Protista Laboratory

A group of very diverse organisms that share some common characteristics contribute to a group referred to as Protists. They are often unicellular, although they may be found in colonies or in bodies with specialized structures. As members of the domain Eukarya some are plant-like autotrophs (algae), some are heterotrophs (protozoan or animal-like) and some are more related to fungi and are absorptive heterotrophs. These organisms exhibit a high degree of complexity. Seventy thousand protozoans have been identified. While generally microscopic, they are found in damp or wet environments, such as lakes, oceans, streams, damp soil, mud puddles and birdbaths. They have an incredibly large impact on ecosystems, economics and health of diverse organisms including Homo sapiens.

Objectives:

1. Discuss general characteristics of protists.
2. Discuss general structure and niche of some important groups.
3. Give examples of some of the clades and explain where members can be found.
4. Compare classification using the 5-kingdom method and 3-domain method.
5. Be familiar with life cycles of *Paramecium* and *Plasmodium*.
6. Differentiate between modes of locomotion in *Amoeba*, *Paramecium* and *Euglena*.
7. In an evolutionary context, describe the connection between Chlorophyta, Charophyta (green algae) and higher plants.

Protista

The origin of eukarya is still debated today. Many scientists support the autogenous theory which suggests that compartmentalization of chemical processes occurred via segregation of enzymes and subsequent infoldings of plasma membrane. The serial endogenous theory proposed by Lynn Margulis suggested that organelles originated in host cells by engulfing smaller cells that had specialized functions, (E.g.: mitochondria and chloroplasts contain extranuclear DNA).

Acritarchs are the oldest fossils of protists. Some have been found in Michigan rocks that are 2.1 billion years old. Protists have been classified quite generally via nutritional modes as algae (plantlike), protozoa (animal-like) and absorptive (fungus-like) heterotrophs. In terms of reproduction, all reproduce asexually while some reproduce sexually via syngamy. Like bacteria, some form cysts when conditions become harsh.

Protists are found in wet or damp environments. They inhabit soil, litter, oceans, ponds and lakes. Some contribute to plankton, the communities of organisms that drift or swim near the water surface. Phytoplankton constitutes the bases of both marine and freshwater environments.

Symbiotic relationships exist between some protists and hosts, which may be classified as mutualistic, commensalistic or parasitic. Some combine photosynthesis and heterotrophic

nutrition and are referred to as mixotrophs. Hosts may be higher organisms, such as vertebrates. Most protists are unicellular. Most eukaryotic lines diverge from protists.

The older five-kingdom method of classification is being replaced by a newer three-domain method of classification. The prokaryotes are placed in the domains Bacteria and Archaea. Reclassification has occurred as more information has been made available which illuminates the biochemistry and further defines relationships of representative organisms. Bacteria and Archaea have been previously presented, thus we will focus on eukaryotic organisms formerly known as Protista.

Excavata

Diplomonadida: 2 haploid nuclei, *Giardia lamblia*.
Parabasala: includes trichomonads, *Trichomonas vaginalis* (STD).
Euglenazoa: characterized by crystalline rod inside flagella.

1. Kinetoplastids contain a large single mitochondria with a mass of DNA inside, the kinetoplast such as *Trypanosoma* which causes sleeping sickness and Chagas disease.
2. Euglenids: pocket at end of cell from which flagella emerge. May be autotrophic, *Euglena*.

SARS

Alveolata: membrane bound sac under plasma membrane.

1. Dinoflagellata: CHO cell wall with two flagella (one wrapped in a groove around the midsection.) Some cause red tide. *Ceratium* is an example
2. Apicomplexa: parasites, many with intricate life cycles such as *Plasmodium* (malaria.)
3. Ciliates: named for the use of cilia for locomotion such as *Paramecium*.

Stramenopila: hairy flagellum (some smooth also.)

1. Bacillariophtes (diatoms): glass-like wall, highly diverse.
2. Chrysophytes (golden algae): yellow and brown carotenoids, *Dinobryon*.
3. Phyophytes (brown algae): many large algae, *Fucus*.
4. Water molds (Oomycota), *Phytophthora*.

Rhizaria: includes many amoeba with pseudopodia.

1. Radiolarians: test made of silica with axopodia extending through pores.
2. Cercazoa: diverse group of pigmented amoeba. Chlorarachniophytes contain nucleomorph.
3. Foraminifera: calcium carbonate porous shells with slender strands of plasma membrane emanating through the test.

Archaeplastida: close relatives to land plants.

1. Rhodophyta: red algae with phycoerythrin, Porphyra.
2. Chlorophyta: green algae. *Ulva*, *Chlamydomonas* and *Volvox*.
3. *Charaphyceans*: link between green algae and green plants. Retain embryos, *Chara*
4. Land plants.

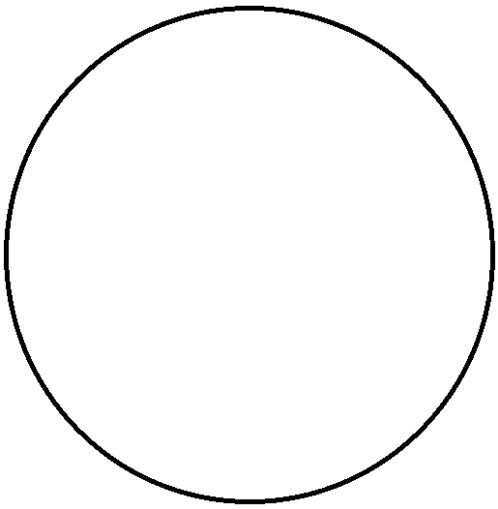
Unikonta: includes protists that are closely related to fungi and animals.

Amoebozoa: lobe-shaped pseudopodia.

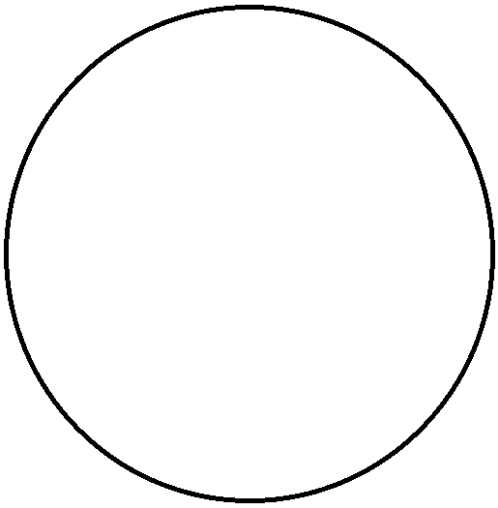
1. Tubulin**s**: diverse group of soil or water-dwellers.
2. Entamoebas**:** parasites such as *Entamoeba histolytica*.
3. Slime molds**:** cellular and plasmodial slime molds: *Dictyostelida* (cellular slime mold) and Plasmodial slime molds, *Physarum*..

Opisthokonts: includes choanoflagellates, nucleariids, animals, fungi.

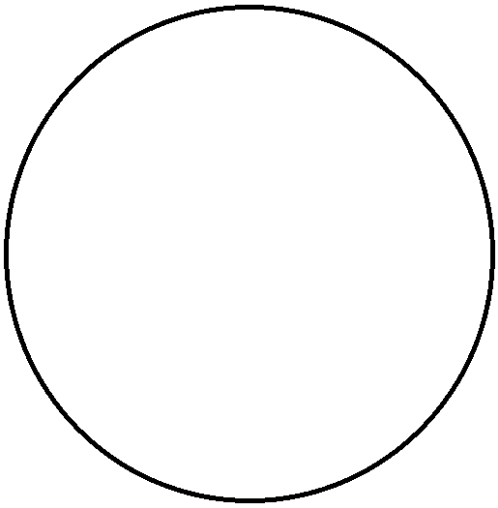
The Supergroup Excavata includes Diplomonads, organisms that lack mitochondria and have two nuclei. One example is *Giardia lamblia*, a diplomonad that causes intestinal distress and even death. The usual mode of infection is drinking water that contains human fecal material. Outbreaks of this disease sometimes occur in daycare centers or other places where good hygiene is in question. Examine prepared slides of *Giardia* and note the two nuclei in this flagellated organism. Draw *Giardia*.



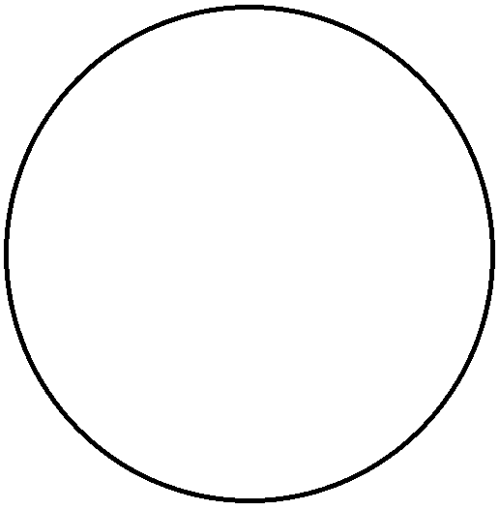
The clade Parabasala includes organisms that lack mitochondria. A common example is *Trichomonas vaginalis* the source of a common STD. This disease can be harbored by males as well as females and is often silent (asymptomatic) in males. It is an interesting organism that possesses both flagella as well as an undulating membrane. Draw *Trichomonas vaginalis*.



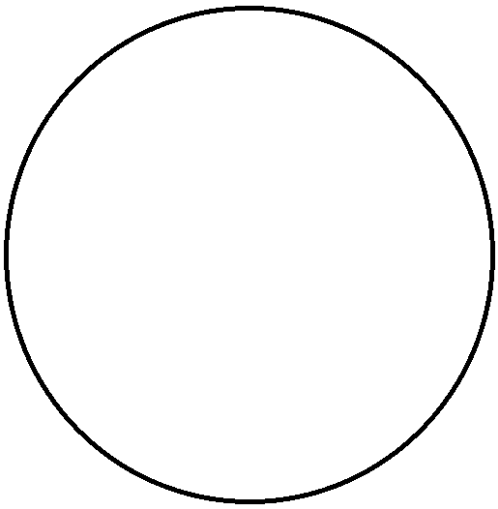
Euglenazoa, includes the pigmented and flagellated *Euglena* as well as another group, the kinetoplastids, a group of parasites that includes *Trypanosoma* (found on prepared slides only). Trypanosomes produce diseases such as sleeping sickness carried by the Tse Tse fly in Africa and Chagas' disease, in South America. It is estimated that a high number of people have been infected with the Chagas" trypanosome and some health experts suggest that our blood supply be examined for the presence of the microbe. Draw a typical trypanosome.



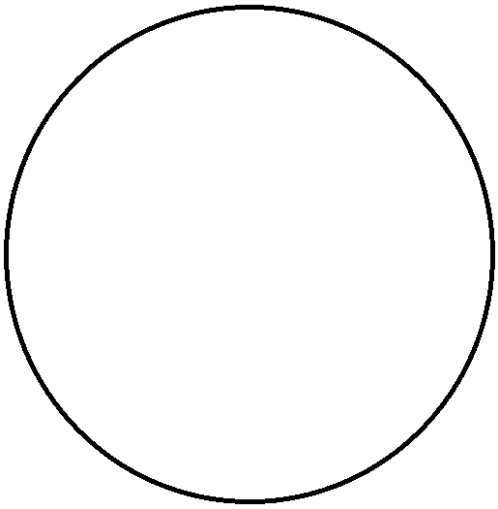
Euglena, generally autotrophic, is typically found in freshwater environs. Euglenazoans are autotrophs, which occasionally function as mixotrophs in the absence of light. Examine both prepared slides and living cultures if available in the laboratory. Living culture media will require a drop of Detain to slow the organism for viewing. (These slides must be then washed after viewing with soapy water as the Detain or Protoslo may be very viscous.) Detain sets up a carbohydrate matrix which organisms become imprisoned in.) Draw *Euglena* in the space provided.



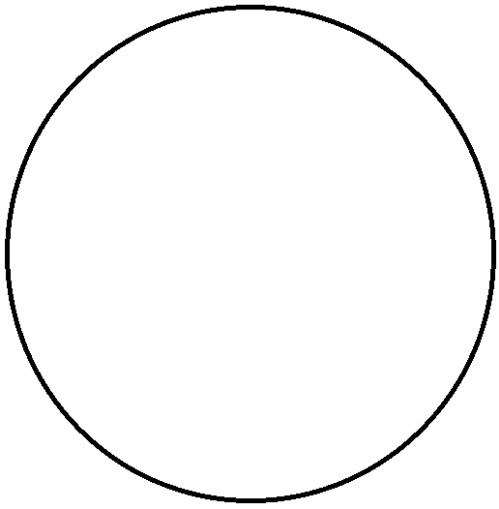
Moving on to the SARS clade (divided into Stramenopiles, Alveolates and Rhizarians.) Alveolata (a monophyletic group) includes Dinoflagellates, Ciliophora and Apicomplexa. Dinoflagellates present internal plates of cellulose, two flagella and are responsible for "Red Tide." Toxins produced by some of the dinoflagellates kill organisms in the waters and are responsible for paralytic seafood poisoning in man. This group includes those that photosynthesize and have external plates of cellulose that exhibit a groove in which one of the flagella can be found. Examine prepared slides of dinoflagellates and draw a representative.



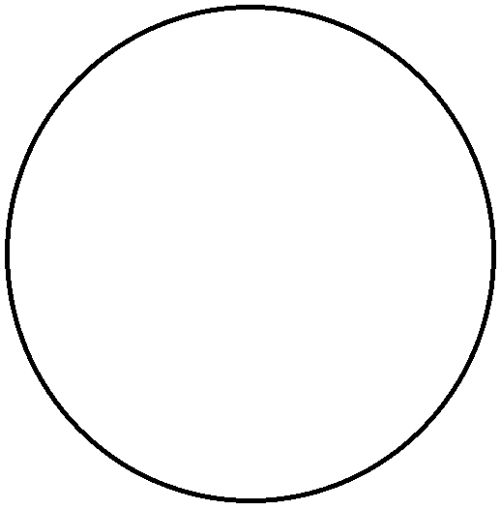
Apicomplexa (parasitic Alveolata) have complicated life cycles as in the case of *Plasmodium*, which causes malaria. Malaria is responsible for the deaths of 30% of the children in Africa. The female *Anolpheles* mosquito carries the infectious sporozoites that are transferred to the human when she bites to draw blood. The *Plasmodial* parasite evades detection by members of the immune system by hiding in cells of the liver. The genetic code of the parasite has been examined and a vaccination has been developed against the protist. Examine slides of *Plasmodium*. Draw representative stages.



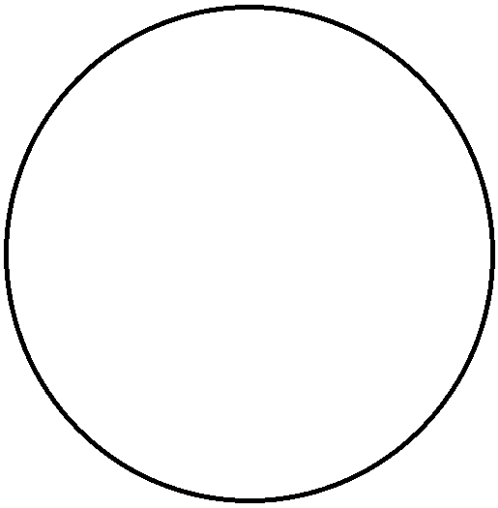
Ciliates (Alveolata) use cilia for motility and feeding. *Paramecium*, *Stentor* and *Vorticella* are examples of this phylum. Examine prepared slides of paramecium. Compare the image with that of cells from a living culture. Use a drop of Detain to slow the motion of the organism. Draw and label a paramecium using your photographic atlas as a guide.



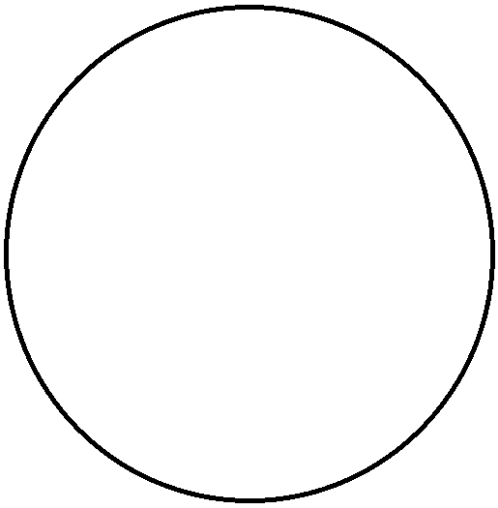
Stramenopila, also found in the SAR clade includes diatoms that have silicon dioxide shells that overlap. The diatoms produce oil, which promotes buoyancy. They also contain a number of pigments. They are used in production of filters and in polishing agents. Obtain a prepared slide of diatoms and draw representatives. These organisms, along with many others, have been used in forensic science to establish locations as the kinds and numbers tend to be fingerprints of aquatic environs. Draw several examples.



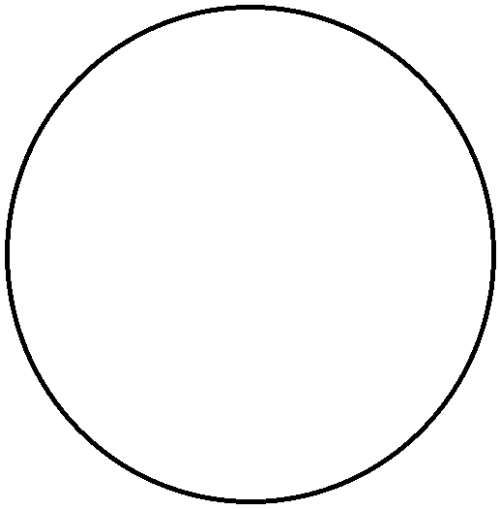
Stramenopila also includes golden algae, Chrysophyta, organisms that are biflagellates, freshwater and colonial. Examples may not be available in the laboratory. Brown algae, Phaeophyta, is also a member of this group. Brown algae are the largest alga. The pigment fucoxanthin produces the brown color. Examples are seaweeds such as Fucus and Sargassum. The basic structure of the algal body is holdfast (root-like structure), stipe (stem) and blade (leaf-like structure.) Extracts of algin, a polysaccharide in the cell wall, are used as thickening agents in food preparation. Examine the examples of brown algae available in the laboratory. Sketch representatives.



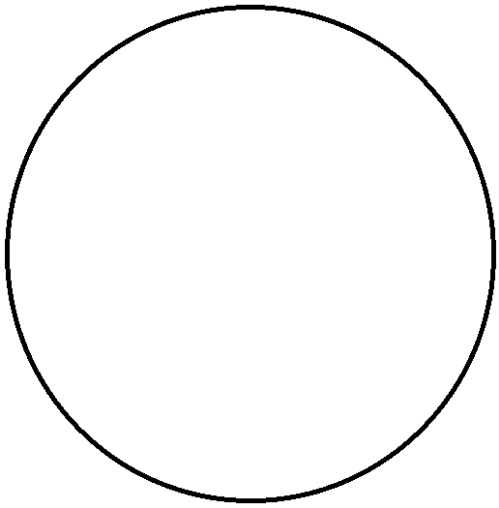
The SAR clade includes Rhizarians which have pseudopodia and are found in planktonic habitats. We find radiolarians in the group. They tend to be spherically symmetrical with axopodia emanating from glass-like tests. Examine prepared slides of radiolarians and draw example of the organism. Fresh cultures will display sun-like structures with axopodial rays. Draw an example of radiolarians.



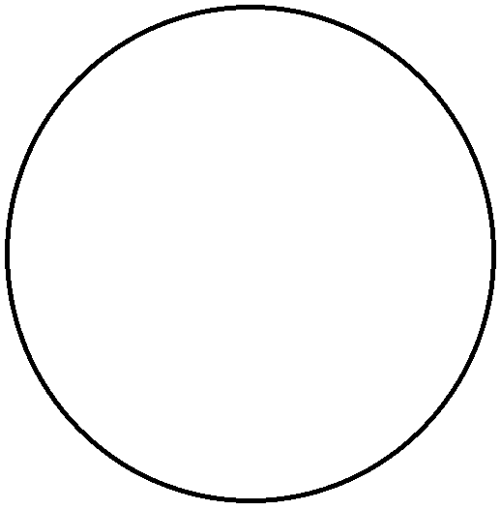
Foraminifera, also found in the Rhizarian clade, are protists that have calcareous compartments with pseudopodia. Historically they are structurally responsible for the for the formation of the White Cliffs of Dover in England. Obtain a slide of forams and draw representatives. Note the various compartments. Examination of forams as well as other representatives has given geologists much information on the formation of landmasses. Draw several.



Archaeplastida includes red algae which survive in deeper water than other algae due to the presence of the pigments phycocyanin and phycoerythrin. These pigments absorb EMR in the green and blue wavelengths. The substance, agar, used in the culture of microbes is obtained from the wall of the red algae. Another extract, carageenan, is used to thicken ice creams, puddings and soups. Red algae are used as wrapping for Sushi. Porphyra is available in the classroom for observation.

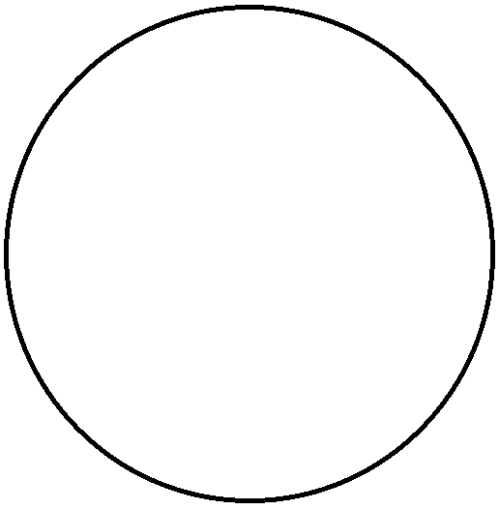


The green algae, Chlorophyta, are generally found in most fresh-water environs. They include unicellular (motile and non-motile) as well as colonial and filamentous forms. Some members form symbiotic relationships in lichens and are thought to be the ancestors of green plants due to similar biochemistry. Examples of Chlorophyta include: *Ulva*, *Spirogyra*, *Chlamydomonas*, *Chara*, *Volvox*. Examine the prepared slides as well as fresh cultures of the above green algae. Draw representatives.



Seaweed has specialized structures: the thallus (body), holdfast (rootlike) and blade (leaflike.) Cell walls of cellulose and gel-forming polysaccharides sometimes with calcium carbonate make them unpalatable but sturdy for intertidal zones. Uses include thickener in foods (algin, agar and carageenan), lubricants, and in microbiological culture medium.

The clade Unikonta contains Amoebozoans which are organisms that use pseudopodia for locomotion, swimming as well as engulfing other microorganisms (lunch). The includes naked and shelled amoebas with broad pseudopodia. The "false feet" are responsible for locomotion as well as engulfing prey. Amoeba move very slowly and detain is not recommended for use in slide preparation. Amoebas are usually colorless and it is often necessary to change both the intensity and angle of the light to observe them successfully. (Manipulate both the light rheostat and iris diaphragm) Draw an amoeba in the space provided.



Slime molds include acellular slime molds (coenocytic plasmodium). These organisms are fungus-like heterotrophs and are often brightly pigmented. The feeding stage is a mass while Dictyostelida's (cellular slime mold) feeding stage is a single haploid cell. The slime molds are probably most related to amoeboid protists. Note the illustrations in the photographic atlas.

Recent revelations concerning the biochemistry of these protists have given cause for reclassification. Many similarities exist between green algae and plants. These will be discussed later. While frustration exists concerning taxonomy in this incredibly diverse group, more information becomes available daily, allowing us the opportunity to reexamine phylogenetic relationships.

Laboratory Questions

1. The ancestors of protists were the first to have organelles and structures such as:

2. Where are protists found?

3. List five protists that photosynthesize.

4. List two protists that resemble absorptive heterotrophs.

5. List three protists that are heterotrophs.

6. Give examples of and distinguishing characteristics of:
    a. Euglena-

    b. Giardia-

    c. Dinoflagellates-

    d. Apicomplexa

    e. Ciliates-

    f. Diatoms

    g. Brown algae-

    h. Red algae-

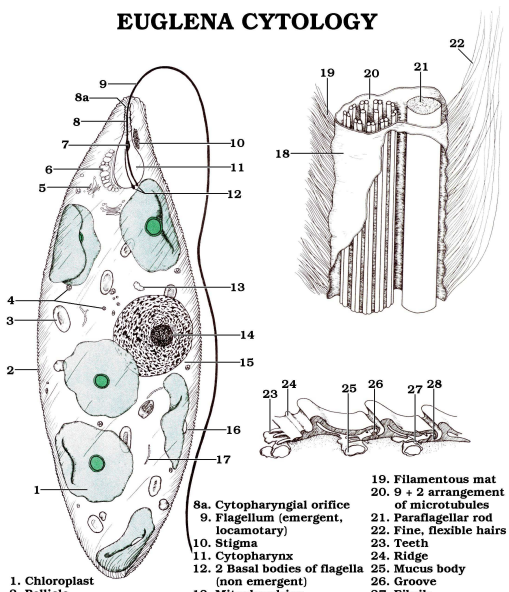
    i. Green algae-

j. Forams-

k. Radiolarians-

7. Describe the general structure of a sea-weed.

EUGLENA CYTOLOGY

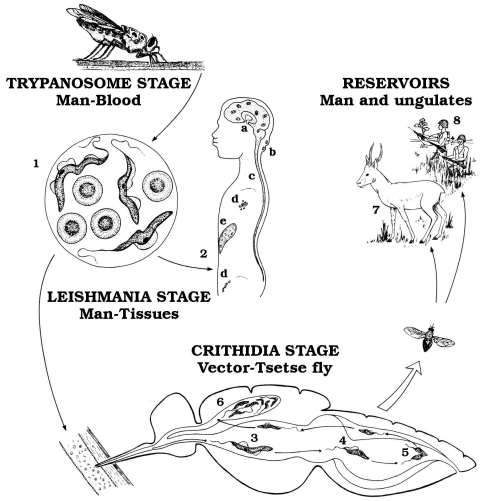


1. Chloroplast
2. Pellicle
3. Paramylon
4. Phospholipid vesicles
5. Golgi body
6. Contractile vacuole
7. Photoreceptor
8. Cytopharyngial canal

8a. Cytopharyngial orifice
9. Flagellum (emergent, locamotary)
10. Stigma
11. Cytopharynx
12. 2 Basal bodies of flagella (non emergent)
13. Mitochondrion
14. Endosome
15. Nucleus
16. Pyrenoid sheathed with paramylon granules
17. Endoplasmic reticulum
18. Flagellar membrane

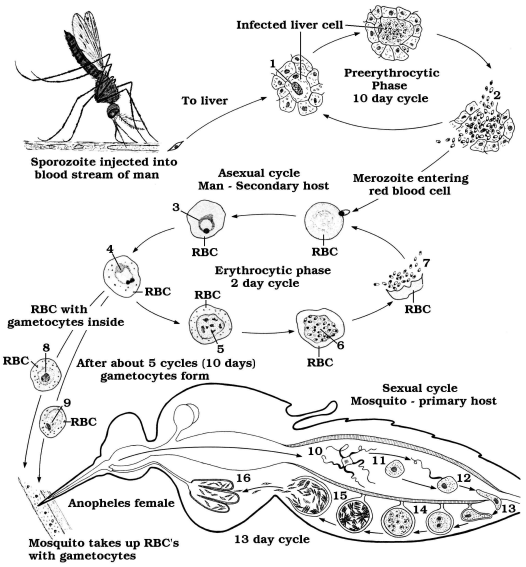
19. Filamentous mat
20. 9 + 2 arrangement of microtubules
21. Paraflagellar rod
22. Fine, flexible hairs
23. Teeth
24. Ridge
25. Mucus body
26. Groove
27. Fibrils
28. Mucus canal

TRYPANOSOME LIFE CYCLE



1. Trypanosomes in blood
2. Trypanosomes in tissues
   a. Brain
   b. Cervical lymph nodes
   c. Spinal cord
   d. Other lymphoid tissue
   e. Spleen
3. Trypanosome (long forms)
4. Crithidia
5. Crithidia (binary fission)
6. Metacyclic trypanosomes
7. Reservoir
8. New host

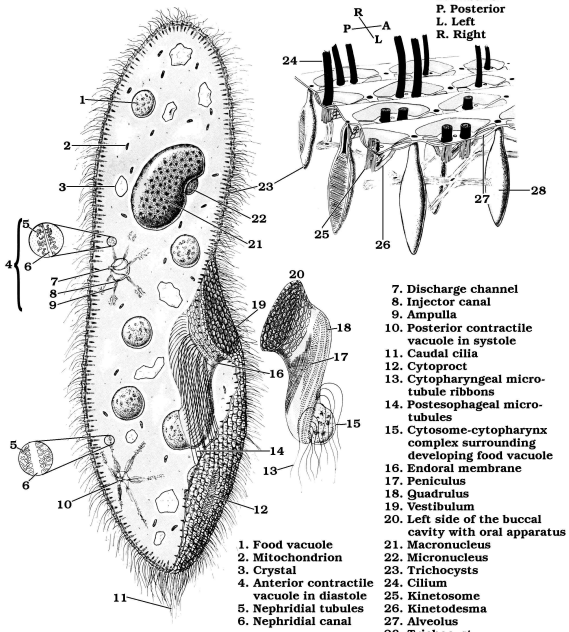
MALARIA LIFE CYCLE



1. Cryptozoite in liver
2. Released merozoite
3. Young trophozoite
4. Older trophozoite
5. Immature schizonts
6. Mature schizonts
7. Merozoites
8. Microgametocyte
9. Macrogametocyte
10. Microgamete
11. Macrogamete
12. Zygote
13. Ookinete
14. Oocyst
15. Oocyst with sporozoites
16. Released sporozoites in salivary gland

PARAMECIUM ANATOMY

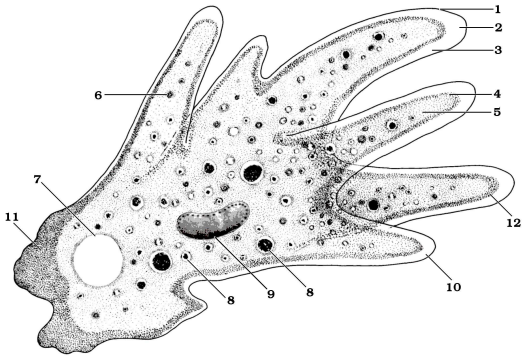
Paramecium caudatum



1. Food vacuole
2. Mitochondrion
3. Crystal
4. Anterior contractile vacuole in diastole
5. Nephridial tubules
6. Nephridial canal
7. Discharge channel
8. Injector canal
9. Ampulla
10. Posterior contractile vacuole in systole
11. Caudal cilia
12. Cytoproct
13. Cytopharyngeal micro-tubule ribbons
14. Postesophageal micro-tubules
15. Cytosome-cytopharynx complex surrounding developing food vacuole
16. Endoral membrane
17. Peniculus
18. Quadrulus
19. Vestibulum
20. Left side of the buccal cavity with oral apparatus
21. Macronucleus
22. Micronucleus
23. Trichocysts
24. Cilium
25. Kinetosome
26. Kinetodesma
27. Alveolus
28. Trichocyst

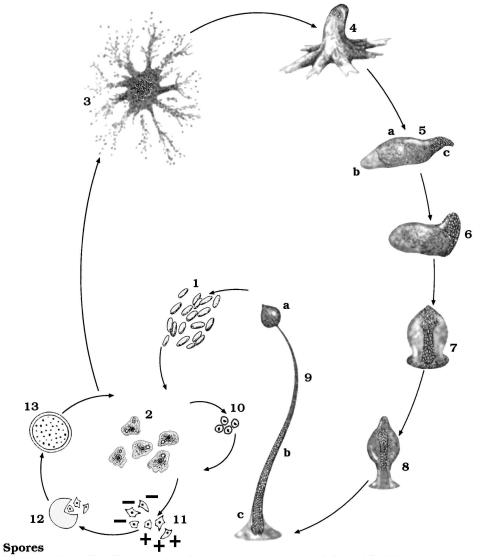
AMOEBA ANATOMY

Amoeba proteus



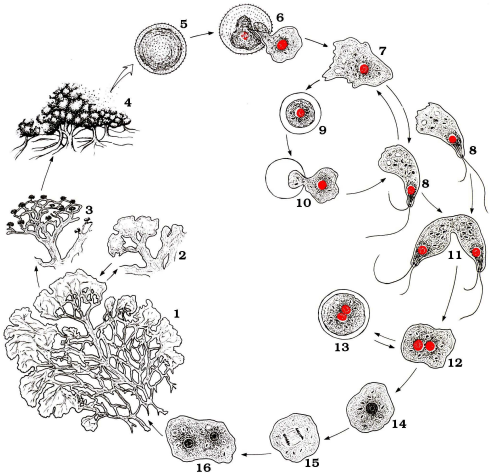
1. Plasmalemma (cell membrane)
2. Hyaline cap
3. Hyaline layer (ectoplasm)
4. Endoplasm—plasmagel
5. Endoplasm—plasmasol
6. Crystal
7. Contractile vacuole
8. Food vacuoles
9. Nucleus
10. Pseudopodium
11. Region of solation
12. Region of gelation

DICTYOSTELIUM LIFE CYCLE



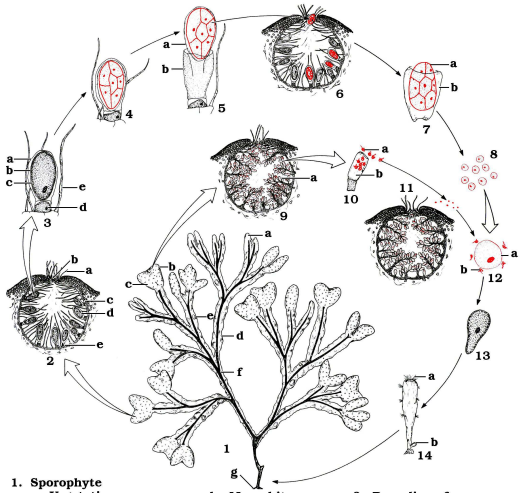
1. Spores
2. Myxamoebae (feeding stage)
3. Aggregation
4. Pseudoplasmodium
5. Migrating slug
   a. Sorus
   b. Stalk cells
   c. Basal disc cells
6. Return to erect position
7. Beginning of sorophore formation
8. Elevation of sorogen
9. Sorocarp
   a. Sorus
   b. Sorophore
   c. Basal disc
10. Microcysts
11. Mating Types (+ and -)
12. Diploid cell engulfing remaining mating types
13. Macrocyst

PHYSARUM LIFE CYCLE



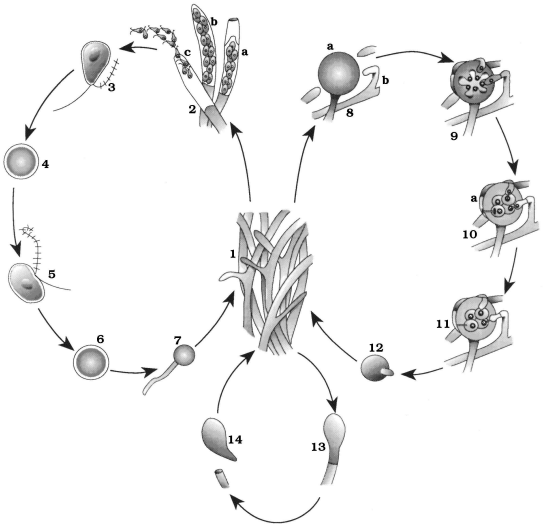
1. Plasmodium
2. Sclerotium
3. Plasmodium forming sporangia
4. Sporangium
5. Spore
6. Germination
7. Amoeboid swarmcell
8. Rotating, flagellated swarmcell
9. Microcyst
10. Emergence swarmcell
11. Posterior fusion of gametes
12. Plasmogamy
13. Binucleate microcyst
14. Nuclear fusion
15. Division
16. Young plasmodium

FUCUS LIFE CYCLE



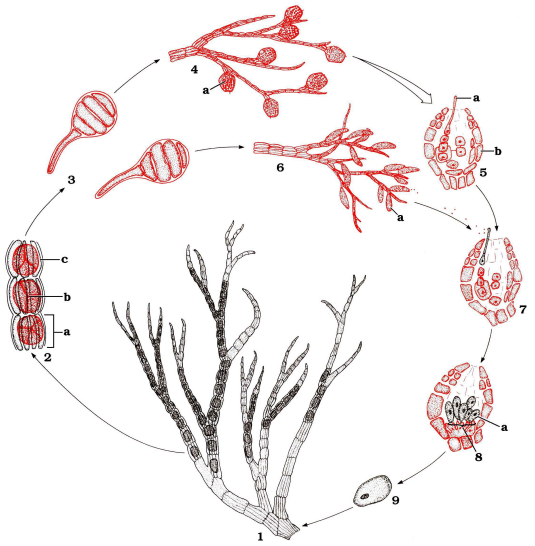
1. Sporophyte
   a. Vegetative apex
   b. Receptacle
   c. Conceptacle
   d. Cryptoblast
   e. Air vesicle or bladder
   f. Midrib
   g. Holdfast
2. Oogonial conceptacle
   a. Paraphysis
   b. Ostiole
   c. Fertile sheet
   d. Oogonium
   e. Stalk cell
3. Oogonium
   a. Exochite
   b. Mesochite
   c. Endochite
   d. Stalk cell
   e. Paraphysis
4. Eggs
5. Liberation of eggs from exochite
   a. Mesochite
   b. Exochite
6. Liberation of eggs from conceptacle
7. Liberation of eggs from mesochite
   a. Endochite
   b. Mesochite
8. Rounding of eggs
9. Antheridial conceptacle
   a. Antheridium
10. Liberation of sperm from exochite
    a. Sperm
    b. Exochite
11. Liberation of sperm from conceptacle
12. Near fertilization
    a. egg
    b. Sperm
13. Developing zygote
14. Sporling
    a. Hairs
    b. Adventitious frond

SAPROLEGNIA LIFE CYCLE



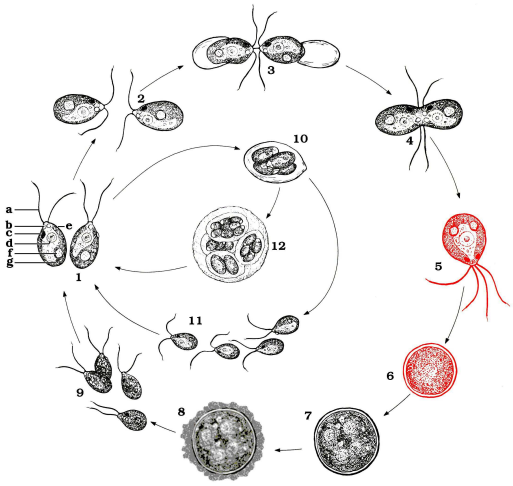
1. Somatic hyphae
2. Sporangia
   a. Proliferation
   b. Mature sporangium
   c. Discharge of primary zoospores
3. Primary zoospore
4. Aplanospore
5. Secondary zoospore
6. Aplanospore
7. Germination
8. Developing sexual structures
   a. Oogonium
   b. Antheridium
9. Cleavage of oospheres
10. Fertilization
    a. Fertilization tube
11. Oospores
12. Germination
13. Gemma
14. Germinating gemma

POLYSIPHONIA LIFE CYCLE



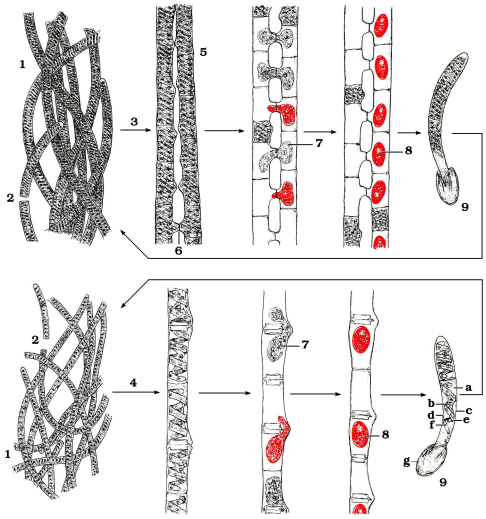
1. Tetrasporophyte
2. Developing tetraspores
   a. Tetraspore mother cell
   b. Tetraspore
   c. Siphon
3. Germinating spores
4. Female gametophyte
   a. Cystocarp
5. Cystocarp, c.s.
   a. Trichegyne
   b. Carpogonium
6. Male gametophyte
   a. Antheridial cluster
7. Cystocarp, fertilization
8. Carposporophyte
   a. Carposporangium
9. Carpospore

CHLAMYDOMONAS LIFE CYCLE



1. Motile cells (n)
   a. Flagellum
   b. Contractile vacuole
   c. Stigma oe eyespot
   d. Nucleus
   e. Cell wall
   f. Ptrenoid
   g. Chloroplast
2. Isogametes
3. Escape of gametes from walls
4. Plasmogamy
5. Quadriflagellate zygote (2n)
6. Young resting zygote
7. Germinating zygote
8. Thick-walled (dormant) zygospore
9. Meiospores
10. Daughter cells
11. Zoospores
12. Palmella stage

SPIROGYRA LIFE CYCLE



1. Vegetative filaments
2. Proliferation by fragmentation
3. Scalariform conjugation
4. Lateral conjugation
5. Alignment of filaments
6. Conjugation tube
7. Migrating protoplast
8. Zygospore
9. Germinating zygospore
   a. Vacuole
   b. Nucleus
   c. Cell wall
   d. Cytoplasm
   e. Chloroplast
   f. Pyrenoid
   g. Spore wall