PROTOZOAN PARASITES

Protozoa are eukaryotic organisms (with a membrane-bound nucleus) which exist as structurally and functionally independent individual cells (= unicellular); including those species which are gregarious or form colonies. None have adopted multicellular somatic organisation characteristic of metazoan organisms. Instead, they have developed relatively complex subcellular features (membranes and organelles) which enable them to survive the rigours of their environments. The unicellular protozoa comprise multiple diverse assemblages, containing motile protozoal protists as well as non-motile algal and fungal protists. Early classification schemes recognized the higher taxon Protozoa (with a capital 'P'), but this was later subsumed to the descriptor protozoa (with a small 'p') in deference to the recognition of Protista (or Protoctista) as a collective group, although most composite members have now been integrated with other eukaryotes into several supergroups (viz. Excavata, SAR and Amorphea).

Protists exhibit enormous diversity in form and function. They are ubiquitous as free-living organisms in terrestrial and aquatic habitats and as symbionts (commensals, mutualists or parasites) of most animals and many plants. The diversity of protistan organisms is well appreciated both in terms of their structural heterogeneity, as well as their species richness and zoogeographic/biogeographic distribution. Most protists are microscopic organisms - only a few grow to a size large enough to be visible to the naked eye. Protistan cells (and indeed all individual metazoan cells) are constrained to microscopic sizes because of the small distances over which macromolecules may be transported for metabolic purposes (all parts of the cell must be accessible). The microscopic cell is therefore the basic unit of life. Individual protistan cells display all the same essential life activities as higher metazoan eukaryotes: they move about to feed, breed and survive.

Protista may be autotrophic (produce their own organic molecules), heterotrophic (derive organic molecules from external sources) or even mixotrophic (alternating between internal and external sources). Phototrophs (or photoautotrophs) synthesize their own organic molecules with energy derived from photosynthesis, while osmotrophs (or saprotrophs) take up dissolved nutrients across their cell membranes by passive and active transport or by pinocytosis. Phagotrophs (or holotrophs) ingest particulate material and use metabolic processes (catabolic decomposition and anabolic synthesis) to derive energy and organic molecules. They may be filter-feeders with simple-to-sophisticated oral structures to sweep food towards the mouth or they may be predatory (actively preying on other organisms) - being bactivorous (ingest bacteria), algivorous (ingest algae), herbivorous (ingest plant material), carnivorous (ingest other microscopic protists or zooplankton) or even histophagous (parasites invading and eating the tissues of larger organisms).

All eukaryotic cells are chimeric; they contain genetic information from multiple ancestral lineages. Modern molecular genealogies have generated complex pictures of eukaryote genomes where ancient lateral gene transfers have tangled their evolutionary history. All eukaryotes have a true nucleus (= 'eu-karyon') whereby the genetic material is enclosed by a membrane - in contrast to archae- and eu-bacteria (formerly referred to collectively, but incorrectly, as 'prokaryotes') where the genetic material lies directly in the cytoplasm. The nuclear membrane of eukaryotic cells is confluent with other endomembrane systems, including endoplasmic reticulum (serving as a scaffold for ribosomes – the sites of protein synthesis) and dictyosomes (Golgi bodies where proteins are processed and packaged). Structural support is provided by cytoskeletal elements (microtubules and microfilaments) which are also involved in motility, by means of undulipodia (including flagella and cilia) or pseudopodia (involving temporary cellular extensions and cytoplasmic streaming), feeding (phagocytosis and pinocytosis) and nuclear division (nuclear spindle formation).

The serial endosymbiosis theory (SET) posits that eukaryotic cells serially acquired various endomembrane and organellar systems from endosymbiotic bacteria over long evolutionary history. Indeed, molecular biological studies have indicated that mitochondria may have originated from α-proteobacteria (similar to rickettsias) and chloroplasts from cyanobacteria. Not all extant protozoa possess the 'full' complement of eukaryotic organelles (some lack dictyosomes, some lack mitochondria, etc.), thus their variable possession of such components makes them basal in eukaryote evolution, or intermediary between bacterial domains and eukaryotes (in some cases, making them the perfect missing links).

Classification schemes

The name ‘proto-zoa’ literally means ‘first animals’ and early classification systems grouped the protozoa with members of the animal kingdom due to their many similarities to eukaryotic metazoan cells. However, they were recognized as a discrete assemblage on the basis of their unicellularity and were assigned to the taxon Protozoa which was considered basal to the Metazoa (they are invariably figured as the trunk of the animal tree of life - befitting their name as the ‘first animals’). Nonetheless, the members of the subkingdom Protozoa are quite disparate. Indeed, the taxon has never been considered a natural assemblage of organisms but rather one of convenience. Various classification schemes have been proposed for protozoan organisms. Since its inception in the 1880’s, the Butschlian scheme has dominated whereby four major assemblages were recognized on the basis of their locomotion using specialized subcellular and cytoskeletal features:

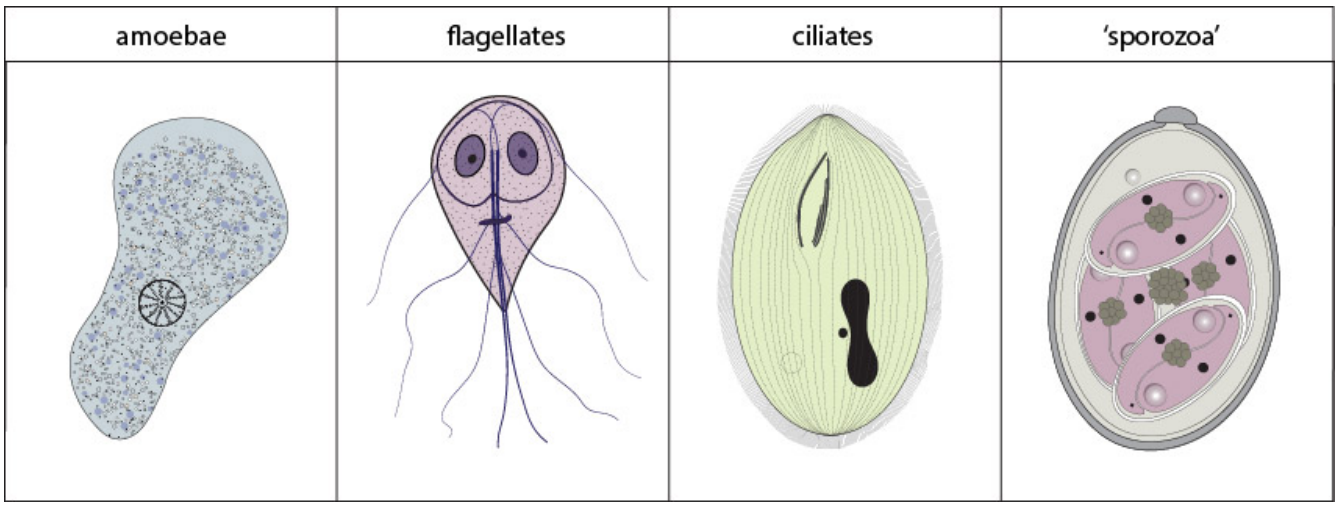
- Sarcodina (amoebae) use pseudopodia (singular: pseudopodium) to creep or crawl over solid substrates. Pseudopodia (or ‘false feet’) are temporary thread-like or balloon-like extensions of the cell membrane into which the protoplasm streams. Similar amoeboid motion has been observed in cells of many life-forms, especially phagocytic cells (e.g. human macrophages).

- Mastigophora (flagellates) use elongate flagella* (singular: flagellum) which undulate to propel the cell through liquid environments. Flagella are ‘whip-like’ extensions of the cell membrane with an inner core of microtubules arranged in a specific 2+9 configuration (2 single central microtubules surrounded by 9 peripheral doublets). This configuration is conserved throughout eukaryotic biology, many organisms produce flagellated cells (e.g. human spermatozoa).

- Infusoria (ciliates) use numerous small cilia* (singular: cilium) which undulate in waves allowing cells to swim in fluids. Cilia are ‘hair-like’ extensions of the cell membrane similar in construction to flagella but with interconnecting basal elements facilitating synchronous movement. Ciliated cells are found in specialized tissues and organs in many other higher life-forms (e.g. human bronchial epithelial cells).

- Sporozoa (‘spore-formers’) were originally recognized not on the basis of their locomotion, but because they all formed non-motile spores as transmission stages. Recent studies, however, have shown that many pre-spore stages move using tiny undulating ridges or waves in the cell membrane imparting a forward gliding motion, but the actual mechanisms involved are not yet known.

*The flagella and cilia of eukaryotes ‘undulate’ (dynein-walking along microtubules) rather than ‘rotate’ like the flagella of archae-/eu-bacterial organisms. Some authorities therefore reserve the term ‘flagellum’ for bacteria and recommend the term ‘cilium’ for eukaryotes, while others favour the collective term ‘undulipodia’ for eukaryotic flagella and cilia.



More recently, many attempts have been made to reconcile the members of these basic but disparate groups into a small number of phyla formally recognized in a consensus classification system. A range of phenotypic characters were used to classify organisms: including morphological (cellular/subcellular, light/electron microscopic); behavioural (*in vivo* host specificity, life cycle, distribution, *in vitro* cultivation requirements); and biochemical (metabolic respiration/digestion, drug sensitivity/resistance, molecular structure/function) characteristics. Several decades ago, the Society of Protozoologists published a revised classification system (Levine *et al*., 1980) and an illustrated guide (Lee *et al*., 1985) to the Protozoa; recognizing eight main phyla, principally on the basis of morphology.

- Sarcomastigophora (flagellates and amoebae) — most free-living, some symbiotic
- Labyrinthomorpha (cells glide on ectoplasmic network) — parasitic on marine algae
- Ciliophora (ciliates, dikaryotic) — most free-living, some symbiotic
- Apicomplexa ('sporozoa', apical complex present) — parasitic in vertebrates/invertebrates
- Microspora (unicellular spores with polar tubes) — parasitic in vertebrates/invertebrates
- Haplosporidia (spores without polar capsules) — parasitic mainly in oysters
- Paramyxea (unique cells within cells configuration) — parasitic mainly in oysters
- Myxozoa (multicellular spores with polar capsules) — parasitic mainly in fish

Over the last two decades, classification schemes have adopted a broader perspective to encompass all unicellular protistan organisms, including traditional protozoal, algal and fungal groups as well as their intermediates. Ultrastructural studies identified many new assemblages with distinctive subcellular features (organelles, membranes and cytoskeletal elements), and contemporary molecular biological studies on gene and protein sequences are now facilitating comparative analyses and the construction of phylogenetic trees whose topologies better reflect evolutionary relationships. The Society of Protozoologists revised their guide to the protozoa (Lee *et al*., 2000) with the recognition of some 40 protistan phyla on the basis of genotypic and phenotypic characters. Lower taxonomic groupings (families, genera and species) are often well supported by comparative genotypic analyses, while their higher taxonomic affinities (orders, classes, phyla) are slowly being resolved amidst some controversy. The Society (now the International Society of Protistologists) recently published a revised classification of eukaryotes (Adl *et al*., 2012, 2018) integrating unicellular and multicellular organisms into several supergroups [note that the taxonomic rank of the assemblages (phylum, class, order, etc.) varies widely according to author, so they are ranked here using dot-points rather than with names to provide an uncluttered overview]:

Domain: Eukaryota (membrane-bound nucleus)

- Supergroup: Excavata (with conspicuous ventral feeding groove)
  - • Metamonad (amitochondriate flagellates with karyomastigonts)
    - •• Fornicata (diplomonads)
    - •• Parabasalia (trichomonads, hypermastigids, retortamonads)
    - •• Preaxostyla (oxymonads)
  - • Discoba (diverse group supported robustly by molecular studies)
    - ••• Heterolobosea (amoebo-flagellates, schizopyrenids or vahlkampfids
    - ••• Euglenozoa (euglenids and kinetoplastids)
- Supergroup: SAR (Stramenopiles + Alveolata + Rhizaria)
  - • Stramenopiles (with heterokont/dissimilar flagella)
    - •• Opalina (opalescent symbiotes in amphibians)
  - • Alveolata (with cortical alveoli)
    - •• Protalveolata (incl. perkinsids parasitic in molluscs)
    - •• Dinoflagellata (mesokaryotic nuclei, autotrophs and heterotrophs)
    - •• Ciliophora (nuclear dualism, hair-like cilia, conjugation)
    - •• Apicomplexa (with apical complex organelles)
  - • Rhizaria (various amoebae and flagellates
    - •• Cercozoa (biflagellated and/or amoeboid, usually with filopodia, plus ascetospora (haplosporidia, paramyxea))
    - •• Retaria (amoebae with reticulopodia (Foraminifera) or axopodia (Radiolaria))

- Supergroup: Amorphea (unikonts, with single flagellum, or nonflagellated amoebae)

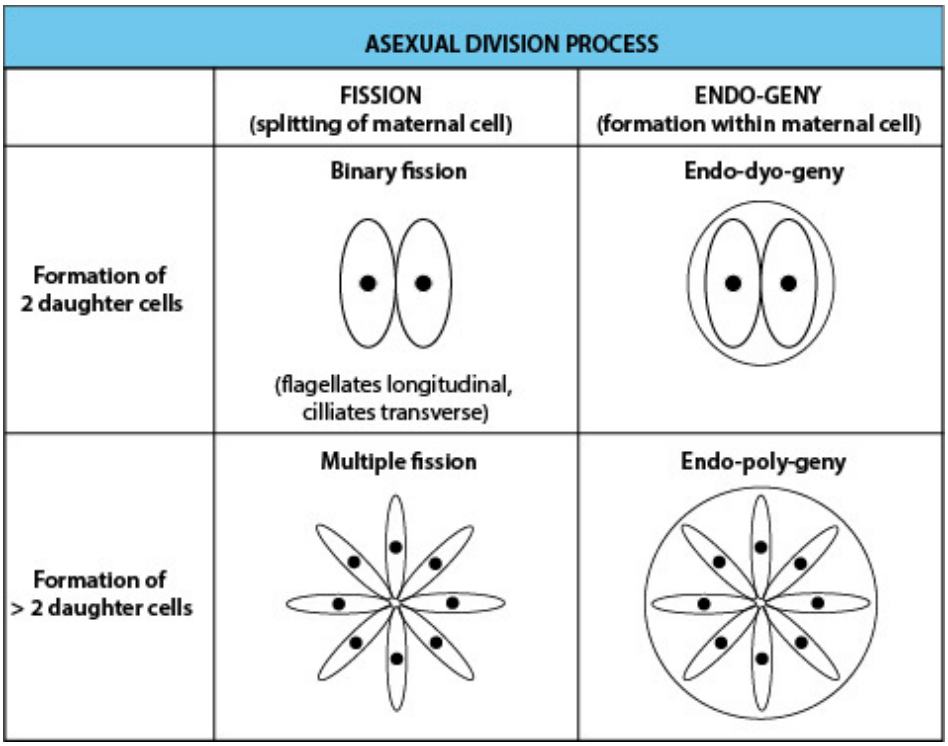
• Amoebozoa			(naked/testate amoebae, noneruptive variable pseudopodia)
	•• Tubulinea		(produce tubular subcylindrical pseudopodia)
	•• Discosea		(never produce tubular pseudopodia)
	•• Archamoebae		(amitochondriate but with nonaerobic organelles)
	•• Gracilipodia		(produce short conical or fine hair-like subpseudopodia)
• Opisthokonta			(stages with single posterior flagellum)
	•• Nucletmycea		(Holomycota, fungi and relatives)
		••• Nucleariа	(round amoebae with filopodia)
		••• Fungi	(fungi, with chitinous walls; incl. microsporidia)
	•• Holozoa		(metazoans, filasterans, ichthyosporeans, choanomonads)
		••• Metazoa	(multicellular heterotrophs, notably animals (incl. myxozoa))

Biodiversity

Protozoan biodiversity (or species richness) includes counts (estimates) of some 32,000 extant (living) species and another 34,000 extinct (fossil) species (especially foraminifera). Of those alive today, some 21,000 species occur as free-living organisms in aquatic or terrestrial environments, whereas the remaining 11,000 species are parasitic in vertebrate and invertebrate hosts. There are approximately 6,900 flagellate species (1,800 parasitic, 5,100 free-living), 11,550 amoebae species (250 parasitic, 11,300 free-living), 7,200 ciliate species (2,500 parasitic, 4,700 free-living) and 5,600 sporozoan species (all parasitic). Estimates of protozoan parasite biodiversity vary considerably because little is known about the host range and specificity of most parasites. Some host species harbour only one or a few protozoan species, while others have many. Some parasite species only occur in one host species, while other parasites have a broad host range. The potential number of parasitic species (N) depends on the number of available host species (A), their host specificity (B = average number of protozoan species per host species) and their host range (C = average number of host species per protozoan species); according to the formula $N = AB/C$. Estimates of N can vary 100-fold because estimates of B and C may vary by as much as 50-fold.

Protozoal diseases

Protists have enormous proliferative/multiplicative potential. While animals and plants divide asexually by open mitosis (extranuclear spindle, with pole bodies), most protists divide by closed mitosis (nuclear envelope does not breakdown, but contains internal spindles), with the exception of parabasalids and dinoflagellates (closed mitosis with external spindles). Protists can divide asexually by splitting (binary or multiple fission) or internal division (endogeny or plasmotomy). Many species are also able to reproduce sexually, either forming conjugated pairs which exchange nuclei (e.g. ciliates) or forming separate male and female gametes (gamogony) which recombine (syngamy) to form zygotes (e.g. apicomplexans).



Parasite pathogenicity (virulence) and disease severity is often positively correlated with their proliferative potential. Protozoan species which quickly multiply within host tissues cause acute transient severe diseases (rapid-onset, high-intensity, short duration). The parasites often exhibit exponential growth until specific host immune responses develop and act against infections (sterile immunity leading to eradication, concomitant or premunitive immunity leading to reduction but not elimination). In most vertebrate hosts, adaptive/acquired immune responses (humoral and cell-mediated) become most effective approximately one week after primary infection. During this time, the parasites may cause significant damage to host cells and tissues leading to overt clinical disease. Some protozoa, however, encyst within host tissues giving rise to chronic protracted diseases.

Host pathology is generally indicated by the four cardinal signs of inflammation: rubor (redness), calor (heat), tumor (swelling) and dolor (pain) due to the hyperaemia and extravasation of phagocytes associated with innate immunity. Inflammation may be focal, local or systemic (indicated by the suffix '-itis', e.g. inflammation of the heart is called carditis, that of the brain encephalitis, etc) and it may be accompanied by other systemic responses (e.g. fever due to the release of endogenous pyrogens which act on the hypothalamic thermostat; ischaemia due to collapse of the microcirculation; circulatory shock due to peripheral constriction to divert blood to vital organs in viscera; toxic shock due to endo- or exo-toxins; hypersensitivities due to hysterical immune responses, etc.).

Disease symptoms (described to clinician) and signs (observed by clinician) depend largely on the host compartment or tissue systems involved. Infections may occur in extracellular compartments (lumen-dwelling or interstitial parasites) but most protozoan species, at some stage, occupy intracellular locations (ultimately causing cell lysis thus structural and functional pathology). Protozoan parasites infecting the gastro-intestinal tract may be lumen-dwelling, mucosal-browsers or invasive in epithelial or subepithelial locations. Their presence causes local inflammation (gastritis and/or enteritis) resulting in vomiting and/or diarrhoea. Protozoa infecting the vascular system (fluid or cellular components) often cause haemolytic anaemia and fever. Parasites infecting specific tissues or organs often cause space-occupying lesions and ultimately organ malfunction.

Life-cycles

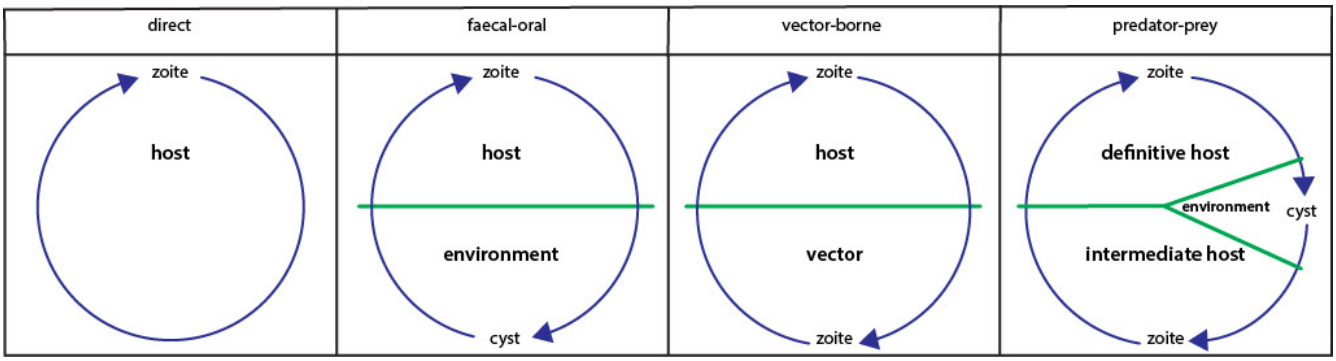
Protozoan developmental stages occurring within hosts generally consist of feeding trophozoites, and they may be found intracellularly (within host cells) or extracellularly (in hollow organs, body fluids or interstitial spaces between cells). While trophozoites are ideally suited to their parasitic mode of existence, they are not very resistant to external environmental conditions and do not survive long outside of their hosts. To move from host-to-host, protozoan parasites use one of four main modes of transmission: direct, faecal-oral, vector-borne and predator-prey transmission.

- direct transmission of trophozoites through intimate body contact, such as sexual transmission (e.g. *Trichomonas* spp. flagellates causing trichomoniasis in humans and bovine infertility in cattle).

- faecal-oral transmission of environmentally-resistant cyst stages passed in faeces of one host and ingested with food/water by another (e.g. *Entamoeba histolytica*, *Giardia duodenalis* and *Balantidium coli* all form faecal cysts which are ingested by new hosts leading to amoebic dysentery, giardiasis and balantidiasis, respectively).

- vector-borne transmission of trophozoites taken up by blood-sucking arthropods (insects or arachnids) and passed to new hosts when they next feed (e.g. *Trypanosoma brucei* flagellates transmitted by tsetse flies to humans where they cause sleeping sickness, *Plasmodium* spp. haemosporidia transmitted by mosquitoes to humans where they cause malaria).

- predator-prey transmission of zoites encysted within the tissues of a prey animal (e.g. herbivore) being eaten by a predator (carnivore) which subsequently sheds spores into the environment to be ingested by new prey animals (e.g. tissue cysts of the sporozoan *Toxoplasma gondii* being ingested by cats, and tissue cysts of the microsporidian *Thelohania* spp. being ingested by crustaceans).

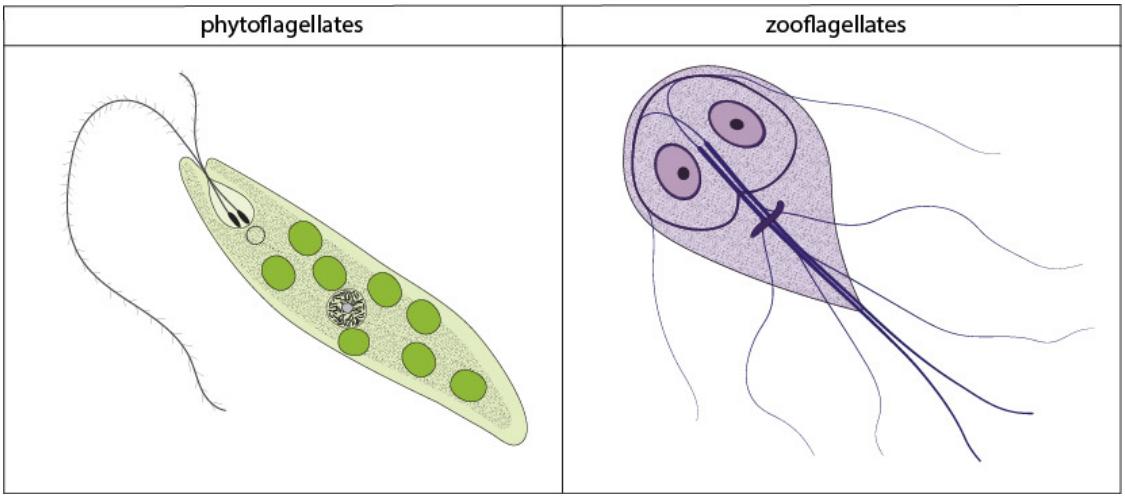


Overview of major protozoan groups parasitizing animals

Flagellates and amoebae are considered to be closely related, because some amoebae form transient flagellated stages (to aid in dispersal) and some flagellates exhibit intermittent amoeboid motion. Two groups of flagellates are recognized on the basis of the presence or absence of chloroplasts:

- Phytoflagellates with chloroplasts derive energy by photosynthesis. Most are free-living aquatic organisms and some exhibit periodic blooms (e.g. red tides). Others contain potent neurotoxins and cause paralytic shellfish poisoning.

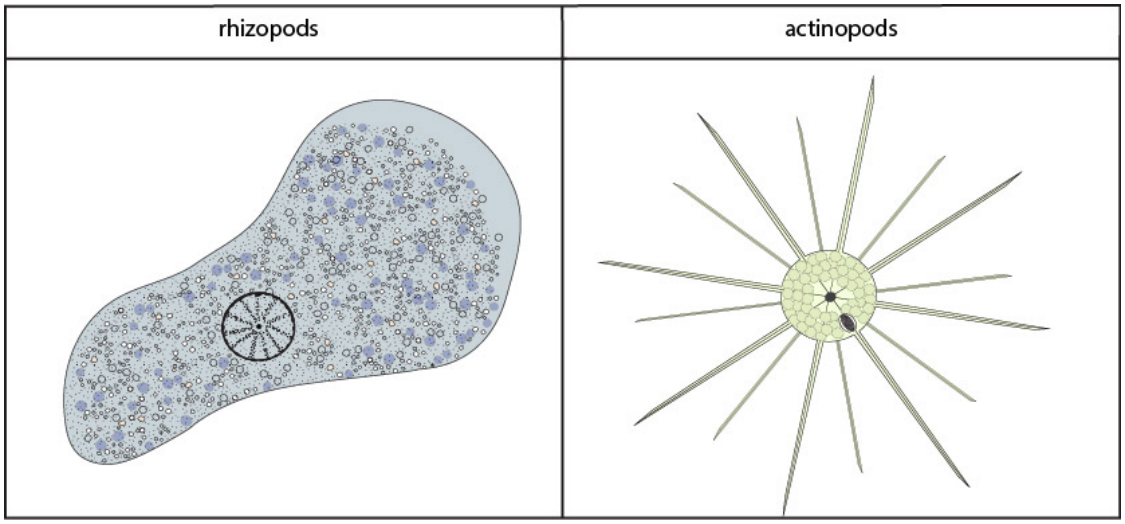
- Zooflagellates without chloroplasts derive energy by the absorption of nutrients or the ingestion of food particles. Many species occur as free-living aquatic organisms whereas others live in insects and some vertebrates as symbiotes, commensals or parasites (several species cause major human diseases such as sleeping sickness, Chagas' disease, kala azar and diarrhoea).



Two groups of amoebae are recognized on the basis of the types of pseudopodia formed with or without regular microtubule arrays:

- Rhizopod amoebae produce broad lobopodia, fine filopodia or net-like reticulopodia which do not contain regular microtubule arrays. Many aquatic species contribute to water quality by consuming bacteria and algae whereas terrestrial species contribute to soil health via nutrient cycling. Some species, such as foraminifera, build unique tests (shells) which contribute to fossil records.

- Actinopod amoebae form radial axopodia which are stiffened by internal arrays of microtubules arising from an organizing centre. All species are free-living planktonic organisms, marine species known as radiolaria, and freshwater species known as heliozoa (or sun animalcules).

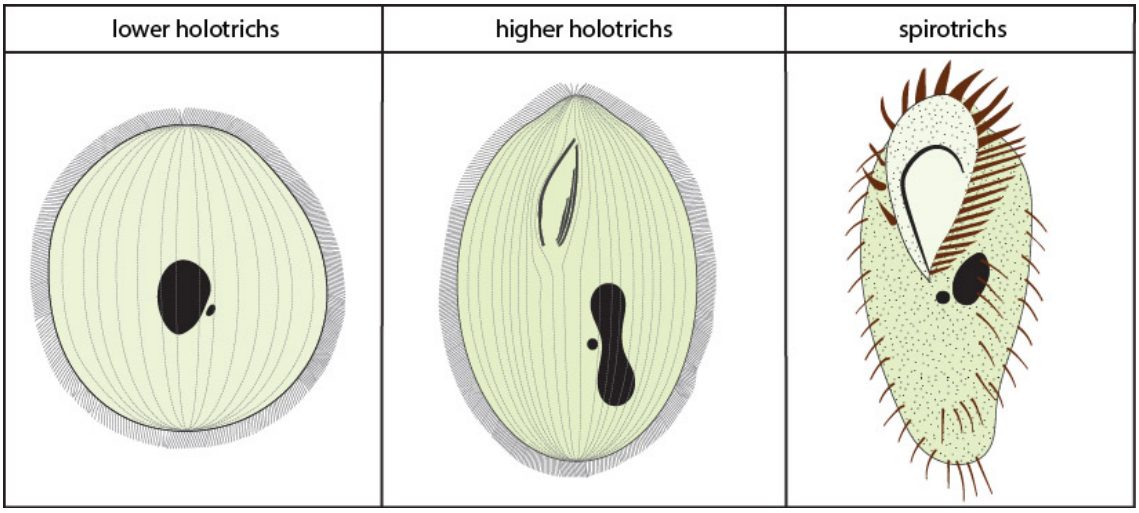


The ciliates are regarded to be quite separate from other groups, more because they possess two types of nuclei (vegetative macronuclei and reproductive micronuclei) than because they possess cilia. Three groups are recognized on the basis of their patterns of somatic (body) and buccal (oral) ciliature:

- Lower holotrichs have simple body and oral ciliature. Most are free-living aquatic species but some are highly specialized symbionts aiding cellulose digestion in herbivores.

- Higher holotrichs have simple body ciliature but more specialized oral ciliature forming membranelles. Most occur as free-living aquatic organisms but some live as commensals or parasites in a range of animals.

- Spirotrichs have reduced body ciliated but well-developed oral ciliature forming an adoral zone of membranelles. The majority are bactivores living in aquatic and terrestrial habitats.

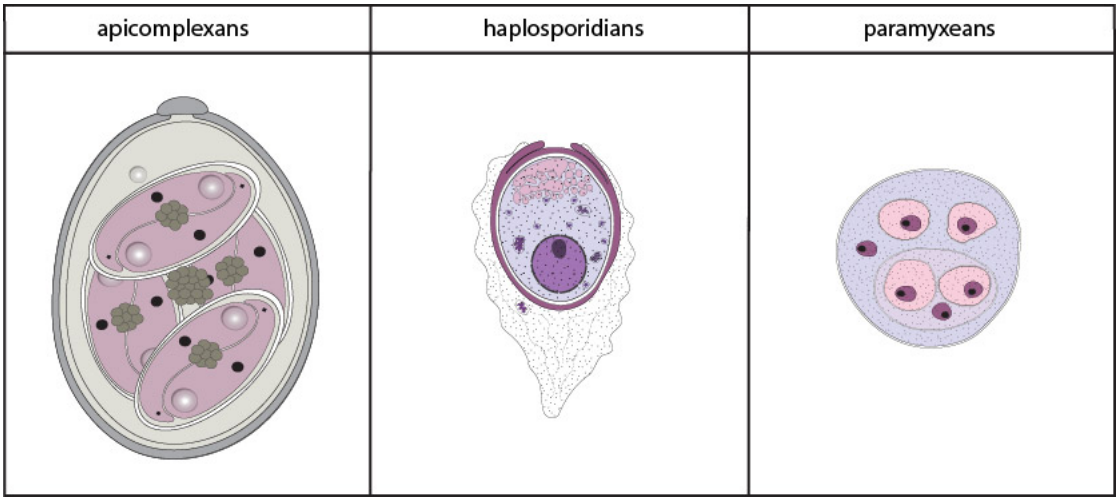


All sporozoa are obligate parasites, they form temporary non-motile spores which contain infective cells. Three major groups are recognized on the basis of different spore morphology:

- Apicomplexan parasites form distinctive oocysts containing infective sporozoites. Many species occur only in invertebrates whereas others may infect vertebrates causing severe diseases (such as malaria, tick fever, diarrhoea or abortion).

- Haplosporidian parasites form unicellular spores without polar filaments in the tissues of aquatic invertebrates. They cause significant morbidity and mortality in oysters throughout the world.

- Paramyxean parasites form unique spore-within-spore arrangements within the tissues of bivalves and polychaetes. They cause QX and Aber disease in oysters



Another two sporozoa-like groups were long considered to be protistan parasites, but contemporary studies have shown them to belong to different kingdoms: microsporidian parasites belonging to the kingdom Fungi, and myxosporean parasites belonging to the kingdom Metazoa (Animalia).

- Microsporidian parasites form unicellular spores containing coiled polar tubes used to infect host cells. Most species infect invertebrates (especially insects) although some form cysts in vertebrates (mainly fish).

- Myxosporean parasites form multicellular spores containing capsulogenic and sporoplasmic cells enclosed within valvulogenic cells. All species are found as coelozoic or histozoic parasites of marine and freshwater fishes; some causing debilitating diseases and tissues lesions.

microsporans	myxozoans