Ultrastructure of *Pyramimonas cyrtoptera* sp.nov. (Prasinophyceae), a species with 16 flagella from northern Foxe Basin, Arctic Canada, including observations on growth rates

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A new species of the genus *Pyramimonas* is reported from the Canadian Arctic, the first record of *Pyramimonas* from northern Foxe Basin, Northwest Territories. The general structure of the cell and six types of scales are described. ***Pyramimonas cyrtoptera*** sp.nov. is remarkable in possessing 16 flagella, two chloroplasts, two pyrenoids, and two pairs of eyespots of unequal size. It is the largest known species of *Pyramimonas*, measuring 38–42 μm in length. The presence of 16 flagella and two chloroplasts sets it apart from all other species of *Pyramimonas*. *Pyramimonas cyrtoptera* belongs to the subgenus *Pyramimonas*. A new type of hair scale is reported for members of this group. The growth responses of *P. cyrtoptera* to variations in temperature and salinity indicate that it is as cold stenothermal and euryhaline.

Key words: Prasinophyceae, *Pyramimonas*, flagella, Arctic Canada, temperature, salinity.

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Les auteurs rapportent une nouvelle espèce de *Pyramimonas* provenant de l'arctique canadien, une première mention rapport pour le genre *Pyramimonas* dans le nord du Foxe Bassin, dans les Territoires du Nord-Ouest. On présente une description de la structure générale de la cellule et de six types d'écaille. Ce qui est remarquable chez le ***Pyramimonas cyrtoptera*** sp.nov. c'est la présence de 16 flagelles, deux chloroplastes, deux pyrénoïdes et deux paires de taches visuelles de dimensions inégales. Il s'agit de la plus grosse espèce connue de *Pyramimonas*, mesurant 38–42 μm de longueur. La présence de 16 flagelles et de deux chloroplastes le distingue de toutes les autres espèces de *Pyramimonas*. Le *P. cyrtoptera* appartient au sous-genre *Pyramimonas*. On décrit un nouveau type d'écaille chez les membres de ce groupe. Les réactions de croissance du *P. cyrtoptera* aux variations de la température et de la salinité montrent qu'il s'agit d'une espèce de conditions froides, sténothermique et euryhaline.

Mots clés : Prasinophyceae, *Pyramimonas*, flagelles, arctique canadien, température, salinité.

[Traduit par la rédaction]

Introduction

The majority of autotrophic flagellates possess one or two flagella or basal bodies. Phytoflagellates with more than two flagella occur only within the Haptophyceae (two species of *Chrysochromulina, C. birgeri* Hällfors & Niemi (Hällfors and Niemi 1974) and *Chrysochromulina* sp. (D. R. A. Hill, personal communication)), the Euglenophyceae (*Eutreptiella, E. braarudii* Throndsen (Throndsen 1969), an undescribed euglenoid (Seguel and McLachlan 1991), and related forms), and the green algae (Chlorophyceae). However, in the Prasinophyceae (sensu Moestrup 1991), which presently comprises 19 genera constituting the presumedly primitive end of the green algae, considerable variation in the number of flagella exists.

One genus of prasinophytes, *Pyramimonas* Schmarda, contains species with different numbers of flagella. Most of the 67 species described (Sym and Pienaar 1991*a*) have four flagella. The following four species have eight flagella: *P. octopus* Moestrup & Aa. Kristiansen (Moestrup et al. 1987), *P. propulsa* Moestrup & Hill (Moestrup and Hill 1991), *P. amylifera* Conrad (Conrad 1939), and *P. octociliata* Carter (Carter 1937). *Pyramimonas amylifera* may rarely occur with four flagella (Norris and Pienaar 1978).

In this paper we report on a species of *Pyramimonas, P. cyrtoptera* sp.nov., with 16 flagella (32 when dividing), the highest reported number of flagella for any known phytoflagellate.

The temperature and salinity profiles in the Foxe Basin ecosystem where *P. cyrtoptera* was observed varied in a characteristic pattern. Adaptation of the new species of *Pyramimonas* to temperature and salinity based on culture experiments is discussed.

Materials and methods

Study area

The sea in the study area was covered by a 1–1.5 m thick layer of ice. *Pyramimonas cyrtoptera* was found at two stations (22A1 and 26A1, Fig. 1) in Hooper Inlet (northern Foxe Basin, 69°23′N and 81°45′W) on 22 and 26 June 1989. During our visit the ice was beginning to melt and the surface water was brackish. Temperature and salinity were measured in situ and vertical profiles at station 22A1 are illustrated in Fig. 2. Both stations had pronounced temperature and salinity gradients. The temperature of the surface water was 0°C, dropping to −1.2°C in the first 10 m and remained constant to the bottom. The surface salinity was 5‰, increasing to 35‰ at a depth of 5 m, and remained constant to the bottom.

Culture

Cells of *P. cyrtoptera* were collected through a lead (22A1) and a seal hole (26A1) with a plankton net (20 μm pore size) connected to a 17 m long string. The samples were enriched with a modified Erdschreiber medium (30‰ salinity) (Throndsen 1978) and clonal cultures were subsequently established by micropipetting. The cultures are maintained in modified Erdschreiber medium at the Institut for Sporeplanter (University of Copenhagen) at 4°C, photon flux density (PFD) 13 μmol m^{-2} s^{-1}, and a 16 h light : 8 h dark cycle.

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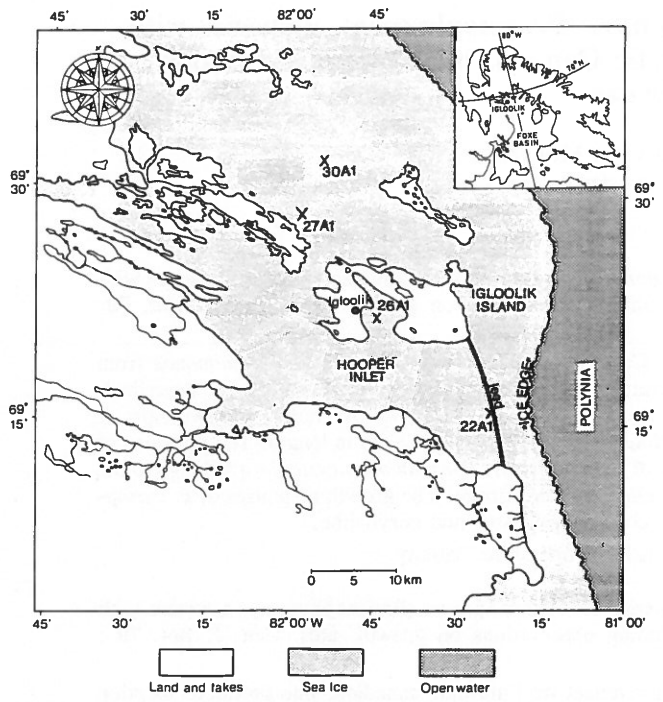


FIG. 1. Map showing sampling sites and location of Igloolik Island, northern Foxe Basin, Canadian Arctic.

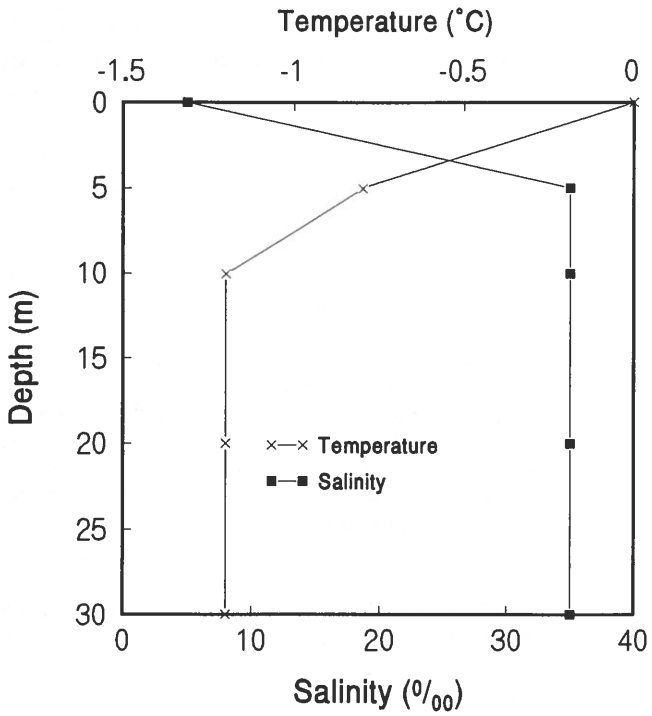


FIG. 2. Vertical profiles of temperature and salinity at station 22A1 in Hooper Inlet, northern Foxe Basin.

Temperature and salinity experiments

Growth of *P. cyrtoptera* was studied at a temperature range between 4.2 ± 0.6 and 18.7 ± 0.3°C. Clonal cultures were grown in modified Erdschreiber medium (25‰) and illuminated by Philips fluorescent tubes, color 29 with a PFD of 15 μmol m^{-2} s^{-1}. A salinity experiment ranging from 5 to 35‰ was done in a 2.6 ± 0.2°C cold cabinet with a PFD of 42 μmol m^{-2} s^{-1} (Luma Thermo WW-LL). Erdschreiber medium of 0‰ was mixed with 34‰ medium to give a salinity gradient in steps of 5‰. The salinity of 35‰ was obtained by evaporation. Each subculture had an initial 2-day period of adaptation in steps of 5‰ until the final salinity was reached. Two experiments were performed for each of the salinities studied.

Every 2nd day for a period of maximum 28 days 2-mL samples were fixed in a drop of 2% Lugol's iodine. Cell densities were determined using a light microscope and a 1.0-mL Sedgewick-Rafter chamber. Calculation of the growth rate (k) during exponential growth was by graphical estimation from semilog plots of successive cell counts, using the equation k (divisions/day) = $3.322/T_{10}$ where T_{10} is the number of days required for a 10-fold increase of the population (Guillard 1979).

Light microscopy

Live cells were examined using an Olympus BH-2 microscope equipped for differential interference contrast, phase contrast, and epifluorescence.

Electron microscopy

Whole mounts for electron microscopy were prepared according to Moestrup and Thomsen (1980). The mounts stained for 30 min in 2% aqueous uranyl acetate and subsequently washed for 30 s in distilled water.

To obtain a high number of recently divided cells, fresh medium was added to a culture of *P. cyrtoptera* 4 days before fixation for thin sectioning. The culture was fixed by adding an equal volume of 2% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.7) containing 0.5 M sucrose. After 1 h at 4°C the material was rinsed for 10 min in buffer and further rinsed for 10 min in buffers of decreasing sucrose concentrations (from 0.5 M sucrose to pure buffer lacking sucrose in steps of 0.1 M sucrose). The cells were postfixed for 1 h in 1% osmium tetroxide in 0.1 M cacodylate buffer, rinsed in buffer for 10 min, and dehydrated in an ethanol series, 15 min in 15, 30, 50, 70, 96 (all at 4°C), and two changes in 100% ethanol at room temperature. After two treatments in propylene oxide (5 min in each) the material was left in a refrigerator overnight in a 1:1 mixture of propylene oxide and Spurr's resin. The embedding medium was then replaced with fresh Spurr's resin at room temperature and transferred 5 h later to an embedding dish and polymerized overnight at 70°C.

A second culture was fixed for 1 h in 1% osmium tetroxide in 0.1 M cacodylate buffer followed by a 10-min rinse in buffer. Dehydration and embedding followed the procedure described above.

Thin sections were cut on a LKB Ultratome V using a diamond knife and mounted on slot grids. Sectioned cells were stained for 30 min in 2% aqueous uranyl acetate, rinsed, and stained in Reynold's lead citrate (Reynolds 1963) for another 20 min. The material was examined using JEOL-100SX (Institut for Sporeplanter) and JEOL-100CX (Genetical Institute, University of Copenhagen) transmission electron microscopes.

Preparations for scanning electron microscopy were fixed for 1 h in 2% glutaraldehyde, followed by 1 h postfixation in 1% osmium tetroxide. Cells were then exposed to increasing concentrations of acetone, from 10 to 100% in steps of 10%. The material was critical point dried, coated with gold, and examined using a 515 Philips scanning electron microscope at the Geological Institute, University of Copenhagen.

Observations

Pyramimonas cyrtoptera Daugbjerg sp.nov.

ETYMOLOGY: From the Greek *cyrtos*, curved, and *pteron*, wing.

HOLOTYPE: Fig. 62.

Cellula 38–42 μm longa, cum alis lateralibus 25–28 μm lata, ante quadrata, truncata, post rotundata, in papillam mediam subito attenuata. Duae partes posteriores conspicue alatae. Quattuor alae ex apice visae circiter quartam ambitus partem

contra horologium curvae, cellula ita torta apparente. Flagella sedecim, cellula paulo longiora, e fovea anteriore profunda orientia. Chloroplasti bini contigui, plastidoma poculiforme longitudinaliter partitum una formantes, quisque ante in quattuor lobos profonde fissus, flavovirens, pyrenoides basale compluribus granulis amyleis circumdatum fovens, duo stigmata intraplastidialia magnitudine binis chloroplastis imparia prope pyrenoides ostendens.

Corpus cellulae squamis trimorphis tectum, interioribus quadrangulis minutis, intermediis capsiformibus perforatis, exterioribus complanate coroniformibus. Flagellis squamae interiores pentagonae, parvae, squamae limuliformes superpositae, piliformes in duas series dispositae.

Cells 38−42 μm long and 25−28 μm wide including the lateral wings. With a square and truncate anterior, and a rounded posterior abruptly tapering to a median tail. The posterior two-thirds of the cell with four conspicuous wings that turn ca. one-quarter of a full anticlockwise turn. The cells thereby appear twisted. Sixteen flagella, slightly longer than the cell, emerge from a deep anterior depression. Two chloroplasts together forming a cup shape, each deeply divided anteriorly into four lobes. Chloroplasts yellow-green, each with a basal pyrenoid surrounded by several starch grains. One pair of intraplastidial eyespots positioned in each chloroplast at the level of the pyrenoids. The two pairs of eyespots differ in size.

Three types of scales cover the cell body: an underlayer of very small square scales, an intermediate layer of perforated box scales, and an outer layer of flattened crown scales. Each flagellum is covered by small pentagonal underlayer scales, limuloid scales and two rows of hair-shaped scales.

Light microscopy

Cell morphology

Cells of *P. cyrtoptera* are yellow-green, 38−42 μm long and 25−28 μm wide including the wings. The general morphology is complex. The apical part is square in outline (Fig. 10), each side 18−20 μm. Four wings emerge from a position about one-third down and continue to the antapical end of the cell (Fig. 3). The wings are approximately at right angles to each other when viewed in optical cross section (Figs. 9 and 11), giving the appearance of a four-leaved *Trifolium pratense*. Each wing turns about one-quarter of a full anticlockwise turn (Fig. 4). The antapical end of the cell bears a more or less distinct median tail (cf. Figs. 5 and 6).

The three-dimensional appearance of the cell is shown in Figs. 16 and 62.

The 16 flagella are slightly longer than the cell (Figs. 3 and 16) and are inserted in an anterior pit, 6−7 μm deep (Fig. 4). When the cell has settled the distal part of each flagellum bends outwards (Fig. 4). The flagella spread out evenly without forming groups (Fig. 9).

Cells of *P. cyrtoptera* contain two chloroplasts that are closely adjoined in the most antapical part of the cell (Fig. 14). Each chloroplast has four deep lobes and the eight lobes closely follow the plasmalemma of the four wings (Fig. 15). Each cell wing contains two lobes (Fig. 11), which at the apical end bend towards the flagellar pit (Fig. 10).

Two pyrenoids are observed at the antapical end of the cell, one in each chloroplast (Fig. 7). The pyrenoids are surrounded by several starch grains (Fig. 6).

Two pairs of basal eyespots (stigmata) are present just above the level of the pyrenoids (Fig. 5). Each pair is located in adjacent chloroplast lobes (Figs. 7 and 8), one pair 3−4 times larger than the other (cf. Figs. 7 and 8).

The scale reservoir and the nucleus are positioned beneath the flagellar pit (Fig. 5).

Four very large vacuoles are present in the lower middle region of the cell (Fig. 6).

Sixteen new flagella form prior to cell division (Fig. 12), each cell receiving eight old and eight new flagella. A division stage with 27 flagella in focus is shown in Fig. 13.

No cysts or amoeboid stages have been observed.

Cell movement

Swimming cells take a slightly curved path. Because of the curved wings a cell turns anticlockwise around its longitudinal axis when moving. It may suddenly stop and change direction. After a few minutes of observation the majority of the cells settle, attaching to the slide with the apical end (see also Fig. 9). When disturbed, the cell quickly swims away, but soon resettles. The swimming and settling behavior of *P. cyrtoptera* is similar to *P. octopus* (Moestrup et al. 1987), indicating that *P. cyrtoptera* is semibenthic, probably associated with the ice surface.

Electron microscopy

General fine structure

Cells of *P. cyrtoptera* have double the number of certain organelles normally encountered, but the organization (Figs. 17 and 18) is otherwise similar to that described for other species of the genus.

The anterior lobes of the chloroplasts curve toward the flagellar pit at the apical end of the cell (Figs. 17 and 21). The antapical tail of the cell contains part of a chloroplast, probably an extension of one of the chloroplast lobes (Fig. 19).

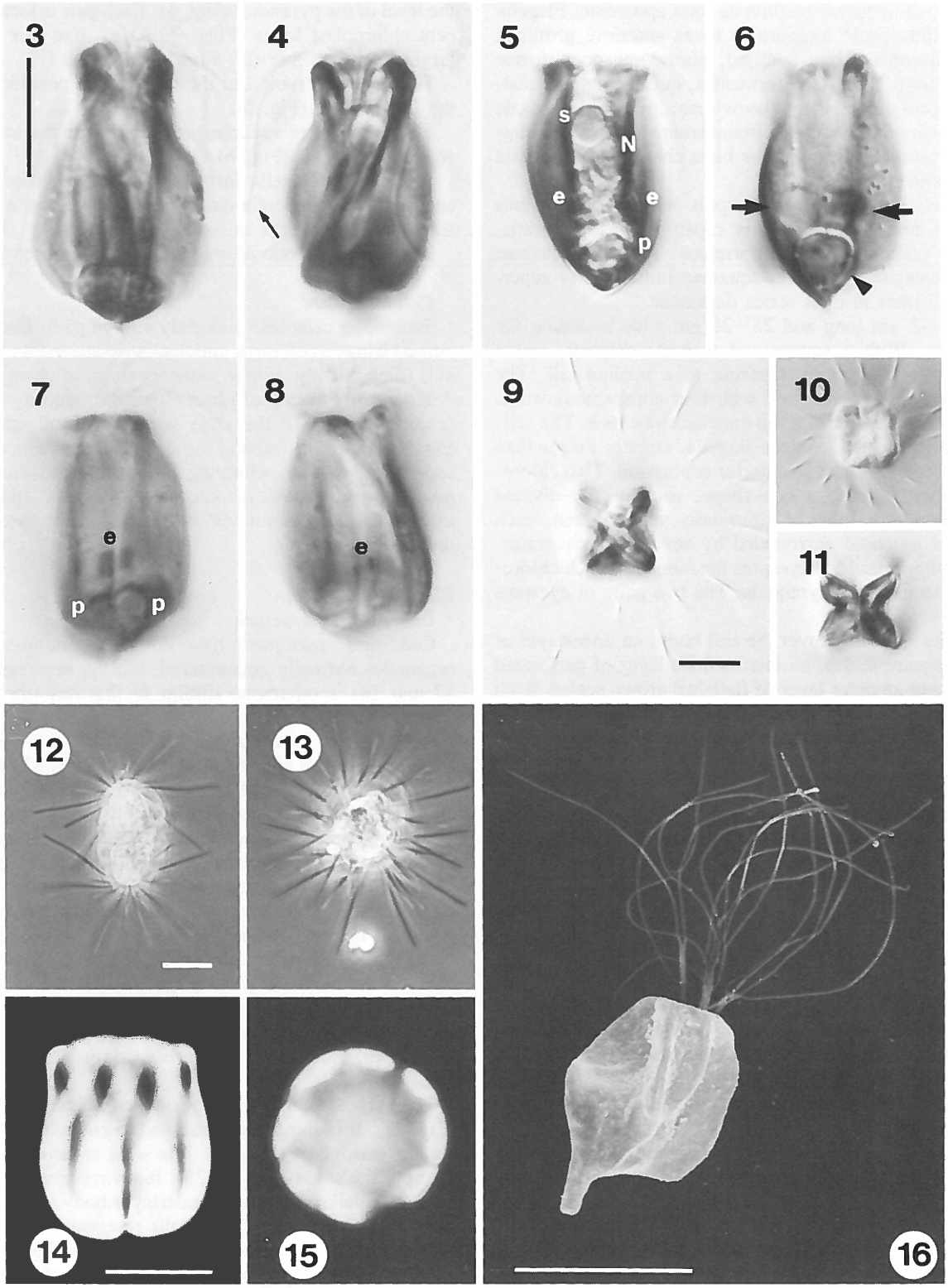
Each pyrenoid is surrounded by three to eight starch grains and the matrix is penetrated by many anastomosing thylakoids (Figs. 25, 29). The pyrenoids correspond to type V of Inouye et al. (1985). A dividing cell in which pyrenoid division is complete is illustrated in Fig. 26.

All four eyespots are composed of a single layer of tightly packed carotenoid droplets (Figs. 24, 27, 28).

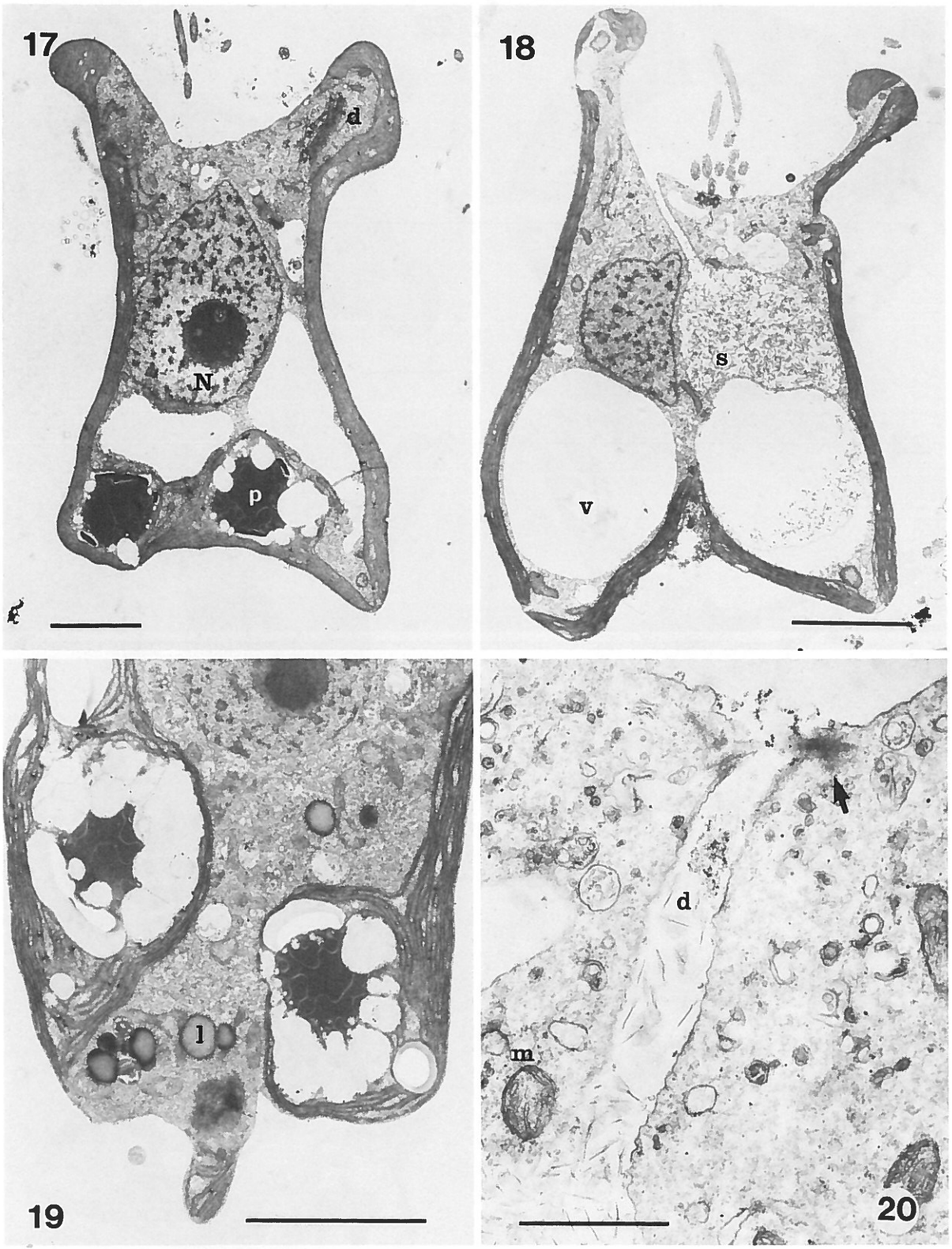
Numerous mitochondrial profiles are seen in serial sections and they probably form a reticulum like that of *P. gelidicola* McFadden, Moestrup & Wetherbee (McFadden and Wetherbee 1982).

The nucleus is lateral and positioned below the basal bodies (Fig. 17). It is sometimes observed to encircle the microbody and the rhizoplasts (Fig. 31). The scale reservoir is located on the opposite side (Figs. 18, 23). It always contains high numbers of flagellar scales and underlayer body scales. The large body scales are seldom seen in the reservoir. Scales are transported from the reservoir through a channel below the flagellar bases to an aperture on the opposite side of the flagellar pit (Fig. 18). The opening is marked by dense material, probably duct fibers (Fig. 20). Release of scales through a channel underneath the basal bodies has also been described in *P. octopus* (Moestrup et al. 1987).

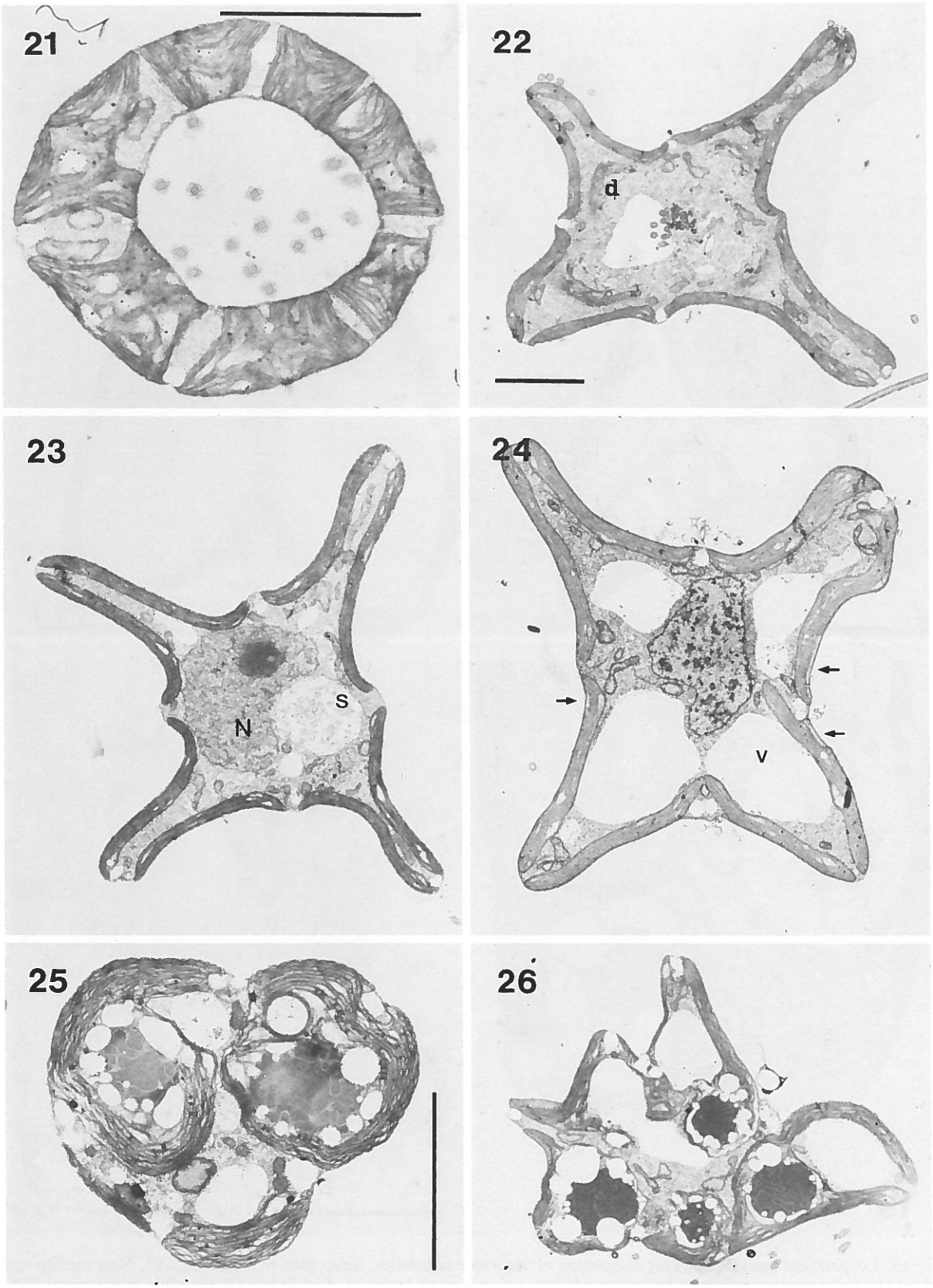
The Golgi apparatus comprises four dictyosomes located in the cell wings at the level of the flagellar pit (Fig. 22). Each dictyosome consists of 15−20 cisternae. Many small vesicles released from the endoplasmic reticulum fuse to form new cisternae at the forming face of each dictyosome.



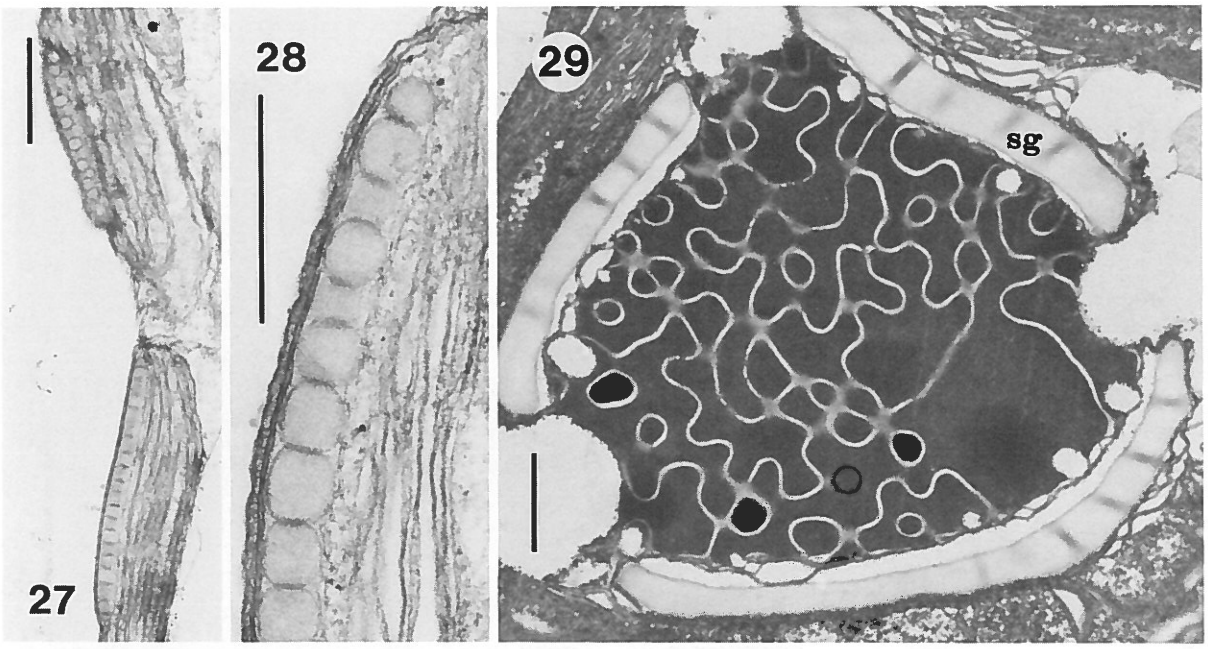
FIGS. 3–15. Light micrographs of *Pyramimonas cyrtoptera* sp.nov. Figs. 3–8. Optical longitudinal sections (DIC). Figs. 3 and 4. Typical cells (note outwards bending of the distal part of a flagellum in Fig. 4, arrow). Fig. 5. Scale reservoir (*s*), nucleus (N), eyespots (*e*), and one of the pyrenoids (*p*). Fig. 6. Pyrenoid surrounded by four starch grains (arrowhead). Two large vacuoles anterior to the pyrenoid (arrows). Figs. 7 and 8. The two pairs of eyespots (*e*) are of unequal size (same cell). Two pyrenoids (*p*) present at the antapical end. Figs. 9–11. Optical cross sections (DIC). Fig. 9. A settled cell. Fig. 10. Apical view of the flagellar pit, showing basal bodies and chloroplast lobes. Fig. 11. View of posterior end, two chloroplast lobes are located in each wing. Figs. 12 and 13. Dividing cells (phase contrast). Fig. 12. Cells adjoining end to end. The newly formed flagella have not yet reached full length. Fig. 13. There are 27 of 32 flagella visible in this micrograph. Figs. 14 and 15. Epifluorescence microscopy. Fig. 14. Lateral view; each chloroplast is deeply divided into four anticlockwise twisted lobes. Fig. 15. Apical view. FIG. 16. Scanning electron micrograph of *P. cyrtoptera* showing the three-dimensional shape of the cell. Note the median posterior tail. Scale bars = 20 μm.



FIGS. 17–19. Longitudinal sections showing disposition of the major organelles. Scale bars = 5 μm. Fig. 17. Near-median section with lateral nucleus (N) and posterior pyrenoids (*p*) surrounded by several starch grains. A dictyosome (*d*) is visible in the apical end. Fig. 18. Oblique section showing nucleus, scale reservoir (*s*), and two large vacuoles (*v*). The scale reservoir duct is very long and opens near the nucleus. Note also chloroplast lobes in the flagellar pit region. Fig. 19. Cell with median posterior tail containing a part of a chloroplast. Two pyrenoids are visible in separate chloroplasts. Note also lipid droplets (*l*). FIG. 20. High magnification showing the aperture of the scale reservoir duct (*d*). Dense fibrillar material (arrow) surrounds the aperture. *m*, mitochondrion. Scale bar = 1 μm.



FIGS. 21–26. Transverse sections of the cell. Fig. 21. The chloroplast lobes bend apically toward the flagellar pit. Fig. 22. Section near the base of the flagellar pit. Four dictyosomes (*d*) are visible. Fig. 23. Section at the level of the nucleus (N) and scale reservoir (*s*). Two chloroplast lobes are present in each cell wing. Fig. 24. Section through eyespots (arrows) and large vacuoles (*v*). Fig. 25. Pyrenoids at the antapical end. Fig. 26. Dividing cell, pyrenoids have duplicated. Scale bars = 5 μm.



FIGS. 27–29. Structure of eyespots and pyrenoids. Fig. 27. Cross section through pair of eyespots located in adjacent chloroplast lobes. Scale bar = 1 μm. Fig. 28. Each eyespot consists of a single layer of tightly packed carotenoid droplets, not separated by thylakoids. Scale bar = 0.5 μm. Fig. 29. Longitudinal section through the pyrenoid matrix, here enclosed by three starch grains (*sg*). Scale bar = 1 μm.

Schematic drawings showing the position of the major organelles are given in Figs. 62–64.

Although the flagellar apparatus has not been examined in detail, it is a primary character for the taxonomy of the genus (Inouye et al. 1985) and a few micrographs are included. The flagellar apparatus and the peripheral cytoskeleton consists of a large number of peripheral microtubules. The flagellar pit is lined by 300–320 microtubules (not shown). Cross sections of a flagellum at various levels reveal the structures typical of the genus (Fig. 30). The eight innermost basal bodies have been numbered according to Moestrup and Hori (1989) while the eight outermost basal bodies have been numbered as shown in Fig. 33. Basal bodies 1 and 2 are connected by a large striated fiber, the synistosome, that is ca. 300 nm long and ca. 125 nm wide (Fig. 33). The 16 basal bodies are further connected by large and thick muscle-like fibers, or small and thin fibers (Figs. 33, 34). Two of the four flagellar roots have been identified to hold five and three over one microtubules, respectively (Fig. 35). There are several rhizoplasts that split before attaching to the proximal part of the basal bodies (Figs. 35, 36). The microbody and the striated nature of the rhizoplasts are shown in Fig. 32.

The flagellar apparatus appears complex but shows affinity to the shifted type designated by Inouye et al. (1985).

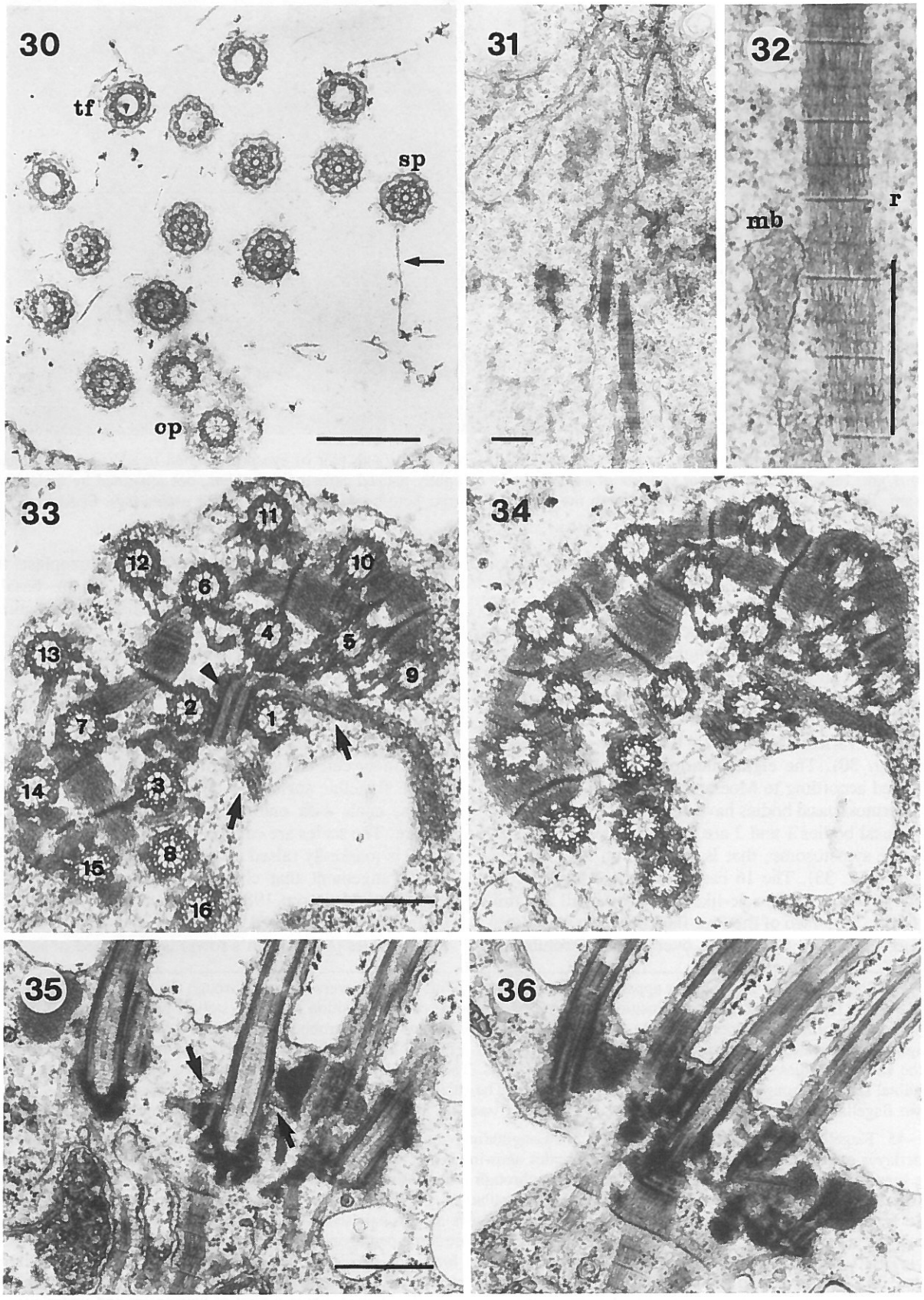
The scaly covering

Cells of *P. cyrtoptera* are covered with six types of scales, three on the cell surface and three on the flagella (Figs. 37–61).

The flagellar surface is covered by a layer of pentagonal scales, each with one very short side, and thus appearing square. The scales are ca. 40–45 nm wide (Fig. 38). A central spine is markedly raised above the sides (Fig. 37). They show the arrangement that characterizes the genus *Pyramimonas* (Hori and Moestrup 1987), i.e., two pairs of opposite longitudinal rows, separated by oblique rows. One of the longitudinal pairs (Melkonian's rows) is illustrated in Fig. 38. Nine

FIGS. 30–36. Some details of the flagellar apparatus of *P. cyrtoptera*. Fig. 30. Transverse section through the proximal end of the flagellar pit, showing various levels of flagella: the transitional fiber (*tf*, arrowhead) of the transition zone, the stellate pattern (*sp*), and the cartwheel pattern (*cp*) of the basal bodies. Note hair scale (arrow). Fig. 31. Rhizoplasts and microbody encircled by the irregularly shaped nucleus. Fig. 32. Higher magnification of a rhizoplast (*r*) and microbody (*mb*). Figs. 33 and 34. Transverse section showing the synistosome (arrowhead) and the 16 basal bodies interconnected by thin and thick fibers. The 1*d* and 1*s* microtubular roots are marked by arrows. Figs. 35 and 36. Longitudinal section through parts of the flagellar apparatus. The rhizoplast splits before reaching the proximal end of the basal bodies. Fig. 35. Two flagellar roots are visible and consist of three over one and four microtubules, respectively (arrows). Scale bars = 0.5 μm.

FIGS. 37–45. Flagellar scales of *P. cyrtoptera*. Fig. 37. Longitudinal section through a flagellum. Note the marked central spine of pentagonal underlayer scales. Fig. 38. Pentagonal flagellar scales showing a pair of Melkonian's rows (arrow). Fig. 39. Stained whole mount of a mature limuloid scale. Figs. 40 and 41. Oblique section through a dictyosome showing two stages in the formation of limuloid scales. Fig. 42. Earliest seen stage of a hair scale composed of at least 59 subunits. Fig. 43. Cross section through a dictyosome illustrating formation of hair scales in five successive cisternae. The upper right corner shows mature hair scales in a vacuole. The midpiece of the hair scales is triangular in cross section (arrow) while the distal part is solid. Note also three pentagonal flagellar scales (arrowhead) and the box scale on the right. Fig. 44. Longitudinal section through a dictyosome showing formation of 13 hair scales in antiparallel rows. The thick midpiece of three mature hair scales is seen in a vacuole in the upper right corner. Inset shows higher magnification of three hair scales. Note the hook-like structure (arrows) at the base of the thin distal part of the hair scales. Fig. 45. Stained whole mount of hair scales and underlayer flagellar scales. A single underlayer body scale is also visible (arrow). Scale bars = 0.2 μm.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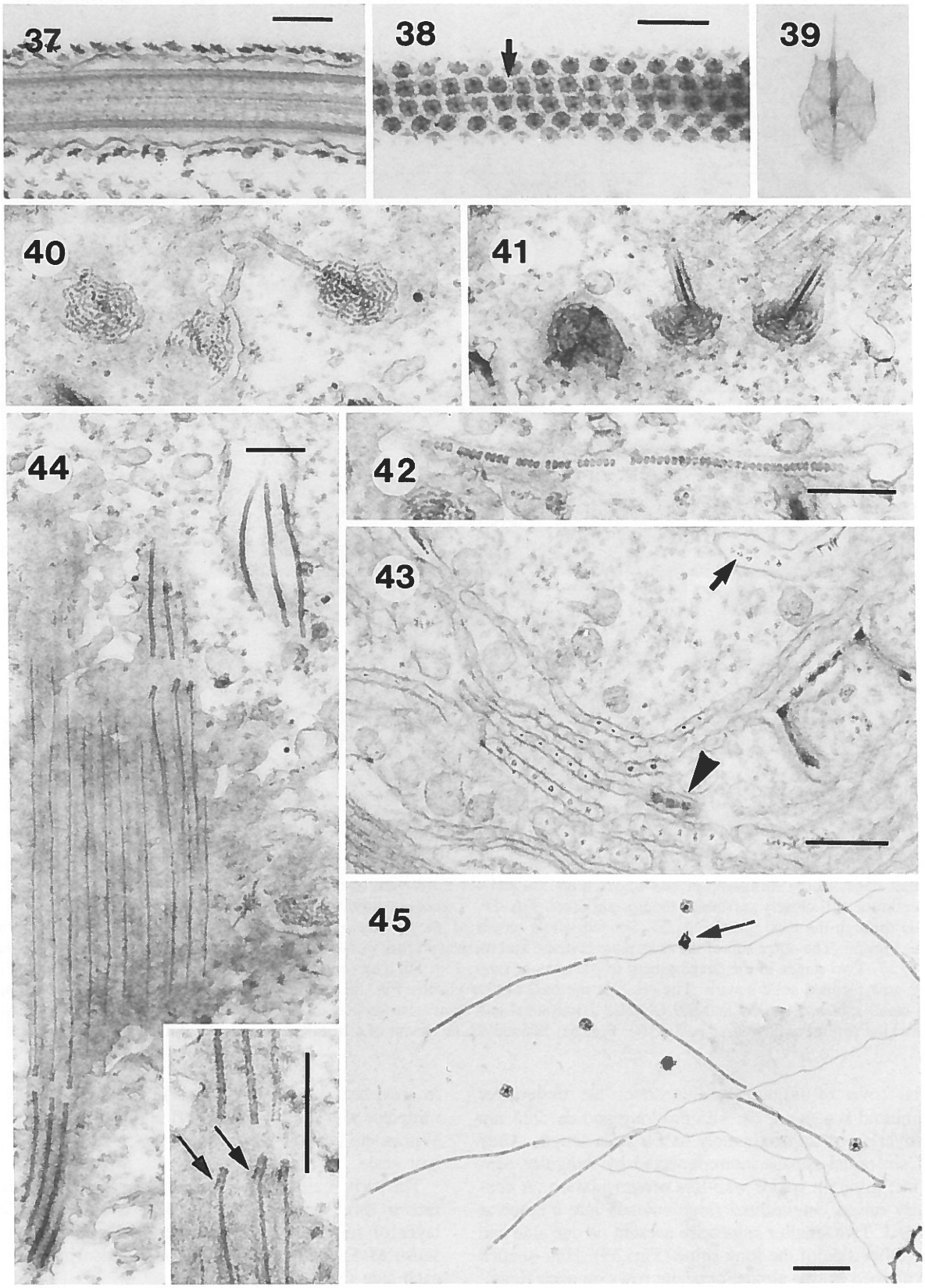
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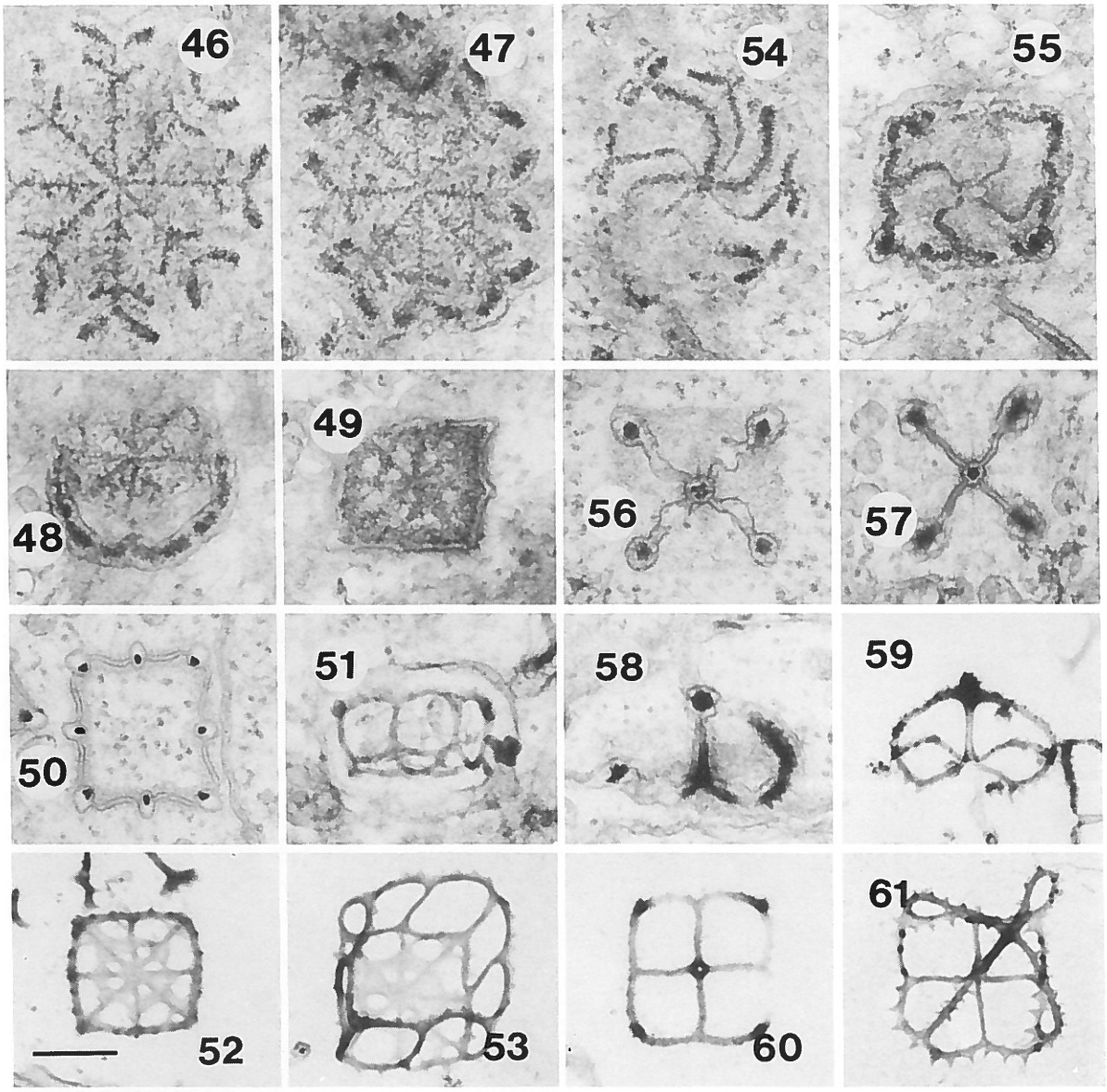
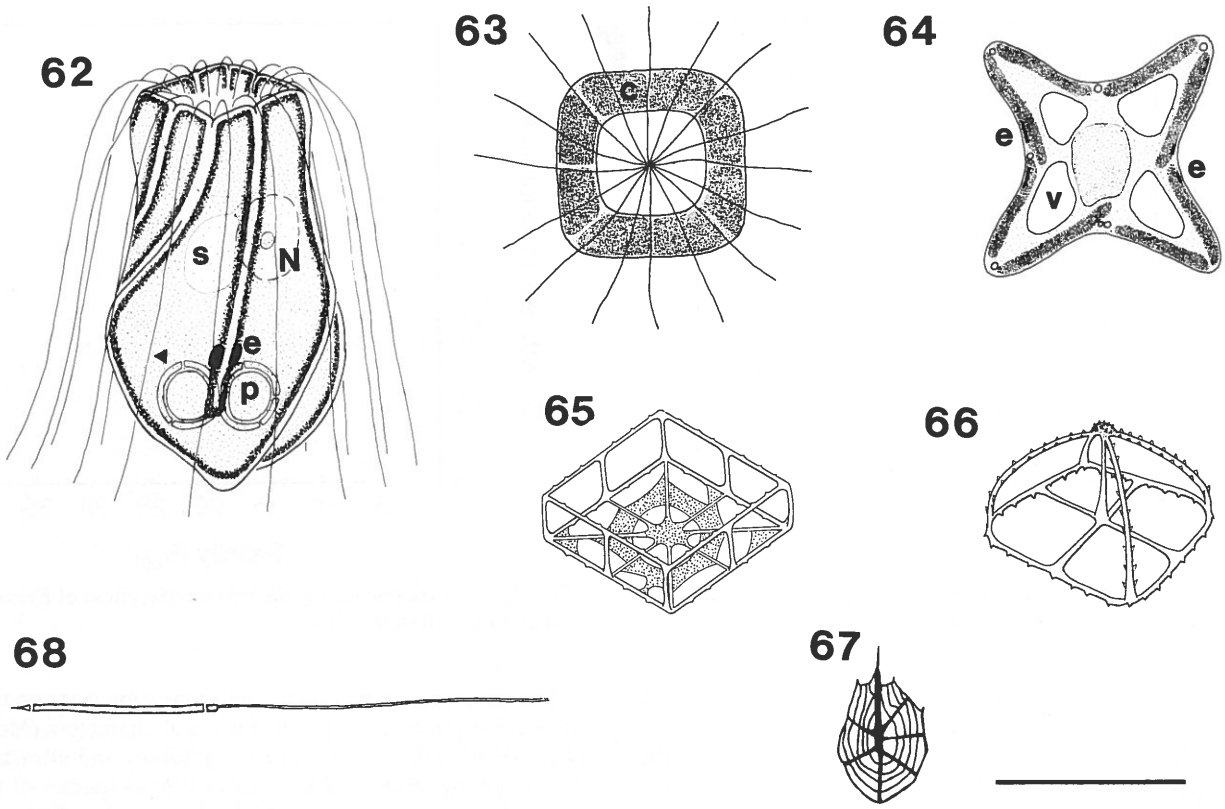


FIGS. 46–61. Large body scales of *P. cyrtoptera*. Figs. 46–53. Box scales. Figs. 54–61. Crown scales. Figs. 46 and 47. Earliest seen developmental stages in the formation of box scales. Figs. 48 and 49. Formation of the base pattern. Fig. 50. Nearly mature box scale, the molding membrane still closely surrounds the upright arms. Fig. 51. Vacuole containing mature box scale and small vesicles. Fig. 52. Transverse section through the base plate. Fig. 53. Stained whole mount of mature box scale. Fig. 54. Earliest seen stage in the formation of a crown scale. Fig. 55. The outer rim of the base plate is dense and the wavy arms which eventually form the cross of the base plate are visible. Figs. 56 and 57. Two stages in the development of the upright arms. Fig. 56. The central strut is seen as four individual dots. Fig. 57. The central strut now forms a solid square. The cross in the base is also visible. Fig. 58. Almost mature crown scale. The distal knob is dense and carries small spines. Figs. 59 and 60. Oblique longitudinal and transverse section through mature crown scales. Note the marked knob in Fig. 59 and the central hollow strut in Fig. 60. Fig. 61. Stained whole mount of a mature crown scale. All × 60 000. Scale bar = 0.2 μm.

longitudinal rows of limuloid scales cover the underlayer scales. Limuloid scales are ca. 425 nm long and ca. 225 nm wide and overlap by approximately half a scale length. They consist of six radial spokes interconnected by irregular concentric arms, giving a spider web-like ornamentation. A central, slightly raised longitudinal ridge extends into a spine at the apical end. Two smaller spines are present on one side and one on the other side of the long spine (Fig. 39). Hair-shaped scales are aligned in two almost opposite rows on each flagellum. Each hair scale is 1.4 μm long and consists of three parts: a proximal triangular part, ca. 50 nm long, a hollow midpiece ca. 500 nm long and ca. 12 nm wide, and a distal part ca. 850 nm long and ca. 6 nm wide. The midpiece is triangular in cross section (Fig. 43, arrow). The distal ca. 30 nm of the midpiece was always broken off in our preparations (Fig. 45). Within the genus *Pyramimonas* this represents a new type of hair scale.

The body scales form a periplast covering the entire cell surface in three separate layers. Close to the plasmalemma is a layer of small square underlayer scales belonging to type 3 sensu McFadden et al. (1986) and measuring 40–45 nm on each side (Fig. 45).

The underlayer scales are covered by box scales (intermediate body scales). These are square, each side ca. 325 nm in width. The base plate is composed of eight radial ribs connected by a thin net (Figs. 52, 53). Some variation exists in



FIGS. 62–68. Schematic drawings of *P. cyrtoptera* and four of the scale types. Fig. 62. Three-dimensional drawing of a cell (holotype), showing position of nucleus (N), scale reservoir (*s*), eyespots (*e*), pyrenoids (*p*) surrounded by starch grains (arrowhead). Fig. 63. Section at the level of the flagellar pit illustrating flagella and chloroplast lobes (*c*). Fig. 64. Section at the level of the eyespots (*e*), four large vacuoles also visible (*v*). Fig. 65. Box scale. Fig. 66. Crown scale. Fig. 67. Limuloid scale. Fig. 68. Hair scale. Scale bar = 20 μm for Figs. 62–64, 0.4 μm for Figs. 65 and 66, and 0.5 μm for Figs. 67 and 68.

the appearance of the net and the number of perforations. Eight vertical struts, each ca. 150 nm high, emerge from the junctions of the radial ribs and the rim. They are attached above to an upper rim. Each side of the rim carries six to eight evenly distributed small spines while each side of the base plate has four small spines (Fig. 53).

The box scales are covered by crown scales (outer body scales). Each crown scale has a square base with slightly rounded corners (Fig. 60). The side is ca. 350 nm wide and ornamented with 8–10 small spines. The base is divided into four quadrants (Fig. 61). A central hollow strut (Fig. 60) arises from the basal cross. It meets with a set of four lateral arms that arises from the corners of the base. These arms are curved, each carrying approximately nine double rows of small spines. The scale is distinctly flattened in side view (Fig. 59) and ca. 220 nm high. A small darkly staining knob with a few spines is present on the distal end of the central strut (Figs. 58, 59).

Reconstructions of the four types of scales are shown in Figs. 65–68.

Scale production

Scale morphogenesis in *P. cyrtoptera* resembles *P. tetrarhynchus* Schmarda (Moestrup and Walne 1979). Scale morphogenesis is not related to certain stages in the cell division cycle, and fixations started at different hours of the day all showed significant scale formation activity in the four dictyosomes.

The earliest seen stage of a limuloid scale bears some resemblance to the snowflake structure of a box scale. It is not symmetrical, however, but has seven rather than eight radiating arms (Fig. 40). One arm is longer and forms the apical spine. The appendages on the radiating arms fuse to form the spider web-like pattern. While in the Golgi cisternae the limuloid scales do not have a fixed orientation (Fig. 41), but the upper side is always directed toward the forming face of the dictyosome.

In the earliest observed stage, a hair scale consists of at least 59 subunits (Fig. 42). Up to 13 hair scales are formed in antiparallel rows in a single cisterna (Fig. 44). Figure 43 shows two hair scales sectioned in the plane of the thick midpiece and one through the thin distal part. A small hook-like structure is observed where the thick part of a hair scale seems to be broken off (inset in Fig. 44). The hook-like structure is not clearly visible in mature scales (Fig. 45).

The first recognizable stage of a box scale resembles a snowflake (Fig. 46). It is symmetrical with eight radiating arms of equal length and thickness. Each arm carries lateral appendages that gradually increase in length toward the distal end. At a later stage the lateral appendages in the distal end thicken and the eight radiating arms adjoin (Fig. 47). The rim of the base is moulded by the surrounding membrane (Fig. 48). In Fig. 49 the eight arms that radiate from a common center toward the corners and the midregion of the base are indistinctly connected by a thin net, but the specific pattern is not yet complete. The eight vertical struts are also moulded by a closely adjoining membrane (Fig. 50).

In the earliest identified stage of a crown scale, eight alternately thick and thin wavy arms radiate toward the corners and the middle of a dense rim (Fig. 54). The four thin arms even-

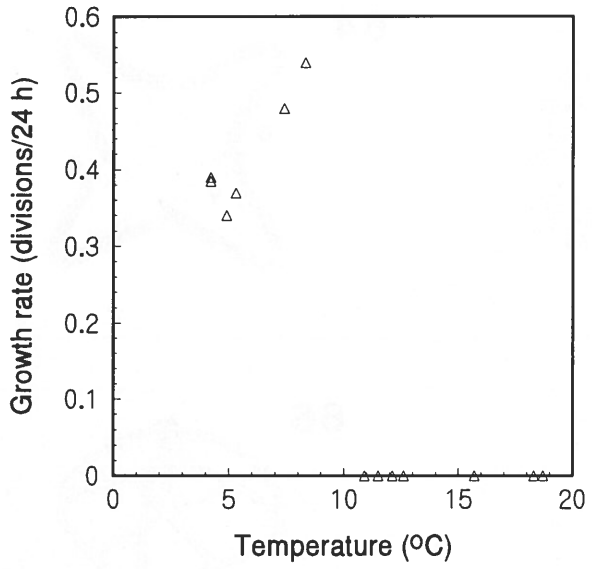


FIG. 69. Growth rate during the exponential phase of *Pyramimonas cyrtoptera* at different temperatures.

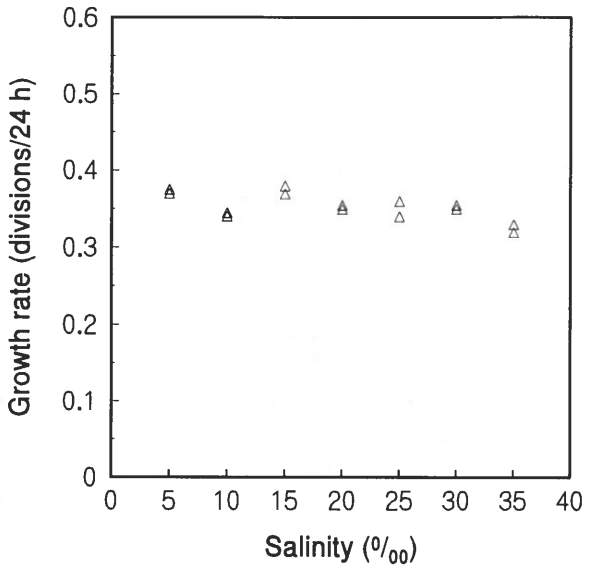


FIG. 70. Growth rate during the exponential phase of *Pyramimonas cyrtoptera* at different salinities.

tually form the cross of the base plate (Fig. 55) while the thick arms make up the four upright arms. In the subsequent developmental stages the proximal part is completed first while the four upright arms form later (Figs. 56, 57). The small distal knob of a crown scale is difficult to discern in whole mounts. In Fig. 56, the four upright arms are surrounded by the cisternal membrane, and the distal part is composed of four individual dots. They subsequently fuse to form a small square (Fig. 57). In an almost mature crown scale (Fig. 58) the knob is solid and small spines are seen on top.

When scales are mature they leave the dictyosomes in vacuoles. Flagellar scales are transported towards the scale reservoir and released by reverse pinocytosis. Box scales and crown scales, either single or together in pairs, are apparently released directly to the plasmalemma in the flagellar pit. Vacuoles containing mature box scales or crown scales also contain vesiculate material (Fig. 51).

Temperature and salinity studies

Owing to mortality the population size within a few days decreased markedly in experiments done at temperatures above 10°C. Growth only occurred at temperatures between 4 and 8°C, and the growth rate ranged between 0.34 and 0.55 divisions/day (Fig. 69). It is important to emphasize that cultures showing the two highest growth rates (temperatures 7.4 and 8.3°C) decreased in cell number after 12 and 10 days, respectively. The decrease is not caused by nutrient limitation since cultures grown at lower temperatures were still in the exponential phase when the experiment was stopped after 20 days, reaching 42 000 – 85 000 cells/mL.

There was very little difference in growth rate of *P. cyrtoptera* within the experimental salinity range (Fig. 70). When the experiments were stopped after 28 days, all populations were still in the exponential growth phase.

Discussion

Pyramimonas cyrtoptera is remarkable in a number of features, whereas the general structure of the cell and the micromorphology of the scales are typical of the genus *Pyramimonas*.

This genus has been divided into four subgenera on the basis of electron microscope and biochemical characters (McFadden et al. 1986, 1987). In its scale morphology and ultrastructure, *P. cyrtoptera* shows a close relationship to species of the subgenus *Pyramimonas* (sensu McFadden et al. 1986) to which four species have been assigned: *P. tetrarhynchus, P. amylifera, P. octopus*, and *P. propulsa*. A comparative study of the scales belonging to this subgenus was reported by Moestrup et al. (1987).

The box scales of *P. cyrtoptera* are nearly identical to the box scales of *P. amylifera* and *P. propulsa*, but small differences exist in details of the perforations between the eight radial ribs.

The crown scales of *P. cyrtoptera* are very similar to those of *P. amylifera*. Both are rather flat but differ in the architecture of the four upright arms; *P. amylifera* lacks rows of small spines. The crown scale of *P. octopus* carries small rows of spines on the four upright arms and a small distal knob is present centrally. However, the form of the distal knob differs from *P. cyrtoptera*.

The limuloid scales of all five species are almost identical and differ only in the size and number of apical spines.

In most descriptions of new species of *Pyramimonas*, the hair scales have been given little attention. Practically no information is available in the subgenus *Pyramimonas*. From whole mounts of *P. propulsa* based on material collected in Danish waters (Limfjorden, Jytland) it has now been confirmed that the hair scales of this species are very similar to those of *P. cyrtoptera* (Ø. Moestrup, unpublished data). The hair scales of the type species *P. tetrarhynchus* differ, however, in morphology (N. Daugbjerg, unpublished data). Another new type of hair scales was recently described in *P. mucifera* Sym & Pienaar (Sym and Pienaar 1991*b*), a species that shows affinity to the subgenus *Punctatae* (sensu McFadden et al. 1987). The hair scales of *P. mucifera* consist of a large number of subunits. The hair scales of *P. olivacea* Carter (type species of the subgenus *Punctatae*) belong to the same category (cf. Fig. 17 in Sym and Pienaar 1991*b* with Fig. 10 in McFadden et al. 1987).

Examination of published micrographs of hair scales from

the subgenus *Vestigifera* (McFadden et al. 1986) reveal only a single type, except in *P. spinifera* Pennick (Pennick 1983), which has two types of hair scales. Hair scales of the subgenus *Trichocystis* (McFadden et al. 1986) have not been examined in detail.

The morphology of hair scales thus varies more than initially expected and it seems probable that the different types fall into groups corresponding to the subgenera, though there are some exceptions. Additional investigations are needed before the value of the hair scale as a taxonomic character can be assessed.

Detailed studies on scale production in *Pyramimonas* have been performed on *P. amylifera* (Manton 1966), *P. tetrarhynchus* (Moestrup and Walne 1979), and *P. pseudoparkeae* Pienaar & Aken (Aken 1985). The initial developmental stages of the large body and limuloid scales are surprisingly identical and the scales can only be distinguished when almost mature.

The earliest seen stages of the large body scales in *P. cyrtoptera* are roughly twice as large as the fully matured scales. The same tendency is indicated from the micrographs of *P. tetrarhynchus* (Moestrup and Walne 1979).

In *P. octopus* five to eight hair scales are assembled in a single cisterna. In *P. orientalis* McFadden, Hill and Wetherbee (Moestrup and Thomsen 1974) and *P. tetrarhynchus* (Moestrup and Walne 1979) the number of hair scales in each cisterna is two to five, and in *P. pseudoparkeae* three to six (Aken 1985). In *P. cyrtoptera* the highest recorded number was 13, but lower numbers have also been observed. The greater number seen in *P. cyrtoptera* probably reflects the higher number required in a cell with 16 flagella. The antiparallel arrangement of the scales in the cisternae was very unexpected and resembles the situation observed during formation of tripartite hairs in the endoplasmic reticulum of heterokont algae (Moestrup 1982). Unlike the situation in the heterokonts, however, the proximal end of the hair scale does not appear to be attached to the cisternal membrane.

The presence of 16 flagella (32 when dividing) is extraordinary for an autotrophic flagellate. It may be speculated that *P. cyrtoptera* has evolved from a *Pyramimonas* with eight flagella, like *P. amylifera*, in which the cell failed to divide after duplication of the flagella and chloroplasts. If *P. cyrtoptera* has evolved as described above, the high number of flagella would have to be considered to be an advanced character.

The presence of two chloroplasts separate *P. cyrtoptera* from most prasinophytes and other primitive green algae. With the exception of *P. urceolata* Conrad & Kufferath, all previously described species of *Pyramimonas* possess one pyrenoid. *Pyramimonas urceolata* is known only from the description by Conrad and Kufferath (1954), however, and the identity of the four organelles of the anterior end tentatively identified as pyrenoids is obscure. *Pyramimonas urceolata* should be reinvestigated using electron microscopy.

Species of *Pyramimonas* possess one or two eyespots. *Pyramimonas mucifera* (Sym and Pienaar 1991*b*) and *P. cyrtoptera* have two pairs of eyespots, but the former may occur with only one pair. No variation was observed in this respect in *P. cyrtoptera*. When *P. mucifera* possesses two pairs of eyespots no difference in size of each eyespot is observed, while they always differ in *P. cyrtoptera*.

Pyramimonas propulsa and *P. cyrtoptera* may be distinguished from other species of *Pyramimonas* by the four lateral wings. In old cultures of *P. propulsa* (Moestrup and Hill 1991) the wings become particularly distinct, while the wings in *P. cyrtoptera* are less conspicuous in old cultures. As noted for a number of species in the subgenus *Vestigifera* (Moestrup and Hill 1991), some morphological variation is noted in the posterior end of the cells and variation in the size of the posterior extension occurs also in *P. cyrtoptera*. *Pyramimonas* species are on the whole difficult to identify at the light microscopical level.

TABLE 1. Species of *Pyramimonas* from which the data for Fig. 71 were extracted, including references

Species	Reference
P. amylifera	Manton 1966
P. cordata	McFadden et al. 1986
P. cyclotreta	Daugbjerg and Moestrup 1992
P. cyrtoptera	This paper
P. disomata	McFadden et al. 1986
P. gelidicola	McFadden et al. 1982
P. grossii	Ø. Moestrup, unpublished
P. longicauda	Inouye et al. 1984
P. lunata	Inouye et al. 1983
P. mantonia	Ø. Moestrup and D. R. A. Hill, unpublished
P. mitra	Moestrup and Hill 1991
P. moestrupii	McFadden et al. 1986
P. mucifera	Sym and Pienaar 1991*b*
P. nansenii	H. A. Thomsen, unpublished
P. nephroidea	McFadden et al. 1986
P. norrisii	Sym and Pienaar 1991*a*
P. obovata	Melkonian 1981
P. octopus	Moestrup et al. 1987
P. orientalis	Moestrup and Thomsen 1974
P. parkeae	Norris and Pearson 1975
P. pseudoparkeae	Pienaar and Aken 1985
P. quadrifolia	N. Daugbjerg and Ø. Moestrup, unpublished
P. tychotreta sp.inedit	N. Daugbjerg, unpublished
P. tetrarhynchus	Ø. Moestrup, unpublished
P. virginica	Ø. Moestrup, unpublished

One of the distinctive characteristics of prasinophytes is the very long basal bodies (Moestrup 1982; Moestrup and Throndsen 1988) and it may be speculated that the length is related to the size of the cell. In species of *Pyramimonas* (Table 1), however, the length of the basal bodies does not show any relation to cell length (Fig. 71) ($r^2 = 0.013$). Plotting the length of the flagellar transitional fiber (coiled fiber) against cell length, however, gives a significant regression ($r^2 = 0.84$). In most cases, the length of the flagella more or less equals the length of the cell. It may be speculated that the transitional fiber acts as a supporting structure, perhaps associated with the rather stiff appearance of the flagella and (or) the very powerful strike of the flagella in *Pyramimonas*.

A similar structure in the prasinophyte *Pterosperma cristatum* Schiller (Inouye et al. 1990) shows no agreement with the regression analysis of species of *Pyramimonas*.

The significance of this structure, presently restricted to the family Halosphaeraceae, thus remains unclear. Also, there is no conclusive evidence that the transitional fiber forms a helix, as in the heterokonts. Most micrographs of *Pyramimonas* show the transitional fiber as two more or less unbroken lines when seen in longitudinal section. In *Pterosperma*, however, the two lines differ distinctly in length, indicating a helix

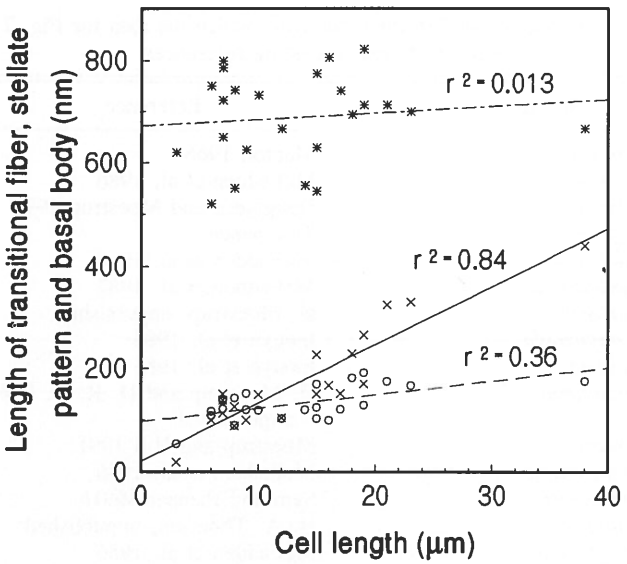


FIG. 71. Regression analysis of the length of the transitional fiber (x), stellate pattern (○), and basal body (*) against the length of the cell in species of *Pyramimonas*.

rather than a cylinder (Inouye et al. 1990, Fig. 11). The helix has a very flat pitch, and the fiber in *Pyramimonas* is probably a similarly flat but much shorter helix (five to six gyres were estimated in *P. obovata* by Melkonian 1982).

The length of the stellate pattern does not show any significant correlation when plotted against cell length ($r^2 = 0.36$).

The temperature and salinity study revealed *P. cyrtoptera* to be cold stenothermal and euryhaline. We conclude that growth of *P. cyrtoptera* becomes limited when temperatures reach 7–8°C. The temperature of the water in northern Foxe Basin varies little throughout the year and never rises above 2°C (Bursa 1961).

For most of the year the salinity in northern Foxe Basin is ca. 32‰ but drops markedly on surface when ice melting begins at the end of June. *Pyramimonas cyrtoptera* grew almost equally well at all tested salinities and is thus well adapted to the ice edge ecosystem.

In the salinity experiment the photon flux density was nearly 3 times the level of the temperature experiment. This, however, did not cause a general increase in growth rate (cf. Figs. 69, 70).

Possible interactions between temperature and salinity was not studied. Such interactions may play an important role when studying the autecology of phytoflagellates.

In *Pyramimonas* and other scale-bearing genera scale morphology has become a very important character for species identification. This requires that scale morphology is not susceptible to environmental changes. If any such variation occurs it may be speculated to take place under stress. Electron microscopical whole mounts were therefore made at regular intervals throughout the autecological study. They revealed no variation in scale morphology, supporting the use of scales as reliable taxonomic markers.

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