

Discussion of Phylogenetic Relationships

The kingdom Fungi is a diverse clade of heterotrophic organisms that shares some characters with animals such as chitinous structures, storage of glycogen, and mitochondrial codon UGA encoding tryptophan. Both animals and fungi have spores or gametes with a single smooth, posteriorly inserted flagellum, but only species of the basal chytrid phyla have retained this primitive character (Barr, 1992; Cavalier-Smith, 1987, 1995). Fungi, animals, and other heterotrophic protist-like organisms such as choanoflagellates and Mesomycetozoa are now considered part of the larger group termed opisthokonts (Cavalier-Smith, 1987) in reference to the posterior flagellum.

The branch uniting the fungi and animals is well-supported based on a number of molecular phylogenetic datasets, including the nuclear small subunit ribosomal RNA gene (Wainwright et al., 1993; Bruns et al. 1993), unique and shared sequence insertions in proteins such as elongation factor 1 α (Baldauf and Palmer, 1993), entire mitochondrial genomes (Lang et al., 2002), and concatenated protein-coding genes (Steenkamp et al., 2006).

Prior classification systems of Fungi based primarily on morphology are in need of updating to more accurately reflect phylogenetic relationships as determined by molecular systematics. Molecular characters have been essential for phylogenetic analysis in cases when morphological characters are convergent, reduced, or missing among the taxa considered. This is especially true of species that never reproduce sexually, because characters of sexual reproduction traditionally have been the basis for classification of Fungi. Use of molecular characters allows asexual fungi to be placed among their closest relatives.

Previous classifications placed early-diverging fungal groups (non-Ascomycota or Basidiomycota) into two phyla: Chytridiomycota and Zygomycota. Numerous phylogenetic studies now suggest that neither is monophyletic, and the latest classification scheme includes six phyla and an additional four unplaced subphyla (Hibbett et al., 2007). At present, because of the ancient divergence times between the fungal phyla, the exact phylogenetic relationships are ambiguous. Chytrids appear to be a paraphyletic group at the base of the fungal phylogeny and merely fungal lineages which have retained the character of flagellated spores. Three phyla of flagellated fungi are proposed (Blastocladiomycota, Chytridiomycota, and Neocallimastigomycota; Hibbett et al., 2007) and two chytrid genera *Olpidium* and *Rozella*, are of uncertain phylogenetic position (James et al., 2006a, 2006b). These genera are interesting because they are both highly reduced endoparasites (living inside the host cell) whose entire thallus consists of only a spherical body absorbing nutrients from the host material that surrounds it. *Rozella* appears in an isolated position in the fungal phylogeny as the very earliest lineage to diverge from the rest of the fungi (James et al., 2006a, 2006b). In contrast, *Olpidium brassicae* appears to have diverged after the majority of chytrids and is more closely related to some zygomycete fungi (James et al., 2006a, 2006b).



Figure 7: The endoparasitic chytrid *Rozella allomyces* inside the hyphae of another chytrid *Allomyces*. Thick spiny spores of the parasite are seen inside some cells while zoospores are produced in other cells. © Timothy Y. James

Fungi with non-septate or irregularly septate hyphae and thick-walled spores were traditionally placed in the phylum Zygomycota. However, evidence for a monophyletic Zygomycota is lacking (Seif et al., 2005), and the deconstruction of the Zygomycota into four unordered subphyla (Entomophthoromycotina, Kickxellomycotina, Mucoromycotina, Zoopagomycotina) has been proposed (Hibbett et al., 2007). The separation of the superficially similar arbuscular mycorrhizal fungi (that lack septa in hyphae but also lack zygospores) into the phylum Glomeromycota has been previously proposed (Schüßler et al., 2001). Whether this phylum is more closely related to the Ascomycota and Basidiomycota lineage or to other zygomycete lineages is controversial (Redecker et al., 2006).

Evidence from shared morphological characters such as regularly septate hyphae and a dikaryotic stage (two separate and different nuclei in a single hyphal segment) in the life cycle usually has been interpreted as support for a close relationship between Basidiomycota and Ascomycota. Numerous phylogenetic studies such as SSU rDNA (Berbee and Taylor, 1992), RNA polymerase genes (Liu et al., 2006), and mitochondrial genome sequencing (Seif et al., 2005) provide strong support for this relationship. A subkingdom termed Dikarya is proposed (Hibbett et al., 2007), creating a division between a highly speciose subkingdom (Dikarya) and the remaining early diverging lineages whose relationships are not precisely known.

Fungal classification is far from static, and even which organisms are actually members of Fungi is changing. For example, the group trichomycetes describes gut inhabitants of arthropods that share similarities with zygomycetes. Molecular phylogenetic studies have demonstrated that two of the four orders of trichomycetes are actually members of the Mesomycetozoa protist group (Benny and O'Donnell, 2000; Cafaro, 2005). Other organisms that were previously considered to be Fungi because of their heterotrophic, mold-like growth forms are now classified as stramenopiles (Oomycota, Hyphochytriomycota, and Labyrinthulomycota) or slime molds (Myxomycota, Plasmodiomycota, Dictyosteliomycota, Acrasiomycota) (Bhattacharya et al., 1992; Leipe et al., 1994; Van der Auwera et al., 1995). More interesting for mycologists are the findings that some species previously considered protozoa are actually Fungi. For example, the species *Hyaloraphidium curvatum* was assumed to be a green alga that had adopted a heterotrophic lifecycle concomitantly with losing its chloroplast. It is now known to be a chytrid fungus related to Monoblephariomycetes but lacking a flagellated stage (Ustinova et al., 2000). Other examples include the parasitic organisms presumed to be protozoa, such as the cockroach parasite *Nepriediophaga* (Wylezich et al., 2004) and the *Daphnia* parasite *Polycarum* (Johnson et al., 2006) recently demonstrated to be members of the fungal kingdom based on SSU rDNA phylogenies.

The most revolutionary addition to the fungal lineage has occurred with phylogenetic evidence indicating the protist group microsporidia is closely related to Fungi—possibly derived from zygomycetes (Keeling, 2003) or sister to the genus *Rozella* on the earliest branch in the fungal kingdom (James et al., 2006a). Microsporidia are highly specialized intracellular parasites (primarily of animals) that lack mitochondria but have chitin and trehalose in their spores (similar to Fungi). All molecular studies have shown that microsporidia evolve at an extremely accelerated rate of evolution, making their placement in the Tree of Life difficult. The relationship with fungi is supported by many single and multiple gene phylogenies (e.g., Liu et al., 2006), but an exact placement within the fungi has not received strong support (Keeling and Fast, 2002).

More recently the nucleariid amoebae have been demonstrated to be a sister group to the Fungi with strong support (Steenkamp et al., 2006). This finding is significant because Nuclearia lacks a cell wall and has phagotrophic nutrition in which the food source (such as a bacterium or algal cell) is engulfed wholly, unlike fungi and microsporidia which utilize absorptive nutrition. Further sampling of basal fungal lineages will be needed to determine whether a Nuclearia-like organism was the cenancestor (most recent common ancestor) of Fungi.

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