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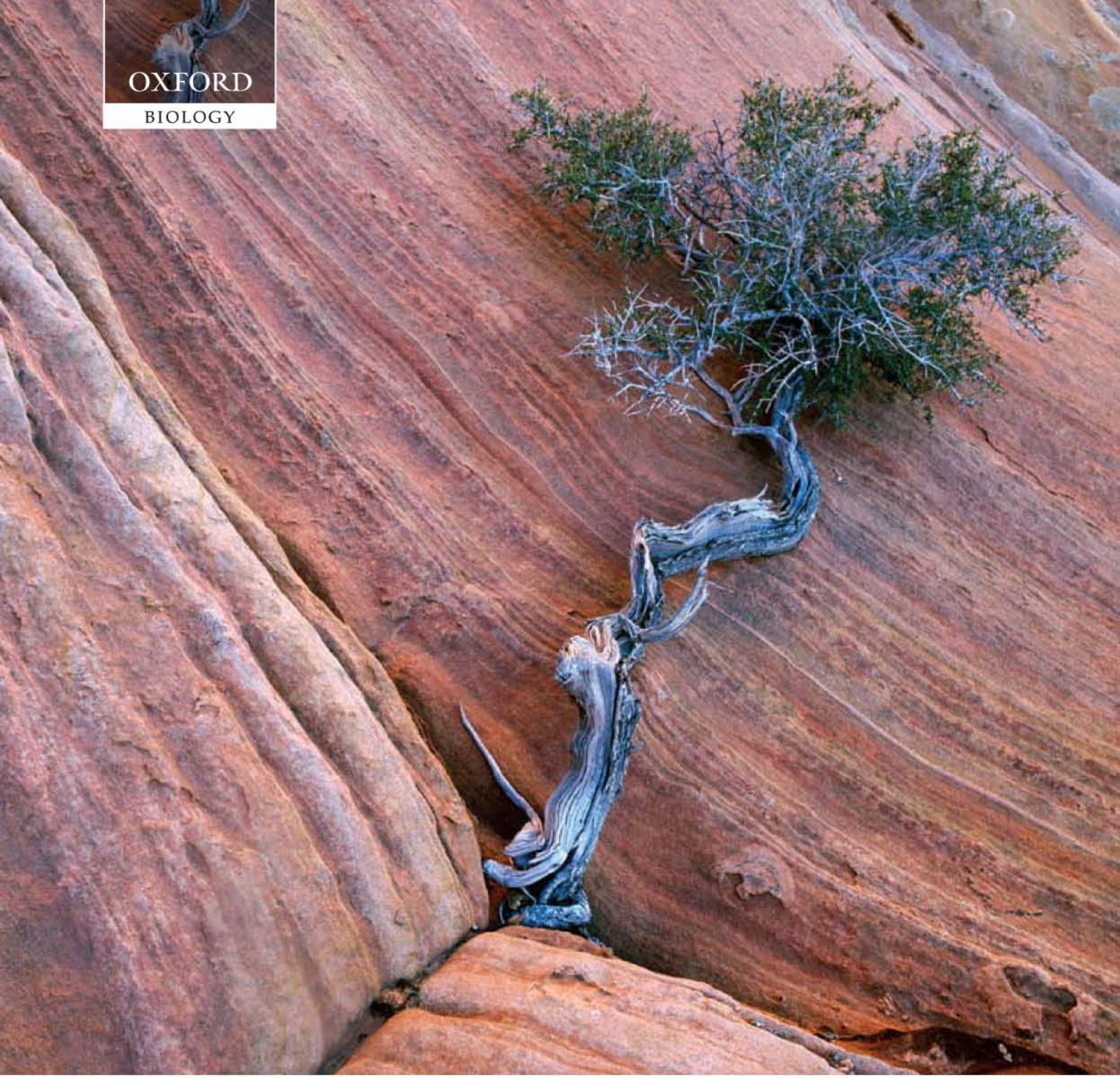

the TIMETREE of LIFE

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Fungi

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Abstract

Fungi are an essential constituent of modern terrestrial ecosystems, partnering with plants and other organisms in a range of symbiotic relationships. Recent phylogenetic analyses of multiple nuclear genes have challenged the traditional division of Fungi into four main groups, and a major reclassification of this kingdom has been proposed. The molecular timetree suggests that at least eight major fungal lineages originated in the Precambrian, before 542 million years ago (Ma), much earlier than their first appearance as fossils in the Ordovician, ~460 Ma. Fungi may have played a major role in the colonization of land by multicellular eukaryotes.

Members of Kingdom Fungi play an integral role in global nutrient cycling and are important symbionts with other eukaryotes and some prokaryotes. They are intimately associated with plants as mutualistic mycorrhizae and as the primary decomposers of lignin. Fungi are most closely related to Metazoa as part of the Opisthokonta, a eukaryotic supergroup which also includes unicellular choanoflagellates, icthyosporeans, and nucleariids (*1, 2*). While estimates vary (*3*), Kingdom Fungi may contain upward of several million species, with only a small fraction (~100,000) being formally described. Here I review the relationships and divergence times among the major fungal lineages.

Fossil evidence for the origin of Fungi is limited, which has confounded molecular time estimates due to a lack of robust fungal calibrations. Putative lichen-like structures first appear in the fossil record around 600 Ma (*4*), and some acritarch assemblages from the early Neoproterozoic have been associated with "higher fungi" (*5*). The first taxonomically identifiable fossils appear in the mid-Ordovician, ~460 Ma, and are similar to modern Glomeromycota (*6*). Diverse fungal remains from a variety of ecological niches then appear in the Devonian, ~400 Ma, including the well-preserved fossils of a probable pyrenomycete (*7, 8*). This fossil species has served as an important and often controversial calibration in some molecular clock studies (*9*). Basidiomycetes with diagnostic hyphal clamp connections are not found until the mid-Pennsylvanian, ~300 Ma (*10*), although evidence for wood decay similar to modern-day basidiomycetous white rot is present in the Upper Devonian (*11*).

Traditionally, fungi have been divided into four major groups: Ascomycota, Basidiomycota, Zygomycota, and Chytridiomycota. With the accumulation of molecular sequence data, it has become clear that both "zygomycetes" (*12*) and "chytrids" (*13, 14*) are not monophyletic assemblages. In addition, some lineages conventionally associated with Kingdom Fungi based on morphological similarities (e.g., oomycetes, slime molds) have been reclassified in separate eukaryotic supergroups (*1*). Conversely, the Microsporidia, obligate intracellular parasites once considered an ancient amitochondriate lineage, are now known to be closely related to, or perhaps nested within, Kingdom Fungi (*15*). Since 2003, a large, community-wide effort has established robust

Fig. 1 A basidiomycete (*Coprinopsis* sp.) from the Eastern United States. Credit: M. E. Hood (Amherst College).

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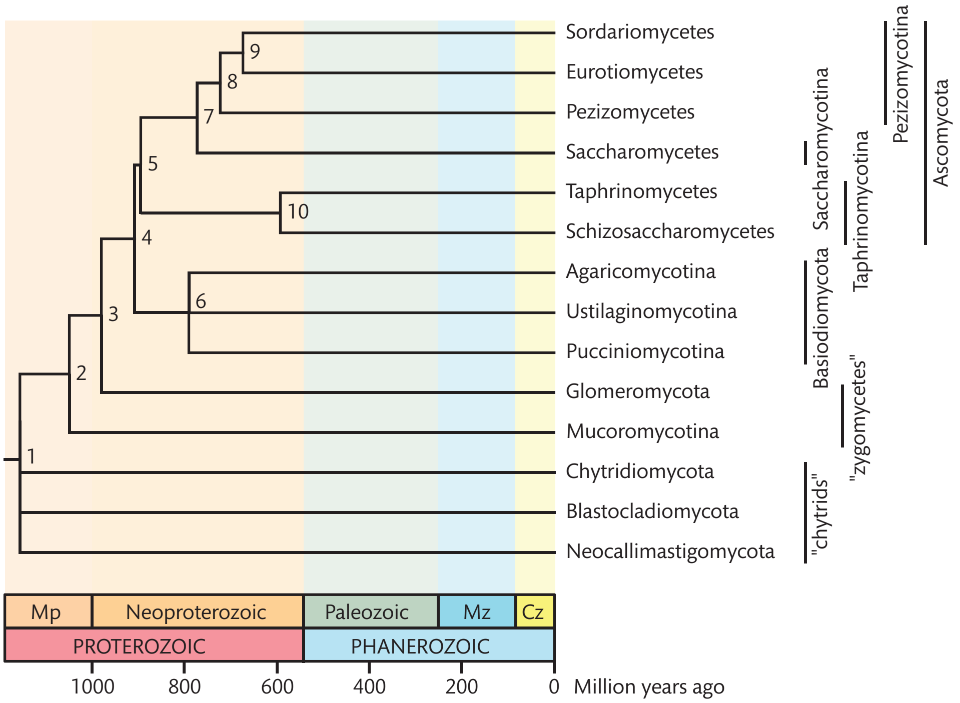

Fig. 2 A timetree of fungi. Taxonomic names are from a revised classification (*18*). Divergence times are shown in Table 1. *Abbreviations*: Cz (Cenozoic), Mp (Mesoproterozoic), Mz (Mesozoic), and Np (Neoproterozoic).

phylogenetic relationships among the main fungal lineages (*16*), as well as within most major groups (*17*). As a result of this phylogenetic reshuffling, a revised classification system for Fungi has been proposed (*18*), and will be followed here.

The majority of fungal species are found in the Phylum Ascomycota, which is divided into three monophyletic subphyla. The Taphrinomycotina, previously known as the "Archiascomycetes," is the most basal of the three subphyla, and contains a variety of ecologically and morphologically diverse taxa, including the model fission yeast *Schizosaccharomyces pombe* (*19*). The Saccharomycotina, or "Hemiascomycetes," are the commonly known yeasts, including beneficial species (e.g., *Saccharomyces cerevisiae*), as well as important human pathogens (e.g., *Candida albicans*). Molecular phylogenies have shown that many genera within the Saccharomycotina are not monophyletic (*20*). The Pezizomycotina, largest of the Ascomycota subphyla, includes filamentous species with diverse ecologically specialties, such as decomposers, plant and animal pathogens, lichens, and mycorrhizal symbionts (*21*). The Pezizomycotina, or "Euascomycetes," are currently divided into 10 classes, although the relationships among these lineages are still unresolved. Taxa previously known as plectomycetes and pyrenomycetes are now found predominantly in the Eurotiomycetes and Sordariomycetes, respectively. In addition, environmental sampling has revealed a distinct lineage of soil-dwelling fungi that may represent a fourth subphylum within Ascomycota (*22*).

The Phylum Basidiomycota (Fig. 1) also has been divided into three subphyla, but the relationships among these lineages remain ambiguous (*23, 24*). The Agaricomycotina, or "Hymenomycetes," includes the largest diversity of mushroom-forming and wood-decaying species (*25*). The Ustilaginomycotina contains many dimorphic plant pathogen "smuts," such as *Ustilago maydis* (corn smut) and *Tilletia indica* (karnal bunt of wheat), which cycle between saprobic yeast and parasitic hyphal stages (*26*). Similarly, the Pucciniomycotina, or "Urediniomycetes," are predominantly plant pathogen "rusts," such as *Puccinia graminis* (cereal rust). Rust fungi often utilize more than one host and can produce multiple spore types during their life cycle, complicating their taxonomic characterization (*27*). The Basidiomycota and Ascomycota form the Subkingdom Dikarya, reflecting their shared derived trait of dikaryotic (i.e., containing two haploid nuclei) and regularly septate hyphae (*28*).

Table 1. Divergence times (Ma) and their confidence/credibility intervals (CI) among fungi.

Timetree		Estimates										
Node	Time	Ref. (*33*)	Ref. (*34*)(a)		Ref. (*34*)(b)		Ref. (*35*)		Ref. (*36*)		Ref. (*37*)	
		Time	Time	CI	Time	CI	Time	CI	Time	CI	Time	CI
1	1156	850	1423	1434–1270	893	928–857	1458	1595–1321	–	–	–	–
2	1049	–	1287	1411–1216	856	909–812	1107	1217–997	947	1047–847	–	–
3	980	600	–	–	–	–	–	–	–	–	–	–
4	908	550	1206	1339–1165	786	848–757	1208	1429–996	968	1195–741	727	837–629
5	895	450	1148	1306–1108	724	812–691	1144	1295–993	1009	1152–866	–	–
6	791	500	1028	1167–966	669	728–630	966	1135–797	–		–	–
7	773	375	1072	1244–1051	657	756–632	1085	1244–926	982	1166–798	466	564–368
8	723	325	972	1163–955	570	686–535	–	–	–	–	–	–
9	673	–	930	1141–921	539	666–510	670	809–531	551	671–431	–	–
10	592	300	928	1189–770	549	689–448	–	–	–	–	–	–

Note: Node times in the timetree represent the mean of time estimates from different studies, except for Nodes 3 and 8 where Node times represent the midpoint between adjacent nodes. Estimates in ref. (*34*) are from a single gene study using a value of 1576 Ma (a) and 965 Ma (b) for the animal–fungal divergence calibration.

Aside from the well-supported Dikarya, the basal lineages of Fungi are currently in a state of phylogenetic and taxonomic flux. As mentioned earlier, the traditional "Zygomycota" phylum has been discarded in the current classification scheme, and its members have been placed in one new phylum, Glomeromycota (*29*), and four subphyla of uncertain placement (Mucoromycotina, Entomophthoromycotina, Zoopagomycotina, Kickxellomycotina). Molecular evidence suggests that Glomeromycota, which includes the arbuscular mycorrhizal symbionts of plants, may be the closest relative of Dikarya (*16, 30*). The Mucoromycotina, a group which includes the common molds *Rhizopus* and *Mucor*, has been shown to be the next closest relative of Dikarya + Glomeromycota (*12, 16*). Species with flagellated motile spores, the "chytrids," are extremely diverse in their ecologies and represent the most basal lineages of Kingdom Fungi. These organisms have been divided among three phyla (Chytridiomycota, Blastocladiomycota, and Neocallimastigomycota), and the relationships among these lineages have not yet been resolved. Microsporidia has recently been classified as a fungal phylum, although it is still unclear whether it is nested within the kingdom (*16*) or as a close relative of Fungi (*14*).

Molecular clock studies have produced a range of time estimates for the origin and diversification of Fungi. Early studies using a single gene, the small subunit ribosomal RNA, generally found young divergence times consistent with the fossil records of fungi and plants, which were used as the source of calibration points (*31, 32*). Rate variation among lineages was noted even in these early studies. A later revision produced slightly older dates (*33*), suggesting the major lineages of fungi had diverged before the colonization of land by plants (Table 1). Also, the origin of the Pezizomycotina was found to be younger than 400 Ma, leading authors to suggest that the Devonian pyrenomycete fossil more likely represented an early "Archiascomycete" (*33*). Issues of rate variation and fossil calibration were addressed in a more comprehensive study of the small subunit ribosomal RNA, which used a rate smoothing clock method to estimate divergence times among 169 fungal taxa (*34*). In this study, two different calibration values for the animal–fungal divergence (1576 vs. 965 Ma) were tested, and produced dramatically different time estimates (Table 1). These authors claim that the use of the older estimate for the animal–fungal calibration may be more appropriate as it accommodates the Devonian pyrenomycete fossil (*34*).

Studies using multiple genes and a variety of molecular clock methods have generally found ancient divergences among the fungal lineages. The first multigene analysis of Fungi (*35*) estimated deep Precambrian divergences for all major lineages. This study also estimated an origin for the land plant lineage ~700 Ma, suggesting that terrestrial ecosystems of multicellular eukaryotes may have existed millions of years before the first fossil evidence of such in the Ordovician (*35*). A later reanalysis (*36*) using additional data and more complex molecular clock methods produced similar estimates (Table 1). An analysis of 129 proteins from 36 eukaryotes suggested younger divergences within Fungi, although only a few fungal lineages were included in this study (*37*). The availability of complete genome sequences from 15 eukaryotes allowed the divergence between Pezizomycotina and Saccharomycotina to be estimated at ~850 Ma (*38*). Analyses of a 50-gene data set also compiled from complete genome sequences illustrated how the use of external versus internal calibrations can affect divergence time estimates both within and among the plant, animal, and fungal kingdoms (*9*).

The molecular timetree shows that, while some phylogenetic uncertainty exists, all major lineages of Fungi likely originated in the Neoproterozoic (Fig. 2). The timetree also supports the interpretation of the Devonian pyrenomycete fossil as a member of the Pezizomycotina, perhaps associated with the Sordariomycetes. There are significant dissimilarities between the molecular clock estimates and the fossil record, which is not unexpected due to the microscopic nature of most fungal species, their poor preservation potential, and the scarcity of mycologically trained paleontologists (*39*). For some groups (e.g., the classes of Basidiomycota, Microsporidia), no molecular time estimates currently exist, leaving only the fossil record to infer their minimum times of origin.

The origin of Kingdom Fungi was almost certainly aquatic, but whether this initial environment was marine or terrestrial remains unclear (*16*). The early-diverging lineages of "chytrid" fungi retain flagellated spores, a trait believed to be homologous with their single-celled Opisthokont relatives (*2, 40*). This character has subsequently been lost multiple times during the evolution of Fungi (*13, 16*), and the vast majority of extant fungal species occupy terrestrial niches with various methods of spore dispersal. The diverse associations between fungal species and other organisms, as lichens, mycorrhizae, pathogens, and decomposers, suggest that Fungi played a crucial role in shaping the paleoecosystem as multicellular eukaryotes colonized land (*41, 42*), perhaps as early as the Neoproterozoic. The rapid accumulation of genome sequence data from a number of Fungi, along with improved molecular clock methods optimized to model rate variation, will allow for further refinement of fungal relationships and divergence times.

Acknowledgments

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