
1 Fungi from PCR to Genomics: The Spreading Revolution in Evolutionary Biology

JOHN W. TAYLOR¹, MARY L. BERBEE²

CONTENTS

I. PCR to Genome Sequencing and a Robust Phylogeny for Fungi.....	1
II. Peering into Variation Among Individuals: Next-Generation Sequencing	3
III. Fungal Species Recognition in Era of Population Genomics	5
IV. Metagenomics and Tools for Identification	5
V. What Is a Fungus? Phenotype and Its Evolutionary Origins	6
A. Discoveries of Protistan Allies Affect Definitions of Fungi and Animals	6
B. Evolutionary Origin of Characters That Define Fungi	8
1. Fungus-Specific Chitin Synthases	8
2. Biosynthesis of Ergosterol, the Characteristic Sterol in Fungal Membranes.....	8
3. Origins of Fungal Lysine Biosynthetic Pathway in Opisthokont Prehistory....	9
4. Hyphae and Absorptive Nutrition Were Missing from Fungal Stem Lineage ..	10
V. Convergent Evolution of Funguslike Protists.....	10
VII. Conclusion.....	11
References.....	12

I. PCR to Genome Sequencing and a Robust Phylogeny for Fungi

In this volume, the fruits of about two decades of molecular systematics are presented. The development of the polymerase chain reaction (PCR) in the late 1980s (Saiki et al. 1988)

made it possible for systematists to become molecular phylogeneticists. Nowhere was the need for additional characters more acute than with fungi, and mycologists responded with pioneering work, none more influential than the development of ribosomal DNA primers, which have been used far beyond Kingdom Fungi (White et al. 1990). Soon thereafter were made fundamental discoveries about the extent of the monophyletic fungal kingdom and the deep divergences in the kingdom (Berbee and Taylor 1992; Bruns et al. 1992; Swann and Taylor 1993). Perhaps the most important discovery from these early investigations was that animals and fungi shared a more recent common ancestor than either did with plants (Wainright et al. 1993). **Animals and fungi, plus related protists, together constitute the opisthokonts** (Lang et al. 2002; Steenkamp et al. 2006) (Fig. 1.1). The single posterior flagellum that gives the opisthokonts their name is, however, retained in only a few clades of fungi that disperse in water (Fig. 1.1, *Rozella* and allied Rozellomycota = Cryptomycota [James and Berbee 2012; Jones et al. 2011; Lara et al. 2010], Chytridiomycota Monoblepharidomycota, and Neocallimastigomycota [see Powell and Letcher 2014], Blastocladiomycota [see James et al. 2014], *Olpidium* [see Benny et al. 2014]).

Once the phylogeny is inferred, however, the biologically interesting fun begins—**unraveling the evolution of phenotype**. In fact, the inaugural use of PCR-amplified DNA sequencing was to infer the evolution of the closed fruiting body of a false truffle from its mushroom ancestors (Bruns et al. 1989). Once phylogenies became broadly inclusive, work began on the evolution

¹Plant and Microbial Biology, University of California, 111 Koshland Hall, Berkeley, CA 94720-3102, USA; e-mail: jtaylor@berkeley.edu

²Department of Botany, University of British Columbia, Vancouver, BC, Canada V6T 1Z4

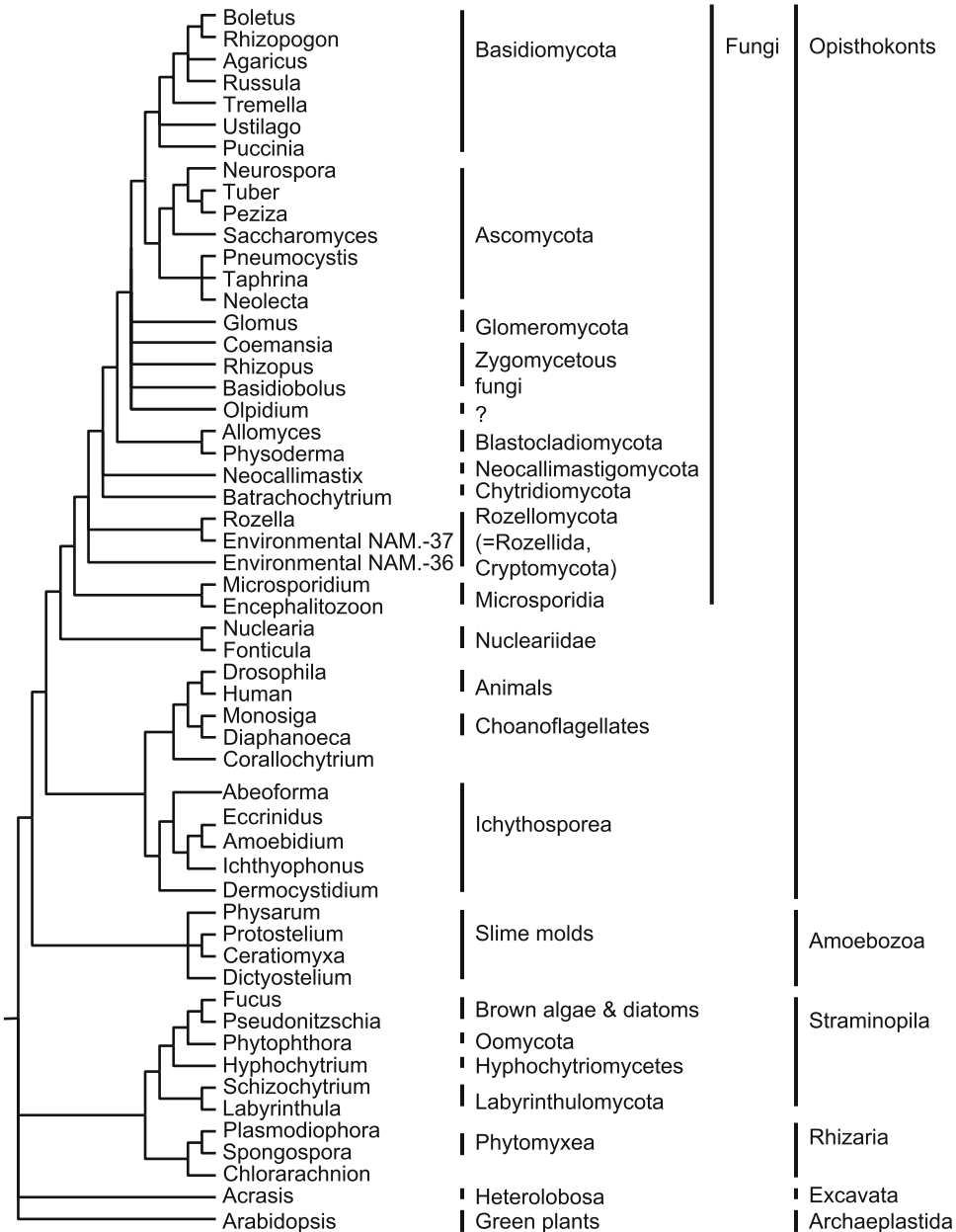


Fig. 1.1 Fungi and funguslike organisms covered in *Mycota* span the eukaryotic diversity diagrammed in this tree, which is based on the Tree of Life Web project (2012) and on references cited in the text

of key phenotypes, such as lichenization (Lutzoni and Pagel 1997), mycorrhizal association (Hibbett et al. 2000), parasitism (Vogler and Bruns 1998), and wood decay (Eastwood et al. 2011). Almost as soon, attempts to fit the new phylogenetic trees to the geologic time scale were made, making it possible to match

evolutionary events in fungi with those in plants and animals, albeit not without controversy (Berbee and Taylor 1993, 2010; Casadevall 2005; Heckman et al. 2001; Simon et al. 1993). As with attempts to fit molecular phylogenies to geological time scales, attempts to fit phenotypes to phylogenies can be controversial, and

conclusions may change as the data sets expand (Hibbett and Matheny 2009; Schoch et al. 2009).

The production of the automated sequencer accelerated the pace of molecular phylogenetics and enabled the first sequence-based studies of **fungal population genetics**. These efforts uncovered cryptic sex (Burt et al. 1996), cryptic species (Koufopanou et al. 1997), and cryptic populations (Fisher et al. 2001). The discovery that the average fungal morphospecies embraced two or more genetically differentiated phylogenetic species improved our understanding of fungal diversity and helped put an end to the notion that all microbial species have global distributions (Taylor et al. 2006). The discovery that fungi are recombining even though we mycologists have not caught them in the act put an end to the notion that a fifth of Kingdom Fungi was asexual (Taylor et al. 1999).

The first eukaryote to have a fully sequenced genome was a fungus, *Saccharomyces cerevisiae* (Goffeau et al. 1996). When the first human genomes were fully sequenced, the large centers that developed to provide the data suddenly had excess capacity and fungi became favored organisms. First, the Institute for Genomic Research (now the J. Craig Venter Institute) and the Broad Institute focused on human pathogens, but soon nonpathogens were sequenced, many of them through the Community Sequencing Project at the Joint Genome Institute. Kingdom Fungi is now the most deeply sequenced eukaryotic kingdom. It is probably impossible to keep abreast of the progress, and we hesitate to list any numbers because they will be hopelessly out of date before this volume is released. As of March 2012, more than 790 fungal genome projects were listed at GOLD (2012), none of which was zygomycetous. A shorter list at Fungal Genomes (2012) included 7 zygomycetous genera, which pushes the number to 800, a total nearly twice that of cordates, land plants, or Archaea. This wealth of data has stimulated **phylogenomics** (Fitzpatrick et al. 2006), where the task has now become to select the best genes for phylogenetic analysis (Townsend 2007) from among an embarrassing wealth of data (Rokas et al. 2005).

II. Peering into Variation Among Individuals: Next-Generation Sequencing

Technologically, the next best thing to emerge was next-generation sequencing, and again fungi are leading the way. The older Sanger sequencing allowed determination of bases of one DNA template at a time, and thousands of separate sequencing reactions were needed to completely sequence a genome. Next-generation sequencing simultaneously determines bases of a huge population of DNA fragments and can, in one lane of a single Illumina run, completely sequence a genome. The population genomics of yeast (Liti et al. 2009), human pathogens (Neafsey et al. 2010), and a model filamentous fungus, *Neurospora* (Ellison et al. 2011), have shown that genetically differentiated fungal populations can be discovered at very young ages, an order of magnitude earlier than even cryptic species, posing yet another challenge to the very definition of a species. Now that sequencing genomes of novel fungi is within the budget of an average research grant, the ultimate data for phylogenetics and molecular systematics are at hand, and the bottleneck has become the computational skills to make use of it.

Not only systematists but other biologists as well have been making use of phylogenetic results. As a result, all fields of mycology have been brought closer together because developmental biologists, industrial microbiologists, and ecologists all use the fruits of **phylogenetics and now phylogenomics as basic tools for tasks as disparate as gene cloning, improving enzyme production, and documenting the diversity of fungal communities**. No field has benefited more from the companionship with molecular systematics than fungal ecology. Mycorrhizal studies led the way, with the startling revelation that species lists from surveys of fruiting bodies bore little relationship to the species actually on the mycorrhizal roots (Horton and Bruns 2001). Even the field of fungal endophytes, which began its rapid emergence based on cultured fungi, has benefited from molecular identification (Arnold and

Lutzoni 2007) and now environmental sequencing (Jumpponen and Jones 2009). Sequencing of environmental DNA from bulk extracts from soil, air, water, or other mixed sources has greatly expanded our knowledge of clades at all taxonomic levels, from species (Suh et al. 2004) all the way up to classes in the Ascomycota (Schadt et al. 2003). For the very deep *Rozella* clade, **environmental sequencing** has shown that one cultivated member is accompanied by many other, previously unknown but highly divergent, species (Jones et al. 2011; Lara et al. 2010). Next-generation sequencing is dramatically enlarging the scope of such studies; witness the study of indoor air fungi that sampled 72 sites on all 6 habitable continents and used large subunit ribosomal DNA sequences to infer the presence of nearly 4,500 fungal species, all without a single culture (Amend et al. 2010).

Study of the evolution of phenotype in fungi has focused on ancient divergences, but next-generation sequencing is making it possible to study adaptation following the most recent divergences. In fact, next-generation sequencing of fungal populations looks to complete the amalgamation of development, evolution, and ecology through the common goal of understanding adaptive phenotypes, this time at the level of genomes.

Understanding the **mechanics of speciation** is suddenly a tractable problem. Dettman et al. (2007) provided experimental evidence for a step in the speciation process, showing that divergent selection may lead to partial reproductive isolation. They applied divergent selection to *S. cerevisiae*, experimentally creating lineages tolerant of either high salt or low glucose. After 500 generations of selection, strains' mitotic growth had improved in each selective environment. However, when crossed, the hybrids between the high-salt and low-glucose lineages had reduced meiotic efficiency. Using next-generation sequencing, Anderson et al. (2010) then tracked down genes related to increased success in each selective environment, including a gene for a proton efflux pump and for a regulator of mitochondrial protein synthesis. However, when alleles of these

two genes that were favorable under opposite selective regimes were combined in the same strain under low-glucose conditions, the consequence was reduced meiotic fitness. If divergent selection leads to reduction in meiotic competence in the laboratory, it may also lead to speciation in nature.

A first stab at detecting genes associated with adaptation and speciation in natural populations has been provided by a study of *Neurospora*, where genomes of 50 individuals from one clade of *N. crassa* revealed two recently diverged populations, one tropical and the other subtropical (Ellison et al. 2011). Comparison of the genomes identified regions of exceptional divergence, in which were found candidate genes that suggested adaptation to cold temperature [an RNA helicase (Hunger et al. 2006) and prefoldin (Geissler et al. 1998)] and differences in light periods [the major circadian oscillator, *frq* (Aronson et al. 1994)]. Comparisons of fitness of wild isolates against those from which the candidate genes had been deleted (Dunlap et al. 2007) failed to reject hypotheses for adaptation to cold shock involving the RNA helicase and the prefoldin. Hypotheses linking these specific genes to adaptation can be further challenged by swapping alleles among individuals from the two populations and by examining other fungi whose populations are also separated by latitudinal gaps. This fungal study of adaptation is different from those previously conducted with animals or plants in that the genetically isolated populations were cryptic, there was no obvious candidate environmental parameter, such as light sand versus dark lava or normal soil versus serpentine soil, and there was no obvious candidate adaptive phenotype, such as mammal coat color (Nachman et al. 2003) or plant growth on serpentine soils (Turner et al. 2010). The "reverse ecological" approach to associating phenotype and genotype detailed for *Neurospora* may prove to be very powerful for natural populations of fungi where a priori identification of adaptive phenotypes can be difficult. Lacking prior bias, this approach also may offer surprises even in systems where candidate phenotypes have been selected.

III. Fungal Species Recognition in Era of Population Genomics

Population genomics has added new complexity to the task of recognizing fungal species. As noted earlier, **phylogenetic species recognition has replaced morphological species recognition** as the method of choice because fungal species typically become genetically isolated in nature long before mycologists can recognize any morphological difference (Cai et al. 2011; Giraud et al. 2008; Taylor et al. 2000). Phylogenetic species recognition has relied on the concordance of several gene genealogies, as described for *Neurospora* species (Dettman et al. 2003a) by an approach that showed good correlation with biological species recognition (Dettman et al. 2003b). However, as described in Sect. II, population genomic analysis of individuals from just one of three *N. crassa* clades revealed that it contained two, genetically distinct, populations (Ellison et al. 2011). Growth rates for the two populations showed a significant difference at low temperature, indicating that a phenotypic difference had evolved between the two populations (Ellison et al. 2011). Individuals from the two populations mate successfully in the lab, but analysis of the population genomic data indicates that intrapopulation gene flow is too low to reverse the genetic differentiation seen between the two populations (Ellison et al. 2011). Low gene flow between species that can be mated in the lab suggests that there is an extrinsic barrier to reproduction in nature. To pose the obvious rhetorical question, if genetic isolation in nature is the criterion for species recognition, should these populations be considered different species? To be sure, **species recognition by population genomics** sounds impossibly impractical today, but it might be worth remembering that species recognition by concordance of gene genealogies sounded impossibly impractical in 1997.

IV. Metagenomics and Tools for Identification

Challenges remain in interpreting the data from environmental and cultivation-independent study of fungi. No one knows how many fungal species exist, but sequencing of environmental DNA will definitely improve the accuracy of the estimate (Hawksworth 2001). To count species or to correlate fungal species across studies requires the development of **sequence identification tools** beyond GenBank. This need has arisen because databases are not populated with enough **vouchered sequences** to permit identification of a majority of environmental sequences. For example, 35 % of the ribosomal internal transcribed spacer sequences shared among the international databases GenBank, EMBL, and DDBJ were not assignable to a named taxon, and only 21 % of ITSs associated with a named taxon were also tied to a vouchered specimen (Ryberg et al. 2009). Interestingly, the rate of deposition of new fungal sequences from the environment now exceeds the deposition of sequences from fungi tied to specimens or cultures, a phenomenon that raises an important nomenclatural challenge (Hawksworth 2001; Hibbett et al. 2011). Sequences from fungi in herbaria and culture collections can be added to the database, and, even if completed for only a fraction of fungi (Hibbett et al. 2011; Jumpponen and Jones 2009), these provide an important link with environmental samples. One point to keep in mind is that, although almost all ecological studies find a very large number of total taxa, the number of commonly encountered taxa can be much smaller, e.g., only 31 common species were found among the 4,500 detected in the aforementioned study of indoor air (Amend et al. 2010). A second problem concerns the accuracy of sequences already in the international databases, of which as many as 20 % are misidentified (Bidartondo et al. 2008;

Nilsson et al. 2006). To avoid compromising its relationship with the original depositors, GenBank refuses annotation by any third party who notices a problem with a sequence. This contrasts with herbaria, which routinely welcome annotations, such as reidentifications from other researchers, contributing to the overall reliability of their data. Imagine a herbarium where no one but the original collector could annotate a specimen, and you can appreciate the problem with the genetic databases. The solution? Eliminating all so-called bad sequences may be politically impossible, but specifically designating good ones is perfectly feasible. The UNITE database provides sequences of carefully identified mycorrhizal fungi (Koljalg et al. 2005). GenBank and the Barcode of Life Database are currently creating curated, public databases of correctly identified sequences where third-party annotations will be invited and quality standards are carefully enforced (Schoch and Seifert 2010).

Having a sequence database, the next step is **developing tools for automatic identifications**. For prokaryotes, automated, reliable identification tools for environmental sequences have revolutionized microbial ecology. Identification is based on (1) a database of curated, correctly identified sequences and (2) a publicly available mechanism for matching environmental sequences to the database, then returning identifications to users. Three Web-based services (Greengenes 2012; Ribosomal Database Project 2012; Silva 2012), each with a slightly different approach (Schloss 2009), provide prokaryotic classifications and are beginning to provide classifications for fungi as well. Fungi lag behind prokaryotes due in part to the ease of aligning the 16S rRNA genes at the core of bacterial identification systems compared to the difficulty in aligning beyond the level of genus or family the more variable ribosomal internal transcribed spacer regions used to identify fungal species. Neither system is perfect. Bacteria are easier to place in a robust phylogeny, but each 16S OTU (operational taxonomic unit) harbors many genetically isolated species (Vos and Velicer 2008; Whitaker et al. 2003); fungi are more easily identified as species-level taxa, but new, divergent sequences may be impossible to link to genera or even families.

Some successful fungal identification databases are therefore genus specific and targeted toward large, economically important genera including *Trichoderma* (TrichOKEY 2 2011; Druzhinina et al. 2005) and *Fusarium* (Park et al. 2011).

V. What Is a Fungus? Phenotype and Its Evolutionary Origins

A. Discoveries of Protistan Allies Affect Definitions of Fungi and Animals

Bringing us closer to the “holy grail” of understanding the evolution of complex organisms with differentiated tissues were discoveries of early diverging protists at the boundary between Kingdom Fungi (Brown et al. 2009; Steenkamp et al. 2006; Zettler et al. 2001) and Kingdom Animalia (Marshall and Berbee 2011; Mendoza et al. 2002). Because these boundary protists evolved before the origin of classical kingdom-level characters, their morphology had been a poor predictor of their relationships. In terms of morphology, perhaps the time has come to invoke Bruns’ law: “There are no [expletive deleted] synapomorphies; get over it.”

Molecular phylogenetics, by accommodating protists that show few fungal or animal traits, provides a simple alternative to defining taxa while offering a framework for exploring how their characters evolved (James et al. 2006). Phylogenetics linked the multicellular animals (Metazoa) and their protist allies together as Holozoa (Lang et al. 2002) within the supergroup Opisthokonta (Fig. 1.1). Protist Holozoa include choanoflagellates, or collar flagellates (Steenkamp et al. 2006), and enigmatic arthropod commensals *Amoebidium* and *Eccrinidus*, which were once considered Trichomycetes (zygomycetous fungi) and are now placed in the Ichthyosporeans or Mesomycetozoa on the animal lineage (Benny and O’Donnell 2000; Cafaro 2005; Mendoza et al. 2002).

Fungi and their unicellular relatives are classified together in the Holomycota, the sister group to the Holozoa within opisthokonts (Liu et al. 2009). Unicellular Holomycota include peculiar amoebae in *Nuclearia* and an

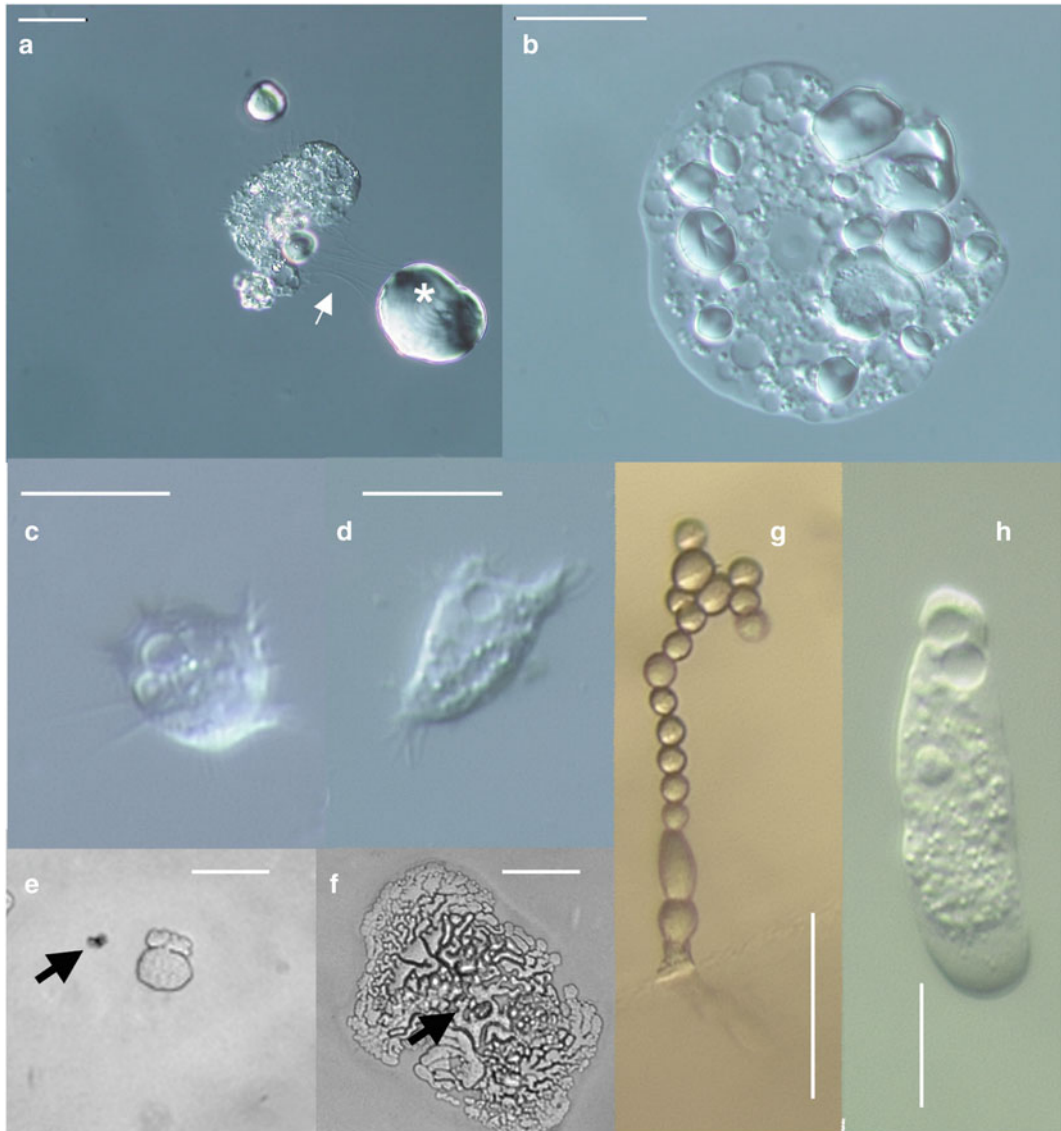


Fig. 1.2 Some of these amoeboid protists that lie phylogenetically outside of Kingdom Fungi may resemble the earliest members of the fungal lineage. *Nuclearia thermophila* (a, b) and *Fonticula alba* (c, d) are members of the sister group to Kingdom Fungi. (a) *N. thermophila* contacting a grain of flour (asterisk) using fine filose pseudopodia (arrow). (b) A *N. thermophila* amoeba that engulfed many flour particles. (c, d) *F. alba* amoebae with filose pseudopodia. (e, f) A living plasmodium of *Abeoforma whisleri* (Ichthyosporea, protist members of animal lineage) that grew from (e) to (f) in 25 h. The

arrow indicates a particle of debris marking the same spot in both images. (g, h) *Acrasis helenhemmesae* is a recently discovered species in a genus once considered a social slime mold. It is in the Excavata along with the photosynthetic flagellate *Euglena* and is only distantly related to familiar social slime molds in *Dictyostelium*. (g) Spore production. (h) Amoeba. Scale bars: (a, b) 25 μm ; (c, d, h) 10 μm ; (e, f) 100 μm ; (g) 50 μm . Photo credits: (a), (b) from Yoshida et al. (2009); (c), (d) from Brown et al. (2009); (e), (f) from Marshall and Berbee (2011); (g), (h) from Brown et al. (2010)

aberrant social slime mold, *Fonticula alba* (Figs. 1.1 and 1.2; Liu et al. 2009). Within Holomycota, phyla from Basidiomycota through

Microsporidia together constitute a monophyletic group (Fig. 1.1). Fungi are therefore easily defined phylogenetically as the sister group to

Fonticula plus *Nuclearia* but are best understood as a dynamic clade of evolving heterotrophs that, parallel to animals, adapted successfully to life on land and in freshwater.

Genomic data, especially from the early diverging taxa, allow a closer appreciation of the evolutionary processes that gave rise to textbook fungal-specific characters. Bearing in mind that all genes but one evolved through modifications of earlier genes, **fungal-specific genes have homologs elsewhere but have diverged in sequence or function.** Phylogenetic analyses of fungal traits increasingly show connections of genes and pathways of Kingdom Fungi and other opisthokonts rather than discrete boundaries across kingdoms. This leads to a much more complete view of fungal origins.

B. Evolutionary Origin of Characters That Define Fungi

1. Fungus-Specific Chitin Synthases

Among the best characterized of potential fungal-specific genes are chitin synthases. Synthases that produce chitin are widespread among eukaryotes, but production of a chitinous wall around actively growing cells is uncommon outside of fungi. **Only fungi, but almost all fungi** including the most divergent, such as *Rozella* and the microsporidium *Encephalitozoon cuniculi*, **share a division 2, class IV chitin synthase** (James and Berbee 2012; Ruiz-Herrera and Ortiz-Castellanos 2010). Although fungi can have more than a dozen chitin synthases, this particular enzyme is implicated in the synthesis of the bulk of the chitin in cell walls (Munro and Gow 2001). Fungi also share one or more additional division 2 chitin synthases that bear a myosin domain at their N-terminal end (James and Berbee 2012; Ruiz-Herrera and Ortiz-Castellanos 2010). While the diatom *Thalassiosira pseudonana* once also seemed to share a myosin domain in a chitin synthase (Durkin et al. 2009), this was probably the result of an error in an early automated gene annotation. More recent gene predictions (e.g., GenBank XP_002295995) no longer show a myosin domain associated with the diatoms' chitin

synthases. The microsporidia lack a myosin-bearing chitin synthase, either because they lost it or because their lineage originated before the enzyme evolved. Clearly, the ancestor of all fungi inherited chitin synthases, which then underwent duplication and divergence to give rise to the distinctive synthases now shared across the kingdom.

2. Biosynthesis of Ergosterol, the Characteristic Sterol in Fungal Membranes

Unlike most animals and plants, the main sterol in fungal plasma membranes is ergosterol. Animals have predominantly cholesterol, and plants have diverse sterols, including campesterol, sitosterol, stigmasterol, and isofucosterol (Schaller 2004). Ergosterol serves as a target for many of the most effective antifungal drugs (Francois et al. 2005). By binding more efficiently to ergosterol in fungal membranes than to cholesterol in human membranes, the important antifungal drug amphotericin B is often able to save people from otherwise fatal fungal infections. Although not all fungi accumulate ergosterol as their predominant sterol, the pathway for its synthesis is widely conserved (Weete et al. 2010). A nice overview of steps involved in ergosterol biosynthesis is available through the Yeast Biochemical Pathway Database (2012). Plant, animal, and fungal sterol biosynthesis pathways begin the same way, using the mevalonate pathway to generate not only sterols but also various other chemicals such as isoprenoids.

Although ergosterol is considered specific to fungi, the enzymes involved in biosynthesis of ergosterol all have close homologs in other organisms. To illustrate this point, we examined sterol 24-C-methyltransferase (EC 2.1.1.41) because differences at this enzymatic step help illustrate why fungi make ergosterol while plants and animals do not. In both animals and fungi, the biosynthetic pathway leading to sterol production proceeds to cyclization of squalene-2,3-oxide producing a lanosterol intermediate, which in fungi and animals is converted to zymosterol, the substrate for sterol 24-C-methyltransferase. The enzyme in *S. cerevisiae* and, presumably, other fungi

(such as the chytrid *Batrachochytrium dendrobatidis* GenBank EGF84453) adds a methyl group to the tetracyclic sterol zymosterol, converting it to fecosterol (Parks et al. 1995). In most multicellular animals, the homologous enzyme lacks the sterol methyltransferase function so that animals cannot add the extra methyl group to carbon-24 of the sterol side chain, as would be necessary for ergosterol biosynthesis (Kaneshiro 2002). However, looking more deeply into early Holozoa, a homolog that seems to have sterol binding sites is present in the sponge *Amphimedon queenslandica* (GenBank XP_003387525.1). Although the differences in sterols across kingdoms are important, they sometimes resulted from loss rather than gain of function, and even then, they resulted from relatively small genetic changes.

3. Origins of Fungal Lysine Biosynthetic Pathway in Opisthokont Prehistory

While most animals must ingest the essential amino acid lysine from their diets, fungi, plants, and bacteria synthesize their own lysine. Synthesis of lysine across all domains of life takes place through one of the three or more alternative, multienzyme pathways of independent evolutionary origin. Fungi synthesize lysine using the **alpha-aminoadipate pathway** (Vogel 1965). The fungal pathway requires seven enzymatic steps (see lysine biosynthesis, Yeast Biochemical Pathway Database 2012). A pathway that also uses an alpha-aminoadipate intermediate, but is of independent evolutionary origin, leads to lysine biosynthesis in the hyperthermophilic bacterium *Thermus thermophilus* and in an anaerobic archaeobacterium *Pyrococcus horikoshii* (Nishida et al. 1999). As reviewed by Zabriskie and Jackson (2000), euglenoids also use alpha-aminoadipate as an intermediate in the synthesis of lysine, and although the genes and enzymes involved have yet to be studied, this group of photosynthetic or phagotrophic protists may also have an alpha-aminoadipate lysine biosynthesis pathway of independent origin. The remaining lysine synthesis pathway, the **diaminopimelic acid pathway**, is widely distributed among prokar-

yotes, protists, oomycetes, and plants (Torruella et al. 2009; Vogel 1961, 1965). Like the fungal pathway, the diaminopimelic acid pathway requires seven enzymatic steps. Remarkably, however, none of the fungal enzymes for lysine biosynthesis are homologous to any of the plant diaminopimelic acid pathway enzymes, and for this reason the alpha-aminoadipate pathway for lysine biosynthesis has been considered a unifying derived characteristic that helped link chytrids to fungi rather than to oomycetes (Vogel 1961).

Vogel's insights still hold; however, closer dissection of the evolutionary relationships of individual enzymes in the pathway, coupled with comparative analysis using new protist genomes, reveals an unexpectedly complex pattern of gene duplication, functional divergence, and loss (Irvin and Bhattacharjee 1998). The first enzymes in fungal biosynthesis are, at a deep level, distant homologs of Krebs' cycle enzymes, while the two last enzymes are related to proteins involved in lysine catabolism (Irvin and Bhattacharjee 1998; Nishida and Nishiyama 2000). Nishida and Nishiyama (2000) carefully tracked the phylogeny of alpha-aminoadipate reductase (EC 1.2.1.31), the fifth gene in the pathway, and, based on sequences available at the time proposed that this enzyme was specific to fungi. However, with new genome sequences, eukaryotic homologs to alpha-aminoadipate reductase were identified in *Corallochytrium limacisporum*, which, despite its name, is not a chytrid fungus but rather a protist that diverged early in the evolutionary history of opisthokonts (Sumathi et al. 2006). Other protists in Holozoa also share the enzyme, and it is even present outside of the opisthokonts, with a homolog in *Dictyostelium discoideum* (Amoebozoa) (Torruella et al. 2009). While the functions of nonfungal homologs to fungal alpha-aminoadipate pathway enzymes have yet to be tested biochemically, it seems likely that the pathway evolved before the Amoebozoa diverged from the opisthokonts (Fig. 1.1). **For fungi, the alpha-aminoadipate pathway for lysine synthesis is a shared primitive character**, and the absence of the pathway in animals represents an evolutionary loss.

4. Hyphae and Absorptive Nutrition Were Missing from Fungal Stem Lineage

The morphology of the opisthokont protists also offers clues into the evolution of fungi. *Fonticula* and *Nuclearia* retain an ancestral habit of ingesting bacteria or algae (Cavalier-Smith 2002). The common ancestor to fungi may have done likewise before the evolution of the multicellular plants and animals that are the nutrient sources of most extant fungi. Wall-less **amoeboid dispersal phases** are common among unicellular opisthokonts and may be an ancestral characteristic of the fungal stem lineage. Amoeboid phases occur in the few Ichthyosporea that grow well in culture (Figs. 1.1 and 1.2; Marshall and Berbee 2011; Whisler 1962) as well as in *Fonticula* (Brown et al. 2009) and *Nuclearia* (Figs. 1.1 and 1.2; Liu et al. 2009; Yoshida et al. 2009; Zettler et al. 2001). Even though Chytridiomycota (Fig. 1.1) seem to have lost the capacity for extensive amoeboid motion, at least some species retain genes encoding animal cell movement proteins (Harris 2011). The movement proteins may still play a role when, for example, zoospores squeeze out of a zoosporangium or are trapped between hyphae (Gleason and Lilje 2009).

Although not ancestral in the kingdom, other classical characters of modern Kingdom Fungi, such as **hyphae, a chitinous cell wall, reproduction by spores, and absorptive nutrition**, had evolved by the time plants colonized land, by 400 million years ago, based on fossil (Taylor et al. 2004) and phylogenetic (Berbee and Taylor 2001) evidence. They must have secreted enzymes across their walls to assimilate nutrients, and they reproduced with walled spores. Most terrestrial fungi lost the flagellated, wall-less zoospore stage of their aquatic predecessors. Suggesting that **flagellar loss** may have taken place convergently and after the origin of hyphae, remnants of what may be a centriole from an ancestral flagellum remain visible in the hyphal zygomycete *Basidiobolus* (Gull and Trinci 1974) and in *Coemansia reversa* (McLaughlin et al. unpublished). Another example of convergent loss of flagella involves *Olpidium*, a flagellated unicellular fungus that disperses by zoospores, clustered phy-

logenetically within the terrestrial fungi and among hyphal zygomycetes (Fig. 1.1; James et al. 2006; Sekimoto et al. 2011). By implying that the ancestor to *Olpidium* was both terrestrial and a flagellate, the phylogeny suggests that early hyphal fungi on land still reproduced by motile spores. Like animals and plants, including mosses and ferns, early terrestrial fungi may have retained swimming flagellated cells as a legacy of their aquatic past.

The Microsporidia (Didier et al. 2014) present a particular challenge to the definition of fungi because their genomes had evolved so rapidly that their phylogenetic history is all but obliterated (Koestler and Ebersberger 2011). They lack shared fungal characters and have no chitin during their assimilative stage, possibly due to derived loss. As obligate parasites, microsporidia cause diseases in animals from *Daphnia* to humans, and in the past they were studied by parasitologists or medical pathologists. More recently, they are catching the attention of evolutionary biologists interested in links between parasitism and rates and modes of evolution (Gill et al. 2010; Keeling et al. 2005).

V. Convergent Evolution of Funguslike Protists

Wisely, the editors and authors have cast their net widely to include not only Kingdom Fungi but also organisms that look or behave like fungi. **Funguslike organisms are found in at least four large clades in addition to the opisthokonts:** Straminopila, Rhizaria, Excavata, and Amoebozoa. Most importantly, socially, is the Straminopila (Beakes et al. 2014), home to the Oomycota, Labyrinthulomycota, and Hyphochytriomycetes. The Oomycota harbors the plant destroyers, literally, *Phytophthora* and relatives. The Labyrinthulomycota also has some plant parasites of grasses, but the Hyphochytriomycetes is, as far as we know, innocent of phytocide. Inspired in part by DeBary's studies of *Phytophthora* species, mycologists and plant pathologists came to value hypothesis-driven experimental research.

In an era when spontaneous generation was still considered a plausible explanation for the appearance of plant disease, DeBary (1863) left a lasting legacy of a higher standard of evidence by using careful observations and experimental inoculations to prove that *Phytophthora infestans* caused late blight of potatoes.

Distinguishing straminopiles from Kingdom Fungi drew on early analysis of cell wall chemistry (Bartnicki-Garcia 1968; Von Wettstein 1921), genetics (Barksdale 1966), biochemistry (Vogel 1965), and microscopy, with molecular phylogenetics offering definitive confirmation of the deep divergence (Gunderson et al. 1987). Von Wettstein (1921) pointed out the striking division between funguslike forms that had chitinous walls versus forms with cellulose walls. He interpreted their cellulose walls as evidence that oomycetes originated relatively recently from algae, in contrast to fungi with chitinous walls, which he felt were an older group of less easily identified origin. Clearly, the nonphotosynthetic straminopiles are related to photosynthetic brown algae and diatoms, and whether the funguslike clades lost chloroplasts or never had them in the first place is still debated (Stiller et al. 2009; Tsui et al. 2009). As in the opisthokonts and plants, the straminopiles evolved into a striking diversity of body plans and ecological functions.

Slime molds, defined by their creeping plasmodium or by social amoebae, span five phylogenetic clades, three in addition to *Fonticula* from the fungal lineage and *Ichthyosporia* (e.g., *Abeoforma whisleri*, Fig. 1.2) on the animal lineage. The Rhizaria (Bulman and Braselton 2014) include the green, photosynthetic amoeba *Chlororachnion* and two funguslike plant pathogens, *Plasmodiophora* and *Spongospora*, scourges of Brassicaceae and potato, respectively (Cavalier-Smith and Chao 2003). The Excavata (Stephenson 2014) is home to a social slime mold, *Acrasis* (Fig. 1.2; Brown et al. 2010). Excavata also includes *Euglena*, a flagellated green photosynthetic protist, and *Naegleria fowleri*, cause of amoebic meningoencephalitis, a rare human disease. If a swimmer has the bad luck to take up a nose full of water containing *Naegleria* amoebae, the amoebae can migrate to and then infect the brain (Cen-

ters for Disease Control and Prevention 2012). Finally, the Amoebozoa (Stephenson 2014) includes slime molds of both the social, cellular type (Dictyosteliomycota) and the plasmodial type (Myxomycota) and relatives, such as *Ceratiomyxa* and *Protostelium*. Dictyosteliomycota are ubiquitous and serve as model systems for research as diverse as cell migration (Ridley et al. 2003) and evolutionary cheating (Strassmann et al. 2000). Myxomycota are also model systems for research as diverse as biological oscillation (Takamatsu et al. 2000) and maze solving (Nakagaki et al. 2000) as well as being stunningly beautiful (Emoto 1977).

VII. Conclusion

The chapters in these volumes detailing phylogenetic relationships of fungi and nonfungi set the stage for future studies of phenotype and adaptation on one hand and ecological diversity on the other. We predict that over the next decade fungi will be among the most attractive targets for research associating genotype and phenotype. Fungi are eukaryotes, but simple ones and with small genomes. Fungi seem to be able to adapt to every environmental parameter. Fungi have evolved many features typical of more complex eukaryotes, including self-/non-self-recognition and even chromosomes determining sexual identity (Heitman et al. 2007; Menkis et al. 2008). Fungi can reproduce both clonally and by recombination. Fungi exchange genes within populations by mating, among populations by introgression, and even among long diverged lineages by horizontal gene transfer (Inderbitzin et al. 2005, 2011; Mehrabi et al. 2011). It would not be surprising if hybridization and introgression among recently diverged populations and species proved to be as important to fungal adaptation as horizontal gene transfer is to bacteria (Juhas et al. 2009; Lacroix et al. 2006). Cultivated fungi are immortal, so experiments that require sacrificing an individual can be replicated with the same individual, a very different situation than with most plants or animals. Cultivated fungi are often haploid, so inbred lines are

unnecessary for studies aimed at associating phenotypic and genotypic variation. Where they are not haploid, fungi often are dikaryotic and function as diploids with the advantage that the dikaryons can often be broken into their haploid components and studied independently. With all of these attributes, we hope that biologists will be attracted to fungi as organisms of choice for their studies aimed at understanding the evolution of phenotype in terms of their genomes.

The phylogenies will have an equally profound effect on studies of fungi in nature. Here, fungi known only from environmental nucleic acid sequences will dominate biodiversity and will likely be among the species most important to ecosystem function. One area where such studies may prove fruitful is in documenting the biological response to global change. Given that the diversity of fungi will far outweigh that of animals or plants in any given ecosystem, it seems likely that some of these fungi will be the best sentinels for recognizing the effects of global change. One can even imagine an automated means of assessing the presence of these sentinel fungi over a region where assessing the effects of global change was a priority.

Acknowledgments Financial support to J. Taylor was provided in part by National Institutes of Health NIH Grant R24 GM081597 and National Science Foundation (NSF) Grant DEB 05-16511 and to M. Berbee by NSF DEB-0732984, Assembling the Fungal Tree of Life, and from a Natural Sciences and Engineering Research Council of Canada Discovery grant.

References

- Amend AS, Seifert KA, Samson R, Bruns TD (2010) Indoor fungal composition is geographically patterned and more diverse in temperate zones than in the tropics. *Proc Natl Acad Sci U S A* 107:13748–13753. doi:10.1073/pnas.1000454107
- Anderson JB, Funt J, Thompson DA, Prabhu S, Socha A, Sirjusingh C, Dettman JR, Parreiras L, Guttman DS, Regev A, Kohn LM (2010) Determinants of divergent adaptation and Dobzhansky-Muller interaction in experimental yeast populations. *Curr Biol* 20:1383–1388. doi:10.1016/j.cub.2010.06.022
- Arnold AE, Lutzoni F (2007) Diversity and host range of foliar fungal endophytes: are tropical leaves biodiversity hotspots? *Ecology* 88:541–549
- Aronson BD, Johnson KA, Loros JJ, Dunlap JC (1994) Negative feedback defining a circadian clock – autoregulation of the clock gene frequency. *Science* 263:1578–1584
- Barksdale AW (1966) Segregation of sex in progeny of a selfed heterozygote of *Achlya bisexualis*. *Mycologia* 58:802–804
- Bartnicki-Garcia S (1968) Cell wall composition and other biochemical markers in fungal phylogeny. In: Harborn JB (ed) *Phytochemical phylogeny*. Academic, New York, NY, pp 83–101
- Beakes GW, Honda D, Thines M (2014) Systematics of the straminipila: labyrinthulomycota, hyphochytriomycota and oomycota. In: McLaughlin DJ, Spatafora JW (eds) *Systematics and evolution: Part A*. Springer, Heidelberg
- Benny GL, O'Donnell K (2000) *Amoebidium parasiticum* is a protozoan, not a Trichomycete. *Mycologia* 92:1133–1137
- Benny GL, Humber RA, Voigt K (2014) Zygomycetous fungi: phylum entomophthoromycota and subphyla kickxellomycotina, mortierellomycotina, mucoromycotina, and zoopagomycotina. In: McLaughlin DJ, Spatafora JW (eds) *Systematics and evolution: Part A*. Springer, Heidelberg
- Berbee ML, Taylor JW (1992) Two ascomycete classes based on fruiting-body characters and ribosomal DNA sequence. *Mol Biol Evol* 9:278–284
- Berbee ML, Taylor JW (1993) Dating the evolutionary radiations of the true fungi. *Can J Bot/Rev Can Bot* 71:1114–1127
- Berbee ML, Taylor JW (2001) Fungal molecular evolution: gene trees and geologic time. In: McLaughlin D, McLaughlin E, Lemke P (eds) *The Mycota, Part B, vol VII*. Springer, Berlin, pp 229–245
- Berbee ML, Taylor JW (2010) Dating the molecular clock in fungi – how close are we? *Fungal Biol Rev* 24:1–16
- Bidartondo MI, Bruns TD, Blackwell M, Edwards I, Taylor AFS, Horton T, Zhang N, Koljalg U, May G, Kuyper TW, Bever JD, Gilbert G, Taylor JW, DeSantis TZ, Pringle A, Borneman J, Thorn G, Berbee M, Mueller GM, Andersen GL, Vellinga EC, Branco S, Anderson I, Dickie IA, Avis P, Timonen S, Kjöller R, Lodge DJ, Bateman RM, Purvis A, Crous PW, Hawkes C, Barraclough T, Burt A, Nilsson RH, Larsson KH, Alexander I, Moncalvo JM, Berube J, Spatafora J, Lumbsch HT, Blair JE, Suh SO, Pfister DH, Binder M, Boehm EW, Kohn L, Mata JL, Dyer P, Sung GH, Dentinger B, Simmons EG, Baird RE, Volk TJ, Perry BA, Kerrigan RW, Campbell J, Rajesh J, Reynolds DR, Geiser D, Humber RA, Hausmann N, Szaro T, Stajich J, Gathman A, Peay KG, Henkel T, Robinson CH, Pukkila PJ, Nguyen NH,

- Villalta C, Kennedy P, Bergemann S, Aime MC, Kauff F, Porras-Alfaro A, Gueidan C, Beck A, Andersen B, Marek S, Crouch JA, Kerrigan J, Ristaino JB, Hodge KT, Kuldau G, Samuels GJ, Raja HA, Voglmayr H, Gardes M, Janos DP, Rogers JD, Cannon P, Woolfolk SW, Kistler HC, Castellano MA, Maldonado-Ramirez SL, Kirk PM, Farrar JJ, Osmundson T, Currah RS, Vujanovic V, Chen WD, Korf RP, Atallah ZK, Harrison KJ, Guarro J, Bates ST, Bonello P, Bridge P, Schell W, Rossi W, Stenlid J, Frisvad JC, Miller RM, Baker SE, Hallen HE, Janso JE, Wilson AW, Conway KE, Egerton-Warburton L, Wang Z, Eastburn D, Ho WWH, Kroken S, Stadler M, Turgeon G, Lichtwardt RW, Stewart EL, Wedin M, Li DW, Uchida JY, Jumpponen A, Deckert RJ, Beker HJ, Rogers SO, Xu JAP, Johnston P, Shoemaker RA, Liu MA, Marques G, Summerell B, Sokolski S, Thrane U, Widden P, Bruhn JN, Bianchinotti V, Tuthill D, Baroni TJ, Barron G, Hosaka K, Jewell K, Piepenbring M, Sullivan R, Griffith GW, Bradley SG, Aoki T, Yoder WT, Ju YM, Berch SM, Trappe M, Duan WJ, Bonito G, Taber RA, Coelho G, Bills G, Ganley A, Agerer R, Nagy L, Roy BA, Laessle T, Hallenberg N, Tichy HV, Stalpers J, Langer E, Scholler M, Krueger D, Pacioni G, Poder R, Pennanen T, Capelari M, Nakasone K, Tewari JP, Miller AN, Decock C, Huhndorf S, Wach M, Vishniac HS, Yohalem DS, Smith ME, Glenn AE, Spiering M, Lindner DL, Schoch C, Redhead SA, Ivors K, Jeffers SN, Geml J, Okafor F, Spiegel FW, Dewsbury D, Carroll J, Porter TM, Pashley C, Carpenter SE, Abad G, Voigt K, Arenz B, Methven AS, Schechter S, Vance P, Mahoney D, Kang SC, Rheeder JP, Mehl J, Greif M, Ngala GN, Ammirati J, Kawasaki M, Gwo-Fang YA, Matsumoto T, Smith D, Koenig G, Luoma D, May T, Leonardi M, Sigler L, Taylor DL, Gibson C, Sharpton T, Hawksworth DL, Dianese JC, Trudell SA, Paulus B, Padamsee M, Callac P, Lima N, White M, Barreau C, Juncai MA, Buyck B, Rabeler RK, Liles MR, Estes D, Carter R, Herr JM, Chandler G, Kerekes J, Cruse-Sanders J, Marquez RG, Horak E, Fitzsimons M, Doring H, Yao S, Hynson N, Ryberg M, Arnold AE, Hughes K (2008) Preserving accuracy in GenBank. *Science* 319:1616
- Brown MW, Spiegel FW, Silberman JD (2009) Phylogeny of the “forgotten” cellular slime mold, *Fonticula alba*, reveals a key evolutionary branch within Opisthokonta. *Mol Biol Evol* 26:2699–2709. doi:10.1093/molbev/msp185
- Brown MW, Silberman JD, Spiegel FW (2010) A morphologically simple species of Acrasis (Heterolobosea, Excavata), *Acrasis helenhemmesae* n. sp. *J Eukaryot Microbiol* 57:346–353. doi:10.1111/j.1550-7408.2010.00481.x
- Bruns TD, Fogel R, White TJ, Palmer JD (1989) Accelerated evolution of a false-truffle from a mushroom ancestor. *Nature* 339:140–142
- Bruns TD, Vilgalys R, Barns SM, Gonzalez D, Hibbett DS, Lane DJ, Simon L, Stickel S, Szaro TM, Weisburg WG, Sogin ML (1992) Evolutionary relationships within the Fungi: analyses of nuclear small subunit rRNA sequences. *Mol Phylogenet Evol* 1:231–241
- Bulman S, Braselton JP (2014) Rhizaria: phytomyxea. In: McLaughlin DJ, Spatafora JW (eds) *Systematics and evolution: Part A*. Springer, Heidelberg
- Burt A, Carter DA, Koenig GL, White TJ, Taylor JW (1996) Molecular markers reveal cryptic sex in the human pathogen *Coccidioides immitis*. *Proc Natl Acad Sci U S A* 93:770–773
- Cafaro MJ (2005) Eccrinales (Trichomycetes) are not fungi, but a clade of protists at the early divergence of animals and fungi. *Mol Phylogenet Evol* 35:21–34. doi:10.1016/j.ympev.2004.12.019
- Cai L, Giraud T, Zhang N, Begerow D, Cai GH, Shivas RG (2011) The evolution of species concepts and species recognition criteria in plant pathogenic fungi. *Fungal Divers* 50:121–133. doi:10.1007/s13225-011-0127-8
- Casadevall A (2005) Fungal virulence, vertebrate endothermy, and dinosaur extinction: is there a connection? *Fungal Genet Biol* 42:98–106. doi:10.1016/j.fgb.2004.11.008
- Cavalier-Smith T (2002) The phagotrophic origin of eukaryotes and phylogenetic classification of Protozoa. *Int J Syst Evol Microbiol* 52:297–354
- Cavalier-Smith T, Chao EEY (2003) Phylogeny and classification of phylum Cercozoa (Protozoa). *Protist* 154:341–358
- Centers for Disease Control and Prevention (2012) <http://www.cdc.gov/parasites/naegleria/>. Cited 13 Apr 2012
- DeBary A (1863) Recherches sur le développement de quelques champignons parasites. *Annales des Sciences Naturelles Sér 4 Botanique* 20:5–148, Pl. 1–13
- Dettman JR, Jacobson DJ, Taylor JW (2003a) A multi-locus genealogical approach to phylogenetic species recognition in the model eukaryote *Neurospora*. *Evolution* 57:2703–2720
- Dettman JR, Jacobson DJ, Turner E, Pringle A, Taylor JW (2003b) Reproductive isolation and phylogenetic divergence in *Neurospora*: comparing methods of species recognition in a model eukaryote. *Evolution* 57:2721–2741
- Dettman JR, Sirjusingh C, Kohn LM, Anderson JB (2007) Incipient speciation by divergent adaptation and antagonistic epistasis in yeast. *Nature* 447:585–588. doi:10.1038/nature05856
- Didier ES, Becnel JJ, Kent ML, Sanders JL, Weiss LM (2014) Microsporidia. In: McLaughlin DJ, Spatafora JW (eds) *Systematics and evolution: Part A*. Springer, Heidelberg
- Druzhinina IS, Kopchinskiy AG, Komon M, Bissett J, Szakacs G, Kubicek CP (2005) An oligonucleotide barcode for species identification in *Trichoderma*

- and *Hypocrea*. *Fungal Genet Biol* 42:813–828. doi:[10.1016/j.fgb.2005.06.007](https://doi.org/10.1016/j.fgb.2005.06.007)
- Dunlap JC, Borkovich KA, Henn MR, Turner GE, Sachs MS, Glass NL, McCluskey K, Plamann M, Galagan JE, Birren BW, Weiss RL, Townsend JP, Loros JJ, Nelson MA, Lambreghts R, Colot HV, Park G, Collopy P, Ringelberg C, Crew C, Litvinkova L, DeCaprio D, Hood HM, Curilla S, Shi M, Crawford M, Koerhsen M, Montgomery P, Larson L, Pearson M, Kasuga T, Tian CG, Bastuerkmen M, Altamirano L, Xu JH (2007) Enabling a community to dissect an organism: overview of the Neurospora functional genomics project. *Adv Genet* 57:49–96. doi:[10.1016/s0065-2660\(06\)57002-6](https://doi.org/10.1016/s0065-2660(06)57002-6)
- Durkin CA, Mock T, Armbrust EV (2009) Chitin in diatoms and its association with the cell wall. *Eukaryot Cell* 8:1038–1050. doi:[10.1128/ec.00079-09](https://doi.org/10.1128/ec.00079-09)
- Eastwood DC, Floudas D, Binder M, Majcherczyk A, Schneider P, Aerts A, Asiegbu FO, Baker SE, Barry K, Bendiksby M, Blumentritt M, Coutinho PM, Cullen D, de Vries RP, Gathman A, Goodell B, Henrissat B, Ihrmark K, Kausered H, Kohler A, LaButti K, Lapidus A, Lavin JL, Lee YH, Lindquist E, Lilly W, Lucas S, Morin E, Murat C, Oguiza JA, Park J, Pisabarro AG, Riley R, Rosling A, Salamov A, Schmidt O, Schmutz J, Skrede I, Stenlid J, Wiebenga A, Xie XF, Kues U, Hibbett DS, Hoffmeister D, Hogberg N, Martin F, Grigoriev IV, Watkinson SC (2011) The plant cell wall-decomposing machinery underlies the functional diversity of forest fungi. *Science* 333:762–765. doi:[10.1126/science.1205411](https://doi.org/10.1126/science.1205411)
- Ellison CE, Hall C, Kowbel D, Welch J, Brem RB, Glass NL, Taylor JW (2011) Population genomics and local adaptation in wild isolates of a model microbial eukaryote. *Proc Natl Acad Sci U S A* 108:2831–2836
- Emoto Y (1977) *The myxomycetes of Japan*. Sangyo Tosho, Tokyo
- Fisher MC, Koenig GL, White TJ, San-Blas G, Negróni R, Alvarez IG, Wanke B, Taylor JW (2001) Biogeographic range expansion into South America by *Coccidioides immitis* mirrors New World patterns of human migration. *Proc Natl Acad Sci U S A* 98:4558–4562
- Fitzpatrick DA, Logue ME, Stajich JE, Butler G (2006) A fungal phylogeny based on 42 complete genomes derived from supertree and combined gene analysis. *BMC Evol Biol* 6:99. doi:[10.1186/1471-2148-6-99](https://doi.org/10.1186/1471-2148-6-99)
- Francois I, Aerts AM, Cammue BPA, Thevissen K (2005) Currently used antimycotics: spectrum, mode of action and resistance occurrence. *Curr Drug Targets* 6:895–907. doi:[10.2174/138945005774912744](https://doi.org/10.2174/138945005774912744)
- Fungal Genomes (2012) <http://fungalgenomes.org/>. Cited 28 Mar 2012
- Geissler S, Siegers K, Schiebel E (1998) A novel protein complex promoting formation of functional α - and γ -tubulin. *EMBO J* 17:952–966
- Gill EE, Lee RCH, Corradi N, Grisdale CJ, Limpright VO, Keeling PJ, Fast NM (2010) Splicing and transcription differ between spore and intracellular life stages in the parasitic Microsporidia. *Mol Biol Evol* 27:1579–1584. doi:[10.1093/molbev/msq050](https://doi.org/10.1093/molbev/msq050)
- Giraud T, Refregier G, Le Gac M, de Vienne DM, Hood ME (2008) Speciation in fungi. *Fungal Genet Biol* 45:791–802. doi:[10.1016/j.fgb.2008.02.001](https://doi.org/10.1016/j.fgb.2008.02.001)
- Gleason FH, Lilje O (2009) Structure and function of fungal zoospores: ecological implications. *Fungal Ecol* 2:53–59. doi:[10.1016/j.funeco.2008.12.002](https://doi.org/10.1016/j.funeco.2008.12.002)
- Goffeau A, Barrell BG, Bussey H, Davis RW, Dujon B, Feldmann H, Galibert F, Hoheisel JD, Jacq C, Johnston M, Louis EJ, Mewes HW, Murakami Y, Philippsen P, Tettelin H, Oliver SG (1996) Life with 6000 genes. *Science* 274:546–567. doi:[10.1126/science.274.5287.546](https://doi.org/10.1126/science.274.5287.546)
- GOLD (2012) http://www.genomesonline.org/cgi-bin/GOLD/phylogenetic_distribution.cgi. Cited 28 Mar 2012
- Greengenes (2012) <http://greengenes.lbl.gov/>. Cited March 28 2012
- Gull K, Trinci APJ (1974) Nuclear division in *Basidiobolus ranarum*. *Trans Br Mycol Soc* 3:457–460
- Gunderson JH, Elwood H, Ingold A, Kindler K, Sogin ML (1987) Phylogenetic relationships between chlorophytes, chrysophytes, and oomycetes. *Proc Natl Acad Sci U S A* 84:5823–5827
- Harris SD (2011) Hyphal morphogenesis: an evolutionary perspective. *Fungal Biol* 115:475–484. doi:[10.1016/j.funbio.2011.02.002](https://doi.org/10.1016/j.funbio.2011.02.002)
- Hawksworth DL (2001) The magnitude of fungal diversity: the 1.5 million species estimate revisited. *Mycol Res* 105:1422–1432
- Heckman DS, Geiser DM, Eidell BR, Stauffer RL, Kardos NL, Hedges SB (2001) Molecular evidence for the early colonization of land by fungi and plants. *Science* 293:1129–1133
- Heitman J, Kronstad JW, Taylor JW, Casselton LA (eds) (2007) *Sex in fungi: molecular determination and evolutionary implications*. ASM Press, Washington, DC
- Hibbett DS, Matheny PB (2009) The relative ages of ectomycorrhizal mushrooms and their plant hosts estimated using Bayesian relaxed molecular clock analyses. *BMC Biol* 7:13. doi:[10.1186/1741-7007-7-13](https://doi.org/10.1186/1741-7007-7-13)
- Hibbett DS, Gilbert LB, Donoghue MJ (2000) Evolutionary instability of ectomycorrhizal symbioses in basidiomycetes. *Nature* 407:506–508
- Hibbett DS, Ohman A, Glotzer D, Nuhn M, Kirk P, Nilsson RH (2011) Progress in molecular and morphological taxon discovery in Fungi and options for formal classification of environmental sequences. *Fungal Biol Rev* 25:38–47
- Horton TR, Bruns TD (2001) The molecular revolution in ectomycorrhizal ecology: peeking into the black-box. *Mol Ecol* 10:1855–1871
- Hunger K, Beckering CL, Wiegshoff F, Graumann PL, Marahiel MA (2006) Cold-induced putative DEAD box RNA helicases CshA and CshB are essential for

- cold adaptation and interact with cold shock protein B in *Bacillus subtilis*. *J Bacteriol* 188:240–248. doi:[10.1128/jb.188.1.240-248.2006](https://doi.org/10.1128/jb.188.1.240-248.2006)
- Inderbitzin P, Harkness J, Turgeon BG, Berbee ML (2005) Lateral transfer of mating system in *Stemphylium*. *Proc Natl Acad Sci U S A* 102:11390–11395
- Inderbitzin P, Davis RM, Bostock RM, Subbarao KV (2011) The ascomycete *Verticillium longisporum* is a hybrid and a plant pathogen with an expanded host range. *PLoS One* 6:e18260. doi:[10.1371/journal.pone.0018260](https://doi.org/10.1371/journal.pone.0018260)
- Irvin SD, Bhattacharjee JK (1998) A unique fungal lysine biosynthesis enzyme shares a common ancestor with tricarboxylic acid cycle and leucine biosynthetic enzymes found in diverse organisms. *J Mol Evol* 46:401–408
- James TY, Berbee ML (2012) No jacket required – new fungal lineage defies dress code. *Bioessays* 34:94–102. doi:[10.1002/bies.201100110](https://doi.org/10.1002/bies.201100110)
- James TY, Kauff F, Schoch CL, Matheny PB, Hofstetter V, Cox CJ, Celio G, Gueidan C, Fraker E, Miadlikowska J, Lumbsch HT, Rauhut A, Reeb V, Arnold AE, Amtoft A, Stajich JE, Hosaka K, Sung G, Johnson D, O'Rourke B, Crockett M, Binder M, Curtis JM, Slot JC, Wang Z, Wilson AW, Schuessler A, Longcore JE, O'Donnell K, Mozley-Standridge S, Porter D, Letcher PM, Powell MJ, Taylor JW, White MM, Griffith GW, Davies DR, Humber RA, Morton JB, Sugiyama J, Rossman AY, Rogers JD, Pfister DH, Hewitt D, Hansen K, Hambleton S, Shoemaker RA, Kohlmeyer J, Volkman-Kohlmeyer B, Spotts RA, Serdani M, Crous PW, Hughes KW, Matsuura K, Langer E, Langer G, Untereiner WA, Lucking R, Buedel B, Geiser DM, Aptroot A, Diederich P, Schmitt I, Schultz M, Yahr R, Hibbett DS, Lutzoni F, McLaughlin DJ, Spatafora JW, Vilgalys R (2006) Reconstructing the early evolution of Fungi using a six-gene phylogeny. *Nature* 443:818–822. doi:[10.1038/nature05110](https://doi.org/10.1038/nature05110)
- James TY, Porter TM, Martin WW (2014) Blastocladiomycota. In: McLaughlin DJ, Spatafora JW (eds) *Systematics and evolution: Part A*. Springer, Heidelberg
- Jones MDM, Forn I, Gadelha C, Egan MJ, Bass D, Massana R, Richards TA (2011) Discovery of novel intermediate forms redefines the fungal tree of life. *Nature* 474:200–203
- Juhas M, van der Meer JR, Gaillard M, Harding RM, Hood DW, Crook DW (2009) Genomic islands: tools of bacterial horizontal gene transfer and evolution. *FEMS Microbiol Rev* 33:376–393. doi:[10.1111/j.1574-6976.2008.00136.x](https://doi.org/10.1111/j.1574-6976.2008.00136.x)
- Jumpponen A, Jones KL (2009) Massively parallel 454 sequencing indicates hyperdiverse fungal communities in temperate *Quercus macrocarpa* phyllosphere. *New Phytol* 184:438–448. doi:[10.1111/j.1469-8137.2009.02990.x](https://doi.org/10.1111/j.1469-8137.2009.02990.x)
- Kaneshiro ES (2002) Sterol biosynthesis in *Pneumocystis*: unique steps that define unique targets. *Drug Resist Updat* 5:259–298
- Keeling PJ, Fast NM, Law JS, Williams BAP, Slamovits CH (2005) Comparative genomics of microsporidia. *Folia Parasitol* 52:8–14
- Koestler T, Ebersberger I (2011) Zygomycetes, Microsporidia, and the evolutionary ancestry of sex determination. *Genome Biol Evol* 3:186–194
- Koljalg U, Larsson KH, Abarenkov K, Nilsson RH, Alexander IJ, Eberhardt U, Erland S, Hoiland K, Kjöller R, Larsson E, Pennanen T, Sen R, Taylor AFS, Tedersoo L, Vrålstad T, Ursing BM (2005) UNITE: a database providing web-based methods for the molecular identification of ectomycorrhizal fungi. *New Phytol* 166:1063–1068. doi:[10.1111/j.1469-8137.2005.01376.x](https://doi.org/10.1111/j.1469-8137.2005.01376.x)
- Koufopanou V, Burt A, Taylor JW (1997) Concordance of gene genealogies reveals reproductive isolation in the pathogenic fungus *Coccidioides immitis*. *Proc Natl Acad Sci U S A* 94:5478–5482
- Lacroix B, Tzfira T, Vainstein A, Citovsky V (2006) A case of promiscuity: *Agrobacterium*'s endless hunt for new partners. *Trends Genet* 22:29–37. doi:[10.1016/j.tig.2005.10.004](https://doi.org/10.1016/j.tig.2005.10.004)
- Lang BF, O'Kelly C, Nerad T, Gray MW, Burger G (2002) The closest unicellular relatives of animals. *Curr Biol* 12:1773–1778
- Lara E, Moreira D, Lopez-Garcia P (2010) The environmental clade LKM11 and Rozella form the deepest branching clade of Fungi. *Protist* 161:116–121. doi:[10.1016/j.protis.2009.06.005](https://doi.org/10.1016/j.protis.2009.06.005)
- Liti G, Carter DM, Moses AM, Warringer J, Parts L, James SA, Davey RP, Roberts IN, Burt A, Koufopanou V, Tsai IJ, Bergman CM, Bensasson D, O'Kelly MJT, van Oudenaarden A, Barton DBH, Bailes E, Ba ANN, Jones M, Quail MA, Goodhead I, Sims S, Smith F, Blomberg A, Durbin R, Louis EJ (2009) Population genomics of domestic and wild yeasts. *Nature* 458:337–341. doi:[10.1038/nature07743](https://doi.org/10.1038/nature07743)
- Liu Y, Steenkamp ET, Brinkmann H, Forget L, Philippe H, Lang BF (2009) Phylogenomic analyses predict sistergroup relationship of nucleariids and Fungi and paraphyly of zygomycetes with significant support. *BMC Evol Biol* 9:272. doi:[10.1186/1471-2148-9-272](https://doi.org/10.1186/1471-2148-9-272)
- Lutzoni F, Pagel M (1997) Accelerated evolution as a consequence of transitions to mutualism. *Proc Natl Acad Sci U S A* 94:11422–11427
- Marshall WL, Berbee ML (2011) Facing unknowns: living cultures (*Pirum gemmata* gen. nov., sp. nov., and *Abeoforma whisleri*, gen. nov., sp. nov.) from invertebrate digestive tracts represent an undescribed clade within the unicellular opisthokont lineage Ichthyosporia (Mesomycetozoa). *Protist* 162:33–57. doi:[10.1016/j.protis.2010.06.002](https://doi.org/10.1016/j.protis.2010.06.002)
- Mehrabi R, Bahkali AH, Abd-Elsalam KA, Moslem M, Ben M'Barek S, Gohari AM, Jashni MK, Stergiou

- poulos I, Kema GHJ, de Wit P (2011) Horizontal gene and chromosome transfer in plant pathogenic fungi affecting host range. *FEMS Microbiol Rev* 35:542–554. doi:10.1111/j.1574-6976.2010.00263.x
- Mendoza L, Taylor JW, Ajello L (2002) The class Mesozozoa: a group of microorganisms at the animal-fungal boundary. *Annu Rev Microbiol* 56:160950. doi:10.1146/annurev.micro.56.012302.160950
- Menkis A, Jacobson DJ, Gustafsson T, Johannesson H (2008) The mating-type chromosome in the filamentous ascomycete *Neurospora tetrasperma* represents a model for early evolution of sex chromosomes. *PLoS Genet* 4:1000030. doi:10.1371/journal.pgen.1000030
- Munro CA, Gow NAR (2001) Chitin synthesis in human pathogenic fungi. *Med Mycol* 39:41–53. doi:10.1080/744118878
- Nachman MW, Hoekstra HE, D'Agostino SL (2003) The genetic basis of adaptive melanism in pocket mice. *Proc Natl Acad Sci U S A* 100:5268–5273. doi:10.1073/pnas.0421157100
- Nakagaki T, Yamada H, Toth A (2000) Maze-solving by an amoeboid organism. *Nature* 407:470
- Neafsey DE, Barker BM, Sharpton TJ, Stajich JE, Park DJ, Whiston E, Hung CY, McMahan C, White J, Sykes S, Heiman D, Young S, Zeng QD, Abouelleil A, Aftuck L, Bessette D, Brown A, FitzGerald M, Lui A, Macdonald JP, Priest M, Orbach MJ, Galgiani JN, Kirkland TN, Cole GT, Birren BW, Henn MR, Taylor JW, Rounsley SD (2010) Population genomic sequencing of *Coccidioides* fungi reveals recent hybridization and transposon control. *Genome Res* 20:938–946. doi:10.1101/gr.103911.109
- Nilsson RH, Ryberg M, Kristiansson E, Abarenkov K, Larsson K-H, Kõljalg U (2006) Taxonomic reliability of DNA sequences in public sequence databases: a fungal perspective. *PLoS One* 1(1):e59
- Nishida H, Nishiyama M (2000) What is characteristic of fungal lysine synthesis through the alpha-aminoadipate pathway? *J Mol Evol* 51:299–302
- Nishida H, Nishiyama M, Kobashi N, Kosuge T, Hoshino T, Yamane H (1999) A prokaryotic gene cluster involved in synthesis of lysine through the amino adipate pathway: a key to the evolution of amino acid biosynthesis. *Genome Res* 9:1175–1183
- Park B, Park J, Cheong KC, Choi J, Jung K, Kim D, Lee YH, Ward TJ, O'Donnell K, Geiser DM, Kang S (2011) Cyber infrastructure for *Fusarium*: three integrated platforms supporting strain identification, phylogenetics, comparative genomics and knowledge sharing. *Nucleic Acids Res* 39:D640–D646. doi:10.1093/nar/gkq1166
- Parks LW, Smith SJ, Crowley SH (1995) Biochemical and physiological effects of sterol alterations in yeast—a review. *Lipids* 30:227–230
- Powell MJ, Letcher PM (2014) Chytridiomycota, monoblepharidomycota and neocallimastigomycota. In: McLaughlin DJ, Spatafora JW (eds) *Systematics and evolution: Part A*. Springer, Heidelberg
- Ribosomal Database Project (2012) <http://rdp.cme.msu.edu/>. Cited 28 Mar 2012
- Ridley AJ, Schwartz MA, Burrridge K, Firtel RA, Ginsberg MH, Borisy G, Parsons JT, Horwitz AR (2003) Cell migration: integrating signals from front to back. *Science* 302:1704–1709
- Rokas A, Kruger D, Carroll SB (2005) Animal evolution and the molecular signature of radiations compressed in time. *Science* 310:1933–1938
- Ruiz-Herrera J, Ortiz-Castellanos L (2010) Analysis of the phylogenetic relationships and evolution of the cell walls from yeasts and fungi. *FEMS Yeast Res* 10:225–243. doi:10.1111/j.1567-1364.2009.00589.x
- Ryberg M, Kristiansson E, Sjökvist E, Nilsson RH (2009) An outlook on the fungal internal transcribed spacer sequences in GenBank and the introduction of a web-based tool for the exploration of fungal diversity. *New Phytol* 181:471–477. doi:10.1111/j.1469-8137.2008.02667.x
- Saiki RK, Gelfand DH, Stoffel S, Scharf SJ, Higuchi R, Horn GT, Mullis KB, Erlich H (1988) Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. *Science* 239:487–491
- Schadt CW, Martin AP, Lipson DA, Schmidt SK (2003) Seasonal dynamics of previously unknown fungal lineages in tundra soils. *Science* 301:1359–1361
- Schaller H (2004) New aspects of sterol biosynthesis in growth and development of higher plants. *Plant Physiol Biochem* 42:465–476
- Schloss PD (2009) A high-throughput DNA sequence aligner for microbial ecology studies. *PLoS One* 4(12):e8230
- Schoch C, Seifert K (2010) The home stretch for fungal barcoding. *IMA Fungus* 1:2–3
- Schoch CL, Sung GH, Lopez-Giraldez F, Townsend JP, Miadlikowska J, Hofstetter V, Robertse B, Matheny PB, Kauff F, Wang Z, Gueidan C, Andrie RM, Trippe K, Ciuffetti LM, Wynns A, Fraker E, Hodkinson BP, Bonito G, Groenewald JZ, Arzanlou M, de Hoog GS, Crous PW, Hewitt D, Pfister DH, Peterson K, Gryzenhout M, Wingfield MJ, Aptroot A, Suh SO, Blackwell M, Hillis DM, Griffith GW, Castlebury LA, Rossmann AY, Lumbsch HT, Lücking R, Budel B, Rauhut A, Diederich P, Ertz D, Geiser DM, Hosaka K, Inderbitzin P, Köhlmeier J, Volkmann-Köhlmeier B, Mostert L, O'Donnell K, Sipman H, Rogers JD, Shoemaker RA, Sugiyama J, Summerbell RC, Untereiner W, Johnston PR, Stenroos S, Zuccaro A, Dyer PS, Crittenden PD, Cole MS, Hansen K, Trappe JM, Yahr R, Lutzoni F, Spatafora JW (2009) The ascomycota tree of life: a phylum-wide phylogeny clarifies the origin and evolution of fundamental reproductive and ecological traits. *Syst Biol* 58:224–239. doi:10.1093/sysbio/syp020

- Sekimoto S, Rochon D, Long JE, Dee JM, Berbee ML (2011) A multigene phylogeny of *Olpidium* and its implications for early fungal evolution. *BMC Evol Biol* 11:331. doi:[10.1186/1471-2148-11-331](https://doi.org/10.1186/1471-2148-11-331)
- Silva (2012) <http://www.arb-silva.de/>. Cited 28 Mar 2012
- Simon L, Bousquet J, Levesque RC, Lalonde M (1993) Origin and diversification of endomycorrhizal fungi and coincidence with vascular land plants. *Nature* 363:67–69
- Steenkamp ET, Wright J, Baldauf SL (2006) The protistan origins of animals and fungi. *Mol Biol Evol* 23:93–106
- Stephenson SL (2014) Excavata: acrasiomycota; amoebzoa: dictyosteliomycota, myxomycota. In: McLaughlin DJ, Spatafora JW (eds) *Systematics and evolution: Part A*. Springer, Heidelberg
- Stiller JW, Huang JL, Ding Q, Tian J, Goodwillie C (2009) Are algal genes in nonphotosynthetic protists evidence of historical plastid endosymbioses? *BMC Genomics* 10:484. doi:[10.1186/1471-2164-10-484](https://doi.org/10.1186/1471-2164-10-484)
- Strassmann JE, Zhu Y, Queller DC (2000) Altruism and social cheating in the social amoeba *Dictyostelium discoideum*. *Nature* 408:965–967
- Suh SO, McHugh JV, Blackwell M (2004) Expansion of the *Candida tanzawaensis* yeast clade: 16 novel *Candida* species from basidiocarp-feeding beetles. *Int J Syst Evol Microbiol* 54:2409–2429. doi:[10.1099/ijs.0.63246-0](https://doi.org/10.1099/ijs.0.63246-0)
- Sumathi JC, Raghukumar S, Kasbekar DP, Raghukumar C (2006) Molecular evidence of fungal signatures in the marine protist *Corallochytrium limacisporum* and its implications in the evolution of animals and fungi. *Protist* 157:363–376. doi:[10.1016/j.protis.2006.05.003](https://doi.org/10.1016/j.protis.2006.05.003)
- Swann EC, Taylor JW (1993) Higher taxa of basidiomycetes: an 18S rRNA gene perspective. *Mycologia* 85:923–936
- Takamatsu A, Fujii T, Endo I (2000) Time delay effect in a living coupled oscillator system with the plasmodium of *Physarum polycephalum*. *Phys Rev Lett* 85:2026–2029
- Taylor JW, Jacobson DJ, Fisher MC (1999) The evolution of asexual fungi: reproduction, speciation and classification. *Annu Rev Phytopathol* 37:197–246
- Taylor JW, Jacobson DJ, Kroken S, Kasuga T, Geiser DM, Hibbett DS, Fisher MC (2000) Phylogenetic species recognition and species concepts in fungi. *Fungal Genet Biol* 31:21–32. doi:[10.1006/fgbi.2000.1228](https://doi.org/10.1006/fgbi.2000.1228)
- Taylor TN, Klavins SD, Krings M, Taylor EL, Kerp H, Hass H (2004) Fungi from the Rhynie chert: a view from the dark side. *Trans R Soc Edinb Earth Sci* 94:457–473
- Taylor JW, Turner E, Townsend JP, Dettman JR, Jacobson D (2006) Eukaryotic microbes, species recognition and the geographic limits of species: examples from the kingdom Fungi. *Philos Trans R Soc Lond B Biol Sci* 361:1947–1963. doi:[10.1098/rstb.2006.1923](https://doi.org/10.1098/rstb.2006.1923)
- Torruella G, Suga H, Riutort M, Pereto J, Ruiz-Trillo I (2009) The evolutionary history of lysine biosynthesis pathways within eukaryotes. *J Mol Evol* 69:240–248. doi:[10.1007/s00239-009-9266-x](https://doi.org/10.1007/s00239-009-9266-x)
- Townsend JP (2007) Profiling phylogenetic informativeness. *Syst Biol* 56:222–231. doi:[10.1080/10635150701311362](https://doi.org/10.1080/10635150701311362)
- Tree of Life web project (2012) <http://tolweb.org/tree/>. Cited 13 Apr 2012
- TrichOKEY 2 (2011) <http://www.isth.info/tools/molkey/index.php>. Cited Jun 2011
- Tsui CKM, Marshall W, Yokoyama R, Honda D, Lippmeier JC, Craven KD, Peterson PD, Berbee ML (2009) Labyrinthulomycetes phylogeny and its implications for the evolutionary loss of chloroplasts and gain of ectoplasmic gliding. *Mol Phylogenet Evol* 50:129–140
- Turner TL, Bourne EC, Von Wettberg EJ, Hu TT, Nuzhdin SV (2010) Population resequencing reveals local adaptation of *Arabidopsis lyrata* to serpentine soils. *Nat Genet* 42:260–263. doi:[10.1038/ng.515](https://doi.org/10.1038/ng.515)
- Vogel HJ (1961) Lysine synthesis and phylogeny of lower fungi – some chytrids versus Hyphochytrium. *Nature* 189:1026–1027
- Vogel HJ (1965) Lysine biosynthesis and evolution. In: Bryson V, Vogel HJ (eds) *Evolving genes and proteins*. Academic, New York, NY, pp 25–40
- Vogler DR, Bruns TD (1998) Phylogenetic relationships among the pine stem rust fungi (*Cronartium* and *Peridermium* spp.). *Mycologia* 90:244–257
- Von Wettstein F (1921) Das Vorkommen von Chitin und seine Verwertung als systematisch - phylogenetisches Merkmal im Pflanzenreich. *Sitzungsberichte Akademie der Wissenschaften in Wien Mathematisch-naturwissenschaftliche Klasse* 130:3–20
- Vos M, Velicer GJ (2008) Isolation by distance in the spore-forming soil bacterium *Myxococcus xanthus*. *Curr Biol* 18:386–391. doi:[10.1016/j.cub.2008.02.050](https://doi.org/10.1016/j.cub.2008.02.050)
- Wainright PO, Hinkle G, Sogin ML, Stickel SK (1993) Monophyletic origins of the Metazoa: an evolutionary link with fungi. *Science* 260:340–342
- Weete JD, Abril M, Blackwell M (2010) Phylogenetic distribution of fungal sterols. *PLoS One* 5(5): e10899. doi:[10.1371/journal.pone.0010899](https://doi.org/10.1371/journal.pone.0010899)
- Whisler HC (1962) Culture and nutrition of *Amoebidium parasiticum*. *Am J Bot* 49:193–199
- Whitaker RJ, Grogan DW, Taylor JW (2003) Geographic barriers isolate endemic populations of hyperthermophilic archaea. *Science* 301:976–978. doi:[10.1126/science.1086909](https://doi.org/10.1126/science.1086909)
- White TJ, Bruns T, Lee S, Taylor J (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ (eds) *PCR protocols. A guide*

- to methods and applications. Academic, San Diego, CA, pp 315–322
- Yeast Biochemical Pathway Database (2012) <http://pathway.yeastgenome.org/>. Cited 10 Apr 2012
- Yoshida M, Nakayama T, Inouye I (2009) *Nuclearia thermophila* sp nov (Nucleariidae), a new nucleariid species isolated from Yunoko Lake in Nikko (Japan). *Eur J Protistol* 45:147–155. doi:[10.1016/j.ejop.2008.09.004](https://doi.org/10.1016/j.ejop.2008.09.004)
- Zabriskie TM, Jackson MD (2000) Lysine biosynthesis and metabolism in fungi. *Nat Prod Rep* 17:85–97
- Zettler LAA, Nerad TA, O’Kelly CJ, Sogin ML (2001) The nucleariid amoebae: more protists at the animal-fungal boundary. *J Eukaryot Microbiol* 48:293–297