Protist Diversity and Eukaryote Phylogeny

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Abstract

The last quarter century has seen dramatic changes in our understanding of the phylogenetic relationships among protist groups and their evolutionary history. This is due in large part to the maturation of molecular phylogenetics, to genomics and transcriptomics becoming widely used tools, and to ongoing and accelerating progress in characterizing the major lineages of protists in the biosphere. As an introduction to the Handbook of the Protists, Second Edition, we provide a brief account of the diversity of protistan eukaryotes, set within the context of eukaryote phylogeny as currently understood. Most protist lineages can be assigned to one of a handful of major groupings ("supergroups"). These include Archaeplastida (which also includes land plants), Sar (including Stramenopiles/Heterokonta, Alveolata, and Rhizaria), Discoba, Metamonada, Amoebozoa, and Obazoa. This last group in turn contains Opisthokonta, the clade that includes both animals and fungi. Many, but not all, of the deeper-level phylogenetic relationships within these groups are now resolved. Additional well-known groups that are related to Archaeplastida and/or Sar include Cryptista (cryptophyte algae and their relatives), Haptophyta, and Centrohelida, among others. Another set of protist lineages are probably most closely related

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to Amoebozoa and Obazoa, including Ancyromonadida and perhaps Malawimonadidae (though the latter may well be more closely related to Metamonada). The bulk of the known diversity of protists is covered in the following 43 chapters of the Handbook of the Protists; here we also briefly introduce those lineages that are not covered in later chapters.

The Handbook is both a community resource and a guidebook for future research by scientists working in diverse areas, including protistology, phycology, microbial ecology, cell biology, and evolutionary genomics.

Keywords

Algae • Alveolata • Amoebozoa • Archaeplastida • Biodiversity • Discoba • Eukaryote • Metamonada • Obazoa • Opisthokonta • Phylogeny • Protist • Protozoa • Rhizaria • Sar • Stramenopiles

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Introduction

It has been more than 25 years since the publication of the Handbook of Protoctista (Margulis et al. eds. 1990). Since then, there have been tremendous advances in our understanding of the diversity and phylogeny of protists/protoctists (i.e., all eukaryotes other than the animals, land plants, and true fungi; we will use the term "protist"). Central to this progress has been the maturation of molecular phylogenetics as a tool for inferring evolutionary relationships, initially using single markers, such as small subunit ribosomal RNA gene sequences, and culminating in "phylogenomic analyses" that incorporate data from dozens or hundreds of genes (van de Peer and De Wachter 1997; Baldauf et al. 2000; Rodríguez-Ezpeleta et al. 2007; Burki et al. 2007; Burki 2014). Genome sequencing (of organellar genomes as well as nuclear genomes), together with transcriptomic surveys, has also greatly enhanced our understanding of the distribution of important cellular and molecular characteristics across the breadth of eukaryotic diversity (e.g., Lang et al. 1997; Ramesh et al. 2005; Hodges et al. 2010; de Mendoza et al. 2014; Wideman and Muñoz-Gómez 2016). At the same time, the discovery of new major lineages of protists (and reinvestigations of known "mystery taxa") has continued apace and even accelerated in recent years. This has resulted in dramatic changes to the catalogue of organisms that are important to consider when inferring the broadscale tree of eukaryote life (e.g., O'Kelly and Nerad 1999; Shalchian-Tabrizi et al. 2006; Not et al. 2007; Yabuki et al. 2010; Glücksman et al. 2011), on top of many important discoveries of novel diversity within major lineages (e.g., Moore et al. 2008; Massana et al. 2014; see numerous other examples below). There have also

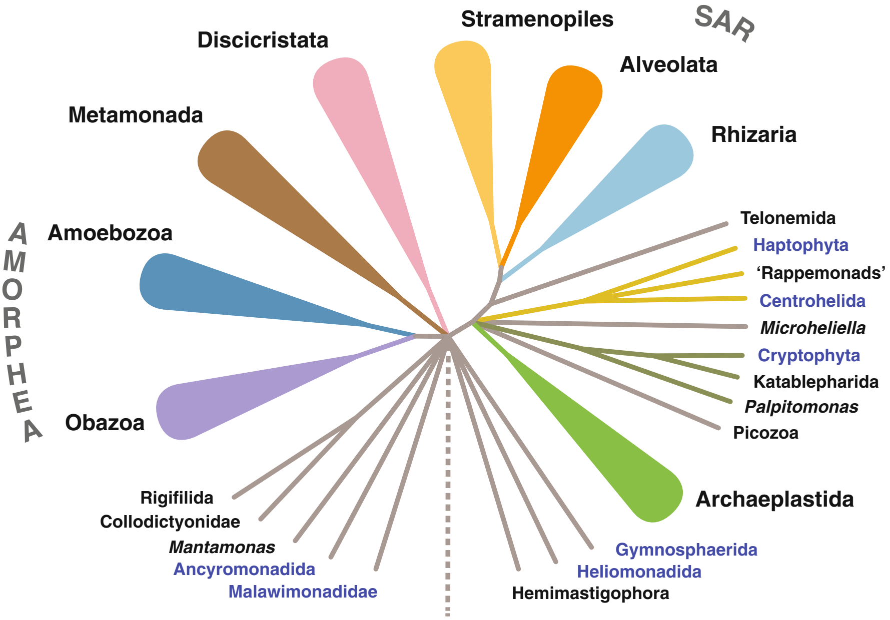

Fig. 1 Phylogeny of eukaryotes, based primarily on Brown et al. (2013), Cavalier-Smith et al. (2014), Kamikawa et al. (2014), Yabuki et al. (2014), Burki et al. (2016), and Leger et al. (2017). Groups with bulbous branches are examined in more detail in Figs. 2–5. Groups with narrow branches do not belong to well-established supergroups and are not illustrated separately; those covered in the Handbook are shown in *blue* and are as follows: ▶ Cryptophyta; ▶ Haptophyta; ▶ Centrohelida; ▶ Ancyromonadida; ▶ Malawimonadidae; ▶ Gymnosphaerida; ▶ Heliomonadida

been a number of important insights gained from electron microscopy studies, especially of the flagellar apparatus and cytoskeleton (e.g., Simpson 2003; Cavalier-Smith 2013; Heiss et al. 2013; Yubuki and Leander 2013).

The current picture of the tree of eukaryotic life can be characterized as largely resolved but with some major points of uncertainty. At present, it is common to divide the vast majority of known eukaryotic diversity into four to eight confirmed (or strongly suspected) monophyletic groups, usually referred to by the informal moniker "supergroups" (Fig. 1; Adl et al. 2012; Burki 2014; Worden et al. 2015; Simpson and Eglit 2016). The precise number and membership of the supergroups varies among accounts, reflecting not just personal taste but also the rapid pace with which important taxa are being added to broad molecular phylogenetic analyses. These supergroups are best thought of as standing well above the rank of "kingdom." For example, the animals and true fungi are generally each considered as a distinct kingdom but belong to the same supergroup (Obazoa, in our listing). The supergroups are often now amalgamated into as few as three or even two still more fundamental assemblages (Adl et al. 2012; Derelle et al. 2015), although this entails some bold assumptions about the position of the root of the tree (see below).

Our current understanding of eukaryote phylogeny confirms and extends a long-understood reality, namely, that most of the basic forms of protists identified by superficial morphology and physiology do not represent evolutionarily cohesive entities. Photosynthetic protists, or "algae," are found within most of the supergroups. In all of these groups (with the arguable exception of Archaeplastida – see below), the algae are intermingled with other kinds of protists, mainly "protozoa" (a term used to describe most heterotrophic protists, other than those that closely resemble fungi). Among the protozoa, "flagellates" (species which have eukaryotic flagella in the main feeding stage of their life history) are found across eukaryote diversity, reflecting the fact that all living eukaryotes descend from a heterotrophic, flagellum-bearing common ancestor. Amoebae, which lack flagella but produce one of the several distinct forms of pseudopodia, have evolved independently on multiple occasions, leading to a dozen or more major radiations of these lifeforms. Special forms of amoebae also have multiple origins. One example is the "heliozoa" – amoebae with many microtubule-supported pseudopodia radiating from a rounded cell body – which have at least three independent origins and likely more (Nikolaev et al. 2004; Bass et al. 2009). The "slime molds" are organisms that live mostly as amoebae (or as giant amoeboid plasmodia) but that also produce stalked structures bearing spores, either by differentiation of a single organism or by numerous amoebae aggregating together: collectively these strategies have evolved several times (Brown et al. 2012; Shadwick et al. 2009). Parasitic protozoa that are passed between hosts via infective spores have also evolved on numerous occasions. Other protists with more-or-less similarity to true fungi (e.g., they produce hyphae-like structures) are found in several different places within the eukaryote tree, mostly very distantly related to true fungi (Taylor and Berbee 2014).

As mentioned above, not all aspects of the deep-level phylogeny and evolutionary history of eukaryotes are well understood at present, which has consequences for any summary of protist diversity. Some important uncertainties and controversies revolve around particularly difficult problems in molecular phylogenetic inference. For example, it remains unclear what the relationships are among "excavate" lineages (Discoba, Metamonada, and Malawimonadidae), which include many groups with high overall rates of sequence evolution. The majority view at present is that they form two or more phylogenetically separate clades (Burki 2014). In a similar vein, phylogenomic analyses have yet to resolve whether the supergroup Archaeplastida truly represents a clade or whether other lineages (especially the Cryptista group) may belong inside it (Yabuki et al. 2014; Burki et al. 2016). The ongoing discovery of new lineages (discussed above) is itself a source of uncertainty, not least because it is unclear how many major lineages remain to be found and characterized. One of the most important open questions in eukaryote evolution concerns the precise history of plastids (chloroplasts). Most major lineages of photosynthetic eukaryotes actually have plastids that were obtained by symbiosis with eukaryotic algae, rather than by symbiosis with cyanobacteria; the number, sequence, and directions of these distinct eukaryote-eukaryote endosymbiotic events are all still unclear (Keeling 2013; Archibald 2015). Finally, one of the most difficult questions for eukaryote phylogeny is locating the "root" of the tree, that is, identifying the very deepest division among the extant eukaryotes. Several mutually incompatible positions have been proposed in recent years, based mostly on sophisticated phylogenomic analyses or the distribution of particular genes across major groups of eukaryotes (e.g., Cavalier-Smith 2010; Derelle and Lang 2012, 2015; Katz et al. 2012; He et al. 2014).

An Overview of Protist Diversity

The remainder of this chapter gives a concise, up-to-date, and (in our view) appropriately cautious summary of the diversity and phylogeny of eukaryotes. The main aim is to provide a broad phylogenetic context for the various other chapters in the Handbook of the Protists, Second Edition (hereafter, "the Handbook"). The majority of these chapters cover a single phylogenetically coherent group and will then have a single placement within the account below. There are a few chapters that instead cover two or more unrelated groups that have historically been considered together (e.g., "heliozoa"); these chapters will be referenced more than once for this reason. Furthermore, we have sought to briefly introduce the important groups of protists that are not covered separately in the Handbook (for reasons of logistics alone; no perception of insignificance should be inferred in these cases). In some of these instances, we direct the reader to recent (2010–onward) publications that are reviews or are reasonably broad in scope. For the sake of brevity, single genera of uncertain phylogenetic position within eukaryotes are omitted (see Adl et al. 2012 for a partial listing), and most lineages known solely as environmental sequences are not discussed.

Archaeplastida (Fig. 2) The supergroup Archaeplastida (meaning "ancient plastids"; sometimes instead called Plantae) consists of the three principal photosynthetic groups with "primary" plastids, in other words eukaryotes whose plastids/chloroplasts were acquired directly through a symbiosis with a cyanobacterium. There is strong phylogenetic evidence, especially from the plastid genome and plastid-associated biochemical features (e.g., the protein import machinery), that true plastids stem from a single event of primary endosymbiosis and thus that all archaeplastids descend from a common primary plastid-containing ancestor (Reyes-Prieto et al. 2007; Price et al. 2012). ▶ Glaucophyta (also known as Glaucocystophyta) is the most obscure of the three archaeplastid lineages. Glaucophytes are rare freshwater algae that mostly associate with surfaces. ▶ Rhodophyta consists of several thousand described species of algae, most of which are marine. They range from a few unicellular species, to diverse filamentous forms, to complex red seaweeds. The third group, Chloroplastida (also known as Chlorobionta or Viridiplantae), includes both the green algae and the land plants. It is divided into two large clades, streptophytes and chlorophytes, with the former including land plants, as well as many green algae; streptophyte green algae are often referred to as "charophytes," and the best studied groups are the ▶ Zygnematophyta, which are unicellular or filamentous freshwater forms, and the ▶ Charophyceae (Charales), which are truly multicellular freshwater "plants." Despite

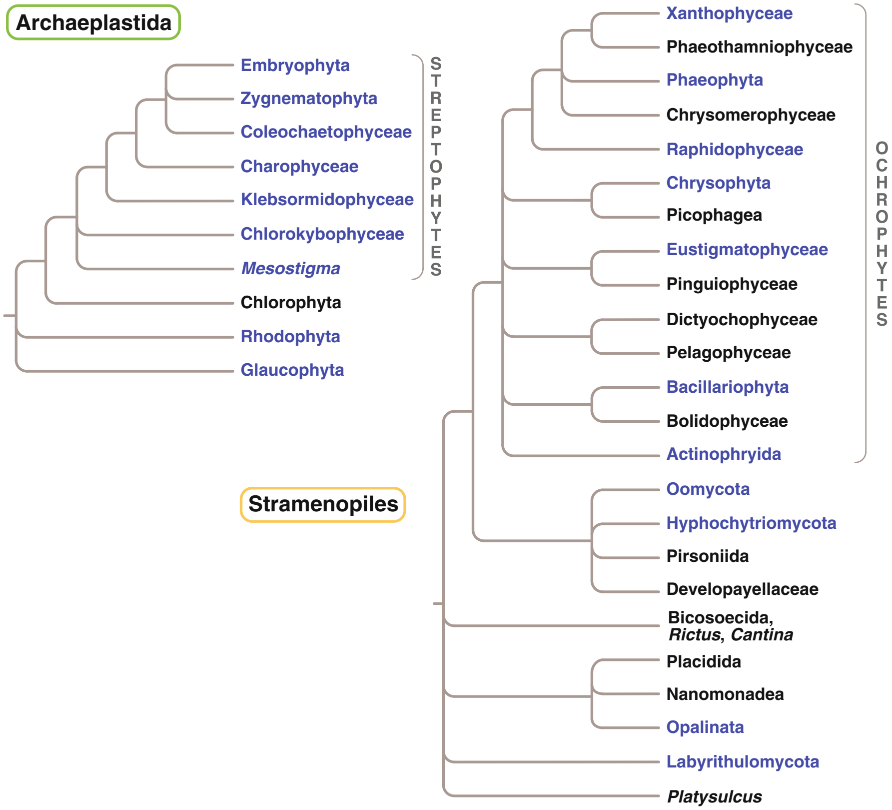

Fig. 2 Summary phylogenetic trees for Archaeplastida and Stramenopiles, based primarily on Leliaert et al. (2012) and Wickett et al. (2014) (Archaeplastida) and Riisberg et al. (2009), Cavalier-Smith and Scoble (2013), Yubuki et al. (2015), Shiratori et al. (2015), and Derelle et al. (2016) (Stramenopiles). Groups covered in Handbook chapters are shown in *blue* and are as follows: Archaeplastida: ► Glaucophyta; ► Rhodophyta; ► Zygnematophyta; ► Charophyceae; ► Chlorokybophyceae, Klebsormidiophyceae, Coleochaetophyceae, *Mesostigma*. Stramenopiles: ► Bacillariophyta; ► Phaeophyta; ► Raphidophyceae; ► Chrysophyta; ► Eustigmatophyceae; ► Xanthophyceae; ► Actinophryida; ► Hyphochytriomycota and Oomycota; ► Labyrinthulomycota; ► Opalinata. MAST clades without described representatives are not shown (see text)

the similarity in complexity between Charophyceae and land plants, recent phylogenetic evidence strongly indicates that land plants are more closely related to Zygnematophyta (Leliaert et al. 2012; Wickett et al. 2014). The remaining charophyte lineages, ► Chlorokybophyceae, Klebsormidiophyceae, Coleochaetophyceae, *Mesostigma*, are all discussed together. Chlorophytes include a wide diversity of unicellular flagellates (and some complex colonial forms), nonflagellated unicells and colonies, filamentous forms, and some more complex macroalgae, including green seaweeds. They are shown as a single branch in Fig. 2, but in reality, they are phylogenetically diverse. The best known subgroups include the Chlorophyceae (e.g., *Chlamydomonas*, *Volvox*), Ulvophyceae (marine macroalgae), and

Trebouxiophyceae. There are several additional distinct lineages, mostly of small flagellates, that collectively are referred to as "prasinophytes." The chlorophyte groups are not covered in the Handbook; the phylogeny and diversity of green algae, especially chlorophytes, is reviewed by Leliaert et al. (2012).

Sar; Stramenopiles (Fig. 2) The supergroup "Sar" (also known as SAR or Harosa) was identified through multigene/phylogenomic analyses (Burki et al. 2007; Hackett et al. 2007) and includes three lineages that are each hugely diverse and speciose in their own right: Stramenopiles, Alveolata, and Rhizaria (SAR is an acronym for these three groups). Stramenopiles, also known as Straminipila or Heterokonta, is distinguished by a characteristic form of rigid tubular flagellar hairs (the group name means "straw hairs"), although these have been lost in many species and several whole subgroups. Stramenopiles includes a wide range of photosynthetic forms as well as many heterotrophs (see Cavalier-Smith and Scoble 2013). Photosynthetic stramenopiles, also known as ochrophytes, have plastids derived ultimately from a red algal donor and form a monophyletic group (Cavalier-Smith and Scoble 2013; Derelle et al. 2016). The best known are the diatoms (▶ Bacillariophyta), which are unicellular/colonial forms with bipartite siliceous "cell walls" that are of huge ecological importance in the marine microplankton (for example), and the filamentous or genuinely multicellular ▶ Phaeophyta (Phaeophyceae), informally known as brown algae. As it happens, neither of these groups are flagellated in the vegetative state; the characteristic stramenopile flagellar hairs are seen only in (some) reproductive stages. Other ochrophyte groups include ▶ Raphidophyceae (Raphidophyta) and ▶ Chrysophyta, which are flagellates (though some famous chrysophytes are colonial and many are no longer photosynthetic), the mostly unicellular ▶ Eustigmatophyceae, and the ▶ Xanthophyceae, which are often filamentous and are among the closest relatives of the brown algae. Other, more obscure, groups of ochrophytes include Phaeothamniophyceae and Chrysomerophyceae (also related to brown algae) plus several groups of mostly unicellular marine forms: Bolidophyceae (the sister group to diatoms), Dictyochophyceae (including the well-known "silicoflagellates"), Pelagophyceae, Pinguiophyceae, and Picophagea (the latter being amoeboid and often non-photosynthetic): None of these are covered independently in the Handbook. Finally, ▶ Actinophryida, a small group of heterotrophic "heliozoan" organisms, belongs phylogenetically among ochrophytes (the exact placement is unresolved).

The heterotrophic stramenopiles are phylogenetically more diverse than the phototrophs and range from fungi-like organisms (most of which nonetheless produce flagellated dispersal stages) through to various kinds of "protozoa." ▶ Hyphochytriomycota and Oomycota are the most fungus-like stramenopiles: they produce (septate) hyphae with cell walls and generally parasitize plants or aquatic organisms. Oomycetes, in particular, cause several major diseases of agricultural crops (e.g., late blight in potatoes) and trees. ▶ Labyrinthulomycota produce non-walled extensions, with the best known, the labyrinthulids, existing as ectoplasmic networks containing numerous cell bodies. Pirsoniida (not covered) is a group of parasitoids of algae that is related to oomycetes and hyphochytrids. Many groups of stramenopiles are heterotrophic flagellates with two flagella or sometimes one. The best known of these is Bicosoecida (sensu lato; also known as Bicosidia); others that are broadly similar but phylogenetically distinct include Placididea, *Cantina*, *Rictus*, *Platysulcus*, and Developayellaceae (the latter is also related to oomycetes and hyphochytrids; Cavalier-Smith and Scoble 2013; Yubuki et al. 2015; Shiratori et al. 2015). Furthermore, environmental sequencing studies have shown that the oceans contain a wide diversity of undescribed lineages of stramenopiles, collectively called "MASTs" (MArine STramenopiles; though some are also found in freshwater), which appear to be largely or entirely heterotrophic flagellates (Massana et al. 2014). In recent years, a couple of species that belong to one MAST lineage have been cultivated or reinvestigated (*Incisomonas* and *Solenicola*), and this group is now known as Nanomonadea (Cavalier-Smith and Scoble 2013). None of these various heterotrophic flagellate groups is covered in the Handbook; a summary of MAST diversity is given by Massana et al. (2014). Finally, the taxon ▶ Opalinata includes a range of inhabitants of animal intestinal tracts, including cells with two to four flagella, the multiflagellated opalinids, and the nonflagellated anaerobe *Blastocystis* (one of the most prevalent protists in the human gastrointestinal tract).

Sar; Alveolata (Fig. 3) Alveolata encompasses three of the most well-known groups of protists, Apicomplexa, Dinoflagellata, and Ciliophora, each represented by a chapter in the Handbook: ▶ Apicomplexa is quintessentially parasitic and includes species that are extremely harmful to humans and animals (e.g., *Plasmodium* spp., *Toxoplasma gondii*, *Cryptosporidium parvum*, etc.). The scope of the Handbook chapter has been extended to include the sister lineages to apicomplexan parasites, namely, colpodellids (which predate upon other protists or parasitize them) and the chromerid algae, which were only discovered this century (Moore et al. 2008). Colpodellids and chromerids are phylogenetically intermingled; recent analyses indicate they may be a clade, "chrompodellids" (Janouškovec et al. 2015). Research on these lineages has resulted in groundbreaking advances in our understanding of the evolution of apicomplexans and their relationships with dinoflagellates. Most notably, chromerids turned out to be the long-sought living descendants of the inferred photosynthetic ancestors of apicomplexans (most of which have non-photosynthetic plastids). ▶ Dinoflagellata includes numerous species that are conspicuous and important components of the marine microplankton, as autotrophs and/or grazers (many are mixotrophs and show both functions). Collectively, dinoflagellates are involved in several phenomena of great ecological importance, such as harmful algal blooms (e.g., *Karenia brevis*, *Alexandrium* spp.), symbioses with reef-forming corals (*Symbiodinium*), and important parasitic associations with animals or with other protists (e.g., *Hematodinium*, *Amoebophrya*). Dinoflagellates are closely related to Perkinsozoa, a small group of aquatic parasites with flagellated spores (not covered separately in the Handbook). While Apicomplexa-chrompodellids and Dinoflagellata-Perkinsozoa are closely related, there is still some uncertainty as to the position and evolutionary significance of several

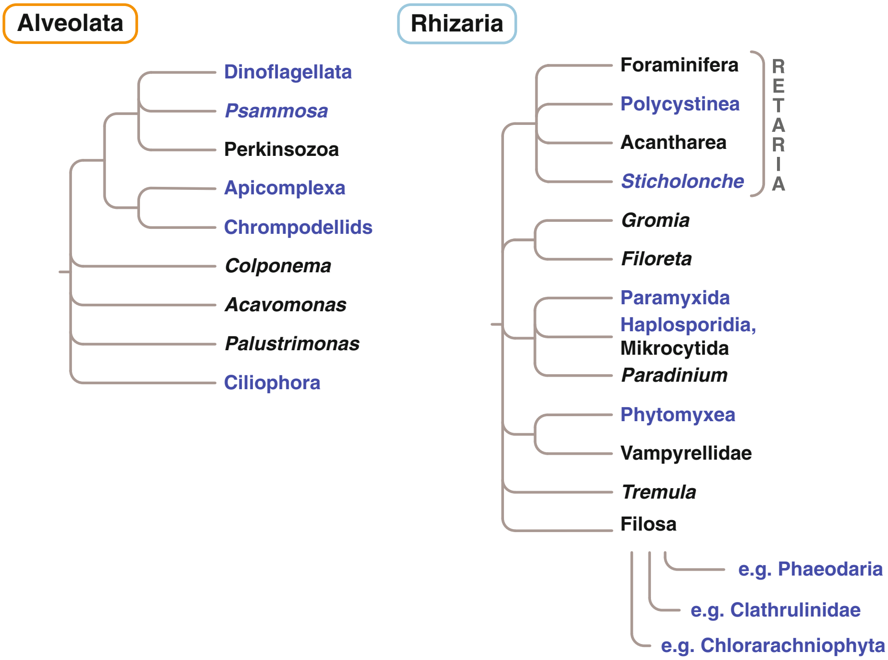

Fig. 3 Summary phylogenetic trees for Alveolata and Rhizaria, based primarily on Tikhonenkov et al. (2014), Janouškovec et al. (2015), Park and Simpson (2015), and Burki et al. (2016) (Alveolata) and Bass et al. (2009), Sierra et al. (2013, 2016), and Krabberod et al. (2017) (Rhizaria). Groups covered in Handbook chapters are shown in *blue* and are as follows: Alveolata: ▶ Apicomplexa (and "Chrompodellids"); ▶ Dinoflagellata (inc. *Psammosa*); ▶ Ciliophora. Rhizaria: ▶ Polycystinea; ▶ *Sticholonche*; ▶ Phaeodaria; ▶ Clathrulinidae; ▶ Chlorarachniophytes; ▶ Phytomyxea; ▶ Paramyxida; ▶ Haplosporidia. Note that Filosa contains many subgroups, and only those few subgroups covered in the Handbook are shown

heterotrophic flagellates lying at and near the split of these two groups (Fig. 3). Of these, *Psammosa* is often considered a basal dinoflagellate, while *Colponema*, *Acavomonas*, and *Palustrimonas* appear to represent one or more sister clades to the whole assemblage (none covered here; Tikhonenkov et al. 2014; Park and Simpson 2015). The third main group of alveolates, ▶ Ciliophora is extremely diverse and probably the most thoroughly studied group of (mostly) free-living heterotrophic protists. Most have large numbers of cilia (i.e., arrays of coordinated eukaryotic flagella), which in many species cover almost the entire cell, and they exhibit a characteristic form of nuclear dimorphism, with somatic macronuclei and germline micronuclei. In spite of the considerable wealth of knowledge on ciliates accumulated to date, the field of ciliate biodiversity is very active, and new environmental sequencing studies indicate that the full diversity of ciliates is far from uncovered.

Sar: Rhizaria (Fig. 3) One of the most morphologically diverse higher-order lineages, Rhizaria is comprised mostly of heterotrophic amoebae, flagellates, and amoeboflagellates, though it also includes some spore-forming parasites and unusual algae. No set of morphological features unites Rhizaria to the exclusion of other eukaryotes; they have emerged as a distinct taxon on the basis of molecular phylogenetic analyses (see Nikolaev et al. 2004). Reticulate or filose pseudopods are often present (thus the name "Rhizaria," referring to their often rootlike appearance), but these may be either actin- or microtubule-supported structures.

The most familiar rhizarians are foraminiferans (Foraminifera) and the radiolarians, most of which are large, often abundant, marine amoebae with microtubule-supported pseudopodia. Most foraminiferans inhabit multichambered tests that are constructed from calcium carbonate or assembled from agglutinated mineral particles; these have left an extensive fossil record extending back to the Cambrian. Foraminifera are not included in the Handbook (but see below). ▶ Radiolaria are subdivided into Polycystinea, usually with silica skeletons, and Acantharea, which have strontium sulfate skeletons (Acantharea are not covered in the Handbook). Foraminifera, Polycystinea, Acantharea, and the peculiar "rowing" radiolarian-like organism ▶ *Sticholonche* are related to one another (as Retaria), but their interrelationships are still unclear (see Sierra et al. 2013; Krabberød et al. 2017).

Much of rhizarian diversity falls within a clade called Filosa. This includes many free-living flagellates, which usually feed using some form of often-fine pseudopodia. The bulk of these flagellates associate with surfaces (e.g., most members of Cercomonadida, Glissomonadida, and Thaumatomonadida) but there are also some free-swimming forms (e.g., Ebriida). A few are parasites/parasitoids (e.g., *Pseudopirsonia*). Filosa also includes several groups of amoebae, the most famous being the filose testate amoebae (Euglyphida), although there is a greater diversity of naked forms (e.g., Bass et al. 2009). The Handbook has accounts of only a couple of groups of these organisms, namely, ▶ Phaeodaria, which are amoebae with siliceous skeletons that until relatively recently were considered to be radiolaria, and the "heliozoan" group ▶ Clathrulinidae. Filosa also includes two photosynthetic lineages, the mostly amoeboflagellate ▶ Chlorarachniophytes (e.g., *Bigelowiella* and *Lotharella*), which possess plastids of green algal secondary endosymbiotic origin, and *Paulinella chromatophora*, which is a euglyphid testate amoeba that harbors a cyanobacterium-derived photosynthetic "chromatophore" of separate origin than canonical plastids (Nowack 2014).

Most other rhizarian groups are various amoebae, including *Gromia*, *Filoreta*, and Vampyrellida (none covered in the Handbook), or are parasites. The latter include ▶ Phytomyxea, which are pathogens of plants and stramenopiles (e.g., *Plasmodiophora brassicae*, *Maullinia ectocarpi*), as well as ▶ Paramyxida and ▶ Haplosporidia, both of which parasitize marine invertebrates (though there are also freshwater haplosporidians). Other parasites infecting marine invertebrates (and not directly covered in the Handbook) include Mikrocytida, such as the oyster parasite *Mikrocytos* (which are very likely related to Haplosporidia or possibly descended from them), and *Paradinium*, which infects crustaceans. It is generally assumed that these parasites of invertebrates are all related, forming a taxon called

Ascetosporea, and this is incompletely supported by molecular phylogenies (e.g., Sierra et al. 2016). The flagellate *Tremula* (not covered) may represent the sister group to (other) Filosa (Howe et al. 2011).

Burki and Keeling (2014) provide a brief overview of the biology and evolution of rhizarian taxa, including some of the more important groups not covered in the Handbook. In addition, recent advances in the systematics of Foraminifera are treated by Pawlowski et al. (2013), and the current systematics of Euglyphida (and some other thecate amoebae within Filosa) was recently summarized by Kosakyan et al. (2016). The report by Howe et al. (2011) illustrates some of the range of free-living flagellates and small amoebae among Filosa. The diversity and phylogeny of Vampyrellida is examined and illustrated by Hess et al. (2012) and Berney et al. (2013). Hartikainen et al. (2014) give the first broad account of mikrocytids.

Other Archaeplastida- and Sar-Related Lineages (Fig. 1) A series of much smaller groups (in terms of the number of described species) are very likely related to Archaeplastida and/or Sar. The taxon Cryptista (sensu stricto) includes the well-known algal group ▶ Cryptophyta and two more obscure relatives, katablepharids and *Palpitomonas* (see Yabuki et al. 2014). Cryptophytes are mostly unicells with two flagella and with a plastid of red algal origin. Quite a few lack photosynthetic capabilities, including goniomonads, which are sister to other cryptophytes and may be ancestrally non-photosynthetic. Katablepharids and *Palpitomonas* are also biflagellated heterotrophs; neither is covered in the Handbook (Yabuki et al. 2010 and Nishimura et al. 2016 essentially summarize the published work on *Palpitomonas*). ▶ Haptophyta also known as Prymnesiophyta, is a major group of unicellular algae, especially in marine systems, where calcite-scale-producing coccolithophorid haptophytes are of regional and global significance in carbon cycling. They too have a plastid ultimately of red algal origin. Haptophyta are probably related to the recently discovered "rappemonads," which are uncultivated unicellular marine algae (not covered here; see Kim et al. 2011), and quite possibly to ▶ Centrohelida, a group of heterotrophic "heliozoan" amoebae with long, radiating microtubule-supported "axopodia" (another recently described heliozoan, *Microheliella*, is currently inferred to be a separate lineage from Centrohelida, but this has not been clearly resolved; Cavalier-Smith et al. 2015). Recent phylogenomic analyses suggest that cryptists are related to Archaeplastida, while haptophytes (and their relatives) are more closely related to Sar (Burki et al. 2016), but these relationships are not yet well resolved (e.g., Yabuki et al. 2014; Cavalier-Smith et al. 2015). Two further groups of obscure heterotrophic flagellates, Telonemida and Picozoa, are most likely related to Sar and Archaeplastida, respectively (Burki et al. 2016). Neither is covered in the Handbook; see Yabuki et al. (2013a), Seenivasan et al. (2013), and Moreira and Lopez-Garcia (2014) for recent publications on these groups.

Discoba (Fig. 4) This clade includes ▶ Jakobida, a group of heterotrophic flagellates best known for their particularly bacterial-like mitochondrial genomes, the recently discovered flagellate *Tsukubamonas*, and a clade called Discicristata which unites the well-known taxa Heterolobosea and Euglenozoa (Hampl et al.

2009; Yabuki et al. 2011; the name Discoba is a portmanteau of Discicristata and *Jakoba*). ▶ Heterolobosea are a collection of amoebae, flagellates, or “amoeboflagellates” with life cycles that include both cell types (the acrasids are also “slime molds” that produce sorocarps). Euglenozoa in turn includes three main subgroups: ▶ Euglenida encompasses many predatory species that glide over surfaces, as well as a large clade of algae with plastids of chlorophyte green algal origin (and very likely a group of specialist anaerobes – Symbiontida). ▶ Kinetoplastea includes a mix of free-living and parasitic flagellates, with the most famous being the trypanosomatids that cause sleeping sickness, Chagas’ disease, and leishmaniases in humans. Diplonemids (Diplonemea) are the sister group to kinetoplastids. While previously obscure, diplonemids have recently been found to be extremely abundant and diverse in ocean waters (Flegontova et al. 2016). They also have remarkable mitochondrial gene expression systems, where genes are encoded as fragments on separate chromosomes and transcripts are trans-spliced together to produce functional mRNAs (and can be extensively edited too; Moreira et al. 2016). Diplonemids are not discussed further in the Handbook but are examined or reviewed in several recent publications (David and Archibald 2016; Flegontova et al. 2016; Gawryluk et al. 2016).

Metamonada (Fig. 4) The metamonads are a large group of anaerobic protozoa, almost all of which are flagellates. They are of contentious phylogenetic placement, with different molecular phylogenetic analyses favoring relationships with Discoba (see above) or Malawimonadidae (see below), this forming a part of the ongoing controversy about the phylogenetic coherence of the “excavates” (Hampl et al. 2009;

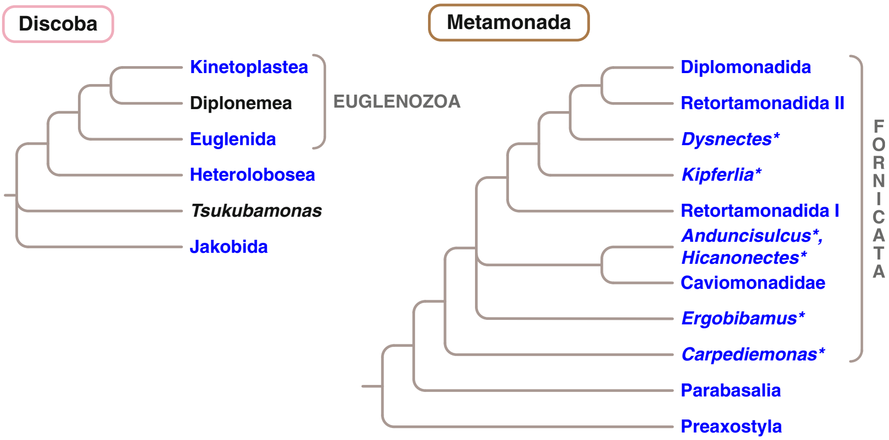

Fig. 4 Summary phylogenetic trees for Discoba and Metamonada, based primarily on Kamikawa et al. (2014) (Discoba) and Leger et al. (2017) and Yubuki et al. (2017) (Metamonada). Groups covered in Handbook chapters are shown in *blue* and are as follows: Discoba: ▶ Jakobida; ▶ Heterolobosea; ▶ Euglenida; ▶ Kinetoplastea. Metamonada: ▶ Preaxostyla; ▶ Parabasalia; ▶ Diplomonadida; ▶ Retortamonadida, Caviomonadidae, and *Carpediemonas*-like organisms (CLOs). CLOs are indicated by *asterisks*

Cavalier-Smith et al. 2014; Derelle et al. 2015). There are three main subgroups within Metamonada. ▶ Preaxostyla includes the oxymonads, which are gut commensals/symbionts, plus the free-living trimastigids. ▶ Parabasalia is a very diverse group (almost entirely) of endobiotic organisms. They range from small parasites (e.g., *Trichomonas vaginalis*, in humans) to giant multiflagellated cells of diverse kinds that are symbiotic in certain wood-eating termites and cockroaches. The third group, Fornicata, includes ▶ Diplomonadida, which are mostly "doubled" cells with two nuclei and flagellar apparatuses (e.g., *Giardia lamblia/intestinalis* in humans). The other members of Fornicata are the commensal/parasitic retortamonads and Caviomonadidae, as well as the various free-living "*Carpediemonas*-like organisms" (e.g., *Carpediemonas*, *Dysnectes*, *Kipferlia*); these are collectively a paraphyletic assemblage of relatives of diplomonads, but covered in a single chapter of the Handbook, ▶ Retortamonadida (with notes on *Carpediemonas*-Like Organisms and Caviomonadidae).

Amorphea; Amoebozoa (Fig. 5) The Amorphea assemblage unites two huge clades that are inferred to be related in most recent global analyses of eukaryotic phylogeny: (i) the animals, fungi, and their immediate protist relatives ("Obazoa"; see below) and (ii) the large grouping of heterotrophic protists called "Amoebozoa" (Adl et al. 2012; Burki 2014). As the name suggests, Amoebozoa mostly (though not entirely) consists of organisms that are amoebae for much or all of their life cycle. Many lineages are various kinds of "slime molds," which also produce a spore-releasing fruiting body. Due to this "fungus-like trait," these have often been studied separately from non-fruiting amoebae and are covered separately in the Handbook. Since "protosteloid" slime molds are phylogenetically intermingled with non-fruiting Amoebozoa, there is an imperfect fit between some chapters of the Handbook and the known phylogeny of the group (which is crystallizing rapidly as insights from phylogenomic analysis are integrated with those from taxon-rich ribosomal RNA gene phylogenies; e.g., Shadwick et al. 2009; Berney et al. 2015; Cavalier-Smith et al. 2016; Tice et al. 2016).

At present there are three reasonably well-accepted groupings that are largely or entirely composed of "lobose" amoebae with no other stages in the lifecycle – Tubulinea, Discosea, and the recently distinguished Cutosea (Cavalier-Smith et al. 2016). Tubulinea includes *Amoeba* itself and many other naked amoebae with thick pseudopodia, as well as the Arcellinida or lobose testate amoebae. Discosea tend to be flatter cells; examples include *Acanthamoeba*, *Vanella*, and *Thecamoeba*. Cutosea is a small group including particular scaled amoebae. The non-fruiting amoebae in these groups are covered in a single chapter, ▶ Amoebozoan Lobose Amoebae (Tubulinea, Flabellinea, and Others). A fourth group, Variosea, includes a few non-fruiting amoebae that often have filose or reticulate pseudopodia but also most of the "protosteloid" slime molds, some of which have flagellated stages as well as amoebae (Variosea also includes a couple of "flagellate-only" taxa, *Phalansterium* and *Multicilia*, that are not covered in the Handbook). A few of the amoebae are explicitly discussed in the lobose amoebae chapter (see above); the protosteloids are treated authoritatively in a separate chapter (▶ Protosteloid Amoebae). This latter

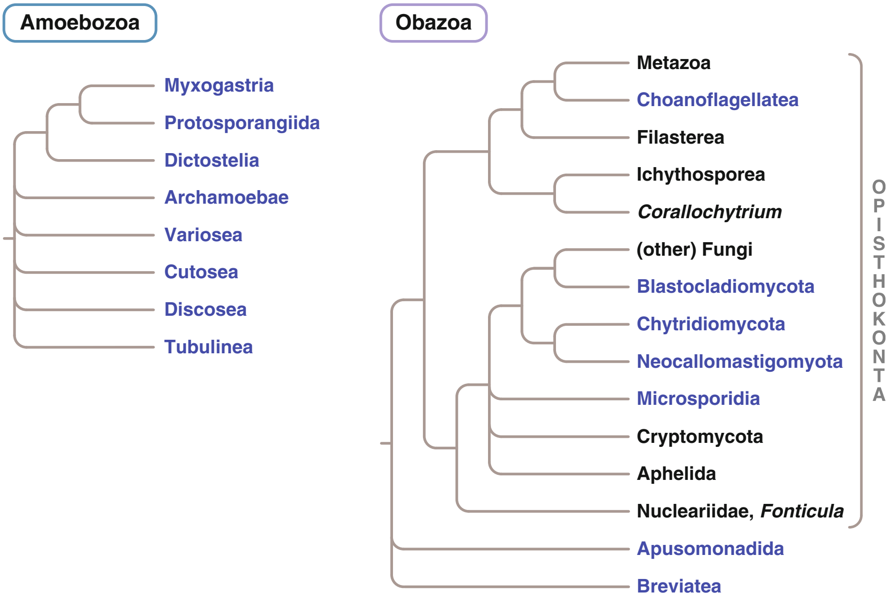

Fig. 5 Summary phylogenetic trees for Amoebozoa and Obazoa, based primarily on Berney et al. (2015), Cavalier-Smith et al. (2016), Tice et al. (2016), and M.W. Brown, pers. comm. (Amoebozoa) and Brown et al. (2013), Cavalier-Smith et al. (2014), Karpov et al. (2014), and Torruella et al. (2015) (Obazoa). Groups covered in Handbook chapters are shown in *blue* and are as follows: Amoebozoa: Many non-fruiting amoebae from Tubulinea, Discosea, Cutosea and Variosea are covered in ▶ Amoebozoan Lobose Amoebae (Tubulinea, Flabellinea, and Others); Protosteloid members of Variosea and Discosea, plus Protosporangiida, are covered in ▶ Protosteloid amoebae; ▶ Archamoebae; ▶ Myxomycetes; ▶ Dictyostelia. Obazoa: ▶ Choanoflagellatea; ▶ Blastocladiomycota; ▶ Chytridiomycota (and Neocallomastigota); ▶ Microsporidia; ▶ Apusomonadida (and Breviatea)

chapter also covers four small groups of fruiting amoebae that belong phylogenetically within Discosea (see above), as well as Protosporangiida, which are actually most closely related to Myxogastria (see below), and the couple of “protosteloid” members of Myxogastria.

The three remaining groups of Amoebozoa are each characterized by distinctive biological traits. Members of ▶ Archamoebae are amoeboid flagellates or amoebae (or cycle between these forms) that are anaerobic and have highly modified mitochondrial organelles (e.g., *Mastigamoeba*, *Pelomyxa*, *Entamoeba*). ▶ Myxomycetes or Myxogastria, is a well-known group of slime molds with complex life cycles that include small amoebae (and flagellates) but also multinucleate plasmodia that are macroscopic in size. It is the plasmodium stage that differentiates into the spore-bearing fruiting body. ▶ Dictyostelia are also slime molds, but unlike protosteloids or myxomycetes, they produce the fruiting body through aggregation of numerous unicellular amoebae. Myxogastria, Protosporangiida, and Dictyostelia are closely related, and they have sometimes been referred to collectively as Macromycetozoa.

Amorphea; Obazoa (Fig. 5) It is now well understood that animals (Metazoa) and Fungi are closely related to one another but also that they are specifically related to a heterogeneous assemblage of protist lineages, collectively forming a group called Opisthokonta (Cavalier-Smith 1987; Brown et al. 2009; Torruella et al. 2015). The protists most closely related to animals are the choanoflagellates (▶ Choanoflagellatea), which are unicellular or colonial flagellates that capture food using a characteristic "collar" of microvilli. Other close animal relatives include the Ichthyosporea (Mesomycetozoea), which are diverse parasites, mostly of aquatic animals and anurans, the isolated genus *Corallochytrium*, and Filasterea, the latter being a small group of free-living or parasitic forms mostly with fine pseudopodia. These groups are not discussed in detail in the Handbook; Ichthyosporea are reviewed by Glockling et al. (2013). Fungi are closely related to the nucleariid amoebae (Nucleariidae), with fine filose pseudopodia, and the slime mold-like organism *Fonticula* (not covered further). The phylogenetic and systematic distinction between Fungi and protists has perpetually been a gray area, and the Handbook contains accounts of groups that are usually considered the deepest branches among the fungi. These include ▶ Blastocladiomycota and ▶ Chytridiomycota with the latter chapter also including a brief account of Neocallimastigomycota; these organisms function as saprotrophs as well as parasites (or symbionts) and typically reproduce via uniflagellate zoospores. The other major taxa at the base of fungi include ▶ Microsporidia, an extensively studied and speciose group of spore-forming intracellular parasites, and the much more poorly known Cryptomycota (Rozellida) and Aphelida (not covered; see reviews by James and Berbee 2012; Karpov et al. 2014). Finally, two obscure groups of free-living heterotrophic flagellates, ▶ Apusomonadida and Breviatea, are now known to be sister taxa to the opisthokonts. It is this clade of all three taxa that is now known as Obazoa (Brown et al. 2013; OBA is an acronym for the three lineages).

Other Lineages (Fig. 1) There are a number of protist lineages that do not belong to any of the taxa listed above. Many are very poorly known groups for which there are very little data (e.g., limited or no electron microscopy data and no molecular sequence information). However, several lineages of free-living heterotrophs appear to be related to Obazoa and Amoebozoa (i.e., Amorphea) in phylogenetic/phylogenomic analyses (Zhao et al. 2012; Cavalier-Smith et al. 2014): Collodictyonidae (also known as diphylleids) are swimming flagellates, while their likely close relatives, Rigidifilida, are partly amoeboid cells without flagella. Ancyromonadida (Planomonadida) and *Mantamonas* are small gliding flagellates. Malawimonadidae is a small group of "excavate" flagellates that closely resemble Jakobida (in Discoba) and *Carpediemonas*-like organisms (in Metamonada) but do not branch with either Metamonada or Discoba in many phylogenomic analyses (though this is a topic of considerable contention; see above). Ancyromonadida is covered in a coda of the chapter on ▶ Apusomonadida and Malawimonadidae in the chapter on ▶ Jakobida Collodictyonidae, Rigidifilida, and *Mantamonas* are not covered in the Handbook, but recent publications on these protists include Zhao et al. (2012), Yabuki et al. (2013b), and Glücksman et al. (2011), respectively.

A different category of unplaced taxa consists of the few well-known groups of several species and genera for which there are substantial morphological data (albeit phylogenetically ambiguous) but no molecular data yet. One conspicuous example is Hemimastigophora, a taxon of multiflagellated cells that consume other smaller protists (Foissner and Foissner, 1993: not covered in the Handbook). We also highlight two groups of "heliozoa," Gymnosphaerida and Heliomonadida (formerly Dimorphida), each of which include several genera. One or both are sometimes placed tentatively within Rhizaria (Bass et al. 2009; Adl et al. 2012), but this awaits testing using molecular phylogenetics. Both are discussed briefly in the chapter on ▶ Centrohelida and Other Heliozoan-Like Protists.

Closing Remarks

Three decades ago, it would have been impossible to provide an accurate phylogenetic framework linking the many groups of protists covered in this edition of the Handbook. We are now able to present a reasonably comprehensive account of that framework, with confidence that most of it is essentially correct. Getting to this position has been a major achievement by the scientific community; this fact should not be lost amid concerns over the parts of the eukaryote tree that remain unresolved, contentious, or unknown. In a similar vein, while it has been necessary in this chapter to emphasize lineages that are not covered by the rest of the book, the Handbook is nonetheless an authoritative account of a substantial majority of known protist diversity. It represents an important collective effort by a large part of the protistology community and a major resource documenting the current state of knowledge on these organisms. We hope that this Handbook has a positive influence on the future direction of protistology, leading to greater depth and breadth in the understanding of our fascinating organisms.

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