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PYRAMIMONAS TYCHOTRETA, SP. NOV. (PRASINOPHYCEAE), A NEW MARINE SPECIES FROM ANTARCTICA: LIGHT AND ELECTRON MICROSCOPY OF THE MOTILE STAGE AND NOTES ON GROWTH RATES[1]

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An undescribed marine prasinophyte, *Pyramimonas tychotreta*, sp. nov., was isolated from a water sample collected near the ice edge in the Weddell Sea (Antarctica) and is characterized by means of light and electron microscopy. This is the second described Antarctic species in the genus and it possesses a cell ultrastructure typical for members of the subgenus *Vestigifera* McFadden. The quadriflagellated cells measure 8–12 μm in length and 6–7 μm in width and are equipped with seven types of organic scales that cover the flagella and cell body. The scale floor of the box scales is ornamented by quadrants of parallel striations running perpendicular to one another. The scale floor is further characterized by a number of randomly positioned perforations. The wall of the box scales may be solid or possess up to five perforations. The base of the crown scales is square with rounded corners. It is formed of two crossed ribs, the extremities of which are interconnected by a peripheral rib. Four upright arms, attached to the peripheral rib in positions slightly offset from its junction with the cross ribs, join up with the distal extremity of a central upright strut. Each arm possesses two spines. The limuloid scales are cross-striped by 10–12 ribs. Some details of the flagellar apparatus are briefly reported. *Pyramimonas tychotreta* is compared with other species of the genus. Experiments were conducted to study the response of growth rate to variations in temperature and salinity in the clonal culture. The best growth rate (0.45 divisions·24 h^{-1}) was found at 4.6° C; growth ceased at temperatures in excess of 12° C. Growth in salinities ranging from 15 to 35 psu was similar, but was arrested at 10 psu. These studies suggest that *P. tychotreta* as a cold stenotherm and euryhaline taxon. New observations are presented on the geographic distribution of previously described species of *Pyramimonas* Schmarda from the Northern Foxe Basin, Canada.

Key index words: **growth rates; marine nanoflagellates; Prasinophyceae; *Pyramimonas tychotreta*; ultrastructure; Weddell Sea**

Abbreviations: **A, B, C, D: chloroplast lobes A, B, C and D; e: eyespots; g: Golgi body; l: lipid droplet; m: mitochondrion; mb: microbody; N: nucleus; p: pyrenoid; pb: proximal band; pf: prominent proximal fiber; r: rhizoplast; r_1, r_2, r_3, and r_4: microtubular flagellar roots 1, 2, 3, and 4; s: starch sheath; 1–4: flagella or basal bodies**

During the past 20 years, a substantial amount of ultrastructural information has been collected on the genus *Pyramimonas*. This has expanded our appreciation of the morphological diversity and distribution records have generated insight into the ecology and biogeography of the genus. Well-circumscribed species of *Pyramimonas*, with the exception of the type species (*P. tetrarhyncus* Schmarda), are marine (Schmarda 1850, Hori et al. 1995). In most seas more than one species is encountered in surface samples. Surveys using electron microscopy for identification purposes give the impression that species diversity is higher in temperate marine waters, but this may change when sampling intensifies in arctic and antarctic waters. Some *Pyramimonas* species are cosmopolitan (e.g. *P. disomata* McFadden, Hill et Wetherbee, *P. grossii* Parke, *P. orientalis* Butcher ex McFadden, Hill et Wetherbee, and *P. virginica* Pennick; see McFadden et al. 1986, Hori et al. 1995) and a few taxa appear to have a narrow biogeographic distribution. Temperature studies on arctic and antarctic species of *Pyramimonas* have demonstrated that cells do not survive temperatures above about 10–12° C (Daugbjerg and Moestrup 1992a, 1992b; present study). Hence, marine species living throughout the year at low temperatures (e.g. *P. aurita* Daugbjerg, *P. cyrtoptera* Daugbjerg, *P. cyclotreta* Daugbjerg, *P. dichotoma* Daugbjerg, *P. gelidicola* McFadden, Moestrup et Wetherbee, *P. igloolikensis* Daugbjerg, *P. quadrifolia* Daugbjerg, and *P. tychotreta* Daugbjerg) are likely to have a circumpolar distribution. On the other hand, the cosmopolitan taxon *P. orientalis* also occurs in arctic waters (Throndsen 1970, Thomsen 1982, Daugbjerg and Moestrup 1993). The arctic population of *P. orientalis* may represent an ecotype adapted to cold environments.

A few studies have addressed the diversity of *Pyramimonas* species from the Arctic, whereas only a single taxon (*P. gelidicola*) has been described from antarctic waters (McFadden et al. 1982). The present study reports on the motile stage of a new species, *P. tychotreta*, isolated into clonal culture from a water sample collected near the ice edge in the northern part of the Weddell Sea, Antarctica. The description is based on a light and electron microscopic investigation of cell morphology and the architecture of the body and

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flagellar scales. The growth response to changes in temperature and salinity was also studied.

MATERIALS AND METHODS

Collection site. During a cruise from November 1988 to January 1989 on-board RV "Polarstern" (EPOS II) Dr. Jacob Larsen collected water samples in the Weddell Sea. *Pyramimonas tychotreta* was isolated into clonal culture from a surface sample collected at station 156 (61° S, 49° W). This station (solid circle in Fig. 1) is located close to the edge of permanent pack ice and over a water column of 2190 m. From 10–300 m the salinity changed from 34.1 to 34.5 psu and the temperature profile from –1.7 to 0.1° C.

Culture. The clonal culture was established by micropipetting from enriched cultures. For the past 10 years *P. tychotreta* has been maintained successfully at 30 psu in modified Erdschreiber medium (Throndsen 1978) and L1 (Guillard and Hargraves 1993) at a temperature of 4° C, a photon flux density of ~13 $\mu mol \cdot m^{-2} \cdot s^{-1}$, and a 16:8 h L:D cycle.

Temperature and salinity experiments. To study the growth response of the clonal culture, cultures were grown at 4.6° C ± 1.6 to 17.5° C ± 0.2 on a temperature-gradient plate. All cultures were grown at a salinity of 30 psu, a photon flux density of 15 $\mu mol \cdot m^{-2} \cdot s^{-1}$, and a 16:8 h L:D cycle. To examine the growth response to changes in salinity, a duplicate series of cultures was grown at 10, 15, 20, 25, 30, and 35 psu (temperature 4° C, photon flux density 13 $\mu mol \cdot m^{-2} \cdot s^{-1}$, and a 16:8 h L:D cycle). Growth experiments were conducted on semicontinuous cultures (total volume 100 mL), as an equal volume of new medium replaced the 5-mL sample removed for cell counts. Live cells were counted using an electronic particle counter (Coulter Counter, Industrial Model D) every second day or at nonregular intervals for a maximum period of 24 d. Growth rates during exponential growth were calculated from semilog plots of successive cell counts, using the equation k (divisions·24 h^{-1}) = $3.322/T_{10}$, where T_{10} is the number of days required for a 10-fold increase of the population (Guillard 1973).

Light and electron microscopy. Live cells were observed using an Olympus Provis AX 70 microscope equipped with Nomarski interference optics. Whole mounts for electron microscopy were either shadow-cast or stained in uranyl acetate according to standard methods (Moestrup and Thomsen 1980). Unfixed cultures for freeze-etching were centrifuged at 1500 rpm for 10 min, frozen in Freon 22, and stored in liquid nitrogen until preparation in a Balzer's 301 apparatus. The material was fractured at –100°C, etched for 3 min, and shadow-cast. Fixation protocols for sectioning of cells were identical to those outlined in Daugbjerg and Moestrup (1992b).

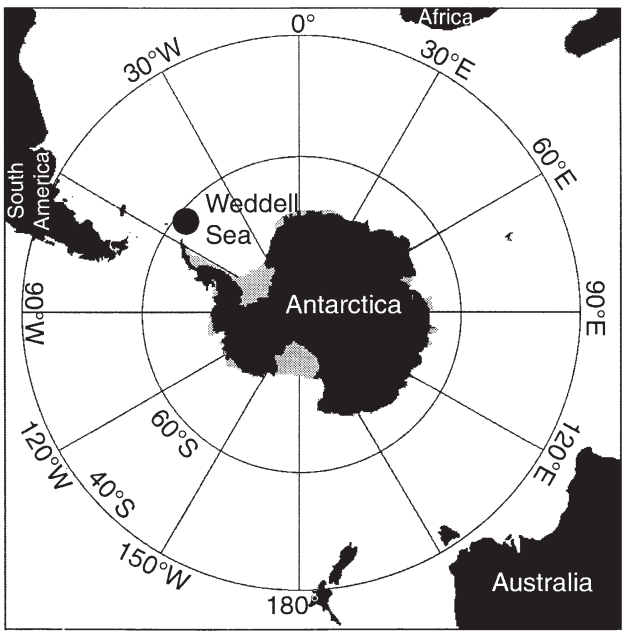


FIG. 1. Map showing the type locality (solid circle) of *Pyramimonas tychotreta* in the Weddell Sea, Antarctica.

RESULTS

Pyramimonas tychotreta Daugbjerg, sp. nov.

Cellulae 8–12 μm longae et 6–8 μm latae lateribus paulo convexis et apice truncato et antapice rotundato. Flagella quattuor depressione apicali emergentia et longitudine paulo cellulam longiora. Chloroplastus graminiviridis cupulatusque et ad apicem cellulae in lobis quattuor divisus. Stigmata duo in lobis chloroplasti propinquis aequatoriaque bistrataque; strata non per thylakoidibus segregata. In extremo antapicali vagina e amylo constata et pyrenoidem excentricam cingens. Pagina flagellorum squamis quinquelateris parvisque squamis limuliformibus striatisque et squamis piliformibus tecta. Corpus cellulae typis quattuor squamarum tectum. Fovea flagellorum squamis minutis quadratisque (ca. 45 nm latis) strati inferioris tecta. Corpus cellulae strato singulari squamae capsiformium (ca. 300 nm latae et 80 nm altae) tectum. Basis squamae capsiformes omnes in directionibus quattuor grammata et cum numero varianti foraminum fortuitorum. Paries squamae capsiformium solidus aut per usque ad foramina quinque perforatus. Squamae vestigiumformes (70 nm longae et 20 nm latae) inter squamas capsiformes distributae. Squamae coroniformes quadratae (ca. 300 nm latae) angulis rotundatis et stratum extimum formantes. Basis squamarum coroniformium omnium costis quattuor crucem formantibus et ad extremum distale labro quadrato connexis. Tigillum verticale centralisque e centro crucis procurrens et ad extremum distale brachiis quattuor curvatis connexum. Brachia omnia deflexa et spinas duas ferentia et prope ad medium lateris uniuscuiusque labri terminantia.

Cells are 8–12 μm long and 6–8 μm wide, with slightly convex sides, a truncate apical end and a rounded antapical end. Four flagella emerge from an apical depression and are slightly longer than the cell. The grass-green chloroplast is cup-shaped and anteriorly divided into four lobes. Two bilayered eyespots without intervening thylakoids are positioned in adjacent chloroplast lobes halfway down the cell. A starch sheath surrounds an eccentric basal pyrenoid. The flagellar surface is covered by small pentagonal underlayer scales, striated limuloid scales and hair-shaped scales. Four scale types cover the cell body. Minute square underlayer scales (~45 nm wide) cover the flagellar pit. Box scales (~300 nm wide and ~80 nm high) cover the cell body in a single layer. The scale floor is ornamented by quadrants of parallel striations running perpendicular to one another and a varying number of unevenly placed perforations. The scale wall is solid or perforated by up to five holes. Footprint scales (70 nm long and 20 nm wide) are dispersed between box scales. Square crown scales (~300 nm wide) with rounded corners form the outermost layer. The base of each crown scale is formed by four ribs in a cross, connected distally to a square rim. A

central strut projects from the center of the cross and connects distally with four curved arms that bend down to terminate near the middle of each side of the rim. Two spines are present on each upright arm.

Etymology: from Greek *tyche*, chance, and *tretos*, perforated. The species epithet refers to the random perforations of the scale floor in the box scales.

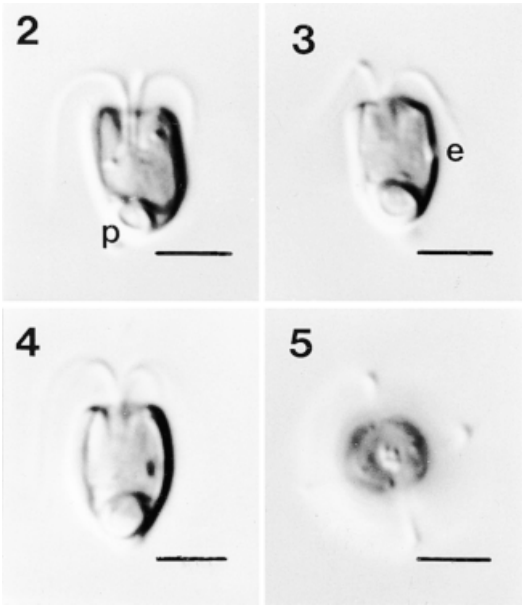
Holotype: Fig. 7.

Isotype: A fixed and embedded sample of the clonal culture has been deposited at the Botanical Museum, Copenhagen, and given the reference number 1569.

Habitat: The type material was collected in the northern part of the Weddell Sea, Antarctica (60° S, 49° W), in December 1988.

Light microscopy. Live cells (length 8–12 μm and width 6–8 μm) are cylindrical and possess four equal flagella that emerge from a flagella pit, ~2 μm deep (Figs. 2–5). At rest the flagella recurve and terminate about halfway down the cell at the level of the two bilayered eyespots (Figs. 2–4). The basal bodies form a diamond (rhombic) shape (Fig. 5). For the past 10 years no palmella or cyst stages have been observed in this isolate. Cells swim in an almost straight path while rotating around their longitudinal axis. They may stop for a short time and change direction. After a few minutes of observation, cells stop swimming and attach to the slide, usually with the apical end. Swimming behavior is similar to that encountered for most species of the subgenus *Vestigifera*.

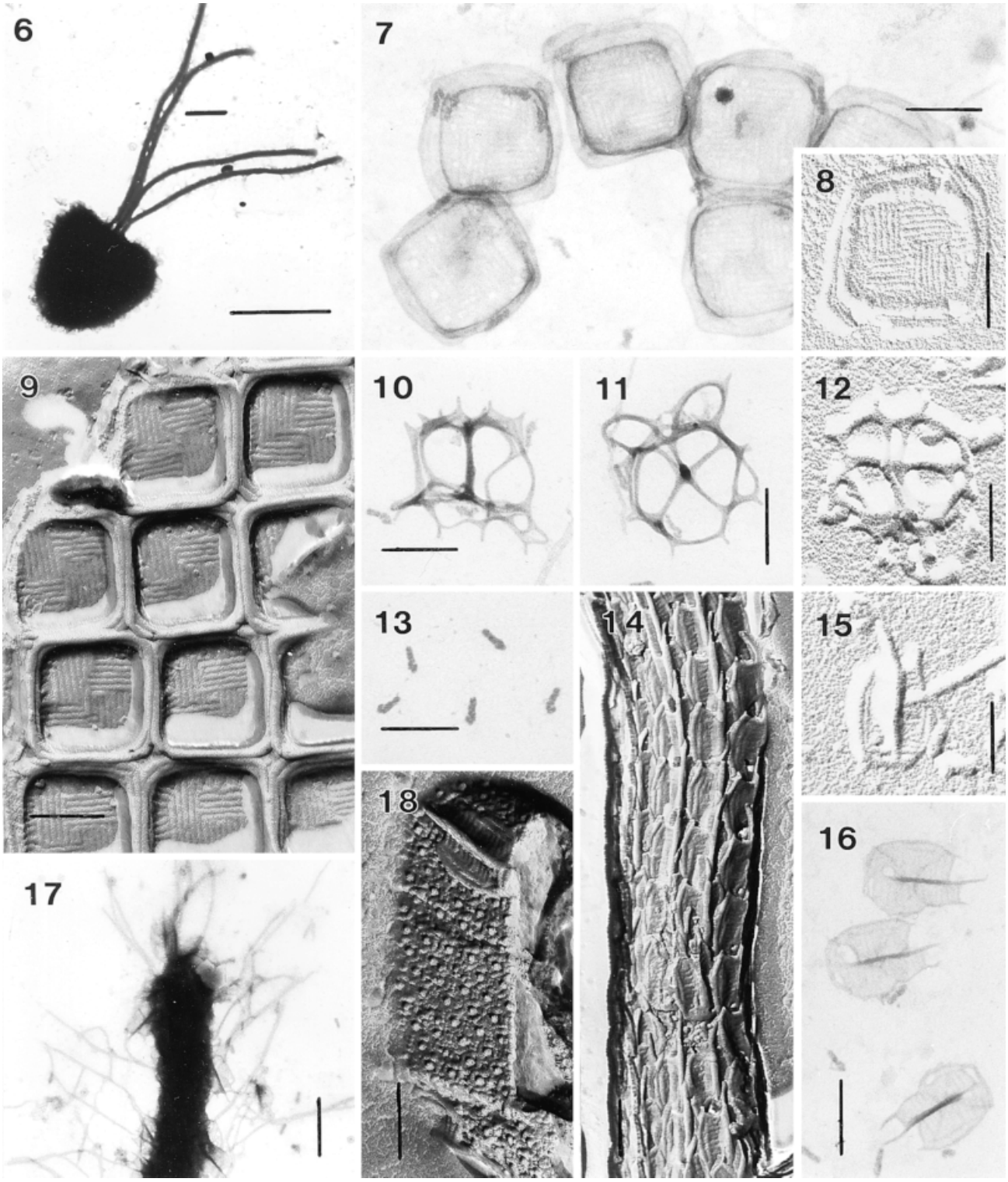
Body and flagellar scales. An osmium-fixed cell with detached body and flagellar scales is shown in Fig. 6. The cell has four types of body scales and three types of flagellar scales (Figs. 7–18). Reconstructions of cells from light microscopy and three body scales and a single flagellar scale based on whole mounts and thin sectioning are illustrated in Figs. 19–23. Details of sectioned scales are shown in Figs. 24–36. As typical for members of the subgenus *Vestigifera*, the flagellar pit in *P. tychotreta* is covered by small underlayer scales. They are similar to type 1 of McFadden et al. (1986) but, as pointed out by Sym and Pienaar (1995), the baseplate of this type of scale has only four perforations (not shown). The square box scales (Figs. 7–9, 29–33) cover the cell surface as a close fitting layer (Fig. 9). The walls of the box scales may be solid (Fig. 30) or have up to five perforations (three and four holes are shown in Figs. 32 and 33, respectively). The walls flare out distally and seem to be interlocked by a narrow flange situated about 1/3 of the scale height from the base (arrows in Fig. 31). The scale floor is ornamented with perpendicular striations in four quadrants, each group composed of 5–6 stripes (Figs. 7–9, 29). The floor is further characterized by randomly positioned perforations (Figs. 7, 29). Footprints scales are scattered between the box scales (Figs. 7, 29–30). Square crown scales (300 nm wide and 300 nm high) with rounded corners form the outermost layer of the periplast (Figs. 10–12, 24–27). Proximally each crown scale comprises a cross whose distal end is interconnected by a rim. Two spines are positioned on each side of the rim (Fig. 11). Slightly displaced from the middle of each side of the proximal rim, four upright arms extend and connect with the distal part of a central strut (Figs. 11, 26–27). Each arm possesses two spines (Fig. 27). Distally the arms join up pairwise, the two pairs connecting by a short distal bar (Fig. 28). This arrangement is also noticeable during the formation of crown scales in the Golgi cisternae (Figs. 24–25).



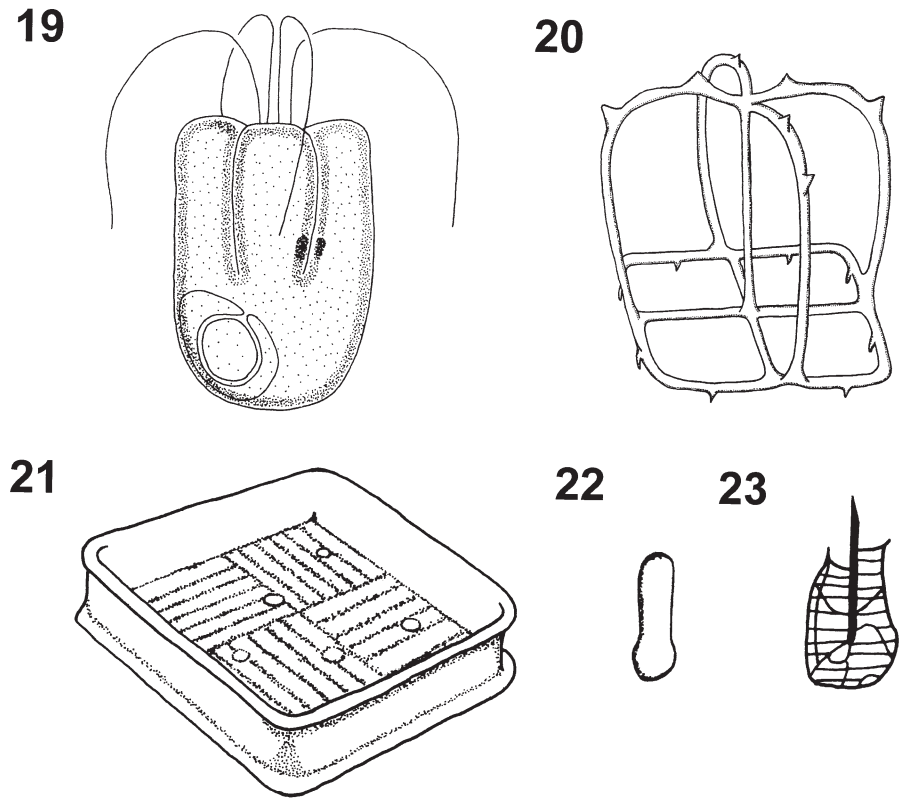
FIGS. 2–5. Live cells of *Pyramimonas tychotreta* sp. nov., differential interference contrast. FIGS. 2–4. Optical longitudinal sections showing position of flagella in resting cells, pyrenoid and eyespots. FIG. 5. Optical transverse section. The four basal bodies are arranged in a diamond-shaped configuration. Scale bars = 5 μm.

Minute pentagonal scales (~40 nm wide) cover the flagellar membrane in the manner typical of other species of *Pyramimonas* (e.g. Figs. 18, 35). Nine rows of limuloid scales cover the pentagonal scales, overlapping each other by half the scale width (Fig. 14). Limuloid scales measure ~313 nm in length and ~190 nm in width, including the apical spine (Figs. 15–16). Four faint ribs radiate to the periphery and two ribs run along the sides. Often two differently sized perforations are present proximally. A small spine is present on each side of the longitudinal rib (Fig. 16). Each scale also bears 10–12 transverse ribs (Figs. 15–16). One side of the scale is longer than the other. Each flagellum bears two rows of almost opposite tubular hair scales, ~1.3 μm long (Figs. 17, 34). They belong to the T-category of Marin and Melkonian (1994).

General fine structure. The organization of major organelles in the cell (i.e. nucleus, chloroplast, eyespots, pyrenoid, Golgi bodies, and mitochondrial profiles) is very similar to other species of the subgenus *Vestigifera* (Figs. 37–42). The cup-shaped chloroplast is appressed to the plasmalemma (Fig. 37) and extends



FIGS. 6–18. Electron micrographs of *Pyramimonas tychotreta,* sp. nov. FIG. 6. Whole cell, some body and flagellar scales noticeable. FIGS. 7–9. Box scales showing perforations of the scale floor and striations in four groups. FIGS. 10–12. Crown scales. FIG. 13. Footprint scales. FIGS. 14–16. Limuloid scales. FIG. 14 shows four of the nine longitudinal rows of limuloid scales surrounding each flagellum. FIG. 17. Flagellar tip with hair scales. FIG. 18. Pentagonal flagellar underlayer scales. A single limuloid scale is also visible. FIGS. 6, 7, 10, 11, 13, 16, and 17: Uranyl acetate stained whole mounts. Figs. 8, 12 and 15: Shadow-cast whole mounts. FIGS. 9, 14, 18: Freeze-etched. Scale bars = 5 μm (FIG. 6); 200 nm (FIGS. 7–18).

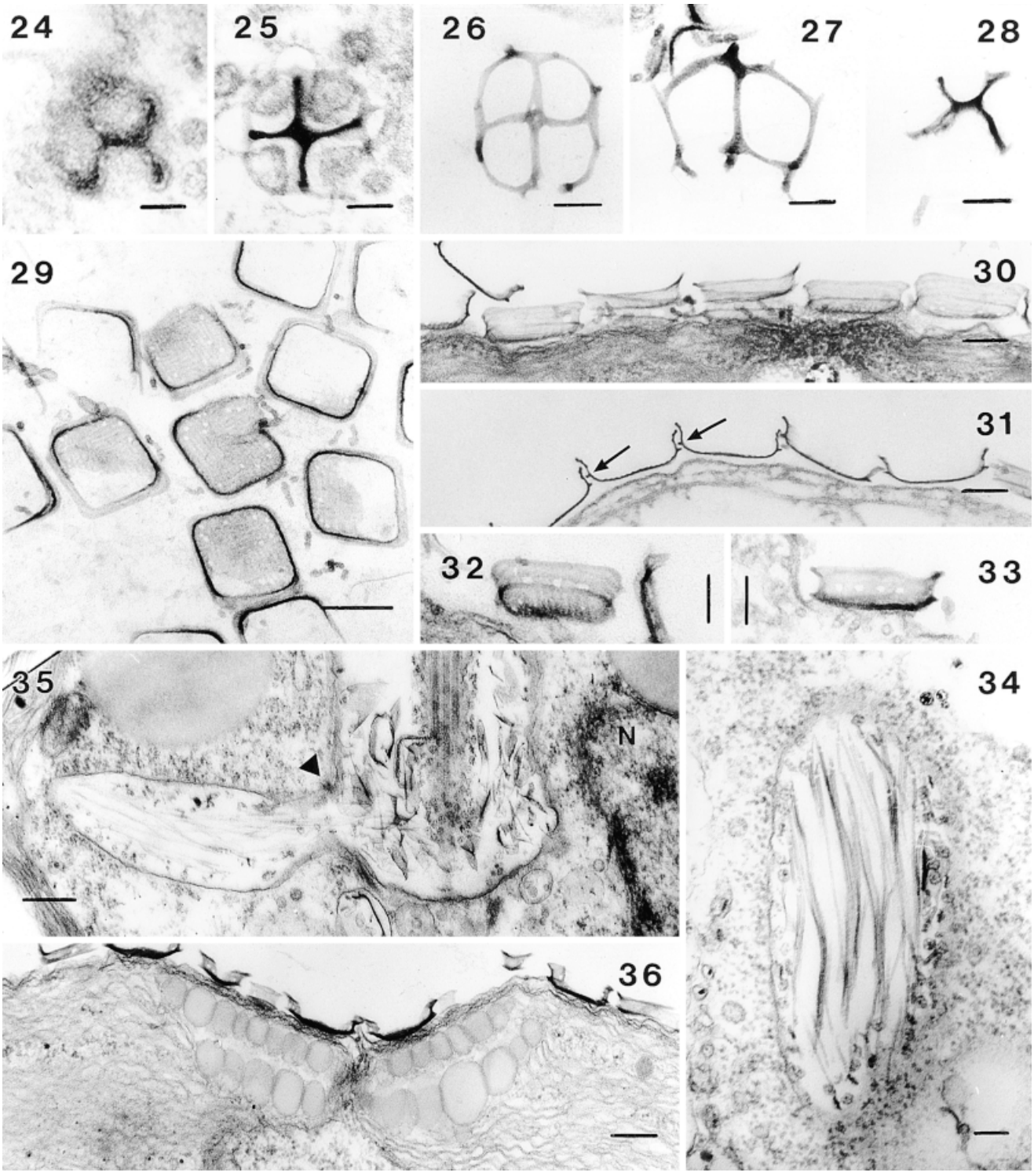


FIGS. 19–23. Schematic drawings of *Pyramimonas tychotreta*, sp. nov. and the most characteristic body and flagellar scales. FIG. 19. Whole cell, showing position of eyespots, pyrenoid, and flagella in a resting cell. FIG. 20. Crown scale. FIG. 21. Box scale with solid walls. Other box scales have walls with up to five perforations. FIG. 22. Footprint scale. FIG. 23. Limuloid scale. Not to scale.

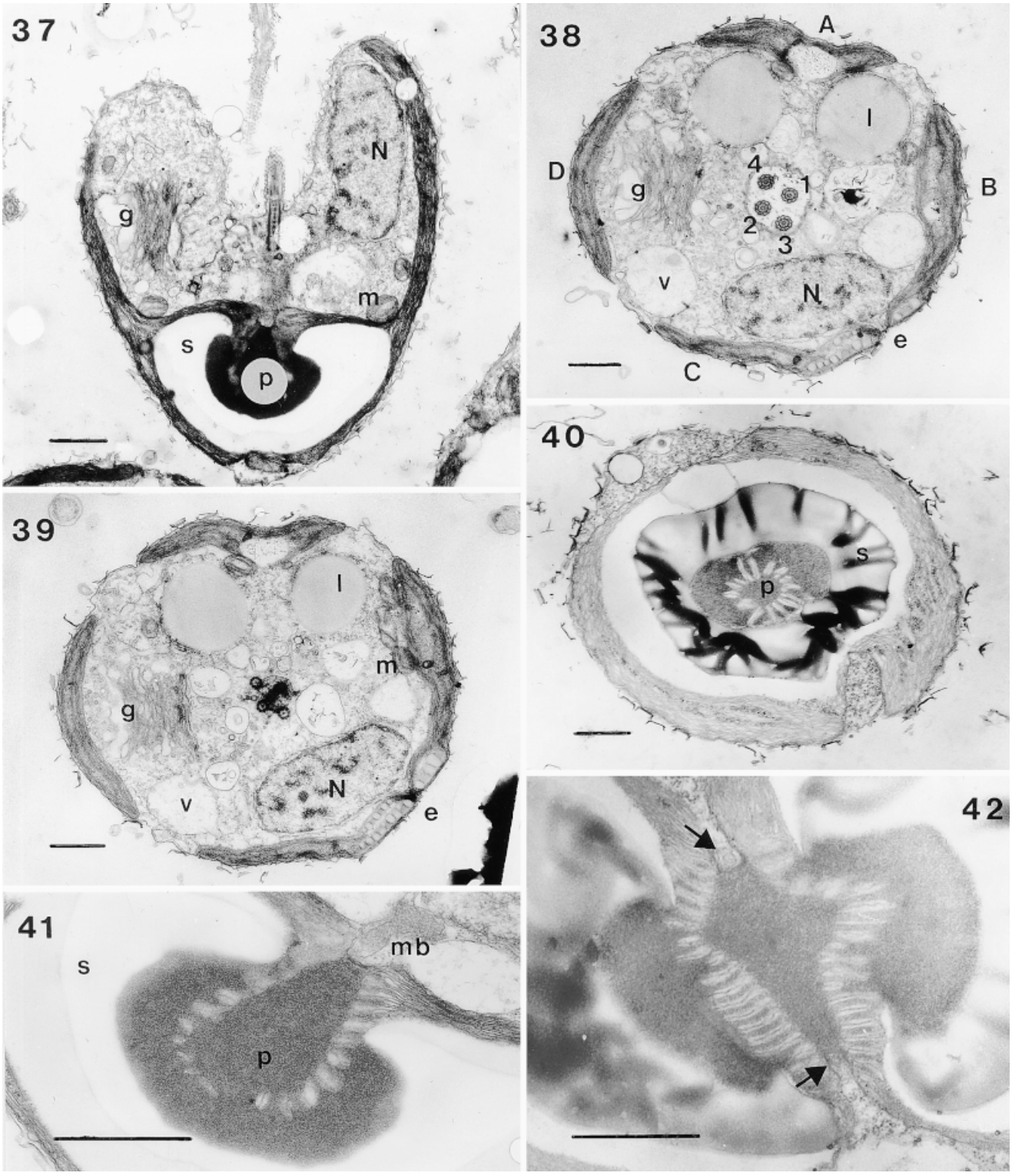
into four lobes (Figs. 38–39). Each eyespot is bilayered, without an intervening thylakoid (Fig. 36) and positioned in closely appressed chloroplast lobes (Fig. 38). Following the terminology of Hori et al. (1995), the eyespots are located in chloroplast lobes B and C, close to the nucleus (=Group III). The pyrenoid (type 1 *sensu* Hori et al. 1995) is enclosed by a starch sheath (Figs. 37, 40). A ring of paired thylakoids invades the pyrenoid matrix from the anterior end (Figs. 40–41). A structure resembling a plastid-dividing ring is observed during chloroplast division (Fig. 42). The plastid-dividing ring is seen as an electron-dense deposit in the division furrow. Before release, the flagellar scales accumulate in a reservoir located opposite the nucleus (Figs. 34–35). Vesicles containing large body scales are often observed (Figs. 38–39). The Golgi apparatus comprises two opposite dictyosomes (only one is visible in Figs. 37–39). Various stages of scale formation are always visible in the cisternae (Figs. 24–25, 37–39).

Flagella and basal bodies. The basal bodies have a discontinuous osmiophilic core (Figs. 37, 43), similar to *P. gelidicola* (McFadden and Wetherbee 1984) and *P. orientalis* (Moestrup and Thomsen 1974). No structures are otherwise present in the lumen apart from numerous ribosomes (see basal body 1 in Fig. 43). The transition region has an undivided stellate pattern. A cylinder is positioned distal to the stellate pattern at the level where the two central microtubules of the flagella terminate (Fig. 43). A weakly developed transverse septum (or plate-like structure) was encountered (Fig. 44). The flagellar pit is lined by 80–100 microtubules (Fig. 47).

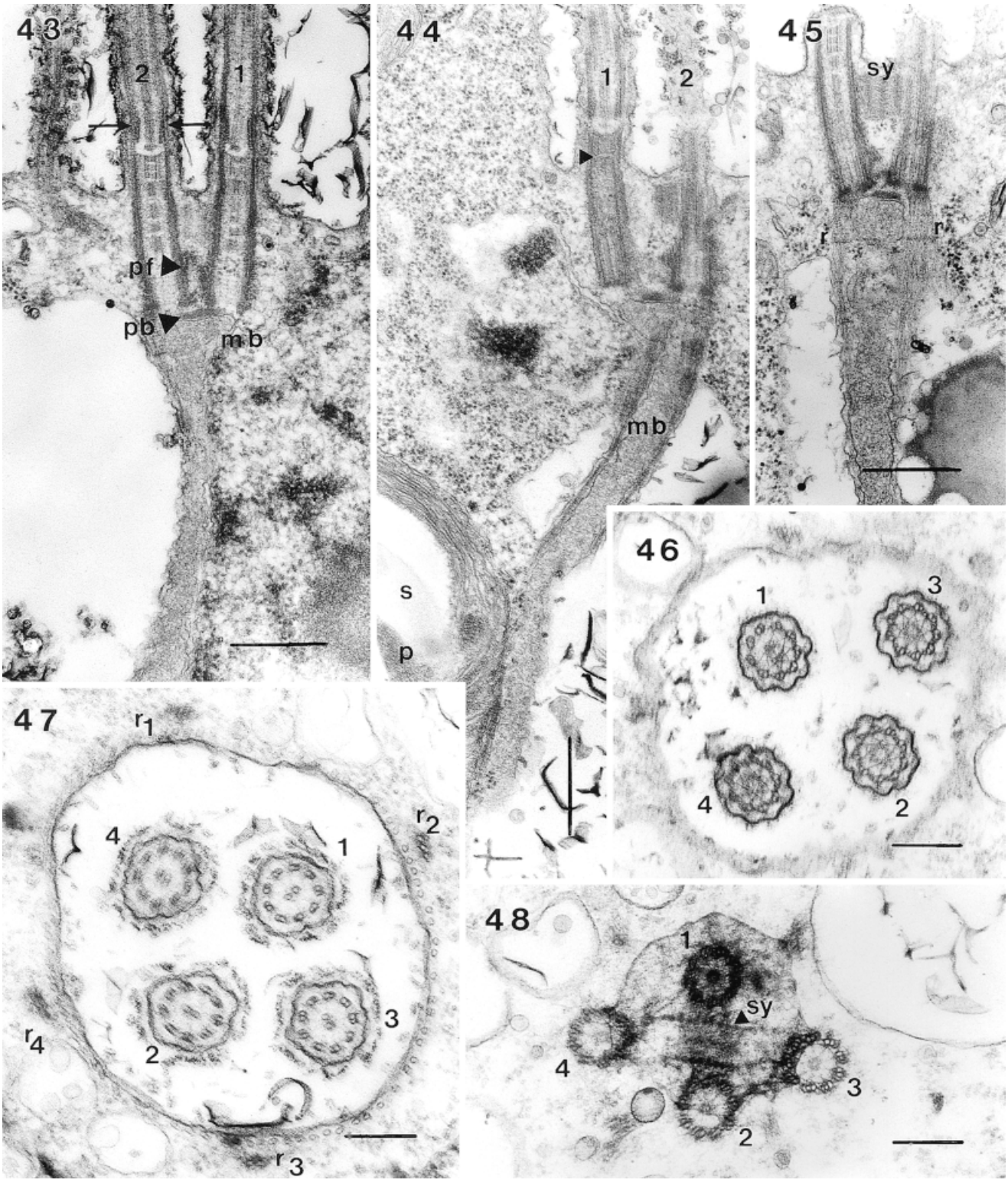
Flagellar apparatus. A detailed reconstruction of the flagellar apparatus was not attempted, but a few details are provided (Figs. 43–48). The flagellar apparatus has a rhombic configuration *sensu* Inouye et al. (1985) and the four basal bodies have been numbered in Figs. 46–48 according to Moestrup and Hori (1989). The synistosome connecting basal bodies 1 and 2 is 200 nm long by 140 nm wide by 160 nm deep (Figs. 45, 48). An intermediate fiber (*sensu* Sym and Pienaar, 1991) was not detected beneath the synistosome. The microtubular root system with roots labeled r_1–r_4 *sensu* Moestrup (2000) (1d, 1s, 2d, and 2s, respectively, in previous terminology) consists of 2, 4 (3/1), 2 (r_1–r_3) (Fig. 47). The r_4 root probably comprises three microtubules, but the exact number was difficult to verify from the sections studied. The microbody and rhizoplast (system II fiber *sensu* Melkonian 1980) are in direct contact and extend from the proximal end of the basal bodies to the pyrenoid (Fig. 44). Proximal to the basal apparatus, the rhizoplast branches to attach to individual basal bodies (Fig. 45).



Figs. 24–36. Sections through body and flagellar scales of *Pyramimonas tychotreta* sp. nov and details of cell ultrastructure. Figs. 24–25. Cross sections of developing crown scales. Fig. 26. Transverse section through the base of a mature crown scale. Fig. 27. Longitudinal section through a mature crown scale showing two small spines on each of the upright arms. Fig. 28. Cross section through the apical end of a mature crown scale showing the short distal bar connecting the four upright arms. Fig. 29. Cross section through the baseplates of box scales depicting the striations in four perpendicular groups. Footprint scales are scattered between box scales. Fig. 30. Vertical section through box scales with solids walls. Fig. 31. Longitudinal section through the central part of box scales. Arrows indicate external flanges, perhaps interlocking adjacent scales. Figs. 32–33. Longitudinal section of box scales having walls with three and four perforations, respectively. Fig. 34. Mature flagellar hair scales in scale reservoir. Hair scales apparently with identical orientation. Fig. 35. Flagellar hair scales and pentagonal underlayer scales in the process of release from the scale reservoir to the flagellar pit. The duct fiber (arrowhead) at the duct aperture is seen as an electron-dense area. Fig. 36. Eyespots in closely appressed chloroplast lobes. Each eyespot consists of two rows of osmiophilic globules not separated by thylakoids. Scale bars = 100 nm (Figs. 24–28, 32–33); 200 nm (Figs. 29–31, 34–36).



Figs. 37–42. General ultrastructure of *Pyramimonas tychotreta* sp. nov. Fig. 37. Longitudinal section showing the disposition of major organelles; nucleus, mitochondria, Golgi body, and pyrenoid surrounded by starch sheath. Figs. 38–39. Transverse sections at the level of the flagellar pit region and basal body region, respectively. Fig. 40. Transverse section through the pyrenoid region. The pyrenoid matrix is invaded by pairs of thylakoids. Fig. 41. Longitudinal section of pyrenoid and starch sheath. The microbody is closely associated with the apical end of the pyrenoid. Fig. 42. Dividing chloroplast and pyrenoid. The arrows indicate the plastid-dividing ring. Scale bars = 1 μm.



FIGS. 43–48. Details of the flagellar apparatus of *Pyramimonas tychotreta* sp. nov. FIGS. 43–45. Longitudinal sections showing the proximal band separating the microbody–rhizoplast complex and the proximal part of the basal bodies. Arrows indicate the transitional fiber. The arrowhead in FIG. 44 marks a weakly developed transitional plate-like structure. FIGS. 46–48. Transverse sections through the flagellar pit and basal body region. The microtubular roots are labeled in FIG. 47. Scale bars = 500 nm (FIGS. 43–45); 250 nm (FIGS. 46–48).

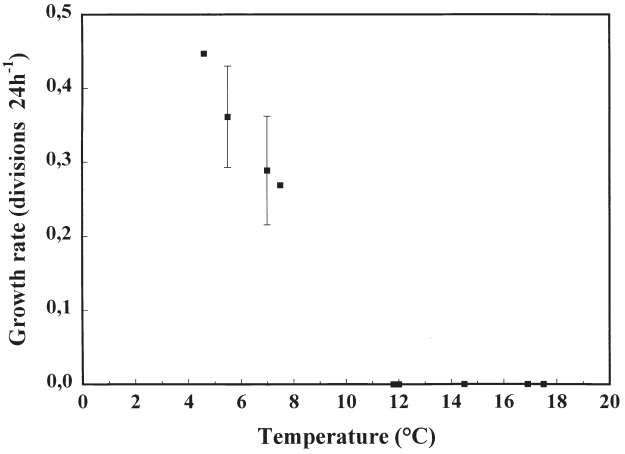


FIG. 49. Growth response of *Pyramimonas tychotreta* to different temperatures during the exponential phase. Error bars represent ±1 SD from the mean growth rate in duplicate sets of cultures. No error bars are given for experiments at 4.6° C and 7.5° C, as duplicates were not performed.

A prominent proximal connective fiber between basal bodies 1 and 2 is seen in Fig. 43.

Temperature and salinity studies. Cells of the clonal isolate of *P. tychotreta* grown at 4.6° C ± 1.6 had a growth rate of 0.45 divisions·24 h^{-1}, giving a generation time of 2.2 d (Fig. 49). Cells grown at 4.6–5.5° C were still in the exponential phase when the experiment was stopped after 21 d (not shown). The growth rate fell to ~0.28 divisions·24 h^{-1} for cultures grown at 7° C ± 0.2 and 7.5° C ± 0.4 and these cells reached the stationary phase after 14 d. No growth occurred at temperatures above 12° C (Fig. 49). Clonal cultures grown in the salinity range of 15–35 psu all reached the exponential phase of growth within 24 d. In exponentially growing cultures, growth rates ranged from 0.42 divisions·24 h^{-1} at 15 psu to 0.59 divisions·24 h^{-1} at 20 psu (Fig. 50). Growth rates were slightly higher at 20–30 psu than at 15 and 35 psu. Cells grown at 10 psu increased in number from about 2000 to 3000 over the first 4 d and then declined to zero by day 11, when the experiment was terminated.

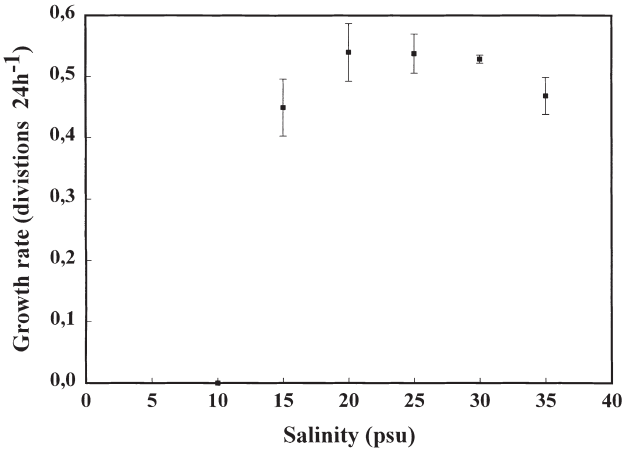


FIG. 50. Growth response of *Pyramimonas tychotreta* to different salinities during the exponential phase. Error bars represent ±1 SD from the mean growth rate (duplicate sets of cultures).

DISCUSSION

Arctic and Antarctic microalgae associated with the sea ice community and open water systems experience fluctuating salinities from seasonal formation, break-up, and melting processes. Although the isolate of *Pyramimonas tychotreta* from the Weddell Sea was observed in open water samples, physiological studies reveal that it is well adapted to an icy environment, being a cold water stenotherm and euryhaline. Autecological experiments on two species of *Pyramimonas* isolated from arctic Canada (Northern Foxe Basin) also revealed that growth stops at temperatures above 10–11° C (Daugbjerg and Moestrup 1992a, 1992b). At temperatures of 2–7° C the maximal growth rate was similar (~0.4 divisions·24 h^{-1}) for the isolates of the three polar species of *Pyramimonas* studied. However, these studies show that they survive and divide at temperatures well in excess of those occurring in polar waters. It remains to be demonstrated whether these polar species occur in subpolar or even temperate regions, as it has been for *P. nansenii* (Braarud) Thomsen, which originally was found in East Greenland Braarud (1935) but since has been observed beneath the ice of a Danish fjord (Thomsen 1988).

Prior to this study, only one species of *Pyramimonas*, *P. gelidicola*, had been successfully isolated from sea ice in Antarctica (McFadden et al. 1982). *Pyramimonas gelidicola* belongs to the subgenus *Vestigifera* and shares many morphological features with *P. tychotreta*. However, a detailed comparison at the ultrastructural level reveals a number of important differences, indicating that the two taxa are separate species. The length-to-breadth ratio in *P. gelidicola* is 1.88, compared with 1.54 in *P. tychotreta*. Both species possess a cup-shaped chloroplast appressed to the plasmalemma and an eccentric pyrenoid surrounded by a starch sheath. The anterior of the chloroplast in *P. gelidicola* divides to form eight widely spaced lobes, whereas only four relatively closely appressed lobes are present in *P. tychotreta*. The eyespots in *P. gelidicola* are separated by an evagination of the nucleus, whereas in *P. tychotreta* the nucleus lies against the eyespots. Limuloid scales on the flagella are markedly different. In *P. tychotreta* they are striated with a few perforations, while those of *P. gelidicola* are longer (400–500 nm, compared with ~313 nm in *P. tychotreta*) with large irregular perforations. The pattern formed by the perforations differs significantly. Box scales are similar in size in the two species and the scale floor has an identical pattern with striations in four groups. However, the randomly positioned perforations in *P. tychotreta* are absent in *P. gelidicola*. In *P. gelidicola* the wall in box scales was described with approximately seven perfo-

rations and solid walls were not observed. The wall in box scales of *P. tychotreta* varies, as some scales possess solid walls but in other scales the walls have to up five perforations. These variations could not be correlated with abiotic factors. Cells exposed to the different temperature and salinity regimes applied here, produced box scales with solid or variably perforated walls. Apart from the difference in number of spines on the upright arms, the crown scales are similar (three per upright arm in *P. gelidicola* and two in *P. tychotreta*).

Variation in the structure of box scales is known also in other species of *Pyramimonas*. Box scales with or without a central spine or boss were reported in *P. cyclotreta* (Daugbjerg and Moestrup 1992a), *P. disomata* (McFadden et al. 1986), *P.* aff. *nephroidea* McFadden (A strain, Sym and Pienaar 1995), and *P. quadrifolia* (Daugbjerg and Moestrup 1993). In a recent study Sym and Pienaar (1995) showed that the scale floor in a clonal culture of *P. norrisii* Sym et Pienaar was identical to that previously reported for *P. moestrupii* McFadden by McFadden et al. (1986) who, despite some other subtle differences in this scale type, considered *P. norrisii* similar enough in most regards to relegate it to synonymy with *P. moestrupii*. Although the large body scales are considered important in species delimitation in *Pyramimonas* (Norris and Pienaar 1978), obviously some degree of morphological variation in the box scales has to be accepted, at least in some vestigiferan species. The architecture of body and flagellar scales in combination with the general ultrastructure of the cell should be used as a basis for species delimitation.

The flagellar and body scales in another *Pyramimonas*, *P. gorlestonae* Pennick et Cann isolated from Gorleston-on-Sea (Norfolk, England) resemble those of *P. tychotreta*. The limuloid scales of *P. gorlestonae* are cross-striped with about 8 striations (Pennick and Cann, 1982); *P. tychotreta* has 10–12 striations. The box scales have four perpendicular groups of striations as in *P. tychotreta*, but the random number of perforations applies only to *P. tychotreta*. The wall in box scales of *P. gorlestonae* is perforated by four or five apertures (Figs. 11–13, 17 in Pennick and Cann 1982); the authors do not mention the presence of solid walls in some box scales. Perforations of the upper rim, one at each corner, in box scales of *P. gorlestonae* have never been observed in *P. tychotreta*. The height of crown scales also differ (160 nm in *P. gorlestonae* and 300 nm in *P. tychotreta*). Each upright arm in *P. gorlestonae* has three spines (although two spines were also mentioned in the text, this was not supported by any micrographs), compared with two in *P. tychotreta*. Spines on the crown scales of *P. gorlestonae* are markedly larger than those of *P. tychotreta*. Although the four ribs extending from the middle of the proximal rim are mentioned and drawn by Pennick and Cann (1982, their Figs. 4 and 5), they are not easily detectable on the micrographs accompanying the paper. The four ribs are drawn with two spines per rib. Such spines are lacking in *P. tychotreta*. *Pyramimonas gorlestonae* measures 7–8.5 μm in length and 5.5–8

TABLE 1. Comparison of *Pyramimonas tychotreta* with three congenerics, *P. gelidicola*, *P. gorlesonae*, and *P. orientalis*.

	P. tychotreta	*P. gelidicola*	*P. gorlestonae*	*P. orientalis*
Length (μm)	8–12	14–18	7–8.5	4–13.6
Width (μm)	6–7	8–9	5.5–8	4–6.8
Length/breadth ratio	1.54	1.88	1.15	1.63
Cell body with lateral wings	No	No	Yes	No
Number of apical chloroplast lobes	4	8	4	4
Number of eyespots	2	2	1	2
Eyespots generated by evagination of nucleus	No	Yes	Not applicable	No
Length of limuloid scales (nm)	~313	400–500	~315	~330
Morphology of limuloid scales	10–12 ribs	Many perforations	~8 ribs	Few perforations
Morphology of box scale floor	Striations in 4 quadrants	Striations in 4 quadrants	Striations in 4 quadrants	~10 parallel striations[a]
Perforations of box scale floor	Random arrangement	None	None	None
Perforations of box scale wall	Solid or up to 5 apertures	~7	4–5	Solid
Perforations of upper rim of box scales	No	No	Yes	No
Height of crown scale (nm)	~300	~300	~160	~200
Number of spines on upright arms of crown sales	2	3	3	0
Geographic distribution	Polar (Antarctica)	Polar (Antarctica)	Temperate (England)	Cosmopolitan
Reference	This study	McFadden et al. 1982	Pennick and Cann 1982	Moestrup and Thomsen 1974, McFadden et al. 1986, Sym and Pienaar 1996

[a]Four of the strains studies by Pennick et al. (1978) and considered allied to *P. orientalis* possessed box scales with striations in quadrant similar to *P. tychotrata*.

μm in width and is therefore smaller than *P. tychotreta*. The length-to-breadth ratio in *P. gorlestonae* is 1.15, compared with 1.54 in *P. tychotreta*. The cell body of *P. gorlestonae* has four lateral wings that extend to the posterior end; wings are absent in *P. tychotreta*. A single bilayered eyespot is present in *P. gorlestonae*, positioned about 1/3 down the cell.

As already mentioned, the transition region and the discontinuous osmiophilic core in *P. tychotreta* are similar to *P. orientalis* and, since this taxon also occurs in polar waters (but restricted to the Northern Hemisphere), a comparison of its scale morphology is included. The comparison is based on isolates of *P. orientalis* studied by Moestrup and Thomsen (1974), McFadden et al. (1986) and Sym and Pienaar (1996). In *P. orientalis* the crown scales lack spines on the four upright arms, and these arms further emanate from the rounded corners of the otherwise square proximal rim. Shadow-cast and freeze-etched material clearly shows box scales with solid walls and the floor with either parallel striations (~8–10 stria) or sometimes with two or three quadrants formed by perpendicular striations. The scale floor lacks perforations. Thus, the comparison of crown scales and box scales possessed by *P. orientalis* and *P. tychotreta* reveals a number of significant differences. Pennick et al. (1978) compared the scale morphology of 12 strains of *Pyramimonas* considered allied to *P. orientalis*. Most of these strains were collected in England and later isolated into culture by Butcher. Although some of these have since been described as new species of *Pyramimonas*, five strains remain as allied to *P. orientalis* (CCAP LB 4/1, 11/92, 67/12, 67/13, and 67/14). The body scales of strain LB 67/13 resemble those present in the isolate of *P. orientalis* studied by Moestrup and Thomsen (1974) and McFadden et al. (1986); the remaining four strains possess body scales with a dissimilar morphology to both *P. orientalis* and *P. tychotreta*. The morphological differences outlined above are significant and *Pyramimonas tychotreta* is recognized as a new taxon. For ease of comparison, major differences of the scale architecture between *P. tychotreta*, *P. gelidicola*, *P. gorlestonae*, and *P. orientalis* are compiled in Table 1.

Pyramimonas tychotreta is not geographically restricted to the Weddell Sea (Daugbjerg, unpublished data). At a few stations visited in the Ross Sea, *P. tychotreta* formed a red-colored bloom in sea ice surface layers (infiltration community). In addition to motiles, cysts and a uniflagellated stage were discovered and considered part of the life history (Daugbjerg, unpublished data). The uniflagellate possessed box, limuloid, and hair scales identical to those of the quadriflagellated stage. It is likely that protists are dispersed over long distances by surface currents in the Southern Ocean and therefore have a circumpolar distribution limited by a species-specific upper temperature boundary.

Another yet undescribed species of *Pyramimonas* is known solely from whole mounts based on osmium fixed material also collected during the EPOS II cruise to the Weddell Sea (Thomsen, personal communication). It possesses trichocysts, indicating that it belongs to the subgenus *Trichocystis*. Two types of flattened box scales somewhat resembling those of *P. aurita* (Daugbjerg and Moestrup 1993) and *P. nansenii* (Thomsen 1988) from ice biota in the Northern Hemisphere further characterize this taxon. Three species of *Pyramimonas* are therefore presently known from antarctic sea ice and open water communities.

Water samples collected in arctic Canada in 1989 and 1992 (Daugbjerg and Moestrup 1992a, 1992b, 1993, Daugbjerg and Vørs 1994) contained an unexpected high number of new species of *Pyramimonas*. It was speculated that these were endemic to Foxe Basin (Daugbjerg and Moestrup 1993). However, cells and body scales of *P. aurita* were later observed in water samples from Disko Bay, West Greenland (D. Grastrup-Hansen, personal communication). Additionally, *P. aurita* and *P. dichotoma* were recorded in water samples from the Greenland Sea and the polynia in the northern part of Baffin Bay and Nares Strait and *P. cyclotreta* was also observed in the Greenland Sea (M. Gammelgaard, personal communication). This illustrates that sampling at remote destinations or even increased sampling frequency at any location may uncover the presence of species formerly considered to be of limited geographic distribution. However, the cold-water species of *Pyramimonas* described from arctic Canada still appear to be confined to cold waters in the Northern Hemisphere and the distribution of the genus in the Arctic and Antarctic ecosystems thus appears distinct.

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