Biology 2004 and Biology 3102

Supplementary Course Material 1

Fall 2020 version

AKA "The Mini-Handbook of Protists"

***Annotated with PDF Bookmarks**

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PART 1: Various topics in eukaryotic cell organisation and evolution

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Biology 2004 and Biology 3102

Supplementary Course Material: Microbial Eukaryotes and Macroalgae

Fall 2020 version

INTRODUCTION

What is the purpose of this material?

These materials are primarily to accompany lectures 8, 9, 10 & 12 of BIOL2004, and Part 1 of BIOL3102.

They are **not** a set of dedicated lecture notes: **It is intended that you treat them like a textbook or reference book**, to access more detailed information than the lecture slides can communicate. These notes cover most of the material that we will go over in these lectures. However, these notes also include material that we will **not** go over in these lectures, or will cover only to a limited extent in the class you are enrolled in. On the other hand, the lectures will contain much more graphical content, and occasionally some other material, that is not in these notes. Further, we may go over subjects in a different sequence to the order they are printed here.

Organisation

The material is divided into three parts:

Part 1 introduces the various basic types of microbial eukaryotes, and includes some discussion on the evolution of eukaryotic cells. It also includes brief supplementary materials on life cycles, and on certain organelles and other components of microbial eukaryote cells that are not normally covered in standard cell biology textbooks.

Part 2 covers the diversity and some basic biology of the major groups of microbial eukaryotes.

Part 3 covers the general biology of macroalgae, and the main phylogenetic groups of macroalgae.

Appendix: Contains a partial classification of eukaryotes, and a summary of some basic properties of plastids in different groups of photosynthetic eukaryotes.

PART 1

1.1 Basic types of eukaryotic cells; Eukaryote diversity.

1.1.1 Protists, protozoa and algae.

There are two 'popular' ways to think about the diversity of eukaryotic organisms.

1) Traditionally, microbial eukaryotes were divided into basic categories according to their nutritional mode. Plant-like photosynthetic forms were called '**algae**'. Animal-like forms that were heterotrophic were mostly called '**protozoa**'. A few heterotrophic forms that were fungi-like in their nutritional mode and/or their morphology were usually treated together with the true fungi.

2) By the 1970s, it became popular to divide eukaryotes up into four groups – Animals, Plants, Fungi and **protists** (Protista). Often these are treated as four distinct "Kingdoms". In this scheme, most eukaryotic microbes are 'protists' (other than those truly within the taxon Fungi).

It is frequently convenient use the terms 'algae', 'protozoa' and 'protists' but it is important to realize that they do NOT correspond to real evolutionary units (i.e. monophyletic groups, also known as clades). The term 'protists' just refers to all those eukaryotes that are NOT animals, plants or fungi. Some protist groups are actually much more closely related to animals than they are to most other 'protists' (choanoflagellates, for example –see Part 2, Section O2.1). The terms 'algae' and 'protozoa' also do not refer to monophyletic groups. There are several groups of 'algae' that are much more closely related to some 'protozoan' groups than to any other algae (several examples will become apparent in Part 2). One reason for this is the complex evolutionary history of plastids (e.g. chloroplasts), including the phenomenon of 'secondary endosymbiosis' (see Section 1.4.5, below).

It is sometimes convenient to recognize a few basic catergories of 'algae' and 'protozoa'. On the algal side, it is common to distinguish between 'microalgae' and 'macroalgae'. **Microalgae** are mostly microscopic, are unicellular or composed of a few cells, and are often motile. '**Macroalgae'** are forms that superficially resemble land plants – usually macroscopic, generally composed of many cells (and often differentiated into many distinct cell types), immotile, and, in most cases, attached to solid substrates. 'Seaweeds' are the most familiar macroalgae. For convenience, we will examine the few groups of macroalgae in Part 3 of these notes, separately from the various groups of microalgae covered in Part 2. Again, it must be stressed that 'macroalgae' and 'microalgae' do not represent evolutionary groups, and the distinction between them is sometimes arbitrary.

In contrast to the algae, almost all protozoa are unicellular or colonial. The most common distinctions of convenience are (or were) based on cell form and motility. '**Flagellates**' (or 'heterotrophic flagellates') have eukaryotic flagella. '**Amoebae**' lack flagella, at least during the normal trophic (feeding) phase of their lifecycle, and produce various kinds of pseudopodia, which are often used for locomotion. '**Ciliates**' have numerous 'cilia' that beat in a co-ordinated fashion (cilia are homologous to eukaryotic flagella). '**Sporozoa**' (rarely used nowadays) referred to parasitic forms that produce immobile 'spores' to infect new hosts. Again, these groupings are assemblages of convenience, except for ciliates, which are in fact a monophyletic group (a clade: see Part 2, Section AV4).

1.1.2 A modern view of eukaryote diversity.

Since about 2004 it has become popular to represent the diversity of eukaryotic organisms, not as four "Kingdoms" (see above), but instead as 5-8 higher order groupings often called 'Supergroups'. A contemporary evolutionary tree of eukaryotes is shown in Figure 1.1. Note that there are still some areas of uncertainty, and a few organisms that cannot be assigned to any of the major groupings.

Where do the familiar kingdoms of 'Animals', 'Plants' and 'Fungi' fit within the scheme? The short answer is that these each fall inside one or other of the 'supergroups': Land plants are one subgroup within 'Archaeplastida', while animals and fungi both fall inside 'Opisthokonta' (Fig. 1.1). The remainder of Opisthokonta and Archaeplastida, plus ALL of the members of other supergroups are the various organisms that used to be considered members of the Kingdom Protista.

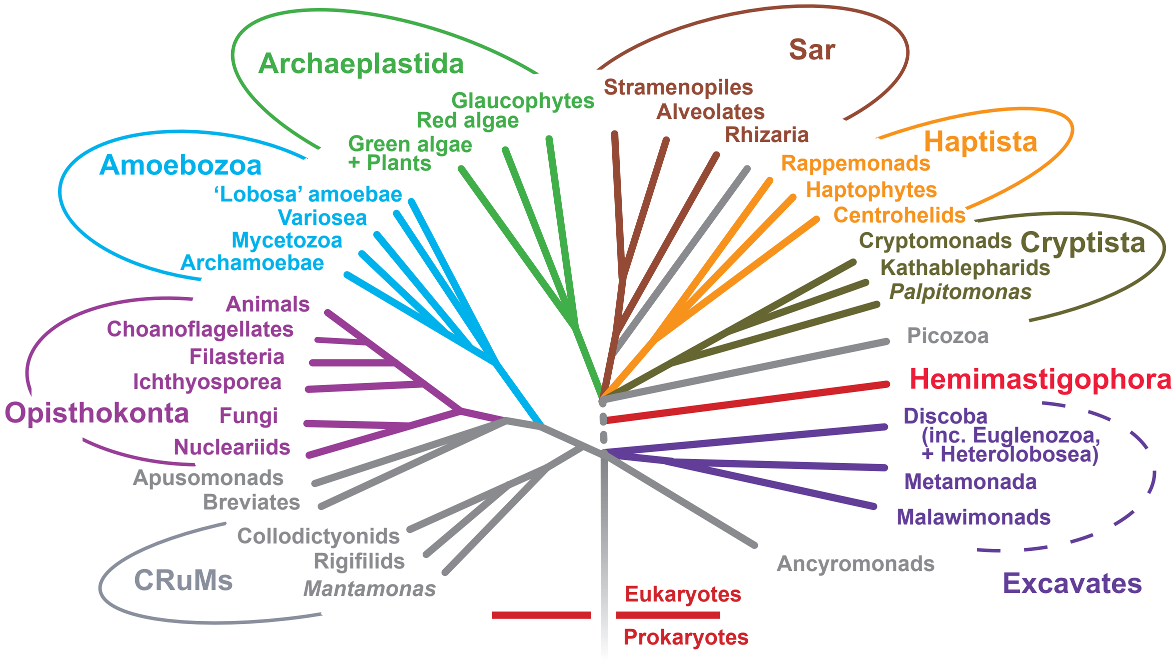


Figure 1.1: Evolutionary tree of eukaryotes, with currently recognized major phylogenetic groups delineated by colour. This tree is based mostly on molecular phylogenetic studies, especially 'phylogenomic' analyses of dozens to hundreds of genes. Some uncertain relationships are indicated by dashed lines. Multifurcations (three or more branches arising at one point) indicate considerable uncertainty about relative branching order. The clades Sar, Haptophyta and Cryptista include the various 'chromalveolate' algae and their heterotrophic relatives (see Part 2, Section CA). Whether 'Excavates' are a single phylogenetic group, or two, is a subject of ongoing controversy (see Part 2, Section E). Groups in grey are small, poorly-known lineages, some which are of uncertain evolutionary affinities. A few such small lineaes are not shown (or not labelled), including *Ancoracysta*, *Microheliella*, *Rhodelphis* and telonemids.

1.2 Organelles and other features of microbial eukaryotes.

Most of our understanding of eukaryotic cell biology is based on the animals, plants and true fungi (e.g. baker's yeast). While these groups collectively illustrate many features of eukaryotic cells, they do not represent the full diversity of structures seen in eukaryotes. For example, most eukaryote lineages actually have tubular or bleb-shaped mitochondrial cristae, rather than the flattened cristae typical of animals, plants and fungi (and a few lineages have discoidal cristae instead). There is also a huge range in the details of mitosis: For example, in many lineages (probably a majority) neither the nuclear envelope nor the nucleolus breaks down during mitosis. In flagellated cells, the most common organisation is actually to have two dissimilar flagella (only one flagellum, or two identical flagella are much more uncommon). There are also entire classes of organelles and structures that are common in microbial eukaryotes, but are not present at all in most animal cells. Figure 1.2 illustrates the general features of a eukaryotic cell, modeled on free-living unicellular eukaryotes, rather than animal or plant cells. The text in Section 1.2 covers certain important features that would not be discussed in depth in a standard cell biology textbook. Note that there are also some comments on plastid structure in Section 1.4, below.

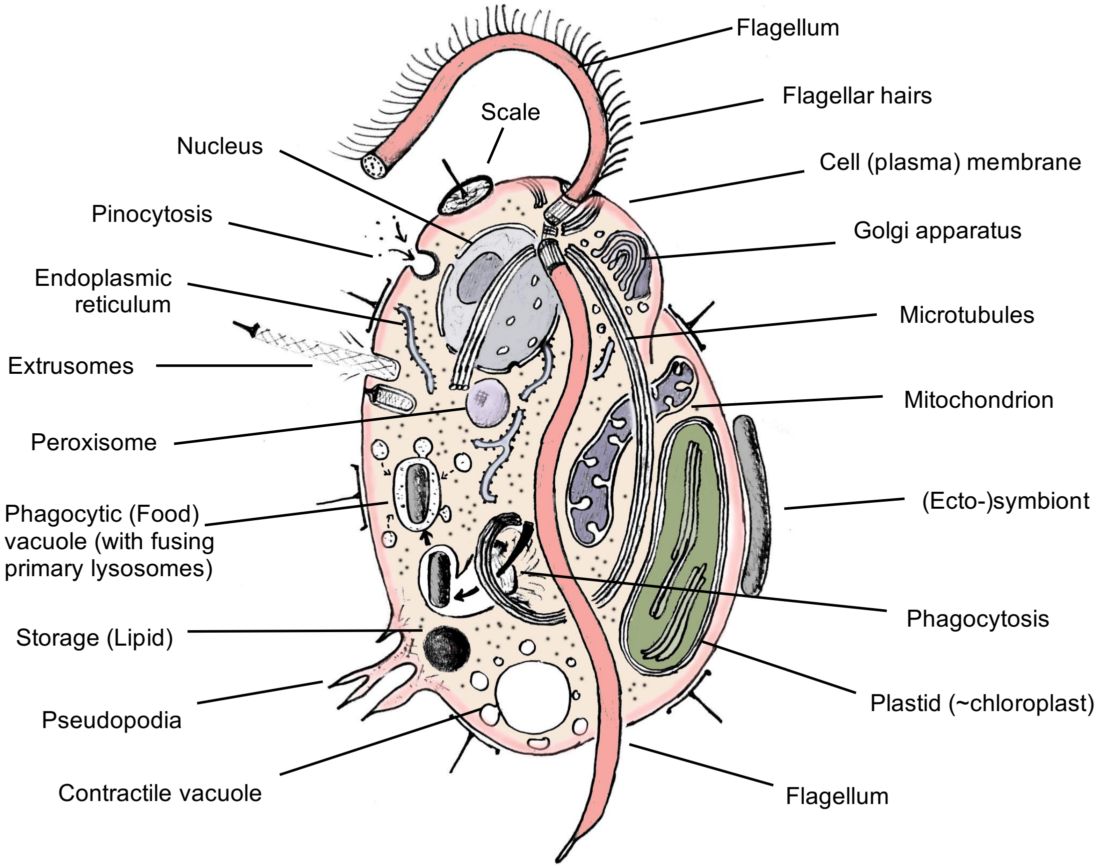


Figure 1.2: Diagram showing major features commonly present in eukaryotic cells, based on various free-living microbial eukaryotes. The diagram does not represent any real species. The cell is depicted engulfing prokaryotic cells by phagocytosis and with a different species of prokaryote as an ectosymbiont (in practice endosymbiotic prokaryotes are probably more common than ectosymbionts). Note also that the cell has two dissimilar flagella - the most common arrangement in microbial eukaryotes. The two flagella emerge from the main body of the cell close together, and are associated with parts of the cytoskeleton, especially microtubular structures. This collection of flagella and connected cytoskeleton is often called the 'flagellar apparatus'.

1.2.1 Extracellular scales, shells, tests and cell walls.

Some unicellular eukaryotes are 'naked', meaning that the cell membrane (= cytoplasmic membrane) is the outer surface that interacts with the environment. Many other eukaryotes, however, have conspicuous extracellular structures. In true fungi and relatives of plants, amongst other groups, the extracellular material forms a continuous close-fitting '**wall**' around the cell (or around the hyphal filament in the case of fungi). In other cells, however, the extracellular material consists of numerous smaller '**scales**'. In still others the cell is contained within a looser-fitting '**shell**', '**test**' or '**lorica**' (plural 'loricae') that includes one or more apertures through which the organism communicates with the environment. In macroalgae there is often a substantial extracellular matrix between adjacent cells that has a major role in determining the structural properties of the organism (as in land plants).

The morphology, composition and biological role of the various extracellular structures varies substantially between groups, and where appropriate, we will discuss these individually in Part 2, when we survey microbial eukaryote diversity. Here are only a few general considerations:

In general, these various extracellular structures are constructed on an organic base, usually composed of complex (and often highly modified) polysaccharides, and/or protein. Two examples of these polysaccharides are cellulose, which is a major component of the cell walls of land plants and their algal relatives, and chitin, which is a major component of the cell walls of fungi. Organic loricae and cell wall precursors are usually secreted from the cell. By contrast, organic scales are generally constructed within the endomembrane system of the cell (primarily the Golgi apparatus), and then exported to the cell surface. Some scales are 'mineralised', that is, chiefly composed of a hard, inorganic material, usually calcium carbonate ($CaCO_3$), or silica (partly hydrated silicon dioxide). In general, this mineral component is also added while the scale is in the endomembrane system (but after it has been released from the Golgi apparatus). This is especially true of silica-containing structures – the silica is laid down within specialised endomembrane compartments called 'silica deposition vesicles'. It is worth noting that some groups of microbial eukaryotes instead have internal mineralized 'skeletons'. The most common mineral for these structures is silica, and again, they are assembled within silica deposition vesicles.

1.2.2 Extrusomes.

'Extrusome' is the general name for an organelle that rapidly extrudes some substance from a microbial eukaryote cell in response to appropriate stimuli. The extruded substance (we shall say 'payload' here) is often a solid structure that expands rapidly when released. In other cases the payload of the extrusome is mucus-like material. In most cases the payload is surrounded by an endomembrane prior to firing, and the extrusome lies immediately below the cell membrane. Firing the extrusome involves fusion of the extrusome membrane and the cell membrane, and is effectively a form of exocytosis. Most extrusomes seem to function either in deterring predation or in capturing prey. Many extrusomes can immobilize or kill other cells (i.e. are toxic), or are adhesive. Other types of extrusomes are used to rapidly coat the cell with a layer that protects against dessication.

Extrusomes are very common in microbial eukaryotes, especially in free-living unicellular forms. There are a bewildering variety of types, and doubtless they have evolved multiple times in evolutionary history (in fact, some unusual extrusomes are actually symbiotic prokaryotes!). The extrusomes of some groups of eukaryotes have a charactistic appearance, and have been useful in helping specialists identify certain evolutionary relationships amongst different microbial eukaryotes.

1.2.3 Contractile vacuoles (water expulsion vesicles).

Cells that live in a hypotonic environment (e.g. freshwater) are subjected to continual osmotic pressure. Water from the external environment tends to migrate into the cell, and unchecked, the cell will swell and could eventually burst (lyse). Two basic coping strategies employed by unicellular eukaryotes are i) having a strong extracellular wall, which exerts mechanical pressure on the expanding cell to counteract osmotic pressure (i.e. essentially the same strategy as that used by most prokaryotes), or ii) having an active system for transporting water back out of the cell. Contractile vacuoles are an active mechanism for expelling water that exploits the endomembrane system of eukaryotic cells. They are present in most freshwater unicellular eukaryotes (interestingly, some freshwater sponges also have contractile vacuoles), as well as certain parasites. Contractile vacuoles also function in excretion of certain ions from the cell (e.g. Ca^{2+}).

There are many kinds of contractile vacuoles. In the simplest forms, water is collected in numerous small endomembrane vesicles or tubules that fuse together to make a single large vacuole, which then merges with the cytoplasmic membrane to expel its contents. This collection and expulsion cycle continues, usually taking a few seconds per cycle in freshwater species. Some cells, such as the large freshwater ciliate *Paramecium*, have more complex contractile vacuoles that include initial collection chambers that the fluid passes through before it is expelled. Often there is a dedicated site of the cell surface for contractile vacuole expulsion, especially in cells where most of the surface is supported by a pellicle, or other rigid structure.

1.2.4 Cell division and reproduction.

Reproduction is the process of making more individuals. As in prokaryotes, the most widespread mode of routine reproduction for unicellular microbial eukaryotes is asexual **binary fission**, where one parent cell divides into two near-identical daughter cells that are genetic copies of the parent. In eukaryotic binary fission, the important organelles of the cell are duplicated and distributed to the two daughter cells. For example, in a 'flagellate' cell with a single nucleus, an early stage in the process leading up to binary fission is the duplication of the flagellar apparatus, which is followed by nuclear division by mitosis. This is necessary because the basal bodies of the flagella in each flagellar apparatus usually also function as the microtubule organising centre for one half of the mitotic spindle. Thus, two copies of the flagellar apparatus are required before mitosis can proceed (Note that the 'centrioles' of animal cells and certain other eukaryotes are homologous to 'basal bodies'). Organelles such as mitochondria and plastids also divide. Finally, actual cell division (cytokinesis) occurs, with one flagellar apparatus and one nucleus going to each daughter cell. Many species without flagella or centrioles also undergo essentially the same process, but use some other type of microtubule-organising centre in the formation of the mitotic spindle.

More complex forms of asexual reproduction and/or development occur in certain groups, mainly through different relative timing of nuclear division and cell division. Some, especially parasitic forms, undergo some type of '**multiple fission**', in which a parent cell rapidly goes through several rounds of mitosis to yield a large multinucleate cell that subsequently divides into numerous daughter cells, each with one nucleus. In other cases, repeated rounds of nuclear division without subsequent cell division results in the growth of large 'cells' with many nuclei (multinucleate cells), rather than in reproduction. Organisms of this type are often described as '**plasmodial**' (especially in parasitic or amoeboid protozoa), or '**syncytial**', or '**coenocytic**' (especially in fungi or algae).

1.2.5 Sexual life cycles.

Sexuality is a widespread feature in eukaryotes, and may be almost universal. In our context, 'sexual' means that genetic information from two parent genomes is combined (in a more-or-less equal admixture), resulting in a novel genome sequence. The parent genomes usually derive from different individuals (though some types show high rates of selfing, or autogamy). Key to sexuality is **meiosis**. Classic meiosis can be thought of as a complex form of nuclear division that reduces ploidy from diploid (two copies of every chromosome), to haploid (one copy of every chromosome), whilst also recombining the two original copies. The 'combination' portion of sexuality then involves two haploid nuclei coming together and fusing to form a new single diploid nucleus. If the haploid nuclei are contained within individual cells, these cells are usually known as **gametes**.

Within this framework, there is a huge variety of ways that eukaryotes incorporate sexuality into the life cycle. Sexuality and reproduction are intrinsically linked in most animals, but this is not the case in many other eukaryotes, especially most microbial forms. Many microbial eukaryotes normally reproduce asexually (e.g. by binary fission) and only very rarely go through a sexual process - hundreds of asexual generations could separate the sexual events. An estimate for one microalgal species suggests that asexual reproduction is a million times more common than sex!

While sexual events are usually associated with reproduction, sex in microbial eukaryotes sometimes involves no reproduction at all. Consider the following scenario as an example: Imagine a diploid unicellular species whose sexual process involved going through meiosis without cell division, then discarding all but one of the haploid nuclei that are produced, resulting in only one cell with a single haploid nucleus. This cell then acts as a gamete: it fuses with another similar haploid cell, and the nuclei fuse, yielding a single daughter cell with a single diploid nucleus. In this scenario, the sexual process has resulted in two parents fusing to form one offspring – this is the opposite of reproduction (i.e. of making more of something).

Furthermore, there is great variation in the relative timing of the event of meiosis (that creates novel haploid genomes), and nuclear fusion (where two haploid genomes are combined to form a diploid genome), leading to a variety of possible life cycles, as discussed in the next few paragraphs.

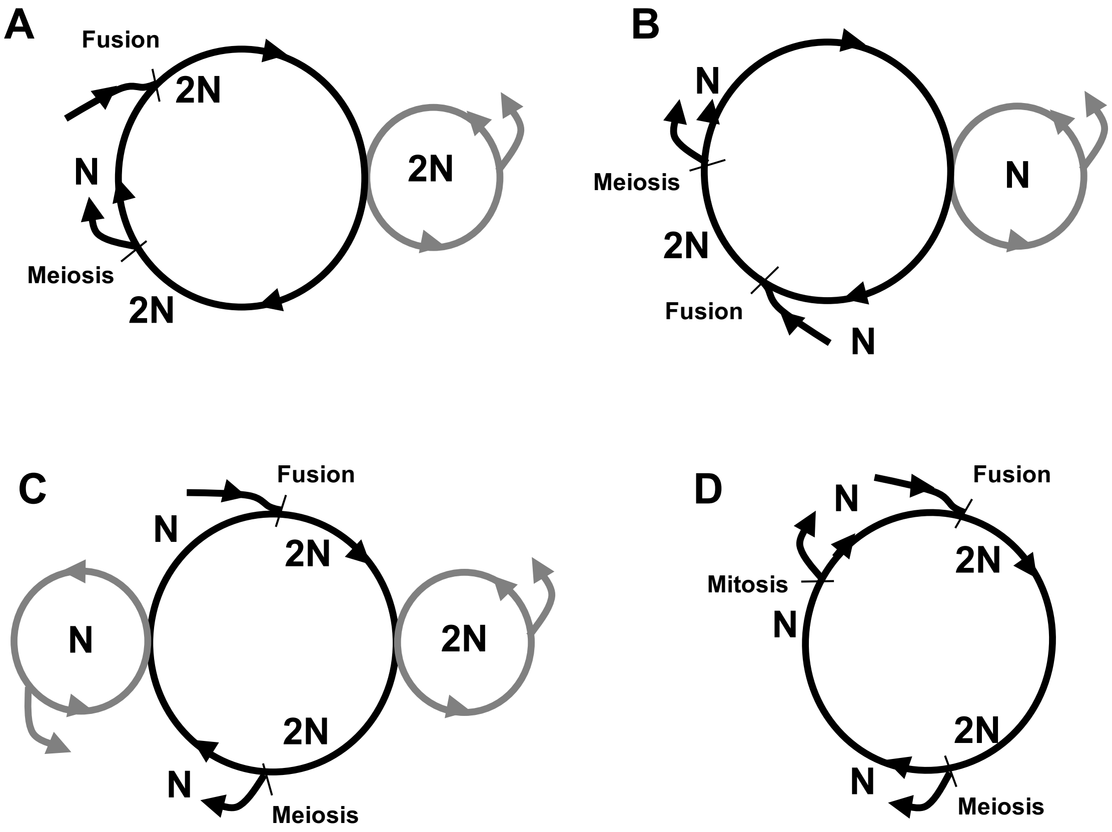


Fig 1.1 Simplified diagrammatic representations of some of the different lifecycles seen in sexual eukaryotes. The large cycles running clockwise represent sexual cycles. The smaller, lighter-coloured circles running anticlockwise represent asexual reproductive cycles (with nuclear division by mitosis). Events of reproduction are shown as bifurcations (note - in many cases more than two progeny would actually result), and gamete fusions are shown by merging lines. **A.** Lifecycle dominated by the diploid phase (diplontic), e.g. diatoms. **B.** Lifecycle dominated by the haploid phase (haplontic), e.g. *Chlamydomonas*. **C.** Life cycle in which both haploid and diploid phases are significant (haplodiplontic). In this case, both the haploid phase and the diploid phase are capable of asexual reproduction (e.g. many haptophytes). **D.** A haplodiplontic lifecycle with strict 'alternation of generations' (e.g. many macroalgae). In the case of a macroalga, the left side of this cycle would represent the 'gametophyte', and the right side would represent the 'sporophyte' (see Part 3).

In some eukaryotes, the main lifecycle phase is diploid. If the organism is capable of asexual reproduction this will involve the production of new diploid nuclei by mitosis, followed by cell division. When the organism undergoes its sexual cycle, meiosis occurs instead, yielding haploid gametes. Gametes then fuse to form a new diploid cell – the zygote (Fig 1.1A). One term for this form of lifecycle is 'diplontic'.

Alternatively, the main life cycle phase can be haploid. Asexual reproduction, if it occurs, involves mitosis to form new haploid nuclei. When the organism enters the sexual cycle, the haploid organism either produces specialised haploid gametes by mitosis, or if the organism is unicellular, existing cells can simply start behaving as gametes. Gametes then fuse to form a zygote, which is the only diploid stage in the lifecycle. The zygote soon undergoes meiosis to produce the new generation of haploid individuals (Fig 1.1B). One term for this form of life cycle is 'haplontic'.

In some eukaryotes both the diploid and haploid phases are significant – a 'haplodiplontic' lifecycle by one terminology. In some cases organisms can go through many rounds of asexual reproduction in either the haploid or diploid state, between occasional events of meiosis (moving from the diploid state to the haploid state) or gamete fusion (going from haploid to diploid - Fig 1.1C). In other cases, especially in many macroalgae, organisms alternate more-or-less strictly between diploid and haploid generations. Mature diploid individuals reproduce by undergoing meiosis and producing many haploid cells, often called 'spores'. Spores then mature into haploid adults. The mature haploid organisms then reproduce by producing numerous haploid gametes by mitosis. Gametes fuse to form diploid zygotes that then mature into the next generation of diploid adults (Fig 1.1D). This life cycle is referred to as an 'alternation of generations', and in macroalgae (and lower land plants!) the diploid and haploid adults are usually referred to as the 'sporophyte' and 'gametophyte' respectively (see Part 3).

1.4 The evolution of eukaryotic cells (and the nature of mitochondria and plastids)

1.4.1 Connections to *Archaea*.

Eukaryotic cells are different from prokaryotes, and in many ways more complex than prokaryotes. How then are eukaryotes and prokaryotes related? Most scientists think that the simpler prokaryotes evolved before the more complex eukaryotes, and that eukaryotes are descended from prokaryotic-like ancestors. This idea is consistent with the fossil record, in which prokaryotes seem to appear earlier than eukaryotes, and with recent discoveries concerning the *Archaea* (see below).

Assuming that eukaryotes did descend from prokaryotes, we can be confident that their closest relatives among living prokaryotes are the *Archaea*. Although having a fundamentally prokaryotic-type cell organisation, *Archaea* differ from other prokaryotes (i.e. *Bacteria*) in numerous features. By contrast, *Archaea* and eukaryotes are similar to each other in several significant ways, particularly in the organization of core life processes such as the transcription and translation apparatuses. Here are just a few examples:

i) The bacterial RNA polymerase complex that transcribes messenger RNA is composed of four subunits, plus a sigma factor, while the main archaeal and eukaryotic RNA polymerases share a very different organization involving at least eight subunits (This is RNA pol II in eukaryotes).

ii) At translation the initiating amino acid in both *Archaea* and eukaryotes is methionine, rather than the modified N-formylmethionine used by *Bacteria*.

iii) There are several ribosomal proteins (proteins that associate with the RNA molecules that make up the functional core of the ribosome, and help to stabilize them) that are shared by *Archaea* and eukaryotes but are not present in *Bacteria*.

The usual interpretation is that these shared features were properties of common ancestors of *Archaea* and eukaryotes after their lineage diverged from *Bacteria*.

In fact, recent phylogenetic studies, and examinations of the genes that are present on the genomes of some atypical *Archaea* indicate that eukaryotes probably evolved from 'within' *Archaea* (i.e. that *Archaea* are a paraphyletic group, and that certain *Archaea* are more closely related to eukaryotes than they are some other *Archaea*). Much of the most important data comes from the 'Asgard archaea', which appear to be the closest relatives of eukaryotes. This is a rapidly moving area of scientific knowledge; most 'Asgard archaea' groups were only discovered within the last five years and until very recently were known only from genomic information extracted from environmental DNA. The first report of successful cultivation of an Asgard archaean was made in mid-2019.

1.4.2 Phagocytosis

Although eukaryotic cells are different in many ways from prokaryotic cells, it is possible that a key innovation that allowed eukaryotic cells to be successful was **phagocytosis**. Phagocytosis allows for the evolution of specialist 'predators' that are substantially larger than their prey – this is a niche that is not really available to prokaryotic cells, it seems. The few *Bacteria* that are considered to be predators (e.g. *Bdellobvibrio*) are usually smaller than their prey, and could almost be considered as parasites. The evolution of phagocytosis is likely to have profoundly altered ecosystems on Earth by allowing the establishment of 'food chains', with several different trophic levels. From the perspective of evolution, phagocytosis doubtless greatly increased the potential for eukaryotic (or proto-eukaryotic) cells to acquire prokaryotic endosymbionts. The endosymbiont that gave rise to plastids was almost certainly originally engulfed via phagocytosis, and it is possible, or even probable, that the mitochondrial symbiosis was also established this way (although there are alternative hypotheses as well).

1.4.3 The endosymbiotic origins of plastids and mitochondria.

'**Endosymbiosis**' refers to a symbiosis when one of the organisms (the symbiont) involved is located inside the cells of the other organism (the host). There is abundant evidence that both **mitochondria** and **plastids** (~ chloroplasts) are of endosymbiotic origin, and were originally bacterial cells. Perhaps the most compelling evidence for this is that both organelles have their own genomes, often in the form of a single chromosome that maps as a circle. This genome contains genes that encode some of the proteins that function in the organelle, which are translated on bacterial-like (70S) ribosomes housed inside the organelle. Some of the ribosomal RNAs for these organellar ribosomes are also encoded on the organellar genome. Evolutionary trees comparing genes from the organelle genomes to the same genes from prokaryote genomes show that plastids are related to *Cyanobacteria*, while mitochondria are related to *Alphaproteobacteria*.

Why do we call mitochondria and plastids 'organelles' and not just 'bacterial symbionts'? A key difference between organelles and symbionts is the degree of dependence on the host genome. The genomes of plastids and mitochondria encode fewer than 10% of the proteins that actually function in the organelle. The remainder – the great majority - are actually encoded on the nuclear genome, are synthesised by cytosolic ribosomes, and these polypeptides must then be imported into the organelle. Some of these imported proteins (though not all, by any means) were originally cyanobacterial or alpha-proteobacterial proteins encoded on the symbiont genome, but the genes that encode them have been transferred to the nuclear genome at some point since the establishment of the endosymbiosis.

1.4.4 Mitochondria early

At the end of the 20th century it was thought that some living eukaryotes had diverged before the acquisition of mitochondria, and that mitochondria had therefore been acquired long after typical features that are present in all eukaryotic groups, such as the nucleus, microtubules, basic endomembrane system, and so on. However, abundant evidence now shows that the mitochondrion was acquired early in eukaryotic evolution, and that all living eukaryotes are descended from a common ancestor that already had a mitochondrion. Any mitochondrion-lacking lineages that diverged prior to the acquisition of mitochondria do not appear to have left any living descendants.

1.4.5 Plastids - primary and secondary endosymbiosis

Plastids were acquired much later than mitochondria and are restricted to some particular groups of eukaryotes. The history of plastids is quite complex. The original acquisition of plastids involved a symbiosis between a eukaryote and a cyanobacterium that eventually evolved into a plastid (Fig. 1.4). This process is called '**primary endosymbiosis**', and it resulted in an organism called a '**primary alga'**. The plastids in a primary alga are surrounded by two membranes, as in mitochondria. In both mitochondria and plastids these two membranes correspond to the cell membrane and outer membrane of the original bacterium – *Cyanobacteria* and *Alphaproteobacteria* are both Gram-negatives.

'**Secondary endosymbiosis**' describes the case where a primary alga becomes an endosymbiont of another eukaryote, and over time is transformed into an organelle, mostly by a process of evolutionary reduction (Fig. 1.5). The resulting organism is called a '**secondary alga**'. The endosymbiosis was probably first established through phagocytosis, and the primary alga remains surrounded by a host endomembrane. The primary alga is also still surrounded by its own cell membrane. Over time, as the symbiosis became permanent and the symbiont became dependent on the host, many components of the symbiont were rendered unnecessary and were lost. The symbiont's cytoplasm became reduced and organelles such as mitochondria could be entirely lost. Unnecessary genes in the symbiont's nuclear genome were lost altogether, while essential genes were gradually transferred to the host's nuclear genome. Eventually, almost every structure in the symbiont other than the plastid itself is lost (but see below), leaving the plastid sitting inside what used to be the symbiont's cell membrane (this membrane is sometimes called the 'periplastid membrane' in such organelles) and the host endomembrane. Since the plastid in the primary algal symbiont was already surrounded by two membranes, this process resulted in an organelle surrounded by four distinct membranes. Sometimes the symbiont cell membrane was lost too, leaving three membranes in total. Such plastids with more than two bounding membranes are sometimes called '**complex plastids'**.

Did secondary symbiosis really happen? Some of the most compelling evidence that it actually occurred comes from two relatively obscure groups of algae in which the process of evolutionary reduction of the symbiont has not progressed to completion. These groups are chlorarachniophytes (a type of rhizarian), and cryptophytes. Both have plastids that are surrounded by four membranes, however, between the inner two and outer two membranes they also have a tiny region of 'cytoplasm' and a membrane-bounded structure called a '**nucleomorph**'. The nucleomorph contains small linear chromosomes encoding a few hundred genes. The nucleomorph is, in fact, the remains of the primary algal symbiont's nucleus (see Figure 1.5C; also Part 2, Figure CA1).

1.4.5.1 Plastid acquisition in eukaryote evolution

It is thought that just **one event of primary endosymbiosis gave rise to the main groups of primary algae** alive today. As plastids still possess a genome, plastid genes can be compared to those from the genomes of cyanobacteria using the same analysis techniques used to compare different prokaryotes. Evolutionary trees of plastid/cyanobacterial genes, and other comparative data indicate that all plastids are more closely related to each other than to any particular group of *Cyanobacteria* – this suggests that they that they all descend ultimately from the one cyanobacterium that became a plastid.

Afterwards, there were **at least three separate events of secondary endosymbiosis**, where different primary algae were acquired as symbionts by different previously non-photosynthetic hosts. This 'one primary, but multiple secondaries' model of plastid evolution explains why algae appear in several separate places on the evolutionary tree of eukaryotes – in essence, secondary endosymbiosis allowed the complex trait of photosynthesis to 'jump' from one group to another, distantly related group. To make matters more complicated, there are also some instances where plastids have been acquired when a secondary alga became an endosymbiont within another eukaryotic cell, and was reduced to the status of an organelle. These events are called **'tertiary endosymbioses'**. In fact some of the organisms that are normally regarded as having secondary plastids may well have actually acquired them through tertiary endosymbiosis (see Part 2, CA1).

In the early 2000s it was confirmed that at least one other primary endosymbiosis has occurred: A rhizarian amoeba, *Paulinella chromatophora,* has a photosynthetic organelle that is clearly derived from a different cyanobacterial ancestor than other plastids (see Part 2, Section R3). This endosymbiosis was established relatively recently (likely within the last 100 million years, whereas the Archaeplastida primary endosymbiosis likely dates to over 1 billion years ago – see 1.4.6), and the organelle's genome (for example) is much less reduced than in 'classic' plastids.

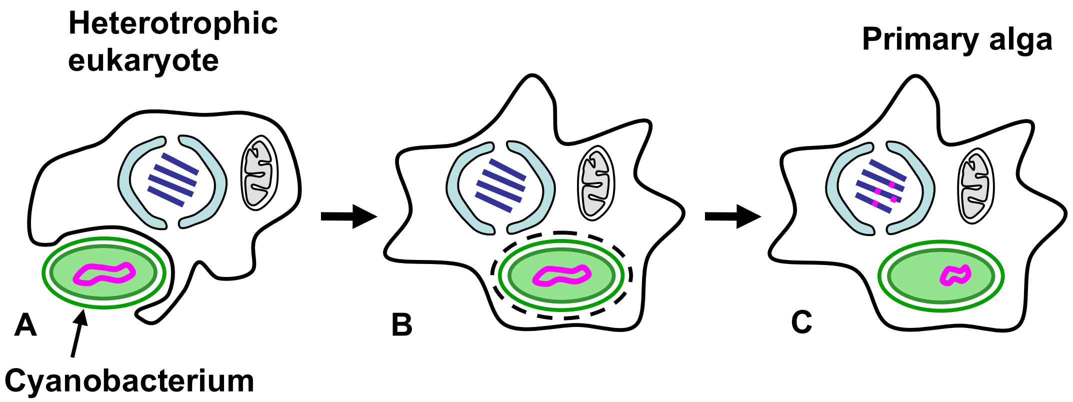


Figure 1.4. Primary endosymbiosis. A heterotrophic eukaryote phagocytoses a cyanobacterium (A), which becomes an endosymbiont. At some point (probably early in the endosymbiosis) the host phagosomal membrane is lost, leaving the endosymbiont surrounded by two membranes, both of symbiont origin (B). Over evolutionary time the endosymbiont becomes dependent on the host, in part because most of the genes encoding proteins that function in the endosymbiont now reside in the host nucleus and not the (highly reduced) endosymbiont genome (C). At some point the endosymbiont is classifiable as an organelle, and the whole organism is called a 'primary alga'.

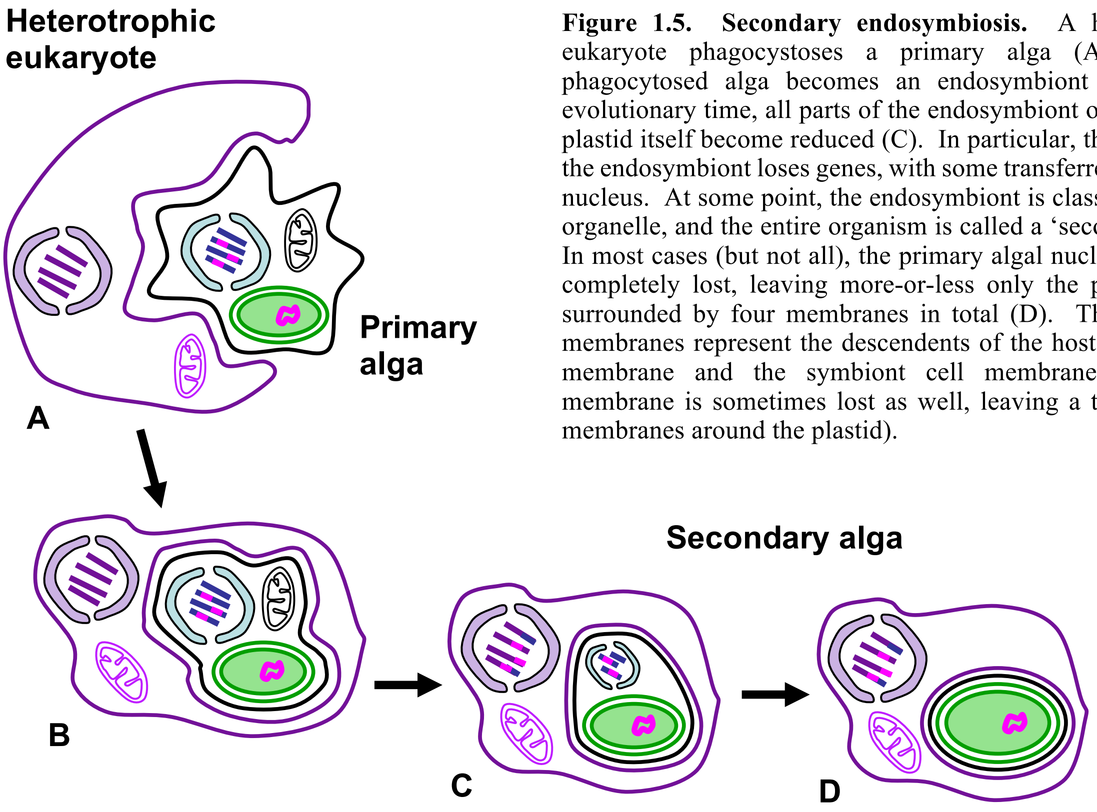


Figure 1.5. Secondary endosymbiosis. A heterotrophic eukaryote phagocystoses a primary alga (A), and the phagocytosed alga becomes an endosymbiont (B). Over evolutionary time, all parts of the endosymbiont other than the plastid itself become reduced (C). In particular, the nucleus of the endosymbiont loses genes, with some transferred to the host nucleus. At some point, the endosymbiont is classifiable as an organelle, and the entire organism is called a 'secondary alga'. In most cases (but not all), the primary algal nucleus has been completely lost, leaving more-or-less only the plastid itself, surrounded by four membranes in total (D). The outer two membranes represent the descendents of the host phagosomal membrane and the symbiont cell membrane (the latter membrane is sometimes lost as well, leaving a total of three membranes around the plastid).

1.4.6 The age of eukaryotes from the fossil record.

There is considerable uncertainty about when eukaryotic cells evolved. Most microbial eukaryotes leave poor fossils at best, and the early fossil record is sparse and/or difficult to interpret. Further, complementary methods such as examining ancient sedimentary rocks for chemical signatures of eukaryotes (biomarkers), and comparing gene sequences from living eukaryotes in order to date ancient evolutionary events (‘molecular clock’ analyses), are quite controversial for various reasons.

The earth has been habitable for about 3.9 billion years. Prokaryotes seem to have appeared at least 2.7 billion years ago – It is from around this time that convincing fossils of prokaryotes and prokaryote biomarkers appear in the geological record, and that oxygen begins to accumulate in the atmosphere (suggesting the presence of oxygenic photosynthesis, as performed by *Cyanobacteria*).

The first fossils *widely* thought to represent possible eukaryotes date from roughly 1.8-2.1 billion years ago. The most compelling of these are filamentous organisms a few millimetres wide that are assigned to the taxon ‘*Grypania’*. These fossils are thought to be eukaryotes almost entirely on the basis of their size – no living prokaryotes are known that are this large. Recently, other larger-than-modern-prokaryotes cellular filaments with putative organelles have been reported from ~1.6. billion year old rocks from India. Further, structures that generally resemple the sheetlike thalli of certain eukaryotic macroalgae (see Part 3) have been reported from ~1.6 billion year old deposits in China. The largest sheets were >20 cm long by several cm wide, and composed of cells about 10 micrometres across, all more consistent with eukaryotic algae (of some kind) than any known type of prokaryote. Other fossils dating from around this time include the earliest ‘acanthomorphic acritarchs’, which are various forms of usually rounded structures that are tens of micrometres in diameter and have projections or spines extending from them. It is suggested that the formation of such projections requires a eukaryote-like complex cytoskeleton, and thus that acanthomorphic acritarchs are the fossil remains of eukaryote life, perhaps cysts or other resistant life history stages.

Another key fossil is a single collection of filamentous structures called *Bangiomorpha*. The *Bangiomorpha* find is now dated at somewhat less than 1.1 billion years old. It represents the earliest fossil that can be placed with confidence within a known group of eukaryotes - *Bangiomorpha* is very similar to some living red algae (Rhodophyta - discussed later in Part 3). *Bangiomorpha* is followed in the geological record by several other fossils that can be equated with living groups. The most clearly identified of these are the ‘vase-shaped microfossils’, or ‘VSM’s. VSMs appear in several deposits dated at 700-800 million years old but many are very similar to the tests (shells) of living lobose testate amoebae – a group of single-celled heterotrophic eukaryotes. This date is only somewhat older than the earliest unambiguous fossils of animals (550+ million years old). Thus, while there is some evidence that eukaryotes may have existed two billion years ago, most modern groups have fossil records that stretch back less than one billion years, often much less (if they even form fossils in the first place).

Clearly more fossil discoveries will be needed before we have reasonable clarity about how old eukaryotes really are, and how long ago it was when the living eukaryote groups actually diverged. Until then we can expect that individual new discoveries will sharply and suddenly alter the landscape of knowledge; for example, in 2017 some multicellular fossils were carefully analysed following the geological formation they are embedded in being redated to 1.6 billion years age. A good case was made they represent florideophycean red algae; If this dating and identification are both correct, it would push the origin of the red algae some 500 million years earlier than *Bangiomorpha*.

PART 2

PART 2, SECTION A: ARCHAEPLASTIDA

A1. Archaeplastida and the history of plastids

Plastids are derived from endosymbiotic prokaryotes, specifically from a subgroup of *Cyanobacteria*. 'Primary endosymbiosis' describes the case where a plastid originates from a symbiosis between a eukaryote and a prokaryote, resulting in a 'primary alga' with a plastid surrounded by two membranes. This is distinct from secondary endosymbiosis, where a eukaryote acquires a primary alga (i.e. another eukaryote) as a symbiont (see Part 1, Section 1.4).

Secondary endosymbiosis has occurred several times, so the living 'secondary algae' are not all closely related. By contrast, it appears that all major groups of living primary algae are descended from a single event of primary endosymbiosis, that is, they derive from a common primary algal ancestor (see Part 1, Section 1.4.5). Here we call this supergroup 'Archaeplastida'.

Archaeplastida includes three very different groups of photosynthetic organisms - **Chloroplastida**, **Rhodophyta** and **Glaucophyta**. Chloroplastida in turn includes the familiar land plants (Embryophyta) plus a wide diversity of macroscopic and microscopic species that are informally called the 'green algae'. Rhodophyta, or 'red algae' are mostly macroalgae, and are most common in marine environments. Glaucophytes are a very small group of freshwater algae of particular evolutionary interest. Other than having a primary plastid, there is little morphological similarity between these three groups, although most have flat mitochondrial cristae (tubular cristae are more common overall across eukaryotes) and few are now capable of phagocytosis (though recent studies confirm that some prasinophyte green algae can phagocytose bacteria). By contrast, *many* secondary algae have retained the ability to consume prey by phagocytosis (see later sections of these notes).

Why is it thought that there was a single event of primary endosymbiosis? As plastids still possess a genome, plastid genes can be compared to those from the genomes of cyanobacteria using molecular phylogenetics and other techniques. If there had been several primary endosymbioses, it would be most likely that the plastids of different groups of eukaryotes would be most closely related to different groups of cyanobacteria, and not to each other. But this is not what is seen - phylogenetic trees of plastid/cyanobacterial genes, and studies of gene order on plastid and cyanobacterial chromosomes, amongst other evidence, indicate that all 'classic' plastids are more closely related to each other than to any particular group of *Cyanobacteria* – this suggests that they all descend from the one cyanobacterium that became a plastid. Another line of evidence concerns the protein import machinery of plastids. Most of the proteins that function in plastids are actually encoded by genes on the nuclear genome, not the plastid genome. These polypeptides are synthesized on cytosolic ribosomes, and are then imported into the plastid, mostly through a complex translocon system within the plastid membranes. Such a system would not be needed by an autonomous cyanobacterium, and thus presumably originated after endosymbiosis. Chloroplastida, Rhodophyta and Glaucophyta all use a clearly homologous translocon system (the 'Tic-Toc' machinery), which suggests strongly that the most recent common ancestor of their plastids was already an endosymbiotic organelle. In addition (and going back to the eukaryotic host), sophisticated phyogenetic studies of nucleus-encoded genes suggest that Chloroplastida, Rhodophyta and Glaucophyta are closely related to each other, if not necessarily each others very closest sister taxa (see A7 and Section CA, below).

A2. A note about names:

Several competing schemes have been suggested for the formal taxon names of various groups of primary algae. Much of the debate is fueled by the semantic question "What is a plant?". Some protistologists consider a 'plant' to be any photosynthetic organism that is directly related to the familiar land plants. They often refer to all primary algae as the "kingdom Plantae", refer to 'land plants plus green algae' as

“Viridiplantae” and refer to land plants as “Embryophyta”. By contrast many botanists equate the taxon Plantae with ‘land plants’ (embryophytes) alone. Still others have referred to ‘land plants plus green algae’ as ‘Plantae’. In other words, there are at least three different groups nested inside one another that different scientists and textbooks will label with the name “Plantae”.

The terminology we use in this course is a compromise suggested by a committee including both protistologists and phycologists (i.e. scientists who specifically study algae). You would never be tested on the different terminologies concerning ‘plants’ – This note is merely to help if you get confused looking at a text, and you cannot find ‘Archaeplastida’ or ‘Chloroplastida’, or you can’t understand why some texts consider green algae part of “Plantae” while others do not.

A3. Plastid organisation within Archaeplastida

The plastids that are most familiar to most biologists are the chloroplasts of the land plants, which belong to Chloroplastida. In fact, the other groups of Chloroplastida have plastids that are similar (but not identical) to those of land plants. These plastids are rather different from those of their extinct ancestors, which presumably resembled typical cyanobacteria. Most cyanobacteria have one main chlorophyll - chlorophyll *a* - and have unstacked thylakoids that bear ‘phycobilisomes’ - assemblies that contain phycobilin accessory pigments which assist in photon absorbtion and that transmit this energy to the thylakoid-embedded photosystems. By contrast, the plastids of Chloroplastida contain two chlorophylls - chlorophyll *a* and chlorophyll *b*, and the thylakoid membranes lack phycobilisomes. The absence of phycobilins (which absorb green-yellow wavelengths of light), together with modest levels of other green-absorbing accessory pigments (e.g. carotenoids) in most chloroplastidan species, gives rise to the grass-green colour typical of chloroplastidans (chloro- = yellow-green). Furthermore, the thylakoids are frequently organised into stacks. These stacks are many layers thick in land plants and some green algae, and are then called ‘grana’.

In contrast to Chloroplastida, both rhodophytes and glaucophytes have plastids that more closely resemble cyanobacteria, at least superficially. Both groups lack chlorophyll *b*. Their thylakoid membranes retain cyanobacterial-like phycobilisomes and are separated rather than stacked (see Appendix II). The phycobilisomes of rhodophytes often contain large amounts of the phycobillin phycoerythrin, leading to the red colour typical of rhodophytes (rhodo- = red). Glaucophyte plastids are often blue-green, similar to the colour of many cyanobacteria, again reflecting the presence of different (longer wavelength-absorbing) cyanobacterial-like phycobilins in addition to chlorophyll *a*. Another interesting feature of glaucophyte plastids is that they retain a thin bacterial-like peptidoglycan wall between the inner and outer plastid membranes, which can be observed by electron microscopy (for this reason the glaucophyte plastids are sometimes called ‘muroplasts’, from the Latin for ‘wall’). This peptidoglycan layer has been lost in most other plastids (and mitochondria), although various pharmacological and genomic evidence indicates that basal plant lineages and perhaps some streptophyte green algae (see Figure A1) may have also retained some form of peptidoglycan surrounding the plastid (and such a layer has been labelled in the moss *Physcomitrella*). The retention of these ancestral features makes it tempting to suppose that glaucophytes might be the earliest diverging of the three main lineages of Archaeplastida – in reality, however, the branching order amongst them is not resolved consistently in phylogenetic studies.

Despite their retaining different levels of cyanobacterial morphology, it is worth emphasising that the plastids of red algae and glaucophytes are similar to those of Chloroplastida in being highly dependent on the eukaryote nucleus: In all three lineages it is estimated that >90% of the proteins that function in the plastid are actually encoded by nuclear genes, rather than genes remaining on the plastid genome (see Part 1, Section 1.4.5).

A4. Glaucophyta

Glaucophytes (also known as Glaucocystophytes) are a very small group – only ~15 species have been formally described so far. They are either unicellular or form small colonies. There is often a cell wall made of polysaccharide. Some species (*Cyanophora* spp.) are free-swimming flagellates; some others produce flagellated dispersal stages. Apart from their key evolutionary position, discussed above (Section A3), they are very obscure. They are found only in freshwater, often on the surfaces of aquatic plants or other (larger) algae. They appear to be rare in the environment.

A5. Rhodophyta

The great majority of rhodophytes are macroalgae, so they have been covered in the macroalgal section of these notes (See Part 3, Sections 'M' and 'RD').

A6. Chloroplastida

Chloroplastida are the most diverse of the archaeplastid groups. Most of their diversity at the level of major lineages is made up by the various groups collectively called 'green algae'. 'Green algae' range from some of the smallest unicellular eukaryotes known (e.g. the flagellate *Micromonas* and the coccoid *Ostreococcus*) to complex multicellular macroalgae (e.g. Charales). There are several thousand species, and they are important in both marine and freshwater environments, and even in terrestrial habitats. Land plants are descended from within the 'green algae', meaning that land plants are also part of the Chloroplastida group. We will briefly discuss the origin of land plants later (Part 3, Section CM), but otherwise concern ourselves with the algal lineages.

Chloroplastidan cells are usually surrounded by an external cell wall or extracellular matrix, or by an arrangement of numerous scales. The flagella are also covered in scales in many groups. Both walls and scales are composed largely of polysaccharides, such as cellulose. Scales are produced within the endomembrane system and exported to the cell surface. By contrast, the cellulose fibres that typically form an important component of the classic cell wall are synthesized at the cell surface by protein complexes that are embedded in the cell membrane (though there are also taxa where a continuous cell wall forms through the fusion of separately synthesized scales).

Many groups of Chloroplastida have flagella, at least for part of the life cycle. Most taxa are 'isokont', meaning that the flagella on a cell are almost identical in morphology and behavior. There are usually two or four flagella, which usually exhibit an identical oar-like (i.e. 'ciliary') beat pattern when propelling the cell. In flagellated cells there is usually a pigmented 'eyespot' that interacts with the flagella to permit phototaxis (orientation to the light during locomotion). This eyespot is actually located within a lobe of the plastid. Probably all eukaryotes produce polysaccharide deposits as an energy store, and in the case of Chloroplastida, this store is primarily in the form of starch. Unusually this storage product is manufactured and accumulated in the plastid, rather than in the cytoplasm, as in other primary algae (and most secondary algae).

A6.1 Chloroplastid diversity.

Studies of cell organisation and molecular phylogenies indicate that there are two main groups within Chloroplastida. These are "**Chlorophyta**" and "**Streptophyta**". Chlorophyta in turn includes Chlorophyceae, Trebuxiophceae and Ulvophyceae, plus many or all of a series of lineages collectively called 'prasinophytes'. Streptophyta includes Zygnematales, Coleochaetales, Charales and the embryophytes, or land plants (there are some additional small groups that we will not consider further). The evolutionary tree of Chloroplastida is summarised in Figure A1.

Many of the subgroups of Chloroplastida are largely or entirely considered as 'microalgae' – that is, they are unicellular or colonial. Others are mostly 'macroalgae'. We will discuss groups dominated by microalgae here, and then return to the macroalgal forms in Part 3 (Section MC).

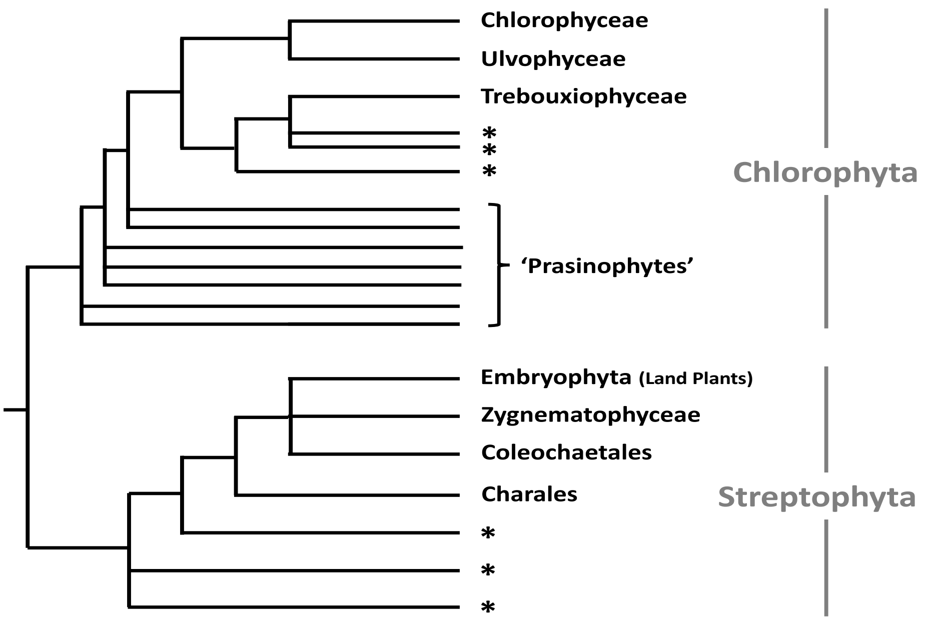


Figure A1. Evolutionary tree of Chloroplastida. Chloroplastida ('green algae + land plants') is divided into two basic monophyletic groups (clades): Chlorophyta and Streptophyta (AKA Streptophytina). Ulvophyceae and Charales are predominantly macroalgal groups that are covered later in Part 3, Section 'CM'. Note the difference between Chloro<u>phyta</u> and Chloro<u>phyceae</u> (Chlorophyceae is one subgroup of Chlorophyta). 'Prasinophytes' are clearly a diverse paraphyletic group within Chlorophyta, but shown somewhat conceptually here. Land plants are one of several subgroups inside Streptophyta. The closest relatives of land plants are Charales, Coleochaetales and Zygnematophyceae. Although Charales is the most complex and superficially plant-like of the three (see Part 3, Section CM3), most recent molecular phylogenetic evidence incidates that Zygnematophyceae are the very closest relatives of land plants. Asterisks (*) indicate minor taxa of streptophytes that are not discussed in detail here. Note that some texts use the taxon name 'Charophyta' (charophyte algae) instead of using 'Streptophyta'. The two names are <u>not</u> synonyms: 'Charophyta' includes the green algae within Streptophyta but <u>not</u> the land plants, and thus represents a paraphyletic group.

A6.2 'Prasinophytes'

'Prasinophytes' are a collection of microalgae within Chloroplastida. They are not all specifically related to each other – they represent a paraphyletic series of early branching lineages within Chlorophyta. It is believed that ancestral chloroplastidans resembled modern day prasinophytes.

Most prasinophytes are marine organisms. They are generally small, unicellular flagellates, typically 2-20 μm in length. Most have 1-4 flagella, occasionally more. The flagella often insert in a depression at the anterior end of the cell (i.e. the cell is vaguely apple-shaped, the stalk representing the placement of the flagella). In most, the cell body and flagella are covered with organic scales. There can be several different types of scales on one cell. A few instead have cell walls, like most other members of Chloroplastida. There is a single plastid per cell. Sexuality is not well documented in prasinophytes.

Some prasinophytes produce c ells without flagella under certain environmental conditions. In some cases the aflagellate forms grow to very large size (>200 μm in some cases) and have very thick walls. These forms are called 'phycomata' (singular, 'phycoma').

Prasinophytes, especially the smallest forms, are an important component of the eukaryotic phytoplankton (photosynthetic plankton) in marine habitats. Very small prasinophytes such as *Micromonas* and *Ostreococcus* can be the dominant photosynthesisers in certain oligotrophic marine waters, making up more of the biomass, and fixing more carbon, than any other type of algae. They can even exceed in importance the cyanobacteria that dominate the phytoplankton in much of the open ocean.

Interestingly, a few particular prasinophytes seem to be able to phagocytose prokaryotes, and perhaps very small eukaryotes, in addition to performing photosynthesis. This ability is not documented in other green algae, nor in red algae or glaucophytes, whereas many secondary algae are phagocytic.

Examples: *Micromonas*, *Nephroselmis, Ostreococcus*.

A6.3 Chlorophyceae

Chlorophyceae are probably the best-known subgroup within Chlorophyta. Chlorophyceae are predominantly freshwater organisms (they can dominate the algal component of freshwater environments) and they range from unicellular flagellates through to complex colonies and a few multicellular forms. Most chlorophyceae have one plastid. Flagellate forms generally have two flagella. There is usually a cell wall.

One iconic chlorophycean is *Chlamydomonas reinhardtii*. This small unicellular flagellate is a ‘model organism’, particularly for cell biological studies of eukaryotic flagella. *Chlamydomonas reinhardtii* is normally haploid and reproduces asexually. However, cultures of opposite ‘mating types’ can be induced to mate, whereby two haploid cells pair up at their anterior ends, then fuse to form a diploid zygote, which encysts and undergoes meiosis to produce new haploid cells. Its haploid nature throughout most of the lifecycle is useful for studies of the phenotypes of mutants. *Chlamydomonas* and similar flagellates (e.g. *Dunaliella*) are common in nature. Some species of *Dunaliella* are amongst the most halophillic (salt-loving) eukaryotes known and can grow even in nearly saturated brine (around 30% w/v salt - seawater is ~3.5% w/v salt).

A6.3.1 Colonial Chlorophyceae. A wide diversity of colony forms is seen in the group, with colonies differing markedly in both morphology, and complexity. Some species produce small colonies with a defined number of cells. *Scenedesmus* and *Desmodesmus*, for example, are common freshwater forms, most species of which form linear colonies of exactly four non-flagellated cells. In these, and many other colonial Chlorophyceae, asexual reproduction involves the production of new colonies from each parental cell by repeated cell division inside the parental cell wall, such that the daughter colonies emerge with the same number of cells as the parent. This form of division is called ‘autocolony formation’. Not surprisingly, the number of individual cells in a colony is almost always from the binary series (2, 4, 8, 16, etc).

Hydrodictyon, the ‘water net’, is a floating colony of numerous elongate cells that each connect to several other cells, thus forming a three-dimensional meshwork. Under appropriate conditions the organism grows rapidly due to the cells within the colony growing to a large size without undergoing cell division. Some Chlorophyceae form filaments or branching filaments, sometimes with specialist holdfast structures to attach themselves to substrates.

A series of chlorophyceans form motile colonies of flagellated cells. Simple forms include a few identical cells (e.g. eight) arranged as a ball. In large colonies, cells inside the colony may not be flagellated (e.g. some species of *Gonium*). The most complex form is *Volvox*, whose colonies contain hundreds or thousands of *Chlamydomonas*-like cells embedded into the surface of a hollow ball of mucilage. There is some differentiation into distinct cell types - A few of the cells, ‘gonidia’ (sing. ‘gonidium’), are larger and non-flagellated cells that are involved in reproduction. Through repeated mitotic cell divisions a gonidium initiates the production of a daughter colony that grows inside the parental sphere. Several daughter colonies are produced at once and are eventually released by rupturing the parental colony surface. There is also a sexual reproductive cycle.

A6.4 Trebouxiophyceae

Trebouxiophyceae are closely related to Chlorophyceae, and include a collection of small algae that lack flagella for most or all of their life cycle. Some are free-living, often living in soils. Other trebouxiophyceans are the algal components (phycobionts) of some lichens (lichens are symbiotic associations between fungi and either algae or cyanobacteria). Other trebouxiophyceans are actually parasites. *Helicosporidium* is an obligate parasite of insects, while *Prototheca* is a soil organism that acts as a facultative parasite in animals including dairy cows and humans. Both are non-photosynthetic but are known or believed to have retained a reduced plastid.

A6.5 Zygnematophyceae

This group of unicellular or filamentous algae is part of Streptophyta (not Chlorophyta), and is closely related to embryophytes (along with Charales and Coleochaetales – see Part 3, Section CM3). Organisms belonging to Zygnematophyceae do not have flagella and are essentially restricted to freshwater.

Filamentous zygnematophyceans are commonly observed algae (they are readily visible to the naked eye), and best display the characteristic conjugative sexuality of the group. The vegetative stage is haploid. During sex, two filaments become aligned and the cell walls of adjacent cells in different filaments are modified to form a connecting tube. The aligned cells, now functioning as gametes, fuse to form a zygote (either one mating type migrates to the other filament, or the two gametes meet in the connecting tube). The zygote forms a resistant wall, and is eventually released from the parental filament walls. It then undergoes meiosis, and one meiotic product survives to germinate and form a new haploid filament. The common filamentous freshwater algae *Spirogyra* and *Mougeotia* are well-known filamentous forms.

The best-known unicellular members of Zygnematophyceae are the placoderm **desmids** (Desmidiales). They are common, distinctive and often relatively large cells, with well-defined morphologies determined by the thick cell wall. The cell is divided into two symmetrical ‘semicells’, with the nucleus sitting in the centre. During normal division, one parental semicell wall is donated to each daughter cell, and the wall of what will become the second semicell is synthesized de novo. Cells range in shape from simple straight or curved rods or ribbons (sometimes superficially resembling pennate diatoms - see Part 2, Section S2.3, below; e.g. *Closterium*), to bi-lobed forms often with the cell wall drawn out elaborate projections (e.g, *Staurastrum* with projections, *Cosmarium* without), to flat forms with sculpted margins (e.g. *Micrasterias*). 3000 or more species have been distinguished.

A7 Other members of the Archaeplastida

In 2019 a new group of free-living heterotrophic flagellates, *Rhodelphis*, was discovered and cultivated. Remarkably, phylogenetic analyses based on large numbers of gene sequences (‘phylogenomics’) established that *Rhodelphis* is a sistergroup to red algae, and thus presumably a member of the Archaeplastida grouping. Although *Rhodelphis* is a heterotroph that consumes other microbes, gene sequence data strongly suggests that it possesses a non-photosynthetic primary plastid, presumably inherited from the primary endosymbiosis in the common ancestor of all Archaeplastida (see A1, above).

PART 2, SECTION CA: THE 'CHROMALVEOLATE' OR 'CHROMIST' LINEAGES: INTRODUCTION, CRYPTOPHYTES, AND HAPTOPHYTES.

CA1 Introduction

The 'chromalveolates' or 'chromists' are general names given to several major groups of eukaryotes, each of which includes algae with plastids that contain chlorophyll *c* in addition to chlorophyll *a.* These groups are Cryptomonada, Haptophyta, Stramenopiles and Alveolata (note: many members of the latter two groups do not have plastids). The plastids of all four of these groups have 3 or 4 bounding membranes and are generally thought to have originated through secondary endosymbiosis. All these plastids are of red algal origin, in contrast to the photosynthetic euglenids and chlorarachniophytes, whose secondary plastids are of green algal origin (see Sections R6 and E8.1).

The evolutionary history of chromalveolates/chromists is the subject of ongoing debate. One hypothesis holds that the plastids of all chromalveolates are the result of a single event of secondary endosymbiosis in a common ancestor (followed by extensive secondary loss of plastids in the various non-photosynthetic groups). The opposing view, currently favoured by most researchers in the field, is that the distribution of plastids amongst chromalveolates is the result of multiple endosymbiotic events – either multiple secondary endosymbioses, or one or more 'tertiary endosymbioses' (see 1.4.5.1), or a combination (and in all cases, rather fewer events of secondary loss of plastids). The first of these hypotheses would be most plausible if the chromalveolate lineages were closely related to one another. At present there is strong phylogenetic evidence that Stramenopiles and Alveolata are closely related, though they are also related to another major group called Rhizaria (Section R; note that no Rhizaria have plastids of red algal origin). This group is referred to as the 'SAR clade' or just 'Sar'. However, it is far from clear whether Sar is closely related to Haptophyta and, especially Cryptophyta. In fact, Cryptophyta usually shows a closer phylogenetic affinity with Archaeplastida (see Section A) than with Sar. Despite the controversy it is convenient to introduce the chromalveolate lineages together, and this is the expedient we will follow here:

CA2 Chromalveolate-type plastids

In secondary endosymbiosis the outer membrane surrounding the plastid was originally the membrane of a phagocytic vacuole (i.e. an endomembrane), and in a way, is still part of the endomembrane system. This affinity is particularly clear in three chromalveolate groups – Cryptophyta, Haptophyta and Stramenopiles. In these groups, the outermost membrane of the four surrounding the plastid closely resembles endoplasmic reticulum, including having attached ribosomes. It is sometimes called '**Plastid Endoplasmic Reticulum**' or 'PER' (alternatively, 'Chloroplast Endoplasmic Reticulum' / 'CER'). In Cryptophyta, Haptophyta and many stramenopiles the plastid is closely associated with the nucleus, and the PER and nuclear envelope are continuous with each other (see Figure CA2A). By contrast, typical dinoflagellate plastids have only three bounding membranes, and the outermost membrane does not resemble ER, and is not connected to the nuclear envelope. The plastids of different algae are compared diagrammatically in Appendix II.

In addition to the novel type of chlorophyll – chlorophyll *c* – chromalveolate plastids have a range of other accessory pigments, mostly carotenoids, which extend the absorption spectrum of the plastid, especially in the blue-green range, and/or protect against excessive light. These pigments typically impart a yellow-to-brown colour to the plastid. Some groups have specific pigments that are characteristic (see below).

CA3 Cryptomonada (inc. Cryptophyta)

Cryptomonads are single-celled organisms ~5-50 micrometres in length. The cells are roughly oval-shaped with an elongate groove or pocket called the 'gullet' or 'furrow' (Figure CA1). There are two fairly short flagella at the anterior end of the gullet/furrow. Each flagellum has flagellar hairs (of different appearance and functionality to those of stramenopiles – see Section S1 below). While almost all other chromalveolates have tubular mitochondrial cristae, cryptomonads have flat cristae. Most cryptomonads have a 'periplast', usually consisting of small rounded or polygonal proteinaceous plates that lie under the cell membrane (each plate is also associated with an extracellular layer as well). Cryptomonad cells also contain very distinctive extrusomes called ‘ejectosomes’. An array of large extrusomes associates with the gullet/furrow, and smaller extrusomes are situated at the corners of the periplast plates (Figure CA1). Both types contain a coiled tape structure. When the extrusome is discharged, this tape rapidly unrolls into an elongate tube. The large extrusomes in the gullet are discharged forwards, incidentally causing the cell to jerk violently backward due to recoil. The extrusomes are probably used as defense against predation.

Almost all cryptomonads are photosynthetic, or are clearly descended from photosynthetic forms by secondary loss of photosynthesis (and retaining a non-photosynthetic plastid, known as a ‘leucoplast’). This assemblage is called **Cryptophyta**, or cryptophytes (or sometimes Cryptomonadales). Cryptophytes usually have one or two plastids. The thylakoid membranes are present in pairs. Cryptophytes are the only chromalveolate group that has retained ‘red algal’ phycobilin accessory pigments, and as a consequence, cryptophyte plastids are often reddish or olive green, rather than the yellow-brown colour typical of other chromalveolate plastids. However, the phycobilin-containing biliproteins are not organized into complex phycobilisomes fixed to the ‘outside’ (stroma-side) of the thylakoid membranes. Instead there is a single soluble phycobilin-containing biliprotein located within the lumen (interior space) of the thylakoids. The most remarkable feature of the cryptophyte plastid is that the original red algal nucleus is still present in reduced form as a ‘nucleomorph’. As with chlorarachniophytes (see Section R6), the nucleomorph of cryptophytes sits between the inner and outer pairs of membranes surrounding the plastids, in a region called the periplastid compartment, or PPC. In cryptophytes this same space is also where the primary storage product (a form of starch) is synthesized and accumulates.

Cryptophytes are found in both marine and freshwater plankton. They tend to be most important in relatively oligotrophic environments (i.e. low in nutrients) and/or colder waters, and to be adapted to lower light levels than most other microalgae. Example cryptophytes include *Cryptomonas* and *Geminigera.*

Although some cryptophytes have been shown to be able to grow osmotrophically, evidence that any can undertake phagotrophy (i.e. consume particulate prey such as prokaryotes) is rather equivocal. However, the sister-group to plastidic cryptophytes, the plastid-lacking cryptomonad *Goniomonas,* is a dedicated phagotroph that consumes bacteria. The closest relatives of cryptomonads are katablepharids and the recently discovered *Palpitomonas,* both of which are also plastid-lacking phagotrophic flagellates.

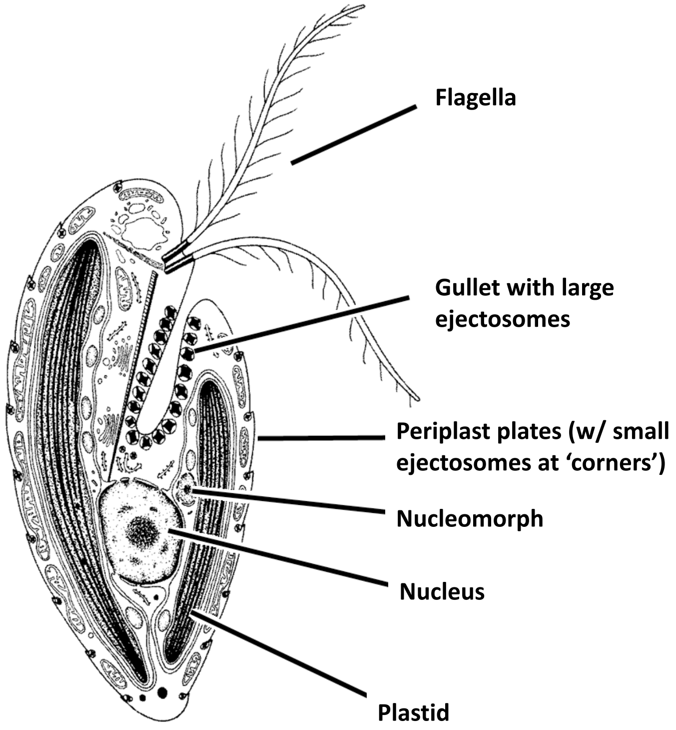


Figure CA1. Cryptophyte. Diagrammatic representation of the cell. Note that the flagellar hairs are different in structure and function from those of stramenopiles, and present on both flagella (single row of hairs on one flagellum, two rows on the other). Image modified from Hausmann et al., 2003.

CA4 Haptophyta

Haptophytes (or 'prymnesiophytes') are a particularly important group of microalgae in marine habitats. They are also present in freshwater, albeit only ~5% of the >300 described species are live there. Haptophytes are small cells (usually <10 micrometres). Most cells have two flagella, usually of similar length, although some species lack flagella in some phases of the life cycle. Most haptophytes have a slender microtubule-supported structure called the **haptonema** that is unique to the group (Figure CA2A). The haptonema projects from the cell near the point of insertion of the flagella, and may be extremely long in some species (sometimes >10x the length of the cell), but is much shorter than the cell body in others, or even barely visible by light microscopy. In cells with a long haptonema the structure may show bending movements, and is also able to rapidly coil up (in 0.01-0.02 seconds) in response to stimulus (see Figure CA2A). The haptonema is used for attachment to surfaces, and may sometimes serve a sensory function. Some species with a long haptonema use it to collect food particles (see below). Haptophyte cells are often covered in scales. The scales are composed of organic material, mostly polysaccharide, but in 'coccolithophorids' the scales are heavily mineralized with calcium carbonate (see Section CA4.1).

Almost all haptophytes are photosynthetic. There is a single plastid (usually), with PER that is connected to the nuclear envelope (Figure CA2A). The thylakoid membranes are arranged in stacks of three. Common accessory pigments include fucoxanthin derivatives like 19' hexanoyloxyfucoxanthin (19' HNOF), which are xanthophylls (a subcategory of carotenioids). Quite a few haptophytes are also capable of phagotrophy, consuming prokaryotes and sometimes very small eukaryotes as well. Prey items collide with and adhere to the haptonema as the cell swims forwards, and are later transferred to the main cell surface for phagocytosis.

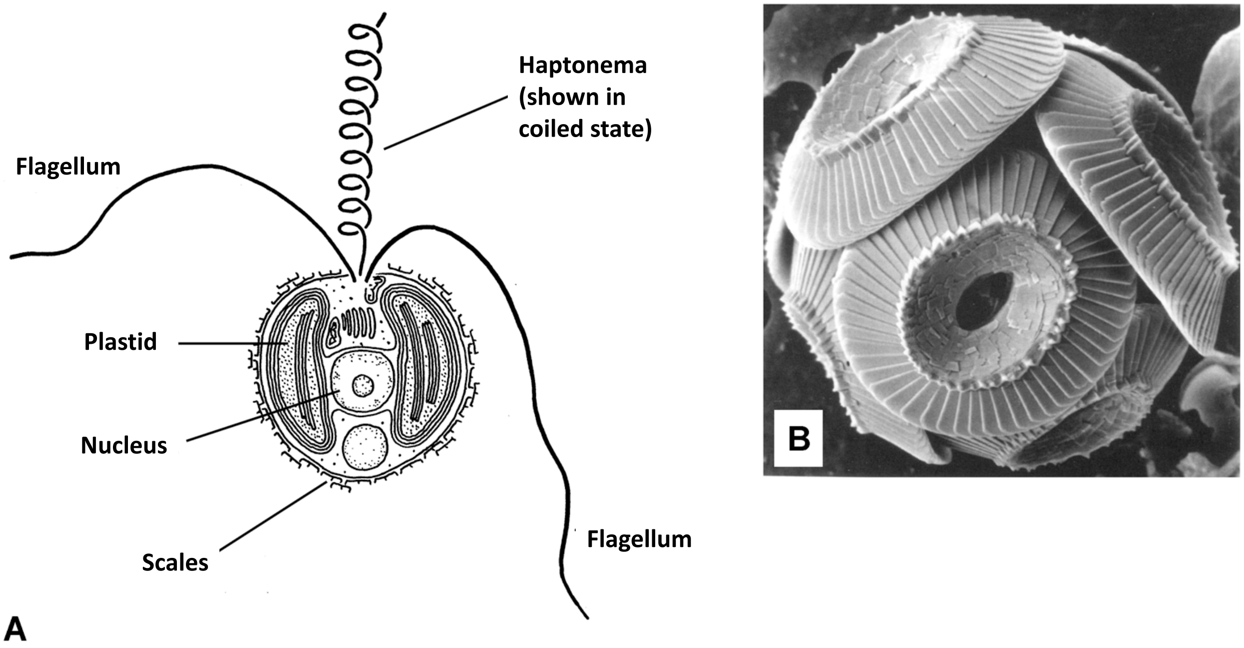


Figure CA2. Haptophytes. A. Diagram of a non-coccolithophorid haptophyte, based on *Chrysochromulina*. The scales in this case would be organic, not mineralised. The haptonema is shown in coiled configuration - when uncoiled this would be several times longer than the cell proper, although most haptophyte species have a much shorter haptonema than that shown here. Note the four membranes around the plastid and the association between the plastid and nucleus (also seen in cryptophytes and many stramenopiles). **B.** Coccoliths of the coccolithophorid *Coccolithus*. The cell itself would be inside this ball (the 'coccosphere'). Scanning electron micrograph. B from Hausmann et al., 2003 (image by K. Callaghan).

CA4.1 Coccoliths and coccolithophores

The most important haptophytes are the coccolithophores (coccolithophorids). These marine forms produce calcium carbonate scales called '**coccoliths**' in at least one stage of their life cycle (Figure CA2B). There are two main types – holococcoliths and heterococcoliths – we only discuss the latter in detail here. Heterococcoliths are produced within the endomembrane system. First, a basal organic scale is synthesized within the Golgi apparatus, after which calcium carbonate is laid down as a series of crystals, and the scale is eventually excytosed from the cell. Most heterococcoliths are round or oval-shaped in profile, with flanges and other elaborate sculpting. They are usually very large compared to the cell – in some species <20 coccoliths are needed to totally enclose the cell in a loose-fitting 'shell' called a 'coccosphere'.

The importance of coccolithophores can be illustrated nicely by considering the well-known species *Emiliania huxleyi*, the most abundant coccolithophore in modern oceans. This species exists either (i) as a free-swimming flagellate with organic scales, or (ii) as a non-motile cell surrounded by heterococcoliths. These two forms are actually the haploid and diploid phases respectively of a haplodiplontic life cycle; See Part 1, 1.2.5). In the coccolith-bearing diploid form, *E. huxleyi* can form dense and widespread 'blooms', which can contain 10^4 cells/ml over thousands of square kilometres (and even denser blooms of 10^5 cells/ml have been recorded within fjords). Massive blooms are common in the North Atlantic, in particular. The huge quantities of coccoliths generated by these blooms are highly reflective - they are easily imaged from space. Furthermore, much of the coccolithophorid material ends up sinking out of the upper ocean water and into the deep ocean. Sinking coccolithophorid material is a major mechanism of carbon export to the deep ocean and ocean sediments (though, conversely, the massive 'unbalanced' calcification seen in *E. huxleyi* blooms also releases CO_2 to the atmosphere, due to the calcification reaction converting dissolved bicarbonate into equal amounts of carbonate and CO_2). Suffice to say that coccolithophores are tremendously important players in the carbon cycle on Earth.

Coccolithophores have a fossil record going back ~225 million years to the late Triassic. Many of the chalk and limestone deposits around the world are derived from discarded coccoliths that accumulated on the ocean floor tens or hundreds of million years ago.

CA4.2 Other ecological importance

The non-coccolithophorid genus *Phaeocystis* contains several species that collectively have a substantial ecological importance. *Phaeocystis* is abundant at high latitudes, and can be bloom-forming. In addition to unicellular flagellates, most species can form colonies in which the cells are embedded in large amounts of extracellular mucilage. During blooms this mucilage can foul beaches, nets etc, and can be hazardous to marine life. At least one species may also be directly toxic to e.g. fish larvae. Conversely, some forms of *Phaeocystis* can enter into symbioses with a number of different radiolaria (a group of planktonic rhizarian amoebae; see R5) to form mixotrophic consortia.

One unusual coccolithophorid (belonging to the taxon *Braarudosphaera*) is the host to the cyanobacterium 'UCYN-A'. UCYN-A is non-photosynthethic, but is diazotrophic (i.e. nitrogen-fixing), and is inferred to be a significant contributer to nitrogen fixation in the ocean. Thus, the consortium of *Braarudosphaera* and UCYN-A is an important player in the marine nitrogen cycle.

Specialist phagotrophic protists generally consume particles at higher rates per cell than do mixotrophic algae. Nonetheless, haptophytes can be ecologically important comsumers of prokaryotes in marine waters, because their abundances may be very high – small haptophytes have been estimated to be responsible to up to 30% of the consumption of prokaryotes some water masses. Interestingly, there is increasing evidence that coccolithophores (which as generally treated as pure phototrophs) may often, even typically, be capable of phagocytosis as well. There is even some evidence that *Emiliania huxleyi* can ingest small prokaryote (-sized particles).

PART 2, SECTION S: STRAMENOPILES

S1 Introduction; Flagellar hairs

Stramenopiles (also called '**heterokonts**') are a large and very diverse group of eukaryotes that includes algae, protozoa, and even some fungi-like organisms. One group – the Phaeophyceae or 'brown algae' – are an important group of macroalgae, and are covered later in Part 3, Section 'P'.

Most stramenopiles have flagella at some stage in their life cycle. Most stramenopile flagellates have either one or two flagella, and one flagellum is directed anteriorly and has two rows of stiff **flagellar hairs** (also known as '**mastigonemes**'). The hair-bearing flagellum is sometimes called the 'tinsel flagellum'. Each flagellar hair has a thickened base, a long, hollow straw-like shaft, and a tip consisting of several fine fibrils. These flagellar hairs reverse the net movement of fluid by the flagellum as it beats, such that the flagellum seems to 'pull', rather than 'push' the cell through the medium when a normal flagellar beat is employed (Figure S1). These 'thrust-reversing' flagellar hairs are distinctive for stramenopiles, and historically have been useful in revealing close evolutionary relationships between organisms with very different life histories, e.g. macroalgae vs fungi-like parasites.

Stramenopiles are an extremely diverse group, and there are numerous types of photosynthetic and mixotrophic stramenopiles, and heterotrophic stramenopiles, often with very different basic biologies. The deepest-level relationships amongst stramenopiles are imperfectly understood, and it is disputed whether the ancestral stramenopile was photosynthetic or heterotrophic (as part of the controversies mentioned above in CA1). Nonetheless, the living photosynthetic stramenopile groups appear to be related to one another, forming a monophyletic group called 'Ochrophyta'. Heterotrophic stramenopiles, do not form a monophyletic group (some are more closely related to Ochrophyta than they are to other heterotrophs). Here, for convenience, we will divide stramenopiles into photosynthetic and heterotrophic forms.

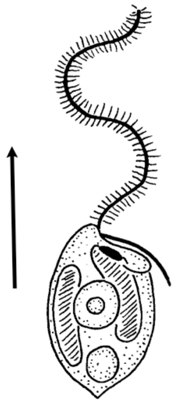


Figure S1. Stramenopile flagellate, during swimming. Diagram based on the chrysophycean *Ochromonas*. The cell body is about 5 micrometres long. The anteriorly-directed flagellum bears distinctive flagellar hairs. During swimming the hair-bearing flagellum moves in front of the cell with a sinusoidal beat, and the cell is 'pulled' forwards (in the direction of the arrow), due to the hydrodynamic properties of the hairs. The dark patch repsresents the 'eyespot' region of the plastid, which is associated with the shorter, non-hairy flagellum.

S2 Photosynthetic stramenopiles (Ochrophytes)

Photosynthetic stramenopiles range from small unicellular organisms through to the largest macroalgae. Phycologists currently divide photosynthetic stramenopiles into about a dozen distinct groups. One group, Phaeophyceae (brown algae), are mostly macroalgae and are covered in Part 3, Section P below. Many, but not all, of the remaining groups are unicellular flagellates, and this probably reflects the ancestral condition of photosynthetic stramenopiles as a whole. We will examine only a couple of example groups in detail here.

The plastids of photosynthetic stramenopiles are similar to those of haptophytes (Sections CA2 and CA4), except that in some larger forms there can be many plastids per cell and the plastids are not necessarily connected to the nuclear envelope. Many stramenopiles have a 'girdle lamella' - one stack of three thylakoid membranes that completely encircles the plastid, just inside the bounding membranes. The suite of accessory pigments includes fucoxanthin (or sometimes vaucheriaxanthin). In many flagellated cells a small part of the plastid is a highly pigmented eyespot, which is closely associated with the non-hairy flagellum and is involved in phototaxis (Figure S1).

The typical storage polysaccharide of photosynthetic stramenopiles is chrysolaminaran, which is synthesized and stored in the cytoplasm. Chrysolaminaran is a glucan, like starch, however the bonds between adjacent glucose units are different (β linkages, mostly between the 1 and 3 carbons, rather than the α 1→4 linkages in starch).

S2.1 Chrysophyceae

The chrysophyceans are unicellular organisms – typically 5-25 μm - that are common in freshwater habitats (though see below). Most chrysophyceans have two flagella, and either swim freely (Figure S1), or are attached to surfaces. Some are colonial. There is a single plastid. Some chrysophyceans are commonly covered in delicate organic scales or silica scales. In some species, the cells secrete an organic lorica (e.g. *Dinobryon*). Many chrysophyceans are known to produce a distinctive form of cyst, called a stomatocyst, which has a siliceous cyst wall and fossilizes well. This cyst is actually produced endogenously (i.e. within the cytoplasm of the cyst-forming cell). Stomatocysts are part of the sexual cycle of some species.

While most groups of photosynthetic stramenopiles are not capable of phagotrophy, many chrysophyceae are, in fact, mixotrophs. They typically consume prokaryotes that are suspended in the water column, and sometimes small eukaryotes as well. The chrysophycean uses its hair-bearing flagellum to create a feeding current, and prey particles are captured and phagocytosed using a specialized hoop-shaped feeding apparatus. Some chrysophytes have small plastids, or completely non-photosynthetic plastids, and rely heavily or entirely on phagotrophy for nutrition (e.g. *Paraphysomonas*). Chrysophyceans are probably most important in oligotrophic (low nutrient) waters, where their ability to phagocytose prey might give them an advantage over pure phototrophs in acquiring nutrients like nitrogen and phosphorous. Recent studies using environmental sequencing approaches suggest that (presumably non-photosynthetic) chrysophyceans are a major part of microbial eukaryote communities in deeper oligotrophic ocean water.

The taxon Synurales are a well-known group of freshwater algae that are now understood to be a subgroup of Chrysophyceae. They have particularly large silicaceous scales (sometimes augmented by modified scales that form large 'spines'). They are not known to engage in phagotrophy. *Mallomonas* is the most commonly encountered unicellular genus, while *Synura* is a free-swimming colonial form.

S2.2 Other flagellated photosynthetic stramenopiles, and their relatives.

There are several other distinct photosynthetic stramenopile groups that are usually flagellated through most of their lifecycle. **Pelagophyceae** and ***Bolidomonas*** are usually very small (some <5 μm) flagellates (or non-flagellated cells) that may be important in marine systems. **Eustigmatophyceae** are strange flagellates or non-flagellated cells mostly from freshwater and soil. **Dictyochophyceae** comprise a variety of marine forms, many of which produce elongate spines, or an entire internal skeleton, constructed of silica. Many members of Dictyochophyceae also produce fine pseudopodia with extrusomes (rather like those seen in some Rhizaria see Section R1), and are either mixotrophic, or exclusively phagotrophic. **Raphidophyceae** are generally larger flagellates (some are >50 μm) with multiple plastids that are found in both freshwater and marine habitats. Some occasionally form harmful blooms (e.g. *Heterosigma akashiwo*). They are naked (i.e. lack scales) but bear large and distinctive extrusomes. Recent studies indicate that raphidophyceans are also able to ingest small particles (prokaryotes).

S2.3 Diatoms (Bacillariophyta)

The photosynthetic stramenopiles generally regarded as being of the greatest ecological importance are the diatoms, also known as Bacillariophyta. Diatoms are unicellular or colonial microalgae, with cells ranging in size from 3 μm to 2 mm (in one exceptional species), mostly 10-100 μm. The male gametes of centric diatoms (see below) are flagellated, but otherwise the cells lack flagella. Diatoms are found at high abundance in freshwater and marine environments, in both planktonic and benthic habitats. Diatoms, especially 'centric' forms (see S2.3.3, below), dominate the 'spring bloom' of 'microphytoplankton' (microalgae >20 μm in size) seen in the photic zone of the ocean, especially in coastal regions. A common estimate is that diatoms are responsible for ~40% of marine primary productivity - this is likely an overestimate, but not dramatically so. There are well over 50,000 named species, including fossils.

S2.3.1 Frustule. The most distinctive feature of diatoms is the '**frustule**' - in effect, a lidded box of silica that encloses the cell. The frustule is composed of two main parts – the bottom portion of the box is called the 'hypotheca', while the 'lid' is called the 'epitheca'. Both the epitheca and hypotheca are constructed of one large valve (the top of the lid, or bottom of the box), and a variable number of 'girdle bands' (forming most of the side-wall). Because of this, the cell looks very different when viewed from the top or bottom ('valve view') as opposed the side ('girdle view') – see Figure S2A. Each valve is often elaborately sculpted and is pierced by numerous small pores called 'areolae', which are required for gas and ion exchange and other communcation with the external environment (usually the areolae each contain a pierced plate, meaning that each is actually small cluster of individual perforations in the realm of 10 nm across).

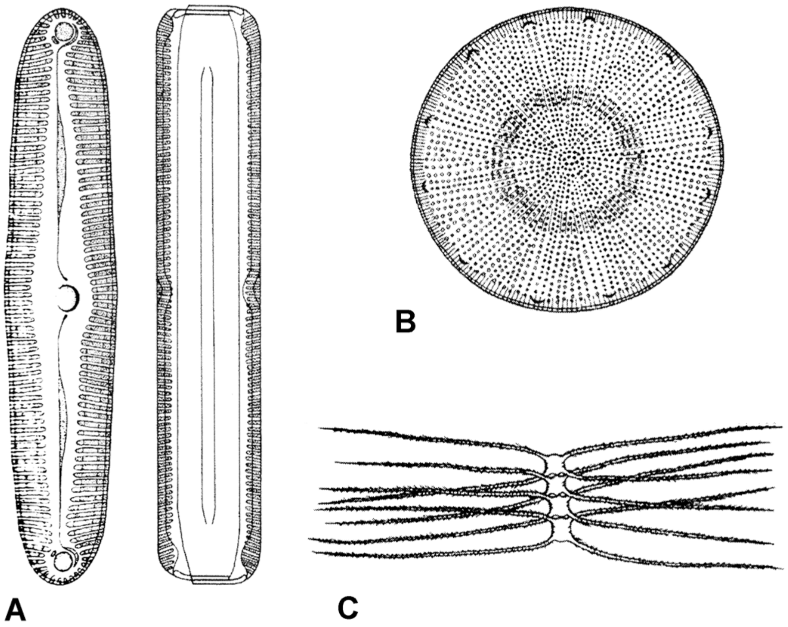


Figure S2. Diatoms. A. Left image: Pennate diatom in valve (top) view. Right image: same cell in girdle (side) view. In the left image, note the structure running down the centre of the valve – this is the 'raphe'. In the right image, note that one half of the frustule fits inside the other (epitheca to the right, hypotheca to the left). **B.** Centric diatom, shown in valve (top) view. **C.** *Chaetoceros* - a colonial centric diatom. Here, three cells are associated valve-to-valve. Each valve bears two huge spines (thus four spines per cell), probably to help deter predation. From Hausmann et al., 2003, after Sitte.

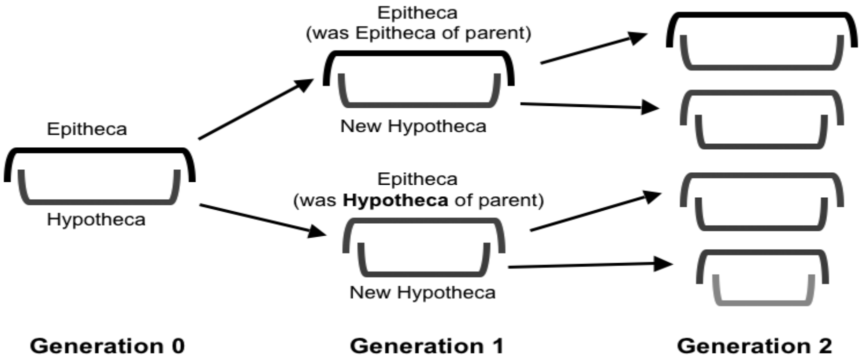


Figure S3. Diatom asexual reproduction, followed through two generations, illustrating reduction in mean size.

S2.3.2 Life cycle. The constraints imposed by the frustule are important in the life cycle of most diatoms. In the asexual phase, cells are diploid. The valves cannot change size, so the cell is restricted to growing along a single axis, by pushing the valves away from one another. The frustule 'keeps pace' by addition of a succession of hypothecal girdle bands. During cell division one half of the frustule – either the epitheca or the hypotheca - is inherited by each daughter cell. However, both parental thecae become epithecae in the daughter cells, and a new hypotheca is produced for each daughter. Since the hypotheca fits inside the epitheca, the average size of cells in a population, as measured by the size of the valves, slowly drops over time (Figure S3). Once the cells reach a 'fertile size' (typically ~1/3rd the maximum cell size) the cells can enter the sexual phase of the lifecycle if triggered (Figure S4). Cells undergo meiosis to form gametes (gamete formation differs in 'centrics' and 'pennates' - see S2.3.3). The gametes fuse and generally then form a large cyst-like cell called an 'auxospore' that usually has a resistant wall (auxospores can sometimes form asexually as well). Auxospores can remain quiescent for some time before germinating. At germination, a new diploid cell develops within the auxospore, produces both an epitheca and hypotheca at one time – this 'initial cell' is large and 'resets' the original cell size for the population.

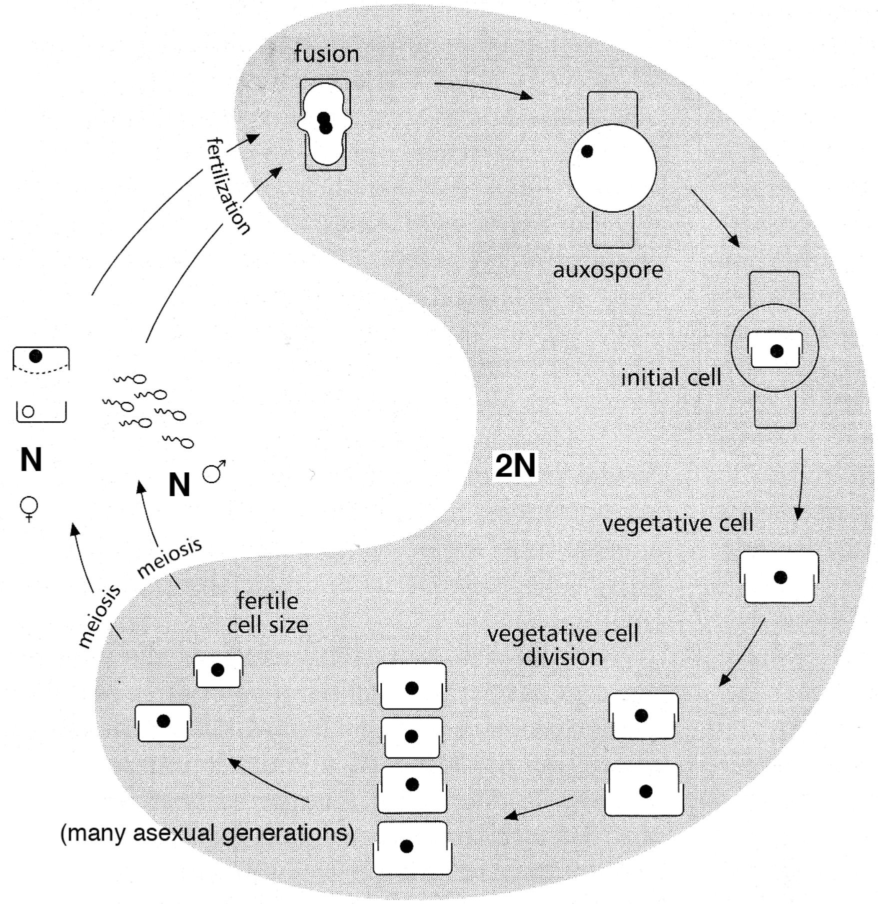


Figure S4. Diatom life cycle (centric diatom example). Typical centric diatoms (shown here) are oogamous (while in pennate diatoms, the gametes are isogamous, large and non-flagellated). Diagram modified from Graham & Wilcox, 2000, originally after Hasle and Syvertsen, 1997).

S2.3.3 Diatom diversity. There are two basic morphologies of diatoms - centric diatoms and pennate diatoms. Centric diatoms are the ancestral form and probably originated in the Jurassic. Pennate diatoms are a monophyletic group that evolved from centric diatom ancestors relatively recently in geological time by microbial eukaryote standards (late Cretaceous: perhaps ~70-80 million years ago).

Centric diatoms generally have valves with radial symmetry and usually a rounded profile (Figure S2B). In some species the valves bear elongate spines, called setae (Figure S2C). There are usually numerous plastids, generally arranged around the periphery of the cell (oftentimes, much of the centre of the cell is taken up by a large vacuole). Most centric diatoms are planktonic and are not capable of independent locomotion, except, potentially, vertical movement by buoyancy regulation. Many species are colonial, usually forming linear chains in which cells are connected valve-to-valve (Figure S2C). Centric diatoms are oogamous. Cells either produce numerous male gametes or 1-2 large, immobile female gametes (Fig. S4). Each male gamete has a single flagellum that bears the distinctive flagellar hairs of stramenopiles (see section S1, above).

Example centric diatoms include the unicellular *Coscinodiscus*, *Cyclotella* and *Thalassiosira*. *Chaetoceros* is an example of a chain-forming colonial diatom with elongate setae. *Skeletonema* is a common colonial centric that lacks setae.

Pennate diatoms generally have valves with bilateral symmetry and a boat-like profile (Figure S2A). Pennate diatoms are predominantly benthic organisms, although there are also important planktonic forms, many of which are colonial. Pennate diatoms often have only a pair of plastids, which run along the sides of the cell. In most species one or both valves have a longitudinal slit-like structure, interrupted at its centre, called the 'raphe' / 'raphe slit'. This plays a role in the distinctive motility shown by most pennates. Cells can adhere to surfaces using mucilage secreted from the raphe, and most can also glide along surfaces. The gliding mechanism is not well understood but seems to involve molecular motors attaching to the mucilage strands and pushing against the internal cytoskeleton of the cell. This is most likely an actin-myosin system.

Pennate diatoms have a different sexual process to that of centric diatoms. Pennate diatoms are generally isogamous and undergo conjugation. Two cells line up next to each other and undergo meiosis, with two meiotic products from each parent surviving to become gametes. One gamete from each original cell exits the frustule, and enters the adjacent frustule to fuse with the non-migratory gamete remaining there. Both of the resulting zygotes then form auxspores (see above). Thus there is no flagellate phase in the lifecycle of pennates. The use of conjugation, and lack of flagellated gametes, forms an interesting parallel with the Zygnematophyceae – a group of chloroplastidans (see Section A6.5).

Typical pennate diatoms include *Navicula*, *Cymbella* and *Pinnularia*. *Pseudo-nitzschia* is an unusal planktonic colonial form, which produces the neurotoxin domoic acid, and causes some human shellfish poisonings (see AV2.7 below for other examples, caused by dinoflagellates).

S3 Non-photosynthetic stramenopiles

Non-photosynthetic stramenopiles are diverse, ranging from small bacterivorous flagellates through to large multiflagellated symbionts of animals (opalinids), through to remarkably fungi-like decomposer organisms and pathogens. Here we discuss a few of the more important groups only:

S3.1 Bicosoecida (and other small heterotrophic flagellates)

Bicosoecids are small heterotrophic cells with two flagella that mostly consume prokaryotes (i.e. are 'bacterivorous'). Many bicosoecids feed using a strategy similar to that of phagotrophic chrysophyceans (see S2.1, above). As with chrysophyceans, many bicosoecids attach to surfaces while feeding or live within an attached lorica. Some have secondarily lost flagellar hairs and generate a feeding current with a different method of flagellar beating, or they instead collect prokaryote prey individually from surfaces.

In addition to bicosoecids there are several other phylogenetically distinct groups of non-photosynthetic stramenopiles that are small bacterivorous flagellates. Some of these have been characterized by culturing, but many have been identified from natural samples using environmental molecular methods (e.g. the 'uncultured MArine STramenopile lineages' or 'MASTs'). Stramenopiles of this kind represent an important fraction of the small heterotrophic flagellates in the oceans.

S3.2 Oomycota (oomycetes)

There are several groups of protists that have a nutritional mode similar to that of true fungi, but have evolved independently. Oomycetes are the most striking example. Like fungi, most oomycetes acquire nutrients through a network of elongate and often branching 'hyphae'. Nutrition is via uptake of small organic molecules (i.e. osmotrophy), rather than phagocytosis, however the organisms mobilize these small molecules by secreting enzymes that break down complex organic compounds (this is sometimes called 'saprotrophy'). Like some true fungi the hyphae of oomycetes are mostly 'coenocytic', that is, not separated into a series of separate cells by cross-walls. However, the cell walls are composed mostly of cellulose and cellulose-like glucan polysaccharides, not chitin (i.e. N-acetyl glucosamine polymers) as in true fungi.

Further differences between oomycetes and true fungi become apparent when the life cycle is examined. Oomycetes are diploid for almost all of the lifecycle (whereas fungi are predominantly haploid). In the sexual phase, differentiated diploid male and female hyphae ('antheridium' and 'oogonium' respectively) grow into contact with each other. Both undergo meiotic divisions to produce haploid cells, and the male gamete penetrates into the oogonium to fuse with a female gamete. The resulting diploid zygote ('oospore') germinates to form a large 'sporangium' that undergoes numerous cell divisions to produce many diploid spores. Again, unlike true fungi (except chytrids and Cryptomycota) the spores are often flagellated (i.e. zoospores), with the typical stramenopile arrangement of one hairy flagellum, and one smooth flagellum. Oomycetes may also reproduce asexually by producing diploid spores.

Oomycetes are important in freshwater, often fulfilling a decomposer role similar to that of true fungi on land, and are also presence in marine systems. However, the best-known oomycetes are parasites of terrestrial plants, which are sometimes infected through the roots by zoospores that travel through wet soil (non-flagellated spores may be wind-dispersed). The oomycete genus *Phytophthora* includes numerous plant parasites. *Phytophthora infestans* is the causative agent of late blight of potatoes, the (biological) cause of the great famine in Ireland (1840s). Other plant-parasitic oomycetes attack grapes, legumes and various trees. Collectively, the plant-parasitic oomycetes have a massive impact on agricultural crops, likely greater than any other protist group, and some tree-infecting species are of major ecological concern. There are also oomycetes that parasitise farmed fish (species of *Achlya* and *Saprolegnia*).

S3.3 Opalinata

This major group of stramenopiles is entirely symbiotic, commensal or parasitic in animals. They lack flagellar hairs, although one taxon (*Proteromonas*) instead has stiff hairs on the surface of the main body of the cell. These hairs are similar in structure to the flagellar hairs of typical stramenopiles, and are homologous. The cell surface is formed into a series of folds in most species, each of which is supported by microtubules. The cell obtains nutrition by pinocytosis (not phagocytosis), at the bottoms of the folds.

The best-known subgroup within Opalinata are the opalinids, which are large cells (some >2 mm long) with numerous flagella in rows along the cell, and either two or numerous identical nuclei. Opalinids are superficially similar to ciliates (see Section AV4, below), but are not closely related - the similarities are the result of evolutionary convergence, and, amongst other differences, opalinids lack the nuclear dualism characteristic of ciliates. Opalinids are found in the lower intestinal tracts of poikilothermic ('cold-blooded') vertebrates, especially anurans (frogs and toads).

Blastocytis is a strange anaerobic cell that is a commensal or parasite of the intestinal tract of humans and other animals. It is, in fact, one of the most common eukaryotes in the intestine of humans, though it is controversial whether it is (ever) pathogenic. The main cell type as almost featureless rounded cell with a large vacuole. Molecular phylogenies revealed that *Blastocystis* is a stramenopile, and that it belongs to the subgroup we now call Opalinata. It is highly sensitive to oxygen, and has a form of anaerobic mitochondrion (for a discussion of other kinds of anaerobic mitochondria, see Section E1.1 below).

S3.4 Labyrinthulomycetes (labyrinthulids)

The labyrinthulids or 'slime-nets' are a bizarre group of heterotrophic stramenopiles. The main life cycle phase consists of numerous spindle-shaped 'cell bodies' that move backwards and forwards within a plasmodial network-like structure – the slime net. The net is cellular – it is bounded by a biological membrane, and contains a rather empty cytoplasm called ‘ectoplasm’. Each spindle-shaped cell body within the ectoplasm is surrounded by an envelope of two layers of membrane and contains a nucleus and other typical organelles. The envelope surrounding each cell body is actually one continuous membrane ‘folded over’ on itself (this is the same topological organisation as in the eukaryotic nuclear envelope). Small pores called ‘bothrosomes (or ‘sagenetosomes’) allow some communication between the cytoplasm within the ‘cell bodies’ and the ectoplasm, and are involved in the formation of new ectoplasm. The inner side of the bothrosome is entremely rich in endomembrane. The organism grows by division of the spindle-shaped cell bodies. The cell bodies can also undergo multiple divisions and release flagellated dispersal cells (zoospores). The anteriorly-directed flagellum of each spore bears stramenopile-type flagellar hairs. It is likely that these flagellated cells are actually gametes (i.e. part of a sexual cycle).

Labyrinthulids are mostly marine or brackish-water organisms that are often associated with seagrasses and macroalgae. They are osmotrophs/ saprotrophs that feed on decaying plant or macro-algal material. A few are actually pathogens, especially of seagrasses (eelgrass).

The strange cell organisation of labyrinthulids traditionally made them difficult to classify, and in the past they have often been given a group of their own at extremely high taxonomic rank. In fact, the presence of the signature stramenopile hairs in the zoospores indicate that labyrinthulids are a ‘merely’ a subgroup of stramenopiles, and this has been also demonstrated convincingly by molecular phylogenetics.

Thraustochrytrids the best known of several small groups of organisms that are closely related to labyrinthulids, but that show a more conventional arrangement of the cell. The main cell body is typically rounded, and enclosed by a single biological membrane, as well a layer of scales. Branching or network-like ectoplasmic strands extend from a bothrosome structure, and extend into the surrounding environment via a gap in the scale layer. Thraustochytrids are most often found in similar environments to labyrinthulids (e.g. colonizing decaying plant/algal material in marine/esturine systems), but are often capable of phagotrophy (in addition presumably to osmotrophy/saproptrophy).

PART 2, SECTION AV: ALVEOLATES

AV1. Introduction

The taxon Alveolata unites three very different groups of eukaryotes – i) dinoflagellates, which are one of the most important groups of larger microalgae, ii) Apicomplexa, arguably the most successful group of protistan parasites on Earth, and iii) ciliates, one of the 'protozoan' groups of greatest ecological significance. Alveolates share a key structural feature - in all three groups there is a layer of flattened membrane sacs called '**alveoli**' or 'cortical alveoli' lying immediately beneath the cell membrane. These sacs are usually subtended in turn by microtubules. The alveoli and microtubules constitute a form of pellicle for the cell, and help to provide structural strength.

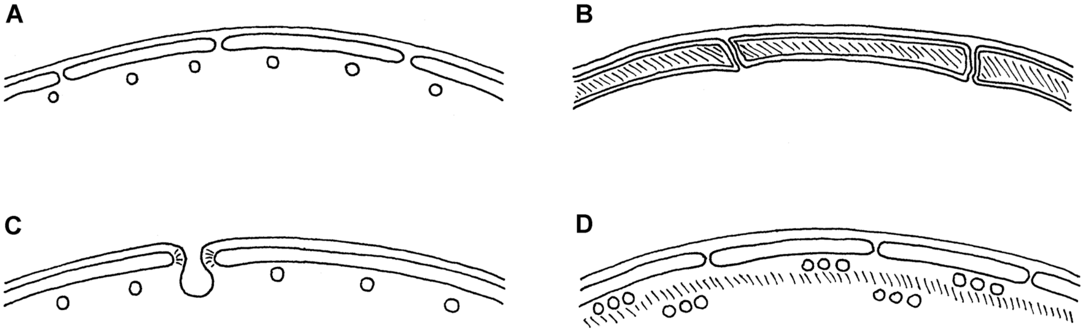


Figure AV1. Alveoli. **A.** General appearance of alveoli, lying immediately under the cell membrane and supported by microtubules (circles). **B.** Alveoli of armoured dinoflagellates, containing thecal plates of cellulose (shaded). **C.** Alveoli of an apicomplexan, with the section through a micropore shown. **D.** Ciliate pellicle, with alveoli underlain by microtubules and epiplasm (shading).

AV2 Dinoflagellata (Dinoflagellates)

Dinoflagellates (sometimes called 'Pyrrhophyta') are the only ***major*** group within Alveolata to include photosynthetic algae (though see AV5, below**)**. There are ~2500 living species described, ranging in size from a few micrometres up to two millimetres. About half of the described species of dinoflagellates are photosynthetic. Most of these are actually mixotrophs that also prey on other organisms, often other eukaryotes. The non-photosynthetic species are generally pure phagotrophs or are parasites.

AV2.1 Plastid

Typical dinoflagellate plastids have chlorophylls *a* + *c*, like those of other 'chromalveolate' algae. However, they differ in that they have only three bounding membranes, with the outermost membrane not being endoplasmic reticulum-like and not connected to the nuclear envelope. The thylakoid membranes are arranged in stacks of three. The signature accessory pigment is peridinin (rather than fucoxanthin, as in most photosynthetic stramenopiles, for example). Bizarrely, the plastid genome of dinoflagellates is broken up into numerous small circles, and the total number of genes is probably the smallest of any photosynthetic plastid genome.

A few species of dinoflagellates have dramatically different plastids, for example, they are surrounded by more than 3 membranes and contain variants of fucoxanthin instead of peridinin, or have chlorophyll *b* instead of chlorophyll *c.* These remarkable cases are the result of various additional events of endosymbiosis that happened more recently than than ancient event that gave rise to the typical 'peridinin-containing' plastid of most photosynthetic dinoflagellates. This is discussed more in Part 3 of Biol3102.

AV2.2 Nucleus

Dinoflagellate usually an extremely unusual nucleus organization, sometimes referred to as ‘dinokaryotic’. Most dinoflagellate nuclei contain a very large amount of DNA, and the genome sizes of dinoflagellates are huge relative to most other microbial eukaryotes - in fact, most dinoflagellates have genomes that are several times larger than the human genome. The chromosomes appear ‘condensed’ throughout the cell cycle – they can be readily distinguished in interphase cells using light microscopy, and the number of chromosomes in the nucleus can even be counted in interphase cells by electron microscopy. The DNA differs from almost all other eukaryotic nuclear DNA in that it is *not* packaged by winding around histone protein complexes to form nucleosomes, and the ratio of protein to DNA within the chromosome structure is dramatically (~10x) lower than in typical eukaryotes. The chromosomes instead have a fibrous appearance when viewed by electron microscopy, reflecting a form of closely ordered packing of lengths of the chromosomal DNA. The bulk of the DNA therefore appears to be essentially inaccesible to enzymes like RNA polymerase, and it is thought that transcription is restricted to relatively small regions of the chromosome that ‘loop out’ from the surface of the main chromosome body, and are therefore accessible. Histones are present in very low amounts, and the main proteins associated with the DNA are a couple of classes of basic proteins that are *not* homologous to histones. Remarkably, this includes DNA-binding proteins called ‘DVNPs’ that are otherwise found in very large viruses with double-stranded DNA genomes.

AV2.3 Flagella

Most dinoflagellates are free-swimming single cells with two flagella. In most, one flagellum is directed longitudinally within a channel called the ‘sulcus’, while the second flagellum runs transverally, and circles the cell in a groove called the ‘cingulum’ (Figure AV2, left image). This ‘transverse flagellum’ within the cingulum beats as a very tight spiral, and imparts rotation as well as much of the forward motion of the cell. There are usually paraxonemal structures in both flagella, and the transverse flagellum in particular bears flagellar hairs. These have a very different structure to the paraxonemal rods of Euglenozoa (see Section E7, below), and flagellar hairs of stramenopiles (for example), and are not homologous in either case.

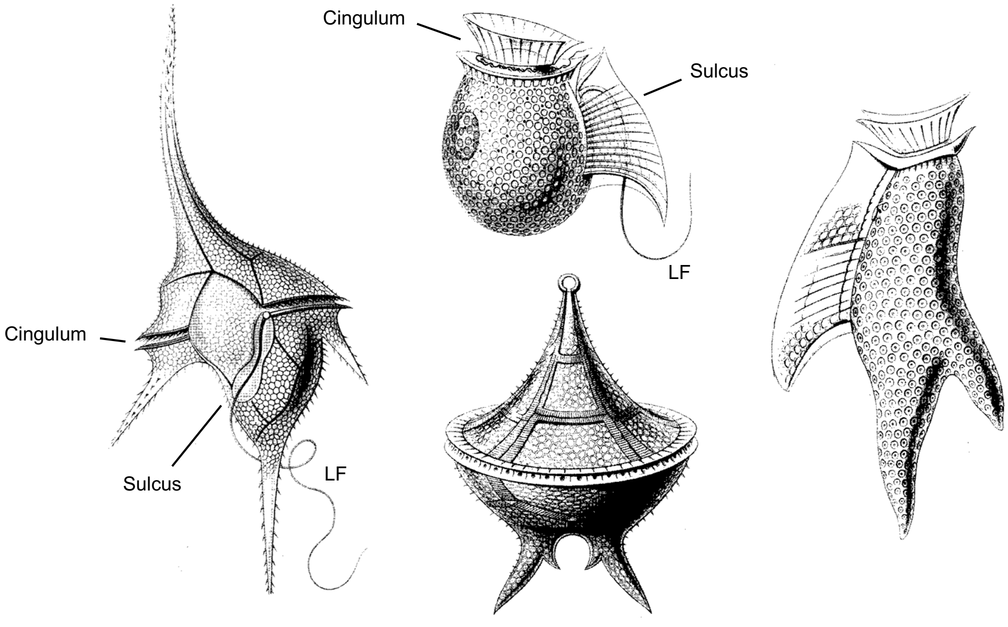


Figure AV2. Dinoflagellates. Light microscopical appearance of some ‘armored’ dinoflagellates from marine plankton. Left - *Ceratium*, Top, Right – *Dinophysis* species, Bottom - *Protoperidinium* (?). Most flagella are note shown in these images. Left and Top images include labels for the cingulum and the sulcus, which houses the longitudinal flagellum (LF). The cingulum in *Dinophysis* (Top, Right) is near the cell anterior, and both cingulum and sulcus are flanked by tall ‘lists’. Drawings by the great 19th century biologist Ernst Haeckel (labels added).

AV2.4 Armoured dinoflagellates

Many dinoflagellates are 'armoured' (also known as 'thecate'). In these species, the alveoli contain plates of cellulose and abut against each other (see Section AV1, and Figure AV1, above), giving the cell a rigid structure. These plates are often called 'thecal plates', but it is important to remember that these plates are located intracellularly, not extracellularly (so the terms 'thecate' and 'thecal plate' are confusing here). The boundaries of the polygonal thecal plates can be visualized readily using various microscopy techniques (see Figure AV2, left, bottom). The plates can be counted, and the patterns of contact between different plates can be determined. These so-called 'tabulation patterns' are an important basis for species identification in dinoflagellates. In some species, the thecal plates extend to form elaborate spines, wings and keel-like structures. These elaborations may help deter predation. Conspicuous armoured dinoflagellates with elaborate thecae include *Dinophysis* and *Ceratium* (see Figure AV2).

AV2.5 Feeding systems

As a group, dinoflagellates specialise in consuming prey that are quite large in comparison to themselves. There are a variety of different adaptations seen in dinoflagellates that permit the consumption of large prey. Many dinoflagellates have a microtubule-supported structure called a 'peduncle' that is extruded from the cell and is used to pierce the cell membrane of another eukaryotic cell. The cytoplasm of the prey is then 'sucked' up through the centre of the peduncle, and collects in a food vacuole that forms at its base. In this way, the dinoflagellate avoids having to completely enclose the entire prey item in order to feed on it. Another, less common feeding structure is the 'palium' – a broad, but very thin, sheet-like pseudopodium that is projected from the cell and used to envelope a prey item and to form a food vacuole. Some dinoflagellates can use palium feeding to consume an entire colony of planktonic diatoms at once, despite the colony being several times longer than the dinoflagellate. The huge (2 mm) marine dinoflagellate *Noctiluca* has a flexible feeding tentacle that is used to capture prey, including small planktonic animals, which are then engulfed in a specialized phagocytic pocket in the main body of the cell.

AV2.6 Lifecycle

Free-living dinoflagellates seem to be haploid and normally divide by binary cell division. During sex, two cells fuse to form a swimming zygote (a planozygote). These can either divide by meiosis to form new haploid cells, or form a thick-walled resting cyst called a 'hypnozygote', which later excysts and undergoes meiosis. Many species also form resistant cysts outside of the sexual cycle.

AV2.7 Importance

Dinoflagellates are of major importance in the environment. As photosynthesiers, they are second only to diatoms in importance in the marine 'microphytoplankton' (i.e. planktonic algae larger than 20 micrometres). They are significant predators of other microbial eukaryotes in the same ecosystems, and seem to be common parasites of marine plankton organisms. Dinoflagellates are perhaps the most widespread algal symbionts of animals and large protozoa (e.g. radiolarians, foraminifera and ciliates), especially in marine systems. These dinoflagellate symbionts are often called 'zooxanthellae', and they take up a non-flagellated rounded (coccoid) form in their hosts. Virtually all reef-building corals worldwide harbour dinoflagellates belonging to the taxon Symbiodiniaceae (e.g. *Symbiodinium*). These symbionts are essential for the long-term survival and maintenance of the corals, and hence, coral reefs. Dinoflagellates are also the most diverse agents of harmful algal blooms (HABs), especially those that cause harm by producing toxins. A common problem in Atlantic Canada is the risk of Paralytic Shellfish Poisoning (PSP) caused by blooms of dinoflagellates of the *Alexandrium tamarense* species complex (e.g. *A. fundyense*). These dinoflagellates produce saxitoxin, a potent and persistent neurotoxin, which accumulates in the bodies of clams and other shellfish that filter water in which the dinoflagellates are abundant. People may then be poisoned (potentially fatally) by eating the shellfish. Monitoring programs assess abundances of these dinoflagellates and trigger bans on shellfish harvesting when necessary.

AV3 Apicomplexa

Apicomplexa is a very large and diverse group of obligate parasites of animals, with ~6,000 described species. There are several that infect humans, including four *Plasmodium* species, the causative agents of malaria. Malaria is one of the three worst infectious diseases of humans in recent history (along with HIV/AIDS and tuberculosis), and currently claims almost half a million people annually (thankfully, the death toll has halved in the last 15 years). Several important diseases of livestock are also caused by species of Apicomplexa, and collectively cause well over a billion dollars of economic loss annually.

Most Apicomplexa are intracellular parasites and produce small infective stages that actively invade cells of their hosts. The following description of the lifecycle and of the operation of the apical complex (AV3.1-2) is based on intracellular species, especially members of the taxa Haemosporidia and Coccidea (see AV3.4). Nonetheless, another major group, the gregarines, are typically quite large and remain in the extracellular milleu of the host, albeit often attached to host cells. Their sexual cycles are also rather different to the general model described in AV 3.1.

AV3.1 Lifecycle

Apicomplexa characteristically have complex lifecycles that include an obligate sexual component. They are fundamentally haploid. The basic lifecycle of most Apicomplexa involves three distinct events of reproduction, each potentially involving production of a large number of progeny (Figure AV3). These events are '**sporogony**', '**merogony**' and '**gamogony**'. Of these 'merogony' is the main phase of asexual reproduction. (In some texts merogony is referred to as 'schizogony' and gamogony can also be referred to as 'gametogenesis').

Sporogony is preceeded by a fusion of gametes, and includes meiotic divisions to produce haploid 'sporozoites'. Sporozoites invade a host cell, and eventually undergo merogony to form numerous 'merozoites'. Merozoites invade other cells, and undergo further rounds of merogony. Sometimes the products of merogony are gametocytes (or gamonts) – cells that are capable of differentiating into large female gametes, or undergoing gamogony to produce large numbers of small male gametes. Male and female gametes fuse to form a diploid cell to complete the lifecycle.

AV3.2 Cell organisation (including apical complex)

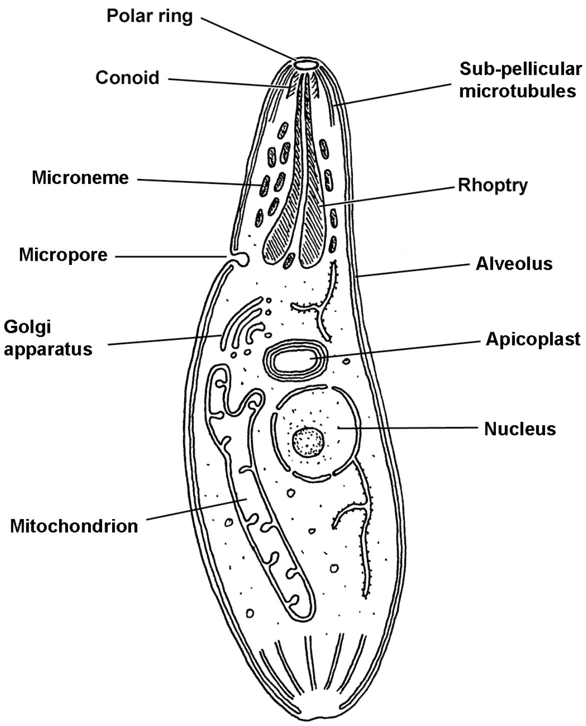
To introduce the apicomplexan cell we will consider the main invasive stages – sporozoites and merozoites. These lack flagella, although many can locomote using a gliding motility (flagella are present only in the male gametes, and only then in certain groups). The cell surface is supported by alveoli and longitudinal microtubules, usually giving the cell an elongate and rather rigid structure. Cells feed via a 'micropore' – an invagination of the cell membrane that passes through a small hole in the alveolar system (structures homologous to micropores are present in other alveolates as well).

The cell is dominated by the '**apical complex**' which has four main components - i) one or two 'polar rings' at the apical end of the cell, ii) a tubulin-based truncated cone-like structure called the 'conoid', iii) a few large secretory vesicles called 'rhoptries', and iv) many small secretory vesicles called 'micronemes' (see Fig. AV3). The conoid is not always present (for example, it is absent in *Plasmodium*).

The apical complex is involved in interaction with the host cell. During invasion, the apex of the sporozoite or merozoite comes into contact with the host cell. The apicomplexan causes the cell membrane of the host to invaginate, forming, in effect, a vacuole around the parasite. This is called the 'parasitophorous vacuole'. Lipids and proteins discharged from the rhoptries (and from another class of secretory organelles called 'dense granules', which prior to invasion are located posterior to the apical complex proper) modify both the contents of the vacuole and the vacuolar membrane, and influence the host cell in various ways (e.g. influence transcription). Proteins secreted from micronemes are involved in attachment, and the motility process of actively 'pushing' into the host cell in the first place.

AV3.3 Apicoplast

Although they are not photosynthetic, most apicomplexans have a highly modified plastid called the 'apicoplast'. The apicoplast is a rounded structure that lacks thylakoid membranes, chlorophylls, etc., but does retain a small genome. It is surrounded by four membranes, and therefore appears to be the product of a secondary or high-order symbiosis (almost certainly the same symbiosis that gave rise to the plastid in peridinin-containing dinoflagellates). The apicoplast is essential for the parasite. It is involved in several vital biosynthetic pathways, such as the production of certain fatty acids, isoprenoids, and heme. Some of these pathways use enzymes that are only found in plastids and prokaryotes. Further, much of the basic maintenance mechanisms for plastids (e.g, in DNA replication, transcription and translation) are prokaryote-like or otherwise distinctive. It is possible that drugs or compounds that inhibit these apicoplast functions would harm the parasite without significantly affecting the animal host cells (since animal cells definitely do not have plastids!). This is an active area of research, and some drugs currently used to combat malaria in fact target functions of the apicoplast. An example is the antibiotic doxycycline, which inhibits translation by bacterial-type ribosomes, such as those of the apicoplast. Doxycycline is used as one prophylactic against malaria, and sometimes also as a component of multidrug malaria treatments.



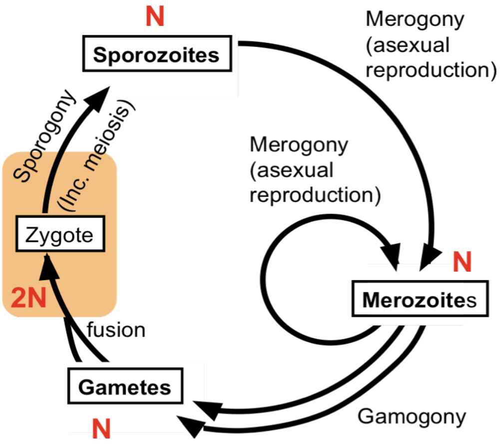


Figure AV3. Left: Cell organization of Apicomplexa, based on the sporozoite stage of *Toxoplasma*. The 'polar ring', 'conoid', 'rhoptries' and 'micronemes' collectively comprise the 'apical complex'. The apicoplast has been drawn over-sized, to show the four bounding membranes. 'Dense granules' are not shown. **Right: Generalised apicomplexan life cycle**, as described in the text.

AV3.4 Diversity – focusing on some Apicomplexan parasites of humans

The apicomplexans of greatest direct importance to humans include *Plasmodium*, *Toxoplasma* and the diarrhea-causing *Cryptosporidium*. We will use the first two as examples to briefly introduce **some** of the diversity within Apicomplexa.

AV3.4.1. *Plasmodium*, the malaria parasite, falls in a group called Haemosporida. Haemosporida are blood-borne parasites of terrestrial vertebrates that are transmitted by biting insects. In the case of human malaria, the insect is the *Anopheles* mosquito. The lifecycle of *Plasmodium* closely follows the generalized lifecycle described above (AV3.2, Figure AV3). The stage transmitted by the mosquito is the sporozoite. Sporozoites enter the bloodstream and invade a liver cell. There they undergo the first round of merogony. Merozoites released from the liver cell then infect red blood cells (erythrocytes), and there undergo another cycle of merogony (albeit producing fewer progeny per infected cell than in the first round). Merozoites released from bursting red blood cells invade other red blood cells and this cycle repeats itself. Some invasions instead result in the production of gametocytes, which remain quiescent withing red blood cells. When an infected human is bitten by another mosquito gametocytes are taken up into the gut of the insect, and develop into male or female gametes. Gametes there fuse, forming a diploid cell (the ookinete), which invades an intestinal cell, and undergoes sporogony to produce haploid sporozoites. Finally, the sporozoites migrate to the salivary gland of the mosquito, ready for another infection.

Malaria in humans is caused by four different species of *Plasmodium*, with different disease characteristics. Most deaths are caused by one species, *Plasmodium falciparum*.

AV3.4.2. ***Toxoplasma*** belongs to a large group of Apicomplexa called Coccidea. Many coccideans infect a single host species, and are passed from host to host via a fecal-oral route (an example is *Eimeria* spp., some of which cause coccidiosis in poultry). The sporozoites, which are packaged within 'oocysts', are the infectious stage. Other coccideans have multiple hosts. Typically an 'intermediate host' species acquires an infection via fecal-oral transmission, and a second, final, host species is infected by eating an infected intermediate host animal (the final host, or 'definitive' host, is where the parasite undergoes the sexual stages of the lifecycle – gamete fusion and sporogony). *Toxoplasma* essentially has a lifecycle of the latter type, although the involvement of intermediate hosts is optional. The final hosts of *Toxoplasma* are cats, and the 'normal' intermediate hosts are probably rodents, however *Toxoplasma* is unusual in that many different bird and mammal species, including humans, can be infected as if they were intermediate hosts. *Toxoplasma* divides to produce long-lived 'tissue cysts' within the intermediate host. These form in many tissues, including within the brain. Intermediate hosts are usually infected by exposure to cat faeces, or by consuming tissue from other intermediate hosts. The very broad range of intermediate hosts capable of being infected by *Toxoplasma*, as well as its ability to be passed between successive intermediate hosts makes it unusual within Coccidea. A large proportion of the world's population have been exposed to *Toxoplasma,* perhaps 20% of us. Human *Toxoplasma* infections are usually benign, but the parasite is serious and potentially fatal in the severely immuno-compromised, or in newborns whose mothers are infected during pregnancy.

AV4 Ciliophora

Ciliophora (ciliates) are one of the most successful groups of 'protozoan' protists on Earth. They are important in freshwater, seawater, hypersaline habitats, benthic habitats and soil, as well as in symbiotic or parasitic niches. Individual cells range in size from around 10 micrometres to several millimetres, depending on the species. Ciliates are the most important predators of small microbial eukaryotes in many habitats, and are also very significant predators of prokaryotes.

There are two principal defining features of ciliates. The first is the presence of numerous **cilia**. 'Cilia' is the name given to eukaryotic flagella when they are present in large numbers and move in a coordinated manner with a distinctive ciliary beat (i.e. an oar-like beat). The second feature is **nuclear dualism** – having two distinctly different types of nuclei within each cell. We will deal with both features in this introduction.

AV4.1. Cilia and the ciliate pellicle

Ciliates have a complex and relatively robust 'pellicle', which imparts a well-defined shape to the cell (although some ciliates are also flexible or contractile). As in other alveolates the cell membrane is underlain by alveoli (see Section AV1, above). Under the alveoli there is usually a protein-rich structural layer called the epiplasm. In many ciliates, the main 'somatic' cilia are distributed all over the cell in longitudinal rows called **kineties**. The basal bodies within a kinety are interconnected by cytoskeletal elements, including microtubular components. Adjacent kineties are similarly connected. Thus, there is an interconnected cytoskeletal 'meshwork' around most of the cell, adding further strength.

The continuity of the pellicle and underlying cytoskeleton is usually interrupted in two main locations. Firstly, there is a distinct ‘cytostome’ where prey is collected and packaged into phagocytic vacuoles (food vacuoles). The cytostome is usually associated with a larger complex called the ‘oral apparatus’ that includes differentiated ciliary structures (see below, and Fig. AV4A). Secondly, there is a ‘cytoproct’ dedicated to the exocytosis of the contents of residual bodies after digestion. In addition, freshwater ciliates have distinct pores associated with the expulsion of contractile vacuoles (the complex contractile vacuoles of ciliates were mentioned briefly in Part 1, Section 1.2.3). Many ciliates are armed with extrusomes (of one of several different kinds), which associate with the cell membrane via small pores within the pellicle.

Ciliates use cilia for swimming, but also for other forms of locomotion and for feeding. At any one time, there are bands of cilia at different points in the ciliary beat cycle across the cell. As a result, the ciliate will swim smoothly since roughly the same number of cilia are executing the effective (power) stroke at any one time. Some ciliates swim very rapidly (millimetres/sec). Many ciliates have bundles or blocks of numerous closely associated cilia that beat in concert with each other. Bundles of cilia are called ‘compound cilia’ or **cirri** (singular ‘cirrus’). More elongate blocks of cilia are often called **membranelles**. Many ciliates have arrays of membranelles as part of the oral apparatus, where they usually generate a feeding current to move food particles towards the cell and the cytostomal region in particular (Fig. AV4B). These membranelles may also act as a filter mechanism to trap suspended food particles and funnel them to the cytostome itself for ingestion.

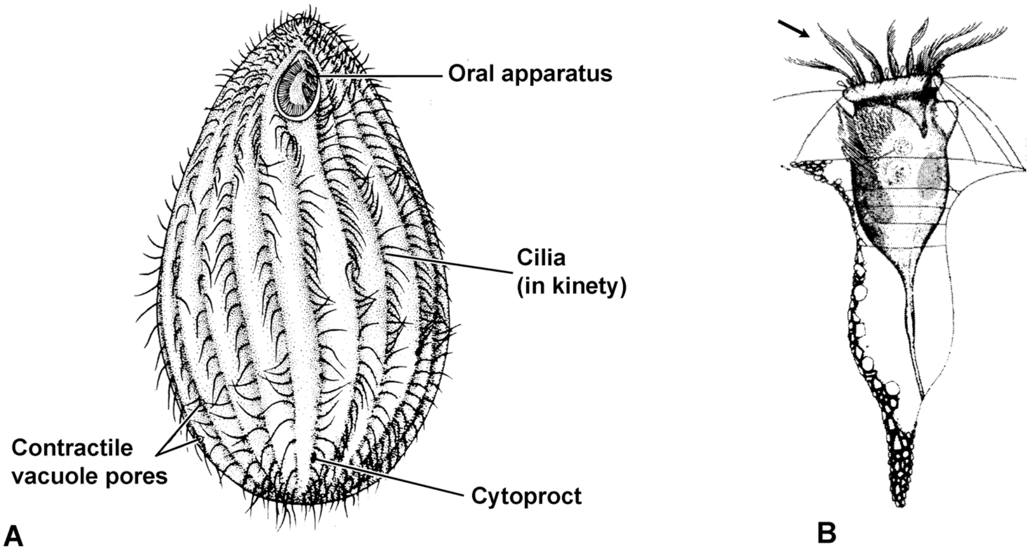


Figure AV4. Example ciliates. A. *Tetrahymena* (Oligohymenophorea). Like many ciliates, the cell is covered in simple cilia organized into rows (‘kineties’). The oral apparatus has a complement of small membranelles. **B.** A tintinnid choreotrich (Spirotrichea). Tintinnids are a major component of the ciliate biotia of the marine plankton. The cell lacks cilia over most of the cell, but has a ring of well-developed oral membranelles (arrow). The cell lives inside a lorica. Modified from Hausmann et al., 2003, after Grell, and MacKinnon and Hawes.

AV4.2 Nuclei

Nuclear dualism is the property of having two different types of nuclei in each cell. They are called **macronuclei** and **micronuclei**. The micronucleus is diploid, is capable of mitosis and meiosis, and contains a genome that is relatively typical for a eukaryote. However, this nucleus is mostly transcriptionally inactive – it can be thought of as the 'germline' for the ciliate. The macronucleus, on the other hand, has a ploidy much greater than two (see below) and is transcriptionally active – in other words, mRNAs are transcribed from the macronucleus and not the micronucleus (the same goes for the ribosomal RNAs). Although the macronucleus contains much more DNA than the micronucleus, the macronuclear genome is actually smaller than the micronuclear genome. In addition to containing less non-coding DNA, the macronuclear genome is broken up into a larger number of chromosomes. In extreme cases, there are thousands of different chromosomes in the macronucleus, each encoding only one or a few genes. Depending on the species of ciliate, and the particular chromosome involved, there are tens-to-thousands of copies of each gene in the macronucleus. In most ciliates the macronucleus divides during each cell division, but the division is not mitotic (i.e. there is no strict segregation of identical chromosomes into the daughter nuclei). As we will see, macronuclei are discarded during sex, and have to be regenerated by transformation of micronuclei.

AV4.3 Reproduction and Sex

Ciliates normally reproduce by asexual binary fission; unusually for eukaryotes this is transversal rather than longitudinal. Occasionally they will instead undergo sex. Most sex in ciliates is conjugative, and involves the fusion of nuclei without the (permanent) fusion of cells. During sex, two ciliates of compatible mating types will pair up and partially fuse. They are then called 'conjugants'. Their macronuclei begin to degrade while the micronuclei of both cells undergo meiotic division, resulting in haploid nuclei. In the model ciliate *Paramecium caudatum*, for example, three of these nuclei degrade, while the fourth undergoes a mitotic division, yielding two genetically identical haploid nuclei (Figure AV5). Then, one haploid nucleus from each conjugant migrates to the other cell, and fuses with the non-migratory nucleus there to produce a zygotic diploid nucleus. The conjugants separate after the nuclear migration, and are then called 'exconjugants'. Obviously, each exconjugant now has a different genotype to that which it had prior to conjugation, even though the cell itself is essentially unchanged in most phenotypic respects.

After conjugation, series of mitotic nuclear divisions takes place in each exconjugant, and some of the micronuclei undergo a developmental process to become new macronuclei (a description of this process is beyond the scope of these notes). Cell divisions (and some more nuclear divisions) eventually result in progeny each with both a micronucleus and a new macronucleus (see Figure AV5).

Most ciliates follow *Paramecium* in that the two conjugants are indistinguishable in size. In some ciliates though, especially sessile forms like peritrichs (see below AV4.4.3 below), the conjugants are very different sizes, usually with the larger remaining sessile and the smaller being motile. In some species the smaller 'male-like' conjugant will completely fuse with (be absorbed by?) its larger conjugating partner.

AV4.4 Ciliate diversity.

The >8000 described species of ciliates cover a huge range of morphologies and life histories. Most ciliates are free-living phagotrophs. Some are free-swimming or free-floating organisms, while others attach temporarily or permanently to surfaces during feeding, e.g. peritrichs, such as *Vorticella*. One unusual case of the latter is seen in the suctorians: species of this group lack cilia in the feeding stage, and trap and consume prey with tube-like extensions of the cell body (though they do usually reproduce and disperse by generating ciliated cells called 'swarmers' or 'buds'). Some surface-attached ciliates form colonies. Some ciliates are parasites of animals – there are important species that parasitise fish and lobsters, for example. There is a large group of anaerobic ciliates that form a major component of the microbiota that live in the rumen of ruminants, such as cattle or sheep. Here we highlight just a couple of important groups:

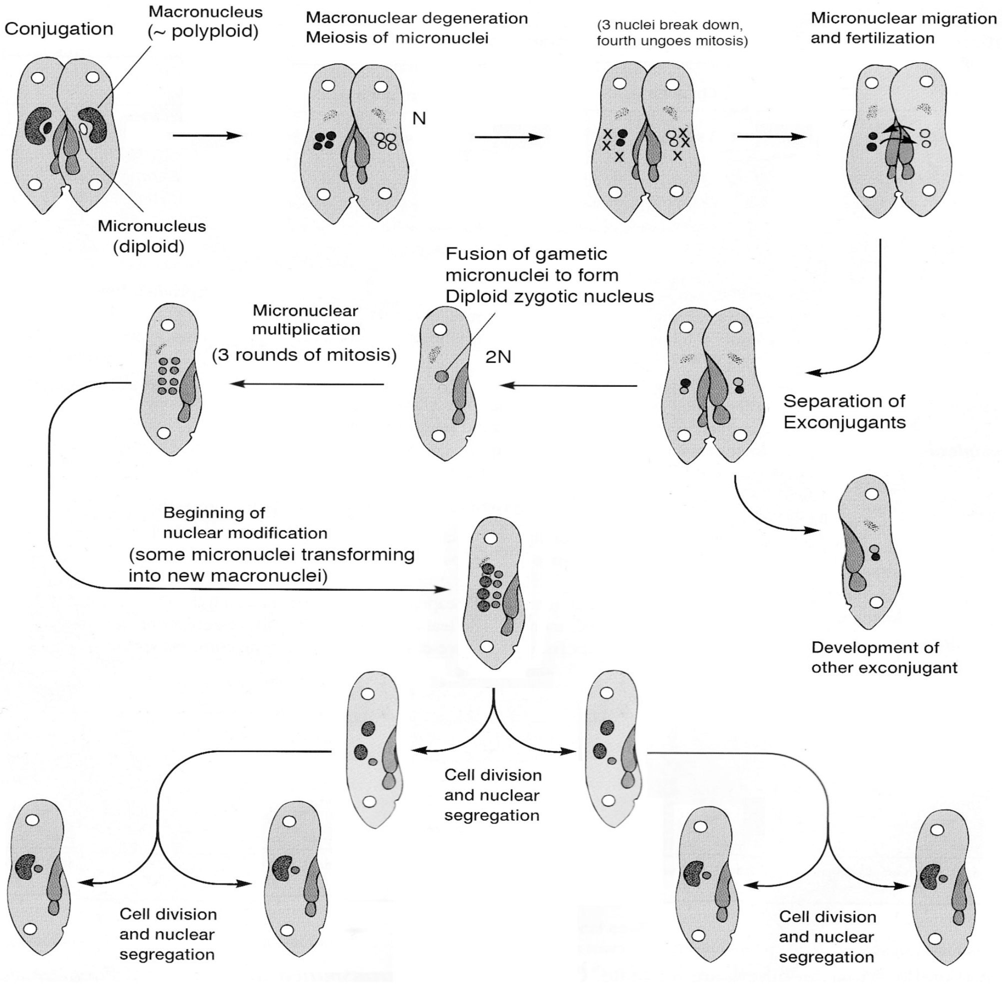


Figure AV5. Conjugative sex and macronuclear regeneration in a model ciliate (*Paramecium caudatum*). Modified extensively from Prescott et al., 2005.

AV4.4.1 *Tetrahymena* and *Paramecium*. The most familiar ciliates are *Paramecium* and *Tetrahymena*. These both belong to a subgroup of ciliates called Oligohymenophorea. Like many ciliates, both *Paramecium* and *Tetrahymena* have simple somatic cilia over the entire cell, and swim through the fluid environment (Fig. AV4A). Species of both *Paramecium* and *Tetrahymena* can be grown readily in the laboratory. They are used for purposes such as bioassays, but are also important model organisms for general cell biology and molecular biology. They are especially important in studies of flagella/cilia and the cytoskeleton. *Tetrahymena,* however, was also employed in the discovery of ribozymes (catalytic RNA molecules), and telomerase (the enzyme that adds telomeres to eukaryotic chromosomes).

AV4.4.2 Spirotrichs. Spirotrichs (Spirotrichea) have a characteristic oral apparatus that includes a long, curving row of membranelles leading to the cytostome. In contrast to *Tetrahymena* and *Paramecium* (for example) many spirotrichs have complex ciliatures, with thick cirri in place of simple cilia on some parts of the cell body, while large areas of the cell surface may have very few cilia or no cilia at all.

Two groups of spirotrichs – Oligotrichia and Choreotrichia, are often the dominant ciliates in ocean plankton, and amongst the most important microplanktonic heterotrophs of any kind. Both tend to have reduced somatic cilia, but the membranelles of the oral apparatus are very well developed, and contain very long cilia (Figure AV4B). These membranelles are used for generating a feeding current, and, in some cases, for swimming as well. Many choreotrichs (the tintinnids) inhabit an organic lorica, which often has various particles associated with it. Tintinnids do not move very much, or are actually attached to larger particles. Choreotrichs and oligotrichs typically feed on small eukaryotes, for example, small microalgae. As with planktonic foraminifera and radiolaria (see Section R, below), many choreotrichs and oligotrichs have algal symbionts, or temporarily retain the plastids from ingested algae, and use them to perform photosynthesis. This latter phenomenon is known as 'kleptoplasty'.

Stichotrichs and hypotrichs are spirotrichs that are commonly associated with surfaces. Many stichotrichs and hypotrichs are flattened. The dorsal face is often poorly ciliated, while the ventral face has numerous cirri that act like legs, such that the ciliate 'walks' on the surface.

AV4.4.3 Some other ciliates. Litostomes (Litostomea) are a diverse taxon of ciliates, many of which specialize in raptorial ('hunting')-type predation of other microbial eukaryotes. *Didinium*, for example, has an anterior oral apparatus without surrounding ciliary structures. It is a specialized predator of *Paramecium* (see above) and was used for some classical predator-prey ecology experiments. Some other litostomes are endobionts of vertebrate animals, such as the entodiniomorphs that are present in abundance in the guts of ruminant mammals (e.g. cattle and sheep). Entodiniomorphs are one of several groups of anaerobes among the ciliates (other example anaerobic protists include metamonads and Archamoebae – covered later). A variety of unrelated ciliates fix to surfaces during feeding, usually generating a current with their oral membranelles while attached. This includes the well known trumpet-shaped *Stentor*, which attaches by its posterior end, but can detach quickly if needed, taking a more ovoid swimming shape. Sessilid peritrichs, meanwhile, are single-celled or colonial forms that lack somatic cilia (while having extensive oral ciliation), and which attach to surfaces more permanently by a long contractile stalk (peritrichs belong to Oligohymenophorea and thus closely related to *Paramecium* and *Tetrahymena*). Perhaps the most bizarre ciliates are the suctoria, which in the feeding state are stationary, attached cells with numerous long, stiff tentacles, but may have no cilia at all. The tentacles trap and ingest prey cells that collide with their ends (the prey contents are actually sucked-up along the tentacles). Most suctoria do produce ciliated swarmer cells, which will swim before attaching somewhere and transforming into the feeding form.

AV5. Other Alveolates

In addition to the three well-known groups discussed above there are several additional alveolate taxa known. **Perkinsids** are aquatic parasites with a flagellated zoospore phase in their lifecycle that are related to dinoflagellates. *Perkinsus marinus* an important parasite of oysters. **Colpodellids** are free-living flagellates that specifically consume other eukaryotic cells, with most species being restricted to certain prey types. They are more closely related to apicomplexan parasites. Interestingly, both perkinsids and colpodellids have structures that closely resemble the apical complex. This apparatus is actually involved in the feeding process in colpodellids. This hints at an ancient origin for the apical complex, perhaps originally with a function in predation rather than host invasion. Even more remarkably, two recently discovered single-celled algae – ***Chromera*** and ***Vitrella*** – have turned out to also be closely related to apicomplexan parasites (they form two separate branches in amongst the colpodellids, and some mysterious parasitic organisms). Both *Chromera* and *Vitrella* have fully photosynthetic plastids, and provide a fascinating window into the evolutionary origins of the non-photosynthetic 'apicoplast' of apicomplexan parasites (See AV3.4, above). Finally, **colponemids** are poorly studied flagellates that prey on other protists, generally other small flagellates, but lack an apical complex (unlike colpodellids – see above). Molecular phylogenies indicate that colponemids are extremely deeply diverging within Alveolata, and represent one or more major phylogenetic divisions in addition to (i) ciliates and (ii) the clade that includes dinoflagellates, perkinsids, apicomplexa, colpodellids, *Chromera* and *Vitrella*.

PART 2, SECTION R: RHIZARIA

R1. Introduction

Rhizaria is a major group of eukaryotes that is largely composed of 'protozoa'. The grouping was recognized through molecular phylogenetics, since members of the group often share little superficial similarity to one other, and share no unique morphological characters. The closest thing to a distinctive morphological feature uniting Rhizaria is the fact that many (not all) produce fine pseudopodia for feeding. However, it must be noted that this feature is found in various other groups of eukaryotes as well. Furthermore, the pseudopodia have different structural bases in different rhizarians - they are supported by actin microfilaments in some taxa, but by microtubules in others.

As with excavates (Section E, below), most members of Rhizaria are heterotrophic, much of the diversity of the group is poorly understood, and the best-known organisms within the group are not particularly representative of the taxon. Rhizaria also includes several parasitic/ commensal lineages, including the fungi-like plasmodiophorids, which primarily infect plants, and the Haplosporidia, which infect marine animals, such as commercially valuable oysters (we will not discuss either of these two groups in detail here). Rhizaria also includes a single group of secondary algae – Chlorarachniophyta – that have a plastid of green algal origin.

Multi-gene phylogenetic analyses have shown that Rhizaria is closely related to stramenopiles and alveolates, forming a major clade called 'Sar' (SAR - 'Stramenopiles, Alveolates, and Rhizaria').

R2. 'Typical' Rhizaria

Many rhizarians are small-to-medium-sized heterotrophic flagellates that glide over surfaces and consume particles such as prokaryotes and other small eukaryotes, which they usually capture and engulf with fine pseudopodia (cercomonads are an example: Figure R1A). Such organisms are very common in benthic and soil habitats, and probably constitute important components of the biota of these systems, but they are not well studied. Other rhizaria are similar, but exist primarily as amoebae (i.e. the main trophic phase does not have flagella). The ancestral rhizarians were probably small flagellates with fine pseudopodia, or perhaps amoebae with a flagellated dispersal stage.

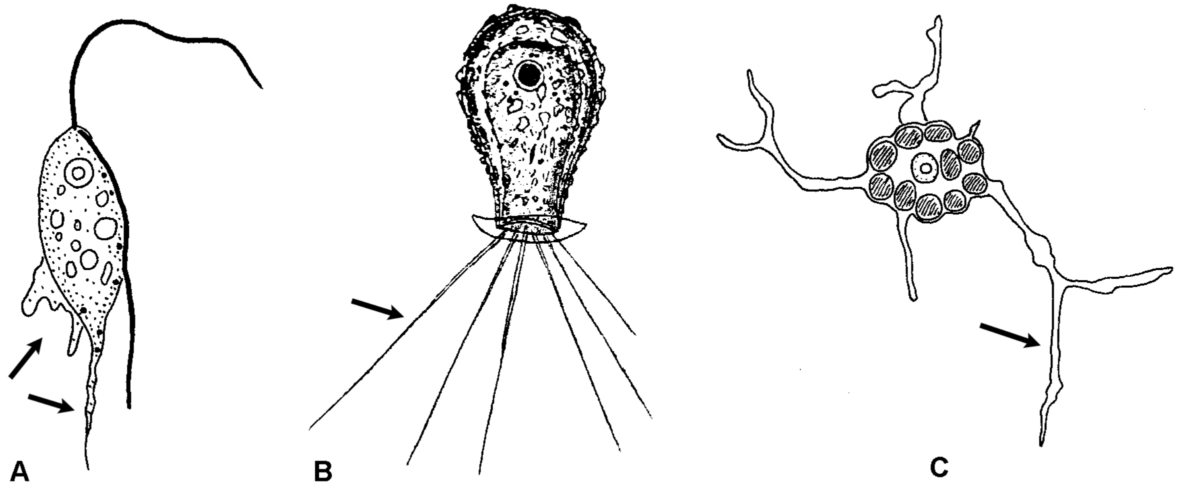


Figure R1. Some Rhizaria. **A.** Cercomonad, a type of flagellate common in soil and sediments (cell body ~10 µm). **B.** Filose testate amoeba (cell body ~30 µm). **C.** Chlorarachniophyte (cell body ~10 µm), a mixotrophic amoeboid alga - shaded organelles represent plastids. Note the presence of (usually) fine pseudopodia in all three organisms (arrows). The pseudopodia are used for prey capture (and locomotion in non-flagellate species). B modified from Lee et al. 2002, after Penard, 1902.

R3. Filose testate amoebae (Euglyphids)

Filose testate amoebae (Figure R1B) are common in freshwater and soils. The cell is protected by a test. Fine, elongate pseudopodia emerge from a single aperture within the test. The best known by far are the Euglyphids, which are a single cohesive group within Rhizaria. In Euglyphids the test is composed of overlapping scales that are made of silica. These scales are produced intracellularly by the amoeba.

Although most euglyphids are heterotrophs, one group, *Paulinella*, includes some species that photosynthesise thanks to possessing a cyanobacterium-derived symbiont/organelle called the 'chromatophore'. The chromatophore represents a kind of primary endosymbiosis, but is not related to the plastids of Archaeplastida (See Section A). *Paulinella* therefore represents a tiny, partial exception to the truism that all eukaryotic algae ultimately obtained their plastids from a single original endosymbiosis.

R4. Foraminifera

Foraminifera (also known affectionately as 'forams') are a diverse group of unicellular amoeboid organisms, most of which are marine cells with a mineralized external shell, or 'test'. A few have a thin organic test or are naked. Foraminifera are of major ecological significance in both planktonic and benthic environments, and are of geological importance. There are ~4,000 described living species, however the fossil record of foraminifera goes back to the Cambrian (>500MYA), and includes ~40,000 described species. Most foraminifera are relatively large cells with tests that are hundreds of micrometres across. Some species have tests that are several mm in diameter, or more!

The pseudopodia of foraminifera are long and thin, and supported internally by microtubules. The pseudopodia branch and rejoin readily to form a network. They are used for prey capture, for movement (in benthic forms), and are involved in test construction in species that assemble the test from foreign particles (see below). The pseudopodia also act as a transport system, with organelles and vesicles being shuttled rapidly in both directions using intracellular transport mechanisms (i.e. dyneins and kinesins that travel along the pseudopodial microtubules).

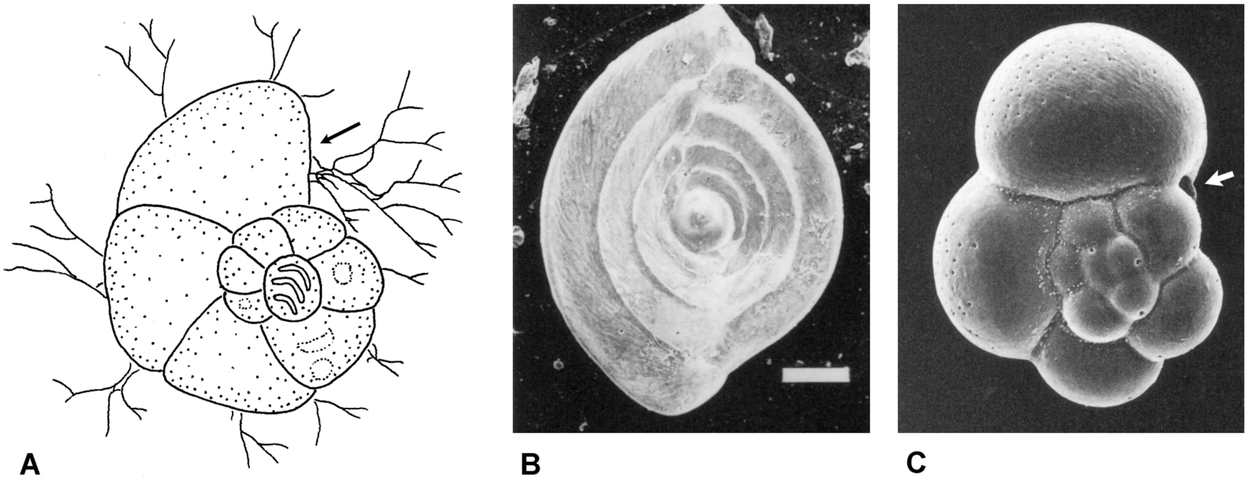


Figure R2. Foraminifera. A. Appearance of a live foraminiferan. Pseudopodia radiate from around the cell. The apeture is not seen in this view – its position is shown by the arrow. **B.** Test of a benthic foram (scanning electron micrograph - SEM). The chambers are arranged in a spiral in this species but each chamber takes up half of one circuit. The test is ~0.5 mm across. **C.** Test of a planktonic foram (SEM). The aperture can just be seen (arrow). This test is ~100 μm across. A redrawn from Lee et al. 2002. B, C From Lee et al., 2002.

The tests of foraminifera are very diverse. In some benthic forms the shell is ‘agglutinated’, that is, composed of mineral particles from the environment (e.g. sand grains), embedded in an organic ‘cement’. In the best-known foraminifera, however, the test is ‘calcareous’- composed of calcium carbonate that is deposited in crystal form by the foraminiferan itself. Calcareous tests can be elaborately ornamented. The test wall itself is generally not solid calcium carbonate, rather it has an open structure, with numerous pores leading to the outside. In most species, the test is enlarged as the organism grows by the addition of larger and larger chambers, with each new chamber being added alongside the previous largest chamber (Figure R2). The chambers are often arranged as a spiral. These chambers are not separate from each other - there are holes called ‘foramina’ connecting adjacent chambers, and in fact the cytoplasm of the cell infiltrates most of the larger chambers of the test. Most of the communication with the external environment is through one or several large ‘apertures’ in the largest chamber. However, the cytoplasm and pseudopodia of the cell usually can spread over the surface of the test, so that the pseudopodial network can extend from around much of the cell, not just from the apertures (Figure R2A).

The lifecycles of foraminifera may be complicated. In the so-called ‘classical’ lifecycle, there is an alternation of haploid and diploid generations (often called the ‘gamont’ and ‘agamont’ respectively). Young haploid individuals grow by the addition of test chambers until they reach a mature size. Multiple rounds of mitosis, then multiple fission, results in numerous haploid gametes that escape the discarded parental test. These gametes are flagellated in many species. Gametes (usually from different parents) fuse, resulting in diploid cells that begin to produce a test. These eventually grow into large multinucleate adult cells. The next cycle of reproduction involves meiosis of these nuclei, and multiple fission to yield numerous small haploid amoebae that evacuate the discarded parental test and begin to grow their own tests, thus completing the life cycle. Sometimes the mature haploid and diploid phases look very different and may be mistaken for different species. The life cycle of large foraminifera can take months or years to complete.

Most groups and species of foraminifera are benthic organisms. Individual amoebae live on or within the sediment and explore the surrounding material with their pseudopodial network to find food particles. They live in habitats ranging from tropical reefs to the Antarctic, and the deep sea. They may be extremely abundant, and can form a large percentage of the total biomass in the sediment. One group of calcareous-shelled foraminifera – globerigerinids - are planktonic (Fig. R2C). They float in the water column and trap prey that collide with their pseudopodia.

Foraminifera will consume all manner of prey, ranging from prokaryotes to other large unicellular eukaryotes, or even small animals. A large fraction planktonic and shallow-water benthic species maintain plastids from algal prey, or harbour endosymbiotic microalgae, and are therefore, functionally mixotrophic (i.e. capable of both photosynthesis and phagotrophy). The endosymbiotic algae harboured by foraminifera include unicellular rhodophytes (red algae), chlorophytes, diatoms, dinoflagellates, and other groups. Foraminifera with endosymbiotic algae have thin tests that transmit light well, or at least have thinned windows in the test under which the algae tend to be located. These symbiotic associations are probably essential for growth in many foraminiferan species.

Both benthic and planktonic foraminifera make huge contributions to sediment production, and thus play a major role in the global carbon cycle. The annual production of calcium carbonate by calcareous benthic foraminifera can be kilograms per square metre of seafloor per year. Parts of the ocean floor are covered in sediments that are dominated by planktonic foraminiferan tests that have sunk from the overlying water column (‘globerigerinan ooze’). Some major limestone formations are essentially massive deposits of foraminiferan tests. One example is the rock from which the Egyptian pyramids were constructed. Being so abundant, fossil foraminiferan tests are also a mainstay of the science of stratigraphy, and are crucial indicators of sediment types and ages in fossil fuel exploration.

R5. Radiolaria

Radiolaria are a distinctive kind of planktonic marine amoebae. They have radiating pseudopodia supported by arrays of microtubules, and they usually have an internal mineral skeleton (Figure R3). Traditionally there are two main types of Radiolaria recognized – acanthareans and polycystines (though it appears that polycystines are actualy a paraphyletic group). The skeleton of acanthareans is composed of strontium sulfate ($SrSO_4$) and consists of 20 or 10 long spines (spicules) radiating from the centre of the cell (Figure R3). The skeletons of polycystines are made of silica and take a variety of radially symmetrical shapes. A few polycystines lack a skeleton and some are colonial.

The cell is organized into two distinct areas – a 'central capsule' containing 'endoplasm' and peripheral area of 'ectoplasm' (Figure R3). The endoplasm contains most of the cell organelles, including nuclei, mitochondria, and so on. The ectoplasm is very diffuse, with most of its volume taken up by large vacuoles, and it is the site of prey digestion. The border between the ectoplasm and endoplasm is defined by an organic capsule wall, which is probably made predominantly of protein. There is limited cytoplasmic communication across the capsule wall via pores or elaborate apertures called 'fusules' (in polycystines). The bundles of microtubules supporting the pseudopodia, and elements of the mineral skeleton also pass though the capsule wall.

Radiolaria show ecological similarities to planktonic foraminifera (see R4, above). They consume other planktonic organisms that encounter their pseudopodia, especially other microbial eukaryotes. Again, however, many radiolarian species harbour algal endosymbionts, usually dinoflagellates or the haptophyte *Phaeocystis* (see CA4.2). Curiously, the symbionts are housed in the endoplasm in acanthareans and the ectoplasm in polycystines. The lifecycles of Radiolaria are poorly understood.

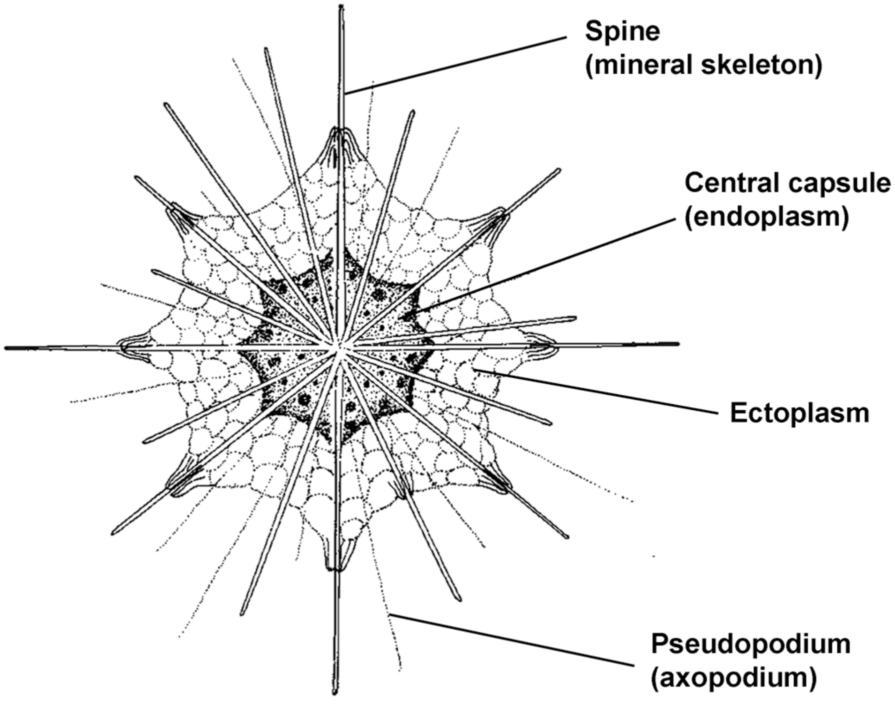


Fig. R3: Radiolarian (an Acantharean). Diagram showing appearance of the living cell. These cells can be very large (0.5 mm across, or more). As in most acanthareans, the skeleton is made up of 20 radiating spines composed of strontium sulfate. Note the 'frothy' nature of the endoplasm, most of the volume of which is taken up by large vacuoles, which probably fulfill a role in buoyancy. Modified from Anderson, 1991.

One very strange radiolarian is an organism called *Sticholonche*. This marine planktonic cell has stiff microtubule-supported axopodia, but unlike typical radiolaria, these rhymthically sweep forwards and backwards to 'row' the cell through the water - an unusual case of a microbial eukaryote legitimately 'swimming' without using flagella. It is also has silica spicules (on its surface, rather than as a skeleton).

Another group of skeleton-bearing amoebae – Phaeodarea - were also traditionally considered to be Radiolaria. Phaeodarea are poorly understood planktonic organisms found in the deeper ocean. They have a silica skeleton, typically with bilateral symmetry. Molecular phylogenetic studies show that Phaeodarea are a separate branch within Rhizaria – more closely related to cercomonads, euglyphids or chlorachniophytes (for example), than to acanthareans and polycystines.

R6. Chlorarachniophyta

The chlorarachniophytes typically have an amoeboid trophic (feeding) phase, but may appear as a rounded (coccoid) cell with a wall, or produce a swimming phase with a single flagellum (all three life history stages are not known for every species). The amoebae are mixotrophic – capable of phagocytosis and photosynthesis. They produce fine pseudopodia that are used to capture prey, but also have functional plastids (Figure R1C). These plastids are green, contain chlorophylls *a* and *b* and are surrounded by four membranes. Phylogenetic analyses of plastid genes confirm that this plastid was acquired by the engulfment of a 'green alga' (i.e. a photosynthetic eukaryote), and represents a case of secondary endosymbiosis. In fact, the nucleus of the engulfed alga is retained in very reduced form as the **nucleomorph**, which sits between the inner pair and outer pair of membranes (see Part 1, Section 1.4).

One other major group of algae – cryptophytes - also retains a nucleomorph, but as a result of a completely different secondary symbiosis (see Section CA3, above). Note also that the secondary endosymbioses that gave rise to chlorarachniophyte plastids was a separate evolutionary event from the event that gave rise to the plastids of green euglenids (see Section E7.1); they involved two different green algal symbionts.

PART 2, SECTION E: EXCAVATES

E1 Introduction:

The 'excavates' are a diverse collection of microbial eukaryotes. Most excavates are heterotrophic unicellular flagellates, although one group (Heterolobosea) are mostly amoebae when in the trophic (feeding) phase, while some members of another group – euglenids - are unicellular microalgae. Many excavates are free-living organisms, however the assemblage includes several major groups of parasites, symbionts and commensals. Several important diseases of humans are caused by excavates – Sleeping sickness, Chagas' disease, Leishmaniasis, Giardiasis and Trichomoniasis.

From an evolutionary perspective, excavates are of special historical interest. In the later 1980s, attempts to reconstruct the evolutionary tree of eukaryotes using sequences of ribosomal RNA genes identified a series of microbial eukaryotes as 'deep branches' that seemed to have diverged early on from other eukaryotes, with the deepest branches of all being cells that appeared to lack mitochondria. The apparent deep branches included Euglenozoa and Heterolobosea (which have mitochondria), and diplomonads and parabasalids (which lack normal mitochondria), all of which are now assigned to one or other of the groups of 'excavates'. It is now known that this model of the eukaryotic evolutionary tree is incorrect: the 'mitochondrion-lacking' lineages are actually all descended from eukaryotes with mitochondria capable of respiration.

Excavates continue to be an evolutionary conundrum. There are three primary subgroups of excavates that researchers are confident represent natural (monophyletic) groups, based on molecular phylogenies: i) **Discoba**, consisting of Euglenozoa, Heterolobosea, jakobids and the obscure *Tsukubamonas*, ii) **Metamonada**, which are all anaerobes, and include diplomonads, parabasalids, oxymonads and several other lineages, and iii) the small flagellate group called Malawimonadida (malawimonads). Some discobids, many metamonads, and all malawimonads share a very similar 'typical excavate' cell structure, suggesting that they are descended from a common 'excavate' ancestor. However, the three groups usually do not branch together in molecular phlyogenies, though, confusingly, some analyses place Metamonada with Discoba (separate from malawimonads), and others place Metamonada with malawimonads (but usually separate from Discoba). One possibility is that 'typical excavates' represent an ancestral state from which many groups of living eukaryotes evolved, however there are also good reasons to suspect that the best molecular phylogenies are still unreliable (and that resolving the evolutionary relationships amongst excavates is a very difficult technical problem).

E1.1. A note on 'mitochondrion-lacking' eukaryotes.

Electron microscopy studies show that some eukaryotes, including many excavates, do not have 'normal-looking' mitochondria with mitochondrial cristae. Instead they either i) have organelles that are surrounded by two membranes, but lack (many) cristae, or ii) no organelle has been observed yet. The organisms with these unusual mitochondria are most found in anoxic (anaerobic) or micro-oxic habitats.

As discussed above, some of these organisms used to be thought of as early-branching eukaryotes that had diverged before the mitochondrial symbiont was acquired. The current understanding, however, is that all of these groups descended from ancestors that already had mitochondria. The organelles found in many 'mitochondrion-lacking' eukaryotes are homologous to mitochondria – they are different versions of the same organelle that are adapted to different functions and conditions. There are two very different types of these mitochondrion-related organelles that we will discuss here: **Mitosomes** and **Hydrogenosomes**.

Mitosomes tend to be very small and they have no known role in energy metabolism. Although they do not generate ATP for the cell they still fulfill some of the other essential functions that are performed by normal mitochondria. The parasitic diplomonad *Giardia* is one of the best studied mitosome-bearing eukaryotes (see Section E4, below). The only known function of *Giardia* mitosomes (from the rest of the cell's perspective) is a major role in the assembly of iron-sulfur clusters. Iron-sulfur clusters are essential components of the active sites of certain enzymes.

Hydrogenosomes were first discovered in the excavate group Parabasala, and are also found in some distantly related eukaryotes, for example, some ciliates. Hydrogenosomes are about the same size as mitochondria, and are involved in energy metabolism. However, they do not have a functioning TCA cycle, they lack most or all of the mitochondrial electron transport machinery, do not use the proton motive force to synthesise ATP and don't respire oxygen – they are actually fermentative. Like mitochondria, hydrogenosomes can utilize the end-product of glycolysis, the 3-carbon molecule pyruvate. Within hydrogenosomes, pyruvate is cleaved to form a 2-carbon portion, and carbon dioxide, with the loss of an electron pair (i.e. pyruvate is decarboxylated and oxidised). The 2-carbon molecule is later released as acetate (CH_3COO^-), yielding one ATP molecule by substrate-level phosphorylation as part of the process. The electrons realized from pyruvate are ultimately accepted by H^+ ions (protons), and hydrogen gas (H_2) is released. Each molecule of pyruvate processed by the hydrogenosome therefore yields one 'extra' ATP.

The waste products of hydrogenosomes (acetate and H_2) are useful for some prokaryotes. For example, H_2 is used as a substrate by methanogenic archaea. Methanogens and hydrogenosome-bearing eukaryotes, including parabasalids, often co-exist in habitats such as the hindguts of termites. Certain other hydrogenosome-bearing eukaryotes harbour endosymbiotic methanogens, which reside within the eukaryote's cytoplasm alongside the hydrogenosomes.

E2. Types of Excavates

Excavates are currently divided into more than a dozen subgroups. Half of these groups are very poorly studied, and include very few described species. They are of evolutionary interest because of the way that they are related to the more familiar groups, and because they retain many morphological features that were probably present in the most recent common ancestor of all excavates. For want of a better term we refer to these here as the 'typical excavates'. The other, more familiar groups are thought to have evolved from ancestors resembling typical excavates, and are covered separately.

E3. 'Typical excavates' – appearance and basic biology.

Typical excavates are unicellular heterotrophic flagellates that usually feed on prokaryotic cells. Depending on the group they vary in size from about 5 µm to around 30 µm, and have either two or four flagella (see Figure E1). Like many small flagellates, the 'flagellar apparatus' is located near the anterior end of the cell, and the nucleus is closely associated with the flagellar apparatus (see Figure 1.2 in Part 1 for a short commentary on the concept of the 'flagellar apparatus'). One flagellum is directed posteriorly, another more-or-less anteriorly (the other two flagella, if present, are directed laterally). The most conspicuous feature of the organism is a groove that extends down most of one side of the cell. The groove is supported primarily by arrays of microtubules that originate from the flagellar apparatus, and lie immediately under the cytoplasmic membrane.

The groove and posterior flagellum form the food-capture apparatus of the cell. The posterior flagellum bears 1-3 broad vanes, and beats within the groove, generating a feeding current. Prokaryotic cells that are suspended in the water column are trapped within the posterior part of the groove and are phagocytosed there.

Jakobids are a group of mitochondrion-bearing 'typical excavates' that belong to the Discoba clade, i.e. they are most closely related to Euglenozoa and Heterolobosea (see E6 and E7, below). Jakobids are small, free-living cells with two flagella. In some jakobid species the cells live within an organic lorica that attaches to surfaces. Interestingly, the mitochondrial genomes of jakobids contain more protein-coding genes than those of other eukaryotes – more than four times as many as the mitochondrial genomes of animals, for example. This gene complement includes several genes that are not found in any other mitochondrial genomes, the most remarkable of which are genes encoding subunits of a bacterial-type RNA polymerase. No other mitochondria are known to use this RNA polymerase; the mitochondrial RNA polymerase in other eukaryotes is a completely different protein related to the RNA polymerases of certain viruses (and is always encoded by a nuclear gene).

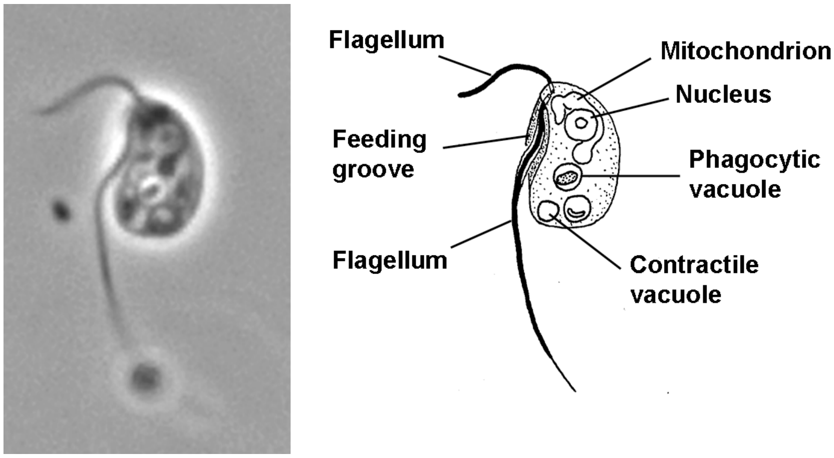


Figure E1. A 'typical excavate' (malawimonad shown). The image on the left is a light micrograph of a living cell. The drawing on the right is an interpretive diagram. The cell body is about 5 μm long. Note the vanes on the posterior flagellum (in practice these are very difficult to see under the light microscope). The labelled phagocytic vacuole contains a prokaryotic cell.

The evolutionarily puzzling group called malawimonads are also typical excavates with aerobic mitochondria (see E1, above and Figure E1).

Other 'typical excavates' belong to the taxon Metamonada and lack normal mitochondria (see E1 above). The best known are **Trimastigids, *Carpediemonas*** (and *Carpediemonas*-like organisms) and **retortamonads**. Trimastigids and *Carpediemonas* are free-living and inhabit environments with little free oxygen, such as water that is highly enriched with organic material (eutrophic), or sediments. Almost all retortamonads inhabit the intestinal tracts of animals, including humans. They are usually regarded as harmless commensals, rather than parasites. *Carpediemonas*-like organisms and retortamonads are closely related to one of the best-known groups of excavates – the diplomonads (see next section, E4).

E4. Diplomonadida

Diplomonads are a remarkable group of heterotrophic flagellates. Each diplomonad cell is symmetrical – each half includes one nucleus that is associated with a flagellar apparatus that usually includes four flagella. Thus there are two near-identical nuclei and two flagellar apparatuses per cell (Figure E2A). In many diplomonads, each flagellar apparatus is associated with a groove, or a tube that encloses one flagellum – these structures are involved in feeding. Free-living diplomonads are commonly seen in many oxygen-poor environments, swimming relatively rapidly, and generally feeding on prokaryotes. Most diplomonads, however, live within the intestinal tract or other tissues of animals. Some cause significant disease, for example, several species of *Spironucleus* cause systemic diseases in various fish, including farmed salmon. Humans are infected by the diplomonad *Giardia*.

E4.1 *Giardia* is an unusual cell, even for a diplomonad. In *Giardia* the two clusters of flagella are located together in the middle of the cell. There is no distinct feeding apparatus and the organisms are incapable of phagocytosis. One side of the cell forms a disk-shaped structure that the cell uses to attach itself to the gut wall of its host (Figure E2B). *Giardia* infects many mammals. The infection is acquired by ingesting 4-nuclei cysts that activate in the upper part of the intestine, releasing cells that undergo division to produce 'trophozoites' (i.e. normal, active cells). The trophozoites attach to the wall of the upper intestine. Trophoziotes divide by binary fission and sometimes transform into new infective cysts that are shed in the feces. The cysts are highly resistant to chemical attack.

The human parasite *Giardia intestinalis* (also known as *Giardia lamblia*), is a diverse species complex. Some subtypes may be passed between animals and humans (i.e. are 'zoonotic'). *Giardia* causes a diarrheal disease in humans, called 'giardiasis' (or 'beaver fever'). The infection is usually acquired from drinking water, or is transmitted between young children. In the first world, giardiasis in adults is popularly associated with camping (presumably zoonotic). There are a couple of million infections per year in the USA and Canada. In the developing world, however, giardiasis (and other diarrheal diseases caused by eukaryotes) are endemic in regions with poor sanitation, and there are literally hundreds of millions of infections every year from regular drinking water supplies. Diarrheal diseases are in fact a significant cause of morbidity and mortality in the developing world.

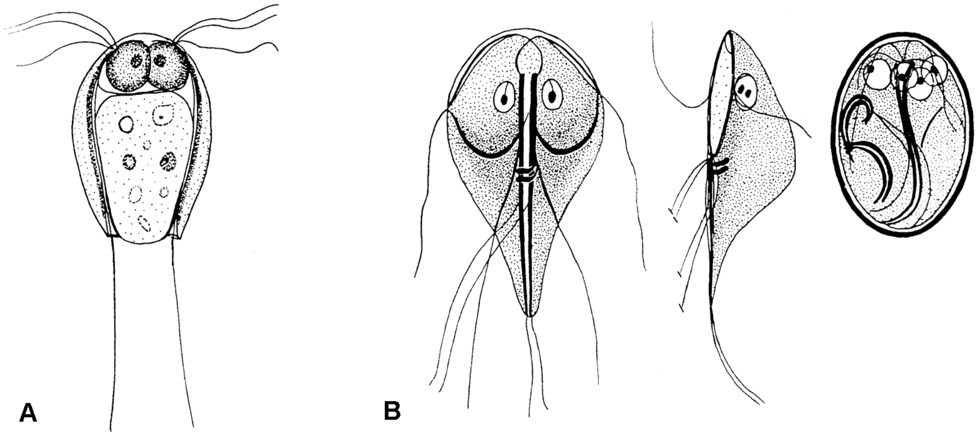


Figure E2. Diplomonads. A. *Hexamita*. The 'goggle eyes' at the anterior end of the cell are actually the two nuclei. Each nucleus is associated with four flagella, one of which runs posteriorly inside a feeding tube. **B.** *Giardia* in ventral view (L), lateral view (C), and in cyst form (R). Note the ventral adhesive disk. From Lee et al., 2002, after Lemmerman, 1910 and Kofoid and Swezy, 1922.

E5. Parabasala

Parabasalids are a diverse group of microbial eukaryotes, almost all of which are commensals or parasites of animals. Parabasalids lack normal mitochondria, but instead have 'hydrogenosomes' that generate ATP through an anaerobic pathway (see E1.1 above).

The simplest parabasalids have a single nucleus associated with the flagellar apparatus, which typically includes 4-5 flagella (Figure E3A). One flagellum is usually attached back to the main cell body, forming an 'undulating membrane'. The cell is supported by the 'axostyle', a bundle of microtubules that extends as a rod down the centre of the cell. A distinctive set of non-microtubular fibres connect the Golgi apparatus to the flagellar apparatus – these are the so-called 'parabasal fibres'. There is no obvious ingestion apparatus - particles are phagocytosed along the cell surface.

In several groups of parabasalids this basic arrangement has been independently modified by great expansion in the number of flagella. This is achieved either i) by having many flagellar apparatuses with ~4 flagella, each associated with a nucleus, or ii) by having only one or a few individual flagellar apparatuses, with many replicates of one particular flagellum forming long rows or fields of parallel flagella (see Figure E3B). While there are many species of large multiflagellated parabasalids, they are found in only one habitat - the hindguts of certain termites and wood-eating cockroaches. There they co-exist with a complex microbial biota that includes many types of prokaryotes, and some other eukaryotes, especially small parabasalids and many **oxymonads**. Oxymonads are another group of metamonads that are found only in animal guts. They often superficially resemble parabasalids, but evolved independently – we will not discuss them separately.

Large parabasalids and oxymonads are important symbionts for the termites. They produce cellulases, enzymes that degrade cellulose, a major component of woody plant material. These cellulases, together with those produced by prokaryotes, are a significant supplement to the cellulases produced by the termite itself. Many large parabasalids and oxymonads phagocytose whole wood particles, which are then digested within the protozoan cell, or are broken down into smaller particles which, when excytosed, are more susceptible to digestion by prokaryotes. Some end products of the anaerobic metabolism of the large protozoa (e.g., acetate and other organic acids) can then be absorbed and utilized by the insect for aerobic energy production. Other products of their metabolism are substrates for various prokaryotes, which in turn release products useable by the insect.

Parabasalids are also responsible for some common and/or important diseases. In cattle, *Tritrichomonas suis* (also known as *Tritrichomonas foetus*) is a sexually transmitted parasite that causes spontaneous abortion, and can result in permanent infertility in cows. *Trichomonas vaginalis* is the most common STD in humans – there are ~250 million infections worldwide each year (2005 estimate). Trichomoniasis is of relatively minor consequence in of itself, and often asymptomatic (especially in males). However, there are indications that *Trichomonas* infection is problematic in pregnancy (e.g. lower average birthwieght) and markedly increases the risk of HIV transmission to women, due to inflammation and damage to the vaginal mucosa. In 2017 there is an outbreak of *Trichomonas gallinae* in wild birds in Halifax. This trichomonad passes between birds via interactions during feeding, or via infected feed (rather than sexual contact, for example), and residents were therefore advised to take down bird-feeders.

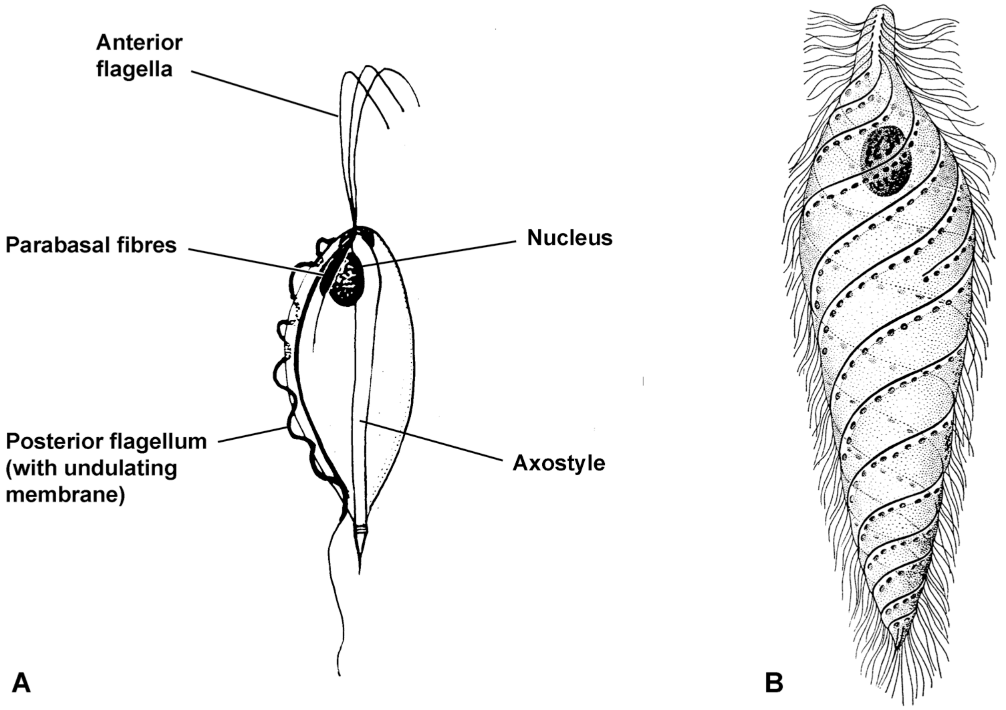


Figure E3. Parabasalids. A. *Tritrichomonas*, about 15 μm long, with four flagella. **B.** *Spirotrichonympha*, about 100 μm long, with hundreds of flagella, from the hindgut of a wood-eating insect. The dark oval is the nucleus. Modified from Lee et al., 2002, after Samuels, 1957 and Grassi, 1971.

E6. Heterolobosea.

Heteroloboseids (also known as 'amoeboflagellates') have two distinct active forms – amoeba and flagellate – with the amoeba also able to transform into an inactive cyst. In most heteroloboseids, the amoeba is the trophic (feeding) stage. The amoebae produce broad pseudopodia for locomotion and feeding - they feed primarily on prokaryotes. The flagellate form, which usually has 2-4 parallel flagella, functions primarily in dispersal. In some species, however, the flagellate form is also capable of feeding, using a groove structure that is somewhat similar to the feeding grooves of 'typical excavates'. Some species lack the flagellate phase altogether, while other species lack an amoeba phase, and exist as heterotrophic flagellates.

Heteroloboseid amoebae are found in diverse locations, but are especially common in soil. In the Simpson lab we have found several species that are very halophilic (salt-loving), and able to grow at salinities at least 5.5 times higher than seawater. However, the most famous (notorious) heterolobose amoeba is *Naegleria fowleri*. This species is a facultative parasite of humans that causes Primary Amoebic Meningoencephalitis (PAM), a brain infection that is almost always fatal (it is difficult to diagnose quickly enough to effect treatment). Infection is generally initiated through the nasal passages after swimming in very warm freshwater, especially when sediment is disturbed. This disease is extremely rare – only a few hundred cases have ever been confirmed.

E7. Euglenozoa.

This diverse group of excavates requires detailed attention. The taxon Euglenozoa includes two rather different main subgroups – euglenids (many of which are photosynthetic) and kinetoplastids (a 'protozoan' group, which includes many important parasites).

Most euglenozoan cells are elongate and supported by parallel microtubules that lie beneath the cell membrane. There is typically a single, often highly branched mitochondrion, and the mitochondrial cristae are usually discoidal in shape. The flagella (there are usually two) insert into a deep depression in the cell surface called a 'flagellar pocket'. The flagella both have 'paraxonemal rods' – cytoskeletal elements that run alongside the flagellar axoneme within the flagellar membrane (Figure E4D). The feeding apparatus is a tubular structure supported by microtubules. In contrast to other excavate flagellates, heterotrophic Euglenozoa typically feed by actively moving through the environment and capturing individual prey particles. Most are adapted to feeding in close association with surfaces, and will glide or 'skid' along a substrate and capture attached particles.

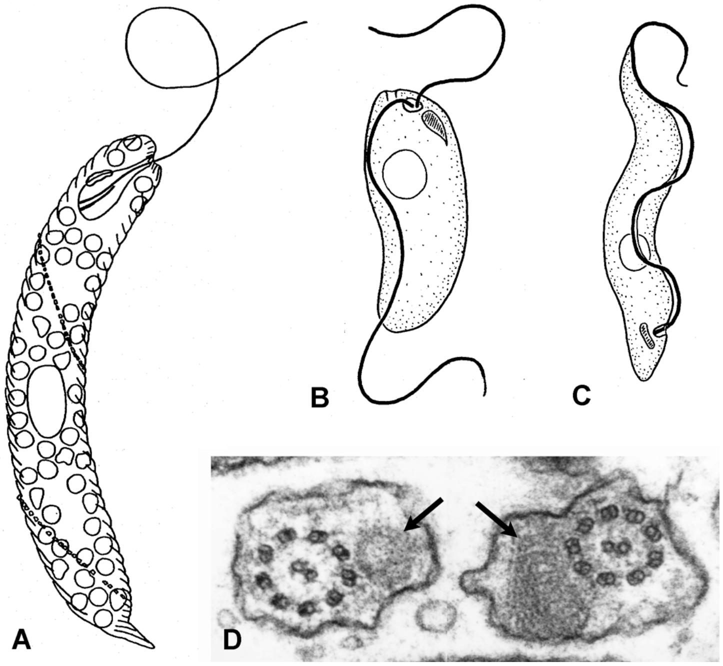


Figure E4. Euglenozoa. A. *Euglena*, a photosynthetic euglenid. The small discs are the plastids. Note the indication of pellicular strips. **B.** *Parabodo*, a free-living kinetoplastid. The indentation at the anterior end is the opening of the feeding apparatus. **C.** *Trypanosoma*, a trypanosomatid parasite. The shaded organelles in B and C represent the kinetoplast, which lies inside the mitochondrion (not shown). **D.** Composite transmission electron micrograph showing transverse sections of the two flagella of a euglenozoan. Paraxonemal rods (arrows) lie alongside the flagellar axoneme inside the flagellar cell membrane. 'A' redrawn from Leedale, 1968.

E7.1 Euglenida. Euglenids have a distinctive 'euglenid pellicle' – a series of abutting proteinaceous strips that lie immediately underneath the cell membrane and, in turn, are supported by the membrane-supporting microtubules (described above). The pellicular strips may be fused to each other, giving the cell a rigid structure, or the strips may be able to slide and are arranged in a spiral around the cell, in which case the cell can actively alter its shape between 'elongate' and 'rounded'. This deformation is often called 'euglenoid motion' (also, confusingly 'metaboly'). Euglenids use a distinctive polysaccharide, paramylon, as a storage product. Paramylon is similar to starch, but with different (β 1→3) chemical linkages between the glucose monomers. There are both heterotrophic and photosynthetic euglenids.

The ancestral euglenids were phagotrophic, and there is a considerable diversity of phagotrophic euglenids on Earth today. Some heterotrophic euglenids are large (up to 100 μm) and collectely they can account for up to 80% of the biomass of heterotrophic flagellates in the top layers of sediments. Most are poor swimmers, but can glide rapidly along surfaces. They mostly feed on attached prey, such as bacteria, or microalgae such as pennate diatoms. The most conspicuous euglenids, however, are the photosynthetic forms (e.g. Figure E4A). Photosynthetic euglenids are the product of a single event of 'secondary endosymbiosis' between a heterotrophic euglenid and a photosynthetic eukaryote – a prasinophyte 'green alga' (see Section A6 – 'Chloroplastida', above). The green alga has now been reduced to a plastid surrounded by three membranes and containing chlorophylls *a* and *b*.

In addition to having plastids, photosynthetic euglenids have a pigmented 'eyespot' that shades a portion of the base of the anterior flagellum, and is involved in phototaxis during swimming (swimming is much more in photosynthetic euglenids than in phagotrophic species). Most photosynthetic forms have a single anterior flagellum that emerges from the flagellar pocket. Most inhabit freshwater and brackish habitats, and they may locally dominate the eukaryotic phytoplankton at benthic sites. Some are highly flexible cells capable of strong euglenoid motion (e.g. *Euglena gracilis*), others are rigid (e.g. *Phacus*) or have flask-like loricae (e.g. *Trachelomonas*). A few have lost the capacity for photosynthesis, although the plastid organelle itself is retained, presumably because it has an essential role, for example, in biosynthetic pathways. *Euglena* has been widely used as a laboratory model organism, and as a paradoxical 'plant-like animal' (or 'animal-like plant') in teaching – of course it is neither an animal nor a plant!

E7.2 Kinetoplastea

Kinetoplastids are identified by the presence of a unique structure called the 'kinetoplast'. The kinetoplast is a massive accumulation of DNA in the mitochondrion that usually lies close to the flagellar basal bodies. The kinetoplast is the physical manifestation of the mitochondrial genome, and this is one of the strangest genomes in nature. Typically, there are two kinds of DNA molecules in the kinetoplast – 'maxicircles' and 'minicircles'. Maxicircles are the closer equivalent of the mitochondrial genomes of other eukaryotes – they contain around a dozen mitochondrial genes - however, the original mRNA transcripts of many of these genes are incomplete. The mRNA must be 'edited' by the insertion (and sometimes deletion) of uracils (U) before it will properly encode a protein. The minicircles are small (usually ~1 kilobase in size) and encode short RNA molecules that 'guide' the editing of maxicircle transcripts, and are therefore called 'guide RNAs'. Each guide RNA is complementary to a small portion of the final, edited sequence of the mRNA. Base-pairing between the guide RNA and the unedited pre-mRNA identifies mismatches that represent the sites where insertion or deletion of uracils is required. A complex editing machinery in the mitochondrion then inserts and deletes uracils at these positions. Dozens of different guide RNAs are required to complete the editing of the several distinct mRNAs produced in the mitochondrion of a kinetoplastid, and most of the mass of the kinetoplast is composed of 5,000-20,000 of these guide-RNA-encoding minicircles.

There are several other unique features of kinetoplastids, but we will mention only one more: Much of the central energy-generating pathway of eukaryotic cells – glycolysis - does not occur in the cytoplasm. Instead, the first two thirds of glycolysis is carried out inside a membrane-bound organelle called the 'glycosome'. The glycosome is probably a highly modified peroxisome.

Most of the diversity of kinetoplastids consists of small (usually <10 μm) free-living cells with two flagella (Figure E4B). These mostly feed on prokaryotes and are particularly common associated with surfaces. They are ubiquitous and extremely abundant in most benthic systems, but are also commonly found in the water column, mostly associated with suspended particles of detritus. Several groups have adopted parasitic habits, infecting various animals. Some of these (e.g. *Cryptobia/Trypanoplasma*) cause significant disease in fish.

E7.2.1 Trypanosomatida. One group of kinetoplastids surpasses all others in its direct importance to humans - Trypanosomatida. Trypanosomatids are a group of obligate parasites, mostly of animals (a few infect plants, but are carried between plants by insect vectors). They have only one flagellum, which usually attaches back to the cell surface along its length, creating an 'undulating membrane' (Figure E4C). Their feeding apparatus is reduced, and is probably capable of pinocytosis, but not phagocytosis.

Some trypanosomatids are 'monogenetic' and infect only one host, usually an insect. 'Digenetic' parasites alternate between two different hosts – in the case of trypanosomatids, one host is a biting insect or a leech, and the other is a vertebrate. The parasite inhabits the intestinal tract or salivary glands of the insect or leech, and is transmitted to the blood or other tissues of the vertebrate host by biting. In some species, including *Trypanosoma cruzi* (see below), transmission is actually caused by the faeces of the biting insect coming into contact with the bite or other wound. In some cases, many different hosts can be infected by one species of trypanosomatid; for example, more than 100 different mammal species reportedly act as hosts to *Trypanosoma cruzi*.

Three different trypanosomatids are among the most serious unicellular eukaryote parasites of humans. i) *Trypanosoma brucei* is the causative agent of African trypanosomiasis, or 'sleeping sickness'. ii) *Trypanosoma cruzi* causes Chagas' disease in South America. iii) Several species of *Leishmania* cause various forms of Leishmaniasis in Africa, the Middle East to India, and South America. Collectively these parasites kill hundreds of thousands of people per year, although the incidence of new *T. cruzi* infections has declined dramatically in recent decades primarily due to public health measures aimed at reducing the number of bites by the insect vector ('kissing bugs').

E7.3 Diplonemids. Diplonemids are a group of Euglenozoa that are the sistergroup of kinetoplastids. Until recently they have been an obscure and little-studied group, and only a few species were formally described. They are small-to-medium sized cells, often highly flexible, and many species are usually found in a feeding stage that has short flagella and glides rather than swims (fully flagellated forms/species have two flagella with paraxonemal rods, and swim rapidly). In 2015 environmental molecular sequencing techniques revealed that there is a spectacularly high diversity of diplonemids in marine waters, and they may well be one of the most important groups of heterotrophic eukaryotes in the world ocean. The described species of diplonemids are predators of other microbes, or (suspected) parasites of animals. Some will invade the frustules of much larger diatoms, or the tissues of decaying plants.

PART 2, SECTION AM: AMOEBOZOA

AM1 Introduction

As the name suggests, Amoebozoa is a huge group that is composed primarily of amoeboid protozoa. Amoebozoa includes most (not all) of the eukaryotes on Earth that produce broad ('lobose') pseudopodia both for locomotion and for phagocytosis of particulate food. The lobose pseudopodia common in Amoebozoa are different from the much thinner, and often microtubule-supported pseudopodia typical of Rhizaria, for example (see Section R). However, fine-branching and network-forming pseudopodia are found in some Amoebozoa as well; while others are flattened cells that extend a broad, flat 'lamellipodial' front. Many members of Amoebozoa are free-living unicellular 'amoebae' that lack flagella, although the group also contains some important parasitic amoebae. In addition, the group includes the 'slime moulds' - amoeboid protists with a dispersal stage that superficially resembles the fruiting bodies of true fungi. There are some minor groups of flagellates within Amoebozoa, but with the exception of pelobionts and lifecycle stages of some slime moulds (see AM3.2.1 and AM3.3, below), we will not discuss these here.

AM2 General organization, and pseudopodia

The amoebozoan cell contains most standard eukaryotic organelles – nuclei, mitochondria with tubular cristae, endomembrane systems, and so on (although, interestingly, amoebozoans don't have complex pellicles or extrusomes, presumably because these features would interfere with amoeboid locomotion – see below). In the largest amoebozoan cells the nucleus (or nuclei) can be huge and contain a massive amount of DNA. In fact, some data hint that large amoebae may have the largest genomes of any eukaryote.

In most amoebozoans, the ability to form pseudopodia provides the mechanism for motility, and for engulfing prey by phagocytosis. Amoeboid cells take characteristic shapes depending on the form of the pseudopodia. Most large amoebozoans are 'polypodial', meaning that they can form several lobose pseudopodia simultaneously (see Figure AM1). Some smaller forms are 'monopodial' and function, in effect, as one large pseudopodium. Some monopodial amoebae advance a very broad, flat pseudopodium (sometimes called a 'lamellipodium' – see above). Some can produce numerous finer and more elongate 'subpseudopodia'. In many amoebozoans, especially lobopodial forms, there is a ruffled, bulb-shaped projection called the 'uroid' at the opposite end of the cell to the advancing pseudopodia. This is probably involved in recycling the material of the contractile cytoskeleton that underlies the pseudopodia.

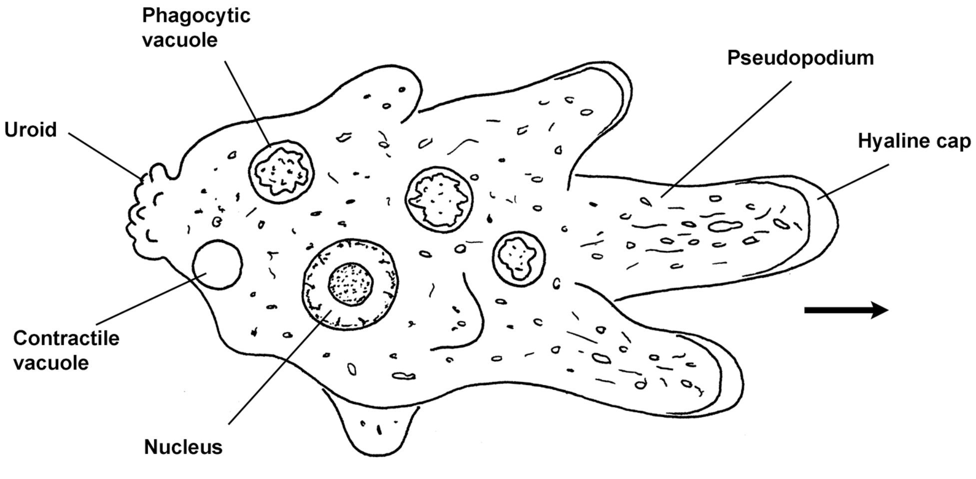


Figure AM1. Amoebozoan cell. Based on a large lobosean amoeba, e.g. *Amoeba*. This cell is 'polypodial', meaning that it produces several pseudopodia at a time. It is moving in the direction of the arrow.

According to most models, the projection of large lobose pseudopodia uses a network of actin and myosin that forms in the cortical region of the cell (i.e. around the edges, rather than the centre of the cell). This network contracts through the molecular motor myosin acting against actin microfilaments (this is the same basic motor-cytoskeleton system as in muscle contraction). This contraction exerts pressure on the central mass of cytoplasm. At the ends of advancing pseudopodia the central cytoplasm is pushed forward by this pressure, somewhat like toothpaste being squeezed from a tube. Models for the extension of lamellipodia, by contrast, invoke actin polymerization within the leading edge of the lamellipodium as the important mechanism powering forward motion.

AM3 Diversity of Amoebozoa

There are three types of Amoebozoa we will consider here – 1) lobosean amoebae (Tubulinea and Discosea), 2) Mycetozoa, or true slime moulds, and 3) Archamoebae. We will not discuss separately the more poorly-studied array of other amoebozoa such as reticulate filose-pseudopodia-producing forms, 'simple' slime molds (protosteloids) and the few true flagellates that lie phylogenetically in Amoebozoa.

AM3.1 Lobosean amoebae

Lobosean amoebae range from very small (< 5 µm across, and very flat) to cells >1 mm long that can be seen with the naked eye, such as '*Amoeba*' and '*Chaos*'. They belong to two large phylogenetic groups within amoebozoa - Tubulinea and Discosea. Most such amoebae are described as 'naked', signifying that they lack an external test (see below). In fact, many 'naked' amoebae have small, extracellular organic structures (e.g. 'microscales') that are attached to the cell surface. Naked amoebae are abundant in sediments and soil. Nutrient-rich marine sediments can contain huge numbers of amoebae, mostly very small amoebozoans – abundances of 10^5 cells per cm^3 have been estimated. Interestingly, the largest naked amoebae are essentially restricted to freshwater – perhaps the equivalent ecological niche in marine sediments is filled by benthic foraminifera (see Section R4).

The members of one subgroup of Tubulinea produce extracellular tests. These amoebae are called the **lobose testate amoebae** (Arcellinida). The test is typically composed of organic material, although many species also incorporate mineral particles from their environment into their test. In some cases, these particles are actually mineral structures sourced from their prey, for example, diatom frustules. The test has an aperture through which the pseudopodia emerge. Lobosea testate amoebae are found in freshwater and soil, and are common in soils, moss beds and other environments in which water is ephemeral or limited. There can be 10^2 testate amoebae per cm^3 of soil, which is an impressive biomass contribution, since that these are reasonably large cells (tests often over 100 µm long or wide). The test probably confers desiccation resistance - circumstantial evidence for this role comes from the fact that the testate amoebae of dry soils tend to have narrower apertures than those from wetter habitats.

Some lobose amoebae are parasites of various animals. A couple of rare diseases of humans are caused by lobose amoebae, however these are probably cases of 'facultative' or 'opportunistic' parasitism by organisms that are normally free-living. *Acanthamoeba*, an amoeba common in soil and sediments, can cause corneal infections (and even blindness) in contact-lens wearers. Occasionally *Acanthamoeba* can also cause dermal infections, and extremely rarely, a lethal brain condition called Granulomatous Amoebic Encephalitis (GAE). A second amoeba, *Balamuthia*, is probably a soil-dwelling organism, but also causes GAE (it was actually first described from brain tissue from a mandrill).

AM3.2 Mycetozoa

Mycetozoa, or 'true slime moulds' are amoeboid organisms that produce a distinctive stalked 'fruiting body', which releases spores. The fruiting bodies of Mycetozoa are superficially similar to the fruiting bodies of true fungi. In the past this has led to the classification of slime moulds with the fungi, despite the fact that the two groups have little in common for the rest of their lifecycle. In fact, the detailed structure and development of the fruiting bodies of slime moulds is very different to those of fungi, and they clearly have independent evolutionary origins.

In essence, slime moulds are actually amoebae that live in terrestrial environments, especially soil, bark, rotting timber, dung etc. The amoebae mostly feed on prokaryotes and fungi. The fruiting body is an adaptation to solve the problem of dispersing in the terrestrial environment (which is often dry and, obviously, devoid of water currents). Within Mycetozoa there are two well-known types, 'acellular slime moulds' and 'cellular slime moulds', that differ markedly both in the way that they produce their fruiting bodies, and in the role of the fruiting body within their lifecycles.

AM3.2.1 Acellular slime moulds. Formal names for this group include 'Myxogastrea' and 'Myxomycota'. Acellular slime molds have a complex sexual lifecycle in which the fruiting body plays a key part (see Figure AM2). In brief, acellular slime moulds alternate between small haploid amoebae and a large **plasmodium** (see Part 1, Section 1.2.4) that contains many diploid nuclei. In general, the amoebae can also transform into haploid flagellates. The plasmodium is an important feeding stage – it overgrows organic material and feeds on it saprotrophically, rather than by phagocytosis (see S3.2, above). The plasmodium is also the stage that actually produces the fruiting bodies. Meiotic division takes place within the fruiting bodies such that numerous haploid spores are produced. After dispersal the spores germinate to produce more amoebae or flagellates. The term 'acellular' refers to the stalk of the fruiting body, which is an extracellular structure in this group (i.e. secreted by the plasmodium).

fruiting body masses are generally macroscopic and are quite frequently seen in the appropriate habitats by field naturalists (though the 'plasmodium' is generally also macroscopic and sometimes readily observable). Fruiting bodies are most commonly seen in forest habitats, forming on detritus or rotting woody plant material, though a variety of other substrates are colonized by different species. Around 1000 species have been described.

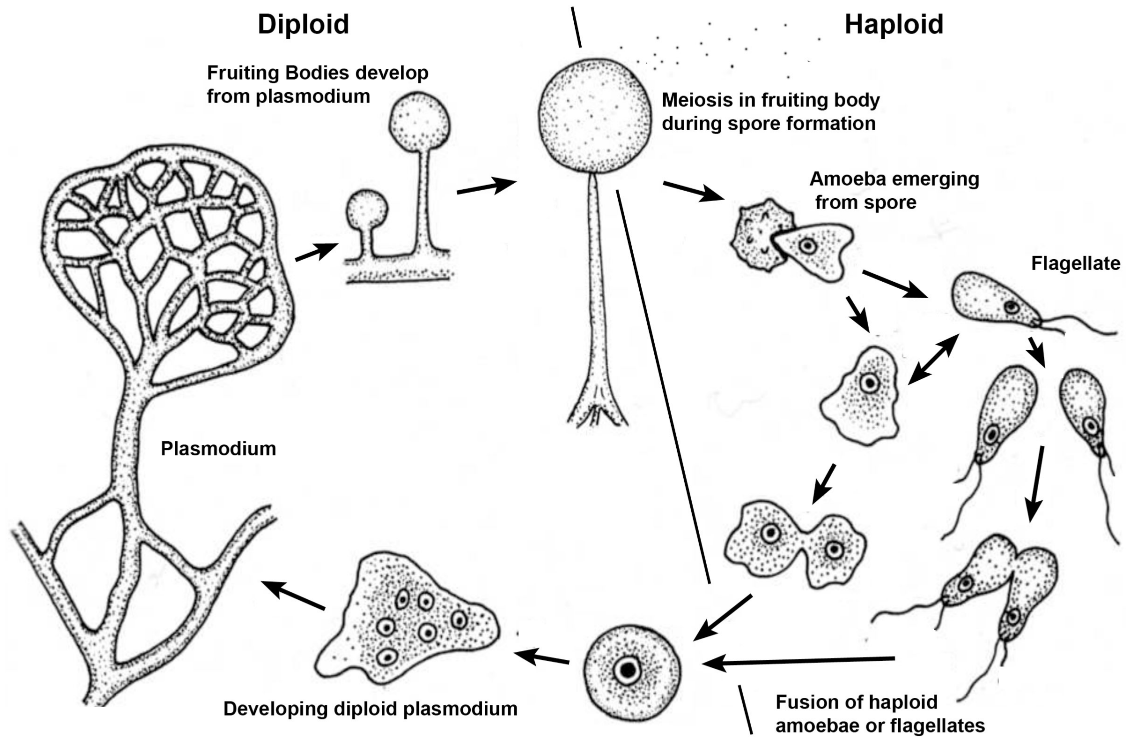


Figure AM2: Generalised life cycle of an Acellular slime mould. Modified from Sleigh (1989)

AM3.2.2 Cellular slime moulds (Dictyostelia). The biology of fruiting body formation in cellular slime moulds is very different to that of acellular slime moulds. As exemplified by the model species *Dictyostelium discoideum* (Figure AM3), the production of fruiting bodies does not involve any cell division, and therefore is not a reproductive process in the literal sense of the term. It is also not part of the sexual cycle (which is not described here). In cellular slime moulds there is an amoeba stage, but no plasmodium stage (and no flagellates). The amoebae (often called 'myxamoebae') grow and divide until the local food supply is exhausted. Numerous amoebae then aggregate to form a mass of cells called a **pseudoplasmodium** (also known as a 'slug' or 'grex'). In some species the pseudoplasmodium will locomote for a time across the substrate. It then forms into a fruiting body, which is constructed entirely of the cells in the pseudoplasmodium. Some of the cells are sacrificed to form the stalk (they expand and harden, dying in the process). Most of the rest of the cells end up in the fruiting body proper (the **sorus**), where they simply encyst to form the spores. The name 'cellular slime mould' refers to the cellular nature of that stalk.

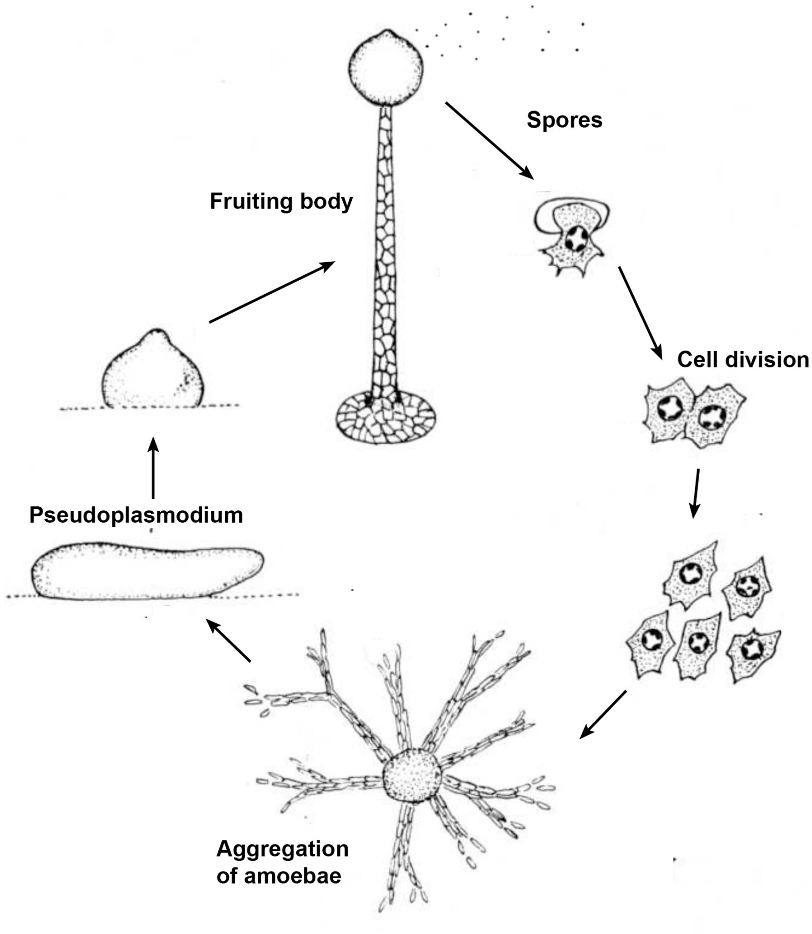


Figure AM3: Production of a fruiting body in the cellular slime mould *Dictyostelium*. Modified from Sleigh (1989)

The basic slime mould habit has actually evolved several times in the history of eukaryotes. For example, there are several, mostly simple slime moulds within Amoebozoa that there not specifically related to true slime moulds and likely (though not certainly) evolved independently. Further, there is a small group of heterolobosean amoebae (see Section E6) called 'acrasids' that also aggregate and form fruiting bodies under stress conditions, similar to true cellular slime moulds. There is also a poorly known slime mould called *Fonticula* that is related to nucleariids (Section O2.4, below), and slime-mold-like organisms amongst the Stramenopiles and Rhizaria (not discussed above). There is even a non-amoeboid organism, the ciliate *Sorogena*, which aggregates to form a fruiting-body-like structure. These organisms are again overwhelmingly found in terrestrial enviroments such as soil, dung and the surfaces of plants.

Many of these distantly related organisms (not all) have life cycles that resemble *Dictyostelium* in that individual cells will aggregate to form a fruit body topped by sorus-like structure, often called the '**sorocarp**'. However, it is not always the case that the stalk will be formed from dead cells, or even from viable cells. For this reason 'cellular slime mold' is not a suitable general term for this broader class of organisms. The term '**sorocarpic protist**' (a protist that makes a sorocarp) is a better alternative.

AM3.3 Archamoebae (Pelobionts and entamoebae).

This monophyletic subgroup of Amoebozoa is characterized by having highly modified mitochondrion-related organelles. They live in environments with little free oxygen, and their mitochondrion-related organelles lack mitochondrial cristae and are usually small relative to typical mitochondria. The organelles of Entamoebae are classified as 'mitosomes' and do not produce ATP. In this respect they are similar to the mitosomes of the unrelated diplomonad *Giardia* (see Section E1.1, above). However, they seem to have no role in iron-sulfur cluster assembly in Entamoebae, since Archamoebae have replaced the standard mitochondrial iron-sulfur cluster assembly machinery with a non-homologous pathway acquired from a bacterial group by horizontal (lateral) gene transfer, and this replacement system functions in the cytoplasm. The organelles of the pelobiont *Mastigamoeba*, by contrast, do house enzymes that support anaerobic ATP production.

Pelobionts are usually free-living cells, most of which have a single flagellum for at least some part of the lifecycle and are capable of swimming (although they still feed using pseudopodia). Entamoebae are non-flagellated amoebae and are commensals or parasites of animals. Several species inhabit the mouth or intestinal tract of humans. One species, *Entamoeba histolytica*, causes serious diarrheal disease (amoebic dysentery). *Entamoeba histolytica* also has the tendency to invade other organs, in particular the liver. It is responsible for an estimated 500 million infections, and 100,000 deaths per year.

PART 2, SECTION O: OPISTHOKONTA

O1. Introduction

The opisthokonts are the supergroup of eukaryotes that includes animals and true fungi, plus some protozoan groups, the best known of which are the choanoflagellates. The most important evidence that opisthokonts represents a monophyletic group has come from molecular phylogenetic studies. Opisthokonts also share an unusual arrangement of their flagella: In most flagellates from other eukaryote groups the flagella emerge from near the anterior end of the swimming cell or emerge laterally. Instead, unicellular opisthokonts with flagella characteristically have two basal bodies but only a single flagellum, and this flagellum emerges from the posterior end of the swimming cell (see inset in Figure O1A). Cells that show this posterior flagellar insertion include the choanoflagellates, the sperm of most animals, and the zoospores of some deep-branching fungi, such as 'chytrids' ('higher' fungi such ascomycotes and basidiomycotes do not have flagella during any part of the lifecycle). This specific arrangement of flagella was likely inherited from a common ancestor of all of these groups and thus was a feature of the first opisthokont. Most opisthokonts also have flattened mitochondrial cristae (although this feature is also found in other eukaryotes that are very distantly related to opisthokonts, such as Archaeplastida and cryptomonads).

O2. Opisthokont diversity

Here we will discuss only the protistan groups within opisthokonts, and omit a couple of small taxa.

O2.1 Choanoflagellata

Choanoflagellates are small unicellular or colonial protozoa. They have a single flagellum that is surrounded by a **collar** (Fig. O1A). The collar consists of a single ring of ~20-50 thin and equally spaced extensions called 'microvilli' that are supported by actin microfilaments. The collar is a food-capture device. During feeding, choanoflagellates are either attached or stationary (see below), and the beating of the flagellum produces a feeding current from 'behind' the cell. Small prey items, mainly prokaryotes, are trapped against the outside of the collar, and are then phagocytosed by small pseudopodia that travel up the microvilli. In some choanoflagellates it is known that the flagellum bears extremely fine delicate vanes of made of extracellular fibres (unlike the vanes supported by intraflagellar lamellae seen in 'typical excavates', for example), and it has recently been inferred from fluid dynamics analyses that this vane is important for the functioning of the collar as an efficient food capture device.

Many choanoflagellates are attached to surfaces by a short stalk. However, cells of one group of marine choanoflagellates – Acanthoecida – produce elaborate openwork loricae made of silica (Fig. O1B). The lorica can be ten times larger than the choanoflagellate cell itself. The units from which the lorica is constructed are fine strips called 'costal strips'. The costal strips are produced intracellularly by the choanoflagellates within silica deposition vesicles (see Part 1, Section 1.2.1). They are then exported to the cell surface, and subsequently assembled end-to-end into longer rods called 'costae'. The loricae may function to greatly increase drag, such that an unattached cell can produce a feeding current while remaining almost stationary within the water column. For hydrodynamic reasons it turns out that being stationary improves the effectiveness of feeding. Loricate choanoflagellates are amongst the most important consumers of prokaryotes in the ocean.

Choanoflagellates are closely related to animals. Over 100 years ago it was noted that choanoflagellates bear a close resemblance to one particular cell type found in sponges - choanocytes. Choanocytes are the feeding cells of sponges, and they have a single flagellum surrounded by a microvillar collar that is morphologically and functionally very similar to the collar of choanoflagellates. Molecular phylogenies have since shown convincingly that choanoflagellates are the closest known protistan relatives of animals.

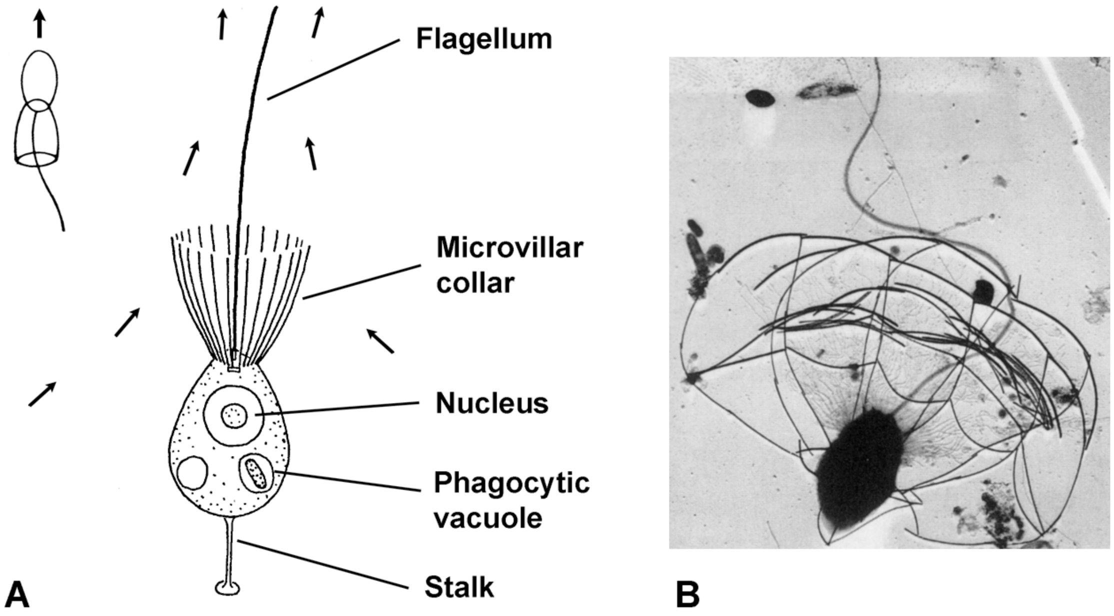


Figure O1. Choanoflagellates. A. Diagram showing the appearance of a non-loricate freshwater choanoflagellate (*Monosiga*) during feeding. The phagocytic vacuole contains a prokaryotic cell. The small arrows indicate the feeding current produced by the flagellum when the cell is attached to a surface by its stalk. The inset at the top left shows the cell when it is swimming (towards the top of the page). **B.** A loricate choanoflagellate, as seen in a transmission electron micrograph whole mount. The cell body – the dark mass the centre bottom of the picture - is about 5 μm long, The basket-like lorica is more than 20 μm across. The flagellum can be seen emerging from the top of the cell body. The flagellum is surrounded at its base by the microvillar collar – the microvilli can be seen only faintly. B. From Lee et al. 2002 (Leadbeater & Thomsen).

O2.2 Ichthyosporea (a.k.a. Mesomycetozoea)

This is a heterogeneous group of parasites, mostly of marine animals. In most cases their lifecycles are incompletely understood. Many produce amoeboid stages that may allow infection of new hosts. A few produce flagellates that might be involved in infection.

Examples: *Ichthyophonus* is a parasite of fish that has been reported in numerous host species (both freshwater and marine), and infects diverse organs. An amoeba-like cell might be the infectious form. *Sphaerothecum* ('rosette agent') is an intracellular parasite of some west-coast salmon. The parasite produces small cells with a single flagellum and these may be the infective stage.

O2.3 Nucleariids and *Fonticula* (Rotosphaerida)

Nucleariids are a small and obscure group of amoeboid organisms that produce fine actin-supported pseudopodia. Some are naked, while in others the cell body is rounded and surrounded by a covering of mineral particles, either harvested from the environment, or small silica granules produced by the amoeba itself (with the pseudopodia projecting from between them). *Fonticula* is a sorocarpic amoeba (see AM3.2.2 above). Molecular phylogenetic studies demonstrate that nucleariids and *Fonticula* are the closest relatives of Fungi (though see O2.4 below). It is likely that more attention will be paid to nucleariids in the future now that the key evolutionary position of this group is better understood.

O2.4 Cryptomycota (and microsporidia)

The evolution of a chitinous cell wall was an important event in the origins of true fungi. It is linked to the ability to simultaneously feed and grow rapidly by extending projections into surrounding material, or into host material in the case of parasites. These projections take the form of tubular 'hyphae' in most Fungi. This ability, in turn, is associated with a loss of phagotrophy (which is incompatible with a complete and robust cell wall) and reliance instead on osmotrophy/saprotrophy, in which small organic molecules are transported across the cell membrane. Recent studies have demonstrated the existence of a more-or-less new group that is the sister group to all other Fungi, called the 'cryptomycota'. Most of the diversity of Cryptomycota is uncultivated, and very poorly understood – it is thought that many might be parasites of other protists, especially algae like diatoms. The best-studied form, *Rozella allomycis*, is an intracellular parasite of a 'chytrid' fungus. *Rozella* produces a tubular chitin-rich infection structure, but not a wall-enclosed hyphal system, and it may feed by a phagocytic process. *Rozella* therefore represents an intriguing potential intermediate in fungal evolutionary history. Like chytrids (which are the deepest-branching fungal lineages after Cryptomycota), these cryptomycetes can have a flagellated stage in their life-cycle.

One of the most highly specialized eukaryote groups on Earth are the microsporidian parasites. These organisms are intracellular parasites of animals (and a few protists) that produce an infective spore stage. The spore has a sophisticated infection apparatus called the 'polar tube'. This initiates infection by violently everting (turning inside-out like a rubber glove) to pierce a host cell. The cell contents of the parasite then pass down the polar tube and are deposited (in a new cell membrane!) within the host cell. Although traditionally treated as protozoa, it is now known that microsporidia are actually Fungi of some form, in the phylogenetic sense. Recent evidence suggests that they are most closely related to Cryptomycota.

Microsporidia mostly infect invertebrates, though they include noted parasites of vertebrates (e.g. *Encephalitozoon cunniculi,* primarily in rabbits). They are of occasional medical significance when they infect immunocompromised patients. Microsporidia are of basic scientific interest as well. They have the smallest genomes of any autonomous eukaryote, and some of the most reduced forms of mitochondria known. The mitochondria of typical microsporidia are 'mitosomes' that play no role in ATP synthesis (see Section E1.1, above). In fact, some microsporidia appear to import ATP directly from their host cell, rather than synthesizing it themselves (even in their own cytoplasm).

O3. Close relatives of opisthokonts (e.g. apusomonads and breviates)

There is strong evidence from phylogenies based on multiple genes that Amoebozoa and Opisthokonta are more closely related to each other than to any of the other major groupings (e.g. Archaeplastida, Sar, Cryptista, the excavates, etc). However, phylogenetic studies that include various poorly known organisms tell a more nuanced story: The very closest relatives of opisthokonts are actually two groups of heterotrophic flagellates; the apusomonads, and the breviates, (see Figure 1.1 on page 3). These organisms are small free-living bacterivorous flagellates that generally feed from surfaces. **Apusomonads** actively locomote across surfaces by gliding (i.e. they do not typically use their two flagella for swimming). The dorsal side of the cell is supported be a pellicle, while the ventral side can produce short pseudopodia that are used in feeding. The anterior flagellum is very short and enclosed in sleeve-like extension of the dorsal cell thickening; it likely functions in food detections/aquisition rather than locomotion. **Breviates** are anaerobic, amoeboid flagellates. They have either one flagellum or two (usually a longer anterior flagellum, and a very short or absent posterior flagellum), and characteristically move along surfaces by producing a succession of fine pseupodia, which connect the cell to the substrate like stilts, then flowing forward over these connections. They also use pseudopodial activity to feed. Some will swim reasonably well, and may transform into an alternate swimming form with a lengthened posterior flagellum (while still keeping the effective anterior flagellum). Both apusomonads and breviates have quite complex microtubular cytoskeletons (these have been documented in detail in work in the Simpson lab). In both of these respects they differ markedly from choanoflagellates, for example, and from the inferred most recent common ancestor of opisthokonts.

It is possible that the study of apusomonads and breviates will help us to understand the evolution of the supergroup Opisthokonta, including ultimately, the evolution of traits like multicellularity that truly define animals (and higher fungi) as distinct forms of life. For example, studies demonstrate the presence of a suite of genes encoding integrin complexes in apusomonads and breviates. These complexes are involved in cell adhesion in animals, which is an important attribute for a multicellular organism, and integrins are not found in typical unicellular eukaryotes. Since apusomonads and breviates are unicellular, integrin complexes are likely used for some other purpose in these cells, and represent a case of exaptation for multicellularity. At present, however, we do not know what their functions are.

PART 3

PART 3, SECTION M: MACROALGAE - INTRODUCTION

M1 Introductory remarks and caveats

Some algal eukaryotes are macroscopic and have complex morphologies, and so bear some resemblance to land plants. Examples include various types of seaweeds found in intertidal and coastal marine habitats, and the 'stoneworts' of freshwater lakes. Collectively, such algae are often called 'macroalgae'.

In these notes we deal with macroalgae separately from other protists. This is because the terminology and biological considerations relevant to macroalgae are often different to those of unicellular and/or microscopic eukaryotes. However the designation of macroalgae is largely a division of convenience, and some cautionary comments must be made at the outset:

Firstly, macroalgae do not represent a natural (monophyletic) group of organisms, nor are they necessarily related to land plants. In fact, there are several major groups of macroalgae that evolved independently from different unicellular ancestors (rhodophytes – red algae; phaeophyceans – brown algae; several different groups of 'green algae'). Only one of these groups (Charales) is closely related to land plants, and even then does not seem to be the *closest* relatives of land plants (see Figure A1, in Part 2, Section A). In fact, one important group of macroalgae - phaeophyceans – are not even part of the supergroup Archaeplastida (the primary algae and land plants). Phaeophyceans belong to the group of eukaryotes called stramenopiles, and have plastids of secondary endosymbiotic origin.

Secondly, the distinction between microalgae and macroalgae is imperfect and artificial. The most complex macroalgae are truly multicellular by any definition, in that they are differentiated into substructures, and each substructure is composed of a different type or types of cells, organized into 2- or 3- dimensional 'tissues'. However, these forms may be closely related to algae without true tissues (e.g. constructed of simple branched filaments one cell wide). Many species have lifecycles that alternate between forms that differ dramatically in complexity.

M2 General features of macroalgae.

In macroalgae, the main body of the organism is usually called the **thallus** (plural 'thalli'). The thallus can have a simple filamentous structure or be organised into moderately complex morphologies, such as the leaf-like 'blades' and stem-like 'stipes' seen in some Phaeophyceae (see Section 'P' below). Like land plants, most macroalgae attach to substrates. The part of the organism that attaches to (or into) the substrate is often differentiated from the (rest of the) thallus and is called the **holdfast** or 'rhizoid'. The holdfast/rhizoid is usually disk-like, or heavily branched (see Figure M2). Large macroalgae, or those that live where they experience strong wave action (e.g. exposed intertidal areas) are very firmly attached. While partly similar to the root systems of higher land plants, holdfasts differ in that they are not, in general, adapted for water and nutrient uptake. Unlike most land plants, the entire thallus of a macroalga is bathed in water that will contain nutrients in dissolved form.

At the level of fine structure, macroalgae rely heavily on the extracellular matrix / cell walls to provide mechanical strength. Cellulose and various other polysaccharides are usually the major components of this matrix. However, a wide variety of macroalgae incorporate large amounts of calcium carbonate into outer cell walls, providing additional strength. Even so, large macroalgae are rarely as rigid as large woody land plants. This reflects the much greater shear forces that macroalgae are typically subjected to because they live in water, which is much denser than the atmosphere. In the absence of rigidity, many large macroalgae use buoyancy as a strategy for maintaining an upright stance in the water column. Buoyancy is provided by gas-filled floats of various kinds. Whereas phagocytosis is quite common in microalgae (see examples in Part 2), macroalgae lack the ability to perform phagocytosis.

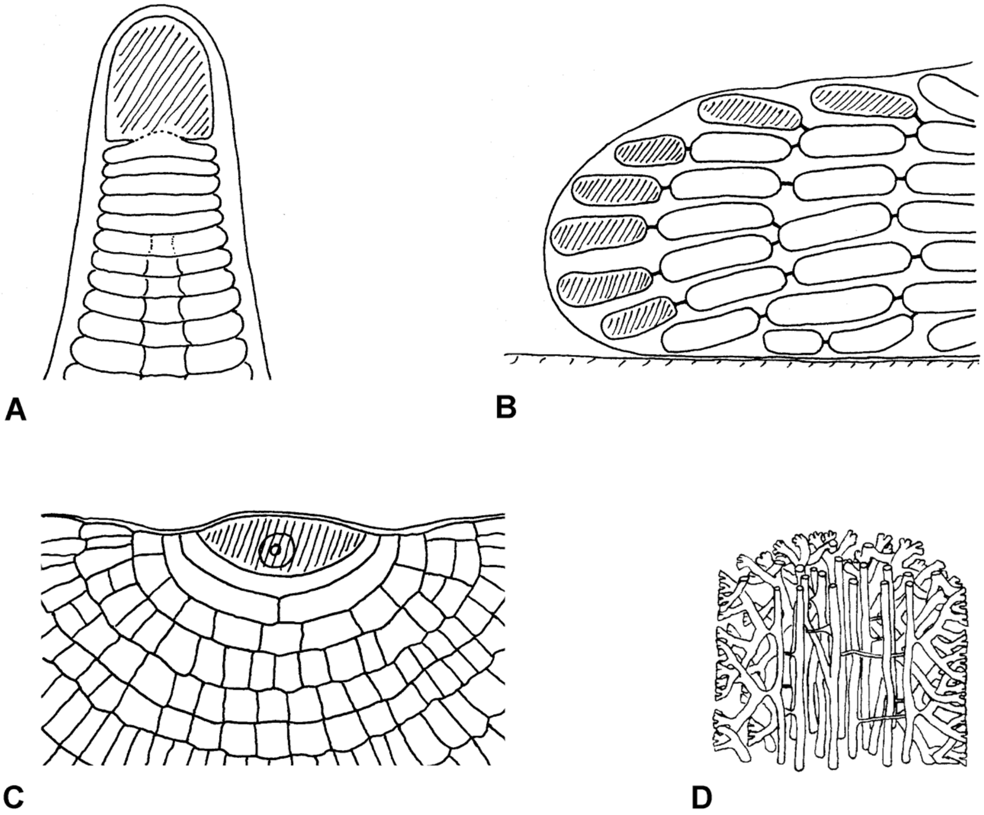


Figure M1. Thallus organisation in macroalgae. A. Uniaxial filament. Illustration shows the tip of the thallus of the red alga *Platysiphonia*. The only cell that divides to elongate the filament is the apical cell (shaded), which undergoes repeated asymmetric divisions. In this species filament cells subsequently undergo a set number of lateral divisions, either to thicken the filament, or to begin lateral branches. Lateral division can be seen towards the bottom of this section of filament. Drawn from Graham and Wilcox, 2000. **B.** Pseudoparenchymatous organisation. Diagram shows the edge of an encrusting non-geniculate coralline red alga (see RD4.3.3, below). The thallus is three-dimensional and tissue-like, but actually grows as a series of branching filaments enclosed in common extracellular material. In this case, the filaments can be traced by following the primary pit plugs that formed between daughter cells during cell division (see RD2.1; secondary pit plugs not shown). Further growth is accomplished by division of the cells at the tips of the filaments (shaded), which collectively form a 'marginal meristem'. **C.** Parenchymatous organisation. Illustration shows the meristematic region of a brown alga with a broad thallus. Growth is initiated by asymmetric division of an apical cell (shaded), and subsequent division along multiple axes. Drawn from Graham & Wilcox, 2000. **D.** Siphonous organisation. Drawing of the cellular material in part of the thallus of a caulerpalean ulvophycean (Chloroplastida). The material is one continuous, many-branched cytoplasm, resulting from growth without cell division. From Graham and Wilcox, 2000, after Friedman and Roth, 1977.

M3 Growth forms, and thallus organisation

Like land plants, the thalli of macroalgae often grow primarily through repeated cell divisions from particular regions called 'meristems'. The simplest thalli are simple **filaments**, one cell wide, or several cells wide. A slightly more complex thallus form consists of **branching filaments**. Filaments are produced as the result of cell growth and/or cell division on one main axis. Often cell division is restricted to the end of a filament (Figure M1A), with an 'apical meristematic cell' that repeatedly divides. Forms where each branch grows by division of a single cell are termed 'uniaxial'. In some algae, tissue-like material is constructed from numerous filaments of cells associated with each other within the extracellular matrix – this habit is termed **pseudoparenchymatous** (Figure M1B). The most complex state is characterized by the production of true tissues by repeated cell division from a meristem along two or more axes. The classic product of such division is material called 'parenchyma', and the general growth habit is called **parenchymatous** (Figure M1C).

Certain groups of macroalgae produce very large cells, which are often multinucleate. In some groups, highly elongate cells develop by growing continuously without ever dividing. When this growth habit dominates the macroalga is often referred to as **siphonous** (Figure M1D). Some of the most complex and differentiated chloroplastid macroalgae, are, in fact, composed of one, or a few extremely elongate and highly branched 'cells' (e.g. Caulerpales – see CM2.2 below). Obviously, such organisms defy neat categorization with respect to multicellularity.

In addition to specialisations associated with reproduction (see M4 below), different cell types can be present within the thalli of multicellular algae. In parenchymatous and pseudoparenchymatous algae, cells in the centre of the thallus are often non-photosynthetic. In many cases cells in the holdfast are also not photosynthetic. Some algae have cells throughout the thallus that are specialised for predator deterrence, while some brown algae have specialised conductive cells within the thallus that are analogous to the sieve tubes of the phloem tissue of tracheophyte land plants (See Section 'P' below).

Figure M2. Holdfast. Photograph shows the branching holdfast of a young specimen of the intertidal brown alga *Laminaria*. The lobes of the holdfast are adhering to the surface of a rock. Photograph by M.D. Guiry (from Algaebase).

M4 Macroalgal lifecycles

Macroalgae usually have sexual lifecycles. In many cases there is an alternation between diploid and haploid generations (see Part 1, Figure 1.1D). The diploid form is usually called the **sporophyte** (this and many other terms for macroalgal lifecycles are similar to, or the same as, those used in land plants). At some point reproductive cells undergo cell division including meiosis, and haploid reproductive/dispersal cells are released. These are usually called **spores** or 'zoospores'. The haploid cells settle and grow into haploid individuals called **gametophytes**. The gametophytes eventually produce **gametes**, which fuse to form diploid zygotes that settle and grow into new sporophytes. While the sporophyte and gametophyte generations are almost indistinguishable in some macroalgae (**isomorphy**), there are many groups in which the sporophyte and gametophyte look completely different to each other (**heteromorphy**). There are also variations in the relative sizes of the gametes (equal size = ‘isogamy’; unequal size = ‘anisogamy’, or ‘oogamy’ if one gamete is huge and immobile). As in land plants, gametes of different mating types (~sexes) can be produced by the different individuals or by the same individual; in the latter case the alga could be described as ‘bisexual’. The general terms most often used, however, are ‘dioecious’ (individuals in the species produce one type of gamete) and ‘monoecious’ (individuals produce both types of gametes).

There are some exceptions to this general life cycle. Some examples include the absence of gametophytes in some brown algae (in which the mature diploid organism produces gametes rather than spores), and the complicated three-part cycles of many red algae, where there are two successive diploid phases. Asexual reproduction (i.e. not associated with changes in ploidy) is common, sometimes occurring through fragmentation of the thallus (with each fragment growing to a new mature individual), or by the production of specialized dispersal cells. In some macroalgae, a gamete that does not manage to pair with another, compatible gamete will instead enact a ‘plan b’ and grow into a new haploid adult (i.e. a gametophyte). Some macroalgae appear to be completely asexual.

M5 Brief comments on the importance of macroalgae

M5.1 Macroalgae in the environment

Macroalgae are the most conspicuous photosynthetic organisms in the oceans, but not the most important in a global sense. Whereas land plants dominate the total photosynthetic output (i.e. new organic carbon) of terrestrial environments, large macroalgae contribute only a small fraction of the total photosynthetic output of the world ocean (the overwhelming majority is performed by microalgae and cyanobacteria). Even in shallow subtidal areas, rapidly growing microalgae and small filamentous forms associated with surfaces are often more important primary producers. Nonetheless, macroalgae are significant and locally dominant components of coastal ecosystems, including the intertidal zone. Being large and often long-lived, they can be important habitat constructors. For example, stands of giant kelp such as *Macrocystis* (Phaeophyceae) anchor complex ecosystems that are important habitats for fish, and are very productive, with a photosynthetic output per unit area rivaling that of rainforests. Forests of smaller kelp are of major importance in coastal Nova Scotia, where they form a habitat for numerous animals, as well as ‘subsidising’ other nearby ecosystems with their primary productivity (exported as detrital biomass). Floating mats of *Sargassum* (a fucoid phaeophycean) harbour specialised biotic communities. In tropical coral reefs, a significant fraction of calcium carbonate deposition (i.e. reef construction) is actually performed by algae, rather than by the corals themselves. Highly calcified non-geniculate members of Corallinales (Rhodophyta – see RD4.3.3, below) are the most important algae in this reef construction process.

M5.2 Macroalgae in industry

The worldwide macroalgal harvest was worth >6 billion US dollars / year in 2012, and is growing fast. Over 70 species in the groups Ulvophyceae, Phaeophyceae and Rhodophyta are cultivated or are collected from the wild, and used as food. Macroalgae are used as foodstuffs primarily in East Asia, but there are seaweed-eating traditions in many other places in the world as well, including Atlantic Canada, where the red alga *Palmaria* is eaten as ‘Dulse’. The single most important foodstuff derived from macroalgae is nori – the sheets of dried seaweed used in sushi. Nori is made from the thallus of the red algae *Porphyra* and *Pyropia* (primarily *Pyropia yezoensis*). Aquacultural production of these algae has been pursued for at least 300 years. It is a huge enterprise in East Asia, in particular, and estimates for the total value of the crop exceed 2.5 billion US dollars per year. The blade-like gametophytes are grown on nets, having been seeded from conchospores produced by sporophytes that are cultured separately (see section RD4.2.1, below). Growth can be very rapid (>3 cm length per day). Nori is made by mincing the thalli, then drying the material in sheets. It is very high in protein, several vitamins and minerals.

Another common foodstuff, Kombu, is made from kelp (laminaralean brown algae), mostly from cultivated *Saccharina japonica*. This crop is worth more than 1 billion US dollars annually.

Various macroalgae are also used in the production of fertilisers and of feed supplements for livestock. For example, in Atlantic Canada there is commercial harvesting of the abundant fucoid brown alga *Ascophyllum* largely for these purposes. The annual harvest is in the tens of thousands of tonnes.

Many gelling compounds are extracted from various brown algae and red algae. Most of these are modified (e.g. sulfated) polysaccharides that are components of the extracellular matrix. 'Alginates' are major components of the extracellular material of brown algae, and are used extensively by the food industry as thickeners, and as components of other domestic/industrial products. The rapidly growing giant kelp *Macrocystis* is a major source of alginates. Natural stands of *Macrocystis* are cropped using mower-like barges on the Pacific coast of the US, and there is also a large harvest in Chile. 'Agars' and 'carageenans', by contrast, are extracted from red algal cell walls. Many different species of red algae are harvested, either from wild stocks, or in aquaculture operations. Agars and carageenans are used in many foodstuffs. Of course, agar is also an indispensable product for microbiology, since it is the basis for most solid media.

PART 3, SECTION CM: CHLOROPLASTID MACROALGAE

CM1 Chloroplastid macroalgae

Several different groups of chloroplastids have evolved a macroalgal habit. The major groups to do so were Ulvophyceae, and Charales (see Figure A1 in Part 2, Section A). A few Chlorophyceae form complex, branched filamentous thalli, but we will not consider them further.

CM2 Ulvophyceae

This large group of chlorophyte green algae (>1000 species) includes a few unicellular and microscopic filamentous forms, but most ulvophyceans are macroscopic and either multicellular or siphonous. Ulvophyceans are predominantly marine organisms, although there are a few freshwater species. They are the dominant green macroalgae in marine environments.

Ulvophyceans usually show an alternation of generations, and both gametes and zoospores are flagellated. Sometimes the flagella have scales similar to those of some unicellular Chloroplastida (see Part 2, Section A6, above). Many ulvophyceans, including the largest and most highly differentiated, are siphonous and are actually composed of one or a few highly elongate multinucleate cells that are usually highly branched. This habit is found in some other macroalgae but is carried to an extreme in ulvophyceans.

Ulvophyceae are very diverse. Here we will highlight just a couple of better known or more remarkable macroalgae in this group.

CM2.1 Ulvales are mostly marine. They have a relatively simple thallus 1-2 cell layers thick that appears as filaments, tubes or blade-like sheets. The sporophyte and gametophyte look almost identical (i.e. are isomorphic).

Examples: *Enteromorpha* has a tubular thallus with walls one cell thick. It forms masses over rocks and other solid structures (including the hulls of boats). Found around the world, the genus appears in a wide range of temperature regimes and salinities. Sporogenesis (production of zoospores) occurs mostly at the ends of the sporophyte thallus, but gametogenesis (the production of gametes) involves most of the gametophyte thallus, destroying it in the process.

Ulva, commonly known as 'sea lettuce' is one of the most common green seaweeds. The blade- or sheet-like thallus may be a metre in length, but is composed of only two layers of cells. Unlike *Enteromorpha*, the gametophyte produces gametes predominantly along the edges of the thallus, and survives gametogenesis.

CM2.2 Caulerpales are mostly marine. They are siphonous, but are often large and morphologically complex. Where known, they are heteromorphic - the gametophyte is smaller than the sporophyte.

Examples: *Codium* is a widespread seaweed that usually has a simply-branched thallus. The branches are relatively thick – a common name for *Codium* spp. is 'Dead Man's Fingers'. The thallus can be a metre in length in some species. A rapidly growing asexual form of *Codium fragile* is an invasive species along the eastern North American coast, where may disrupt stands of native seaweeds.

Caulerpa is a highly differentiated alga that produces a horizontal 'runner' from which arises a row of vertical structures with a 'stem and leaf' appearance.

Halimeda is one of several members of Caulerpales whose thalli become highly calcified (i.e. incorporate calcium carbonate). *Halimeda* itself has a branching thallus consisting of rigid, calcified segments that are linked by flexible joints. *Halimedia* (and other related algae) disintegrate with the release of gametes, and can go through several generations a year, thus contributing a large amount of new calcium carbonate to the sediment. It is found mostly in tropical waters.

Other Ulvophyceae of note include the tiny unicellular/siphonous *Acetabularia*, and the marine/freshwater filamentous form *Cladophora.*

CM3 Charales

The second major group of macroalgae belonging to the group Chloroplastida is the taxon Charales. This group belongs to Streptophyta (or Charophyta), rather than Chlorophyta (see Figure A1, in Part 2, Section A). Charalean algae are fairly closely related to land plants. Similarities between streptophyte/charophyte algae and plants may be discussed in the 'Plant' section of the Biology 2004 course.

The thallus of Charalean algae consists of a central erect shoot with tufts of feathery branches emerging from **nodes** that are regularly spaced along the central shoot (Figure CM1A). The nodes are composed of parenchymatous tissue, while each internode is a single undivided cell up to 15 cm in length. The organism grows from an apical meristematic cell – each new cell produced at the meristem undergoes a further round of cell division, with the upper one then dividing laterally to form the node, while the lower one does not divide, but instead elongates greatly to form the internode. In some species rings of long nodal cells grow upwards or downwards from each node, forming a one-cell-wide sheath of narrower cells around each internodal cell.

Reproduction involves the development of conspicuous round 'gametangia' in node regions. Charales are oogamous - the gametangia produce either a single large ovum that remains in place, or large numbers of motile sperm. After fertilization by a sperm, the ovum forms a zygote with a thick, resistant wall and sinks to the substrate. It is thought that the zygote germinates and immediately undergoes meiosis, and thus there is no real alternation of generations (and the thallus is haploid).

Charales are common in many freshwater habitats, where they may dominate the freshwater macroalgal biota. They sometimes also occur in brackish water. Charales often accumulate considerable amounts of calcium carbonate in their cell walls, earning the colloquial name 'stoneworts'. Charales are a major source of calcium carbonate deposition in freshwater sediments. There are a couple of hundred species in total. Example genera are *Chara* and *Nitella.*

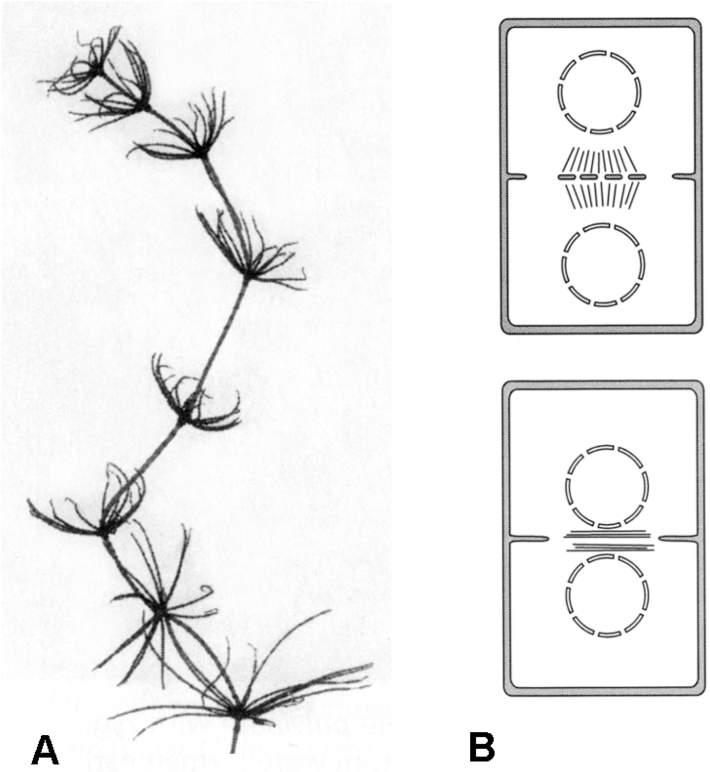


Figure CM1. Charales. A. Portion of the thallus of the charalean *Chara*. Note the whorls of lateral branches, denoting the nodes, separated by internodes. **B.** Comparison of the morphology of cell division in different Chloroplastida. The 'phragmoplast' of land plants, Charales and Coleochaetales (a small group of green algae that is probably even more closely related to land plants) is depicted on top. Some Zygnematales (Part 2 A2.6.5) also develop a small phragmoplast. The 'phycoplast', characteristic of most other 'green algae', is shown at bottom. The straight lines represent microtubules. From Graham and Wilcox, 2000.

PART 3, SECTION RD: RHODOPHYTA

RD1 Introduction

Rhodophyta, or red algae, is a group that is dominated by marine macroalgae. There are 7000 or more species described, only 100-150 of which inhabit freshwater. Some red algae are notable for living at considerable depth (~250 metres), and correspondingly, extremely low light conditions. One curious unicellular form, *Cyanidium*, is amongst the most extremophillic of eukaryotes, living in acidic hot springs at 57°C and pH 2. Some red algal species are parasites of other red algae.

Red algae are one of the largest groups of eukaryotes that completely lack flagella and basal bodies (and centrioles) for their entire life cycle. The storage product is a starch-like polysaccharide called 'floridean starch', which is synthesized and stored in the cytoplasm (not in the plastid, as in Chloroplastida). The plastids of rhodophytes are discussed in Part 2, Section A3, above. Red algae have complex cell walls that contain cellulose, but also other complex and modified polysaccharides, such as sulfated polygalactans. Some of these cell wall components are called 'agars' and 'carrageenans' and are harvested for industrial purposes (see Section M5.2, above).

RD2 Thallus organisation

Most red macroalgae are fundamentally composed of linear or branching filaments that grow by cell division in apical meristems, usually from a single apical meristematic cell (see Figure M1A, above). Many construct filaments that are more than one cell wide, for example, by a precisely defined number of subsequent lateral divisions. Many red algae have thickened and complex thalli, but these nonetheless are usually constructed from branching filaments and so are considered to be 'pseudoparenchymatous' (see Section M3 and Figure M1B above). The blade-like thalli of the gametophytes of the well-known red algae *Porphyra* and *Pyropia* are atypical (see RD4.2.1, below).

Leaving aside cells involved in reproduction, there is a limited degree of cell differentiation in red algae. Rhodophytes with thick thalli are often differentiated into an outer region of pigmented photosynthetic cells - the 'cortex', and a central region of colourless, non-photosynthetic cells – the 'medulla'. Some thalli include specialized 'hair cells' that form into an elongate projection, and 'gland cells' that have secretory functions, possibly associated with deterring grazing by animals.

Two other distinctive features of red algal cells are important within the context of the organisation of multicellular forms: **Pit plugs** and **cell fusion**.

RD2.1 Pit plugs.

At each cell division, a new region of cell wall material – a cross-wall - is formed between the two daughter cells. In most red algae, the cells themselves completely separate, but the cross-wall is not completed. Instead, a small, central region of the cross-wall is filled by a proteinaceous structure called a 'pit plug' (specifically, a '**primary pit plug**') that attaches together the two recently divided cells (Figure RD1A). It is not clear whether pit plugs allow any communication between the cytoplasm of these cells. It is probable that their main purpose (or sole purpose) is to solidly anchor the cells together. In this way they may differ in role from the plasmodesmata found in land plants and some other Chloroplastida (see Section CM3.3), and in Phaeophyceae (see Section P1 below).

RD2.2 Cell fusion.

Most red algae exhibit a remarkable capacity for vegetative **cell fusion** (This is nothing to do with gamete fusion during sex – the nuclei do not fuse). One manifestation of this ability is seen in wound repair. In some filamentous red algae, the death of a cell in the centre of a filament triggers the cells either side to divide. The two new cells either side of the position of the dead cell then grow towards each other. When the cells meet, they fuse to form a single cell that now takes the place of the dead cell. Fusion is also used by some red algae to connect close-lying cells that are not immediate daughter cells, for example, cells in adjacent filaments within a pseudoparenchymatous thallus. A cell from one filament undergoes a very unequal cell division, with a pit plug beginning to form between the two cells. Then the small daughter cell fuses with a different cell (for example, a cell in the adjacent filament). At the end of the process, two large cells remain, connected by the pit plug. Pit plug connections formed by this process are called '**secondary pit plugs**'.

The capacity for cell fusion is exploited by parasitic red algae, which use the cell fusion apparatus to insert nuclei and cytoplasm into cells of their host, taking over each cell from the inside. In ~90% of these associations the parasite is very closely related to the host species – presumably the parasite cell is similar enough to be misidentified as a host cell, allowing cell fusion to proceed.

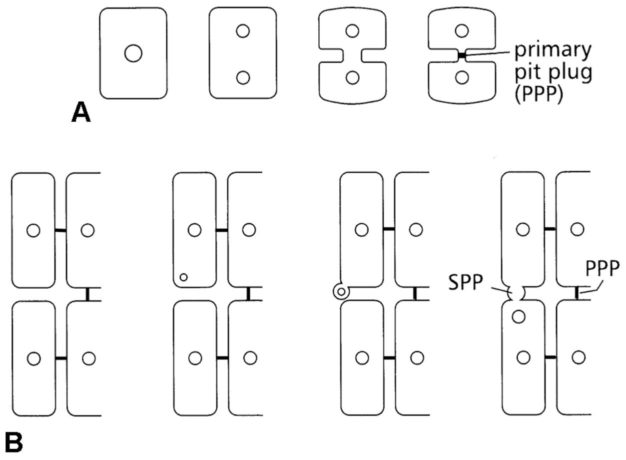


Figure RD1. Pit plugs. A. Formation of a primary pit plug during cell division. **B.** The formation of a secondary pit plug (SPP) to connect two cells that are not adjacent within a filament. The circles represent nuclei. One cell (top left of this group) undergoes unequal cell division. As a pit plug forms between the daughter cells, the smaller of the two fuses with another cell. From Graham and Wilcox, 2000.

RD3. Life cycles

The basic sexual life cycle involves an alternation between haploid and diploid forms, but in the major group of red algae, the Florideophyceae (see RD4.3, below) there is a **triphasic life cycle**, with two distinct diploid phases that follow one after the other (Figure RD2). This complex lifecycle is used here to illustrate red algal life cycles, although it should be remembered that some red algae, including the well-known *Porphyra* and *Pyropia*, have simpler cycles (see RD4.2.1, below).

In the classical triphasic life cycle of Florideophyceae there is a haploid gametophyte phase followed by the two diploid phases - the '**carposporophyte**', then the '**tetrasporophyte**'. We shall begin with the gametophyte phase: The gametophyte is usually the largest (or equal largest) adult stage. Gametes are produced by gametophytes, generally by differentiation at the tips of filaments (usually lateral branches, when these are present). Most red algae are dioecious and oogamous (see Section M4, above). The female gamete – or '**carpogonium**' - is large and remains attached to the gametophyte. Usually the carpogonium has an elongate projection called the 'trichogyne', which acts as a collection device. The male gametes – 'spermatia'- are small and usually produced in large numbers, often from particular regions of the thallus (spermatangia). Since rhodophytes lack flagella, spermatia are usually dependent on water currents for dispersal. Fertilization is initiated after a spermatium contacts and adheres to the trichogyne (see the top part of Figure RD2).

After fertilization, the diploid carposporophyte is formed. This develops *in situ*, remaining connected to the maternal gametophyte. Transfer of nutrients from the maternal gametophyte to the carposporophye has been documented. Many red algae use cell fusion (see RD2.2, above) to transfer diploid nuclei to different cells within the maternal gametophyte, and take them over, such that many genetically identical carposporophytes can develop on different parts of the parental gametophyte from a single fertilization event. The mature carposporophyte is typically a small filamentous mass. It produces numerous diploid '**carpospores**' from the apical cells of filaments.

Carpospores germinate into the second diploid phase called the 'tetrasporophyte'. Usually the tetrasporophyte grows as a free-living alga, independent of the maternal gametophyte. Unlike the carposporophyte, the tetrasporophyte can be similar in size and complexity to the gametophyte, and is often almost indistinguishable (isomorphic). It is the tetrasporophyte that finally produces haploid spores by meiosis at particular regions of the thallus. These spores are produced in bundles of four and are called '**tetraspores**'. The tetraspores are released and germinate into new gametophytes.

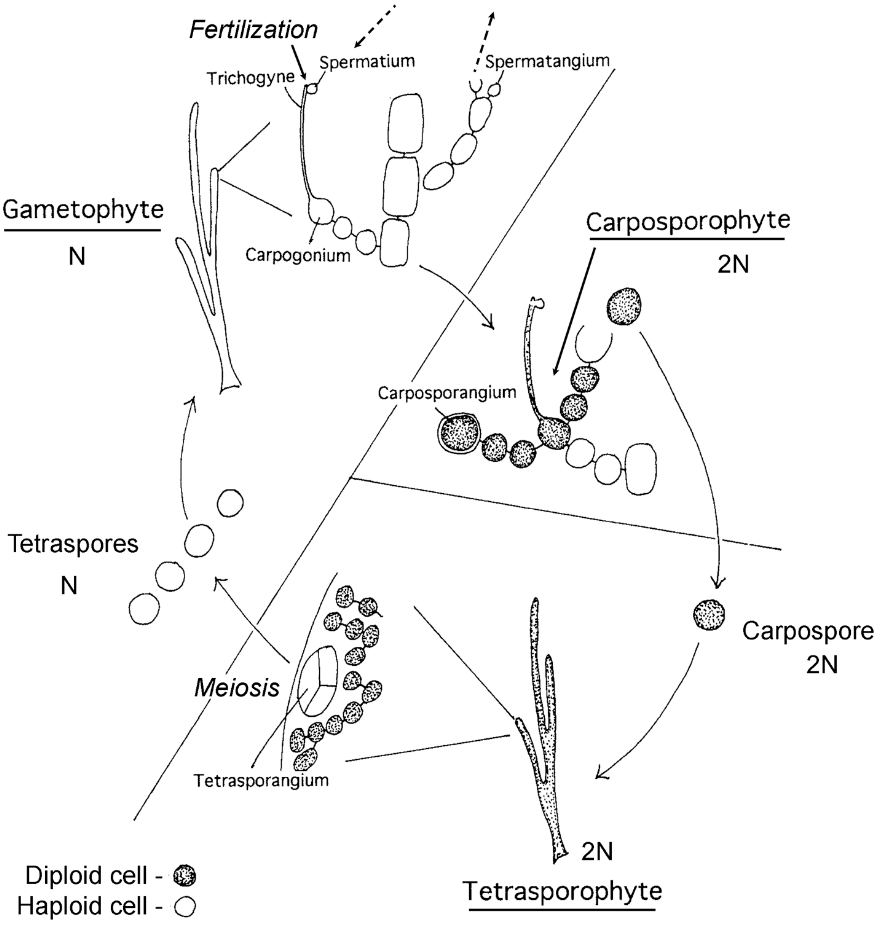


Figure RD2. Triphasic lifecycle of florideophycean red algae. The three adult phases are underlined. The first diploid stage – the carposporophyte - grows on the maternal gametophyte, since the female gamete (carpogonium) remains attached even after fertilization. The carposporophyte produces diploid spores (carpospores) that germinate and grow into diploid tetrasporophytes. These undergo meiosis to produce the haploid spores (tetraspores) that settle and grow into new gametophytes. Note that the monecious (~bisexual) habit shown here would be atypical. Modified from Lee, 1999.

RD4 Red algal diversity

Traditionally, red algae are divided into two groups, Florideophyceae and 'bangiophytes'. In fact 'bangiophytes' are a paraphyletic assemblage, with Florideophyceae descended from bangiophyte ancestors. One group of unicellular species, Cyanidiales, is perhaps the deepest branch within red algae. It is often treated as part of the 'bangiophytes', but will be considered separately here.

RD4.1 Cyanidiales

Cyanidiales are not macroalgae - they are unicellular. Usually they are rounded with a single large plastid. Many cyanidialeans are thermophillic and acidophilic. *Cyanidium caldarum* tolerates 57°C and pH 2-4, and can form an algal monoculture in the runoff from geothermal hot springs. Molecular phylogenetic studies suggest that Cyanidiales represents the deepest branch within rhodophytes. It is possible that they diverged from other red algae prior to the evolution of multicellularity in the rhodophyte lineage.

RD4.2 'Bangiophytes'

There are many fewer species of 'bangiophytes' than of Florideophyceae. The assemblage includes various filamentous forms as well as blade-like algae, and there are a few unicellular species. Bangiophytes never have triphasic life cycles and often lack pit plugs.

RD4.2.1 *Porphyra* and *Pyropia*. The best-known bangiophyte algae are *Porphyra* and *Pyropia*. Species are common and relatively conspicuous intertidal algae around the world, and several are harvested for food, including 'ori' production (see Section M5.2, above).

The conspicuous (and edible) phase of *Porphyra/Pyropia* is actually the gametophyte. The thallus is sheet-like, usually a broad blade, that can approach a metre in length in some species, but is only 1-2 cells thick (Figure RD3A). Very unusually for a red alga, the gametophyte thallus is tissue-like, rather than being composed of multiple filaments (see RD2, above), but cell division occurs throughout the blade, rather than being restricted to a meristematic region. There is also a small holdfast, with which the alga attaches to rocks and other substrates, or to other algae.

The lifecycle of *Porphyra* and its close relatives differs in several ways from the classic triphasic lifecycle described in Section RD3. The blade-like gametophyte produces both male and female gametes from different parts of the margins of the blades (i.e. it is monecious, not dioecious, but see below). After the female gametes are fertilized the new diploid zygotes are dispersed as 'carpospores'. Each carpospore germinates into a sporophyte, which is an inconspicuous branching filamentous alga, very different in appearance to the gametophyte (i.e. the generations are heteromorphic). The sporophyte is often called the 'conchocelis' phase, since these were originally classified as a separate genus – '*Conchocelis*' - before it was realized that they were merely one part of the lifecycle of an already-described alga. The sporophyte produces 'conchospores' from apical cells. Unusually, these spores are still diploid when released from the sporophyte. They undergo meiosis to produce haploid cells only after settlement and attachment, and it is these haploid cells that divide and develop into the new gametophyte. Like some other red algae, *Porphyra* also reproduces asexually quite readily, with the gametophyte phase producing unicellular 'monospores' that develop into new gametophytes, but we will not discuss this further.

One consequence of meiosis being delayed until after settlement is that the gametophyte can include cells that derive from (up to) all four of the cells produced by that meiosis. Since the products of meiosis would be genetically different, this means that the gametophyte is usually a mosaic of different genotypes, rather than a genetically uniform individual. Cells bearing the different genotypes can be distributed in longitudinal bands in the thallus, and in fact, cells derived from different meiotic products produce the male and female gametes.

In commercially harvested *Porphyra* and *Pyropia*, the sporophyte grows on different substrates (e.g. mollusc shells) and potentially at a greater depth than the gametophyte. The sporophyte was not linked to the harvested gametophyte until the lifecycle of these algae was elucidated in 1949 by British phycologist Kathleen Drew Baker. This discovery transformed the nori industry. Now the sporophyte phase is cultured, and used to reliably 'seed' a crop of gametophytes. This allows much more control over recruitment and breeding.

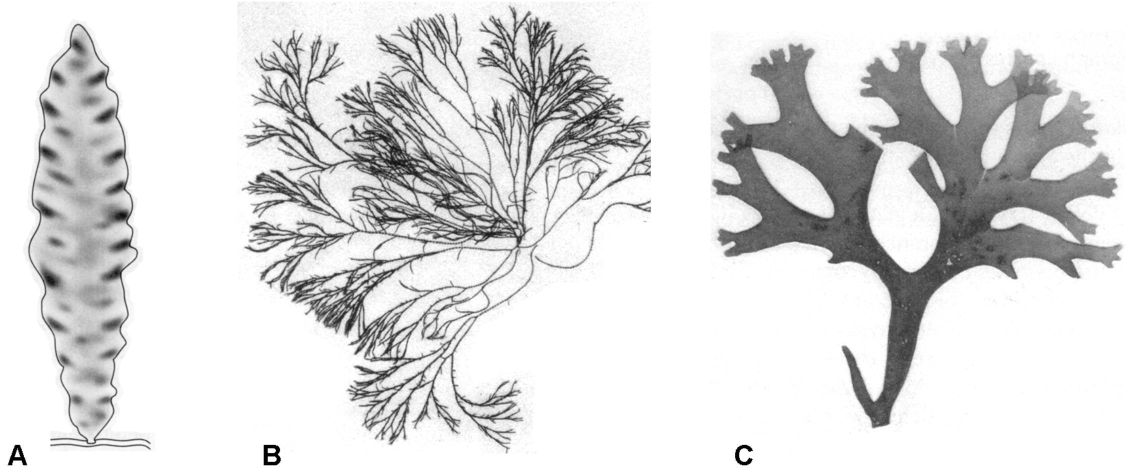


Figure RD3. Some common red algae. **A.** *Porphyra* gametophyte. **B.** *Ceramium*, A filamentous Ceramialean. C. Fragment of *Chondrus,* with a more fleshy thallus (fragment is about 5 cm across). From Graham and Wilcox, 2000.

RD4.3 Florideophyceae.

The triphasic lifecycle is characteristic of Florideophyceae, although it has been lost secondarily in a few taxa. There is a tremendous diversity of florideophycean algae. We will highlight only a few important or well-known taxa here as representatives.

RD4.3.1 Ceramiales is one of many groups of red algae with branching filamentous thalli. The filaments derive from a single meristematic cell (i.e. are uniaxial) but are generally more than one cell wide, and are composed of multiple distinct cell types. For example, there can be a single axis of 'axial cells' surrounded by a ring of 'periaxial cells', which are formed by lateral cell division during development. As with many rhodophytes, Ceramiales are generally dioecious, and have isomorphic gametophyte and tetrasporophyte generations.

Ceramiales is a large group with many described genera. *Ceramium* (Figure RD3B), for example, is a common intertidal red alga with a global distribution. *Polysiphonia* is another common and relatively well-studied intertidal Ceramialean.

RD4.3.2 *Chondrus* is a common intertidal/subtidal alga in much of the northern hemisphere. The thallus is broad and flattened with a branching habit (Figure RD3C) – each branch is composed of numerous cell filaments embedded in a common extracellular matrix. It forms thick growths in both gametophyte and tetrasporophyte phases (the group is isomorphic). *Chondrus crispus*, or "Irish Moss" is a long-exploited source of carrageenans.

RD4.3.3 Corallinales. The Corallinales characteristically have considerable depositions of calcium carbonate in their cell walls. In general they have a thickened thallus of pseudoparenchymatous organisation. There are two basic forms: 'Geniculate' corallines have an upright branching thallus consisting of thick, highly calcified segments with short flexible joints, or 'genicula' (very similar to some ulvophycean 'green algae' such as *Halimeda* – see Section CM2.2, above). 'Non-geniculate' forms lack joints and are usually very low-growing forms that encrust the substrate – rocks, other algae, etc. They generally grow very slowly, but are highly resistant to grazing and mechanical damage. The reproductive cells of Corallinales are buried within the thallus, within chambers called 'conceptacles' that communicate with the environment via a small pore (the 'ostiole'). Example corallines include *Corallina* (geniculate) and *Lithophyllum* and *Lithothamnion* (non-geniculate).

PART 3, SECTION P: PHAEOPHYCEAE

P1. Introduction.

The final major group of macroalgae is the Phaeophyceae or 'brown algae'. There are about 1500 species, most of which are marine, and they range in scale and complexity from small, unbranched filaments through to large, differentiated organisms with parenchymatous thalli. Some brown algae - the kelps - rival land plants in size, and degree of tissue formation and specialisation. Ironically, Phaeophyceae are the group of macroalgae that are the most *distantly* related to land plants.

Unlike the macroalgae we have examined so far, Phaeophyceae are not part of Archaeplastida. They actually belong to Stramenopiles (see Part 2, Section S). Like other photosynthetic stramenopiles, phaeophyceans are (at least) secondary algae, not primary algae, and have 'complex' plastids that are surrounded by four membranes and contain chlorophylls *a* and *c*. As with some other stramenopiles (e.g. diatoms) the plastids contain a considerable amount of the accessory pigment fucoxanthin, which is primarily responsible for their brown colour. The carbohydrate storage product is chrysolaminaran (laminaran), which accumulates in the cytoplasm.

The cell walls of phaeophyceans include some cellulose, but the distinctive components are modified (sulfated) polysaccharides called 'alginates', which can give the thallus a tough but flexible character. The cell walls are usually not calcified. Adjacent cells are connected by plasmodesmata, which function like the plasmodesmata of land plants, allowing communication between the cytoplasms of the adjacent cells. However, the plasmodesmata of Phaeophyceae do not contain desmotubules, unlike those of land plants (and Charales).

P2. Thallus organisation

Filamentous brown algae develop by division in one plane from meristems located either at the tip of the filament or, somewhat unusually, at the base of the filament. A few brown algae have pseudoparenchymatous growth, or diffuse growth across the entire thallus. However, most of the more complex brown algae are parenchymatous, producing true tissues by division in multiple planes from a meristematic region. In algae with broad, flat thalli these meristems are often at the apex (tip) or margins of the thallus. By contrast, in kelps with a stem-like 'stipe', there is generally an '**intercalary meristem**' between the stipe and blade, allowing elongation of both structures (see P4.2, below). Thus the blades of kelp grow from their bases, not their tips.

P3. Life Cycle

Most Phaeophyceae show a classical alternation of haploid and diploid generations. The generations may be isomorphic, but brown algae include some extreme cases of heteromorphy, with large sporophytes alternating with microscopic, barely multicellular (or even unicellular) gametophytes (see P4.2). The diploid sporophyte undergoes meiosis and produces haploid zoospores (usually called 'meiospores'), which are flagellated. They settle to produce gametophytes, which are usually dioecious. The gamete organisation ranges from isogamous or anisogamous flagellated gametes, through to oogamy, with flagellated male 'sperm'. Some brown algae, especially the Fucules (see P4.3) lack gametophytes - instead the diploid thallus undergoes meiosis to produce gametes that fuse and develop into new diploid adults. Some brown algal sporophytes can also reproduce asexually by producing diploid flagellated zoospores. Flagellated zoospores and gametes usually have two flagella, one with the flagellar hairs characteristic of stramenopiles (Part 2, Section S1).

P4. Diversity of Phaeophyceae

We will discuss only some examples of the diversity of brown algae here.

P4.1 Ectocarpales.

Ectocarpales include a variety of simpler and generally smaller brown algae with filamentous or broad thalli. They grow on a variety of surfaces – including, often, other macroalgae. *Ectocarpus* is a widespread filamentous form. Like some other brown algae, *Ectocarpus* has a well-developed asexual cycle in which the sporophyte produces numerous diploid zoospores, which settle to grow into new sporophytes. *Ectocarpus* species colonise a large variety of substrates, and can be a significant problem as a biofouling agent on the hulls of ships.

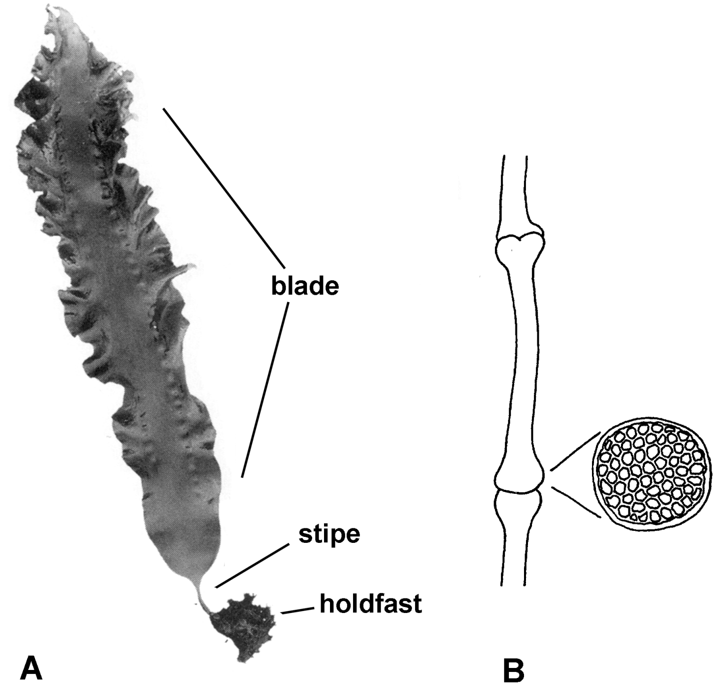


Figure P1. Laminariales (kelps). A. *Saccharina,* a kelp of moderate size and relatively simple overall morphology. **B.** Drawing showing a single connected chain of trumpet hyphae (sieve tubes) - the phloem-like solute transport cells of Laminiariales. The connections between trumpet hyphae are perforated, as shown in the transverse section (right), allowing transport of sugar/nutrient solutions along the network of these vessels. After Graham and Wilcox, 2000.

P4.2 Laminariales

Laminariales are the brown algae known as ‘kelps’. They are arguably the most complex macroalgae on earth and some species attain a huge size – over 50 metres in length. The major lifecycle phase is the sporophyte. The sporophyte thallus is typically composed of a stem-like **stipe** surmounted by leaf-like **blades** (Figure P1A). The stipe is usually unbranched, but is branched in some taxa. Most kelp live in the intertidal zone or the high subtidal, however, some species have adapted to live in deeper water and have gas-filled floats called **pneumatocysts** or **bladders** associated with the stipe, usually at the base of each blade. These floats help to maintain the primary photosynthetic tissues - the blades - near the water surface. The blades themselves can be plain, ornately patterned or have a central strengthening midrib. There is a large, usually branched holdfast at the base of the stipe (Figure P1A, and M2, above).

Both the blades and stipe generally contain specialised tissues and cell types. Firstly, there are distinct cortical and medullary tissue types. Typically the outer layer of the cortex is pigmented and photosynthetically active, while the internal tissue is colourless. Secondly, some of the internal cells – known as **trumpet hyphae** or ‘sieve tubes’ – are enlarged, highly elongate and specialised for transporting solutes – they are analogous to the solute transport system in land plants (Figure P1B). A system of trumpet hyphae that are connected end-to-end runs longitudinally throughout much of the thallus, and transports sugars and amino acids from the photosynthetically most active regions (e.g. blades) to other parts of the organism, such as the intercalary meristem (see P2, above). Note that 'trumpet hyphae' have no similarity to the hyphae of fungi, in spite of the suggestive name.

In Laminariales haploid spores (meiospores) are produced from regions of tissue that develop either along the blades or on specialised reproductive structures called 'sporophylls' that form along the stipe. In most cases, spores are released and quickly reach the bottom to settle, however, large deep-water kelp such as *Nereocystis* and *Macrocystis* (see below) have evolved various ways to liberate their spores near the substrate. In *Macrocystis* the spores are produced by the lower-most blades. The blades of *Nereocystis* float along the surface of the water, some 20 metres from the seabed. Rather than have the unicellular spores swim to the bottom to settle, the fertile region tears out of the blade prior to spore release. After settling the spores germinate as tiny (microscopic) filamentous gametophytes, thus kelp are extremely heteromorphic. Kelps are oogamous, with biflagellated sperm.

Common genera include *Laminaria* and *Saccharina,* which are often abundant in the sublittoral and intertidal worldwide. These have a short stipe that gives rise to very long blade that reaches a several metres in length in the largest species (Figure P1A). The blade is broad but 'dissected' into several strips in many *Laminaria*. Extensive beds of these forms are major features of the Altantic coast of Nova Scotia. Other kelp are huge and complex: The giant kelp *Macrocystis* can be 50 meters in length and has a branched stipe that bears numerous blades, which resemble large leaves. The blades have pneumatocysts at their bases to keep the massive thallus erect within the water column. *Macrocystis* can form extensive 'forests' in coastal waters, especially in the Pacific and in the southern hemisphere. *Nereocystis*, another form important in the Pacific, has a thick, flexible stipe, sometimes 20 meters in length, topped by a single pneumatocyst (=bladder) about 10-15 cm across, surmounted in turn by numerous long, thin blades, which themselves can be 10 – 20 metres in length. While many large kelp are long-lived (some >10 years), *Nereocystis* is an 'annual'. It achieves large size by having a growth rate of >10 cm/day (extremely high growth rates are common in large kelp). Other important large kelp include *Alaria,* and *Ecklonia* and *Egregia*.

P4.3 Fucales

Fucales rival Laminariales in complexity if not size. Fucales have a thickened thallus and usually a branching habit, with growth from apical meristems. The thallus shows a parenchymatous organization, and is differentiated into cortical and medullary tissues. Gas floats (akin to pneumatocysts) are common. Interestingly, it is likely that Fucales and Laminariales evolved independently from non-parenchymatous brown algal ancestors. Fucales are unusual amongst brown algae in having no distinct gametophyte phase – the adults are diploid, and produce gametes directly by meiotic division. Gametes fuse to form a zygote that develops directly into a new diploid adult. Fucales are either monecious or dioecious, and usually oogamous.

Fucus is a common intertidal fucoid brown alga around much of the northern hemisphere. It usually has flattened blade-like branches, and can grow to a metre in length. Another fucoid, *Ascophyllum*, is the most common macroalgae along the coast of Nova Scotia, forming extensive mats in the intertidal during low tide and a canopy for fish and invertebrates during high tide. *Ascophyllum* can reach two or three metres in length. Tens of thousands of tonnes of *Ascophyllum* are harvested annually in Nova Scotia.

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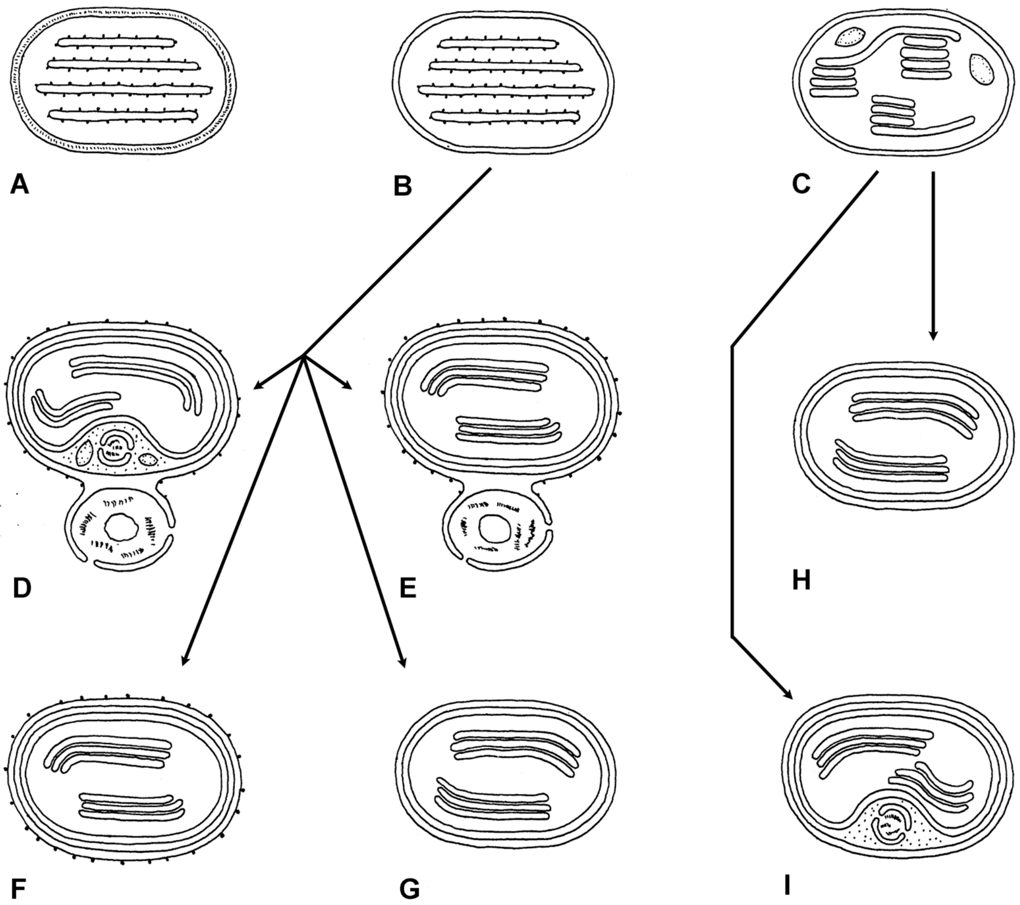
APPENDIX 1: A partial classification of eukaryotes.

Only important groups included. Bold names are taxon names (unless in inverted commas), names in parentheses are equivalent informal names. Example genera for some groups are listed on the right

Archaeplastida	
Chloroplastida (‘green algae + land plants’)	
Chlorophyta (chlorophytes)	
Chlorophyceae (chlorophyceans)	*Chlamydomonas*
Ulvophyceae *	*Ulva*
Streptophyta	
Charales *	*Chara*
Zygnematophyceae	
Embryophyta (land plants) **	
Rhodophyta * (red algae)	
‘**Bangiophytes**’ *	*Porphyra, Pyropia*
Florideophyceae *	
Glaucophyta	
Cryptomonada	
Cryptophyta (cryptophytes)	
Haptophyta (haptophytes, including. coccolithophorids)	*Emiliania*
Sar (‘SAR clade’)	
Stramenopiles/Stramenopila	
Chrysophyceae	
Bacillariophyta (diatoms)	
Phaeophyceae (brown algae) *	
Laminariales (kelp)*	*Macrocystis*
Bicosoecida	
Oomycota (oomycetes, inc. water moulds)	*Phytophthora*
Alveolata (alveolates)	
Dinoflagellata (dinoflagellates)	
Apicomplexa	*Plasmodium*
Ciliophora (ciliates)	*Paramecium, Tetrahymena*
Rhizaria	
Foraminifera (forams)	
Radiolaria	
Euglyphida (~’filose testate amoebae’)	*Paulinella*
Chlorarachniophyta (chlorarachniophytes)	
Metamonada ***	
Diplomonadida (diplomonads)	*Giardia*
Parabasala (parabasalids)	*Trichomonas*
Discoba ***	
Jakobida (jakobids)	
Heterolobosea	
Euglenozoa	
Euglenida (euglenids)	*Euglena*
Kinetoplastea (kinetoplastids, inc. trypanosomatids)	*Trypanosoma*
Amoebozoa	
Tubulinea and **Discosea** (‘lobose amoebae’)	*Amoeba*
Mycetozoa (slime moulds)	
Myxogastrea (acellular slime moulds)	
Dictyostela (cellular slime moulds)	*Dictyostelium*
Archamoebae	*Entamoeba*
Opisthokonta	
Choanoflagellata (choanoflagellates)	
Metazoa (true animals) **	
Fungi (true fungi) **	

* Predominantly macroalgal group, covered in the macroalgal sections
** Traditional multicellular ‘kingdom’
*** Often grouped together as ‘Excavata’, but this is controversial at present

APPENDIX 2: A diagrammatic summary of plastid organisation in eukaryotes.



A-C. Plastids of primary algae.

- **A. Glaucophyta** (chlorophyll *a*, phycobilisomes, remnant peptidoglycan wall).
- **B. Rhodophyta** – red algae (chlorophyll *a*, phycobilisomes).
- **C. Chloroplastida –** green algae and land plants **-** based on Charales or land plants (chlorophylls *a* and *b*; starch synthesis in plastid).

D-G. Plastids of secondary/tertiary algae, with plastids of red algal origin ('chromalveolates' – all with chlorophylls *a* and *c*; note that a single ultimate origin of these plastids does not necessarily mean that the algae that contain them are closely related: see Part 2, Section CA).

- **D. Cryptophyta** (nucleomorph, starch synthesis in 'primary host cytoplasm', connection to nucleus; Plastid Endoplasmic reticulum - PER).
- **E. Haptophyta** (connection to nucleus; PER).
- **F. Stramenopiles**, based on centric diatoms, girdle lamella not shown (PER; Note – many stramenopiles also have their plastids attached directly to the nucleus, as in figure E).
- **G. Dinoflagellates** (note only three surrounding membranes)

H, I. Plastids of secondary algae, with plastids of chloroplastidan origin, due to separate evolutionary events (chlorophylls *a* and *b*).

- **H. Photosynthetic euglenids**, *e.g. Euglena* (note only three surrounding membranes)
- **I. Chlorarachniophytes** (nucleomorph)