

A multigene phylogeny of the Dothideomycetes using four nuclear loci

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Abstract: We present an expanded multigene phylogeny of the Dothideomycetes. The final data matrix consisted of four loci (nuc SSU rDNA, nuc LSU rDNA, *TEF1*, *RPB2*) for 96 taxa, representing five of the seven orders in the current classification of Dothideomycetes and several outgroup taxa representative of the major clades in the Pezizomycotina. The resulting phylogeny differentiated two main dothideomycete lineages comprising the pseudoparaphysate Pleosporales and paraphysate Dothideales. We propose the subclasses Pleosporomycetidae (order Pleosporales) and Dothideomycetidae (orders Dothideales, Capnodiales and Myriangiales). Furthermore we provide strong molecular support for the placement of Mycosphaerellaceae and Piedraiaceae within the Capnodiales and introduce Davidiellaceae as a new family to accommodate species of *Davidiella* with *Cladosporium* anamorphs. Some taxa could not be placed with certainty (e.g. Hysteriales), but there was strong support for new groupings. The clade containing members of the genera *Botryosphaeria* and *Guignardia* resolved well but without support for any relationship to any other described orders and we hereby propose the new order Botryosphaeriales. These data also are consistent with the removal of Chaetothyriales and Coryneliales from the Dothideomycetes and strongly support their placement in the Eurotiomycetes.

Key words: bitunicate asci, hamathecium, loculoascomycetes, pseudoparaphyses

INTRODUCTION

Members of the Dothideomycetes often are found as pathogens, endophytes or epiphytes of living plants and also as saprobes degrading cellulose and other complex carbohydrates in dead or partially digested plant matter in leaf litter or dung. Combinations of these niches can be occupied by a single fungus as it passes through its life cycle; for example several fungi initiate their life cycles on living plants and switch to saprobic states when the plant dies or leaves are lost. The nutritional modes are not limited to associations with plants and several species are lichenized, while others occur as parasites on fungi or members of the kingdom animalia.

Although to a casual observer there is little to distinguish the flask-, spherical- or disk-shaped fruiting bodies of the Dothideomycetes from several other ascomycete groups, they share a distinctive pattern of development. The asci bearing the sexual spores develop in locules already formed lysigenously within vegetative hyphae. This, defined as ascolocular development, is in contrast to ascohymenial development found in the majority of other fungal classes. Ascohymenial development generates asci in a broad hymenium interspersed with apically free paraphyses and the reproductive structure is derived from cells after fertilization.

Building on earlier descriptions of ascolocular development Nannfeldt (1932) proposed the group “Ascoloculares” and in 1955 this was formally proposed as a class “Loculoascomycetes” by Luttrell (1955). The importance of ascus morphology and dehiscence, in addition to the presence of surrounding elements inside the ascostromata, was emphasized (Luttrell 1951). The bitunicate ascus remains a defining character in modern dothideomycete taxonomy. It consists of a thick extensible inner layer (endotunica) and a thin inextensible outer layer (ectotunica). Most species release their ascospores by the extension of the inner ascus wall and the rupture of the outer wall, similar to a jack-in-the-box (fissitunicate), but variations are numerous. Another character of note, the centrum, defined as the tissues and cells occupying the cavity of the sexual structure, was expanded by Luttrell when he described three different ascostromatal developmental types exemplified by the genera *Dothidea*, *Pleospora* and *Elsinoë* forming part of the currently accepted orders, Dothideales, Pleosporales and Myriangiales (see tolweb.org/Dothideomycetes for details). The ha-

mathecium (Eriksson 1981) (i.e. the sterile centrum tissues existing between the asci) is one of the most reliable characters used to delineate ordinal classifications within the Dothideomycetes. The presence of pseudoparaphyses (sterile cells extending down from the upper portion of the ascoma, initially attached at both ends, although the upper part may become free) is a notable character for the Pleosporales, together with mainly ostiolate flask-shaped pseudothecia. Conversely the absence of pseudoparaphyses and the presence of fascicles of asci are important in the Dothideales. The Myriangiales also do not have pseudoparaphyses but produce single globose asci in multiple locules. Several additional orders currently accepted are defined by combinations of centrum and ascomal characters. For a summary of different centrum types and features see Kirk et al (2001 p 224–225).

The different classification systems proposed thus far exhibited an emphasis on varying characters. For instance, the presence and morphology of characters in the hamathecium, together with ascostroma shape were used as the main characters to define ordinal groups by Luttrell (1955), while von Arx and Müller (1975) emphasized the form of the ascus and the specific opening of the ascoma. Although basing her classification on the work of Luttrell, Barr (1987) employed additional characters such as the morphology of pseudoparaphyses.

The best studied species in this group tend to be plant pathogens on important agricultural crops. Therefore a large body of work in dothideomycete taxonomy and systematics concerns descriptions of anamorphs, the predominant morphological state encountered on agricultural crops; in fact several families in this class (e.g. Pleosporaceae, Mycosphaerellaceae, Tubeufiaceae) include a high proportion of anamorphic species. These include both hyphomycetes and coelomycetes. Many of the hyphomycetes have sympodially proliferating conidiogenous cells. *Phoma*-like and other coelomycetes occur in several families (e.g. Leptosphaeriaceae, Lophiostomataceae); these have ostiolate pycnidia lined with phialidic, annellidic or holoblastic conidiogenous cells and produce small, aseptate conidia in slime. Other important species include the group now informally referred to as the “black yeasts” (some of which also belong to the Eurotiomycetes) characterized by the production of dark, slimy colonies and sporulation patterns that resemble the budding of true yeasts but actually are reduced versions of phialidic, annellidic or sympodially proliferating conidiogenous cells (de Hoog 1974). A selection of the variety of morphological structures exhibited by teleomorph and anamorph forms in the Dothideomycetes is shown (FIG. 1).

The refinement of character state homologies and the development of morphology-based classifications into a phylogenetic classification system are accelerating with the advent of molecular data. Initial analyses using DNA sequence data from the small subunit ribosomal RNA gene did not support the monophyly of the Loculoascomycetes (Spatafora et al 1995, Berbee 1996). A more recent phylogeny produced from protein gene coding data (Liu and Hall 2004) was inferred as supporting the taxonomic concepts for a monophyletic lineage for ascostromatic taxa, but the ontogenetic designations were considered oversimplified by some (Lumbsch et al 2005). Other studies combining data from protein-coding genes and the ribosomal operon have shown the paraphyly of ascostromatic, bitunicate lineages (Lutzoni et al 2004, Reeb et al 2004). An example is the group of fungi that recently were transferred to the Eurotiomycetes based on nuclear small subunit ribosomal sequences, the “black yeasts” of the Chaetothyriales (Winka et al 1998). Together with the Verrucariales and Pyrenulales these bitunicate taxa have been placed within a separate subclass, the Chaetothyriomycetidae (Miadlikowska and Lutzoni 2004), which is sister of the Eurotiomycetidae (Lutzoni et al 2004, Reeb et al 2004) in the class Eurotiomycetes (also see Geiser et al in this issue).

Several studies provide the groundwork for a phylogenetically based classification for the Dothideomycetes. Most have used nuclear small subunit ribosomal data, but nuclear large subunit ribosomal and mitochondrial small subunit sequences also were used (Lindemuth et al 2001, Lumbsch and Lindemuth 2001). This allowed for the reassessment of specific morphological characters proposed in earlier work. Specifically, poor support for phylogenetic groups based on the morphology of pseudoparaphyses was found while phylogenetic correlation of their presence or absence was well supported (Liew et al 2000, Lumbsch and Lindemuth 2001), although a single exception to this was noted (Silva-Hanlin and Hanlin 2000). In spite of these recent examples of interordinal, molecular-based phylogenetic studies, a large number of species within the ascostromatic Ascomycota remain listed as Dothideomycetes or Chaetothyriomycetes incertae sedis (Eriksson 2006). Furthermore the question of whether Dothideomycetes represents a natural group derived from a single ancestor is not settled and the need to investigate its relationships to a number of the bitunicate lichen species such as the currently separate class Arthoniomycetes remains essential. The main focus of this study however is to provide an extension of previous ribosomal DNA-based phylogenetic studies and combine a number of smaller phylogenetic analyses

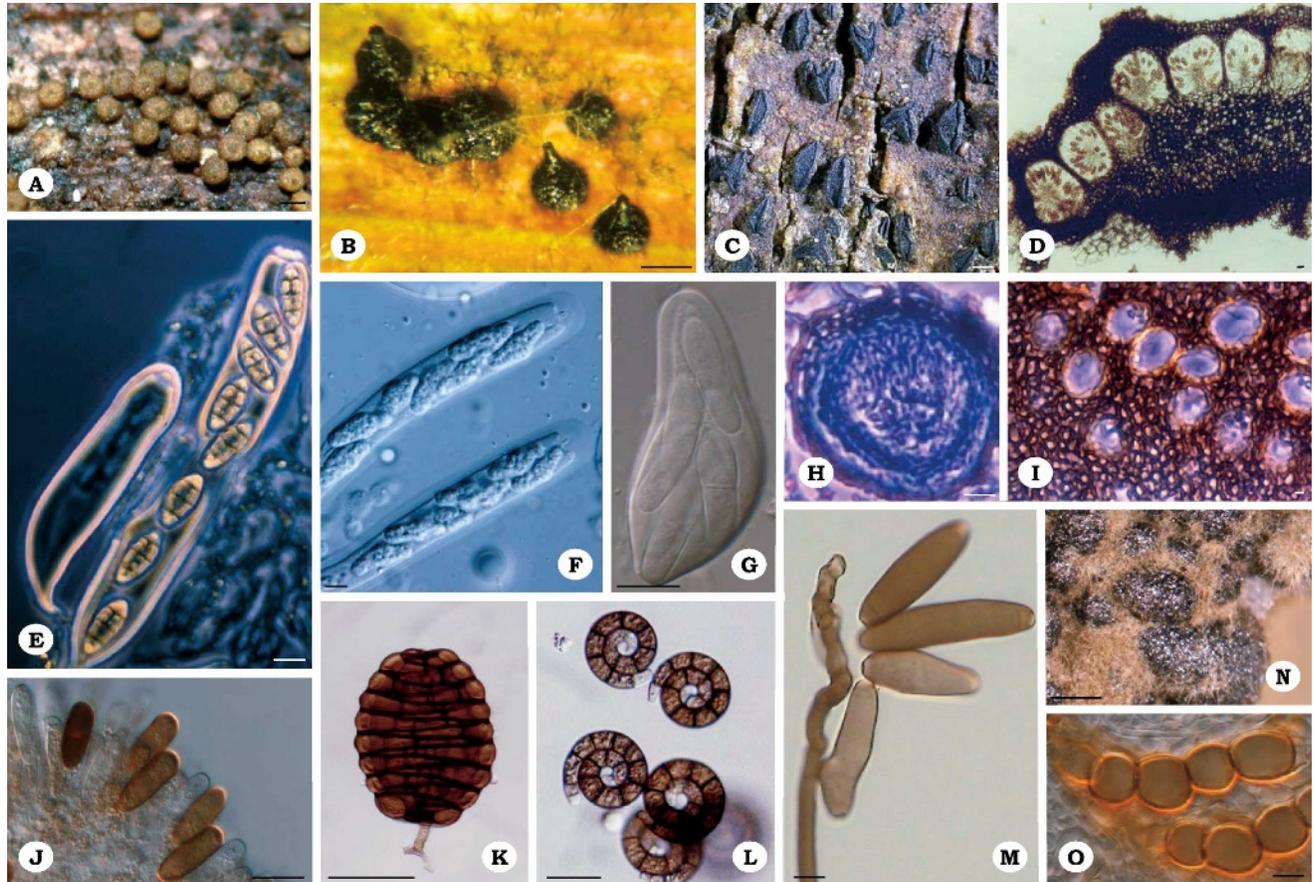


FIG. 1. A selection of dothideomycete morphological forms. Teleomorphs, ascostromata: A. Light-colored, flask-shaped pseudothecia of *Tubeufia cerea* (Tubeufiaceae) on wood. B. Dark pseudothecia of *Cochliobolus heterostrophus* (Pleosporales) on corn leaf. C. Hysterothecia of *Hysteropatella prostii* (Hysteriales), with slit-like openings. Teleomorphs, asci and locules: D. *Stylodothis puccinioides* (Dothideales), multiascus locules. E. *Pyrenophora brizae* (Pleosporales) bitunicate asci, one with broken ectotunica. F. *Guignardia magniferae* (Botryosphaerales) asci with ascospores. G. Bitunicate ascus of *Davidiella tassiana* (Capnodiales). H. *Phaeosphaeria avenaria*, juvenile ascoma with pseudoparaphyses. I. *Myriangium duriaei* (Myriangiales), monascus locules in stroma. Anamorphs: J. Conidia borne in pycnidium of *Dothiorella* sp. (Botryosphaerales). K, L. Helical conidia, in two different dimensions, of *Helicoon* and *Helicoma* spp. (Tubeufiaceae). M. Conidia and conidiophore of *Bipolaris* sp. (Pleosporales). N. Stroma of *Trimmatostroma abietis* (Capnodiales) bearing conidia in culture. O. Chlamydozoospores of *Trimmatostroma abietis* (Capnodiales). Scale bars are approximations obtained from published sources; the bar indicates 10 μm except in A, B, C and N where it indicates 200 μm . Photo credits, courtesy of: Jean-Paul Priou (A), B. Gillian Turgeon (B), Hans-Otto Baral (C), Robert A. Shoemaker (D, E, H, I), Gary Samuels (F), Pedro W. Crous (G, J, N, O), Clement K.M. Tsui (K, L), Keith A. Seifert (M).

within the framework of a multiple gene analysis showing intraordinal relationships in the Dothideomycetes.

MATERIALS AND METHODS

Sampling and alignments.—Sequence data were obtained from GenBank and the Assembling the Fungal Tree of Life Project (AFTOL; <http://ocid.nacse.org/research/aftol/>). All strains and sequences used in this study are listed (SUPPLEMENTARY TABLE I). DNA alignments are available from the AFTOL Web site and TreeBASE (SN2913-11828). A number of sequences generated by the AFTOL project and available from the AFTOL Web site as well as from

GenBank were used. Newly generated DNA sequences were deposited at GenBank (TABLE I supplement). Genes used were nuclear small subunit ribosomal RNA gene DNA (nuc SSU), nuclear large subunit rDNA (nuc LSU), elongation factor la gene (*TEF1*), and the second largest subunits of RNA polymerase II gene (*RPB2*). Herbaria and culture collections where strains and specimens used in this study are deposited are listed (TABLE I supplement).

Phylogenetic analysis.—Maximum and weighted parsimony (MP and WP) analyses were performed on a combined dataset with a total of 117 taxa that included 96 Dothideomycetes. Nineteen taxa contained data for only three loci to maximize taxon sampling. The majority of the missing data were in the terminal branches of the tree, and care was

taken to include complete data sampling for taxa on branches underpinned by the more basal nodes. Two taxa with only ribosomal data (AFTOL ID 1856 *Phoma herbarum* and AFTOL ID 1864, *Didymella cucurbitacearum*) also were included to clarify the position of the clade surrounding *Phoma herbarum*. Removal of these taxa did not significantly affect support values in other parts of the tree. Likewise a comparison of a parsimony and Bayesian analysis with and without complete sets of characters yielded trees with congruent topologies. DNA sequences from a single strain (*Leptosphaeria maculans* DAOM 229267) inadvertently were included twice in the final analysis but were left in the final tree to ensure correct comparison across all approaches. We rooted the tree with three taxa from the class Pezizomycetes as outgroups (*Pyronema domesticum*, *Caloscypha fulgens* *Gyromitra californica*) (not shown in figure).

For the WP analyses the unambiguously aligned regions were subjected to symmetric step matrices for eight partitions (i.e. nuc SSU rDNA, nuc LSU rDNA and six codon positions of *TEF1* and *RPB2*) to incorporate the differences in substitution rates and patterns as described in Lutzoni et al (2004). MP and WP analyses were performed with only parsimony informative characters with these settings: 100 replicates of random sequence addition, TBR branch swapping and MULTREES in effect. Maximum likelihood was performed with PHYML (Guindon and Gascuel 2003) using a GTR+I+ Γ model of evolution. In all preceding cases nodal support was verified by nonparametric bootstrapping under the conditions mentioned, using 500 replicates.

Initial incongruence in the single gene trees for the taxa used was tested by examining single gene analyses with WP under the conditions previously mentioned for a set of taxa containing data for all four loci. A 70% majority rule consensus tree was compared in each case. Phylogenetic analysis using Bayesian inference of maximum likelihood was performed with a parallelized version of MrBayes v 3.1.2 across four processors (Altekar et al 2004). MrBayes was run with these parameters: a general time reversible model of DNA substitution (GTR) with gamma-distributed rate variation across sites (invariance, partitioning across genes and codons). A Markov chain Monte Carlo (MCMC) analysis with metropolis coupling was run starting from a random tree for 5×10^6 generations, sampling every 100th cycle. Four chains were run simultaneously with the initial 1000 cycles discarded as burn-in. Two additional runs with 5×10^6 generations were compared to confirm that stationarity in likelihood values was reached and compared. The phylogenies obtained in all cases were congruent. A 50% majority rule tree from a total of 45 000 trees obtained from a single run is presented (FIG. 2).

RESULTS AND DISCUSSION

Data analyses.—The alignment for the phylogenetic analyses, after excluding introns and ambiguously aligned regions, consisted of 5098 base pairs, 1882 of which were parsimony informative. The reciprocal comparisons of 70% bootstrap trees from each gene

with 61 core taxa did not reveal any incongruence (data not shown). Therefore all of 109 taxa in the current taxon sampling were used in the combined analyses. The heuristic search in MP and WP analyses yielded six MPTs with 20 917 steps (CI = 0.204, RI = 0.535) and three MPTs with 34 319.54 steps, respectively. In model-based methods, ML heuristic search analysis resulted in a tree of $-94 457.67$ log likelihood and resulted after the GTR model was applied with a gamma value of 0.395 across four rate categories with a proportion of invariant sites equal to 0.287. The Bayesian analysis converged on the plateau of the log-likelihood on a mean value of $-93 955$. The tree from Bayesian analyses is shown (FIG. 2) with all of the bootstrap proportions as well as the Bayesian posterior probabilities. Internodes were considered strongly supported if they received all of bootstrap proportions $\geq 70\%$ and posterior probabilities $\geq 95\%$ (Lutzoni et al 2004).

Overview.—The tree (FIG. 2) contains representatives of the major classes in the Ascomycota, as defined previously (Eriksson 2001). The supraclass relationships in our analysis indicated no support for a close relationship between the Dothideomycetes and Sordariomycetes, alluded to in an earlier study (Lutzoni et al 2004) and the sister relationships of the Sordariomycetes and Leotiomycetes are supported in agreement with recent data (Lumbsch et al 2005). A few taxon pairs containing isolates used in previous works have remarkably high similarity to each other over all four loci. Two examples noted in this analysis were incorrectly identified strains, namely “*Clathrospora diplospora*” CBS 174.52 = *Alternaria alternata* and “*Epipolaeum longisetosum*=*Raciborskomyces longisetosum*” CBS 180.53 = *Cladosporium herbarum*.

Non-Dothideomycete bitunicate groups. Several lineages historically associated with the loculoascomycetes, such as the two species representing the Coryneliales, also were included. The placement of *Caliciopsis orientalis* together with *Caliciopsis pinea* (FIG. 2) indicates a close relationship with the Eurotiomycetidae (Geiser et al this issue). Other ordinal groups traditionally associated with the Dothideomycetes and now placed in the Eurotiomycetes were mentioned earlier. These groups share a number of centrum characters with members of the Dothideomycetes, such as the presence of periphysoids (Verrucariales, Chaetothyriales) and periphysate ostioles (Verrucariales, Chaetothyriales, Pyrenulales). The phylogeny (FIG. 2) confirms the separation of the Chaetothyriales and Verrucariales from the Dothideomycetes.

Dothideomycetes-Arthoniomycetes clade. The relationship of the Dothideomycetes and Arthoniomycetes (node A) is well supported by Bayesian and

maximum likelihood but not parsimony, although in an analysis without third codon positions, support by MP bootstrap and WP bootstrap increased. The internal node supporting the monophyly of the Dothideomycetes (node B) also had higher support in maximum likelihood and the two parsimony processes when the more saturated third codon positions were omitted. In more complete analyses containing characters from the RPB1 locus, this node was moderately supported and the *Trypethelium* strain is shown inside the Dothideomycetes (see Spatafora et al this issue).

Although taxon sampling for the Arthoniomycetes is sparse in our dataset, these levels of support (FIG. 2) largely agree with other recent large analyses where the Dothideomycetes is resolved as monophyletic but with low statistical support (Lumbsch et al 2005). A possible sister relationship of Dothideomycete/Arthoniomycetes has been proposed (Barr 1987, Tehler 1990) and there is some phylogenetic support for this (Lumbsch et al 2005, Lutzoni et al 2004). Clear differences between the groups exist, such as the ascohymental type development of the Arthoniomycetes apothecium (Henssen and Thor 1994). More thorough sampling of Arthoniomycetes will test the monophyly of its relationship with the Dothideomycetes. It is premature to comment on the ultimate monophyly of the Dothideomycetes, but it seems quite reasonable that increased sampling of taxa and genes could increase support for this node. As pointed out by Lumbsch et al (2005), most of the large scale interclass relationships have been in conflict in recent publications and taxon sampling should be an important consideration before making major classification changes.

Dothideomycetes. The addition of protein gene data illustrates that the lineages clustering around the core orders Pleosporales and Dothideales correlate with the presence or absence of pseudoparaphyses and other centrum characteristics. The node supporting the Dothideales, Capnodiales, Myriangiales and Mycosphaerellaceae (C) is strongly supported. This node was unaffected when third base codon positions were removed, but a small increase in parsimony bootstrap support was noted at node M, combining the Dothideales and Myriangiales, although ML bootstrap decreased. Saturation and the specific evolutionary model applied might have influenced this. Node C might indicate a single loss of pseudoparaphyses in all the terminal clades. However previous molecular phylogenies based on nuc SSU rDNA data have shown the presence of members of the aparaphysate genus *Leptosphaerulina* nested within the Pleosporales (Silva-Hanlin and Hanlin 2000), which could imply multiple, isolated losses of this character in other parts of the tree.

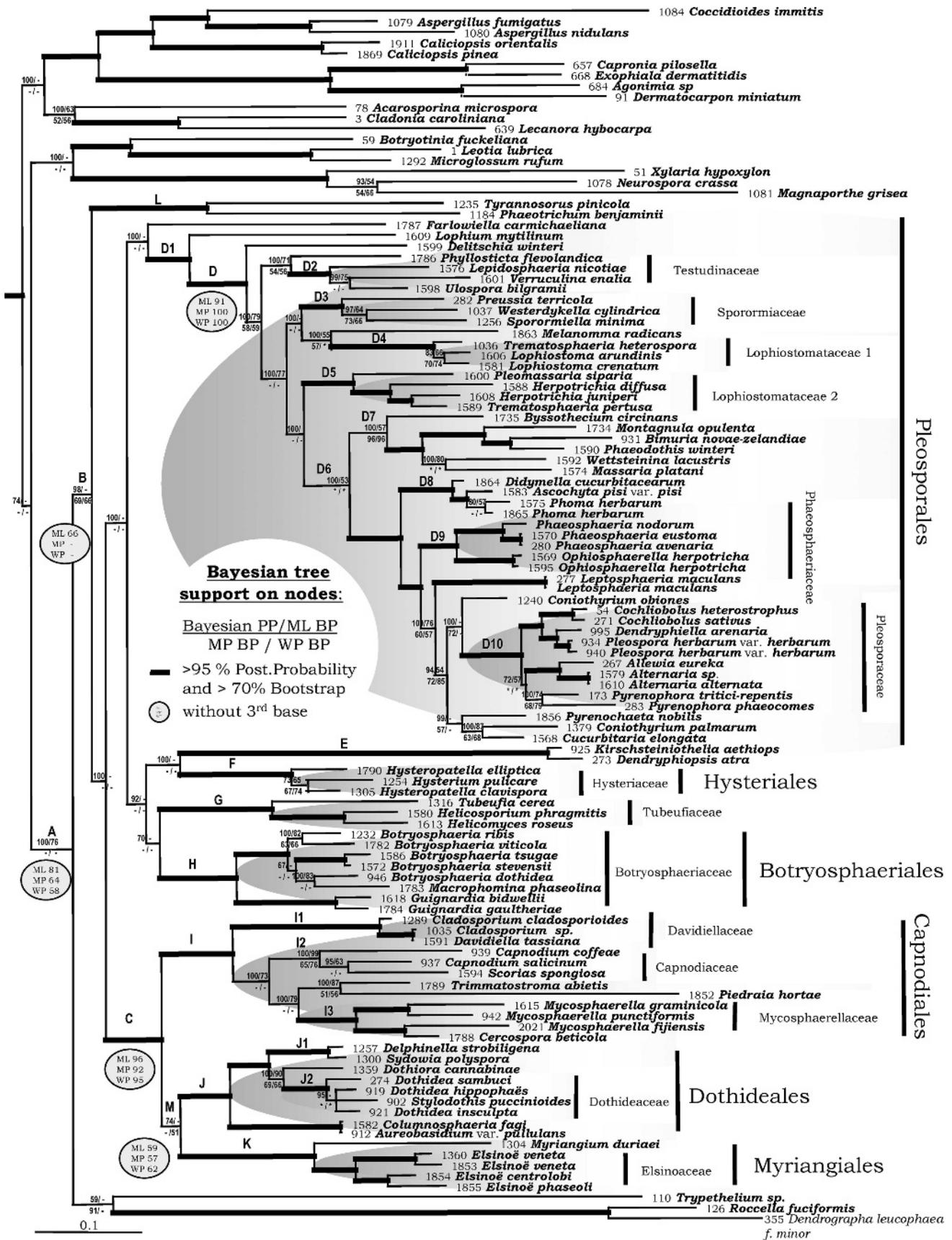
Anamorphs play an important role in the life cycles of many orders of Dothideomycetes. Many are coelomycetes, especially phialidic, *Phoma*-like anamorphs, which may be a plesiomorphic anamorph character in the class, perhaps serving some kind of spermatial function. In the Pleosporaceae and Mycosphaerellaceae hyphomycetes with sympodially proliferating conidiogenous cells with scars, and dry conidia, are particularly common and strictly anamorphic species may comprise the majority in these families. The Capnodiales, with their multitude of hyphomycete and coelomycete synanamorphs, and the helicoconidial anamorphs of the Tubeufiaceae, contain particularly distinctive anamorph groups. The anamorph genera of both hyphomycetes and coelomycetes, lacking teleomorph connections, continue to be examined for their phylogenetic relationships, many of them undoubtedly will be found to be associated with the Dothideomycetes. Several clades are well supported (FIG. 2) and will be discussed in more detail below.

Aparaphysate Dothideomycetes.—We hereby propose an emendation of the subclass Dothideomycetidae (*nom. nud.*) (Kirk et al 2001), which has been superceded by the Dothideomycetes O.E. Erikss. and Winka (2000). Dothideomycetidae *sensu* Lutzoni et al (2004) also was included in the Sordariomycetes as subclass Dothideomycetidae along with the subclass Sordariomycetidae (*syn. Sordariomycetes s. str.*) and Arthoniomycetidae (*syn. Arthoniomycetes*), although there was no strong statistical support for this broadened concept of Sordariomycetes. We validate and emend the concept of Dothideomycetidae *sensu* Kirk et al (2001) to include the bitunicate orders Dothideales, Capnodiales and Myriangiales, which lack paraphyses, pseudoparaphyses and paraphysoids. This emended subclass overlaps with the Loculoparenchymatomycetidae (Barr 1983) but differs by including the Myriangiales and excluding the Asterinales, now listed under its constituent families as Dothideomycetes et Chaetothyriomycetes incertae sedis by Eriksson (2006).

Dothideomycetidae P.M. Kirk, P.F. Cannon, J.C. David & J.A. Stalpers, ex Schoch, Spatafora, Crous et Shoemaker, **subclass nov.**

≡ *Dothideomycetidae* P.M. Kirk, P.F. Cannon & J.C. David & J.A. Stalpers, in Kirk et al, Dictionary of Fungi, 9th ed., p 165, 572. 2001 (*nom. nud.*).

Ascomata immersa vel erumpentia vel superficialia, minuta vel magnitudine media, separata vel in stromate basilari aggregata, unilocularia vel plurilocularia, ostiolata, nonnumquam periphysata. Pseudoparaphyses absentes, periphysoideae nonnumquam praesentes. Asci globosi vel ellipsoidei vel clavati vel



subcylindrici. Ascospores hyalinae vel subhyalinae vel fuscae, unicellulares vel pluriseptatae vel muriformes. Anamorphoses seu coelomycetes seu hyphomycetes.

Ascomata immersed, erumpent or sometimes superficial, minute, small or medium-sized; separate or merged or grouped on basal stroma, uni- to multi-loculate apical pore mostly present, when present ostiolar canal at times periphysate, stromatic tissues may contain pseudoparenchymatous cells. Pseudoparaphyses lacking, periphysoids may be present; Asci globose, subglobose, ovoid to ellipsoid, saccate, oblong, clavate or subcylindrical, Ascospores hyaline, subhyaline or dark brown, variable in shape and size, one celled or one to several septate or muriform.

Anamorphs coelomycetous and/or hyphomycetous.

Type order. Dothideales (1897) Lindau in Engler & Prantl, Nat. Pflanzenfam. 1(1):373. 1897.

Represented orders. Dothideales Lindau 1897, Capnodiales Woron. 1925, Myriangiiales Starbäck 1899.

Dothideales. Species from this order generally have smaller ascomata and fewer asci than the pseudoparaphysate Pleosporales (node D) and traditionally have been segregated because of the absence of pseudoparaphyses in their pseudothecia. The species included in this order encompass saprotrophs, hemibiotrophs and biotrophs. It is represented by eight species in our analysis, including the recent epitype isolate of *Dothidea sambuci*, the type of the genus *Dothidea* (Shoemaker et al 2003). The family Dothideaceae includes biotrophs, necrotrophs and saprobes on plant tissue. *Stylothis puccinoides* was redescribed as a separate species from *Dothidea* but remains closely associated with the genus in our phylogeny.

Three members of the Dothioraceae are polyphetic in the tree. The so-called black yeast anamorphs associated with Dothideomycetes tend to occur in this family, with *Aureobasidium pullulans* (probably an anamorph species complex based on the ITS sequences deposited in GenBank), and the micro-morphologically similar *Hormonema dematioides* (teleomorph *Sydowia polyspora*, perhaps also a complex of anamorph species) (de Hoog 1974). These species are found commonly on moist surfaces of plants and can convert from yeast to meristematic growth under

nutritional stress. Some progress in the resolution of the nature of *Aureobasidium pullulans* has been made here with the linkage of *Columnosphaeria fagi* (H.J. Hudson) M.E. Barr to a “neotype” culture CBS 584.75 of *A. pullulans* var. *pullulans* (SUPPLEMENTARY TABLE I).

Capnodiales. The node I is well supported in this multigene analysis. This same node is present in a ribosomal rDNA phylogeny containing “*Raciborskiomyces longisetosus*” as erroneous name for a *Cladosporium* species with *Capnodium citri* (Lumbsch and Lindemuth 2001). Synapomorphies are limited in this expanded order and these taxa have not been grouped together before. The presence of short, paraphyses-like cells in the ostiolar pore of some genera of the Capnodiales such as *Capnodium* also are reported from other families, including the Mycosphaerellaceae (von Arx and Müller 1975) and might be a synapomorphy uniting these taxa. We hereby propose an expansion of the current Capnodiales to include the Mycosphaerellaceae and Piedriaceae. The constituent families are discussed below.

Capnodiaceae. An ascostromatal family without pseudoparaphyses, the Capnodiaceae are leaf epiphytes associated with the honeydew of insects. Also known as sooty molds, they tend to live in complex communities, with multiple species, and often multiple fungal parasites of those species, inhabiting a common, sooty mass. They are noted for the production of darkly pigmented hyphae, often of very characteristic morphology (Hughes 1976, Reynolds 1998). The members of this order have superficial ascostromata with ovoid asci in fascicles and hyaline to dark, one to multiseptate ascospores. The sooty molds are highly pleomorphic and often highly pleoanamorphic. The order includes many anamorphic species, all dematiaceous, including several conidiomatal, mycelial (often with dry-spored, blastic phragmo- or dictyoconidia) or presumably spermatial (usually phialidic) hyphomycete genera or pycnidial synanamorphs (Hughes 1976).

Mycosphaerellaceae. The *Mycosphaerellaceae* is characterized by small pseudothecial ascomata that are immersed in host tissue, single and superficial, or imbedded in a pseudoparenchymatal stroma, papil-

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FIG. 2. Dothideomycete phylogeny. 50% majority rule consensus tree of 45 000 trees obtained by Bayesian inference and MCMCMC under GTR+I+ Γ applied across seven partitions. Only orders and families with more than two members under the current classification of Eriksson (2005) are shown in shadow. Bar indicates the nucleotide substitutions per site. Nodes of interest are labeled alphabetically and support values are shown above and below. Bayesian PP = posterior probability, ML BP = maximum likelihood bootstrap, MP BP = maximum parsimony bootstrap, WP = weighted parsimony bootstrap. Gaps (–) show a collapsed node and asterisks show the presence of a differently resolved node under the specific statistical sampling method used.

late, ostiolate, lacking interascal tissue. Asci vary from ovoid to saccate to subcylindrical, usually stipitate, with or without an apical chamber, lacking any other apical mechanism. Ascospores are hyaline to slightly pigmented, 1-septate, but in some cases also 3-septate, and sometimes are enclosed in a sheath. *Mycosphaerella* has close to 30 anamorph genera associated with it, most of which have cicatrized, sympodially proliferating conidiogenous cells and single or acropetally catenate, dry conidia. The two clades delineated within *Mycosphaerella* here also were recognized in a separate study employing multiple genes to resolve relationships in *Mycosphaerella* (Hunter et al 2006). Node II contains the type of *Mycosphaerella*, *M. punctiformis*, and the bulk of *Mycosphaerella* species, while the second clade (above I4) appears to contain more extremotolerant species (Crous et al unpubl data).

Mycosphaerella is distinguished from *Davidiella* (*Cladosporium* anamorphs) by lacking irregular lumens or inclusions in its ascospores and not having anamorphs with protruberant, thickened, darkened, *Cladosporium*-like scars (Braun et al 2003, Aptroot 2006). As shown in this study *Davidiella* with its *Cladosporium* anamorphs (type species *Davidiella tassiana*, anamorph *Cladosporium herbarum*) clusters in a well supported clade apart from *Mycosphaerella* s.str. (*Mycosphaerellaceae*), and thus a new family is proposed for clade II.

Davidiellaceae Schoch, Spatafora, Crous et Shoemaker, **fam. nov.**

Ascomata *Mycosphaerellae* similia, sed lumen ascoporum forma variabile et anamorphe *Cladosporium*.

Ascomata immersed to erumpent, small or medium-sized; separate or aggregated, uniloculate, apical pore present, periphysate; wall of several layers of brown, thickened, pseudoparenchymatal cells. Pseudoparaphyses lacking. Asci bitunicate, 8-spored, ovoid to ellipsoid or subcylindrical, fasciculate, with or without apical chamber. Ascospores hyaline to pale brown, smooth to somewhat roughened, mucous sheath sometimes present, one-septate, thick-walled, with irregular lumens. Anamorphs are species of *Cladosporium*.

Typus. *Davidiella tassiana* (De Not.) Crous & U. Braun, Mycol. Prog. 2:8. 2003.

The position of a single representative of the Piedraiaceae, *Piedraia hortae*, is refined here as associated with the Capnodiales and allies but not the Myriangiales as reported earlier (Lindemuth et al 2001). This species was described with an ascus containing only one thin wall (Shoemaker and Egger 1982). The specialized parasites in this family are

almost exclusively associated with human hair in tropical regions. It is shown with low parsimony bootstrap support (I3) with *Trimmatostroma abietis*, a meristematic anamorph species isolated from conifer needles and rock surfaces. This species was shown to be closely related to *Mycosphaerella* and its allies in a recently published molecular phylogeny (Selbman et al 2005).

Myriangiales. The Myriangiales are reported to be related to the Dothideales (node M), although without any significant bootstrap support for this placement. They generally have ascostromata without ostioles in monoascal locules. The species of the type genus, *Myriangium*, has globose asci scattered at many levels in an undifferentiated stromatic mass (Sivanesan 1984). The order includes saprobic, epiphytic or biotrophic organisms. The anamorphs of this order, when known, generally are acervular coelomycetes with polyphialidic conidiogenous cells, such as the *Sphaceloma* anamorphs of *Elsinoë* species (Kirk et al 2001).

Paraphysate Dothideomycetes.

We hereby propose a new subclass for the pseudoparaphysate taxa supported by node D1.

Pleosporomycetidae Schoch, Spatafora, Crous et Shoemaker, **subclass nov.**

Ascomata perithecialia vel hysterothecialia vel cleistothecialia, immersa vel erumpentia. Hamathecii pseudoparaphyses cellulares vel trabeculatae, maturae nonnumquam deliquescentes. Asci bitunicati, plerumque basilares, nonnumquam lateraliter extendentes, cylindrici vel clavati vel oblongi vel saccati. Ascosporae colore, forma septisque variabiles, plerumque heteropolares sed nonnumquam etiam symmetricae.

Ascomata perithecioid, hysterothecioid or cleistothecioid, conchate or dolabrate, immersed, erumpent or superficial; globose, sphaeroid, turbinate, ovoid, obpyriform, conoid, doliiform, dimidiate. Hamathecium of wide to narrow cellular or trabeculate pseudoparaphyses, deliquescing at maturity in some. Asci bitunicate, usually basal, at times extending laterally, cylindric, clavate, oblong or saccate. Ascospores variable in pigmentation, shape and septation, usually with bipolar asymmetry, but some symmetrical.

Type order. Pleosporales Luttrell ex M.E. Barr.

Represented order. Pleosporales Luttrell ex M.E. Barr.

Pleosporales. The Pleosporales is the largest order in the Dothideomycetes. It contains several well known plant pathogens such as *Cochliobolus heterostrophus*, the causative agent for southern blight on corn, *Leptosphaeria maculans*, causing black leg on rape seed and

Phaeosphaeria nodorum causing stagonospora blotch in cereals. In this analysis a strain of *Delitschia winterti* is placed above node D, supporting the rest of the Pleosporales according to Eriksson's broad concept (2001). *Delitschia* shares features common to several bitunicate species occurring on dung; they are darkly pigmented, usually strongly constricted ascospores with germ slits (Barr 2000). The family Delitschiaceae was described by Barr (2000) for species previously placed in the Sporormiaceae. The delineation is based on an ostiole containing periphyses and asci with wide outer ascus walls and an ocular chamber containing refractive rods. This placement was confirmed with nuc SSU rDNA sequence comparisons (Kruys 2005). A combined nuc SSU analysis of *Delitschia winterti* grouped it close to another species of the genus, *D. didyma* (AF242264), confirming the identification of the strain used (results not shown). Members of this family are hypersaprotrophic on old dung and exposed wood.

There was also strong support for the monophyly of Pleosporales, with *Lophium mytilinum* branching at its most basal node (D1). This species is found as a saprobe on wood and on cones of conifers and is listed incertae sedis as part of the Mytiliniaceae (Eriksson 2006). The family contains species with characteristic conch shaped ascomata. Analyzing additional taxa from the Mytiliniaceae and related groups also will be important to investigate ancestral character states for the Pleosporales but they should be placed as Pleosporomycetidae incertae sedis for now.

The morphology of ascospores has played an important role in delimiting families in the Pleosporales. However, as noted from some of the first molecular based phylogenies of the Dothideomycetes, several family relationships might be poorly supported (Lindemuth et al 2001). Perhaps the strains chosen are not good exemplars for their families or are derived from misidentified specimens. However it seems unlikely that this can account for all the relationships (FIG. 2) and a reassessment at this level of classification seems urgent. Here we will discuss only briefly a selection of highlighted families (FIG. 2).

The most basal node inside the Pleosporales (D2) supports two members of the Testudinaceae, provisionally included among Ascomycota incertae sedis by Eriksson (2006). Members of this family are mainly isolated from soil and produce reduced, cleistothecoid ascostromata. This clade unexpectedly contains the ostiolate marine species, *Verruculina enalia* (Didymosphaeriaceae) as also noted in an earlier phylogenetic analysis (Kruys 2005). The next well supported clade above node D3 supports the Spor-

ormiaceae. These fungi are found commonly on dung but some occur on other substrates (e.g. wood, soil and plant debris). A large number of species in this group have germ slits. This morphological variability was confirmed in a phylogenetic study using DNA sequences from multiple ribosomal loci (Kruys 2005).

The Lophiostomataceae and Melanommataceae are inferred as paraphyletic in the next set of clades (above D4 and D5), with one clade including two species of *Lophiostoma* (Lophiostomataceae 1). This clade also contains one species of *Trematosphaeria heterospora*, which was classified as *Lophiostoma heterosporum* (Barr 1992). The second clade (Lophiostomataceae 2) includes members of the Lophiostomataceae and Pleomassariaceae as well as Melanommataceae. Node D5 contains a diverse group of species isolated from diseased and decaying plants as well as soil (each currently classified under a different family). This overlapped with relationships reported before, using molecular-based phylogenies (Liew et al 2000, 2002), but like many of the other clades will require more intense sampling to address family and genus descriptions.

The more terminal branches in the Pleosporales (D6) include well studied families containing important plant pathogens, saprobes and animal pathogens with numerous anamorphs. *Didymella cucurbitacearum* forms a clade with the anamorphs *Ascochyta pisi* and *Phoma herbarum* (D8), parasites on agricultural crops. *Leptosphaeria* (Leptosphaeriaceae), shown on a single branch, is a large genus with pale to dark brown and septate ascospores. Members of this family have flask-shaped pseudothecia with narrow asci and a characteristic thin apex. Many species are associated with coelomycetous anamorphs. *Phoma* anamorphs are particularly common (Camara et al 2001, Verkley et al 2004). The Phaeosphaeriaceae (D9) are distinguished from the Leptosphaeriaceae by ascomal wall morphology and all have pycnidial coelomycetes, mostly classified in *Stagonospora*, characterized by holoblastic or sometimes annellidic conidiogenesis and the production of phragmoconidia. Unnamed pycnidial microconidial anamorphs also are reported in some species (Leuchtman 1984). In a poorly supported clade a trio of species without any clear phylogenetic placement are noted. Two of these species are anamorphs, *Coniothyrium palmarum* and *Pyrenochaeta nobilis*, linked to the teleomorphs *Leptosphaeria* and *Herpotrichia*.

The next well supported node (D10) contains the Pleosporaceae, which have ascostromata that are mainly flask-shaped pseudothecia embedded in the substrate with 1-septate to muriform ascospores. In

addition to species found in marine environments and as parasites on animals a number of important grass and cereal crop parasite genera, *Cochliobolus*, *Pyrenophora* and *Lewia*, are included in this family. The sexual states are normally well linked with single anamorph genera. Important anamorph species include the well known genera *Alternaria* (with *Ulocladium* paraphyletic within it), *Stemphylium*, the so-called helminthosporia (*Bipolaris*, *Curvularia*, *Drechslera*, *Exserohilum*) and a few other genera such as *Dendryphion* and *Dendryphiopsis*.

Dothideomycetes incertae sedis.—A number of orders could not be placed in any of the two subclasses defined and will be discussed in more detail. Two orders, Jahnulales and Patellariales, currently listed by Eriksson (2006) are not included in this analysis but a separate comparison using deposited sequences from nuc SSU obtained from GenBank combined with our complete taxa revealed them to be separate from the groups referred to in this paper (data not shown).

Members of Hysteriales have been reported with pseudoparaphyses in apothecoid ascomata with elongated openings (von Arx and Müller 1975, Barr 1987, Luttrell 1974) and are often saprobes on wood or weak parasites of woody plants. Four members of the Hysteriales agreeing mainly with Luttrell's original definitions are included (FIG. 2) and it is clear that these are not a monophyletic group, a proposition also mentioned by Luttrell (1973). *Farlowiella carmichaeliana* could not be resolved with any certainty.

The phylogeny also supports a relationship between the dung fungus *Phaeotrichum benjaminii* and *Tyrannosorus pinicola* (FIG. 2). *Phaeotrichum* is characterized by dark-brown, septate spores and cleistothecoid ascostromata. *T. pinicola* produces ostiolate ascostromata with characteristic long, sharp spines and have been isolated from wood and plant material. The multiple germ slits that were described for *T. pinicola* may be linked to the terminal germ pores characteristic of *P. benjaminii*.

Node E supports *Kirschsteiniothelia aethiops* with its *Dendryphiopsis atra* anamorph. These two species also appear unrelated to other species in the genus (Shearer 1993) based on nuc SSU rDNA data and the genus is reportedly heterogenous (Hawksworth and Eriksson 2003). *K. aethiops* does not have close associations with the Pleosporaceae and should be placed in a separate family.

The Tubeufiaceae clade (above node G) contains species with a variety of nutritional modes. They often are reported as saprobes from terrestrial and freshwater environments, but some species are hyperparasites and others can parasitize insects. Teleomorphs consist of brightly colored ascostromata, with long,

hyaline, multiseptate ascospores (Rossman 1987). The best-known anamorphs of the Tubeufiaceae are helicosporous hyphomycetes and well known genera include *Helicodendron*, *Helicomycetes* and *Helicoon*. Recent DNA sequence-based comparisons did not find strong correlation between these anamorph forms and phylogenetic groups. (Tsui et al 2006). Combining recent focused phylogenies into a large scale dataset is required before placement of this group in the current classification.

Botryosphaeriaceae. The position of the Botryosphaeriaceae (H) within the Dothideomycetes has been enigmatic. The taxonomy of this group of plant-associated fungi has relied mostly on anamorph descriptions; sequence data recently have linked several anamorph genera to the genus *Botryosphaeria* (Jacobs and Rehner 1998). Associated anamorphs were divided into two groups, those with thin-walled, hyaline conidia (*Fusicoccum*), and those with thick-walled, pigmented conidia (*Diplodia*) (Denman et al 2000). In a recent phylogenetic study employing LSU sequence data to resolve relationships among members of the Botryosphaeriaceae, Crous et al (2006) segregated *Botryosphaeria* into several genera, supported by morphological differences of their anamorphs. From the phylogenetic results obtained in this study, it is clear that the Botryosphaeriaceae deserves an order separate from the Pleosporales and Dothideales, which is introduced below.

Botryosphaeriales Schoch, Crous & Shoemaker, **ord. nov.**

Family. Botryosphaeriaceae Theiss. & P. Syd., Ann. Mycol. 16:16 (1918).

Type. Botryosphaeria Ces. & De Not., Comment. Soc. Crittog. Ital. 1:211 (1863)..

Type species. B. dothidea (Moug.:Fr.) Ces. & De Not., Comment. Soc. Crittog. Ital. 1:212 (1863).

Ascomata unilocularia vel plurilocularia, pariete multistratosa fusca inclusa, singularia vel aggregata, raro in stromate submersa. Asci bitunicati, endotunica crassa, stipitati vel sessiles, clavati, camera apicali distincta, pseudoparaphysibus hyalinis, septatis, ramosis vel simplicibus intermixti. Ascospores hyalinae vel pigmentatae, unicellulares vel septatae, ellipsoideae vel ovoideae, nonnumquam appendicibus vel tunica gelatinosis praeditae. Anamorphoses: conidiomata pycnidialia, unilocularia vel multilocularia, saepe in stromate submersa, cellulis conidiogenis phialidicis, conidia hyalina vel pigmentata, tenui- vel crassitunicata proferentibus, quae nonnumquam appendicibus vel tunica gelatinosis praedita sunt.

Ascomata uni- to multilocular with multilayered dark brown walls, occurring singularly or in clusters, frequently embedded in stromatic tissue. *Asci* bituni-

cate, with a thick endotunica, stalked or sessile, clavate, with a well developed apical chamber, intermixed with hyaline, septate pseudoparaphyses, branched or not. *Ascospores* hyaline to pigmented, septate or not, ellipsoid to ovoid, with or without mucoid appendages or sheath. *Anamorphs* have uni- to multilocular pycnidial conidiomata, frequently embedded in stromatic tissue, with hyaline, phialidic conidiogenous cells, and hyaline to pigmented, thin- to thick-walled conidia, which sometimes have mucoid appendages or sheaths.

Conclusion.—This multigene phylogeny contributes to the overall phylogenetic classification of the Dothideomycetes. We emend a previously proposed subclass, the Dothideomycetidae, and propose a new one, the Pleosporomycetidae, based on the presence or absence of pseudoparaphyses as defined by Barr (1987) based on Luttrell (1955). The orders according to Eriksson (2006) are largely upheld with the exception of the Hysteriales, but we also expand this classification with an additional order, the Botryosphaerales, and redefine the Capnodiales to include the currently defined Mycosphaerellaceae and Piedraiaceae. A new family, the Davidiellaceae, is proposed to accommodate *Davidiella* species with *Cladosporium* anamorphs. Several clades did not correlate with familial relationships under Eriksson's classification (2006) and should be addressed in subsequent analyses. Similarly a number of small clades are incertae sedis and remain to be addressed in the future. The strains used in this study, although validated by morphological examinations in previous publications (e.g. Berbee 1996) as well as by comparisons with sequences from GenBank, should continue to be validated by more intensive taxon sampling in a number of clades. The value of additive sampling in this study, where two strains used in previous studies could be shown to be misidentified, supports this.

One large gap in this analysis is the absence of lichenized lineages. A single unidentified *Trypethelium* species was included, but numerous lichenized ascostromatic bitunicate species (such as those in the Pyrenulales) remain candidates for placement in the Dothideomycetes. In fact a study by Del Prado et al (2006) shows good support for a placement of the lichenized Trypetheliaceae within the Dothideomycetes. In addition, numerous lineages remain unresolved in this class. For example the current classification of Eriksson (2006) contains 23 families placed in orders but more than 40 families remain listed as Chaetothyriomycetes et Dothideomycetes incertae sedis. It appears likely that, in the process of combining the comprehensive body of work already done on the biology, ontogeny and morphol-

ogy of these fungi within a molecular-based phylogenetic context, they will continue to surprise and challenge us well into the future.

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SUPPLEMENTARY TABLE I. The list of species used in this study

AFTOL No.	Taxon	Source*	GenBank accession numbers				
			SSU	LSU	RBP2	EF	
78	<i>Acarosporina microspora</i>	CBS 338.39	AY584667	AY584643	AY584682	DQ782890	
684	<i>Agonomia sp</i>	NY 808041	DQ782885	DQ782913	DQ782874	DQ782917	
267	<i>Alleuvia eureka</i>	DAOM 195275	DQ677994	DQ678044	DQ677938	DQ677883	
1610	<i>Alternaria alternata</i>	CBS 916.96	DQ678031	DQ678082	DQ677980	DQ677927	
1579	<i>Alternaria sp. (as 'Clathrospora diplospora')</i> ^a	CBS 174.52	DQ678016	DQ678068	DQ677964	DQ677911	
1583	<i>Ascochyta pisi var. pisi</i>	CBS 126.54	DQ678018	DQ678070	DQ677967	DQ677913	
1079	<i>Aspergillus fumigatus</i>	TIGR	AB008401	AFU438345	Genome	Genome	
1080	<i>Aspergillus nidulans</i>	Broad	ENU77377	AF454167	Genome	Genome	
912	<i>Aureobasidium pullulans var. pullulans</i>	CBS 584.75	DQ471004	DQ470956	DQ470906	DQ471075	
931	<i>Bimuria novae-elandiae</i>	CBS 107.79	AY016338	AY016356	DQ470917	DQ471087	
946	<i>Botryosphaeria dothidea</i>	CBS 115476	DQ677998	DQ678051	DQ677944	DQ767637	
1292	<i>Botryosphaeria ribis</i>	CBS 115475	DQ678000	DQ678053	DQ677947	DQ677893	
1572	<i>Botryosphaeria stevensii</i>	CBS 431.82	DQ678012	DQ678064	DQ677960	DQ677907	
1586	<i>Botryosphaeria tsugae</i>	CBS 418.64	AF271127	DQ767655	DQ767644	DQ677914	
1782	<i>Botryosphaeria viticola</i>	CBS 117009	DQ678036	DQ678087	DQ677985	DQ677914	
59	<i>Botryotinia fuckeliana</i>	OSC 100012	AY544695	AY544651	DQ247786	DQ471045	
1735	<i>Byssothecium circinans</i>	CBS 675.92	AY016339	AY016357	DQ767646	DQ471111	
1911	<i>Caliciopsis orientalis</i>	CBS 138.64	DQ471039	DQ470987	DQ470939	DQ677937	
1869	<i>Caliciopsis pinea</i>	CBS 139.64	DQ678043	DQ678097	DQ677992	DQ471054	
152	<i>Caloscypha fulgens</i>	OSC 100062	DQ247807	DQ247799	DQ247787	DQ471089	
939	<i>Capnodium coffeae</i>	CBS 147.52	DQ247808	DQ247800	DQ247788	DQ677889	
937	<i>Capnodium salicinum</i>	CBS 131.34	DQ677997	DQ678050	DQ677998	DQ840565	
657	<i>Capronia pilosella</i>	DAOM 216387	DQ823106	DQ823099	AF107798	DQ677932	
1788	<i>Cercospora beticola</i>	CBS 116456	DQ678039	DQ678091	DQ677932	DQ782888	
3	<i>Cladonia caroliniana</i>	DUKE Lutzoni 01.26.03-2	AY584664	AY584640	AY584684	DQ677898	
1289	<i>Cladosporium cladosporioides</i>	CBS 170.54	DQ678004	DQ678057	DQ677952	DQ677891	
1035	<i>Cladosporium sp. (as 'Raciborskiomyces longisetosum')</i> ^b	CBS 180.53	AY016351	AY016367	DQ677945	Genome	
1084	<i>Coccidioides immitis</i>	TIGR	Genome	Genome	Genome	Genome	
54	<i>Cochliobolus heterostrophus</i>	CBS 134.39	AY544727	AY544645	DQ247790	DQ497603	
271	<i>Cochliobolus sativus</i>	DAOM 226212	DQ677995	DQ678045	DQ677939	DQ677932	
1582	<i>Colmotoysphaeria fagi (as 'Discosphaeria fagi')</i> ^c	CBS 171.93	AY016342	AY016359	DQ677966	DQ677895	
1240	<i>Coniothyrium obiones</i>	CBS 453.68	DQ678001	DQ678054	DQ677948	DQ677903	
1379	<i>Coniothyrium palmarum</i>	CBS 400.71	DQ678008	DQ767653	DQ677956	DQ677904	
1568	<i>Cucurbitaria elongata</i>	CBS 171.55	DQ678009	DQ678061	DQ677957	DQ677918	
1591	<i>Davidiella tassiana (as anamorph Cladosporium herbarum)</i>	CBS 399.80	DQ678022	DQ678074	DQ677971	DQ677922	
1599	<i>Delitschia winteri</i>	CBS 225.62	DQ678026	DQ678077	DQ677975	DQ471100	
1257	<i>Delphinella strobiligena</i>	CBS 735.71	DQ471029	DQ470977	DQ677951	DQ471100	
355	<i>Dendrographa leucophaea f. minor</i>	DUKE Ornduff 10,070	AF279381	AF279382	AY641034		

SUPPLEMENTARY TABLE I. Continued

AFTOL No.	Taxon	Source*	GenBank accession numbers				
			SSU	LSU	RPB2	EF	
995	<i>Dendryphiella arenaria</i>	CBS 181.58	DQ471022	DQ470971	DQ470924	DQ677890	
273	<i>Dendryphiopsis atra</i>	DAOM 231155	DQ677996	DQ678046	DQ677940	DQ677884	
91	<i>Dermatocarpon minutum</i>	DUKE 9702	AY584668	AY584644	DQ782863	DQ782893	
	<i>Didymella cucurbitacearum</i>	IMI 373225	AY293779	AY293792			
919	<i>Dothidea hippophaës</i>	DAOM 231303	U42475	DQ678048	DQ677942	DQ677887	
921	<i>Dothidea insculpta</i>	CBS 189.58	DQ247810	DQ247802	AF107800	DQ471081	
274	<i>Dothidea sambuci</i>	DAOM 231303	AY544722	AY544681	DQ522854	DQ497606	
1359	<i>Dothiora cannabinae</i>	CBS 737.71	DQ479933	DQ470984	DQ470936	DQ471107	
1854	<i>Elsinoë centrolobi</i>	CBS 222.50	DQ678041	DQ678094		DQ677934	
1855	<i>Elsinoë phaeoli</i>	CBS 165.31	DQ678042	DQ678095		DQ677935	
1360	<i>Elsinoë veneta</i>	CBS 164.29	DQ678007	DQ678060	DQ782385	DQ677902	
1853	<i>Elsinoë veneta</i>	CBS 150.27	DQ767651	DQ767658		DQ767641	
668	<i>Exophiala dermatitidis</i>	CBS 207.35	DQ823107	DQ823100	DQ840562	DQ840566	
1787	<i>Farlowiella carnichaadina</i>	CBS 206.36	AY541482	AY541492	DQ677989	DQ677931	
1618	<i>Guignardia bidwellii</i>	CBS 237.48	DQ678034	DQ678085	DQ677983		
1784	<i>Guignardia gaultheriae</i>	CBS 447.70	DQ678089	DQ678089	DQ677987	DQ677930	
176	<i>Gyromitra californica</i>	OSC 100068	AY544717	AY544663	DQ470891	DQ471059	
1613	<i>Helicomyces roseus</i>	CBS 283.51	DQ678032	DQ678083	DQ677981	DQ677928	
1580	<i>Helicosporium phragmitis</i>	CBS 245.49	DQ767649	DQ767654	DQ767643	DQ767638	
1588	<i>Herpotrichia diffusa</i>	CBS 250.62	DQ678019	DQ678071	DQ677968	DQ677915	
1608	<i>Herpotrichia juniperi</i>	CBS 200.31	DQ678029	DQ678080	DQ677978	DQ677925	
1254	<i>Hysterium pulicare</i>	CBS 239.34	DQ678002	DQ678055	DQ677949	DQ677896	
1305	<i>Hysteropatella clavispora</i>	CBS 247.34	DQ678006	AY541493	DQ677955	DQ677901	
1790	<i>Hysteropatella elliptica</i>	CBS 935.97	EF495114	DQ767657	DQ767647	DQ767640	
925	<i>Kirschsteiniothelia aethiops</i>	CBS 109.53	AY016344	AY016361	DQ470914	DQ471084	
639	<i>Lecanora hybocarpa</i>	DUKE 03.07.04-2	DQ782883	DQ782910	DQ782871	DQ782901	
1	<i>Leotia lubrica</i>	OSC 100001	AY544687	AY544644	DQ470876	DQ471041	
1576	<i>Lepidosphaeria nicotiae</i>	CBS 101341	DQ678015	DQ678067	DQ677963	DQ677910	
277	<i>Leptosphaeria maculans</i>	DAOM 229267	DQ470993	DQ470946	DQ470894	DQ471062	
1606	<i>Lophiostoma arundinis</i>	CBS 269.34	DQ782383	DQ782384	DQ782386	DQ782387	
1581	<i>Lophiostoma crenatum</i>	CBS 629.86	DQ678017	DQ678069	DQ677965	DQ677912	
1609	<i>Lophium mytilinum</i>	CBS 269.34	DQ678030	DQ678081	DQ677979	DQ677926	
1783	<i>Macrophomina phaseolina</i>	CBS 227.33	DQ678037	DQ678088	DQ677986	DQ677929	
1081	<i>Magnaporthe grisea</i>	Broad	AB026819	AB026819	Genome	Genome	
1574	<i>Massaria platani</i>	CBS 221.37	DQ678013	DQ678065	DQ677961	DQ677908	
	<i>Melanomma radicans</i>	ATCC 42522	U43461	U43479	AY485625		
1292	<i>Microglossum rufum</i>	OSC 100641	DQ471033	DQ470981	DQ470933	DQ471104	
1734	<i>Montagnula opulenta</i>	CBS 168.34	AF164370	DQ678086	DQ677984	DQ677984	
2021	<i>Mycosphaerella fijensis</i>	OSC 100622	DQ767652	DQ678098	DQ677993	DQ677993	

SUPPLEMENTARY TABLE I. Continued

AFTOL No.	Taxon	Source*	GenBank accession numbers				
			SSU	LSU	RPB2	EF	
1615	<i>Mycosphaerella graminicola</i>	CBS 292.38	DQ678033	DQ678084	DQ677982		
942	<i>Mycosphaerella punctiformis</i>	CBS 113265	DQ471017	DQ470968	DQ470920	DQ471092	
1304	<i>Myriangium duriaei</i>	CBS 260.36	AY016347	DQ678059	DQ677954	DQ677900	
1078	<i>Neurospora crassa</i>	Broad	X04971	AF286411	XM_324476	Genome	
1569	<i>Ophiosphaerella herpotricha</i>	CBS 620.86	DQ678010	DQ678062	DQ677958	DQ677905	
1595	<i>Ophiosphaerella herpotricha</i> (as syn. <i>Ophiobolus herpotrichus</i>)	CBS 240.31	DQ767650	DQ767656	DQ767645	DQ767639	
1590	<i>Phaeodothis wintarii</i>	CBS 182.58	DQ678021	DQ678073	DQ677970	DQ677917	
280	<i>Phaeosphaeria avenaria</i>	DAOM 226215	AY544725	AY544684	DQ677941	DQ677885	
1570	<i>Phaeosphaeria eustoma</i>	CBS 573.86	DQ678011	DQ678063	DQ677959	DQ677906	
	<i>Phaeosphaeria nodorum</i>	Broad	Genome	Genome	Genome	Genome	
1184	<i>Phaeotrichum benjamini</i>	CBS 541.72	AY016348	AY004340	DQ677946	DQ677892	
1575	<i>Phoma herbarum</i>	CBS 276.37	DQ678014	DQ678066	DQ677962	DQ677909	
1865	<i>Phoma herbarum</i>	ATCC12569	AY293778	AY293791			
1786	<i>Phyllosticta fleviolandica</i>	CBS 998.72	DQ678038	DQ678090	DQ677988		
1852	<i>Piedraita hortae</i>	CBS 480.64	AY016349	AY016366	DQ677990		
1600	<i>Pleomassaria siparia</i>	CBS 279.74	DQ678027	DQ678078	DQ677976	DQ677923	
934	<i>Pleospora herbarum</i> var. <i>herbarum</i>	CBS 714.68	DQ676748	DQ678049	DQ677943	DQ677888	
940	<i>Pleospora herbarum</i> var. <i>herbarum</i>	CBS 541.72	DQ247812	DQ247804	DQ247794	DQ471090	
282	<i>Preussia terricola</i>	DAOM 230091	AY544726	AY544686	DQ470895	DQ471063	
1856	<i>Pyrenochaeta nobilis</i>	CBS 407.76	DQ678096	DQ677991	DQ677936	DQ677936	
283	<i>Pyrenophora phaeocomes</i>	DAOM 222769	DQ499595	DQ499596	DQ497614	DQ497607	
173	<i>Pyrenophora tritici-repentis</i>	OSC 100066	AY544716	AY544672		DQ677882	
949	<i>Pyronema domesticum</i>	CBS 666.88	DQ247813	DQ247805	DQ247795	DQ471093	
126	<i>Rocella fuciformis</i>	DUKE 15572	AY584678	AY584654	DQ782866		
1594	<i>Scorias spongiosa</i>	CBS 325.33	DQ678024	DQ678075	DQ677973	DQ677920	
1256	<i>Sporormiella minima</i>	CBS 524.50	DQ678003	DQ678056	DQ677950	DQ677897	
902	<i>Sylodothis puccinioides</i>	CBS 193.58	AY016353	AY004342		DQ677886	
1300	<i>Sydowia polyspora</i>	CBS 116.29	DQ678005	DQ678058	DQ677953	DQ677899	
1036	<i>Trematosphaeria heterospora</i>	CBS 644.86	AY016354	AY016369	DQ497615	DQ471049	
1589	<i>Trematosphaeria pertusa</i>	CBS 400.97	DQ678020	DQ678072	DQ677969	DQ677916	
1789	<i>Trimmatostroma abietis</i>	CBS 459.93	DQ678040	DQ678092		DQ677933	
110	<i>Trypethelium</i> sp.	DUKE 0000007	AY584676	AY584652	AY584690	DQ782896	
1316	<i>Tubefuia cerea</i>	CBS 254.75	DQ471034	DQ470982	DQ470934	DQ471105	
1235	<i>Tyrannosonus pinicola</i>	CBS 124.88	DQ471025	DQ470974	DQ470928	DQ471098	
1598	<i>Ulospora bigramii</i>	CBS 110020	DQ678025	DQ678076	DQ677974	DQ677921	
1601	<i>Verruculina enalita</i> (as <i>Lojkania enalita</i>) ^d	CBS 304.66	DQ678028	DQ678079	DQ677977	DQ677924	
1037	<i>Westerdykella cylindrica</i>	CBS 454.72	AY016355	AY004343	DQ470925	DQ497610	
1592	<i>Wettsteinina lacustris</i>	CBS 618.86	DQ678023		DQ677972	DQ677919	
51	<i>Xylaria hypoxylon</i>	OSC 100004	AY544719	AY544676	DQ247797	DQ471042	

Notes:

^a Culture CBS 174.52 was initially identified as *Clathrospora diplospora*. The original isolations by E.G. Simmons were of two types: *Clathrospora diplospora* that produced the teleomorph in culture (without any conidia) (his type I) and of an *Alternaria* that did not produce even initials of ascogonia (his type II). When an isolate of *Clathrospora diplospora* was sent to CBS there was no indication recorded at CBS as to whether it was type I or type II. This and other anamorph-teleomorph connections were disowned in a later publication (Simmons 1986; page 286). Based on this and sequence data it seems clear that this isolate is an *Alternaria* species.

^b The strain was initially deposited as *Raciborskioomyces longisetosum* (CBS 180.53). This species was described with the pseudoparaphyses present, arguing against an association with the Mycosphaerellaceae. The high similarity with *Cladosporium herbarum*, a common aerial contaminant strongly suggested that this is not the correct culture.

^c The strain CBS 171.93, *Columnosphaeria fagi* (Barr 2001) was initially given as *Discosphaeria fagi*. This is shown closely related to *Aureobasidium pullulans* in the tree and this species was previously described with this anamorph (Hudson 1965). However, the type species for the genus, *Discosphaeria discophora*, was described with unilocular asci (Holm et al 1999); therefore the generic name for 'Discosphaeria' teleomorphs associated with *A. pullulans* is incorrect.

^d This species was transferred from *Didymosphaeria enalia* to the new genus *Verruculina* (Kohlmeier & Volkmann-Kohlmeier 1990). In the same year this *Didymosphaeria* species was also transferred to *Lojkania* (Barr 1990). Subsequently Aptroot stated that placement of *D. enalia* within *Lojkania* is satisfactory (Aptroot 1995). However, due to differences in peridium and ascus characters (Kohlmeier & Volkmann-Kohlmeier, personal communication) the genus *Verruculina* remains validly listed in the Didymosphaeriaceae as part of the latest classification of the Ascomycota (Eriksson 2006).

References in notes

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* Source abbreviations:

Culture collections and herbaria
 ATCC = American Type Culture Collection, Manassas, Virginia
 CBS = Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands
 DAOM = National Mycological Herbarium, Department of Agriculture, Ottawa, Ontario, Canada
 DUKE = Duke University Herbarium, Durham, North Carolina
 IMI = International Mycological Institute, Surrey, U.K.
 NY = The New York Botanical Garden, Bronx, New York
 OSC = Oregon State University Herbarium, Corvallis, Oregon
 Genome Databases
 Broad = Broad Institute, Cambridge, Massachusetts
 TIGR = The Institute for Genomic Research, Rockville, Maryland