Resolving the phylogenetic position of the Wallemiomycetes: an enigmatic major lineage of Basidiomycota

P. Brandon Matheny, Jasmin A. Gossmann, Polona Zalar, T.K. Arun Kumar, and David S. Hibbett

Abstract: The Wallemiomycetes includes three species of molds from the genus *Wallemia*. These fungi are adapted to environments of high osmotic stress, contaminate various foods, cause respiratory disease, and have an unusual mode of asexual reproduction. *Wallemia* was recently proposed as a new class based on 18S ribosomal RNA gene sequences to accommodate the isolated position of the clade in the Basidiomycota. We analyzed the phylogenetic position of the Wallemiomycetes using 3451 nucleotide characters of the 18S, 25S, and 5.8S ribosomal RNA genes and 1282 amino acid positions of *rpb1*, *rpb2*, and *tef1* nuclear protein-coding genes across 91 taxa. Different gene regions and methods of phylogenetic inference produce mildly conflicting placements of the Wallemiomycetes. Parsimony analyses of nrDNA data suggest that the Wallemiomycetes is an early diverging lineage of Basidiomycota, occupying a basal position near the Entorrhizomycetidae. Ultrastructural data, some Bayesian analyses, and amino acid sequences suggest the Wallemiomycetes may be the sister group of the Agaricomycotina or Ustilaginomycotina. The combined gene tree supports the Wallemiomycetes as a lineage basal to a core clade of Pucciniomycotina, Ustilaginomycotina, and Agaricomycotina with robust measures of branch support. This study reinforces the isolated position of *Wallemia* in the Basidiomycota using molecular data from six nuclear genes. In total, five major lineages of Basidiomycota are recognized: the Agaricomycotina, Ustilaginomycotina, Pucciniomycotina, Entorrhizomycetidae, and the Wallemiomycetes.

Key words: Basidiomycota, elongation factor 1-alpha, fungi, molds, RNA polymerase II, systematics.

Résumé : Les Wallemiomycètes incluent trois espèces de moisissures du genre *Wallemia*. Ces champignons sont adaptés à des environnements osmotiques très stressants, contaminent divers aliments, causent des maladies respiratoires, et ont un mode de reproduction sexuelle bien particulier. On a récemment proposé *Wallemia* comme une nouvelle classe, basée sur les séquences génétiques de l'ARN 18S ribosomal, pour accommoder la position isolée de ce clade, chez les Basidiomycota. Les auteurs ont analysé la position phylogénétique des Wallemiomycètes en utilisant 3451 caractères nucléotidiques des gènes ARN ribosomal 18S, 25S, et 5.8S, ainsi que 1282 positions d'acides aminés des gènes codant pour des protéines *rpb1, rpb2* et *tef1,* chez un ensemble de 91 taxons. Différentes régions des gènes et méthodes d'inférence phylogénétique produisent des localisations légèrement conflictuelles pour les Wallemiomycètes. Les analyses en parcimonie des données nrADN suggèrent que les Wallemiomycètes constituent une lignée divergente précoce des Basidiomycota, occupant une position basale près des Entorrhizomycetidae. Les données ultrastructurales, certaines analyses bayésiennes et des séquences d'acides aminés suggèrent que les Wallemiomycètes pourraient être un groupe frère des Agaricomycètes ou des Ustilaginomycotina. L'arbre génétique combiné supporte le taxon des Wallemiomycètes comme une lignée basale du noyau Pucciniomycotina, Ustilaginomycotina et Agaricomycotina, avec des mesures solides supportant les branches. Cette étude consolide la position isolée du genre *Wallemia* chez les Basidiomycota, en utilisant les données moléculaires de six gènes nucléiques. Au total, on reconnaît cinq lignées majeures de Basidiomycota : Agaricomycotina, Pucciniomycotina, Enthorrhizomycetidae et Wallemiomycètes.

Mots clés : Basidiomycota, facteur d'élongation 1-alpha, champignons, moisissures, polymérase II de l'ARN, systématique.

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Introduction

The Basidiomycota is generally regarded as having three major clades (Swann and Taylor 1995; Lutzoni et al. 2004; Taylor et al. 2004; Matheny et al. 2007): the Agaricomycotina (= Hymenomycetes), the Ustilaginomycotina (= Ustilaginomycetes), and the Pucciniomycotina (= Urediniomycetes). A fourth major lineage, however, may have been identified, composed of three species of xerophilic molds from the genus *Wallemia* (Zalar et al. 2005). These microorganisms grow slowly in culture, have been detected worldwide in hypersaline saltern water (Zalar et al. 2005), and are typically involved in contamination of low-moisture foods. *Wallemia sebi*, for example, has been isolated from cake, jam, rice, flour, and dried fish (Wood et al. 1990). This species is also important medically because it has been identified as an allergen, an agent of respiratory disease, and a producer of toxic metabolites (Sakamoto et al.1989; Wood et al. 1990; Lappalainen et al. 1998; Moss 1998; Frank et al. 1999; Reboux et al. 2001; Roussel et al. 2005). For these reasons, rapid detection of *W. sebi* from environmental samples has become a priority (Zeng et al. 2004).

Septal pore ultrastructure data support the placement of the Wallemiaceae R. T. Moore among the basidiomycetes (Moore 1986, 1996), although sexual states (i.e., production of basidia) have not been observed. *Wallemia* possesses a dolipore type of septum, first reported by Terracina (1974), which is indicative of many members of the Basidiomycota and has been reported in a few Ascomycota (Kreger-van Rig and Veenhuis 1971). However, interpretations of *Wallemia* septal pore morphology have differed (Terracina 1974; Moore 1986), which obscures the evolutionary affinities *Wallemia* might share with other lineages of fungi. Terracina (1974) observed the lack of a parenthosome or pore cap, a curved double membrane about both sides of the septal pore swelling, the morphology of which has been used to segregate groups of Basidiomycota (Wells 1994; Moore 1996; Bauer et al. 1997; Fell et al. 2001; Weiss et al. 2004). Moore (1986), however, reported a parenthosome in *W. sebi* and described it as single layered and vesiculate. Debate has also ensued over the karyology of conidiogenesis, a process by which asexual spores are produced. Studies on conidium ontogeny (Hashmi and Morgan-Jones 1973; Madelin and Dorabjee 1974) suggest that *Wallemia* is a mitosporic group of molds, but Moore (1986) has speculated that the mode of reproduction in *W. sebi* could entail meiosis.

Phylogenetic analysis of the 18S rRNA gene supported an isolated position of the Wallemiaceae within the Basidiomycota (Zalar et al. 2005). As a result, a clade of three *Wallemia* species was proposed as a new class and new order, the Wallemiomycetes and Wallemiales, respectively. The taxon sampling strategy of this study, however, was limited to 14 exemplars of the Ustilaginomycotina and 22 exemplars of the Agaricomycotina (Zalar et al. 2005). Here, we investigated the phylogenetic position of the Wallemiomycetes by sampling six nuclear genes for a 91-taxon data set and assembling a nuclear ribosomal RNA data set for 182 taxa to test the hypothesis that the class is the clade sister to an inclusive grouping of the Ustilaginomycotina and Agaricomycotina as was found in Zalar et al. (2005). To evaluate this

Fig. 1. Intron distribution in *rpb1* between domains A and C and *rpb2* domains 5 and 7 for major groups of Basidiomycota. Intron positions are numbered and marked by arrows according to Matheny et al. (2002) and Matheny et al. (2007). Dark gray filled bars represent *rpb1*. Light gray bars represent *rpb2*. The *tef1* intron in *Wallemia* occurs seven amino acid positions downstream of "intron 1" in Matheny et al. (2007). Bars are not drawn to scale.

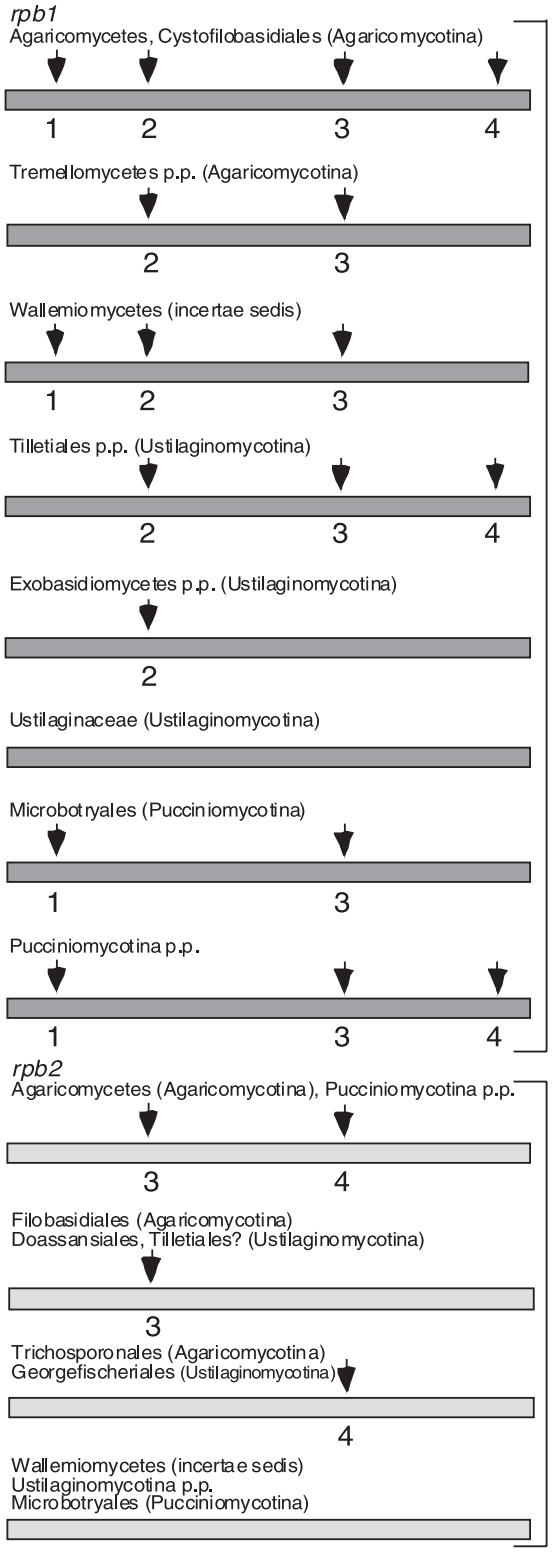


Fig. 2. The phylogeny of the Wallemiomycetes assessed by separate rDNA and protein-coding gene summary cladograms. The Entorrhizomycetidae is excluded. Cladograms were produced from equally weighted MP bootstrap analyses. The number of taxa, number of included characters, and parsimony bootstrap values are indicated for each data set. Black filled circles indicate BP support equal to or greater than 70%; gray filled circles indicate BP support between 69% and 50%. (A) Nuclear rDNA cladogram. The Wallemiomycetes is positioned outside the "core" Basidiomycota, which contains the subphyla Pucciniomycotina, Ustilaginomycotina, and the Agaricomycotina . (B) Combined protein cladogram of 1289 amino acid characters. Note the conflicting position of the Wallemiomycetes within the "core" Basidiomycota. (C) The *rpb2* summary bootstrap cladogram. The Wallemiomycetes receives moderate support near the Tremellomycetes and Agaricomycetes. (D) The *rpb1* summary bootstrap cladogram. (E) The *tef1* summary bootstrap cladogram. The names Agaricomycetes, Dacrymycetes, and Tremellomycetes are provisional.

hypothesis, we used a more inclusive taxon sampling approach to include representatives of the Pucciniomycotina, rusts and allies, and the Entorrhizomycetidae, a small group of fungi that produce sori (clusters of spores) as galls in root nodules of Juncaceae and Cyperaceae (Vánky 2002). The Entorrhizomycetidae has previously been placed among the Ustilaginomycotina (Bauer et al. 2001).

Materials and methods

Taxon sampling

All taxa and sequences used in this study are provided in the supplementary data (Table S1).[2] *Wallemia* strains are maintained in the Culture Collection of Extremophilic Fungi (EXF), Biotechnical Faculty, Department of Biology, SI-1000 Ljubljana, Slovenia. Most sequences of the Ustilaginomycotina and some Pucciniomycotina have been produced for a separate study that is currently in preparation (J.A. Gossmann, P.B. Matheny, and O.S. Hibbett, unpublished data). Ninety-one taxa were assembled for a six gene region analysis. Three species of *Wallemia*, *W. sebi*, *W. muriae*, and *W. ichthyophaga* (type), represent all known taxa of the Wallemiomycetes (Zalar et al. 2005) and are included here. The data set also includes 39 Agaricomycotina, 25 Ustilaginomycotina, 17 Pucciniomycotina, 5 Ascomycota, and 2 outgroups of the Glomeromycota. This subphylum classification follows that proposed in Aime et al. (2006), Bauer et al. (2006), and the Assembling the Fungal Tree of Life classification project site at http://www.clarku.edu/faculty/dhibbett/AFTOL/AFTOL.htm. Extensive taxon sampling of 176 Basidiomycota using nuclear ribosomal DNA of the 18S, 25S, and 5.8S ribosomal RNA genes (nrDNA) was also carried out to further evaluate the position of the Wallemiomycetes. This expanded nrDNA data set is represented by 56 Pucciniomycotina, 55 Agaricomycotina, 62 Ustilaginomycotina, and the 3 Wallemiomycetes.

Gene sampling

Three nuclear protein-coding genes are sampled here. The *rpb1* gene encodes the largest subunit of RNA polymerase II (Stiller and Hall 1997, 1998). The highly variable region between conserved domains A and C of *rpb1* (Matheny et al. 2002) was sequenced for this study. The *rpb2* gene encodes the second largest subunit of RNA polymerase II (Liu et al. 1999). Sequences between conserved domains 5 and 11 were analyzed, which include the highly variable region between domains 6 and 7. For the Wallemiomycetes, we were only able to obtain sequences between domains 5 and 7 (approx. 1100 bp). The *tef1* gene encodes the transcription elongation factor 1-alpha, which is necessary for ribosomal protein synthesis (Wendland and Kothe 1997). This gene is composed of a single domain. In total, the combined analysis includes 62 sequences of *rpb1*, 83 sequences of *rpb2*, and 78 sequences of *tef1*. All taxa are represented by at least one protein-coding gene, except for *Entorrhiza*. Eight taxa include nrDNA and *rpb2* sequences only, and three are represented by only nrDNA and *tef1* sequences (Table S1[2]). The *Wallemia* species are represented by all three protein-coding genes.

Only nuclear rDNA data could be generated for *Entorrhiza* aff. *fineranae*, a member of the monogeneric subclass Entorrhizomycetidae (Fineran 1971; Bauer et al. 1997, 2001), which has been classified in the Ustilaginomycotina. Combined analyses were done with and without *Entorrhiza* to determine sensitivity of topologies to missing data.

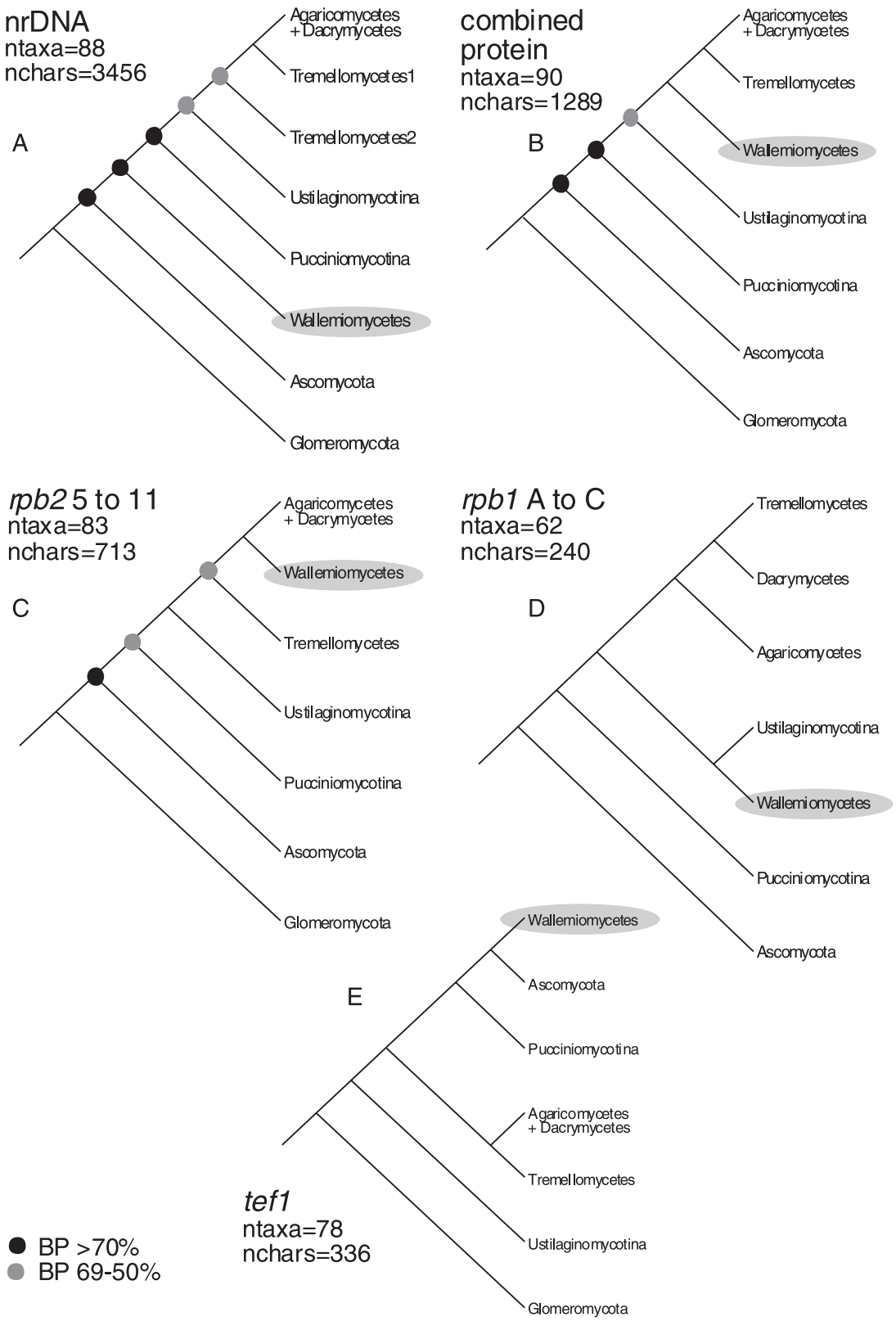
DNA extraction, PCR, and sequencing

Protocols for DNA extraction, PCR, PCR purification, cloning, and sequencing are outlined in Matheny et al. (2007). PCR primers gAf and fCr (Matheny et al. 2002) were used to target a region of *rpb1* between 800 and 1400 bp in size, depending on the presence, absence, and size of spliceosomal introns. Primers for *rpb2* and *tef1* are provided in Matheny et al. (2007). The first 1400 bp of the 25S rRNA gene, approximately 1790 bp of the 18S rRNA gene, and 158 bp of the 5.8S rRNA gene were also sequenced. PCR and sequencing primers used to obtain these regions are listed in Matheny et al. (2007).

Phylogenetic analyses

Sequences of each gene were aligned separately in MacClade 4.0 (Maddison and Maddison 2000). Alignments are available online at the AFTOL database and at TreeBASE (S1602). Group I introns and ambiguously aligned regions were excluded before analysis. Separate gene regions were analyzed under the Maximum Parsimony (MP) criterion in PAUP* (Swofford 2003) with 200 bootstrap replicates to inspect data for strongly supported conflict (>70% bootstrap proportions (BP)) before assembling sequences into supermatrices. MP bootstrapping of combined data sets entailed 1000 replicates with gaps treated as "missing". Starting trees for bootstrapping were obtained via stepwise addition using 20 random addition sequences holding a single tree at each step during stepwise addition. The subtree-pruning-regrafting

[2] Supplementary data for this article are available on the journal Web site (http://canjbot.nrc.ca) or may be purchased from the Depository of Unpublished Data, Document Delivery, CISTI, National Research Council Canada, Building M-55, 1200 Montreal Road, Ottawa, ON K1A 0R6, Canada. DUD 5093. For more information on obtaining material refer to http://cisti-icist.nrc-cnrc.gc.ca/irm/unpub_e.shtml.



(SPR) branch-swapping algorithm was used with the steepest descent and "MulTrees" options not in effect.

A General-Time-Reversible model was used for Bayesian nucleotide analyses across each nrDNA gene region in combination with rate heterogeneity parameters allowing a proportion of invariable sites with remaining sites modeled according to gamma-distributed rates in the parallel version of MrBayes 3.1.2 (Ronquist and Huelsenbeck 2003; Altekar et al. 2004). Independent runs were conducted in MrBayes for two million to five million generations, sampling trees every 200 to 500 generations, respectively. Amino acid partitions were modeled under a single model of amino acid

Fig. 3. The 50% majority-rule MP bootstrap consensus tree (shown as a phylogram) plus other clades consistent with this tree produced from combined amino acid sequences of *rpb1*, *rpb2*, and *tef1* (1289 included characters). The Wallemiomycetes is the sister group to the Agaricomycotina with a significant posterior probability (PP) but with weak bootstrap support. Note that the Wallemiomycetes is not positioned outside the clade of the three subphyla of Basidiomycota as in Fig. 2A.

evolution estimated by MrBayes. The model also allowed for optimization of both rate heterogeneity parameters. After an assessment of intergene conflict, nucleotide nrDNA regions and amino acid sequences of *rpb1*, *rpb2*, and *tef1* were combined for single phylogenetic analyses. For this treatment, data were partitioned according to nrDNA nucleotides and amino acids and run for two million generations sampling trees every 200 generations. These settings, in combination with partitioned heterogeneity parameters, allowed a GTR model to be applied to the nrDNA nucleotides partition, and an amino acid model to be estimated for the protein-coding partition. The first 10%–20% of sampled trees were discarded after inspection of stationary curves where likelihood scores were plotted against the number of generations in a spreadsheet program.

Results

Distribution of spliceosomal introns in the Wallemiomycetes

Spliceosomal intron placement tends to be conserved and can be of phylogenetic utility in the Basidiomycota for some clades (Matheny et al. 2007). Four *rpb1* introns tend to be conserved by position between domains A and C in many Agaricomycotina (Fig. 1). *Wallemia ichthyophaga*, *W. sebi*, and *W. muriae* share three of these four intron positions. Only the Wallemiomycetes, Agaricomycotina, and Ustilaginomycotina share *rpb1*-intron 2. The Wallemiomycetes, most Agaricomycotina, and Pucciniomycotina possess *rpb1*-intron 1.

Sequences between domains 5 and 7 indicate *rpb2*-introns 3 and 4 are absent in the Wallemiomycetes (Fig. 1). Absence of both of these introns is similar to most Ustilaginomycotina and a few Pucciniomycotina (Microbotryales), whereas most Agaricomycetes possess both introns. The intron pattern among several Tremellomycetes and Ustilaginomycotina is variable or incompletely known, with either intron 3 or 4 absent.

Gene structure in *tef1* is also unique in *Wallemia*. All three species share the same *tef1* intron position between amino acid motifs IKNMITGTSQ and ADCGIL, seven positions downstream of "intron 1" in Matheny et al. (2007). No other introns are present in the region sequenced. General absence of introns is a feature shared with *tef1* sequences of Ustilaginomycotina, some Tremellomycetes, and Dacrymycetales. The possession of *tef1* "intron 5" appears to characterize the Agaricomycetes (Matheny et al. 2007). This intron does not occur in the Wallemiomycetes, nor is it present in the Dacrymycetales and exemplars of the Tremellomycetes such as *Cryptococcus gastricus* and *Cystofilobasidium capitatum*. Overall, there is no distinctive similarity among intron positions in *Wallemia* and any particular major lineages of Basidiomycota.

Nucleotide polymorphisms in the same individual are low (zero to three polymorphic sites) in sequences of *rpb1* and *rpb2*. Polymorphic sites are inferred by observation of mixed peaks in sequence chromatograms or mismatched base pairs in consensus sequences of multiple clones. However, *W. sebi* (strain EXF483) displays 17 silent polymorphic sites (1.7% of total sequence length) in exon regions of *tef1*. The *tef1* intron is also polymorphic in length in *W. sebi* and *W. muriae* (strain EXF 1054), which are sister taxa. *Wallemia sebi* is also polymorphic at the ITS locus. Zalar et al. (2005) identified two strongly supported, but phenotypically indistinguishable, ITS clades of *W. sebi*.

Separate nrDNA and amino acid phylogenetic analyses

Figure 2 depicts five summary cladograms of relationships among the major lineages of Basidiomycota. A combined 88-taxon nrDNA tree, with *Entorrhiza*, *Cantharellus*, and *Tulasnella* excluded (Fig. 2A), shows a strong bootstrap proportion (88% BP) that anchors the Wallemiomycetes as the group sister to the remaining Basidiomycota, which is monophyletic with 76% BP. This stands in contrast, however, to a Bayesian analysis of these data that put the Wallemiomycetes sister to the Ustilaginomycotina with a significant posterior probability (0.99 PP). When *Entorrhiza* is included, the Bayesian topology shifts to that shown in Fig. 2A with 0.97 PP. *Entorrhiza* subtends the Wallemiomycetes branch with a 1.0 PP. The combined protein-coding tree (Figs. 2B, 3) places the Wallemiomycetes with 50% bootstrap support and 0.99 PP within a clade containing the Agaricomycotina (Agaricomycetes, Dacrymycetes, and Tremellomycetes) and Ustilaginomycotina. Its position as the sister group to the Agaricomycotina is poorly supported. The taxa included in this tree are shown in Fig. 3.

Of the separate protein-coding genes, only *rpb2* produces moderate support for the placement of the Wallemiomycetes (Fig. 2C). Sequences of *rpb2* suggest the monophyly of Wallemiomycetes with the Agaricomycotina with 52% BP. Data from *rpb1* weakly support a relationship between the Wallemiomycetes and the Ustilaginomycotina (Fig. 2D), similar to Bayesian analysis of the nrDNA data. The bootstrap tree of *tef1* sequences (Fig. 2E) is unable to resolve the monophyly of the Basidiomycota and weakly suggests a relationship between the Wallemiomycetes and the Ascomycota. This result appears to be sensitive to taxon sampling and (or) method of phylogenetic inference. Neither is it consistent with Bayesian analyses of *tef1* amino acid and exon sequences (Matheny et al. 2007), nor with MP bootstrap analyses of 183 *tef1* sequences, which place the Wallemiomycetes as the sister group of the Agaricomycotina with weak BP support (results not shown). Nevertheless, there is no strongly supported conflict among the protein-coding genes, and between the combined amino acid tree and nrDNA nucleotide tree.

Combined nrDNA and amino acid phylogenetic analyses

18S, 25S, and 5.8S ribosomal DNA sequences were combined with *rpb1*, *rpb2*, and *tef1* protein-coding regions to

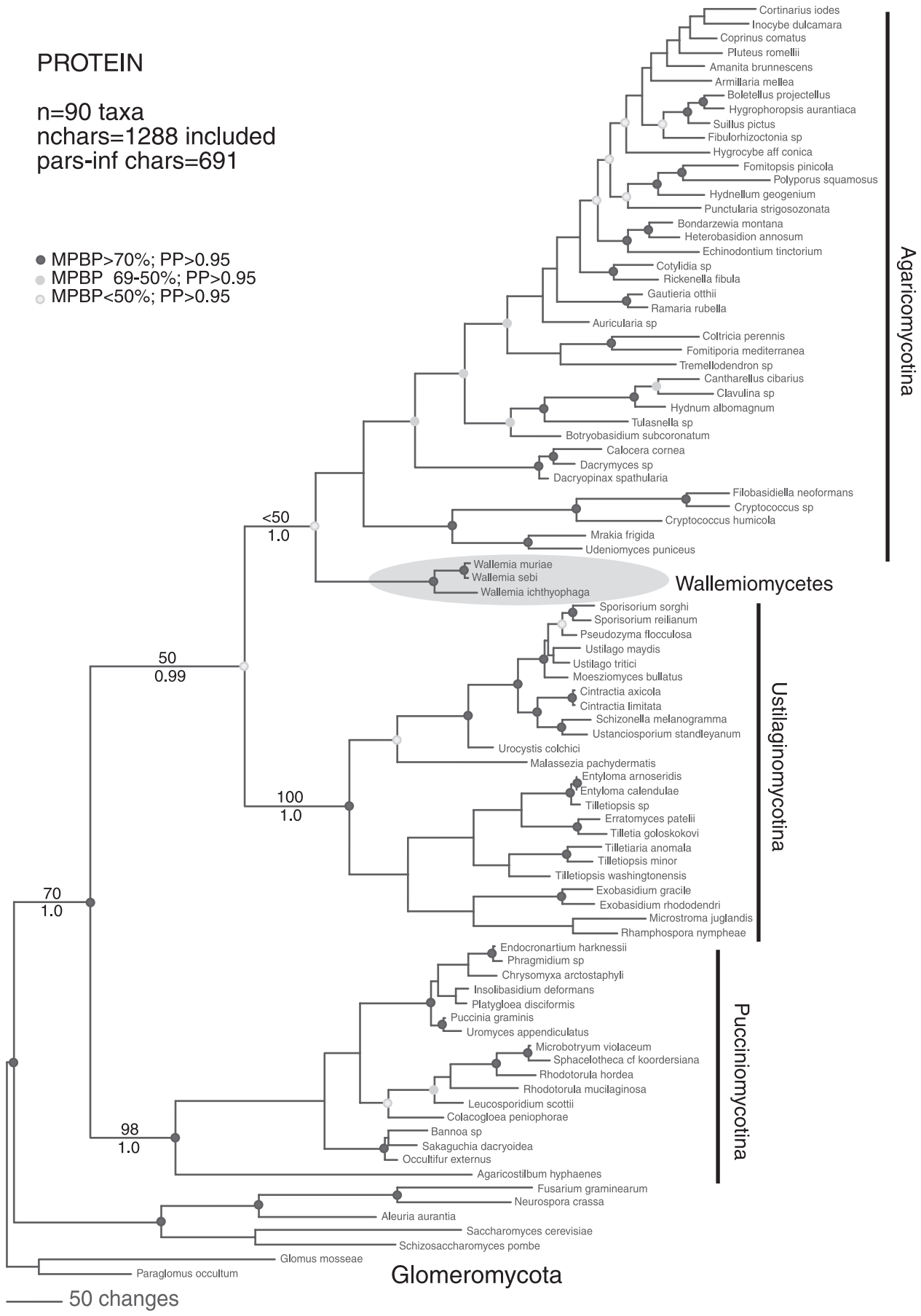


Fig. 4. The 50% majority-rule MP bootstrap consensus tree (shown as a phylogram) plus other clades consistent with this tree produced from combined amino acid sequences of *rpb1*, *rpb2*, and *tef1* and nucleotides of 18S, 25S, and 5.8S rRNA (4745 included characters). The Wallemiomycetes is positioned strongly outside the "core" clade of the three subphyla of Basidiomycota with high bootstrap support and a significant posterior probability. This topology is recovered whether nrDNA sequences of *Entorrhiza* are included or not. Bootstrap values for major clades only are provided above branches for analysis excluding *Entorrhiza* and below branches in a gray box for the analysis including *Entorrhiza*. The position of *Entorrhiza* (Entorrhizomycetidae) is superimposed as a dashed branch not drawn to scale. This tree conflicts with the amino acid tree illustrated in Fig. 3 with a moderate level of support.

produce a supermatrix for parsimony and Bayesian analyses. Ribosomal DNA sequences of *Cantharellus* and *Tulasnella* were excluded because of unusual patterns of sequence substitution found in the nuclear rDNA gene regions of these taxa and their tendency to cluster together in strong conflict with respect to protein-coding and mitochondrial rDNA phylogenies (Moncalvo et al. 2007; Matheny et al. 2007). In sum, this data set contains 4745 included sites, 2813 of which are parsimony-informative.

The MP bootstrap tree (as a phylogram) of the six gene region data set is shown in Fig. 4. Despite moderate conflict between nrDNA nucleotide data and combined amino-acid sequences (Figs. 2A and 2B), this "total evidence" tree mirrors the nrDNA phylogeny (Fig. 2A) with respect to the early position of the Wallemiomycetes with strong measures of branch support. The position of the three *Wallemia* species is consistently resolved outside the "core" Basidiomycota ((Agaricomycotina, Ustilaginomycotina), Pucciniomycotina) whether *Entorrhiza* is included in the analysis or excluded. When included, *Entorrhiza* and *Wallemia* cluster together to represent the first branch of Basidiomycota with low bootstrap support (51%). Exclusion of *tef1* sequences produces the same topology with neglible differences in BP support values.

Phylogenetic analyses of extended nrDNA taxon sampling

182 taxa, including four outgroups from the Ascomycota and two outgroups from the Glomeromycota, were assembled into a single data set composed of 18S, 25S, and 5.8S nrDNA partitions. A total of 6005 characters was included. After exclusion of group I introns and ambiguously aligned regions, 3451 characters were analyzed to determine if the phylogenetic position of the Wallemiomycetes was consistently resolved as in Fig. 2A. Sequences of *Entorrhiza* species were included in this analysis.

A Bayesian topology of the 50% majority-rule consensus tree is shown in Fig. 5. Five major clades of Basidiomycota are inferred. The earliest branch of the Basidiomycota is the Entorrhizomycetidae. Its position outside the "core" Basidiomycota is significantly supported (1.0 PP). To maintain the monophyly of the Ustilaginomycotina, the Entorrhizomycetidae must be excluded. The MP bootstrap tree produces strong support (95%) for the monophyly of the Agaricomycotina, Ustilaginomycotina, and Pucciniomycotina to the exlusion of the Wallemiomycetes and Entorrhizomycetidae (not shown). In contrast, the Bayesian analysis of the same data nests the Wallemiomycetes within the "core" Basidiomycota, where it shares a weakly supported branch (0.73 PP) sister to the Ustilaginomycotina. This topology is similar to Bayesian analysis of the nrDNA 88-taxon data set and the parsimony bootstrap tree of *rpb1* sequences (Fig. 2D), both of which exclude *Entorrhiza*. The Entorrhizomycetidae is continuously supported as the earliest branch of Basidiomycota.

Discussion

Wallemia is a Basidiomycete lineage

Recent synoptic overviews of the Basidiomycota make no remarks about the systematic placement of *Wallemia* (McLaughlin et al. 2001; Weiss et al. 2004; Bauer et al. 2006), a medically important arthrospore-producing mold with no known sexual state and unusual xerophilic physiology. Indeed, current classifications, for example, the online systems Index Fungorum, at http://www.indexfungorum.org, and the National Center for Biotechnology Information (NCBI), at http://www.ncbi.nlm.nih.gov/, currently classify *Wallemia* as an anamorphic ascomycete genus.

Moore (1996) first suggested *Wallemia* was related to the Filobasidiales (e.g., *Filobasidiella neoformans* and allies) in the Basidiomycota by virtue of similar septal pore characters. The septal pore swelling is termed a "dolipore", and its possession appears unique to many Basidiomycota (Kirk et al. 2001). Independent studies have confirmed that the septal pore apparatus in *Wallemia* is characterized by a dolipore (Terracina 1974; Moore 1986). These ultrastructural data, independent of gene sequences, support *Wallemia* as a basidiomycete, as was later confirmed by Zalar et al. (2005) using 18S rDNA sequences. Additional genes (*rpb1*, *rpb2*, 18S, 25S, and 5.8S) unequivocally support *Wallemia* in the Basidiomycota. A sixth gene, *tef1*, does so upon an increase in taxon sampling.

Is *Wallemia* an early-diverging lineage of Basidiomycota?

Analyses of combined data of the 91 taxon supermatrix (with or without *Entorrhiza*) strongly support *Wallemia* as an early-diverging branch of the Basidiomycota, independent of the "core" Basidiomycota, which includes the rusts and allies (Pucciniomycotina), true smuts and allies (Ustilaginomycotina), and the mushroom-forming fungi and allies (Agaricomycotina). This result, however, conflicts moderately with amino acid sequences of the protein-coding phylogeny (Figs. 2B, 3) and Bayesian analyses of the nrDNA data alone (Fig. 5).

To complicate matters, Terracina (1974) observed no parenthosome in *W. sebi*, but did observe a "distinctly banded substructure of the electron-dense regions about the pores", a trait unique to *W. sebi*. In contrast, Moore (1986) observed a vesiculate parenthosome about the dolipore of *W. sebi* and found it to be composed of a single membrane. If the nrDNA parsimony topology of the 88 taxon data set is correct (Fig. 2A), then this would suggest that there have been independent gains or multiple losses of the dolipore in line-

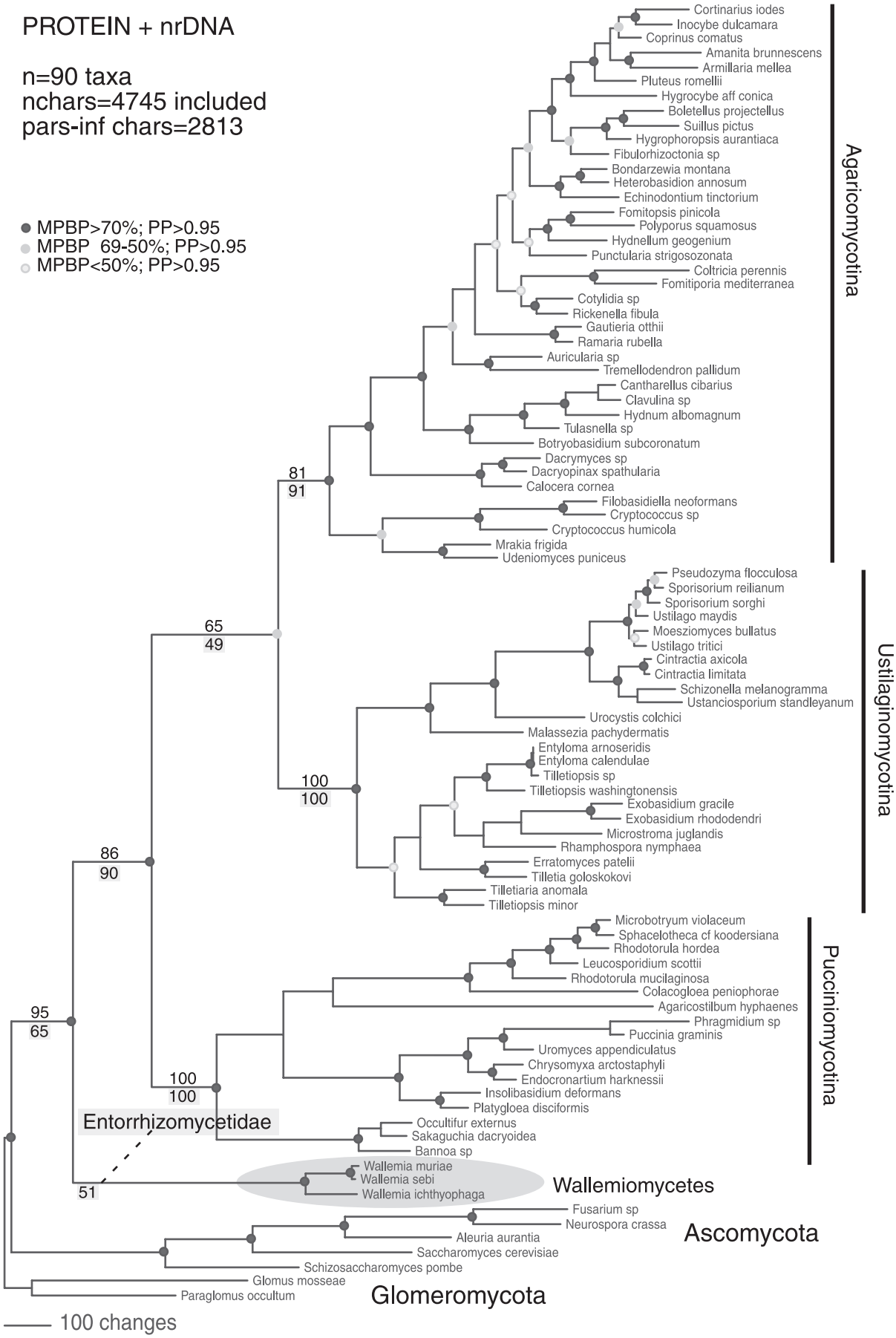


Fig. 5. The nrDNA (18S, 25S, 5.8S) phylogeny of the Basidiomycota based on 182 taxa. The topology is a 50% majority-rule consensus tree of 16 000 trees sampled from a Bayesian analysis. The Wallemiomycetes is resolved among the "core" Basidiomycota with a significant posterior probability (PP) but poorly placed (0.73 PP) as the lineage sister to the Ustilaginomycotina (similar to the *rpb1* cladogram shown in Fig. 2D). The MP analysis of these same data suggest the Wallemiomycetes, together with the Entorrhizomycetidae (61% BP), is the sister group to the "core" Basidiomycota (results not shown), similar to the cladogram in Fig. 2A. In both MP and Bayesian analyses, the Entorrhizomycetidae clusters outside the "core" Basidiomycota. Branches interrupted by slashes indicate they have been shortened to fit the figure.

ages of Basidiomycota. The various interpretations of the parenthosome (absent or present as a vesiculate structure) in *Wallemia* do not help to confirm the placement of the group. Bauer et al. (1997) indicate that the dolipore septum has evolved independently on several occasions, at least once in the Entorrhizomycetidae and again in the Tilletiales. However, if Terracina (1974) is correct (and a parenthosome is absent in *Wallemia*), then the Wallemiomycetes might share the absence of such a structure with the Entorrhizomycetidae, with which it forms a monophyletic group in some analyses (e.g., Fig. 4).

The mode of conidiogenesis in *Wallemia*, whereby arthrospores are produced from a meristematic conidiogenous cell, has been studied in detail (Hashmi and Morgan-Jones 1973; Hill 1974; Madelin and Dorabjee 1974) and it has been suggested that this mode of conidiogenesis is unique to the group (Barron 1968). Multiple investigators have concluded that the reproductive mode is asexual (Vuillemin 1906; Von Arx 1970; Carmichael et al. 1980), and karyological studies support this view (Hashmi and Morgan-Jones 1973). Moore (1986) challenged this idea and instead speculated that the arthrospores might be products of meiosis produced from a "basidiophore" rather than conidiophore. The correct interpretation of this character could also prove pivotal to understand the conflicting molecular phylogenetic positions of the Wallemiomycetes.

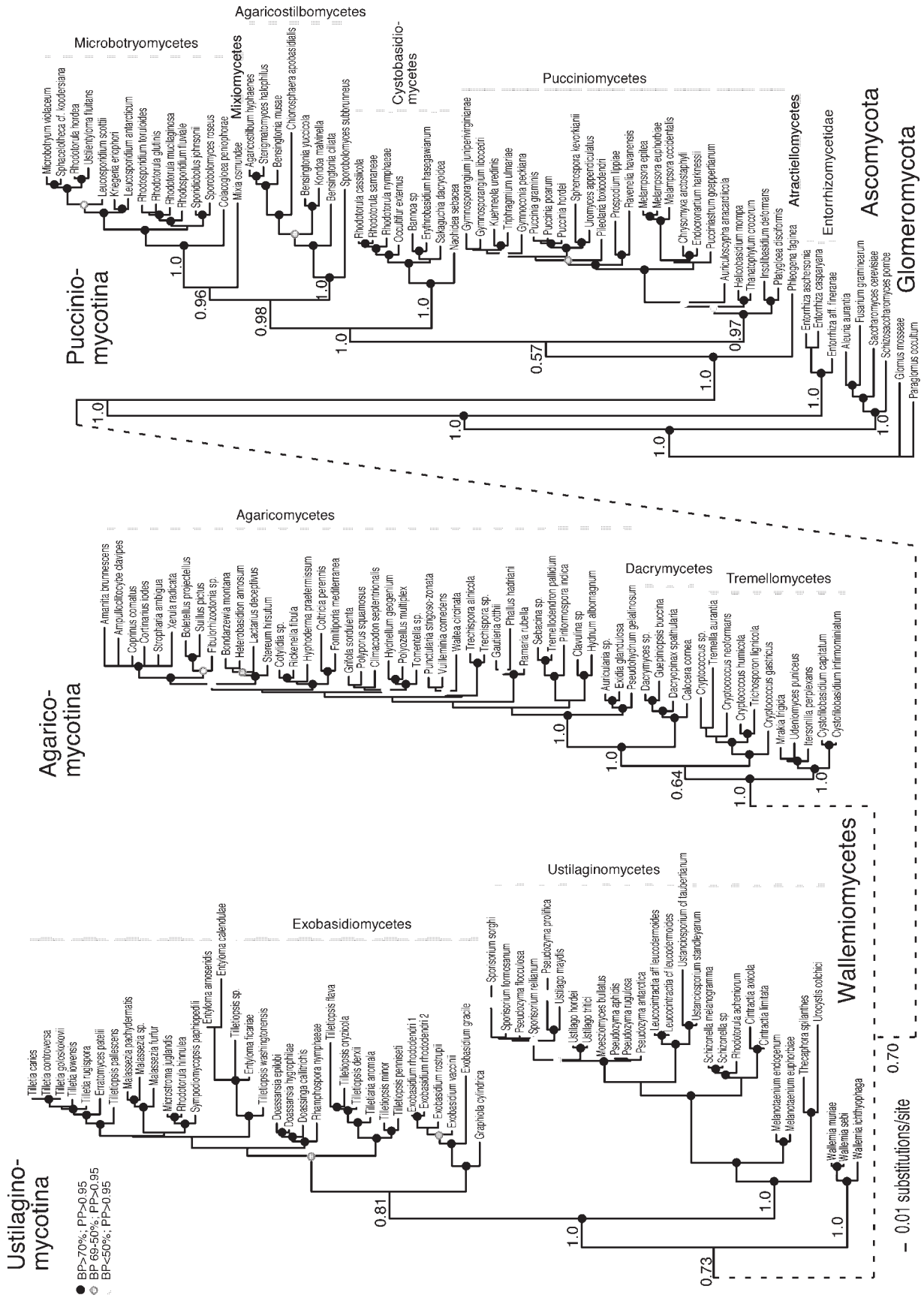
If the combined protein-coding gene phylogeny (Figs. 2B, 3) is correct, then this enables the interpretation of the evolution of the parenthosome type (in this case vesiculate as interpreted in Moore 1986 and Moore 1996) as fairly straightforward. This type of septal pore ultrastructure is similar to that expressed in members of the Tremellales and Filobasidiales (Moore 1978, 1996) and some Ustilaginomycotina (Bauer et al. 1997) and does not appear terribly inconsistent with the Wallemiomycetes branching just before the split of the Tremellomycetes (*Filobasidiella neoformans* and allies) and the remaining Agaricomycotina. The single membrane parenthosome would be unique to the Wallemiomycetes, while the double membrane parenthesome would have evolved either earlier or on independent occasions. In addition, Moore's interpretation that the reproductive mode as a sexual state would suggest another similarity between the Wallemiomycetes and Filobasidiaceae (Moore 1986), but these are not sister taxa and a karyological study refutes this view (Hashmi and Morgan-Jones 1973). An independent assessment of the septal pore apparatus and karyology of reproduction in *Wallemia* is necessary to settle this debate.

Phylogenetic conflict between nuclear ribosomal DNA and nuclear protein-coding genes

Conflict between rDNA and single-copy nuclear protein-coding genes in fungi is infrequent, but has been shown to affect placement of major lineages (rather than distribution of species at the finer tips of phylogenetic trees). Several species-level studies of Basidiomycota that entail multiple gene comparisons (Matheny et al. 2002; Aime and Phillips-Mora 2005; Frøslev et al. 2005; Matheny 2005) depict no, or rare, instances of strongly supported gene conflict. However, independent protein-coding phylogenies have contradicted rDNA higher-level placement of major lineages like microsporidia (Hirt et al. 1999; Keeling et al. 2000), which are now supported in the Fungi by protein-coding genes. In another example, Redecker and Raab (2006) show that rDNA based-analysis supports a grouping of the Ascomycota, Basidiomycota, and Glomeromycota, a clade referred to as the "Symbiomycota" in Tehler et al. (2003). Yet, Redecker and Raab's (2006) analysis of protein-coding genes suggests the placement of the Glomeromycota with other Zygomycota. Phylogenetic analyses of nrDNA sequences of Cantharellaceae and *Tulasnella* in the Cantharellales produce artifactual phylogenies when assessed independently by protein-coding genes and mitochondrial rDNA (Moncalvo et al. 2007; Matheny et al. 2007). Conflicting placements of the Strobilomycetaceae in the Boletales offer yet another example of strongly supported conflict between nrDNA and protein-coding genes (Matheny et al. 2007). Thus, there is a precedent for rDNA being misleading in fungal phylogenetic studies in some cases. Consequently, we are reluctant to draw firm conclusions about the placement of the Entorrhizomycetidae, which is represented only by nrDNA sequences in this study.

In the example documented here, combined amino acid data produce a moderate level of support for the nested placement of the Wallemiomycetes among the "core" Basidiomycota and weak support as the sister group to the Agaricomycotina. This finding appears congruent with ultrastructural data of *Wallemia* as interpreted by Moore (1986, 1996). In conflict with this assessment, parsimony analysis (Fig. 2A) of nuclear rDNA sequences strongly positions the Wallemiomycetes outside the "core" Basidiomycota. Yet, these sequences are not marked by unusual inserts in their nrDNA sequences and are not problematic to align. Intron placement in *Wallemia* is unique overall, but cannot be used to predict a relationship to the other three major lineages of Basidiomycota (Fig. 1).

At least two avenues of research are available to address the lack of a consensus for the phylogenetic placement of the Wallemiomycetes: (1) extend *rpb1* and *rpb2* sequences for *Wallemia* and other taxa in an effort to produce more amino acid characters (and thereby dilute the potentially profound influence of rDNA characters) and (or) sequence additional single-copy protein-coding or mitochondrial rRNA genes; and (2) extend the nrDNA taxon sampling. Option 2 has demonstrated the possibility that, at least under



a Bayesian scenario (Fig. 5), the Wallemiomycetes may indeed be nested within the "core" Basidiomycota as suggested by the protein-coding genes, but this result is not strongly supported. At this time, based on the total molecular data available, the most strongly supported placement of *Wallemia* is as the sister group to the rest of the Basidiomycota, either as an isolated branch or possibly joining a clade with the Entorrhizomycetidae (Fig. 4).

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