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A five-gene phylogeny of Pezizomycotina

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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-1α) 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

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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 sac-shaped 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-

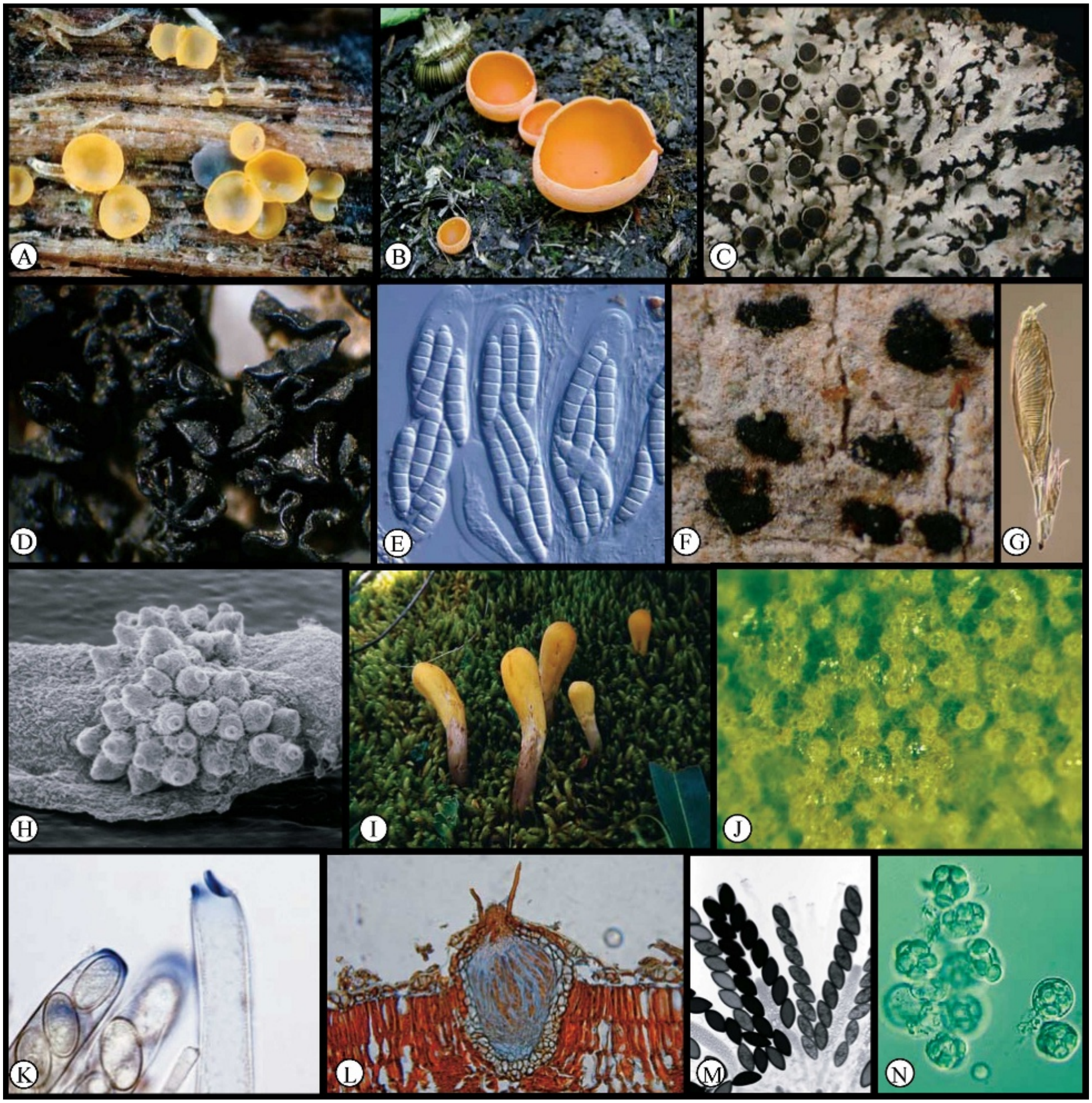

FIG. 1. Exemplar ascomatal and ascal morphologies for 10 classes of the Pezizomycotina. a. Apothecia (yellow) of *Orbilia*, Orbiliomycetes (J.H. Petersen/MycoKey). b. Apothecia of *Aleuria*, Pezizomycetes (J.H. Petertsen/MycoKey). c. Thallus of *Ophioparma* w/apothecia, Lecanoromycetes (B. McCune, Oregon State University). d. Thallus of *Lichinella*, Lichinomycetes (B. McCune, Oregon State University). e. Bitunicate asci of *Thaxteriella*, Dothideomycetes (S. Huhndorf, Field Museum). f. Thallus of *Arthonia* with apothecia, Arthoniomycetes (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-1α, 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-1α, 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.

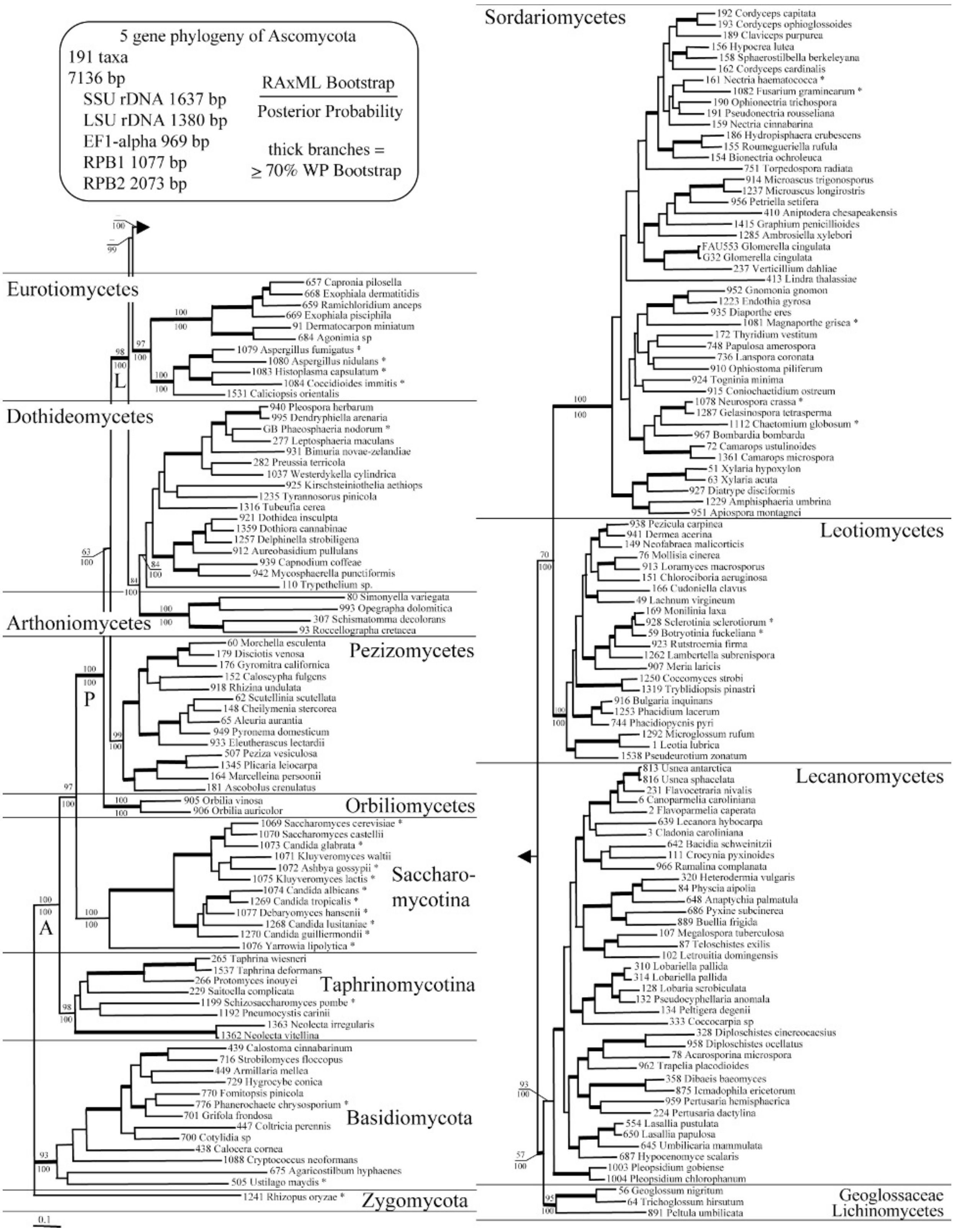

FIG. 2. Bayesian consensus tree of the Pezizomycotina from 90 000 credible trees. ML-BS values ≥ 70% and PP ≥ 95 are provided respectively above and below major branches, and all branches that received WP-BS ≥ 70% are thickened. Asterisks denote species with annotated genomes. Nodes for higher taxa are 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, thin-walled, 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 cup-shaped (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. *Hymenoscyphus*) 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 plant-associated 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.

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