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Early Diverging Fungi:
Diversity and Impact at the
Dawn of Terrestrial Life

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Abstract

As decomposers or plant pathogens, fungi deploy invasive growth and powerful carbohydrate active enzymes to reduce multicellular plant tissues to humus and simple sugars. Fungi are perhaps also the most important mutualistic symbionts in modern ecosystems, transporting poorly soluble mineral nutrients to plants and thus enhancing the growth of vegetation. However, at their origin over a billion years ago, fungi, like plants and animals, were unicellular marine microbes. Like the other multicellular kingdoms, Fungi evolved increased size, complexity, and metabolic functioning. Interactions of fungi with plants changed terrestrial ecology and geology and modified the Earth's atmosphere. In this review, we discuss the diversification and ecological roles of the fungi over their first 600 million years, from their origin through their colonization of land, drawing on phylogenomic evidence for their relationships and metabolic capabilities and on molecular dating, fossils, and modeling of Earth's paleoclimate.



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Fungi: kingdom including mushrooms, molds, and allied protists; sister clade to Holozoa

Weathering: Processes that break down rocks into minerals; contributes to controlling rates of plant growth and atmospheric CO₂ drawdown

INTRODUCTION

This review summarizes phylogenomic and paleomycological evidence for the evolution of Fungi starting with their ancestral origin as unicellular microbes and continuing through their diversification into wide-ranging saprotrophs and ecologically vital decomposers and symbionts. Because key transitions in fungal biology were ancient, we focus on events between 1,000 and 400 million years ago (Mya). Over this vast 600 million years of geological time, fungi, plants, and animals first colonized the land, developed entwined symbiotic relationships, and set the stage for the evolution of our species. Our aims are to outline phylogenetic and fossil evidence for the diversification of fungal clades, relating the evolution of form in fungal bodies to changing environmental opportunities over geological time. Fungi are heterotrophs, and we consider evidence of food sources that could have constrained or facilitated their diversification. Finally, extant fungi participate in geological weathering and global biogeochemical cycles, and we discuss evidence for similar roles of ancestral fungi in deep time.

ANIMALS, FUNGI, AND ANCIENT CLADES OF DIVERSE PROTISTS SHARED A COMMON ANCESTOR ~1,481 TO 900 MILLION YEARS AGO

As members of Holozoa, we are close relatives of Fungi (2, 23, 136) and our lineages independently evolved multicellularity from a unicellular ancestor (**Figure 1**; 108, 118). Somewhere between 1,481 and 900 Mya (26, 93) that common ancestor had a single flagellum that beat to propel it forward (92, 131). “Opisthokonta,” the name for the clade of Holozoa plus Fungi, refers to this single posterior flagellum (13).

The earliest fungi did not leave an interpretable fossil record so indirect inferences are necessary to put them into a paleontological perspective. Cytoskeletal and ecological prerequisites for diversification were already established in eukaryotic microorganisms fossilized nearly 1,500 Mya (54). Geochemical constraints and quantitative models put boundaries on animal, and by extension fungal, diversification times. The spatial and temporal variability in surface ocean O₂ levels suggests that marine environments might have limited the emergence and large-scale proliferation of motile multicellular life ~1,800–800 Mya (102). Molecular dating, using housekeeping genes, predicted that crown group metazoans originated deep in the Cryogenian period (850–635 Mya), whereas most modern animals’ lineages radiated during the Cambrian (541–485 Mya) (31). The metazoan age estimates, despite their imprecision, offer a minimum age for Fungi and confirm that Fungi were evolving at the dawn of terrestrial life.

Earliest Diverging Fungal Branch(es) Were Phagotrophic and Endoparasitic

Most modern fungi are osmotrophs, growing as filaments of cells (hyphae) into their food, secreting digestive enzymes across their cell walls, and absorbing dissolved nutrients. In contrast, the microscopic, unicellular ancestors of Fungi and Holozoa were wall-less during their trophic phases. Being without a constraining cell wall, many extant protistan Opisthokonta and by extension their common ancestor are characterized by phagotrophic nutrition (13). On the animal side, extant phagotrophic protists include the marine *Ministeria marisola* (95) as well as freshwater and marine collar flagellates (21). As in fungi, some holozoan protists convergently lost phagotrophy; e.g., *Sphaeroforma* spp. (**Figure 1**) (82) absorb nutrients from the animal guts they inhabit.

Some 1,000 Mya the fungal stem lineage gave rise to the nuclearioid line of phagotrophs represented by extant nuclearioid amoebae (141) and by *Fonticula*, a peculiar genus of social slime molds (10, 139). These organisms use slender, thread-like lobes to capture bacteria, yeast (94, 139), or, in some species, cyanobacteria, prey of size ranges that would have been available at the time of their origin.

Following the divergence of the free-living nuclearioid line, the fungal stem lineage gave rise to Aphelida and the endoparasitic taxa Cryptomycota and Microsporidia, some of which maintain phagotrophic nutrition. Three pivotal observations are stimulating active research on these deeply diverse organisms. Firstly, James et al. (51) discovered that the genus *Rozella* (**Figure 2a,b**) formed the basal-most branch of the chitinous fungi. Secondly, Jones et al. (55) revealed that diverse environmental DNAs related to *Rozella* constitute a new and variously named clade we shall, for convenience, call Cryptomycota (56). Lastly, James et al. (52) and Karpov et al. (60) showed that the Microsporidia, endoparasites so dependent on their host for primary metabolism that they lack mitochondria, form a clade with Cryptomycota and aphelids.

Mounting evidence shows that Microsporidia are derived through increasing specialization and genomic reduction from a *Rozella*-like ancestor (43, 52, 84). Phylogenetic intermediates with *Rozella* (Cryptomycota) include *Paramicrosporidium*, an intranuclear parasite of amoebae originally

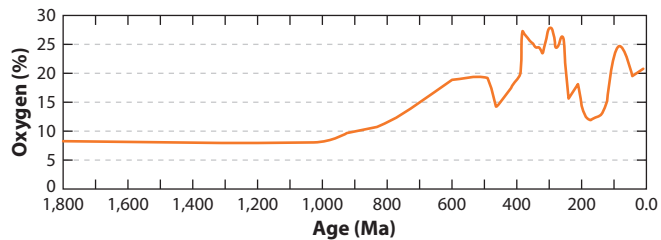
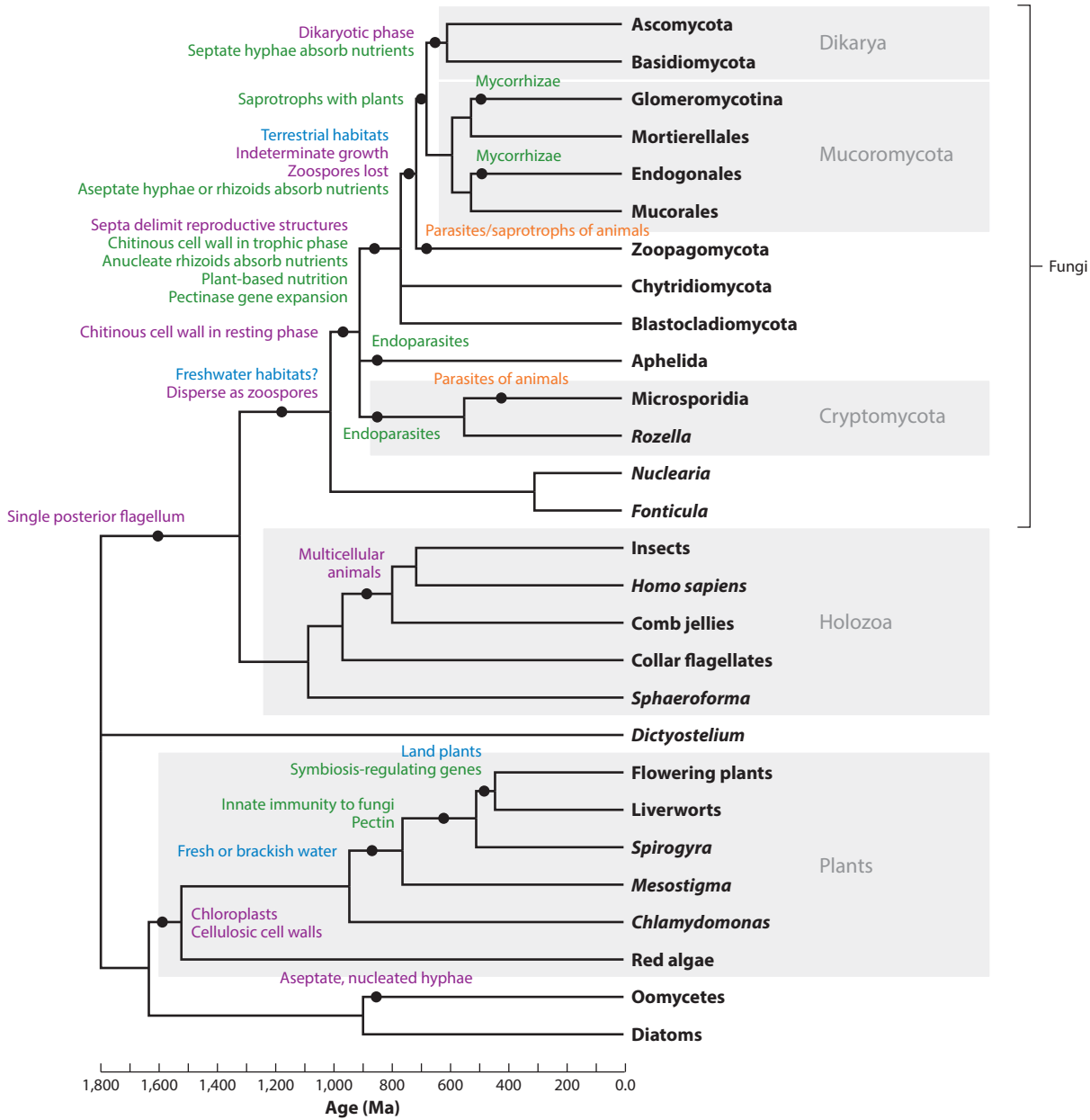
Holozoa:

multicellular animals and allied protists

Osmotroph:

an organism that absorbs dissolved nutrients that diffuse across its plasma membrane as opposed to engulfing food particles by phagocytosis

Hyphae: nucleated filaments that form the bodies of most fungi; characterized by tip growth and a chitinous cell wall



considered a microsporidium (18), and *Mitosporidium daphniae*, morphologically similar to microsporidia but with genomic similarities to *Rozella*.

Genomes of Cryptomycota and Microsporidia underwent reduction from about 6,500 proteins to fewer than 2,500 with loss even of genes for glycolysis (17). Microsporidia have the smallest genomes of any eukaryotes (61). Unresolved however, is the genomic content of the last universal common fungal ancestor since dramatic gene loss characterized all of the earliest diverging lineages. Traits that allowed osmotrophy across a chitinous fungal cell wall could have evolved in the common ancestor and been lost in endoparasitic lineages, or they may have evolved in the fungal stem after the endoparasites diverged. That the cryptomycotan *Rozella* has a full suite of fungus-specific, paralogous chitin synthases supports the possibility that the absence of a cell wall in its trophic stage is a derived adaptation (49, 131). What is known is that the genes important for plant-derived carbon nutrition are mostly lacking in the *Rozella* genome and appear to blossom in later branches (15). Added taxon sampling through single-cell genomics from uncultured lineages may yield further insights into the earliest steps in fungal evolution.

The divergence of Aphelida, Cryptomycota, and Microsporidia predates the major diversification of multicellular organisms and colonization of land, and it is no surprise that Cryptomycota and aphelid endoparasites lack invasive growth that would ramify through multicellular tissue; rather, they insert their phagotrophic or absorptive protoplasm into one host cell at a time. Aphelids are exclusively known as parasites of algae, including green algae species that may be used for biofuel production (73). Cryptomycota parasitize various microscopic hosts, including water molds, slime molds, amoebae, and algae (41). Few can be cultured, and it is easy to understand why they long remained cryptic.

Fossil evidence of host diversification is consistent with inferences about the great age of early protistan fungi. Phylogenomic molecular dating suggests unicellular planktonic cyanobacteria evolved in freshwater environments 1,600 Mya to 1,000 Mya (110). The geochemical record points to possible subaerial bacterial mats of cyanobacteria and eukaryotic green algae 850 Mya (138). Putative fossils of freshwater chlorophytes from Australia provide evidence of green algae from the Late Precambrian (3).

Terrestrialization: How Did the Earliest Fungi Colonize Land?

Terrestrialization could have occurred directly from the sea (marine route) (77), or colonization could have been first to freshwater and subsequently to land (freshwater route) (40). The salt-to-freshwater transition was difficult, based on its limited frequency in phylogenies (14, 21). Lozano-Fernandez et al. (80) merged molecular and fossil evidence to elucidate the deepest history of the terrestrial arthropods, of Myriapoda, Hexapoda, and Arachnida. Analyzing available fossil records of stem terrestrial lineages and their sister groups, they showed that a marine route to the colonization of land was the most likely scenario for independent Paleozoic terrestrialization in each of the three groups. This kind of approach might be used to improve understanding of the origins of fungal lineages.

Figure 1

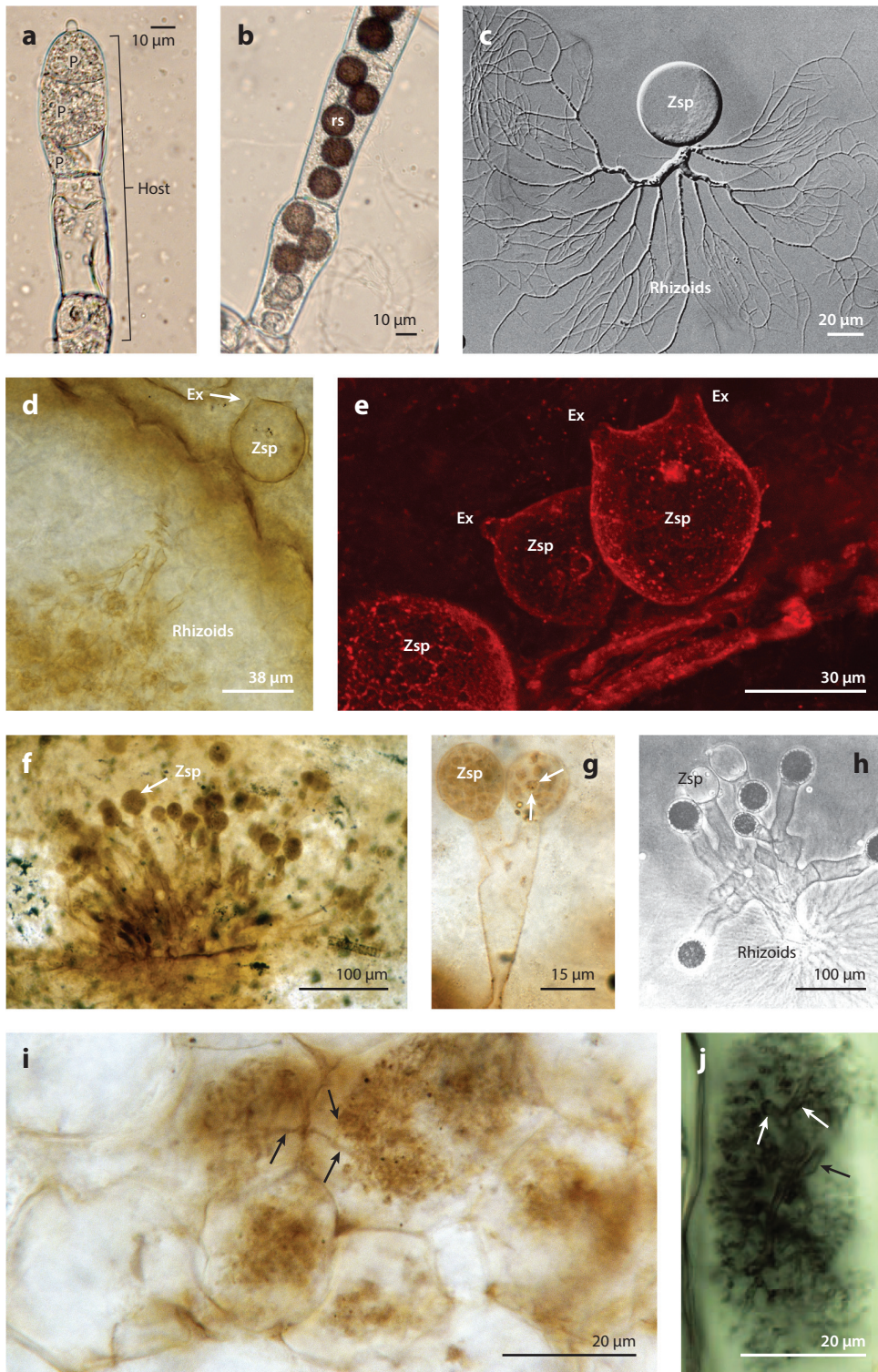
Early evolutionary events in fungi as related to eukaryotic hosts and symbionts. Text color designates the nature of evolutionary changes from a marine, unicellular, flagellated, and phagotrophic eukaryotic ancestor: blue, change in habitat; green, nutrition from plants or other nonanimal sources; orange, nutrition from animals; purple, change in form or genetics. Ages of fungi are from Chang et al. (15); others are from Parfrey et al. (93) or if not estimated elsewhere are arbitrary (e.g., Aphelida, Microsporidia, *Nuclearia*, *Fonticula*). O₂ levels are from Berner (7), Glasspool et al. (37), and Blamey et al. (9).

Chitin:

Tough polymer of *N*-acetylglucosamine that contributes rigidity to fungal cell walls and animal exoskeletons

Precambrian: eon from the origin of the Earth to 541 Mya

Terrestrialization: process through which aquatic organisms adapt to a subaerial or aerial lifestyle (76)



The fungal route to terrestrial colonization began in the seas, but phylogenetic habitat fidelity is difficult to discern among early diverging lineages because of inadequate sampling of habitats and taxa and perhaps also because of convergent adaptation over a vast amount of time. Fungi from early diverging clades have usually been cultured from freshwater, whereas they are increasingly detected among environmental DNA sequences from marine as well as freshwater sources (20, 60, 79, 105, 139). Possibly, single-cell genome sequencing followed by phylogenomic analysis of organisms that are known as yet only from environmental ribosomal DNAs will improve phylogenetic resolution and reconstruction of ancestral habitats of early fungi.

Relationships, Not Characters, Define Fungi

By one definition, the kingdom Fungi is characterized by osmotrophic nutrition across a chitinous cell wall (**Figure 1**), but this would partially or completely exclude the early diverging taxa in the fungal clade that may have lost chitinous walls as an adaptation to parasitism, while failing to exclude osmotrophic members of the Holozoa (131). Walls may initially have surrounded only resting stages, as in modern microsporidia; subsequently in early fungi, they may have taken on a role in infection or penetration (52). In *Rozella* and many other parasitic fungi, cell walls likely constrain and direct turgor pressure to help penetrate the cell wall of a host (109). Defining fungi based on walls requires a somewhat awkward suprankingdom taxon to include all species that contribute to interpretation of patterns of evolution over time. Excess terminology and unfamiliar names being impediments to appreciation of evolution and biology, we suggest simply defining the kingdom Fungi as the sister taxon of Holozoa (**Figure 1**).

ORIGINS OF OSMOTROPHY AND ITS IMPLICATIONS

The evolution of osmotrophy and chitinous cell walls in the shared ancestor of Chytridiomycota and Blastocladiomycota some 750 Mya restricted movement in search of food. Indicating that broad biosynthetic capabilities were ancestral, fungi, plants, and bacteria share homologous biosynthetic enzymes (12, 96). Multicellular animals lost enzymes for synthesis of essential amino acids (96) and vitamins (87), perhaps because they move and select food. Fungal species from deeply divergent clades, e.g., *Mucor pusillus* (Mucoromycota) (114) and *Aspergillus nidulans*

Figure 2

Early diverging fungi, (*a-c, b, j*) extant and (*d-g, i*) fossils from Rhynie Chert, aged 407 Ma, including (*a, b*) *Rozella* sp. (Cryptomycota) and host fungus *Allomyces* sp., (*c-e*) Chytridiomycota, (*f-b*) Blastocladiomycota, and (*i-j*) Glomeromycotina. (*a*) Three endoparasitic thalli and (*b*) spherical resistant sporangia of *Rozella* sp. in host fungus *Allomyces* sp. (*c*) *Rhizidium phycophilum*. Immature zoosporangium. (*d*) *Cultoraquaticus trewini*. Zoosporangium attached to, and producing rhizoids within, the wavy-walled, rounded structure of unknown affinity. (*e*) *C. trewini*. Confocal laser scanning image of fungal zoosporangia showing discharge papillae. (*f*) *Paleoblastocladia milleri*. Well-developed fungal tuft showing numerous zoosporangia. (*g*) *P. milleri*. Two zoosporangia; arrows indicate differentiating zoospore. (*h*) *Blastocladopsis elegans*. (*i*) Arbuscules in *Aglaophyton major*. Each polygonal plant cell contains one arbuscule. (*j*) Arbuscule in root cell. Arrows indicate coarse dichotomous hyphal branches toward the proximal end of an arbuscule. Abbreviations: Ex, discharge papillae; P, endoparasitic thalli; rs, spherical resistant sporangia; Zsp, zoosporangium. Photo credits: (*a, b*) Timothy James; (*c*) figure 1 in Reference 97, reprinted with permission from *Mycologia*, ©The Mycological Society of America; (*d, e*) Reference 119 (*PLOS ONE*); (*f, g*) Hans Kerp (103), reprinted with permission from *American Journal of Botany*, ©The Botanical Society of America; (*h*) figure 3 in Reference 106 (*Canadian Journal of Botany*); (*i*) Hans Kerp (129), reprinted with permission from *Mycologia*, ©The Mycological Society of America; (*j*) figure 11 in Reference 135 (*Physiologia Plantarum*).

Rhizoids: anucleate fungal filaments narrowing toward their tips, surrounded by a chitinous cell wall; function in anchorage and nutrient uptake

Streptophytes: land plants including bryophytes and flowering plants, along with their closest green algal relatives, such as *Chara* and *Spirogyra*

(Ascomycota) (5), grow on chemically defined media without added amino acids or vitamins. A long series of osmotrophic ancestral fungi had limited means to select their diet and consequently retained wide-ranging synthetic capabilities.

Chytridiomycota: Microscopic Osmotrophs

In contrast to the cylindrical, nucleated hyphae of most other fungi, the unicellular body plan of the Chytridiomycota (**Figures 1** and **2c,d**) typically includes radiating anucleate, walled rhizoids, each branching and narrowing toward a slender, acute tip (35, 59, 116). Like hyphae, rhizoids extend into a food source, secrete enzymes, and then absorb dissolved nutrients. The rhizoids feed a growing, roughly spherical or cylindrical zoosporangium that initially contains dividing nuclei and eventually produces zoospores. Growth is usually determinate, and rhizoids and the zoosporangium degenerate after a single round of zoospore release (116). Zoospores, once released, swim with their posterior flagellum and navigate using chemical cues (38) to find substrates such as pollen grains and unicellular algae that offer just enough calories to support one round of asexual reproduction (35). Over the course of their long evolutionary history, a few lineages of Chytridiomycota adapted independently to take advantage of multicellular food using indeterminate invasive growth (19). Most Chytridiomycota known from cultures have been collected by baiting—adding pollen, onion skin, or other substrates to water and then isolating the fungi from the bait into pure culture (35). This approach leaves much uncertainty about the range of substrates these fungi use for their nutrition in nature.

Genome Sequences Provide Evidence for Ancient Fungal Assault on the Ancestors of Land Plants

Indicating a long history of plant-based nutrition, early expansions of gene copies of fungal carbohydrate active enzymes targeting plant cell walls predate the divergence of Chytridiomycota from Ascomycota (**Figure 1**). Cellulose and pectin are important components in plant but not fungal cell walls, and so fungal cellulases and pectinases have unambiguous functions in nutrition, either breaking plant cells open to allow digestion of cell contents or solubilizing wall polymers as a direct source of nutrition. Many Chytridiomycota species degrade cellulose or cellobiose (39). Chytridiomycota, Ascomycota, and Basidiomycota share multiple homologs to several cellulase gene families (113).

Pectinases are good markers for plant-based nutrition because pectins are narrowly restricted to streptophytes (85, 115). The maximum age of pectins is constrained by the timing of streptophyte algal diversification; while open to debate, the age of pectin-containing streptophytes is estimated by Parfrey et al. (93) to be ~750 Mya. Chytridiomycota is the earliest diverging fungal lineage with diverse pectinases. The *Gonapodya prolifera* genome has 27 pectinases representing 5 of the known 7 classes of fungal pectin-specific enzymes (15). Tracking the genealogies of the pectinases shows that many are shared between the chytrids and Dikarya. The maintenance of the pectinase homologs through the hundreds of millions of years of evolutionary history suggests that most of the ancestors of modern fungi evolved using plant-based nutrition. Assuming that pectin-containing streptophytes evolved ~750 Mya (93) and that fungi developed the ability to digest pectins sometime later, this constrains the common ancestor of chytrids and the terrestrial fungi to have evolved more recently than 750 Mya (15).

Research into the streptophyte algae and land plants' conserved genetic machinery for fungal recognition corroborates a story of ancient evolutionary interkingdom interaction. Streptophyte plants share molecular sensors of fungal cell wall components, specifically, oligomers of the

N-acetylglucosamine molecules that make up chitin (75). To accomplish detection, plants ranging from advanced streptophyte algae to angiosperms share lysin motif receptor-like kinases and calcium- and calmodulin-dependent protein kinases (22, 137). To interact with their streptophyte algal hosts, early fungi must have inhabited fresh or brackish water. That freshwater algae had already evolved innate immunity to fungi stands as a record of ancient assault by fungi, not just saprotrophy or commensalism. The earliest fungi in Chytridiomycota and their sister clade were not just saprotrophs; at least some were also plant pathogens.

Ancient Fungi Would Have Survived “Snowball Earth” Global Glaciations 850–635 Mya

Metagenomic studies show that some Chytridiomycota survive extreme environments perhaps analogous to frigid Precambrian habitats during glacial intervals. Chytrids are associated with pollen or blooms of algae on alpine snow (11, 33, 34). Chytrid DNAs have been surprisingly abundant and diverse in metagenomic samples from arctic seawater (16). In sea ice samples, chytrids parasitize diatoms (44). How eukaryotic lineages survived global glaciations is unknown. However, the resilience of modern chytrids suggests that brine pools in sea ice and unicellular algal food may have sufficed to support the endurance of ancestral fungi through the “snowball Earth” glacial periods 850–650 Mya (140).

Fossils Document Ancient Chytridiomycota on Algae, Plants, and Fungi

Precambrian microfossils have been interpreted as, or directly compared to, chytrids (125), but they cannot be conclusively attributed to this group. Chytrid-like globular forms connected with filaments (rhizoids) are associated with aquatic algae, land plants, and land plant and fungal spores from the 407 Mya Rhynie Chert (**Figure 2d,e**; 66, 119, 125 and references therein, 127). Relatively complete epibiotic zoosporangia and endobiotic rhizoidal systems are evident in (a) *Krispiromyces discoides* (126) associated with the freshwater streptophyte alga *Palaeonitella cranii*, (b) *Cultoraquaticus trewini* (**Figure 2d,e**; 119) associated with an enigmatic freshwater organism, and (c) *Illmanomyces corniger* (66) colonizing a fungal spore, probably glomeromycotan. While its environment was not detailed, *I. corniger* was found in a loose accumulation of fragments of *P. cranii* and degraded land plant tissue suggestive of a freshwater environment. *C. trewini* and *I. corniger* have been compared with modern Chytridiomycota orders Spizellomycetales (4) and Rhizophydiales (74). *C. trewini*, with its aquatic habitat and narrow rhizoidal tips, is similar to Rhizophydiales (119). However the possibility that these organisms were Hyphochytridiomycetes (Heterokonta, a clade including oomycetes and brown algae) cannot be excluded. Some hyphochytrids have polycentric bodies, in which a broad hypha-like germ tube emerges, branches, and produces multiple zoosporangia (Hyphochytriaceae, e.g., *Hyphochytrium*; 58). One option in the future might be to document the composition of the cell wall at the submicrometer scale using focused ion beam (FIB) ultrathin sections extracted from rock specimens observed by transmission electron microscopy (TEM) and synchrotron-based scanning transmission X-ray microscopy (STXM) (e.g., 1) to detect chitin.

Blastocladiomycota as a Distinct Lineage of Chytrids

Blastocladiomycota (**Figure 2f–b**) is distinguished from other groups of chytrids, i.e., Chytridiomycota and Cryptomycota, on the basis of zoospore ultrastructure, life cycle, and phylogenetic position (51, 53). Its 14 described genera range from plant/algal pathogens to mosquito/midge

Indeterminate

growth: characteristic of hyphae that continue growing indefinitely, as long as nutrients and environmental conditions permit

Ordovician:

485–443 Mya; age of the earliest fossil evidence of terrestrial plants and fungi

Silurian:

443–419 Mya; age of the earliest fossil evidence of land plants with vascular tissue

Rhynie and**Windyfield cherts:**

Devonian rocks (~407 Mya) containing exquisitely preserved bacterial, algal, plant, animal, and fungal fossils

parasites to saprotrophs. No marine species of Blastocladiomycota are known. Some species are identical in morphology to the Chytridiomycota described above, while others have true hyphae with indeterminate growth (e.g., *Catenaria* and *Allomyces* species). Indeterminate growth may have evolved early in the clade, as it is observed in the diploid phase of the basal lineage *Physoderma*. Blastocladiomycota life cycles, where known, involve sporic meiosis with an alternation of a dominant diploid and shorter haploid generation. However, it is becoming increasingly clear that the early diverging zoosporic lineages of fungi (e.g., Chytridiomycota, Cryptomycota) are not haploid as expected based on the paradigm of an ancestral fungal life cycle with zygotic meiosis (52, 88). Most hyphal fungi clearly have zygotic meiosis.

Resolving the position of Blastocladiomycota within Fungi has dramatic implications for understanding the evolution of the kingdom. Blastocladiomycota present a combination of putatively ancestral (sporic meiosis and small number of genes for carbohydrate active enzymes) and derived [hyphal growth; regular septation; closed mitosis; and an apical secretion body, the Spitzkörper (134)] characters. A fully resolved phylogeny would help clarify which characters are homologous with the terrestrial fungi. Yet, even with multiple nuclear and mitochondrial genomes sequenced and phylogenomic analyses, it is unclear whether Blastocladiomycota diverged before or after Chytridiomycota (15, 71, 78, 117).

Fossils of Blastocladiomycota

The earliest convincing fossils of Blastocladiomycota also come from the Rhynie Chert (**Figure 2f,g**). *Paleoblastocladia milleri*, in the order Paleoblastocladiales, shares characters with Blastocladiomycota including alternation of gametothalli with sporothalli bearing terminal zoosporangia and resting sporangia (103, 128). It colonized partially degraded axes of the plant *Aglaophyton major*. Unlike the case of modern Blastocladiomycota (**Figure 2b**), preformed discharge sites were missing, as were chains of reproductive structures that have sympodial branches and gametothalli bearing single terminal zoosporangia. *Palaeozoosporites renaultii*, another fungus recently found at this site, had similar morphological features and colonized the inner cortex of the small rootlets arising from the rhizome of the plant *Asteroxylon mackiei* (121). Its thalli had branches lacking any septation or pseudoseptation and divided more or less isotomously to produce globose to elongated zoosporangia in small clusters and sympodially to produce resting sporangia possibly showing exit pores. The two fungal species colonized degraded plant tissues that might have been submerged by water, suggesting that the two fungi developed in freshwater.

Fossils of Hosts of Osmotrophic, Zoosporic Fungi

During the early Paleozoic, tasmanitids (resembling modern prasinophyte cysts) accumulated in transitional, brackish environments as the oldest recorded algal blooms (83). The most abundant Paleozoic freshwater chlorophytes, the Chlorococcales and chlorococcalean colonies, occurred in the Ordovician and achieved higher levels of organization in the Silurian (123), whereas charophytes radiated in the Silurian (83). These potential nutrient sources for fungi have also been recorded from Rhynie and Windyfield cherts (132). The (possibly brackish) freshwater was rich in cyanobacteria (30, 63, 65) and algae [*Palaeonitella cranii* (63) and prasinophytes (24, 70)]. Freshwater pools were likely steeped in the decomposing remains of plants from along their edges. These elements from Silurian–Early Devonian freshwater environments, although younger than the estimated ages of origin of Chytridiomycota and Blastocladiomycota, would have supported continuing diversification of zoosporic fungi.

HYPHAL FUNGI EVOLVED INVASIVE GROWTH AS TERRESTRIAL NUTRIENTS EXPANDED

From a common ancestor ~700 Mya, hyphal fungi diversified, giving rise to the ancient phylum Zoopagomycota and to Dikarya and its sister clade Mucoromycota (**Figure 2**). The hyphal form can be interpreted as an adaptation to invasive, heterotrophic growth. At hyphal tips, cell walls are not yet highly cross-linked, and as a result, they are extensible. Back from the tip, hyphal walls form rigid and inflexible cylinders, roughly 5 to 10 μm wide. Turgor pressure contained by the rigid walls contributes to driving elongation of growing tips. In addition, fungal protoplasm migrates, amoeba-like, usually toward the elongating tip (45, 46). The fungus deploys exploratory hyphae to search for fresh food and then forces hyphal tips filled with actively metabolizing organelles into nutrient-rich, living or dead plant or animal tissues (107). Like a car or a truck, a growing, exploratory hypha of a fungus has to have enough energy to proceed to the next fueling station. Hyphal fungi could not have diversified before the origin of food sources big enough to justify exploration and fuel successful reproduction.

Zoopagomycota: Animal Associates, Early Diverging Fungi

The phylum Zoopagomycota, recently erected based on phylogenomic analysis, encompasses three subphyla formerly included in Zygomycota: Entomophthoromycotina, Kickxellomycotina, and Zoopagomycotina (117). Zoopagomycota rely mostly on nonplant nutrition and are pathogens, parasites, or commensals of insects, microinvertebrates, amoebae, or other fungi or saprobes on dung or soil (6, 48). None are marine, with the exception of some gut-inhabiting Kickxellomycotina. While putative members of the group are plant parasites (e.g., the fern gametophyte parasite *Completozia*), their classification has not been confirmed with molecular sequences (48). Zoopagomycota may be coenocytic, lacking regular crosswalls in their hyphae, or they may have regular septa lacking any perforation (e.g., *Basidiobolus*) (42) or septa with complex pore structure (e.g., Kickxellomycotina) (122).

Species reproduce sexually with zygosporangia or asexually by aerial merosporangia or conidia. Given Zoopagomycota's deep phylogenetic distance from Mucoromycota and Dikarya, its aerial spore dispersal may represent independent innovation. Gain of aerial spores is hypothesized to be linked to loss of flagellated zoospores (50). *Olpidium*, an enigmatic zoosporic fungus lacking either hyphae or rhizoids appears in multilocus phylogenies at the base of Zoopagomycota (50, 112), which if supported by phylogenomic analysis, would strengthen the argument that hyphae and aerial spores of this clade evolved independently.

Fossils of Zoopagomycota are as yet unknown. The oldest fossils of their possible food—freshwater arthropods—are from the 407-Mya Rhynie and Windyfield cherts (111), too young to have supported their earliest origins. However, molecular dating is consistent with an assortment of older animal life that may have supported Zoopagomycota's early evolution (31).

Mucoromycota Tracked New Opportunities for Diversification as Streptophyte Algae Evolved into Land Plants

Spatafora et al. (117) erected Mucoromycota to unite former Zygomycota (Glomeromycotina, Mortierellales, Endogonales, and Mucorales) into a clade that is strongly supported in all recent phylogenomic analyses (15, 117, 130). Most of its species are irregularly septate to coenocytic and reproduce sexually with zygosporangia. Asexual reproduction differs from that of Dikarya and Zoopagomycota in that sporangia typically undergo internal cleavage into spores.

Arbuscular mycorrhiza (AM): mutualistic

Mucoromycota-plant symbiosis; the root receives minerals, which commonly improves growth, while the fungus receives photosynthate

Biotrophs: species that extract their nutrients from living hosts

Arbuscules: a highly branched, specialized hypha within a plant root cell wall and site of plant-fungus nutrient exchange

Phylogenomic and fossil evidence (**Figure 2i**) suggest that Mucoromycota used photosynthate from land plants for nutrition. Paradoxically, the estimated age of the first divergences in Mucoromycota from molecular dating (578 Mya) predates land plants by over 100 million years (**Figure 1**). The Mucoromycota age estimate may simply be too early. However, some evidence suggests that the significant accumulation of terrestrial photosynthate began 850 Mya, long before land plants appeared as fossils (64). Low $^{13}\text{C}/^{12}\text{C}$ ratios in carbonates modified by water runoff may reflect biomass created by microbial crusts (64, 69). This biomass could also have supported early diversification of hyphal fungi.

Fungi in arbuscular mycorrhizae (AM) facilitate plant growth (47, 133). When hyphae invade soil or rock, they secrete organic acids that expedite weathering, dissolving phosphorus, iron, and calcium (47). If involved in mutualistic symbioses, the hyphae can convey these otherwise poorly soluble minerals directly to plant cells. Fungal species in both the Endogonales and the Glomeromycotina form symbioses with early diverging embryophytes (8, 32). While the Mortierellales are not known to form mycorrhizae, they sometimes associate with roots (90) and can dissolve otherwise insoluble rock phosphate (91). The first land plants lacked roots, but experimental studies document that liverworts, extant rootless plants, can form mycorrhiza-like mutualisms with their thalli. Quirk et al. (100) showed experimentally that modern mutualistic fungal partners increased liverwort growth and P weathering two- to sevenfold compared with nonmycorrhizal controls. If liverworts can form mutualistic relationships with fungi, early, rootless land plants could have done likewise.

As biotrophs, mutualistic fungi typically secrete few enzymes, presumably because of selective pressure against release of effector molecules that would activate host plant defenses (27). The genome of the Glomeromycotina species *Rhizophagus irregularis* shows the tracks of a lineage long adapted to biotrophy, encoding exceptionally few secreted enzymes and possibly even relying on plant enzymes to allow it to enter into host cells to produce the highly branched fungal arbuscules that are the site of host-fungus nutrient transfer (**Figure 2i,j**; 130).

Evidence from genetic machinery shared among land plants for regulating symbiosis testifies to a long geological history of mycorrhizal relationships that might have been initiated with early Mucoromycota. Orthologs to the set of plant genes with known roles in establishing symbiosis with fungi (following more ancient initial signaling events described earlier) are missing from algal streptophytes but are widely shared by embryophytes, by land plants ranging from liverworts to flowering plants (22). This suggests that after a long period of assault by fungi on their close algal ancestors, early land plants tamed some of their attackers, leading to mutually beneficial AM relationships. Assuming that genes regulating symbiosis are restricted to plants that had evolved with mutualistic fungal partners, the age of the most recent common ancestor of embryophytes, ~471 Ma (29, 81) is also the minimum age for the mutualistic fungi, broadly consistent with molecular dating of Endogonales and Glomeromycotina.

Mucoromycota Fossils: Arbuscules and Spores Document Ancient Fungus-Plant Mutualism

Rhynie Chert fossils document mycorrhizal and spore structures typical of AM fungi in Glomeromycotina associated with rootless early land plants from 407 Mya (62, 125). Remy et al. (104) showed the first unequivocal evidence of arbuscules (**Figure 2i**) in the Rhynie Chert plant *Aglao-phyton major*. Later named *Glomites rhyniensis* (129), the fungus colonized the aerial, sinuous axes of the sporophyte. *Glomites sporocarpoides* (57) produced vesicles in the outer cortex of aerial axes of *Rhynia gwynne-vaughanii*; however, arbuscules were not observed. *Palaeoglomus boullardii* from

Horneophyton lignieri (120) resembled *Glomites* colonization in *Aglaophyton* and was also similar to mutualistic fungi observed in extant thalloid liverworts.

Recent discoveries reveal more diversity than assumed hitherto, overturning the long-held paradigm that the early endophytes were exclusively Glomeromycotina (120). A second mycorrhizal fungus from *H. lignieri*, *Palaeoendogone gwynne-vaughaniae* represented Endogonales; it packed the intercellular spaces in the corm (a rounded underground stem base) with thin-walled, aseptate hyphae and thick-walled fungal structures and was present as intracellular coils (120). Colonization by *P. gwynne-vaughaniae* showed some similarities with the symbiotic features described in *G. rhyiniensis* in *Nothia aphylla* (68) and resembled that observed in mutualisms of Endogonales with several modern plants (e.g., *Treubia*, several hornworts, *Lycopodium clavatum*, and *Lycopodium cernuum*) (120).

German & Podkovyrov (36) suggested that 1,000 Mya microfossils (*Mucorites tipheicus*) from Siberia resembled the modern mucoralean *Mucor tenuis* in morphological and developmental characters; however, it is difficult to confirm this affinity based on the images. Spores and hyphae from the Middle Ordovician (460 Mya) represented the oldest fossils of Glomeromycotina (101), and the oldest recorded terrestrial fungus. Four fungal reproductive units covered by hyphal mantles with possible affinities with the Glomeromycota or Endogonales have been documented from the Rhynie Chert (67 and references therein), as have spores showing a germination shield typical of Glomeromycotina (e.g., 25).

Hyphal Fungi, Weathering, and CO₂ Drawdown

Through mineral weathering, the common saprotrophic and mutualistic fungi in early environments (125) must have contributed to global carbon cycles, although the level of their effect is actively debated (28, 98, 100). Lenton et al. (72) extrapolated from moss microcosms and used climate modeling to suggest that Ordovician cation mobilization by nonvascular plants and their symbionts led to CO₂ drawdown that cooled the earth, explaining Ordovician glaciation. On the other hand, Quirk et al.'s (100) analyses using a liverwort model indicated that effects of cation release by ancient nonvascular plants would have been too small and too highly localized to account for dramatic climate change, whereas Mitchell et al. (86) argued that a diverse range of associations among cyanobacteria, fungi, and bryophytes rather than a single organism or association model would be needed to establish expectations. Porada et al. (98) countered with further studies using communities of artificial species similar to lichens and bryophytes, and resulting models predicted cooling enough to permit Ordovician glaciations.

While the climate-altering effects of fungi and rootless plants remain controversial, the trees that first appeared in the Middle Devonian (~395 Mya) and their fungal symbionts are acknowledged as important global bioengineers (89, 99, 124). By using extant mycorrhizal trees representing taxa of past forests, Morris et al. (89) demonstrated that in atmosphere conditions of elevated CO₂, as in the Middle Devonian, trees support larger hyphal networks and AM fungi increase physical alteration of silicate mineral surfaces. These studies show the benefits of a cross-disciplinary approach integrating paleontological, mineralogical, and geochemical analyses of paleosol sequences.

COMMENT: “FUNGI” 2,400 MILLION YEARS OLD VERSUS EVIDENCE FOR AGE OF ORIGIN OF HYPHAE

Bengtson et al. (5a) describe branching, 2,400-Ma-old filaments in basalt from deep marine volcanoes, which they interpret as possible fungi or extinct fungus-like organisms. They correctly point

out that the filaments are far older than any other corroborating evidence for opisthokont origins. We are skeptical about interpreting the filaments as fungi, because the deep marine habitat and morphology of the fossils are inconsistent with predictions about early fungi from phylogenetic and phylogenomic analyses. Further, the fossils lack organic components that would support a biological affinity, and a 1,900-Ma gap separates the filaments in basalt from the oldest convincing fungal fossils. Convincing fungal fossils from the Ordovician and Silurian, ~460–419 Mya, had organic walls and were fossilized in a presumed shallow marine setting (101) or were originally terrestrial, “occasionally washed into estuarine or marine settings” (112a). From philosophical and practical perspectives, disproving the possibility of an extraordinarily early age for hyphal fungi is difficult, but we look forward to future research that will undoubtedly take up the challenge.

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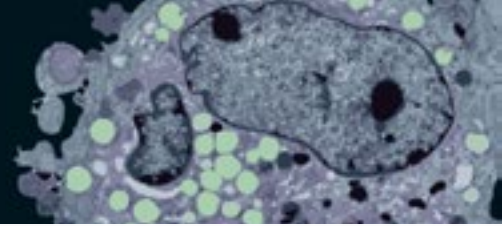
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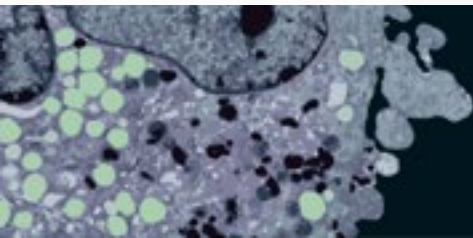
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