Life history stages of *Pyramimonas tychotreta* (Prasinophyceae, Chlorophyta), a marine flagellate from the Ross Sea, Antarctica

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SUMMARY

During a summer cruise to the Ross Sea (Antarctica) areas of snow-covered sea ice were red-coloured due to high concentrations of the recently described *Pyramimonas tychotreta* Daugbjerg. Light microscopy of living material revealed that the population was comprised of quadriflagellate motile cells and thick-walled cysts. The red colour was due to large numbers of secondary carotenoid-containing granules, positioned in the periphery of motile cells and cysts. Mature cysts also contained numerous starch grains and lipid droplets. Cells from a red-coloured field sample turned green overnight as the secondary carotenoids disappeared when cells were placed in low light conditions. The sample then exhibited the typical grass-green colour of motile cells observed in water samples from the area. Under reduced light motile cells showed strong positive phototaxis. The encystment process involved the asexual transformation of quadriflagellate cells into cysts. A single type of square cyst scale, with perforated floors and walls, replaced the body scales of motile cells. A marked extension, often ending in a hook was at each corner of the cyst scales. Germinating cysts produced four motile cells. Electron microscopy showed the cyst wall to be tri-layered, with a thin, electron-dense inner layer, a thick middle layer and a thin outer layer. Sea ice samples with dense populations of motile cells and cyst stages also contained elongate uniflagellate cells. These cells were covered with box scales, foot-print scales, an underlayer of pentagonal scales, limuloid scales and flagellar hair scales identical to those present on the quadriflagellate stage. We tentatively suggest that the uniflagellate stage represents a gamete and its presence implies the occurrence of sexual reproduction. Although, fusion of gametes was not observed, a biflagellate cell with a larger volume was seen which may have been a zygote. How this stage fits into of the life history remains to be explained.

Key words: cyst ultrastructure, life history, Prasinophyceae, *Pyramimonas tychotreta*, Ross Sea.

INTRODUCTION

At present, more than 30 species of *Pyramimonas* Schmarda have been characterized ultrastructurally. Three additional species are known only from whole mount preparations (Daugbjerg and Moestrup 1993). Only a few studies have reported cyst formation in any of the species, and observations on sexual reproduction are still undocumented. Parke (1949) and Parke and den Hartog-Adams (1965) provided some of the first descriptions of species of *Pyramimonas* that produce cysts (*Pyramimonas grossii* Parke and *Pyramimonas amylifera* Conrad, respectively), indicating that these had a more complex life history. Hargraves and Gardiner (1980) subsequently showed that clonal cultures of *Pyramimonas amylifera*, isolated from tidal salt marshes, produced cysts with large starch grains when placed in the dark. Box and crown scales, similar to those of the motile stage, covered the surface of the cysts (Hargraves and Gardiner 1980). The cultured material also revealed several stages considered to represent different stages of the life history, including bi-, quadri-and octoflagellate, and multilobate cells. They observed cysts containing two types of flagellate cells, but determination of the number of flagella was not possible.

Another species, *Pyramimonas pseudoparkeae* Pienaar et Aken, was isolated from tidal pools on the South African coast. Thick-walled cysts were found in old cultures (Pienaar and Aken 1985). Pienaar and Aken's detailed study included observations on cysts using light and electron microscopy. Motile, so-called 'L-cells', produced cysts with a bi-layered cell wall after the shedding of the four flagella. The cysts always contained large amounts of storage material (starch and lipid droplets), and when cysts were transferred to fresh medium, four quadriflagellate cells were released. No scales were reported on the cyst surface (Aken and Pienaar 1981; Pienaar and Aken 1985).

Pyramimonas gelidicola McFadden is the first recorded species of *Pyramimonas* from Antarctic sea ice (McFadden *et al.* 1982). More recently van den Hoff *et al.* (1989) established unialgal cultures of *P. gelidicola* from Ace Lake and Ellis Fjord in the Vestfold Hills (Antarctica) that produced cysts similar to those observed in field material. Electron microscopy showed the thick-walled cysts to be covered with a single and unusual type of scale not present on the motile vegetative stage. Unfortunately, all attempts to induce cyst germination failed (van den Hoff *et al.* 1989).

Here we report on the ultrastructure of the cyst stage and part of the life history, including encystment, germination and an enigmatic uniflagellate stage in *Pyramimonas tychotreta* Daugbjerg, from Antarctic waters. All observations are based on field populations collected in the Ross Sea (Antarctica, January 1999).

MATERIALS AND METHODS
Study area

Dense populations consisting of motile and cyst stages of *P. tychotreta* were observed in first-year ice along longitude 165°W during a cruise to the Ross Sea, Antarctica. *Pyramimonas tychotreta* was present in surface layers of snow-covered sea ice and confined to more or less circular patches, often not wider than 20 cm. Usually *P. tychotreta* was observed associated with a brine tube system penetrating through the sea ice to the water column below. The material examined was collected at 165°25′W, 73°1′S (Fig. 1). The temperature of the sea ice was −1.4°C and salinity 25 psu.

Melting of sea ice samples and light measurements

In order to reduce the impact of osmotic changes, sea ice samples were melted by adding an equal volume of filtered seawater collected from a depth of *ca* 1000 m using Niskin bottles (Garrison and Buck 1986). Sterile seawater was obtained by gravity filtration through 0.2-μm filters. Photon flux densities were measured using a QSL 100 Biospherical light meter (Biospherical Instruments, Inc., USA).

Light and electron microscopy

Examinations of motile cells and cysts were made using a Leitz Dialux 20 microscope equipped with DIC, phase contrast and an electronic flash (Leitz, Germany), and an Olympus Provis AX70 microscope with DIC, phase contrast and epifluorescence (Olympus, Tokyo, Japan). Shadow cast and uranyl acetate-stained whole mounts and material for thin sectioning were prepared as outlined in Daugbjerg (1996).

RESULTS
Light and electron microscopy of motile cells and cyst stages

Sea ice samples with a distinct red colour consisted of motile cells and cysts. The red colour was due to numerous secondary carotenoid granules dispersed in the periphery (i.e., outside the chloroplast) of both motile cells and cysts. The quadriflagellate stage of *P. tychotreta* from the Ross Sea was 10–13 μm long and 7–8 μm wide (Figs 2–6), similar in size to a clonal culture established from a water sample collected in the Weddell Sea (Daugbjerg 2000). The body and flagellar scales of the Ross Sea isolate were identical to those of the Weddell Sea isolate and a detailed description of the quadriflagellate stage is given in Daugbjerg (2000). Here we briefly describe and illustrate the large body and flagellar scales considered important for species identification of the quadriflagellate stage from the Ross Sea (Figs 7–11). The box scales were square and measured approximately 300 nm in width (Figs 7–8). The scale floor possessed quadrants of parallel striations running perpendicular to one another. The floor was perforated by a number of randomly positioned perforations. The scale wall was either solid or perforated (not shown). The crown scales were also
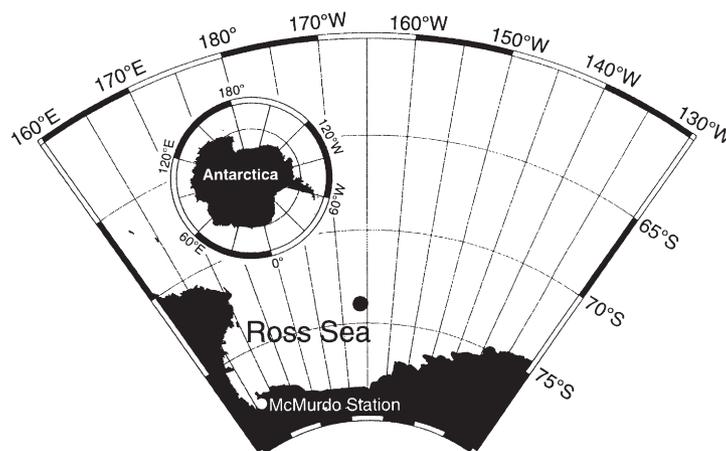
Fig. 1. Map showing the area where motile cells, cyst stages and the uniflagellate stage of *Pyramimonas tychotreta* were collected from the Ross Sea (modified from original made by Chris Masters, ASA).
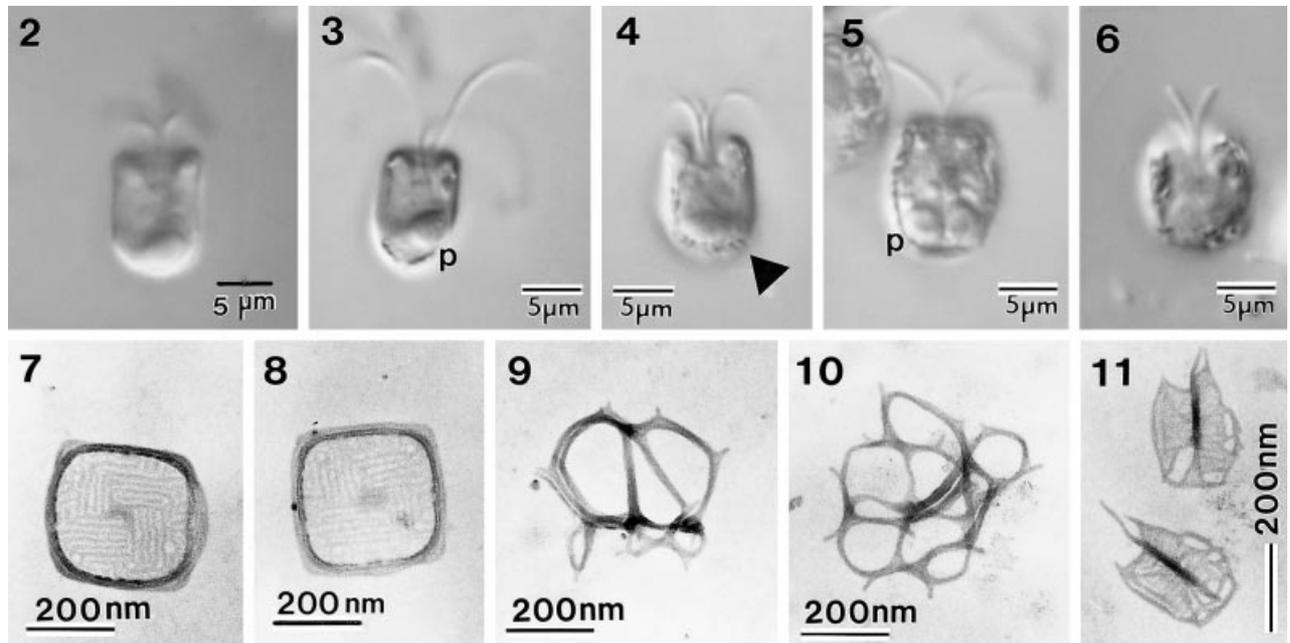
Figs 2–11. Light (differential interference contrast) and electron micrographs of *Pyramimonas tychotreta*. 2. Live cell placed in a cool room overnight with reduced light. There are no traces of secondary carotenoid granules. 3–6. Motile cells in a freshly collected sample showing the presence of numerous secondary carotenoid granules at the periphery of the cell (arrowhead in 4). 5. A dividing cell with two pyrenoids. 7–8. Uranyl acetate-stained box scales. 9–10. Uranyl acetate stained crown scales. 11. Uranyl acetate-stained limuloid scales. p, pyrenoid.

square, but with rounded corners. They measured approximately 300 nm in width (Figs 9–10) and 300 nm in height. (Fig. 9) Four upright arms extended slightly displaced from the middle of a proximal rim and connected distally with a central strut (Fig. 9). Two spines were present on each of the upright arms (Fig. 9). Two spines were also present on each side of the proximal rim (Fig. 10). The limuloid scales were approximately 340 nm long and 190 nm wide, thus slightly longer compared to the limuloid scales of the isolate from the Weddell Sea (Daugbjerg 2000). The scales bore 10–12 transverse ribs and often more than two perforations were present proximally (Fig. 11).

The motile cells showed positive phototaxis as they were observed to concentrate in the corner of a 4-L plastic container closest to the artificial lighting in the ship's cool room. The diameter of mature, thick-walled cysts was 13–18 µm, whereas germinating cysts were larger (compare Fig. 14 with Figs 16–18). Hence, the cyst diameter increases either prior to or during the germination process. The cysts always contained large numbers of starch grains and lipid droplets (Figs 12–14) in addition to the carotenoids (Figs 12,13). Cyst stages in freshly collected, red-coloured sea ice samples were seen to contain numerous small droplets of secondary carotenoids dispersed along the periphery (Fig. 12 and inset). When kept at reduced light (4.8 µmol photons m^{-2} s^{-1}) the droplets had aggregated to form a cluster some hours later (Fig. 13). The following day the sample was green and all traces of secondary carotenoids had disappeared (compare Figs 12,13 with Fig. 14). Another red-coloured sea ice sample consisting of mostly motile cells also turned green overnight as the secondary carotenoids disappeared (compare Fig. 2 with Figs 3–6). The photon flux density was approximately 1990 µmol photons m^{-2} s^{-1} below a 7-cm snow layer covering the sea ice at the time the samples were collected and, therefore, cells were exposed to more than 400 times the light level measured in the ship's cool room. Using epifluorescence microscopy the cysts autofluoresced red, indicating chlorophyll pigmentation (not shown).

Encystment and ultrastructure of the cyst stage

Whole mounts and sectioned material showed quadriflagellate cells in the process of transformation to cysts (Figs 19–21). The process of encystment involved replacement of body scales with a single type of cyst scale (Fig. 22). This was apparently initiated at the apical end, where vacuoles containing cyst scales were observed (Fig. 21). Additionally, sections of flagellate cells in the process of encystment showed them to be surrounded by cyst scales, except at the antapical end that was still covered by box scales (Fig. 19). Motile cells covered mostly by cyst scales still divided (cell with two pyrenoids in Fig. 19) and division appeared to continue until the formation of the thick cyst wall.
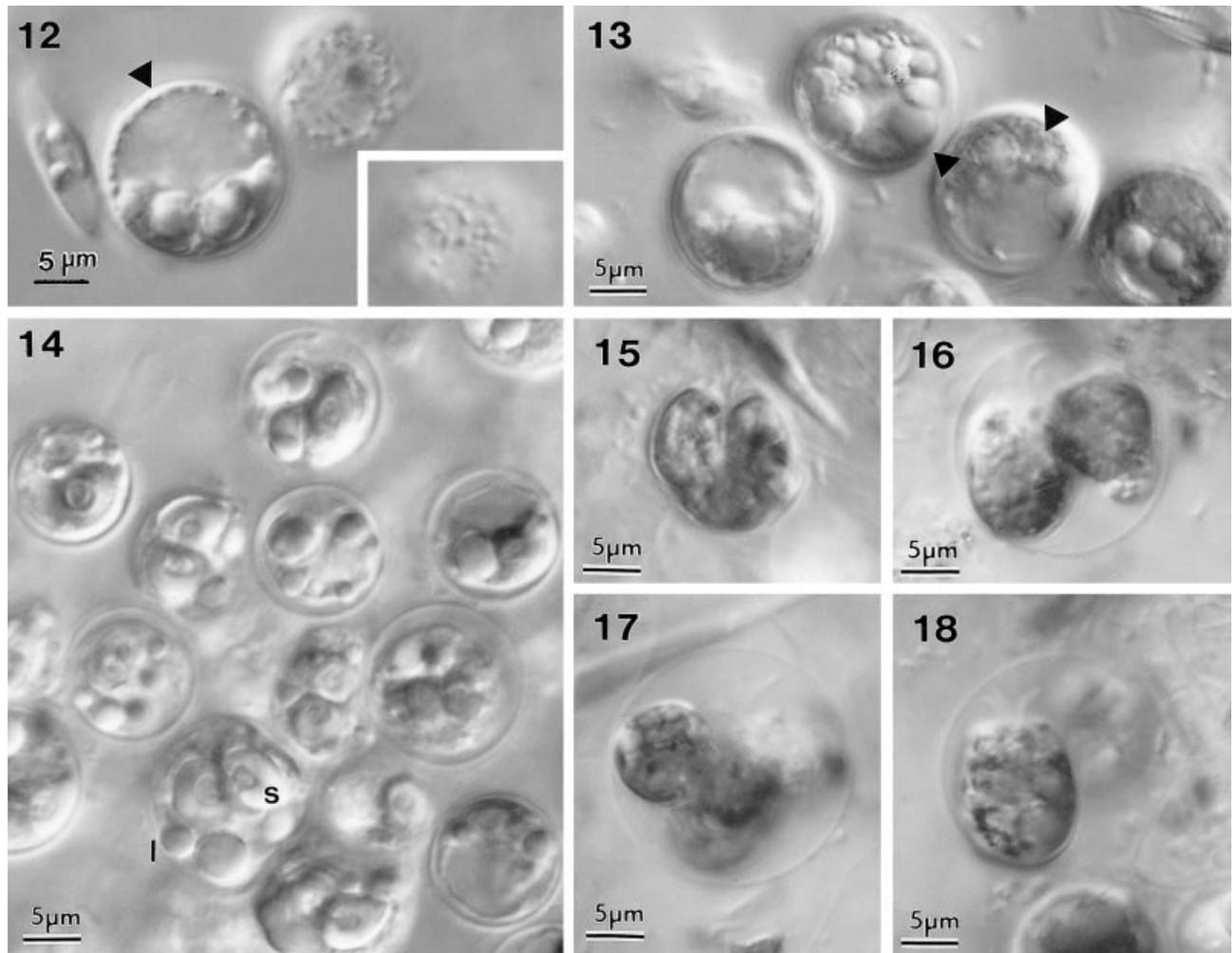
Figs 12–18. Light microscopy (differential interference contrast) of live cysts stages of *Pyramimonas tychotreta*. 12. Cysts from a freshly collected sample. The surface of the cysts is densely packed with secondary carotenoid granules (arrowhead). The inset shows the carotenoids at the cyst surface. 13. Cyst sample placed for a few hours under reduced light. Secondary carotenoid granules have relocated to form a more or less tight cluster (arrowhead). 14. Cysts placed in a cool room overnight. Numerous lipid droplets and starch grains are visible, but the secondary carotenoid granules are no longer present. 15. Probably an early stage in cyst germination showing the first division (cleavage of the cyst contents). 16. Germinating cyst with two motile cells. 17. Germinating cyst with four motile cells. 18. Two mature motile cells still left in the cyst wall. l, lipid droplets; s, starch grains.

Cysts were covered only by cyst scales (Fig. 22). The cyst scales were square, approximately 150 nm wide and with an approximately 60-nm high rim (Figs 23–25). They were only half the width of the box scales and crown scales present in the flagellated stage (Daugbjerg 2000). The scale wall was perforated by a few holes (Figs 19–21), while the scale floor was traversed by a few relatively large and many smaller perforations (Fig. 27). A distinct horn-like extension was present in each corner (Figs 23–25). These extensions were 300–500 nm high and often terminated in a hook. Immature cysts, in which the cyst wall was not fully developed, contained starch grains and lipids droplets. The wall in mature cysts possessed three layers: a thin outer layer (about 50 nm thick), a thick middle layer (about 225 nm thick) and an electron dense inner layer approximately 50 nm thick (Fig. 26).

A uniflagellate stage

The freshly collected, red-coloured sea ice samples also contained numerous elongate cells, each bearing a single flagellum (Figs 28–32). The cells were 14–16 μm long and 3–5 μm wide at the apical end. The apical end was truncate and the posterior end either rounded or pointed (compare Fig. 28 with Fig. 30). The length of the flagellum approximated the cell length. The single chloroplast was positioned in the posterior end of the cell as verified by epifluorescence microscopy (not shown). Cells stained with 4′6-diamidino-2-phenylindole (DAPI) showed that the nucleus was positioned centrally (not shown). Video-recorded cells showed an unusual beat of the single flagellum. The cell swims forward while the flagellum beats in circles. During swimming the apical and antapical cell ends
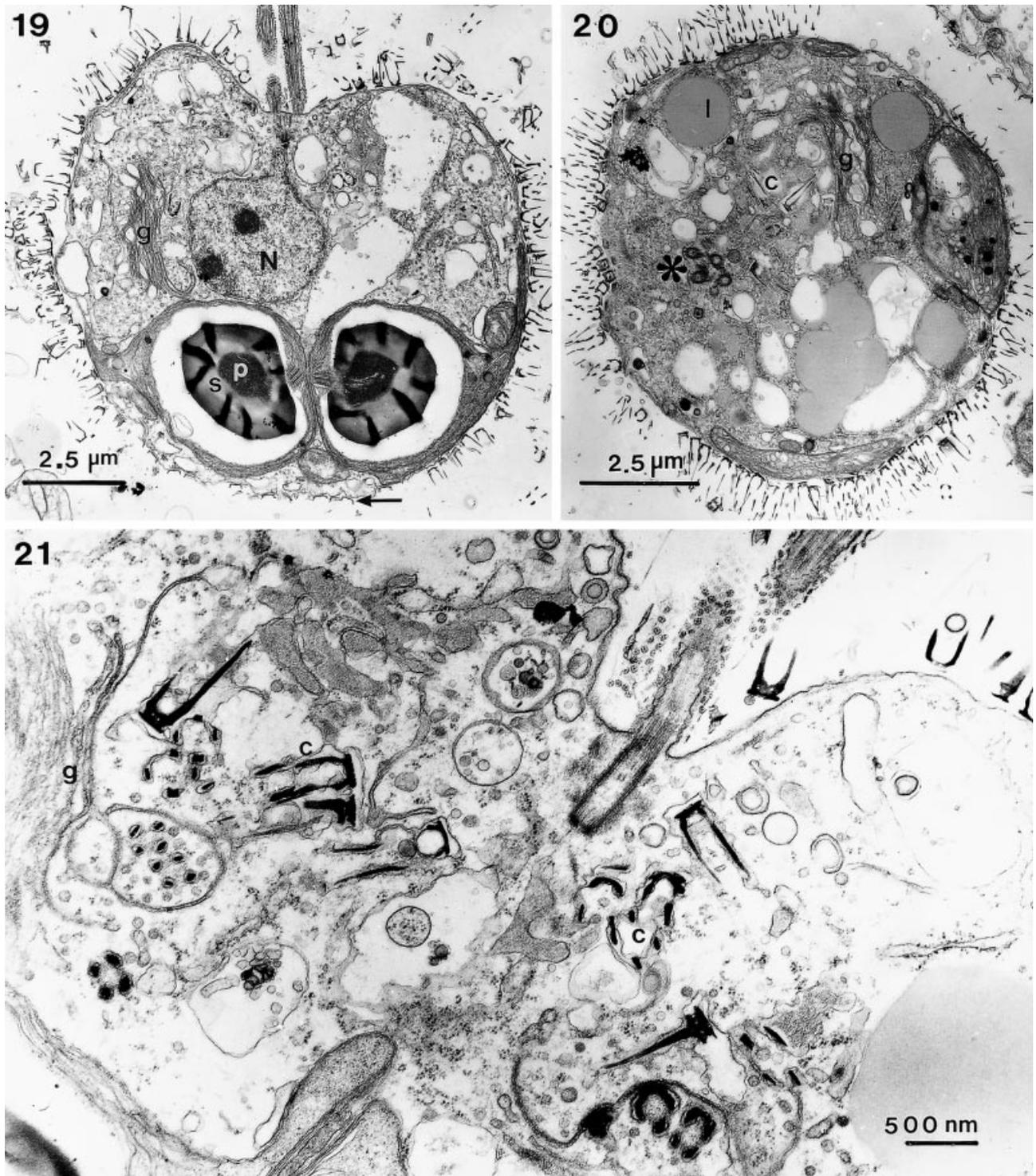
Figs 19–21. Ultrastructure of cyst formation in *Pyramimonas tychotreta*. 19. Longitudinal section through a cell completely covered with cyst scales except for the antapical end that still possesses box scales typical of motile cells (arrow). 20. Transverse section through the basal body region (asterisk) showing newly formed cyst scales in vacuoles and numerous lipid droplets. 21. Longitudinal section of the apical end showing cyst scales formed in the region between the Golgi bodies and the flagellar apparatus. c, cyst scales in vacuoles; g, Golgi body; l, lipid droplets; N, nucleus; p, pyrenoid; s, starch grains.

form circular motions while the middle of the cell does not oscillate. The cells rotate around their longitudinal axis. When cells are at rest the proximal part of the flagellum often takes a curved position (Figs 30–32).

The cell body is completely enclosed by box scales (Figs 33–35,37–39). The box scales are identical in morphology and size to those of the quadriflagellate cells of *Pyramimonas tychotreta* from the Weddell Sea
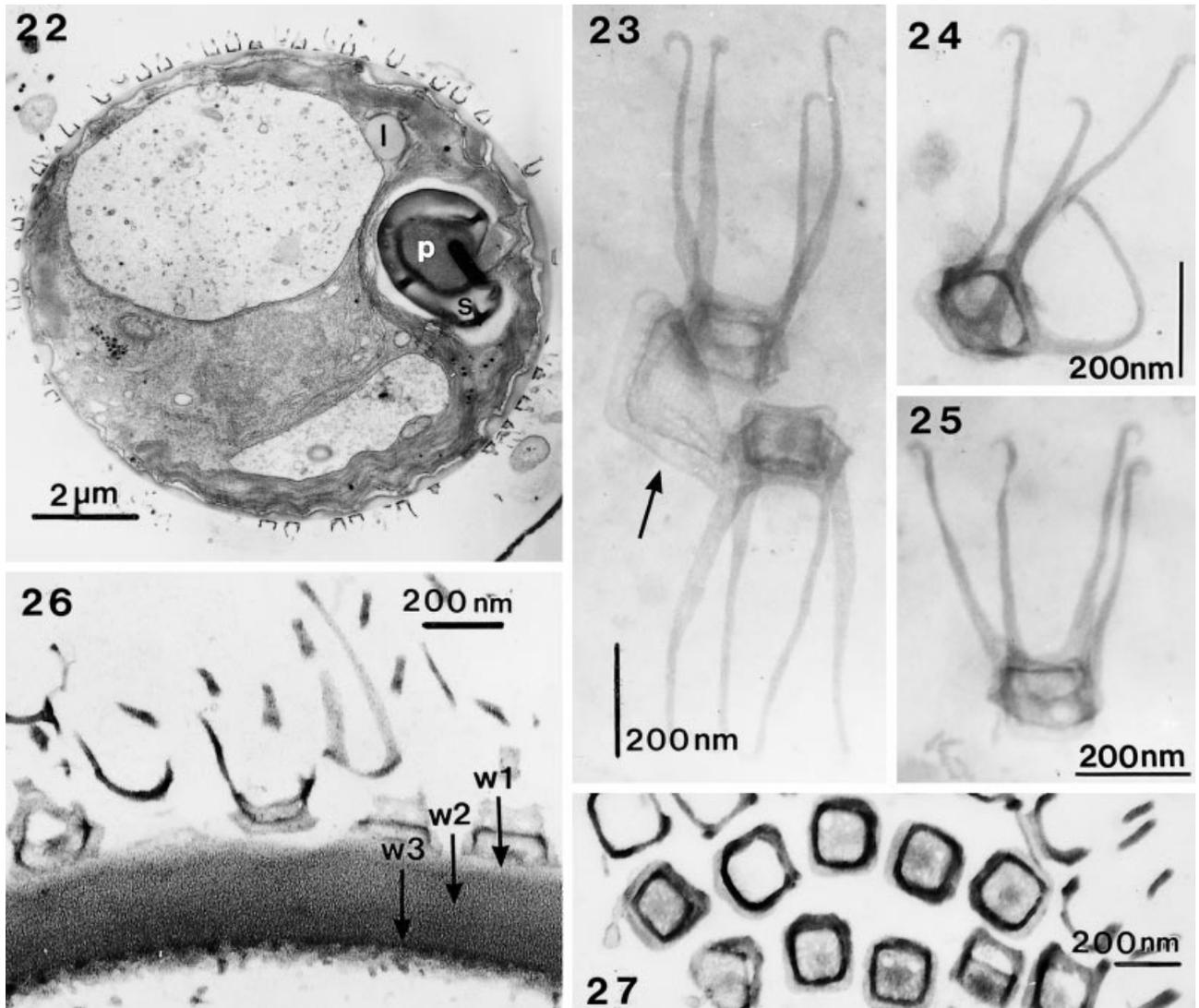
Figs 22–27. Cyst and cyst scales in *Pyramimonas tychotreta*. 22. Section through an immature cyst. The cyst wall is not yet fully developed, but the characteristic cyst scales are seen to cover the cyst in a tight layer. Note a single lipid droplet (l) and the pyrenoid (p) surrounded by a starch grain (s). 23–25. Uranyl acetate stained cyst scales. A box scale from the motile stage is also present in 23 (arrow). 26. Section through the thick tri-layered wall of a mature cyst. Cyst scales cover the surface of the cyst. w1, thin outer layer of cyst wall; w2, thick middle layer of cyst wall; w3, electron dense inner layer of cyst wall. 27. Transverse section through the base plate in cyst scales. Note the irregularly sized perforations.

(Daugbjerg 2000). Only box scales with perforated walls and foot-print scales were observed. Foot-print scales were interspersed between box scales as in the quadriflagellate stage (Fig. 35). Crown scales were not observed, but as they are known to drop off easily during preparation, such scales may have been present. Whole mounts and sectioned material confirmed that this stage possessed only a single flagellum (Fig. 38). It emerged from a narrow apical depression, and it was covered by pentagonal underlayer scales, limuloid scales and hair scales identical to those on the flagella of the motile stage of *P. tychotreta* (Figs 36,38).

The general organization of cell organelles is depicted in Figs 37–39. The Golgi body was positioned in the apical end and has been observed to produce box scales (not shown). Starch grains surrounded the pyrenoid in the antapical end (Fig. 39). The single basal body is approximately 850 nm long and did not appear to be associated with a rhizoplast/microbody complex (not shown). The flagellar apparatus has not been examined in detail, but at least one flagellar root was present (not shown). The uniflagellate stage was only observed in the red-coloured sea ice samples that comprised large numbers of motile cells and cyst stages of *Pyramimonas tychotreta*. Light microscopy of live material did not show uniflagellates in the process of fusion, but an elongate cell with two beating flagella was observed and video recorded. The cell is not shown
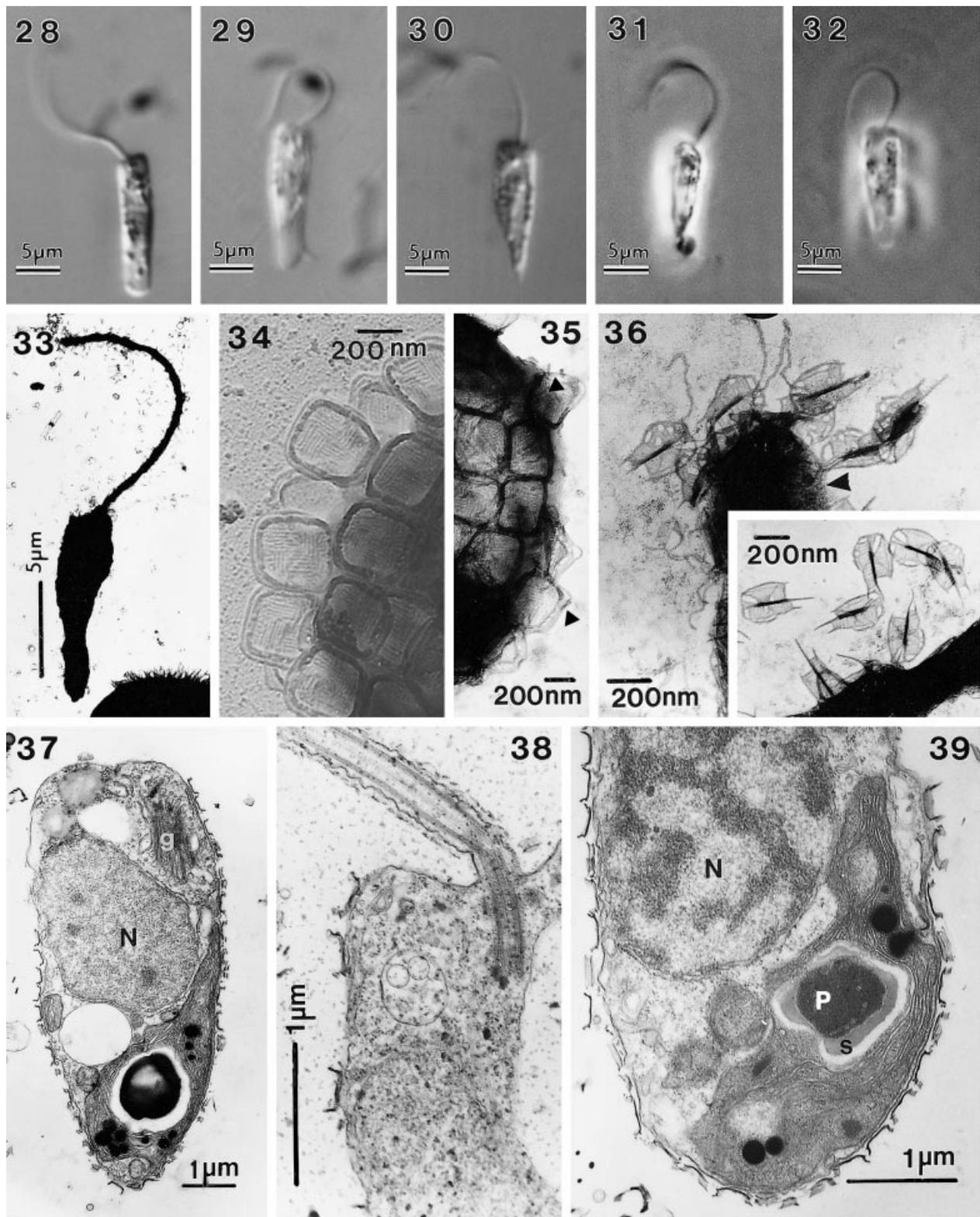
Figs 28–39. Light and electron micrographs of the uniflagellate stage of *Pyramimonas tychotreta*. 28–30. Live cells, differential interference contrast. 31–32. Live cells, phase contrast. 33–36. Whole mounts. 37–39. Longitudinal sections. 33. Whole cell stained with uranyl acetate. Body and flagellar scales may be discernable. A mature cyst is partly visible at the bottom. 34. The cell body covered by box scales (shadow cast). 35. Foot-print scales are scattered among the box scales (arrowheads). 36. Flagellar tip with limuloid scales, hair scales and pentagonal underlayer scales (arrowhead). Inset shows limuloid scales. 37. Section through the uniflagellate stage showing the general disposition of organelles: nucleus (N), chloroplast, pyrenoid and Golgi body (g). 38. The single flagellum is inserted in a depression. Note the long basal body. 39. High magnification of the posterior end. A starch grain (s) surrounds the eccentric pyrenoid (p). Box scales with perforated walls cover the cell body.
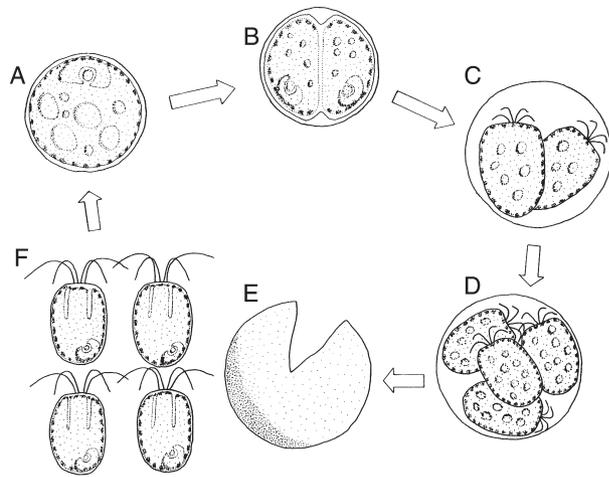
Fig. 40.   Proposed life history of *Pyramimonas tychotreta* based on field material collected in the Ross Sea. A. Cyst with pyrenoid, starch grains and lipid droplets. B. Division of cyst contents resulting in the formation of two cells (first step in the germination process). C. Cyst wall containing two vegetative cells each with four short flagella. D. Cyst wall with four motile cells formed from division of cells in C. E. Broken cyst wall. F. Four mature flagellate cells released from the cyst case. Encystment (F–A) involves (i) loss of flagella; (ii) replacement of the body scales with a single type of cyst scales; and (iii) the formation of a tri-layered cyst wall. Not drawn to scale.

due to the poor quality of the digitally captured images, but it can be obtained electronically upon request from the first author. This cell, which we consider a zygote, had the same body length as the uniflagellate stage, but was wider, indicating that it was likely to be the result of fusion between uniflagellates. This stage has not been observed in any of the cultures brought back to our laboratory, hence it is only known from field material.

Life history

Field samples were comprised of a number of stages which we considered part of the alternating stages in its life history. The quadriflagellate cells constituted the main part of the life cycle and divided by binary fission. The motile cells may have developed into mature cysts through a process that involves shedding of the four flagella, a replacement of body scales with cyst scales and the formation of a tri-layered cyst wall (encystment). Cysts are considered asexual as they did not form following fusion of motile cells. The contents of the thick-walled spherical cysts divided and produced two quadriflagellate cells (Figs 15,16). Division of these two cells produced four motile cells within the cyst wall (Fig. 17). Recently divided flagellate cells appeared smaller, but grew to the size of motile cells before escaping from the ruptured cyst wall (Fig. 18).

Figure 40 illustrates those stages in the life history of *P. tychotreta* that have been documented in detail. Unfortunately, our observations on field material and established cultures did not allow us to determine how the uniflagellate stage is formed and what happens to the zygote. Thus, it is premature to in include it in Fig. 40.

DISCUSSION

Secondary carotenoids

Algae inhabiting high-altitude mountains and polar environments are typically confronted with rapid changes of abiotic factors (e.g. solar radiation, temperature and nutrients), and thus live under extreme conditions. High visible light exposure is known to cause photodamage and photoinhibition. Secondary carotenoid droplets have been suggested as protecting cells from such injuries, as they reduce the otherwise sublethal or lethal amount of light (e.g. Neale 1987; Rau 1988; Yong and Lee 1991; Bidigare *et al.* 1993). Secondary carotenoids are synthesized from β–carotene and accumulate outside the chloroplast (Czygan 1968). At the time of sampling, motile cells and cysts had been subjected to extreme solar radiation, capable of inflicting lethal photodamage and photoinhibition. The photon flux density measured on the snow surface was thus 4485 µmol photons $m^{-2} s^{-1}$, and still as high as 1993 µmol photons $m^{-2} s^{-1}$ below the 7 cm snow cover. A dispersal of the extrachloroplastic secondary carotenoids to cover the surface of motile cells and cysts may have thus been an adaptation to mitigate the effect of strong light. In a laboratory experiment, Yong and Lee (1991) also observed a relocation of secondary carotenoids under the influence of light in *Haematococcus lacustris* Girod-Chantrans. Bidigare *et al.* (1993) showed that secondary carotenoids in snow algae (mostly *Chlamydomonas* spp.) were not involved in light capture for photosynthesis and further that accumulation of these compounds (astaxanthin) was linked to availability of nitrogen. We do not have any field data to demonstrate such a relationship.

Pyramimonas tychotreta in the Ross Sea

The sea ice cruise to the Ross Sea onboard R/V *Nathaniel B. Palmer* cruise number 99-01 (NBP 99-01) involved an intensive sampling program along longitudes 165°, 150° and 135°W. The ice condition changed from open water with small ice floes at 65°S to land-fast ice at 77°S. However, most of the sea ice encountered was relatively large ice floes or vast, contiguous masses of less than 2-meter thick, first-year ice. The dense population of motiles and cyst stages of *P. tychotreta* was recorded at 165°W and 73°S. For unknown reasons *P. tychotreta* was not observed in the sea ice at any stations visited along 150° and 135°W,

despite seemingly similar sea ice conditions. Other autotrophic taxa, for example diatoms, dinoflagellates and prymnesiophytes, were recorded at all three transects and did not appear to be restricted to a single area. This may be coincidental as specimens of *P. tychotreta* were recorded in water column samples from 150° and 135°W.

Sea ice stations along 150–76°W and 135–76°W were visited up to 3 weeks later than those at 165–76°W. The fact that the melting process was thus more progressed may perhaps explain the observed difference between transects. Further recordings of changes in the physical and chemical parameters during the late austral summer are required to fully understand the nature of sea ice surface blooms of *P. tychotreta* in the Ross Sea.

Pyramimonas in Antarctica

Pyramimonas tychotreta is the second species of *Pyramimonas* from Antarctic sea ice in which cyst formation has been observed; *P. gelidicola* was the first. Cysts of both possess a complete and uniform cover of a single type of scale that is not present in the quadriflagellate stage. Superficially the cyst scales of the two species were somewhat similar. However, the width of the base plate in *P. tychotreta* was approximately 150 nm as compared with 230 nm in *P. gelidicola* (van den Hoff *et al.* 1989) and the scale height also differed (60 nm in *P. tychotreta* and 80 nm in *P. gelidicola*). The scale floor in cyst scales of *P. gelidicola* was identical to that of the box scales in the motile stage (McFadden *et al.* 1982; van den Hoff *et al.* 1989). In *P. tychotreta* the morphology of the scale floor differed in cyst scales and box scales (Fig. 23). The scale floor in both species possessed four horns that extend from each corner of the square cyst scales. The length of the horns was in the same size range (approximately 400 nm). The spherical cysts have a similar diameter: 15–20 µm in *P. gelidicola* (van den Hoff *et al.* 1989; however, approximately 40 µm according to their Figs 16,17) and 13–18 µm in *P. tychotreta*. According to van den Hoff *et al.* (1989) the mature cyst wall in *P. gelidicola* was bi-layered, whereas sections through mature cysts of *P. tychotreta* revealed three appressed layers. Examination of Fig. 9 in van den Hoff *et al.* (1989) reveals the cyst wall to be comprised of three layers. The unaccounted-for layer in mature cysts of *P. gelidicola* is the thin, electron-dense inner layer. Hence, the organization of layers constituting the thick-walled cysts in *P. gelidicola* and *P. tychotreta* is indistinguishable. The cyst ultrastructure with large amounts of reserve material (lipid and starch grains) also appears identical. Cysts produced by South African strains of *P. pseudoparkeae* resemble those observed in the Antarctic species. Sections through the cyst wall in *P. pseudoparkeae* show a similar tri-layered arrangement, though only two layers were reported (see Fig. 59 in Pienaar and Aken 1985). The cyst surface in *P. pseudoparkeae* was without a scaly covering (Pienaar and Aken 1985). Cysts produced by *P. parkeae*, *P. grossii* and *P. tetrarhynchus* are in need of a more thorough study before a detailed comparison with cysts from *P. gelidicola*, *P. pseudoparkeae* and *P. tychotreta* becomes feasible. At present three types of cyst covering can be distinguished in species of *Pyramimonas*: (i) those with unique cyst scales (*P. gelidicola* and *P. tychotreta*); (ii) those with identical scales on motile cells and cysts (*P. amylifera*); (iii) those with a non-scaly covering (*P. pseudoparkeae*).

Cyst formation

Formation of cysts in *P. pseudoparkeae* was also considered asexual because fusion of cells was not observed (Pienaar and Aken 1985). Pienaar and Aken induced cyst germination by transferring cysts to fresh medium. Four quadriflagellate cells were formed. This scenario thus matches cyst formation in *P. tychotreta*, except that encystment and excystment seem to be separated in time in *P. pseudoparkeae*. At the time we collected our samples, both encystment and germination of *P. tychotreta* had occurred, indicating that no single abiotic factor regulates the formation of these stages. This somewhat contradicts observations on encystment and germination in other algae (e.g. chlorophytes, chrysophytes, dinoflagellates and raphidophytes). Cyst germination usually requires a dormancy period in these algal groups. Observations on encystment and germination of *P. tychotreta* still convey only a fragmented picture of what regulates the transformation from one stage to the next. Factors to be studied include changes in photon flux density, nutrient concentrations and the presence of solid surfaces (sea ice).

Sexual reproduction

We consider it unlikely that the uniflagellate cell belongs to a different species, as this would have required an unprecedented degree of morphological convergence of body and flagellar scales. Based on circumstantial evidence such as morphology of box scales, foot-print scales, the scaly covering of the flagellum, the pyrenoid matrix penetrated by thylakoids and surrounded by starch and the long basal bodies we, therefore, link the uniflagellate stage with *P. tychotreta* rather than reporting it as a new species. We tentatively suggest that the uniflagellate stage represents a gamete although fusion of individual uniflagellates was not seen. However, an elongate bi-flagellated cell, approximately twice the width of a

single uniflagellate cell, was, in fact, observed in field samples. The presence of a uniflagellate stage and possibly a bi-flagellate zygote implies the existence of sexual reproduction in *P. tychotreta*. Observations indicating a likely occurrence of sexual reproduction have never been reported, although more than 30 species of *Pyramimonas* have been cultured and studied in great detail.

The uniflagellate cells are not regarded as abnormal stages of the quadriflagellate stage. Scrutiny of the red-coloured sea ice samples collected in the Ross Sea contained only quadriflagellate cells, various stages of encysting cells, low numbers of germinating cells or, on a few occasions, numerous cells of the uniflagellate stage. Only little morphological variation of the uniflagellate stage was observed. The variation pertained to either the rounded or pointed posterior end. No other morphological forms were observed, which would be expected if abnormal forms were present in the populations of *P. tychotreta* that we examined from the field. Abnormal forms of the quadriflagellate stage of *Pyramimonas* are known to occur in senescent cultures. We have never observed the uniflagellate stage in old cultures of *P. tychotreta* established from samples collected in the Weddell Sea and the Ross Sea. This indicates that it is not formed as an abnormal stage under the culture conditions provided in our laboratory. Our unsuccessful attempts to initiate the development of either cyst stages or uniflagellate cells in culture have further hampered our understanding of the various stages that form the life history of *Pyramimonas tychotreta*.

Few studies have reported the occurrence of sexual reproduction in prasinophytes. At present it has been convincingly demonstrated in *Nephroselmis olivacea* stein (Suda *et al.* 1989). The vegetative cell is haploid and this stage may act as gametes. A zygote is produced when a male gamete fuses with a female gamete that has attached to a substratum. Two subsequent meiotic divisions result in four haploid daughter cells. This haplont life history appears simple in comparison with *P. tychotreta* that has two flagellate stages. A uniflagellate stage capable only of backward swimming is known in *Cymbomonas* (Sym and Pienaar 1993). In culture this stage co-occurs with cysts (Ø Moestrup, pers. comm.). This indicates that gametes are also part of the life history in *Cymbomonas* and that sexual reproduction in prasinophytes may not be as rare an event as hitherto conceived.

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