

## An overview of the higher level classification of Pucciniomycotina based on combined analyses of nuclear large and small subunit rDNA sequences

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**Abstract:** In this study we provide a phylogenetically based introduction to the classes and orders of Pucciniomycotina (=Urediniomycetes), one of three

subphyla of Basidiomycota. More than 8000 species of Pucciniomycotina have been described including putative saprotrophs and parasites of plants, animals and fungi. The overwhelming majority of these (~90%) belong to a single order of obligate plant pathogens, the Pucciniales (=Uredinales), or rust fungi. We have assembled a dataset of previously published and newly generated sequence data from two nuclear rDNA genes (large subunit and small subunit) including exemplars from all known major groups in order to test hypotheses about evolutionary relationships among the Pucciniomycotina. The utility of combining nuc-18S sequences spanning the entire D1-D3 region with complete nuc-28S sequences for resolution and support of nodes is discussed. Our study confirms Pucciniomycotina as a monophyletic group of Basidiomycota. In total our results support eight major clades ranked as classes (Agaricostilbomycetes, Atractiellomycetes, Classiculomycetes, Cryptomycocolacomycetes, Cystobasidiomycetes, Microbotryomycetes, Mixiomycetes and Pucciniomycetes) and 18 orders.

**Key words:** basidiomycetes, molecular phylogenetics, parasitic fungi, rDNA systematics, Urediniomycetes

### INTRODUCTION

One-third of described Basidiomycota belong to subphylum Pucciniomycotina (=Urediniomycetes *sensu* Swann and Taylor 1995). Roughly 7000 of these, or ~90%, belong to a single order, Pucciniales (=Uredinales G. Winter) or the rust fungi (Kirk et al 2001). The remaining fungi within the Pucciniomycotina are remarkably diverse ecologically, biologically and physiologically; only with the relatively recent advent of ultrastructure, biochemical and molecular systematic studies have the relatedness of the varied fungi now placed in the Pucciniomycotina become apparent. These include Microbotryales, phytopathogens once classified in the Ustilaginomycotina (true smuts and relatives, Ustilaginomycetes *sensu* Bauer et al 2001); numerous basidiomycetous yeasts formerly allied with Auriculariales and other groups of Agaricomycotina (mushroom-forming fungi and related taxa; Hymenomycetes *sensu* Swann and Taylor 1993); and at least one species, *Mixia osmundae*, formerly classified within the Ascomycota (Nishida et al 1995).

Most species of Pucciniomycotina are parasitic and the group includes phytopathogens (e.g. Pucciniales, Microbotryales), mycoparasites (e.g. *Tuberculina*, *Spiculogloea*) and entomopathogens (e.g. Septobasidiales). A few are putatively saprotrophic or are of unknown trophic habit. Pucciniomycotina species are present in most habitats including freshwater (Bauer et al 2003) and marine environments (Swann et al 2001). Dimorphism, cryptic fructifications, heteroecism and multiple spore types all have evolved in at least some lineages of Pucciniomycotina, hampering the collection of complete biological and life cycle data for many taxa. Yet species of Pucciniomycotina are causal agents of some of the most devastating diseases of crops, may cause opportunistic disease in humans and have shown potential as biological control organisms of invasive plants and of other pathogenic fungi (e.g. Evans 1993, Swann et al 2001).

A few Pucciniomycotina species produce basidiocarps, these typically being simple stipitate-capitate (stilboid) or resupinate (FIG. 1b) structures. Some Pucciniomycotina species form hyphae (filamentous) or yeasts or both (dimorphic) (FIG. 1d). Members of the Pucciniales produce spore-filled fruiting structures termed sori (FIG. 1f). Basidia of teleomorphic species may take the form of teliospores, holobasidia or phragmobasidia, and basidiospores may be sessile, gasteroid or forcibly discharged. The Pucciniomycotina is distinguished from the other two major clades of Basidiomycota by the possession of simple septal pores that lack membrane-bound caps (Swann et al 2001, Weiß et al 2004 and references therein). In addition the cell wall sugar composition of the Pucciniomycotina differs from that in the Ustilaginomycotina and Agaricomycotina (Prillinger et al 2002).

Diverse approaches have been used to delimit taxa within the Pucciniomycotina, including studies of ultrastructural, physiological, biochemical and ecological characters. Molecular characters have had a major impact on our understanding of the phylogenetic relationships of Pucciniomycotina. As early as 1985 Gottschalk and Blanz showed that the secondary structure of 5S ribosomal RNA in some species of Pucciniomycotina differs from that of some other lineages of Basidiomycota. The pioneering studies of Swann and colleagues (Swann and Taylor 1993, 1995; Swann et al 1999) used nuclear small subunit (nuc-ssu) rDNA sequences to support the monophyly of the Pucciniomycotina and a number of major clades. Other studies have employed a region of about 500–650 bp at the 5' end of the nuclear large subunit (nuc-lsu) rDNA, containing the highly variable divergent domains D1 and D2 (Hassouna et al 1984) providing resolution for many clades within the

Pucciniomycotina (e.g. Fell et al 2001, Weiß et al 2004, Bauer et al 2006). However D1–D2 sequences have provided only weak support for monophyly of Pucciniomycotina (Begerow et al 1997, Berres et al 1995), or have resolved the group as a paraphyletic assemblage from which the Ustilaginomycotina and Agaricomycotina have been derived (Weiß et al 2004). Nor were analyses of a third gene, that coding for  $\beta$ -tubulin, able to recover a monophyletic Pucciniomycotina (Begerow et al 2004). Because of the published discrepancies in tree topologies and support values, monophyly for the group has yet to be established conclusively by molecular analyses.

Current major classifications of Pucciniomycotina include those of Kirk et al (2001), Swann et al (2001), Weiß et al (2004) and Bauer et al (2006). The latter three classifications have many similarities (SUPPLEMENTARY TABLE I). However there are also differences between these systems in both resolution and composition of taxa. Kirk et al (2001) divide the Pucciniomycotina (as Urediniomycetes) into five orders and do not include subclasses; thus their classification is quite different from the others.

The goal of the present study is to reassess the higher level systematics of the Pucciniomycotina with combined analyses of nuc-lsu rDNA and nuc-ssu rDNA sequences. Two other studies have included a simultaneous analysis of nuc-lsu and nuc-ssu rDNA sequences of Pucciniomycotina (Lutzoni et al 2004, Bauer et al 2006). However the former was focused on relationships across the Fungi and it included only 14 species of Pucciniomycotina, of which 10 were members of the Pucciniales and the combined analysis in the latter employed only 25 species of Pucciniomycotina and did not cover all major lineages. In the present study we compile and analyze datasets containing new and previously published sequences that represent 174 species from all major groups of Pucciniomycotina. We assess the ability of different rDNA genes and gene regions to recover and support each clade, and we provide an overview of the classes and orders of Pucciniomycotina.

#### MATERIALS AND METHODS

This study uses both newly generated sequences and sequences that were retrieved from GenBank from more than 80 studies (SUPPLEMENTARY TABLE II). Some Pucciniales sequences were generated with the methods described in Aime (2006); sequences of *Jola* spp. were generated as in Frieders (1997). Descriptions of laboratory methods pertaining to the majority of new sequences produced at Clark University can be found online (SUPPLEMENTARY APPENDIX 1).

The entire data matrix contains 208 operational taxonomic units (OTUs) including six Ascomycota that were



FIG. 1. Representatives of the Pucciniomycotina. a. *Jola* cf. *javensis* (Platyglloeales) fruiting on *Sematophyllum swartzii*. b. *Septobasidium burtii* (Septobasidiales) fungal mat completely covering scale insects. c. *Eocronartium muscicola* (Platyglloeales) fruiting on undetermined moss. d. Yeast and filamentous cells of *Sporidiobolus pararoseus* (Sporidiobolales). e. Cultures of two *Sporidiobolus* species in the *S. pararoseus* clade (Sporidiobolales). f. *Phragmidium* sp. (Pucciniales) on *Rosa rubiginosa*.

used for rooting purposes, nine Ustilaginomycotina, four Agaricomycotina and 189 Pucciniomycotina that represent 174 species (26 species of Pucciniomycotina are represented by two OTUs and one species is represented by three OTUs). One OTU, *Septobasidium* sp., is represented by a nuc-lsu rDNA sequence deposited as *Septobasidium* sp. and a nuc-ssu rDNA sequence deposited as *Septobasidium canescens*. All other OTUs include sequences from the same putative species and where possible from the same collection or isolate (SUPPLEMENTARY TABLE II). Alignments are available from the first and/or last author on request. We performed analyses using two sets of OTUs. Dataset 1 contains the 128 OTUs that have both nuc-lsu and nuc-ssu rDNA, including all the Ascomycota, Ustilaginomycotina and Agaricomycotina, and 109 Pucciniomycotina that include representatives of all the major groups in the classifications of Swann et al (2001), Weiß et al (2004) and Bauer et al (2006) except the Cryptomycocolacomycetes. Dataset 2 contains all 208 OTUs, 74 of which have only nuc-lsu rDNA, including *Cryptomycocolax abnormis* (Cryptomycocolacomycetes), and six OTUs that have only the nuc-ssu rDNA. To compare the phylogenetic utility of the short (D1–D2 region only) versus long (D1–D3 region, see SUPPLEMENTARY APPENDIX 1) nuc-lsu rDNA sequences, as well as various combinations of data, we assembled a third dataset (Dataset 3) that contains 20 Pucciniomycotina and two other Basidiomycota that have nuc-lsu rDNA sequences of at least 1117 bp and nuc-ssu rDNA sequences of at least 1667 bp (indicated by an asterisk in SUPPLEMENTARY TABLE II). Datasets were analyzed with equally weighted maximum parsimony (MP); Dataset 1 also was analyzed with neighbor joining (NJ) and Bayesian methods. (Details of analytical methods are provided online in SUPPLEMENTARY APPENDIX 1.)

#### RESULTS AND DISCUSSION

MP analyses of Dataset 1 recovered 36 trees of 8215 steps (CI = 0.345, RI = 0.731), one of which is depicted (FIG. 2, an expanded version is available in online supplementary materials). MP analyses of Dataset 2 recovered 1000 trees of 9748 steps (CI = 0.310, RI = 0.729); the strict consensus tree for Dataset 2 is presented (SUPPLEMENTARY FIG. 3). Detailed results and discussion of analyses for all three datasets are provided online (SUPPLEMENTARY APPENDIX 2).

This work brings together data from more than 80 studies along with newly generated data to provide the first multigene molecular analyses for all major clades of Pucciniomycotina. The present study strongly supports monophyly of the Pucciniomycotina (FIG. 2). We recover eight major clades containing 18 subclades (SUPPLEMENTARY FIG. 3), which most closely correspond to the classification of Bauer et al (2006). All higher taxa of Pucciniomycotina recognized in the present study are supported by bootstrap values of at least 90% in one or more of our analyses

and have Bayesian posterior probabilities of at least 0.98 with the exception of the Sporidiobolales, Agaricostilbomycetes including Spiculogloeales, and the Cryptomycocolales which is represented by only a single nuc-lsu sequence (SUPPLEMENTARY FIG. 3).

The names of higher taxa used here follow the classification formalized in Bauer et al (2006). The classification of Bauer et al (2006) is different from earlier classifications (SUPPLEMENTARY TABLE I) in that more groups are named and recognized as formal taxa (e.g. Cystobasidiomycetes); groups formerly ranked as subclasses were elevated to the rank of class (e.g. Microbotryomycetes); and higher level names prefixed “Uredinio-” or “Uredin-” have been replaced by names based on *Puccinia*, the largest genus within the Pucciniomycotina (Kirk et al 2001).

Many of the genera of Pucciniomycotina are not monophyletic, notably anamorphic yeasts such as *Rhodotorula* and *Sporobolomyces* (Weiß et al 2004, FIG. 2). One of the obvious obstacles to diagnose Pucciniomycotina taxa is the homoplasious nature of the micromorphological characters available (Bauer and Oberwinkler 1991a, Bauer et al 2006). Ultrastructural characters, for example, structure of spindle pole body or the presence of colacosomes in many Microbotryomycetes (Swann et al 1999) appear promising for delimiting natural groups. However at present the major obstacle to applying meaningful diagnoses to these groups is the dearth of ultrastructural, ecological and life-cycle data for most Pucciniomycotina members.

*Descriptions of higher level taxa.—Pucciniomycetes.* This is the most speciose class of Pucciniomycotina. We have resolved four orders (Helicobasidiales, Platygloeales *s. str.*, Pucciniales and Septobasidiales). A fifth order, Pachnocybales (Bauer et al 2006), is represented by a single species, *Pachnocybe ferruginea* (SUPPLEMENTARY FIG. 3) although molecular evidence from other studies places this taxon within the Septobasidiales (Berres et al 1995, Frieders 1997, Henk and Vilgalys 2006). With the exception of *P. ferruginea*, which as a holobasidia-producing saprotroph (Kropp and Corden 1986, Bauer and Oberwinkler 1990) holds an isolated position within the class, and *Platygloea disciformis*, which may be saprotrophic, all known members are parasitic and produce phragmobasidia or teliospores.

The members of the Helicobasidiales produce a dikaryophase (*Helicobasidium*) that is parasitic on plant roots and a haplophase (*Tuberculina*) that is parasitic on rust fungi (Lutz et al 2004b). Species of *Tuberculina* are capable only of infecting rusts in the haploid stage of development (Thirumalachar 1941) and the host-specificity of some species (Lutz et al

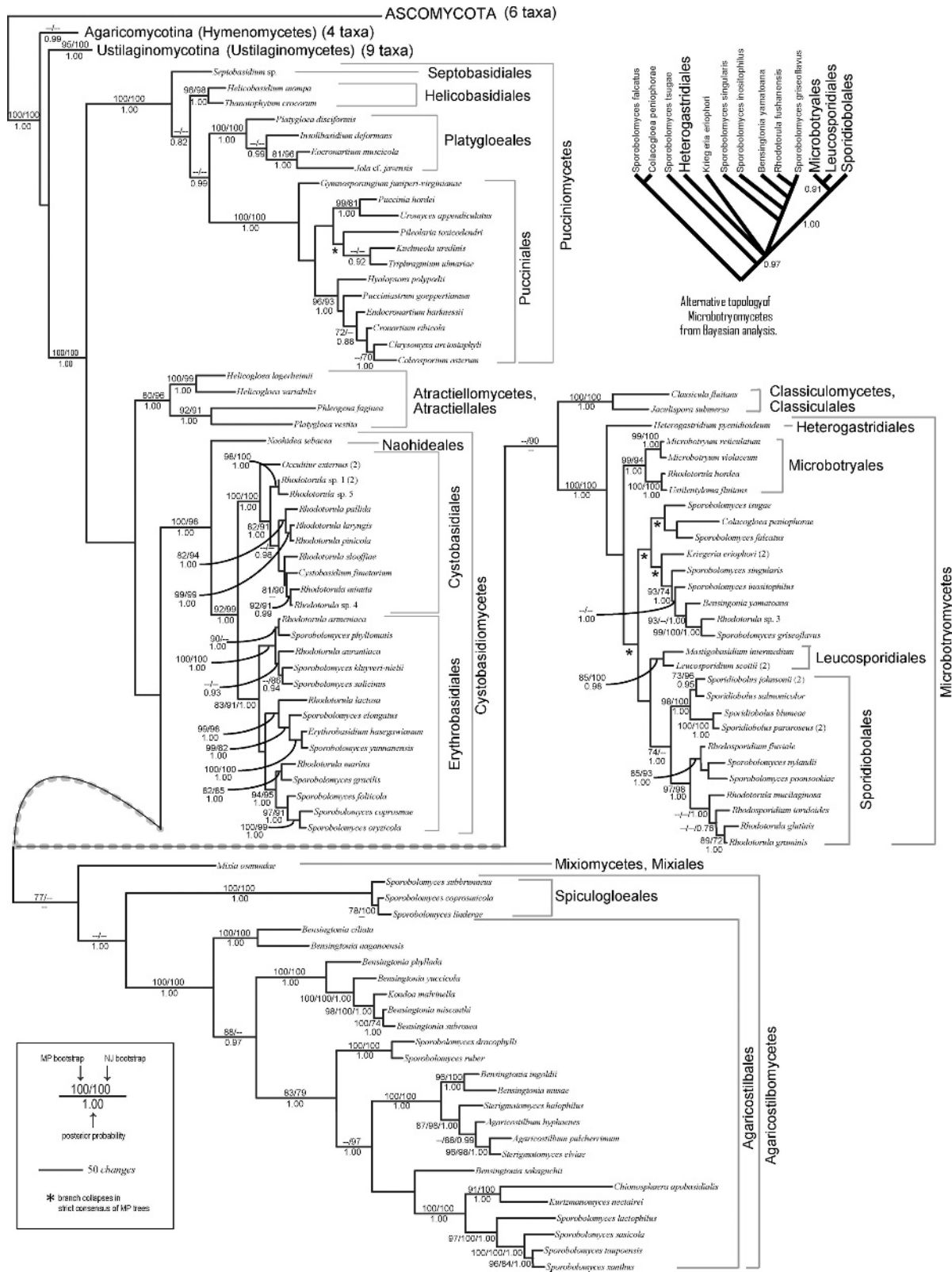


FIG. 2. Phylogenetic relationships in the Pucciniomycotina based on analyses of Dataset 1 (128 OTUs, each with nuc-18S rDNA and nuc-28S rDNA sequences). Phylogram representing one of 36 equally most parsimonious trees (8215 steps, CI = 0.345, RI = 0.731). Nodes with asterisks in the strict consensus of equally most parsimonious trees. Support values include MP bootstrap frequencies above 70% (first value, before slash), NJ bootstrap frequencies above 70% (second value)

2004a) might make them candidates for biocontrol. Lutz et al (2004c) discuss the confused taxonomic history of *Tuberculina*. The position of *H. corticioides* could not be confirmed with nuc-ssu data alone (SUPPLEMENTARY FIG. 3).

Until relatively recently all basidiomycetes with transversely septate basidia (termed auricularioid) were classified in the rusts, smuts (Ustilaginales) or Auriculariales (Agaricomycotina). Moore (1990) created the Platygloales by removing those species that produced simple (rather than dolipore) septal pores from the Auriculariales. The order now is considered to contain mostly phytoparasitic species in the genera *Platyglöea*, *Insolibasidium*, *Herpobasidium*, *Eocronartium* and *Jola*. These last two genera include species that are parasitic on mosses, produce conidia and develop basidiomata which, in the case of *Eocronartium*, are clavarioid fruiting bodies that may reach up to 15 mm tall (Atkinson 1902, Frieders and McLaughlin 2001) (FIG. 1a, c). Fitzpatrick (1918) provides descriptions and illustrations of the life cycle, cytology, host-range and taxonomy of *E. muscicola*, and the host-parasite interaction is described in Boehm and McLaughlin (1988). The columnar type of fructification (FIG. 1c) similar to that of the rust genus *Cronartium* led Atkinson (1902) to hypothesize a close relationship between *Eocronartium* and the Pucciniales, which is supported by molecular analyses (FIG. 2). The unsupported association of *Platyglöea pustulata* with the Septobasidiales (SUPPLEMENTARY FIG. 3) might be an artifact of our supermatrix approach (see SUPPLEMENTARY APPENDIX 2) for which additional gene sampling might be necessary to resolve.

Species of Septobasidiales are unique in that they are parasites of scale insects. This is the second largest order of Pucciniomycotina, with more than 170 described species (Kirk et al 2001), nearly all of which belong to the genus *Septobasidium*. The principal reference is Couch's 1938 publication "The Genus *Septobasidium*". These fungi are found as mats of hyphae covering infected scale insects on plants (FIG. 1b). The life cycle (SUPPLEMENTARY FIG. 4) of *Septobasidium* has been relatively well characterized and includes the only known occurrence of a yeast stage in the Pucciniomycetes. Members of the order live primarily as dikaryons and undergo meiosis

within probasidia (Couch 1938, Olive 1943). *Auriculoscypha*, *Coccidioidictyon*, *Uredinella* and *Ordonia* are the other genera included in the Septobasidiales (Oberwinkler 1989, Henk and Vilgalys 2006).

Pucciniales species are obligate parasites of vascular plants (FIG. 1f) and have developed the most complex life cycles of any Fungi (Cummins and Hiratsuka 2003) (SUPPLEMENTARY FIG. 5). Rusts are phenotypically and genetically plastic organisms with many characters that set them apart from other Basidiomycota including the development of spermatogonia, heteroecism and the occurrence of up to six different spore types (Laundon 1973, Hennen and Buriticá 1980, Cummins and Hiratsuka 2003). Approximately 115 (Cummins and Hiratsuka 2003) to 163 (Kirk et al 2001) genera of Pucciniales currently are recognized and family classification remains contentious (Hennen and Buriticá 1980, Ono and Hennen 1983, Kirk et al 2001). Three recent studies of molecular data from nuc-lsu (Maier et al 2003), nuc-ssu (Wingfield et al 2004) and combined nuc rDNA genes (Aime 2006) have provided confirmation for some families (e.g. Melampsoraceae) and highlighted others requiring revision (e.g. Pucciniaceae). The origins of the Pucciniales and their relationship to other Basidiomycota have been a ripe field for conjecture. Many early hypotheses of basidiomycete evolution consider Pucciniales as the most "primitive" Basidiomycota from which all other lineages are derived (e.g. Linder 1940, Savile 1955). Our results confirm other molecular studies (Prillinger et al 2002, Lutzoni et al 2004) that show the Pucciniales as derived from lineages that include insect and non-vascular plant parasites (FIG. 2).

*Cystobasidiomycetes*.—Our data resolve two orders of Cystobasidiomycetes. A third order, Naohideales, has been proposed (Bauer et al 2006) but it is here represented solely by nuc-lsu sequences of *Naohidea sebacea*, a mycoparasite (SUPPLEMENTARY FIG. 3). Three mostly monotypic taxa, *Sakaguchia* (isolated from marine habitats), *Cyrenella* and *Bannoa*, cannot be placed into orders with our data. The Cystobasidiales include both anamorphic yeasts and dimorphic yeasts that produce clamp connections and auricularioid basidia without basidiocarps. Genera included in this order are *Occultifur* (*O. internus* is a mycoparasite

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and Bayesian posterior probabilities above 0.70 (below branch). Outgroup taxa are condensed into a single branch for Ascomycota (six taxa represented); Agaricomycotina (four taxa represented); Ustilaginomycotina (nine taxa represented). Duplicate sequences have been collapsed into a single branch, which are indicated in parentheses after the taxon name, e.g. *Occultifur externus* (2). The small cladogram depicts the topology of Microbotryomycetidae obtained in Bayesian analyses, with posterior probabilities indicated only for strongly supported nodes that conflict with the MP topology. (An expanded version of FIG. 2 is presented in the online supplementary materials).

of Dacrymycetales, Bauer et al 2006), *Cystobasidium* (which like *Occultifur* produces tremelloid haustorial cells that are indicative of mycoparasitism) and some *Rhodotorula* spp. (anamorphic yeasts). The Erythrobasidiales include many yeasts currently placed in *Rhodotorula* pro parte (p.p.) and *Sporobolomyces* p.p., as well as the monotypic mitosporic yeast *Erythrobasidium*. Other studies (Sampaio 2004, Bauer et al 2006) support the inclusion of *Bannoa* within the Erythrobasidiales. No teleomorph has been found in this order.

*Atractiellomycetes*.—The Atractiellomycetes contain a single order, Atractiellales, originally erected to accommodate gasteroid auricularioid species with simple septa formerly placed in the Auriculariales (Oberwinkler and Bandoni 1982). Walker (1984) and Gottschalk and Blanz (1985) recognized the artificiality of this group, which, as supported by our data, is limited to include the genera *Atractiella*, *Saccoblastia*, *Hobsonia*, *Pleurocolla*, *Helicogloea*, *Platyglaea* p.p. and *Phleogenia*. No yeast state is known for these fungi (Swann et al 2001) but some may produce conidia. *Atractiella* and *Phleogenia* produce stilboid fruitbodies. All members of this class studied thus far possess unique ultrastructural organelles of unknown function termed symplechosomes (Oberwinkler and Bauer 1989, McLaughlin 1990, Bauer and Oberwinkler 1991b).

*Agaricostilbomycetes*.—Our data strongly support a monophyletic Agaricostilbales that is supported as sister of the Spiculogloeales in Bayesian analyses (FIG. 2). Blanz and Gottschalk (1986) found that structure of the 5S rRNA in *Agaricostilbum pulcherrimum*, a small gasteroid fungus found worldwide on palms, was unique from all examined basidiomycetes and posited that this taxon held an isolated position within the heterobasidiomycetes. *Agaricostilbum* is also unusual in that the nuclei divide in the parent cell rather than in the bud cell (Frieders and McLaughlin 1996), and a similar mitotic pattern was found in the closely related *Stilbum vulgare* but not in the more distantly related (SUPPLEMENTARY FIG. 3) yeast *Bensingtonia yuccicola* (McLaughlin et al 2004). The yeast phase of the life cycle of *A. pulcherrimum* is illustrated in Frieders and McLaughlin (1996). Other genera placed in the Agaricostilbales include *Bensingtonia*, *Kondoa*, *Sterigmatomyces*, *Sporobolomyces* p.p., *Chionosphaera*, *Stilbum*, *Mycogloea* p.p. and *Kurtzmanomyces*. These include gasteroid yeasts with auricularioid basidia (e.g. *Agaricostilbum hyphaenes*), gasteroid yeasts with holobasidia (e.g. *Chionosphaera apobasidialis*), and teliospore-forming yeasts (e.g. *Kondoa malvinella*). *Chionosphaera* species produce clamps and microscopic stipitate-capitate fruiting

structures and are mycophilic, associated with lichens and beetle gallery fungi (Kirschner et al 2001a).

The order Spiculogloeales has been placed within the Agaricostilbomycetes by Bauer et al (2006) and contains *Mycogloea* p.p., *Spiculogloea* and some anamorphic *Sporobolomyces* spp. Characters shared by the teleomorphic species of this order include dimorphism, mycoparasitism and tremelloid haustorial cells subtended by clamp connections (Bandoni 1998). *Mycogloea* does not appear monophyletic with these data (SUPPLEMENTARY FIG. 3) and additional molecular (combined nuc-lsu and nuc-ssu sequences could not be obtained for any *Spiculogloea* or *Mycogloea* species for this study) and taxonomic sampling, including the type species of *Mycogloea*, are needed to resolve their placement.

*Microbotryomycetes*.—This group includes mycoparasites, phytopathogens and putative saprotrophs with a diversity of micromorphological characters. Many species contain organelles, termed colacosomes, that are associated with mycoparasitism (Bauer and Oberwinkler 1991a). Four orders are recognized: Heterogastridiales (Oberwinkler et al 1990b), which are mycoparasites and include the aquatic genus *Heterogastridium*; Leucosporidiales (Sampaio et al 2003), which includes the teliospore-forming yeast *Leucosporidium* and two other genera, *Mastigobasidium* and *Leucosporidiella*; the Sporidiobolales (Sampaio et al 2003), including the genera *Rhodotorula* p.p., *Sporidiobolus* (FIG. 1d, e), *Sporobolomyces* p.p. and *Rhodosporeidium* (a teliospore-forming yeast illustrated in SUPPLEMENTARY FIG. 6); and the Microbotryales (Bauer et al 1997). Members of Microbotryales are teliospore-forming phytopathogens that once were classified within the Ustilaginomycotina and include the genera *Bauerago*, *Fulvisporium*, *Aurantiosporium*, *Rhodotorula* p.p., *Ustilentyloa*, *Liroa*, *Microbotryum* and *Sphacelotheca*. *Microbotryum violaceum* has become an important model organism for co-evolutionary and population genetics studies between pathogens and their hosts (e.g. Antonovics et al 2002).

Additional taxa that belong to this class but could not be assigned to an order are *Colacogloea* (a monotypic, mycoparasitic genus that possesses colacosomes, Oberwinkler et al 1990a), *Kriegeria* (a monotypic sedge parasite with an anamorphic yeast stage and unique multiperforate septa, Doubles and McLaughlin 1991, Swann et al 1999), *Camptobasidium* (a monotypic aquatic hyphomycete associated with decaying leaf litter, Marvanová and Suberkropp 1990), *Rhodotorula* p.p., *Sporobolomyces* p.p., *Bensingtonia yamatoana* and *Leucosporidium antarcticum*.

*Classiculomycetes*.—This class contains a single order, the Classiculales, containing aquatic fungi of fresh-

water habitats (Bauer et al 2003). *Classicula fluitans* is the teleomorph of the hyphomycete *Naiadella fluitans* and produces auricularioid basidia and clamp connections. *Jaculispora submersa* is an aquatic hyphomycete for which no teleomorph has been discovered. *Classicula* is unique in forming subapically swollen sterigmata (Bauer et al 2003). *Classicula fluitans* forms tremelloid haustorial cells that were observed to occasionally self-parasitize in culture, which might indicate a mycoparasitic habit for this fungus (Bauer et al 2003).

*Cryptomycocolacomycetes*.—This class contains a single order, Cryptomycocolales, represented by the genus *Cryptomycocolax*, a mycoparasitic fungus that infects ascomycete sclerotia (Oberwinkler and Bauer 1990) and has been placed in the Ustilaginomycotina (Kirk et al 2001). Our study confirms the placement of this clade within the Pucciniomycotina (SUPPLEMENTARY FIG. 3). Cryptomycocolales species are the only fungi outside the Microbotryomycetes that possess colacosomes, which might indicate a sister group relationship between the two. *Cryptomycocolax* has a unique mode of basidium development that on the other hand might be indicative of a basal position within the Pucciniomycotina (Oberwinkler and Bauer 1990). Transmission electron microscopy studies of another genus of colacosome-producing mycoparasites, *Colacosiphon*, indicate that it belongs in the Cryptomycocolales (Kirschner et al 2001b). Both genera are monotypic.

*Mixiomycetes*.—This class, containing the Mixiales, is monotypic containing a single fern parasite, *Mixia osmundae*. Until recently *M. osmundae* was classified within the Ascomycota (Nishida et al 1995) and remains isolated within the Pucciniomycotina with no known close relatives (Swann et al 1999, 2001; FIG. 2). *Mixia osmundae* is a blastosporic yeast, although it has not been established whether it produces meiospores or mitospores. The hyphae are multinucleate containing few septa (Kramer 1958, Nishida et al 1995, Swann et al 2001).

*Incertae sedis*.—*Reniforma strues* was isolated from biofilms in a wastewater treatment plant in West Virginia (Pore and Sorenson 1990). It is a yeast that, unlike any other known basidiomycete, produces kidney-shaped (reniform) somatic cells. Its classification within the Basidiomycota is confirmed with these data, yet its position within the Pucciniomycotina could not be fully resolved with nuc-lsu data alone (SUPPLEMENTARY FIG. 3).

*Concluding remarks*.—While our results provide the first support for many of the higher level clades of Pucciniomycotina formalized in the classification of

Bauer et al (2006), many nodes, especially along the backbone, still are not resolved. Future molecular systematic studies aimed at filling in missing data in Dataset 2 and targeting additional gene regions for exemplar taxa from all the groups recovered by this study should be the next steps toward recovering a robust phylogenetic hypothesis for these fungi. Additional taxonomic sampling might be necessary before a fully resolved phylogeny for the Pucciniomycotina is attainable. However, excluding the Pucciniales, we have sampled 54 genera, or nearly the entire known generic diversity in the subphylum (Kirk et al 2001). The Pucciniales is often considered one of the best documented groups of fungi. Yet recent estimates indicate that only 10–30% of their diversity in the neotropics alone has been discovered (Hennen and McCain 1993). Many genera of Pucciniomycotina have been discovered and described only since the 1990s, often from specialty niches (e.g. beetle galleries, biofilms) or neotropical habitats (e.g. Oberwinkler and Bauer 1990; Oberwinkler et al 1990a, 1990b; Pore and Sorenson 1990; Kirschner et al 2001a, 2001b; Sampaio et al 2003). Furthermore many of these are representative of lineages that are monotypic or problematic with regard to phylogenetic resolution (e.g. *Reniforma strues*). This would indicate that a tremendous amount of diversity still awaits discovery within this ubiquitous group of fungi.

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## APPENDIX 1

### MATERIAL AND METHODS

*DNA extractions, PCR, and sequencing.*—New sequences were obtained from live or lyophilized cultures, herbarium material and fresh collections. DNA was isolated with the EZNA Fungal DNA Kit or EZNA Forensic DNA Kit (Omega Bio-tek, Doraville, Georgia) following the manufacturer's protocols. Primer sequences for nuc-ssu are from White et al (1990) and O'Donnell et al (1998) and can be found online at <http://aftol.org/>. Primer pairs PNS1/NS41 and NS19b/NS8 were used to generate two nuc-ssu rDNA products, sequences of which overlap and yield a total product size of about 1780 bp. On some occasions NS51 was paired with ITS2 to amplify the 3' end of the gene when the primer combination NS19b/NS8 was not optimal. Additional primers for sequencing included NS19bc, NS51 and NS6. Due to the presence of multiple introns only partial nuc-ssu rDNA sequences were obtained for *Colacogloea peniophorae* (sample No. 3) and *Entyloma holwayi*. Only the 18S region between PNS1/NS41 (~1200 bp) at the 5' end of the gene was obtained for *Gymnosporangium juniperi-virginianae*, *Kondoa malvinella*, *Kuehneola uredinis*, *Malassezia pachydermatis*, *Sporisorium formosanum* and *Tilletia controversa*. Nuc-lsu rDNA sequences of approximately 1400 bp were amplified with primers LR0R and LR7 and sequenced with these primers and LR3R, LR16 and LR5 (Vilgalys and Hester 1990; <http://www.biology.duke.edu/fungi/mycolab/primers.htm>). This region includes the first three divergent domains (D1–D3) of the large subunit rDNA (Hassouna et al 1984, Hopple and Vilgalys 1999) as opposed to most published Pucciniomycotina nuc-lsu rDNA sequences, which consist of the first ~600 bp of the region containing the first two divergent domains (D1–D2). In several instances, especially for taxa of the Pucciniales, LR16 was not a suitable sequencing primer. For *Pileolaria toxicodendri* individual nuc-lsu rDNA amplicons were cloned with the TOPO TA cloning kit (Invitrogen, Carlsbad, California) but in all other cases the PCR products were sequenced directly. DNA sequencing reactions were performed with ABI Prism dye terminator chemistry and were run on an ABI 377XL or 3700 automated DNA sequencer (the latter at the DNA sequencing facility of the Duke University Biology Department, R. Vilgalys laboratory).

*Assessing conflict.*—The nuc-lsu and nuc-ssu rDNA are linked in the nuclear rDNA repeat, which reduces the chances of phylogenetic conflicts among the loci due to hybridization, lineage sorting or horizontal gene transmission. Nevertheless the majority of operational taxonomic units (OTUs) combine sequences obtained from different studies (SUPPLEMENTARY TABLE II) so misidentifications, laboratory errors or differing species concepts could be sources of incongruence. To assess conflict we performed 1000 replicate bootstrapped neighbor joining (NJ) analyses of the nuc-lsu and nuc-ssu rDNA partitions independently, using PAUP\* 4.0b12 (Swofford 2002) with Kimura two-

parameter distances. OTUs with differing placements that received at least 70% NJ bootstrap support in both data partitions were considered to demonstrate conflict. Potential conflicts were detected between the nuc-lsu and nuc-ssu rDNA partitions in *Filobasidiella neoformans*, *Melampsora euphorbiae* and *Mycogloea* sp. (trees not shown). One of the conflicting nodes involving *F. neoformans* was strongly supported only marginally (bootstrap = 73%) so the data for that taxon were combined. The data for *M. euphorbiae* and *Mycogloea* sp. were not combined and only nuc-lsu data were used for these OTUs in Dataset 2.

*Data analyses.*—Datasets 1 and 2 were analyzed with equally weighted maximum parsimony (MP) and bootstrapped MP. Dataset 1 also was analyzed with neighbor joining (NJ) and Bayesian methods. MP analyses were implemented in PAUP\* with a two-step search strategy consisting of an initial search with 1000 heuristic searches (each with a starting tree generated by taxon addition with a random sequence, TBR branch swapping, and keeping up to two trees per replicate) followed by a second heuristic search using the shortest trees found in the initial search as starting trees, with TBR branch swapping and MAXTREES set to 1000. Bootstrapped MP analyses used 1000 replicates, each with one heuristic search with a starting tree generated by random taxon addition, TBR branch swapping, and keeping up to 10 trees per replicate.

Modeltest 3.7 (Posada and Crandall 1998) was used to evaluate models of molecular evolution for use in NJ and Bayesian analyses. Optimal models were identified for the nuc-ssu rDNA alone, nuc-lsu rDNA alone and the combined dataset. For each data partition the optimal model according to the Akaike information criterion was the general time reversible model, with a proportion of invariable sites and among-site rate heterogeneity modeled with a discrete gamma distribution (i.e. the GTR+G+I model). However hierarchical likelihood ratio tests selected the TIM+I+G model for the nuc-ssu rDNA alone and the combined dataset and the TrN+I+G model for the nuc-lsu rDNA data partition.

NJ and bootstrapped NJ analyses (with 1000 replicates) were implemented in PAUP\* with the BIONJ algorithm (Gascuel 1997) with maximum likelihood distances that were obtained using the GTR+I+G model. Model parameters were fixed based on values obtained from Modeltest, as follows: base frequencies A = 0.2498, C = 0.2045, G = 0.2772, T = 0.2685; substitution rates A↔C = 1.1857, A↔G = 2.4999, A↔T = 1.5885, C↔G = 1.0903, C↔T = 4.7743, G↔T = 1.0000; proportion of invariable sites = 0.2680; gamma distribution shape parameter = 0.5577 (with four rate classes).

Bayesian analyses were performed using MrBayes 3.0 (Ronquist and Huelsenbeck 2003) with the GTR+I+G model with all parameter values allowed to vary. Model parameters for the nuc-lsu rDNA and nuc-ssu rDNA partitions obtained with Modeltest were similar (values not

shown), so the data were not partitioned. Three independent Metropolis-coupled Markov chain Monte Carlo analyses were performed, each with four chains, three of which were "heated" (with default temperature settings). Each analysis was run  $1 \times 10^6$  generations, with trees sampled every 100 generations. A total of 8500 trees were uploaded from each run after a burn-in of the first 1501 trees. Posterior probabilities were calculated from the 25 500 trees produced by each of the three independent runs.

To assess the phylogenetic resolution and support afforded by different rDNA regions we performed two sets of partitioned analyses. The first set of partitioned analyses used MP, bootstrapped MP and bootstrapped NJ analyses of the nuc-lsu rDNA and nuc-ssu rDNA data partitions of Dataset 1, with the same analytical settings as the combined nuc-lsu/nuc-ssu rDNA analysis. The nuc-lsu rDNA data partition in Dataset 1 contains many sequences that include only the D1–D2 region, as well as sequences up to 1778 bp (SUPPLEMENTARY TABLE II). To compare the phylogenetic utility of the short (D1–D2) versus long (D1–D3) nuc-lsu rDNA sequences, as well as various combinations of data, we assembled a third dataset (Dataset 3) that contains 21 species of Pucciniomycotina and two other Basidiomycota that have nuc-lsu rDNA sequences of at least 1117 bp and nuc-ssu rDNA sequences of at least 1667 bp (OTUs selected for Dataset 3 are indicated by an asterisk in SUPPLEMENTARY TABLE II). We performed analyses of Dataset 3 with six different data partitions, including: (i) The first approximately 650 bp of nuc-lsu rDNA containing the D1–D2 region, with the same set of included positions used in analyses of Datasets 1 and 2; (ii) The D1–D2 region of nuc-lsu rDNA but including two hypervariable regions that were excluded in analyses of Datasets 1 and 2 (the hypervariable regions were aligned with Clustal X 1.83 [Thompson et al 1997] with default settings); (iii) Longer D1–D3 nuc-lsu rDNA sequences (excluding the hypervariable regions); (iv) Complete nuc-ssu rDNA sequences; (v) Combined D1–D2 and nuc-ssu rDNA sequences (excluding the hypervariable regions of nuc-lsu rDNA); and (vi) combined D1–D3 nuc-lsu rDNA and nuc-ssu rDNA sequences (excluding the hypervariable regions of nuc-lsu rDNA).

Each data partition was analyzed with MP, bootstrapped MP and bootstrapped NJ. Analytical settings were the same as described above, except that the MP analysis used a single-step heuristic search with 1000 replicates, each with a starting tree generated with a random taxon addition

sequence, TBR branch swapping, and MAXTREES set to autoincrease.

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## APPENDIX 2

### RESULTS AND DISCUSSION

The newly generated nuc-lsu rDNA sequences range from 917 to 1778 bp (SUPPLEMENTARY TABLE II). Seventy percent (140) of the nuc-lsu rDNA sequences in the complete dataset (Dataset 2) are between 501 and 648 bp, reflecting the prevalence of studies that solely use the region containing the highly variable divergent domains D1 and D2 (Hassouna et al 1984). The newly generated nuc-ssu rDNA sequences range from 829 to 2146 bp. Eighty percent (107) of the nuc-ssu rDNA sequences in the Dataset 2 are more than 1600 bp (SUPPLEMENTARY TABLE II) and contain divergent domains D1–D3. Introns ranging from 255 to 416 bp are present in the nuc-lsu rDNA sequence of *Entyloma holwayi* and in the nuc-ssu rDNA sequences of *Bensingtonia ciliata*, *Colacogloea peniophorae*, *Entyloma holwayi* and *Ustilago maydis*. The aligned length of the complete nuc-lsu rDNA sequences is 2069 bp. The analyzed region of nuc-lsu rDNA (excluding introns and regions that we considered to be of ambiguous alignment) contains 1357 positions, of which 699 are variable and 550 (40.5%) are parsimony informative (in the context of Dataset 1). The nuc-ssu rDNA has an aligned length of 3736 positions. Excluding introns and regions of ambiguous alignment the nuc-ssu rDNA contains 1843 positions, including 1007 variable positions and 710 (38.5%) parsimony informative positions (in the context of Dataset 1; SUPPLEMENTARY TABLE III).

*Analyses of Dataset 1.*—MP analyses of Dataset 1 recovered 36 trees of 8215 steps (CI = 0.345, RI = 0.731; SUPPLEMENTARY TABLE III). Thirty-one of the most parsimonious trees were found in 125 of the 1000 heuristic searches in the first part of the analysis. The strict consensus of all equally most parsimonious trees is highly resolved but there is a large polytomy at the base of the Microbotryomycetes (FIG. 2). The Pucciniomycotina was supported as monophyletic with 100% MP and NJ bootstrap support and posterior probability of 1.0 (FIG. 2). The Atractiellomycetes received MP bootstrap support of 80%, but the other five classes for which there were at least two OTUs each received MP bootstrap support of 100% (FIG. 2 and SUPPLEMENTARY TABLE IV). All six classes with more than two OTUs received NJ bootstrap support of at least 96% and posterior probabilities of 1.0. In total 52 nodes in the Pucciniomycotina received MP bootstrap support greater than 90%, 62 nodes received NJ bootstrap support greater than

90% and 100 nodes received Bayesian posterior probabilities greater than 0.95 (FIG. 2). However the basal nodes in the “backbone” of the Pucciniomycotina are weakly supported (FIG. 2).

MP analyses of Dataset 1 using only the nuc-lsu rDNA recovered 1000 trees of 3799 steps (CI = 0.324, RI = 0.695); 33 nodes received MP bootstrap support exceeding 90% and 27 nodes received NJ bootstrap support exceeding 90% (results summarized in SUPPLEMENTARY TABLE III). MP analyses of Dataset 1 using only the nuc-ssu rDNA recovered 1000 trees of 4136 steps (CI = 0.373, RI = 0.766); 44 nodes received MP bootstrap support exceeding 90% and 50 nodes received NJ bootstrap support exceeding 90% (SUPPLEMENTARY TABLE III).

*Analyses of Dataset 2.*—MP analyses of Dataset 2 recovered 1000 trees of 9748 steps (CI = 0.310, RI = 0.729). Four trees were recovered in two of the 1000 heuristic searches in the first part of the analysis. Despite the large number of equally parsimonious trees the strict consensus is highly resolved (SUPPLEMENTARY FIG. 3). For the most part the same major groups that are strongly supported in analyses of Dataset 1 were resolved in analyses of Dataset 2, but MP bootstrap support values in analyses of Dataset 2 are generally lower than those in analyses of Dataset 1 (SUPPLEMENTARY TABLE IV). For example, MP bootstrap support for the Pucciniomycotina dropped from 100% (Dataset 1) to 83% (Dataset 2) and support for the Microbotryomycetes dropped from 100% to 74% (SUPPLEMENTARY TABLE IV).

*Analyses of Dataset 3.*—Dataset 3 was analyzed with six different data partitions (SUPPLEMENTARY TABLE III). The D1–D2 region alone had the worst performance, with only four (MP) or five (NJ) nodes receiving bootstrap support of 90% or greater. The Clustal X-aligned hypervariable subregions of the D1–D2 region had an aligned length of 153 bp, with 97 variable positions and 81 (53%) parsimony informative positions. Addition of these data to the more conserved part of the D1–D2 dataset resulted in seven nodes receiving bootstrap support of at least 90%. Independent analyses of the longer (D1–D3) nuc-lsu rDNA and nuc-ssu rDNA regions alone yielded similar results, with 8–10 nodes receiving at least 90% bootstrap support. Combined analyses of D1–D2 or longer nuc-lsu sequences and nuc-ssu rDNA sequences yielded 10 or 11 nodes supported by at least 90% of the bootstrap replicates.

*Combined vs. independent analyses of nuc-lsu rDNA and nuc-ssu rDNA.*—Combined analyses of nuc-lsu rDNA and nuc-ssu rDNA sequences provide stronger support for the phylogenetic relationships of Pucciniomycotina than is attained through analyses of either region alone (SUPPLEMENTARY TABLES III and IV). In analyses of Dataset 1 (with 128 OTUs) combined analyses recovered about twice as many strongly supported nodes as analyses of nuc-lsu rDNA alone, and about 1.2–1.4 times as many strongly supported nodes as analyses of nuc-ssu rDNA alone. Combined analyses of Dataset 1 also provide greater resolution than the single-gene analyses; both single-partition analyses of Dataset 1 recovered 1000 equally most parsimonious trees (this was the MAXTREES setting, so there are probably many more equally parsimonious trees), whereas the combined analysis recovered only 36 trees.

The nuc-lsu rDNA data partition in Dataset 1 includes taxa with a range of nuc-lsu sequence lengths (SUPPLEMENTARY TABLE II). Analyses of Dataset 3 allow assessment of the impact of the longer nuc-lsu rDNA sequences which incorporate divergent domains D1–D3 compared to shorter nuc-lsu sequences covering only D1–D2, as well as the resolving power of different combinations of data (SUPPLEMENTARY TABLE III). The D1–D2 region is the smallest data partition analyzed, and not surprisingly it provides the weakest resolution. Inclusion of the hypervariable parts of the D1–D2 region increases the number of strongly supported nodes (SUPPLEMENTARY TABLE III) but the extreme variability of these regions also increases the danger of making incorrect hypotheses of homology when aligning the sequences. The strongest results were obtained in the combined analyses of the nuc-lsu and nuc-ssu rDNA sequences. There was essentially no difference in the strength of support in analyses that combined nuc-ssu rDNA sequences with the D1–D2 regions vs. those that combined nuc-ssu rDNA sequences with extended-length nuc-lsu rDNA sequences (SUPPLEMENTARY TABLE III). Nevertheless the extended-length nuc-lsu rDNA sequences have about 1.4 times as many variable and parsimony-informative

sites as the D1–D2 region alone, and the extended-length nuc-lsu rDNA provides stronger resolution than the D1–D2 region when those regions are analyzed independently (SUPPLEMENTARY TABLE III).

Analyses of Datasets 1 and 3 indicate that combining nuc-lsu rDNA and nuc-ssu rDNA sequences improves phylogenetic resolution and support and also that the utility of D1–D2 sequences can be enhanced significantly by extending them to include the complete LR0R–LR7 fragment covering D1–D3. Another strategy for building on the existing data would be to combine them with a supermatrix approach. Such approaches, where taxon sampling is extensive but not all taxa have been sampled at all loci resulting in a large amount of missing data, nonetheless have shown potential for phylogenetic reconstruction in large datasets (Driskell et al 2004). Analyses of Dataset 2 (in which 74 OTUs have only nuc-lsu rDNA sequences and six OTUs have only nuc-ssu rDNA sequences) resolve the same major clades as analyses of Dataset 1 (FIG. 2 and SUPPLEMENTARY FIG. 3), but many more equally most parsimonious trees were derived from Dataset 2 than were obtained in analyses of Dataset 1 and the bootstrap values are lower in Dataset 2 (FIG. 2, SUPPLEMENTARY FIG. 3, and SUPPLEMENTARY TABLE IV). Thus supermatrix analyses might provide a useful means to construct comprehensive phylogenetic trees that incorporate the large database of D1–D2 sequences, but they probably will not provide the resolution and support that could be attained through combined analyses that have no missing data.

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SUPPLEMENTARY TABLE I. Comparison of classifications of higher taxa of Pucciniomycotina. Exact compositions of taxa vary across classification. Major lineages in each classification are indicated by boldface.

Swann et al (2001)	Weiß et al (2004)	Bauer et al (2006)
UREDINIOMYCETES	UREDINIOMYCETES	PUCINIOMYCOTINA
<b>Urediniomycetidae</b>	<b>Urediniomycetidae</b>	<b>Pucciniomycetes</b>
Uredinales	Uredinales	Pucciniales
<i>Helicobasidium</i>	<i>Helicobasidium</i> subgroup	Helicobasidiales
Platyglloeales <i>s. str.</i>	Platyglloeales	Platyglloeales
Septobasidiales	Septobasidiales	Septobasidiales
Pachnocybaceae <sup>a</sup>	Pachnocybaceae	Pachnocybales
<b><i>Erythrobasidium</i><sup>b</sup> clade</b>	<b>Cystobasidiaceae lineage</b>	<b>Cystobasidiomycetes</b>
<i>Cystobasidium</i> <sup>c</sup>	Cystobasidiaceae group	Cystobasidiales
<i>Erythrobasidium</i>	<i>Erythrobasidium</i> subgroup	Erythrobasidiales
<i>Naohidea</i>	<i>Naohidea</i> subgroup	Naohideales
<b>Atractiellales</b>	<b>Atractiellales lineage</b>	<b>Atractiellomycetes</b>
		Atractiellales
<b>Agaricostilbomycetidae</b>	<b>Agaricostilbomycetidae</b>	<b>Agaricostilbomycetes</b>
Agaricostilbales	Agaricostilbales	Agaricostilbales
<i>Mycogloea</i>	<i>Mycogloea</i> group	Spiculogloeales
<b>Microbotryomycetidae</b>	<b>Microbotryomycetidae</b>	<b>Microbotryomycetes</b>
Heterogastridiales	Heterogastridiales	Heterogastridiales
Sporidiobolaceae p.p.	Leucosporidiales	Leucosporidiales
Microbotryales	Microbotryales	Microbotryales
Sporidiobolaceae p.p.	Sporidiobolales	Sporidiobolales
Camptobasidiaceae	Camptobasidiaceae group	incertae sedis <sup>d</sup>
Cryptomycocolacales	Cryptomycocolacales	<b>Cryptomycocolacomycetes</b>
		Cryptomycocolacales
<i>Naiadella</i> <sup>c</sup>	<b>Classiculales lineage</b>	<b>Classiculomycetes</b>
		Classiculales
<b>Mixiaceae</b>	<b>Mixiaceae lineage</b>	<b>Mixiomycetes</b>
		Mixiales

<sup>a</sup> Placed within Septobasidiales.

<sup>b</sup> Full name: *Erythrobasidium*, *Naohidea*, *Sakaguchia* clade.

<sup>c</sup> Placed incertae sedis within Urediniomycetes.

<sup>d</sup> Placed within Microbotryomycetes.

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SUPPLEMENTARY TABLE II. GenBank numbers and sources for sequences analyzed in this study. Dataset 1 contains those taxa with both nuc-1su and nuc-ssu sequences. Dataset 2 contains all sequences. Taxa used for Dataset 3 are indicated by an asterisk. Double asterisks indicate a nuc-ssu sequence derived from a different isolate/ collection than the nuc-1su sequence.

Species	nuc-1su rDNA			nuc-ssu rDNA		
	GenBank no.	length (bp)	Reference (cited in Genbank)	GenBank no.	length (bp)	Reference (cited in Genbank)
<b>ASCOMYCOTA</b>						
<i>Leotia tubrica</i> (Scop.) Pers.	AY544644	1396	Lutzoni et al (2004)	AY544687	1729	Lutzoni et al (2004)
<i>Morchella cf. elata</i> Fr.	AY544665	1386	Lutzoni et al (2004)	AY544709	1729	Lutzoni et al (2004)
<i>Neoctea vitellina</i> (Bres.) Korf & J.K. Rogers	AF279401	1252	Bhattacharya et al (2000)	Z27393**	1722	Landvik et al (1993)
<i>Saccharomyces cerevisiae</i> Meyen ex E.C. Hansen	J01355	3911	Bayev et al (1981)	J01353	1798	Rubtsov et al (1980)
<i>Sordaria fimicola</i> (Roberge ex Desm.) Ces. & De Not.	AY545728	1343	Lutzoni et al (2004)	X69851**	1796	Wilmitte et al (1993)
<i>Taphrina viresneri</i> (Ráthay) Mix	AY548292	1411	Lutzoni et al (2004)	AY548293	1725	Lutzoni et al (2004)
<b>BASIDIOMYCOTA</b>						
<b>AGARICOMYCOTINA</b>						
<i>Auricularia</i> sp.	AY634277	1433	Matheny et al (2004) unpublished	DQ234542	1764	Matheny et al (2005) unpublished
<i>Botryobasidium botryosum</i> (Bres.) J. Erikss.	DQ089013	1297	Nilsson et al (2005) unpublished	AY662667	1766	Nilsson et al (2005) unpublished
<i>Calocera cornea</i> (Batsch) Fr.*	AY701526	1367	Matheny et al (2004) unpublished	AY771610	1764	Matheny et al (2004) unpublished
<i>Filobasidiella neoformans</i> Kwon-Chung	AJ551290	724	Kidd (2003) unpublished	X60183**	1769	Van de Peer et al (1992)
<b>USTILAGINOMYCOTINA</b>						
<i>Entyloma holuwayi</i> Syd.	AY745721	1778	Matheny et al (2004) unpublished	DQ234562	2045	Matheny et al (2005) unpublished
<i>Malassezia furfur</i> (C.P. Robin) Baill.*	AY745725	1373	Matheny et al (2004) unpublished	AY083223**	1628	McIlhatton and Curran (2002) unpublished
<i>Malassezia pachydermatis</i> (Weidman) C.W. Dodge	AY745724	1389	Matheny et al (2004) unpublished	DQ457640	1179	this study
<i>Sporisorium formosanum</i> (Sawada) Vánky	AY740134	594	Stoll et al (2005)	DQ832226	1096	this study
<i>Tilletia controversa</i> J.G. Kühn	DQ832244	1373	this study	DQ832245	829	this study
<i>Tilletia anomala</i> Bandoni & B.N. Johri	AY745715	1381	Matheny et al (2004) unpublished	AY803752	1771	Matheny et al (2004) unpublished
<i>Tilletiopsis minor</i> Nyland	AY745713	1404	Matheny et al (2004) unpublished	D83190	1727	Takahima and Nakase (1996)
<i>Tilletiopsis washingtonensis</i> Nyland	AY745714	1397	Matheny et al (2004) unpublished	D83192	1730	Takahima and Nakase (1996)
<i>Ustilago maydis</i> (DC.) Corda	AF453938	1115	Piepenbring et al (2002)	X62396**	2183	De Wachter et al (1992)
<b>Pucciniomycotina</b>						
<i>Agaricostilbum hyphaenes</i> (Har. & Pat.) Oberw. & Bandoni*	AY634278	1369	Matheny et al (2004) unpublished	AY665775	1774	Matheny et al (2004) unpublished
<i>Agaricostilbum pulcherrimum</i> (Berk. & Broome) B.L. Brady, B. Sutton & Samson	L20277	539	Berres et al (1995)	AY373391**	1760	McLaughlin et al (2004)
<i>Atracella solani</i> (Cohn & J. Schröt.) Oberw. & Bandoni	AY512831	554	Begerow et al (2003) unpublished			
<i>Aurantiosporium subnitens</i> (J. Schröt. & Henn.) M. Piepenbr., Vánky & Oberw.	AF009846	560	Begerow et al (1997)			
<i>Bannoa habajimensis</i> Hamam., Thanh & Nakase	AB082571	648	Nagahama et al (2002) unpublished			



SUPPLEMENTARY TABLE II. Continued

Species	nuc-lsu rDNA			nuc-ssu rDNA		
	GenBank no.	length (bp)	Reference (cited in Genbank)	GenBank no.	length (bp)	Reference (cited in Genbank)
<i>Bensingtonia changbaiensis</i> F.Y. Bai & Q.M. Wang	AY233342	639	Wang et al (2003)			
<i>Bensingtonia ciliata</i> Ingold*	AY745730	1402	Matheny et al (2004) unpublished	D38233	2093	Takashima et al (1995a)
<i>Bensingtonia ingoldii</i> Nakase & Itoh	AF189888	631	Fell et al (2000)	D38234	1746	Takashima et al (1995a)
<i>Bensingtonia miscanthi</i> (Nakase & M. Suzuki) Nakase & Boekhout	AF189891	640	Fell et al (2000)	D38236	1749	Takashima et al (1995a)
<i>Bensingtonia musae</i> M. Takash., S.O. Suh & Nakase	AY512833	628	Begerow et al (2003) unpublished	D43946**	1739	Takashima et al (1995b)
<i>Bensingtonia naganoensis</i> (Nakase & M. Suzuki) Nakase & Boekhout	AF189893	634	Fell et al (2000)	D38366	1753	Takashima et al (1995a)
<i>Bensingtonia phyllada</i> (Van der Walt & Y. Yamada) Van der Walt, Nakagawa & Y. Yamada ex Boekhout	AY512836	642	Begerow et al (2003) unpublished	D38237***	1748	Takashima et al (1995a)
<i>Bensingtonia sakaguchii</i> Sugita, M. Takash., Hamam. & Nakase	AF363646	599	Scorzetti et al (2002)	AB001746	2653	Sugita et al (1997)
<i>Bensingtonia sorbi</i> F.Y. Bai & Q.M. Wang	AY233345	631	Wang et al (2003)			
<i>Bensingtonia subrosea</i> (Nakase & M. Suzuki) Nakase & Boekhout	AF189895	640	Fell et al (2000)	D38238	1744	Takashima et al (1995a)
<i>Bensingtonia yamatoana</i> (Nakase, M. Suzuki & Itoh) Nakase & Boekhout	AF189896	619	Fell et al (2000)	D38239	2658	Takashima et al (1995a)
<i>Bensingtonia yuccicola</i> (Nakase & M. Suzuki) Nakase & Boekhout*	AY745727	1388	Matheny et al (2004) unpublished	DQ234543	1775	Matheny et al (2005) unpublished
<i>Camptobasidium hydrophilum</i> Marvanová & Suberkr. [1]	AY212991	570	Sampaio et al (2003)			
<i>Camptobasidium hydrophilum</i> [2]	AY512837	541	Begerow et al (2003) unpublished			
<i>Chionosphaera aphobasidiatis</i> D.E. Cox [1]	AF393470	487	Kirschner et al (2001)			
<i>Chionosphaera aphobasidiatis</i> [2]	AF177407	632	Sampaio et al (1999b)	U77662**	1658	Swann et al (1999)
<i>Chionosphaera cuniculicola</i> R. Kirschner, Begerow & Oberw.	AF393473	483	Kirschner et al (2001)			
<i>Chrysomyxa arctostaphyli</i> Dietel*	AY700192	1180	Matheny et al (2004) unpublished	AY657009	1667	Matheny et al (2004) unpublished
<i>Classicula shuitans</i> R. Bauer, Begerow, Oberw. & Marvanová	AY512838	559	Begerow et al (2003) unpublished	AY124478	1516	Bauer et al (2003)
<i>Colacogloea peniophorae</i> (Bourdot & Galzin) Oberw., R. Bauer & Bandoni [1]	AF189898	621	Fell et al (2000)			
<i>Colacogloea peniophorae</i> [2]	AY512839	616	Begerow et al (2003) unpublished			
<i>Colacogloea peniophorae</i> [3]*	AY629313	1315	Matheny et al (2004) unpublished	DQ234565	2146	Matheny et al (2005) unpublished
<i>Colosporium asterum</i> (Dietel) Syd. & P. Syd.	AF522164	951	Szaro and Bruns (2002) unpublished	AY123286	1760	Wingfield et al (2004)
<i>Gronartium ribicola</i> J.C. Fisch.	AF522166	963	Szaro and Bruns (2002) unpublished	M94338	1753	Bruns et al (1992)
<i>Cryptomycolax abnormis</i> Oberw. & R. Bauer	AY512841	609	Begerow et al (2003) unpublished			
<i>Cyrenella elegans</i> Goch.	AY512842	573	Begerow et al (2003) unpublished			

SUPPLEMENTARY TABLE II. Continued

Species	nuc-1su rDNA			nuc-ssu rDNA		
	GenBank no.	length (bp)	Reference (cited in Genbank)	GenBank no.	length (bp)	Reference (cited in Genbank)
<i>Cystobasidium fimetarium</i> (Schumach.) P. Roberts	AY512843	633	Begerow et al (2003) unpublished	AY124479	1624	Bauer et al (2003)
<i>Endocronartium harknessii</i> Hiras.*	AY700193	1200	Matheny et al (2004) unpublished	AY665785	1764	Matheny et al (2004) unpublished
<i>Eocronartium muscicola</i> (Pers.) Fitzp. [1]	AF014825	914	Bruns and Szaro (1997) unpublished	AY123323	1763	Wingfield et al (2004)
<i>Eocronartium muscicola</i> [2]	AY512844	622	Begerow et al (2003) unpublished			
<i>Erythrobasidium hasegavianum</i> Hamam., Sugiy. & Komag. [1]	AF131058	603	Sampaio et al (1999a)			
<i>Erythrobasidium hasegavianum</i> [2]	AF189899	598	Fell et al (2000)	D12803**	1755	Suh and Sugiyama (1993)
<i>Fulvisporium restifaciens</i> (D.E. Shaw) Vánky	AF009860	561	Begerow et al (1997)			
<i>Gymnosporangium juniperi-virginianae</i> Schwein.	AY629316	1348	Matheny et al (2004) unpublished	DQ667158	1145	this study
<i>Helicobasidium corticioides</i> Bandoni				U75303	1747	Swann and McLaughlin (1996) unpublished
<i>Helicobasidium longisporum</i> Wakef.	AY222046	1132	Lutz et al (2004b)			
<i>Helicobasidium mompa</i> Tanaka [1]	AY254178	1128	Lutz et al (2004a)			
<i>Helicobasidium mompa</i> [2]*	AY254179	1117	Lutz et al (2004a)	U77064	1657	Swann et al (1999)
<i>Helicobasidium purpureum</i> (Tul.) Pat. [1]	AY222049	1106	Lutz et al (2004b)			
<i>Helicobasidium purpureum</i> [2]	AY254180	1121	Lutz et al (2004a)			
<i>Helicogloea lagerheimii</i> Pat.	AY512849	510	Begerow et al (2003) unpublished	AY124476	1721	Bauer et al (2003)
<i>Helicogloea variabilis</i> K. Wells	L20282	557	Berres et al (1995)	U78043	1769	Swann et al (1999)
<i>Herpobasidium filicinum</i> (Rostr.) Lind [1]	AF426193	506	Maier et al (2003)			
<i>Herpobasidium filicinum</i> [2]	AY512850	619	Begerow et al (2003) unpublished			
<i>Heterogastrium pycnidioideum</i> Oberw. & R. Bauer	AY512851	599	Begerow et al (2003) unpublished	U41567**	1651	Swann and Taylor (1995)
<i>Hobsonia mirabilis</i> (Peck) Linder				AF289663	1720	Sikaroodi et al (2001)
<i>Hyalospora polypodii</i> (Pers.) Magn.				AB011015	1747	Sjamsurizal et al (1999)
<i>Insolobasidium deformans</i> (C.J. Gould) Oberw. & Bandoni*	AY512852	516	Begerow et al (2003) unpublished			
<i>Jaculispora submersa</i> H.J. Huds. & Ingold	AY646099	1413	Matheny et al (2004) unpublished	AY123292**	1756	Wingfield et al (2004)
<i>Jola</i> cf. <i>javensis</i> Pat.	AY512853	595	Begerow et al (2003) unpublished	AY124477	1669	Bauer et al (2003)
<i>Jola hookerianum</i> A. Möller	DQ416207	570	this study	DQ416206	1717	this study
<i>Kondoa aaria</i> A. Fonseca, J.P. Samp. & Fell	DQ416208	568	this study			
<i>Kondoa matumella</i> (Fell & I.L. Hunter) Y. Yamada, Nakagawa & I. Banno	AF189902	638	Fell et al (2000)			
<i>Kondoa</i> sp.	AY745720	1440	Matheny et al (2004) unpublished	DQ667164	1190	this study
<i>Kriegeria eriophori</i> Bres. [1]*	AF189904	637	Fell et al (2000)			
<i>Kriegeria eriophori</i> [2]	AY745728	1397	Matheny et al (2004) unpublished	DQ419918	1766	this study
<i>Kuehneola uredinis</i> (Link) Arthur	AF189905 <sup>b</sup>	619	Fell et al (2000)	U77063***	1684	Swann et al (1999)
<i>Kurtzmanomyces insolitus</i> J.P. Samp. & Fell	AY745696	1385	Matheny et al (2004) unpublished	DQ092919	1310	Matheny et al (2005) unpublished
	AF177408	630	Sampaio et al (1999b)			

SUPPLEMENTARY TABLE II. Continued

Species	nuc-1su rDNA			nuc-ssu rDNA		
	GenBank no.	length (bp)	Reference (cited in Genbank)	GenBank no.	length (bp)	Reference (cited in Genbank)
<i>Kurtzmanomyces neclatirei</i> (Rodr. Mir.) Y. Yamada, Itoh, H. Kawas., I. Banno & Nakase	AF177409	629	Sampaio et al (1999b)	D64122**	1739	Suh et al (1996b)
<i>Kurtzmanomyces tardus</i> Gim-Jurado & Uden	AF177410	629	Sampaio et al (1999b)			
<i>Leucosporidiella creatinivora</i> (Golubev) J.P. Samp.	AF189925 <sup>c</sup>	619	Fell et al (2000)			
<i>Leucosporidiella fragariae</i> (J.A. Barnett & Buhagiar) J.P. Samp.	AY512879	594	Begerow et al (2003) unpublished			
<i>Leucosporidiella muscorum</i> (Di Menna) J.P. Samp.	AY213008	589	Sampaio et al (2003)			
<i>Leucosporidiella yakutica</i> (Golubev) J.P. Samp.	AY213001	583	Sampaio et al (2003)			
<i>Leucosporidium antarcticum</i> Fell, Statzell, I.L. Hunter & Phaff	AY512855	622	Begerow et al (2003) unpublished			
<i>Leucosporidium fellii</i> Gim-Jurado & Uden	AY512856	617	Begerow et al (2003) unpublished			
<i>Leucosporidium scottii</i> Fell, Statzell, I.L. Hunter & Phaff [1]	AF070419	619	Fell et al (1998)	X53499**	1765	Hendriks et al (1991)
<i>Leucosporidium scottii</i> [2]*	AY646098	1389	Matheny et al (2004) unpublished	AY707092	1803	Matheny et al (2004) unpublished
<i>Liroa emodensis</i> (Berk.) Cif. [1]	AY512858	565	Begerow et al (2003) unpublished			
<i>Liroa emodensis</i> [2]	AY212992	587	Sampaio et al (2003)			
<i>Mastigobasidium intermedium</i> Golubev [1]	AF189889	619	Fell et al (2000)			
<i>Mastigobasidium intermedium</i> [2]	AY512859	603	Begerow et al (2003) unpublished			
<i>Melampsora euphorbiae</i> (C. Schub.) Castagne	DQ437504	1100	this study	D38235**	1744	Takahima et al (1995a)
<i>Microbotryum cordae</i> (Liro) G. Deml & Prillinger	AY213002	589	Sampaio et al (2003)			
<i>Microbotryum reticulatum</i> (Liro) R. Bauer & Oberw.	AY213003	586	Sampaio et al (2003)	U79566**	925	Swann et al (1999)
<i>Microbotryum violaceum</i> (Pers.) G. Deml & Oberw.	AY512864	620	Begerow et al (2003) unpublished	U77062**	1727	Swann and Taylor (1995)
<i>Mixia osmundae</i> (Nishida) C.L. Kramer [1]	AB052840	600	Sjamsuridzal et al (2002)	D14163	1780	Nishida et al (1995)
<i>Mixia osmundae</i> [2]	AY512867	613	Begerow et al (2003) unpublished			
<i>Mycogloea macrospora</i> (Berk. & Broome) McNabb				U41848	1601	Swann and Taylor (1995)
<i>Mycogloea nipponica</i> Bandoni	AY159650	602	Kirschner et al (2002) unpublished			
<i>Mycogloea</i> sp.	AY512868	629	Begerow et al (2003) unpublished			
<i>Naohidea sebacea</i> (Berk. & Broome) Oberw. [1]	AF131061	541	Sampaio et al (1999a)			
<i>Naohidea sebacea</i> [2]*	AF522176	1203	Szaro and Bruns (2002) unpublished	AY123302	2094	Wingfield et al (2004)
<i>Occultifur externus</i> J.P. Samp., R. Bauer & Oberw. [1]*	AY745723	1423	Matheny et al (2004) unpublished	DQ457639	1774	this study
<i>Occultifur externus</i> [2]	AF189911	629	Fell et al (2000)	AY124475**	1629	Bauer et al (2003)
<i>Pachnocybe ferruginea</i> Berk.	L20284	554	Berres et al (1995)			

SUPPLEMENTARY TABLE II. Continued

Species	nuc-1su rDNA			nuc-ssu rDNA		
	GenBank no.	length (bp)	Reference (cited in Genbank)	GenBank no.	length (bp)	Reference (cited in Genbank)
<i>Phleogenia faginea</i> (Fr.) Link	AY512869	537	Begerow et al (2003) unpublished	AY373392**	1833	McLaughlin et al (2004)
<i>Pileolaria toxicodendri</i> (Berk. & Ravenel) Arthur [1]	AY745699	1173	Matheny et al (2004) unpublished			
<i>Pileolaria toxicodendri</i> [2]	AY745698	1414	Matheny et al (2004) unpublished	DQ092921	1774	Matheny et al (2005) unpublished
<i>Platygoea disciformis</i> (Fr.) Neuhoff*	AY629314	1355	Matheny et al (2004) unpublished	DQ234563	1763	Matheny et al (2005) unpublished
<i>Platygoea pustulata</i> G.W. Martin & Cain	AY512871	585	Begerow et al (2003) unpublished	AY124480	1602	Bauer et al (2003)
<i>Platygoea vestita</i> Bourdot & Galzin	AY512872	593	Begerow et al (2003) unpublished			
<i>Pleurocolla compressa</i> (Ellis & Everh.) Diehl	AY382581	501	Kirschner et al (2004) unpublished			
<i>Puccinia arundinariae</i> Schwein.	DQ415277	1042	this study			
<i>Puccinia hordei</i> G.H. Outh	DQ354527	1074	Aime (2006)	DQ415278		this study
<i>Pucciniastrum goeppertianum</i> (Kuehn) Kleb.	AF522180	940	Szaro and Bruns (2002) unpublished	AY123305	1754	Wingfield et al (2004)
<i>Reniforma strues</i> Pore & Sorenson	AF189912	557	Fell et al (2000)			
<i>Rhodosporiidium diobovatum</i> S.Y. Newell & I.L. Hunter	AF189914	597	Fell et al (2000)			
<i>Rhodosporiidium fluviale</i> Fell, Kurtzman, Tallman & J.D. Buck [1]	AF070422	604	Fell et al (1998)			
<i>Rhodosporiidium fluviale</i> [2]*	AY745719	1362	Matheny et al (2004) unpublished	U77395**	1737	Swann et al (1999)
<i>Rhodosporiidium sphaerocarpum</i> S.Y. Newell & Fell	AY512875	569	Begerow et al (2003) unpublished	D12806**	1759	Suh and Sugiyama (1993)
<i>Rhodosporiidium toruloides</i> Banno	AF070426	603	Fell et al (1998)			
<i>Rhodotorula armeniaca</i> R.G. Shivas & Rodr. Mir.	AF189920	629	Fell et al (2000)	AB126644	1783	Nagahama et al (2003) unpublished
<i>Rhodotorula aurantiaca</i> (Saito) Lodder	AY512877	626	Begerow et al (2003) unpublished	AB030354**	1757	Takahama et al (2000)
<i>Rhodotorula bogoriensis</i> (Deinema) Arx & Wejman	AF189923	592	Fell et al (2000)			
<i>Rhodotorula cresolica</i> Middelhoven & Spaaij	AF189926	619	Fell et al (2000)			
<i>Rhodotorula diffusa</i> (Ruinen) T. Haseg., Banno & Yamauchi	AY512878	621	Begerow et al (2003) unpublished			
<i>Rhodotorula foliorum</i> (Ruinen) Rodr. Mir. & Wejma	AF317804	619	Fell (2000) unpublished			
<i>Rhodotorula glutinis</i> (Fresen.) F.C. Harrison [1]	AF070430	604	Fell et al (1998)			
<i>Rhodotorula glutinis</i> [2]*	AY646097	1349	Matheny et al (2004) unpublished	DQ832194	1762	this study
<i>Rhodotorula graminis</i> Di Menna	AF070431	604	Fell et al (1998)	X83827**	1803	Cai et al (1996)
<i>Rhodotorula hordea</i> Rodr. Mir. & Wejman [1]	AY512881	623	Begerow et al (2003) unpublished	AY657013	1777	Matheny et al (2004) unpublished
<i>Rhodotorula hordea</i> [2]*	AY631901	1412	Matheny et al (2004) unpublished	D45366	1748	Suh et al (1996a)
<i>Rhodotorula lactosa</i> T. Haseg.	AF189936	601	Fell et al (2000)	AB126649	1782	Nagahama et al (2003) unpublished
<i>Rhodotorula laryngis</i> Reiersøl	AF189942	629	Fell et al (2000)			
<i>Rhodotorula marina</i> Phaff, Mrak & O.B. Williams	AF189944	629	Fell et al (2000)	AB126645	1784	Nagahama et al (2003) unpublished
<i>Rhodotorula minuta</i> (Saito) F.C. Harrison	AF189945	629	Fell et al (2000)	D45367	1752	Suh et al (1996a)

SUPPLEMENTARY TABLE II. Continued

Species	nuc-1su rDNA			nuc-5su rDNA		
	GenBank no.	length (bp)	Reference (cited in Genbank)	GenBank no.	length (bp)	Reference (cited in Genbank)
<i>Rhodotorula mucilaginosa</i> (A. Jörg.) F.C. Harrison	AF070432	600	Fell et al (1998)	AB021668**	1760	Hamamoto and Nakase (2000)
<i>Rhodotorula pallida</i> Lodder	AF189962	629	Fell et al (2000)	AB126651	1784	Nagahama et al (2003) unpublished
<i>Rhodotorula piniicola</i> F.Y. Bai, L.D. Guo & J.H. Zhao	AF444293	627	Zhao et al (2002)	AB126652	1783	Nagahama et al (2003) unpublished
<i>Rhodotorula slooffiae</i> E.K. Novák & Vörös-Felkai	AF189968	629	Fell et al (2000)	AB126653**	1781	Nagahama et al (2003) unpublished
<i>Rhodotorula sonchii</i> (Hopsu-Havu, Tunnela & Yarrow) Rodr. Mir. & Weijman [1]	AF189969	627	Fell et al (2000)			
<i>Rhodotorula sonchii</i> [2]	AY213009	592	Sampaio et al (2003)			
<i>Rhodotorula</i> sp. 1	AB055196 <sup>d</sup>	636	Sjamsuridzal et al (2001) unpublished	AB055191 <sup>d</sup>	1742	Sjamsuridzal et al (2001) unpublished
<i>Rhodotorula</i> sp. 2	AB055197 <sup>d</sup>	636	Sjamsuridzal et al (2001) unpublished	AB055192 <sup>d</sup>	1743	Sjamsuridzal et al (2001) unpublished
<i>Rhodotorula</i> sp. 3	AB176591 <sup>e</sup>	591	Nakase et al (2004) unpublished	AB176530 <sup>e</sup>	1783	Nakase et al (2004) unpublished
<i>Rhodotorula</i> sp. 4	AB055194 <sup>f</sup>	637	Sjamsuridzal et al (2001) unpublished	AB055189 <sup>f</sup>	1736	Sjamsuridzal et al (2001) unpublished
<i>Rhodotorula</i> sp. 5	AB055195 <sup>g</sup>	634	Sjamsuridzal et al (2001) unpublished	AB055190 <sup>g</sup>	1719	Sjamsuridzal et al (2001) unpublished
<i>Saccharastasia farinacea</i> (Höhn.) Donk	AJ406401	903	Langer (2000) unpublished			
<i>Sakaguchia dacryoidea</i> (Fell, I.L. Hunter & Tallman) Y. Yamada, K. Maeda & Mikata [1]	AF189972	571	Fell et al (2000)			
<i>Sakaguchia dacryoidea</i> [2]	AF189973	571	Fell et al (2000)			
<i>Septobasidium burtii</i> Lloyd	L20289	548	Berres et al (1995)	AY373388	1430	McLaughlin et al (2004)
<i>Septobasidium canescens</i> Burt	AY254182	570	Lutz et al (2004a)	AY373389	1787	McLaughlin et al (2004)
<i>Septobasidium carexianum</i> Bres.						
<i>Septobasidium</i> sp. <sup>h</sup>				AY123320**	1729	Wingfield et al (2004)
<i>Sphaelotheca polygomi-persicariae</i> G. Deml & Oberw.	AF189974	619	Fell et al (2000)			
<i>Sphaelotheca polygomi-serrulati</i> Maire	AY512884	620	Begerow et al (2003) unpublished			
<i>Sphenospora kevoikiamii</i> Linder	DQ354521	1205	Aime (2006)			
<i>Spiculogloea</i> sp.	AY512885	601	Begerow et al (2003) unpublished			
<i>Sporidiobolus johnsonii</i> Nyland [1]	AY167608	604	Alvarez-Rodriguez et al (2003)	L22261**	1760	Swann and Taylor (1993)
<i>Sporidiobolus johnsonii</i> [2]*	AY745718	1371	Matheny et al (2004) unpublished	AB021672**	1807	Hamamoto and Nakase (2000)
<i>Sporidiobolus pararoseus</i> Fell & Tallman [1]	AF070437	601	Fell et al (1998)	AB021689**	1759	Hamamoto and Nakase (2000)
<i>Sporidiobolus pararoseus</i> [2]	AF189978	601	Fell et al (2000)	AB021694**	1758	Hamamoto and Nakase (2000)
<i>Sporidiobolus salmonicolor</i> Fell & Tallman	AF070439	604	Fell et al (1998)	AB021697	1781	Hamamoto and Nakase (2000)
<i>Sporobolomyces blumeae</i> M. Takash. & Nakase	AY213010	573	Sampaio et al (2003)	AB030321	1756	Takahima and Nakase (2000)
<i>Sporobolomyces coprosmae</i> Hamam. & Nakase	AF189980	629	Fell et al (2000)	D66880	1757	Hamamoto and Nakase (2000)
<i>Sporobolomyces coprosmicola</i> Hamam. & Nakase [1]						
<i>Sporobolomyces coprosmicola</i> [2]	AF189981	630	Fell et al (2000)	D66879	1749	Hamamoto and Nakase (2000)
<i>Sporobolomyces dracophylli</i> Hamam. & Nakase	AY512888	632	Begerow et al (2003) unpublished			
	AF189982	630	Fell et al (2000)	D66882	1757	Hamamoto and Nakase (2000)

SUPPLEMENTARY TABLE II. Continued

Species	nuc-lsu rDNA			nuc-ssu rDNA		
	GenBank no.	length (bp)	Reference (cited in Genbank)	GenBank no.	length (bp)	Reference (cited in Genbank)
<i>Sporobolomyces elongatus</i> R.G. Shivas & Rodr. Mir.	AF189983	601	Fell et al (2000)	AB021669	1761	Hamamoto and Nakase (2000)
<i>Sporobolomyces falcatus</i> Nakase, Itoh & M. Suzuki	AF075490	621	Fell et al (1999)	AB021670	1758	Hamamoto and Nakase (2000)
<i>Sporobolomyces foliicola</i> R.G. Shivas & Rodr. Mir.	AF189984	629	Fell et al (2000)	AB021671	1781	Hamamoto and Nakase (2000)
<i>Sporobolomyces gracilis</i> Deryx	AF189985	629	Fell et al (2000)	AB178480	1787	Nagahama et al (2004) unpublished
<i>Sporobolomyces griseoflavus</i> Nakase & M. Suzuki	AF207888	610	Hong et al (2000)	D66884	1758	Hamamoto and Nakase (2000)
<i>Sporobolomyces inositophilus</i> Nakase & M. Suzuki	AF189987	619	Fell et al (2000)	AB021673	1781	Hamamoto and Nakase (2000)
<i>Sporobolomyces kluyveri-nidii</i> Van der Walt	AF189988	629	Fell et al (2000)	AB021674	1753	Hamamoto and Nakase (2000)
<i>Sporobolomyces lactophilus</i> Nakase, Itoh, M. Suzuki & Bandoni	AF177411	621	Sampaio et al (1999b)	AB021675	1668	Hamamoto and Nakase (2000)
<i>Sporobolomyces linderae</i> Nakase, M. Takash. & Hamam. [1]	AF189989	631	Fell et al (2000)			
<i>Sporobolomyces linderae</i> [2]	AF207890	621	Hong et al (2000)	D66885	1753	Hamamoto and Nakase (2000)
<i>Sporobolomyces nylandii</i> M. Takash. & Nakase	AF387123	573	Valerio et al (2002)	AB030319	1756	Takashima and Nakase (2000)
<i>Sporobolomyces onyzicola</i> Nakase & M. Suzuki	AF189990	629	Fell et al (2000)	AB021677	1756	Hamamoto and Nakase (2000)
<i>Sporobolomyces phyllomatus</i> Van der Walt & Y. Yamada	AF189991	629	Fell et al (2000)	AB021685	1761	Hamamoto and Nakase (2000)
<i>Sporobolomyces poonsookiae</i> M. Takash. & Nakase	AF387124	570	Valerio et al (2002)	AB030320	1757	Takashima and Nakase (2000)
<i>Sporobolomyces ruber</i> (Nakase, G. Okada & Sugiy.) Boekhout	AF189992	626	Fell et al (2000)	AB021686	2072	Hamamoto and Nakase (2000)
<i>Sporobolomyces salicinus</i> (B.N. Johri & Bandoni) Nakase & S. Ito	AF189995	625	Fell et al (2000)	AB021687	1760	Hamamoto and Nakase (2000)
<i>Sporobolomyces sasicola</i> Nakase & M. Suzuki	AF207889	613	Hong et al (2000)	AB021688	2088	Hamamoto and Nakase (2000)
<i>Sporobolomyces singularis</i> Phaff & Carmo Souza	AF207885	609	Hong et al (2000)	AB021690**	1763	Hamamoto and Nakase (2000)
<i>Sporobolomyces subbrunneus</i> Nakase & M. Suzuki*	AY745717	1431	Matheny et al (2004) unpublished	AB021691	1744	Hamamoto and Nakase (2000)
<i>Sporobolomyces taupoensis</i> Hamam. & Nakase	AF177413	623	Sampaio et al (1999b)	D66886	2044	Hamamoto and Nakase (2000)
<i>Sporobolomyces tsugae</i> (Phaff & Carmo Souza) Nakase & Itoh	AF189998	621	Fell et al (2000)	AB021692	1760	Hamamoto and Nakase (2000)
<i>Sporobolomyces xanthus</i> (Nakase, G. Okada & Sugiy.) Boekhout	AF177414	616	Sampaio et al (1999b)	D64118	1730	Suh et al (1996b)
<i>Sporobolomyces yunnanensis</i> F.Y. Bai, M. Takash., Hamam. & Nakase	AY335162	601	Bai et al (2004)	AF229176	1777	Bai et al (2001)
<i>Stenignatomyces etviae</i> Sonck & Yarr [1]	AF189999	632	Fell et al (2000)			
<i>Stenignatomyces etviae</i> [2]	AF190000	632	Fell et al (2000)	AY373390**	1047	McLaughlin et al (2004)

SUPPLEMENTARY TABLE II. Continued

Species	nuc-1su rDNA			nuc-ssu rDNA		
	GenBank no.	length (bp)	Reference (cited in Genbank)	GenBank no.	length (bp)	Reference (cited in Genbank)
<i>Sterigmatomyces halophilus</i> Fell*	AY745716	1400	Matheny et al (2004) unpublished	D64119	1749	Suh et al (1996b)
<i>Stilbum vulgare</i> Tode				AY373387	1799	McLaughlin et al (2004)
<i>Thanatophyllum crocorum</i> (Pers.) Nees	AY885168 <sup>f</sup>	917	Matheny et al (2005) unpublished	D85648**	1742	Kunimaga (1996) unpublished
<i>Triphragmium ulmariae</i> (DC.) Link	AF426219	512	Maier et al (2003)	AY125401	1698	Wingfield et al (2004)
<i>Tuberculina maxima</i> Rostr. [1]	AY222044	1119	Lutz et al (2004b)			
<i>Tuberculina maxima</i> [2]	AY292408	1108	Lutz et al (2004a)			
<i>Tuberculina sbrozzi</i> Cavara & Sacc. [1]	AY222045	593	Lutz et al (2004b)			
<i>Tuberculina sbrozzi</i> [2]	AY292425	593	Lutz et al (2004a)			
<i>Uromyces appendiculatus</i> (Pers. ex Pers.) Unger*	AY745704	1368	Matheny et al (2005) unpublished	DQ354510	1775	Aime (2006)
<i>Ustilentyloma fluitans</i> (Liro) Vánky	AF009882	579	Begerow et al (1997)	AY124481	2059	Bauer et al (2003)

\* as *Bensingtonia phylladus* Y. Yamada, Nakagawa & Van der Walt.

<sup>b</sup> as *Zymoxenogloea eriophori* D.J. McLaughlin & Doublés.

<sup>c</sup> as *Rhodotorula creatinivora* Golubev.

<sup>d</sup> as *Rhodotorula cassicola* (Sjamsuridzal et al unpublished).

<sup>e</sup> as *Rhodotorula fushanensis* (Nakase et al unpublished).

<sup>f</sup> as *Rhodotorula nymphaeae* (Sjamsuridzal et al unpublished).

<sup>g</sup> as *Rhodotorula samanaeae* (Sjamsuridzal et al unpublished).

<sup>h</sup> 25S is from *Septobasidium* sp.; 18S is from *S. canescens*.

<sup>i</sup> as *Halicobasidium purpureum*.

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SUPPLEMENTARY TABLE III. Resolution and support afforded by different data partitions using Datasets 1 and 3

	Dataset 1 (128 OTUs)				Dataset 3 (22 OTUs)				
	nuc-lsu long (D1-D3)	nuc-ssu complete	nuc-ssu complete + nuc-lsu long	nuc-lsu short + hyper-variable regions	nuc-lsu short (D1-D2)	nuc-lsu long	nuc-ssu complete	nuc-ssu complete + nuc-lsu short	nuc-ssu complete + nuc-lsu long
Aligned length	2069	3736	5805	993	840	2069	3736	4576	5805
Included positions	1357	1843	3200	700	557	1357	1843	2400	3200
Variable positions	699	1007	1706	368	271	521	567	838	1088
Informative positions	550 (40%)	710 (38%)	1260 (40%)	278 (40%)	197 (35%)	369 (27%)	352 (19%)	549 (23%)	721 (22%)
No. MP trees	1000	1000	36	1	1	3	6	1	1
MP tree length	3799	4136	8215	1186	775	1351	1247	2039	2616
CI	0.324	0.373	0.345	0.515	0.528	0.554	0.621	0.581	0.583
nodes with MP bs >90%	33	44	62	7	4	8	9	11	11
nodes with MP bs >70%	58	65	75	12	12	13	10	14	15
nodes with NJ bs >90%	27	50	68	7	5	10	10	10	10
nodes with NJ bs >70%	44	69	84	13	12	16	14	17	17

SUPPLEMENTARY TABLE IV. Support for major groups of Pucciniomycotina in this and other studies. Key to support values: bs = bootstrap %; MP = maximum parsimony; NJ = neighbor joining; ML = maximum likelihood; ME = minimum evolution; Bpp = Bayesian posterior probability; - = group is not resolved as monophyletic in optimal or consensus tree(s); NA = not applicable because group was not sampled or is monotypic. If the delimitation of the group in the cited work conflicts with that in the present study, the support values in this table correspond to the group as delimited here, except as noted.

	This study Dataset 1				This study Dataset 2				Other studies			
	No. OTUs	MPbs	NJbs	Bpp	No. OTUs	MPbs	Study	Data	No. OTUs	Support <sup>1</sup>		
<b>Pucciniomycotina</b>	109	100	100	1.00	189	83	Bauer et al (2006) Bauer et al (2006) Weiß et al (2004)	lsu lsu + ssu lsu	87 25 42	- NJbs 100 NJbs - Bpp - NJbs 100 MPbs 100 NJbs		
							Swann et al (1999)	ssu	25	100 MPbs		
							Lutzoni et al (2004)	lsu + ssu	15	1.00 Bpp 100 NJbs		
							Berres et al (1995)	lsu	9	37 MPbs 65 MLbs - MEbs		
<b>Pucciniomycetes</b>	19	100	100	1.00	41	86	McLaughlin et al (1995, fig 2) Bauer et al (2006) Bauer et al (2006) Weiß et al (2004)	morph lsu lsu + ssu lsu	7 16 3 12	- MP 99 NJbs 100 NJ 1.00 Bpp 92 NJbs		
							Sampaio (2004, fig. 1) Swann et al (1999)	lsu ssu	12 4	1.00 Bpp 100 MPbs 100 NJbs		
							Lutzoni et al (2004)	lsu + ssu	11	1.00 Bpp 100 NJbs		
<b>Septobasidiales</b>	1	NA	NA	NA	4	49						
<b>Helicobasidiales</b>	2	96	98	1.00	10	87						
<b>Platyglouales</b>	4	100	100	1.00	8	99	Weiß et al (2004)	lsu	3	1.00 Bpp 99 NJbs		
<b>Pucciniales</b>	12	100	100	1.00	16	88	Weiß et al (2004)	lsu	6	1.00 Bpp 99 NJbs		
<b>Pachnocybales</b>	0	NA	NA	NA	1	NA	Wingfield et al (2004)	ssu	72	<50 MPbs		

SUPPLEMENTARY TABLE IV. Continued

	This study Dataset 1				This study Dataset 2			Other studies			
	No. OTUs	MPbs	NJbs	Bpp	No. OTUs	MPbs	Study	Data	No. OTUs	Support <sup>1</sup>	
<b>Cystobasidiomycetes</b>	27	100	96	1.00	33	90	Bauer et al (2006) Bauer et al (2006) Weiß et al (2004)	lsu lsu + ssu lsu	10 2 5	90 NJbs 100 NJ 0.74 Bpp – NJbs	
Cystobasidiales	12	100	100	1.00	12	98	Sampaio (2004, fig. 1) Sampaio (2004, fig. 2) Fell et al (2001) Weiß et al (2004)	lsu lsu lsu lsu	11 26 19 2	0.92 Bpp 0.98 Bpp 98 MPbs 0.96 Bpp	
Erythrobasidiales	14	83	91	1.00	15	43	Sampaio (2004, fig. 2) Fell et al (2001) Sampaio (2004, fig. 2) Fell et al (2001)	lsu lsu lsu lsu	8 5 18 13	1.00 Bpp 100 MPbs 1.00 Bpp 69 MPbs	
Naohideales	1	NA	NA	NA	2	98	Bauer et al (2006)	lsu	15	– NJbs	
<b>Agaricostilbomycetes</b>	25	–	–	1.00	41	–	Bauer et al (2006)	lsu + ssu	4	89 NJ	
Agaricostilbales	22	100	100	1.00	34	98	Weiß et al (2004)	lsu	3	0.94 Bpp <50 NJbs	
Spicutigloceales	3	100	100	1.00	7	74	Sampaio (2004, fig. 1) Sampaio (2004, fig. 2) Swann et al (1999) Fell et al (2001) Bauer et al (2006) Bauer et al (2006) Weiß et al (2004)	lsu lsu ssu lsu lsu lsu + ssu lsu	7 23 3 24 3 2 2	1.00 Bpp 1.00 Bpp 100 MPbs 100 NJbs 64 MPbs – NJbs 90 NJbps 1.00 Bpp	
							Sampaio (2004, fig. 2) Fell et al (2001)	lsu lsu	3 3	1.00 Bpp 100 MPbs	

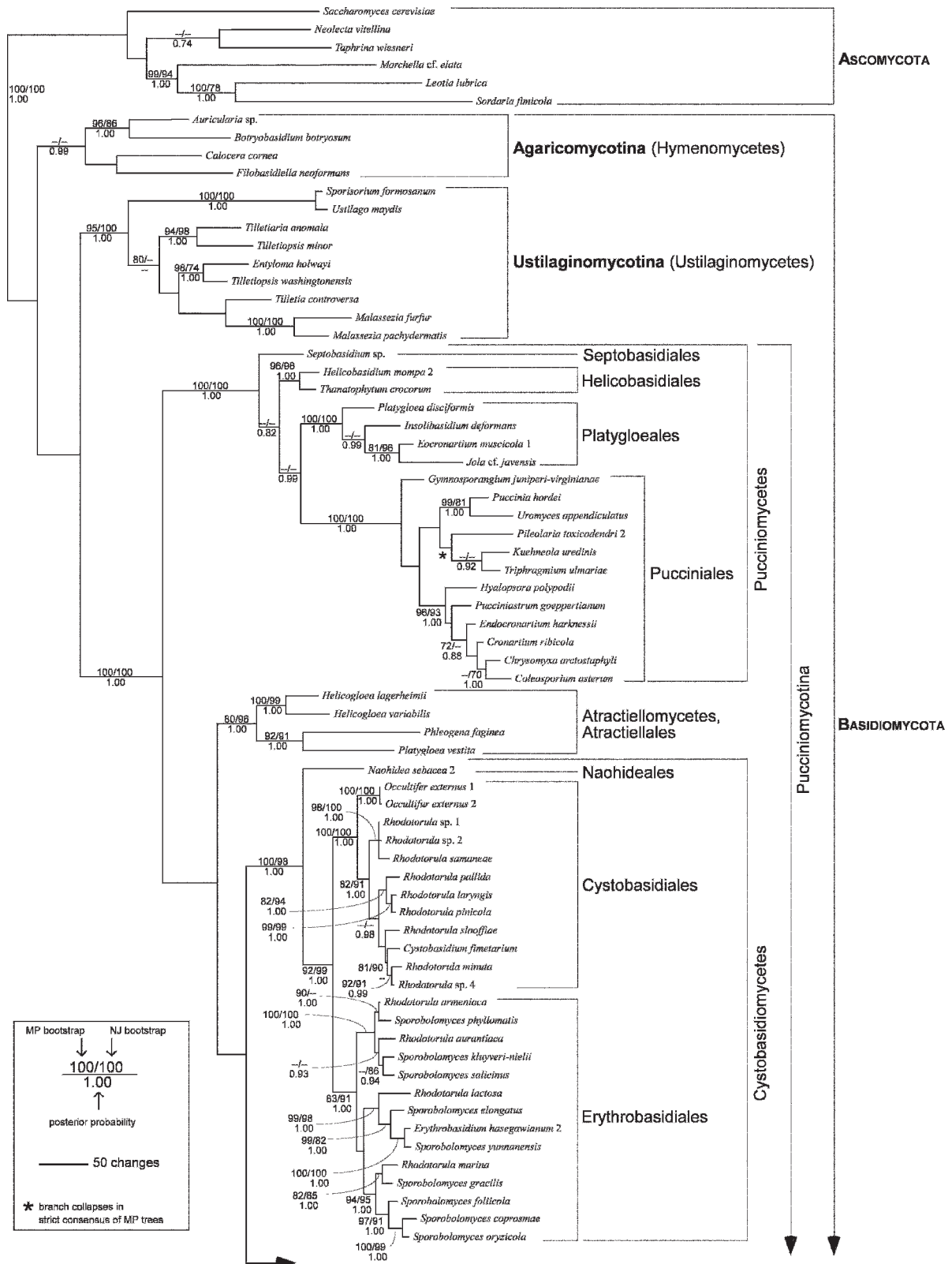
SUPPLEMENTARY TABLE IV. Continued

	This study Dataset 1				This study Dataset 2				Other studies			
	No. OTUs	MPbs	NJbs	Bpp	No. OTUs	MPbs	Study	Data	No. OTUs	Support <sup>1</sup>		
<b>Microbotryomycetes</b>	31	100	100	1.00	60	74	Bauer et al (2006)	lsu	32	85 NJbs		
							Bauer et al (2006)	lsu + ssu	7	100 NJbs		
							Weiß et al (2004)	lsu	13	0.83 Bpp		
							Sampaio (2004, fig. 1)	lsu	10 <sup>a</sup>	0.66 Bpp		
							Sampaio (2004, fig. 2)	lsu	49 <sup>a</sup>	0.87 Bpp		
							Swann et al (1999)	ssu	15	100 MPbs		
							Fell et al (2001)	lsu	78	100 NJbs		
Heterogastridiales	1	NA	NA	NA	1	NA	Weiß et al (2004)	lsu	6	- Bpp		
Microbotryales	4	99	94	1.00	12	82	Swann et al (1999)	ssu	2	- NJbs		
							Fell et al (2001)	lsu	4	91 MPbs		
							Weiß et al (2004)	lsu	2	100 NJbs		
Leucosporidiales	3	85	100	0.98	9	67	Sampaio (2004, fig. 2)	lsu	4	81 MPbs		
							Fell et al (2001)	lsu	2	- Bpp		
							Swann et al (1999)	ssu	6	69 NJbs		
Sporidiobolales	13	74	68	1.00	17	69	Fell et al (2001)	lsu	4	1.00 Bpp		
							Fell et al (2001)	lsu	6	<50 MPbs		
							Sampaio (2004, fig. 2)	lsu	20	0.98 Bpp		
							Swann et al (1999)	ssu	6	- MPbs		
							Fell et al (2001)	lsu	22	- NJbs		
							Fell et al (2001)	lsu	29	52 MPbs		
							Bauer et al (2006)	lsu	87	- MPbs		
							Bauer et al (2006)	lsu + ssu	25	- NJbs		
							Weiß et al (2004)	lsu	3	81 NJ		
							Bauer et al (2006)	lsu	2	- Bpp		
							Weiß et al (2004)	lsu	1	- NJbs		
<b>Classiculomycetes</b>	2	100	100	1.00	2	100	Bauer et al (2006)	lsu	2	100 NJbs		
							Bauer et al (2006)	lsu + ssu	1	NA		
							Weiß et al (2004)	lsu	2	1.00 Bpp		
							Bauer et al (2006)	lsu	2	99 NJbs		
<b>Mixiomycetes</b>	1	NA	NA	NA	2	100	Bauer et al (2006)	lsu	2	100 NJbs		
<b>Cryptomycocolacomycetes</b>	0	NA	NA	NA	1	NA	Bauer et al (2006)	lsu	2	100 NJbs		
<i>Reniiforma sbrues</i>	0	NA	NA	NA	1	NA	Bauer et al (2006)	lsu	2	100 NJbs		

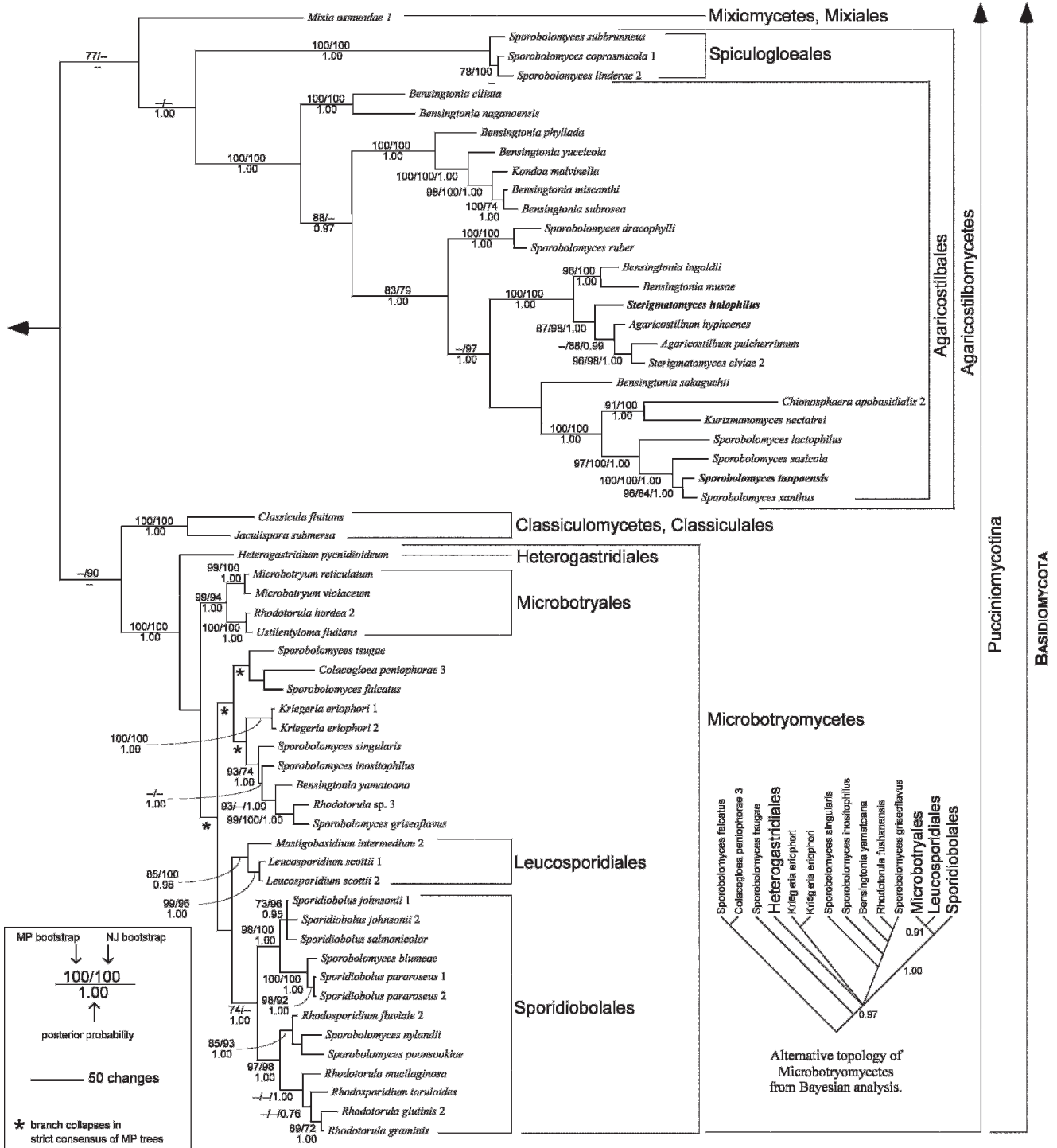
<sup>1</sup> Support ranges result from different analyses with different alignment programs.

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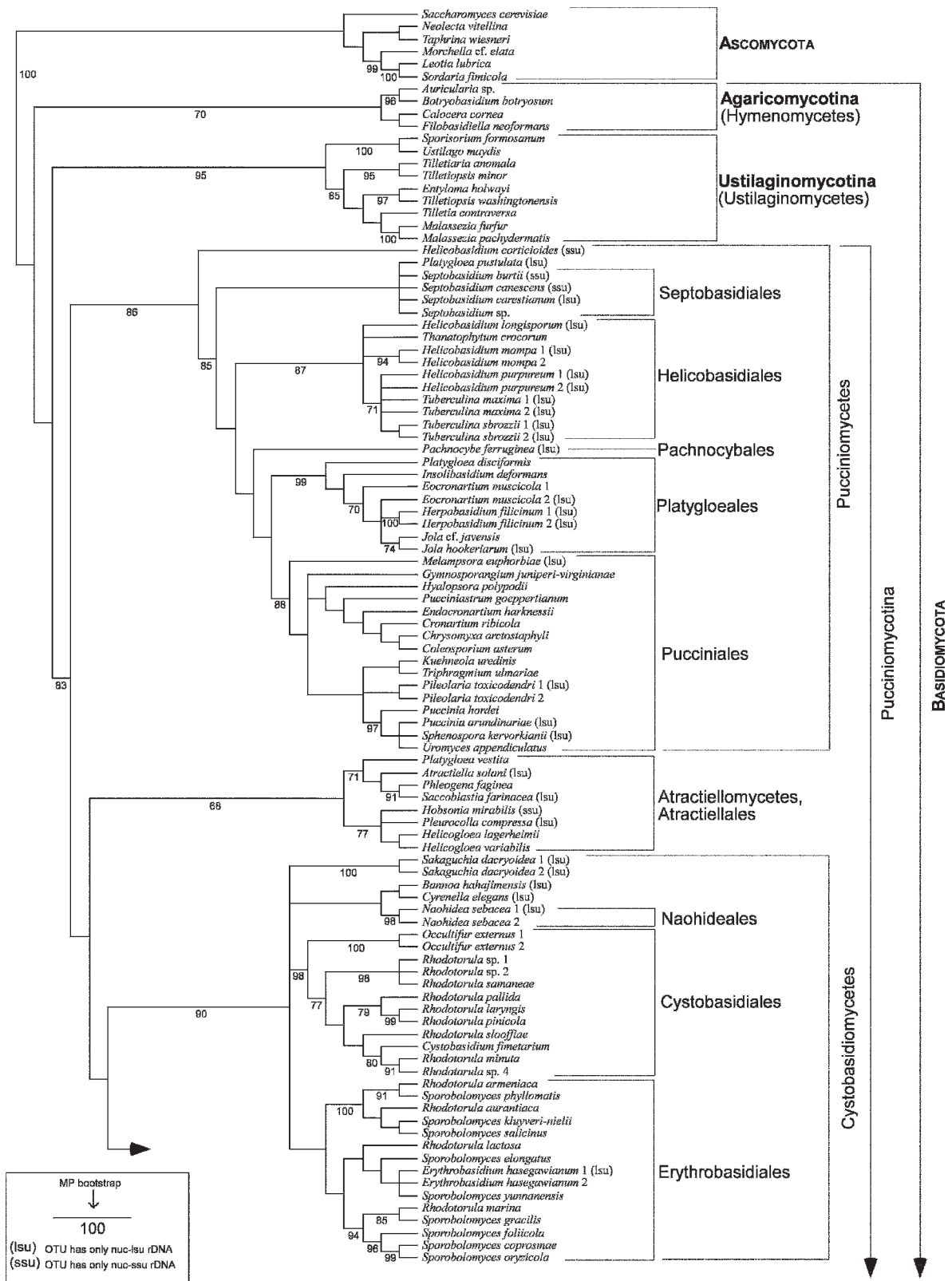


SUPPLEMENTARY FIG. 2A (Expanded). Phylogenetic relationships of Pucciniomycotina based on analyses of Dataset 1 (128 OTUs, each with nuc-18S rDNA and nuc-28S rDNA sequences). Phylogram representing one of 36 equally most parsimonious trees (8215 steps, CI = 0.345, RI = 0.731). Nodes with asterisks collapse in the strict consensus of equally most parsimonious trees. Support values include MP bootstrap frequencies above 70% (first value, before slash), NJ bootstrap frequencies above 70% (second value) and Bayesian posterior probabilities above 0.70. A. Ascomycete outgroups, Ustilaginomycotina, Agaricomycotina, and Pucciniomycotina p.p.

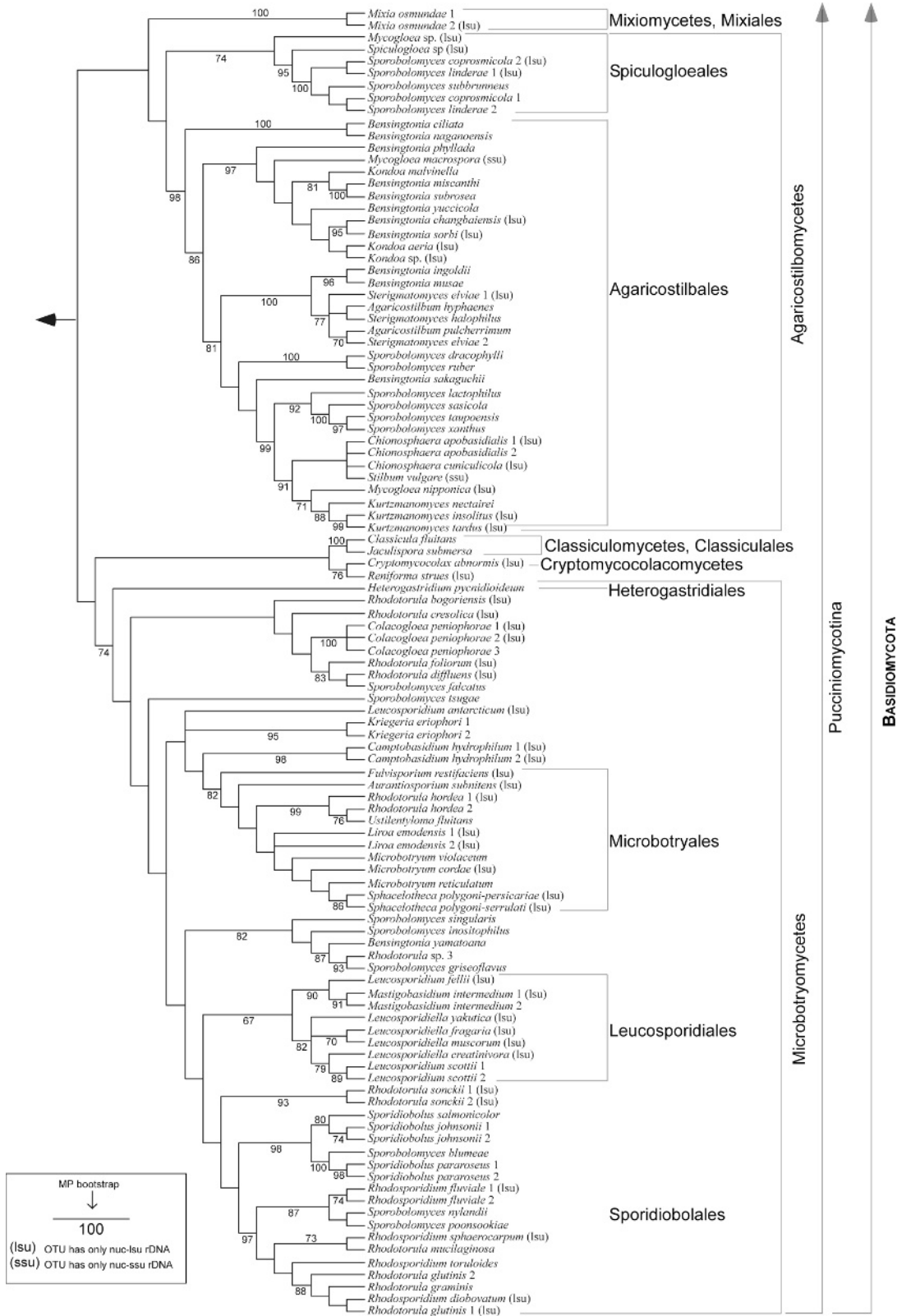


SUPPLEMENTARY FIG. 2B (Expanded). Phylogenetic relationships of Pucciniomycotina based on analyses of Dataset 1 (128 OTUs, each with nuc-lsu rDNA and nuc-ssu rDNA sequences). Phylogram representing one of 36 equally most parsimonious trees (8215 steps, CI = 0.345, RI = 0.731). Nodes with asterisks collapse in the strict consensus of equally most parsimonious trees. Support values include MP bootstrap frequencies above 70% (first value, before slash), NJ bootstrap frequencies above 70% (second value) and Bayesian posterior probabilities above 0.70. B. Pucciniomycotina p.p. The small cladogram depicts the topology of Microbotryomycetidae obtained in Bayesian analyses, with posterior probabilities indicated only for strongly supported nodes that conflict with the MP topology.

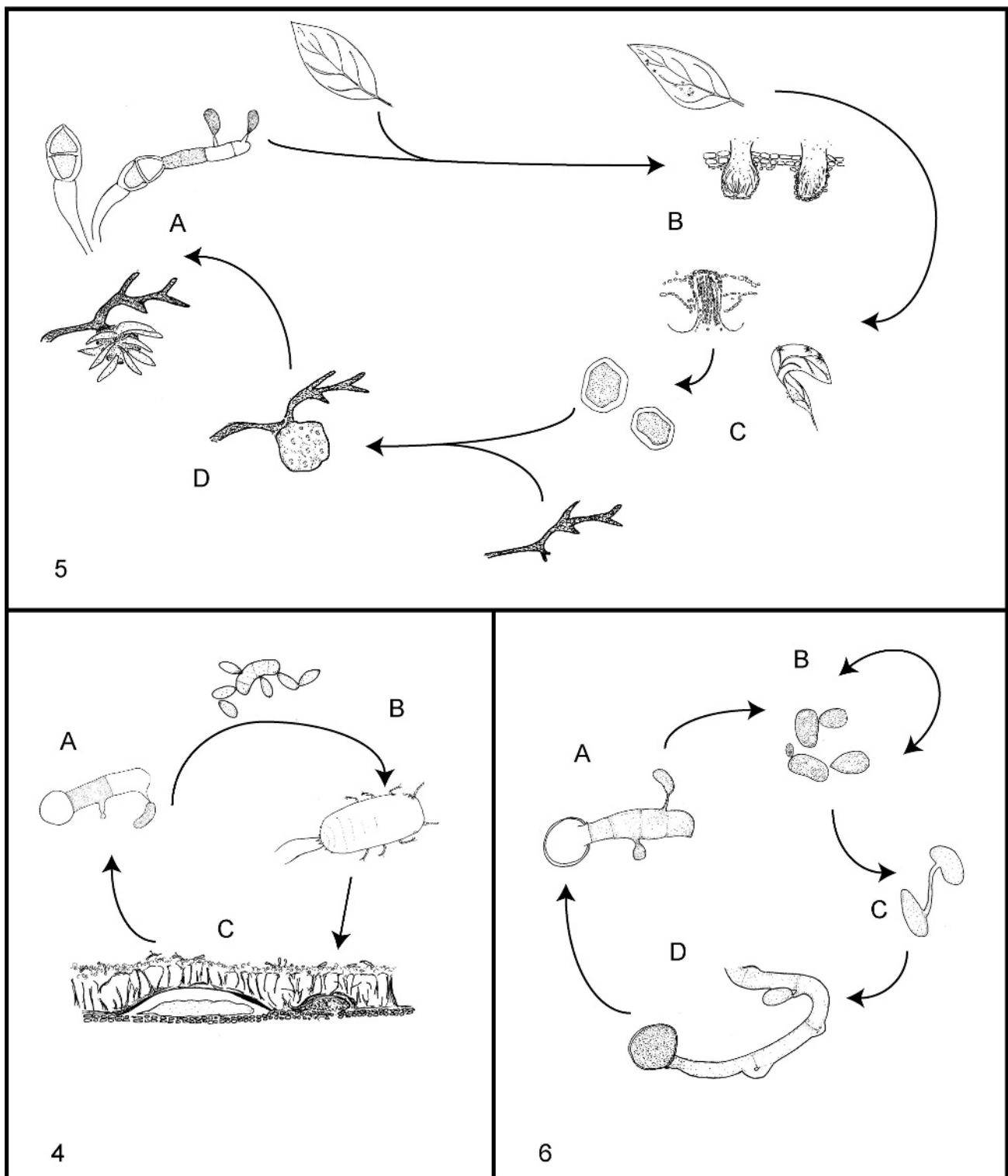




SUPPLEMENTARY FIG. 3A. Phylogenetic relationships in the Pucciniomycotina based on analyses of Dataset 2 (208 OTUs, 128 with nuc-lsu rDNA and nuc-ssu rDNA sequences, 74 with only nuc-lsu rDNA sequences, indicated by “(lsu)” after taxon name, and 6 with only nuc-ssu rDNA sequences, indicated by “(ssu)” after taxon name). Strict consensus of 1000 equally most parsimonious trees (9748 steps, CI=0.31, RI=0.729). MP bootstrap values above 70% are indicated. A. Ascomycete outgroups, Ustilaginomycotina, Agaricomycotina, and Pucciniomycotina p.p.



SUPPLEMENTARY FIG. 3B. Phylogenetic relationships in the Pucciniomycotina based on analyses of Dataset 2 (208 OTUs, 128 with nuc-lsu rDNA and nuc-ssu rDNA sequences, 74 with only nuc-lsu rDNA sequences, indicated by “(lsu)” after taxon name, and 6 with only nuc-ssu rDNA sequences, indicated by “(ssu)” after taxon name). Strict consensus of 1000 equally most parsimonious trees (9748 steps, CI=0.31, RI=0.729). MP bootstrap values above 70% are indicated. B. Pucciniomycotina p.p.



SUPPLEMENTARY FIG. 4. Life cycle of *Septobasidium meredithae* (Septobasidiales). A. Transversely septate basidium gives rise to forcibly discharged spores. B. Spores are subdivided by septa, and each section gives rise to bud cells that also bud to form yeast colonies in culture. C. When spores encounter a young scale insect, the clusters of bud cells form hyphae that penetrate the cuticle of the scale insect and begin to form coiled structures called haustoria. Infected scale insect settles on the host plant and becomes completely invaded by the fungus. The fungus grows out of the insect to form a mycelial mat that covers the host plant and adjacent uninfected scale insects, producing basidia along the upper surface. SUPPLEMENTARY FIG. 5. Life cycle of the rust *Gymnosporangium juniperi-virginianae* (Pucciniales). A. Teliospores germinate to produce basidia and haploid basidiospores that are wind-borne to the leaf of a rosaceous host. B. Spermogonia containing haploid spermatia on the adaxial leaf surface. C. The products from compatible spermatia fuse to form limited dikaryotic hyphae that emerge from the abaxial leaf surface forming spore-filled sori known as aecia. The aecia contain long chains of dikaryotic aeciospores that are wind-borne to the alternate host. D. *Juniperus* spp. are infected with aeciospores, producing galls from which gelatinous columns of telia emerge. Karyogamy occurs within the teliospores. SUPPLEMENTARY FIG. 6. Life cycle of *Rhodosporidium toruloides* (Sporidiobolales). A. A transversely septate basidium gives rise to spores. B. The spores bud and persist as yeasts. C. Yeast cells of the proper mating types fuse via a thin hyphal connection to form a dikaryon. D. The dikaryon forms hyphae that will eventually give rise to teliospores.