Protist Evolution and Phylogeny

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The beginning of this century brought spectacular changes in our understanding of eukaryote phylogeny, especially the early evolution of microeukaryotic lineages commonly called protists. Phylogenomic studies based on analysis of more than 200 genes produced a relatively well-resolved tree of eukaryotes. Metagenetic studies using environmental deoxyribonucleic acid (eDNA) revealed an extraordinary diversity of protist lineages, highlighting their great ecological and evolutionary importance. A new higher level classification of eukaryotes combining the results of microscopic and various 'omics' studies has been proposed. In this classification, the majority of eukaryotes are placed in seven monophyletic supergroups: Amoebozoa, Opisthokonta, Archaeplastida, Alveolata, Stramenopiles, Rhizaria and Excavata. Phylogenetic relationships between these supergroups and 11 independent eukaryotic lineages are relatively well established. However, there is no consensus concerning the position of the root of eukaryotic tree. Further single-cell microscopic and genomic studies are also necessary for exploring the extraordinary diversity of protistan phyla revealed by the eDNA surveys.

Introduction

Protists is an informal term describing all eukaryotes other than animals, plants and fungi. The majority of protists, also called 'microeukaryotes', 'microbial eukaryotes' or 'unicellular eukaryotes', are unicellular and microscopic. However, small size and their single-celled nature can hardly define all protists. Some of them can reach extraordinary dimensions, such as xenophyophorean foraminifera, whose sponge-like tests pave the deep-sea bottom, or the laminarian seaweeds, whose plant-like thalli become several metres long and form dense forests in coastal regions. Moreover, aggregative multicellularity is commonly found in some protist groups (e.g. cellular slime moulds).

From an evolutionary perspective, protists are a paraphyletic group, that is, a group that shares its ancestor with other groups. In fact, the last common ancestor of protists is also the ancestor of all other eukaryotes. For convenience, the traditional 'five kingdoms' system is used to separate typically microscopic and unicellular protists from macroscopic and multicellular animals, plants and fungi. However, this separation has no other justification than our anthropocentric perspective. As shown by modern phylogenetic analyses, the three dominant kingdoms are in reality just small branches amidst a vast radiation of eukaryotes. Genetic distance between the ancestor of animals and fungi is as large as the distance between two distant groups of amoebas. For these reasons the 'five kingdoms' system was abandoned and replaced by a much more objective and phylogenetically coherent system of eukaryotic supergroups. The new system is widely accepted now and its historical development and current interpretation are presented here.

Ribosomal Tree of Eukaryotes

The first molecular trees of eukaryotes were based exclusively on analyses of ribosomal genes. This was mainly due to the exceptional conservation of these genes across all eukaryotic phyla. From a practical point of view, ribosomal genes represented by thousands of identical or nearly identical copies in every single cell were the easiest to amplify and to sequence. Therefore, it was not surprising that in the early days of molecular systematics, all phylogenetic studies of prokaryotes and eukaryotes were based on ribosomal genes (Pace *et al.*, 1986; Sogin, 1991).

As prokaryotes phylogeny was based on the small subunit 16S ribosomal ribonucleic acid (rRNA) gene, eukaryotic phylogenetists predominantly used the small subunit 18S rRNA gene. Indeed, this gene still constitutes a gold

standard for eukaryotic species identification and phylogenetic inferences at a lower taxonomic level (Pawlowski *et al.*, 2012). The Protist Ribosomal Reference (PR2) database http://ssu-rrna.org/ comprises currently approximately 130 000 18S ribosomal deoxyribonucleic acid (rDNA) sequences that represent all eukaryotic phyla (Guillou *et al.*, 2012). In addition, thousands of 18S sequences generated by high-throughput environmental DNA surveys are submitted yearly to DNA databases.

Although ribosomal genes remain a reference for studying protist diversity, their current use for inferring eukaryotes phylogeny is much more limited. This is mainly due to artefacts caused by an excessive heterogeneity of evolutionary rates across eukaryotic phyla. Early ribosomal trees of eukaryotes have a characteristic ladder structure with longer branches at the bottom and shorter branches in the crown of the tree (Sogin, 1991). As most of the amitochondrial parasitic lineages have been found at the base of the tree, it gave rise to the Archeozoa hypothesis, suggesting that early eukaryotes, such as diplomonads, trichomonads and microsporidians, originated before the event of mitochondrial endosymbiosis (Cavalier-Smith, 1993). Although this hypothesis was very appealing, it did not last for long because several studies demonstrated that most of these putative early eukaryotic lineages contain residual mitochondrial genes, suggesting that they have lost mitochondria due to their parasitic mode of life. Moreover, the genomic studies showed that some of them (e.g. microsporidia) were misplaced due to rapid evolutionary rates of their ribosomal genes. Hence, the group of Archeozoa was finally abandoned, remaining the most famous example of long-branch attraction artefact in eukaryotic phylogeny (Philippe *et al.*, 2000).

Yet, not all phylogenetic relationships inferred from 18S rRNA gene were false. In fact, most of the currently recognised eukaryotic supergroups have been already present in ribosomal phylogenies (Cavalier-Smith, 1998, 2002). Some of them, such as Alveolates and Stramenopiles (Heterokonta), have been established very early based on ultrastructural and molecular evidence. Ribosomal trees also show evidence for close relationships between animals and fungi and a common origin of green and red algae. However, these relationships were not strongly supported. The most problematic groups were amoeboid protists, whose 18S rRNA genes evolved at highly variable rates; lobose amoebae, for example, were scattered across eukaryotic ribosomal phylogenies, whereas foraminifera were consistently placed at the base of the trees far from other amoeboid protists.

Phylogenomic Adjustments

The currently accepted structure of the eukaryotic tree appeared with the advent of multigene phylogenies. Early consensus multigene trees, such as the ones published by Simpson and Roger (2002) and Baldauf (2003), comprise almost all currently recognised supergroups, albeit some of them are under different names or are subdivided into smaller groups. These trees were principally based on analysis of four proteins (actin, α-tubulin, β-tubulin, and EF1), combined with analysis of ribosomal genes (Baldauf *et al.*, 2000). Further development of multigene phylogenies led to a reduction in the number of supergroups, obtained by introducing larger assemblages, such as Unikonts (grouping Opisthokonts with Amoebozoa) and Chromalveolates (grouping Alveolates, Stramenopiles, Haptophytes and Cryptophytes).

The support for these groups in early multigene phylogenies composed usually of less than 10 genes was relatively weak, leading to a critical evaluation of the new eukaryotic system (Parfrey *et al.*, 2006). Nevertheless, with the development of new phylogenomic tools, the analysis of large datasets became much more accurate and the detection and correction of various biases more efficient (Rodrguez-Ezpeleta *et al.*, 2007). Moreover, the database of protistan genomes grew rapidly. The major progress was obtained by analysis of transcriptomic sequences developed within the Protist EST Program (PEP) and other similar projects. The access to EST data allowed analysis of more than 100 genes and obtaining much more robust phylogenies (Burki *et al.*, 2008).

Among several issues raised by the development of eukaryotic phylogenomics, the most controversial one was the Chromalveolate hypothesis suggesting a unique origin of red algal secondary endosymbiosis (Keeling, 2009). From a phylogenetic perspective, this hypothesis was confirmed by the robust clade of Stramenopiles and Alevolates. However, the clustering of these two groups with Haptophytes and Cryptophytes was never strongly supported. A serious doubt about the existence of Chromalveolates appeared when it became evident that the sister group to Stramenopiles and Alveolates is Rhizaria, which is known to comprise no phototrophes, except for Chlorarachniophytes bearing chloroplasts of green algal origin (Burki *et al.*, 2007). The introduction of Stramenopiles + Alveolates + Rhizaria (SAR) and the growing support for this assemblage with an increasing number of analysed genes were difficult to reconcile with the Chromalveolate hypothesis.

Another negative evidence for Chromalveolates hypothesis came from the phylogenomic analyses of Haptophytes and Cryptophytes. In early studies, both groups were grouped together with other orphan protistan lineages, such as Telonemia and Centroheliozoa, in new putative supergroups called cryptomonads, centrohelids, telonemids, haptophytes (Burki *et al.*, 2009) or Hacrobia (Okamoto *et al.*, 2009). However, there was no strong support for these groupings and further analyses based on more than 200 genes showed that Cryptophytes, Haptophytes, Centrohelids, Telonemids and some other protistan lineages branch independently in the eukaryotic tree (Burki *et al.*, 2012). The Chromalveolate hypothesis was finally abandoned and the current structure of the eukaryotic tree comprises 4–7 supergroups and a few microkingdoms, as illustrated in **Figure 1**.
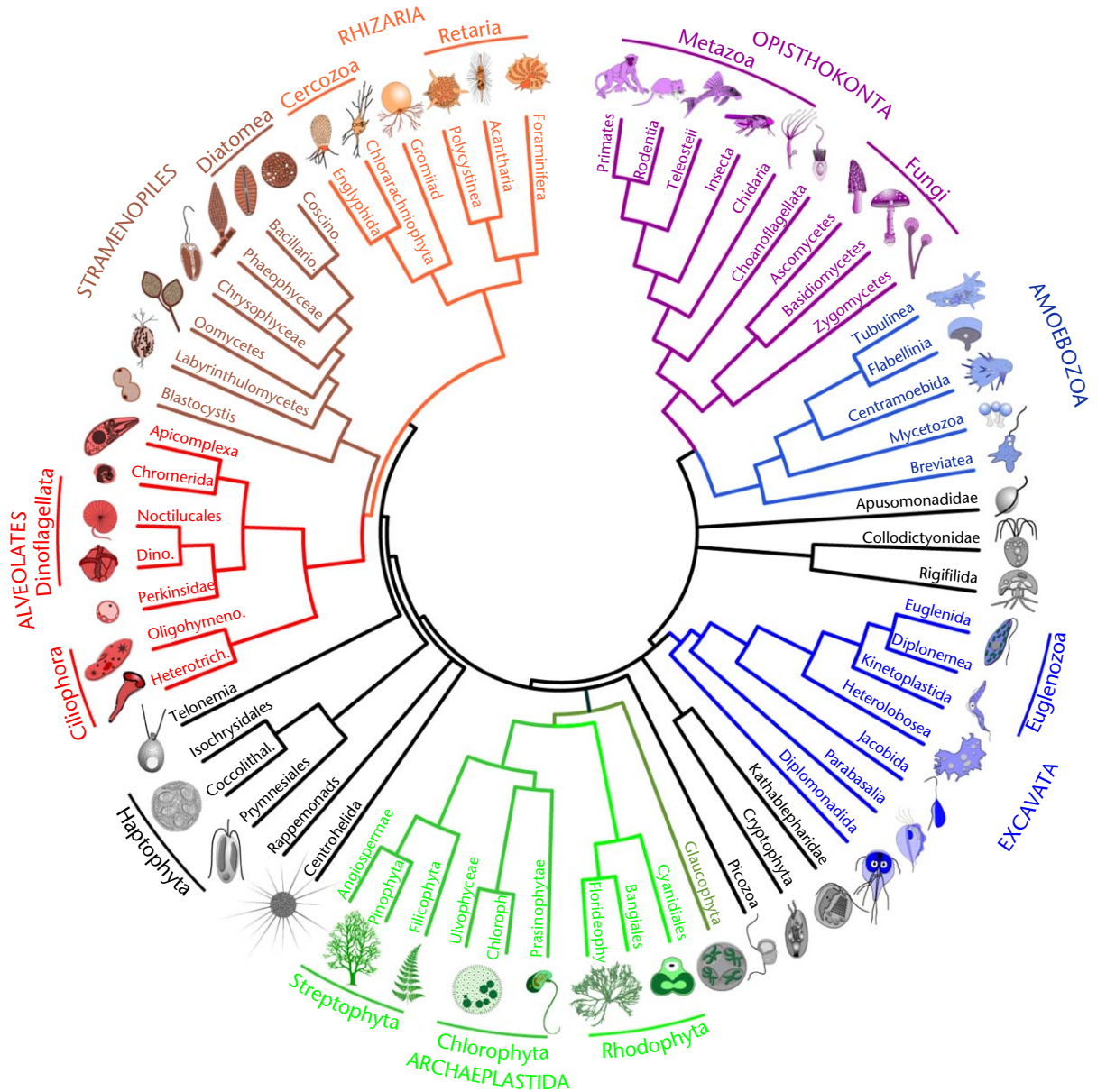
Phylogenomic tree of eukaryotes

Figure 1 Phylogenomic tree of eukaryotes. Modified after the analysis of 258 genes by Burki *et al.* (2012) and other recent publications.

Metagenetic Explosion

In parallel to phylogenomic attempts to reinforce the support for eukaryotic phylogenies, a particular effort was also made to assess the diversity of eukaryotic phyla by metagenetic studies. These studies consist of sequencing ribosomal gene fragments obtained by PCR amplification of environmental DNA (eDNA) using more or less specific eukaryotic primers. Early metagenetic studies were based on the cloning of ribosomal amplicons, which strongly limited the number of obtained sequences. The development of

high-throughput sequencing technologies removed these limitations and we are currently witnessing an explosion of metagenetic data.

Metagenetic studies showed right from the beginning that the traditional view of protistan diversity is strongly underestimated (Moreira and Lopez-Garcia, 2002). Most of the huge diversity of protistan ribotypes revealed by eDNA surveys could not be assigned to known taxa. As highlighted by some studies, this astonishing richness could partly be due to technical artefacts, such as chimera (Berney *et al.*, 2004). Yet, a careful examination of eDNA

ribotypes shows that many of them represent true eukaryotic lineages that either are new to science or have not been sequenced yet. **See also**: Microbial Diversity

The upsurge of protist diversity played a pivotal role in phylogenomics. The eukaryotic tree was enriched by hundreds of environmental lineages branching often at the base of protistan phyla (Bass and Cavalier-Smith, 2004). Some of them (e.g. Rappemonads and Picozoa – formerly Picobiliphytes) could not be assigned to any supergroup (Not *et al.*, 2007; Kim *et al.*, 2011). Others formed new highly diversified picoeukaryotic phyla within known supergroups, such as marine stramenopiles (MAST) and marine alvolates (MALV). Cryptic diversity was also observed at lower taxonomic level, with practically all protistan species subdivided into a number of genetically distinct phylotypes or operational taxonomic units.

Characteristics of Major Eukaryotic Supergroups

The International Society of Protozoology has recently published a new version of eukaryotic classification (Adl *et al.*, 2012). According to this paper, signed by 25 experts in protist taxonomy, the eukaryotes are divided into five supergroups: Amoebozoa, Opisthokonta, Excavata, SAR and Archaeplastida, plus some independent taxa. Based on phylogenetic and phylogenomic analyses, Amoebozoa and Opisthokonta are combined into the assemblage of Amorphea, previously referred to as 'Unikonta', whereas the Archaeplastida, SAR, Cryptophyceae, Centrohelida, Telonemia and Haptophyta are grouped into the assemblage of Diaphoretickes.

For reasons of clarity, the large assemblages (Amorphea, Diaphoretickes and SAR) are omitted here. The composition of the remaining seven supergroups is presented on the basis of the latest phylogenetic studies. The independent lineages are grouped according to their phylogenetic position and described separately as *incertae sedis* eukaryotes. The major taxa of each supergroup, including their catalogued and estimated diversity, are indicated in **Table 1**.

Amoebozoa

As indicated by its name, this supergroup is composed mainly of amoebae. However, only the amoebae bearing lobopodia, traditionally classified as Lobosea, belong to this supergroup; filose and reticulose amoebae branch either within Rhizaria or Stramenopiles. In addition to the lobosean amoebae, Amoebozoa comprise a few flagellates, including the uniciliate genus *Phalansterium* and the multiciliated species *Multicilia marina*. Another amoeboflagellate species, *Breviata anathema*, branches as sister group to Amoebozoa in phylogenomic analyses (Minge *et al.*, 2009) and is also included in this supergroup.

A recent phylogenetic revision divided amoebozoans into five major groups: Tubulinea, Discosea, Variosea, Archamoebea and Mycetozoa (Smirnov *et al.*, 2011). The first two groups comprise most of the free-living naked and testate species, differing in pseudopodial morphology and type of cytoplasmic flow: monoaxial in Tubulinea and polyaxial or without a pronounced axis in Discosea. Archamoebae include the amitochondrial free-living pelobionts and parasitic entamoebids, encompassing the human parasite *Entamoeba histolytica*. In some phylogenetic trees, the archamoebae are related to Mycetozoa (slime moulds), which comprises Dictyostelia and Myxogastria; however, there is no support for this grouping (Fiore-Donno *et al.*, 2010). The protostelid mycetozoans have been shown to form a polyphyletic assemblage (Shadwick *et al.*, 2009). The phylogenetic position of Variosea, which include flagellate species, and some other enigmatic amoebozoans, such as *Trichosphaerium*, remained unresolved. **See also**: Amoeba

Opisthokonta

Metazoa and Fungi dominate this supergroup, which comprises relatively few protistan taxa. These taxa are of pivotal evolutionary importance for explaining the origin of multicellularity and therefore have been the subject of many phylogenomic studies. There is strong genomic evidence for Choanoflagellates being the sister group of Metazoa. A recent phylogenomic study (Suga *et al.*, 2013) suggests that *Capsaspora owczarzaki*, an endosymbiontic amoeba of a pulmonate snail, is the closest relative to Metazoa + Choanoflagellates clade. However, genomic data for other unicellular opisthokonts branching at the base of Metazoa, especially *Ministeria* and ichtyosporean fish parasites, are lacking and their phylogenetic position is not well resolved (Steenkamp *et al.*, 2006). Similarly, there is no genomic data for unicellular lineages branching at the base of Fungi, such as *Nuclearia*, *Fonticula* and *Rozellida*, the last one forming a huge clade composed mainly of environmental sequences (Lara *et al.*, 2010).

Archaeplastida

This supergroup has been introduced for the three main lineages of primary photosynthetic organisms: glaucophytes, rhodophytes (red algae) and chlorophytes (green algae), the latter forming the group of Chloroplastida together with vascular plants. The three groups harbour plastids uniquely derived from cyanobacterial endosymbiosis, according to single- or multigenes phylogeny of plastid genes (Rodríguez-Ezpeleta *et al.*, 2005). However, phylogenetic support for the Archaeplastida is weak, especially if trees are based on nuclear genes (Deschamps and Moreira, 2009). In particular, the glaucophytes, which are a small group of freshwater algae, have a tendency to branch separately (Burki *et al.*, 2012).

Table 1 Current classification of eukaryotes, following Adl *et al.* (2012), including major phyla and their representative genera. Catalogued diversity according to Pawlowski *et al.* (2012)

Supergroups	Major taxa	Representative genera	Catalogued diversity
Amoebozoa	Tubulinea + Arcellinida	*Amoeba, Arcella*	1100
	Discosea	*Vannella, Paramoeba*	180
	Variosea	*Phalansterium, Multicilia*	20
	Mycetozoa	*Dictyostelium, Physarum*	1062
	Archamoebae	*Pelomyxa, Entamoeba*	>248
	Breviatea	*Breviata*	120.5
Opisthokonta	Metazoa	*Trichoplax, Drosophila*	1 200 000
	Fungi	*Chytridium, Saccharomyces*	377 200
	Choanoflagellida	*Diaphanoeca, Monosiga*	250
	Mesomycetozoa	*Capsaspora, Ministeria*	470.5
Archaeplastida	Chlorophyta (Streptophyta)	*Chlamydomonas, Ulva*	9000
	Streptophyta	*Chara, Pinus, Rosa*	350 000
	Rhodophyta	*Porphyridium, Bangia*	5000
	Glaucophyta	*Cyanophora*	50.5
Alveolata	Ciliophora	*Paramecium, Tetrahymena*	8000
	Dinophyceae	*Alexandrium, Symbiodinium*	2280
	Apicomplexa	*Plasmodium, Toxoplasma*	6000
	Perkinsea	*Perkinsus*	?
	Syndiniales	*Amoebophrya*	?
	Chromerida	*Chromera*	10.5
Stramenopiles	Bacillariophyta	*Thalassiosira, Nitschia*	20 000
	Bicosoecida	*Bicosoeca, Cafeteria*	72
	Chrysophyceae-Synurophyceae	*Chromulina, Ochromonas Synura*	1200
	Dictyochophyceae	*Dictyocha*	15
	Eustigmatales	*Vischeria*	15
	Hyphochytriales	*Hyphochytridium*	25
	Labyrinthulomycetes	*Labyrinthula, Amphitrema*	40
	Oomycetes	*Saprolegnia, Phytophthora*	676
	Opalinata	*Opalina*	400
	Pelagophyceae	*Aureococcus*	12
	Phaeophyceae	*Fucus, Laminaria*	1750
	Phaeothamniophyceae	*Phaeothamnion*	25
	Pinguiophyceae	*Pinguiochrysis*	5
	Raphidophyceae	*Goniostomum*	20
	Xanthophyceae	*Botrydium, Vaucheria*	6000.5
Rhizaria	Cercozoa	*Cercomonas, Euglypha*	600
	Foraminifera	*Ammonia, Allogromia*	12 000
	Acantharea	*Acanthometra*	160
	Polycystinea	*Collozoum, Saturnalis*	850
	Taxopodida	*Sticholonche*	1
	Ascetosporea	*Bonamia, Marteilia*	43
	Phytomyxea	*Plasmodiophora*	41
	Vampyrellida	*Arachnula, Vampyrella*	?
	Gromida	*Gromia*	90.5
Excavata	Euglenozoa	*Euglena, Trypanosoma*	1520
	Fornicata	*Giardia, Hexamita*	146
	Heterolobosea	*Naegleria, Vahlkampfia*	80
	Jakobida	*Jakoba, Andalucia*	10
	Malawimonadidae	*Malawimonas*	3
	Parabasalia	*Trichomonas, Trichonympha*	4660.5
*Incertaea sedi*s	Apusomonadidae	*Apusomonas, Ancyromonas*	12
	Centrohelida	*Raphidiophrys*	150
	Cryptophyta	*Cryptomonas, Rhodomonas*	70

Table 1 Continued

Supergroups	Major taxa	Representative genera	Catalogued diversity
	Haptophyta	*Emiliania*, *Coccolithus*	350
	Katablepharidophyta	*Roombia*	9
	Picozoa	*Picomonas*	1
	Telonemia	*Telonema*	2

Alveolata

This supergroup is composed of three major phyla: Ciliates, Dinoflagellates and Apicomplexa. Ciliates are mainly free-living protists, characterised by the presence of abundant cilia on their body surface, nuclear dualism and a conjugation stage during the sexual phase of the life cycle. They are one of the few classical protistan taxa, whose monophyly has never been questioned by molecular studies. There is also good evidence for the monophyly of dinoflagellates, characterised by two flagella in the motile stage, although this group is much more heterogeneous. Some dinoflagellates are phototrophes, whereas others are parasitic (e.g. *Alexandrium*) or endosymbiotic (e.g. *Symbiodinium*). Closely related to dinoflagellates are Apicomplexa, including among others the malarian parasite *Plasmodium*, a scourge of humanity. All apicomplexans are obligate parasites characterised by the presence of an apical complex that helps with the attachment and initial penetration of the host. In addition to these three major groups, the Alveolates comprise some independent lineages, such as the parasitic Syndiniales, whose huge diversity was revealed by metagenetic studies; the Perkinsinidae, which are well-known parasites of shellfish; and the phototrophic Chromeridae, recently isolated from corals. **See also**: Ciliophora; *Gymnodinium* and Related Dinoflagellates

Stramenopiles

This supergroup is possibly the largest and most diverse group of eukaryotes and is also often referred to as Heterokonta. It is characterised by the presence of typically two morphologically different cilia, one of them bearing two rows of tripartite hair (mastigonemes). Like Alveolates, the Stramenopiles have been introduced at the dawn of molecular phylogenetic studies. They are composed of several algal groups containing plastids resulting from secondary endosymbiosis, such as diatoms (Bacillariophyta), brown algae (Phaeophyceae) and golden algae (Chrysophyceae). The Stramenopiles also comprise a wide range of heterotrophic groups, including parasitic Oomycetes (water moulds and downy mildews, previously classified as Fungi) and endocommensal opalinids as well as free-living bicosoecids, amoeboid labyrinthulids, amphitremids and actinophryid heliozoans. Moreover, this supergroup also includes a highly diversified assemblage of marine uncultured picoeukaryotes (MAST), revealed by metagenetic studies (Logares *et al.*, 2012). **See also**: Diatoms

Rhizaria

Contrary to others, the definition of this supergroup is based exclusively on molecular data (Cavalier-Smith, 2002). Its monophyly is well supported by multigene phylogenies (Nikolaev *et al.*, 2004; Burki *et al.*, 2010) and by some molecular signatures, such as an amino acid insertion in the polyubiquitin gene (Bass *et al.*, 2005). Although any ultrastructural characters that would unite the main rhizarian taxa are not known, most of the organisms included here possess filose or reticulose pseudopodia, the feature after which the supergroup is named. At present, the Rhizaria are composed of two major assemblages (Cercozoa and Retaria) and a number of independent lineages. The Cercozoa form a diverse clade composed of biciliated heterotrophic taxa, such as cercomonads, the phototrophic chlorarachniophytes with reticulate pseudopodia, and the euglyphid filose amoebae with silicate outer shells (Cavalier-Smith and Chao, 2003). They also comprise taxa that have silica endoskeleton, such as ebriids and phaodarians, the latter one traditionally classified with Radiolaria (Polet *et al.* (2004)). The Retaria is a monophyletic group that comprises two of the most important microfossil groups: Foraminifera and Polycystinea. It also includes the radiolarian class Acantharea and the enigmatic Taxopodida, represented by a single formally described species *Sticholonche zanclea* and possibly many more species revealed by metagenetic studies. The characteristic feature of Retaria is a particular type of β-tubulin, which may be responsible for the unusually active pseudopodial network and the extraordinary diversity of foraminiferal and radiolarian skeletons. In phylogenetic trees of Rhizaria, Retaria and Cercozoa are separated by some independent taxa, including marine filose Gromiida, amoeboid Vampyrellida, the plant parasites Phytomyxea and Ascetosporean parasites of shellfish and other invertebrates. These independently branching groups are often combined in the assemblage Endomyxa (Bass *et al.*, 2005), but phylogenomic analyses do not confirm its monophyly (Sierra *et al.*, 2013).

Excavata

Most organisms belonging to this supergroup possess a conspicuous 'excavated' ventral feeding groove. In spite of

this apparent synapomorphy, the monophyly of Excavates is not well supported even in multigenic trees (Hampl *et al.*, 2009). The Excavates comprise several groups of amitochondriate parasites, including diplomonads, retortamonads, oxymonads and parabasalids; some of them possess hydrogenosomes or mitosomes instead of mitochondria. Together with a few free-living taxa (e.g. *Carpediemonas*) they are classified into the group Metamonada. Another large monophyletic assemblage of Excavates is formed by Discoba. This grouping contains free-living Jakobida, amoeboflagellate Heterolobosea and Euglenozoa that comprises Euglenida (some are phototrophic), heterotrophic Diplonemea and parasitic Kinetoplastea (including the important human parasites *Trypanosoma* and *Leishmania*). In addition, the Excavates also comprise a very unusual free-living genus *Malawimonas* that usually branches separately from Metamonada and Discoba in molecular phylogenies (Simpson *et al.*, 2006).

Incertae sedis eukaryotes

Phylogenomic analyses reveal 11 orphan lineages that cannot be confidently placed in any of the existing supergroups. As shown in **Figure 1**, these taxa can be grouped into three paraphyletic assemblages. One of these assemblages is composed of four lineages (Apusomonadidae, Ancyromonadidae, Collodictyonidae and Rigidifilida) that branch close to Amoebozoa (Zhao *et al.*, 2012). All of them are heterotrophic free-living protists with different morphologies. The Apusomonadidae and Ancyromonadidae feature gliding cells with two unequal cilia and short pseudopodia; Collodictyonidae have two or four apical cilia; whereas Rigidifilida have branching pseudopodia arising from a ventral depression (Yabuki *et al.*, 2013).

The second assemblage of *incertae sedis* protists includes Cryptophyta, Katablepharidae and Picozoa. The first two lineages group together, whereas the Picozoa branch close to Glaucophyta. Cryptophytes are autotrophes with chloroplasts and residual nucleomorphs that issue from red algal secondary symbiosis – Katablepharids are free-swimming heterotrophs with two cilia inserted subapically (Okamoto *et al.*, 2009). Picozoa, formerly known as Picobiliphyta, are represented by many environmental sequences. Only recently the first picobiliphytes (*Picomonas judraskeda*) was successfully cultivated and formally described; surprisingly, its detailed study showed no evidence of autotrophy (Seenivasan *et al.*, 2013). **See also**: Cryptomonads

The third assemblage branching close to SAR is composed of Haptophyta, Centrohelida, Telonemia and Rappemonads. The largest of these groups are Haptophytes, which are named after the presence of a unique anterior appendage, the haptonema, used for adhesion and prey capture. Some marine haptophyte species are protected by calcareous scales (coccoliths) and capable of producing massive blooms (e.g. *Emiliania huxleyi*). Centrohelida are a

particular group of heliozoans with typical axopodia, whereas *Telonema* is a marine heterotrophic flagellate genus with two flagella and very specific ultrastructural features. Rappemonads are marine picoeukaryotes, known only from environmental DNA sequences (Kim *et al.*, 2011).

Future Challenges

Although the basal scaffold of eukaryotic phylogeny seems to be solidified by now, there are still a lot of unresolved issues concerning the phylogenetic position of *incertae sedis* microkingdoms. In many cases, phylogenetic relationships within the supergroups are not resolved either. Compared with metazoans or plants, the number of sequenced protistan genomes is relatively small, for example, genomic data are available only for one species of Rhizaria (Curtis *et al.*, 2012). Furthermore, transcriptomic data are scarce or not available for many groups. Nevertheless, given the extraordinary progress of sequencing technologies, we can expect that these limitations will be rapidly overcome and the number of sequenced nonmodel organisms will increase substantially.

A more difficult issue related to eukaryotic phylogeny might be the positioning of the root. This question has been recently addressed by analysing mitochondrial genes, but there is no unambiguous solution (Derelle and Lang, 2012). Two major hypotheses have been formulated: One proposes that the root is situated between Amorphea (Amoebozoa + Opisthokontes) and other eukaryotes (Stechmann and Cavalier-Smith, 2003), the other points to the Excavates as the most basal group (Cavalier-Smith, 2010).

Further phylogenomic studies of known genera may help in resolving this question, but we cannot exclude that we are still missing some pivotal lineages. Therefore, it is very important to increase the effort of characterising new protistan lineages. As shown by the recent study of Picozoa, a cultivation approach remains the most efficient way to accurately describe hitherto unknown organisms (Seenivasan *et al.*, 2013). However, this traditional approach is extremely time consuming and may not be appropriate for many uncultivable protists. More rapid single-cell microscopic and genomic approaches are now available (Yoon *et al.*, 2011) and future studies will probably use these new tools for exploring the extraordinarily diverse protistan phyla.

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