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, Jørgensen 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 (-mycotidae) 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 Linnaean 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, Gernandt 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.

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

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Key words
Bayesian inference
cybrid 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 Leotia, Mitrella, Cudonia, Microglossum, Thueemi, Spalhatulária 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 ‘Saccharomyctea’, ‘Dothideomyctea’, and ‘Sordariomyctea’ for supraordinal nodes, within the current phylogeny, acting as rankless taxa. As part of this revision, the continued use of ‘Leotiomycota’, now as a rankless taxon, is proposed.

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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 outgroup 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 ascomal 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: Zhuliang 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 invariant 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 maximum likelihood (ML) 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 replications 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 invariant sites. A metabolism 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 Taphrinales 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 -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 ascomatal development, ultrastructure of ascus apical apparatus, mossy habitat, and our multilocus gene phylogeny, Geoglossomycetidae cl. & ord. nov. is justified here as incertae sedis in Pezizomycotina and ‘Leotiomycota’.

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

Ascomata solitaria vel gregaria, capitata, stipitata; stipe cylin- drical, dark-brown, blackish to hyaline, septate. Paraphyses filiformes, pullae vel hyalinae. Distributio generalis, terrestrial, habituale locus fere uliginoso et muscoso.

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

Ascomata scattered to gregarious, capitata, stipitata; stipe cylindrical, black, smooth to furfuraceous. Asci clavate, inoperculate, poro parvum in iodo caerulecentes. Ascosporae elongatae, fusca, pullae vel hyalinae, multi-septatae. Paraphyses filiformes, pullae vel hyalinae. Distributo generalis, terrestrial, habituale locus fere uliginoso et muscoso.

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 Leotiomyceta, 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, ‘Saccharomycota’, ‘Dothideomycota’ and ‘Sordariomycota’. 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 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-porical ascii 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 ‘Saccharomycceta’

‘Saccharomycceta’ 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, ascomata 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 ‘Saccharomycceta’ 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 ‘Leotiomycota’

We apply ‘Leotiomycota’ as a rankless taxon containing the majority of fungi with a diversity of inoperculate ascii (e.g., fissitunicate, poricidal, deliquescent). ‘Leotiomycota’ excludes the earliest diverging classes of Pezizomycotina, Pezizomycetes and Orbiliomycetes. 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 Persson (1794). Persson described it as club-shaped, with unilicate, inoperculate ascii, with the type species given as Geoglossum glutarium Pers. Trichoglossum...
| AFTOL no. | Class | Order | Voucher | Taxon | nSSU | nLSU | mSSU | RPB1 | RPB2 | TE1 |
|----------|-------|-------|---------|-------|------|------|------|------|------|-----|
| 1241     | Zygomycota | outgroup | GB      | Rhizopus oryzae | AF113440 | AY213626 | AY883212 | Genome | Genome | Genome |
| 438      | Basidiomycota | outgroup | GEL 539 | Calocera cornea | AY771610 | AY710526 | AY857980 | AY536286 | AY81019 |
| 439      | Basidiomycota | outgroup | AW 136 | Calostoma cinnabarum | AY665773 | AY665754 | AY679117 | AY857980 | AY80939 |
| 1088     | Basidiomycota | outgroup | GB      | Cryptococcus neoformans | Genome | Genome | Genome | KM_507943 | KM_507943 | Genome |
| 770      | Basidiomycota | outgroup | MB 03-036 | Fomitopsis pinicola | AY705967 | AY861464 | FJ436112 | AY868474 | AY786056 | AY885152 |
| 701      | Basidiomycota | outgroup | DSH s.n. | Grifola frondosa | AY705960 | AY663618 | AY846564 | AY846876 | AY786057 | AY885153 |
| 126      | Arthoniomycetes | | D6 echinata 15572 | Roccella fuciformis | DQ883705 | DQ883696 | FJ727240 | DQ83713 | DQ83733 |
| 93       | Arthoniomycetes | | BG Printzen1981 | Roccellagapha cretacea | DQ883696 | FJ727240 | DQ83713 | DQ83733 |
| 307      | Arthoniomycetes | | DUKE 00475 | Schismatostoma decolorans | AY548809 | AY548815 | DQ83718 | DQ83715 | DQ83725 |
| 946      | Dothideomycetes | | Bdorpsphaeriales | | | | | | | |
| 156       | Dothideomycetes | | CBS 115476 | | | | | | | |
| 1618     | Dothideomycetes | | CBS 230.48 | | | | | | | |
| 1784     | Dothideomycetes | | CBS 447.70 | | | | | | | |
| 939      | Capnidiaceae | | CBS 147.52 | | | | | | | |
| 1289     | Capnidiaceae | | CBS 170.52 | | | | | | | |
| 1591     | Capnidiaceae | | CBS 398.80 | | | | | | | |
| 2021     | Capnidiaceae | | O3 100622 | | | | | | | |
| 1615     | Capnidiaceae | | CBS 292.38 | | | | | | | |
| 942      | Capnidiaceae | | CBS 113265 | | | | | | | |
| 1594     | Capnidiaceae | | CBS 325.33 | | | | | | | |
| 274      | Dothideomycetes | | D瘤1 231303 | | | | | | | |
| 1359     | Dothideomycetes | | CBS 737.71 | | | | | | | |
| 1300     | Dothideomycetes | | CBS 116.29 | | | | | | | |
| 1300     | Dothideomycetes | | CBS 114601 | | | | | | | |
| 1300     | Dothideomycetes | | EB 032 | | | | | | | |
| 1613     | Dothideomycetes | | EB 0249 | | | | | | | |
| 1580     | Dothideomycetes | | CBS 238.51 | | | | | | | |
| 1580     | Dothideomycetes | | CBS 245.49 | | | | | | | |
| 1583     | Myriangiales | | CBS 150.28 | | | | | | | |
| 1304     | Myriangiales | | CBS 327.18 | | | | | | | |
| 1304     | Myriangiales | | EB 0248 | | | | | | | |
| 1304     | Myriangiales | | CS 1003 | | | | | | | |
| 1304     | Myriangiales | | EB 0248 | | | | | | | |
| 1304     | Myriangiales | | CBS 260.9 | | | | | | | |
| 1304     | Myriangiales | | EB 0248 | | | | | | | |
| 1304     | Myriangiales | | CS 135.4 | | | | | | | |
| 1304     | Myriangiales | | EB 0248 | | | | | | | |
| 267      | Pleosporales | | DAOM 195275 | | | | | | | |
| 1583     | Pleosporales | | CBS 128.56 | | | | | | | |
| 54       | Pleosporales | | CBS 134.29 | | | | | | | |
| 1599     | Pleosporales | | CBS 225.60 | | | | | | | |
| 1576     | Pleosporales | | CBS 101341 | | | | | | | |
| 277      | Pleosporales | | CBS 249.19 | | | | | | | |
| 1575     | Pleosporales | | CBS 276.37 | | | | | | | |
| 1600     | Pleosporales | | CBS 279.74 | | | | | | | |
| 940      | Pleosporales | | CBS 541.72 | | | | | | | |
| 283      | Pleosporales | | CBS 279.74 | | | | | | | |
| 1256     | Pleosporales | | CBS 10020 | | | | | | | |
| 1958     | Pleosporales | | CBS 304.66 | | | | | | | |
| 1037     | Pleosporales | | CBS 454.72 | | | | | | | |
| 1063     | Pleosporales | | CBS 275.9 | | | | | | | |
| 1033     | Pleosporales | | CBS 198.61 | | | | | | | |
| 671      | Pleosporales | | CBS 157.67 | | | | | | | |
| 1911     | Eurotiomycetes | | CBS 134.66 | | | | | | | |
| 5007     | Eurotiomycetes | | CBS 665.74 | | | | | | | |
| 2014     | Eurotiomycetes | | CBS 339.97 | | | | | | | |
| ORBITALIACEAE | CBS 397.93 | Persoonia – Volume 22, 2009 |
|-------------|-----------|--------------------------|
| ORBITALIACEAE | CBS 917.72 | Artrobrya elegans |
| PEZIZOMYCETES | OSC 10018 | Aleuaria aurantia |
| PEZIZOMYCETES | HK-00-08 | Ascochytara carbonaria |
| PEZIZOMYCETES | OSC 100062 | Calocypis fucigenis |
| PEZIZOMYCETES | CBS 666.88 | Pyronema domesticum |
| PEZIZOMYCETES | CBS 472.80 | Saccobolus dilatellus |
| PEZIZOMYCETES | CBS 733.68 | Scoriaspora lactata |
| PEZIZOMYCETES | OSC 100049 | Scoriaspora crassa |
| PEZIZOMYCETES | OSC 10015 | Sclerotinia scutellata |
| PEZIZOMYCETES | NRRR 2233 | Verpa conica |
| SACCHAROMYCETES | GB | Candia glabrata |
| SACCHAROMYCETES | GB | Candida tropicalis |
| SACCHAROMYCETES | GB | Debaryomyces hansenii |
| SACCHAROMYCETES | GB | Eremothecium gosspii |
| SACCHAROMYCETES | GB | Saccharomyces cerevisiae |
| SCHIZOCHYTRIOMYCETES | GB | Schizosaccharomyces pombe |
| SCOLOPHAERIACEAE | CBS 115999 | Calosphaeria pulchella |
| SORDARIOMYCETES | SMH4420 | Bretia moriformis |
| SORDARIOMYCETES | CBS 171.69 | Cryptospora hypodermia |
| SORDARIOMYCETES | CBS 109767 | Diaporthes eae |
| SORDARIOMYCETES | CBS 112915 | Endothia cygnus |
| SORDARIOMYCETES | CBS 199.53 | Gnomonia gnomon |
| SORDARIOMYCETES | JG 17-32 | Boletia aureofulva |
| SORDARIOMYCETES | GAM 1285 | Claviceps purpurea |
| SORDARIOMYCETES | OSC 93607 | Cordycps cardinis |
| SORDARIOMYCETES | OSC 71233 | Elaphomyces cristatus |
| SORDARIOMYCETES | OSC 106405 | Elaphomyces hyophodes phlogosis |
| SORDARIOMYCETES | ATCC 56429 | Epichloé typhina |
| SORDARIOMYCETES | ATCC 20838 | Hypocre a lutea |
| SORDARIOMYCETES | CBS 140055 | Nectria cinnabarina |
| SORDARIOMYCETES | FAU 553 | Glomarella cinclata |
| SORDARIOMYCETES | ATCC 16535 | Verticillium dahliae |
| SORDARIOMYCETES | JK 590A | Lindra thallae |
| SORDARIOMYCETES | JK 468 | Luworthia grandispora |
| SORDARIOMYCETES | JK 5285 | Gueaumarnyces maderarius |
| SORDARIOMYCETES | Broad | Magnaporthe grisea |
| SORDARIOMYCETES | ATCC 15515 | Malsenoprasa tiffanyana |
| SORDARIOMYCETES | TCH C89 | Ceratostys fimbrata |
| SORDARIOMYCETES | 728a | Coriolopsis maritima |
| SORDARIOMYCETES | CBS 12611 | Gondwanomyces caperata |
| SORDARIOMYCETES | CBS 197.60 | Halkohyphae ascophaea |
| SORDARIOMYCETES | CBS 139.51 | Ophiostoma stenoceras |
| SORDARIOMYCETES | Broad | Neurospora crassa |
| SORDARIOMYCETES | CBS 15-973 | Sordaria fimicola |
| SORDARIOMYCETES | XOS 10004 | Xylaria hypoxylon |
| SORDARIOMYCETES | CBS 356.35 | Taphrina deformans |
| SORDARIOMYCETES | IAM 14515 | Taphrina wiesenii |

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, Arthro-romycetes and Dothideomycetes. Arthoniomycetes consists of ± 1 600 species of lichenised and lichenicolous fungi with fissitunicate asci and exposed hymenia (Grube 1998, Erz et al. 2009). Unlike other species with fissitunicate asci, these taxa have ascohyllenial development, prompting their placement in a transitory group, or ‘Zwischengruppe’ that is intermediate between ascohymenial and ascolocular 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, Botryosphaeriales (Schoch et al. 2006). Placement of Botryosphaeriales within subclass Pleosporomycetidae is well supported, as is a close relationship with the unplaced family Tubefiaceae (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 ascomata produced by the division and enlargement of ascospores that are difficult to characterize in existing ascomatal 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 (Taveraes 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.

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