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The Fungal Tree of Life: from Molecular Systematics to Genome-Scale Phylogenies

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ABSTRACT The kingdom Fungi is one of the more diverse clades of eukaryotes in terrestrial ecosystems, where they provide numerous ecological services ranging from decomposition of organic matter and nutrient cycling to beneficial and antagonistic associations with plants and animals. The evolutionary relationships of the kingdom have represented some of the more recalcitrant problems in systematics and phylogenetics. The advent of molecular phylogenetics, and more recently phylogenomics, has greatly advanced our understanding of the patterns and processes associated with fungal evolution, however. In this article, we review the major phyla, subphyla, and classes of the kingdom Fungi and provide brief summaries of ecologies, morphologies, and exemplar taxa. We also provide examples of how molecular phylogenetics and evolutionary genomics have advanced our understanding of fungal evolution within each of the phyla and some of the major classes. In the current classification we recognize 8 phyla, 12 subphyla, and 46 classes within the kingdom. The ancestor of fungi is inferred to be zoosporic, and zoosporic fungi comprise three lineages that are paraphyletic to the remainder of fungi. Fungi historically classified as zygomycetes do not form a monophyletic group and are paraphyletic to Ascomycota and Basidiomycota. Ascomycota and Basidiomycota are each monophyletic and collectively form the subkingdom Dikarya.

INTRODUCTION

In 1996 the genome of *Saccharomyces cerevisiae* was published and marked the beginning of a new era in fungal biology (1). Since then, rapid advancements in both sequencing technologies and computational biology have resulted in the sequencing of genomes for more than 800 species (e.g., http://genome.jgi.doe.gov/fungi/). These genomes represent a windfall of data that are informing evolutionary studies of fungi and the search for biological solutions to alternative fuels, bioremediation, carbon sequestration, and sustainable agriculture and forestry (2). Indeed, the marriage between genomics

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and phylogenetics occurred early, both in the use of phylogenetic techniques to study genome evolution and in the use of genome-scale data to infer evolutionary relationships. In this article we will review the impact of genomic-scale phylogenies, along with standard molecular phylogenies, on our understanding of the evolution of the fungal tree of life and the classification that communicates it.

Genomic data provide the maximum amount of discrete genetic information available for phylogenetic analyses, and hundreds to thousands of genes have been identified as useful phylogenetic markers (3). Markov clustering algorithms have been proven powerful tools to identify orthologous clusters of proteins that can be filtered for single-copy clusters that are useful in phylogenetic analyses (4). This approach has transformed phylogenetics by no longer requiring selection of an *a priori* set of markers (e.g., rDNA, RPB2, etc.), but rather promotes the mining of a data set of genomes for the largest set of appropriate markers. Furthermore, hidden Markov models have proven to be valuable tools for identifying and retrieving these markers in newly sequenced genomes and rapidly growing genome-scale phylogenetic data sets (5).

The estimation of species trees from genome-scale data sets is not without challenges, however. Phylogenetic analyses of genomic data have revealed that different genes within a genome can have different evolutionary histories, i.e., phylogenetic conflict (6). Sources of conflict include incomplete lineage sorting (or deep coalescence), hybridization, and horizontal gene transfer, and the detection and characterization of this conflict in the context of phylogenetic inference are still in their infancy (7). The application of standard measures of topological support, such as the bootstrap partition, can also be difficult to interpret, due to the observation that nodes that resolve differently in different gene data sets can have high or maximum bootstrap partition values in a subset of analyses (e.g., 8, 9). At the time of the writing of this manuscript the majority of genome-scale phylogenetic analyses focus on the analysis of concatenated superalignments, but development and use of supertree methods, gene tree-species tree reconciliations, and alternative measures of nodal support are increasing (e.g., 8, 10) and will be developed further over the coming years.

Despite the challenges mentioned above, phylogenetic analyses of genome-scale data sets, and more traditional multigene data sets, have greatly advanced our understanding of fungal evolution. Historically, the fungi were divided into more or less four groups—chytridiomycetes, zygomycetes, ascomycetes, and basidiomycetes—defined by morphological traits associated with reproduction. (Note: The suffix "-mycetes" is used to denote a class-level taxonomic group in fungal nomenclature, e.g., Agaricomycetes. Its use as a lowercase noun, however, signifies an informal name and not an explicit taxonomic rank.) The chytridiomycetes, or zoosporic fungi, were recognized based on their production of zoospores, characterized by a single posterior, smooth flagellum. The zygomycetes were characterized by gametangial conjugation and the production of zygospores, coenocytic hyphae, and typically asexual reproduction by sporangia. The ascomycetes and basidiomycetes were identified by the production of asci and basidia, respectively, possession of regularly septate hyphae, and a dikaryotic nuclear phase in their life cycle. The classification of the kingdom Fungi used here recognizes eight phyla (Fig. 1, Table 1), with the zoosporic fungi comprising the first three lineages of the kingdom—Cryptomycota/Microsporidia, Chytridiomycota, and Blastocladiomycota—since the divergence from the last universal common ancestor (LUCA) of Fungi.

The resolution of zoosporic fungi as paraphyletic rejects the flagellum as a diagnostic trait (synapomorphy) for a monophyletic group of flagellated fungi. Rather, it is an ancestral (symplesiomorphic) trait inherited from the LUCA of the kingdom Fungi. Most extant species of fungi are nonflagellated and are the result of multiple losses of the flagellum during fungal evolution. Two losses of the flagellum have occurred, giving rise to the Microsporidia and the most recent common ancestor (MRCA) of the remaining phyla of zygomycetes, ascomycetes, and basidiomycetes. Inferences of additional losses of the flagellum are required for the placement of nonflagellated species among the Chytridiomycota (11) and possibly for the placement of the enigmatic zoosporic genus *Olpidium* among zygomycetes (12), but the absolute number of losses is unclear. The zygomycetes are also paraphyletic and are classified in two phyla: Zoopagomycota and Mucoromycota (13). This classification rejects the zygospore as a synapomorphy for the zygomycetes; rather, it was inherited from the MRCA of terrestrial fungi and lost in the MRCA of ascomycetes and basidiomycetes. The monophyly of ascomycetes and basidiomycetes has been confirmed, and they are classified as the phyla Ascomycota and Basidiomycota, respectively, of the subkingdom Dikarya (14). More information on character evolution will be highlighted throughout this article.

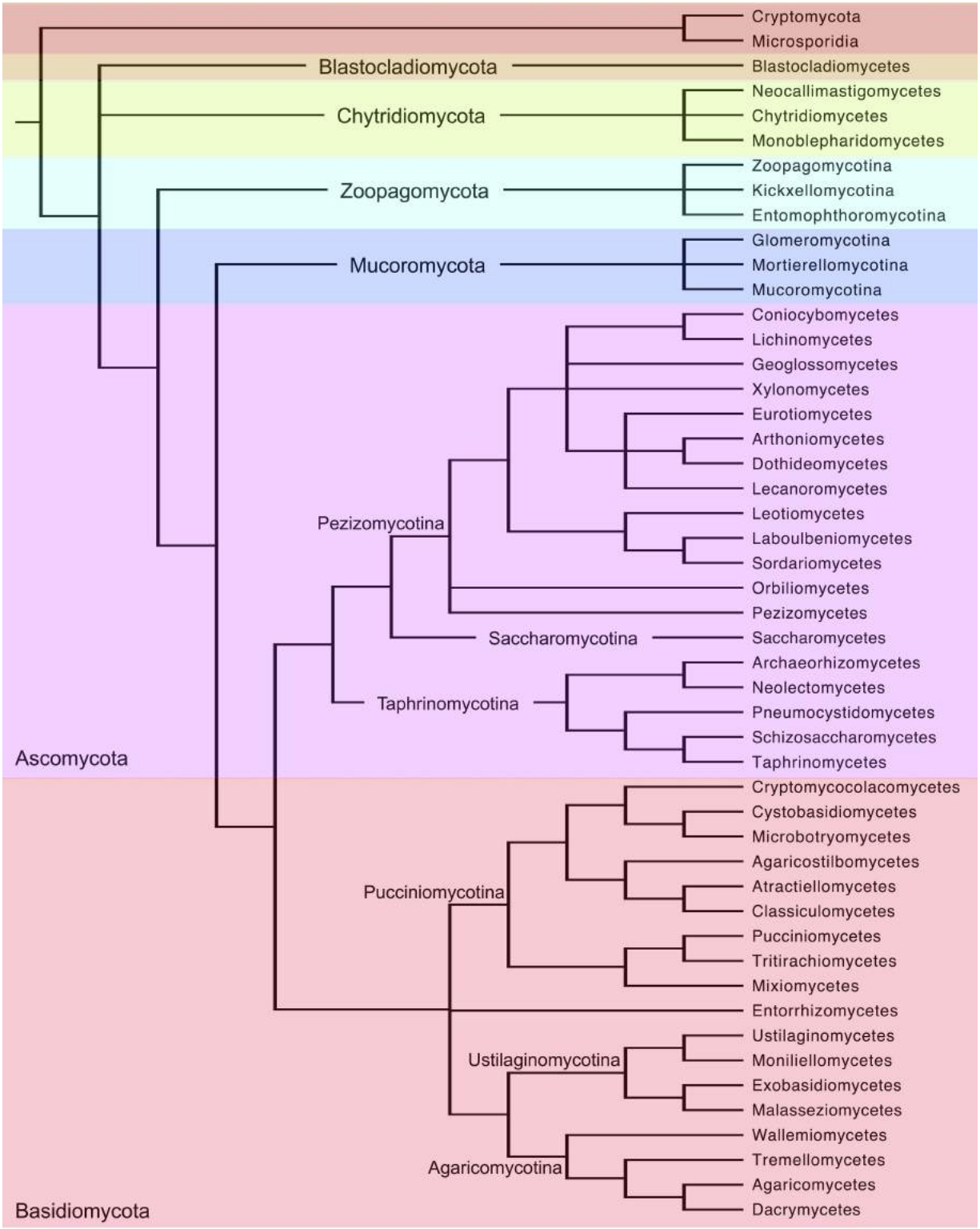

FIGURE 1 Fungal tree of life. Cladogram of the kingdom Fungi based on published multi-gene and genome-scale phylogenies (11–14, 17, 18, 32, 33, 83, 98, 109, 112, 167, 168). Polytomies represent regions of the tree currently unresolved by molecular and genomic data.

TABLE 1 Classification of the kingdom Fungi

Cryptomycota M.D.M. Jones & T.A. Richards 2011 [=Rozellomycota Doweld (2011)]
Microsporidia
Blastocladiomycota T.Y. James (2007)
  Blastocladiomycetes Doweld (2001)
Chytridiomycota Hibbett et al. (2007)
  Chytridiomycetes Caval.-Sm. (1998)
  Monoblepharidomycetes J.H Schaffner (1909)
  Neocallimastigomycetes M.J. Powell (2007)
Zoopagomycota Gryganski et al. (2016)
  Zoopagomycotina Benny (2007)
  Kickxellomycotina Benny (2007)
  Entomophthoromycotina Humber (2007)
    Basidiobolomycetes Doweld (2001)
    Neozygitomycetes Humber (2012)
    Entomophthoromycetes Humber (2012)
Mucoromycota Doweld (2001)
  Glomeromycotina Spatafora & Stajich (2016)
    Glomeromycetes Caval.-Sm. (1998)
  Mortierellomycotina Hoffm., K. Voigt & P.M. Kirk (2011)
    Moretierellomycetes Caval.-Sm. (1998)
  Mucoromycotina Benny (2007)
Ascomycota (Berk.) Caval.-Sm. (1998)
  Pezizomycotina O.E. Erikss. & Winka (1997)
    Arthoniomycetes O.E. Erikss. & Winka (1997)
    Coniocybomycetes M. Prieto & Wedin (2013)
    Dothideomycetes O.E. Erikss. & Winka (1997)
    Eurotiomycetes O.E. Erikss. & Winka (1997)
    Geoglossomycetes Zheng Wang, C.L. Schoch & Spatafora (2009)
    Laboulbeniomycetes Engler (1898)
    Lecanoromycetes O.E. Erikss. & Winka (1997)
    Leotiomycetes O.E. Erikss. & Winka (1997)
    Lichinomycetes Reeb, Lutzoni & Cl. Roux (2004)
    Orbiliomycetes O.E. Erikss. & Baral (2003)
    Pezizomycetes O.E. Erikss. & Winka (1997)
    Sordariomycetes O.E. Erikss. & Winka (1997)
    Xylonomycetes Gazis & P. Chaverri (2012)
  Saccharomycotina O.E. Erikss. & Winka (1997)
    Saccharomycetes G. Winter (1880)
  Taphrinomycotina O.E. Erikss. & Winka (1997)
    Archaeorhizomycetes Rosling & T.Y. James (2011)
    Neolectomycetes O.E. Erikss. & Winka (1997)
    Pneumocystidomycetes O.E. Erikss. & Winka (1997)
    Schizosaccharomycetes O.E. Erikss. & Winka (1997)
    Taphrinomycetes O.E. Erikss. & Winka (1997)
Basidiomycota R.T. Moore (1980)
  Agaricomycotina Doweld (2001)
    Agaricomycetes Doweld (2001)
    Dacrymycetes Doweld (2001)
    Tremellomycetes Doweld (2001)
    Wallemiomycetes Zalar, de Hoog & Schroers (2005)
  Pucciniomycotina R. Bauer, Begerow, J.P. Samp., M. Weiss & Oberw. (2006)
    Agaricostilbomycetes R. Bauer, Begerow, J.P. Samp., M. Weiss & Oberw. (2006)
    Atractiellomycetes R. Bauer, Begerow, J.P. Samp., M. Weiss & Oberw. (2006)
    Classiculomycetes R. Bauer, Begerow, J.P. Samp., M. Weiss & Oberw. (2006)
    Cryptomycocolacomycetes R. Bauer, Begerow, J.P. Samp., M. Weiss & Oberw. (2006)
    Cystobasidiomycetes R. Bauer, Begerow, J.P. Samp., M. Weiss & Oberw. (2006)
    Microbotryomycetes R. Bauer, Begerow, J.P. Samp., M. Weiss & Oberw. (2006)
    Mixiomycetes R. Bauer, Begerow, J.P. Samp., M. Weiss & Oberw. (2006)
    Pucciniomycetes R. Bauer, Begerow, J.P. Samp., M. Weiss & Oberw. (2006)
    Tritirachiomycetes Aime & Schell (2011)
  Ustilaginomycotina Doweld (2001)
    Exobasidiomycetes Begerow, M. Stoll & R. Bauer 2007
    Malasseziomycetes Denchev & T. Denchev 2014
    Moniliellomycetes Q.M. Wang, F.Y. Bai & Boekhout (2014)
    Ustilaginomycetes E. Warming (1884)
  Incertae sedis
    Entorrhizomycetes Begerow, Stoll & R. Bauer (2007)

One of the greatest challenges in evolutionary biology of fungi is accurately estimating geologic dates of the origin of the kingdom Fungi, emergence of the major phyla, and diversification of extant lineages (15). Our knowledge of the fossil record of fungi is less than that of plants and animals, but there does exist an increasing number of fossils that can be assigned to major groups of fungi based on their morphological similarity to extant taxa (16). An important observation is that morphologies associated with Blastocladiomycota, Chytridiomycota, Mucoromycota, and Ascomycota are present in the early Devonian and are associated with the earliest known land plant flora of the Rhynie chert. This observation, in combination with relaxed molecular clock analyses (e.g., 17, 18), suggests that the common ancestors of the phyla of Fungi are in fact old and may have been among the first terrestrial organisms. The interpretation of fungus-like fossils can be challenging, however, as it is difficult to interpret some morphologies that are not present among extant lineages as definitive representatives of the kingdom Fungi (19).

In this article we will highlight the major phyla of fungi based on the current understanding of the fungal tree of life. In doing so, we will outline their phylogenetic diversity and classification, provide examples of important taxa for each group, and discuss advancements in our understanding of morphological and ecological evolution through the analysis of genomic and molecular data. There are many specialized terms used in this article, and we are unable to fully define all of them here. However, Fig. 2 to 5 provide examples of taxa and morphologies discussed herein, but the reader is directed to more traditional textbooks in mycology for more detailed discussions.

ZOOSPORIC FUNGI (FIG. 2)

Before we consider zoosporic fungi, a brief discussion of some unique aspects of their development and morphology is necessary. The morphology of zoosporic

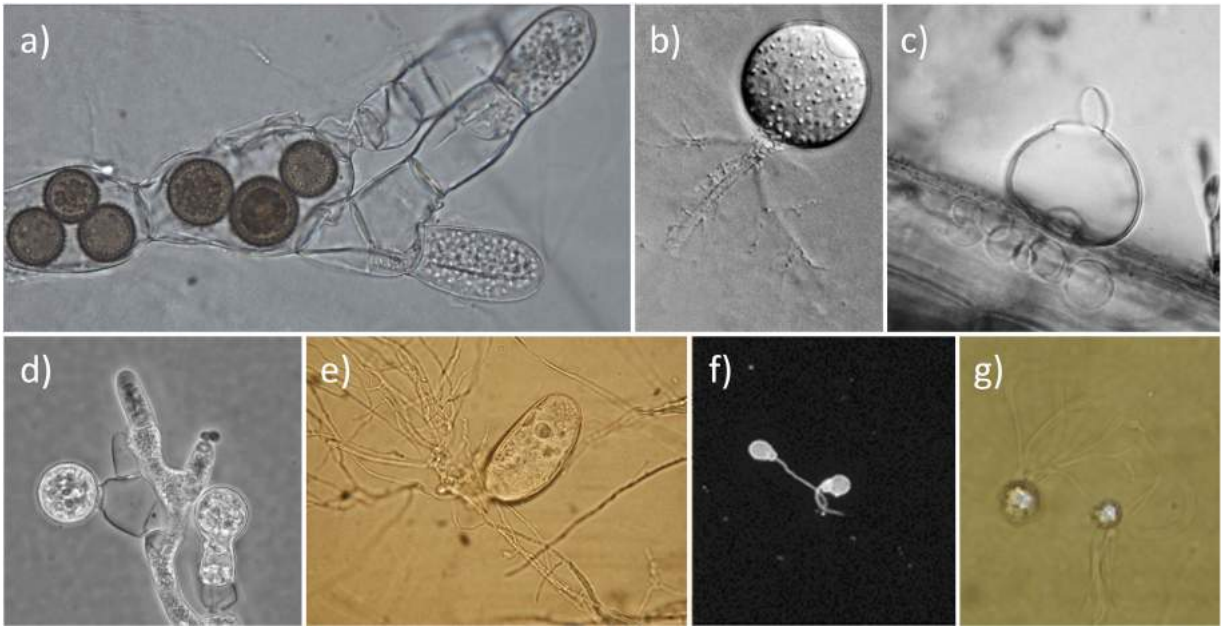

FIGURE 2 Examples of zoosporic fungal diversity. **(a)** *Rozella allomycis* (Cryptomycota) parasitizing hyphae of *Allomyces* (Blastocladiomycota). Chytridiomycota: **(b)** *C. hyalinus* (Chytridiomycetes) monocentric, operculate zoosporangium with rhizoids; **(c)** *Catenochytridium* sp. (Chytridiomycetes) monocentric, operculate zoosporangium with rhizoids; **(d)** *Monoblepharis polymorpha* (Monoblepharidomycetes) mature zygote or oospore, empty and mature antheridia and antherozoids or male gametes emerging from antheridium (by Marilyn M. N. Mollicone); **(e)** *Neocallimastix* sp. (Neocallimastigomycetes) monocentric thallus with rhizoids (by Gary Easton); **(f)** *Olpidium bornovanus* (*incertae sedis*) zoospores (photo by D'Ann Rochon); **(g)** *Neocallimastix* sp. (Neocallimastigomycetes) multiflagellate zoospores (photo by Gary Easton).

fungi varies depending on the extent of their thallus development, number and position of reproductive structures, and position in the substrate. Thalli may exist as single cells with sparse rhizoidal systems to more extensive networks of rhizoids (rhizomycelia) and mycelial thalli. Endobiotic chytrids are partially or completely immersed in their substrate, while only the rhizoids of epibiotic chytrids are immersed. Often, the thalli of single-celled chytrids are converted entirely to thin-walled zoosporangia or thick-walled resting sporangia (holocarpic condition), and others have thalli that are only partially converted to reproductive structures (eucarpic condition). Other terms describe the number of reproductive structures produced by an individual: a single sporangium on a thallus is a monocentric thallus, and multiple sporangia on a rhizomycelium or mycelium are termed polycentric. Zoosporangia, in which zoospores are produced, are asexual reproductive structures; resting sporangia that germinate by release of flagellated cells and resting spores that germinate by germ tubes may be asexual or sexual structures.

Cryptomycota/Microsporidia

Cryptomycota plus Microsporidia are sister to the remaining lineages of Kingdom Fungi. (Note: Rozellomycota [20] is another name for Cryptomycota [21] based on the genus *Rozella* and the principle of autotypification [14]. *Cryptomyces* is a genus in Ascomycota and cannot be used to typify Cryptomycota.) Cryptomycota consists of a handful of described taxa and taxa that are known only from environmental samples. One described taxon is *Rozella* (Fig. 2a), a biotrophic intracellular parasite of other zoosporic fungi of Chytridiomycota and Blastocladiomycota and oomycetes of the kingdom Stramenopila (22). There are few additional described genera and species of Cryptomycota, but environmental sampling using molecular markers has revealed a phylogenetically diverse assemblage of fungi detected in soils, marine and freshwater sediments, and oxygen-depleted environments (23, 24). Some environmental Cryptomycota produce zoospores with a single, smooth flagellum, but chitin, a cell wall carbohydrate produced by most fungi, was not originally detected in

the life history stages first observed (25). A more recent study detected chitin restricted to the cyst phase that attaches to the *Allomyces* hyphae and in the inner wall of the resting sporangia (26). The ecology of the environmental Cryptomycota is largely conjecture at this time, because they have not been cultured, an observation frequently invoked in fungal systems to infer an obligate biotroph. Recently, molecular data have determined that *Paramicrosporidium* (20), nonflagellated parasites of amoebae, and *Amoeboaphelidium*, an algal parasite, are members of Cryptomycota (27), lending support to parasitism as an ecological signature of the phylum.

Microsporidia are intracellular parasites of all major groups of animals. They are particularly well known from insects, crustaceans, and fish but are also known to occur in mammals, including humans (28). Microsporidia produce unique spores that infect host cells through a harpoon-like organelle that pierces the host cell membrane and provides a conduit for the injection of the parasite's cytoplasm (29). Once inside the host cell, a spore, which includes an inner chitin-containing wall, is ultimately formed. The phylogenetic affinity of Microsporidia has been difficult to determine, with past classifications placing it among the polyphyletic protists, and early multigene phylogenies placing it in different parts of the kingdom Fungi (reviewed in reference 30). Genome-scale phylogenies have also proven problematic due to highly reduced genomes and fast rates of nucleotide substitution (31), but multiple analyses have garnered increasing support for its close relationship to Cryptomycota (32, 33). Comparative genomic analyses have demonstrated that both groups share genomic traits including a nucleotide transporter that Microsporidia use for obtaining ATP from their hosts (33). This phylogenetic placement of Microsporidia provides further evidence for the early origins of parasitism and more than one loss of the flagellum within the kingdom Fungi.

Blastocladiomycota

The remaining two phyla of zoosporic fungi are Blastocladiomycota and Chytridiomycota. The branching order of these two lineages is unresolved (Fig. 1), and both have been inferred as the sister lineage to the terrestrial, nonflagellated fungi in large multigene and genome-scale phylogenetic analyses (11, 13, 18). The failure to resolve this branching order appears to be attributed to low phylogenetic signal, rather than strong conflict among competing individual gene trees (18), although only two species of Blastocladiales have been sequenced at this time. Resolution of this branching is central to accurately understanding the changes in life cycles and morphology, especially the loss of flagellum that accompanied the transition onto land. Additional taxon sampling and analysis of changes associated with genome content (e.g., phylogenetic mapping of gene birth events, rare genomic changes, etc.) will be required.

Blastocladiomycota contains a single class and order, Blastocladiomycetes and Blastocladiales, respectively. Fossils of both sporothalli and gametothalli have been described from the Lower Devonian (Rhynie chert) as *Paleoblastocladia* (34), supporting it as an ancient lineage, but current molecular data are consistent with extant species sharing a recent MRCA as compared to other phyla of fungi. Blastocladiomycota exhibit a range of growth morphologies from monocentric with limited thallus development to polycentric with the production of robust, coenocytic hyphae. Furthermore, most known species exhibit a true alternation of generation with free-living haploid and diploid life stages. In *Allomyces*, for example, free-living haploid thalli produce male and female gametangia that can usually be differentiated through color and size; the male gametangia are typically pigmented with carotenoids and are smaller in size than female gametangia. Both male and female gametangia produce motile gametes (planogametes) that fuse to form a diploid zygote, which germinates to form a free-living diploid thallus. This thallus produces sporangia that yield diploid zoospores that are released into the environment and germinate to form other diploid thalli. The diploid thallus ultimately matures and produces resistant sporangia that are the sites of meiosis and production of haploid zoospores, which when released into the environment germinate into haploid thalli, completing the life cycle. Meiosis associated with spore formation (sporic) is unknown in any other fungi.

Well-studied genera within Blastocladiales include *Allomyces*, *Blastocladiella*, *Coelomomyces*, and *Physoderma*, which exhibit saprobic and parasitic ecologies associated with animals and plants. *Allomyces* is frequently isolated from soil and serves as an excellent instructional model of polycentric development (mycelial development with multiple sporangia) and alternation of generations. Its ease of growth on synthetic media with simple sugar sources is consistent with a saprobic life history. *Blastocladiella* is another saprobic species isolated from soil, but it displays monocentric development, with a simple thallus consisting of a single sporangium anchored to its substrate by rhizoidal elements. *Coelomomyces* is a parasite of mosquitoes and copepods, with alternate generations produced in different

hosts. Haploid zoospores infect copepods and produce one- to two-celled wall-less hyphal bodies within the host. These hyphal bodies give rise to flagellated gametes that fuse inside or outside of the copepod host, resulting in a diploid flagellated zygote that infects mosquito larvae. Inside the mosquito, the fungus develops a limited diploid thallus that produces resting sporangia, which are the site of meiosis and production of haploid zoospores. *Physoderma* includes stem and foliar pathogens of plants (e.g., corn, alfalfa, etc.), which is relatively rare for zoosporic species of the kingdom Fungi. Species of *Physoderma* produce monocentric epibiotic sporangia that infect a single host cell, but as the infection progresses, polycentric growth develops, producing large endobiotic sporangia that occupy much of the host cell.

Chytridiomycota

Chytridiomycota includes three classes of fungi: Chytridiomycetes, Monoblepharidomycetes, and Neocallimastigomycetes. Although some classifications recognize the latter two classes as phyla (e.g., 14), the three taxa collectively form a well-supported monophyletic clade in genome-scale analyses, but their relationship to each other is uncertain (18). Chytridiomycota may have been the earliest fungi in terrestrial environments, but it is not clear if certain Precambrian microfossils actually represent species of the phylum. Spherules and flask-shaped fossils from chert lenses of the Early Devonian Rhynie chert formation have been interpreted as thalli and zoosporangia of Chytridiomycota. The study of plants and plant parts, including pollen, has been a productive means of discovering and identifying increasing numbers of Chytridiomycota fossils and the presumptive reactions of their hosts 412 million years ago (16).

Chytridiomycetes

Chytridiomycetes are water-inhabiting fungi, often parasitic on algae and oomycetes, or soil inhabitants, some of which are parasitic on vascular plants. A few chytrids parasitize animal eggs and protozoa, while others are saprobic on the decaying remains of plants. Multigene phylogenetic analyses, new culture techniques, and additional collections of Chytridiomycetes have revealed greater diversity and led to increased numbers of orders in which to classify about 700 species in under 90 genera (e.g., 11, 14). Today there are 10 described orders of Chytridiomycetes: Chytridiales, Spizellomycetales, Cladochytriales, Rhizophydiales, Polychytriales, Rhizophlyctidales, Lobulomycetales, Synchytriales, Gromochytriales, and Mesochytriales (e.g., 35–39).

An exemplar life cycle is that of *Chytriomyces hyalinus*, which forms a well-developed rhizoidal system within its substrate (Fig. 2b). The sporangium that develops from the encysted zoospore has a saucer-shaped operculum from which zoospores escape into a fibrous vesicle of overlapping filaments where the cells complete their maturation and then escape (40). The zoospores encyst and germinate to form new sporangia and rhizoids (asexual thalli) or to function as sexual thalli. This is one of the few members of the Chytridiomycetes in which sexual reproduction has been well documented. The rhizoids of the two thalli come into contact and fuse, and a resting spore forms at the junction of the rhizoidal anastomosis. The resting sporangium develops a thick wall and eventually germinates by production of zoospores, apparently after meiosis (41). Other well-studied members of Chytridiomycetes include *Synchytrium endobioticum*, a pathogen that causes potato wart disease, and *Nowakowskiella*, an operculate, polycentric genus of aquatic saprobes of decaying plant materials. Species of *Nowakowskiella* are often isolated from pond water by using cellulosic baits. The most widely studied chytrid is *Batrachochytrium dendrobatidis*, the fungus associated with amphibian declines (42, 43). The disease was first detected in Australia and Panama, but it now has been found on all continents except Antarctica. Estimates predict that 30% of the world's amphibian species will be affected by severe population decline or extinction (44–47). Zoospores attack the keratinized parts of the amphibian, such as larval mouthparts and the first two layers of adult skin, to cause the infection. The flagella are resorbed, and the *B. dendrobatidis* cells enlarge within the infected host cells. Growth of the somatic cells and their conversion to zoosporangia cause the hyperplasia and hyperkeratosis that are symptomatic of the disease. Severe infections reduce the efficiency of cutaneous respiration and osmoregulation. Evolutionary analyses of the *B. dendrobatidis* genome revealed that it has evolved from saprobic ancestors and that its unique ecology of being a vertebrate pathogen is correlated with lineage-specific expansion of multiple gene families of proteases (48).

Monoblepharidomycetes

Monoblepharidomycetes consists of only about 30 species in 6 genera, most of which are saprobes that grow in fresh water on submerged twigs and fruits. The fungi can be isolated by baiting soil samples in water with hemp or sesame seeds. Genera are distinguished in part by a polycentric mycelial thallus (*Gonapodya*,

Monoblepharella, and *Monoblepharis*) or a uniaxial thallus (*Oedogoniomyces*, *Harpochytrium*, and *Hyaloraphidium*). When hyphae are present in Monoblepharidales, they appear foamy or reticulate because the protoplasm is highly vacuolated. Monoblepharidales is of special interest because of its unique method of sexual reproduction using a nonmotile female gametangium and a flagellated male gamete, unlike any other fungus.

Monoblepharis polymorpha has a well-developed branched thallus consisting of hyphae with a foamy appearance. Elongated zoosporangia are borne singly at the hyphal tips subtended by a septum. Zoospores are released from the tip of the sporangium, swim for a time, become rounded, and germinate by a germ tube to form a new mycelium. The same thallus that bears sporangia also produces gametangia when subjected to higher temperatures. The elongated male gametangium is borne on the large, rounded egg-like female gametangium (Fig. 2d). Uniflagellate gametes formed within the male gametangium mature and are released. A single male gamete fertilizes the enlarged female gamete, resulting in cytoplasmic fusion and production of a diploid zygote. The zygote functions as a resting spore and germinates by producing a hypha to initiate a new thallus. The site of meiosis has not been demonstrated but probably occurs in early divisions of the zygote nucleus (49, 50).

Neocallimastigomycetes

The zoospores of members of Neocallimastigomycetes were once considered to be flagellated protozoa, but DNA sequences placed them in a distinct group of the core chytrids (11). The class consists of about 20 species placed in 6 genera, including *Neocallimastix*, *Orpinomyces*, and *Piromyces* (Fig. 2e). All species inhabit anaerobic regions of the rumen and hindgut of herbivores, and they produce cellulases and xylanases that help to degrade the dietary fibers from plant cell walls (51, 52). Phylogenomic analyses revealed that many of these plant cell wall-degrading enzymes are of bacterial origin and represent a horizontal gene transfer from bacteria that presumably co-occur with Neocallimastigales in the herbivore digestive tract (53). All species are obligate anaerobes, and although a few other fungi (e.g., *Blastocladia* and certain yeasts in Saccharomycetales) are facultatively anaerobic, among fungi only members of Neocallimastigales require completely anaerobic environments. As a group, these fungi grow on a range of simple and complex carbohydrates and exhibit mixed acid fermentation (54), and renewed attention is aimed at their potential in applied industrial mycology (55).

ZYGOMYCETE FUNGI (FIG. 3)

As mentioned previously, genome-scale phylogenies do not support the monophyly of zygomycetes and reject the zygospore as a synapomorphy for them (13). Rather, the zygospore is best interpreted as arising in the MRCA of Zoopagomycota, Mucoromycota, Ascomycota, and Basidiomycota and lost in the MRCA of Dikarya (Ascomycota + Basidiomycota). Most zygomycetes are characterized by coenocytic hyphae and sporangial asexual reproduction, but lineages characterized by septate or compartmentalized hyphae and/or asexual reproduction by formation of conidia exist. Importantly, it is with the emergence of the zygomycete fungi that we observe a loss of the fungal flagellum and the rise of the terrestrial, filamentous fungi. It is generally assumed that this loss of the flagellum in the kingdom Fungi corresponds to the transition to a terrestrial environment and the dawn of early terrestrial ecosystems. As such, zygomycetes represent an important group of fungi for ecological studies of host association and diversification of nutritional modes and cell biology studies regarding the evolution of centrosomes, organelles associated with hyphal growth and differentiation, and multicellularity.

Zoopagomycota

Zoopagomycota is the sister to Mucoromycota+Dikarya. It comprises three subphyla: Zoopagomycotina, Kickxellomycotina, and Entomophthoromycotina. The primary ecologies of members of the phylum include pathogens and commensals of animals, parasites of other fungi and amoebae, and rarely, as plant associates. The phylogenetic placement of Zoopagomycota as sister to the remainder of nonflagellated fungi is important for numerous reasons, but two are highlighted here. First, diversification with animals and nonplant hosts occurred at least as early as diversification with terrestrial plants. This suggests that fungi were among the first terrestrial organisms and that fossils of the first land animals should be examined with greater scrutiny for fungal associations, potentially providing a more complete picture of early terrestrial fungi. Second, the loss of the flagellum in fungi corresponds to other modifications, including the loss of the centriole. Most nonflagellated fungi of Mucoromycota, Basidiomycota, and Ascomycota possess an organelle unique to fungi, the spindle pole body, which serves as the microtubule attachment necessary for chromosome segregation during nuclear division. It has been hypothesized that the spindle pole body is derived from centrioles through the loss of the 9+2 microtubular system, though there is no support for this homology based on detectable sequence similarity. In contrast,

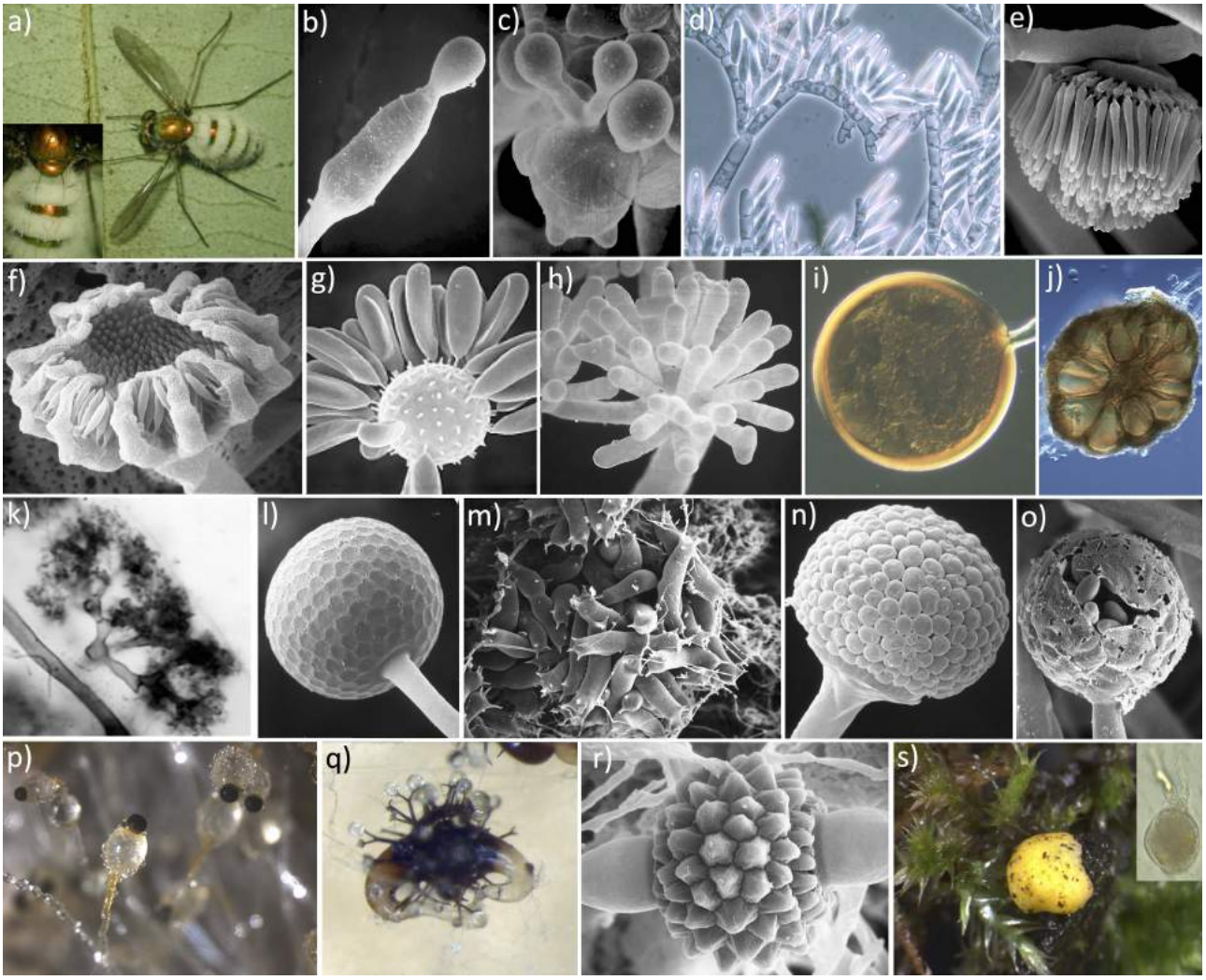

FIGURE 3 Examples of zygomycete fungal diversity. Zoopagomycota: **(a)** adult fly infected by a species of Entomophthorales; **(b)** *Basidiobolus* conidium; **(c)** *Conidiobolus* conidia; **(d)** *Zygopolaris* thallus with trichospores (photo by R.W. Lichtwardt); **(e)** *Linderina* sporangium; **(f)** *Kickxella* sporangium; **(g)** *Rhopalomyces* sporangium; **(h)** *Piptocephalis* sporangium. Mucoromycota: **(i)** *Glomus* spore (photo from American Society for the Advancement of Science); **(j)** *G. sinuosum* sporocarp (photo by D. Redeker); **(k)** *G. mosseae* arbuscule (photo by K. Wex); **(l)** *Mortierella* sporangium; **(m)** *Lobosporangium* sporangium; **(n)** *Rhizopus* sporangium; **(o)** *Thamnidium* sporangium; **(p)** *Pilobolus* sporangium; **(q)** *Phycomyces* zygosporangium; **(r)** *Cunninghamella* zygosporangium; **(s)** *Endogone* sporocarp, zygospore (inset).

Zoopagomycota lineages retain a functional centrosome that possesses a degenerate 9+2 microtubular system (56). Furthermore, there is some evidence that the genus *Olpidium*, a zoosporic fungus that retains its flagellum and infects nematodes and plant roots of Brassicaceae, may be a member of—or closely related to—Zoopagomycota (12). Collectively, these observations suggest that Zoopagomycota is critical to understanding the evolution of fungi as they transitioned to terrestrial ecosystems.

Zoopagomycotina contains a single order, Zoopagales. Species in this order include predators of nematodes (e.g., *Stylopage*) and nematode eggs (e.g., *Rhopalomyces* [Fig. 3g]), predators of amoebae (e.g., *Stylopage*, *Zoopage*), and mycoparasites of mucoralean fungi (e.g., *Syncephalis*). Hyphae are small in diameter, coenocytic, and they form haustoria on or within their hosts. Asexual reproduction is by conidia or sporangia according to species, and where known, sexual reproduction is by production of zygospores. Many of these

fungi are obligate symbionts and thus are difficult to obtain in axenic culture, and for this reason there is a paucity of molecular and genomic data. Furthermore, these fungi are underrepresented in environmental sequence data (57), presumably due to the inadequacy of existing environmental sampling techniques (e.g., primer bias, insufficient reference data, etc.), and their internal transcribed spacer sequences are longer than most fungi, contributing to underrepresentation (58).

Kickxellomycotina comprises four orders: Asellariales, Dimargaritales, Harpellales, and Kickxellales. Species of Kickxellomycotina possess hyphae that are regularly compartmentalized by bifurcate septa that are occluded by a lenticular plug. Asellariales and Harpellales are associated with digestive tracts of aquatic stages of arthropods and comprise two of the four orders that have been treated previously as Trichomycetes (59); the other two orders, Amoebidiales and Eccrinales, are members of Mesomycetozoea, not the kingdom Fungi (60, 61). Asellariales has filamentous, branched thalli and reproduces asexually by disarticulation of the thalli into arthrospores. They occur in the digestive tracts of marine, aquatic, and terrestrial species of isopods and Collembola, where they are thought to function as commensals (62). Harpellales has branched or unbranched filamentous thalli, and they reproduce by trichospores, which are asexual spores with hair-like appendages (Fig. 3d). They attach to the hindgut of aquatic stages of arthropods via a holdfast and are generally considered to be in a commensal relationship with their host (59). Dimargaritales species are haustorial parasites of other fungi, with the best-known species occurring on mucoralean hosts (63), and Kickxellales includes mycoparasites and saprobes isolated from soil (64). Dimargaritales and Kickxellales, as well as Zoopagales, produce unique sporangia called merosporangia. These are cylindrical sporangia that arise from a bulbous structure, and one or more sporangiospores may occur in chains within the sporangium (Fig. 3e–h).

Entomophthoromycotina contains three classes, each with a single order: Basidiobolomycetes and Basidiobolales, Entomophthoromycetes and Entomophthorales, and Neozygitomycetes and Neozygitales (13, 65, 66). These fungi are associated with animals as either commensals isolated from animal dung or as pathogens and parasites of insects. Many species are commonly isolated from soil and maintained in pure culture, which is consistent with a saprobic life cycle phase. Basidiobolales is commonly isolated from amphibian dung, although species are known to occur on the dung of many vertebrates. They produce primary conidia that forcibly eject a single spore which if it lands on an appropriate substrate will germinate to form a mycelium or otherwise will undergo repetitive germination, producing a secondary conidium (Fig. 3b). Under some conditions nonforcibly discharged capilliconidia are produced from forcibly discharged conidia. These adhere to the outer surface of insects (67). Dispersal is then achieved when the insects are ingested by insectivorous animals, and after surviving gut passage, the fungus is excreted with the feces. In a few cases gut infections are known in various marine and terrestrial vertebrates, and human gut infections have been mistaken for Crohn's disease (68). The ecological function of Basidiobolales is speculative at this time because no definitive functional experiments have been performed, but its specificity to animal dung and placement in Zoopagomycota support an interaction with animal hosts in the digestive system. The phylogenetic placement of Basidiobolales with molecular- and genome-scale data is problematic. In all current data sets, it is characterized by long and unstable branches, and its relationship to other Entomophthoromycotina is unambiguous at this time (69).

Entomophthorales, or literally, insect destroyers, comprises pathogens of insects (Fig. 3a). They infect their hosts via spores and multiply within the host as one- to two-celled hyphal bodies, which also can function as gametangia. Upon the host's death, the fungus ruptures through the cuticle segments, producing forcibly discharged primary conidia (Fig. 3a). Frequently, infected hosts alight in perched or elevated positions, a phenomenon known as summit disease, which is thought to be an induced behavior or adaptation for spore dispersal of the pathogen (20, 70, 71). Neozygitales are pathogens of insects and mites. They were classified as a family within Entomophthorales but were distinguished from Entomophthorales based on the shape and size of chromosomes (65), although inadequate molecular data currently exist to test this hypothesis. *Neozygites* produces adhesive capilliconidia similar to those of *Basidiobolus* (72).

Mucoromycota

Mucoromycota consists of the subphyla Glomeromycotina, Mortierellomycotina, and Mucoromycotina. Unlike Zoopagomycota, Mucoromycota is characterized by plant associations and plant-based ecologies (e.g., mycorrhizae, root endophytes, decomposers, etc.). Some exist as parasites of animals and other fungi, but these all represent opportunistic infections of hosts with compromised immune systems or relatively recent derivations from saprobic ecologies (73). Mucoromycota is the sister group to Dikarya, which is also

characterized by dominant plant-associated life styles, suggesting that the MRCA of Mucoromycota and Dikarya corresponds to the origin of modern fungus-plant associations, or at least the evolutionary potential for such relationships.

Glomeromycotina consists of the arbuscular mycorrhizae and *Geosiphon*, a symbiont of cyanobacteria (74). Arbuscular mycorrhizae (Fig. 3i–k) are the most common form of mycorrhizae on the planet, and arbuscule fossils are present among the first land plant fossils (75–77), confirming an ancient symbiosis. As such, they are a central taxon in the development of hypotheses concerning the evolution of early land plants and terrestrial ecosystems (78–80). Despite this importance, they have been an enigma with respect to phylogenetics of the kingdom Fungi. Morphologically, they resemble zygomycetes in the production of coenocytic hyphae and terminal or subterminal spores that resemble azygospores (Fig. 3i), asexually formed zygospore-like structures produced terminally on a single hypha or suspensor cell (81). Sexual reproduction has never been observed for the group, preventing analysis of morphological characters traditionally used in classifications. Early molecular phylogenies based on the small subunit ribosomal DNA resolved the arbuscular mycorrhizae—with varying statistical support depending on the analysis—as separate from the zygomycetes and sister to Dikarya (40, 82). However, genome-scale phylogenies and genome content analyses strongly support the arbuscular mycorrhizae as members of Mucoromycota (13, 83). Recently, mating type genes have been identified in the Glomeromycotina genomes, and their structure and diversity are consistent with a functional sexual reproductive cycle without morphological evidence of sex. Interestingly, the *MAT* genes are more similar to those of basidiomycetes than those in other fungal groups, although this is still a topic of debate (84). So while we have learned much about Glomeromycotina from genomic data, where and when these fungi undergo sexual reproduction remains a mystery. Currently, there are four orders of Glomeromycotina, Archaeosporales, Diversisporales, Glomerales, and Paraglomerales, with *Geosiphon* being classified in Archaeosporales (74).

The relationship of Glomeromycotina to the other subphyla of Mucoromycota is unresolved, with some analyses resolving it as sister to Mortierellomycotina and Mucoromycotina, while others resolved it as the sister group to Mortierellomycotina. The taxon sampling for both Glomeromycotina and Mortierellomycotina is sparse, and expanded taxon sampling is needed to fully test these rival hypotheses. Mortierellomycotina, and its sole order Mortierellales, are commonly isolated soil fungi. They produce zygospores and sporangia similar to some species of Mucorales (Fig. 3l and 3m), the order in which they were previously classified, but molecular phylogenetics (85) and genome-scale (13) phylogenies both strongly support the taxon as representing a distinct subphylum. Interestingly, even earlier studies of sterols separated the two orders because Mortierellales contain membrane sterols other than ergosterol (86). These fungi have been demonstrated as root endophytes of plants (87, 88), but their effect on the host fitness remains unknown. Some species are reported to have distinctive odors, perhaps associated with animal dispersal. Mortierellales are also prolific producers of fatty acids, in particular arachidonic acid, and *Mortierella* species are used in its industrial production (89). Both Glomeromycotina and Mortierellomycotina possess intimate relationships with bacteria, and while facultative, show high levels of specificity and cospeciation (90, 91), and the fungus tends to grow better when cleared of the bacterium (92). Because of their relationship to Glomeromycotina, demonstrated plant associations, and industrial applications including potential alternative fuels, Mortierellomycotina are the subject of significant genomic inquiries.

Mucoromycotina contains the remainder of known zygomycete species and is classified in three orders: Mucorales, Umbelopsidales, and Endogonales (13). Mucorales is one of the more commonly isolated groups of fungi, because many are fast-growing, early colonizers of carbon-rich substrates. Because many species culture relatively easily, Mucorales are well represented in culture collections, and their zygospores and sporangia (Fig. 3n–r) are well documented (93). They include taxa that cause economically significant pre- and postharvest diseases of fruits (e.g., *Gilbertella*, *Mucor*, *Rhizopus*). They also significantly impact humans both beneficially through their use in industrial production of food (e.g., tempeh, *Rhizopus*) and compounds used as food supplements (e.g., beta-carotene, *Blakeslea*), and antagonistically as rare but increasingly diagnosed human mycoses (e.g., *Mucor*, *Apophysomyces* [94]). It is among Mucorales that sexual reproduction in fungi was first demonstrated (95), and numerous species of Mucorales exhibit phototropic responses to light (96), making them important eukaryotic model organisms (e.g., *Mucor mucedo*, *Phycomyces blakesleeanus*). Umbelopsidales was recently described for *Umbelopsis* (13), a genus of soil-inhabiting fungi that also occurs as root endophytes (87). Like *Mortierella*, it was formerly

classified in Mucorales, but genomic and molecular analyses support it as a distinct taxon (85). Endogonales are saprobic or ectomycorrhizal depending on the species (81). Saprobic species occur in heavily decayed woody substrates, while mycorrhizal species associate with both nonvascular and vascular plants (78). They have been argued as important organisms in the colonization of land by green plants (79) and represent an independent origin of mycorrhizae relative to both Glomeromycotina and Dikarya. It is within Mucoromycota that the first multicellular sporocarps were produced. These include independent origins in *Endogone* (Mucoromycotina [78]) (Fig. 3s) and *Modicella* (Mortierellomycotina [97]), and as aggregations of spore-producing hyphae and spores in species of Glomeromycotina (Fig. 3j) (74). Multicellular sporocarps are not produced by Zoopagomycota, suggesting that the genetic potential for complex thallus development did not arise until the MRCA of Mucoromycota and Dikarya and then resulted in multiple independent inventions of complex spore-producing structures in Mucoromycota, Ascomycota, and Basidiomycota (98).

DIKARYA

Dikarya is the only described subkingdom of Fungi and comprises the phyla Ascomycota and Basidiomycota. The name is based on the nuclear condition of possessing two genotypically distinct nuclei within the thallus at some point in the life cycle. The hyphae of Dikarya are regularly septate, and if a hyphal compartment contains one nucleus or more than one nucleus but all of the same genotype, it is referred to as monokaryotic or homokaryotic, respectively. If a hyphal compartment contains two nuclei of different genotypes, it is referred to as dikaryotic or heterokaryotic. In Basidiomycota, basidiospores typically possess one type of nucleus and germinate to form a monokaryon. Monokaryons fuse (plasmogamy) with other compatible monokaryons, producing the dikaryotic state that is maintained by the products of the mating type genes. The dikaryon constitutes the major vegetative phase of the life cycle for most Basidiomycota, and it is as a dikaryon that most Basidiomycota function in nature (e.g., wood decay, mycorrhizae, pathogens, etc.). Karyogamy occurs in unique cells, termed basidia, resulting in a short-lived diploid zygote that immediately undergoes meiosis to produce haploid nuclei that are incorporated into basidiospores. Importantly, plasmogamy is separated from karyogamy and meiosis both temporally and spatially. In Ascomycota the dikaryotic state is restricted to the sexual reproductive cells, with the vegetative mycelium being homokaryotic. Female gametangia (ascogonia) are fertilized by male gametangia (antheridia) or gametes (spermatia), resulting in a dikaryotic state (ascogenous hyphae). A number of rounds of conjugate nuclear divisions occur prior to karyogamy. Karyogamy and meiosis follow shortly afterward in the young ascus cell, resulting in homokaryotic ascospores. Thus, although both phyla of Dikarya have inherited the dikaryotic state from a common ancestor, they are expressed differently in the life cycles of Ascomycota and Basidiomycota. For more on life cycles of Ascomycota and Basidiomycota, the reader is directed to general mycology textbooks (e.g., 41).

More genomes have been sequenced for species of Ascomycota and Basidiomycota than other phyla of the kingdom Fungi (http://genome.jgi.doe.gov/fungi/), resulting in an increased resolution of phylogenetic relationships and evolutionary processes that have shaped phylogenetic and ecological diversity in Dikarya. Ascomycota consists of three subphyla—Taphrinomycotina, Saccharomycotina, and Pezizomycotina—as does Basidiomycota: Pucciniomycotina, Ustilaginomycotina, and Agaricomycotina. These subphyla include the majority of described species of fungi, and most are associated with plants as symbionts or decomposers of plant materials, although there are numerous independent transitions to other hosts and materials (e.g., animals). It is within Dikarya that we see the apex of multicellular sporocarp production, complex multicellularity, yet sporocarps are distributed sporadically across separate subphyla. Major innovations in sporocarp formation can be found primarily in Pezizomycotina and Agaricomycotina (98), but also within a single genus of Taphrinomycotina (*Neolecta* [99]) and scattered within Pucciniomycotina (e.g., *Septobasidium* [100], *Eocronartium*, and *Jola* [101]). When considering the diversity across the kingdom Fungi, there are a minimum of seven clades of sporocarp-forming fungi: three in Mucoromycota (discussed above), two in Ascomycota, and two in Basidiomycota. Genomic analyses of evolutionary development, or EvoDevo, of sporocarp formation are in their infancy, but a recent study involving Ascomycota paints a complex picture (102). Genes hypothesized to function in complex multicellularity were present in the MRCA of Ascomycota and diversified in Pezizomycotina but were lost in parallel in Saccharomycotina, the budding yeasts, and the yeast lineages of Taphrinomycotina. While present, genes that may be necessary for complex multicellularity did not expand in copy number or show substantial diversification within *Neolecta*, thus com-

plicating a simple explanation of gains and losses of sporocarps.

Ascomycota (Fig. 4)

Ascomycota is a diverse phylum of fungi that includes decomposers associated with a myriad of substrates (e.g., dung, wood, soil), symbionts and associates of plants and animals, and inhabitants of marine and terrestrial ecosystems. Plant-associated species range from antagonistic pathogens to beneficial symbionts (e.g., mycorrhizae) to foliar, root, and wood endophytes whose true functions remain unknown. They have impacted our civilization since the dawn of humans with both positive and negative outcomes. Some ascomycetes were among the first organisms domesticated by humans and have been used in fermentation of foods and beverages for over 9,000 years (103). They are the source of numerous lifesaving drugs including antibiotics, statins, and immunosuppressants. Unfortunately, they are also the causal agents of disease, especially of plants, that have changed continents by removing entire species from the landscape after introduction by humans (e.g., chestnut blight [104]) or have resulted in billions of dollars of loss in modern agriculture (e.g., *Fusarium* head blight [105]). Indeed, our relationship with Ascomycota is one that has profound effects on our planet and our species.

Taphrinomycotina

The subphylum Taphrinomycotina includes about 140 species with both yeast and filamentous growth forms and is a sister group to the remaining Ascomycota. Taphrinomycotina is classified into five classes—Taphrinomycetes, Schizosaccharomycetes, Pneumocystidiomycetes, Neolectomycetes, and Archaeorhizomycetes—each with a single order. Taphrinomycetes includes plant pathogens such as *Taphrina* and *Protomyces*, which are characterized by saprobic, monokaryotic yeast and pathogenic, dikaryotic filamentous life stages. Taphrinales has played an important role in the development of evolutionary hypotheses of the kingdom Fungi and has been considered a possible evolutionary link between Ascomycota and Basidiomycota, due to the life cycle described above, which is similar to that of some smut and other microfungal basidiomycetes of Ustilaginomycotina and Pucciniomycotina. Based on numerous molecular phylogenetic studies, however, it is strongly supported as a member of Ascomycota, and it is unclear if the similarity in life cycles with some Basidiomycota is homologous or not.

Schizosaccharomycetes includes the fission yeasts, which display an unusual form of cell division for fungi. Rather than dividing by budding, as is true for all other yeast forms, cells of Schizosaccharomycetales elongate and divide into equal cells through the formation of a fission plate. *Schizosaccharomyces pombe* is the best-studied species and was originally isolated from millet beer in East Africa (106). Pneumocystidiomycetes comprises pulmonary pathogens of mammals, including humans. Species of *Pneumocystis* are found in soils associated with rodent dens. *Pneumocystis jirovecii* is an opportunistic pathogen of humans and is the causal agent of pneumocystic pneumonia in people with compromised immune systems. Neolectomycetes includes the only sporocarp-producing species of Taphrinomycotina. The genus *Neolecta* forms earth tongue-like sporocarps with a hymenium of asci produced from uniquely branched hyphae (107). The ecology of the group is unknown, but the inability to maintain it in axenic culture suggests a symbiotic association in nature (108). Ancestral character state reconstruction analyses resolved *Neolecta* as an independent origin of sporocarp formation in Ascomycota, unique to that of Pezizomycotina (98, 109), but comparative genomic analyses support the possession of common genetic multicellular machinery between *Neolecta* and Pezizomycotina (102). Archaeorhizomycetes is the most recently described class of Taphrinomycotina. It is commonly detected in environmental sampling of soil and was initially known informally as Soil Clone Group 1 (110, 111). One species was serendipitously described from a culture collection of root-associated fungi; it and a second recently described species are associated with the surfaces of tree roots (112, 113).

Saccharomycotina

The subphylum Saccharomycotina includes about 1,000 species in a single order, Saccharomycetales, and 10 major families and several undescribed clades (114). Saccharomycetales include the majority of the ascomycete yeasts and are characterized by budding in asexual reproduction (115). Yeasts, however, have evolved multiple times in the kingdom Fungi, most likely through parallel diversification of novel transcription factors (116). In Saccharomycotina, somatic cells and hyphae may be haploid or diploid, and diploid cells undergo meiosis to convert to gametes, which fuse to form a zygote and develop into asci to produce one to eight ascospores. Ascogenous hyphae are not produced as in Pezizomycotina. Two other traits that distinguish Saccharomycetales yeasts from other ascomycetes occur during ascosporogenesis. These are meiotic divisions with perpendicular spindles developing within the

FIGURE 4 Examples of Ascomycota diversity. **(A)** Apothecia (yellow) of *Orbilia*, Orbiliomycetes (J. H. Petersen/MycoKey). **(B)** Apothecia of *Aleuria*, Pezizomycetes (J. H. Petertsen/MycoKey). **(C)** Thallus of *Ophioparma* with apothecia, Lecanoromycetes (B. McCune, Oregon State University). **(D)** Thallus of *Lichinella*, Lichinomycetes (B. McCune, Oregon State University). **(E)** Bitunicate asci of *Thaxteriella*, Dothideomycetes (S. Huhndorf, Field Museum). **(F)** Thallus of *Arthonia* with apothecia, Arthoniomycetes (B. McCune, Oregon State University). **(G)** Thallus of *Prolixandromyces*, Laboulbeniomycetes (A. Weir, SUNY-ESF). **(H)** Perithecia of *Neurospora*, Sordariomycetes (N. B. Raju, Stanford University). **(I)** Earth-tongue apothecia of *Cudonia*, Leotiomycetes (Z. Wang, Iowa State University). **(J)** Cleistothecia of *Eupenicillium*, Eurotiomycetes (D. Geiser, Penn State University). **(K)** Operculate ascus of *Peziza* (J. H. Petersen/MycoKey). **(L)** Ascostroma of *Venturia*, Dothideomycetes (T. Volk, University of Wisconsin at La Crosse). **(M)** Unitunicate asci *Neurospora* (N. B. Raju, Stanford University). **(N)** Prototunicate ascus of *Eurotium* (D. Geiser, Penn State University).

nuclear envelope and formation of ascospores within a common ascus vesicle instead of ascospores developing in individual ascus vesicles as in Pezizomycotina (41). Although they are relatively similar in morphology, Saccharomycotina yeasts are metabolically diverse. In common with Taphrinomycotina, Saccharomycotina lack the septal pore-blocking Woronin bodies characteristic of Pezizomycotina. Earlier yeast taxa were distinguished by the application of about 100 physiological tests. Previously, hypotheses of relationships were difficult to address because of the lack of distinctive morphological characteristics. Now DNA analysis distinguishes yeast species rapidly, as well as providing phylogenetic hypotheses (114, 115). To expand our knowledge of biotechnologically important yeasts, a variety of additional species were sequenced across the known phylogeny, especially from outside of the better-known *S. cerevisiae* clade. This study revealed several transitions in the early evolution of the ascomycetes, the synteny of the *MAT* loci across Ascomycota, and a genetic code change from CUG-Ser to CUG-Ala in one lineage (117, 118). Phylogenomic analyses have also revealed that *S. cerevisiae* and its relatives are the product of a whole-genome duplication (119, 120) that is the result of an ancient hybridization event between two ancestral species (121).

Yeasts occupy a wide variety of natural (e.g., desert and forest plants, insect guts, and environments) and human-provided (e.g., pickle vats, breweries) habitats. *S. cerevisiae* is the most widely known yeast in the world due to its role in bread making and brewing of alcoholic beverages and as a model organism. This was the first eukaryotic species to have its entire genome sequenced, which was justified by its industrial and biological importance. *S. cerevisiae* and many other yeasts are closely associated with insects, including bees, dipterans, and beetles. Certain yeasts display specific associations with insects and plants, including dipterans and cacti in American deserts (122), nitidulid beetles and ephemeral flowers around the world (123), and widely distributed wood-feeding beetles (124). A distinctive group of previously unknown yeasts related to *Suhomyces tanzawaensis* are associated with fungus-feeding beetles and drosophilids (125). Sexual reproduction and overwintering of *S. cerevisiae* and its hybrids are promoted in the gut of social wasps (126). The bodies of human beings and many mammals are substrates for animal pathogenic yeasts. *Candida albicans* and relatives inhabit the digestive tracts of healthy individuals but may become invasive in those with suppressed immune systems. Infections can be localized (esophageal candidiasis, or thrush; genital candidiasis, or "yeast infection") or invasive in the brain, heart, bones, and eyes. *Candida* can be spread in the blood, a fairly common infection in hospitals where candidemia has been estimated to be fatal in as many as 19 to 24% of cases (127). *Candida auris*, recently recognized as an emerging fungal disease in the United States, is multidrug-resistant (https://www.cdc.gov/fungal/diseases/candidiasis/candida-auris.html). Few yeasts are plant pathogens, but *Ashbya gossypii* is well known to be destructive to fruit development in cotton (128). This species is also industrially important because it overproduces riboflavin (129). Interestingly, *A. gossypii*, and occasionally *S. cerevisiae*, are insect-associated.

Pezizomycotina

The subphylum Pezizomycotina contains many more species than the two other ascomycete subphyla combined; the 63,000 or so species are placed in 13 classes and 67 orders. Pezizomycotina are mostly filamentous (130), although many are capable of dimorphic growth. With the exception of *Neolecta* (Taphrinomycotina), they include all of the sporocarp-forming Ascomycota, but the life cycles of many are only known from their hyphal and asexual reproductive states. Past classifications of the group have emphasized sporocarp morphology and development and ascus morphology and dehiscence. Ascomycete sporocarps, or ascomata, were categorized into four basic types. Apothecia have an exposed hymenium of asci and are typically cup-shaped or spathulate (Fig. 4a–d). Perithecia enclose the hymenium in a flask-shaped ascoma, and the ascospores are released through an opening called an ostiole (Fig. 4h). Cleistothecia completely enclose an ill-defined hymenium of scattered asci, and no pore or opening is present (Fig. 4j). (Note: Chasmothecia are another form of completely enclosed ascomata, but the hymenium exists as a single basal fascicle of asci.) All three forms of these ascomata are assumed to be formed after fertilization of the ascogonium, a form of development called ascohymenial. The fourth major group of ascomata is ascostromata (Fig. 4l). In these fungi, ascogonia are fertilized in preformed locules, which mature into ascostromata containing asci and ascospores, a form of development called ascolocular.

Asci of Pezizomycotina may be operculate or inoperculate. The apex of operculate asci contains a preformed lid, like a manhole cover, that is opened for ascospore release (Fig. 4k), while inoperculate asci lack an operculum. Inoperculate asci can be further categorized based on the nature of the ascus walls. Bitunicate

or fissitunicate asci possess more than one functional wall layer, an ectoascus and an endoascus (Fig. 4e). Ascospores are released by the endoascus rupturing through and extending beyond the ectoascus in a manner reminiscent of a jack-in-the-box. Unitunicate asci possess a single functional wall layer, and the ascus apex usually possesses a pore or canal through which ascospores are released (Fig. 4m). Prototunicate asci are typically globose and possess a thin ascus wall that breaks down, or deliquesces, at ascospore maturity (Fig. 4n). Species that produce prototunicate asci do not forcibly eject their ascospores. The combinations of ascomatal and ascal characters can be informative in describing the overall morphology of a species, but most traits have been gained and lost multiple times during the evolution of Pezizomycotina, with a few notable exceptions (e.g., the operculate ascus of Pezizomycetes).

In addition to the sexual morphs, Pezizomycotina reproduce prolifically through asexual reproduction in which the nuclei of spores are products of mitotic divisions. A species can only have one sexual state but may have many asexual states, a phenomenon called pleomorphy. Many Pezizomycotina are only known from their asexual morphs, and it is common for a species to reproduce asexually throughout the growing season but reproduce sexually only once. Historically, unique Latin binomials were given to asexual and sexual states, a system referred to as the dual system of nomenclature, and thus a single species could have several names. The reason for this was manifold, but the main argument was that the sexual and asexual states of Pezizomycotina could be separated in space and time, so when one observed an asexual state, it could be difficult to link it to a sexual state. Perhaps the fungus was only observed in its asexual form by chance; perhaps the sexual form occurs on a different host or in a different geographic region; perhaps the fungus only reproduces asexually in axenic culture; or perhaps the fungus is truly asexual. The advent of molecular data and phylogenetic analyses provided a mechanism to link sexual and asexual morphs and to identify them as conspecific. For this reason, the dual system of nomenclature was abolished recently (131), and all fungi must now be called by only one name. Needless to say, determining what name to call a fungus that has many names is no trivial task and is the subject of much debate, but today students only need to learn one name for a single species.

Nonlichenized apothecial fungi are classified into four classes of Pezizomycotina: Orbiliomycetes, Pezizomycetes, Leotiomycetes, and Geoglossomycetes. Orbiliomycetes and Pezizomycetes are sister to the remainder of the subphylum, but their branching order is unresolved (132). Orbiliomycetes produce small apothecia with inoperculate asci. Apothecia typically fruit on wood or soil, and the asci are small and release ascospores through an apical slit. The group is best known for its asexual states of *Arthrobotrys*, which are predators of nematodes. These fungi form hyphal rings or loops that constrict and capture nematodes as they crawl through the rings. Pezizomycetes produce a diversity of ascomatal types with operculate asci. Apothecia may be minute, as in the coprophilous *Ascobolus*, or large, as in the type genus *Peziza*. Some taxa produce apothecia elevated on a stipe, as in *Helvella*, or below ground, as in the prized culinary truffles of *Tuber*. The asci of all these taxa are operculate and have a positive amyloid reaction, with the exception of species that form truffles, which have lost their ability to forcibly discharge their spores. Leotiomycetes is the largest class of inoperculate apothecial fungi (133). They produce a myriad of apothecial morphologies ranging from cup fungi to stipitate earth tongues to hysteriate apothecia that are resupinate and expose the hymenium as a long and slender slit. At one time all fungi that produce apothecia with inoperculate asci were classified in Leotiomycetes. Species of the class include important plant pathogens (*Sclerotinia*), foliar (*Rhytisma*) and root (*Phialocephala*) endophytes, and mycorrhizae of ericaceous plants (*Rhizoscyphus*), to name a few. Animal pathogens are also known, including the recently emerged white nose syndrome of bats (*Pseudogymnoascus destructans*), which is devastating brown bat populations of North America (134). Geoglossomycetes comprise a subset of earth tongue species (Fig. 4i). These fungi were classified in Leotiomycetes along with other earth tongue genera *Leotia* and *Spathularia*. Molecular systematic analyses revealed that *Geoglossum* and its relatives represent a separate origin of the earth tongue morphology, however, and a unique class of fungi (135).

Sordariomycetes includes nonlichenized, perithecial species with inoperculate, unitunicate asci, but species possessing cleistothecia and/or prototunicate asci are known to be derived from within perithecial lineages (133). Perithecia may be produced on well-developed, stipitate stromata (e.g., as in *Xylaria* and *Cordyceps*), embedded in resupinate (e.g., *Hypoxylon*) or cushion-shaped stromata (e.g., *Hypocrea*), or superficially in an aggregated or scattered manner (e.g., *Neurospora*, Fig. 4h). Species of the class include some of the most devastating plant pathogens known. Chestnut blight, *Cryphonectria parasitica*, essentially eliminated the American chestnut from the forests of eastern North

America and contributed to the development of the Plant Quarantine Act of 1912. Dutch elm disease, *Ophiostoma ulmi*, devastated the American elm and significantly impacted its presence and abundance on the landscape and its use as an important urban tree (136). *Fusarium graminearum*, head scab of wheat, is a global pathogen of wheat, one of the most important grains in human agriculture, and results in losses of billions of dollars in crop production (105). In addition to plant pathogens, Sordariomycetes are frequently identified as endophytes through culture and amplicon-based environmental sampling studies (137). Sordariomycetes also includes insect pathogens of *Beauveria* and *Metarhizium*, which are some of the most promising biological control agents of insect pests in agricultural ecosystems (138). Some species have recently been identified as having the potential to grow as an endophyte, suggesting a role in plant protection against insect pests and a nutritional role of transferring nitrogen to the plant host (e.g., 72, 139).

Eurotiomycetes are cleistothecial species that produce prototunicate asci and ascostromatic species that produce bitunicate or fissitunicate asci (140). Cleistothecial taxa include important industrial and medical species of *Aspergillus* and *Penicillium* (Eurotiales) and human pathogens of *Coccidioides* (Onygenales) that cause valley fever. These fungi produce both some of the most potent mycotoxins known to science (aflatoxins of *Aspergillus*) and lifesaving antibiotics that changed the course of history (penicillin of *Penicillium*). There even exists an independent origin of ectomycorrhizae among Eurotiomycetes, the genera *Elaphomyces* and *Pseudotulostoma*, which form large truffle-like or stipitate-capitate fruiting bodies, respectively (141). Although *Pseudoulostoma* and *Elaphomyces* are members of Eurotiales, the carbohydrate metabolism of *Elaphomyces granulatus* has been shown to be more similar to animal pathogens in Onygenales than to other members of its order (142). Ascostromatic species include the black yeasts (Chaetothyriales) that result in opportunistic cutaneous and subcutaneous infections. Lichenized species are also represented among the Eurotiomycetes by the order Verrucariales. These lichens produce perithecioid ascostromata with bitunicate to fissitunicate asci, representing one of the rare combinations of reported ascohymenial development and bitunicate asci. A third and unique clade of Eurotiomycetes is Mycocaliciales, which are saprobes or commensals on lichens (143).

Dothideomycetes is a large and ecologically diverse class and represents the core of ascostromatic fungi with bitunicate asci (144). Ascostromata are frequently perithecioid in appearance and are referred to as pseudothecia (Fig. 4l), but there are apothecioid and cleistothecioid morphologies, as well. The majority of species are associated with terrestrial plants, and they are commonly identified as endophytes in environmental sampling studies based on cultures and DNA barcodes (e.g., 137, 145, 146). Numerous taxa diverged from this ecology, however, including marine fungi of mangroves, insect pathogens (e.g., *Myriangium*), mycoparasites (e.g., *Ampelomyces*), lichens (e.g., *Trypethelium*), and ectomycorrhizae (147). *Cenococcum geophilum* is one of the most abundant ectomycorrhizae-formers on the planet and represents another independent origin of ectomycorrhizae within the kingdom Fungi (148). Functional genomic studies have demonstrated that *C. geophilum* plays a role in drought tolerance of host trees, where it manipulates the host's response to water stress (149). The class is best known, however, for its large number of virulent plant pathogens including *Bipolaris maydis* (the causal agent of southern corn blight), *Zymoseptoria tritici* (Septoria wheat blotch), and *Mycosphaerella fijiensis* (black sigatoka of banana), to name a few. Species and races of these fungi frequently produce host-selective toxins that function as pathogenicity factors for selective host species and genotypes (150, 151).

Because of their importance in agriculture and relative ease of culturing, more than 100 Dothideomycetes genomes have been sequenced (http://genome.jgi.doe.gov/programs/fungi/). Published analyses of Dothideomycetes genomes revealed that the structural evolution of chromosomes is mostly a product of intrachromosomal rearrangements, a phenomenon called mesosynteny (152). Furthermore, disposable chromosomes, which may be present or absent within an isolate, are common, but their function is mostly unknown. Comparative analyses of multiple genomes across a phylogenetic and ecological diversity of Dothideomycetes support the idea that the plant pathogen "play book" is particularly rich in enzymes for secondary metabolite production, carbohydrate-active enzymes, small secreted proteins (SSPs), peptidases, and lipases (153). In addition, genes that encode for effector proteins, which have a role in pathogenicity, occur often in close proximity to transposable elements (154), suggesting that transposable elements may play a role in their evolutionary diversification.

The remaining two classes of nonlichenized Pezizomycotina are Laboulbeniomycetes and Xylonomycetes. Laboulbeniomycetes is an enigmatic class that is primarily composed of ectoparasites of insects (155–157). In Laboulbeniales, minute thalli develop from an asco-

spore through a definitive number of cell divisions and adhere to the exoskeleton through the production of a holdfast cell (Fig. 4g). Certain species tend to be host- and position-specific, with some species being transmitted through behaviors associated with sexual reproduction (158). Members of the second order, Pyxidiophorales, are mostly mycelial mycoparasites with ascospore dispersal by arthropods (159). Xylonomycetes is one of the more recently described classes of Pezizomycetes and contains wood endophytes in *Xylona* (160) and endosymbionts of beetles in *Symbiotaphrina*, the latter of which may benefit the insect by detoxifying noxious plant compounds (161).

The remaining four classes of Pezizomycotina are Arthoniomycetes, Coniocybomycetes, Lecanoromycetes, and Lichinomycetes and consist almost entirely of lichenized species (144, 162). Lichens are stable symbioses between a fungus (mycobiont) and photosynthetic green alga and/or cyanobacterium (photobiont) that result in the formation of a thallus unique to the symbiosis. Lichens are some of the more successful fungal symbioses on the planet and are conspicuous members of terrestrial ecosystems, where they play important roles in carbon and nitrogen cycles. The classic definition of a lichen being formed by one fungus has been challenged recently, however, by environmental sampling and microscopy data (163) which demonstrated that basidiomycete yeasts of Cystobasidiomycetes (Pucciniomycotina) were embedded in the cortex. Furthermore, the presence and abundance of the yeasts correlated with variations in phenotype. Although there are fewer genomic studies of lichenized than nonlichenized fungi, current comparative genomic analyses have shed some light on the formation and maintenance of lichen symbiosis. For example, species of lichenized Pezizomycotina possess an ammonium transporter/ammonia permease family that was horizontally transferred from Archaea but is absent in nonlichenized species (164). Functional analyses using the lichen-forming fungus *Endocarpon pusillum* and its algal partner identified genes involved in the nitrogen and carbon transfer between symbionts and lectins required for symbiotic recognition (165).

Lecanoromycetes is the largest and best-studied class of lichens (162). It includes most of the common forest lichens and such growth forms as foliose, fruticose, and crustose lichens. Lichinomycetes and Coniocybomycetes are two smaller classes that form a monophyletic group. Lichinomycetes form gelatinous thalli in which the cyanobacterial photobiont is not sequestered in a defined layer of the lichen. Coniocybomycetes form mazedia, which are stalked ascospore-producing structures. Mazedial lichens are also known from Caliciales of Lecanoromycetes, and mazedia are known from several groups of nonlichenized Pezizomycotina (166). Arthoniomycetes is the second-largest class of lichens, and they possess bitunicate asci, although their ascomata have been interpreted as ascohymenial (144).

Molecular systematics has provided great clarity to the phylogenetic relationships of the classes of lichenized and nonlichenized Pezizomycotina (109, 167), but genomic sampling of lichens is still sparse and is heavily biased toward nonlichenized fungi. Orbiliomycetes and Pezizomycetes are sister to the other classes of Pezizomycotina, with the remaining classes informally referred to as Leotiomyceta to designate their monophyly. Within Leotiomyceta, Sordariomycetes, Laboulbeniomycetes, and Leotiomycetes form a clade, as do Arthoniomycetes and Dothideomycetes. Eurotiomycetes is sister to Arthoniomycetes+Dothideomycetes, and the three classes may form a more inclusive clade with Lecanoromycetes, Coniocybomycetes, Lichinomycetes, and Xylonomycetes, but the relationships are not unequivocally supported by current data (109, 167). Finally, Geoglossomycetes appears to represent an isolated lineage of Pezizomycotina (135), but genomic data are lacking.

Basidiomycota (Fig. 5)

The phylum Basidiomycota is defined by the synapomorphies of basidium and basidiospore. Basidia are modified terminal hyphal cells that are the site of karyogamy and meiosis. They are typically produced in hymenial tissues such as gills or pores. Basidiospores are, with few exceptions, formed on sterigmata, outgrowths of basidia, and typically contain a single haploid nucleus. Basidiospores can either be forcibly ejected from the sterigma (ballistospores) or passively dispersed (statismospores) by water, wind, or animals. Most Basidiomycota have a filamentous thallus that is compartmentalized by regularly distributed septations. Basidiomycota consists of three subphyla: Pucciniomycotina, Ustilaginomycotina, and Agaricomycotina. Although reconstruction of the earliest nodes of Basidiomycota has been problematic, genomic studies tend to support Ustilaginomycotina (smuts and relatives) and Agaricomycotina (fleshy basidiomycetes) as a monophyletic group that shares an MRCA relative of Pucciniomycotina (e.g., 168). Pucciniomycotina includes yeasts and filamentous taxa, with the best-known species being the plant-pathogenic rusts of Pucciniales. Pucciniomycotina and Ustilaginomycotina were formerly classified in the obsolete class Teliomycetes based on the produc-

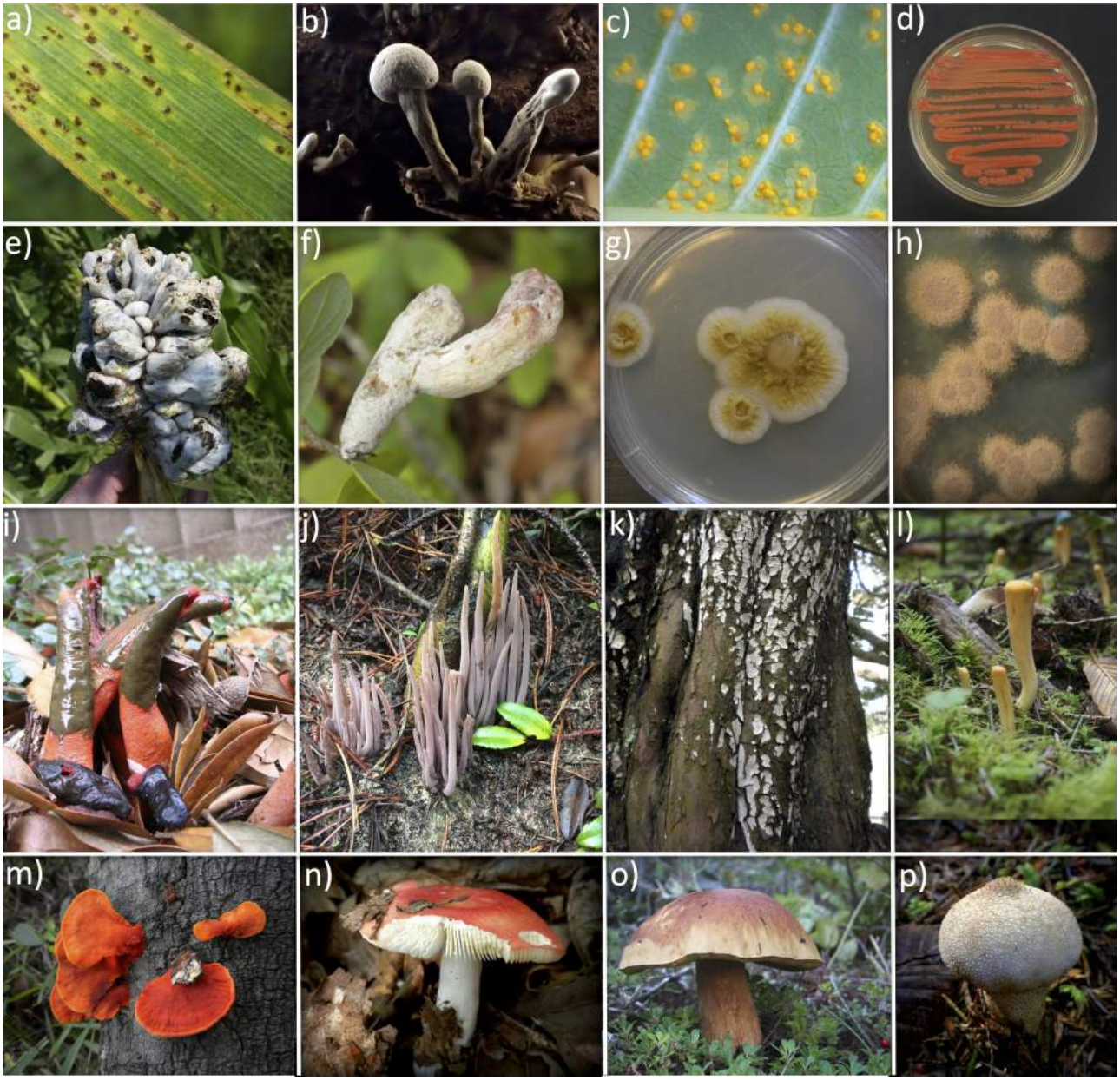

FIGURE 5 Examples of Basidiomycota diversity. Pucciniomycotina: **(a)** uredinia of *Puccinia iridis*; **(b)** fruiting body of *Phleogena faginea*; **(c)** aecia of *Coleosporium*; **(d)** yeast state of *Symmetrospora oryzicola*. Ustilaginomycotina: **(e)** smut galls of *Ustilago maydis*; **(f)** gall of *Exobasidium*; **(g)** culture of *Moniliella* sp. Agaricomycotina: **(h)** culture of *Wallemia*; **(i)** stinkhorn fruiting body of *Phallus* (photo by Nu Nguyen). **(j)** coral fruiting body of *Clavaria*; **(k)** crust fruiting body of *Amylostereum*; **(l)** club fruiting body of *Clavariadelphis*; **(m)** polypore, conk fruiting body of *Pycnoporus*; **(n)** gilled mushroom fruiting body of *Russula*; **(o)** pored mushroom fruiting body of *Boletus*; **(p)** puffball fruiting body of *Lycoperdon*.

tion of teliospores, which germinate to produce basidia (as promycelia) and basidiospores, rather than the production of a hymenium.

Former higher-level classifications of Basidiomycota emphasized the morphology of the basidia and the nature of basidiospore germination. Phragmobasidia were described as basidia with some form of septation, whereas holobasidia lacked septations (169). Heterobasidiomycetes produced basidiospores that could germinate either directly via a germ tube or indirectly via

secondary spore production or repetitive germination. Homobasidiomycetes produced basidiospores that germinated directly through germ tube formation only (170). There is considerable positive correlation between the two systems in that most taxa that produce phragmobasidia are heterobasidiomycetes, and most taxa that produce holobasidia are homobasidiomycetes, but with exceptions. When considered in light of current phylogenetic classifications, heterobasidiomycetes are found in all three subphyla of Basidiomycota, and homobasidiomycetes are predominantly found in Agaricomycotina. Interestingly, the current phylogenetic classification of subphyla is most consistent with septum morphology (171, 172). Pucciniomycotina possess a simple septum. Agaricomycotina possess a dolipore septum characterized by a septum wall that swells near the septal pore and is bounded by a septal pore cap that is derived from the endoplasmic reticulum. Ustilaginomycotina possess a range of septum types from simple pores coupled with membranous pore caps to swollen pore margins that lack a septal pore cap.

One final important trait in Basidiomycota taxonomy that we will emphasize here is the clamp connection. Most basidiomycetes possess a vegetative mycelium that is dikaryotic, meaning that each hyphal compartment contains two nuclei. Clamp connections occur in the dikaryon and function in maintenance of the dikaryotic nuclear state. Clamp connections are short, modified hyphal branches that grow away from the hyphal tip and undergo self-fusion with the main hyphae from which they branched. Clamp connection formation involves synchronized nuclear divisions and septum formation and results in a clamp-like morphology at the septum of the main hypha. Clamp connections are found only in Basidiomycota but are not found in all species or all tissue types (e.g., monokaryon versus dikaryon, vegetative hyphae versus sporocarps, etc.).

Molecular phylogenetic and phylogenomic analyses find greater support for the relationship of Pucciniomycotina as the sister to Ustilaginomycotina and Agaricomycotina than for other arrangements and are consistent with phragmobasidia and repetitive spore germination being symplesiomorophic characters inherited from the MRCA of Basidiomycota. As is also true of Ascomycota, sporocarp formation may have evolved multiple times within Basidiomycota, being rare but scattered throughout Pucciniomycotina and common among most lineages of Agaricomycotina (e.g., jelly fungi, mushrooms, polypores, etc.). The oldest definitive fossil data for Basidiomycota are septate hyphae with clamp connections from the Pennsylvanian (300 to 360 million years ago) that occur associated with gymnosperms and ferns (173, 174). There also exist fossils of mushrooms from the Cretaceous through the Tertiary (reviewed in reference 16).

Pucciniomycotina

One significant outcome of molecular phylogenetics is the discovery of the true phylogenetic, morphological, and ecological diversity of Pucciniomycotina (101, 175). Currently there are 9 classes and over 8,000 species in which yeast and dimorphic growth forms are common. Basidiocarp formation is rare, but fleshy clavarioid or crust-like forms occur in Pucciniomycetes (e.g., *Eocronartium*, *Septobasidium*), and smaller stilboid and pulvinate basidiocarps are formed in some Atractiellomycetes (e.g., *Phleogena*, *Hobsonia*; Fig. 5b), Agaricostilbomycetes (e.g., *Agaricostilbum*), and Microbotryomycetes (e.g., *Pycnopulvinus*), which presumably represents an independent origin of sporocarps from that of Agaricomycotina. Basidia are almost always phragmobasidia, but unicellular forms (holobasidia) are known. The unifying character of the subphylum is the simple septum, which is morphologically similar to the simple septum of Ascomycota that lacks any septal pore cap. The majority of species are plant-associated as pathogens, endophytes, and phylloplane fungi, but there also exist insect pathogens, orchid mycorrhizae, mycoparasites, and freshwater and marine yeasts. Environmental sequencing studies have revealed unknown Pucciniomycotina diversity in anoxic deep sea habitats, Arctic ice, and other extreme environments including niches characterized by extreme osmotic pressures (reviewed in reference 176). Indeed, the modern concept of Pucciniomycotina comprises fungi that occupy a diversity of ecological niches comparable to Agaricomycotina and Pezizomycotina.

The largest class in Pucciniomycotina is Pucciniomycetes, and the best-studied species are plant pathogens in the order Pucciniales, the "rust" fungi, which collectively attack a wide range of plants from grasses to trees (Fig. 5a and 5c). These fungi exhibit the most complex life cycles among the kingdom Fungi, which include multiple (up to five) spore stages that can occur on more than one host. For example, *Puccinia graminis*, rust of wheat and other grasses, produces monokaryotic spermatia and dikaryotic aeciospores on the alternate host *Berberis*. Aeciospores infect the primary host, *Triticum*, resulting in the production of dikaryotic urediniospores and teliospores. Karyogamy and meiosis occur in teliospores, which overwinter in wheat stubble and germinate in the spring to produce basidia and

basidiospores, which reinfect the alternate host. Eradication of the alternate host has been applied as one control method for serious disease caused by rust fungi, the most famous example being the Barberry Eradication Program for stem rust of wheat that was in effect in the United States from 1918 to 1980. This program had a positive impact by reducing the amount of inoculum available for infecting wheat in the United States during its implementation (177). This life cycle of occurring on two hosts is referred to as heteroecious, and the production of all five spore stages is termed macrocyclic. Not all Pucciniales life cycles are this complex, however; some only occur on one host (autoecious), and some have lost certain spore stages (demicyclic and microcyclic). Septobasidiales, also in Pucciniomycetes, represents one of the more divergent ecologies of Pucciniomycotina in that its members parasitize scale insects (100, 178). The fungus parasitizes adults of the insect colony, sterilizing them but not killing them, at least initially. The insect continues to feed on the host plant, providing nutrients to the fungus, which in turn provides shelter to other free-living members of the insect colony.

In addition to Pucciniomycetes, Pucciniomycotina includes eight other classes that exhibit a range of ecologies and morphologies (175). Members of Agaricostilbomycetes were originally described as ascomycetes but are now known to be Pucciniomycotina and include plant saprobes and mycoparasites. Atractiellomycetes display diverse ecological strategies including mycorrhizae of neotropical orchids. Classiculomycetes include hyphal species that associate with leaf litter in freshwater environments but may be capable of mycoparasitism, as well. Cryptomycocolacomycetes are mycoparasites and have been isolated from bark beetle galleries. Cystobasidiomycetes are predominantly mycoparasitic yeasts and include some commonly isolated basidiomycete red yeasts formerly placed in the anamorphic genera *Rhodotorula* and *Sporobolomyces* (Fig. 5d), as well as Cyphobasidiales—a yeast lineage recently identified as comprising a third symbiotic partner in many common lichen species (163).

Microbotryomycetes, the second-largest class, contains primarily plant pathogens and saprobic phylloplane fungi. The plant pathogens include the "anther smuts," *Microbotryum violaceum* and relatives, which were originally classified in Ustilaginomycotina because of the smut-like appearance of the teliospores, a convergent habit of infecting host reproductive tissues. Most orders of the class contain yeast species that are common in environmental sampling and represent a large and poorly characterized diversity (reviewed in reference 179), including the ubiquitous red yeasts of the genera *Rhodotorula*, *Sporobolomyces*, and *Sporidiobolus*. Mixiomycetes are an enigmatic monotypic class that was originally classified in ascomycetes based on the production of a sac-like spore-producing structure, although it appears that these spores are mitotic in origin. *Mixia osmundae* is an intracellular parasite of ferns but has also been detected in environmental sequencing of angiosperms, including bamboo and beach (180). Finally, Tritirachiomycetes are another group that was originally classified as ascomycete molds. No sexual state has been observed for the group. It is isolated from dead plant material and indoor environments and is suspected of being a mycoparasite of ascomycete molds, such as *Penicillium* (181).

Genomic sequencing of Pucciniomycotina is rapidly advancing and includes some of the largest (*Melampsora allii-populina*, 335 Mb; http://genome.jgi.doe.gov/) and some of the smallest (*Mixia*, 13.6 Mb [180]) filamentous fungal genomes sequenced to date. Comparison of pathogenic species of Pucciniomycetes revealed genomic features related to their biotrophic ecology including a large number of SSPs, diminished nitrogen and sulfur assimilation pathways, and expanded families of membrane transporters (182). SSPs along with secreted hydrolytic enzymes and membrane transporters are upregulated *in planta*, consistent with functions in host infection and nutrient acquisition.

Ustilaginomycotina

The subphylum Ustilaginomycotina includes the smut fungi and relatives. The majority of species are plant pathogens, and the term "smut fungi" refers to the black and powdery masses of teliospores produced on the host plant. The smut morphology has been derived convergently in Ustilaginomycotina and Pucciniomycotina (see Microbotryomycetes, Pucciniomycotina) and also occurs in Entorrhizomycetes. This last class contains a small cohort of sedge- and rush-associated smut fungi that cause spore-filled galls to form on host roots. Traditionally placed within Ustilaginomycotina, this group has recently been elevated to phylum status (183), although robust phylogenetic and genomic data are needed to definitively resolve their relationship to other Dikarya. In Entorrhizomycetes, the septal pore is of the dolipore type and lacks a septal pore cap, similar to that of the smut *Tilletia* (Ustilaginomycotina, Exobasidiomycetes [184]).

The cell walls of Ustilaginomycotina are unique in that they contain high proportions of glucose and an absence of xylose, distinguishing them from the rest of

the Basidiomycota (185). Their hyphal septa can be swollen near the septal pore, reminiscent of dolipores, and although they lack a septal pore cap, they may possess a membranous pore cap associated with the septal pore (reviewed in 186). They also possess a characteristic host-parasite interaction zone defined by fungal exocytosis of interaction vesicles (179). The genomes of Ustilaginomycotina are some of the smallest among the Basidiomycota, ranging from ~8 Mb and 4,000 genes in animal-associated yeasts of *Malassezia* (187) to 24 Mb and 8,400 genes in phylloplane species of *Tilletiopsis* (http://genome.jgi.doe.gov/programs/fungi/).

The subphylum includes important plant-pathogenic fungi that occur on angiosperms, especially grasses and sedges, with some exceptions. The majority of Ustilaginomycotina species exhibit a dimorphic life cycle that includes a haploid, saprobic yeast phase and a dikaryotic, filamentous biotrophic or pathogenic phase. Yeast phases can be found on numerous plant substrates, and the filamentous phase is initiated by the mating event. Young basidia become thick-walled and darkly pigmented and develop into teliospores. Teliospores germinate promycelia that may be septate (e.g., phragmobasidia of *Ustilago*) or not (e.g., holobasidia of *Tilletia*), depending on the species. The majority of smut fungi produce basidiospores that are capable of repetitive germination, giving rise to secondary spores called sporidia. Sporidia grow and divide as yeasts until mating between two sporidia reconstitutes the filamentous phase.

Ustilaginomycotina consists of four classes: Exobasidiomycetes, Malasseziomycetes, Moniliellomycetes, and Ustilaginomycetes. Most species of Exobasidiomycetes produce holobasidia, and teliospores may be present or absent according to group. *Exobasidium* species (Exobasidiales) are biotrophic primarily on members of Ericaceae (Fig. 5f); they lack a teliospore and sporulate by producing long holobasidia through stomata or from the epidermis (188). *Tilletia* (Tilletiales) is a particularly notorious plant pathogen responsible for karnal bunt of wheat and diseases of other grasses including barley and rice. It is not known to be dimorphic, and basidiospores often conjugate on the basidium, directly giving rise to filamentous dikaryotic growth. Infections can cause large losses in production, and contamination of grains with *Tilletia* teliospores has had a profound impact on agricultural trade due to plant quarantine regulations (189).

Malasseziomycetes are lipophilic yeasts. They are unusual among Ustilaginomycotina in that they are not plant associated but are found on the skins of mammals including humans. *Malassezia* species are sometimes referred to as dandruff fungi, and the genomes of multiple species have been sequenced with the first, *M. globosa*, sequenced by Procter & Gamble, the manufacturers of Head & Shoulders shampoo (187). Comparative genomics of multiple species have revealed that these fungi are incapable of fatty acid biosynthesis and are dependent upon a lipid-rich diet. To compensate for its inability to synthesize fatty acids, *Malassezia* possesses numerous secreted lipases and other hydrolases for securing host lipids (187, 190). And while *Malassezia* species are only known as asexual yeasts, their genomes do possess the genes necessary for mating. Recently, environmental sampling studies have identified *Malassezia* as a commonly encountered marine fungus, although its function in these ecosystems is unknown (191). Moniliellomycetes also consists of lipophilic yeasts (Fig. 5g) that were traditionally classified with other fungi now placed in Agaricomycotina. However, recent molecular phylogenies suggest it is a distinct class-level taxon in Ustilaginomycotina (192). Most species are known from industrial settings, foods, fats, oils, or substrates with low water activity. The septal pore apparatus of *Moniliella oedocephalis* is of the dolipore type but without pore caps (193), which may further support the current placement within Ustilaginomycotina.

Most Ustilaginomycetes species are dimorphic plant pathogens and usually produce teliospores in the reproductive organs of their host. *Ustilago maydis*, corn smut, is the best-studied species due to corn's importance in agriculture as food and biofuel feedstock. The fungus infects the plant through the developing ovaries but can colonize all parts of the host, resulting in chlorosis, anthocyanin formation, and reduced growth. The production of teliospores occurs in kernels of corn that have been infected by the fungus and coopted for spore production. The result is that a kernel is transformed into a large gasteroid "tumor" or gall filled with teliospores (Fig. 5e). Teliospores are darkly pigmented and produce four-celled phragmobasidia with basidiospores. Basidiospores divide by budding, producing a haploid and saprobic yeast phase. Two yeasts of opposite mating types conjugate to initiate the dikaryotic and pathogenic filamentous phase of the life cycle. Although the pathogens can be quite destructive on grains, some, such as the galls of *U. maydis*, which are a delicacy in Mesoamerica called huitlacoche, are edible and have gained increasing popularity in adventure eating in other parts of the world (194).

Genome analyses of *Ustilago* revealed a small genome of approximately 20 Mb encoding less than 7,000 genes with no known pathogenicity factors (195). Rather,

SSPs, which exist in small gene families, were demonstrated to function in pathogenicity. These SSPs are coregulated and expressed in infected tissue, and SSP mutants exhibited a range of phenotypes from reduced to increased virulence. The discovery of SSPs and demonstration of their role in infection and pathogenicity have since transformed the study of biotrophic fungi including pathogens and beneficial symbionts (196).

Agaricomycotina

Agaricomycotina is currently divided into four classes: Wallemiomycetes, Tremellomycetes, Dacrymycetes, and Agaricomycetes. Wallemiomycetes is sister to the remainder of Agaricomycotina and consists of ascomycete-like molds that do not produce sporocarps and are capable of withstanding conditions of high osmotic stress (Fig. 5h) (168). Tremellomycetes and Dacrymycetes include the majority of fungi with gelatinous sporocarps, or jelly fungi, while Agaricomycetes includes some jelly fungi and the remainder of fleshy, sporocarp-producing species (e.g., mushrooms). Tremellomycetes is sister to Dacrymycetes and Agaricomycetes and contains three orders: Cystofilobasidiales, Filobasidiales, and Tremellales. Cystofilobasidiales and Filobasidiales comprise species that are yeasts or are dimorphic, with sporocarp production being unknown. Tremellales includes many of the well-known jelly fungi (e.g., *Tremella mesenterica*, witches butter) and important human pathogens such as *Cryptococcus*. Species of *Tremella* fruit from wood and produce phragmobasidia with longitudinal septations dividing the basidium into four equal compartments with long, slender sterigmata. Many species in the class are either known or suspected to be mycoparasites and likely play a role in parasitizing wood-inhabiting fungi. *Cryptococcus* is a common inhabitant of soils, plant material (e.g., bark), and bird guano. It grows primarily as yeast in host tissue but is dimorphic, producing holobasidia with long chains of basidiospores. *Cryptococcus neoformans* is an important human pathogen, especially of people with compromised immune systems, but *Cryptococcus gattii* is known to infect immunocompetent people who were otherwise healthy (197). Dacrymycetes includes a single order, Dacrymycetales, and is the sister group to Agaricomycetes. Species of this class produce Y-shaped basidia in gelatinous sporocarps that fruit from wood. Dacrymycetales are characterized as brown rot fungi in which wood decay involves the breakdown of cellulose and hemicellose, but not lignin.

Agaricomycetes comprise the majority of fleshy, sporocarp-producing basidiomycetes. The diversity of fruiting bodies includes mushrooms and boletes, polypores and conks, crusts, coral fungi, puffballs and truffle-like fungi, and stinkhorns. Historically, these fungi were classified as hymenomycetes and gasteromycetes in a system attributed to Elias Fries in what is frequently referred to as the Friesian system (198). Hymenomycetes (hymenial fungi) produce basidia and basidiospores on a basidia-producing tissue called the hymenium that is exposed to the environment and forcibly eject their basidiospores. These fungi include the mushrooms, boletes, corals, crusts, polypores, and conks. Gasteromycetes (stomach fungi) produce basidia in an enclosed region of the sporocarp, the gleba, and do not forcibly eject their basidiospores. Spores may be dispersed by wind and rain, as in the puffballs (Fig. 5p), or by animal mycophagy or phoresis, as in truffles and stinkhorns (Fig. 5i), respectively. We now understand that hymenomycetes and gasteromycetes are artificial taxa and that these forms are intermixed through the phylogeny of Agaricomycetes. Molecular phylogenetic analyses resolve the evolutionary transition in spore dispersal from forcibly discharged to passively discharged basidiospores, with gasteromycetes being derived from hymenomycetes on multiple occasions (199, 200). Moreover, most sporocarp morphologies have been derived multiple times, including the mushroom morphology characterized by a stipe, a gilled or pored hymenium, and a cap (Fig. 5n). The evolutionary plasticity of the sporocarp is likely a result of strong evolutionary selection pressures on production and dispersal of basidiospores.

The modern understanding of Agaricomycetes evolution is the result of numerous studies of molecular phylogenetics (e.g., 201) and evolutionary genomics (e.g., 17, 202), with the outcome being significant revisions to the premolecular taxonomy of the class. Currently, there are 21 orders of Agaricomycetes, with 6 orders classified in the subclass Agaricomycetidae, 4 in Phallomycetidae, and the remaining 11 treated as *incertae sedis*. Auriculariales, Sebacinales, and Cantharellales represent some of the first orders to diverge since the MRCA of Agaricomycetes, but the branching order of these taxa is unresolved. Auriculariales and Sebacinales include species that produce gelatinous sporocarps and, in some species, phragmobasidia, providing further evidence that these traits are ancestral for Agaricomycetes. Cantharellales is best known for prized edible forest mushrooms of *Cantharellus*, but the order includes numerous morphologies including toothed fungi (e.g., *Hydnum*), coral fungi (e.g., *Clavulina*), and crusts (e.g., *Botryosphaeria*). Phallomycetidae is one of the more morphologically diverse clades and contains four orders:

Phallales (the stinkhorns; Fig. 5i), Geastrales (earthstars), Gomphales (coral fungi of *Ramaria* and cantherelloid fungi of *Gomphus*; Fig. 5j and 5l), and Hysterangiales (basidiomycete truffles). Agaricomycetidae contains many of the best-known mushroom-forming taxa, including Agaricales and Boletales, but the modern definition of these orders includes numerous other morphologies. For example, Agaricales also includes bird's nests of *Nidularia*, puffballs of *Lycoperdon* (Fig. 5p), coral fungi of *Clavaria* (Fig. 5j), truffles of *Hydnangium*, and oyster mushrooms of *Pleurotus*. Likewise, in addition to the pored mushrooms of boletes (Fig. 5o), Boletales includes truffles of *Rhizopogon*, earthballs of *Scleroderma*, and resupinate fungi of *Serpula*.

Other orders of Agaricomycetidae include crust-forming fungi (Atheliales and Jaapiales) and clavarioid basidiolichens (Lepidostromatales).

The remaining orders of Agaricomycetes mostly include nongilled fungi that were once accommodated in the concept of Aphyllophorales, an old order name meaning "without gills" (203). These include the polypores (Fig. 5m), conks, and shelf fungi (Gloeophyllales, Hymenochaetales, Polyporales, Stereopsidales, Thelephorales, Trechisporales) and the crust or parchment fungi (Corticiales). Perhaps the most remarkable order of Agaricomycetes is Russulales. This order of fungi contains all known major morphologies of fleshy sporocarps, including mushrooms (*Russula* and *Lactarius*), polypores (*Bondarzewia*), tooth fungi (*Auriscaplium*), crusts (*Aleurodiscus*), coral fungi (*Clavicorona*), and truffles (*Gymnomyces*), and it demonstrates the "tricks" that evolution has played on fungal taxonomists. Interestingly, most of the fungi of Russulales form basidiospores with wall ornamentation that stain blue to black in iodine solution, the positive amyloid reaction. For a more complete review of Agaricomycetes systematics and taxonomy see Hibbett et al. (204).

Agaricomycetes are dominant forest fungi, where they function as ectomycorrhizal symbionts, tree pathogens, and agents of wood and litter decay. Comparative and evolutionary genomics have provided significant insight into the evolution of these ecologies. Saprobic, or plant-decomposing, Agaricomycetes appear to be the ancestral ecology, with symbiotic lifestyles such as ectomycorrhizae being more derived. Wood-decay Agaricomycetes are categorized into two major groups, white rot and brown rot, although comparative genomic analyses of wood-decay species reveal the inadequacy of a simple two-category system (205). White rot fungi are capable of breaking down cellulose, hemicellulose, and lignin at roughly equal rates. Brown rot fungi do not break down lignin to any appreciable degree. The ability to efficiently break down lignin is attributed to major innovations of wood- and lignin-degrading enzymes, the fungal class II peroxidases (PODs), in the common ancestor of Agaricomycetes (17). Phylogenomic analyses support a diversification of PODs in the late Permian, leading to the hypothesis that the rise of wood-decay fungi and the diversification of their enzymatic machinery resulted in dramatic decreases in lignified coal deposits at the end of the Permian (17). Brown rot fungi have been derived multiple times through the loss of fungal PODs and the ability to degrade lignin. Major white rot orders include Auriculariales, Hymenochaetales, Corticiales, Polyporales, Russulales, and Agaricales, while brown rot has evolved independently in Gloeophyllales, Polyporales, and Boletales.

Ectomycorrhizae ("outer" + "fungus" + "root") are fungi that form symbioses with forest trees, especially species of Pinaceae and Fagaceae. They associate with the fine roots of the plant, but they do not penetrate the cells of the plant—thus the term "ecto." Rather, they form a sheath around the cortex cells of the fine roots. The symbiosis is based on an exchange of common goods, with the fungus providing water and mineral nutrients (e.g., phosphorus, nitrogen, etc.) to the plant and the plant providing sugars (e.g., glucose) to the fungus. Ectomycorrhizae have been derived numerous times during the evolution of Agaricomycetes, including within Agaricales, Boletales, Russulales, Hymenochaetales, and Cantharellales, and from both white rot and brown rot ancestors. Comparative genomics have revealed some consistent themes that allow a fungus to adopt an ectomycorrhizal lifestyle (197). First, these fungi lose much of the enzymatic machinery (carbohydrate active enzymes), especially cellulases, associated with the breakdown of plant cell walls. Second, they have evolved SSPs that interact with the plant's host defense system. Together these attributes allow the fungus to colonize plant roots and not be identified by the plant as a hostile intruder. Also, many ectomycorrhizal fungi exhibit significant genome expansions, but not in gene content. These genome expansions are due to an increase in the abundance of transposable elements. The function of these transposable elements is unknown, but it hypothesized that they may promote genomic adaptations.

SUMMARY

Molecular and genomic analyses of the fungal tree of life have shown that numerous morphologies empha-

sized in premolecular classification (e.g., zoospores, zygospores, fruiting body morphology, etc.) are not diagnostic of monophyletic groups. Rather, the taxa that possess these traits experienced more complicated patterns of diversification and are frequently paraphyletic. The first three lineages to diverge since the LUCA of kingdom Fungi comprise mostly zoosporic fungi, Cryptomycota, Blastocladiomycota, and Chytridiomycota. While losses of flagellum are known within the zoosporic clades and presumably in the MRCA of the remaining phyla of nonflagellated fungi, the definitive number and placement of flagellum losses are currently disputed due to insufficient taxon and data sampling (e.g., the genus *Olpidium*). Zygomycete fungi comprise two separate phyla of nonflagellated fungi: Zoopagomycota and Mucoromycota. They display differences in host and substrate associations—Zoopagomycota with animals and fungi, Mucoromycota with plants and plant substrates—and may represent morphologies and lifestyles of the first terrestrial fungi. Ascomycota and Basidiomycota form the Dikarya and possess the most derived traits, representing the apex of morphological complexity among the fungi.

Our understanding of fungal evolution has been significantly influenced by molecular and genome technologies, in both unraveling the aforementioned phylogenetic relationships and illuminating processes of adaptation and diversification. The sequencing of fungal genomes is quickly becoming routine, representing the starting point for an increasing number and types of studies. This is resulting in a rapid increase in the number of species that can be incorporated into genome-scale phylogenies, as evidenced by MycoCosm, with more than 800 fungal genomes (http://genome.jgi.doe.gov/fungi/). Continued taxon sampling should exploit existing resources of biological culture collections to continue populating the fungal tree of life with genome data from unsampled species and lineages. Doing so will not only incorporate the global collecting effort of mycologists across generations, but it will also add significant value to existing isolates, making them more amenable to inclusion in a wider range of research. The next wave of genome sampling must also incorporate a greater diversity of fungi that cannot be maintained in culture. This will require sampling of sporocarps and spores and will in most cases represent metagenomes with resident populations of bacteria and other eukaryotes. While this approach is more computationally intensive, extraction of phylogenetically informative markers is tractable (139). Furthermore, significant progress has been made in grouping sequences by organism to achieve accurate assemblies of the composite of individuals within a metagenome (206, 207), and increased sporocarp sampling will provide more data examples for refinement of algorithms and computational pipelines. Continued advancements in genome-scale phylogenies of fungi are not completely dependent upon sampling alone, however. Advancements in models of evolution are needed to understand conflict among data partitions more accurately and better discern between complicated processes such as incomplete lineage sorting and insufficient phylogenetic signal (6).

All phylogenetic analyses are inherently biased by what has been called the "invisible dimension of fungal diversity" (208). Environmental sampling of ecosystems and niches is consistent with the existence of numerous unknown higher-level lineages (209). While some of these have been brought into culture and expanded our knowledge of sparsely populated lineages (e.g., 25, 111), an urgent need to culture more of these fungi exists, resulting in biological resources necessary for functional analyses. Currently, the incorporation of unknown fungi into phylogenetic analyses is limited to a few loci of limited phylogenetic informativeness that can be obtained through amplicon-based sampling approaches. The holy grail of metagenomic sampling would be the sequencing of, and access to, the complete genomes of all or most of the organisms in a sample. Such data would have considerably more explanatory power within and beyond phylogenetics (e.g., ecological genomics, EvoDevo, etc.).

Finally, a greater need for integration of other types of data (e.g., fossils, ecology, physiology, etc.) with genomic data and genome-scale phylogenies exists. Fossils of fungi are more rarely reported than those of plants and animals, but the number of researchers studying paleomycology is as much of a limiting factor as the fossilization rate of fungi. As more fossils are described, greater effort should be given to integration of these data in phylogenies and the development of relaxed clock methods so that major events in fungal evolution can be incorporated more accurately into other biological and geological events of the Earth's history. Fungal ecology and ecological genomics are rapidly growing, and significant advancements have been made in understanding evolutionary transitions to mycorrhizal symbioses, wood decay, and plant pathogenesis. Systematic integration of metagenomic (e.g., short sequence read libraries, metatranscriptomes, etc.) with genome-derived phylogenies is needed, however, to better understand the distribution of fungi and fungal metabolic traits across ecosystems and ecological niches.

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