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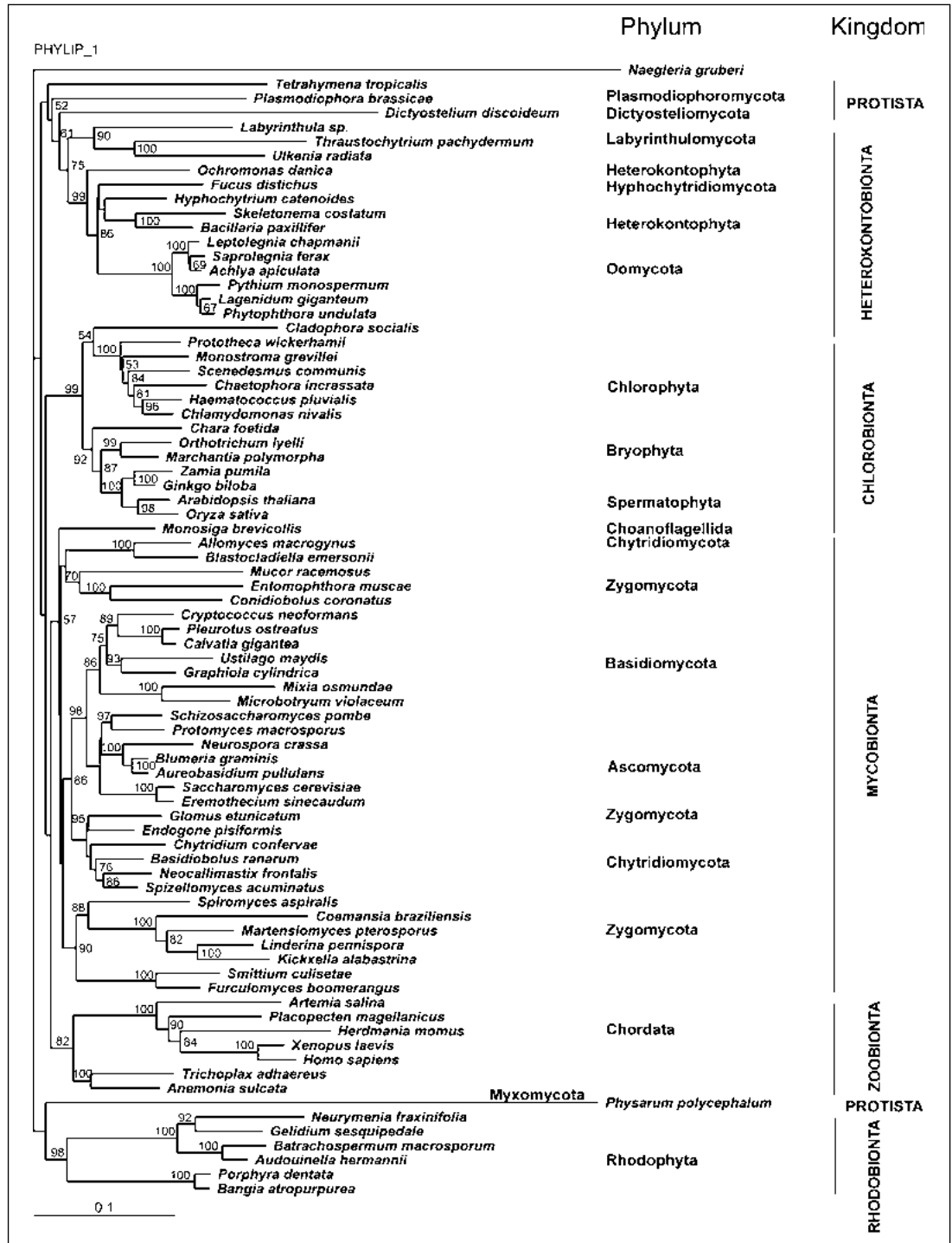
Phylogeny and Systematics of the Fungi with Special Reference to the Ascomycota and Basidiomycota

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In 1965, Zuckerkandl and Pauling [1] argued that sequence comparison of informational macromolecules permits the evaluation of evolutionary relatedness, thereby fomenting a phylogenetic revolution, especially in prokaryotic organisms and protists [2, 3]. Protista were once considered as a distinct third kingdom besides animals and plants by Haeckel [4]. Kimura's neutral theory of molecular evolution also had an impact on studies of the phylogeny and evolution, especially of microorganisms with an inadequate fossil record [5–7]. Meanwhile, molecular systematics has revolutionized our understanding of the microbial world. Currently, phylogenies of the Eukarya depend principally on small [3, 8, 9–17] or large [18–24] ribosomal RNA (rRNA) subunits, although 5S rRNA [25, 26] and a number of protein sequences [27, 28] also influence phylogenetic interpretations. Based on 18S rDNA sequencing, the Ascomycota and Basidiomycota form monophyletic clades within the kingdom Mycobionta or chitinous Fungi (fig. 1) [14, 29–31]. Defined by a membrane-bounded nucleus, the kingdom Mycobionta is one of several kingdoms within the crown groups of the Eukarya or eukaryotes (fig. 1).

Dedicated to Dr. C.P. Kurtzman on the occasion of his 60th birthday and for his valuable help in establishing the VIAM culture collection.



The Eukarya constitute one of the three principal domains of life [32]. According to Knoll [33], the Eukarya are an ancient group, as old as the prokaryotic Bacteria and Archea, or nearly so [2, 8]. Paleontological and biogeochemical data suggest that Eukarya were significant organisms of ecosystems at least as early as 1,700–1,900 million years ago [33]. Sequence analyses of protein-encoding genes that duplicated before the divergence of the domains now suggest that the general tree of life should be rooted between the Bacteria and Archea, with the Eukarya bearing a specific phylogenetic relationship to the Archea [34, 35].

Within the Eukarya, the earliest diverging organisms (not shown in fig. 1) are aerotolerant anaerobes, most of which live parasitically or symbiotically within animal hosts (microsporidia, diplomonads, oxymonads, hypermastigids, parabasalids and some others) [3, 8, 36]. Microsporidia contain many promising species for biological control of harmful insects. It is remarkable that microsporidia cluster within the kingdom Fungi or Mycobionta (fig. 1) if gene sequences encoding the largest subunit of the RNA polymerase II or the elongation factors EF-1 α and EF-2 are used [37]. Doolittle [28] stresses the importance of lateral gene transfers in prokaryotic and eukaryotic evolution. This, however, may complicate phylogenetic interpretations. The diplomonad *Giardia* infects the human intestine and can cause diarrhea, a disease known as giardiasis, or 'hiker's diarrhea'. These organisms have a well-defined nucleus and flagellum apparatus, but no mitochondria or chloroplasts and are included in the kingdom Archezoa [38]. Representatives of the Archezoa have relatively simple cytoskeletons and exhibit a number of ultrastructural (e.g. extranuclear pleuromitosis) [39–41] and biochemical characters more similar to those of prokaryotes than to other eukaryotes [38].

Protists occupying the middle branches of the phylogenetic tree [33] of the Eukarya commonly contain mitochondria, but no chloroplasts. Euglenids are the exception; about one third of them are photosynthetic. Euglenid chloroplasts may be derived from symbiotic green algae [13], implying a relative late acquisition of photosynthesis within this group. The amoebaflagellate *Naegleria* (fig. 1) is considered to be one of the earliest diverging protists with mitochondria. Nuclear rRNA phylogenies support this view. Heterolobosea emerge at

Fig. 1. Phylogenetic tree of eukaryotic organisms based on the primary structure of the 18S rRNA gene. Complete sequences of the 18S rRNA gene were aligned by means of the CLUSTALX program [44]. Software package PHYLIP [45] was used for phylogenetic inferences. Distance matrix was constructed in the DNADIST program (Kimura 2 parameter model) and the FITCH program was used for calculating phylogeny. The phylogenetic tree was displayed in TREEVIEW [46]. Branch lengths are proportional to nucleotide differences and the numbers given on branches represent the percentage of frequencies with which a given branch appeared in 100 bootstrap replications. The sequences were retrieved from the nucleotide sequence libraries (EMBL, GenBank and DDBJ).

the base of mitochondria-bearing eukaryotes, together with trypanosomids and euglenoids. All three taxa feature extraordinarily long branch lengths [3] (fig. 1). Although predominantly aerobic, organisms in this part of the phylogenetic tree commonly thrive under relatively oxygen-poor conditions [42].

Most eukaryotic diversity is nested within the densely branched crown of the phylogenetic tree (fig. 1) [3, 13, 33, 43]. Major clades that branch near a common point include the kingdoms (fig. 1) Zoobionta or Animalia (Metazoa + unicellular relatives), Chlorobionta (green algae and terrestrial plants), Mycobionta (chitinous or true fungi), Heterokontobionta (Stramenopila or chromophyta: golden brown algae, diatoms, brown algae, oomycetes, slimenets), Rhodobionta (red algae) and Alveolobionta (Alveolates: ciliates, dinoflagellates, apicomplexans; not shown in fig. 1) [3]. Because of rapid diversification, branching order within the crown group of Eukarya remains uncertain.

Mitochondria and chloroplast genomes have molecular sequences that ally them to the Bacteria (proteobacteria and cyanobacteria) [47, 48]. The sequence data are congruent with ultrastructural and biochemical evidence supporting the endocytobiotic theory for the origins of these organelles [49, 50]. Molecular data are in agreement with a multiple origin of plastids, with some plastids originating from prokaryotic (simple plastids: chloroplasts, cyanelles, rhodoplasts) and others from eukaryotic (complex plastids: cryptophytes, haptophytes, heterokontophytes, euglenophytes, chlorarachniophytes, dinoflagellates) algae [13, 43].

The Kingdom Mycobionta (Eumycota) or True Fungi

Among earlier phylogenetic speculations extensively discussed in Jahrmann and Prillinger [51] and Barr [52], the concept of Cavalier-Smith [53, 54] and Prillinger [41, 51, 55] is noteworthy. Based on a framework of data on cell wall chemistry, biosynthetic pathway of lysine, storage carbohydrates, ultrastructure of mitochondrial cristae, type of motile cells and ploidy of vegetative hyphae, Cavalier-Smith and Prillinger only considered the chytridiomycetes, zygomycetes, ascomycetes and basidiomycetes as true or chitinous fungi and included them in the kingdom fungi or Eumycota. These four fungal groups are characterized by chitinous cell walls [56, 57], the α -amino adipic acid lysine biosynthetic pathway [58, 59], glycogen as storage carbohydrate [54], nondiscoid plate-like mitochondrial cristae [54], the absence of heterocont flagella, and the absence of diploid vegetative hyphal compartments in the higher Ascomycota and Basidiomycota (exceptions: forced heterokaryons: e.g. *Aspergillus*, *Penicillium*; solopathogenicity in the smuts: *Ustilago*; Hymenomycetes: *Armillaria* [41, 60]; see Oomycota [61]). The primarily heterotrophic origin of this group is extensively discussed by Jahrmann and Prillinger [51]. Within the Eumycota the

chytridiomycetes are considered basal, the Entomophthorales (zygomycetes) evolved from a chytridiomycete by loss of the flagella [54]. Based on a single posterior flagellum (opisthokont), flattened, nondiscoid mitochondrial cristae, a chitinous exoskeleton, storage of glycogen instead of starch, lack of chloroplasts, and the code UGA for tryptophan, not chain termination, in their mitochondria, Cavalier-Smith [54] suggests a common origin of the true fungi with animalia and choanoflagellate protozoa (fig. 1).

The oomycetes, hyphochytrids, labyrinthuloids, and thraustochytrids are included in the kingdom Heterokontobionta or pseudofungi [54] based on the presence of cellulose in their cell walls, a tubular mitochondrial crista, heterokont flagella, one decorated with tripartite hairs, and the α , β -diaminopimelic acid lysine biosynthetic pathway. The slime molds were classified into the kingdom Protozoa [38].

Evidence from complete 18S rDNA sequence divergence (fig. 1) [29, 62] put an end to the discussion on the kingdom Eumycota or true fungi and corroborated the existence of four naturally related phyla or divisions: the Chytridiomycota, the Zygomycota, the Ascomycota and the Basidiomycota within the kingdom Fungi or Mycobionta (fig. 1) [63, 64] or Eumycota [41, 52]. Specific acyclic polyols [65], an exclusively absorptive or lysotroph nutrition [66] and a distinct ultrastructure of the flagellar apparatus of the Chytridiomycota [52] are additional characteristics which support the kingdom Mycobionta.

Based on the complete sequence of the 18S rRNA gene and the amino acid sequence of the elongation factor, the Animalia or Zoobionta appeared as a sister group of the Mycobionta or true fungi (fig. 1) [3, 67–72]. Nikoh et al. [73] come to a similar conclusion from a phylogenetic analysis of 23 different proteins. A closer phylogenetic relationship of Zoobionta and Chlorobionta, however, becomes apparent from homologous comparisons of ribosomal proteins [74]. The protozoal Choanoflagellida are phylogenetically closely related to the Zoobionta and Mycobionta (fig. 1) [52].

Prototheca is a ubiquitous achlorophyllous green alga (fig. 1) that lives on decaying organic matter and exhibits a yeast-like growth pattern. Human infection usually involves the skin and underlying tissues. *P. wickerhamii* (fig. 1) is recovered most often from human specimens, while *P. zopfii* usually is associated with infections in animals [75].

In the phylogenetic trees of Bruns et al. [29] and Sugiyama [14], the phagotrophic plasmodial slime molds (Myxomycota) and cellular slime molds (Dictyosteliomycota) diverged prior to the terminal radiation of eukaryotes (fig. 1). Presently no data are available on the cellular Acrasiomycota. In contrast, parsimony analysis of amino acid sequences of EF-1 α , a protein involved in the translation of messenger RNA, strongly supports a monophyletic origin of the Dictyosteliomycota and Myxomycota and the amoebflagellate protostelid

Planoprotostelium (kingdom Mycetozoa). Among the multicellular eukaryotes, the Mycetozoa appear closer to Animalia and true fungi than to green plants [27]. The use of EF-1 α emphasizes the importance of developing multiple sequence data sets. As a conclusion, the phylogeny of the Acrasiomycota, Dictyosteliomycota and Myxomycota remains uncertain at the moment, and additional sequence data are urgently needed. Based on 18S ribosomal DNA sequencing, the plant parasitic slime mold *Plasmodiophora brassicae* (Plasmodiophoromycota), a severe pathogen of crucifers, may be more closely related to the Alveolobionta than to any of the fungi [76].

The Oomycota (fig. 1, *Achlya*, *Lagenidium*, *Leptolegnia*, *Phytophthora*, *Pythium*, *Saprolegnia*), Hyphochytridiomycota (fig. 1, *Hyphochytrium*) and net slime molds or Labyrinthulomycota (fig. 1, *Labyrinthula*, *Thraustochytrium*, *Ulkenia*) form a clade with brown algae (Phaeophyceae), diatoms (Bacillariophyceae), Chrysophyceae, Xanthophyceae, and Chloromonadophyceae. These organisms have heterokont flagella, one decorated with tripartite hairs; autotrophic species contain chlorophylls a and c, and are classified within the kingdom Chromista [38], Heterokontobionta [64] or Stramenopila [63]. The Oomycota lack acyclic polyols [65] and differ in sterol biosynthesis from the true fungi [77]. Based on the biosynthesis of sterols, Berg and Patterson [77] suggest a heterotrophic origin of the Oomycota. The labyrinthuloids appear to be basal to other heterokont algae, Oomycota and Hyphochytridiomycota within the Heterokontobionta (fig. 1) [78, 79].

The division Oomycota mainly consists of two orders. The order Saprolegniales comprises aquatic species, some of which are pathogenic to fish. Representatives of the order Peronosporales mostly occur in soil or as parasites of plants [63]. The latter order comprises one species of clinical significance, *Pythium insidiosum* [80]. Two members of the order Peronosporales, *Phytophthora infestans* and *Plasmopara viticola*, have been implicated as allergenic fungi [81].

Morphological Differentiation within the Kingdom Mycobionta

Figure 2 shows a phylogenetic and ontogenetic scheme accounting for the range of morphological organization in the kingdom Mycobionta. Figure 2 is based on a scheme proposed by Pascher [82] for algae, but, unlike the latter, envisages evolution from polykaryotic, via oligokaryotic to mono- and dikaryotic systems [41]. Different types of morphological organization are extensively discussed in Jahrmann and Prillinger [51] and Prillinger [41, 83]. The basal position of the flagellate and rhizopodial types was further corroborated by a compilation of ultrastructural data [41] and sequencing of the ribosomal RNA genes [3, 84]. In figure 2 we used the term flagellate instead of monadal

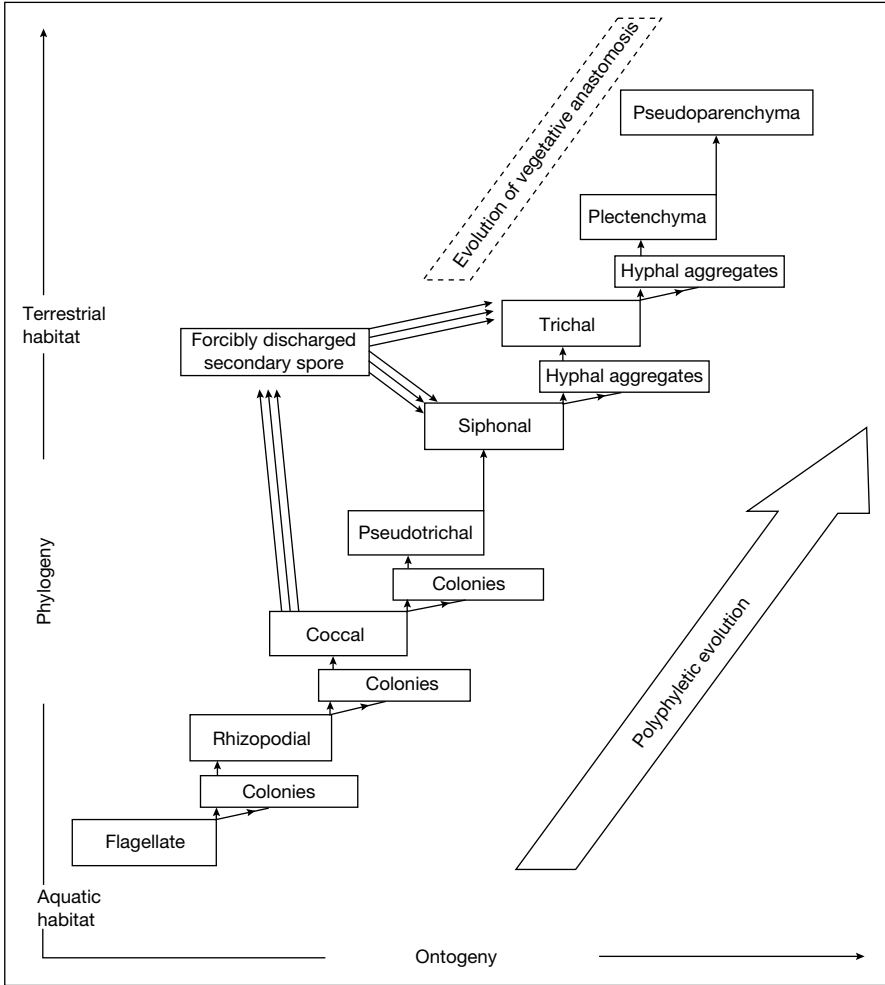


Fig. 2. Evolutionary scheme for morphological differentiation within the kingdom Mycobionta. Modified from Prillinger [83].

because the first Eucarya was most probably already a chimera of two prokaryotic organisms [49]. Presently, it is not clear whether the Amoebidiales with free-living rhizopodial or amoeboid stages belong to the Zygomycota. The yeast form, denoted by the term ‘coccal’ (i.e. a unicellular organism having a rigid cell wall outside its plasma membrane), occupies a basal position among the Zygomycota, Ascomycota, and Basidiomycota [Oberwinkler, pers. obs.], but seems to be derived in the Chytridiomycota (e.g. *Basidiobolus*) [83, 85, 86].