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Fine Structure of *Pyramimonas cyclotreta* sp. nov. (Prasinophyceae) from Northern Foxe Basin, Arctic Canada, with some Observations on Growth Rates

Niels Daugbjerg and Øjvind Moestrup
Institut for Sporeplanter, Copenhagen, Denmark

SUMMARY

An undescribed species of *Pyramimonas*, *P. cyclotreta* from Northern Foxe Basin (Arctic Canada) has been examined by light and electron microscopy of cultured material. Particular emphasis is given to the fine structure of the cell and the periplast. *Pyramimonas cyclotreta* is a typical member of the subgenus *Vestigifera* but differs in scale structure. It possesses a type of footprint scales not encountered before in the genus. *Pyramimonas cyclotreta* also differs in certain cytoplasmic features such as the structure of the posterior end of the cell and the very distinct connection between the flagellar apparatus and the microbody system. The response of growth to different temperatures reveals *P. cyclotreta* as cold stenothermal. *Pyramimonas cyclotreta* shows some morphological similarity to *P. torta*, a species from Belgian waters.

Introduction

In the Canadian arctic, research in marine ecosystems has recently been focused on production dynamics, biomass estimates and spatial and seasonal variations [e.g., 2, 6, 7, 9, 11, 13–15]. Only on rare occasions have phytoflagellates been the subject of detailed taxonomic studies involving modern light and electron microscopic technics [4, 18, 19].

Using light and inverted microscopes only, Bursa [2] in a year-long survey (September 1955 – September 1956) of the succession of the marine protist plankton in Northern Foxe Basin recorded diatoms, dinoflagellates, chlorophytes, coccolithophorids, choanoflagellates and cilates. He did not encounter any prasinophytes. With the exception of *Dolichomastix nummulifera* [18] and *Pyramimonas grossii* [12] no prasinophytes have to our knowledge been reported previously from Arctic Canada. From EM whole mounts based on wild material and crude cultures collected in the vicinity of Igloolik Island (Northern Foxe Basin) we have now observed prasinophytes belonging to three genera: *Micromonas* (*M. pusilla* (Butcher) Manton and Parke), *Mantoniella* (*M. squamata* (Manton and Parke) Desikachary) and two known and seven undescribed species of the genus *Pyramimonas* [5, Daugbjerg and Moestrup, in prep.].

The present paper describes one of the species of *Pyramimonas* established in unialgal culture, using light and electron microscopy. The response of growth to variations in temperature was also studied.

Material and Methods

Culture

Clones of *P. cyclotreta* were isolated by micropipetting from enriched water samples. The water samples were collected in June 1989 at station 22A1, 27A1 and 30A1, Northern Foxe Basin. Data on sampling area, temperature and salinity characteristics of the water column are given in Daugbjerg and Moestrup [5]. Cultures of *P. cyclotreta* are maintained at Institut for Sporeplanter in a modified Erdschreiber medium (30‰ S) [33] at 4 °C, light quantity 13 µmol m^{-2} sec^{-1} and a 16 : 8 h L : D cycle.

Temperature Study

Temperatures between 5.6 and 15 °C were obtained using a gradient plate with a light quantity of 35 µmol m^{-2} sec^{-1}. The temperatures of 2.4 and 3.6 °C were obtained in two cold rooms with light quantities of 38 µmol m^{-2} sec^{-1} and 35 µmol m^{-2} sec^{-1}, respectively.

The cultures were grown in dishes containing 100 ml modified Erdschreiber medium (30‰ S). The temperature was measured

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every second day of the experiments using a Viking 2000 digital thermometer.

For counting, a 1.5 ml sample was taken every 48 h and fixed in a drop of 2% Lugol's iodine. Cells were counted in a 1.0 ml Sedgewick-Rafter chamber. Estimates of growth rates (k) were calculated when batch cultures were in the exponential phase using the equation k (divisions/24 h) = $3.322/T_{10} \cdot T_{10}$ = days required for a tenfold increase of the population [8].

Light Microscopy

Live cells were observed on an Olympus BH-2 microscope equipped with Nomarski interference contrast and electronic flash.

Electron Microscopy

Whole mounts for electron microscopy were prepared according to Moestrup and Thomsen [23] and they were stained for 30 min in 2% aq. uranyl acetate followed by a brief rinse (30 sec) in distilled water.

Material for thin sectioning was fixed using two different protocols (Fix. 1 and 2):

Fix. 1. Cells were fixed for 1 h in 2% cold glutaraldehyde in 0.1 M cacodylate buffer (pH 7.7) containing 0.5 M sucrose. The material was rinsed for 10 min in cold buffer of decreasing sucrose concentration (from 0.5 M sucrose to pure buffer in steps of 0.1 M sucrose). The cells were postfixed for 1 h in cold 1% osmium tetroxide in 0.1 M cacodylate buffer and rinsed twice in pure buffer for 10 min. Dehydration was in an ethanol series: 15%, 30%, 50%, 70%, 96% and 2 × absolute ethanol, 15 min in each change. The material was heated to room temperature when in absolute alcohol. Dehydration was completed by 2 × 5 min in propylene oxide (PO). The material was stored in a refrigerator overnight in PO and Spurr's resin (ratio 1 : 1). This mixture was then replaced with fresh Spurr's resin and 6 h later transferred to an embedding dish for polymerization at 70 °C overnight.

Fix. 2. Cells were fixed for 1 h in 1% osmium tetroxide in 0.1 M cacodylate buffer. The material was then rinsed twice in buffer for 10 min. The subsequent dehydration and embedding schedules were those described for Fix. 1.

Thin sections were cut with a diamond knife on a LKB ULTRATOME V and mounted on 100 mesh grids. Sections were stained for 30 min in 2% uranyl acetate, rinsed, and stained in Reynold's lead citrate for 20 min. Sectioned cells were examined on a JEOL-100 SX electron microscope at the Institut for Sporeplanter.

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

Results

P. cyclotreta Daugbjerg sp. nov.

Etymology: from Greek cyclos (circle) and tretos (perforated).

Holotype: Fig. 9.

Diagnosis

Cellula 15–18 μm longa, ad apicem 7–8 μm lata, ex apice visa quadrata lateribus paulum cavis, ex antapice visa elliptica vel orbicularis. Quattuor flagella cellulae paene aequilonga e fovea apicali orta, inter quietem juxta cellulam retroflexa. Vesiculae obscurae haud raro in ultimo antapice sitae membranas foventes globulares inanes sese induentes. Chloroplastus laete viridis, poculiformis, in quattuor lobos anticos divisus, in parte basali pyrenoides unicum eccentricum fovens testa amylea continua urniformi circumdatum, thylacoidibus geminatis per ostium penetratum. Stigma anterius bipartitum, in marginibus contiguis binorum loborum chloroplasti situm, guttulis substantiae carotenoidis in duo strata dispositis, nullis thylacoidibus intermissis. Squamae corpus tegentes tetramorphae, intimae parvae solum in fovea flagellari praesentes, intermediae capsiformes laminis basalibus striatis 6–8 foraminibus parvis orbicularibus in parte media pertusis, eis et in fovea flagellorum et alibi squamae intermixtae vestigiiformes in medio constrictae, externae coroniformes. Flagellis squamae intimae parvae pentagonae, superpositae squamae limuliformes et squamae piliformes.

Cells 15–18 μm long and 7–8 μm wide at the apical end. The apical part is square, slightly indented anteriorly while the antapical end is elliptical to rounded. Four flagella emerge from an apical depression. The flagella almost equal the cell in length. They are directed downwards along the cell when the cell has settled. The posterior tail may contain darkly coloured vesicles with concentric membranes. The light green chloroplast is cup-shaped and divided into four lobes. The basal part of the chloroplast possesses an eccentric pyrenoid, invaded anteriorly by pairs of thylakoids and surrounded by a single-lobed starch grain. Two anterior eyespots are located in adjacent chloroplast lobes. Each eyespot consists of two rows of carotenoid droplets, not separated by thylakoids.

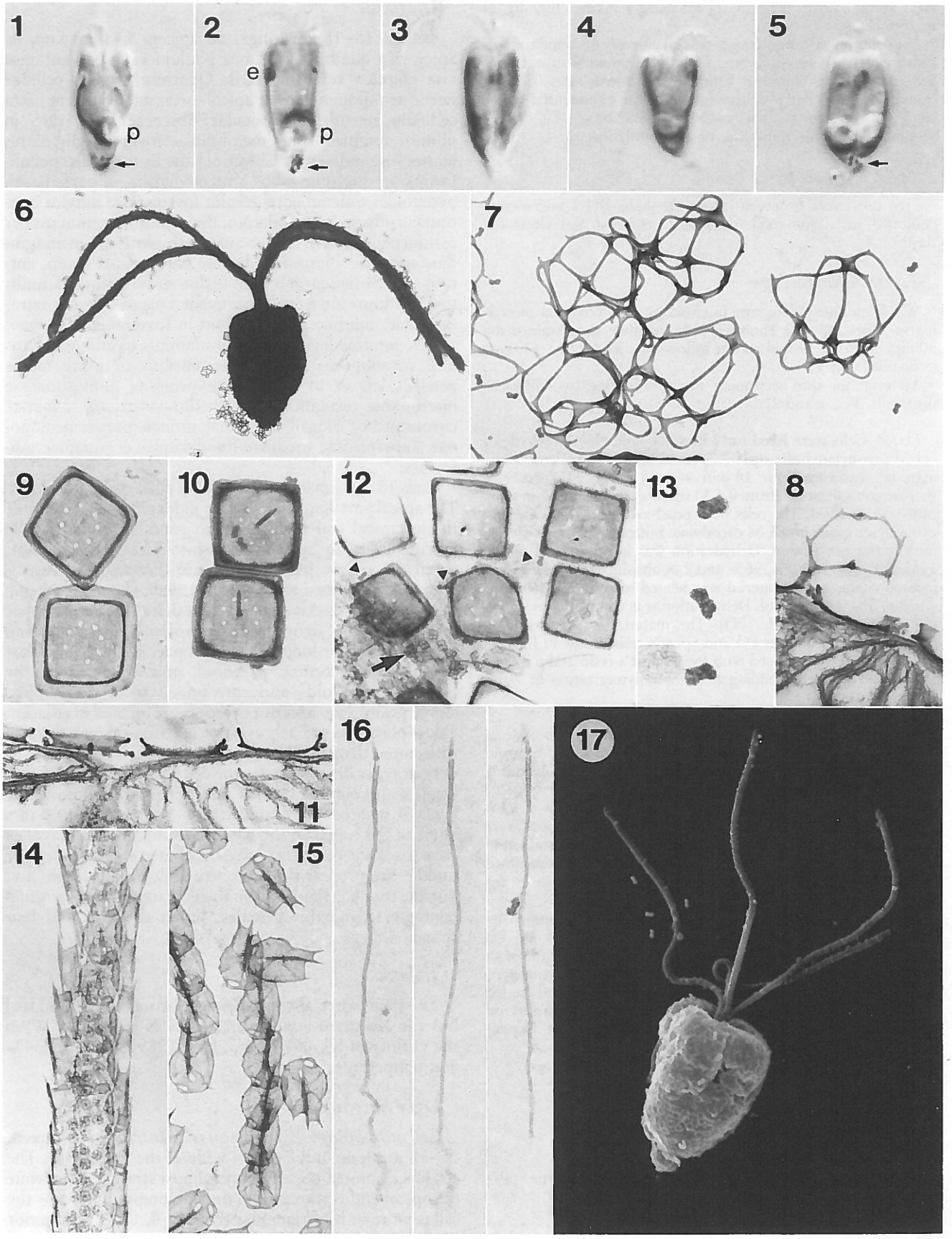
Four types of scales cover the cell body: an underlayer of small scales confined to the flagellar pit, an intermediate layer of striated box scales with 6–8 holes arranged in a circle in the center of the base plate and an outer layer of crown scales. Footprint scales with a constriction at the middle are present between box scales both within and outside the flagellar pit. The flagella are covered by small pentagonal underlayer scales, limuloid scales and hair shaped scales.

Habitat

The type material was collected through leads and seal holes in Northern Foxe Basin (69°23′N and 81°45′W) in the vicinity of Igloolik Island, June 1989. Salinity c. 34‰ and temperature c. −1.5 °C.

Light Microscopy

Cell morphology. Pyramimonas cyclotreta is light green, 15–18 μm long and 7–8 μm wide at the apical end. The cell has a pointed posterior and almost straight sides while the apical end is truncate. In optical longitudinal view the cell is more or less triangular (Figs. 1–4, 17). The anterior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part is square and slightly indented. Four flagella are inserted in a c. 2–3 μm deep anterior depression, the flagellar pit (Fig. 1). The flagella approximately equal the cell in length. When the cell has settled the flagella lie along the sides of the cell (Figs. 1–5). The shape of the posterior end of the cell varies, even in cells from the same clone. Some cells carry a number of darkly stained vesicles (Figs. 1–2, 5, arrows) while other cells lack the vesicles (Fig. 4). In the former the posterior end of the chloroplast is located c. 3 μm from the antapical end of the cell (Figs. 1–2), while the chloroplast continues to the posterior end in cells without the vesicles (Fig. 4). Posteriorly the cup-shaped chloroplast contains an eccentric pyrenoid surrounded by a single-lobed starch grain (Figs. 1–2). A dividing cell with two pyrenoids is illustrated by Fig. 5. The eyespot is double, each eyespot positioned anteriorly in adjoining chloroplast lobes (Fig. 3), opposite the pyrenoid (Fig. 2). A diagrammatic representation of the cell is illustrated in Fig. 34.

Cell movement. Cells of *P. cyclotreta* may swim for long periods of time in almost straight lines. They stop suddenly and attach by their flagella to the slide. After a brief period swimming is resumed in a new direction.

Electron Microscopy

The scaly covering. Pyramimonas cyclotreta possesses seven types of scales (some of which may be seen in Fig. 6). The periplast contains four types while the flagella are covered by three types. Four types of scales have been reconstructed based on whole mounts and sectioned material (Figs. 35–38).

The flagella are covered by pentagonal underlayer scales, 40–45 nm wide (Fig. 14). The scales show the arrangement which characterises the genus [10]. Limuloid scales surround the pentagonal underlayer scales in nine non-helical rows (Fig. 14). A limuloid scale is c. 310 nm long and c. 200 nm wide. Four curved ribs radiate to the periphery and two ribs run along the sides. Two perforations of different size are present posteriorly. The central slightly raised longitudinal spine extends c. 40 nm beyond the anterior part of the scale. One small spine is present on each side of the spine. Anteriorly one side of a limuloid scale is longer than the other side (Fig. 15).

Two almost opposite rows of hair shaped scales are attached to each flagellum at the two pairs of "Melkonian's rows". A hair scale is c. 1.2 μm long and divided into three morphological regions (Fig. 16). The proximal part forms a triangle, c. 60 nm long and c. 15 nm wide. The midpiece is c. 140 nm long and c. 15 nm wide, gradually decreasing in width to the distal part. The latter is c. 1 μm long and c. 11 nm wide (Fig. 16).

The cell membrane of the flagellar pit is covered by small underlayer body scales (Fig. 12) similar to type 1 sensu McFadden et al. [21]. The scales are square, c. 45 nm wide.

The cell body is covered throughout by box scales (Figs. 9–12). Each box scale is c. 360 nm wide and c. 75 nm high with solid walls (Figs. 11–12). The base is ornamented with striations in four groups, each group composed of 5–6 stripes (Figs. 9–10). 6–8 perforations form a circle in the middle of the base (Figs. 9–11). Some variation exists in the morphology of the box scales: a central vertical spine (c. 120 nm long) is present in some cells (Fig. 10), but lacking in others (Fig. 9). Whole mounts of both wild material and cultured cells have revealed both variants of box scales on the same cell (not shown).

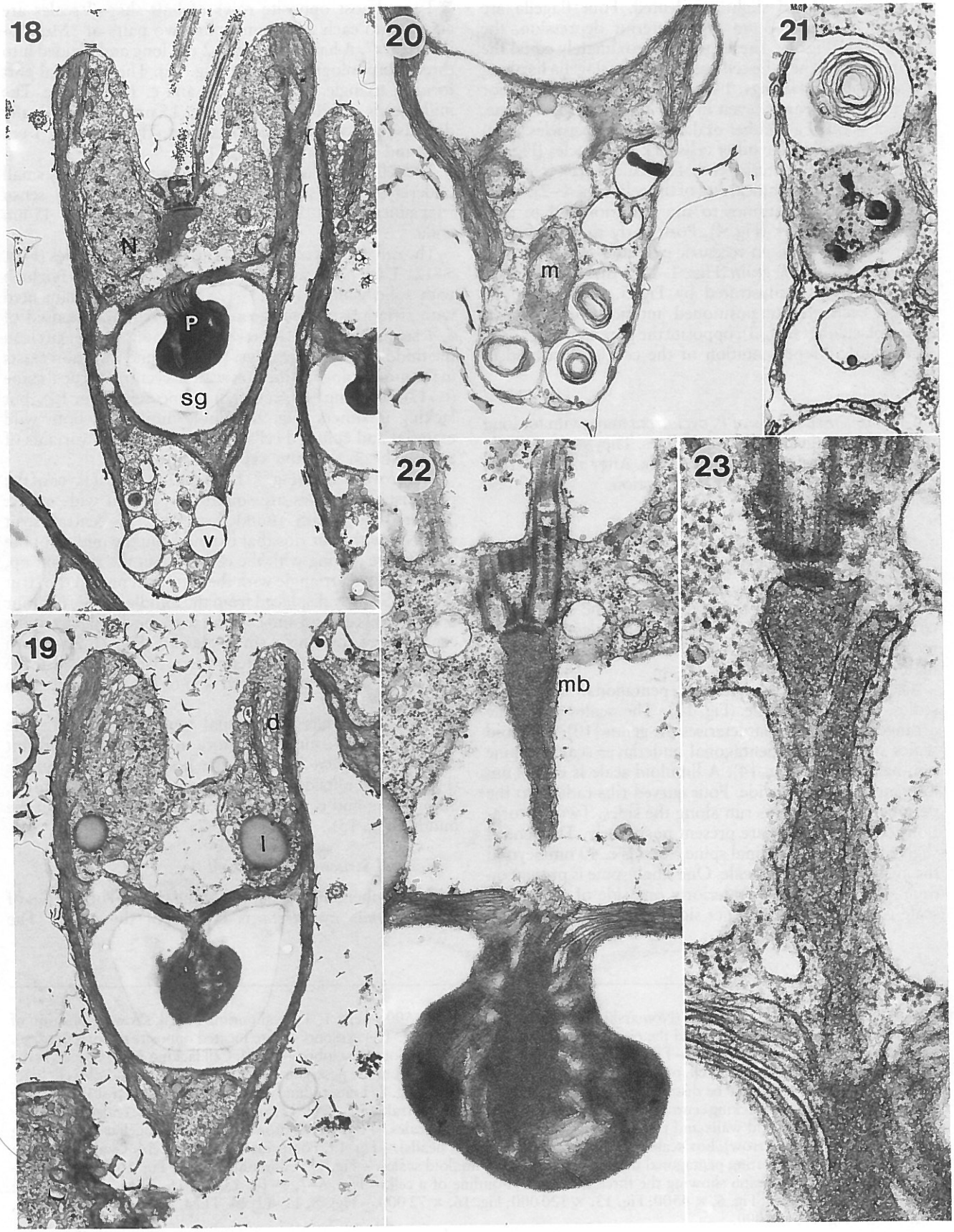
The crown scales (Fig. 7) form the outermost layer of the periplast. The scales are square (c. 360 nm wide and c. 360 nm high) with rounded corners. A central strut connects with four ribs that extend from the middle of the rim. Before joining with the central strut the ribs split up, each forming a triangle with the proximal part of the strut (Fig. 7). Slightly displaced from the middle of the rim, four upright arms extend and connect with the distal part of the central strut forming a small plateau (Fig. 7). Each arm possesses two spines of different size, the smallest near the top of the scale (Fig. 8). Each side of the rim carries two small spines.

P. cyclotreta has an unusual type of footprint scale (Fig. 13) not encountered before in the genus. Footprint scales are present between the box scales both inside (Fig. 12) and outside the flagellar pit (Fig. 11). They are c. 50 nm long and c. 30 nm wide with a constriction in the middle (Fig. 13).

General Structure of the Cell

The number and disposition of the internal organelles of *Pyramimonas cyclotreta* is typical of the genus. The

◀ Figs. 1–5. Live cells of *P. cyclotreta* (Nomarski interference contrast), × 1500. – Fig. 1. Typical outline of cell, showing position of flagella, chloroplast, pyrenoid (p) and the posterior vesicles (arrow). – Fig. 2. The eyespots (e) are located opposite the pyrenoid (p). Arrow pointing to posterior vesicles. – Fig. 3. Eyespots in closely appressed chloroplast lobes. – Fig. 4. Cell lacking dense vesicles at the antapical end. – Fig. 5. Dividing cell, pyrenoid division complete. Note vesicles at the posterior end (arrow). – Fig. 6. Whole cell, body scales and flagellar scales may be distinguished. – Fig. 7. Crown scales. – Fig. 8. Longitudinal section through crown scale and box scales. – Fig. 9. Box scales lacking central spine. – Fig. 10. Box scales with central spine. – Fig. 11. Section through box scales outside the flagellar pit. Note the solid walls and the footprint scales between box scales. – Fig. 12. Section through the flagellar pit, showing underlayer body scales (arrow), box scales and footprint scales (arrow heads). – Fig. 13. Footprint scales. – Fig. 14. Glancing section through flagellum, illustrating pentagonal underlayer scales and limuloid scales. – Fig. 15. Limuloid scales. – Fig. 16. Hair scales. – Fig. 17. Scanning micrograph showing the three dimensional outline of a cell. – Figs. 6–7, 9–10, 13, 15–16. TEM, stained whole mounts, × 42 000, except Fig. 6, × 4500; Fig. 13, × 120 000; Fig. 16, × 72 000. – Figs. 8, 11–12, 14. TEM, thin sectioned material, × 42 000. – Fig. 17, × 4000.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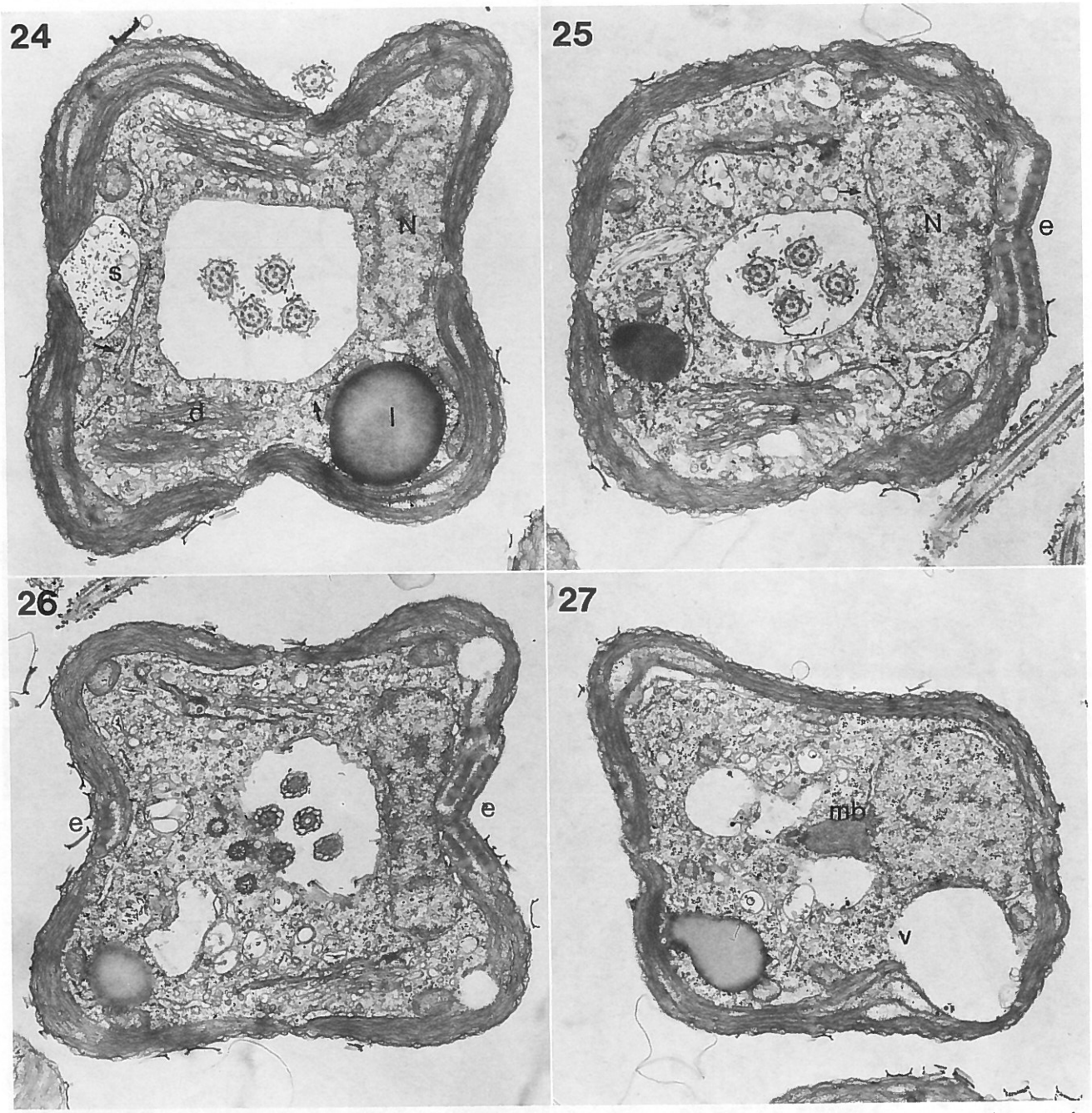
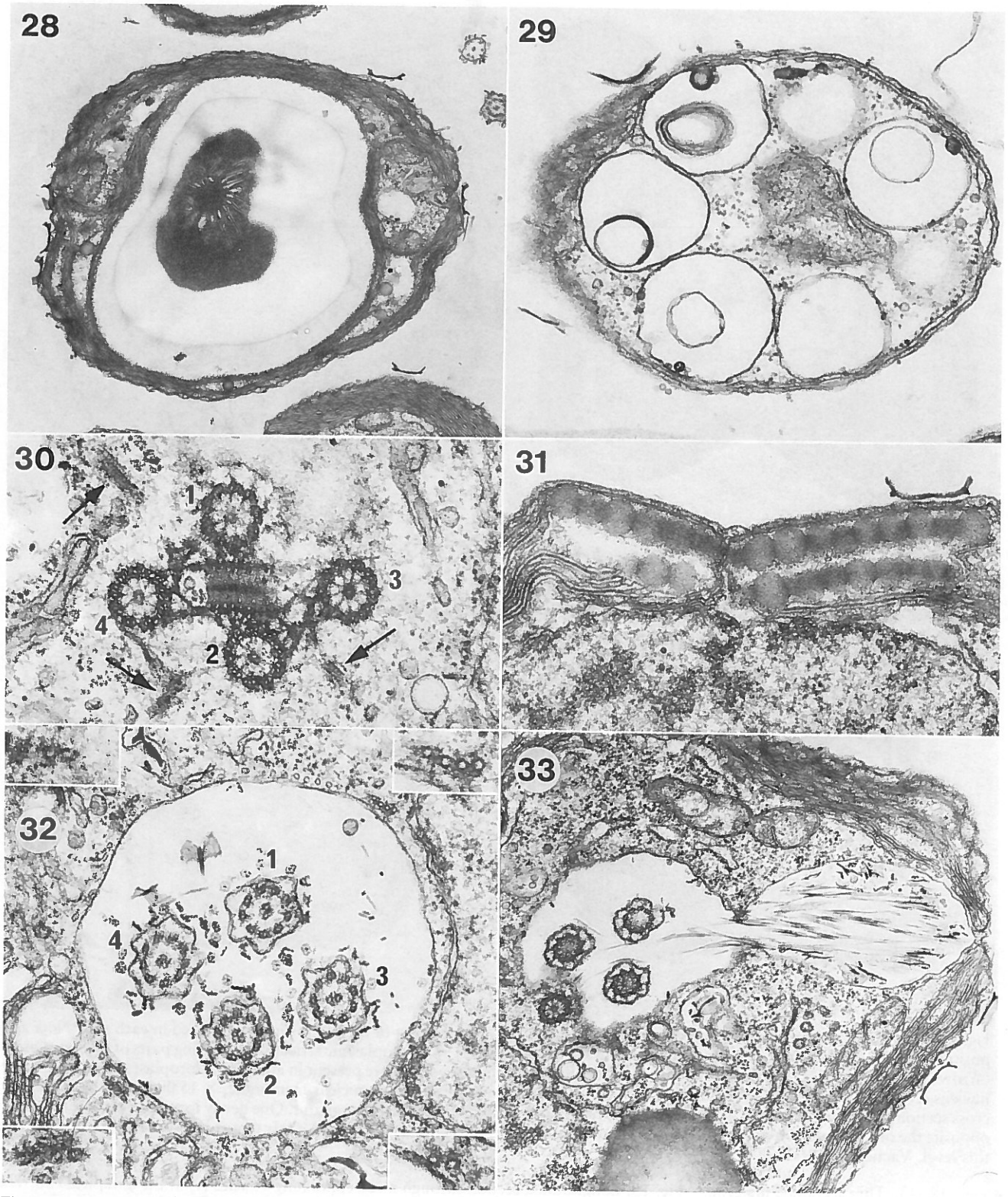


Figs. 24–27. Transverse sections at different levels. – Fig. 24. The anterior part of the cell is slightly indented in each side. Note also position of nucleus (N), scale reservoir (s), dictyosomes (d), lipid droplets and endoplasmic reticulum encircling parts of the flagellar pit (arrows), × 15 000. – Fig. 25. The proximal part of the flagellar pit. Eyespots (e) are present in adjacent chloroplast lobes, close to the nucleus (N). The endoplasmic reticulum (arrows) forms an extension of the outer nuclear membrane, × 15 000. – Fig. 26. Oblique cross section of dividing cell with four newly formed basal bodies surrounding the original four. One newly formed eyespot (e) is visible opposite the old eyespots (e), × 13 000. – Fig. 27. Section at the level of the microbody (mb). Only two chloroplast lobes are present at this level. Vacuole (v), × 12 000.

◀ Figs. 18–23. Thin sectioned material. – Fig. 18. Longitudinal section through a cell containing vesicles (v) at the antapical end. The nucleus (N) and the pyrenoid (p) surrounded by a single-lobed starch grain (sg) are also visible, × 8500. – Fig. 19. Longitudinal section through a cell lacking posterior vesicles. Dictyosomes (d) and lipid droplets (l) are also visible, × 9500. – Fig. 20. Antapical end showing vesicles with concentric pieces of membrane. Mitochondrion (m), × 22 500. – Fig. 21. Similar vesicles, probably different stages in the formation of the concentric membranous material, × 40 000. – Fig. 22. Median section through basal bodies, microbody (mb) and pyrenoid. Note paired thylakoids penetrating the anterior part of the pyrenoid and the distinct dense plate between the basal bodies and the microbody, × 24 000. – Fig. 23. Branching microbody extending to the anterior end of the pyrenoid, × 60 000.



Figs. 28–33. Details of *P. cyclotreta*. – Fig. 28. Cross section at the posterior end showing the pyrenoid surrounded by a single starch grain, × 14 500. – Fig. 29. Cross section through the antapical end showing the vesicles and a centrally positioned mitochondrion. Compare with Figs. 20, 21, × 36 000. – Fig. 30. The four basal bodies form a rhombic figure. Three flagellar roots are also visible (arrows), × 48 000. – Fig. 31. Each eyespot consists of two tiers of carotenoid droplets not separated by thylakoids, × 36 000. – Fig. 32. The flagellar pit surrounded by microtubules and flagellar roots, × 40 000. Insets illustrate the four roots, × 90 000. – Fig. 33. Scale reservoir releasing scales to the flagellar pit via a short duct, × 24 000.

longitudinal section in Fig. 18 shows the characteristic tapering seen in some cells and the location of the major organelles. Transverse sections through the anterior end of the cell show a slight indentation in the middle of each side, i.e. each side is concave (Fig. 24). However, a few sections deeper, but still in the flagellar pit, the cell becomes square (Fig. 25). The square outline is also visible in the scanning electron micrograph (Fig. 17). The cell becomes rounded posteriorly (Figs. 28–29).

The chloroplast closely adjoins the plasmalemma. It extends to the apical end (Figs. 18–19) and to the vesicle containing part of the antapical end (Fig. 18). Figure 19 illustrates a longitudinal section through a cell which lacks the dense vesicles. The chloroplast here reaches the antapical end of the cell (compare with Fig. 18). Anteriorly the chloroplast is divided into four lobes fusing to form two large lobes further down (compare Figs. 24–26 and Fig. 27).

The eccentric pyrenoid occupies the posterior part of the chloroplast (Fig. 18). It is surrounded by a single-lobed starch grain and invaded anteriorly by paired thylakoids (Fig. 22). The pyrenoid equals type I of Inouye et al. [17].

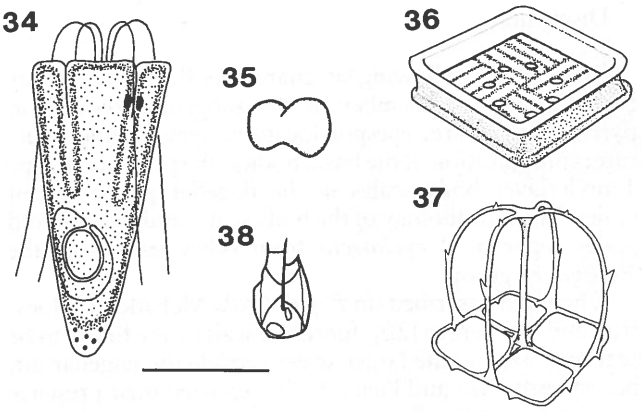
The two eyespots are located in adjacent chloroplast lobes close to the nucleus and near the base of the flagellar pit (Figs. 25–26). Each stigma consists of two rows of carotenoid droplets, not separated by pairs of thylakoids (Fig. 31).

The nucleus and the scale reservoir are located on opposite sides of the flagellar pit (Figs. 24–25). The scale reservoir mostly contains flagellar scales. It opens into the flagellar pit via a short duct (Fig. 33). The flagellar pit is supported by 90–100 microtubules.

The Golgi apparatus consists of two anteriorly positioned dictyosomes (Figs. 24–25). A dictyosome has 10–15 cisternae and scale morphogenesis takes place here. Endoplasmic reticulum (ER) is present around parts of the flagellar pit (Fig. 24, arrows). ER also forms an extension of the outer nuclear membrane (Fig. 25, arrows).

The darkly coloured vesicles at the posterior end of the cell contain concentric membranes or appear empty (Fig. 20). The vacuole in Fig. 21, center, probably represents an early stage in the formation of concentric membranous material as seen in the uppermost vesicle. The vesicles surround a mitochondrion (Fig. 29) also seen in Figs. 19–20.

The flagellar apparatus has been examined in some detail. The basal bodies are arranged in a rhombic configuration (Fig. 30), and numbering of individual basal bodies is according to Moestrup and Hori [24]. The arrangement of the basal bodies equals type I of Inouye et al. [17]. Basal body 1 and 2 are connected by a rectangular synistosome (Fig. 30) c. 340 nm long, c. 190 nm wide and c. 230 nm high. The four microtubular roots of the flagellar apparatus forms a 4 2 4 2 system (Fig. 32). The right roots (1 d and 2 d) contain two microtubules, respectively, and the left roots (1 s and 2 s) four microtubules, respectively. The combination of the flagellar roots is typical of species assigned to the subgenus *Vestigifera*. The microbody extends from the anterior end of the pyrenoid



Figs. 34–38. Diagrammatic representation of *Pyramimonas cyclotreta* and four scale types. – Fig. 34. Optical longitudinal section showing position of flagella in a settled cell, eyespots, pyrenoid and posterior vesicles. – Fig. 35. Footprint scale. – Fig. 36. Box scale lacking central spine. – Fig. 37. Crown scale. – Fig. 38. Limuloid scale. Scale bar = 8 µm (Fig. 34); 75 nm (Fig. 35); 340 nm (Figs. 36–37); 250 nm (Fig. 38).

(Fig. 23) and attaches to the proximal part of the basal bodies (Fig. 22). The attachment is marked by a plate of electron dense material, probably a species-specific character of *P. cyclotreta*.

Temperature Study

No growth of *P. cyclotreta* occurred at temperatures above 10 °C (Fig. 39). The average growth rate for cells in the batch-cultures grown at 2.4–6.6 °C was 0.42 divisions/day (0.39–0.46) (Fig. 39) giving a generation time of c. 2.4 days (2.2–2.6). Dividing cells were still in the exponential phase when the experiment was stopped after 22 days.

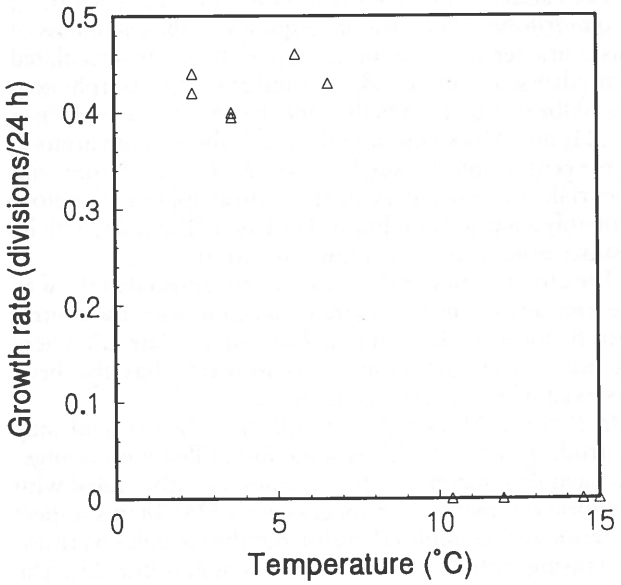


Fig. 39. Growth rate of *P. cyclotreta* at different temperatures. All cultures are in the exponential phase of growth.

Discussion

Based on the following six characters *P. cyclotreta* may be assigned as a member of the subgenus *Vestigifera:* pyrenoid structure; eyespot location; synistosome structure; configuration of the basal bodies; the presence of type 1 underlayer body scales in the flagellar pit; footprint scales. The morphology of the body scales and the limuloid scales separate *P. cyclotreta* from other species of the *Vestigifera* group.

When first described (in *P. gelidicola* McFadden, Moestrup and Wetherbee [20]) footprint scales were found to be scattered around the larger scales outside the flagellar pit, but recently Sym and Pienaar [31] reported their presence also inside the flagellar pit in *P. norrisii* Sym and Pienaar.

The footprint scales of *P. cyclotreta* are c. 30 nm shorter than those of *P. gelidicola* [20] and show a central constriction. The footprint scales of *P. gelidicola* are more or less clubshaped.

The box scales of *P. cyclotreta* bear some resemblance to the box scales of *P. disomata* Butcher [21], *P. gelidicola* [20], *P. gorlestonae* Pennick [29] and *P. spinifera* Pennick [30]. In all species the base is striated in four sets of parallel lines. Each wall-side is perforated by 7 holes in *P. gelidicola,* 5 in *P. gorlestonae,* 5–7 in *P. spinifera* while they are solid in *P. cyclotreta* and *P. disomata.* However, four perforations are present at the base margins in some box scales of *P. disomata* [21]. None of these species have a base plate with perforations forming a ring as observed for *P. cyclotreta.*

The variation in the occurrence of a slender central spine has also been noted in box scales of *P. disomata* [21] and in *Pyramimonas quadrifolia* sp. ined, a species from Arctic Canada (Daugbjerg and Moestrup, in prep.). No such variation has been reported in species with a central boss such as *P. moestrupii* McFadden [21], *P. norrisii* [31], *P. obovata* Carter [27] and *P. olivacea* Carter [22].

The variation noted in the central spine of *P. cyclotreta, P. quadrifolia* and *P. disomata* questions the usefulness of this character as a taxonomic marker. It may be speculated that adverse culture conditions influence the morphogenesis of the box scales. On the other hand both McFadden et al. [21] and Moestrup (unpublished) observed a variation of the central spine in single cells of *P. disomata* from wild material. The variability of the central spine is therefore probably a natural condition. The base of the scale, with its distinct ornamentation, shows no variation.

The crown scales of *P. cyclotreta* are unusual in the way the four arms split up before connecting with the central strut to form small triangles. The distal "plateau" where the four upright arms join the central strut has also been observed in *P. quadrifolia* sp. ined.

In *P. mitra* Moestrup and Hill the posterior end may protrude as a tail, which is sometimes filled with orange-red vacuolate material. The vacuoles are either filled with membranous material or appear empty [25]. In this respect *P. cyclotreta* resembles *P. mitra,* but the vacuoles were not seen fusing with the cell membrane as in *P. mitra* [25]. The fusion of the membranous material with the cell membrane in *P. mitra* indicates membrane recycling.

In *P. longicauda* Van Meel emend. Inouye and Chihara the long tail-like extension held a number of dark red granules or appeared colourless [21]. Sectioned cells of *P. longicauda* shown in Inouye et al. [16] did not expose the content of the vacuoles. A number of red granules have also been observed in cells of *P. disomata* [21, Moestrup, unpublished]. Stellate formed vacuoles which appear empty are seen at the posterior end of *P. norrisii* [31].

While it has been possible in some cases to link electron microscopical studies of species of *Pyramimonas* with species described previously by light microscopy only (e.g., *P. amylifera, P. disomata, P. grossii, P. longicauda, P. nansenii, P. olivacea, P. orientalis, P. tetrarhynchus*), the literature still holds a number of species only seen by light microscopists. One of these, *P. torta* Conrad and Kufferath [3], shows some resemblance to *P. cyclotreta.* Both species have a pointed posterior and the cell is almost triangular. *P. torta* is 8–11 μm long and 4–5 μm wide, about half the length and width of *P. cyclotreta.* The tail of *P. torta* is colourless and twisted, the character used in naming the species. In *P. cyclotreta* the tail of some cells is colourless but any twisting was never seen. The chloroplast of *P. torta* is divided into four lobes clearly separated by cytoplasm as illustrated in the three drawings of the cell (Pl. VIII, Fig. 5A, B and C by Conrad and Kufferath [3]). A single eyespot is positioned anteriorly in one of the lobes. The chloroplast lobes of *P. cyclotreta* are always closely adjoined and cells possess two eyespots. The way in which the cell holds its flagella is to a certain extent species specific [26]. When settled, the flagella of *P. torta* are not parallel with the cell body as in *P. cyclotreta.* Ecological data are also different. The type locality of *P. torta* (Lillo, Belgium) had a salinity of 4–5‰ and a temperature of 17 °C [3]. Our temperature experiment showed *P. cyclotreta* to be cold stenothermal, not having growth potential at temperatures above 10 °C. In conclusion, we consider *P. torta* and *P. cyclotreta* to be different species based on morphological differences, the higher salinity of the type locality of *P. cyclotreta* (34‰ opposed to 3.4‰) and the results of the temperature study.

Conrad and Kufferath [3] described eight haline species of *Pyramimonas* from Belgian waters and two of these (*P. nanella* and *P. pisum*) have recently been linked with *P. grossii,* a species well characterised electron microscopically [25]. There is an urgent need to sample at the localities used by Conrad and Kufferath and to reinvestigate the many species described by these authors, using modern techniques.

Some of the limiting factors for marine phytoplankton in Northern Foxe Basin are the ice cover, the total darkness during the 3–4 winter months, the low temperature, and the continuous freezing and thawing of the ice, the latter affecting salinity. The temperature of Northern Foxe Basin never rises above 2 °C [3]. The coldest temperature measured by Daugbjerg et al. [4] was −1.5 °C. *Pyramimonas cyclotreta* appears to be well adapted to the temperature conditions in the Northern Foxe Basin, and its temperature response resembles that reported for another new species of *Pyramimonas* found in the area, *P. cyrtoptera* [5].

The effect of temperature on growth rates have only been examined for a few species of *Pyramimonas*. Throndsen [32] studied the temperature response of *P. disomata*, a common and widespread species, and calculated a growth rate of c. 1.5 divisions/day at temperatures of 14 and 22 °C. No growth occurred at 9 and 29 °C. Aken [1] in the larger species *P. pseudoparkeae* Pienaar and Aken found a growth rate of c. 0.28 divisions/day at 25 °C. Growth stopped when the temperature reached 30 °C. The lower temperature limit for *P. pseudoparkeae* was not studied. *P. cyclotreta* and *P. cyrtoptera* only grew at low temperatures and the growth rate was estimated to 0.42 and 0.38 divisions/day, respectively.

Only one species of *Pyramimonas*, *P. grossii*, a widespread species, has been reported previously from Northern Canada (Eastern Baffin Island) [12], but this needs electron microscopical confirmation. *P. grossii* and *P. cirolanae* Pennick [28] cannot be distinguished in the light microscope. Investigations of prasinophytes in the marine waters of arctic Canada are still in their infancy, but the few studies done recently have drawn our attention to the occurrence of an unexpectedly high number of unknown species of *Pyramimonas* in this area.

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Niels Daugbjerg, Institut for Sporeplanter, Øster Farimagsgade 2 D, DK-1353 Copenhagen K, Denmark