A five-gene phylogeny of Pezizomycotina

Joseph W. Spatafora¹ Gi-Ho Sung Desiree Johnson Cedar Hesse Benjamin O'Rourke Maryna Serdani Robert Spotts Department of Botany and Plant Pathology, Oregon State University, Corvallis, Oregon 97331

François Lutzoni

Valérie Hofstetter Jolanta Miadlikowska

Valérie Reeb

Cécile Gueidan

Emily Fraker

Department of Biology, Duke University, Box 90338, Durham, North Carolina 27708

Thorsten Lumbsch Robert Lücking

Imke Schmitt

Kentaro Hosaka

Department of Botany, Field Museum of Natural History, Chicago, Illinois 60605

André Aptroot

ABL Herbarium, G.V.D. Veenstraat 107, NL-3762 XK Soest, The Netherlands

Claude Roux

Chemin des Vignes vieilles, FR - 84120 MIRABEAU, France

Andrew N. Miller

Illinois Natural History Survey, Center for Biodiversity, Champaign, Illinois 61820

David M. Geiser

Department of Plant Pathology, Pennsylvania State University, University Park, Pennsylvania 16802

Josef Hafellner

Institut für Botanik, Karl-Franzens-Universität, Holteigasse 6, A-8010, Graz, Austria

Geir Hestmark

Department of Biology, University of Oslo, P.O. Box 1066 Blindern, NO-0316 Oslo, Norway

A. Elizabeth Arnold

Department of Plant Sciences, University of Arizona, Tucson, Arizona 85721

Accepted for publication 13 August 2006.

¹Corresponding author. E-mail: spatafoj@science.oregonstate.edu

Burkhard Büdel Alexandra Rauhut Department of Biology, University of Kaiserslautern, Kaiserslautern, Germany David Hewitt Harvard University Herbaria, Harvard University, Cambridge, Massachusetts 02138 Wendy A. Untereiner Department of Botany, Brandon University, Brandon, Manitoba, Canada Mariette S. Cole 2017 Thure Avenue, St Paul, Minnesota 55116 Christoph Scheidegger Swiss Federal Institute for Forest, Snow and Landscape Research, WSL Zürcherstr. 111CH-8903 Birmensdorf, Switzerland Matthias Schultz Biozentrum Klein Flottbek und Botanischer Garten der Universität Hamburg, Systematik der Pflanzen Ohnhorststr. 18, D-22609 Hamburg, Germany Harrie Sipman Botanischer Garten und Botanisches Museum Berlin-Dahlem, Freie Universität Berlin, Königin-Luise-Straße 6-8, D-14195 Berlin, Germany Conrad L. Schoch Department of Botany and Plant Pathology, Oregon State University, Corvallis, Oregon 97331 Abstract: Pezizomycotina is the largest subphylum of Ascomycota and includes the vast majority of filamentous, ascoma-producing species. Here we report the

results from weighted parsimony, maximum likelihood and Bayesian phylogenetic analyses of five nuclear loci (SSU rDNA, LSU rDNA, RPB1, RPB2 and EF-1a) from 191 taxa. Nine of the 10 Pezizomycotina classes currently recognized were represented in the sampling. These data strongly supported the monophyly of Pezizomycotina, Arthoniomycetes, Eurotiomycetes, Orbiliomycetes and Sordariomycetes. Pezizomycetes and Dothideomycetes also were resolved as monophyletic but not strongly supported by the data. Lecanoromycetes was resolved as paraphyletic in parsimony analyses but monophyletic in maximum likelihood and Bayesian analyses. Leotiomycetes was polyphyletic due to exclusion of Geoglossaceae. The two most basal classes of Pezizomycotina were Orbiliomycetes and Pezizomycetes, both of which comprise species that produce apothecial ascomata. The seven

remaining classes formed a monophyletic group that corresponds to Leotiomyceta. Within Leotiomyceta, the supraclass clades of Leotiomycetes s.s. plus Sordariomycetes and Arthoniomycetes plus Dothideomycetes were resolved with moderate support.

Key words: ascoma, Ascomycota, ascus, evolution, fungi, systematics

INTRODUCTION

Ascomycota is the largest phylum of kingdom Fungi and includes approximately 33 000 described species (Kirk et al 2001). It is characterized by the production of ascospores (meiospores) in a specialized sacshaped meiosporangium, the ascus (Alexopoulos et al 1996). The current classification of Ascomycota (Outline of Ascomycota, Eriksson 2006, now hosted at http://www.fieldmuseum.org/myconet) consists of four early diverging classes, Neolectomycetes, Pneumocystidiomycetes, Schizosaccharomycetes and Taphrinomycetes, and two subphyla, Saccharomycotina and Pezizomycotina. The former were classified previously in Taphrinomycotina (Eriksson et al 2003), a taxon that is not currently recognized due to the lack of statistical support for its monophyly in single gene and multigene phylogenies (Eriksson 2006). Saccharomycotina comprises the single class Saccharomycetes and order Saccharomycetales and includes the majority of ascomycetous yeast species (e.g. Candida albicans, Saccharomyces cerevisiae). Pezizomycotina (= Ascomycetes sensu Kirk et al 2001, = Euascomycetes sensu Alexopoulos et al 1996) is the largest subphylum of the Ascomycota with more than 32 325 described species. It includes all filamentous, ascoma-producing species, with the exception of Neolecta. Pezizomycotina is ecologically diverse with species functioning in numerous ecological processes and symbioses (e.g. wood and litter decay, animal and plant pathogens, mycorrhizae and lichens) and occurring in aquatic and terrestrial habitats.

Before molecular phylogenetics, supraordinal classifications of Pezizomycotina were based on varying interpretations of morphology and development of ascomata and asci. The four main ascoma morphologies include apothecia, perithecia, cleistothecia and ascostromata (Alexopoulos et al 1996, FIG. 1). Apothecia are typically disk-shaped to cup-shaped to spathulate and produce asci in a well defined layer, a hymenium, exposed to the environment. Perithecia and cleistothecia are partially or completely enclosed ascomata, respectively, with ascus production occurring within the central cavity or centrum of the ascoma. Perithecia are described as "true" ascomata whereby at least the inner ascoma wall forms concurrently with development of ascogenous hyphae

(ascohymenial development, Luttrell 1951). It is generally presumed that ascomatal development initiates after fertilization of the ascogonium. The asci are produced in a defined hymenium often interspersed with sterile (hamathecial) tissue of paraphyses, which originate from the subhymenial region of the ascoma, and ostioles are typically periphysate. Absence of true paraphyses is known in several ascohymenial lineages (e.g. Hypocreales), however. Ascostromata differ in that asci are formed in preformed locules (ascolocular development; Nannfeldt 1932, Luttrell 1973), which often develop in flask-shaped (pseudothecia) or open, cup-shaped (hysterothecia and thyriothecia) stromatic tissue that superficially resemble perithecia or apothecia, respectively. It is presumed generally that initiation of ascostromata development occurs before fertilization of ascogonia. Asci, while sometimes produced in a fascicle, are typically not produced in a hymenial layer interspersed with paraphyses, although hamathecial tissue may be present (Luttrell 1955, Liew et al 2000). Cleistothecia have been described as ascohymenial or ascostromatic according to species (Malloch 1985a, b; Barr 1987).

The major ascus types include operculate, inoperculate, prototunicate, unitunicate and bitunicate. The different types are based primarily on the number and thickness of functional ascus walls, which appear multilayered in transmission electron microscopy, and mechanisms of dehiscence (FIG. 1; Eriksson 1981, Alexopoulos et al 1996, Kirk et al 2001). Operculate asci are restricted to apothecial fungi. They release ascospores through a defined operculum that is formed either terminally or subterminally at the ascus apex. Inoperculate asci are produced by apothecial, cleistothecial and perithecial fungi and are typically thin-walled and release their spores through a pore or canal, by rupturing of the ascus apex or by disintegration of the ascus wall. Prototunicate asci are produced by apothecial, cleistothecial and perithecial fungi. They are thin-walled, typically globose to broadly clavate and release their ascospores passively by disintegration of the ascus wall. Bitunicate asci are conspicuously thick-walled and characterized by possessing two, often separable, functional ascus walls, the exotunica and a distinct endotunica. They are produced by ascostromatic lichenized and nonlichenized species (e.g. Dothideales, Barr 1987) and ascohymenial lichens (e.g. Arthoniales, Henssen and Thor 1994). In the traditional definition of bitunicate asci, fissitunicate ascus dehiscence occurs when the endotunica ruptures through the exotunica in a jack-in-the-box manner (Eriksson 1981). Additional modes of dehiscence exist among "bitunicate" ascus morpholo-



FIG. 1. Exemplar ascomatal and ascal morphologies for 10 classes of the Pezizomycotina. a. Apothecia (yellow) of *Orbilia*, Orbiliomycetes (J.H. Petersen/MycoKey). b. Apothecia of *Aleuria*, Pezizomycetes (J.H. Petersen/MycoKey). c. Thallus of *Ophioparma* w/apothecia, Lecanoromycetes (B. McCune, Oregon State University). d. Thallus of *Lichinella*, Lichinomycetes (B. McCune, Oregon State University). d. Thallus of *Lichinella*, Lichinomycetes (B. McCune, Oregon State University). g. Thallus of *Prolixandromyces*, Laboulbeniomycetes (A. Weir, SUNY-ESF). h. Perithecia of *Neurospora*, Sordariomycetes (N.B. Raju, Stanford University). i. "Earth-tongue" apothecia of *Cudonia*, Leotiomycetes (Z. Wang, Iowa State University). j. Cleistothecia of *Eupenicillium*, Eurotiomycetes (D. Geiser, Penn State University). k. Operculate ascus of *Peziza* (J.H. Petersen/MycoKey). l. Ascostroma of *Venturia*, Dothideomycetes (T. Volk, University of Wisconsin at La Crosse). m. Unitunicate asci *Neurospora* (N.B. Raju, Stanford University). n. Prototunicate ascus of *Eurotium* (D. Geiser, Penn State University).

gies, including semifissitunicate, pseudofissitunicate, rostrate and bilabiate (Eriksson 1981, Hafellner 1988), which involve little to no ascus wall separation and are especially prevalent among lichenized taxa. Due to the nonfissitunicate mechanisms of dehiscence and relatively thin ascus walls, operculate, inoperculate and prototunicate asci are collectively referred to as unitunicate (Luttrell 1951).

A complete review of the past major classifications is not possible here; rather we attempt to provide a concise overview and direct the reader to McLaughlin et al (2001) for more thorough reviews and historical treatments. Classification of the major groups of Ascomycota into the modern concepts of Discomycetes, Plectomycetes and Pyrenomycetes, based respectively on apothecia, cleistothecia and perithecia, has its foundation in Anton de Bary's classic work on comparative morphology of fungi (de Bary 1887). In the early 20th century this system was refined and expanded, most notably by von Höhnel (1907), Gäumann (1928) and Bessey (1935). Nannfeldt (1932) proposed the distinction between ascolocular (= ascostromatic) and ascohymenial forms of development and defined a fourth major group, Ascoloculares, which later was modified to Loculoascomycetes (Luttrell 1973). Discomycetes traditionally were divided into various higher taxa (e.g. subclasses, orders, etc.) based on operculate and inoperculate ascus morphology (Seaver 1942, 1951; Kimbrough 1970; Korf 1973; Pfister and Kimbrough 2001) with lichenized apothecial species classified in separate higher taxa (e.g. orders) from nonlichenized species (e.g. Hawksworth and Hill 1984, Poelt 1973). Pyrenomycetes included a host of perithecial orders that were centered around taxa we recognize today as Hypocreales, Sordariales and Xylariales (Miller 1949, Luttrell 1951, Wehmeyer 1975, Samuels and Blackwell 2001), but in various treatments the class also has included lichenized species of Pyrenulales (Bessey 1961), including Porinaceae which is now part of Ostropomycetidae, cleistothecial species of Erysiphales (Yarwood 1973) and ascostromatic species of Dothideales (Bessey 1939). Plectomycetes traditionally included cleistothecial taxa of Eurotiales and Onygenales (Fennell 1973, Geiser and Lobuglio 2001), but it also has been used to accommodate taxa that produce prototunicate asci in a scattered manner in either cleistothecial or perithecial ascomata (e.g. Ophiostoma, Luttrell 1951, Benny and Kimbrough 1980).

With the advent of molecular phylogenetics it became apparent that certain morphologies were derived many times and were the product of convergent evolution (e.g. cleistothecium, prototunicate asci, Berbee and Taylor 1992), while others likely represent ancestral traits for the Pezizomycotina (e.g. apothecium, Gernandt et al 2001). These and other studies collectively reflect the impact that phylogenetic analyses of ribosomal DNA (rDNA) sequences have had on our understanding of fungal phylogeny and the classification of kingdom Fungi. The current classification of Ascomycota (Eriksson 2006) is based largely on rDNA phylogenies, with Pezizomycotina

divided into 10 classes with corresponding ascomatal and ascus character states as follows: Arthoniomycetes (apothecia; bitunicate), Dothideomycetes (ascostromata; bitunicate), Eurotiomycetes (perithecia, cleistothecia, ascostromata; bitunicate, prototunicate), Laboulbeniomycetes (perithecia; prototunicate), Lecanoromycetes (apothecia, perithecia; bitunicate, inoperculate, prototunicate), Leotiomycetes (apothecia, cleistothecia; inoperculate, prototunicate), Lichinomycetes (apothecia; bitunicate, inoperculate, prototunicate), Orbiliomycetes (apothecia; inoperculate), Pezizomycetes (apothecia; operculate) and Sordariomycetes (perithecia, cleistothecia; inoperculate, prototunicate). Here we analyze data from multiple genes, including rDNA and protein-coding loci, from a broad sampling of taxa to test current classifications and provide additional insight into the evolution of Pezizomycotina.

MATERIALS AND METHODS

Taxon and character sampling.—Data were sampled both to provide a broad and inclusive taxon sampling of Pezizomycotina and to include the maximum number of genes with the minimum amount of missing data. A total of 196 taxa were sampled, including 14 outgroup taxa (1 Zygomycota and 14 Basidiomycota) and 182 Ascomycota (8 'Taphrinomycotina', 12 Saccharomycotina and 162 Pezizomycotina). Sequence data from five nuclear genes (SSU rDNA, LSU rDNA, EF-1 α , RPB1 and RPB2) were obtained from the Assembling the Fungal Tree of Life (AFTOL) and GenBank sequence databases. Eleven of the taxa possess sequence data for only three or four of the five genes. A complete list of taxa included in this study along with AFTOL and GenBank accession numbers are provided (SUPPLEMENTARY TABLE I).

Phylogenetic analyses.—Sequence alignments for each gene were performed in Clustal X and refined by direct examination. Regions designated as ambiguously aligned were excluded from analyses. To test for potential conflict, parsimony bootstrap analyses were performed on each individual dataset and 70% bootstrap consensus trees were examined for conflict (Lutzoni et al 2004). The resulting combinable data were concatenated into a single alignment and delimited into 11 partitions, including SSU rDNA, LSU rDNA, and the first, second and third codon positions of EF- 1α , RPB1 and RPB2, with unique models applied to each partition. The nexus dataset is available on the AFTOL Website (aftol.org) and TreeBASE (SN2929).

Weighted parsimony (WP) analyses were performed with step matrices according to Lutzoni et al (2004). A total of 100 heuristic replicates of random sequence addition (RSA) were performed with PAUP* v10 (Swofford 2002) with TBR branch swapping and MULTREES in effect. Maximum likelihood was performed with RAxML-VI-HPC and RAxML-VI ver.1.0 using a GTRCAT model of evolution with 50 rate categories (Stamatakis et al 2005). Bayesian Metropolis coupled Markov chain Monte Carlo (B-MCMCMC) analyses were conducted with MrBayes 3.1 with GTR+I+ Γ (Ronquist et al 2005). Searches were conducted with four chains (three cold, one hot) with trees sampled every 100 generations. Three 10 000 000-generation analyses were conducted to verify likelihood convergence and burn-in parameter.

Nodal support in WP and RAxML analyses was determined by nonparametric bootstrapping. One hundred WP bootstrap replicates were performed with the same search options as described previously, but with five heuristic RSA per bootstrap replicate. Two hundred fifty RAxML bootstrap replicates were performed with the same search options as described previously. Nodal support in B-MCMCMC analyses was estimated as posterior probabilities calculated from the posterior distribution of trees excluding burn-in trees (Ronquist et al 2005). For purposes of this manuscript support for individual nodes were considered weak, moderate or strong when receiving WP bootstrap (WP-BS), RAxML bootstrap (ML-BS) and B-MCMCMC posterior probabilities (B-PP) as follows: weak support WP-BS and ML-BS <50%and/or B-PP < 0.95, moderate support WP-BS and/or ML-BS 50–69% and B-PP > 0.94, strong support WP-BS and/or ML-BS 70–100% and B-PP > 0.94.

RESULTS AND DISCUSSION

Phylogenetic analyses.—Significant levels of conflict were detected among independent gene trees for five taxa (881 Opegrapha varia LSU rDNA, 101 Anisomeridium polypori LSU rDNA, 1014 Pannaria mediterranea RPB1, 230 Polychidium sp. RPB1 and 105 Gyalideopsis vulgaris SSU and LSU rDNA, RPB1), which were removed from subsequent analyses. The final dataset consisted of 191 taxa and 7136 included nucleotide positions (1637 SSU, 1380 LSU, 969 EF-1a, 1077 RPB1, 2073 RPB2). Weighted parsimony analyses were based on 4199 parsimony informative characters (675 SSU, 705 LSU, 551 EF, 845 RPB1, 1405 RPB2) and resulted in a single most parsimonious tree of 171 419.23 steps with little bootstrap support for deep nodes. RAxML produced a tree identical to the Bayesian phylogeny with minor exceptions at terminal nodes and increased bootstrap support for some deep nodes. All three Bayesian analyses converged on a set of most likely trees after approximately 1 000 000 generations. The consensus of the 90 000 Bayesian trees from one of the three MrBayes analyses (harmonic mean 562 704.78) after excluding 10 000 burn-in trees is provided (FIG. 2).

Deep nodes of Ascomycota.—Although weakly supported by the data, taxa classified in Saitoëlla, Neolectomycetes, Pneumocystidiomycetes, Schizosaccharomycetes and Taphrinomycetes were resolved as a monophyletic group corresponding to Taphrinomycotina (Eriksson et al 2003) in all analyses. While we propose no nomenclatural changes we do recognize these taxa as representatives of early diverging branches of Ascomycota. Saccharomycotina and Pezizomycotina were inferred both to be monophyletic and collectively formed a strongly supported superphylum clade (FIG. 2).

Basal branches of Pezizomycotina.—Orbiliomycetes and Pezizomycetes. These classes are the two most basal or early diverging branches of Pezizomycotina. Orbiliomycetes is resolved as the most basal branch of Pezizomycotina but the node separating it and Pezizomycetes is weakly supported (FIG. 2). Orbiliomycetes consists of a single order (Orbiliales) with two genera Orbilia and Hyalorbilia (Eriksson et al 2003). It is characterized by waxy, minute, colored to white to translucent apothecia that are formed on soil or wood. It is perhaps best known for being linked to nematophagous anamorphs of the genus Arthrobotrys, but other anamorphs are known as well (Pfister 1997). Asci are minute with branched bases and truncate apices that possess or lack a conspicuous apical pore according to species. Orbiliales previously were classified in Helotiales based on the characters of apothecial ascomata and inoperculate asci. Molecular phylogenetic studies of rDNA (Pfister 1997, Gernandt et al 2001) did not support these characters as being diagnostic of monophyletic groups and a new order and class were erected to accommodate this taxon (Eriksson et al 2003). These analyses are consistent with Orbilia's exclusion from Leotiomycetes.

Pezizomycetes comprises one order, Pezizales, which includes all species of Ascomycota that form operculate asci (Landvik et al 1997, Eriksson 2006). Most species produce apothecial ascomata with the exception of truffle-forming, prototunicate taxa (e.g. Tuber), which originated many times during the evolution of the clade (O'Donnell et al 1997, Hansen et al 2001). Pezizomycetes has never been strongly supported in rDNA phylogenies with different studies producing both paraphyletic and monophyletic groupings (Landvik et al 1997, Gernandt et al 2001, Lutzoni et al 2004). These analyses expand the character sampling and provide moderate support for the class and the diagnostic trait of operculate asci. These data do provide strong support for the existence of three major subclades within Pezizales, a finding consistent with Landvik et al (1997) (FIG. 2). The majority of Ascomycota species that form ectomycorrhizae are members of Pezizomycetes, but the definitive ecology and nutritional mode for many species of this class is not well characterized and is largely extrapolated from other taxa and ascomatal fruiting patterns. Based on its wide range of habitats (e.g. forests, urban landscaping, etc.), substrates (e.g. soil, wood, dung, etc.) and life history strategies (e.g.



FIG. 2. Bayesian consensus tree of the Pezizomycotina from 90 000 credible trees. ML-BS values \geq 70% and PP \geq 95 are provided respectively above and below major branches, and all branches that received WP-BS \geq 70% are thickened. Asterisks denote species with annotated genomes. Nodes for higher taxa are labeled: A = Ascomycota, P = Pezizomycotina and L = Leotiomyceta.

reliance on old growth forests, fruiting after fires, etc.), Pezizomycetes likely encompasses a range of saprobic to ectomycorrhizal ecologies similar to Agaricales.

The crown clade of Pezizomycotina, Leotiomyceta.—The remaining seven classes of the Ascomycota form a well supported clade, Leotiomyceta (Eriksson and Winka 1997, Lumbsch et al 2005), although the supraclass nomenclature has not been used widely. This clade includes all inoperculate (except for Orbilia) and bitunicate taxa of Pezizomycotina. Sordariomycetes and Leotiomycetes s.s. are resolved with moderate support in RAxML and Bayesian analyses as sharing a most recent common ancestor, a finding consistent with the results of rDNA analyses of Lumbsch et al (2005) and phylogenomic analyses of Robbertse et al (2006). The Sordariomycetes/ Leotiomycetes s.s. clade includes the majority of nonlichenized species that form relatively thin-walled, inoperculate, unitunicate asci with ascus apices possessing either canals or pores, or derived prototunicate forms. Geoglossaceae is a member of Leotiomyceta and is an exception to this character state distribution (see below).

Sordariomycetes. This class includes the majority of perithecial taxa, although cleistothecia have been derived numerous times through the loss of the ostiolar canal (Samuels and Blackwell 2001). The dominant ascus morphology is inoperculate, thinwalled, unitunicate with a diversity of ascus tip morphologies according to taxa (e.g. thickened ascus tip of Cordyceps). Prototunicate asci have been derived numerous times presumably under selection for insect (e.g. Ophiostoma) and/or water (e.g. Corollospora) dispersal of ascospores or correlated with the production of cleistothecia (Blackwell 1994, Spatafora et al 1998). Sordariomycetes is strongly supported by these analyses with the monophyly of the class confidently resolved across a range of taxon and gene samplings (Lutzoni et al 2004). It contains three subclasses, Hypocreomycetidae, Sordariomycetidae and Xylariomycetidae (Eriksson 2006), each of which is resolved as monophyletic but with varying levels of support (FIG. 2). Based on the current sampling production of true paraphyses is restricted to Sordariomycetidae and Xylariomycetidae, with members of Hypocreomycetidae either possessing apical, lateral or centripetal pseudoparaphyses (periphysoids, Eriksson 1981), pseudoparenchyma (Luttrell 1951) or lacking paraphyses altogether (Luttrell 1951, Spatafora and Blackwell 1994). Major ecologies represented in the clade include wood decay (e.g. Xylariales), saprobes (e.g. Hypocreales), coprophiles (e.g. Sordariales), endophytes (e.g. Sordariales), mycoparasites (e.g. Hypocreales), plant pathogens (e.g. Diaporthales,

Ophiostomatales, Hypocreales), aquatic (e.g. Halosphaeriales, Lulworthiales) and insect mutualists (e.g. Microascales) and pathogens (e.g. Hypocreales).

Leotiomycetes. Leotiomycetes sensu Eriksson (2006) includes the majority of nonlichenized, inoperculate, unitunicate apothecial species except for Neolecta, Orbilia and Ostropales s.s. Monophyly of the class was not recovered by previous analyses of rDNA analyses (Gernandt et al 2001, Lutzoni et al 2004) and the data present herein did not support the monophyly of Leotiomycetes. The inclusion of protein coding data provided strong support for the core clade Leotiomycetes s.s., but Geoglossum and Trichoglossum (Geoglossaceae) were rejected as being members of the class (FIG. 2). They grouped with strong support with the sole representative of Lichinomycetes, but sampling is insufficient to comment beyond that they are not member of Leotiomycetes s.s. Ordinal and familial representatives of Leotiomycetes s.s. sampled here are Helotiales, Leotiales, Rhytismatales and Pseudeurotiaceae, which collectively exhibit cupshaped (e.g. Sclerotinia), earth-tongue (e.g. Leotia) and hysteriate (e.g. Coccomyces) apothecia and cleistothecia (e.g. Pseudeurotia). Additional taxa that are supported as members of Leotiomycetes s.s. based on rDNA data, but for which protein-coding data were lacking, include Erysiphales and Cyttariales (Wang et al this volume), which have been classified respectively as Pyrenomycetes (Yarwood 1973) and Discomycetes (Korf 1973). Leotiomycetes s.s. comprises plant-associated fungi whose ecologies range from pathogens (e.g. Sclerotinia), to endophytes (e.g. Phacidium), to saprobes (e.g. Lachnum), to mycorrhizae (e.g. Hymenos*cyphus*) and a large number of taxa whose ecology and nutritional modes are poorly understood but are assumed to be plant based.

Dothideomycetes and Arthoniomycetes. Dothideomycetes closely corresponds to Loculoascomycetes (Barr 1987, Berbee 1996) excluding Chaetothyriales and related taxa (FIG. 2). It includes the majority of nonlichenized, ascostromatic, bitunicate fungi, although lichenized members are known (e.g. Arthopyreniaceae, Trypetheliaceae; Lumbsch et al 2005). Dothideomycetes is resolved as monophyletic with Arthoniomycetes as sister group (FIG. 2). Dothideomycetes includes a pseudoparaphysate Pleosporales clade, a core aparaphysate Dothideales, Capnodiales and Myriangiales clade, and other residual bitunicate taxa (e.g. Tyrannosorus, Kirschsteiniothelia and the lichenized Trypetheliaceae) (FIG. 2, Schoch et al this volume). Ecologically it is represented by plantassociated fungi that range from highly virulent plant pathogens (e.g. Cochliobolus, Mycosphaerella) to relatively benign saprobes (e.g. Cochliobolus, Aureobasidium) to marine fungi most frequently isolated from

mangroves (e.g. *Verruculina*), to lichenized fungi in mostly (sub)tropical environments. Arthoniomycetes contains the single, mostly lichenized order, Arthoniales, and consists of chiefly tropical and subtropical microlichens that produce bitunicate asci in ascohymenial apothecia (Henssen and Thor 1994). Monophyly of the Arthoniomycetes-Dothideomycetes clade is resolved in all analyses with moderate support and is consistent with previous classifications of Santesson (1952), Barr (1987) and Tehler (1990).

Eurotiomycetes. Eurotiomycetes comprises a diverse group of taxa that includes cleistothecial, perithecial and ascostromatic fungi, which exhibit saprobic, parasitic, pathogenic and lichenized adaptations. Molecular phylogenetic studies based on different gene and taxon samplings have produced trees that have been interpreted as both rejecting (Liu et al 2004) and supporting the hypothesis (Lutzoni 2004, Lumbsch et al 2005). These data resolve Eurotiomycetes as a strongly supported monophyletic class (FIG. 2) and support its recognition as defined in Eriksson (2006). The class contains two subclasses, Eurotiomycetidae and Chaeothyriomycetidae (but see Geiser et al this volume). Eurotiomycetidae includes Eurotiales and Onygenales, two cleistothecial orders whose close relationship is undisputed. In addition Caliciopsis of Coryneliales is well supported as a member of Eurotiomycetidae, a finding consistent with the placement of Corynelia based on rDNA (Winka 2000) and protein data (Geiser et al this volume). Coryneliales is an enigmatic order of bitunicate Ascomycota whose asci have been described as intermediate between bitunicate and prototunicate (Read and Beckett 1996). While its supraordinal affiliation has been problematical the placement of Coryneliales is consistent with the sister group relationship of Chaeothyriomycetidae and Eurotiomycetidae. The former includes bitunicate fungi including the ascostromatic nonlichenized Chaetothyriales and the ascohymenial lichenized Verrucariales, which share the synapomorphy of evanescent periphysoids and periphysate ostioles (Barr 1987). Additional studies have demonstrated that Pyrenulales, a mainly tropical group of ascohymenial perithecial lichens with paraphyses and periphysate ostioles, is also a member of the Chaetothyriomycetidae (Lumbsch et al 2004, Schmitt et al 2005). As such these findings support the hypothesis that cleistothecial, prototunicate fungi of Eurotiomycetes were derived from a bitunicate ancestor (Berbee 1996).

Lecanoromycetes. This class represents the largest group of lichenized Ascomycota. The majority of species produce apothecial ascomata, although perithecial ascomata are known for members of Porinaceae, Thelenellaceae and related families

(Grube et al 2004, Schmitt et al 2005). Asci are typically bitunicate (although of a different type compared to the other bitunicate classes), often multilayered, but also sometimes prototunicate (Caliciaceae) or unitunicate (Porinaceae, Coenogoniaceae), with the majority of taxa displaying rostrate, semifissitunicate and bilabiate modes of dehiscence (Eriksson 1981, Hafellner 1988). Numerous multigene phylogenetic studies of the class have been conducted recently and have greatly increased our understanding of this large and diverse taxon (Reeb et al 2004, Grube et al 2004, Lumbsch et al 2004, Miadlikowska and Lutzoni 2004, Schmitt et al 2005). Lecanoromycetes currently includes three subclasses, Acarosporomycetidae, Ostropomycetidae and Lecanoromycetidae, which are resolved here, and additional lineages (e.g. Umbilicariaceae) whose relationship in the group is poorly defined in molecular phylogenies (Lutzoni et al 2004, FIG. 2). Previous studies have identified Eurotiomycetes as the closest relative of the Lecanoromycetes (Lutzoni et al 2001), but the analyses presented here resolved the placement of Eurotiomycetes differently albeit weakly supported (FIG. 2). While these two classes may be closely related, these analyses strongly support Peltula (Lichinomycetes) and Geoglossaceae as more closely related to Lecanoromycetes. Based on the inability of the current dataset to confidently resolve a number of basal nodes of the Pezizomycotina phylogeny (see asterisks in FIG. 2), the complexity of this clade and associated taxa likely will require more intensive phylogenetic analyses on a genomic scale (Rokas et al 2005, Robbertse et al 2006).

Undersampled and unsampled classes of the Pezizomycotina. Lichinomycetes is a class of lichenized Ascomycota that includes a single order, Lichinales, with four families that associate almost exclusively with cyanobacteria. The sampling here included a single species of *Peltula* (Peltulaceae), which possesses a lecanoralean ascus and is arguably not representative of the more common prototunicate ascal species of Lichinales. Phylogenetic analyses of rDNA data however supported its monophyly with Lichinaceae and Heppiaceae of Lichinales (Schultz et al 2001). Peltula formed a well supported clade with Geoglossaceae that was resolved as closely related to Lecanoromycetes (FIG. 2), but increased taxon sampling is needed for Lichinales to test its monophyly and its relationship to both Geoglossaceae and Lecanoromycetes. The only unsampled class of Pezizomycotina is Laboulbeniomycetes, which comprises ectoparasites of insects (Laboulbeniales) and mycoparasites and coprophiles (Pyxidiophorales). The two orders possess drastically different ascomatal morphologies but produce similar ascospores characterized by holdfasts (Blackwell 1994). The close relationship between the two orders was

confirmed based on SSU rDNA (Blackwell 1994), supporting the homology of ascospores, but their placement within Pezizomycotina remains elusive.

Conclusion.—In general these data support the class level taxonomy of Eriksson (2006) with some notable refinements: (i) Geoglossaceae is removed from Leotiomycetes and is treated as a family incertae sedis; (ii) Coryneliales is placed as a member of Eurotiomycetidae, providing further confirmation for a close relationship between some bitunicate ascostromatic taxa and Eurotiales and Onygenales; (iii) Arthoniomycetes plus Dothideomycetes and Sordariomycetes plus Leotiomycetes respectively form monophyletic groups with moderate support. The increase in support for some nodes previously noted in ribosomal based phylogenetic analyses suggests that the addition of protein coding data will continue to improve resolution for certain nodes. This is especially true for Eurotiomycetes, a controversial taxon that was inferred as monophyletic with strong statistical support. It seems likely however that resolution of several deep nodes of the Pezizomycotina will require phylogenomic analyses involving tens to hundreds of genes (Rokas et al 2005, Robbertse et al 2006) and thus sequencing of additional fungal genomes.

As of this publication, 29 Ascomycota genomes were sequenced and annotated from six classes (1 Dothideomycetes, 8 Eurotiomycetes, 2 Leotiomycetes, 6 Sordariomycetes, 11 Saccharomycetes and 1 Schizosaccharomycetes). Phylogenomic analyses of these classes (Robbertse et al 2006) supported a number of findings presented here, including strong support for the monophyly of Pezizomycotina plus Saccharomycotina, the monophyly of Pezizomycotina and the monophyly of Leotiomycetes plus Sordariomycetes, but some taxa remained problematic (e.g. Eurotiomycetes and Dothideomycetes). This might be due to either inadequate taxon sampling or that the backbone of the Ascomycota is most accurately explained as a hard polytomy. Future sampling of Ascomycota genomes therefore should prioritize sequencing of unsampled classes of Ascomycota (e.g. Arthoniomycetes, Orbiliomycetes, etc.) to ensure more global coverage of known phylogenetic diversity.

ACKNOWLEDGMENTS

We thank these people for providing material in support of this manuscript: A. Amtoft, I. Brodo, W. Buck, P. Crittenden, P. Diederich, D. Ertz, S. Hambleton, D. Hillis, S.M. Huhndorf, S. Joneson, I. Martinez, R. Oliver, M. Garcia Otalora, C. Printzen, M. Saunders, R. Shoemaker, R. Yahr and A. Zavarzin; D. Hibbett, P.B. Matheny, M. Binder and the Basidiomycota AFTOL group for outgroup sequences; P. Crous and Centraal Bureau voor Schimmelcultures for providing isolates; and S. Given and C. Sullivan in the CGRB at Oregon State University for assistance in parallel computing. We also acknowledge support from the National Science Foundation (DEB-0228725, Assembling the Fungal Tree of Life to J.W. Spatafora and DEB-0090301, Research Coordination Network: A Phylogeny for Kingdom Fungi to M. Blackwell, J.W. Spatafora and J.W. Taylor).

LITERATURE CITED

- Alexopoulos CJ, Mims CW, Blackwell M. 1996. Introductory mycology. 4th ed. New York: John Wiley & Sons.
- Barr ME. 1987. Prodromus to Class Loculoascomycetes. Amherst, Massachusetts: Newell Inc.
- Benny GL, Kimbrough JW. 1980. A synopsis of the orders and families of plectomycetes with keys to genera. Mycotaxon 39:43–184.
- Berbee ML, Taylor JW. 1992. Convergence in ascospore discharge mechanism among Pyrenomycete fungi based on 18S ribosomal RNA gene sequence. Mol Phylogen Evol 1:59–71.
- ———. 1996. Loculoascomycete origins and evolution of filamentous ascomycete morphology based on 18S rDNA gene sequence data. Mol Biol Evol 13:462–470.
- Bessey EA. 1935. A textbook of Mycology. Philadelphia: P. Blakiston's Son & Co. Inc.
- ——. 1961. Morphology and taxonomy of fungi. New York: Hafner Publishing Co.
- Blackwell M. 1994. Minute mycological mysteries: the influence of arthropods on the lives of fungi. Mycologia 86:1–17.
- de Bary A. 1887. Morphology and biology of the fungi, mycetozoa and bacteria. Oxford, UK: Clarendon Press.
- Eriksson OE. 1981. The families of bitunicate Ascomycetes. Opera Botan 60:1–209.
- ——, ed. 2006. Outline of Ascomycota 2006. Myconet 12: 1–82.
- ——, Winka K. 1997. Supraordinal taxa of Ascomycota. Myconet 1:1–16.
- ——, Baral H-O, Currah RS, Hansen K, Kurtzman CP, Rambold G, Laessoe T, eds. 2003. Outline of Ascomycota 2003. Myconet 7:1–103.
- Fennell DI. 1973. Plectomycetes: Eurotiales. In: Ainsworth GC, Sparrow FK, Sussman AS, eds. The fungi. Vol. IVA. New York: Academic Press. p 45–68.
- Gäumann EA. 1928. Comparative morphology of fungi. New York: McGraw-Hill Book Co. Inc.
- Geiser DM, LoBuglio KL. 2001. The monophyletic Plectomycetes: Ascosphaerales, Onygenales, Eurotiales. In: McLaughlin DJ, McLaughlin EG, Lemke PA, eds. The Mycota. Vol 7A. Systematics and evolution. NewYork: Springer-Verlag. p 201–220.
- Gernandt DS, Platt JL, Stone JK, Spatafora JW, Holst-Jensen A, Hamelin RC, Kohn LM. 2001. Phylogenetics of Helotiales and Rhytismatales based on partial small subunit nuclear ribosomal DNA sequences. Mycologia 93:915–933.
- Grube M, Baloch E, Lumbsch HT. 2004. The phylogeny of

Porinaceae (Ostropomycetidae) suggests a neotenic origin of perithecia in Lecanoromycetes. Mycol Res 108:1111–1118.

- Hafellner J. 1988. Principles of classification and main taxonomic groups. In: Galun M, ed. CRC handbook of lichenology. Boca Raton: CRC Press. vol 3: 4–52.
- Hansen K, Laessøe T, Pfister DH. 2001. Phylogenetics of the Pezizaceae, with an emphasis on Peziza. Mycologia 93: 958–990.
- Hawksworth DL, Hill DJ. 1984. The lichen-forming fungi. Glasgow and London: Blackie.
- Henssen A, Thor G. 1994. Developmental morphology of the "zwischengruppe" between Ascohymeniales and Ascoloculares. In: Hawksworth DL, ed. Ascomycete systematics: problems and perspectives in the nineties. New York: Plenum Press Press. p 43–56.
- Kimbrough JW. 1980. Current trends in the classification of Discomycetes. Bot Rev 36:91–161.
- Kirk PM, Cannon PF, David JC, Stalpers JA. 2001. Ainsworth and Bisby's dictionary of the fungi. 9th ed. Wallingford, UK: CAB International.
- Korf RP. 1973. Discomycetes and Tuberales. In: Ainsworth GC, Sparrow FK, Sussman AS, eds. The fungi. Vol. IVA. New York: Academic Press. p 249–319.
- Landvik S, Egger KN, Schumacher T. 1997. Toward a subordinal classification of the Pezizales (Ascomycota): phylogenetic analyses of SSU rDNA sequences. Nord J Bot 17:403–418.
- Liew CY, Aptroot A, Hyde KD. 2000. Phylogenetic significance of the pseudoparaphyses in loculoascomycete taxonomy. Mol Phylogen Evol 16:392–402.
- Liu YJ, Hall BD. 2004. Body plan evolution of ascomycetes, as inferred from an RNA polymerase II phylogeny. Proceedings of the National Academy of Sciences, USA 101:4507–4512.
- Lumbsch HT, Schmitt I, Palice Z, Wiklund E, Ekman S, Wedin M. 2004. Supraordinal phylogenetic relationships of Lecanoromycetes based on a Bayesian analysis of combined nuclear and mitochondrial sequences. Mol Phylogen Evol 31:822–832.
 - —, —, Lindemuth R, Miller A, Mangold A, Fernandez F, Huhndorf S. 2005. Performance of four ribosomal DNA regions to infer higher-level phylogenetic relationships of inoperculate euascomycetes (Leotiomyceta). Mol Phylogen Evol 34:512–524.
- Luttrell ES. 1951. Taxonomy of the Pyrenomycetes. U Missouri Stud 3:1–120.
- ——. 1973. Loculoascomycetes. In Ainsworth GC, Sparrow FK, Sussman AS, eds. The fungi. Vol. IVA. New York: Academic Press. p 135–219.
- Lutzoni F, Pagel M, Reeb V. 2001. Major fungal lineages are derived from lichen symbiotic ancestors. Nature 411: 937–940.
- —, Kauff F, Cox CJ, McLaughlin D, Celio G, Dentinger B, Padamsee M, Hibbett D, James TY, Baloch E, Grube M, Reeb V, Hofstetter V, Schoch C, Arnold AE, Miadlikowska J, Spatafora J, Johnson D, Hambleton S, Crockett M, Shoemaker R, Sung G-H, Lücking R, Lumbsch T, O'Donnell K, Binder M, Diederich P, Ertz D, Gueidan C, Hansen K, Harris RC, Hosaka K, Lim Y-

W, Matheny B, Nishida H, Pfister D, Rogers J, Rossman A, Schmitt I, Sipman H, Stone J, Sugiyama J, Yahr R, Vilgalys R. 2004. Assembling the fungal tree of life: progress, classification and evolution of subcellular traits. Am J Bot 91:1446–1480.

- Malloch D. 1985. The Tricochomaceae: relationships with other Ascomycetes. In: Samson RA, Pitt JI, eds. Advances in Penicillium and Aspergillus systematics. New York: Plenum Press. p 365–382.
- McLaughlin D, McLaughlin EG, Lemke P, eds. 2001. The Mycota. Vol 7A. Systematics and evolution. New York: Springer-Verlag.
- Miadlikowska J, Lutzoni F. 2004. Phylogenetic classification of peltigeralean fungi (Peltigerales, Ascomycota) based on ribosomal RNA small and large subunits. Am J Bot 91:449–464.
- Miller JH. 1949. A revision of the classification of the Ascomycetes with special reference to the Pyrenomycetes. Mycologia 41:99–127.
- Nannfeldt JA. 1932. Studien über die Morphologie und Systematik der nicht-lichenisierten inoperculaten Discomyceten. Nova Acta Regiae Soc Sci Upsal IV 8:1–368.
- O'Donnell K, Cigelnik E, Weber NS, Trappe JM. 1997. Phylogenetic relationships among ascomycetous truffles and the true and false morels inferred from 18S and 28S ribosomal DNA sequence analysis. Mycologia 89:48–65.
- Pfister DH. 1997. Castor, Pollux and life histories of fungi. Mycologia 89:1–23.
- —, Kimbrough JW. 2001. Discomycetes. In: McLaughlin DJ, McLaughlin EG, Lemke PA, eds. The Mycota. Vol 7A. Systematics and evolution. New York: Springer-Verlag. p 257–281.
- Poelt J. 1973. Classification. In: Ahmadjian V, Hale ME, eds. The lichens. New York and London: Academic Press. p 599–632.
- Read ND, Beckett A. 1996. Centenary review: ascus and ascospore morphogenesis. Mycol Res 100:1281–1314.
- Reeb V, Lutzoni F, Roux C. 2004. Multilocus phylogenetic circumscription of the lichen-forming fungi family Acarosporaceae and its position within the Ascomycota. Mol Phylog Evol 32:1036–1060.
- Robbertse B, Reeves J, Schoch C, Spatafora JW. 2006. A phylogenomic analysis of the Ascomycota. Fungal genetics and biology (In press).
- Rokas A, Krüger D, Carroll SB. 2005. Animal evolution and the molecular signature of radiations compressed in time. Science 310:1933–1938.
- Ronquist F, Huelsenbeck JP, van der Mark P. 2005. MrBayes 3.1. http://mrbayes.csit.fsu.edu/index.php.
- Samuels GJ, Blackwell M. 2001. Pyrenomycetes—fungi with perithecia. In: McLaughlin DJ, McLaughlin EG, Lemke PA, eds. The Mycota. Vol 7A. Systematics and evolution. New York: Springer-Verlag. p 221–255.
- Santesson R. 1952. Foliicolous lichens I. A revision of the taxonomy of the obligately foliicolous, lichenized fungi. Symb Bot Ups 12:1–590.
- Schmitt I, Mueller G, Lumbsch HT. 2005. Ascoma morphology is homoplaseous and phylogenetically mis-

leading in some pyrenocarpous lichens. Mycologia 97: 362–374.

- Schultz M, Arendholz W-R, Büdel B. 2001. Origin and evolution of the lichenized ascomycete order Lichinales: monophyly and systematic relationships inferred from ascus, fruiting body and SSU rDNA evolution. Plant Biol 3:116–123.
- Seaver FJ. 1942. The North American cup fungi (Operculates). New York: published by the author.
 - ——. 1951. The North American cup fungi (Inoperculates). New York: published by the author.
- Spatafora JW, Blackwell M. 1994. Cladistic analysis of partial ssrDNA sequences among unitunicate, perithecial ascomycetes and its implication on the evolution of centrum development, In: Hawksworth DL, ed. Ascomycete systematics. London: Plenum Press Press. p 233– 242.
 - ——, Volkmann-Kohlmeyer B, Kohlmeyer J. 1998. Independent terrestrial origins of the Halosphaeriales (marine Ascomycota). Am J Bot 85:1569–1580.
- Stamatakis A, Ludwig T, Meier H. 2005. RAxML-III: a fast

program for maximum likelihood-based inference of large phylogenetic trees. Bioinformatics 21:456–463.

- Swofford DL. 2002. PAUP*: phylogenetic analyses using parsimony (*and other methods). Version 4. Sunderland, Massachusetts: Sinauer Associates.
- Tehler A. 1990. A new approach to the phylogeny of Eucascomycetes with a cladistic outline of Arthoniales focussing [sic] on Roccellaceae. Can J Bot 68:2458– 2492.
- von Höhnel F. 1907. Fragmente zur Mykologie III. Sitzungsber. Akad Wiss, Wien, Math Nat Kl I Abt 116: 83–162.
- Wehmeyer LE. 1975. The pyrenomycetous fungi. Mycologia memoir No. 6.
- Winka K. 2000. Phylogenetic relationships within the Ascomycota based on 18S rDNA sequences [Doctoral dissertation]. Umeå, Sweden: Umeå University.
- Yarwood CE. 1973. Pyrenomycetes: Erysiphales. In: Ainsworth GC, Sparrow FK, Sussman A, eds. The Fungi, Vol. IVA. New York: Academic Press. p 71– 86.

AFTOL						
no.	Species	SSU	LSU	tefl	RPB1	RPB2
1	Leotia lubrica	AY544687	AY544644	DQ471041	DQ471113	DQ470876
2	Flavoparmelia caperata	AY584663	AY584685	DQ883763	DQ883778	AY584685
3	Cladonia caroliniana	AY584664	AY584640	DQ782888	DQ782816	AY584684
6	Canoparmelia caroliniana	AY584658	AY584634	DQ782889	DQ782817	AY584683
49	Lachnum virgineum	AY544688	AY544646	DQ497602	DQ842030	DQ470877
51	Xylaria hypoxylon	AY544692	AY544648	DQ471042	DQ471114	DQ470878
56	Geoglossum nigritum	AY544694	AY544650	DQ471044	DQ471115	DQ470879
59	Botryotinia fuckeliana	AY544695	AY544651	DQ471045	DQ471116	DQ247786
60	Morchella esculenta cf.	AY544708	AY544664	DQ471046	DQ471117	DQ470880
62	Scutellinia scutellata	DQ247814	DQ247806	DQ471047	DQ479935	DQ247796
63	Xylaria acuta	AY544719	AY544676	DQ471048	DQ471118	DQ247797
64	Trichoglossum hirsutum	AY544697	AY544653	DQ471049	DQ471119	DQ470881
65	Aleuria aurantia	AY544698	AY544654	DQ466085	DQ471120	DQ247785
72	Camarops ustulinoides	DQ470989	DQ470941	DQ471050	DQ471121	DQ470882
76	Mollisia cinerea	DQ470990	DQ470942	DQ471051	DQ471122	DQ470883
78	Acarosporina microspora	AY584667	AY584643	DQ782890	DQ782818	AY584682
80	Simonyella variegata	AY584669		DQ782891	DQ782819	DQ782861
84	Physcia aipolia	DQ782876	DQ782904	DQ782892	DQ782820	DQ782862
87	Teloschistes exilis	AY584671	AY584647	DQ883764	DQ883779	DQ883759
91	Dermatocarpon miniatum	AY584668	AY584644	DQ782893	DQ782821	DQ782863
93	Rocellographa cretacea	DQ883705	DQ883696	DQ883733	DQ883716	DQ883713
102	Letrouitia domingensis	AY584672	AY584648	DQ883765	DQ883734	DQ883747
105	Gyalideopsis vulgaris	AY584673	AY584649	DQ883766	DQ883780	DQ883760
107	Megalospora tuberculosa	AY584674	AY584650	DQ471073	DQ883781	DQ883761
110	Trypethelium sp.	AY584676	AY584652	DQ782896	DQ782824	AY584690
111	Crocynia pyxinoides	AY584677	AY584653	DQ883767	DQ883735	DQ883748
128	Lobaria scrobiculata	AY584679	AY584655	DQ883768	DQ883736	DQ883749
132	Pseudocyphellaria anomala	DQ883785	DQ883794	DQ883769	DQ883737	DQ883750
134	Peltigera degenii	AY584681	AY584657	DQ782897	DQ782826	AY584688
148	Cheilymenia stercorea	AY544705	AY544661	DQ471052	DQ471123	EF080826
149	Neofabreae malicorticis	AY544706	AY544662	DQ847414	DQ471124	DQ470885
151	Chlorociboria aeruginosa cf.	AY544713	AY544669	DQ471053	DQ471125	DQ470886
152	Caloscypha fulgens	DQ247807	DQ247799	DQ471054	DQ471126	DQ247787
154	Bionectria ochroleuca	AY489684	AY489716	AY489611	DQ842031	DQ522415
155	Roumeguieriella rufula	DQ522561	DQ518776	DQ522355	DQ522403	DQ522461
156	Hypocrea lutea	AF543768	AF543791	AF543781	AY489662	DQ522446
158	Sphaerostilbella berkeleyana	AF543770	U00756	AF543783	AY489671	DQ522465
159	Nectria cinnabarina	U32412	U00748	AF543785	AY489666	DQ522456
161	Nectria haematococca	AY489697	AY489729	AU489624	AY489660	DQ518180
162	Cordyceps cardinalis	AY184973	AY184962	DQ522325	DQ522370	DQ522422
164	Marcelleina persoonii	DQ470991	DQ470943	DQ471055	DQ471127	DQ470887
166	Cudoniella clavus cf.	DQ470992	DQ470944	DQ471056	DQ471128	DQ470888
169	Monilinia laxa	AY544714	AY544670	DQ471057		DQ470889
172	Thyridium vestitum	AY544715	AY544671	DQ471058	DQ471129	DQ470890
176	Gyromitra californica	AY544717	AY544673	DQ471059	DQ471130	DQ470891
179	Disciotis venosa	AY544711	AY544667	DQ471060	DQ471131	DQ470892
181	Ascobolus crenulatus	AY544721	AY544678	DQ471061	DQ471132	DQ470893
186	Hydropisphaera erubescens	AY545722	AY545726	DQ522344	DQ522390	AY545731
189	Claviceps purpurea	AF543765	AF543789	AF543778	AY489648	DQ522417
190	Ophionectria trichospora	AF543766	AF543790	AF543779	AY489669	DQ522457
191	Pseudonectria rousseliana	AF543767	U17416	AF543780	AY489670	DQ522459
192	Cordyceps capitata	AY489689	AY489721	AY489615	AY489649	DQ522421
193	Cordyceps ophioglossoides	AY489691	AY489723	AY489618	AY489652	DQ522429
224	Pertusaria dactylina	DQ782880	DQ782907	DQ782899	DQ782828	DQ782868
229	Saitoella complicata	AY548297	AY548296	DQ471133	DQ471133	AY548300

SUPPLEMENTARY TABLE I. List of species used in this study

AFTOL						
no.	Species	SSU	LSU	tef1	RPB1	RPB2
921	Flanocotrania mizualis	DO883786	DO883705	DO882770	DO883738	DO882751
231 927	Verticillium dablige	AV480705	DQ003735	AV480629	AV480673	DQ509751
257	Tabhrina missneri	AV548903	AV548909	DO471184	DO471184	AV548908
205	Protomocas in ouvri	AV548905	AV548904	DQ471134	DQ471134	AV548900
200	I obtosthamia magulans	DO470003	DO470046	DQ471155	DQ471135	DO470804
999	Deprosphaena macutans	DQ470995	DQ470940	DQ471002	DQ471130	DQ470894
202	Sahiamatamma dagalarang	A1544720	A1544080	DQ471003	DQ471137	DQ470895
307 910	Lobariella balliola	A1340009	A1340013	DQ883725	DQ003710	DQ883715
310 814	Lobariella palliola	DQ883787	DQ883790	DQ883771	DQ883739	DQ883752
290	Loodnella pallola	DQ003700	DQ003797	DQ883772	DQ883740	DQ883755
299	Diblocabistas sin magazina	DQ883789	DQ883738	DQ883773	DQ883741	DQ883754
328 999	Diploschistes cinereocaesius	DQ665790	DQ003799	DQ003774	DQ003742	DQ003755
222 950	Dibasia basanyan	DQ005791	DQ000000	DQ865775	DQ003743	DQ003750
338 410	Aritta dana akara kan harain	AF113/12	AF 279303	DQ642006	DQ642011	A1041037
410	Anipioaera chesapeakensis	U40670	U40002	DQ471004	DQ471136	DQ470890
413	Linara thalassiae	DQ470994	DQ470947	DQ471005	DQ471159	DQ470897
438	Calocera cornea	AY//1010	AY/01520	AY881019	A1857980	AY550280
439	Catostoma cinnabarinum	A1003773	A1043034	A10/911/	A1037979	A1700939
447	Cottricia perennis	AF020505	AF287854	AY885147	AY804807	AY218520
449	Armillaria mellea	AY/8/21/	AY/00194	AY881023	AY/88849	AY/80938
505	Ustilago maydis	AY854090	AF453938	AY885160	XM_401478	AY487030
507	Peziza vesiculosa	DQ470995	DQ470948	DQ4/1066	DQ471140	DQ470898
554	Lasallia pustulata	DQ883700	DQ883690	DQ883726	DQ883719	DQ883707
639	Lecanora hybocarpa	DQ782883	DQ782910	DQ782901	DQ782829	DQ782871
642	Bacidia schweinitzii	DQ782884	DQ782911	DQ782902	DQ782830	DQ782872
045 649	Umbilicaria mammulata	50659902	DQ782912	DQ782903	DQ782831	DQ782873
648 650	Anaptychia palmatula	DQ883792	DQ883801	DQ883776	DQ883744	DQ883757
650	Lasallia papulosa	DQ883701	DQ883691	DQ883727	DQ883720	DQ883708
657	Capronia pilosella	DQ823106	DQ823099	DQ840565	DQ840554	DQ840561
659	Ramichloridium anceps	DQ823109	DQ823102	DQ840568	DQ840557	DQ840564
668	Exophiala dermatitidis	DQ823107	DQ823100	DQ840566	DQ840555	DQ840562
669	Exophiala pisciphila	DQ823108	DQ823101	DQ840567	DQ840556	DQ840563
675	Agaricostilbum hyphaenes	AY665775	AY634278	AY879114	AY/88845	AY/89033
684	Agonimia sp.	DQ782885	DQ782913	DQ782917	DQ782853	DQ782874
686	Pyxine subcinerea	DQ883793	DQ883802	DQ883777	DQ883745	DQ883758
687	Hypocenomyce scalaris	DQ782886	DQ782914	DQ782918	DQ782854	DQ782875
700	Cotylidia sp.	AY705958	AY629317	AY885148	AY864868	AY883422
701	Grifola frondosa	AY705960	AY629318	AY885153	AY864876	AY/86057
716	Strobilomyces floccopus	AY662661	AY684155	AY883428	AY858963	AY/86065
729	Hygrocybe conica	AY752965	AY684167	AY883425	AY860522	AY803/4/
730	Lanspora coronata	DQ470996	U40889	DQ471067	DQ4/1141	DQ470899
744	Potebniamyces pyri	DQ470997	DQ470949	DQ471068	DQ471142	DQ470900
748	Papulosa amerospora	DQ470998	DQ470950	DQ471069	DQ471143	DQ470901
751	Torpedospora radiata	DQ470999	DQ470951	DQ471070	DQ471144	DQ470902
770	Fomitopsis pinicola	AY705967	AY684164	AY885152	AY864874	AY/86056
//0	Phanerochaete chrysosporium	Genome	Genome	Genome	Genome	Genome
813	Usnea antarctica	DQ883702	DQ883692	DQ883728	DQ883721	DQ883709
816	Usnea sphacelata	DQ883703	DQ883693	DQ883729	DQ883722	DQ883710
875	Icmadophila ericetorum	DQ883704	DQ883694	DQ883730	DQ883723	DQ883711
889	Buellia frigida	DQ883699	DQ883695	DQ883731	DQ883724	DQ883712
891	Pettula umbilicata	DQ/82887	DQ832334	DQ/82919	DQ/82855	DQ832335
905	Orbilia vinosa	DQ471000	DQ470952	DQ471071	DQ471145	DOIFOOOS
906	Orbiha auricolor	DQ471001	DQ470953	DQ471072	DO451142	DQ470903
907	Meria laricis	DQ471002	DQ470954	DQ842026	DQ471146	DQ470904
910	Ophiostoma piliferum	DQ471003	DQ470955	DQ471074	DQ471147	DQ470905
912	Aureobasidium pullulans	DQ471004	DQ470956	DQ471075	DQ471148	DQ470906

SUPPLEMENTARY	TABLE I.	Continued

AFTOL						
no.	Species	SSU	LSU	tef1	RPB1	RPB2
913	Loramyces macrosporus	DO471005	DO470957	DO471076	DO471149	DO470907
914	Microascus trigonosporus var. trigonosporus	DQ471006	DQ470958	DQ471077	DQ471150	DQ470908
915	Coniochaetidium ostreum	DQ471007	DQ470959	DQ471078	DQ471151	DQ470909
916	Bulgaria inquinans	DQ471008	DQ470960	DQ471079	DQ471152	DQ470910
918	Rhizina undulata	DQ471009	DQ470961	DQ471080	DQ471153	DQ470911
921	Dothidea insculpta	DQ247810	DQ247802	DQ471081	DQ471154	DQ247792
923	Rutstroemia firma	DQ471010	DQ470963	DQ471082	DQ471155	DQ470912
924	Togninia minima	DQ471011		DQ471083	DQ471156	DQ470913
925	Kirschsteiniothelia aethiops	AY016344	AY016361	DQ471084	DQ471157	DQ470914
927	Diatrype disciformis	DQ471012	DQ470964	DQ471085	DQ471158	DQ470915
928	Sclerotinia sclerotiorum	DQ471013	DQ470965	DQ471086		DQ470916
931	Bimuria novae-zelandiae	AY016338	AY016356	DQ471087	DQ471159	DQ470917
933	Eleutherascus lectardii	DQ471014	DQ470966	DQ471088	DQ471160	DQ470918
935	Diaporthe eres	DQ471015	AF408350	DQ479931	DQ471161	DQ470919
938	Pezicula carpinea	DQ471016	DQ470967	DQ479932	DQ842032	DQ479934
939	Capnodium coffeae	DQ247808	DQ247800	DQ471089	DQ471162	DQ247788
940	Pleospora herbarum	DQ247812	DQ247804	DQ471090	DQ471163	DQ247794
941	Dermea acerina	DQ247809	DQ247801	DQ471091	DQ471164	DQ247791
942	Mycosphaerella punctiformis	DQ471017	DQ470968	DQ471092	DQ471165	DQ470920
949	Pyronema aomesticum	DQ247815	DQ247805	DQ471095	DQ471100	DQ247795
991	Apiospora moniagnei	DO471010	DQ 471018	DQ642027	DQ642055	DQ470921
952	Botriella estifma	DQ471019	Ar406301	DQ471094	DQ471107	DQ470922
950	Dibloschistes occiliatus	DQ471020 AE028877	DQ470909	DQ850911	DQ842034	DQ850885
950	Dipiosinisies occiuitus Partusaria hemisehaarica	DO009340	AF381556	DQ300251	DQ300232	DQ300233
969	Trapelia placodioides	AF119500	AF974103	DQ366958	DQ366959	DQ366960
96 <u>6</u>	Ramalina complanata	DO883784	DO883783	DQ300238	DQ300233	DQ300200
967	Romhardia homharda	DQ003704	DQ883783	DQ883805	DQ805782	DQ003702
993	Obeorapha dolomitica	DQ883706	DQ110510	DQ171033	DQ883717	DO883714
995	Dendryphiella arenaria	DO471022	DO470971	DO677890	DO842036	DO470924
1003	Pleopsidium gobiense	DO525573	DO883698	DO883804	DO883746	DO525452
1004	Pleopsidium chlorophanum	DO525541	DO842017	DO782920	DO782858	DO525442
1037	Westerdykella cylindrica	AY016355	AY004343	DO497610	DO471168	DO470925
1069	Saccharomyces cerevisiae	SCYLR154C	SCYLR154C	Genome	x96876	SCYOR151C
1070	Saccharomyces castellii	Z75577	Genome	Genome	Genome	Genome
1071	Kluyveromyces waltii	Genome	Genome	Genome	Genome	Genome
1072	Ashbya gossypii	AE016820	AE016820	Genome	NM_209535	AE016819
1073	Candida glabrata	AY198398	AY198398	Genome	XM_447415	XM_448959
1074	Candida albicans	Genome	Genome	Genome	XM_714321	XM_713346
1075	Kluyveromyces lactis	Genome	Genome	Genome	XM_455310	XM_451784
1076	Yarrowia lipolytica	AB018158	AJ616903	Genome	XM_501909	XM_502376
1077	Debaryomyces hansenii	DHA508273	AF485980	Genome	XM_456921	CR382139
1078	Neurospora crassa	X04971	AF286411	Genome	XM_959004	XM_324476
1079	Aspergillus fumigatus	AB008401	AY660917	Genome	XM_747744	XM_741647
1080	Aspergillus nidulans	ENU77377	AF454167	Genome	XM_653321	Genome
1081	Magnaporthe grisea	AB026819	AB026819	Genome	XM_362207	XM_362269
1082	Fusarium graminearum		AY188924	Genome	XM_381092	Genome
1083	Histoplasma capsulatum	Genome	Genome	Genome	Genome	Genome
1084	Coccidioides immitis	Genome	Genome	Genome	Genome	Genome
1088	Cryptococcus neoformans	Genome	Genome	Genome	XM_570943	XM_570204
1112	Chaetomium globosum	AY545725	AY545729	Genome	Genome	Genome
1192	Pneumocystis carinii	S83267.1	AF047831	C	Genome	AY485631
1199	Schizosaccharomyces pombe	X54866	Z19136	Genome	X56564	D13337
1223	Endothia gyrosa	DQ471023	DQ470972	DQ471096	DQ471169	DQ470926

_

SUPPLEMENTARY	Table I.	Continued
---------------	----------	-----------

=

AFTOL						
no.	Species	SSU	LSU	tef1	RPB1	RPB2
1234	Taphrina deformans	DQ471024	DQ470973	DQ471097	DQ471170	DQ470927
1235	Tyrannosorus pinicola	DQ471025	DQ470974	DQ471098	DQ471171	DQ470928
1237	Microascus longirostris	DQ471026	AF400865	-	DQ471172	-
1241	Rhizopus oryzae	AF113440	AY213626	Genome	Genome	Genome
1250	Coccomyces strobi	DQ471027	DQ470975	DQ471099	DQ471173	DQ470929
1253	Phacidium lacerum	DQ471028	DQ470976		DQ471174	
1257	Delphinella strobiligena	DQ471029	DQ470977	DQ471100	DQ471175	DQ677951
1262	Lambertella subrenispora	DQ471030	DQ470978	DQ471101	DQ471176	DQ470930
1265	Glomerella cingulata	AF543762	AF543786	AF543773	AY489659	DQ522441
1268	Candida lusitaniae	M55526	Genome	Genome	Genome	Genome
1269	Candida tropicalis	M55527	Genome	Genome	Genome	Genome
1270	Candida guilliermondii	AB013587	Genome	Genome	Genome	Genome
1285	Ambrosiella xylebori	DQ471031	DQ470979	DQ471102	DQ471177	DQ470931
1287	Gelasinospora tetrasperma	DQ471032	DQ470980	DQ471103	DQ471178	DQ470932
1292	Microglossum rufum	DQ471033	DQ470981	DQ471104	DQ471179	DQ470933
1316	Tubeufia cerea	DQ471034	DQ470982	DQ471105	DQ471180	DQ470934
1319	Tryblidiopsis pinastri	DQ471035	DQ470983	DQ471106	DQ471181	DQ470935
1345	Plicaria leiocarpa	DQ842039	DQ842029	DQ842028	DQ842037	DQ842038
1359	Dothiora cannabinae	DQ479933	DQ470984	DQ471107	DQ471182	DQ470936
1361	Camarops microspora	DQ471036	AY083821	DQ471108	DQ471183	DQ470937
1362	Neolecta vitellina	DQ471037	DQ470985			AAF19058
1363	Neolecta irregularis	DQ842040	DQ470986	DQ471109		
1415	Graphium penicillioides	DQ471038	AF027384	DQ471110	DQ471184	DQ470938
1911	Caliciopsis orientalis	DQ471039	DQ470987	DQ471111	DQ471185	DQ470939
1912	Pseudeurotium zonatum	DQ471040	DQ470988	DQ471112	DQ471186	DQ470940
	Glomerella cingulata	U48427	U48428	AF543772	DQ858454	DQ858455
	Phaeosphaeria [®] nodorum	Genome	Genome	Genome	Genome	Genome