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Ultrastructural studies on marine Prymnesiales (Prymnesiophyceae)

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2015-01-09
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flagella (Figs 53, 60 - 61). The PB1 is more ventrally situated, with the PB2 lying between the PB1 and the AB1.

Microtubular roots

The R1 root originates from an amorphous cushion of material between the left basal body and the haptonematal base, immediately distal to, or at the level of, the proximal connectives (Figs 41, 46, 50, 56). At its origin this root is composed of only two or three microtubules (Fig. 56), but additional microtubules are added as the root ascends anteriorly, so that up to thirteen microtubules can be seen lying between the inner membrane of the peripheral endoplasmic reticulum and the left chloroplast, above the level of the insertion of the appendages (Fig. 64, and compare Figs 45 - 46, 54 - 55). In the region of the distal band, amorphous material connects the root to the left basal body and the haptonema (Figs 45, 51, 56). Glancing sections indicate that the R1 microtubules apparently curve from the dorsal to ventral cell regions (Figs 41, 47 - 48, 62), ascending over the left chloroplast and descending ventrally enclosed in a tongue of cytoplasm that extends through the peripheral endoplasmic reticulum to the plasmalemma (Figs 20, 56, 58 - 59, 62 - 63, 67). Before breaching the lumen of the peripheral endoplasmic reticulum, the descending microtubules are closely associated with a mitochondrial branch on the inner cytoplasmic face of the left chloroplast (Figs 56, 58, 64). No fibrous root has yet been seen associated with the cytoplasmic tongue, but the constituent microtubules are clearly interconnected at their proximal ends (Fig. 67).

In some cells, a single microtubule was seen to extend from the left basal body directly towards the ventral mitochondrial branch (Fig. 55). It is uncertain if this microtubule, which is not always present and which lies variably in the distal region of the flagellar apparatus in different cells, actually contributes towards the microtubules of the cytoplasmic tongue.

Root R2 consists of three microtubules which originate near the left basal body between the two flagellar bases (Figs 41, 52, 55). The microtubules of this root emerge from beneath the ventral extension of the DB, and follow a course ventrally between the two chloroplasts (Fig. 42).

Root R3 originates as a pair of microtubules (Fig. 53) at the base of the right basal body, and travels anteriorly along the ventral side of the long axis of this basal body (Figs 41, 50 - 52, 55,

57). Between the proximal and distal sets of connective bands, another two microtubules are added, so that the R3 consists of a tiered arrangement of paired microtubules (Figs 47, 56, 65 - 66). Anteriorly the root curves into a more horizontal plane, to line the ventral, inner face of the right chloroplast (Figs 55 - 56).

The single microtubule of root R4 originates on the ventral side of the AB1, near the haptonematal base (Figs 51, 52). It slopes upwards (anteriorly) towards and past the right basal body (Figs 55, 56, 48, 65) to underlie and follow the pathway of the two pairs of R3 microtubules (Fig. 66).

DISCUSSION

Taxonomy

There are a number of species of ***Chrysochromulina*** that have scales with relatively short spines (i.e. where spine length is of a similar magnitude to scale diameter), namely ***C. acantha*** Leadbeater *et* Manton, ***C. alifera*** Parke *et* Manton, ***C. brevifilum***, ***C. breviturrita*** Nicholls, ***C. ephippium*** Parke *et* Manton and ***C. kappa*** Parke *et* Manton. Unlike ***C. brevifilum***, three of these species (***C. acantha***, ***C. alifera*** and ***C. ephippium***) are true to the generic description in being saddle-shaped cells with long, coiling haptonemata (Parke *et al.*, 1956; Leadbeater & Manton, 1971). Although sphaeroidal in shape, ***C. kappa*** has a haptonema minimally longer than the flagella, but also differs more obviously from ***C. brevifilum*** in having: muciferous bodies concentrated at the non-flagellar pole; spine scales confined to the region of flagellar insertion; struts of the spine extending to the periphery of the base plate; large plate scales with an erect rim; and extremely tiny (0.3 μm x 0.4 μm) small plate scales (Parke *et al.*, 1955). ***C. breviturrita*** is very like ***C. brevifilum*** in shape, but is slightly larger, and being lacustrine possesses an obvious contractile vacuole (Nicholls, 1978). The spined scales of ***C. breviturrita*** are supported by a variable number of struts - two or more cited in the type description (Nicholls, 1978) and five or six described in scale studies on isolates of ***C. breviturrita*** (Brown *et al.*, 1986). Both spine length and base plate diameter are smaller in ***C. breviturrita*** than in

C. brevifilum, and the plate scales of the former are small (0.2 μm x 0.3 μm - 0.3 μm x 0.6 μm) and do not conform to the description of distal ornamentation given by Manton for *C. brevifilum* (Manton, 1972; Brown *et al.*, 1986). Although the present study clearly does not deal with ***C. breviturrita***, it is interesting to note that this is the only other species of ***Chrysochromulina*** in which a fibrillar flagellar coating has been described (Day *et al.*, 1986).

The organism of this study agrees in all respects with the type description of the motile cells of ***C. brevifilum***, although the range of cell and flagellar dimensions in the type is wider than that observed in the present isolate, and of course no plate scales were described initially (Parke *et al.*, 1955). The *C.* aff. ***brevifilum*** described by Moestrup (1979) is probably ***C. kappa***, given that the sizes of both spined and plate scales are comparable (and compare Moestrup, 1979, Fig.8, with Parke *et al.*, 1955, Fig.35). When compared with the type description of ***C. brevifilum***, the spine scale illustrated and described as *C.* ***brevifilum*** by Hallegraeff (1983, Figs 7 and 8) has a smaller base plate, fewer radial ridges, a spine half as short in length, four struts that are adnate to the scale surface along their entire length and which also extend right to the periphery of a scale that lacks an erect rim. This scale clearly does not originate from *C.* ***brevifilum***, particularly as the scales of this species are said to be constant in size, morphology and patterning (Leadbeater, 1972b).

Flagellar apparatus

Information pertaining to the transitional region of members of the Prymnesiales (reviews by Moestrup, 1982; Preisig, 1989) is based entirely on the work of Manton on ***Prymnesium parvum*** Carter (Manton, 1964). More recent publications differ from this initial description in that they indicate that the distal plate commonly contains an axosome in species of ***Chrysochromulina*** and ***Prymnesium*** (Gregson *et al.*, 1993; Birkhead & Pienaar, 1994 in press; present study). Also, a stellate pattern between the two transitional plates similar to that of ***P. parvum*** (Manton, 1964) has yet to be clearly demonstrated. Whether or not the 'spoked' arrangement in ***C. brevifilum*** is unique to the order can only be determined when information on more species becomes available (although a survey of published micrographs has not revealed a similar structure in any

other member of the order or the class).

The angles of insertion of the ***C. brevifilum*** appendages, although variable, resemble those of ***Prymnesium*** (Green & Hori, 1990; Birkhead & Pienaar, 1994 in press) more closely than those of ***Chrysochromulina*** (Moestrup & Thomsen, 1986; Gregson *et al.*, 1993). This is probably due to the apical insertion into the truncate apex of a spherical cell, on contrast to a subapical - ventral insertion into the concave face of a saddle-shaped cell. It is also conceivable that the presence of a cytoplasmic tongue may be correlated with cell shape, as this structure has only been observed in cells that are roughly spherical and which are not dorsi-ventrally flattened, e.g. various species of ***Pleurochrysis*** (Gayral & Fresnel, 1983; Beech & Wetherbee, 1988; Fresnel & Billard, 1991), ***Prymnesium nemamethecum*** Pienaar *et* Birkhead (Birkhead & Pienaar, 1994 in press), ***Chrysochromulina brevifilum*** (present study) and possibly also in ***C. mactra*** Manton (Manton, 1972, Fig.6) and less convincingly, in ***Prymnesium calathiferum*** Chang *et* Ryan (Chang & Ryan, 1985, Fig.5).

Within the genus, the pathways of the microtubular roots of ***C. brevifilum*** resemble those of ***C. apheles*** Moestrup *et* Thomsen (Moestrup & Thomsen, 1986) more closely than those of ***C. acantha*** (Gregson *et al.*, 1993), although the number of consituent microtubules is always greater with the exception of the perpendicularly aligned portion of the broad root, which if present in ***C. brevifilum*** has only one microtubule, but has two in ***C. apheles*** (Moestrup & Thomsen, 1986). The 2 + 2 + 1 (R3 + R4) arrangement is common to both ***C. brevifilum*** and ***C. acantha*** (and to at least two other species of ***Chrysochromulina***, pers. obs.), but ***C. acantha*** lacks a root that arises from beneath the distal band in a ventral position (the root commonly designated as the R2 by Inouye & Pienaar, 1985; Green & Hori, 1986; Beech & Wetherbee, 1988; Green & Hori, 1990; Fresnel & Billard, 1991; Roberts & Mills, 1992). In ***C. acantha***, the two microtubules lining the anterior, left side of the R1, have no analogue in either ***C. apheles*** or in ***C. brevifilum***, but the accessory fibre is the same as one of the distal band extensions described in ***C. brevifilum*** and as part of the "..somewhat diffuse contours of the distal fibre" of ***C. apheles*** (Moestrup & Thomsen, 1986, p.601, and see Fig.48). The varied interpretations are at least partially due to the difficulties encountered in reconstructing such asymmetrically arranged structures.

The basic pattern of the flagellar apparatus of the Prymnesiales is consistent with that described by Preisig (1989), but the variations on this theme are as numerous as the species studied, when comparing the two species of ***Prymnesium*** (Green & Hori, 1990; Birkhead & Pienaar, 1994 in press) and the three species of ***Chrysochromulina*** (Moestrup & Thomsen, 1986; Gregson *et al.*, 1993; present study). For example, the range in the number of microtubules in the R1 extends from 4+2 in ***C. apheles***, to 13(+1?) in ***C. brevifilum***, to 20+4 in ***C. acantha***, to 25 in ***P. patelliferum*** and ***P. nemamethecum***; ***C. brevifilum*** has an R2 root resembling that of the two ***Prymnesium*** species, ***C. apheles*** has a relatively reduced R2, while ***C. acantha*** lacks this root altogether; both ***Prymnesium*** species possess an intermediate connecting band (across the ventral sides of the basal bodies) which is apparently absent in all three of the ***Chrysochromulina*** species (but which has been seen, pers. obs., in a species of ***Chrysochromulina*** called the 'eyelash' ***Chrysochromulina*** by Moestrup, 1979); there is a compound R1 root in ***P. nemamethecum*** but not in any other described ***Prymnesium*** or ***Chrysochromulina*** species (although compound roots have also been observed in other ***Chrysochromulina*** cells, pers. obs., M. Kawachi pers. comm.); the distal, striated connective from the ventral side of the right basal body towards the right chloroplast (distal band extension in ***C. brevifilum***, auxiliary connecting root of the two ***Prymnesium*** species) is not found in either ***C. apheles*** or ***C. acantha***. Thus, there is no particular feature of the flagellar apparatus which can be used to distinguish between species of ***Prymnesium*** and ***Chrysochromulina***, as the two genera are currently understood. This illustrates that the taxonomy of these two genera needs attention and that the prymnesialean flagellar apparatus is at present not as useful a phylogenetic indicator as, for example, flagellar features in the green algae.

ACKNOWLEDGEMENTS

R.N.P. is funded by the University of the Witwatersrand and by the Foundation for Research Development of the CSIR. M.B. would like to thank Julia Birkhead for her support.

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Figs 1 - 9. Light microscopy of *C. brevifilum*.

Fig.1. Slightly compressed, attached cell to illustrate the two pyrenoids (arrowheads). Scale bar = 5 μm.

Fig.2. Compressed cell to show the median/posterior nucleus (arrow) overlying the posterior vacuole. Scale bar = 5 μm.

Fig.3. Two rows of muciferous bodies (arrows) line the edges of the chloroplasts. Note the loosely coiled haptonema (arrowhead). Scale bar = 5 μm.

Fig.4. Rapidly swimming cell with posterior end directed forwards. Scale bar = 8 μm.

Fig.5. Slowly swimming cell with anterior end forwards and haptonema extended. Scale bar = 8 μm.

Fig.6. Slowly swimming cell with coiled haptonema. Scale bar = 10 μm.

Fig.7. Attached cell with posteriorly directed flagella. Scale bar = 10 μm.

Fig.8. Quivering laterally positioned flagella of an attached cell. Scale bar = 10 μm.

Fig.9. Attached cell with anteriorly undulating flagella. Scale bar = 10 μm.

Figs 10 - 18. Shadowcast scale preparations.

Fig.10. Osmicated cell with loosely coiled haptonema (H). Scale bar = 13μm.

Fig.11. Portion of the scale periplast to show the small plate scales (arrows) sandwiched between the large plate scales (arrowheads) and the spined scales. Scale bar = 0.5 μm.

Fig.12. Distal surface of a spined scale with collapsed spine and peripheral wall. Shadow reveals struts raised from the scale surface. Scale bar = 0.5 μm.

Fig.13. Distal surface of spined scale with collapsed struts. Scale bar = 0.5 μm.

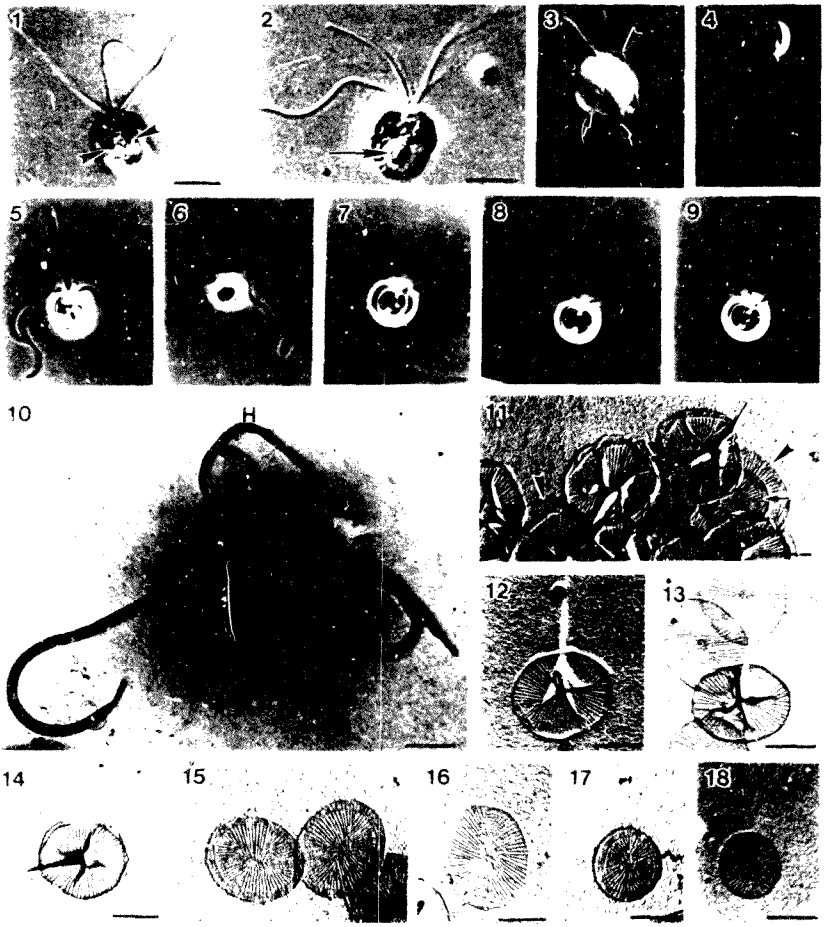
Fig.14. Proximal surface of a spined scale. Scale bar = 0.5 μm.

Fig.15. Distal surfaces of large plate scales. Scale bar = 0.5 μm.

Fig.16. Proximal surface of a large plate scale. Scale bar = 0.5 μm.

Fig.17. Distal surface of a small plate scale. Scale bar = 0.5 μm.

Fig.18. Proximal surface of a small plate scale. Scale bar = 0.5μm.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Figs 19 - 23. General ultrastructure of *C. brevifilum*.

Fig.19. Longitudinal section through the more dorsal part of the cell. Note the muciferous bodies (arrowheads). Scale bar = 1 μm.

Fig.20. Transverse section through the anterior part of a cell to show the dorsal (D) positioning of the appendages and the ventral position (V) of the cytoplasmic tongue (arrow). Scale bar = 1 μm.

Fig.21. Transverse section through a pyrenoid. Scale bar = 0.4 μm.

Fig.22. Transverse section through a Golgi body with the forming face (arrowheads) on the left side of the cell. Scale bar = 0.5 μm.

Fig.23. Myelin-like, concentric mitochondrial membranes. Note the periplastidal reticulum (arrowheads). Scale bar = 0.5 μm.



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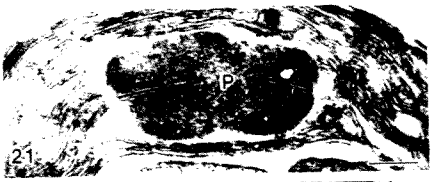

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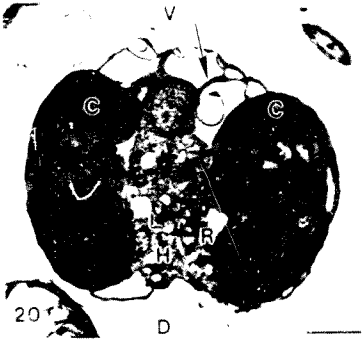

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Figs 25 - 40. Ultrastructure of the appendages.

Fig.25. Insertion of the three appendages into the truncate apex of the cell. Note the interrupted osmiophilic lining (arrows) of the outer surface of the chloroplast. Scale bar = 1 μm.

Fig.26. Flagellar hairpoint. Scale bar = 0.3 μm.

Fig.27. Shadowcast preparation with fine fibrils attached to the flagellar membrane. Scale bar = 0.3 μm.

Fig.28. Two consecutive sections through a flagellum to show the tubular rings (arrowheads) lying beneath the membrane. Scale bar = 0.4 μm.

Fig.29. Longitudinal section through an intact, but autotomising, flagellum showing the distal (arrowhead) and the proximal (arrow) transitional plates, as well as the extent of the 'spoked' configuration (dotted line). Scale bar = 0.2 μm.

Fig.30. The region between the distal plate (arrowhead) and the central pair of microtubules, that contains the 'spoked' structures. Scale bar = 0.2 μm.

Figs 31 - 37. Consecutive serial sections transverse through a flagellum, from distal to proximal parts of the transitional region. All scale bars = 0.2 μm.

Fig.31. The 'spoked' arrangement.

Fig.32. Distal transitional plate.

Fig.33. Central axosomal connection (arrow) and indistinct cylinder inside the peripheral doublets.

Fig.34. Distended membrane, central axosomal connection, thin cylinder.

Fig.35. Proximal transitional plate.

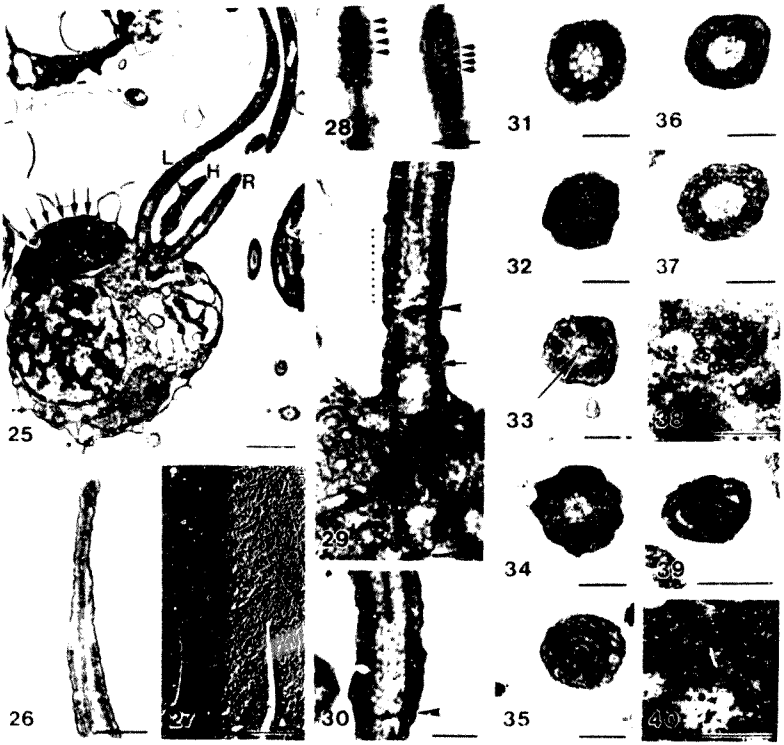
Fig.36. Diaphragm with slight central thickening, immediately below the proximal plate.

Fig.37. Flagellum prior to insertion.

Fig.38. Basal body triplet arrangement with central core. Scale bar = 0.2 μm.

Fig.39. Transverse section through the free part of the haptonema. Scale bar = 0.2 μm.

Fig.40. Nine microtubules at the base of the haptonema. Scale bar = 0.2 μm.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Figs 42 - 46. Distal structures of the flagellar apparatus.

Figs 42 - 44. Consecutive serial sections transverse to the long axis of the left basal body. Scale bars = 0.4 μm.

Fig.42. Note two of the R2 microtubles (small arrows) and the fibrous material (arrowhead) extending from between the left basal body and the haptonema towards the R1 microtubules (large arrows).

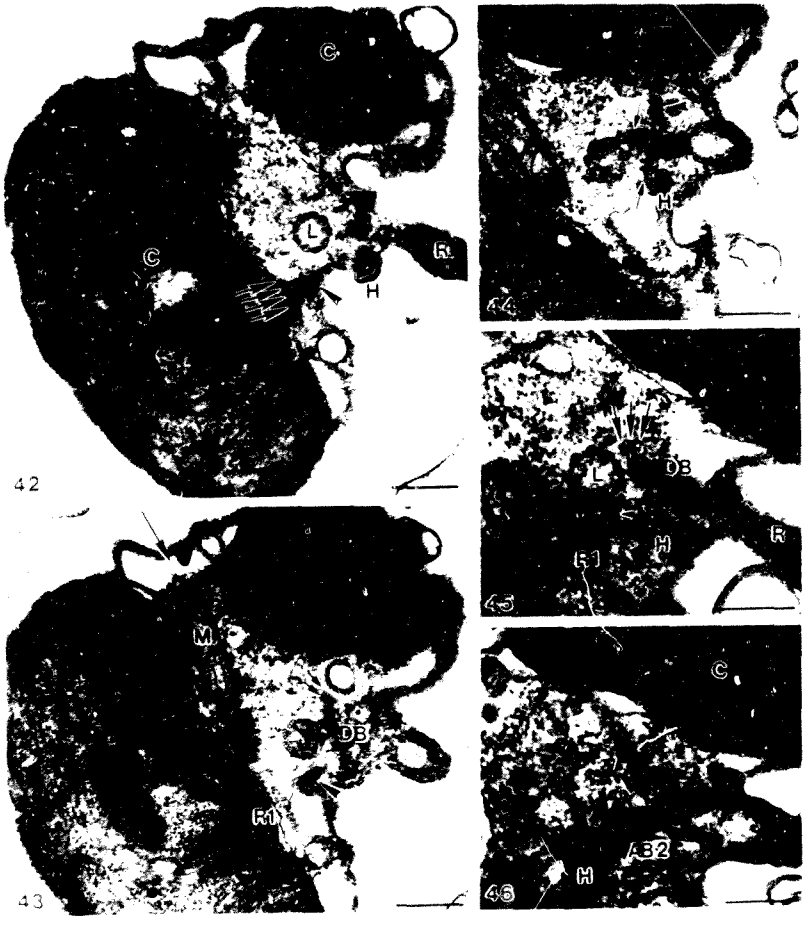
Fig.43. The fibrous connective (arrowhead) to the R1, the beginning of the distal band (DB) from beneath which the R2 microtubules emerge (small arrows). Note the mitochondrial branch (M) against which the R1 microtubules will become aligned before entering the ventral PER (large arrow) as the cytoplasmic tongue.

Fig.44. The striated distal band (arrows) with an extension (arrowhead) towards the right chloroplast.

Figs 45 & 46. Serial sections (numbers 1, 3) almost transverse to the left basal body. Scale bars = 0.3 μm.

Fig.45. Note the amorphous material (arrowhead) connecting the R1 to the left basal body and the haptonema, the striated distal band (DB), and the three microtubules of the R2 (arrows).

Fig.46. Note the right basal body-chloroplast connection (arrow) and the regularly striated accessory band, AB2.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M
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Figs 47 & 48. Consecutive serial sections transverse to the long axis of the haptonema. Scale bars = 0.4 μm.

Fig.47. The three appendages connected by the striated distal band. Note the microtubules of the R1 passing over the left chloroplast (small arrows), the ventrally directed microtubules of the R2 (arrowhead), and the inflated muciferous bodies overlying the chloroplast (large arrows).

Fig.48. The pathways of all four microtubular roots are evident in this section immediately below the distal band.

Figs 49 - 53. Serial sections (numbers 1-5) perpendicular to the right basal body. All scale bars = 0.4 μm.

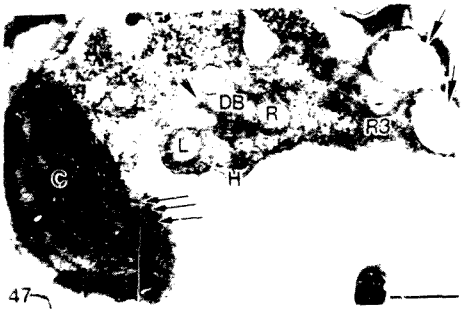
Fig.49. The DB with a connection (arrow) to the R1. Note the osmiophilic rings (arrowheads) beneath the flagellar membrane.

Fig.50. The striated AB2 (arrowhead), the R3 (small arrowhead) and the R4 (arrow).

Fig.51. The ventral and right DB extensions (large arrowheads). Note the single microtubule of the R4 (arrow) ventral to the AB1, the R3 (small arrowhead) and the amorphous material (large arrow) between the haptonema, the left basal body and the R1.

Fig.52. The R2 microtubules (arrows), the initial two microtubules of the R3 (large arrowheads) and the R4 (small arrowhead).

Fig.53. The proximal connectives of the apparatus, the AB1, PB1 (large arrowhead) and PB2 (small arrowhead). The initial two R3 microtubules (arrows) are just visible.



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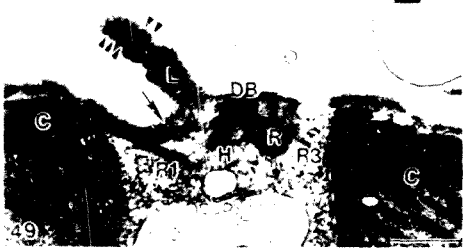

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Figs 54 - 55. Serial sections (numbers 1, 3) transverse / tangential to the left basal body. Scale bars = 0.2 μm.

Fig.54. The distal connectives (DB and AB2). Note the two main striations (arrowheads) of the DB and the ventral PER through which the cytoplasmic tongue will pass.

Fig.55. Proximal to the DB the AB2 is still present , and the striated extension (large arrow) from the DB towards the right chloroplast is evident. Note the microtubule (small arrows) stretching ventrally away from the left basal body at right angles to the other R1 microtubules.

Figs 56 - 59. Serial sections (numbers 1, 2, 6, 7) transverse to the left basal body. Scale bars = 0.2 μm.

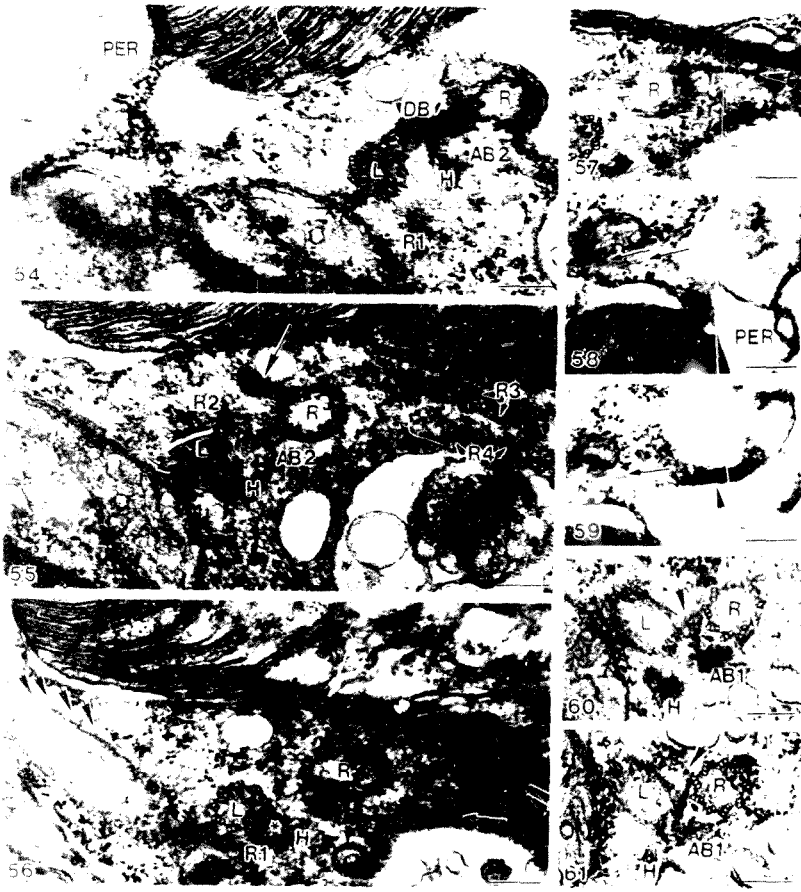
Fig.56. Note the R3 microtubules (paired arrowheads) running along the right chloroplast, the R4 microtubule (arrow), and the descending R1 microtubules (four arrowheads) passing the mitochondrial branch as they approach the ventral PER. The asterisk (*) marks the amorphous material near the origin of the R1 between the left basal body and the haptonematal base.

Fig.57. Part of the R3 (arrowhead) shown attached to the base of the right basal body.

Fig.58. The microtubules (arrows) lining the mitochondrial profile near the ventral PER.

Fig.59. The beginning of the cytoplasmic tongue (large arrow) formed by the R1 microtubules (small arrow).

Figs 60 - 61. Two consecutive sections through the proximal region of the apparatus, showing the AB1 and the two proximal bands, PB1 (large arrowhead) and PB2 (small arrowhead). Scale bar = 0.2 μm.


PER
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R
AB 2
L
H
R1
54
R2
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H
R1
56
R
57
PER
58
59
L
R
AB1
H
60
L
R
AB1
H
61

Figs 62 - 67. Detail of the microtubular roots.

Fig.62. Glancing section to show the arching R1 microtubules (arrowheads). Scale bar = 0.4 μm.

Fig.63. Serial section following Fig.62, to show the initial, more anterior development of the cytoplasmic tongue from the R1 microtubules (arrowheads). Scale bar = 0.4 μm.

Fig.64. The R1 lining the left chloroplast, with some of its microtubules (arrowheads) passing ventrally over the mitochondrial profile. Scale bar = 0.4 μm.

Fig.65. Anterior, glancing section through the two pairs of R3 microtubules (large arrows) which are joined by the single microtubule of the R4 (small arrow). Scale bar = 0.4 μm.

Fig.66. Transverse section through the R3 (large arrows) and the R4 (small arrow) as they extend laterally along the right chloroplast. Scale bar = 0.2 μm.

Fig.67. Transverse section through the posterior end of a cell. The large posterior vacuole (PV) is transected by a cytoplasmic tongue composed of interconnected microtubules (arrows). Scale bar = 0.5 μm. *Inset: the same micrograph enlarged to show the microtubules (arrows) of the cytoplasmic tongue more clearly.*


PER
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63
L
R1
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V
PER
PV
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CHAPTER 6

THE TAXONOMY AND ULTRASTRUCTURE OF *CHRYSOCHROMULINA SIMPLEX* (PRYMNESIOPHYCEAE)

ABSTRACT

The type description of ***Chrysochromulina simplex*** Estep *et al.* (Prymnesiophyceae) is emended, based on clonal cultures from the southern Indian Ocean. Cells of this species possess a single scale type (0.3 μm - 0.58 μm) with faint concentric markings on the distal face and 21 - 36 radiating ridges with an excentric cross on the proximal face. The long, coiling haptonema (max. 79 μm) and the two heterodynamic flagella (11 μm - 16 μm) are inserted on the concave, ventral face of the saddle-shaped cells. The flagellar apparatus consists of four microtubular roots (R1 of 6 + 4, a single stranded R2, a bifurcated R3 of 2/2 + 1, a single stranded R4) and various fibrous connectives (distal band with extensions to the R1 and over the R2, a proximal band, three accessory bands). The ultrastructure of the flagellar apparatus shares features with both ***C. apheles*** Moestrup & Thomsen and ***C. acantha*** Leadbeater & Manton, and intra- and intergeneric comparisons are made.

INTRODUCTION

From a mixed plankton sample from Kaikoura, New Zealand, Moestrup illustrated a field of scales from an undescribed species of ***Chrysochromulina*** Lackey (Moestrup 1979, Fig. 26).

These scales belonged to a cell with a coiling haptonema, and in a personal communication from H. Thomsen, were recognised as being identical to culture 384 in the Plymouth Culture Collection. Thomsen also reported the presence of this scale type in England and Denmark (pers. comm. to Moestrup, Moestrup 1979). In 1983, Hallegraeff illustrated and described a scale type identified as "***Chrysochromulina*** Plymouth 384, Moestrup" from the East Australian current (Hallegraeff 1983, Figs 18a, 18b). These scales however, differed markedly in size and were slightly differently patterned when compared with those found by Moestrup (Moestrup 1979). Then in 1984, Estep *et al.* described a new species of ***Chrysochromulina***, ***C. simplex*** Estep *et al.* in mixed nanoplankton samples from the North Atlantic (Estep *et al.* 1984). The small cells (2 - 6 μm) each possess a long, coiling haptonema and are covered by a single, but extremely variable, scale type that exhibits a greater degree of variability with respect to size and patterning, than any other species of ***Chrysochromulina***. This extreme variability is acknowledged by Estep *et al.* (1984, p. 623), but in support of it, reference is made to the scale variability described by Leadbeater (1972; but misquoted by Estep *et al.* as Leadbeater 1972b instead of 1972a). In ***Chrysochromulina chiton*** Parke & Manton, scale variability relates to both size and patterning and is due to genetic mutation (Leadbeater 1972). This variation should therefore be reflected taxonomically as subspecies or varieties. However, in all other species the described scale variation refers only to size but not to patterning. Even in the two species cited by Leadbeater (1972) as extremely variable, viz. ***C. ericina*** Parke & Manton and ***C. microcylindra*** Leadbeater, the patterning of the scales remains consistent. The patterning of the plate scales of all clones of ***C. campanulifera*** Manton & Leadbeater was identical, despite the variation in the cup-shaped scales that had from 1 - 3 rows of 'windows' depending on the scale size (Manton & Leadbeater 1974). The type description for ***C. simplex*** is therefore unacceptable as it encompasses three different forms of patterning on the plate-like scales (Estep *et al.* 1984, compare Figs 15, 16 & 18). The purpose of this paper is to redefine and describe ***C. simplex*** based on light and electron microscope studies of clonal cultures.

Whether or not this species resembles the undescribed Plymouth culture (Plymouth 384, not "Plymouth 383" as stated by Estep *et al.* 1984, p 623) is of little importance, as this culture no longer exists (J.C. Green, pers. comm.). Gregson *et al.* (1993) briefly described the flagellar

apparatus of Plymouth 384 based on a negatively-stained, detergent-extracted basal apparatus.

MATERIALS AND METHODS

Clonal cultures of ***Chrysochromulina simplex*** were established by single cell isolation from seawater samples collected from the following localities in the southern Indian Ocean : Durban, South Africa (Isolates U15, U16); Mauritius (Isolate M7); Reunion Island (R17). All cultures were maintained at 20°C, 18:6 light:dark cycle in 35‰ seawater enriched with half-strength Provasoli (8) and soil extract (McLachlan in Stein 1979). Only isolate U16 is presently alive, but all four isolates were used to obtain the full range of cell and scale dimensions. All the data were combined as there were no statistically valid differences between isolates from the different geographical localities.

Phase contrast and Nomarski interference optics were used with flash photography on a Zeiss Axiophot photomicroscope, for light microscopical observations.

For electron microscopy, 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, cultures (in 35‰ 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 resuspended in drops of cooling 0.2% purified agar. Agar pieces were rinsed in culture medium five times at 15 min intervals, followed by 60 min post-fixation in 2% OsO_4. The samples were again repeatedly rinsed in culture medium, 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, double-stained for 20 min in saturated aqueous uranyl acetate followed by lead citrate, and viewed with a Jeol 100S transmission electron microscope.

Terminology

Parke *et al.* (1956) first defined the concave surface of saddle-shaped cells as the ventral side. In ***Chrysochromulina simplex*** the appendages are inserted on the ventral, concave side and

transverse sections indicate that the nucleus lies on the convex dorsal side of the cell. This is in agreement with the cell and appendage orientation first described by Beech & Wetherbee (1988) and subsequently applied to all flagellar studies on the prymnesiophycean group. The root and accessory band numbering follow that of Green & Hori (1990). For comparative purposes within the genus ***Chrysochromulina***, it should be noted that the structures described in this paper correspond to those of *C. acantha* (Gregson *et al.* 1993) as follows : DB (distal band) = df; AB1 (accessory band 1) = hf1; AB2 (accessory band 2) = hf3; AB3 (accessory band 3) = hf2; R1 = R1; R2 = not present in *C. acantha*; R3 = R2; R4 = R3; PB (proximal band) = pf. In the present study, the large accessory fibre (af) of Gregson *et al.* (1993) is considered to be an extension of the distal band and not a separate structure.

Abbreviations used in the text and in the figures

AB1, AB2, AB3 - accessory bands; C - chloroplast; D - dorsal side of the cell; DB - distal band; F - food vacuole; G - Golgi body; H - haptonema; L - left flagellum or basal body; M - mitochondrion; N - nucleus; P - pyrenoid; PB - proximal band; R - right flagellum or basal body; R1, R2, R3, R4 - microtubular roots; V - ventral side of the cell.

OBSERVATIONS

Light microscopy

The small cells (3.5 μm x 3.5 μm) - 3.9 μm x 3.8 μm - 5.1 μm x 4.2 μm - (6.9 μm x 5.4 μm) are saddle-shaped, but often become spherical when fixed or when observed for more than several minutes beneath a coverslip (Figs 1-10). When viewed laterally the cells are roughly oval in shape (Fig. 2), but have an obliquely truncate anterior end when seen from the dorsal or ventral sides (Figs 1, 3, 5, 6). The three appendages are inserted into the concave, ventral side of the cell, in a median to posterior position (Figs 1, 2, 10). The two heterodynamic flagella (9.3 μm) - 11 μm - 16 μm - (19.4 μm) are usually equal in length (Fig. 1), but when unequal the difference between them never exceeds 3.4 μm, although the lopsided posture of the

cell exaggerates the apparent difference. (Fig. 3). The long, coiling haptonema varies in length with the age of the cell, rarely being less than 28 μm twelve hours after cell division. The maximum recorded haptonematal length is 79 μm.

There are two pale golden, parietal chloroplasts in each cell, with oil droplets / leucosin granules evident in some older cells (Fig. 8). Only motile cells have been observed in culture.

During slow gliding, the haptonema is extended obliquely forwards with the flagella undulating behind (Figs 1-3). The cell body rotates slowly around the long axis of the cell. Occassional spurts of speed accompanied by vigorous cell body rotation, are due to a more rapid beating of one flagellum, with the second flagellum held stiffly behind the cell (Fig. 4). Infrequently, cells may swim with the posterior end of the cell forwards and the extended haptonema trailing behind the cell which exhibits wide looping movements around the direction of forward swimming. The cells may swim with the haptonema extended for a few minutes, then change direction abruptly with a concurrent coiling of the haptonema. Gliding may then be resumed once the haptonema is unfurled (Fig. 5) or the cells may dart for a short distance with the haptonema tightly coiled into the groove of the saddle on the ventral cell surface (Fig. 7). During less rapid swimming the haptonema is partially coiled and may be held forwards in the direction of swimming (Fig. 9) or trailed posteriorly (Fig. 6). During darting and slower swimming, the cell body rotates almost strobe-like around the axis of forward movement.

The position and action of the appendages during the fastest swimming behaviour cannot be elucidated, but the cells are seen to flick almost instantaneously along a curved pathway for a short distance, in a manner reminiscent of a ***Nephroselmis*** Stein cell. When attached to a slide, the cells spiral around the point of attachment with the flagella undulating or quivering. Flagellar movement becomes increasingly heterodynamic and asynchronous, as cells attempt to detach knotted and partially coiled haptonemata (Fig. 8).

Electron microscopy

The single scale type is a flattened, rimless plate that is circular to slightly ovate in shape. The scales vary in size from a minimum diameter of 0.34 μm to a maximum of 0.51 μm, although the patterning of the scales is identical in all four isolates. The smooth distal surface is

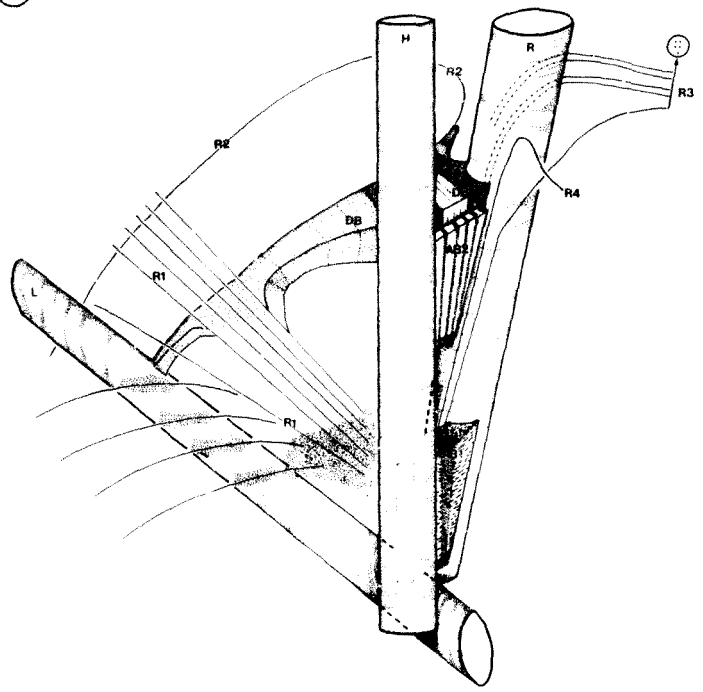

Fig. 21. Schematic reconstruction of the flagellar apparatus of *Chrysochromulina simplex*.
The proximal band (PB) and the AB3 are not visible in this view. Not drawn to scale.

interrupted by random concentric markings (Fig. 11). On the proximal surface there are 21 - 26 straight, radiating ridges that overlie very fine, concentrically-arranged fibrils (Figs 12, 13). Centrally there is a faint cross whose arms are excentrically positioned relative to the ridges that divide the scale into quarters (Figs 12, 13). The scales usually detach during fixation, but seem to occur most commonly in two layers around the cell, although up to six layers have been observed (Fig. 14).

The appendages are ventrally inserted between the two parietal chloroplasts and are laterally displaced towards the left chloroplast (Fig. 15). The Golgi body, in which the scales are produced (Figs 18, 46), is polarised around the appendage bases, with the forming face on the left side of the cell and the maturing face opening into the area between the right flagellum and the right chloroplast (Fig. 15). The ventral inner face of each chloroplast is lined by a mitochondrial lobe in the region of appendage insertion (Figs 15, 16). The nucleus lies dorsally in a median to anterior position, connecting the ends of the two chloroplasts (Figs 16, 17, 19). The nuclear envelope and the chloroplast endoplasmic reticulum are confluent, and both also contribute to the forming face of the dictyosome (Figs 17, 18). Each chloroplast has a well developed periplastidal reticulum lining the central portion of the inner face (Fig. 18), as well as an embedded pyrenoid traversed by single, inflated thylakoids (Figs 15, 18, 20).

Material from the enrichment that provided isolate U16 indicates that cells of ***C. simplex*** are capable of phagotrophy, due to the presence of a large food vacuole (Fig. 20).

The flagella and haptonema are highly angled in their insertion and although the relative positions of the appendage bases is slightly variable, the left basal body typically lies almost perpendicular to the paths of the haptonema and right basal body (Figs 21-32). This asymmetry makes a precise interpretation of the flagellar apparatus difficult, but a schematic reconstruction is presented in Fig. 21. Of the three appendages, the haptonema is situated most dorsally with the right basal body lying most ventrally. Between them (ventral to the haptonema, dorsal to the right basal body) is the left basal body that originates on the right side of the haptonema base and then slants across the cell body to emerge on the left side of the cell. The left basal body is marginally longer than the right (a difference of 15 - 90 nm) and therefore penetrates more

deeply into the cell body. There are two microtubular roots associated with the left, ventral side of the cell (R1, R2) and two roots associated with the right, dorsal side of the cell (R3, R4).

There are two main distal connectives and three proximal connecting bands which hold the flagellar apparatus together and maintain its position in the cell. The large, centrally-striated distal band (DB) connects all three appendages: the dorsal side of the left basal body, the ventral side of the haptonematal base and the left side of the right basal body (Figs 21, 23, 27, 29, 40). A tongue-like portion of the DB extends to the microtubules of the R1, or broad root, on the left side of the cell (Figs 21, 27, 33, 34, 36, 46). On the ventral side of the cell the DB produces a short, narrowly-tapered, striated extension that overlies the emergence of the R2 root from beneath the DB (Figs 21, 47, 49). The other distal connective is an accessory band, AB2, that links the haptonema with the right basal body (Figs 21, 24, 30, 37, 39, 47). The striated AB2 is extremely narrow in transverse section (Fig. 37), but appears as a well developed fibrous sheet when sectioned along the long axes of the haptonema/right flagellar base (Fig. 30).

Proximally there are two accessory bands that connect the haptonematal base with each flagellar base: the haptonematal - right basal body connection or AB1 (Figs 21, 26, 30, 36); the haptonematal - left basal body linkage, the AB3 (Figs 30, 35). The AB1 is obviously striated and in transverse section is seen to taper from a median swelling to a delicate tail that attaches to two adjacent triplets of the right basal body (Fig. 36). Slightly tangential sections indicate that this accessory band is also linked to the other flagellar base, as it passes dorsally over the left basal body to approach the haptonema (Figs 21, 26, 30-32).

The AB3 is not obviously striated and in transverse section is seen to attach to two triplets of the left basal body (Fig. 30).

A proximal band, PB, links the bases of the two flagella (Figs 25, 29, 31, 32). It appears to have two components but it has proved impossible to determine whether or not these components are actually separate structures, so they will both be considered as part of one PB. The more ventrally positioned portion of the PB is a large, loosely fibrous sheet with two striations (Figs 25, 29, 32). The dorsal portion of the PB is a dense, shorter and more compact connective (Figs 25, 29, 31).

The R1 microtubular root originates between the bases of the haptonema and the left flagellum (Figs 21, 25, 38), becoming associated with the mitochondrial lobe that underlies the left chloroplast (Figs 22-25, 27, 29, 33, 38, 39, 46). As the R1 moves distally to approach the region of appendage insertion, four microtubules splay back ventrally past the left basal body from the main part of the root (Figs 21-25, 39-41, 43). These splayed microtubules, which are attached to the left basal body by amorphous material (Figs 21, 24, 25, 43), lie in a thin band of cytoplasm that extends through the peripheral endoplasmic reticulum to attach to the most ventral portion of the plasmalemma (Figs 28, 39, 40, 42). The R1 microtubules that constitute the main root component form a sheet along the inner face of the mitochondrion (Figs 22, 29, 41) and subsequently fan out over the adjacent chloroplast for a short distance (Figs 38, 43, 45). These microtubules are linearly arranged in a closely-packed group of 5, with a sixth microtubule being slightly displaced away to the left (Figs 21, 22, 41). Immediately below the level of left flagellar insertion the five microtubules lie parallel to the left basal body, with the sixth microtubule beginning to veer ventrally (Fig. 22).

The R2 consists of a single microtubule that arises beneath the distal band between the haptonema and the right basal body (Figs 21-23, 47, 49). It then curves sharply backwards towards the ventral side of the cell, passing the right side of the left basal body in the plane perpendicular to the long axis of this basal body (Figs 22, 31, 41, 44). This short root terminates at the ventral plasmalemma.

The R3 root is bifurcate around the base of the right basal body (Figs 21, 47, 48) and so consists of two parts : a single microtubule that begins on the ventral side of the AB1 (Fig. 36), moves distally on a pathway roughly parallel to the dorsal side of the right basal body (Figs 26, 28, 37, 46), then joins the other R3 component to extend to the dorsal side of the cell beneath the peripheral endoplasmic reticulum (Figs 47, 48, 50); the second part of the R3 consists of two pairs of tiered microtubules, initially one pair (Figs 31, 33), that pass up the ventral side of the right basal body and then veer towards the dorsal side of the cell (Figs 26, 28, 47-50). The entire R3 microtubular complement (2/2 + 1) travels to the dorsal side of the cell beneath the plasmalemma and above the right chloroplast (Figs 46, 47).

The R4 is a single stranded microtubular root that arises in a similar position to the single microtubule of the R3, that is ventral to the AB1 between the right basal body and the

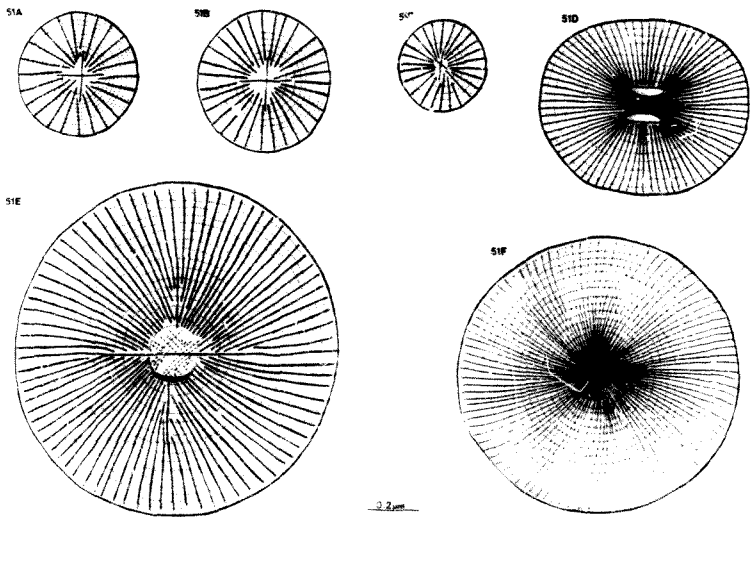

Fig. 51. Proximal patterning of scale types previously assigned to *Chrysochromulina simplex*. A - present study; B - Moestrup 1979, Fig.26; C - Estep *et al.* 1984, Fig.15; D - Estep *et al.* 1984, Fig.16; E - Hallegraeff 1983, Fig.18a; F - Estep *et al.* 1984, Fig.18. (Figs 55B - 55F copied from the published micrographs).

haptonematal base (Fig. 36). It also lies parallel to the dorsal side of the right basal body to which it appears attached in the region of the distal connectives (Fig. 37). This short root then slants towards the haptonema and extends towards the dorsal side of the cell until it reaches the Golgi body (Figs 25, 46, 48).

DISCUSSION

A comparison of the various scale types previously assigned to the species ***C. simplex*** are presented in Fig. 51. The scales illustrated by Moestup (19[illegible] are clearly the same as those described in this paper, despite being slightly larger (up to 0.53 μm with 36 radiating ridges). Those of Hallegraeff (1983) are not identical in that the size difference is rather large and consequently the number of radiating ridges on the proximal surface more than doubled (80 in Fig. 18a, 75-80 in Fig. 18b). This size difference (between the African isolates and the Australian scales) is double that described for the plate scales of ***C. ericina*** (Leadbeater 1972) and is therefore deemed unacceptable. More importantly, the central area of the Australian scales is raised with a centric cross on the proximal surface and a fibrillar grid-like patterning on the distal surface. This scale type is therefore not included in the range of scale variability for the species. Fig. 15 of Estep *et al.* (1984) contains scales that resemble those of Moestrup (1979) and the present study in terms of both size and patterning (0.3 μm with 27 radiating ridges and an excentric cross), but it is not possible to see the proximal central patterning of the larger scales from this cell. The distal patterning consists of faint concentric markings and the variation in scale size (from 0.3 μm to 0.58 μm) does not far exceed that found between the four isolates of the present study and the scales found by Moestrup (1979), so this micrograph (Estep *et al.* 1984, Fig. 15) should be retained as the holotype. Figs 16 and 18 (Estep *et al.* 1984) represent two other undescribed species.

Close similarities in the flagellar apparatus of the three described species, ***Chrysochromulina***, *C. **apheles*** (Moestrup & Thomsen 1986), *C. **acantha*** (Gregson *et al.* 1993) and ***C. simplex***

(present study) are to be expected, as all three species have saddle-shaped cells with long, coiling haptonemata. This is in contrast to the ***Chrysochromulina*** species with shorter haptonemata inserted into cells that are not typically saddle-shaped, e.g. ***C. brevifilum*** Parke et Manton and the 'eyelash' ***Chrysochromulina***, which tend to also resemble species of ***Prymnesium*** Conrad in their flagellar / haptonematal arrangement (pers. obs.).

C. simplex appears to be intermediate between ***C. apheles*** and ***C. acantha***, in that it has an R1 of 6 + 4 compared with the 4(5) + 2 of ***C. apheles*** (Moestrup & Thomsen 1986) and the 16 + 4 of ***C. acantha*** (Gregson *et al.* 1993); it has a single stranded R2 like ***C. apheles*** while ***C. acantha*** lacks this root altogether; the insertion angles of the appendages are more similar between ***C. simplex*** and ***C. apheles*** than either is to ***C. acantha***; ***C. simplex*** and ***C. apheles*** apparently share a more simple, but very similar system of connectives relative to ***C. acantha***; neither ***C. simplex*** nor ***C. apheles*** has microtubules running parallel to the termination of the R1 microtubules; ***C. simplex*** has an R3 of 2/2+1 like ***C. acantha*** but ***C. apheles*** has an R2 of 1/2+1; both ***C. simplex*** and ***C. acantha*** have a short root (here designated the R4) extending dorsally between the haptonema and the right basal body, while ***C. apheles*** lacks this root.

This study confirms some of the observations made by Gregson *et al.* (1993) on the flagellar apparatus of Plymouth 384 (assuming that this culture corresponds to ***C. simplex*** as defined here), in that the R1 has 6 + 4 microtubules, there is a tiered R3 (but it is bifurcate with a 2/2+1 composition) and there is a striated accessory band between the right basal body and the haptonema base (although there is also another accessory band between these two appendages, as well as several other connecting fibres). In the extracted apparatus illustrated by Gregson *et al.* (1993, Fig. 28) it is possible that the R2 is visible as a short microtubular profile between the distal ends of the haptonema and the right basal body.

The flagellar apparatus of the ***Chrysochromulina*** species has already been succinctly compared with those of other prymnesiophycean cells (see Gregson *et al.* 1993). There is one other point of interest, which is that the splayed R1 microtubules of ***C. simplex*** seem homologous with the cytoplasmic tongue / contractile root of some coccolith-bearing cells (Beech & Wetherbee 1988, Fresnel & Billard 1991): the R1-derived microtubules extend ventrally, enclosed in a thin band of cytoplasm. Obviously the structure is extremely reduced in the ***Chrysochromulina*** species,

as is the associated fibrous root which is probably confined to the amorphous attachment material between the left basal body and the R1-microtubules.

Emended description of *Chrysochromulina simplex* Estep *et al.*

Saddle-shaped cells (3.5 μm - 6.9 μm) with two heterodynamic flagella (9.3 μm - 19.4 μm) and a long, coiling haptonema (max. 79 μm) inserted into the ventral side of the cell. Cells with a single type of plate-like scale (0.3 - 0.58 μm) with faint concentric markings on the distal face and 21 - 36 radiating ridges, fine concentric fibrils and a central cross on the proximal face. The arms of the proximal cross are excentrically positioned relative to the radiating ridges.

ACKNOWLEDGEMENTS

This research was funded by the University of the Witwatersrand and the Foundation for Research and Development of the CSIR. M.B. would like to thank Julia Birkhead and Niki Papadopoulos for their help and for providing some of the water samples.

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Figs 1 - 14. Light microscopy and scales of *Chrysochromulina simplex*.

Fig. 1. Ventral view of gliding cell with extended haptonema. Arrowheads indicate points of flagellar insertion.

Fig. 2. Lateral view of gliding cell showing median-posterior appendage insertion.

Fig. 3. Dorsal view of cell with heterodynamic flagella and extended haptonema.

Fig. 4. Lateral view of rapidly twirling cell with extended haptonema and one trailing flagellum.

Fig. 5. Stationary, rounded cell with partially uncoiled haptonema.

Fig. 6. Saddle-shaped, actively swimming cell trailing its loosely coiled haptonema.

Fig. 7. Rapidly swimming cell viewed from the dorsal surface so that the tightly coiled haptonema is not visible.

Fig. 8. Attached cell with tangled haptonema.

Fig. 9. Actively swimming cell with loosely coiled haptonema extended forwards in the direction of cell movement.

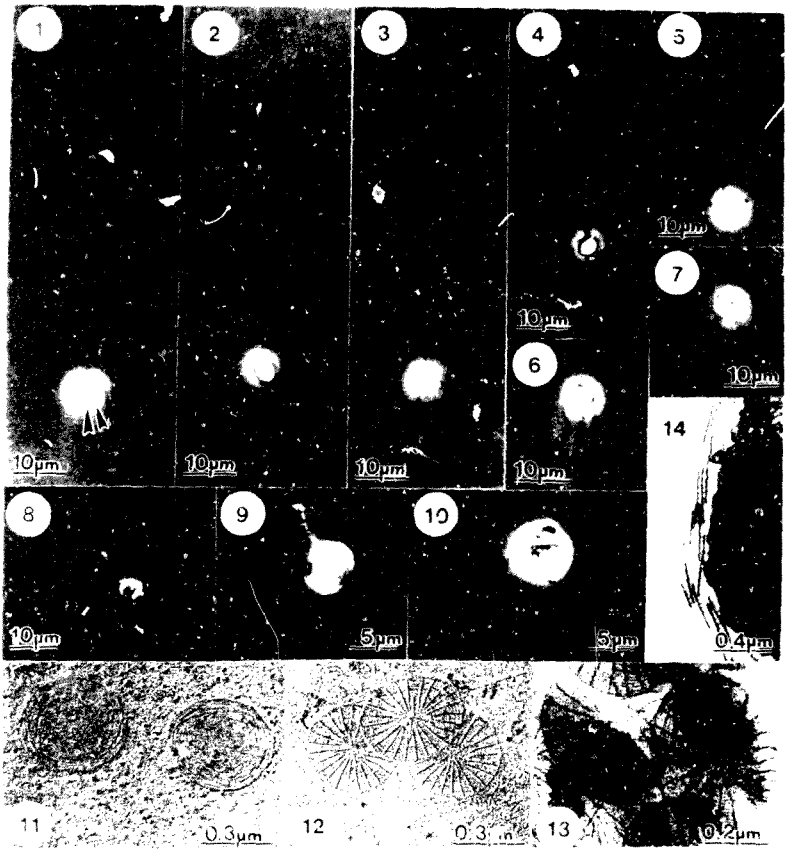
Fig. 10. Rapidly swimming cell showing a tightly coiled haptonema tucked into the concave face of the cell.

Fig. 11. Distal surface of single scale type. Shadowcast preparation.

Fig. 12. Proximal surface of scales. Shadowcast preparation.

Fig. 13. Sectioned scales showing radiating ridges, excentric cross and concentric fibrils of the proximal scale face.

Fig. 14. Layering of scales around a sectioned cell.


1
10μm
2
10μm
3
10μm
4
10μm
5
10μm
7
10μm
6
14
0.4μm
8
10μm
9
5μm
10
5μm
11
0.3μm
12
0.3μm
13
0.2μm

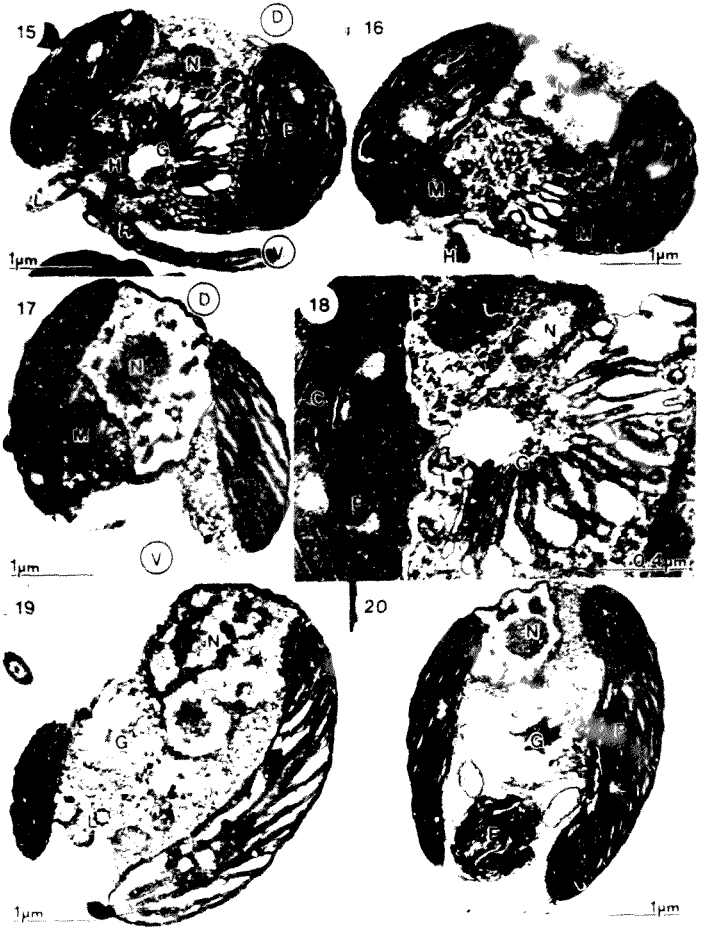
Figs 15 - 20. General ultrastructure of *Chrysochromulina simplex*.

Figs 15-17. Transverse serial sections (nos. 1, 3, 7) sloping from the ventral up to the dorsal cell side, to show the ventrally inserted appendages, the polarised Golgi body and the nucleus situated towards the anterior, dorsal, convex cell surface.

Fig. 18. The forming face of the dictyosome is associated with the chloroplast endoplasmic reticulum (small arrowheads) and the outer membrane of the nucleus (arrow). Note the scale (large arrowhead) inside the Golgi cisterna.

Fig. 19. Longitudinal section through a cell, angled from ventral to dorsal surfaces.

Fig. 20. Longitudinal section through a cell with a food vacuole (F), from an original water sample.


15
D
N
G
F
V
1μm
16
M
H
1μm
17
D
N
M
V
1μm
18
C
F
N
G
0.4μm
19
N
G
C
1μm
20
N
G
F
1μm

Figs 22 - 28. Flagellar apparatus of *Chrysochromulina simplex*.

Figs 22-26. Serial sections (nos 1, 2, 4, 5, 6) transverse to the long axis of the left basal body (L), from distal to proximal levels.

Fig. 22. The R1 of 5 aligned microtubules with the sixth veering ventrally (small arrow). Note the splayed R1 microtubule (large arrow) and the R2 (arrowheads) curving past the right side of the left basal body.

Fig. 23. A central striation through the DB as it lies between the three appendages. Note the splayed microtubule (arrow).

Fig. 24. Dense material (arrow) lies between the left basal body and the splayed microtubules of the R1.

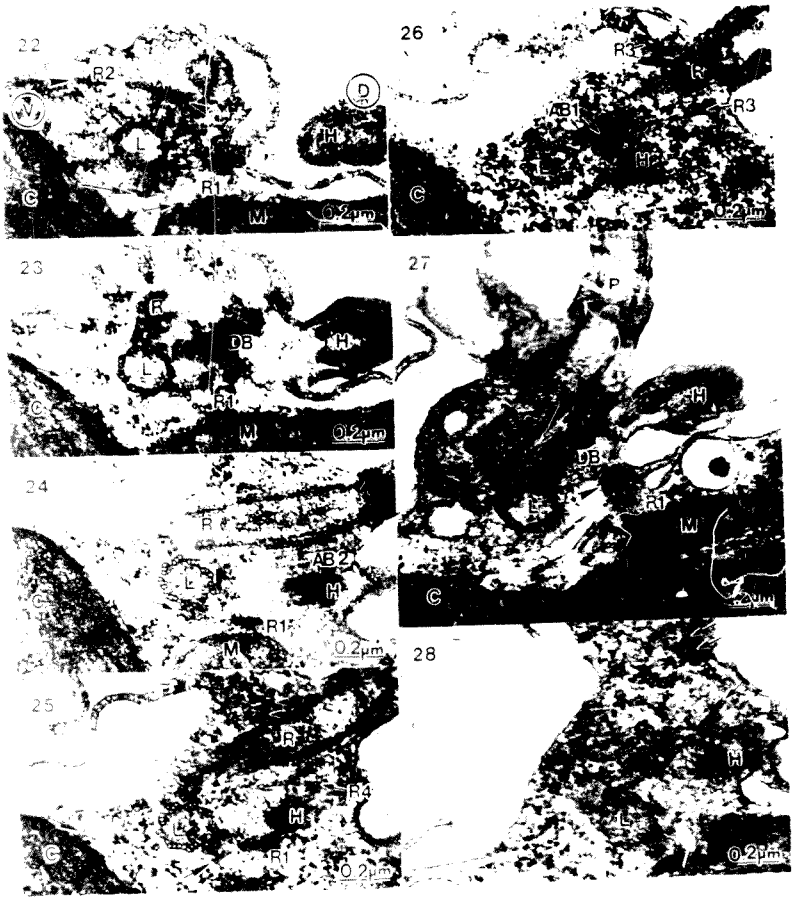
Fig. 25. The two basal bodies are joined by a PB (* = larger striated component, + = dense, compact component). Microtubules from three roots are present: the R1 and its splayed microtubules (large arrowheads); the R4 as it curves towards the dorsal side of the haptonema; two of the R3 microtubules (small arrowheads) on the right side of the right basal body.

Fig. 26. The two components of the R3 on either side of the right basal body, with the single R3 microtubule arising from the region of the AB1.

Figs 27, 28. Serial sections (nos 1, 5) almost transverse to the long axis of the left basal body.

Fig. 27. The DB connects all three appendages and has a tongue-like extension (arrowhead) towards the R1.

Fig. 28. Three of the R3 microtubules (arrows) and one of the splayed R1 microtubules (arrowhead) moving ventrally in a narrow band of cytoplasm.


22
R2
D
M
H
L
C
R1
M
0.2μm
26
R3
R
R3
AB1
H2
L
C
0.2μm
23
R
DB
H
L
R1
C
M
0.2μm
27
P
H
DB
R1
M
C
0.2μm
24
R
AB2
H
L
C
R1
M
0.2μm
25
R
R4
H
L
C
R1
0.2μm
28
H
L
0.2μm

Figs 29 - 38. Connecting fibres and roots of *Chrysochromulina simplex*.

Figs 29, 30. Serial sections (nos 1, 4) almost perpendicular to the long axis of the left basal body, but sloping up from ventral to dorsal cell sides.

Fig. 29. The PB (large, fibrillar component = small arrow; small, dense component = large arrow) join the bases of the flagella.

Fig. 30. Two accessory bands, the proximal AB1 (→) and the distal AB2 (*), connect the right basal body and the haptonema. The AB3 (small arrows) links the haptonema and the left basal body.

Fig. 31. A glancing section through the single-stranded R2, the initial pair of R3 microtubules, the ventrally situated PB and the base of the AB1.

Fig. 32. A transverse section through the right basal body with the left basal body almost longitudinally sectioned. They are connected by the striated PB.

Fig. 33. A tongue-like fibrous extension (arrow) from the PB near the right basal body, to the R1.

Fig. 34. A glancing section showing the DB - R1 extension (arrow) and a small portion of the R2 (arrowheads) just beneath the DB.

Fig. 35. The AB3 that links the proximal portion of the left basal body to the haptonematal base.

Figs 36, 37. Serial sections (nos 1, 3) from proximal to distal levels, transverse to the right basal body.

Fig. 36. The single R4 microtubule (small arrow) and part of the R3 (arrowhead) lie immediately ventral to the proximal accessory band.

Fig. 37. Both the R4 (small arrow) and the one R3 microtubule (arrowhead) lie dorsal to the narrow, striated distal accessory band, AB2.

Fig. 38. A glancing section showing the R1 microtubules (arrowheads) fanning out over a mitochondrial profile towards the left chloroplast, from between the left basal body and the haptonematal base.


29
R
L
DB
H
R1
M
0.2μm
30
R
L
H
0.2μm
31
L
PB
AB1
R
R2
R3
0.2μm
32
M
R1
H
L
PB
AB1
R
0.2μm
33
C
DB
R1
R3
R1
M
H
0.2μm
34
L
R
DB
AB2
H
R1
0.2μm
35
L
R1
AB3
H
0.2μm
36
DB
H
0.2μm
37
DB
R
H
AB1
DB
R
H
AB2
0.2μm
38
H
L
0.2μm
0.2μm

Figs 39 - 45. Microtubular roots of *Chrysochromulina simplex*.

Figs 39, 40. Consecutive serial sections transverse to the left basal body to show the R1 microtubules (arrows) splaying past the left basal body to extend ventrally in a narrow band of cytoplasm.

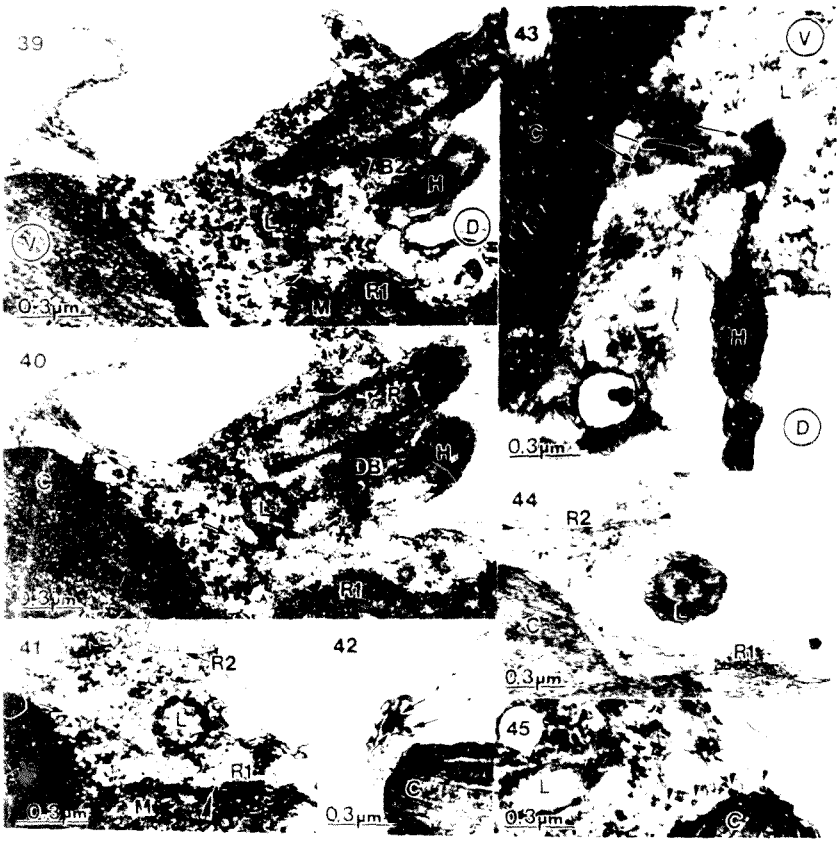
Fig. 41. The sixth microtubule (arrowhead) of the R1 is displaced from the other five as it begins to veer ventrally, at the level of left basal body insertion. Note the splayed R1 microtubule (arrow).

Fig. 42. Three R1 microtubules (arrows) in the cytoplasmic band attached to the ventral part of the plasmalemma.

Fig. 43. Four R1 microtubules (arrows) splay sharply ventrally from amorphous material between the haptonematal and left flagellar bases, while the remaining six R1 microtubules (arrowheads) curve towards the left chloroplast.

Fig. 44. The R2 root extends ventrally past the right side of the left basal body.

Fig. 45. R1 microtubules (arrowheads) forming out over the left chloroplast.


39
AB2
H
L
D
V
R1
M
0.3µm
40
R
H
DB
C
L
R1
0.3µm
41
R2
L
R1
M
0.3µm
42
C
0.3µm
43
V
L
C
H
D
0.3µm
44
R2
L
C
R1
0.3µm
45
L
C
0.3µm

Figs 46 - 50. The R3 and R4 roots of *Chrysochromulina simplex*.

Figs 46, 47. Consecutive serial sections tangential to all three appendages.

Fig. 46. Three parallel microtubules of the tiered R3 pass between the scale-containing Golgi vesicle and the right chloroplast, approaching the ventrally-situated appendage bases. The R4 slants towards the haptonema, dorsal to the AB2. Note the DB - R1 extension.

Fig. 47. The components of the R3 bifurcate on either side of the right basal body, having traversed the breadth of the cell immediately beneath the peripheral endoplasmic reticulum. A tapering extension from the DB can be seen overlying the single microtubule of the R2 (arrowhead).

Figs 48, 49. Consecutive serial sections angled to the right basal body and the haptonematal base.

Fig. 48. The R4 (arrowheads) extends dorsally from between the right basal body and the haptonematal base, terminating abruptly at the edge of the Golgi body. The R3 is bifurcate around the right basal body.

Fig. 49. The R2 passes from beneath the DB, between the right basal body and the haptonematal base, extending ventrally to the right side of the left basal body.

Fig. 50. Transverse section through the R3 microtubules (arrowheads) with two pairs and a single microtuoule.


46
L
R
C
DB
R1
H
R4
M
G
C
R3
0·4μm
47
R2
L
R
C
DB
H
AB2
R3
N
48
DB
R
H
R4
G
R3
0·3μm
49
R2
L
DB
R
H
R3
0·3μm
50
R
R3
R3
0·3μm
0·4μm

CHAPTER 7

GENERAL DISCUSSION AND CONCLUSION

This study has confirmed that there is certainly overlap between the genera ***Chrysochromulina*** and ***Prymnesium*** in their present delineation. Until the description of ***P. nemamethecum***, ***Prymnesium*** was a cohesive grouping of cells with subapical insertion of the appendages, a short haptonema and two types of scales, one a plate scale and the other with a species-specific distal ornamentation that is most elaborate in ***P. calathiferum***. These cells are extremely similar to the motile cells of ***Platychrysis*** (differing only in the subapical appendage insertion as opposed to apical insertion and possibly in ultrastructural features of the flagellar apparatus) which means that the existance of the two genera is, to a large extent, reliant on knowledge of the life history. This highlights the importance of basing species descriptions on clonal isolates, although given the fact that geographical and genotypic scale variation exists, it is preferable to also have access to wild material from a number of localities. This descriptive approach, which combines two sources of information, is the most preferable but is often thwarted because of the difficulty in culturing some prymnesialean species. In addition, the fact that a clonal isolate has been obtained does not ensure that the life cycle will be elucidated at all, and in fact may be as misleading as the observation of individual cells from mixed nanoplankton hauls. This is because it is tempting to accept that whatever life history stage has been obtained in culture, is the dominant one, but this may not be so. For example, the most commonly encountered form of ***Phaeocystis*** in natural populations is the mucilagenous colonial form, but when this is cultured it invariably fragments and the culture transforms into a self-sustaining culture of one of the two forms of motile cell (Parke *et al.* 1971). (If the motile cell form obtained lacks the three 'H'-like filaments, the cells could easily be identified as a ***Platychrysis*** motile cell).

However, in all three descriptions of ***Platychrysis*** from cultured material (Chrétiennot 1973; Gayral and Fresnel 1983a), the dominant amoeboid/palmelloid benthic phase is present, with the transitory production of motile cells being readily prompted by changing the culture medium. These motile cells always settle to produce the non-motile phase again, so it was on this basis that the species of ***Prymnesium*** described in this study was assigned to its given genus (as it did not seem to have any sort of viable non-motile phase that could re-establish the presumably dominant, motile stage). At the light microscope level it resembled other ***Prymnesium*** species, and was therefore placed in this genus regardless of its ultrastructural details, as generic identification at the light microscope level is still the most practical approach at present. Obviously electron microscopy is necessary for species identification, but the presence of a unique scale complement of three scale types in ***P. nemamethecum*** was, in itself, not considered a problem in terms of generic inclusion. However, this feature, when combined with details of the flagellar apparatus, suggested some disparity between ***P. nemamethecum*** and at least two other species of ***Prymnesium*** (***P. parvum*** and ***P. patelliferum***). As the extent of intrageneric variation that may exist in the prymnesiophycidean flagellar / haptonematal apparatus has not been determined yet, it was deemed premature to create a new genus before other species that may belong to this new genus (i.e. certain species of ***Chrysochromulina***) had been investigated ultrastructurally in order to determine the full range of variation that would need to be incorporated. Perhaps this caution will merely compound the taxonomic problems within the Prymnesiaceae, as the same criterion (for more information) was initially stated by Parke *et al.* (1955, p.580) " We therefore propose to describe most of our species under the one genus ***Chrysochromulina***, and to amend the generic diagnosis to incorporate the new knowledge at the end of the series." Unfortunately, the number of species in this one genus proliferated so rapidly, each species exhibiting yet more extraordinary variation, that an amended generic description has never been subsequently provided. Little did these phycologists realise what sort of taxonomic triffid would result from their pioneering research!

Having placed something of an ultrastructural incongruity within the genus ***Prymnesium*** (see chapters 2 and 3), representatives of the closely related genus ***Chrysochromulina*** were

investigated in order to try to elucidate groupings both between the two genera and within this obviously paraphyletic genus. It was essential to study ***Chrysochromulina*** cells with different shapes and therefore different regions of appendage insertion, from slightly ***Prymnesium***-like cells (***Chrysochromulina*** sp. = 'eyelash' ***Chrysochromulina***, see chapter 3), to rounded cells with apical appendage insertion (***C. brevifilum***, see chapter 4), to a 'typical' saddle-shaped cell with ventral appendage insertion (***C. simplex***, see chapter 5).

On the basis of these observations, and also considering the ***Chrysochromulina*** species descriptions which are often very limited in terms of comparative information, it may be possible to speculate on the existance of a number of apparently natural groupings within the genus. Species for which too little information is provided in the type description are not included in this discussion, while in some cases, details that are not mentioned in the descriptions are assumed after observation of the published micrographs. As mentioned in the introduction to this thesis, the use of scale types alone cannot be used to circumscribe a group, a point well illustrated in the following example. Cells that bear both plate scales and larger, open-ended, cylindrical scales (***C. brachcylindra***, ***C. cyathophora***, ***C. ericina***, ***C. mactra***, ***C. megacylindra***, ***C. microcylindra***, ***C. pachycylindra***) are traditionally considered to form a composite group (e.g. see Manton *et al.* 1981). However, although there is no information pertaining to their flagellar apparatus, there is variation in the shape of the cells from conical with a flattened anterior end (***C. ericina***, ***C. megacylindra***) to subspherical (***C. brachycylindra***, ***C. cyathophora***, ***C. microcylindra***) to globose (***C. mactra***, ***C. pachycylindra***). Haptonematal coiling is present in all species except ***C. megacylindra***, and the haptonema may be five times longer than the flagella (***C. ericina***), equal to flagellar length (***C. brachcylindra***, ***C. mactra***, ***C. microcylindra***) or shorter than the flagella (***C. cyathophora***, ***C. megacylindra***, ***C. pachycylindra***). It is therefore not possible to accept scale features as the unifying concept of a group, especially considering that in ***C. polylepis*** scale morphology varies between two different motile forms (Edvardsen and Paasche 1992). (This validates one of the reasons why ***P. nemamethecum*** was included within the genus ***Prymnesium***).

The natural groupings that can be described are as follows:

1. The saddle-shaped cells with ventral insertion of the long, coiling haptonema and two equal-subequal flagella, a morphology that allegedly typifies the genus. Cells may be freshwater or marine, and may have a single type of plate scale (*C. parva*, *C. simplex*): two types of plate scale, one of which has a raised rim (*C. apheles*); two scale types, one of which is plate like, the other which is cup-shaped (*C. camella*, *C. campanulifera*, *C. cymbium*, *C. leadbeaterii*, *C. strobilus*); two scale types, one of which is a plate scale, the other being a small spined scale in which the length of the spine is approximately equal to scale diameter (*C. acantha*, *C. alifera*, *C. ephippium*). The flagellar apparatus of these cells is the simplest (or most reduced) among the Prymnesiophycidae, and has been reconstructed for three species *C. apheles* (Moestrup and Thomsen 1986) *C. acantha* (Gregson *et al.* 1993a) and *C. simplex* (see chapter 5; Gregson *et al.* 1993a), with superficial observations on *C. parva* (Moestrup and Thomsen 1986) and *C. camella* (pers. obs.) being consistent with these complete descriptions. *C. apheles* and *C. simplex* are more similar to each other than to *C. acantha* in terms of cell size, relative length ratios of appendages, scale complement, swimming behaviour and flagellar apparatus, while *C. acantha* is a larger cell with a shorter haptonema, more complex scales and a more complex flagellar apparatus. If the genus is ever amended, then this group of species should constitute the genus *Chrysochromulina* Lackey.

There are numerous species with coiling haptonemata longer than the flagella, variably inserted into cells that may be one of a number of described shapes, but because these cells have coiled haptonemata and usually an elaborate scale complement (with the exceptions of *C. chiton* with only two types of plate scales and *C. kappa* with plate scales and only a small spined scale to distinguish it), the describing authors have had no problem in the generic or specific placing of these cells, and in most cases there are very few, if any, other distinguishing details that could be used to naturally separate or group these cells.

2. The ovate/strawberry-shaped cells of *C. brevifilum* could probably be grouped with other *Chrysochromulina* species which are subspherical and which often have a slightly truncate anterior end. These cells have two equal-unequal flagella and a haptonema that is shorter than

the flagellar length, with the appendages being apically inserted (but not in a depression). The reduced length of the haptonema has resulted in the coiling ability being most frequently described as 'limited' and haptonematal gyres are rarely seen in fixed wholemounts. The scale complements are typically plate-like (*C. adriatica*, *C. bergenensis*, *C. herdlensis*, *C. inornamenta*, *C. laurentiana*, *C. minor*) with some species also bearing a short spined scale (*C. brevifilum*, *C. breviturrita*). Where described, swimming behaviour is similar among species, but no flagellar apparatus comparisons can be made. Again, both freshwater and marine species constitute the group. The flagellar apparatus of *C. brevifilum* is probably more similar to that of *C. simplex* than to any other described prymnesialean cell, but could be construed as being equally similar, for different reasons, to both *Prymnesium patelliferum* and *C. acantha*. This feature certainly does not provide an obvious distinction between species of the two genera, nor does scale complement for this particular grouping, which leaves apical appendage insertion and swimming behaviour as the main generic distinctions.

3. Among the *Chrysochromulina* cells that have very short, non-coiling haptonemata, there are two groups. One group consists of fusiform cells with apical appendage insertion, non-coiling haptonemata and spined scales so large that they are visible with the light microscope. Crystalline compound roots have been observed in some of these species by Kawachi (M. Kawachi, pers. comm). This group consists of *C. mantoniae*, *C. parkeae* and *C. spinifera*. The second group consists of cells with subapical appendage insertion into a ventral groove, a short haptonema that does coil although this is only infrequently observed, and scales that are basically flattened but with beautiful distal elaborations (*C. polylepis*, *Chrysochromulina* 'eyelash' sp.). These two species are of most interest because they resemble *Prymnesium nemamethecum* in general morphology and region of appendage insertion, swimming behaviour (rarely darting with the posterior pole directed forwards and the appendages trailing, as is typical of so many *Chrysochromulina* species) and lack of elaborate spine scales. In *C. polylepis* it is probable that the narrow, elliptical plate scales may occur in a group near the flagellar pole (Manton and Parke 1962), while preliminary observations on *Chrysochromulina* sp. indicate that this species also has small plate scales only around the insertion region. In addition, the

flagellar apparatus of ***Chrysochromulina*** sp. is also very similar to that of ***P. nemamethecum***, as is the transition region. In ***Chrysochromulina***, compound crystalline roots seem to be associated with robust cells that are not spherical and which bear very short haptonemata (for example, two compound roots have been demonstrated in ***C. parkeae*** by Kawachi, pers. comm). In this regard it is almost certain that ***C. polylepis*** will be found to have a compound root. Whether or not the two different motile stages of ***C. polylepis*** have the same flagellar apparatus ultrastructure would also assist in determining what the intrageneric limits of variation in the flagellar apparatus may be, as without some guideline it is inadvisable to delimit a new genus based largely on flagellar apparatus similarities. Given the lack of information on life histories in other ***Chrysochromulina*** species, the only practical way to solve the problem of what constitutes intrageneric variation is to obtain molecular data. However, if a new genus was erected to contain the three species mentioned above (***P. nemamethecum***, possibly ***C. polylepis*** subject to further information and ***Chrysochromulina*** sp.) it would be characterised by having cells with appendages inserted in a subapical depression on the ventral side of the cell; a haptonema shorter than the flagella with coiling ability determined by its length, but usually limited, often to flexing/bending; an elaborate scale complement composed of scales that are essentially flattened or plate-like but with some sort of unusual distal ornamentation (*i.e.* not just a simple spine or cylinder or raised patterning) and a scale type restricted to the flagellar region; a flagellar apparatus containing at least one crystalline root in the interphase cell and an intermediate band between the ventral sides of the flagellar bases; swimming patterns that typically occur with the anterior end of the cell directed forwards. In many of these features, the resemblance to the genus ***Prymnesium*** is unmistakeable, but the differences include the larger size of the scales and the scale arrangement, the range of length ratios of haptonema:flagella or haptonema:cell, and the presence of a compound microtubular root. (In this respect it is interesting to note that of all the ***Chrysochromulina*** species, only *C.* ***parkeae*** has appendage:cell length ratios that fall into the range listed by Chang and Ryan (1985) for ***Prymnesium***).

One other species should be mentioned here, *C.* ***birgeri*** (Hallfors and Niemi 1974; Hallfors and Thomsen 1979). This species was assigned to the genus ***Chrysochromulina*** on the basis of a

haptonema that coiled, but this only occurred in dying/fixed cells - as it does in the coccolithophorid *Syracosphaera pulchra*. Apart from this structural difference that is not functionally reflected in living cells, the only differences between *C. birgeri* and species of *Prymnesium* are the size of the large scales forming the distal scale layer (four times bigger than any described species of *Prymnesium*) and one of the two described modes of swimming. In this rapid swimming, cells move with the posterior end forwards, at other times they swim in a manner reminiscent of *Prymnesium* cells. Cell shape and the insertion of the appendages into a groove in the obliquely truncate, anterior end of the cell are features common to both *C. birgeri* and *Prymnesium* species. It would be most interesting to know what the flagellar apparatus of this species of '*Chrysochromulina*' looks like as it is difficult to determine which available features are more important in generic distinction: scale size and swimming behaviour, or haptonematal coiling ability, cell shape and appendage insertion. If the latter group is accepted, then *C. birgeri* would become a *Prymnesium* with a relatively long, but non-coiling haptonema. This would expand the length ratio range for *Prymnesium* to 0.46 for haptonema:flagella, compared with the present upper limit of 0.33 in *P. patelliferum*. *C. inornamenta* (Wujek and Gardiner 1985) fits comfortably into this range, and given the fact that this species has a single, simple type of plate scale (?), and has a non-coiled haptonema inserted into the truncate apex of the cell, it is more than possible that *C. inornamenta* is actually a species of *Prymnesium*.

To reiterate, there are species of *Chrysochromulina* that certainly do not belong in the genus, but which share features of both *Prymnesium* and *Chrysochromulina*, as presently defined. There appears to be more of a continuum of characters between the genera than any clear cut distinctions, with the exception of the saddle-shaped *Chrysochromulina* cells. The creation of a new genus in order to remove some of the variability from the borders between the two existing genera, is substantiated by the extremely thin dividing lines that presently separate genera within the Prymnesiaceae. Features of the flagellar apparatus of this new genus would overlap with both genera, but this situation seems to exist within the entire Prymnesiophycidae, for which flagellar features are currently a disappointing phylogenetic indicator - as are other

presumably conservative features such as mitosis. It is apparent that ultrastructure alone may not provide the answers to the phylogenetic questions posed by the Prymnesiophycidae, and assistance from molecular biologists will have to be enlisted before a natural taxonomy can be developed.

CHAPTER 8

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APPENDIX 1

List of genera and species referred to in the text (directly or in a cited publication). Names in apostrophes are no longer considered valid and represent a stage in a life history.

***'Apistonema'* Pascher**

***Calyptrosphaera* Lohmann**

Calyptrosphaera sphaeroidea Schiller

Chrysochromulina Lackey

Chrysochromulina acantha Leadbeater & Manton

Chrysochromulina adriatica Leadbeater

Chrysochromulina alifera Parke & Manton

Chrysochromulina apheles Moestrup & Thomsen

Chrysochromulina bergensis Leadbeater

Chrysochromulina birgeri Hällfors & Niemi

Chrysochromulina brachycylindra Hällfors & Thomsen

Chrysochromulina brevifilum Parke & Manton

Chrysochromulina breviturrita Nicholls

Chrysochromulina camella Leadbeater & Manton

Chrysochromulina campanulifera Manton & Leadbeater

Chrysochromulina chiton Parke & Manton

Chrysochromulina cyathophora Thomsen

Chrysochromulina cymbium Leadbeater & Manton

Chrysochromulina discophora Manton

Chrysochromulina elegans Estep, Davis, Hargraves & Sieburth

Chrysochromulina ephippium Parke & Manton

Chrysochromulina ericina Parke & Manton

Chrysochromulina fragilis Leadbeater

Chrysochromulina herdlensis Leadbeater

Chrysochromulina hirta Manton

Chrysochromulina inornamenta Wujek & Gardiner

Chrysochromulina kappa Parke & Manton

Chrysochromulina latilepis Manton

Chrysochromulina laurentiana Kling

Chrysochromulina leadbeateri Estep, Davis, Hargraves & Sieburth

Chrysochromulina mactra Manton

Chrysochromulina mantoniae Leadbeater

Chrysochromulina megacylindra Leadbeater

Chrysochromulina microcylindra Leadbeater

Chrysochromulina minor Parke & Manton

Chrysochromulina novae-zelandiae Moestrup

Chrysochromulina orbiculata Rouchijanien

Chrysochromulina pachycylindra Manton, Oates & Course

Chrysochromulina parkeae Green & Leadbeater

Chrysochromulina parva Lackey

Chrysochromulina pelagica Estep, Davis, Hargraves & Sieburth

Chrysochromulina polylepis Manton & Parke

Chrysochromulina pontica Rouchijanien

Chrysochromulina pringsheimii Parke & Manton

Chrysochromulina pyramidosa Thomsen

Chrysochromulina simplex Estep, Davis, Hargraves & Sieburth

Chrysochromulina spinifera (Fournier) Pienaar & Norris

Chrysochromulina strobilus Parke

Chrysochromulina tenuispina Manton

Chrysochromulina tenuisquama Estep, Davis, Hargraves & Sieburth

Chrysochromulina vexillifera Manton & Oates

Chrysotila Anand

Chrysotila lamellosa Anand = ***Ruttnera spectabilis*** Geitler

Coccolithus **Schwarz**

Coccolithus pelagicus (Wallich) Schiller

Corymbelius **Green**

Corymbellus aureus Green

'Crystallolithus' **Gaarder & Markali**

Crystallolithus hyalinus (Gaarder) Markali

Cruciplacolithus **Hay & Mohler**

Cruciplacolithus neohelis (McIntyre & Bé) Reinhardt

Diacronema **Prauser**

Diacronema vlkianum Prauser

Dicrateria **Parke**

Dicrateria inornata Parke

Emiliania **Hay & Mohler**

Emiliania huxleyi (Lohm.) Hay & Mohler

Exanthemachrysis **Lepailleur**

Exanthemachrysis gayraliae Lepailleur

***Hymenomonas* Stein**

Hymenomonas coronata Mills

Hymenomonas globosa (Magne) Gayral & Fresnel

Hymenomonas roseola Stein

***Imantonia* Reynolds**

Imantonia rotunda Reynolds

***Isochrysis* Parke**

Isochrysis galbana Parke

Isochrysis litoralis (Anand) Billard & Gayral

***Jomonlithus* Inouye & Chihara**

Jomonlithus littoralis Inouye & Chihara

***Ochrosphaera* Schussnig**

Ochrosphaera neopolitana Schussnig = *Ochrosphaera verrucosa* Schussnig

***Papposphaera* Tangen**

***Pavlova* Butcher**

Pavlova calceolata van der Veer

Pavlova ennorea van der Veer & Leewis = *Exanthemachrysis ennorea* (van der Veer & Leewis) Gayral & Fresnel

Pavlova granifera (Mack) Green = *Chrysocapsa granifera* Mack

Pavlova gyrans Butcher

Pavlova helicata van der Veer

Pavlova lutheri (Droop) Green = *Monochrysis lutheri* Droop

Pavlova noctivaga (Kalina) van der Veer & Leewis =
Corconiochrysis noctivaga Kalina
Pavlova pinguis Green
Pavlova salina (Carter) Green = *Nephrochloris salina* Carter =
Pavlova mesolychnon van der Veer
Pavlova virescens Billard

***Phaeocystis* Lagerheim**
Phaeocystis globosa Lagerheim
Phaeocystis pouchetii (Hariot) Lagerheim
Phaeocystis scrobiculata Moestrup

***Platychrysis* Geitler**
Platychrysis neustophila Norris
Platychrysis pienaarii Gayral & Fresnel
Platychrysis pigra Geitler
Platychrysis simplex Gayral & Fresnel

***Pleurochrysis* Pringsheim** emend. Gayral & Fresnel
Pleurochrysis carterae (Braarud & Fagerland) Christensen =
Hymenomonas carterae
Pleurochrysis haptonemofera (Inouye & Chihara) Gayral & Fresnel =
Cricosphaera roscoffensis var. *haptonemofera*
Pleurochrysis placolithoides Fresnel & Billard
Pleurochrysis pseudoroscoffensis Gayral & Fresnel
Pleurochrysis roscoffensis (Gayral & Fresnel) Fresnel & Billard =
Cricosphaera roscoffensis
Pleurochrysis scherffelii Pringsheim

***Prymnesium* Massart ex Conrad**

Prymnesium annuliferum Billard

Prymnesium calathiferum Chang & Ryan

Prymnesium czosnowskii Starmach

Prymnesium minutum Carter

Prymnesium nemamethecum Pienaar & Birkhead

Prymnesium parvum Carter

Prymnesium patelliferum Green, Hibberd & Pienaar

Prymnesium saltans Conrad

Prymnesium zebrinum Billard

***Syracosphaera* Lohmann** emend. Gaarder

Syracosphaera pulchra Lohmann

***'Turrisphaera'* Manton, Sutherland & Oates**

***Umbilicosphaera* Lohmann**

Umbilicosphaera sibogae (Weber-van Bosse) var. *foliosa* (Kamptner) Okada & McIntyre

'Zygosphaera' (Kamptner) Heimdal

APPENDIX 2

List of micrographs used in publications but not taken by the author.

Chapter 1. Figs 4, 8, 9-12, 19, 22, 29.

Chapter 2. None.

Chapter 3. Figs 1, 2, inset of Fig 47.

Chapter 4. Figs 1, 5.

Chapter 5. Figs 13, 20

Author Birkhead Monica
Name of thesis Ultrastructural studies on Marine Prymnesiales (Prymnesiophyceae) 1994

PUBLISHER:
University of the Witwatersrand, Johannesburg
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