INFORMATION TO USERS

This manuscript has been reproduced from the microfilm master. UMI films the text directly from the original or copy submitted. Thus, some thesis and dissertation copies are in typewriter face, while others may be from any type of computer printer.

The quality of this reproduction is dependent upon the quality of the copy submitted. Broken or indistinct print, colored or poor quality illustrations and photographs, print bleedthrough, substandard margins, and improper alignment can adversely affect reproduction.

In the unlikely event that the author did not send UMI a complete manuscript and there are missing pages, these will be noted. Also, if unauthorized copyright material had to be removed, a note will indicate the deletion.

Oversize materials (e.g., maps, drawings, charts) are reproduced by sectioning the original, beginning at the upper left-hand corner and continuing from left to right in equal sections with small overlaps. Each original is also photographed in one exposure and is included in reduced form at the back of the book.

Photographs included in the original manuscript have been reproduced xerographically in this copy. Higher quality 6" x 9" black and white photographic prints are available for any photographs or illustrations appearing in this copy for an additional charge. Contact UMI directly to order.

UMI®

Bell & Howell Information and Learning
300 North Zeeb Road, Ann Arbor, MI 48106-1346 USA
800-521-0600

DISSERTATION

SYSTEMATICS AND ULTRASTRUCTURE OF NEW AND RARE CHRYSOPHYTES FROM COLORADO AND WYOMING LAKES

Submitted by

Rosane Aguiar

Biology Department

In partial fulfillment of the requirements

for the Degree of Doctor of Philosophy

Colorado State University

Fort Collins, Colorado

Fall 2000

COLORADO STATE UNIVERSITY

SEPTEMBER 21, 2000

WE HEREBY RECOMMEND THAT THE DISSERTATION PREPARED UNDER OUR SUPERVISION BY ROSANE AGUIAR ENTITLED SYSTEMATICS AND ULTRASTRUCTURE OF NEW AND RARE CHRYSOPHYTES FROM COLORADO AND WYOMING LAKES BE ACCEPTED AS FULFILLING IN PART REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY.

Committee on Graduate Work

David D. Steingraber

R B Reeves

John P. Chen

Paul Kugrens

Advisor

Joan M. Hall

Department Head

ABSTRACT

SYSTEMATICS AND ULTRASTRUCTURE OF NEW AND RARE CHRYSOPHYTES FROM COLORADO AND WYOMING LAKES

The focus of this study is a description of the Light (LM), Scanning (SEM) and Transmission (TEM) electron microscopic characteristics of nine chrysophytes or golden-brown algae from Colorado and Wyoming.

Phaeaster pascheri var. *avesiculosa* var. nov. was isolated from Colorado and Wyoming lakes. Cells are compressed in an anterior-posterior indicating a somewhat reniform shape in lateral view. Cells are biflagellate based on TEM but the second flagellum is not evident with either light microscopy or scanning electron microscopy. Cells are covered by oblong non-mineralized scales without ornamentation. Cells have a single, golden-brown lobed chloroplast with a central pyrenoid that has several tubular invaginations of the chloroplast envelope. The nucleus lies near the inner face of the chloroplast and is adjacent to the pyrenoid. This isolate appears similar to *Phaeaster pascheri* Scherffel, but it lacks the rhizopodial extensions around the flagellar pit, contractile vacuoles and a wide sheath around the long flagellar base. Its systematic position is discussed and a new variety is proposed.

A strain of *Diacronema vlkianum* from Colorado was studied by light and electron microscopy. This is a first report of this alga from the United States. The differences between this isolate from Colorado and a freshwater strain described previously are compared with respect to cell size, flagellar length, and presence of vacuoles. These differences, however, do not merit a separate species designation at this time. The taxonomic position of this golden-brown flagellate was uncertain, and this study corroborates and expands on earlier findings which support its current taxonomic placement.

An isolate of *Hymenomonas* Stein from Colorado is described using light and electron microscopic techniques. Cells usually occurred in a non-motile or palmelloid stage and are covered by non-calcified and calcified scales, the latter known as coccoliths. The coccoliths are distributed in a single layer and each coccolith possesses a basal-plate that is unmineralized. The calcified scales consists of an average of 11 calcium carbonate elements situated between the margin and the raised rim of the base-plate scale. Several layers of non-mineralized scales lie beneath the coccolith layers and are oval and rounded with ornamentations. The pattern of ornamentation on the proximal surface consists of radiating fibrils while the distal surface shows a pattern of concentric fibrils. Four plastids generally are present per cell, each with a bulging pyrenoid that is traversed by paired thylakoids. A single anterior Golgi apparatus often contains developing scales. Flagella and haptonema were not observed in this isolate since the flagellate stage was rare. The ultrastructure of palmelloid cells is compared with cells of *H. roseola* from Europe.

The ultrastructure and systematics of two new freshwater prymnesiophytes are described. An isolate of *Prymnesium* from a freshwater lake in Wyoming was examined by light and electron microscopy, and this isolate represents the first representative of this genus from a freshwater habitat. Cells vary in morphology, ranging from elongate to subspherical to spherical, with a rounded posterior end and an obliquely truncate anterior end. Two equal or subequal flagella and a short, non-coiling haptonema arise subapically from a groove or depression in the truncate portion of the cell. Two types of organic scales that differ in size, shape, and pattern of ornamentation cover the cell. Chloroplasts vary in number, ranging from one to seven. Each chloroplast has a pyrenoid that protrudes toward the cell interior and is partially traversed by thylakoids.

The second new prymnesiophyte is *Chrysochromulina asquamosa* sp.nov. and *C. asquamosa* was collected from five different lakes in Colorado. It is proposed as a new species because it lacks scales. Cells are obovate, spherical, and usually with two chloroplasts, although cells with three to five chloroplasts are common. Each chloroplast has an embedded pyrenoid that is not invaded by thylakoids. The long haptonema (15 μm) and the two subequal flagella are inserted subapically on the concave side of the obovate cell. The flagella lack hairs but both flagella have narrow terminal portions. *C. asquamosa* resembles *C. parva* and *C. breviturrita,* but *C. asquamosa* differs from both by its smaller cells and lack of scales. Since scale morphology is the most important criterion for delineating *Chrysochromulina* species, this character provides the basis for proposing a new species.

Three colonial chrysophycean flagellates belonging to the genus *Uroglena* were examined with the electron microscope, and their structure was compared with data other *Uroglena* species. Scanning electron microscopy (SEM) of *U. volvox* reveals a colonial structure that is different from any previously described. It is a spherical colony with several hundred cells that are attached by their pointed tails to a confluent sheet of mucilage, rather than being embedded within a mucilaginous colonial envelope. The spherical colonial mucilage is hollow, and SEM shows that the confluent mucilage consists of a meshwork of fibrils. Cells are obovoid with acutely pointed posterior ends, and a large chrysolaminarin vacuole occupies the posterior one-third of the cell. Each cell has a single sheet-like, spiral chloroplast with a stigma and a pyrenoid. The pyrenoid is adjacent to the nucleus and projections from the nucleus protrude into the pyrenoid. The stigma is in the anterior end of the chloroplast in a specialized lobe, and is associated with the short flagellum and its flagellar swelling. A descending microtubular root of 11 microtubules begins at the base of the long flagellum and extends around the periphery of the cell, terminating in the cell's posterior. A second *Uroglena* species is smaller and has up to 32 cells per colony, but an investing colonial envelope or a confluent envelope also is absent. Cells are obovoid with a short pointed end, and the cells are connected to each other by dichotomously branched hollow stalks or tubes. Cells have a single U-shaped chloroplast with a stigma, but lack a pyrenoid. *Uroglena articulata* is the third colony, and its cells also are connected to each other by sympodially branched stalks but an investing colonial envelope is absent. Cells usually have two chloroplasts with a stigma in one of the chloroplast lobes but pyrenoids are lacking. In addition, an unusual bacterial

endosymbiosis was discovered. A rod-shaped bacterium, with its wall intact, grows inside the nuclear envelope of all cells examined. The bacterium did not appear to be pathogenic, but each is a permanent resident of these cells. All three isolates also differ in the fine structure of the flagellar swelling that is associated with the eyespot. The ultrastructure of cells from the colonial chrysophyte *Uroglena articulata* and the presence of bacteria in the nuclear envelope space are described and discussed. The bacteria appear to be rod-shaped and possess a wall. They are not located near the chloroplasts, and it is postulated that these bacteria either provide some essential nutrient to the alga.

The ultrastructure of *Ochromonas pleiomorpha* sp. nov. is described. Cells are variable in shape, but all possess a single U-shaped chloroplast with lobes of different lengths, and a stigma located in the dorsal lobe. The dorsal lobe usually is shorter than the ventral lobe. A pyrenoid is located toward the inside of the chloroplast and is adjacent to the nucleus on the concave side of the chloroplast. The pyrenoid is invaded by several tubular chloroplast envelope invaginations. A chrysolaminarin vesicle is posterior to the nucleus, and the vesicle membrane is in contact with the posterior portion of the nuclear envelope. The ventral portion of the cell has a concentration of small vesicles that presumably are pre-lysosomal vesicles and/or secondary lysosomes. Light and scanning microscopy revealed that cells may form numerous cytoplasmic extensions (rhizopodia, pseudopodia, filipodia) which may attach cells to a substrate by a posterior extension. Furthermore, the cytoplasmic extensions may be branched. These extensions may form from extrusive vesicles located underneath the plasma membrane. The contents of the

vesicles are dark staining and upon discharge the contents become more diffuse. Neither actin microfilaments nor microtubules were observed in these extensions. *O. pleiomorpha* is an active mixotroph, feeding on diatoms and on its own cells. Diatoms and *O. pleiomorpha* cells are digested in food vacuoles. After the contents of diatoms are digested in the food vacuoles, empty frustules are releasedby exocytosis.

Rosane Aguiar
Biology Department
Colorado State University
Fort Collins, CO 80523
Fall 2000

ACKNOWLEDGEMENTS

I owe the largest debt to my advisor, Dr. Paul Kugrens, and wish to express my sincerest gratitude for his supervision, patience, and encouragement throughout this work. He not only gave me excellent guidance but also was a good friend who was available to be involved in my research. I feel extremely fortunate to have worked with someone who is so willing to share his time and his considerable expertise.

I am grateful to Dr. Brent Reeves, Dr. David Steingraeber and Dr. John Chandler for their contributions, suggestions, encouragement, confidence and friendship during this work.

I would like to thank CAPES (Federal Agency for Post-graduate Education), and the Federal University of Viçosa, Viçosa, Minas Gerais, Brazil, for their encouragement and financial support.

Thanks to my parents, Jose Ferreira de Aguiar and Dione Linhares Aguiar , for instilling in me an appreciation for a good education and determined effort. Their love and influence are felt more than they know.

Thanks to my American and Brazilian friends for sharing their time, laughter, hopes and experiences to help make this journey enjoyable.

DEDICATION

I dedicate this dissertation to my husband, Paulo Euclydes, my sons, Rodrigo A. Cipriano, and Leonardo A. Cipriano, and my daughter, Paula A. Cipriano. Without their love, caring, encouragement, and enlightenment it would not have been possible. I will always strive to return their sacrifices and provide the same support and love through their own endeavors in life.

This is our Ph.D, and I am excited by the possibilities which lie ahead for us.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES LEGENDS

GENERAL INTRODUCTION

INTRODUCTION

Chromophyte algae constitute a significant component of phytoplankton populations in marine and freshwater habitats. They contain chlorophyll *a* & *c* and include diatoms, dinoflagellates, cryptomonads, prymnesiophytes, phaeophytes and chrysophytes. They are also called heteronkont algae, or autotrophic stramenopiles, with the latter name based on the presence of tubular flagellar hairs (Cavalier-Smith, Chao & Alsopp 1995). The chromophyte algae are a biologically diverse group consisting of an estimated one million species, representing 13 taxonomic categories (Andersen, 1992, 1993), and they appear to have phylogenetic affinities with some aquatic fungi and zooflagellates (Saunders, Potter, Paskind & Andersen 1995). Ecologically, the marine planktonic chromophytes account for approximately 50% of oceanic primary productivity (Werner 1977, Shapiro & Guillard 1986) and this phytoplankton *in toto* account for up to 40% of the global primary production (Bolin, Degens, Duvigneaud & Kemp 1977; Berger, Smetacek & Wefer 1989). These algae range in size from minute picoplankton (1-2 μm) to the large kelps or brown seaweeds, whose size may exceed 40 M or more.

Two of these Chromophyta phyla, the Chrysophyta and Prymnesiophyta have been examined extensively from the marine environment, but freshwater representatives need considerably more studies on their systematics, ecology and cell structure. The

lack of information may be due to the tendency to study lake systems that have been impacted by human activities, and these algae are relatively rare in these types of lakes. Representatives from these two divisions are common in oligotrophic lakes and reservoirs in the Rocky Mountain region, especially in Colorado and Wyoming, preferring highly alkaline (pH 8.4), oligotrophic, and colder waters. This region is a prime area for the study of chrysophyte phytoplankton because of the lack of information about these freshwater algae.

Since there is a paucity of information on the ultrastructure and systematics of freshwater chrysophytes and prymnesiophytes, representatives from these groups were isolated and maintained in the Colorado State University Algal Culture Collection. Selected species were used in this study because of their availability, because several had not been reported from the USA, and became some had not been described previously. Electron microscopy (EM) was used for studying these algae so that generic and species details and cell structure could be described properly.

The objectives of this research were to examine selected chrysophycean and prymnesiophycean algae from Colorado and Wyoming, using several electron microscopic techniques to accomplish the following: (1) to provide additional information on rare and new chrysophytes; (2) to contribute additional data regarding the biodiversity of this group by discovering and describing new taxa; (3) to isolate, culture, and describe chrysophytes or prymnesiophytes that represent the first reports of these algae from the USA; (4) to obtain SEMs of chrysophytes, since information about the surface configuration of these cells is lacking; (5) to provide additional information

on mixotrophy since some were observed to be mixotrophic; and (6) to clarify and enhance the systematics of some genera and species.

The Divisions Chrysophyta and Prymnesiophyta were the focus of this work. A discussion of these chrysophytes and prymnesiophytes is provided to acquaint the reader with these algae. Since there are several classification schemes for chrysophytes and prymnesiophytes, the most current scheme proposed by Preisig (1995), was used in this study.

Division Chrysophyta

This division consists of the Classes Chrysophyceae, Pedinellophyceae and Dictyochophyceae (Kristiansen 1990). The morphology ranges from single-celled to colonial forms. Chloroplasts contain chlorophylls *a* and *c* and the accessory pigment fucoxanthin. Chrysolaminarin (-1,3-glucan) is the major storage product. Motile cells have heterokont flagella (unequal flagella length) where one flagellum has tubular hairs and the other is smooth.

Class Chrysophyceae. This class has unicells and colonial forms. Cells are naked and bilaterally symmetrical, with a pair of anteriorly directed flagella of unequal length that are inserted subterminally into the cell at an oblique angle to each other (Hibberd 1976). The longer flagellum bears tubular flagellar hairs, and the shorter flagellum has a swelling, usually with electron-dense contents, near the proximal end. This swelling lies in a shallow depression in the cell surface, directly over the eyespot. The flagellar transition region has a transitional helix (Hibberd 1979) and a transverse

partition with a central axonemal thickening at the level of the cell surface. There are two types of flagellar roots, one consisting of relatively small numbers of microtubules that run superficially, and the other is a large cross-banded root that extends deeply into the cell, with branches ramifying over the surface of the nucleus. Cells are uninucleate and each nucleus is somewhat pyriform with its narrow end directed toward the flagellar basal bodies. The outer membrane of the nuclear envelope forms a double membraned investiture called the chloroplast endoplasmic reticulum (CER). There are one or two chloroplasts per cell with a peripheral girdle lamella and thylakoids arranged in groups of three. An eyespot is at the margin of the chloroplast, with this region being pressed closely against the flagellar depression. A single large Golgi body usually is near the nucleus in the anterior end of each cell, sometimes in a concavity of the nuclear envelope. Chrysolaminarin is stored in large vesicles, usually occupying the posterior part of the cell. When present, a contractile vacuole system typically is in the extreme anterior end of the cell.

The Class Chrysophyceae, as presently described by Preisig 1995, comprises six orders - the Bicosoecales, the Chromulinales, the Hibberdiales, the Hydrurales, the Sarcinochrysidales, and the Chrysomeridales. This modern concept of classification is based on the flagellar apparatus, mitosis, cytokinesis processes and other details of cell ultrastructure, as well as on photosynthetic pigments and other biochemical and physiological data.

(a) Order Bicosoecales, Grassé (1926). Members of this order are heterokont flagellates lacking a chloroplast, and the group is more likely to represent, or be related to, the ancestral type of autotrophic chrysophytes such as *Ochromonas*. Electron microscopic examination has shown considerable similarities between the flagellar apparatus of bicosoecids and chrysophytes (Moestrup 1995). The main difference is the absence of a silicified cyst, the stomatocyst (found in many of the phototrophic chrysophytes).

(b) Order Chromulinales, Pascher (1910). This order is a combination of the Order Chromulinales (Pascher 1910) and Ochromonadales (Pascher 1910). Members of this order are predominantly unicellular, with occasional rhizopodial or palmelloid stages. Cells have two heterokont flagella and are naked, or, in a few species, are covered by simple organic scales. Members whose vegetative cells are aflagellate are characterized by the formation of *Chromulina*-like or *Ochromonas*-like zoospores sometime during their life history, and their flagellar apparatus and other cellular components are closely related to chromulinalean chrysophytes. Cells typically are pigmented, but a few colorless genera also occur. Moreover, the characteristic stomatocysts or statospores of many chrysophycean algae are not known for this group. Until more detailed studies become available on the ultrastructure, biochemistry and molecular genetics, the taxonomy of many members of this order will remain uncertain.

(c) Order Hibberdiales, Andersen (1989). Members of this order and its families were established by Andersen (1989) who described the structure of the flagellar apparatus of *Hibberdia* and found that the flagellar apparatus differs from *Chromulina*, *Ochromonas*

and related genera. The flagella are inserted at a much wider angle, and the flagellar roots do not form a loop under the second flagellum (Andersen 1991). This genus is also unique in its pigmentation, because it contains antheraxanthin and fucoxanthin as a light-harvesting carotenoids. The two different photosynthetic light- harvesting carotenoids, are considered unique among algae (Preisig 1995). Furthermore, preliminary observations on mitosis in *Hibberdia* suggest that the behavior of the nuclear envelope during mitosis is similar to that of *Hydrurus* (Hoffman, Vesk & Pickett-Heaps 1986), but it differs from other Chrysophyceae such as *Ochromonas*, *Poterioochromonas* and *Uroglena / Uroglenopsis*. The nuclear envelope appears to be more or less intact during metaphase except for polar fenestrae, where the spindle microtubules penetrate (Andersen, 1989). Unlike *Hydrurus*, however, the flagellar apparatus lies at the spindle poles and the spindle microtubules originate near the basal bodies.

(d) Order Hydrurales, Pascher (1913). This order includes only the family Hydruraceae, represented by *Hydrurus, Celloniela* and *Phaeodermatium*. The zoospores produced by these genera differ from other chrysophytes by being tetrahedral in shape. Furthermore, the flagellar roots do not form a loop under the second flagellum (vestigial flagellum) as in chromulinalean chrysophytes. This unique shape of the zoospores is associated with a complex skeletal system of microtubules. In contrast with other members of the Chrysophyceae, there is no photoreceptor system, but there are many contractile vacuoles and a Golgi apparatus. The stomatocysts or statospores differ from the normal chrysophycean type. They are ellipsoid and have a delicate wing extension

in the periphery. As mention previously, mitosis and cytokinesis in this order also are different from that of other Chrysophyceae, but are similar to those found in the family Hibberdiaceae.

(e) Order Sarcinochrysidales, Gayral & Billard (1977). This order includes genera with phaeophycean affinities (Preisig 1995), it is exclusively marine (or brackish), and stomatocysts have never been observed. Further investigations demonstrated the existence of two natural groups within this order, which were split in to the Sarcinochrysidales *sensu stricto* and Chrysomeridales (Kristiansen 1990). The Order Sarcinochrysidales lacks an eyespot in motile cells, and pyrenoids are stalked, a characteristic that is similar to phaeophycean zooids. Diatoxanthin and diadinoxanthin are the major accessory pigments. Plastid DNA occurs as nucleoids that are scattered throughout the chloroplast. The major sterol is C_{29}- sterol. The flagellar root system also differs from other Chrysophyceae because it lacks a helical structure in the basal body transition region, and the basal plate is elevated above the plane of the plasmalemma.

(f) Order Chrysomeridales, Kristiansen 1990. Members of this order have a tendency to form lobed or branched cytoplasmic extensions. The vegetative cells are ameboid (rhizopodial organization) during most of their life history. Flagellate stages occur in *Amphichrysis*, *Chrysamoeba*, and *Rhizoochromonas*, whereas flagellate stages have not been observed in *Chrysarachnion, Rhizochrysis* and *Leukochrysis*. Chloroplasts have eyespots and the accessory pigment violaxanthin. When zoospores are produced, the flagella are more or less laterally inserted into the cell. The similarities in zoospores between these algae and brown algae have led to the speculation that the Phaeophyceae

probably evolved from an alga similar to *Giraudyopsis* (O'Kelly, 1989, Saunders, Potter & Andersen 1997b).

Division Haptophyta (Prymnesiophyta)

The division Haptophyta (Hibberd 1976) includes diverse organisms known principally from marine environments, but a few freshwater and terrestrial representatives also have been reported (Stein 1878, Lackey 1939, Parke, Lund & Manton 1961, Green 1973, Green & Parke 1975, Nicholls 1978, Kling 1981, Wujek & Gardiner 1985). This division includes unicellular and colonial forms, some of which are motile, while others are non-motile. Non-motile forms occur as palmellae, filaments, or gelatinous colonies. The most familiar representatives of the Haptophyta are the coccolithophorids, which have an investiture of calcified plates called coccoliths.

The feature most readily associated with the Haptophyta is the haptonema. This is a filiform organelle whose length may be many times that of the cell, as in *Chrysochromulina strobilus* (Parke, Manton & Clarke 1958) or may be reduced, as in species of *Isochrysis, Chrysotila* (Green & Parke 1975), *Diacrateria* and *Imantonia,* and *Diacronema* where the haptonema is detectable only by electron micrographs as a small proboscis between the flagella (Green & Pienaar 1977).

Many members of the Haptophyta also have unmineralized scales on the cell surface. The scales, in their simplest form, consist of a two-layered plate, with each layer composed of fibrils arranged in a radial pattern. Such scales, when present, often

occur alone or as an underlayer beneath coccoliths. In most coccolithophorids the coccoliths themselves are formed on a base-plate scale (Leadbeater 1994). Since haptonemata, coccoliths, and two-layered fibrillar scales are found only in the Haptophyta, taxonomic studies of the group have been largely based on variations in these structures (Jordan & Green 1994).

Most members of Haptophyta are photosynthetic, but at least one non-photosynthetic coccolithophorid, *Balaniger balticus* Stein, is known (Thomsen & Oates 1978). A number of freshwater algae also have been included in the Haptophyta by some authors (Starmach 1985) on the basis of their flagellar arrangement, but it is now known that many of these algae have affinities elsewhere (Jordan & Green 1994).

Blooms of toxic planktonic haptophytes occurred in Scandinavian waters during 1988 causing a mass mortality of marine life and severe economic losses, and was attributed to species of *Chrysochromulina* (Underdahl, Skulberg, Dahl & Aune, 1989, Tangen 1991). Previously, only localized fish mortalities, caused by species of *Prymnesium,* had been recorded from other parts of the world, such as northern Europe and the Middle East, particularly Israel (Green, Hibberd & Pienaar 1982, Jordan & Green 1994).

Class Prymnesiophyceae: The prymnesiophyceae are primarily marine organisms, although there are some freshwater representatives. They make up a major part of the marine nanoplankton and constitute about 45% of the total phytoplankton cells

in the middle latitudes of the South Atlantic (Lee 1999). They decrease in frequency toward the poles, although some still occur in polar waters (Manton, Sutherland, & Oates 1977).

One of the main structural characteristics of the group is the presence of a haptonema, a filiform organelle that occurs between two flagella. Parker, Manton & Clarke (1958) demonstrated that the "third flagellum" was not a flagellum, but a novel organelle, which they named the haptonema. They described the presence of unmineralized scales, in some cases very elaborate in structure, forming an investment around the cell.

Electron microscopic (EM) observations resulted in the separation of chrysophytes possessing a pair of more or less equal length flagella into a separate class known as Prymnesiophyceae or Haptophyceae. Originally, Pascher (1910) included algae with this type of flagellation in a separate group within the Class Chrysophyceae, and placed them in the order Isochrysidales, but subsequently, Bourrelly (1965, 1968) placed them in the subclass Isochrysophycidae. The official separation of the Prymnesiophyceae from the Chrysophyceae was made by Christensen (1962), and it has been strongly supported by additional electron microscopic data. The recognition of this new class is now almost universal, but its acceptance was slow, probably because of the lack of comparative data on the Chrysophyceae. A detailed comparison of the Prymnesiophyceae and Chrysophyceae was published by Hibberd (1976), and it remains essentially unmodified, except for the new data on the structure and formation of prymnesiophycean cysts (Green, Hibberd & Pienaar 1982), and these support the molecular data (Cavalier-

Smith, Allsopp, Häuber, Gothe, Chao, Couch & Maier 1996, Larsen & Medlin 1997, Larsen & Edvardsen 1998, Larsen 1999).

The general characteristics of the Prymnesiophyceae follow. Cells are spherical to oval, flattened or saddle-shaped. Except for species of the Order Pavlovales, prymnesiophytes bear two equal, subequal or unequal flagella, which characteristically lack flagellar appendages. The flagellar transitional region has a characteristic structure with two relatively widely spaced cross partitions that have a stellate pattern. Flagellar roots are either simple or compound and microtubule groups and cross-banded fibrillar roots are absent. The haptonema may be long and capable of coiling or it may be short and stiff. It may be short and narrow, and it may be vestigial as in *Diacronema*, *Isochrysis*, and *Diacrateria*. In some species, such as *Emiliania huxleyi* and *Pleurochrysis carterae* the haptonema can be completely absent (Green & Pienaar 1977, Johansen, Doucette, Barclay & Bull 1988).

Haptonemata typically are composed of a core of cytoplasm with a ring of microtubules. It appears as a ring of 6 or 7 microtubules usually surrounded by three concentric unit membranes. The outermost membrane is the plasmalemma, which is continuous with the cell membrane, while the remaining pair represents a tube of endoplasmic reticulum continuous with the peripheral ER of the cell body. The ring of microtubules is embedded in the cytoplasm core of the organelle and a layer of cytoplasm occurs between the outermost ER membrane and the plasmalemma (Hibberd 1980).

Cells are covered by one to several layers of delicate unmineralized scales that usually are visible only with an electron microscope. These scales are simple to

extremely complex in shape and are composed of a cellulose matrix and pectic polysaccharides (Hibberd 1980). In addition, coccolithophorids have a peripheral covering of coccoliths or calcified scales. Each cell usually possesses one or two golden-brown chloroplasts without girdle lamellae, and the chloroplasts are surrounded by chloroplast endoplasmic reticulum (CER). Each chloroplast usually contains a single pyrenoid, and its matrix is penetrated by doublet-thylakoid lamellae. Photosynthetic pigments include a, c_1 and c_2, carotene, fucoxanthin, diadinoxanthin and diatoxanthin. The storage product generally is chrysolaminarin. Usually, there is no flagellar swelling associated with the eyespot (Green & Hibberd 1977). A single nucleus lies immediately posterior to the large Golgi body. Many species possess a peripheral layer of ER underlying the plasmalemma called the peripheral endoplasmic reticulum (Hibberd 1980).

The Class Prymnesiophyceae is further divided into two orders (Green & Jordan 1994):

(a) Order Prymnesiales: Cells have two equal smooth flagella, a haptonema, and scales, but lack eyespots.

(b) Order Pavlovales: Cells have unequal flagella, often bearing a tomentum of long fine hairs in which the long flagellum usually has a regular array of small dense bodies which vary in form between different species (Green & Manton 1970, Van der Veer 1972, 1976, Green 1976 and Billard 1983). The haptonema originates between the flagella. The long flagellum is usually covered by small cylindrical to club-shaped scales (Green & Manton 1970). Eyespots may be present (Green & Jordan 1994). The

Order Pavlovales is considered to be the most primitive order in the Prymnesiophyceae (Green 1980).

REFERENCE

Andersen, R.A. 1991. The cytoskeleton of chromophyte alga. Protoplasma **164**:143-159.

Andersen, R.A. 1992. Biodiversity Conserv. **1**:267-292.

Andersen, R.A. 1993. The chrysophytes: a review. Protoplasma **164**:143-159.

Andersen, R.A. 1989. The Synurophyceae and their relationship to other golden algae. Nova Hedwigia Bei. **95**:1-26.

Berger, W.H., Smetacek,V.S. & Wefer, G. 1989. **In**: Productivity of the Ocean: Present and Past, edited by Berger W.H., Smetacek V.S. & Wefer, G. New York: Wiley, 471p.

Billard, C. 1983. Recherches sur les Chrysophyceae marines de l órdre Sarcinochrysidales: biologie, systématique, phylogénie. Doctoral thesis, University of Caen. 233pp.

Bolin, B., Degens E.T., Duvigneaud P., & Kempe S. 1977. **In**: The Global Carbon Cycle, Bolin, B., Degens E.T., Kempe, S. & Ketner, M (Eds.). New York: Wiley, pp. 1 - 53.

Bourrelly, P. 1965. La classification des Chrysophycées: ses problèmes. Revue algol. **1**: 56 - 60.

Bourrelly, P. 1968. Les algues d'eau douce, 2: Algues Jaunes et Brunes. Paris: Boubée et Cie.

Cavalier-Smith, T., Chao E.E., & Allsopp, M.T.E.P. 1995. Ribosomal RNA evidence for chloroplast loss within Heterokonta: pedinellid relationship and a revised classification of algae. Arch. Protistenk.**145**:209 - 220.

Cavalier-Smith, T., Allsopp, M.T.E.P., Häuber, M.M., Gothe, G., & Chao E.E. 1996. Chromobiote phylogeny: the enigmatic alga *Reticulosphaera japonensis* is an aberrant haptophyta, not a heterokont. Eur. J. Phycol. **31**: 255 – 263.

Christensen, T. 1962. Alger. **In**: Bötanik Bd. 2, Systematisk Botanik., Böcher, M.L.T.W., & Sorensen, T. (Eds.). Munksgaard, Copenhagen. pp. 1 - 178.

Green, J.C. & Manton, I. 1970. Studies in the fine structure and taxonomy of flagellates in the genus *Pavlova*. I. A revision of *Pavlova gyrans*, the type species. J. Mar. Biol. Assoc. U.K. **50**: 1113-1130.

Green, J.C. 1973. Studies in the fine structure and taxonomy of flagellates in the genus *Pavlova*. II. A freshwater representative, *Pavlova granifera* (Mack) comb.nov. Brit. Phycol. J. **8**: 1-12.

Green, J.C., & Parke, M. 1975. New observations upon members of the genus *Chrysotila*, with remarks upon their relationships with the Haptophyceae. J. Mar. Biol. Ass. U.K. **55**: 109-121.

Green, J.C. 1976. Notes on the flagellar apparatus and taxonomy of *Pavlova mesolychnon* Van Der Veer, and on the status of *Pavlova* Butcher and related genera within the Haptophyceae. J. Mar. Biol. Assoc. U.K. **56**: 595-602.

Green, J.C. & Hibberd, D. 1977. The ultrastructure and taxonomy of *Diacronema vlkianum* (prymnesiophyceae) with special reference to the haptonema and flagellar apparatus. J. Mar. Biol. Assoc. U.K. **57**:1125-1136.

Green, J.C. & Pienaar, R.N. 1977. The taxonomy of the order Isochrysidales (Prymnesiophyceae) with special reference to the genera *Isochrysis* Parke, *Dicrateria* Parke and *Imantonia* Reynolds. J. Mar. Biol. Assoc. U.K. **57**: 7- 17.

Green, J.C. 1980. The fine structure of *Pavlova pinguis* and a preliminary survey of the order Pavlovales (Prymnesiophyceae). British Phycol. J. **15**:151-191.

Green, J.C. Hibberd, D.J. & Pienaar, R.N. 1982. The taxonomy of *Prymnesium* (Prymnesiophyceae) including a description of a new cosmopolitan species, *P. patellifera* sp. nov., and further observations on *P. parvum* N. Carter. Br. Phycol. J. **17:** 363 – 382.

Green, J.C. & Jordan, R.W. 1994. Problems in the taxonomy and terminology of living coccolithophorids. INA Newsletter, **13**(2):52-53.

Hibberd, D.J. 1972. Observations on the ultrastructure of flagellar scales in the genus *Synura* (Chrysophyceae). Arch. Mikrobiol.**89**:291-304.

Hibberd, D.J. 1976. The ultrastructure and taxonomy of the Chrysophyceae and Prymnesiophyceae (Haptophyceae): a survey with some new observations on the ultrastructure of the Chrysophyceae. Bot. J. Linn. Soc.**72**: 55 - 80.

Hibberd, D.J. & Chretiennot-Dinet, M.J. 1979. The ultrastructure and taxonomy of *Rhizochromulina marina* gen. et sp. nov. an ameboid marine chrysophyte. J. Mar. Biol. Ass. U.K. **59:**197-193.

Hibberd, D.J. 1980. Prymnesiophytes (= Haptophytes). **In**: Phytoflagellates, Elsevier (Eds.), North Holland, Inc., p. 273 - 317.

Hoffman, L.R., Vesk, M. & Pickett-Heaps J.D. 1986. The cytology and ultrastructure of zoospores of *Hydrurus foetidus* (Chrysophyceae). Nord. J. Bot. **6**: 105 – 122.

Johansen, J.R., Doucette, G.J., Barclay, W.R. & Bully, J.D. 1988. The morphology and ecology of *Pleurochrysis carterae* var. *dentate* var. nov. (Prymnesiophyceae), a new coccolithophoriod from and Inland saline pond in New Mexico, USA. Phycologia **27** (1): 78-88.

Jordan, R.W. & Green, J.C. 1994. A check-list of the extant Haptophyta of the world. J. Mar. Biol. Ass. U.K. **74**: 149-174.

Kling, H.J. 1981. *Chrysochromulina laurentiana*: an electron microscopic study of a new species of Prymnesiophyceae from Canadian Shield lakes. Nord. J. Bot. **1**: 551 – 555.

Kristiansen, J. 1990. Phylum Chrysophyta. **In**: *Handbook of Protoctista*. Margulis, L, Corliss J.O., Melkonian, M. & Chapman D.J.E. Boston: Jones & Bartlet, pp. 438 - 453.

Lackey, J.B. 1939. Notes on plankton flagellates from the Scioto River. Loyldia **2**: 128-143.

Larsen, A. & Medlin, L.K. 1997. Inter-and intraspecific genetic variation in twelve Prymnesium (Haptophyceae) clones. J. Phycol. **33**: 1007-1015.

Larsen, A. & Edvarsen. 1988. Relative ploidy levels in *Prymnesium parvum* and *P. patelliferum* (Haptophyta) analyzed by flow cytometry. Phycologia **37** (6): 412-424.

Larsen, A. 1999. *Prymnesium parvum* and *P. patelliferum* (Haptophyta)- one species. Phycologia **38** (6): 514-543.

Leadbeater, B.S.C. 1994. Cell coverings. In: *The Haptophyte Algae*, edited by J. C. Green & Leadbeater, B.S.C. Oxford: Claredon Press. 132p.

Lee, R. E. 1999. *Phycology*. Cambridge: Cambridge University Press. 614p.

Manton, I., Sutherland, J. & Oates, K. 1977. Artic coccolithophorids: *Wigwammia artica* gen. et sp. nov. from Greenland and artic Canada. *W. annulifera* sp. nov. from South Africa and S. Alaska and *Calciarcus alaskensis* gen. et sp. nov. from S. Alaska. Proc. R. Soc. Lond. [B] **197**: 145 - 168.

Moestrup, Ø. 1995. Current status of chrysophyte "splinter groups": synurophytes, pedinellids, silicoflagellates. In: Chrysophyte Algae. Sandgren, C.D., Smol, J.P. & Kristiansen, J. (Eds.). Cambridge: Cambridge University Press. pp. 75-91.

O'Kelly, C.J. 1989. The evolutionary origin of the brown algae: information from studies of motile cell ultrastructure. In: Green, J.C., Leadbeater, B.S.C. & Diver, W.L. (Eds.). The Chromophyte Algae: Problems and Perspectives. Systematics Association Special Volume 38. Claredon Press, Oxford. pp. 255-78.

Parke, M., Manton, I. & Clarke, B. 1958. Studies on marine flagellates. IV. Morphology and microanatomy of a new species of *Chrysochromulina*. J. Mar. Biol. Assoc. U.K. **37**:209 – 228.

Parke, M., Lund, J.W.G. & Manton, I. 1961. Observations on the biology and fine structure of the type species of *Chrysochromulina* (*C. parva*. Lackey) in the English Lake District. Archiv f:ur Mikrobiol. **42**: 333-352.

Pascher, A. 1910. Chrysomonaden aus dem Hirschberger GroBteiche. Untersuchungen über die Flora des Hirschberger GroBteiches. I. Teil. Monogr. Abh. Int. Rev. Gesamten Hydrobiol. Hydrogr. **1**: 1-66.

Preisig, H.R. 1995. A modern concept of chrysophyte classification. **In**: *Chrysophyte Algae*. Sandgren, C.D., Smol, J.R.A. & Kristiansen, J. Cambrigde: Cambridge University Press. pp. 46 - 74.

Saunders, G.W., Potter D., Paskind, M.P. & Andersen, R.A. 1995. Cladistic analyses of combined traditional and molecular data sets reveal an algal lineage. Proc. Natl. Acad. Sci. USA. **92**: 244-248.

Saunders, G.W., Potter, D. & Andersen, R.A. 1997b. Phylogenetic affinities of the *Sarcinochrysidales* and *Chrysomeridales* (*Heterokont*) based on analyses of molecular and combined data. J. Phycol. **33**: 310-318.

Shapiro, L.P., & Guillard, R.R.L. 1986. **In**: *Phothosynthetic Picoplankton*. Paltt, T. L. & Wilkers, K.W. (Eds.). Ottawa: Can. Bull. of Fisheries and Aquatic Sciences, p. 371 - 389.

Starmach, K. 1985. Chrysophyceae und Haptophyceae. **In**: *Süsswasserflora von Mitteleuropa*, Ettl, H., Gerloff, J., Heynig H. & Mollenhauer, D.E. Stuttgart: Fisher, p. 1 - 515.

Stein, F.V. 1878. Der Organismus der Infusionsthiere. *Leipzig*. 3.

Tangen, K. 1991. Serious fish kills due to algae in Norway. Red Tide Newsletter (Sherkin Island) **4**: 9-10.

Thomsen, H.A. & Oates, L.D. 1978. Ultrastructural studies of the flagellate and cysts stages of *Pseudopedinella tricostata* (Pedinellales, Chrysophyceae). Br. Phycol. J. **23**:1-16.

Underdahal, B., Skulberg, O.M., Dahl, E. & Aune, T. 1989. Disastrous blooms of *Chrysochromulina polylepis* (Prymnesiophyceae) in Norwegian coastal waters 1988 mortality in marine biota. Ambio **18**: 265-270.

Van der Veer, J. 1972. *Pavlova helicata* (Hatophyceae), a new species from the Frisian Island Schiermonnikoog, The Netherlands. Nova Hedwigia **23**:131-143.

Van der Veer, J. 1976. *Pavlova calceolate* (Haptophyceae), a new species from the Tamar Estuary, Cornwall, England. J. Mar. Biol. Assoc. U.K. **56**:21-30.

Werner, D. 1977. **In**: The Biology of Diatoms. Werner, D. E. (Eds.). London: Blackwell. pp.1-17.

Wujek, D.E. & Gardiner, W. E. 1985. Chrysophyceae (Mallomonadaceae) from Florida. II. New species of *Paraphysomonas* and the prymnesiophyte *Chrysochromulina.* Flor. Scient. 48: 59-63.

CHAPTER 1

PHAEASTER, DIACRONEMA AND *HYMENOMONAS* (CHRYSOPHYTA) FROM COLORADO AND WYOMING: ULTRASTRUCTURE AND NEW RECORDS FOR THE UNITED STATES.

1.1: *PHAEASTER PASCHERI* VAR. *AVESICULOSA*

ABSTRACT

Phaeaster pascheri var. *avesiculosa* var. nov. was isolated from Colorado and Wyoming lakes. Cells are compressed in an anterior-posterior direction resulting in a somewhat reniform shape in lateral view. Cells are biflagellate, but the second flagellum is not obvious with either light microscopy or scanning electron microscopy. Cells are covered by oblong non-mineralized scales without ornamentation. Cells have a single, golden-brown lobed chloroplast with a central pyrenoid that has several tubular invaginations of the chloroplast envelope. The nucleus lies near the inner face of the chloroplast and is adjacent to the pyrenoid. This variety appears similar to *Phaeaster pascheri* Scherffel, but lacks rhizopodial extensions around the flagellar pit, contractile vacuoles and a wide sheath around the long flagellar base. Its systematic position is discussed, and a new variety is proposed.

1.1. *PHAEASTER PASCHERI* VAR. *AVESICULOSA*

INTRODUCTION

Phaeaster pascheri Scherffel is a freshwater member of the Chrysophyceae that was originally described by Scherffel (1927). It was not observed again until Korshikov (1941) collected this alga from the Kovda River and provided a detailed account. Korshikov's alga appeared identical to *Phaeaster pascheri* described by Scherffel (1927), and it had a pyrenoidal region in the chloroplast which Scherffel described as a hole in the chloroplast. At that time, the pyrenoidal region was interpreted by Korshikov (1941) as a mass of leucosin (Belcher 1969).

Later, Skuja (1948) established the genus *Monochrysis* for three species of planktonic flagellates observed from Swedish lakes. In his descriptions, he referred to small cells that were dorso-ventrally flattened, irregularly heart-shaped or angular, with a single flagellum inserted sub-apically. He described *M. aphanaster*, *M. vesiculifera*, and *M. maior*. These species were separated by differences in cell shape and size, number and shape of chloroplasts, and the presence or absence of cytoplasmic vesicles. *M. aphanaster* was the first to be described, and it had both motile and mucilaginous non-motile stages and a stellate chloroplast with a central pyrenoid. Based on these characteristics this species appeared to be *Phaeaster pascheri* rather than a *Monochrysis*.

Bourrelly (1957) transferred all of Skuja's *Monochrysis* species, along with *M. lutheri* Droop, to *Phaeaster*, because of the structural similarity of Skuja's *M. aphanaster* to *P. pascheri* Scherffel (Belcher & Swale 1971).

To date, these species are known mainly from their original descriptions (Preisig, Vørs & Hällfors 1991). Therefore, Patterson & Zölffel (1991) consider them as flagellates of uncertain taxonomic position, because the original descriptions of the genus were made from light microscopy. They suggested that more detailed ultrastructural studies are necessary to ascertain an accurate systematic and phylogenetic relationship.

During investigations on lakes in Northern Colorado and Southern Wyoming, numerous small chrysophytes and cryptophytes were found to dominate lakes with high pHs (8.4). Numerous taxa that had not been reported from the United States were isolated and examined with electron microscope. In this section the ultrastructure of *Phaeaster pascheri* Scherffel is described.

RESULTS

General Description of Cells

Light micrographs are not included because cells had some distortion or discoloration when fixed or their movements were too rapid for microphotography. Nevertheless, descriptions of cells are provided.

The cells of *P. pascheri* are oval in anterior view and reniform in lateral view. Thus, the cells are compressed in an anterior-posterior direction. Cells measure 5.0 - 6.0 μm in length and 3.0 - 4.5 μm in width, and they appear uniflagellate, with the flagellum measuring 6.0 μm in length. Living organisms swim rapidly in a spiral or circular fashion. The cell has a single golden-brown lobed or stellate chloroplast. A pyrenoid is located in the posterior end of the cell and the lobes of the chloroplast radiate from the region of the pyrenoid, but the pyrenoid is difficult to discern. The stigma is located toward the inside of the chloroplast.

Scanning electron microscopy

SEM confirms that cells are compressed in an anterior- posterior direction. They generally are oval in anterior view, and reniform in lateral view. The cells have a single emergent flagellum. The flagellum arises from a distinct pit or depression in the middle of the concave cell (Figs.1-5). Furthermore, the emergent flagellum originates from one side of the flagellar pit and bears tubular hairs (Figs. 2,4). The emergent flagellum sometimes adheres to the cell surface, or the flagellar pit (Fig.3). The cell covering

appears somewhat irregular, perhaps due to the oval or elliptical unmineralized scales (Fig. 6) that will be described in the TEM results.

Transmission Electron Microscopy

Shadow-cast preparations.

Shadowed cells reveal a single type of body-scale that appears unmineralized. There appear to be several layers of these scales (Fig.6). The scales are thin and delicate, oval to oblong, with a thickened rim, and measure approximately 1.1 μm in length and 0.5 μm in width. They do not exhibit any ornamentation or sculpturing.

The flagellum bears tubular flagellar hairs measuring 3.2 μm in length. The flagellar hairs are composed of a tapered basal region (200 nm long x 40 nm wide), a shaft (700 nm long) and a long terminal filament (500 nm long) (Fig. 7).

General ultrastructure

A second non-emergent flagellum was revealed in sectioned material. The non-emergent flagellum is enclosed in an invagination of the cytoplasm (Figs. 8,9). The angle formed between the insertions of the two flagella is approximately 45^0. The emergent flagellum is directed anteriorly while the short flagellum is directed laterally within the invagination. The short flagellum appears smooth and measures 0.7 μm in length, as interpreted from sectioned material. Furthermore, the short flagellum has a flagellar swelling that is closely associated with an eyespot (stigma) that is located in a modified lobe of the chloroplast (Figs. 9-10). This lobe projects toward the center of the cell. The flagellar swelling contains granular material (Fig. 10).

Chloroplast and Chloroplast Endoplasmic Reticulum (CER).

A single, parietal chloroplast with 4-10 lobes is located in the posterior and peripheral regions of the cell (Figs. 11-12). An invagination of the outer membrane of

the nuclear envelope forms the endoplasmic reticulum around the chloroplast called the chloroplast endoplasmic reticulum (CER) (Figs. 12,14). The hypertrophy of this CER probably represents a fixation artifact and could not be eliminated regardless of the fixation techniques utilized. The formation of tubular flagellar hairs occurs in the CER cisterna (Figs. 10,13), and could cause part of this hypertrophy.

As is typical in Chrysophyceae, thylakoids occur in groups of three. Scattered osmophilic droplets are present in the chloroplast stroma (Figs. 11,13). A pyrenoid is located in the center of the chloroplast in the posterior of the cell (Figs. 12, 14), and several tubular invaginations, of the chloroplast envelope (0.2 μm long to 0.1 μm wide), penetrate the pyrenoid. Thylakoids do not penetrate the pyrenoid (Fig.14). The pyrenoid is angular and tends to impart this angularity to the posterior portion of the cell. The prominent oval stigma is located in an inflated lobe of the chloroplast (Figs. 9-11,13, 15). The stigmatic granules are in a single layer, and are arranged ovally in face view (Fig.16).

Nucleus / Golgi apparatus / Mitochondria

A flattened nucleus is anterior, and adjacent to the pyrenoid (Figs. 11-12,14), and is located in the convex side of the cell posterior to the Golgi apparatus. The single, large Golgi apparatus (Figs. 12,13) measures approximately 1.8 μm long x 0.2 μm wide. Presumably the scales are produced in the Golgi vesicles, as in other scaly chrysophycean algae, although this was not observed in *P. pascheri.*

A reticulate mitochondrion is present in each cell, and usually is near the flagellar apparatus, but serial sections were not obtained that would verify the reticulate nature of the mitochondrion.

Contractile Vacuoles

Contractile vacuoles are common in freshwater chrysophycean; however, contractile vacuoles were not observed in this isolate.

Flagellar apparatus.

The complete configuration of the flagellar apparatus has been described previously for *P. pascheri* (Inouye, Zhang, Enomoto & Chihara 1990), and this study confirmed many of the same features. In our isolate, the long flagellum arises in the flagellar groove of the cell (Figs. 18,19). In its transitional region, the transitional helix has four gyres (Figs. 9, 17, 18). The short flagellum is situated toward the right side of the long flagellum at about a 45^0 angle to the long flagellum (Figs. 9,17). As mentioned previously, the short flagellum has a flagellar swelling with dense material. A transitional helix was not observed in the short flagellum. However, a transverse partition

at the level of the cell surface with a central thickening can be seen in both flagella (Figs. 17-18).

There is a connecting band between the basal bodies (Figs. 17,18). As described for *P. pascheri* (Inouye, Zhang, Enomoto & Chihara 1990), the basal body of the long flagellum has two microtubular roots termed R1 and R2, while the basal body of the short flagellum also has two microtubular roots, termed R3 and R4. In sections from our isolate, R1 was the only one observed. It arises from the left side of the basal body of the long flagellum (Fig. 17), and extends anteriorly and is parallel to the cell contour of the left margin of the flagellar groove (Fig. 17). In Figure 18, four microtubules can be seen in transverse section (Fig. 15 arrow), which originate from the R1 root. As suggested by Inouye, Zhang, Enomoto & Chihara (1990), this R1 root carries a microtubule organizing center (MTOC) that may contribute to maintaining the anterior cell shape (Andersen, 1985, 1987).

DISCUSSION

Uniflagellate, single celled chrysophytes generally are placed in the Order Chromulinales (Bourelly 1968), and due to their small size, many chrysophyte unicells are difficult to identify using light microscopy, thus requiring careful electron microscopic observations. Even after SEM examination only a single emergent flagellum was evident in *Phaeaster pascheri*, but a second, vestigial flagellum, was found after TEM examination, within an invagination of the cytoplasm. Whether the remaining species described by Skuja (1948) also have a similar depression with a hidden second

flagellum remains to be determined. In fact, before the Order Chromulinales was combined with the Order Ochromonadales, its major diagnostic feature was a single flagellum. However, EM thus far has demonstrated that there are always two flagella present (Patterson & Zölffel 1991, Preisig, Vϕrs & Hällfors 1991).

Green (1975) examined a marine *Monochrysis*, which had been described as *M. lutheri* Droop (Droop 1953), and he found that it also had a haptonema in addition to two flagella. He subsequently transferred it to the genus *Pavlova* because of the presence of the haptonema. Another marine *Monochrysis* sp. is in the CCMP culture collection (Andersen 1989) and it also is placed in the Prymnesiophyceae, although it has not been examined with the electron microscope. Because of these two instances it is currently assumed that all *Monochrysis* species belong to the Prymnesiophyceae, and the freshwater species are referred to *Phaeaster* due to the absence of a haptonema. The isolate used in this study, which corresponds to Skuja's description of *M. aphanaster*, clearly belongs in the Order Chromulinales and appears to be synonymous with *Phaeaster pascheri*. Due to some differences found in this alga, an emended description of *Phaeaster pascheri* follows.

Phaeaster pascheri* var. *avesiculosa

Aguiar,R. & Kugrens,P.

DIAGNOSIS

Biflagellate cell, ovoid, flattened with one surface convex and the other concave, 5.0- 6.0 μm long. Flagella are unequal. A single emergent flagellum that is longer than

the cell bears tubular hairs. The other flagellum is short and smooth and is within an invagination of the cytoplasm. Cells are covered by oval or elliptical unmineralized scales. The single chloroplast is lobed, with a central pyrenoid and a stigma in a specialized chloroplast lobe. The nucleus is flattened and is anterior and adjacent to the pyrenoid. Contractile vacuoles are absent.

P. pascheri Scherffel was described as biflagellate (Belcher & Swale 1971), having a rhizopodial arrangement around the flagellar pit. Contractile vacuoles were located between lobes of the chloroplast and a wide sheath surrounded the base of the long flagellum. These three features, however, were not observed in *P. pascheri* var. *avesiculosa*. While this may justify the erection of a new species, I have chosen to amend the description for the species and refer to it as *P. pascheri* variety *avesiculosa*.

P. pascheri, collected from mud samples has a dominant stage that is palmelloid (Inouye, Zhang, Enomoto & Chihara 1990). These authors also interpreted some features, such as the configuration of basal bodies, flagella and microtubular flagellar roots to be unusual and distinct from typical chrysophyte cell organization. There are clear relationships with our isolate such as stellate chloroplasts with a highly differentiated morphology to accommodate the eyespots, pyrenoid with tubular invaginations, a long flagellum bearing two rows of tubular hairs, and a short flagellum hidden in the flagellar pocket. However, there were some differences between our isolate and theirs. The dominant life stage in our isolate was unicelled, it had unmineralized scales without any

specific ornamentation, the transitional region in the long flagellum has four gyres instead of three, and contractile vacuoles were not found.

Membranous components within pyrenoids are common in different groups of algae. These may be cytoplasmic invaginations, as in *Lobocharacium* (Kugrens, Clay & Aguiar 2000), *Oedogonium* (Hoffman 1961) and the prasinophyte *Tetraselmis* (Hori, Norris & Chihara 1986), or thylakoid traversals or penetrations as in many green algae (Moestrup & Hoffman 1973, Pickett-Heaps 1975), some cryptomonads (Gantt, Edwards, & Provasoli 1971, Kugrens & Lee 1991, Brett & Wetherbee 1996), some red algae (Hara 1971, Lee 1974, Waaland, Waaland & Bates 1974), and some diatoms (Jeffrey & Vesk 1977, Medlin, Williams & Sims 1993). In *Phaeaster pascheri* there are tubular invaginations of the chloroplast envelope into the pyrenoid, and these are similar to those found in some *Ochromonas* species (Andersen, 1982). While membranous components in pyrenoids are common, a function has not been ascribed to these structures. Based on a recent hypothesis regarding the chloroplast envelope and chloroplast endoplasmic reticulum (Lee and Kugrens 1998, 1999, 2000), a function for these pyrenoidal membranes also can be proposed. It has been established that pyrenoids contain large amounts of ribulose-1, 5-biphosphate carboxylase-oxygenase (Rubisco), the enzyme that fixes carbon dioxide (Rawat, Henk, Lavigne & Morney 1996, Süss, Prokhorenko & Adler 1995), and CO_2 is the only form of dissolved inorganic carbon (DIC) that can be catalyzed by Rubisco. DIC can exist as different forms, depending on the pH, and DIC occurs as CO_2 only under acidic pH. It has been demonstrated by Vrieling, Gieskes & Beelen (1999) that the chloroplast thylakoids and envelope space are acidic and could

function as reservoirs for CO_2. By an extension of this hypothesis, a similar function can be proposed for the pyrenoidal lumen. Invaginations of the chloroplast envelope would increase the volume of the chloroplast envelope, thereby resulting in a corresponding increase in the amount of CO_2 being stored within these pyrenoidal lumens. As a result more CO_2 would be in proximity to Rubisco within the pyrenoid and would enhance carbon fixation. This would be especially important in high pH waters, where DIC would not be in the form of CO_2 (Lee & Kugrens 1998, 2000). Having a large pool of DIC within the pyrenoid would provide a ready source of CO_2 for carbon fixation by Rubisco. This increased source of CO_2 would permit the alga to be more efficient in fixing carbon and further provide a competitive advantage over non-CER algae growing in high pH waters. In this context it is important to note that *Phaeaster pascheri* was isolated from a lake whose pH was 8.4. It is suggested that *Phaeaster pascheri var. avesiculosa* has utilized this strategy to increase the pool of CO_2 in the pyrenoid and be more competitive in high pH water.

REFERENCES

Andersen, R.A. 1982. A light and electron microscopical investigation of *Ochromonas sphaerocystis* Matvienko (Chrysophyceae): the statospores, the vegetative cell and its peripheral vesicles. Phycologia **21**: 390-398.

Andersen, R.A. 1985. The flagellar apparatus of the golden alga *Synura uvella*: four absolute orientations. Protoplasma **128**: 94-106.

Andersen, R.A. 1987. Synurophyceae classis nov., a new class of algae. Amer. J. Bot. **74**(3): 337-353.

Andersen, R.A. 1989. Absolute configuration of the flagellar apparatus of *Hiberdia magna* comb. Nov. (Chrysophyceae). Nord. J. Bot. **8**:653-669.

Belcher, J.H. 1969. A re-examination of *Phaeaster pascheri* Scherffel in culture. Br. Phycol. J. **4**: 191-197.

Belcher, J.H. & Swale, M.F. 1971. The microanatomy of *Phaeaster pascheri* Scherffel (Chrysophyceae). Br. Phycol. J. 6 (2): 157-169.

Bourrelly, P. 1957. Recherches sur les Chrysophycées. Morphologie, Phylogénie, Systématique. Revue algologique, Mémoire Hors-Série, No. **1**: 1-142.

Bourrelly, P. 1968. Les algues d'eau douce. II. Les algues jaunes et brunes. Paris.

Brett, S.J., & Wetherbee, R. 1996. Periplast development in Cryptophyceae. II. Development of the inner periplast component in *Rhinomonas pauca, Proteomonas sulcata* [haplomorph], *Rhodomonas baltica,* and *Cryptomonas ovata*. Protoplasma **192**: 40-48.

Droop, M.R. 1953. On the ecology of flagellates from some brackish and freshwater rockpools of Finland. Acta Bot. Fenn. **51**:1-52.

Gantt, E., M.R. Edwards, & Provasoli, L. 1971. Chloroplast structure of the Cryptophyceae. Evidence for phycobiliproteins within the intracellular spaces. J. Cell Biol. **48**: 280-290.

Green, J.C. 1975. The fine-structure and taxonomy of the haptophycean flagellate *Pavlova lutheri* (Droop) comb. nov. (= *Monochrysis lutheri* Droop). J. Mar. Biol. Assoc. U.K. **55**: 785-793.

Hara, Y. 1971. An electron microscopic study on the chloroplasts of the Rhodophyta. Proc. 7th Intern. Seaweed Symp.: 153-158.

Hoffman, L.R. 1961. Studies on the Morphology, Cytology and Reproduction of *Oedogonium* and *Oedocladium*. Ph.D. Dissertation. Austin. University of Texas.

Inouye, I., Zhang, X., Enomoto, M. & Chihara, M. 1990. Cell structure of an unusual chrysophyte *Phaeaster pascheri* with particular emphasis on the flagellar apparatus architecture. Jpn. J. Phycol. **38**:11-24.

Jeffrey, S. W., & Vesk, M. 1977. Effect of blue-green light on photosynthetic pigments and chloroplast structure in the marine diatom *Stephanopyxis turris*. J. Phycol. **13**:271-279.

Korshikov, A. A. 1941. On some new or little known flagellates. Arch. Protistenk. **95**: 22-44.

Kugrens, P., & Lee, R.E. 1991. Organization of cryptomonads. **In:** The Biology of Free-living Heterotrophic Flagellates. (P.J. Patterson & J. Larsen; Eds). Oxford, Claredon Press: 219-233.

Kugrens, P., Clay, B. & Aguiar, R. 2000. Ultrastructure of *Lobocharacium coloradoense*, gen. et sp. nov. (Chlorophyta, Characiosiphonaceae), an unusual coenocyte from Colorado. J. Phycol. **36** (2): 421-432.

Lee, R.E. 1974. Chloroplast structure and starch grain production as phylogenetic indicators in the lower Rhodophyceae. Br. Phycol. J. **9**: 291-295.

Lee, R.E. & Kugrens P. 1998. The ecological advantage of chloroplast E.R. The ability to outcompete at low dissolved CO_2 concentrations. Protist **149**:341-345.

Lee, R.E. & Kugrens, P. 1999. Acidity of the thylakoid lumen in plastids makes sense from an evolutionary perspective. Photosynthetica **37**(4): 609-614.

Lee, R.E. & Kugrens, P. 2000. Ancient atmospheric CO_2 and the timing of evolution of secondary endosymbiosis. Phycologia **39** (2): 167-172.

Medlin, L.K., Williams, D.M. & Sims, P.A. 1993. The evolution of the diatoms (Bacillariophyta). I. Origin of the group and assessment of the monophyly of its major divisions. Eur. J. Phycol. **28**:261-275.

Moestrup,ϕ. & Hoffman, L.R. 1973. Ultrastructure of the green alga *Dichotomosiphon tuberosus* with special reference to the occurrence of striated tubules in the chloroplast. J. Phycol. **9**: 430-437.

Patterson, J.D. & Zölffel, M. 1991. Heterotrophic flagellates of uncertain taxonomic position. **In:** The Biology of Free-living Heterotrophic Flagellates. (J. D. Patterson & J. Larsen). Oxford, Claredon Press: 427-476.

Pickett-Heaps, J.D. 1975. Green Algae, Structure, Reproduction and Evolution in Selected Genera. Sunderland, MA, Sinauer Assoc.

Preisig, H. R., Vϕrs, N. & Hällfors, G. (1991). Diversity of heterotrophic heterokont flagellates. **In**. The Biology of Free-living Heterotrophic Flagellates. (D. J. Patterson and J. Larsen.; Eds.) Oxford, Claredon Press: 361-399.

Rawat, M., Henk, M.C., Lavigne, L.L. & Morney, J.V. 1996. *Chlamydomonas reinhardtii* mutants without ribulose-1,5 bisphosphate carboxylase/oxygenase lack a detectable pyrenoid. Planta (Berl.) **189**: 263-270.

Scherffel, A. 1927. Beitrag zur Kenntnis der Chrysomonadinen. II. Arch. Protistenk. **57**: 331-361.

Skuja, H. 1948. Taxonomie des Phytoplanktons einiger Seen in Upsala, Schweden. Symbol.Bot. Upsal. **9**:3-399.

Süss, K. H., Prokhorenko, I. & Adler, K. 1995. *In Situ* association of Calvin cycle enzymes, ribulose-1,5 biphosphate carboxylase/oxygenase activase, ferredoxin NADP+ reductase, and nitrate reductase with thylakoid and pyrenoid membranes of *Chlamydomonas reinhardtii* chloroplast as revealed by immunoelectron microscopy. Plant Physiol. **107**: 1387-1397.

Vrieling, E.G., Gieskes, W.W.C. & Beelen, T.P.M. 1999. Silicon deposition in diatoms: Control by the pH inside the silicon deposition vesicle. J. Phycol. **35**:438-559.

Waaland, J.R., Waaland, S.D. & Bates, G. 1974. Chloroplast structure and pigment composition in the red alga *Griffithsia pacifica*: Regulation by light intensity. J. Phycol. **10**:193-199.

1.2. *DIACRONEMA VLKIANUM* FROM COLORADO

ABSTRACT

A strain of the prymnesiophyte *Diacronema vlkianum* from Colorado was studied by light and electron microscopy, and represents a first report of this alga from the United States. The differences between this Colorado isolate and a freshwater strain described from Europe are compared with respect to cell structure. Slight differences in cellular structure were noted, however, these differences do not merit a separate species designation for this alga. The taxonomic position of this golden-brown flagellate has been uncertain, and this study expands upon earlier findings to help clarify its taxonomic status.

1.2. *DIACRONEMA VLKIANUM* PRAUSER FROM COLORADO

INTRODUCTION

The freshwater *Diacronema* Vlk was originally observed by Vlk (1938), but was validly described as *D. vlkianum* by Prauser (1958). Vlk (1938) concluded that the organism occupied a taxonomic niche somewhat apart from typical chrysophycean algae because the organism he described had flagella without any hairs. Prauser (1958) used electron microscopy of shadow cast cells and light microscopy of living material and confirmed the absence of flagellar hairs. He also discussed the taxonomy of *Diacronema* in relation to both the Ochromonadales with heterokont flagella and the genus *Chrysochromulina* Lackey, which has equal, smooth flagella. He suggested that the genus could be placed in a new family, the Diacronemataceae, which might have originated from an ochromonad ancestor, and might possibly represent a unique line within the chrysomonads (Green & Hibberd, 1977).

Since then, the taxonomic position of *D. vlkianum* has been uncertain. However, Bourrelly (1968) included *Diacronema* in the Family Diacronemataceae, in the Order Isochrysidales, Sub-class Isochrysophycidae, based on the characteristic of the smooth flagellum, but he did not add any new structural information. Fournier (1969) confirmed the placement of this species in the Sub-class Isochrysophycidae.

Subsequently, Green & Pienaar (1977), placed *D. vlkianum* in the Class Prymnesiophyceae, Order Pavlovales, Family Pavlovaceae, on the basis of its overall similarity with other members of the Order Pavlovales (Green 1976). *D. vlkianum* most closely resembles *Pavlova lutheri* (Droop) Green (Green & Hibberd 1977) because of its strongly compressed shape, a large stigma underlying the short flagellum, and other fine structural features. However, *D. vlkianum* lacks scales and the characteristic dense bodies found on the long flagellum of all other species in the Family Pavlovaceae.

This study was undertaken, initially, to determine if the isolate represented *Boeklovia* or a new species, to provide new information on its surface features, and to provide additional information for proper classification of this alga.

RESULTS

Light microscopy.

Diacronema cells are flattened in an anterior-posterior direction. In anterior view the cells are slightly heart-shaped to rounded (Figs. 20-22). Cell length can vary from 15 to 18 μm, and cell width can range from 13 to 20 μm. Two heterokont flagella arise from the anterior or concave side of the cell where there is a slight depression. With careful observation both flagella can be seen with the light microscope (Figs. 20,22). An olive-yellow parietal chloroplast, which appears as two lobes or two chloroplasts is visible in the cell but a stigma is not evident (Fig. 20).

Scanning electron microscopy

SEM confirms that cells typically are compressed and have an anterior depression and the various cell shapes are illustrated in Figures 23-27. In addition, a short haptonema measuring 0.5 μm in length is evident, and it arises from an anterior concave depression between both flagella (Figs. 23-26). The two flagella are unequal. The long flagellum measures 8 μm in length and the short flagellum 5 μm in length (Fig.27).

Transmission electron microscopy.

Shadow-casting preparations

Shadowed preparations of whole cells demonstrated that the long flagellum bears tubular hairs. Tubular hairs consist of a short shaft, which ends with two terminal filaments (Figs. 28-29). Both flagella have narrow terminal regions (Figs. 28,30). The short flagellum appears smooth and lacks hairs. Shadowed cells also exhibited a thick, external mucilage (Figs. 28-30).

Ultrastructure of Sectioned Cells

The flattened shape of the cells also is evident in longitudinally sectioned material. Furthermore, the mitochondrion is situated near the basal bodies and the chloroplast is located in the posterior and lateral regions of the cell (Figs. 31,33, 41). The chloroplast may have one or two lobes (Figs. 20,31,33,41). The chloroplast lacks a

pyrenoid, but there is a small stigma (Figs. 31,33,38-41) that is not set in its location in the chloroplast.

A conspicuous feature of every cell is the membrane bound space in the peripheral or cortical cytoplasm. Each cell has a dilated space that probably represents a modified peripheral endoplasmic reticulum (Figs. 31-41). Whether this is an artifact of fixation in *Diacronema* is not known. Quick freezing and freeze fracture were attempted to resolve this question but information that could have resolved this question could not be obtained.

The two flagella and the vestigial haptonema are inserted in the anterior of the cell. The haptonema forms a small papilla between the flagella (Figs. 35,42). The angle between the flagellar basal bodies is approximately 90^0 (Fig.34-35,42), which is unusual for prymnesiophytes, but is characteristic for chrysophytes. A proximal expanded part or "wing" was observed in the short flagellum, on the side adjacent to the cell, which represents a flagellar swelling and contains some granular dense material (Figs.34). The swelling was associated with the stigma whenever the stigma occurred on that side of the chloroplast. The transitional region consists of a single transverse partition located slightly above the basal body in both flagella (Figs. 35,37,41-42). A transverse partition also is present in the haptonema (Fig. 35,39).

Chloroplast

The two lobes of the chloroplast are evident along the dorsal (convex) surface of the cell (Figs.31,33-34,38,41). The lobes are connected by a narrow bridge (Fig. 33,41).

Grouping of the lamellae is usually three, but with crowding there may be more thylakoids appressed to each other, which creates large central areas of lamella-free stroma. Pyrenoids were not observed in this alga.

Nucleus/ Golgi apparatus/ Mitochondrion

The position of the nucleus is variable (Figs. 31-33,35,38,40). It may occur in the ventral lobe of the cell just above the middle region of the chloroplasts (Figs. 31,38), in the center, beneath the concave depression (Figs. 33,35), or near the short flagellum (Figs. 34,39-41). A chloroplast endoplasmic reticulum that is continuous with the nuclear envelope surrounds the chloroplast (Fig. 32).

A single large Golgi body is anterior to the nucleus and lateral to the basal bodies (Figs. 31,39-41). Since scales are not produced by these cells, the function of the Golgi body is unknown.

Several serial sections indicate that the mitochondrion is single and branched. It is located in the anterior of the cell near to flagellar bases (Figs. 31,33-34,36-37). Cristae are tubular.

Stigma

The location of the stigma varies. It may be located in the anterior portion of the chloroplast (Figs. 38,40-41), it may be in the posterior side of the chloroplast (Figs. 31,33) or it may be in the proximity to the short flagellum (Figs. 39,41). It usually consists of a single layer of ovoid stigmatic granules (Figs.31,33,38-41).

Vacuoles

Contractile vacuoles were not observed in *Diacronema*, however, one or two small vacuoles often are near the Golgi body, or posterior to the nucleus (Figs. 32,41). The content and function of these vacuoles are unknown.

Flagellar apparatus.

The axoneme of the long flagellum has a slight lateral swelling (Figs.35, 42). The short flagellum has a proximal "wing" on the side adjacent to the cell (Figs.34,42), and contains some dense material (Fig.34) on the side toward the cell body.

Both flagella have a transition region with a single transverse partition located above the level of the cell surface. This partition consists of a central electron-translucent region (Figs. 35,37,41-42). At the level of this partition the flagellar membrane of the long flagellum is constricted and is linked to the axoneme by amorphous electron-dense material (Figs. 35, 41).

Basal bodies are connected by a cross-striated fibrous band (Figs. 35-36,39-40,42). In addition, one set of microtubules extends from the short flagellum to the right ventral lobe of the plastid (Figs.34-35,37).

The haptonema is a non-coiling, short vestigial protrusion (0.9 – 1.2 μm), and is simple in structure. It consists of an evagination of the plasmalemma and tubular endoplasmic reticulum that extends into the protrusion (Figs.34-36,39,42). Microtubules were not observed in haptonemata.

The peripheral endoplasmic reticulum is near the flagellar bases. In this region there is a ring of material that surrounds the flagellar bases and haptonema (Figs. 32,37,39-40), perhaps to provide additional support to the cell (Green & Hibberd 1977).

DISCUSSION

This study has provided the first SEMs that illustrate the surface features of *Diacronema*. Since SEM is becoming increasingly important in phytoplankton identification, these SEMs should provide valuable basic information for future reference on chrysophyte identification.

The ultrastructure of *Diacronema* sp. from Colorado is compared to the *Diacronema vlkianum* Prauser (Green & Hibberd 1977) and similarities and differences are shown in Table 1. The main differences between *D. vlkianum* from Colorado and *D. vlkianum* Prauser (Green & Hibberd 1977) from Europe are the presence of the tubular hairs on the long flagellum, which consist of a bifurcated shaft ending with two terminal fibrils; the material in the peripheral endoplasmic reticulum that surrounds the flagellar and haptonemal bases; the absence of contractile vacuoles; and the variable location of the stigma in the chloroplast. None of these features were reported previously (Green & Hibberd 1977, Fournier 1969) for *Diacronema vlkianum* Prauser from freshwater/marine habitats.

Fournier (1969) investigated a marine *D. vlkianum,* and he did not find flagellar hairs. He suggested that if flagellar hairs were present in *Diacronema,* then this organism would have to be considered a member of the Chrysophyceae, but the absence of hairs in

his alga was an indication of a strong affinity with the Haptophyceae (Christensen 1962) despite the absence of a haptonema in his material. Fournier (1969) also reported that a small reddish-orange body (stigma) could be observed anywhere the ventral surface, usually in proximity to the plastid and could be present in both lobes, which differed from Green & Hibberd's (1977) description. Conversely, Green & Hibberd (1977) reported the presence of non-tubular hairs on both flagella, a pit or canal penetrating the cell beneath the long flagellum where the contractile vacuole discharges into the canal, and the ventral position of the stigma underlying the short flagellum in one lobe of the chloroplast. They also described a denticulate structure in the flagellar swelling near the stigma. The findings in this study suggest that *Diacronema* is a unique chrysophyte.

Green & Hibberd (1977) compared also the structure of the flagellar transitional region between *Prymnesium* and *Diacronema* and they reported that *Diacronema* differs from the general pattern because there was only a single partition across the axoneme, which presumably corresponds to the most distal of the two in *Prymnesium*. This also was the case for *Diacronema* from Colorado.

The expanded PER observed in all cells of *Diacronema* from Colorado might represent a modified chrysolaminarin vesicle. As a cell flattens in the anterior- posterior direction, the posterior vesicle also could become compressed and displaced along the periphery of the cell. Biochemical studies will have to be performed in order to determine whether this is the case. Furthermore, freeze fracture studies also should be conducted to determine whether the putative dilated PER is an artifact. Several attempts at freezing fracturing these cells failed to provide this information.

Diacronema vlkianum was assigned to the Class Prymnesiophyceae *sensu* Hibberd (1976) (= Haptophyceae *sensu* Christensen 1962), Order Pavlovales and Family Pavlovaceae, on the basis of its overall structural similarity with other members of this group (Green 1976). With its strongly compressed shape, the large stigma underlying the posterior flagellum and other fine structural features, it resembles *Pavlova lutheri* (Droop) Green (Green & Hibberd 1977). However, significant differences include the complete absence of dense bodies found on the anterior flagellum and cell body of most species in the family. *P.lutheri* differs from *D. vlkianum* by the denticulate structures located below the axoneme and associated diffuse material in the region of the flagellum that lies adjacent to the eyespot. This structure so far is unique, and has not been recorded for the Prymnesiophyceae or any other class of algae (Green & Hibberd 1977). Based on these differences, Green & Hibberd (1977) considered these to be of sufficient taxonomic value to retain *Diacronema* and *Pavlova* as separate genera. In *Diacronema* from Colorado, a similar denticulate structure was observed below the axoneme of the short flagellum as well as dense material in this region.

The differences presented in this study for *D. vlkianum* might indicate valid specific differences that could justify the formation of a new species. The overlapping ordinal and class features need to be re-evaluate based on these differences. However, additional studies on its biochemistry, molecular biology and microanatomy should be undertaken to confirm whether there differences justify a new species or classification.

REFERENCES

Bourrelly, P. 1968. *Les algues d'eau douce, 2: Algues Jaunes et Brunes.* Paris. Boubée et Cie.

Christensen, T. 1962. Alger. **In**: *Bötanik* Bd. 2, *Systematisk Botanik.* M. L. T.W. Böcher, and T. Sorensen. Munksgaard, Copenhagen. **Nr. 2:** pp. 1-178.

Fournier, R.O. 1969. Observations on the flagellate *Diacronema vlkianum* Prauser (Haptophyceae). Brit. Phycol. J. **4**: 185-190.

Green, J.C. 1976. Notes on the flagellar apparatus and taxonomy of *Pavlova mesolychnon* Van Der Veer, and on the status of *Pavlova* Butcher and related genera within the Haptophyceae. J. Mar. Biol. Assoc. U.K. **56**: 595-602.

Green, J.C. & Hibberd, D.J. 1977. The ultrastructure and taxonomy of *Diacronema vlkianum* (Prymnesiophyceae) with special reference to the haptonema and flagellar apparatus. J. Mar. Biol. Assoc. UK **57**: 1125-1136.

Green, J.C. & Pienaar, R.N. 1977. The taxonomy of the order Isochrysidales (Prymnesiophyceae) with special reference to the genera *Isochrysis* Parke, *Dicrateria* Parke and *Imantonia* Reynolds. J. Mar. Biol. Assoc. UK **57**: 7-17.

Hibberd, D.J. 1976. The ultrastructure and taxonomy of the *Chrysophyceae* and *Prymnesiophyceae (Haptophyceae)*: a survey with some new observations on the ultrastructure of the *Chrysophyceae*. Bot. J. Linn. Soc. **72**: 55-80.

Prauser, H. 1958. *Diacronema vlkianum*, eine neue Chrysomonade. Archiv. für Protistenk. **90**: 117-128.

Vlk, W. 1938. Über den Bau der Geissel. Archiv. für Protist **90**: 448-448.

1.3: *HYMENOMONAS* FROM COLORADO

ABSTRACT

An isolate of *Hymenomonas* Stein from Colorado is described using light and electron microscopic techniques. Cells usually occurred in a non-motile stage, and these were covered by uncalcified and calcified scales, with the latter known as coccoliths. The coccoliths are distributed in a single layer over the uncalcified scales, and each coccolith possesses a basal-plate that is unmineralized. The calcified portion consists of an average of 11 calcium carbonate elements situated between the margin and the raised rim of the base-plate scale. Several layers of unmineralized scales lie beneath the coccolith layers. These are oval and rounded with ornamentations. The ornamentation on the proximal surface consists of radiating fibrils while the distal surface has of concentric fibrils. Four plastids generally are present per cell, each with a bulging pyrenoid that is traversed by paired thylakoids. A single anterior Golgi apparatus often contains developing scales. Flagella and haptonemata were not observed in this isolate since the flagellate stage was rare. The ultrastructure of palmelloid cells is compared with the flagellate *H. roseola*.

1.3. *HYMENOMONAS* FROM COLORADO

INTRODUCTION

Coccolithophorids are a major group of marine phytoplankton whose cell covering consists of minute calcite scales called coccoliths. The majority of species are marine, and inland forms are rare. They are particularly important in tropical and temperate waters. They are the most prolific calcium carbonate producing organisms on earth (Tappan 1980), and their coccoliths are useful in determining the biostratigraphy of sediments from the Jurassic through the Pleistocene (Tappan 1980; Holligan, Viollier, Harbour & Champagne- Phillippe 1983; Perch-Nielsen 1985; Groom & Holligan 1987). However, a number of genera consistently occur in near-shore waters. These are mostly representatives of the Family Hymenomonadaceae and include the genera *Hymenomonas* (Braarud, 1954; Lecal 1965; Manton & Peterfi 1969; Pienaar 1976; Vaulot & Olson, 1987), *Cricosphaera* (Manton & Leedale 1969; Blackwelder, Weiss & Wilbur 1976; Beuffe 1979; Gayral & Fresnel 1979; Pienaar 1969a, 1969b), *Pleurochrysis* (Leadbeater 1971; Gayral & Fresnel, 1983; Inouye & Pienaar 1985; Johansen, Doucette, Barclay & Bull 1988), *Ochrosphaera* (Inouye & Chihara 1980; Lefort 1975), and *Cruciplacolithus* (Johansen, Doucette, Barclay & Bull 1988).

Hymenomonas roseola Stein is the most commonly reported inland species and is freshwater. Another freshwater species is *Pleurochrysis* (Johansen, Doucette, Barclay &

Bull 1988). Marine species often have alternate stages in their life history but alternate life stages have not been described for freshwater representatives. The alternative stages in marine species are benthic stages and flagellate stages, and thereby demonstrate a heteromorphic life history (Jordan & Kleijne 1993).

Coccolithophorids (algae with coccoliths) belong to the Division Haptophyta (Prymnesiophyta). However, they were originally assigned to the Order Isochrysidales by Papenfuss (1955). Later Round (1973) transferred then to the Order Prymnesiales. Subsequently, Hibberd (1976) divided the prymnesiophycean algae into four orders, the Pavlovales, Isochrysidales, Prymnesiales and Coccolithales. Since the criteria used in the separating orders are inconsistent, Jordan & Green (1994) retained only two orders. These orders are Pavlovales (in the sub-class Pavlovophycidae) and the Prymnesiales (in the sub-class Prymnesiophycidae), and this scheme is followed in this chapter.

Several coccolithophorids have now been studied in more detail, and some of the motile forms possess a haptonema (Parke, Manton & Clarke 1955, Braarud 1960, Manton & Peterfi 1969, Pienaar 1976, Johansen et al 1988), while in others have either a reduced haptonema or, it is absent (Manton 1967; Green & Pienaar 1977). During studies on algal diversity and characterization of freshwater chrysophytes in Rocky Mountain Lakes from Colorado and Wyoming, a coccolithophorid was isolated from Cowdrey Lake. After an initial ultrastructural examination, differences in structure between this coccolithophorid and *Hymenomonas roseola* were noted. This prompted a more detailed investigation, and this chapter provides detailed descriptions of this freshwater species of *Hymenomonas* from Colorado. General cell comparisons with *H. roseola* also are provided.

RESULTS

Light Microscopy

Cells are aflagellate and non-motile. They are spherical to oval in shape, and they form clusters of cells. Each cell measures 15-22 μm in length and 11-20 μm in width. Neither flagella nor haptonemata are present in vegetative cells. The cells are covered by a layer of coccoliths that are readily visualized with light microscopy. Most cells possess four chloroplasts.

Scanning electron microscopy

Cells and flagella and haptonemata are covered with a single layer of coccoliths (Fig. 43-50. A transparent, delicate membrane is lies over the coccolith layer (Fig. 45). When the membrane ruptures, coccoliths are exposed (Figs. 43-45). An air-dried cell (Figure 46) shows that coccoliths cover the entire cell. The majority of coccoliths are oval, sometimes round in shape, and they have a "crown-like" or coronate structure (Figs. 47-50). Their sizes vary slightly. Several detached coccoliths remained near the cells (Figs. 47-48).

Transmission electron microscopy

Shadow- cast preparation

Cells have a single layer of coccoliths (Fig. 51) and they are similar in structure to those described previously for *Hymenomonas roseola* (Manton & Peterfi 1969, Braarud 1999).

Coccoliths are 2.0-2.5 μm in length, 1.5-2.0 μm in width and 1.5 μm in height and each possesses an elliptical, unmineralized basal-plate scale (Fig.52). Each coccolith element is attached to the outer face of an unmineralized plate.

Unmineralized scales have two distinct surfaces. The pattern on the proximal surface (i.e. the one facing the cell) consists of radiating fibrils, while the distal surface (i.e. the one facing away from the cell) shows a pattern of somewhat concentric fibrils (Fig. 52). These scales vary from 0.8-2.8 μm in length to 0.5-2.5 μm in width and have an inflexed rim on both surfaces.

The coccoliths average 11 calcium carbonate elements that are situated between the margin and the raised rim of the base-plate scale (Figs. 53-54). Each coccolith has a characteristic "crown-like" shape that consists of a basal region, an upright vertical region, and a distally pointed region. In lateral view, the distal flange is more pronounced than the proximal flange. The distal flange is pointed thus the coccoliths appear jagged. The parallel vertical sides and jagged top are characteristics of the tremalith type of coccolith. The average height of a coccolith is about 0.9 μm. Individual calcium carbonate elements have a "fence-like"structure (Figs. 53-54) with parallel bands external to the other elements. The presence of a continuous ring of calcium carbonate supporting individual elements (Fig. 53) also was observed.

Several layers of rimless, circular sometimes oval, unmineralized scales occur immediately external to the cell membrane and beneath the single layer of coccoliths (Fig. 54). Both the proximal and distal surfaces are covered with a system of concentric fibrils. The average dimensions of these scales are 11.0 x 9.5 μm.

Transmission electron microscopic observations

Cells are surrounded by several layers of unmineralized scales and one layer of coccoliths (Fig. 55). The coccoliths appear as rimmed scales distributed in a single layer (Figs. 55,57-60), and, when viewed from the top, each coccolith is composed of on average of 11 calcium carbonate elements (Fig. 56). In addition to these layers there is a columnar fibrillar deposit (Figs. 55,57,60) and a network of tubular material external to the plasma membrane (Figs. 55,57,60).

Cells usually possess four parietal chloroplasts, with bulging pyrenoids (Figs.55,59). Three of the four chloroplasts are evident in Figure 55. The plastids are somewhat lobed and surrounded by a layer of chloroplast endoplasmic reticulum (Fig. 57). The pyrenoids protrude toward the inside of the cell (Fig. 59). Several paired thylakoids traverse each pyrenoid (Figs. 55,57-59).

The nucleus is located in the posterior end of the cell, immediately behind the anterior Golgi body (Figs. 58-59). The single mitochondrion probably is reticulate and is situated towards the periphery of the cell. Mitochondria have tubular cristae (Figures 55, 58-59).

Contractile vacuoles were not observed; however, there are some vesicles in the cell that possess fibrillar elements resembling those found in the columnar layer (Figs. 55, 59-60).

DISCUSSION

This study has provided additional information on a freshwater *Hymenomonas*, which represents the first report of this genus from the United States. The coccolith morphology suggests that this is *H. roseola*. Whether the stage investigated during this study represents an alternate phase or the only phase was not determined and awaits further study. A few flagellate stages were observed; however, the cells were rare and there was not enough material for ultrastructural study. It was observed that one cell divides to form four zoospores within a parent cell and in some other instances, the parent cells formed flagella and became large zoospores. Obviously, the interrelationships among these different stages needs to be resolved before this isolate can be assigned to an appropriate genus or species.

Hymenomonas from Colorado is compared to *H. roseola* in Table 2. A continuous outer investment, which is a delicate membrane or "skin" that completely covered the coccoliths and the unmineralized scales was observed in *Hymenomonas* from Colorado. Manton & Leedale (1963), Klaveness (1973) and Rowson & Leadbeater (1986) had found a similar structure in *Crystallolithus hyalinus*, *Calyptrosphaera sphaeroidea* J. Schiller and *Coccolithus pelagicus* respectively. However, this structure

was not observed and described in *Hymenomonas roseola* by Manton & Peterfi (1969) and its function remains unknown.

The coccoliths and scales of *Hymenomonas* from Colorado superficially resemble those of the *H. roseola*. However, there were significant differences in scale morphology between *Hymenomonas roseola* (Manton & Peterfi 1969) and *Hymenomonas* examined in this study. The coccolith base-plate of *H. roseola* has a characteristic boat-shape with a circular outline of the coccolith base, but there is a discontinuity in the contour of the upper edge. This peculiar structure was not noted in the coccolith base-plate of *Hymenomonas* from Colorado. Rather, the base plate is uniformly oval and has a continuous margin. In both organisms the base-plates are similar, since they have marked bilateral symmetry and the coccoliths in both species have an irregular jagged distal region. A significant difference could be observed in the flanging of the proximal and distal region of the calcified elements. In *H. roseola* the coccoliths are composed of 13 calcified elements, and the upper parts are irregularly or obliquely cut off and the coccoliths resemble a crown with irregular indentations. In *Hymenomonas* from Colorado coccolith structure is more regular, and it is composed of 11 elements, all having regular indentations in the distal region.

In *H. roseola,* the unmineralized scales that underlie the single coccolith layer are different from those observed in *Hymenomonas* from Colorado. In *Hymenomonas* from Colorado these scales vary in shape from round to oval. In the proximal and distal surfaces, they have the same concentric fibrillar patterns. Conversely, in *H. roseola* scales

usually are oval with the proximal surface having radiating ridges and distal surface having concentric fibrils. Both scale types have a thin inflexed rim.

The main differences between the *H. roseola* and *Hymenomonas* from Colorado are the absence of flagella and haptonemata and the number of chloroplasts per cell. In *H. roseola* there is a short, club shaped haptonema and flagella are constantly present, emerging from a depression in the cell surface (Manton & Peterfi 1969). The haptonema is highly reduced and is covered by small rimless scales. It is attached to the cell by a slender stalk. The flagellar and the haptonemal base were described as a flagellar root system of an unusually complex kind. Since flagella were not observed in *Hymenomonas* from Colorado, comparisons were not possible.

The two parietal chloroplasts of *H. roseola* (Manton & Peterfi 1969) have parallel, thylakoids grouped in twos, threes and fours, that pass through the pyrenoid. Although the same pattern was observed in *Hymenomonas* from Colorado, most cells observed had four chloroplast per cell (Manton & Peterfi 1969).

Since scale morphology is a primary diagnostic feature for species designation in prymnesiophytes, the *Hymenomonas* examined in this study could be erected as a new species based on the mineralized scales. However, additional features of its reproduction need to be determined before such a proposed is made.

REFERENCES

Blackwelder, P.L., Weiss, R.E., & Wilbur, K.M. 1976. Effects of calcium, strontium, and magnesium on the coccolithophorid *Cricosphaera (Hymenomonas) carterae* I. Calcification. Mar. Biol. **34**: 11-16.

Beuffe, H. 1979. Une Coccolithophoraceae marine nouvelle: *Cricosphaera gayraline* nov. sp. Protistologica **14**: 451-458.

Braarud, T.G. 1954. Coccolith morphology and taxonomic position of *Hymenomonas roseola* Stein and *Syracosphaera carterae* Braarud & Fagerland. Nytt. Mag. Bot. **3**:1-4.

Braarud, T.G. 1960. On the coccolithophorid genus *Cricosphaera* n. gen. Nytt. Mag. Bot., **8**:211-212.

Gayral, P. & Fresnel J. 1979. Révision du genre *Hymenomonas* Stein - à propos de l'étude comparative de deux Coccolithacees: *Hymenomonas globosa* (Magne) Gayral et Fresnel et *Hymenomonas lacuna* Pienaar. Rev. Algol. , N.S. **14**: 117-125.

Gayral, P. & Fresnel J. 1983. Description, sexualité et cycle de developpement d' une nouvelle Coccolithophoracée (Prymnesiophyceae): *Pleurochrysis pseudoroscoffensis* sp. nov. Protistologica **19**: 245-261.

Green, J.C. & Pienaar, R. N. 1977. The taxonomy of the order Isochrysidales (Prymnesiophyceae) with special reference to the genera *Isochrysis* Parke, *Dicrateria* Parke and *Imantonia* Reynolds. J. Mar. Biol. Assoc. U.K. **57**: 7-17.

Groom, S. B & Holligan, P.M. 1987. Remote sensing of coccolithophore blooms. Adv. Space Res. **7 (2)**: 72-78.

Hibberd, D.J. 1976. The ultrastructure and taxonomy of the *Chrysophyceae* and *Prymnesiophyceae (Haptophyceae)*: a survey with some new observations on the ultrastructure of the *Chrysophyceae*. Bot. J. Linn. Soc. **72**: 55-80.

Holligan, P.M., Viollier, M., Harbour, D.S. & Champagne-Phillippe. 1983. Satellite and ship studies of coccolithophore production along a continental sheld edge. Nature **304(5924)**: 339-342.

Inouye, I. & Chihara M. 1980. Laboratory culture and taxonomy of *Hymenomonas*

coronata and *Ochrosphaera verrucosa* (Class Prymnesiophyceae) from the Northwest Pacific. Bot. Mag. Tokyo **93**: 195-208.

Inouye, I. & Pienaar R.N. 1985. Ultrastructure of the flagellar apparatus in *Pleurochrysis* (Class Prymnesiophyceae). Protoplasma **125**: 24-35.

Johansen, J.R., Doucette, G.J.B., Barclay, W.R. & Bull, J.D. 1988. The morphology and ecology of *Pleurochrysis carterae* var. *dentata* var. nov. (Prymnesiophyceae), a new coccolithophorid from an inland saline pond in New Mexico, USA. Phycologia **27 (1)**: 78-88.

Klaveness, D. 1973. The microanatomy of *Calyptrosphaera sphaeroidea*, with some supplementary observations on the motile stage of *Coccolithus pelagicus*. Norw. J. Bot. **20**:151-162.

Leadbeater, B.S.C. 1971. Observations on the life history of the haptophycean alga *Pleurochrysis scherffelli* with special reference to the microanatomy of the different types of motile cells. Ann. Bot. **35**: 429-439.

Lecal, J. 1965. Un novel *Hymenomonas: H. prenantii* n.sp. (coccolithophoridés). An. de Limn. **1**: 156-162.

Lefort, F. 1975. Etude de quelques Coccolithophoracees marines rapportées aux genres *Hymenomonas* et *Ochrosphaera*. Cah. Biol. Mar. **16**: 213-233.

Manton, I. & Leedale, G.F. 1963. Observations on the fine structure of *Prymnesium parvum* Carter. Arch. Mikrobiol. **45**: 285-303.

Manton, I. 1967. Further observations on the fine structure of *Chrysochromulina chiton* with special reference to the haptonema, "peculiar" Golgi structure and scale production. J. Cell Sci. **2**: 265-272.

Manton, I. & Leedale, G.F. 1969. Observations on the microanatomy of *Coccolithus pelagicus* (Wall.) Schiller and *Cricosphaera carterae* (Braarud & Fagerland) Braarud with special reference to the origin and nature of coccoliths and scales. J. Mar. Biol. Ass. U.K. **1969**: 1-16.

Manton, I. & Peterfi, L.S. 1969. Observations on the fine structure of coccoliths, scales and the protoplast of a freshwater coccolithophorid, *Hymenomonas roseola* Stein, with supplementary observations on the protoplast of *Cricosphaera carterae*. Proc. R. Soc. London Ser. B **172**: 1-15.

Papenfuss, G.F. 1955. Classification of the algae. A century of progress in the natural sciences. San Francisco, California Academy of Sciences.: 115 - 224.

Parke, M., Manton, I. & Clarke, B. 1955. Studies on marine flagellates. II. Three new species of *Chrysochromulina*. J. Mar. Biol. Assoc. U.K. **34**: 579-609.

Perch-Nielsen, K. 1985. Cenozoic calcareous nannofossils. Plankton Stratigraphy. H. M. S. Bolli, J.B. and Perch-Nielsen, K. eds. Cambridge: 427-554.

Pienaar, R.N. 1969a. The fine structure of *Cricosphaera carterae*. I. External morphology. J. Cell Sci. **4**: 561-567.

Pienaar, R.N. 1969b. The fine structure of *Hymenomonas (Cricosphaera) carterae*. II. Observations on scale coccolith production. J. Phycol. **5**: 321-331.

Pienaar, R.N. 1976. The microanatomy of *Hymenomonas lacuna* sp. nov. (Haptophyceae). J. Mar. Biol. Ass. U.K **56**: 1-11.

Round, F.E. 1973. The biology of the algae. London, Edward Arnold.

Rowson, J.D. & Leadbeater, B.S.C. 1986. Calcium carbonate deposition in the motile (*Crystallolithus*) phase of *Coccolithus pelagicus* (Prymnesiophyceae).

Tappan, H. 1980. The paleobiology of Plant Protists. San Francisco, W. H. Freeman & Co.

Vaulot, D. & Olson, R.J. 1987. Cell-cycle response to nutrient starvation in two phytoplankton species, *Thalassiosira weissflogii* and *Hymenomonas carterae*. Mar. Biol. **95**: 625-630.

CHAPTER 2

PRYMNESIUM WYOMINGI SP.NOV. AND *CHRYSOCHROMULINA ASQUAMOSA* SP. NOV. TWO NEW SPECIES OF PRYMNESIOPHYTES FROM COLORADO AND WYOMING.

ABSTRACT

The ultrastructure and systematics of two new freshwater prymnesiophytes are described. An isolate of *Prymnesium* from a freshwater lake in Wyoming was examined by light and electron microscopy, and it represents the first isolate of this genus from a freshwater habitat. Cells vary in morphology, ranging from elongate to subspherical to spherical, with a rounded posterior end and an obliquely truncate anterior end. Two equal or subequal flagella and a short, non-coiling haptonema arise subapically from a groove or depression in the truncate portion of the cell. Two types of organic scales, which are differentiated by size, shape, and pattern of ornamentation cover the cell. Chloroplasts vary in number, ranging from one to seven. Each chloroplast has a pyrenoid that is partially traversed by doublet thylakoids.

The second new prymnesiophyte is *Chrysochromulina asquamosa* sp.nov. *C. asquamosa* was collected from five different lakes in Colorado. It is described as a new species because it lacks scales. Cells are obovate, spherical, and usually with two chloroplasts, although cells with three to five chloroplasts are common. The long haptonema (15 μm) and the two subequal flagella are inserted subapically on the concave face of the obovate cell. The flagella lack hairs but both flagella have narrow terminal portions. *C. asquamosa* resembles *C. parva* and *C. breviturrita,* but *C. asquamosa* differs from both in having smaller cells and lacking scales. Since scale

morphology is the most important criterion for delineating *Chrysochromulina* species, this character provides the basis for proposing a new species.

PRYMNESIUM WYOMINGI SP. NOV. AND *CHRYSOCHROMULINA ASQUAMOSA*, SP. NOV. TWO NEW SPECIES OF PRYMNESIOPHYTES FROM COLORADO AND WYOMING

INTRODUCTION

The Division Haptophyta (Jordan & Green 1994) contains a single class, the Prymnesiophyceae (Hibberd 1976). Representatives from the Class Prymnesiophyceae are unicellular or colonial chrysophytes and generally are of marine plankton. The cells are yellow-brown, and the chloroplasts contain chlorophyll *a* & two or three different chlorophyll *c*'s (Jeffrey 1990). There have been a few records of prymnesiophytes from freshwater and terrestrial environments (Green & Jordan 1994), specifically *Chrysochromulina parva* Lackey emend. Parke et al. (Parke, Lund & Manton 1962), *Hymenomonas roseola* Stein (Manton & Peterfi 1969), *Corcontochrysis noctivaga* Kalina (Kalina 1970, 1975), *Diacronema vlkianum* Prauser (Green & Hibberd 1977), *Pleurochrysis carterae* (Johansen, Doucette, Barclay & Bull 1988) and *Chrysochromulina breviturrita* (Nicholls 1978).

One of the unique characteristics of this group is the haptonema, a filiform organelle that is located between two flagella. The length of the haptonema is used as a genus or species characteristic (Leadbeater, 1972). However, some coccolithophorids,

such as *Emiliana huxleyi* lack a haptonema or it is rudimentary, as in *Diacronema*, *Isochrysis* and *Dicrateria* (Green & Pienaar 1977).

Cells of most prymnesiophytes are covered with unmineralized, carbohydrate scales, whose structure is species specific (Green, Hibberd & Pienaar 1982). In addition, the organic scales may also be partially calcified to form scales called coccoliths, and delineate the group known as coccolithophorids (Kleijne 1993).

In contrast to the rapid advances in knowledge about marine Prymnesiophyceae (Moestrup 1979), there is a lack of information on freshwater species. Small, fragile forms are present in this group, and they rupture readily when placed in fixation. As a result, they have been overlooked or are poorly represented in phytoplankton counts.

During surveys of phytoplankton from several Rocky Mountain Lakes two new freshwater members of the *Prymnesium* and *Chrysochromulina* were discovered. Since both differ from any described species, this chapter provides data in support of proposing new species for both genera.

RESULTS

DIAGNOSIS

Prymnesium wyomingi sp. nov.

Aguiar,R & Kugrens,P.

Cellulae natants 8-10 μm longae, 8 - 9.5 μm latae, subsphericae vel ovales, anteriore parte oblique truncata, posteriore rotundata vel raro contracta. Flagella duo aequalia vel subaequalia heterodynamica ad apices attenuata (6.5-11 μm) longae; haptonema flexible sed spiram non formans (2-3 μm) in tenuem depressionem apicaliter insertae anteriore parte oblique truncata. Squamae coporis duarum formarum typus. Typus 1: squamae ovales (c. 0.4 x 0.4 μm) aut marginibus inflexis, angustum, area centralis inflatum, aspectus proximalis atque distalis cum fibrillas radiantes. Typus 2: squamae proximales ellipticae (c. 0.2 – 0.4 μm) unum, duo strata, aspectus proximales cum fibrillas radiantes, marginem erectum, aspectus distalis marginem erectum et fibrillas concentricorum annulorum. Chloroplasti variabilis numerous, laterales atque parietales, fulvi, lobati vel dissecti, uterque pyrenoids immersa. Nucleus centralis inter chloroplastos, corpusculum Golgianum parabasale. Materia penaria in vacuola postica condita, "chrysolaminarin". Corpuscula mucigera peripherica priesente.

Swimming cells 8-10 μm long x 8- 9.5 μm wide; mostly sub-spherical to elongate with more or less parallel sides and rounded posterior end; anterior end obliquely truncate. Two equal or sub-equal heterodynamic flagella, 6.5-11 μm in length, and a short, 2-3 μm, flexible non-coiling haptonema arising sub-apically from a groove or depression in the truncate face. Cells covered by two scale types. *Type1* scales are rounded (c. 0.4 x 0.4 μm) with a narrow inflexed rim, a central thickening on the distal faces, and a radial fibrillar pattern on proximal and distal faces. *Type 2* scales are proximal elliptical (c. 0.2-0.4 μm) in one or two layers, the proximal surface with

radiating fibrils and a tall upright rim, the distal surface with a tall upright margin and concentric fibrils. Chloroplasts variable in number, parietal, golden-brown, lobed or dissected, each with a protruding pyrenoid. Nucleus is central, and Golgi apparatus is parabasal. Reserve metabolite is chrysolaminarin and is contained in a large posterior vesicle. Peripheral muciferous bodies may be present in the space between the nucleus and chrysolaminarin vesicles.

Light microscopy

Cultures of *Prymnesium wyomingi* are golden-brown, but may darken slightly with age. The elongated, ovoid cells vary between 8-10 μm in length and 7- 9.5 μm in width. Cells typically have a truncated apex and a posterior end that is rounded, and somewhat pointed or angular (Figs. 1-3). In old cultures, cells tend to be more rounded (Fig. 3). The short flagellum average 8.0 μm in length, and the long flagellum average 11 μm in length. The non-coiling haptonema average 3.0 μm in length. The flagella and haptonema are subapically inserted in the anterior portion of the cell (Fig.1). Cells contain two or more chloroplasts that are oriented parallel to the axis of the cell (Figs.2-3). A large muciferous body often is present, particularly in older cells (Figs. 1-3).

Scanning electron microscopy.

Most cells are elongate, oval, or rounded (Figs.4-12). The two subequal flagella and the haptonema arise from a deep subapical depression, or a groove that is approximately 2.5 μm in depth (Figs. 5, 8-10). The two flagella have narrow terminal

portions (Figs. 4-7, 9-10), probably formed by the inner pair of axonomal microtubules extending into this region. The haptonema apparently is able to bend slightly (Figs.6-8,11-12).

Transmission electron microscopy

Shadow-cast preparations

Both flagella have narrowed terminal regions (Fig. 13), and both flagella and haptonema bear two rows of delicate, long, non-tubular hairs (Figs. 17-18). The hairs are approximately 1.3 μm in length.

The cell surface, including the depression where the flagella and haptonema are inserted, is covered by unmineralized scales that are arranged in several layers (Figs.13, 15,17). The scales are rounded or oval to elliptical. All scales have a radial fibrillar pattern extending to the periphery on the proximal face. However, there is a major shape difference between the two types of scales. The most proximal scales are rounded, generally measuring 0.4 μm in diameter. In the proximal face, these scales are flattened with a central thickening and radiating ridges extending to the periphery. In the distal face, they have concentric ridges and an inflexed rim (Figs. 14,17).

The second scale type varies from oval to elliptical and measures approximately 0.4 μm in length and 0.2 μm in width. This scale type lies external to the Type 1 scales and is the most abundant scale covering the cell. In the proximal face these scales are flattened with radiating ridges extending to the periphery, and tall, upright rims. In the

distal face, the scales have a sharply inflexed, smooth rim without ornamentation, and sometimes the rim is upright with several concentric rings of fibrils in the central area (Figs.15,17).

General ultrastructure

The general arrangement of organelles in *P. wyomingi* (Figs. 19, 21) resembles that of *P. parvum* f. *patelliferum* (Manton & Leedale 1963; Green, Hibberd & Pienaar 1982; Larsen 1999), except for some specific differences. First, longitudinal sections also show that the two flagella and the haptonema are subapically inserted in a depression or groove (Figs.20, 22). The anterior depression into which the three appendages are inserted is flanked by the anterior portions of the chloroplasts, which probably are responsible for creating the deep furrow that surrounds these appendages (Figs.19, 21). Secondly, there are generally 2 or more parietal chloroplasts per cell, but the number varies from 2 to 7 (Figs.19, 21). Each chloroplast has a pyrenoid that protrudes toward the central cytoplasm and is partially traversed by one to several thylakoid lamellae (Figs. 19, 21). A chloroplast endoplasmic reticulum (Fig.19) surrounds the chloroplast (Billard 1983).

The nucleus is situated in the posterior of the cell (Fig.21), and the Golgi body is situated in the anterior part of the cell between the nucleus and the flagellar basal bodies (Figs. 19, 22, 29). Figures 23-24 show that unmineralized scales are produced within the Golgi vesicles; hence, the maturing face of the Golgi apparatus often consists of large

vesicles that contain forming scales. Scale releasing vesicles occur in the vicinity of the release site (Fig. 24).

A contractile vacuole was not observed in this study. However, large vesicles with dark staining contents were common (Fig.21). They may represent the reddish bodies that are seen with the light microscope. Mixotrophy was not observed in this species.

Flagellar apparatus

As already described from SEM, the two flagella originate in a flagellar pit or depression, and are inserted at an acute angle to each other (Figs. 25-27). The transitional region consists of two widely spaced partitions, both lying above the level of the flagellar insertion (Figs. 25-26, 32). The flagellar membrane appears slightly inflated between these partitions (Figs. 25,28). The tubular roots consist of a single sheet of microtubules (Figs. 25-26, 29-30), which originate near the flagellar basal bodies, around the flagellar depression, and extend into the cell, appearing to make contact with the inner face of the chloroplast (Figs. 29-30).

The haptonema originates between the two flagella (Figs. 20, 22, 25-27, 31-32), and it has the typical structure of a ring of 7 microtubules surrounded by three concentric unit membranes (Fig. 31). The outermost membrane is the plasmalemma, and the remaining pair of membranes represents a sheath of endoplasmic reticulum that is continuous with the peripheral ER of the cell body (Fig. 32). In the haptonema, only one transverse plate occurs in the transitional region, and it is above the level where the haptonema attaches to the cell (Figs. 26, 31-32).

***Chrysochromulina asquamosa* sp. nov.** (asquamosae n. Latin = without scales)

Aguiar,R. & Kugrens,P.

DIAGNOSIS

Cellulae 4 –5 μm longae, 4 – 4.5 μm latae, obovale, anteriore atque posteriore parte oblique truncata. Flagella atque haptonema subapicaliter insertae. Flagella duo subaequalia vel non aequalia (14 – 17 μm, 21 – 28 μm) longae atque haptonema longum extensum, retractum in spiram convolutis. Chloroplast duo vel sex, fulvi, uterque pyrenoide immersa. Cellulae asquamosae.

Cells 4-4.5 μm in length and 4-5 μm in width, ovoid, subspherical being obliquely truncate anteriorly. Two subequal to unequal flagella (14-17μm and 21-28 μm long) and a long, coiled haptonema originating subapically from a depression. The haptonema is fully extended or may be coiled into a helix when retracted. Two to six golden-brown chloroplasts, each with a bulging pyrenoid. Scales are absent.

Isolates from all localities appeared to be identical in structure, especially in their lack of scales. Cells are spherical, or obovate, with the anterior end often slightly truncate. When the haptonema is coiled it is barely detectable with light microscopy. Cells contain one to several golden-brown, parietal chloroplasts. Pyrenoids are not visible with light microscope. Oil droplets and other vesicles are scattered throughout the cytoplasm, and often there is a small vacuole in the posterior end of the cell.

Scanning electron microscopy.

Cells are spherical but when viewed laterally, they are somewhat oval to obovate, and they have an obliquely truncate anterior end with a slight anterior depression (Figs. 33-40).

The two flagella and haptonema originate subapically from a depression (Figs.34-40). The flagella are equal in length and each has a narrow terminal region (Figs. 33, 39). The haptonema may form a tight coil (Figs.33, 35, 38) or it may be in various stages of uncoiling (Figs. 36, 39-40). The cell surface appears smooth (Figs. 33- 40).

Transmission electron microscopy

Shadow-cast preparations.

Observations of hundreds of cells from different localities clearly demonstrated that *Chrysochromulina asquamosa* cells lack scales (Figs. 41-43), and this is not a variation caused by culture conditions.

The flagella have narrow terminal regions and they lack hairs of any type (figs.41-43). The loosely coiled haptonema can form several gyres (Fig. 41), and it also lacks hairs. An extended haptonema can be seen in Figure 42, showing that it is several times longer than the cell length.

General ultrastructure

Cells generally have one or more parietal chloroplasts, each with an immersed, small pyrenoid that is not traversed by thylakoids (Figs. 44-45). Higher magnification shows that the pyrenoid appears crystalline (Figs. 44-46).

A nucleus is in the posterior end of the cell and forms a compartment of ER around the chloroplast, which constitutes the CER (Figs.44, 46). Many cells are binucleate, but whether this is a prelude to cell divison or an abnormal situation is not known. A nascent Golgi body occurs near the flagellar bases (Figs. 45, 47, 51).

The mitochondrion is situated internal to the chloroplasts and near the flagellar bases (Figs. 44, 46-48). Some adjacent sections indicate that the mitochondrion is reticulate.

Flagellar apparatus

While portions of the flagellar apparatus were examined in this study, a complete reconstruction of the flagellar apparatus was not conducted and will need to be done in the future.

The flagellar apparatus tends to be situated somewhat subapically and is closer to the left than to the right chloroplast (Figs. 47-51). The transitional region of the flagella in *C. asquamosa* is characterized by two transverse plates, both distal to the junction of the flagellum and the cell body, and approximately 0.2 μm apart (Figs.47, 49). A network of microtubular roots originates from the right basal body and extends along the cell

periphery (Figs.48,51). These microtubules probably are associated with the cytoskeletal microtubules to provide support and maintain cell shape.

The haptonema has the typical structure observed in other prymnesiophytes and consists of a plasmalemma, and a tubular endoplasmic reticulum surrounding six microtubules (Fig. 52). The coiled haptonema has a flanged basal swelling that bulges along the dorsiventral cell axis (Fig. 46).

DISCUSSION

This chapter provided cytological details of two new freshwater species of prymnesiophytes, *Prymnesium wyomingi* and *Chrysochromulina asquamosa*. For comparative purposes, selected features of *Prymnesium* species (Table 3) and selected features of *Chrysochromulina* species (Table 4) are summarized in Appendix 2.

This study has provided a new characteristic for distinguishing among species of *Prymnesium*, namely the presence of non-tubular hairs on both flagella and haptonema and two different types of unmineralized scales that are distributed in several layers. Prior to this study, species of *Prymnesium* had not described from a freshwater environment.

Chrysochromulina asquamosa is erected as a new species due to the absence of scales. This is the first time that a lack of scales has been confirmed for *Chrysochromulina*. Numerous isolates from different localities were examined and this feature was consistent and not attributable to culture conditions. *C. asquamosa* is the third freshwater species to be described together with *C. parva* Lackey and *C. breviturrita* Kenneth.

Prymnesium wyomingi. The discovery that two species of *Prymnesium* (*P. calathiferum* and *P. nemamethecum*) produce more than one type of scale re-opened the question of the taxonomic distinction between *Prymnesium* and *Chrysochromulina* (Pienaar & Norris 1979). The systematics for *Prymnesium* had been addressed by Green, Hibberd & Pienaar (1982) and Billard (1983). They suggested that *Prymnesium* species are most easily distinguished by their surface scales. All have two equal or unequal flagella and a short haptonema. Cell sizes range from 6.0-8.5 μm x 3.5 μm in the smallest species, *P. minutum*, to 11-18 μm x 5-7.5 μm in the largest species, *P. saltans*. However, there is considerable size overlap between species and comparative features between described species are provided in Table 3.

The specimens described in this study differ in several respects from the others. First, *P. wyomingi* was isolated from a freshwater environment, although it is well known that species of *Prymnesium* can have wide salinity tolerances (Mc Laughlin 1958; Chang & Ryan 1985). Second, it has long non-tubular hairs on both flagella and the haptonema. Third, *P. wyomingi* has two types of unmineralized scales, whereas other species have three, as in *P. nemamethecum* (Pienaar & Birkhead 1994).

Chang & Ryan (1985) suggested that cell morphology can also be used to distinguish among species of *Prymnesium* and considered that the ratio of flagellar length to cell length and the ratio of haptonemal length to flagellar length are important diagnostic features for species of *Prymnesium*. Table 3 indicates that *P. wyomingii* has the same flagella to cell length ratio (1.7) and the same haptonema to flagellar length ratio as *P.annuliferum* (0.25). However, cell dimensions, flagellar length and haptonema

length are significantly greater in *P. annuliferum* than in *P. wyomingi*. Ultrastructurally, *P. wyomingi* resembles other members of the genus in the arrangement and ultrastructure of organelles (Manton & Leedale 1963; Green, Hibberd & Pienaar 1982) except for the variable number of chloroplast per cell. In summary, this new species of *Prymnesium* expands the existing generic limits.

Chrysochromulina asquamosa. *C. asquamosa* is the third described freshwater member that can be included in the group of saddle-shaped species of *Chrysochromulina,* which includes marine species such as *C. acanthi*, *C. alifera*, *C. apheles*, *C. camella* Leadbeater et Manton, *C. campanulifera* Manton et Leadbeater, *C. cymbium* Leadbeater et Manton, *C. ephippium*, *C. parva* Lackey, *C. pontica* Rouchijajnen, *C. simplex*, *C. strobilus*, and *C. throndsenii* Eikrem (Eikrem 1999). *C. asquamosa* clearly belongs to the genus *Chrysochromulina*. It was discovered during studies on *Katablepharis ovalis* (Lee & Kugrens 1991), which feeds on this alga, and it was assumed to be *C. parva*. This prey alga lacked scales, and, as a result of these observations, several additional lakes were sampled and additional isolates were examined. All isolates lacked scales, prompting the description of a third freshwater species.

Chrysochromulina species usually are described as having one or more elaborate types of scales and a long, often coiling haptonema. The type species *C. parva* (Lackey 1939) was the only species described with only one type of body-scale (Parke, Lund & Manton 1962). However, some species, such as *C. spinifera* (Fournie) Pienaar ET Norris

and *C. parke* Green et Leadbeater, may have a short non-coiling haptonema (Leadbeater 1972), and their placement in this genus appears tennuos.

The function of the haptonema may be for surface adhesion, and the cell may then glide autonomously with an attached haptonema (Leadbeater 1972a). The participation of the haptonema in phagocytosis also has been suggested, because some species of *Chrysochromulina* possess a phagotrophic ability to capture various particles with the haptonema, such as graphite, bacteria and nanoplanktonic organisms such as *Chlorella* and *Aureococcus* species (Kawachi, Inouye, Maeda & Chihara 1991). Sleigh (1989) stated that the primary function of the haptonema possibly is the capture of food particles, which are then brought near the cell surface by the coiling of this organelle. He concluded that the phagotrophic activity of different species might correlate with the length of their haptonema. A tactile or similar sensory role for the haptonema was also suggested by Leadbeater (1972), but no evidence was presented to substantiate this suggestion (Kawachi, Inouye, Maeda & Chihara 1991). The haptonema does not beat like a flagellum, but it is capable of bending and coiling and adhering to a substratum. If the haptonema is short, it still may have an adhesive function, even if it does not coil (Kawachi, Inouye, Maeda & Chihara 1991).

In species of *Chrysochromulina*, scale morphology has been demonstrated to be a consistent character for identification (Eikrem & Moestrup 1998; Eikrem & Throndsen 1998). *Chrysochromulina asquamosa* shares a number of features with other species as shown in Table 4. Ultrastructurally, they resemble other members of the genus in the arrangement and ultrastructure of the organelles. However, when compared with other

described species and *C. parva, C. asquamosa* has the smallest cell size. When comparing the length of flagella and the length of haptonema between *C. parva* and C. *asquamosa*, both from freshwater, there are noticeable differences. In *C. parva* the flagellar length is three times longer, the cell is wider, and the haptonema length is longer than in *C. asquamosa.* Leadbeater (1972) suggested that these features are important as a genus or species characteristic.

REFERENCES

Billard, C. 1983. *Prymnesium zebrinum* sp. nov. et *P. annuliferum* sp. nov., deux nouvelles espèces apparentées à *P. parvum* Carter (Prymnesiophyceae). Phycologia **22**: 141-151.

Chang, F.H. & Ryan, KG. 1985. *Prymnesium calathiferum* sp. nov. (Prymnesiophyceae), a new isolated from Northland, New Zealand. Phycologia **24**: 191-198.

Eikrem, W. & Moestrup O. 1998. Structural analysis of the flagellar apparatus and scaly periplast in *Chrysochromulina scutellum* sp. nov. (Prymnesiophyceae, Haptophyta) from the Skagerrak and the Baltic. Phycologia **37**: 132-153.

Eikrem, W. & Throndsen, J. 1998. Toxic prymnesiophytes in Norwegian coastal waters. **In:** Toxic phytoplankton blooms in the sea. Proc. 5th Intern. Conf. Tox. Mar.Phytopl., Newsport, R.I., 28 October- 1 November 1991. Smayda, T.J. & Shimizu, Y. (Eds.). Elsevier Science Publishers, Amsterdam. pp. 687-672

Eikrem, W., Larsen, A. & Paasche, E. 1999. Growth and toxicity in *Prymnesium patelliferum* (Prymnesiophyceae) isolated from Norwegian waters. Can. J. Bot. **71**:1357-1362.

Green, J.C. & Hibberd, D.J. 1977. The ultrastructure and taxonomy of *Diacronema vlkianum* (Prymnesiophyceae) with special reference to the haptonema and flagellar apparatus. J. Mar. Biol. Assoc. U.K **57**: 1125-1136.

Green, J.C. & Pienaar R.N. 1977. The taxonomy of the order Isochrysidales (Prymnesiophyceae) with special reference to the genera *Isochrysis* Parke, *Dicrateria* Parke and *Imantonia* Reynolds. J. Mar. Biol. Assoc. U.K **57**: 7-17.

Green, J.C., Hibberd, D.J. & Pienaar, R.A. 1982. The taxonomy of *Prymnesium* (Prymnesiophyceae) including a description of a new cosmopolitan species, *P. patellifera* sp. nov., and further observations on *P. parvum* N. Carter. Br. Phycol. J. **17**: 363-382.

Green, J.C. & Jordan R.W. 1994. Systematic history and taxonomy. The Haptophyte Algae. J. C. L. Green, B. S. C. (Eds.). Oxford, Clareton Press. **Special Vol. 51:** p. 1-22.

Hibberd, D.J. 1976. The ultrastructure and taxonomy of the *Chrysophyceae* and *Prymnesiophyceae (Haptophyceae)*: a survey with some new observations on the

ultrastructure of the *Chrysophyceae*. Bot. J. Linn. Soc. **72**: 55-80.

Jeffrey, S.W. 1990. Chlorophyll *c* pigments and their distribution in the chromophyte algae. The Chromophyte Algae. J. C. Green, B. S. C. Leadbeater and W. L. Diver. Oxford, Claredon Press: 13-36.

Johansen, J.R., Doucette, G.J.B., Barclay, W.R. & Bull, J.D. 1988. The morphology and ecology of *Pleurochrysis carterae* var. *dentata* var. nov. (Prymnesiophyceae), a new coccolithophorid from an inland saline pond in New Mexico, USA. Phycologia **27 (1)**: 78 -88.

Jordan, R.W. & Green, J.C. 1994. A check-list of the extant Haptophyta of the world. J. Mar. Biol. Ass. U.K **74**: 149-174.

Kalina, T. 1970. *Corcontochrysis noctivaga* gen. et sp. nov. (Chrysophyceae). Preslia **42**: 297-302.

Kalina, T. 1975. Taxonomie und ultrastruktur der Art *Corcontochrysis noctivaga* (Haptophyceae). Preslia **47**: 1-13.

Kawachi, M., Inouye, I., Maeda, O. & Chihara, M. 1991. The haptonema as a food-capturing device: observations on *Chrysochromulina hirta* (Prymnesiophyceae). Phycologia **30 (6)**: 563-573.

Kleijne, A. 1993. Morphology, taxonomy and distribution of extant coccolithophorids (calcareous nannoplankton), Drukkerij FEBO B. V.

Lackey, J. B. 1939. Notes on plankton flagellates from the Scioto River. Lloydia **2**: 128-143.

Larsen, A. 1999. *Prymnesium parvum* and *P. patelliferum* (Haptophyta) - one species. Phycologia **38**: 541-543.

Leadbeater, B.S.C. & Manton I. 1969. *Chrysochromulina camella* sp. nov. and *C. cymbium* sp. nov., two relatives of *C. strobilus* Parke and Manton. Archiv. für Mikrobiol. **68**: 116-132.

Leadbeater,B.S.C. 1972. Fine structural observations on six species of *Chrysochromulina* (Haptophyceae) from Norway. Sarcia **49**: 65-80.

Leadbeater, B.S.C. 1972a. Identification, by means of electron microscopy, of flagellates nanoplankton from the coast of Norway. Sarcia **49**: 107-124.

Lee, R.E. & Kugrens, P. 1991. *Katablepharis ovalis*, a colorless flagellate with

interesting cytological characteristics. J. Phycol. **27**: 505-513.

Manton, I. & Leedale G.F. 1963. Observations on the fine structure of *Prymnesium parvum* Carter. Arch. Mikrobiol. **45**: 285-303.

Manton, I. & Peterfi L.S. 1969. Observations on the fine structure of coccoliths, scales and the protoplast of a freshwater coccolithophorid, *Hymenomonas roseola* Stein, with supplementary observations on the protoplast of *Cricosphaera carterae*. Proc. R. Soc. London Ser. B **172**: 1-15.

McLaughlin, J.J.A. 1958. Euryhaline chrysomonads: nutrion and toxigenis in *Prymnesium parvum*, with notes on *Isochrysis galbana* and *Monochrysis lutheri*. J. Protozool. **5**: 75-81.

Moestrup, Ø. 1979. Identification by electron microscopy of marine nanoplankton from New Zealand , including the description of four new species. New Zealand J. Bot. **17**: 61-95.

Nicholls, K.H. 1978. *Chrysochromulina breviturrita* sp. nov., a new freshwater member of the Prymnesiophyceae. J. Phycol. **14**: 499-505.

Parke, M., Lund, J.W.G. & Manton, I. 1962. Observations on the biology and fine structure of the type species of *Chrysochromulina* (*C. parva* Lackey) in the English Lake District. Arch. Mikrobiol. **42**: 333-352.

Pienaar, R.N. & Norris R.E.1979. The ultrastructure of the flagellate *Chrysochromulina spinifera* (Fournier) comb. nov. (Prymnesiophyceae) with special reference to scale production. Phycologia **18**: 99-108.

Pienaar, R.N. & Birkhead, M. 1994. Ultrastructure of *Prymnesium nemamethecum* sp. nov. (Prymnesiophyceae). J. Phycol. **30**:291-300.

Sleigh, M.A. 1989. Protozoa and other protists. London, Arnold.

CHAPTER 3

COMPARATIVE ULTRASTRUCTURE OF THREE *UROGLENA* SPECIES FROM COLORADO.

ABSTRACT

Three colonial chrysophycean flagellates were examined with the electron microscope, and their structure was compared with data on other *Uroglena* species. Scanning electron microscopy (SEM) of *U. volvox* reveals a colonial structure that is different from any described. It is a spherical colony with several hundred cells that are attached by their pointed tails to a confluent sheet of mucilage, rather than being embedded within a mucilaginous colonial envelope. The spherical colonial mucilage is hollow, and SEM indicates that the confluent mucilage consists of a meshwork of fibrils. Cells are pyriform with acutely pointed posterior ends, and a large chrysolaminarin vacuole occupies the posterior one-third of the cell. Each cell has a single sheet-like, spiral chloroplast with a stigma and a pyrenoid. The pyrenoid is adjacent to the nucleus and projections from the nucleus protrude into the pyrenoid. The stigma occurs in the anterior end of the chloroplast in a specialized lobe, and it is associated with the short flagellum and its flagellar swelling. A descending microtubular root of 11 microtubules begins at the base of the long flagellum and extends around the periphery of the cell, terminating in the cell's posterior. A second *Uroglena* species is smaller and has up to 32 cells per colony, but an investing colonial envelope or a confluent envelope also is absent. The obovoid cells have a short pointed end, and the cells are connected to each other by dichotomously branched hollow stalks or tubes. Cells have a single U-shaped chloroplast with a stigma, but without a pyrenoid. The third colony is *Uroglena*

articulata and its cells also are connected to each other by sympodially branched stalks. An investing colonial envelope is absent. Cells usually have two chloroplasts with a stigma in one chloroplast but pyrenoids are lacking. In addition, an unusual bacterial endosymbiosis was discovered. A rod-shaped bacterium, with its wall intact, grows inside the nuclear envelope of all cells examined. The bacteria did not appear to be pathogenic, but they are a permanent resident of these cells. All three isolates differ in the fine structure of the flagellar swelling that is associated with the eyespot.

COMPARATIVE ULTRASTRUCTURE OF THREE *UROGLENA* SPECIES FROM COLORADO

INTRODUCTION

Ultrastructural studies in the Division Chrysophyta, Class Chrysophyceae, have focused on unicellular representatives, although the ultrastructure and systematics of synurophycean colonial representatives, such as *Synura* spp., have been examined extensively (Andersen 1987). In contrast, the systematics of colonial chrysophycean flagellates have been limited and are in need of electron microscopic investigation. Some dendroid colonies such as *Anthophysa* (Belcher and Swale 1972) and *Dinobryon* (Herth and Zugenmayer 1979, Owen, Mattox and Stewart 1990a, Sandgren 1980, Sheat, Hellebust and Sawa 1975) have been examined, but these are easily identified, even with the light microscope. The spherical colonial types are difficult to distinguish taxonomically, since cells appear similar when viewed with light microscope and colonial organization is not evident. Only a putative *Uroglena americana* and a marine *Syncrypta* (Clarke and Pennick 1975) have been examined ultrastructurally, but neither of these studies provided pertinent features of colony organization relating to the classical light microscopic descriptions (Bourelly 1968, Huber-Pestalozzi 1947 & Starmach 1985). Therefore numerous taxonomic questions exist regarding the colonial genera and species in this class because the characteristics used in delineating the genera have been

contradictory, non-existent, or undocumented. Whether this group should be separated into several genera or combined into one or two genera also remains unresolved.

The following brief discussion indicates how genera are separated on the basis of colonial organization and how chloroplast shapes and number are used in this assessment. *Uroglena* is delineated by its cells being ellipsoid or ovoid and connected to each other by prominent dichotomously branched stalks. In addition, the cells are embedded in an investing colonial envelope with the flagella extending beyond this colonial envelope. *Synochromonas* is a spherical colony with its cells bound together by an investing, mucilaginous colonial envelope, but lacking the dichotomous stalks between cells. *Volvochrysis* is characterized by an internal spherical colonial mucilage that is a solid mass, with cells embedded by their posterior ends along the periphery of this mucilage. Cells possess two chloroplasts. *Uroglenopsis* has a confluent sheath in which cells are embedded, but does not have individual stalks connecting individual cells. To complicate matters, Bourelly (1968) suggested combining all of these genera, except *Uroglena*, into *Syncrypta* since he believed that the differences in colony structure were too minor to retain them as distinct genera. These, and other suggestions, were made without reinvestigation of the genera and without the use of electron microscopy. Furthermore, it is apparent that electron microscopy has to be used to determine which characters should be used and how these genera and species should be delineated.

This chapter reports on three colonial isolates from Colorado using electron microscopic characters and provides novel information that could be useful for delineating species within the genus *Uroglena*.

RESULTS

Uroglena volvox

The identification of this colony is tenuous and is based primarily on cell shape and disposition within the colony, and on diagrams provided in various keys. Since cultured material was not available, it was impossible to ascertain whether this strain matches the earlier descriptions.

Light Microscopy

Colonies range from a few cells to several hundred cells per colony. At maturity these spherical colonies consist of several hundred cells per colony (Figs. 1, 2), with mature colonies measuring 250-400 μm in diameter. Larger non-motile cells probably represent stomatocysts, or resistant spores (Fig. 2). As seen in Figures 2 and 3, the cells are not touching and appear to radiate from the colony. In fact, the cells within the colony exhibit individual movement and often detach from the colony and swim away, probably to establish a new colony (Figs. 3-4). However, while attached, the movement of each individual cell is restricted to moving back and forth. Figure 4 shows the typical shapes of the cells, which are ovoid with a pointed posterior. The colonial mucilage is not evident. The heterokont flagellation can also be seen in some cells (Figs. 3-4)

Electron microscopy

Scanning electron microscopy. SEM confirms that cells are not surrounded by a colonial envelope (Figs. 5 -6). Furthermore, they are not connected to each other by dichotomously branched stalks. Rather, they are attached to a central confluent mucilage, and cells are mainly on the outside of the material that holds the colony together (Fig. 5). This mucilage appears fibrillar and forms a meshwork that is hollow, as indicated when colonies rupture to expose the mucilage (Fig. 6).

The cell shape generally is pyriform and slightly curved on one side, and all cells protrude from the mucilage (Figs. 7-10). Only the cell posteriors are attached to the mucilaginous material (Figs. 7,8). For the most part, the cells do not touch each other, except in areas where the mucilage apparently has been compressed or new cells have recently formed (Fig. 7). The mucilage appears fibrillar and forms a meshwork of fibrils (Figs 7-10). The posterior ends of cells are acutely pointed (Figs. 8-9), and these short points could represent short cellular stalks, observed with LM, that anchor cells to the internal colonial mucilage, but this could not be resolved with SEM. Although they could not be detected in fixed material of this species, such stalks are demonstrated for the other two species.

Flagella are inserted on one side of the cell and there appears to be a depression where the short flagellum is located (Figs. 7-10). A flagellar swelling is evident on the small flagellum in this depression (Fig. 10).

Transmission electron microscopy

Shadow-casting. Cells have heterokont flagella (Fig. 11). The long flagellum is approximately four times the cell length, whereas the short flagellum is approximately as long as the cell. The long flagellum bears tripartite hairs and the short flagellum has fine, non-tubular hair (Figs. 12, 13-14). The cells lack scales (Fig. 14).

General Ultrastructure

The general ultrastructure of *U. volvox* is remarkably similar to the cell structure for *Uroglena americana* (Owen, Stewart, Mattox 1990), however, the authors did not specifically deal with either the cell or colony structure, but focused on the flagellar apparatus. Cells range in length from 11-15 μm and 6-8 μm in diameter. The cells possess the typical insertion of flagella for the Class Chrysophyceae, whereby flagella are not inserted parallel to each other, but rather have at least a 45^0 angle between them. The smaller flagellum typically has a flagellar swelling that is associated with the stigma.

In sectioned material, the polarity of the cells is evident, with a chrysolaminarin vacuole located in the posterior one third of the cell (Figs. 15, 25), and the other organelles located in the anterior of the cell. A centrally situated nucleus and a chloroplast that adheres to, and wraps around, the nucleus are the most prominent organelles in the anterior end. The chloroplast is enclosed within a chloroplast endoplasmic reticulum that is continuous with the outer membrane of the nuclear envelope membrane (Fig. 15). An anterior eyespot or stigma is in a special lobe of the chloroplast in the anterior end (Figs. 15- 18). The stigma consists of several layers of carotenoid granules and there are no thylakoids interspersed among the granules (Figs 17,

18). A swelling of the short flagellum is associated with the stigma (Figs. 16-18) and its internal structure can be deduced from various sections (Figs. 17-19). Small spherical structures that appear interconnected by fine fibrils occupy the region nearest the axoneme and three striated lamellae are on the ventral side nearest the plasma membrane in this swelling (Figs. 17-19). In Figure 19 the transition region of the short flagellum shows that there are five helical gyres.

A Golgi apparatus generally is lateral to the nucleus (Figs 15, 16, 21), rather than being anterior to the nucleus. In Figure 16, it appears that the Golgi vesicles are forming a larger vesicle. In addition to the Golgi vesicles, there are two other types of vesicles in these cells. One type contains small dark staining granules, which are located in the anterior region of the cell (Figs. 15, 16, 21,23, 26). The second type appears to contain dark staining solid material with some spaces within the material, and by their appearance, the latter may be lipid storing vesicles (Figs.15, 25-27). However, the function for both is unknown.

One of the characteristics of *Uroglena americana* is a descending complex microtubular rootlet (Owen, Stewart, Matox 1990) that originates ventral to the long flagellum and extends around the periphery of the cell, terminating in the posterior end where the cells attach to the mucilage. *U. volvox* also has a similar rootlet and Figures 20-24 show various aspects of this structure. The location of the descending rootlet is parallel with the basal body of the long flagellum (Figs. 20-22) where the microtubules of the descending root are shown in transverse or slightly oblique section. A longitudinal section (Fig. 22) indicates the location of a part of the descending composed of 11

microtubules. These microtubules curve slightly as they descend toward the posterior of the cell (Fig. 24) and these are illustrated as they pass through the peripheral cytoplasm. As will be discussed later Owen, Stewart and Mattox (1990) believe that this is a significant characteristic that could be used in systematics of this group.

The outer portion of the entire pyrenoid lies adjacent to the nucleus, and large nuclear projections routinely protrude into the pyrenoid (Figs. 15, 25, 26). These nuclear protrusions are oval in cross section and the chloroplast endoplasmic reticulum membranes, the periplastidal space, and the nuclear envelope can be discerned (Fig. 25). The pyrenoid also appears oval in end view (Fig. 27).

Uroglena americana has been reported to be strongly mixotrophic, but the putative *U. volvox* used in this study only showed a slight tendency toward mixotrophy. To illustrate that mixotrophy does occur, a cell with an ingested prokaryote is shown in Figure 25, and the cell in the food vacuole appears to be a cyanobacterium. The vesicle also contains other debris.

Uroglena sp.

This isolate could not be identified to species since it does not possess any combination of characteristics that have been used previously to delineate species.

Light Microscopy

Uroglena sp. generally has fewer than 100 cells per colony, with colonies ranging in size from 25 μm to < 100 μm in diameter. Cells are 10-15 μm in length and 5-8 μm in

diameter. Generally, pairs of cells are attached to a longer stalk (Figs. 28, 29), which is attached to other stalks radiating from the center (Fig. 28). A colonial envelope is lacking and individual cells can rotate independently but are constricted in their movements by their attachment to the stalks. Flattened colonies (Figs. 28, 29) show the general shape of the cells, which are oblong and tapered toward the posterior. The posterior of each cell is flattened where the cells attach to their short stalks (Figs. 28, 29).

Each cell contains one contractile vacuole, and it is approximately one fourth the distance from the anterior end of the cell (not depicted). The dilation and expulsion of the contractile vacuole were observed routinely with the light microscope but sectioned material did not preserve any contractile vacuoles. Cells contain a single chloroplast, as determined when cells rupture. A stigma was not evident when viewed with the light microscope.

Electron microscopy

Scanning electron microscopy

Figures 30-35 are SEM micrographs showing variations in cell numbers per colony and surface features of the cells. Variations in the number of cells per colony are common, ranging from three cells (Fig. 31) to approximately 17 cells (Fig. 34). Few colonies with more than 20 cells per colony were found. Cells appeared ovoid to almost spherical and all have a cone-shaped posterior end (Figs. 30, 31, 35). A flattened region in this area may correspond to the flat posterior portion observed with light microscopy.

Intact stalks were not preserved in most colonies, although some extracellular material shown in Figures 39 and 42 probably represents remnants of stalks since the fixation procedures probably distorted some of the remaining stalk material. Each cell has two heterokont flagella with the long flagellum generally three times as long as the cell length. Cells have a shallow anterior, circular depression from which the flagella originate on one side, near the rim of this depression (Figs. 33, 33). A flagellar swelling on the short flagellum also is evident in Figure 33.

Transmission electron microscopy

Shadowed whole cells (Figs. 36- 38) show the typical heterokont flagella of the chrysophyceae. The long flagellum bears two rows of tripartite tubular hairs, and the short flagellum is naked. Cells are naked and no scales were observed.

A low magnification EM (Fig. 39) shows the arrangement of some cells in the colony, including stalks. The chrysolaminarin vacuoles are prominent in the posterior one third of the cell, as are the delicate stalks that radiate from a central point and attach to the cell posterior. The stalks appear thin walled delicate and hollow (Figs. 40, 41), with some lamellae evident in the stalks. Cell posteriors appear somewhat flattened where the stalks attach to the cell (Figs. 39, 41).

An oblique section of a cell provides the typical arrangement of organelles anterior to the chrysolaminarin vacuole (Fig. 42). In this section it appears that there are two chloroplasts in the cell but these are merely lobes of a single U-shaped chloroplast that partially encircles the nucleus when viewed in a cross section of a cell (Fig. 43). A

stigma, which was not evident in light microscopic observations, is present in one lobe of the chloroplast and the stigmatic pigment granules are small and occur as a single row (Figs. 42, 44-46). The stigma is associated with the flagellar swelling of the short flagellum. The flagellar swelling has two components ventral to the axoneme. The area nearest the axoneme consists of large granules, similar to those in *U. volvox*, but the area nearest the stigma is granular. Furthermore, the stigma is not in a special lobe of the chloroplast, although there is a depression in the chloroplast, and often the thylakoids are more irregular in shape near the stigma (Figs. 44-46). This anterior depression in the cell may be the circular depression seen with SEM (Figs. 32, 33).

A Golgi apparatus is located anterior to the nucleus (Figs. 42, 47), and it usually appeared to be inactive. As is typical of chromophyte algae, a chloroplast endoplasmic reticulum surrounds the chloroplast in all cells, and often there are dilated areas in this CER where tubular flagellar hairs are being assembled (Fig. 43).

The flagellar apparatus will be addressed briefly. In longitudinal section, part of the flagellar apparatus possesses a dark staining granular material that attaches the flagellar base to the anterior of the nucleus (Figs.47, 48). A striated root or rhizoplast is embedded within this granular material (Fig. 47). An extensive descending microtubular root, as described for *U. americana* (Owen, Stewart and Mattox 1990), and also observed in *U. volvox*, was not observed in *Uroglena* sp. This is usually prominent near the basal body of the long flagellum when viewed in cross section (Figs. 47, 49), but numerous observations failed to reveal a descending microtubular root.

Uroglena articulata

Light Microscopy

Colonies are spherical, measuring 70-100 μm in diameter and consisting of 8 to 100+ cells per colony. Most mature colonies range in cell number from approximately 32 - 64 cells per colony. Since colonies are not coenobic, it is difficult to provide a definite number. Cells are obovoid with a narrower posterior (Figs. 50-53). Cells have two chloroplasts that may be in different positions within cells (Fig. 50). As in the previous colonies, the cells have a prominent posterior chrysolaminarin vacuole (Figs. 50-53).

Flattened colonies (Fig. 50) show that the cells are connected to each other by stalks that are sympodially branched. The flattening of colonies results in cells compacting and causing the cells to appear more elongate than non-compacted cells. Nevertheless, the posterior ends of the cells are flat and a short cone-shaped individual cell stalk is evident in each cell (Figs. 51, 52).

During observations on flattened colonies, the release of chrysolaminarin vacuoles was observed (Fig. 52), and the process may be an alternate means of cell release to form new colonies. As seen in Figures 52 and 53, portions of chrysolaminarin vesicles are extruded and released. Extrusion of the chrysolaminarin vacuole contents causes a separation of the cells from the stalks, and they are squeezed out of the colony by adjacent cells. Once the cells are free, they become spherical and swim away. Whether this actually occurs naturally in unflattened colonies is speculative, but the

released cells swim actively and conceivably could divide to form new colonies. More chrysolaminarin would need to be secreted to reconstitute the large chrysolaminarin vacuole and to restore the cells to their original structure.

Electron microscopy

Scanning electron microscopy

All cells are biflagellate and the flagella are heterokont. The cell posteriors have a cone-like region or extension that could represent the individual cell stalks that attach to the colonial stalks in the light micrographs. The colonial stalks are not as distinct as seen in Figure 51, although remnants of the stalks are evident in Figures 54-57. The cells have a slight anterior depression from which the flagella originate (Figs. 54, 55).

Transmission electron microscopy

Shadow-cast preparations.

Cells again have the typical heterokont flagellar structure, with the long flagellum possessing two rows of opposing tripartite tubular hairs (Fig. 59) and the short flagellum is naked. The long flagellum is approximately four times as long as the cell length.

The typical polar appearance of the cells, with the posterior chrysolaminarin vacuole and the other cell structures located anterior to this vacuole, is shown in a longitudinal section of the cell (Fig. 60). Two chloroplasts flank the nucleus, and both

are surrounded by chloroplast endoplasmic reticulum. A cross section of a cell confirms that there are two chloroplasts per cell. The chloroplasts lack pyrenoids but as will be discussed later, one of the chloroplasts possesses an anterior stigma. The posterior and ventral portions of the nuclear envelope are dilated due to bacteria that occur within the nuclear envelope compartment or space (Figs. 60-61). The bacteria appear to be living, rather than being digested, as would be the case in a food vacuole. Further discussion of this bacterial association and their possible roles within the cell are discussed in Chapter 4. The posterior end of each cell is flattened and is attached to a hollow delicate stalk, which in turn is attached to branches of other stalks (Fig.62).

A stigma occurs at the anterior end of one of the chloroplasts (Figs. 63, 64, 67) and there may be several layers of stigmatic granules. The stigma is not located in a specialized lobe in the chloroplast but rather it occurs at the periphery of the anterior end of the chloroplast (Figs. 63, 64). A flagellar swelling is associated with the stigma and the swelling contains striated lamellar material (Fig. 63) that is periclinal to the curvature of the swelling.

The long flagellum is inserted at almost a 90^{0} angle to the short flagellum (Fig. 63). A cross section of the basal body from this flagellum shows that it does not have a DR associated with it, but there are longitudinally oriented microtubules in the peripheral cytoplasm (Figs. 66, 67). While this may not be a distinct DR in *U. articulata* there are numerous peripheral microtubules that extend longitudinally (Fig. 67) but their points of origin were not determined and will constitute a portion of future studies on the flagellar apparatuses of these genera. Posterior to the flagellar apparatus there is a dark-staining

granular mass associated with the striated rhizoplast (Fig 65), which appears to be attached to the outer membrane of the nuclear envelope. Furthermore, the flagella have five helical gyres (Fig. 66).

DISCUSSION

It is apparent from the literature that the colonial chrysophycean algae are in need of detailed investigation. Excluding the Synurophytes, *Dinobryon* and *Anthophysa*, the genera that are not clearly delineated are *Uroglena*, *Uroglenopsis*, *Syncrypta*, *Synochromonas*, and *Volvochrysis*. *Uroglena* consists of spherical colonies with cells that are attached to dichotomously or sympodially branched stalks by their pointed ends and often a colonial envelope surrounds the cells in the colony. The latter was not observed in this study and if this remains a diagnostic feature, the isolates used in this study have to be described as new genera. Cells are ovoid to elliptical in shape, although some species have pyriform cells, such as *U. volvox* (Huber-Pestalozzi 1941). *Uroglenopsis* colonies are spherical and their cells have a drawn out tail that joins other cells at the colony center, and stalks are absent. A colonial envelope surrounds all cells. In addition, the cells have a rather long smooth flagellum, without tripartite hairs. *Volvochrysis* colonies have cells that are attached by their pointed tails to the periphery of a solid sphere of mucilage, and the cells have two chloroplasts. *Synochromonas* has spherical colonies with cells that are pyriform and attached to each other by their tails. Bourrelly (1957) proposed to incorporate all of the other genera, except *Uroglena*, into *Syncrypta* since he considered differences in the colony organization, specifically the mucilage arrangement, to be minor. In *Syncrypta* the cells are united in the center by their tails (Bourrelly 1957), and the only species that has been examined with the EM has scales (Clarke and

Pennick 1975), so its relation to the Chrysophyceae may be tenuous, and it may be related to *Synura*.

Preisig, Vørs and Hällfors (1991) state that *Uroglena* has cells that are *Ochromonas*-like, in colonies, arranged radially at the periphery of a spherical to ellipsoid or more irregular gelatinous matrix. The interior of the matrix is rather homogeneous or contains a system of fine radiating and branched threads to which the cells are attached by their pointed posterior ends. However, a diagram of the colony on page 396 of Preisig, Vors & Hällford (1991), shows that the cells are not pointed, and that the colonial envelope extends around the cells.

In his broad treatise on chrysophycean algae, Starmarch (1985) recognized 15 species of *Uroglena*. Based on these generic characteristics, our isolates of *Uroglena* sp. and *Uroglena articulata* can be placed in *Uroglena* since the cells are connected by branched stalks. However, the putative *U. volvox* remains problematic. The tails of the cells are attached to an inner hollow sphere of mucilage, a feature that has not been described previously. The cell structure closely resembles that of *U. americana* (Casper 1972, Owen, Stewart & Mattox 1990) but details of colony architecture were not provided in these studies. The diagrams in Huber-Pestalozzi of *U. volvox* are similar to the material that we examined, therefore the name *U. volvox* was tentatively assigned to this isolate.

Micrographs provided by Owen, Stewart & Mattox (1990) of *U. americana* indicate that the general cell structure bears a remarkable similarity to the cells of *U. volvox*. However, they note that their cells were ovoid or ellipsoid rather than

being pyriform. Thus the cellular structure, aside from cell shape, is similar in *U. americana* and *U. volvox*, but the cell shape and colony organization apparently differ. They also noted that the descending microtubular rootlets in *U. americana* and those of *Anthophysa* (Belcher & Swale 1972) and *Chrysonephele* (Pipes, Tyler & Leedale 1989) are similar and the unique DR that was found in *Uroglena* could define a group of chrysophytes separate from those that have the typical chrysophyte type rootlet. However, neither *Uroglena* sp. or *Uroglena articulata* has this descending microtubular rootlet. In *U. volvox* there is an extensive microtubular root that extends the length of the cell and consists of 13-17 microtubules, but *Uroglena* sp. lacks these microtubules. *U. articulata* does not have a discrete descending root but there are more than 60 longitudinally oriented microtubules around the cell periphery. It is possible that this variation could be used as another characteristic upon which species could be delineated. It is also important to note that the cell structure in *Uroglena* sp and *U. articulata* differ considerably from *U. volvox* and *U. americana*. Therefore erecting new genera or species for these two chrysophyceans may be justified in the future.

Since the characters used to delineate *Uroglena volvox* were not found in the isolate used in this study, the diagnostic features might have to be modified based on the SEM observations, or a new species will need to be erected. Instead of being interconnected by prominent dichotomously branched stalks and embedded in colonial mucilage, SEM showed that the complex of cells is not surrounded by mucilage. In mature colonies of *U. volvox*, cells are not connected by dichotomous stalks, but form a confluent matrix that is sheet-like, fibrous and hollow. It is possible that young colonies

might have distinct stalks but since the colonies could not be grown in culture this aspect remains speculative.

The confusion about the colonial chrysophytes is further illustrated by the following examples. In their textbook, Graham and Wilcox (1999) depict a colony that is similar to *U. volvox* described in this chapter, but they refer to it as *Uroglenopsis* sp., and obviously it is not that genus. In another study, the colonial structure of *Uroglena dendracantha* (Hollowday 1993) appears identical to *U. volvox* described in this chapter, except that the resistant spores, called stomatocysts, are spiny, unlike the smooth stomatocysts in *U. americana* or *U. volvox*. However, they also note that stomatocysts may be smooth in young colonies of *U. dendracantha*. Except for SEMs of the stomatocysts, other electron microscopic studies were not conducted, thus the cellular features of *U. dendracantha* remain to be examined.

This study has provided some additional aspects that need to be considered in the classification of these algae, and it has provided variations in structure that need to be evaluated when additional isolates are studied. General cell structure among two of the three species studied is similar but there are some notable differences, and these are listed in Table 5. All three isolates are biflagellate and have heterokont flagella whose structure is typical for the Class Chrysophyceae (Moestrup and Andersen 1991), with the short flagellum having a swelling that is associated with the eyespot. However, flagellar swellings were remarkably different among the three species and could be another variation that could be used in delineating species.

Colony architecture for *Uroglena sp.* and *Uroglena articulata* are similar in that cells are attached by dichotomously or sympodially branched stalks, which are articulated. However, there generally are fewer cells per colony in *Uroglena* sp than in the other two. Furthermore, in culture most *Uroglena sp.* cells remain as unicells and small colonies of 2-8 cells are common, although colonies up to 60 cells may occur but are rare. Finally, *Uroglena articulata* cells contain two chloroplasts, but pyrenoids are lacking, thus separating it from *U. volvox* and *Uroglena* sp. Finally, *U. volvox* has a prominent pyrenoid that is invaded by portions of the nucleus.

With TEM the attachment material or stalks in *Uroglena* sp. and *U. articulata* appear thin, hollow, and segmented. It is not clear how this posterior mucilage is produced or formed since there is a large chrysolaminarin vacuole that occupies the posterior 1/3 - 1/2 of the cell. This large vesicle would block the Golgi vesicles from depositing this material. It is possible that the anteriorly situated Golgi apparatus exocytoses the mucilage, and the stalk material is transported posteriorly across the surface of the plasma membrane. At any rate, the mucilage is quite extensive in mature colonies, as evidenced with SEM, and it serves to hold the posterior of the cells in the colony.

Colony reproduction in *Uroglena* spp. apparently can be accomplished in two ways. In one case, *U. articulata*, individual cells are released from the colony by an extrusion of the chrysolaminarin vesicle, forming a spherical cell that eventually forms a new chrysolaminarin vacuole, then divides, with the subsequent daughter cells remaining attached to a common mucilage that is formed during this time. Second, entire colonies

can fragment into two colonies, with the mucilage in each daughter colony closing to form two new daughter colonies. This was observed frequently in *U. volvox*. Finally, cells may detach and form new colonies. All three types of asexual reproduction were observed either in natural samples or in culture.

Based on the flagellar arrangements in the species investigated during this study, they belong in the Class Chrysophyceae, Order Chromulinales, because the flagella are not parallel in insertion and the shorter flagellum is associated with the stigma. A flagellar swelling occurs on the short flagellum and is in contact with the cell membrane where the stigma occurs. The stigma may be in a lobe of the chloroplast, as in *U. americana*, or it may occur in the anterior end of a chloroplast without any differentiated region, which was the case in the other two species.

Bacteria occur in the nuclear envelope space in all cells of *Uroglena articulata*. A similar endobacterial association was reported for the colorless chrysophyte *Paraphysomonas* (Preisig, Vørs, Hällfors 1991), but a possible function for these endobacteria was not proposed. It is possible that these bacteria provide a continuous and sustainable metabolic system by providing some essential nutrient or nutrients so that the alga no longer has to rely on the vagaries of mixotrophy to obtain the essential nutrients. It is also possible that the bacteria are nitrogen fixers and can provide a steady supply of nitrogen for various metabolic pathways. This would enhance growth and allow the alga to outcompete other phytoplankton in nitrogen- limited aquatic systems. Obviously this avenue of research merits further research and it is the intent to pursue this aspect in the future.

The studies by Kimura and Ishida (1985, 1986, 1989) and Kimura, Ishida and Kadota (1986) indicated that *U. americana* can not grow photosynthetically without the presence of bacteria and was dependent on mixotrophy for proper growth. However, ingestion of bacteria was rarely observed in *U. volvox,* therefore this species was not an obligate mixotroph and probably does not require phagocytosis of bacteria for proper growth, although this aspect was not tested. Nevertheless, some metabolic factor was missing since this alga was unable to grow in culture.

In conclusion, this study has provided new ultrastructural observations on a putative *U. volvox* and on two additional species *Uroglena.* Furthermore, the observations also reinforce the fact that electron microscopy is essential in determining cellular and colony features that are pertinent to classifying this group of phytoflagellat

REFERENCES

Andersen, R. A. 1987. Synurophyceae classis nov., a new class of algae. Am. J. Bot. **74**:337-353.

Belcher, J. H. and Swale, E. M. F. 1972. The morphology and fine structure of the colorless colonial flagellate *Anthophysa vegetans* (O. F. Müller) Stein. Br. Phycol. J. **7**:335-346.

Bourrelly, P. 1957. Recherches sur les Chrysophycées. Revue Algol., Mém. Hors. Sér., **1**:1-142.

Bourrelly, P. 1968. Les Algues d'eau Douce. II. Algues Jaunes et Brunes. Boubee et Cie, Paris, 438 p.

Casper, S. J. 1972. Zum feinbau der Geisseln der Chrysomonaden. I. *Uroglena americana* Calkins. Arch Protistenk. **114**:65-82.

Clarke, K. J. & Pennick, N. C. 1975. *Syncrytpa glomerifera* sp. nov., a marine member of the Chrysophyceae bearing a new form of scale. Br. Phycol. J. **10**:363-370.

Herth, W. and Zugenmayer, P. 1979. The lorica of Dinobryon. J. Ultrastr. Res. **69**:262-272.

Hollowday,. 1993. *Cephalodella edax*, sp. nov. a rotifer parasitic in the motile colonial *Uroglena volvox* Ehrenberg. Hydrobiologia **255/256**:445-448.

Huber-Pestalozzi, G. 1947. Das Phytoplanton des Susswassers. 2. Teil. Chrysophyceen. Farblose Flagellaten, Hetrokonten. E. Schweizerbart'sche Verlagsbuchhandlung, Stuttgart. 365 pp.

Kimura B. & Ishida, Y. 1985. Photophagotrophy in *Uroglena americana*, Chrysophyceae. Jpn. J. Limnol. **46**:315-318.

Kimura B. & Ishida, Y. 1986. Possible phagotrophic feeding of bacteria in a freshwater red tide chrysophyte *Uroglena americana*. Bull. Jap. Fish Soc. Sci. and Fisheries. **52**:697-701.

Kimura B. & Ishida, Y. 1989. Phospholipid as a growth factor of *Uroglena americana*, a red tide chrysophyceae in Lake Biawa. Nippon Suisan Gakkaishi **55**:799-804.

Kimura, B., Ishida, Y. and Kadota, H. 1986. Effect of naturally collected bacteria on growth of *Uroglena americana*, a freshwater red tide chrysophyte. Bull. Jap. Soc. Sci and Fisheries **52**:691-696.

Moestrup, O. & Andersen, R. A. 1991. Organization of heterokonts. *In The Biology of Free-living Heterotrophic Flagellates* (Patterson D. J. and Larsen, J. eds). The Systematics Association. pp.333-360.

Owen, H. A., Mattox, K. R. and Stewart, K. D. 1990a. Fine structure of the flagellar apparatus of *Dinobryon cylindricum* (Chrysophyceae). J. Phycol. **26**:131-141.

Owen, H. A., Stewart, K. D., Mattox, K. R. 1990. Fine structure of the flagellar apparatus of *Uroglena americana* (Chrysophyceae) J. Phycol. **26:**142-149.

Pipes, L., Tyler, P. A. & Leedale, G. F. 1989. *Chrysonephele palustris* Gen et sp. nov. (Chrysophyceae), a new colonial chrysophyte from Tasmania. Beih. Nov. Hedwigia **95**:81097.

Preisig, H. R., Vors, N., and Hällfors, G. 1991. Diversity of heterotrophic heterokont flagellates. In Prescott, G. W. 1962. Algae of the Western Great Lakes Region. Wm. C. Brown Publishers, Dubuque, Iowa. 977 p.

Sandgren, C. D. 1980. An ultrastructural investigation of resting cyst formation in *Dinobryon cylindricum* Imhof (Chrysophyceae, Chrysophyta). Protistologica. **16**:259-276.

Sheat, R. G., Hellebust, J. A. and Sawa, T. 1975. The statospore of *Dinobryon divergens* Imhod: formation and germination in a subarctic lake. J. Phycol. **11**:131-138.

Starmach, K. 1985. Chrysophyceae und Haptophyceae. In: Ettl, H., Gerloff, J., Heynig, H. & Mollenhauer, D. (eds), Süsswasserflora von Mitteleuropa 1. VEB Gustav Fischer, Stuttgart. 515pp.

CHAPTER 4

ENDOSYMBIOTIC BACTERIA IN THE NUCLEAR SPACE AND ENDOPLASMIC RETICULUM OF *UROGLENA ARTICULATA* (CHRYSOPHYTA) FROM COLORADO

ABSTRACT

The ultrastructure of cells from the colonial chrysophyte *Uroglena articulata* and the presence of bacteria in the nuclear envelope space are described and discussed. The bacteria appear rod-shaped and possess a wall. They are not located near the chloroplasts, and it is postulated that these bacteria either provide some essential nutrient to the alga or the bacteria are nitrogen fixers.

INTRODUCTION

Non-pathogenic endosymbiotic bacteria (endobacteria) are common in algae (Moestrup and Andersen 1991, Preisig 1995), and they have been reported in euglenoids (Leedale 1969, Surek & Melkonian 1983), green algae (Lee & Kochert 1976, Turner & Friedmann 1974, Colombo 1978, Dawes & Lohr 1978, Wujek, Gardiner & Dawes 1982, Nozaki 1989, Liddle, Carvalho, Meinesz 1998), dinoflagellates (Gold & Pollingher 1971, Silva 1978, Silva & Franca 1985, Chesnick & Cox 1986), *Cryptomonas* (Schnepf & Melkonian 1990), chloromonads (Heywood 1978) and chrysophytes (Wujek 1978, 1982, Preisig 1995). Usually, the bacteria occur in the cytoplasm but they have also been reported occurring in the nucleus (Leedale 1969), in the endoplasmic reticulum (Wujek 1982) and in the nuclear envelope (Preisig 1995).

During an ultrastructural survey of Colorado and Wyoming chrysophytes, an ultrastructural examination of *Uroglena articulata* Skuja revealed that all cells contained endobacteria within the nuclear envelope space, which seems an unlikely location for endosymbionts. In this paper the occurrence of these bacteria is reported and structural details of this association are provided. Hypotheses on their possible role in the cell are also provided.

RESULTS

Uroglena articulata is a colonial chrysophycean alga, whose colony is formed by cells that are interconnected by sympodially branched stalks (Fig.1). Transverse sections (Fig. 2) show that, invariably, all cells contain bacteria within the nuclear envelope space. The nuclear envelope is dilated wherever bacteria are present. Each chloroplast is surrounded by chloroplast endoplasmic reticulum, but the bacteria never occur adjacent to the chloroplasts, as is shown more readily in a longitudinal section (Fig. 3) where the bacteria occur in the space opposite the chloroplast. Figure 4 clearly shows that the bacteria are located in the nuclear envelope space and that the nuclear envelope is continuous with the chloroplast endoplasmic reticulum. In addition to occurring in the nuclear envelope, bacteria also were found sometimes in inflated rough endoplasmic reticulum cisternae, which are continuous with the nuclear envelope (Fig. 5). Tubular hairs (stramenopiles) are typically formed in the CER (Bouck 1971), and in this case within the same compartment as the bacteria (Fig. 5). Thus bacteria apparently do not impede stramenopile formation.

The bacteria are rod-shaped and possess a wall that appears crenulated, probably caused by fixation (Figs. 6-8). Some bacteria were dividing within this compartment, which indicates that they are not being digested in this space (Fig. 7).

DISCUSSION

Mixotrophy is common in chrysophytes (Aaronson 1973, 1974, Andersson, Falk, Samuelsson, Hageström 1989, Bennett, Saders, Porter 1990, Berniger, Caron, Sanders 1992, Boraas, Estep, Johnson, Sirburth,1988, Boraas, Selae, Holen 1992, Caron, Porter, Sanders 1990, Daley, Morris, Brown 1973, Dubboursky 1974, Holen 1999, Jones Leadbeater, Green 1993, Kimura, Ishida 1985, 1989, Preisig 1995, Sanders 1991). Several *Uroglena* spp. have been reported to be mixotrophic (Kimura, Ishida 1985,1989), and at first, the presence of bacteria in *Uroglena articulata* was interpreted as mixotrophy, but additional observations indicated that the bacteria were localized within the nuclear envelope, and therefore *Uroglena articulata* did not exhibit mixotrophy. Mixotrophy may have been present at some time in this alga, but obviously the need for mixotrophy as an auxiliary type of nutrition is no longer necessary since the cells harbor bacteria that probably can supply the metabolic factors required by *Uroglena*.

The bacteria are rod-shaped and surrounded by a cell wall, which is unusual for an endosymbiont. Based on the wall presence, this may indicate an incipient, or a recent symbiosis, where the relationship has not progressed to a stage where a wall has been eliminated. Without exception, all cells of every colony examined contained endocytobacteria, and the cells appeared to be healthy except for the dilated nuclear envelope where the bacteria were located.

Since the culture was not bacteria free, the free-living bacteria in the culture medium also were surveyed to determine whether the endosymbiont occurred in the free-living state. Bacteria that resembled those in the *Uroglena* cells were not found.

The location of bacteria within the nuclear envelope space is unusual and has been reported only for *Paraphysomonas*, which is a colorless non-photosynthetic unicelled, distant relative (Preisig 1995). It is interesting that the location of bacteria within this space is not near the chloroplasts and is localized since the CER is continuous around the chloroplasts and nuclear envelope. These bacteria could be concentrated anywhere in this endomembrane system. If the hypothesis that this space represents an acidic compartment is correct (Lee & Kugrens 1999), then these bacteria would have to be acidophiles.

While endocytobacteria have been reported in a variety of algae, possible functions for these have not been proposed in any publications. The bacteria do not appear to be pathogenic, and based on hypotheses proposed for mixotrophs, similar functions can be attributed to these permanent intracellular inhabitants. If bacteria persist within cells there must be some selective advantage in this association. Since the host cell has to provide organic nutrients to the bacteria, this endoplasmic reticulum connected compartment could serve as a rich source of organics for the bacteria. The bacteria in turn would provide some essential metabolite to the host. It has been proposed that bacteria are capable of concentrating nutrients or inorganic ions in their cells and this sequestering is especially significant in oligotrophic waters. It has been proposed that prokaryotes and not eukaryotic phytoplankton possess sequestering structures called siderophores (Abd-

Alla 1999, Granger 1999, Yun, Tiedeman, Moore, Philpott 2000, Wilhelm, Maxwell & Trick 1996) for concentrating iron (Estep, Davis, Hargraves & Sieburth 1984). Thus mixotrophs would have a selective advantage by ingesting the bacteria, with the bacteria extracting iron or other inorganic nutrients to enhance *Uroglena*'s metabolism.

The bacteria also could be nitrogen fixers. As was the case with siderophores, bacteria and cyanophytes are the only organisms that can fix atmospheric nitrogen (Gergman, Gallon, Rai, Stal 1997, Carpenter & Price 1977, Capon & Carpenter 1999, Fogg, 1942, James 2000, Sanches, Cardenas & Quinto 1999, Singh 1961, Zahran 1999, Zerh, Carpenter & Villareal 2000). Nitrogen fixation requires anaerobic conditions, the enzyme nitrogenase, a supply of protons and the involvement of molybdenum and iron (Lee 1999). The space could be anaerobic to facilitate this conversion, since nitrogenase activity is inhibited by the presence of oxygen, and this could explain why bacteria are not near chloroplasts. Even if the space is not anaerobic then nitrogen fixation still could occur during the dark growth periods. Assuming the space is acidic, there would be an abundance of protons in this compartment that would facilitate the reduction of nitrogen to ammonia. For either hypothesis, the establishment and maintenance of a permanent bacterial population within the cells could provide a constant and reliable source of essential metabolites. Populations with obligate mixotrophs would exhibit fluctuations because the availability of bacteria for mixotrophy would be intermittent and undependable. To test either of these hypotheses, separate growth experiments without essential nutrients need to be conducted. Furthermore, experiments on nitrogen fixation are being planned in collaboration with individuals who

have the expertise and experience in nitrogen fixation to test whether the endobacteria are nitrogen fixers.

REFERENCES

Abd-Alla, M.H. 1999. Growth and siderophore production in *Bradyrhizobium* strains under iron limitations. Folia Microbiol. **44**(2):196-200.

Aaronson, S. 1973. Particle aggregation and phagotrophy by *Ochromonas*. Arch. Mikrobiol. **92**:39-44.

Aaronson, S. 1974. The biology and ultrastructure of phagotrophy in *Ochromonas danica* (Chrysophyceae: Chrysomonadida). J. Gen. Microbiol. **83**:21-29.

Andersson, A., Falk, S., Samuelsson, G. & Hageström, A. 1989. Nutritional characteristics of a mixotrophic nanoflagellate *Ochromonas* sp. Microbial Ecol. **17**:252-262.

Bennett, S. J., Sanders, R. W., & Portyer, K. C. 1990. Heterotrophic, autotrophy and mixotrophic nanoflagelaltes: seasonal abundances and bacterivory in eutrophic alke. Limnol. Oceanogr. **35**:1821-1832.

Bergman, B., Gallon, J.R., Rai, A.N. & Stal, L.J. 1997. N_2 fixation by non-heterocystous cyanobacteria. FEMS Microbiol. Rev. **19**:139-185.

Berninger, U.-G., Caron, D.A. & Sanders, R. W. 1992. Mixotrophic algae in three ice-covered lakes of the Pocono Mountains, U.S/A. Freshwater Biol. **28**:263-272.

Boraas, M. Estep, K. W., Johnson, P. W. and Sirburth, J. McN. 1988. Phagotrophic phototrophs: the ecological significance of mixotrophy. J. Protozool. **35**:249-252.

Boraas, M. E., Selae, D. B. & Holen, D. 1992. Predatory behavior of *Ochromonas* analyzed with video microscopy. Arch. Hydrobiol. **123**:459-468.

Bouck, G.B. 1971. The structure, origin, isolation and composition of the tubular mastigonemes of the *Ochromonas* flagellum. J. Cell Biol. **50**:362-384.

Capon,, D. & Carpenter, E.J. 1999. Nitrogen fixation by marine cyanobacteria: History and global perspectives. Bul. De L'Inst. Oceanog. 235-256.

Caron, D. A., Porter, K. G. & Sanders, R. W. 1990. Carbon, nitrogen and phosphorous budgets for the mixotrophic phytoflagellate *Poterioochromonas malhamensis* (Chrysophyceae) during bacterial ingestion. Limnol. Ocenogr. **31**:646-650.

Carpenter, E.J., Montoya, J.P., Burns, J., Mulholland, M.R., Subramaniam, J., Capone, D.G. 1999. Extensive bloom of N_2-fixing diatoms/ cyanobacterial association in the tropical and subtropical Atlantic ocean. Mar. Ecol. Prog. Ser.**(185)**:273-283.

Carpenter, E.J. & Price, C.C. 1977. Nitrogen fixation, distribution, and production of *Oscillatoria (Trichodesmium)* spp. In the western Sargasso and Caribean seas. Limnol. & Oceanog. **22**:60-72.

Chesnick,J.M. & Cox, E.R. 1986. Specialization of endoplasmic reticulum architecture in response to a bacterial symbiosis in Peridinium balticum (Phyrrhophyta). J. Phycol. **22**:291-298.

Clay, B. & Kugrens P. 1999. Systematics of the enigmatic Kathablepharids, including EM characterization of the type species, *Kathablepharis phoenikoston*, and new observations on *K. remigera* comb. nov. Protist **150**: 43-59.

Cole, G. T. & Wynne, M. J. 1974. Endocytosis of *Microcystis aeruginosa* by *Ochromonas danica*. J. Phycol. **10**:397-410.

Colombo, P.M. 1978. Occurrence of endosymbiotic bacteria in siphonus algae. Phycologia **17**:148-151.

Daley, R. J., Morris, G. P., & Brown, S. R. 1973. Phagotrophic ingestion of a blue-green alga by *Ochromonas*. J. Protozool. **20**:58-61.

Dawes, C.J. & Lohr, C.A. 1978. Cytoplasmic organization and endosymbiotic bacteria in the growing points of *Caulerpa prolifera*. Rev. Algol. **13**:309-314.

Dubboursky, N. 1974. Selectivity of ingestion and digestion in the chrysomonad flagellate *Ochromonas malhamensis*. J. Protozool. **21**:295-298.

Estep, K. W., Davis, P. G., Hargraves, P. E. & Sieburth, J. McN. 1984. Chloroplast sustaining flagellates in natural populations of North Atlantic nanoplankton, their identification and distribution; including a description of five new species of *Chrysochromulina* (Prymnesiophyceae). Protistologica **20**:613-634.

Fogg, G.E. 1942. Studies on nitrogen fixation by blue-green algae. I. Nitrogen fixation by *Anabaena cylindrical* Lemm. J. Exp. Biol. **19**:78-87.

Gergman, D.E, Gallon, H., Rai, P. & Stal, M.P. 1997. Mechanisms of Fe acquisition from siderophores by microorganisms and plants. **In:** Chen, Y., Hadar, Y. (eds.) Iron Nutrition and Interactions in Plants. Kluwer Academic Publishers, Dordrecht, The Netherlands, pp 213-232.

Gold, K. & Pollingher, U. 1971. Occurrence of bacteria in marine dinoflagellates. J. Phycol. 7:264-265.

Granger, J. 1999. The importance of siderophore in iron nutrition of heterotrophic marine bacteria. Limnol. & Oceanog. **44**(3):541-555.

Heywood,P. 1978. Intracellular bacteria in *Gonyostomum semen* (Chloromonadophyceae) J. Phycol. **14**:121-122.

Holen, D. 1999. Effects of prey abundance and light intensity on the mixotrophic chrysophyte *Poterioochromonas malhamensis* from a mesotrophic lake. Freshwater Biol. **42**:445-455.

James, E.K. 2000. Nitrogen fixation in endophytic and associative symbiosis. Field Crops Res. **65**(2-3):197-209.

Jones, H. L. J., Ledbeater, B. S. C. & Green, J. C. 1993. Mixotrophy in marine species of *Chrysochromulina* (Prymnesiophyceae): Ingestion and digestion of a small green flagellate. J. Mar. Biol. Assoc. U. K. **73**:383-296.

Kimura B. & Ishida, Y. 1985. Photophagotrophy in *Uroglena americana*, Chrysophyceae. Jpn. J. Limnol. **46**:315-318.

Kimura B. & Ishida, Y. 1989. Phospholipid as a growth factor of *Uroglena americana*, a red tide chrysophyceae in Lake Biawa. Nippon Suisan Gakkaishi 55:799-804.
Kochert, G. & Olseon, L.W. 1970. Endosymbiotic bacteria in *Volvox carteri*. Trans. Am. Microsc. Soc. **89**:475-478.

Lee, R. E. 1999. *Phycology*. Cambridge: Cambridge University Press.

Lee, W.S.B. & Kochert, G. 1976. Bacterial endosymbionts in *Volvox carteri* (Chlorophyceae). J. Phycol. **12**:194-197.

Lee, R. E., & Kugrens, P. 1999. The ecological advantage of chloroplast E.R. The ability to outcompete at low dissolved CO2 concentrations. Protist **149**: 341 - 345,

Leedale, G.F. 1969. Observations on endonuclear bacteria in euglenoid flagellates. Österr. Bot. Zeitschrift **116**:279-294.

Liddle, L.B., Carvalho, N. & Meinesz, A. 1998. Use of immunofluorescence microscopy to compare small nuclei in two populations of *Caulerpa taxifolia* (Caulerpales, Chlorophyta). Phycologia **37**:310-312.

Moestrup, O. & Andersen, R.A. 1991. Organization of heterotrophic heterokonts. In: Patterson, D.J. & Larsen, J. (eds.) *The Biology of Free-Living Heterotrophic Flagellates*. Clarendon Press, Oxford. Pp. 333-360.

Nozaki,H. 1989. *Pleodorina japonica* sp. nov. (Volvocales, Chlorophyta) with bacteria-like endosymbionts. Phycologia **28**:252-267.

Preisig,, H.R. 1995. A modern concept of chrysophyte classification. In *Chrysophyte Algae*, ed. C.D. Sandgreen, J.R. Smol, and J. Kristiansen, pp. 46-74. Cambridge: Cambridge University Press.

Sanches,F., Cardenas, L. & Quinto, C. 1999. Biological nitrogen fixation and future challenges of agriculture. The endophytic connection. Adv. Exp. Med Biol. **464**:107-115.

Sanders, R. W. 1991. Mixotrophic protists in marine and freshwater ecosystems. J. Protozool. **38**:76-81.

Schnepf, E. & Melkonian, M. 1990. Bacteriophage-like particles in endocytic bacteria of *Cryptomonas* (Cryptophyceae). Phycologia **29**:338-343.

Silva, E.S. 1978. Endonuclear bacteria in two species of dinoflagellates. Protistologica **14**:113-119.

Silva, E.S. & Franca, S. 1985. The association dinoflgellate-bacteria: their ultrastructural relationship in two species of dinoflagellates. Protistologica **21**:429-446.

Singh, R.N. 1961. *The Role of Blue-Green Algae in Nitrogen Economy of Indian Agriculture*. Indian Council for Agricultural Research. New Delhi, India.

Surek, B. & Melkonian, M. 1983. Intracellular bacteria in Euglenophyceae: prolonged axenic culture of an algal-bacterial system. In: Endocytobiology II (Ed. By H.E.A. Schenk & W. Schwemmler), pp. 475-486. Walter de Gruyter. Berlin & New York.

Turner, J.B. & Friedmann, E.I. 1974. Fine structure of capitular filaments in the coenocytic green alga *Penicillus*. J. Phycol. **10**:125-134.

Wilhelm, S.W., Maxwell, D.P. & Trick, C.G. 1996. Growth, iron requirements, and siderophore production in iron-limited *Synechococcus* PCC 7002. Limnol. Oceanog. **41**:89-97.

Wujeck, D. E. 1978. Ultrastructure of flagellated chrysophytes. III *Mallomonas caudata*. Trans. Kansas Acad. Sci. **81**:327-336.

Wujeck, D.E. 1982. Bacteria in the perinuclear cisternae of the chrysophytes *Chrysosphaerella*. Trans. Kansas Acad. Sci. 85.

Wujeck, D.E., Gardiner, W.E., & Dawes, C.J. 1982. Intracellular bacteria in the green scaly prasinophyte *Tetraselmis*. Cryptogamie. Algologie **2**:121-127.

Yun, G.Y., Tiedeman, M.N., Moore, J.P. & Philpott, T.C. 2000. Investigation of the mechanism of iron acquisition by marine bacterium Alteromonas luteovilaceous: characterization of siderophore production. Limnol. Oceanogr. **36**:1783-1792.

Zahran, H.H. 1999. *Rhizobium*-legume symbiosis and nitrogen fixation under severe conditions and in an arid climate. **63**(4):968-989.

Zher, J.P., Carpenter, E.J. & Villareal, T.A. 2000. New perspectives on nitrogen-fixing microorganisms in tropical and subtropical oceans. Trends Microbiol. **8**(2):68-73.

CHAPTER 5

OCHROMONAS PLEIOMORPHA, SP. NOV. FROM WYOMING: A PREDATOR OF DIATOMS AND A CANNIBAL

ABSTRACT

The ultrastructure of *Ochromonas pleiomorpha* sp. nov. is described. Cells are variable in shape, but all possess a single U-shaped chloroplast with lobes of different lengths, and a stigma located in the dorsal lobe. The dorsal lobe usually is shorter than the ventral lobe. A pyrenoid is located toward the inside of the chloroplast and is adjacent to the nucleus on the concave side of the chloroplast. The pyrenoid is invaded by several tubular chloroplast envelope invaginations. A chrysolaminarin vesicle is posterior to the nucleus, and the vesicle membrane is in contact with the posterior portion of the nuclear envelope. The ventral portion of the cell has a concentration of small vesicles that presumably are pre-lysosomal vesicles and/or secondary lysosomes. Light and scanning microscopy revealed that cells may form numerous cytoplasmic extensions (rhizopodia, pseudopodia, filipodia) and may attach to a substrate by a posterior extension. Furthermore, the cytoplasmic extensions may be branched. These extensions may form from extrusive vesicles located underneath the plasma membrane. The contents of the vesicles are dark staining and upon discharge the contents become more diffuse. Neither actin microfilaments nor microtubules were observed in these extensions. *O. pleiomorpha* is an active mixotroph, feeding on diatoms and on its own cells. Diatoms and *Ochromonas* cells are digested in food vacuoles, which compress the chrysolaminarin vesicle and dislocate it toward the posterior of the cell. After the

contents of ingested diatoms are digested, empty frustules are released from the food vacuole.

INTRODUCTION

Ochromonas is a common unicelled flagellate belonging to the Class Chrysophyceae of the Division Chrysophyta, or golden-brown algae, and it is thought to be the primitive cell type in the chrysophyte phylogenetic lineage (Andersen 1982). More than 50 species have been described but only three have been examined ultrastructurally (Hibberd 1970; Aaronson 1974; Andersen 1982 and Andersson, Falk, Samuelsson & Hageström 1989). *Ochromonas* occurs in freshwater, brackish water and marine habitats and is characterized by naked cells with two heterokont flagella. The shorter flagellum is associated with an eyespot or stigma. *Ochromonas* is usually planktonic, although it also can take several other growth forms, depending on the species. Some species may form palmelloid colonies after the flagella are withdrawn (Huber-Pestalozzi 1941), while others may attach to substrates by their tail ends (Huber-Pestalozzi 1941). All species are photosynthetic, but some ingest bacteria in addition to being photosynthetic, and thus are classified as mixotrophs. *Ochromonas* is difficult to assess taxonomically as to whether many other genera should be placed in this genus or whether some *Ochromonas* species should be classified as other genera since a broad investigation has not been conducted. For instance, *Poterioochromonas malhamensis*, initially described as *Ochromonas* (Pringsheim 1952), can form rhizopodia and ingest a variety of bacteria and algae (Holen 1999). It was separated from *Ochromonas* after electron microscopic studies revealed that it had a thin chitinous lorica (Peterfi 1969) by

which it attaches to a substrate. A lorica is a cell covering that is not intimately associated with the cell membrane and is comprised of non-cellulosic material.

A chrysophyte was isolated that resembled *Poterioochromonas*, but this tentative identification was based on light microscopic observations. It was isolated from a reservoir in Wyoming, and like *Poterioochromonas*, it is phagocytic, and it can attach to a substrate, but it routinely ingests diatoms. Subsequent EM observations revealed that it lacked a lorica, and attached to the substrate by a rhizopodium and therefore it is obviously an *Ochromonas*. Since it routinely forms rhizopodia, it differs from other *Ochromonas* species and probably justifies the establishment of a new species. Its description and ultrastructure are presented in this chapter, and the proposed species name is *Ochromonas pleiomorpha*, because it changes shape constantly.

RESULTS

Light Microscopy

Cells are variable in shape, and they can be either free-swimming or attached to a substrate by long stalks (Fig. 1). Each cell has a single, U-shaped chloroplast (Fig.1). Cells are generally 12-14 μm in length and 4 - 9 μm in diameter. A broader diameter usually indicates ingested particulates, specifically diatoms. A series of light micrographs shows the same cells extending and retracting their rhizopodia (Figs. 2-4). These rhizopodia are used to attached the cells to a substratum and facilitate the capture of particulates.

Electron microscopy

Scanning electron microscopy

Cells of *O. pleiomorpha* are highly variable in shape, as illustrated by the SEMs. The basic cell shape is pyriform (Fig. 13) and depending on ingested material or feeding activity, the cell changes shape accordingly (Figs. 7, 8, 9, 17, 18). Sometimes the cells become ovoid (Fig. 5), obovoid (Fig. 6), conical (Figs. 9, 11, 12. 116), oblong (Figs.19, 20), or almost spherical (Figs. 11, 14, 18). Many cells have cytoplasmic extensions (Figs. 7, 8) by which they may attach to substrates, or conceivably use to capture prey organisms. Due to this variability in cell morphology, cells from field material could be misidentified as different species.

There appear to be two types of diatoms in the culture. Both form short filaments (Figs. 21-25), but they also occur as individual cells (Figs.22-23). Cells produce a thick mucilage that surrounds the whole cell or filament and attaches them to the substrate. The pennate diatoms have a bilateral symmetry (Fig. 22), and while the centric diatoms have a radial symmetry with several labiate processes in the hypotheca (Fig. 23). Several attempts were made to clear the frustules; however, the methods employed were not successful. Thus, the diatoms could not be identified to genus.

Transmission electron microscopy

The basic cell structure of *O. pleiomorpha* is shown in Figure 26. Cells that lack food particulates are mostly pyriform to oblong in longitudinal section and the posterior end generally is pointed. The nucleus is located in the anterior portion of the cell and the single chloroplast appears as two chloroplasts in sectioned material, but these represent two lobes. One of the lobes generally is shorter in length but both lobes partially flank the anterior portion of chrysolaminarin vesicle. The outer membrane of the nuclear envelope extends around the chloroplast as a double membraned structure, forming the chloroplast endoplasmic reticulum (Figs. 26-32). The dorsal side of the cell has a concentration of small vesicles, and this concentration of vesicles invariably is on the side that contains the stigma and the short flagellum that is associated with the stigma. This side would represent the dorsal side of the cell. The small vesicles might be pre-lysosomal vesicles, since some appear to fuse and form a larger vesicle. In addition, a chrysolaminarin vacuole is located posterior to the nucleus, and its membrane in the anterior region usually is in contact with the nuclear envelope. A striated rhizoplast extends from the longitudinally oriented flagellum and attaches to the nuclear envelope (Fig.28). A Golgi apparatus is ventral to the striated rhizoplast and on the side opposite the vesicles (Figs. 27, 28). Based on its location and general inactive appearance, it does not appear likely that the Golgi apparatus is responsible for vesicle formation.

The chloroplast is U-shaped in transverse section and a pyrenoid occurs nearest the nucleus (Figs. 30, 32). The pyrenoid is on the internal side of the U-shaped chloroplast (Figs. 30, 32), and it has membranous invaginations of the chloroplast envelope. In a transverse view the pyrenoid is semicircular, and in a longitudinal view it

is somewhat rectangular in shape, appearing as a bridge between the lobes of the chloroplast at this level of sectioning.

An eyespot or stigma is located in the anterior end of the dorsal lobe of the chloroplast near the region of the vesicles (Figs. 31, 33), on the side opposite the Golgi apparatus. Triplet thylakoids generally are absent in this lobe (Figs. 31, 33).

During isolation of *O. pleiomorpha* two diatom contaminants also were included, but these went undetected for several years until EM examinations of *O. pleiomorpha* were conducted. The ingestion of diatoms was first observed with an inverted light microscope. Cells were attached to the bottom of the culture vessel by stalks and the cells rotated around the axis of this attachment. Additionally, the cells' flagella were creating a vortex that drew particulates into the anterior region of the cell. When a diatom was drawn near the dorsal side of the cell, it was rapidly ingested and became visible within a vacuole in the cell. This process was observed several times, in different cells, and within a few minutes, which prompted this study. An ingested diatom is seen in the food vacuole in Figures 34-36, in various stages of digestion. The food vacuole is on the dorsal side where the vesicle accumulation was noted previously. The contents of the diatom are digested, leaving the frustule in the food vacuole. Eventually the diatom frustule is expelled from the food vacuole by exocytosis (Fig. 37). The diatom in the culture is either a raphid naviculoid diatom or centric diatom. Both form pseudofilaments, but neither has been identified. A raphe of the naviculoid diatom is visible in the epitheca and hypotheca when viewed in transverse sections (Fig. 38). SEM confirms the presence of a

raphe and some ornamentation can also be seen, but to date the clearing of the diatom, by using either 30% H_2O_2 or concentrated H_2SO_4, has not been successful.

In addition to ingesting diatoms, this alga ingested cells of its own species (Figs. 39, 40). Even though the cells were in an advanced state of digestion, the characteristic pyrenoid of this species (Figs. 39, 40) was still identifiable within the food vacuole, specifically by the invaginations of the chloroplast envelope. The large food vacuole with its contents displaces the chrysolaminarin vacuole (Fig. 39) toward the posterior or ventral side of the cell. The food vacuole forms on the side where the small vesicles are concentrated. (Figs. 39,40).

This alga routinely forms cytoplasmic extensions or rhizopodia (Figs. 7, 8). These extensions are fairly common in cells observed with SEM, but the structures are difficult to identify in thin sections. Nevertheless, elevations or extensions were commonly noted along the periphery of cells in sectioned material (Figs. 41, 42), and these may represent precursors to the extensions. All are vesicular, and in the undischarged state they appear to contain dark staining reticulate material. Expanded or discharged vesicles contain less of the darkly staining material and frequently the dark staining contents are no longer present. Perhaps the dark staining material represents stored membranes that would expand during the expansion. If this is the case, then this represents a new type of extrusosome for algae. The function of these extensions may be food capture or substrate attachment.

***Ochromonas pleiomorpha* sp. nov.**

Aguiar, R. & Kugrens, P.

DIAGNOSIS

Cellulae motiles, variabiles formes, et pyrifome aut spherice, ovale, conicae, plerumque 12-15 μm longae, 6-9 μm latae. Flagella duo inaequalia approximate infronte depressiona inserta. Plastus unus, luteo-brunneus, une pyrenoide. Stigma unum rubrum, figura disculi plani vel concavi, ad extremum anticum in pare speciali incrassata lipoidlibus circumcinctus. Nucleus unus, anterioris in parte antica cellulae. Vacuola contractile non observata. Unacellulae vesculis chrysolaminri posteriore. Mixotrophicae, diotomae ingestione.

Cells motile, variable in form and may be pyriform, spherical, oval or conical, 12-15μm in length and 6-9 μm in width. Two unequal flagella are inserted in an anterior depression of the cell. Cells have a single plastid with a pyrenoid. The stigma is red and convex, located in the extreme anterior and consists of lipid granules. A single nucleus is located in the center of the cell, mainly in the anterior one-third of the cell. A contractile vacuole was not observed. A single chrysolaminarin vesicle occurs in the posterior one third of the cell.

DISCUSSION

Evidence supports the fact that mixotrophy is an important factor in aquatic food webs (Azam, Fenchel, Field, Gray, Meyer-Reil & Thihgstad 1983, Fenchel 1987, Jones & Ilmavirta 1988, Sherr, Sheer, & McDaniel 1991, Sanders 1991) but during the last five to seven years there has been a decline in the study and survey of organisms with this type of nutrition. Mixotrophy is a common mode of nutrition among chrysophytes (Boraas, Estep, Johnson & Sieburth 1988) and is defined as a type of nutrition where a photosynthetic alga also exhibits phagotrophy and has the ability to change from one nutritional mode to another, depending on changes in the environment (Boraas, Estep, Johnson & Sieburth 1988, Boraas, Seale and Holen 1992, Sanders, Porter, Bennett & DeBiase 1989). Mixotrophic chrysophyte genera are *Dinobryon* (Boraas, Estep, Johnson & Sieburth 1988, Berninger, Caron & Sanders 1992), *Uroglena* (Kimura & Ishida 1989), *Chrysostephanosphaera* (Sanders, Porter, Bennett and DeBiase 1989), *Pedinella* , *Cyrtophora*, *Ochromonas*, (Boraas, Selae, Holen 1992, Cole and Wynne 1974, Daley, Morris, and Brown 1973, Dubousky 1974, Sanders, Porter, Bennett and DeBiase 1989), *Poterioochromonas* (Caron, Porter, & Sanders 1990, Herth, Kuppel, and Schnepf 1977, Holen 1999, Holen and Boraas 1995, Pringsheim 1951,) and several prymnesiophytes such as *Chrysochromulina* (Estep, Davis, Hargraves and Sieburth 1984, Jones, Leadbeater and Green 1993, Sanders 1991, Sanders, Porter and Caron 1990). To properly understand the ecological role of mixotrophs, detailed systematic analyses are needed to determine which organisms are mixotrophic, which organisms are selectively

preyed upon, and how efficient these mixotrophs are in affecting or depleting prey populations. Colorado and Wyoming have not been surveyed with respect to mixotrophic organisms and this is an opportune region for additional studies on biodiversity of mixotrophs and other algae. As a result of numerous isolations of mixotrophic algae, an expanded study of mixotrophs and their roles in local lakes is being conducted. This is the first report of a new mixotroph from Wyoming waters, and it appears to be a new species of *Ochromonas*.

Ochromonas is a ubiquitous, common and important genus in freshwater, brackish and marine habitats, but species comparisons remain difficult since so few *Ochromonas* spp. have been examined ultrastructurally. This level of resolution is required to determine the differences in characters among the species. Thus it becomes problematic when a new species is proposed since only diagrams based on light microscopic observations exist for the remaining species. After a careful examination of the published diagrams of known species, the description of *O. pleiomorpha* as a new species appears justified. None of the described species have cytoplasmic extensions, and cells are not as variable in cell shape.

This study has documented, for the first time, mixotrophy in *Ochromonas* that involves the ingestion of diatoms, although diatom ingestion by small colorless flagellates has been reported (Suttle, Chan, Taylor & Harrison 1986). Furthermore, exocytosis eliminates the frustule from the cell and thus represents a primitive excretory system in a unicelled alga. The diatom initially proliferates until the population of *O. pleiomorpha* increases and begins to feed actively on the diatoms. The ingestion of

diatoms is more rapid than their ability to reproduce, consequently the entire diatom population often is eliminated. Thus one more aspect of mixotrophy is added and that is the influence that *O. pleiomorpha* can exert on the phytoplankton abundances in a given body of water.

This study also has shown that mixotrophy can be inferred from cell structure, due to the concentration of small vesicles that may represent small lysosomal vesicles, and they are located in a specific region of the cytoplasm. Once the prey is ingested, these vesicles fuse and form a larger food vacuole where the prey is digested. A specialized feeding apparatus was not observed thus the dynamics of phagocytosis remain unknown.

Surprisingly, few *Ochromonas* spp. have been examined with the electron microscope, although there are more than 50 described species. Only *Ochromonas danica* (Pringsheim 1952), *O. tuberculatus* (Hibberd 1970), *O. sphaerocystis* (Andersen 1982), and *O. minuta* (Hill & Outka 1974) have been examined and described. The cellular structures and their arrangements suggest that this isolate belongs to *Ochromonas*. The flagella are heterokont, they are not inserted parallel to each other, and there is a flagellar swelling on the short flagellum in the region where it is associated with the stigma. The pyrenoid structure differs from previously described *Ochromonas* species, but the importance of the pyrenoid variations needs to be determined in the future.

The major difference that justifies the establishment of new species is the occurrence of cytoplasmic extensions. There have been several reports of cytoplasmic extensions for *Poterioochromonas malhamensis* (Peterfi 1969), *Pedinella*, *Apedinella* and

Chrysameba, and they have been termed rhizopodia. In these genera, the rhizopodial extensions contain rod-like filaments that might be microfilaments. No similar structures were found in *O. pleimorpha.* Instead there were small vesicles that contain a dark staining reticulate material that could be the precursors that form the cytoplasmic extensions seen with SEM. These vesicles appeared to bulge from the cell and were thin. The plane of sections did not go through a more elongated extension but these are likely candidates for the structures observed with SEM. The function of these extensions might be attachment to a substrate so that diatoms can be captured and ingested or they may be used in attaching to the prey and pulling the prey to the cell for ingestion. However, attachments to diatoms were not observed.

Light microscopic observations clarified the capture of the prey organisms. Two mechanisms aid in the capture of particulates. The first involves a vortex created by the beating of the long flagellum. Particles are drawn into this vortex but most are not taken into the cell. When a diatom was drawn into the vortex, it seemed to attach quickly to the anterior dorsal side of the cell and drawn in immediately. The second vortex involves the cell being attached to a substrate. By the beating of its long flagellum the cell rotates in a wider circle around the stalk thus increasing the area for capturing particulates.

There have been several reports of cannibalism in *Ochromonas* (Aaroson 1974, Andersen & Moestrup 1991) but electron micrographic evidence had not been provided. In this study thin sectioned cells routinely contained remnants of *O. pleiomorpha* in the food vacuoles. Even though the prey cell had been digested in the food vacuole, the pyrenoidal structure remained recognizable so that the contents could be identified as *O.*

pleiomorpha. Whether the cells ingest weakened or dead cells from the culture could not be determined, but it is probable that this might be the case.

The specific need for phagotrophy in photosynthetic species appears to be varied, and one function cannot be ascribed to all mixotrophs. In one instance there is a loss of the ability to synthesize a compound essential for growth, and the mixotroph compensates for this loss by obtaining the required compound from ingesting an appropriate organism. Specifically these mixotrophs may be unable to synthesize vitamin B_1 or other B group vitamins or phospholipids (Kimura & Ishida 1985, 1989). This inability to synthesize phospholipids occurs in *Uroglena Americana,* and it cannot grow without ingesting bacteria. Photosynthesis is essential but without phagocytosis of bacteria, growth does not occur, even in the presence of added vitamins.

Mixotrophy also is a means of providing a carbon source for energy in the absence of light (Bird and Kalff 1987). In the daytime, the mixotroph photosynthesizes and fixes its own carbon whereas at night additional carbon is obtained by ingesting bacteria or other organisms.

Minerals, such as phosphorous and nitrogen can be obtained in a concentrated form from prokaryotes (Boraas, Estep, Johnson & Sirburth 1988). Bacteria and cyanobacteria have nutrient concentrating capabilities that eukaryotic organisms do not possess. The acquisition of nutrients and growth factors may be particularly important in oligotrophic environments. In some of these oligotrophic waters mixotrophs, such as *Ochromonas* and *Chrysameba*, make up more than 65% of the phytoplankton population (Estep, Davis, Hargraves, & Sieburth 1984).

In the thousands of cells that were examined, bacteria were rarely found in the food vacuole, which is rather unusual. Bacterivory is common in all of the other mixotrophs that have been studied where bacteria supply a variety of essential inorganics or organics, such as vitamins and phospholipid, to promote and/or sustain the growth of the food organism. In addition, the bacteria provide an alternate source of carbon. Diatoms and *O. pleiomorpha* cells probably contribute only to the carbon flux, unless they also contain vitamins or other organics that cannot be synthesized by *Ochromonas*.

Considering the importance of *Ochromonas* in mixotrophy, it is important to study, in detail, the systematics and structure of a broad spectrum of *Ochromonas* and *Chromulina* species to determine the variations among species in these two genera, and whether the separate genera are justified. For instance *O. pleiomorpha* initially was incorrectly identified to be *Chromulina* species because only one flagellum was evident, but upon closer examination a second flagellum was found. A literature search on the ultrastructure of these two genera yielded limited and non-comparable information because so few species have been investigated.

REFERENCES

Aaronson, S. 1974. The biology and ultrastructure of phagotrophy in *Ochromonas danica* (Chrysophyceae: Chrysomonadida). J. Gen. Microbiol. **83**:21-29.

Andersen, R.A. 1982. A light and electron microscopical investigation of *Ochromonas sphaerocysts* Mativenko (Chrysophcyeae): The statospore, vegetative cell and its peripheral vesicles. Phycologia **21**:390-398.

Andersen, R.A. & Moestrup, O. 1991. Microtubules of the flagellar apparatus are active during prey capture in the chrysophycean alga *Epipyxis pulchra*. Protoplasma 166:8-20.

Andersson, A., Falk, S., Samuelsson, G. & Hageström, A. 1989. Nutritional characteristics of a mixotrophic nanoflagellate *Ochromonas* sp. Microbial Ecol. **17**:252-262.

Azam, F., Fenchel, T., Field, J. G., Gray, J. S., Meyer-Reil, L. A. & Thingstad, F. 1983. The ecological role of water-column microbes in the sea. Mar. Ecol. Prg. Ser. **10**:257-263.

Berninger, U.-G., Caron, D.A. & Sanders, R. W. 1992. Mixotrophic algae in three ice-covered lakes of the Pocono Mountains, U.S.A. Freshwater Biol. **28**:263-272.

Bird, D. F. and Kalff, J. 1987. Algal phagotrophy: Regulating factors and importance relative to photosynthesis in *Dinobryon* (Chrysophyceae) Limnol. Oceanogr. **32**: 277-284.

Boraas, M. Estep, K. W., Johnson, P. W. and Sirburth, J. McN. 1988. Phagotrophic phototrophs: the ecological significance of mixotrophy. J. Protozool. **35**:249-252.

Boraas, M. E., Selae, D. B. & Holen, D. 1992. Predatory behavior of *Ochromonas* analyzed with video microscopy. Arch. Hydrobiol. **123**:459-468.

Caron, D.A., Porter, K.G. & Sanders, R.W. 1990. Carbon, nitrogen and phosphorous budgets for the mixotrophic phytoflagellate *Poterioochromonas malhamensis* (Chrysophyceae) during bacterial ingestion. Limnol. Ocenogr. **31**:646-650.

Cole, G.T. & Wynne, M.J. 1974. Endocytosis of *Microcystis aeruginosa* by *Ochromonas danica*. J. Phycol. **10**:397-410.

Daley, R. J., Morris, G. P., & Brown, S. R. 1973. Phagotrophic ingestion of a blue-green alga by *Ochromonas*. J. Protozool. **20**:58-61.

Dubboursky, N. 1974. Selectivity of ingestion and digestion in the chrysomonad flagellate *Ochromonas malhamensis*. J. Protozool. **21**:295-298.

Estep, K. W., Davis, P. G., Hargraves, P. E. & Sieburth, J. McN. 1984. Chloroplast sustaining flagellates in natural populations of North Atlantic nanoplankton, their identification and distribution; including a description of five new species of *Chrysochromulina* (Prymnesiophyceae). Protistologica **20**:613-634.

Fenchel, T. 1982. Ecology of protozoa. The Biology of Free-living Phagothrophic Protist. 1-197. Science Tech Publishers, Madison, WI.

Herth, W., Kuppel, A. & Schnepf, E. 1977. Chitinous fibrils in the lorica of the flagellate chrysophyte *Poterioochromonas stipitata* (Syn. *Ochromonas malhamensis*). J. Cell Biol. **73**:311-321.

Hibberd, D. J. 1970. Observations on the cytology and ultrastruture of *Ochromonas tuberculatus* sp. nov. (Chrysophyceae), with special reference to the discobolocysts. Br. Phycol. J. **5:**119-143.

Hill, F. G. & Outka, D. E. 1974. The structure and origin of mastigonemes in *Ochromonas minuta* and *Monas* sp. J. Protozool. **21**:299-312

Holen, D. 1999. Effects of prey abundance and light intensity on the mixotrophic chrysophyte *Poterioochromonas malhamensis* from a mesotrophic lake. Freshwater Biol. **42**:445-455.

Holen, D. & Boraas, M. E. 1995. Mixotrophy in chrysophytes. Chrysophyte Algae (Eds. C. D. Sandgren, Smol, J. P. & Kristiansen, J. Cambridge Univ. Press, Cambridge. pp. 119-140.

Huber-Pestalozzi, G. 1941. Das Phytoplanton des Susswassers. 2. Teil. Chrysophyceen. Farblose Flagellaten, Hetrokonten. E. Schweizerbart'sche Verlagsbuchhandlung, Stuttgart. 365 pp.

Jones, H.L.J., Ledbeater, B.S.C. & Green, J.C. 1993. Mixotrophy in marine species of *Chrysochromulina* (Prymnesiophyceae): Ingestion and digestion of a small green flagellate. J. Mar. Biol. Assoc. U. K. **73**:383-296.

Kimura B. & Ishida, Y. 1985. Photophagotrophy in *Uroglena americana*, Chrysophyceae. Jpn. J. Limnol. **46**:315-318.

Kimura B. & Ishida, Y. 1989. Phospholipid as a growth factor of *Uroglena americana*, a red tide chrysophyceae in Lake Biawa. Nippon Suisan Gakkaishi **55**:799-804.

Peterfi, L.S. 1969. The fine structure of *PoterioOchromonas malhamensis* (Pringsheim) comb. nov. with special reference to the lorica. Nova Hedwigia **17**:93-103.

Pringsheim, E.G. 1952. On the nutrition of *Ochromonas*. Q.J. Microsc. Sci **93**:71-96.

Sanders, R.W. 1991. Mixotrophic protists in marine and freshwater ecosystems. J. Protozool. **38**:76-81.

Sanders, R.W., Porter, K.G., Bennett, S.J. & DeBiase, A. E. 1989. Seasonal patterns of bacterivory by flagellates, ciliates, rotifers, and cladocerans in a freshwater planktonic community. Limnol. Oceanogr.**34**:673-687.

Sanders, R.W., Porter, K.G. & Caron, D.A. 1990. Relationship between phototrophy and phagotrophy in the mixotrophic chrysophyte *Poterioochromonas malhamensis*. Microb. Ecol. **19**:97-109.

Sheer, E.B., Sheer, B.F. & McDaniel, J. 1991. Clearance rates of less than 6 μm fluorescently labeled algae (FLA) by estuarine protozoa: potential grazing impact of flagellates and ciliates. Mar. Ecol. Prog. Ser. **69**:81-82.

Suttle, C. A., Chan, A. M., Taylor, W. D. & Harrison, P. J. 1986. Grazing of planktonic diatoms by microflagellates. J. Plank. Res. **8**:393-398.

APPENDIX 1: MATERIALS AND METHODS

MATERIALS AND METHODS

Collection Sites for chrysophytes used in this study.

1. *Phaeaster pascheri* var. *avesiculosa* were collected from Dowdy Lake, Larimer County, Colorado (40^0 47'49'' N, 105^0 33'21'' W).

2. *Diacronema vlkianum* were collected from Diamond Lake, Carbon County, Wyoming (41^0 36'29'' N, 1060 39'48'' W) and South Delaney Buttes Lakes, Jackson County, Colorado (40^0 42'09'' N, 106^0 27'37'' W).

3. *Hymenomonas* sp. were collected from Cowdrey Lake, Jackson County, Colorado (40^0 51'28'' N, 105^0 51'28'' W).

4. *Prymnesium wyomingii* were collected from Twin Buttes Lake, Wyoming (41^0 14'06'' N, 105^0 51'32'' W).

5. *Chrysochromulina asquamosa* were collected from Dowdy Lake, Larimer County, Colorado (40^0 47'49'' N, 105^0 33'21'' W), Lake John, Larimer County, Colorado (40^0 46'34'' N, 106^0 28'18'' W), Rolland Moore Park Pond, Larimer County, Colorado (40^0

33'32'' N, 105^0 05'48'' W), South Delaney Buttes Lakes, Jackson County, Colorado (40^0 42'09'' N, 106^0 27'37'' W).

6. *Uroglena volvox* were collected from North Shields Ponds, Larimer County, Colorado (40^0 36'09'' N, 105^0 05'45'' W).

7. *Uroglena* sp. were collected from South Delaney Buttes Lakes, Jackson County, Colorado (40^0 42'09'' N, 106^0 27'37'' W).

8. *Uroglena articulata* were collected from Dowdy Lake, Larimer County, Colorado, (40^0 47'49'' N, 105^0 33'21'' W), and

9. *Ochromonas pleiomorpha.* were collected from Diamond Lake, Carbon County, Wyoming (41^0 36'29'' N, 106^0 39'48'' W).

All samples were kept on ice and transported to the laboratory for isolation and culturing.

Cultures

Cells were isolated into unialgal culture by the serial dilution micropipette technique (Hoshaw & Rosowski 1973). Unialgal cultures were grown in 125 ml Erlenmeyer flasks and maintained in sterilized lake water enriched with Alga-Gro

concentrate (Carolina Biological Supply, Co.) at 40 ml Alga-Gro/L of water. Cultures were maintained in an environmental growth chamber at 18 °C, in 16:8 light /dark regimes.

Scanning electron microscopy (SEM)

For scanning electron microscopy, cells were fixed according to the method described by Parducz (1967) and modified by Clay & Kugrens (1999). Four ml of culture were transferred to a fixative solution that consisted of three parts 4% osmium tetroxide, three parts of distilled water, and one part saturated mercuric chloride. Following a 1 h fixation, the cells were filtered through a Nucleopore membrane (pore size = 2 μm), rinsed several times with distilled water to remove salts and fixatives, dehydrated in an ethanol series, dried in a BioRad critical point dryer using liquid carbon dioxide, sputter-coated with gold (17 nm) in a Hummer VII sputter-coater, and viewed in a Philips 505 scanning electron microscope with accelerating voltage = 20 KeV.

For shadow casting, a drop of actively growing culture was placed on formvar coated copper grids. Cells were exposed to osmium tetroxide vapors for 15 to 30 seconds (Clay & Kugrens 1999), and allowed to settle. Water was removed by placing filter paper on the edge of the grid. Grids were shadowed with platinum and carbon at an angle of 45° in a Kinney Vacuum – Model KDTG - 3P. Cells were examined with an AEI 801 transmission electron microscope.

For transmission electron microscopy (TEM), cells were fixed either for 2 h (*Phaeaster pascheri* var. *avesiculosa, Hymenomonas* sp., *Uroglena volvox, Uroglena* sp. and *Ochromonas pleiomorpha*) or 1.5 h (*Diacronema vlkianum*, *Prymnesium wyomingii, Chrysochromulina asquamosa* and *Uroglena articulata*) in 2.5% glutaraldehyde buffered at pH 7.8 with 0.1M Na-cacodylate. Cells were centrifuged and the resultant pellet was embedded in agar. Following three rinses in buffer, cells were post-fixed in buffered 2% osmium tetroxide for 2 hr, dehydrated in an acetone series, embedded in Spurr's Epoxy, and polymerized in an oven at 70 °C for 8-12 hr. Thin sections were obtained with a diamond knife mounted on a Sorvall MT-2 ultramicrotome. Sections were post-stained with 1% uranyl acetate followed with Reynold's lead citrate using different times for different species. These are indicated by the following.

(1) 10 minutes in 1% uranyl acetate followed by 18 minutes in Reynold's lead citrate solution *Prymnesium wyomingi*

(2) 10 min. in 1% uranyl acetate followed by 2 min. in 0.2% Reynold's lead citrate solution: *Phaeaster pascheri* var. *avesiculosa, Hymenomonas* sp., and *Uroglena* sp.,

(3) 7 min. in 1% uranyl acetate followed by 3 minutes in 0.2% Reynold's lead citrate solution *Ochromonas pleiomorpha*

(4) 6 min. in 1% uranyl acetate followed by 2 min. in 0.2% lead citrate: *Uroglena volvox* and *U. articulata,*

(5) 4 min. in 1% uranyl acetate followed by 2 minutes in 0.2% Reynold's lead citrate solution, and *Diacronema vlkianum* and *Chrysochromulina asquamosa.*

The following cultures were stained in block with 1% uranyl acetate for 10 minutes.: *Phaeaster pascheri* var. *avesiculosa, Diacronema vlkianum,Prymnesium wyomingi., Chrysochromulina asquamosa, Ochromonas pleiomorpha* and *Uroglena sp.* Thin sections were viewed with an AEI 801 transmission electron microscope.

Light Microscopy

Light microscopic examinations were made with a Reichert microscope using Normarski differential interference contrast (DIC) optics. Kodak T-Max 100 film was used for photomicrography.

REFERENCES

Clay, B. & Kugrens, P. 1999. Description and ultrastructure of *Kathablepharis tenuis* sp. nov. and *K. obesa* sp. nov.-two new freshwater Kathablepharids (Kathablepharididae) from Colorado and Wyoming. Europ. J. Protistol. **35**:435-447.

Hoshaw, R.W. & Rosowski, J.R. 1973. Isolation and purification. 3: Methods for microscopic algae. Handbook of Phycological Methods. J. E. Stein. New York, Cambridge University Press: 53-67.

Parducz, B. 1967. Ciliary movement and coordination in ciliates. Int. Rev. Cytol. **21**: 91 - 128.

APPENDIX 2: TABLES

TABLE 1. Comparative Morphology of *Diacronema vlkianum* Prauser (Grenn & Hibberd 1977) and *Diacronema vlkianum* from Colorado (Aguiar & Kugrens 2000)

CHARACTERS	*D. vlkianum* (Green & Hibberd 1977)	*D. vlkianum* (Aguiar & Kugrens 2000)
Habitat	Marine / Freshwater	Freshwater
Dimensions (μm)	3.5 – 7.5 x 3.5 – 7.0	2.0 – 5.0 x 2.5 – 4.5
Shape (μm)	Broadly oval	Oval, reniform
Compression (μm)	Dorsiventrally	Dorsiventrally
Dilated PER	Absent	Present
Flagellar insertion	Median (Concave face)	Median (Concave face)
Long flagellum (μm)	7.0	7.0
Short flagellum (μm)	6.0	3.0
Haptonema length (μm)	Present (1.0)	Present (0.5)
Haptonema tip	Narrowed	Narrowed
Flagellar hairs		
Long flagellum (LF)	Non-tubular hairs	Tubular hairs
Short flagellum (SF)	Absent	Absent
Flagellar narrow tips	Present	Present
Stigma	Present (Single layer)	Present (Single or Group)
	Variable in location	Variable in location
Flagellar swelling (SF)	Present	Present
Plastids	1 – 3 lobes, parietal	1 –3 lobes, parietal
Pyrenoid	Absent	Not observed
Contractile vacuole	1	Absent

Table 2. Selected Comparative Features of *Hymenomonas roseola* Stein and *Hymenomonas sp. nov.* from Colorado.

CHARACTER	***Hymenomonas roseola***	***Hymenomonas*** from Colorado
Life Stage	Motile and Non-motile	Non-motile
Filamentous stage	Present	Absent
Presence of an outer membrane	Absent	Present
Flagella	Present	Absent
Haptonema	Present	Not observed
Type of coccolith	Tremaliths	Tremaliths
Base-plate contours	Discontinuous	Continuous
Shape of coccoliths	Crown	Crown
Number of coccolith layers	1	1
Type of unmineralized scales		
(underneath coccolith layer)	2	2
Pattern of fibrils		
Proximal surface	Radiating fibrils	Concentric fibrils
Distal surface	Concentric fibrils	Concentric fibrils
Type of rim	Broad inflexed rim	Narrow rim
Columnar deposit	Present	Present
Number of chloroplast/cell	2	4
Number of traversing thylakoids	2, 3 or 4	2, 3 or 4
Contractile vacuole	Present	Not observed
Habitat	Freshwater	Freshwater

Table 3. A comparison of cell sizes, flagella and haptonema length, ratio of flagellum length to cell length (F/C), and ratio of haptonema length to flagellum length (H/F) in the genus *Prymnesium*

Species	Cell dimensions (µm)	Flagella length (µm)	Haptonema length (µm)	F/C	H/F	Reference
Prymnesium wyomingi	5.0 x 6.5	6.5 – 11	2 - 3	1.7	0.25	Aguiar & Kugrens (2000)
P. parvum	6.0 – 12 x 3.5 – 8.0	10 – 14.5	3 – 5	1. 4 – 1.5	0.33	Green et al (1999)
P. annuliferum	10 – 14 x 6.5	20 – 22	5 – 6	1 .8	0.26	Billard (1983)
P. zebrinum	10 – 12 x 5.5	15 – 16	3	1. 41	0.19	Billard (1983)
P.calathiferum	6.2 – 10.3 x 4.3 -7.9	11.4 – 18.2	2.2 – 2.8	2.15 –3.03	0.16	Chang & Ryan (1985)
P. saltans	11 – 18 x 5 – 7.5	–	2.5 – 5.0	2.0	–	Massart ex Conrad (1926)
P. minutum	6.0 – 8.5 x 3.5	–	–	1.7	–	Carter (1937)

Table 4. The saddle-shaped species of *Chrysochromulina* presently described.

Species	Cell Dimensions Length x width (µm)	Length of flagella (µm)	Length of haptonema (µm)	Scales types	Habitat
C. asquamosa	4 - 5 (long) 4 – 4.5 (wide)	14 - 17 (short) 21 - 28 (long)	c. 60 – 80 10 - 20	Scales lacking	Freshwater
C. strobilus	6(5)-10(12)	2.0 – 3.0 12 (10) - 30 (36)	12 -18(20) x cell 82-180(240) c. 150	Plate, cup	Marine
C. cymbium	6-8	c. 20	c. 60 (20-30 gyres) c. 160	Plate, cup	Marine
C. camella	14 x 16	c.25	c. 160	Plate, cup	Marine
C. campanulifera	10 x 10	c. 25	hapt. >> flag.	Plate, cup	Marine
C. throndsenii	5 - 6	c. 12	32-50	Plate (2)	Marine
C. ephippium	6(4.5)-10(12)	3-4 18(13.5) –40(48)	12-14 x cell 72(54)-140(168)	Plate, spine	Marine
C. alifera	6(4)-10(12)	2 – 2.5	10-12 x cell	Plate, spine	Marine
C. scutellum	4.0 – 9.0 long 4.0 – 8.0 wide 4.0 – 5.0 dorsoventral	12 – 20	13-80	Plate, spine (2)	Marine
C. acanthi	6.0 – 10.0	c. 16	c. 40	Plate, spine	Marine
C. apheles	3.0 – 4.0	7-10, 11-15	20-40	Plate (2)	Marine
C. simplex	3.9 x 3.8 (3.5 x 3.5) - 5.1 x 4.2 (6.9 x 5.4)	11(9.3)-16(19.4) sometimes unequal	max. 79	Plate	Marine
C. parva (type species)	7 – 8 x 8 -11	10	c. 80	Plate	Freshwater
C. pontica (LM Obs. only)	3. 6 x 4.8 – 4.8 x2.3	c. 9 - 12	c. 70	Not observed	Marine

Table 4. Continued

Species	N° of MT in emergent part of Haptonema	Microtubular flagellar root	Immersed pyrenoids	Peculiar Golgi body	References
C. asquamosa	6	R1	Not traversed by thylakoids	Present	Aguiar & Kugrens 2000
C. strobilus	6	No obs.	Present	Present	Leadbeater & Manton 1969b
C. cymbium	6	No obs.	Present	Present	Leadbeater & Manton 1969b
C. camella	6	No obs.	Traversed by thylakoids	Present	Manton & Leadbeater 1974
C. campanulifera	6	R1= c. 4 + c. 7 MT	Traversed by tubules	Not present	Eikrem 1996
C. throndsenii	No obs.	No obs.	No obs.	No obs.	Parke et al. 1956
C. ephippium	7	No obs.	Traversed by thylakoids	No obs.	Manton & Leadbeater 1974
C. alifera	No obs.	No obs.	No obs.	No obs.	Parke et. al. 1956
C. scutellum	7	R1=7+4 MT R2= 3-4 MT R3= 4 MT R4= 1MT	Traversed by thylakoids	Not present	Eikrem & Moestrup 1998
C. acanthi	7	R1= c. 10-20 MT R2= 2 MT R4= 2+2+1 MT	Present	No obs.	Leadbeater & Manton 1971 MTGregson et al 1993
C. apheles	6	R1= 5+1+ 1 MT R2= 1 MT	Traversed by tubules	Present	Moestrup & Thomsen 1986
C. simplex	7	R1= 4+5+1MT R2= 1 MT R4= 1 MT	Traversed by tubules	Present	Estep et al 1984 Birkhead & Pienaar 1995a
C. parva (type species)	7	R1= 7 MT	Present	No obs.	Parke et al 1962
C. pontica (LM 0bs. only)	No obs.	No obs.	No obs.	No obs.	Rouchijajnen 1966

Table 5. Comparative Characteristics of Three *Uroglena* species.

CHARACTER	***U. volvox***	***Uroglena* sp.**	***U. articulata***
Number of Cells/Colony	Several 100+	< 100	6-100+ (~32 usual)
Colonial Envelope	Present	Absent	Absent
Colonial Stalks	Absent	Present	Present
Chloroplasts	One	One	Two
Pyrenoid	Present	Absent	Absent
Endobacteria	Absent	Absent	Present
Chloroplast Shape	Spiral	Spiral	Fusiform
Mixotrophy	Slight	Absent	Absent
Flagellar Swelling	Globules/ Lamellate	Globules/ Granular	Striated
Descending Root	Present	Absent	Absent
Elaborate Diffuse Cytoskeleton	Absent	Absent	Present
Stigma	Several Layers	One Layer	Two layers
Special Stigma Lobe	Present	Absent	Absent

APPENDIX 3: FIGURES AND LEGENDS

ABBREVIATIONS USED IN FIGURES

AX = Axoneme	NE = Nuclear envelope
B = Bacteria	NI = Nuclear invagination
BB = Basal body	NTH = Non-tubular hairs
BP = Base plate scale	OM = Osmiopholic material
BW = Bacteria cell wall	P = Pyrenoid
C = Chloroplast	PB = Polyphosphate body
CB = Connecting band	PER = Peripheral endoplasmatic reticulum
CC = Coccolith	PM = Plasma membrane
CD = Columnar deposit	RER = Rough endoplasmic reticulum
CER = Chloroplast endoplasmic reticulum	RH = Rhizoplast
CEX = Cytoplasmic extension	S = Scales
CH = Chrysolaminarin vacuole	SB = Spherical body
CI = Chloroplast invagination	SK = Stalks
CY = Cyanobacterium	SL = Striated lamellae
D = Diatom	SM = Striated material
DG = Dark granules	SF = Short flagellum
EP = Epitheca	ST = Stigma
ER = Endoplasmic reticulum	SV = Secretory vesicles
F = Flagella	TH=Tubular hairs
FP = Flagellar pit	TI = Tubular invagination
FR = Flagelar root	TR = Transitional region
FS = Flagellar swelling	V = Contractile vacuole
FV = Food vacuole	
G = Golgi apparatus	
GB = Granular body	
GY = Gyres	
H = Haptonema	
HMT = Haptonema microtubule	
HY = Hypotheca	
LB = Lipid body	
LF = Long flagellum	
LM = Lamellae	
M = Mitochondrion	
MT = Microtubules	
MU = Mucilage	
N = Nucleus	

FIGURE LEGENDS

CHAPTER 1

Phaeaster pascheri* var. *avesiculosa

Figures 1-5. Scanning electron micrographs (SEM) of *P. pascheri*.
Scale bar = 2 μm.

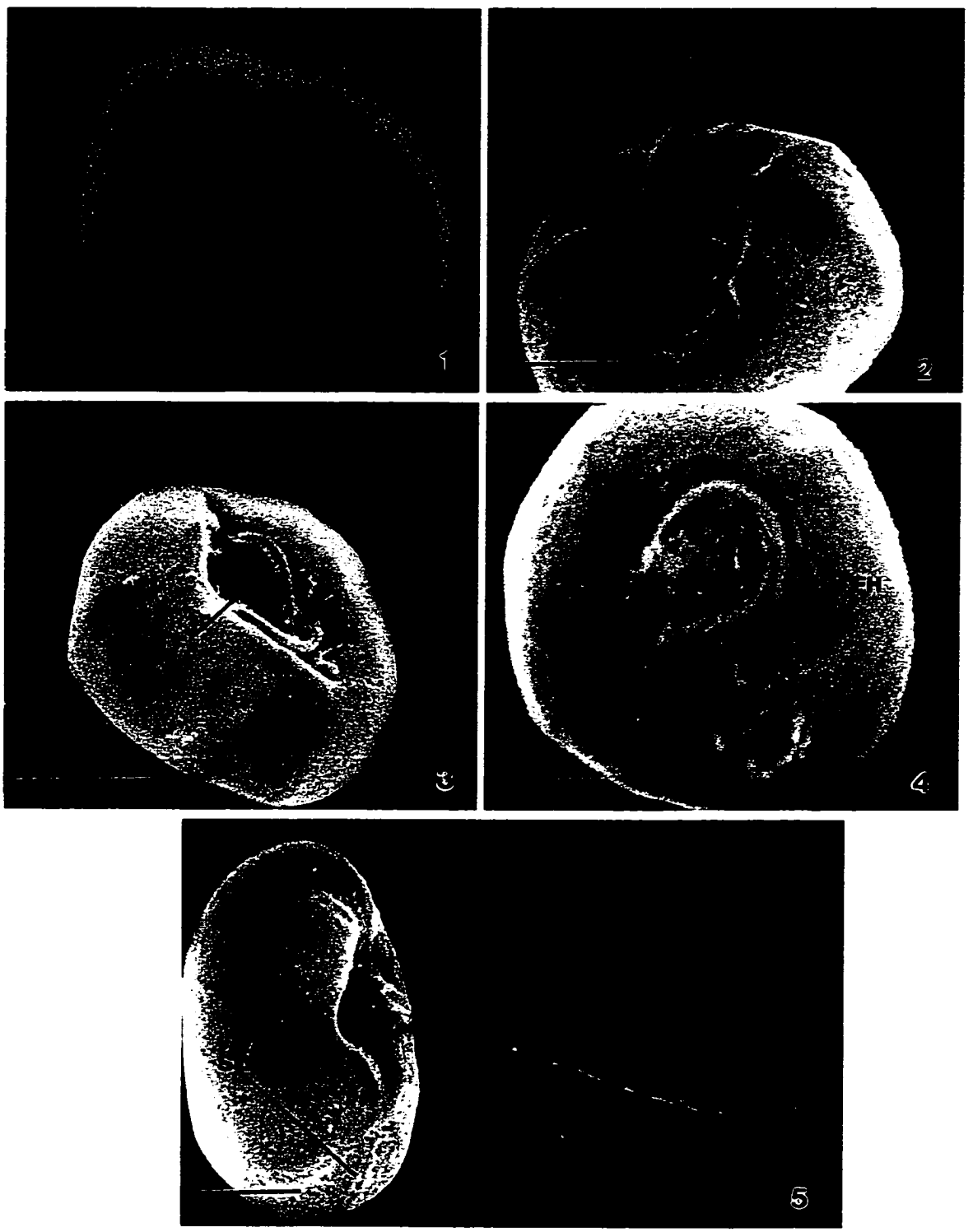
Fig. 1. Ventral view of cells showing the long flagellum arising in the middle of the concave face from a flagellar depression or pit.

Fig. 2. Long flagellum with tubular flagellar hairs.

Fig. 3. Cell with the long flagellum coiled inside the flagellar depression.

Fig. 4. Cell with flagellum close to the surface of the cell.

Fig. 5. Lateral view of a cell showing an irregular surface that probably represents unmineralized scale layers.



Figures 6 -7. Shadow casting preparations of *P. pascheri.*

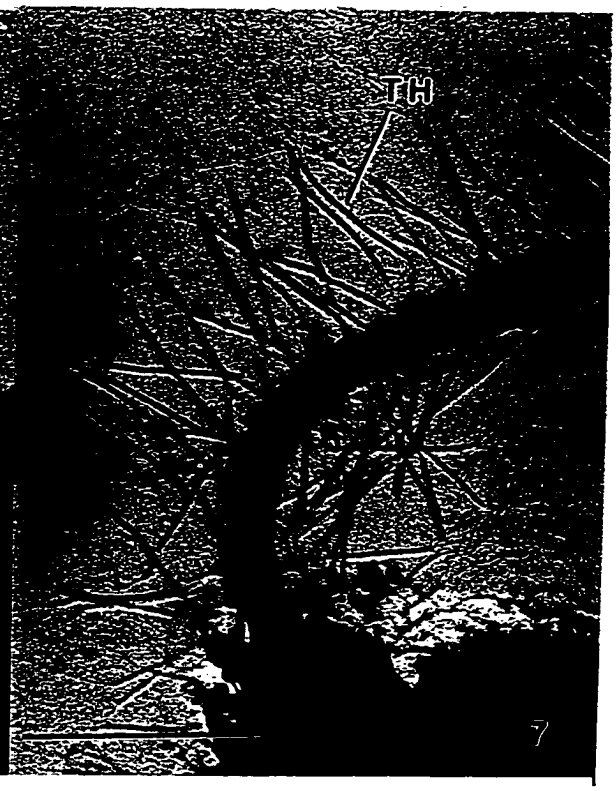
Fig. 6. Micrograph showing the entire cell surrounded by several layers of unmineralized scales. Scale bar = 2 μm.

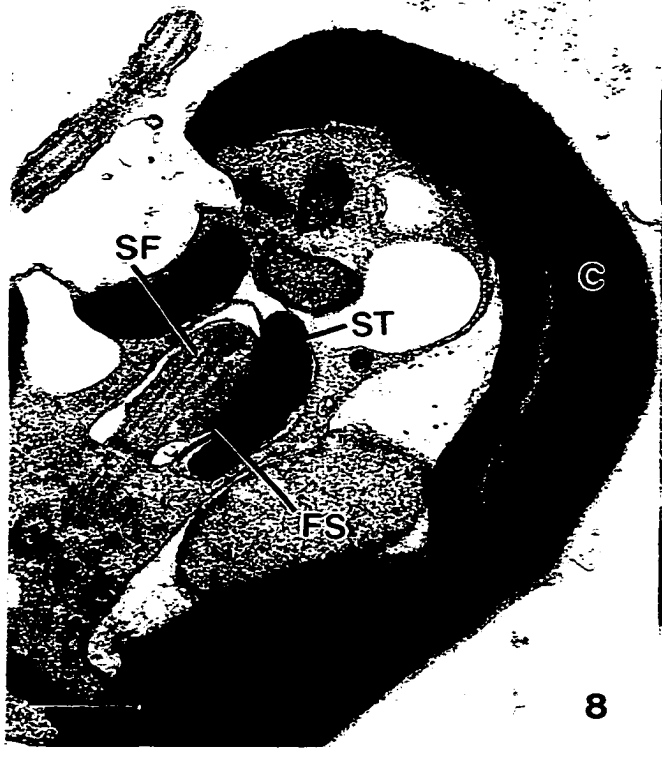
Fig. 7. Long flagellum with two rows of tubular hairs.
Scale bar = 1 μm

Figures 8 - 9. Transmission electron micrographs (TEM) of *P. pascheri.*

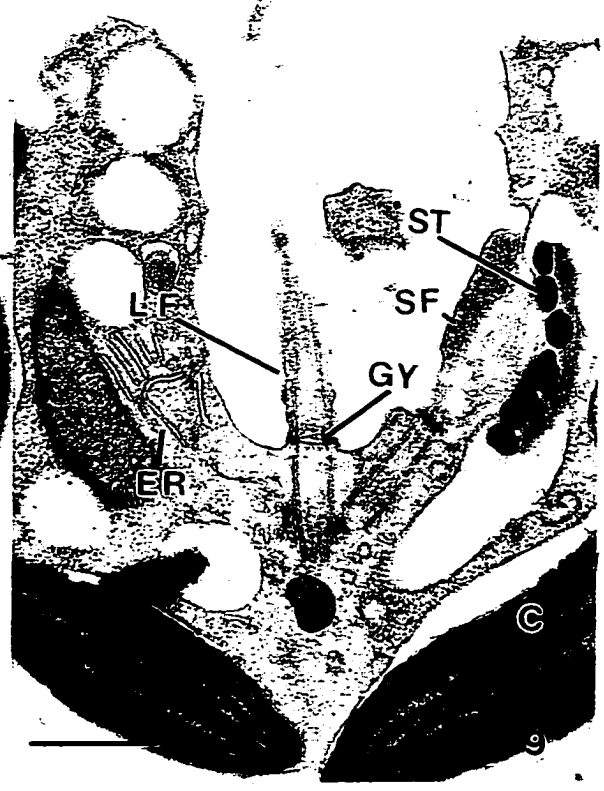
Fig. 8. Longitudinal section showing the cytoplasmic invagination (pocket) that contains the short flagellum. Note the association of the short flagellum and its flagellar swelling close to the stigma. Note the lobed chloroplast. Scale bar = 1μm.

Fig. 9. Longitudinal section showing the orientation of both flagella. Note the four gyres in the transition region of the long flagellum. The short flagellum associated with the stigma. The endoplasmic reticulum forms a network in the cytoplasm. Chloroplast. Scale bar = 0.5 μm.





8



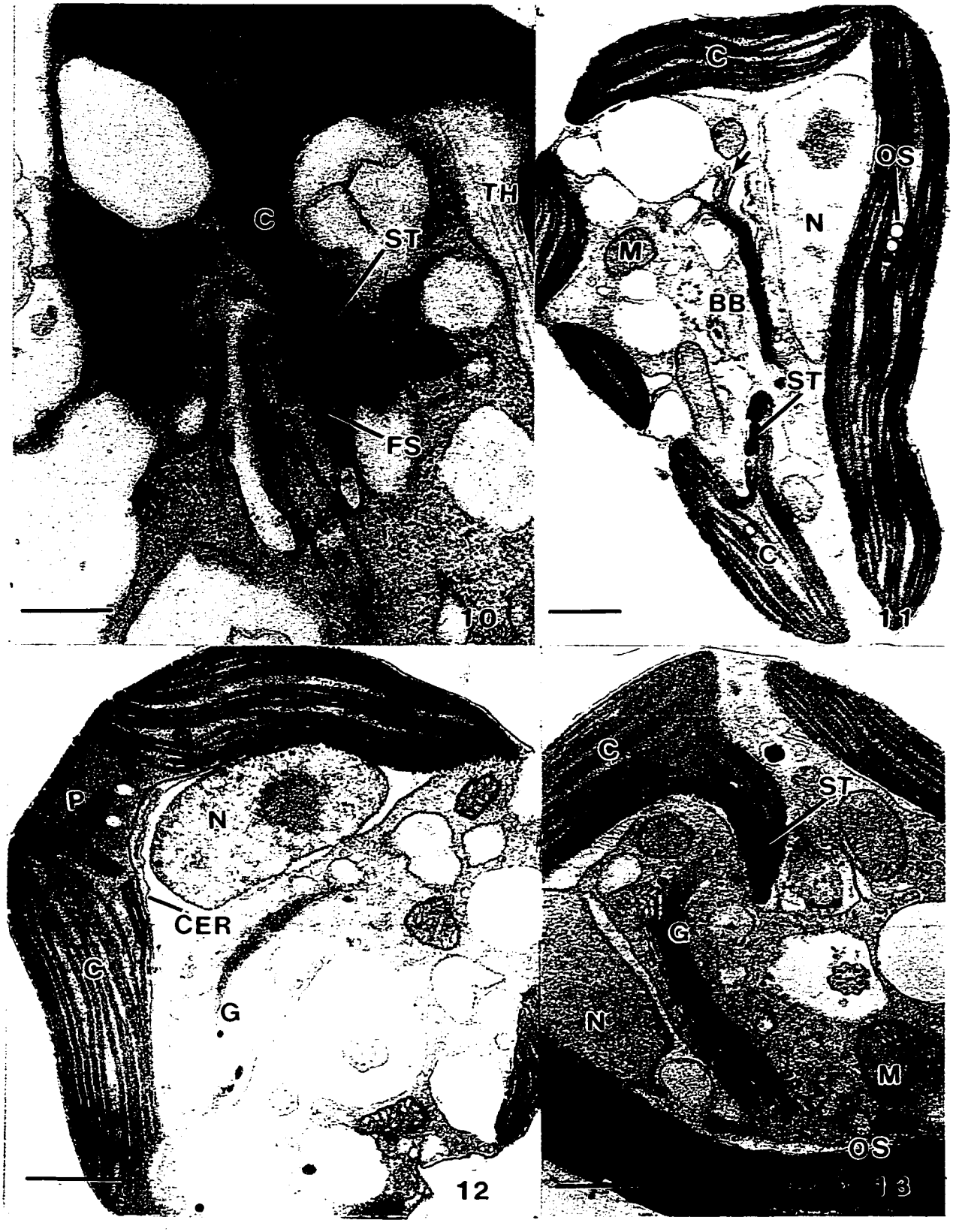
Figures 10-13. Transmission electron micrographs of *P. pascher.*

Fig. 10. Longitudinal section of a cell through the anterior region of the chloroplast showing a modified portion of the chloroplast and the stigma. Higher magnification of the flagellar swelling and the tubular hairs in a dilated portion of the chloroplast endoplasmic reticulum. Scale bar = 0.5 μm.

Fig. 11. Longitudinal section of a cell with a lobed chloroplast. Note the lateral position of the nucleus, which is anterior to the pyrenoid in the convex side of the cell. Basal bodies of the long and the short flagellum are in the middle of the cell. The Golgi apparatus, is anterior to the nucleus and is forming several sizes of vesicles (arrow). A mitochondrion is shown near the basal bodies. Lipid bodies. Scale bar = 0.5 μm.

Fig. 12. Oblique section of a cell showing a single parietal chloroplast in the convex surface of the cell and a prominent pyrenoid with tubular invaginations originating from the chloroplast envelope and projecting into the pyrenoid. The outer membrane of the nuclear envelope forms the chloroplast endoplasmic reticulum. A flattened nucleus is anterior to the pyrenoid and posterior to the Golgi apparatus. Scale bar = 0.5 μm.

Fig. 13. Transverse section of a cell showing the modified chloroplast and the stigma. Golgi body. Mitochondrion. Nucleus. Lipid bodies. Scale bar = 1μm.



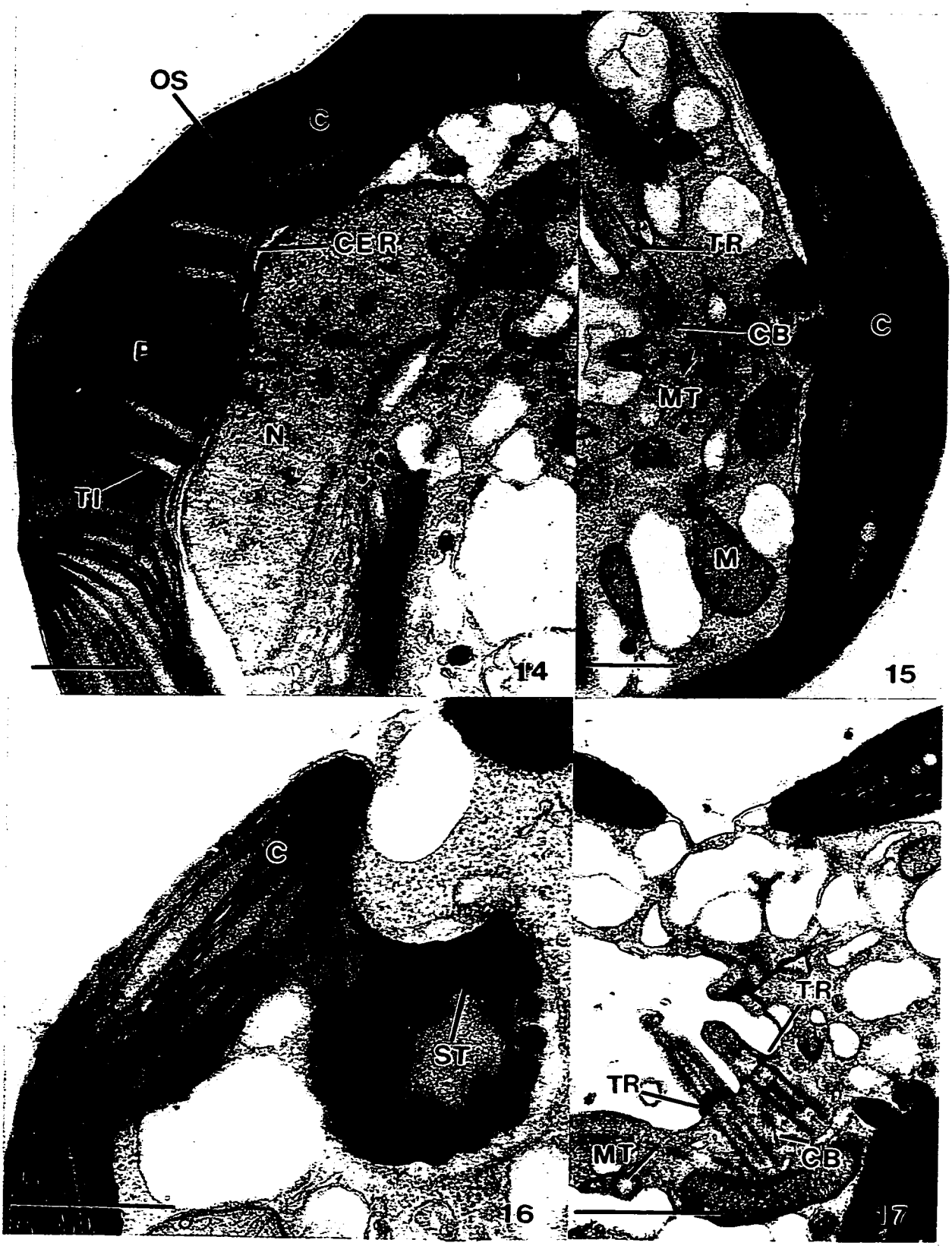
Figures 14 -17. Transmission electron micrographs of *P. pascheri*.

Fig. 14. Longitudinal section of a cell showing a prominent pyrenoid with several tubular invaginations. Note the lipid bodies in the chloroplast, chloroplast endoplasmic reticulum, and nucleus. Scale bar = 0.5 μm.

Fig. 15. A portion of the flagellar apparatus. The transitional region is visible in both flagella. A connecting band is located between the two basal bodies. Four microtubules (arrow) are viewed in cross section, on the left side of the long flagellum. Note several mitochondria around the flagellar base and a parietal chloroplast. Scale bar = 0.5 μm.

Fig. 16. Longitudinal section through the chloroplast showing a modified lobe of the chloroplast with a stigma in the anterior portion of the lobe. Scale bar = 0.5 μm.

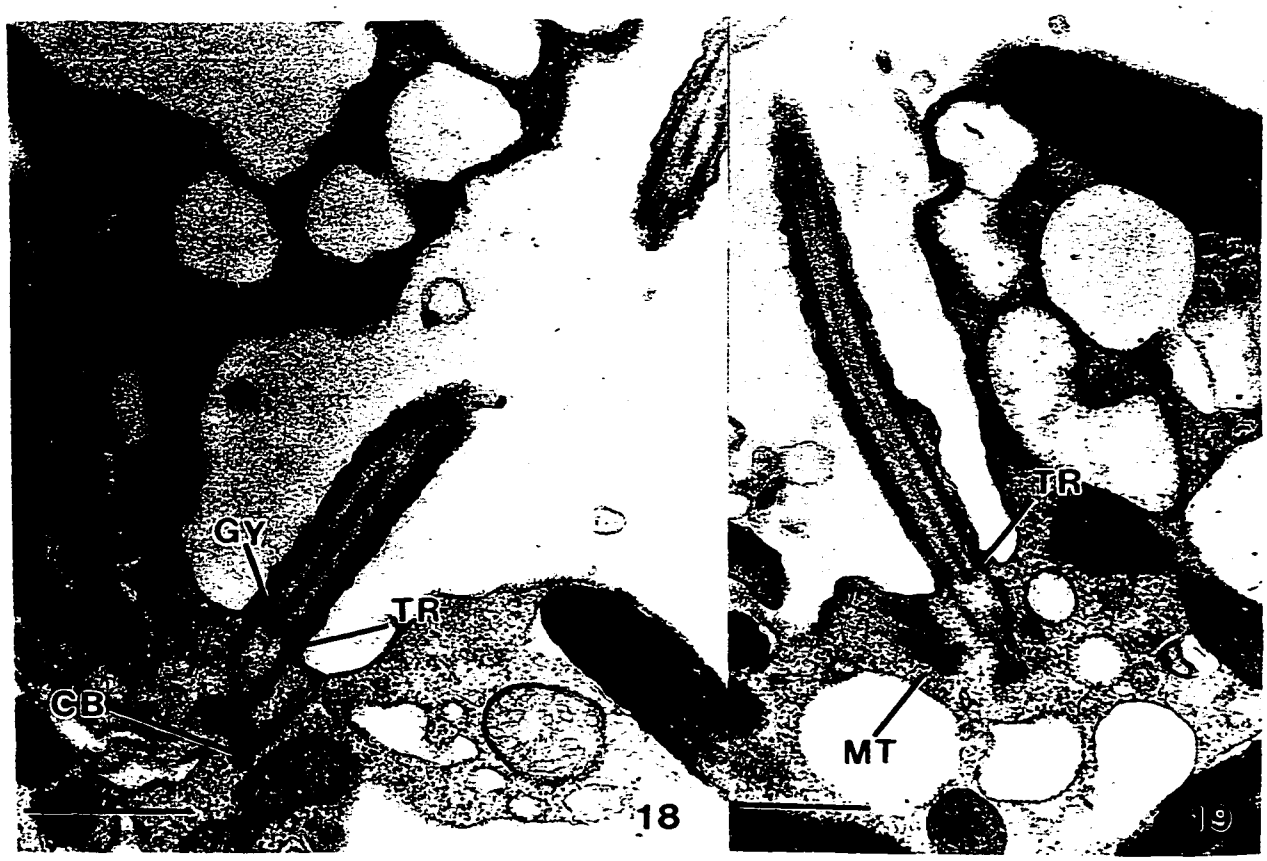
Fig. 17. Longitudinal section of a cell showing the connecting band associated with both flagellar bases and longitudinal microtubules running along the periphery of the cell. Scale bar = 0.5 μm.



Figures 18-19. Transmission electron micrographs of *P. pascheri.*

Fig. 18. Longitudinal section of a cell showing the transitional region with four gyres. A connecting band is shown on the right side of the long flagellum.
Scale bar = 0.5 μm.

Fig. 19. Longitudinal section through the flagellar base showing a microtubules running around the periphery of the cell. Transitional region showing an electron dense material.
Scale bar = 0.5 μm.



Diacronema vlkianum

Figures 20-22. Light micrographs (LM) of *D. vlkianum*.
Scale bar = 10 μm.

Fig. 20. A broadly oval cell with a long and short flagellum.

Fig. 21. A dorso-ventrally flattened cell showing a long flagellum.

Fig. 22. A rounded cell showing the long and short flagellum arising from the middle portion of the cell.

Figures 23-27. Scanning electron micrographs (SEM) of *D. vlkianum*.
Scale bar = 0.5 μm.

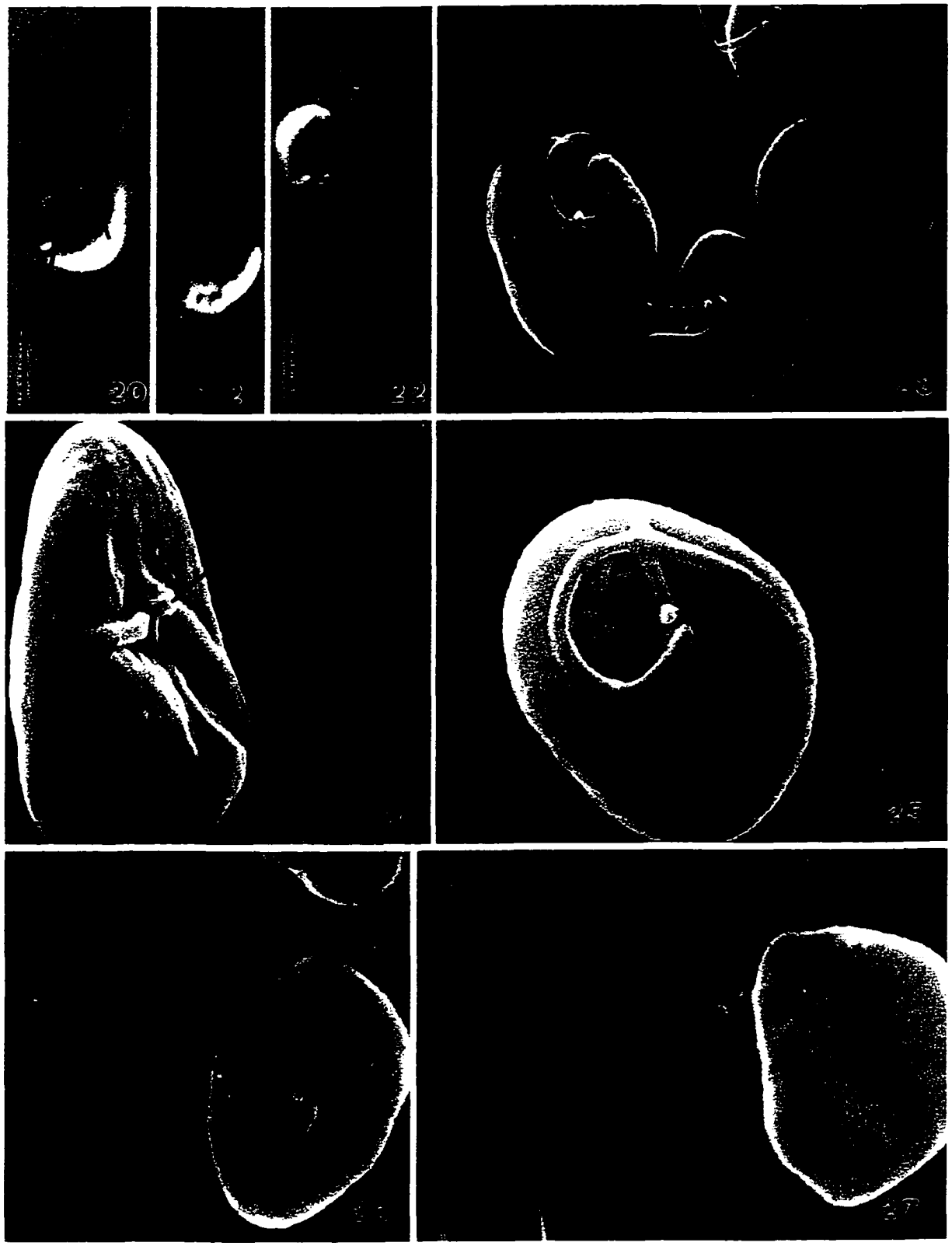
Fig. 23. Group of cells showing two flagella and short haptonema arising from the middle of the concave face of the cell. Note the different shapes of cells, varying from broadly oval to round.

Fig. 24. A dorso-ventrally compressed cell showing the short flagellum with a narrow tip and the reduced haptonema between the flagella.

Fig. 25. Micrograph showing the smooth surface of the cell, long flagellum, short flagellum, and haptonema.

Fig. 26. Anterior view of a cell showing narrow tips in both flagella and the reduced haptonema arising between the flagella.

Fig. 27. Posterior view of a cell showing the two unequal flagella and their narrow tips.

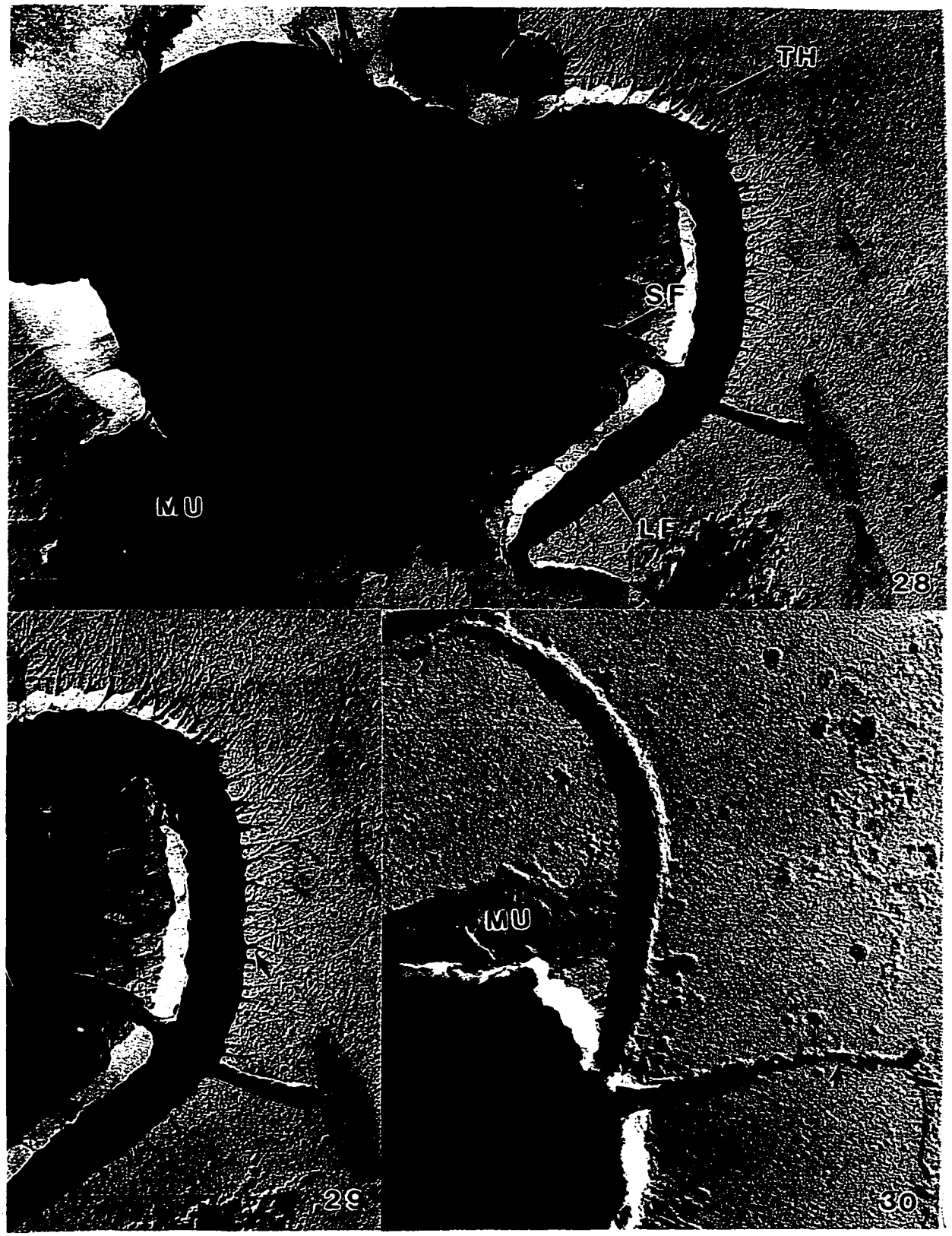


Figures 28-30. Shadow casting preparations of *D. vlkianum.*

Fig. 28. Shadowed whole mount of a cell showing the long flagellum with bifurcated tubular hairs. Note the mucilage surrounding the cell. Scale bar = 2 μm.

Fig. 29. Higher magnification of tubular hairs showing a short bifurcate shaft with two terminal filaments (arrow). Scale bar = 2.4 μm.

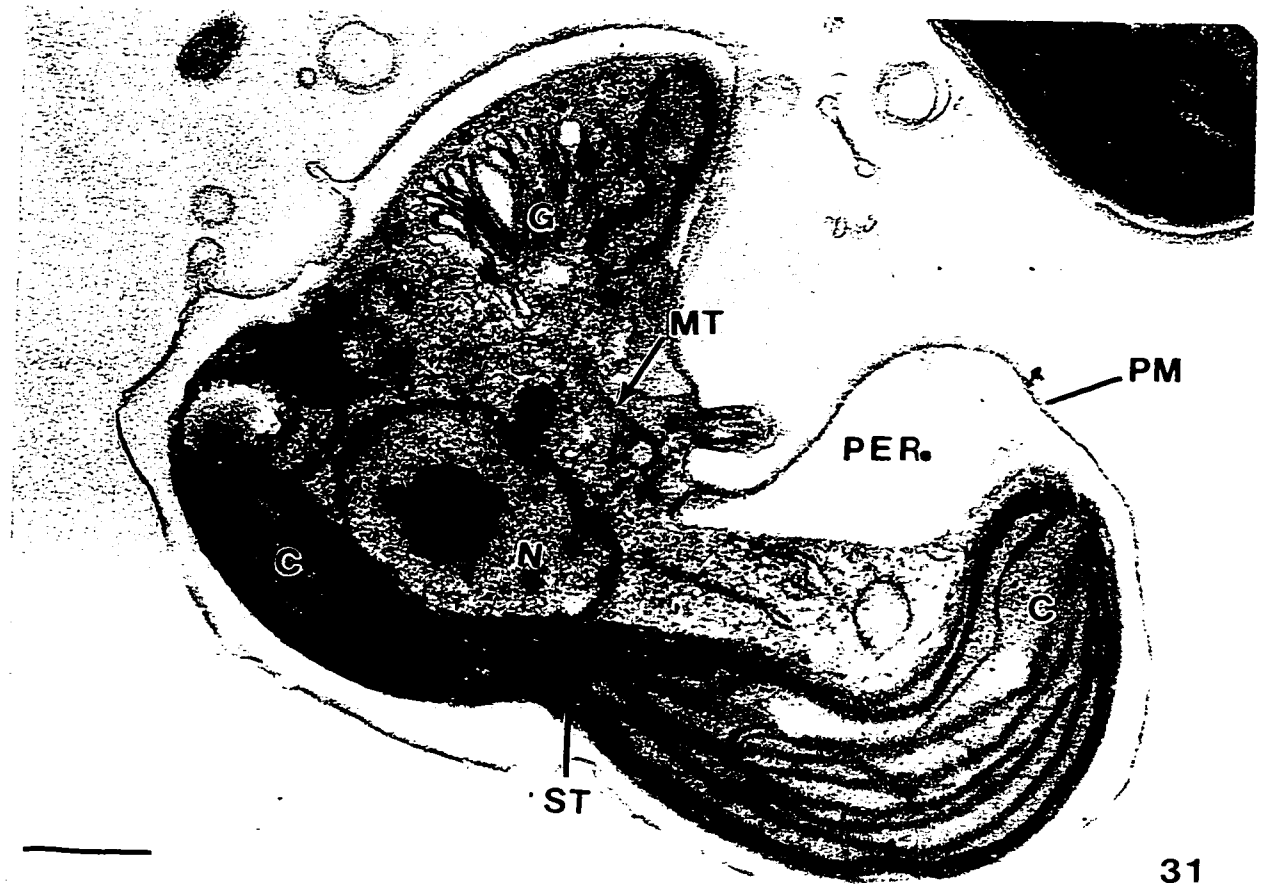
Fig. 30. Shadowed cell with unequal flagella and narrow tips (arrows). Note the mucilage. Scale bar = 0.5 μm



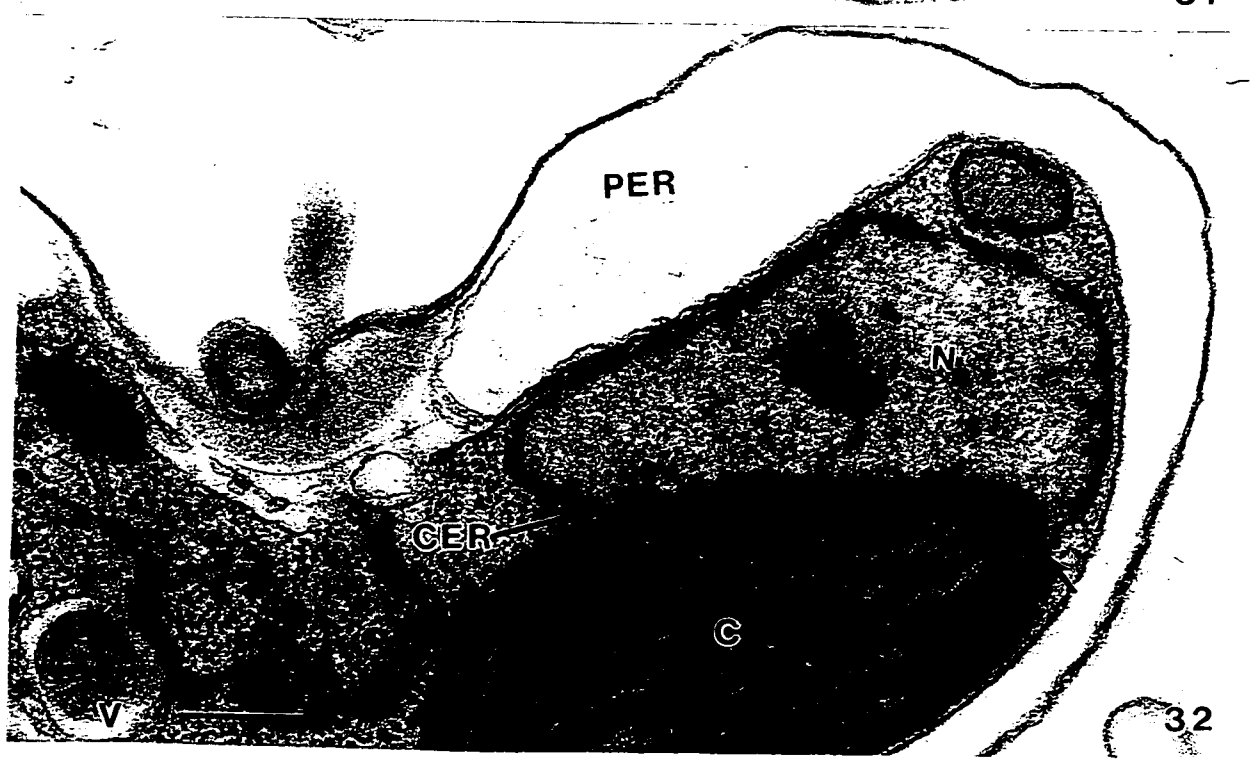
Figures 31-32. Transmission electron micrographs (TEM) of *D. vlkianum.*
Scale bar = 0.5 μm.

Fig. 31. A longitudinal section of a cell showing the plasma membrane and the dilated peripheral endoplasmic reticulum. Note the bilobed chloroplast in the dorsal side of the cell, and a prominent nucleus anterior to the chloroplast. A transverse section through the Golgi apparatus shows stacks of cisternae with releasing vesicles. A single microtubule arises from the basal body and runs parallel to the periphery of the cell.

Fig. 32. Micrograph through the anterior portion of a cell showing the chloroplast endoplasmic reticulum. Note the peripheral endoplasmic reticulum surrounding the cell and a vacuole with a concentric material of unknown nature. A lobed chloroplast is in the dorsal side of the cell.



31

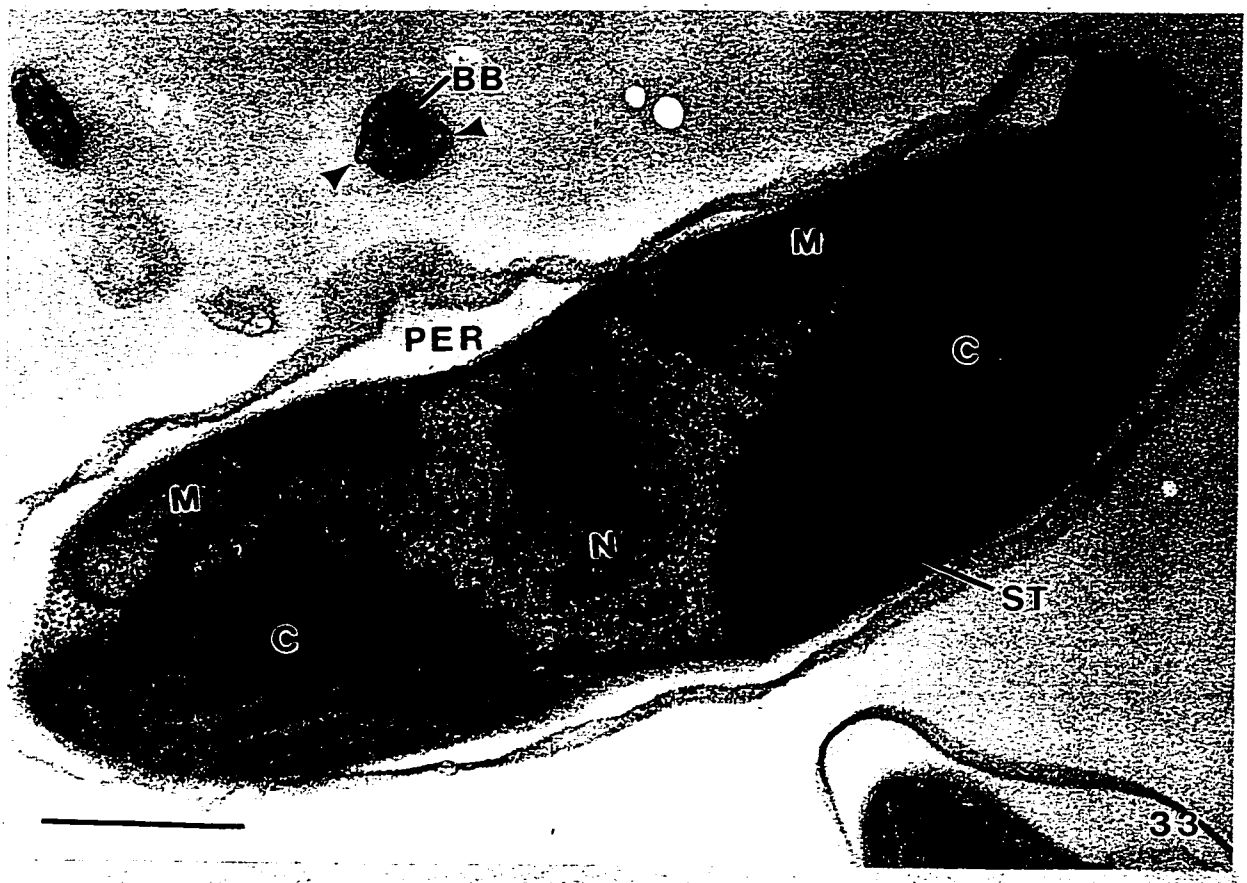


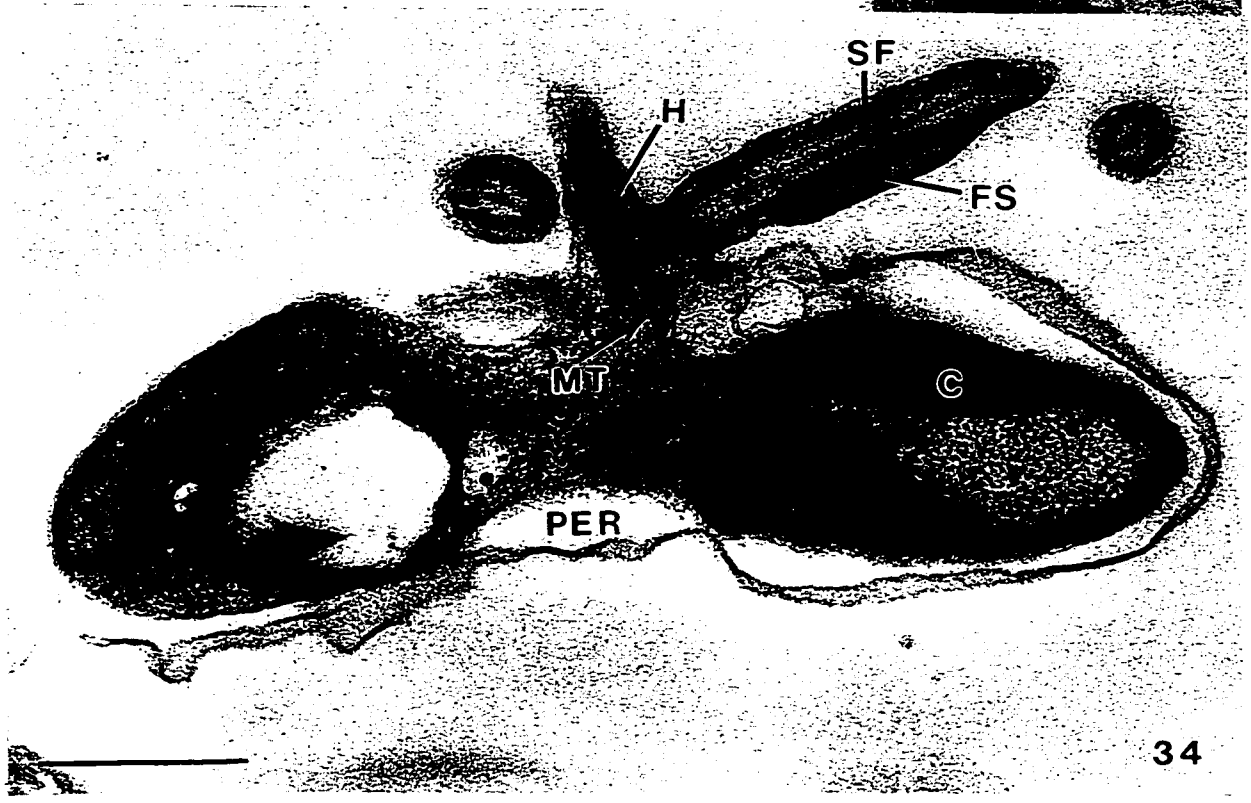
32

Figures 33-34. Transmission electron micrographs of *D. vlkianum.*
Scale bar = 0.5 μm.

Fig. 33. Longitudinal section through a cell showing the bilobed chloroplast and stigmatic granules in the posterior end of the chloroplast near to the membrane. A mitochondrion with tubular cristae is located laterally, and a central conspicuous nucleus is in the middle of the convex side (dorsal) of the cell. In cross section the axoneme of the long flagellum is shown with lateral dilations.

Fig. 34. A longitudinal section through the short flagellum showing the flagellar swelling and with dense material. A single microtubule runs from the short flagellum basal body to the ventral side of the chloroplast. The haptonema with endoplasmic reticulum profiles is in oblique section. The peripheral endoplasmic reticulum surrounds the cell.





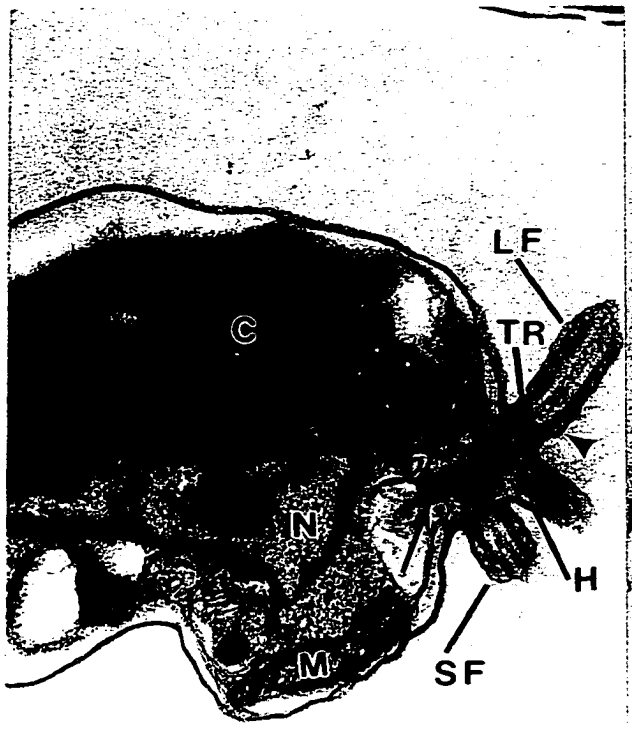
Figures 35-38. Transmission electron micrographs of *D. vlkianum*.
Scale bar = 0.5 μm.

Fig. 35. Longitudinal section through the flagella and haptonema showing the insertion of flagellar bases at almost a right angle. Note the transitional region and the constriction in the long flagellum (arrow), chloroplast, mitochondrion, and nucleus.

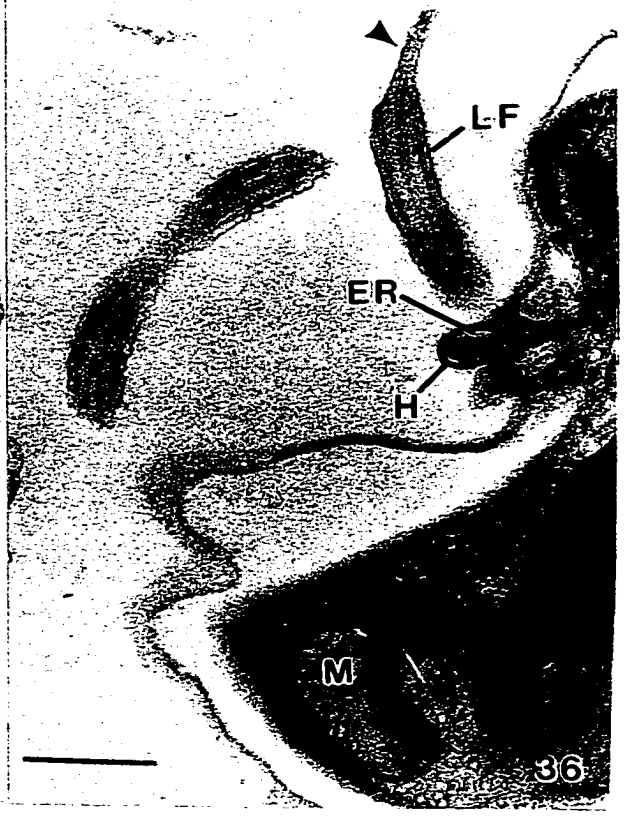
Fig. 36. Longitudinal section through the haptonema showing the endoplasmic reticulum profiles and the long flagellum with narrow tip (arrowhead).

Fig. 37. Longitudinal section through the basal body of the long flagellum showing a granular material surrounding the flagellar bases that forms an arc (arrow). Note the transitional region and a single microtubule running from the basal body of the long flagellum to the chloroplast lobe. Mitochondrion is also present.

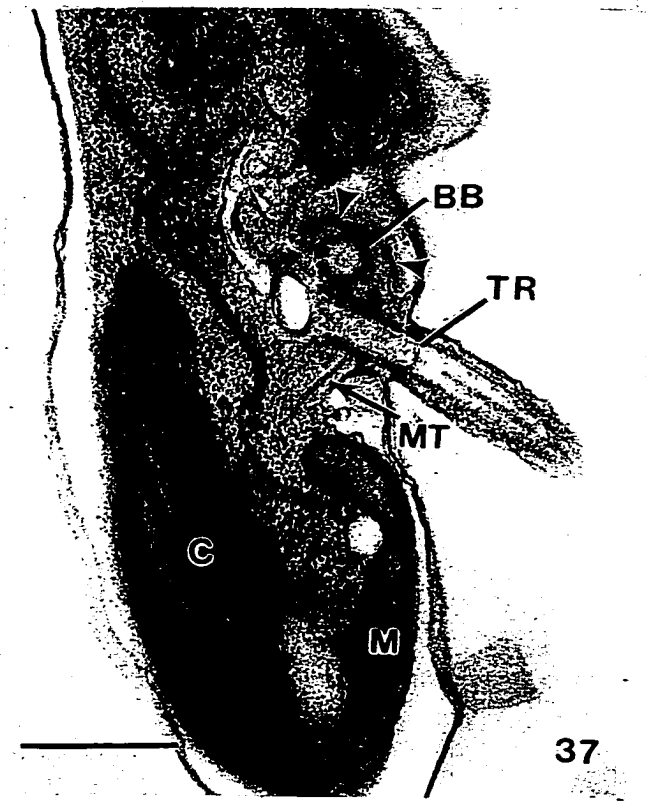
Fig. 38. Longitudinal section of a cell through the anterior region of the chloroplast showing a single layer of stigma granules. Nucleus is present.



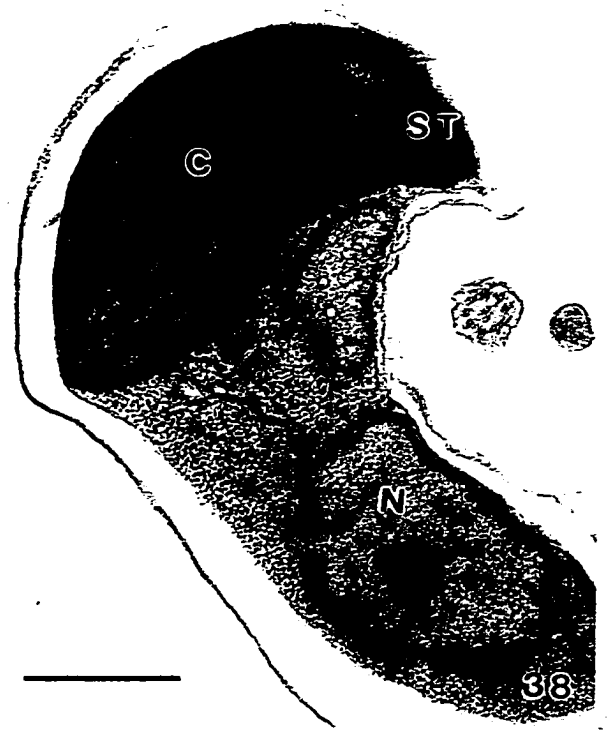
35



36



37



38

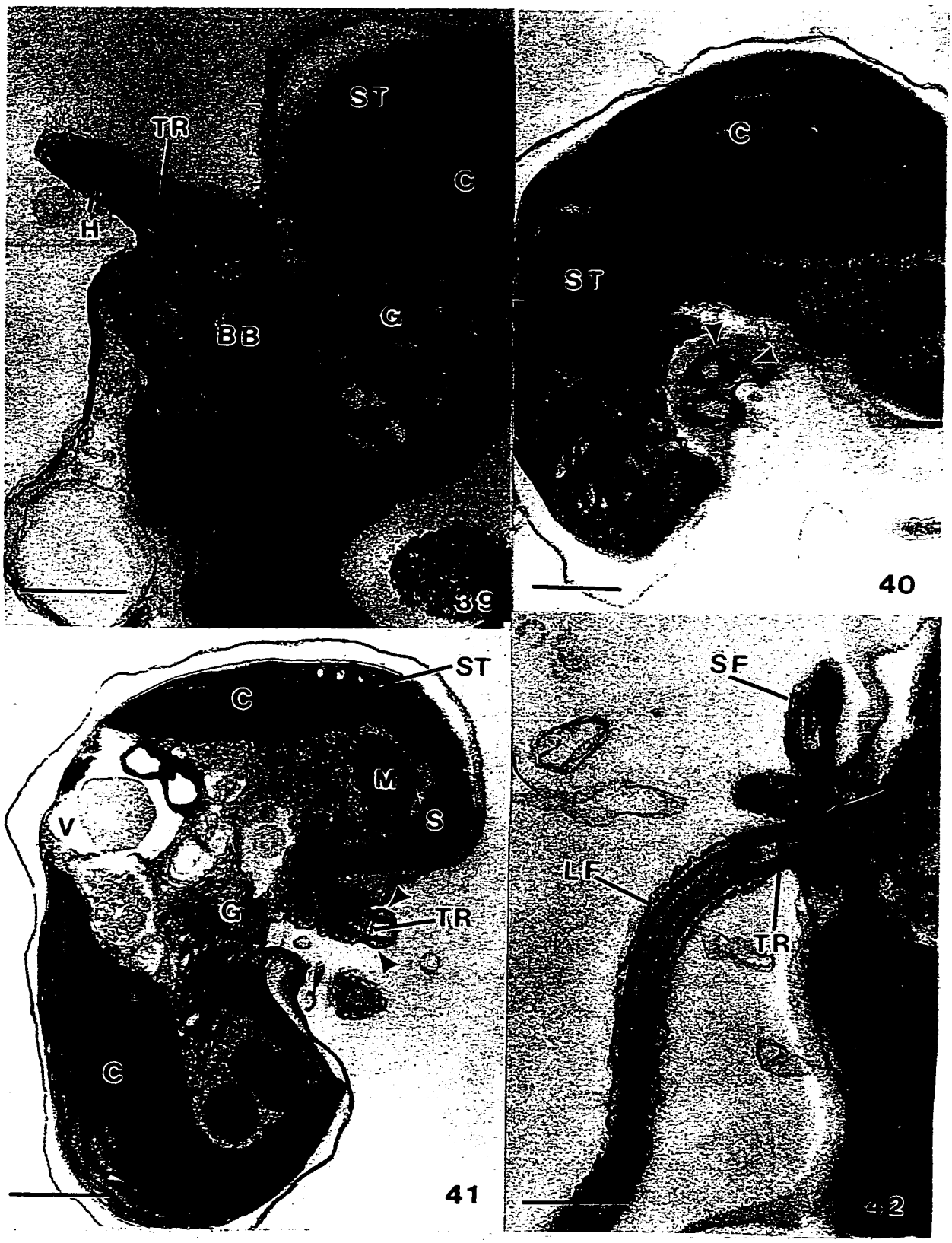
Figures 39-4 2. Transmission electron micrographs of *D. vlkianum.*
Scale bar = 0.5 μm.

Fig.39. Longitudinal section of a cell through the anterior region showing details of the stigma in the anterior portion of the chloroplast. The Golgi apparatus is positioned lateral to the chloroplast. The transitional region in the haptonema is evident. Basal bodies are also present.

Fig. 40. Longitudinal section through the chloroplast showing a group of stigmatic granules in the anterior portion of the lobe. Note the semi-arc of material around the flagellar bases (arrowhead).

Fig. 41. Longitudinal section of a cell showing the transitional region in the long flagellum and a slight swelling above the transitional region (arrowhead). Note the vacuoles in the convex side of the cell near the Golgi apparatus. Chloroplast is also present.

Fig. 42. Longitudinal section of a cell through the anterior region showing the insertion of the flagella and a dense material between the bases (arrowhead). Note the mitochondrion profile near the flagellar base and the transitional region in the long flagellum .



Hymenomonas from Colorado

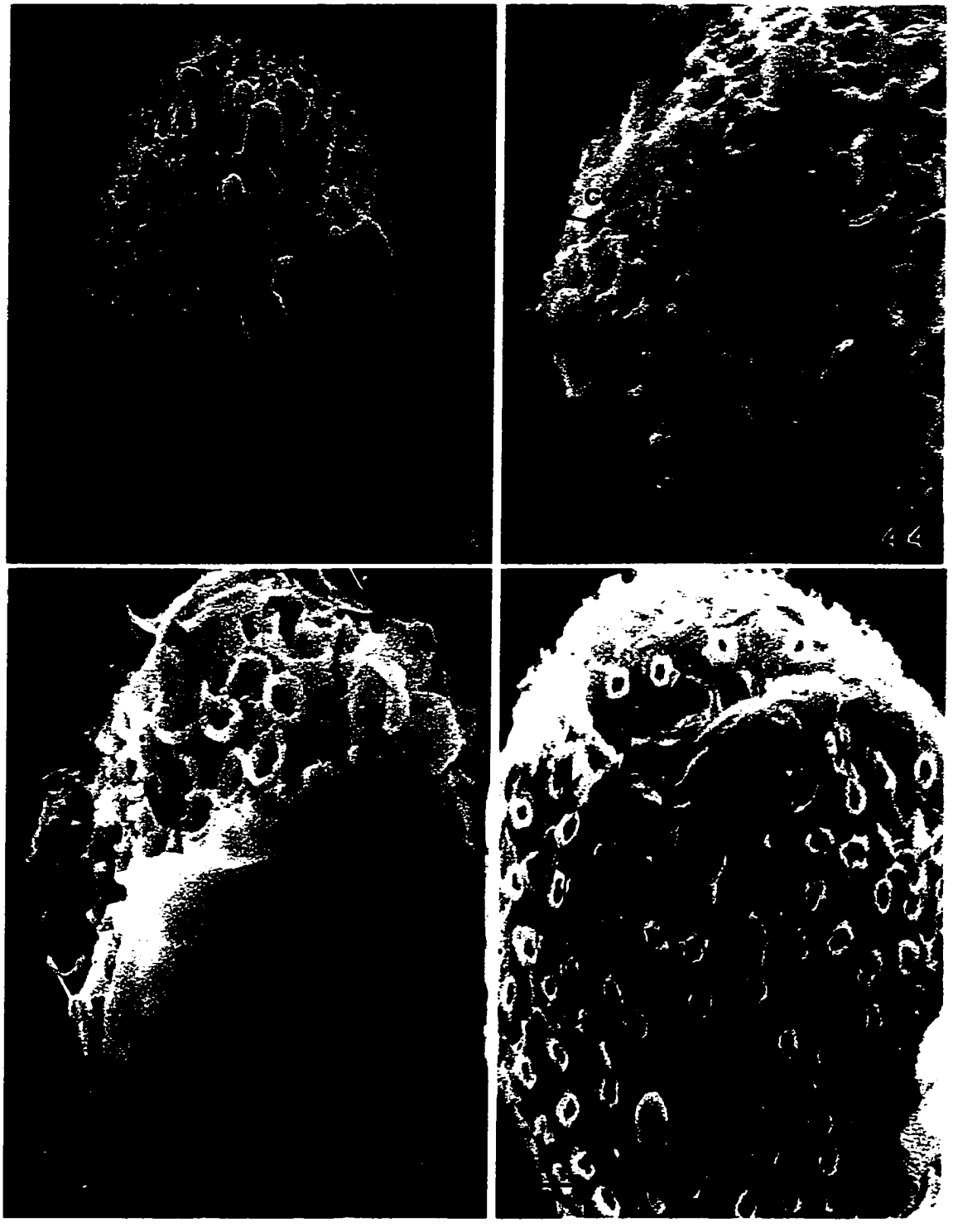
Figs. 43-56. Scanning electron micrographs (SEM) of *Hymenomonas* sp.
Scale bar = 5 μm.

Fig. 43. SEM of a cell showing coccoliths covering the cell.

Fig. 44.At higher magnification details of coccoliths.

Fig. 45. Cell with a portion of a semi-transparent membrane covering the coccolith layer.

Fig. 46. Micrographs showing the general appearance of the cell covered by coccoliths.



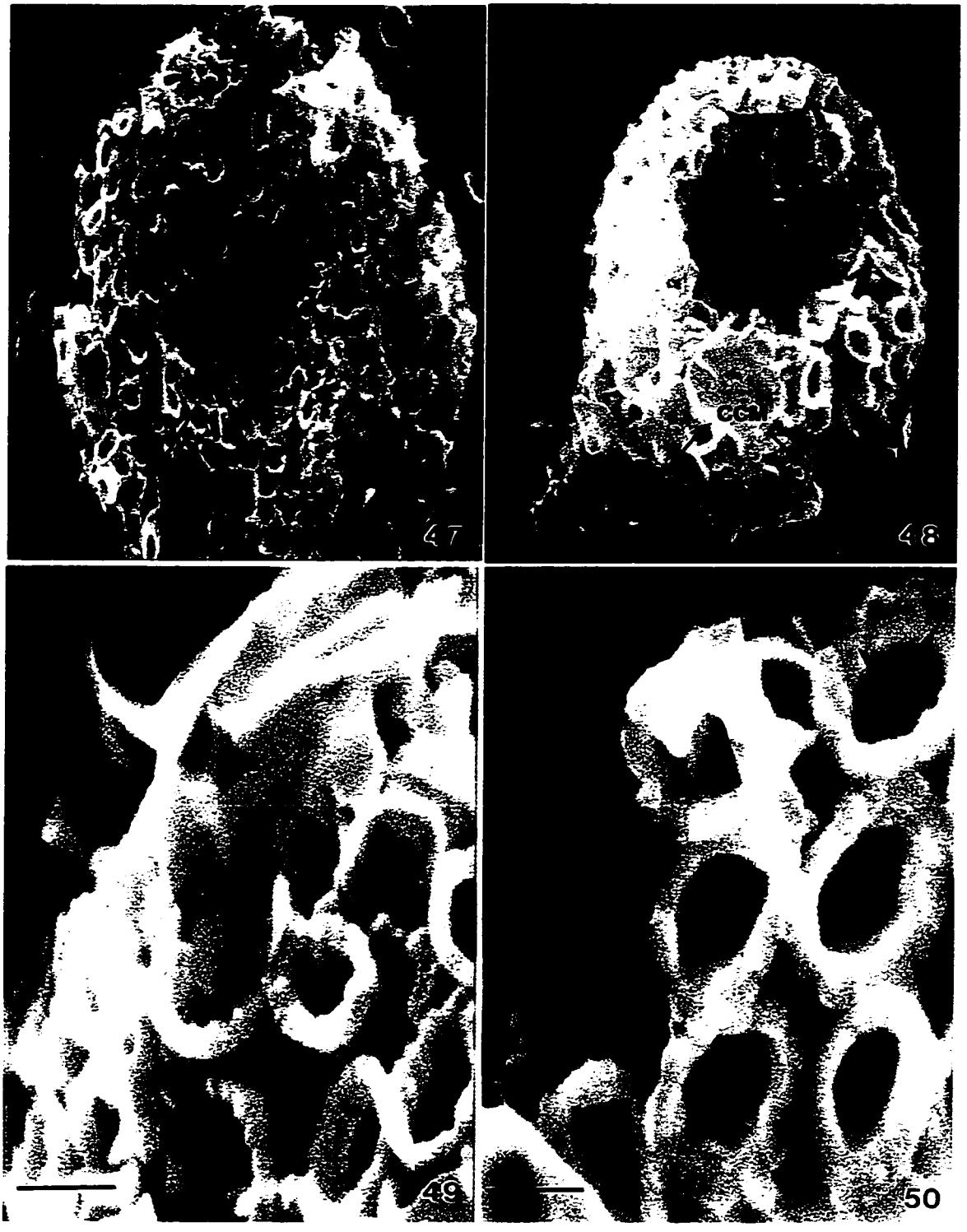
Figures 47-50. Scanning electron micrographs of *Hymenomonas* sp.
Scale bar = 5 μm.

Fig. 47. Coccoliths on cell partially covered by a membrane.

Fig. 48. A cell showing remnants of a membrane adhering to the coccoliths.

Fig. 49. Lateral view of a "crown" shaped coccolith (arrows).

Fig. 50. Higher magnification of the coccolith elements and the "fence-like" structure of the elements (arrows).



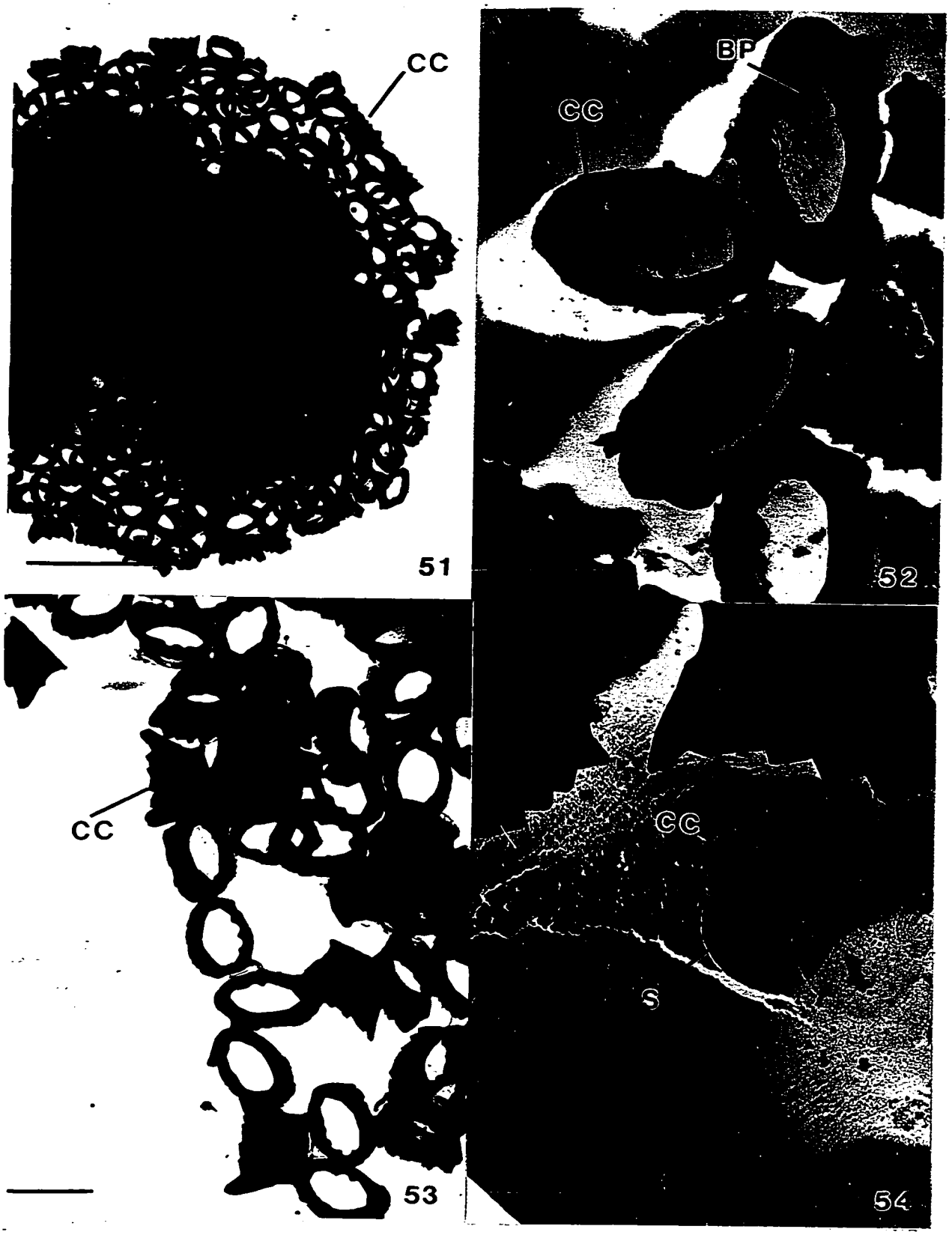
Figures 51-54. Shadow cast preparations of *Hymenomonas* sp.
Scale bar = 0.5 μm.

Fig. 51. Shadowed cells of *Hymenomonas* with a single coccolith layer.

Fig. 52. Higher magnification of a shadowed cell showing details of coccolith with a dense calcified rim. The base-plate scale and uncalcified scales. Note the radial pattern in the proximal face of the uncalcified scales.

Fig. 53. Calcified scales with an upright rim. Note the "crown"-like shape and the "fence"-like structure.

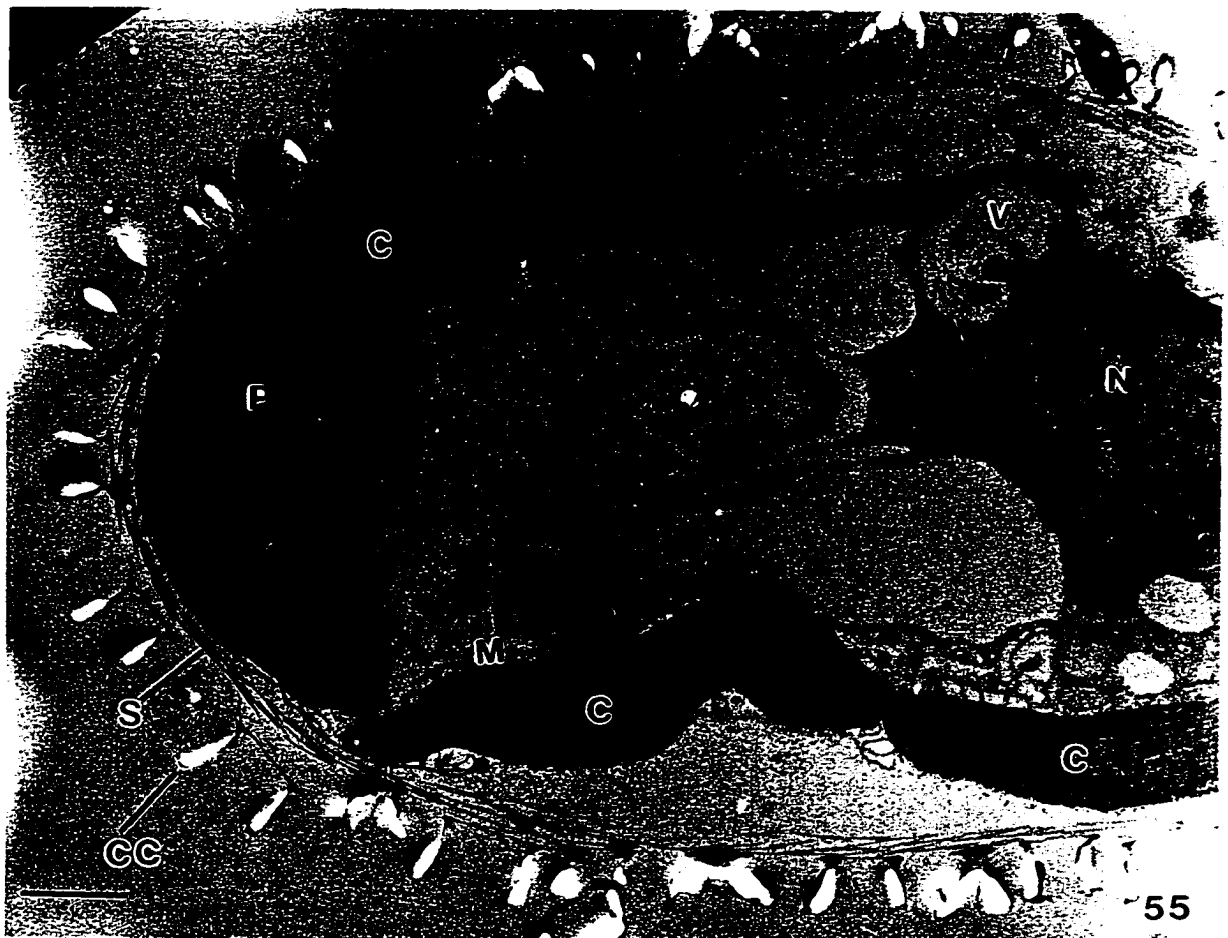
Fig. 54. Unminralized oval scales with concentric rings in the distal face. Scales have a narrow rim. Details of the elements in the coccolith .

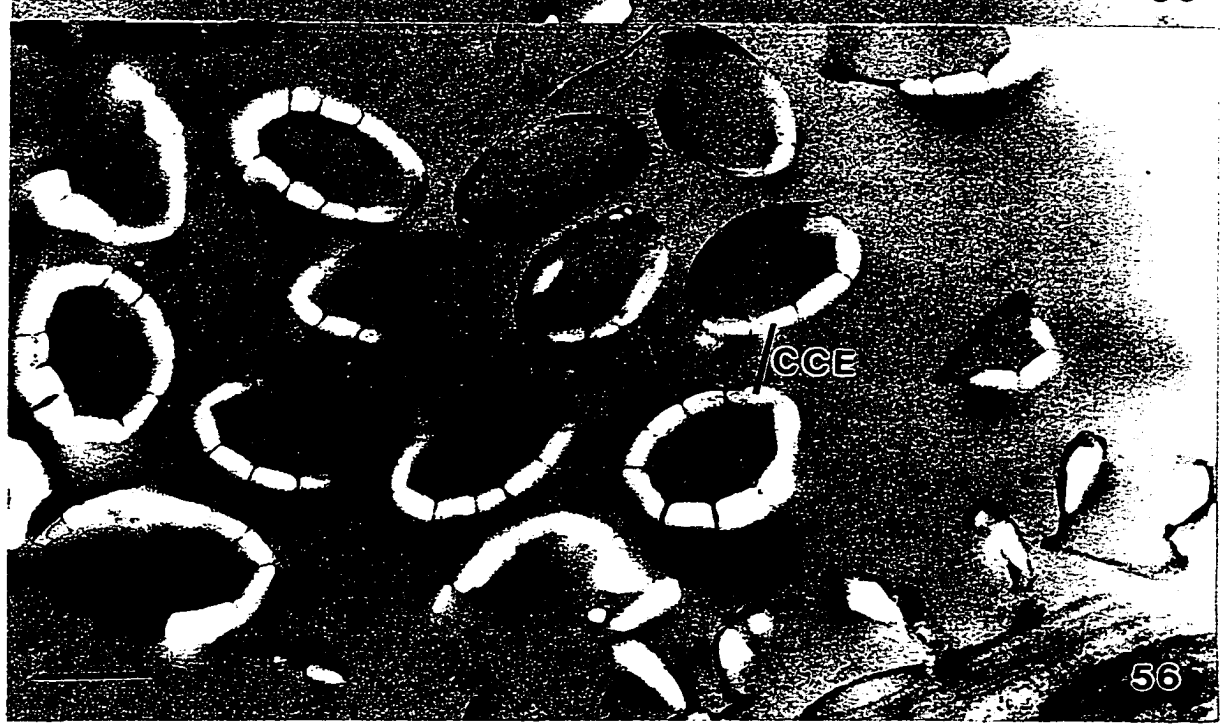


Figures 55-56. Transmission electron micrographs (TEM) of sectioned material of *Hymenomonas*.
Scale bar = 1 μm

Fig. 55. Longitudinal section of a *Hymenomonas* cell with a single coccolith layer and several unmineralized scale layers beneath the coccolith layer. The cell has three parietal chloroplasts with a bulging pyrenoid. Posterior nucleus and contractile vacuoles with unknown contents are also shown.

Fig. 56. Frontal section through a coccolith layer showing an average of 11 calcified elements.





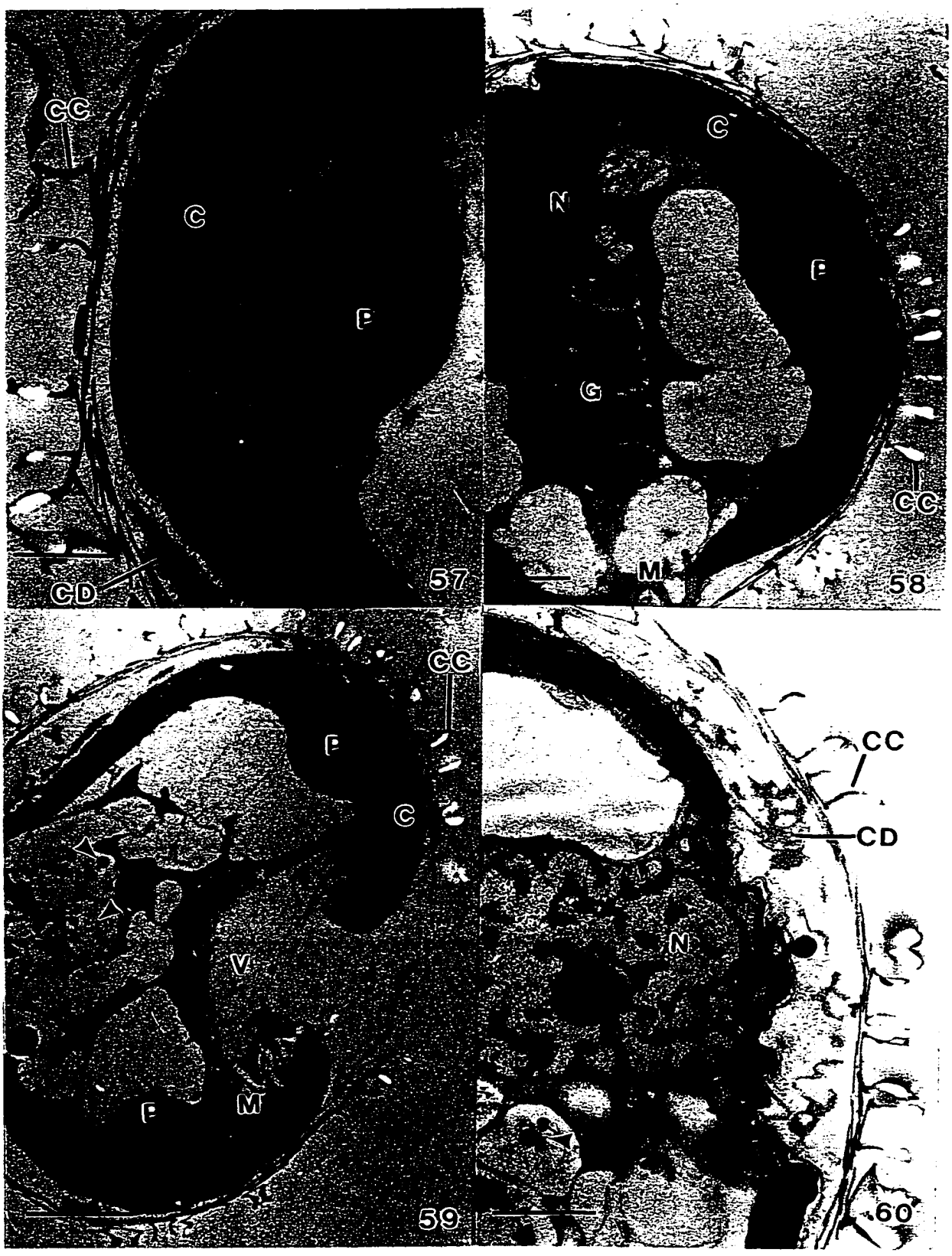
Figures 57-60. Transmission electron micrographs of *Hymenomonas* sp.

Fig. 57. A cell showing details of the chloroplast and the pyrenoid traversed by thylakoids. Note that the outermost layer of the cell has a single coccolith layer. A columnar dense deposit lies beneath the unmineralized scale layer. Scale bar = 1μm.

Fig. 58. A general view of a cell with a parietal chloroplast, pyrenoid, nucleus, Golgi apparatus and several small mitochondria. Scale bar = 1μm.

Fig. 59. Longitudinal section showing two chloroplasts each with, a bulging pyrenoid. Vacuoles with unknown materials are also present (arrows). Scale bar = 0.5 μm.

Fig. 60. Higher magnification of a longitudinal section through the posterior region showing the single layer of coccoliths, the columnar dense deposit, the nucleus and the vacuole contents (V). Scale bar = 0.5 μm.



CHAPTER 2

FIGURE LEGENDS

Prymnesium wyomingi

Figures 1-3. Light micrographs (LM) of *P. wyomingi.*
Scale bar = 4 μm.

Fig. 1. Cell showing two unequal flagella and the haptonema.

Fig. 2. Cell with two parietal chloroplasts.

Fig. 3. Cell with several small granular bodies in the periphery of the cell.

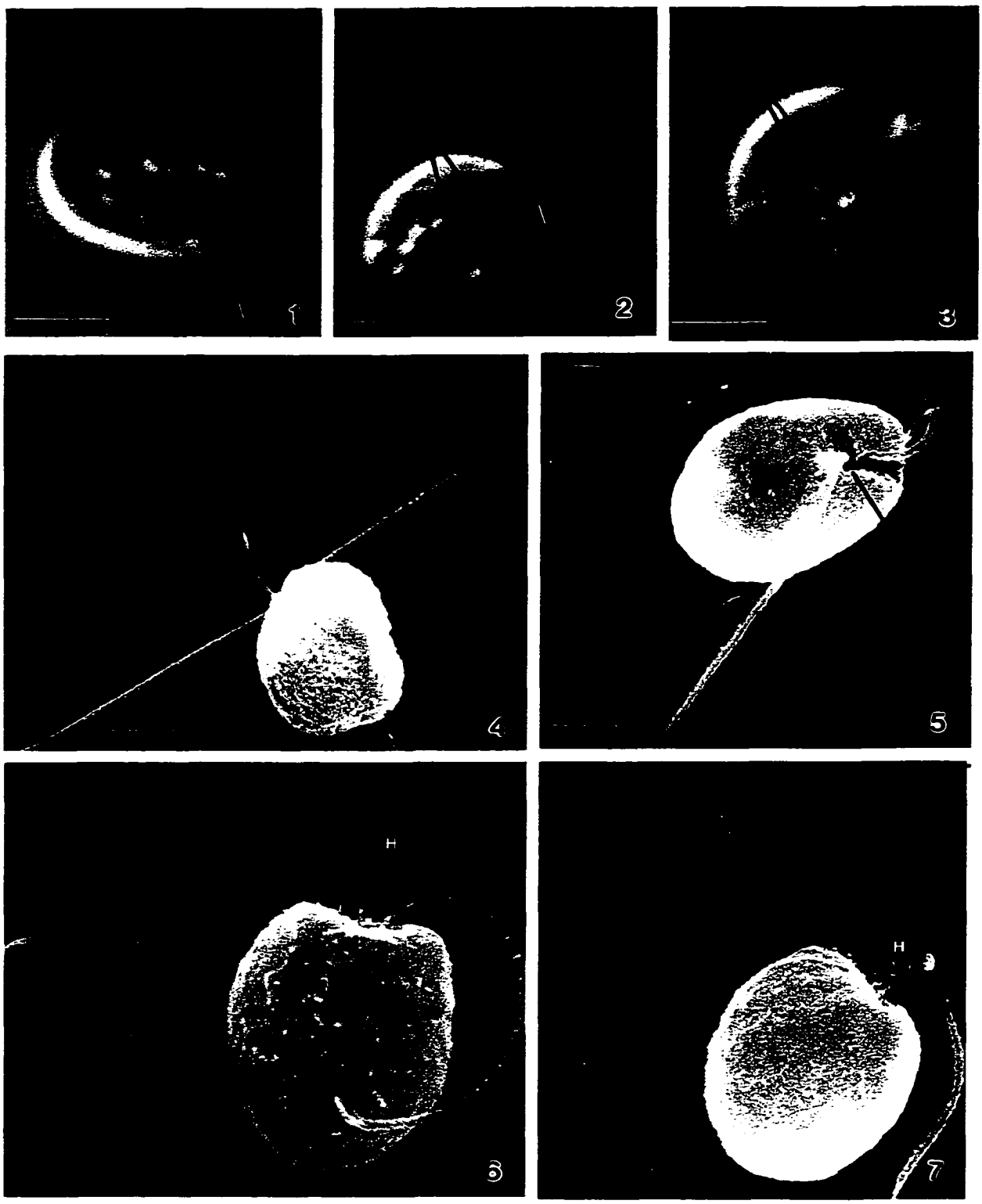
Figures 4-7. Scanning electron micrographs of *P.wyomingi.*
Scale bar = 2 μm.

Fig. 4. Cell with two flagella and an uncoiled haptonema between the flagella. Note the rough surface of the cell that might be due to scales.

Fig. 5. Cell showing the subapical flagellar insertion in a flagellar pit or groove.

Figs. 6. Sub-spherical cell showing the obliquely truncate anterior and the rounded posterior of the cell.

Fig. 7. The haptonema is bent slightly. Note the narrow tips in both flagella.



Figures 8-12. Scanning electron micrographs of *P. wyomingi.*
Scale bar = 2 μm.

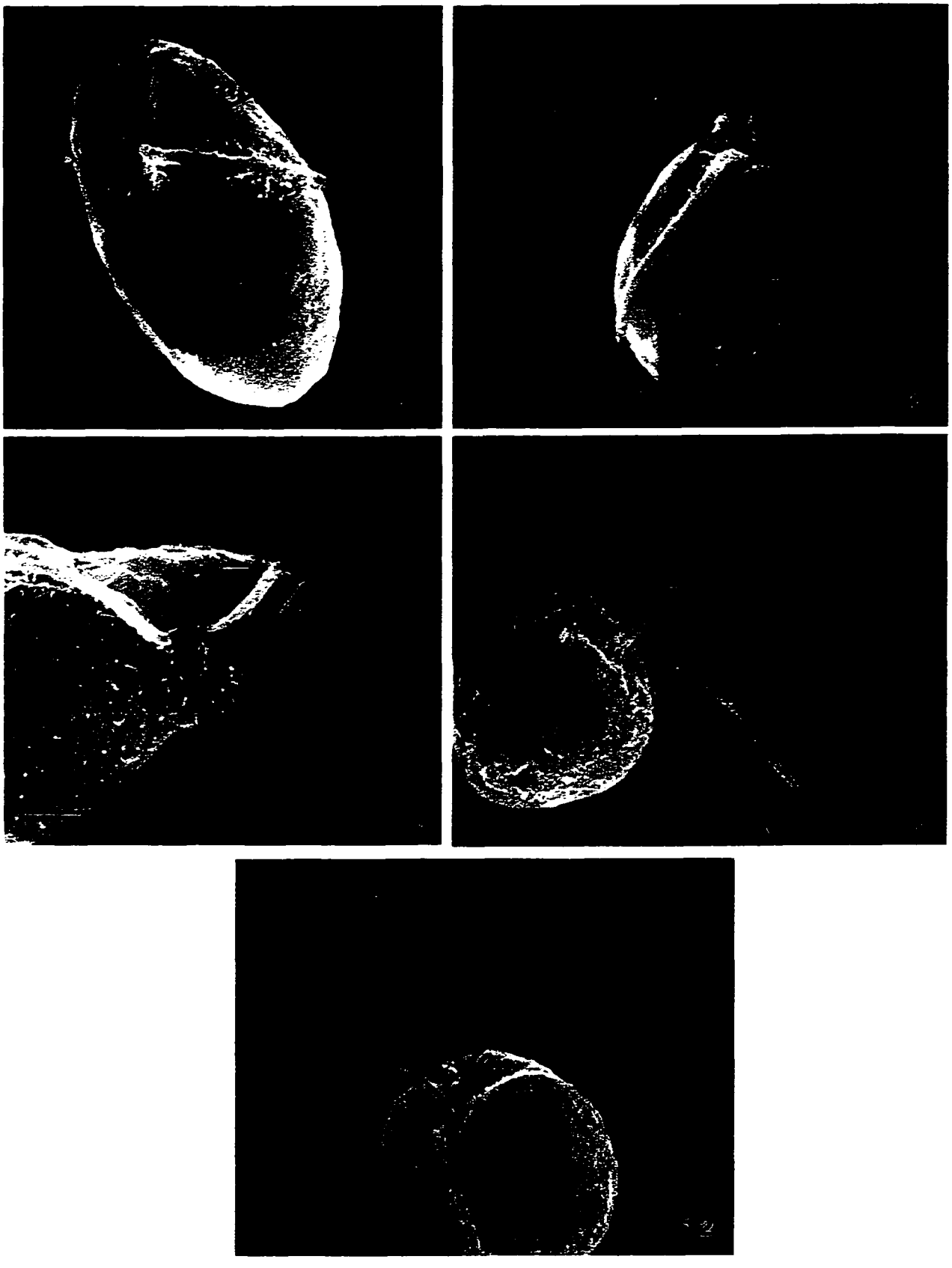
Fig. 8. Higher magnification of a cell showing the flagellar pit from which the flagella and haptonema arise.

Figs. 9. Cell showing the sub-apical pit (arrows).

Fig. 10. Higher magnification of the flagellar pit.

Figs. 11. Cells with a slightly bent haptonema.Note the narrow tip of the short flagellum.

Fig. 12. A cell with a bent haptonema.



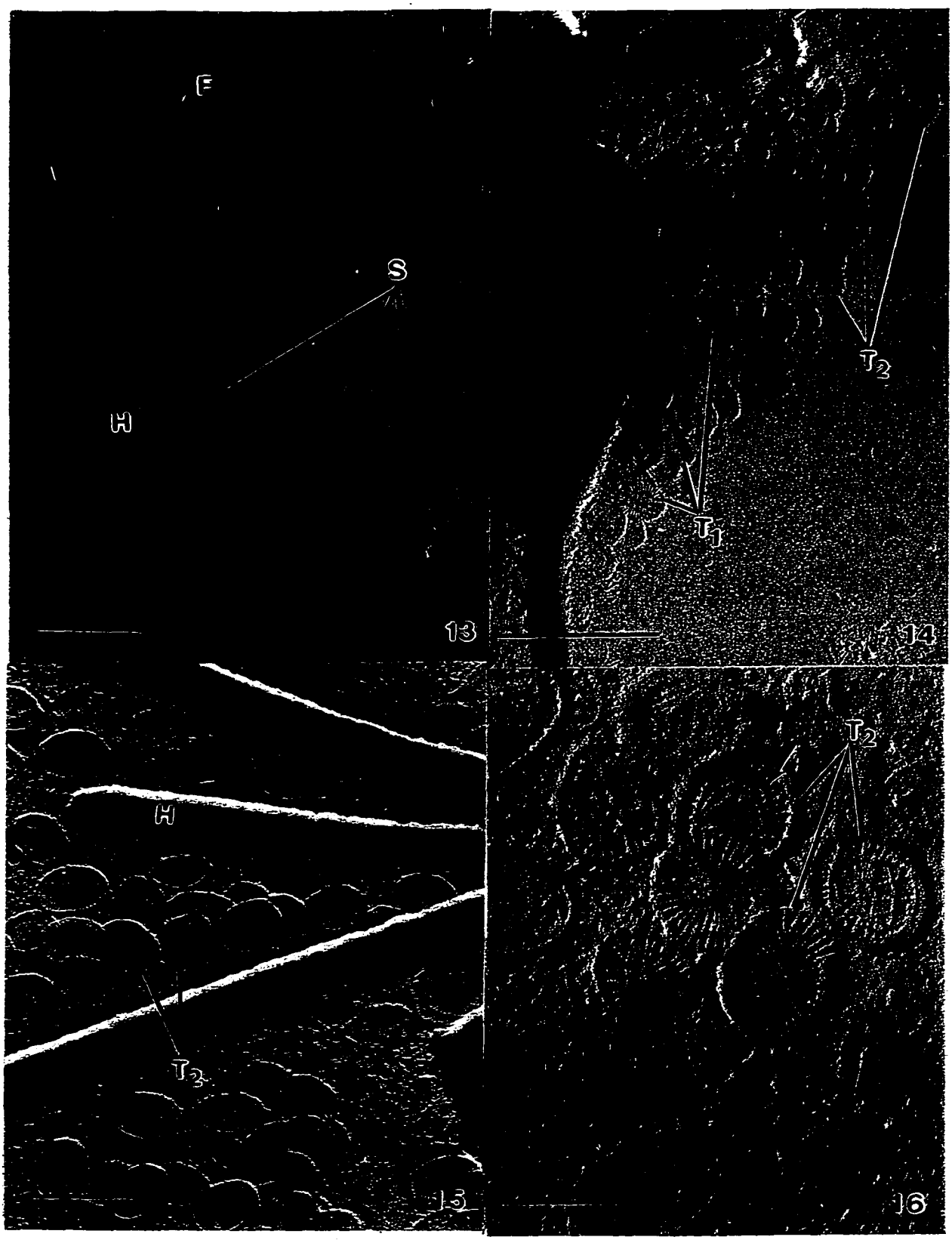
Figs. 13-16. Shadow whole mount of cells of *P. wyomingi*.
Scale bar = 0.5 μm.

Fig. 13. General view of *P. wyomingi* cell. Several layers of unmineralized scales are near the cell and the flagella. Note the narrow terminal regions in both flagella.

Fig. 14. Cell showing a general view of Type 1 and Type 2 scales. Type 1 scales are rounded, flattened with inflexed rims and a radiating pattern of fibrils in both proximal and distal faces.

Fig. 15. Type 2 scales. Scales are elliptical with radiating fibrils in the proximal face and several concentric rings in the distal face. Note that the scales surround flagella and haptonema.

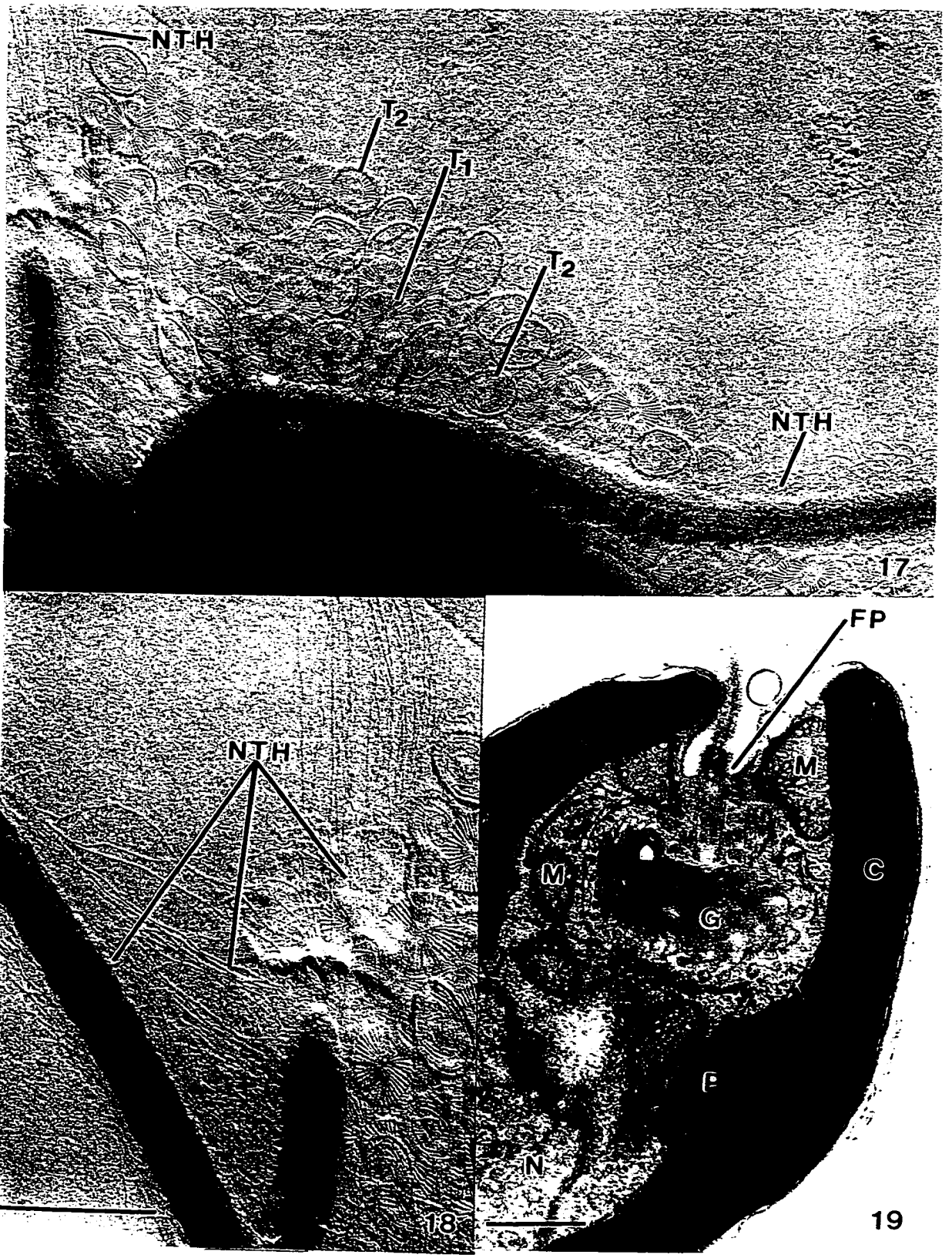
Fig. 16. Details of Type 2 scales showing the radial pattern in the proximal face and a diamond-like structure surrounded by concentric fibrils in the distal face (arrow).



Figures 17-18. Shadowed cast preparations of *P. wyomingi* . and Fig. 19. is a TEM. Scale bar = 0.5 μm.

Fig. 17. Details of the two type scales: Type 1 and Type 2 scales. Type I is in the innermost scale layer and Type 2 is in the outermost scale layer. Note that the flagellum bears non-tubular hairs. Fig. 18. Cell showing flagella and haptonema bearing long, non-tubular hairs.

Figure 19. Longitudinal section through a cell showing the parietal chloroplast with bulging pyrenoids traversed by thylakoids. A flagellar pit can be seen surrounding by mitochondrion profiles. The Golgi apparatus is located immediately beneath the flagellar apparatus. Note several layers of unmineralized scales surrounding the cell.



Figures 20-23. Transmission electron micrographs of *P. wyomingi*.
Scale bar = 0.5 μm.

Fig. 20. Oblique section of a cell through the anterior portion showing the anterior depression with the long flagellum, short flagellum, and haptonema partially sectioned. Note the scales surrounding the cell and also present in the flagellar depression. Chloroplasts surround the depression.

Fig. 21. Longitudinal median section through a cell showing the general location of organelles. There are three chloroplasts with bulging pyrenoids, and a posterior prominent nucleus, and a large polyphosphate body.

Fig. 22. Oblique section of a cell showing a prominent Golgi apparatus positioned immediately beneath the flagellar depression, with several small and large vesicles being formed in the maturing face. Note the approximate 45^0 angle between the flagella and haptonema (H).

Fig. 23. Longitudinal section of the anterior portion of the cell through the Golgi body showing details of its maturing face where mature vesicles contain developing scales (arrows). Two parietal chloroplasts are present.

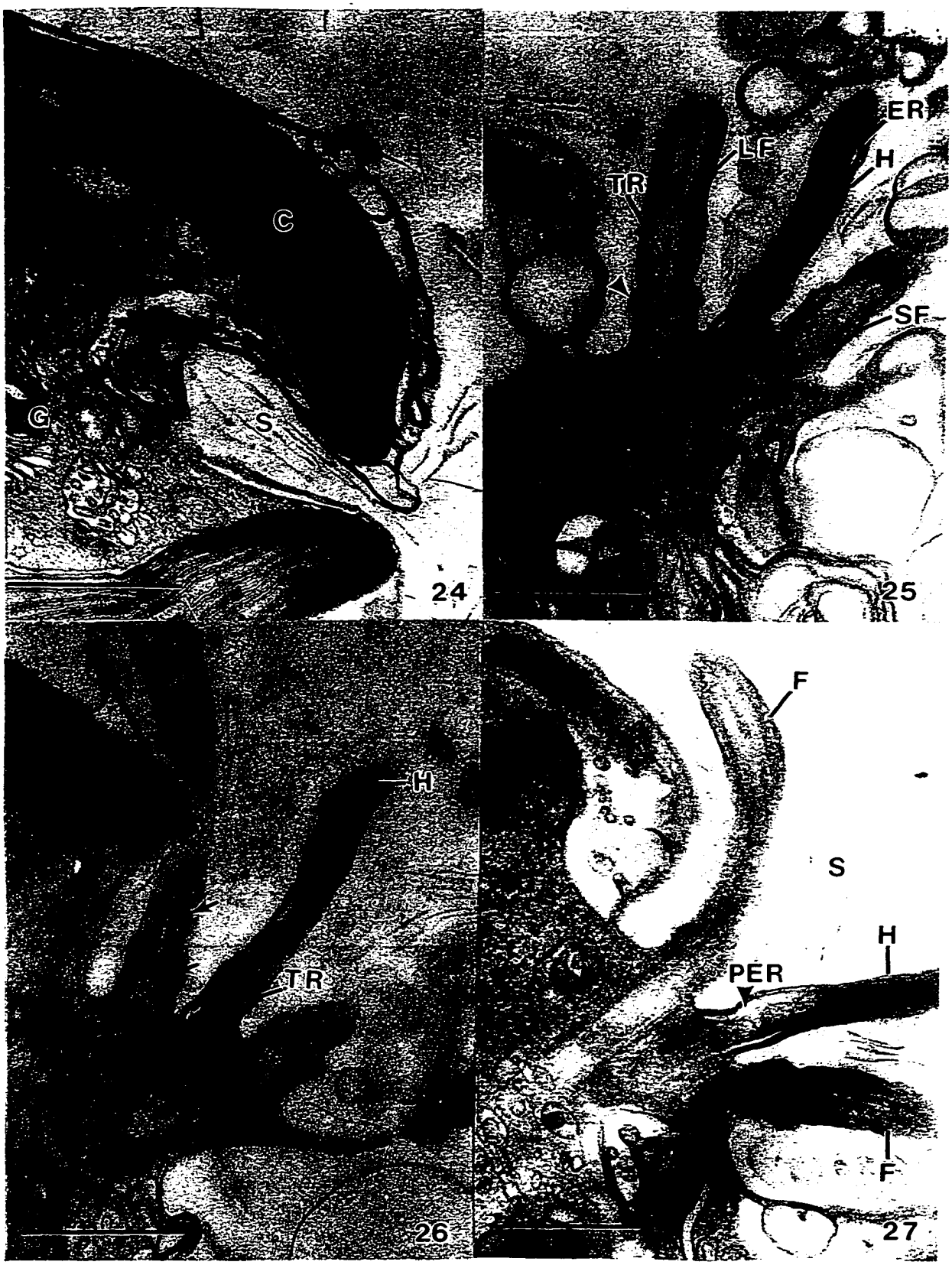
Figures 24-27. Transmission electron micrographs of *P. wyomingi*.
Scale bar = 0.5 μm.

Fig. 24. Higher magnification of a portion of cell showing a large Golgi vesicle releasing scales, and a parietal chloroplast.

Fig. 25. Longitudinal section of a cell through the flagellar apparatus showing the transitional region of the long flagellum. Note that the haptonema between the flagella only has one partition. The long flagellum has a lateral dilation in both sides between the partitions (arrow). The endoplasmic reticulum is continuous with the peripheral endoplasmic reticulum in the anterior end of the haptonema. Microtubules run from the flagellar bases to inside the cell.

Fig. 26. Longitudinal section of a cell showing details of the haptonema with one partition in the transitional region. Two partitions can be seen in the long flagellum (arrows).

Fig. 27. Oblique section of a cell through a haptonema (H) showing part of its internal structure The haptonema has a layer of cytoplasm between the peripheral endoplasmic reticulum (PER) and plasmalemma (arrow).



Figures 28-32. Transmission electron micrographs of *P. wyomingi*.
Scale bar = 0.5 μm.

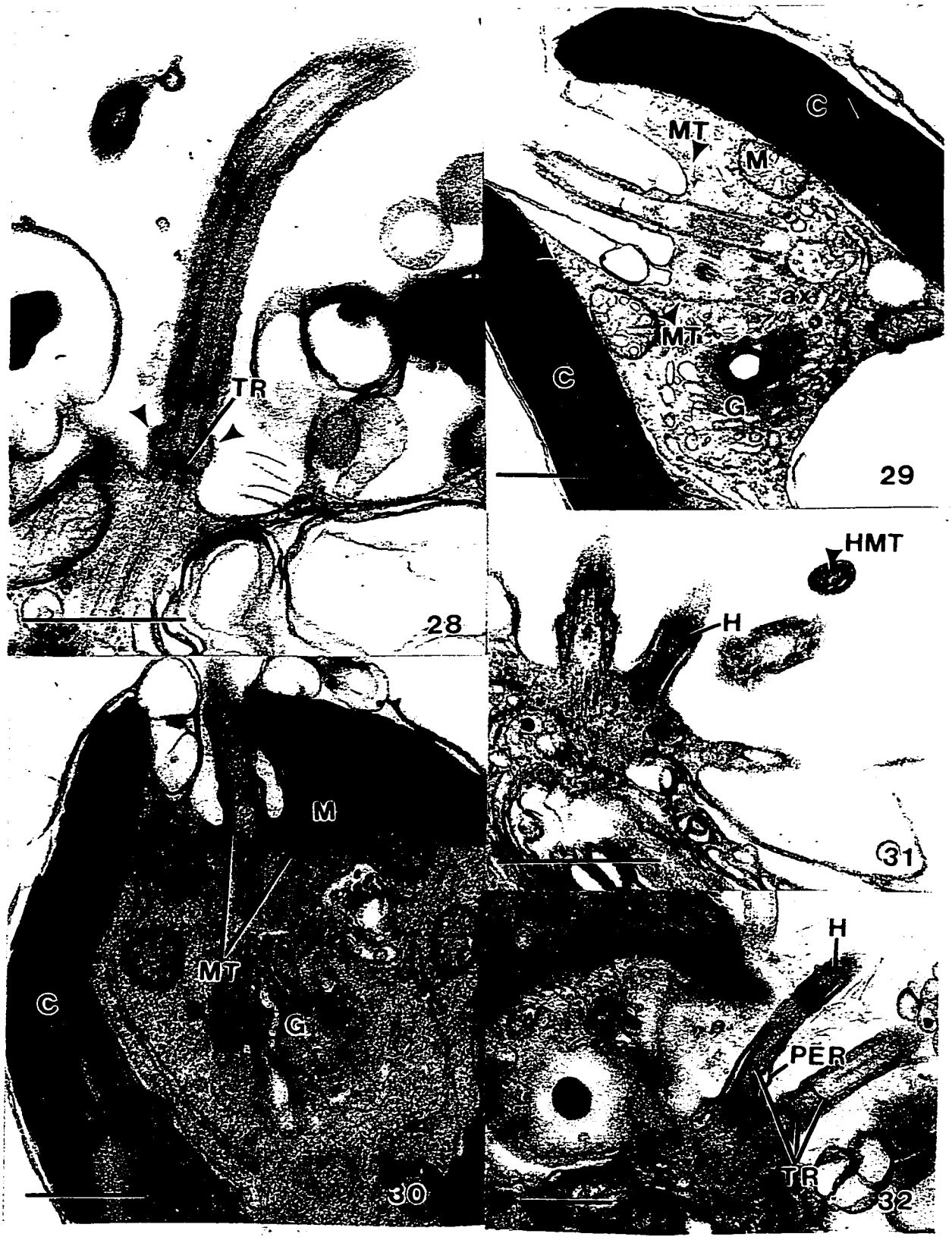
Fig. 28. A longitudinal section through the long flagellum showing the inflated flagellar membrane (arrowheads) between the partitions.

Figs. 29. A longitudinal section of a cell through the basal body of the long flagellum showing the tubular rootlet with a single sheet of microtubules running laterally from the flagellar basal bodies to the periphery of the cell.

Fig. 30. A longitudinal section of a cell showing microtubules, mitochondria that are near the basal bodies, Golgi body, and chloroplast with a pyrenoid.

Fig. 31. Transverse section of a haptonema showing the internal arrangement of the microtubules. Seven microtubules are surrounded by the ER and the plasmalemma.

Fig. 32. Longitudinal section of a cell through the flagellar apparatus showing the peripheral endoplasmic reticulum in the haptonema. Note the transitional region in both short flagellum and haptonema.



Chrysochromulina asquamosa

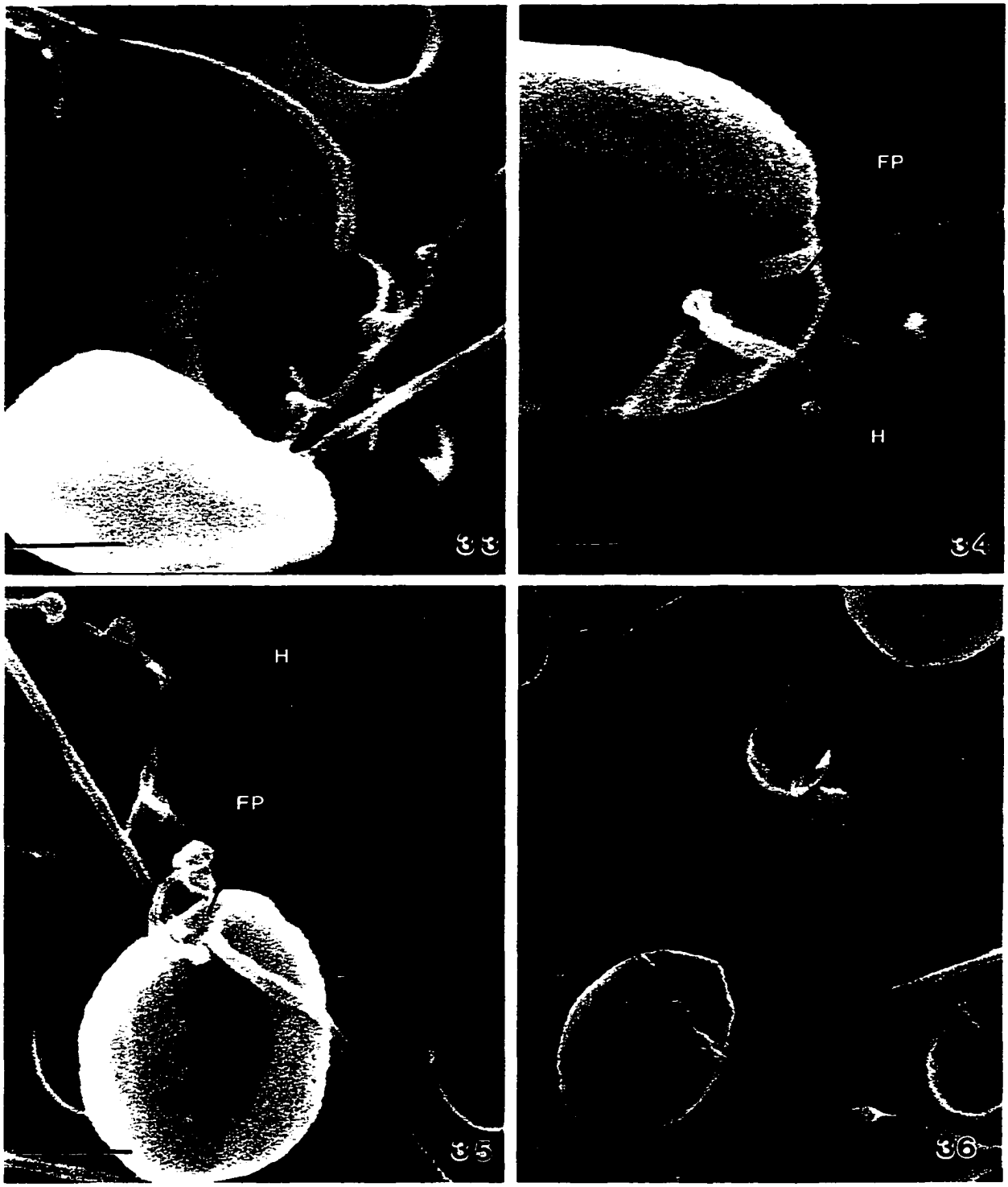
Figures 33-36. Scanning electron micrographs of *C. asquamosa*
Scale bar = 2 μm.

Fig. 33. An obovate cell with posterior and anterior truncate ends showing the flagella (F) and a long, uncoiled haptonema between the two flagella. Note that the cell surface is smooth.

Fig. 34. Cell with a coiled haptonema. Note the flagellar pit on the truncate anterior end of the cell.

Fig. 35. Cell showing the anterior truncate end and the subapical flagellar insertion (FP). Note the coiled haptonema (H).

Fig. 36. Cell showing the flagellar pit.



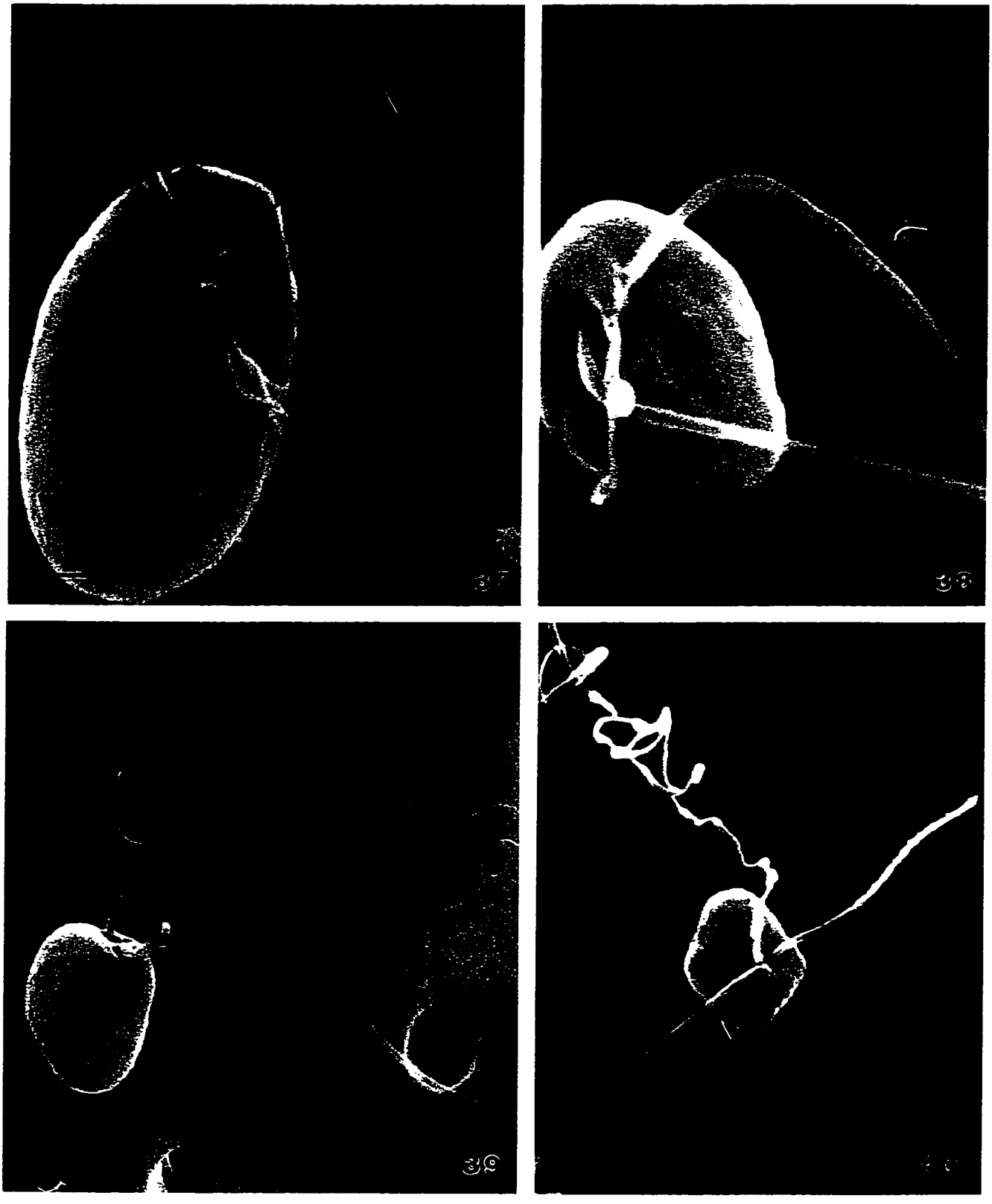
Figures 37-40. Scanning electron micrographs of *C. asquamosa.*
Scale bar = 2 μm.

Fig. 37. Higher magnification of a flagellar pit showing the flagellar insertion,and uncoiled haptonema.

Fig. 38. Cell with a short, coiled haptonema (H).

Figs. 39 Low magnification of cells showing partially coiled haptonemata (H) and both flagella (F).

Fig. 40. Top view of a cell showing the falgellar insertion and a partially coiled haptonema.



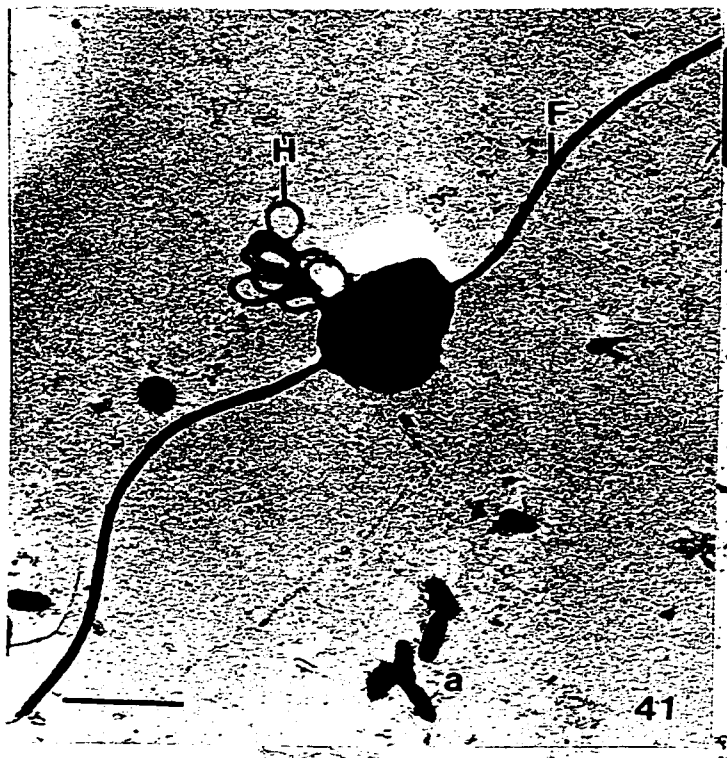
Figures 41- 42 and 44. Shadowed whole cells of *C. asquamosa.* Fig. 43. is TEM of sectioned material.
Scale bar = 0.25 μm.

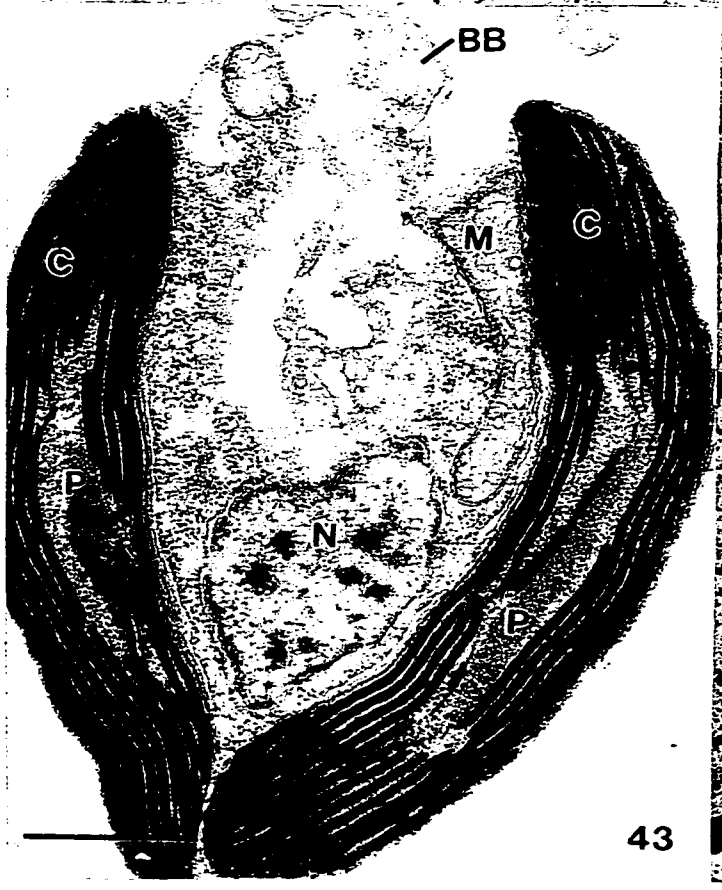
Fig. 41. Oval shaped cell with a coiled haptonema and flagella. Note that the cells lack scales. Scale bar = 0.25 μm.

Fig. 42. Cell with an uncoiled haptonema. Scale bar = 0.25 μm.

Fig. 43. Transmission electron micrographs (TEM) of *C. asquamosa.*
Cell with two parietal chloroplasts. Each chloroplast has an immersed pyrenoid that is not traversed by thylakoids. Note the basal bodies in the anterior end of the cell, a nucleus lying in the posterior end of the cell and a mitochondrion close to the flagellar basal bodies. Scale bar = 1 μm.

Fig. 44. Shadowed cell displaying the subapical insertion of the flagella and haptonem. Scale bar = 0.25 μm.





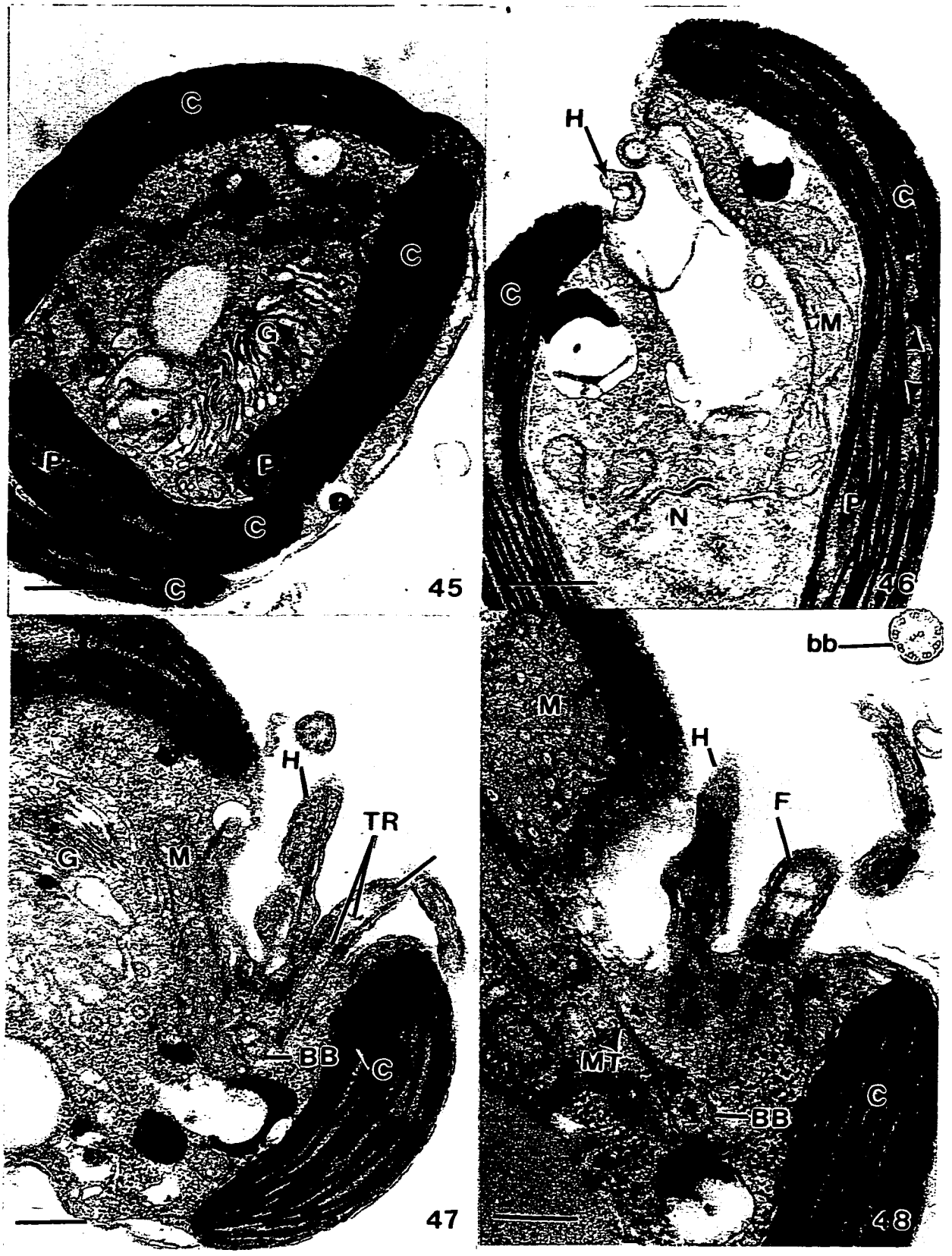
Figures 45-48. Transmission electron micrographs of *C. asquamosa*.

Fig. 45. Longitudinal section of a cell. The Golgi apparatus has the forming face on the left side of the cell and the maturing face on the right side of the cell. Note the four chloroplasts with immersed pyrenoids shown in three of the chloroplasts. Scale bar = 0.5 μm.

Fig. 46. Longitudinal section of a cell. The flagellar basal bodies and haptonema originate near a depression. A large mitochondrion is locate on one side of the cell. Chloroplasts with some osmiophilic bodies can be inside the chloroplast. Scale bar = 0.5 μm.

Fig. 47. Longitudinal section of a cell through the flagellar depression (arrow) showing the flagellar transitional region and two partitions. A haptonema shows the peripheral endoplasmic reticulum and a microtubule. A mitochondrion is near the flagellar base. The long flagellum basal body, chloroplast, and Golgi body are also shown.
Scale bar = 1 μm.

Fig. 48. Longitudinal section of a cell showing the microtubular root running laterally from the flagellar basal body to the periphery of the cell. A short flagellum and a haptonema are inserted subapically. In cross section. A basal body is showing the 9 triplet organization. Scale bar = 0.5 μm.



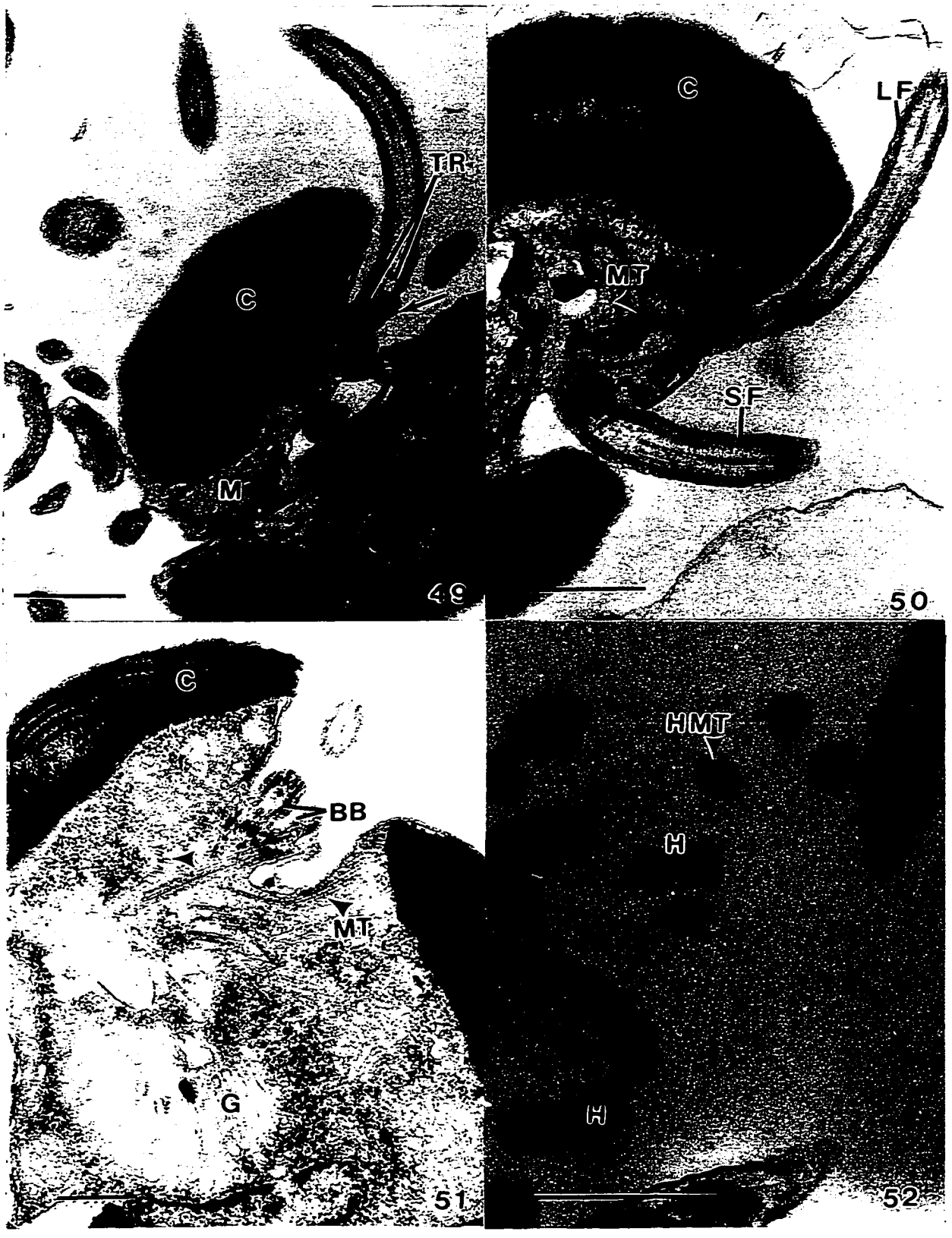
Figures 49-52. Transmission electron micrographs of *C. asquamosa.*

Fig. 49. Longitudinal section of a cell through the long flagellum showing the transitional region and two partitions. The base of the long flagellum is slightly dilated (arrow). Chloroplasts, and mitochondria also are shown. Scale bar = 0.5 μm.

Fig. 50. Anterior portion of the cell showing the long and short flagellum orientation. The angle formed is approximately at 45^0 . Microtubule runs from the long flagellum basal body (arrowhead). Chloroplast is also present. Scale bar = 0.5 μm.

Fig. 51. Longitudinal section of the anterior portion of the cell showing a network of microtubular roots running from the flagellar basal bodies to both sides of the cell (arrows). Note a single microtubule in the periphery of the cell on the right side (arrows). It is oriented in the direction of the anterior lobe of the chloroplast. Golgi body (G). Scale bar = 0.5 μm.

Fig. 52. Transverse section of a haptonema showing details of the internal organization. Six microtubules are surrounded by the peripheral endoplasmic reticulum and the plasmalemma. Scale bar = 0.5 μm.



CHAPTER 3

FIGURE LEGENDS

Uroglena volvox

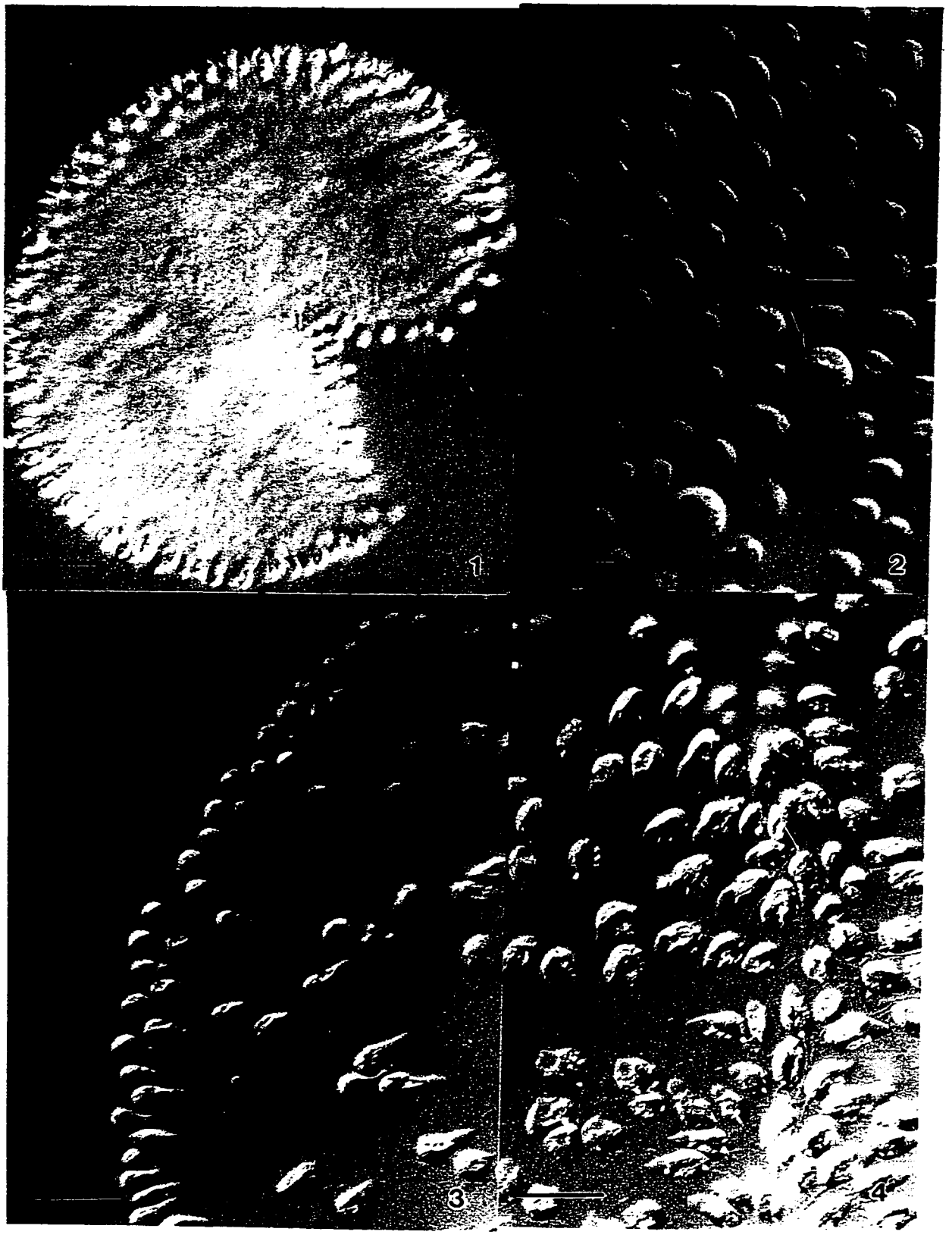
Figures. 1-4. Light micrographs (LM) of *Uroglena volvox*.
Scale bar = 10 μm.

Fig. 1. A colony with hundreds of cells. The colony was flattened by a cover slip, and the colonial integrity was disrupted, as indicated by the rupture of the colonial mucilage on the right.

Fig. 2. Higher magnification of a portion of *U. volvox* colony showing cells from the colony. Note the presence of a stomatocysts (arrow).

Fig. 3. A colony showing the pyriform shapes of cells. The pointed posteriors are toward the colonial matrix.

Fig.4. Higher magnification of a colony showing individual cells and their features, specifically their heterokont flagellation.



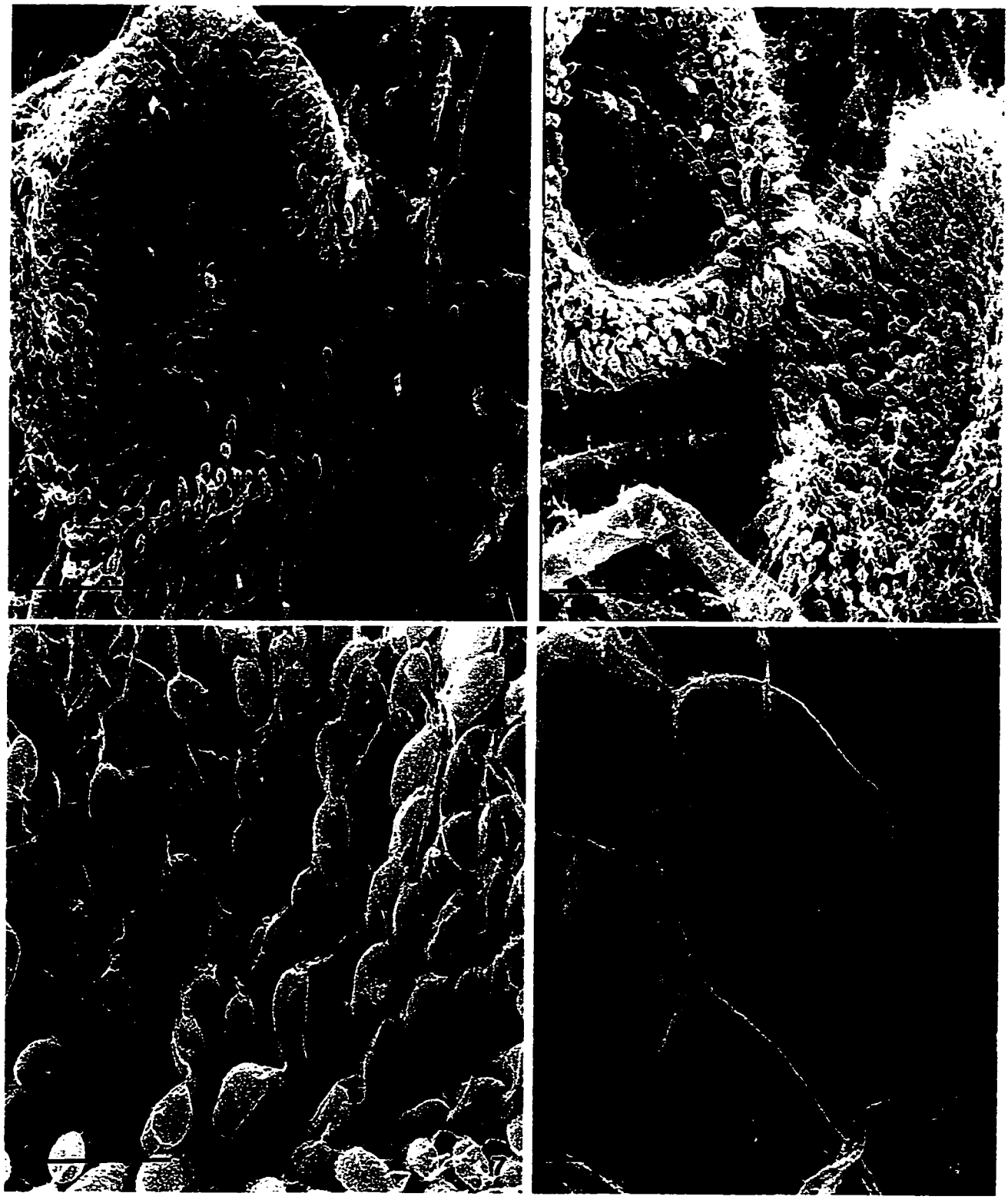
Figures 5-8. Scanning electron micrographs of *U. volvox* colonies.
Scale bar = 10 μm.

Fig. 5. Low magnification of a *U. volvox* colony with cells that extend out from a central colonial matrix.

Fig. 6. Ruptured colony showing the hollow nature of the mucilage to which cells attach. (arrow).

Fig. 7. Higher magnification of a portion of a colony showing the individual cells attached to a fibrillar matrix. Note the heterokont flagella in each cell (arrow).

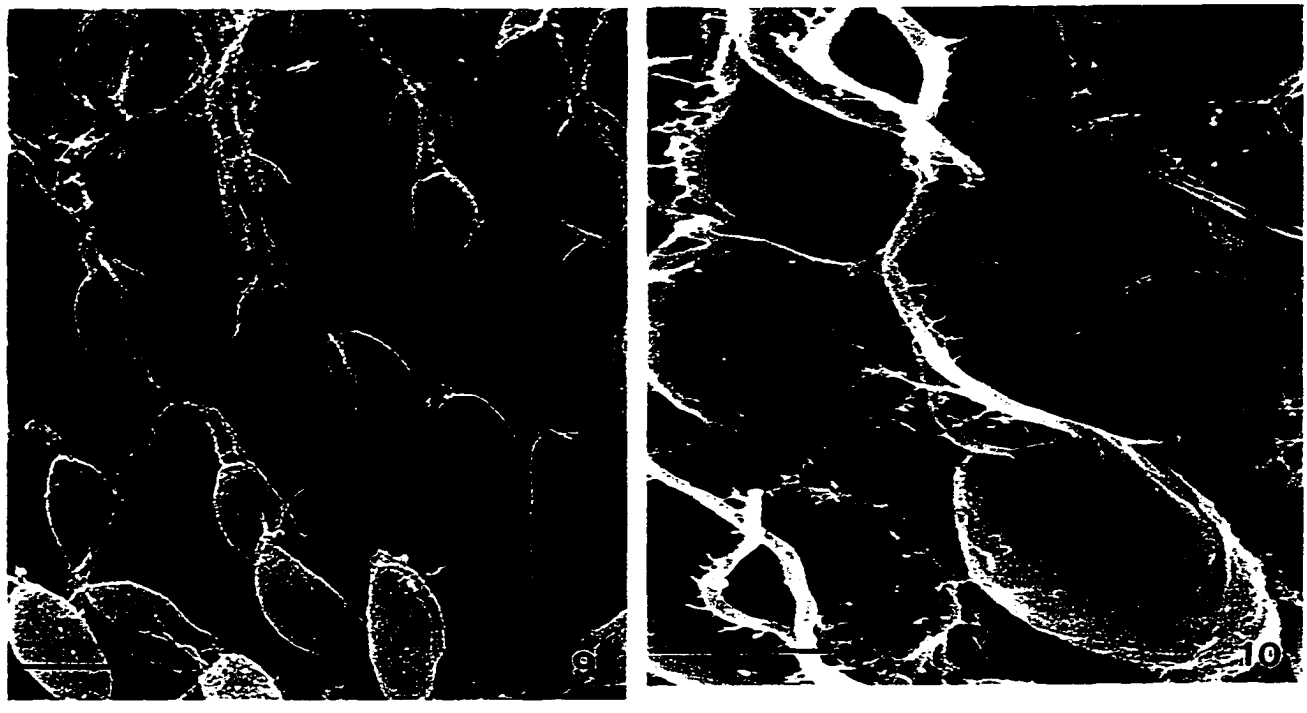
Fig. 8. Cells attached to the matrix of the colony. Note the length of the flagella and the distinctive, slightly curved shapes of the cells with their pointed ends.



Figures 9-10. Scanning electron micrographs of *U. volvox*.
Scale bar = 10 μm.

Fig. 9. Details of cells showing their attachment to the colonial matrix by their posterior ends, which may represent individual cell stalks.

Fig. 10. Higher magnification SEM of the anterior of cells showing a depression where the short flagellum is oriented at right angles to the long flagellum. A flagellar swelling is evident on the short flagellum (arrow).



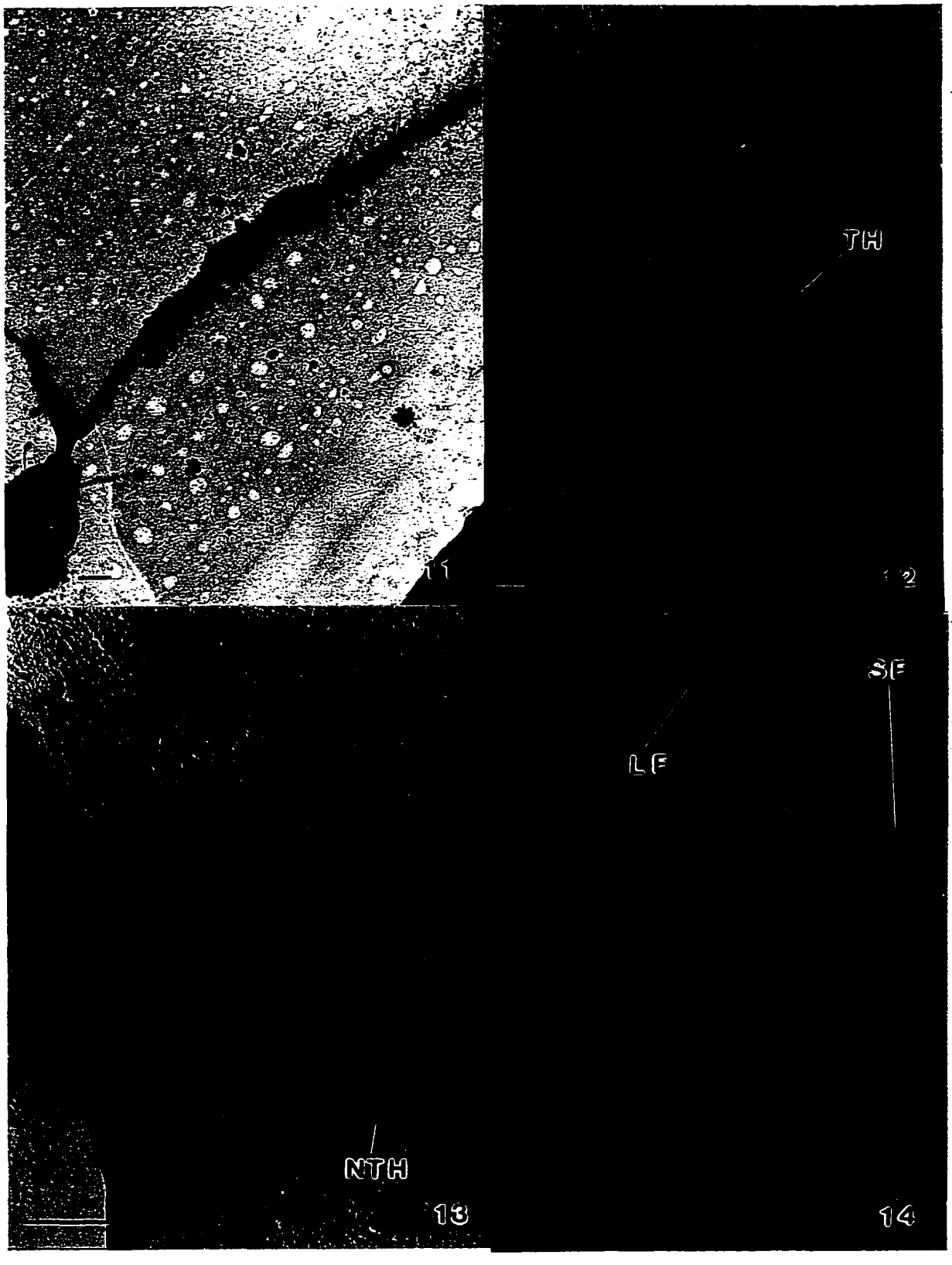
Figures 11-14. Shadowed whole mount of cells of *U. volvox*.
Scale bar = 1 μm.

Fig. 11. Cell showing the heterokont flagella. Note the relative length of the flagella.

Fig. 12. Flagella with tripartite hairs on the long flagellum.

Fig. 13. Higher magnification of flagella showing the fine non-tubular hairs on the short flagellum.

Fig. 14. Overview of a cell to demonstrate the absence of scales on the cell body. Note the tripartite tubular hairs on the long flagellum. A portion of the short flagellum also is visible.



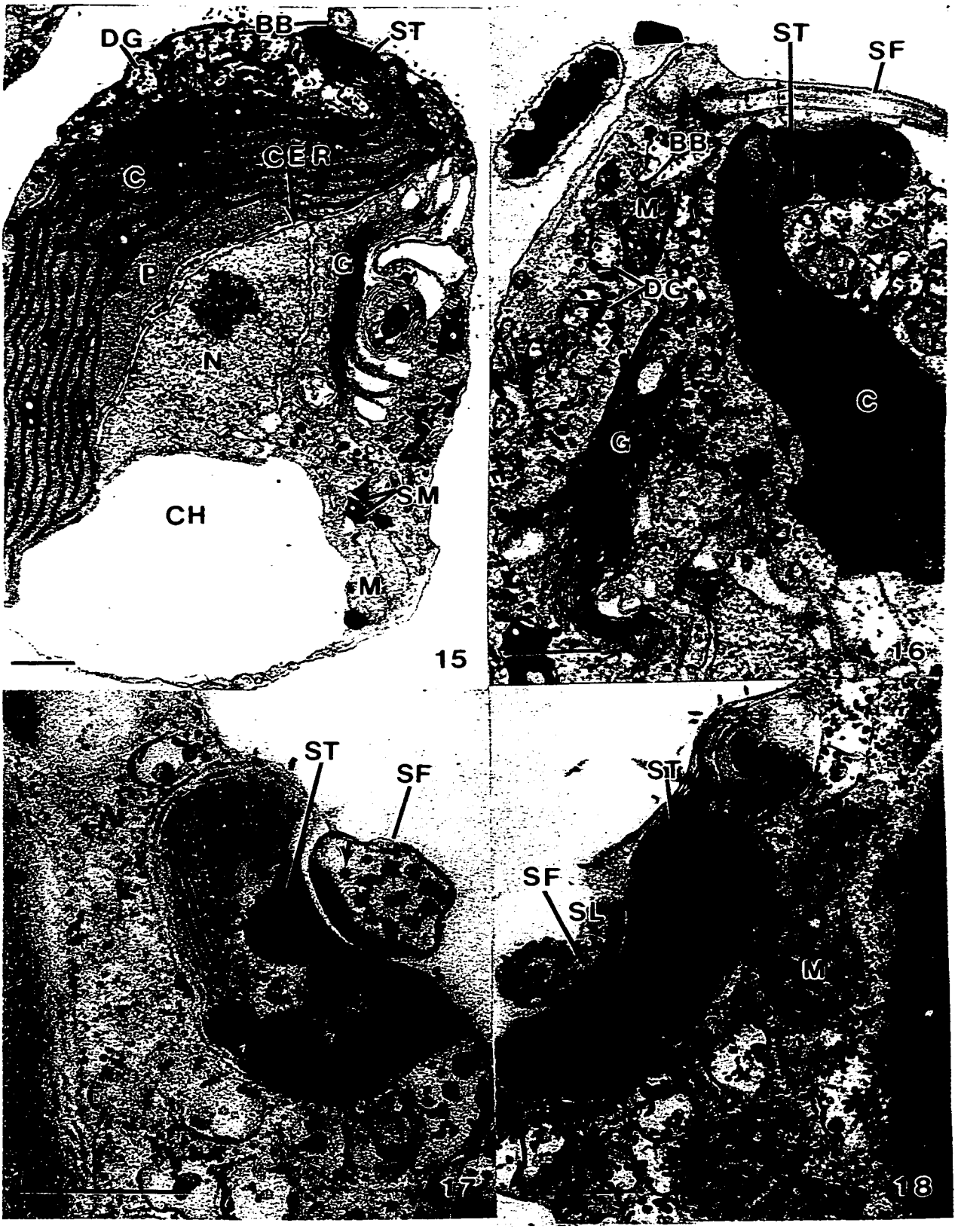
Figures 15-18. Transmission electron micrographs (TEM) of *U. volvox*.
Scale bar = 1 μm.

Fig. 15. Longitudinal section of a *U. volvox* cell. Note the cell polarization with a chrysolaminarin vacuole occupying the posterior one third of the cell. The chloroplast surrounds the nucleus and a portion of it is anterior to the nucleus. The pyrenoid is in contact with the nuclear membranes. The Golgi apparatus is positioned lateral to the nucleus. Small vesicles with dark staining granules are common in the anterior region of the cell.

Fig. 16. Longitudinal section of a cell through the anterior portion showing flagellar bases at right angles and the association of the flagellar swelling of the short flagellum with the stigma. A chloroplast, dark staining granules, Golgi body, and mitochondrion are also shown.

Fig. 17. Transverse section through the flagellar swelling in the short flagellum and the components found in the swelling. Note the spherical and striated laminar structures and their relative positions (arrows). Stigma is also present.

Fig. 18. Oblique section through the flagellar swelling showing the axoneme, spherical bodies and the striated lamellae. Note the connections between the spherical bodies (arrow). Short flagellum basal body , stigma , and mitochondrionare also shown.

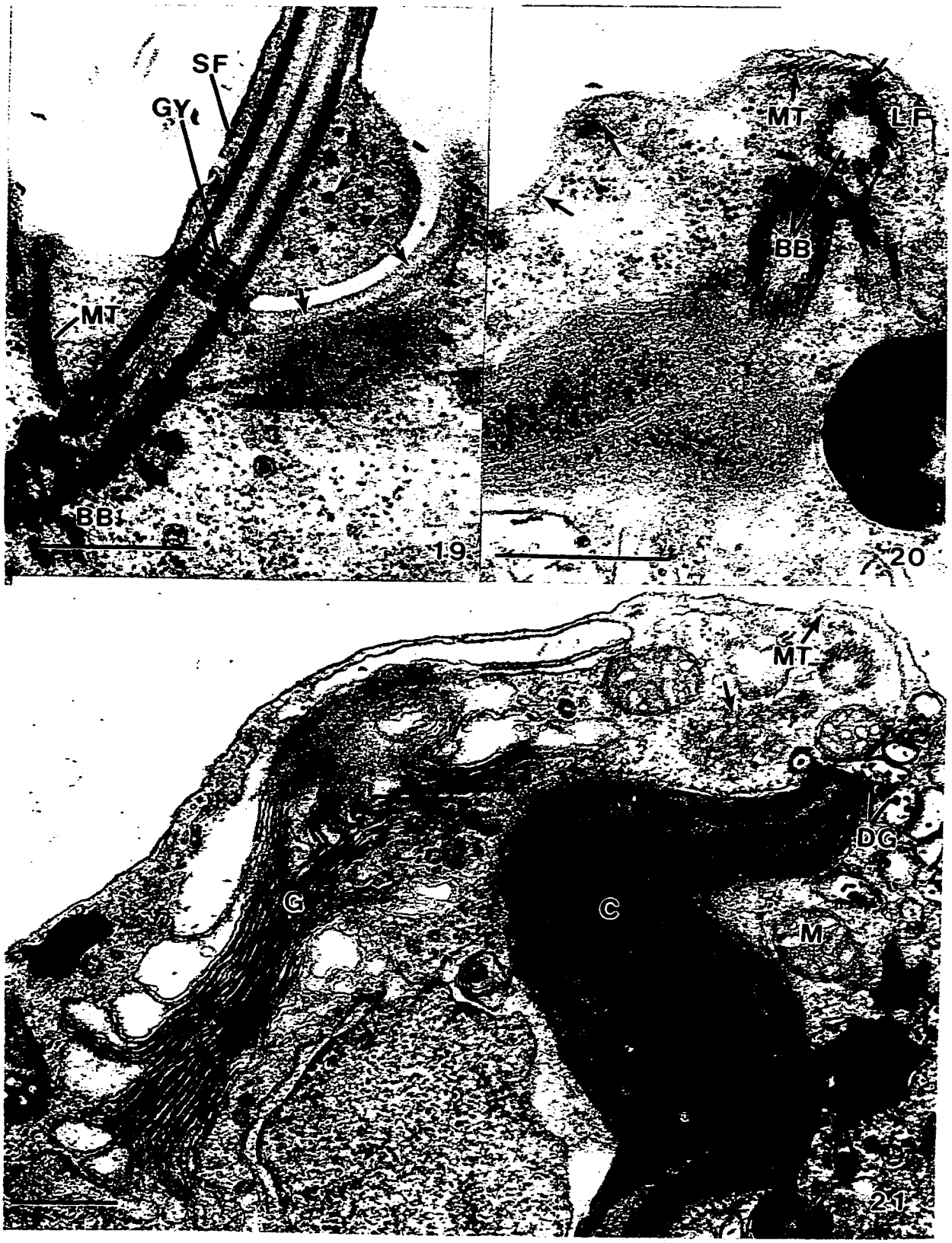


Figures 19-21. Transmission electron micrographs of *U. volvox*.
Scale bar = 1 μm refers to Figures 19-21.

Fig. 19. Longitudinal section through the short flagellum showing the spherical bodies (arrows)in the flagellar swelling and five helical gyres in the transition region of the flagellum.

Fig. 20. Higher magnification of the basal bodies and descending microtubular root (arrows). Note the perpendicular orientation of the basal bodies in relation to each other.

Fig. 21. Longitudinal section of a cell with Golgi vesicles apparently fusing to form a larger flattened vesicle beneath the plasma membrane (arrow). A basal body from the long flagellum is present and the long descending microtubular root is shown in transverse section (arrow).

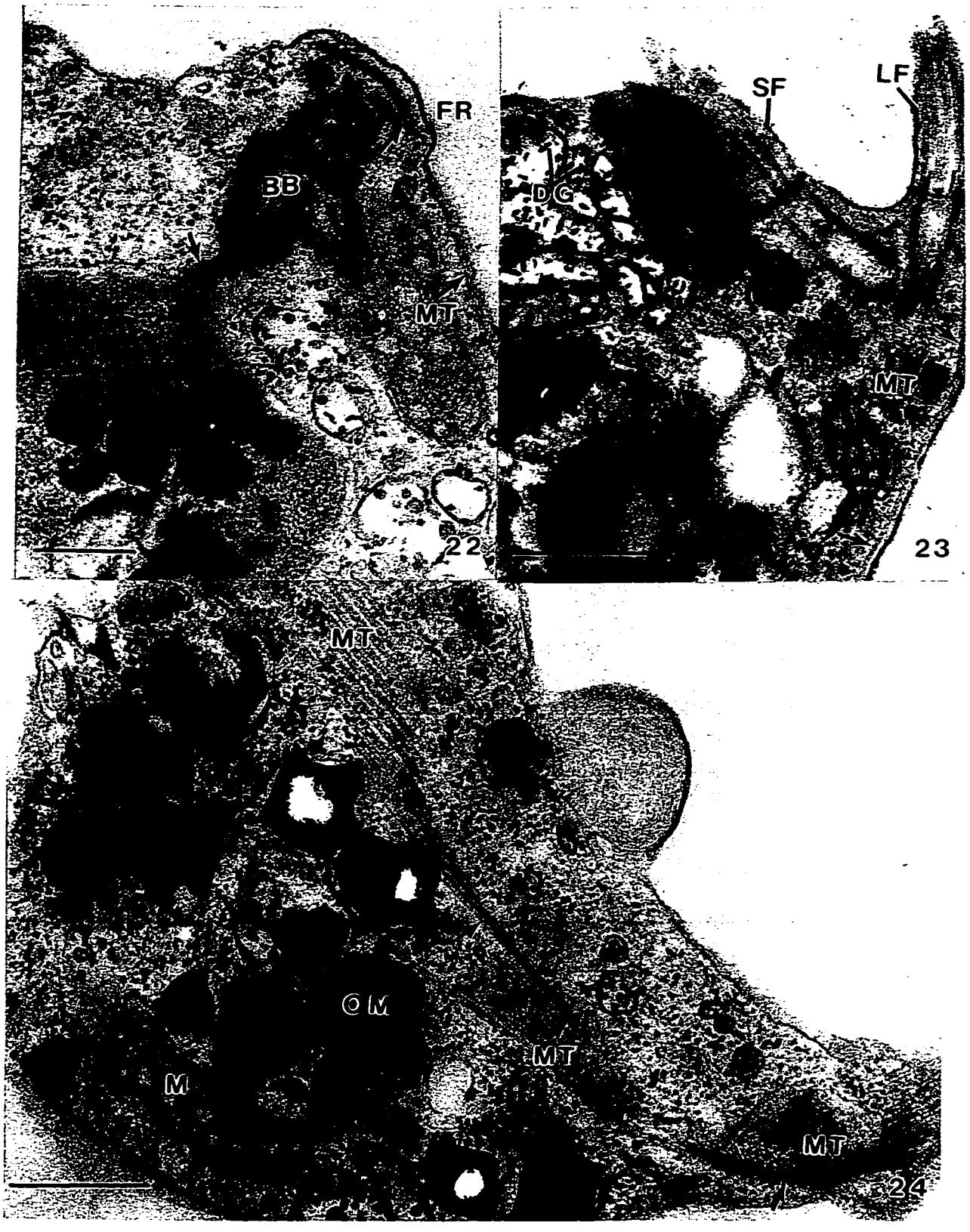


Figures 22-24. Transmission electron micrographs of *U. volvox*.
Scale bar = 1 μm.

Fig. 22. Cross section of the descending flagellar rootlet and the connecting fiber (arrow) between the basal bodies.

Fig. 23. Longitudinal section through the long flagellum and short flagellum showing the orientation of both flagella and a portion of the descending root beneath the plasma membrane (arrow). Dark staining granules are also shown.

Fig. 24. Glancing longitudinal section showing the descending microtubular root through the cytoplasm just beneath the plasma membrane. A mitochondrion and osmiophilic material occurs in small vacuoles.

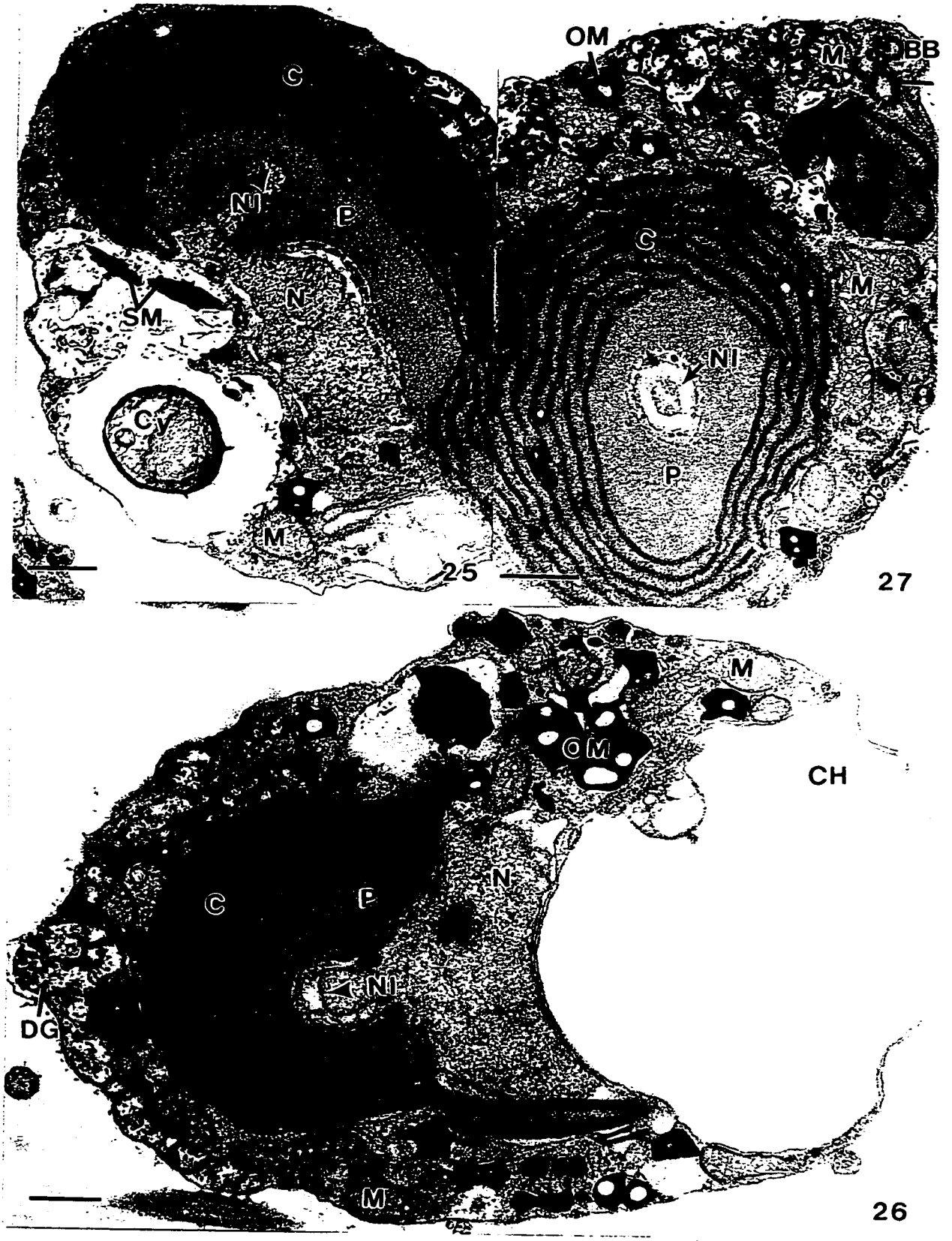


Figures 25-27. Transmission electron micrographs of *U. volvox*.
Scale bar = 1 μm refers to Figures 25-27.

Fig. 25. Cross section of a cell with a food vacuole and a cyanobacterium and other debris in the food vacuole. Note the nuclear invagination into the pyrenid. Chloroplast, mitochondrion, and nucleus are also shown.

Fig. 26. Longitudinal section of a cell with a protrusion of the nucleus into the pyrenoid. Chloroplast, chrysolaminarin vesicle, dark staining granules, mitochondrion, nucleus, and solid muciferous bodies are also shown.

Fig. 27. Longitudinal section of a cell at the level of the chloroplast showing the shape of the pyrenoid in end view and the round-oval nuclear invagination in transverse section. Basal bodies, chloroplast, mitochondrion, and solid muciferous bodies are present.

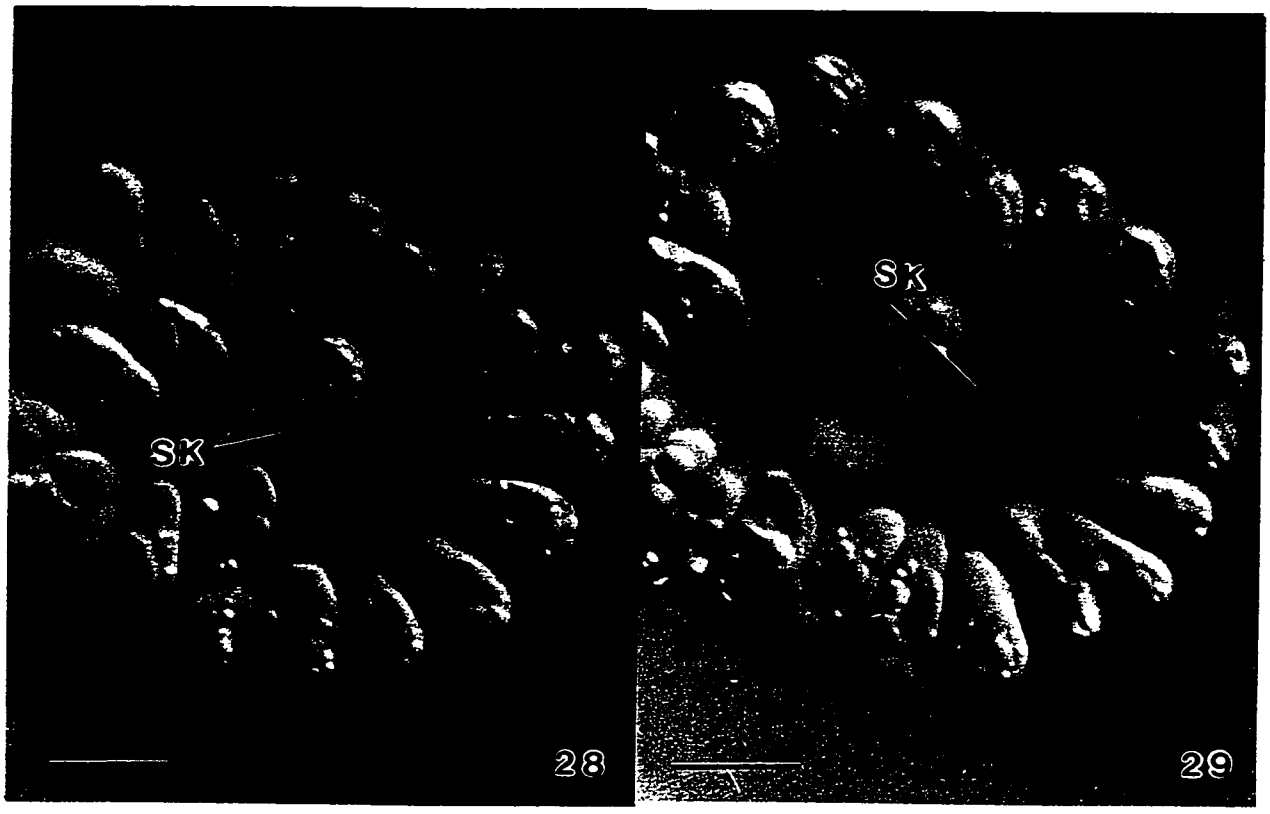


***Uroglena* sp.**

Figures 28-29. Light micrographs of *Uroglena* sp.
Scale bar = 10 μm.

Fig. 28. A colony of *Uroglena* sp. Note the cells with flattened posteriors and the attachment to individual short stalks.

Fig. 29. Flattened colony of *Uroglena* sp. showing the arrangement of cells on stalks (SK) in the colony. Note the cell shapes and the arrangement of stalks where pairs of cells share the same major stalk in the dichotomously branched stalks.



Figures 30-35. Scanning electron micrographs of *Uroglena* sp.
Scale bar = 10 μm.

Fig. 30. Colony showing cells with cone-like protusions.

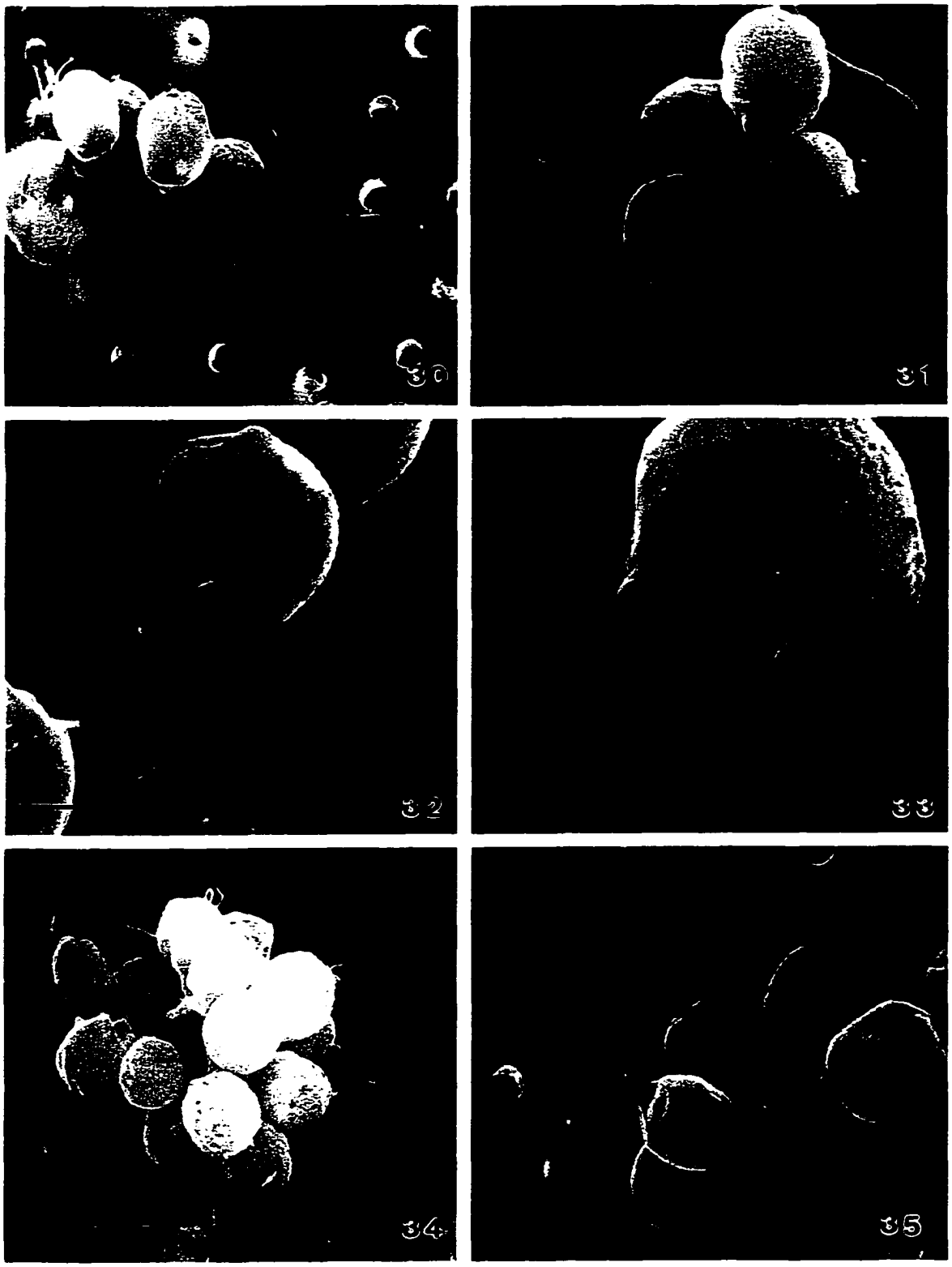
Fig. 31. Five celled colony showing heterokont flagellation and cone-like protrusions on the cell posterior (arrow).

Fig. 32. Individual cells showing the characteristic anterior end, with a circular depression and the lateral insertion of the flagella (arrow).

Fig. 33. Higher magnification of the anterior end of a cell showing the circular depression and lateral insertion of flagella (arrow).

Fig. 34. Twenty two celled colony.

Fig. 35. Six celled colony showing some remnants of stalk material (arrow) and the cone shaped posterior of the cells.

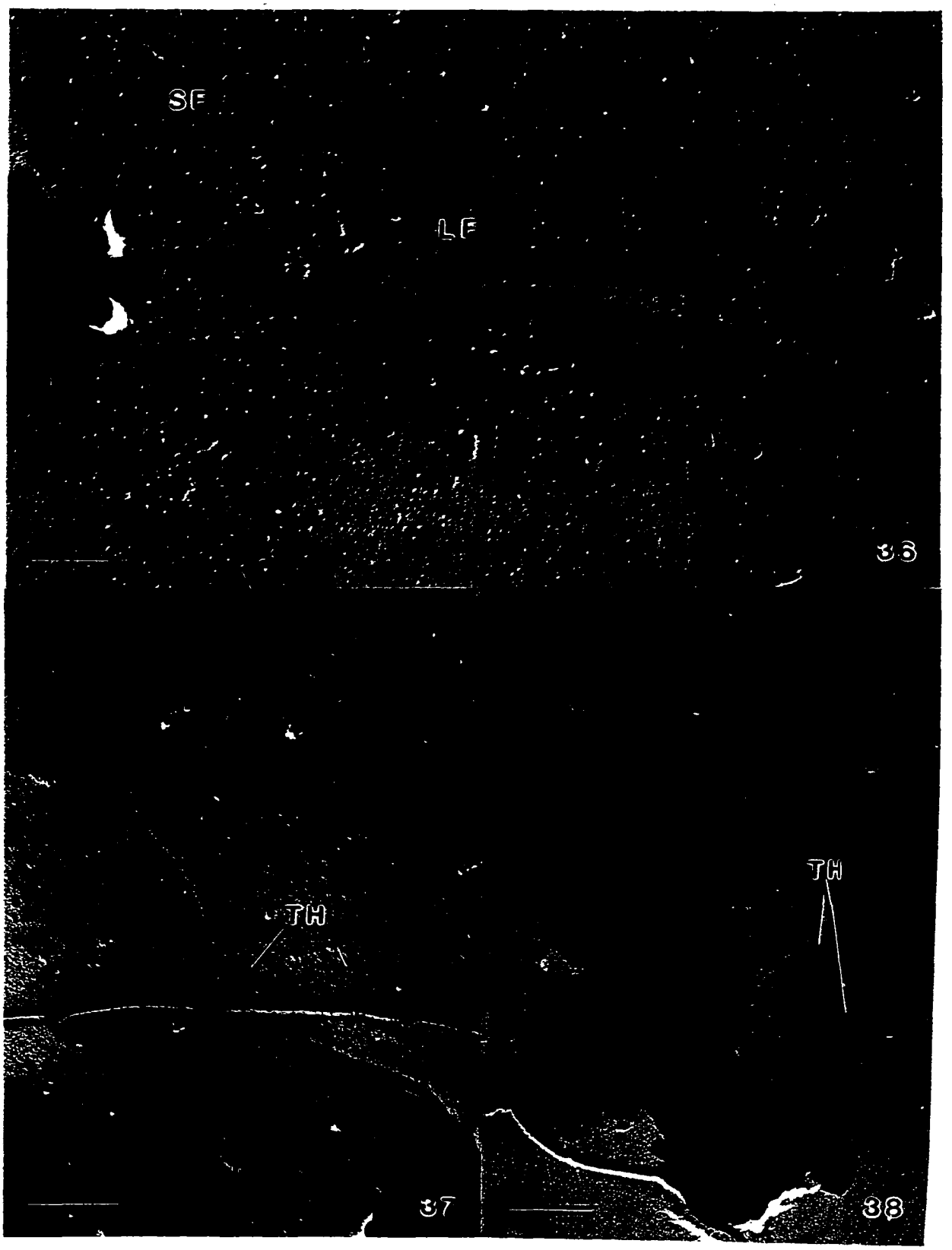


Figures 36-38. Shadowed cells of *Uroglena* sp.
Scale bar = 1 μm.

Fig. 36. Cell showing the heterokont flagella, with the long flagellum bearing tubular hairs.

Fig. 37. Higher magnification of a shadowed cell showing the typical heterokont flagellation and the tubular hairs only on the long flagellum.

Fig. 38. Higher magnification of several cells a shadowed colony showing the tubular hairs on the long flagellum.

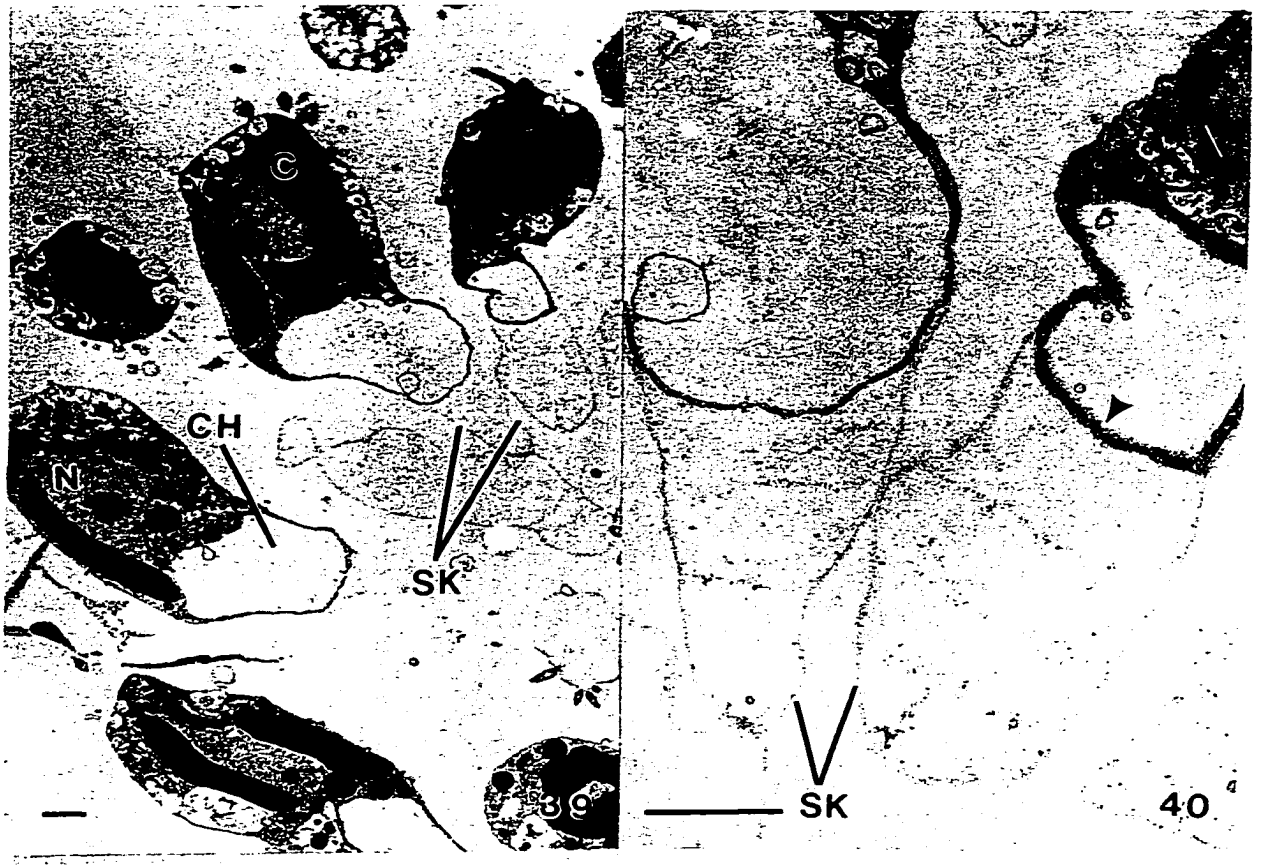


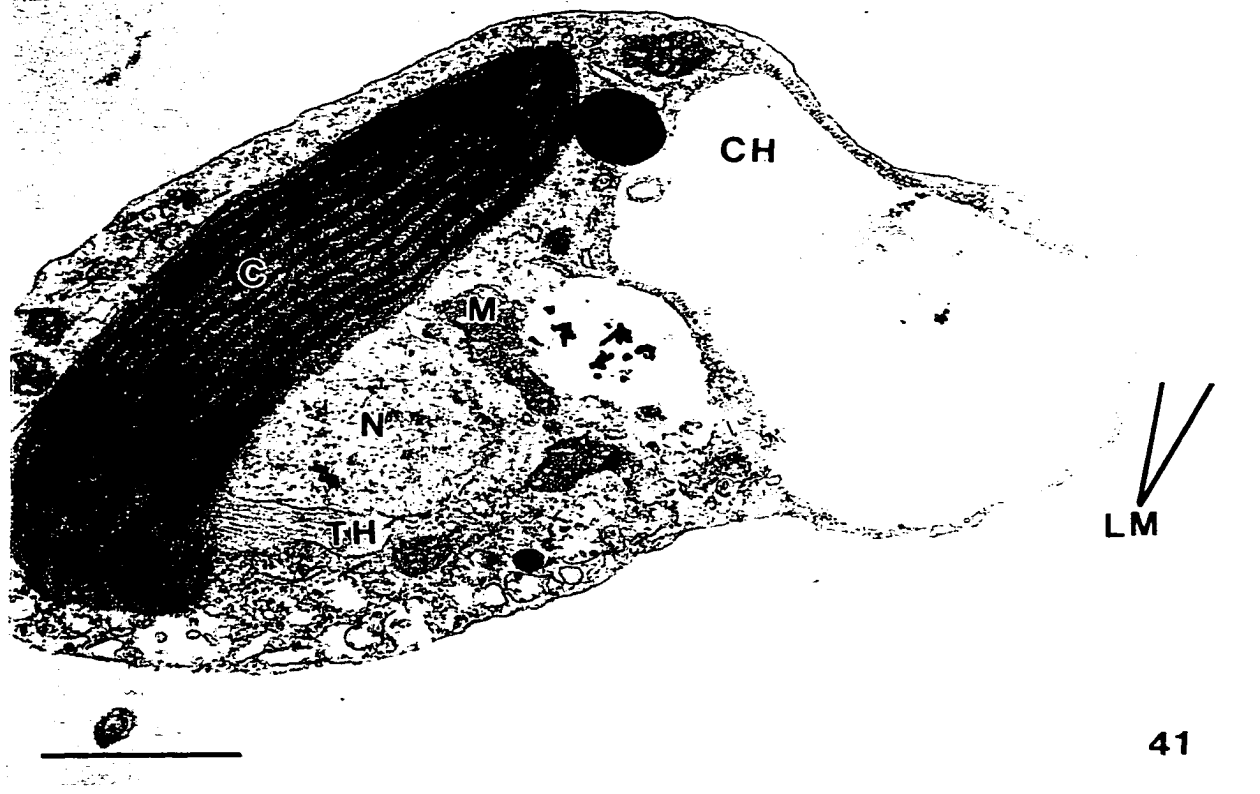
Figures 39-41. Transmission electron micrographs (TEM) of *Uroglena* sp.
Scale bar = 1 μm.

Fig. 39. Portion of a colony showing the general structure of cells and the stalks . Chloroplast, chrysolaminarin vacuole , and nucleus are also shown.

Fig. 40. Longitudinally sectioned cells with layered stalks attached to the posterior ends of the cell.

Fig. 41. Longitudinal section of a cell , with a large chrysolaminarin vacuole in the posterior, attached to thin-walled hollow stalks. Chloroplast, lamellae, mitochondrion nucleus, and tubular hairs are also shown.

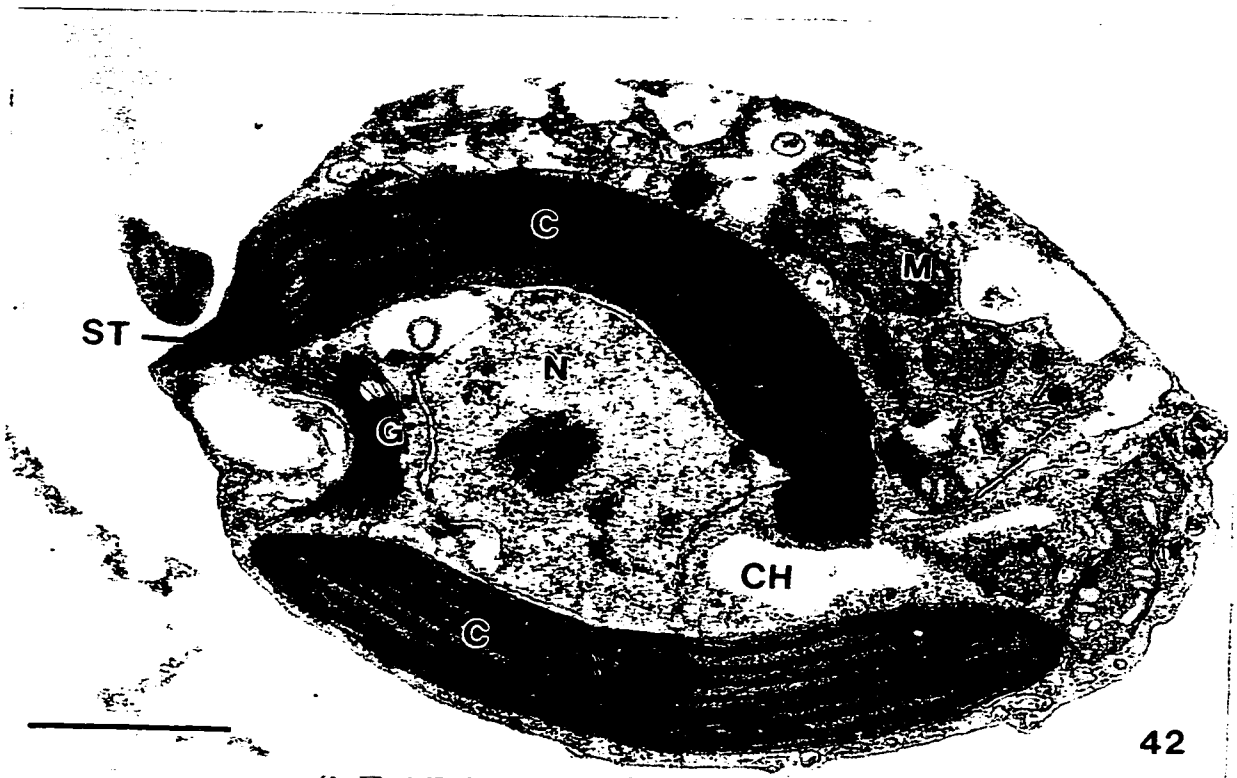




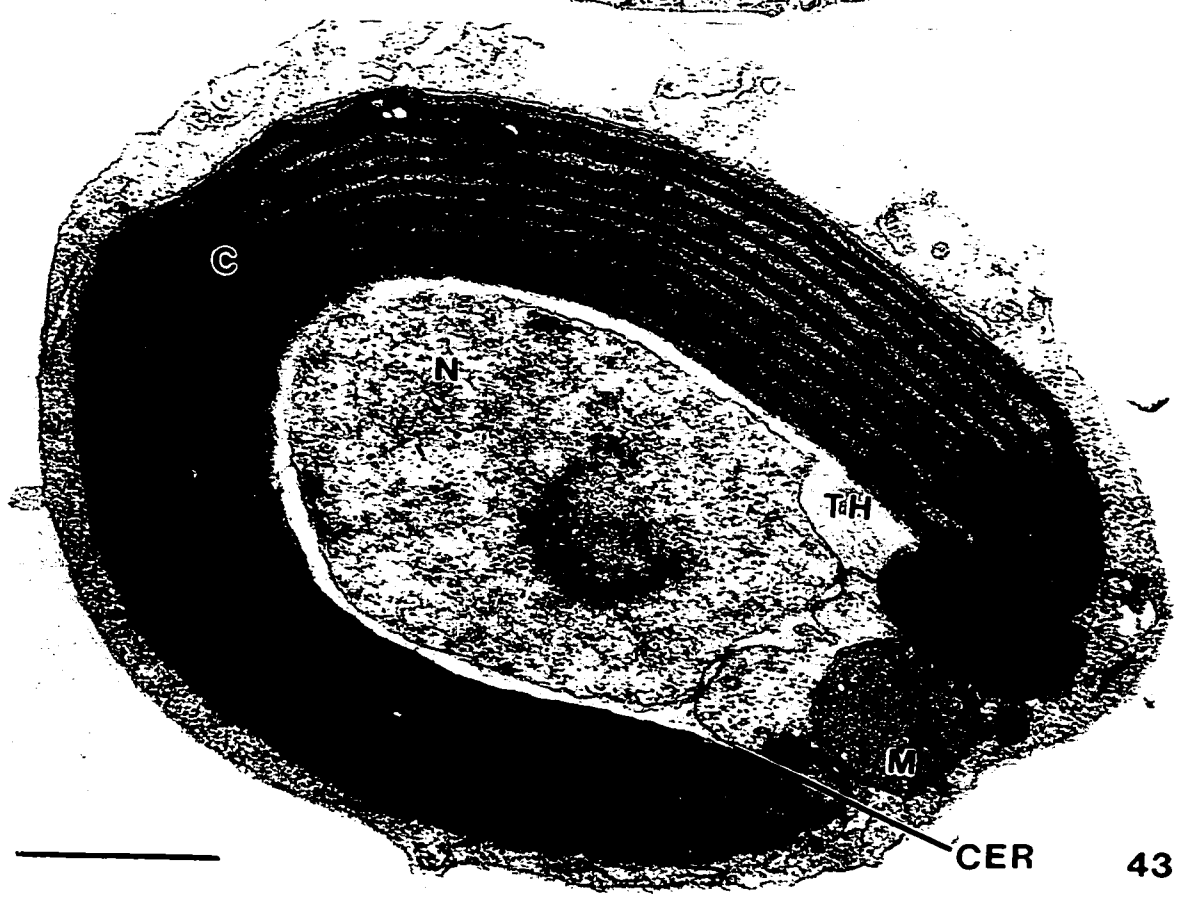
Figures 42-43. Transmission electron micrographs of *Uroglena* sp.
Scale bar = 1 μm.

Fig. 42. Oblique section of a cell illustrating the relative positioning of organelles. The nucleus is flanked by chloroplast lobes and the Golgi apparatus is anterior to the nucleus. Chrysolaminarin vacuoles, mitochondrion, and Stigma are also shown.

Fig. 43. Transverse section of a cell showing the single U-shaped chloroplast. Note some tubular hair formation in a dilated portion of the chloroplast endoplasmic reticulum (CER). Nucleus and mitochondrion are also shown.



42



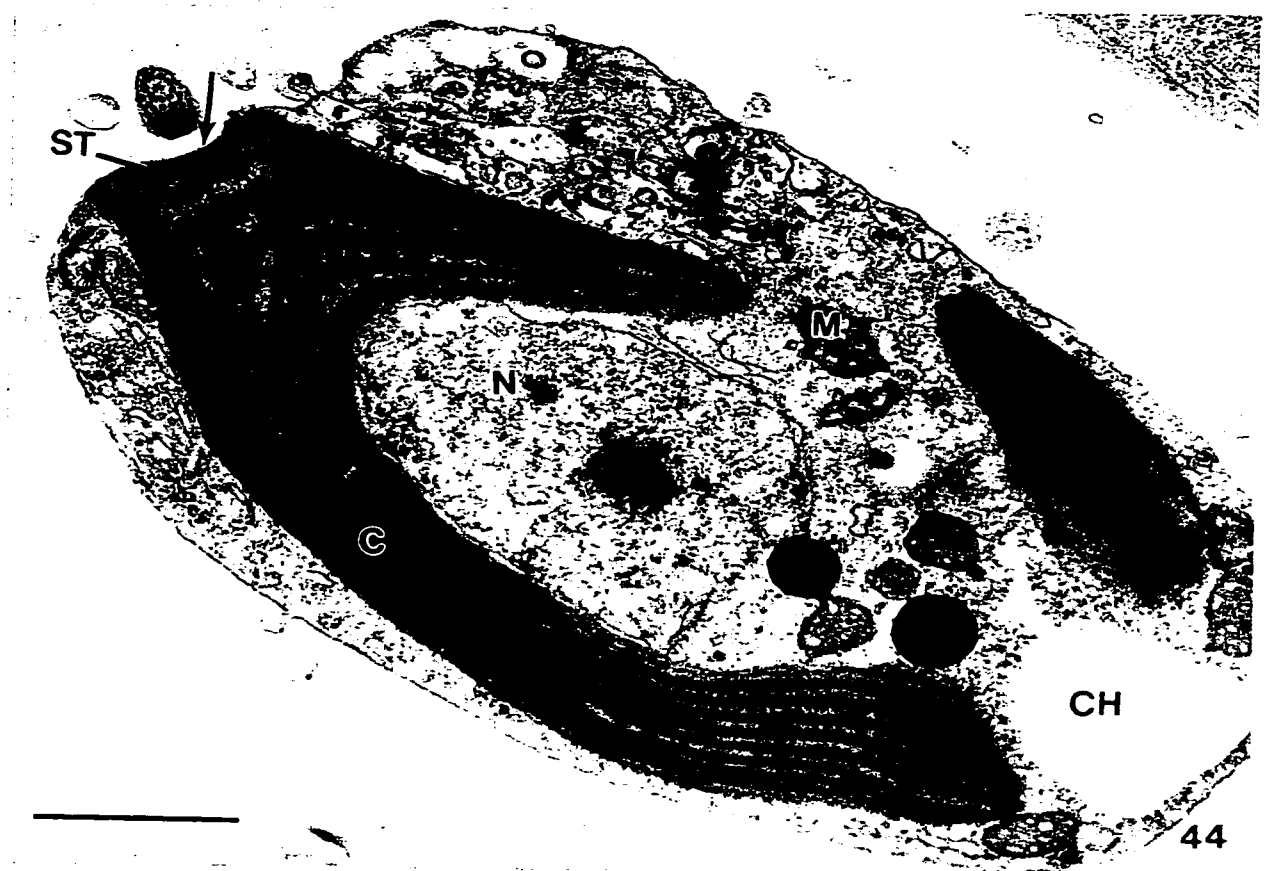
43

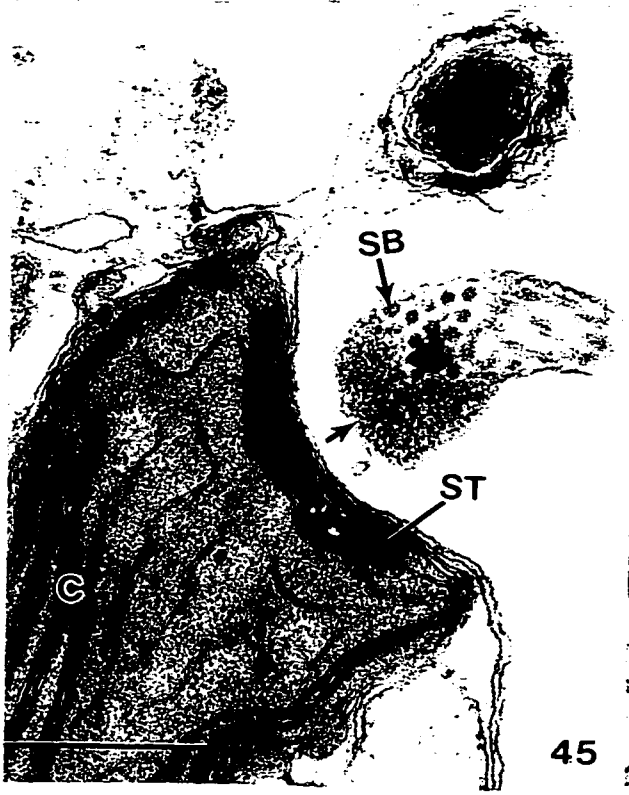
Figures 44-46. Transmission electron micrographs of *Uroglena* sp.
Scale bar = 1 μm.

Fig. 44. Longitudinal section of a cell with a stigma in the anterior portion of the chloroplast. Note the depression in the area of the stigma (arrow).

Fig. 45. Higher magnification of the stigma and a transverse section of the flagellar swelling, which has spherical bodies and a granular matrix ventral to the spherical bodies (arrow). Note that the stigmatic granules occur in a single layer and that the thylakoids are not organized into groups of three in this area. Chloroplast is also shown.

Fig. 46. Portion of the anterior of a cell to show the stigma and the flagellar swelling constituents (arrows). Chloroplast, and Golgi body are also shown.



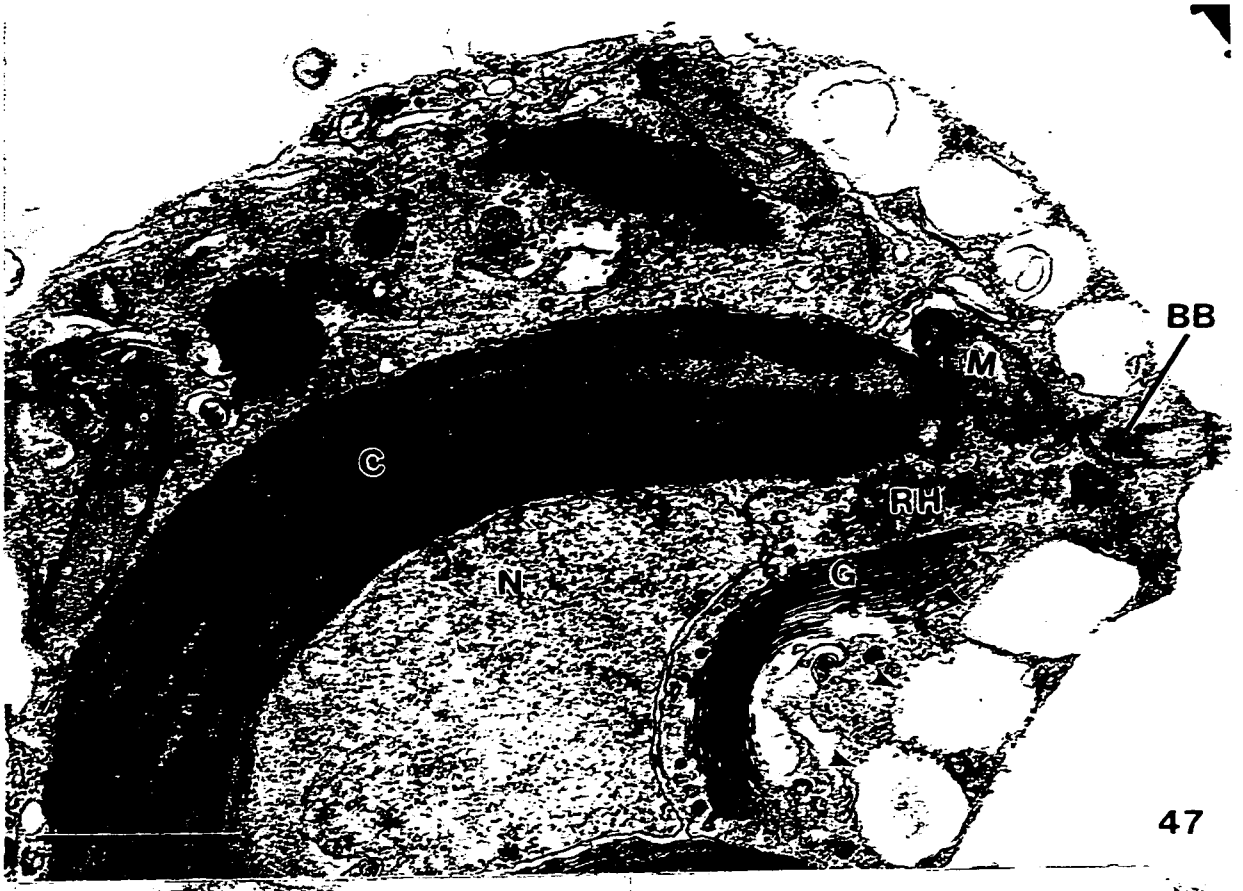


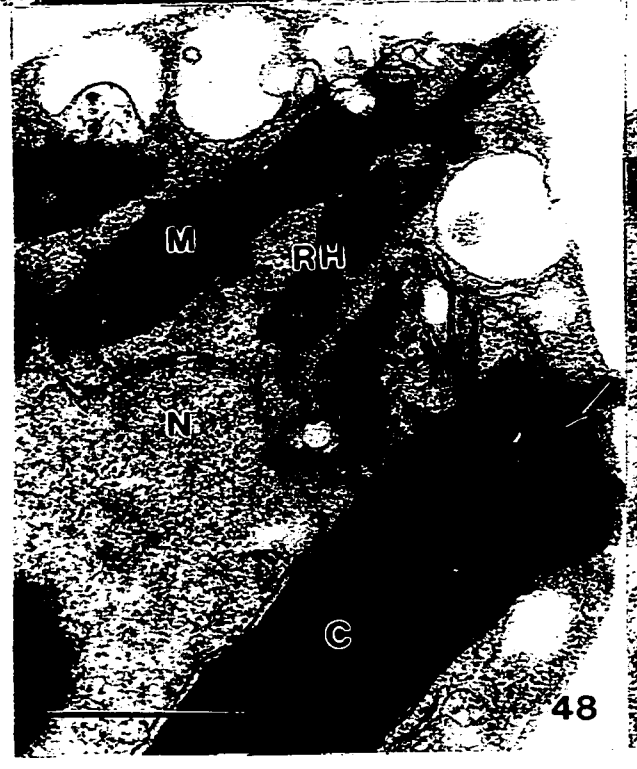
Figures 47-49. Transmission electron micrographs of *Uroglena* sp.
Scale bar = 1 μm.

Fig. 47. Oblique section of a cell with a nucleus and chloroplast surrounded by chloroplast endoplasmic reticulum and a Golgi apparatus that is anterior to the nucleus and appears to be forming large vesicles (arrows). Note the rhizoplast extending from the area of the basal bodies. Mitochondrion is also shown.

Fig. 48. Higher magnification of the anterior of a cells with a rhizoplast that shows the granular and striated components. Note the areas of the chloroplast where thylakoids are not organized into triplet aggregations (arrow).

Fig. 49. Anterior end of a cell showing a cross section of the basal body of the long flagellum, but a descending root is not present. Chloroplast, nucleus , and stigma are also shown.





Uroglena articulata

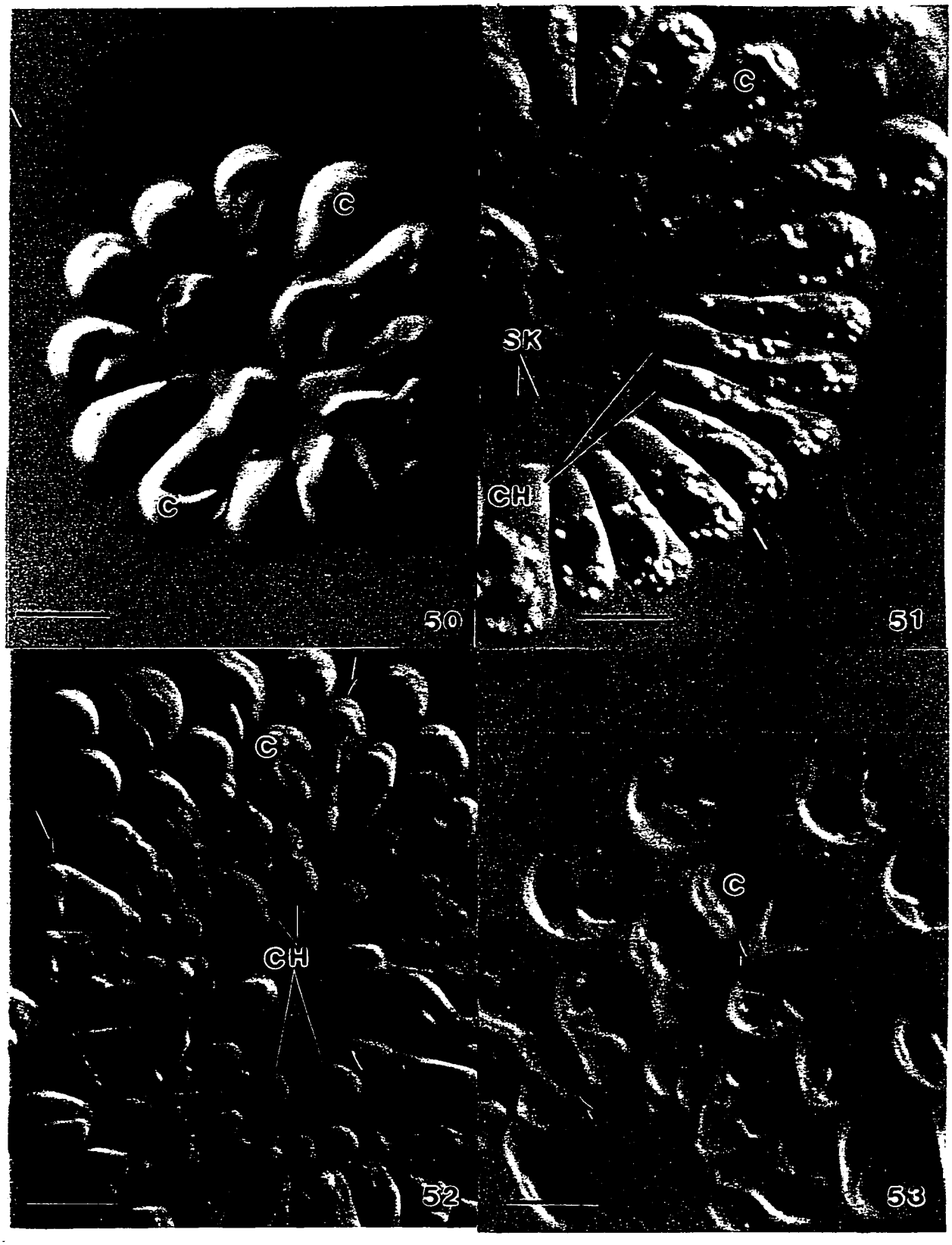
Figures 50-53. Light micrographs (LM) of *Uroglena articulata.*
Scale bar = 10μm.

Fig. 50. A 15 celled colony. Several cells with two chloroplasts per cell are distinguishable. Note that the chloroplasts are not necessarily parallel and flanking the nucleus.

Fig. 51. Flattened colony showing the stalks, their sympodial branching pattern and their attachment to cell posteriors.

Fig. 52. Cells in colony showing extrusion of chrysolaminarin vesicles, allowing cells to be squeezed from the colony (arrows).

Fig. 53. Peripheral region of a colony showing released cells (arrows) and some that are being ejected during their release of the chrysolaminarin vesicle (arrowhead).



Figures 54-58. Scanning electron micrographs of *Uroglena articulata.*
Scale bar = 0.5 μm.

Fig. 54. Thirty two celled colony showing its general morphology.

Fig. 55. Higher magnification SEM of a colony showing some attachment stalks (arrow). Note the cone shaped posteriors on some cells.

Fig. 56. Fourty celled colony.

Fig. 57. Five celled colony with some attachment stalks (arrow) in the posterior end of the cell.

Fig. 58 Portion of a colony showing three of the cells attached by stalks (arrows).

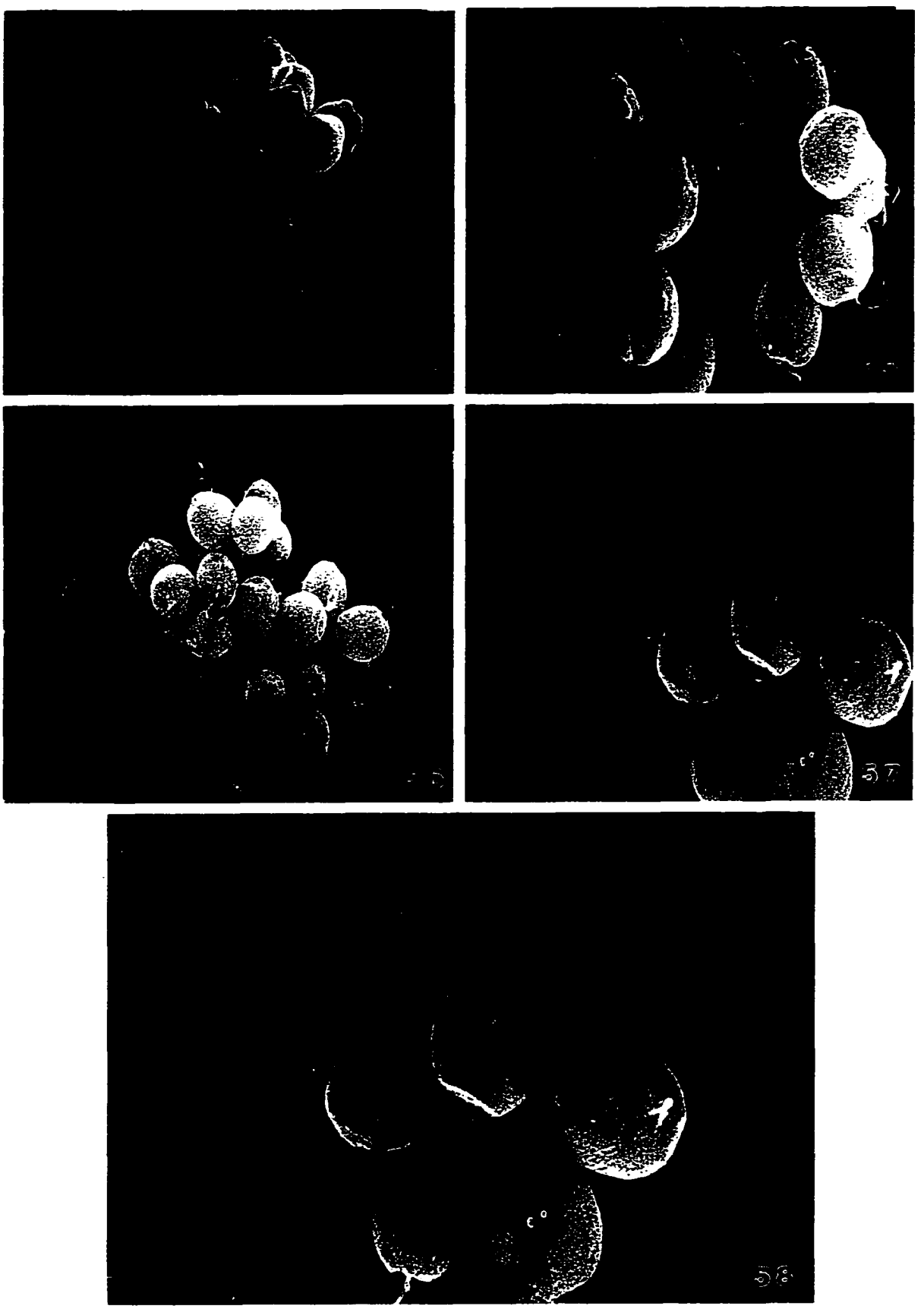


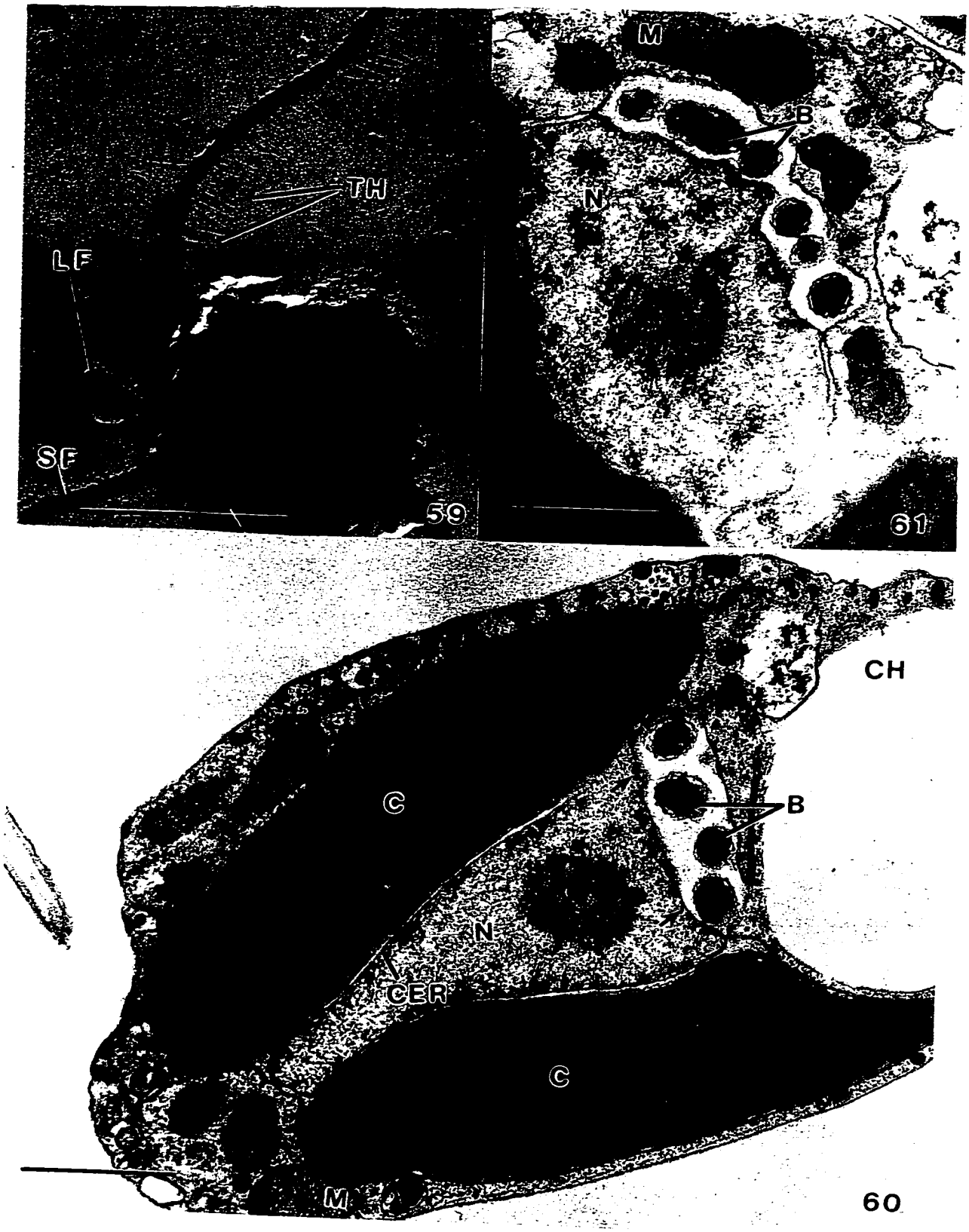
Figure 59. Shadowed cell of *Uroglena articulata*
Scale bar = 1 μm.

Fig. 59. Cell showing the heterokont flagellation, long flagellum and short flagellum and the long flagellum bears tubular hairs whereas the short flagellum does not.

Figures 60-61. Transmission electron micrographs (TEM) of *Uroglena articulata.*
Scale bar = 1 μm.

Fig. 60. Longitudinally sectioned cell showing the typical arrangement of cell components. Two chloroplasts flank the nucleus which is cone-shaped or elongate, there is a posterior chrysolaminarin vesicle, mitochondria are in the anterior portion and endobacteria occur in the nuclear envelope compartment. Note that the chloroplast endoplasmic reticulum is continuous with the outer membrane of the nuclear envelope.

Fig. 61. Transverse section of a cell confirming the presence of endobacteria (B) in a dilated region of the nuclear envelope (N). Mitochondrion (M).

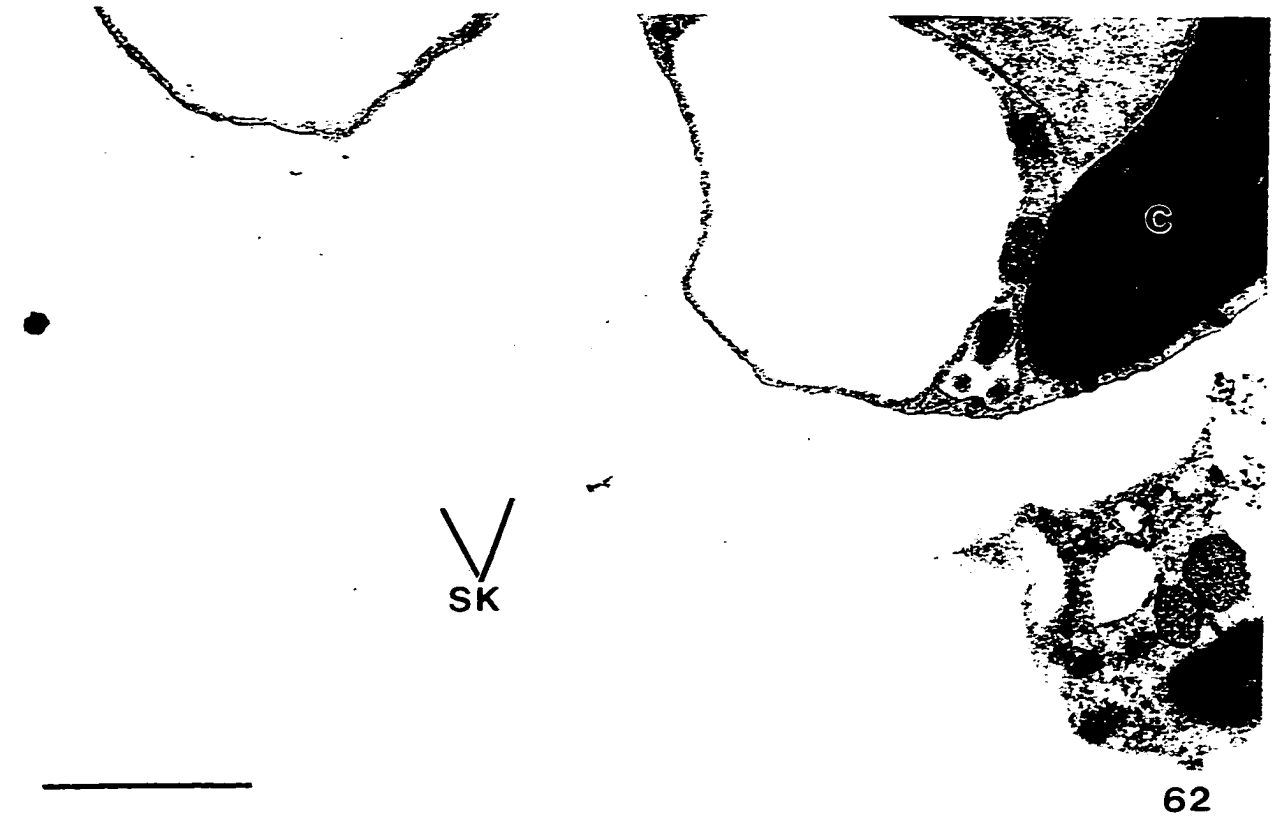


Figures 62-64. Transmission electron micrographs of *U. articulata.*
Scale bar = 1μm.

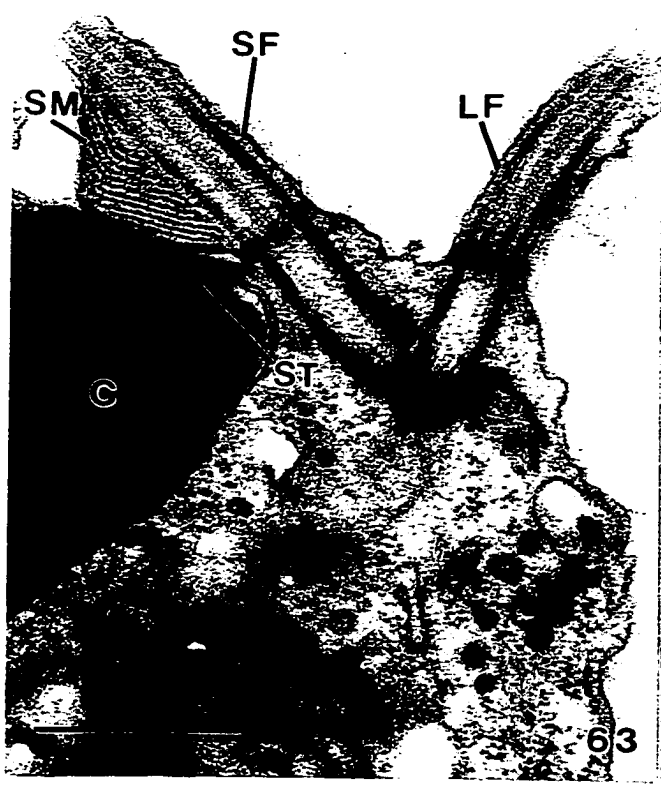
Fig. 62. Cell posteriors with the flattened ends that are attached to stalks. Note the stalks with thin walls and the stalks which appear to be hollow. Chloroplastis also shown.

Fig. 63. Anterior of a cell with a stigma and flagella. The long and short flagellum are oriented anticlinally to each other. Note the distinct flagellar swelling containing layers of plate-like material.

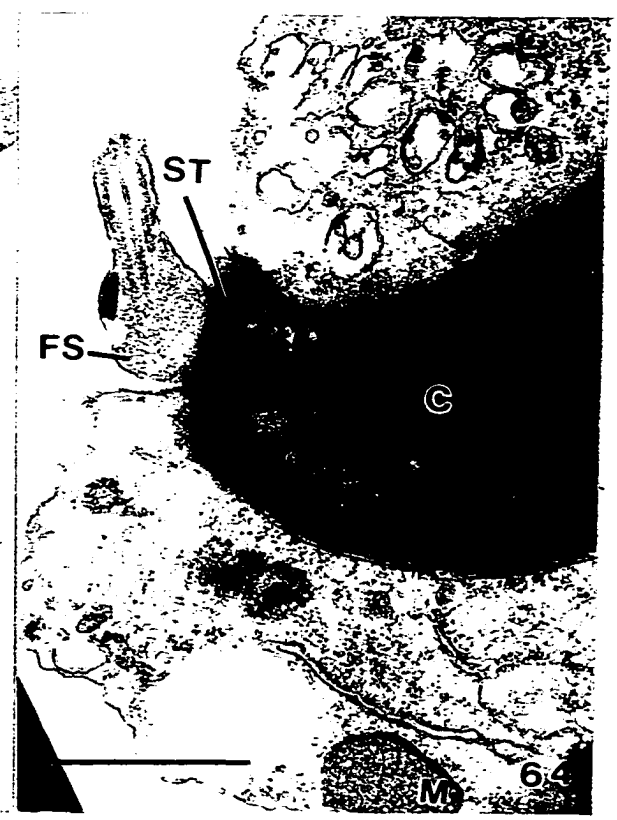
Fig. 64. Oblique section of a cell showing the absence of a descending root at the anterior end of the cell. Chloroplast, flagellar swelling, mitochondrion, and stigma are also shown.



62



63



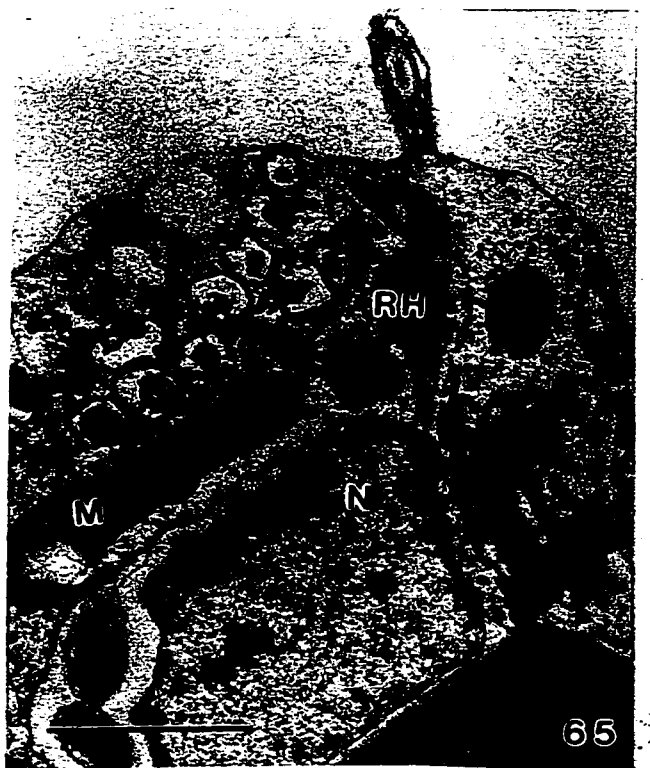
64

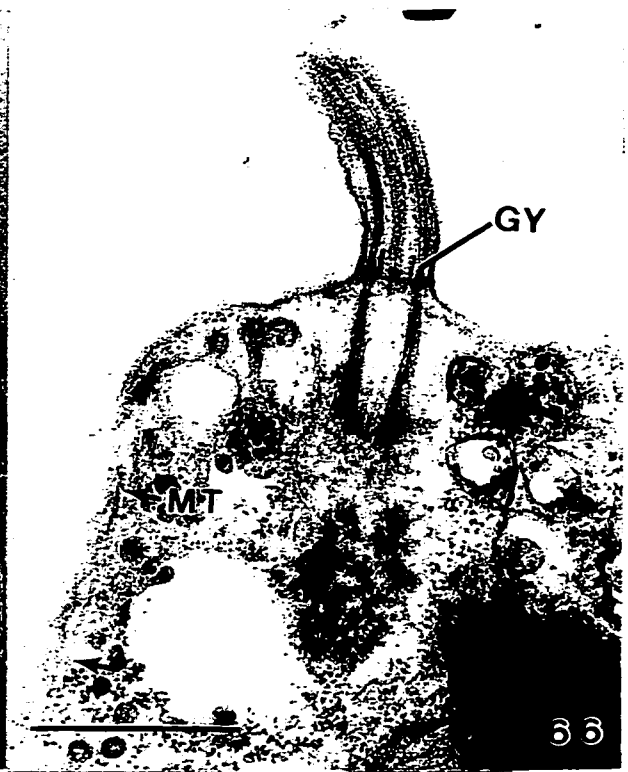
Figures 65-67. Transmission electron micrographs of *U. articulata*.
Scale bar = 1μm.

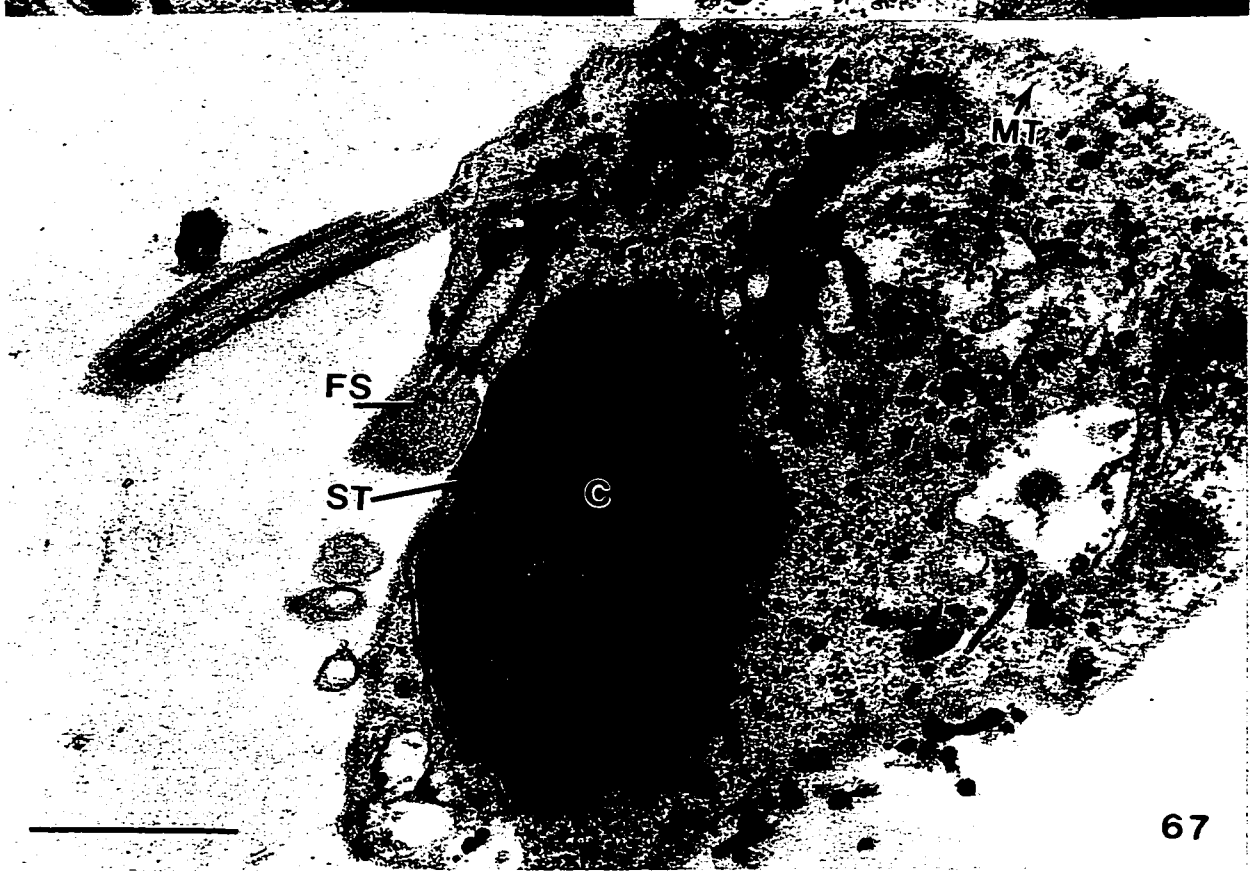
Fig. 65. Cell with rhizoplast and granular material directed toward, and touching the nucleus.

Fig. 66. Long flagellum showing five helical gyres (GY).

Fig. 67. Oblique view of a cell with two flagella and numerous cytoplasmic microtubules surrounding the periphery of the cell (arrows). Chloroplast, flagellar swelling, and stigma are also shown.







CHAPTER 4

FIGURE LEGENDS

Bacterial Endosymbiosis – *Uroglena articulata*

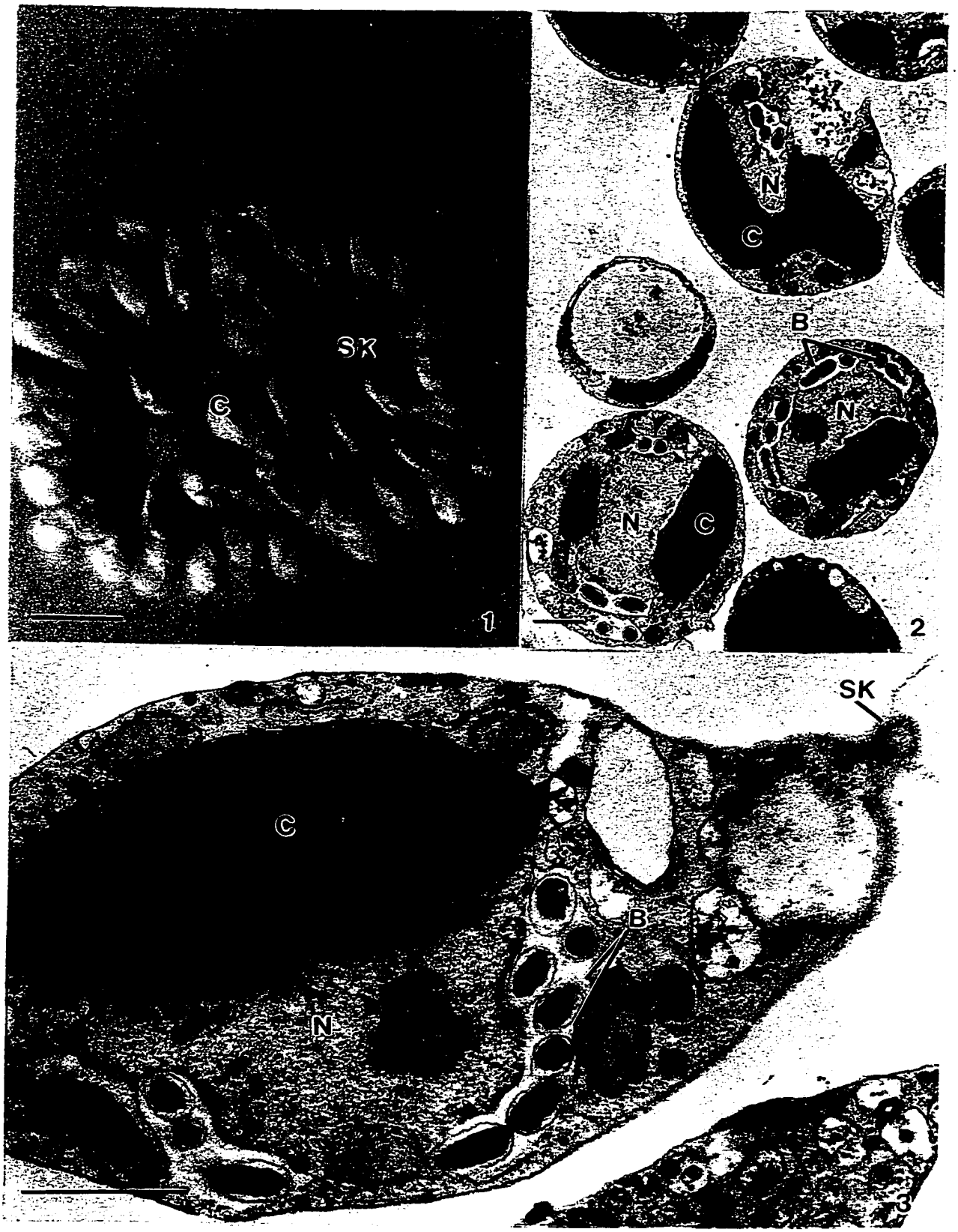
Figure 1. Light micrograph of a colony of *Uroglena articulata*.
Scale bar = 10μm.

Fig.1. Colony with cells interconnected by delicate sympodially branched stalks. Chloroplast.

Figures 2-3. Transmission electron micrographs (TEM) of Bacterial Endosymbiosis – *Uroglena articulata.*
Scale bar = 1μm.

Fig.2. Transverse sections through cells showing bacteria within nuclear membrane spaces. Note that the bacteria do not occur adjacent to the chloroplast.

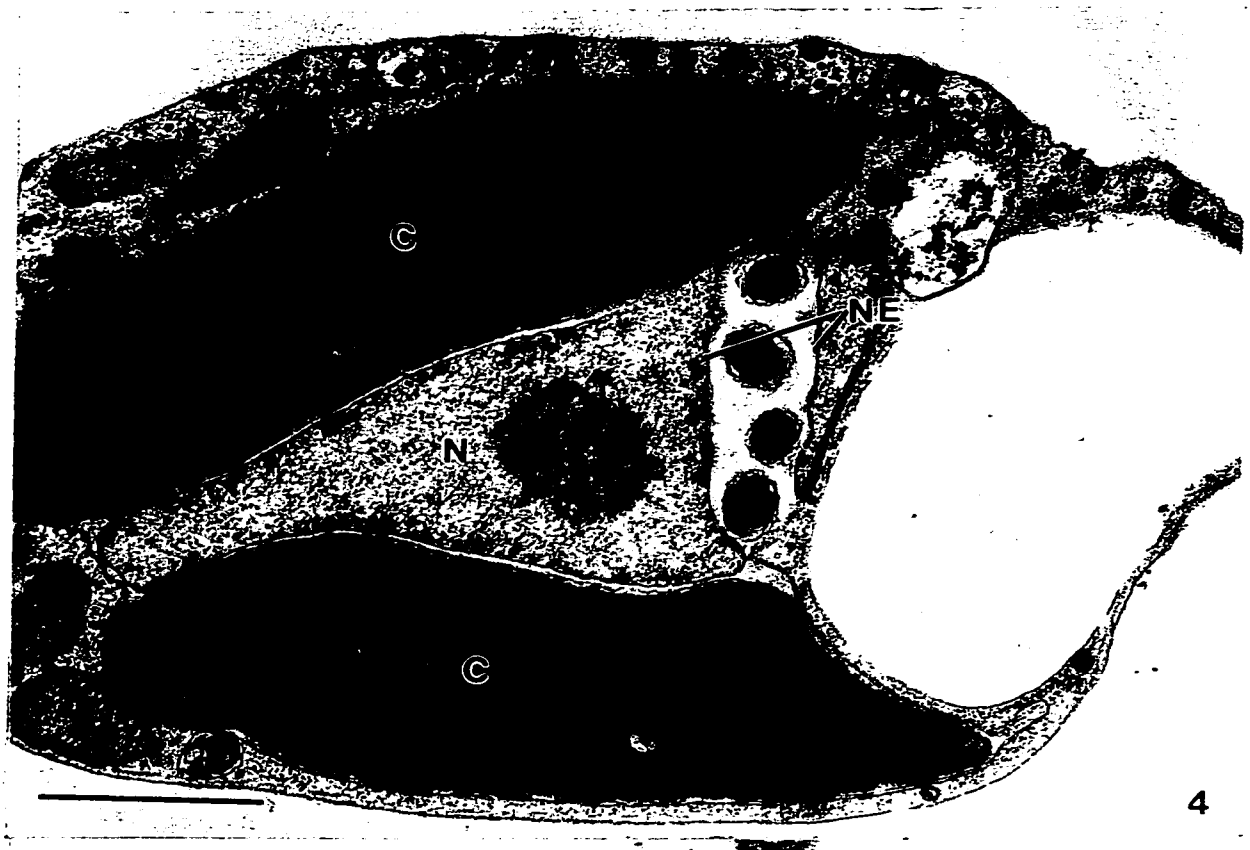
Fig. 3. Longitudinal section of a cell showing the bacteria in the space opposite the chloroplast. Nucleus and stalk are also shown.



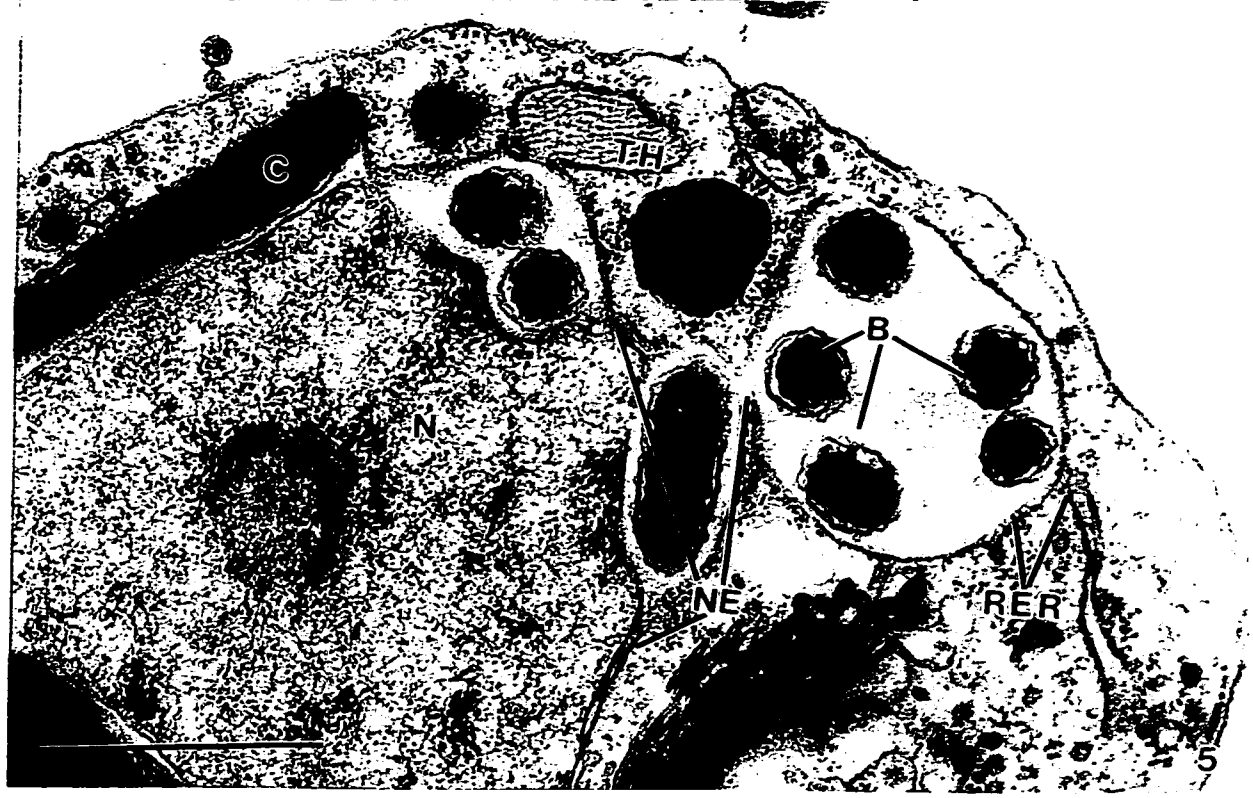
Figures 4-5. Transmission electron micrographs of Bacterial Endosymbiosis – *Uroglena articulata.*
Scale bar = 1μm.

Fig. 4. Details of the bacteria in the nuclear envelope space.

Fig. 5. Bacteria in a rough endoplasmic reticulum cisterna. Note that the bacteria are located within the same compartment where the tubular hairs are being formed in the chloroplast endoplasmic reticulum.



4



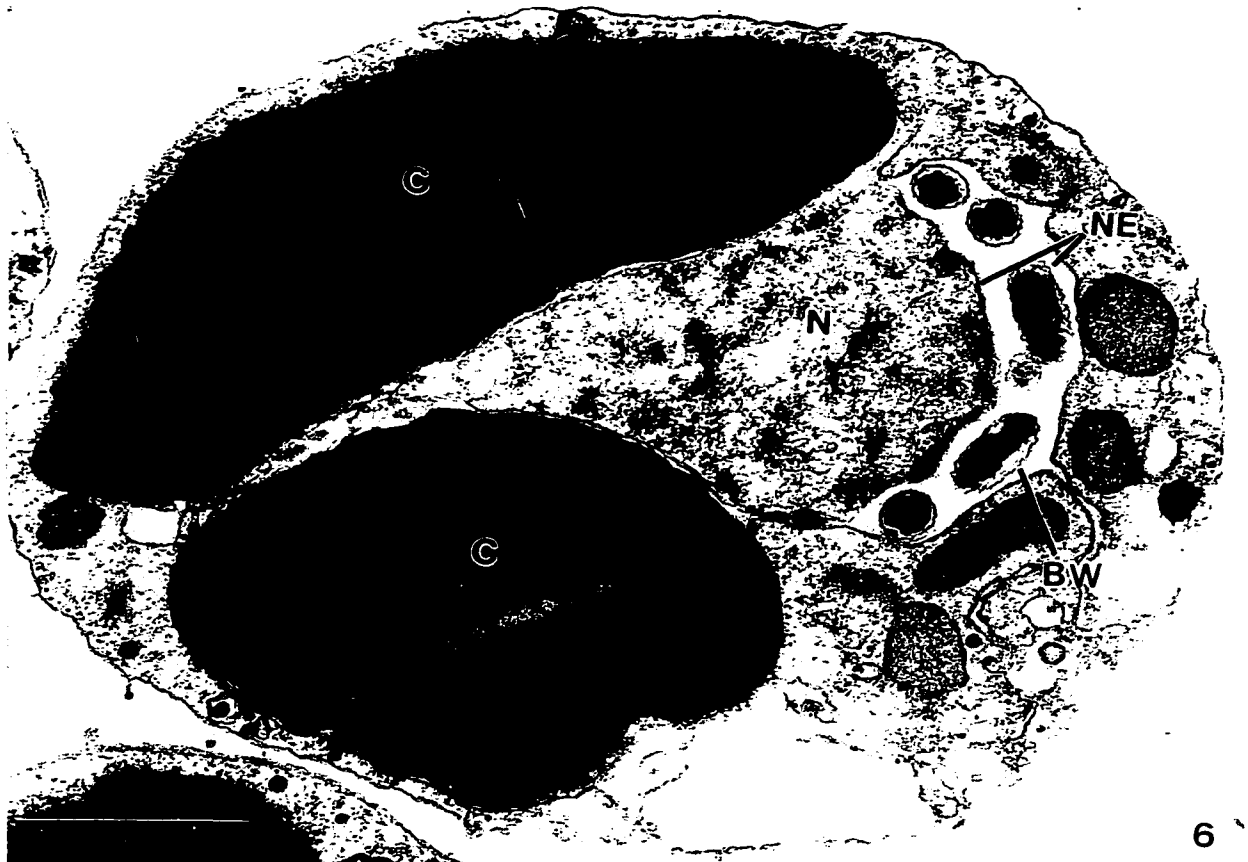
5

Figures 6-8. Transmission electron micrographs of *Uroglena articulata* with bacterial endosymbionts
Scale bar = 1 μm.

Fig. 6. Bacteria in dilations of the nuclear envelope.

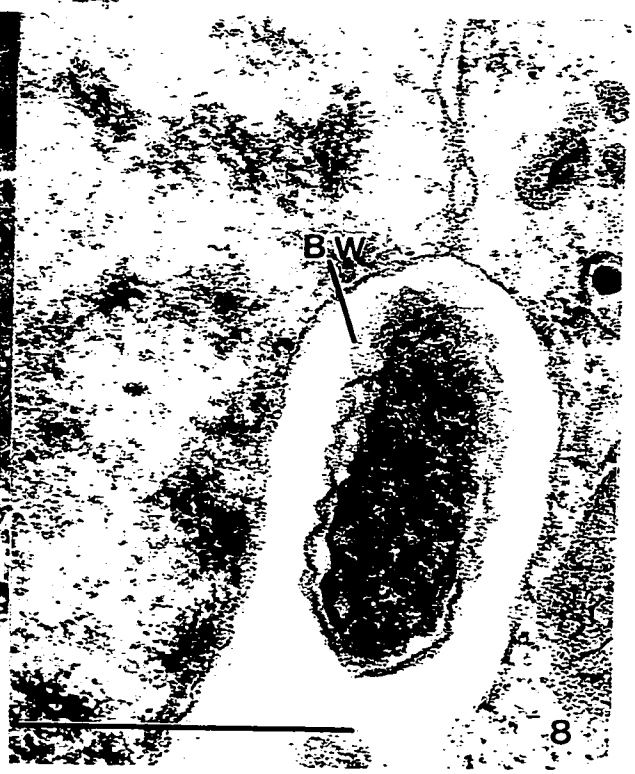
Fig. 7. Higher magnification of bacteria showing details of a dividing bacterium in the compartment (arrows).

Fig. 8. Rod-shaped bacterium with a crenulated cell wall of a rod-shaped bacteria.



6

7



8

CHAPTER 5

FIGURE LEGENDS

Ochromonas pleiomorpha

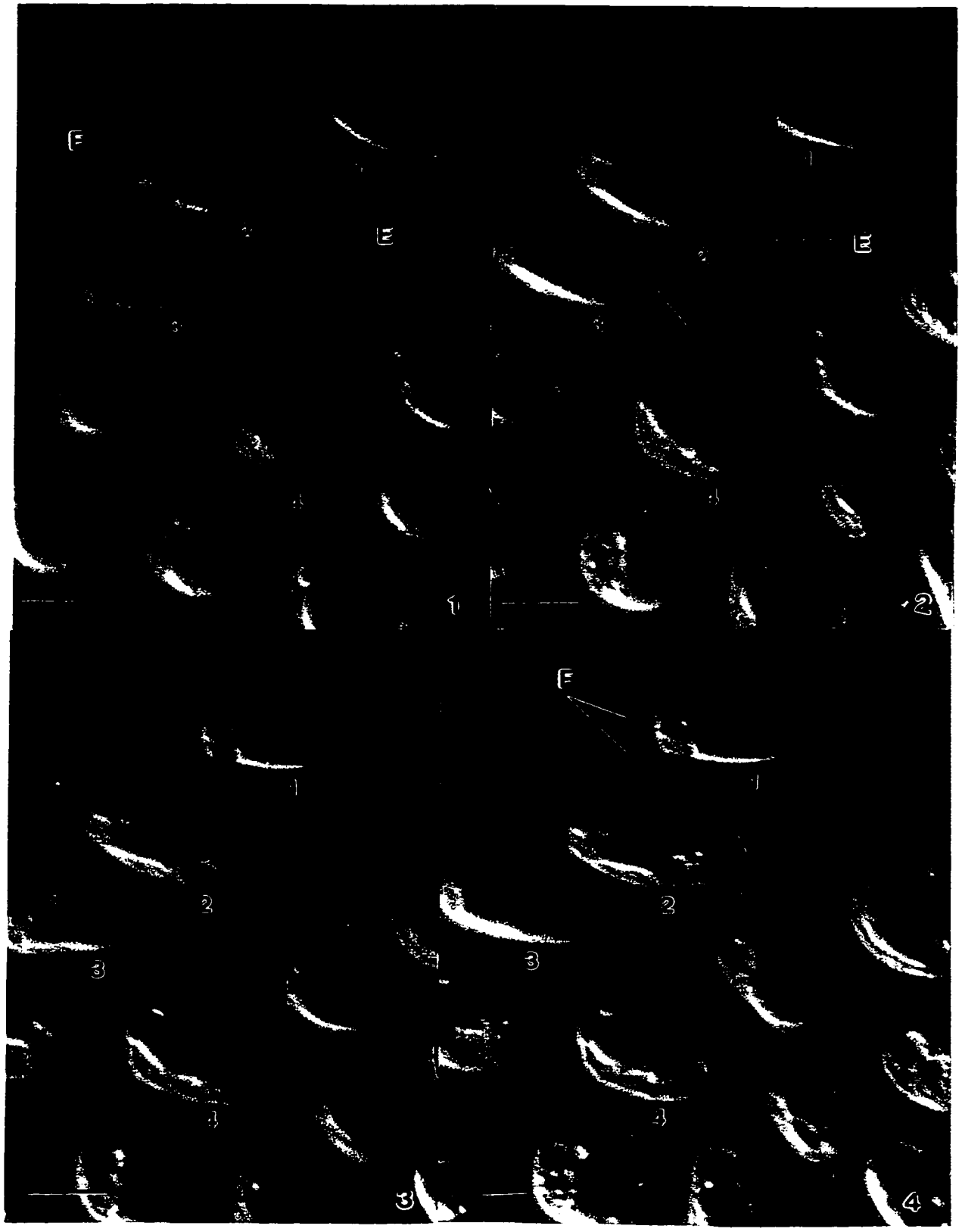
Figures 1-4. Light micrographs of *Ochromonas pleiomorpha.* Figs. 1-4. Four cells (1, 2, 3, and 4) illustrating extension and retraction of cytoplasmic extension in the posterior end of the cell. These micrographs were taken over a three minute period.

Fig. 1. Cytoplasmic extension and heterokont flagella in cell # 2.

Fig. 2. Cells with cytoplasmic extensions. Note that the cytoplasmic extension in cell # 2 is shorter than in Fig. 1 and an extension is forming in cell # 1.

Fig. 3. Cells with various shapes. Compare cell shapes in Figs. 1,2, and 4 to illustrate variability in cell shapes. Note absence of cytoplasmic extension in cell #2.

Fig. 4. Cells with cytoplasmic extensions (# 1) and flagella are evident in the first. Compare with previous figures.
Scale bars = 4 μm.



Figures 5-10. Scanning electron micrographs (SEM) of *Ochromonas pleiomorpha.* Scales bars = 2 μm, except for Fig. 5 where it is 1 μm and Fig. 6 where it is 0.7 μm.

Fig. 5. Ovoid cell with small elevations visible.

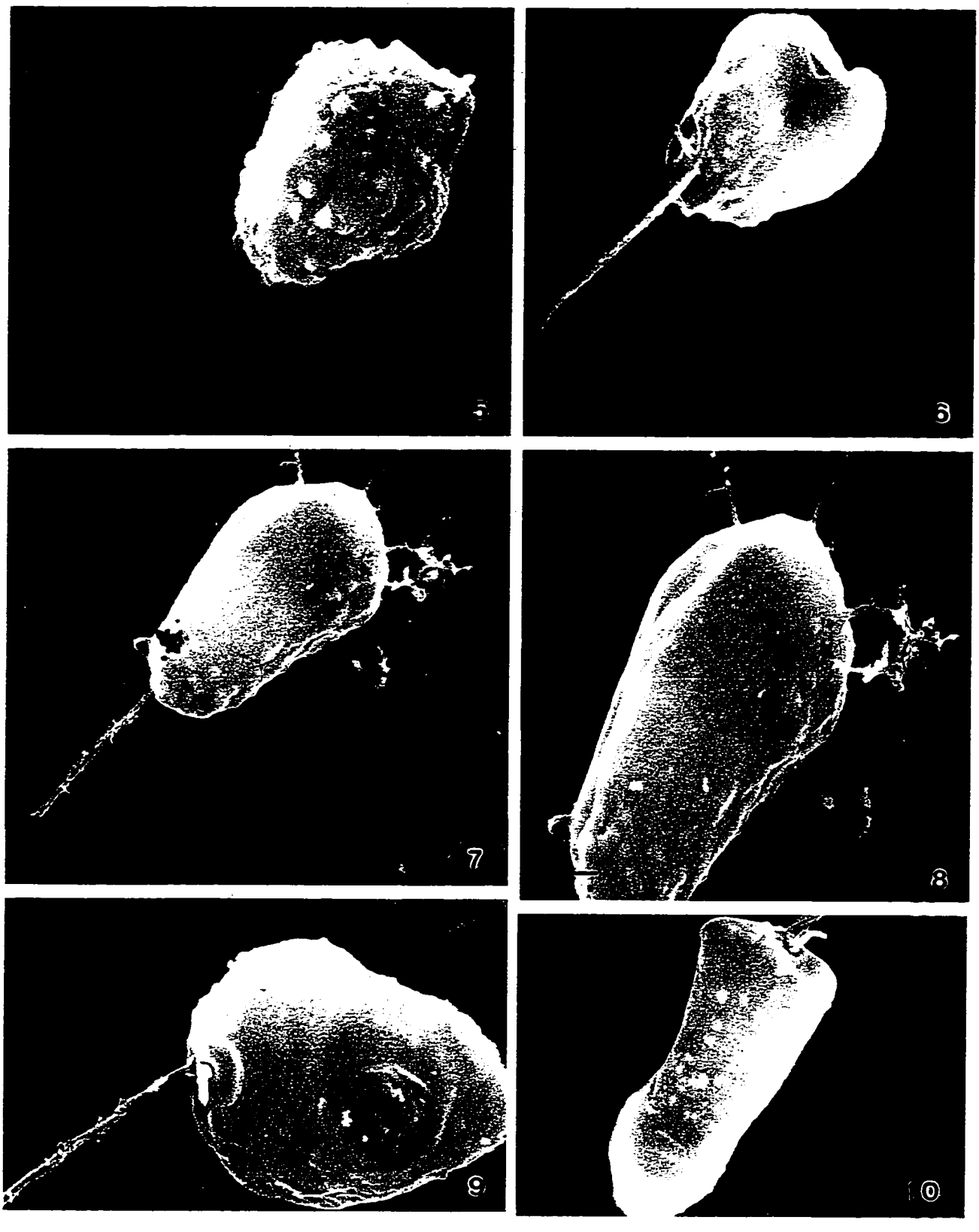
Fig. 6. Obovoid cell with the posterior broader than the anterior end.

Fig. 7. Obovoid cell with cytoplasmic extensions, some of which are branched (arrow).

Fig. 8. Higher magnification of cell in Fig. 7 with cytopalsmic extensions (arrow).

Fig. 9. Conoid shaped cell with a surface contour in a portion of the cell that conforms to an ingested diatom .

Fig. 10. Elongate cell with truncated anterior end.



Figures 11-15. Scanning electron micrographs of *O. pleiomorpha*.
Scale bar = 2 μm, except for Fig.11 where it is 5 μm.

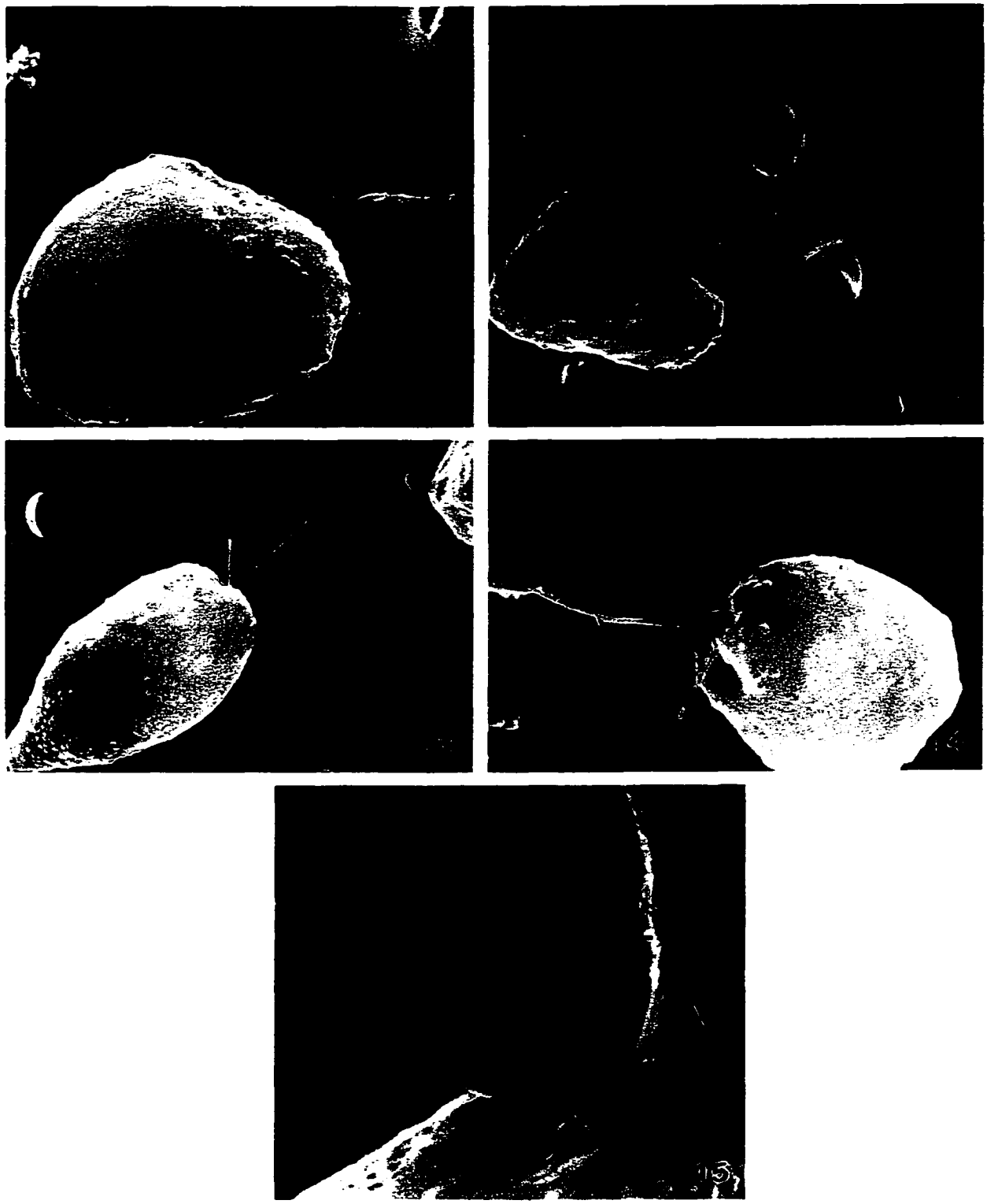
Fig. 11. Subspherical cell with small surface elevations.

Fig. 12. Short conoid cell with surface "bumps" or elevations.

Fig. 13. Pyriform cell with a distinct conoid tail.

Fig. 14. Spherical cell.

Fig. 15. Higher magnification of the anterior end of a cell showing flagellar. Scale bar = 2 μm.



Figures 16-20. Scanning electron micrographs of *O. pleiomorpha*.
Scale bars = 2 μm in Figs. 16,and 19 and 1 μm in Figs.17,18 and 20.

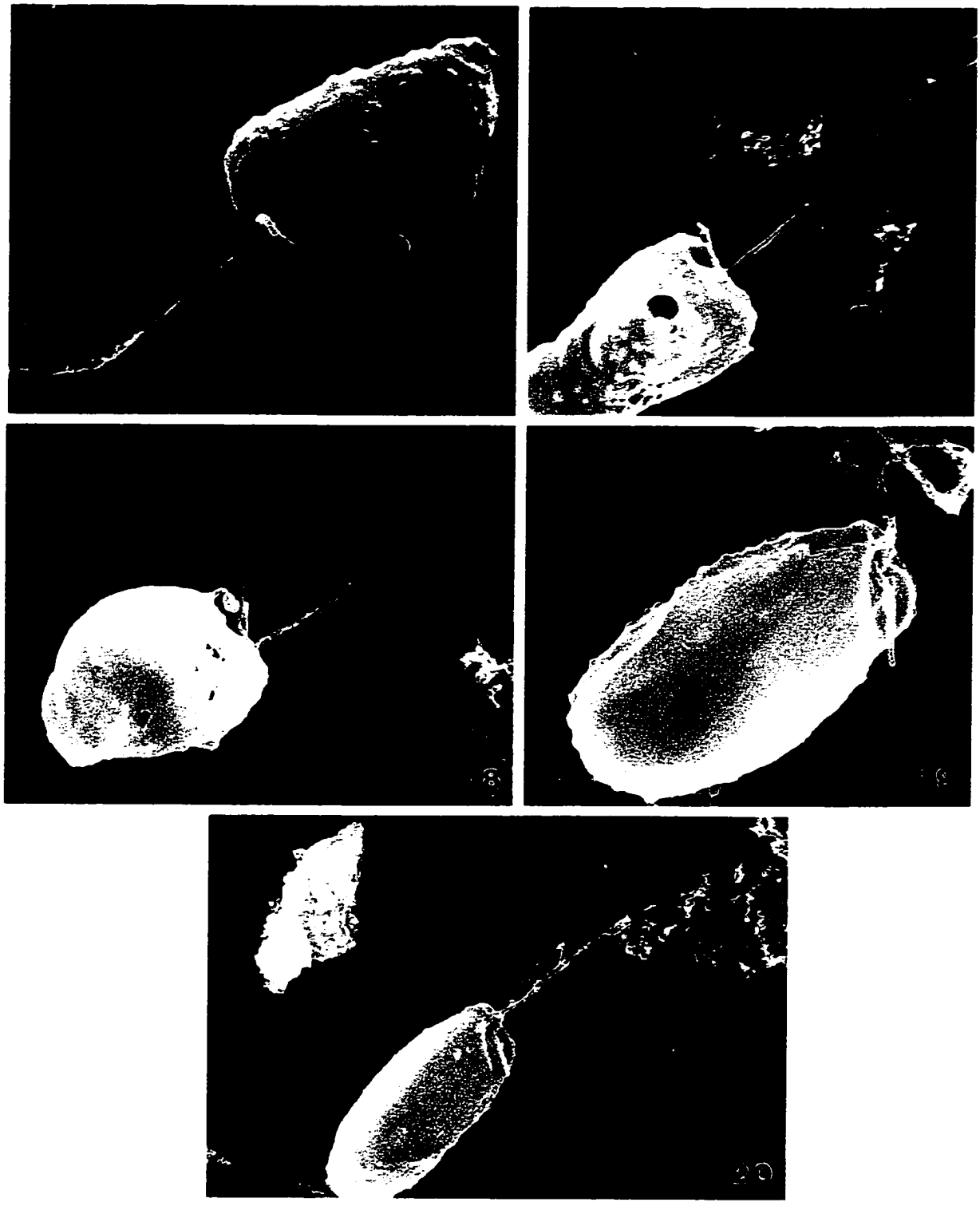
Fig. 16. Conoid shaped cell.

Fig. 17. Conoid elongate cell with truncated anterior.

Fig. 18 Small conoid cell.

Fig. 19. Ellipsoid cell.

Fig. 20. Elongate ellipsoid cell.



Figures 21-25. Scanning electron micrographs of diatom cells.
Scale bars = 10 μm except for Fig. 22 where it is 1 μm.

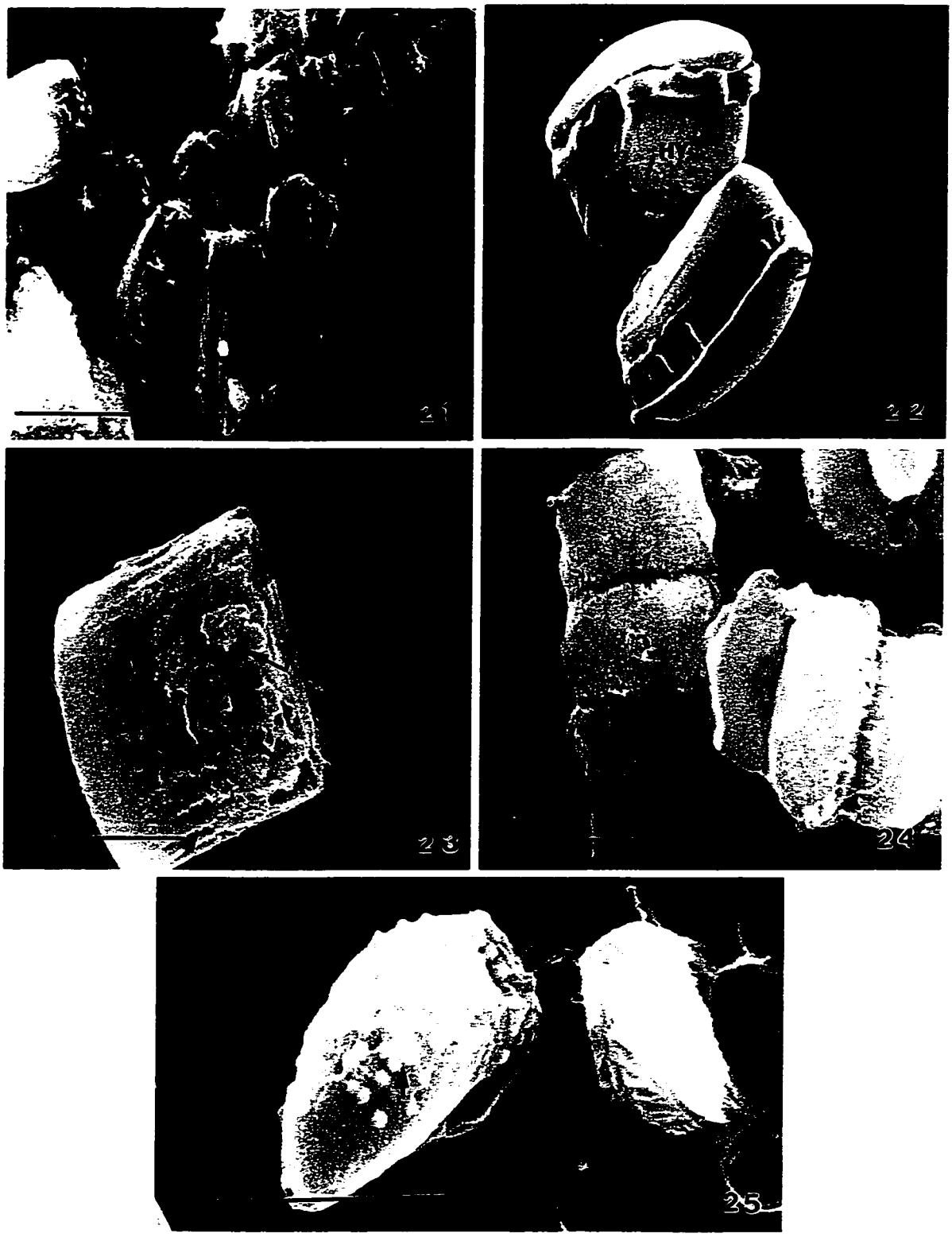
Fig. 21. A pseudofilament pennate diatom showing the arrangement of the cells and the extracellular mucilage attached to the frustules.

Fig. 22. Girdle view of pennate diatom cells showing details of their frustules such as the epitheca and hypotheca. Note the connecting band between the edges of the valves.

Fig. 23. Girdle view of a centric diatom showing the hypotheca with a marginal ring of labiate processes (arrows). Note that the labiate processes extend laterally along the frustule.

Fig. 24. A colonial centric diatom in a filamentous stage.

Fig. 25. Cell of *O. pleimorpha* near a diatom cell. Several small vesicles are visible in the dorsal side of the *O. pleiomorpha* (arrow). Note the mucilage strands around the diatom, with attach the cell to the substrate (arrow).

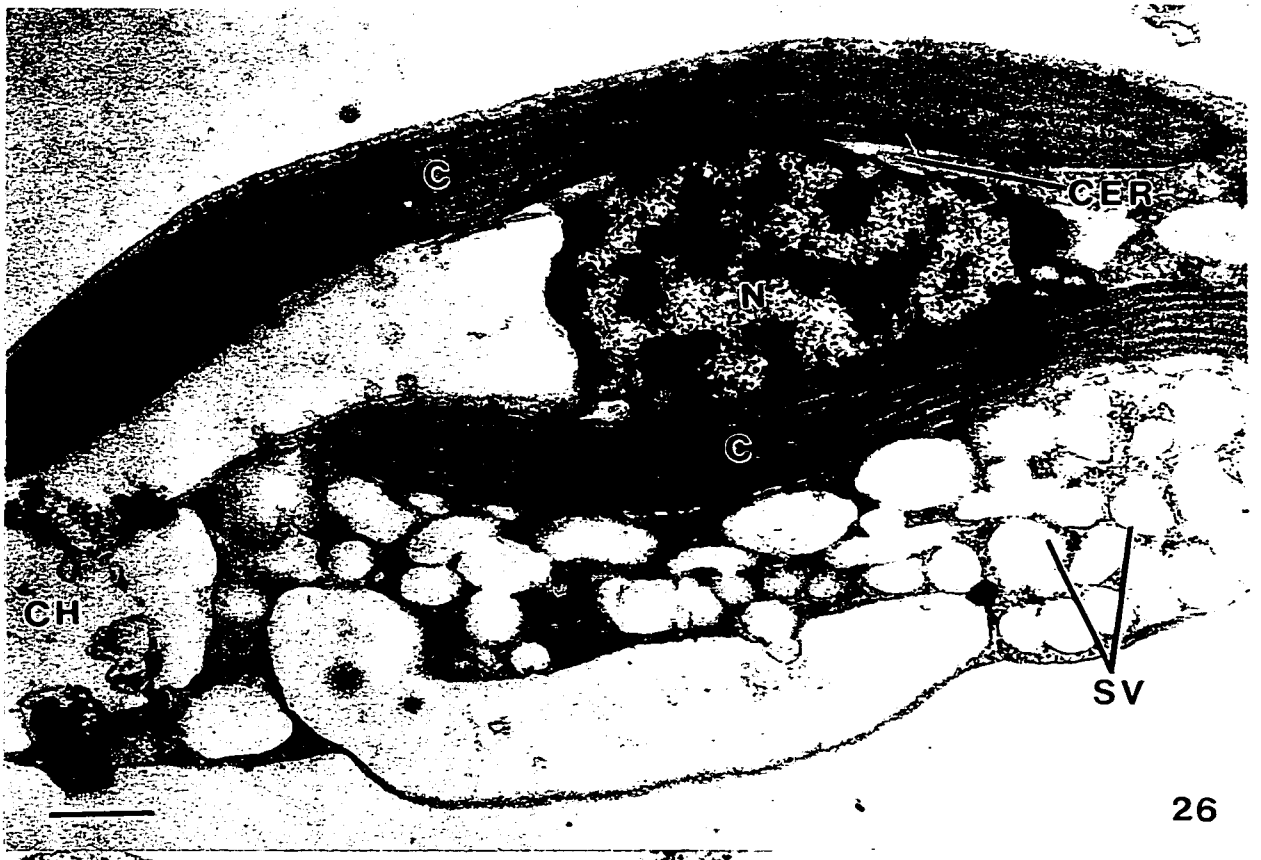


Figures 26-28. Tranmission electron micrographs of *Ochromonas pleiomorpha.* Scale bar = 1μm.

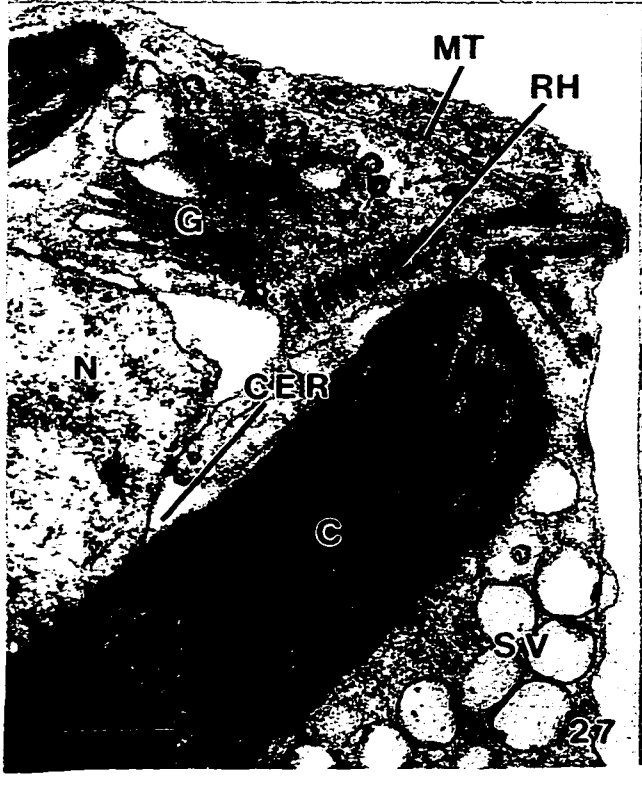
Fig. 26. Longitudinal section of a cell showing the nucleus and chloroplast complex, the chrysolaminarin vacuole and small fusing vesicles. The nucleus is located between two lobes of the chloroplast and anterior to the chrysolaminarin vacuole. The lobes of the chloropalst are unequal in length. The vesicles designate the dorsal portion of the cell. Note the larger vesicle near the periphery of the cell.

Fig. 27. Longitudinal section of a cell with an anterior Golgi apparatus the lobes of the chloroplast and secretory vesicles. Note the striated rhizoplast that extends from, one of the basal bodies and adheres the nuclear envelope at the anterior end. Note the chloroplast endoplasmic reticulum.

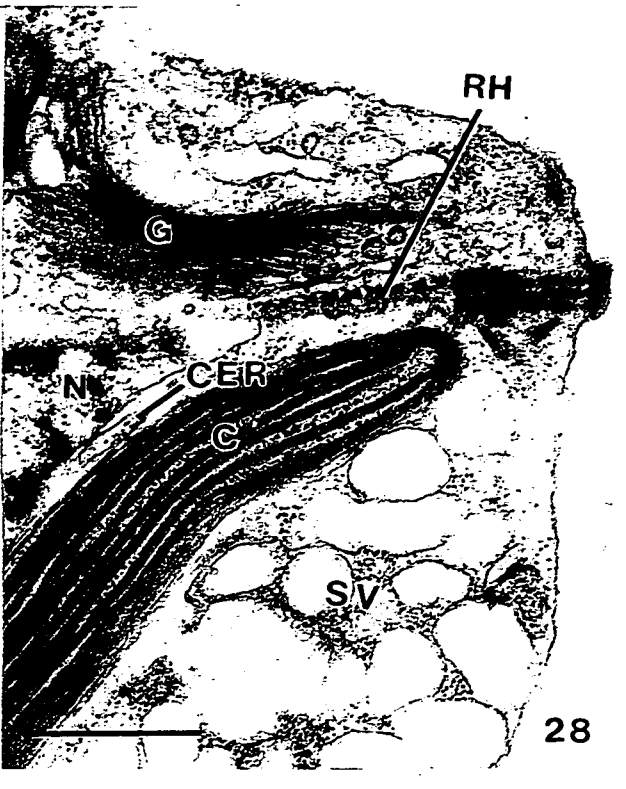
Fig. 28. Anterior of a cell showing an inactive Golgi apparatus, a portion of the basal body and a portion of the striated rootlet of the rhizoplast. Note the chloroplast endoplasmic reticulum, chloroplast, nucleus and digestive vesicles.



26



27



28

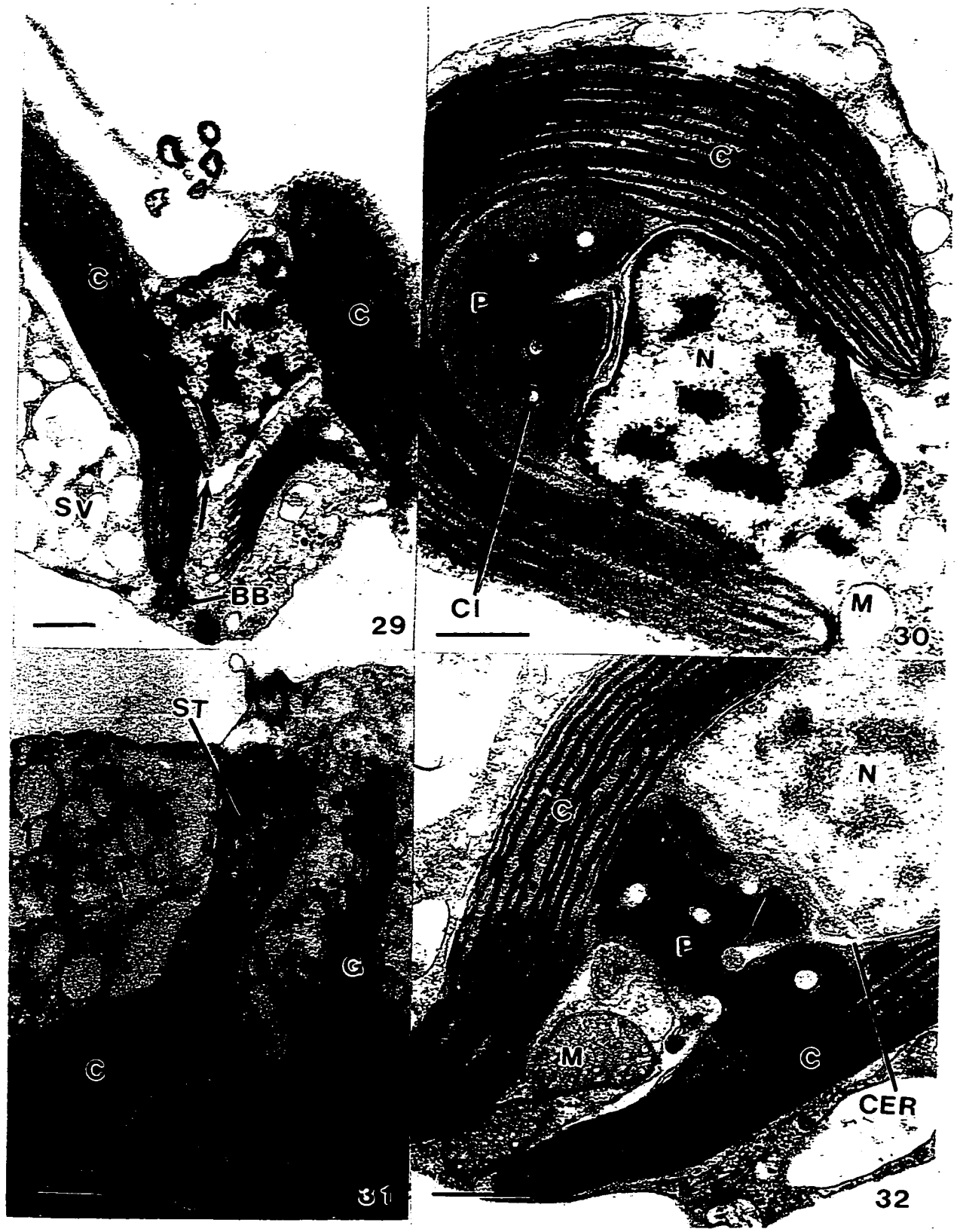
Figures 29-32. Transmission electron micrographs of *O. pleiomorpha*.
Scale bar = 1μm.

Fig. 29. Oblique section of a cell showing the basal body of the ventral flagellum near the anterior of one of the lobes of the chloroplast. The Golgi apparatus appears inactive. The chloroplast endoplasmic reticulum is evident (arrow).

Fig. 30. Longitudinal section of the cell showing the pyrenoid and the chloroplast. Note the membranous invaginations of the chloroplast envelope into the pyrenoid. Mitochondrion and nucleus are also shown.

Fig. 31. Longitudinal section through a chloroplast lobe that contains the stigmatic granules. The basal body of the short flagellum is near the stigma.

Fig. 32. Transverse section of a cell showing the pyrenoid adjoining the nucleus and occupying the inner concave region of the chloroplast. Membranous invagination of the chloroplast into the pyrenoid is also shown (arrow).



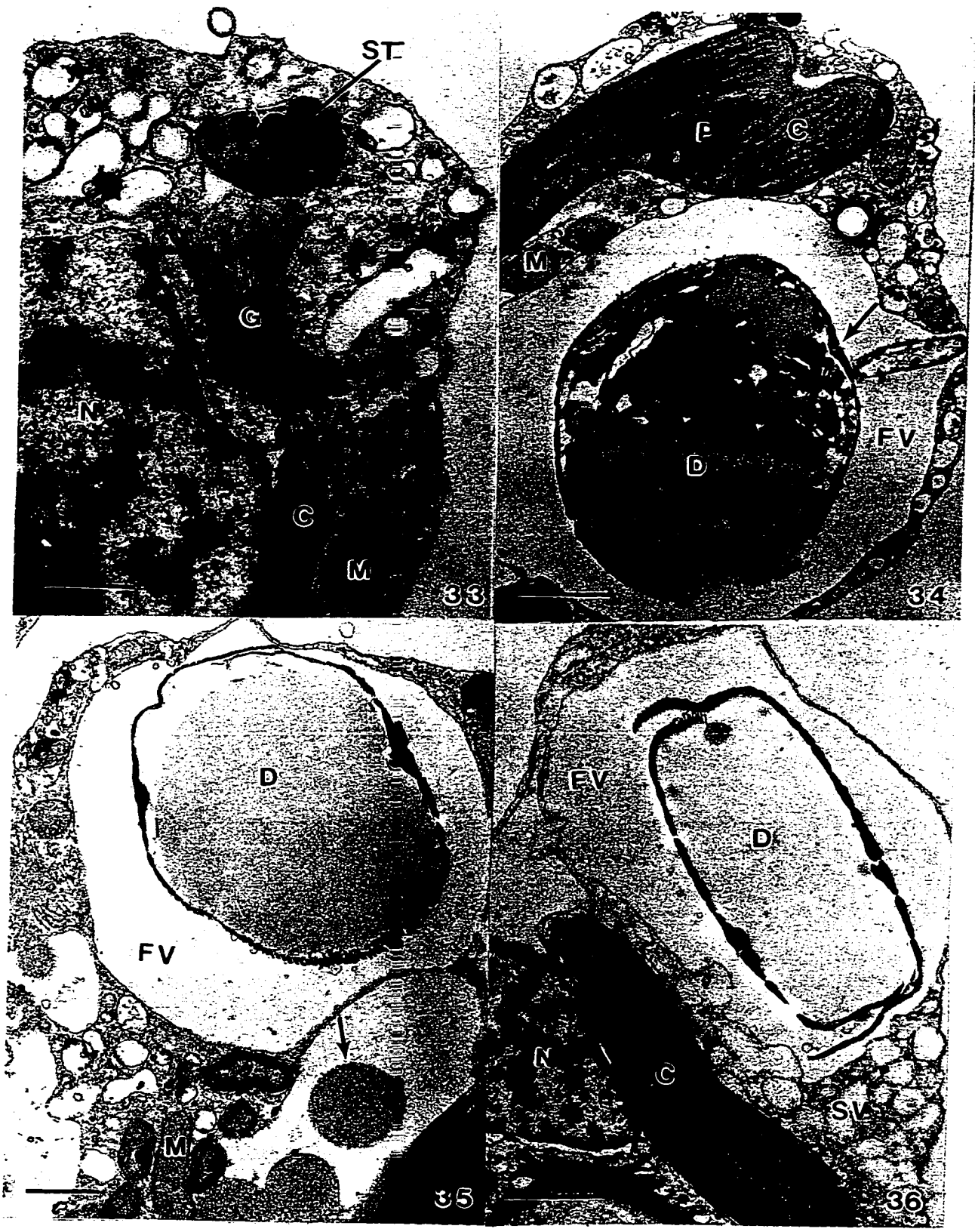
Figures 33-36. Transmission electron micrographs of *O. pleiomorpha*.
Scale bar = 1μm.

Fig. 33. Oblique section of a cell showing stigmatic granules near the transition region of the short flagellum. Note that the free portion of the axoneme is not intimately associated with the stigma.

Fig. 34. Transverse section of a cell with a food vacuole and a partially digested diatom inside the vacuole. The raphe is evident in one of the valves, presumably the hypovalve (arrow). Chloroplast, mitochondrion, and pyrenoid are also shown.

Fig. 35. Cell with a food vacuole containing a diatom in an advanced state of digestion. A chrysolaminarin vacuole is adjacent to the food vacuole (arrow).

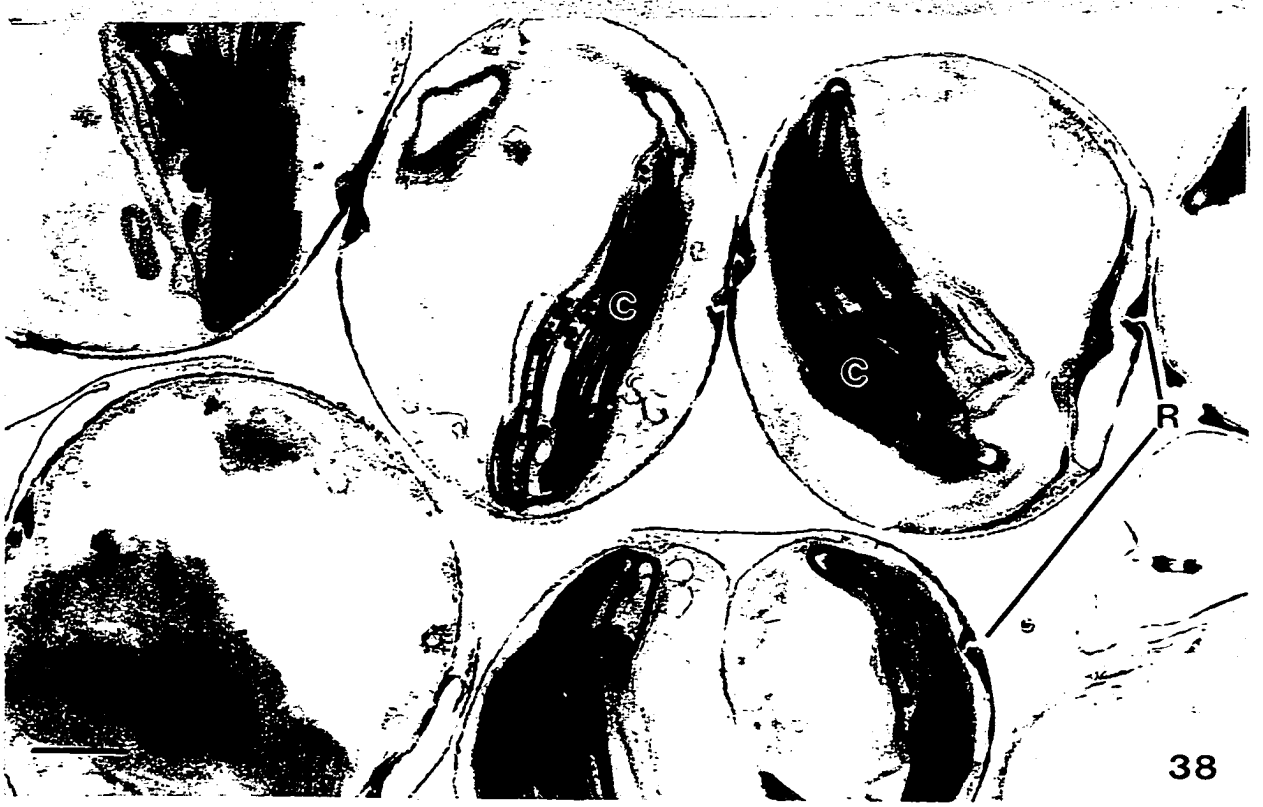
Fig. 36. Cell with a food vacuole containing a diatom with completely digested cytoplasm. Chloroplast, nucleus, secretory vesicles are also shown.



Figures 37-38. Transmission electron micrographs of *O. pleiomorpha*.
Scale bar = 1μm.

Fig. 37. Cell releasing the frustule of a digested diatom. Note the large opening created by the food vacuole fusing with the plasma membrane.

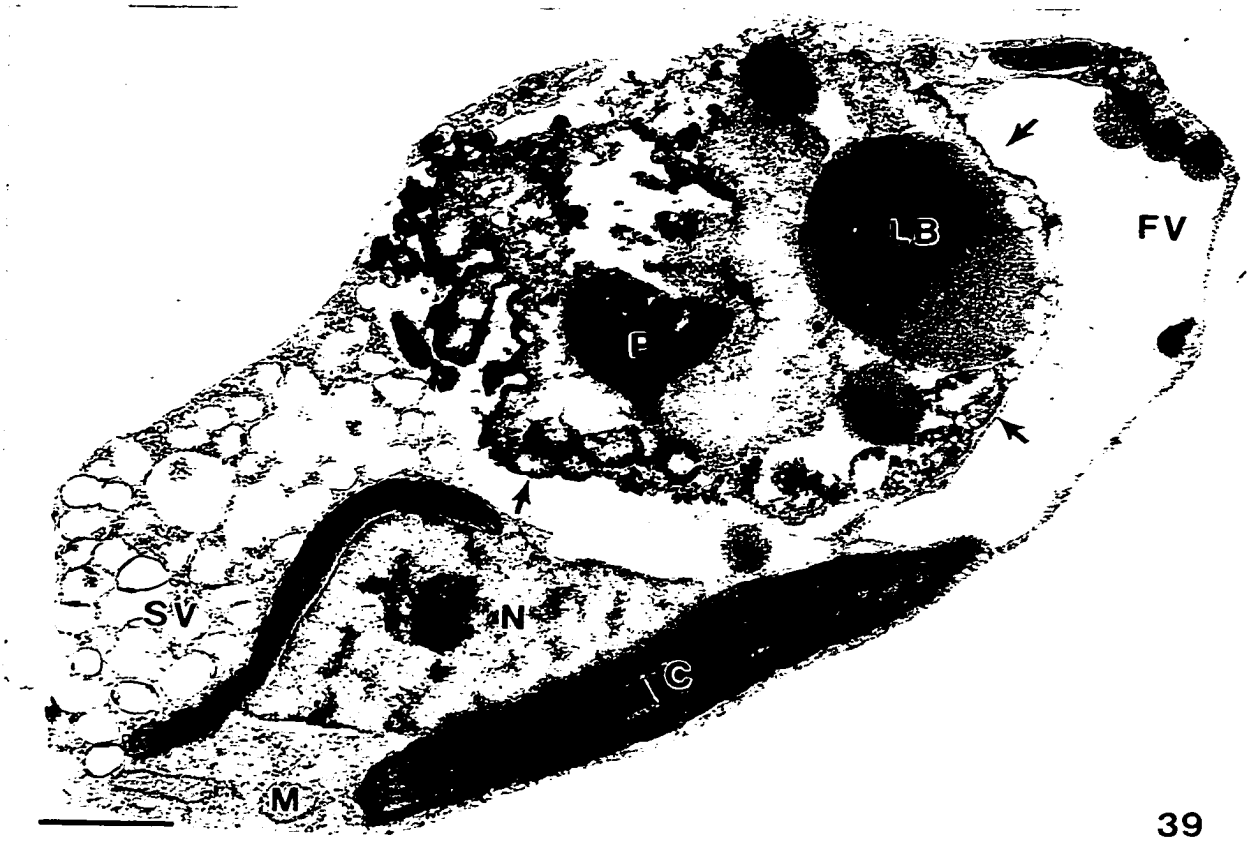
Fig. 38. Raphe pennate diatom from an actively growing culture showing the cellular characteristics of the diatom cells. Note the raphe and chloroplast.



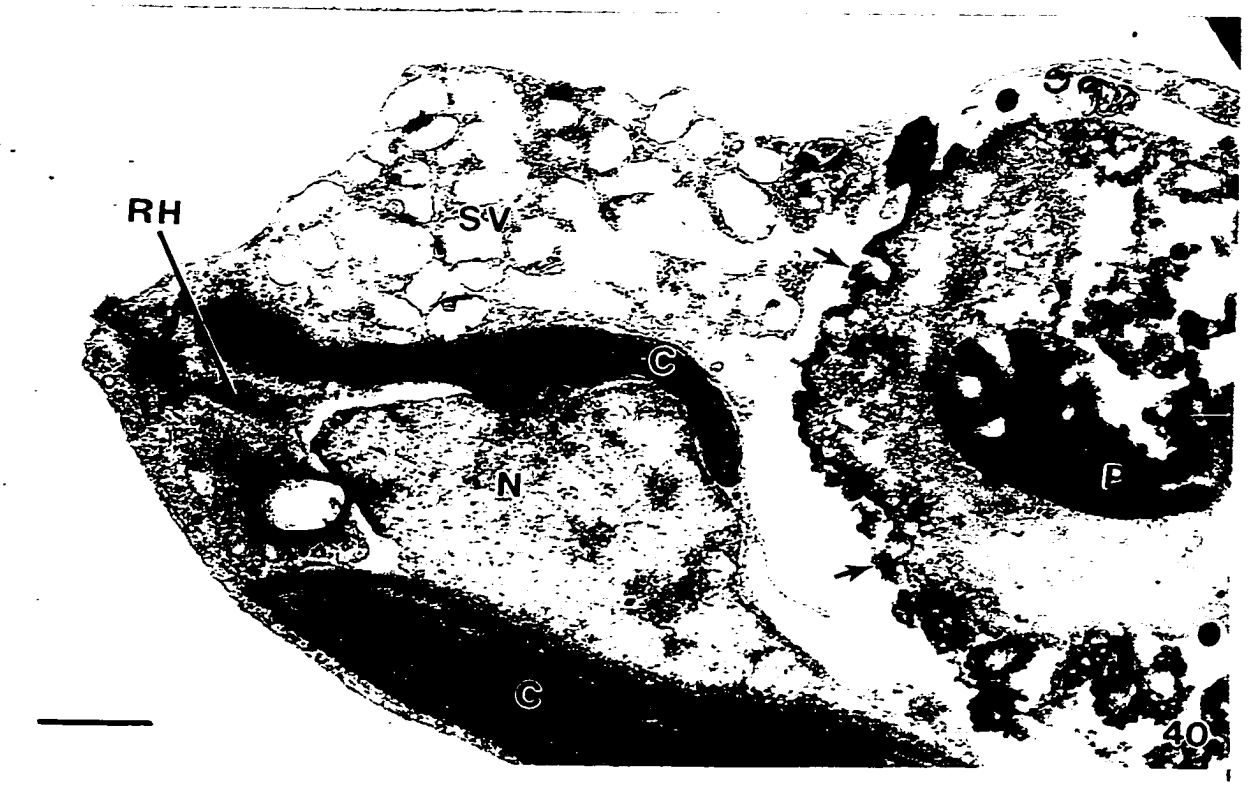
Figures 39-40. Transmission electron micrographs of *O. pleiomorpha*.
Scale bar = 1μm.

Fig. 39. Cell with a food vacuole that contains a partially digested cell of another *O. pleiomorpha* cell (arrows). Note the structure of the pyrenoid for comparisons with Figures 30 and 32, to support the suggestion that this is *O. pleiomorpha*. The food vacuole is located posterior to the nucleus and the smaller digestive vesicles.

Fig.40. Cell with a less digested *O. pleiomorpha* in its food vacuole (arrows).
Note rhizoplast attached to the anterior of the nucleus. Chloroplast, pyrenoid, and secretory vesicles are also shown.



39



40

Figures 41-42. Transmission electron micrographs of *O. pleiomorpha*.
Scale bar = 1μm.

Fig. 41. Cell with membranous cytoplasmic extensions originating from vesicles just beneath the plasma membrane. The chloroplast endoplasmic reticulum adjacent to the lower extension is in the process of forming tubular hairs (arrow).

Fig.42. Protruding vesicles with variable contents. One has dark staining contents (arrow) whereas the other contains lighter staining membranous components.