



Geoglossomycetes cl. nov., Geoglossales ord. nov. and taxa above class rank in the Ascomycota Tree of Life

C.L. Schoch¹, Z. Wang², J.P. Townsend², J.W. Spatafora³

Key words

Bayesian inference
hybrid classification
maximum likelihood

Abstract Featuring a high level of taxon sampling across *Ascomycota*, we evaluate a multi-gene phylogeny and propose a novel order and class in *Ascomycota*. We describe two new taxa, *Geoglossomycetes* and *Geoglossales*, to host three earth tongue genera: *Geoglossum*, *Trichoglossum* and *Sarcoleotia* as a lineage of 'Leotiomyceta'. Correspondingly, we confirm that these genera are not closely related to the genera *Neolecta*, *Mitula*, *Cudonia*, *Microglossum*, *Thuemenidium*, *Spathularia* and *Bryoglossum*, all of which have been previously placed within the *Geoglossaceae*. We also propose a non-hierarchical system for naming well-resolved nodes, such as 'Saccharomyceta', 'Dothideomyceta', and 'Sordariomyceta' for supraordinal nodes, within the current phylogeny, acting as rankless taxa. As part of this revision, the continued use of 'Leotiomyceta', now as a rankless taxon, is proposed.

Article info Received: 16 April 2009; Accepted: 11 May 2009; Published: 5 June 2009.

INTRODUCTION

The multi-gene sequence datasets generated by the research consortium 'Assembling the Fungal Tree of Life' (AFTOL) have resulted in several multi-gene phylogenies incorporating comprehensive taxon sampling across Fungi (Lutzoni et al. 2004, Blackwell et al. 2006, James et al. 2006). AFTOL generated a data matrix spanning all currently accepted classes in the *Ascomycota*, the largest fungal phylum. The phylogenies produced by AFTOL prompted the proposal of a phylogenetic classification from phylum to ordinal level in fungi (Hibbett et al. 2007). Although the Botanical Code does not require the principle of priority in ranks above family (McNeill et al. 2006), this principle was nevertheless followed for all taxa. The following ranked taxa were defined: subkingdom, phylum (suffix -mycota, except for Microsporidia), subphylum (-mycotina), class (-mycetes), subclass (-myctidae) and order (-ales). As in Hibbett et al. (2007), several phylogenetically well-supported nodes above the rank of order could not be accommodated in the current hierarchical classification system based on the International Code of Botanical Nomenclature. To remedy this deficiency, rankless (or unranked) taxa for unambiguously resolved nodes with strong statistical support was proposed (Hibbett & Donoghue 1998). Hybrid classifications that include both rankless and Linnaean taxa have since been discussed elsewhere (Jørgensen 2002, Kuntner & Agnarsson 2006), and applied to diverse organisms from lichens (Stenroos et al. 2002) and plants (Sennblad & Bremer 2002, Pfeil & Crisp 2005) to spiders (Kuntner 2006). These studies all attempt to create a comprehensive code for phylogenetic nomenclature that retains the current Linnean hierarchical codes.

In keeping with the practice of previous hybrid classifications, we propose to use names corresponding to clades of higher taxa that were resolved in this phylogeny as well as preceding studies.

The proposed informal, rankless names for well-supported clades above the class level in our phylogeny agrees with the principles of the PhyloCode (<http://www.ohio.edu/phylocode/>). It is our hope that such names should function as rankless taxa, facilitating the naming of additional nodes/clades as they become resolved. Eventual codification will follow the example of Hibbett et al. (2007) by applying principles of type names and priority. A number of published manuscripts already provide background on other supraordinal relationships of Fungi; for more complete treatments of the various classes, see Blackwell et al. (2006).

During the AFTOL project a data matrix was generated spanning all currently accepted classes in the *Ascomycota*, the largest fungal phylum. A multi-gene phylogeny was recently inferred from these data, demonstrating relevant patterns in biological and morphological character development as well as establishing several distinct lineages in *Ascomycota* (Schoch et al. 2009). Here we test whether the relationships reported in Schoch et al. (2009) remain valid by applying both maximum likelihood (ML) and Bayesian analyses on a more restricted but denser set of taxa, including expanded sampling in the *Geoglossaceae*.

We will therefore address the taxonomic placement of a group of fungi with earth tongue morphologies that are shown to be unrelated to other known classes. This morphology is closely associated with the family *Geoglossaceae* (Corda 1838). With typical inoperculate asci and an exposed hymenium, *Geoglossaceae* has long been thought to be a member of *Leotiomycetes*, though the content of the family itself has experienced many changes (Nannfeldt 1942, Korf 1973, Spooner 1987, Platt 2000, Wang et al. 2006a, b). It is currently listed with 48 species and 6 genera in the Dictionary of the Fungi (Kirk et al. 2008). Several analyses using molecular data supported a clade including three earth tongue genera, *Geoglossum*, *Trichoglossum* and *Sarcoleotia* (Fig. 1), and cast doubt upon their positions in *Leotiomycetes* (Platt 2000, Gernhardt et al. 2001, Lutzoni et al. 2004, Sandnes 2006, Spatafora et al. 2006, Wang et al. 2006b). Here we present a comprehensive phylum-wide phylogeny, including data from protein coding genes. We can confidently place the earth tongue family as separate from currently accepted classes in *Ascomycota*.

¹ National Center for Biological Information (GenBank), National Library of Medicine, National Institute of Health, 45 Center Drive, MSC 6510, Bethesda, Maryland 20892-6510, USA;
corresponding author e-mail: schoch2@ncbi.nlm.nih.gov

² Department of Ecology and Evolutionary Biology, Yale University, 165 Prospect Street, New Haven, CT 06520, USA.

³ Department of Botany and Plant Pathology, Oregon State University, Corvallis, OR 97331, USA.

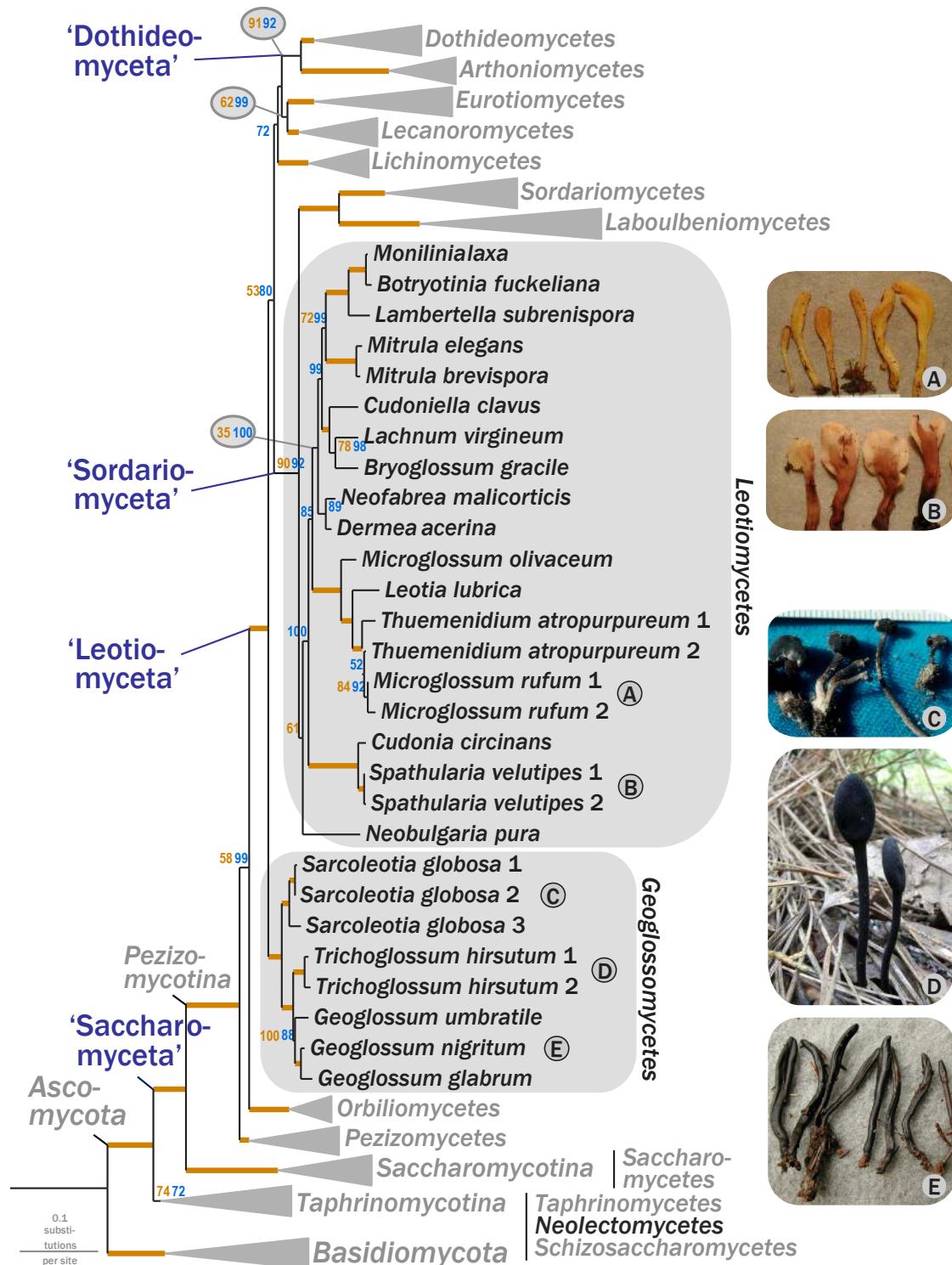


Fig. 1 A most likely tree obtained by RAxML for Ascomycota. Subphyla, class and rankless taxa are indicated. Classes containing fungi designated as earth tongues are indicated in black. The tree was rooted with outlier *Rhizopus oryzae* (not shown). Bootstrap values are shown in orange and Bayesian posterior probabilities in blue. Orange, bold branches are supported by more than 80 % bootstrap and 95 % posterior probability, respectively. The full phylogeny, without collapsed clades, are shown in Fig. 2. The inset figures illustrate morphological ascospore diversity in the earth tongues. The species are as follows: A: *Trichoglossum hirsutum*; B: *Geoglossum nigritum*; C: *Microglossum rufum*; D: *Spathularia velutipes*; E: *Geoglossum nigritum*. Photo credits: A: Zhiliang Yang; B, D, E: Kentaro Hosaka; C: Dan Luoma.

MATERIALS AND METHODS

Data were extracted from the complete data matrix obtained from the WASABI database (www.aftol.org), incorporating representatives for all currently accepted classes, and maximizing the number of orders and available data. Following the approach of James et al. (2006) we performed a combined analysis, with both DNA and amino acid data, while allowing for missing data. This data was supplemented with additional ribosomal sequences from earth tongue genera obtained and

deposited in GenBank from two previous studies (Wang et al. 2006a, b). To further minimise poorly aligned areas, 219 additional columns, which proved variable when viewed in BioEdit with a 40 % shade threshold, were excluded from the original AFTOL inclusion set. The refined dataset consisted of 161 taxa (including outgroups) and 4 429 characters for six different loci: the nuclear small and large ribosomal subunits (nSSU, nLSU), the mitochondrial small ribosomal subunit (mSSU) and fragments from three proteins: transcription elongation factor 1 alpha (*TEF1*) and the largest and second largest subunits of

RNA polymerase II (*RPB1*, *RPB2*). A complete table with the published GenBank numbers is listed in Table 1.

The phylogenetic analysis was run in RAxML v7.0.0 (Stamatakis 2006), partitioning by gene (six partitions) and estimating unique model parameters for each gene, as in Schoch et al. (2009). Models of evolution were evaluated as in Schoch et al. (2009) with the same models selected. For DNA sequences, this resulted in a general time reversible model (GTR) with a discrete gamma distribution composed of four rate classes plus an estimation of the proportion of invariable sites. The amino acid sequences were analysed with a RTREV model with similar accommodation of rate heterogeneity across sites and proportions of invariant sites. In addition, protein models for *TEF1* and *RPB2* incorporated a parameter to estimate amino acid frequencies. The tree shown in Fig. 1 was obtained by using an option in RAxML running a rapid bootstrap analysis and search for the best-scoring ML tree in one single run. This meant the GTRCAT model approximation was used, which does not produce likelihood values comparable to other programs. The full tree is shown here as Fig. 2 and was deposited in TreeBASE (www.treebase.org). We also ran 100 repetitions of RAxML under a gamma rate distribution option. The best scoring tree was included in TreeBASE.

A second analysis was run using Bayesian inference of maximum likelihood in MrBayes v3.1.2 (Huelsenbeck & Ronquist 2001, Altekar et al. 2004) using models and parameters that were comparable to the maximum likelihood run. Data were similarly partitioned and amino acids were analysed, so that a mixture of models with fixed rate matrices for amino acid sequences could be evaluated. In all cases rate heterogeneity parameters were used by a discrete gamma distribution plus an estimation of the proportion of invariable sites. A metropolis coupled Markov Chain Monte Carlo analysis was run for 9 million generations sampling every 200th cycle, starting from a random tree and using 4 chains (three heated and one cold) under default settings. Two separate runs were confirmed to converge using Tracer v1.4.1 (<http://tree.bio.ed.ac.uk/software/tracer/>). The first 10 000 sampled trees (2 million generations) were removed as burn in each run. A 50 % majority rule consensus tree of 70 000 Bayesian likelihood trees from the two combined runs was subsequently constructed, and average branch lengths and posterior probabilities determined. The numbers of nodes shared with the most likely tree in Fig. 1 was determined and plotted on the branches. This tree was deposited in TreeBASE, along with the inclusive character set.

RESULTS

The phylogeny presented in Fig. 1 supports 15 classes (11 in *Pezizomycotina*, 1 in *Saccharomycotina*, 3 in *Taphrinomycotina*) with good statistical support (both ML bootstrap and Bayesian posterior probability) for 14. Phylogenies with all lineages in the analysed data matrix are included in Fig. 2. A run with 100 repetitions of RAxML under a gamma rate distribution option resulted in a best scoring tree with a log likelihood of -111983. This tree shared the same supported nodes with the one presented in Fig. 1 but had changes in poorly supported nodes regarding placement of the *Eurotiomycetes* and *Dothideomycetes*. The two Bayesian runs produced trees with harmonic means of likelihood values of -112094 and -112076, respectively, with similar topological differences in poorly supported nodes.

As can be seen in Fig. 1, we continue to find low bootstrap and posterior probability support for *Leotiomycetes* as a monophyletic clade using a combined analysis of protein and nucleic acids. In our analysis, this includes *Neobulgaria pura* as the earliest diverging lineage. The node internal from this lineage

is found in all ML bootstrap trees, suggesting that this taxon is unstable in our analyses. No conflicts were detected in *Neobulgaria* genes under a previous study and missing data did not affect important nodes (Schoch et al. 2009). A repeat run under maximum likelihood was done with *Neobulgaria pura* removed under the same settings but with only 100 bootstrap repetitions. This trimmed dataset yielded a congruent phylogeny with increased bootstrap for *Leotiomycetes* (78 %; data not shown). The instability of the placement of *Neobulgaria pura* does not compromise any of the conclusions we present here and may be due to various reasons. Improved taxon sampling will likely help to resolve its placement in future analyses.

We find support for numerous backbone nodes in *Ascomycota*, as did Schoch et al. (2009). Our phylum-wide sampling of *Ascomycota* classes in this study, combined with the results of a previous study (Schoch et al. 2009), facilitated addressing the placement of the previously problematic and unsampled lineages such as the *Geoglossaceae* in relation to all currently accepted *Ascomycota* classes.

Taxonomy

Given their unique ascromatal development, ultrastructure of ascus apical apparatus, mossy habitat, and our multilocus gene phylogeny, *Geoglossomycetes* cl. & ord. nov. is justified here as incertae sedis in *Pezizomycotina* and 'Leotiomyceta'.

Geoglossomycetes, Geoglossales Zheng Wang, C.L. Schoch & Spatafora, cl. & ord. nov. — MycoBank MB513351, MB513352

Ascomata solitaria vel gregaria, capitata, stipitata; stipe cylindricus, atrum, glabrum vel furfuraceus. Regio hymenali capitata, clavata vel pileata, indistinctum ex stipe; hymenium atrum, continuatum stipe ad praematu incrementi grado. Asci clavati, inoperculati, octospori, poro parvo in iodo caerulecentes. Ascospores elongatae, fuscae, pullae vel hyalinae, multi-septatae. Paraphyses filiformes, pullae vel hyalinae. Distributio generalis, terrestris, habita locis fere uliginoso et musoso.

Type genus. *Geoglossum* Pers., Neues Mag. Bot. 1: 116. 1794; *Geoglossaceae*.

Ascomata scattered to gregarious, capitate, stipitate; stipe cylindrical, black, smooth to furfuraceous. Ascigerous portion capitate, club-shaped to pileate, indistinguishable from stipe. *Hymenium* surface black, continues with stipe at early development stage. Asci clavate, inoperculate, thin-walled, J+, usually 8-spored. Ascospores elongate, dark-brown, blackish to hyaline, septate when mature. Paraphyses filiform, blackish to hyaline. Global distribution, terrestrial, habitat usually boggy and mossy.

DISCUSSION

In keeping with the phylogeny presented in Fig. 1, we endorse use of the -myceta suffix in order to circumscribe well-supported clades above class. The numbers of these clades are limited, and the use of such taxa will continue to become more practical as our biological knowledge base broadens. Use of this suffix will also allow for the continued use of *Leotiomycetes*, a taxon that has already been defined with a Latin diagnosis provided as a ranked superclass (Eriksson & Winka 1997) and remains in use (Lumbsch et al. 2005, Wang et al. 2006a). We propose its continued use, but as a rankless taxon together with the newly proposed rankless taxa, 'Saccharomyceta', 'Dothideomyceta' and 'Sordariomyceta'. Since these taxa are not currently accepted under the Code (McNeill et al. 2006), we will refrain from formal designations. The relevant clades are discussed below with the informal designations indicated in single quotations.

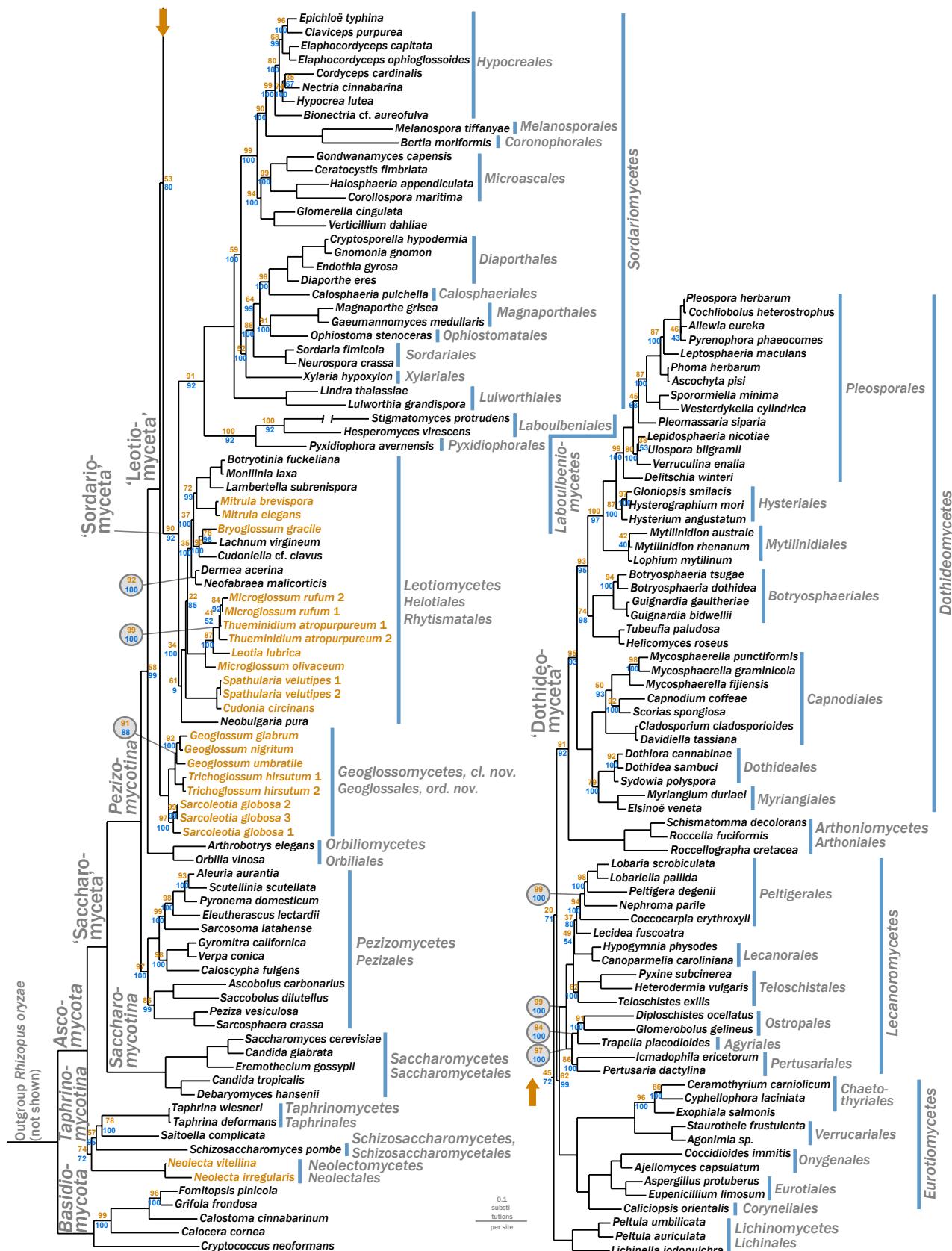


Fig. 2 A most likely tree obtained by RAxML for Ascomycota (as in Fig. 1). Phyla, subphyla, class, order and rankless taxa are indicated. Taxa designated as earth tongues are indicated in orange. The tree is displayed as two subtrees – orange arrows indicate where the subtrees were joined. The tree was rooted with outgroup *Rhizopus oryzae* (not shown). Bootstrap values are shown in orange above nodes and Bayesian posterior probabilities in blue below. Numbers were removed for nodes with 100 % bootstrap and 100 % posterior probability.

Subphylum Taphrinomycotina

As in recent studies using large multi-gene datasets (Spatafora et al. 2006, Sugiyama et al. 2006, Liu et al. 2009, Schoch et al. 2009), we find ML bootstrap support here for the monophyly of the *Taphrinomycotina*. The addition of sequences from protein coding genes has been vital to the establishment of statistical support for this grouping. Recent work has shown that the short generation times characteristic of species in this group make phylogenetic analyses particularly susceptible to long branch attraction artefacts (Liu et al. 2009). The placement of *Neolecta* in this subclade is also confirmed here. The club-shaped apothecia of the members of *Neolecta* share superficial similarity with those of the *Geoglossaceae*. *Neolecta* was long thought to be included in the *Geoglossaceae* until molecular work proved otherwise (Landvik 1996). In support of its placement in this early diverging group, *Neolecta* has several presumably ancestral features, such as simplified non-poricidal ascospores without croziers and the absence of paraphyses (Redhead 1979, Landvik et al. 2003). With additional sampling of both taxa and genes we find here moderate support for the monophyly of *Taphrinomycotina*, and thus demonstrate that the earliest diverging clade of the *Ascomycota* was dimorphic, with both filamentous and yeast growth forms. Nevertheless, it remains apparent that this part of the *Ascomycota* tree remains under sampled. This lack of adequate sampling is supported by the recent description of a clade labelled 'Soil Clone Group I' (SCGI). SCGI is ubiquitous in soil and is only known from environmental sequence data (Porter et al. 2008). It appears possible that they form a novel early diverging lineage outside of *Taphrinomycotina*. Very little remains known about their ecology, morphology and general biology.

Rankless taxon 'Saccharomyceta'

'Saccharomyceta' includes the two remaining subphyla of *Ascomycota*, *Saccharomycotina* and *Pezizomycotina*. *Saccharomycotina* comprises the 'true yeasts' (e.g., *Saccharomyces cerevisiae*), although hyphal growth has been documented in some taxa (e.g., *Eremothecium*). The *Pezizomycotina* consists of the majority of filamentous, ascospore producing species, but numerous species are additionally capable of yeast and yeast-like growth phases. Thousands of species are only known to reproduce asexually. These two subphyla form a well-supported, monophyletic group that has been recovered in a large number of studies across a diversity of character and taxon sets. The recognition of 'Saccharomyceta' highlights the shared common ancestry of these two taxa and the inaccurate characterisation of *Saccharomycotina* as a primitive or basal lineage of the *Ascomycota*. Rather, its small genome size (Dujon et al. 2004) and dominant yeast growth phase can be characterized as derived traits for this subphylum.

Rankless taxon 'Leotiomyceta'

We apply 'Leotiomyceta' as a rankless taxon containing the majority of fungi with a diversity of inoperculate ascospores (e.g., fissitunicate, poricidal, deliquescent). 'Leotiomyceta' excludes the earliest diverging classes of *Pezizomycotina*, *Pezizomycetes* and *Orbillomycetes*. It was first defined as a superclass (Eriksson & Winka 1997). This definition has remained in use (Lumbsch et al. 2005, Spatafora et al. 2006). Included in this clade are the informal, rankless taxa 'Dothideomyceta', 'Sordariomyceta', as well as the classes *Eurotiomycetes*, *Lecanoromycetes*, *Lichinomycetes*, and a newly proposed class, *Geoglossomycetes*.

The type genus of *Geoglossaceae*, *Geoglossum* was initially proposed by Persoon (1794). Persoon described it as club-shaped, with unitunicate, inoperculate ascospores, with the type species given as *Geoglossum glabrum* Pers. *Trichoglossum*

have historically been classified in *Geoglossaceae*, and *Sarcoleotia* has historically been classified in the *Helotiaceae* (*Leotiomycetes*). These inoperculate *Discomycetes* produce terrestrial, stipitate, clavate ascomata, commonly referred to as earth tongues, which include *Leotia*, *Microglossum*, *Cudonia*, and *Spathularia*. In terms of ascospore development, species of *Geoglossum*, *Trichoglossum*, and *Sarcoleotia* possess a hymenium that freely develops towards the base, while other earth tongue fungi feature a distinct ridge to their hymenium, implying a developmental stage during which the hymenium is enclosed (Schumacher & Sivertsen 1987, Spooner 1987, Wang et al. 2006b). An enclosed hymenium has been observed as well in several other lineages, such as *Cyttaria*, *Erysiphales* and *Rhytismatales* in the *Leotiomycetes* (Korf 1983, Gargas et al. 1995, Johnston 2001). Although the name earth tongue implies these fungi are terrestrial and have no direct association found with other organisms, *Trichoglossum*, *Geoglossum* and *Sarcoleotia globosa* have often been recorded in boggy habitats abundant with bryophytes (Seaver 1951, Dennis 1968, Schumacher & Sivertsen 1987, Spooner 1987, Jumpponen et al. 1997, Zhuang 1998). Ascospore apical morphology is one of the major features in distinguishing higher ascomycetes, and operculate ascomycetes as members of *Pezizales* have an apical or subapical operculum which is thrown back at spore discharge while a definite plug is present in the thickened 'inoperculate' ascospore apex as in species of the *Helotiales* (Korf 1973). Ultrastructure of the ascospore apical apparatus suggested no close relationship between *Leotia lubrica* and species of *Geoglossum* and *Trichoglossum*. A structure known as a tractus connects the uppermost spore to the apical wall and the spores to each other in *Trichoglossum hirsutum*, but is never found in other species of the *Helotiales* and is possibly homologous to structures in *Sordariomycetes* and *Pezizomycetes* (Verleyen 1994). Recent molecular phylogenetic analyses (Sandnes 2006, Wang et al. 2006a, b) confirmed that the earth tongue fungi are not monophyletic. At least two origins occurred in *Leotiomycetes*: in *Leotia* and allies in *Helotiales*, and in *Cudonia* and allies in *Rhytismatales*. *Geoglossum*, *Trichoglossum*, *Sarcoleotia* (*Geoglossomycetes* as we define it) represent a third, independent lineage of earth tongues, which we confirmed does not belong within the *Leotiomycetes*.

DNA-only and combined model analyses produced conflicting placements of *Geoglossaceae* within *Pezizomycotina*. Previous analyses applying nucleotide sequences only placed the order as a sister group to the *Lichinomycetes* (Lutzoni et al. 2004, Spatafora et al. 2006), which includes a small number of lichenised species mainly associated with cyanobacteria (Reeb et al. 2004). Our sampling of *Lichinomycetes* includes two genera, *Peltula* and *Lichinella* that encompass at least some of the ascospore diversity, i.e., rostrate and deliquescent, present in the class. In contrast, our combined amino acid and nucleotide model analyses resolved *Geoglossaceae* as an isolated, unique lineage of 'Leotiomyceta' with no supported sister relationship, in agreement with Schoch et al. (2009). Different levels of missing data underlie these two conflicting topologies, and several phenomena can potentially explain this conflict, ranging from model misspecification to long-branch attraction. Regardless of these concerns, our conclusion that the *Geoglossaceae* is a monophyletic lineage, unaligned with members of the *Leotiomycetes* and any of the other large fungal classes remains strongly supported.

Eurotiomycetes and *Lecanoromycetes* are the two remaining classes in 'Leotiomyceta'. *Eurotiomycetes* is arguably the most ecologically diverse class within *Ascomycota* including lichenised species, saprobes and pathogens of animals and plants. As currently defined, this class incorporates several distinct orders and three subclasses spanning virtually all known fun-

Table 1 Taxa and sequences used in this study.

AFTOL no.	Class	Order	Voucher ¹	Taxon	nSSU	nLSU	nSSU	nLSU	RPB1	RPB2	TEF1
1241	Zygomycota outgroup		GB GEI_5359	<i>Rhizopus oryzae</i> <i>Calocera cornea</i>	AF113440 AY771610	AY213626 AY701526	AY863212	AY857980 AY857979	Genome Genome	AY56286 AY780939	Genome
438	<i>Basidiomycota</i> outgroup		AV 136	<i>Calostoma cinnabarinum</i>	AY665773	AY645054		XM_570943		XM_570204	AY879117
439	<i>Basidiomycota</i> outgroup		GB	<i>Cryptococcus neoformans</i>		Genome		AY864874		AY786056	Genome
1088	<i>Basidiomycota</i> outgroup		MB 03-036	<i>Fomitopsis pinicola</i>	AY705967	AY684164	FJ436112			AY885152	AY885153
770	<i>Basidiomycota</i> outgroup		DSH s.n.	<i>Grifola frondosa</i>	AY705960	AY629318	AY786057	AY864876	DQ782825	DQ782866	
701	<i>Basidiomycota</i> outgroup		Diederich 15572	<i>Roccellographa cretacea</i>	AY594654	AY704082			DQ883733	DQ883733	
126	<i>Arthoniomycetes</i>		BG Prinzen1981	<i>Schismatomma decolorans</i>	DQ883705	DQ883696	FJ772240		DQ883715	DQ883725	
93	<i>Arthoniomycetes</i>		DUKE 0047570	<i>Botryosphaeria dothidea</i>	AY548809	AY548816	DQ677944		DQ676737	DQ7677944	
307	<i>Arthoniomycetes</i>		CBS 115476	<i>Botryosphaeria tsugae</i>	DQ677998	DQ678051	FJ190612		DQ7677914	DQ6777914	
946	<i>Dothideomycetes</i>		CBS 418.64	<i>Botryosphaeriales</i>	AF271127	DQ767655	DQ767644		DQ677798	DQ677798	
1586	<i>Dothideomycetes</i>		1618	<i>Botryosphaeriales</i>	DQ678034	DQ678085	FJ190646		DQ677987	DQ677987	
			1784	<i>Botryosphaeriales</i>	DQ678089	DQ678098	FJ190609		DQ677788	DQ677788	
			939	<i>Capnodiales</i>	DQ247808	DQ247800	FJ190604		DQ677952	DQ677952	
			1289	<i>Capnodiales</i>	DQ678004	DQ678057	FJ190628		DQ677791	DQ677791	
			1591	<i>Capnodiales</i>	DQ678022	DQ678074	DQ677971		DQ6777920	DQ6777920	
			2021	<i>Capnodiales</i>	DQ767652	DQ678098	FJ190656		DQ677993	DQ677993	
			1615	<i>Capnodiales</i>	DQ678033	DQ678084	DQ470920		DQ677982	DQ677982	
			942	<i>Capnodiales</i>	DQ471017	DQ470968	FJ190611		DQ470920	DQ470920	
			1594	<i>Capnodiales</i>	DQ678024	DQ678075	DQ677973		DQ6777920	DQ6777920	
			274	<i>Dothideomycetes</i>	DQ544681	DQ544681	AY544739		DQ497606	DQ497606	
			1359	<i>Dothideomycetes</i>	DQ479933	DQ479984	FJ190636		DQ471182	DQ471182	
			1300	<i>Dothideomycetes</i>	DQ677905	DQ678058	FJ190631		DQ677953	DQ677953	
			EB 0324	<i>Dothideomycetes</i>	FJ161135	FJ161174	FJ161114		FJ161091	FJ161091	
				<i>Hysteriales</i>	FJ161167	FJ161207	FJ161129		FJ161111	FJ161111	
				<i>Dothideomycetes</i>	FJ161155	FJ161196	FJ161104		DQ677981	DQ677981	
				<i>Incertae sedis</i>	DQ678032	DQ678083	DQ677928		DQ677643	DQ677643	
				<i>Incertae sedis</i>	DQ767649	DQ767654	DQ471179		DQ677793	DQ677793	
				<i>Myriangiales</i>	DQ767651	DQ767658	FJ161135		FJ161114	FJ161114	
				<i>Mytilinidales</i>	DQ767651	DQ767659	FJ161167		FJ161129	FJ161129	
				<i>Pleosporales</i>	DQ678059	DQ678059	FJ161128		FJ161104	FJ161104	
				<i>Dothideomycetes</i>	DQ677954	DQ677954	DQ677900		DQ6777928	DQ6777928	
				<i>Dothideomycetes</i>	FJ161128				DQ471182	DQ471182	
				<i>Dothideomycetes</i>					DQ677953	DQ677953	
				<i>Dothideomycetes</i>					DQ677793	DQ677793	
				<i>Dothideomycetes</i>					DQ677794	DQ677794	
				<i>Dothideomycetes</i>					DQ677795	DQ677795	
				<i>Dothideomycetes</i>					DQ677796	DQ677796	
				<i>Dothideomycetes</i>					DQ677797	DQ677797	
				<i>Dothideomycetes</i>					DQ677798	DQ677798	
				<i>Dothideomycetes</i>					DQ677799	DQ677799	
				<i>Dothideomycetes</i>					DQ677800	DQ677800	
				<i>Dothideomycetes</i>					DQ677801	DQ677801	
				<i>Dothideomycetes</i>					DQ677802	DQ677802	
				<i>Dothideomycetes</i>					DQ677803	DQ677803	
				<i>Dothideomycetes</i>					DQ677804	DQ677804	
				<i>Dothideomycetes</i>					DQ677805	DQ677805	
				<i>Dothideomycetes</i>					DQ677806	DQ677806	
				<i>Dothideomycetes</i>					DQ677807	DQ677807	
				<i>Dothideomycetes</i>					DQ677808	DQ677808	
				<i>Dothideomycetes</i>					DQ677809	DQ677809	
				<i>Dothideomycetes</i>					DQ677810	DQ677810	
				<i>Dothideomycetes</i>					DQ677811	DQ677811	
				<i>Dothideomycetes</i>					DQ677812	DQ677812	
				<i>Dothideomycetes</i>					DQ677813	DQ677813	
				<i>Dothideomycetes</i>					DQ677814	DQ677814	
				<i>Dothideomycetes</i>					DQ677815	DQ677815	
				<i>Dothideomycetes</i>					DQ677816	DQ677816	
				<i>Dothideomycetes</i>					DQ677817	DQ677817	
				<i>Dothideomycetes</i>					DQ677818	DQ677818	
				<i>Dothideomycetes</i>					DQ677819	DQ677819	
				<i>Dothideomycetes</i>					DQ677820	DQ677820	
				<i>Dothideomycetes</i>					DQ677821	DQ677821	
				<i>Dothideomycetes</i>					DQ677822	DQ677822	
				<i>Dothideomycetes</i>					DQ677823	DQ677823	
				<i>Dothideomycetes</i>					DQ677824	DQ677824	
				<i>Dothideomycetes</i>					DQ677825	DQ677825	
				<i>Dothideomycetes</i>					DQ677826	DQ677826	
				<i>Dothideomycetes</i>					DQ677827	DQ677827	
				<i>Dothideomycetes</i>					DQ677828	DQ677828	
				<i>Dothideomycetes</i>					DQ677829	DQ677829	
				<i>Dothideomycetes</i>					DQ677830	DQ677830	
				<i>Dothideomycetes</i>					DQ677831	DQ677831	
				<i>Dothideomycetes</i>					DQ677832	DQ677832	
				<i>Dothideomycetes</i>					DQ677833	DQ677833	
				<i>Dothideomycetes</i>					DQ677834	DQ677834	
				<i>Dothideomycetes</i>					DQ677835	DQ677835	
				<i>Dothideomycetes</i>					DQ677836	DQ677836	
				<i>Dothideomycetes</i>					DQ677837	DQ677837	
				<i>Dothideomycetes</i>					DQ677838	DQ677838	
				<i>Dothideomycetes</i>					DQ677839	DQ677839	
				<i>Dothideomycetes</i>					DQ677840	DQ677840	
				<i>Dothideomycetes</i>					DQ677841	DQ677841	
				<i>Dothideomycetes</i>					DQ677842	DQ677842	
				<i>Dothideomycetes</i>					DQ677843	DQ677843	
				<i>Dothideomycetes</i>					DQ677844	DQ677844	
				<i>Dothideomycetes</i>					DQ677845	DQ677845	
				<i>Dothideomycetes</i>					DQ677846	DQ677846	
				<i>Dothideomycetes</i>					DQ677847	DQ677847	
				<i>Dothideomycetes</i>					DQ677848	DQ677848	
				<i>Dothideomycetes</i>					DQ677849	DQ677849	
				<i>Dothideomycetes</i>					DQ677850	DQ677850	
				<i>Dothideomycetes</i>					DQ677851	DQ677851	
				<i>Dothideomycetes</i>					DQ677852	DQ677852	
				<i>Dothideomycetes</i>					DQ677853	DQ677853	
				<i>Dothideomycetes</i>					DQ677854	DQ677854	
				<i>Dothideomycetes</i>					DQ677855	DQ677855	
				<i>Dothideomycetes</i>					DQ677856	DQ677856	
				<i>Dothideomycetes</i>					DQ677857	DQ677857	
				<i>Dothideomycetes</i>					DQ677858	DQ677858	
				<i>Dothideomycetes</i>					DQ677859	DQ677859	
				<i>Dothideomycetes</i>					DQ677860	DQ677860	
				<i>Dothideomycetes</i>					DQ677861	DQ677861	
				<i>Dothideomycetes</i>					DQ677862	DQ677862	
				<i>Dothideomycetes</i>					DQ677863	DQ677863	
				<i>Dothideomycetes</i>					DQ677864	DQ677864	
				<i>Dothideomycetes</i>					DQ677865	DQ677865	
				<i>Dothideomycetes</i>					DQ677866	DQ677866	
				<i>Dothideomycetes</i>					DQ677867	DQ677867	
				<i>Dothideomycetes</i>					DQ677868	DQ677868	
				<i>Dothideomycetes</i>					DQ677869	DQ677869	
				<i>Dothideomycetes</i>					DQ677870	DQ677870	
				<i>Dothideomycetes</i>					DQ677871	DQ677871	
				<i>Dothideomycetes</i>					DQ677872	DQ677872	
				<i>Dothideomycetes</i>					DQ677873	DQ677873	
				<i>Dothideomycetes</i>					DQ677874	DQ677874	
				<i>Dothideomycetes</i>					DQ677875	DQ677875	
				<i>Dothideomycetes</i>					DQ677876	DQ677876	
				<i>Dothideomycetes</i>					DQ677877	DQ677877	
				<i>Dothideomycetes</i>					DQ677878	DQ677878	
				<i>Dothideomycetes</i>					DQ677879	DQ677879	
				<i>Dothideomycetes</i>					DQ677880	DQ677880	
				<i>Dothideomycetes</i>					DQ677881	DQ677881	
				<i>Dothideomycetes</i>					DQ677882	DQ677882	
				<i>Dothideomycetes</i>					DQ677883	DQ677883	
				<i>Dothideomycetes</i>					DQ677884	DQ677884	
				<i>Dothideomycetes</i>					DQ677885	DQ677885	
				<i>Dothideomycetes</i>					DQ677886	DQ677886	
				<i>Dothideomycetes</i>					DQ677887	DQ677887	
				<i>Dothideomycetes</i>					DQ677888	DQ677888	
				<i>Dothideomycetes</i>					DQ677889	DQ677889	
				<i>Dothideomycetes</i>					DQ677890	DQ677890	
				<i>Dothideomycetes</i>					DQ677891	DQ677891	
				<i>Dothideomycetes</i>					DQ677892	DQ677892	
				<i>Dothideomycetes</i>					DQ677893	DQ677893	
				<i>Dothideomycetes</i>					DQ677894	DQ677894	
				<i>Dothideomycetes</i>					DQ677895	DQ677895	
				<i>Dothideomycetes</i>					DQ677896	DQ677896	
				<i>Dothideomycetes</i>					D		

1083	<i>Eurotiomycetes</i>	GB	<i>Ajellomyces capsulatum</i>	Genome	Genome
1084	<i>Eurotiomycetes</i>	TIGR	<i>Coccidioides immitis</i>	Genome	Genome
684	<i>Eurotiomycetes</i>	NYBG 808041	<i>Agromyia</i> sp.	DQ782913	DQ782917
697	<i>Eurotiomycetes</i>	DUKE 0047959	<i>Staurothele frustulenta</i>	DQ823098	DQ840560
56	<i>Geoglossomycetes</i>	OSC 60610	<i>Geoglossum glabrum</i>	AY789316	DQ471044
64	<i>Geoglossomycetes</i>	OSC 100009	<i>Geoglossum nigritum</i>	AY544694	DQ470879
	<i>Geoglossomycetes</i>	Mycorec1840	<i>Geoglossum umbratile</i>	AY789302	DQ47115
	<i>Geoglossomycetes</i>	HMAS 71956	<i>Sarcocolla globosa</i> 1	AY789299	DQ471133
	<i>Geoglossomycetes</i>	OSC 63633	<i>Sarcocolla globosa</i> 2	AY789409	DQ471049
	<i>Geoglossomycetes</i>	MBH 52476	<i>Sarcocolla globosa</i> 3	AY789428	DQ470881
	<i>Geoglossomycetes</i>	OSC 100017	<i>Trichoglossum hirsutum</i> 1	AY544697	DQ471119
	<i>Geoglossomycetes</i>	OSC 61726	<i>Trichoglossum hirsutum</i> 2	AY789312	DQ471133
229	<i>Incertae sedis</i>	IAM 12963	<i>Saitoella complicata</i>	AY548297	DQ471133
	<i>Laboulbeniomycetes</i>	GB	<i>Hesperomyces virescens</i>	AF298235	DQ471049
2197	<i>Laboulbeniomycetes</i>	CBS 657-82	<i>Sigmatomycetes protrudens</i>	AF298232	FJ238412
962	<i>Laboulbeniomycetes</i>	GB	<i>Pixidiophorales</i>	FJ176839	DQ366258
589	<i>Lecanoromycetes</i>	DUKE 0047522	<i>Trapelia placodioides</i>	AF19500	DQ366260
6	<i>Lecanoromycetes</i>	DUKE 0047740	<i>Lecidea fuscoatra</i>	DQ912310	DQ912355
195	<i>Lecanoromycetes</i>	DUKE 0047550	<i>Canoparmelia caroliniana</i>	AY584658	DQ782889
958	<i>Lecanoromycetes</i>	Lumsbch 995	<i>Hypogymnia physodes</i>	DQ973006	DQ366251
1349	<i>Lecanoromycetes</i>	JK 5548K	<i>Diploschistes ocellatus</i>	AF05077	DQ247793
128	<i>Lecanoromycetes</i>	DUKE 0047503	<i>Glomerobolbus gelineus</i>	DC247803	DQ247793
314	<i>Lecanoromycetes</i>	DUKE 0047520	<i>Lobaria scrobiculata</i>	AY584679	DQ883768
131	<i>Lecanoromycetes</i>	DUKE 0047548	<i>Lobariella pallida</i>	DQ883788	DQ883772
134	<i>Lecanoromycetes</i>	DUKE 0047504	<i>Nephroma parile</i>	DQ973061	FJ772246
333	<i>Lecanoromycetes</i>	DUKE 0047747	<i>Peltigera degenerii</i>	AY584681	DQ782897
875	<i>Lecanoromycetes</i>	DUKE 0047641	<i>Coccocarpia erythroxili</i>	DQ912294	DQ883756
224	<i>Lecanoromycetes</i>	DUKE 0047506	<i>Icmadophilus erectorum</i>	DQ883694	DQ883723
320	<i>Lecanoromycetes</i>	DUKE 0047507	<i>Pertusaria dactylina</i>	DQ782907	DQ782899
686	<i>Lecanoromycetes</i>	DUKE 0047544	<i>Heterodermia vulgaris</i>	46411424	DQ973075
87	<i>Lecanoromycetes</i>	DUKE 0047925	<i>Pyxine subcinerarea</i>	AY584657	DQ883748
59	<i>Leotiomycetes</i>	OSC 100012	<i>Teloschistes exilis</i>	DQ883793	DQ883745
	<i>Leotiomycetes</i>	MBH 52481	<i>Botryotinia fuckeliana</i>	DQ772245	DQ883759
	<i>Leotiomycetes</i>	OSC 100054	<i>Bryoglossum gracile</i>	DQ782880	DQ883773
	<i>Leotiomycetes</i>	CBS 161-38	<i>Cudoniella cf. clavus</i>	DQ883789	DQ883754
	<i>Leotiomycetes</i>	OSC 100002	<i>Dermea acerina</i>	DQ883793	DQ883758
	<i>Leotiomycetes</i>	CBS 811-85	<i>Lachnum vigneum</i>	DQ883802	DQ883754
	<i>Leotiomycetes</i>	OSC 100001	<i>Lambertia subrenispora</i>	AY584671	DQ883773
	<i>Leotiomycetes</i>	FH-DSH -97103	<i>Leotia lubrica</i>	DQ782907	DQ883759
	<i>Leotiomycetes</i>	Ingo-Clark-Geo163	<i>Microglossum rufum</i> 1	AY544644	DQ471116
	<i>Leotiomycetes</i>	OSC 100641	<i>Microglossum rufum</i> 2	DQ257359	DQ247786
	<i>Leotiomycetes</i>	ZW02-012	<i>Mitula brevispora</i>	DQ470981	DQ471045
	<i>Leotiomycetes</i>	WZ-Geo47-Clark	<i>Mitula elegans</i>	AY789292	DQ471056
	<i>Leotiomycetes</i>	OSC 100063	<i>Monilinia laxa</i>	AY789334	DQ471091
	<i>Leotiomycetes</i>	CBS 477-97	<i>Neobulgaria pura</i>	AY544670	DQ497602
	<i>Leotiomycetes</i>	OSC 10036	<i>Nectobraea malicorticis</i>	FJ176865	DQ471101
	<i>Leotiomycetes</i>	1100803	<i>Thuemindium atropurpureum</i> 1	AY544706	DQ470876
	<i>Leotiomycetes</i>	1136126	<i>Thuemindium atropurpureum</i> 2	AY789307	DQ471041
	<i>Leotiomycetes</i>	DUKE 0047585	<i>Cudonia circinans</i>	AY533013	AY641033
	<i>Leotiomycetes</i>	OSC 100640	<i>Spathularia velutipes</i> 1	FJ997861	FJ997863
	<i>Leotiomycetes</i>	ZW Geo58	<i>Spathularia velutipes</i> 2	AY789356	DQ832327
	<i>Leotiomycetes</i>	Schultz 163-9a	<i>Lichenella iodopulchra</i>	DQ832330	DQ832331
	<i>Leotiomycetes</i>	DUKE 0047648	<i>Peltula auriculata</i>	DQ782887	DQ832335
	<i>Leotiomycetes</i>	DUKE 0047527	<i>Peltula umbilicata</i>	DQ470986	DQ471109
	<i>Leotiomycetes</i>	DAH-3	<i>Neolecta irregularis</i>	DQ471037	AAF19058
	<i>Nelectomycetes</i>	DAH-11	<i>Nelecta vitellina</i>		

Table 1 (cont.)

AFTOL no.	Class	Order	Voucher ¹	Taxon	nSSU	mSSU	RPB1	RPB2	TEF1
1252	Orbillomyces	Oribiliales	CBS 397.93	<i>Athroborys elegans</i>	FJ176864	FJ238349	DQ471071	FJ238395	
905	Orbillomyces	Oribiliales	CBS 917.72	<i>Orbilia vinoso</i>	DQ470952	DQ471145	DQ471120	DQ466085	
65	Pezizomyces	Pezizales	OSC 100018	<i>Aleuria aurantia</i>	AY544698	AY544654	FJ238423	FJ247785	
70	Pezizomyces	Pezizales	KH-00-08	<i>Ascobolus carbonarius</i>	AY544720	DQ247807	DQ471126	DQ471054	
152	Pezizomyces	Pezizales	OSC 100062	<i>Caloscypha fulgens</i>	DQ247799	FJ190606	DQ471160	DQ471088	
933	Pezizomyces	Pezizales	CBS 626.71	<i>Eleutherascus lectardi</i>	DQ471014	AY544677	DQ471130	DQ471059	
176	Pezizomyces	Pezizales	OSC 100063	<i>Gyromitra californica</i>	AY544717	AY544673	DQ470995	DQ471066	
507	Pezizomyces	Pezizales	TL-6398	<i>Peziza vesiculosa</i>	DQ470948	DQ471140	DQ471166	DQ471093	
949	Pezizomyces	Pezizales	CBS 666.88	<i>Pyronema domesticum</i>	DQ247813	FJ190613	FJ238436	FJ238402	
1299	Pezizomyces	Pezizales	CBS 472.80	<i>Saccobolus dilutellus</i>	FJ176814	FJ176870	FJ176860	FJ238392	
954	Pezizomyces	Pezizales	CBS 733.68	<i>Sarcosoma latahense</i>	FJ176806	AY544668	FJ238424	FJ238399	
153	Pezizomyces	Pezizales	OSC 100049	<i>Sarcosphaera crassa</i>	DQ247814	DQ247806	FJ190587	DQ471047	
62	Pezizomyces	Pezizales	OSC 100015	<i>Scutellinia scutellata</i>	AY544710	AY544666	AY544761	AY544761	FJ238395
74	Pezizomyces	Pezizales	NRRL 223388	<i>Verpa conica</i>	AY198398	AY198398	XM_447415	XM_448959	Genome
1073	Saccharomyces	GB	Candida glabrata	<i>Candida tropicalis</i>	M55527	Genome	Genome	Genome	
1269	Saccharomyces	GB	<i>Debaromyces hansenii</i>	DHA508273	AF485980	XM_456921	CR382139	Genome	
1077	Saccharomyces	GB	<i>Eremothecium gossypii</i>	AE016820	AF442353	NM_209535	AE016819	Genome	
1072	Saccharomyces	GB	<i>Saccharomyces cerevisiae</i>	SCYLR154C	AF442281	X96876	SCYOR151C	Genome	
1069	Saccharomyces	GB	<i>Schizosaccharomyces pombe</i>	X54866	X54421	X56564	D13337	Genome	FJ238421
1199	Schizosaccharomyces	GB	<i>Calbosphaeria pulchella</i>	AY761071	AY761075	AY761075	AY780151		
5086	Sordariomyces	SMH4320	Berlia moriformis	<i>Cryptosporae hypoderma</i>	DQ862049	DQ862028	DQ862018	DQ862034	
2124	Sordariomyces	CBS 115999	<i>Diaporthe eres</i>	DQ471015	AF408350	FJ190607	DQ470919	DQ47931	
935	Sordariomyces	CBS 109767	<i>Endothia gyroza</i>	DQ471023	DQ470972	DQ471169	DQ470926	DQ471096	
1223	Sordariomyces	CBS 112915	<i>Gnomonia gnoman</i>	DQ471019	AF408361	FJ190615	DQ471167	DQ471094	
952	Sordariomyces	CBS 199.53	<i>Bionectria cf. aureofulva</i>	DQ862044	DQ862027	FJ713625	DQ862013	DQ862029	
187	Sordariomyces	GJS 71-328	<i>Claviceps purpurea</i>	AF543765	AF543789	AY489648	DQ522417	AF543778	
189	Sordariomyces	GAM 12885	<i>Cordyceps cardinalis</i>	AY184962	EF469007	DQ522325	DQ522370	DQ522422	
162	Sordariomyces	OSC 93609	<i>Elaphocordyceps capitata</i>	AY489689	FJ713628	AY489649	DQ522421	AY489615	
192	Sordariomyces	OSC 71233	<i>Elaphocordyceps ophioglossoides</i>	AY489691	FJ713629	AY489652	DQ522429	AY489618	
193	Sordariomyces	OSC 106405	<i>Epichloë typhina</i>	U17396	FJ713624	DQ522440	DQ522440	AF543777	
163	Sordariomyces	ATCC 56429	<i>Hypocreale lutea</i>	AF543768	FJ713620	AY489662	DQ522446	AF543781	
156	Sordariomyces	ATCC 208838	<i>Neotria cinnabarinia</i>	U32412	U00748	FJ713622	AY489666	AF543785	
159	Sordariomyces	CBS 114055	<i>Glomerella cingulata</i>	AF543762	AF543786	AY489659	DQ522441	AF543773	
1265	Sordariomyces	FAU 553	<i>Verticillium dahliae</i>	AY489705	DQ470945	FJ713630	AY489673	AY489632	
237	Sordariomyces	ATCC 16535	<i>Lindra thalassiae</i>	DQ470994	DQ470947	FJ190593	DQ471139	DQ471065	
413	Sordariomyces	JK 5090A	<i>Lulworthia grandispora</i>	DQ522856	DQ522856	FJ190595	DQ518181	DQ497608	
747	Sordariomyces	JK 4686	<i>Gaeumannomyces medullaris</i>	FJ176801	FJ176854	Genome	Genome	Genome	
734	Sordariomyces	JK 5528S	<i>Magnaporthe grisea</i>	AB026819	AB026819	AY015637	AY015637		
1081	Sordariomyces	ATCC 15515	<i>Melanospora tiffanyae</i>	AY015619	AY015630	FJ238372	FJ238415		
1906	Sordariomyces	TCH C89	<i>Ceratocystis fimbriata</i>	U32418	U17401	FJ190660	FJ238381		
5011	Sordariomyces	728a	<i>Corollospora maritima</i>	FJ176846	FJ176888	FJ238373	FJ238390		
1907	Sordariomyces	CBS 122611	<i>Gondwanamyces capensis</i>	FJ176834	U46885	DQ836904	DQ836912	Genome	
409	Sordariomyces	CBS 197.60	<i>Halosphaeria appendiculata</i>	U46872	DQ838897	X04971	XM_959004		
1038	Sordariomyces	CBS 139.51	<i>Ophiostoma stenoreras</i>	AY545728	AY545724	AY544692	DQ470878	DQ518175	
1078	Sordariomyces	Broad	<i>Neurospora crassa</i>	AY544688	AY544648	AY548292	DQ471042	DQ471042	
216	Sordariomyces	CBS 15-5973	<i>Sordaria fimicola</i>	DQ470973	DQ470973	AY548293	DQ471097	DQ471097	
51	Sordariomyces	OSC 100004	<i>Xylaria hypoxylon</i>	FJ713610	DQ471114	AY548291	DQ471114	DQ471114	
1234	Taphrinomyces	CBS 356.35	<i>Taphrina deformans</i>	AY548293	DQ471170	DQ471170	DQ471170	DQ471170	
265	Taphrinomyces	IAM 14515	<i>Taphrina wiesneri</i>					AY548298	

¹ voucher GB = obtained from GenBank, or genome databases without clear voucher numbers.

gal ecological niches (Geiser et al. 2006). *Lecanoromycetes* contain the majority of the lichenised fungi (Miadlikowska et al. 2006). Earlier large-scale phylogenies (e.g. Lutzoni et al. 2004) have suggested a sister relationship between these two classes, but we find that such a relationship remains without strong statistical support (Fig. 1). Despite this, internal nodes are well supported enough to provide good support for the hypothesis that lichenisation evolved multiple times in the *Ascomycota*, with losses being rare (Gueidan et al. 2008, Schoch et al. 2009).

The remaining classes are discussed in relation to their respective rankless taxa listed below.

Rankless taxon 'Dothideomyceta'

This taxon is well supported, with ML bootstrap of 91 % and a moderate Bayesian posterior probability of 92 %. It includes two classes of fungi which produce fissitunicate asci, *Arthoniomycetes* and *Dothideomycetes*. *Arthoniomycetes* consists of \pm 1 600 species of lichenised and lichenicolous fungi with fissitunicate asci and exposed hymenia (Grube 1998, Ertz et al. 2009). Unlike other species with fissitunicate asci, these taxa have ascohymenial development, prompting their placement in a transitory group, or 'Zwischengruppe' that is intermediate between ascohymenial and ascocolocular development (Henssen & Jahns 1974). The class is resolved as sister to *Dothideomycetes*, consistent with recent studies (Lutzoni et al. 2004, Spatafora et al. 2006, Wang et al. 2006a). *Dothideomycetes* is a large class containing two subclasses, *Dothideomycetidae* and *Pleosporomycetidae* (Schoch et al. 2006). Our analysis contains members of all known orders in the class, including recent additions (Boehm et al. 2009). This broad representation yields increased resolution in the placement of an order previously labelled incertae sedis, *Botryosphaerales* (Schoch et al. 2006). Placement of *Botryosphaerales* within subclass *Pleosporomycetidae* is well supported, as is a close relationship with the unplaced family *Tubeufiaceae* (Fig. 2).

Rankless taxon 'Sordariomyceta'

'*Sordariomyceta*' contains three classes, *Leotiomycetes*, *Laboulbeniomycetes* and *Sordariomycetes*. We find similar resolution for this clade as for the 'Dothideomyceta'. These three classes are characterised by the production of unitunicate, poricidal asci, or derivatives of such asci (e.g., deliquescent asci). *Leotiomycetes* and *Sordariomycetes* include numerous fungi associated with plants as pathogens, endophytes and epiphytes. The sordariomycete phylogeny is comparatively well resolved with 15 orders and 3 subclasses named (Zhang et al. 2006, Kirk et al. 2008). In contrast, the leotiomycete classification still poorly matches its inferred phylogeny. A recent class-wide effort to assess morphological and ecological data in a phylogenetic context continued to find high levels of diversity unaccounted for in the current classification (Wang et al. 2009). In addition to the aforementioned two classes, Fig. 1 also supports the placement of the *Laboulbeniomycetes* reported in Schoch et al. (2009) as part of a monophyletic lineage. The relationship between the *Sordariomycetes* and *Laboulbeniomycetes* is also well supported but we will refrain from naming this node until sampling can be expanded for the *Laboulbeniomycetes*. The class *Laboulbeniomycetes* encompasses an enigmatic lineage of insect symbionts and mycoparasites that have long proved problematic with respect to placement in higher-level classification schemes. *Laboulbeniomycetes* comprises two orders, *Laboulbeniales* and *Pyxidiophorales*, that are united by an ascospore synapomorphy of a darkened holdfast region and by molecular data (Weir & Blackwell 2001, 2005). Members of *Pyxidiophorales* possess globose perithecia with a single

layer of wall cells, and long perithecial necks that release their ascospores passively in droplets at the tips of their necks; this mechanism is repeatedly derived within *Ascomycota* for insect dispersal of ascospores (Blackwell 1994). For this reason, they have been likened to other insect-dispersed perithecial ascomycetes (e.g., *Ophiostomatales*) that now are strongly supported as members of *Sordariomycetes*. *Laboulbeniales* includes ectoparasites of insects and displays morphological traits not found elsewhere in the *Ascomycota*. They form apomorphic ascocarps produced by the division and enlargement of ascospores that are difficult to characterize in existing ascocarpal terms. *Laboulbeniales* feature an ostiole, however, which is consistent with perithecia produced by hyphal growth. Determinate growth of the ascospore with a series of predictable cell divisions produces a thallus of a finite number of cells that is characteristic at the genus and species level (Tavares 1979). The analyses presented here strongly support *Laboulbeniomycetes* as sister to *Sordariomycetes*. This placement corresponds with the terminology originally applied to this group (Thaxter 1896). It is interesting to note that while species of *Pyxidiophorales* are endowed with a diverse group of anamorphs, members of *Laboulbeniales* are mainly known to reproduce sexually.

Summary

In conclusion, we propose two monotypic formal taxa and describe continued support for four informal rankless taxa. Important improvements in the resolution of deep nodes within the *Ascomycota* may be attributed to multi-gene sequence data produced by AFTOL and other projects during the last 5 years. The accelerating accumulation of genome-scale sequence data will continue to challenge and improve existing phylogenetic hypotheses. However, in order to direct limited resources towards under-sampled areas in the fungal phylogeny, an accurate, up-to-date classification is required. By placing three earth tongue genera in a separate newly described class, we underscore and communicate the genetic diversity that is found in the fungi producing these very convergent morphologies.

Acknowledgements We are grateful to Kentaro Hosaka, Dan Luoma and Zhiliang Yang for the photographs used. We acknowledge funding provided by NSF through a grant (DEB 0717476) to J.W.S. and C.L.S. (while at Oregon State). C.L.S. was also supported in part by the Intramural Research program of the National Institutes of Health. Z.W. was supported by the Yale University Anonymous Postdoctoral Fellowship.

REFERENCES

- Altekar G, Dwarkadas S, Huelsenbeck JP, Ronquist F. 2004. Parallel Metropolis coupled Markov chain Monte Carlo for Bayesian phylogenetic inference. Bioinformatics 20: 407–415.
- Blackwell M. 1994. Minute mycological mysteries: the influence of Arthropods on the lives of Fungi. Mycologia 86: 1–17.
- Blackwell M, Hibbett DS, Taylor JW, Spatafora JW. 2006. Research Coordination Networks: a phylogeny for kingdom Fungi (Deep Hypha). Mycologia 98: 829–837.
- Boehm EWA, Schoch CL, Spatafora JW. 2009. On the evolution of the Hysteraceae and Mytilinidiaceae (Pleosporomycetidae, Dothideomycetes, Ascomycota) using four nuclear genes. Mycological Research 113: 461–479.
- Corda ACJ. 1838. Abbildungen der Pilze und Schwaemme. Icones Fungorum, Hucusque Cognitorum 2: 1–43.
- Dennis RWG. 1968. British ascomycetes. Cramer Verlag, Lehre, Germany.
- Dujon B, Sherman D, Fischer G, Durrens P, Casaregola S, et al. 2004. Genome evolution in yeasts. Nature 430: 35–44.
- Eriksson OE, Winka K. 1997. Supraordinal taxa of Ascomycota. Myconet 1: 1–16.
- Ertz D, Miadlikowska J, Lutzoni F, Desseine S, Raspe O, et al. 2009. Towards a new classification of the Arthoniales (Ascomycota) based on a three-gene phylogeny focussing on the genus *Opegrapha*. Mycological Research 113: 141–152.

- Gargas A, DePriest PT, Grube M, Tehler A. 1995. Multiple origins of lichen symbioses in Fungi suggested by SSU rDNA phylogeny. *Science* 268: 1492–1495.
- Geiser DM, Gueidan C, Miadlikowska J, Lutzoni F, Kauff F, et al. 2006. Eurotiomycetes: Eurotiomycetidae and Chaetothyriomycetidae. *Mycologia* 98: 1053–1064.
- Gernandt DS, Platt JL, Stone JK, Spatafora JW, Holst-Jensen A, et al. 2001. Phylogenetics of Helotiales and Rhytismatales based on partial small sub-unit nuclear ribosomal DNA sequences. *Mycologia* 93: 915–933.
- Grube M. 1998. Classification and phylogeny in the Arthoniales (Lichenized Ascomycetes). *The Bryologist* 101: 377–391.
- Gueidan C, Ruibal CV, Hoog GS de, Gorbushina AA, Untereiner WA, Lutzoni F. 2008. An extremotolerant rock-inhabiting ancestor for mutualistic and pathogen-rich fungal lineages. *Studies in Mycology* 62: 111–119.
- Henssen A, Jahns HM. 1974. Lichenes. Georg Thieme Verlag, Stuttgart.
- Hibbett DS, Binder M, Bischoff JF, Blackwell M, Cannon PF, et al. 2007. A higher-level phylogenetic classification of the Fungi. *Mycological Research* 111: 509–547.
- Hibbett DS, Donoghue MJ. 1998. Integrating phylogenetic analysis and classification in Fungi. *Mycologia* 90: 347–356.
- Huelsenbeck JP, Ronquist F. 2001. MrBayes: Bayesian inference of phylogenetic trees. *Bioinformatics* 17: 754–755.
- James TY, Kauff F, Schoch CL, Matheny PB, Hofstetter V, et al. 2006. Reconstructing the early evolution of Fungi using a six-gene phylogeny. *Nature* 443: 818–822.
- Johnston PR. 2001. Monograph of the monocotyledon-inhabiting species of *Lophodermium*. *Mycological Papers* 176: 1–239.
- Jørgensen PM. 2002. Rankless names in the Code? *Taxon* 53: 162.
- Jumpponen A, Weber NS, Trappe JM, Cazares E. 1997. Distribution and ecology of the ascomycete *Sarcoleotia globosa* in the United States. *Canadian Journal of Botany* 75: 2228–2231.
- Kirk PM, Cannon PF, Minter DW, Stalpers JA. 2008. Ainsworth and Bisby's dictionary of the Fungi. CAB International, Wallingford, UK.
- Korf RP. 1973. Discomycetes and Tuberales. In: Ainsworth GC, Sparrow FK, Sussman AS (eds). *The Fungi: an advanced treatise*. Vol. IVA: 249–319. Academic Press, New York.
- Korf RP. 1983. Cyttaria (Cyttariales) – Coevolution with *Nothofagus*, and evolutionary relationship to the Boedijnopezizeae (Pezizales, Sarcoscyphaceae). *Australian Journal of Botany Suppl.* 10: 77–87.
- Kuntner M. 2006. Phylogenetic systematics of the Gondwanan nephilid spider lineage Clitaetrinae (Araneae, Nephilidae). *Zoologica Scripta* 35: 19–62.
- Kuntner M, Agnarsson I. 2006. Are the linnean and phylogenetic nomenclatural systems combinable? Recommendations for biological nomenclature. *Systematic Biology* 55: 774–784.
- Landvik S. 1996. Neolecta, a fruit-body-producing genus of the basal ascomycetes, as shown by SSU and LSU rDNA sequences. *Mycological Research* 100: 199–202.
- Landvik S, Schumacher TK, Eriksson OE, Moss ST. 2003. Morphology and ultrastructure of Neolecta species. *Mycological Research* 107: 1021–1031.
- Liu Y, Leigh JW, Brinkmann H, Cushion MT, Rodriguez-Ezpeleta N, Philippe H, Lang BF. 2009. Phylogenomic analyses support the monophyly of Taphrinomycotina, including *Schizosaccharomyces* fission yeasts. *Molecular Biology and Evolution* 26: 27–34.
- Lumbsch HT, Schmitt I, Lindemuth R, Miller A, Mangold A, et al. 2005. Performance of four ribosomal DNA regions to infer higher-level phylogenetic relationships of inoperculate euascomycetes (Leotiomyceta). *Molecular Phylogenetics and Evolution* 34: 512–524.
- Lutzoni F, Kauff F, Cox CJ, McLaughlin D, Celio G, et al. 2004. Assembling the fungal tree of life: progress, classification, and evolution of subcellular traits. *American Journal of Botany* 91: 1446–1480.
- McNeill JF, Barrie F, Burdet HM, Demoulin V, Hawksworth DL, et al. (eds), 2006. International Code of Botanical Nomenclature (Vienna Code). Regnum Vegetabile. Koeltz Scientific Books, Königstein.
- Miadlikowska J, Kauff F, Hofstetter V, Fraker E, Grube M, et al. 2006. New insights into classification and evolution of the Lecanoromycetes (Pezizomycotina, Ascomycota) from phylogenetic analyses of three ribosomal RNA- and two protein-coding genes. *Mycologia* 98: 1088–1103.
- Nannfeldt JA. 1942. The Geoglossaceae of Sweden (with regard also to the surrounding countries). Almqvist & Wiksell, Stockholm.
- Persoon CH. 1794. Neuer Versuch einer systematischen Eintheilung der Schwamme. *Neues Magazin für die Botanik*, Römer 1: 63–80.
- Pfeil BE, Crisp MD. 2005. What to do with Hibiscus? A proposed nomenclatural resolution for a large and well known genus of Malvaceae and comments on paraphyly. *Australian Systematic Botany* 18: 49–60.
- Platt JL. 2000. Lichens, earth tongues, and endophytes: evolutionary patterns inferred from phylogenetic analyses of multiple loci. PhD dissertation. Botany and Plant Pathology, Oregon State University, Corvallis, Oregon, USA.
- Porter TM, Schadt CW, Rizvi L, Martin AP, Schmidt SK, Scott-Denton L, Vilgalys R, Moncalvo JM. 2008. Widespread occurrence and phylogenetic placement of a soil clone group adds a prominent new branch to the fungal tree of life. *Molecular Phylogenetics and Evolution* 46: 635–644.
- Redhead SA. 1979. Mycological observations: 1, on *Cristulariella*, 2, on *Valdensinia*; 3, on *Neolecta*. *Mycologia* 61: 1248–1253.
- Reeb V, Lutzoni F, Roux C. 2004. Contribution of RPB2 to multilocus phylogenetic studies of the euascomycetes (Pezizomycotina, Fungi) with special emphasis on the lichen-forming Acarosporaceae and evolution of polyspority. *Molecular Phylogenetics and Evolution* 32: 1036–1060.
- Sandnes ACS. 2006. Phylogenetic relationships among species and genera of Geoglossaceae (Helotiales) based on ITS and LSU nrDNA sequences. Cand.scient. dissertation. The University of Oslo, Oslo.
- Schoch CL, Shoemaker RA, Seifert KA, Hambleton S, Spatafora JW, Crous PW. 2006. A multigene phylogeny of the Dothideomycetes using four nuclear loci. *Mycologia* 98: 1041–1052.
- Schoch CL, Sung G-H, López-Giráldez F, Townsend JP, Miadlikowska J, et al. 2009. The Ascomycota Tree of Life: A phylum wide phylogeny clarifies the origin and evolution of fundamental reproductive and ecological traits. *Systematic Biology*: doi 10.1093/sysbio/syp020.
- Schumacher T, Sivertsen S. 1987. *Sarcoleotia globosa* (Sommerf.: Fr.) Korf, taxonomy, ecology and distribution. In: Larsen GA, et al. (eds), *Arctic Alpine Mycology*. Plenum Press, New York & London: 163–176.
- Seaver FJ. 1951. The North American cup-fungi (in-operculate). Seaver (published by the author), New York.
- Sennblad B, Bremer B. 2002. Classification of Apocynaceae s.l. according to a new approach combining Linnaean and phylogenetic taxonomy. *Systematic Biology* 51: 389–409.
- Spatafora JW, Johnson D, Sung G-H, Hosaka K, O'Rourke B, et al. 2006. A five-gene phylogenetic analysis of the Pezizomycotina. *Mycologia* 98: 1018–1028.
- Spooner BM. 1987. Helotiales of Australasia: Geoglossaceae, Orbiliaceae, Sclerotiniaceae, Hyaloscyphaceae. *Bibliotheca Mycologica* 116: 1–711.
- Stamatakis A. 2006. RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics* 22: 2688–2690.
- Stenroos S, Hyvönen J, Myllys L, Thell A, Ahti T. 2002. Phylogeny of the genus *Cladonia* s.lat. (Cladoniaceae, Ascomycetes) inferred from molecular, morphological, and chemical data. *Cladistics* 18: 237–278.
- Sugiyama J, Hosaka K, Suh S-O. 2006. Early diverging Ascomycota: phylogenetic divergence and related evolutionary enigmas. *Mycologia* 98: 996–1005.
- Tavares II. 1979. The Laboulbeniales. *Mycological Memoirs* 9: 1–627.
- Thaxter R. 1896. Contribution towards a monograph of the Laboulbeniaceae. *Memoirs of the American Academy of Arts and Science* 12: 187–429.
- Verley GJM. 1994. Ultrastructure of the apical apparatus in *Leotia lubrica* and some Geoglossaceae (Leotiales, Ascomycotina). *Persoonia* 15: 405–430.
- Wang Z, Binder M, Schoch CL, Johnston PR, Spatafora JW, Hibbett D. 2006a. Evolution of helotialean fungi (Leotiomycetes, Pezizomycotina): A nuclear rDNA phylogeny. *Molecular Phylogenetics and Evolution* 41: 295–312.
- Wang Z, Johnston PR, Takamatsu S, Spatafora JW, Hibbett DS. 2006b. Towards a phylogenetic classification of the Leotiomycetes based on rDNA data. *Mycologia* 98: 1065–1075.
- Wang Z, Johnston PR, ang ZL, Townsend JP. 2009. Evolution of reproductive morphology in leaf endophytes. *PLoS ONE* 4: e4246.
- Weir A, Blackwell M. 2001. Molecular data support the Laboulbeniales as a separate class of Ascomycota, Laboulbeniomycetes. *Mycological Research* 105: 1182–1190.
- Weir A, Blackwell M. 2005. Phylogeny of arthropod ectoparasitic ascomycetes. In: Vega FE, Blackwell M (eds), *Insect-fungal associations: Ecology and evolution*: 119–145. Oxford University Press, Oxford, UK.
- Zhang N, Castlebury LA, Miller AN, Huhndorf S, Schoch CL, et al. 2006. An overview of the systematics of the Sordariomycetes based on a four-gene phylogeny. *Mycologia* 98: 1076–1087.
- Zhuang W-Y. 1998. *Fungal Flora of China* Vol. 8. Sclerotiniaceae and Geoglossaceae. Science Press, Beijing, China.