The PROTOZOA

Introduction to PROTOZOOLOGY

JOHN N. FARMER, Ph.D.

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with 600 illustrations

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Cover illustration is a modification of a scanning electron micrograph of *Peridinium leonis.* (With permission from Dodge, J. D. The fine structure of algal cells. Copyright 1973 by Academic Press Inc. [London] Ltd.)

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Preface

Why study protozoa? This question has been directed to me in my role as an advisor by students inquiring about the merit of courses offered in my department. My answer is to point out the importance of protozoa in food chains, as parasites of humans, to the economy as parasites of domestic animals, and as research organisms for cytologists, developmental biologists, electron microscopists, and geneticists. Invariably my discourse ends with the comment "and besides, they are beautiful!" Although not a scientific remark, it is a fond tribute to the kindling of my own interest in protozoa, fired by my major professor, the late Elery R. Becker. He was, of course, primarily concerned with parasitic protozoa. However, in the protozoology course he taught, Dr. Becker took a little time to introduce his students to the major groups of free-living protozoa. As is often the case in such courses, we looked at the wet mounts made from a variety of samples and attempted to identify the rapidly moving contents. Dr. Becker leaned against the edge of a desk at the front of the room, observing the process. On seeing an upraised hand, he would bound across the room (he never did anything slowly), peer into the microscope, and exclaim, "Beeeautiful!" And indeed they are.

In 1967, I was invited to teach a five-week course in "field" protozoology at Iowa Lakeside Laboratory, a biological station in northwest Iowa. Courses at the laboratory are attended by undergraduates, advanced undergraduates, graduate students, high-school teachers, and sometimes by college professors. In view of the variety of backgrounds, I thought it was important to choose an appropriate textbook. None of the texts available and none of the excellent new books and series of books dealing with protozoa that have become available has truly fulfilled the needs of such a course in protozoology.

Such a broad discipline is difficult to confine within the covers of a single text and necessitates a reduction of coverage of certain areas. Parasitic protozoa are usually allotted little space, and function is often emphasized at the expense of description. Moreover, there is a tendency to assume that the reader already has a working knowledge of the morphological details of major protozoan groups. This may be true for the advanced undergraduate or graduate student toward whom most protozoology courses are directed. However, it is my experience that the initial question asked by the student microscopist is "How do I know what I'm looking at?" The role of the laboratory instructor becomes one of constantly pointing out characters used for the identification of the organisms in question.

Accordingly, I set out to write a protozoology text organized for the less-advanced student who may have become interested in protozoa mentioned only briefly in introductory biology, invertebrate zoology, or microbiology courses. The writing has taken up most of my leisure time for the past eleven years, a period during which there has been an unprecedented surge in research with protozoa. Sophisticated techniques employed by only a few in previous years have become refined and commonly available for use by numerous investigators interested in cellular structure and function. No longer are protozoological studies confined to morphological descriptions, although new species are being described almost daily. Now the protozoa are used in biochemical studies concerning enzyme chemistry

and chloroplast function. Advances in electron microscopy have permitted greater insight concerning the structure and related function of protozoan organelles. The ease of culturing protozoa makes possible the study of ciliary, flagellar, and protoplasmic movement; flux across membranes; morphogenesis; and genetics. Environmental concerns have caused an increased interest in research relative to the role and biological importance of protozoa in ecosystems, particularly as possible indicators of water quality.

Because of this increased interest in protozoa, it is inevitable that organisms of interest to individual investigators and specialists have been omitted or paid short shrift. I apologize for these omissions, whether intentional or not. However, *The Protozoa: Introduction to Protozoology* was written with the student in mind and not as a reference text. It has been my experience that students enroll in protozoology courses because they have already been intrigued by protozoa. Their curiosity has been piqued by a life cycle or by the phenomenon of slime mold aggregation, the functioning of contractile vacuoles, and sometimes even by the morphology of protozoa. These students wish to know more about protozoa, how they function, and, recently, their role in ecosystems. The students are much less interested in taxonomy. To maintain this curiosity, I have chosen many common examples readily available for observation in the laboratory. Other examples are included because of their medical importance or their use as research organisms. Finally, questions are raised concerning systematic, ecological, biochemical, and physiological problems that still need to be solved.

I have attempted to organize material in the book so that it may be used in two ways. First, as a supplement to lecture presentation, the initial four chapters deal generally with structure, function, reproduction, and the role of protozoa in ecosystems. The remaining thirteen chapters deal more specifically with the morphology, ultrastructure, biology, and ecology of particular protozoan groups. A second use of *The Protozoa* would be as a laboratory supplement. The classification keys at the beginning of later chapters may be used to identify the genus of organisms observed during laboratory exercises. These keys are unconventional in that they are not arranged in couplets. Characteristics of all classes being considered are compared within the subphylum. A number in parentheses by the name of the class refers the reader to the next lower rank, the orders within the class. By selecting the appropriate characters, the genus of the organism being observed can be determined. Details of the biology of the organism and its relatives may then be located in the body of the chapter.

Concerning taxonomy, because of the rapidity with which new information about protozoa is being disseminated, it is impossible to present a classification scheme acceptable to all who work with these organisms. Students are always dismayed by this apparent inability of so-called experts to agree on a common scheme. "If the experts can't agree, how do you expect us to organize the material?" is a question familiar to many, I am sure. However, because classification methods are considered to be dynamic, they are subject to change as new information becomes available. The problem is that this information is being published at a staggering rate. Moreover, protozoa are an extremely diverse assemblage of organisms with only one thing in common: organization within a single and independent cell. It is difficult for many taxonomists to consider such a diverse group to be related closely enough to be considered a phylum. A current view is to elevate the Protozoa to the level of a subkingdom within the phylum Protista. This gives the taxonomist more flexibility and enables the elevation of the traditional class or superclass designation to the level of phylum. Thus flagellates, amoebae, sporozoa, and ciliates would be ranked as phyla Mastigophora, Sarcodina, Sporozoa, and Ciliophora, respectively. Certain justification exists for this approach, since each group is distinctive enough to merit phylum ranking. However, I have chosen to retain the classification proposed by the Committee on Taxonomic Problems of the Society of Protozoologists* for the

*Honigberg, B. M.; Committee. J. Protozool. **11**:7-20; 1964.

simple reason that it was the most logical scheme available at the time this project was started. For several more recent publications dealing with the taxonomy of particular groups and, in some cases, in which revisions are proposed, the student is directed to the following:

Ciliates—Corliss, J. O. Syst. Zool. **23**:91-138; 1974; J. Protozool. **21**:207-220; 1974; Trans. Am. Microsc. Soc. **96**:104-140; 1977.

Sporozoa—Frenkel, J. K. J. Parasitol. **63**:611-628; 1977; Levine, N. D. J. Parasitol. **56**:208-209; 1970.

Amoebae—Page, F. C. An illustrated key to freshwater and soil amoebae. Cumbria, England: Freshwater Biological Association; 1976.

Flagellates—Leedale, G. F. Euglenoid flagellates. Englewood Cliffs, N.J.: Prentice-Hall, Inc.; 1967.

Parasitic flagellates—Hoare, C. A. The trypanosomes of mammals: a zoological monograph. Oxford, England: Blackwell Scientific Publications, Ltd.; 1972; Lumsden, W. H. R.; Evans, D. A., eds. The biology of the Kinetoplastida. New York: Academic Press, Inc.; 1976.

All parasitic groups—Levine, N. D., ed. Protozoan parasites of domestic animals and of man. 2d ed. Minneapolis: Burgess Publishing Co.; 1973.

All groups—Jahn, T. L.; Bovee, E. C.; Jahn, F. F. How to know the Protozoa. 2d ed. Dubuque, Iowa: William C. Brown Co., Publishers; 1979.

One last comment: Protozoa watching is catching! All one needs to become addicted is some pond or lake water, a microscope, and curiosity. Wide-mouthed jars of varying sizes can be used for collecting samples. To ensure a variety of protozoa, fill jars approximately a third full with vegetation, floating leaves, and scrapings from rocks. Distribute these samples in finger bowls, and place them in lighted areas but not in direct sunlight. Add a few rice or wheat grains to ensure bacterial growth, a nutrient source for many protozoa.

One of the problems facing the novice protozoologist is the rapidity of protozoan movement. Protozoa can be slowed by adding methylcellulose to the drop of water on the slide, which permits the viewer to better see the morphological characters of the organisms. Vital dyes can also demonstrate internal organization: crystal violet, Janus green, methylene blue, and neutral red dyes diluted in water (0.01% to 0.001%) are commonly used. Drops of sample are added to a region of dried dye solution. Nuclei, food vacuoles, and, in some cases, mitochondria can be observed with Janus green.

A relatively simple method for affixing protozoa to slides for permanent slide mounts is the nigrosin–mercuric chloride–formalin technique.* This method employs a stain affixative applied to fresh samples and results in permanently stained cells within 15 minutes. The stain affixative is prepared as follows: saturated mercuric chloride, 10 ml; glacial acetic acid, 2 ml; formalin, full strength, 2 ml; tertiary butanol, 10 ml; formol-nigrosin solution, 2 ml. The formol-nigrosin solution is formulated as follows: formalin, full strength, 20 ml; soluble nigrosin, 4 g; distilled water, 100 ml.

Place a culture of protozoa on a clean slide. Pipette a drop of stain affixative from a height of 2 to 3 cm onto the sample. With additional stain, wash the resulting mixture to an end of the slide. After approximately 15 seconds, move the slide through a dehydrating series of ethyl alcohols, 35% to 100%. Clear in xylene, and cover with mounting medium. Ciliary structures and nuclei will be clearly differentiated against a gray background. Silver staining is another method used to demonstrate ciliary fields. Affix ciliates to a slide with the formol–mercuric chloride–tertiary butanol–glacial acetic acid solution described previously. Immerse these treated slides for 20 minutes in a 3% silver nitrate solution at 5° to 10° C. Wash the slides in cold distilled water, submerge in water, and expose to sunlight for 30 minutes. Dehydrate in a graded alcohol series, clear in xylene, and mount. For a more complete discussion of the collection, cultivation, and observation of protozoa, the reader is referred to the text by Kudo.**

*Borrow, A. C. Marine flora and fauna of northeastern United States: protozoa, ciliophora. Washington, D.C.: U.S. Government Printing Office; 1973. (National Oceanic and Atmospheric Administration Technical Report, National Marine Fisheries Service circular no. 378.)

**Kudo, R. R. Protozoology. 5th ed. Springfield, Ill.: Charles C Thomas, Publisher; 1966.

A text by a single author which covers such a broad field is a task that cannot be completed without the help and support of many. I wish to thank the students, colleagues, and friends who have encouraged and gently cajoled me toward the completion of this book. Although the list is incomplete, I am especially indebted to the following persons for their time, kindly criticism, and expert review and counsel: A. Baker, H. Blankespoor, R. Bovbjerg, R. Breitenbach, L. Congdon, L. Cortez, J. Dodd, D. Farish, D. Frederickson, L. Jackson, H. James, N. Levine, R. McGuire, P. Pappas, C. Reimer, B. Ridgeway, J. Rooney, and D. Vesole. These people have reviewed the entire manuscript, have read and corrected sections covering their area of specialization, have provided material for photography, or were simply there to listen to ideas. They were responsible for the removal of mistakes and the clarification of ideas. Any errors of fact or of interpretation that remain are solely my responsibility. My thanks are also extended to those who gave permission for use of illustrations, some of which have not been published previously. These figures are credited, and their use is greatly appreciated. My warm thanks go also to Blaise Brazos and Kent Loeffler, who helped with the culture, staining, and photography of selected protozóa. Many of the line illustrations are my own, having been drawn from living material, stained specimens, or descriptions or freely redrawn from other sources; in cases of single sources, credit is given.

Finally, my personal thanks are due to Mary Keyes, my typist, who maintained her humor through twenty-one legal pads, two cut-and-paste drafts, and the final manuscript copy; to my graduate students for understanding the meaning of deadlines; to J. Hobart and I. Herbert of the University College of North Wales–Bangor for providing office space during my sabbaticals; to the students, both past and present, at Iowa Lakeside Laboratory, Milford, for their curiosity and youthful enthusiasm; to Dick Bovbjerg, a special friend and colleague and Director of Iowa Lakeside Laboratory, who has provided space for writing, as well as advice and often-needed encouragement; and to my wife, Margaret, who has often wondered when it would all end but who has been most patient and understanding.

John N. Farmer

Contents

CHAPTER 1

Introduction

WHAT ARE PROTOZOA?

Although it would appear to be a simple task to describe a protozoan as a microscopic, unicellular, independent organism, in reality, clearly defining a protozoan is difficult. The problem is one of immense variety. All of what have been considered as protozoa do not, for example, fit within neat morphological parameters, such as uniform shape and microscopical size, or physiological parameters involving heterotrophic nutrition. As more information accumulates, a miscellaneous description becomes necessary.

For example, so-called protozoa come in many shapes and sizes; although one usually thinks of them as being microscopical, some can be seen easily without the aid of a lens. For example, ciliates such as *Stentor polymorphus* (Fig. 1-1) measure between 1 and 2 mm, whereas *Spirostomum ambiguum* (Fig. 1-2) can develop to a length of 3 mm. The calcium carbonate **tests*** of certain foraminifera measure about 4 mm in diameter. Indeed, the remains of foraminifera found in the limestone of the Pyramids measure more than 100 mm, whereas in the present, *Chaos (Pelomyxa)* sp., a giant amoeba (Fig. 1-3), can extend to 5 mm. At the lower end of the scale, **trophozoites** of malaria may be only several micrometers (μm) in diameter (Fig. 1-4); those of certain parasitic amoebae such as *Dientamoeba* sp. and *Endolimax* sp. are only 5 μm. *Oikomonas termo* (Fig. 1-5), a tiny free-living flagellate, is about the same size. However, size is an extremely unreliable taxonomic characteristic. The environment may well affect the organism's development. *Tetrahymena vorax*, a well-known example, can vary in size remarkably, depending on its diet. Small-mouthed bacteria feeders can transform into voracious, carnivorous, large-mouthed forms, depending on the availability of nutrients. Accordingly, size is a relative characteristic in regard to identifying protozoa.

From a morphological standpoint, it is difficult to be definitive concerning the shape of protozoa. Floating forms, heliozoa (Fig. 1-6), for example, are spherical, whereas those relying on active swimming to acquire food, as *Paramecium* does, tend to be elongate. On the other hand, hypotrichs are flattened (Fig. 1-7), enabling them to browse among bottom debris to feed, whereas attached ciliates or flagellates have evolved in a conical or ovoid form.

Beyond these generalities, the shape of protozoa is diverse. An *Amoeba* organism is constantly changing its form, whereas the flagellated *Phacus* sp. (Fig. 1-8) is limited by a rigid **pellicle.** Others retain a basic body form, yet are capable of changing shape, as in *Distigma* sp., or a wormlike twisting of the body, as in *Euglena deses* (Fig. 1-9). A vaselike or cuplike structure produced by the protozoan and within which it resides is common. *Arcella vulgaris*, a testate amoeba (Fig. 1-10), synthesizes a symmetrical envelope about itself; the amoeba *Difflugia urceolata* (Fig. 1-11) employs debris to accomplish the same thing. Some protozoa, like *Trachelomonas hispida* (Fig. 1-12), are free swimming and take their capsules wherever they go. In

*Terms in boldface type are defined in the Glossary at the end of the text.

Text continued on p. 7.

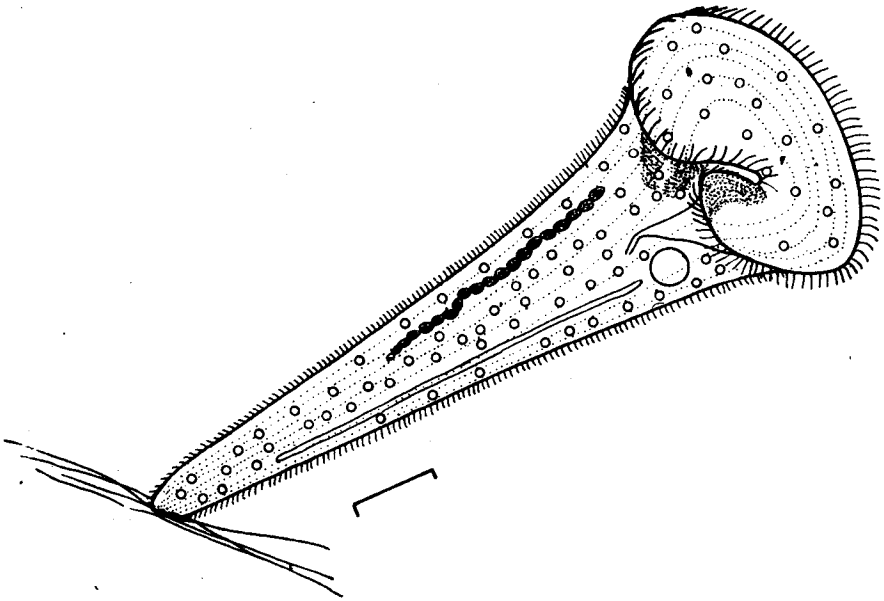

Fig. 1-1. *Stentor polymorphus*. Cytoplasmic inclusions are green chlorellae; a contractile vacuole and radial canal are usually conspicuous. (Scale = 100 μm.)

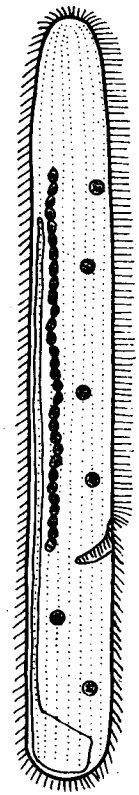

Fig. 1-2. *Spirostomum ambiguum*. A large contractile vacuole is located at the posterior end. The oral opening (cytostome) is located approximately one fourth the length of the body from the posterior end. (Scale = 500 μm.)

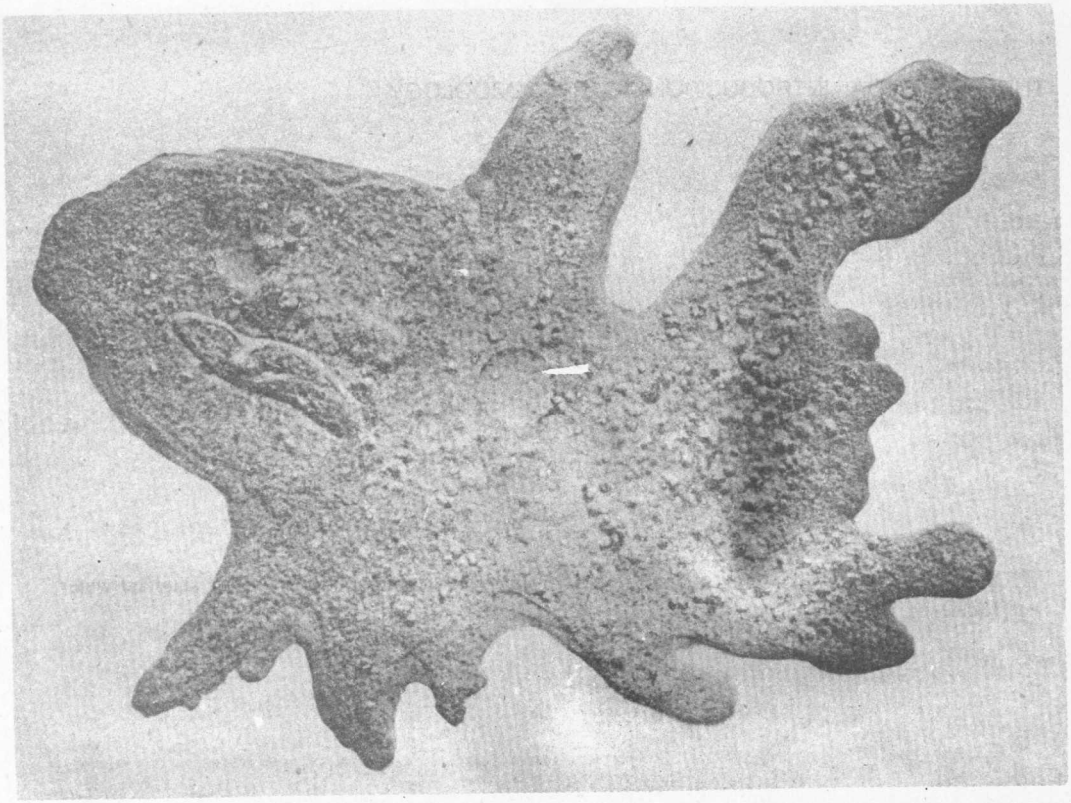

Fig. 1-3. *Chaos (Pelomyxa)* sp., a giant amoeba, is 1 to 5 mm in length. Compare the size of the *Chaos* sp. with that of the ingested *Paramecium* organism. (Courtesy Carolina Biological Supply Co., Burlington, N.C.)

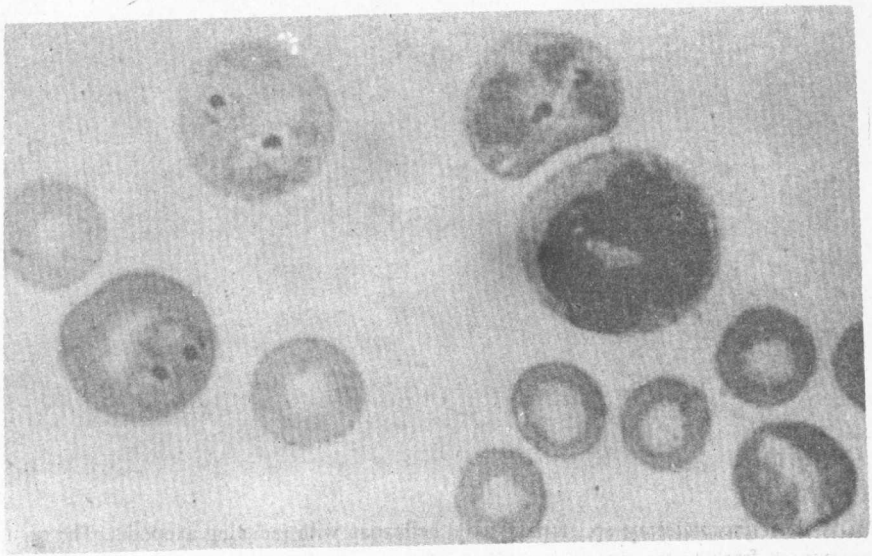

Fig. 1-4. Trophozoites of *Plasmodium berghei,* which causes rodent malaria, parasitizing mouse erythrocytes. The large nucleated cell is a neutrophil for comparison of size. (×1500.)

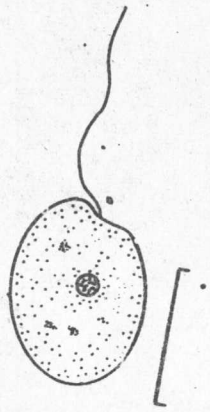

Fig. 1-5. *Oikomonas termo,* a small free-living flagellate commonly observed in stagnant water. (Scale = 10 μm.)

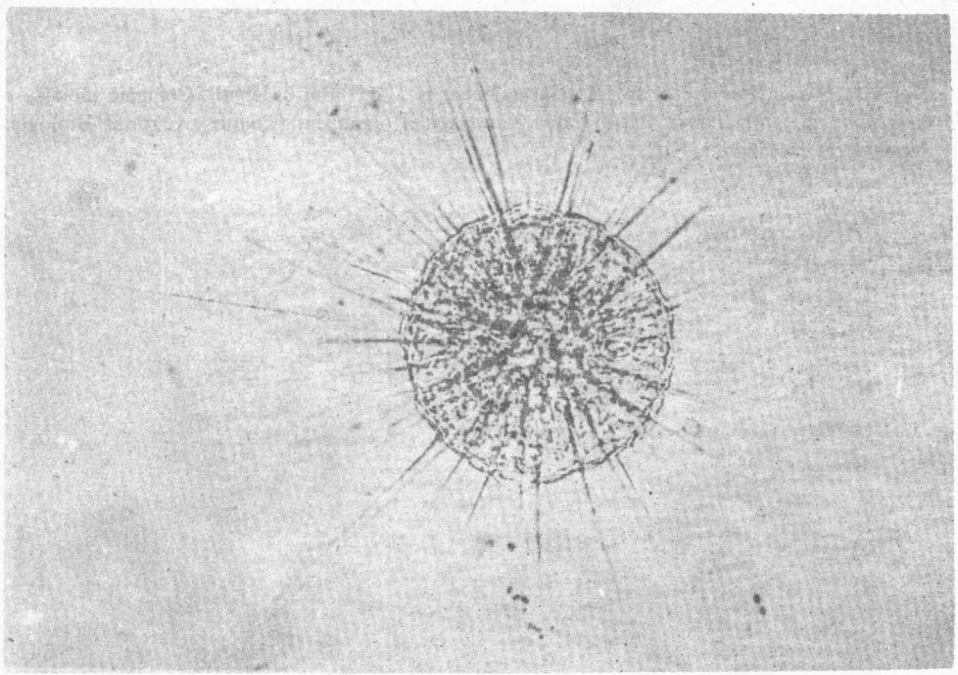

Fig. 1-6. *Actinosphaerium* sp., a planktonic heliozoan with radiating axopodia. The organism is common in fresh water among aquatic vegetation. The body is approximately 200 μm long. (Courtesy Carolina Biological Supply Co., Burlington, N.C.)

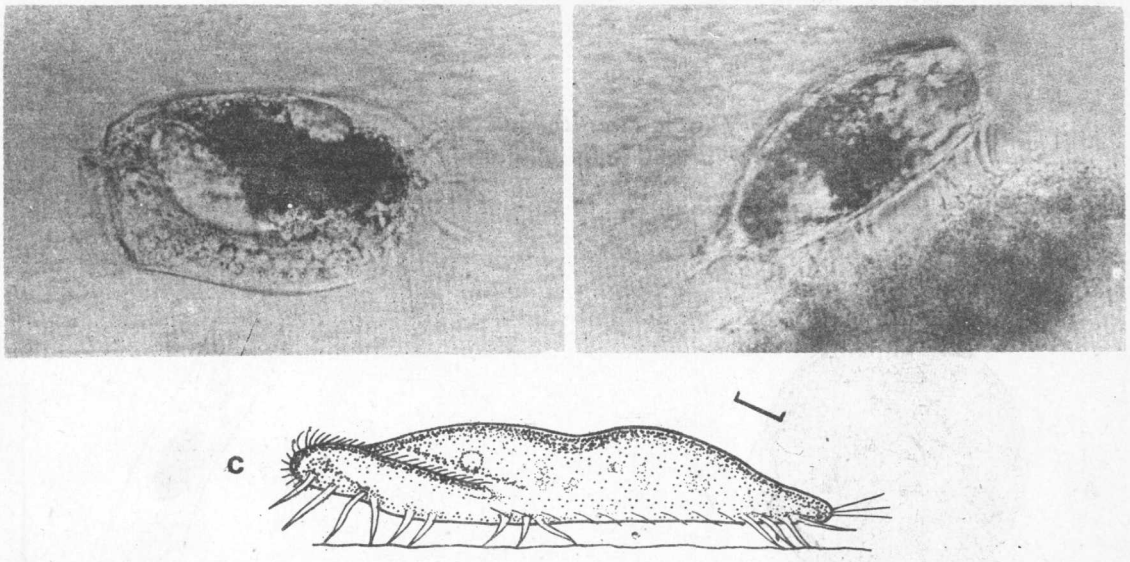

Fig. 1-7. A, Surface view of *Euplotes* sp., illustrating the caudal cirri and spiraling row of anterior cilia associated with feeding. (Living preparation, phase-contrast photomicrograph.) **B,** Side view of *Stylonychia* sp. "walking" on debris. (Living preparation, phase-contrast photomicrograph.) **C,** *Stylonychia* sp., a hypotrich ciliate, is capable of using cirri to browse among benthic debris. (Scale = 10 μm.)

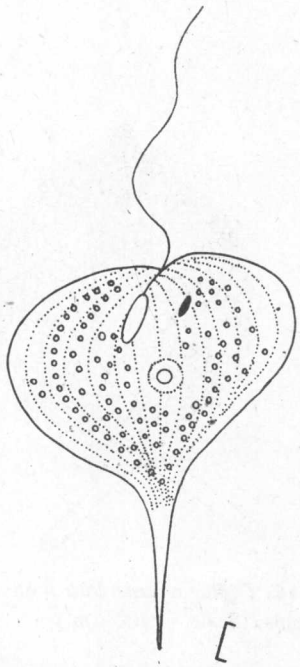

Fig. 1-8. *Phacus longicauda,* a euglenoid flagellate with a rigid pellicle drawn out as an oblique projection posteriorly. (Scale = 25 μm.)

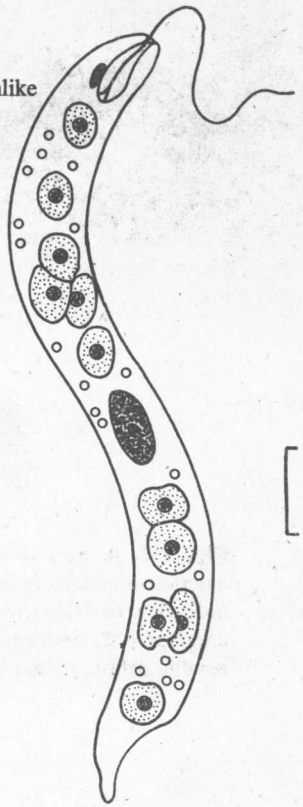

Fig. 1-9. *Euglena deses,* characterized by its wormlike crawling movement. (Scale = 10 μm.)

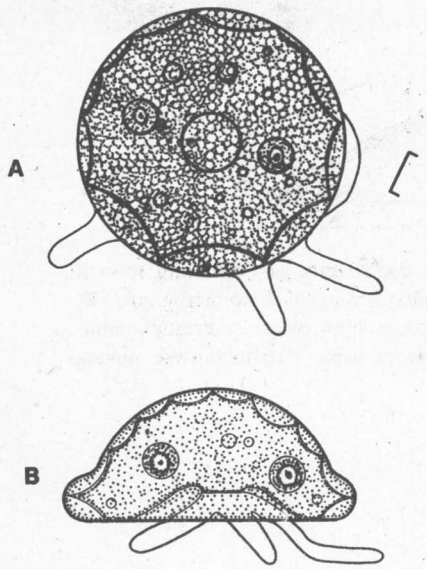

Fig. 1-10. *Arcella vulgaris,* a testate amoeba. **A,** Surface view. The central opening at the bottom can usually be seen through the test, which is often brown in color. Pseudopodia extend through this opening. (Scale = 10 μm.) **B,** Side view.

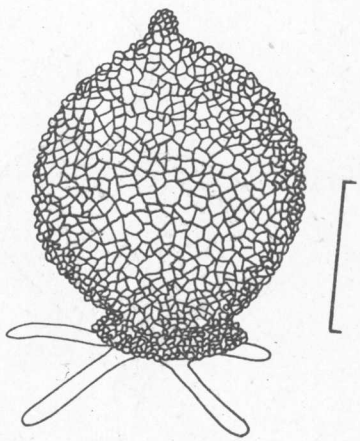

Fig. 1-11. *Difflugia urceolata* with a test formed from sand grains. (Scale = 100 μm.)

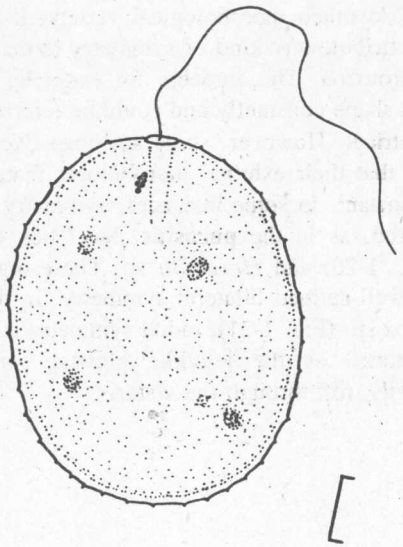

Fig. 1-12. *Trachelomonas hispida,* a testate euglenoid. (Scale = 10 μm.)

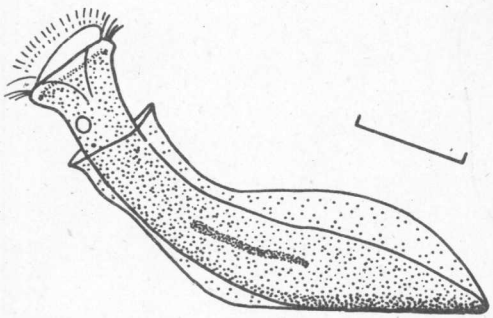

Fig. 1-13. *Platycola* sp., a loricate peritrich ciliate. The covering is referred to as a lorica, since it is not a closely fitting protective covering. (Scale = 20 μm.)

fact, it is possible to squash these organisms under a coyer glass, thereby destroying the protective covering. The "naked" flagellate will swim away, presumably to regenerate another capsule. Although *Platycola* sp. (Fig. 1-13), a ciliated peritrich, lives in its secreted structure, it is not free swimming, since the covering is attached to the **substratum.** Cellulose coverings, grooved to accommodate **flagella,** are characteristic of the nonarmored dinoflagellates. In some armored forms an additional plate-like layer is synthesized (Fig. 1-14). When observed under a microscope, these armored forms, churning through the water, remind one of pictures of World War I tanks.

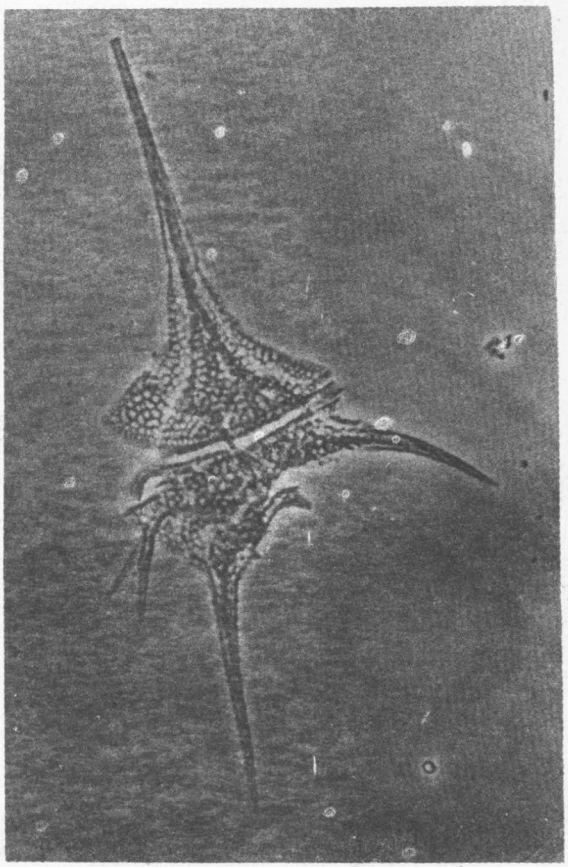

Fig. 1-14. *Ceratium hirundinella,* a thecate dinoflagellate. The body, covered with distinct plates, is divided into two regions by a transverse groove, or girdle, and is approximately 250 μm long.

Some colonial flagellates are stalked and **sessile.** To feed, they have evolved delicate cuplike or vaselike collars. Food is drawn toward and eventually trapped by these structures. Sessile Suctoria such as *Tokophrya quadripartita* (Fig. 1-15), are oval or conical and equipped with sucking tentacles to facilitate feeding. The trumpet-shaped *Stentor amethystinus* (Fig. 1-16), the leaflike *Trypanosoma musculi* (Fig. 1-17), the amoeboflagellate transformations of *Dimorpha* sp., the stellate appearance of "stressed" *Amoeba* organisms (Fig. 1-18), and the barrel shape of *Coleps octospinus* (Fig. 1-19) are examples of the variations in shape.

With so much morphological variety, it is difficult to attribute any kind of symmetry to the majority of protozoa. The amoeba, for example, usually changes shape constantly and could be referred to as asymmetrical. However, some amoebae live within tests so that their external morphologic features remain constant. In some instances, symmetry can be recognized, as in the **parasitic** flagellates *Giardia* sp. (Fig. 1-20) and *Hexamita* sp. These organisms have a well-defined bilateral symmetry. In the case of *Volvox* sp. (Fig. 1-21), radial symmetry is clearly differentiated as the jellylike globes, containing many cells, roll through the water.

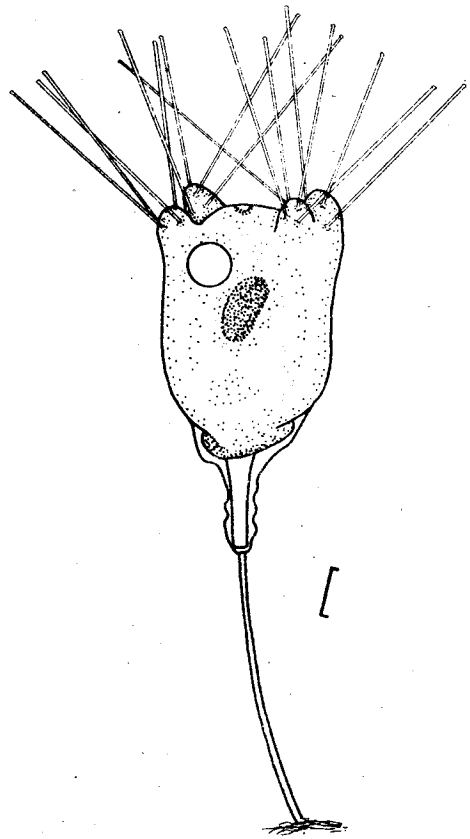

Fig. 1-15. A suctorian ciliate, *Tokophrya quadripartita*. The sessile stage, as shown, feeds by using the hollow capitate tentacles to extract cytoplasm from its prey. (Scale = 25 μm.)

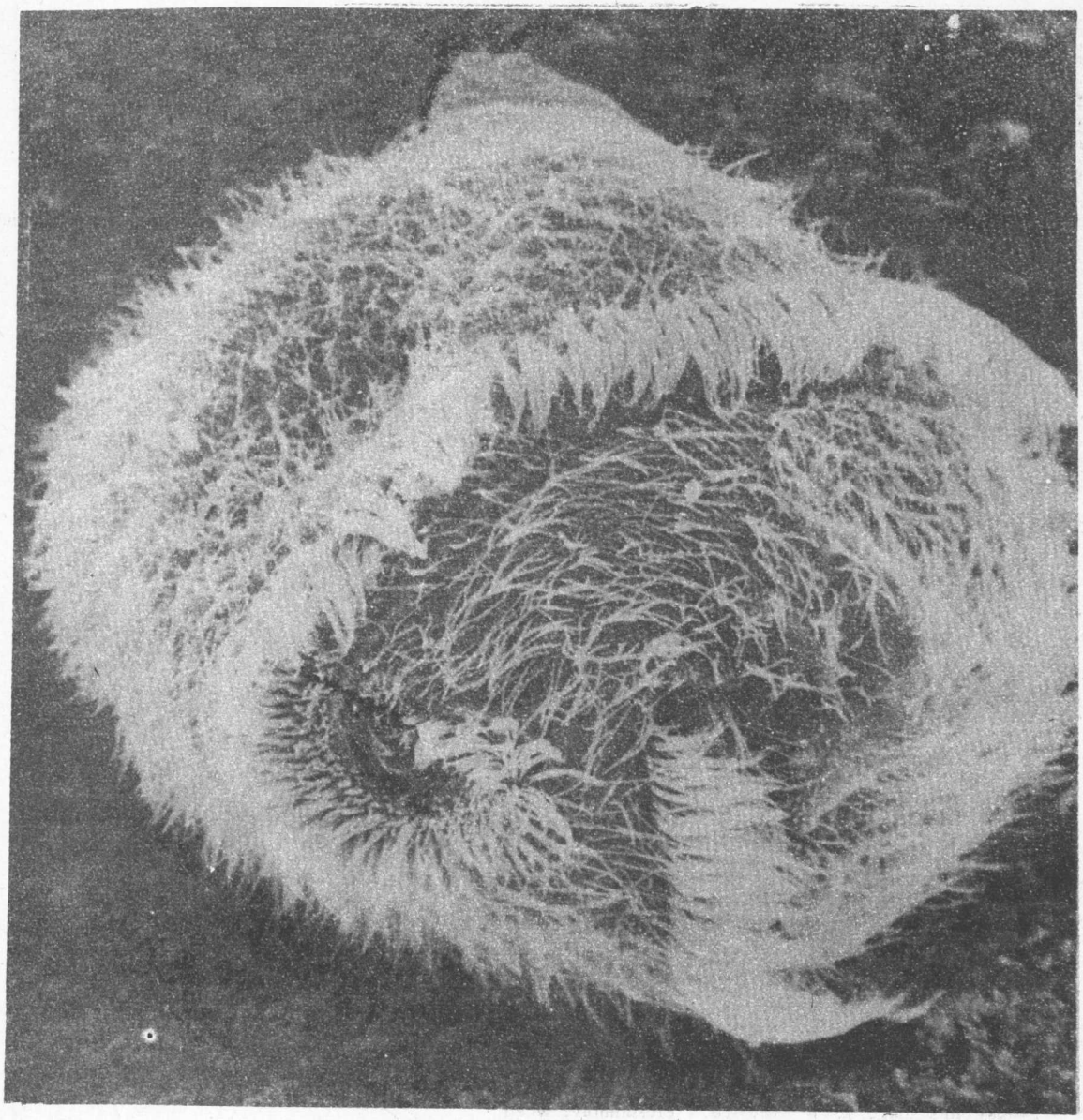

Fig. 1-16. A scanning electron micrograph of the peristomal region of *Stentor amethystinus*. The body is uniformly ciliated, as is the edge of the peristome. The cytostome is conspicuous at the end of the spiraling peristomal cilia on the right. (~ × 1600.) (Courtesy H. Blankespoor, Hope College, Holland, Mich.)