

# Protozoology

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# Protozoology

*by*

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R. P. HALL

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# I

## General Morphology of the Protozoa

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**T**HE PROTOZOA include a variety of microorganisms which, by general agreement of protozoologists, are currently assigned to the phylum. More specific characterization of the Protozoa is difficult and even the name of the phylum, as applied to the groups it conventionally includes, is not entirely appropriate. Many flagellates—those usually listed as Phytomastigoda, Phytomastigina, or Phytomastigophora—are commonly considered algae by botanists. Also, the Mycetozoida (Mycetozoa)

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of protozoologists are nothing else than the slime-molds of botanists, and the Sarcosporidia, usually considered Sporozoa, are believed by some workers to be molds.

This situation, which suggests that protozoologists are unable to distinguish animals from plants, is somewhat disconcerting to those who favor consistency in taxonomy. Consequently, various taxonomic reforms have been suggested. The old term, *Protista*, recalls such an effort by Haeckel, but the Protista were only a heterogeneous collection of micro-organisms with the plant and animal labels obscured. A more positive reform was proposed by Calkins (17) in his decision to eliminate the chlorophyll-bearing flagellates from the Phylum Protozoa. On the face of it, the proposal seemed to be an admission that zoologists had been in error in laying claim to the "Phytomastigophora." However, some of the more interesting colorless phytoflagellates were saved from a botanical fate by arbitrary transfer to the "Zoomastigophora." The resulting mixtures could not be justified on the basis of sound taxonomic criteria; hence, this innovation has not been generally accepted. The basic classification of Copeland (33) recognizes a separate Kingdom Protocista which includes the Protozoa and various groups of algae and fungi. While this suggestion sidesteps the problem of deciding which Protozoa are animals and which are plants, it seems to imply that such Protozoa as the ciliates are more closely related to the red algae and related organisms than they are to the Kingdom Animalia.

At present, many protozoologists continue to list the phytoflagellates and slime-molds as Protozoa, although they realize that botanists have no objections to placing these groups in the plant kingdom. While the current practice is a bit confusing taxonomically, there is the advantage that botanists and protozoologists can legitimately maintain equal interest in these groups which apparently represent mergers of the plant and animal kingdoms.

From the morphological standpoint, Protozoa are often referred to as *unicellular* animals, in contrast to the multicellular Metazoa. The small size and simple structure of many Protozoa tend to justify this designation. On the other hand, some Protozoa are not so small and are measurable in millimeters, or even centimeters, instead of microns. Furthermore, the uninucleate condition is far from universal. Many species possess more than one nucleus, and the numbers range from two to many hundreds. Examples are found in each of the major taxonomic groups. Structural complexity often extends beyond a mere increase in number of nuclei. Mycetozoon protoplasm, as noted in *Physarum* (167), is traversed by channels through which a liquid, containing many granules, flows back and forth in a sort of primitive circulatory system. Multiplicity of flagellar units is associated with multinuclearity in Mastigophora. The result may be many nucleo-flagellar units (karyomastigonts), as in certain Calonym-



phidae (Fig. 1. 10, D). In addition to normally multinucleate Protozoa, many species are uninucleate in one phase of the life-cycle and multinucleate in another.

Such structural diversity has led protozoologists into difficulties with the Cell Theory. Dobell (45), who suggested that Protozoa are *non-cellular* organisms, was one of the first to revolt against strict application of the Cell Theory to this group. Such an interpretation has appealed to some zoologists. A different concept, favored by Kofoid (138) for example, is that some Protozoa are unicellular while others are multicellular. Protozoan "multicellularity" is considered analogous to metazoan multicellularity as seen in syncytial tissues. According to this view, the Protozoa are the phylum in which multicellularity originated in animals.

The evolutionary transition from Protozoa to Metazoa involved differentiation beyond the separation of reproductive and somatic cells. Hyman (98) has stressed the characteristic establishment of an axis along which morphological and physiological differentiation has occurred. Such colonial types as *Volvox*, in spite of their specialized somatic and reproductive "cells," are usually considered Protozoa. The distinction is mainly one of degree, since *Volvox* has several attributes of an organism in the metazoan sense. The colony moves as a unit, with apparently coordinated flagellar activity, and exhibits some degree of polarity with functional differentiation. The colony may produce daughter colonies asexually or it may develop gametes. The zygote develops into a young colony in a manner not unlike that in which a fertilized egg produces a young metazoan individual. The Myxosporida, another exceptional group, show somatic differentiation in that some cells produce spore-membranes while others give rise to the polar capsules of the myxosporidian spore. In other words, the separation of Protozoa from Metazoa in borderline cases may involve somewhat arbitrary decisions influenced to some extent by factors of taxonomic convenience.

#### VARIATIONS IN FORM OF THE BODY

Protozoa range from approximately spherical forms to bizarre shapes not readily explained on a functional basis. Symmetry is often poorly defined. Most active swimmers show spiral torsion in some degree and this tendency toward asymmetry is presumably correlated with the usual spiral course in locomotion (62, 136). However, universal symmetry and radial symmetry may be noted in various floating and sessile species, respectively, and bilateral symmetry is apparent in such genera as *Giardia* and *Octomitus*. In Protozoa which are not spherical, form of the body may be rather characteristic of a given species under particular conditions. However, form is often relatively constant rather than absolutely so, and within specific limits, may be modified by environmental



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conditions and activities of the organism. Even the nature and quantity of the available food may influence form as well as size of the body. Such a relationship is striking in *Tetrahymena vorax* (Fig. 1. 1) when strains are fed on different diets (118). In addition to the usual variations, attrib-

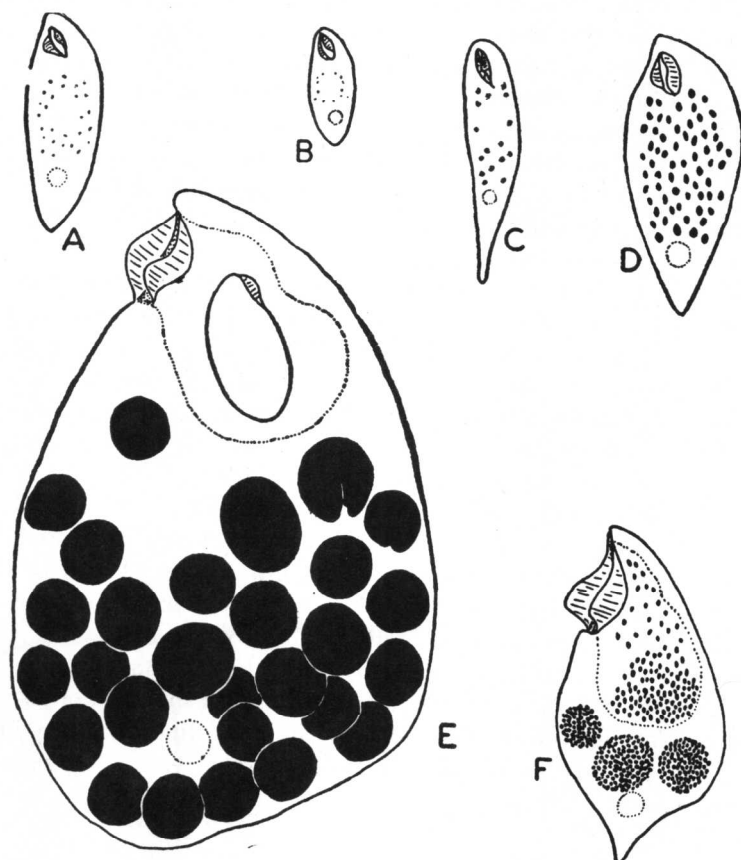


Fig. 1. 1. Influence of diet on form and size in *Tetrahymena vorax*. A. Organism from young broth culture (saprophytic nutrition). B. Specimen from older broth culture. C. A ciliate fed on *Aerobacter cloacae*. D. A ciliate fed on killed *Tetrahymena geleii*. E. A large carnivore from a culture fed living *T. geleii*. F. A carnivore after transfer to a culture of living yeast. Ingested food, peristomial area, and contractile vacuole are indicated diagrammatically but cilia are not shown. x450 (after Kidder, Lilly, and Claff).

utable to environmental or inherent factors, dimorphic and polymorphic life-cycles include two or more different morphological stages. *Naegleria gruberi* (Chapter V), for example, exhibits both flagellate and amoeboid stages. Although adaptive trends may be assumed, specific correlation of form with habitat is impossible in many instances. Yet certain generaliza-

tions are permissible for sessile, floating, swimming, and creeping types. Floating types, free from the usual stresses of locomotor activity, often approach a spherical form. Active swimmers are usually elongated, with a major axis more or less parallel to the path of locomotion. Creeping

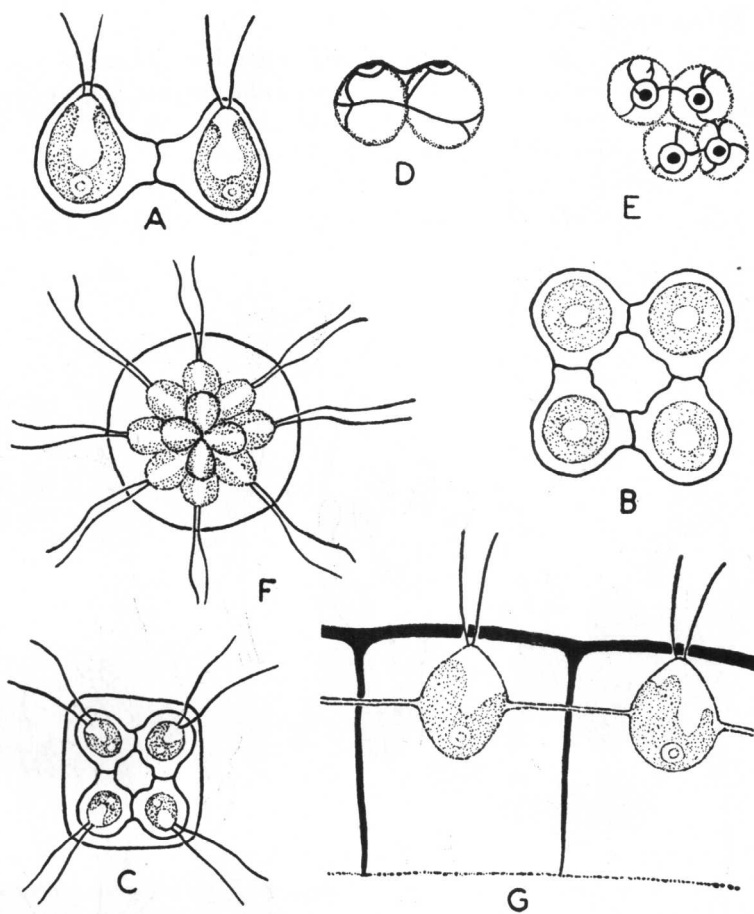


Fig. 1. 2. A-C. *Gonium sociale*: side view (A); surface view (B); colony with superficial continuous matrix (C); x900 (after Pascher). D, E. *Gonium* sp., portions of colonies showing supposed protoplasmic connections impregnated with silver; x760 (after Klein). F. *Syncrypta volvox*; x580 (after Stein). G. Protoplasmic connexions of somatic flagellates in *Volvox*; x1800 (after Janet).

forms are frequently flattened and may show differentiated dorsal and ventral surfaces. Sessile ciliates and flagellates are often more or less conical, attached to the substratum directly or by a secreted stalk.

In individual Protozoa, form of the body may be maintained by a thickened cortex (the differentiated outer zone of cytoplasm), by various

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secreted layers (pellicle, theca, lorica, test, and shell of particular groups), and by internal structures such as radiolarian skeletons. The gross morphology of protozoan aggregates and colonies depends upon the means by which the individual organisms are bound together.

### COLONIAL ORGANIZATION

The usual colony consists of similar organisms joined together in some particular pattern so that the form of the mature colony is characteristic of the genus or species. As a rule, any member of the colony may undergo fission or budding. In the Phytomonadida, this is true in *Gonium*, *Pandorina*, and *Platydorina* but apparently not in *Eudorina*, *Pleodorina*, and *Volvox*. However, flagellates isolated from colonies of

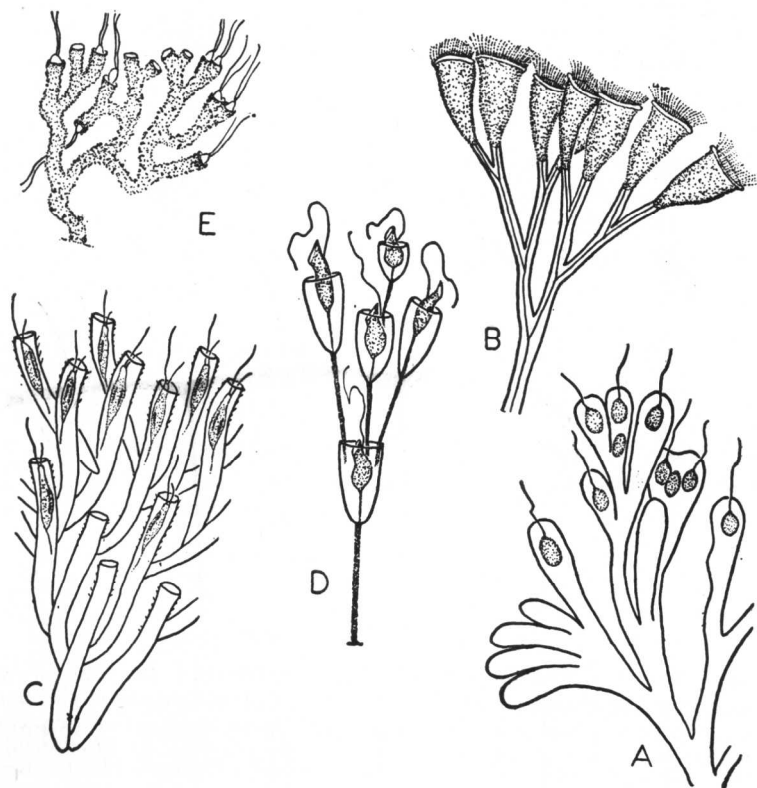


Fig. 1. 3. Arboroid colonies. A. *Phalansterium digitatum*, branching matrix; x290 (after Lemmermann). B. *Zoothamnium adamsi*, portion of colony showing stalk with continuous branching fibril; diagrammatic (after Stokes). C. *Hyalobryon ramosum*, loricate type; x720 (after Awerinzew). D. *Poteriodendron petiolatum*; each lorica with stalk; x290 (after Lemmermann). E. *Cladomonas fruticulosa* with continuous branching "lorica"; x290 (after Lemmermann).

*Eudorina*, *Gonium*, and *Pandorina* may undergo fission and produce daughter colonies (11). The component flagellates of the *Volvox* colony are differentiated into somatic and reproductive individuals and the former are believed to lose their reproductive ability when the colony reaches maturity.

Protozoan colonies are usually classified on the basis of their organization. *Spheroid* and *discoïd* colonies, containing a matrix secreted by the associated organisms during development of the colony, are represented by such ciliates as *Ophrydium* and various flagellates—*Syncrypta*, *Gonium*, *Pandorina*, *Volvox*, and others. In *Gonium sociale*, for example, the matrix shows two components (Fig. 1. 2, C), a "cell wall" enclosing each flagellate and a continuous outer gelatinous layer. In some specimens (Fig. 1. 2, A, B) the outer layer is lacking. Each flagellate in the *Volvox* colony is enclosed in a thin cell wall and a thick outer sheath. Except in *V. aureus*, the boundaries of the individual sheaths are readily distinguished. The flagellates appear to be joined by protoplasmic strands in certain species of *Volvox* (Fig. 1. 2, G) and apparently also in *Eudorina*, *Gonium*, and *Pandorina* (11). Dried colonies of *Gonium* (Fig. 1. 2, D, E), after silver impregnation, show "silver-line" connections between adjacent flagellates (131).

In *arboroid* colonies (Fig. 1. 3), the individual organisms are arranged in a branching pattern. Stalks are characteristic of many arboroid colonies. In different species, each organism may have its own stem which is attached to a common stalk, or each stalk of the framework may bear more than one organism. Such stalks may be gelatinous or sometimes solid and relatively firm, and in certain cases they are elastic tubes containing contractile fibrils. In other arboroid types, colonial organization is maintained by attachment of one lorica to another (Fig. 1. 3, C, D), or by a continuous tubular "lorica" in which the organisms are located at the tips of the branches (Fig. 1. 3, E).

#### NON-COLONIAL GROUPINGS

Certain other aggregates are not colonies in the strict sense. So-called *catenoid* colonies have been described in dinoflagellates (Fig. 1. 4, D) and certain astomatous ciliates (Fig. 1. 4, C). These chains arise in repeated fission without prompt separation of daughter organisms and are temporary groupings rather than true colonies. *Palmella* stages (Fig. 1. 4, A) of certain flagellates develop in much the same manner as spheroid and discoïd colonies. However, the palmella does not show a well defined range in size, the number of organisms varies with size of the mass, and the flagellates lack flagella. The term, *gleocystis* stage, is sometimes applied to similar aggregates in which an individual gelatinous layer surrounds each organism (Fig. 1. 4, B).

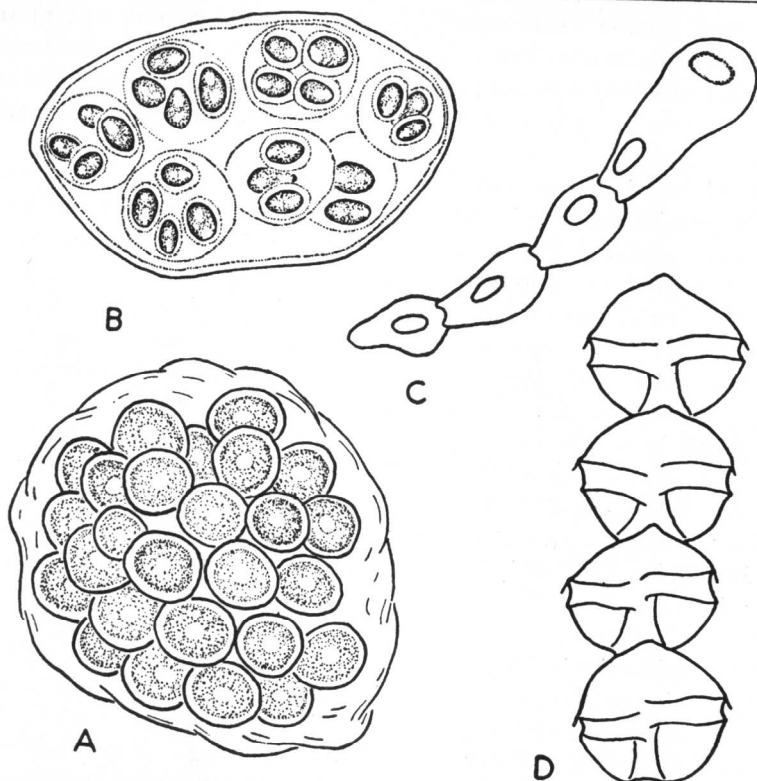


Fig. 1. 4. A. Palmella stage, as seen in *Haematococcus* and related *Phytomonadida*; diagrammatic (after Wollenweber). B. Gleocystis stage, as found in various *Chlamydomonadidae*; diagrammatic (after Goroschan-kin). C. Chain ("catenoid colony") of *Haptophrya michiganensis*; x90 (after Bush). D. Chain formed in fission of *Gonyaulax catenella*; x580 (after Whedon and Kofoid).

#### CORTEX, SECRETED COVERINGS, AND SKELETONS

No well developed cortex is apparent in simple flagellates or typical amoebae. The superficial cytoplasmic layer of *Amoeba proteus* is formed from, and gives rise to endoplasm continuously during amoeboid activity and thus lacks the relative permanence of the cortex in more specialized Protozoa. However, some amoeboid organisms have a thin pellicle similar to that of *Amoeba verrucosa*. In this species, the pellicle maintains itself under mechanical stress in microdissection (96).

At the other extreme, the relatively thick cortex of a ciliate may contain basal granules, fibrils, myonemes, mitochondria, and other inclusions, and sometimes trichocysts. Although often flexible, the layer is at least firm enough to maintain a typical body form in the swimming ciliate. The pellicle covering the surface of ciliates seems to be a distinct

layer, and *Blepharisma undulans* is said to shed its pellicle after treatment with strychnine. The cilia are withdrawn and the body retracted, leaving a space beneath the pellicle, and the ciliate later emerges through the old cytostomal area or the region of the posterior contractile vacuole (169).

Surface layers of flagellates range from a delicate periplast or pellicle, similar to that of certain amoebae, to thick tests or shells. The flexible

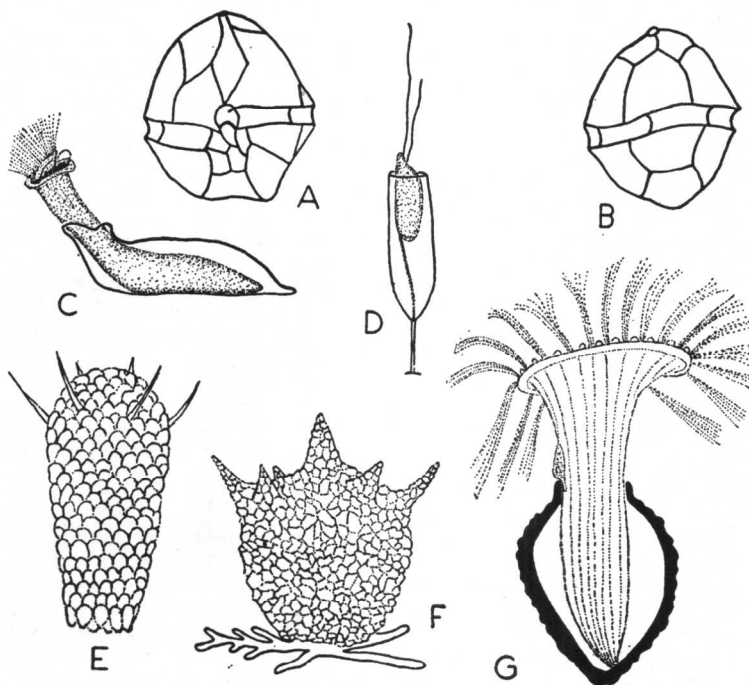


Fig. 1. 5. A, B. Ventral and dorsal thecal plates in *Gonyaulax acatenella*; x560 (after Whedon and Kofoed). C. *Vaginicola longicollis*, optical section of lorica; x140 (after Penard). D. *Stokesiella lepteca*, stalked lorica; x1060 (after Stokes). E. Test of *Euglypha alveolata*; x350 (after Leidy). F. *Difflugia corona*; x135 (after Leidy). G. *Tintinnopsis nucula*, optical section of lorica; diagrammatic; x570 (after Campbell).

periplast of many Euglenida permits a characteristic euglenoid movement ("metaboly"), but tends to maintain a characteristic form in the swimming flagellate. This periplast presumably is a secreted layer, since it becomes separated from the underlying cytoplasm in plasmolysis (22). Thickened pellicular layers, as seen in *Lepocinclis* and *Phacus*, may be so firm that the body shows little change in shape. Such membranes are often decorated with ridges, papillae or other markings.

The *theca* of many Phytomonadida and Dinoflagellida is a secreted

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covering applied directly to the surface of the body and is comparable to the thick cell wall found in higher plants. The flagella emerge through pores in the theca. A theca may be somewhat flexible, allowing slight changes in form, or it may be rigid. The firmness imparted by cellulose or pectins is sometimes increased by impregnation with inorganic salts to produce a hard covering, as in *Phacotus*, *Trachelomonas*, and some of the dinoflagellates. The theca of many dinoflagellates is differentiated into a number of plates (Fig. 1. 5, A, B), the pattern varying with the species.

*Lorica*, *test*, and *shell* are terms applied to coverings which often fit less closely than the theca and hence are less comparable to the typical cell wall of plants. A lorica (Fig. 1. 5, C, D) is usually a tubular or vase-like structure with an opening through which the anterior part of the

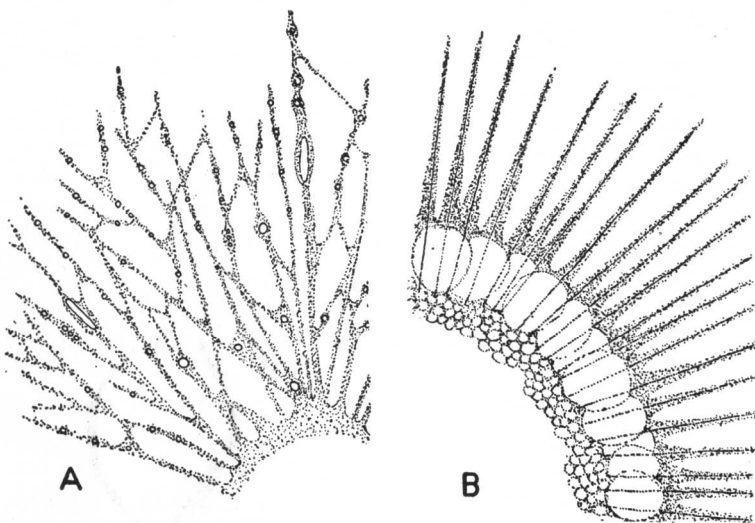


Fig. 1. 6. Groups of myxopodia (A) and axopodia (B); diagrammatic.

body or its appendages can be extended. The base of the lorica, in sessile species, may be attached directly to the substratum or may end in a stalk. In colonial types (Fig. 1. 3, C, D), one lorica may be attached to another directly or by means of a stalk. A lorica may be composed entirely of secreted material or may be reinforced with diatom shells, sand grains, or other foreign particles.

The tests (or shells) of many Sarcodina vary widely in form and composition. Some appear to be homogeneous. Others consist mainly of separate elements cemented together, as in *Euglypha* and *Diffugia* (Fig. 1. 5, E, F). The test of *Euglypha* is composed of plates, formed within the body prior to fission; that of *Diffugia* is made of sand grains embedded in a secreted cement. The comparable arenaceous tests of certain Foraminiferida (Chapter V) are built of sand grains, discarded tests, sponge



spicules, or other materials cemented together over a thin chitinous test. The composition of other foraminiferan tests varies from group to group. That of the Allogromiidae is typically chitinous, while the majority of the multichambered tests are calcareous. Siliceous tests also have been reported in a few Foraminiferida. In many species at least, the foraminiferan test is not really external; instead, it is normally enclosed within a thin layer of cytoplasm.

The simplest skeletons of Radiolarida are represented by scattered siliceous spicules, while the more complicated types are structures unique among the Protozoa (Chapter V). In the Acantharina long spines radiate in definite patterns from the center of the body. To these elements is often added a lattice-work shell, joining and supported by the spines. Siliceous skeletons of other Radiolarida are quite varied in structure. Spherical types may be composed of several concentric lattice-work shells, and sometimes of spicules in addition. Bilateral types, conical forms, and other departures from radial symmetry are fairly common.

### PSEUDOPODIA

Pseudopodia are temporary organelles which can be retracted and formed anew, depending upon activities of the organism. Four major types may be distinguished—*lobopodia*, *filopodia*, *myxopodia*, and *axopodia*.

Lobopodia, which have relatively dense outer layers and more fluid inner zones, are relatively broad pseudopodia with rounded tips. Short or slender lobopodia may be hyaline, but larger ones usually show a clear ectoplasm enclosing a granular endoplasm. Lobopodia are characteristic of amoebae, certain flagellates, and certain testate rhizopods (Fig. 1. 5, F).

Filopodia are slender hyaline pseudopodia which taper from base to pointed tip and also tend to branch and anastomose. In addition, filopodia may fuse locally to produce thin webs of cytoplasm. The absence of circulating granules helps to distinguish filopodial from myxopodial nets.

Myxopodia (rhizopodia, or reticulopodia), characteristic of the Foraminiferida, are filamentous structures (Fig. 1. 6, A) which branch and anastomose into complex networks often covering a wide area. Such nets are efficient food-traps and are fairly effective locomotor organelles. In addition, the digestive activities of myxopodia are usually marked in Foraminiferida (Chapter V). The comparatively dense inner zone of the myxopodium has been considered fibrillar in structure (198). The fluidity of the outer layer is indicated by the active circulation of cytoplasmic granules, as illustrated by *Elphidium* (*Polystomella*) *crispum* (103).

Axopodia (Fig. 1. 6, B) tend to radiate singly from the surface of more or less spherical organisms (Heliozoidea, Radiolarida). The axial filament of a typical axopodium has been described as a fibrillar tube enclosing a

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homogeneous core (193, 195). In contrast to the axial filament, the outer cytoplasm is a sol, as indicated by the movement of inclusions. Axial filaments may converge in a *central granule* (*Acanthocystis* and related genera) or they may end separately in the cytoplasm (*Actinosphaerium*).

### FLAGELLA AND ASSOCIATED STRUCTURES

#### Flagella

These organelles are found in Mastigophora and in flagellate stages of Sarcodina and Sporozoa. A typical flagellum is composed of a *sheath*, which may be circular, elliptical, or flattened in cross-section, and an inner *axoneme*. The latter, according to some workers, is the active portion of the flagellum while the sheath is merely protective. Others think that the axoneme is only an elastic support for a contractile sheath. The axoneme arises from a granule, the *blepharoplast*, and may or may not extend beyond the sheath as a distal end-piece (Fig. 1. 7, F). A terminal knob (Fig. 1. 7, H), instead of a filament, is evident in silver preparations of *Trypanosoma rhodesiense* (127). The anterior flagella of *Hexamitus pulcher* (130) also are unusual in that they arise from external rod-like structures (Fig. 1. 7, E) of uncertain significance.

The finer structure of the flagellum<sup>1</sup> is incompletely known, although investigations with the electron microscope (13, 56, 180, 199) have supplemented earlier observations. The axoneme may be composed of one, two (*Astasia*, *Euglena*), three (*Peranema*), or perhaps more fibrils, while the sheath apparently contains a spirally coiled filament in certain species.

The sheath in some flagella shows lateral filaments (Fig. 1. 7, A, C), the *mastigonemes* (43) or "Flimmer," the nature of which is uncertain. Although observed in living *Mallomonas acaroides* in dark-field (217), they may be artifacts (173) or may represent fibrils of the sheath which are frayed out laterally under certain conditions (180). At any rate, such filaments appear consistently in some species and not in others. In the *stichonematic* flagellum (43), a single row of filaments extends along one side of the sheath (Fig. 1. 7, A), as in *Astasia* and *Euglena* (13). In the *pantonematic* type there are two or more rows of mastigonemes. Only a terminal filament is present in the *acronematic*, or "lash" flagellum (174), while the *pantacronematic* type shows both a terminal filament and one or more rows of mastigonemes. A *simple* type, found in Cryptomonadida and Dinoflagellida (174), shows neither terminal filament nor mastigonemes. These characteristics of the flagellum seem to be constant within various groups and may furnish significant information in studies on taxonomy and phylogeny (174, 217).

In the majority of flagellates, the flagellum extends forward from its

<sup>1</sup> This subject has been reviewed in several papers (13, 174, 180, 217).