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New Observations on the Fine Structure of *Chrysochromulina strobilus* Parke and Manton with Special Reference to Some Unusual Features of the Haptonema and Scales

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Summary. A new isolate of *C. strobilus* Parke and Manton has been investigated with light and electron microscopy to add significant details not previously studied. With the aid of a ciné record, to be described more fully elsewhere, some of the more important haptonema movements have been accurately timed. With the aid of improved technical methods of electron microscopy, new observations have been made on the haptonema base, and on the structure and mode of origin of the scales. The presence of 6 microtubules (instead of the more usual 7) in the very long haptonema of this species has been confirmed but the elaborate changes in tubule number and arrangement which take place in the haptonema base are shown to be essentially like those of other species. The morphology and arrangement of the two types of scale present on the cell are as previously described though information on several new and taxonomically valuable surface features have been added. The cup-shaped scales in particular are shown to be more different from those of other species now known in the genus than might previously have been recognised. This difference extends to the position of nascent scales within the Golgi system which is described in a preliminary way. Further discussion is deferred until some other new isolates representing related taxa have been described and named.

When first described (Parke *et al.*, 1959) this species was remarkable for the extreme length of its haptonema ("12—18 or exceptionally 20 times the body length") and for certain details of scales and microanatomy, some of which have subsequently been found again in related species and genera whilst others remain unique. The type culture was subsequently lost.

In the years which have elapsed since 1959 much more has become known about the fine structure of members of the group Haptophyceae (sensu Christensen, 1962). The application of improved methods of fixation has clarified certain features, notably the peculiarities of the Golgi structure first detected in *C. strobilus* but now known to be widespread within the group, while an intensive series of studies directed to the elucidation of haptonema structure in a number of species, of which

Prymnesium parvum and *Chrysochromulina chiton* are the best known (for literature see MANTON, 1968), has shown that many details are of general occurrence regardless of the length of the organelle.

Nevertheless there are two known characters in which *C. strobilus* remains aberrant within the genus, namely on the one hand the special type of cup-shaped scales, about which more below, and on the other the possession of 6 microtubules in the haptonema axis instead of the customary 7. It is true that outside the genus *Chrysochromulina* 6 microtubules have also sometimes been found, notably among certain coccolithophorids (MANTON and LEEDALE, 1963; MANTON and PETERFI, 1969) but in at least some of these the morphology of the haptonema is also aberrant and interpretable as perhaps degenerate. *C. strobilus* therefore remains the only example at present known in which a very long and fully mobile haptonema has this reduced number of component microtubules.

The exact part played by individual microtubules in haptonema movement is not a topic which at present lends itself to direct investigation. Cognate problems involving the haptonema base are more approachable, given suitable material. Recent observations on *Prymnesium parvum* (MANTON, 1964) and *Chrysochromulina chiton* (MANTON, 1968) have shown that within the cell some complex and at present inexplicable changes take place in an identical manner in both species. Thus at the point of entry into the cell or shortly before it the ring of 7 microtubules becomes compacted into a close-set arc. After entry into the cell the arc becomes a curved zigzag and the number of microtubules changes to 8. These close up still further to form two superposed arcs of 4 microtubules in each, after which a 9th tube is added converting the arrangement into that of hexagonal close-packing before the whole group is abruptly discontinued. These changes seem unconnected with either the length or other attributes of the free part of the organelle since in *Prymnesium* the haptonema is very short and unable to coil while that in *C. chiton* is longer than the flagella and is habitually retracted into a helix of 6—8 gyres, at least when the cell is killed. Comparative information about the nature of the haptonema base in a species such as *C. strobilus* possessing only 6 microtubules in the fully formed haptonema is not yet available but would be of interest.

The opportunity of investigating *C. strobilus* from this point of view, in spite of loss of the type culture, arose as an unexpected consequence of an experimental study of haptonema movement in general, undertaken by one of us (BL) with the aid of ciné photography of living cells. It was necessary for this purpose to test all the available cultures that could be supplied from the collection at Plymouth, to find cells of the right size, neither too large nor too small, with long haptonemata

robust enough to perform their movements whilst under observation, without either being discarded or withdrawn into the cell. After completion of the film the selected cultures were further investigated to confirm and in some cases to elucidate the taxonomic identity of the clones used. Somewhat unexpectedly no less than seven different isolates attributable to three or four species proved to belong to the *C. strobilus* affinity although only one proved to be *C. strobilus* itself. This new isolate is the basis of the present communication.

Material and Methods

The new isolate, known as 43 A in the Plymouth collection, was obtained by Miss R. JOWETT from a seawater sample taken at international station E1 (50°02′N, 04°22′W) 3 m depth on 25. 1. 1967.

In addition, some embedded material of the type culture used in the original description (PARKE *et al.*, 1959) has been re-investigated for comparison with the new material. Although embedded in methacrylate, application of stains has made it possible to see details of patterning on the scales in a manner not previously possible. In spite of considerable damage caused by the earlier form of processing there is no doubt that the specific identification of our new isolate as *C. strobilus* Parke and Manton is correct.

Standard techniques for whole mounts, sections and for light microscopy have been used throughout. The most important fixative for sections was 5% glutaraldehyde (used for 2 h) followed by 5 h in 2% osmium tetroxide, both reagents being made up in cacodylate buffer at pH 7, 1/4 molar sucrose having been added to the glutaraldehyde. For certain purposes other variants were used with acceptable results. Thus fixation in 2% osmium tetroxide in cacodylate buffer at pH 7 used for 30 min followed by rapid dehydration gave better results for details of sculpturing on the scales. Equally good for scales though somewhat better for the cell interior, was the use of a mixture of 5% glutaraldehyde and 2% osmium tetroxide each made up as for the single fixative but mixed together in equal volumes immediately before use. This was applied for 15 min on ice followed by 2 rinses (15 min each) in buffer before completion of fixation by 2 h post-osmication. We are indebted to Dr. R. M. BROWN, JR., for information on the details of this method.

Embedding into epon 812 was standard. Sections were cut with a diamond knife and double stained with uranyl acetate and lead citrate. They were examined with a Siemens Elmiskop I or an A.E.I. EM6B electron microscope. These two instruments may be distinguished by the initial D before the exposure number in the case of the Siemens and some other letter (usually L or Z) before the exposure number in the case of the EM6B.

The photographs of fixed cells reproduced in Figs. 1—3 were taken by means of anoptral contrast light microscopy on a Reichert Zetopan microscope.

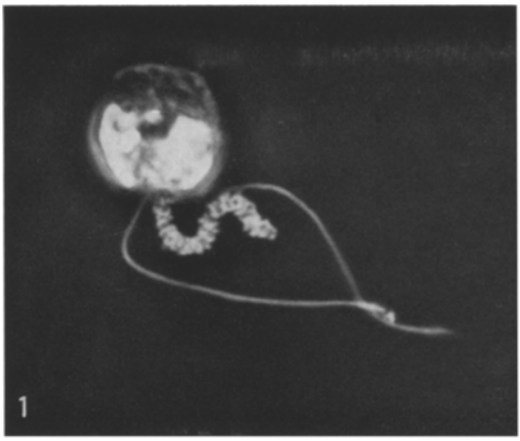
New Observations on the Haptonema

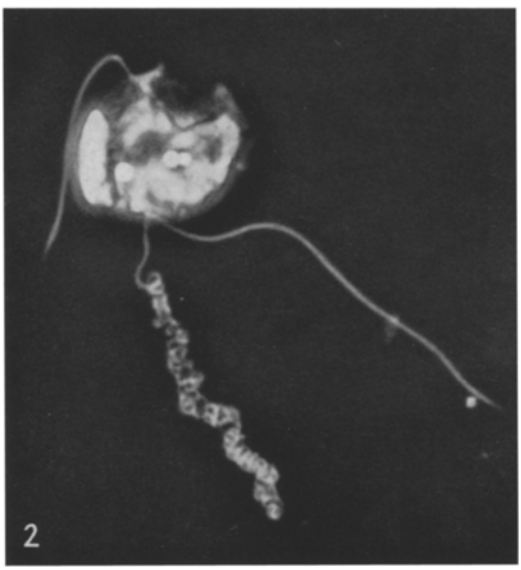
Although this species, among others, had been selected for its ability to coil and uncoil its haptonema without signs of distress whilst under the conditions needed for prolonged observation, we have found no method of killing a cell which will retain the haptonema in the outstretched condition. A dead cell, no matter how carefully fixed, has its

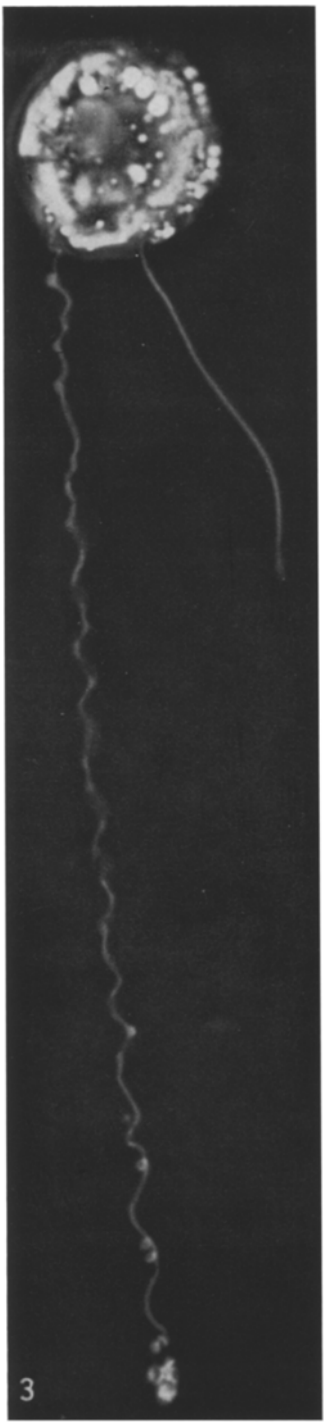
haptonema coiled. All preparations of apparently extended haptonemata involving such cells will therefore necessarily display attitudes which are to some extent artifacts although such artifacts can be very informative. This will be obvious from Figs. 1—3 which show cells killed with osmic vapour and examined at once under slightly different conditions of coverslip pressure. Fig. 1 is relatively undisturbed though the helix of its coiled haptonema is bent into an S shape which may or may not be natural (living cells do not permit determination of this feature). In Fig. 2 the helix has been slightly extended by gentle coverslip pressure, permitting a rough estimate of the number of gyres to be made. In Fig. 3 on the other hand the cell has been subjected to lateral movement of the coverslip as well as slightly greater pressure. The haptonema has in consequence become considerably extended although the gyres have not been completely effaced. The overall length of the organelle can now be much more clearly appreciated but the undulated and looped condition, representing residual gyres, would not be seen in a living extended haptonema which is normally straight. A similar interpretation must be applied to the very elegant examples of apparently extended haptonemata on dried cells after killing with osmic vapour illustrated by PARKE *et al.* (1959).

As recorded in the ciné film (LEADBEATER, 1969) the uncoiling movement is slow, taking 2—10 sec though the recoil is so rapid that the eye cannot follow it. The time required for recoil can however be analysed with the aid of high speed ciné microscopy as occupying less than $^1/_{50}$th sec. Under the conditions of filming, cells can remain stationary for long periods with the flagella beating gently and the haptonemata fully extended without being visibly attached to slide or coverslip. In this condition the haptonema is extremely sensitive to mechanical stimuli; even light tapping near the microscope will elicit recoil without necessarily disturbing the position of the cell.

The dimensions of the cell and appendages agree closely with those provided in the type description (PARKE *et al.*, 1959). In our clone, the average cell length and breadth is 10 μm though the ranges are slightly different, 6—12 μm being the range in length and 7—12 μm the range in breadth. The average flagellar length is 20 μm and the average haptonema length c. 100 μm. The appendages arise from the concave ventral surface of the saddle-shaped cell near to the posterior end; when swimming they are usually all directed posteriorly whether the haptonema is coiled or uncoiled though the latter can sometimes be directed forwards when the cell is stationary. A coiled haptonema is commonly partly enclosed by the incurved flanges of the saddle, each of which contains a chromatophore; the number of gyres in the helix is within the range 25—45. Other cell characteristics are as previously described (loc. cit.).







Figs. 1—3. Three cells killed with osmic vapour and photographed with anoptral contrast light microscopy; the coiled haptonemata showing various degrees of mechanical expansion caused by the handling. For further explanation see text.
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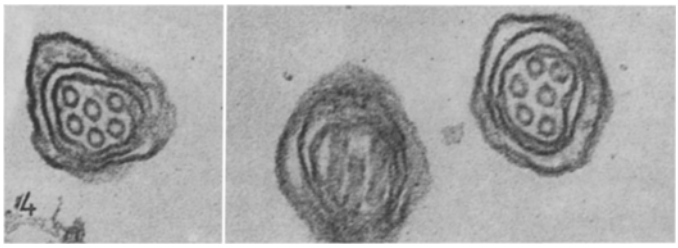
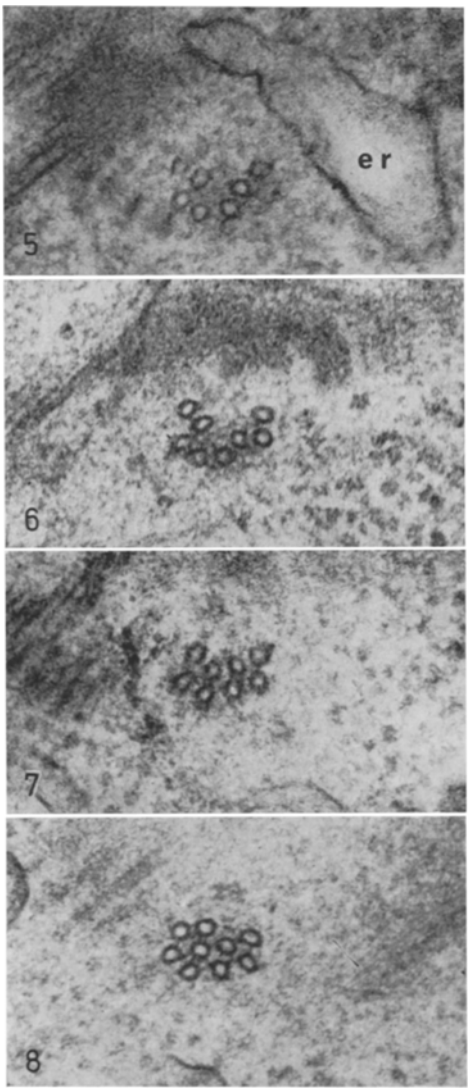


Fig. 4

Haptonema structure in three adjacent gyres of one specimen showing the 6 microtubules surrounded by the three membranes. Electron micrograph
D8 510 × 100,000



Figs. 5—8

Transverse sections showing the number and arrangement of microtubules characteristic of different levels in a haptonema base (compiled from two different specimens) arranged in descending order from the cell surface. In the uppermost section (Fig. 5) a cisterna of endoplasmic reticulum (*er*) approaching the group of 7 fibers before surrounding it; for the final stage in the external parts of the haptonema see Fig. 4. For further description see text.
Micrographs D8507, D8524, D8532, D8521 × 100,000

In sections it is usual to find the coiled haptonemata detached from the subtending cells and with their gyres somewhat disarranged. It was however not difficult to verify the presence of 6 and not 7 microtubules within the usual three membranes. The cavity between the two inner membranes is now known to represent a cisterna of endoplasmic reticulum (MANTON, 1968). These features can be seen in two gyres of one specimen in Fig. 4.

Loss of the haptonema by breakage impedes investigation of the transition region but does not prevent effective investigation of the base within the cell. Sections characteristic of the different levels are illustrated in Figs. 5—8, the micrographs being selected from two different series which however repeat each other exactly. Near to the cell surface (Fig. 5) there are already 7 and not 6 microtubules, arranged in a zig-zag arc. Below this (Fig. 6) an eighth tubule is added though the configuration is still that of a zig-zag arc. Below this again (Fig. 7) the arrangement becomes more compact to give the appearance of two concentric arcs each with four microtubules. Finally (Fig. 8) addition of the ninth tubule produces the hexagonally close-packed group characteristic of the extreme base. This is therefore exactly as already described for *Prymnesium parvum* and *Chrysochromulina chiton* in spite of the lower tubule number (6 instead of 7) present in *C. strobilus* in the free part of the organelle.

New Observations on Scales

In the original description of the species (PARKE *et al.*, 1959) it had been necessary to supply a diagram based on a section to illustrate the scale shapes and relative positions since without staining they were so faint as to be visible only with difficulty. They were also too small for much detail to be seen in direct preparations. The new observations amplify the previous information while confirming the essential correctness of the previous descriptions as far as they went.

Most cells carry an outermost layer of mucilage which tends to obscure the underlying scales in a rapidly dried direct preparation; thus Fig. 9 compares very closely in this respect with Fig. 25 in the previous paper (loc. cit.). When the mucilage is removed by more effective washing, loose scales of two morphological types can be found mixed together (Fig. 11). Some are oval flat plates with a pattern of ridges on their surfaces while others (Figs. 10 and 11) are the cup-shaped scales which can just be seen emerging through the mucilage in Fig. 9. The details are nevertheless better seen in sections.

The arrangement of scales on the cell surface is in two layers, the flat scales being undermost. This can be seen in Figs. 12—15. Morphologically the cups can be described as composed of three parts namely a short

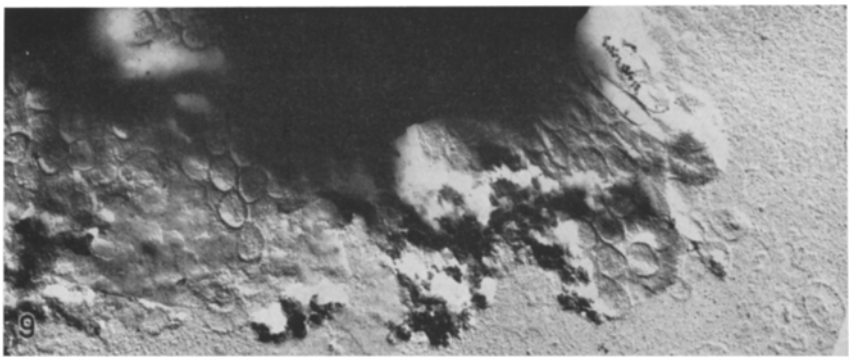


Fig. 9. Edge of a dried cell from a whole mount showing the scaly covering partly concealed by mucilage. Micrograph Z901 × 20,000

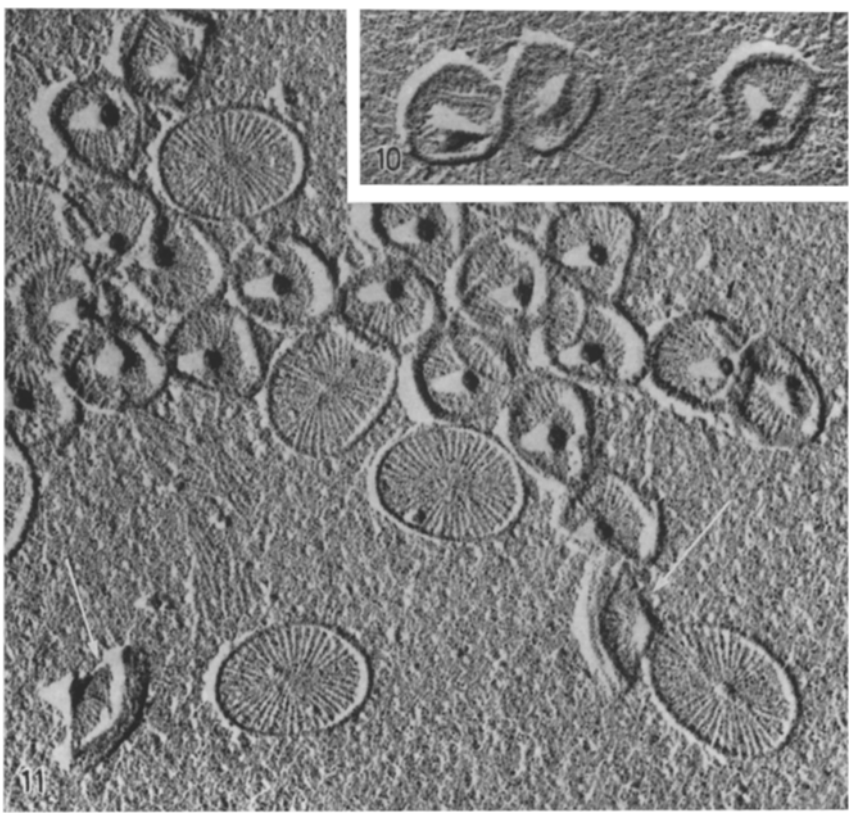


Fig. 10. Shadow-cast direct preparation of three cup-scales the two on the left with their concave surfaces facing upwards and the one on the right with the convex surface facing upwards. Micrograph L1108 × 60,000

Fig. 11. Field of scales from a shadow-cast direct preparation with mucilage removed. Most cup-scales showing the lower surface but two seen laterally at bottom left and right (arrows); plate scales also present. Micrograph L1107 × 60,000

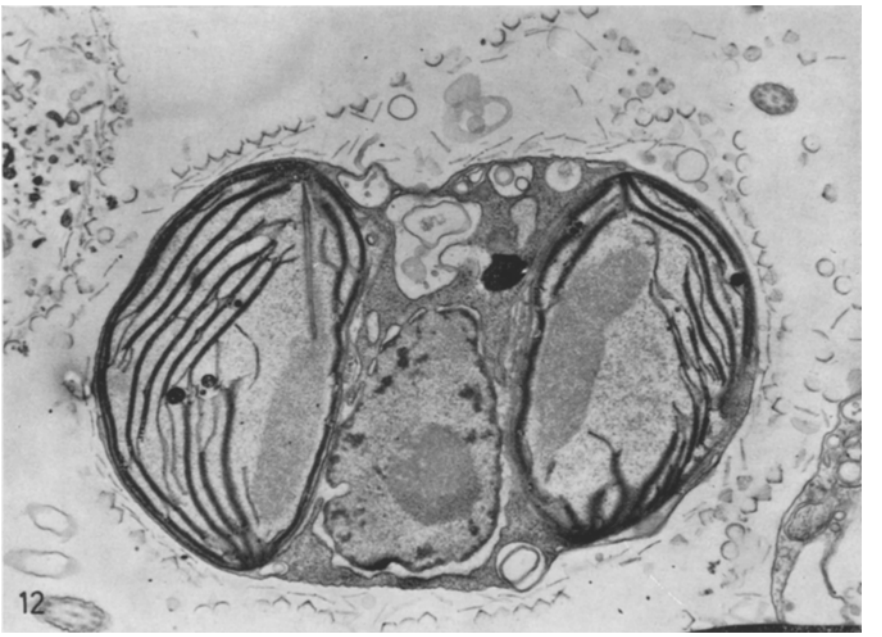
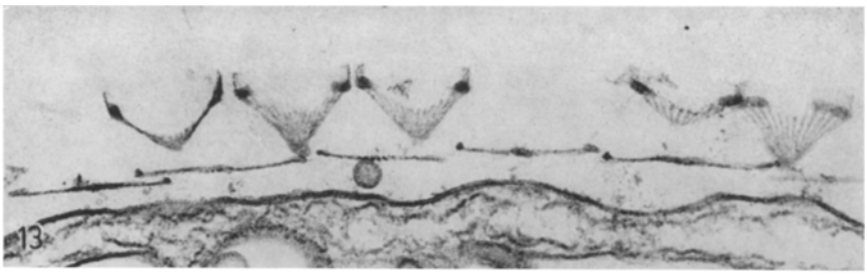


Fig. 12. Section of a cell after glutaraldehyde fixation showing the nucleus, two chloroplasts and vesicles, some with dense contents; the surface covering of scales still almost in position. Micrograph D8932 × 15,000

dense "stalk", a wide conical bowl and a straight-sided distal margin. The base of the bowl and the straight-sided margin is patternless but the outer surface of the rest of the bowl is delicately striated with a single row of apparent perforations or "windows" below the junction with the smooth distal edge. Transverse sections through the bowl at any level give circular profiles on which the same details of sculpturing may be seen (compare especially Figs. 14 and 15). There are nevertheless slight differences in the cup-scales from one and the same culture when treated with different fixatives. Figs. 14 and 15 shows the normal appearance, in epon, after post-osmicated glutaraldehyde which can be matched in methacrylate sections of the type culture after osmic fixation, allowance being made for some additional distortion.

On the other hand Fig. 13 shows the appearance, in epon, after very short fixation in osmium tetroxide (30 min) while Fig. 17, which is very similar, illustrates the appearance in epon after 15 min fixation in the mixture of glutaraldehyde and osmium tetroxide described last on p. 107 above. In both Figs. 13 and 17 a ring of dense material located exactly along the joint between the wide end of the conical bowl and the straight-



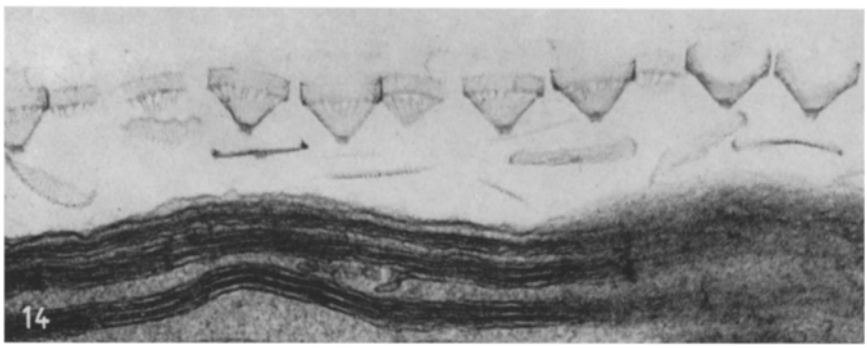
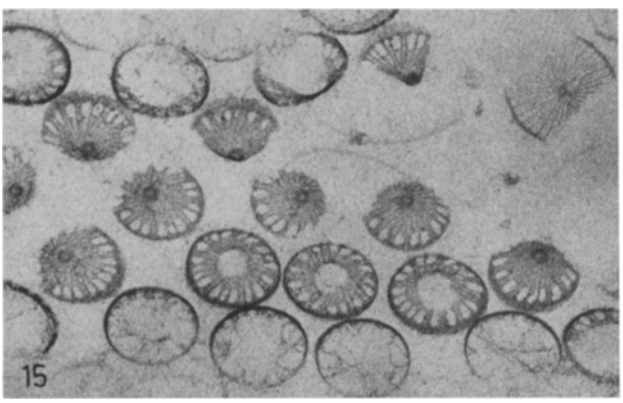


Fig. 13. Scales in position on a cell surface after osmic fixation. Note the under layer of plate scales and the ring of dense material retained near the upper edge of each cuscale after this treatment. Micrograph D9639 × 70,000

Fig. 14. Scales in position on a cell surface after glutaraldehyde fixation. Note the finely fibrillar traces of mucilage in the field beyond the cup-scales. Micrograph D8511 × 60,000



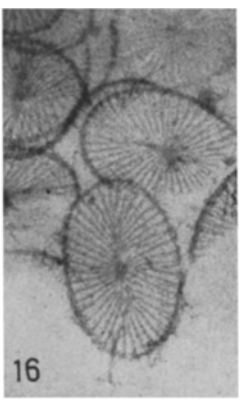


Fig. 15. Tangential section near a slightly curved cell surface showing profiles of cup-scales in face view. Note the single row of "windows" on the forward edge of the bowl (compare with Fig. 14). Glutaraldehyde fixation. Micrograph D9564 × 60,000

Fig. 16. Plate scales in face view from a section after osmic fixation. Micrograph Z2169 × 70,000

sided forward edge has been preserved on the concave inner face. This is absent from Figs. 14 and 15 but when present it is accompanied by less unevenness in the spacing of the striations on the bowl. Since under these circumstances the appearance of a nascent scale not yet liberated to the surface (Fig. 20) is identical in these features with external scales, it seems necessary to suppose that minor loss of material may be the cause of distortion in Figs. 14 and 15 to an extent which might not otherwise have been suspected. These observations are unimportant in themselves since the details involved are trivial. They are nevertheless perhaps noteworthy because scales in the genus Chrysochromulina are usually rather resistant to mechanical and other damage and the fact that the cup-scales of *C. strobilus* are as sensitive as they appear to be suggests that they must in fact be more delicately constructed than is usual among the other described species.

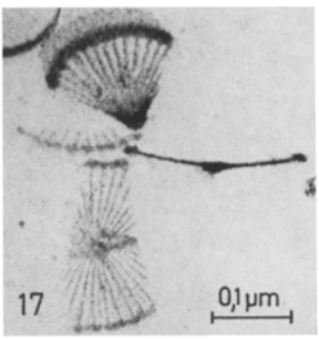


Fig. 17. Parts of some detached scales after short fixation with glutaraldehyde/osmium-tetroxide mixture; note the very regular "windows" in the cup-scale after this treatment. Micrograph D9631 × 100,000

The oval plate-scales require less comment. They show a pattern of radiating ridges on both faces, the ridges converging to a plain centre. The scale margin is thickened and there is also a slight thickening in the centre, more especially on the lower surface (Fig. 11 bottom right and Fig. 17). The surface details stain up well after osmic fixation and are best seen in Fig. 16. The ribs are very slender and fairly widely spaced, sometimes with traces of a more delicate striation alternating with the ribs. The substance of the whole scale is very thin and except for minor surface features in the centre of a scale the two faces are similar.

New Observations on Golgi Activity

C. strobilus was the species in which certain manifestations of Golgi structure were seen for the first time (loc. cit. 1959). We now know that slow (1 h) osmic fixation breaks down some of the cisternal membranes in the interior of the Golgi stack and that the "peculiar" structure as first described was imperfectly preserved. A fuller study of *Chrysochromulina chiton* has already been published (MANTON, 1967a) correcting these shortcomings with respect to that species by the use of glutaraldehyde fixation and epon embedding (the methods used here). Since

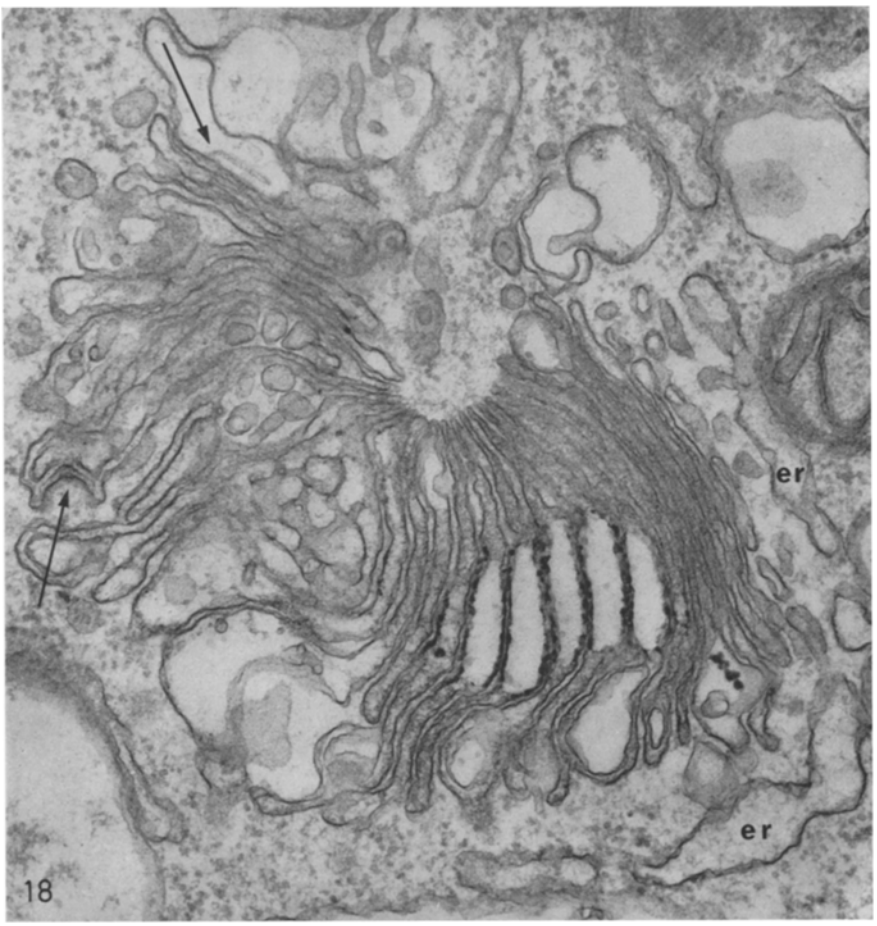


Fig. 18. Golgi system in relation to subtending endoplasmic reticulum (*er*). Quiescent cisternae (right); asymmetrical cisternae strongly polarised towards a clear area from which they radiate (centre), with 6 intercalary dilations; and (left) distended cisternae with tubular contents and traces of scale production, arrows. For further description see text. Micrograph D8940 × 50,000

C. strobilus is essentially similar, it is enough to draw attention to Fig. 18 as an example of the true structural basis of the peculiarity. The Golgi cisternae in this as in other species of the group are asymmetrical and strongly polarised towards one side, the stack itself being curved. At one end of the curved stack (right in Fig. 18) a layer of endoplasmic reticulum partly envelopes the Golgi stack at a fairly constant distance from it. The Golgi cisternae at this end are flattened and with mainly

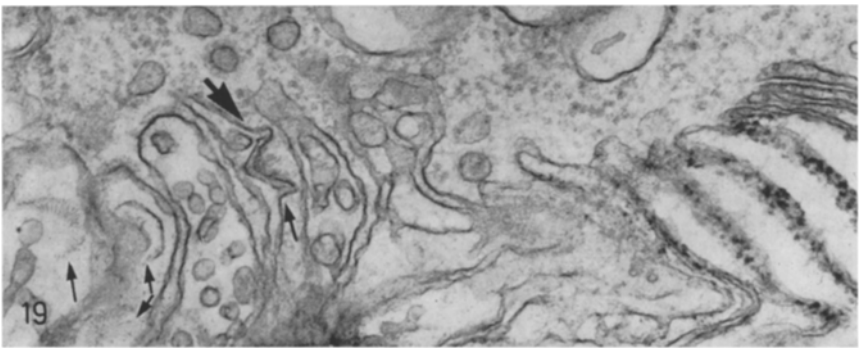


Fig. 19. Part of the edge of a Golgi stack, showing "peculiar" dilations (right) and scale production (arrows). Micrograph D8,934 × 50,000

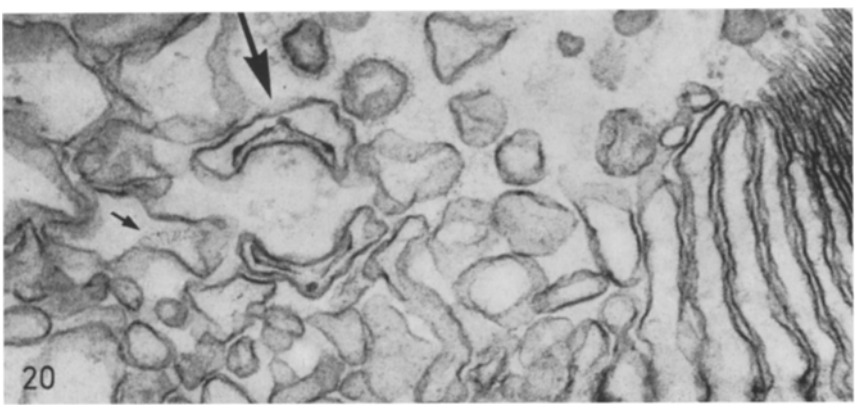


Fig. 20. Scales in vesicles becoming separated from the Golgi stack. For structural details of the cup-scales compare with Fig. 13. Micrograph Z1283 × 60,000

undifferentiated contents. In the centre of the field some six adjacent cisternae display conspicuous central dilations which are the basis of the peculiarity referred to. After osmic fixation the cisternal walls in this region break down giving the appearance of a space with vesicular contents. After glutaraldehyde fixation the walls remain intact but they are lined with fragments of a dense metabolite of unknown nature which is generated only in this region, some other metabolite being presumably responsible for the local dilation of the lumen.

At the other end of the Golgi stack (left in Fig. 18) the cisternae become dilated locally in a different manner and their contents take on the characters already known in other species. Thus proliferation of the cisternal membranes into the cisternae which was traced as a late

stage in *C. chiton* (MANTON, 1967b) giving rise to reticular, tubular or vesicular contents can be seen in these various forms on the left-hand side of Fig. 18. Such cisternae will sometimes contain scales though in this material the scales can only be recognised after their morphological development is complete. Two examples are marked by arrows, one a cup scale and the other a plate scale. Both are almost or quite ready for discharge. Fig. 19 illustrates part of another specimen with several mature scales in the left-hand cisternae but with dilated cisternae of the "peculiar" type included in the field on the right. Finally two fully mature cup scales which have left the stack apparently enclosed in separate vesicles are visible in Fig. 20. The morphology of these scales can usefully be compared with those illustrated in Figs. 13 and 17.

A detail that can be ascertained from both Figs. 18 and 19 is that before a cup scale has begun to move away from the site of formation the orientation with respect to the subtending Golgi stack is unusual. In the best known representatives of Haptophyceae in which this matter has been studied (*Prymnesium* Manton 1966, *C. chiton* Manton 1967b and coccolithophorids Manton and Leedale 1969, MANTON and PETERFI, 1969) the arrangement hitherto encountered without exception has been such that the morphologically outer surface of a nascent scale is directed away from the Golgi centre unless the subtending cisterna has been deformed or displaced, as it is in certain very large scales such as those of the normal form of *C. chiton* or the large coccoliths of *Coccolithus pelagicus*. In *C. strobilus* however we seem to have the converse, namely that the morphologically outer surface (with respect to the cell) of a very small scale i.e. the concavity of the cup, faces towards the centre of the Golgi stack in both the specimens illustrated in Figs. 18 and 19. We do not propose to offer any explanation of this situation at present though it is noteworthy as adding yet another item to the growing list of characters in which *C. strobilus* is anomalous within the genus.

Discussion

The new observations recorded above have amplified the descriptive facts for this species almost up to the level already attained for *Prymnesium parvum* and *Chrysochromulina chiton*. Compared with these and other species in the genus *Chrysochromulina*, *C. strobilus* stands out as even more distinct than was previously thought. The haptonema is nevertheless clearly in agreement with that of other species developmentally, although the progressive diminution of microtubule number in passing from the base of the organelle towards the cell surface goes one step further in this species than in the others. This is nevertheless probably not a major difference and it is scarcely if at all reflected in the functioning of the organelle.

The scales on the other hand remain at least as unusual as they were previously thought to be. Some of the structural and developmental details will come up for further discussion after more of the related species mentioned on p. 4 have been described. It is nevertheless already clear that the evidence provided from a considerable range of taxa, including coccolithophorids (for a recent discussion see MANTON and LEEDALE, 1969), that a primitive scale type underlying many of the most bizarre and complex shapes, namely the two-layered plate with or without a rim, does not apply to *C. strobilus* in any obvious form. In several other cases, for example. *C. pringsheimii* (PARKE and MANTON, 1962) and an aberrant form of *C. chiton* (MANTON, 1967a, 1967b) one of the two layers of material believed to have been present in the primordial primitive scale has become secondarily suppressed or greatly reduced. In such cases, however, direct or indirect evidence has always been forthcoming to show that reduction has indeed taken place phyletically and that no fundamental departure in basic construction has been involved. No such evidence is at present available for *C. strobilus* and the anomalous position within the Golgi cisternae is perhaps further indication of greater divergence from a common ancestral pattern than scale morphology alone might have suggested.

Further discussion on these lines must be deferred pending fuller study of the developmental processes involved and of comparable facts for related taxa which have not yet been described. We can nevertheless perhaps usefully conclude by summarising the present and former findings in the form of an emended diagnosis in which certain details have been omitted and others added or expanded. It should be noted that this does not imply disagreement with anything that was previously stated but rather that with fuller knowledge than was available in 1959 we can more clearly discern which details are likely to be of special importance as taxonomic indicators at the specific level. In the following emended diagnosis only these have been stressed.

C. strobilus (PARKE and MANTON) emend

Biflagellate monads c. 10 μm in length and breadth, with two equal flagella, c. 20 μm long and a haptonema c. 100 μm long when fully extended, forming a helix of 25—45 gyres when coiled. Cell variable in shape, usually saddle-shaped with the two incurved lateral flanges containing the chromatophores; the external appendages arising close together from the concave lower surface near one end. Cell covered externally with mucilage partially enveloping scales of two morphological types, individually visible only with an electron microscope. A lower layer of thin oval plates each with a thickened margin and a sparse surface pattern of simple striations radiating from a cruciform

centre; dimensions: 0.14—0.20 μm wide × 0.24—0.40 μm long. An outer layer of cup-shaped scales, 0.15—0.20 μm wide and 0.09 to 0.12 μm deep, with the outer surface of the bowl marked by delicate ridges between which transparent or perforate areas forming a single row of apparent windows; the base of the bowl and the straight-sided margin patternless. Within the cell usually two pale golden brown chromatophores each with a central storage region; other cell details as in the type description including the presence of a single Golgi body containing the peculiar dilations now known to be characteristic of Haptophyceae. Other stages of life history as in the type description. Habitat: open sea near Plymouth in surface waters.

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