Four new species of *Pyramimonas* (Prasinophyceae) from arctic Canada including a light and electron microscopic description of *Pyramimonas quadrifolia* sp. nov.

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A preliminary survey of the marine nanoplankton of Northern Foxe Basin, Canada, based on observations of field material and crude cultures, has revealed nine species of *Pyramimonas*. Only two of these (*P. nansenii* and *P. orientalis*) have been reported previously, *P. nansenii* from Greenland and Denmark and *P. orientalis* from many parts of the world.

In this paper we describe, using light and electron microscopy techniques, a species obtained in culture, *Pyramimonas quadrifolia* sp. nov. *Pyramimonas quadrifolia* is unique in scale morphology and flagellar root structure but otherwise displays characters typical of the subgenus *Vestigifera*. During division of the chloroplast, a structure similar to the "plastid-dividing (PD) ring" was observed.

We also report on three new species known only from EM whole mounts of field material: *Pyramimonas aurita* sp. nov., *Pyramimonas dichotoma* sp. nov. and *Pyramimonas igloolikensis* sp. nov. *Pyramimonas* sp. is only known from its large body scales.

Key words: *Pyramimonas*, Prasinophyceae, ultrastructure, marine nanoplankton, arctic Canada.

Introduction

Among the genera of Prasinophyceae (Chlorophyta), the genus *Pyramimonas* Schmarda is one of the best circumscribed and comprises five subgenera (McFadden, Hill & Wetherbee, 1986, 1987; Hori, Moestrup & Hoffmann, 1993).

Species occur in most marine waters and usually more than one taxon is present; this applies to polar waters (e.g. McFadden, Moestrup & Wetherbee, 1982; Thomsen, 1982; Daugbjerg & Moestrup, 1992a, b), temperate waters (e.g. McFadden, Hill & Wetherbee, 1986; Throndsen, 1969), subtropical waters (e.g. Leadbeater, 1974) and tropical waters (Moestrup, unpublished; Thomsen, unpublished). *Pyramimonas* species do not generally contribute significantly to the total biomass of primary producers. Thus, only a few species are reported to form blooms: *P. lunata* Inouye, Hori & Chihara, *P. propulsa* Moestrup & Hill (as *P.* aff. *amylifera*), *P. disomata* McFadden, Hill and Wetherbee (Inouye, Hori & Chihara, 1983), and *P. nansenii* Braarud (Thomsen, 1988).

Many species of *Pyramimonas* are geographically widespread (e.g. *P. disomata*; *P. grossii* Parke; *P. orientalis* McFadden, Hill & Wetherbee) and these may be considered truly cosmopolitan. Only a few studies have included autecological data but some species are evidently restricted to cold water (Daugbjerg & Moestrup, 1992a, b).

Whole mounts of field material and crude cultures based on water samples collected in Northern Foxe Basin (Canadian Arctic) have shown that at least seven undescribed and two previously described species occur in this area (Daugbjerg & Moestrup, 1992a, b). Here we describe one of the species obtained in culture, *Pyramimonas quadrifolia* sp. nov. We also describe three new species known solely from EM whole mounts of field material (*Pyramimonas aurita* sp. nov., *Pyramimonas dichotoma* sp. nov., *Pyramimonas igloolikensis* sp. nov.). A fourth new species (*Pyramimonas* sp.) is known only from its large body scales.

Materials and methods

The material for this study was collected in the vicinity of Igloolik Island (arctic Canada). Whole mounts of field material were made on location by gravity filtration through 3 μm Millipore filters of 1 l water samples from various depths. For information on the area studied and vertical profiles of temperature and salinity, see Daugbjerg & Moestrup (1992a).

Clonal cultures of *P. quadrifolia* were established by micropipetting from enriched crude cultures. The cultures are maintained at the Institut for Sporeplanter in modified Erdschreiber medium (30‰ S) (Throndsen, 1978) at 4°C, photon flux density 13 μmol m^{-2} sec^{-1}, and a 16:8 h L:D cycle.

Live cells were observed using an Olympus BH-2 microscope equipped with Nomarski interference contrast and electronic flash. Photographs were taken with Kodak technical pan film.

Whole mounts for electron microscopy were shadowcast with gold/palladium according to Moestrup &

Thomsen (1980), or stained for 30 min in 2% aqueous uranyl acetate followed by rinsing for $\frac{1}{2}$ min in distilled water.

Three different fixation protocols (Fix. 1, 2 and 3) were used for material to be thin sectioned.

Fix. 1.—Cells were fixed for 1 h at 4°C 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 decreasing concentrations of sucrose, from 0·5 M sucrose to pure buffer in steps of 0·1 M sucrose. The cells were postfixed for 1 h in 1% cold osmium tetroxide in 0·1 M cacodylate buffer, rinsed in buffer for 10 min, and dehydrated in an ethanol series (15 min in each of cold 15, 30, 50, 70 and 96% alcohol followed by 2× absolute alcohol at room temperature). Following dehydration, cells were treated for two 5 min periods in propylene oxide (PO) and left in a refrigerator overnight in a 1:1 mixture of PO 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 for polymerization at 70°C overnight.

Fix. 2.—Following postomication and a rinse in buffer, the material was dehydrated for 1 h in 2,2-dimethoxypropane (DMP) and then transferred to a 1:1 mixture of DMP and Spurr's resin. Subsequent replacement with fresh Spurr's resin and polymerization is as described for Fix. 1.

Fix. 3.—Fixation was for 1 h in cold 1% osmium tetroxide in 0·1 M cacodylate buffer. The material was then rinsed in buffer for 10 min. Dehydration and embedding protocols were identical to those of Fix. 1.

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

Cells for scanning electron microscopy were fixed for 1 h in 2% glutaraldehyde and postfixed for 1 h in osmium tetroxide. The material was then dehydrated in increasing concentrations of acetone (in steps of 10%) from 10% to 100% acetone. Cells were critical point dried, coated with gold, and examined in a 515 Phillips scanning electron microscope at the Geological Institute, University of Copenhagen.

Results

Pyramimonas quadrifolia Daugbjerg sp. nov.
Etymology: from latin quadrifolia, quadri (four), folium (leaf).

Diagnosis

Cellula 15–16 μm longa, 8–9 μm lata, lateribus parallelis vel paulum convexis, ante truncata, post rotundata vel in caudam mediam parvam protrudens. Flagella quaterna cellula paulo longiora, in fovea apicali inserta, dimidiis partibus apicalibus cellulae sedentis retroflexis. Chloroplastus laete viridis, in quattuor lobos profunde divisus, in parte posteriore pyrenoides eccentricum fovens ante thylacoidibus geminis penetratum, testa amylea continua circumdatum. Stigmata bina in lobis contiguis chloroplastic circiter tertiam partem cellulae infra apicem sita, quidque duo strata guttularum substantiae carotenoidis ostendens nullis thylacoidibus separata. Corpus cellulae in fovea flagellari nihilo praeter squamas parvas alibi subjacentes tectum, ceterum omnino squamis capsuliformibus et squamis coroniformibus. Squamae capsuliformis quodque latus quinque fenestris quadratis perforatum, lamina basalis in media parte quattuor gregulis striarum parallelarum ornata, extra striis brevibus cum lateribus angulos rectos formantibus. Lamina basalis squamae coroniformis quattuor fenestras exteriores magnas ostendens et partem mediam quadratam quattuor foraminibus ellipticis (unde nomen specificum) et quattuor triangulis cum iis alternantibus pertusam. Extra fovea squamae vestigiiformes inter capsiformes praesentes. Flagella squamas parvas pentagonas subjacentes, limuliformes et piliformes superpositas gerentia.

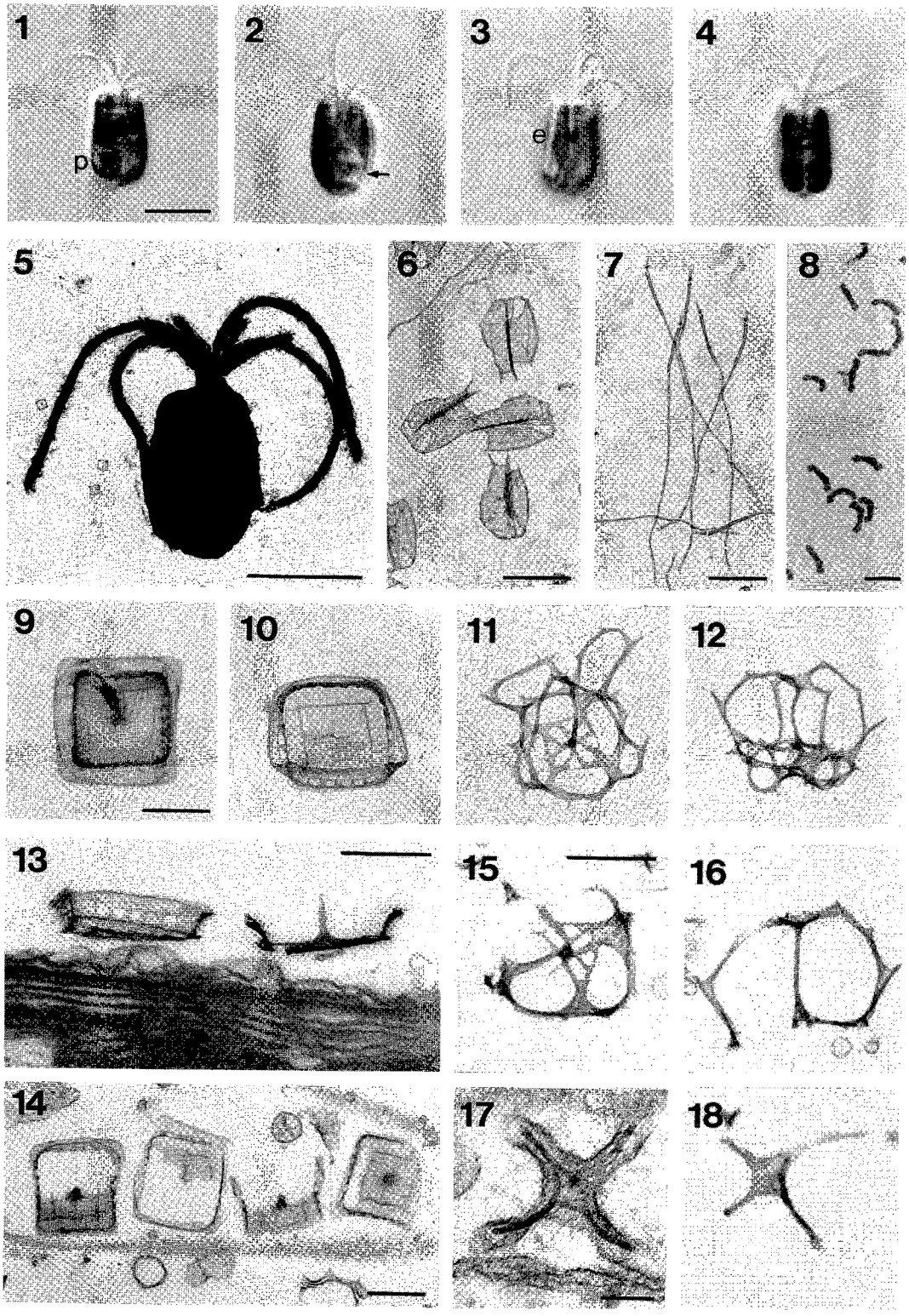
Cells 15–16 μm long and 8–9 μm wide, with parallel to slightly convex sides. The anterior end truncate, the posterior end rounded or protruding into a small median tail. The four flagella somewhat longer than the cell, inserting in an apical depression. When the cell has settled, the distal half of each flagellum bends back. The light green chloroplast is deeply divided into four lobes. The posterior end of the chloroplast with an eccentric pyrenoid penetrated anteriorly by pairs of thylakoids and surrounded by a single-lobed starch grain. Two eyespots situated c. $\frac{1}{3}$ down the cell in closely appressed chloroplast lobes. Each eyespot with two rows of carotenoid droplets, not separated by thylakoids. Cell surface covered by box scales and crown scales. Each side of a box scale with five square perforations. The base plate is divided into an inner part ornamented by stripes in four directions and an outer part ornamented by short stripes at right angles to the sides of the scale. The crown scale base has four peripheral perforations and an inner quadrant perforated alternatingly by four elliptical and four triangular holes. Footprint scales present between box scales outside the flageller pit. The flagellar pit covered by small underlayer scales beneath the box scales. The flagella covered by small pentagonal underlayer scales, limuloid scales and hair shaped scales.

Holotype: Fig. 11.

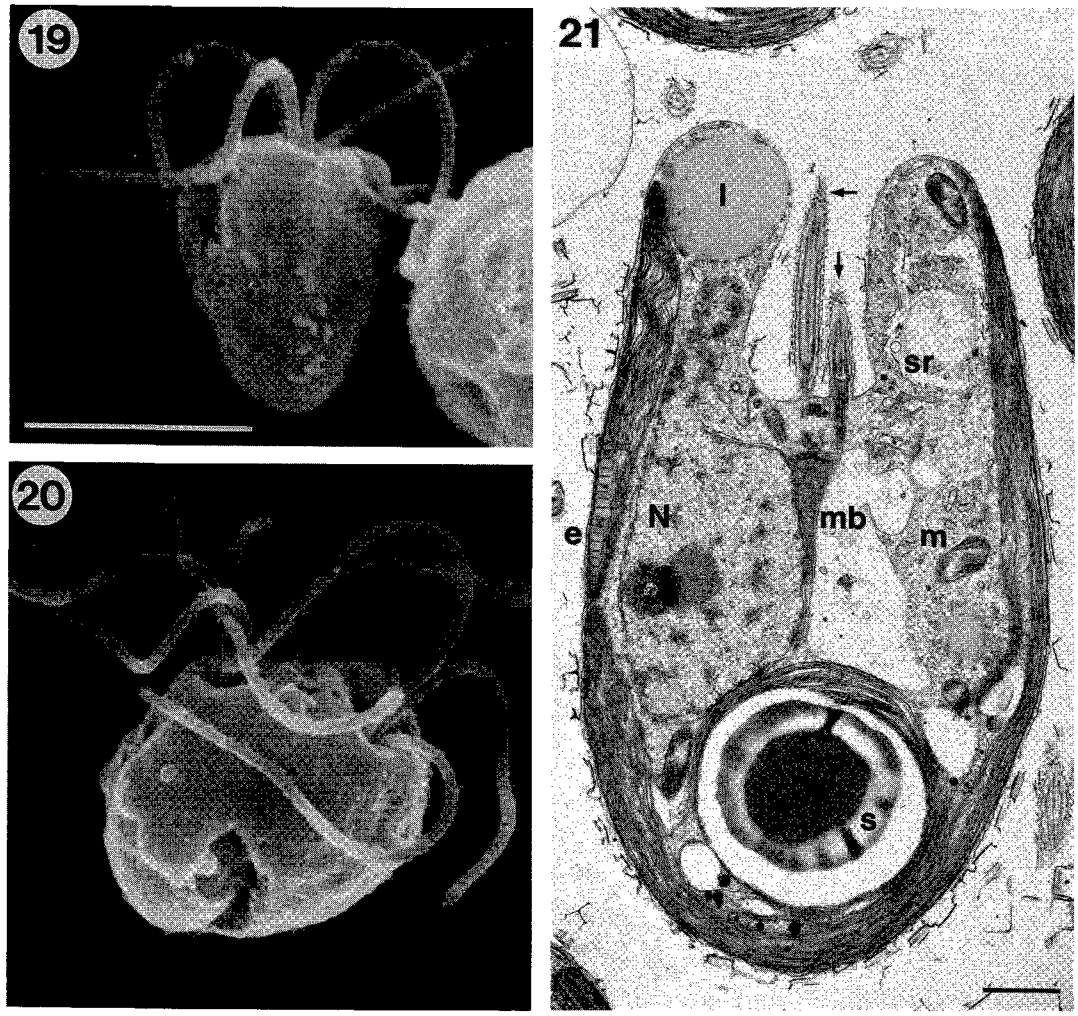
Habitat: The type material was collected from leads and seal holes at Hooper Inlet and Richard's Bay in the vicinity of Igloolik Island (69°23'N and 81°45'W) in June 1989. Salinity was c. 33‰ and temperature c. −1·5°C.

Cell morphology

Cells of *Pyramimonas quadrifolia* are 15–16 μm long and



Figs 1–18. Cell morphology, flagellar and body scales of *Pyramimonas quadrifolia*. Figs 1–4. Nomarski interference contrast. Optical longitudinal sections. Fig. 1. Antapical end protruding into a small tail. An eccentric pyrenoid (p) also visible. Fig. 2. Cell without antapical tail. The pyrenoid is surrounded by a single starch grain (arrow). Fig. 3. Typical position of flagella in a resting cell. Two eyespots (e) are located in closely appressed chloroplast lobes. Fig. 4. Dividing cell, pyrenoid division complete. Figs 5–12. Uranyl acetate stained whole mounts. Fig. 5. Dividing cell, the newly formed flagella not yet at full length. Fig. 6. Limuloid scales. Fig. 7. Hair scales. Fig. 8. Footprint scales. Fig. 9. Box scale with central spine. Fig. 10. Box scale lacking a central spine. Figs 11–12. Crown scales. Figs 13–18. Sections of the large body scales. Fig. 13. Longitudinal section through box scales. Fig. 14. Glancing section through box scales. Fig. 15. Cross section through the base plate of a crown scale. Fig. 16. Longitudinal section through a crown scale. Figs 17–18. Sections showing the distal plateau in an immature scale in a dictyosome and in a mature scale, respectively. Scale bar=10 μm (Figs 1–4); 5 μm (Fig. 5); 250 nm (Figs 6–7, 9–14); 100 nm (Figs 8, 17–18).



Figs 19–21. Scanning (Figs 19–20) and transmission (Fig. 21) electron microscopy of *Pyramimonas quadrifolia*. Fig. 19. Three dimensional appearance, note position of flagella (cf. Fig. 3). A few box scales with a central spine are also visible. Fig. 20. Dividing cell. Cell cleavage proceeds from the posterior end and from the area between the flagellar pits. Fig. 21. Longitudinal section showing eyespot (e); lipid droplet (l); mitochondrion (m); nucleus (N); microbody (mb); pentagonal flagellar scales (arrows); pyrenoid (p); starch grain (s); scale reservoir (sr). Scale bar=5 μm (Figs 19–20); 1 μm (Fig. 21).

8–9 μm wide. The cell possesses almost parallel (Fig. 1) or slightly convex sides (Fig. 2). The apical end is truncate (Figs 1, 3) while the shape of the antapical end varies even in cells from the same clone. It may be rounded (Fig. 2) while in other cells it protrudes into a small median tail (Fig. 1) which appears empty. The four flagella emerge from a flagellar pit, 3–4 μm deep (Fig. 1). They are somewhat longer than the cell and the distal half of each flagellum bends backwards when the cell has settled (Fig. 3). The light green chloroplast is divided anteriorly into four distinct lobes and possesses an eccentric pyrenoid at the posterior end (Fig. 1). The pyrenoid is surrounded by a single starch grain (Fig. 2). A dividing cell with two pyrenoids is illustrated in Fig. 4. Two eyespots are positioned in adjacent chloroplast lobes c. $\frac{1}{3}$ down the cell (Fig. 3), in the side opposite the pyrenoid. A diagrammatic representation of the cell is illustrated in Fig. 49. The three dimensional appearance of the cell and the position of the flagella is shown well in scanning micrographs (Fig. 19). A dividing cell is shown in Fig. 20, the new flagellar pit with four emerging flagella has been formed. The cleavage proceeds from both the anterior and the posterior ends.

Cell movement

Under the light microscope, cells may swim for several minutes before suddenly settling and attaching to the slide by the flagella. They may resume movement in a new direction. Cells swim in an almost straight line or in a slightly curved path; they never swim backwards.

The scaly covering

The cell body of *P. quadrifolia* is covered by four types of scales (some of which are visible in Fig. 5) and each flagellum by three types. Five types of scales have been reconstructed based on whole mounts and sectioned material (Figs 50–54).

The innermost part of the periplast is composed of small underlayer scales c. 40–45 nm wide identical to type 1 sensu McFadden, Hill & Wetherbee (1986). They are restricted to the flagellar pit.

Box scales cover the underlayer scales of the flagellar pit and the plasmalemma outside the pit. Each box scale is c. 375 nm wide, the sides are c. 100 nm high. Each side possesses five square perforations (Figs 10, 13). The central part of the base is striated in four directions. This part is surrounded by four slightly more prominent lines forming an inner square which measures c. 250 nm on each side. C. 13 short stripes are present between and at right angles to the inner square and the outer margin (Figs 9–10, 14). A spine (c. 115 nm high) is present in some box scales (Figs 9, 13) but lacking in others (Fig. 10).

The crown scales form the outermost layer of the periplast and cover the entire cell surface. The base of each scale is square with rounded corners. A crown scale is c. 375 nm wide and c. 360 nm high (Figs 11–12). The base is reticulate. It comprises four large lateral perforations and an inner square connected to the rim. The inner square is perforated by alternate elliptical and triangular holes (four of each) (Figs 11, 15). Four upright arms emanate from near the corners of the rim and join with a central vertical strut to form a small distal plateau (Figs 12, 17–18). Three spines of different sizes are present on each of the uprights. The spine closest to the base is larger than the two distal spines (Figs 12, 16). The basal rim bears a spine at each corner and one at the middle of each side (Fig. 11).

Footprint scales are present between the box scales outside the flagellar pit. They are c. 90 nm long and c. 20 nm wide (Fig. 8), clubshaped and identical to those described for *P. gelidicola* McFadden, Moestrup & Wetherbee (McFadden, Moestrup & Wetherbee, 1982).

The flagella are covered by pentagonal underlayer scales (not shown). One side is very short, c. 17 nm, while the others are c. 50 nm wide. The scales are arranged in the way typical for the genus (Hori & Moestrup, 1987).

Limuloid scales cover the underlayer flagellar scales in nine longitudinal rows. The scales are c. 325 nm long and c. 210 nm wide (Fig. 6). They are ornamented with c. eight ribs, and three perforations of different size are present at the antapical end. A small apical spine is present on each side of the slightly raised central spine.

Each flagellum bears two opposite rows of hair shaped scales, each c. 1·3 μm long (Fig. 7). They are identical to those of *Pyramimonas cyclotreta* Daugbjerg (Daugbjerg & Moestrup, 1992b).

General ultrastructure

In general structure *P. quadrifolia* is similar to most other species of the genus.

The four-lobed chloroplast closely adjoins the plasmalemma (Fig. 21). The pyrenoid (Figs 21, 26, 28) is surrounded by a single starch grain and invaded anteriorly by pairs of thylakoids (Figs 33–34). It is similar to type I of Inouye, Hori & Chihara (1985).

During division of the chloroplast a cellular structure similar to the plastid-dividing (PD) ring of the unicellular red alga *Cyanidium caldarium* (Tilden) Geitler (Mita & Kuroiwa, 1988) is observed (Fig. 27, arrows). It is seen as an electron dense deposit on the outer envelope, located in the division furrow. The diameter of the PD ring is c. 400 nm. It disappears after completion of chloroplast division (Fig. 28).

The two eyespots are positioned in closely appressed chloroplast lobes near the nucleus (Figs 21, 24). Each eyespot is composed of two rows of carotenoid droplets. It is not traversed by thylakoids (Fig. 30). In surface view the droplets form hexagonal rows (Fig. 31).

The Golgi apparatus consists of two apically located dictyosomes (Fig. 22). The endoplasmic reticulum is present at the forming face of each dictyosome (Fig. 22) and connected to the outer nuclear membrane (Figs 21–22, 24–25. The nucleus is located in the middle part of the cell, closely associated with the rhizoplast and the microbody system (Fig. 21).

The scale reservoir is situated near the apical end of the cell (Figs 21–22, 24–25). Flagellar scales (rarely body scales) are present in the reservoir. They are released to the flagellar pit via a very short duct (Fig. 32), which opens among the microtubules of the flagellar pit (Fig. 23, top). The aperture of the duct is marked by electron dense material (Fig. 23, arrowhead). The reservoir in Fig. 32 is in the process of releasing scales to the flagellar pit, mostly hair scales. The hair scales are orientated in opposite directions (antiparallel).

The flagellar pit is lined by 100–120 microtubules (Fig. 23) which form part of the microtubular cytoskeleton.

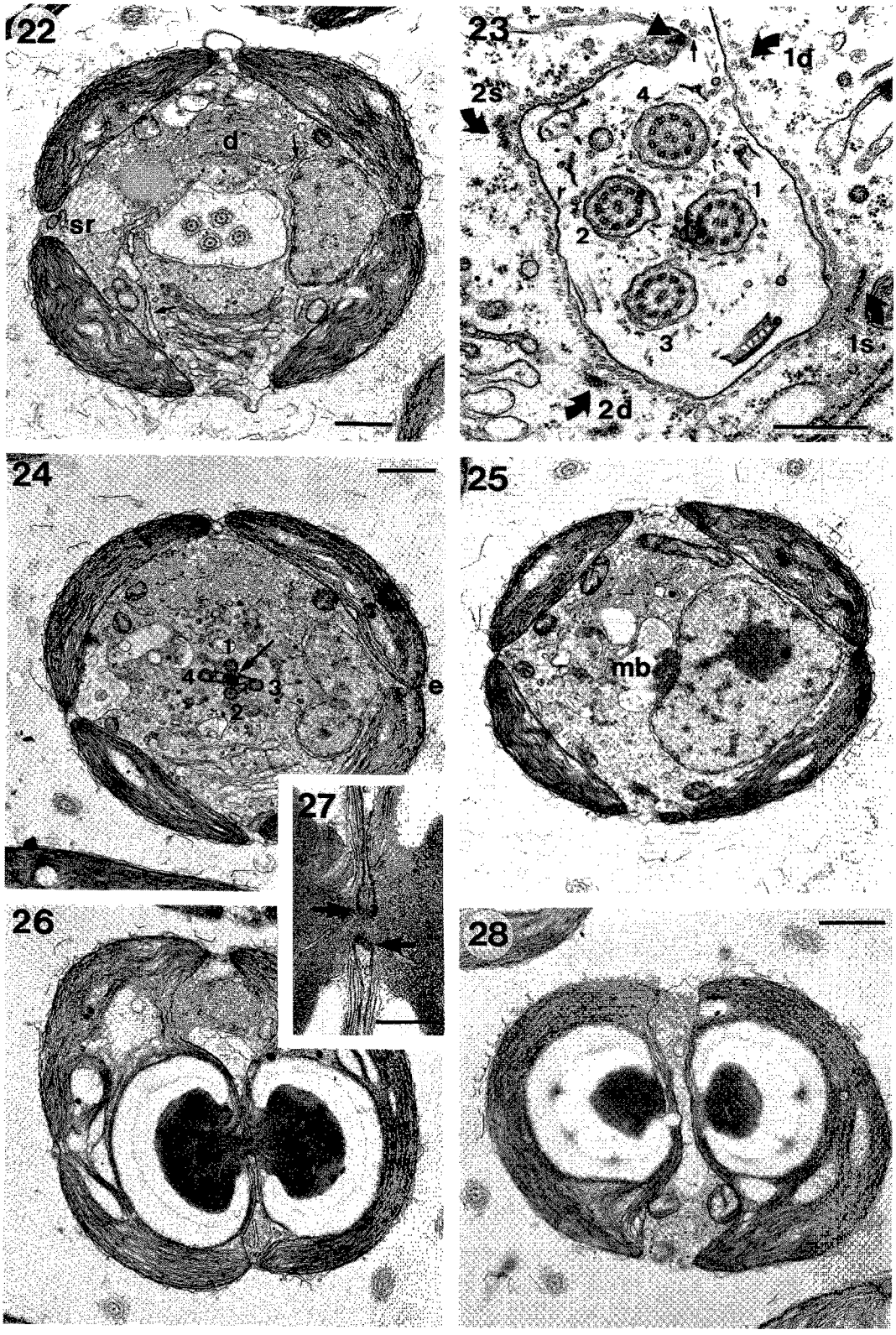
Mitochondrial profiles are observed in most sections (Figs 21–26). They probably form a single reticulum as in *P. gelidicola* (McFadden & Wetherbee, 1982).

The flagellar apparatus has not been studied in detail, but a few notes will be given. The four basal bodies form a rhombic figure (Fig. 24) which is equivalent to the rhombic type of Inouye, Hori & Chihara (1985). Numbering of individual flagella (Figs 23–24) is according to Moestrup & Hori (1989). The rectangular synistosome connecting basal bodies 1 and 2 is c. 285 nm long, c. 160 nm wide and c. 215 nm deep. The four flagellar roots consist of 4-2-5-2 microtubules, respectively. The 2-stranded roots (1d and 2d) are situated between basal bodies 1 and 4 and between 2 and 3, respectively. The 4-stranded root (1s) appears between basal bodies 1 and 3, the 5-stranded root (2s) between basal bodies 2 and 4 (Fig. 23).

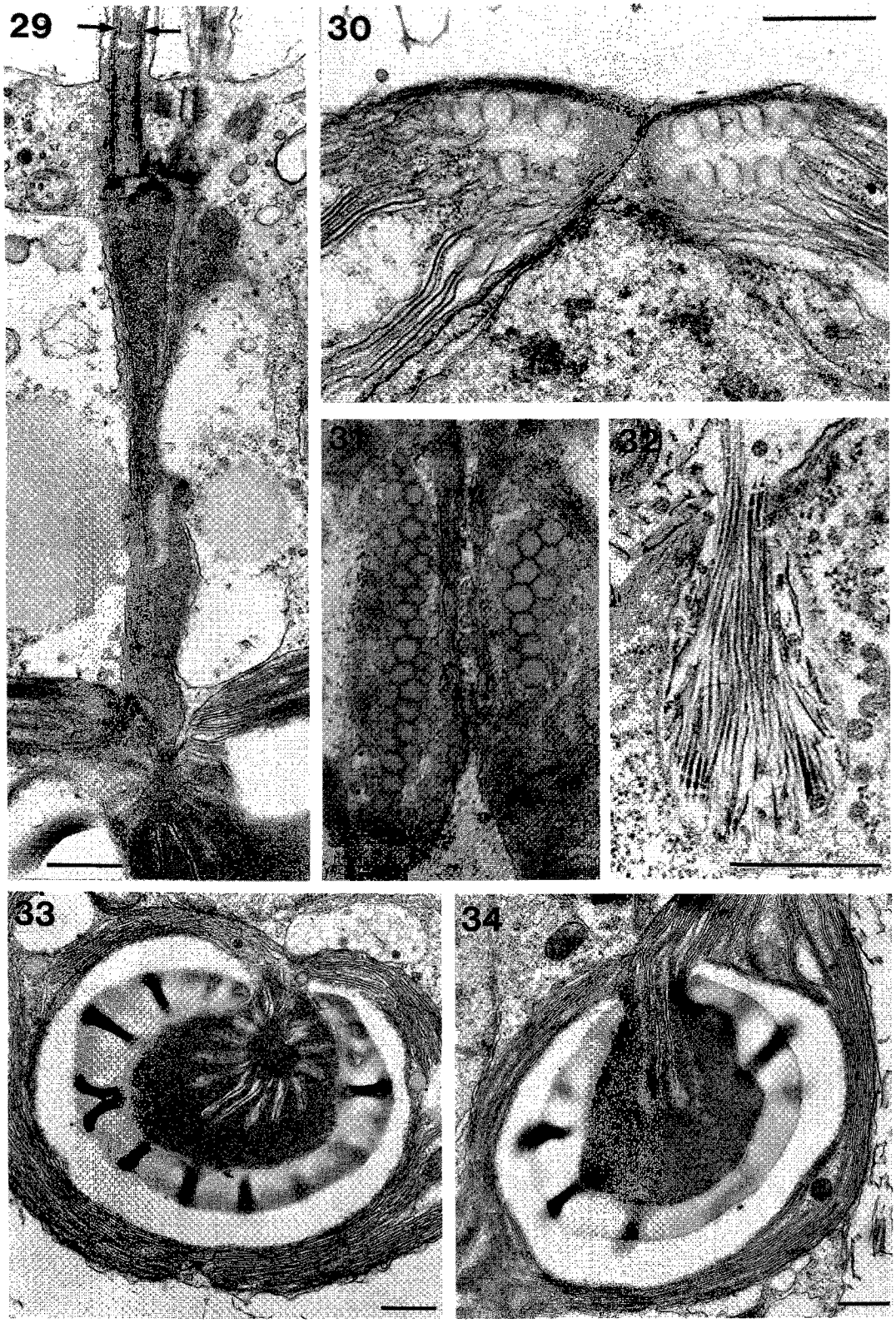
The rhizoplast and microbody system is closely associated with the anterior end of the pyrenoid (Fig. 29). Both the rhizoplast and the microbody branch before reaching the proximal end of the basal bodies (Fig. 29). A branching microbody is also shown in Fig. 25.

Species of *Pyramimonas* from field material

Based on whole mounts of field material four additional new species of *Pyramimonas* will also be described. Despite



Figs 22–28. Transverse sections through *Pyramimonas quadrifolia*. Fig. 22. Section at the flagellar pit region. Golgi apparatus (d); endoplasmic reticulum (arrows); scale reservoir (sr). Fig. 23. Higher magnification of the flagellar pit region, showing cytoskeletal microtubules, four flagellar roots (curved arrows), pentagonal flagellar scales (arrow) and electron dense area of the duct aperture (arrowhead). Fig. 24. Section at the level of the basal bodies. Eyespots (e), synistosome (arrow). Fig. 25. Section showing branching microbody (mb). Fig. 26. Dividing chloroplast and pyrenoid. Fig. 27. Higher magnification of the chrloroplast invagination in Fig. 26. The plastid-dividing ring is marked by arrows. Fig. 28. Chloroplast and pyrenoid division complete. Scale bar=1 μm (Figs 22, 24–26, 28); 500 nm (Figs 23, 27).



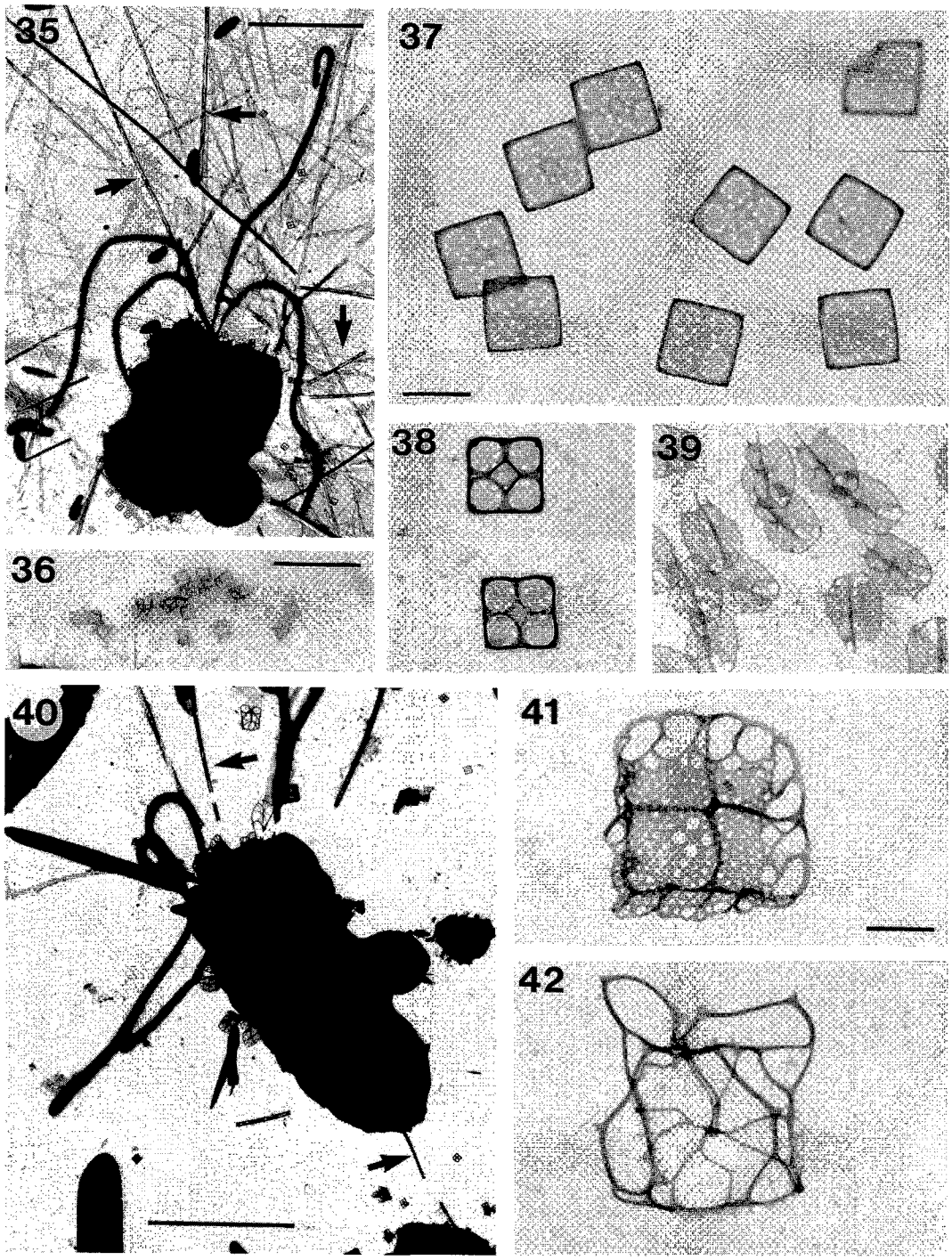
Figs 29–34. Details of *Pyramimonas quadrifolia*. Fig. 29. Longitudinal section of the flagellar apparatus, showing rhizoplast and microbody system. The transitional fibre of the transition zone is marked by arrows. Fig 30. Cross section through eyespots in closely appressed chloroplast lobes. Each eyespot is composed of two rows of carotenoid droplets not separated by thylakoids. Fig. 31. Glancing section through eyespots. Some droplets are hexagonal in shape. Fig. 32. Scale reservoir releasing mainly hair scales to the flagellar pit. Note bundles of hair scales in antiparallel rows. Figs 33–34. Section through the pyrenoid. The pyrenoid is surrounded by a starch grain, the matrix is traversed by pairs of thylakoids. Scale bars=500 nm.

the rather limited material, a formal description is given for three of these. The description is based mainly on scale structure but certain other characters are also provided.

Pyramimonas aurita Daugbjerg sp. nov.
Etymology: from latin auritus: with ears, cf. *Aurelia aurita*, the common jellyfish, the gonads of which form four ear-like figures very similar to those seen in one type of box scale.

Diagnosis

Cellula in sicco complanata circiter 7–8 μm longa, circiter 5–6 μm lata, trichocystibus armata, quattuor flagellis natans cellulae aequilongis. Squamae corporis subjacentes



Figs 35–42. *Pyramimonas aurita* and *P. dichotoma*. Figs 35–39. *Pyramimonas aurita*. Fig. 35. Au/Pd shadowcast whole mount showing whole cell and discharged trichocysts (arrows). Other trichocysts originate from a cryptomonad. Figs 36–39. Uranyl acetate stained scales. Fig. 36. Underlayer body scales of type 2. Figs 37–38. Two types of box scales. Fig. 39. Limuloid scales.

Figs 40–42. *Pyramimonas dichotoma*. Fig. 40. Whole cell. Note large body scales and discharged trichocysts (arrows). Fig. 41. Box scale. Fig. 42. Crown scale. Scale bars=5 μm (Figs 35, 40); 250 nm (Figs 36–39, 41–42).

parvae, quadratae, simplices (typo a McFadden *et al.* 1986 secundo nominato attribuendae). Superpositae majores, capsiformes, complanatae, dimorphae. Alterarum quaeque laminam basalem praebens circiter octo foraminibus pertusam circulum centralem formantibus et in quoque angulo 9–12 foraminibus circulum ad centrum versus aperte interruptum describentibus. Alterarum cuique machina superposita e quattuor brachiis formata in quadratulum superius centrale convergentibus. Flagella squamis limuliformibus longitudinaliter striatis tecta.

Dried cells c. 7–8 μm long, c. 5–6 μm wide. Trichocysts present. The four flagella equal the cell in length. Cell surface covered by type 2 underlayer body scales and two types of flat box scales. In one type the base is perforated by c. eight holes in a circle around the centre. Each corner perforated by 9–12 holes forming a half circle which opens toward the centre of the base. The other scale type with a superstructure of four ribs which form a small square distally. Flagella covered by longitudinally striped limuloid scales.

Holotype: Fig. 37.

Electron microscopy of Pyramimonas aurita

Only whole mounts of *P. aurita* are available. It occurred in a mixed culture together with the cryptomonad *Teleaulax* but died before embedding was attempted. In whole mounts, dried cells of *P. aurita* are 7–8 μm long and 5–6 μm wide. *Pyramimonas aurita* possesses four flagella about twice the length of dried cells. Each cell possesses a number of trichocysts which have been discharged in the cell in Fig. 35. The cell surface is covered by c. 45 nm wide underlayer scales (Fig. 36) identical to type 2 sensu McFadden, Hill & Wetherbee (1986). The underlayer body scales are covered by two types of box scales but their relative disposition could not be ascertained. One type, illustrated in Fig. 37, is square, each side measuring c. 290 nm in width. The base plate is perforated by c. eight holes that form a median circle. Near each corner 9–12 perforations form a half circle which opens toward the centre of the base plate. The scale appears to be very flat judged from Fig. 37 (upper right corner).

The other type of box scale is c. 275 nm wide. It is also square and perforated by small circular holes, however, the pattern could not be determined with any accuracy (Fig. 38). Four ribs extend from the middle of each side to form a small central square. The latter is c. 75 nm wide and rotated 45° compared to the base plate. The limuloid scales on the flagella are c. 300 nm long and c. 200 nm wide. The base is striped longitudinally by 6–7 ribs. A small spine is present on each side of the slightly raised median spine (Fig. 39). The antapical end is perforated by one large and four smaller holes. Due to the presence of type 2 underlayer scales and trichocysts *P. aurita* is assigned to subgenus *Trichocystis* sensu McFadden, Hill & Wetherbee (1986).

Pyramimonas dichotoma Daugbjerg sp. nov.
Etymology: from greek dichotomos: divided equally.

Diagnosis

Cellula in sicco complanata circiter 10–11 μm longa, circiter 4–5 μm lata, trichocystibus armata, quattuor flagellis natans cellula brevioribus. Corpus squamis capsiformibus et squamis coroniformibus tectum. Squama capsiformis circiter 550 nm lata. Lamina basalis ejus foraminibus circularibus variae magnitudinis pertusa. 16 cancelli e margine laminae surgentes, trabem horizontalem circumcurrentem suffulgentes et inde quattuor costas crucem superiorem formantes. Squama coroniformis circiter 575 μm lata. Pars basalis ejus e trabe marginali et quattuor costis horizontalibus dichotomis e centro radiantibus eae affixis formata. Quattuor brachia deorsum bifurcata e trabe marginali surgentia supra coalita.

Dried cells c. 10–11 μm long, c. 4–5 μm wide. Trichocysts present. The four flagella shorter than the cell. Box scales large, c. 550 nm wide. The base perforated by circular holes of different sizes. Sixteen vertical ribs emanate from the base margin and meet with an upper horizontal rim. The rim carries four ribs forming a cross. Crown scales equally large, c. 575 nm wide. Four horizontal dichotomously divided ribs emanate from the centre of the base and connect to the rim. Four downwards bifurcated arms join upwards with a central vertical strut.

Holotype: Fig. 41.

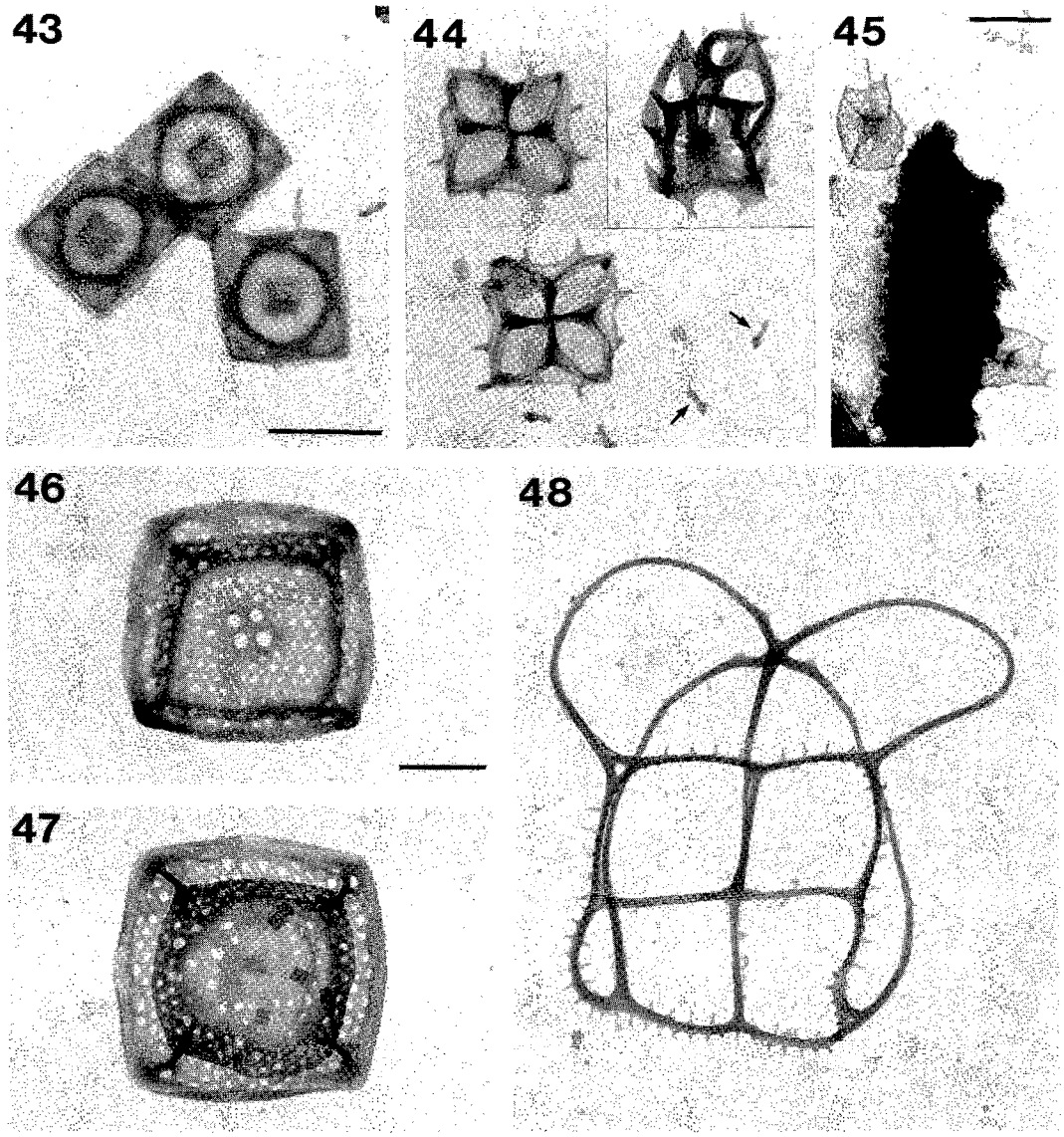
Electron microscopy of Pyramimonas dichotoma

The dried cell illustrated in Fig. 40 is 10–11 μm long and 4–5 μm wide. The cell carries four flagella shorter than the cell length. *Pyramimonas dichotoma* possesses trichocysts which have been discharged in the cell in Fig. 40.

The square box scales are c. 550 nm wide and c. 170 nm high (Fig. 41). The base plate is mostly perforated by small circular holes but four larger circular holes are present in the centre. In addition, other box scales possess a few larger circular holes arranged more randomly. The lateral margins of the base plates are also perforated by larger holes. An upright rib from each corner and three evenly distributed upright ribs from each side of the base meet with a square rim. From the middle of each side of the rim four ribs project towards the centre of the scale, forming a cross.

The crown scales are c. 575 nm wide and c. 425 nm high (Fig. 42). The square base carries c. 10 small spines on each side. Four horizontal ribs emanate from the centre of the base. Each rib branches dichotomously. The proximal end of the four upright arms is bifurcated and each arm is ornamented with two small spines. The upright arms join with a central vertical strut.

The underlayer body scales could not be referred to type.



Figs 43–48. Scale morphology of *Pyramimonas igloolikensis* and *Pyramimonas* sp.

Figs 43–45. Scale morphology of *Pyramimonas igloolikensis*. Fig. 43. Box scales. Fig. 44. Crown scales and footprint scales (arrows). Fig. 45. Distal tip of flagellum with pentagonal underlayer scales and limuloid scales. Figs 46–48. Scale morphology of *Pyramimonas* sp. Fig. 46. Distal (upper) surface of box scale. Fig. 47. Proximal surface of box scale. A few underlayer scales also present. Fig. 48. Crown scale. Scale bars=250 nm.

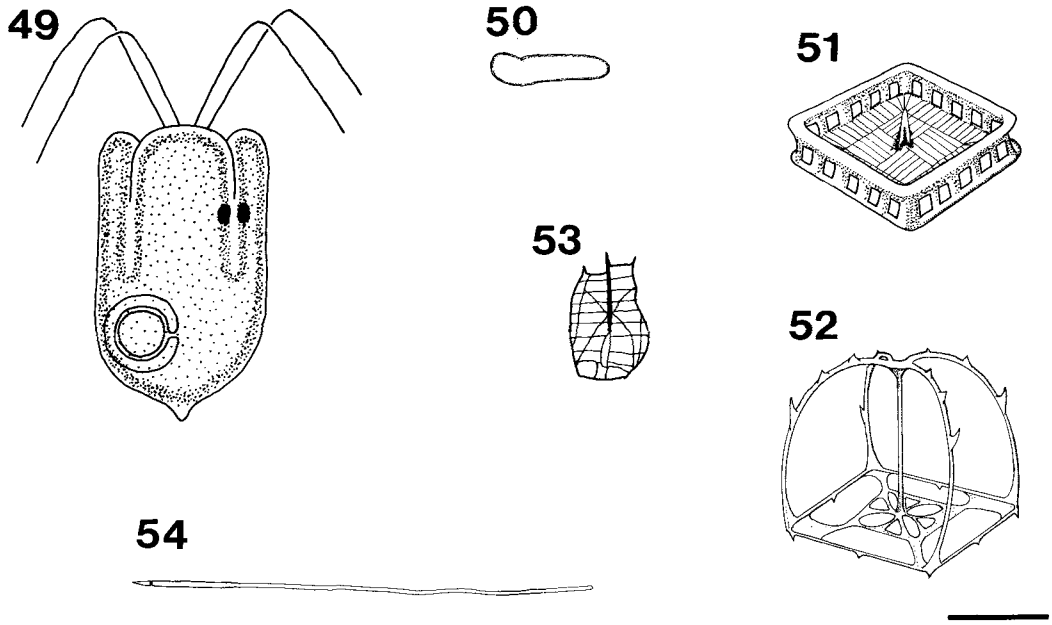
Pyramimonas dichotoma should probably be assigned to the subgenus *Trichocystis* (McFadden, Hill & Wetherbee) due to the presence of trichocysts.

Pyramimonas igloolikensis Daugbjerg sp. nov.
Etymology: from the village name Igloolik and ensis: inhabiting.

Diagnosis

Cellula in sicco complanta circiter 9 μm longa, quattuor flagellis cellula brevioribus natans. Corpus imprimis squamis capsiformibus et squamis coroniformibus tectum. Squama capsiformis circulo ornata paene ejusdem ut squamae diametri et quadrangulo medio minore. Squama coroniformis laminam basalem quadrangulam paene continentem praebens et quattuor brachia inde surgentia supra coalita, quoque latere laminae binis spinis armato. Squamae vestigiiformes praesentes. Flagella squamis pentagonis subjacentibus et squamis limuliformibus superioribus tecta, lamina basali squamae limuliformis quattuor costis notata crucem formantibus.

Dried cells c. 9 μm long. The four flagella shorter than the cell. Cell surface covered by box scales with a circular structure of almost the same diameter as the width of the base plate. A quadri angular structure is situated in the middle. Crown scales with almost solid base plates and four upright arms. The rim ornamented with two large spines on each side. Footprint scales present. Flagella



Figs 49–54. Schematic drawings showing the cell and five scale types of *Pyramimonas quadrifolia*. Fig. 49. Whole cell showing position of major organelles and flagella at rest. Fig. 50. Footprint scale. Fig. 51. Box scale with central spine. Fig. 52. Crown scale. Fig. 53. Limuloid scale. Fig. 54. Hair scale. Scale bar=5 μm (Fig. 49); 75 nm (Fig. 50); 250 nm (Figs 51–54).

covered by pentagonal underlayer scales and limuloid scales. The base of each limuloid scale carries four ribs forming a cross.

Holotype: Fig. 43.

Electron microscopy of Pyramimonas igloolikensis

Dried cells of *P. igloolikensis* are c. 9 μm long (not shown). The square box scales are c. 280 nm wide (Fig. 43). Each scale is characterised by a circular structure of almost the same diameter as the base plate. An 80 nm wide square is situated in the middle of the base. The hight of the scale could not be ascertained. The crown scales are c. 250 nm wide and c. 220 nm high. Each side possesses two distinct spines (Fig. 44). A characteristic feature is the almost solid base plate. The base is, however, perforated by eight narrow elongated holes. The four upright arms join with a central upright strut. Footprint scales are c. 70 nm long (Fig. 44, arrows). The flagella are covered by pentagonal underlayer scales typical for the genus (Fig. 45) and by limuloid scales, c. 345 nm long and c. 200 nm wide. Limuloid scales carry a small spine on each side of the apically directed spine. The base is without striations but four ribs radiate from the centre of the base, forming a cross.

Due to the presence of footprint scales *P. igloolikensis* is assigned to the subgenus *Vestigifera* sensu McFadden, Hill & Wetherbee (1986).

Electron microscopy of Pyramimonas *sp.*

No cells were observed of this species and no formal description will therefore be made.

The box scales somewhat resemble a basket. The base is c. 540 nm wide and perforated by holes of slightly different size (Figs 46–47). In some scales four large circular holes are present centrally (Fig. 46), less distinct or probably lacking in others (Fig. 47). The sides are c. 190 nm high and 750 nm wide and also perforated. Underlayer body scales probably similar to type 1 sensu McFadden, Hill & Wetherbee (1986) are visible in Fig. 47. The crown scales (Fig. 48) are square with slightly rounded corners. They are c. 780 nm wide and c. 750 nm high. The basal rim is ornamented with double rows of small spines. Four horizontal ribs divide the base into four quadrants. Four upright arms extend from the corners of the base to meet with a central strut. The proximal part of each upright is usually dichotomously branched. Each upright carries a rather indistinct single spine near the central strut.

Discussion

By comparison with marine species of *Pyramimonas* known solely from light microscopy, it is evident that *P. quadrifolia* is not identical to any previously described species. Differences include cell dimensions, position of flagella at rest, and the small posterior tail present in some cells.

The micromorphology of the box scales of *P. quadrifolia* shows similarity to *P. spinifera* Pennick (Pennick, 1983), *P. gorlestonae* Pennick & Cann (Pennick & Cann, 1982), *P. disomata* (Pennick, 1984) and *P. gelidicola* (McFadden, Moestrup & Wetherbee, 1982). These species all possess more or less square perforations in the walls of the box scales and the base plate is striated in four groups at right angles to each other. An inner square is also seen in the base plate of *P. moestrupii* McFadden but the basal floor of

this species possesses radiating perforations rather than striations (McFadden, Hill & Wetherbee, 1986). In *P. mitra* Moestrup & Hill the base of each box scale has two slightly different sized inner squares. The base plate is otherwise without sculpturing (Moestrup & Hill, 1991).

The variation in *P. quadrifolia* regarding presence or absence of a central spine in the box scales is identical to the situation in *P. disomata* (McFadden, Hill & Wetherbee, 1986) and *P. cyclotreta* (Daugbjerg & Moestrup, 1992b).

The crown scales of *P. quadrifolia*, notably the unique base plate, are very different from crown scales described in other species of *Pyramimonas*.

As in most other species assigned to the subgenus *Vestigifera*, *P. quadrifolia* has footprint scales scattered between the box scales outside the flagellar pit. However, two recently described species, *P. norrisii* Sym & Pienaar (Sym & Pienaar, 1991a) and *P. cyclotreta* (Daugbjerg & Moestrup, 1992b), possess footprint scales between the box scales both within and outside the flagellar pit.

The transversely striated limuloid scales of *P. quadrifolia* are similar to those of *P. spinifera* (Pennick, 1983) and *P. gorlestonae* (Pennick & Cann, 1982).

In many aspects the general ultrastructure of *P. quadrifolia* resembles that observed in other species of the subgenus *Vestigifera*. Only a few ultrastructural details will therefore be discussed in more detail.

When located inside the scale reservoir the hair scales apparently do not have a definite orientation (antiparallel in Fig. 32). We conclude that the hair scales are manipulated after or during release to the flagellar pit to the correct orientation on the flagella.

The PD ring observed at the isthmus of the dividing chloroplast in *P. quadrifolia* is known also from a few other algae, as well as from mosses and higher plants. It was observed in a species of *Pyramimonas* by Hori *et al.* (personal communication cited in Kuroiwa, 1989) and in *P. mitra* (Moestrup, unpublished results). In *Cyanidium caldarium* the PD ring apparently consists mainly of actin-like proteins (Mita & Kuroiwa, 1988). The PD ring is thought to contract in the plane of division of the chloroplast and to generate the mechanical force necessary to divide the chloroplast (Mita & Kuroiwa, 1988). The molecular mechanism is still to be resolved.

Flagellar roots of many *Pyramimonas* species conform to a cruciate 4-2-4-2 system. Deviations from this general pattern have been considered by Sym & Pienaar (1991b), who concluded that the X-2-X-2 system varies more than initially expected. In *P. quadrifolia* the flagellar roots form a 4-2-5-2 system; the 5-stranded (2s) has a 2-over-3 configuration.

McFadden and Wetherbee (1984) found that the number of microtubules between the plasmalemma and the chloroplast of *P. gelidicola* equals the number surrounding the flagellar pit (excluding the flagellar roots). In *P. quadrifolia* 100–120 microtubules encircle the flagellar pit and the number of microtubules under the periphery of the cell almost equals this number. The number of microtubules equals that observed in other species of similar cell dimensions, e.g. *P. cyclotreta* (90–100, Daugbjerg & Moestrup 1992b); *P. olivacea* (130, McFadden, Hill & Wetherbee 1987). Large species apparently possess higher numbers: *P. octopus* (c. 16 μm long) has 185 microtubules (Moestrup, Hori & Kristiansen, 1987), *P. cyrtoptera* (c. 40 μm long) 300–320 microtubules (Daugbjerg & Moestrup, 1992a).

Apart from *P. quadrifolia*, which was established in unialgal culture, the description of new species in this paper are based solely on electron microscopical whole mounts. This raises the question whether some of the species are identical to the species described from other parts of the world—often in a very incomplete way—by light microscopists without access to electron microscopy. Thirteen such species were listed by Moestrup & Hill (1991), mainly from northern temperate waters. The true identity of these 13 species can probably never by proved, but if the names are to be used they should be applied to species which (1) agree reasonably well with the light microscopical descriptions, but differ in scale and perhaps other ultrastructural details from other species and (2) are collected at or near the type locality. It is unlikely that the new species from the northern part of Foxe Basin are conspecific with any of the 13 "light microscopical species" from temperate waters. Most of the species from Foxe Basin appear to be cold water species, and only 2 *Pyramimonas* species known from elsewhere were in fact found in Foxe Basin: *P. nansenii* (a cold water species known from Greenland and from an ice-covered Danish inlet) and *P. orientalis*, which occurs world wide. We therefore decided to apply new names to the species observed in the vicinity of Igloolik. These will be compared with other species below.

The scales of *Pyramimonas aurita* resemble those of *P. nansenii* (Thomsen, 1988). Both species lack crown scales but possess two types of flat box scales, one of which is characterised by cross bars while the other lacks this superstructure. The limuloid scales of the two species are similar. They show longitudinal striations and are perforated by somewhat irregularly positioned holes at the antapical end.

Pyramimonas aurita is the fourth species known to possess two types of flat box scales (see Thomsen 1988 and Moestrup & Hill 1992).

The large body scales of *P. dichotoma* are approximately twice as wide as scales of most other *Pyramimonas* species. Apart from the size difference, the box scales show resemblance to the box scales in species assigned to the subgenus *Pyramimonas*. The base plate is different but the scale margin of vertical ribs which attach to an upper rim is similar. However, in *P. dichotoma* the number of vertical ribs is 16 compared to 8 in other species of the subgenus and cross bars of the upper rim are present only in *P. dichotoma*. The dichotomous branching of the ribs in the basal cross and the bifurcated four uprights are unusual features of crown scales in the genus *Pyramimonas*.

The box scales of *Pyramimonas igloolikensis* show little resemblance to box scales of other *Pyramimonas* species. Box scales of *P. norrissii* also possess a central diamond-shaped structure (Sym & Pienaar, 1991) but the architecture is otherwise different. The almost solid base plate in crown scales of *P. igloolikensis* differs from all other species of *Pyramimonas*. The footprint scales resemble those of other species assigned to the subg. *Vestigifera* except *P. cyclotreta* (Daugbjerg & Moestrup, 1992b).

Pyramimonas sp. is known only from EM whole mounts but we have reasons to believe that the box and crown scales of Figs 46–48 belong to the same species. In whole mounts of wild material these scales were always observed on the same grids. Furthermore the length of box and crown scales from species of *Pyramimonas* always fall in the same size range and the same applies to the scale types of *Pyramimonas* sp. which represent some of the largest scales reported for any species of *Pyramimonas*.

In size and three dimensional appearance, scales of *Pyramimonas* sp. resemble those of *P. longicauda* (Inouye, Hori & Chihara, 1984). In both types the base plate and the walls are perforated by numerous circular holes. The walls are supported by upright ribs, twelve in *P. longicauda*, four in *Pyramimonas* sp. The crown scales of *Pyramimonas* sp. do not resemble those of *P. longicauda*. They are extremely large but the architecture is similar to crown scales in many other species of the genus.

Cells of *Pyramimonas nansenii* were also encountered in whole mounts from Northern Foxe Basin (not shown). This species was originally described by Braarud (1935) from the pack ice in East Greenland. Thomsen (1988) in an ice-covered Danish fjord observed a species of *Pyramimonas* which he considered identical to *P. nansenii*. The finding of *P. nansenii* in similar environmental conditions in arctic Canada confirms *P. nansenii* as a cryophilic (cold water) species.

Prior to this investigation *Pyramimonas orientalis* had only been encountered at two other arctic localities: West Greenland (Thomsen, 1982) and northern Norway (Throndsen, 1970). It occurs also in many other parts of the world (see references in McFadden, Hill & Wetherbee, 1986).

Our survey of the nanoflagellates in Northern Foxe Basin has shown an unexpectedly large number of previously unknown species of *Pyramimonas*. So little is known about the nanoplankton of other arctic regions that it is impossible to predict whether these species have a circumpolar distribution or are restricted to the Canadian Arctic.

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