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ULTRASTRUCTURAL STUDIES ON MARINE PRYMNESIALES (PRYMNESIOPHYCEAE)

Monica Birkhead

A Dissertation submitted to the Faculty of Science
University of the Witwatersrand, Johannesburg
for the Degree of Doctor of Philosophy

Johannesburg 1994

this is for all my gorgeous ones

DECLARATION

This thesis is my own unaided, original research and has not been previously submitted for any other degree purposes. The thesis is submitted as a collection of papers for publication, with introductory and closing chapters. All five papers for publication were researched, written and prepared for publication by myself, but as co-author, my supervisor Prof. R.N. Pienaar has contributed some of the micrographs. These are listed in Appendix 2.

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11 April 1994

SUMMARY

This thesis consists of eight chapters, with the first chapter being a general introduction to and literature review of the Prymnesiophyceae, the seventh chapter being an overall discussion and conclusion, and the eigth being the references cited in the two general chapters. The main body of the thesis comprises five papers for publication. These include the type description of a new species of ***Prymnesium***, ***P. nemamethecum***; the flagellar/haptonematal apparatus of this species; the flagellar/haptonematal apparatus of an undescribed species of ***Chrysochromulina*** that bears 'eyelash' scales; the flagellar/haptonematal apparatus of ***Chrysochromulina brevifilum*** and elucidation of the identifying features of this species; the ultrastructure of ***Chrysochromulina simplex*** with a flagellar reconstruction and an amended description. The flagellar apparatus of ***C. simplex*** resembles that of another described species of ***Chrysochromulina***, ***C. apheles***. The other two species discussed in this study are both unique within the genus, with ***C. brevifilum*** having the simpler flagellar apparatus of the two, although still more complex than that of ***C. simplex***. The ***Chrysochromulina*** sp. shares features with species of both ***Chrysochromulina*** and ***Prymnesium***, particularly ***P. nemamethecum***. In terms of flagellar features, ***P. nemamethecum*** differs in a only a few details from ***P. patelliferum***, but the generic concept of ***Prymnesium*** is broadened by the addition of this new species as it has three scale types with one type restricted to the haptonematal surface.

ACKNOWLEDGEMENTS

Hierarchical lists of the recipients of my gratitude do not appeal to me, but I owe an enormous debt of thanks and appreciation to all my friends and my family for helping me in innumerable ways. I would not have got here without you all!

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TABLE 1. Classic taxonomic system for the Prymnesiophyceae, outlined by Parke and Green (1976).

Isochrysidales

Motile cells with two equal or subequal flagella, haptonema reduced or absent. Four families, [illegible] genera including:

Chrysotila, ***Cricosphaera***, ***Dicrateria***, ***Emiliania***,
Gephyrocapsa, ***Imantonia***,***Isochrysis***, ***Ochrosphaera***,
Pleurochrysis

Prymnesiales

Motile cell with two equal or subequal flagella and an obvious haptonema that may be long and coiling. Two families, six (only five currently accepted) including:

Chrysochromulina, ***Corymbellus***, ***Phaeocystis***, ***Platychrysis***.
Prymnesium

Coccosphaerales

Coccolith-bearing, motile or non-motile cells which may be a stage in a polymorphic life history, haptonema may be present. Nine families, twenty eight genera including:

Braarudosphaera, ***Calyptrosphaera***, ***Coccolithus***,
Helicosphaera, ***Pappsphaera***, ***Pontosphaera***, ***Syracosphaera***,
Zygosphaera

Pavlovales

Motile cells with two anisokont flagella (the longer flagellum bearing hairs and knob-like structures), haptonema reduced. One family, three genera being:

Diacronema, ***Exanthemachrysis***, ***Pavlova***

CHAPTER 1

GENERAL INTRODUCTION TO THE PRYMNESIOPHYCEAE

The Prymnesiophyceae (= Haptophyceae; Table 1) is a class of organisms that are found predominantly in marine environments (for reviews see Hibberd 1980; Green *et al.* 1989a) but which may also occur in brackish (e.g. Carter 1937), freshwater (Stein 1878; Lackey 1939; Nicholls 1978; Kling 1981) and terrestrial habitats (Green and Parke 1974). Although the majority of species are motile unicells, there are also colonial and palmelloid species (Lagerheim 1893; Billard and Gayral 1972; Green and Parke 1974, 1975; Green 1976a; Gayral and Billard 1977; Gayral and Fresnel 1979) and species with a pseudofilamentous, benthic stage in the life history (e.g. Leadbeater 1970, 1971a; Fresnel and Billard 1991).

THE HAPTONEMA

In many of the species, the motile cells possess a haptonema ('attaching thread' of Parke *et al.* 1955), hence the descriptive class nomenclature. This structure occurs between the two flagella, with all three appendages being inserted into the cell either anteriorly, subapically or ventrally (see Green *et al.* 1989a). The ultrastructure of the haptonema (Fig. 1) was elucidated initially by Manton (1964b, 1967, 1968; Manton and Leedale 1963; Leadbeater and Manton 1969a), research that provided an excellent base for further comparative work (e.g. Green and Pienaar 1977; Green 1980; Moestrup and Thomsen 1986; Gregson *et al.* 1993a, 1993b). Typically (at least in the Prymnesiales, particularly species of ***Chrysochromulina*** and ***Prymnesium***[1]), the free part of the haptonema consists of a cytoplasmic core in which 6 - 7 microtubules are arranged

[1] Generic and specific authorities listed in Appendix 1.

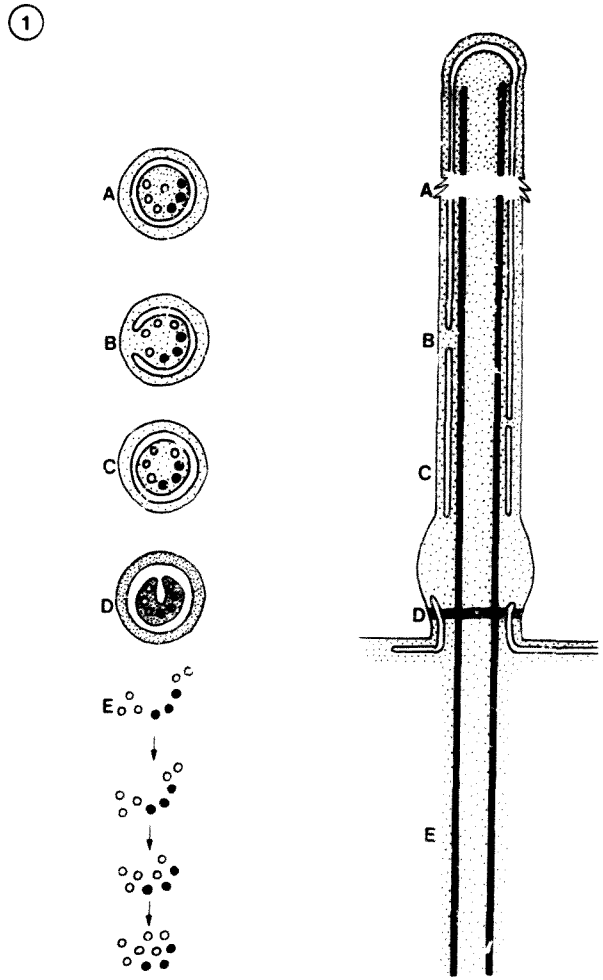

FIG. 1. **The ultrastructure of the haptonema.** A - region of coiling with asymmetrically arranged microtubules; B - region with fenestrated endoplasmic reticulum; C - non-coiled region with three concentric, uninterrupted membranes surrounding the microtubules; D - finger-like peg of endoplasmic reticulum extending into the microtubular arc immediately above the level of haptonematal insertion into the cell; E - haptonematal base with changing microtubular arrangements. (Drawn from Moestrup and Thomsen 1986; Gregson *et al.* 1993a).

in a ring. Surrounding the central microtubular arrangement is a perforated sheet of endoplasmic reticulum that is continuous with the peripheral endoplasmic reticulum underlying the plasmalemma of the entire cell body. This peripheral layer of endoplasmic reticulum is another characteristic feature of prymnesiophyte cells (Hibberd 1980). Near the haptonematal insertion into the cell, there is often a cytoplasmic swelling that creates a haptonematal bulge. Below this, the microtubules reorientate to form an arc that is penetrated by a finger-like extension of the endoplasmic reticulum, while in the haptonematal base the microtubules are rearranged in two rows. A maximum of two microtubules may be added in the haptonematal base to form a hexagonal pack with two lateral microtubules. There are many variations on this 'typical' haptonematal structure, which have been used for ordinal separation within the class (Table 1), for example:

In the Isochrysidales *sensu* Parke & Green (see Green and Parke 1975, Green and Pienaar 1977) the haptonema is not well developed and may be:

- absent (***Dicrateria inornata***);
- so reduced as to be apparently absent at the light microscope level, although electron microscopy reveals profiles of endoplasmic reticulum in a short protuberance (***Imantonia rotunda***);
- rudimentary, being evident at the light microscope level as a short proboscis, that ultrastructurally is shown to have endoplasmic reticulum profiles with three microtubules in the emergent part and four microtubules in the haptonematal base (***Isochrysis galbana***) or three microtubules along the entire haptonematal length (***Chrysotila lamellosa***);

In the Pavlovales (see Green 1980), the haptonema is reduced with only 1 - 2 microtubules in the free part, although there are 7 microtubules around the finger of endoplasmic reticulum distal to the insertion region and 8 microtubules in the haptonematal base (except in ***Diacronema vlkianum*** (Green and Hibberd 1977) where a maximum of only 7 microtubules is present).

In the Coccosphaerales (Gayral and Fresnel 1976; Inouye and Chihara 1983, Inouye and Pienaar 1984, 1988; Kori and Green 1985c), haptonematal variation extends from:

- complete absence (all life-history stages of ***Emiliania huxleyi***);
- to a vestigial, non-emergent haptonema with a haptonematal base of either 5 microtubules (e.g. ***Hymenomonas globosa***, ***Jomonlithus littoralis***, ***Pleurochrysis roscoffensis***) or 8 microtubules (***Umbilicosphaera sibogae*** var. ***foliosa***);
- to a prominent haptonema that can be bulbous or slender in form, with either 6 microtubules in the free part and 8 in the basal portion (***Calyptrosphaera sphaeroidea*** and in the motile '***Crystallolithus***' phase of ***Coccolithus pelagicus***) or 7 microtubules in the free part and 8 in the base (***Syracosphaera pulchra***).

In the Prymnesiales (see Manton and Leedale 1963; Manton 1964b, 1968; Leadbeater and Manton 1969a, 1969b; Parke *et al.* 1971; Chrétiennot 1973; Moestrup and Thomsen 1986; Green and Hori 1990; Gregson *et al.* 1993a, 1993b) an obvious haptonema is always present and the haptonema shows its greatest development (up to 160 μm long in the 14 μm cells of ***Chrysochromulina camella***). In the free part of the haptonema there may be either 6 microtubules (at least six species of ***Chrysochromulina***; ***Corymbellus aureus***; ***Phaeocystis***) or seven microtubules (species of ***Chrysochromulina***, ***Platychrysis*** and ***Prymnesium***), with a reduction to only three microtubules at the most distal tip (***Chrysochromulina simplex***). Numerous vesicular/membranous profiles have been described in the short haptonema of ***Phaeocystis*** (Parke *et al.* 1971). The haptonematal base consists of eight or nine microtubules (***Prymnesium*** and ***Chrysochromulina***). Haptonemata that are long, relative to cell body length, are capable of rapid coiling, a feature associated with many species of ***Chrysochromulina***. Obviously haptonemata that are short (as in cells of ***Prymnesium*** or ***Platychrysis*** for example), do not have the necessary length to form gyres and are restricted to slight flexing or bending. The only prymnesiophyte that does not belong in the Prymnesiales *sensu* Parke & Green, but which exhibits haptonematal coiling on death, is the coccolithophorid ***Syracosphaera pulchra*** (Inouye and Pienaar 1988). Many ***Chrysochromulina*** cells that do not have coiling haptonemata when living, are seen to exhibit this feature when dead / fixed (Manton and Leadbeater 1974).

The coiling of long haptonemata, which is considered to be either sensory and part of an

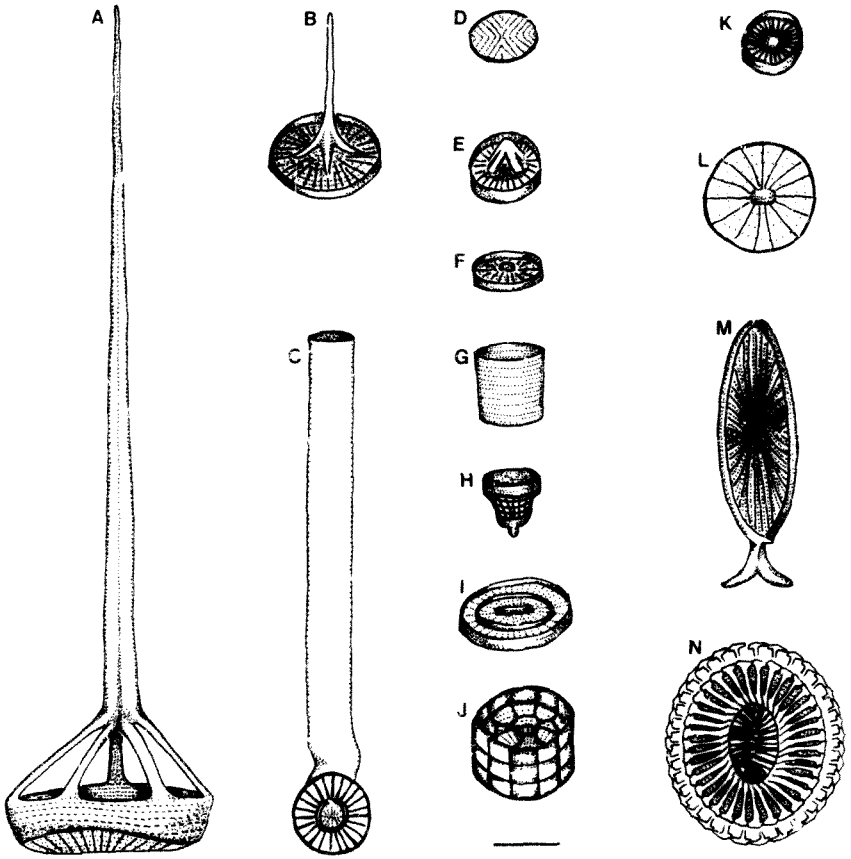

FIG. 2. A variety of scales and coccoliths from prymnesiophycean cells. A - spine scale of *Chrysochromulina pelagica*; B - spine scale of *Chrysochromulina brevifilum*; C - cylinder scale of *Chrysochromulina microcylindra*; D - body scale of *Isochrysis litoralis*; E - body scale type of *Corymbellus aureus*; F - distal scale type of *Platychrysis simplex*; G - distal scale type of *Platychrysis pigra*; H - cup-shaped scale of *Chrysochromulina camella*; I - distal scale type of *Prymnesium annuliferum*; J - distal scale type of *Prymnesium calathiferum*; K - distal scale type of *Phaeocystis pouchetii*; L - scale of *Imantonia rotunda*; M - one of four scale types from *Chrysochromulina polylepis*; N - heterococcolith from *Emiliania huxleyi*. Scale bar = 0.3 μm except for fig.M (= 0.8μm) and fig.N (= 1μm).

avoidance reaction in response to obstacles or to assist in reducing drag during swimming, has recently been shown to be a Ca^{2+}-mediated process (Gregson *et al.* 1993b; Kawachi & Inouye 1994). In addition, the haptonema also serves as an attachment device in all species, although this is not its principle function (Leadbeater 1971b). Estep and MacIntyre (1989) postulated that the adhesive qualities of haptonemata were important in prey capture, and were integral to a form of parasitism which they named 'dasmotrophy'. In an extremely elegant study, Kawachi *et al.* (1991) demonstrated the role of the haptonema in food capture and transport to the posterior phagocytic region of a mixotrophic species of ***Chrysochromulina, C. hirta***. This study also confirmed many of the observations on haptonematal behaviour that were first described by Leadbeater (1971b), e.g. the movement of 'swellings' up and down the haptonema.
The phenomenon of phagotrophy in photosynthetic prymnesiophytes is apparently related to the nutritional status of the habitat, particularly phosphate limitation (Jones *et al.* 1993; Nygaard and Tobiesen 1993; and for a review on phagotrophy in prymnesiophytes see Green 1991).

SCALES AND COCCOLITHS

With the exceptions of all members of the Pavlovales and ***Dicrateria inornata*** of the Isochrysidales, prymnesiophytes typically have a scale/coccolith covering (Fig. 2). This has been extensively used in the taxonomy of the class members and has recently been shown to be of use in determining diploidy in the life history stages of some coccolithophorids (Fresnel 1989). The scale covering may be composed of one to several layers of similarly or differently patterned types of unmineralised body scales that may also be found over the haptonema and/or calcified coccoliths (e.g. Conrad 1914; Parke *et al.* 1955, *et seq.*; Manton and Leedale 1969; Parke 1971; Leadbeater 1971a; for reviews see Hibberd 1980; Green *et al.* 1989a). The unmineralised scales are composed of protein and cellulosic carbohydrate (Green and Jennings 1967; Brown *et al.* 1969; Allen and Northcote 1975; for review see Romanovicz 1981), while the coccoliths consist of an organic base plate, similar to the unmineralised scales, on which crystals of calcium carbonate are arranged (except in ***Emiliania huxleyi*** which has coccoliths without a base plate and no unmineralised scales, Klaveness 1972a). The basic form of the

unmineralised scales is a double-layered plate with a pattern of radiating ridges on the proximal face and c[illegible]ric patterning on the distal face (Manton and Leedale 1969). The distal face may be elab[illegible]rough, to be rimmed, spined, extended as a cylindrical structure, or variously patterned with raised/upright configurations. If a species possesses only plate scales, it is principally the ornamentation on the distal face of the outermost scale layer that is used in identification. Although the plate scales are not visible with the light microscope, many of the distal elaborations are sufficiently large to enable this to be possible (e.g. the spine scales of a number of species of ***Chrysochromulina***). In some species (e.g. ***Isochrysis galbana***, ***Corymbellus aureus***, ***Prymnesium nemamethecum***, several coccolithophorids and species of ***Chrysochromulina***) certain scale types are restricted to a particular region of the cell, typically at the poles or around the area of appendage insertion.

Given their relatively robust structure, coccoliths are usually visible at the light microscope level. This, together with the presence of coccoliths as fossilised, calcareous nanoplankton deposits that have been studied since the late nineteenth century (see Tappan 1980; Green *et al.* 1989a for reviews), has resulted in all coccolithophorids being classified according to the structure of their coccoliths. The variation in their structural conformation has necessitated the development of a complex terminology, with the basic distinction being made between coccoliths composed of only one type of calcite element (holococcoliths) and those constituted by several different types of calcite crystals (heterococcoliths). Although this taxonomic approach is the only practical one available to palaeobotanists, research on living coccolithophorids has revealed a number of inadequacies in a system based purely on coccolith morphology (e.g. Parke and Adams 1960; Leadbeater 1971a; Thomsen *et al.* 1991; see section on life histories, this chapter). In addition, there are reports of genotypic variation and variation in both size and degree of calcification, within and between strains of ***Emiliania huxleyi*** (Young and Westbrook 1991). Similarly, in the organic scales of non-coccolith-bearing species, both genotypic variation (***Chrysochromulina chiton***, Manton 1967) and geographical variation relating to size only, but not to patterning, has been described (e.g. ***C. apheles***, Moestrup and Thomsen 1986; ***C. ericina***, Leadbeater 1972b; ***C. hirta***, Manton 1978; ***C. microcylindra***, Leadbeater 1972b; ***C.***

pachycylindra, Manton *et al.* 1981; *C. parkeae*, Green and Leadbeater 1972).

Various speculations have been made concerning the function of scales / coccoliths, all of which conclude some protective advantage, e.g. as light-deflecting structures, as a buffer between the external medium and the plasmalemma, as additional support for an otherwise fragile cell membrane, or as toxin absorbing structures (Estep and MacIntyre 1989; Green *et al.* 1989a).

Although an outer scaly covering is typical of most members of the Prymnesiophyceae, other external features are rare and have been infrequently described. These include the presence of knob-like structures on the flagella and the cell body of species of ***Pavlova*** Butcher (Green 1980); vermiform, membranous or columnar deposits/ tomentose material associated with the scales in some species of ***Chrysochromulina***, ***Prymnesium***, ***Platychrysis*** and in some coccolithophorids (Manton and Leedale 1969; Manton and Leadbeater 1974; Green *et al.* 1982; Gayral and Fresnel 1983a) and a mucilagenous coating or a continuous membranous skin may cover the scale layers entirely (e.g. Manton and Peterfi 1969; Parke *et al.* 1971; Manton and Leadbeater 1974; Moestrup 1979; Van Emberg *et al.* 1986). In addition, Parke (1971), Green and Parke (1974) and Green and Course (1983) reported the presence of extracellularly-formed, calcified structures in strains of ***Chrysotila***. These crystalline structures accrete in the mucilage surrounding the cells, eventually enclosing the cells and forming spherulites that resemble calcareous nanofossils such as ***Prinsiosphaera*** (Green and Course 1983).

Due to the pioneering work of Manton (1966a, 1967; Manton and Leedale 1969; Leadbeater and Manton 1969a, 1969b), it is now well known that the production of scales and coccoliths occurs in the Golgi cisternae (for review see Hibberd 1980). This is not surprising, as Golgi-derived scales are found in many other flagellates (Cox 1980), but what is unusual in the prymnesiophytes is the organisation of the Golgi body (see Hibberd 1976 for review). In all prymnesiophytes the single Golgi body lies between the nucleus and the flagellar bases, being polarised around the latter with the forming face on the left side of the cell and associated with profiles of endoplasmic reticulum / chloroplast endoplasmic reticulum, while the maturing face

with vesicles containing scales / coccoliths / vermiform membranous profiles, opens on the right side of the cell. Between the two dictyosomal faces there is generally a series of cisternae with median dilations, first noted by Parke *et al.* (1959) and subsequently termed 'peculiar' dilations.

FLAGELLA

The ultrastructure of the flagella and flagellar apparatus has been reviewed (Moestrup 1982; Preisig 1989) with other publications emphasizing the transitional region (Hibberd 1979) or the cytoskeletal arrangement (Andersen 1991). Subsequent additions to flagellar information include publications on the flagellar apparatus of ***Isochrysis galbana*** (Hori and Green 1991), ***Hymenomonas coronata*** (Roberts and Mills 1992), ***Chrysochromulina acantha*** and ***C. simplex*** (Gregson *et al.* 1993a).

Excluding the Pavlovales, prymnesiophycean motile cells are characterised by two, equal to slightly subequal flagella, which lack stiff, tubular mastigonemes and which are considered to be naked (Moestrup 1982). However, a tomentose surface coat, composed of delicate fibrils with serological similarities to fungal fimbriae, occurs in ***Chrysochromulina breviturrita*** (Day *et al.* 1986) and, in sectioned cells of many species belonging to the Prymnesiales, there is often a layer of material with a mucilagenous appearance, found around each flagellum (e.g. ***Phaeocystis pouchetii***, Parke *et al.* 1971, figs. 29-35; ***Platychrysis pienaarii***, Gayral and Fresnel 1983a, fig. 16; ***Prymnesium calathiferum***, Chang and Ryan 1985, figs. 18, 19).

In the Pavlovales, fine hairs coat the long flagella of the anisokont cells, and small knob-like structures may also be found here in species of ***Pavlova*** (Green 1980).

The transitional region of prymnesiophytes is not well documented and, until 1985, review descriptions were based on the work of Manton (1964a) on ***Prymnesium parvum***. In this species, and probably all other prymnesialean species, there are two interconnected, transitional plates distal to the level of flagellar insertion that are separated by a stellate pattern. Subsequent

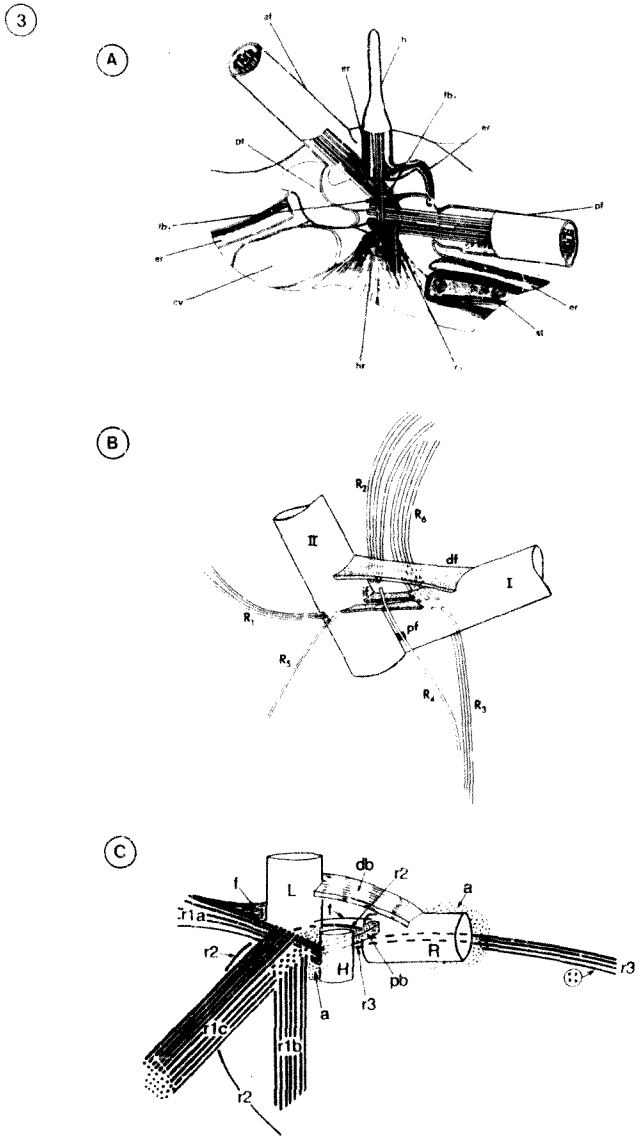

FIG. 3. Published reconstructions of prymnesiophycean flagellar / haptonematal systems.

A - *Diacronema vkianum* (Green and Hibberd 1977); B - *Imantonia rotunda* (Green and Hori 1986); C - *Isochrysis galbana* (Hori and Green 1991);

research (e.g. Gregson *et al.* 1993a) indicates that the stellate pattern may not be typical of the order, but also that central axosomes are present in both transitional septa and that the proximal plate is the more elaborately constructed of the two septa. No detailed descriptions are available though.

In the coccolith-bearing cells, the transitional region of ***Pleurochrysis carterae*** has been detailed (Beech and Wetherbee 1988). At a level immediately above the plasmalemma, there is a single septum with a robust axosome, distally positioned helical bands lying between the peripheral doublets and proximal transitional rings. A similar organisation is evident in ***Pleurochrysis*** sp. (Inouye and Pienaar 1985) and ***Hymenomonas coronata*** (Roberts and Mills 1992). Some of the longitudinal sections through the flagella of ***Prymnesium parvum*** (Manton 1964a) suggest that transitional rings may also be present here, but this requires confirmation.

In the Pavlovales there also appears to be a single transitional septum, often with a central axosome (Green 1980). The transitional region of ***Diacronema vlkianum*** has been thoroughly described (Green and Hibberd 1977), but as is the case in other prymnesiophytes, there are few studies available for comparison.

The ultrastructure of the flagellar apparatus is so variable within the class, that Preisig (1989) was compelled to include three diagrams, as opposed to the single illustration required for each of the heterokont classes covered in his review. For comparative purposes, all published reconstructions of prymnesiophycean flagellar/haptonematal systems are presented in Fig. 3 and an explanation of the abbreviations used in these reconstructions is presented in Table 2. (In continuing this overview, reference will be made to publications not describing the flagellar apparatus directly, but this is done with caution as the components of the flagellar apparatus are known to alter dramatically with different stages in the cell cycle - see Beech *et al.* 1991). To avoid unnecessary repetition, the following publications are the ones to which reference is made:

Isochrysidales - ***Imantonia rotunda***, Green and Hori 1986

Isochrysis galbana, Hori and Green 1991

Prymnesiales - ***Chrysochromulina acantha***, Gregson *et al.* 1993a

C. apheles, Moestrup and Thomsen 1986

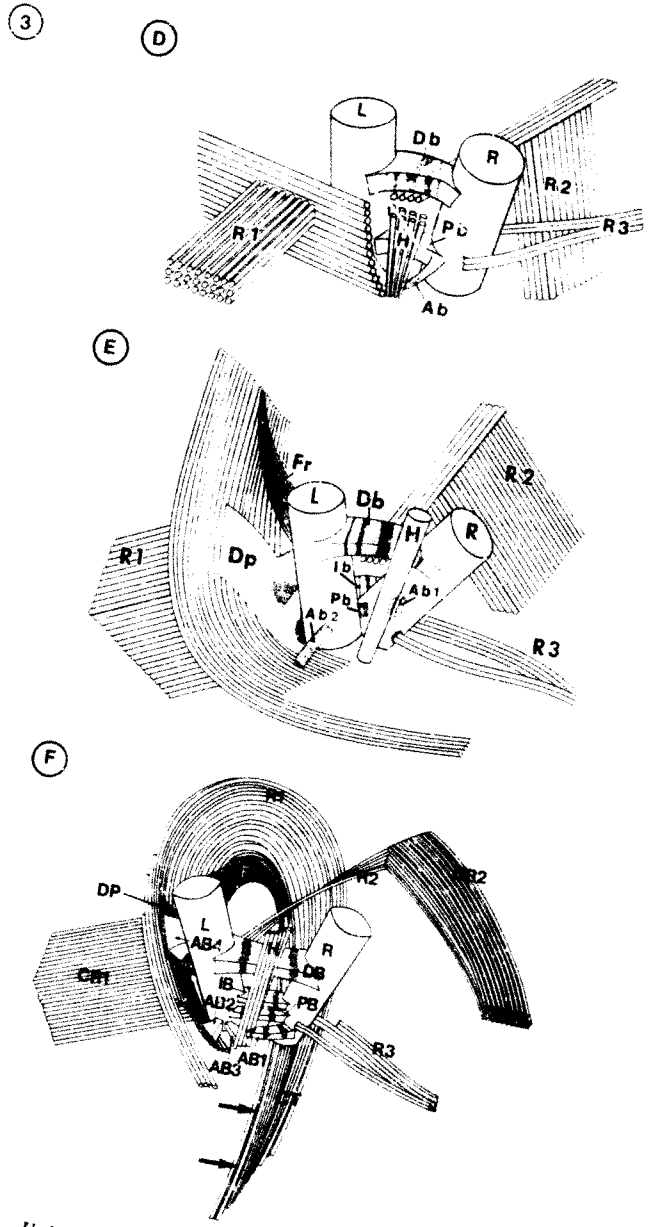

FIG. 3. (ctd). D - *Umbilicosphaera sibogae* var. *foliosa* (Inouye and Pienaar 1984); E - *Pleurochrysis* sp. (Inouye and Pienaar 1985); F - *Pleurochrysis carterae* (Beech and Wetherbee 1988);

C. simplex, Gregson *et al.* 1993a
Corymbellus aureus, Green 1976a
Phaeocystis pouchetii, Parke *et al.* 1971
Platychrysis pienaarii, Gayral and Fresnel 1983a
Platychrysis pigra, Chrétiennot 1973
Platychrysis simplex Gayral and Fresnel 1983a
Prymnesium parvum, Manton 1964a, 1964b
Prymnesium patelliferum, Green and Hori 1990

Coccosphaerales - *Hymenomonas coronata*, Roberts and Mills 1992
Jomonlithus littoralis, Inouye and Chihara 1983
Ochrosphaera neopolitana, Inouye and Chihara 1980
Pleurochrysis carterae, Beech and Wetherbee 1988
Pleurochrysis placolithoides, Fresnel and Billard 1991
Pleurochrysis pseudoroscoffensis, Gayral and Fresnel 1983b
Pleurochrysis sp., Inouye and Pienaar 1985
Syracosphaera pulchra, Inouye and Pienaar 1988
Umbilicosphaera sibogae var. *foliosa*, Inouye and Pienaar 1984

The flagella have been termed 'left' and 'right', with the open arms of the arc of haptonematal microtubules facing the left flagellum. *Pleurochrysis carterae* is the only species in which it has been determined that the left flagellum is the longer of the two (Beech and Wetherbee 1988), and that it may also be numbered as the number 1 flagellum as it is in the mature developmental state (Beech *et al.* 1988). Unfortunately, neither of the flagella in this species exhibits the autofluoresence present in some other prymnesiophycean flagella (Kawai and Inouye 1989; see also Lindholm and Virtanen 1992), as this would be another useful distinguishing characteristic between the flagella.

The conventions for cell orientation are that the haptonema is most dorsally inserted, followed by the left basal body and then the right basal body (Beech and Wetherbee 1988). A broad root, R1, is always present and originates from dense material between the haptonematal and left

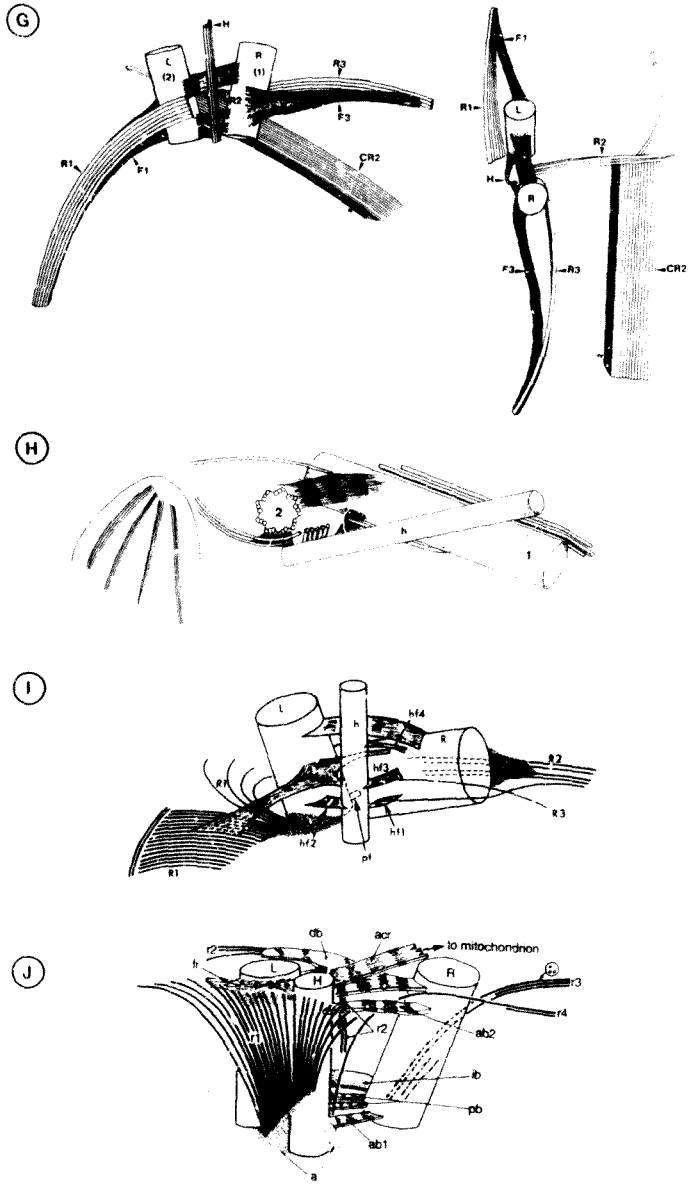

FIG. 3. (ctd). G - *Hymenomonas coronata* (Roberts and Mills 1992); H - *Chrysochromulina apheles* (Moestrup and Thomsen 1986); I - *Chrysochromulina acantha* (Gregson *et al.* 1993a); J - *Prymnesium patelliferum* (Green and Hori 1990).

flagellar bases. However, due to the diversity in composition, the other microtubular roots are usually numbered sequentially in any given publication, which is logical but which makes direct comparisons more difficult. Terminology relating to connective fibres also varies (see Andersen *et al.* 1991; also Fig.2) It is unfortunate that the system used by Green and Hori (1990), where, for example 'accessory bands' referred only to connectives between the haptonema and one basal body, has not been generally adopted (see, for example, Gregson *et al.* 1993a).

Once again, the Pavolvales are distinct from other prymnesiophytes (Green 1980) and have only two microtubular roots associated with the posterior, left basal body (typically of 7 and 2 microtubules). Fibrous root, a fibrillar haptonematal root which may or may not be striated, a striated distal band and a striated proximal band. The left flagellum is inserted most deeply into the cell, almost at right angles to the haptonema and the right flagellum which are inserted along more similar pathways (Green and Hibberd 1977).

There is considerable overlap in features of the flagellar apparatus in the non-pavlovean members, so a generalised pattern can be discerned. There are typically three or four microtubular roots: the broad root (R1) which is often sheet-like and is found between the haptonematal and the left flagellar bases; a root (R2) found between the two flagellar bases on their ventral sides; one, one bifurcated, or two roots associated with the right basal body and the ventral/dorsal right sides of the cell. Fibrous, unstriated roots have been reported, but these are associated with the flagellar bases and either the R1/R3 roots (compared with the fibrous root in the Pavlovales which is not associated with any microtubular root). In some species the R1/R2 are compound, being associated with an additional crystalline component.

In the coccolithophorids, the appendages are usually apically inserted in the spherical cells. The bases of the appendages are arranged at similar angles to each other, if slightly unequally positioned, then with the haptonematal base and the right basal body more similarly aligned (see for example Beech and Wetherbee 1988; Roberts and Mills 1992). However, in the spherical cells of the Isochrysidales (viz. ***Imantonia rotunda*** and ***Isochrysis galbana***) the bases of the appendages are markedly angled, with the left and right basal bodies almost perpendicular to

TABLE 2. Abbreviations used in the flagellar/haptonematal apparatus reconstructions in Figure 3.

a - amorphous material

AB1-AB4, ab1-ab4 - accessory bands 1 to 4

acr - auxiliary connective root

af - anterior flagellum

CT - cytoplasmic tongue

DB, db - distal band

df - distal fibre

DP - dense plate

F, f - fibrous root

fr - fibrous root

F1, F3 - fibrous roots 1 and 3

fb1, fb2 - fibrous band 1, 2

H, h - haptonema

hf1-hf4 - haptonematal fibres 1 - 4

hr - haptonematal root

if - intermediate fibre

L - left basal body

PB, pb - proximal band

pf - proximal fibre; or posterior flagellum (*Diacronema*)

pt - pit

R - right basal body

R1-R6, r1-r6 - numbered microtubular roots

each other, and in ***Isochrysis galbana*** the haptonematal base lies parallel to the left basal body. In the Prymnesiales, the appendages may be either apically inserted (several species of ***Chrysochromulina***, e.g. ***C. brevifilum***, ***C. breviturrita***, ***C. kappa***, ***C. parkeae***, ***C. pringsheimii***, ***C. spinifera***) or subapically / ventrally inserted (e.g. ***Chrysochromulina acantha*** and ***C. apheles***, ***Prymnesium parvum***, ***P. patelliferum***) usually in a pit or depression (e.g. ***Chrysochromulina pyramidosa***, ***Corymbellus aureus***, ***Phaeocystis pouchetii***, all three species of ***Platychrysis***). In these prymnesialean species, the angles of the bases of the appendages are usually different and become increasingly marked the more ventrally the appendages are inserted. So, for example, in ***Prymnesium patelliferum*** the left basal body and haptonematal base are aligned roughly in parallel, with the right basal body laterally offset, while in the two saddle-shaped species of ***Chrysochromulina***, one of the basal bodies lies almost perpendicular to both the haptonematal base and the other basal body. (In ***C. apheles*** it is the left basal body that is markedly angled, but it is the right basal body in ***C. acantha***, although angles in the latter species are variable).

The connectives of the flagellar apparatus are most simple in ***Isochrysis galbana*** where there are only two, a striated distal band between the flagella and an unstriated proximal band connecting the bases of all three appendages. The other described member of the Isochrysidales, ***Imantonia rotunda***, has a more complex system consisting of four connectives: a striated distal band, two unstriated intermediate bands and one proximal band. In the coccolithophorids the connectives typically include a large, striated distal band between all three appendages; one striated proximal band between the basal bodies; an accessory band between the haptonematal base and the right basal body. There is variation in the presence of both an accessory band between the left basal body and the haptonematal base (e.g. present in ***Hymenomonas coronata*** and ***Pleurochrysis carterae***, but absent in ***Pleurochrysis*** sp. and ***Umbilicosphaera sibogae*** var. ***foliosa***), and of a striated intermediate band that connects the ventral sides of the basal bodies (present in ***Hymenomonas coronata*** and at least two species of ***Pleurochrysis***, but absent in ***Syracosphaera pulchra*** and ***Umbilicosphaera sibogae*** var. ***foliosa***). The system of connectives of ***Prymnesium patelliferum*** (and as far as can be determined from Manton 1964b, also ***Prymnesium parvum***)

is more complex than any of the described coccolithophorids, in that it is constituted by: a striated distal band which has a ventral extension associated with the R2 root, as well as an association with a large, distal auxiliary connective that extends from the R1 across to a mitochondrial profile on the right side of the cell; a non-striated intermediate band; two striated proximal bands; a distal and a proximal striated accessory band between the right basal body and the haptonematal base. ***Chrysochromulina acantha*** is equally complex in terms of its connectives, despite the absence of an intermediate band and a second proximal band. This complexity is due to the possession of a third accessory band, unstriated, between the right basal body and the haptonematal base; a proximal accessory band between the left basal body and the haptonematal base; and instead of the distal auxiliary connective described in ***Prymnesium patelliferum***, it has a more proximal auxiliary connective that extends from the R1 across the cell to the right basal body, but with no organelle connection. The distal and proximal bands of ***C. acantha*** are considered to be unstriated (but see Gregson *et al.* 1993a, fig. 26b). Of the prymnesialean cells described, ***Chrysochromulina apheles*** appears to have the simplest system of connectives (a striated distal band; a striated distal and a striated proximal accessory band between the haptonematal base and the right basal body; a small proximal band).

The R1 root, usually associated with mitochondrial profiles, is variously developed and may consist of as few as 2 or 6 microtubules (***Imantonia rotunda*** and ***Chrysochromulina apheles*** respectively) to more than 20 microtubules (***C. acantha***, ***Prymnesium patelliferum***, species of ***Platychrysis*** and ***Pleurochrysis***). In most coccolithophorids (***Hymenomonas coronata***, at least four species of ***Pleurochrysis***, ***Umbilicosphaera sibogae*** var. ***foliosa***), the R1 microtubules ascend distally in a plane that is roughly perpendicular to the long axes of the bases of the appendages. However, in species where the R1 microtubules lie almost parallel to the long axis of the left basal body (species of ***Chrysochromulina***, ***Platychrysis***, ***Prymnesium*** and ***Syracosphaera pulchra***) some of these R1 microtubules splay sharply backwards past this basal body, towards the ventral cell surface. These microtubules have either been labelled as a separate root (R1 and R5 of ***Imantonia rotunda***), as a bipartite root (4(5) + 2 of ***Chrysochromulina apheles***) or simply incorporated into the total number of microtubules for the

species (20 microtubules in ***C. acantha*** of which 4 veer ventrally). The pathway of the majority of R1 microtubules is generally arched, first anteriorly (upwards) then curving towards the ventral/left side of the cell, but it is only in some coccolithophorids that the complete pathway of these microtubules has been determined. In these cases (***Hymenomonas coronata***, species of ***Pleurochrysis***), the interconnected R1 microtubules are seen to extend to the most posterior portion of the cell, enclosed in a strand of cytoplasm and associated with a fibrous root that extends from the right side of the left basal body. This structure was first described as a "languette cytoplasmique" by Gayral and Fresnel (1983a), in a beautifully illustrated publication on ***Pleurochrysis pseudoroscoffensis***. The association of the cytoplasmic tongue with the peripheral endoplasmic reticulum, was demonstrated by Beech and Wetherbee (1988), and the contractile nature of the entire structure was further discussed by Fresnel and Billard (1991), where they recommended that the cytoplasmic tongue be renamed a 'contractile root'. A fibrous root that extends from the ventral side of the left basal body towards the R1, has also been seen in ***Isochrysis galbana*** (although this species does not seem to have a cytoplasmic tongue), and in all described species with a fibrous root there is also a dense plate that connects the R1 microtubules to the left basal body. In organisms that lack a fibrous root, there are still connections to the R1 microtubules, in the form of osmiophilic material lying between the R1 and the left basal body (in ***Prymnesium patelliferum*** this is considered to represent an extremely reduced fibrous root, although it does not extend from the ventral side of the left basal body). ***Prymnesium patelliferum*** (Green and Hori 1990) is the only described instance of the R1 microtubules arching anteriorly, although it may also occur in *C. **acantha*** (see Gregson *et al.* 1993a, figs. 11-13). The R1 microtubules may:

- not be associated with any other microtubules (e.g. as in ***Chrysochromulina apheles***, ***C. parva***, ***C. simplex***, ***Hymenomonas coronata***, ***Prymnesium parvum*** and ***P. patelliferum***, ***Syracosphaera pulchra***);

be associated with a pair of perpendicularly-aligned microtubules (***C. acantha***);

- be associated with a perpendicularly-aligned, crystalline bundle of up to 200 microtubules, the CR1 (only reported in some coccolithophorids, viz. ***Jomonlithus littoralis***, ***Ochrosphaera neopolitana***, all investigated species of ***Pleurochrysis***,

Umbilicosphaera sibogae var. ***foliosa***; and possibly present in species of ***Platychrysis*** although these cells may have been dividing);

- be associated with two, small, crystalline bundles of microtubules, the R1b and R1c (***Isochrysis galbana***).

In all cases, the crystalline roots are not superficial (as are the R1 microtubules that underlie the peripheral endoplasmic reticulum), but penetrate into the cell cytoplasm usually becoming associated with the inner face of an organelle (either mitochondrial / chloroplastic / nuclear).

Similarly, the R2 root which originates above (distal to) the proximal connectives and emerges from beneath (proximal to) the major distal connective, may be associated with a crystalline, microtubular bundle (found in all coccolithophorids investigated as yet, with the exception of ***Syracosphaera pulchra***). The R2 itself may be absent (***C. acantha***); may consist of a single microtubule (***Chrysochromulina apheles***, ***Isochrysis galbana***), three microtubules (***Imantonia rotunda***, ***Prymnesium patelliferum***), four microtubules (species of ***Pleurochrysis***, ***Syracosphaera pulchra***, ***Umbilicosphaera sibogae*** var. ***foliosa***), or even five (***Jomonlithus littoralis***). The R2 attains its greatest development in ***Hymenomonas coronata***, in which it consists of 5-6 microtubules with 2 extra microtubules curving towards the left, ventral cell surface in the opposite direction to the large, associated, crystalline root. The pathway of the R2 microtubules in most other coccolithophorids is directly ventral (away from the haptonema), or if slightly curved, then towards the right, ventral side of the cell. This is in contrast to ***Chrysochromulina apheles*** and ***Prymnesium patelliferum***, both of the Prymnesiales, in which the R2 microtubules curve ventrally towards the left side of the cell (and one of the three R2 microtubules in ***Prymnesium patelliferum*** then curves posteriorly to become associated with the Golgi body). Members of the Isochrysidales differ from each other and from the other genera that have been examined, in that the R2 in ***Isochrysis galbana*** consists of a single microtubule that recurves around the ventral side of the left basal body towards the left, dorsal side of the cell, while in ***Imantonia rotunda*** the three R2 microtubules curve ventrally but towards the right, anterior cell surface. It is possible that the two extra microtubules that attach to, and then curl away from the R2 in ***Hymenomonas coronata***, may be present in ***Platychrysis simplex*** (see Gayral and

Fresnel 1983a, fig. 30).

The R3 root is associated with the right basal body, and follows a path anteriorly towards the right, ventral side of the cell (***Chrysochromulina acantha***, ***C. apheles***, ***Isochrysis galbana***, ***Jomonlithus littoralis***, ***Umbilicosphaera sibogae*** var. ***foliosa***) or to the right, dorsal side of the cell (***Hymenomonas coronata***, ***Imantonia rotunda***, species of ***Pleurochrysis***, ***Syracosphaera pulchra***), in both cases immediately underlying the peripheral endoplasmic reticulum. The R3, which is rarely associated with a fibrous root (only described in ***Hymenomonas coronata*** and ***Syracosphaera pulchra***), consists of a varying number of microtubules that are typically arranged in tiers (with the exception of the 2-stranded R3 of ***Imantonia rotunda***), for example, a 1/2 arrangement in ***Prymnesium patelliferum***, a 1/2/1 arrangement in ***Chrysochromulina apheles***, a 2/2 arrangement in ***Isochrysis galbana*** and ***Jomonlithus littoralis***, a 2/4 arrangement in species of ***Pleurochrysis***, a 2/2/1 arrangement in ***Chrysochromulina acantha***. With the exceptions of ***Hymenomonas coronata***, ***Isochrysis galbana*** and ***Prymnesium patelliferum***, the lower (proximal) R3 tier consists of microtubules that originate on the dorsal side of the right basal body, either in association with that basal body (as is common amongst the coccolithophorids) or from between the proximal connectives (***Chrysochromulina acantha***, ***C. apheles***). These latter microtubules, which arise from the more dorsal side of the proximal connectives, have been numbered as the R4 root by Green and Hori (1985, 1990). The R4 in ***Chrysochromulina acantha*** and ***Prymnesium patelliferum*** extends dorsally and therefore follows a different pathway relative to the ventrally-directed R3 microtubules, but in ***Imantonia rotunda***, both the single-stranded R4 and the two R3 microtubules follow the same dorsal pathway. (If the terminology of Green and Hori was generally applied, then, for example, ***Chrysochromulina apheles*** would have an R3 root of 1/2 and a single-stranded R4 that follows the same course as the R3 microtubules, but which originates among the proximal connectives). The only recorded absence of an R3 root is in ***Cruciplacolithus neohelis*** (Fresnel 1986).

Unusual roots not found in the majority of species include a 3-stranded root that is found only in ***Imantonia rotunda*** and which is designated the R6. The R6 originates from the ventral side

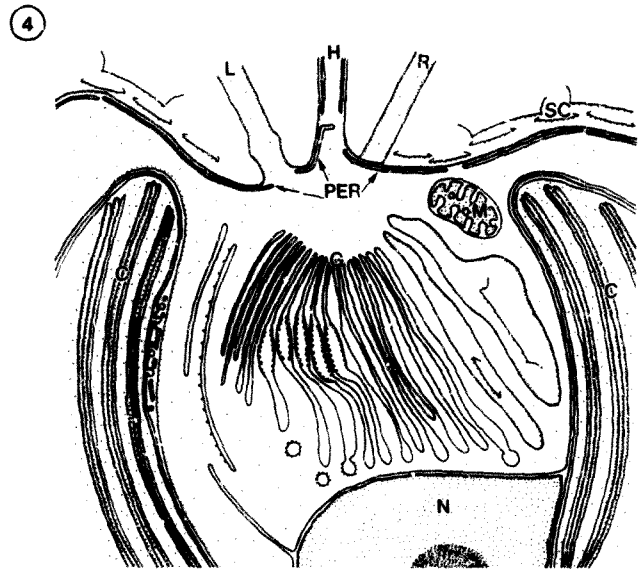

FIG. 4. Characteristic ultrastructural features of prymnesiophycean motile cells. C - chloroplast; H - haptonema; L - left flagellum; G - Golgi body; M - mitochondrial profile; N - nucleus; PER - peripheral endoplasmic reticulum; R - righ: flagellum; SC - scales. (Redrawn from Hibberd 1976).

of the right basal body adjacent to the origin of the R3, but in contrast to the R3 microtubules though, the R6 microtubules extend anteriorly and ventrally. There are also a few, very short microtubules associated with the proximal right side of the right basal body in ***Prymnesium patelliferum***, and it is possible that these are homologous with the well developed, but hitherto undescribed, root structure visible in ***Platychrysis simplex*** (Gayral and Fresnel 1983a, fig. 29).

This overview of flagellar features in the Prymnesiophyceae, certainly seems to support the conclusion of Roberts and Mills (1992, p. 642): " This perplexing nonallegiance of flagellar apparatus architecture to cell morphological features compounds existing systematic turmoil."

OTHER ULTRASTRUCTURAL FEATURES

The polarised Golgi body with its median dilations is the most distinct internal organelle in prymnesiophyte cells (Fig. 4). However, the ultrastructure of the chloroplasts also distinguishes members of the Prymnesiophyceae from many other algal classes, in that each chloroplast has thylakoids in groups of three and lacks a girdle lamella. Pyrenoids, usually traversed by paired thylakoids but infrequently by swollen single thylakoids, are found in the chloroplasts and may be embedded or may bulge from the inner face of each plastid into the central cytoplasmic region (Hibberd 1980). A crystalline matrix within the pyrenoid has only been described in ***Chrysochromulina acantha*** (Leadbeater and Manton 1971), ***C. apheles*** (Moestrup and Thomsen 1986) and ***Emiliania huxleyi*** (Klaveness 1972a), while ***C. chiton*** (Manton 1966b) is unique in having a crystalline cap covering the outer surface of the bulging pyrenoid. The "delicate bounding layer" first described around the pyrenoids of ***Phaeocystis*** motile cells (Parke *et al.* 1971, p. 932) has also been illustrated in a number of other prymnesialean genera (species of ***Platychrysis***, Gayral and Fresnel 1983a; several ***Chrysochromulina*** species, see Moestrup and Thomsen 1986). In many cells, the continuity between the chloroplast endoplasmic reticulum (for reviews of this structure see Gibbs 1981, Billard 1985) and the nuclear envelope, occurs most typically over the pyrenoid-containing area of the chloroplast. No functional explanation for this arrangement has been elucidated yet.

In many other chromophyte classes, there are intraplastidial eyespots (stigmata), and the apparent absence of this structure in prymnesiophyte cells was considered to be a distinguishing characteristic of the class. However, intraplastidial stigma-like structures, consisting of a layer of osmiophilic granules, have been described in members of the Pavlovales (Green 1980). An eyespot has also been recorded (but not illustrated) in one species of ***Chrysochromulina***, *C. **inornamenta*** (Wujek and Gardiner 1985), although the species description is poor and the organism is probably not a species of ***Chrysochromulina***.

All prymnesiophytes have mitochondria with tubular cristae, but the presence of a large, reticulate mitochondrion has only been demonstrated in ***Pleurochrysis carterae*** (Beech and Wetherbee 1984), although a single mitochondrion is probably also present in some members of the Pavlovales (Green and Hibberd 1977). The mitochondrial profiles in prymnesiophytes are typically associated with the inner faces of the chloroplasts (Hibberd 1980).

Within the Prymnesiophyceae, a feature common among members of the Prymnesiales is the presence of muciferous bodies (e.g. species of ***Chrysochromulina***, for review see Estep and MacIntyre 1989; species of ***Platychrysis***, Chrétiennot 1973; Gayral and Fresnel 1983a; species of ***Prymnesium***, Green *et al.* 1982; Billard 1983; Chang and Ryan 1985). The contents of these structures are produced within the Golgi cisternae, but the mature bodies are found within the lumen of the peripheral endoplasmic reticulum. Similar structures appear to be present in the motile cells of ***Chrysotila lamellosa*** (Green and Parke 1974, plate III), but this needs confirmation. Under chemical stimulation the muciferous bodies are extruded from the cell as globules or strands. Estep and MacIntyre (1989) believe that the function of these structures is to release prymnesialean toxins into the surrounding medium.

TOXICITY

Support for the theory of Estep and MacIntyre (1989, mentioned above) lies in the fact that it is only members of the Prymnesiales that are responsible for mass mortality of fish and

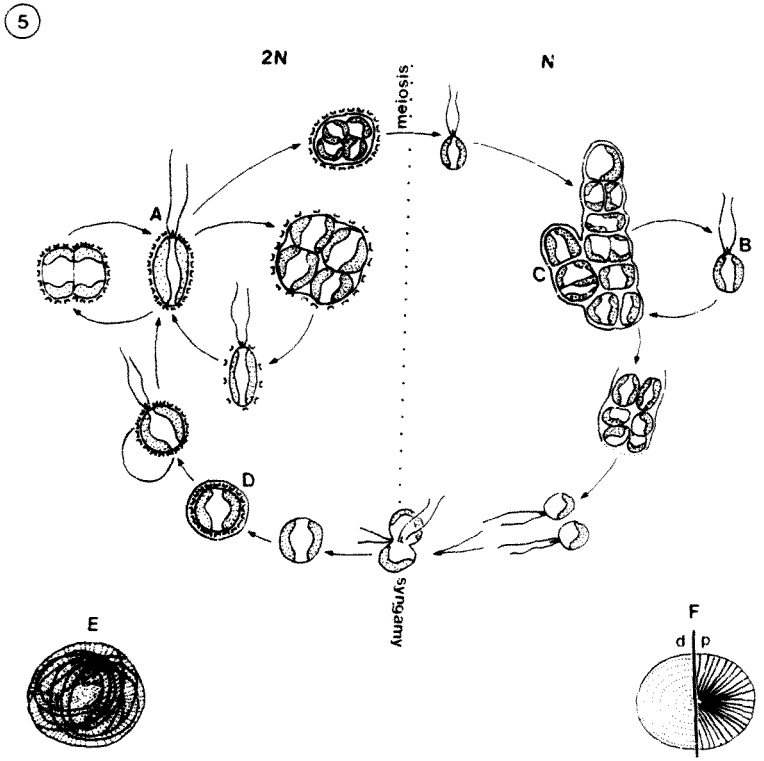

FIG. 5. Life history of *Pleurochrysis pseudoroscoffensis*. A - diploid, coccolith-bearing motile cell; B - haploid, non-coccolith-bearing motile cell; C - haploid, benthic filamentous phase; D - encysted zygote; E - patterning of the unmineralised organic scales of the diploid cells, distal and proximal faces similar; F - distal (d) and proximal (p) faces of the organic scales of the haploid motile cells. (Redrawn from Gayral and Fresnel 1983b; Fresnel 1989).

crustaceans. Species of ***Chrysochromulina*** and ***Prymnesium*** are the most commonly reported causal agents (for a review see Green *et al.* 1989a; and for several more recent examples see Cosper *et al.* 1989; Hallegraeff 1992; Lindholm and Virtanen 1992; Rhodes and Hubbs 1992; Holmquist and Willen 1993), while blooms of ***Phaeocystis*** colonies cause a number of problems including animal mortality (e.g. see Boalch 1987; Davies *et al.* 1992).

LIFE HISTORIES

One of the main reasons that the taxonomy of the Prymnesiophyceae is so confused at the lower taxonomic levels, is because of the lack of information regarding life histories. As this information becomes available, certain taxonomic blunders have become apparent. For example, life cycles in the coccolithophorids are now known to involve coccolith polymorphy in pairs of cells that have been taxonomically distinguished. The holococcolithophorid ***Crystallolithus hyalinus*** is the motile phase of the heterococcolithophorid ***Coccolithus pelagicus*** (Parke and Adams 1960); similarly, holococcolithic species of ***Turrisphaera*** alternate with particular species of the heterococcolithic ***Pappospĥaera*** genus, and the heterococcolithophorid ***Syracosphaera*** alternates with the holococcolithophorid ***Zygosphaera*** (Kleijne 1991; Thomsen *et al.* 1991, and also for further examples); species of ***Ochrosphaera*** may produce motile cells either with a coccolith complement resembling that of ***Pleurochrysis*** species, or non-coccolith-bearing motile cells that can settle to form a pseudofilament that resembles ***Apistonema***, an alga previously classified within the Isochrysidales (Lefort 1975).

This latter sort of life history cycle, but without the alternation between different coccolith-bearing forms, has been completely elucidated for the genus ***Pleurochrysis*** (Rayns 1962; Leadbeater 1970, 1971a; Gayral and Fresnel 1983b; Fresnel and Billard 1991). In this organism (see Fig. 5), the diploid coccolith-bearing stage alternates with a haploid non-coccolith-bearing cell that gives rise to a benthic, filamentous stage. The benthic phase produces haploid motiles (facultative gametes) that can settle to produce another filament or that can fuse to form a zygote that germinates to produce a diploid coccolithophorid. The organic plate scales of the diploid

and the haploid cells have been shown to differ in patterning, with the haploid scales being typically prymnesiophycean and the diploid scales being similarly patterned on both faces. This ploidy-dependant patterning on the plate scales also occurs in the alternation of generations in ***Ochrosphaera*** and ***Hymenomonas*** which are coccolithophorids that have life histories with an alternation between coccolith-bearing cells and non-coccolith-bearing cells (Fresnel 1989) and appears to also exist in coccolithophorids that exhibit life cycles with coccolith polymorphy (alternation between two different coccolith-bearing forms), so plate scale patterning is an extremely useful indicator of ploidy. If this feature can be applied to other prymnesiophytes, it suggests that most members of the Prymnesiales and species of ***Isochrysis*** are haploid (with the possible exception of some species of ***Chrysochromulina*** e.g. ***C. tenuisquama***, Estep *et al.* 1984, fig.20). This is entirely speculative, however, as no studies on ploidy in these organisms have been successfully completed.

Ultrastructural studies on different stages of the life history of ***Emiliania huxleyi***, have also indicated taxonomic affinities not apparent in the present taxonomic position of this coccolithophorid (grouped with the other coccolith-bearers). In addition to its unusual haptonematal, scale and flagellar features (mentioned above), the non-motile heterococcolithophorid stage (probably diploid) can produce haploid, non-coccolith-bearing, motile cells that are either ***Isochrysis***-like or devoid of any organic scale covering (Klaveness 1972b, Hibberd 1980). The resemblance of the scaly motile cells to ***Isochrysis***, together with similarities in alkenone and sterol composition (Marlowe *et al.* 1984), has led some authors (Hori and Green 1991) to suggest that this coccolithophorid is more closely related to isochrysidalean genera (with which they were originally grouped by Parke and Green 1976) than to other coccolith-bearing cells. This is yet another example of the taxonomic problems resulting from a system based on scale/coccolith morphology.

There are no substantiated reports on sexuality in any member of the Prymnesiophyceae other than the coccolithophorids. In most cases the only described reproductive mode is presumably asexual (Billard and Gayral 1972; Chrétiennot 1973; Green and Parke 1975; Gayral and Fresnel 1979, 1983a; Green 1980; Green *et al.* 1982).

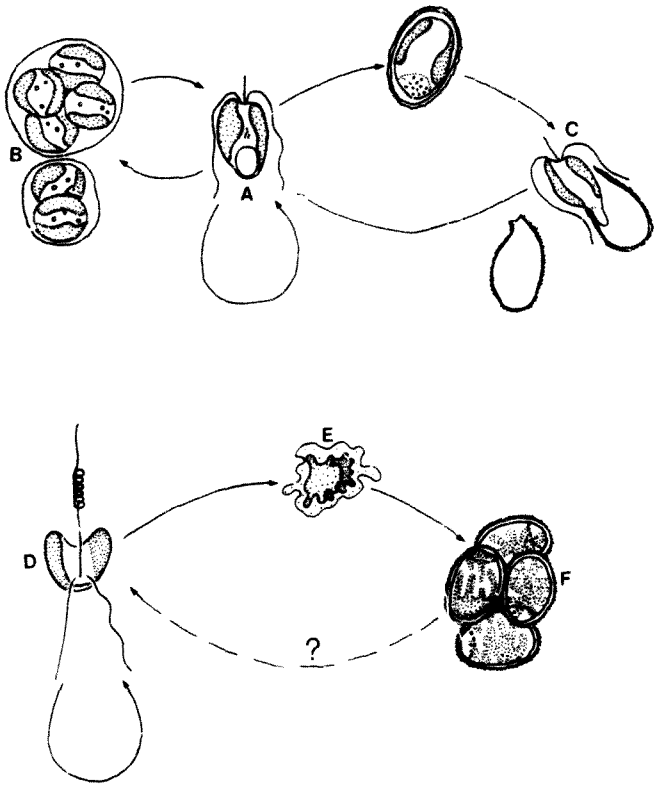

FIG. 6. Life histories of *Chrysochromulina* and *Prymnesium* species. A - motile cell of *Prymnesium*; B - palmelloid phase; C - encysted stage; D - motile cell of *Chrysochromulina*; E - amoeboid phase; F - encysted stage. (A, B, C - redrawn from Carter 1937; D, E, F - redrawn from Parke *et al.* 1955).

i) motile cells divide mitotically to produce more motile cells (as in ***Imantonia rotunda***, ***Isochrysis galbana***, species of ***Chrysochromulina*** and ***Prymnesium***, some species of ***Pavlova***). Recent research has suggested that there may be an alternation between different stages of motile cells, in that two distinct forms, possibly with differing DNA content, have been found in populations of ***Chrysochromulina polylepis*** (Edvardsen and Paasche 1991).

ii) in the predominantly motile organisms (see Fig. 6), there are also records (Conrad 1941; Parke 1949; Parke *et al.* 1955, 1956; Manton and Parke 1962; Green *et al.* 1982) of non-motile amoeboid or pseudofilamentous stages (***Chrysochromulina***) and amoeboid, palmelloid and encysted stages (***Prymnesium***). Observations made by Parke (1949) on encystment in ***Dicrateria inornata*** and ***Isochrysis galbana*** were based on non-unialgal cultures and subsequently have been retracted (see Hibberd 1976), so the reported existance of a sexual cycle in these isochrysidalean cells requires confirmation.

iii) in ***Phaeocystis***, the colonial planktonic stage which can replicate by fragmentation, alternates with typical prymnesiophyte motile cells which have haptonemata and organic scales (Kornmann 1955; Parke *et al.* 1971). Some of the motile cells may also produce external, filamentous, star-like structures, and both types of motile cell are capable of maintaining themselves without the development of the colonial stage. Although the release of both types of motiles from colonial forms apparently occurs, there is no irrefutable account of the production of colonial stages by the star-bearing motile form. Kornmann (1955) also surmised that the smaller sized motile cells that were formed by the colonies, could be gametes, but no information about their respective DNA contents is available. There is also no further substantiation of the report of a benthic phase in the life history (Kayser 1970).

iv) a dominant non-motile phase can give rise to prymnesiophycean motile cells, usually as a result of changing environmental conditions (e.g. ***Exanthemachrysis gayraliae***, ***Isochrysis litoralis***, species of ***Pavlova*** and ***Platychrysis***).

An interesting observation was made by Manton and Leadbeater (1974) in which they suggested

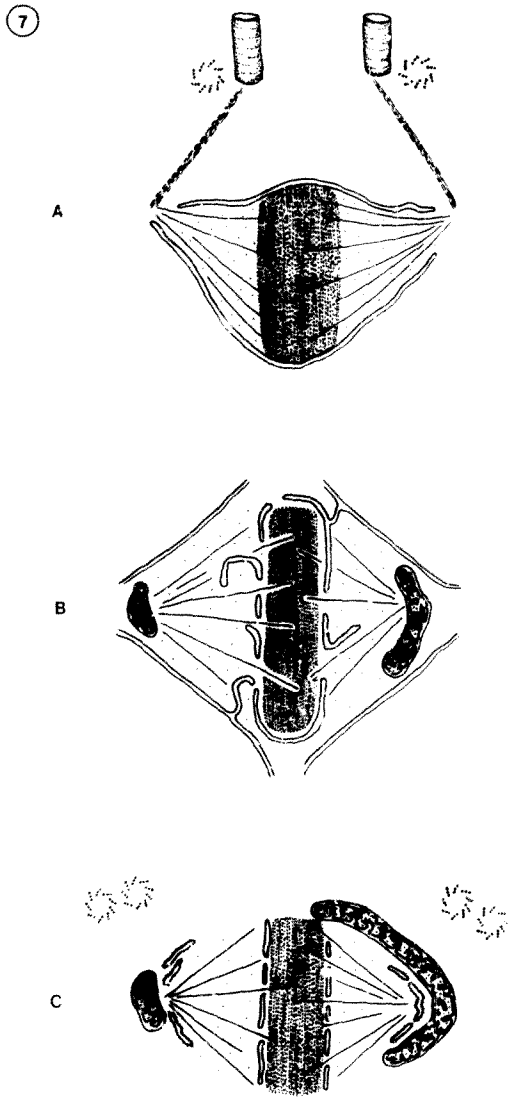

FIG. 7. Schematic metaphase mitotic patterns in the Prymnesiophyceae. A - *Pavlova*; B - *Chrysochromulina*, C - a coccolithophorid.

that ***Chrysochromulina bergenensis*** could be an uncalcified life history stage of an as yet, unidentified coccolithophorid. This was based on the presence of columnar deposits between the plasmalemma and the scales, a skin-like layer covering the entire cell including the scale layers, and a short haptonema with only 6 microtubules in the free part. This combination of features is also found only in the motile stage of ***Coccolithus pelagicus***.

MITOSIS IN THE PRYMNESIOPHYCEAE

Mitosis in the Pavlovales (Green and Hori 1988; Green 1989) is characterised by the fibrous flagellar root acting as a microtubule organising centre and by an obviously V - shaped spindle, as opposed to other prymnesiophytes (see Green 1989 for a review) in which the spindle axis is straight and in which no flagellar-associated MTOCs have been reported (Fig. 7). However, spindle microtubules may arise from a ribosome-free region in ***Chrysochromulina chiton*** (Green *et al.* 1989b), and also appear to be associated with the inner face of a mitochodrial profile in ***Chrysochromulina chiton*** (Green *et al.* 1989b, fig. 6), ***Imantonia rotunda*** (Hori and Green 1985a), and in ***Pleurochrysis haptonemofera*** (Hori and Inouye 1981, fig. 10). Mitosis is preceded by duplication of the chloroplasts, followed by basal body replication, then haptonematal division and doubling of the Golgi body. Mitotic microtubules develop which subsequently penetrate the nuclear region, the nuclear envelope may begin to become disrupted and numerous vesicles aggregate around the spindle. By anaphase, the nuclear envelopes of the daughter nuclei have formed on the poleward faces of the chromatin masses. Abscission of the interzonal microtubules then occurs and the nuclear envelopes become complete. Cytokinesis typically involves vacuoles and/or invaginations of the peripheral endoplasmic reticulum.

There are minor variations that relate to, for example, the behaviour of the outer nuclear membrane. This structure may

i) separate from the chloroplast endoplasmic reticulum as the nucleus migrates anteriorly in preprophase cells of ***Pleurochrysis haptonemofera*** (Hori and Inouye 1981) or it may remain intact without nuclear migration during chloroplast replication in preprophase cells of ***Isochrysis galbana*** (Hori and Green 1985b);

ii) be conserved throughout mitosis (***Imantonia rotunda***, Hori and Green 1985a; ***Pavlova***, Green and Hori 1988), be partially broken down (***Emiliania huxleyi***, Hori and Green 1985c) or be completely broken down, with or without some remaining connections to the chloroplast endoplasmic reticulum ('***Apistonema***' stage of an unknown coccolithophorid, Mesquita and Santos 1983; ***Chrysochromulina chiton***, Green *et al.* 1989b; ***Isochrysis galbana***, Hori and Green 1985b; ***Pleurochrysis haptonemofera*** and ***Pt. carterae***, Hori and Inouye 1981, Stacey and Pienaar 1980; ***Prymnesium parvum***, Manton 1964c). In this latter group, the nuclear envelope begins to reform by metaphase so that an open or a partially open mitosis occurs, and the remainder of the new outer nuclear membrane is often derived from the chloroplast endoplasmic reticulum either during telophase (***Chrysochromulina chiton***, ***Emiliania huxleyi***, ***Isochrysis galbana***, ***Imantonia rotunda***) or after cytokinesis ('***Apistonema***', ***Prymnesium parvum***).

Some of the other minor variations may be found in details of:

i) the spindle microtubules, for example the distribution and number of microtubules that lie in the cytoplasmic channels perforating the metaphase plate (Green 1989);

ii) the pole-to-pole distance during anaphase, which typically increases (e.g. ***Chrysochromulina chiton***, ***Isochrysis galbana***, ***Pleurochrysis carterae***, ***Prymnesium parvum***) but which is reduced in ***Pleurochrysis haptonemofera*** (Hori and Inouye 1981) and unchanged in ***Imantonia rotunda*** (Hori and Green 1985a).

It should be noted that in cells with crystalline compound roots, the constituent microtubules contribute towards spindle formation, and new daughter roots are synthesized *de novo* by metaphase (Beech *et al.* 1988).

HISTORICAL DEVELOPMENT OF THE CLASS PRYMNESIOPHYCEAE

Klebs (1892) delimited the Chysomonadina by grouping together golden-brown flageliates with one or two flagella. These cells stored oil and leucosin (not starch), and many species formed plugged endogenous cysts. When Pascher first introduced the Chrysophyceae (Pascher 1914), these siliceous endocyst-forming chrysomonads constituted the largest portion of the new class. They were placed in three different orders on the basis of their flagellation, a distinguishing feature employed by Pascher in his previous classifications of the Chrysomonadinae (e.g. Pascher 1910, 1912, Pascher and Lemmerman 1913). Members of the Chromulinales had one flagellum, those of the Ochromonadales had two unequal flagella and, in the Isochrysidales (which included the coccolithophorids), motile cells had two equal or subequal flagella. In subsequent taxonomic systems, Pascher (1925, 1931) placed all the vegetatively flagellate members of the Chrysophyceae into one order, the Chrysomonadales, on the grounds that in other algal classes, all flagellate cells were grouped together at the ordinal level, e.g. Volvocales of the Chlorophyceae.

However, the taxonomic systems of other phycologists (Papenfuss 1955, Bourrelly 1968) continued to base the subdivisions of the Chrysophyceae on flagellation. For example, Bourrelly's Chrysophyceae (Bourrelly 1968) which is characterised by endogenously formed, siliceous cysts, is divided into four groups:

1) Acontochrysophycideae (no flagellate stages)
2) Isochrysophycideae (two isokont flagella)
3) Heterochrysophycideae (one flagellum or two anisokont, heterokont flagella)
4) Craspedomonadophycideae (one flagellum and a pseudopodial collar)

The discovery of apparently triflagellate golden-brown cells (***Chrysochromulina*** Lackey, ***Phaeocystis*** Pnaeocystis Lagerheim, ***Platychrysis*** Geitler, ***Prymnesium*** Massart ex Conrad - Scherffel 1900, Büttner 1911, Massart 1920, Carter 1937, Lackey 1939, Conrad 1941; Conrad and Kufferath 1954) did little to disrupt the Chrysophyceae as a class, the triflagellate cells generally being separated from other members with isokont flagellation and grouped together at

the ordinal level (Prymnesiales) or the family level (Prymnesiaceae, or Prymnesiophycideae and Platychrysideae).

Although Massart (1920) had noticed that the short 'third flagellum' (the 'pseudopodium' of Wislouch 1924) differed from the other two flagella in that it was capable of attaching the cells to the surface of a slide, it was only with the advent of electron microscopy that it was possible to show that this 'third flagellum' was structurally and functionally different from true flagella. The outstanding pioneering work in this field was undertaken by Parke and Manton. In the first of a series of papers on new species of ***Chrysochromulina*** (Parke *et al.* 1955) the 'third flagellum' was termed a haptonema (attaching thread). In addition, this paper revealed the presence of organic body scales which had species-specific patternings. With the development of EM techniques, many more features were revealed and, by 1961, Parke had begun to discern two groups within the Chrysophyceae. The first group had:

i) a pleuronematic ("flimmer") flagellum, and sometimes an acronematic (smooth) flagellum which showed varying degrees of development in different genera

ii) scales, when present, impregnated with silica. The proximal scale face was smooth, punctate or poroid; the distal surface was variously sculptured with ribs, pores and spines. This structure was suggestive of diatom affinities.

The second group had:

i) only acronematic flagella

ii) scales, when present, without any mineral impregnation. Proximally patterned with radial ridges; distally patterned with a criss-cross lattice or roughly concentric circular ridges

iii) a haptonema.

These flagellar and scale features pointed towards a coccolithophorid affinity.

The only controversial genus was ***Pavlova*** Butcher, a heterokont flagellate with a haptonema-like appendage. Parke (1961) suggested that this structure was not a haptonema because it contracted instead of coiling, and it therefore probably had a different internal structure. She therefore included ***Pavlova*** in the first group of chrysophytes along with genera such as ***Mallomonas*** Perty, ***Ochromonas*** Wyssotzki, ***Paraphysomonas*** De Saedeleer and ***Synura*** Ehrenberg. The

second group was constituted by both naked (***Diacronema***, ***Dicrateria***, ***Isochrysis***) and scaly (***Chrysochromulina***, ***Phaeocystis***, ***Prymnesium***) members (Parke 1961).

The distinction made between these two groups by Parke (1961), was evident when a new class, the Haptophyceae, was erected by Christensen (1962). He combined species possessing a haptonema, with those having two equal, smooth (acronematic) flagella (including the coccolithophorids). The Haptophyceae contained two orders, the Isochrysidales and the Prymnesiales (which was constituted by the Prymnesiaceae, the Coccolithophoraceae and the Phaeocystaceae). This class was rejected by both Bourrelly (1968) and Fott (1974), but for different reasons.

i) Fott objected to the class status of the Haptophyceae because the rank of class should represent an evolutionary line of increasingly complex vegetative morphology, from flagellates to higher forms - this being the classic theory first presented by Pascher (1914). Leedale (1976) and Hibberd (1976) both consider this viewpoint to be counter productive, in view of (a) the unnatural classes that could result from rigid implementation of this class concept;(b) the features found to be most relevant in phylogenetic systems: vegetative habit is one of the less important features and even when flagellates are linked to more 'typical' algae as a result of a class concept based on morphological lines of advance, the motile cell structure has to be uniform at all the different morphological levels of organisation within the class. (i.e. recognising that cell flagellation / flagellar apparatus is one of the major phylogenetic tools)

ii) Bourrelly felt that the presence or absence of a haptonema was insufficient to separate the members of two classes, and the only other distinguishing feature between the Chrysophyceae and the Haptophyceae was the possession of acronematic, isokont flagella by the Haptophyceae. However, ***Pavlova*** is heterokont but also possesses a haptonema, so flagellation was not a valid distinguishing feature. In addition, the feature that was initially so important in identifying a chrysomonad (an urn-shaped, endogenous, siliceous cyst) was common to both classes, as this feature had been described in both ***Dicrateria*** and ***Isochrysis*** by Parke (1949). Subsequently, these cysts were said not to belong to the organsims described (Parke pers. comm. in Hibberd 1976) and prymnesiophycean cysts are now known to be fundamentally different from those of the Chrysophyceae, in that

they are not "plugged" and are formed by the deposition and accretion of siliceous material on the distal face of the outermost layer of scales (Pienaar 1980).

Bourrelly's system is, however, based on light microscopy only, and despite the practicality of such an identification system, it does not reflect true taxonomic groupings. It also ignores the wealth of taxonomic information available from electron microscopy, and as information on ultrastructural studies accumulated (largely due to the work of the British phycologists - e.g. Green 1973, 1975; Green and Leadbeater 1972; Green and Manton 1970; Green and Parke 1974, 1975; Leadbeater 1972a, 1972b; Leadbeater and Manton 1969a, 1969b, 1971; Manton 1964a, 1964b, 1964c,. 1966a, 1966b, 1967, 1968, 1972a, 1972b; Manton and Leadbeater 1974; Manton and Leedale 1961, 1963, 1969; Manton and Parke 1962; Parke 1961, 1971; Parke *et al.* 1955, 1956, 1959, 1962, 1971;), it became increasingly clear that the existence of the Haptophyceae was well supported by microanatomical details. Hibberd (1976) summarised much of this information and changed the descriptive class name, Haptophyceae, to the typified name, Prymnesiophyceae. The most important ultrastructural features noted by Hibberd (1976; see Fig. 4) were the haptonema; the two equal/subequal flagella with, at most (in the Pavlovales), fine hairs; no transitional helix in the flagella; only microtubular roots; no girdle lamellae in the chloroplasts; a characteristically polarised Golgi body between the flagellar bases and the nucleus; unmineralised/$CaCO_3$-containing scales.

With the conceptual development of the Prymnesiophyceae as a class separate from the Chrysophyceae, Christensen (1962) placed both classes within the division Chromophyta. As the name indicates, this was a division composed of coloured (but not green), algae that lacked chlorophyll *b* and which produced motile cells (Christensen 1980). This name has continued to be used, but less as an applied taxonomic category and increasingly as a broad term covering a number of algal groups that are derived independently from non-photosynthetic ancestors (Christensen 1989). This group of chlorophyll *c* - containing organisms traditionally included the Bacillariophyceae, Chrysophyceae, Cryptophyceae, Dinophyceae, Eustigmatophyceae, Phaeophyceae, Prymnesiophyceae, Raphidophyceae and Xanthophyceae. More recently, four more classes have been added: the Dictyochophyceae (Silva 1980; Moestrup and Thomsen

1990); the Pedinellophyceae (Cavalier-Smith 1986; Kristiansen 1989 - not validly described); the Pelagophyceae (Andersen *et al.* 1993) and the Synurophyceae (Andersen 1987, but which is not recognised by Chrétiennot-Dinet *et al.* 1993). In addition, a number of classes have been raised to the level of division (see Bold and Wynne 1985). One of the most important characteristics for inclusion in the Chromophyta *sensu lato* is the possession of stiff, tubular mastigonemes ('retronemes' of Cavalier-Smith 1989; see Leadbeater 1989 for review), a feature that Manton *et al.* (1952) first suggested could be of notable phylogenetic importance. Other important characters include the presence of a chloroplast endoplasmic reticulum (terminology of Bouck 1965; see also Gibbs 1981, Billard 1985); tubular mitochondrial cristae (Taylor 1976), a pigment complement consisting of at least one chlorophyll-*c* molecule (Jeffrey 1989) and a number of carotenoids including ß,ß-carotene (Bjornland and Liaaen-Jensen 1989). Members of the Prymnesiophyceae do have tubular mitochondrial cristae and a chloroplast endoplasmic reticulum, in addition to pigment complements that indicate affinities to the Bacillariophyceae and Chrysophyceae (Jeffrey 1989) or to the Dinophyceae, and to a lesser extent, the Chrysophyceae, Phaeophyceae, Bacillariophyceae and Raphidophyceae (Bjornland and Liaaen-Jensen 1989). However, prymnesiophycean motile cells are distinct within the Chromophyta in lacking mastigonemes, and unique in their possession of a haptonema and/or unmineralised scales composed of two layers, with a pattern of radiating ridges on the proximal face and concentric patterning on the distal face (Hibberd 1980, Green *et al.* 1989a). As a result, many taxonomists (Parke and Green 1976, Christensen 1980, Tappan 1980, Cavalier-Smith 1989, Jordan and Green 1994) currently place the Prymnesiophyceae (= Haptophyceae of Parke and Green 1976, Cavalier-Smith 1989; = Coccolithophyceae of Tappan 1980) in its own division, the Haptophyta (= Haptomonada of Cavalier-Smith 1989). The '*ultrastructural identity*' (terminology of Patterson and Sogin 1992) of the Prymnesiophyta is represented in Fig. 4. The absence of prymnesiophycean mastigonemes is presumed to be the only evolutionary loss of these structures which are considered by both Cavalier-Smith (1989) and Patterson (1989) to be the monophyletic, unifying feature of all chromophytes (Round 1989).

At the higher taxonomic levels, the original emphasis on pigmentation (apparent even in the name 'Chromophyta') has decreased, due to the accumulation of substantial ultrastructural and molecular evidence that links the chromophytes (many of which are facultative heterotrophs)

both with the fungal oomycetes and hyphochytridiomycetes (for example see Beakes 1989, Barr and Désaulniers 1989. Andersen 1991) and with several protozoan taxa, particularly the labyrinthulids and thraustochytrids (Cavalier-Smith 1989, 1993; Patterson 1989, Patterson and Sogin 1992). This rather interdisciplinary grouping has been variously named the kingdom Chromista (Cavalier-Smith 1981, and revised by Cavalier-Smith 1986, 1989, 1993), the kingdom Protista (Corliss 1984) or the Stramenopiles (Patterson 1989, Patterson and Sogin 1992).

TAXONOMY WITHIN THE PRYMNESIOPHYCEAE

Opinions differ about the various ordinal groupings within the Prymnesiophyceae, due largely to the earlier over emphasis of haptonematal and scale/coccolith features. By 1976, Parke and Green had established a system of four orders (Isochrysidales, Prymnesiales, Coccosphaerales, Pavlovales), which was commonly accepted as the most practical working system and is still used as a basic taxonomic framework by some phycologists. (e.g. Chrétiennot-Dinet *et al.* 1993). However, a number of researchers consider the differences between the members of the Pavlovales and all other prymnesiophytes, to be of greater importance than suggested by ordinal status alone, and the Pavlovales have been separated into their own sub-class, the Pavlovidae of Cavalier-Smith (1989) or the Pavlovophycidae of Jordan and Green (1994). Cavalier-Smith (1993) subsequently erected the class Pavlovea to incorporate these organisms, grouping all other prymnesiophytes together in another class Patelliferea (with three orders Isochrysidales, Prymnesiales and Coccosphaerales). The reasons for this pavlovean supra-ordinal separation are well justified (see also Van Valkenburg *et al.* 1977), as the Pavlovales have a unique prymnesiophycean ultrastructural and chemical identity in terms of

i) flagella (two markedly unequal flagella, with the longer flagellum always bearing fine hairs and often small knob-like structures, except in ***Exanthemachrysis gayraliae***, ***Pavlova noctivaga***, ***Diacronema vlkianum***. The latter species is also unusual in having a flagellar swelling containing teeth-like structures attached to the B microtubules)

ii) flagellar root systems which consist of only two microtubular roots and a non-straited fibrous

root

iii) short haptonemata with, in at least some species, an associated haptonematal root

iv) absence of a non-mineralised scale covering but with small knobbed structures similar to those found on the flagella covering the cell body (and also covering the haptonema in ***Pavlova lutheri***)

v) presence of stigmata in most species

vi) mitosis involving a V-shaped spindle and a MTOC associated with the fibrous root

vi) synthesis of the reserve metabolite, paramylon

vii) sterol and fatty acid composition.

This circumscription of the group has developed mainly due to the work of Green (1973, 1975, 1976b, 1980; Green and Hibberd 1977; Green and Hori 1988; Green and Manton 1970) and Van Der Veer (1969, 1972, 1976; Van Der Veer and Leewis 1977; but see also Gayral and Fresnel 1979; Volkman *et al.* 1991).

There is one, more recent exception to the supra-ordinal separation of the Pavlovales, where Christensen (1980) groups them as one of eight families within the Prymnesiales, the only order in the entire class Prymnesiophyceae.

In a number of taxonomic systems, the remainder of the prymnesiophytes (i.e. excluding the Pavlovophycidae) are still ordinally grouped as first outlined by Parke and Green (1976). For example Cavalier-Smith retains the three orders Isochrysidales, Prymnesiales and Coccosphaerales, as originally conceived. Tappan (1980) also retains the original Isochrysidales and Prymnesiales, but the six orders erected to contain the coccolithophorids perhaps reflects some bias in interest, while in Chrétiennot-Dinet *et al.* (1993) there are still three orders, the Isochrysidales, Prymnesiales and Coccolithophorales (as opposed to the Coccosphaerales), but all coccolith-bearing cells have been placed within the Coccolithophorales regardless of whether or not they produce ***Isochrysis***-like motile cells or ***Apistonema***-like benthic pseudofilaments. This system is indubitably practical, but given the overlap in so many features (as outlined in the preceding sections), it quite obviously does not reflect the phylogeny of the group.

Although Christensen (1980) ignored the overwhelming evidence that supported the separation of the Pavlovales from other prymnesiophytes, he also appears to have acknowledged the overlap

between the members of the remaining three orders, by splitting them between a number of families within one order. This approach has also been adopted by Jordan and Green (1994), as they have only one order, the Prymnesiales (sub-class Prymnesiophycidae), containing all isochrysidalean, prymnesialean and coccosphaeralean cells. This amalgamation is entirely justified, given the present understanding and lack of knowledge pertaining to life histories, ploidies, variation in flagellar apparatus and molecular data. Much of this has been reviewed above, but it is pertinent to point out that certain features that have been successfully employed in creating phylogenies within the green algae (with particular reference to mitotic features and the flagellar apparatus), do not correspond in any way with the original ordinal groupings (*sensu* Parke and Green 1976) found within the Prymnesiophycidae. The most important reason for this is probably the ignorance related to life histories - it is difficult to piece together a phylogeny when there is little idea of what life cycle stage is being observed, or where it fits into the overall scheme! In addition, there are prymnesiophycean life histories that involve two different motile stages, which may have differing flagellar apparatus arrangements. This creates the problem of deciding which arrangement should be considered the phylogenetically valid one. In this regard, it is important to actually obtain descriptive data of different motile stages (e.g. the ***Isochrysis***-like motile stages of some coccolithophorids, the two different motile stages of ***Chrysochromulina polylepis***). It is tempting to speculate that certain ultrastructural features which are conservative within the green algae, are not so highly conserved in evolutionary lines stemming from chlorophyll-*c* containing protists.

THE PRYMNESIALES

Although the erection of the sub-class Prymnesiophycidae is fully supported in the present study, it is relevant to review the Prymnesiales *sensu* Parke and Green in order to place the research done for this thesis in its correct (original) context.

As initially conceived, the Prymnesiales consisted of five genera which are always separated into two families, regardless of which taxonomic system is being used. The two families are the Phaeocystaceae, with one genus ***Phaeocystis*** Lagerheim, and the Prymnesiaceae with four

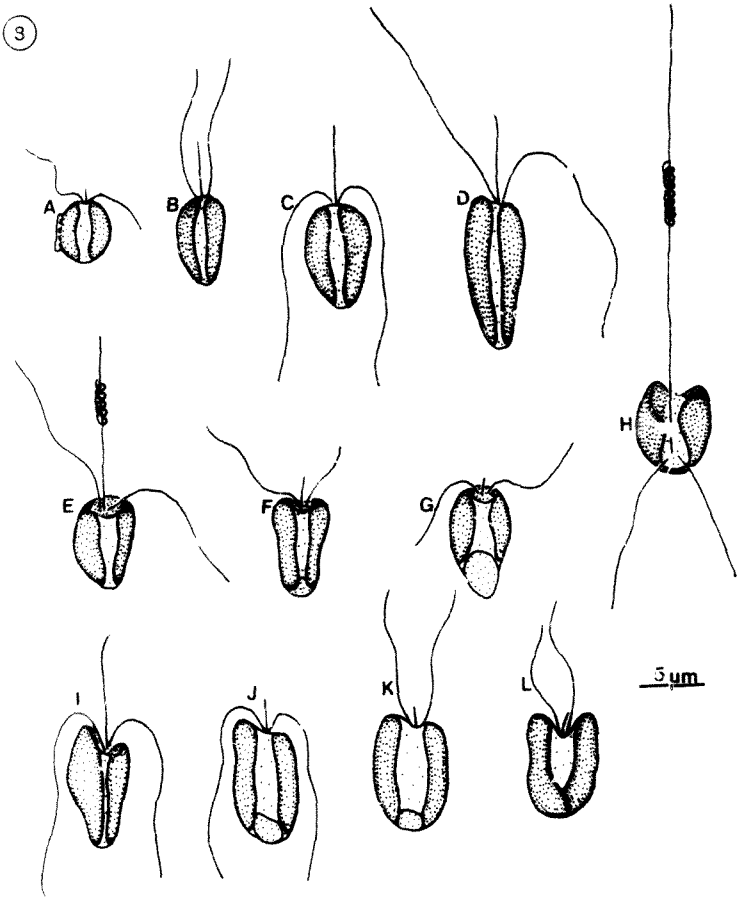

FIG. 8. Selected representatives of the Prymnesiales. Drawn from the type illustrations/descriptions. A - *Phaeocystis pouchetii* (Parke *et al.* 1971); B - *Chrysochromulina mantoniae* (Leadbeater 1972a); C - *Chrysochromulina breviturrita* (Nicholls 1978); D - *Chrysochromulina birgeri* (Hallfors and Niemi 1974); E - *Chrysochromulina chiton* (Parke *et al.* 1958); F - *Chrysochromulina spinifera* (Fournier 1971); G - *Platychrysis pigra* (Carter 1937); H - *Chrysochromulina alifera* (Parke *et al.* 1956); I - *Chrysochromulina polylepis* (Manton and Parke 1962); J - *Prymnesium annuliferum* (Billard 1983); K - *Prymnesium calathiferum* (Chang and Ryan 1985); L - *Corymbellus aureus* (Green 1976a).

genera, ***Chrysochromulina*** Lackey, ***Corymbellus*** Green, ***Platychrysis*** Geitler and ***Prymnesium*** Massart ex Conrad (see Fig. 8). A fifth genus, ***Chrysocampanula***, was added to the Prymnesiaceae by Fournier (1971), but has been subsequently incorporated into ***Chrysochromulina*** (see Pienaar and Norris 1979).

Phaeocystis is the only genus in the family Phaeocystaceae, but the number of species is a source of contention and has been most recently discussed by Sournia (1988), with only two species generally being accepted as valid. As mentioned in the preceding section on life histories, ***Phaeocystis*** occurs as a planktonic, mucilagenous colony composed of cells (lacking appendages and scales) embedded around the periphery (Chang 1984). The shape of the colonies is environmentally variable (Kornmann 1955) and has therefore been dismissed as a valid species characteristic (Sournia 1988). The life history includes the production of prymnesialean motile unicells, which have been ultrastructurally described from ***P. pouchetii*** (Parke *et al.* 1971) and from ***P. scrobiculata*** (Moestrup 1979). The latter is a species described on the basis of the motile cells alone, without reference to a colonial phase. The motile cells have short haptonemata, appendages inserted into a depression and two scale types that are used to distinguish between the two species. An additional specific characteristic, unique among members of the Prymnesiophyceae, is the thread-like, intracellularly-produced, filamentous structure that is released from the motile cells and forms a stellate pattern with either five rays (***P. pouchetii***) or 9 rays (***P. scrobiculata***). However, not all motile cells produce these thread-like structures (Parke *et al.* 1971 described this phenomenon in ***P. pouchetii*** as being dependent on the culture strain), although the cell morphology and scale complement are identical whether or not the 'stars' are produced. Swimming behaviour of different strains of ***P. pouchetii*** is the same, with flagellar movement being markedly heterodynamic. One flagellum beats with an anterior wave-like motion while the other extends laterally and exhibits a stiff, jerky motion. Cells swim with the anterior end of the cell directed forward, with helical rotation of the cell body around the direction of movement (Parke *et al.* 1971).

It is of interest that the first diagrams of a haptonema-bearing cell were drawn by Scherffel (1900) from the motile cells of ***P. globosa***.

Corymbellus aureus (Green 1976a) is the most easily distinguished genus of the Prymnesiaceae, consisting as it does of motile, spherical colonies. These are composed of a number of cells grouped together in a mucilagenous substance. The individual cells each have a short, non-coiling haptonema which is inserted, together with the two subequal flagella, into an apical depression. There are two types of scales, the smaller plate scales being confined to the apical groove, while the larger scales, which have both an upturned rim and a central spine on the distal surface, are found around the entire cell body. Flagellar motion is usually heterodynamic in cells constituting a colony, but becomes homodynamic when cells separate from each other. Homodynamic flagellar movement is associated with cells swimming rapidly with the posterior end directed forwards and the appendages trailing.

Individual cells of ***Corymbellus*** resemble the motile cells of three described species of ***Platychrysis*** (see Chrétiennot 1973 for the type species ***P. pigra***; Gayral and Fresnel 1983a for ***P. pienaarii*** and ***P. simplex***; the only other species is ***P. neustophila*** Norris for which there is only limited information). This similarity lies in the fact that all three species have short, non-coiling haptonemata inserted into an apical groove, as well as two scale types one of which is plate-like, the other with at least a marginal rim on the distal surface. However, the plate scales are evenly distributed around the cell body beneath the outer layer of specifically ornamented scales, although a more obvious difference between the two genera is the dominant phase of the life history, as ***Platychrysis*** species are not motile colonies, but typically occur as a benthic amoeboid / palmelloid phases (Chrétiennot 1973; Gayral and Fresnel 1983a). Although the dominant phase is non-motile, the flagellar apparatus is retained by the constituent amoeboid cells of ***P. pigra***. It is interesting to note that it was only after observation of the motile cells produced from the benthic phase, that Carter (1937) realised the prymnesialean affinity of ***Platychrysis***, and withdrew it from the benthic chrysophyte group where Geitler (1930) had placed it.

The genera ***Platychrysis*** and ***Prymnesium*** are separated only on the basis of the dominant life

history stage, which is non-motile in ***Platychrysis*** but motile in ***Prymnesium*** (Massart 1920; Conrad 1926, 1941; Green *et al.* 1982). There has been some doubt about the validity of this identifying feature, as the motile cells of the two genera are superficially similar and both possess short, non-coiling haptonemata and two scale types. Despite this, the appendages of ***Prymnesium*** species are subapically, not apically, inserted in a groove on the obliquely truncate anterior end of the cell (Carter 1937; Green *et al.* 1982; Billard 1983; Chang and Ryan 1985). In addition, details of the flagellar apparatus of ***Platychrysis*** species, (although not fully elucidated), are apparently different from those of ***Prymnesium patelliferum*** and ***Prymnesium parvum***. Swimming behaviour in cells of both genera is, however, similar, with heterodynamic flagellar movement as the cells rotate around their long axis and swim with the anterior end directed forwards. This heterodynamic movement is not as obviously different as that exhibited by either ***Phaeocystis*** or ***Corymbellus***, as it is usually the relative positions of the two flagella that are different although the beating motion is the same.

It is with the last, and largest genus of the Prymnesiaceae, ***Chrysochromulina***, that real taxonomic problems appear. Of the 47 species of ***Chrysochromulina*** currently listed (see Estep and MacIntyre 1989), 19 have been described from living and fixed material, 2 from living cells only, and 25 from fixed material. Of these 25 latter species, 17 have been described only from shadowcast wholemounts.

As the type species, ***C. parva*** Lackey, is a saddle-shaped cell with a haptonema far longer than the flagella and capable of coiling, the generic concept developed with this as the 'typical' *Chrysochromulina* cell. Subsequent species descriptions (Parke *et al.* 1955, 1956, 1959) concentrated more on the length and the coiling ability of the haptonema than on cell shape, and were admittedly "provisional" (Parke *et al.* 1955, p.579). The importance of haptonematal length and coiling ability has persisted as part of ***Chrysochromulina***'s generic description, although not actually part of any formalised diagnosis. For example "Our species is clearly referrable to the genus ***Chrysochromulina*** Lackey on the ground of its coiling haptonema, which is too long for a ***Prymnesium***." Hallfors and Niemi (1974, p.93), or, coiling is "at present regarded as a significant taxonomic criterion for separating species of ***Chrysochromulina*** from

those of at least three other genera (***Phaeocystis*** Lagerheim, ***Prymnesium*** Conrad and ***Chrysocampanula*** Fournier)." Manton and Leadbeater (1974, p.19); the coiling haptonema of ***Chrysochromulina*** is "a significant taxonomic criterion for separating species of ***Chrysochromulina*** from species of ***Prymnesium*** and ***Phaeocystis***." Thomsen (1979, p.74). There are, however, many species of ***Chrysochromulina*** in which the haptonema is shorter than the flagella and in which coiling is absent (***C. mantoniae***, ***C. spinifera***), only partial (***C. tenuisquama***), limited to bending and flexing (***C. bergenensis***, ***C. birgeri***, ***C. breviturrita***, ***C. herdlensis***, ***C. parkeae***) or has not been verified, but is not evident in the published micrographs (***C. fragilis***, ***C. laurentiana***, ***C. megacylindra***, ***C. polylepis***, ***C. pyramidosa***, ***C. tenuispina***). In addition, some reports that describe coiling haptonemata in fixed cells, may not necessarily be true of the situation in living cells. This is because when fixed, the haptonemata of cells of ***Chrysochromulina*** automatically coil (Manton and Leadbeater 1974; see also, for example, Hallfors and Niemi 1974; Nicholls 1978; Moestrup and Thomsen 1986), so the practice of describing new species of ***Chrysochromulina*** on the basis of shadowcast wholemounts from mixed nanoplankton hauls, creates additional generic problems.

The second important contribution to ***Chrysochromulina*** taxonomy made by Parke *et al.* (1955), was at the species level. They recognised that the species-specific organic scales "would provide excellent diagnostic characters....for the delimitation of species" (Parke *et al.* 1955, p.580), and this observation proved to be correct. Although geographical variation in scales has been reported in some species, this relates to scale size only, as the patterning remains consistent. The presence of more than one scale type in species of ***Chrysochromulina*** was initially used as a means of distinguishing between this genus and ***Prymnesium***, as the type species of ***Prymnesium***, ***P. parvum*** was originally believed to possess only one scale type (Manton and Leedale 1963). It was later shown that all species of ***Prymnesium*** actually have two scale types (Green *et al.* 1982; Billard 1983; Chang and Ryan 1985), while the type species of ***Chrysochromulina*** had only one simple type of plate scale (Parke *et al.* 1962). By this stage, however, numerous species of ***Chrysochromulina*** had already been described with scale complements of remarkable complexity.

When cells of ***Chrysochromulina*** are fixed, not only do the haptonemata coil, but fixation also results in a tendency for the cells to shrink (up to 50% of their original size) and round up (Manton and Leadbeater 1974). Of the 17 species described only from shadowcast wholemounts, 11 are described as being globose or spherical (***C. discophora***, ***C. elegans***, ***C. latilepis***, ***C. laurentiana***, ***C. leadbeateri***, ***C. novae-zelandiae***, ***C. pachycylindra***, ***C. pelagica***, ***C. simplex***, ***C. tenuispina***, ***C. tenuisquama***), 3 are reportedly subspherical (***C. adriatica***, ***C. brachycylindra***, ***C. cyathophora***), 1 is 'nearly isodiametric'(***C. vexillifera***), 1 is not described (***C. hirta***), while there is only a single cell described as being saddle-shaped (***C. pyramidosa***). This confirms the fact that cell shape and region of insertion of the appendages have not been considered to be very important features (although mentioned by Green *et al.* 1982 for ***Chrysochromulina*** as being saddle-shaped with ventral insertion). So the range of cell types that has been assigned to ***Chrysochromulina*** has expanded well beyond the range of any other prymnesialean genus.

Chrysochromulina species with long coiling haptonemata do not present identification problems at the generic level. All the cells with haptonemata greater than five times the flagellar length (e.g. ***C. alifera***, ***C. camella***, ***C. campanulifera***, ***C. parva***, ***C. strobilus***) are saddle-shaped and exhibit ventral flagellar insertion. This is also true of other members of the 'strobilus' group (i.e. ***C. apsteler***, ***C. cymbium***) as well as ***C. acantha*** and ***C. ephippium***. Of species with haptonemata between two and five times the flagellar length, both ***C. ericina*** and ***C. kappa*** have apical insertion of the appendages, while all the cells with relatively short haptonemata (equal to or less than the flagellar length) have apical insertion and are not saddle-shaped. In this category the living cells studied include ***C. birgeri***, ***C. brevifilum***, ***C. breviturrita***, ***C. chiton***, ***C. minor***, ***C. parkeae***, ***C. polylepis***, ***C. pringsheimii***, ***C. spinifera***. In four of these species (viz. ***C. birgeri***, ***C. chiton***, ***C. ericina***, ***C. polylepis***) the flagella and haptonemata arise from a central depression or groove in the obliquely truncate anterior end of the cell - the arrangement that typifies ***Prymnesium***. The overlap between the two genera therefore includes cell shape, in addition to details of the scale complement and the haptonematal ability to coil. Swimming behaviour is also not generically consistent within ***Chrysochromulina***, as although flagellar movement is most often described as being homodynamic (e.g. Green *et al.* 1982), there are

numerous reports of heterodynamic flagellar movement (e.g. see Parke *et al.* 1955, *et seq.*). Direction of swimming also varies in ***Chrysochromulina***, as slow swimming occurs with the appendages positioned as in ***Prymnesium*** (flagella trailing, haptonema extended forwards), while rapid movement often occurs with the posterior end of the cell directed forwards and the appendages trailing (as in cells of ***Corymbellus***).

Some phycologists (e.g. Green and Leadbeater 1972; Pienaar and Norris 1979) have questioned whether or not haptonematal ability to coil warrants generic separation. A negative response to this question resulted in the genus ***Chrysocampanula*** being incorporated into ***Chrysochromulina***, as the former had been separated on the basis of a short, non-coiling haptonema (***Prymnesium***-like) but very elaborate scales (***Chrysochromulina***-like). However, Green *et al.* (1982) concluded that either a coiling haptonema and/or an elaborate scale complement could distinguish a species of ***Chrysochromulina*** from one of ***Prymnesium***. Although this definition results in most species of ***Chrysochromulina*** being retained within the genus, the problem has not been entirely resolved: some ***Chrysochromulina*** species without coiling haptonemata (e.g. ***C. adriatica***, ***C. herdlensis***, ***C. laurentiana***) have scales that are no more elaborate than those of a ***Prymnesium*** species such as ***P. calathiferum***.

The overlap in haptonematal, scale and cell shape features between species of ***Chrysochromulina*** and ***Prymnesium*** has led to some uncertainty. "It seems that the genera ***Prymnesium*** and ***Chrysochromulina*** are not quite clearly defined and separable in their present delimitation" Hallfors and Niemi (1974, p.93). Despite the generic distinctions outlined by Green *et al.* (1982), taxonomic wariness still occurred "The separation of both genera [***Prymnesium*** and ***Platychrysis***] from ***Chrysochromulina*** also warrants careful consideration in the future." Thomsen (1987, p.137).

Added to this is the more recent observation that the flagellar apparatus of some species of ***Chrysochromulina*** may be as dissimilar to each other as they are to ***Prymnesium*** (comparing the simple system in ***C. apheles*** with the more complex, but unique systems of ***C. acantha*** and ***P. patelliferum***).

AIM OF THIS STUDY

This study was conducted in order to contribute to the descriptive data relating to the ultrastructure of prymnesiophycean motile cells, particularly with respect to features of the flagellar/haptonematal apparatus. The two genera selected for the study were ***Chrysochromulina*** and ***Prymnesium***, because although together they constitute two of the largest prymnesiophycidean genera, little detail is known about their flagellar features: from a possible 55 species, there is only one complete description of a transitional region and three reconstructions of the flagellar/haptonematal apparatus, each of which is a different variation on the prymnesiophycean flagellar theme. A further aim of this study was to use the ultrastructural information obtained, in an attempt to answer the question of whether or not the present generic separation between the two genera ***Chrysochromulina*** and ***Prymnesium*** is supported by features of the flagellar/haptonematal apparatus. In order to do this, it was necessary to consider species from across the wide morphological range existing within the genus ***Chrysochromulina*** (from less 'typical' species which are not saddle-shaped and which have relatively short haptonemata with subapical or apical appendage insertion, to saddle-shaped species with long coiling haptonemata and ventral insertion into the concave face of the saddle-shaped cells).

The research undertaken for this thesis is presented in the five papers that constitute the following chapters. Three of these papers are in press (chapter 2 in the Journal of Phycology, chapter 3 in Phycologia; chapter 5 in the European Journal of Phycology) while the remaining two have been submitted to the Journal of Phycology (chapter 4) and Phycologia (chapter 6) and are currently under review.

CHAPTER 2

ULTRASTRUCTURE OF *PRYMNESIUM NEMAMETHECUM* SP. NOV. (PRYMNESIOPHYCEAE)

ABSTRACT

Based on material collected from Cape Town, a new sand-dwelling, marine species of ***Prymnesium*** is described. Using light and electron microscopy, ***Prymnesium nemamethecum*** sp. nov. has been found to resemble other species of the genus in size, organelle arrangement and swimming behavior. It differs from other described species in that it has three types of scales, one of which is confined to the region of appendage insertion and forms a sheath of simple plate scales over the haptonema. In addition, the scales constituting the proximal body scale layer(s) are unusual because they are not simple plate scales, but are specifically ornamented.

INTRODUCTION

During an ongoing study of the inshore nanoplankton of the South African coast, a new species of the genus ***Prymnesium*** Massart ex Conrad was isolated. Since the overview of this genus by Green *et al.* (1982), only three new species have been described, viz. ***P. annuliferum*** Billard, ***P. zebrinum*** Billard (Billard 1983) and ***P. calcithecum*** Chang & Ryan (Chang and Ryan 1985). With the exception of the type species ***P. saltans*** Conrad (Conrad 1926, 1941), the mode of

swimming is similar in all species, as is the general arrangement of cell organelles. As a result, present species descriptions are based on cell and appendage morphometry, and on scale ornamentation. In all species described using the electron microscope, the innermost scale layer/s is/are composed of typically prymnesiophycean scales (Hibberd 1980), having radiating ridges on the proximal surface and a rimmed distal surface with concentric or radial fibrillar patterning (Green *et al.* 1989). The single, outermost scale layer, the distal layer, is the one that possesses species specific ornamentation. In this paper, we describe a new species of ***Prymnesium*** in which there is a generically atypical distribution of three scale types. This organism has been briefly introduced by Pienaar and Gold (1989, 1990).

MATERIALS AND METHODS

In September 1988, a water and sand sample from the bottom of the tidal swimming pool at St. James, near Cape Town, was enriched with half-strength Provasoli-a culture medium (McLachlan in Stein 1979) in $36^0/_{00}$ seawater (pH 7.8) and incubated at 20°C, with a 16:8h L:D cycle. A number of single ***Prymnesium*** cells were subsequently isolated from this enrichment, with clonal cultures (Q16 - Q21) being housed in the culture collection at the University of the Witwatersrand. Four years later, water samples from the type locality again produced the same species of ***Prymnesium***. The cultures are presently maintained under conditions similar to the original enrichment, but with the addition of soil extract to the culture medium (McLachlan in Stein 1979). Although this new species is euryhaline, growth is maximized using seawater of $35^0/_{00}$ (Gold 1990).

A Zeiss Axiophot microscope equipped with phase and Nomarski interference optics was used for studying cell shape, size and swimming behavior.

For electron microscopy (EM), scale preparations were made by osmicating drops of cell culture on 0.3% formvar-coated, copper grids, and then shadow-coating at 30° with gold palladium. For sectioned material, two methods were used. Cells were fixed either by the procedure described by Sym and Pienaar (1991), or as follows. Cell cultures (in $35^0/_{00}$ seawater, pH 7.8, with PES and soil extract) were fixed in 1.25% glutaraldehyde for 40 min. The sample

was then centrifuged and the pellet was resuspended in drops of cooling 0.2% purified agar. Agar pieces were repeatedly rinsed in culture medium followed by 60 min post-fixation in 2% OsO_4. The samples were again rinsed in culture medium and then dehydrated in a graded aicohol series at 15 min intervals, prior to embedding in Spurr's resin (Spurr 1969). Sections were cut on a Reichert ultracut E microtome, stained for 20 min in saturated aqueous uranyl acetate followed by lead citrate, and viewed with a Jeol 100S or Jeol 100C transmission electron microscope.

NOTE: Abbreviations used in the figures : C, chloroplast; CER, chloroplast endoplasmic reticulum; G, Golgi body; H, haptonema; L, left flagellum; PER, peripheral endoplasmic reticulum; PV, posterior vacuole; Py, pyrenoid; R, right flagellum; SV, scale-containing vesicle.

OBSERVATIONS

Prymnesium nemamethecum sp nov.

Cellulae natantes elongatae (8-18.5μm x 6-11μm) non complanata cum parte antica oblique truncata et parte postica metabolo. Appendices in depressione subapicale insertae. Flagella duo heterodynamica et subaequalia vel inaequalia (16-25μm et 9.5-20μmm); haptonema (2.5-6.3μm) flexile sed spiram non formans. Cellulae obtectae corporibus muciferibus, numerosis et fortuitis. Cellulae tribus typis squamarum tectae. Usque ad sex strata squamae proximalium in corpore. Squamae proximales magnae, ovales et catilliformes cum cristis radiantibus in aspectu proximali (circa 30 per quadrantem) et cum labro inflexo atque ornamento secundario et coroniformi super cristae radiantes in aspectu distali. Stratum distale unicum, squamas ellipticas (0.65μm x 0.34μm) continens. Hi squamae cum cristis radiantibus (circa 20 per quadrantum) in aspectu proximali et distali visibilibus. Superficies distalis cum parietе peripherali et erecto atque spissescente in medio secus axem longum cum ramis lateralibus et alternatibus et ad parietem peripheralem extentibus. Typus tres squamae catilliformis et ellipticus (0.61μm x 0.31μm), cum cristis radiantibus (circa 20 per quadrantum) in superficiebus ambabus, sed in aspectu distali margine angusta et inflexa cum umbone centrali. Typus tres squamae ad regionem flagellorum

limitatus et fortasse in latu distale per axem longum concavus. Cellulae sine motu subito natantes cum flagellis postice reflexis. Modus natandi rectus, cellulis circum axem longitudinalem revolventibus et cum polo antico spiram latam describente. Chloroplasti duo, mellei, parietales per longitudinem cellulae, lobati; pyrenoidibus internis satis visibilibus; nucleus centralis inter chloroplastos; corpus Golgi parabasale; vacuola postica corpora oleosa praesentes.

HOLOTYPUS: Figura nostra 9.

Cells elongate (8-18.5μm x 6-11μm), not compressed, being obliquely truncate anteriorly and posteriorly metabolic. Appendages subapically inserted in a depression and comprise two heterodynamic, subequal to unequal flagella (16-25μm and 9.5-20μm) and a short, non-coiling, but flexible, haptonema (2.5-6.3μm). Cells covered by numerous, randomly - scattered, muciferous bodies, and by three scale types. Up to six layers of proximal body scales occur. These proximal body scales are large, oval plate scales (0.84μm x 0.6μm), with proximal radiating ridges (about 30 per quadrant), and distally an inflexed rim and secondary coronate ornamentation on top of the radiating fibrils. The single distal scale layer consists of elliptical scales (0.65μm x 0.34μm) with proximal radiating ridges (about 20 per quadrant), which are also evident on the distal surface. The distal surface is further elaborated by a peripheral, upright wall and a long, central thickening that gives rise to alternating side branches. The third scale type is a simple plate scale (0.61μm x 0.31μm) with radiating ridges both proximally and distally (about 20 per quadrant), but distally also having an inflexed, narrow rim and a small, central thickening. These scales may be distally concave along their long axis and occur only in the anterior region of appendage insertion, around the haptonema. Cells exhibit a smooth swimming behavior with the flagella directed posteriorly. Cells - forward revolving around the long axis with the anterior pole describing a wider helix. Two golden - orange lobed chloroplasts, each with an obvious pyrenoid, lie parietally along the length of the cell. Other cell contents include the median nucleus between the chloroplasts, the parabasal Golgi body, a posterior vacuole, and scattered lipid droplets.

HOLOTYPE: Figure 9.

ORIGIN: Seawater (35$^0/_{00}$) from the sediment of the tidal pool at St. James, Cape Town. Type

material collected from this locality in September 1988. Culture Q16 in the phycology culture collection of the University of the Witwatersrand.

ETYMOLOGY: Greek *nema* (from haptonema) meaning thread, and *me* + *theca* with a sheath.

Light microscopy

Cultures of ***P. nemamethecum*** are a golden - orange colour which may darken slightly with age. After some four weeks, many cells settle to the bottom of the flask on the side nearest the light source, to form a dark orange-brown pellet. These old cells remain motile and were never seen to produce encysted stages. Vegetative reproduction occurs most commonly in freely swimming cells. Active cells may also occasionally shed their scale casing (Fig. 7), but as they retain their original shape and behavior, they cannot be described as amoeboid. In wild material, the cells are gregarious and attach to sand grains and shell fragments (Fig. 8).

The elongate, ovoid cells, 12μm (8.0 - 18.5μm) X 8μm (6 - 11μm), are typically broader anteriorly and taper posteriorly (Fig. 1). All cells have a truncate apex (Figs. 1, 2), but metaboly occurs posteriorly, resulting in rounded, pointed or angular posterior ends. Older cells and flattened cells (that have been viewed for several minutes under a coverslip) are rounder than normal (Figs. 3, 4), and the old cells found on the bottom of the culture vessels are often completely spherical. The two subequal to unequal flagella, 21μm (16 - 25μm) and 14.5μm (9.5 - 20μm), and the non-coiling haptonema, 4.1μm (2.6 - 6.3μm), are subapically inserted in an anterior pit that runs laterally across the cell (Fig. 2). (All cell orientations follow those of Beech and Wetherbee 1988 and Green and Hori 1990). The length ratios of the haptonema to the two unequal flagella are 0.20 and 0.28, and the ratios of flagellar lengths to cell length are 1.75 and 1.21.

Both Nomarski interference and phase contrast optics demonstrate that each cell is surrounded by a translucent layer (Fig. 1) which is continuous with a conical sheath that encloses the haptonema (Fig. 4). EM observations reveal that this layer is composed of scales). The cell membrane is randomly stippled with numerous muciferous bodies (Fig. 6). The cells each contain two lobed, parietal, chloroplasts that lie laterally along the length of the cell (Figs. 1 - 4), but which may fill only the anterior third of old or stressed cells. Associated with each

chloroplast is a pyrenoid, which is readily apparent even though it is partially immersed in the plastid (Fig. 4). The cytoplasm contains a number of lipid globules and a large posterior vacuole (Figs. 1-4) that increases in size with increasing age. Active, living cells may also have a single, brightly birefringent, amorphous granule located over the chloroplasts, the central cytoplasmic region or, most commonly, over the posterior vacuole (Figs. 2, 5a).

The cells move smoothly, either for short distances in straight lines or, more frequently, for longer periods in a circular pathway (the diameter of which is four to five cell lengths). In both cases, however, the cells rotate around their long axes with the anterior pole describing the widest helix. During normal forward swimming the flagella are held obliquely backwards down the sides of the cell, with the longer flagellum undulating away from the cell body and the shorter flagellum flicking (Fig. 2). When cells attach, flagellar movement may be homodynamic with both flagella undulating gently, either laterally or posteriorly positioned. This type of flagellar action is temporary though, and movement always tends to become obviously heterodynamic (Fig. 5a, b). The longer flagellum continues to undulate at about 45° to the cell body, the speed varying from a gentle wave-like motion to a rapid whipping movement to a frenzied rippling. The shorter flagellum is either closely adpressed around the cell or is held out laterally (approximately 90° to the long axis) where it flicks intermittently, sometimes quivering, sometimes coiling into a few gyres. Both flagella may coil tightly when the cell becomes stressed (Fig. 3), and just prior to autotomy the two flagella may straighten out directly in front of the cell. During swimming the haptonema extends forwards, but when attached it may bend slightly towards the shorter flagellum (Fig. 5a).

Electron microscopy

Prymnesium nemamethecum possesses three different types of scales (Figs. 9 - 28). 1) Scale type 1 (Figs. 10 - 19) is the most proximal and lines the cell body with up to six imbricate layers of scales (Figs. 9, 14). The proximal surface of these ovoid scales (0.84μm x 0.6μm) bears a pattern of radiating ridges in quadrants, *c.* 30 lines per quadrant (Fig. 11). The distal surface (Figs. 10, 12, 13) has a sharply inflexed, smooth rim (0.04μm wide), radiating ridges and a central, slightly raised, coronate ornamentation which is only partially visible when viewed

from the proximal surface (Fig. 11). This ornamentation generally consists of a central circle flanked by two smaller semicircles, all of which are bisected by the arms of a centrally positioned cross (Figs. 10, 12). This cross lies along the quadrant divisions in the center of the scale. The longer component (along the long axis of the scale) extends beyond the semicircles in the form of a tapering spine. The shorter arm of the cross runs through the middle of the large circle (which is therefore divided into four subunits). It too extends beyond the circle on each side, to form a short spine. Each of the four central compartments also has a small spine extending outwards, at about 45° to the arms of the basic cross. These spines tend to give the four subunits a slightly angular outline (Figs. 10, 12). The central coronate ornamentation is surprisingly variable and it may occur as a single, long arm of the cross with incomplete side arms (Fig. 15) or as a long arm of the cross with an incomplete large circle (Fig. 16). Some scales have a central cross but an incomplete large circle (Fig. 17) or a central ornamentation lacking one semicircle (Fig. 18), while other scales have one large circle and three semicircles (Fig. 19). Further variation has been seen in material from Bass Strait, south-eastern Australia, (D.R.A. Hill, pers comm.), where the large central circle is surrounded by numerous semicircles in two layers, creating a honey-comb effect.

2) Scale type 2 (Figs. 9, 14, 20 - 24) occurs as a single distal layer (Figs. 9, 14) of elongate, ovate scales (0.65μm x 0.34μm). The proximal surface (Fig. 21) displays radiating fibrils (*c.* 20 per quadrant). These radiating ridges are also visible on the base of the distal surface (Figs. 20, 22) but may be obscured by the 0.11μm high, thin peripheral wall (Figs. 23, 24) which tends to collapse in shadowed material (Fig. 20). Distally, along the length of each scale, is a raised thickening (0.14μm high) which subtends branches alternately to each side (Figs. 20, 22, 23). The 17 - 22 side-branches extend to the distal wall but are only 45nm high;

3) Scale type 3 (Figs. 9, 25 - 28) is only found around the haptonema (Fig. 9) and is responsible for the haptonematal hyaline sheath evident at the light microscope level. These scales are similar to scale type 2, in that they are elongate elliptics (0.61μm x 0.31μm) with radiating ridges (*c.* 20 per quadrant) on both the proximal and the distal surfaces (Figs. 25, 26, 27). Here, however, the distal surface has a narrow (30nm), smooth, inflexed rim (Figs. 25, 27, 28). When sectioned, these scales frequently appear to be distally concave along their long axes (Fig. 28).

The internal arrangement of cell organelles in ***P. nemamethecum*** resembles that of other described species (eg. Manton and Leedale 1963, Green *et al.* 1982). The anterior groove into which the three appendages are inserted (Figs. 29, 30, 31, 37, 41) is enclosed largely by portions of the two chloroplasts which are anteriorly lobed (Figs. 30, 31, 35). Each chloroplast contains a pyrenoid that protrudes into the central cytoplasm, and which is variably traversed by 4 - 10 thylakoid pairs (Figs. 29, 30, 32). Limited amounts of periplastidal reticulum lie along the inner face of each chloroplast (Fig. 34), generally in the region of the pyrenoids. Posteriorly, the chloroplast endoplasmic reticulum (CER) over the pyrenoids is continuous with the nuclear membrane (Fig. 32) in a manner now regarded as typical for the Prymnesiophyceae (Billard 1985).

Anteriorly, on the left side of the cell, the CER contributes to the development of the forming face of the Golgi body (Fig. 35). This organelle is polarized around the flagellar bases (Figs. 35, 36) and possesses typical 'peculiar dilations' (Figs. 29, 35). At the maturing face of the Golgi body, which is usually associated with concentrically-arranged membranes (Figs. 36, 37), a large scale-containing vesicle fuses with the plasmalemma on the right side of the anterior groove (Figs. 36 - 42). The region of fusion between vesicle and groove forms a slightly constricted mouth that is lined by root microtubules on its ventral, posterior side (Figs. 36, 40). In addition to the scale-releasing vesicle, there are only two other 'openings' directly connecting the cell cytoplasm to the surrounding medium (at least in cells that have been successfully fixed so that the peripheral endoplasmic reticulum remains intact). These two openings are both hairy diverticula associated with the Golgi body. One pit lies on the dorsal side of the cell, between the haptonema and right flagellum, at the base of the broad root (R1 of Green and Hori 1991) (Figs. 36, 40, 42). The other ventrally-situated pit is associated with the left flagellum and lies immediately ventral to the R2 microtubular root, very close to the adjacent chloroplast lobe (Figs. 38, 41). These two diverticula appear to correspond in position to those described by Manton (1966) in ***P. parvum*** Carter but are not directly involved in the release of scales as she hypothesized. In addition to scale production, many of the Golgi cisternae contain dense osmiophilic deposits (Figs. 35, 36, 38) which are ultimately extruded into the peripheral endoplasmic reticulum (Figs. 29 - 32), where they are apparent at the LM level as muciferous bodies.

The nucleus lies in a median position above the large posterior vacuole and is frequently flanked by large lipid reserves that protrude into the vacuole (Figs. 29, 30). A cytoplasmic tongue (as first described by Gayral and Fresnel 1983a) runs posteriorly through the vacuole (Figs. 30, 33).

DISCUSSION

At the light microscope level there is no doubt that the cells of this organism belong to the genus ***Prymnesium*** because of their shape, the short non-coiling haptonema, the way they swim, and the absence of a dominant non-motile life stage as in ***Platychrysis*** Geitler (Gayral and Fresnel 1983b). Ultrastructurally they resemble other members of the genus in the arrangement and ultrastructure of their organelles (Manton and Leedale 1963, Green *et al.* 1982) and in the length ratios of flagella/haptonema/cell (for the generic range of values see Chang and Ryan 1985). They also possess both typical plate scales (most similar to those of ***P. patelliferum***, Green *et al.* 1982, in having distal radiating ridges and a narrow rim) and a distal scale layer of upright-rimmed, distally ornamented scales of a similar size to the plate scales. ***Prymnesium nemamethecum*** differs from other members of the genus in having the following : 1) The simple plate scales cover the haptonema and so are confined to the region of appendage insertion. In all other species of ***Prymnesium***, the simple plate scales form the proximal scale layer(s) around the cell. The phenomenon of distinct haptonematal scales has been previously described in ***Isochrysis*** Parke (Parke 1971) and a number of coccolith-bearing cells (e.g. Leadbeater 1971, Fresnel and Billard 1991); 2) Three types of scales, one of which constitutes a specifically-ornamented proximal body scale layer(s), and which is much larger than the other two scale types. The distal scale layer and the haptonematal scales correspond to the two scale types typical of other species, because all other species have dimorphic scales with similar scale base sizes (see Chang and Ryan 1985). However, the proximal body scale type of ***P. nemamethecum*** is the largest and most variably ornamented scale ever described for the genus.

Neither of these two features presents an obstacle to generic inclusion, as another genus in the Prymnesiales, ***Chrysochromulina*** Lackey, contains both species with varying numbers of scale types (Green et al. 1990) and species which may exhibit specialization of scales anteriorly (Parke *et al.* 1955, Parke and Manton 1962). ***Chrysochromulina*** is, however, undoubtedly a polyphyletic assemblage, so a perhaps less controversial genus that could be used to illustrate this taxonomic point is the well described prasinophyte genus, ***Pyramimonas*** Schmarda, which contains species with a scale type confined to the flagellar pit and species with differing numbers of scale types (McFadden *et al.* 1986).

The unique ornamentation of the three scale types obviously differentiates this taxon from any other species described at the EM level, viz. **P. *parvum*** Carter (Manton and Leedale 1963), ***P. patelliferum*** Green *et al.* (Green *et al.* 1982), ***P. annuliferum***, ***P. zebrinum*** (Billard 1983), and ***P. calatiziferum*** (Chang and Ryan 1985). The three remaining members of the genus have been described only with the light microscope. Cell morphometry can be used to distinguish between ***P. nemamethecum*** and both the very small ***P. minutum*** Carter and the small, globose ***P. czosnowskii*** Starmach (Green *et al.* 1982). This leaves the type species, ***P. saltans*** Conrad, which was illustrated and described by Massart (1900, 1920) and Conrad (1926, 1941) and reported once since then by Heynig (1978). As the size range of most species of ***Prymnesium*** overlaps, Green *et al.* (1982) consider the peculiar swimming mode of ***P. saltans*** to be its primary identifying characteristic. Both Conrad (1941) and Heynig (1978) described an erratic, jerky locomotion with the flagella extended obliquely forwards during swimming. In contrast, ***P. nemamethecum*** swims smoothly with backwardly - directed flagella. The only time the flagella are held forwards is in attached cells, immediately prior to autotomy. The rippling flagellar beat of ***P. saltans***, illustrated by Massart (1920) and noted by Green *et al.* (1982), occurs in attached cells of ***P. nemamethecum*** too. This particular flagellar phenomenon may not be specifically characteristic, however, as Heynig (1978) has also illustrated this movement in cells that were identified as ***P. parvum*** and which did not swim like ***P. saltans*** (e.g. Figure 1j, p.226). Other differences between ***P. saltans*** and ***P. nemamethecum*** include the facts that the flagella of ***P. nemamethecum*** are markedly subequal to unequal, whereas those of ***P. saltans*** are equal (Conrad 1941); ***P. saltans*** has one or two contractile vacuoles (Conrad 1941, Heynig

1978), which are absent in ***P. nemamethecum***; and there are two visible pyrenoids in ***P. nemamethecum***, none in ***P. saltans***.

Clearly this is a new species of ***Prymnesium*** that expands the existing generic limits. It is possible that its specific peculiarities, with regard to both its scales and to its complex flagellar root system (Birkhead and Pienaar, unpubl.) warrant the erection of a suitable subgenus.

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FIGS. 1 - 8. Light microscopy of *Prymnesium nemamethecum* sp. nov. All scale bars = 10μm unless otherwise stated.

Fig. 1. Ventral view of cell, with two heterodynamic flagella and a non-coiling haptonema inserted in the obliquely truncate, anterior end of the cell. Cell contents include two parietal chloroplasts, a posterior vacuole and scattered lipid droplets.

Fig. 2. Flash photography of an actively swimming cell, illustrating flagellar movement, subapical appendage insertion in an anterior depression, and a birefringent granule (arrow) in the posterior vacuole.

Fig. 3. Stressed cell with coiling flagella and rounded cell body.

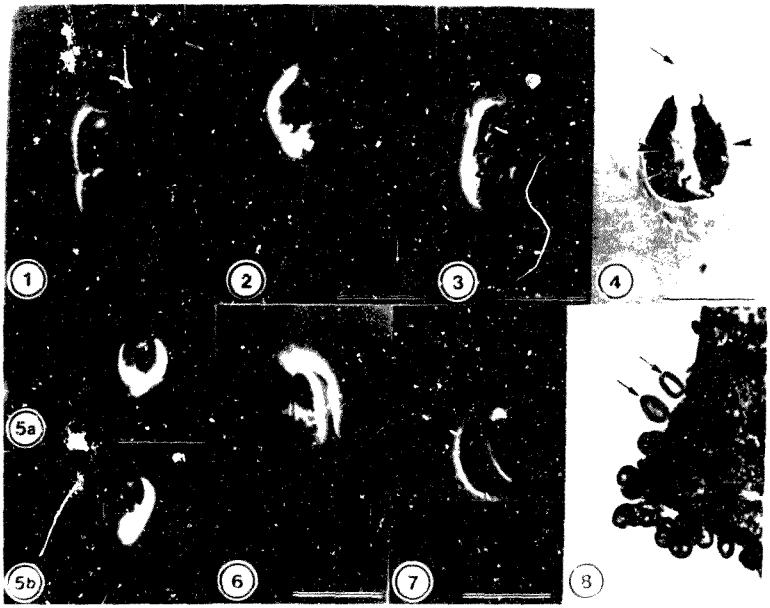
Fig. 4. Compressed cell revealing the two median pyrenoids (arrowheads) and the haptonematal sheath (arrow).

Fig. 5a, b. Sequential flash photomicrographs of an active, attached cell. Note the curving of the haptonema (arrowhead) towards the shorter flagellum.

Fig. 6. Surface view of cell to show randomly-arranged muciferous bodies. Scale bar = 8μm.

Fig. 7. Empty scale case.

Fig. 8. Two cells (arrows) attached to a sandgrain in the original enrichment. Scale bar = 30μm.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2
3
4
5a
5b
6
7
8

FIGS. 9 - 28. Scales of *P. nemamethecum* sp. nov.

Fig. 9. Longitudinal section through the anterior end of a cell, with scales (sc3) forming a sheath around the haptonema. The arrowheads indicate the apical extent of the distal (sc2) and proximal (sc1) body scale layers Scale bar = 1μm.

Figs. 10 - 19. Proximal body scale type, scale type 1. All scale bars = 0.28μm.

Fig. 10. Distal surface (shadowcast preparation).

Fig. 11. Proximal surface (shadowcast preparation).

Fig. 12. Horizontally sectioned scale.

Fig. 13. Cross-section through the shorter scale axis.

Fig. 14. Arrangement of the two types of body scales over the cell surface : a single layer of distal scales (arrowheads) and several layers of proximal scales.

Figs. 15 - 19. Variation in the central ornamentation on the distal surface of the proximal body scales.

Figs. 20-24. Distal body scale type, scale type 2. All scale bars = 0.25μm.

Fig. 20. Distal surface (shadowcast preparation).

Fig. 21. Proximal surface (shadowcast preparation).

Fig. 22. Horizontal section through the base of the scale.

Fig. 23. Transverse section across the shorter scale axis.

Fig. 24. Section grazing the peripheral wall of the scale.

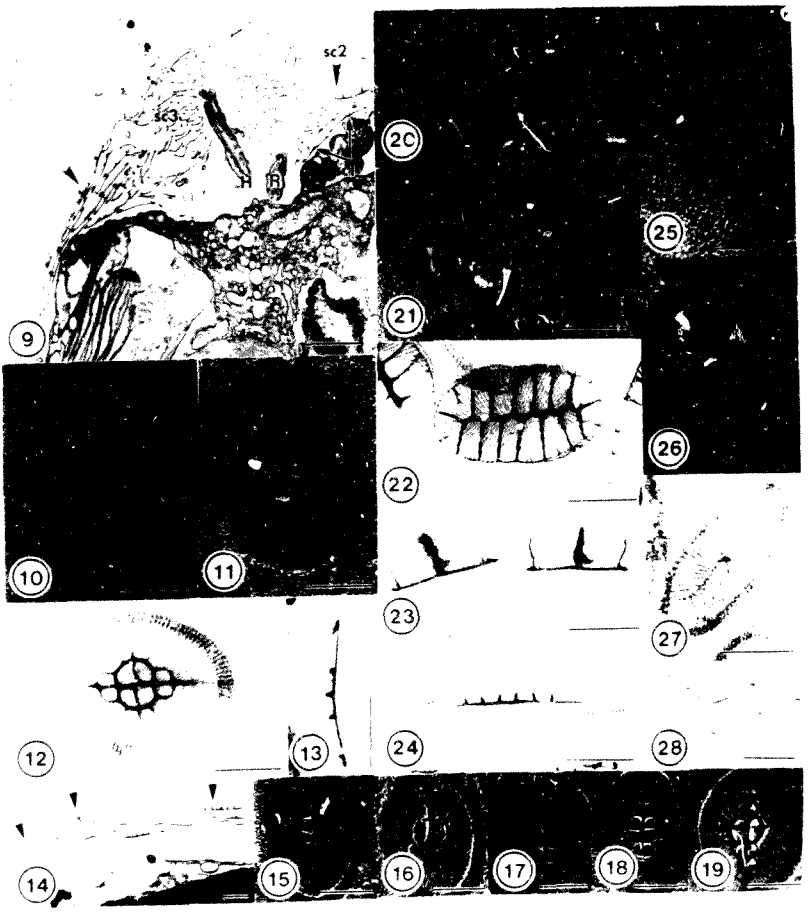
Figs. 25-28. Haptonematal sheath scales, scale type 3. All scale bars = 0.25μm.

Fig. 25. Distal surface (shadowcast preparation).

Fig. 26. Proximal surface (shadowcast preparation.

Fig. 27. Horizontal section through base of scale.

Fig. 28. Cross-section through shorter scale axis.


sc2
sc3
H
R
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10
11
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18
19

FIGS. 29 - 32. General cell structure of *P. nemamethecum* sp. nov.

Fig. 29. Longitudinal section in a dorso-ventral plane through the haptonema. Note the muciferous bodies (arrowheads) that disrupt the PER. Scale bar = 2μm.

Fig. 30. Longitudinal section in the lateral plane through all three appendages. Note the cytoplasmic tongue (arrowheads) that extends across the posterior vacuole. Scale bar = 2μm.

Fig. 31. Transverse section through the anterior end of a cell to show the lobed chloroplasts lined by the PER externally and by the CER on their inner faces. The haptonema lies between the left and right flagella in the anterior depression (lateral limits of this depression indicated by arrows). Scale bar = 1μm.

Fig. 32. Tangential transverse section through the median plane of a cell. Note the continuity of the outer nuclear membrane with the CER overlying the pyrenoid (arrowheads). Scale bar = 2μm.


H
PV
29
H
L
PV
30
R
CER
PER
31
32

FIGS. 33 - 37. Cytoplasmic tongue, periplastidal reticulum, Golgi body and Golgi-derived structures in *P. nemamethecum* sp. nov.

Fig. 33. Transverse section through the regularly-arranged microtubules (arrowheads) of the cytoplasmic tongue as it passes through the posterior vacuole. Scale bar = 1.2μm.

Fig. 34. Tubular periplastidal reticulum (arrows) lining the inner face of a chloroplast. Scale bar = 0.25μm.

Fig. 35. The CER lining the anterior part of the lobed chloroplast contributes (arrowheads) to the forming face of the Golgi body. Scale bar = 0.5μm.

Figs. 36, 37. Tangential serial sections (numbers 1 and 4) through the anterior end of a cell to illustrate the fusion of a scale-containing vesicle with the anterior groove in which the appendages are inserted. Arrowheads indicate the various microtubular roots present at this level. Note that the scales (e.g. S2) are Golgi-derived. Scale bars = 0.5μm.


PV
33
34
L
H
R
SV
S2
36
L
H
SV
35
37

FIGS. 38 - 42. Scale vesicles and hairy pits in *P. nemamethecum* sp. nov.

Figs. 38-40. Serial sections (numbers 1, 3, 5) through the flagellar bases, haptonematal base, and the associated microtubular roots (arrowheads), to show the fusion of the two coated diverticula (arrows) and the scale-containing vesicle, with the anterior depression. Scale bars = 0.5μm.

Figs. 41, 42. Serial sections (numbers 1 and 4) through the anterior groove to clearly reveal the points of fusion of the ventral (Fig. 41) and the dorsal (Fig. 42) hairy diverticula (arrowheads) in relation to the three appendages. Scale bars = 0.5μm.


38
B
L
39
SV
R
H
L
40
SV
B
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41
L
R
42
L
R
H

CHAPTER 3

THE FLAGELLAR APPARATUS OF *PRYMNESIUM NEMAMETHECUM* (PRYMNESIOPHYCEAE)

ABSTRACT

The flagellar apparatus of ***Prymnesium nemamethecum*** sp. ined. (Pienaar & Birkhead in press) is more similar to that of *P.* ***patelliferum*** Green *et al.* than to any other prymnesiophyte yet described. This includes the arrangement of the appendages, the origins and pathways of the microtubular roots and the system of fibrous and striated connectives. There is however a major difference in the presence of a compound broad root in interphase cells of ***P. nemamethecum***. Other differences include the number of constituent microtubules in some roots, the extent of development of a root (herein designated R7) associated with the right basal body, and an additional accessory band (AB3) in ***P. nemamethecum***. The transitional region of the flagella is also described. It resembles that of ***P. parvum*** Carter, as both species have two transitional plates separated by a form of stellate structure. ***P. nemamethecum*** also has a distal axosome and a series of tubular rings between the outer doublets and the flagellar membrane. The transitional regions of both species appear completely different from those described in the Isochrysidales and the Coccosphaerales.

INTRODUCTION

The ultrastructure of the flagellar apparatus has proved to be an invaluable tool for developing green algal phylogenies (Irvine & John 1984), so it seems appropriate to use this feature to clarify the relationships within other algal classes. Although the Prymnesiophyceae is a distinctly divergent class among the chromophyte organisms (Cavalier-Smith 1986; Andersen 1991), the taxonomy and phylogeny of its constituent members is frequently unclear using the present systems of classification. The Prymnesiophyceae is composed of four orders (*sensu* Parke and Green, in Parke & Dixon 1976) of which the Pavlovales is the most coherent grouping and is considered to be phylogenetically distanced from the remaining three orders (Green 1980; Green *et al.* 1989). However, the taxonomic confusion and overlap presently existing between the Isochrysidales, Prymnesiales and Coccosphaerales has already been confirmed by the relatively few studies on the flagellar apparatus (review by Preisig 1989; also Hori & Green 1991; Roberts & Mills 1992, Gregson *et al.* 1993). Taxonomies that reflect phylogeny depend on the amount of available ultrastructural or molecular data. The primary purpose of this paper is to contribute to the flagellar information pertaining to the Prymnesiales, whilst also completing the description of ***Prymnesium nemamethecum*** sp. ined. (Pienaar & Birkhead in press). This new species of ***Prymnesium*** is unusual in having three scale types, one of which forms a sheath around the haptonema.

MATERIALS AND METHODS

Cells of ***P. nemamethecum*** were isolated from a seawater sample from an intertidal swimming pool at St. James on the Cape Peninsula. The same clonal culture was used for this study and for the type description. Cells were fixed, embedded and serial sectioned as previously described (Pienaar & Birkhead in press). Only cells in interphase and without any signs of impending division were used to interpret the flagellar apparatus. Flagellar and microtubular

root numbering follow Beech & Wetherbee (1988) and Green & Hori (1990), while the numbering of the accessory bands follows Green & Hori (1990). Given that silver sections are approximately 80nm thick, the thickness of many structures, particularly the connectives, has been determined from serial transverse silver sections, and corroborated by longitudinal sections where possible.

Abbreviations used in the text and the figures

AC - auxiliary connective; C - chloroplast, D - dorsal side of the cell; DB - distal band; G - Golgi body; H - haptonema; IB - intermediate band; L - left flagellum or basal body; M - mitochondrion; PV - posterior vacuole; R1-R7 - microtubular roots; R - right flagellum or basal body; SV - scale-containing vesicle; V - ventral side of the cell.

RESULTS

Arrangement and insertion of the appendages

The two unequal flagella [(16 μm) - 21 μm - (25 μm) and (9.5 μm) - 14.5 μm - (20 μm)] and the haptonema [(2.5 μm) - 4.1 μm - (6.3 μm)] are inserted into a sub-apical groove on the ventral side of the obliquely truncate apex of the cell (Figs 1, 2). This groove is lined by chloroplast lobes which overlie branches of the mitochondrial reticulum (Figs 2-5, 32, 43, 58). The long axis of the groove runs from left to right across the cell, perpendicular to the dorsi-ventral cell axis, with the free parts of the three appendages being linearly positioned as they enter the groove (Figs 2, 3). However, their insertion into the cell is angled and asymmetric, so that they form a triangle more proximally (compare Fig. 3 with Fig. 7, and Fig. 43 with Fig. 48). When viewed from the cell's dorsal surface, the haptonema base lies closest to the dorsal cell surface, with the left flagellar basal body behind it (more ventrally situated) and the right basal body laterally displaced towards the right side of the cell (Fig. 7). The paths of the haptonema base and the left basal body are similar, while that of the right flagellum is more highly angled to them both.

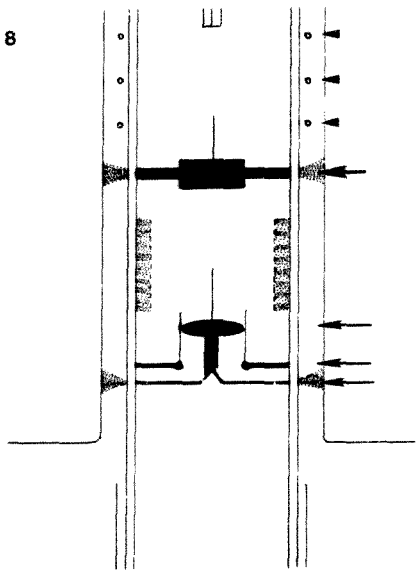


Fig. 8. Schematic longitudinal section through the flagellar transitional region in *Prymnesium nemamethecum*. Note the tubular rings (arrowheads), the distal plate (large arrow) and the three components of the proximal plate (small arrows).

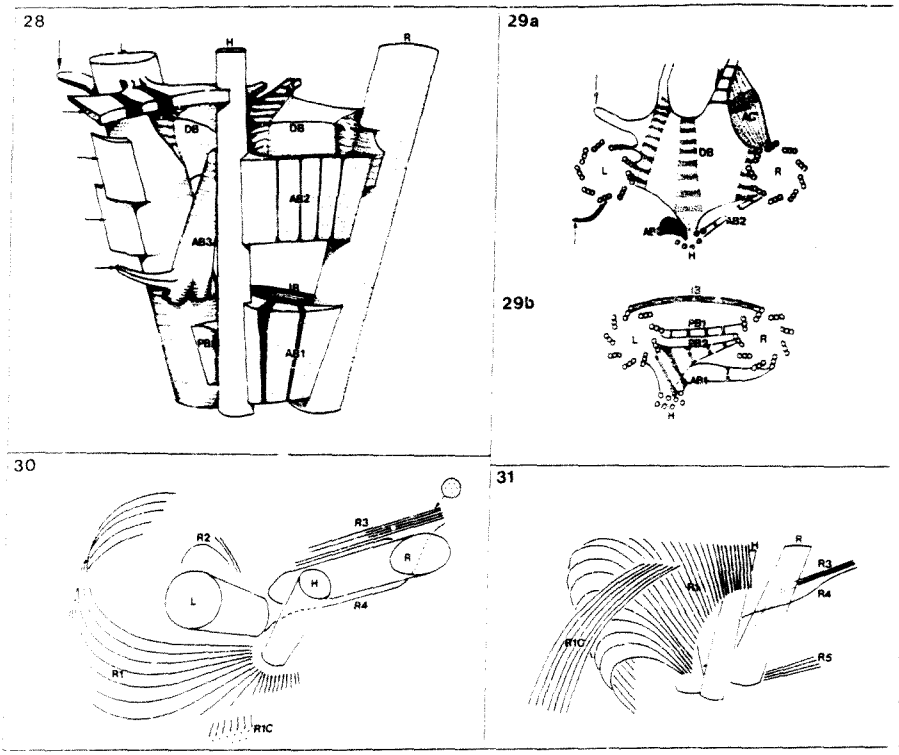
Flagellar and haptonematal ultrastructure

A schematic representation of the flagellar ultrastructure is presented in Fig. 8. The free part of each flagellum possesses the typical 9+2 axonemal structure and lacks a fibrillar surface coat (Figs 9-13), but may be surrounded by a thin, mucilage-like layer (Figs 22-25). Between the outer doublets and the flagellar membrane of both flagella, is a series of small tubular rings (Figs 8-11, 13) which extends from the insertion region for at least 1.3 μm up the proximal length of the flagellar shaft.

The transitional region is characterised by two transverse plates. These are not linked (Figs 8, 11-13, 18-22), although a slight bulging of the flagellar membrane may occur between the plates (Figs 11-13, 21). The distal plate is robust with a knob-like central thickening evident in transverse and in median longitudinal section (Figs 11, 12, 18). From this thickening, a thin thread extends distally towards the central pair of microtubules (Figs 8, 11). Osmiophilic material lies between and on the outside of the 9 outer doublets in the region of the distal septum (Figs 11, 12, 18, 19). The proximal plate is delicate and more complex, forming a tripartite structure (Figs 8, 11, 13, 22-24). A very thin, proximal plate rises centrally to a small cone that extends through the central hole of a sturdy, perforated septum, and bears an ovoid 'head'. The edges of the head are supported by a thin, cylindrical collar that rims the hole of the perforated plate and continues distally for some 160 nm (Figs 8, 13, 20, 21). A fine, antenna-like structure protrudes distally from the centre of the head to lie in the central space of the cylindrical collar (Figs 13, 20). Transverse sections through the cylindrical collar (Fig. 21) reveal an arrangement reminiscent of, but not identical to, a stellate structure. It could not be determined however, whether or not the fine connections link alternate A-tubules of the outer doublets as in the stellate structures of the green algae (see Andersen *et al.* 1991).

At the point where the flagella enter the cell, a faintly staining line of material sometimes lies across the lumen of each basal body (Fig. 11 lowermost arrow, Fig. 12). The basal bodies are generally 660 nm long and may contain a central core of osmiophilic material (Figs 7, 13, 36). These cores seem to occur most frequently in the left flagellar base. The triplet region is terminated by a short (100 nm) cartwheel structure. In only one cell, a septum was seen immediately above (distal to) the cartwheel region (Fig. 12).

The free part of the non-coiling haptonema consists of 7 microtubules surrounded by a



Figs 28 - 31. Schematic reconstructions of the flagellar apparatus of *P. nemamethecum*. Not drawn to scale. **Figs 28-29b.** Connectives of the apparatus. **Fig. 28.** Dorsal view of the connectives. Note the DB extension over the pathway of the R2 (large arrow) and the connections that extend towards the R1 (small arrows). **Fig. 29a.** Transverse section through the connectives at the distal level. Note the extensions towards the R1 (small arrow) and over the R2 pathway (large arrow). (The three appendages have been drawn as though arranged in parallel). **Fig. 29b.** Transverse section through the proximal connectives. **Figs 30, 31.** Pathways of the microtubular roots. **Fig. 30.** Anterior view, tilted slightly ventrally, of the three appendages and the associated roots. **Fig. 31.** Dorsal view of the microtubular roots.

perforated sheath of endoplasmic reticulum (Figs 14, 15, 26). In longitudinal sections in the lateral plane, the haptonemal membrane bulges out towards the left side of the cell, in the region where the 7 microtubules form an arc interrupted by a finger-like peg of endoplasmic reticulum (Figs 14, 15). The basal portion of the haptonema consists of 7 microtubules for at least three quarters of its length (Figs 5-7, 59), with an eighth microtubule being added only in the most proximal portion (Fig. 27).

Connectives of the flagellar apparatus

The three appendages are linked to each other by an array of striated and fibrous structures (Figs 28, 29a, 29b). The largest of these is the distal band (DB) which, when viewed from the cell's anterior end, is a plate-like sheet of material (c. 160 nm thick) with transverse striations running along the plane of the dorsi-ventral cell axis (Figs 6, 33, 34, 45, 46). The DB joins the right side of the left basal body, the ventral side of the haptonema base and the left side of the right basal body (Figs 6, 28, 29a). Two other minor connectives are associated with the DB: (1) a small extension that curves round the ventral side of the left basal body, briefly overlying the pathway of the R2 microtubular root (Figs 5, 28, 29a, 34, 35, 40, 45, 46);

(2) a series of at least three attachments that are associated either with the ventral chloroplast lobe nearest the right basal body, or with a mitochondrial profile that lies immediately below this chloroplast lobe. Two of these attachments (one fibrous, one striated) occur in the same plane as, and are continuous with, the major portion of the DB (Figs 28, 29a, 33, 34). The third, larger attachment is confluent with the DB, but may also continue to a slightly more proximal level. It extends from a triplet of the right basal body towards the chloroplast/mitochondrial lobes, and is characteristically fibrous with a single striation across the widest, central portion (Figs 29a, 32, 34-36, 47). This attachment is homologous with the large auxiliary connecting root of ***Prymnesium patelliferum*** Green *et al.* (Green & Hori 1990), and will therefore be termed an auxiliary connective (AC).

Given the asymmetry of the flagellar apparatus and the apparent positional alterations depending on the plane of section, it is difficult to determine whether these minor connectives are merely components of the DB, or whether they are discrete entities.

Three other connectives occur distally, although they are not confined to this area, but may continue proximally into the region above the proximal connectives:

(1) extending from the level of appendage insertion to that of the DB is a striated connective tongue that links both the haptonema and the left basal body to the broad root, R1 (Figs 4, 28, 32, 39, 44). Delicate, non-striated, fibrous connections are also present between the left basal body and the microtubules of the R1, from the level of the DB down to the proximal connectives (Figs 5, 6, 28, 29a, 34, 40). It is difficult to distinguish between the most distal of these fibrous connectives and the transitional fibres (e.g. Fig. 5);

(2) an extremely narrow, striated, accessory band AB2, links the haptonema base to the right basal body, from the level of the proximal half of the DB down to the proximal connectives (Figs 28, 29a, 34-36, 46-48);

(3) a densely-staining, non-striated accessory band AB3, linking the haptonema base to the left basal body (Fig. 28). The AB3 originates immediately above (distal to) the proximal connectives as a bipartite structure (Fig. 7) attached to at least two triplets of the left basal body (Figs 47, 48) and which contributes towards R1 connectives (Fig. 48). The AB3 tapers as it extends distally up to the haptonema, so that distally it appears as a small dark body attached to the haptonemal microtubules in the plane of the DB (Figs 6, 29a).

The four proximal connectives occur in the proximal 400 nm of the appendage bases :

(1) a delicate intermediate band (IB) which is thin (*c.* 160 nm), fibrous and unstriated, and links the two basal bodies on their ventral sides (Figs 28, 29b, 37, 42). The IB usually lies some 40 - 70 nm more distally than the other three proximal connectives;

(2) dorsal to the IB is a small (*c.* 160 nm thick), striated proximal band, PB1, that connects the two basal bodies (Figs 29b, 37, 42);

(3) a larger (*c.* 240 nm thick) proximal band, PB2, more dorsally positioned than PB1 (Figs 29b, 37, 42). The PB2 tapers from a widely-striated, loosely-fibrous structure lying between the haptonema base and the left basal body, to a densely-staining, tail-like portion that attaches to a triplet of the right basal body;

(4) a robust (*c.* 320 nm thick), widely-striated accessory band (AB1) with a granular appearance (Figs 29b, 37, 38, 42). The AB1 joins the right basal body to the haptonema base. The point

of attachment of the AB1 to the haptonema is extremely close to, and apparently overlaps that of, the PR , although the AB1 consistently attaches to a pair of adjacent triplets of the right basal body which are one triplet away from the point of PB2 attachment (Figs 29b, 42).

Microtubular roots

Three microtubular roots are associated with each of the basal bodies : the compound broad root (R1 and R1c) and the R2 with the left basal body; the R3, R4 and R7, with the right basal body (Figs 30, 31).

When viewed from the left side of the cell's dorsal surface, the R1 appears as a microtubular sheet in the s e of an asymmetric arch (Figs 30, 31, 52). Initially consisting of 3 or 4 microtubules (Figs 50, 59), the R1 begins proximally between the haptonema base and the left basal body, from a cushion of amorphous material (Figs 37, 38). As it ascends distally, numerous microtubules are added on the right side of the root (Figs 4-7, 31, 43-48), so that some 25 microtubules are seen to line the dorsal side of the anterior groove in the region of appendage insertion (Figs 43, 53) where they are closely adpressed to a mitochondrial branch (Figs 5, 44). These microtubules curve towards the left anterior edge of the cell and then descend posteriorly and ventrally (Figs 30, 31, 43, 50, 52) between the left dorsal and left ventral chloroplast lobes. The ascending microtubules lie almost parallel to the long axis of the left basal body, until they veer away latero-ventrally to form the hooped arrangement (Fig. 52). A few short microtubules also splay sharply leftwards in the more proximal region of the R1 (Figs 47, 50). In contrast to the closely-aligned, flanged, ascending microtubular component of the R1 (Figs 53, 56), the descending microtubular portion of the R1 tends to have more widely spread microtubules with faint connections discernible between them (Fig. 50), and they penetrate deep into the posterior region of the cell, well beyond the proximal level of the appendage bases (Fig. 62). The descending microtubules are arranged within a small cytoplasmic tongue, being associated with the dorsal face of a vesicle of peripheral endoplasmic reticulum between the left chloroplast lobes, and ultimately extending through the large posterior vacuole of the cell body (Figs 50, 62). This cytoplasmic tongue lacks the associated fibrous root that occurs in some coccolithophorids (e.g. Beech & Wetherbee 1988).

A thin line of osmiophilic material occassionally occurs between the R1 and the plasmalemma of the anterior groove distal to the plane of appendage insertion (Fig. 53). Serial sections reveal that this structure is formed by the adpression of the membranes of the peripheral endoplasmic reticulum, and that it is not a 'dense plate' as described in some coccolith-bearing cells (e.g. Beech & Wetherbee 1988).

Associated with the R1 is a small compound root, the R1c, consisting of a cluster of about 20 microtubules (Figs 30, 31, 40, 43, 51). The R1c attaches to a thin sheet of material (Fig. 53) at right angles to the R1, just distal to the level of appendage insertion (Figs 32, 39-41, 43). It extends over and between mitochondriai profiles (Figs 41-43), curving down slightly towards the left inner face of the dorsal chloroplast (Fig. 62). In interphase cells, the depth to which the R1c penetrates the cytoplasm varies. It may terminate at the level of the distal connectives, or extend down to the most proximal level of the appendage bases. The R1c is so named, as it is not truly crystalline and is clearly homologous with the r1c of ***Isochrysis galbana*** Parke (Hori & Green 1991). In dividing cells, the R1c enlarges tremendously (in terms of number of constituent microtubules and length), and becomes bifurcate around the nucleus (not shown).

The R2 root consists of three microtubules (initially two) which originate on the right side of the left basal body, dorsal to and slightly distal to the IB (Figs 35, 36, 41, 54 inset). They curve up and around the ventral side of the left basal body, passing beneath the ventrally-positioned extension of the DB, to fan out slightly towards the left ventral surface of the cell (Figs 30, 34-36, 41). One microtubule then curves sharply downwards to become associated with the forming face of the Golgi body, which is found on the left side of the cell (Figs 54-56). The remaining pair of microtubules runs laterally along the inner face of the left, ventral chloroplast for a short distance.

The R3 microtubular root begins at the level of the proximal connectives, in the same transverse plane as, or immediately distal to, the IB. Two microtubules lie on the ventral side of the right basal body (Fig. 41), with another two being added as the root moves distally (Fig. 60), with all four microtubules arranged in a line. As the root approaches and passes beneath the AC, two more microtubules are added above the original four, with another, single microtubule appearing beneath the four (Fig. 61). As this tiered arrangement of microtubules curves laterally to

underlie the peripheral endoplasmic reticulum lining the ventral edge of the anterior groove, the microtubule closest to the right basal body peels away from the remaining three central microtubules (Figs 30, 61). The resultant 2/3/1 arrangement continues for some distance over the chloroplast lobe lining the anterior depression, persisting at least to a level that corresponds to the transitional region of the right flagellum. In only one cell investigated, the singleton microtubule was absent, to give a 2/3 R3 arrangement.

The single microtubule of the R4 begins just inside the AB1, between the AB1 and the PB2, at the most distal extremities of the proximal connectives (Figs 30, 31, 37, 60). Initially associated more closely with the haptonema base and the left basal body, the R4 immediately slants upwards to the right side of the cell, passes over the AB2, and follows a highly angled path past the dorsal side of the right basal body (Figs 34, 47, 57). Distally it approaches the microtubules of the R3, by crossing from the dorsal to ventral side of the right basal body (Figs 43-45) immediately beneath the opening into the anterior depression of a large, scale-containing vesicle (Fig. 49, and see Pienaar & Birkhead in press for further details). Thereafter, the R4 appears to follow the R3 microtubular pathway beneath the peripheral endoplasmic reticulum.

The R7 consists of a bundle of four to six microtubules that originates on the most proximal, right side of the right basal body (Figs 31, 37-41, 58, 59). It is a short root (*c.* 1 μm) that stretches towards the right side of the cell, running dorsal to a mitochondrial profile that lines the adjacent chloroplast region (Figs 58, 59). Transverse sections through cells reveal that the R7 does not curve up or down, but is limited to a plane of some 100 nm in depth. This root has not been numbered sequentially to avoid any confusion with the R5 or R6 of ***Imantonia rotunda*** Reynolds (Green & Hori 1986).

DISCUSSION

A comparison of the transitional regions of members of the Prymnesiales suggests that this order may be typified by having two transitional plates which lie between the termination of the central

pair of microtubules and the beginning of the triplets of the basal body. The exquisite work done by Manton on ***Prymnesium parvum*** Carter (Manton 1964a, 1964b; Manton & Leedale 1963) reveals a transitional region similar to that of ***P. nemamethecum***, but with a more distinct 'stellate' pattern and lacking both an axosome in the centre of the distal plate and tubular rings beneath the flagellar membrane. Although there are no other detailed descriptions of prymnesialean transitional regions, it appears that a similar structure occurs in ***Phaeocystis*** Lagerheim (Parke *et al.* 1971) and some species of ***Chrysochromulina*** Lackey (Gregson *et al.* 1993; ***C. brevifilum***, ***C. simplex***, the 'eyelash' ***Chrysochromulina***, pers. obs.). In contrast, members of the Isochrysidales seem to have a proximal plate and a distal plug that is traversed by the central pair of microtubules (Green & Pienaar 1977; Green & Hori 1986; Hori & Green 1991), while coccolithophorids that have a life history stage related to the Isochrysidales tend to have a single robust plate, helical bands and transitional rings (Beech & Wetherbee 1988; Roberts & Mills 1992).

There are obvious similarities between the flagellar apparatus of ***Prymnesium nemamethecum*** and that of ***P. patelliferum*** (Green & Hori 1990), the most important being: (1) the orientation and the insertion angles of the three appendages; (2) the system of connectives which proximally appears identical (compare Fig. 42 with Green & Hori 1990 fig. 7a, p.198) but with slight differences distally (which in part may be dependent on interpretation of particular series of sections); (3) the size and position of the R1 root; (4) a three-stranded R2 with, as far as can be determined, similar microtubular pathways; (5) R3 and R4 microtubular roots having the same origins and following the same pathways; (6) a root, the R7, associated with the proximal part of the right basal body. Probable similarities which have yet to be confirmed in ***P. patelliferum*** are the presence of a reduced cytoplasmic tongue, and the R3/R4 microtubular array underlying the opening of a large, scale-containing Golgi vesicle into the anterior depression.

The major differences include: (1) the presence of a compound R1 root component, the R1c, in ***P. nemamethecum***; (2) a greater number of constituent microtubules in the R3 in *P. **nemamethecum*** (six compared with three in ***P. patelliferum***, Green & Hori 1990); (3) more

extensive development of the R7 in ***P. nemamethecum***, regarding both the length and the number of microtubules; (4) absence of a second microtubule in the R4 in ***P. nemamethecum***; (5) an additional dense connective, the aB3, between the left basal body and the haptonema base in ***P. nemamethecum***.

A comparison between ***Prymnesium nemamethecum*** and other genera in the Prymnesiales indicates an affinity to both ***Platychrysis*** Geitler and to ***Chrysochromulina*** Lackey. Although the flagellar apparatus of a ***Platychrysis*** species has yet to be reconstructed, both ***Platychrysis simplex*** Gayral & Fresnel and ***Platychrysis pienaarii*** Gayral & Fresnel appear to have at least one compound root (Gayral & Fresnel 1983, Figs 12, 34), possibly two in ***Platychrysis pienaarii*** (Gayral & Fresnel 1983, Fig. 17). However, caution must be exercised in interpreting single micrographs particularly of cells that could be dividing, as compound roots may be dynamic structures and their presence is dependent on cell cycle stage in ***Pleurochrysis carterae*** (Braarud & Fagerland) Christensen (Beech *et al.* 1988) and ***Chrysochromulina spinifera*** (Fournier) Pienaar & Norris (M. Kawachi, pers. comm.). Another possible similarity between ***Platychrysis simplex*** and both ***Prymnesium potelliferum*** and ***Prymnesium nemamethecum***, is in the presence of an R7 root associated with the proximal part of the right basal body (Gayral & Fresnel 1983, Fig. 29). However the arrangement of the microtubules of the R2 of ***Platychrysis simplex*** (Gayral & Fresnel 1983, Fig. 30) appears more similar to that of ***Hymenomonas coronata*** Mills (Roberts & Mills 1992) than to either species of ***Prymnesium***.

The flagellar apparatus of two species of ***Chrysochromulina*** has been described, *C.* ***apheles*** Moestrup et Thomsen (Moestrup & Thomsen 1986) and *C.* ***acantha*** Leadbeater et Manton (Gregson *et al.* 1993). There are a number of intrageneric and intergeneric similarities and differences: In both ***Chrysochromulina*** and ***Prymnesium*** species there is a broad R1 root with ventrally splayed microtubules and a microtubular root complex associated with the right basal body (R3+R4 of this study); in both species of ***Chrysochromulina*** the angles of appendage insertion are far wider than those encountered in the two species of ***Prymnesium*** (Green & Hori 1990; present study); the R1 of *C.* ***acantha*** is more similar to the R1 of the ***Prymnesium*** species than it is to that of *C.* ***apheles***, although the orientation of the R1 microtubules relative to the

appendage bases is different between the genera; ***C. acantha*** has a more complex system of connective fibres than does ***C. apheles***, although these connectives differ from both ***Prymnesium*** species; ***C. apheles*** has a root (R2) extending ventrally from beneath the distal fibre, a root homologous with the R2 in the ***Prymnesium*** species, but ***C. acantha*** lacks this root altogether; ***C. acantha*** is the only described cell to have two microtubules perpendicular to the left lateral edge of the R1; both ***C. acantha*** and ***P. patelliferum*** have a root associated with the right dorsal side of the cell, but this root is absent in C. apheles and ***P. nemamethecum***; ***P. nemamethecum*** is the only one of the four species to have a compound R1.

Compound roots have been found in some coccolith-bearing cells (e.g. Gayral & Fresnel 1976; Inouye & Chihara 1983; Inouye & Pienaar 1984, 1985; Beech & Wetherbee 1988; Fresnel & Billard 1991; Roberts & Mills 1992) and in ***Isochrysis galbana*** (Hori & Green 1991). A comparison of ***P. nemamethecum*** with ***Pleurochrysis***, ***Umbilicosphaera*** or ***Hymenomonas*** suggests that despite the presence of compound roots in all cases, the differences are greater than those found when comparing ***P. nemamethecum*** with another species of ***Prymnesium*** (viz. ***P. patelliferum***). For example : the array of connectives in ***P. nemamethecum*** is far more complex than in any coccolithophorid yet described; the position of the R1 differs with the constituent microtubules angled almost perpendicular to the long axis of the haptonema base of the coccolithophorids, but almost parallel in ***P. nemamethecum***; the coccolithophorids have an R3 bifurcated at its origin, while ***P. nemamethecum*** has distinct R3 and R4 roots (with the R4 not originating on the dorsal side of the right basal body); there does not appear to be a root equivalent to the R7 of ***P. nemamethecum*** in any of the flagellar reconstructions of the coccolithophorids; the transitional region of ***P. nemamethecum*** is very different from that of the only coccolith-bearing cell described in detail, viz. ***Pleurochrysis carterae*** (Beech & Wetherbee 1988). Even the point of similarity between ***P. nemamethecum*** and these coccolithophorids differs, because despite the homology, the compound root of ***P. nemamethecum*** is reduced relative to the coccolithophorids, so too the cytoplasmic tongue and the R1c component which is too small to warrant being termed 'crystalline'.

There is only one other consideration that provides an interesting link between ***P. nemamethecum*** and some coccolith-bearing cells, and it relates to the haptonema. The haptonema is covered by specific haptonemal plate scales (see Pienaar & Birkhead in press, and compare, for example, with Leadbeater 1971), and the haptonema base does not have a full complement of 9 microtubules as originally described for ***P. parvum*** Carter and ***Chrysochromulina chiton*** Parke & Manton (Manton 1968). Only eight microtubules are present in ***P. nemamethecum***, ***Pleurochrysis*** sp. (Inouye & Pienaar 1985) and in ***Syracosphaera pulchra*** Lohmann (Inouye & Pienaar 1988). The fact that these three organisms have very different flagellar apparatus configurations suggests that the number of basal haptonemal microtubules is not of major phylogenetic importance. This idea is supported by the fact that in the Isochrysidales, ***I. galbana*** also has a reduced number of microtubules in the haptonema base, in addition to possessing haptonemal scales (Parke 1971; Hori & Green 1991). Other points of resemblance between ***P. nemamethecum*** and ***I. galbana*** are an R1c component of a tripartite R1, and peripheral rings in the transitional region, although in ***Isochrysis*** these rings are limited to a distal plug region (Hori & Green 1991). In all other respects (root origin; pathway and number of microtubules in R1, R2, R3; absence of R4, R7 and any connectives apart from a distal band and one proximal band) ***Isochrysis*** is very different from ***P. nemamethecum***. ***Imantonia rotunda*** Reynolds (Green & Hori 1986) is also very different from ***P. nemamethecum***, except that both organisms have two roots originating from the right basal body.

It is difficult to determine which flagellar apparatus features should be considered more important in generic and ordinal comparisons within the Prymnesiophyceae. Considerable intraordinal variation has already been shown to exist, for example within the Coccosphaerales, the coccolith-bearing cells appear to fall into at least three groupings - ***Pleurochrysis carterae*** (Beech & Wetherbee 1988), ***Umbilicosphaera sibogae*** var. ***foliosa*** (Kamptner) Okada & McIntyre (Inouye & Pienaar 1984), ***Jomonlithus littoralis*** Inouye & Chihara (Inouye & Chihara 1983) versus ***Hymenomonas coronata*** (Roberts & Mills 1992) versus ***Syracosphaera pulchra*** (Inouye & Pienaar 1988). There is also much variation in the flagellar apparatus of the two

described isochrysidalean species, ***Isochrysis galbana*** (Hori & Green 1991) and ***Imantonia rotunda*** (Green & Hori 1986), and between members of the Prymnesiales (as described above). Intraordinal variation can therefore be extensive. However, should intrageneric variation be as wide as that found between ***P. patelliferum*** and ***P. nemamethecum***? The only available comparison here is between ***Pleurochrysis carterae*** (Beech & Wetherbee 1988) and an undescribed species of ***Pleurochrysis*** (Inouye & Pienaar 1985), these two representatives being very similar indeed. This implies that the two species of ***Prymnesium*** could be separated at the generic level, although this seems impetuous being based on only one other example. In addition, the variation in the flagellar apparatus of members of the closely-related, but possibly polyphyletic, genus ***Chrysochromulina*** Lackey is far greater, ranging from relatively simple arrangements as in ***C. apheles*** (Moestrup & Thomsen 1986) to complex compound roots in a number of the species with shorter haptonemata (pers. obs.; M. Kawachi pers. comm.). It is possible that ***P. nemamethecum*** may be combined with some of these latter Chrysochromulina species, when more data is available - but such alteration to the present taxonomic system needs to be based on a wider framework of information.

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Figs 1 - 7. Light and electron microscopy of *Prymnesium nemamethecum*.

Fig. 1. Sub-apical appendage insertion on the ventral (V) side of the obliquely truncate cell (Nomarski optics).

Fig. 2. Transverse section through the anterior end of a cell to show appendage insertion in the anterior groove which is displaced towards the ventral side of the cell. Note the large vesicle (SV) associated with right side of the anterior groove.

Figs 3-7. Serial sections (nos 1, 4, 5, 6, 9) transverse to the long axis of the haptonemal base.

Fig. 3. The three appendages arranged linearly in the anterior groove, above the insertion region.

Fig. 4. The striated connective (arrowhead) clearly attaching the left basal body and the arc of haptonemal microtubules to the R1, at the insertion level.

Fig. 5. The striated DB with its ventral extensions (arrowheads). Note the fibrous connection (arrow) from the left basal body to the R1.

Fig. 6. The most distal portion of the AB3 (arrowhead) which is attached to two haptonemal microtubules. Fibrous material (arrow) lies between the left basal body and the R1.

Fig. 7. More proximally the AB3 (arrowhead) appears bipartite.



D
V
5μm
3
V
H
R
D
6μm
2
V
L
H
R
SV
D
1μm
4
L
H
R
R1
2μm
5
R3
L
R
H
2μm
6
DB
L
R
H
R1
2μm
7
L
R
R1
H
2μm

25×10

Figs 9 - 27. Flagellar and haptonematal ultrastructure in *Prymnesium nemamethecum*.

Figs 9, 10. Serial sections (nos 1,3) through the flagellar axoneme to show the tubular rings (arrows) beneath the flagellar membrane.

Fig. 11. Longitudinal section through a flagellum with a distal transverse plate (arrowheads), a proximal plate (large arrows) and a line of amorphous material across the basal body (small arrow). The number 18 refers to the figure of the corresponding transverse section.

Fig. 12. Flagellum with disc of amorphous material at appendage insertion level (arrow) and an unusual septum (arrowhead) above cartwheel triplets.

Fig. 13. Longitudinal section through a flagellum (not median to the distal plate) detailing the structure of the proximal transverse plate. Numbers 20-25 correspond to the appropriate transverse sections. Note the osmiophilic core (asterisk) in the basal body.

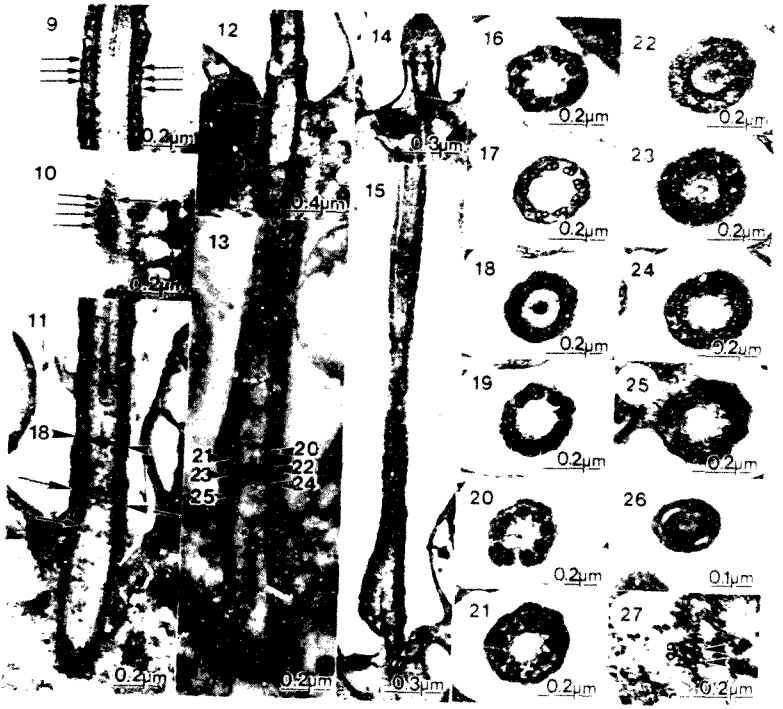
Fig. 14. Glancing section through the haptonema showing an ER extension into the microtubular arc.

Fig. 15. Longitudinal section in a lateral plane, through a haptonema.

Figs 16-25. Consecutive transverse serial sections through the transitional region of a flagellum.

Fig. 26. Transverse section through the free part of a haptonema.

Fig. 27. Transverse section through the most proximal portion of a haptonemal base to show the two arcs of microtubules (arrowheads). Note the delicate connections present between some of the microtubules.


9
12
14
16
22
0.2μm
0.3μm
0.2μm
0.2μm
10
15
17
23
0.4μm
0.2μm
0.2μm
13
0.2μm
18
24
0.2μm
0.2μm
11
19
25
18
21
20
0.2μm
0.2μm
23
22
25
24
20
26
0.2μm
0.1μm
21
27
0.2μm
0.2μm
0.2μm
0.3μm
0.2μm

Figs 32 - 42. Ultrastructure of the flagellar apparatus of *P. nemamethecum*.

Fig. 32. Glancing longitudinal section in a dorsi-ventral plane through the anterior cell region, illustrating the anterior groove lined by chloroplast and mitochondrial lobes, the three appendages (L, H & R), some of the distal connectives (large arrows) and most of the microtubular roots (R1, R1c, R3 - arrowhead, R4 - small arrow, R7 - asterisk).

Figs 33-38. Serial transverse sections (nos 1, 2, 3, 4, 7, 8) down the right basal body, from distal to proximal levels.

Fig. 33. The distal portion of the distal band (DB) with a striated connective (arrow) joining it to a chloroplast lobe.

Fig. 34. The major portion of the DB with a ventral extension (large arrow) overlying the R2, a filtrous connective to the chloroplast lobe (small arrow), and part of the auxiliary connective (AC). The single arrowhead indicates the R4, the paired arrowheads point to two tiers of R3 microtubules.

Fig. 35. The two distal accessory bands, AB3 (small arrow) and AB2 (large arrow). Note the fanned microtubules of the R2 (arrowheads).

Fig. 36. Connections between the AB3 (small arrow) and the left basal body, the haptonema and the base of the R1. The large arrow points to the striated AB2.

Fig. 37. Proximal connectives include the IB (large arrow), the two proximal bands (PB1 +, PB2 *) and the AB1 (small arrow), with the latter two enclosing the R4 (large arrowhead). There is a single layer of R3 microtubules (small arrowhead).

Fig. 38. The most proximal region where the IB is no longer present. The bases of the R1, the R3 (arrowhead) and the R7 are evident.

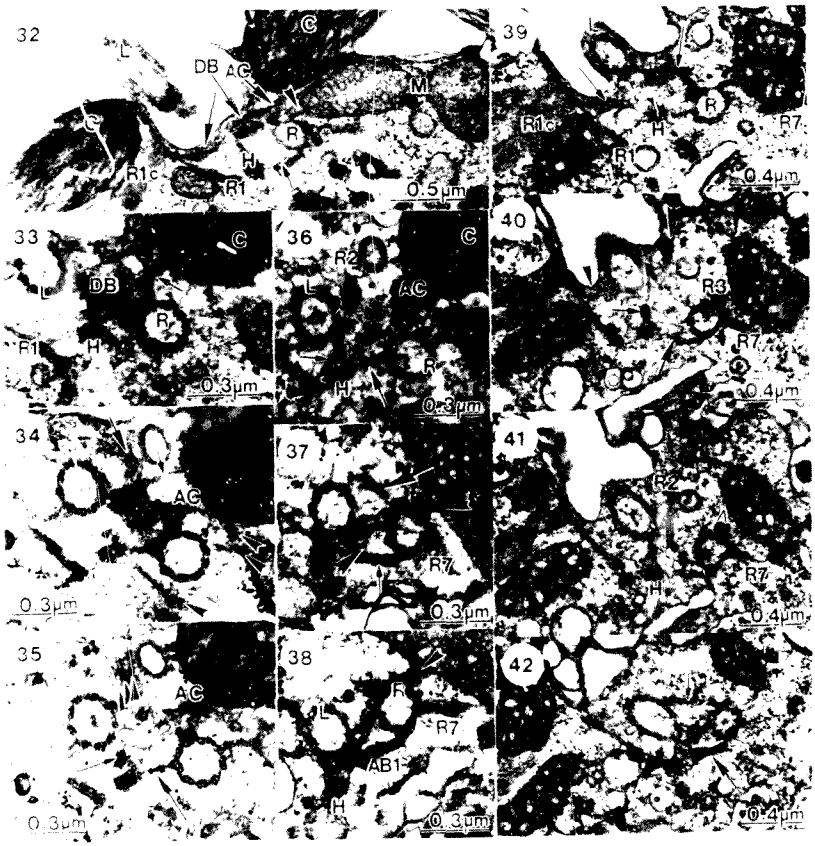
Figs 39-42. Serial transverse sections down the right flagellum (nos 1, 3, 4, 6) from distal to proximal levels.

Fig. 39. Portions of the connectives associated with the DB (arrows).

Fig. 40. The AB2 (large arrow) and the AB3 (small arrow). Note the connection between the left basal body and the R1 (small arrowhead) and the ventral DB extension (large arrowhead).

Fig. 41. The origin and pathway of two of the R2 microtubules, the two initial microtubules of the R3 (^), and the R7.

Fig. 42. Proximal connectives with the IB (small arrow), PB1 (+), PB2 (*) and AB1 (large arrow).


32
C
DB
AC
M
C
R
H
R1c
R1
0.5μm
33
C
DB
R
R1
H
0.3μm
34
AC
0.3μm
35
AC
0.3μm
36
C
R2
L
AC
R
H
0.3μm
37
R7
0.3μm
38
R
L
R7
AB1
H
0.3μm
39
L
R
H
R7
R1c
R
0.4μm
40
R3
R7
0.4μm
41
R2
R7
H
0.4μm
42
0.4μm

Figs 43 - 49. Ultrastructure of the flagellar apparatus of *P. nemamethecum*.

Figs 43-48. Serial sections (nos 1, 3, 5, 6, 8, 9) transverse to the long axis of the left basal body.

Fig. 43. Appendage insertion into the anterior groove which is lined on its dorsal and left sides by the R1 microtubules (arrows), and by the R3 and R4 microtubules on its right ventral side.

Fig. 44. The striated connective (arrow) between the left basal body, the haptonema and the R1. Note the microtubules of the R3 and R4 roots.

Fig. 45. The DB connecting the three appendage bases, with the ventral-R2 overlay (arrow). The R4 is moving dorsally away from the R3.

Fig. 46. A more proximal level of the DB with the AB2 (large arrow), AB3 (small arrow) and part of the AC also present.

Fig. 47. The AC lies over some of the R3 microtubules, the AB3 (small arrow) connects to the left basal body by fine filaments, the R4 moves towards the AB2. Note the splayed microtubule of the R1 (large arrow).

Fig. 48. A fibrous connective (arrow) extends from the AB3 towards the R1.

Fig. 49. Transverse section through the anterior groove (asterisks) to show the fusion of a scale-containing vesicle with the right side of the anterior groove. The opening of the vesicle is lined on the ventral side by the single microtubule of the R4 (arrowhead) as it approaches the pathway of the R3 (arrow).


43
L
H
R
R3
0.5µm
R1c
R4
44
R3
R4
0.3µm
45
DB
R3
H
R4
0.3µm
46
C
R
H
0.3µm
47
AC
R3
AB2
0.3µm
R4
48
M
C
L
R
0.3µm
H
49
C
H
R
R1
SV
0.4µm

Figs 50 - 55. The R1, R1c and R2 roots of *P. nemamethecum*.

Fig. 50. R1 microtubules (arrows) splay ventrally and posteriorly to run down the long axis of the cell.

Fig. 51. A portion of the R1c in transverse section.

Fig. 52. A tangential longitudinal section revealing the arching microtubules of the R1 (arrowheads).

Fig. 53. Transverse section of the R1 slightly above the region of appendage insertion. Note the fine line of material (arrow) at the juncture between the R1 and the R1c (arrowheads).

Figs. 54, 55. Serial sections (nos 1, 4) tracing the pathway of one microtubule (arrows) of the R2 which is associated with the forming face of the Golgi body. Fig. 54 inset: the two microtubules of the R2 at its origin.


50
52
C
C
M
L
0.4 µm
53
54
L
M
M
L
R1
H
G
0.4 µm
0.2 µm
51
C
55
H
0.2 µm
0.2 µm
0.4 µm

Figs 56 - 62. Microtubular roots of *P. nemamethecum*.

Fig. 56. A tangential longitudinal section in the dorsi-ventral plane through the left basal body, to show the single R2 microtubule associated with the Golgi body.

Figs. 57, 58. Serial sections (nos 1, 5) passing from dorsal to ventral cell regions.

Fig. 57. The single microtubule of the R4 (arrow) arising from between the proximal connectives near the base of the left basal body, and extending up to the right side of the cell towards the tiered R3 microtubules (arrowheads).

Fig. 58. The R3 (arrow) approaching its origin on the ventral side of the right basal body.

Fig. 59. Section perpendicular to the haptonema in the proximal region, to show the origins of the R1 and the R7. Note that there are still only 7 microtubules in the haptonemal base.

Figs. 60, 61. Serial transverse sections (nos 1, 5) through the R3 and R4.

Fig. 60. The four microtubules of the R3 (arrow) and the single microtubule of the R4 (arrowhead).

Fig. 61. The R4 (arrowhead) still on the dorsal side of the right basal body, while the R3 has become stacked (short arrows) with one microtubule (long arrow) peeling away from the central trio.

Fig. 62. Glancing section through a cell to show how the R1 microtubules (arrows) fan down posteriorly from the anterior groove to extend through the posterior vacuole (PV). Part of the cytoplasmic tongue has pulled away from the area of attachment near the chrysolaminarin-containing vesicle (arrowheads).


56
L
R1
G
0.4 µm
58
C
R
R2
0.5µm
59
R
L
R2
R1
H
0.3µm
57
L
H
R
0.5µm
60
H
R
0.2 µm
61
R
0.2 µm
62
C
R1c
C
PV
0.3µm

CHAPTER 4

THE FLAGELLAR APPARATUS OF *CHRYSOCHROMULINA* SP. (PRYMNESIOPHYCEAE)

ABSTRACT

The flagellar apparatus of an undescribed species of ***Chrysochromulina*** that bears 'eyelash' scales, is reconstructed. The transitional region consists of two transitional plates each with an axosome, with no stellate pattern between them. Fine, osmiophilic rings lie between the flagellar membrane and the outer doublets in the transitional region. The three appendages are inserted in a subapical depression that is lined ventrally by a spine-like projection formed by one of the parietal chloroplasts. The angles of insertion are similar to those of some other ***Chrysochromulina*** Lackey species in that the haptonema and the right basal body both lie at an extreme angle to the left basal body. The connectives of the apparatus consist of a striated distal band with a dorsal extension to the R1 and a ventral extension overlying the R2, a striated distal accessory band, an auxiliary connective from the right basal body to the adjacent ventral chloroplast, a well-developed intermediate band, two striated proximal bands and a striated proximal accessory band. Of the microtubular roots in this ***Chrysochromulina*** species, three are associated with the left side of the cell (an R1 of 8+3, a small crystalline compound root the R1C associated with the R1, an R2 of three microtubules) and two are associated with the right basal body (an R3 of 2/2 with which the single-stranded R4 converges to form a 2/2+1 and then a 2/3 tiered arrangement). Comparisons are drawn with other species in the genus and

related genera, particularly ***Prymnesium***.

INTRODUCTION

The type species of the genus ***Chrysochromulina***, ***C. parva*** Lackey, is a saddle-shaped cell with a long, coiling haptonema and a single type of simple plate scale (Lackey 1939, Parke *et al.* 1962). Since this description, more than 45 other species have been described, many of which are not saddle-shaped, do not have haptonemata that coil in the living cell, or have very elaborate scale complements. Certainly some of these species have been assigned to the genus ***Chrysochromulina*** for lack of a generic alternative, with the result that this genus is an artificial, and probably polyphyletic, grouping. However, it is presently accepted that a cell with either a long, coiling haptonema and/or elaborate scales should be assigned to ***Chrysochromulina*** (Green *et al.* 1982). Accordingly, Moestrup bestowed the name 'the eyelash ***Chrysochromulina***' on the organism with a previously undescribed, beautifully elaborate prymnesiophycean scale found in a nanoplankton sample from New Zealand (Moestrup 1979, Fig. 24). Similar 'eyelash' scales have been seen from the Gulf of Elat (H.A. Thomsen, pers. commun. to Moestrup 1979), the south-eastern coast of South Africa (Pienaar and Bandu 1984) and Japan (M. Kawachi, pers. commun.).

This paper describes the flagellar apparatus of the 'eyelash' ***Chrysochromulina*** (***Chrysochromulina*** sp.) based on observations made on a clonal culture from Japan, that was graciously provided by M. Kawachi and I. Inouye. Other descriptions of the flagellar apparatus of ***Chrysochromulina*** refer to the saddle-shaped cells of ***C. apheles*** Moestrup & Thomsen, ***C. acantha*** Leadbeater & Manton and ***C. simplex*** Estep *et al.* (Moestrup and Thomsen 1986, Gregson *et al.* 1993, Birkhead and Pienaar unpubl.) all of which have long, coiling haptonemata, and to the strawberry-shaped cells of ***C. brevifilum*** Parke & Manton which have a coiling haptonema that is shorter than the flagella (Birkhead and Pienaar 1994a).

MATERIALS AND METHODS

A clonal culture of ***Chrysochromulina*** sp., culture CP0, was obtained from M. Kawachi in Japan. The culture is maintained in half-strength Provasoli-a culture medium with soil extract (Mc' achlan in Stein 1979) in 35°/$_{00}$ seawater (pH 7.8) at 18°C, with a 18:6 h L:D cycle.

A Zeiss Axiophot photomicroscope equipped with Nomarski optics was used for light microscopy, with flash photography.

For electron microscopy, cell cultures (in 35°/$_{00}$ seawater, pH 7.8, with PES and soil extract) were fixed in 2.5% glutaraldehyde for 45 min. The sample was then gently centrifuged and the pellet was resuspended in drops of cooling 0.2% purified agar. Agar pieces were rinsed in culture medium five times at 15 min intervals, followed by 90 min post-fixation in 1.5% OsO_4. The samples were again repeatedly rinsed in culture medium and then dehydrated in a graded alcohol series at 15 min intervals, prior to embedding in Spurr's resin (Spurr 1969). Serial sections were cut on a Reichert Ultracut E microtome, double stained in saturated uranyl acetate and lead citrate, and viewed on a Jeol 100S transmission electron microscope. Only cells that were in interphase and showed no signs of impending division were used for the flagellar reconstruction. Shadowcast preparations were made by osmicating drops of cell culture on 0.3% formvar-coated copper grids, drying, rinsing with distilled water and shadowing with gold palladium at 30°.

The terminology used follows that of Beech and Wetherbee (1988) for cell and appendage orientation, and Green and Hori (1990) for the microtubular roots and the fibrous connectives.

NOTE: Abbreviations used in the figures and in the text: AB1 - proximal accessory band; AB2 - distal accessory band; AC - auxiliary connective; C - chloroplast, D - dorsal side of the cell; DB - distal band; G - Golgi body; H - haptonema; L - left flagellum or basal body; M - mitochondrion; N - nucleus; PB1 - ventral proximal band; PB2 - dorsal proximal band; PV - posterior vacuole; R - right flagellum or basal body; R1, R1C, R2, R3, R4 - microtubular roots; SG - subapical groove; V - ventral side of the cell.

OBSERVATIONS

General ultrastructure

The cells of ***Chrysochromulina*** sp. have a characteristic shape with a spine-like projection on the ventral side of the cell (Fig. 1) and a unique scale complement (Fig. 2). The three appendages are inserted in a subapical groove that lies from left to right across the base of the narrow ventral projection, with the bulk of the cell body occurring dorsal to this appendage insertion groove (Figs. 1, 3-5). The groove is not centrally positioned along the left - right lateral axis but is displaced towards the left side of the cell (Figs. 1, 3, 5). On the right side of the groove, the maturing face of the Golgi body releases vesicles/scales (Fig. 5). The ventral 'spine' is formed almost entirely by one of the two chloroplasts (Figs. 3-5), so the ventral side of the groove is lined by this parietal chloroplast, while extensive mitochondrial profiles occur immediately beneath the plasmalemma on the dorsal side of the groove (Figs. 3, 5, 22, 24). The nucleus is median but more dorsally positioned, with the posterior end of the cell occupied by a posterior vacuole (Fig. 4). The general ultrastructure of the cell, including details of the strange peripheral structures (visible in Figs. 3, 5; and said to contain DNA - I. Inouye, pers. comm.), will be more fully described elsewhere.

Flagellar and haptonematal ultrastructure

Each flagellum bears a finely fibrous tomentum around its outer surface (Figs. 6, 9). Immediately beneath the flagellar membrane, but between the membrane and the outer pairs of doublets, is a series of osmiophilic rings which in some sections appear to be helically arranged (Figs. 6, 8). As far as can be determined, these rings are limited to the transitional regions of the flagella and do not extend much beyond the beginning of the 9+2 microtubular arrangement. The transitional region of each flagellum consists of two transitional plates (Fig. 6): the distal plate has a small, central axosome that is distally continuous with a central strand of material that extends towards, but does not reach, the central pair of microtubules (Figs. 6, 10-12); the proximal plate consists of a robust septum that is centrally interrupted by a cylinder which is plugged distally by a dense axosome (shaped like a flying saucer) that has a short, distal projection (Figs. 6, 14, 15). The two transitional plates are not interconnected, but in the

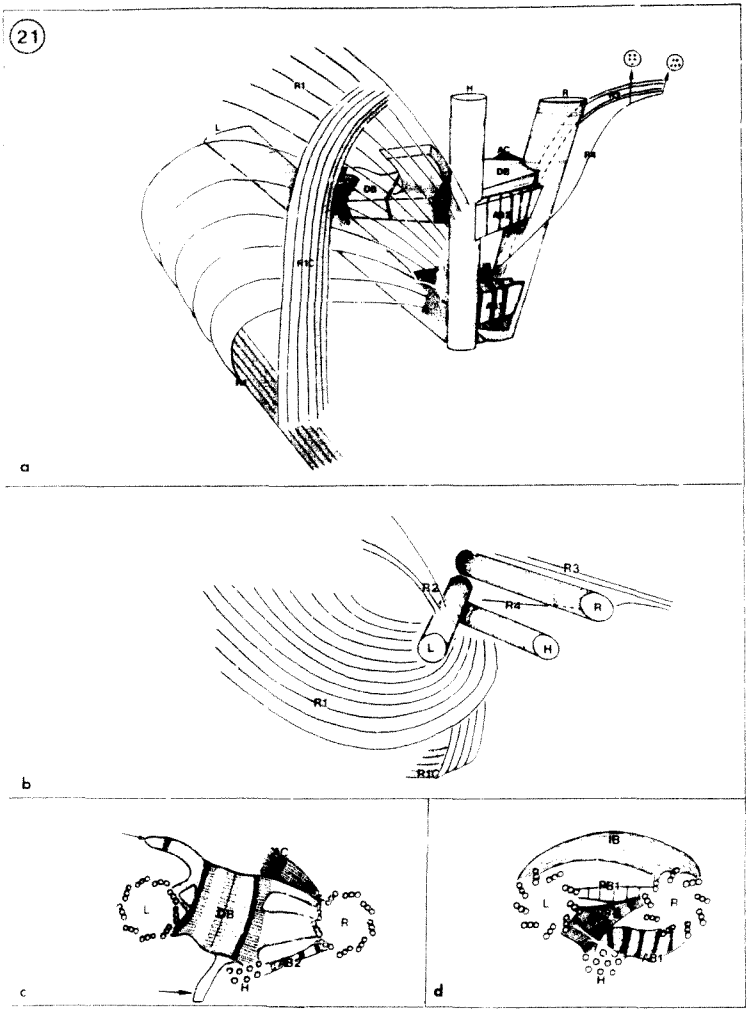


Fig. 21. Schematic reconstruction of the flagellar apparatus of *Chrysochromulina* sp. Not drawn to scale. **Fig. 21a.** Dorsal view of the apparatus. **Fig. 21b.** Anterior view of the pathways of the microtubular roots. **Figs 21c, 21d.** Transverse sections through the connectives of the apparatus. The appendage bases have been drawn as though arranged in parallel, to clearly show attachment points to individual triplets. **Fig. 21c.** Distal connectives including the distal DB extension to the R1 (large arrow) and the ventral extension over the R2 (small arrow). **Fig. 21d.** Proximal connectives of the apparatus.

regions of both septa the outer doublets are joined by osmiophilic material (Figs. 6, 12, 13, 16). The basal bodies (*ca.* 600 nm long) do not possess dense cores that fill the entire lumen (e.g. Fig. 19), but the triplets are connected along their inner surfaces by very fine, fibrillar material (Fig. 17).

The coiling haptonema has a flanged basal swelling that bulges along the dorsiventral cell axis (Figs. 7, 20, 22). When longitudinally sectioned along the left-right cell axis, the haptonema is simply cylindrical. There are seven microtubules in the free part of the haptonema (Fig. 20), with nine at the base of the structure (Fig. 18).

The flagellar apparatus

A reconstruction of the flagellar apparatus is presented in Fig. 21. A complex series of fibrous/striated connectives link the appendages, and the entire structure is anchored into the cell by five microtubular roots: the R1, R1C and R2 associated with the left basal body; the R3 and R4 associated with the right basal body.

Although the three appendages are almost linearly positioned as they enter the subapical depression (Figs. 3, 5, 22) they are inserted at such angles that at their bases, the haptonema is most dorsally positioned, followed by the left basal body with the right basal body being most ventrally positioned (Figs. 30, 38). The positions of the three appendages relative to each other is variable, but the paths of the haptonema and the right basal body are more similar to each other than either is to the left basal body, and in some cells they lie almost perpendicular to the left basal body (Figs. 34-38).

A striated distal band (DB) connects the ventral side of the haptonema to the right side of the left basal body and the left side of the right basal body (Figs. 21a, 21c, 25, 26, 31). It is a sheet of material (*ca.* 190 nm thick) with two dark striations running ventrally from the attachment point of the left basal body and the haptonema, and enclosing a finer central striation (Figs. 25, 26). There are no obvious striations in the region between the haptonema and the right basal body, in any plane of section. There are two extensions of the DB: a striated, narrow ventral extension that overlies the emergence of the R2 microtubular root (Figs. 21c, 26, 32, 45); a densely staining tongue that extends distally upwards from the DB's haptonematal

striation to the R1 (Figs. 21a, 21c, 23, 24, 34). Immediately below (proximal to) the DB, a fan-shaped striated connective reaches from the ventral side of the right basal body towards the ventrally positioned right chloroplast (Figs. 21c, 26-29, 33, 35). This connective has been called the auxiliary connective, AC, as it is a discrete structure which is probably homologous with the large auxiliary connective described in ***Prymnesium patelliferum*** Green *et al.* (Green & Hori 1990). Amorphous, granular material continues from beneath the AC (proximally) between the right basal body and the chloroplast (Figs. 35-38). The other main distal connective is a striated accessory band, AB2, that connects the haptonema to the right basal body (Figs. 21a, 21c, 25-27, 35). The AB2 is very narrow in transverse section, but extends for some 240 nm from distal to more proximal lengths of the two appendage bases.

Four different connectives join the more proximal portions of the appendages (Fig. 21d):

1) from an intermediate level down to their most proximal regions (approximately 250 nm), the ventral sides of the two flagellar bases are linked by a robust, non-striated fibrous connective, the intermediate band IB (Figs. 29, 30, 35-37). On the right basal body, the IB attachment is immediately proximal to the AC attachment, to some of the same triplets (Figs. 28, 29);

2) a loosely fibrous, regularly striated accessory band, AB1, joins the haptonema base to the dorsal side of the right basal body (Figs. 30, 39, 46). Some sections seem to suggest that the AB1 also links to the left basal body (Fig. 30);

3) a lightly staining, striated proximal band, PB1, that connects the right side of the left basal body to the left side of the right basal body (Figs. 37, 39);

4) a second striated proximal band, PB2, that is more dorsally positioned than the PB1 and more densely staining, links the two flagellar bases (Figs. 30, 37, 39, 42).

The broad root, R1 (Figs. 21a, 21b), originates on the dorsal side of the cell between the left basal body and the haptonematal base, in an osmiophilic cushion of material (Figs. 21a, 28-30, 35, 36). Initially consisting of four microtubules (Fig. 35), additional microtubules are added as the root ascends distally so that at least eight microtubules (a maximum of eleven) are present at the level of appendage insertion (Figs. 17, 19, 34). At this level, the R1 microtubules are enclosed in a narrow band of cytoplasm that lies in the peripheral endoplasmic reticulum (Figs.

17, 24, 34, 50) but which is incorporated into the cell body both distally and proximally (Figs. 22, 25, 35, 51). The R1 microtubules are initially aligned almost parallel to the long axis of the left basal body, and are attached to this basal body and the haptonematal base by amorphous material (Figs. 27, 32). The transitional fibres of the left basal body also connect to the microtubules of this root (Fig. 24). The R1 microtubules follow a slightly ventral, curved pathway towards the left cell surface (Figs. 28, 34, 46, 50, 51), and some microtubules (up to three in number) appear to veer very sharply away from the remaining microtubules (Figs. 19, 23) although this angle varies with the plane of section. While in the region of the appendages, this root is adpressed to the ventral side of a mitochondrial profile that lines the subapical groove (Figs. 19, 22-30, 51). As the microtubules arch leftwards and posteriorly, they continue to align themselves along and between the mitochondrial profiles as they approach the left inner sides of the two chloroplasts (Figs. 46, 51). The R1 microtubules then continue posteriorly to a level well below the termination of the appendage bases, until they penetrate the posterior vacuole enclosed in a cytoplasmic strand (Figs. 46, 47).

Associated with the R1 is a small compound root, the R1C (Figs. 21a, 21b, 22, 23, 45, 48, 49) which consists of a bundle of 26-30 microtubules (Figs. 18, 52 inset). The R1C microtubules are perpendicularly aligned to the R1 microtubules at the juncture of the two roots (Figs. 48, 49) which occurs at least 600 nm above (distal to) the level of appendage insertion. From this origin, the R1C microtubules move over a mitochondrial branch towards the dorsal, left side of the cell (Figs. 22, 24, 52), dropping sharply in a plane almost parallel to the long axis of the haptonematal base (Fig. 18). The R1C extends posteriorly until it reaches the anterior portion of the nucleus, where the microtubules apparently merge with the nuclear membrane (Fig. 53). One of the first signs of incipient cell division is an increase in the size of the R1C, which forms two constituent microtubular bundles from the origin at the R1, with these bundles bifurcating around the nucleus (Fig. 54).

The R2 (Fig. 21b) consists of three microtubules that arise between the two flagellar bases immediately distal to the intermediate band (Fig. 33 inset). The microtubules fan out ventrally from beneath the DB extension, with one, probably two, microtubule(s) curving past the ventral left side of the left basal body to become associated with the endomembrane system - the forming face of the Golgi body (Figs. 19, 28, 33, 45). The remaining microtubule passes

directly ventrally to approach the chloroplast lining the subapical groove (Fig. 33).

The R3 (Figs. 21a, 21b) begins as a pair of microtubules attached by densely-staining material to the ventral side of the right basal body, at the most distal level of the proximal connectives (Figs. 40, 46). These microtubules emerge from beneath the point of attachment of the IB to the right basal body (Fig. 42), then pass ventrally past the proximal part of the AC (Figs. 27-30). A second pair of microtubules appears above the initial two at a more distal level (Figs. 31, 41-43), and the R3 continues along the right, ventral side of the subapical groove just below the plasmalemma (Fig. 42).

The single microtubule of the R4 (Figs. 21a, 21b) arises proximally between the AB1 and the PB2 (Fig. 40). It then moves distally across the dorsal side of the right basal body (Figs. 29, 33, 41, 42) travelling towards the ventral side of the subapical groove to converge with the R3 microtubules (Figs. 27, 28, 31, 32, 42-44). This convergence occurs below (proximal to) the fusion of Golgi-derived vesicles with the right side of the subapical groove (Figs. 31-33). Initially the arrangement is a 2/2+1 pattern (Fig. 43) but as the combined roots proceed further along the right ventral side of the cell, the R4 aligns itself in the same plane as the lower pair of R3 microtubules (Fig. 44).

DISCUSSION

The transitional region of members of the Prymnesiales is consistent in having two transitional plates. As yet, all species of ***Chrysochromulina*** have an axosome in each septum, with the proximal septum being the more structurally complex of the two (Green*son et al.* 1993, Birkhead and Pienaar 1994a). The stellate pattern found between the two transitional septa in ***Prymnesium parvum*** Carter (Manton 1964) remains an isolated occurrence within the class. The presence of the helical/ring structures beneath the flagellar membrane has only been observed in this study and in ***C. brevifilum*** (Birkhead and Pienaar 1994a), as well as in ***Prymnesium neotamethecum*** Pienaar & Birkhead (Birkhead and Pienaar 1994b). These rings do not occur in ***C. acantha*** Leadbeater & Manton, ***C. simplex*** Estep *et al.* or ***C. camella*** Leadbeater &

Manton (Gregson *et al.* 1993, pers. obs.). Apparently similar structures occur in ***Hymenomonas coronata*** Mills (Roberts and Mills 1992, Fig. 2) although it is stated that this figure is misleading as the helical bands do not extend beyond the peripheral doublets. In contrast, the helical bands of ***Pleurochrysis carterae*** (Braarud & Fagerland) Christensen are not homologous with the subplasmalemmal prymnesialean bands, as they definately occur only within the peripheral doublets (Beech and Wetherbee 1988).

Although the cell shape of ***Chrysochromulina*** sp. is not typical for the genus, strong similarities exist with several other species. For example, the appendages of ***C. chiton*** Parke & Manton are inserted slightly "off centre" in a polar depression (Parke *et al.* 1958, p. 211), while the cell shape of ***Chrysochromulina*** sp. is remarkably similar to that of ***C. polylepis*** Manton & Parke (Manton and Parke 1962). ***C. polylepis*** is an unusual species recently shown to have two motile stages with different scale morphologies (Edvardsen and Paasche 1992), while a pseudofilamentous life history stage is mentioned in the type description (Manton and Parke 1962). It would be interesting to determine whether the flagellar apparatus of ***C. polylepis*** resembles that of ***Chrysochromulina*** sp., as this ultrastructural feature differs from other descriptions for the genus in a number of respects. Obviously the most striking feature is the presence of a compound root, R1C, in interphase cells. Compound roots have been demonstrated in several other genera in the Prymnesiophyceae, including ***Isochrysis galbana*** Parke (Hori and Green 1991), numerous coccolith-bearing cells (e.g. see Gayral and Fresnel 1983a, Inouye and Chihara 1983, Beech and Wetherbee 1988, Roberts and Mills 1992), ***Platychrysis simplex*** Gayral & Fresnel and ***Platychrysis pienaarii*** Gayral & Fresnel (Gayral and Fresnel 1983b), and ***Prymnesium nemamethecum*** (Birkhead and Pienaar 1994b). The widespread occurrence of a compound (crystalline) root suggests that this feature may have little taxonomic or phylogenetic relevance, a suggestion supported by the dynamic nature of compound roots. Marked changes in the CR1 of ***Pleurochrysis carterae*** during the cell cycle have been demonstrated by Beech *et al.* (1988) and the same compound root bifurcation around the nucleus of preprophase cells has been illustrated here in ***Chrysochromulina*** sp.. Whether or not a compound root could develop *de novo* only during cell division and then disintegrate, as has been suggested by Kawachi after observation of ***C. spinifera*** (Fournier) Pienaar & Norris (M.

Kawachi, pers. commun.) would certainly diminish the importance of this ultrastructural feature as a means of distinguishing between different prymnesiophycean groups. Previously, the genus ***Chrysochromulina*** was distinct in having the most simple flagellar apparatus, but the presence of compound roots in some species of ***Chrysochromulina*** (present study, ***C. parkeae*** Green & Leadbeater and ***C. spinifera***, M. Kawachi pers. commun.) does link the genus more closely to other prymnesiophyceaen genera. Not surprisingly, this linkage has occurred via cells that are not typical of the genus (ie. not saddle shaped with long, coiling haptonemata), which supports the opinion that this group is polyphyletic.

The only other flagellar apparatus difference between ***Chrysochromulina*** sp. and all other described species is the presence of a large intermediate band connecting the ventral sides of the flagellar bases. A similar unstriated connective has been found only in species of ***Prymnesium***, ***P. patelliferum*** Green *et al.* (Green and Hori 1990) and ***P. nemamethecum*** (Birkhead and Pienaar 1994b), but is not as well developed in either of these two organisms. Minor differences between the ***Chrysochromulina*** species include the number of constituent root microtubules in the R1 4(5)+2 in ***C. apheles***, 16+4 in ***C. acantha***, 6+4 in ***C. simplex***, 8+ in ***Chrysochromulina*** sp.); the presence of a ventrally directed root associated with the DB, (the R2), which is single-stranded in ***C. apheles*** but consists of three microtubules in both ***Chrysochromulina*** sp. and ***C. brevifilum*** (Birkhead and Pienaar 1994a) and is absent altogether in ***C. acantha***; the presence of two microtubules perpendicularly aligned to the R1 microtubules only in ***C. acantha***; a proximally situated accessory fiber in ***C. acantha*** that extends from all three appendages to the R1, whereas this R1 connection is distal to the DB and is considered to be an extension of the connective in ***Chrysochromulina*** sp., ***C. simplex*** and ***C. brevifilum*** (Birkhead and Pienaar 1994a); a 2/2+1 tiered R3 arrangement in ***C. acantha***, ***C. brevifilum***, ***C. simplex*** and ***Chrysochromulina*** sp. but only 1/2+1 in ***C. apheles***; a single-stranded root extending dorsally from between the haptonema and the right basal body, which is found only in ***C. acantha***; the angles of appendage insertion are variable both within and between species, although the almost perpendicular angle of the left basal body to both the haptonematal base and the right basal body, is common to ***Chrysochromulina*** sp., ***C. apheles*** (Moestrup and Thomsen 1986) and ***C. simplex*** (pers. obs.), while ***C. acantha*** (Gregson *et al.* 1993) and ***C. brevifilum***

(Birkhead and Pienaar 1994a) are more similar to species of ***Prymnesium*** (Green and Hori 1990, Birkhead and Pienaar 1994b) in having the left basal body and the haptonematal base more similarly aligned with the right basal body angled to them both. Similarities within the genus ***Chrysochromulina*** (Moestrup and Thomsen 1986, Gregson *et al.* 1993) include the arrangement of the R1 microtubules, a tiered root associated with the right basal body, a distal band, a distal accessory band AB2 (Moestrup and Thomsen 1986, Fig. 29) and a proximal accessory band AB1 (Moestrup and Thomsen 1986, Figs. 47-50).

An interesting comparison can be made between ***Chrysochromulina*** sp. and the coccolith-bearing cells in which the R1 microtubules descend posteriorly as a cytoplasmic tongue / contractile root (e.g. see Beech and Wetherbee 1988, Fresnel and Billard 1991). There is an obvious homology between the structure found in the ***Chrysochromulina*** sp. cell and the coccolithophorid cytoplasmic tongue, but ***Chrysochromulina*** sp. exhibits a great reduction in the development of the contractile root and apparently also lacks the associated fibrous root. The pathway of the R1 microtubules towards the left posterior side of the cell in ***Chrysochromulina*** sp., is more similar to that found in ***Hymenomonas coronata*** Mills (Roberts and Mills 1992) than in ***Pleurochrysis carterae*** (Beech and Wetherbee 1988) where the R1 microtubules make a sweeping curve down the ventral side of the cell. In this respect, it is interesting to note the similarity between ***Chrysochromulina*** sp. and ***Prymnesium nemamethecum*** which also has a compound R1 in interphase cells. However, in this latter species the R1C joins to the R1 at a level closer to the insertion region of the appendages and the pathway of the crystalline root does not descend as steeply posteriorly as it does in ***Chrysochromulina*** sp. (Birkhead and Pienaar 1994b).

With every publication on the flagellar apparatus of a prymnesiophycean motile cell, more variation either within the genus, or at higher taxonomic levels, is illustrated. So much more fundamental description is necessary before a phylogenetically-based taxonomy can be developed, but even before the flagellar apparatus can be used reliably as a prymnesiophycean phylogenetic tool, an understanding of the life histories is desirable. No evidence concerning a sexual cycle has been provided for ***Chrysochromulina***, although if the ploidy-dependent patterning of coccolithophorid plate scales (Fresnel 1989) also occurs in ***Chrysochromulina***, then

it is possible that some species, e.g. ***C. tenuisquama*** Estep *et al.* (Estep *et al.* 1984, Fig.21) may be diploid. The possibility of ***C. bergenensis*** Manton & Leadbeater being a life history stage of a coccolithophorid has also been suggested (Manton and Leadbeater 1974). If it continues to prove so difficult to determine complete life histories for the prymnesiophytes, then at least it would be most interesting to know whether the flagellar ultrastructure is the same in the genotypically variable forms of ***Chrysochromulina chiton*** Parke & Manton (Leadbeater 1972), or in the two different motile stages of ***C. polylepis*** (Edvardsen and Paasche 1992).

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Figs 1 - 5. *Chrysochromulina* sp.

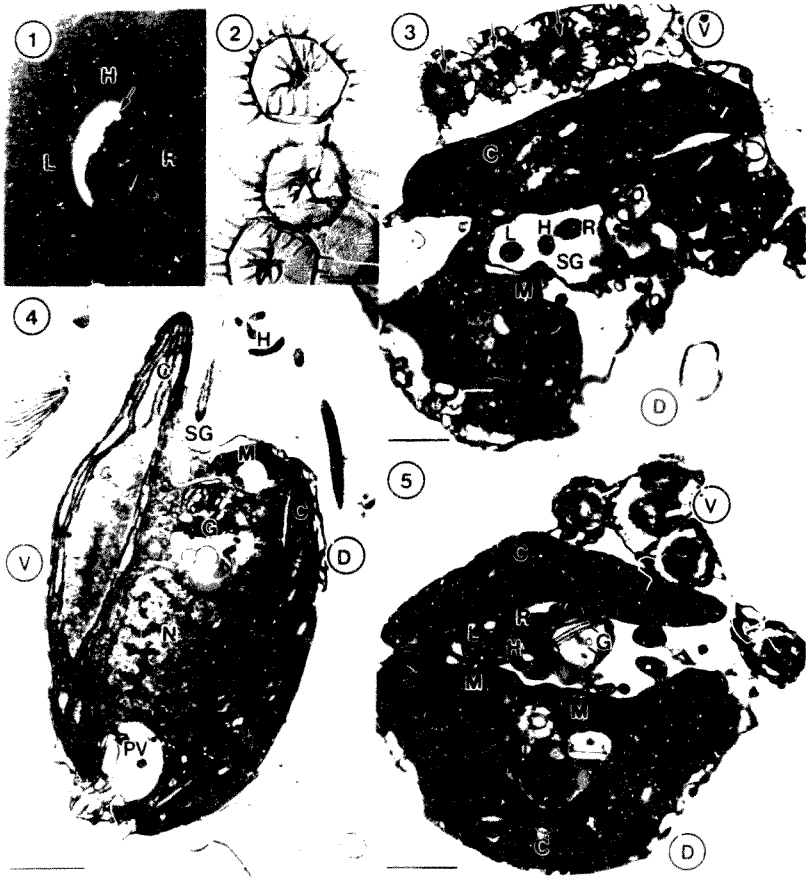
Fig. 1. Dorsal view of actively swimming cell showing subapical appendage insertion displaced towards the left side of the cell. Note the ventral cell projection (arrow). Scale bar = 5 μm.

Fig. 2. Shadowcast preparation of the distal surface of three 'eyelash' scales, with large plate scales also visible. Scale bar = 0.36 μm.

Fig. 3. Transverse section through the anterior end of a cell showing subapical appendage insertion in a depression (SG). The ventral (V) side of the cell is delineated by a parietal chloroplast that is lined by strange peripheral structures (arrows). Scale bar = 1 μm.

Fig. 4. Longitudinal section in a dorsoventral plane showing the subapical groove (SG) with the ventral projection formed by a chloroplast. Scale bar = 2 μm.

Fig. 5. Transverse section immediately below the level of appendage insertion, with scale-containing Golgi veiscles on the right side of the cell. Note the relative sizes of the dorsal (D) and the ventral (V) areas of the cell. Scale bar = 1.5 μm.


1
H
L
R
2
3
V
C
L
H
R
SG
M
D
4
H
C
SG
M
G
V
D
N
PV
5
V
C
R
L
H
G
M
M
C
D

Figs 6 - 20. Flagellar and haptonematal ultrastructure. All scale bars = 0.2 μm.

Fig. 6. Longitudinal section through the transitional region of a flagellum. Arrows indicate transversely - sectioned osmiophilic rings, the numbers mark the corresponding figures of the transverse section at that point.

Fig. 7. Longitudinal section in a dorsoventral plane through a haptonema.

Fig. 8. Tangential section across a flagellum to show the osmiophilic rings (arrows) lying immediately below the flagellar membrane.

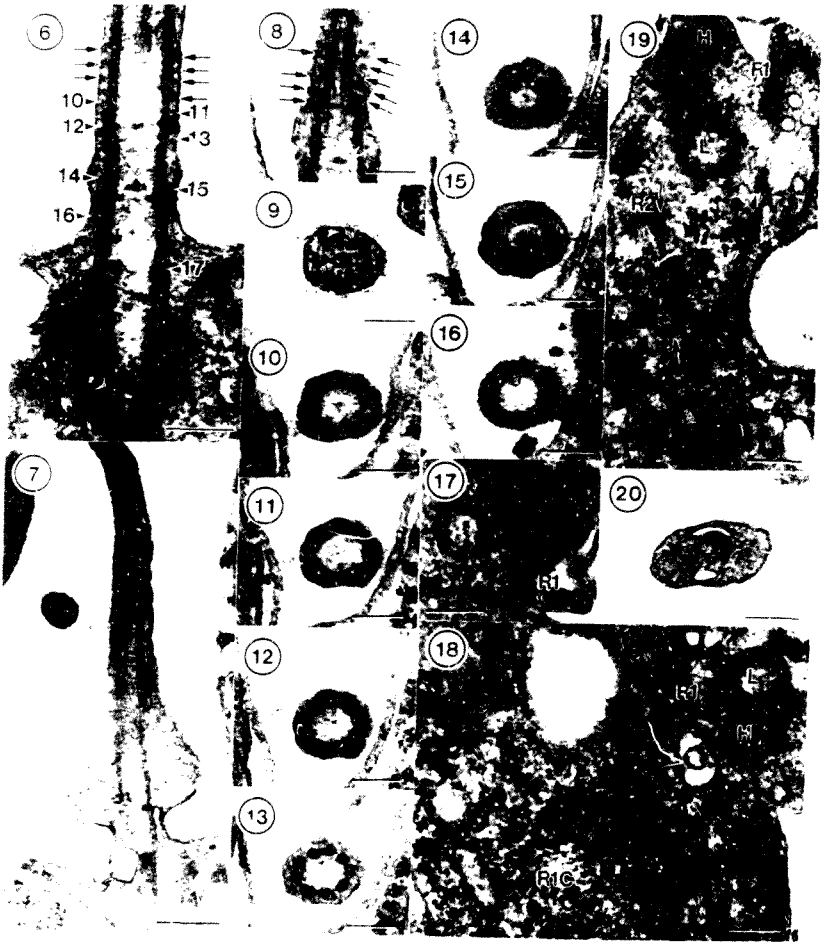
Figs 9-16. Consecutive serial transverse sections through a flagellum. Note particularly the distal transitional plate (Figs. 11, 12) and the proximal transitional plate (Figs. 14, 15, 16).

Fig. 17. Triplet region of a basal body.

Fig. 18. Transverse section through the base of the haptonema.

Fig. 19. Sheared section through the left basal body. Arrowheads indicate the splayed R1 microtubules. Note the R2 microtubule (arrows) associated with a membranous profile.

Fig. 20. Transverse section through the flanged haptonema with an arc of seven microtubules.


6
8
14
19
10
12
11
13
14
15
16
17
9
15
16
10
7
11
17
20
R1
12
18
13
R1C

Figs. 22 - 27. Transverse serial sections (nos 1, 6, 7, 8, 9, 10) through the distal region of a cell. (This series continues as Figs. 28-30).

Fig. 22. The R1C approaches the R1 distal to the insertion region. The ventral side of the cell contains only a chloroplast, the dorsal side of the cell is lined by a mitochondrial reticulum. Scale bar = 0.5 μm.

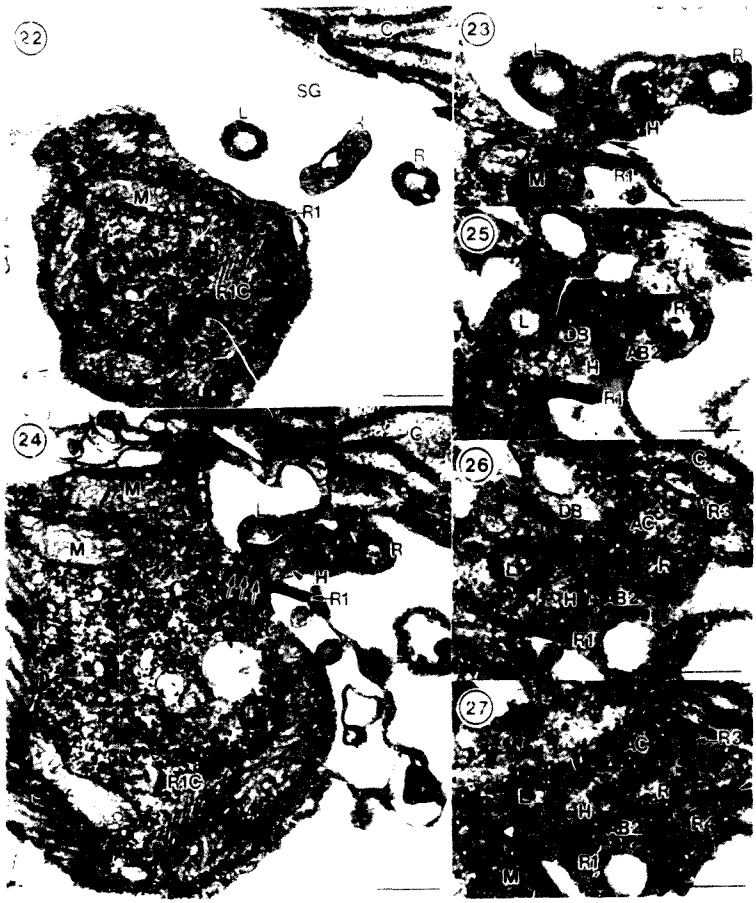
Fig. 23. At the level of appendage insertion the R1 lies in a cytoplasmic band in the peripheral endoplasmic reticulum, with a fibrous connection from the haptonema (arrow). The R1 microtubules curve leftwards and ventrally (arrowhead). Scale bar = 0.3 μm.

Fig. 24. The R1 is connected to the left basal body by transitional fibres (arrows) and to the haptonema by a distal extension of the DB (arrowhead). Scale bar = 0.5 μm.

Fig. 25. Distal connectives include the striated DB and the AB2. Scale bar = 0.3 μm.

Fig. 26. The DB with a left ventral extension (arrow) and the auxiliary connective AC projecting from the right basal body. Scale bar = 0.3 μm.

Fig. 27. The striated AC stretches ventrally towards the chloropiast. Note the ventrally positioned R3 and the more dorsal R4, as well as the connective material between the R1, the left basal body and the haptonematal base. Scale bar = 0.3 μm.


22
C
SG
L
R
M
R1
R1C
23
L
R
H
M
R1
25
L
R
DB
AB2
H
R1
24
C
M
M
R
H
R1
R1C
26
C
DB
AC
R3
L
R
H
AB2
R1
27
AC
R3
L
R
H
AB2
R4
R1
M

Figs. 28 - 33. Tangential serial sections through the appendage bases. All scale bars = 0.3 μm.

Figs. 28-30. A continuation of the series from Figs.22-27, being section numbers 11, 12 and 13.

Fig. 28. Both the R1 and the R2 (arrows) curve leftwards past the left basal body.

Fig. 29. The intermediate band IB connects the ventral surfaces of the two flagellar bases.

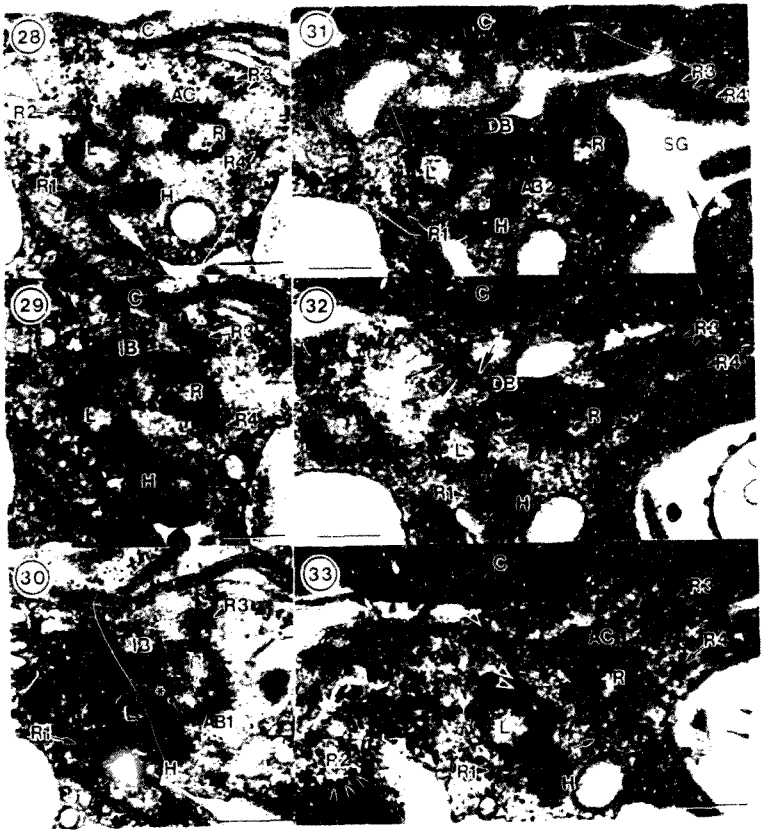
Fig. 30. Three of the proximal connectives, the IB, the AB1 and the PB2 (*).

Figs. 31-33. Consecutive serial sections.

Fig. 31. The striated DB and the AB2 connect the appendages distally. The R1 microtubules splay leftwards, the R3 and R4 lie ventral to the opening (arrow) of a scale-containing vesicle into the subapical groove (SG).

Fig. 32. The narrow ventral extension (arrows) from the DB. Note the amorphous material lying between the R1, the left basal body and the haptonema.

Fig. 33. The pathways of the three microtubules of the R2 (arrowheads, and inset) and the R4 (arrows). Note the heavily-striated AC.


28
C
R3
AC
R2
R
L
R4
R1
H
29
C
IB
R3
R
L
R4
H
30
R3
IB
L
AB1
R1
H
P1
31
C
R3
R4
DB
R
SG
L
AB2
R1
H
32
C
R3
R4
DB
R
L
R1
H
33
C
R3
AC
R4
R
L
P2
R1
H

Figs. 34 - 45. Connectives and microtubular roots. All scale bars = 0.2 μm.

Figs 43-38. Serial sections (nos 1, 5, 6, 7, 8) transverse to the long axis of the left basal body. Scale bars = 0.2 μm.

Fig. 34. One of the dense striations of the DB (arrow) extends towards the R1 with its curving microtubules.

Fig. 35. Lightly-staining, granular material (arrowheads) extends from the AC towards the ventral chloroplast.

Fig. 36. The attachment of the IB to the ventral side of the right basal body.

Fig. 37. The evenly-striated PB1 (*) and the more dense PB2 (+) connect the proximal ends of the flagellar bases.

Fig. 38. The dark PB2 (+) and the beginning of the loosely fibrous AB1 (arrow) appear to overlap slightly.

Fig. 39. The proximal connectives the fibrous IB (arrows), the striated PB1 (*), the osmiophilic, striated PB2 (+) and the loosely-structured striated AB1.

Fig. 40. The origin of the R4 microtubule (arrowhead) between two proximal connectives and the initial pair of R3 microtubules (arrows) attached to the ventral side of the right basal body by dense material.

Fig. 41. Two pairs of R3 microtubules with a fine connection between them and granular material linking them to the right basal body. The R4 is still on the dorsal side of the basal body.

Fig. 42. The convergence of the R3 and the R4 on the ventral right side of the subapical groove. The asterisk marks the dense PB2.

Figs. 43, 44. Serial sections (nos 1, 5) transverse through the R3 to show the movement of the R4 microtubule as it approaches and joins the tiered R3.

Fig. 45. The microtubular roots associated with the left basal body are the R1, R1C and the R2. Overlying the R2 is the dense material (arrow) of the ventral extension of the DB.


34
DB
H
R2
L
R1
35
C
IB
AC
R
AB2
H
R1
36
IB
R
L
H
37
L
R
H
38
R
H
39
R
L
R1
AB1
H
40
PB2
R
AB1
41
R
R4
42
L
IB
H
R
SG
R3
43
R3
R4
44
R3
R4
45
R1C
R2
R1

Figs. 46 - 51. R1 and R1C roots.

Fig. 46. Tangential transverse section sloping posteriorly on the left side. The R1 microtubules (arrows) pass across the ventral face of a mitochondrial profile, to enter a cytoplasmic strand that penetrates the most anterior portion of the posterior vacuole. Scale bar = 0.4 μm.

Fig. 47. Longitudinal section through a cell to show the cytoplasmic strand (arrow) containing the R1 microtubules that is associated with the posterior vacuole. Scale bar = 1.5 μm. INSET: A transverse section through the posterior portion of the cytoplasmic tongue to show the microtubules (arrows) in the narrow cytoplasmic strip which has contracted away from the inner lining of the posterior vacuole.

Figs. 48, 49. Juncture of the R1 and the R1C, transverse (Fig. 48) and longitudinal (Fig. 49) to the R1. Scale bars = 0.2 μm.

Figs. 50, 51. Sequential serial sections almost transverse to the long axis of the haptonema showing the left ventral pathway of the R1 microtubules (arrows) as they curve over and between the mitochondrial profiles. Scale bars = 0.2 μm.


46
PV
M
R3
R4
R
L
AB1
H
47
C
PV
48
R1
R1C
49
R1
R1C
50
M
L
DB
R1
H
R1C
51
M
L
M
R1
H

Figs. 52 - 54. The R1C of *Chrysochromulina* sp.

Fig. 52. The sweep of the microtubular bundle constituting the R1C as it passes across to the dorsal side of the cell. INSET: the R1C in transverse section. Scale bar = 0.5 μm.

Fig. 53. The R1C (arrow) associated at its most proximal extremities with the anterior edge of the nuclear envelope. Scale bar = 0.2 μm.

Fig. 54. The R1C (arrows) is bifurcate around the tip of the nucleus in a preprophase cell. Scale bar = 0.2 μm.


52
C
R1C
G
53
54
C
N
N

CHAPTER 5

THE ULTRASTRUCTURE OF *CHRYSOCHROMULINA BREVIFILUM* (PRYMNESIOPHYCEAE)

ABSTRACT

The identifying features of ***Chrysochromulina brevifilum*** are clarified, based on a detailed ultrastructural investigation. The light microscopical observations in the type description are confirmed, and the first description of all three scale types (spine scales, large and small plate scales) is provided. The general ultrastructure and arrangement of the organelles is no different from that of most other prymnesialean motile cells, but the transitional region of each flagellum has an unusual 'spoked' arrangement distal to the two transitional plates. The flagellar apparatus has two main distal connectives (with extensions from the distal band ventrally over the R2 microtubular root, anteriorly towards the R1 broad root, and posteriorly towards the left chloroplast), three proximal connectives, and four different microtubular roots of which the R1 descends posteriorly and ventrally as a cytoplasmic tongue. This flagellar apparatus is compared with others in the genus and order, but the variation between species and genera is too wide to enable any phylogenetic conclusions to be made.

INTRODUCTION

In the first of a series of papers on new species of ***Chrysochromulina*** Lackey, Parke *et al.* (1955) described *C.* ***brevifilum*** Parke *et* Manton. The generic identification was "provisional"

(Parke *et al.*, 1955, p.579) and based on the fact that the third filiform appendage which Parke *et al.* (1955) named a haptonema, was much longer than that of species of either ***Prymnesium*** Conrad or ***Platychrysis*** Geitler, and was capable of coiling. Species-specific organic scales were also described in ***Chrysochromulina*** for the first time in this innovative publication and are presently the main species characteristic. The type description of *C. **brevifilum*** identifies a single scale type that has a central spine subtended by four decurrent ridges. Manton later mentions the presence of plate scales (Manton, 1972), and these scales are described by Leadbeater (Leadbeater, 1972a - on the basis of a personal communication from Manton) as having quadrants of radiating ridges visible on both the proximal and the distal surfaces, with a band of concentrically striated material bordering the distal side of each scale. No illustrations or dimensions of the plate scales were given.

The species has been recorded subsequently from Norway (Leadbeater, 1972b), Denmark (Manton & Leadbeater, 1974), eastern Australia (Hallegraeff, 1983) and, tentatively as *C.* aff. ***brevifilum***, from New Zealand (Moestrup, 1979). However, there is doubt about the identification in both reports from the Southern hemisphere, as these are taxonomically dubious and probably represent other species.

The purpose of this study, therefore, is to delineate clearly the species concept of *C. **brevifilum***, and to describe its ultrastructural features. These include the flagellar apparatus which is considered to be phylogenetically important (Preisig, 1989), but which has been described for only two other species of ***Chrysochromulina*** (Moestrup & Thomsen, 1986; Gregson *et al.*, 1993).

MATERIALS AND METHODS

A water sample was collected from the inshore coastal waters of the Indian ocean, near Durban on the Natal coast (29°58'S 31°30'E), on 23 July 1989. The sample was passed through a series of nylon meshes to concentrate the nanoplankton, but no nutrients were added to enrich the suspension. A single cell isolate was obtained by micropipetting, and the culture is currently

maintained at 20°C, with a light:dark cycle of 16:8 hours, a light intensity of 40-60 $\mu E\ m^{-2}\ s^{-1}$, in $35^{o}/_{oo}$ seawater enriched with soil extract and half-strength Provasoli-a culture medium (McLachlan, 1979).

A Zeiss Axiophot microscope equipped with phase and Nomarski interference optics was used for studying cell shape, size and swimming behaviour.

For electron microscopy, scale preparations were made by placing drops of cell culture on 0.3% formvar-coated, copper grids, osmicating in OsO_4 vapour for 5 min, followed by shadow-coating at 30° with gold palladium. A minimum of 50 scales was measured for each scale type, although the standard error was within 10% of the mean after only 23 - 27 scales had been measured. After nine months in culture the isolate no longer produced any scales, and because an adequate fixation procedure had not yet been accomplished, details about the precise scale arrangement are limited.

Several methods of fixation for sectioned material were tried, including a paraformaldehyde fixation (Karnovsky, 1965) and a sucrose/cacodylate buffered fixation (Moestrup & Thomsen, 1986 ... with a series of different sucrose concentrations being tested). After experimenting with a variety of fixative concentrations, buffers, and fixation times, the following method produced the best results: Cell cultures (in enriched $35^{o}/_{oo}$ seawater, pH 7.8) were fixed in 1.25% glutaraldehyde for 40 min. The sample was then centrifuged and the pellet was resuspended in drops of cooling 0.2% purified agar. Agar pieces were rinsed in culture medium five times at 15 min intervals, followed by a 60 min post-fixation in 2% OsO_4. The samples were again rinsed five times in culture medium over a period of 2 hours, and then dehydrated in a graded alcohol series at 20 min intervals, prior to embedding in Spurr's resin (Spurr, 1969). Serial sections were cut on a Reichert ultracut E microtome, stained for 20 minutes in saturated aqueous uranyl acetate followed by lead citrate, and viewed with a Jeol 100S transmission electron microscope.

The numbering of the flagella and the cell orientation are in accordance with the conventions established by Beech & Wetherbee (1988). The labelling of the connectives and the microtubular root numbering follow that of Green & Hori (1990).

Abbreviations used in the figures

AB1 - proximal accessory band 1; AB2 - distal accessory band 2; C - chloroplast; D - dorsal; DB - distal band; G - Golgi body; H - haptonema; L - left flagellum/basal body; M - mitochondrial branch; N - nucleus; P - pyrenoid; PB1 & PB2 - proximal bands 1 and 2; PER - peripheral endoplasmic reticulum; PV - posterior vacuole; R - right flagellum/basal body; R1, R2, R3, R4 - numbered microtubular roots; V - ventral.

RESULTS

Light microscopy

Living cells are sphaeroidal, sometimes strawberry - shaped, with the anterior end, into which the three appendages are inserted, often being slightly truncate (Figs 1 - 9, also 25). The cell size is 4.2 μm - 9.7 μm, though cell dimensions are generally 5.1 μm - 6.6 μm (length and breadth being equal given the cell shape). The two equal flagella are 13 μm - 17 μm long (range of 11.3 μm - 19.5 μm). In recently divided cells the flagella may be subequal, but the difference in length never exceeds 2 μm. The haptonema is always shorter than the flagella, and depending on the stage of development may be as short as 3 μm, although the maximum recorded length is 14.2 μm and the average range is between 7.6 μm - 9.5 μm. Length ratios were found to be useful in differentiating between species of ***Prymnesium*** (Chang & Ryan, 1985), but in *C.* ***brevifilum*** these are so highly variable that they can be of little taxonomic value. The average length ratio of flagella to cell is 2.57, and of haptonema to flagella is 0.46.

Each cell typically contains two parietal chloroplasts (Fig. 9), seldom one or four. Each plastid has a small pyrenoid that is not easily visible unless the cell is squashed (Fig. 1). The chloroplasts may be lobed either anteriorly prior to cell division, or posteriorly below the pyrenoid-containing region. Oil droplets and other vesicles are scattered throughout the cytoplasm (Fig. 5), and there is a posterior vacuole of variable size over which the nucleus, which lies in a median - posterior position, may sometimes be observed (Fig. 2). The plasmalemma is stippled with muciferous bodies which are often found along the periphery of

the chloroplasts (Fig. 3), but which may occasionally also be seen to be randomly scattered (Figs 1, 2).

The cells are honey-coloured but become tinged with green as they age, so in old cultures a dark green pellet of stationary phase cells forms on the illuminated side of the flask. No amoeboid or encysted life history stages have been observed.

During rapid swimming (which always occurs as a result of coverslip pressure, but is otherwise infrequently observed), the posterior end of the cell is directed forwards with the three appendages trailing behind (Fig. 4). The cells swim very fast in straight lines, with a scissor-like flagellar movement that results in the cell body flicking sharply to rotate around the axis of forward movement. Slow swimming occurs in wide, sweeping arcs with a lethargic rotation of the cell body. As a cell reaches the end of an arc, it stops moving, then abruptly changes direction to move languorously along the next, apparently randomly chosen, pathway. The flagellar beat is homodynamic and the haptonema is generally extended forwards (Fig. 5), although sometimes it may be coiled and not detectable (Fig. 6). Although capable of coiling (Fig. 3), the haptonema of ***C. brevifilum*** is rarely seen contracted into tight gyres, even in dead/fixed material (Figs 10, 19). Haptonematal coiling is not seen when swimming cells are viewed under a coverslip, the pressure apparently being sufficient to prevent coiling.

When cells are attached to the slide by means of their haptonemata, the haptonema is extended straight out (Figs 7 - 9) or, less frequently, exhibits some degree of bending or flexure (Figs 1, 2). The heterodynamic flagellar movement varies from gentle undulation when the flagella are held posteriorly (Fig. 7) or anteriorly (Fig. 9), to a vigorous quivering when held laterally (Fig. 8).

Electron microscopy

Scales

Shadowed preparations reveal that ***C. brevifilum*** has three distinguishable types of body-scale (Fig. 11): spined scales, large plate scales and small plate scales. The spined scales have an

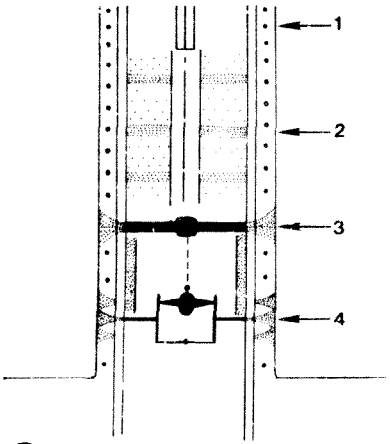
ellipsoidal base measuring 0.8 μm x 0.9 μm (0.75 μm x 0.84 μm - 0.89 μm x 0.98 μm), and a central spine of 0.63 μm - 0.72 μm in height. The rim around the distal circumference is 75 nm (Figs 11 - 13). Both proximal and distal surfaces display quadrants of radiating ridges (58 - 62 in number) which overlie very fine, concentrically arranged fibrils (Figs 11, 12). On the distal surface the central spine, which tends to collapse in shadowed preparations, is subtended by four decurrent struts which do not extend to the periphery of the scale, and which are not adnate to the scale surface along their entire length (Figs 12, 13). The peripheral wall on the distal surface is probably upright, but this needs to be confirmed with sectioned material.

The large plate scales are similar to the spined scales in size (0.72 μm x 0.9 μm; range 0.68 μm x 0.76 μm - 0.92 μm x 1.02 μm) and in the ridged patterning on both distal and proximal surfaces, with 58 - 64 radiating ridges (Figs 15, 16). However, the plate scales lack the distal spine and upright wall, having instead a narrow, smooth border (70 nm wide) on their distal surfaces (Fig. 15). This border is probably inflexed, but again this needs to be confirmed with sectioned material. The scale shape occassionally tends towards circular as opposed to elliptical (not shown).

The small plate scales have the same patterning as the large plate scales (Figs 17, 18), but differ in size (0.6 μm x 0.7 μm; range 0.53 μm x 0.6 μm - 0.66 μm x 0.71 μm) and therefore in the number of radiating ridges (44 - 48 per scale). There is no overlap in size between the large and small plate scales, and there are four times more of the former than the latter. There are equal numbers of spined and large plate scales in the scale casing of each cell, and no scale type is restricted to a particular region of the cell body. It appears that the small plate scales lie between the outer spined scales and the more proximally situated, large plate scales (Fig. 11), but this needs to be confirmed with sectioned material.

General ultrastructure

Sectioned material confirms and expands observations made with the light microscope. There are two parietal chloroplasts (Figs 19, 20), each with an immersed pyrenoid that is penetrated by paired thylakoids (Fig. 21); a Golgi body polarized around the bases of the appendages, with the forming face on the left side of the cell and the maturing face opening apically on the right side of the cell (Fig. 22); a nucleus beneath the dictyosome in a slightly dorsal position (Figs



(24)

Fig. 24. **Diagrammatic reconstruction of a longitudinal section through the transitional region of a flagellum in *C. brevifilum*.** Numbered arrows indicate: 1 - the 9+2 axonematal arrangement; 2 - the 'spoked' arrangement; 3 - the distal transitional plate; 4 - the tri-partite proximal plate.

19, 25); and a posterior vacuole that is more ventrally situated (Fig. 67). A thin layer of dense, osmiophilic material lines the outer surfaces of the chloroplasts, usually as a continuous covering (Figs 19, 20, 42) but occasionally as a punctate series of discs (Fig. 25). The chloroplast endoplasmic reticulum includes periplastidial reticulum along the inner cytoplasmic faces of the plastids (Fig. 23), and continuity of the outer membrane between the nuclear envelope and the chloroplasts (Figs 19, 25). The muciferous bodies are apparent as small, membranous bubbles along the outer membrane of the peripheral endoplasmic reticulum (Figs 19, 25, 47). Ventrally, the peripheral endoplasmic reticulum is associated with a cytoplasmic tongue or contractile root (Figs 20, 59, 63, 67), as first described in ***Pleurochrysis pseudoroscoffensis*** Gayral *et* Fresnel (Gayral & Fresnel, 1983).

A number of cells from exponentially growing cultures possessed myelin-like structures of concentrically arranged membranes (Fig. 23). Serial sections and the infrequent fortuitous section revealed that these configurations are actually formed by the inner mitochondrial membrane (Fig.23). The mitochondria of higher plants and animals are generally believed to be recycled by autophagocytosis (De Priester, 1984), and the only homologous structures have been recorded in ageing insect muscle (Sacktor & Shimada, 1972).

Ultrastructure of the appendages

The three appendages are apically inserted in the truncate apex of the cell (Fig. 25). Each flagellum is terminated by a short hair-point (65 nm) containing an extension of the central pair of microtubules (Figs 10, 26). The outer surface of the flagellar membrane is covered by an extremely delicate, fibrillar tomentum (Figs 26, 27, 31) which is usually lost during the preparation of shadowed specimens, but which is always present in sectioned material, if only as a short, fuzzy layer. Between the flagellar membrane and the peripheral doublets is a series of small tubular rings (Figs 28, 49), which extends for more than 7 μm from the point of flagellar insertion towards the flagellar tip.

The transitional region (Fig. 24) lies between the beginning of the central pair of microtubules and the triplet arrangement of the basal body (Fig. 29). In *C. **brevifilum*** the transitional region is typified by two transitional plates and an unusual spoked-doublet configuration. Above (distal to) the distal plate, and below the central pair of microtubules, there is a region (0.35 μm in

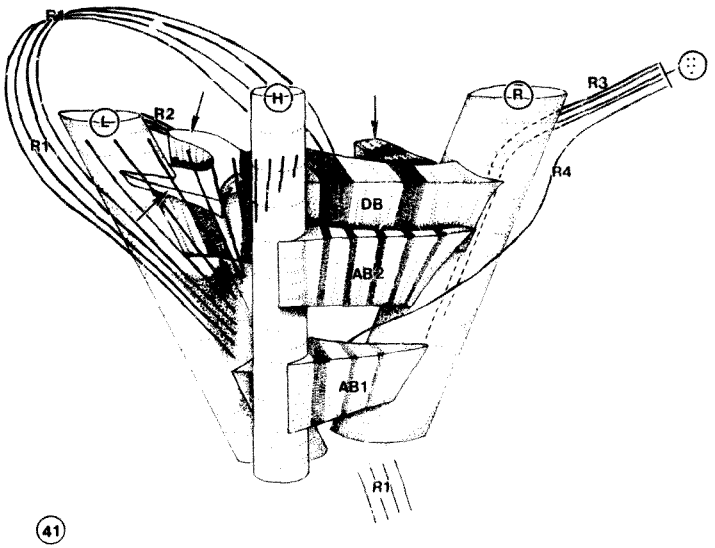


Fig. 41. Diagrammatic reconstruction of the flagellar apparatus of *C. brevifilum* when viewed from the dorsal side of the cell. (The two proximal bands are not visible from this angle). The three arrows mark the three extensions of the large distal band (DB). For clarity, the pathway of only four of the R1 microtubules has been drawn. The relative sizes of the connectives are in proportion, but the entire system has not been drawn to scale.

length) with a central cylindrical core that is attached to the A-microtubule of each peripheral doublet by fine threads (Figs 24, 29, 30, 31). A single thin thread also occurs in the centre of this cylindrical core (Fig. 30). The sturdy distal plate (Figs 24, 29, 32) has a well defined, central axosome, and osmiophilic material connects the nine doublets to both the flagellar membrane and to each other. Between the two transitional plates, the flagellar membrane is often distended (compare Fig. 34 with Figs 32 and 35), and there is evidence of a fine cylinder that links the doublets centripetally (Figs 33, 34). The central osmiophilic structure inside this cylinder connects the axosomes of the two transitional septa. As yet, no stellate pattern has been seen here. The proximal plate (Figs 24, 29, 35, 36) consists of a centrally - discontinuous septum which encircles a short cylinder. The proximal axosome is found in the distal portion of this cylinder (Fig. 35), while the base of the cylinder is sealed by a thin diaphragm that has a central thickening (Fig 36). Again the outer doublets are connected by densely-staining material in the region of the transitional plate.

The basal bodies are c. 700 nm long with a triplet region containing a dense core (Fig. 29) that attaches to each of the nine triplets (Fig. 38). Each basal body proximally contains a short (140 nm long) cartwheel region (Fig. 29).

The ultrastructure of the haptonema is unremarkable : seven microtubules occur in the free part of this appendage (Fig. 39), and on insertion, the arrangement of these microtubules, as well as the two that are subsequently added (Fig. 40), follows the pattern described for other ***Chrysochromulina*** species (e.g. Manton, 1968, Leadbeater & Manton, 1969, Moestrup & Thomsen, 1986).

Insertion of the appendages

A reconstruction of the flagellar apparatus is given in Fig. 41.

When viewed from the dorsal cell surface, the haptonema is the first appendage to be inserted, followed by the left flagellum, with the right flagellum being most ventrally positioned (Figs 41, 42 - 44, 47). The flagella are laterally displaced on each side of the haptonema, the right flagellum being displaced further away from the haptonema than the left flagellum. Anteriorly, immediately above the cell body, the three appendages are almost in a line and diverge only

slightly from the left-right lateral plane, but at their bases they form a small triangle. The angles between the bases vary, but roughly follow an isosceles pattern in that the LHR and the HRL angles are of a similar magnitude, with the HLR always being greater than 95°. The long axes of the two flagella slope upwards at 50° - 70° to the horizontal.

Connectives

The most extensive connective of the flagellar apparatus, the distal band (DB), lies just beneath the plasmalemma and connects all three appendages (Figs 41, 44, 47, 49, 54). The distal band is a thin sheet of material and is striated with the two most obvious striations being associated with the right side of the left basal body and the left side of the haptonema (Figs 44, 54). The distal band also has three extensions: a fibrous flange that passes anteriorly between the left basal body and the haptonema, to attach to the microtubules of the broad root (R1), above (anterior to) the main portion of the distal band (Figs 41, 42 - 43, 48, 49); a thin, narrow, ventral extension that briefly overlies the pathway of the R2 microtubular root as the microtubules emerge from beneath the distal band (Figs 43, 47, 51), this extension also being anterior to the bulk of the distal band; a fibrillar, but striated, band that is associated with the ventral side of the right basal body (Figs 41, 44, 45, 51, 55). This band originates at the level of the DB then curves posteriorly to attach to the chloroplast adjacent to the right basal body.

Another connective, the accessory band AB2, is also situated in the distal region of the flagellar apparatus (Figs 41, 46, 50, 54 - 55), at a level corresponding to the most proximal portion of the distal band and extending proximally for c. 80 nm beyond the lower boundary of the distal band. This narrow, regularly striated accessory band joins the haptonema to a triplet of the right basal body (Fig. 50).

There are three proximal connectives (Figs 41, 51 - 53, 60 - 61). A robust, irregularly striated accessory band, (AB1), links the bases of the haptonema and the right basal body (Fig. 52), but which also seems to reach the base of the left basal body at its most proximal level (Figs 53, 61). The AB1 tapers from a wide, faintly granular structure at the haptonematal base, to a slender, pointed portion that is attached to two adjacent triplets of the right basal body (Fig. 60). In addition, two delicately striated proximal bands (PB1 and PB2) connect the bases of the two

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