitunicati, plerumque annulo parvo apicali praediti, qui iodo non reagit. Ascospores hyalinae, unicellulares, leves, nonnumquam curvatae. Anamorphe

Colletotrichimodo phialidica; conidia unicellularia, mucida, guttulata.

Typus *Glomerella* Spauld. & H. Schrenk

Sordariomycetidae.—Members of this subclass possess light to dark colored perithecia. Asci are nonamyloid, amyloid or lack apical rings, and true paraphyses are present in some members. The subclass consists of six orders, all of which are recognized as monophyletic groups in this study (FIG. 2). The clade representing the Coniochaetales is well supported and the Coniochaetales remains as an unsupported sister group of the clade containing the Sordariales, Boliniales and Chaetosphaeriales. The internode uniting the Chaetosphaeriales with the Boliniales also is supported strongly. The Diaporthales forms a sister group relationship with the economically important family Magnaporthaceae (e.g. *Magnaporthe grisea* and *Gaeumannomyces graminis*). The Ophiostomatales is an unsupported sister group of the clade containing the representative taxa of the Annulatasceae and Papulosaceae.

Boliniales.—Members of the Boliniales occur primarily on wood. It is the only order in the Sordariomycetidae that contains taxa with large, stromatic, carbonaceous ascomata. Other taxa in the group may possess soft-textured stromata that may be brightly colored. Perithecia may be polystichous or monostichous wherein they often will be distinctively vertically elongate. Members of this order possess small asci and brown, ellipsoidal, frequently flattened ascospores that often have polar germ pores. No anamorphs are known. The Boliniales in the four-gene phylogeny is represented by four species of *Camarops* encompassing disparate morphologies and more strongly allied with *Lasiosphaeriella nitida* and *Limocarpon appendiculatum* (taxa that currently are placed in Sordariomycetidae inc. sed.) (FIG. 2).

Chaetosphaeriales.—The Chaetosphaeriales includes saprobic, often wood-inhabiting fungi. Small, dark perithecial ascomata that are often setose are found in a number of genera in the group. Ascomata typically are associated with dematiaceous, hyphomycetous anamorphs. Members of the Chaetosphaeriales have filiform paraphyses and cylindrical asci, usually with a pronounced apical refractive ring. Ascospores are often hyaline. Ascospores can range from ellipsoidal, nonseptate to elongate, almost filiform, and septate in species of *Chaetosphaeria*, with fusiform, 1–3-septate ascospores being typical of most members of the order (Fernández and Huhndorf 2005).

Sordariales.—This order consists of mostly wood- and dung-inhabiting species with relatively large, erumpent or superficial ascomata with large celled, membranous or coriaceous ascomatal walls. Filiform paraphyses are present in some taxa as are subapical globules within the asci. Ascospores in this group show variation on a distinct developmental theme and range from cylindrical, hyaline ascospores to ellipsoid, brown ascospores, often with appendages or sheaths. Many of the species in this order lack anamorphs, but some have lightly pigmented, phialidic, *Phialophora*-like anamorphs, or the ascospores germinate by directly producing phialides (Gams 2000, Rėblová and Winka 2000). The most important discovery in these four-gene analyses is that the internode representing the Sordariales is strongly supported by both bootstrap and posterior probability, which was not shown in previous studies (Huhndorf et al 2004b, Miller and Huhndorf 2005). The present taxon sampling encompasses some of the great morphological variation within the group, but the use of four genes does not distinguish any well supported new lineages. Previous studies uncovered well supported clades that correlated with distinct ascomal wall characters (Miller and Huhndorf 2005). The Sordariaceae (e.g. *Neurospora* and *Sordaria*) remains a well supported monophyletic group, and this is the only traditional family recognized in the order. While *Chaetomium* is monophyletic with strong support, the genera *Podospora* and *Cercophora* are polyphyletic.

Coniochaetales.—Members of this order typically possess small, setose perithecial ascomata and occur on wood, dung or soil. The asci are more or less clavate without an apical ring. Ascospores are brown, ellipsoidal, fusoid or discoid, and most possess a germ slit. The relatively simple ascomatal morphology can cause members of the group to be confused with taxa in the Chaetosphaeriales or the Sordariales. The known anamorphs of this order are classified as species of *Lecythophora*. These are phialidic hyphomycetes with the phialide often reduced to a collarette lateral on a hyphal cell (Gams 2000).

Diaporthales.—The Diaporthales is a strongly supported monophyletic order (FIG. 2) and includes primarily plant-associated fungi such as the agent of chestnut blight (*Cryphonectria parasitica*) and dogwood anthracnose (*Discula destructiva*). The order is characterized by black perithecial fruiting bodies that may or may not be aggregated and immersed in a stroma, evanescent interthelial elements, and asci that float free at maturity and often have conspicuous refractive apical rings. The ascospores vary from colorless, allantoid (*Valsa*), one-septate, ellipsoidal

(*Diaporthe*), or elongated (*Cryptosporella*) to large, multiseptate, and black (*Melanconis*). Commonly encountered anamorphs are coelomycetes such as *Cytospora* (*Valsa*), *Phomopsis* (*Diaporthe*) and *Melanconium* (*Melanconis*), producing pycnidia with phialidic conidiogenous cells, often on the same stroma as the sexual state.

Ophiostomatales.—This order includes fungi associated with wood and bark, such as the agents of Dutch elm disease (*Ophiostoma ulmi* and *O. novo-ulmi*) and blue stain of hard- and softwood timber (*O. piliferum*). Most species of the order however are saprobic and usually are associated with beetle dispersers, while related anamorphs (i.e. *Sporothrix schenckii*) are reported to cause diseases in humans (Summerbell 1999). The order is characterized by solitary, black perithecia, and most species have long necks from which sticky ascospores ooze and are transported by insects to new substrates. The asci are globose and dissolve early in development, while the ascospores often have ornamenting sheaths, ridges or wings. Many of the hyphomycete genera once associated with the Ophiostomatales have been simplified by the recognition that the apparent variety in conidium ontogeny represents minor variation of a common pattern. In genera such as *Leptographium* and *Pesotum* a percurrent proliferation of the conidiogenous cells and delayed secession of conidia gives a false impression of sympodial proliferation. In addition some species have exclusively sympodially proliferating, denticulate conidiogenous cells that are classified in *Sporothrix* (Wingfield et al 1993). *Ophiostoma* once was considered synonymous with *Ceratocystis* in the Microascales and their long-necked, fleshy perithecia with sticky ascospores associated with insects in wood represent a morphological homoplasy (Cain and Weresub 1957, Samuels and Müller 1978, Spatafora and Blackwell 1994).

Magnaporthaceae, Annulatascaceae and Papulosa-ceae.—These families, each with one representative species included in this phylogenetic study (FIG. 2), were grouped in the Sordariomycetidae. Their ordinal placement is unsettled due to their distinctive morphological characters and paucity of sequence data. The Magnaporthaceae is characterized by nonstromatic black perithecia, usually with long hairy necks, tapering paraphyses, persistent asci and filiform ascospores. The anamorphs are hyphomycetous. Members of the Annulatascaceae are found in rotten wood in tropical freshwater habitats. Nonstromatic perithecia are dark brown to black, sometimes long-necked, paraphyses are persistent, asci are typically cylindrical with large nonamyloid apical rings, and ascospores often have polar appendages or a

gelatinous sheath (Wong et al 1998). The obligate marine fungus *Papulosa amerospora* grows in leaves of the halophyte *Juncus roemerianus*. It is characterized by dark brown, nonstromatic perithecia, long periphysate necks, lateral paraphyses, persistent cylindrical asci with apical amyloid rings and aseptate, ellipsoidal, verruculose ascospores covered by a gelatinous sheath.

Order of uncertain position.—The placement of the Lulworthiales, although taxon sampling has been limited, suggests a new subclass lineage and is treated as an order of uncertain position in this study. This recently described order was segregated from the Halosphaeriales based on molecular data (Kohlmeyer et al 2000). The Lulworthiales is characterized by dark ostiolate ascomata, with or without a subiculum, and the absence of interascal tissue although the centrum is filled initially with a thin-walled pseudoparenchyma. Asci are thin-walled and deliquesce early, and ascospores are usually filamentous (FIG. 1O), mostly with mucus-containing apical chambers or appendages. The members of the Lulworthiales degrade wood and marsh plants in marine and estuarine environments. Two species of an enigmatic genus *Spathulospora*, obligate parasites on red algae, recently were placed in this order based on nuclear ribosomal data (Inderbitzin et al 2004). It was noted by Inderbitzin et al (2004) that *Spathulospora* shares the apical mucus-filled chambers of the ascospores with several lulworthialean species. However major features of *Spathulospora* species, absent in other members of Lulworthiales, are presence of sterile ascomatal hairs, antheridia and trichogynes. *Haloguignardia*, another genus with algae-inhabiting species and ascospores characterized by mucus-filled apical chambers, also is nested within the Lulworthiales (Campbell et al 2005). The only reported anamorphs in this order are the hyphomycetes, *Zalerion maritimum* (teleomorph *Lulworthia uniseptata*, Nakagiri 1984), with helically coiled conidia, and *Anguillospora marina* (teleomorph *Lindra obtusa*, Nakagiri and Tubaki 1983) with filiform conidia.

Evolution of morphology and ecology.—A synapomorphy of the Sordariomycetes is the perithecial ascoma, which is evolved from the apothecium of ancestral Pezizomycotina (see Spatafora et al this issue). However taxa in a number of unrelated lineages of the Sordariomycetes have lost ostioles, which usually is associated with the loss of forcible discharge of ascospores (Malloch 1981, Suh and Blackwell 1999). Most members of the Xylariomycetidae and some of the Sordariomycetidae have dark perithecia, amyloid asci, true paraphyses and periphysate ostioles. We hypothesize that these traits are plesiomorphies in the

Sordariomycetes, although the relationships among the three subclasses still are not resolved confidently. Most of the sampled taxa of the Hypocreomycetidae have light colored perithecia, nonamyloid apical rings when apical rings are present and the absence of true paraphyses.

The majority of members of the Sordariomycetes are terrestrial, and life in aquatic habitats is considered a derived character for the class (Samuels and Blackwell 2001). The Halosphaeriales, Sordariales, Diaporthales, Xylariales and Magnaporthaceae contain freshwater species, while most marine species are classified in the Halosphaeriales and Lulworthiales. Most of these fungi break down lignin and cellulose from plant debris in intertidal and subtidal zones, very rarely also in the deep sea. All the major lineages in the Sordariomycetes contain aquatic species (Shearer 1993, Spatafora et al 1998). This study further supports the conclusion that the move to aquatic environments occurred many times in the class. The Xylariomycetidae comprises saprobes and plant pathogens, which are also abundant in the other two subclasses. Therefore the saprobic and plant parasitic habits might be the ancestral states of the Sordariomycetes. Most mycoparasites and insect associates are derived from the Hypocreomycetidae, and the Sordariomycetidae is rich in coprophilous taxa.

CONCLUSION

We have tested the hypotheses of the systematics of the Sordariomycetes based on multigene phylogenetic analyses. While the phylogeny is largely consistent with the subclass and ordinal classification of Eriksson (2006), the Melanosporales is recognized as a distinct order in the Hypocreomycetidae. The phylogenetic analyses strongly support monophyly of the three subclasses, and 12 orders in the study are recognized as monophyletic groups except for the Microascales. Results from this study, coupled with 400 000 000 y old fossil record (Taylor et al 2005), also provide an insight into the long and diverse evolution of the nutritional mode/fungal symbioses in the Sordariomycetes. Because its ancestral nutritional mode is hypothesized to be saprobic or plant pathogenic, our data indicate multiple independent origins of other nutritional ecologies (e.g. insect associates and mycoparasites) and provide a foundation for developing hypotheses on the dynamic process of evolutionary patterns, such as marine colonization.

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