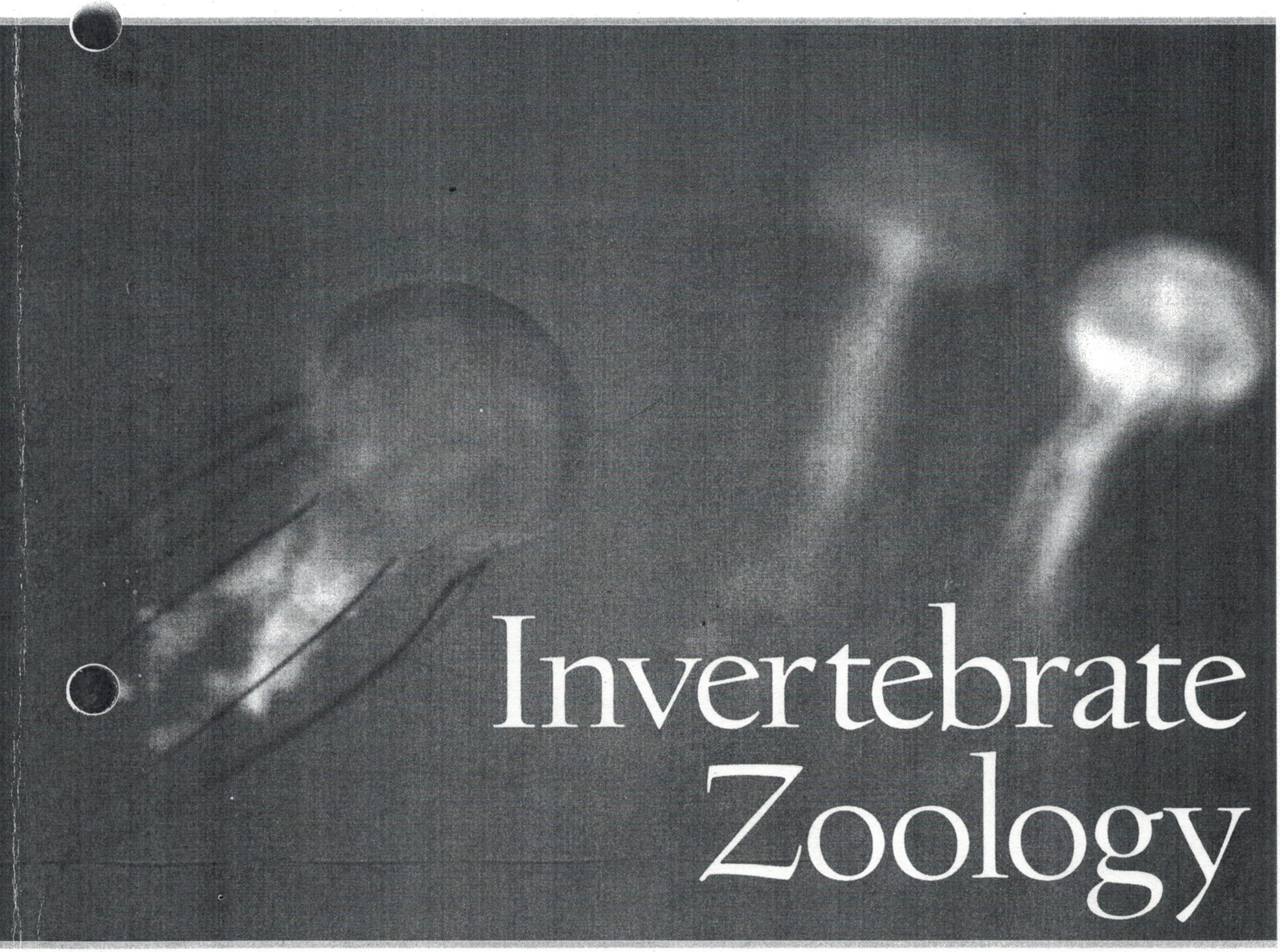

Invertebrate Zoology

A Laboratory Manual

Sixth Edition

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EXERCISE 1

The Protozoans

Over 60,000 species of flagellates, amebas (or amoebas), opalinids, spore formers, and ciliates, often called collectively the protozoa, represent some of the most fascinating organisms to both novice and experienced microscopists. The tremendous number of individuals found in one drop of water can be overwhelming. Until recently, these mostly microscopic and primarily unicellular organisms were grouped in a single Phylum Protozoa [PRO-to-ZO-ah; G., *proto*, first + G., *zoon*, animal]. Most protozoologists now agree that this grouping constituted a heterogenous assemblage of distantly related forms. Many biologists place the protozoans within the Subkingdom Protozoa (Kingdom **Animalia**) containing at least six, probably polyphyletic phyla (Levine et al. 1980). Others prefer the term *protists* instead of *protozoa* and follow Whittaker (1969) in grouping the subkingdom under the Kingdom **Protista**. However, it is generally recognized that protozoans are not invertebrates. Nevertheless protozoans play important roles in the web of life and are fascinating and challenging organisms to study. For these reasons, and because they have traditionally been studied in invertebrate zoology courses, we cover them here.

Few places on the earth are devoid of protozoans. Free-living forms occur in aquatic habitats, moist soils, and decaying organic matter. Many protozoans are important parasites of plants, animals, and humans. Nearly one-half of the 60,000 protozoan species are fossils; some have been in existence at least since the **Precambrian** (Fig. 1.1).

The basic body plan is the single eukaryotic cell representing a functionally complete organism that performs all physiological processes found in multicellular animals or the **Metazoa** (G., *meta*, after or between + G., *zoa*, animal). Some biologists prefer to call protozoans acellular [G., *a*, without] organisms instead of unicellular, to emphasize the complete, functional organismal viewpoint.

Protozoan organelles tend to be more specialized than those found in the typical cell of metazoans. Some of the common organelles (together with their functions) are as follows: **food vacuole** or **phagosome** (digestion), **contractile vacuole** or water expulsion vesicle (water regulation), **myoneme** (contractile), **paraflagellar swelling** and **stigma** (sensory), **extrusome** (food getting and defense), and **pseudopodium, flagellum,** and **cilium** (food getting and locomotion). The traditional designation of protozoans as amebas, flagellates, spore formers, and ciliates was based primarily on their locomotory organelles.

Some species are colonial and can be seen with the unaided eye. However, there are no tissues, organs, or germ layers. Digestion is intracellular, occurring within food vacuoles. Respiration and excretion are accomplished by diffusion across the cell membrane. Reproduction is by **budding, fission, conjugation,** or **syngamy**. The motile form is often called a **trophozoite** (trophont), whereas the nonmotile form is called a **cyst.**

Selected representatives of the major phyla will be studied in the following exercises. Your instructor may provide additional examples for study.

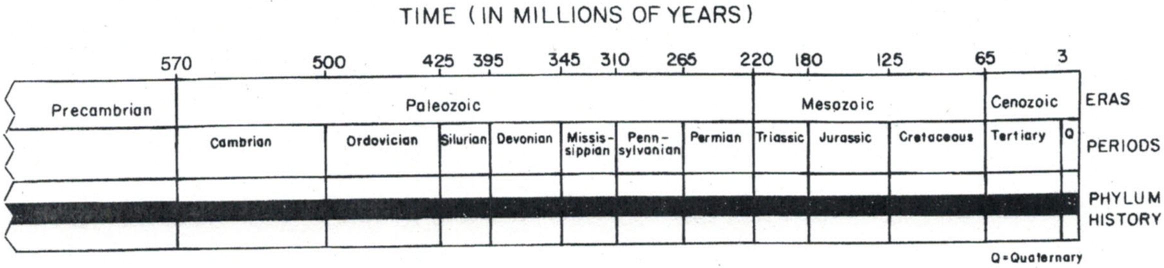

Figure 1.1. Geologic history of the Subkingdom Protozoa. Based on geological and biochemical evidence we know that the origin of protist, dates to at least 2.7 billion years ago.

Classification

The higher classification of protozoans has undergone many changes in the past decade. Authors of textbooks of Invertebrate Zoology, General Zoology, and General Biology do not always agree what higher taxa (e.g., phyla, subphyla, class, and order) should be used for a particular group. In this edition of our manual we present a classification that reflects the opinions of many authors; however, we fully realize that some instructors will not agree totally with the system given below. The major changes in this edition from the 5th edition occur in separating the flagellates, amebas, and opalinids into separate phyla, instead of the single Phylum Sarcomastigophora. Also, the Phylum Ciliophora has undergone some substantial modifications.

a. Flagellated Protozoans. Main organelle for movement is the flagellum. Nucleus monomorphic.

(a.) Plantlike Flagellates (Phytoflagellates)

The bodies of these protozoans or protists typically possess chloroplasts (organelles with chlorophyll) and eyespots. Botanists regard these flagellates to be algae.

1. **Phylum Dinophyta (Dinoflagellata).** Dinoflagellates. Body with a transverse groove and a ventral groove, each containing a flagellum. Cell body naked or covered with cellulose plates. Colorless or with yellow or brown chromoplasts. Marine and freshwater. Examples: *Glenodinium*, *Gymnodinium*, *Gonyaulax*, and *Ceratium*.

2. **Phylum Euglenophyta.** Euglenid Flagellates. Cells with one or two flagella that arise anteriorly within the reservoir. Both chloroplast-containing and colorless forms present. Stigma present in colored forms. Most are freshwater. Examples: *Euglena* and *Peranema*.

3. **Phylum Chlorophyta.** Volvocid Flagellates. Cells usually with two flagella, one chloroplast, and two contractile vacuoles. Protists with chlorophyll *a* and *b*. Solitary or colonial forms are known. Examples: *Chlamydomonas*, *Gonium*, *Eudorina*, and *Volvox*.

(b.) Animal-like Flagellates (Zooflagellates)

These organisms typically lack chloroplasts and eyespots.

4. **Phylum Choanoflagellida.** Choanoflagellates. Flagellates with a single anterior flagellum encircled by a funnel-shaped collar of microvilli. Both solitary and colonial forms are known. Choanoflagellates are similar to choanocytes (collar cells) of sponges (Phylum Porifera. Exercise 2). Marine or freshwater. Examples: *Monosiga*, *Codosiga*, and *Proterospongia*.

5. **Phylum Metamonada.** (Order Diplomonadida). Bilateral symmetrical flagellates parasitic primarily in alimentary canal of host. Trophozoites with one or two karomastigonts (e.g., complex of flagellum, nucleus, and associated organelles), each with one to four flagella. Examples: *Giardia* and *Hexamita*.

6. **Phylum Parabasalia.** (Order Hypermastigida). Gut inhabitants of termites, cockroaches, and woodroaches, possessing many flagella. The motile cell usually is uninucleate. Example: *Trichonympha*.

7. **Phylum Kinetoplastida.** Parasitic, endocommensal, and free-living protozoans with cells possessing one to two flagella. The kinetoplast is the largest extranuclear repository of DNA for any cell type. Life cycle involves vertebrate and invertebrate hosts. Examples: *Leishmania*, *Bodo*, and *Trypanosoma*.

b. Opalinid Protozoans. Ovoid protozoans with numerous cilia in oblique rows covering the entire body. Cytosome and cytopharynx are absent.

8. **Phylum Opalinata.** Opalinids. Two or many monomorphic nuclei. Reproduction is usually asexual, longitudinal binary fission. Most are gut commensals of frogs and toads. Example: *Opalina*.

c. Ameboid Protozoans. Primary means of locomotion is ameboid movement involving pseudopodia typically on the trophozoite. Cell body is naked, with a test, or with an internal skeleton. Reproduction is primarily by binary fission.

9. **Phylum Rhizopoda (Rhizopodea).** Sarcodines that use lobopodia, filopodia, or reticulopodia.

(1.) **Class Lobosea.** Sarcodines with pseudopodia lobose to filiform in shape. Cells are usually uninucleate. Cell body either naked or in a test. Both free-living and parasitic forms are known. Examples: *Amoeba proteus, Chaos carolinense (=Pelomyxa carolinensis), Entamoeba histolytica, Arcella*, and *Difflugia*.

(2.) **Class Filosea.** Filiform pseudopodia that often branch and sometimes anastomose. No flagellate stage known. Cell body is naked or in a test. Examples: *Lecythium* and *Euglypha*.

(3.) **Class Granuloreticulosea.** (Order Foraminiferida). Foraminiferidans (forams). Amebas primarily with delicate granular reticulopodia and a test of one to many chambers. Tests usually calcareous. Most are marine. Examples: *Globigerina, Elphidium*, and *Allogromia*.

10. **Phylum Actinopoda.** Radiolarians. Mostly planktonic amebas with actinopods (axopods) and delicate filopods radiating from a spherical body. Capsular membrane between ectoplasm and endoplasm present in most forms.

(1.) **Class Acantharea.** Radiolarians. Skeleton of strontium sulfate with centrally joined radial spines. Mostly planktonic; all marine. Examples: *Acanthometra* and *Collosphaera*.

(2.) **Class Polycystinea.** Radiolarians. Planktonic marine radiolarians with skeleton of silica and a perforated capsular membrane. Example: *Thalassicolla*.

(3.) **Class Phaeodarea.** Radiolarians. Planktonic marine radiolarians with skeletons, when present, of silica and organic matter. Capsular membrane with three large pores. Spines of skeleton hollow. Examples: *Aulacantha* and *Circoporus*.

(4.) **Class Heliozoea.** Sun Animalcules. Primarily freshwater amebas with a skeleton, when present, of siliceous or chitinoid scales and spines. Central capsule with membrane absent. Examples: *Actinosphaerium* and *Actinophrys*.

d. Spore-forming Protozoans. Parasitic protozoans with apical complex at some stage in the life cycle.

11. **Phylum Sporozoa (Apicomplexa).** Entirely parasitic forms lacking cilia or flagella (except for flagellated microgametes). Nucleus is vesicular. Both asexual and sexual phases present in the life cycle. Spores lacking polar filaments.

(1.) **Class Gregarinea.** Gregarines. Mature trophozoites large. Parasites in guts and body cavities of annelids and arthropods. Examples: *Gregarina* and *Monocystis*.

(2.) **Class Coccidea (Sporozoea).** Coccidian Sporozoans. Mature trophozoites are small and intracellular. Examples: *Eimeria, Plasmodium*, and *Toxoplasma*.

(3.) **Class Piroplasmea.** Parasites of red blood cells of vertebrates. Transmitted by ticks. Spores lacking. Example: *Babesia*.

12. **Phylum Microspora.** Intracellular parasites mainly of insects. Spores with a polar filament. Example: *Nosema*.

13. **Phylum Myxozoa (Myxosporidia).** Obligate, extracellular parasites of annelids and poikilothermic vertebrates (mainly fish). Spores with polar filament and encased in several valves. Usually two polar capsules present. (Recent analyses suggest that these organisms may be degenerate metazoans.) Example: *Myxosoma*.

e. Ciliate Protozoans. Protozoans with cilia.

14. **Phylum Ciliophora.** Ciliate Protozoans. Cells typically with cilia and dimorphic nuclei: macronucleus and micronucleus. Reproduction is primarily by asexual transverse binary fission (homothetogenic) and sexual conjugation.

(1.) **Class Kinetofragminophora.** Oral region of body bearing cilia, but lacking compound ciliary organelles.

(a) **Subclass Gymnostomata.** Cell mouth at or near body surface and located anterior or laterally. Body ciliation mostly uniform. Examples: *Coleps, Didinium, Dileptus,* and *Lacrymaria.*

(b) **Subclass Vestibulifera.** Cell mouth within a vestibule and bearing distinct ciliation. Examples: *Balantidium* and *Colpoda.*

(c) **Subclass Suctoria.** Suctorians. Sessile and generally stalked protozoans. Tentacles at the distal end. Adults lack cilia which are present in the free-swimming larva. Mostly ectosymbionts on aquatic invertebrates. Examples: *Acineta, Ephelota,* and *Podophrya.*

(2.) **Class Oligohymenophora.** Compound ciliary organelles associated with an oral apparatus.

(a) **Subclass Hymenostomata.** Body ciliation uniform. Oral structures not conspicuous. Examples: *Colpidium, Paramecium, Pleuronema,* and *Tetrahymena.*

(b) **Subclass Peritricha.** Oral ciliary band usually conspicuous. Most are sessile with reduced body ciliation. Example: *Vorticella.*

(3.) **Class Polyhymenophora.** Buccal membranelles at oral region. Body ciliation uniform; some with compound organelles (e.g., cirri).

(a) **Subclass Spirotricha.** Characteristics of the class. Examples: *Blepharisma, Bursaria, Spirostomum, Stentor, Euplotes,* and *Stylonychia.*

A. Flagellated Protozoans

The flagellum is the primary locomotory organelle of this large and diverse group. Flagellates are mostly free-living; however, there are a number of parasitic forms that cause sickness, and even death, to man and other animals. This diverse group of protozoans are often divided into plantlike (Phytoflagellates) and animal-like (Zooflagellates) forms.

These plantlike flagellates have a cell wall of cellulose, chloroplasts, and stigma. The usual type of nutrition is holophytic (autotrophic). Representatives of the following three orders will be emphasized: Order Dinoflagellida *(Ceratium, Glenodinium,* and *Gymnodimium),* Order Euglenida *(Euglena and Peranema),* and Order Volvocida *(Chlamydomonas and Volvox).*

Order Dinophyta (Dinoflagellida). Dinoflagellates are widely distributed in marine, brackish, and freshwater habitats. Most are free living. Dinoflagellates have an unequal pair of flagella. One is ribbon-like and typically lies in a transverse surface furrow called the **cingulum** or **girdle** (Fig. 1.2). The second flagellum is directed posteriorly and usually in a longitudinal furrow, the **sulcus**. Reproduction is by binary fission and syngamy.

The region above the cingulum in armored forms is the **epitheca** (epicone in unarmored ones); below the cingulum is the **hypotheca** (hypocone in unarmored ones). Unarmored or naked species (e.g., *Gymnodinium*) have a cell covering of membranes, whereas thecal plates of cellulose or other polysaccharides occur on armored species (e.g., *Ceratium* and *Glenodinium*). Many dinoflagellates (e.g., *Noctiluca, Gonyaulax,* and *Pyrocystis*) are bioluminescent and their bluish-green glow can be seen for some distance in the oceans. Certain genera, such as *Gonyaulax* and *Gymnodinium,* may occur in hugh numbers; these blooms are often called **red tides**. Millions of cells per liter of water may be produced. Blooms of *Gymnodinium breve* produce toxins that directly kill fish and some invertebrates. The toxins of other species may be concentrated in the bodies of clams, oysters, and scallops, rendering these organisms unsafe for human consumption. Deaths of manatees in coastal waters of Florida have been blamed on dinoflagellate toxins.

■ Observational Procedure: Order Dinoflagellida

1. Make a small ring, using a thick suspension of methyl cellulose, on a glass slide. Add a drop of the culture in the center of the ring, cover with a coverslip, and observe under low power of a compound microscope. After the organism is located, change to high magnification.

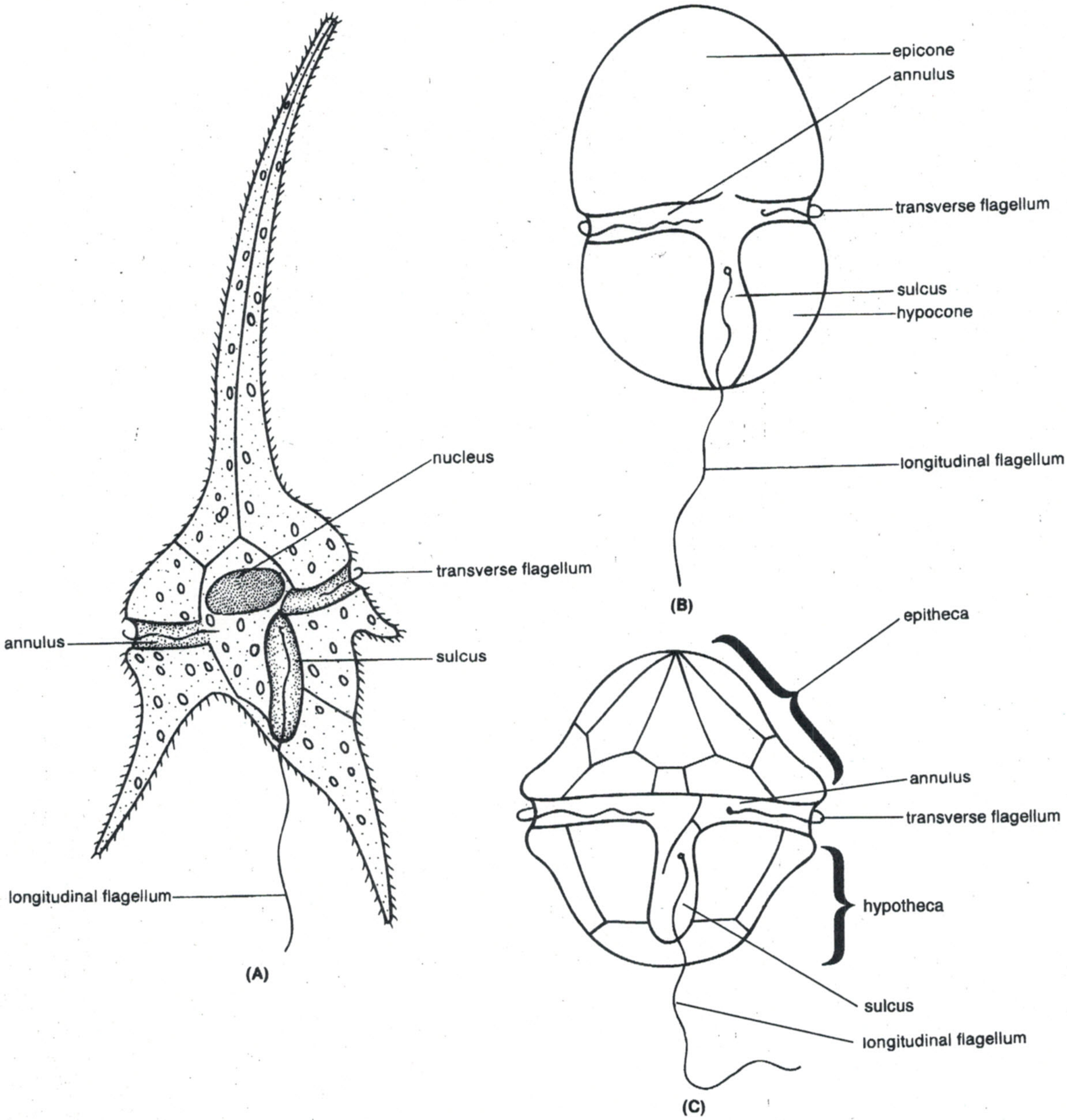

Figure 1.2. Dinoflagellates: (A) Ceratium. (B) Gymnodinium. (C) Glenodinium.

2. Describe the locomotion of these protozoans. Attempt to locate the cingulum and sulcus in a stationary individual. Can you see flagella beating? What variations in the body shape, size, and structure do you see, especially in *Ceratium*, which has hornlike extensions (Fig. 1.2A)? What might cause these variations?

3. Examine prepared slides of several species of dinoflagellates (e.g., *Ceratium, Glenodinium, and Gymnodinium*). Note the nature of the body covering. *Ceratium* has one apical horn and one to three antapical horns. The nucleus should be evident. Locate the cingulum and the sulcus. ■

Phylum Euglenophyta. Euglenoid flagellates are primarily freshwater organisms. Over 800 species in at least 37 genera have been described. Most species are bilaterally symmetrical with bodies elongated, ovoid, spherical, or leaflike. Both chloroplast-containing and

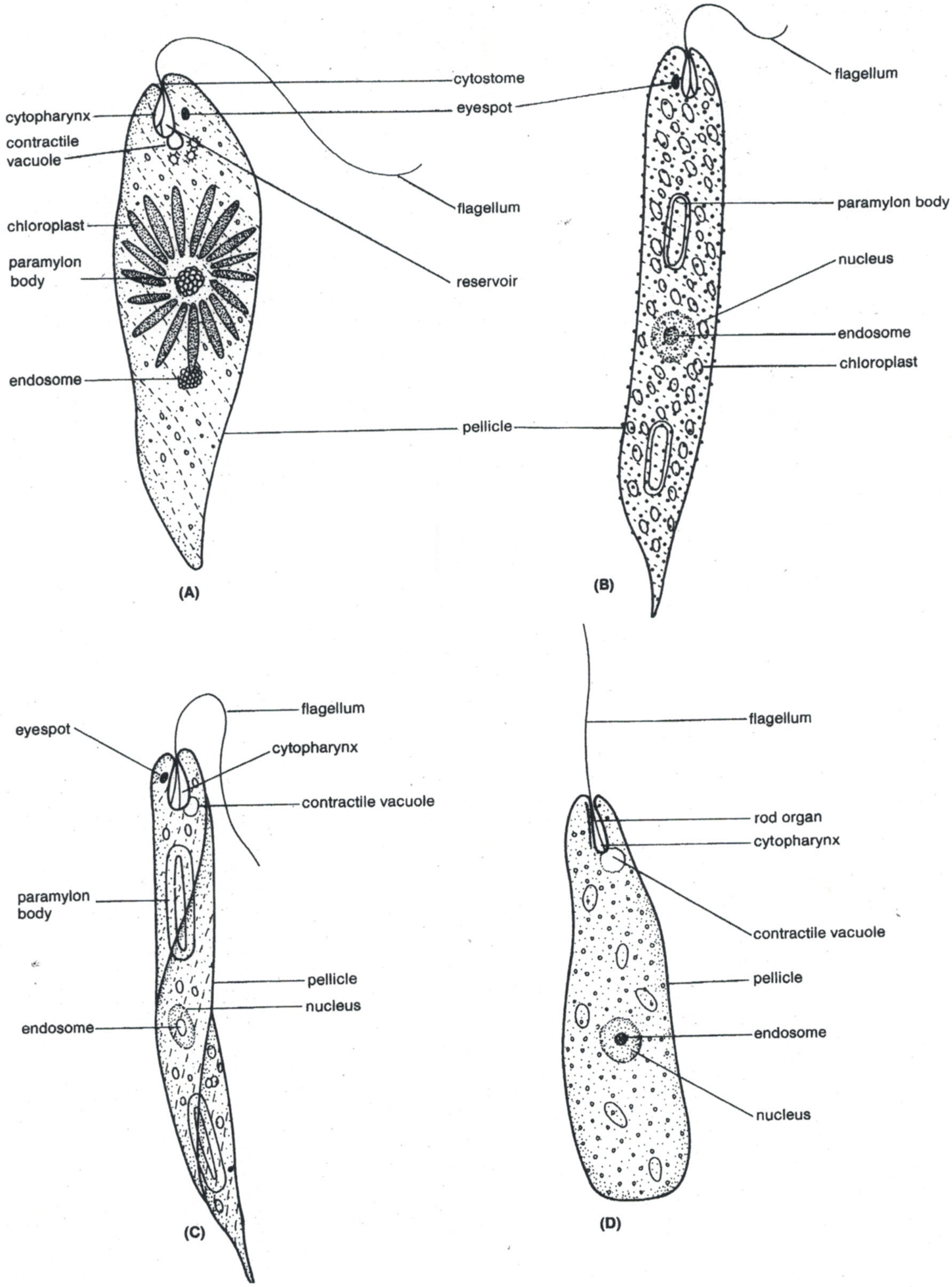

Figure 1.3. Selected protozoans. (A) *Euglena viridis.* (B) *E. spirogyra.* (C) *E. oxyuris.* (D) *Peranema.*

colorless forms are common. Chloroplasts vary in number and shape according to the species.

The anterior end of the cell is invaginated forming a flask-shaped **reservoir** (Fig. 1.3A). The narrow part of the reservoir is sometimes called the **cytopharnyx** and its opening the **cytostome** (cell mouth). Most euglenoid flagellates have two flagella (generally unequal in length) and a contractile vacuole near the reservoir. All species with an **eyespot** (stigma) have the photoreceptor or **paraflagellar swelling** near the base of the longer, emergent flagellum and opposite to the eyespot. Surrounding the cell is a **pellicle**, consisting of interlocking proteinaceous strips and microtubules beneath the cell membrane. Rapid changes of body shape, called euglenoid movement, are seen in many euglenoids. A single nucleus with **endosome** (nucleolus) is present. Reproduction is primarily by longitudinal fission.

■ Observational Procedure: Order Euglenida

1. Add a drop of the *Euglena* culture in the center of a ring of methyl cellulose and cover with a coverslip.
2. Examine under low power of a compound microscope to locate the organism and then change to high power (Fig. 1.3). Describe *Euglena's* movement and body shape. Does the cell rotate, creep, or glide? Do all individuals move in the same direction? Are any of the individuals showing **euglenoid movement?** Euglenoid movement in *E. viridis* is somewhat jerky. *Euglena gracilis* exhibits pronounced euglenoid movement, but not all *Euglena* species perform this movement.
3. The outer covering of the organism is the pellicle. The forward end is generally less pointed than the trailing end. Carefully focus on the forward end with the fine adjustment to see movement of the whiplike flagellum. You might want to make another wet mount and add a drop of Lugol's iodine solution before applying the coverslip. The solution will stain the flagellum, but will kill the organism. Near the reservoir is a contractile vacuole and a reddish eyespot (stigma). The eyespot is more easily seen in strains lacking pigment. The paraflagellar swelling at the base of the long flagellum will not be observed.
4. Much of the organism's cytoplasm contains chlorophyll-containing chloroplasts. Is your specimen greenish in color? The shape and position of the chloroplasts vary with the species. What shape are the chloroplasts in your species? In *E. viridis* the chloroplasts radiate from a common **paramylon body** (carbohydrate storage area). In the chloroplasts, there may be **pyrenolds**, proteinaceous bodies containing starch reserves. Although chlorophyll allows *Euglena* to manufacture its own food, this organism also has saprozoic nutrition; that is, it absorbs nutrients across the body surface in the absence of light. Holozoic nutrition, the ingesting of whole organisms, has never been demonstrated in *Euglena*; however, it does occur in *Peranema*, a relative of *Euglena*.
5. Attempt to locate the ovoid nucleus of the organism. This organelle is often obscured by chloroplasts and is best observed in colorless phytoflagellates. If a drop of acidified methyl-green stain is added to the culture, the nucleus will stain bright green. The nucleus can be observed in the prepared slide.
6. Examine a prepared slide of *Euglena* under high power of a compound microscope. Note the body shape. Locate the nucleus. Can you see the nucleolus (endosome) within the nucleus? Are chloroplasts evident? Carefully focus on the anterior end of a specimen. Attempt to see the flagellum, reservoir, eyespot, cytostome, and cytopharynx. These are more easily seen in the larger species of *Euglena*. *Euglena* reproduces by longitudinal binary fission. Are any individuals undergoing division? ■

Peranema. This freshwater, euglenoid phytoflagellate is similar to *Euglena*, but is colorless, lacks the eyespot, has a long trailing flagellum. Nutrition, is holozoic. The two rodlike structures (**rod organ**) near the reservoir are for food-catching (Fig. 1.3).

■ Observational Procedure: *Peranema*

Mount a drop of the culture on a slide as before. Observe the shape and movement of *Peranema*. Can you see striations in the pellicle? *Peranema* undergoes pronounced euglenoid movement. Observe a prepared slide of *Peranema* and compare the organism with *Euglena*. ■

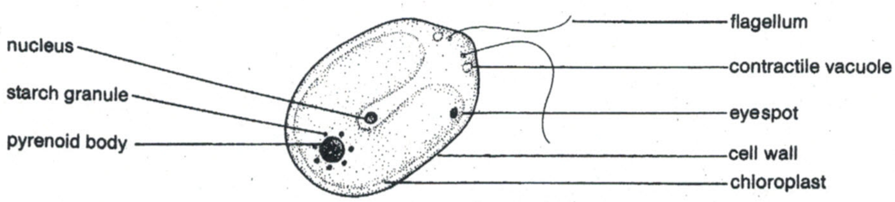

Figure 1.4. *Chlamydomonas*, a unicellular volvocida flagellate.

Phylum Chlorophyta. Most species occur in still freshwater ponds and ditches. The organisms usually have two flagella of equal length directed anteriorly, two contractile vacuoles, one large chloroplast, a red eyespot, and a centrally located nucleus (Figs. 1.4 and 1.5). The chloroplast is cup-shaped, usually grass green, and contains chlorophylls *a* and *b* and other pigments. Associated with the chloroplast is a single basal pyrenoid body with stored starch. Asexual and sexual means of reproduction are present.

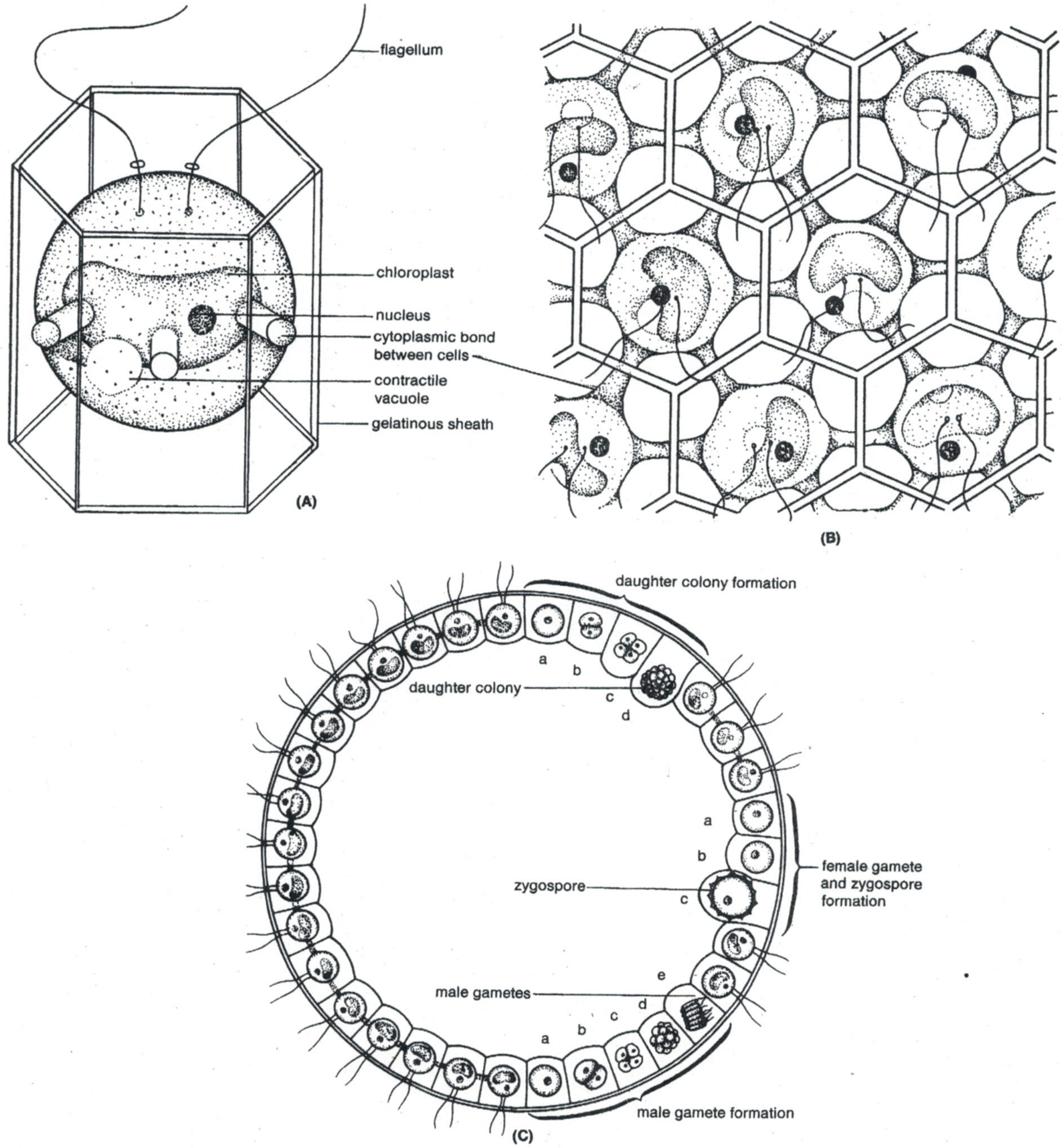

Figure 1.5. Volvox. (A) One zooid (cell). (B) Surface view of coenobium showing position of zooids in relation to cytoplasmic bonds. (C) Daughter colony formation, gametogenesis, and zygospore formation (diagrammatic). Lower case letters indicate sequence of development for three different processes: clockwise—daughter colony formation, female gamete and zygospore formation, and male gamete formation.

Chlamydomonas. *Chlamydomonas* is a small (9–16 µm), solitary volvocid. Over 500 species, mostly freshwater, belong to this genus (Fig. 1.4).

■ Observational Procedure: *Chlamydomonas*

Prepare a wet mount of *Chlamydomonas* as before. Note the ellipsoid shape and bilateral symmetry. Examine a stained slide of *Chlamydomonas*. Not all structures in Fig. 1.4 will be observed. Near each flagellum is a contractile vacuole. Observe the single, large chloroplast and nucleus. Attempt to see the eyespot in the anterior half of the chloroplast. The single pyrenoid body probably will not be seen. ■

Volvox. *Volvox* is a green, spherical or ellipsoidal colony (**coenobium**) surrounded by a cellulose cell wall. The colony may reach 1.5 mm in diameter. The phytoflagellate is common in ponds, ditches, and pools. Colony formation in *Volvox* is similar to embryonic development in some metazoans.

The organism consists of many cells or **zooids** (500–60,000) embedded in a single surface layer in a gelatinous matrix (Fig. 1.5). The colony consists of mainly somatic (vegetative) and some reproductive zooids. Each somatic zooid is chlamydomonas-like with two flagella directed outward; the zooids are often attached laterally by protoplasmic connections. The few fertile zooids (**gonidia**) are somewhat larger than the somatic ones. Vegetative reproduction results in a hollow sphere, the **daughter colony**, formed by repeated mitotic division of a gonidium that has lost the flagella. When the parent dies, the daughter colony is released in the water where it forms a new colony. Colonies of *Volvox* are monoecious or dioecious. Sexual maturation involves formation of female and male gonidia. One egg (**macrogamete**) develops from the female gonidia. Those of the male result in sperm packets (**plakeas**) of 16 to 512 biflagellate **microgametes**. One sperm cell penetrates the colony and fertilizes the egg. A thick, protective wall surrounds the zygote, forming a **zygospore**. When the parent colony disintegrates, the zygospore is released. Inside the zygospore repeated division occurs and during the spring a new colony is formed.

■ Observational Procedure: *Volvox*

Prepare a wet mount of the *Volvox* culture using methyl cellulose. Raise the coverslip with clay or other supports. Study the organism under low power and then change to high power of a compound microscope. Describe movement of the colony. Observe the daughter colonies if present. How many are there? Do they move about inside the colony or remain stationary? Can you tell if the colony possesses sexual stages? The zooids are very small and details of structure will not be observed. Examine a prepared slide of *Volvox*. Observe the general anatomy of the colony and compare it with the living organism. ■

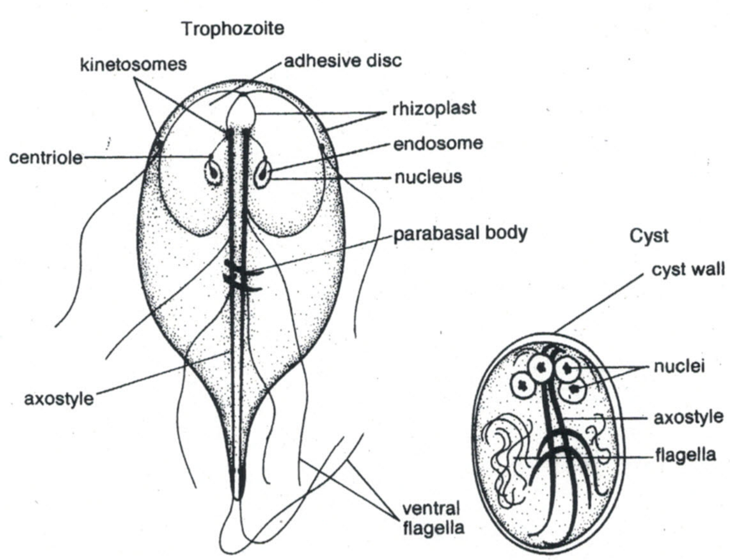

Figure 1.6. *Giardia intestinalis*, trophozoite and cyst.