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STUDIES ON MARINE FLAGELLATES

V. MORPHOLOGY AND MICROANATOMY OF *CHRYSOCHROMULINA STROBILUS* SP.NOV.

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(With total of 44 Figures in text and on Plates I–VIII)

CONTENTS

INTRODUCTION

The new species to be described here is very common in the English Channel, though like other members of this genus it is so fragile that it needs to be cultured to be effectively detected. This particular species has been under observation for some years, but publication has been deferred until sections of it could be made available. The observations involved in the taxonomic description have been based on two early isolates numbered 4 and 43 in the Plymouth collection. More recently, however, it has been encountered

frequently in routine sea-water samples brought into temporary culture to record the nanoplankton forms present, and we are therefore able for the first time to give tables of seasonal and depth distributions for the incidence of the species, at various stations. In Table 1 of Appendix (p. 187) its occurrences are listed, all being from water-bottle samples, except those taken on 23 August 1950 and 6 November 1957 (fine tow-net) and on 19 June 1957 (very fine tow-net). Table 2 (p. 188) gives densities at different depths sampled on one day at Hydrographic Station E 1.

The other technical methods involved are substantially as before except for minor variants in fixing and embedding procedures. As on previous occasions the electron micrographs of external morphology were carried out on the old Philips microscope in the Leeds Botany Department. A few of the high-power details, notably those on haptonema structure contained in Pl. VIII, were obtained on the Siemens Elmiskop I at the Rockefeller Institute which was made available during a 2-week visit at Christmas 1957. The remaining anatomical micrographs have been taken on the new Siemens Elmiskop I recently installed in the Leeds Botany Department by means of a grant from the Rockefeller Foundation.

Very grateful thanks are due to Dr K. R. Porter of the Rockefeller Institute for his unfailing courtesy and help during flying visits to his laboratory, also to the Rockefeller Foundation for removing the necessity for such visits. For help with the Latin diagnosis we have to thank Dr T. Christensen of Copenhagen. We have also to thank Miss I. Adams for assistance in the routine examination of samples, Mr F. G. C. Ryder for designing and building the special apparatus used for the growth of the very numerous temporary cultures from which distribution records have been obtained, Miss D. Ballantine (Mrs B. Hepper) for testing this organism for its possible toxicity to fish, Dr L. H. N. Cooper, Mr E. I. Butler, Dr T. J. Hart ('Discovery II'), Mr D. Vaux and Mr A. C. Burd ('Sir Lancelot') for the collection of sea-water samples.

FORMAL TAXONOMIC DIAGNOSIS

Chrysochromulina strobilus sp.nov. Parke & Manton

(Gr. *στρόβιλος*—a pirouette)

Motile cells showing considerable metaboly; dorsi-ventrally flattened, convex on the dorsal surface, flat or concave ventrally; when stationary or gliding slowly body saddle-shaped or appearing truncate-ovate in dorsal or ventral view; when swimming rapidly bell-shaped, obovoid or depressed-globose; 6–10 (exceptionally 5–12) μ in size. Two flagella and one haptonema arising fairly close together from the ventral surface, usually one-third cell length from rounded end in a centre line; flagella subequal to equal, very fine,

smooth, tapered to a small knob (E.M. observation), appearing homodynamic when cell moving rapidly and heterodynamic when cell moving slowly or stationary, 2–3 times body length; the haptonema capable of attaching along its whole length, half the thickness of the flagella, 12–18 (exceptionally 20) times the body length when fully extended, with a swollen tip and an internal structure of three concentric membranes surrounding a ring of six 'fibres'. The periplast, pectic in nature, showing a surface pattern of tightly packed angular 'cup' scales 0·15–0·2 μ in diameter; additional very thin, transparent, circular to oval, sculptured scales, 0·3–0·4 μ in diameter, with a pattern of radiating ridges, present beneath the 'cup' scales.

Cells uninucleate, no stigma. Chromatophores faintly striated on outer face, 2 or 4, occasionally 1 or none, golden brown; in cells of motile phase parietal, saucer-shaped to oblong, lacking an external pyrenoid but with a well-marked internal storage region; in non-motile phase pale gold and very finely lobed. Lipids and leucosin produced. Ejectile muciferous bodies small, distributed in peripheral cytoplasm, more numerous on dorsal and ventral surface of back of saddle but their position changing with the metaboly of the body. Nutrition phototrophic and/or phagotrophic. Non-toxic to fish.

In motile phase asexual reproduction by fission into two daughter-cells, usually of equal size; in non-motile phase by successive fission of amoeboid cells to produce 4 ovate daughter-cells with thin walls; motile phase liberated from walled daughter-cell through pore.

Habitat. The sea at position lat. N. 49° 21′, long. W. 04° 54′ (9 May 1950, type culture) at surface. Type culture (Plymouth no. 43) deposited with the Culture Collection of Algae and Protozoa, Cambridge.

Cellula in statu erratico satis metabola, depressa, dorso convexo, ventre plano vel cavo; dum quieta lenteve prolabens ephippioides seu a dorso vel ventre truncato-ovata visa; dum cito natans campanuliformis seu obovata seu depresse globularis; 6–10 (raro 5–12) μ longa. Flagella duo haptonemaque unicum in facie ventrali sat conferte inserta, plerumque mediana, tertia cellulae longitudinis parte ab apice rotundato remota; flagella paene vel plane aequalia, tenuissima, glabra, ad apices attenuata, nodulo quidque terminatum (per microscopium electronicum viso), cellula 2–3 plo longiora, inter motum citum homodynamica, inter lente movendum ut inter quietem heterodynamica visa; haptonema flagellis dimidio tenuius, extensum cellula 12–18 (raro –20) plo longius, apice incrassatum, in sectione transversa tres membranas tubiformes concentricas ostendens fibras 6 in orbem dispositas induentes, in tota longitudine adhaerendi potens. Periplastum pecticum, squamis dense angulate congestis, 0·15–0·2 μ diametro, marginibus adscendentibus, discis intus mucronato-incrassatis obtectum, alteris illis suppositis delicatulis, hyalinis, orbicularibus vel ovalibus, 0·3–0·4 μ diametro, costis radiantibus ornatis.

Nucleus unicus; stigma nullum. Chromatophora 2 vel 4, interdum unum vel nullum, fulva, in facie externa striatula, inter statum erraticum cellulae parietalia, catilliformia vel oblonga, pyrenoidibus externis carentia, sed regione penaria interna manifesta quidque instructum; inter statum sedentarium pallide aurea, subtilissime lobata. Synthemata lipoida et leucosinea. Corpora mucifera ejectilia parva, in strato

externo cytoplasmatis distributa, in facie dorsali et ventrali posterioris partis ephippii crebriora, inter metabolam situs mutantia.

Alga et phototropha et phagotropha seu alterutro solum victu alta; piscibus non venenosa.

Propagatio vegetativa in statu erratico bifissione effecta, cellulis filialibus plerumque aequalibus; in statu sedentario fissione iterata cellulae amoeboidis, cellulis filialibus 4, ovatis, parietibus subtilibus indutis, quaque earum cellulam erraticam per porum liberante.

Typus die 9. Maji 1950 in summo mari lat. bor. 49° 21′, long. occ. 04° 54′ lectus, in Plymouth Angliae sub numero 43 cultus, postea in vivario Cantabrigiensi depositus.

OBSERVATIONS WITH THE LIGHT MICROSCOPE

Description and behaviour of the living cell

Chrysochromulina strobilus (Figs. 1–12) is somewhat similar to *C. ephippium* and *C. alifera* (Parke, Manton & Clarke, 1956) in shape, size and in the behaviour of the flagella and haptonema when an individual is stationary or when slowly gliding; it also exhibits phagotrophism in common with all our species so far described.

It differs from *C. ephippium* and *C. alifera* in the position of origin of the flagella and haptonema (Fig. 8), the body attitude and the behaviour of the

Legends to Text-figs. 1–12

Chrysochromulina strobilus sp.nov.

(Figs. 1–7, × 1250; Figs. 8–12, × 5000)

Fig. 1. Cell anchored by fully extended haptonema which is bent and attaching only by swollen tip.

Fig. 2. Anchored saddle-shaped cell with flagella showing heterodynamic movement; haptonema attached along most of its length.

Fig. 3. Saddle-shaped cell gliding slowly with haptonema lying away from and in front of body.

Fig. 4. Early fission stage rotating slowly and moving with haptonema fully extended in front of body.

Fig. 5. Fission stage with four flagella and two haptonemata in attitude adopted for fairly rapid swimming.

Fig. 6. Individual swimming with flagella in the position characteristic for the species for fairly rapid movement; haptonema extended but coiled up on itself and trailing behind body.

Fig. 7. Individual gliding without rotating with flagella in position characteristic for the species during gliding movement.

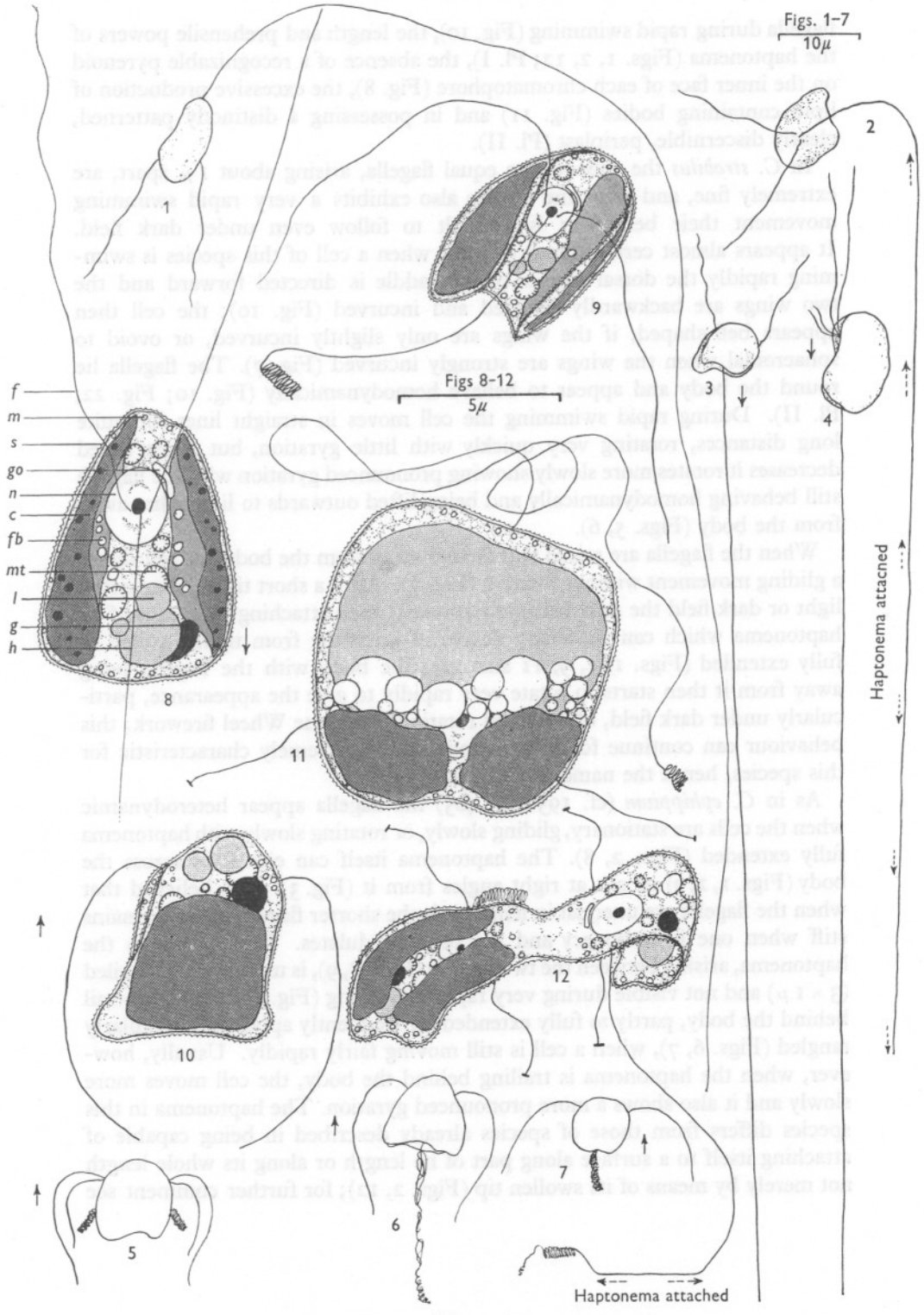
Fig. 8. Kite-shaped cell (ventral surface) moving slowly with haptonema fully extended in front of body, one flagellum slowly undulating, the other stiff or gently vibrating. *c*, chromatophore containing saturated lipid globules stained by Sudan Black; *f*, flagellum; *fb*, lipid globules; *g*, graphite; *go*, golgi; *h*, haptonema; *l*, leucosin vesicle; *m*, muciferous body; *mt*, mitochondrion; *n*, nucleus; *s*, 'cup'-shaped scale.

Fig. 9. Anchored cell with haptonema partly coiled and flagella lying out from body.

Fig. 10. Individual swimming with flagella and body in the position characteristic for the species during very rapid swimming.

Fig. 11. Cell containing numerous lipid globules and a large leucosin vesicle from a culture grown in strong light for 10 days; haptonema nearly fully extended, coiled on itself and attached only at tip.

Fig. 12. Late fission stage, one daughter-cell without chromatophores.


Figs. 1–7
10μ
Figs 8–12
5μ
f
m
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1
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8
9
10
11
12
Haptonema attacned
Haptonema attached

Figs. 1–12

flagella during rapid swimming (Fig. 10), the length and prehensile powers of the haptonema (Figs. 1, 2, 12; Pl. I), the absence of a recognizable pyrenoid on the inner face of each chromatophore (Fig. 8), the excessive production of lipid containing bodies (Fig. 11) and in possessing a distinctly patterned, clearly discernible, periplast (Pl. II).

In *C. strobilus* the subequal to equal flagella, arising about 1 μ apart, are extremely fine, and since the species also exhibits a very rapid swimming movement their behaviour is difficult to follow even under dark field. It appears almost certain, however, that when a cell of this species is swimming rapidly the dorsal surface of the saddle is directed forward and the two wings are backwardly directed and incurved (Fig. 10); the cell then appears bell-shaped, if the wings are only slightly incurved, or ovoid to sphaeroidal when the wings are strongly incurved (Fig. 7). The flagella lie round the body and appear to behave homodynamically (Fig. 10; Fig. 22, Pl. II). During rapid swimming the cell moves in straight lines for quite long distances, rotating very quickly with little gyration, but when speed decreases it rotates more slowly showing pronounced gyration with the flagella still behaving homodynamically and being lifted outwards to lie further away from the body (Figs. 5, 6).

When the flagella are raised still farther away from the body the cell shows a gliding movement without rotation (Fig. 7). After a short time under either light or dark field the cells become stationary, each attaching by means of its haptonema which can show any degree of uncoiling from tightly coiled to fully extended (Figs. 1, 2, 9, 11 and 12); the body with the flagella lying away from it then starts to rotate very rapidly to give the appearance, particularly under dark field, of a lighted rotating Catherine Wheel firework; this behaviour can continue for long periods and is extremely characteristic for this species, hence the name *C. strobilus*.

As in *C. ephippium* (cf. 1956, p. 405) the flagella appear heterodynamic when the cells are stationary, gliding slowly, or rotating slowly with haptonema fully extended (Figs. 2, 8). The haptonema itself can either lie across the body (Figs. 1, 2, 9) or out at right angles from it (Fig. 3). It is believed that when the flagella are unequal in length it is the shorter flagellum that remains stiff when one is stationary and the other undulates. In *C. strobilus* the haptonema, arising between the two flagella (Figs. 8, 9), is usually tightly coiled (3×1 μ) and not visible during very rapid swimming (Fig. 10) but it can trail behind the body, partly to fully extended, or frequently appearing irregularly tangled (Figs. 6, 7), when a cell is still moving fairly rapidly. Usually, however, when the haptonema is trailing behind the body, the cell moves more slowly and it also shows a more pronounced gyration. The haptonema in this species differs from those of species already described in being capable of attaching itself to a surface along part of its length or along its whole length not merely by means of its swollen tip (Figs. 2, 12); for further comment see

p. 177. In this species also it took a great deal of time and patience to obtain measurements of 100 fully extended haptonemata since a cell with a fully extended haptonema when brought into the light or dark field for measurement will immediately retract its haptonema and swim away. Although the species lacks an obvious stigma it shows a definite phototactic reaction.

In an actively growing culture 65% of the cells are between 6 and 9 μ in size and a culture at the peak of growth contains up to $\frac{1}{2}$ million cells per ml. Occasionally cells lacking chromatophores are seen in the stock cultures (Fig. 12), but in cultures treated with penicillin and streptomycin the colourless cells are not uncommon; they are usually smaller (4–6 μ) than those possessing chromatophores.

Colourless forms, undoubtedly belonging to this species, have also been recorded from sea-water samples as well as their frequent occurrence being recorded from mixed cultures grown from sea-water samples.

Phagotrophy occurs very frequently (Figs. 8, 12; Fig. 18, Pl. I; Fig. 35, Pl. VI), the cell ingesting graphite, bacteria and plant cells usually up to 4×3 μ but in a mixed culture set up from a sea-water sample a cell of *C. strobilus* was seen to have engulfed a 15 μ long Naviculoid diatom, but the ends of the diatom were sticking out of the body. Direct examination of sea-water samples has also shown that *C. strobilus* does exhibit phagotrophy under natural conditions.

Contents of the body

Unlike *C. chiton* (1958), there is no external pyrenoid attached to the inner face of the chromatophore in *C. strobilus* and the lamellae in the chromatophores are less obvious, but, as in *C. chiton*, saturated lipid globules are present between the lamellae of the outer face of the chromatophore. Additional globules of unsaturated lipid material are also present in the body, as in *C. chiton* (1958, p. 221), but in *C. strobilus* they are generally more numerous. In the cells from an actively growing culture of *C. strobilus* there are from 1 to 4 of these lipid globules (*ca.* 0·5 μ) lying near to or against the inner face of each chromatophore (Fig. 8; Fig. 28, Pl. III; Fig. 34, Pl. IV). As growth in a culture slows down or when a culture has been grown in strong light (112 ft.c.) for 10 days the number and size of these globules increases very greatly so that a cell can contain from 10 to 20 (Fig. 11), some measuring up to 1·5 μ in diameter. This great increase in lipid material in the cell may be due to low nitrogen content of the medium (see Fogg, 1956). A control culture grown in weak light (8 ft.c.) for the same period showed that the cells still had only 1 to 4 small globules to each chromatophore.

All lipid globules stain orange to orange red with Sudan IV but only those free in the body react with osmium tetroxide becoming yellowish brown in the centre and dark brown or black on the outside. Using an unpublished

method for the staining of phospholipids given to one of us (M.P.) by Dr G. Y. Kennedy of Sheffield University the following results were obtained. The small globules between the lamellae of the outer face of the chromatophores gave a reaction for lipids of the 'cephalin' group whereas the larger globules free in the body (originating from the inner storage face of the chromatophore?) sometimes gave a staining characteristic for lecithins in the centre while a layer round the outside of the globule gave a reaction for cephalins. Two or three fairly small vesicles of leucosin are generally present in this species, lying in the body usually towards the non-flagellar end of the saddle. When a cell is moving rapidly, however, the leucosin can frequently be seen pushed to the centre of the dorsal surface and forming a slight bulge in the front of the cell while the chromatophores are drawn back into the wings (Fig. 10). The cells which showed a great increase in the number and size of the lipid globules after being grown in very strong light also showed the production of very large leucosin vesicles which sometimes filled nearly half the volume of the cell (Fig. 11).

The nucleus, ovoid and up to $3 \times 2\ \mu$ in size, lies towards the ventral surface of the saddle sometimes centrally in the body but more frequently excentrically, to the left of the centre when looking down on the ventral surface.

A dark bar-shaped body lying to one side of the nucleus and visible in the living cell stains up bright red with cresyl blue and as blue-green stripes with Janus green; this is almost certainly the golgi area (Figs. 8, 9, see p. 182 and Pls. V–VII). Four to six mitochondria can be distinguished by the use of Janus green; two or four, ($0{\cdot}5$–$0{\cdot}75\ \mu$), lie fairly close to the nucleus and two others, usually larger (1–$1{\cdot}5\ \mu$), lie towards the non-flagellar end of the saddle (Figs. 8, 9).

The muciferous bodies are small (*ca.* $0{\cdot}25\ \mu$) and not very conspicuous in this species (Fig. 8; Pl. V). They may be of a different structure to those described for other species (1955, 1956, 1958) since it is difficult to make them eject their contents even with the use of vital stains. *In situ* they stain a true clear blue round the outside with cresyl blue, but the contents do not appear to take up the stain. Most usually when ejection does occur with the use of dilute cresyl blue, the ejected mass appears to consist of two parts—a thicker part close to the body which becomes yellow to lime green and a much thinner mass which gradually balloons out from the thicker part and becomes a pale mauve colour; this suggests that the whole body may be ejected on a neck and that the thicker part is the organ itself—possibly not a natural procedure as the ejection is so difficult to obtain. Very occasionally, however, the ejection of short threads has been observed and, as in *C. ericina* (1956, p. 395), a small disk remains attached to the distal end of the thread.

When the cells are fixed with osmium tetroxide the contents shrink leaving the 'pellicle' or scale covering clearly visible; under high power it appears to

be covered by punctae. With cresyl blue and methylene blue the pellicle frequently bursts and the cell contents are extruded leaving the 'pellicle' practically complete (cf. *C. chiton*, 1958, and see p. 181).

Reproduction

Fission in the motile phase occurs as in *C. alifera* (1956, p. 413) and takes place most frequently in late afternoon (Figs. 4, 5, 12). Non-motile stages similar to those already described for other species (1955, 1956, 1958) are then produced. The large amoeboid and walled cells with very finely lobed chromatophores, as in *C. alifera* (1956), measure from 14×11 to $17 \times 12\ \mu$. The ovate to sphaeroidal daughter-cells have thin walls and finely lobed chromatophores and measure from 4×3 to $7 \times 5\ \mu$ in size. The partial but not the complete release of the motile stage from the walled daughter-cells has been observed.

OBSERVATIONS WITH THE ELECTRON MICROSCOPE—MORPHOLOGY

In addition to the observations already described from the light microscope, which include among others the measurement of relative length of the haptonema and its capacity, when alive, to become attached to a surface at any point along its length and not merely at the tip as in our previous species, the use of shadowcast whole mounts has added several significant facts by which this species is peculiar. One of these is the extreme elegance with which the haptonema uncoils, as this is expressed in dried specimens in a partly uncoiled condition. Several examples are included in Pl. I with greater detail of one of these in Fig. 27, Pl. II. The original coil itself is faintly visible in the undried cell of Fig. 18, Pl. I. The very regular configuration of two loops alternating to right and to left, often associated with the presence of apparent constrictions occurring at regular intervals along the haptonema, at first suggested to us that the whole organ might perhaps be ribbon-shaped rather than cylindrical and owe some of its properties to this cause. This suggestion is not borne out by sections, however, and therefore although in the dried condition the organ is undoubtedly flat, this flattening must in itself be attributed to the drying process. Nevertheless, it is possible that a tendency to become flattened may be greater in this than in any of our previous species, a circumstance which, if it were true, might explain the unusual ability of the haptonema to become attached throughout its length. For further discussion see p. 179.

Another peculiarity is the firmness with which the scales remain in position. In all our other species the scales have been so loosely attached both to each other and to the cell that they commonly litter the field either singly or in clusters which bear no relation to their original position. In this species

scales are only rarely encountered singly. With fresh material killed directly onto the formvar film (our usual method) the scales generally appear as compact uniform sheets often still in relation to the cell body (Figs. 22, 25, Pl. II), and with the component scales held in a very regular close-packed arrangement (Fig. 25) suggesting that they are partially embedded in some kind of a matrix, the whole forming a coherent pellicle-like mat which tends to behave as a unit. When cells are not killed in this way but treated in any other of our occasional procedures such as with iodine as a preliminary to stripping from glass, the scale-mat may cohere completely to the body surface and be undetectable (Fig. 23). In embedded material, on the other hand, pieces of 'scale-mat' with the scales still in position are abundant and easily recognized (for further details see p. 181); we may therefore be certain that the close-packing seen in whole mounts is here not accidental.

There are undoubtedly two types of scale present. This is not obvious from the surface view of a scale mat which (Fig. 25) generally shows only a compact and even array of small featureless scales with raised rims. But occasionally loose scales of slightly larger diameter and with faint surface striations are encountered (Figs. 25 (top right), 26). Once seen these can with care always be detected near sufficiently dismembered cells, but they are so very thin that it is never easy to determine their finer details. Nothing can be ascertained with certainty from whole mounts about their arrangement on the cell body.

OBSERVATIONS WITH THE ELECTRON MICROSCOPE—ANATOMY

The haptonema

The great length of this organ in our present species compared with *C. chiton* (1958) is at once revealed by the clusters of much more numerous component sections which can be encountered; compare, for example, Fig. 30, Pl. IV, with Pl. IV of our previous paper. Since the structure of this organ was the most important first object of inquiry and the only one for which the American microscope was used (cf. p. 170) it will be convenient to describe it first.

As may be seen at a glance in Fig. 30, Pl. IV, the haptonema here, as in our previous species, is only about half the width of a flagellum. The actual dimensions can be directly measured either in Fig. 30 or in several of those included in Pl. VIII, all of which indicate that, disregarding obvious distortions, the average width of cross-section is of the order of 0·2 μ. It should, however, perhaps be pointed out that higher magnifications than were achieved previously have been used for most of Pl. VIII and only the inset pictures on Fig. 39 are exactly comparable with some of those reproduced on Pl. IV of our previous paper.

Within the haptonema the salient anatomical features are comparable, though not identical, with those of *C. chiton*. As before, the most conspicuous

components are the three concentric membranes (using the term membrane rather loosely and without implications as to whether each is in fact single or compound) surrounding a ring of fibres (or tubes) and a central space. As before, the number of component fibres in the ring is extremely constant, having in this case been ascertained without any variation in thirteen specimens. It is, however, six and not seven as in the other species. Representative sections illustrating this number from three different cells are included in Pl. VIII (Figs. 39–41, Fig. 42 and Fig. 43 respectively), though the clearest individual section is doubtless that of Fig. 41. The diameter of each of the central fibres is of the order of 200–250 Å.

The three membranes of the haptonema wall are not all alike; the outermost one is undoubtedly compound. Its total thickness is rather less than 100 A, but when accurately cut a three-layered sub-structure consisting of two dense surface layers separated by a lighter central layer can be clearly resolved (see especially between the arrows in Figs. 41 and 40), the thickness of each of these components being of the order of 30 Å. or somewhat less. The other two membranes contained in the haptonema wall cannot be similarly resolved. They appear to be thinner than the outer triple membrane (see especially Fig. 41) and they are certainly more fragile since they often break under the action of the fixative (cf. Fig. 42). In so doing they frequently roll up and cease to be individually recognizable (see Fig. 39*c* and various parts of Fig. 40), an artifact which prevents effective discussion of the possible presence of an additional component which is not membranous. Nevertheless, certain sections (e.g. Fig. 43 uppermost LS) do suggest rather strongly that there may be a fibrous component in the haptonema wall arranged roughly at right angles to the longitudinal direction marked by the position of the central fibres. Further evidence will, however, be required before this component, if genuine, can be located with certainty.

A minor peculiarity which might perhaps be connected with the special properties referred to in the first paragraph of this section is that the central fibres seem less firmly attached to the innermost membrane than was the case in *C. chiton*. In our former species the union was so close that we were at first in doubt whether the fibres themselves were in fact thickenings or intuckings of the inner membrane and it was only in specimens considerably distended by 'blistering' that their separation could be clearly demonstrated (as in Fig. 42 here). In our present species it is the exception rather than the rule for the inner fibres to be found still touching the inner membrane, e.g. in insets *a* and *b* in Fig. 39. More usually, e.g. Figs. 41 and 43, the fibres are free from the membrane and are tending to become clustered together in the centre of the organ thus partially obliterating the cavity. Since it is an unavoidable condition of working with killed cells that we can only observe structure in relaxed coiled haptonemata and not in extended ones, it is impossible to know whether this difference from *C. chiton* is trivial or of functional significance.

The uppermost section included in the right-hand top corner of Fig. 39 perhaps represents the region of the thickened haptonema tip. It contains no interpretable structure except a solid centre surrounded by one membrane. If its nature had been more fully demonstrated, these observations would be important, but until they can be confirmed by additional, or by better, specimens we can only quote this one as possibly of this nature. We have no observations on the structure of the basal end of the haptonema.

Explanation of Plates I–IV

Chrysochromulina strobilus sp.nov.

I

Fig. 14. A cell dried on glass and photographed with the light microscope. × 1000.
Fig. 15. As Fig. 14, another cell. × 1000.
Fig. 16. Flagella and haptonema from the cell of Fig. 15 photographed by phase contrast. × 2000.
Fig. 17. The specimen of Figs. 15 and 16 after transfer to formvar, viewed with the low power of the electron microscope. Micrograph M 166.9, × 3000.
Fig. 18. A cell killed after ingesting graphite and photographed in a liquid mount under oil immersion; the central pellet of graphite and two lateral plastids appear dark, the coiled haptonema attached to the lower part of the body appears light. × 2000.
Fig. 19. As Fig. 14, another cell. × 1000.
Fig. 20. As Fig. 14, another cell. × 1000.
Fig. 21. The cell of Fig. 20 after transfer to the electron microscope. Micrograph M. 152.23, × 3000. For further details from this specimen see Fig. 25, Pl. II.

II

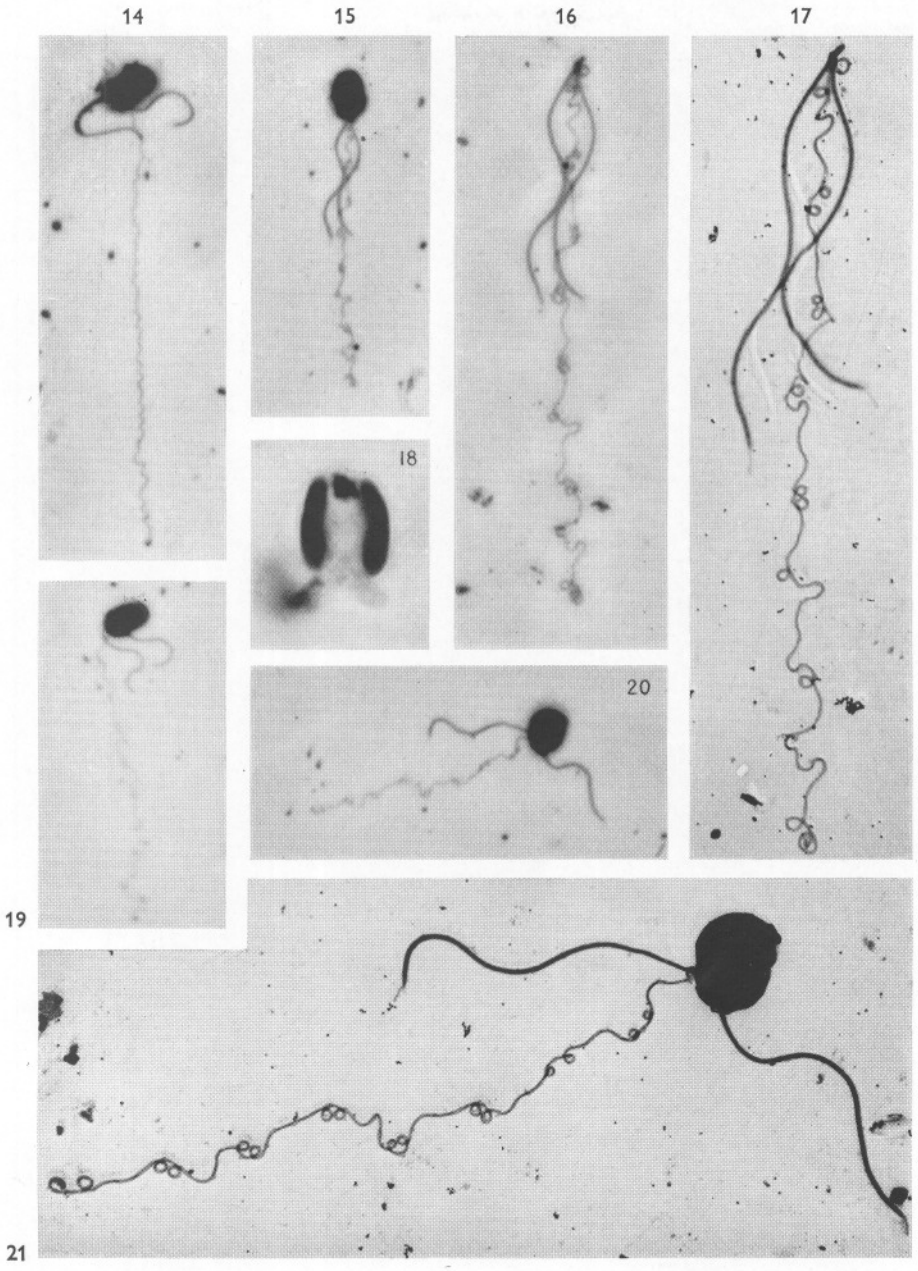
Fig. 22. A flattened cell seen with the electron microscope showing the layer of scales still in position outside the more opaque body. Micrograph M. 327.3, × 3000.
Fig. 23. Part of a cell with flattened appendages, stripped from a glass preparation. The partial decomposition of the haptonema has revealed a slender axis within translucent material (cf. with Fig. 27). × 10,000.
Fig. 24. Tip of a flagellum. M. 284.16, × 10,000.
Fig. 25. The edge of a scale-mat from a specimen comparable to that of Fig. 22, the cup-shaped scales still in position and apparently embedded in some amorphous material; two separate plate-scales appearing at the top of the figure. Micrograph M. 135.4, × 20,000.
Fig. 26. Free scales of the two types, the plate-scales in the upper part of the figure very transparent and difficult to record, the cup-shaped scales in the lower part of the figure projecting slightly from the surface of the mount. Micrograph M. 336.13, × 30,000.
Fig. 27. Tip of the haptonema of Fig. 21, more highly magnified, showing the swollen tip and configuration characteristic of uncoiling, in a less damaged specimen than that of Fig. 23. Micrograph M. 152.25, × 10,000.

III

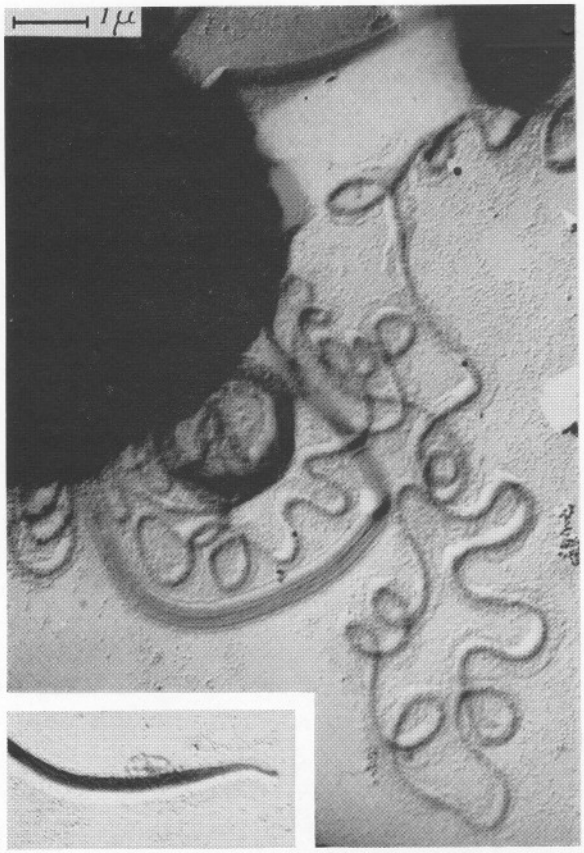
Fig. 28. Section of a cell with scales on the surface, showing nucleus (*N*) 2 plastids (*P*), a flagellar base (*fb*), two black lipoid bodies and part of a third, mitochondria, golgi area and miscellaneous vesicles. Micrograph H. 1837, × 15,000.
Fig. 29. Surface of a similar cell with scales in position (for further details see Fig. 44, Pl. VIII). Micrograph H. 1733, × 30,000.

IV

Fig. 30. Section of a cell with related haptonema (*H*) and flagella (*f*) near two detached pieces of scale mat (*S*), one inverted with respect to the other. Micrograph H. 1830, × 15,000.
Fig. 31. Part of Fig. 30 to show details of the storage region in the plastid; arrows point to sections of paired tubes which traverse it. Micrograph H. 1831, × 30,000.



(*Facing p.* 180)



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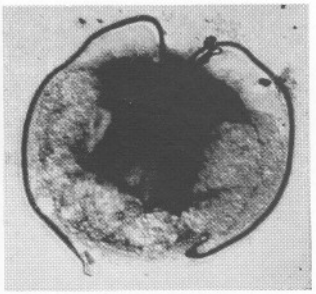

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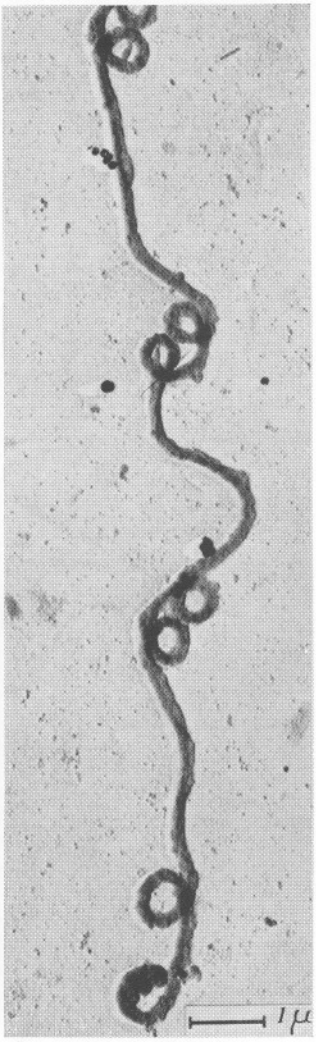

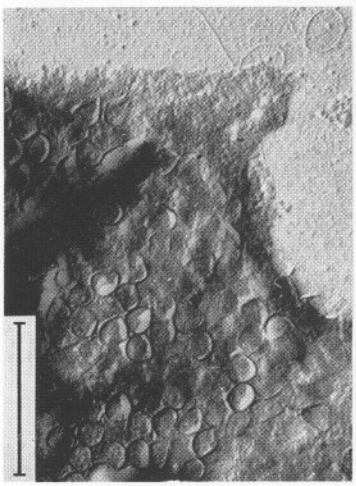

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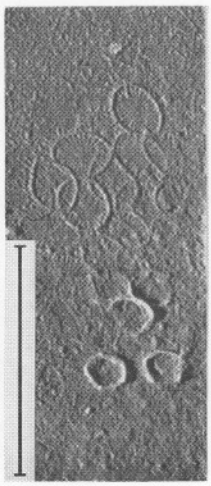

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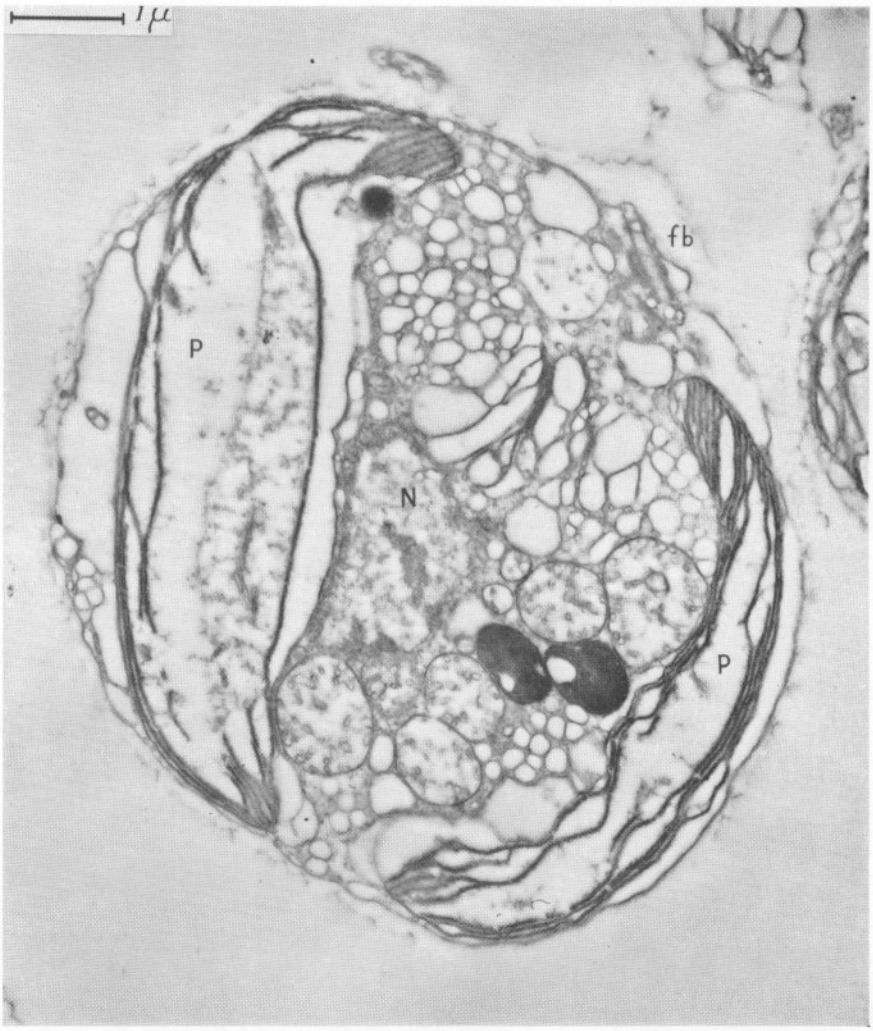

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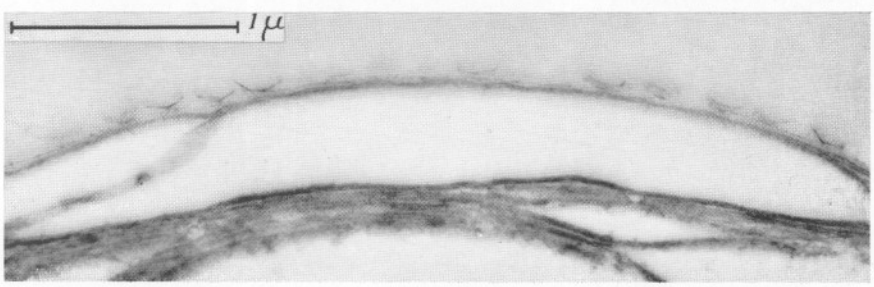

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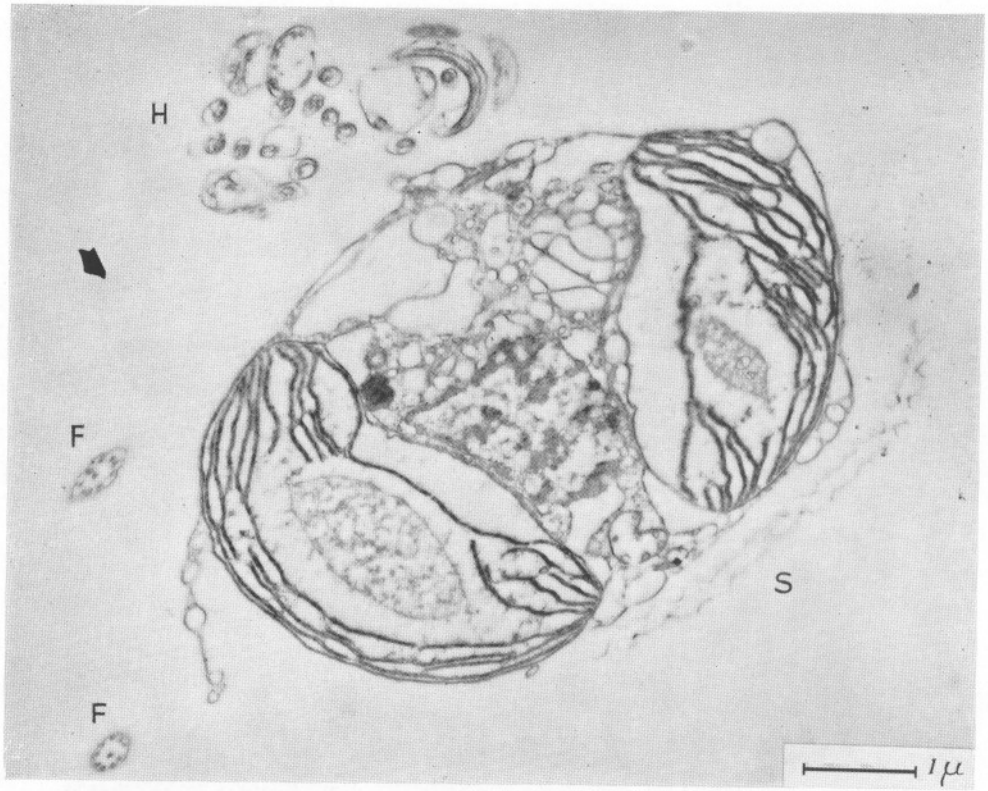

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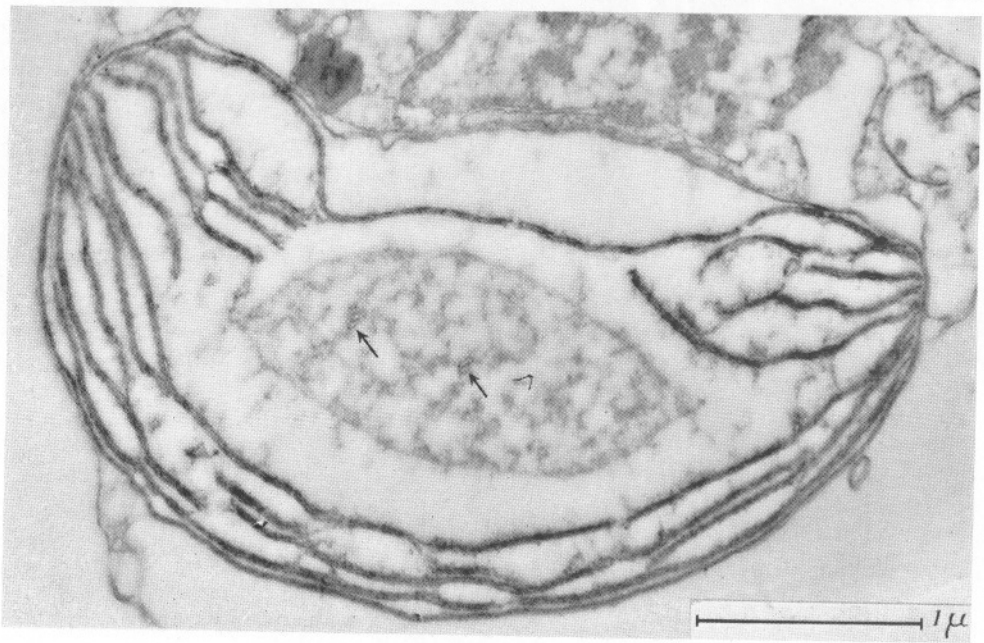


31

The scales

Fig. 44, Pl. VIII (for explanatory diagram see Fig. 13), shows a section of scales in position on the body at a magnification comparable to that of the adjacent haptonema sections (i.e. 60,000 as in Figs. 42 and 43). The cup-shaped scales with conical thickened base are arranged in a very regular layer, as was to be expected from the external view. Beneath them are the plate scales, also in a single layer; each is a very delicate sheet with a slight central thickening and a rim projecting up from both surfaces like the edge of a coin. Other less highly magnified sections showing the scales still in position on the cell are contained in Pl. III and elsewhere.

Fig. 13. Diagram traced over a photographic print to show the arrangement of scales in Fig. 44, Pl. VIII, magnification of the diagram × 80,000.

Detached scales, still arranged in sheets, are common. Two such sheets, one inverted with respect to the other, are situated near the letter S on Fig. 30, Pl. IV. Neither can be directly related to the adjacent cell since the sheet nearest to the body surface is upside down with respect to it. When cut transversely the conical cup-scales, arranged side by side, produce an effect as of a delicate zig-zag line at this low power. The amorphous material partially investing the scales on dried material is no longer visible in sections but this is likely to be due to solution in the methacrylate since the regular array of scales in each sheet is undisturbed. This can be seen no less clearly when a sheet is cut tangentially as in the top corner (right) of Fig. 32, Pl. V. In such an aspect the scales appear circular with a central opaque area; there is again no sign of intercalary material between them but they are in exactly the same serial arrangement as in Fig. 25, Pl. II.

Beneath the scales is a membrane, as in *C. chiton*, and sometimes though not always a layer or layers of small vesicles. The diameter of these vesicles is of the same order as that of the scales. Since there were no superficial vesicles in *C. chiton* as small as this we may perhaps find this fact to be significant when developmental processes can be more seriously considered.

The internal organs

A glance at the low-power views of complete sections reproduced in Pls. III–V will sufficiently indicate the general positions of the more important body organs. The chromatophores, nucleus, mitochondria and food vacuole are labelled as such in Fig. 32, Pl. V. In addition the lipid bodies in the cell interior and the muciferous bodies on the surface are conspicuous by their

opacity, a property connected with their chemical effect on the osmic fixative. Leucosin vacuoles or equivalent spaces bounded by a membrane appear empty.

Some of these organs or organelles resemble those of *C. chiton* sufficiently closely to be passed over without further comment; the nucleus, mitochondria and food vacuole fall into this category. Differences of a fairly striking kind do nevertheless exist and since we are still without sufficient knowledge to be able to distinguish characters which may be of generic importance from others which only delimit taxa of a lower order, it will be necessary to enumerate differences as fully as possible. The following comments will therefore be restricted to this topic.

The pyrenoid

The most conspicuous difference between the internal organs of *C. chiton* and our present species concerns the pyrenoid. In *C. chiton* this was a subspherical body filled with dark contents and attached by a narrow neck to the inner face of a chromatophore. No such bodies can be detected in our present species. Instead there is an internal storage region within the chromatophore, often of considerable extent and containing similar dark contents. A good example is illustrated in Pl. IV and parts of others will be detectable elsewhere. The stored material is traversed by some highly characteristic narrow paired tubes which are almost certainly continuous with some of the lamellations of the pigmented part of the chromatophore although we have not on this occasion reproduced any micrographs illustrating this continuity. Examples of the tubes in question, cut in slightly oblique TS are indicated by arrows in Fig. 31, Pl. IV.

The flagellar bases

These have not been studied in great detail here but it is necessary to locate them before attention can be given to the organelle to be discussed in the next paragraph. They differ a little from the situation in *C. chiton* by the extreme obliquity with which they are inserted. This is clearly indicated in Fig. 28, Pl. III, where a flagellar base (fb) in almost median LS is lying almost parallel to the cell surface. This is a highly characteristic position and we have never encountered in this species the almost vertical orientation of flagella to the surface which was illustrated, for example, in Fig. 34, Pl. IX, of our previous paper. When both flagellar bases are contained in the same section they make a very obtuse angle with each other as in Fig. 34, Pl. VI.

Golgi

Immediately below the attachment of the flagellar bases is an area occupied by vesicles of the characteristic kind previously diagnosed as golgi. The golgi area is commonly bounded by the nucleus, mitochondria and chromatophores

on the sides towards the cell (e.g. Fig. 28, Pl. III and Fig. 32, Pl. V), though it is never separated from the flagellar bases by any of these organs, and it is not delimited by a membrane. When cut tangentially or merely grazed, only the compacted vesicles and traces of flattened paired membranes are visible (e.g. Figs. 28, 32). When cut more nearly centrally, however, a surprising complexity of structure is revealed. Pls. VI and VII are inserted to illustrate this.

In Pl. VI are two cells cut in planes approximately at right angles to each other but both passing through the central region of the golgi area. A more highly magnified view of one of these cells is reproduced in Fig. 38, Pl. VII, while the two upper figures on Pl. VII are similarly magnified views of two different sections from one specimen resembling Fig. 34 in plane of cutting. Pls. VI and VII thus illustrate the golgi structure in three different cells.

The central part of the golgi area is most easily interpreted as a cavity occupying the concave side of the strongly curved outer paired membranes (flattened cisternae) into which many of the inner paired membranes are budding off vesicles. These vesicles are commonly somewhat distorted and perhaps burst as a fixation artifact leaving numerous fragments of membrane distributed through colourless liquid. For further discussion see p. 185.

Muciferous bodies

The only additional details to add about these organelles are firstly that they are not very numerous. A section displaying as many as appear in Fig. 32, Pl. V, is an exception selected for reproduction for this reason; the more usual condition is as in the other plates in which these bodies are only encounted here and there or not at all. In structure they recall those of *C. chiton* though they may perhaps be simpler. There is no suggestion here of any obvious modification of the outer wall, an undischarged body merely projecting a little above the surface of the cell (Fig. 33) without other detectable structural features. The contents appear slightly darker than in our micrographs of *C. chiton*, but a minor difference in fixation procedure could perhaps explain this.

DISCUSSION

While the more obvious comparisons, namely those with *C. chiton* (Parke, Manton & Clarke, 1958), have already been made, a few general comments remain. It is clear that the structural differences recorded fall into two rather different categories. On the one hand there are clearly determined divergences as in the pyrenoids and numerical details within the haptonema, on the other are observations which could depend in part on fuller knowledge of our present species. It may be recalled that for our previous paper there was only very limited access to high resolution microscopes and the amount of material surveyed was in consequence severely restricted. We cannot therefore be quite

certain without further investigation of *C. chiton* whether, for example, the very peculiar structure encountered in the centre of the golgi area in our present species is actually absent from our previous one or whether we failed to detect it for the reason given.

Explanation of Plates V–VIII

Chrysochromulina strobilus sp.nov.

V

Fig. 32. Section of a cell to show muciferous bodies (black) in the outermost layer. In addition the nucleus (*N*), mitochondria (*m*), a food vacuole (*FV*), spaces which could have contained leucosin, golgi vesicles near the base of the appendage (uncertain whether this is a flagellum or a haptonema). Arrow in the top right-hand corner points to a scale mat seen in surface view; elsewhere scales in position on the body and sections of bacteria in the ground. Micrograph H. 1637, × 18,000.

Fig. 33. Part of the surface of a similar cell to show the way in which full muciferous bodies (dark) project above the cell surface. Micrograph H. 1969, × 20,000.

VI

Fig. 34. Section of a cell to show golgi structures between the two plastids (*P*), and below the flagellar bases (*fb*). In addition (dark) lipid bodies, mitochondria and vesicles. Micrograph H. 1706, × 15,000.

Fig. 35. A similar cell cut in a plane roughly at right angles to the preceding showing the nucleus (*N*), a large food vacuole (*FV*) and the golgi area immediately above it; for further details see Fig. 38, Pl. VII. Micrograph H. 1833, × 15,000.

VII

Figs. 36, 37. Two sections, some distance apart, of a cell cut somewhat as in Fig. 34, to show the structure of the golgi area when cut near its edges (Fig. 36) and more centrally (Fig. 37). Signs of the flagellar bases (*fb*) visible in Fig. 36. Two fat bodies and the edges of adjacent plastids, arranged as in Fig. 34 visible laterally (see especially Fig. 36), in addition several mitochondria (*m*) and scattered vesicles. Micrographs H. 1619 and H. 1624, × 25,000.

Fig. 38. Detail of the golgi area in Fig. 35 more highly magnified, lettering as in that figure. Micrograph H. 1834, × 25,000.

VIII

Fig. 39. General view of a haptonema with inset details of three component sections (a, b, c) from the places marked by the upper arrows; lower arrow a significant region for Figs. 40 and 41; *fb* a flagellar base on what is probably the subtending cell. Micrographs RS. 80, × 25,000, and RS. 378, × 50,000 (insets).

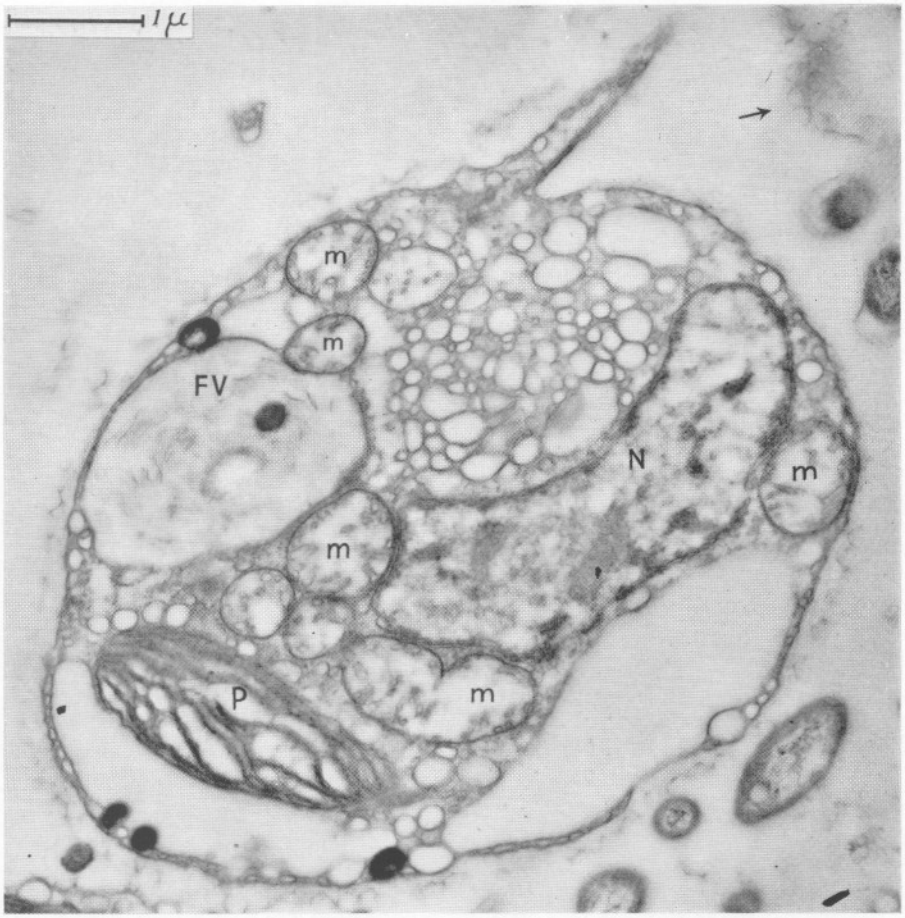
Fig. 40. Part of another section through the haptonema of Fig. 39 more highly magnified. Micrograph RS. 390, × 70,000.

Fig. 41. Part of the preceding more highly magnified and placed in a different attitude on the page; the six central fibres and three concentric membranes very clearly displayed with also (between the arrows) evidence that the outermost membrane is double. Micrograph RS. 390, × 100,000.

Fig. 42. Part of another specimen exhibiting 'blistering' but showing the three membranes and six central fibres very clearly when the inner membranes have split. Micrograph RS. 400, × 60,000.

Fig. 43. Part of another specimen to show a similar general anatomy in TS (lower arrow) and parts of an LS (upper arrow). Micrograph H. 1611, × 60,000.

Fig. 44. Section showing the two types of scale in position on a cell, the cup-shaped scales forming an outer layer and the plate-shaped scales below. Micrograph RS. 60, × *ca.* 60,000.



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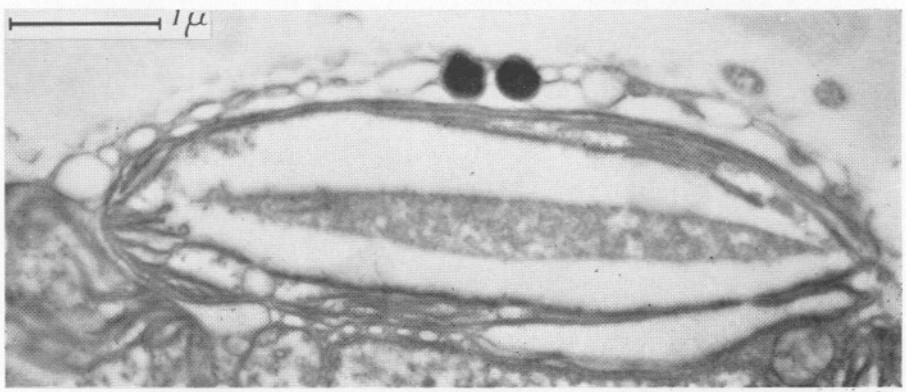

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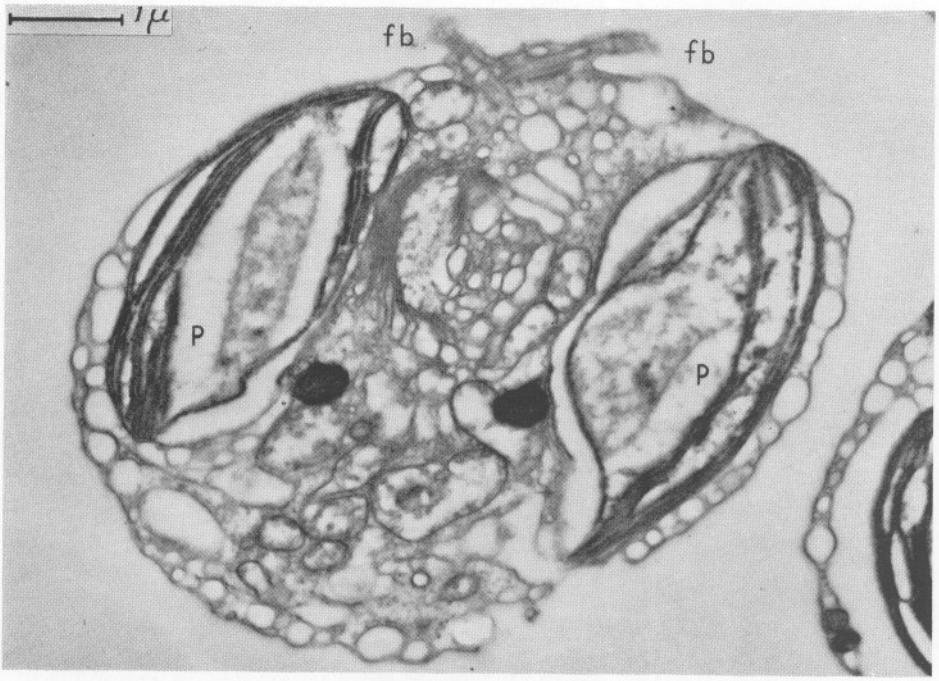

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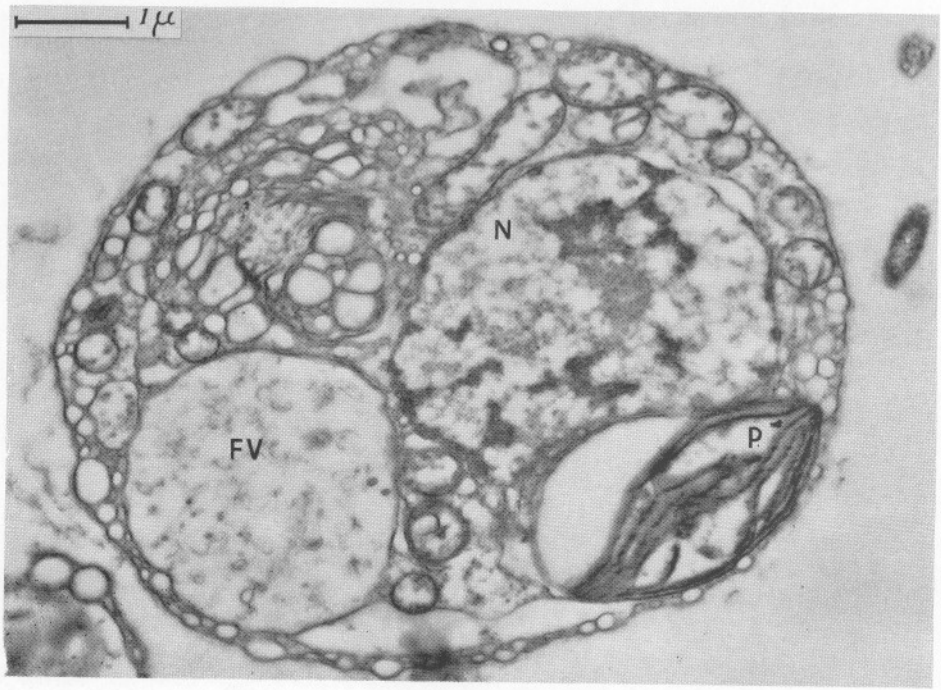

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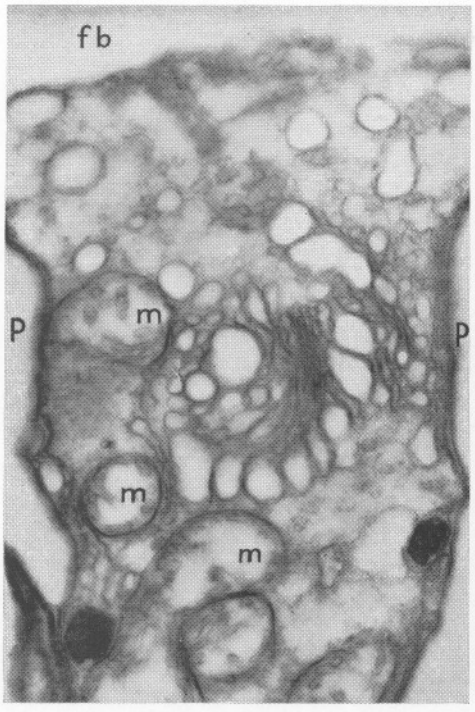

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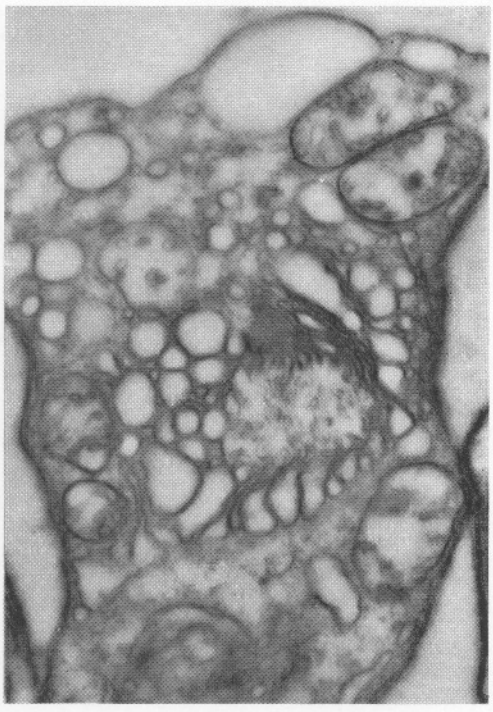

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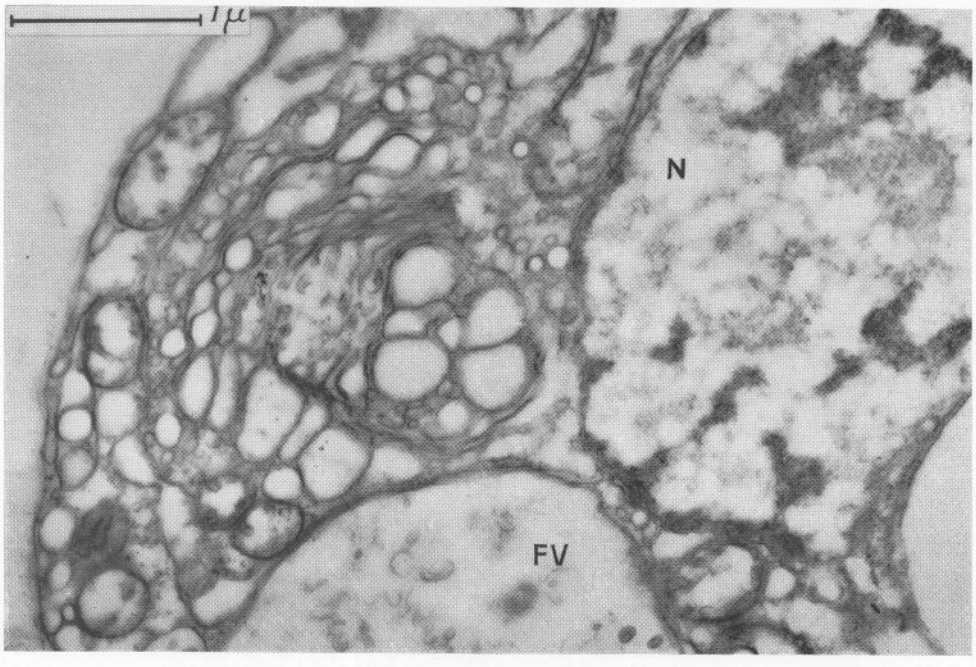

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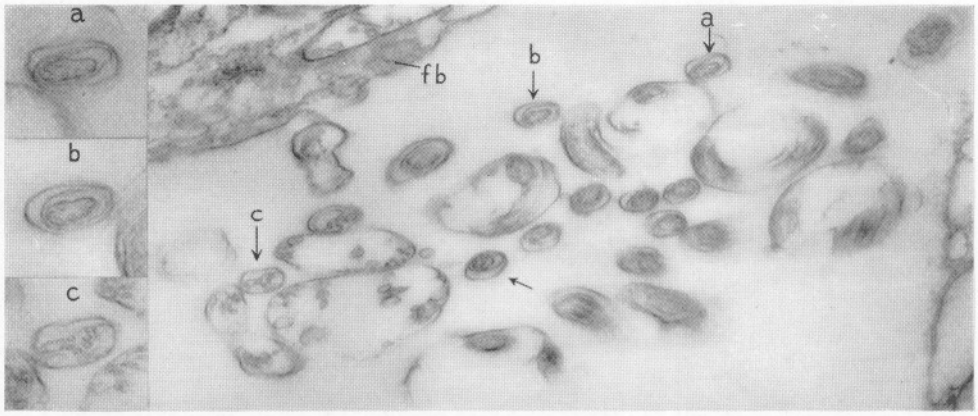

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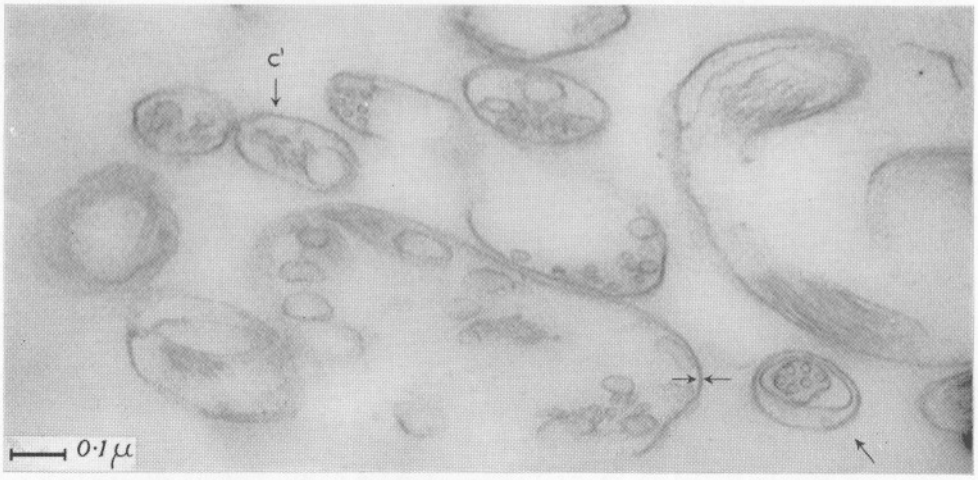

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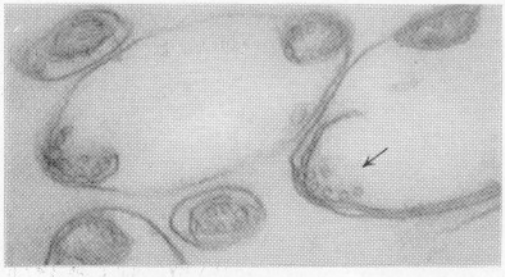

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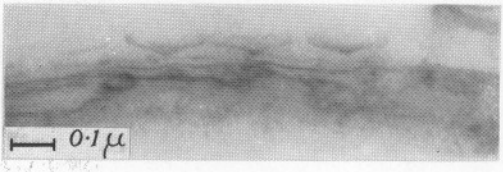

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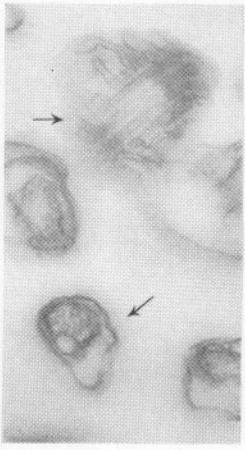

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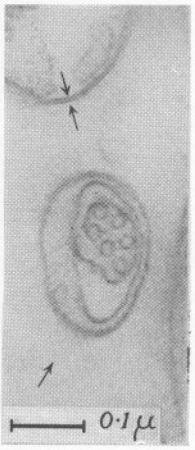

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There is, however, no doubt that the observations recorded for the centre of the golgi area in our present species are unusual, and for this reason we have at once taken steps to ascertain whether or not we are here dealing with an idiosyncrasy confined to one species. Preliminary sections of two other of our previously described species, *C. ericina* and *C. ephippium* of Parke, Manton & Clarke, 1956, have, however, at once revealed exactly comparable details, and since at least one of these species seems to be a more favourable object for study than our present one we propose to defer further discussion of this organ until more evidence can be surveyed.

We are on firmer ground with regard to the pyrenoid and haptonema for which positive differences from *C. chiton* have been demonstrated. A pyrenoid of the type encountered here recalls very closely that recently described for *Hydrurus* by Hovasse & Joyon (1957). We can say further (Manton, unpublished) that similar pyrenoids, differing only in minor features of size and position, are present also in *C. ericina* and *C. ephippium*. It is therefore possible that in this character *C. strobilus* rather than *C. chiton* may represent the norm within the group.

With regard to the haptonema structure, external comparisons are not yet possible since knowledge is at present limited to the two species under discussion. Comparison between these species is, however, instructive in more than one way. The numerical difference in fibre number, 7 in *C. chiton* and 6 in *C. strobilus*, is not to be explained away as a sampling error since both numbers have been determined with equal certainty through a range of specimens. Each number seems to have the constancy of a specific character, but this only underlines the fundamental difference between the haptonema and a flagellum for which complete uniformity of internal structure prevails, not merely from species to species but right through the plant and animal kingdoms. The resemblances, on the other hand, are no less important since here we may believe that the two species are both contributing essential information towards a descriptive understanding of this complex and peculiar organ, which though still incomplete, is significantly enhanced by the new facts contributed by *C. strobilus*. The triple structure of the outermost membrane of the haptonema wall is most unlikely to be a specific peculiarity confined to *C. strobilus* since similar membranes are now known to be a normal covering to many external protoplasmic organs, e.g. cilia and flagella. This very fact, however, indicates fairly clearly that the special properties possessed by the haptonema are likely to be determined by the internal parts about which less is known.

With regard to the scales the presence of two distinct types arranged one beneath the other in separate layers seems to remove entirely the possibility of continuous production of scales from the body surface at all times. It seems necessary to conclude that scale production must in some way be a cyclic phenomenon, a conclusion which was also, though less definitely, formulated in connexion with the rather different scale arrangement in *C. chiton*.

SUMMARY

A new species of *Chrysochromulina*, *C. strobilus*, characterized by a very long haptonema capable of attaching at any point along its length and by very small scales of characteristic pattern has been described. The anatomy of the haptonema consists, as in a previous species, *C. chiton*, of three concentric membranes surrounding a ring of fibres and a central space. The outermost membrane is three-layered and there are traces of some intercalary material between this and the middle membrane. The number of fibres in the inner ring is 6 and not 7 as in *C. chiton*; each fibre is of the order of 200–250 A. in width. The organs internal to the cell show two major differences from those of our previous species, notably in the pyrenoid and golgi area. The pyrenoid is inside the chromatophore; its structure is described. The golgi area shows a central structure which is so unusual that a full description is reserved until more species have been examined.

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APPENDIX

Table 1. RECORDED DISTRIBUTION OF *CHRYSOCHROMULINA STROBILUS* SP.NOV.

Date	Position N.	Position W.	Depth (m.)
12. xi. 48	50° 19·5′	04° 10′*	10
1. ii. 49	50° 19·5	04° 10′	10
8. vi. 49	50° 19·5′	04° 10′	10
12. iv. 50	49° 25′	03° 47′†	0·5
13. iv. 50	50° 15′	04° 13′	0·5
4. v. 50	50° 15′	04° 13′	0·5
9. v. 50	49° 21′	04° 54′†‡	0·5
23. viii. 50	Tamar Estuary off Torpoint		—
20. ix. 52	Off the Azores§		0·5
17. i. 56	50° 02′	04° 22′	20
29. v. 56	50° 06′	04° 21′	5
29. xi. 56	50° 07′	04° 23·5′	0·5
2. i. 57	50° 03′	04° 04·5′	0·5
19. vi. 57	50° 09′	04° 15′	—
16. vii. 57	50° 02′	04° 22′	0·5
24. vii. 57	50° 11′	04° 14′	0·5
17. ix. 57	50° 02′	04° 22′	0·5, 5, 10, 20, 70
17. ix. 57	50° 06′	04° 21′	0·5
	50° 11′	04° 13′	0·5
	50° 15′	04° 13′	0·5
	50° 18′	04° 11′	0·5
	50° 20′	04° 10′	0·5
15. x. 57	50° 11′	04° 13′	0·5
	50° 15′	04° 13′	0·5
6. xi. 57	Tamar Estuary off Forder Mill		—
3. xii. 57	50° 02′	04° 22′	5, 10, 20, 70
3. xii. 57	50° 06′	04° 21′	0·5
3. xii. 57	50° 11′	04° 13′	0·5
	50° 15′	04° 13′	0·5
	50° 18′	04° 11′	0·5
	50° 20′	04° 10′	0·5
21. i. 58	50° 02′	04° 22′	20, 50
21. i. 58	50° 06′	04° 21′	0·5
	50° 15′	04° 13′	0·5
	50° 15′	04° 11′	0·5
	50° 20′	04° 10′	0·5
18. ii. 58	50° 02′	04° 22′	0·5
			5, 10, 20, 50, 70
18. ii. 58	50° 06′	04° 21′	0·5
	50° 15′	04° 13′	0·5
18. ii. 58	50° 18′	04° 11′	0·5
	50° 20′	04° 10′	0·5
16. iii. 58	47° 40′	07° 13′	10, 20
18. iii. 58	47° 46′	07° 05′	20
19. iii. 58	50° 02′	04° 22′	0·5, 5, 10, 20, 50, 70
19. iv. 58	46° 30′	08° 00′	10
20. iv. 58	47° 30′	07° 18′	10
	47° 38′	07° 10′	10, 225
21. iv. 58	47° 46′	07° 05′	10, 20, 163
22. iv. 58	50° 02′	04° 22′	0·5, 5, 10, 20, 50, 70
20. v. 58	50° 02′	04° 22′	5, 10, 20, 50, 70
20. v. 58	50° 06′	04° 21′	0·5
	50° 11′	04° 13′	0·5
	50° 15′	04° 13′	0·5
	50° 18′	04° 11′	0·5
	50° 20′	04° 10′	0·5
10. vi. 58	50° 02′	04° 22′	0·5, 5, 10, 15, 20, 50, 70
10. vi. 58	50° 06′	04° 21′	0·5
	50° 15′	04° 13′	0·5
	50° 18′	04° 11′	0·5
9. vii. 58	50° 02′	04° 22′	0·5, 5, 10, 70
9. vii. 58	50° 11′	04° 13′	0·5
	50° 15′	04° 13′	0·5
	50° 18′	04° 11′	0·5
26. viii. 58	50° 02′	04° 22′	0·5, 5, 20, 30, 50, 70
26. viii. 58	50° 06′	04° 21′	0·5
1. x. 58	50° 02′	04° 22′	0·5, 5, 10, 20, 50
22. x. 58	50° 02′	04′ 22′	0·5, 10, 20, 50, 70
	50° 20′	04′ 10′	0·5
18. xi. 58	50° 02′	04° 22′	0·5
	50° 06′	04° 21′	0·5
	50° 11′	04° 13′	0·5
	50° 15′	04° 13′	0·5
	50° 18′	04° 11′	0·5

* Strain of *Chrysochromulina strobilus* (Plymouth no. 4) isolated from this sample.

† Samples brought in by 'Sir Lancelot'.

‡ Type culture strain of *Chrysochromulina strobilus* (Plymouth no. 43) isolated from this sample.

§ Sample brought in by 'Discovery II'.

TABLE 2. SEASONAL DISTRIBUTION OF *CHRYSOCHROMULINA STROBILUS* SP.NOV.

	International Station E 1								Plymouth Laboratory Stations				
									L6	L5	L4	L3	L2
Depth (m) ...	0·5	5	10	15	20	30	50	70	0·5	0·5	0·5	0·5	0·5
Date													
17. ix. 57	3	3	3	—	3	—	0	1	2	3	2	2	3
15. x. 57	0	0	0	—	0	—	0	0	0	1	1	0	0
6. xi. 57	1	—	0	—	0	—	—	—	—	—	—	—	—
3. xii. 57	0	2	3	—	5	—	0	2	1	1	1	1	1
21. i. 58	0	0	0	—	2	—	1	0	1	0	2	1	2
18. ii. 58	2	3	3	—	4	—	1	3	2	0	3	2	3
20. iii. 58	3	3	4	—	4	—	5	5	—	—	—	—	—
22. iv. 58	6	6	6	—	4	—	3	3	—	—	—	—	—
20. v. 58	0	2	3	—	3	—	2	2	3	4	4	2	1
10. vi. 58	5	5	6	4	3	—	2	4	5	0	1	1	0
9. vii. 58	1	2	1	0	0	—	0	1	0	2	2	2	0
26. viii. 58	4	1	0	—	1	3	3	3	4	0	0	0	0
1. x. 58	2	2	3	—	1	—	1	0	—	—	—	—	—
22. x. 58	2	0	1	—	1	—	1	1	0	0	0	0	2
18. xi. 58	1	—	0	—	0	—	0	0	2	1	1	4	0

6, min. no. *C. strobilus* per l. 1000 — E 1 50° 02′ N., 04° 22′ W.
5, min. no. *C. strobilus* per l. 800 — L 6 50° 06′ N., 04° 21′ W.
4, min. no. *C. strobilus* per l. 600 — L 5 50° 11′ N., 04° 13′ W.
3, min. no. *C. strobilus* per l. 400 — L 4 50° 15′ N., 04° 13′ W.
2, min. no. *C. strobilus* per l. 200 — L 3 50° 18′ N., 04° 11′ W.
1, min. no. *C. strobilus* per l. 10 — L 2 50° 20′ N., 04° 10′ W.
0, Absent from sample