

The most abundant predators on earth are not animals like lions or ants, but the microscopic, unicellular protozoa. When I was a student, protozoa were treated as one phylum in the animal kingdom, with just four classes: amoebae, flagellates, ciliates and the purely parasitic Sporozoa. Now they are recognized as a separate kingdom comprising 11 phyla. Ciliates remain as one phylum, Ciliophora, which also includes the highly specialized Suctoria that lack cilia in their sedentary adult stages, having evolved elaborate tentacles with explosive organelles for trapping prey. But amoebae and flagellates are abandoned as formal taxa, while Sporozoa have been drastically refined, by removal of microsporidia to Fungi and Myxozoa to Animalia, in a radical overhaul of protozoan systematics that lasted for three decades but is now settling down, after extensive lively controversies, into a period of broad consensus.

As a student in the 1960s I realized that electron microscopy showed protozoan cells as architecturally far more diverse than animal or plant cells. Classically, the boundary between protozoa and algae was fuzzy, with several groups claimed both by zoologists and botanists, in particular dinoflagellates, euglenoids, cryptomonads and chryomonads, some of which are photosynthetic algae while others are predatory heterotrophs; a few, notably some dinoflagellates, that feed both ways were an especial problem for early classifications based just on nutrition and motility. The first major break from the simple 19th century fourfold division occurred 25 years ago when I established the kingdom Chromista for a mixture of algae and former protozoa and treated the remaining Protozoa as a separate kingdom, in which flagellates were split into several phyla (Fig. 1). By then it was widely accepted that chloroplasts originated by the enslavement of a cyanobacterium and that all eukaryote

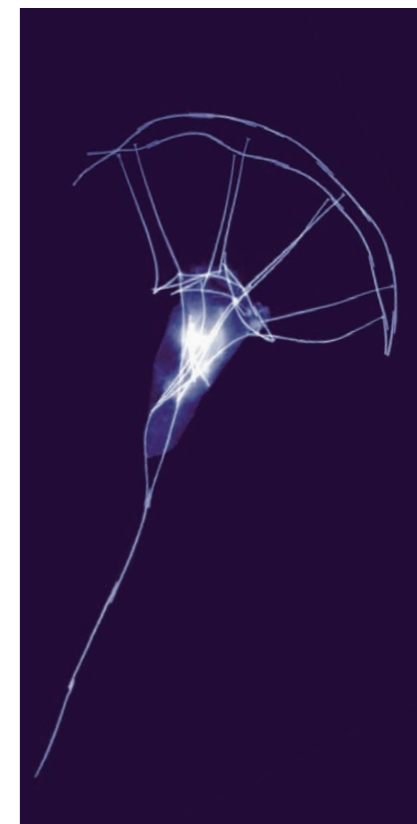
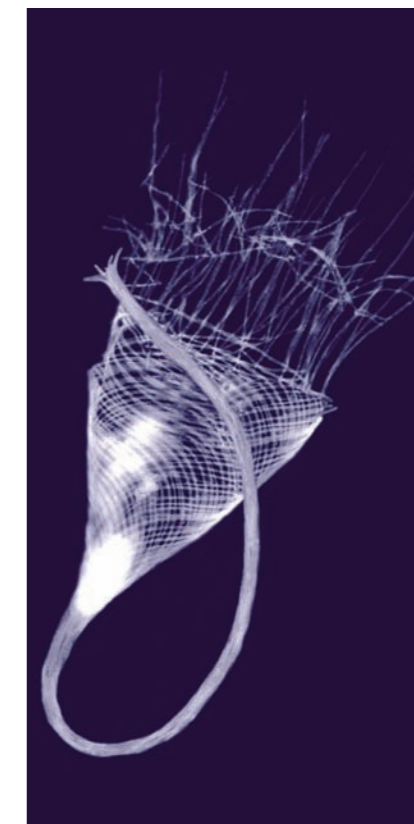
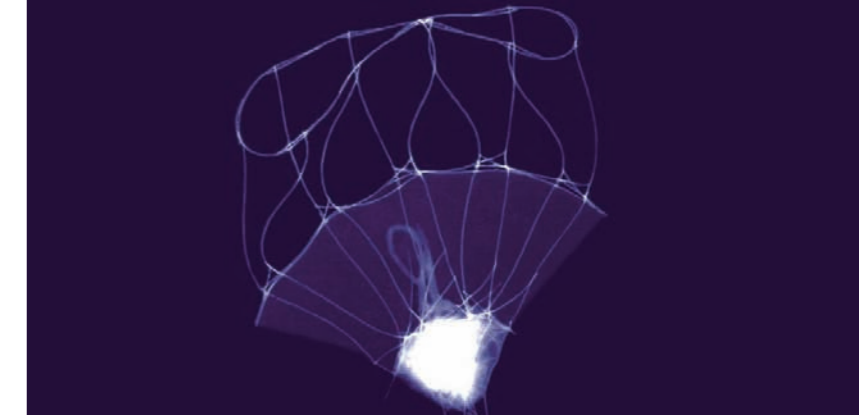
algae evolved ultimately from heterotrophic ancestors. We now know that the primary diversification of eukaryote cells took place among zooflagellates: non-photosynthetic predatory cells having one or more flagella for swimming, and often also generating water currents for pulling in prey.

Evolution of early eukaryotes
Cilia of ciliates and oviducts, and flagella of flagellates and sperm, are homologous structures that grow from centrioles with a beautiful 9-triplet microtubule structure; I here adopt the increasing tendency to refer to both as cilia. The traditional distinction that cilia are short and many, whereas flagella are long and few, is trivial and often fails, as does the idea of a distinct beat pattern. Cilia share no evolutionary relationship with the rotary extracellular flagella of bacteria, but are specializations of the eukaryote cytoskeleton that probably evolved at the same time as the nucleus and the enslavement of an alphaproteobacterium as the first

The kingdom Protozoa embraces an immense diversity of single-celled predators and numerous parasites causing diseases like malaria, sleeping sickness, and amoeboid dysentery. As **Thomas Cavalier-Smith** explains, they were the first eukaryote cells, giving rise to all higher kingdoms, and there has recently been a revolution in their high-level systematics.

mitochondrion. Thus the first eukaryote cell was a flagellate, very likely one with amoeboid tendencies, not a rigid pellicle – and probably only one cilium. Amoeboid protozoa arose several times from flagellate ancestors by evolving highly varied pseudopodia for creeping and crawling; some, notably the secondarily anaerobic mastigamoebae and *Multicilia* (both Amoebozoa) retained a cilium or cilia, but others lost them altogether or kept them only for wider dispersal, suppressing them during crawling/feeding.

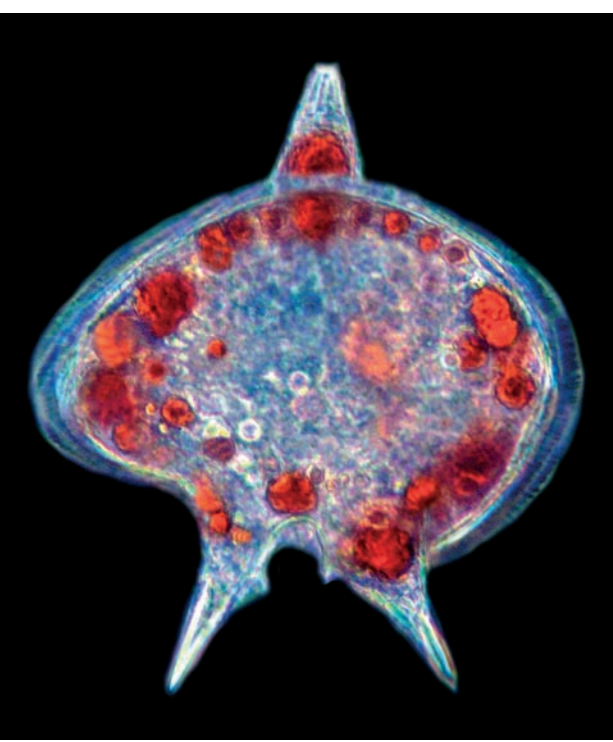
The role of molecular systematics
Some thoughtful 19th century protozoologists suspected that flagellates preceded amoebae, as just asserted. But until DNA sequencing and molecular systematics burgeoned in the 1980s and 1990s the often more popular idea of Haeckel that amoebae came first and flagellates were more advanced could not be disproved. Sequences enabled calculation of phylogenetic trees based on differing degrees of gene sequence divergence. Equally importantly, they reveal much rarer and more substantial discrete molecular changes, e.g. gene fusions, splitting of genes, insertion and loss of introns, gene duplications and indels (insertions or deletions). Such characters, treatable by the cladistic reasoning that morphologists used for centuries, are sometimes more decisive



than sequence trees. Gene sequence trees excel in establishing close clusters of strongly related organisms, but sequence evolution is more complex and variable than sometimes thought, so is sometimes extremely misleading, yielding partially wrong topologies. The test of a good phylogeny and classification is that different independent lines of evidence yield congruent conclusions. We are approaching this happy state of affairs for protozoan large-scale systematics, though problems and controversies remain.

Diversification and diversity
The emerging picture (Fig. 1) shows the primary diversification of eukaryotes as involving changes in the development

- ▲ Transmission electron micrographs of choanoflagellate protozoa. Choanoflagellates feed on bacteria by trapping them with a collar of microvilli around the base of the cilium, much as do the remarkably similar internal feeding cells of sponges, the most primitive animals. It is probable that animals evolved from a choanozoan protozoan similar to a choanoflagellate. Top *Cosmoeca* sp.; lower left *Acanthoeca spectabilis*; lower right *Parvicorbicula* sp. Dr Per R. Flood / Natural History Museum Picture Library
- ◀ Dark field light micrograph of a live specimen of the predatory dinoflagellate *Protoperidinium depressum*. Dr Per R. Flood / Natural History Museum Picture Library



Protozoa: the most abundant predators on earth

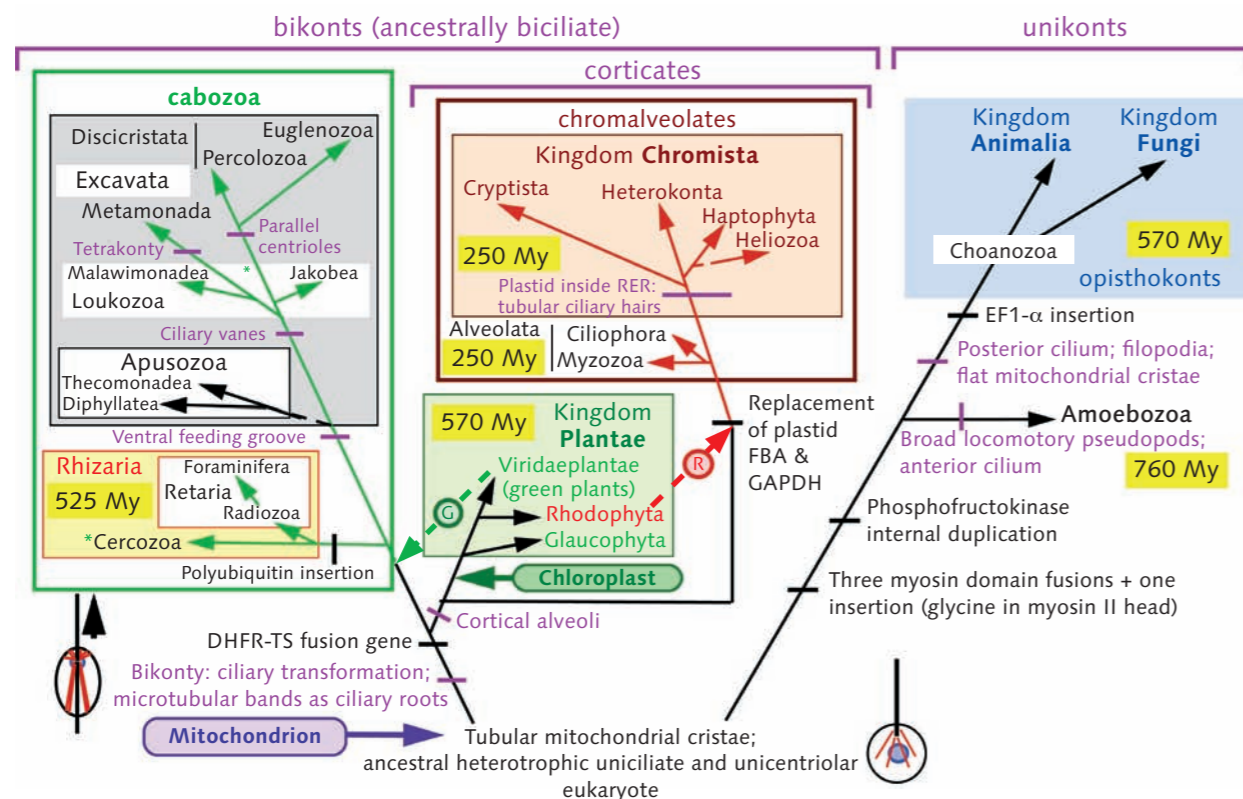


Fig. 1. The eukaryote evolutionary tree. Taxa in black comprise the basal kingdom Protozoa. The thumbnail sketches show the contrasting microtubule skeletons of unikonts and bikonts in red. The dates highlighted in yellow are for the most ancient fossils known for each major group. Major innovations that help group higher taxa are shown by bars. Four major cell enslavements to form cellular chimaeras are shown by heavy arrows (enslaved bacteria) or dashed arrows (enslaved eukaryote algae). Four features of the tree need more research. (1) Are centrohelid Heliozoa (non-photosynthetic and non-flagellate predators that catch prey by slender radiating axopodia supported by microtubules) really sisters of Haptophyta; do they even belong in Chromista? (2) Are Apusozoa perhaps sisters of all other bikonts and not really early diverging excavates? (3) Was a green alga (G) really enslaved by the ancestral cabozoa rather than separately by Cercozoa and euglenoids (asterisks)? (4) Are Rhizaria and Excavata really sisters? T. Cavalier-Smith

of cilia and structure of microtubular roots that attach them to the rest of the cell. Unikonts comprise animals, fungi and the protozoan phyla closest to them: Choanozoa (e.g. choanoflagellates) and Amoebozoa (typical amoebae with broad pseudopods, slime moulds like *Dictyostelium* and mastigamoebae), which all share the same myosin II as our muscles, which is absent from all bikonts. Bikonts comprise the plant and chromist kingdoms, three protozoan infrakingdoms (Alveolata, Rhizaria, Excavata), and the small zooflagellate phylum Apusozoa, whose closest relatives are unclear. Ancestrally bikonts had two diverging cilia: the anterior undulates for swimming or prey attraction; the posterior serves for gliding on surfaces in many groups (likely its ancestral condition), but secondarily adapted for swimming in others (or quite often was lost to make secondary uniciliates, not to be confused with genuine unikonts, which confusingly are sometimes secondarily biciliate). All major eukaryote groups except Ciliophora include severely modified organisms that lost cilia; some became highly amoeboid.

Among bikonts emphasis on pseudopodia is greatest in Rhizaria, comprising two phyla (Cercozoa, Retaria) in which pseudopods are typically very thin (filopodia: 'thread feet') and often branch or fuse together in elaborate feeding networks: especially well developed

as nets (reticulopodia) in Foraminifera (predominantly benthic Retaria, the most abundant seafloor predators) and Radiozoa (major micropredators of oligotrophic oceans: acantharia and polycystine radiolaria). Cercozoa include flagellates, the peculiar chlorarachnean algae formed by the enslavement of a green alga (G in Fig. 1), amoebae with filopodia (many with shells that evolved independently of shells of some Amoebozoa) and amoebiflagellates; they are the most abundant predators in soils, yet were only recognized as a phylum in 1998; they also include parasites of commercially important shellfish (e.g. oysters) and crops (e.g. sugarbeet and cabbages).

Parasitism developed more strongly in phylum Myxozoa, named after their widespread feeding by myxocytosis (sucking out the interior of cells rather than engulfing them whole by phagocytosis). Sporozoa *sensu stricto*, e.g. malaria parasites, are all parasites and are grouped with some free-living sucking predatory zooflagellates as sub-phylum Apicomplexa, within Myxozoa

together with Dinozoa (dinoflagellates and relatives), also often parasites. Myxozoa and Ciliophora comprise Alveolata, typically with well-developed cortical alveoli: smooth vesicles with skeletal and calcium segregating functions. Alveolates are sisters of kingdom Chromista, being jointly called chromalveolates. The ancestral chromalveolate flagellate had phagotrophic nutrition and chloroplasts surrounded by extra membranes arising from their origin by intracellular enslavement of a red alga (Rhodophyta; Fig. 1). Chromalveolates comprise chromophyte algae, whose chloroplasts bear chlorophyll c2, and many derived lineages that lost photosynthesis and sometimes plastids altogether, e.g. ciliates, oomycete chromists. Chromists differ from alveolates in that the phagocytic vacuole membrane around the enslaved red alga fused with the nuclear envelope, placing their plastids inside the rough endoplasmic reticulum. This contrasts markedly with kingdom Plantae, where chloroplasts lie in the cytosol and were never lost, even in chlorophyll-free plants.

The test of a good phylogeny and classification is that different independent lines of evidence yield congruent conclusions.

Within excavates, the strictly non-amoeboid Loukozoa ('groovy animals') have a well developed ventral feeding groove with a scooped out, 'excavated', look that gives the infrakingdom its name; their mitochondrial genome retains more bacterial genes than in other eukaryotes. Percolozoa are predominantly amoebiflagellates (Heterolobosea, e.g. the human pathogen *Naegleria fowleri*), sometimes pure amoebae (having lost cilia and groove), rarely pure flagellates. Other excavates are largely non-amoeboid flagellates. Metamonada are secondary anaerobes, having converted mitochondria into hydrogenosomes or mitosomes, with a marked tendency to multiply cilia and/or nuclei, e.g. *Giardia*, *Trichomonas*. Euglenozoa include the seriously pathogenic trypanosomatids (e.g. sleeping sickness agents), the ubiquitous bodonid zooflagellates (the weeds of eukaryotic microbiology), and the euglenoids (with complex pellicular strips: phototrophs like *Euglena*, phagotrophs like *Peranema* or saprotrophs like *Rhodomonas*).

Bikonts share a complex pattern of ciliary development with the anterior cilium being younger and transforming in the next cell cycle into a posterior cilium, often structurally and behaviourally different. This anterior-to-posterior ciliary transformation is unique to bikonts; it probably evolved in their common ancestor as an adaptation to gliding on surfaces, using the posterior cilium as a skid. Bikonts have two distinct posterior bands of microtubules as ciliary roots and ancestrally a structurally different anterior band. This cytoskeletal asymmetry was similarly adaptive, becoming secondarily more symmetrical in many algal lineages after they became planktonic and abandoned eating prey. Thus past nutritional history is reflected in cell structure of protozoa and their modern descendants. But the picture was confusing, because of repeated evolutionary losses of organelles, until molecular evidence helped sort it out.

In marked contrast to bikonts, unikonts ancestrally had only one centriole and no microtubular bands; instead a simple cone of microtubules anchored the centriole to

the nucleus and the rest of the cell. I consider this simpler structure the ancestral state for eukaryotes that evolved together with the cell nucleus. In only slightly modified form this ancestral unikont pattern is still seen in zoospores of lower fungi, choanoflagellates, the best model for animal ancestors, and our own sperm – such is the force of stasis in cellular evolution, much of which we can reconstruct by studying those most fascinating of all organisms, the protozoa, our relatives and ancestors.

Thomas Cavalier-Smith FRS

President, British Society for Protist Biology
(www.protist.org.uk)

Department of Zoology, University of Oxford, South Parks Road, Oxford OX1 3PS, UK (e tom.cavalier-smith@zoo.ox.ac.uk)

Further reading

- Bass, D. & Cavalier-Smith, T. (2004). Phylum-specific environmental DNA analysis reveals remarkably high global biodiversity of Cercozoa (Protozoa). *Int J Syst Evol Microbiol* 54, 2393–2404.
- Cavalier-Smith, T. (2003). Protist phylogeny and the high-level classification of Protozoa. *Eur J Protistol* 39, 338–348.
- Cavalier-Smith, T. (2004). Chromalveolate diversity and cell megaevolution: interplay of membranes, genomes and cytoskeleton. In *Organelles, Genomes and Eukaryote Phylogeny Systematics Association Special Volume 68.*, pp. 75–108. Edited by R.P. Hirt & D.S. Horner. London: CRC Press.
- Cavalier-Smith, T. (2006). Cell evolution and earth history: stasis and revolution. *Phil Trans R Soc Lond B* 361, 969–1006.
- Cavalier-Smith, T. (2006). The tiny enslaved genome of a rhizarian alga. *Proc Natl Acad Sci U S A* 103, 9739–9780.
- Richards, T. & Cavalier-Smith, T. (2005). Myosin domain evolution and the primary divergence of eukaryotes. *Nature* 436, 1113–1118.