Protist Systematics

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Online posting date: 15th January 2014

Protist systematics is concerned with the classification of the typically microscopic organisms found in abundance nearly everywhere in the Earth's biosphere. Such organisms include the algae, the protozoa and certain lower fungi. Protists were assigned to the taxon Protista by Haeckel. However, it wasn't until the technological innovation of the electron microscope and the repopularisation by Margulis of the theory of serial endosymbiosis to explain the origin of eukaryotes that the discipline of protistology achieved a critical mass. By the end of the twentieth century, protistologists agreed that the Protista was not a natural assemblage. Data from both the ultrastructure of the flagellar/ciliary apparatuses of diverse lineages and later gene/genome sequences confirmed this. Currently, a consensus is emerging that there are possibly three major assemblages of eukaryotes into which the majority of protists can be assigned: Adl *et al.* (2012) have named these the Amorphea, Excavata and Diaphoretickes.

Introduction

More than 130 years ago, several biologists suggested and named a 'third kingdom' of organisms to contain principally the microorganisms known at that time. Their general belief was that such organisms did not fit at all well into the groups of macroscopic organisms – the conventional plants and animals of Linnaeus. The most lasting proposed name has been the Protista of Ernst Haeckel (1866, 1878) even though this name does not have nomenclatural priority (e.g. Protoctista was suggested by Hogg (1860)). This is because the universally accepted rules of taxonomic nomenclature do not apply to names of ranks other than the family, genus and species. **See also**: History of Taxonomy

Today, 'protist' has come to embrace essentially the conventional high-level taxonomic assemblages known commonly as the protozoa or heterotrophic protists, the algae or phototrophic protists and the zoosporic and plasmodial fungal groups. However, it is no longer regarded by most protistologists as a monophyletic grouping. How to treat this highly diverse collection of species, slightly over half of which are fossil species, systematically is the main subject of this article. **See also**: Algae: Phylogeny and Evolution; Fossil Record; Fungi and the History of Mycology; Protozoa; Protozoan Evolution and Phylogeny

Historical Considerations

For many centuries, microorganisms, generally several orders of magnitude larger than the totally invisible bacterial forms of life, have been noted, if not identified or given scientific names. Aristotle and other great natural philosophers in Greece and elsewhere were quite aware of the tests or shells of foraminifera and of the luminescence or discolouration of seas, snows and ponds. However, they did not understand the significance of these shells or the causes of these discolourations. It was not until the seventeenth century were 'protists' actually observed and described by the early microscopist from Delft, Holland – Antoni van Leeuwenhoek (Dobell, 1932). **See also**: Foraminifera; Leeuwenhoek, Antoni van

During the 100 years following Leeuwenhoek's remarkable descriptions, few new observations of 'protists' were made, with the most notable exceptions being the works of Louis Joblot in France and John Hill in England. Then, the important monographic taxonomic treatises of O. F. Müller of Denmark appeared in the 1770s and 1780s. However, it was really not until the first half of the nineteenth century that studies of algae, protozoa and lower fungi began to be carried out in earnest and on a large scale because microscopes had been technically improved (Corliss, 1992). **See also**: Fungi and the History of Mycology; History of Parasitology; Phycology

By the late nineteenth century, universities were establishing separate Departments of Botany and Zoology to study plants and animals, respectively. These administrative divisions established the academic study of the
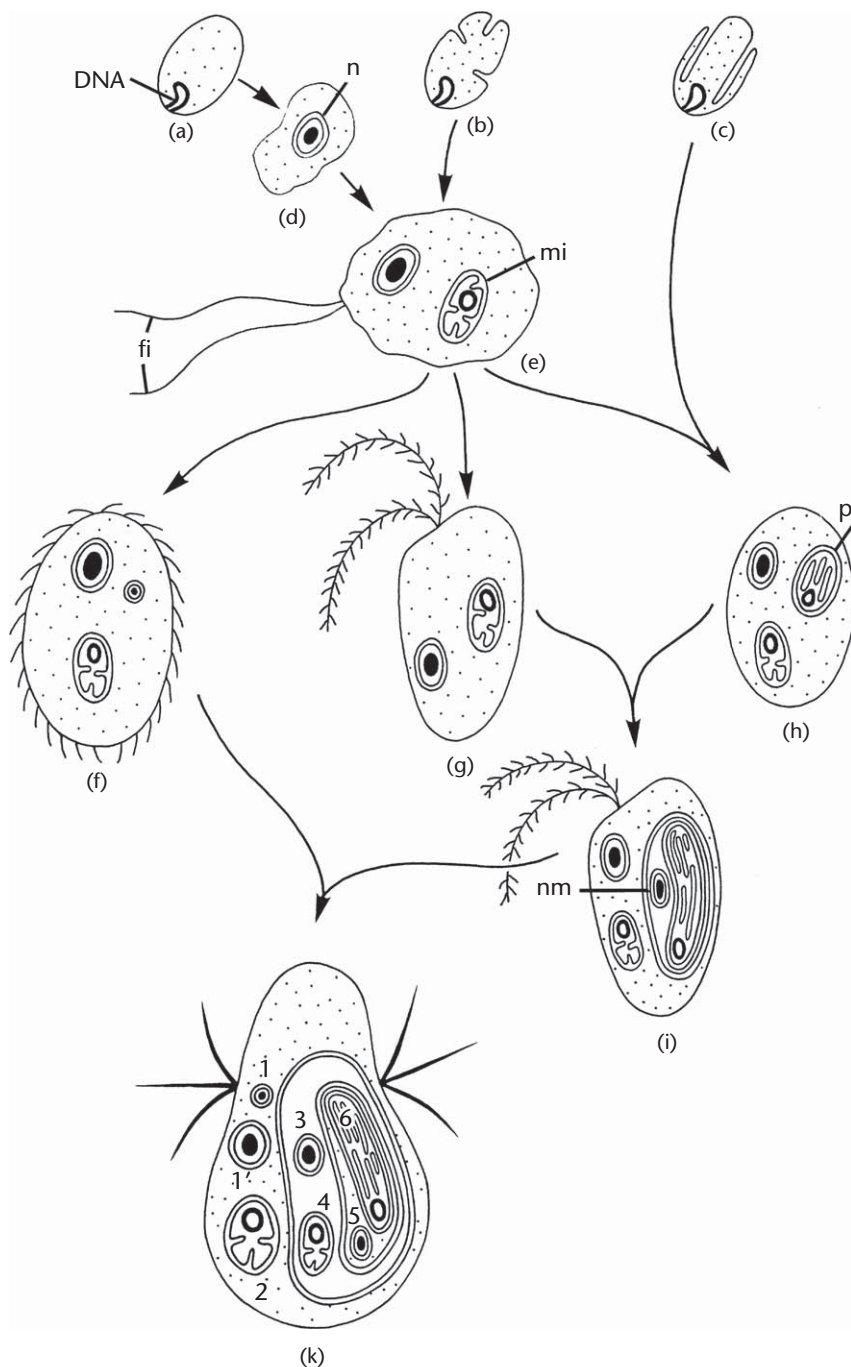
Figure 1 A model of eukaryogenesis through serial endosymbioses. A prokaryote ancestor (a) evolves as a nucleated (n) proeukaryote (d) that becomes ciliated/flagellated (fl) and ingests (e) an α-proteobacterium (b), which evolves as the first mitochondrion (mi). Later this ancestral eukaryote (e) ingests a cyanobacterium (c), which evolves as the first plastid (pl), and in some forms loses the flagellum to become an immotile phototrophic form, like a red alga (h). The heterotrophic ancestral eukaryote gave rise to a variety of lineages, such as ciliates with their micronucleus and macronucleus (f) and cryptomonad-like flagellates (g). Some cryptomonads ingested a red algal cell (i), and this is a secondary endosymbiosis. The ciliate *Mesodinium rubrum* (k) ingested a cryptomonad, making this a tertiary endosymbiosis that has six compartments containing DNA (1–6). Reproduced with permission of Hausmann, Hülsmann and Radek. © Schweizerbar'sche Verlag.

animal-like protists or protozoa by zoologists and the study of plant-like protists or algae by botanists. Even though Cell Theory argued that these organisms were similar as cells, there was not a united effort to recognise the many similarities among 'protists'. Thus, when Haeckel (1866, 1878) established his Protista, it was not enthusiastically embraced by zoologists and botanists. This continued even up to the mid-twentieth century when

the striking differences between bacteria and all other organisms resulted in the taxonomic naming of bacteria as Prokaryota and all other forms of life as Eukaryota (Corliss, 1986). In the mid-twentieth century, two important developments occurred – one technological and the other conceptual – which laid the groundwork for a refocus on the unifying features of 'protists'. The technological innovation was the development of techniques to enable use of the electron microscope on biological material. This not only revealed a whole new set of characters that increased our understanding of 'protist' structural diversity but also revealed similarities at this level of organisation that suggested that protozoa and algae were not monophyletic groups. The conceptual development was the repopularisation of the origin of eukaryotes through serial endosymbioses with various prokaryotic ancestors (Margulis, 1970, 1981). These two developments aided the establishment of a new research field – eukaryogenesis (i.e. the 'coming-into-being' or evolutionary appearance of eukaryotes), which aimed to understand the evolution of the first unicellular eukaryotes or 'protists' and how these later evolved into the multicellular/multitissued forms (**Figure 1**). **See also**: Eukaryotes and Multicells: Origin; Margulis, Lynn; Prokaryotic Systematics: a Theoretical Overview

Protistology emerged now as an independent investigative field in its own right. Interest in Copeland (1956) was revived, while the five-kingdom system of Whittaker (1969), popularised by Margulis and Schwartz (1998), 'reestablished' the Kingdom Protista as a formal taxonomic grouping, further highlighting the significance of this grouping of eukaryotes as distinct from bacteria, fungi, plants and animals. Scientific research in evolutionary protistology was profiled further by the founding of The International Society for Evolutionary Protistology by L. Margulis, F. J. R. Taylor and others. Nevertheless, even as Whittaker (1969) argued for his new vision of five kingdoms he would write:

> "The protists are a complex of variously interconnected evolutionary lines, of many evolutionary developments in parallel and convergence, and of phyla which have been difficult to delimit and some of which are doubtless polyphyletic." (p. 158)

By the end of the twentieth century, the Handbook of Protoctista (Margulis *et al.*, 1990) highlighted the tremendous diversity of 'protists', which had already led some to suggest alternate ways of viewing the classification of life: Cavalier-Smith (1998) revised his six-kingdom classification of life! The revolution brought to biology by molecular genetics has had a tremendous impact on our understanding of the major lines of eukaryote evolution. Sequencing of the ribosomal ribonucleic acid (rRNA) genes and now sequencing of genomes is uncovering the deep phylogenetic relationships among eukaryote lineages. This new understanding has in turn influenced our classification of life, including the placements of the

Figure 2 The chlorophycean *Chlamydomonas reinhardtii,* at one time classified in the Phytoflagellata of the Sarcomastigophora. Reproduced with permission of Wolfgang Bettighofer. © Wolfgang Bettighofer.

Figure 3 The euglenophycean *Euglena viridis,* at one time classified in the Phytoflagellata of the Sarcomastigophora. Reproduced with permission of William Bourland. © William Bourland.

'protist' groups. Although views are converging, there is still not complete agreement on how to classify the major monophyletic groups of eukaryotes (e.g. Adl *et al.*, 2012; Cavalier-Smith, 2012).

How to Classify Protists?

Classification is the activity of grouping organisms together at different taxonomic levels on the basis of shared, derived or homologous characters. As one would expect, the classification of eukaryotic microorganisms is strongly influenced by the technology that is used to observe them: the optical light microscopes of the seventeenth century revealed far less detail than their sophisticated technological descendants of the twentieth century and electron microscopes revealed even more detail.

Up until the mid-twentieth century, 'protists' were generally classified by significant features of their cytology or cell structure. For example, they might be pigmented or not: pigmented forms, such as green algae, were grouped together based on their colour, the assumption being that they shared the presence of plastids containing the green photosynthetic pigment chlorophyll (**Figure 2** and **Figure 3**). Other kinds of algae were distinguished based on their plastid pigments – brown and red – and these were

typically studied by botanists. Those 'protists' that were unpigmented were often observed to be heterotrophic and so were considered to be 'microscopic animals' or protozoans, and these were typically studied by zoologists.
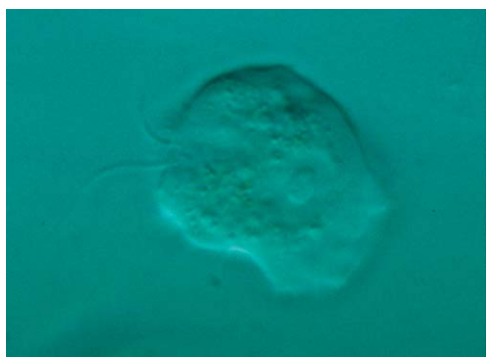

Figure 4 The amoebozoan *Planoprotostelium aurantium*, at one time classified in the Sarcodina of the Sarcomastigophora. Reproduced with permission of Frederick W. Spiegel. © Frederick W Spiegel.

Figure 6 The kinetoplastean *Trypanoplasma borreli*, at one time classified in the Zooflagellata of the Sarcomastigophora. Reproduced with permission of David J. Patterson, Linda Amaral-Zettler, M. Pegler and Thomas Nerad. © David J. Patterson, Linda Amaral-Zettler, M. Pegler, and Thomas Nerad.
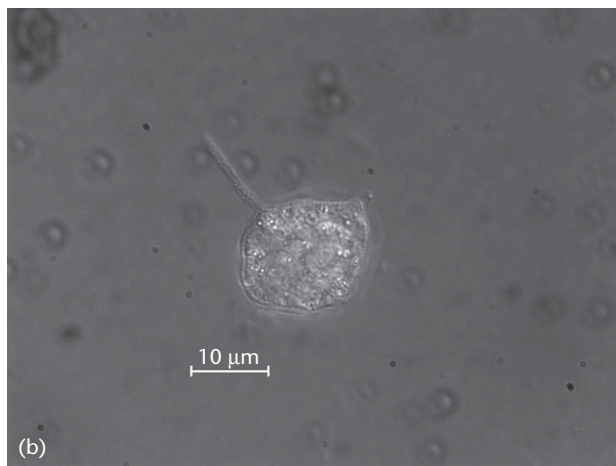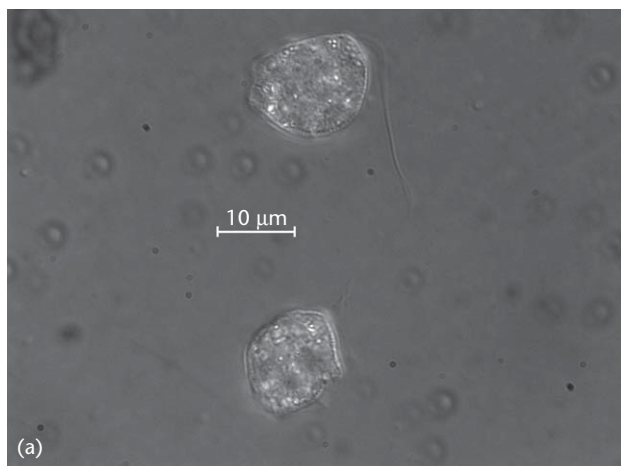

Figure 5 An undescribed rhizarian amoeboflagellate, probably a *Cercomonas* species, showing a flagellated (a) and amoeboid stage with a single pseudopodium (b). At one time it would have been classified in the Sarcodina of the Sarcomastigophora. Reproduced with permission of Francisco Amaro Torres. © Francisco Amaro Torres.

Figure 7 The parabasalian *Metadevescovina* sp. from the termite *Neotermes jouteli*, at one time classified in the Zooflagellata of the Sarcomastigophora. This protist has three flagella extending from the anterior end on the lower left, whereas the other 'filaments' are actually ectosymbiotic bacteria. Reproduced with permission of Patrick Keeling and Erick James. © Patrick Keeling and Erick James.

Figure 8 The oligohymenophorean ciliate *Paramecium caudatum*. Reproduced with permission of William Bourland. © William Bourland.

Figure 9 The spirotrich ciliate *Euplotes eurystomus*. Reproduced with permission of William Bourland. © William Bourland.

As well as pigmentation, protistologists used both body form and means of locomotion as significant characters: amoebae (**Figure 4** and **Figure 5**), flagellates (**Figure 6** and **Figure 7**), ciliates (**Figure 8** and **Figure 9**) and 'sporozoans' (**Figure 10** and **Figure 11**). Amoebae and flagellates were later united into one group as some amoeboid forms also carried flagella, whereas some flagellates could transform into amoebae under certain environmental conditions (e.g. *Naegleria*) (Hausmann *et al.*, 2003).

Zoological classifications of protists often recognised these groups as the highest ranks: amoebae (i.e. Sarcodina) and flagellates (i.e. Mastigophora) as Sarcomastigophora, ciliates as Ciliophora and spore-forming protozoa as Sporozoa (e.g. Manwell, 1961).

Table 1 Classification of the higher ranks of eukaryotes

Diaphoretickes
  Sar (**Figure 15**)
    Rhizaria
      Retaria
        Polycystinea (**Figure 15**)
        Foraminifera (**Figure 15**)
        Acantharia
      Cercozoa (**Figure 15**)
    Alveolata (**Figure 15**)
      Protoalveolata (P)
      Dinoflagellata
      Apicomplexa
      Ciliophora
    Stramenopiles
      Opalinata
      Blastocystis
      Bicosoecida
      Placidida
      Labyrinthulomycetes
      Hyphochytriales
      Peronosporomycetes
      Actinophryidae
      Bolidomonas
      Chrysophyceae
      Dictyochophyceae
      Eustigmatales
      Pelagophyceae
      Phaeothamniophyceae
      Pinguiochrysidales
      Raphidophyceae
      Synurales
      Xanthophyceae
      Phaeophyceae
      Schizocladia
      Diatomea
  Archaeplastida (**Figure 15**)
    Glaucophyta (**Figure 15**)
    Chloroplastida (**Figure 15**)
    Rhodophyceae (**Figure 15**)
Excavata (**Figure 15**)
  Discoba (**Figure 15**)
  Malawimonadidae (**Figure 15**)
  Metamonada (**Figure 15**)
Amorphea
  Amoebozoa (**Figure 15**)
    Dictyostelia (**Figure 15**)
    Tubulinea (**Figure 15**)
    Discosea
    Archamoebae
    Gracilipodida
    Multicilia
    Protosteliida
    Cavosteliida
    Protosporangiida
                    (*continued*)

Table 1 Continued

      Fractovitelliida
      Schizoplasmodiida
      Myxogastria
    Opisthokonta (**Figure 15**)
      Nucletmycea
        Fungi (**Figure 15**)
        Nucleariida (**Figure 15**)
        Fonticula
        Rozella
      Holozoa
        Ichthyosporea (**Figure 15**)
        Choanomonada (**Figure 15**)
        Metazoa, Animalia (**Figure 15**)
        Filasterea
        Aphelidea
        Corallochytrium
Incertae Sedis
  Apusomonadida (**Figure 15**)
  Ancyromonadida (**Figure 15**)
  Breviatea (**Figure 15**)
  Collodictyonidae (**Figure 15**)
  Mantamonas
  Rigidifilida (**Figure 15**)
  Cryptophyceae (**Figure 15**)
    Cryptomonadales
    Kathablepharidae (**Figure 15**)
    Goniomonas
  Centrohelida (**Figure 15**)
  Haptophyta (**Figure 15**)
  Telonemia (**Figure 15**)
  Palpitomonas
  Picobiliphytes
  Rappemonads
  Spironemidae

Note: For a more detailed information, see text, Figure 15, and Adl *et al*. (2012). P, paraphyletic.

During the period of intense research on protist ultrastructure, between 1950 and 1990, many new characters were discovered, especially related to the flagellar or ciliary apparatus. Various microtubular and fibrous rootlets were associated with the basal body or kinetosome and appeared to function to anchor and stabilise this organellar complex. Sufficient comparative data have now been acquired to demonstrate that these three zoological divisions of 'protists' are not monophyletic; for examples, the patterns of flagellar rootlets are not consistent within them (**Figure 12** and **Figure 13**; Moestrup, 2000). Even within major groups, such as the ciliates, groups established by optical light microscopic characters were not confirmed as monophyletic by features revealed by electron microscopy (Lynn, 2008). **See also**: Ciliophora

From the 1980s until today, molecular biology has played an increasingly significant role in deepening our

Figure 10 The spores, actually oocysts, of three apicomplexans – *Eimeria* (top left) from the intestinal tract of a chicken, *Cytoisospora* (bottom left) from the intestinal tract of a dog and *Monocystis agilis* (right) from the seminiferous vesicles of the earthworm *Lumbricis terrestris*. At one time these would have been classified in the Sporozoa. Reproduced with permission of Jan Slapeta. © Jan Slapeta.
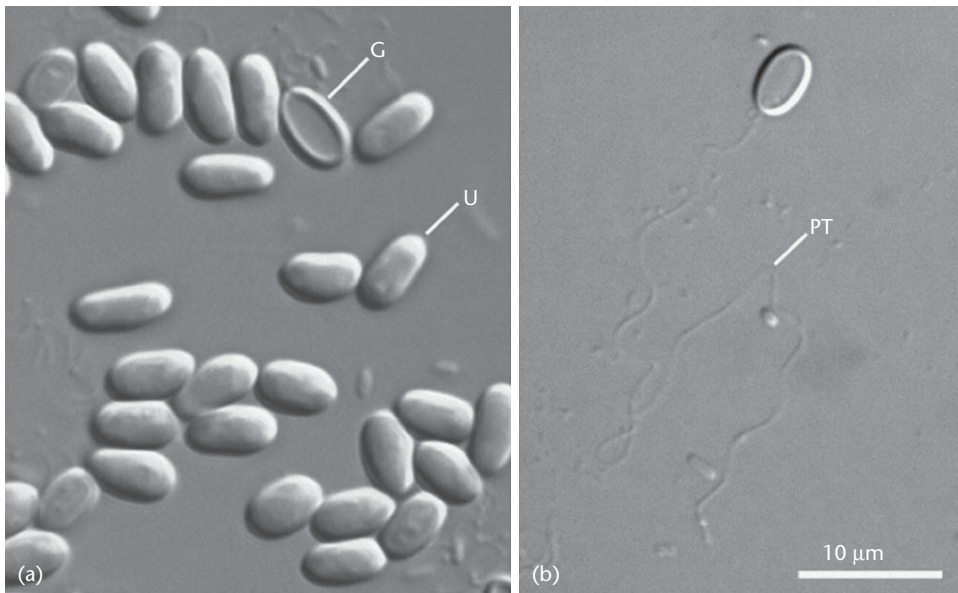

Figure 11 The spores of the microsporidian *Antonospora locustae*, a parasite of the locust, showing in (a) ungerminated spores (U) and germinated ones (G), and in (b) a germinated spore with its everted polar tube (PT). At one time it might have been classified in the Sporozoa. Reproduced with permission of Keeling (2009). © PLoS.
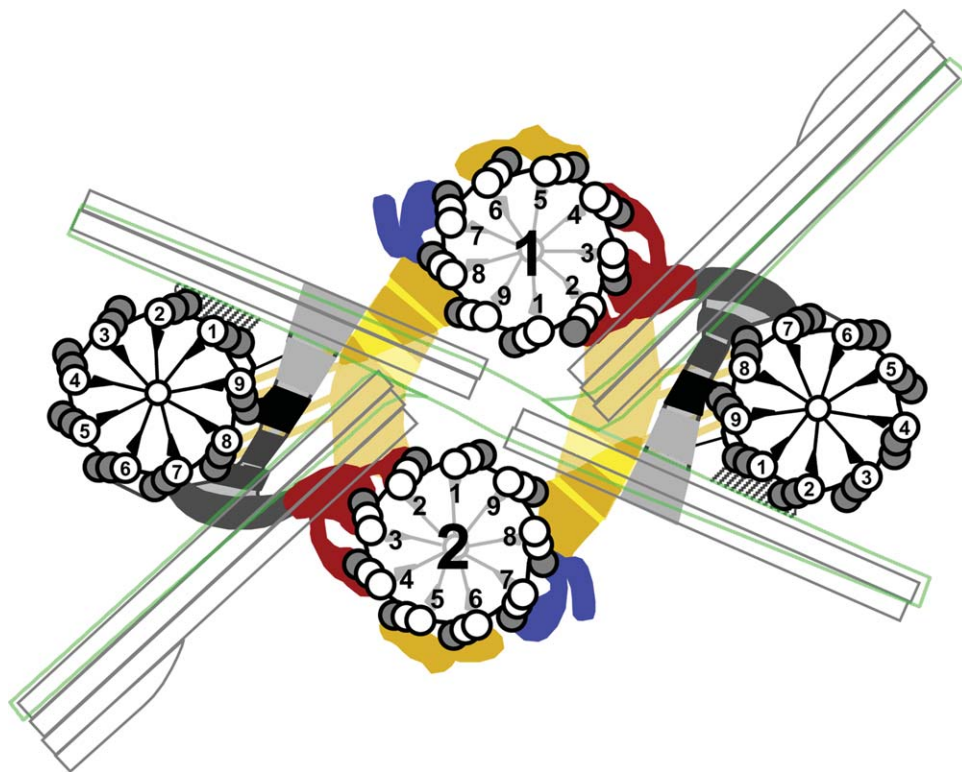
Figure 12 Flagellar apparatus of *Chlamydomonas reinhardtii* at a level below the cruciate microtubular rootlets. This flagellate has two ciliated basal bodies or kinetosomes (1, 2), and associated with them are two probasal bodies. This arrangement is quite different from its former 'phytoflagellate' relative *Euglena* (cf. **Figure 13**). Reproduced and adapted with permission from Geimer and Melkonian (2004). © The Company of Biologists Ltd.

understanding of relationships among and within protists. The first gene chosen, coding for the RNA that forms the skeleton of the small subunit of the ribosome – small subunit rRNA, confirmed some of the new lineages recognised by electron microscopy and suggested others that had only a 'genetic fingerprint' (Lynn, 2008; Sogin *et al*., 1996). In recent years, sequences of hundreds of genes that represent a larger sampling of the genome have revealed even deeper relationships among 'protists' and all eukaryotic life (**Figure 14**; Burki *et al*., 2012; Hampl *et al*., 2009; Parfrey *et al*., 2010). These kinds of studies have enabled us to revise our views on what are now regarded as the major groups of protists (Adl *et al*., 2012; Cavalier-Smith, 2012).

Major Groups of Protists

In this section, a brief characterisation of the major groups of protists follows (**Figure 15**; **Table 1**), based on the revised classification of Adl *et al*. (2012). This classification is a hierarchical one, but it does not use ranks or categories (i.e. phylum, class, order) as Adl *et al*. (2012) have argued that the diversity of life and the pattern of its evolution are too complex and varied to be captured by the traditional ranks used in classification.

First, a tree is emerging, which suggests that eukaryotic diversity may be divided into three major assemblages: (group 1) the Archaeplastida, Sar and some minor Incertae Sedis lineages (e.g. Telonemia, Haptophyta, Centrohelida, Cryptophyceae and Kathablepharidae); (group 2) the Excavata; and (group 3) the Amoebozoa, Opisthokonta and some other minor Incertae Sedis lineages (e.g. Apusomonadida, Ancyromonadida and Breviatea) (**Figure 15**). Of these three, only the Excavata can be characterised by details of morphology: these protists typically have a suspension-feeding groove, which is supported by specific sets of microtubular ribbons and which is used for the capture and ingestion of small particles brought to the groove by the current created by a posteriorly directed flagellum (Simpson *et al*., 2006 and references therein, but see a contrary view by Cavalier-Smith, 2012). The other two groups are defined by their placements in phylogenies generated by multigene sequences. Group 1 has been named the Amorphea (*a*, Gr. – without; *morphos*, Gr. – form, shape), relating to the fact that the cells of most of the included groups do not have a fixed form unless they are restricted by an external layer, such as a cell wall, lorica or test. Group 3 has been named Diaphoretickes (*Diaforetikés*, Gr. – diverse), relating to the fact that this assemblage is extremely diverse in morphology and basic cellular features (Adl *et al*., 2012). Apart from these major groups, there is an emerging consensus that there are four
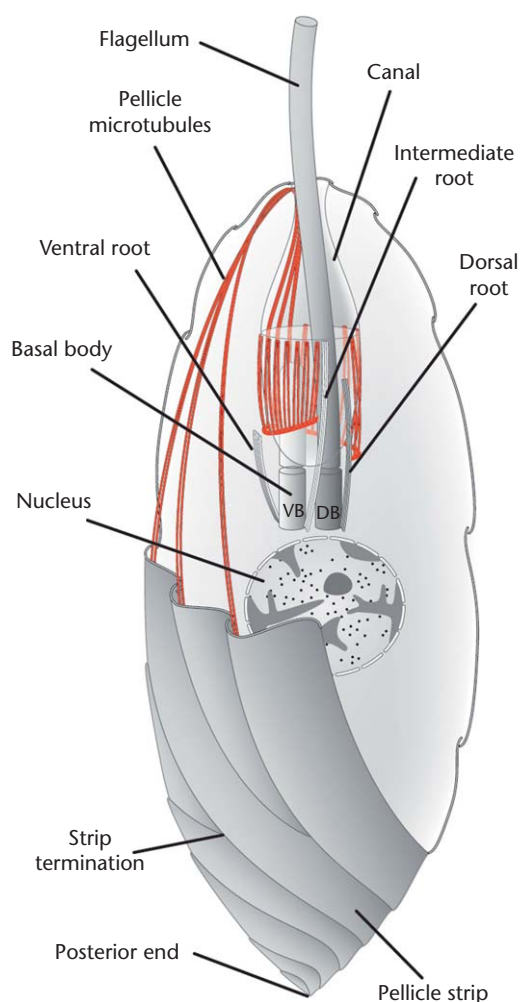
Figure 13 Flagellar rootlets and cytoskeletal apparatus of *Euglena gracilis* showing the origin of the microtubular rootlets near the dorsal (DB) and ventral (VB) basal bodies or kinetosomes. This arrangement is quite different from its former 'phytoflagellate' relative *Chlamydomonas* (cf. **Figure 12**). Reproduced with permission from Yubuki and Leander (2012). © Springer.

significant subgroups, which will be briefly commented upon below (**Figure 15**).

Before doing this, the Excavata deserves a little more attention. This group was first established to include a morphologically diverse group of typically endosymbiotic flagellates, which shared features of the microtubular rootlets associated with the flagellar apparatus and the feeding groove. The Excavata has now been broadened to include several free-living groups and even some amoeboid forms (e.g. acrasid slime moulds) (Simpson *et al.*, 2006).

The Archaeplastida is the major group that now includes the higher plants. The name of the group refers to our understanding that the ancestral primary endosymbiosis with a cyanobacterium occurred in this lineage to give rise to the photosynthetic plastid with chlorophyll *a* (Adl *et al.*, 2012). There are three major archaeplastida subgroups differentiated by features of the plastid: (1) the

Glaucophyta is the only group to have a plastid that has a peptidoglycan wall between its two membranes, much like the cyanobacterial ancestor of this organelle; (2) the Rhodophyceae or red algae have plastids with unstacked thylakoids and phycobilisomes and a special cytoplasmic carbohydrate reserve called floridean starch and (3) the Chloroplastida have plastids with chlorophylls *a* and *b* and typically a cell wall made of cellulose. **See also**: Embryophyta (Land Plants)

Amoebozoa is a diverse grouping of amoeboid forms that use noneruptive pseudopodia for locomotion. Many of the included groups disperse and/or reproduce by one or other of the two methods of 'fruiting': (1) sporocarpic fruiting in which a single amoeboid cell differentiates into a stalked structure at the top of which one to several spores are formed (e.g. Protosteliida) or (2) sorocarpic fruiting in which many amoebae aggregate into a multicellular mass that develops into a multicellular fruiting body (e.g. Dictyostelia and Myxogastria). **See also**: Slime Moulds

Opisthokonta are characterised by having a single posterior flagellum, which never carries flagellar 'hairs' or mastigonemes. The flagellum is present typically in at least one stage of the life cycle, although gene sequences suggest that some included taxa have lost flagellated stages. This group includes the two 'older' kingdoms – Fungi and Animalia: the former is now placed in the subgroup Nucletmycea and the latter is placed in the subgroup Holozoa; both Nucletmycea and Holozoa are only defined in relation to multigene-based phylogenies (Adl *et al.*, 2012; Brown *et al.*, 2009).

Sar is the last major group, again defined with reference to multigene-based phylogenies (Adl *et al.*, 2012; Burki *et al.*, 2012). It is considered to include three major subgroups – Stramenopiles, Alveolata and Rhizaria, which provide the acronym SAR on which the group's name is based. Stramenopiles are typically flagellates with two different flagella, hence heterokonts, although some stramenopiles have lost their flagella (e.g. *Blastocystis*). The anterior flagellum has tripartite mastigonemes arranged in two opposite rows, whereas the posterior or trailing flagellum is typically smooth. The Alveolata includes three major groups – Dinoflagellata, Apicomplexa and Ciliophora – and an assemblage of minor groups whose relationships are uncertain. Nevertheless, they all are assumed to have or have had cortical alveoli, which are membrane-bound sacs that underlie the plasma membrane. These alveoli show varying degrees of differentiation, from being very flattened to swollen and bag-like. The Rhizaria is a diverse grouping of amoeboid forms that have fine pseudopodia, varying from simple fine extensions to complex branched and anastomosing networks (e.g. Foraminifera). Some of the included taxa have been related by gene sequences only as they appear to have lost the amoeboid stage in the life cycle and are only flagellated (Adl *et al.*, 2012). **See also**: Apicomplexa; Ciliophora; Dinoflagellates; Foraminifera; Radiolaria

A number of lineages have been illustrated in grey in **Figure 15**, and these are considered Incertae Sedis or of
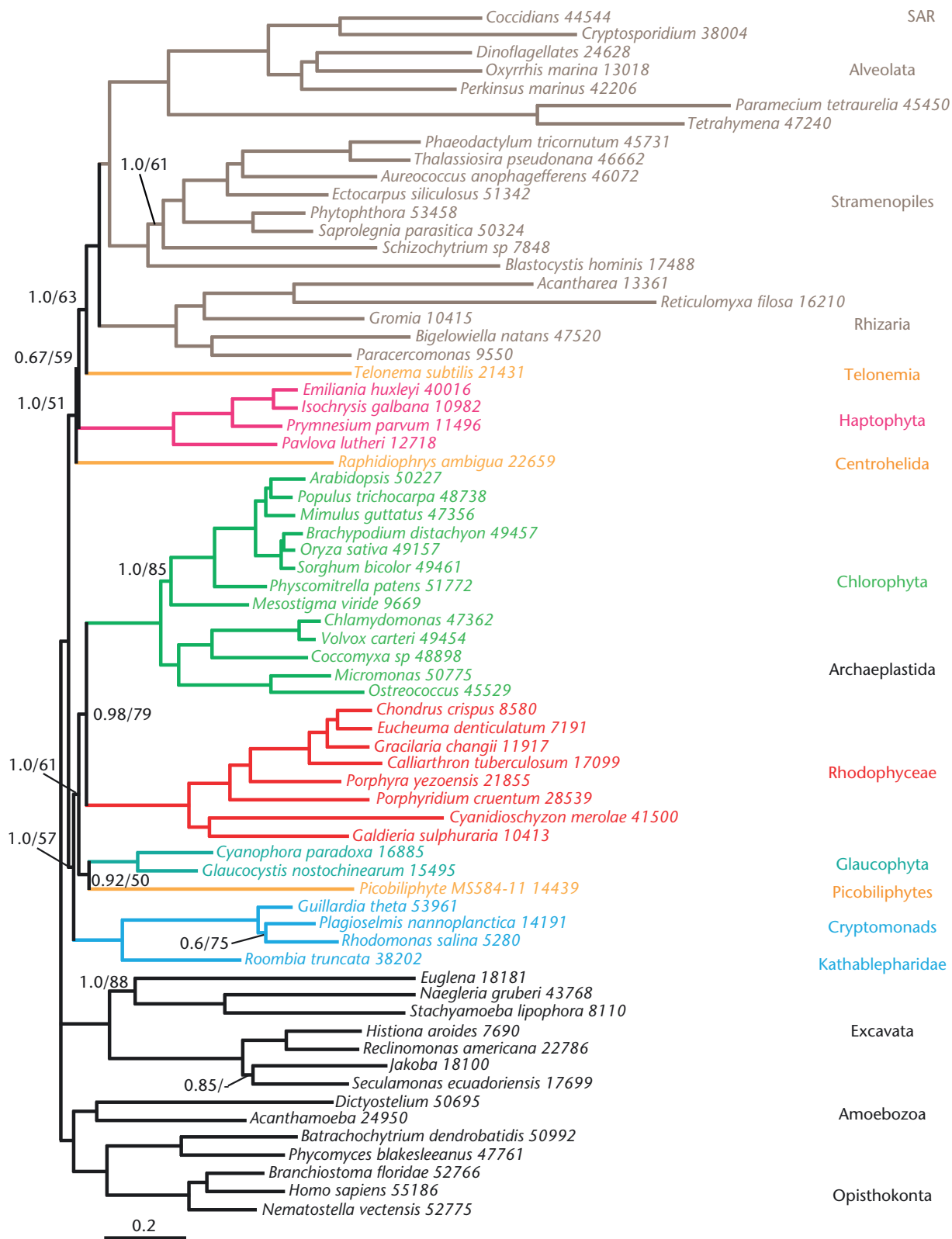
Figure 14 Global phylogeny of eukaryotes based on an alignment of 258 genes and inferred using the CAT+4 model. The numbers at the nodes are support values if the branch was not supported in all analyses. Reproduced and adapted with permission of Fabien Burki. © Fabien Burki.
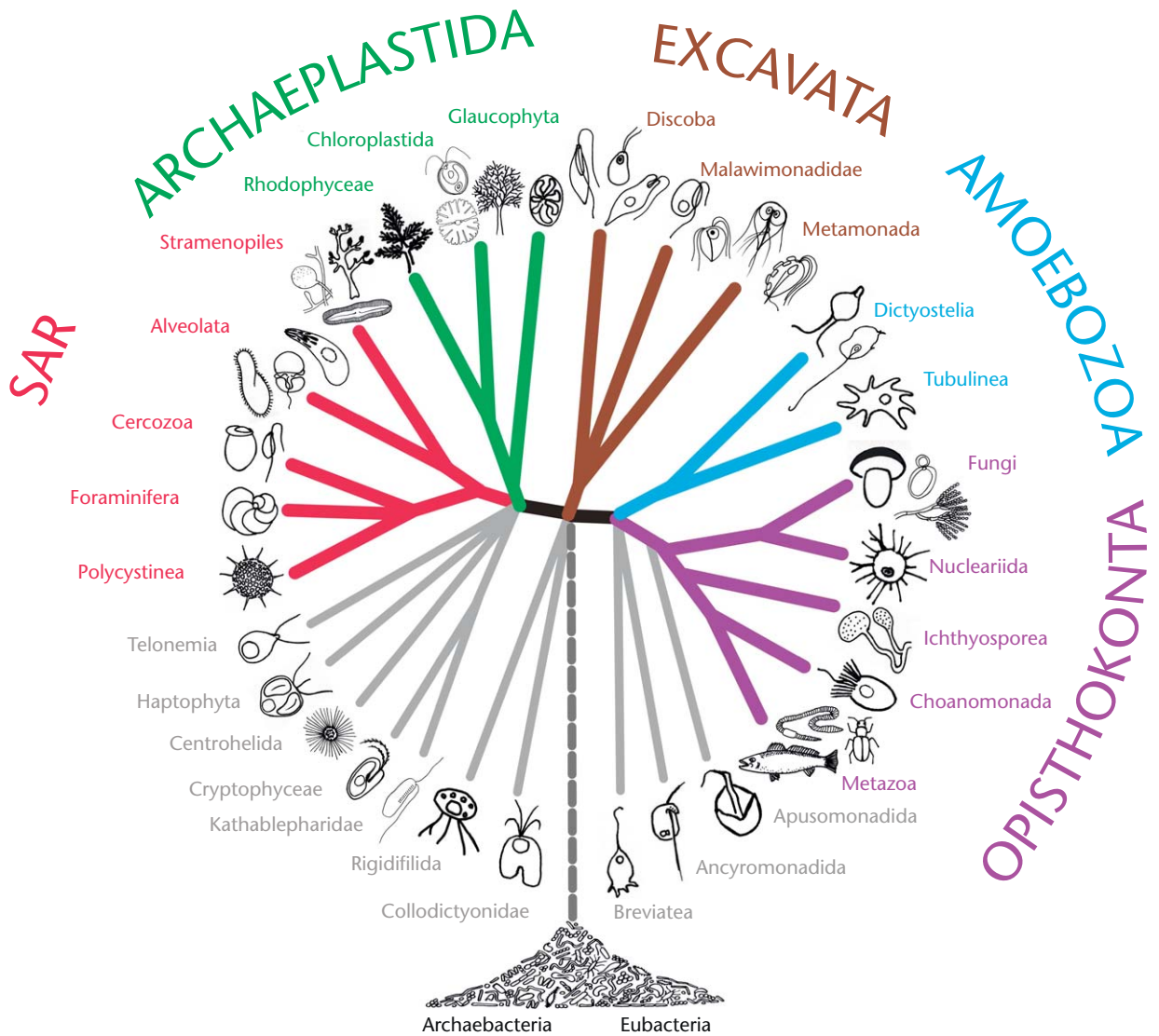
Figure 15 Schematic representation of the eukaryote 'Tree of Life' outlining the major groups in the revised classification of eukaryotes proposed by Adl *et al.* (2012). See also **Table 1** for further detail. Revised tree provided with permission by Sina Adl. © Sina Adl.

uncertain placement at the moment. Their locations on the tree are meant to suggest our 'best guess' as to which of the major lineages they are most closely related. Additional genetic and/or morphological information will be needed to confirm the placement of these groups.

Macroevolution and Microevolution

The above section 'Major Groups of Protists' has focussed on the 'megasystematics' of the major groups of protists, groups above the species. This is what has also been called the study of macroevolution – evolution above the species level. However, systematists are also interested in the process of evolution at the levels of populations and

species, which can also be called microevolution. **See also**: Microevolution and Macroevolution: Introduction

The microevolutionary study of protists is a relatively new subject area, which is also a hotly debated one. This is because with larger organisms, one of the major mechanisms driving speciation is the separation of populations by geographic boundaries that prevent the movement of animals: this is called allopatric speciation. Some protistologists argue that no such boundaries exist for protists, and so they predict low genetic diversity within protist species and no geographic patterns for protist populations.

In fact, morphological species of protists, contrary to this prediction, do show moderate to high levels of genetic diversity (Gentekaki and Lynn, 2012 and references therein; Watts *et al.*, 2011 and references therein). In some cases, there are no geographic patterns of genetic variation

(Gentekaki and Lynn, 2012 and references therein), whereas in other cases, geographic patterns of genetic variation map to regions that have been previously identified through studies on larger animals and plants (Watts *et al*., 2011 and references therein). As more data accumulate in this area, one will undoubtedly have more detailed explanations for the patterns or lack of patterns of genetic variation of protists.

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