12

John W. Taylor
Joseph Spatafora
Kerry O'Donnell
François Lutzoni
Timothy James
David S. Hibbett
David Geiser
Thomas D. Bruns
Meredith Blackwell

The Fungi

Pages 171-194 in:
Cracraft, J. and M. J. Donoghue. 2004.
Assembling the Tree of Life.
Oxford University Press.

The fungi contain possibly as many as 1.5 million species (Hawksworth 1991, 2001), ranging from organisms that are microscopic and unicellular to multicellular colonies that can be as large as the largest animals and plants (Alexopoulos et al. 1996). Phylogenetic analyses of nuclear small subunit (nSSU) ribosomal DNA (rDNA) put fungi and animals as sister clades that diverged 0.9 to 1.6 billion years ago (Wainright et al. 1993, Berbee and Taylor 2001, Heckman et al. 2001). The grouping of fungi and animals as sister taxa is controversial, with some protein-coding genes supporting the association and others not (Wang et al. 1999, Loytynoja and Milinkovitch 2001, Lang et al. 2002). Assuming that fungi and animals are sister taxa, a comparison of basal fungi (Chytridiomycota) with basal animals and associated groups (e.g., choanoflagellates and mesomycetozoa) should shed light on the nature of the last common ancestor of animals and fungi (fig. 12.1). It must have been unicellular and motile, indicating that multicellularity evolved independently in the two clades, and again in the several differently pigmented plant clades (M. Medina, A. C. Collins, J. W. Taylor, J. W. Valentine, J. H. Lips, L. Amaral-Zettler, and M. L. Sogin, unpubl. obs.). Fungi, like animals, are heterotrophs but, unlike animals, fungi live in their food. They do so as unicellular yeasts or as thin, filamentous tubes, termed hyphae (hypha, singular), which absorb simple molecules and export hydrolytic enzymes to make more simple molecules out of complex polymers, such as carbohydrates, lipids, proteins, and nucleic acids. Fungi have been spectacularly successful in the full range of heterotrophic interactions—decomposition, symbiosis, and parasitism. Fungi are well known to decay food stored too long in the refrigerator, wood in homes that have leaky roofs, and even jet fuel in tanks where condensation has accumulated. In nature, apart from fire, almost all biological carbon is recycled by microbes. The hyphae of filamentous fungi do the hard work in cooler climes and wherever invasive action is needed, as in the decay of wood.

Fungi enter into many symbioses, three of the most widespread and enduring are with microbial algae and cyanobacteria as lichens, with plants as mycorrhizae, and again with plants as endophytes. These symbioses are anything but rare. Nearly one-fourth of all described fungi form lichens, and lichens are the last complex life forms seen as one travels to either geographic pole (Brodo et al. 2001). Almost all plant species form mycorrhizae, and there is good fossil and molecular phylogenetic evidence that the first land plants got there with fungi in their rhizomes (Smith and Read 1997). There probably is not a plant that lacks a fungal endophyte, and there is good evidence that the endophytes improve plant fitness by deterring insect and mammalian herbivores and affect plant community structure (Clay 2001). Fungi are not limited to symbioses with autotrophs. Symbioses with animals are also prevalent, with partners ranging from ants and other insects to the gut of many ruminate animals and other herbivores (Blackwell 2000). Many insects may have been able to occupy new habitats due to associations with gut yeasts that provide digestive enzymes (Suh et al. 2003).

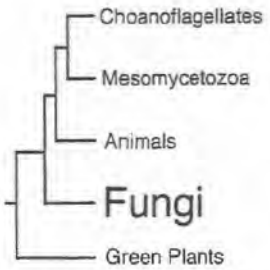

Figure 12.1. Phylogenetic tree showing relationships of the fungi, animals, and green plants based on nSSU rDNA.

Fungi also are well-known parasites. The stories of the spread of plant pathogens such as wheat rust, chestnut blight, and Dutch elm disease are biological and social tragedies, often initiated by intercontinental transport of pathogenic fungi (Agrios 1997). Fungi also plague humans, with athlete's foot and ringworm being the relatively benign end of a spectrum that ends in coccidioidomycosis, histoplasmosis, and other systemic and sometimes fatal diseases (Kwon-Chung and Bennett 1992). In the era of immune suppression, many yeasts and filamentous fungi, heretofore considered not to be serious human pathogens, have been found to cause grave systemic disease, among them *Aspergillus fumigatus* and *Candida albicans*. The close relationship of fungi and animals brings with it a similarity in metabolism that has made it difficult to find pharmaceuticals that attack the fungus and not the host.

Fungi have life histories that are far more interesting than those of most animals. Typically, fungi can mate and use meiosis to make progeny that have recombined genotypes, and they also can reproduce clonally via mitosis to make progeny with identical genotypes (Alexopoulos et al. 1996). Reproduction involves spore formation, with both mitotic and meiotic spores often facilitating long-distance transport and resistance to adverse environmental conditions. Huge numbers of spores can be produced, with the record annual spore release of several trillion being held by giant puffballs and the large fruiting bodies of wood-rotting Basidiomycota. Reproduction often is triggered by exhaustion of the food supply. Before mating, individuals find partners by chemical communication via pheromones, which range from complex organic compounds in Chytridiomycota and Zygomycota to oligopeptides in Ascomycota and Basidiomycota. Spores germinate to produce hyphae or germinate by budding to produce yeasts; in both cases the cell wall is composed of glucose polymers, the best known being chitin, a polymer of N-acetylglucosamine. Most fungi are not self-motile, the exception being the Chytridiomycota, which produce unicellular zoospores that have one typical eukaryotic flagellum inserted posteriorly.

Humans have domesticated yeasts to make bread, beer, wine, and fermentations destined for distillation. They have done the same with a number of filamentous fungal species, with species of *Penicillium* being the best known because of their role in making the camembert and roquefort families of cheese, dry-cured sausage, and the life-saving antibiotic penicillin. Biologists also have exploited several fungi as model organisms for genetics, biochemistry, and molecular biology, among them, *Neurospora crassa*, *Saccharomyces cerevisiae*, and *Schizosaccharomyces pombe*—Nobel Prize winners all.

Within the monophyletic Fungi, four major groups generally are recognized: Chytridiomycota, Zygomycota, Basidiomycota, and Ascomycota (fig. 12.2). Analysis of nSSU rDNA shows the Ascomycota and Basidiomycota to be monophyletic, but the Zygomycota and Chytridiomycota are not easily made into monophyletic groups, and their monophyly, or lack thereof, is controversial (Nagahama et al. 1995). The earliest divergences within Fungi involve certain Chytridiomycota and Zygomycota. The hyphae of these fungi typically lack the regularly spaced, cross walls (septa) typical of Ascomycota and Basidiomycota. In Chytridiomycota and Zygomycota, haploid nuclei are brought together by mating and fuse without delay. One of the clades radiating among the Chytridiomycota and Zygomycota leads to the Glomales + Ascomycota + Basidiomycota clade. Again, the placement of the Glomales on this branch may be controversial (James et al. 2000). Together, the Ascomycota and Basidiomycota form an informal group, the dikaryomycetes, which have regularly spaced cross walls in their hyphae, oligopeptide mating pheromones, and, because of an extended period between mating and nuclear fusion, pairs of genetically dissimilar nuclei in mated hyphae (i.e., a dikaryon). In the following sections, each of these groups is discussed, beginning with the largest and most familiar ones: Ascomycota, Basidiomycota, Zygomycota, and Chytridiomycota. Mycologists study more organisms than are found in the monophyletic Fungi, but inclusion of these organisms is beyond the scope of this chapter; some are covered elsewhere in this volume and are treated in mycology textbooks (Alexopoulos et al. 1996). These "fungal" groups include the water molds (Oomycota, Straminipila), home of the infamous plant pathogen *Phytophthora infestans*, cause of late blight of potato; the cellular slime molds (Dictyosteliomycota), home of the model social microbe *Dictyostelium discoideum*; the plasmodial slime molds (Myxomycota), home of the cell biology model organism *Physarum polycephalum*; and a myriad of other myxomycetes having beautiful sporangia. Conversely, some organisms not presently classified as Fungi may belong there, especially the microsporidia, a group of obligate animal parasites that branch deeply on the eukaryote branch in rDNA trees, but close to, or within, the fungi in some protein gene trees (Keeling et al. 2000, Tanabe et al. 2002).

Ascomycota

The Acomycota, or sac fungi (Gr. *ascus*, sac; *mycetos*, fungi), are the largest of the four major groups of Fungi in terms of number of taxa. With approximately 45,000 sexual and

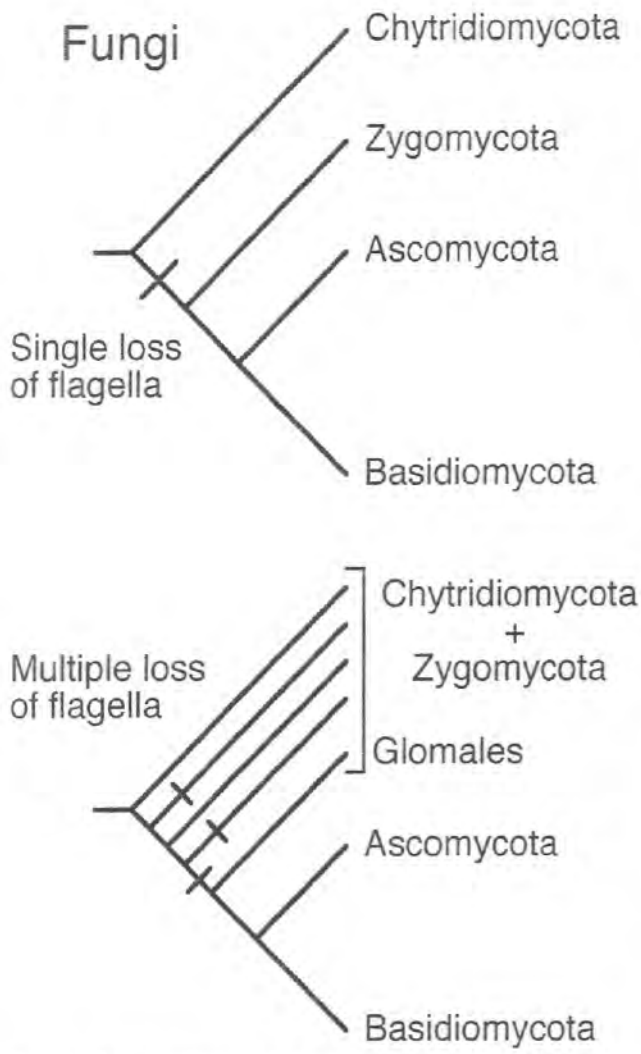

Figure 12.2. Alternative phylogenetic trees showing the relationships among the major groups of fungi. Each branch is monophyletic if flagella have been lost just once in the evolution of fungi, but both Zygomycota and Chytridiomycota are nonmonophyletic if flagella have been lost independently.

asexual species, it accounts for about 65% of all described fungi (Hawksworth et al. 1995, Kirk et al. 2001). This group is characterized by the production of meiospores (ascospores) within sac-shaped cells (asci). It includes more than 98% of the fungi that combine with green algae or cyanobacteria or both to form lichens, as well as the majority of fungi that lack morphological evidence of sexual reproduction (mitosporic fungi). Ascomycota include many well-known fungi that have transformed civilization through food and medicine and that serve as model organisms through which major advancements in science have been made (Taylor et al. 1993). Some examples of these fungi include *Saccharomyces cerevisiae* (the yeast of commerce and foundation of the baking and brewing industries, not to mention molecular genetics), *Penicillium chrysogenum* (producer of the antibiotic penicillin), *Tolypocladium inflatum* (producer of the immunosuppressant drug cyclosporin A, which revolutionized the field of organ transplantation), *Morchella esculenta* (the edible morel), and *Neurospora crassa* (the "one-gene-one-enzyme" organism). There are also many notorious members of Ascomycota that cause disease in humans and in many ecologically and economically important organisms. Some of these examples include *Aspergillus flavus* (producer of aflatoxin, the fungal contaminant of nuts and stored grain that is both a toxin and the most potent known natural carcinogen), *Candida albicans* (cause of thrush, diaper rash, and vaginitis), *Pneumocystis carinii* (cause of a pneumonia in people with compromised immune systems), *Magnaporthe grisea* (cause of rice blast disease), and *Cryphonectria parasitica* (responsible for the demise of 4 billion chestnut trees in the eastern United States; Alexopoulos et al. 1996).

Characteristics

The shared derived character state that defines members of the Ascomycota is the ascus (fig. 12.3). It is within the ascus that nuclear fusion (karyogamy) and meiosis ultimately take place. In the ascus, one round of mitosis typically follows meiosis to produce eight nuclei, and eventually eight ascospores; however, numerous exceptions exist that result in asci containing from one to more than 100 ascospores, depending on the species. Ascospores are formed within the ascus by the enveloping membrane system, a second shared derived character unique to Ascomycota. This double membrane system packages each nucleus with its adjacent cytoplasm and organelles and provides the site for ascospore wall formation. These membranes apparently are derived from the ascus plasma membrane in the majority of filamentous species, and the nuclear membrane in the majority of "true yeasts," and are assumed to be homologous (Wu and Kimbrough 1992, Raju 1992).

Within Ascomycota, two major growth forms exist. Species that form a mycelium consist of filamentous, often branching, hyphae. Hyphae exhibit apical growth and in Ascomycota are compartmentalized by evenly spaced septations that originate by centripetal growth from the cell wall. These septations are relatively simple in morphology and possess a single pore through which cytoplasmic connectivity may exist between hyphal compartments. Numerous examples exist, however, in which the pores become plugged, preventing or at least regulating movement between adjacent hyphal compartments. Hyphae also are the basic "cellular" building blocks for the different types of fungal tissues (e.g., the meiosporangia or fruiting bodies termed ascomata). The second major type of growth form found within Ascomycota is the yeast, a single-celled growth form that multiplies most commonly by budding. Both yeasts and hyphae have cell walls made of varying proportions of chitin and β-glucans (Wessels 1994). It is important to note that neither the hyphal (filamentous) morphology nor the yeast morphology is indicative of phylogenetic relationships. In fact, many spe-

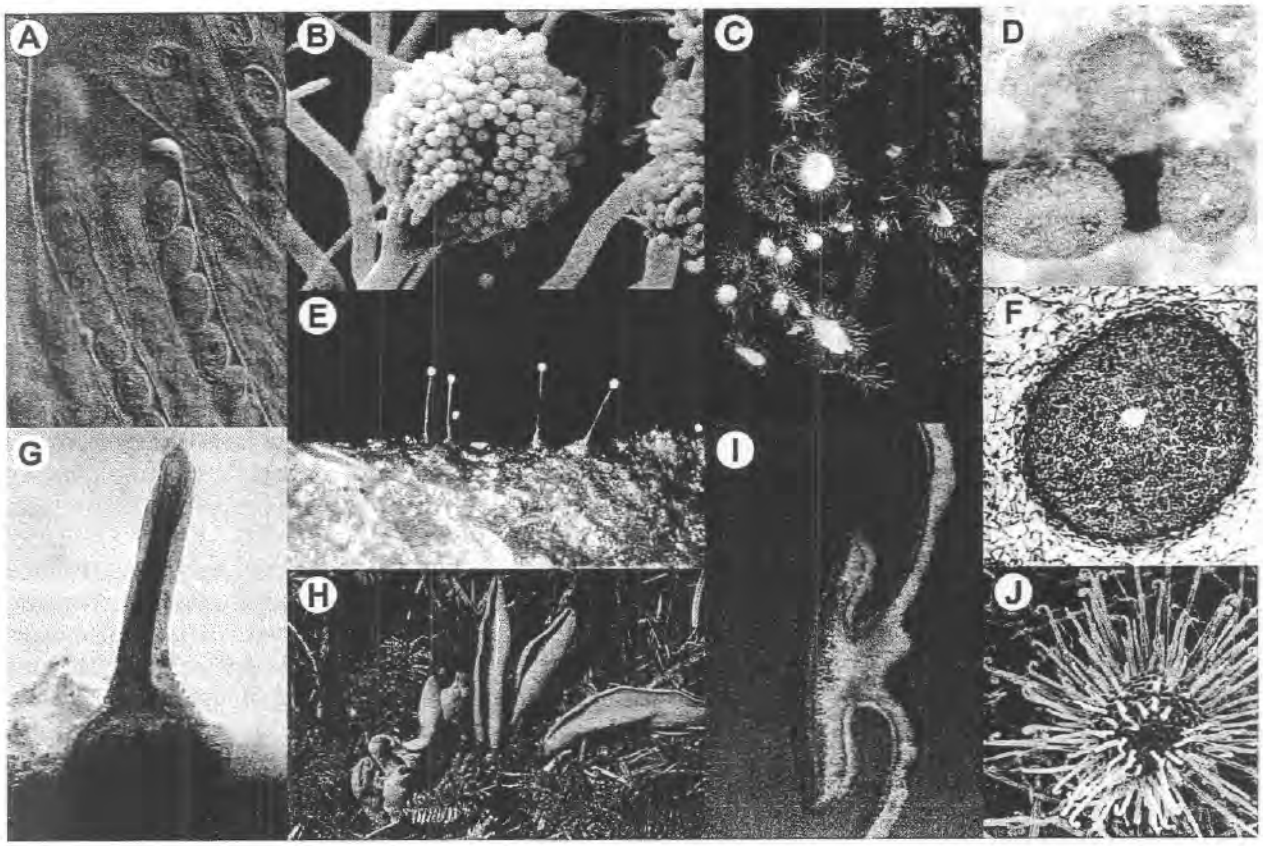

Figure 12.3. Macroscopic and microscopic images of meiotic and mitotic stages of Ascomycota. (A) Young asci and ascospores of *Otidea* (courtesy of J. W. Spatafora). (B) Scanning electron micrograph of conidia and conidiophores of *Aspergillus* (courtesy of C. W. Mims). (C) Lichen thallus of *Usnea* showing apothecia (courtesy of S. Sharnoff). (D) Perithecia of *Nectria* (courtesy of J. W. Spatafora). (E) Dungscape showing perithecial necks of *Sphaeronaemella fimicola* emerging from dung substrate (courtesy of D. Malloch and M. Blackwell). (F) Cross section of cleistothecium of *Talaromyces* with asci dispersed throughout central cavity of cleistothecium (courtesy of T. Volk). (G) *Kathistes calyculata* perithecium with basal asci and terminal, incurved setae (courtesy of D. Malloch and M. Blackwell). (H) Ear-shaped apothecia of *Otidea* (courtesy of W. Colgan III). (I) Cross section of *Lobaria* thallus showing arrangement of green algal layer (courtesy of S. Sharnoff). (J) Scanning electron micrograph of cleistothecium of *Uncinula* with hooked appendages (courtesy of C. W. Mims).

cies of Ascomycota are dimorphic, producing both hyphal and yeast stages at certain points in their life cycle. Regardless of the growth form, all members of Ascomycota are eukaryotes, typically possessing a single haploid nucleus, or several identical haploid nuclei, per hyphal compartment or yeast cell, although examples exist of diploid species of Ascomycota (e.g., *Candida albicans*) or species possessing long-lived diploid stages (e.g., *Saccharomyces cerevisiae*).

Reproduction and Life Cycle

Like much of life apart from the vertebrates, fungi have more than one reproductive option, a phenomenon termed pleomorphy (Sugiyama 1987). This phenomenon is arguably most pronounced among members of Ascomycota. The textbook Ascomycota example can make spores sexually (ascospores or meiospores) and asexually (conidia or mitospores; fig. 12.4), although many species are known to reproduce only by ascospores, and many more are known to reproduce only by conidia. After meiosis, the ascospores take shape inside the ascus with new cell walls synthesized de novo in association with the aforementioned enveloping membrane system. Conidia contain mitotic nuclei, and their cell wall is a modification or extension of a preexisting hyphal or yeast wall. In hyphal Ascomycota, conidia may be produced by specialized hyphae that range from structures scarcely differentiated from vegetative mycelium (*Geotrichum candidum*) to hyphae consisting of elaborate heads of ornamented condida (*Aspergillus niger*; Cole and Kendrick 1981). Classification of Ascomycota is based on characteristics of sexual reproduction (i.e., ascomata and asci), and for this reason species that reproduce only asexually have been problematic in their integration into the classification of Ascomycota. In older systems of classification, all asexual members of

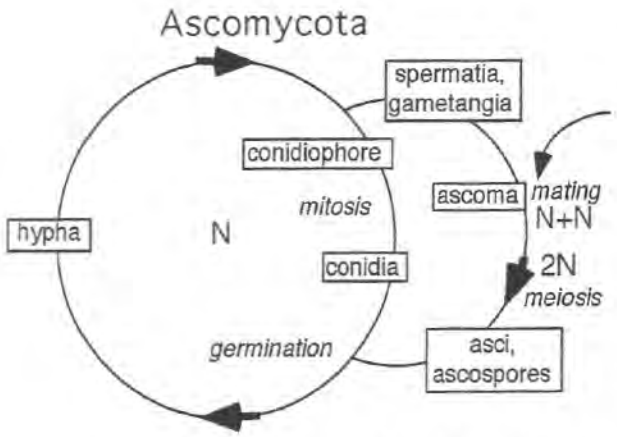

Figure 12.4. Generalized Ascomycota life cycle. The thallus (body) typically is hyphal and haploid. Vegetative hyphae can differentiate into reproductive structures for clonal (conidiophores, conidia) or sexual reproduction (spermatia, gametangia) or both. Sexual reproduction involves mating to produce, in a limited set of hyphae, a short-lived dikaryotic phase (N+N). Typically, the dikaryon is surrounded by a developing haploid ascoma. Karyogamy produces a zygote and is followed immediately by meiosis to produce ascospores. Both ascospores and conidia germinate to produce haploid hyphae.

Ascomycota were placed in the admittedly artificial Deuteromycota. This classification scheme has since been abandoned, and with the advent of molecular phylogenetics, sexual and asexual taxa can be integrated into a common system of classification based on comparison of gene sequences that are ubiquitously distributed across their genomes (Taylor 1995).

Ascospores and conidia are propagules whose main functions are dispersal to and colonization of appropriate substrates or hosts. Ascospores may or may not be forcibly ejected from an ascus. With forcible ejection, turgor pressure builds within the ascus, resulting in the eventual violent eruption of the ascospores from the ascus. In these systems, wind is the primary dispersal agent. Other members of Ascomycota do not forcibly eject their ascospores. In these systems the ascus wall breaks down, passively releasing the ascospores into the environment. This latter mechanism is especially common among Ascomycota that rely on arthropods and water to disperse their ascospores (Ingold 1965). In an analogous manner, conidia also may be produced in a relatively dry mass and be dispersed by wind, or may be produced in wet or sticky heads and be dispersed by water or arthropods (fig. 12.3). In most species, both ascospores and conidia are capable of germination, restoring the dominant haploid mycelial stage (fig. 12.4).

Species of Ascomycota may be either self-fertile (homothallic) or self-sterile (heterothallic), with the latter form requiring a separate and mating-compatible partner for sexual reproduction. Genetic regulation of sex expression and mating is well understood in several model members of Ascomycota, such as budding yeast (*Saccharomyces cerevisiae*), fission yeast (*Schizosaccharomyces pombe*), and *Neurospora crassa*; there are two sexes, and mating is coordinated by the aforementioned oligopeptide pheromones (Marsh 1991, Glass and Lorimer 1991). In yeast species, individual yeast cells function as gametangia and fuse to form the zygote, which eventually becomes the ascus after karyogamy and meiosis. In hyphal species, female gametangia (ascogonia) are produced and are fertilized either by male gametangia (antheridia) or by minute conidia that function as spermatia. In this latter example, cytoplasmic fusion (plasmogamy) may not be immediately followed by karyogamy, leading to a short phase where two genetically different nuclei occupy the same hyphal segment, as mentioned in the introductory remarks. These dikaryotic hyphae may be protected and nourished by differentiated haploid hyphae, which form a fruiting body (the ascoma; plural, ascomata; fig. 12.3). It is within the ascomata that asci eventually are produced from the dikaryotic hyphae originating from sexual reproduction. Asci exhibit a range of morphologies across Ascomycota with unitunicate asci possessing a single functional wall layer and bitunicate asci possessing two functional wall layers that operate much like a "jack-in-the-box" (Luttrell 1951, 1955). Unitunicate asci may be operculate and possess an apical lid (operculum) through which ascospores are released, or they may be inoperculate and release their ascospores through an apical pore or slit. As discussed below, ascus morphology does correlate with phylogeny. Ascospores are released from the asci as described above and germinate to form a new haploid mycelium, which will go on to produce hyphae, conidia, and ascospores that are characteristic of the species.

Nutrition, Symbioses, and Distribution

Like other fungi, members of Ascomycota are heterotrophs and obtain nutrients from dead (saprotrophism) or living (ranging from mutualism through parasitism) organisms (Griffin 1994, Carroll and Wicklow 1992). If water is present, as saprotrophs they can consume almost any carbonaceous substrate, including jet fuel (*Amorphotheca resinae*) and wall paint (*Aureobasidium pullulans*), and play their biggest role in recycling dead plant material. As symbionts, they may form obligate mutualistic associations with photoautotrophs such as algae and cyanobacteria (lichens; Brodo et al. 2001, Lutzoni et al. 2001, Nash 1996; fig. 12.3), plant roots (mycorrhizae; Varma and Hock 1999), and the leaves and stems of plants (endophytes; Arnold et al. 2001, Carroll 1988, 1995). Other Ascomycota form symbiotic associations with an array of arthropods, where they can line beetle galleries and provide nutrition for the developing larvae (*Ceratocystis* and *Ophiostoma*) or inhabit the gut of insects to participate in sterol and nitrogen metabolism (*Symbiotaphrina* and other yeasts and yeastlike symbionts). In return, the insects maintain pure cultures of the fungi and provide for their trans-

port (Benjamin et al. in press, Currie et al. 2003). As parasites and pathogens, ascomycetes account for most of the animal and plant pathogenic fungi, including those mentioned in the introduction to the Ascomycota section and many others, such as *Ophiostoma ulmi*, the Dutch elm disease fungus that is responsible for the demise of elm trees in North America and Europe (Agrios 1997). Numerous species are known from marine and aquatic ecosystems, where they are most frequently encountered on plant debris but may also be parasites of algae and other marine organisms (Kohlmeyer and Kohlmeyer 1979, Spatafora et al. 1998).

Ascomycota can be found on all continents and many genera and species display a cosmopolitan distribution (*Candida albicans* or *Aspergillus flavus*). Others are found on more than one continent (*Ophiostoma ulmi* or *Cryphonectria parasitica*), but many are known from only one narrowly restricted location. For example, the white piedmont truffle (*Tuber magnatum*) is known from only one province of northern Italy.

Relationships of Ascomycota to Other Fungi

The Ascomycota are the sister group to Basidiomycota. This relationship is supported by the aforementioned presence in members of both groups of regularly septate hyphae, and pairs of unfused haploid nuclei present in some stage of the thallus after mating and before nuclear fusion (dikaryons). Further support comes from the apparent homology between structures that coordinate simultaneous mitosis of dikaryotic nuclei (Ascomycota croziers and Basidiomycota clamp connections). Finally, numerous molecular phylogenetic studies all support the hypothesis that Ascomycota and Basidiomycota share a more recent common ancestor with one another than with any other major group (e.g., Zygomycota, Chytridiomycota) in Fungi (e.g., Bruns et al. 1992, Berbee and Taylor 1993, Tehler et al. 2000).

Phylogenetic Relationships within Ascomycota

Comparison of the genes that encode for the nuclear ribosomal RNAs (rRNAs) and the gene family of RNA polymerase, especially RNA polymerase II subunit B, supports a monophyletic Ascomycota that possesses three major subgroups (fig. 12.5; Berbee and Taylor 1993, Bruns et al. 1992, Spatafora 1995, Liu et al. 1999, Lutzoni et al. 2001). In the most recent classification (Eriksson et al. 2003), the three groups are designated subphylum Taphrinomycotina (= class Archiascomycetes), subphylum Saccharomycotina (= class Hemiascomycetes), and subphylum Pezizomycotina (= class Euascomycetes).

Taphrinomycotina are a group recently discovered from comparison of nucleic acid sequences and contains several species previously thought to be Saccharomycotina (Nishida and Sugiyama 1994). Some species, such as the fission yeast, *Schizosaccharomyces pombe*, are unicellular, but others grow as hyphae as well as single cells (e.g., *Taphrina* species). Members of Taphrinomycotina do not produce ascomata with the exception of the genus *Neolecta*. *Neolecta* produces stipitate, club-shaped ascomata and once was classified among the Pezizomycotina. Recent molecular phylogenetic studies of independent gene data sets do not support the placement of *Neolecta* within the Pezizomycotina (Landvik 1996, Landvik et al. 2001). Rather all are consistent with its placement in the Taphrinomycotina, suggesting that the ability to form ascomata arose early in the evolution of Ascomycota. Monophyly of the Taphrinomycotina is not strongly supported by current analyses, however, and it is possible that the genera in question arose independently, possibly during the early radiation of Ascomycota.

Saccharomycotina consist of organisms most biologists recognize as yeasts or "true yeasts" and is home to one of the best-known species of fungi, *Saccharomyces cerevisiae*, better known as the baker's yeast. Although most Saccharomycotina are primarily unicellular, numerous species do make abundant hyphae, but none produce ascoma (Barnett et al. 1990). Phylogenies within the Saccharomycotina are among the most developed in the fungi because the taxon sampling is very dense (Kurtzman and Robnett 2003).

Pezizomycotina contain well more than 90% of the members of Ascomycota. Most species exhibit a dominant hyphal growth form, with almost all of the sexually reproducing forms possessing ascomata. Members of Pezizomycotina fall into two major categories: ascohymenial, which form after the initial sexual fertilization event, and ascolocular, which form before the initial sexual fertilization event. Ascohymenial ascomata may be closed (cleistothecium), open by a narrow orifice (perithecium), or broadly open like a cup (apothecium; see fig. 12.3). They may be less than a millimeter in diameter in the case of perithecia and cleistothecia, or up to 10 cm in diameter in the case of some apothecia. The common names often used to denote groups possessing ascohymenial ascomata include "plectomycetes" for the cleistothecial species, "pyrenomycetes" for the "perithecial" species, and "discomycetes" for the apothecial species. The ascolocular ascomata are referred to as ascostromata, and the common name given to these fungi is the "loculoascomycetes." Most current phylogenetic hypotheses propose that the apothecium (discomycetes in fig. 12.5) is the most primitive ascomatal morphology within the Pezizomycotina (Gernandt et al. 2001, Eriksson et al. 2003) and that the remaining ascomatal morphologies are more derived, in some cases through numerous independent events of convergent and parallel evolution (fig. 12.5, Berbee and Taylor 1992, Spatafora and Blackwell 1994, Suh and Blackwell 1999, Lutzoni et al. 2001). Pezizomycotina contain species of all ecologies, including plant pathogens (e.g., *Pyrenophora tritici-repentis*), animal pathogens (e.g., *Cordyceps militaris*), mycorrhizae (e.g., *Tuber melanosporum*), endophytes (e.g., *Rhytisma acerinum*), and innumerable plant decay fungi. Importantly, Pezizomycotina include more than 98% of fungi that are lichenized. Lichenized fungi are an amazingly suc-

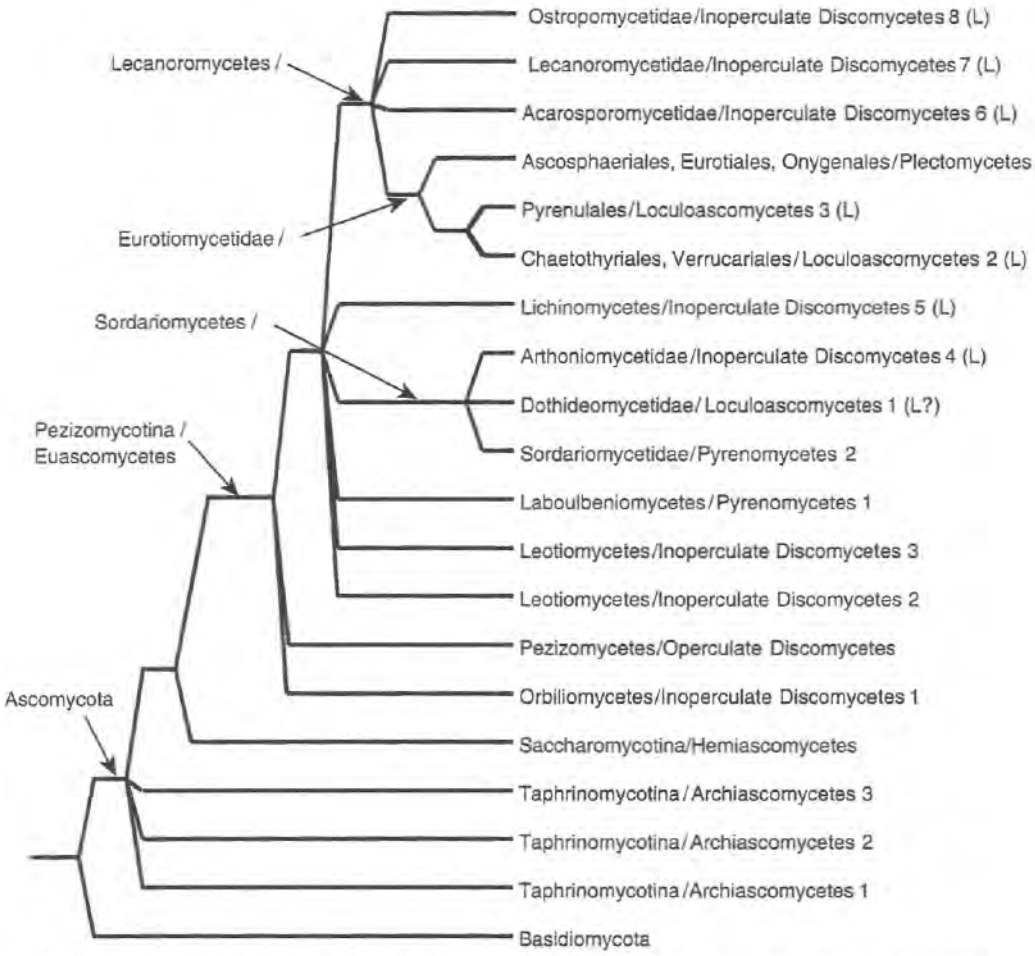

Figure 12.5. Depiction of the current understanding of relationships among members of Ascomycota, sister group to the Basidiomycota (adapted from Suh and Blackwell 1999, Bhattacharya et al. 2000, Platt and Spatafora 2000, Gernandt et al. 2001, Kirk et al. 2001, Lutzoni et al. 2001, McLaughlin et al. 2001, Kauff and Lutzoni 2002). Higher taxa of Eriksson et al. (2003) and "common names" are shown on the tree before and after "/," respectively. Taxa listed at the tips of terminal branches that include lichen-forming species are denoted "(L)." Note the phylogenetic uncertainty among several groups, including Taphrinomycotina (= Archiascomycetes) and within the Pezizomycotina (= Euascomycetes). Common groups such as the "inoperculate discomycetes" (e.g., Orbiliomycetes, Leotiomycetes, Lecanoromycetidae, and Ostropomycetidae) and "loculoascomycetes" (e.g., Chaetothyriales, Dothideomycetidae, Verrucariales, and Pyrenulales) do not denote monophyletic groupings. Most cleistothecial fungi ("plectomycetes") occur in a monophyletic group (Ascospheriales, Eurotiales, Onygenales; Geiser and LoBuglio 2001), whereas others are derived members of other groups such as the Sordariomycetes ("pyrenomycetes"). The vast majority of "pyrenomycetes" are members of Sordariomycetes, with a few unique and poorly known perithecial species among Laboulbeniomycetes (Weir and Blackwell 2001). The Lecanoromycetes, a recently established group of mostly lichen-forming species, include four major subgroups of Ascomycota: Acarosporomycetidae, Eurotiomycetidae, Lecanoromycetidae, and Ostropomycetidae.

cessful group, accounting for approximately 42% of all described species of Ascomycota and probably close to 50% of the known members of Pezizomycotina. Lichens are ecologically important organisms that cover as much as 8% of Earth's land surface, serve as important food sources for animals in harsh arctic environments, and function as pollution indicators in industrialized parts of the world. Lichens were widely believed to have arisen independently multiple times, accounting for the high diversity and mixed occurrence of lichenized and nonlichenized fungal species within Ascomycota (Gargas et al. 1995). A recent comparative phylogenetic study reported that lichens may have evolved earlier than previously believed within Pezizomycotina, and that independent gains of lichenization have occurred one to three times during Ascomycota evolution but have been followed by multiple independent losses of the lichen symbiosis

(Lutzoni et al. 2001). As a consequence, major Ascomycota groups of exclusively non-lichen-forming species, which include the medically important species *Exophiala* and *Penicillium* (e.g., Chaetothyriales and Plectomycetes), would have been derived from lichen-forming ancestors (fig. 12.5).

Although most of the recent molecular phylogenetic efforts have been directed at the Pezizomycotina, interrelationships of the major groups within Pezizomycotina are still poorly understood and not confidently resolved by phylogenetic analyses of the current data. Figure 12.5 presents the most current understanding of the relationships of the major groups within the Pezizomycotina; detailed discussion is available in Alexopoulos et al. (1996), Holst-Jensen et al. (1997), Berbee (1998), Liu et al. (1999), Eriksson et al. (2003), Gernandt et al. (2001), Lutzoni et al. (2001), and Miadlikowska and Lutzoni (in press), to name a few.

Basidiomycota

The Basidiomycota (Gr. *basidion*, small base or pedestal; *mykes*, fungi) contain roughly 22,000 described species, which is approximately 35% of the known species of fungi (Hawksworth et al. 1995, Kirk et al. 2001). Basidiomycetes include some of the most familiar and conspicuous of all fungi, namely, mushrooms and polypores, as well as yeasts (single-celled forms) and other relatively obscure taxa. Some basidiomycetes are economically important edible species, including button mushrooms (*Agaricus bisporus*), shiitake mushrooms (*Lentinula edodes*), and chanterelles (*Cantharellus cibarius*), whereas others are deadly poisonous (e.g., *Amanita phalloides*) or hallucinogenic (*Psilocybe* spp.). The latter play important roles in traditional shamanic cultures of Central America (Wasson 1980).

The overwhelming majority of basidiomycetes are terrestrial, but some species can be found in marine or freshwater habitats, including many basidiomycete yeasts (Fell et al. 2001). Some basidiomycetes have free-living, saprotrophic (decomposer) lifestyles, whereas others live in symbiotic associations with plants, animals, and other fungi. The oldest fossils of the group are hyphae with diagnostic clamp connections from the Pennsylvanian period [~290 million years ago (Mya)], but recent molecular clock estimates suggest that the common ancestor of all modern basidiomycetes lived at least 500 Mya, and maybe 1.0 billion years ago (Dennis 1970, Berbee and Taylor 2001, Heckman et al. 2001).

Tremendous progress has been made in basidiomycete phylogenetics through the use of molecular characters. Three major groups are now recognized, the Urediniomycetes, Ustilaginomycetes, and Hymenomycetes (Swann and Taylor 1995), and the major clades within these groups largely have been delimited (fig. 12.6). Nevertheless, many aspects of the relationships within and among the major groups remain poorly understood.

Characteristics and Life History

The dominant phase of the life cycle in most basidiomycetes is a heterokaryotic mycelium, which is a network of hyphae, in which each cell contains two different types of haploid nuclei resulting from the mating of two monokaryotic (haploid, uninucleate) mycelia (fig. 12.7). Historically, it has been very difficult to determine the longevity and spatial distribution of mycelia, but recently molecular markers have been used to study this phase of the life cycle—with astonishing results. In the "honey mushroom," *Armillaria* (Hymenomycetes), mycelia have been discovered that inhabit continuous patches of forest of many acres. One giant *Armillaria* mycelium in a Michigan forest was estimated to be about 1500 years old, with a mass of around 10,000 kg (Smith et al. 1992). *Armillaria* is a wood-decaying timber pathogen that forages along the forest floor using rootlike rhizomophs. Most other basidiomycetes, especially those that colonize patchy, ephemeral resources (e.g., dung) or that lack rhizomorphs, probably have much more limited mycelia.

Sexually reproducing basidiomycetes produce cells called basidia (from which the group derives its name), in which the two haploid nuclei fuse, immediately undergo meiosis, and give rise to haploid spores (fig. 12.7). Thus, there is usually only a single diploid cell in the entire life cycle. In most species, the spores are discharged from the basidia by a forcible mechanism termed ballistospory that is unique to basidiomycetes. Ballistospory has been secondarily lost in puffballs and their relatives (which produce spores within enclosed fruit bodies), as well as in aquatic species and in most smut fungi. Basidia often are produced in elaborate, multicellular fruiting bodies (the basidioma; plural, basidiomata), although some species produce basidia directly from single-celled yeasts. Fruiting bodies are the most visible stage of the life cycle and encompass an amazing diversity of forms, including mushrooms, puffballs, bracket fungi, false truffles, jelly fungi, and others.

Numerous variations on the basic life cycle described above have evolved in basidiomycetes. In many groups, asexual spores are produced, from either monokaryotic or heterokaryotic hyphae, and some basidiomycetes have no known sexual stage at all (fig. 12.7). Some basidiomycetes are heteromorphic, alternating between a yeast phase and a filamentous phase. The most complex life cycles in basidiomycetes are those of the plant pathogens called rusts (Urediniomycetes), which have multiple spore-producing stages that may be formed on two, unrelated plant hosts.

Ecological Importance

Basidiomycetes play diverse ecological roles, but the decay of wood and other plant tissues may be the single most important process performed by the group. Although other fungi, particularly certain groups in the ascomycetes, can digest cellulose and lignin (the major components of plant

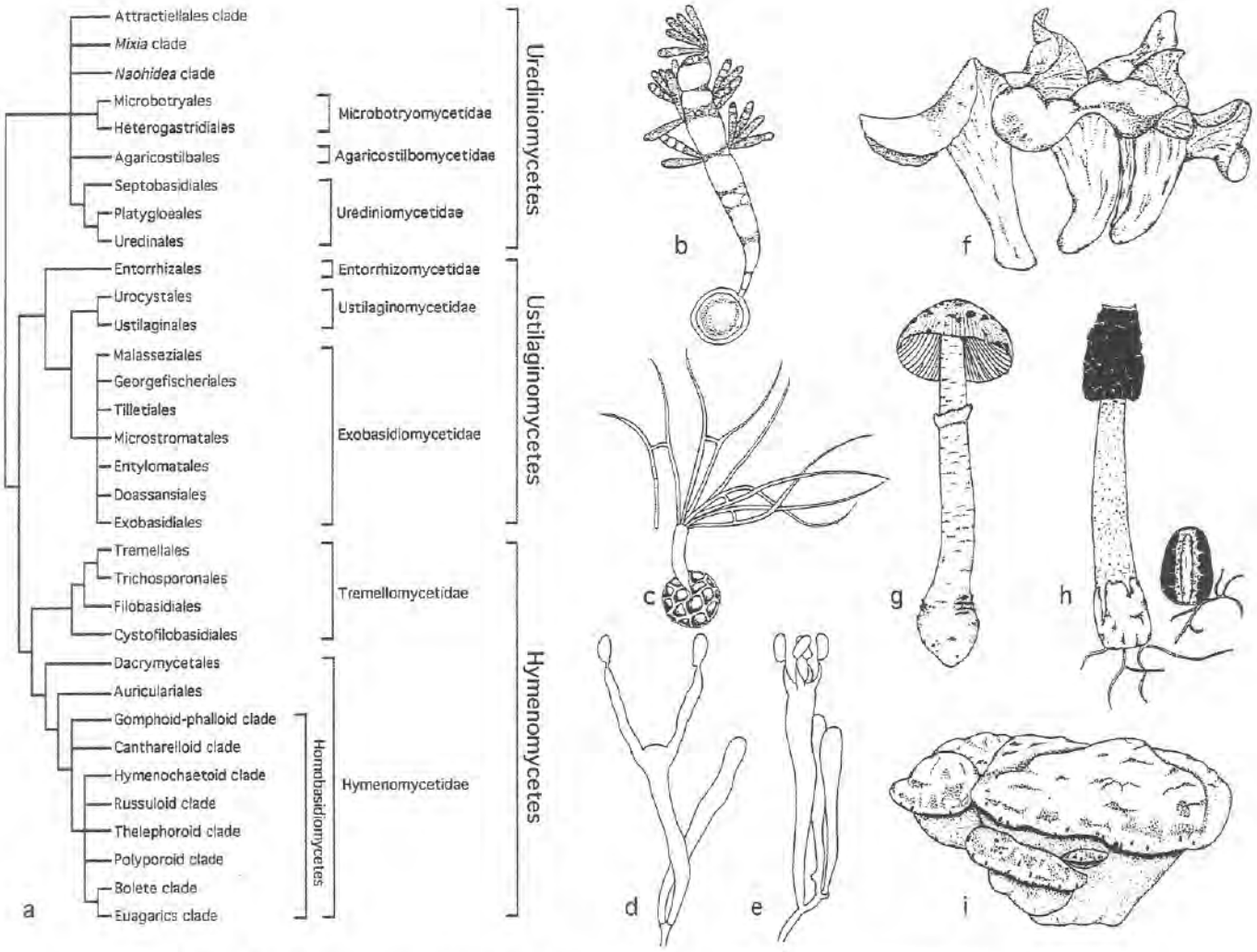

Figure 12.6. Phylogenetic relationships, basidia, and fruiting bodies of basidiomycetes. (A) Phylogenetic relationships of basidiomycetes, based on trees and classifications published by Swann et al. (2001: fig. 1); Swann and Taylor (1995: figs. 1–2); Bauer et al. (2001: figs. 33, 34); Hibbett and Thorn (2001: figs. 1F, 2); Fell et al. (2001: fig. 19B); and Wells and Bandoni (2001). Several minor clades of uncertain placement are not shown. (B–E). Diversity of basidia. (B) *Leucosporidium fellii* (Urediniomycetes; after Fell et al. 2001: fig. 3). (C) *Tilletia caries* (Ustilaginomycetes; after Oberwinkler 1977: fig. 24). (D) *Dacrymyces stillatus* (Hymenomycetes; after Wells and Bandoni 2001: fig. 13). (E) *Cantharellus cibarius* (Hymenomycetes; after Oberwinkler 1977: fig. 28). (F–I) Diversity of fruiting bodies in the Hymenomycetes. (F) *Phlogiotis helvelloides*. (G) *Amanita* species. (H) *Phallus* species (primordium on right). (I) *Inonotus dryadeus*. Drawings by Zheng Wang.

cell walls), this ability is best developed in the Hymenomycetes (Rayner and Boddy 1988, Hibbett and Thorn 2001, Hibbett and Donoghue 2001). With few exceptions, the major timber pathogens and saprotrophic wood decayers are basidiomycetes—this role makes their impact on forest systems substantial from both ecological and management perspectives (Edmonds et al. 2000, Rayner and Boddy 1988). Basidiomycetes use a diverse array of enzymes to digest wood and plant debris in leaf litter and soil (Cullen 1997, Reid 1995). Because of their enzymatic capabilities, basidiomycetes have come under scrutiny for possible applications in bioremediation and biopulping (involved in paper production). A recent project to sequence the genome of the wood-decaying basidiomycete *Phanerochaete chrysosporium* (Hymenomycetes) was motivated, in part, by the potential of its enzymes for degrading recalcitrant substrates.

Ectomycorrhizal symbiosis (an association involving fungal hyphae and the roots of trees) is another major role that is well developed within the basidiomycetes. Ectomycorrhizal basidiomycetes have been shown to scavenge mineral nutrients directly from organic matter, thereby providing their host trees exclusive access to nutrient pools

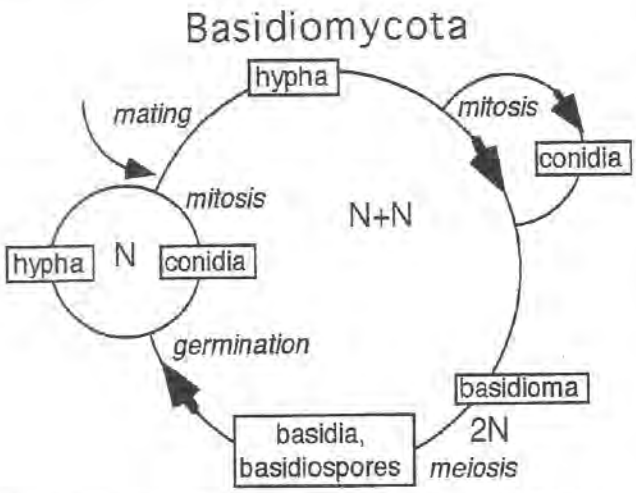

Figure 12.7. Basidiomycota life cycle. The haploid hyphal individual mates early in the life cycle and then persists as a dikaryon, so basidiomycetes found in nature are most often dikaryons. Both haploid and dikaryotic individuals are able to reproduce clonally via conidia in some species. Completion of the sexual cycle involves nuclear fusion in basidia, followed immediately by meiosis to produce basidiospores. Basidia and basidiospores in some groups are produced on basidioma made of dikaryotic hyphae, for example, mushrooms. Conidia and basidiospores germinate to produce hyphae.

that are unavailable to most plants (Haselwandter et al. 1990, Perez-Moreno and Read 2000). In return, ectomycorrhizal basidiomycetes receive sugars from their plant hosts. More than 6000 species of Hymenomycetes are known or suspected to be ectomycorrhizal, as well as a handful of ascomycetes and even zygomycetes (Molina and Trappe 1982, Smith and Read 1997). The plants that are involved in ectomycorrhizal symbioses include pines, oaks, poplars, chestnuts, birches, dipterocarps, eucalypts, and caesalpinoid legumes—that is, the dominant tree species in many temperate and some tropical forest ecosystems. There is strong evidence that ectomycorrhizal basidiomycetes have been derived multiple times from saprotrophic ancestors (Bruns et al. 1998, Gargas et al. 1995), and some analyses suggest that reversions to saprotrophy also have occurred (Hibbett et al. 2000).

Plant parasitism is phylogenetically the most widespread ecological niche within the basidiomycetes. The rusts (Urediniomycetes), with more than 7000 described species, are a particularly successful group. Wheat rusts, coffee rust, and fusiform and blister rust of pines are excellent examples of species that have a major economic impact on agriculture and forestry (Edmonds et al. 2000, Swann et al. 2001). Rusts use angiosperms, gymnosperms, lycopods, and pteridophytes as hosts, whereas closely related taxa parasitize mosses and scale insects. The smuts, which comprise a polyphyletic group composed of members of both the Ustilaginomycetes and Urediniomycetes (fig. 12.6), are important parasites that attack a huge diversity of angiosperms. *Ustilago* and *Tilletia* species (e.g., *U. hordei*, *U. tritici*, *U. maydis*, *T. caries*, and *T. controversa*) that occur on cereal crops cause large agricultural losses. In both the rusts and smuts there is widespread phylogenetic tracking of hosts, but jumps to unrelated hosts are well documented (Bauer et al. 2001, Sjamsuridzal et al. 1999, Vogler and Bruns 1998).

Saprotrophy, ectomycorrhizal symbiosis, and plant parasitism are by no means the only lifestyles represented in basidiomycetes. Basidiomycetes also parasitize other fungi and animals—an example is the human parasite *Filobasidiella neoformans*, causative agent of cryptococcosis. Basidiomycota form symbioses with insects, such as bark beetles and the leaf-cutter ants of the neotropics (Chapela et al. 1994). They also attack and digest bacteria and microscopic invertebrates, apparently as a means by which they acquire additional nitrogen (Barron 1988, Thorn and Barron 1984, Klironomos and Hart 2001). Basidiomycota also enter into lichenized symbioses with photosynthetic algae (Gargas et al. 1995, Lutzoni and Pagel 1997). These examples demonstrate some of the ecological diversity of basidiomycetes but hide the fact that we actually know very little about the basic ecology of the majority of species in this clade. For example, numerous basidiomycete yeasts can be isolated from soil and plant and animal substrates and grown on synthetic media, but little is known about how they function in nature (Fell et al. 2001). Even within the mushroom-forming basidiomycetes, our knowledge is limited usually to where they grow, if that, and the details about what they do and how they manage to successfully establish and compete often remain obscure.

Phylogeny

The traditional taxonomy of basidiomycetes was based largely on the morphology of fruiting bodies and basidia. Since the late 1980s, understanding of the phylogenetic relationships of basidiomycetes has been revolutionized through the use of molecular characters, especially sequences of ribosomal genes (rDNA). Three major clades are recognized now: Urediniomycetes, Ustilaginomycetes, and Hymenomycetes (fig. 12.6; Swann and Taylor 1995). The branching order among these three groups is not well resolved by rDNA data; however, this is one area where additional data from genome studies may help add resolution.

The Urediniomycetes consist of roughly 7400 (34%) of the described species of basidiomycetes (Swann et al. 2001, Hawksworth et al. 1995, Kirk et al. 2001). Members of Urediniomycetes include yeasts and filamentous forms, which function as saprotrophs and pathogens of plants, animals, and fungi. When they occur, fruiting bodies in this group usually are small and inconspicuous (Swann et al. 2001). Monophyly of Urediniomycetes appears to be supported by biochemical features of cell wall composition (cell wall sugars; Prillinger et al. 1993), ultrastructural aspects of the hyphal septa, and

other characters that are visible only with transmission electron microscopy (Swann et al. 1999, 2001).

The Urediniomycetes are divided into six major clades (fig. 12.6). Relationships among the clades, however, are poorly resolved by rDNA data. By far the largest clade in Urediniomycetes is the Urediniomycetidae, which includes more than 7000 species, most of which are the plant pathogenic rusts (Uredinales). One intriguing member of Urediniomycetidae is *Septobasidium*, which parasitizes colonies of living scale insects as they feed on plant sap. Some groups now recognized as Urediniomycetes were formally classified among distantly related groups of fungi. For example, the Microbotryomycetidae include anther smuts that were formerly placed along with true smuts in Ustilaginomycetes (fig. 12.6). Similarly, *Mixia osmundae*, a fern parasite, was once thought to be a member of the ascomycetes, but rDNA data clearly place it in the Urediniomycetes (Nishida et al. 1995). Recognition of the monophyletic Urediniomycetes is a triumph of fungal molecular systematics. Nevertheless, the lack of resolution among the major clades remains a barrier to understanding pathways of morphological and ecological evolution in this group.

The Ustilaginomycetes contain about 1300 (6%) of the described species of basidiomycetes (Bauer et al. 2001, Hawksworth et al. 1995, Kirk et al. 2001) and includes plant parasites, which often are dimorphic with a saprotrophic yeast phase. Smuts of corn, barley, and wheat are economically important members of this group. Corn smut (*Ustilago maydis*) produces a large gall on maize ears that is eaten in the traditional cuisine of Mexico, as cuitlacoche. Monophyly of Ustilaginomycetes has received strong support in analyses of nSSU rDNA sequences (Swann and Taylor 1993) but only moderate support in more densely sampled studies of nuclear large subunit rDNA sequences (Begerow et al. 1997). The composition of cell wall sugars and ultrastructural aspects of host–fungus interaction provide additional characters that support monophyly of the Ustilaginomycetes (Bauer et al. 2001).

Three major clades have been recognized within Ustilaginomycetes: Entorrhizomycetidae, Ustilaginomycetidae, and Exobasidiomycetidae (fig. 12.6). The Exobasidiomycetidae are not strongly supported as monophyletic by rDNA data, however, and the branching order among the three clades is not well resolved. Bauer et al. (2001) have developed a detailed classification of Ustilaginomycetes (fig. 12.6) and have inferred patterns of evolution of morphological characters and host associations.

The Hymenomycetes include about 13,500 (60%) of the described species of basidiomycetes (Swann and Taylor 1993, Hawksworth et al. 1995, Kirk et al. 2001). A unifying character for this group is the production of a "dolipore" septum between cells. Typically, the dolipore septum is flanked by a membrane bound structure termed a parenthesome, the configuration of which is useful for delimiting major groups within Hymenomycetes. Diverse fruiting bodies are formed in Hymenomycetes, including some of the most complex forms that have evolved within the fungi.

The Hymenomycetes consist of seven main clades; six of them (Tremellales, Trichosporonales, Filobasidiales, Cystofilobasidales, Dacrymycetales, and Auriculariales) include many members of the heterobasidiomycetes *sensu* Wells and Bandoni (2001), and the seventh (homobasidiomycetes) includes the better known mushrooms, shelf fungi, and puffballs (fig. 12.6). The heterobasidiomycetes encompass a tremendous range of morphologies, including yeasts and filamentous forms, and a wide range of ecological modes, including saprotrophs and parasites of fungi and animals. Fruiting bodies of heterobasidiomycetes are typically gelatinous and translucent, giving rise to the common name "jelly fungi." Familiar examples include "witches butter" (*Tremella mesenterica*) and the edible wood-ear (*Auricularia auricula-judae*), which is cultivated in Asia.

The homobasidiomycetes include more than 90% of the species in Hymenomycetes, suggesting that this group has undergone an increase in diversification rate relative to heterobasidiomycetes. Homobasidiomycetes include the mushroom-forming fungi, which display an incredible diversity of fruiting body forms. Yeast phases are generally absent from this group. Traditionally, taxonomy of homobasidiomycetes depended on morphological and anatomical characters of fruiting bodies. This group has been sampled intensively by fungal systematists (Bruns et al. 1998, Moncalvo et al. 2002, Hibbett et al. 2000). Although many aspects of morphology-based classifications have been upheld, there have also been major rearrangements, especially concerning the placement of the taxonomically enigmatic gasteromycetes, such as puffballs, false truffles, earthstars, and stinkhorns (Hibbett et al. 1997). Hibbett and Thorn (2001) proposed a classification of the homobasidiomycetes that includes eight major clades (fig. 12.6). Relationships among the clades are generally not well resolved, however, and recent analyses suggest that there are also some additional minor clades of homobasidiomycetes (Hibbett and Binder 2002).

Conclusions

Taxonomy of basidiomycetes has progressed dramatically in recent years, but significant questions remain. Relationships within and among major clades are often unresolved, which limits understanding of the pathways of evolution in basidiomycetes, and their role in the evolution of ecosystems. One major class of questions concerns the causes of the different patterns of apparent species richness observed from clade to clade. For example, why are homobasidiomycetes and rusts so diverse? The diversity seems too great simply to be due to the ease with which large mushrooms are recognized or to the intense economic interest in rusts. Did these two groups diversify in response to some environmental change, such as the rise of angiosperms, or are there intrinsic properties of these groups that contributed to their success?

Zygomycota

Species of the Zygomycota (Gr. *zygos*, marriage pairing; *mykes*, fungi) are remarkable for their morphological and ecological diversity (Hawksworth et al. 1995, Kirk et al. 2001), even though they account for fewer than 2% of all described fungal species. This group includes fast-growing molds responsible for storage rots of fruits, such as peaches and strawberries. Other species can cause life-threatening infections in humans and other animals, especially in immunocompromised or artificially immunosuppressed patients and diabetics (Rinaldi 1989). Most of the approximately 1000 described members of Zygomycota, however, are not encountered by humans and lack common names because of their microscopic size coupled with the fact that approximately half of the species cannot be cultured axenically. Economically and ecologically, the most important zygomycetes are represented by Glomales, whose members are all asexual, obligate symbionts of the great majority of vascular plants (Sanders 1999, Redecker et al. 2000b, Schüßler et al. 2001). This specialized fungus–plant root symbiosis (mycorrhizae; Gr. *mykes*, fungi; *rhiza*, root) functions as an auxiliary root system that is critical for ecosystem function and plant diversity. The mycorrhizal symbiosis is vital for phosphate uptake by plants, especially in nutrient-poor soils. In addition, such fungi are hypothesized to have been instrumental in the colonization of land by the first terrestrial plants (Pirozynski and Malloch 1975, Simon et al. 1993). Molecular clock estimates indicate that Glomales diverged after the divergences among zoosporic fungi (Chytridiomycota), at least 600 Mya and possibly as much as 1.2–1.4 billion years ago (Heckman et al. 2001, Berbee and Taylor 2001). Extant glomalean species are remarkably similar to fossils from the Ordovician period 460 Mya (Redecker et al. 2000a).

Beneficial species within Mucorales are used in the production of the traditional east Asian soybean-based fermented foods sufu (i.e., Chinese cheese) and tempeh. Another species within the Murorales, *Phycomyces blakesleeanus*, is used as a model system for understanding the genetics of phototropism and sensory transduction, in part because it responds to light over the same range as the human eye (Eslava and Alvarez 1996). Species within the Entomophthorales (Gr. *entoma*, insect; *phthora*, destroyer) have enormous potential as natural biological control agents of pest insects.

Characteristics and Life Cycle

Although there are relatively few species of Zygomycota, compared with Ascomycota and Basidiomycota, they exhibit a remarkable diversity of life history strategies and ecological specializations. Zygomycota species function as ecto- and endomycorrhizal symbionts of vascular plants, obligate mycoparasites, entomopathogens, endocommensials of aquatic arthropods, terrestrial saprobes, and endo- or ectoparasites of protozoa, nematodes, and other invertebrates (Benjamin 1979). A generalized life cycle is presented in figure 12.8. Hyphal thalli typically consist of branched or unbranched tubular filaments (fig. 12.9A) that either are predominately nonseptate (i.e., coenocytic: Mucorales, Entomophthorales, Glomales, and some Zoopagales and Endogonales) or are regularly septate (Kickxellales, Dimargaritales, Harpellales, and some Zoopagales). Where known, thalli have cell walls composed of chitin plus chitosan or chitin plus β-glucan (Bartnicki-Garcia 1987). Septa or cross walls are simple partitions in hyphae, except in the Harpellales, Kickxellales, and Dimargaritales, where they are flared with a plugged central pore. Species-specific differences in the mating system determine whether thalli are self-fertile (i.e., homothallic) or self-sterile (i.e., heterothallic, requiring the union of thalli of different mating types). Sexual reproduction, where known, involves the fusion of differentiated (fig. 12.9B) or undifferentiated hyphae followed by the development of a variously enlarged unicellular zygosporangium (fig. 12.9C–E), within which is formed a single zygospore. The zygospore is the only diploid stage in the life cycle and the site of meiosis. Relatively few studies have documented meiosis and zygospore germination, in part because these thick-walled spores require a dormancy period before they germinate to give rise to a haploid mycelium. Although this group derives its name from the sexual stage, phylogenetic studies are needed to assess whether the zygospore is synapomorphic for this group. Zygomycota also are united by the production of asexual nonflagellated mitospores in uni- to multispored sporangia (fig. 12.9F–O). Asexual spores also can be produced as intercalary or terminal modifications of the vegetative mycelium, or very rarely as a yeastlike phase. Mitospores are passively released, except in Entomophthorales, where they frequently are ejected forcibly (fig. 12.9k), and in the coprophilic mucoralean genus *Pilobolus* (Gr. *pileos*, hat; *bolus*, to throw), where the entire sporangium is discharged as far as 2 m toward light.

Although members of the largest order, Mucorales, comprise only one-third of all described Zygomycota taxa, they represent the overwhelming majority of zygomycetous species in axenic culture because they all grow saprobically (O'Donnell 1979). Representatives of the other seven orders account for less than half of all members of Zygomycota in culture, in part because they include obligate parasites (Dimigaritales, Zoopagales, and many Entomophthorales), obligate arthropodphilous symbionts (Harpellales), and ecto- and endomycorrhizal species (Endogonales and Glomales, respectively). Except for one mycoparasitic species, all Kickxellales species can be cultivated axenically. Mycoparasitic species of Dimargaritales and Zoopagales typically are cultured on their mucoralean hosts, but some of these species can be grown axenically on specialized media (Benjamin 1979). Specific culture collections have been established for Entomophthorales (Humber and Hansen 2003) and Harpellales (Lichtwardt et al. 2001). In addition, several phylogenetically diverse collections of the

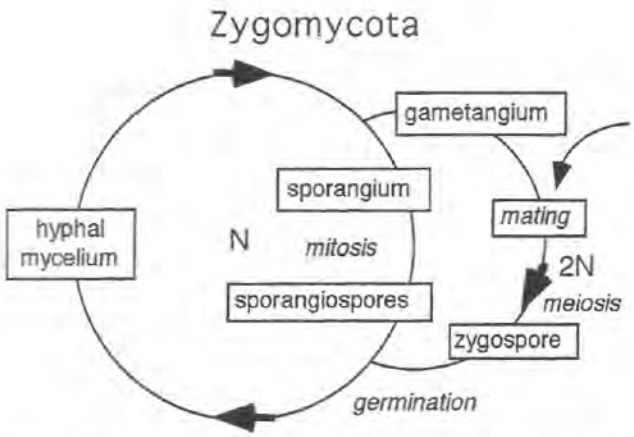

Figure 12.8. Generalized Zygomycota life cycle. Individuals in nature typically are hyphal and haploid. Vegetative hyphae can differentiate into reproductive structures for clonal (sporangia, sporangiospores) or sexual reproduction (gametangia). Sexual reproduction involves mating by gametangial fusion to produce a diploid zygote. In almost all cases, there is no fruiting body surrounding the zygospores. Both mature zygospores and conidia germinate to produce haploid hyphae. In the case of zygospores, the germinating hypha immediately differentiates to make a sporangium and sporangiospores.

obligately mycorrhizal Glomales are available (http://invam.caf.wvu.edu, http://res2.agr.ca/ecorc/ginco-can/ and http://www.ukc.ac.uk/bio/beg/). In these collections, Glomales species are maintained *in vivo* in host plants, stored as dried inoculum, or kept as cryogenically preserved material, or accessioned by all three methods.

Phylogenetic Relationships and Taxonomic Implications

Zygomycota appear to be non-monophyletic in most SSU rRNA and some β-tubulin gene analyses. However, the monophyly of this group has not been tested fully through analyses of the available molecular phylogenetic data. These analyses are based primarily on SSU rRNA (Bruns et al. 1992, Gehrig et al. 1996, James et al. 2000, Jensen et al. 1998, Nagahama et al. 1995, Schüßler et al. 2001, Tanabe et al. 2000), β-tubulin (Keeling et al. 2000) and several protein-coding genes within the mitochondrial genome (Forget et al. 2002, Lang 2001). Interestingly, Zygomycota may be monophyletic, if the putative long-branch taxon *Basidiobolus ranarum* (Entomophthorales), which clusters with Chytridiomycota in unconstrained SSU rRNA analyses, is excluded from the analysis [see James et al. (2000) for more information on *Basidiobolus*; see the section on Chytridiomycota below).

Relationships among orders of Zygomycota are poorly resolved by SSU rRNA phylogenies, except for a Harpellales + Kickxellales + *Spiromyces* clade (Gottlieb and Lichtwardt 2001, O'Donnell et al. 1998), with Zoopagales as a putative sister group (Tanabe et al. 2000). Overall, the available SSU data suggest that the orders as presently circumscribed, based on morphological apomorphies, nutritional mode, and ecological specialization, are monophyletic except for Mortierellaceae, which may not form a monophyletic group with the Mucorales (Gehrig et al. 1996). Three orders of Zygomycota described recently (Cavalier-Smith 1998) are not accepted here, however, because Geosiphonales appears to be nested within Glomales, and too few data are available to assess the phylogenetic validity Mortierellales and Basidiobolales. Also, a new group, Glomeromycota, proposed to accommodate Glomales *sensu* Schwarzott et al. (2001), is based primarily on SSU rRNA data. It should be considered provisional until more robust molecular phylogenetic data become available.

Recent molecular phylogenies have advanced our knowledge of Zygomycota by providing novel hypotheses of evolutionary relationships within Glomales (Simon et al. 1993, Gehrig et al. 1996, Redecker et al. 2000b, Schüßler et al. 2001, Schwarzott et al. 2001), Harpellales and Kickxellales (Gottlieb and Lichtwardt 2001, O'Donnell et al. 1998), Entomophthorales (Jensen et al. 1998), Mucorales (O'Donnell et al. 2001), and Dimargaritales and Zoopagales (Tanabe et al. 2000). Two classes have been recognized in all recent taxonomic schemes for Zygomycota (Benny 2001, Benny et al. 2001): Trichomycetes (Gr. *thrix*, hair; *mykos*, fungi), represented by four arthropodophilous orders, Amoebidiales, Harpellales, Eccrinales and Ascellariales (Lichtwardt 1986); and Zygomycetes. However, polyphyletic Trichomycetes is not accepted here. Molecular phylogenetic analyses based on SSU rRNA indicate members of Amoebidiales are protists (Ustinova et al. 2000, Benny and O'Donnell 2000), as long suspected because their cell walls lack chitin and they produce amoeboid cells, which otherwise are unknown in Fungi (although some zoospores of Chytridiomycota can exhibit amoeboid movement). Phylogenetic evidence from SSU rRNA data also has identified Harpellales as a sister group to a *Spiromyces* + Kickxellales clade or to *Spiromyces* within Zygomycetes (Gottlieb and Lichtwardt 2001, James et al. 2000, O'Donnell et al. 1998). Lastly, Eccrinales and Asellariales are treated as *incertae sedis* until their phylogenetic relationships are resolved.

Chytridiomycota

Chytridiomycota are a relatively poorly known group at the base of the fungal tree, accounting for 1% or 2% of described fungal species. Chytridiomycetes, or chytrids, as they commonly are known, are microscopic and have a simple morphology. The distinguishing feature of the group is reproduction through a motile zoospore. The chytridiomycete zoospore typically possesses a single, smooth flagellum that is inserted on the cell posterior to the direction of motility. The chytridiomycetes have been variously classified through the years with other fungi and protists; as recently as 1990 Chytridiomycota were placed in Protoctista (Barr 1990). Because they produce zoospores, chytrids are generally thought to be aquatic fungi.

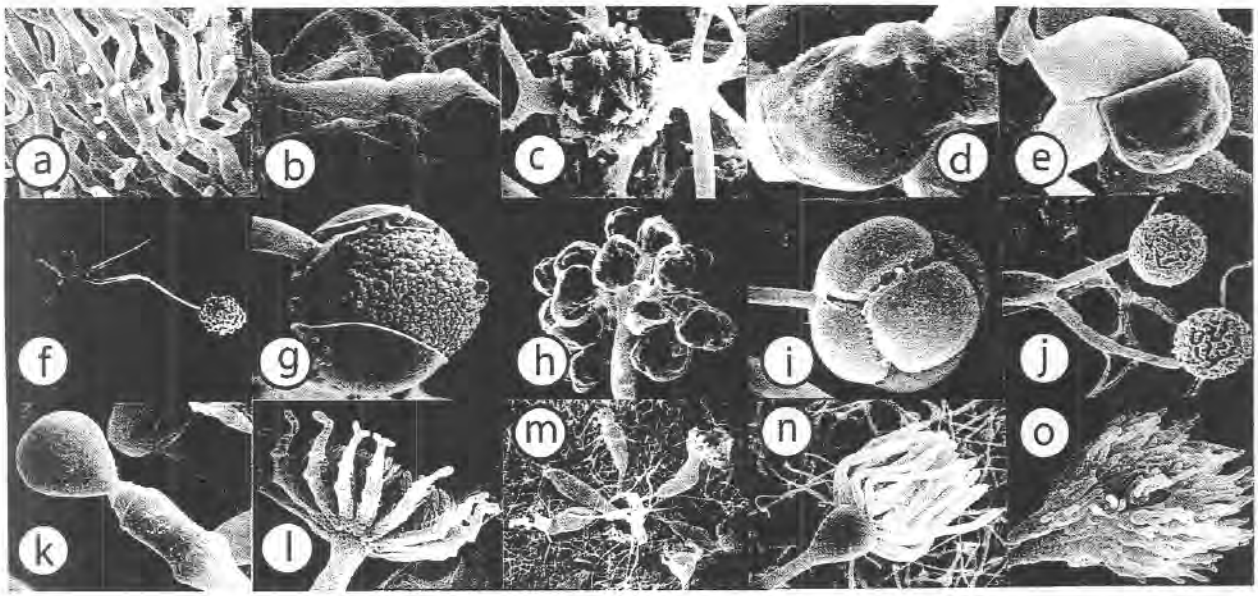

Figure 12.9. Scanning electron micrographs of Zygomycota. (A) Coenocytic mycelium with aerial hyphae beginning to form. (B–E) Sexual reproduction. (B) Gametangial fusion. (C–E) Zygosporangia. (F–O) Asexual reproduction. (F) Aerial, terminal multispored sporangium with basal rhizoids. (G) Multispored sporangium. (H and I) Few-spored sporangia. (J–L) Unispored sporangia. (M) Vesiculate mycoparasite growing on mucoraceous host. (N) Terminal fertile vesicle of mycoparasite. (O) Terminal fertile branch of a mycoparasite with two-spored sporangia.

This characterization is inaccurate, because they readily are isolated from soil. Originally described in the 19th century as curious "asterospheres" in living algae, these fungi have a strong habitat association as parasites and saprophytes on algae (Sparrow 1960). Chytrids, however, also play an important role in the decomposition of recalcitrant substrates, such as chitin, keratin, pollen, insect exuviae, plant debris, and so forth (Powell 1993). As a group, chytrids are ubiquitous in lakes, ponds, and soil. Many can be cultured, and the current study of chytrids generally involves observations of species in pure culture, whereas past descriptions focused on "gross culture" or their study on freshly collected substrates. Chytrids easily can be isolated from environmental samples by baiting with appropriate substrates, for example, pollen, cellophane, purified shrimp exoskeletons, and snake skin (Barr 1987).

The chytridiomycetes may be regarded as the economically least important major group of fungi, but there are several notable exceptions. Neocallimastigales are a clade of chytrids whose members are found in the rumen and hindgut of mammalian herbivores, where they aid in the digestion of plant fibers (Orpin 1988). Other economically important chytrids are the generalist plant pathogens *Synchytrium* and *Physoderma*. Species in both genera cause agricultural diseases in tropical climes, and *Synchytrium endobioticum* causes plant disease in the temperate zone. This parasite causes a malformation of potato tubers known as black wart. As recently as 2000, it was responsible for a one-year total quarantine on the importation of potatoes from Prince Edward Island into the United States, resulting in a loss of at least $30 million to Canadian farmers. Finally, chytrids are parasites also on metazoans, primarily on soil invertebrates, such as nematodes and tardigrades. A notable exception is the vertebrate pathogen *Batrachochytrium dendrobatidis*, which infects frogs and has been associated with the recent global trend of amphibian declines (Berger et al. 1998, Longcore et al. 1999). If *Basidiobolus ranarum* truly is a chytrid (see below), then this amphibian and sometimes human pathogen would join *B. dendrobatidis* as a chytrid pathogen of vertebrates.

Taxonomy

Chytridiomycota consist of five orders, containing approximately 120 genera and 1000 species (Longcore 1996). Blastocladiales include *Allomyces macrogynus*, well known for studies on its cytology, genetics, and physiology, and *Coelomomyces stegomyiae*, a parasite of mosquito larvae. Fungi in this clade are distinguished by zoospores with a prominent "nuclear cap" of ribosomes. Monoblepharidales embrace only five genera; these aquatic chytrids are rarely seen but can be collected on decaying plant material such as fruits and twigs. Monoblepharids are distinguished by oogamous sexual reproduction (i.e., the female gamete is not motile and is larger than the uniflagellate male gamete) and vacuolate cells. Members of Spizellomycetales are ubiquitous in soil; one distinguishing feature is the amoeboid movement of zoospores during swim-

ming (Barr 2001). Neocallimastigales are reserved for chytrids that inhabit anaerobic, rumen, and hindgut environments. These fungi either are uniflagellate or possess multiple flagella. The final and largest order, Chytridiales (~80 genera), contains a diversity of morphological forms. Most of the algal parasites are found in this clade.

Morphology

Chytridiomycete classification, traditionally, has been based on characteristics of vegetative growth and reproductive structures. The primary reproductive structure is the sporangium, a saclike structure whose contents are cleaved internally into zoospores (fig. 12.10A,B). Sporangia generally are subtended by a system of rhizoids that penetrate the substrate and facilitate anchoring and nutrient absorption. In some chytrids, the rhizoid system develops into an indeterminate, interconnected group of filaments, termed a rhizomycelium. Numerous sporangia can be produced from a rhizomycelium, which typically is coenocytic and lacks true septa. At maturity, zoospores are released from sporangia either through a small rounded opening (papillus) or a discharge tube. In some chytrids, the presence of a lidlike cover at the site of zoospore release can be seen clearly. This structure, the operculum, played an important role in previous classifications of chytrids (fig. 12.10B; Sparrow 1960, Karling 1977). A final, distinguishing character of many chytrids is the production of a resting spore. These thick-walled spores are desiccation resistant and can germinate into a sporangium after many years of dormancy. Although sexual reproduction generally results in the production of a resting spore, these spores also are produced asexually.

Life Cycle

Sexual reproduction has been observed in very few chytrids, but the variety of described mating systems is excitingly varied. Different modes of reproduction include the fusion of zoospores, gametangia, or rhizoids with subsequent transformation of the zygote into a resting spore (wherein meiosis is believed to occur; Doggett and Porter 1996). Oogamous reproduction occurs in Monoblepharidales, as mentioned above. In some species of Blastocladiales, an alternation of generations occurs between diploid sporophytes and haploid gametophytes. *Allomyces* species are hermaphoditic in that both male and female gametangia are produced on the same thallus. Sexual reproduction has been observed neither in Spizellomycetales nor in Neocallimastigales (Barr 2001). A representative Chytridiales life cycle is shown in figure 12.11.

Ultrastructure

Most chytrids have a simple and variable body plan that presents few characters on which to base a phylogentically meaningful taxonomy. Consequently, their ultrastructure as revealed by the transmission electron microscope is important in classification. Useful characters have been discovered in the zoospore (Lange and Olsen 1979); this special spore has proven to be exceptionally informative because of its internal complexity and conserved features (fig. 12.12). The zoospore is bounded by a membrane but lacks a cell wall. The zoospore of most chytrids contains a nucleus associated with an electron dense microbody and one to several lipid globules (fig. 12.12). The arrangement of these organelles is called the microbody–lipid globule complex and was used to group chytridiomycete zoospores

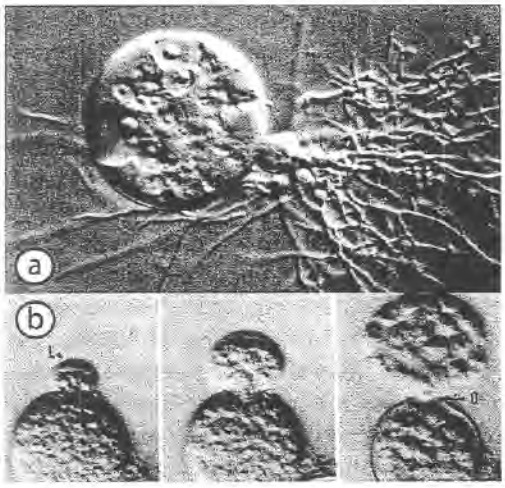

Figure 12.10. (A) Light micrograph of a developing sporangium with rhizoids of *Chytriomyces hyalinus*. (B) Light micrographs of zoospore discharge in *Chytriomyces hyalinus* showing an operculum (O) and a lenticular, expanding net of fibers (L) that constrains the zoospores for a brief period before they mature and swim away. From Taylor and Fuller (1981).

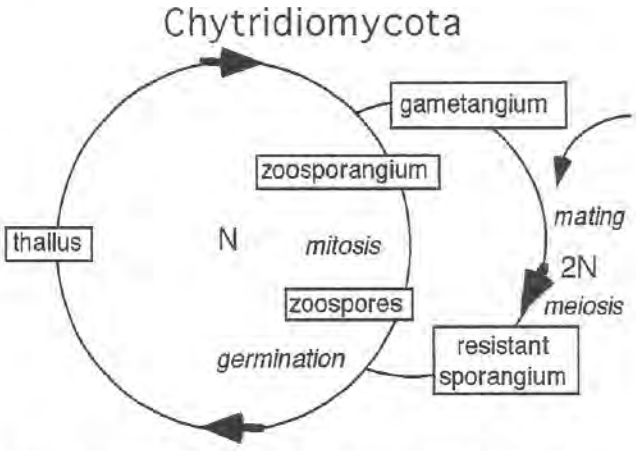

Figure 12.11. Generalized Chytridiomycota life cycle. The haploid thallus can differentiate to produce a zoosporangium with clonal zoospores, or to mate and produce a resistant sporangium. The resistant sporangium may germinate to release zoospores. Upon finding a suitable substrate, zoospores form cysts and the cysts germinate to produce a new thallus.

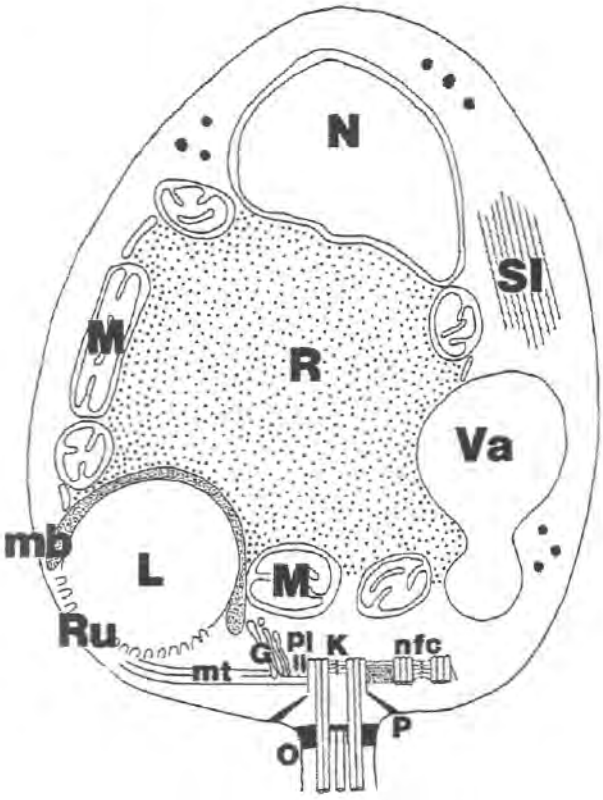

Figure 12.12. Ultrastructure of a typical Chytridiales zoospore as exemplified by *Podochytrium dentatum*. G, Golgi apparatus; K, functional kinetosome at the base of the flagellum; L, lipid globule; M, mitochondrion; mb, microbody; mt, microtubules; N, nucleus; nfc, second (nonfunctional) kinetosome; O, transition-zone plug; P, prop; pl, plates; R, ribosomes; Ru, rumposome; SI, striated inclusion; Va, vacuole. From Longcore (1992).

into broad taxonomic categories (Powell 1978). Another important feature of the zoospore is the rumposome, a fenestrated membrane located near the posterior portion of the zoospore adjacent to the spore membrane (Fuller and Reichle 1968). This organelle has been observed only in members of Chytridiales and Monoblepharidales. More recently, emphasis has been placed on the fine details of the flagellar apparatus (Barr 1990, 2001, James et al. 2000). Important characters include the connection of the nonflagellated centriole to the kinetosome (base of the flagellum) and the arrangement of microtubules and other kinetosomal roots. Zoospore ultrastructure currently is the only phenotypic means of accurately classifying chytrids into orders and even genera (Barr 1980, 2001).

Phylogenetic Relationships

Although the chytridiomycetes were recently classified in the Protoctista (Barr 1990), the link between Chytridiomycota and other members of Fungi already had been suggested by the presence of chitinous cell walls, use of glycogen as a storage molecule, and presence of flattened mitochondrial cristae (Cavalier-Smith 1987, Powell 1993). Early phylogenies based on nSSU rDNA confirmed that Chytridiomycota are part of a monophyletic Fungi and are basal within Fungi (Förster et al. 1990, Dore and Stahl 1991, Bowman et al. 1992). The basal position of Chytridiomycota in Fungi suggests that the common ancestor of all fungi possessed motile zoospores. Therefore, the retention of a zoospore stage by the chytrids is considered a pleisiomorphy (ancestral character), which makes tenuous the unification and classification of chytrids based on the presence of a zoospore, because multiple independent losses of the flagellum may have occurred. For this reason, it is possible that Chytridiomycota is not a monophyletic group.

At present, few molecular phylogenetic data are available for the chytrids. Relationships of Chytridiomycota to other fungi have been examined, using primarily the SSU rRNA gene (Li and Heath 1992, Bruns et al. 1992, Nagahama et al. 1995, Jensen et al. 1998, James et al. 2000, Tanabe et al. 2000). These data are unclear as to whether the chytrids are monophyletic, because Blastocladiales typically groups with Zygomycota, rendering Chytridiomycota paraphyletic. In addition, placement of the putative zygomycete *Basidiobolus ranarum* within Chytridiomycota in SSU rRNA phylogenies has raised the possibility that some zygomycete orders may be chytrids that have experienced independent losses of the flagellum (Nagahama et al. 1995, Jensen et al. 1998). In support of the multiple independent losses of flagella is the observation that *Basidiobolus* species, which lack flagella, harbor an organelle resembling the centriole-like kinetosome found at the cellular end of flagella in Chytridioimycota; no such organelle is found in Zygomycota (McKerracher and Heath 1985). Confusing the picture is the placement of *B. ranarum* in Chytridiales by nSSU rDNA analyses but in Zygomycota by using β-tubulin analyses (Keeling et al. 2000). One possible explanation is that tubulin molecules evolve in similar ways when the constraint of flagellar function is lost, as might have occurred in *B. ranarum* and Zygomycota. The resolution of the possible non-monoplyly of Chytridiomycota awaits further sampling of genes and taxa.

Only one molecular phylogenetic study has heavily sampled taxa within Chytridiomycota (James et al. 2000). The authors of this study concluded that zoospore ultrastructure was concordant with the SSU rRNA phylogeny and that the five orders of chytrids seem to be monophyletic, with the exception of the largest order, Chytridiales. Within Chytridiales, well-supported clades were found, and these were consistent with groupings based on zoospore ultrastructure. However, relationships among clades of Chytridiales as well as among the orders were unresolved. Molecular phylogenies also confirmed the suspicion that chytrid gross morphology is of little use in classification. Indeed, pure culture studies have shown plasticity of devel-

opmental characters previously thought to be important in chytrid classification (Roane and Paterson 1974, Powell and Koch 1977). In contrast, zoospore ultrastructure has proven to be quite informative, and further investigation of these characters is warranted.

Studies of other gene regions also have shed some light on phylogenetic relationships of the chytridiomycetes. As mentioned above, analyses of β-tubulin gene sequences conflict with nSSU rDNA analyses over the placement of *Basidiobolus* (Keeling et al. 2000). Unfortunately, β-tubulin sequences show minimal variation among chytrids and provide little resolution of relationships among orders, making it imperative to examine other protein-coding genes to understand relationships of Chytridiomycota and Zygomycota. One promising development is the effort of the Fungal Mitochondrial Genome Project, which has sequenced the entire mitochondrial genome of several chytrids (Paquin et al. 1997, Forget et al. 2002, Bullerwell et al. 2003). Their analyses with concatenated mitochondrial proteins suggest a Spizellomycetales + Chytridiales clade, with Monoblepharidales as a sister group. These data also show a paraphyletic Chytridiomycota because *Allomyces* (Blastocladiales) again groups with the nonzoosporic fungi (including Zygomycota). Unfortunately, analysis of whole mitochondrial genomes must exclude the amitochondriate Neocallimastigales. In analyses of SSU rRNA, however, these fungi appear to be allied to Spizellomycetes, the order in which they previously were placed (Heath et al. 1983).

Based on current knowledge, it is possible to suggest a plausible phylogenetic hypothesis for Chytridiomycota for future testing (fig. 12.13). We may have been conservative in treating Chytridiomycota and Zygomycota as monophyletic groups and not as non-monophyletic groups, as shown in figures 12.2 and 12.13. However, until data from additional genes and taxa are available, we prefer to consider the treatment in figure 12.13 to be a hypothesis. In addition, more diversity continues to be uncovered as new chytrids are described and investigated with the electron microscope (Nyvall et al. 1999). Characterizing this diversity at the molecular level may result in the discovery of new major clades.

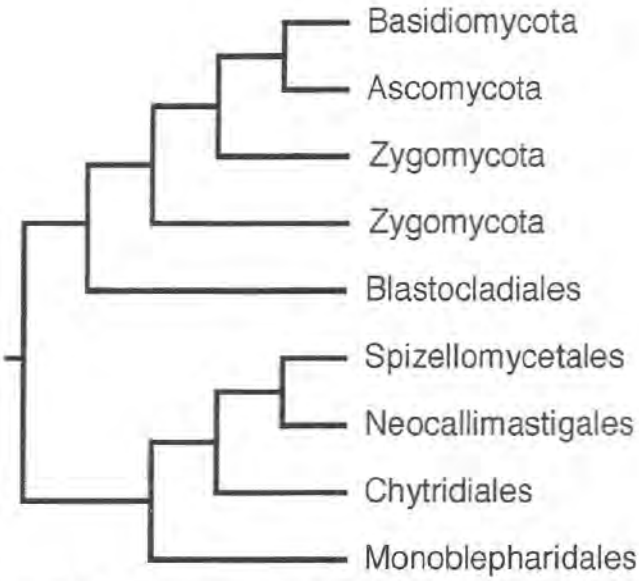

Figure 12.13. Phylogenetic relationships of Chytridiomycota orders to other fungi.

Fungi and Geologic Time

Our knowledge of the geologic history of Fungi is the subject of debate, mostly because of a lack of good fossils. The fossil record for fungi is based on very few specimens compared with that for plants and animals, probably because of a combination of factors: (1) fungi are mostly microscopic and are therefore easy to miss, (2) their tissues do not preserve very well, and (3) there are relatively few paleontologists looking for fungal fossils. Indeed, many of the best fossils are known only in association with a preserved plant or animal host. Some very well preserved fossils have been discovered, but they provide only a few, hazy pictures of the long history of fungi. The oldest convincing fossils of Fungi were discovered in the Ordovician (~460 Mya) of Wisconsin, as hyphae and spores that strongly resemble modern structures in the genus *Glomus* (Redecker et al. 2000a). Otherwise, the vast majority of the oldest fungal fossils come from a single site, the lower Devonian (~400 Mya) Rhynie Chert of Scotland. A wide variety of fossils have been taken from this location, mostly members of Zygomycota and Chytridiomycota (Taylor and Taylor 1997). These fossils include zygomycete lichens associated with probable cyanobacterial photobionts (Taylor et al. 1995a, 1997), chytrid fungi resembling members of the modern genera *Allomyces* (Blastocladiales; Taylor et al. 1994, Remy et al. 1994a) and *Entophlyctis* (Chytridiales; Taylor et al. 1992), and glomalean fungi (Remy et al. 1994b, Taylor et al. 1995b). Most surprising, fossils morphologically very similar to extant members of Sordariomycetes (Ascomycota) were identified in the Rhynie Chert associated with the early land plant *Asteroxylon* (Taylor et al. 1999). The Rhynie Chert fossils indicate that a wide variety of fungi were present in the early Devonian period, including some resembling modern taxa thought to have evolved much more recently.

With few fossils available, analysis of DNA sequence is an attractive and powerful tool for inferring the times of origin for the major groups of Fungi. Different sets of molecular data have been used for these analyses and different analyses have used different calibration times for the divergence of animals and fungi; their results are summarized in table 12.1. Most approaches to date divergence times of organisms assume a molecular clock, where a rate of sequence evolution is identified for a particular gene region, and use a known calibration point, for example, the age of a known

fossil or an independently estimated divergence time for fungi and animals. With these assumptions and data, divergence times between fungal divergences can be estimated. The first comprehensive attempts to date fungal divergences used nSSU rDNA and dated the origin of terrestrial fungi from the aquatic chytrids at approximately 550 Mya, in the Cambrian (Berbee and Taylor 1993). Using the knowledge that Fungi and Animalia probably share a common ancestor (Wainright et al. 1993), and a date of 965 Mya for that divergence (Doolittle et al. 1996), Berbee and Taylor (2001) revised their estimates based on nSSU rDNA and found that most inferred divergence times were pushed 50–100 million years earlier. Using the revision of Feng et al. (1997) for the divergence of animals and fungi, from 965 to 1200 Mya, would only have increased that effect. Berbee and Taylor (2001) used one gene for which sequences from many taxa were available, but more recent studies have used the ever-expanding DNA sequence databases to analyze more genes from fewer taxa. Wang et al. (1999) used amino acid sequences from 50 genes to explore the origin of animals, plants, and fungi. Although the majority of genes supported animals and fungi as closest ancestors, others supported animal and plant or plants and fungi as closest relatives, with an estimate of approximately 1576 Mya for the origin of these three kingdom-like clades. Using this and other molecular calibration points, Heckman et al. (2001) used amino acid sequences from 119 genes to estimate the divergence times of the major groups of fungi and inferred that most major groups evolved deep in the Precambrian, long before the points from which we have good fossils. These authors note that nSSU rDNA data give a similar result, provided that a date of about 1576 Mya is used for the divergence of animals and fungi. This result leaves us to wonder what fungi were doing on Earth for a billion years before they were preserved as the fossils we know to exist. A point strongly in favor of the older estimate for the divergence of animals and fungi is the multiple gene estimate of ~670 Mya for the divergence of Sordariomycetes, which accommodates the discovery of a 400 Mya sordariomycete fossil from the lower Devonian. The age of this fossil is in conflict with the SSU estimate of ~310 Mya for the sordariomycete divergence, which is calibrated by a divergence of animals and fungi of 900 Mya (table 12.1).

In summary, both newly discovered fossils and molecular data have pushed back our estimates of the origins of the major fungal groups (Taylor et al. 1999, Redecker et al. 2000a, Berbee and Taylor 2001, Heckman et al. 2001). Ancient origins of fungi strongly suggest that fungi played an important role in the early colonization of land by plants and animals, both by changing the physical and chemical environment and by establishing mutualistic symbioses such as mycorrhizae and lichens (Selosse and Le Tecon 1998, Redecker et al. 2000b, Lutzoni et al. 2001, Heckman et al. 2001). The discrepancies between the history of fungi told by the fossil record and that by a molecular clock suggest that far more data are needed. Precambrian sources should be analyzed further for fungal fossils, and reports of Silurian fossils of Ascomycota (Sherwood-Pike and Gray 1985) deserve renewed attention. New methods of analysis that can

Table 12.1
Divergence Times within Major Fungal Groups.

Groups compared (reference group in parentheses)	*rDNA[a] estimate (Mya)*	*rDNA[b] estimate (Mya)*	*119 protein gene[c] estimate (Mya)*	*Age of oldest known fossil in ref. group (Mya)*
(Chytridiomycota) versus Zygomycota + Ascomycota + Basidiomycota	~550	~660	1458 ± 70	~400[d]
Chytridiomycota + (Zygomycota) versus Ascomycota + Basidiomycota	~490	~590	1107 ± 56[e]	~460[f]
(Ascomycota) versus Basidiomycota	~390	~560	1208 ± 108	~400[g]
(Hymenomycetes) versus Ustilaginomycetes	~380	~430	966 ± 86	~290[h]
(Taphrinomycotina) versus Saccharomycotina + Pezizomycotina	~320	~420	1144 ± 77	None
Saccharomycotina versus (Pezizomycotina)	~310	~370	1085 ± 81	~400[g]
Eurotiomycetes versus (Sordariomycetes)	~290	~310	670 ± 71	~400[g]

[a]Molecular clock calibrated using fungal fossils (Berbee and Taylor 1993).

[b]Molecular clock calibrated using fungal fossils and divergence time of fungi vs. animals estimated at 965 Ma (Doolittle et al. 1996, Berbee and Taylor 2001).

[c]Molecular clocks calibrated using divergence of plants, animals, and fungi estimated at 1576 Mya, divergence of nematodes and arthropods at 1177 Mya, and arthropods and chordates at 993 Mya, each of which was in turn based on a 75-gene molecular clock calibrated with the vertebrate fossil record (Heckman et al. 2001).

[d]Several different fossilized chytrids from Rhynie Chert (Taylor and Taylor 1997).

[e]No glomalean fungi were included in this study. The Glomales, which represent the oldest reliable fungi in the fossil record, are probably the most recently derived major clade of the Zygomycota.

[f]Fossilized glomalean spores and hyphae from the Ordovician period (Redecker et al. 2000a).

[g]Fossilized Pyrenomycete from Rhynie Chert (Taylor et al. 1999).

[h]Fossilized hyphae with clamp connections (Dennis 1970).

accommodate rate variation among lineages (e.g., Sanderson 2002) should be investigated and compared with other methods. Our current estimates of the timing of events in fungal evolution undoubtedly are crude and are sure to be improved as data and methods improve. However, it is essential that they be made, even knowing that they can be improved, because time is the common currency of evolutionary biologists, and only by making such estimates can events in the history of Fungi be compared with those in the other major kingdom-like groups. We hope that those who design museum displays will note these efforts and include fungi in their work.

Last Word

Looking back on a dozen years of fungal molecular phylogenetics, it is clear that no approach since microscopy has had such a profound influence on our understanding of fungal evolution. Owing to their microscopic size and ability to live in their food, fungi are cryptic in a way that no angiosperm or vertebrate can imitate. This fact has made the research of fungal molecular phylogenetics even more valuable, because it enables ecologists, finally, to add the fungi to their studies. Over the next decade, we look forward to the improved phylogenetic resolution that genomics and improved analytical methods promise, and to the application of microarray technology to ecological studies. The latter should automate fungal identification and make it possible to more accurately estimate fungal biodiversity. That information should provide some further surprises and it seems sure to close the gap between the 100,000 described fungi and the 1.5 million estimated to exist in nature.

Acknowledgments

We thank Joyce Longcore for advice on the manuscript and Zheng Wang for the illustrations used in figure 12.6. We acknowledge the support of the National Science Foundation (Research Coordination Networks in Biological Sciences: A Phylogeny for Kingdom Fungi; NSF 0090301).

Literature Cited

Agrios, G. N. 1997. Plant pathology. 4th ed. Academic Press, San Diego.

Alexopoulos, C. J., C. W. Mims, and M. Blackwell. 1996. Introductory mycology. John Wiley and Sons, New York.

Arnold, A. E., Z. Maynard, and G. S. Gilbert. 2001. Fungal endophytes in dicotyledonous neotropical trees: patterns of abundance and diversity. Mycol. Res. 105:1502–1507.

Barnett, J. A., R. W. Payne, and D. Yarrow. 1990. Yeasts: characteristics and identification. Cambridge University Press, Cambridge.

Barr, D. J. S. 1980. An outline for the reclassification of the Chytridiales, and for a new order, the Spizellomycetales. Can. J. Bot. 58:2380–2394.

Barr, D. J. S. 1987. Isolation, culture and identification of Chytridiales, Spizellomycetales, and Hyphochytriales. Pp. 118–120 *in* Zoosporic fungi in teaching and research (M. S. Fuller and A. Jaworski, eds.). Southeastern Publishing, Athens, GA.

Barr, D. J. S. 1990. Phylum Chytridiomycota. Pp. 454–466 *in* Handbook of Protoctista (L. Margulis, J. O. Corliss, M. Melkonian, and D. J. Chapman, eds.). Jones and Bartlett, Boston.

Barr, D. J. S. 2001. Chytridiomycota. Pp. 93–112 *in* The mycota VIIA, systematics and evolution (D. J. McLaughlin, E. G. McLaughlin, and P. A. Lemke, eds.). Springer-Verlag, Berlin.

Barron, G. L. 1988. Microcolonies of bacteria as a nutrient source for lignicolous and other fungi. Can. J. Bot. 66:2505–2510.

Bartnicki-Garcia, S. 1987. The cell wall: a crucial structure in fungal evolution. Pp. 389–403 *in* Evolutionary biology of the fungi (A. D. M. Rayner, C. M. Brasier, and D. Moore, eds.). Cambridge University Press, Cambridge.

Bauer, R., D. Begerow, F. Oberwinkler, M. Piepenbring, and M. L. Berbee. 2001. Ustilaginomycetes. Pp. 57–84 *in* The mycota VIIB, systematics and evolution (D. J. McLaughlin, E. G. McLaughlin, and P. A. Lemke, eds.). Springer-Verlag, Berlin.

Begerow, D., R. Bauer, and F. Oberwinkler. 1997. Phylogenetic studies on nuclear large subunit ribosomal DNA sequences of smut fungi and related taxa. Can. J. Bot. 75:2045–2056.

Benjamin, R. K. 1979. Zygomycetes and their spores. Pp.573–616 *in* The whole fungus. The sexual-asexual synthesis (B. Kendrick, ed.), vol. 2. Museums of Canada and the Kananaskis Foundation, Ottawa.

Benjamin, R. K., M. Blackwell, I. Chapella, R. A. Humber, K. G. Jones, K. A. Klepzig, R. W. Lichtwardt, D. Malloch, H. Noda, R. A. Roeper, J. W. Spatafora, and A. Weir. In press. The search for diversity of insects and other arthropod associated fungi. *In* Biodiversity of fungi: standard methods for inventory and monitoring (G. M. Mueller, G. F. Bills, and M. Foster, eds.). Academic Press, New York.

Benny, G. L. 2001. Zygomycota: Trichomycetes. Pp. 147–160 *in* The mycota VIIA, systematics and evolution (D. J. McLaughlin, E. G. McLaughlin, and P. A. Lemke, eds.). Springer-Verlag, Berlin.

Benny, G. L., R. A. Humber, and J. B. Morton. 2001. Zygomycota: Zygomycetes. Pp. 113–146 *in* The mycota VIIA, systematics and evolution (D. J. McLaughlin, E. G. McLaughlin, and P. A. Lemke, eds.). Springer-Verlag, Berlin.

Benny, G. L., and K. O'Donnell. 2000. *Amoebidium parasiticum* is a protozoan, not a Trichomycete. Mycologia 92:1133–1137.

Berbee, M. L. 1998. Loculoascomycete origins and evolution of filamentous ascomycete morphology based on 18S rRNA gene sequence data. Mol. Biol. Evol. 13:462–470.

Berbee, M. L., and J. W. Taylor. 1992. Convergence in ascospore discharge mechanism among Pyrenomycete fungi based on 18S ribosomal RNA gene sequence. Mol. Phylogenet. Evol. 1:59–71.

Berbee, M. L., and J. W. Taylor. 1993. Dating the evolutionary radiations of the true fungi. Can. J. Bot. 71:1114–1127.

Berbee, M. L., and J. W. Taylor. 2001. Fungal molecular evolution: gene trees and geologic time. Pp. 229–245 in The mycota VIIB, systematics and evolution (D. J. McLaughlin, E. G. McLaughlin, and P. A. Lemke, eds.). Springer-Verlag, Berlin.

Berger, L., R. Speare, P. Daszak, D. E. Green, A. A. Cunningham, C. L. Goggin, R. Slocombe, M. A. Ragan, A. D. Hyatt, K. R. McDonald, et al. 1998. Chytridiomycosis causes amphibian mortality associated with population declines in the rain forests of Australia and Central America. Proc. Natl. Acad. Sci. USA 95:9031–9036.

Bhattacharya, D., F. Lutzoni, V. Reeb, D. Simon, and F. Fernandez. 2000. Widespread occurrence of spliceosomal introns in the rDNA genes of Ascomycetes. Mol. Biol. Evol. 17:1971–1984.

Blackwell, M. 2000. Perspective: Evolution: Terrestrial life—fungal from the start? Science 289:1884–1885.

Bowman, B. H., J. W. Taylor, J. L. Brownlee, S.-D. Lu, and T. J. White. 1992. Molecular evolution of the fungi: relationship of the Basidiomycetes, Ascomycetes, and Chytridiomycetes. Mol. Biol. Evol. 9:258–296.

Brodo, I. M., S. Duran Sharnoff, and S. Sharnoff. 2001. Lichens of North America. Yale University Press, New Haven, CT.

Bruns, T. D., T. M. Szaro, M. Gardes, K. W. Cullings, J. Pan, D. L. Taylor, and Y. Li. 1998. A sequence database for the identification of ectomycorrhizal fungi by sequence analysis. Mol. Ecol. 7:257–272.

Bruns, T. D., R. Vilgalys, S. M. Barns, D. Gonzalez, D. S. Hibbett, D. J. Lane, L. Simon, S. Stickel, T. M. Szaro, W. G. Weisburg, and M. L. Sogin. 1992. Evolutionary relationships within the Fungi: analyses of nuclear small subunit rRNA sequences. Mol. Phylogenet. Evol. 1:231–241.

Bullerwell, C. E., L. Forget, and B. F. Lang. 2003. Evolution of monoblepharidalean fungi based on complete mitochondrial genome sequences. Nucleic Acids Res. 31:1614–1623.

Carroll, G. 1988. Fungal endophytes in stems and leaves: from latent pathogen to mutualistic symbiont. Ecology 69:2–9.

Carroll, G. 1995. Forest endophytes: pattern and process. Can. J. Bot. 73:S1316–S1324.

Carroll, G. C., and D. T. Wicklow. 1992. The fungal community: its organization and role in the ecosystem. Marcel Dekker, New York.

Cavalier-Smith, T. 1987. The origin of Fungi and pseudofungi. Pp. 339–353 in Evolutionary biology of the fungi (A. D. M. Rayner, C. M. Brasier, and D. Moore, eds.). Cambridge University Press, Cambridge.

Cavalier-Smith, T. 1998. A revised six-kingdom of life. Biol. Rev. 73:203–266.

Chapela, I., S. A. Rehner, T. R. Schultz, and U. G. Mueller. 1994. Evolutionary history of the symbiosis between fungus-gorwing ants and their fungi. Science 266:1691–1694.

Clay, K. 2001. Symbiosis and the regulation of communities. Am. Zool. 41:810–824.

Cole, G. T., and B. Kendrick. 1981. Biology of conidial fungi. Academic Press, New York.

Cullen, D. 1997. Recent advances on the molecular genetics of ligninolytic fungi. J. Biotechnol. 53:273–289.

Currie, C. R., B. Wong, A. E. Stuart, T. R. Schultz, S. A. Rehner, U. G. Mueller, G. H. Sung, J. W. Spatafora, and N. A. Straus. 2003. Ancient tripartite coevolution in the attine ant-microbe symbiosis. Science 299:386–388.

Dennis, R. L. 1970. A Middle Pennsylvanian basidiomycete mycelium with clamp connections. Mycologia 62:578–564.

Doggett, M. S., and D. Porter. 1996. Sexual reproduction in the fungal parasite, *Zygorhizidium planktonicum*. Mycologia 88:720–732.

Doolittle, R. F., D. F. Feng, S. Tsang, G. Cho, and E. Little. 1996. Determining divergence times of the major kingdoms of living organisms with a protein clock. Science 271:470–477.

Dore, J., and D. A. Stahl. 1991. Phylogeny of anaerobic rumen Chytridiomycetes inferred from small subunit ribosomal RNA sequence comparisons. Can. J. Bot. 69:1964–1971.

Edmonds, R. L., J. K. Agee, and R. I. Gara. 2000. Forest health and protection. McGraw-Hill Series in Forestry. McGraw-Hill, Boston.

Eriksson, O. E., H.-O. Baral, R. S. Currah, K. Hansen, C. P. Kurtzman, G. Rambold, and T. Laessøe. 2003. Pp. 1–89 in Outline of Ascomycota—2003. Myconet Umeå, Umeå University. Available: http://www.umu.se/myconet/Myconet.html. Last accessed 3 December 2003.

Eslava, A. P., and M. I. Alvarez. 1996. Genetics of Phycomyces. Pp. 385–406 in Fungal genetics: principles and practice (C. J. Bos, ed.). Marcel Dekker, New York.

Fell, J. W., T. Boekhout, A. Fonseca, and J. P. Sampaio. 2001. Basidiomycetous yeasts. Pp. 3–35 in The mycota VIIB, systematics and evolution (D. J. McLaughlin, E. G. McLaughlin, and P. A. Lemke, eds.). Springer-Verlag, Berlin.

Feng, D. F., G. Cho, and R. F. Doolittle. 1997. Determining divergence times with a protein clock; update and reevaluation. Proc. Natl. Acad. Sci. USA 94:13028–13033.

Forget, L., J. Ustinova, Z. Wang, V. A. R. Huss, and B. F. Lang. 2002. The "colorless green alga" *Hyaloraphidium curvatum*: a linear mitochondrial genome, tRNA editing, and an evolutionary link to lower fungi. Mol. Biol. Evol. 19:310–319.

Förster, H., M. D. Coffey, H. Elwood, and M. L. Sogin. 1990. Sequence analysis of the small subunit ribosomal RNAs of three zoosporic fungi and implications for fungal evolution. Mycologia 82:306–312.

Fuller, M. S., and R. E. Reichle. 1968. The fine structure of *Monoblepharella* sp. zoospores. Can. J. Bot. 46:279–283.

Gargas, A., P. T. DePriest, M. Grube, and A. Tehler. 1995. Multiple origins of lichen symbioses in fungi suggested by SSU rDNA phylogeny. Science 268:1492–1495.

Gehrig, H., A. Schüßler, and M. Kluge. 1996. *Geosiphon pyriforme*, a fungus forming endocytobiosis with *Nostoc* (Cyanobacteria), is an ancestral member of the Glomales: evidence by SSU rRNA analysis. J. Mol. Evol. 43:71–81.

Geiser, D. M., and K. F. LoBuglio. 2001. The monophyletic Plectomycetes: Ascosphaeriales, Onygenales, Eurotiales. Pp. 201–219 in The mycota VIIA, systematics and evolution (D. J. McLaughlin, E. G. McLaughlin, and P. A. Lemke, eds.). Springer-Verlag, New York.

Gernandt, D. S., J. L. Platt, J. K. Stone, J. W. Spatafora, A. Holst-Jensen, R. C. Hamelin, and L. M. Kohn. 2001. Phylogenetics of Helotiales and Rhytismatales based on partial small subunit nuclear ribosomal DNA sequences. Mycologia 93:915–933.

Glass, N. L., and I. A. J. Lorimer. 1991. Ascomycete mating types. Pp 193–216 *in* More gene manipulations in fungi (J. W. Bennett and L. L. Lasure, eds.). Academic Press, Orlando, FL.

Gottlieb, A. M., and R. W. Lichtwardt. 2001. Molecular variation within and among species of Harpellales. Mycologia 93:66–81.

Griffin, D. H. 1994. Fungal physiology. 2nd ed. Wiley-Liss, New York.

Haselwandter, K., O. Bolbleter, and D. J. Read. 1990. Degradation of 14C-labelled lignin and dehydropolymer of coniferyl alchohol by ericoid and ectomycorrhizal fungi. Arch. Microbiol. 153:352–354.

Hawksworth, D. L. 1991. The fungal dimension of biodiversity: magnitude, significance and conservation. Mycol. Res. 95:641–655.

Hawksworth, D. L. 2001. The magnitude of fungal diversity: the 1.5 million species estimate revisited. Mycol. Res. 105:1422–1432.

Hawksworth, D. L., P. M. Kirk, B. C. Sutton, and D. N. Pegler. 1995. Ainsworth and Bisby's dictionary of the fungi. 8th ed. CAB International, Wallingford, UK.

Heath, I. B., T. Bauchop, and R. A. Skipp. 1983. Assignment of the rumen anaerobe *Neocallimastix frontalis* to the Spizellomycetales (Chytridiomycetes) on the basis of its polyflagellate ultrastructure. Can. J. Bot. 61:295–307.

Heckman, D. S., D. M. Geiser, B. R. Eidell, R. L. Stauffer, N. L. Kardos and S. B. Hedges. 2001. Molecular evidence for the early colonization of land by fungi and plants. Science 293:1129–1133.

Hibbett, D. S., and M. Binder. 2002. Evolution of complex fruiting body morphologies in homobasidiomycetes. Proc. R. Soc. Lond. B 269:1963–1969.

Hibbett, D. S., and M. J. Donoghue. 2001. Analysis of character correlations among wood decay mechanisms, mating systems, and substrate ranges in homobasidiomycetes. Syst. Biol. 50:215–242.

Hibbett, D. S., L. B. Gilbert, and M. J. Donoghue. 2000. Evolutionary instability of ectomycorrhizal symbioses in basidiomycetes. Nature 407:506–508.

Hibbett, D. S., E. M. Pine, E. Langer, G. Langer, and M. J. Donoghue. 1997. Evolution of gilled mushrooms and puffballs inferred from ribosomal DNA sequences. Proc. Natl. Acad. Sci. USA 94:12002–12006.

Hibbett, D. S., and R. G. Thorn. 2001. Basidiomycota: Homobasidiomycetes. Pp. 121–168 *in* The mycota VIIB, systematics and evolution (D. J. McLaughlin, E. G. McLaughlin, and P. A. Lemke, eds.). Springer-Verlag, Berlin.

Holst-Jensen, A., L. M. Kohn, and T. Schumacher. 1997. Nuclear rDNA phylogeny of the Sclerotiniaceae. Mycologia 89:885–899.

Humber, R. A., and K. S. Hansen. USDA-ARS Collection of Entomopathogenic Fungal Cultures (ARSEF). Available: http://www.ppru.cornell.edu/mycology/Insect_mycology.htm. Last accessed 3 December 2003.

Ingold, C. T. 1965. Spore liberation. Clarendon Press, Oxford.

James, T. Y., D. Porter, C. A. Leander, R. Vilgalys, and J. E. Longcore. 2000. Molecular phylogenetics of the Chytridiomycota supports the utility of ultrastructural data in chytrid systematics. Can. J. Bot. 78:336–350.

Jensen, A. B., A. Gargas, J. Eilenberg, and S. Rosendahl. 1998. Relationships of the insect-pathogenic order Entomophtorales (Zygomycota, Fungi) based on phylogenetic analyses of nuclear small subunit ribosomal DNA sequences (SSU rDNA). Fungal Genet. Biol. 24:325–334.

Karling, J. S. 1977. Chytridiomycetarum Iconographia. Lubrecht and Cramer, Monticello, NY.

Kauff, F., and F. Lutzoni. 2002. Phylogeny of the *Gyalectales* and *Ostropales* (Ascomycota, Fungi): among and within order relationships based on nuclear ribosomal RNA small and large subunits. Mol. Phylogenet. Evol. 25:138–156.

Keeling, P. J., M. A. Luker, and J. D. Palmer. 2000. Evidence from beta-tubulin phylogeny that microsporidia evolved from within the fungi. Mol. Biol. Evol. 17:23–31.

Kirk, P. M., P. F. Cannon, J. C. David, and J. A. Stalpers. 2001. Ainsworth and Bisby's dictionary of the fungi. 9th ed. CAB International, Wallingford, UK.

Klironomos, J. N., and M. M. Hart. 2001. Animal nitrogen swap for plant carbon. Nature 410:651–652.

Kohlmeyer, J., and E. Kohlmeyer. 1979. Marine mycology. The higher fungi. Academic Press, New York.

Kurtzman, C. P., and C. J. Robnett. 2003. Phylogenetic relationships among yeasts of the "Saccharomyces complex" determined from multigene sequence analyses. FEMS Yeast Res. 1554:1–16.

Kwon-Chung, K. J., and J. E. Bennett. 1992. Medical mycology. Lea and Febiger, Philadelphia.

Landvik, S. 1996. *Neolecta*, a fruit-body-producing genus of the basal ascomycetes, as shown by SSU and LSU rDNA sequences. Mycol. Res. 100:199–202.

Landvik, S., O. E. Eriksson, and M. L. Berbee. 2001. *Neolecta*—a fungal dinosaur? Evidence from β-tubulin amino acid sequences. Mycologia 93:1151–1163.

Lang, B. F. 2001. Fungal Mitochondrial Genome Project. Http://megasun.bch.umontreal.ca/People/lang/FMGP/.

Lang, B. F, C. O'Kelly, T. Nerad, M. W. Gray, and G. Burger. 2002. The closest unicellular relative of animals. Curr. Biol. 12:1773–1778.

Lange, L., and L. W. Olson. 1979. The uniflagellate phycomycete zoospore. Dansk Bot. Arkiv. 33:7–95.

Li, J., and I. B. Heath. 1992. The phylogenetic relationships of the anaerobic chytridiomycetous gut fungi (Neocallimasticaceae) and the Chytridiomycota. I. Cladistic analysis of rRNA sequences. Can. J. Bot. 70:1738–1746.

Lichtwardt, R. W. 1986. The trichomycetes, fungal associates of arthropods. Springer-Verlag, New York.

Lichtwardt, R. W., M. J. Cafaro, and M. M. White. 2001. The Trichomycetes: rev. ed. Available: http://www.nhm.ku.edu/%7Efungi/monograph/text/mono.htm. Last accessed 3 December 2003.

Liu, Y. J., S. Whelen, and B. D. Hall. 1999. Phylogenetic relationships among ascomycetes: evidence from an RNA polymerse II subunit. Mol. Biol. Evol. 16:1799–1808.

Longcore, J. E. 1992. Morphology, occurrence, and zoospore ultrastructure of Podochytrium dentatum sp. nov. (Chytridiales). Mycologia 84:183–192.

Longcore, J. E. 1996. Chytridiomycete taxonomy since 1960. Mycotaxon 60:149–174.

Longcore, J. E., A. P. Pessier, and D. K. Nichols. 1999. *Batracho-*

chytrium dendrobatidis gen. et sp. nov., a chytrid pathogenic to amphibians. Mycologia 91:219–227.

Loytynoja, A., and M. C. Milinkovitch. 2001. Molecular phylogenetic analyses of the mitochondrial ADP-ATP carriers: the Plantae/Fungi/Metazoa trichotomy revisited. Proc. Natl. Acad. Sci. USA 98:10202–10207.

Luttrell, E. S. 1951. Taxonomy of the Pyrenomycetes. Univ. Mo. Stud. 24:1–120.

Luttrell, E. S. 1955. The ascostromatic Ascomycetes. Mycologia 47:511–532.

Lutzoni, F., and M. Pagel. 1997. Accelerated evolution as a consequence of transitions to mutualism. Proc. Nat. Acad. Sci. USA 94:11422–11427.

Lutzoni, F. L., M. Pagel, and V. Reeb. 2001. Major fungal lineages are derived from lichen symbiotic ancestors. Nature 411:937–940.

Marsh, L. 1991. Signal transduction during pheromone response in yeast. Annu. Rev. Cell Biol. 7:699–728.

McKerracher, L. J., and I. B. Heath. 1985. The structure and cycle of the nucleus-associated organelle in two species of *Basidiobolus*. Mycologia 77:412–417.

McLaughlin, D. J., E. G. McLaughlin, and P. A. Lemke. 2001. The mycota VIIA: systematics and evolution. Springer-Verlag, New York.

Miadlikowska, J., and F. Lutzoni. In press. Phylogenetic classification of peltigeralean fungi (Peltigerales, Ascomycota) based on ribosomal RNA small and large subunits. Am. J. Bot.

Molina, R., and J. M. Trappe. 1982. Patterns of ectomycorrhizal host specificity and potential among pacific northwest conifers and fungi. Forest Sci. 28:423–458.

Moncalvo, J. M., R. Vilgalys, S. A. Redhead, J. E. Johnson, T. Y. James, M. C. Aime, V. Hofstetter, S. J. W. Verduin, E. Larsson, T. J. Baroni, et al. 2002. One hundred and seventeen clades of Euagarics. Mol. Phylogenet. Evol. 23:357–400.

Nagahama, T., H. Sato, M. Shimazu, and J. Sugiyama. 1995. Phylogenetic divergence of the entomophthoralean fungi: evidence from nuclear 18s ribosomal RNA gene sequence. Mycologia 87:203–209.

Nash, T. H. 1996. Lichen biology. Cambridge University Press, Cambridge.

Nishida, H., K. Ando, Y. Ando, A. Hirata, and J. Sugiyama. 1995. *Mixia osmundae*: transfer from the Ascomycota to the Basidiomycota based on evidence from molecules and morphology. Can. J. Bot. 73:S660–S666.

Nishida, H., and J. Sugiyama. 1994. Archiascomycetes: detection of a major new linage within the Ascomycota. Mycoscience 35:361–366.

Nyvall, P., M. Pedersén, and J. E. Longcore. 1999. *Thalassochytrium gracilariopsidis* (Chytridiomycota), gen. et sp. nov., endosymbiotic in *Gracilariopsis* sp. (Rhodophyceae). J. Phycol. 35:176–185.

Oberwinkler, F. 1977. Das neue System der Basidiomyceten. Pp. 59–105 *in* Biologie der niederen Pflanzen (H. Frey, H. Hurka, and F. Oberwinkler, eds.). G. Fischer, Stuttgart.

O'Donnell, K. 1979. Zygomycetes in culture. Department of Botany, University of Georgia, Athens.

O'Donnell, K., E. Cigelnik, and G. L. Benny. 1998. Phylogenetic relationships among the Harpellales and Kickxellales. Mycologia 90:624–639.

O'Donnell, K., F. M. Lutzoni, T. J. Ward, and G. L. Benny. 2001. Evolutionary relationships among mucoralean fungi (Zygomycota): evidence for family polyphyly on a large scale. Mycologia 93:286–296.

Orpin, C. G. 1988. Nutrition and biochemistry of anaerobic Chytridiomycetes. Biosystems 21:365–370.

Paquin, B., M.-J. Laforest, L. Forget, I. Roewer, Z. Wang, J. Longcore, and F. Lang. 1997. The fungal mitochondrial genome project: evolution of fungal mitochondrial genomes and their gene expression. Curr. Genet. 31:380–395.

Perez-Moreno, J., and D. J. Read. 2000. Mobilization and transfer of nutrients from litter to tree seedlings via the vegetative mycelium of ectomycorrhizal plants. New Phytol. 145:301–309.

Pirozynski, K. A., and D. W. Malloch. 1975. The origin of land plants: a matter of mycotropism. Biosystems 6:153–164.

Platt, J. L., and J. W. Spatafora. 2000. Evolutionary relationships of nonsexual lichenized fungi: molecular phylogenetic hypotheses for the genera *Siphula* and *Thamnolia* from SSU and LSU rDNA. Mycologia 92:475–487.

Powell, M. J. 1978. Phylogenetic implications of the microbody-lipid globule complex in zoosporic fungi. Biosystems 10:167–180.

Powell, M. J. 1993. Looking at mycology with a Janus face: a glimpse at Chytridiomycetes active in the environment. Mycologia 85:1–20.

Powell, M. J., and W. J. Koch. 1977. Morphological variations in a new species of *Entophlyctis*. II. Influence of growth conditions on morphology. Can. J. Bot. 55:1686–1695.

Prillinger, H., F. Oberwinkler, C. Umile, K. Tlachac, R. Bauer, C. Dörfler, and E. Taufratzhofer. 1993. Analysis of cell wall carbohydrates (neutral sugars) from ascomycetous and basidiomycetous yeasts with and without derivatization. J. Gen. Appl. Microbiol. 39:1–34.

Raju, N. B. 1992. Genetic control of the sexual cycle in *Neurospora*. Mycol. Res. 96:241–262.

Rayner, A. D. M., and L. Boddy. 1988. Fungal decomposition of wood: its biology and ecology. John Wiley, Chichester, UK.

Redecker, D., R. Kodner, and L. E. Graham. 2000a. Glomalean fungi from the Ordovician. Science 289:1920–1021.

Redecker, D., J. B. Morton, and T. D. Bruns. 2000b. Ancestral lineages of arbuscular mycorrhizal fungi (Glomales). Mol. Phylogenet. Evol. 14:276–284.

Reid, I. D. 1995. Biodegradation of lignin. Can. J. Bot. 73:S1011–S1018.

Remy, W., T. N. Taylor, and H. Hass. 1994a. Early Devonian fungi—a Blastocladalean fungus with sexual reproduction. Am. J. Bot. 81:690–702.

Remy, W., T. N. Taylor, H. Hass, and H. Kerp. 1994b. 4–Hundred-million-year-old vesicular-arbuscular mycorrhizae. Proc. Natl. Acad. Sci. USA 91:11841–11843.

Rinaldi, M. G. 1989. Zygomycosis. Infect. Dis. Clin. N. Am. 3:19–41.

Roane, M. K., and R. A. Paterson. 1974. Some aspects of morphology and development in the Chytridiales. Mycologia 66:147–164.

Sanders, I. R. 1999. No sex please, we're fungi. Nature 399:737–739.

Sanderson, M. J. 2002. Estimating absolute rates of molecular evolution and divergence times: a penalized likelihood approach. Mol. Biol. Evol. 19:101–109.

Schüßler, A., D. Schwarzott, and C. Walker. 2001. A new fungal phylum, the Glomeromycota: phylogeny and evolution. Mycol. Res. 105:1413–1421.

Schwarzott, D., C. Walker, and A. Schüßler. 2001. *Glomus*, the largest genus of the arbuscular mycorrhizal Fungi (Glomales), is nonmonophyletic. Mol. Phylogenet. Evol. 21:190–197.

Selosse, M. A., and F. Le Tacon. 1998. The land flora: a phototroph-fungus partnership? Trends Ecol. Evol. 13:15–20.

Sherwood-Pike, M. A., and J. Gray. 1985. Silurian fungal remains: probable records of the Class Ascomycetes. Lethaia 18:1–20.

Simon, L., J. Bousquet, R. C. Lévesque, and M. Lalonde. 1993. Origin and diversification of endomycorrhizal fungi and coincidence with vascular land plants. Nature 363:67–69.

Sjamsuridzal, W., H. Nishida, H. Ogawa, M. Kakishima, and J. Sugiyama. 1999. Phylogenetic positions of rust fungi parasitic on ferns: evidence from 18S rDNA sequence analysis. Mycoscience 40:21–27.

Smith, M. L., J. N. Bruhn, and J. B. Anderson. 1992. The fungus *Armillaria bulbosa* is among the largest and oldest living organisms. Nature 356:428–434.

Smith, S. E., and D. J. Read. 1997. Mycorrhizal symbiosis. Academic Press, San Diego.

Sparrow, F. K. 1960. Aquatic phycomycetes. 2nd rev. ed. University of Michigan Press, Ann Arbor.

Spatafora, J. W. 1995. Ascomal evolution among filamentous ascomycetes: evidence from molecular data. Can. J. Bot. S811–S815.

Spatafora, J. W., and M. Blackwell. 1994. The polyphyletic origins of ophiostomatoid fungi. Mycol. Res. 98:1–9.

Spatafora, J. W., B. Volkmann-Kohlmeyer, and J. Kohlmeyer. 1998. Independent terrestrial origins of the Halosphaeriales (marine Ascomycota). Am. J. Bot. 85:1569–1580.

Sugiyama, J. 1987. Pleomorphic fungi: the diversity and its taxonomic implications. Elsevier, Amsterdam.

Suh, S.-O., and M. Blackwell. 1999. Molecular phylogeny of the cleistothecial fungi placed in Cephalothecaceae and Pseudeurotiaceae. Mycologia 91:836–848.

Suh, S.-O., C. Marshall, J. V. McHugh, and M. Blackwell. 2003. Wood ingestion by passalid beetles in the presence of xylose-fermenting gut yeasts. Mol. Ecol. 12:3137–3145.

Swann, E. C., E. M. Frieders, and D. J. McLaughlin. 1999. *Microbotryum*, *Kriegeria* and the changing paradigm in basidiomycete classification. Mycologia 91:51–66.

Swann, E. C., E. M. Frieders, and D. J. McLaughlin. 2001. Urediniomycetes. Pp. 37–56 *in* The mycota VIIB, systematics and evolution (D. J. McLaughlin, E. G. McLaughlin, and P. A. Lemke, eds.). Springer-Verlag, Berlin.

Swann, E. C., and J. W. Taylor. 1993. Higher taxa of basidiomycetes: an 18S rRNA gene perspective. Mycologia 85:923–936.

Swann, E. C., and J. W. Taylor. 1995. Phylogenetic perspectives on basidiomycete systematics: evidence from the 18S rRNA gene. Can. J. Bot. 73:S862–S868.

Tanabe, Y., K. O'Donnell, M. Saikawa, and J. Sugiyama. 2000. Molecular phylogeny of parasitic Zygomycota (Dimargaritales, Zoopagales) based on nuclear small subunit ribosomal DNA sequences. Mol. Phylogenet. Evol. 16:253–262.

Tanabe, Y., Watanabe, M. M., and J. Sugiyama. 2002. Are Microsporidia really related to fungi? A reappriasal based on additional gene sequenc es from basal fungi. Mycol. Res. 106:1380–1391.

Taylor, J. W. 1995. Making the Deuteromycota redundant: a practical integration of mitosporic and meiosporic fungi. Can. J. Bot. 73:S754–S759.

Taylor, J. W., B. Bowman, M. L. Berbee, and T. J. White. 1993. Fungal model organisms: phylogenetics of *Saccharomyces*, *Aspergillus* and *Neurospora*. Syst. Biol. 42:440–457.

Taylor, J. W., and M. S. Fuller. 1981. The Golgi apparatus, zoosporogenesis, and development of the zoospore discharge apparatus of *Chytridium confervae*. Exp. Mycol. 5:35–59.

Taylor, T. N., H. Hass, and H. Kerp. 1997. A cyanolichen from the Lower Devonian Rhynie Chert. Am. J. Bot. 84:992–1004.

Taylor, T. N., T. Hass, and H. Kerp. 1999. The oldest fossil ascomycetes. Nature 399:648–648.

Taylor, T. N., H. Hass, W. Remy, and H. Kerp. 1995a. The oldest fossil lichen. Nature 378:244–244.

Taylor, T. N., W. Remy, and H. Hass. 1992. Fungi from the Lower Devonian Rhynie Chert—Chytridiomycetes. Am. J. Bot. 79:1233–1241.

Taylor, T. N., W. Remy, and H. Hass. 1994. Allomyces in the Devonian. Nature 367:601–601.

Taylor, T. N., W. Remy, H. Hass, and H. Kerp. 1995b. Fossil arbuscular mycorrhizae from the Early Devonian. Mycologia 87:560–573.

Taylor, T. N., and E. L. Taylor. 1997. The distribution and interactions of some Paleozoic fungi. Rev. Palaeobot. Palynol. 95:83–94.

Tehler, A., J. S. Farris, D. L. Lipscomb, and M. Källersjo. 2000. Phylogenetic analyses of the fungi based on large rDNA data sets. Mycologia 92:459–474.

Thorn, R. G., and G. L. Barron. 1984. Carnivorous mushrooms. Science 224:76–78.

Ustinova, I., L. Krienitz, and V. A. R. Huss. 2000. *Hyaloraphidium curvatum* is not a green alga, but a lower fungus; *Amoebidium parasiticum* is not a fungus, but a member of the DRIPs. Protist 151:253–262.

Varma, A., and B. Hock. 1999. Mycorrhiza: structure, function, molecular biology, biotechnology. Springer-Verlag, New York.

Vogler, D. R., and T. D. Bruns. 1998. Phylogenetic relationships among the pine stem rust fungi (*Cronartium* and *Peridermium* spp.). Mycologia 90:244–257.

Wainright, P. O., G. Hinkle, M. L. Sogin, and S. K. Stickel. 1993. Monophyletic origins of the Metazoa: "an evolutionary link with fungi." Science 260:340–342.

Wang, D. Y. C., S. Kumar, and S. B. Hedges. 1999. Divergence time estimates for the early history of animal phyla and the origin of plants, animals and fungi. Proc. R. Soc. Lond. B 266:163–171.

Wasson, G. 1980. The wondrous mushroom: mycolatry in Mesoamerica. McGraw-Hill, New York.

Weir, A., and M. Blackwell. 2001. Molecular data support the Laboulbeniales as a separate class of Ascomycota, Laboulbeniomycetes. Mycol. Res. 105:715–722.

Wells, K., and R. J. Bandoni. 2001. Heterobasidiomycetes. Pp. 85–120 *in* The mycota VIIB, systematics and evolution (D. J. McLaughlin, E. G. McLaughlin, and P. A. Lemke, eds.). Springer-Verlag, Berlin.

Wessels, J. G. H. 1994. Developmental regulation of fungal cell wall formation. Annu. Rev. Phytopathol. 32:413–437.

Wu, C. G., and J. W. Kimbrough. 1992. Ultrastructural studies of ascosporogenesis in *Ascobolus immersus*. Mycologia 84:459–466.