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An overview of the phylogeny and diversity of eukaryotes

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Abstract Our understanding of eukaryote biology is dominated by the study of land plants, animals and fungi. However, these are only three isolated fragments of the full diversity of extant eukaryotes. The majority of eukaryotes, in terms of major taxa and probably also sheer numbers of cells, consists of exclusively or predominantly unicellular lineages. A surprising number of these lineages are poorly characterized. Nonetheless, they are fundamental to our understanding of eukaryote biology and the underlying forces that shaped it. This article consists of an overview of the current state of our understanding of the eukaryote tree. This includes the identity of the major groups of eukaryotes, some of their important, defining or simply interesting features and the proposed relationships of these groups to each other.

Key words biodiversity, eukaryotes, molecular evolution, phylogeny, systematics, tree of life.

Eukaryotes are only one of the three domains of life, along with Bacteria and Archaea, yet we are particularly intrigued by eukaryotes. This is at least partly because they include the organisms we can see. However, the vast diversity of eukaryotes are single celled organisms, and their importance to our understanding of ourselves, our world and our history are immense. Knowledge of the morphological, functional and ecological diversity of microbial eukaryotes is essential for numerous practical reasons, but also because they teach us about the must fundamental rules of biology. For every rule we think we know, there is some organisms some where that bends or breaks those rules, and these breaks and bends hold important clues to how biology works.

Eukaryotes are by definition complex-celled organisms. Even the "simplest" have nuclei with highly structured chromatin, introns and large spliceosomal complexes to remove them (Collins & Penny, 2005), and complex membrane pores to control traffic in and out (Jékely, 2005). The cytoplasm is structured by an extensive cytoskeleton facilitating intracellular traffic, endo- and exocytosis, amoeboid locomotion (Cavalier-Smith, 2002). There is a vast array of organelles usually including, at a minimum, a mitochondrion or its derivative (Embley & Martin, 2006) and a golgi apparatus for synthesizing and recycling membranes and modifying their proteins (Mironov et al., 2007). Eukaryotic flagella are large complex intracellular structures unrelated to the simple bacterial structures of the same name (Pazour et al., 2005). Life histories also tend to be complex in eukaryotes, often with multiple highly distinct forms, sometimes including complex multicellular ones.

On the other hand, eukaryotes are fairly metabolically uniform. This is in contrast to bacteria, whose metabolic diversity is vast and quite possibly largely unknown (Frias-Lopez et al., 2008). Eukaryotes mostly rely on endosymbiotic former bacteria, the mitochondrion and chloroplast, for the bulk of their ATP production. The former are probably universal among extant eukaryotes, although they have been drastically functionally reduced multiple times (Embley & Martin, 2006). Chloroplasts arose later and, with one known exception (Nowack et al., 2008), probably all trace to a single endosymbiotic event early in the evolution of the former "Plantae" (now, Archaeplastida). Nonetheless, photosynthesis is widespread in eukaryotes. This most often takes the form of temporarily acquired plastids that must be replaced every generation from external sources (Fehling et al., 2007). This is found in nearly every major group of eukaryotes except amitochondriate excavates, including animals from that are essentially algae (Fehling et al., 2007). This has evolved into permanent secondary endosymbioses at least three times, and many of the most ecologically and economically important algae photosynthesize with permanently stolen plastids ("kleptoplasts"; Archibald, 2005). Eukaryotes are also involved in a wide variety of other endosymbioses for which there seems to be a continuum of host-symbiont interdependency (Moya et al., 2008).

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Our understanding of deep eukaryote phylogeny has begun to coalesce around data from large scale sequencing projects (Burki et al., 2007; Hackett et al., 2007; Rodriguez-Ezpeleta et al., 2007). As a result, most at least moderately well studied eukaryotes can now be assigned to a small number of major groups (Fig. 1). However, there are at least three important caveates to this. First, there are still many groups of eukaryotes, including whole major divisions, about which we know very little (Adl et al., 2005) including their true diversity (e.g., Bass & Cavalier-Smith, 2004). Second, we still have a very poor understanding of the cryptic diversity of eukaryotes, which could be vast (e.g., Slapeta et al., 2005). Finally, we are only just beginning to uncover the vast diversity of bacterial-sized (pico- and nano-) eukaryotes, first discovered in clone libraries derived by PCR amplification of pooled "environmental" DNAs (culture independent PCR or ciPCR) (Moreira & Lopéz-Garcia, 2002). Some of these species are as small as 1 μm or less in diameter, and they appear to include whole new divisions of eukaryotes (Massana et al., 2006).

1 Overview of the tree

Most well studied eukaryotes can now be assigned to one of four to five major groups. These are (1) Unikonts, (2) Archaeplastida, (3) Rhizaria+ Alveolates+Stramenopiles (RAS), and (4) Excavates, which are probably at least two distinct groups referred to here as the 1.4.1) mitochondriate Excavates, and 1.4.2) core (amitochondriate) Excavates. Unikonts include all eukaryotes thought to be primitively uniflagellate, that is, Opisthokonts (including animals and fungi) and Amoebozoa (Cavalier-Smith, 2002). The RAS group was only recently recognized and includes most of the former "chromalveolates" plus Rhizaria (Burki et al., 2007; Hackett et al., 2007). Archaeplastida is the group in which eukaryotic photosynthesis first arose (Adl et al., 2005; Archibald, 2005). Mitochondriate excavates include the former discicristates and core Jakobids. They are probably not directly related to the amitochondriate excavates, a collection of highly derived taxa with simplified internal cell structure and lacking aerobic mitochondria.

1.1 Unikonts

1.1.1 Opisthokonts The close evolutionary relationship between animals and fungi is now firmly established (Rodriguez-Ezpeleta et al., 2005; Hackett et al., 2007; Yoon et al., 2008). Since the earliest branches of both lineages are single celled organisms, it is also clear and not particularly surprising that multicellularity evolved independently and fundamentally quite differently in the two groups. In fact, both lines have at least two unicellular sister taxa—mesomycetozoa and choanoflagellates in the case of Metazoa (Steenkamp et al., 2006; Ruiz-Trillo et al., 2008) and nucleariid amoebas and chytrids in the case of Fungi (Medina et al., 2003; Steenkamp et al., 2006). Intriguingly, some data split the mesomycetozoa, placing *Capsaspora owczarzaki*, which was once classified as a nucleariid, as the earliest branch of Holozoa (Ruiz-Trillo et al., 2008). If correct, this would suggest that the last common ancestor of animals and fungi was a nucleariid-like amoeba.

The first branches of true fungi are chytridiomycetes, which appear to be paraphyletic (James et al., 2006). These aquatic unicells with pseudohyphae are the only fungi with flagella, which occur singly on their zoospores. All other fungi are multicellular, hyphal organisms with absorptive nutrition, probably all at least capable of producing multicellular fruiting bodies. The Glomales (arbuscular mycorrhizal fungi) are found exclusively in symbioses with plants, invading the outer root cells to develop highly branched tree-like structures to facilitate nutrient exchange with their hosts. This symbiosis probably dates to very early in land plant evolution and was probably an important factor in the successful invasion of land by plants (Read et al., 2000; Wang & Qiu, 2006). The overall outline of the fungal tree of life is now coming clear (James et al., 2006), although the number of undiscovered species is probably immense (Vandenkoornhuyse et al., 2002) and is estimated by some to be as much as 95% of all extant species (James et al., 2006).

The earliest known branch(es) of Holozoa are the mesomycetozoa, which may or may not be paraphyletic (see above), followed by the choanoflagellates, which are the closest sister group to Metazoa (Steenkamp et al., 2006; Ruiz-Trillo et al., 2008). Mesomycetozoa are parasites or symbionts and include pseudohyphal, flagellate and amoeboid forms. Choanoflagellates, known for their resemblance to the collared cells of sponges (Porifera), encode metazoan developmental proteins (King et al., 2008). Some data indicate that the enigmatic Ministeriids are the closest sister taxa to Metazoa (Cavalier-Smith & Chao, 2002; Steenkamp et al., 2006). However, only one species (*Ministeria vibrans*) has been examined, and it always forms a long branch in phylogenetic trees. Although a second species has been described, it has been lost from culture and not seen since (Simpson & Patterson,

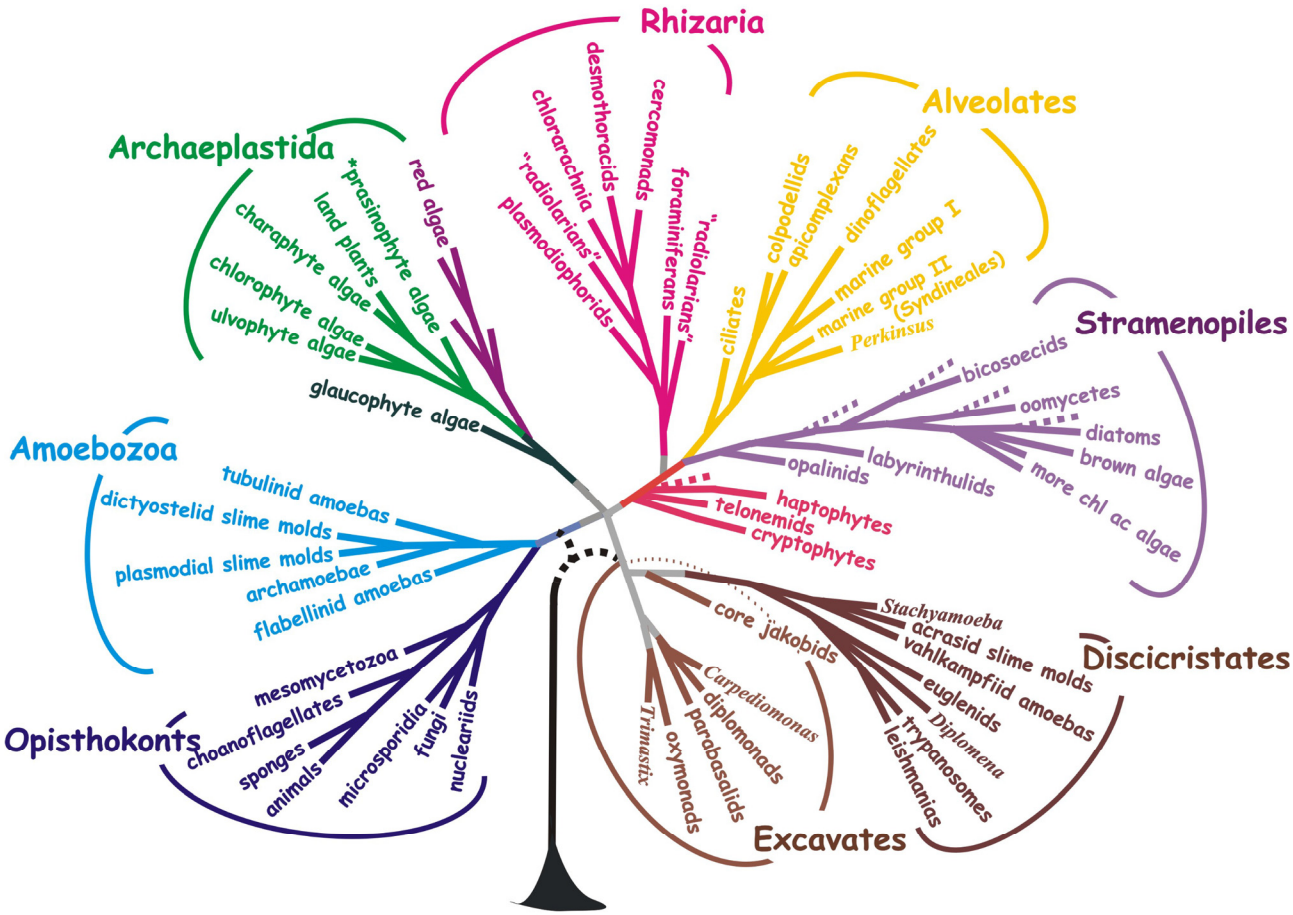

Fig. 1. A consensus phylogeny of the major groups of eukaryotes based on published molecular phylogenetic and ultrastructural data (adapted from Baldauf, 2003). Dotted lines indicate positions of major lineages of Stramenopiles known primarily from ciPCR (Massana et al., 2006). The two currently proposed positions for the eukaryote root are also indicated.

2006). Dire warnings to the contrary (Rokas et al., 2005), large molecular data sets and careful analysis has led to tremendous recent progress in resolving the deepest branches of Metazoa (Dunn et al., 2008).

1.1.2 Amoebozoa The Amoebozoa include several divisions of free-living amoebas as well as amitochondriate amoeboflagellates (Archamoebae) and social (Mycetozoa) amoebas, which may be each other's closest relatives (Smirnov et al., 2005; Nikolaev et al., 2006). There are also many species of uncertain affinity, and the higher-order phylogeny of the group is very uncertain. This is due partly to the difficulty in isolating and identify these species and because of a general lack of molecular data of any kind for most of them. Amoebozoan amoebae tend to have lobose or tube-like pseudopods, a single nucleus, and tubular branched mitochondrial cristae (Adl et al., 2005). They range in size from a few microns to several millimeters, and many smaller forms probably remain to be discovered. Cyst formation to survive desiccation or to invade hosts is common. Most taxa are free-living in soil where they are important as bacterial predators. The common soil amoebae of the Arcellinidae are the only amoebozoans to form tests, which they construct from organic material.

Medically the most important amoebozoans are the Entamoebae, which are tentatively grouped together with "pelobionts" as the Archamoebae (Bapteste et al., 2002; Cavalier-Smith et al., 2004). Archaemobids tend to live in low-oxygen environments and have mitochondria that are reduced to genome-free mitosomes (Tovar et al., 1999), which continue to function in the production of heme and other compounds (Embley & Martin, 2006). Pelobionts are amoeboflagellates that can be as large as 3 mm long (*Pelomyxa*) and have 1-many non-motile flagella (Adl

et al., 2005). Entamoebae are small, non-flagellate and mostly commensal or parasitic, living in the mouth and intestinal tracts of various Metazoa. *Entamoeba histolytica*, causative agent of amoebic dysentery, appears to have developed this life style very recently. It is morphologically and molecularly nearly indistinguishable from the harmless commensal, *E. dispar*, and many of the enzymes required for its anaerobic life style were acquired relatively recently by horizontal gene transfer from bacteria (Clark et al., 2007).

The most dramatic amoebozoans are the Macromycetozoa, the myxogastrid (plasmodial) and dictyostelid (cellular) slime molds. These have very different trophic (feeding) stages but similar fruiting bodies, albeit formed in very different ways. Since their discovery ~150 years ago, Dictyostelia and Myxogastria have been variously classified, together or separately, as plants, animals, or fungi. However, abundant molecular data confirm that they are amoebozoans (Bapteste et al., 2002; Yoon et al., 2008). Their closest relatives are protostelid slime molds, although it is not clear if these are monophyletic.

There are over 6,000 described species of Myxogastria, also known as "giant amoebas". Most of what is known about their life cycle comes from studies of *Physarum polycephalum.* Following mating, these amoeboflagellates transform into plasmodia that can grow to 100+ decimeters in diameter (Olive & Stoianovich, 1975). Plasmodia are motile, can contain 10,000s of synchronously dividing nuclei and may have thickened branching channels throughout their cytoplasm. However, there are never any internal cell membranes and the nuclei remain undifferentiated, even when the plasmodia break down to form fruiting bodies (sporophores) (Marwan et al., 2005; Gloeckner et al., 2008). Nonetheless, these macroscopic, often colorful structures can appear highly complex with multiple distinct tissue-like layers, internal structures and ornamentation. This is in striking contrast to Dictyostelia or social amoebas (~100 described species; Schaap et al., 2006), which spend most of their life cycle as solitary amoebas. Under appropriate conditions 10^4–10^5 amoebae aggregate, generally to form 2–5 mm long motile "slugs". The "head" of the slug senses environmental stimuli, directs slug migration, and forms the inert cellulosic stalk of the relatively inconspicuous sporophores. The tail cells then rise to the top of the stalk and form the live spores (Strmecki et al., 2005; Romeralo et al., in press). Less is known about the protostelids, which form simple largely microscopic sporophores (Olive & Stoianovich, 1975; Spiegel et al., 1995).

1.2 Archaeplastida

This is the group in which eukaryotic photosynthesis first arose (Adl et al., 2005), and all species in the group are photosynthetic with the exception of a few minor parasitic lines. There are three highly distinct lineages—Rhodophyta, Glaucophyta and Chloroplastida (green algae and land plants)—and, so far, no apparent intermediate branches between them. All eukaryotic photosynthesis originates from this group, with one very recent exception (Nowack et al., 2008), and many different lines of evidence support monophyly of archaeplastid chloroplasts (Reyes-Prieto et al., 2007). Molecular phylogenetic support for the monophyly of their nuclear genomes has been more elusive (Rodriguez-Ezpeleta et al., 2005), possibly due to the antiquity of the group and/or extremely sparse molecular sampling of rhodophyte and glaucophyte taxa.

Glaucophytes are unicells that vary from biflagellates to coccoid non-flagellates to palmelloid forms (non-motile cells in a mucilaginous matrix). Their plastids (cyanelles) resemble those of red algae in that they have phycobiliproteins and unstacked thylakoids but lack chlorophyll *b*. However cyanelles are also unique in that they have a bacterial-like peptidoglycan cell wall sandwiched between their inner and outer membranes (Steiner et al., 2005). Rhodophytes vary from large seaweeds to crustose mats that, to the naked eye look more like rocks than living plants. Two major subgroups are recognized, Bangiophyta and Florideophyta, both of which probably invented multicellularity independently. Chloroplastida include the Chlorophyta, Ulvophyta, Prasinophyta and Strepsystera (Charaphyta and land plants), although prasinophytes maybe para- or even polyphyletic. There are probably multiple inventions of multicellularity in Chloroplastida, as Chlorophytes, Ulvophytes, and Strepsystera are all mixtures of uni- and multicellular forms.

1.3 RAS Group

RAS (or SAR) unites three of the largest, most diverse divisions of eukaryotes, the Rhizaria, Alveolates and Stramenopiles (formerly, Heterokonts) (Burki et al., 2007; Hackett et al., 2007). There was very little evidence to suggest the existence of this supergroup until the very recent acquisition of substantial EST (expressed sequence tag) data from Rhizaria (Burki et al., 2007; Hackett et al., 2007). The group may also include the remaining chlorophyll *c* algae, the Haptophytes and Cryptophytes, but molecular phylogenetic support for this is still very weak (Burki et al., 2007; Hackett et al., 2007).

1.3.1 Rhizaria These are largely, but not entirely, amoeboid forms. The amoebas tend to have fine pointed (filose) pseudopodia and to build shells (tests) from various materials. The most famous divisions are Radiolaria, Foraminifera, Plasmodiophora, Heliozoa and Cercozoa (Nikolaev et al., 2004). The Radiolaria were popular subjects of Haeckel, who designed striking lithographs based on their almost snowflake-like tests. These amoebae are exclusively marine and have internal mineralized "skeletons" from which radiate long arm-like microtubular rays (axopodia). They include the Acantharia, with skeletons composed of strontium sulfate, and the Polycistinae, whose skeletons vary from simple spicules to complex helmet-shaped structures. The third traditional group of "Radiolaria", the Phaeodaria, evolved independently and have siliceous skeletons usually made of hollow radial spines (Nikolaev et al., 2004).

Foraminifera are widely distributed in all types of marine environments but also occur in freshwater and on land. Their amoebae have reticulated pseudopods with bidirectional cytoplasmic flow. Most also build tests, which are organic, agglutinated or calcareous and with one or more chambers (Lee et al., 2000). Both foraminiferan (Polycistinae) and radiolarian (Phaeodaria) skeletons contribute substantially to the microfossil record extending back to the Cambrian. These fossilized tests are used in micropaleontology as biostratigraphic markers and in paleoceanography as indicators of ancient water temperatures, ocean depths, circulation patterns, and the age of water masses.

The remainder of the Rhizaria form a large heterogeneous assemblage, the Cercozoa, which includes various amoebae, some formerly classified as heliozoans (desmothoracids) or radiolarians (Phaeodarea), as well as flagellates, amoeboflagellates and plasmodial parasites (Nikolaev et al., 2004). They also include chlorarachniophytes, which are closely related to heterotrophic amoeboflagellates (cercomonads, Archibald, 2005) but have acquired photosynthesis. This they have done by secondary endosymbiosis of a green alga, and they retain a highly reduced version (nucleomorph) of the original alga's nucleus to help service their plastids (Archibald, 2007). Cercozoa also include common freshwater and/or terrestrial species, such as euglyphids, which have silica tests, and the plasmodial parasites Haplosporidians (endoparasites of freshwater and marine invertebrates) and Plasmodiophorids. The latter are important endoparasites of plants or stramenopile algae (Nikolaev et al., 2004).

1.3.2 Stramenopiles Stramenopiles are characterized by flagella with rows of stiff, tripartite hairs (stramenopiles), which reverse the flow around the flagellum so that the cell is dragged forward rather than pushed along. Most also possess a second, shorter, smooth flagellum (hence the alternative name "heterokont"). This is an extraordinarily diverse group including numerous lineages of single-celled heterotrophs (bicosoecid, pseudociliates), plasmodial parasites (oomycetes), cosmopolitan and often highly abundant single-celled algae (diatoms, ochromonads) and large to giant multicellular algae (xanthophytes, phaeophytes). Environmental sampling (ciPCR) suggests that there may be additional major divisions of the group, consisting largely, if not entirely, of ultra-small species (Moreira & Lopéz-Garcia, 2002).

There are at least five known lineages of non-photosynthetic stramenopiles. Oomycetes (water molds and downy mildews) were previously classified as fungi and include numerous extremely destructive plant parasites such as *Phytophthora infestans*, the cause of potato blight. The bicosoecids are small heterotrophic biflagellates, such as *Cafeteria*, possibly the world's most abundant predator (Moreira & Lopéz-Garcia, 2002). Labyrinthulids (slime nets) form filamentous "railway-like" networks patrolled by amoeboid-like cells. Opalinids look almost like ciliates, except that their numerous flagella have stramenopile hairs. The taxonomically enigmatic *Blastocystis* spp. are commensals in the guts of cold-blooded animals and appear to be in the process of converting their mitochondrion into a hydrogenosome (Stechmann et al., 2008).

Photosynthetic stramenopiles constitute at least eleven distinct lineages, including some of the most important and abundant algae. Diatoms have intricately patterned bipartite silica tests that fit together like lidded boxes. They are ubiquitous and often abundant in marine and fresh water, with ~11,000 described and possibly as much as 10^7+ undescribed species (Fehling et al., 2007). Phaeophytes (brown algae) are particularly widespread in temperate intertidal and subtidal zones. They have true parenchyma and build "forests" in near-shore waters, supporting complex ecosystems including fish and marine mammals. Xanthophytes (giant sea kelps) are the keystone species of deep sea kelp forests, another set of complex marine ecosystems. Environmental sampling also suggests that there are at least eight additional major divisions of extremely small (pico- and nano-sized) stramenopiles, which are widespread in occurrence but so far known only from ciPCR libraries (Massana et al., 2006).

1.3.3 Alveolates These include ciliates, dinoflagellates, and apicomplexans, which are united by molecular phylogeny and by the presence of cortical alveoli underlying their plasma membranes (Hausmann et al., 2003), and two divisions of picoeukaryotes (<10 μm diameter) largely known only from ciPCR (Lopéz-Garcia et al., 2001). Ciliates are highly speciose aquatic unicells characterized by an abundance of flagella and dimorphic nuclei, that is, micro- and macronuclei. The micronucleus is a transcriptionally inactive germ nucleus, with genes that can consist of numerous fragments, sometimes arranged in scrambled order and even distributed over multiple loci. The situation is not lethal because transcription occurs in the macronuclei, which are, in the most extreme cases, essentially cDNA libraries consisting of multiple, correctly processed copies of the micronuclear genes (Prescott, 2000; Dalby & Prescott, 2004).

Dinoflagellates are a diverse, predominantly unicellular group with characteristic often-elaborate plates or armor and two unequal flagella that give rise to a unique rotatory swimming motion. Although the group was probably primitively photosynthetic only about half of the extant species still are. They also have extremely reduced plastid genomes, with most genes relocated to the cell nucleus (Bachvaroff et al., 2004). This may explain why they are particularly adept at acquiring and retaining exogenous plastids (Yoon et al., 2002; Archibald, 2005). Dinoflagellates are important symbionts of coral and other hydrozoans and the main source of harmful algal blooms (HABs; e.g., red tides), where they produce some of the most potent neurotoxins known. They also have some of the largest known nuclear genomes, with large amounts of repetitive DNA, which makes the prospect of a full dinoflagellate genome sequence unlikely for some time, if ever. This repetitive DNA may play a structural role in compensating for the nearly complete lack of histones in dinoflagellate nuclei (Hackett et al., 2005).

Apicomplexa are the sister group to the dinoflagellates and include some of the most important protozoan disease agents of both invertebrates and vertebrates. Nearly all are obligate intracellular parasites, including the causative agents of malaria (*Plasmodium* spp.) and toxoplasmosis. Their name derives from their characteristic apical complex, which functions in the attachment and initial penetration of the host cell. All species retain a vestigial plastid (apicoplast), most likely of red algal origin (Fast et al., 2001) and required for heme, lipid and isoprenoid biosynthesis (Waller & McFadden, 2005).

One of the most striking features of Alveolates and Stramenopiles is the widespread occurrence of secondary chloroplasts (kleptoplasts). Stealing plastids is a complex process, since ~95% of chloroplast proteins are encoded in the host nucleus (Reyes-Prieto et al., 2007). Thus, the secondary host must acquire not just a plastid, but the ~3600 nuclear genes needed to maintain it. This presumably takes quite a while, during which time a working remnant of the primary host nucleus must be maintained in the new host cell (Archibald, 2005). Such a remnant has now been observed in chlorarachniophytes (see above) and in cryptophytes and haptophytes (see below).

1.3.4 Haptophytes and cryptophytes These are both primarily chlorophyll *c* algae. However, although their plastids clearly share a common origin with the chlorophyll *c* plastids of stramenopiles, there is little evidence that their nuclear genomes do. The group of Cryptophytes + Haptophytes, if it indeed it is a group, is potentially quite large. Two major new lineages have been discovered recently—the Telonemids (Shalchian-Tabrizi et al., 2007) and Klephablepharids (Not et al., 2007).

Haptophytes are named for their haptoneme, an anterior appendage used for adhesion and prey capture. They include coccolithophorids, which are unicells covered in overlapping calcium carbonate scales (coccoliths). Blooms of the coccolithophore *Emiliana huxleyi* can be 1,000+ miles across and visible from space. These massive blooms substantially affect the temperature and optical qualities of ocean waters, and when they die, they release enough dimethyl sulfoxide to seed clouds (Buitenhuis et al., 1996). Blooms end in massive die-offs caused by a marine virus, and the resulting limestone deposits are the largest inorganic reservoirs of carbon on Earth.

The cryptophytes are relatively small (mostly 2–10 μm diameter) unicells primarily found in cold or deep waters. Similar to the chlorarachniophytes, their plastids are accompanied by a remnant of the primary host nucleus. Both the cryptophyte and chlorarachniophyte nucleomorphs encode some of the proteins required for plastid function. However, mostly they encode proteins needed to main the nucleomorph itself (Lane et al., 2007). Cryptophytes are abundant and ubiquitous and are commonly involved in temporary endosymbioses (Fehling et al., 2007).

1.4 Excavates

Taxa classified as “excavates” are unicells with an often-large excavated groove at their anterior end into which they trap food particles with the aid of a

flagellum (suspension feeding; Simpson, 2003). While molecular evidence strongly suggests that this is a paraphyletic, if not a polyphyletic assemblage, the name and attendant hypotheses are at least convenient and will probably persist for some time. The organisms grouped into this category fall into two or three very distinct and quite possibly unrelated groups (Simpson et al., 2006). However, their phylogeny is challenging, as they also tend to have extremely fast rates of molecular evolution. For convenience, they are divided here into the "mitochondriate excavates", which is well supported and includes Euglenozoa, Heterolobosea and core Jakobids (Simpson et al., 2006), and "core excavates", which includes two possibly-unrelated divisions (Simpson et al., 2006).

1.4.1 Mitochondriate excavates

1.4.1.1 Euglenozoa The Euglenozoa include Kinetoplastids, Diplonemids and Euglenids (von der Heyden et al., 2004). Kinetoplastid genera include *Trypanosoma, Bodo, Leishmania.* These are small uni- or biflagellated cells, many of which are parasites including the causative agents of sleeping sickness, Chagas disease and leishmaniases. Euglenids are also uni- or biflagellate cells, but are mostly free-living and have a characteristic thickened pellicle made of proteinaceous strips. Phagotrophic euglenids can ingest whole eukaryotic cells and a subset, *E. gracilis* and its relatives, have acquired a green algal chloroplast. All examined euglenozoans have striking molecular biology in their nuclear, mitochondrial and, where present, plastid genomes. Self-splicing twintrons, where an inner intron must be spliced out before the outer intron can assume its own catalytically competent secondary structure, were discovered in the *E. gracilis* plastid genome (Hallick et al., 1993). RNA editing was discovered in the *Trypanosoma* mitochondrial genome. Here the genes are essentially encoded in shorthand, and the initial transcripts require extensive nucleotide modification and oligonucleotide insertion to encode a functional protein (Lukes et al., 2005). There are also many unusual molecular features of euglenozoan nuclear genomes (von der Heyden et al., 2004).

1.4.1.2 Heterolobosea The Heterolobosea are mostly amoebae, although many have flagellate phases in their life cycles (Simpson & Patterson, 2006). They are abundant, ubiquitous and their ecological importance is poorly understood but probably very substantial. They are naked amoebae, and they differ from lobosan amoebae in that their pseudopods develop and move in a sporadic, "eruptive" manner. Most are soil or freshwater bacterivores, although one, *Naegleria fowleri*, is a rare but often fatal facultative human pathogen. They also include the acrasid "slime molds", which were reclassified from amoebozoa to heterolobosea based on molecular trees (Baldauf et al., 2000). This is consistent with much earlier observations of their very unamoebozoan-like pseudopodia (Olive & Stoianovich, 1975).

1.4.1.3 Jakobids These small free-living bacterivores are particularly noted for their variable mitochondrial morphology (O'Kelly, 1993) and, more recently, their bacterial-like mitochondrial genomes (Lang et al., 1997). While most eukaryotes have fewer than 20 protein-coding genes remaining in their mitochondrial genomes, Jakobids retain more than 100. Also unlike other eukaryotes, these genes are arranged in bacterial like operons (Lang et al., 1997), consistent with an alpha-proteobacterial ancestry for mitochondria.

1.4.2 Amitochondriate (core) excavates

The core excavates consist of two distinct lineages of uncertain affinity. The Fornicata includes Diplomonads, Retortamonads, *Carpediemonas* and possibly also Parabasalids (Simpson, 2003). Axostyla consists of Oxymonads and *Trimastix* (Simpson, 2003). This huge and possibly ancient group is known only as unicells living in anaerobic or micro-aerobic habitats, often as commensal or parasites. Their simplified internal cell structure and apparent lack of mitochondria or mitochondrially-derived organelles gave rise to the Archaezoa hypothesis, which maintained that these were remnants of early, pre-mitochondriate eukaryote lineages (Cavalier-Smith & Chao, 1996). However, genes of mitochondrial ancestry have been found in their nuclear genomes, and highly reduced mitochondrial relicts have recently been discovered in many of them (Embley & Martin, 2006). Thus the Archaezoa hypothesis is now defunct. Nonetheless, these taxa still appear as the earliest branches in rooted molecular trees (Bapteste et al., 2002; Arisue et al., 2005), although this is widely, albeit not universally, interpreted as a long-branch attraction artefact (Philippe & Germot, 2000).

The Diplomonads typically have a striking "mirrored morphology", looking like an incompletely divided cell with two sets of nuclei, flagella and cytoskeletons arranged back to back. *Giardia intestinalis* is a common human gut parasite sometimes found in remote seemingly pristine habitats. *Spironucleus* is a parasite and plague of fish farms (Bernard et al., 2000). Retortamonads are intestinal commensals, roughly resembling half of a diplomonad cell (Silberman et al., 2002). Oxymonads are flagellated gut

symbionts of animals, including termites (see below).

Parabasalids are mostly parasites and symbionts, characterized by a complex parabasal apparatus involved in host cell attachment. They include Hypermastigids and Trichomonads (Dacks et al., 2001). Hypermastigids are large (100+ μm) cells that appear multiflagellate due to the presence of a dense covering of elongate ectosymbiotic bacteria. They are found almost exclusively in the hindguts of termites and wood-eating cockroaches where they form part of a complex microfauna responsible for the breakdown of cellulose. Trichomonads are small teardrop-shaped cells with four to six flagella and cause trichomoniasis, the most common human sexually transmitted disease. It is quite likely that major lineages of these groups remain to be discovered (e.g., Yubuki et al., 2007).

1.5 *Incertae sedis*

In 1999, Patterson identified 230 cultured protists of uncertain affinity (Patterson, 1999). In 2005 that number had only decreased to 204 (Adl et al., 2005), so much remains to be done. Most of these are small free-living heterotrophic flagellates or amoebae, or they are parasites of various kinds. Many will undoubtedly turn out to fall within one or more of the groups described above. However, ciPCR surveys suggest the existence of major undiscovered eukaryotic lineages as well (Moreira & Lopéz-Garcia, 2002), much of which probably consists of nano- and picoeukaryotes. These are cells as small as 1 μm in diameter that have previously escaped detection by light microscopy because, at this level they are all but indistinguishable from bacteria. Even supposedly known species may be the sole representatives of what are in fact major unsuspected lineages. For example recent ciPCR surveys show that Apusomonads, which also include Ancyromonads and Mastigamoeba are a large and diverse group (Cavalier-Smith et al., 2004). Multigene phylogenies also suggest that they may be the sister group to Opisthokonts (Kim et al., 2006).

2 The root of the eukaryote tree

Probably the single most outstanding question in eukaryote evolution is the location of the root of the tree. For a long time, the predominant theory was that species lumped together in the now-defunct Archaezoa lay near the root of the eukaryote tree, as they tended to form the deepest branches in molecular trees (Arisue et al., 2005; Baldauf et al., 1996; Bapteste et al., 2002; Stiller & Harrell, 2005; Ciccarelli et al., 2006). However, there has been growing distrust in the ability of molecular phylogeny to resolve the deepest branches in the tree of life, because of the problem of long branch attraction (Philippe & Germot, 2000).

A radically different placement of the eukaryote root is suggested by the fusion of the genes for dihydrofolate reductase and thymidylate synthase. These genes are adjacent and co-transcribed in bacteria, separate in Opisthokonts, and fused in representatives of all other major eukaryote groups except core Excavates and Amoebozoa, although the latter mostly lack the genes entirely (Stechmann & Cavalier-Smith, 2003). Since gene fusions are rare, and gene fissions undoubtedly rarer, this suggests that Archaeplastida, RAS and Excavates, if amitochondriate and core excavates are assumed to be a group (Simpson et al., 2006), share a unique common ancestor excluding Opisthokonts and possibly Amoebozoa. Thus, this root divides all eukaryotes into two supergroups—Unikonts (Opisthokonts + Amoebozoa) and bikonts (everything else).

However, the near complete absence of these genes in Amoebozoa is disconcerting, and lateral transfer among eukaryotes and between eukaryotes and bacteria appears to be an on-going and not too infrequent process. Therefore, additional data are needed to confirm this new rooting, of which little seems to be forthcoming. Thus it is possible that this critical question may remain outstanding for sometime.

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