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A six-gene phylogenetic overview of *Basidiomycota* and allied phyla with estimated divergence times of higher taxa and a phyloproteomics perspective

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Abstract In this paper, we provide a phylogenetic overview of *Basidiomycota* and related phyla in relation to ten years of DNA based phylogenetic studies since the AFTOL publications in 2007. We selected 529 species to address phylogenetic relationships of higher-level taxa using a maximum-likelihood framework and sequence data from six genes traditionally used in fungal molecular systematics (nrLSU, nrSSU, 5.8S, tef1-α, rpb1 and rpb2). These species represent 18 classes, 62 orders, 183 families, and 392 genera from the phyla *Basidiomycota* (including the newly recognized subphylum *Wallemiomycotina*) and *Entorrhizomycota*, and 13 species representing 13 classes of *Ascomycota* as outgroup taxa. We also conducted a molecular dating analysis based on these six genes for 116 species representing 17 classes and 54 orders of *Basidiomycota* and *Entorrhizomycota*. Finally we performed a phyloproteomics analysis from 109 *Basidiomycota* species and 6 outgroup taxa using amino-acid sequences retrieved from 396 orthologous genes. Recognition of higher taxa follows the criteria in Zhao et al (Fungal Divers 78:239–292, 2016): (i) taxa must be monophyletic and statistically well-supported in molecular dating analyses, (ii) their respective stem ages should be roughly equivalent, and (iii) stem ages of higher taxa must be older than those of lower level taxa. The time-tree indicates that the mean of stem ages of *Basidiomycota* and *Entorrhizomycota* are ca. 530 Ma; subphyla of *Basidiomycota* are 406–490 Ma; most classes are 358–393 Ma for those of *Agaricomycotina* and 245–356 Ma for those of *Pucciniomycotina* and *Ustilaginomycotina*; most orders of those subphyla split 120–290 Ma. Monophyly of most higher-level taxa of *Basidiomycota* are generally supported, especially those taxa introduced in the recent ten years: phylum *Entorrhizomycota*, classes *Malasseziomycetes*, *Moniliellomycetes*, *Spiculogloeomycetes*, *Tritirachiomycetes* and orders *Amylocorticiales*, *Golubeviales*, *Holtermanniales*, *Jaapiales*, *Lepidostromatales*, *Robbauerales*, *Stereopsidales* and *Trichosporonales*. However, the younger divergence times of *Leucosporidiales* (*Microbotryomycetes*) indicate that its order status is not supported, thus we propose combining it under *Microbotryales*. On the other hand, the families *Buckleyzymaceae* and *Sakaguchiaceae* (*Cystobasidiomycetes*) are raised to *Buckleyzymales* and *Sakaguchiales* due to their older divergence times. *Cystofilobasidiales* (*Tremellomycetes*) has an older

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divergence time and should be amended to a higher rank. We however, do not introduce it as new class here for *Cystofilobasidiales*, as DNA sequences from these taxa are not from their respective types and thus await further studies. Divergence times for *Exobasidiomycetes*, *Cantharellales*, *Gomphales* and *Hysterangiales* were obtained based on limited species sequences in molecular dating study. More comprehensive phylogenetic studies on those four taxa are needed in the future because our ML analysis based on wider sampling, shows they are not monophyletic groups. In general, the six-gene phylogenies are in agreement with the phyloproteomics tree except for the placements of *Wallemiomycotina,* orders *Amylocorticiales, Auriculariales, Cantharellales, Geastrales, Sebacinales* and *Trechisporales* from *Agaricomycetes.* These conflicting placements in the six-gene phylogeny vs the phyloproteomics tree are discussed. This leads to future perspectives for assessing gene orthology and problems in deciphering taxon ranks using divergence times.

Keywords Fungi · Systematics · Taxonomy · *Wallemiomycotina*

Introduction

The phylum *Basidiomycota* R.T. Moore (1980) contains 16 classes, 52 orders, 177 families, 1589 genera and more than 30,000 species (Kirk et al. 2008). It is estimated that approximately 32% of the described fungal taxa belong to this phylum (Dai et al. 2015). The *Basidiomycota* includes the well-known mushrooms, bracket fungi, puffballs, toadstools, but also some microfungi, such as rusts, smuts and yeasts. Although there is an extraordinary diversity within this group, molecular studies indicate there are numerous taxa yet to be described (e.g. Kemler et al. 2009; Wannathes et al. 2009; Zhao et al. 2011; Wu et al. 2014; Li et al. 2016; Hyde et al. 2016). Traditionally *Basidiomycota* are typically characterized by the presence of basidia and basidiospores. Recent research shows that some variable morphological characters are an expression of a long evolutionary history within the different level taxa of *Basidiomycota*, such as diversity of cellular constructions in hyphal systems, meiosporangia, the basidiocarps, and ultrastructural characters such as septal pores (Garnica et al. 2007; Van Driel et al. 2009; Yang 2011; Oberwinkler 2012). This indicates that defining higher taxa (e.g. classes, orders) using unique or rather limited morphological characters is subjective.

Basidiomycota species are possibly the main contributors to wood and litter degradation, including degradation of the different components of wood, and they play a key process in carbon recycling (Peláez et al. 1995; Pointing 2001; Pointing et al. 2005; Oberwinkler 2012). *Basidiomycota* are also an important food source for both humans and animals (Zhang et al. 2015). Mushrooms have been a vital source of food for humans mainly because they are rich in carbohydrates (structural polysaccharides) and proteins, including essential amino acids necessary for humans and contain less fat (Zhang et al. 2015). *Basidiomycota* can produce a number of biochemical compounds that can be either beneficial or toxic to humans. Thus, they are used as traditional medicines to cure cancer, diabetes and other ailments (De Silva et al. 2012a, b, 2013), but are also the cause of serious food poisoning (Liu 2004; Dai et al. 2009; Chen et al. 2014). *Basidiomycota* also include plant pathogens, such as rusts and smuts (Sinclair et al. 1987; Wang and Zhuang 1998; Zhuang 2003, 2005, 2012; Dai et al. 2007; Hyde et al. 2014), and also form mutualistic associations with a variety of other organisms, such as ectomycorrhizas with plants (e.g. Bonfante and Genre 2008; Rinaldi et al. 2008), symbioses with liverworts (e.g. Kottke et al. 2003), lichen symbioses (e.g. Lawrey et al. 2007), and fungus-farming (fungiculture) by ants and termites (e.g. Chapela et al. 1994; Mueller et al. 2005).

The phylum *Basidiomycota* was shown to be a monophyletic group sister to *Ascomycota* (James et al. 2006; Hibbett et al. 2007). Currently, *Basidiomycota* includes three major clades—*Agaricomycotina* (mushrooms, jelly fungi, bracket fungi and others), *Pucciniomycotina* (rusts), and *Ustilaginomycotina* (smuts and others) (Hibbett et al. 2007)—as well as *Wallemiomycetes* (Matheny et al. 2007a; Padamsee et al. 2012). A higher-level classification of *Basidiomycota* was published around ten years ago, based mainly on a series phylogenetic studies, such as the Deep Hypha and AFTOL projects (Blackwell et al. 2006; James et al. 2006; Hibbett et al. 2007; Lutzoni et al. 2004). However, there has been considerable advances since this time and the classification needs updating. For example, the class *Entorrhizomycetes* has been split from *Basidiomycota* and raised to phylum *Entorrhizomycota*. New classes (*Malasseziomycetes*, *Moniliellomycetes*, *Spiculogloeomycetes*, *Tritirachiomycetes*), and new orders (*Holtermanniales*, *Trichosporonales*, *Golubeviales*, *Robbauerales*, *Unilacrymales*, *Amylocorticiales*, *Jaapiales*, *Stereopsidales* and *Lepidostromatales*) have been introduced (Binder et al. 2010; Boekhout et al. 2011; Schell et al. 2011; Wuczkowski et al. 2011; Shirouzu et al. 2013; Hodkinson et al. 2014; Sjökvist et al. 2014; Weiss et al. 2014; Wang et al. 2014, 2015c). All of the recently introduced higher-level taxa (hereon refers to orders and above) were studied alongside related taxa. No recent study has combined all high-ranked taxa in a single phylogeny to establish whether they are well-resolved. In this study, we selected 529 taxa across all higher ranks of *Basidiomycota* in standard phylogenetic and molecular dating analyses, as well as phylogenetic reconstruction with hundreds of proteins, to establish if they are well-supported.

Current systematic and taxonomic research generally focuses on organizing and classifying taxa using a combination of characters, including chemical, molecular, morphological, ecological, physiological and biogeographical data (e.g., Hyde et al. 2013; Kuhnert et al. 2015; Maharachchikumbura et al. 2015, 2016). Taxon distinction is based on the recognition of monophyly (e.g. Vilgalys et al. 1994; Taylor et al. 2000), however, the rank of any taxon at any level is subjective and lacks universal criteria (Avise and Johns 1999; Liu et al. 2016). This is apparent both within (e.g. *Ascomycota* and *Basidiomycota*) and outside of the Fungi (e.g. across animals, fungi and plants, Samarakoon et al. 2016). Researchers usually rank monophyletic lineages based on their distinct phylogenetic position, plus a combination of morphological and or other characters, and the level of ranking may thus follow that of their known sister clades.

Researchers have attempted to use other criteria in taxon ranking and a polyphasic approach is generally recommended. This is because the use of morphological, phenotypic or molecular characters may be misleading and differ from group to group. For example, Talavera et al. (2013) have designated an age interval of 4–5 Ma to define genera of blue butterflies using sequence data from nine genes. The arrangement of higher-level taxa has been subjective and has led to many hostile and futile arguments in the past (Liu et al. 2016). The concept of using divergence times as a universal criterion for taxa ranking was first proposed by Hennig (1966). He suggested that a taxonomic rank should reflect its geological age, and divergence time could be used as a universally standardized criterion in the systematics of all known organisms (Hennig 1966). In Hennigs' day this was not possible, but the estimation of organism divergence times has now become feasible, due to advances of molecular biology techniques and analytical methods. The latter can convert molecular change into evolutionary time (Robinson and Robinson 2001; Drummond et al. 2006, 2012). The first attempt that aimed at reconciling taxonomic ranks with divergence time estimates in fish, arthropods and fruit flies is that of Avise and Johns (1999). They found a notable disparity in the ages of lineages and their taxonomic rank. Since it was established, molecular clock analysis have largely been used in biogeographic and phylogeographic studies (Hickerson et al. 2010; Zhao et al. 2013; Stefani et al. 2014), but not in taxonomic ranking.

Several recent reports have established divergence times in the fungi. For example, *Basidiomycota* are estimated to have evolved during the Palaeozoic, around 500 million years ago (Ma), and is a sister group to *Ascomycota* being of similar age (Berbee and Taylor 2010; Oberwinkler 2012; Hibbett 2014). *Agaricomycetes* diverged ca. 290 Ma (Floudas et al. 2012). The ages of some groups within *Agaricomycetes*, such as *Boletales* (Skrede et al. 2011; Wilson et al. 2012), *Agaricales* (Matheny et al. 2009; Ryberg and Matheny 2012), and brown-rot lineages (Garcia-Sandoval et al. 2011) have been also estimated, as were fungal epiphytes (Hongsanan et al. 2016).

The first attempt at establishing a taxonomic system in the Fungi based on divergence time was a reconstruction of a taxonomic system for *Agaricus* (Zhao et al. 2016). These authors produced a multigene phylogeny based on combined ITS, nrLSU, tef1-α, and rpb2 sequence data. Divergence times within the multigene phylogeny were calculated and used to standardize the ranking of taxa into subgenera and sections. The following criteria were used to recognize taxa above species level: (i) they must be monophyletic and statistically well-supported in the molecular dating analyses; (ii) their respective stem ages should be roughly equivalent, and higher taxa stem ages must be older than lower level taxa stem ages; and (iii) they should be identifiable phenotypically, whenever possible. Based on those criteria some subgenera or sections were split or rejected, and new ones were discovered and named.

In the present study, we produce a phylogeny of representative species from almost all classes and orders of *Basidiomycota* and related phyla using six-gene data commonly used in fungal systematics. Divergence times are estimated, and used as a criterion for ranking of higher-level taxa. We also compare this six-gene phylogeny with a tree produced from amino acid data extracted from published genomes (115 taxa and 396 proteins).

Materials and methods

Six-gene sampling

Our dataset comprises nrLSU, nrSSU, 5.8S, tef1-α, rpb1 and rpb2 sequence data from 529 specimens or cultures representing 18 classes, 62 orders, 183 families, 392 genera and 503 species of *Basidiomycota* and *Entorrhizomycota*. In addition, 13 species of *Ascomycota* belonging to 13 different classes, orders, families and genera were chosen as outgroup taxa (Beimforde et al. 2014). Multigene sequence data from 81 specimens are new taxonomic contributions from this study, all of which have been morphologically examined in detail following the methods of Largent (1986a, b). The other sequences were downloaded from GenBank, and their details are listed in Supplementary Table 1.

PCR amplification and sequencing

Genomic DNA was extracted from dried specimens using an E.Z.N.A. Forensic DNA Kit (OMEGA Bio-Tek,

Norcross, GA, USA). We used primers ITS4 and ITS5 (White et al. 1990) for the ITS1-5.8S-ITS2 region (ITS) of the nuclear ribosomal DNA repeat, primers LROR and LR5 (Moncalvo et al. 2000) for the nuclear LSU-rDNA region, primers EF1-983F and EF1-1567R (Morehouse et al. 2003) for translation elongation factor alpha (tef1-α), and primers b6F and b7.1R for RNA polymerase II subunit II (rpb2) (Matheny et al. 2007b) and primers RPB1-Ac RPB1-Cr for rpb1 (Matheny et al. 2002). Genes were amplified by polymerase chain reaction (PCR) using the procedures mentioned in those studies (Moncalvo et al. 2000; Morehouse et al. 2003; Matheny et al. 2007b; Zhao et al. 2011). The PCR products were sent to a commercial biotech company (Baimaide Biotechnique Company, Beijing) for sequencing.

Phylogenetic analysis

The sequence data from each gene was aligned using Muscle 3.6 with default settings (Edgar 2004a, b), then manually adjusted in Mesquite 3.2 (Maddison and Maddison 2016). Regions with ambiguous alignment were excluded, then all sequence data from each gene were combined into a single, concatenated datamatrix. Single-gene phylogenies were constructed to detect possible significant conflicts among the single-gene trees. The final alignment has been submitted to TreeBase (submission No. 20837). Maximum-likelihood (ML) analyses were performed using RAxML v7 (Stamatakis 2006) using a GTR + G+I substitution model. To assess the statistical support of clades we ran 1000 fast-bootstrap (BS) replications under the GTR-CAT approximation.

Divergence-time analysis

We selected 116 taxa for dating analysis based on the phylogenetic results from the six-gene phylogeny and sequence data completeness. The selected taxa represent 17 classes and 54 orders of Basidiomycota and two species of Entorrhizomycota as outgroup taxa (Supplementary Table 1).

We used two fossils for calibration: Archaeomarasmius leggetti Hibbett et al., an agaricoid fruiting body preserved in 90 Ma Dominican amber (Hibbett et al. 1997) as representative of the minimum age of Agaricales; and Quatsinoporites cranhamii S.Y. Sm. et al., a poroid fruiting body from Apple Bay on Vancouver Island from 113 Ma (Smith et al. 2004) as representative of the minimum age of Hymenochaetales. In addition, we used a genomic study that concluded that Agaricomycetes are 290 Ma old (Floudas et al. 2012). Divergence times were estimated in BEAST v1.8.4 (Drummond et al. 2012). We first constructed an XML file with BEAUTI v1.8.4 with a combined six-gene alignment. As substitution models, we used the GTR + G as suggested by jModelTest v2 (Darriba et al. 2012). We used the uncorrelated lognormal relaxed

clock model (Drummond et al. 2006; Lepage et al. 2007), specifying a gamma distribution prior (offset = 0, scale = 0.001, shape = 1) on the ulcd.mean parameter of each gene partition. For calibration, we specified a gamma distribution prior (scale = 20, shape = 1) on the Agaricales (offset = 90 Ma), and Hymenochaetales (offset = 113 Ma) clades (Sánchez-Ramírez et al. 2015; Zhao et al. 2016). In addition, we constrained the Agaricomycetes to be 230 Ma (mean) by applying a normal distribution prior (SD = 1), based on Floudas et al. (2012). We ran two independent Monte Carlo Markov Chains of 100 million generations, logging states every 10,000 generations. Log files were checked for convergence and mixing in Tracer v1.6 (Rambaut et al. 2013; http://tree.bio.ed.ac.uk/software/tracer/). An ultrametric maximum-clade-credibility (MCC) tree was summarized using TreeAnnotator 1.8.4, discarding 10% of states as burn-in and annotating clades with ≥ 0.8 posterior probability.

Phyloproteomics analysis

One-hundred and fifteen published Basidiomycota and outgroup proteomes were downloaded from JGI and Ensembl (Supplementary Table 2). All-vs-all comparisons were conducted using Diamond BLAST with maximum sensitivity (Buchfink et al. 2015). In-paralogs were identified using a Python script based on the assumption that these should be a better match to another than to any sequence from any other species, and all clusters of in-paralogs were reduced to a single representative sequence. BLAST output was pruned to remove instances of in-paralogs and used for MCL-based identification of orthologs with an inflation parameter of 3.0 (van Dongen 2000). Single-copy orthologs were selected with a minimum taxa occupancy of 70, aligned using Muscle (Edgar 2004a, b), and trimmed using trimAl with the "strict" heuristic option (Capella-Gutiérrez et al. 2009). 396 alignments with trimmed lengths between 100 and 600 sites were selected and used to generate a partitioned super-matrix with 82,536 sites. Maximum likelihood phylogenetic analysis was conducted using ExaML (Kozlov et al. 2015) with 100 bootstraps, gamma rate distribution, and evolutionary models for all partitions determined by maximum likelihood during the analysis.

Results and discussion

Six-gene phylogeny and general dating analysis

The combined nrLSU, nrSSU, 5.8S, tef1-α, rpb1 and rpb2 dataset comprised 529 species with 3957 bps, after excluding the ambiguous regions. The best scoring RAxML trees with all species and BS support are shown in

Fig. 1. (Fig. 1a, b). Figure 1a is a concatenated tree from Fig. 1b and highlights the relationships among classes). The MCC tree is shown in Fig. 2, which is based on 116 taxa with 95% highest posterior density (HPD) of divergence time estimates and PP values at the nodes.

The phylum *Basidiomycota* is monophyletic with 1.0 PP support in the MCC tree (Fig. 2) and 75% BS support in the ML tree (Fig. 1). *Basidiomycota* have a stem age of 530 Ma. In this tree, *Basidiomycota* is composed of four main clades which correspond to *Agaricomycotina*, *Pucciniomycotina*, *Ustilaginomycotina* and *Wallemiomycotina* with the divergence time period of ca. 406–490 Ma. *Entorrhizomycota* is sister to *Basidiomycota* at the divergence time of 530 Ma.

The four subphyla of *Basidiomycota*, *Pucciniomycotina*, *Ustilaginomycotina* and *Wallemiomycotina* are well-supported in both trees, however, *Agaricomycotina* has 55% BS support in the ML tree (Fig. 1), but 1 PP support in the MCC tree (Fig. 2). *Agaricomycotina* comprises three classes: *Agaricomycetes*, *Dacrymycetes* and *Tremellomycetes*. These subphyla were estimated to have diverged 406 to 430 Ma ago. Most classes of *Basidiomycota* have 99% or 100% BS support, with the exceptions of *Agaricostilbomycetes* with 61% BS support, while *Exobasidiomycetes* is polyphyletic in the ML tree (Fig. 1).

Phyloproteomics analysis
Results of our phyloproteomics analysis are depicted in Fig. 3. Overall, there is a high level of congruence with the six-gene phylogeny (Figs. 1, 2). One striking exception, however, is the placement of *Wallemiomycotina* (represented by its holotype *Wallemia*), which is sister group to *Agaricomycotina* in the phyloproteomics tree (Fig. 3) whereas it is sister group to *Agaricomycotina* + *Ustilaginomycotina* + *Pucciniomycotina* in the six-gene phylogeny (Figs. 1, 2).

At the order level, this phyloproteomic analysis also reveals inconsistent phylogenetic positions from the six-gene phylogeny for orders *Amylocorticiales*, *Auriculariales, Cantharellales Geastrales, Sebacinales* and *Trechisporales* of *Agaricomycetes* (Figs. 1, 2, 3).

Taxonomy
From the results depicted in Figs. 1, 2, 3 and the divergence times of higher taxa summarized in Table 1 we propose to treat subphyla, classes and related orders as follows.

Phylum: *Basidiomycota* R.T. Moore, Bot. Mar. 23(6): 371 (1980)
    Subphylum *Agaricomycotina* Doweld, Prosyllabus Tracheophytorum, Tentamen Systematis Plantarum Vascularium (Tracheophyta) (Moscow): LXXVIII (2001)

    Subphylum *Pucciniomycotina* R. Bauer, Begerow, J.P. Samp., M. Weiss & Oberw., Mycol. Progr. 5(1): 45 (2006)
    Subphylum *Ustilaginomycotina* Doweld, Prosyllabus Tracheophytorum, Tentamen Systematis Plantarum Vascularium (Tracheophyta) (Moscow): LXXVIII (2001)
    Subphylum *Wallemiomycotina* Doweld, Index Fungorum 73 (2014)

1. Subphylum *Agaricomycotina*
    Class *Agaricomycetes* Doweld, Prosyllabus Tracheophytorum, Tentamen Systematis Plantarum Vascularium (Tracheophyta) (Moscow): LXXVIII (2001)
    Class *Dacrymycetes* Doweld, Prosyllabus Tracheophytorum, Tentamen Systematis Plantarum Vascularium (Tracheophyta) (Moscow): LXXVIII (2001)
    Class *Tremellomycetes* Doweld, Prosyllabus Tracheophytorum, Tentamen Systematis Plantarum Vascularium (Tracheophyta) (Moscow): LXXVIII (2001)

Phylogenetic support
The subphylum *Agaricomycotina* comprises three lineages: *Dacrymycetes*, *Agaricomycetes* and *Tremellomycetes* (Figs. 1, 2). The classes *Agaricomycetes* and *Dacrymycetes* cluster together with 55% BS support, and are sister to *Tremellomycetes* with poor statistical support in the ML tree (Fig. 1). In the MCC tree (Fig. 2) and phyloproteomic tree (Fig. 3) there is the same topology within this subphylum, but *Agaricomycotina* has 1 PP/100% BS support (Fig. 2). The mean of stem age of *Agaricomycotina* is 406 Ma and the classes diverged from 358 Ma to 393 Ma (Table 1).

Discussion
The subphylum *Agaricomycotina* contains approximately 70% of the known basidiomycetes (Kirk et al. 2008), including mushrooms, shelf fungi, puffballs and others. Historically the subphylum has been named as "*Hymenomycetes*" using single gene sequences (Swann and Taylor 1993, 1995). This lineage has been well-accepted as a subphylum based on analysis of nrLSU and nrSSU sequence data, and the integrated analysis of ultrastructural features, such as form and behaviour of the spindle pole bodies and types of host–parasite interaction (Bauer et al. 2006).

A phylogenetic overview of *Agaricomycotina* has been published using sequence data from nuclear rRNA and protein coding genes. The subphylum contains three major clades (*Agaricomycetes*, *Dacrymycetes* and *Tremellomycetes*; Hibbett 2006). In Hibbett's study, *Agaricomycotina* is well-supported using combined nuclear rRNA and protein-coding-gene sequence data from 125 taxa when
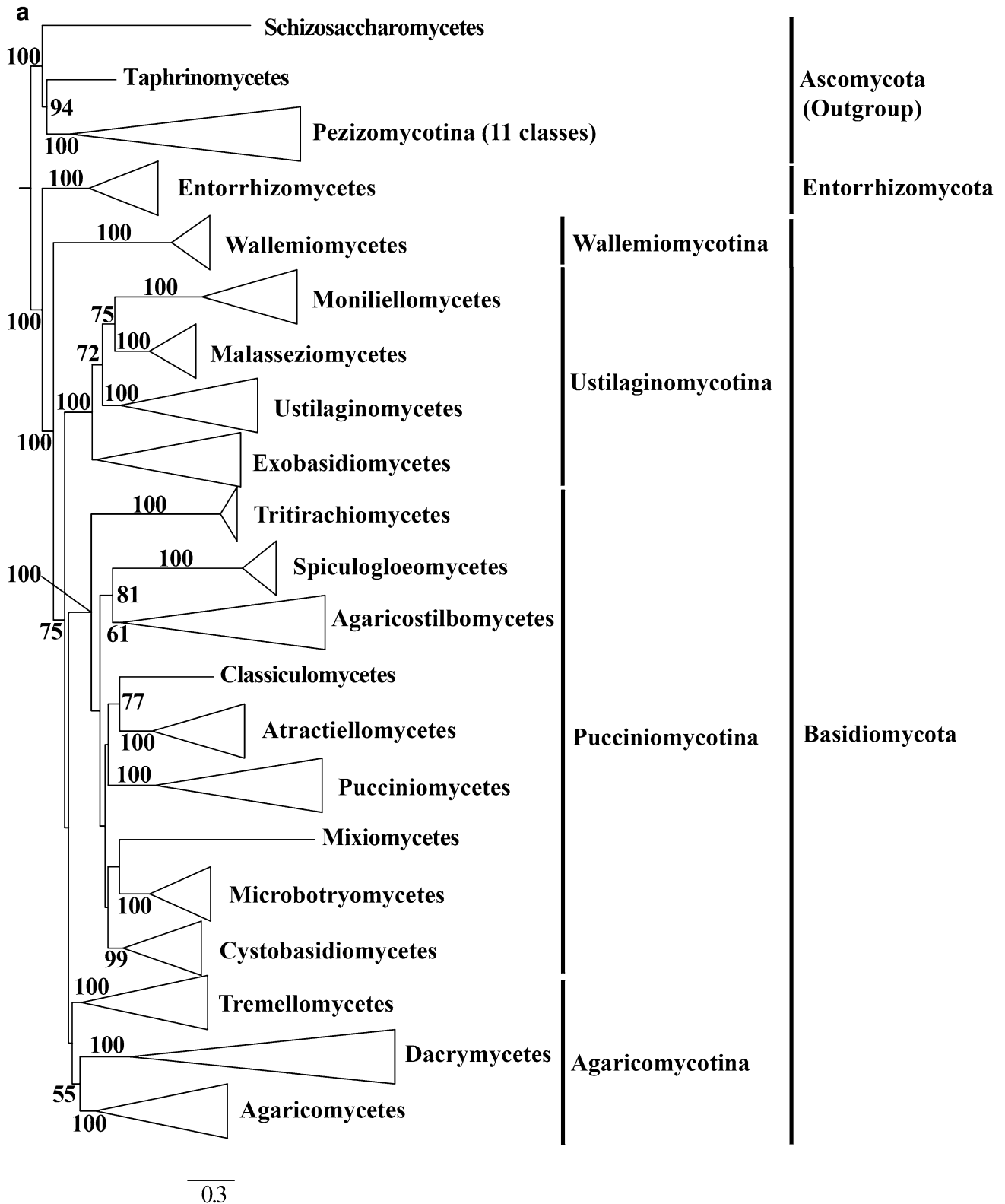
a

Fig. 1 A concatenated tree showing the relationships among classes from the phylogenetic analysis of *Basidiomycota* and allied phyla generated from maximum likelihood analysis of nrLSU, nrSSU, 5.8S, tef1-α, rpb1 and rpb2 sequence data, rooted with 13 species from *Ascomycota*. Bootstrap support (BS) values >50% are given at the internodes. The *thickened branch* indicates the order with a BS values >80% (**a** A concentred tree from the completely ML tree, in order to show the highlights of the relationships among classes; **b** The completely ML tree)
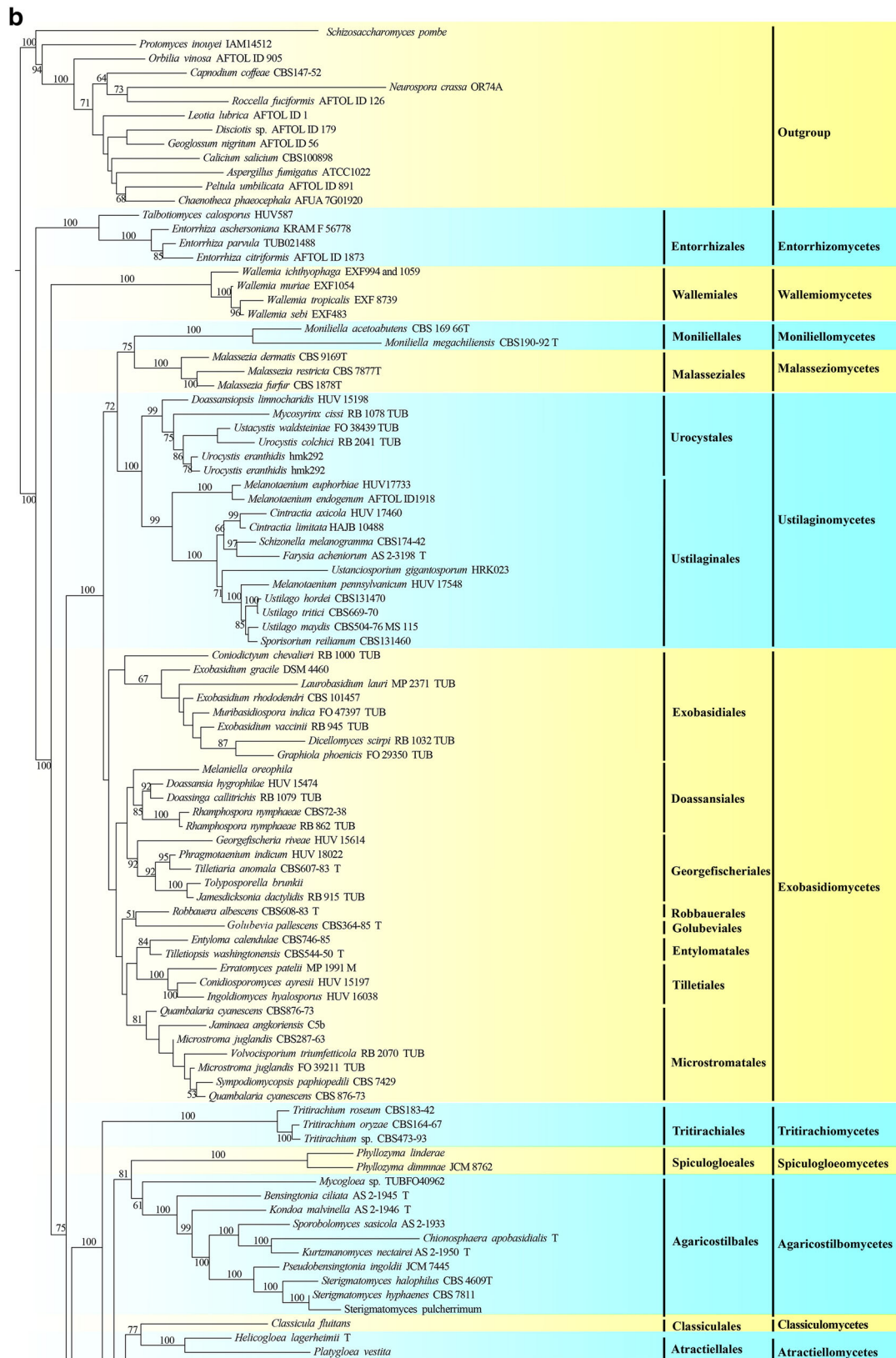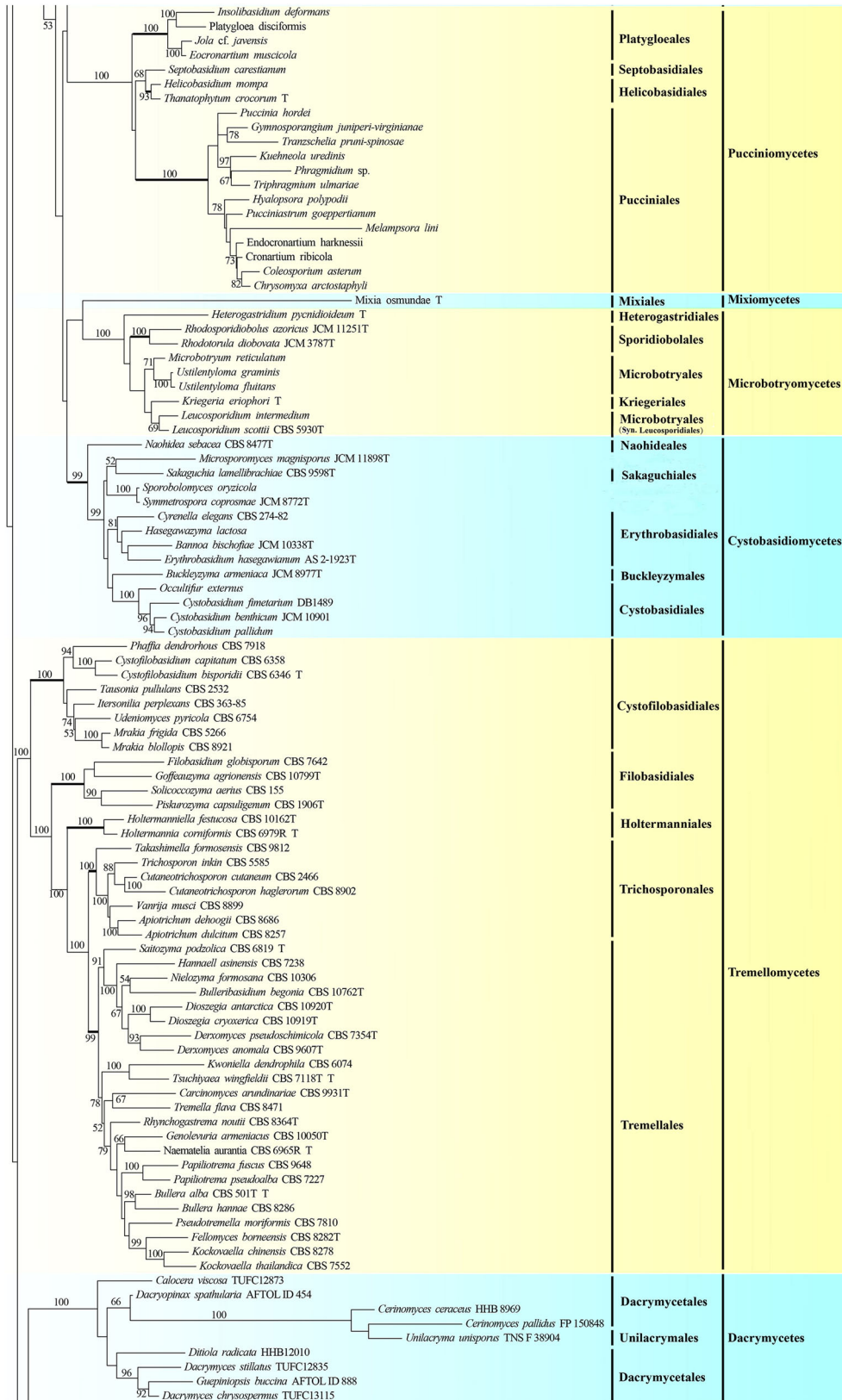
b

Fig. 1 continued
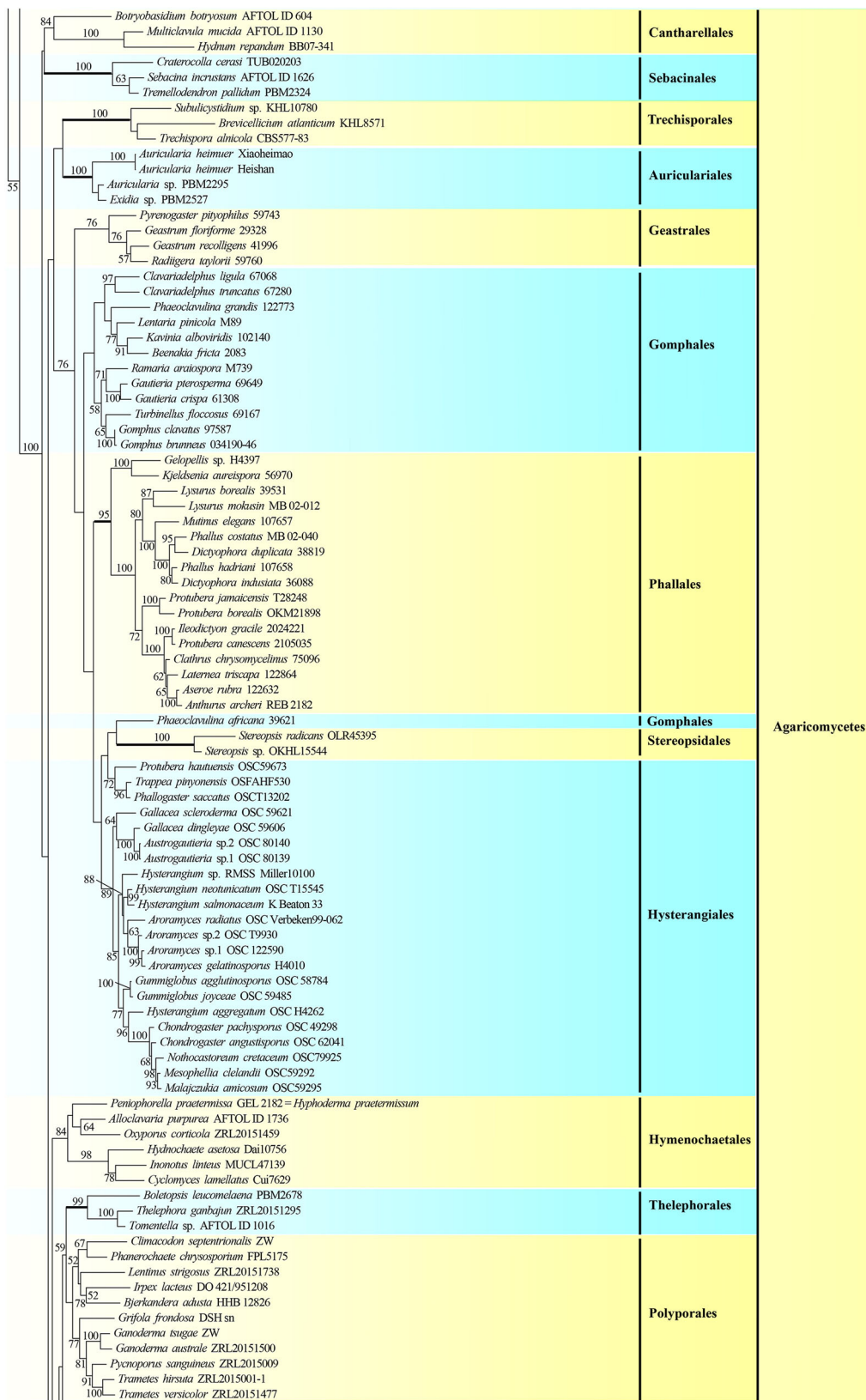
Fig. 1 continued
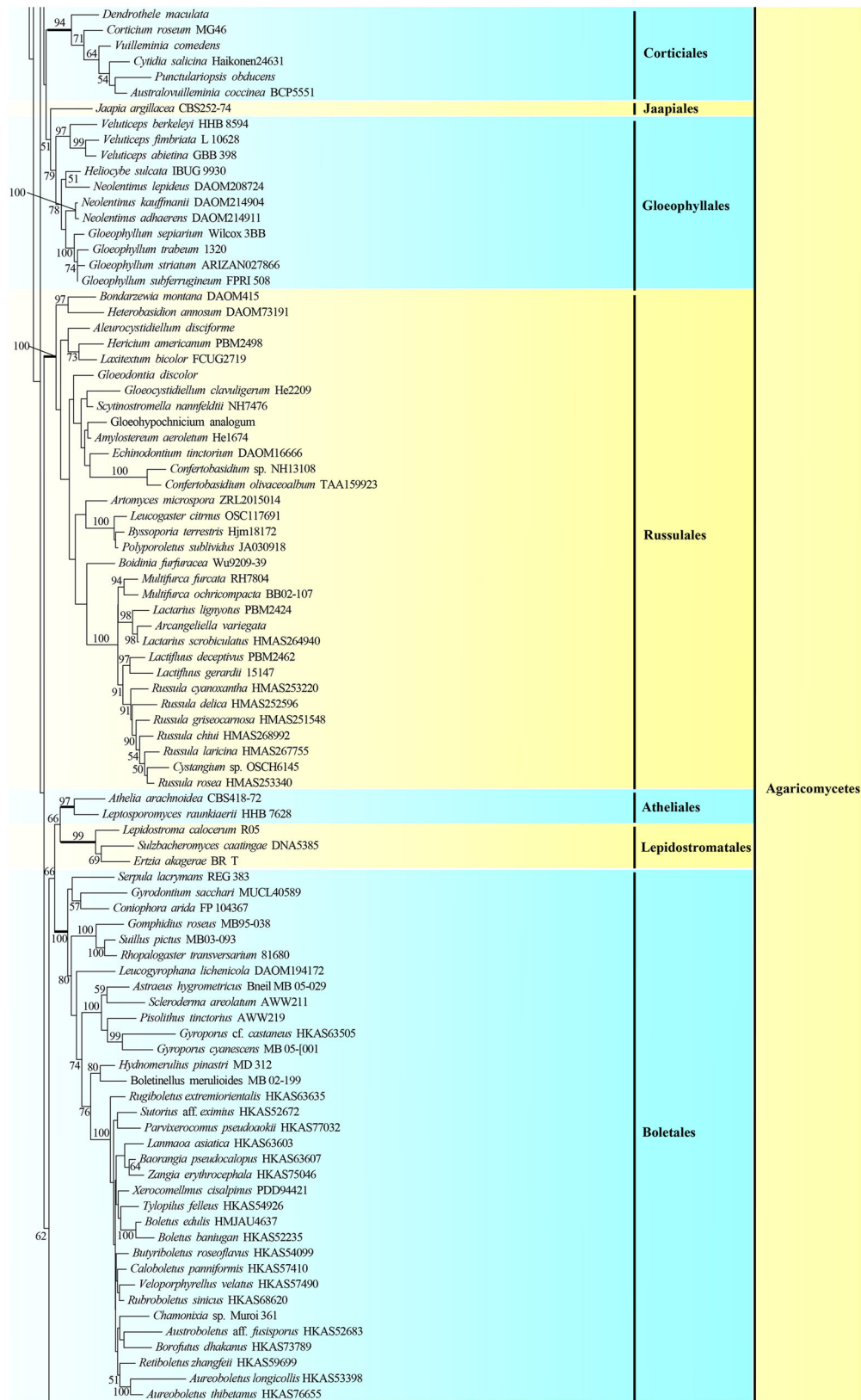
Fig. 1 continued
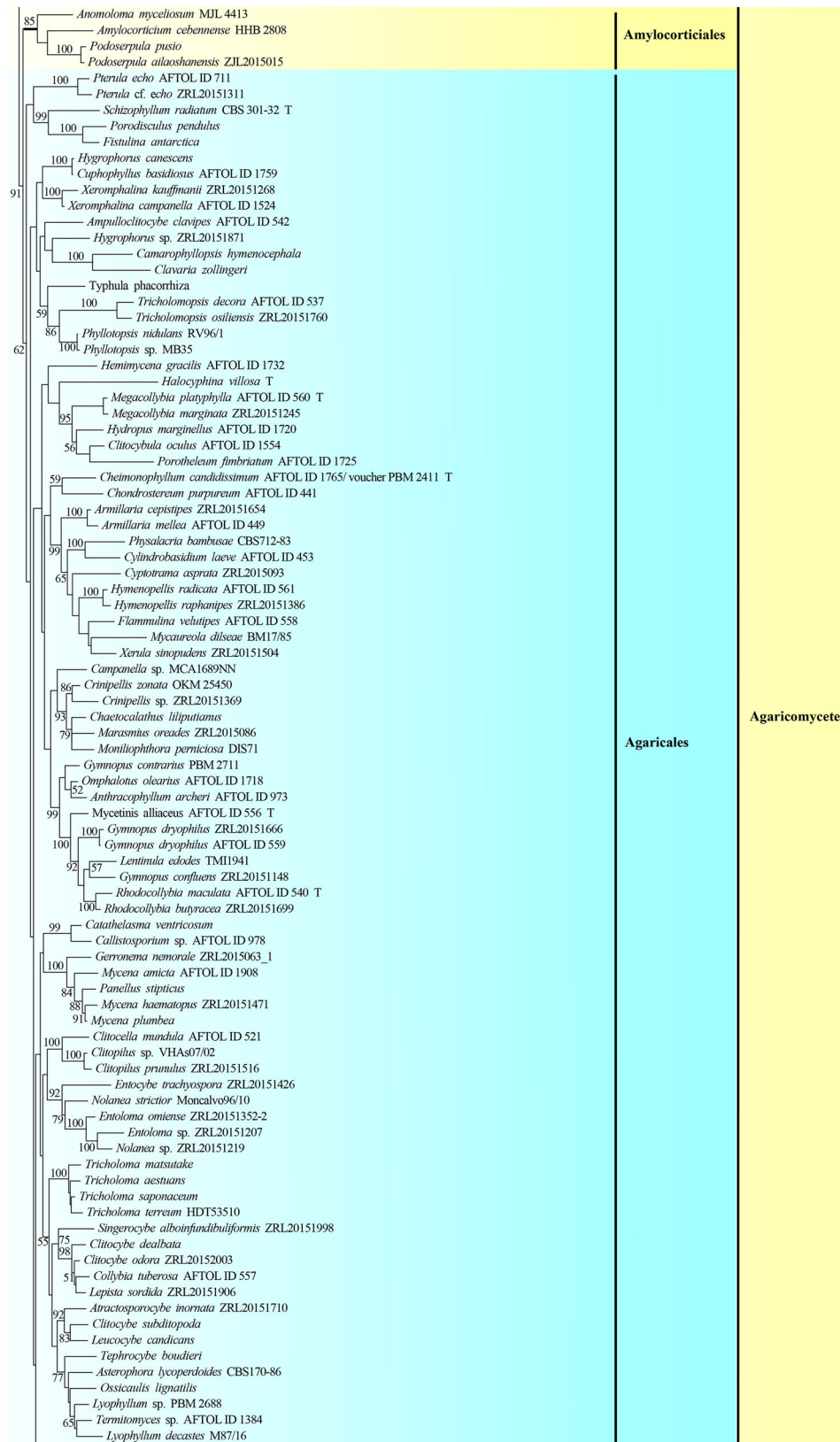
Fig. 1 continued
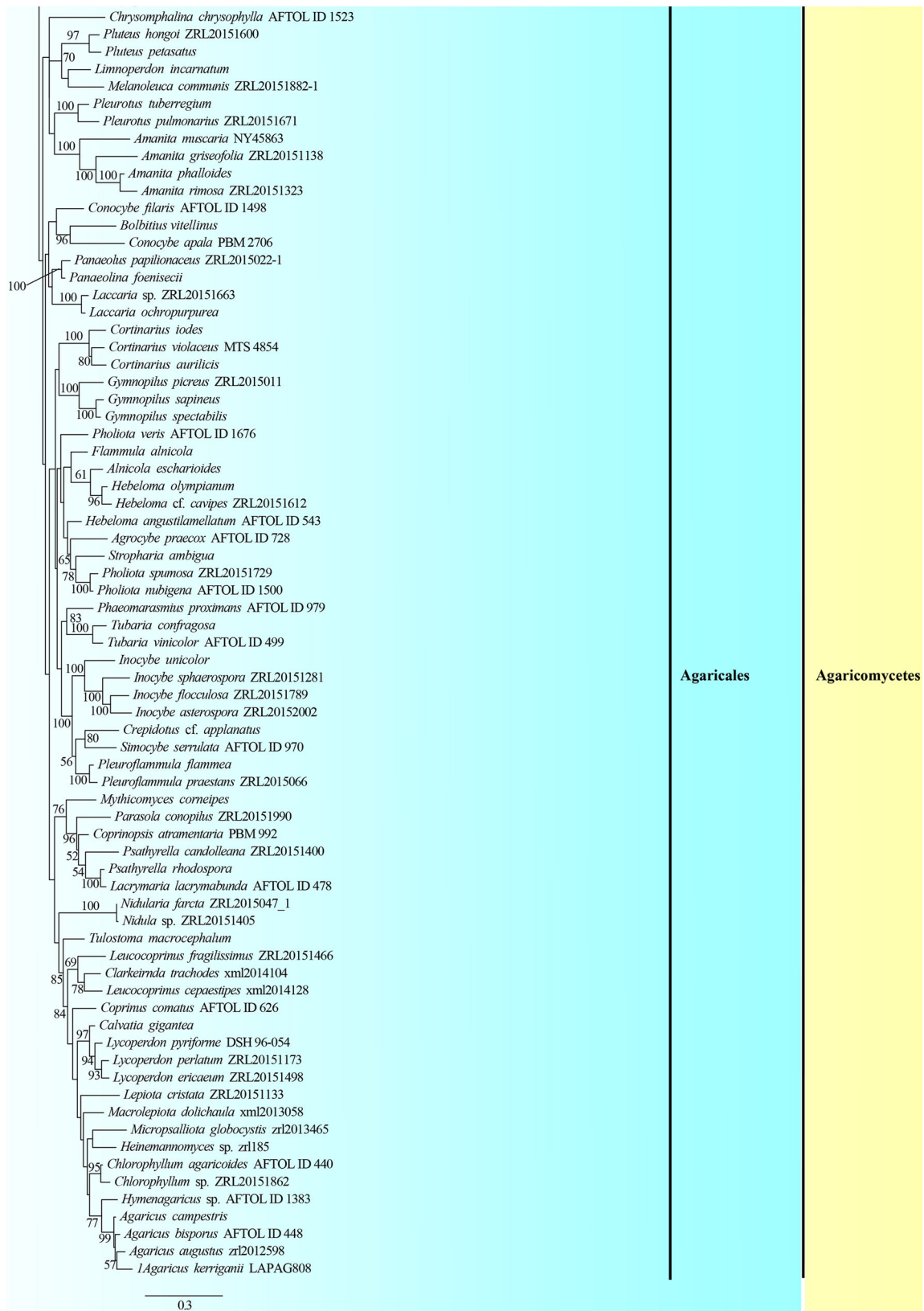
Fig. 1 continued

Fig. 1 continued
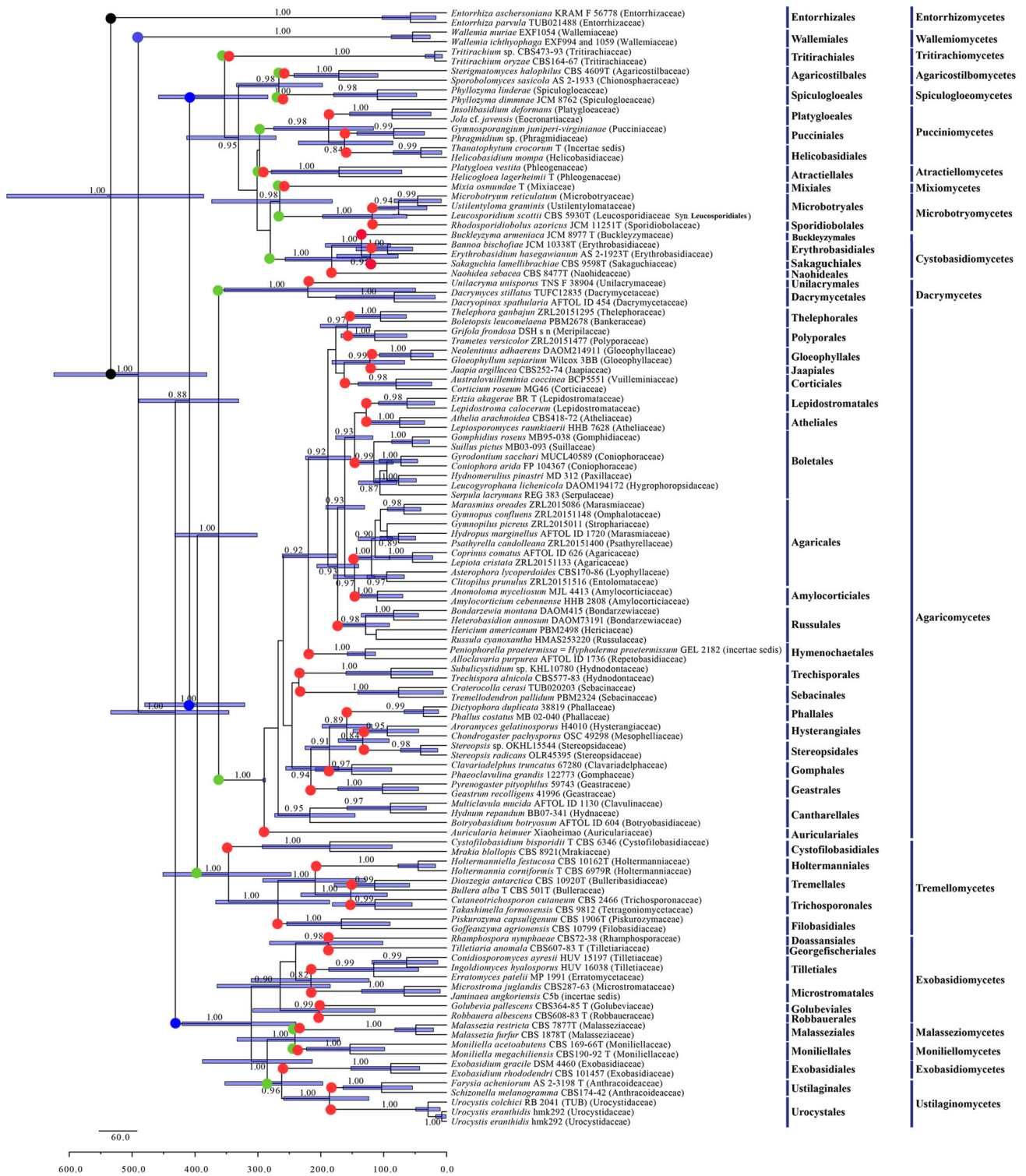
Fig. 2 Maximum Clade Credibility tree of *Basidiomycota* and allied phyla based on nrLSU, nrSSU, 5.8S, tef1-α, rpb1 and rpb2 sequence data with the outgroups *Entorrhizomycota*. Posterior probabilities which are equal or greater than 80% are annotated at the internodes. The 95% highest posterior density of divergence time estimates are marked by horizontal bars. The *coloured dots* ●●●● refer to the positions of the mean stem age of phyla, subphyls, classes and orders respectively

Fig. 3 Phyloproteomic tree of 109 *Basidiomycete* and six outgroup species, based on 396 protein alignments and a total of 82,536 sites. Statistical support values are based on maximum likelihood, and are shown only for nodes with lower than 100% support

Table 1 Subphyla, classes, subclasses, orders and families of Basidiomycota concluded in this study

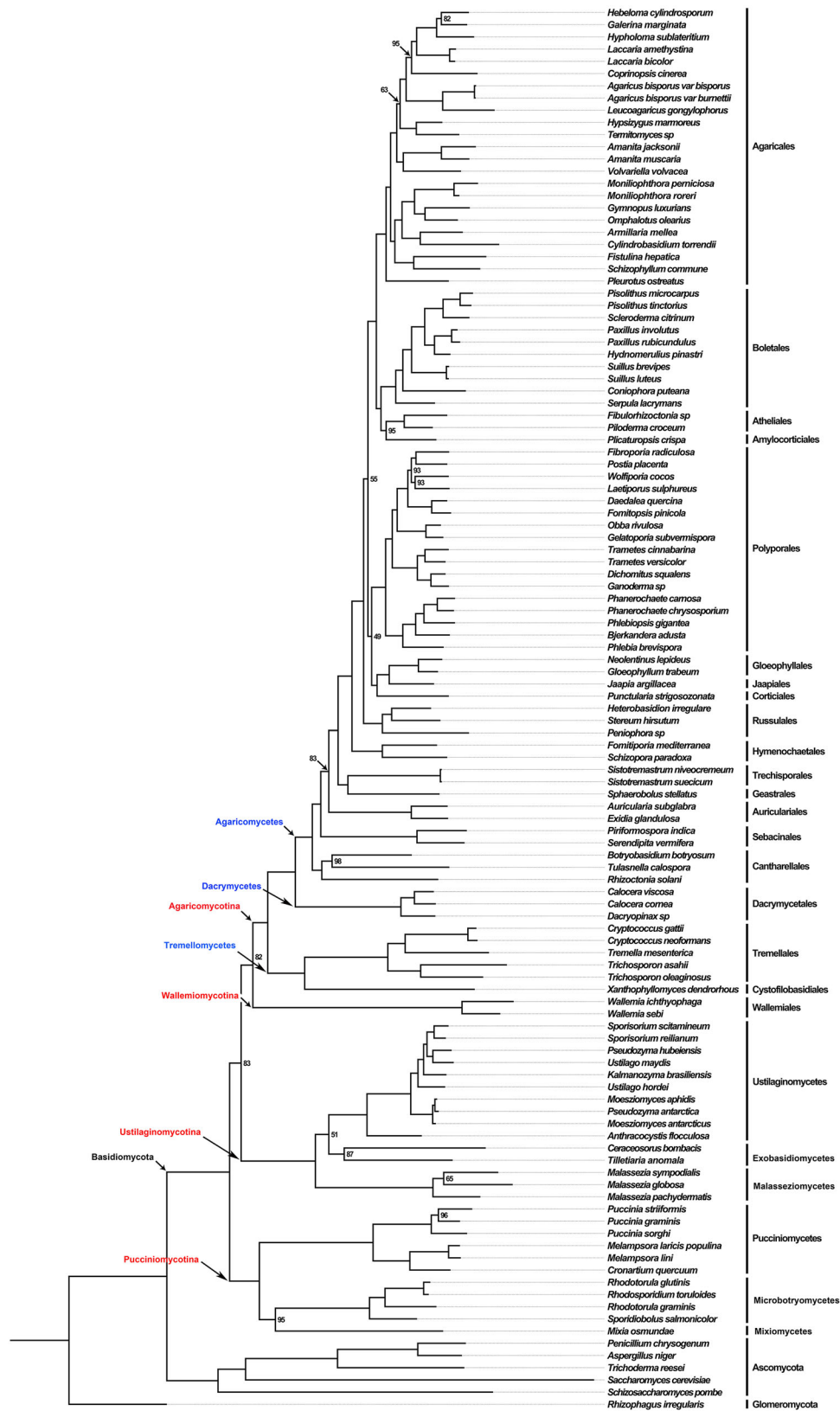| Subphylum	Class/subclass	Order (number of families in this study/the total known families)	Families recognized in this study	Mean of stem age (Ma) in MCC tree	BS values in ML tree
Agaricomycotina				406	***
	Agaricomycetes			358	100%
	Agaricomycetidae			173	62%
		Agaricales (30/32)		147	62%
			Agaricaceae		***
			Amanitaceae		100%
			Bolbitiaceae		***
			Clavariaceae		100%
			Cortinariaceae		**
			Cyphellaceae		59%
			Hygrophoraceae		**
			Entolomataceae		***
			Fistulinaceae		100%
			Hydnangiaceae		100%
			Marasmiaceae		**
			Hygrophoraceae		**
			Inocybaceae		100%
			Limnoperdonaceae		*
			Lyophyllaceae		77%
			Mycenaceae		**
			Niaceae		*
			Omphalotaceae		**
			Physalacriaceae		99%
			Pleurotaceae		100%
			Pluteaceae		97%
			Porotheleaceae		*
			Psathyrellaceae		76%
			Pterulaceae		100%
			Schizophyllaceae		*
			Strophariaceae		**
			Tricholomataceae		**
			Tubariaceae		*
			Typhulaceae		*
		Amylocorticiales (1/1)		147	85%
			Amylocorticiaceae		85%
		Atheliales (1/1)		146	97%
			Atheliaceae		97%
		Boletales (12/16)		146	100%
			Boletaceae		100%
			Boletinellaceae		*
			Coniophoraceae		57%
			Diplocystaceae		*
			Gomphidiaceae		*
			Gyroporaceae		***
			Hygrophoropsidaceae		*
			Paxillaceae		*
			Rhizopogonaceae		*
			Sclerodermatineae		*

Table 1 continued

Subphylum	Class/subclass	Order (number of families in this study/the total known families)	Families recognized in this study	Mean of stem age (Ma) in MCC tree	BS values in ML tree
			Serpulaceae		*
			Suillaceae		*
		Lepidostromatales (1/1)		146	99%
			Lepidostromataceae		99%
	Phallomycetidae			***	76%
		Geastrales (1/1)		217	76%
			Geastraceae		76%
		Gomphales (2/3)		187	***
			Clavariadelphaceae		97%
			Gomphaceae		**
		Hysterangiales (5/5)		133	**
			Hysterangiaceae		88%
			Mesophelliaceae		**
			Gallaceaceae		100%
			Phallogastraceae		**
			Trappeaceae		*
		Phallales (3/3)		159	95%
			Claustulaceae		100%
			Phallaceae		100%
			Phallogastraceae		**
	subclass *incertae sedis*	Auriculariales (1/7)		290	100%
			Auriculariaceae		100%
		Cantharellales (3/7)		290	84%
			Botryobasidiaceae		*
			Clavulinaceae		*
			Hydnaceae		*
		Corticiales (3/3)		189	94%
			Corticiaceae		**
			Punctulariaceae		*
			Vuilleminiaceae		*
		Gloeophyllales (1/1)		124	79%
			Gloeophyllaceae		79%
		Hymenochaetales (3/3)		221	84%
			Hymenochaetaceae		98%
			Repetobasidiaceae		*
			Schizoporaceae		*
		Jaapiales (1/1)		124	*
			Jaapiaceae		*
		Polyporales (6/10)		158	78%
			Ganodermataceae		100%
			Lentinaceae		*
			Meripilaceae		*
			Meruliaceae		52%
			Phanerochaetaceae		67%
			Polyporaceae		91%
		Russulales (11/12)		172	100%
			Albatrellaceae		100%

Table 1 continued

Subphylum	Class/subclass	Order (number of families in this study/the total known families)	Families recognized in this study	Mean of stem age (Ma) in MCC tree	BS values in ML tree
			Amylostereaceae		*
			Auriscalpiaceae		*
			Bondarzewiaceae		97%
			Echinodontiaceae		*
			Gloeocystidiellaceae		*
			Gloeodontiaceae		*
			Hericiaceae		73%
			Peniophoraceae		100%
			Russulaceae		***
			Stereaceae		*
		Sebacinales (1/1)		290	100%
			Sebacinaceae		100%
		Stereopsidales (1/1)		133	100%
			Stereopsidaceae		100%
		Thelephorales (2/2)		158	99%
			Bankeraceae		*
			Thelephoraceae		100%
		Trechisporales (1/1)		290	100%
			Hydnodontaceae		100%
	Dacrymycetes			358	100%
		Dacrymycetales (2/2)		225	**
			Dacrymycetaceae		100%
			Cerinomycetaceae		**
		Unilacrymales (1/1)		225	*
			Unilacrymaceae		*
	Tremellomycetes			393	100%
		Cystofilobasidiales (2/2)		350	100%
			Mrakiaceae		***
			Cystofilobasidiaceae		94%
		Filobasidiales (2/2)		271	100%
			Piskurozymaceae		90%
			Filobasidiaceae		***
		Holtermanniales (1/1)		211	100%
			Holtermanniaceae		100%
		Tremellales (8/12)		153	100%
			Bulleraceae		**
			Bulleribasidiaceae		67%
			Carcinomycetaceae		*
			Cryptococcaceae		*
			Cuniculitremaceae		99%
			Naemateliaceae		*
			Trimorphomycetaceae		*
			Tremellaceae		*
		Trichosporonales (2/2)		153	100%
			Trichosporonaceae		100%
			Tetragoniomycetaceae		*
Pucciniomycotina				406	100%
	Agaricostilbomycetes			268	61%

Table 1 continued

Subphylum	Class/subclass	Order (number of families in this study/the total known families)	Families recognized in this study	Mean of stem age (Ma) in MCC tree	BS values in ML tree
		Agaricostilbales (1/3)		268[#]	61%
			Agaricostilbaceae		100%
			Chionosphaeraceae		100%
			Kondoaceae		**
	Atractiellomycetes			330	100%
		Atractiellales (1/3)		330[#]	100%
			Phleogenaceae		100%
	Classiculomycetes			–	*
		Classiculales (1/1)			*
			Classiculaceae		*
	Cystobasidiomycetes			330	99%
		Buckleyzymales (1/1)		136	*
			Buckleyzymaceae		
		Cystobasidiales (1/?)		–	100%
			Cystobasidiaceae		96%
		Erythrobasidiales (1/?)		122	**
			Erythrobasidiaceae		**
		Naohideales (1/?)		185	*
			Naohideaceae		*
		Sakaguchiales (1/1)		122	*
			Sakaguchiaceae		*
		Order *incertae sedis*	Microsporomycetaceae		*
			Symmetrosporaceae		*
	Microbotryomycetes			264	100%
		Heterogastridiales (1/1)		–	*
			Heterogastridiaceae		*
		Kriegeriales (1/2)		–	*
			Kriegeriaceae		*
		Leucosporidiales (1/1) (Syn. of Microbotryales in this study)		74	69%
			Leucosporidiaceae		69%
		Microbotryales (3/3)		118	***
			Microbotryaceae		*
			Ustilentylomataceae		100%
			Leucosporidiaceae		69%
		Sporidiobolales (1/1)		118	100%
			Sporidiobolaceae		100%
	Mixiomycetes			264	*
		Mixiales (1/1)		264[#]	*
			Mixiaceae		*
	Pucciniomycetes			330	100%
		Helicobasidiales (1/1)		162	93%
			Helicobasidiaceae		93%
		Platygloeales (2/?)		187	100%
			Eocronartiaceae		100%
			Platygloeaceae		100%
		Pucciniales (8/15)		162	100%
			Coleosporiaceae		82%

Table 1 continued

Subphylum	Class/subclass	Order (number of families in this study/the total known families)	Families recognized in this study	Mean of stem age (Ma) in MCC tree	BS values in ML tree
			Cronartiaceae		**
			Melampsoraceae		*
			Phragmidiaceae		**
			Pucciniaceae		**
			Pucciniastraceae		**
			Raveneliaceae		*
			Uropyxidaceae		*
		Septobasidiales (1/?)		–	*
			Septobasidiaceae		*
	Spiculogloeomycetes			266	100%
		Spiculogloeales (1/1)		266#	100%
			Spiculogloeaceae		100%
	Tritirachiomycetes			356	100%
		Tritirachiales (1/?)		356#	100%
			Tritirachiaceae		100%
Ustilaginomycotina				430	100%
	Exobasidiomycetes			***	**
		Microstromatales (3/3)		216	81%
			Microstromataceae		**
			Quambalariaceae		**
			Volvocisporiaceae		*
		Doassansiales (3/3)		187	85%
			Doassansiaceae		92%
			Melaniellaceae		*
			Rhamphosporaceae		100%
		Entylomatales (1/1)		–	84%
			Entylomataceae		84%
		Exobasidiales (4/4)		265	***
			Brachybasidiaceae		*
			Cryptobasidiaceae		**
			Exobasidiaceae		**
			Graphiolaceae		*
		Georgefischeriales (3/4)		187	92%
			Georgefischeriaceae		*
			Gjaerumiaceae		*
			Tilletiariaceae		**
		Golubeviales (1/?)		202	*
			Golubeviaceae		*
		Robbauerales (1/?)		202	*
			Robbaueraceae		*
		Tilletiales (2/2)		216	100%
			Erratomycetaceae		*
			Tilletiaceae		100%
	Malasseziomycetes			245	100%
		Malasseziales (1/1)		245#	100%
			Malasseziaceae		100%
	Moniliellomycetes			245	100%
		Moniliellales (1/1)		245#	100%

Table 1 continued

Subphylum	Class/subclass	Order (number of families in this study/the total known families)	Families recognized in this study	Mean of stem age (Ma) in MCC tree	BS values in ML tree
			Moniliellaceae		100%
	Ustilaginomycetes			265	100%
		Urocystales (3/?)		187	99%
			Doassansiopsidaceae		*
			Mycosyringaceae		*
			Urocystidaceae		86%
		Ustilaginales (4/9)		187	99%
			Anthracoideaceae		66%
			Melanopsichiaceae		*
			Melanotaeniaceae		100%
			Ustilaginaceae		85%
Wallemiomycotina				487	100%
	Wallemiomycetes			487[#]	100%
		Wallemiales (1/1)		487[#]	100%
			Wallemiaceae		100%

Bold indicates families used in both ML and BEAST analyses

* Refers to the taxa represented by a single species in this study

** Refers to taxa found to be either para- or polyphyletic in this study

*** Refers to taxa with <50%/0.8 BS/PP support

[#] Refers to the divergence times of this class/order is following those of its higher-level taxa, because this class/order is the unique class/order within its higher taxa. Those kinds of divergence times of class/order are excluded in generating the universal divergence in this study

data for *Wallemiomycetes* and *Entorrhizomycetes* was not available. However, once sequence data from *Wallemiomycetes* and *Entorrhizomycetes* are included, this subphylum is well-supported using nuclear rRNA genes; but lacks strong BS support when using combined nuclear rRNA and protein coding genes (Hibbett 2006).

In our analyses, we used rRNA and protein coding genes and include more than 500 species: *Agaricomycotina* lacks high BS support in the ML tree (Fig. 1), but has 1 PP/100% BS support in the molecular dating and phyloproteomic analyses (Figs. 2, 3). We therefore accept *Agaricomycotina* with three classes: *Agaricomycetes, Dacrymycetes* and *Tremellomycetes.*

Class Agaricomycetes

Phylogenetic support
This class is monophyletic with 100% BS and 1 PP support (Figs. 1, 2, 3). In the ML tree (Fig. 1), 12 orders (*Amylocorticiales, Atheliales, Auriculariales, Boletales, Corticiales, Lepidostromatales, Phallales, Russulales, Sebacinales, Stereopsidales, Thelephorales, Trechisporales*) out of 21 orders have more than 85% BS support, and six orders (*Agaricales, Cantharellales, Geastrales,*

Gloeophyllales, Hymenochaetales, and *Polyporales*) have 62–84% BS support. The problematic orders in this ML study are *Gomphales* and *Hysterangiales*. The former lacks statistical support and the latter is polyphyletic. In the molecular dating analysis, all those 21 orders of *Agaricomycetes* are monophyletic with 0.95 or 1 PP support. In the phyloproteomic analysis, a total of 15 orders are included and those six weak-supported orders (*Agaricales, Cantharellales, Geastrales, Gloeophyllales, Hymenochaetales,* and *Polyporales* with 62–84% BS support in six-gene analysis) get 100% BS support (Fig. 3).

Based on the molecular dating analysis (Fig. 2), *Agaricomycetes* has a mean stem age of 358 Ma. The 21 orders within *Agaricomycetes* diverged at different times, but mostly within the mean of stem ages ranging from 124 Ma to 290 Ma (Table 1). In that analysis the order *Auriculariales* is basal (Figs. 1, 2; Table 1). However, the phyloproteomic analysis, the order *Cantharellales* has the basal position, followed by *Sebacinales* and *Auriculariales* (Fig. 3).

Discussion
The members of *Agaricomycetes* are mostly mushrooms, and include cultivated, saprotrophic and ectomycorrhizal species used as sources of food and medicine (De Silva et al. 2012a, b, 2013; Hibbett et al. 2014). Some species

also cause poisoning or psychoactivity when eaten (Chen et al. 2014). Bioactive components from these mushrooms can be used in the modern medical industries (Ruiz-Dueñas and Martínez 2009). This class previously contained 17 orders, more than 100 families, 1000 genera and 21,000 species, which is one-fifth of all known fungi (Hibbett et al. 2007; Kirk et al. 2008).

The early recognized orders have been resolved as monophyletic groups using analysis of rRNA genes, and support for some orders were weak or absent, such as *Cantharellales* (Binder and Hibbett 2002; Binder et al. 2005; Moncalvo et al. 2006). In part due to elevated rates of evolution in rRNA genes, protein coding genes have been introduced in fungal phylogenetic analyses, such as in Matheny et al. (2006). Several weakly supported orders based on rRNA gene analysis are strongly supported in Matheny et al. (2006). This includes *Agaricales*, *Polyporales*, and *Cantharellales* (excluding species of *Tulasnella*) and the russuloid and gomphoid-phalloid clades (Binder and Hibbett 2002; Binder et al. 2005; Moncalvo et al. 2006). Since 2010, the orders *Amylocorticiales*, *Jaapiales*, *Stereopsidales* and *Lepidostromatales* have been introduced within *Agaricomycetes* (Binder et al. 2010; Hodkinson et al. 2014; Sjökvist et al. 2014).

In this study, we included all 21 orders and in the ML analysis using rRNA and protein coding gene sequences from many more species than in Matheny et al. (2006). When combining the ML and molecular dating analysis (Figs. 1, 2; Table 1) as well as previous studies, the orders *Agaricales*, *Amylocorticiales*, *Atheliales*, *Auriculariales*, *Boletales*, *Corticiales*, *Geastrales*, *Gloeophyllales*, *Hymenochaetales*, *Jaapiales*, *Lepidostromatales*, *Polyporales*, *Phallales*, *Russulales*, *Sebacinales*, *Stereopsidales*, *Thelephorales*, and *Trechisporales* are well-supported. All are monophyletic groups within similar divergence times in our analyses (Figs. 1, 2, 3; Table 1). Within those orders, *Jaapiales* was established in 2010 based on analysis of a six-locus nuclear dataset of 191 species, and contains a single resupinate genus *Jaapia* comprising two species (Binder et al. 2010). In that study, *Jaapiales* is sister to *Boletales*. However in our study, *Jaapia* is sister to *Gloeophyllales* in all our analyses, including the ML (Fig. 1), molecular dating analysis (Fig. 2) and phyloproteomic analysis (Fig. 3).

The main problems in the present taxonomic system of *Agaricomycetes* are as follows:

(i) *Redefinition of Cantharellales. Cantharellales* has been thought to be a monophyletic group, represented by members of *Cantharellaceae*, *Clavulinaceae* and *Hydnaceae* (Matheny et al. 2006). *Botryobasidiaceae* was included in this order by Moncalvo et al. (2006), appearing to be sister to

the core cantharelloid clade when using analysis of rRNA genes (Moncalvo et al. 2006). The most recent phylogenetic study on *Cantharellales* based on ITS sequences from six families, also included *Botryobasidiaceae* (Veldrea et al. 2013). In our ML analysis, we used rRNA and protein coding gene sequence data from members of *Clavulinaceae, Hydnaceae* and *Botryobasidiaceae*. The phylogeny shows this order is monophyletic with 84% BS support. However, this order has a 1.0 PP and 100% BS statistical support in the molecular dating and phyloproteomic analysis, but it does not have a wide taxon sampling. Thus, more comprehensive studies are needed to redefine this order in future work.

(ii) An improved phylogenetic analysis emphasizing on *Gomphales* is needed. In the ML analysis, this order is paraphyletic as our sample of *Phaeoclavulina africana* (collection number 39,621) did not cluster with the other twelve taxa of *Gomphales,* which formed a monophyletic clade, but without statistical support (Fig. 1). However, in the molecular dating analysis this order has 0.97 PP support (Fig. 2) and has a divergence time of 187 Ma. Further studies are needed because this dating analysis is only based on two species sequences.

(iii) A more comprehensive phylogenetic analysis of *Hysterangiales* is needed. Similar to *Gomphales*, *Hysterangiales* is monophyletic with 0.95 PP support, and a divergence time of 133 Ma based on two species sequences. However this order is paraphyletic in the ML analysis when using sequences from 22 species.

Class Dacrymycetes

Phylogenetic support
This class is represented by nine species and is supported by 100% BS (Fig. 1), 1.0 PP in the molecular dating analysis (Fig. 2) and 100% BS in the phyloproteomics analysis (Fig. 3). This class is sister to *Agaricomycetes* with 57% BS support (Fig. 1). The recently introduced order *Unilacrymales*, represented by a single species of *Unilacryma*, nests in the clade comprising *Cerinomycetaceae* (*Dacrymycetales*) with 100% BS support (Fig. 1), but is sister to *Dacrymycetales* in the molecular dating analysis (Fig. 2). The mean stem age for *Dacrymycetes* is 358 Ma, while *Dacrymycetales* and *Unilacrymales* are both 225 Ma (Fig. 2; Table 1).

Discussion

Dacrymycetes comprises the jelly fungi, and traditionally contains a single order *Dacrymycetales*, one family *Dacrymycetaceae*, nine genera and more than 100 species (Hibbett 2006; James et al. 2006; Hibbett et al. 2007; Kirk et al. 2008). Members of this class are mainly brown rot and rarely white rot fungi. The divergence time for this class was estimated at 280–360 Ma (Dentinger et al. 2010), so the class is regarded as one of the earliest wood decomposers to diverge in the *Basidiomycota* as compared to the later-evolved wood decomposers in *Agaricomycetes* (Shirouzu et al. 2013); such as the order *Polyporales* (*Agaricomycetes*), which is about 114 Ma old (Fig. 2). Recent molecular phylogenetic studies support the monophyly of *Dacrymycetes* and show it has a sister relationship to *Agaricomycetes* (Hibbett 2006; James et al. 2006; Hibbett et al. 2007).

Cerinomycetaceae (Jülich 1981; Larsson et al. 2004) is a second family of *Dacrymycetales* introduced by Shirouzu et al. (2009). A new genus *Unilacryma* was introduced with a single species *U. unisporus*; this species always occupies a sister position to other species of *Dacrymycetes*. To accommodate this taxon, a new family *Unilacrymaceae* and a new order *Unilacrymales* were introduced (Shirouzu et al. 2013).

However, in our study, *Unilacrymales* clusters in the clade of *Cerinomycetaceae* of *Dacrymycetales* with 100% BS support in ML analysis (Fig. 1), but sister to *Dacrymycetales* in the molecular dating analysis (Fig. 2). Their divergence times are both 225 Ma (Table 1). Then we accept *Unilacrymales* as an order in this study, but a more detailed study should be conducted using more genes.

Class Tremellomycetes

Phylogenetic support

In this study, *Tremellomycetes* as represented by 43 species, is monophyletic with 100% BS support in the ML tree (Fig. 1). *Tremellomycetes* is sister to the classes *Agaricomycetes* and *Dacrymycetes* and they form a larger clade which respect to the subphylum *Agaricomycotina* with the BS values less than 50% support. However, in the MCC and phyloproteomic trees (Figs. 2, 3), *Tremellomycetes* is sister to the clade of *Agaricomycetes* + *Dacrymycetes*, and all cluster within *Agaricomycotina* with 1.0 PP/100% BS support.

Both analyses show the same topology within the class *Tremellomycetes* (Figs. 1, 2): the order *Cystofilobasidiales* and the orders *Filobasidiales*, *Holtermanniales*, *Trichosporonales* and *Tremellales* represent a deep dichotomy; *Filobasidiales* is sister to the clade composed of *Holtermanniales*, *Trichosporonales* and *Tremellales.* All orders have 99–100% BS and 0.99–1.0 PP support.

The mean of stem age of *Tremellomycetes* is 393 Ma, and the mean of stem ages of its orders mostly range from 153 Ma to 270 Ma with the exception of order *Cystofilobasidiales* is older (350 Ma, Table 1).

Discussion

Tremellomycetes, the jelly fungi, have gelatinous fruiting bodies. However, the classes also contain yeasts and dimorphic fungi. The phylogenetic position of *Tremellomycetes* has been well-studied (Fell et al. 2000; Matheny et al. 2006; Hibbett et al. 2007), and three orders *Cystofilobasidiales*, *Filobasidiales* and *Tremellales* have been widely accepted within this class (Kirk et al. 2008). *Holtermanniales* and *Trichosporonales* are additional orders (Boekhout et al. 2011; Wuczkowski et al. 2011; Weiss et al. 2014), and a related robust framework and updated taxonomic system of this class has been provided using analysis of combined sequence data from seven genes (Liu et al. 2015a,b).

In our study, *Tremellomycetes* is strongly supported and it contains five well-supported orders (Figs. 1, 2; Table 1). However, this class is loosely related to the subphylum *Agaricomycotina* in the ML analysis (Fig. 1), this is similar to the phylogenetic overview of *Agaricomycotina* (Hibbett 2006): the protein-coding gene sequences reduce the support of *Tremellomycetes* as a member of subphylum *Agaricomycotina*. However, in the molecular dating and phyloproteomic analysis, *Tremellomycetes* is strongly supported as a member of *Agaricomycotina*. On the other hand, the older age of order *Cystofilobasidiales* may indicate that it could eventually be raised to a higher taxon.

2. **Subphylum *Pucciniomycotina*** R. Bauer, Begerow, J.P. Samp., M. Weiss & Oberw., Mycol. Progr. 5(1): 45 (2006)

Class *Agaricostilbomycetes* R. Bauer, Begerow, J.P. Samp., M. Weiss & Oberw., Mycol. Progr. 5(1): 45 (2006)

Class *Atractiellomycetes* R. Bauer, Begerow, J.P. Samp., M. Weiss & Oberw., Mycol. Progr. 5(1): 45 (2006)

Class *Classiculomycetes* R. Bauer, Begerow, J.P. Samp., M. Weiss & Oberw., Mycol. Progr. 5(1): 46 (2006)

Class *Cryptomycocolacomycetes* R. Bauer, Begerow, J.P. Samp., M. Weiss & Oberw., Mycol. Progr. 5(1): 46 (2006) (not involved in this study)

Class *Cystobasidiomycetes* R. Bauer, Begerow, J.P. Samp., M. Weiss & Oberw., Mycol. Progr. 5(1): 46 (2006)

Class ***Microbotryomycetes*** R. Bauer, Begerow, J.P. Samp., M. Weiss & Oberw., Mycol. Progr. 5(1): 47 (2006)

Class ***Mixiomycetes*** R. Bauer, Begerow, J.P. Samp., M. Weiss & Oberw., Mycol. Progr. 5(1): 47 (2006)

Class ***Pucciniomycetes*** R. Bauer, Begerow, J.P. Samp., M. Weiss & Oberw., Mycol. Progr. 5(1): 48 (2006)

Class ***Spiculogloeomycetes*** Q.M. Wang, F.Y. Bai, M. Groenew. & Boekhout, Stud. Mycol. 81: 172 (2015)

Class ***Tritirachiomycetes*** Aime & Schell, in Schell, Lee & Aime, Mycologia 103(6): 1339 (2011)

Phylogenetic support

This subphylum is a monophyletic clade with 100% BS support (Fig. 1), and sister to subphylum *Agaricomycotina*. Nine of the ten classes of *Pucciniomycotina* were included in this study. *Cryptomycocolacomycetes* is not included as it lacks reliable sequence data. All nine classes are monophyletic with 99% BS or 1.0 PP support, except for *Agaricostilbomycetes* with 61% BS support in the ML analysis (Fig. 1). Eight classes (*Classiculomycetes* is excluded due to limited sequence data) are included in the molecular dating analysis. All classes are monophyletic, and have 0.95–1.0 PP support (Fig. 2; Table 1). In the phyloproteomic analysis, this subphylum is fully supported and represented by three classes (*Pucciniomycetes*, *Microbotryomycetes* and *Mixiomycetes*), however it is sister to the *Agaricomycotina* + *Ustilaginomycotina* clade (Fig. 3).

The orders *Classiculales, Heterogastridiales, Kriegeriales, Mixiales, Naohideales, Sakaguchiales ord. nov.* and *Septobasidiales* are represented by a single species, so they lack statistical support (Fig. 1). The orders *Atractiellales, Cystobasidiales, Helicobasidiales, Platygloeales, Pucciniales, Spiculogloeales, Sporidiobolales* and *Tritirachoales* have 93% to 100% BS support; and the orders *Agaricostilbales, Erythrobasidiales, Leucosporidiales* (proposed as a synonym of *Micorbotryales* in this study) and *Microbotryales* have 61, 81, 69 and 71% BS support respectively (Fig. 1). In the class *Cystobasidiomycetes,* the genera *Buckleyzyma, Microsporomyces, Sakaguchia* and *Symmetrospora* are represented by a single type specimen in the ML analysis.

In the molecular dating analysis, the mean of stem age of *Pucciniomycotina* is 406 Ma, its classes have mean stem ages mostly from ca. 264 Ma to 356 Ma. The mean stem ages of the orders are mainly from 118 Ma to 187 Ma, however *Leucosporidiales* and *Microbotryales* have a similar stem age (74 Ma), which is much younger than those of other orders (Fig. 2; Table 1).

Discussion

There are more than 8000 species in *Pucciniomycotina*, which makes up one third of the described *Basidiomycota*

(Aime et al. 2006, Bauer et al. 2006). Previous systematic studies mainly compared ultrastructural features, such as septal pore apparatus, form and behaviour of the spindle pole bodies, types of host-parasite interaction, presence or absence of colacosomes, symplechosomes, atractosomes and cystosomes as well as analyses of nuclear rRNA gene sequence data. A taxonomic arrangement has been proposed with eight classes: *Agaricostilbomycetes, Atractiellomycetes, Classiculomycetes, Cryptomycocolacomycetes, Cystobasidiomycetes, Microbotryomycetes, Mixiomycetes,* and *Pucciniomycetes* (Hamamoto and Nakase 2000; Nakase 2000; Fell et al. 2000; Scorzetti et al. 2002; Aime et al. 2006, 2014; Bauer et al. 2006; Hibbett et al. 2007; Boekhout et al. 2011. Among these classes, *Pucciniomycetes* includes the most species from this subphylum (around 8000 species, Kirk et al. 2008) and most are plant pathogens. The class *Tritirachiomycetes* is based on a single genus *Tritirachium* Limber, which are hyphomycetes recently placed as *incertae sedis* within the *Pezizomycotina* (Ascomycota). Now the genus is placed in *Tritirachiomycetes* and as the ninth class of *Pucciniomycotina* (Schell et al. 2011). The tenth class, *Spiculogloeomycetes* Q.M. Wang, F.Y. Bai, M. Groenew. & Boekhout, comprises yeast and yeast-like species which were introduced by Wang et al. (2015a, c).

All classes are monophyletic groups based on the phylogenies of the ML and molecular dating analyses. The recently introduced classes *Tritirachiomycetes* and *Spiculogloeomycetes* are well-supported, not only from the phylogenetic analysis, but also have similar divergence times.

Most orders of *Pucciniomycotina* are well-supported in this study. Exceptions are: (i) In class *Microbotryomycetes*, *Leucosporidiales* which is represented by its type species and type specimen *Leucosporidium scottii* in this study. The molecular dating analysis shows this order has a very young divergence time (74 Ma) and is sister to *Microbotryales* in molecular dating analysis. In the ML analysis *Leucosporidiales* is sister to *Kriegeriales* without statiscal support, then both of them are sister to *Microbotryales* without statiscal support too. Based on the younger divergence time of *Leucosporidiales* and its clustering with *Microbotryales* under 1.0 pp value support, we propose combining *Leucosporidiales* under *Microbotryales*. Its related orders (such as *Kriegeriales*) therefore need further studies. (ii) In class *Cystobasidiomycetes,* the genera *Buckleyzyma, Microsporomyces, Sakaguchia* and *Symmetrospora* are the only genera representing the families *Buckleyzymaceae, Microsporomycetaceae, Sakaguchiaceae* and *Symmetrosporaceae* respectively, and are placed in *Cystobasidiomycetes incertae sedis* at the ordinal level (Wang et al. 2015c). In the ML analysis, the type specimens of those four genera

do not cluster with any known orders with good statiscal support. *Buckleyzyma armeniaca* (type JCM 8977) and *Sakaguchia lamellibrachiae* (type CBS9598) are included in the molecular dating analysis and are positioned as sister to *Erythrobasidiales* with divergence times of 136 Ma and 122 Ma respectively. From these results we introduce the new orders *Buckleyzymales* and *Sakaguchiales* to accommodate the families *Buckleyzymaceae* and *Sakaguchiaceae*. Sequence data for members of the families *Microsporomycetaceae* and *Symmetrosporaceae* were not available for molecular dating and phyloproteomic analyses in this study; we therefore keep them in the *Cystobasidiomycetes*.

Buckleyzymales R.L. Zhao & K. D. Hyde, **order nov.**
Fungal Names: FN570465
Type Family: *Buckleyzymaceae* Q.M. Wang, F.Y. Bai, M. Groenew. & Boekhout, Stud. Mycol. 81:176 (2015)
Type genus: *Buckleyzyma* Q.M. Wang, F.Y. Bai, M. Groenew. & Boekhout. Stud. Mycol. 81:176 (2015)

The diagnosis of the order *Buckleyzymales* is based on the description of family Buckleyzymaceae, which is based on the description of the genus *Buckleyzyma*: "Sexual reproduction not known. Colonies brownish-orange or orange and butyrous. Budding cells present. Hyphae and pseudohyphae present or not. Ballistoconidia present or not, ellipsoidal allantoid to amygdaliform" (Wang et al. 2015c).

Sakaguchiales R.L. Zhao & K.D. Hyde, **order nov.**
Fungal Names: FN570466
Type family: *Sakaguchiaceae* Q.M. Wang, F.Y. Bai, M. Groenew. & Boekhout, Stud. Mycol. 81:177 (2015)
Type genus: *Sakaguchia* Y. Yamada et al., Biosc. Biotechn. Biochem. 58:102. 1994. emend. Q.M. Wang, F.Y. Bai, M. Groenew. & Boekhout., Stud. Mycol. 81:177 (2015)

The diagnosis of the order *Sakaguchiales* is based on the description of the family *Sakaguchiaceae*, which is based on the genus *Sakaguchia*: "Sexual reproduction in some species. Clamp connections present. Teliospores laterally or terminally on the hyphae. Teliospores germinate with two- to four-celled metabasidium with lateral and terminal basidiospores. Colonies red or orange-red and butyrous. Budding cells present. Pseudohyphae or true hyphae present or not. Ballistoconidia not produced" (Wang et al. 2015c).

Microbotryales R. Bauer & Oberw., Can. J. Bot. 75:1309 (1997)
= *Leucosporidiales* J.P. Samp. et al., Mycol. Prog. 2: 61 (2003)

Type family: *Microbotryaceae* R.T. Moore. Can. J. Bot. 75:1309 (1997)

3. Subphylum *Ustilaginomycotina* Doweld, Prosyllabus Tracheophytorum, Tentamen Systematis Plantarum Vascularium (Tracheophyta) (Moscow): LXXVIII (2001)
Class *Exobasidiomycetes* Begerow, M. Stoll & R. Bauer, Mycologia 98(6): 908 (2007) [2006]
Class *Malasseziomycetes* Denchev & T. Denchev, Index Fungorum 145: 1 (2014)
Class *Moniliellomycetes* Boekhout, Q.M. Wang & F.Y. Bai, Persoonia 33:46 (2014)
Class *Ustilaginomycetes* R. Bauer, Oberw. & Vánky, Can. J. Bot. 75: 1311 (1997)

Phylogenetic support
Ustilaginomycotina is a monophyletic group with 100% BS and 1.0 PP support (Figs. 1, 2, 3). The classes *Malasseziomycetes*, *Moliliellomycetes* and *Ustilaginomycetes* have 100% BS and 0.96–1.0 PP support in both analyses, however *Exobasidiomycetes* is polyphyletic in the molecular dating analysis and lacks good support in the ML analysis. *Ustilaginomycotina* diverged at 430 Ma, and the classes later diverged between 245 and 265 Ma (Fig. 2; Table 1). The orders of *Ustilaginomycetes*, *Malasseziomycetes* and *Moliliellomycetes* are monophyletic with 100% BS and 1.0 PP support (Figs. 1, 2, 3; Table 1). The orders *Exobasidiales* and *Doassansiales* of *Exobasidiomycetes* do not have high BS support in the ML analysis, but have 1 PP support in the molecular dating analysis, which only includes a small number of species. The mean of stem age of all orders in this subphylum are generally 187–265 Ma (Fig. 2; Table 1). Two recently proposed orders *Golubeviales* and *Robbauerales* of *Exobasidiomycetes* are divergent at 202 Ma (Fig. 2; Table 1).

Discussion
Ustilaginomycotina contains more than 1000 species, and comprises plant pathogenic fungi (smuts), which are mostly dimorphic and have a yeast-like stage during their life cycle. Traditionally *Ustilaginomycotina* contained two classes: *Ustilaginomycetes* and *Exobasidiomycetes* (Begerow et al. 2006; Matheny et al. 2007a; Hibbett et al. 2007). *Malassezia* grows on skin of animals and *Moniliella* (syn. *Trichosporonoides*) is a yeast-like taxon representing *Moliliellomycetes*. Those two genera and related orders are maintained in *Ustilaginomycotina incertae sedis* for a long time because their variable phylogenetic positions in different gene trees. They nest into *Exobasidiomycetes* or in *Ustilaginomycetes* using rDNA sequence data alone or in combination with protein genes (Begerow et al. 2000, 2006; Bauer et al. 2001; Weiß et al. 2004; Matheny

et al. 2007a). Until 2014, different analyses based on the sequences of six-gene confirmed that *Malassezia* and *Moniliella* belong to *Ustilaginomycotina*, and forming deep and well-supported lineages. The classes *Malasseziomycetes* and *Moniliellomycetes* were therefore proposed for these lineages, with additional support from phenotypic characters (Wang et al. 2014). In a recent phylogenetic study on *Ustilaginomycotina* using seven genes, *Exobasidiomycetes* resulted paraphyletic (Wang et al. 2015b). Two orders *Golubeviales* and *Robbauerales* were introduced in *Exobasidiomycetes*, so that there are eight orders in this class (Wang et al. 2015b).

In our study, the recently introduced classes *Malasseziomycetes* and *Moniliellomycetes* along with *Ustilaginomycetes* are well-supported and their divergence times fall within ranges of 245–330 Ma which is the most classes have. The class *Exobasidiomycetes* is paraphyletic as in the previous studies (Wang et al. 2014, 2015b). Two recently proposed orders *Golubeviales* and *Robbauerales* of *Exobasidiomycetes* are also supported in our study.

4. Subphylum *Wallemiomycotina* Doweld, in Index Fungorum no. 73 (2014)

Type class: *Wallemiomycetes* Zalar, de Hoog et Schroers, Antonie van Leeuwenhoek, 87:322 (2005)

Type order: *Wallemiales* Zalar, de Hoog & Schroers, Antonie van Leeuwenhoek 87(4): 322 (2005)

Type family: *Wallemiaceae* R.T. Moore, Rhizoctonia Species, Taxonomy, Molecular Biology, Ecology, Pathology and Disease Control (Dordrecht): 20 (1996)

Type genus: *Wallemia* Johan-Olsen. designated by Doweld in Index Fungorum no. 73 (2014)

Type species: *Wallemia ichthyophaga* Johan-Olsen 1887, designated by Zalar et al., Antonie van Leeuwenhoek, 87:322 (2005)

Phylogenetic support
Wallemiomycota is represented by four species in the ML analysis (Fig. 1). Sequences from the type strain of *Wallemia tropicalis* (stain number EXF_8739, from Jančič et al. 2016), and those of voucher stains for *W. ichthyophaga*, *W. muriae* and *W. sebi* from previous studies (Zalar et al. 2005; Matheny et al. 2007a) are included in the analyses. All *Wallemia* species cluster together with 100% BS and 1.0 PP support (Figs. 1, 2, 3). *Wallemiomycotina* is sister to other three subphyla of *Basidiomycota* in the ML and molecular dating analyses with 100% BS and 1.0 PP support (Figs. 1, 2) respectively. The mean of stem age of *Wallemiomycotina* is 487 Ma (Fig. 2). However in the phyloproteomics analysis it is sister to subphylum *Agaricomycotina*, then clade of *Agaricomycotina* + *Wallemiomycotina* is sister to the subphylum *Ustilaginomycotina* (Fig. 3).

Discussion
Subphylum *Wallemiomycotina* was proposed with a brief morphological description in Index Fungorum (Doweld 2014).

Based on a previous study, *Wallemia* is classified in the only family *Wallemiaceae* under the only order *Wallemiales* and the only class *Wallemiomycetes* based on phylogenetic analysis of 18S sequence data (Zalar et al. 2005). Four more species have recently been described (Jančič et al. 2016). Species of this class are xerophilic molds and have an unusual mode of asexual reproduction. Matheny et al. (2007a) investigated the phylogenetic placement using six nuclear genes. Their combined gene tree supports this class as being an isolated lineage and its position is basal to the core clade of *Pucciniomycotina*, *Ustilaginomycotina* and *Agaricomycotina* in *Basidiomycota* (Matheny et al. 2007a).

Our analysis, based on a larger sampling from *Basidiomycota* and *Entorrhizomycota* (*Ascomycota* as outgroups), confirms its distinct phylogenetic position, which is sister to other three subphyla of *Basidiomycota* with 100% BS and 1.0 PP support (Figs. 1, 2). While in the phyloproteomic analysis it shows up as sister to the *Agaricomycotina* (Fig. 3), which is in agreement with previous genomic studies (Zajc et al. 2013; Padamsee et al. 2012). In this study, we accept the class *Wallemiomycetes* to subphylum level and place it under *Wallemiomycotina* (Doweld 2014) to better fit its evolutionary history.

Phylum *Entorrhizomycota* R. Bauer, Garnica, Oberw., K. Riess, M. Weiß & Begerow, PLoS ONE 10(7): 10 (2015)

Phylogenetic support
Entorrhizomycota is represented by four species in our analysis, having 100% BS and 1.0 PP support, and is sister to the clade of *Basidiomycota* + *Wallemiomycota* (Figs. 1, 2). *Entorrhiza* and *Talbotiomyces* represent a deep dichotomy within the *Entorrhizomycota* in the ML Tree (Fig. 1). The stem age of this phylum is 530 Ma and is represented by two *Entorrhiza* species (Fig. 2).

Discussion
This phylum was established for a single genus, *Entorrhiza,* which is a small group of unusual teliosporic root parasites on *Juncaceae* and *Cyperaceae* (Begerow et al. 2006). Traditionally, species of *Entorrhiza* were considered as smut fungi and classified in *Tilletiaceae* (Zundel 1953). More recently based on ultrastructure and molecular phylogeny, *Entorrhiza* was accommodated in the distinct family, order and class, *Entorrhizaceae*, *Entorrhizales* and *Entorrhizomycetes* in the subphylum *Ustilaginomycotina* (Bauer et al. 1997; Begerow et al. 1997, 2006) and phylum *Basidiomycota*. It was recently separated from

Basidiomycota and established as a novel phylum *Entor-rhizomycota* containing the only class *Entorrhizomycetes* (Bauer et al. 2015). Molecular phylogenetic analyses as well as morphological and ecological data indicate that *Talbotiomyces calosporus* belongs to *Entorrhizomycota,* and a novel order *Talbotiomycetales* K. Riess, R. Bauer, R. Kellner, Kemler, Piątek, Vánky & Begerow and family *Talbotiomycetaceae* K. Riess, R. Bauer, R. Kellner, Kemler, Piątek, Vánky & Begerow were established based on this genus (Riess et al. 2015). Our study accepts this phylum based on the phylogeny and deep divergence.

Conclusions

Phylogenetic overview of Basidiomycota

In this study, we used sequence data from six-gene and a broad sampling that represents all classes of *Basidiomycota* except the *Cryptomycocolacomycetes* which lack proper sequence data. Almost all orders from each class have been included and are represented by all or most of known families. Exceptions are *Auriculariales* and *Cantharellales* (*Agaricomycetes*), *Atractiellales* (*Classiculomycetes*) and *Ustilaginales* (*Ustilaginomycetes*). We evaluated the relevance of higher-level taxa by considering whether or not they are monophyletic and have similar relative divergence times using the mean of stem age.

In our six-gene phylogenetic analysis (Figs. 1, 2), most higher-level taxa are monophyletic with sufficient statistical support (BS > 50% and PP > 0.8). Taxa that lacked high support included the *Agaricomycotina*, *Gomphales* and *Exobasidiales* (Figs. 1, 2); while some resulted polyphyletic/paraphyletic: *Exobasidiomycetes*, *Cantharellales* and *Hysterangiales* (*Agaricomycetes*), and *Unilacrymales* (*Dacrymycetes*). The recently introduced order *Unilacrymales* nests in the *Cerinomycetaceae* clade of *Dacrymycetales* in ML tree. Some of these unsupported taxa agree with previous studies, such as the polyphyletic groups in *Exobasidiomycetes* (Wang et al. 2015a), but the others differ from previous studies (*Cantharellales* in Veldrea et al. 2013; *Hysterangiales* in Hosaka et al. 2006; *Unilacrymales* in Shirouzu et al. 2013). Thus, further comprehensive studies are needed to clarify these disagreements.

Our results widely agree with dating estimates reported by previous researchers. For example, the phylum *Basidiomycota* originated more than 500 Ma according to Floudas et al. (2012), compared to 530 Ma in this study; *Agaricomycotina* diverged 429 Ma according to Floudas et al. (2012), compared to 406 Ma in this study. The divergence estimates for *Agaricomycetidae* were 149 Ma in Floudas et al. (2012) and 203–250 Ma in Garcia-

Sandoval et al. (2011), versus 173 Ma in our study. The class *Dacrymycetes* originated 280–360 Ma according to Dentinger et al. (2010), versus 358 Ma in this study. The orders *Boletales* and *Gloeophyllales* originated during the early Cretaceous (around 66–146 Ma in Garcia-Sandoval et al. 2011) and in our study, they are divergent at 146 and 124 Ma respectively. However, in our study, *Polyporales* has a younger divergence time compared to the estimates of Garcia-Sandoval et al. (2011), which estimated the order *Polyporales* to have evolved during the late Jurassic, about 203–250 Ma.

Based on our study, the phyla and subphyla of *Basidiomycota* formed ca. 530 Ma and 406–490 Ma respectively, so we accept these divergence times ranges as supplementary criteria in ranking phyla and subphyla. These estimated divergence times of classes within *Basidiomycota*, were variable in different subphyla. For example, divergence times of classes in *Agaricomycotina* were estimated in a narrow range of 358–393 Ma. However, divergence times for classes of *Pucciniomycotina* and *Ustilaginomycotina* are mostly estimated 245–356 Ma, which are much wider than those classes of *Agaricomycotina*. However, the estimated divergence times of orders within those different subphyla remain between 120 and 290 Ma (Table 1). We use these divergence time ranges as criteria for the ranking of phyla, subphyla, classes and orders within *Basidiomycota* in this study.

Based on our relative divergence time estimates and phylogenetic analyses, we conclude that most higher-level taxa in *Basidiomycota* are well-supported, especially the recently introduced taxa: phylum *Entorrhizomycota*, classes *Malasseziomycetes*, *Moniliellomycetes*, *Spiculogloeomycetes*, *Tritirachiomycetes* and orders *Holtermanniales*, *Trichosporonales*, *Golubeviales*, *Robbauerales*, *Amylocorticiales*, *Jaapiales*, *Stereopsidales* and *Lepidostromatales*.

However, some taxa are not well-supported. The problems are found at the order level as follows: (1) *Unilacrymales* (*Dacrymycetes*) nests in the clade of *Cerinomycetaceae* of *Dacrymycetales* with 100% BS in ML tree (Fig. 1), however it is sister to *Dacrymycetales* with divergence time of 225 Ma in the MCC tree (Fig. 2). In this study we accept *Unilacrymales* but further scrutiny is needed. (2) *Leucosporidiales* (*Microbotryomycetes*) has a divergence of 74 Ma, which is much younger than other orders (120–290 Ma), thus we propose combining *Leucosporidiales* under the older name of *Microbotryales*. (3) Two new orders *Buckleyzymales* and *Sakaguchiales* are introduced to accommodate the families *Buckleyzymaceae* and *Sakaguchiaceae* (*Cystobasidiomycetes*) due to their older divergence times. (4) The oldest divergence time and distinct phylogenetic position of *Cystofilobasidiales* of *Tremellomycetes* indicates it should be raised to a higher

rank. We however, do not introduce a new class here, as DNA sequence data from these taxa are not from their respective types, thus we await further studies. (5) Monophyletic evaluation and divergence time estimates for some higher-level taxa (i.e. *Exobasidiomycetes, Cantharellales, Gomphales,* and *Hysterangiales*) were based on limited species sequences in this study, therefore, await further scrutiny.

The tree topology of our phyloproteomic analysis largely agrees with the six-gene trees (Figs. 1, 2, 3). The main exceptions are followings: (1) the monophyletic *Wallemiomycetes* is sister to three subphyla of *Basidiomycota* (*Agaricomycotina* + *Pucciniomycotina* + *Ustilaginomycotina*) in the six-gene analysis, however, it sister to subphylum *Agaricomycotina* in phyloproteomic analysis. In this study we recognize this group at the subphylum level (*Wallemiomycotina*) but further studies are needed. (2) the positions of the *Agaricomycetes* orders *Amylocorticiales, Auriculariales, Cantharellales, Geastrales, Sebacinales* and *Trechisporales* are inconsistent between the six-gene phylogeny and phyloproteomic analyses, therefore, also need further scrutiny (Figs. 1, 2, 3).

Discussion on taxa rank using divergence times

The divergence time of a lineage could be used as a universal criterion for ranking taxa. However, there are still some obstacles to overcome, and one is how to choose proper calibration points in the dating analysis. Fossil isotopic ages aid estimation of molecular divergence time by providing points of calibration (Berbee and Taylor 2010). Several mushroom fossils have been discovered and these are used as calibration points in modern molecular clock analysis within *Basidiomycota*. Fossils included are *Archaeomarasmius leggetti* within the order *Agaricales* (Hibbett et al. 1995, 1997) and *Quatsinoporites cranhamii* within the order *Hymenochaetales* (Smith et al. 2004). These fossils calibrations have been used in several studies (Gueidan et al. 2011; Skrede et al. 2011, Floudas et al. 2012; Sánchez-Ramírez et al. 2015; Zhao et al. 2016; see Sánchez-Ramírez et al. 2017 for other potentially useful fossils). However, the fossil record for such a large group as *Basidiomycota* is relatively poor. Other sources that have the potential to address ages, are vicariant events and fossils of obligate symbionts of ectomycorrhiza hosts (Hibbett 2001; Hibbett and Matheny 2009; Matheny et al. 2009; Wilson et al. 2012; Sánchez-Ramírez et al. 2017), such as the putative suilloid ectomycorrhiza (LePage et al. 1997). The genera *Termitomyces* and *Amylostereum* which are associated with termite and sawflies respectively, could be dated through these arthropods (Slippers et al. 2003; Mikheyev et al. 2010; Nobre et al. 2011). Reliably identified fossils are still thought to be too few to establish reliable dates (Berbee and Taylor 2010; Hibbett 2014;

Sánchez-Ramírez et al. 2017). Different researchers may also choose different calibration points in their dating analysis, and this may result in inconsistent divergence times for the same group (Sánchez-Ramírez et al. 2017). For example, the divergence estimates for *Inocybaceae* (*Agaricales*) was 143 (99–191) Ma according to Matheny et al. (2009), whereas the common ancestor of *Inocybaceae* and *Crepidotaceae* evolved ca. 45 (30–60) Ma according to Skrede et al. (2011).

Another difficulty in dating analysis is the diversification rates. In cladistics, a lineage consists of a stem and a crown group. The stem group refers to those of origin, which may include not only the most recent common ancestor of all of its members and their descendants, but also extinct lineages. The crown group comprises the last common ancestor of a living clade, plus all of its descendants (Budd and Jensen 2000; Budd 2001). Reflection of groups in divergence times are stem and crown age respectively. This means that for any given group of taxa the crown age is always younger than the stem age (Stadler et al. 2014). Some researchers have determined that the length of branch between stem ancestor and crown clade depends on factors, such the timescale, the net diversification rate, and the species richness of the clade (McPeek and Brown 2007; Stadler et al. 2014). At the species level, the most recent research has synthesized a global time tree of life from 50,632 species, and examined the pattern and rate of diversification, as well as the timing of speciation using genomic data. The rate of diversification in eukaryotes has been mostly constant (Hedges et al. 2015). In the present and our previous study (Zhao et al. 2016), we use stem ages to reflect divergence times.

Other factors which may affect the reliability of divergence time estimates are the exorbitant data sizes, the difficulty in correctly modelling the rate heterogeneity in highly diverse taxonomic groups, and the uncertain evolutionary rates for most groups of species. Evolutionary rates vary extensively among lineages when following autocorrelated and uncorrelated models (Berbee and Taylor 2010; Tamura et al. 2012). Some researchers have attempted to estimate the dating of evolutionary events using relaxed molecular clock analyses, which could compensate for the rate heterogeneity (Drummond et al. 2006; Garcia-Sandoval et al. 2011).

Tamura et al. (2013) proposed a method of relative time estimates (RelTime) for determining the relative ordering and spacing of evolutionary events, identifying lineages with significantly slower or faster evolutionary rates, diagnosing the effect of selected calibrations on absolute divergence times, and estimating absolute times of divergence when highly reliable calibration points are available. Divergence times from BEAST and RelTime methodologies are becoming universal criteria in taxa ranking.

Although challenges in dating analysis are apparent as discussed above, the introduction of divergence times into systematics research under the framework of a robust phylogeny, will provide extra evidence to evaluate the application of higher taxon ranks. Presently, these may not be perfect clocks that reflects perfect lineages, but they provide extra quantitative evidence as compared to the known limitations expressed in morphologies in present modern systematics arrangements.

Phylogenomics perspectives

The examination of more than 400 articles reveals that aprox. 40% of the phylogenetic studies that used more than one gene have reported incongruence among gene phylogenies (Rokas and Chatzimanolis 2010). Thus, the phylogeny based on single-gene data is still insufficient to reconstruct consistent and accurate phylogenetic hypotheses due to the conflicting gene trees. Phylogenetic reconstructions from genome-scale data could eventually solve these problems (Rokas et al. 2003; Delsuc et al. 2005; Bushley and Turgeon 2010; Medina et al. 2011; Capella-Gutiérrez et al. 2012; Shelest and Voigt 2014).

In this study, we used 396 orthologous genes from 109 taxa with published genomes in *Basidiomycota* to detect the phylogenetic positions of the most higher-level taxa in this phylum. The results largely agree to those of phylogenetic topology based on six-gene sequences. However, there are some inconsistencies, such for the subphylum *Wallemiomycotina,* orders *Amylocorticiales, Auriculariales, Cantharellales, Geastrales, Sebacinales* and *Trechisporales* from *Agaricomycetes*. The increasing availability of genomic data will enable researchers to overcome those phylogenetic problems in the future. On the other hand, those inconsistent positions would provide the clues to demonstrate potential horizontal gene transfer, introgression, hidden paralogs and lineage sorting throughout the evolution of the *Basidiomycota*.

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