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STUDIES ON MARINE FLAGELLATES

VI. *CHRYSOCHROMULINA PRINGSHEIMII* SP.NOV.

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(With total of 36 Figures in text and on Plates I–VI)

The most striking feature of our present species is the elaborately constructed scaly casing from which the monad can readily disengage itself and swim away leaving the empty case apparently intact. Such discarded cases are easily seen with the light microscope and they make very rewarding objects of study with the electron microscope. Our main attention has indeed been devoted to them since in other respects the monad is unremarkable. It possesses the normal two flagella and haptonema of a *Chrysochromulina*, practices phagotrophic feeding both when encased and when naked, and as far as the internal details can be ascertained with the light microscope is a typical member of this genus.

It should, however, be noted at the outset that this interpretation may perhaps need revision if ever it becomes possible to elicit the full life-history. Under existing cultural conditions the only stage manifested is that of the free-swimming monad, and we do not find the normal accumulation of dormant walled cells on the bottom of the flask characteristic of the other marine species described (Parke, Manton & Clarke, 1955–59). Since it is difficult to believe that any plankton flagellate could survive in nature without some provision for dormancy to tide over adverse conditions, we cannot exclude the possibility that we may have here a motile phase of some entirely different organism rather than a normal species of *Chrysochromulina* (cf. Parke & Adams, 1960; Parke, 1961). This possibility, nevertheless, does not detract from the intrinsic interest of the monad in itself nor alter the necessity of treating it provisionally as a *Chrysochromulina* on our present knowledge.

In choosing the name *pringsheimii*, we wish to record the debt that algology owes to a great algologist, Professor E. G. Pringsheim.

MATERIAL AND METHODS

Methods are exactly as in previous papers in this series, except that the absence of a resting stage makes it necessary to subculture this particular species very frequently (about every 10 days), or populations die out.

It was originally isolated from two different localities (see p. 400) in 1957, both isolations being examined electron-microscopically in a preliminary way in the same year and found to be indistinguishable. Only two micrographs from this preliminary work are reproduced here (Fig. 12, Pl. I, and Fig. 15, Pl. II) but these are sufficient to establish the constancy of the morphological characters in culture. In the fuller working out which was carried out in 1961 on descendants from the original isolates these have been treated as equivalent for all practical purposes, and they will not be separately distinguished in the description which follows.

Our thanks are due, among others, to Miss Irene Adams for her contribution to the original isolations and the subsequent maintenance of cultures, to Mr B. Clarke for some of the preliminary observations, Mr K. Oates for making the shadow-cast preparations and for much help in other aspects of the electron microscopy, Miss Sheila Wright for help in assembling the plates for publication, Miss D. Ballantine (Mrs B. Hepper) for testing this organism for its possible toxicity to fish, and the staff of the Plymouth Laboratory for the collection of sea-water samples. Finally, we are indebted to Dr T. Christensen of Copenhagen for translating the taxonomic description into Latin.

GENERAL MORPHOLOGY OF THE MONAD

The more obvious morphological features of this organism can be ascertained by perusal of Pl. I. Figs. 1–8 show various aspects of the behaviour in life taken with the light microscope. Figs. 6 and 7 illustrate phagotrophically ingested graphite. The body shape of an encased cell is best seen in Fig. 3. An extended haptonema on a swimming cell appears in Fig. 1 with various degrees of coiling in Figs. 2 and 3. Figs. 4 and 5 show characteristic attitudes of cells detaching themselves (backwards) from their scale-cases which can usefully be compared with the drawings, Figs. 35–36, on p. 399. Finally, Fig. 8 shows a living division stage actively swimming to the right and with the flagella and haptonemata of the two daughter-cells in attitudes characteristic of forward swimming (right-hand cell) and backward swimming (left-hand cell). These can also profitably be compared with the drawings on p. 399.

When mounts of whole cells are prepared for the electron microscope the appendages, unlike the scale-case, are so fragile that we have not been able to present a complete cell with all its parts intact. Figs. 9–12, nevertheless, give sufficient information regarding the two equal flagella and the relatively short

haptonema, as well as of the change of body shape in naked cells (Fig. 12) as compared with encased cells (Figs. 9 and 3).

THE SCALY CASING

With the light microscope and working with a fresh liquid mount it is possible to distinguish the terminal spines on swimming encased cells without difficulty (Figs. 1, 2, 4 and 8, Pl. 1; Fig. 18, Pl. IV) and also, within empty cases, a brightly refringent lining layer (Fig. 13, Pl. II). If the preparation has been dried the lining layer apparently vanishes.

With the electron microscope (Figs. 14, 15, Pl. II, et seq.) the same observations can be made though of course with much greater elaboration of detail. The number of terminal spines is liable to be reduced by damage during drying, but since it is unlikely to be increased by this means the maximum numbers of large spines on otherwise intact cases are significant. They range from three to eight (up to fourteen on early fission stages) and the two ends of the case can be similar or different.

The smaller spines, which cannot be individually distinguished with the light microscope, cover the whole surface of the casing, as may be seen in Figs. 9, 14 and 15. They conceal the lining layer except where this is either folded or presented in optical section. In damaged specimens of suitable type (Pl. III) the inner layer may be exposed with the scales still in position. They can then be seen to be spineless plates each approximating in area to that of the bases of the spined scales and with a surface patterning which will be described in the next section.

In addition to the more obvious components of the complete cases there are a few, much smaller, oval scales commonly present in a group near the bases of the long spines at the flagellar pole. The position of these small scales has been ascertained so often that there seems little doubt that they are normally present at one end only, and that this end is that through which the flagella and haptonema emerge. Further structural details will be given below.

DETAILS OF INDIVIDUAL SCALES

Very large numbers of all four scale types are commonly scattered, singly or in groups, over the surfaces of shadow-cast preparations, doubtless derived from broken cases. This is not surprising, since there is no sign of any cementing material around or between the scales which can be credited with retaining them in position and abandoned cases fall apart rapidly when handled. Though such free scales tell us nothing about the relative positions on the cell they are often excellent for supplying information on the finer constructional details, some of which are illustrated in Pls. IV–VI.

The small plate-scales (average dimensions $0{\cdot}8 \times 0{\cdot}5\,\mu$). Though relatively few and inconspicuous, the smallest scales are conveniently considered first

since they are in some ways the most straight-forward. A group is illustrated between the bases of two large spines in Fig. 21, Pl. IV, and there are two beside some large plate-scales in Fig. 25, Pl. V. Both these figures indicate that there is a difference of pattern on the two surfaces of these scales of the kind very commonly encountered in plate-scales of other species of the genus (e.g. *C. chiton*, *C. ericina*, *C. ephippium*, *C. alifera*). So similar are they indeed that on this evidence alone it would be safe to conclude with little risk of error that the pattern of radiating lines (right-hand small scale in Fig. 25) is the side towards the cell surface and the pattern of loosely woven concentric lines (left-hand small scale in Fig. 25) faces outwards.

The large plate-scales (average dimensions $1{\cdot}7 \times 1{\cdot}3\,\mu$). The imbricated arrangement of these *in situ* is well shown by Pl. III, and the finer details of individual scales are illustrated in Figs. 25, Pl. V, and 28, Pl. VI. These scales are undoubtedly thinner than the little ones last mentioned, since, when superposed on other scales they do not completely occlude the pattern of striations

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Explanation of Plates I–II

Chrysochromulina pringsheimii sp.nov.

I

Fig. 1. A cell swimming with haptonema fully extended forwards; spines visible faintly at both ends of the cell. × 1000.

Fig. 2. A cell, killed with iodine, showing the flagella and partly uncoiled haptonema; spines visible faintly at the flagellar end of the cell. × 1000.

Fig. 3. Cell showing the elongated shape characteristic of the monad whilst in its case, the latter otherwise invisible. × 1000.

Figs. 4 and 5. Two cells in the act of withdrawing backwards from their scale case (C). × 1000. (cf. Figs. 35 and 36.)

Figs. 6 and 7. Two cells immobilized by uranyl acetate and showing ingested graphite, each cell with a fully extended haptonema (very faint), scale cases still present but invisible in the photographs. × 500.

Fig. 8. Living cell, in the act of fission, swimming towards the right; spines faintly visible near the point of attachment of the two daughter-cells. × 500.

Fig. 9. Dried cell still within its case showing spines (large and small), one flagellum and the haptonema still almost in position. Micrograph B3473, × 3000.

Fig. 10. A fully extended haptonema beside scales from the flagellar pole of another specimen. Micrograph B3475, × 5000.

Fig. 11. Flagellar pole of another specimen, still within its case and with all three appendages (two flagellar and the haptonema) broken off but still near the body, one flagellum almost complete and showing the attenuated distal tip. Micrograph B3539, × 5000.

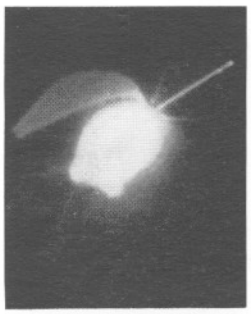
Fig. 12. Free monad after escape from its case showing rounded body and the two equal flagella. Micrograph M528.6, × *c.* 2000.

II

Fig. 13. An empty case seen in a liquid mount under dark ground illumination with the light microscope. × 1000.

Fig. 14. A dried empty case as seen with the electron microscope. Micrograph B3535, × 2000, reversed print.

Fig. 15. One end of another specimen more highly magnified. Micrograph M524.11, *c.* × 7000.



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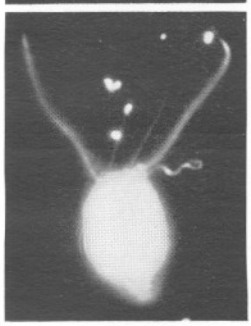

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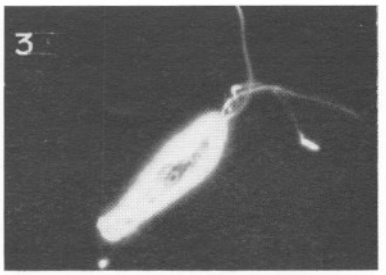

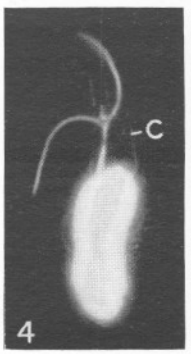

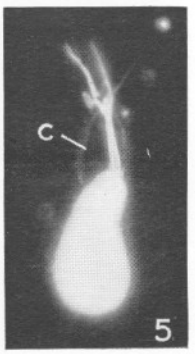

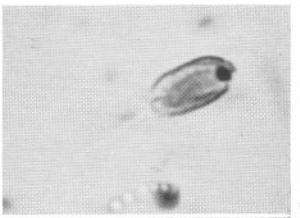

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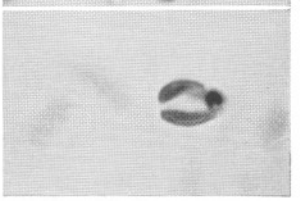

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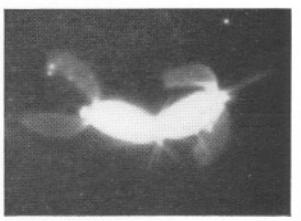

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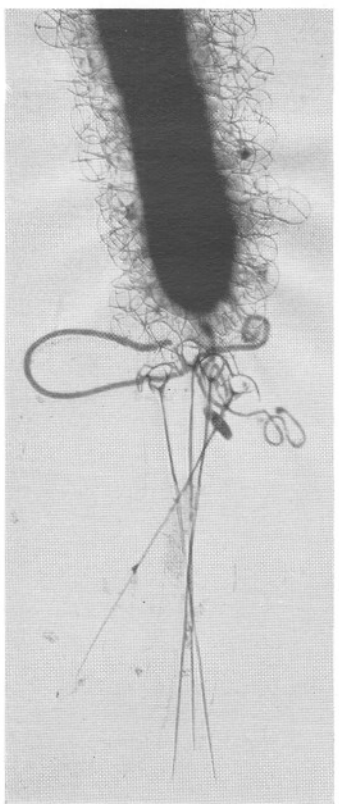

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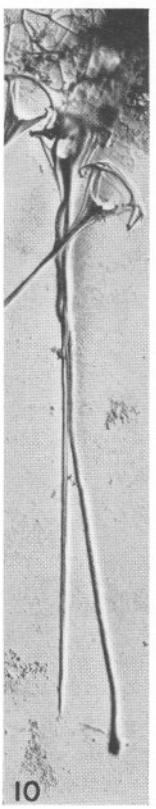

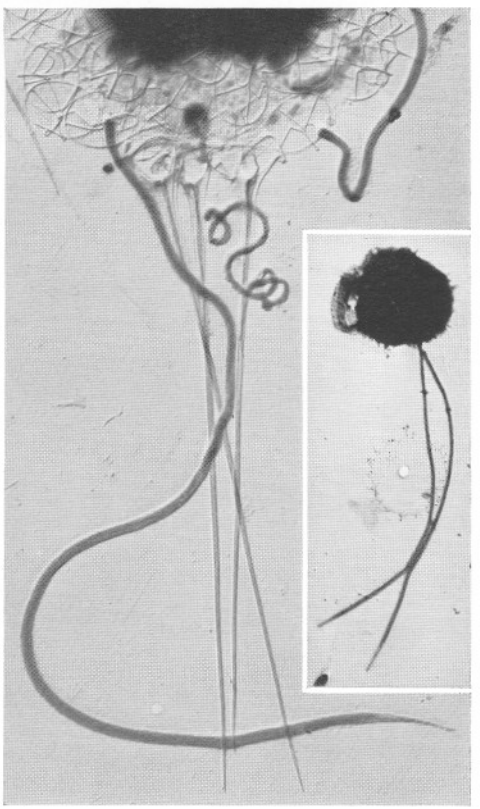

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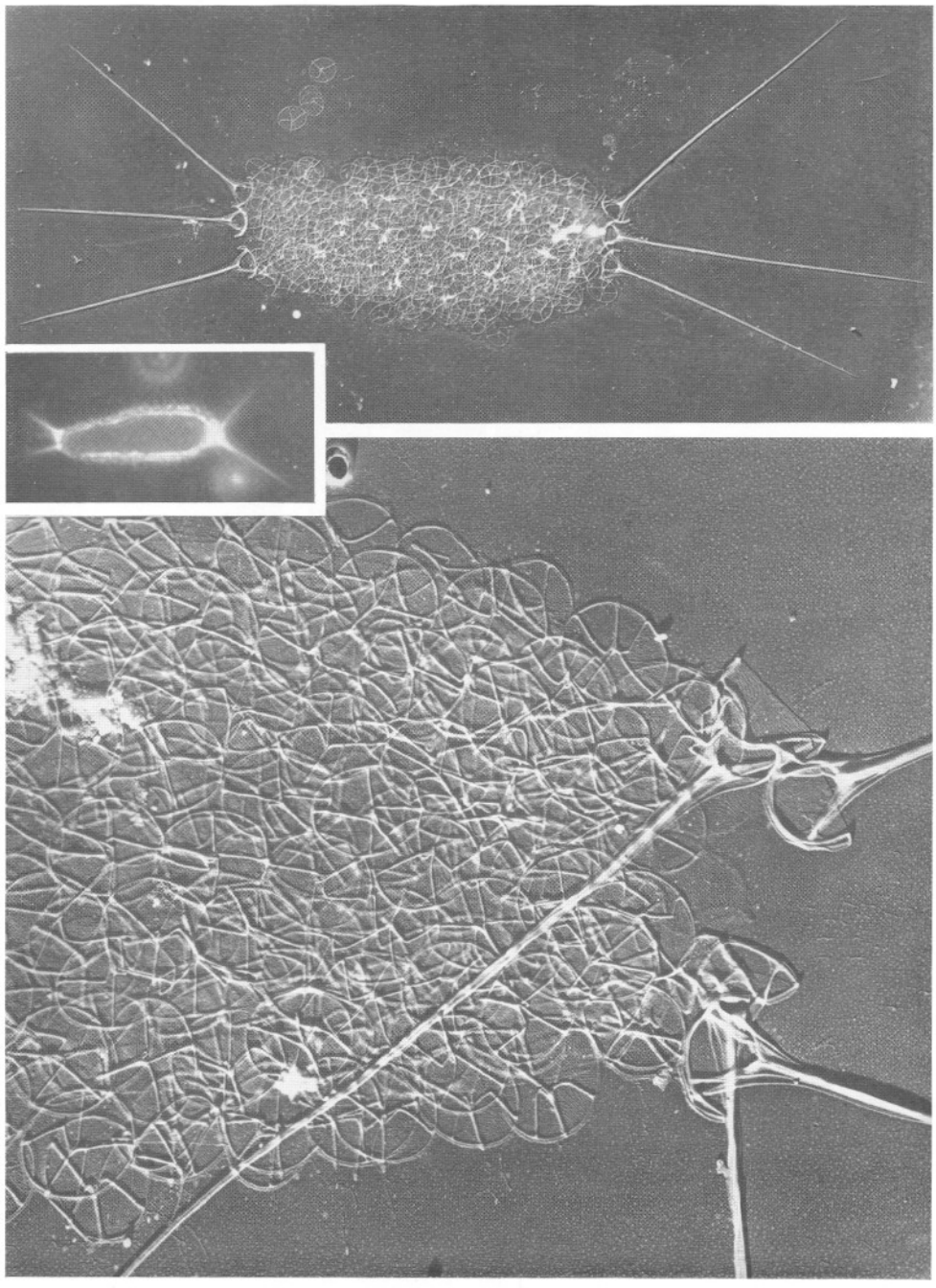

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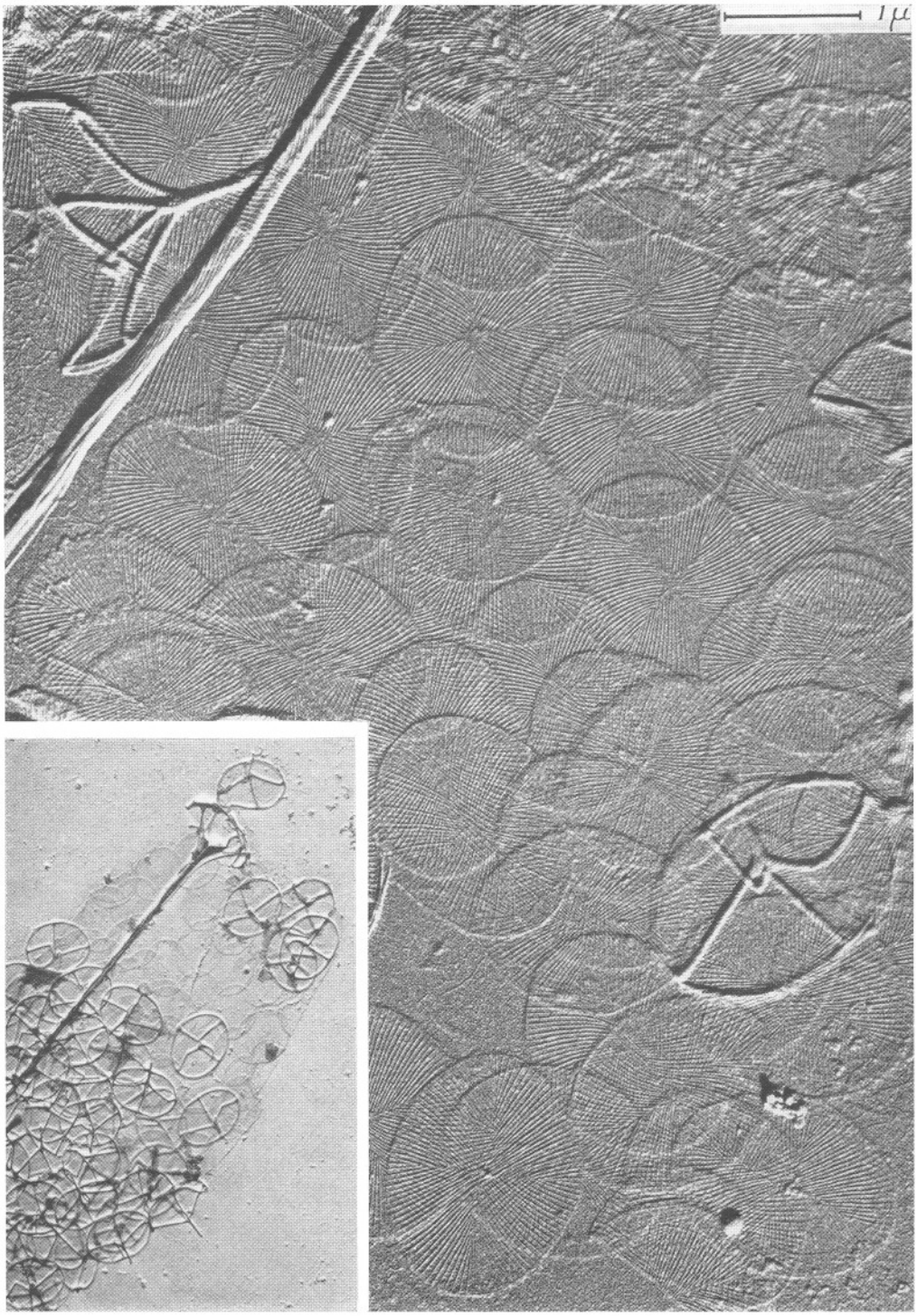

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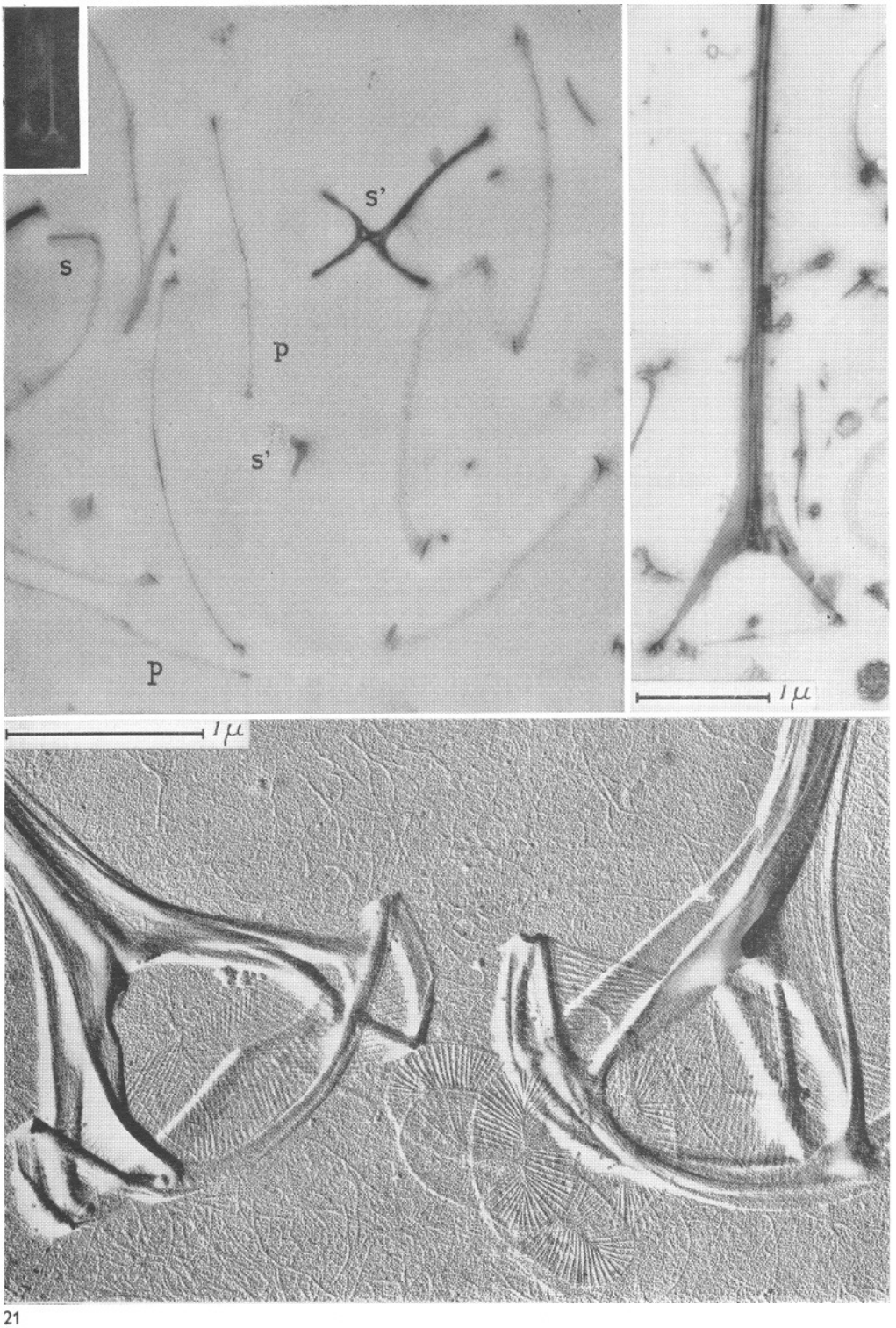

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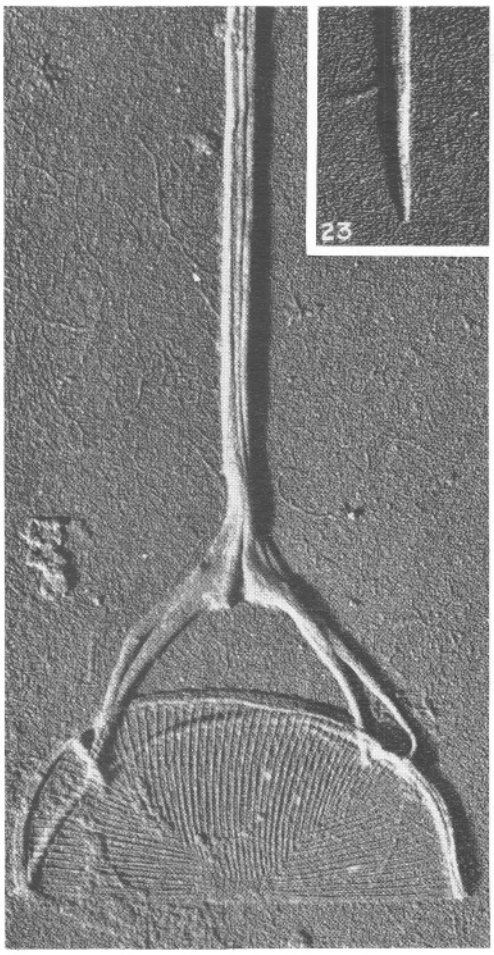

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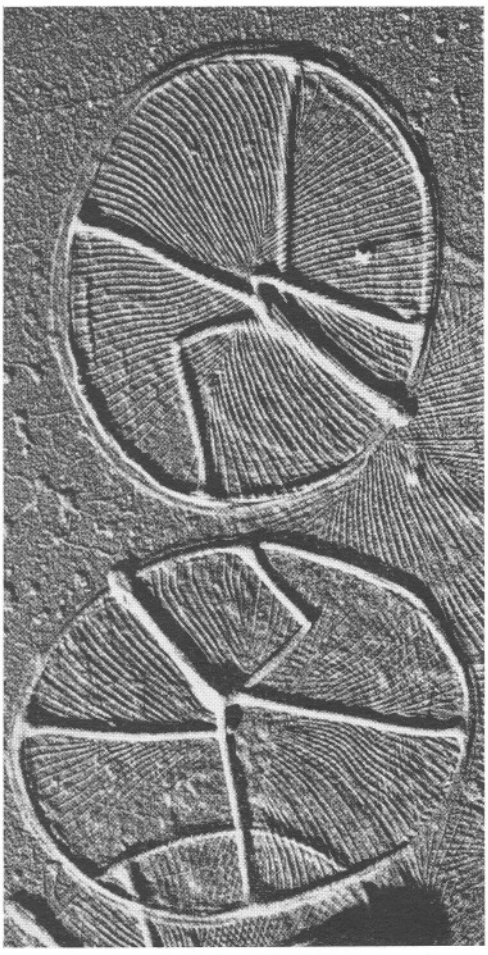

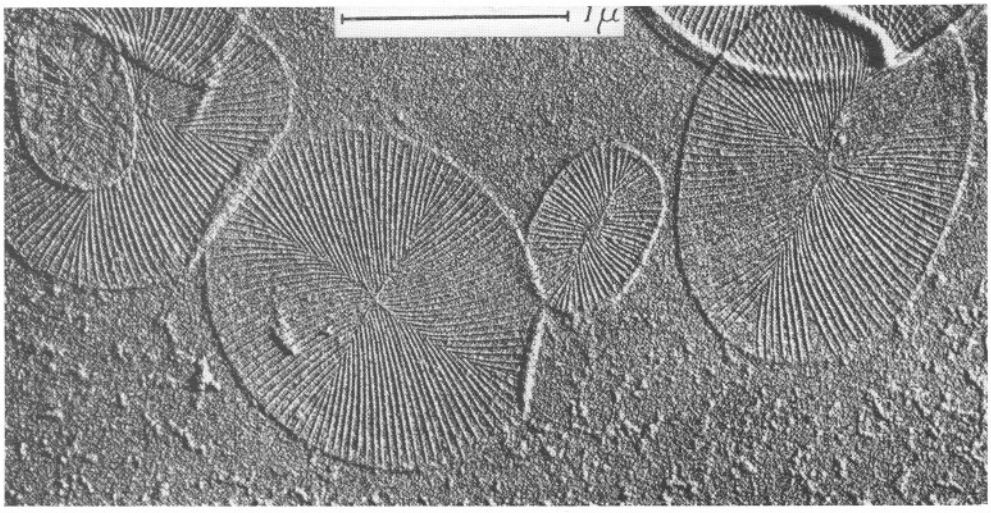

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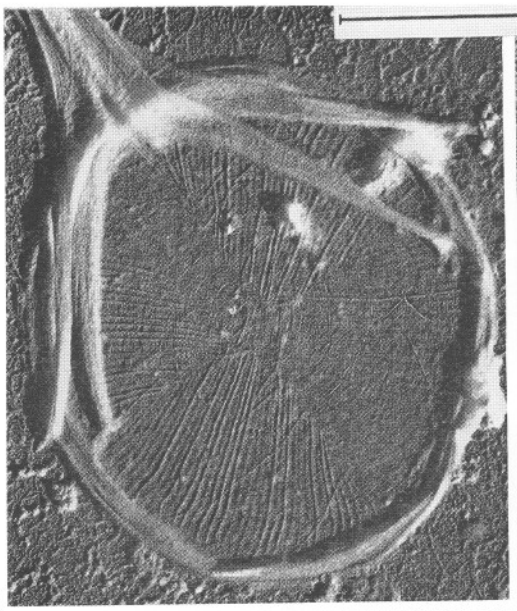

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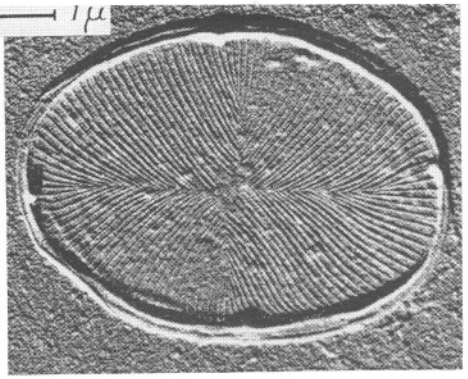

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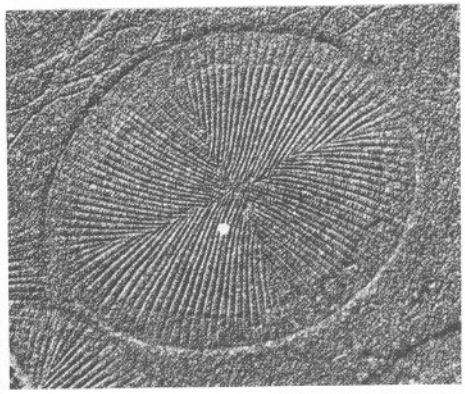

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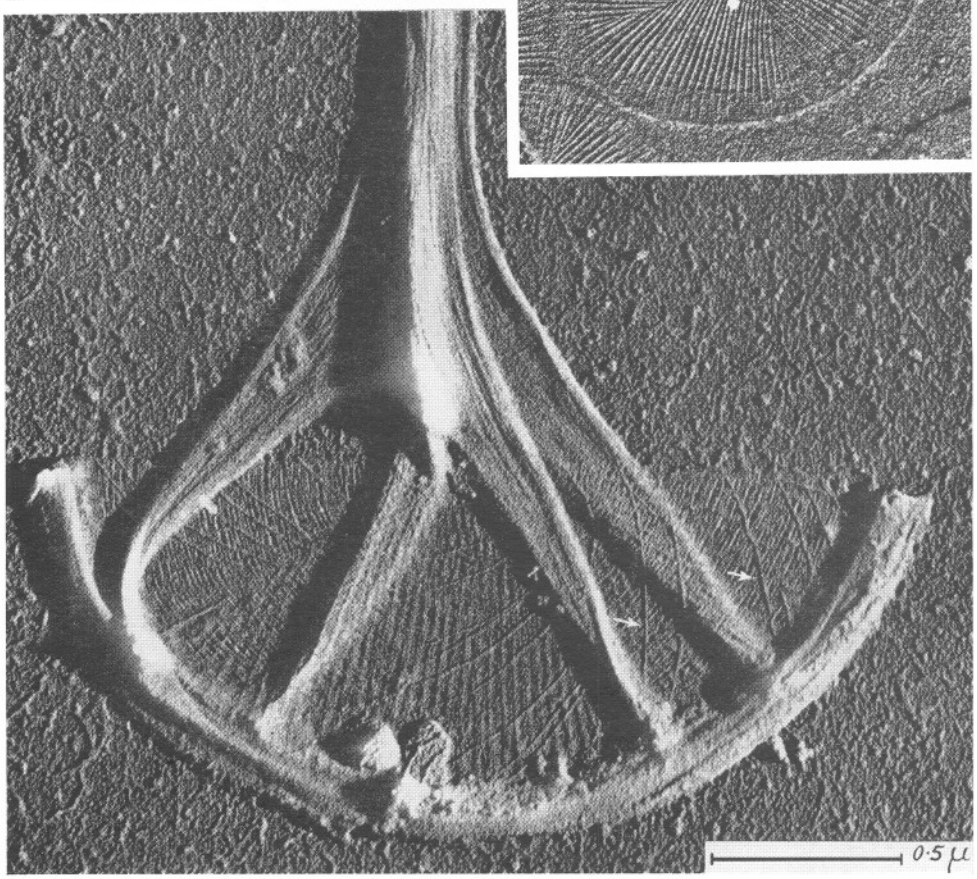

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on the scale so covered; the little ones in contrast, are not, or scarcely, transparent at all (see especially left-hand small scale in Fig. 25). The two surfaces of the large scales are very similar to each other in pattern and it is only by careful comparison of numerous fields or the occasional favourable specimen in which the edge may be bent back to expose part of the other surface (as at

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Explanation of Plates III–VI

Chrysochromulina pringsheimii sp.nov.

III

Fig. 16. An empty case with the outer layer of spined scales removed over a considerable area, exposing the lining layer of plate-scales. Micrograph B3477, × 5000.

Fig. 17. Part of the lining layer from another specimen, most scales seen from the inner side. Micrograph B3458, × 20,000.

IV

Fig. 18. Two large spines near an abandoned case photographed with the light microscope under anoptral contrast and faintly revealing the struts and base-plate at the bottom of the spine. × 1500.

Fig. 19. Field of detached scales seen in section, the cruciform object being a slightly oblique transverse section of a large spine near the base; a small spine (s) transected by the edge of the field (left) with various views of plate scales (p) and large spined scales (S′) elsewhere in the field. Micrograph B2495, × 30,000.

Fig. 20. LS of a large spine beside a TS of a flagellum (bottom right) among fragments of other sorts of scales. Micrograph H671*a*, × 20,000.

Fig. 21. Bases of two large spines, both with the folded base-plates showing the upper surface, with a group of the smaller plate-scales between them; probably from the flagellar pole of a broken case. Micrograph B3528, × 30,000.

V

Fig. 22. A large spine with the folded base-plate exposing the lower surface (contrast with Fig. 21). Micrograph B3523, × 30,000, reversed print.

Fig. 23. Tip of a large spine showing the tapered and pointed apex. Micrograph B3527, × 30,000.

Fig. 24. Two of the smaller spined-scales, the upper one exposing the lower (inner) face and the lower one exposing the outer face. Note the close similarity of the markings on the two surfaces. Micrograph B3452, × 30,000.

Fig. 25. Group of plate-scales showing the two sizes; the three larger ones all exposing the lower faces, the right-hand small scale showing the lower face and the left-hand small scale the upper face; note the considerable differences of pattern on the two sides of the small scales. Micrograph B4040, × 30,000.

VI

Fig. 26. The base-plate of a large spine showing its upper surface, without folding. Micrograph B2652, × 30,000.

Fig. 27. Base-plate of a small spine with the spine and struts broken off exposing the upper surface. Micrograph B4021 , × 30,000.

Fig. 28. A plate-scale showing the upper surface (contrast with Fig. 25), the very incomplete outer layer represented by a flat border not reaching the centre. Micrograph B4034, × 30,000.

Fig. 29. Base of a large spine comparable in attitude to those in Fig. 21 but more highly magnified to show among other details some individual fibres crossing the surface of the base-plate, and individually measurable as less then 50Å in width. Micrograph B2653, × 50,000.

bottom right of Fig. 17, Pl. III) that any differences can be detected. It is then found that scales with the striated pattern extending uninterruptedly to the edge as in Fig. 25 are exposing their lower (inner) surface, while others, exhibiting a thin flat border partly covering without completely occluding the radiating lines near the edge of the plate (Fig. 28, Pl. VI), are showing their outer side. That this apparent border represents a local thickening near the edge of the plate is clearly indicated in sections such as that of Fig. 19, Pl. IV, in which two plate-scales (p) have been transected approximately at right angles and both show a thickened border but no raised rim.

The small spined scales (average dimensions, $1{\cdot}9 \times 1{\cdot}4\,\mu$, base-plate, 1–$1{\cdot}5\,\mu$, spine length). These are very numerous, and instructive views can be obtained from Pls. II, III and V. Fig. 24, Pl. V, is selected from a group because it happens to show two such scales side by side, the upper one lying with its inner surface exposed and the lower one with its outer surface exposed. Each scale consists of a plate with a conspicuous rim and a pattern of radiating lines present on both faces. Attached to the junction of rim and plate at four points are the four slender struts which support the base of the spine. These struts are slightly longer than the radii of the base-plate and in life they are arched above it as may be seen near the lower right-hand edge of Fig. 16, Pl. III, or more highly magnified in Fig. 17 (top left). When dried the struts and spine have commonly collapsed against the surface of the base-plate but it is not difficult to reconstruct the true condition, which can also be attested by sections.

Fig. 27, Pl. VI, is inserted to show more completely the pattern on the base-plate of a small spined scale from which the struts have been broken off at points which can be clearly seen. The close resemblance between this and the plate-scales of Figs. 25 and 28 is obvious though the presence of a true raised rim no less than the remains of the broken struts will at once distinguish them. The oval outline of the base-plate of the spine (Fig. 27) is characteristic of these structures which are not radially symmetrical but bilateral.

The large spines (average dimensions *c.* $2\,\mu$, base-plate, $\times 18\,\mu$, spine length). Bilateral symmetry is still more marked in the large spines than in the small ones. There are again four struts which are not exactly at right angles though arranged in a cruciform manner (Fig. 19, Pl. IV). When cut longitudinally (Fig. 20) the struts are seen to be raised high above the flat base-plate but when flattened by drying the base-plate is usually folded, generally to expose the upper surface as in Fig. 21, but sometimes exposing the lower surface as in Fig. 22, Pl. V. Both these types of folding are represented among the large spines on the specimen of Fig. 15, Pl. II, but the fact that they are by no means equally common is likely to reflect the intrinsic asymmetry of the scales which come to rest more easily with the long axis, rather than the short axis, of the base parallel to the mount.

An exceptional specimen in which the base-plate has broken away from the struts to expose the whole of its upper face without folding is illustrated in

Fig. 26, Pl. VI. It is larger than the base-plate of the small spines and has an even larger raised rim (cf. Fig. 27). In other features they are very much alike.

The large spines, compared with every other scale which we have so far encountered in the genus, are exceptionally massive in construction. The nearest equivalent, namely, the spines of *C. ericina* (Parke *et al.* 1956) recently re-investigated by Manton & Leedale (1961), differ markedly in this respect. In that species the spines, though 9–12 μ or exceptionally 15 μ long, were hollow, thin-walled, truncate at the tip and joined to the base-plate continuously along the whole margin. In *C. pringsheimii* the spine itself is also hollow, showing either two cavities (Fig. 19, Pl. IV) or one, according to the level sectioned, but this cavity represents only a small part of the total thickness of the spine. The tip is pointed and apparently solid (Fig. 23), the struts are also solid although the arched space between adjacent struts is empty. The resemblance to the spines of *C. ericina* is thus by no means a close one.

FINE STRUCTURE OF SCALES

While it is sufficient, and indeed necessary, to limit the present description to scale morphology, there is one detail of fine structure which is so conspicuous that it would be foolish to ignore it. On the upper face of base-plates of spined scales, both large and small, it is often found that a few slender threads stray over the surface in an unoriented manner. This does not happen on the lower face, and deductions could probably be made from this about the order in which the scale material is laid down. At present the only observation that we wish to record is a measurement. As may be seen in the most highly magnified micrograph reproduced here (Fig. 29, Pl. VI) such threads are of the order of 40Å wide. They represent undoubtedly one of the components from which the base-plate is built up, and we hope to report further about them on a later occasion.

ADDITIONAL OBSERVATIONS WITH THE LIGHT MICROSCOPE

Like the type species of the genus, *C. parva* Lackey (see Parke, Lund & Manton, 1962), this organism usually shows no phototactic response whatsoever; but, unlike the type species, it does not produce really thick cultures, the maximum density observed being about one-quarter million cells per ml.

The cells can swim with the flagella either behind or in front of the body (Fig. 8, Pl. I; Figs. 30, 31). When enclosed in their scaly covering the cells swim only for short periods before becoming stationary with their appendages held characteristically in the position visible in Fig. 32; they may then move off in a different direction. Such encased cells move comparatively slowly with a slow rotation of the body, sometimes accompanied by considerable gyration. A characteristic gliding movement without gyration is also some-

times seen, usually with the flagella directed forwards. Short periods of anchorage are common, during which the haptonema may remain coiled throughout most of its length, uncoiling only the distal tip for attachment, in which state the attached cell may show a deceptive resemblance to the genus *Prymnesium* (Fig. 32); at other times the haptonema can be fully extended and may be attached along part or the whole of its length (Fig. 33).

When strongly illuminated, as for observation with the light microscope, the scaly covering is readily abandoned, the cell emerging backwards and withdrawing the flagella last after the body has emerged (Figs. 4, 5, Pl. I; Figs. 35, 36). Occasionally the flagella may break off and remain within the casing. At other times the cell will swim away changing the body shape, as it swims, from elongated to pyriform, or even to subspherical. The subsequent behaviour of these naked cells has been ascertained in a general way for both strains of the organism by isolating in clonal culture the pyriform cells that have been freshly liberated from their scaly coverings and watching for the reappearance of encased cells. These can always be detected in such cultures within 4 to 6 weeks.

Phagotrophy (Figs. 6 and 7, Pl. I) is frequent, even with encased cells, which are able to ingest objects at the nonflagellar end of the cell up to $2 \times 1\,\mu$ in size; the naked cells ingest larger objects up to $3 \times 4\,\mu$.

The scales stain violet with dilute cresyl blue. Janus green used as a vital stain shows up four bodies inside the cell, two about $1\,\mu$ and two about $0{\cdot}5\,\mu$ which are almost certainly mitochondria (Fig. 30) A curved body close to the nucleus is also stained which is probably the Golgi body; two can be detected

Legends to Text-figs. 30–36

Chrysochromulina pringsheimii sp.nov.

Fig. 30, × 5000; Figs. 31–36, × 1250)

Fig. 30. Individual swimming with flagella and haptonema in front of body, haptonema fully extended. *c*, chromatophore; *f*, flagellum; *fb*, lipid globule; *go*, golgi; *h*, haptonema; *l*, chrysose (leucosin); *m*, muciferous body; *mt*, mitochondrion; *n*, nucleus; *s*, plate-scale; *sss*, small-spined scale; *lss*, long-spined scale.

Fig. 31. Individual swimming with flagella and haptonema behind body in the position characteristic for the species during rapid swimming; early fission stage with four flagella (two daughter-flagella short), showing increase in number of long-spined scales, four present at front and ten at back of body.

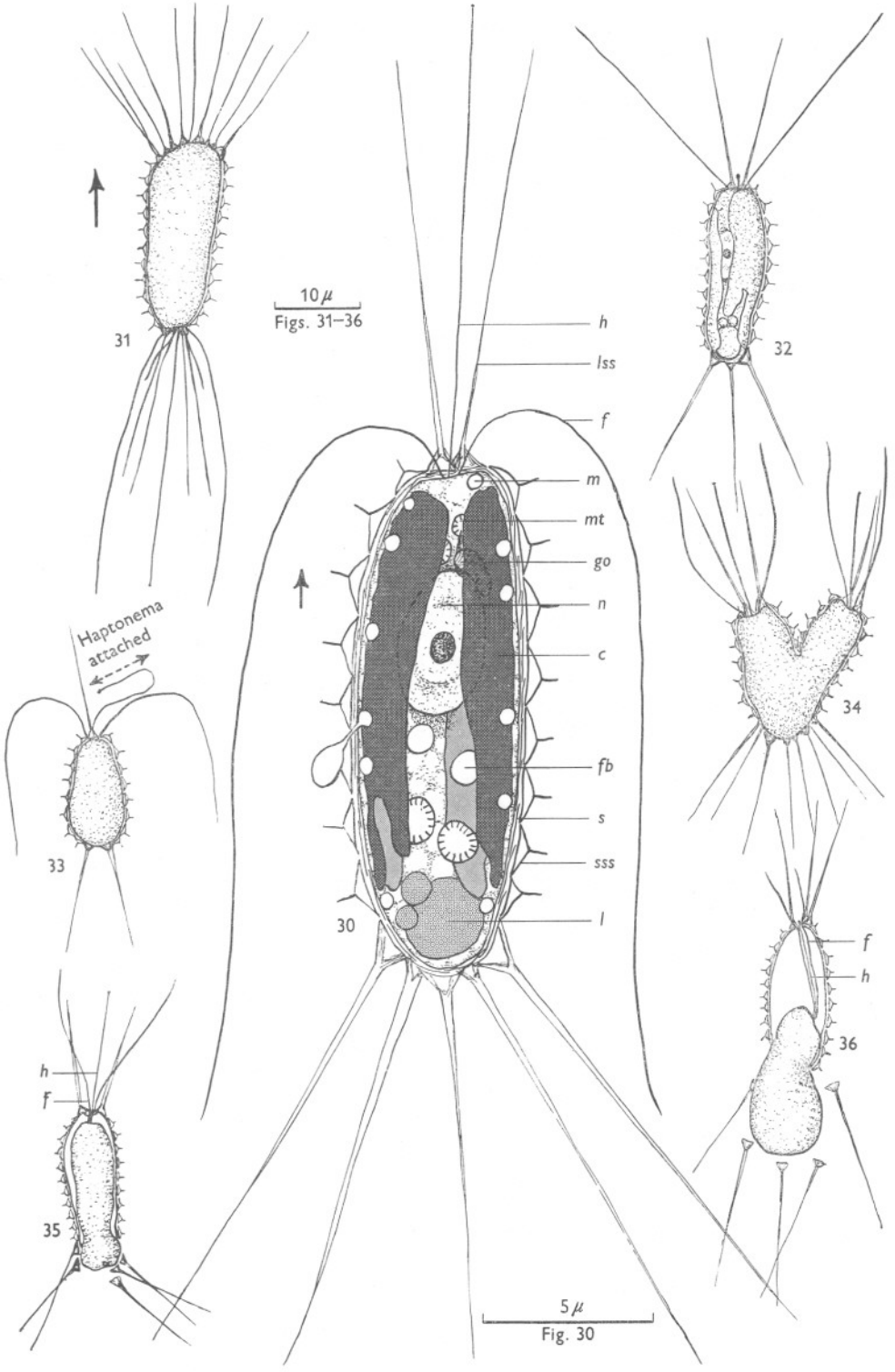
Fig. 32. Anchored cell with flagella stiff and lying in a very characteristic attitude for this species when an individual is stationary; haptonema attached by tip; remainder coiled and lying beneath body (cf. *Prymnesium*); two long-spined scales at front and three at back of body.

Fig. 33. Anchored cell forming new scale-casing after withdrawing from original casing; one long-spined scale at front and two at back of body; haptonema attached along part of its length.

Fig. 34. Late fission stage.

Fig. 35. Individual just starting to withdraw from scale casing.

Fig. 36. Individual just completing withdrawal of body from scale casing; flagella and haptonema not yet out of casing.



Text-figs. 30–36

in early fission stages. Ejectile muciferous bodies (blue with cresyl blue), 0·5–0·75 μ, are scattered over the cell surface and appear to be more numerous at the flagellar end of the cell. Lipid bodies (*c.* 1 μ) can also be distinguished, usually one or two being visible in each cell. Chrysose (leucosin) vesicles, 1–3 in number and usually 2–3 μ in diameter, are commonly encountered close to the non-flagellar pole.

Fission occurs in both the encased and the 'naked' cells; in the encased cells the scaly covering becomes increased and redistributed to cover both daughter-cells, which are usually of equal size (Fig. 8, Pl. I; figs. 31, 34). No other stages of the life-history are known.

FORMAL TAXONOMIC DESCRIPTION

Chrysochromulina pringsheimii sp.nov.

Motile cells with an easily visible (light microscope) scaly covering; encased cells, usually elongated, cylindrical, clavate or fusiform, occasionally ovoid, with flagellar end often obliquely truncate, when without visible scale-covering cells generally pyriform. Cells strongly metabolic; 14–20 (exceptionally 12–24) μ in length, 5–8 (exceptionally 4–9) μ in breadth. Two flagella and one haptonema arising close together centrally from one end; the flagella subequal to equal, smooth, fairly robust, homodynamic, 1½–2 times body length; the haptonema, capable of attaching along its whole length, half the thickness of the flagella, commonly 1–2 times body length when fully extended, but occasionally longer than the flagella. Scales in two layers, the lining layer of ovate plate-scales (average dimensions 1·7 × 1·3 μ), the outer layer of ovate spined scales (spine 1–1·5 μ long on base 1·9 × 1·4 μ), the spine attached to the edge of the ovate base by four arched struts. Terminal spines very large (12–20 μ long on bases *c.* 2 μ wide), commonly 3 (2–4) at flagellar pole and 5 (3–6) at non-flagellar pole. An inconspicuous group of small two-layered plates (0·8 × 0·5 μ) present near the flagellar bases.

Chromatophores usually two or four, occasionally one, golden brown, oblong sometimes lobed and bifid towards the non-flagellar end, parietal, each with an internal pyrenoid. Lipid and chrysose (leucosin) produced. Ejectile muciferous bodies distributed in peripheral cytoplasm, usually more frequent towards the flagellar end. Nutrition phototrophic and/or phagotrophic. Non-toxic to fish.

In motile phase asexual reproduction by fission into two daughter-cells, usually of equal size; other stages unknown.

Habitat. Marine to estuarine—the St German's River (25 June 1957, type culture, Plymouth No. 165) from a townet sample and a second strain from the sea near the Eddystone lighthouse at position lat. N. 50° 11′, long. W. 04° 13′ (24 July 1957, Plymouth No. 166) at surface. It has been recorded from many other stations off Plymouth from the surface down to 70 m during the spring and summer months of the years 1957 to 1959 though it was rare or absent from late October to late April in those years. It was not recorded again until 4 October 1961, when it occurred in a sample of sea water brought in by Dr A. J. Southward, from position lat. N. 64° 57·5′, long. W. 06° 02′, taken at the surface. Type culture deposited with the Culture Collection of Algae and Protozoa, Cambridge.

Cellula in statu erratico plerumque squamis per microscopium luminarium bene conspicuis induta, elongata, cylindrica vel clavata vel fusiformis, interdum ovalis, apice saepe oblique truncata; rarius squamis conspicuis destituta, dum ita nuda

pyriformis; semper valde metabola; 14–20 (raro 12–24) μ longa, 5–8 (raro 4–9) μ lata. Flagella bina ex apice conferte orientia, subaequalia vel aequalia, glabra satis, crassa, homodynamica, cellula $1\frac{1}{2}$–2 plo longiora; haptonema ibidem oriens, potestate haerendi in tota longitudine donatum, dimidiae flagellorum crassitudinis, extensum plerumque cellula 1–2 plo longius, sed interdum flagella superans.

Squamarum duo strata; interiores planae, ovatae, ca. $1{\cdot}7 \times 1{\cdot}3$ μ diam.; extoriores ovatae, $1{\cdot}9 \times 1{\cdot}4$ μ diam. spinam quaeque gerens 1–1·5 μ longam quattuor trabeculis suffultoriis margini squamae affixis portatam; apicales 2–4, plerumque 3, et posticae 3–6, plerumque 5, ca. 2 μ diam. spinas gerentes 12–20 μ longas; prope bases flagellorum squamae parvae, $0{\cdot}8 \times 0{\cdot}5$ μ diam., e duobus stratis compositae.

Chromatophora plerumque 2 vel 4, raro 1, fulva, oblonga, interdum in margine postico lobata vel bifida, parietalia, pyrenoïde faciei interiori cuiusque apposito. Synthemata lipoïda et chrysosea (leucosinea). Corpora mucifera ejectilia in strato externo cytoplasmatis distributa, plerumque ad apicem versus crebriora.

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

Propagatio vegetativa in statu erratico bifissione effecta, cellulis filialibus plerumque aequalibus. Alii status ignoti.

Typus die 25. Junii 1957 in ostio fluvii anglici St. German's River lectus, in Plymouth sub numero 165 cultus, viva prole Vivario Cantabrigiensi tradita.

DISCUSSION

The only details in the description of this organism which are fundamentally new to the genus are those associated with the escape of the monad from its case. This behaviour is doubtless in part a consequence of the dimensions and character of the scaly covering, and as such it is not a very elaborate adjustment. The capacity to engulf solid food through the casing is not peculiar to this species, since all our phagotrophic species seem unimpeded by their scales.

The diversity of form and details of arrangement of the scales are probably less different from those of other species than the spectacular appearance at first suggests. We are accustomed to finding two different sorts of scale present together, commonly spined and spineless though sometimes of other shapes, and the extension to four scale types is perhaps only a difference of degree. This seems the more probable in that all four seem to be related to simple two-layered plates, a condition still retained by the smallest scales, the outer of the two layers in the others being either incompletely formed (as in the large plate-scales) or moulded into the spine with its four struts. This kind of differentiation is exactly comparable with that already found in a more limited degree in species such as *C. ericina* (Manton & Leedale, 1961), *C. ephippium* and *C. alifera* (Parke *et al.* 1956).

With regard to the distribution of the scales on the cell, the tendency of spines, when present, to be arranged near the ends of the cell has been encountered to some extent already in *C. kappa* (Parke *et al.* 1955) though here the spines are small and confined to the flagellar pole, and *C. ericina* (Parke *et al.* 1956) in which the much larger spines are more scattered but are characteristic-

ally at the non-flagellar pole. It is inherently probable that any motile, rotating cell carrying a limited number of very large spines would bear them terminally, and we need not therefore be surprised by this condition in *C. pringsheimii*. A stratified arrangement with two morphologically different types of scale in superposed layers over the body surface has also been encountered before, in *C. strobilus* (Parke *et al.* 1959), although the scales themselves in that species are different and much smaller; there is a layer of very small plate-scales covered by a layer of close-packed cup-shaped scales apparently embedded in an amorphous matrix which presumably keeps them in place. In *C. strobilus* the scales collectively form a close-fitting skin over the cell surface which can become detached in coherent sheets with handling but which the monad does not normally leave. The absence of a visible cementing substance in *C. pringsheimii* suggests that the forces holding the components of the case together are here perhaps frictional and determined in part by scale size, shape and patterning, as well as by their imbricated arrangement, all details which differ in degree but not in kind from commonplace occurrences elsewhere.

Direct observations on scale production have not yet been made in *C. pringsheimii* and may indeed not be possible owing to its sensitiveness to mechanical disturbance. As in *C. strobilus*, however, it seems necessary to postulate a rhythmic or cyclical production of the different types of scale to explain their stratified arrangement, and any such cycle may be expected to be linked in some way with cell division. If this is so one must believe that the little group of small plate-scales encountered at the flagellar pole must mark either the beginning or the end of such a growth cycle, the former being the more probable since the formation of new flagella and the separation of a new flagellar pole is known to precede cell cleavage in all these species. Whatever the relation, to the nuclear cycle—and of the latter we have no knowledge at all—it is of some interest that for a brief period in each division cycle the organism apparently reverts to the production of a primitive type of scale, which the uncomplicated two-layered plate undoubtedly is.

This fact seems significant when the evolutionary aspect is considered. We can at present neither know nor usefully speculate on how a state of complexity such as that exhibited by *C. pringsheimii* can come about. Once it is there, however, some possible consequences are obvious. Among the easiest categories of genetically controlled changes to bring about in many groups of organisms are those involving the rates of processes or the deletion of single stages in them. A slowing down of the scale cycle relative to the growth and mitotic cycles, so that all four scale types were no longer produced by one individual but became separated in time, would at once destroy the scale case as we see it and would lead to a sequence of individuals carrying different arrangements or types of scales. Should these become spaced out so far that a succession of mitoses were to intervene between the production of each

successive scale type we should have a life-history resembling morphological alternation of generations though not necessarily limited to two phases. If any one stage became so fixed as to be perpetuated indefinitely with the permanent loss of capacity for the others the resulting populations would become species, if able to establish themselves against the pressure of natural selection. Such developments, if they occurred, could be rapid since complex sequences needing perhaps millenia to develop can certainly be stopped by single mutations. One species such as this is therefore potentially capable of explosive development into a cluster of species differing in structure and/or life-history. This possibility is not as fanciful as it may perhaps appear to a non-algologist since one of the surprises which study of algae in culture has occasioned is the demonstration that organisms so different as to have been described and classified in separate genera or even subfamilies, may only be stages in one life-history (cf. the relation recently established by Parke & Adams, 1960, between *Crystallolithus hyalinus* and *Coccolithus pelagicus*).

We are unlikely ever to know whether *C. pringsheimii* itself will actually do all this, or to be able to recognize, among other species, which ones may have originated in this way. The mechanisms involved in species formation among apparently sexless unicells are, nevertheless, so little understood that it is worth suggesting how great an evolutionary potential they may in fact possess, regardless of whether, in any one instance, it is likely to be used or not. Since no other species of *Chrysochromulina* so far described has presented the right assemblage of facts to bring such a situation to light, this is an additional reason for wishing to describe and name it while living material of this very delicate and culturally troublesome organism is still available.

SUMMARY

A new species of *Chrysochromulina* with an unusual type of cocoon-like scaly covering has been described with the light microscope. The morphology and arrangement of the scales has been elucidated with the electron microscope. Salient features are the presence of four different types of scale (large and small plates and large and small spines) in characteristic positions in or on the case, the small plates having the normal two layers characteristic of primitive scales in this group, the other three types being specialized modifications of this. Some developmental and evolutionary consequences of the morphological observations are discussed. A preliminary observation on the thickness of micro-fibrils composing the base-plates of spined scales is given.

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