THE PROTIST

Sina M. Adl
51 Campus Drive, University of Saskatchewan, Saskatoon, SK, Canada

Keywords: Algae, Apicomplexa, Brown algae, Cercozoa, Ciliate, Cryptomonad, Diatoms, Dinoflagellate, Euglenid, Foraminifera, Fungi, Green algae, Haptophyte, Heliozoa, Heterolobosea, Kinetoplastid, Oxymonad, Parabasalia, Protist, Protozoa, Radiolaria, Red algae, Rhizaria, Stramenopile.

Contents

8.1. Protoalveolates
8.1.1. Colpodellida
8.1.2. Perkinsidae
8.1.3. Colponemidia
8.2. Dinoflagellata
8.2.1. Syndiniales
8.2.2. Dinophyceae
8.3. Apicomplexa
8.3.1. Aconoidasida
8.3.2. Conoidasida
8.4. Ciliophora
9. Rhizaria
9.1. Gymnosphaeridae
9.2. Cercozoa
9.2.1. Cercomonadida
9.2.2. Paracercomonadida
9.2.3. Glissomonadida
9.2.4. Viridiraptoridae
9.2.5. Pansomonadidae
9.2.6. Sainouroidea
9.2.7. Thecofilosea
9.2.8. Granofilosea
9.2.9. Chlorarachnea
9.2.10. Imbricatea
9.3. Endomyxa
9.3.1. Vampyrellida
9.3.2. Phytomyxea
9.3.3. Ascetosporea
9.4. Radiolaria
9.4.1. Taxopodida
9.4.2. Acantharea
9.4.3. Polycystinea.
9.5. Foraminifera
9.5.1. Monothalamea
9.5.2. Tubothalamea
9.5.3. Globothalamea
10. Amorphea and Obazoa
11. Amoebozoa
11.1. Tubulinea
11.2. Evosea
11.3. Discosea
12. Discoba
12.1. Jakobida
12.2. Heterolobosea
12.3. Euglenozoa
12.3.1. Euglenida
12.3.2. Symbiontida
12.3.3. Diplonemea

Summary

The protists are eukaryotes with a unicellular organization. Growth forms are diverse and can vary between life history stages. These include single motile or immotile cells, colonial forms, filamentous forms, cells that aggregate into more or less parenchymatous tissue, and cells that aggregate for reproduction. Cell differentiation into other stages includes dormant (resting) and dispersal cysts, sexual cells, spores from conjugation for dormancy or dispersal, motile non-feeding dispersal cells (tomites), and symbiotic and parasitic forms. Cellular differentiation of vegetative cells (trophonts) to specialized function occurs in some parenchymatous forms. Multicelluar clades which evolved from protists include the plants (Embryophta), the animals (Animalia, syn. Metazoa), and the macrophyte brown algae in the Fucales. The fungi are treated separately. Protists form two main clades called the Amorphea and the Diaphoretickes (Figure 1). The Amorphea include the Amoebozoa, the Opisthokonta (fungi and animals), and several small basal clades. The Diaphoretickes include the green algae, the red algae, the Stramenopiles and brown algae, the Alveolata (Ciliophora, Dinoflagellata, Apicomplexa), and the Rhizaria (Opalozoa, Silicofilosea, Endomyxa, Foraminifera). There are two clades that stand on their own, the Metamonada and the Discoba. In addition, there are several smaller basal clades to the Amorphea and Diaphoretcikes presented briefly. Each group is thought to be monophyletic based on recent molecular phylogenies and the current classification of protists from the International Society of Protistology. This overview of the protists updates the previous educational text on the topic in 2003 by K. Hausmann, N. Hülsmann, and R. Radek called Protistology.

1. Introduction

Protists are eukaryotic cells with a unicellular level of organization. They are distinguished from prokaryotes (the Bacteria and Archaea) by a considerable amount of cell biology and biochemistry, but most simply, eukaryotes have a nucleus with an endomembrane network. The origin of the eukaryotic cell is from a merging symbiosis of an α-Proteobacteria with an "Asgard" Achaea. The morphology of extant protists is diverse, ranging from unicells that are immotile, gliding, amoeboid, or swimming, to taxa that form colonies, aggregate into pluricellular structures, or form large macroscopic algae. The oldest fossils are of red algae filaments dated to about 1.1 billion years ago. Thus simpler eukaryotes would have existed long before. About 7,200 genera are recognized across 7 principal clades, and a small number of deep-branching harder-to-place genera. The number of protist species is uncertain because

environmental sequences include many novel sequences that remain to be studied. The most recent estimate of the number of eukaryote species was by Mora, Tittensor, Adl, and Worm in 2010, which obtained about 8.7 million eukaryote species (±1.3 million SE).

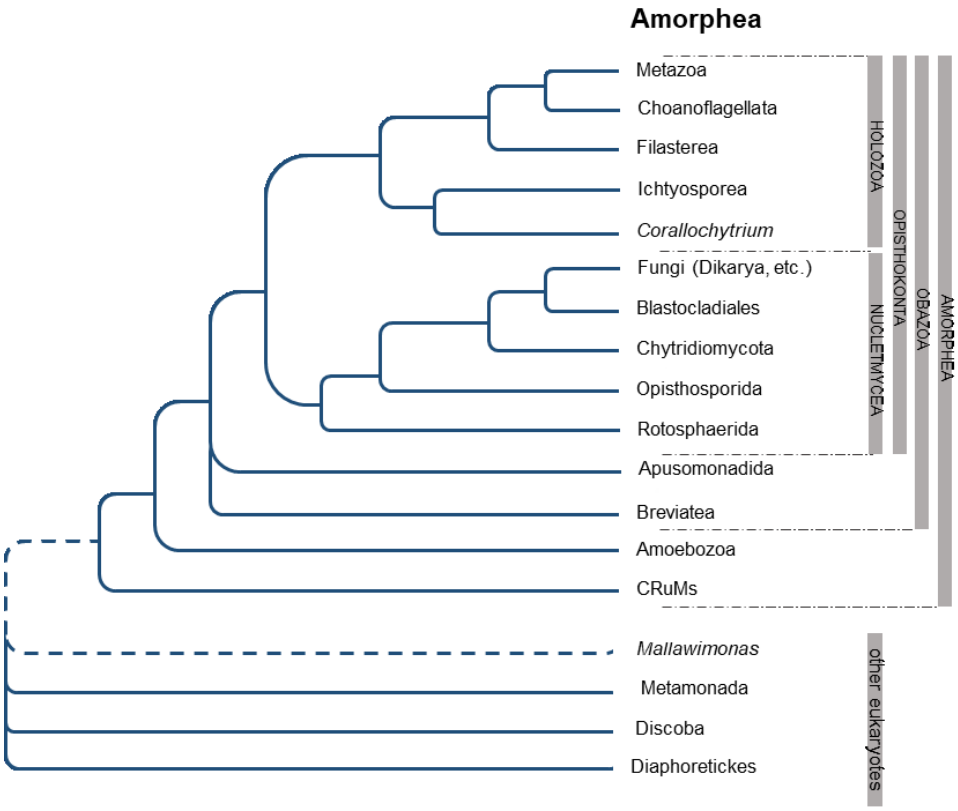

Figure 1. Phylogeny of the Kingdom Amorphea with the Kingdom Diaphoretickes and other eukaryotes

Nowadays, probably all protistologists recognize the limits of species identification by microscopy alone. Irrespective of its size, from the tiniest unicells at <10 µm to macrophytes, which genus or family has not had to be rethought, redefined, synonymized, or deleted, with species moved or renamed? It seems that most of the past fourty years consisted of continuously updating and modifying the learnt classification and phylogenic arrangement, to accommodate new research findings that altered some part of the tree. This imposes challenges to educators but also to anyone trying to keep-up with specialists. Most try to keep-up with at least one part of the phylogenetic tree that concerns them but not necessarily all of it.

The lesson from DNA sequence analysis is that species described by light microscopy based on visible morphology (fixed-stained and live descriptions) led to incorrect lumping of "similar" species into genera or families. Sometime, a species has been moved from phylum to phylum looking for a better fit. Many of the classification systems tended to arrange species incrementally from simpler to more complex, often erroneously. The electron microscope's contributions to ultrastructural morphological information during the second half of the 20th century contributed a massive amount of

new data. Our understanding of evolutionary trends across the diversity of protists was improved tremendously. However, elucidating the correct trend remained elusive for two reasons. First, most of the thinking was tainted by preconceptions, inherited from two or three centuries of past practice in classification and nomenclature, and disciplinary subcultures often with their particular jargon. Second, in the final decades of the 20th century the bioinformatics for molecular phylogenies was in its infancy, prone to erroneous conclusions. A combination of insufficient taxon sampling, insufficient understanding of tree behaviour, with an adolescent bioinformatics discipline led to numerous erroneous conclusions still at the beginning of this century. There were doubts about how much statistical resolution could be extracted from DNA sequence information.

Taxon sampling has improved in the marine photic environment, particularly from expeditions such as the Tara Ocean project. The terrestrial environment is better sampled but not nearly sufficiently, and many regions are unsampled. Deeper ocean pelagic waters and the benthos will remain under-sampled for a long time yet. Bioinformatics has become a more confident science, and phylogenetic conclusions are reached more cautiously. Nonetheless, some fundamental questions remain to be addressed, and I will raise three here. These are, a) what constitutes a species in protistology? b) what is the biogeographic distribution of species across ecosystems? and c) was sex already evolved at the origin of eukaryotic cells? If so, we are left with an awkward question, of why was it lost so many times? The reverse situation, of sex having evolved multiple times is not easier to confront.

1.1. What is a Protist Species?

A couple of decades ago, at the turn of the century, some stated that protists (in fact, that all species <1 mm) were cosmopolitan with a global distribution. Unfortunately, the argument was based on light microscopic observations that morphospecies here could be found there. It was met with considerable skepticism then, and refuted by a number of arguments. First, it was already generally recognized that at that scale, a morphotype did not mean one species but likely a collection of similar-to-identical-looking species. Sina Adl and V.V.S.R. Gupta remarked in 2007 that with information already available at the turn of the century, species that were well-known with extensive sampling and resampling showed that each morphotype represented a collection of cryptic species. Second, as Wilhelm Foissner elaborated in 2006, when considering dispersal and biogeographic distribution, it is the size of the dispersal particle that matters, not the size of the mature organism. By that reasoning, all spores, pollen, cysts, and dispersal particles <1 mm would include plants (including mosses and ferns), algae and macrophytes, fungi, protists, and a variety of microinvertebrates and coral organisms. Yet it is evident to any specialist from those disciplines that indeed, many species do have biogeographic limits. Using a different argument, Helmut Hillenbrand showed in a series of papers since 2001 that it is statistically unlikely that species do not have a biogeography based on meta-analysis of available data.

The evidence that morphospecies hide a collection of cryptic species comes mostly from species that have been domesticated for the laboratory, and that have been sampled globally from many places, such as parasites. Some examples follow, and readers can

probably think of many more. In the Ciliophora, *Tetrahymena* is a cluster of more than 20 morphologically indistinguishable species. Several *Euplotes* species and *Paramecium aurelia* are identical and could be distinguished only by mating-type clusters. Similarly, a study of marine ciliates revealed specialization within morphotypes. Even in oxytrichids, hypotrich ciliates with many morphological traits in their oral and somatic ciliature, Foissner's classification based on morphology was shown to be insufficient to distinguish species or genera correctly. Identical isolates of *Sterkiella histriomuscorum* from different geographical regions were also referred to as *Oxytricha fallax* or *Histriculus muscorum* in the literature. After careful study, these morphologically identical morphotypes were found to have differences in feeding preferences, in cyst morphology, in mating types and sexuality, and differences in the position and sequence of introns. Similarly, molecular sequence data and biochemistry indicate that the green algal genus *Chlamydomonas* (Chlorophyta: Chlorophyceae) and the brown algal genus *Nannochloropsis* consist of many morphologically similar or identical species. Isolates of the common soil *Acanthamoeba* genus (Discosea; Centramoebia) showed it was a cluster of morphologically identical but related species that could not be visually distinguished from *A. castellani*, an opportunistic human pathogen. The same is true for many parasitic genera, such as the *Entamoeba* (Evosea; Archamoebea) and *Trypanosoma* blood parasites. There does not seem to be many examples of single morphotypes from a broad geographical distribution representing a single species of protist. One must accept that a safe working hypothesis is that a morphotype probably represents a cluster of species. These species have niche adaptations for prey or micro-habitats that set them apart. These species, if sexual, usually have differences in conjugation, autogamy, and compatible mating types.

Species consist of populations that may become segregated over time, and diverge over time. In sexual species it can lead to mating type clusters that cannot mate with all mating types. In asexual (exclusively clonal) species, by cumulative successive mutations, populations diverge to adopt new niches. Not all changes will reflect in sequence change in genes used for molecular phylogenies. Not all species or genes change at the same rate. These make it harder to identify populations of similar species. The term "operational taxonomic unit" (OTU) used to infer species reflects the DNA sequences of a chosen DNA region from populations from a location. Cultures in laboratories also represent a clonal culture from isolates of a population from a location. How much difference between populations does reflect a separate species? These are not easily delineated, especially in non-sexual species. Studies of natural history of isolates that describe food and microhabitat preferences are essential to delineate species, and this is rarely done. It is prudent and sound practice to refer to strains from locations and laboratories where they are maintained and sub-cloned from. The term species has practical utility but shouldn't be used without an understanding of its limitations.

1.2. Biogeography and Dispersal

Our understanding of what constitutes a species is tightly linked to our ability to discuss its biogeography. A different approach with statistical analysis of metadata cautioned against claims that microbial species are ubiquitous in distribution. To appreciate the scope of cryptic species within morphospecies we must rely on well-known species that

have been studied and reisolated from diverse geographic locations. The pattern leans toward the existence of species limited in biogeography, without excluding that some species are cosmopolitan. The debate shifts to a discussion of whether most species have biogeographic limits or not. Since it becomes an issue of how many are cosmopolitan and how many are endemic with range limitations, this can be approached with existing ecological concepts about niche, and life history strategy as *r* or *k* adapted organisms. There are several recent papers describing the biogeography of algae, as it is more tractable than for soil species.

1.3. Sex: Multiple Origins or Multiple Losses?

The question of whether eukaryotes are ancestrally sexual is important for verifying the species concept, based on sexual isolation. Looking across the phylogeny, taxa where sex is known are scattered around the tree, so that we can make a couple of obvious hypotheses. One, sex was acquired multiple times independently; or sex existed at the origin of eukaryotes and was subsequently lost multiple times. Both options are problematic. The benefits of sex to sexual species has been known since Emile Maupas' classic paper in 1900 "*Sur le rajeunissement karyogamic chez les ciliés*". The paper establishes that in a sexual population, if sex does not occur regularly, the culture demonstrates signs of ageing (less vigorous growth, slower cell cycles, increased mortality, morphological and biochemical defects) and will eventually collapse. A history of studies on the benefits of sex to prevent clonal ageing was provided by Graham Bell in his now classic 1988 book "Sex and Death in Protozoa".

In contrast to these studies, many non-sexual species do not exhibit signs of clonal ageing. Clonal cultures are maintained in incubators and at culture centres for decades without any evidence of sex or ageing. In these species, clonal ageing seems to be managed or tolerated without the dire consequences of accumulating mutations cell cycle after cell cycle (called Muller's ratchet), by rare lethal mutants simply dying. Yet, many biologists hold the dogmatic position that sex must be taking place when you are not looking. At some point, and at the end of the day, evidence for sex must be provided where none seem to exist.

The last common ancestor of eukaryotes is supposed to have been sexual for several reasons. One idea proposed in 2013 by Elvira Hörandl and Franz Hadacek (see Hörandl and Speijer, 2016) is that acquiring an endosymbiont that became the mitochondrion also created an abundance of reactive oxygen species causing an enhanced mutation rate. Thus, the DNA repair mechanism of the host archaeon would have to adapt to its new endosymbiont pre-mitochondrion. Some adaptations include the peroxisomes, additional anti-oxidant mechanisms, and maintaining the chromatin separate from the metabolic cytoplasm by the nuclear double membrane. The homologous DNA sequence recombinations characteristic of sex would have evolved from the archaeon DNA repair mechanism. One example is the archaeal topoisomerase VI homolog Spo11 that has lost its ligase function but retains the double-strand cut function. Some Archaea are known to fuse cells and show chromosome or oligonucleotide recombination. The presence of two types of genomes (archaeon host and the α –Proteobacteria pre-mitochondrion) would necessitate a multinucleate polygenomic cell that would be plasmodial or siphonous until a eukaryotic cell evolves. Homologous sequence recombination with

cohesin proteins, permitting a synapsis, might be the step from mitosis to meiosis. The process of chromosome separation is more conserved than the process of eukaryote cell division (mitosis and cytokinesis).

It is generally accepted amongst protistologists that sexual protists are clonally reproducing with episodic sex triggered by some stress factor. Nutrient depletion is often one of the triggers. In parasitic species, the nutrient depletion is often at time of leaving the host to disperse. Those that promote this idea also avoid the more difficult question of why so many clades have become asexual, by stating that truly clonal species are rare. This escape hatch is naïve and false. In fact, scanning the protist diversity one observes about an equal number of clades, large and small (i.e., from species or populations within genera, to classes and phyla) are clonal or sexual.

In fact, rare sex means populations are more likely to diverge and become sexually incompatible, and possibly eventually asexual. It can also lead to complicated multiple mating type clusters. The extrapolation of finding divergent homologous DNA sequences between clades as being functional for sex is nothing short of a dogmatic belief that asexual organisms are inconvenient and should not exist.

Earlier this century, a series of papers showed that genes associated with sex and the meiotic apparatus in yeast (*Saccharomyces cerevisiae*) and other sexual model organisms existed in the genome of apparently all eukaryotic lineages. This was considered sufficient evidence that if some of the genes required for meiosis were in the genome, then it must be a sexual clade at its origin. This is a very weak argument for a cell biologist. It is akin to claiming an animal oncogene that causes cancer must be causing cancer in protists. Although one should not be surprised to find oncogenes such as *src* or *myb* in all eukaryotes, it would be foolish to assume variants are cancer-causing in a protist. This is looking at evolution backwards.

There are two ways to look at this. One, that the genes are translated into proteins that correctly assemble to carry out their function in meiosis, despite divergent DNA sequences. The other is that the genes have some function that may even be related to cell division, but they are not involved in anything sexual. The important question is, are these genes still functional for sex or slowly degenerating away from their ancestral function? That distantly related sequences across eukaryotic clades assemble into the same protein complexes or organelles is a leap of faith that peer-reviewed cell biology publications would not accept without evidence. The evidence is usually in substituting the gene in one organism with that of another and expect homologous function. Alternatively, one needs to show that the proteins in the same organism do in fact assemble or function together to do something in that organism. One good place to look is in clonal non-sexual species, to consider what roles these (presumably) functioning genes do. Another is to look for evidence of chromosome synaptic recombinations within populations, as was done for example in trypanosomes. The experimental evidence that would be convincing to an evolutionary minded cell biologist does not exist.

The second problem is that if sex is necessary to avoid clonal ageing, why was it lost in so many lineages that have persisted for hundreds of millions of years – or even, just for

decades? Clearly, at least in some sexual species, sex is prerequisite to chromosome rejuvenation. But also clearly, many species (botanical, protistological, zoological) do not have a need for rejuvenation. Chromosome recombination at meiosis is a source of genetic variation that is complementary to genetic drift by accumulation of point mutations and sequence variation in purely clonal asexual species. The fraction of mutations that are lethal is small so that Muller's ratchet of increasing mutation load acts on a tiny portion of a clonal cells population, with most mutations being neutral on their own. Genetic drift is selected by randomness as well as adaptive functions. Both mitosis and meiosis can generate genetic variation in protists, as long as the asexual cells give rise to sexual cells.

This situation endures in plants and macrophyte algae as somatic cells in the vegetative organisms differentiate into sexual cells at designated locations. Any acquired mutation will be incorporated into the sexual cells derived from those cells. In animal species, somatic cells are not involved in sexual reproduction. Any variations accumulated in the somatic cells during growth and adulthood are genetically isolated from the sexual cells involved in genetic inheritance. (These observations have a long history since August Weissmann elaborated on these; for example, in his collected lectures of 1902, "*Vorträge über Deszendenztheorie*"). In multicellular animal species, the cells that become the sexual cells (germ cells) are set aside during embryogenesis or before adulthood. In animal clades with early germ line determination (differentiation of sexual cells from somatic cells), the germ cells are quiescent until needed and set aside early during embryogenesis. This creates the odd situation whereby the germ cells are differentiated in the early embryo while it is still in the mother. This grand-mother effect on the grand-children in genetic inheritance can be substantial as it represents the environment of the grandmother. Heavy metal pollution, the health of the nucleoplasm, stress and malnutrition will leave their mark, at least by epigenetics, on the 3rd generation.

In late germ line determination, somatic cells will have formed much of the embryo, or of the organism, prior to differentiation of the sexual cells. This allows for some somatic mutations to make it into the next generation. This issue was elaborated by Leo Buss in 1987 in his essay on "*The Evolution of Individuality*". Early germline determination is a safeguard against change from somatic mutations. In contrast, somatic cells differentiating later during the life of an organism increases genetic variation due to environmental conditions. There is a competition between vegetative cells to become the germ cells. The more cells are descended from one cell, the more chance it will become germline. Cancer cells have escaped control and regulation to become more numerous, and more successful. It is a success for those somatic cells, a disaster for coordinated task-sharing by functionally specialized and differentiated cells in the multicellular organism, and genetically futile if the germ line cells are already set-aside, as they are in animals.

In protists, the more numerous clonal cells will retain the sexual advantage. Nonetheless, as I noted above, in sexual protists genetic variation accumulates from both vegetative and sexual cell cycles. Why did some species lose sex, or why did sex evolve independently so many times? Clearly, the second option is unlikely to be correct, leaving us to seek an explanation for the first option. The answer might be in

the ecological strategy, as *r* or *k* or *s* adapted species. The "stable-environment *k*-adapted species" would be more conservative (thus sexual) and restrained to change more slowly than species that benefit from continuously generating change (thus clonal and asexual) to accommodate a dynamic fluctuating environment. Sex imposes many restrictions on change because it requires two complementary types to recognize each other, and for two sets of parent chromosomes to be homologous for pairing. Any change in the molecular signals for initiating a sexual cell cycle, mating type pairing, cell and nuclear fusion (membrane attached complementary type proteins), chromosome changes, and courtship behavior is more likely to prevent or interrupt sex. Any haploid nefarious mutation would further eliminate variations. Asexual species are free from all those barriers to genetic variation, and do not need haploid generations to enforce genome stability. Instead, haploidy permits continuous adaptation and selection.

2. A Very Brief History

Pioneering microscopists became aware of the existence of tiny living organisms which were called animalcules in English by Henry Oldenburg (the editor of the Philosophical Transactions of the Royal Society, London), the translator of Antonie van Leeuwenhoek. Leeuwenhoek used the Dutch diminutives of animals, often preceded by the words small or very small, meaning "little animals" using dierken, diertgens, or diertjes. The origin seems to be a letter to the Royal Society dated 16th of October 1676. These observations were hard to accept at first by the Royal Society (where Robert Hooke was a member) who considered the observations for a year before publishing it. During this time, Robert Hooke was also playing with his microscope and is credited for using the word *cell*, although not in its modern biological context (Micrographia, 1665). Leeuwenhoek's microscope had a magnification of about x270, and Hooke's microscope had a magnification of about x300, and both had a resolution of 1.35 µm, which is adequate for larger microscopic organisms. Animalcule was similar in meaning to the German *Urthiere* (*Urtiere, Urtierche*) introduced by Lorenz von Oken in 1805 in his discussion of cell generation "*Die Zeugung*". The term infusoria was also used for microscopic creatures but later in the 19th century became synonymous with the Ciliophora. Infusoria was introduced by Martin Frobenius Ledermüller between 1760-1763 as *Aufgußtierchen* – animals from an infusion, referring to emergence of encysted forms from adding an infusion. However cysts were unknown and that contributed to support for the creation of life from rotting organic matter. Another early term that infiltrated protistology is monad, which lends itself as a second root to protist names as *something*monad. Its origins are in Gottfried Wilhelm Leibniz's metaphysical description of the world (1714), as the smallest indivisible part of things, which contained both the soul and matter of things. It was adapted for naming unicells, for example an early use was *Monas* O.F. Müller 1786. Microscopes didn't improve significantly until the 1880s when Carl Zeiss and his team began making changes to the optics.

It is important to understand the cytology and microscopy at the time to appreciate the difficulty of making sense from microscopic observations. The research was conducted without knowledge of the molecules and chemistry of organelles, of which organelle in one thing was the same as a similar organelle in another thing, or the same function as a different-looking organelle in yet other things. Many doctoral theses were written on

imaginary constructions, illusions, and incorrect interpretation of the observations. Such misinterpretations led to ideas such as the *homunculus*, and many similar false routes. Cytochemistry and histology developed in parallel, with descriptions of patterns in which stain or dye colours what in which organism. There was gradual, slow development in understanding the composition and chemistry of these stained molecules and organelles. Fixation artifacts were common, especially in the fragile animal tissues, where cell boundaries were not always visible. Very similar difficulties were encountered by microscopists using transmission electron microscopy in its pioneering days in mid-20th century. What is fixation artifact, and what is being stained with the heavy metals? In the early 1980's there were still debates about the existence of the endoplasmic reticulum and its "true" arrangement and purpose. Similarly, the labile and fragile cytoskeletal elements would show differently with different buffers and fixation techniques. Which was the correct fixation and interpretation? (It remains a good student exercise to prepare the same set of ultrathin sections with different buffers and modified fixative preparation). These discussions continued to the very end of the 20th century.

In the 19th century, the idea of a basic unit of life had been around for generations and the evolution of the idea of *a cell* as that basic unit was long in development. Unicells with walls and algae were easier to observe compared to the fragile animal tissues. In parenchymatous or filamentous growth the wall was believed to be shared between adjacent units. They were thought to be a bunch of cells in a common matrix, similar to a sarcinoid colony. Johann Heinrich Friedrich Link (1807) showed that plant tissues consisted of individual cells and not an agglomeration of tissue with a cellular structure.were recognized in 1804 for resolving the nature of cells with a prize from the University of Göttingen. It was Henri Dutrocher in his 1824 classic "*Recherches anatomiques et physiologiques sur la structure intime des animaux et des végétaux*" that first provided the wording, context and discussion of the cell as the basic unit in plants and animals. Barthelemy Dumortier (in "*Recherches sur la structure comparée et le développement des animaux et des végétaux*", 1832), working with filamentous algae, and Robert Remak ("*Über extracellulare Entstehung thierischer Zellen und über Vermehrung derselben durch Theilung*", 1852), working with animal cells, both were the first to recognize cell division for what it was (others had observed it, but failed to appreciate its universality). Dumortier's work was used but not credited by Matthias Jakob Schleiden. Schleiden had correctly concluded that plant cells formed by cell division forming two nuclei, one for each new cell. A lunch conversation with Theodor Schwann in 1837 led to a mutual advance in linking the nucleus and its division to forming new cells, which composed tissues. Both Schleiden and Schwann published separately in 1838-1839 landmark works on cells as the fundamental unit which divided to form new cells, in all animals and plants. Remak's conclusion that all cells come from pre-existing cells provided sense and explanation for Louis Pasteur's results with microbes and pasteurization in 1860-64. It would be unfair to omit Lazzaro Spallanzani's 1786 contributions to this debate ("*Experiencias Para Servir a La Historia de La Generación De Animales y Plantas*"); he argued vociferously with experimental evidence of the effect of heating, that spontaneous generation (George Buffon in 1749) or abiogenesis (John Needham also in 1749) was false. Pasteur's results were conclusive to the debate about origin of microbes and pathogens. Rudolph Virchow, known as the father of pathology ("*Die Cellularpathologie*" in 1858), used

Remak's conclusion uncredited, but elegantly stated *Omnis cellula e cellula* ("All cells come from cells").

The term 'protozoa' was introduced by Georg August Goldfuβ in 1818, presupposing an ancestral simple growth form that preceded animals, and in the same spirit as Leeuwenhoek's *sehr kleine dierken*. It included unicells along with microscopic metazoans. However, the word is little used until Carl Theodor von Siebold gives it its then modern connotation (in 1845) of animals that can live as a single cell. The study of algae as seaweeds and distinct from plants and other forms of life begins with separation of pigmented unicells, filaments and macrophytes into separate categories. The Latin etymology of algae exists since Roman times for the macrophytes, but phycology as a discipline of biology doesn't really come to maturity until mid-19th century. In Carl Linnaeus' system (Systema Naturae 1735-1770, 13 editions) algae were classified as *Cryptogamae* (together with mosses, ferns, lichens, and fungi) as one of the 24 classes of plants (*Regnum Vegetabile*). For the better part of the 18th century, there was debate about what was plant and what was animal, with the coral organisms (invertebrates and algae) treated as plants at first, but increasingly disputed. Jean Vincent Félix Lamouroux separated the algae by pigmentation in 1813, and Rudolph Amandus Philippi moved the coralline algae from animals to the algae in 1837. Both Lamouroux and William Henry Harvey made early contributions to trying to subdivide the algae into groups during the first part of the 19th century. Criticism of the classification and duality of lumping everything into a botanical or zoological realm in mid-19th century led John Hogg ("*On the distinctions of a plant and an animal and on a fourth kingdom of Nature"*, 1860) to bring together the unicellular red algae with the protozoa that were known at the time, into a group called protoctista under a vaguely defined kingdom called Primigenal for all organisms (including bacteria) that would have given rise to the plants and animals.

Ernst Haeckel developed the idea of phylogeny, based on Charles Darwin's evolutionary idea, prioritizing evolution by adaptation from a common ancestor. He defined the Protista kingdom in 1866 ("*Generelle Morphologie der Organismen*"), bringing together all the unicellular organisms, along with several basal animal clades, but distinct from the bacteria (Kingdom Monera). However, he was not the first to separate unicellular heterotrophic organisms from animals, as that must be credited to Rudolf Leuckart ("*Über die Morphologie und Verwandtschaftsverhältnisse der wirbellosen Tiere*", 1848). In Haeckel's subsequent revisions, the Protista were separated from the basal animal lineages. The definition proposed then by Haeckel is similar to the one used by Robert Harding Whittaker in his five Kingdom classification in 1969 ("*New concepts of kingdoms or organisms*"), and by protistologists today ("*The new higher level classification of eukaryotes with emphasis on the taxonomy of protists*", 2005). Protists are defined by not having an embryology that leads to tissue differentiation. In other words, multicellularity in its developmental biology and embryology sense, is an evolved trait from a protist ancestor. Some have used the term multicellular loosely, outside of its formal biological meaning, to refer to any aggregation of cells into filaments, colonies, sheets, sarcinoid, plasmodial, or pseudoparenchymatous organization. In fact, an embryology is known only in the animals (Animalia Linnaeus 1758, syn. Metazoa Haeckel 1874, emend. Adl et al. 2005), in the plants (Embryophyta Engler 1886, emend. Lewis and McCourt 2004), and in the large brown algae (Phaeophyceae Hansgirg 1886, the Fucales). The macrophyte red

algae show some differentiation of somatic cells from the apical cell but lack an embryology. For a modern definition of terms and names in the classification, readers are referred to Phylonemes by De Queiroz, Cantino, and Gauthier 2020, (CRC Press).

3. Diversity, Classification, and Nomenclature

The eukaryotes form two main clades, the Diaphoretickes and the Amorphea, with possibly a third clade (Metamonada and Discoba) which has not yet been positioned in phylogenies with sufficient resolution. The reference classification and explanation of terms and nomenclature are the latest revision from the International Sociey of Protistologists (2019). Protists constitute the Archaeplastida (red and green algae), Ameobozoa (mostly cells with primarily amoeboid locomotion), Cryptista (cryptomonads), Haptista (haptomonads and centrohelids), Discoba (jakobids, heterolobosean amoebae, and Euglenozoa), Metamonada (primarily micro-auxic and anaerobic groups), Rhizaria (cerocozoa, filose testate amoebae, foraminifera, radiolaria), Stramenopiles (brown algae and derived clades secondarily heterotrophic, diatoms, oomycetes), and Alveolata (apicomplexan parasites, dinoflagellates, ciliates). The animals and fungi (together the Opisthokonta) emerge in the Amorphea (Fig. 1); while the plants emerge from the green algae in the Diaphoretickes (Figure 2). The Diaphoretickes regroups most of the other clades, with the exception of a few basal genera, and the two clades, the Metamonada and the Discoba (previously grouped as Excavata with *Malawimonas*). We will first address the Diaphoretickes, followed by the Amorphea, and the basal eukaryotic clades. Ironically, these two principal divisions, if correct, repeat an Aristotelian description of life into botanical and zoological forms (but with most of the Fungi migrated to zoology, and others moved to the Stramenopiles) that was continued by Carl Linnaeus.

Diaphoretickes: The Kingdom Diaphoretickes (Figure 2, Table 1), meaning diverse, includes the green algae, the red algae, the brown algae and other stramenopiles, as well as the cryptomonads, the haptophytes and centrohelids, and the sub-Kingdom SAR that groups four phyla, the ciliophores, dinoflagellates, apicomplexa, and rhizaria, with the first three grouped in a monophyletic lineage called the alveolates.

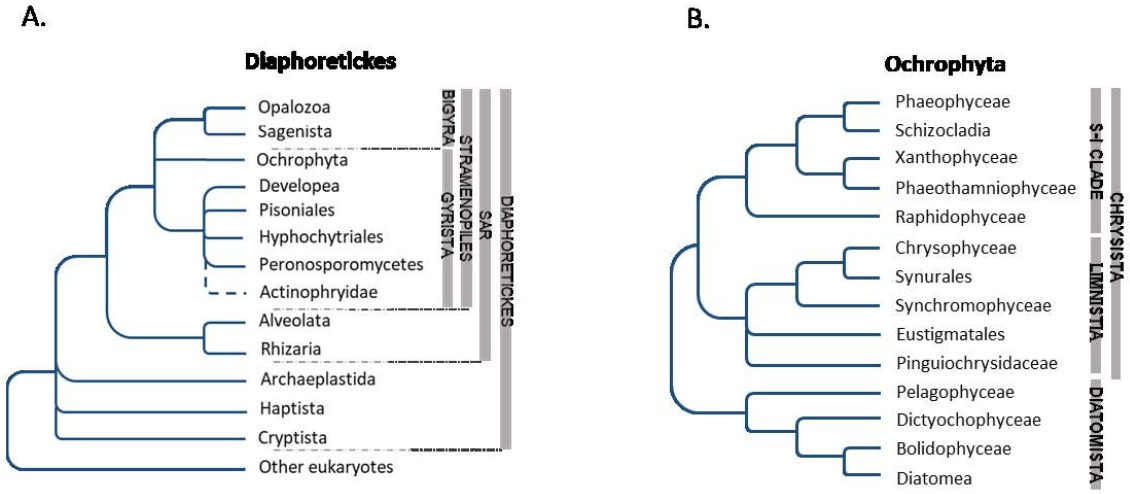

Figure 2. A. Phylogeny of the Diaphoretickes; B. Representation of the phylogeny of the Ochrophyta within the Diaphoretickes.

Sub-Kingdom	Super-Phylum	Phylum, Class	Birth-clade
	Archaeplastida (SP)	Rhodophyceae (P)	
		Chloroplastida (P)	Embryophyta (K)
		Cryptista (C)	
		Haptista (P)	
SAR (SK)		Stramenopiles (P)	Ochrophyta (P)
	Alveolata (SP)	Dinoflagellata (P)	
		Apicomplexa (P)	
		Ciliophora (P)	
		Rhizaria (P)	

Table 1. Kingdom Diaphoretickes and its sub-groups, as indicated.

4. Archaeplastida

The Archaeplastida (Figure 3) incude the red algae and green algae Phyla, and the Glaucophyta. These are photosynthetic due to an ancient endosymbiosis with cyanobacteria. The closest extant cyanobacteria are *Gloeomargarita lithophora*, related to a basal *Synechococcus* clade. In clades outside the Archaeplastida, some organisms have secondarily acquired photosynthesis by acquiring a photosynthetic eukaryote endosymbiont. In Archaeplastida it is called a primary endosymbiosis as it is the ancestral clade that established the chloroplast from a single endosymbiosis. There is a tertiary symbiosis by acquiring an endosymbiont that obtained its chloroplast from a eukaryote with secondary endosymbiosis. Clades that acquired a chloroplast (primary, secondary, tertiary endosymbiosis) tend to obtain three characters in addition to metabolic capabilities. These are the ability to produce starch (an α–glucan) or a β–glucan as a reserve material, the ability to synthesize cellulose for cell walls, and the addition of mastigonemes on cilia. One or more of these are often retained after secondary loss of the plastid, due to gene transfer to the nuclear genome.

4.1. Glaucophyta

This is a small clade related to the red algae within the Archaeplastida. They are important because of a photosynthesis plastid that retains ancestral characters of an ancient endosymbiont bacteria. There are 4 genera, *Cyanophora, Cyanoptyche, Glaucosystis, and Gloechaete*, with about 15 described species. However, many more will probably be described from environmental sampling. Known species are found in fresh-water in the plankton or benthos. Cells occur with a pair of cilia with mastigonemes, and without cilia as coccoid colonies or single cells. Cell shapes tend to be dorso-ventrally flat, with apical cilia when present (one trailing and one pulling). The motile dispersal cells of *Gloechaete* do not feed or divide. Vegetative cells of *Gloeochaete* have short stiff cilia called pseudo-cilia emerging apically, while *Glaucocystis* has a pair of reduced cilia, and none in *Cyanoptyche*. The cruciate kinetid has 4 multilayered structures (MLS) but 2 in *Cyanophora*. Mitochondria have flat

cristae. The plastid retains the most ancestral characters of any protist, thus of any eukaryote. A loose peptidoglycan (murein) layer persists in the space between the two plastid membranes, reminiscent of cyanobacteria and Gram negative cell walls. This plastid is given a special name, the cyanelle or muroplast. The discovery of the cyanelle as a plastid with ancestral morphology, a sort of living fossil, was critical in developing and accepting the endosymbiosis theory of eukaryogenesis. In *Cyanophora paradoxa* the murein layer is synthesized as in *E. coli*. Transport of nuclear encoded proteins into the cyanelle is by the Tic-Toc mechanism with specific polypeptide transit peptide. An additional signal sequence targets the thylakoid. Thylakoid membranes form many layers with chlorophyll *a*, β-carotene, zeaxanthin, β-cryptoxanthin, allophycocyanin, and C-phycocyanin pigments. Photosynthesis is with both photosystems I and II. The photosynthesis pigment and enzyme clusters are called phycobillisomes, and are similar to those in red algae and in Cyanobacteria. Seven core phycobilliproteins remain plastid encoded and regulate its assembly. The bacteria carboxysome (site of RuBisCo enzyme aggregation) occurs, but it is called the pyrenoid in protists and other eukaryotes. The storage compound is starch which accumulates as granules in the cytoplasm. Genomic analysis identified genes for fermentation enzymes, but anaerobic respiration is unstudied. Beneath the cell membrane, flat vesicles (lacunae) reminiscent of Alveolates are associated with a single layer of loose microtubules. The mitotic spindle is open, without centriole or phycoplast. Sex or conjugation is unknown.

4.2. Rhodophyceae

The red algae (Table 2) are a large and diverse group often treated as a phylum, with three main subgroups. About 7,100 species are known but many more remain to be discovered. They are important because the oldest known eukaryote fossils are of filamentous red algae dated at over 1 billion years ago. They are also important for the production of sea-weed as food, food additives and thickeners such as agars, and carrageenan. Another odd character is a triphasic life cycle (in the higher red algae) with two distinct diploid stages and one haploid stage. Red algae have a relatively small genome (Table 3) compared to other eukaryotes. This is due to a genome reduction event causing the loss of several typical eukaryotic characters, such as motility. Species occur as single cells, filamentous forms, or large macrophyte algae with thallus. Red algae occur primarily in marine photic environments, but their habitat extends deeper that green or brown algae. There are freshwater genera (about 5 % of described species), and even a couple of terrestrial species (Cyanidiophyceae) from coastal caves.

Growth forms include unicellular species, palmelloid colonies, filamentous, multiseriate (bundle of parallel filaments), filamentous (with or without branching) or multiseriate growth, microscopic pseudoparenchymatous branching forms, and foliose macrophytes with blade or fine fronds and thallus. Many of the pseudoparenchymatous and foliose forms have specialised differentiated cells, and can be referred to as multicellular. Some forms produce a calcified skeleton and are called crustose. The majority of described red algae (95%) belong to the florideophyte sub-group. These are marine macrophytes, with some extending to estuaries, and some in the Batrachospermales are found in freshwater. Some Bangiales occur at the upper end of the inter-tidal, but most form the understory in algal beds, and some tropical species can occur 200 m below the surface in clear waters.

Cyanidales Christensen 1962 (O)	
Proterhodophytina Munó-Gómez et al. 2017	Compsopogonales Skuja 1939
	Pophyridiophyceae Yoon HS et al. 2006
	Rhodellophyceae Cavalier-Smith 1998
	Stylonematales Drew K 1956
Eurhodophytina Saunders GW and Hommersand 2004	Bangiales Nägeli 1847
	Florideophycidae Cronquist 1960

Table 2. The main subgroups of the red algae, Rhodophyceae (Phylum), (can be considered super-Class and Classes, except Cyanidiales is an Order)

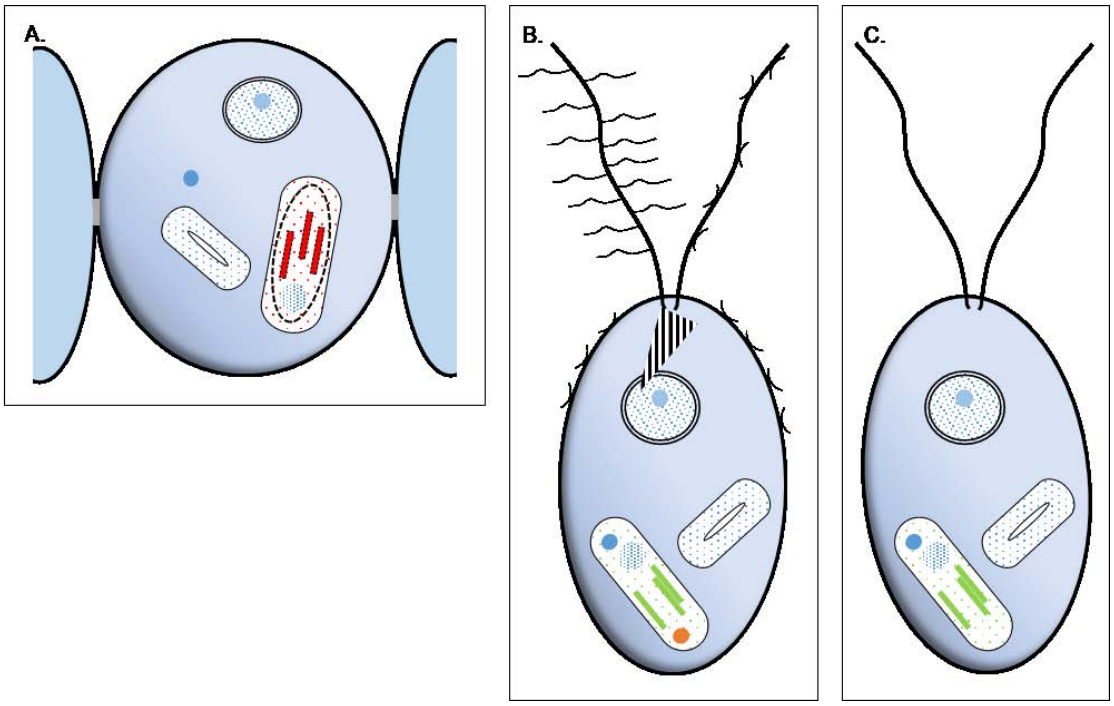

Figure 3. Archaeplastida. A. Rhodophyceae, with pit connections between adjacent cells. B. Chlorophyta (Chloroplastida) (only some clades with scales). C. Streptophyta (Chloroplastida). This figure and the following ones are stylized and modular to represent organelles and structures, but do not reflect sizes or number in each cell.

Mitochondria have flat cristae. Characteristically, in red algae the mitochondrion is near the cis-face of a Golgi apparatus, although it is not exclusively found there. The chloroplast has unstacked thylakoids. The accessory pigments aggregate as phycobilisomes on the outer thylakoid membrane, passing the energy to Chlorophyll *a*. As for the Glaucophytes, this arrangement reflects the cyanobacterial origin of the plastid. The storage product accumulates as starch granules in the cytoplasm. The starch consists of amylopectin, without amylose thus distinguishing it from green algae and plants. It is more similar to animal glycogen with α-1-4 linked glucan and frequent α-1-6 branching. Aggregation of CO_2 fixation by RuBisCo enzymes into a pyrenoid occurs

throughout the red algae but unlike green algae where it is common, it is rare. Pyrenoid ultrastructure offers several useful morphological traits. There is no periplastid endoplasmic reticulum.

A characteristic trait of red algae is the absence of centrioles, kinetosomes and cilia. Therefore, there are no motile stages in red algae. There is an amorphous MTOC (the polar ring) that forms at spindle poles in mitosis and meiosis. The microtubules of the spindle do not converge at the poles, instead forming a broad wide spindle. The nuclear envelope persists through mitosis (closed mitosis), and it is surrounded by an endoplasmic reticulum membrane, called the nuclear ER. However, cells are capable of directional taxis by gliding on secreted mucus by an otherwise unexplained mechanism.

In filamentous and parenchymatous forms, adjacent cells remain connected by a structure called the pit-connection. Due to incomplete cell wall synthesis at the end of cytokinesis in almost all multicellular genera, the open connection between dividing cells is filled with a proteinaceous plug. The pit-plug may have a membrane lining it in many genera. There is no evidence of transport or communication across the plug.

Specialized cells occur in multicellular forms by differentiation from dividing apical cells. These include sexual cells for sporogenesis, rhizoids, gland cells, hair cells, and epithelial cells in the coralline algae. These epithelial cells are terminally differentiated, senesce, and slough-off. They are replaced by the underlying meristem cells.

Coralline red algae. The calcified species occur in three sub-groups of the Florideophycidae, the Corallinales, Hapalidiales, and Sporolithales. They grow as articulated algae (geniculate) or rock-like as crustose algae. These contribute to the coral structure in reefs. The coralline algae, especially the crustose forms, dominate overgrazed algal beds, as the uncalcified algae are grazed. Coralline turfs in the intertidal form an entangled mesh of branched articulated filaments and fronds that become a porous sponge. It helps retain moisture in the exposed phase protecting other sea-life from desiccation, and provides some protection from mechanical wave action. These coralline turfs are therefore high in micro-invertebrate biodiversity. They also release a compound that attracts larvae of molluscs by chemotaxis, thus promoting coral growth through colonization (Tebben et al., 2015). There are two types of coralline red algae dominated sea-weed ecosystems in the ocean. They are the rhodolith beds and the crustose red algae dominated tropical reefs. The other two are the kelp forests (brown algae) and the seagrass meadows. As a result, the coralline red algae are important ecosystem engineers in the ocean.

Biological calcification is a useful marker of past climate and paleontological and biogeochemical processes. Current climate change and ocean acidification trends are having a non-trivial effect through solubilisation of the calcareous ocean coral reefs, past geological and benthic deposits, including of ancient sea-beds that are now in terrestrial environments. Acidification reduces growth, reproduction, and survival of the coralline algae. The magnitude of carbonate production and accumulation by red algae is significant, and we can call them major global $CaCO_3$ biofactories. For example, southwestern Atlantic tropical reefs near Brazil contribute 0.3 % of the global carbonate production, and the stocks are comparable to existing large deposits of carbonates.

The biogeography of the red algae is not clear and requires additional exploration. Molecular studies show that morphological species are in fact, often, species complexes similar to what has been found in many other protist lineages. Thus, species geographical distribution is uncertain. Nonetheless, species have a distribution influenced by temperature and regional factors. There is agreement that red algae originated from the Pacific Ocean, and spread to the Arctic Ocean and the north Atlantic Ocean. Red algae now occur across the globe at all latitudes.

Commercial consumption of red algae has been on the rise with popularization of Japanese and Korean food around the world. The main seaweed aquaculture productions are in the western Pacific Asian coastlines, with a few genera comprising most of the cultivation (*Eucheuma, Kappaphycus, Gracilaria, Porphyra, Pyropia*). However, as cultivation expands, uses for derived extracts and possible medicinal compounds are increasing. Most of the cultivation is for seaweed in food, hydrogels as food additives, or functional food ingredients.

Life history. Asexual reproduction by mitosis leads to vegetative growth. Fragmentation of filaments and macrophytes leads to dispersal. Vegetative dispersal spores are produced by specialised cells of gametophyte or tetrasoprophyte. Some Florideophycidae lack meiosis (apomictic) or the fusion of gametes to form a zygote does not occur (apogamic).

Sex and conjugation. Early-diverging red algae possess a typical biphasic life cycle, with a haploid generation alternating with a diploid generation. Higher taxa have a haploid generation followed by two morphologically distinct diploid generations. These taxa are in the Florideophycidae subgroups Hildenbrandiaceae and Nemaliophycidae. There are variations on the basic pattern, but one can generalize a common triphasic life cycle. The female gametophyte (1 n) produces reproductive branches (carpogonial branch) that end with an egg cell (carpogonium) that has a thin extension (trichogyne) to receive the spermatium. The haploid male gametophyte (1 n) produces gametic dispersal cells called spermatia. The released spermatia that attach to the female gametophyte, initiate fertilization and the zygote begin the first diploid generation. The diploid nucleus of the carpogonium begins mitotic divisions that form a filamentous diploid organism, the gonimoblast. (Sometimes the diploid nucleus is passed to an adjacent auxiliary cell where mitotic divisions begin). The gonimoblast forms specialised differentiated cells (carposporophytes) at regular intervals that will release dispersal spores (carpospores). Thus, this first diploid generation grows out from the female gametophyte. The released carpospores settle and grow into a mature macrophyte called the tetrasporophyte (2 n) organism that forms differentiated reproductive cells (tetrasporangium) where meiosis occurs, producing a tetrad of haploid (2 male, 2 female) small cells (tetraspores (1 n)) for dispersal. Each tetraspore settles to grow into a haploid gametophyte. The gametic (male and female) generation form isomorphic organisms. In some, the gametic and tetrasporophyte are also isomorphic, but in others heteromorphic organisms depending on the lineage. There can be great size differences between the generations. For example, in the Palmaria the male gametophyte and tetrasporophyte are macrophytes while the female gametophyte is microscopic.

Parasitic red algae. Parasitism evolved multiple times in the red algae and there are more than 120 described parasitic species. The parasite species tend to be closely related to their host species, and often belong to the same genus. Incompatibility between host and parasite cytoplasm limits host diversity, with most restricted to <3 host species. It is believed that a secondary pit connection from the parasite to the host is essential for organelle transfer, and to control the host cell. These species are difficult to identify because they are colourless and form a discrete thallus on the host thallus. Too few have been studied in sufficient detail to elaborate on the parasitism.

Genome reduction. Molecular phylogeny and comparative genomics indicate a significant gene-loss event early in the diversification and evolution of red algae. This phenomenon occurs in species that become intracellular parasites that adopt a parasitic life history and become obligate symbionts. In free-living organisms, the phenomenon occurs in taxa that are in a narrow niche with invariant environment, yet the red algae are very diverse and occur in many environments between the poles and the tropics. One idea is that in their evolutionary history, some time ago, they suffered a genome reduction due to their environmental adaptation then. Some have speculated on what that might have been with scarce evidence. In part speculation revolves around the habitat of the Cyanidiales, which are the earliest-diverging red algae. The Cyanidales are asexual, unicellular, that occur at pH 0-4 and a temperature of 25-55 C, in places near hot springs and acidic sulphurous water. It is not clear if they acquired a plastid before or after occupying this habitat. Their adaptation to this environment is due to significant lateral gene transfer (about 1 % of the genes) from prokaryotes. This very specialised group has three morphological genera, *Cyanidium, Cyanidioschyzon,* and *Galderia*. More recent sampling and DNA sequencing reveals a much greater diversity. On average red algae have retained about 10,000 genes, and probably lost upwards of 1,000 genes. The consequent lost functions involve the absence of cilia and kinetosome, phytochromes, glycosylphosphatidylinositol anchor, macro-autophagy pathway, and the Ni-dependent urease metabolic pathway.

Species	Genome size	Number of genes, estimated
Escherichia coli	4.6 x 10^6 bp	[a]4,288
Nostoc puctiforme	9.0 x 10^6	[a]7,432
Saccharomyces cerevisiae	12 x 10^6 bp	[b]6,294
Caenorhabditis elegans	97 x 10^6 bp	[c]19,000
Drosophila	175 x 10^6 bp	[c]13,600
Mus musculus	2,700 x 10^6	[d]20,210
Homo sapiens	3,200 x 10^6	[d]100,000

Table 3. Genome sizes

4.3. Chloroplastida

The green algae are even more diverse than the red algae. They are characterised by having both photosystem I and II with chlorophyll *a* and *b*, and all the RuBisCo small

subunits encoded in the nucleus rather than in the plastid. Motile dispersal cells and sexual conjugation with a biphasic life history are common. The main subdivisions are the Chlorophyta (about 4,300 species) and Streptophyta with the remainder (more than 8,000 species known). Most species are marine or freshwater, with a small number of genera that occur in terrestrial environments. The Charophyceae (in the Phragmoplastophyta) are the ancestral sister-group to the plants (Embryophyta Engler 1896 emend Lewis and McCourt 2004), which have about 400,000 species.

Chlorophyta Pascher 1914, emend Lewis and McCourt 2004	Ulvophyceae Mattox and Stewart 1984 (C)
	Trebouxiophyceae Friedl 1995 (C)
	Chlorophyceae Christensen 1994 (C)
	Chlorodendrophyceae Fritsch 1917 (O)
	Pedinophyceae Moestrup 1991 (C)
	Chloropicophyceae Lopes dos Santos and Eikrem 2017 (C)
	Pyramimonadales Lopes dos Santos and Eikrem 2017 (C)
	Mamiellophyceae Marin and Melkonian 2010 (C)
	Nephroselmidaceae Skuja ex P.C.Silva (F)
	Pycnococcaceae Guillard 1991 emend Fawley 1999 (F)
	Palmophyllophyceae Leliaert et al., 2016 (C)
Streptophyta Bremer and Wanntorp 1981	*Chlorokybus* Geitler 1942 (C)
	Mesostigma Lauterborn 1894 (C)
	Klebsormdiophyceae, van den Hoek et al., 1995 (F)
	Phragmoplastophyta Lecointre and Guyander 2006 (C) (includes Zygnemataceae (F), Coleochaetaceae (F), Charales (O), Embryophyta (K))

Table 4. The main subgroups of the green algae.

4.3.1. Chlorophyta

Species are unicellular with an even number of cilia, or colonies of cells with cilia, or palmelloid non-motile colonies, non-motile coccoid unicells, sarcinoid (clump of cells), filamentous, multiseriate, foliose macrophyte with thallus, or syphonous growth. The oldest precursor cells at the base of the green alga lineage are believed to have been similar to unicells of the Palmophyllophyceae and *Nephroselmis.* These include the prasinophyte clade VI (Prasinococcales Guillou et al., 2004), which are marine planktonic unicells without cilium, with cellulosic cell wall, a single cup-shaped chloroplast with pyrenoid, and unequal cell division whereby one cell retains the parent cell wall and the other makes one *de novo*. The Palmophyllaes are the other group of Palmophyllophyceae, comprising marine benthic macrophyte algae with crustose or erect sarcinoid cell growth, with a single cup-shaped chloroplast without pyrenoid. One

of the ancestral traits of all green algae is the presence of Golgi cisternae derived proteinaceous scales 40-50 nm covering the surface of cells and cilia. These occur in the Palmophyllophyceae and in tomites and gametes of the Streptophyta. For example, *Nephroselmis* (prasinophyte clade III) are bi-ciliated unicells covered with square-ish scales in most species, and a single cup-shaped chloroplast with pyrenoid and stigma. These basal clades consist of the older prasinophyte classification sub-divided into 8 named clades in addition to Picocystophyceae Lopes dos Santos and Eikrem 2017, which includes *Picocystis* as clade VII-C. One small group are the Pedinophyceae Moestrup 1991 emend Fawley et al., in Adl et al., 2012. They are worth mentioning because they are unicellular with a single cilium, which is unusual. The cilium is covered with thin hairs called mastigonemes.

The remaining three groups of Chlorophyta are derived from the prasinophyte (informal term) clades. The **Ulvophyceae** Mattox and Stewart 1984 might be polyphyletic and need further phylogenetic analysis. They grow as multinucleate unicells (Dasycladales), as filaments sometimes branching, or in leaf-like parenchymatous forms. Swimming cells have one or two pairs of cilia with rhizoplast. The 4 ciliary roots from kinetosomes are cruciate (cross-shaped) in counter-clockwise orientation, and this arrangement is an important morphological traits in the evolution of green algae. Cell division is by closed mitosis, without phragmoplast. Species are sexual, with biciliated gametes and quadri-ciliated dispersal meiospores. Gametophytes can be iso- or heteromorphic. One genus, *Cephaleuros,* causes red-rust disease on leaves of leafy tropical and sub-tropical plants, affecting important crops such as coffee, guava, mango, and tea. The cell grows by dichotomous branching on leaf surfaces but penetrates into the leaf tissue. Interestingly, the genus can also form a symbiosis with certain lichens.

One sub-group, the **Dasycladales**, are siphonous, consisting of one giant cell with nuclei throughout the cytoplasm. The cell wall consists of cellulose and mannan, sometimes encrusted with calcium carbonate. One species, *Acetabularia acetabulum*, has been used as a model organism for molecular biology.

The **Trebouxiophyceae** are mostly freshwater species (although there are soil and marine species) and have differences from the Ulvophyceae. The kinetosomes have an additional structure, the MLS, and a prominent rhizoplast. Cell division is by closed or semi-closed mitosis, and cytokinesis involves a phycoplast. Asexual reproduction is by dispersal swimmers and cysts (autospores). Sexual conjugation has been reported but not observed. Many genera are symbiotic, for example *Coccomyxa* and *Symbiochloris*. *Chlorella* are a clade of cosmopolitan species found in freshwater and terrestrial habitats that also occur as a common endosymbiont in many protists (ciliates, amoebae, testate amoebae, heliozoans, and foraminifers), in many freshwater sponges and cnidarians. The association with protists is not necessarily permanent, as there are examples of transient association. Another similar genus, *Trebouxia* is a common symbiont in lichens. *Prototheca* has lost photosynthesis and is a rare green algal opportunistic parasite of mammals. It is most probably a natural mutant of *Chlorella* (losing the cell wall and photosynthesis) that became saprophytic and osmotrophic in soil. It can invade moist surfaces of the intestines and mouth, nose, eyes, and mammary glands, and even reach kidneys. One terrestrial species, *Helicosporidium parasiticum,* has also lost photosynthesis and exists as a parasite of diverse metazoans (collembolan, mites,

beetles, dipteran, lepidopteran, cladocerans, and trematodes). Anoher parasitic genus is *Phyllosiphon* which affects angiosperm leaves.

The **Chlorophyceae** are the last of the three clades in the Chlorophyta. They contain eight sub-groups, of which we will present two that are important, both for their diversity and for evolutionary significance, the Chlamydomonadales (Volvocales) and the Chlorococcales. The latter has about 780 species, mostly from freshwater and terrestrial habitats. Species grow as coccoid unicells without cilia, some are colonial, sarcoid, filamentous, or syphonous. The motile ciliated cells are restricted to reproductive dispersal cells. However, all cells retain a kinetosome and its roots.

The **Chlamydomonadales** cells are based on the *Chlamydomonas* cell structure, with a pair of cilia and four cruciate microtubular roots. Kinetosomes are directly opposed or displaced clockwise, and rhizoplast connects kinetosomes and extends to the nucleus. Cell division is by closed mitosis with phycoplast at cytokinesis. Two genera (*Polytoma* and *Polytomella*) have secondarily lost the chloroplast. Plasmodesmata connections form between adjacent cells in colonial forms. Ciliary beating is co-ordinated for the whole colony. Colonial species are held together by a mucilaginous matrix. Cells at the apical end remain vegetative while cells at the posterior become differentiated sexual cells. Asexual dispersal is by cysts called aplanospores, akinetes, or autospores. Sexual conjugation is by isogamy, anisogamy, or oogamy. The sub-groups within the Chlamydomonadales represent incremental increased complexity in colony organization. *Chlamydomonas* is an example of permanently unicellular motile species. *Tetrabaena* consist of 4 adjoined cells; *Gonium* forms planar colonies of 8 or 16 cells; *Pandorina* forms spherical colonies of 16 cells; *Eudorina* forms spherical colonies of 16 or 32 cells; *Pleodorina* forms colonies of 64 or 128 cells; *Volvox* forms colonies of several hundred cells.

4.3.2. Streptophyta

General features include asymmetric motile cells with a pair of cilia. Kinetosomes with microtubular rootlet and cytoskeletal anchor. Mitosis is open with a phycoplast at cytokinesis, and a phragmoplast in some. Filamentous forms have primary plasmodesmata between adjacent cells. Several enzymes are characteristic, such as glycolate oxidase, Cu/Zn superoxide dismutatse, and ciliary peroxisome.

There are four small but important groups that form motile dispersal unicells very similar to the male gameophyte of plants. Two are represented by a single species each, *Chlorokybus atmophyticus,* and *Mesostigma viridae.* The Klebsormidiophyceae include three genera, *Hormidiella, Klebsormidium,* and *Interfilum,* with about 24 species but additional genera may become included. The fourth is Choleochaetophyceae with two genera, *Chaetosphaeridium* and *Coleochaete*. Interestingly, although these occur in freshwater, many are also found in terrestrial environments or exclusively so. The terrestrial species have amino acids that absorb ultra-violet light, which are lacking in the aquatic species; they also have better resistance to desiccation and form cysts as zygospores. Only the Choleochaetophyceae are sexual, with oogamous reproduction forming a diploid zygote that is the only diploid form in the life history. The cell wall of *Coleochaete* most resembles the plant cell wall in composition, even having small

amounts of lignin and plant pectic polysaccharides. They even have a rosette cellulose-synthesizing complex assemble similar to plants. The other genera tend to have less cellulose and more callose. *Mesostigma* in contrast has no cell wall but three layers of proteinaceous scales and a pectic polysaccharide found in plants. These genera are unicellular, sarcinoid, colonial sarcinoid clumps, filamentous, sometimes branching. The Choleochaetophyceae have a sheathed hair-like extension from some cells.

There are two diverse groups more closely related to plants, the Zygnematophyceae and the Charophyceae, in a clade called the Phragmoplastophyta.

The **Zygnematophyceae** (commonly called desmids) form beautiful shapes and are extremely diverse with at least 4,000 species, occurring mostly in freshwater. Cells grow as unicells or filamentous species. They have large variously-shaped chloroplasts. The cell wall is arranged in three layers, which has been useful in classification. They lack cilium and kinetosome at all stages of asexual and sexual life history. Sexual conjugation is triggered by nutrient-poor conditions. Adjacent filaments that are sexually compatible will allow a male amoeboid gamete to escape its cell wall and penetrate a female cell. Two haploid gametic cells fuse to form a zygospore, which proceeds with meiosis and releases haploid vegetative cells. (Note that the mating systems vary).

It is thought a filamentous zygnematophyte-like ancestor is the most likely common ancestor of Zygnematophycae, Charophyceae, and plants (Embryophyta), with plants arising from the charophytes. The likely terrestrial (or freshwater edge) ancestor of plants are basal to extant charophytes, and the derived and simplified desmids form a sister clade.

The **Charophyceae** form large parenchymatous algae in freshwater that can reach ½ m in length. In some habitats they can be the dominant aquatic vegetation. Some species occur in brackish water. The thallus forms a stem with fronds branching at regular nodes as whorls of branchlets, anchored by a rhizoid. Adjacent cells are connected by true plasmodesmata. The stem is a multinucleate syncytium with mononucleate cells at the nodes. Growth is from apical cells at the tip of the stem and branchlets. Many species accumulate calcium carbonate in the cell wall. Thalli can be monoecious or dioecious. In monoecious forms both reproductive structures can occur at the same node, or at alternating nodes. Sexual conjugation is oogamous, with male antheridia releasing sperm cells, and female manubrium holding an egg cell. There are six extant genera with two being common, *Chara* and *Nitella*. The number of species is disputed as it is unclear what the extent of sub-species and synonymy really is, but hundreds are described; there are probably about a hundred species each. Many other genera are known only from the fossil record.

-

-

Bibliography

Adl S., Simpson A.G.B., Farmer M.A., Andersen R.A., Anerson O.R.A., Barta J., Bowser S.S., Brugerolle G., Fensome R.A., Fredericq S., James T.Y., Karpov S., Kugrens P., Krug J., Lane C.E., Lewis L.A., Lodge J., Lynn D.H., Mann D.G., McCourt R.C., Mendoza L., Moestrup Ø., Mozley-Standridge S.E., Nerad T.A., Shearer C.A., Smirnov A.V., Spiegel F.W., Taylor F.J.R. (2005). The new higher level classification of eukaryotes with emphasis on the taxonomy of protists. *Journal of Eukaryotic Microbiology* 52: 399-451. [This paper provides a synthesis of the eukaryote classification resulting from phylogenetic analysis, with brief clade descriptions; the introduction to the tables provides an important reading to understand some of the issues that were problematic, and how the classification was assembled without named ranks].

Adl S., Bass D., Lane C.E., Lukes J., Shoch C.L., Smirnov A., Agatha S., Berney C., Brown M.W., Burki F., Cárdenas P., Cepicka I., Chistyakova L., del Campo J., Dunthorn M., Edvardsen B., Eglit Y., Guillou L., Hampl V., Heiss A.A., Hoppenrath M., James T.Y., Karnkowska A., Karpov S., Kim E., Kolisko M., Kudryatsev A., Lahr D.J.G., Lara E., le Gall L., Lynn D.H., Mann D.G., Massana R., Mitchell E.A.D., Morrow C., Park J.S., Pawlowski J., Powell M.J., Richter D.J., Rueckert S., Shadwick L., Shimano S., Spiegel F.W., Torruella G., Youssef N., Zlatogursky V., Zhang Q. (2019). Revisions to the classification, nomenclature, and diversity of eukaryotes. *Journal of Eukaryotic Microbiology* 66: 4-119. [A follow-up to the 2005 and 2012 classification revisions with updated descriptions of clades; this monograph is important in introducing trophic functional designations, primers useful for each clade, and modernising the taxonomy in Chinese script; the introduction provides context for understanding the current state of the classification; it is the most recent complete description of protist clades].

Adl, M.S. and Blaine Mathison. 2019. Taxonomy and classification of human eukaryotic parasites. In: *Manual of Clinical Microbiology* (12th ed). American Society of Microbiologists. Chapter 135, 2379-2388. [Revised by a collaborative, international, interdisciplinary team of editors and authors, this seminal reference text continues to set the standard for state-of-the-science laboratory practice as the most authoritative reference in the field of clinical microbiology; the chapters on medically important protists are updated every two years].

Adl, M.S. and Gupta, V.S., 2006. Protists in soil ecology and forest nutrient cycling. Canadian Journal of Forest Research, 36(7): 1805-1817.

Archibald J.M., Simpson A.G.B., Slamovits C.H. (2017). *Handbook of the Protists* (2nd ed.) Springer. 1657 pp. [This two volume book provides a great up-to-date overview of what we know in most groups of protists. However, it is missing several groups and would need a third volume to complete it].

Baroin A., Perasso R., Qu, L.-H., Brugerolle G., Bachellerie J.-P., Adoutte A. (1988). Partial phylogeny of the unicellular eukaryotes based on rapid sequencing of a portion of 28S ribosomal RNA. *Proceedings of the National Academy of Science* 85: 3474-3478. [A historical paper that first described eukaryote diversity with a molecular phylogeny].

Bell, G. 1988. Sex and Death in Protozoa: the history of obsession. Cambridge University Press. 216 pp.

Bringloe T.T., Starko S., Wade R.M., Vieira C., Kawai H., de Clerk O., Cock J.M., Coelho S.M., Destombe C., Valero M., Neiva J., Pearson G.A., Faugeron S., Serrão E.A., Verbruggen H. (2020). Phylogeny and evolution of the brown algae. *Critical Reviews in Plant Science* 39: 281-321. [A recent paper that sheds some light on a diverse group of Stramenopile that were incompletely explained by phylogenetic analysis].

Buss, L.W. 1988. The Evolution of Individuality. Princeton University Press, Princeton, NJ, xvi, 203 pp.

Foissner, W., 2006. Biogeography and dispersal of micro-organisms: a review emphasizing protists. Acta protozoologica, 45(2): 111-136.

Garg S.G., Martin W.F. (2016). Mitochondria, the cell cycle, and the origin of sex via a syncytial eukaryote common ancestor. *Genome Biology and Evolution* 8: 1950-1970. [This paper provides a nice summary of the various issues around the origin of a eukaryotic cell, and how it assembled into organelles].

Graf L., Yang E.C., Han K.W., Küpper F.C., Benes K.S., Oyadomari J.K., Herbert R.J.H., Verbruggen H., Wetherbee R., Andersen R.A., Yoon H.S. (2020). Multigene phylogeny, morphological observation

and re-examination of the literature lead to the description of the Phaeosacciophyceae classis nova and four new species of the Heterokontophyta SI clade. *Protist* 171: 125781. [This recent paper describes the current phylogeny of brown algae].

Harris H. (1995).*The Cells of the Body*. Cold Spring Harbor Laboratory Press, New York. [Includes an overview of the history of the cell theory].

Hausmann K., Hülsmann N., Radek R. (2003). *Protistology* (3rd ed.) E. Schweizerbart'sche, Stuttgart. 379 pp. [This introductory textbook provides an accessible overview of protistology, although the classification is dated, and it is beautifully illustrated].

Hörandl E., Speijer D. (2016). How oxygen gave rise to eukaryotic sex. *Proceedings of the Royal Society B* 285: 20172706.

Lee, J.J., Leedale G.F., Bradbury P. 2000. *An illustrated guide to the protozoa* (2nd ed.). Society of Protozoologists. 1432 pp. [This book, though dated, remains an important reference for descriptions of genera but the classification uses terms no longer in use].

Ribatti D. (2018). A historical note on the cell theory. *Experimental Cell Research* 364: 1-4. [A short well researched paper on the history of understanding cells].

Starko S., Gomez M.S., Darby H., Demes K.W., Kawai H., Yotsukurai N., Lindstrom S.C., Keeling P.J., Graham S.W., Martone P.T. (2019). A comprehensive kelp phylogeny sheds light on the evolution of an ecosystem. *Molecular Phylogenetics and Evolution* 136:138-150. [A significant paper in elucidating the phylogeny of kelp, showing how it provides valuable information on ecosystem evolution over geological periods and modern biogeography of the clade].

Biographical sketch

Professor Sina Adl graduated from the University of British Columbia with training in natural history and diversity, cell and developmental biology, genetics, and protistology. His mentors were Professors J. Beisson (Ciliate Genetics), J.D. Berger (Protistology, Genetics), T. Cavalier-Smith (Protistology), J. Cohen (Molecular Biology), L. Edelstein-Keshet (Mathematical Biology), B. Green (Protistology), L. Harrison (Pattern Formation), J. Maze (Botany), Max Taylor (Protistology), N. Towers (Plant physiology & biochemistry). He defended his PhD thesis in 1998 on cell cycle regulation under the supervision of J. D. Berger. His research has been on soil ecology, with small microcosms using stable isotopes to trace nutrient flow and food web interactions. Other research focused on protist ecology in soils. Field research included experiments with sustainable agriculture, organic agriculture, grassland management and succession, post-mining remediation of soils, and utilization of municipal organic waste composts as fertilizer. One project analysed microfossils in amber to reconstruct the food web of a 100 million year old forest system. The principal current project is on global soil biodiversity of eukaryotes, focusing on protists and micro-invertebrates. Ongoing interests and papers include work on systematics, classification, and nomenclature of protists. He published one book (The Ecology of Soil Decomposition, 2003), four book chapters, and about 100 peer-reviewed papers. He is the founding editor (2016) and Editor-in-Chief of Rhizosphere.