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STUDIES ON MARINE FLAGELLATES

III. THREE FURTHER SPECIES OF *CHRYSOCHROMULINA*

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(With total of 76 Figures in text and on Plates I–IX)

CONTENTS

INTRODUCTION

Two of the new species of *Chrysochromulina* to be described here are somewhat more like the type species (*C. parva* Lackey) than were any of those included in our last communication (Part II of this series—Parke, Manton & Clarke, 1955). The diagnostic generic character of the presence of three filiform appendages arising close together is shared by all, and as before two of the appendages are flagella of equal or almost equal length and the third is a special organ to which the name *haptonema* has been given (Gr. ἅπτω to attach, νῆμα pl. νῆματα, a thread) (1955, p. 581). In our previous species the haptonemata when fully extended were relatively short, being not very different in length from the flagella. When not extended it was coiled in a flat spiral. Our three new species all have much longer haptonemata, of the order of twice the length of the flagella in *C. ericina* and three to five times the length of the flagella in *C. ephippium* and *C. alifera*. In all three species the haptonema is distinctly thinner than the flagella, a character probably connected with the absence of a conspicuous sheath (contrast with *C. kappa* and *C. minor* of our previous paper) and when not extended it is coiled in a solenoid. Several of these characters agree fairly closely with those given by Lackey for *C. parva*, as will be explained in

connexion with *C. ephippium* (p. 406), though the comparison cannot yet be made in full detail since *C. parva* is an American freshwater species which has only been found once (Lackey, 1939) and it has not yet been available to us for study.

Our principal comparisons are therefore with *C. kappa*, *C. minor* and *C. brevifilum* of our previous paper, and here there is marked agreement in the main features of structure and life-history. As before we are dealing with pigmented flagellates showing many of the metabolic characteristics of the photosynthetic Chrysophyceae combined with the habit of phagotrophic feeding. Ingestion of graphite has been demonstrated with the light microscope in all three new organisms and evidence is included in the plates. As before the body surface is apparently covered by translucent scales without mineral impregnation, which take up cresyl blue. Their details can only be accurately seen with the electron microscope but they provide important criteria for the specific diagnoses. The very long spines of *C. ericina* can, however, with difficulty be seen individually with the light microscope.

Though we are still ignorant of the functional significance and mode of formation of the scales, our three new species all suggest that they may contain two layers of material. All the spineless scales show a characteristic and different pattern on their two surfaces, and since similar patterns occur on the flat parts of the spined scales (with special clarity in *C. ephippium*) we have been able to identify the outer and inner surfaces respectively. These differences were not detectable in our previous species.

Another detail of difference from our previous species, the significance of which cannot yet be wholly evaluated, is that the point of emergence of the three appendages is somewhat asymmetrical in relation to the rest of the cell. In *C. ericina* the point of emergence is no more than slightly 'off centre' and the two flagella seem isodynamic in function as well as equal in length. In the other two species the point of emergence is more definitely ventral under certain conditions, though the capacity for the body to change its shape hampers exact description. We have not been able to detect in these any marked or constant difference in either the length or the structure of the two flagella, but when the organisms are studied alive the flagella are definitely heterodynamic in behaviour. The significance, if any, of this observation will be discussed on a later occasion, but it is perhaps of importance to draw attention to the facts as described for *C. ephippium* (pp. 403–5) and *C. alifera* (p. 412) and to the preliminary discussion on p. 413.

With regard to life history, an advance on our previous work has been the more detailed observation of the emergence of cells of the motile phase from the dormant, walled, cells of the non-motile phase in *C. ephippium* (pp. 405, 406). In all our other species we have described empty membranes with a circular opening as providing strong suggestive evidence that such a transition takes place and the new observations demonstrate the truth of this supposition.

In presenting the facts for individual species we have necessarily had to do so in considerable detail, since it cannot yet be known with certainty which characters are limited to, and therefore diagnostic of, single species, and which will be found common to assemblages of species when more are known. We have already evidence that the remarkable spines of *C. ericina*, for example, are not an isolated phenomenon but that they will recur in various ways in the specific descriptions of other organisms which have not yet been fully studied. We have therefore placed on record everything that we have been able to ascertain about the species to be described, as studied in unialgal cultures, apart from certain fundamental characters common to all species of *Chrysochromulina* that have already been discussed (1955).

Our methods of study have been exactly as enumerated before (1955, pp. 281–3).

As before, our grateful thanks are due to Dr J. E. Morton, of Queen Mary College, for translating our specific diagnoses into Latin, and to Miss D. Ballantine for testing these organisms for their possible toxicity to fish.

Specific Descriptions

Chrysochromulina ericina n.sp., Parke & Manton

(Lat. *Ericinus*—like a hedgehog)

Diagnosis

Motile cells usually ovoidal to nearly oblong, very slightly flattened in one plane, showing marked metaboly, 6–10 (exceptionally 5–12) μ in length, 5–8 (exceptionally 4–10) μ in breadth; flagellar pole obliquely truncated with a slight, almost central depression. Two flagella and one haptonema arising close together not quite centrally from the depression: the flagella equal, homodynamic, 2–2$\frac{1}{2}$ times body length, smooth, gradually attenuated to a small knob (E. M. observation); the haptonema thinner than the flagella, 4–5 times body length when fully extended with a small basal swelling, a club-shaped tip but no clearly marked translucent sheath obvious under the electron microscope. The periplast of a pectic nature covered by very thin transparent, sculptured, dimorphic scales, details visible only under the electron microscope. Scales without spines very numerous, $0{\cdot}5 \times 0{\cdot}6\,\mu$ to $0{\cdot}7 \times 0{\cdot}9\,\mu$, with a pattern of radiating ridges on one side and a slightly raised rim surrounding irregular crossed striations on the other. Spined scales, 28–30 in number, the spine abruptly truncated and slightly tapering, 9–12 (exceptionally 15) μ long, 0·2–0·3 μ wide, arising from a circular (or conical?) base, 1–1·4 μ wide, marked with concentric striations on its outer side.

Cells uninucleate, no stigma. Chromatophores usually 2 or 4, sometimes 6 or 8, deep golden brown; in motile phase saucer-shaped, ellipsoid, or oblong, frequently bifid towards non-flagellar pole, parietal, with a single globular body (pyrenoid?) on inner face placed near margin towards the non-flagellar pole; in non-motile phase deeply lobed or stellate. Oil and leucosin produced. Ejectile muciferous bodies generally distributed in peripheral cytoplasm. Nutrition phototrophic and/or phagotrophic. Non-toxic to fish.

In motile phase asexual reproduction by fission into 2 daughter-cells of equal or unequal size; in non-motile phase reproduction (asexual?) by successive fission of amoeboid cells to produce 4 ovate daughter-cells with walls, walls faintly brownish and slightly rugose on the exterior; motile phase probably liberated from walled daughter-cells through a pore.

Habitat: the sea at position (Plymouth Laboratory Station L4) Lat. N. 50° 15′, Long. W. 4° 13′ (15 May 1949, type culture) at surface; and at position (International Station E1) Lat. N. 50° 02′, Long. W. 04° 22′ (13 July 1955) at 20 m. Type culture (Plymouth no. 25) deposited with the Type Culture Collection, Cambridge; preserved material and photographs lodged with the Marine Biological Association, Plymouth, England.

Cellula motili, generaliter ovoidali aut ferme oblongio, paululum planato in uno aspectu, formam conspicue mutanti, longitudine 6–10 μ (rare 5–12 μ), latitudine 5–8 μ, rare 4–10 μ; apice quo inserta flagella oblique truncato, leviter depresso prope centrum. Duobus flagellis et unico haptonemate conjunctim exorientibus e depressione; flagellis aequis, homodynamicis, longioribus 2 ad 2½ quam cellula, teretibus, paulatim attenuatis et acutis; haptonemate teneriore quam flagellis, longiore 4–5 quam cellula, cum maxime extensus, leviter tumescenti prope originem, clavato extremitate sed nulla tunica externa semidiaphana ut videtur per microscopiam electronicam. Periplasto, pectico natura, induto delicatissimis sculptis squamis diaphanis, sculptura invisibili nisi per microscopiam electronicam, manifestis sub duobis formis: altera forma, sine spinulis, pernumerosis, longis 0·6–0·9 μ, latis 0·5–0·7 μ, ornatis radiantibus striis ad inferiorem aspectum, margine paululum elevata circumdanti irregulares decussatas strias ad superiorem aspectum: altera forma, squamis 28–30 numerosis, spinulis praeditis, quoque spinulo subfastigiato sed et abrupte truncato ad extremum, 9–12 μ (rare 15 μ) longo, 0·2–0·3 μ lato, exoriente e basi circulari aut conica, ornata concentricis striis ad aspectum superiorem.

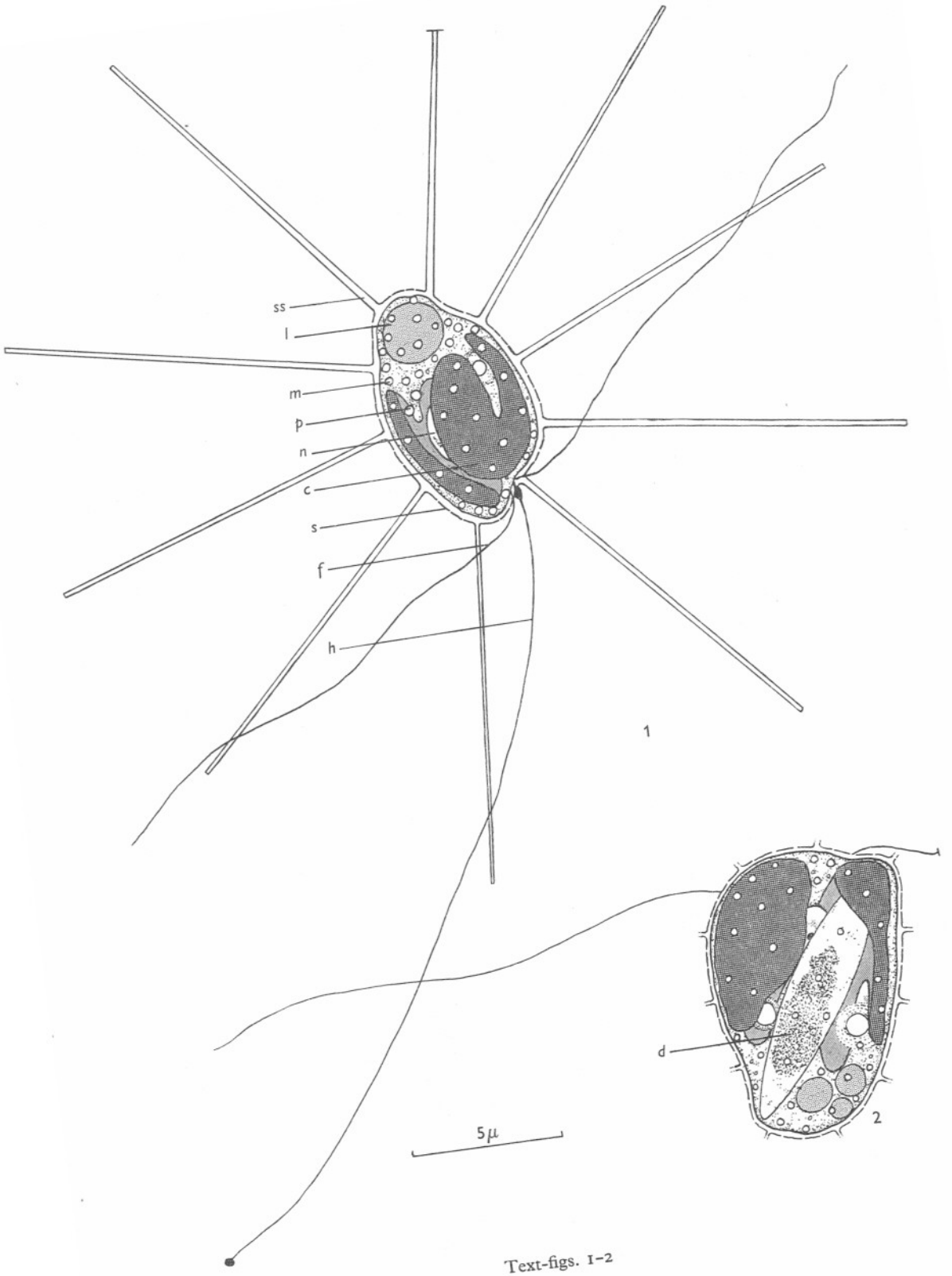
Nucleo unico, nullo stigmate, chromatophoris ex norma 2 aut 4, nonnunquam 6 aut 8, profunde aureo-brunneis; in statu motili cellulae crateriformibus, ellipsoidalibus aut oblongis, saepe bifidis versus apicem cellulae quo desunt flagella, parietalibus;

Legends to Text-figs. 1–2

Chrysochromulina ericina n.sp. (× 5000)

Fig. 1. Individual with two dividing chromatophores anchored by haptonema which is partly extended; the flagella are in the characteristic position adopted when the species is stationary. *c*, chromatophore; *f*, flagellum; *h*, haptonema; *l*, leucosin vesicle; *m*, muciferous body; *n*, nucleus; *p*, pyrenoid-like body; *s*, scale; *ss*, spined scale.

Fig. 2. Individual anchored by coiled haptonema which is hidden below protruding lobe of flagellar pole; cell contains an ingested *Navicula salinicola* Hust. (*d*).



Text-figs. 1–2

unico corpore globulari (? pyrenoidali) in concavo aspectu prope marginem chromatophori posito versus apicem quo desunt flagella; in statu non-motili cellulae chromatophoris profunde lobatis vel stellatis. Cellula oleum leucosinumque parienti; corporibus muciferosis et ejectilibus undique distributis in cytoplasmate superficiali. Nutritione phototrophica necnon phagotrophica. Non toxica piscibus.

Generanti asexualiter in statu motili per fissionem in duas cellulas filiolas vel aequas vel inaequas magnitudine; in statu non-motili generanti (?asexualiter) per fissiones subsequentes cellularum amoeboidalium ad 4 cellulas filiolas ovoidales producendas, parietibus leve brunneis et paulum rugosis externe; maxime potest ut cellulae in statu motili ex cellulis pariete praeditis per foramen liberentur.

Habitat mare prope Plymouth ad locationem Lat. N. 50° 15′, Long. W. 4° 13′ (15 Mai 1949—cultura typica) ad summum mare; necnon ad locationem Lat. N. 50° 2′, Long. W. 4° 22′ (13 Jul. 1955).

Description

The form range of the motile cells is illustrated in Figs. 1–5 and in the photographs of Pl. I. The slight flattening of the body can be most easily observed when looking down on the non-flagellar pole (Fig. 3) and the pronounced metaboly is most obvious when individuals are ingesting or have ingested cells of other species (Figs. 2, 4, 5). In an actively growing culture 85% of the cells are from 6 to 10 μ in length, while 5% are between 5·0 and 6·0 μ. The remaining 10% are incipient fission stages from 10 to 12 μ in length.

The flagella and haptonema arise close together slightly to one side of a shallow depression at the obliquely truncated pole (Fig. 13, Pl. I; Figs. 1, 4, 5). The flagella (Figs. 14, 15, Pl. II) are very thin as in *C. kappa* Parke & Manton,

Legends to Text-figs. 3–9

Chrysochromulina ericina n.sp. (× 5000)

Fig. 3. Characteristic position of an anchored cell when the haptonema is not extended; view is looking down on non-flagellar pole with flagella lying straight out below body; bacteria (*b*) in vacuole adjacent to pyrenoid-like body.

Fig. 4. Individual with four chromatophores swimming with flagella and haptonema behind body in the position characteristic for the species during rapid swimming; muciferous organelles exuding contents; recently ingested *Chlorella stigmatophora* cell (*i*) at non-flagellar pole and an empty wall of a *Chlorella* cell immediately after ejection from the end of a colourless tube.

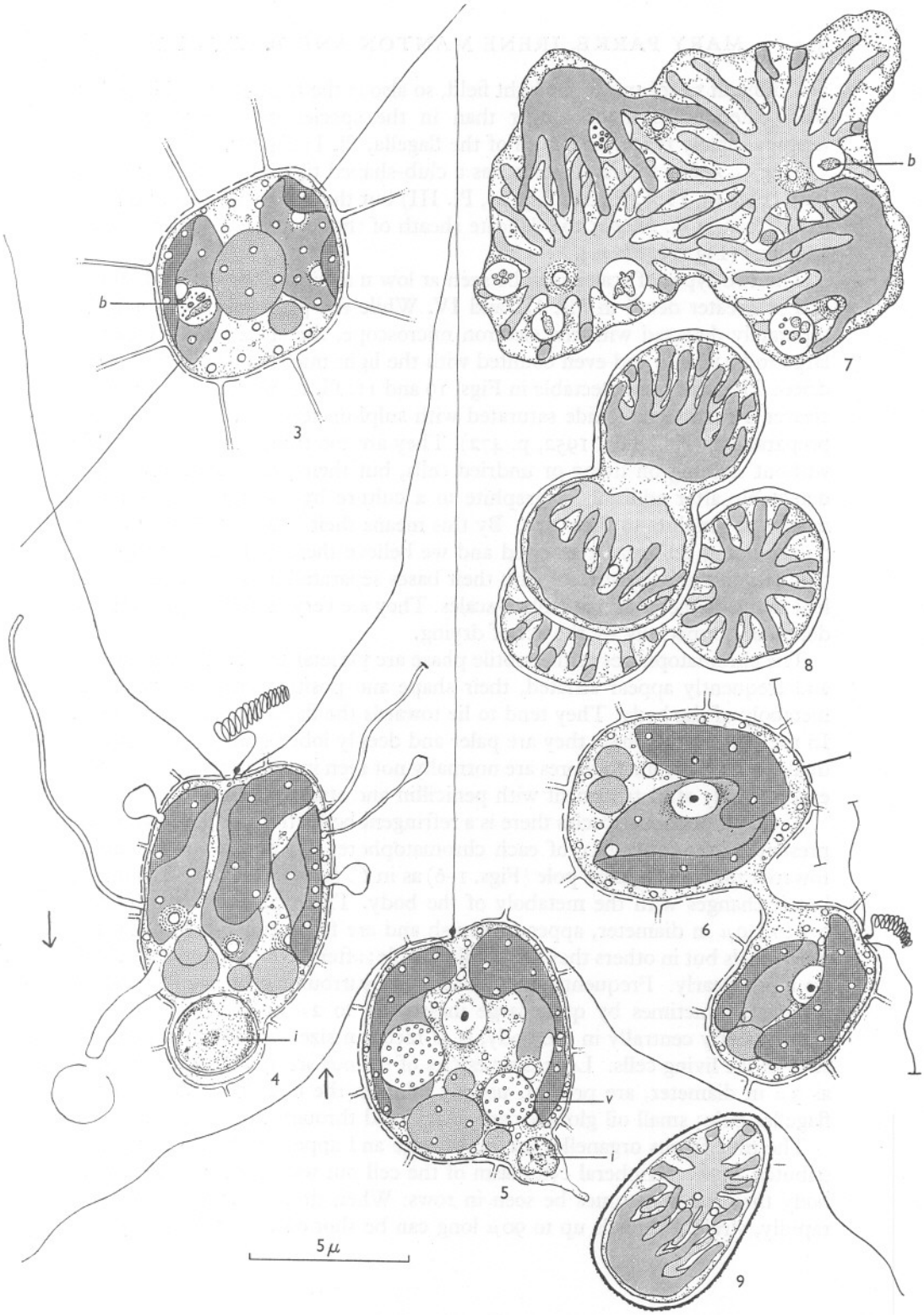
Fig. 5. Individual swimming with flagella and haptonema in front of the body in characteristic position, haptonema fully extended; a cell of *Oicomonas* (*i*) being ingested and large vacuoles (*v*) containing granules showing Brownian movement adjacent to both pyrenoid-like bodies.

Fig. 6. Late fission stage just before separation of daughter-cells.

Fig. 7. Large amoeboid individual with four deeply lobed, pale chromatophores and four pyrenoid-like bodies surrounded by non-refringent material; ingested bacteria (*b*) dancing in vacuoles.

Fig. 8. Second fission of a large walled cell almost completed, to give four small, walled daughter-cells.

Fig. 9. Small rugose walled daughter-cell with two stellate chromatophores and two pyrenoid-like bodies.



Text-figs. 3–9

and difficult to see under the light field, so also is the haptonema which when fully extended is much longer than in the species previously described (approximately twice the length of the flagella, Pl. I; Fig. 5).

Like *C. kappa* the haptonema has a club-shaped tip (Pl. II) and a swollen base (Fig. 13, Pl. I; Pl. II; Fig. 17, Pl. III) but the base is smaller and more ovoid than in *C. kappa*; the delicate sheath of the haptonema is very inconspicuous (Fig. 16, Pl. II).

The two types of scales may be seen at low magnifications in Pls. I and II and in greater detail in Pls. III and IV. While the plate-scales can only be separately detected with the electron microscope, the spines are sufficiently large to be visible and even counted with the light microscope if the cells are dried. They are just detectable in Figs. 10 and 11, Pl. I, and they become even clearer if methylene iodide saturated with sulphur at 30° C. is added to dry preparations (Hollande, 1952, p. 472). They are too translucent to be visible without staining on living or undried cells, but their presence is sometimes detectable after addition of graphite to a culture by the adhesion of small masses of graphite to their tips. By this means their length and distribution on the living cell can be assessed and we believe them to be uniformly distributed on the body surface with their bases separated by a distance equivalent to the diameter of 2 or 3 plate-scales. They are very readily displaced after death, and especially by the act of drying.

The chromatophores of the motile phase are parietal and deeply pigmented and frequently appear striated, their shape and position changing with the metaboly of the body. They tend to lie towards the flagellar pole (Figs. 1–6). In the non-motile phase they are paler and deeply lobed (Figs. 7–9). Individuals lacking chromatophores are normally not seen in cultures of this species except rarely after treatment with penicillin and streptomycin.

As in our previous species there is a refringent body, the so-called pyrenoid, present on the inner face of each chromatophore. Its position is eccentric, towards the non-flagellar pole (Figs. 1–6) as in *C. brevifilum* Parke & Manton, but it changes with the metaboly of the body. These pyrenoid-like bodies, 0·5 to 1·0 μ in diameter, appear greenish and are fairly conspicuous in some individuals but in others they are hardly visible; after osmic fixation they show up more clearly. Frequently these bodies are surrounded by non-refringent material, sometimes by quite large masses up to 2 μ across. The nucleus, placed nearly centrally in the body, is of medium size and can sometimes be seen in the living cells. Leucosin vesicles of various sizes, sometimes as large as 3 μ in diameter, are present, usually lying in the body towards the non-flagellar pole; small oil globules are distributed through the cytoplasm.

The muciferous organelles are quite large and appear to be generally distributed in the peripheral cytoplasm of the cell but with the metaboly of the body they can sometimes be seen in rows. When they expel their contents rapidly, straight threads up to 90 μ long can be shot out, but when they dis-

charge slowly, as for instance when kept at a temperature of 22–24° C or when certain other organisms have been added to the culture, then the contents exude either as small balloons or as thin waving threads (Fig. 4) frequently showing what appears to be a small flat colourless disk sticking to them (Fig. 4). When extremely dilute cresyl blue is added to the living cells one sees almost immediately what appear to be minute disks shot out from the cell surface, which dance about for a time and then disappear. It could not be ascertained whether these disks were caps covering the organelles, as in Hovasse's discobolocysts (Hovasse, 1949), or surface scales pushed off during the discharge, but their capacity to stain a deep blue with cresyl blue suggests that they are probably caps, not scales, as the latter usually stain a pale violet colour. Immediately after the liberation of disks the contents of some of the organelles are discharged, some as small balloons, others as fine threads (Fig. 4).

Some distinct granules, possibly mitochondria, occur generally distributed in the peripheral region of the cell. They stain an intense blue with cresyl blue and under oil immersion can be seen to be connected together by a very fine blue network.

This species moves comparatively slowly with a fairly even rotation and little gyration. There is a marked phototactic reaction in spite of the absence of a stigma. As in the three species already described, swimming is most rapid when the flagella and haptonema are directed backwards and the haptonema is tightly coiled (Fig. 14, Pl. II; Fig. 4).

The rate of movement decreases with an increase of temperature up to 22–24° C. when movement becomes extremely slow, the individual then generally swimming with the haptonema in front of the body.

When not tightly coiled, the haptonema may be fully extended (Fig. 5), in front of, or behind the body, or only partly extended, the remainder appearing as a blob at the distal end (Fig. 11, Pl. I). When individuals swim with the haptonema foremost the flagella are held as in Fig. 5, but when they swim with the haptonema behind the body the flagella are held as in Fig. 4 with their free ends farther apart than the width of the body. The cells do not swim for long in one direction. They can stop suddenly by bringing the flagella to the position shown in Fig. 1, or they can suddenly change and move in the opposite direction by a flick of the flagella from the position of Fig. 5 to the position of Fig. 4 and vice versa. When moving with the haptonema extended in front of the body (Fig. 5) cells are frequently seen to jump back suddenly as if the haptonema had touched something obnoxious. The haptonema can also be bent over from side to side.

Quite long periods of anchorage are common, but the tip of the haptonema can seldom actually be seen attached to a surface, the cell body usually lying over it. The most characteristic position adopted by attached cells is shown in Fig. 3; the haptonema is tightly coiled below the body while the

flagella appear as a straight line on either side. The flagella can either remain quite still or vibrate slowly, causing the body to show a slight dancing movement. Individuals can frequently be seen in the act of attaching with the haptonema nearly fully extended (Fig. 1). The haptonema may remain fully extended or it may coil up drawing the cell down to the surface of attachment (Fig. 2). In the latter position the haptonema becomes hidden owing to the asymmetry of the body. When the distal end of an attached haptonema can be seen, it is sometimes coiled in a flat spiral appearing as a disk with the point of attachment in the centre (Fig. 1). In other cases the haptonema can appear very short (and thicker?), as in *Prymnesium* species, without a visible disk at the attached end (Fig. 6). In these it is perhaps only the distal tip of the haptonema that is extended, the remainder lying coiled and hidden below the body since we have no evidence suggesting that the haptonema can actually contract, as opposed to coiling.

Phagotrophy is of common occurrence, the individuals ingesting bacteria and other organisms, usually up to a size of 3μ but occasionally larger, the maximum ingested size observed being a diatom cell of $9 \times 3\mu$. In addition to graphite (Fig. 12, Pl. I), a number of cultures of different sized organisms were also used with the following results: ingestion of *Oicomonas* sp. $1–2\mu$

Explanation of Plates I–IV

Chrysochromulina ericina n.sp.

I

Fig. 10. A cell killed with osmic vapour and dried on a glass slide, with some detached scales marked by arrows. Photographed dry without a coverslip. Magnification × 1000.

Fig. 11. Two cells of the same.

Fig. 12. Three cells killed with osmic vapour after graphite feeding and photographed in a liquid mount with oil-immersion lens and visual light. Magnification × 2000.

Fig. 13. A low-power view of a cell dried and shadowed after vapour killing, on a formvar film, seen with the electron microscope. Micrograph M. 128·1, magnification × 3000.

II

Fig. 14. A cell showing flagella and a coiled haptonema. Electron micrograph M. 239·26, magnification × 5000.

Fig. 15. Tip of left-hand flagellum of Fig. 14, magnification × 10,000.

Fig. 16. A haptonema from the cell of Fig. 13 more highly magnified. Electron micrograph M. 128·2, magnification × 50 000.

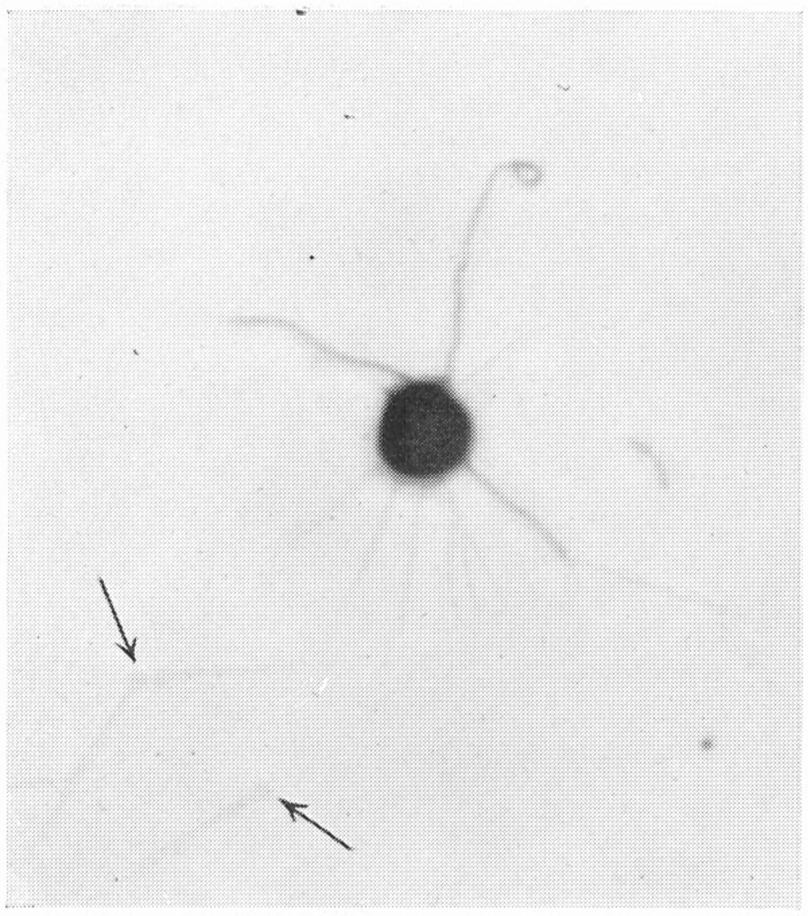
III

Fig. 17. The body of the cell of Fig. 13 more highly magnified to show scales and the bases of spines. Electron micrograph M. 128·3, magnification × 10,000.

Fig. 18. A group of detached spines and scales. Electron micrograph M. 128·9, magnification × 5000.

IV

Fig. 19. Part of Fig. 18 more highly magnified to show details of the scales and bases of the spines. Electron micrograph M. 128·13, 40 kV, magnification × 20,000.



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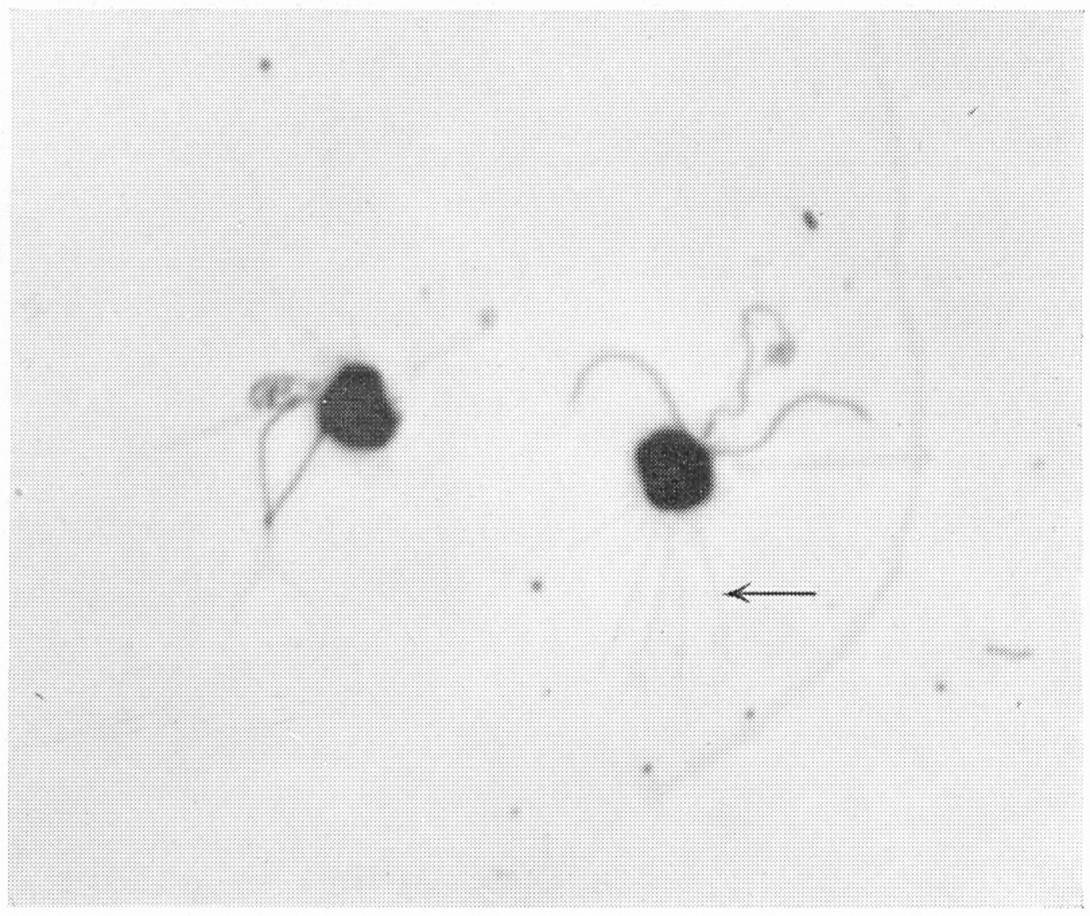

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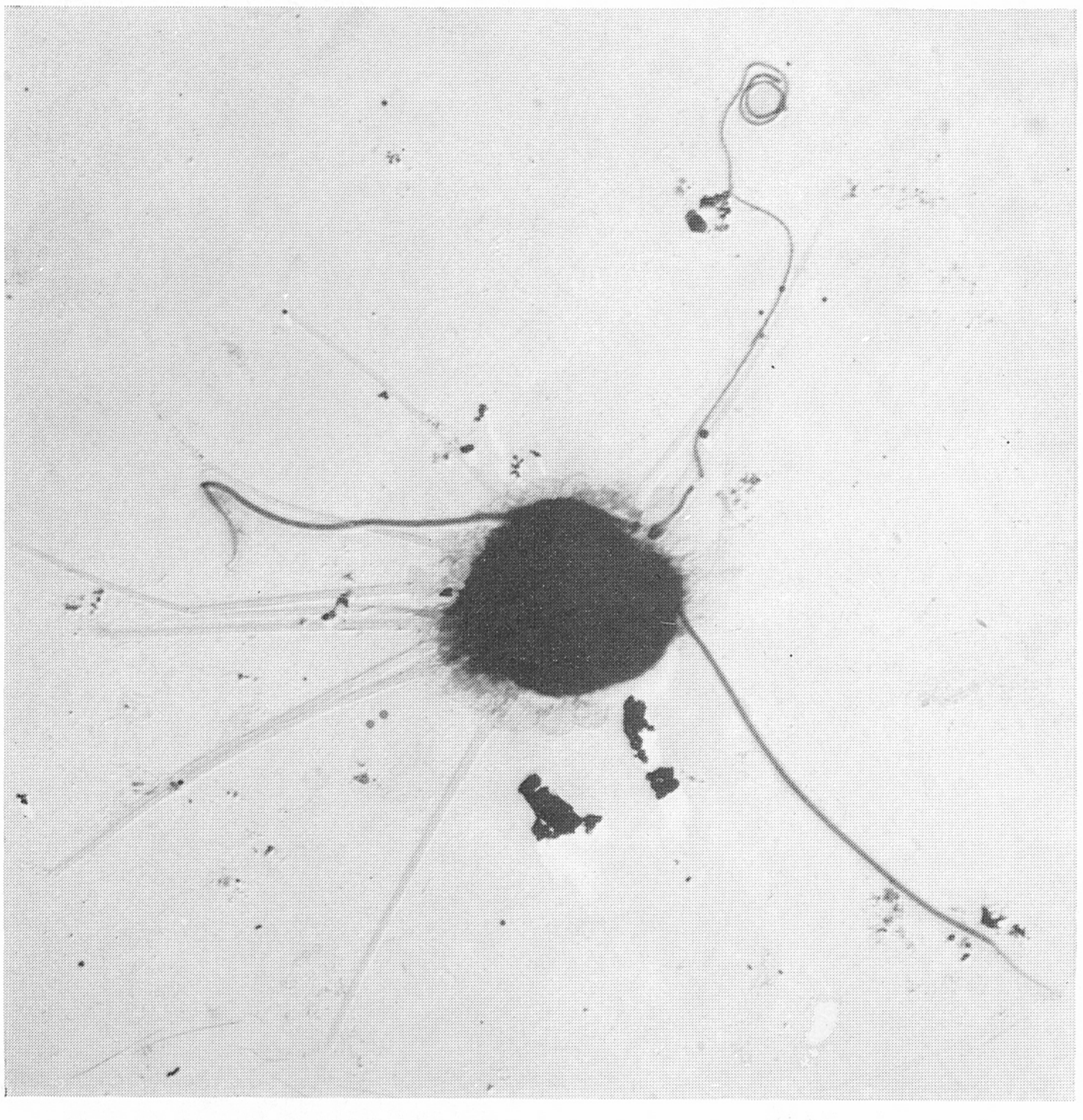

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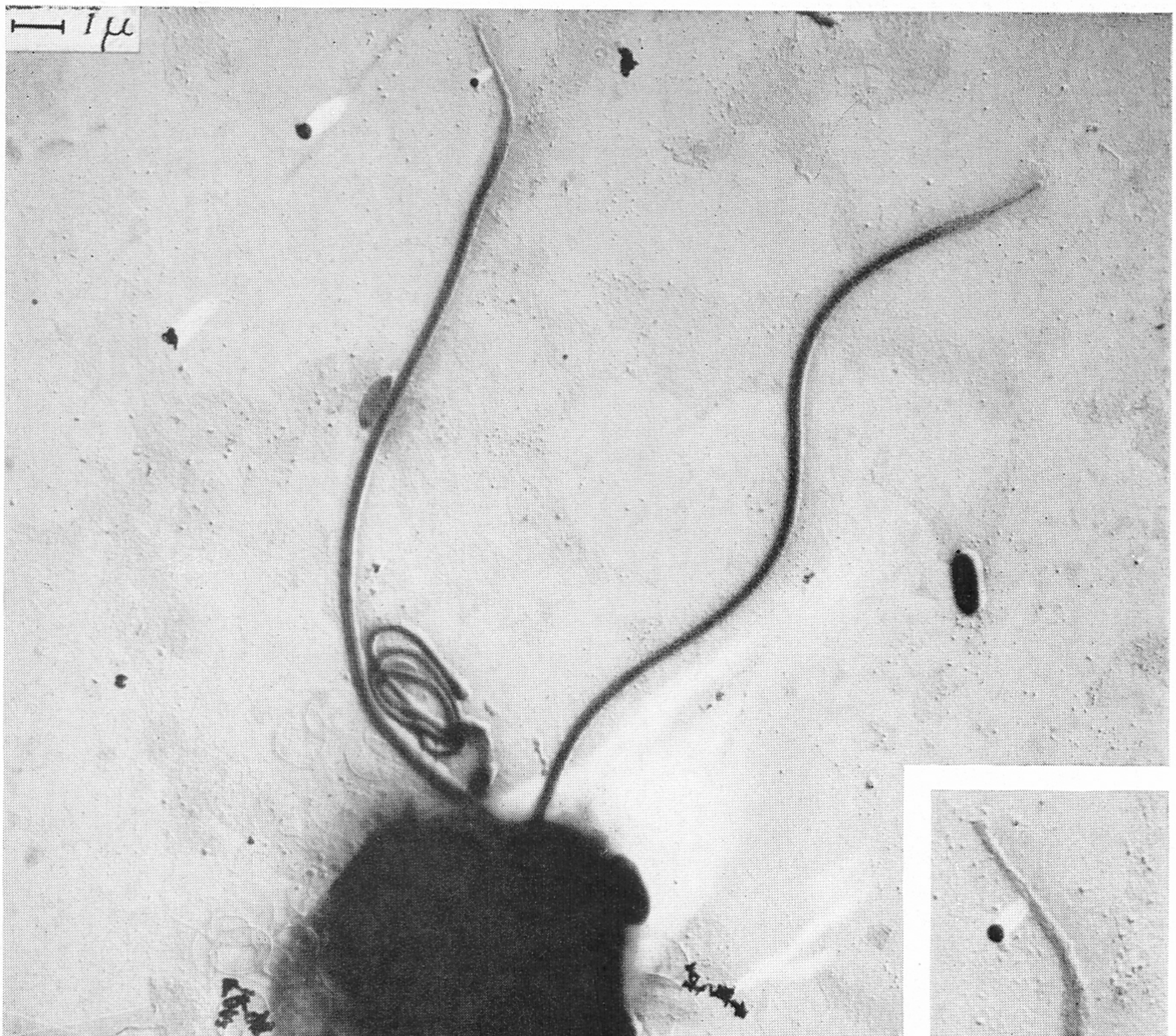

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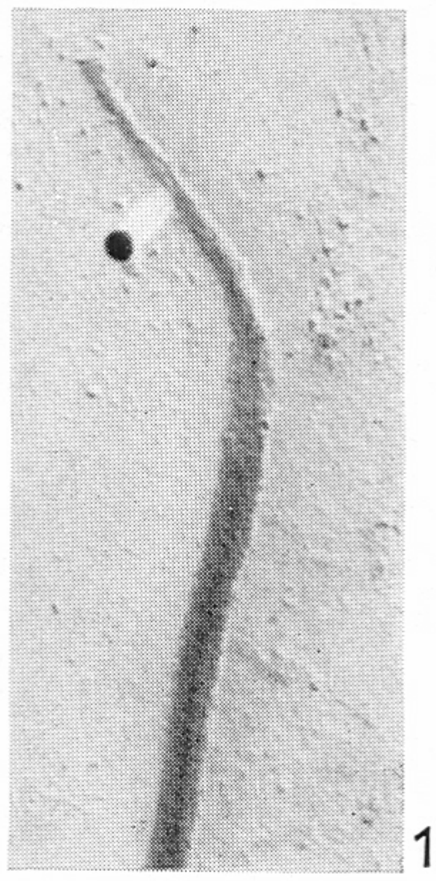

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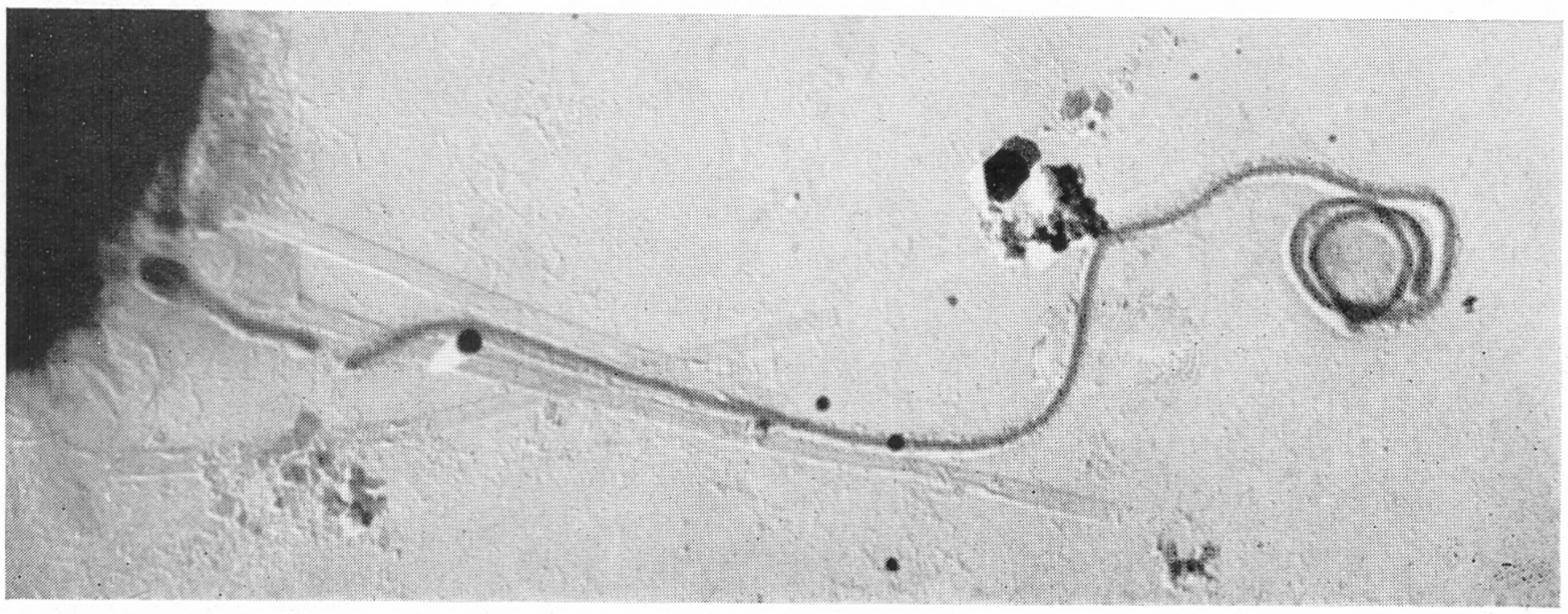

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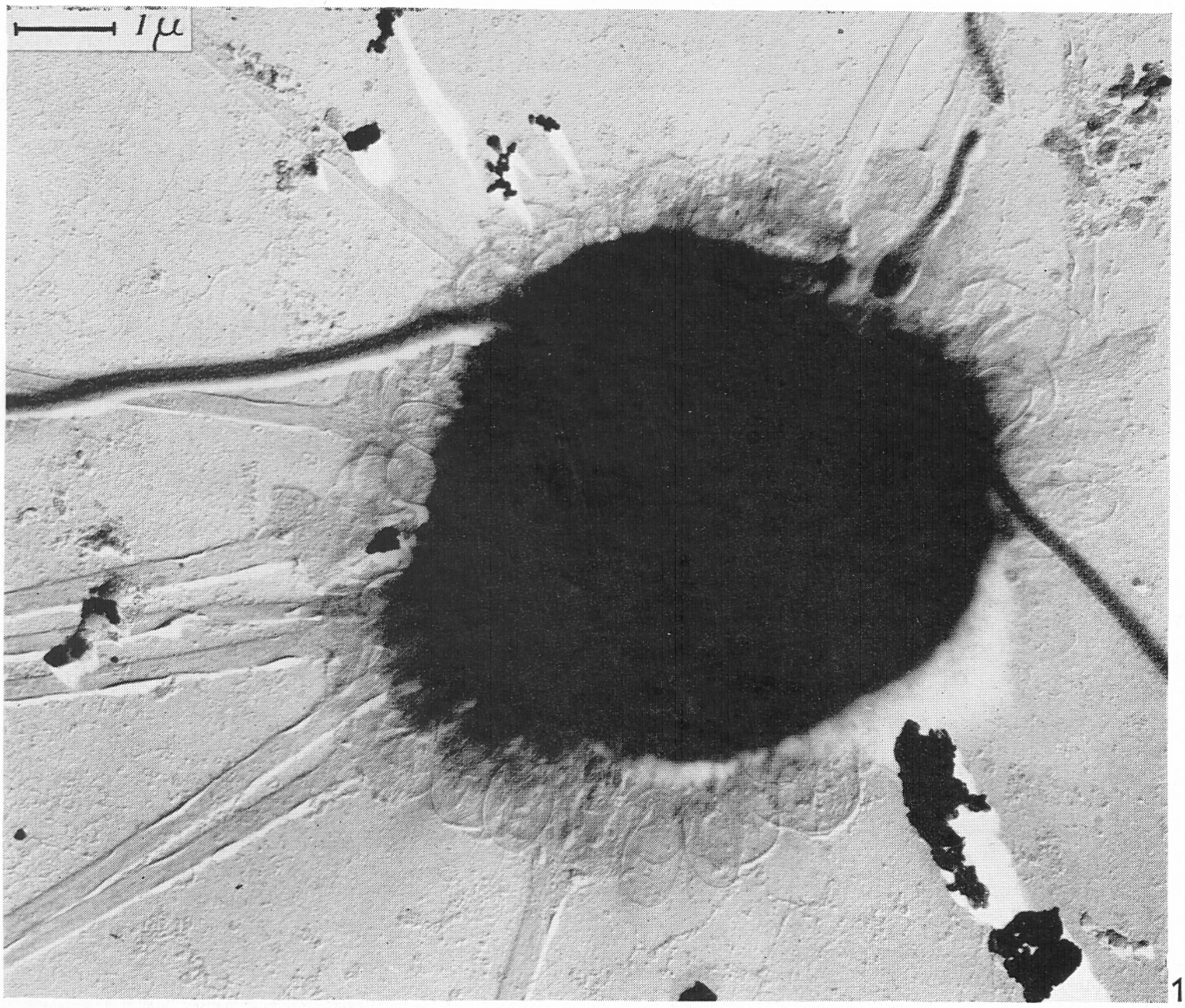

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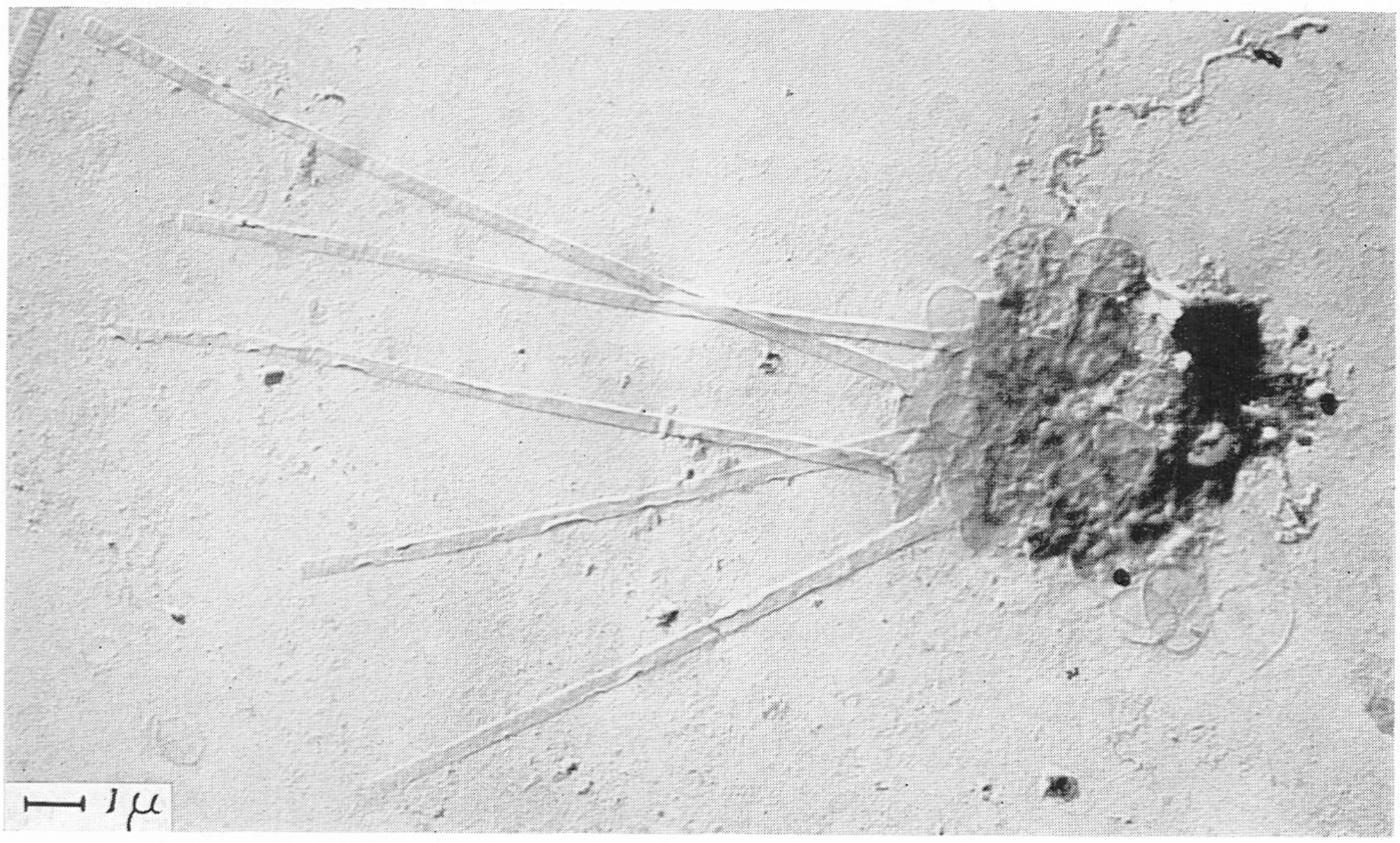

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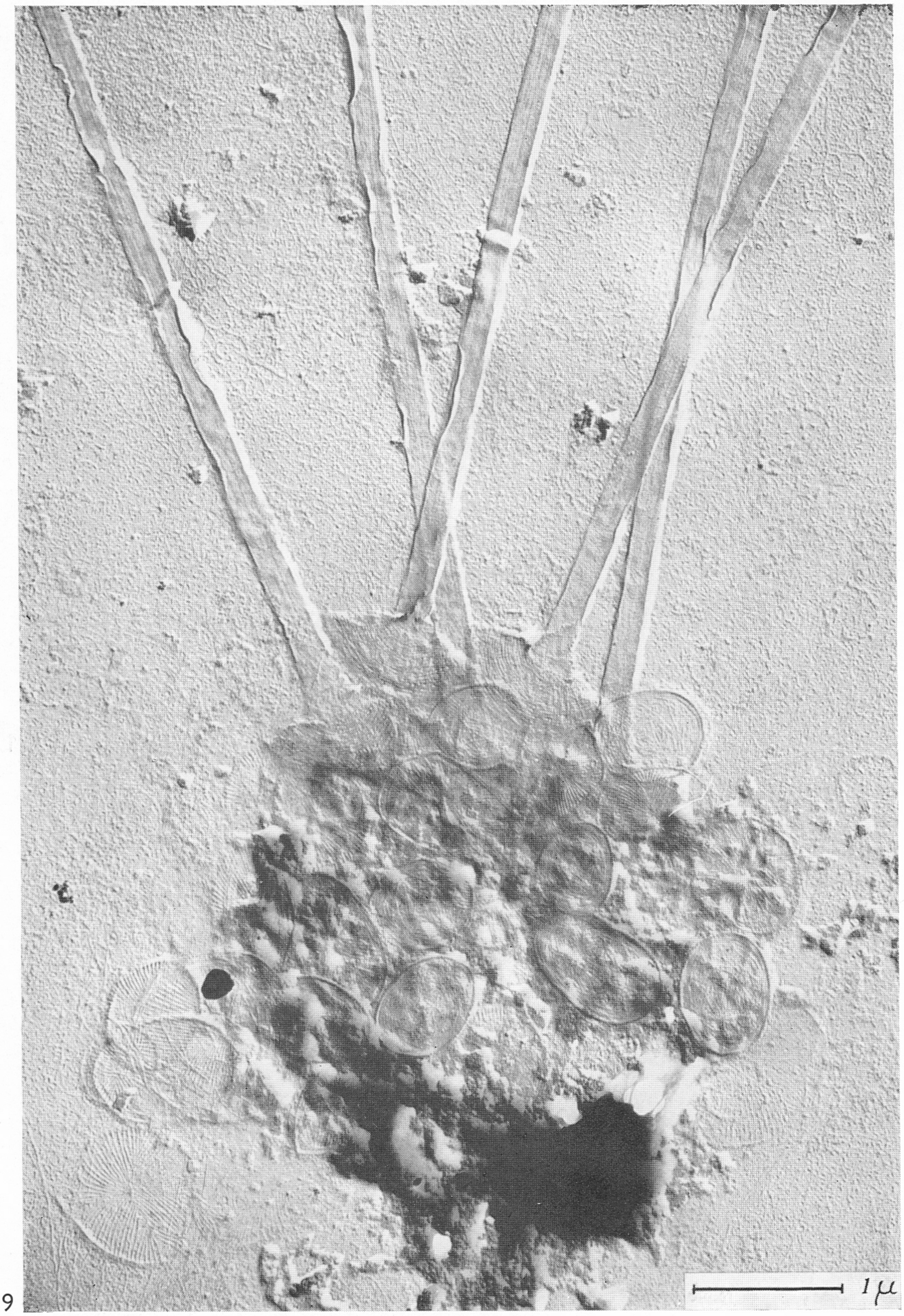

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(Fig. 5), *Stichococcus cylindricus* Butcher 3–5 × 2 μ, Plymouth no. 55 (possibly a *Stichochrysis* sp.) 3–10 × 3 μ, and *Chlorella stigmatophora* Butcher 2·5–4·5 μ (Fig. 4) was fairly frequent; ingestion of the smaller individuals of *Nitzschia gotlandica* A. Cleve-Euler 6–10 μ L. and *Porphyridium cruentum* (Ag.) Näg. 4–12 μ diam. was not uncommon, but the ingestion of *Navicula salinicola* Hust. 9–10·5 μ L. was seen only once (Fig. 2) when a cell 9 × 3 μ had been ingested by an individual 10 × 6 μ. The following species were tested for ingestion with negative results: two *Dunaliella* spp. 6–12 μ, *Phaeodactylum tricornutum* Bohlin 8–35 μ and *Nannochloris atomus* Butcher 2–3 μ. One of the *Dunaliella* sp. (Plymouth no. 81) and the *Nannochloris* appeared to have an adverse effect on the *Chrysochromulina ericina*, whilst *Chrysochromulina* cells which had actually ingested *Chlorella* cells were believed to disintegrate afterwards, but the evidence is not yet absolutely conclusive. With the addition of certain unialgal cultures (e.g. *Dunaliella*, *Chlorella*, *Phaeodactylum*, *Navicula*) to the *Chrysochromulina*, the muciferous organelles of the *Chrysochromulina* cells were seen to exude their contents (Fig. 4) as they did when the cultures were kept at a temperature of 22–24° C. General exudation from the muciferous organelles was never seen when graphite was added, although a few hours after the addition practically every individual had ingested a certain amount—from minute particles to masses up to 4·5 μ (Fig. 12, Pl. I). Small discharges from the organelles were sometimes observed accompanying ejection of the graphite.

The actual ingestion of material occurs always at the non-flagellar pole (Fig. 12, Pl. I; Figs. 4, 5). The ingested material, if sufficiently small, is then moved close to one of the 'pyrenoids'. The whole process was followed in detail in a culture of *Chrysochromulina*, which had been cleaned by utilizing the phototactic properties of the species (Droop, 1954), until the bacterial contaminants were reduced to one species, in this case a species distinctly bottle-shaped and about 1 μ in length. The *Chrysochromulina* was then observed to take in bacteria by surrounding them with a clear or slightly granular substance which flowed out from the body enclosing one or more bacteria. Almost immediately afterwards the bacteria could be detected in a vacuole (Fig. 3) close to one of the 'pyrenoids' which were sometimes masked by the vacuole (Fig. 5). The bacteria then began to show dancing movements inside the vacuole and in a matter of 2–3 minutes were broken up into minute granules (Fig. 5) which continued to show Brownian movement for several more minutes. Similar vacuoles, full of minute particles in Brownian movement, were seen by Parke (1949) in *Chromulina pleiades*, but for *Prymnesium parvum* and *P. minutum*, Carter (1937) records the presence of a large number of minute granules in active Brownian movement in 'an ill-defined region', not in clearly delimited vacuoles. In this culture, with only one bacterial contaminant, the phagotrophic nature of the large amoeboid non-motile phase of the *Chrysochromulina* was also demonstrated; a number

of vacuoles containing bacteria could be seen quite clearly lying close to the pyrenoid-like bodies (Fig. 7).

When the motile phase ingests cells with definite walls the cell contents are absorbed but the walls are not; a colourless tube containing the wall flows out, usually from the side of the body, and discards the wall from its tip (Fig. 4); the tube is then withdrawn into the body. Empty walls of *Chlorella*, *Stichococcus*, '*Stichochrysis*', *Porphyridium* and *Nitzschia gotlandica* have been seen thrown out of the body in this manner.

Reproduction follows the same pattern as that described for *Chrysochromulina kappa*, but in the motile phase no double-fission stages have so far been observed. The second haptonema and the two new flagella can be formed before the cell broadens for the actual fission, which can produce daughter-cells of equal or very unequal size (Fig. 6). In the species previously described, the daughter-cells remain attached by a small connexion at the non-flagellar pole when the fission is nearly completed, but in *C. ericina* the connexion was frequently seen to be between the sides of the daughter-cells (Fig. 6) towards the flagellar pole.

At the peak of growth a culture produces $1\frac{1}{2}$–2 million cells per ml. Non-motile stages, similar to those described in detail for *C. kappa*, have been observed, forming a dark olive-green to brownish skin on one side of the bottom of the flask after the peak of growth has been passed. The large amoeboid cells, up to $14 \times 9{\cdot}5\,\mu$, with four very finely lobed chromatophores, frequently show large numbers of ingested bacteria (Fig. 7) while the tetrads of walled daughter-cells (Fig. 8), each with 2 stellate or finely lobed chromatophores, were distinguishable by deeper pigmentation. The free, walled daughter-cells (Fig. 9), in which the pyrenoid-like bodies could sometimes be seen, were usually ovoid, measuring from $4 \times 2{\cdot}5\,\mu$ to $7 \times 4\,\mu$. They differed only from those previously described in having a slightly thicker wall which appeared faintly brownish and was delicately rugose on the outside, somewhat as described by Carter (1937) for *Prymnesium parvum*. A thick culture of the motile phase can be obtained from the non-motile phase in 6–9 days after addition of fresh culture medium, the dark skin disappearing from the bottom of the flask.

Chrysochromulina ephippium n.sp., Parke & Manton

(Gr. ἐφίππιον—a saddle)

Diagnosis

Motile cells showing considerable metaboly, approximately saddle-shaped when moving slowly or stationary, bell-shaped to spheroidal when swimming rapidly, 6–10 (exceptionally 4·5–12) μ in size (length of back of saddle). Two flagella and one haptonema arising close together from the ventral concave

surface near to one margin in a centre line; flagella equal, smooth, gradually attenuated to a hair point (E. M. observation), usually heterodynamic, occasionally appearing homodynamic, 3 to 4 times cell size in length; the haptonema, thinner than the flagella, 12 to 14 (exceptionally 16) times body size in length when fully extended, a club-shaped tip but no obvious translucent sheath visible with the electron microscope. The periplast, pectic in nature, showing a covering of very thin transparent circular to oval sculptured, dimorphic scales, visible only under the electron miscroscope. Scales without spines 0·5–0·7 μ, with a pattern of radiating ridges on one side and crossed striations within a wide raised rim on the other. Scales with spines 0·3–0·6 μ, with a pattern of radiating ridges on the side towards the body and a narrow raised rim surrounding concentric markings on the outer side, the slender tapering spine, approximately equal to scale diameter in length, attached by 4 decurrent ridges extending to scale margin. Distribution of two types of scales on body unknown.

Cells uninucleate, no stigma. Chromatophores appearing striated, 1 or 2, pale golden brown; in cells of motile phase parietal, saucer-shaped to oblong, with a single inconspicuous globular body (pyrenoid?) placed eccentrically on inner face of each; in cells of non-motile phase coarsely lobed. Oil and leucosin produced. Ejectile muciferous bodies small, localized in groups in peripheral cytoplasm, but their position changing with the metaboly of the body. Nutrition phototrophic and/or phagotrophic. Not 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 very thin walls; motile phase almost certainly liberated from walled daughter-cells through a pore.

Habitat: the sea at position (Plymouth Laboratory Station L4) Lat. N. 50° 15′, Long. W. 4° 13′ (13 Sept. 1950, type culture) from a townet sample. Type culture (Plymouth no. 31) deposited with the Type Culture Collection, Cambridge; preserved material and photographs lodged with the Marine Biological Association, Plymouth, England.

Cellula motili formam satis mutanti, fere ephippioidea cum lente natat aut restat immotilis, cupuliformi aut sphaeroidali cum natat rapiditer, longitudine 6–10 μ (rare 4·5–12 μ) per dorsum ephippii. Flagellis duobus et unico haptonemate conjunctim exorientibus ex aspectu concavo ventrali, prope lineum medium; flagellis aequis, teretibus, paulatim attenuatis sicut ad capilli extremitatem, ut videtur per microscopiam electronicam, generaliter heterodynamicis, nonnunquam homodynamicis, longioribus 3 vel 4 quam cellula. Haptonemate teneriore quam flagellis, 12–14 (rare 16) longiore quam cellula, cum maxime extensus, apice clavato sed nulla tunica externa semidiaphana ut videtur per microscopiam electronicam. Periplasto, pectico natura, induto delicatissimis squamis diaphanis, circularibus aut ovalibus, sculptis, invisibilibus nisi per microscopiam electronicam; squamis manifestis sub duabus formis: altera forma, sine spinulis, 0·5–0·7 μ longis, sculptis radiantibus rugis ad aspectum inferiorem, et striis decussatis circumdatis ab lata margine elevata ad aspectum superiorem; altera forma, praeditis spinulis longis 0·3–0·6 μ, sculptis rugis radiantibus

ad aspectum inferiorem, et angusta margine elevata circumdanti rugas concentricas ad aspectum superiorem. Spinulis teneribus, longitudine ferme aequis latitudine squamae, affixis per quattuor costas decurrentes, extendentes ad marginem squamae. Ignotum est quomodo duae formae squamarum distributae sint in superficie cellulae.

Nucleo unico, nullo stigmate, chromatophoris striatis ut videntur, 1 aut 2, pallide aureo-brunneis; in cellula in statu motili, crateriformibus aut oblongis, praeditis unicis corporibus globularibus inconspicuosis (?pyrenoidalibus) locatis ex centro in aspectu concavo; in cellula in statu non-motili rude lobatis. Cellula oleum leucosinumque parienti; corporibus parvis ejectilibus et muciferosis, aggregatis in cytoplasmate superficiali, situ tamen mutanti secundum mutationem formae cellulae. Nutritione phototrophica necnon phagotrophica. Non toxica piscibus.

Generanti asexualiter in statu motili per fissionem in duobus cellulis filiolis, generaliter aequis magnitudine; in statu non-motili per fissiones subsequentes cellularum amoeboidalium ad 4 cellulas filiolas producendas, cum tenerrimis parietibus. Ferme certum est quod cellulae in statu motili ex cellulis filiolis per foramen liberantur.

Habitat mare prope Plymouth ad locationem Lat. N. 50° 15′, Long. W. 4° 13′ (13 Sept. 1950—cultura typica).

Description

The position of the haptonema differs from all those previously described in lying across the body during slow swimming (though not during rapid swimming) instead of projecting out from it. In this condition, or when anchored, the cells are roughly saddle-shaped with smooth curved sides. When seen from above or below the cells appear squarish or oblong with the

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Legends to Text-figs. 20–29

Chrysochromulina ephippium n.sp.

(Figs. 20–22 × 1250; Figs. 23–29 × 5000)

Fig. 20. Cell in shape adopted during rapid swimming; flagella and haptonema behind body in position characteristic for the species in this state.

Fig. 21. Saddle-shaped cell gliding slowly with haptonema fully extended in front of the body.

Fig. 22. Similar to Fig. 20 but sides of saddle overlapping differently and flagella in position for slower movement.

Fig. 23. Ventral view (concave surface) of saddle-shaped cell, haptonema loosely coiled; one flagellum still, the other undulating. *c*, chromatophore; *f*, flagellum; *g*, graphite; *h*, haptonema; *l*, leucosin vesicle; *m*, muciferous body; *n*, nucleus; *p*, pyrenoid-like body; *s*, scale; *v*, vacuole containing ingested particles in Brownian movement.

Fig. 24. Lateral view of saddle-shaped cell, haptonema fully extended; bacterium in vacuole.

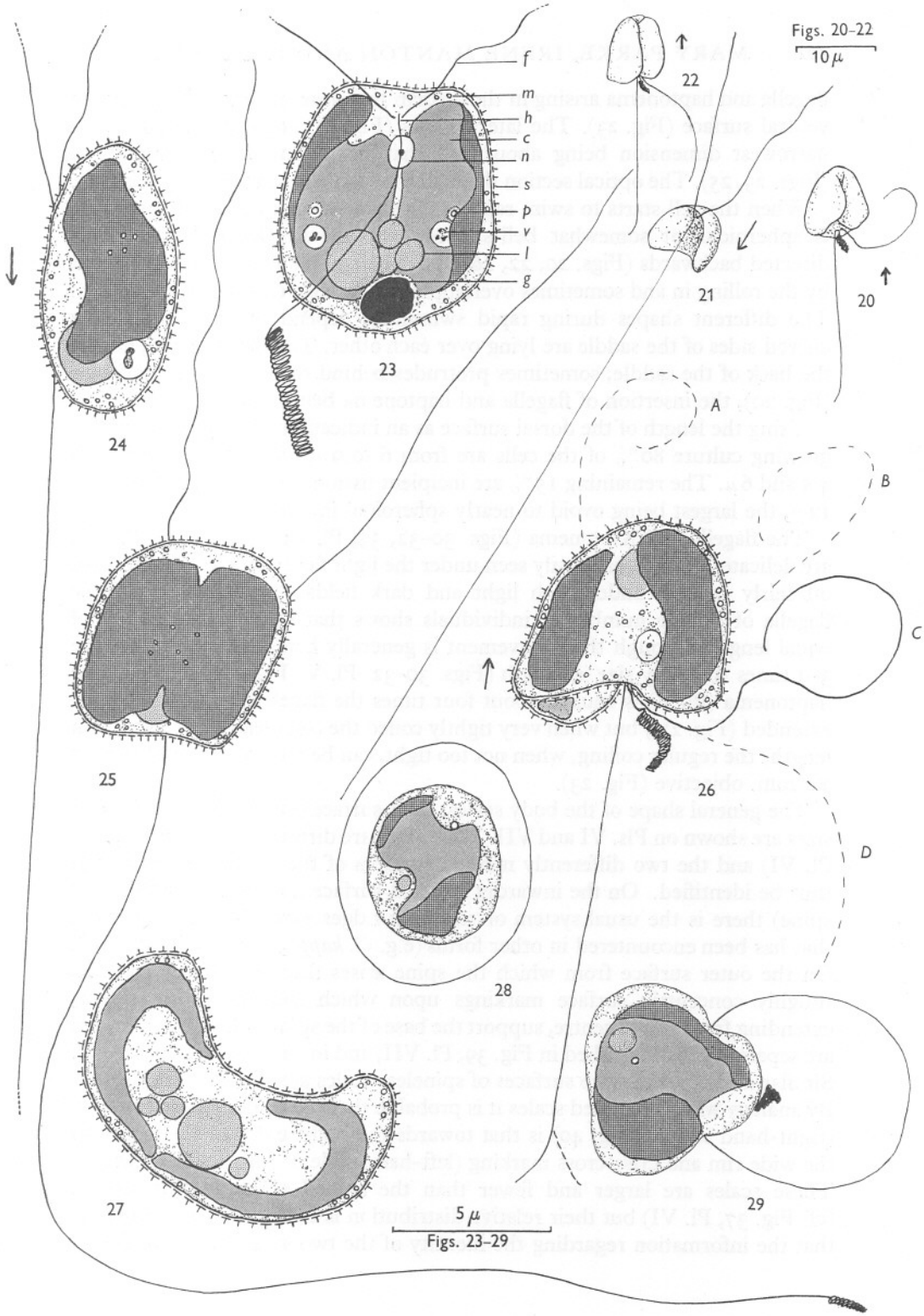
Fig. 25. Dorsal view (convex surface) of anchored saddle-shaped cell, chromatophore dividing, anchored haptonema bent and partly extended; flagella undulating at different speeds.

Fig. 26. Individual with sides of saddle overlapping; flagella and haptonema behind body in position characteristic for the species during very rapid swimming; positions *A* and *B*, less rapid swimming than in position *C*; position *D*, slower movement than in positions *A* or *B*.

Fig. 27. Optical section of large saddle-shaped individual with two chromatophores.

Fig. 28. Walled daughter-cell with contents shrunk away from the wall and with chromatophore similar to those of the motile phase.

Fig. 29. Contents of walled daughter-cell partly released through pore, flagella not detected.



Text-figs. 20–29

flagella and haptonema arising in the median line near one end of the concave ventral surface (Fig. 23). The lateral views (Fig. 24) are somewhat oval, the narrowest dimension being about half that of the dorsal or ventral views (Figs. 23, 25). The optical section through the saddle is bean-shaped (Fig. 27).

When the cell starts to swim rapidly the shape changes to a half-ovoid, or is spheroidal, or somewhat bell-shaped, with the flagella and haptonema directed backwards (Figs. 20, 22, 26). The change in shape is brought about by the rolling in and sometimes overlapping of the curved sides of the saddle. The different shapes during rapid swimming depend on how tightly the curved sides of the saddle are lying over each other. The flagellar end, now at the back of the saddle, sometimes protrudes behind the body as a small lobe (Fig. 20), the insertion of flagella and haptonema being clearly seen.

Using the length of the dorsal surface as an indication of size, in an actively growing culture 80% of the cells are from 6 to 9 μ, while 5% are between 4·5 and 6 μ. The remaining 15% are incipient fission stages and are from 9 to 12 μ, the largest being ovoid to nearly spheroidal in shape.

The flagella and haptonema (Figs. 30–32, 34, Pl. V; Figs. 35, 36, Pl. VI) are delicate and not very easily seen under the light field; they are also thrown off fairly quickly under both light and dark fields. Measurement of the flagella of a large number of individuals shows that the two flagella are of equal length although their movement is generally heterodynamic; they are 3–4 times the body size in length (Figs. 30–32, Pl. V; Figs. 20, 25, 26). The haptonema (Pl. V) is usually about four times the flagella length when fully extended (Fig. 21), but when very tightly coiled the coil measures 1·5–2·0 μ in length; the regular coiling, when not too tight, can be seen quite clearly under a 2 mm. objective (Fig. 23).

The general shape of the body scales, their surface sculpturing and relative sizes are shown on Pls. VI and VII. The spines are directed outwards (Fig. 35, Pl. VI) and the two differently marked surfaces of the subtending scale can thus be identified. On the inwardly directed surface (i.e. that away from the spine) there is the usual system of radiating ridges extending to the margin that has been encountered in other forms (e.g. *C. kappa*, *C. minor*, *C. ericina*). On the outer surface from which the spine arises there is a raised rim and roughly concentric surface markings upon which four cruciform ridges, extending from rim to centre, support the base of the spine. These two surfaces are separately distinguished in Fig. 39, Pl. VII, and in various parts of Fig. 38. Similar details for the two surfaces of spineless scales are contained in Fig. 40. By analogy with the spined scales it is probable that the rimless ridged surface (right-hand scale of Fig. 40) is that towards the body and that the face with the wide rim and criss-cross marking (left-hand scale of Fig. 40) is outwards. These scales are larger and fewer than the spined scales in this species (cf. Fig. 37, Pl. VI) but their relative distribution is unknown. It is probable that the information regarding the identity of the two surfaces will be found

applicable to other species in which direct evidence has not so far been obtained, notably for the flat scales of *C. ericina*.

The chromatophores, one in smaller individuals, two in larger, are clearly striated and their position changes very considerably with the metaboly of the body. When the cell is saddle-shaped the chromatophores lie close to the dorsal surface (Figs. 23, 25) and curve round on to the ventral surface (Figs. 23, 27). They sometimes appear ribbon-shaped, the two edges nearly meeting ventrally if there is one chromatophore, or both dorsally and ventrally if there are two. When the cell changes shape for rapid swimming, the chromatophores tend to elongate, also becoming narrower (Fig. 26).

Completely colourless cells have not yet been detected in this species, but some peculiar small orange-brown chromatophores borne singly in a few otherwise unpigmented small cells are suspected to have been ingested fragments of degenerating cells. It is therefore probable that specimens lacking chromatophores are occasionally formed.

In the motile phase the pyrenoid-like bodies are very inconspicuous, measuring only about 0·5 μ in diameter. In many individuals they could not be seen at all, but when observed each one appeared to lie on the inner face of a chromatophore towards one margin and slightly towards the non-flagellar end of the cell (Fig. 23); they sometimes appeared to be surrounded by a small mass of non-refringent material. The medium-sized nucleus is occasionally visible in the living cells lying in the body towards the ventral surface near the point of insertion of flagella and haptonema. In this species fairly small vesicles of leucosin are produced, generally one to three in each cell, and they lie in the body towards the dorsal surface away from the flagellar end (Figs. 23–25). Small oil globules could also be detected distributed throughout the cytoplasm. The refringent ejectile muciferous bodies are not very conspicuous and are localized in groups of 5 to 7, scattered over the body in the peripheral cytoplasm (Figs. 23–26). Their contents are generally exuded quickly as short threads, but sometimes slowly as small globules.

Movement is generally extremely rapid, the individuals swimming in straight lines for long periods. In spite of the absence of a stigma there is a marked phototactic reaction.

Figs. 20, 22 and 26 illustrate the body shape and the position of the flagella and haptonema during the most rapid swimming. The body rotates very quickly as the cell moves forward in the water, showing only slight gyration. One flagellum trails behind the body showing little movement except possibly near the tip, which appears to beat from side to side, though this appearance is probably due to the rotation of the body. The other flagellum adopts the various attitudes labelled *A* to *D* in Fig. 26, the position *C* being that of the most rapid motion, *A* and *B* being less rapid and *D* still slower. During very rapid swimming the haptonema is coiled. The various degrees of uncoiling exhibited by Figs. 30–32, Pl. V, are probably fixation

effects but the attitudes of the flagella shown in these figures are highly characteristic of the normal slow swimming in the directions indicated by the arrows.

By putting the flagella straight out stiffly from the body, a cell can stop abruptly from rapid swimming. It then usually reassumes the saddle shape and becomes anchored by the end of the haptonema. Under dark field the uncoiling of the haptonema can be followed quite easily. It unrolls, sometimes quite slowly, the unrolling starting from the body until it lies out stiffly like a rod: it then attaches. Alternatively the haptonema can anchor at any stage of the uncoiling or when not uncoiled at all. If the haptonema does not uncoil when the cell anchors, the cell rotates very rapidly with the flagella either lying out from the body or curved inwards at their distal ends and under dark field recalling a catherine wheel firework. If the haptonema uncoils partly or completely the body is seen usually with the dorsal (convex) surface uppermost, the flagella projecting in the opposite direction to the haptonema (Fig. 25). When the cell is attached with the haptonema extended both

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Explanation of Plates V–VII

Chrysochromulina ephippium n.sp.

V

Fig. 30. A cell killed with iodine in KI and dried on a glass slide; photograph of the dry specimen taken without a coverslip. Magnification × 1000.

Fig. 31. Another cell, as Fig. 30.

Fig. 32. Another cell as Fig. 30 after transfer from glass to a quartz slide, examined in a liquid mount (water, with a trace of iodine) and photographed on the ultraviolet microscope with a glycerine-immersion monochromet (wave length 2750 A) Exposure no. UV 256·3*b*. Magnification × 3000.

Fig. 33. A cell killed with osmic vapour after graphite feeding, photographed in the cultur fluid with an oil-immersion lens. Magnification × 2000.

Fig. 34. A cell killed on a formvar film with osmic vapour, shadowed, and examined with the electron microscope; the appendages more disarranged than in Figs. 30–32 but visible. Electron micrograph M 115·20, magnification × 3000.

VI

Fig. 35. Central portion of the cell of Fig. 34, Pl. V, more highly magnified to show scales in position on the body. Electron micrograph M. 115·21, magnification × 10,000.

Fig. 36. Tip of a flagellum. Electron micrograph M. 273·11, magnification × 10,000.

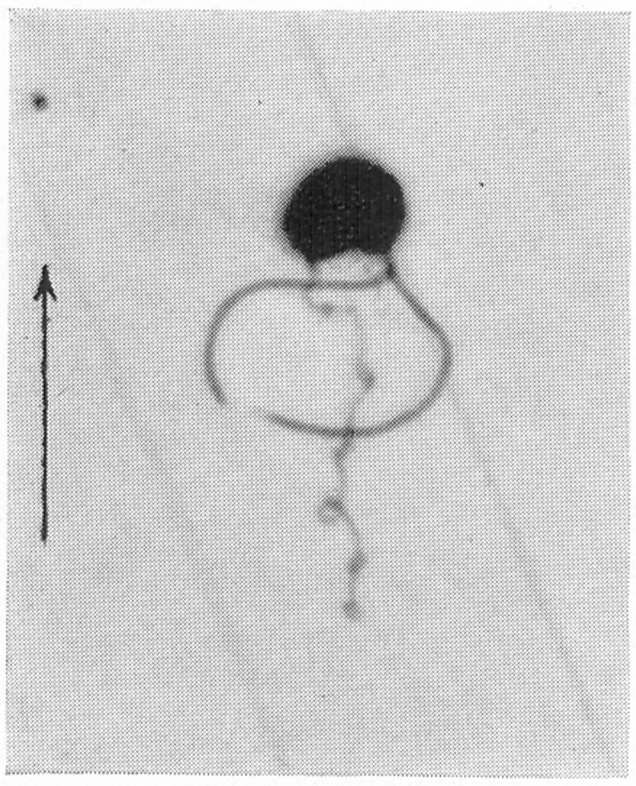
Fig. 37. Scales near the body of another cell. Electron micrograph M. 251·15, magnification × 20,000.

VII

Fig. 38. Scales near the body of another cell showing details of both surfaces of plate scales and spined scales; for further description see text p. 402. Electron micrograph M. 251·4, magnification × 30,000.

Fig. 39. Details of two spined scales from the field of Fig. 37, Pl. VI, to show the two different faces, left-hand scale showing inner face, right-hand scale showing outer face. Electron micrograph M. 251·15, reversed print, magnification × 30,000.

Fig. 40. Details of two plate scales from another cell showing the two faces, left-hand scale showing outer face, right-hand scale showing inner face. Electron micrograph M. 251·8, reversed print, magnification × 30,000.



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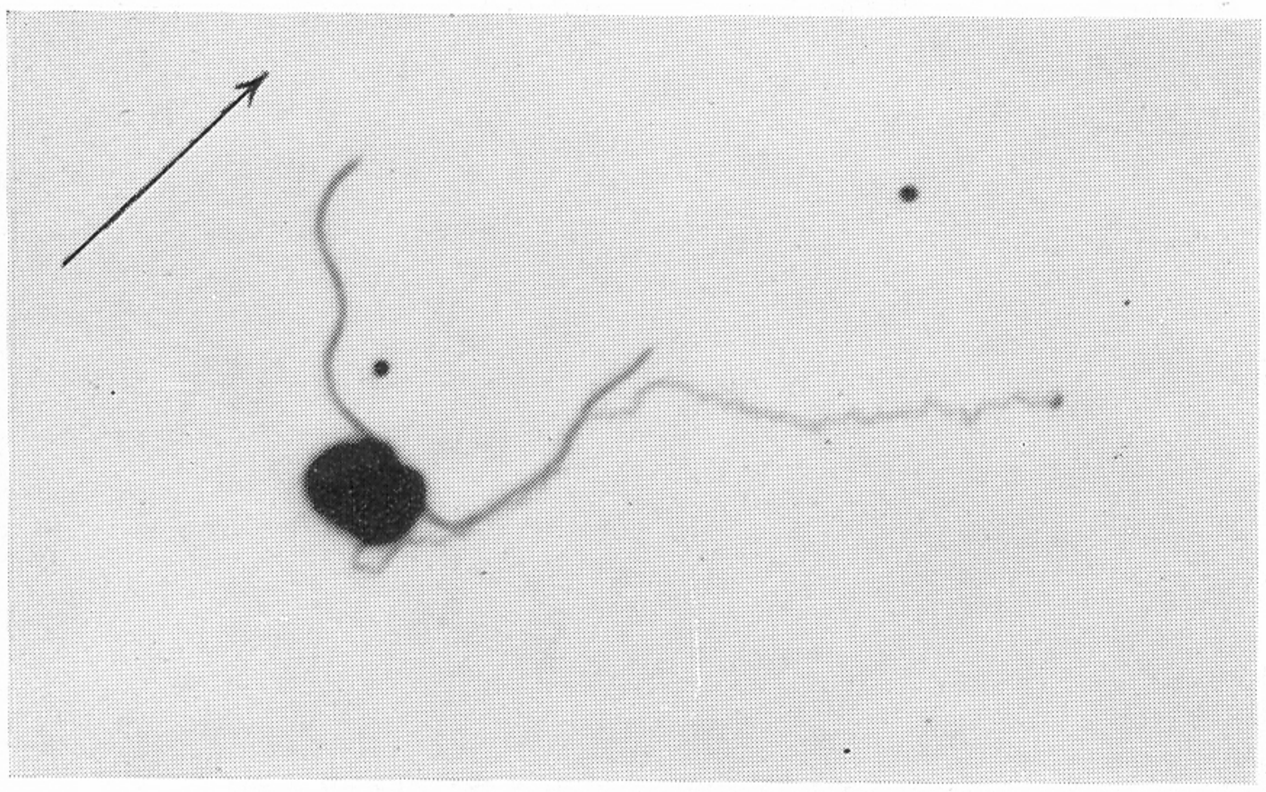

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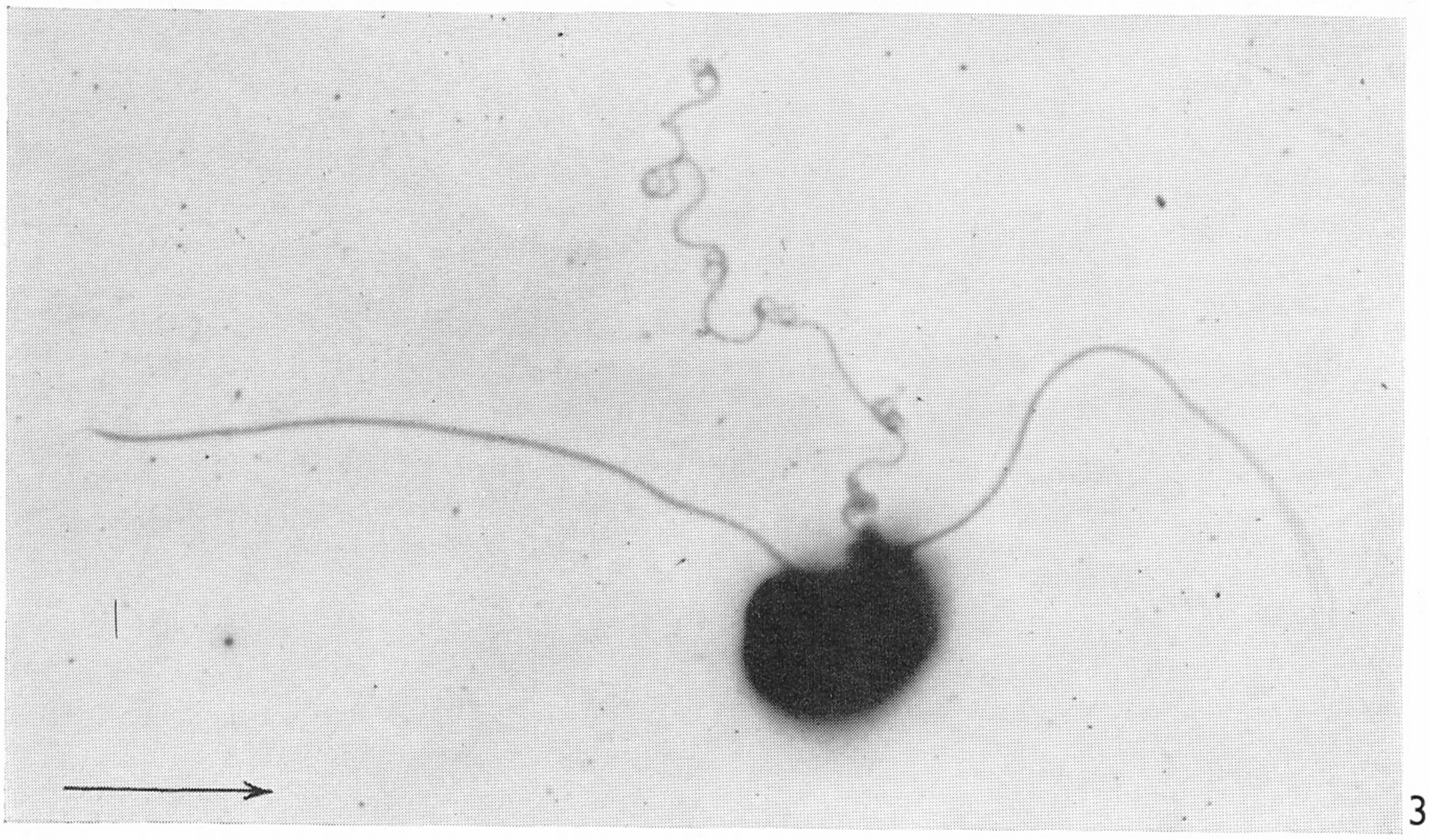

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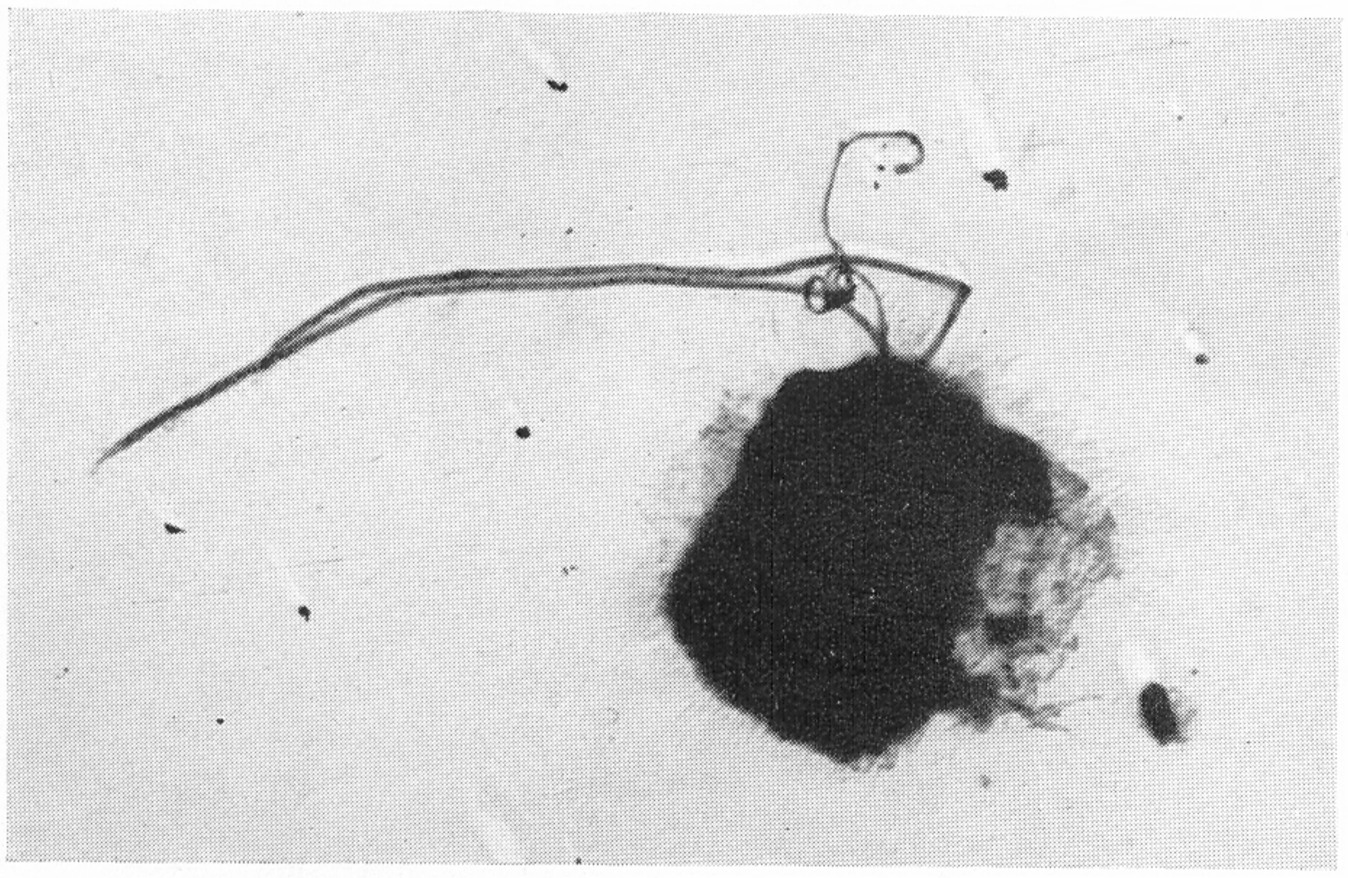

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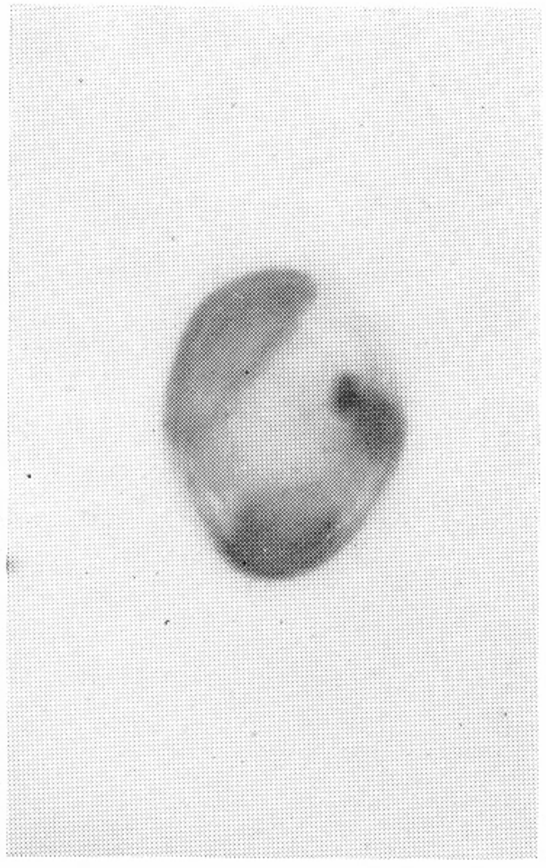

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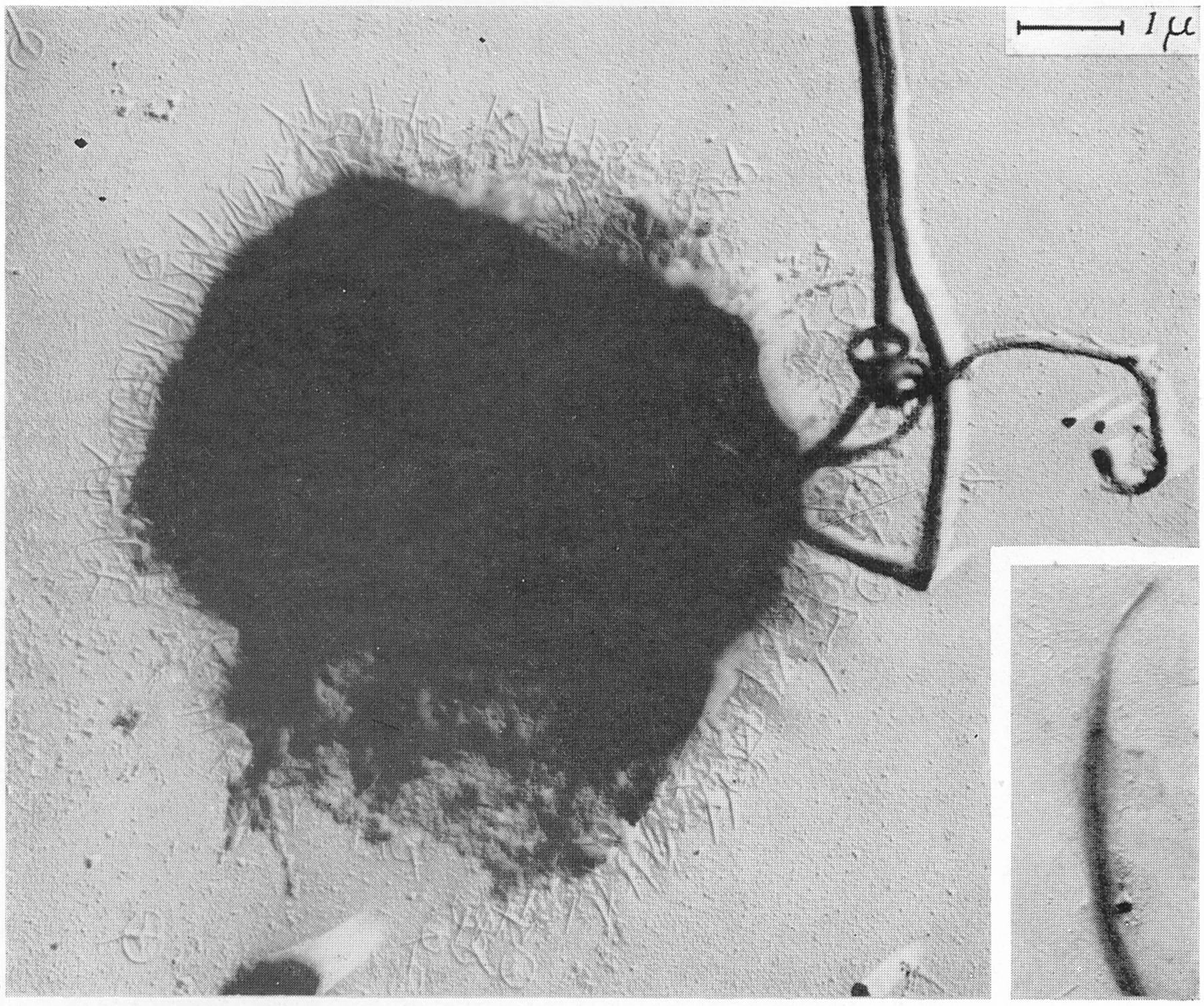

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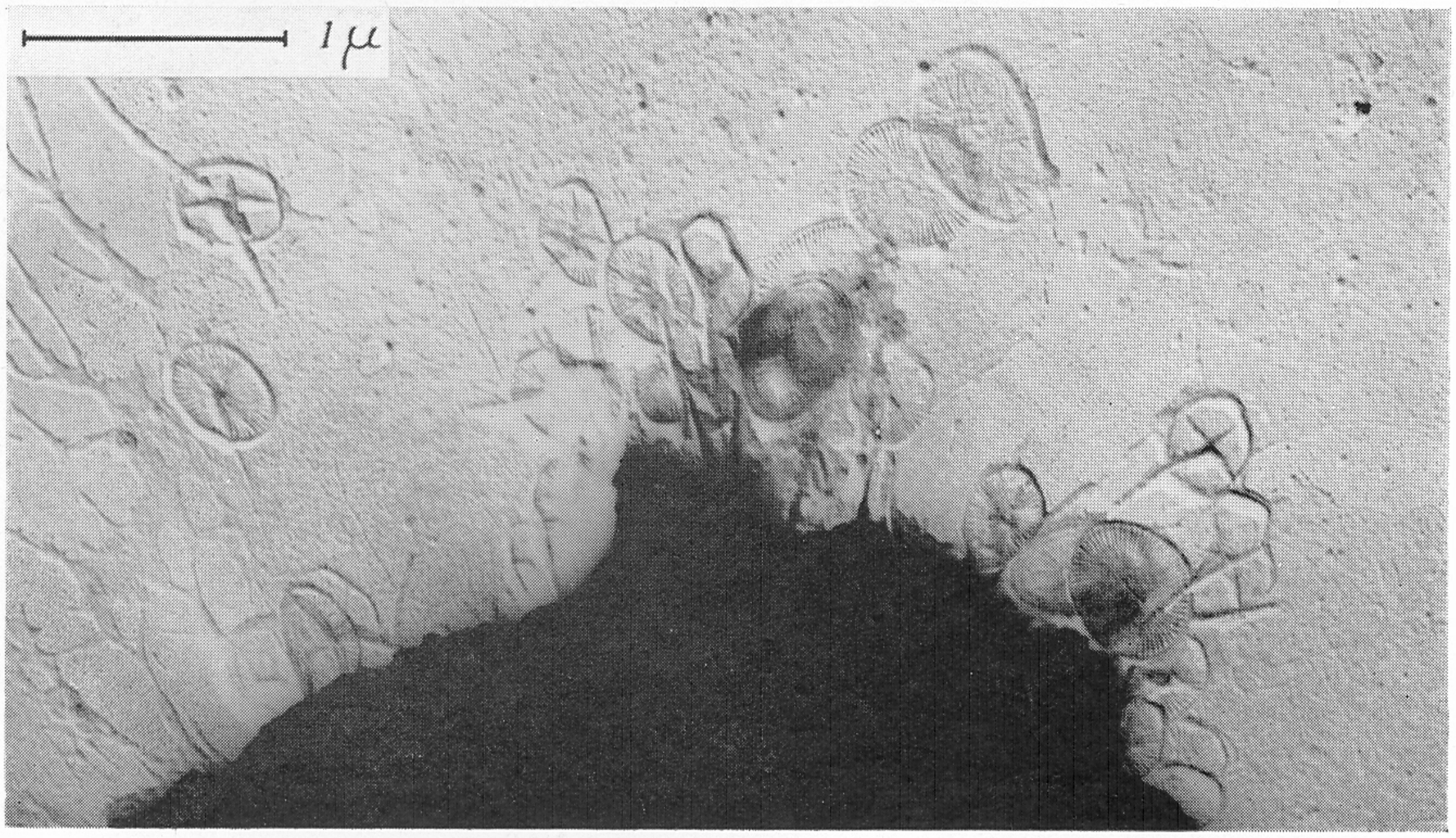

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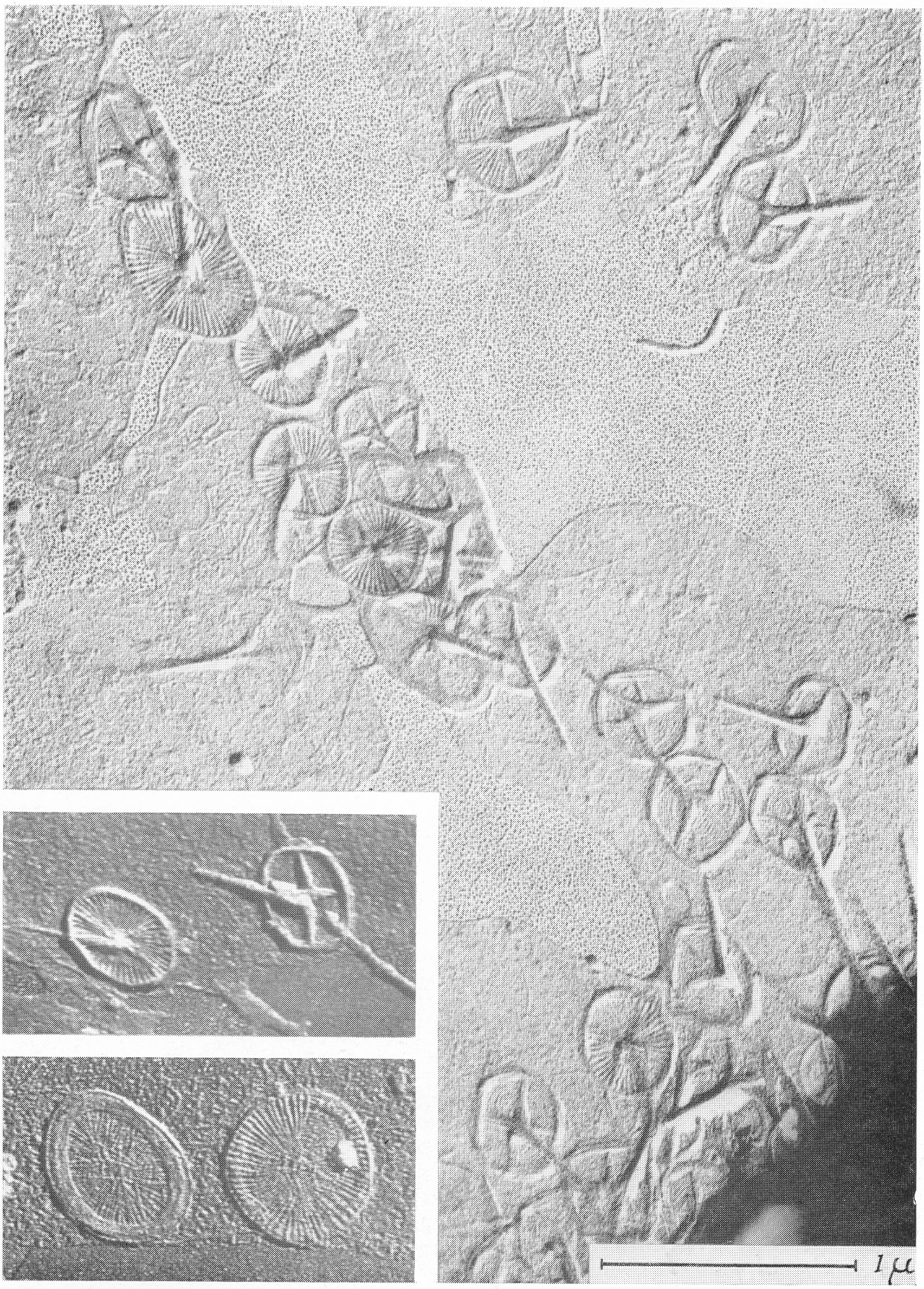

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flagella can undulate at the same rate, or appear to do so, or one can undulate more slowly than the other with undulations of larger amplitude (Fig. 25). In the extreme case, which is quite common, one flagellum can undulate and the other remain still or move stiffly in a short back and forward dithering stroke. When attached with the haptonema extended the body can sway about on the attached haptonema and the haptonema itself can bend over (Fig. 25) so that sometimes the body is near the point of attachment of the haptonema.

Saddle-shaped cells are frequently seen gliding through the water and rotating very slowly with the haptonema fully extended forwards and lying across the body in the direction of motion (Figs. 21, 24). The flagella thus project backwards and both can either undulate slowly, usually at a slightly different rate, or one can remain stiff while the other undulates (Fig. 21). If another cell is encountered, or for no apparent reason, the haptonema may be withdrawn with a sudden jerk and coiled up so quickly that the act cannot be followed. The body then resumes the shape and characteristics of rapid swimming.

Phagotrophy is of common occurrence, the cells ingesting graphite, bacteria and other organisms up to a size of $2{\cdot}5\,\mu$. Ingested material lies at the non-flagellar end towards the dorsal surface (Figs. 23, 24). In a few instances small vacuoles containing either bacteria or graphite have been seen lying close to the pyrenoid-like bodies (Fig. 23). As in *C. ericina*, Brownian movement can be seen inside the vacuoles which, after a short time, suddenly disappear.

Before fission the motile cells become more ovoid to spheroidal in shape, the incipient fission stages being from 9–12 μ in diameter. The second pair of flagella, frequently seen as very short ones, and the second haptonema develop before the actual fission which passes from dorsal to ventral surface, giving usually daughter-cells of equal size but occasionally ones of unequal size.

In culture, this species produces from 1 to 2 million cells per ml. at the peak of growth, after which, as in the other species, non-motile stages are produced. The large naked phagotrophic amoeboid cells, up to $16 \times 9\,\mu$, have lobed chromatophores but they are rather coarsely lobed in this species. The four daughter-cells with stellate chromatophores, product of the fission of the large walled cells up to $14 \times 10\,\mu$, are generally ovoidal with a very thin smooth wall, and range in size from $5 \times 3{\cdot}5$ to $8 \times 6\,\mu$. In a four-month-old culture many of these small, walled, cells were present and in some of them the contents had shrunk quite considerably leaving a clear area inside the wall. The cells with the shrunk contents (Fig. 28) were more deeply pigmented than the others and therefore conspicuous. On examination it was found that the chromatophores although striated were no longer lobed but had resumed the appearance of those of the motile phase (Figs. 28, 29); the pyrenoid-like body and small leucosin vesicles could also be seen in some of these cells. Only the part-release of the contents of a number of these daughter-cells has

been seen so far; in one, the cell was seen to come partly out of the wall through the pore and what was almost certainly the haptonema could be seen on that part of the body still inside the wall, but no flagella could be detected. Numerous empty walls with circular pores were found on the bottom of the flask containing the four-month-old culture.

In shape this species is very similar to the type species of the genus, *C. parva* Lackey, but it is larger, has a relatively longer haptonema which is thinner instead of thicker than the flagella. It also lacks a contractile vacuole and, when saddle-shaped, has the flagella and haptonema projecting in the opposite direction to that shown by Lackey for *C. parva*.

Chrysochromulina alifera n.sp., Parke & Manton

(Lat. *Ala*—a wing + *fero*—I bear)

Diagnosis

Motile cells showing extreme metaboly, approximately saddle-shaped with large lateral curved wings when moving slowly or stationary; bell-shaped, oblong, ovoid or spheroidal when swimming rapidly; 6–10 (exceptionally 4–12) μ in length of back of saddle. Two flagella and one haptonema arising close together from ventral concave surface near to one margin in a centre line; flagella smooth, of equal length or subequal, gradually attenuated to a hair point (E. M. observation), usually heterodynamic, occasionally appearing homodynamic, 2–2½ times cell size in length; the haptonema thinner than the flagella, 10 to 12 (exceptionally 14) times body size in length when fully extended, with a swollen tip but no obvious translucent sheath visible under the electron microscope. The periplast, pectic in nature, showing a covering of very thin transparent circular to oval sculptured, dimorphic scales, visible only under the electron microscope; scales without spines 0·25 to 0·45 μ, sculpturing similar to those of *C. ephippium*; scales with spines 0·28 to 0·45 μ, the spine slightly less than scale diameter in length attached centrally by 2–4 short decurrent ridges not extending to the margin. Distribution of the two types of scales on body unknown.

Cells uninucleate, no stigma. Chromatophores striated, 2 or 4, occasionally one or none, intense golden brown; in cells of motile phase saucer-shaped to square or oblong, with single inconspicuous globular body (pyrenoid?) placed near the margin towards the non-flagellar end; in non-motile phase finely lobed. Oil and leucosin produced. Ejectile muciferous bodies small, localized in groups mainly towards the non-flagellar end of the cell. Nutrition phototrophic and/or phagotrophic. Not toxic to fish.

In motile phase asexual reproduction by fission into two daughter-cells of equal or unequal size; in non-motile phase by successive fission of amoeboid cells to produce 4 ovate daughter-cells with exceptionally thin walls; motile

phase probably liberated from walled daughter-cells through a pore. Habitat: the sea at position (Plymouth Laboratory Station L4) Lat. N. 50° 15′, Long. W. 4° 13′ (4 May, 1950, type culture) at surface. Type Culture (Plymouth no. 34) deposited with the Type Culture Collection, Cambridge; preserved material and photographs lodged with the Marine Biological Association, Plymouth, England.

Cellula motili, maxime formam mutanti, fere ephippioidea, praedita magnis alis lateralibus curvatis cum lente motilis aut immotilis, cupuliformi, oblonga, ovoidali aut sphaeroidali cum natat rapiditer; longi 6–10 μ (rare 4–12 μ) per dorsum ephippii. Flagellis duobus et haptonemate unico conjunctim exorientibus e concavo aspectu ventrali prope marginem in medio lineo; flagellis teretibus, longitudine aequis aut subaequis inter se, paulatim attenuatis sicut ad capilli extremitatem ut videtur per microscopiam electronicam; generaliter heterodynamicis, nonnunquam homodynamicis, ut videtur, longioribus 2–2½ quam cellula; haptonemate teneriore quam flagellis, longiore 10–12 (rare 14) quam cellula, cum maxime extensus, apice tumescenti sed nulla tunica externa semi-diaphana apparente, ut videtur per microscopiam electronicam. Periplasto, pectica natura, induto delicatissimis diaphanis squamis circularibus aut ovalibus, sculptis, manifestis sub duabus formis, invisibilibus nisi per microscopiam electronicam; altera forma, squamis sine spinulis, 0·25–0·45 μ longis, sculptis simili modo squamis *C. ephippii*; altera forma, squamis praeditis spinulis, longis 0·28–0·45 μ quoque spinulo longo minusquam squamae latitudo, affixo ad centrum squamae per 2–4 breves costas decurrentes, non extendentes usque ad marginem squamae. Ignotum est quomodo duae formae squamarum distributae sint in superficie cellulae.

Nucleo unico, nullo stigmate. Chromatophoris striatis, 2 aut 4, rare unico aut absente, profunde aureo-brunneo; in cellulis in statu motili, crateriformibus, rectangularibus aut oblongis, unico corpore inconspicuoso globulari (?pyrenoidali) locato prope marginem cellulae, versus apicem quo desunt flagella; in cellulis in statu non-motili chromatophoris delicate lobatis. Corporibus ejectilibus muciferosis parvis, locatis versus apicem cellulae quo desunt flagella. Nutritione phototrophica necnon phagotrophica. Non toxica piscibus.

Generanti asexualiter in statu motili per fissionem in duas cellulas filiolas magnitudine aequas aut inaequales; generanti in statu non-motili per fissiones subsequentes cellularum amoeboidalium ad 4 ovatas cellulas filiolas producendas, parietibus extreme delicatissimis. Fere certum est quod cellulae in statu motili liberantur per foramen.

Habitat mare prope Plymouth ad locationem Lat. N. 50° 15′, Long. W. 4° 13′ (4 Mai 1950—cultura typica) ad summum maris.

Description

The details which distinguish this species from the preceding include body shape, the relatively shorter flagella and haptonema, some details of the swimming movements, the position of the pyrenoid, the greater average number of chromatophores and the smaller and slightly simpler scales.

The exceptional form-range is illustrated in Figs. 41–59. In general character this species is somewhat similar to *C. ephippium*, though the form range is greater, the body thinner (Fig. 65) and the wings larger and more curved (Figs. 46, 60, 61). It was impossible to get an exact measurement of the thickness of the body, but it is not more than 1·5–2·0 μ.

When a cell glides slowly through the water without rotating and with the haptonema extended, or when it is anchored by the extended haptonema, the body shape is characteristically as in Fig. 60 (see also Fig. 70, Pl. VIII). When the cell moves fairly slowly with the haptonema extended and the body rotating the sides curl in slightly and frequently overlap (Figs. 41, 42). Sometimes the sides fold in, one above the other, as in Fig. 49. When the cells are swimming rapidly (Figs. 55–57, 62, 63) all shapes from spheroidal, ovoidal, half an ovoid, oblong, bell-shaped to umbrella-shaped can be seen, depending on how much and at what angle the wings are overlapping.

The flagella (Pls. VIII, IX) are comparatively shorter and a little sturdier than in *C. ephippium*, but even so neither the flagella nor the haptonema are easily seen under the light field, particularly when the cells are moving rapidly; the flagella are also thrown off very quickly under both light and dark fields. Measurement of the flagella of a large number of cells showed that in the majority they were of equal length, as in the previous species, but in a few, one flagellum was slightly longer (1–3 μ) than the other; their movement is generally heterodynamic as in *C. ephippium*. The haptonema is usually

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Legends to Text-figs. 41–59

Chrysochromulina alifera n.sp. (× 1250)

Fig. 41. Saddle-shaped cell moving slowly with haptonema fully extended, one flagellum slowly undulating, the other stiff or gently vibrating; wings incurved, one slightly overlapping the other.

Fig. 42. As Fig. 41 but wings incurved and not overlapping, body rotating slowly.

Fig. 43. As Fig. 41 but wings straight, body not rotating and haptonema lying close to ventral surface of body.

Fig. 44. As Fig. 41 but wings lying close together but straight not incurved, haptonema lying away from body.

Fig. 45. Anchored cell with haptonema coiled and flagella lying out from body, two new flagella developing, wings of body curved in and one slightly overlapping the other; body rotating very rapidly giving the impression of a catherine wheel firework when looking down on it under dark field.

Fig. 46. Stationary cell with wings straight out and haptonema slowly uncoiling.

Figs. 47–48. Cells anchored by partly extended haptonemata.

Fig. 49. Cell swimming with flagella and haptonema in front of the body, body elongated and wings rolled in one above the other.

Figs. 50–51. Stationary cells with incurved wings.

Fig. 52. Early fission stage with overlapping wings and with four flagella and two haptonemata behind body, characteristic position for rapid swimming.

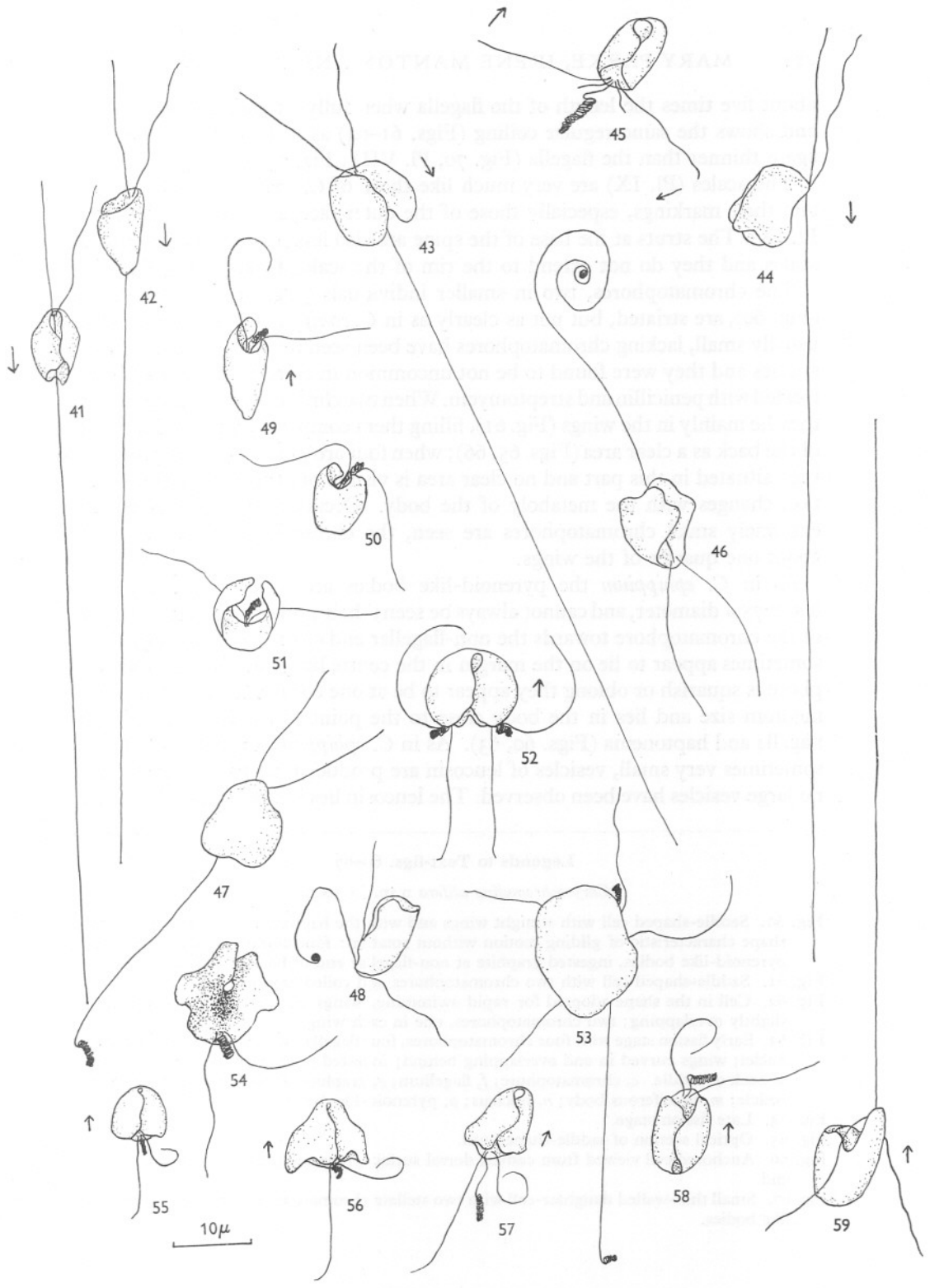
Fig. 53. Early fission stage with four flagella and two haptonemata, body anchored by one haptonema, the other coiled up close to body.

Fig. 54. Cell in process of overlapping wings to produce shape seen in Fig. 56 looking down on ventral surface which is folded inside in Fig. 56.

Figs. 55–57. Variously shaped cells with flagella and haptonemata behind body in positions characteristic for the species during rapid swimming.

Fig. 58. Cell swimming with flagella and coiled haptonema in front of the body.

Fig. 59. Same cell as Fig. 58 just stopped swimming, body shape slightly changed and haptonema extended. It then started gliding with the extended haptonema in front of the body.



Text-figs. 41–59

about five times the length of the flagella when fully extended (Figs. 41–44) and shows the same regular coiling (Figs. 61–64) as in *C. ephippium*, and is again thinner than the flagella (Fig. 70, Pl. VIII; Fig. 73, Pl. IX).

The scales (Pl. IX) are very much like those of *C. ephippium* but smaller, and their markings, especially those of the outer face, less distinct (Fig. 76, Pl. IX). The struts at the base of the spine are also less massive on the spined scales and they do not extend to the rim of the scale (Figs. 74, 76, Pl. IX).

The chromatophores, two in smaller individuals (Fig. 61), four in larger (Fig. 60), are striated, but not as clearly as in *C. ephippium*. Occasional cells, usually small, lacking chromatophores have been seen in stock cultures of this species and they were found to be not uncommon in cultures which had been treated with penicillin and streptomycin. When two chromatophores are present they lie mainly in the wings (Fig. 61), filling them completely and leaving most of the back as a clear area (Figs. 65, 66); when four are present (Fig. 60) two are then situated in this part and no clear area is visible but their shape and position changes with the metaboly of the body. Occasionally cells possessing extremely small chromatophores are seen, the chromatophores filling only about one quarter of the wings.

As in *C. ephippium* the pyrenoid-like bodies are small (Figs. 60, 63), 0·5–0·75 μ diameter, and cannot always be seen; their position is on the margin of the chromatophore towards the non-flagellar end of the cell (Fig. 66); they sometimes appear to lie on the margin in the centre line, but if the chromatophore is squarish or oblong they appear to be at one corner. The nucleus is of medium size and lies in the body close to the point of the insertion of the flagella and haptonema (Figs. 60, 63). As in *C. ephippium* a number of small, sometimes very small, vesicles of leucosin are produced, up to 5 in a cell, but no large vesicles have been observed. The leucosin lies in the central clear area

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Legends to Text-figs. 60–67

Chrysochromulina alifera n.sp. (× 5000)

Fig. 60. Saddle-shaped cell with straight wings and with the haptonema fully extended, the shape characteristic of gliding motion without rotation: four chromatophores and four pyrenoid-like bodies, ingested graphite at non-flagellar end of back of saddle.

Fig. 61. Saddle-shaped cell with two chromatophores and coiled haptonema.

Fig. 62. Cell in the shape adopted for rapid swimming, wings in the front, incurved and slightly overlapping; two chromatophores, one in each wing.

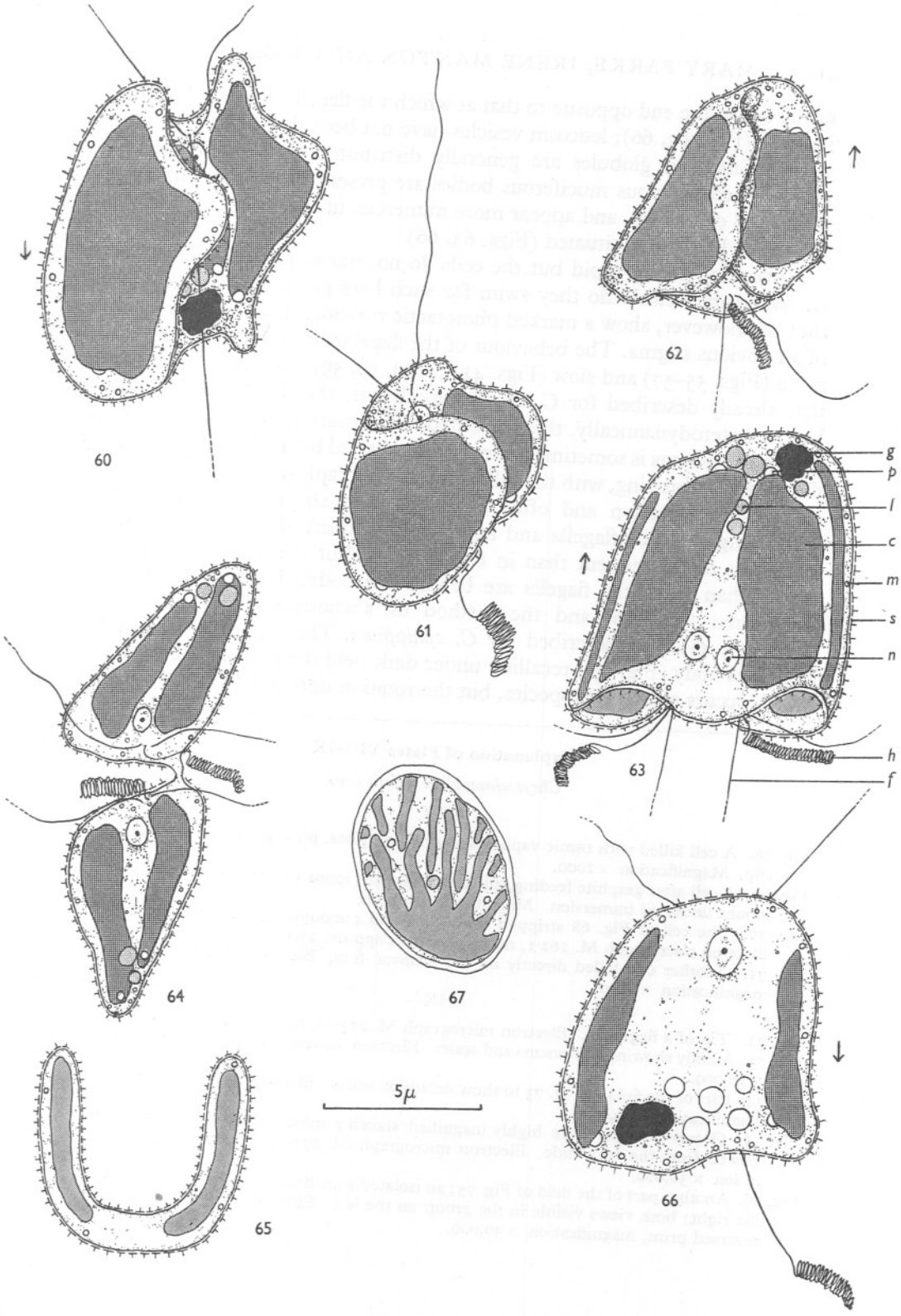
Fig. 63. Early fission stage with four chromatophores, four flagella, two haptonemata, and two nuclei; wings curved in and overlapping behind; ingested graphite at non-flagellar end of back of saddle. *c*, chromatophore; *f*, flagellum; *g*, graphite; *h*, haptonema; *l*, leucosin vesicle; *m*, muciferous body; *n*, nucleus; *p*, pyrenoid-like body; *s*, scale.

Fig. 64. Late fission stage.

Fig. 65. Optical section of saddle-shaped cell.

Fig. 66. Anchored cell viewed from convex dorsal surface, ingested graphite at non-flagellar end.

Fig. 67. Small thin-walled daughter-cell with two stellate chromatophores and two pyrenoid-like bodies.



Text-figs. 60–67

of the cell at the end opposite to that at which the flagella and haptonema are inserted (Figs. 63, 66); leucosin vesicles have not been detected in the lateral wings. Small oil globules are generally distributed throughout the cytoplasm. Inconspicuous muciferous bodies are present in small groups in the peripheral cytoplasm, and appear more numerous in that part of the body in which the leucosin is situated (Figs. 63, 66).

Movement is very rapid but the cells do not move quite so quickly as in *C. ephippium*, neither do they swim for such long periods in one direction; they do, however, show a marked phototactic reaction in spite of the absence of an obvious stigma. The behaviour of the flagella and their position during rapid (Figs. 55–57) and slow (Figs. 41–44, 49, 58, 59) movement is similar to that already described for *C. ephippium*; that is, the flagella nearly always behave heterodynamically, though sometimes appearing to be homodynamic.

The haptonema is sometimes seen partly extended behind the body (Fig. 57) during fast swimming, with the cells rotating very rapidly, sometimes showing considerable gyration and often changing their shape whilst in motion. Swimming with the flagella and haptonema in front of the body (Figs. 49, 58, 59) is more frequent than in *C. ephippium* but the movement is much slower than when the flagella are behind the body. The uncoiling of the haptonema (Fig. 46) and the method of anchorage of the cell by it (Figs. 47, 48) is as described for *C. ephippium*. The rapid rotation of the anchored body (Fig. 45), recalling under dark field the motion of a catherine wheel, occurs also in this species, but the rotation does not last for such long

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Explanation of Plates VIII–IX

Chrysochromulina alifera n.sp.

VIII

Fig. 68. A cell killed with osmic vapour and dried on glass, photographed without a coverslip. Magnification × 1000.

Fig. 69. A cell after graphite feeding, killed with osmic vapour and photographed in a liquid mount under oil immersion. Magnification × 2000.

Fig. 70. The cell of Fig. 68 stripped from glass and remounted for electron microscopy. Electron micrograph M. 162·1, magnification × approx. 2300.

Fig. 71. Another cell killed directly on the formvar film. Electron micrograph M. 179·7, magnification × 3000.

IX

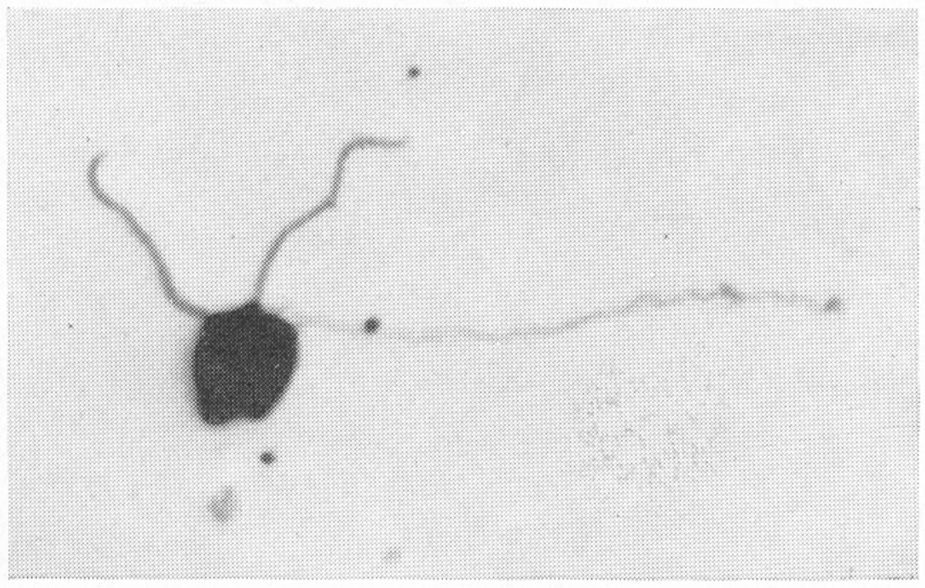
Fig. 72. Tip of a flagellum. Electron micrograph M. 273·11, magnification × 10,000.

Fig. 73. A body showing haptonema and scales. Electron micrograph M. 179·8, magnification × 10,000.

Fig. 74. Part of the field of Fig. 73 to show details of scales. Electron micrograph M. 179·12, magnification × 20,000.

Fig. 75. Group of scales more highly magnified showing spined and spineless scales, most viewed from the body side. Electron micrograph M. 277·12, reversed print, magnification × 30,000.

Fig. 76. Another part of the field of Fig. 75; an isolated spined scale showing the outer face on the right; both views visible in the group on the left. Electron micrograph M. 277·12, reversed print, magnification × 30,000.



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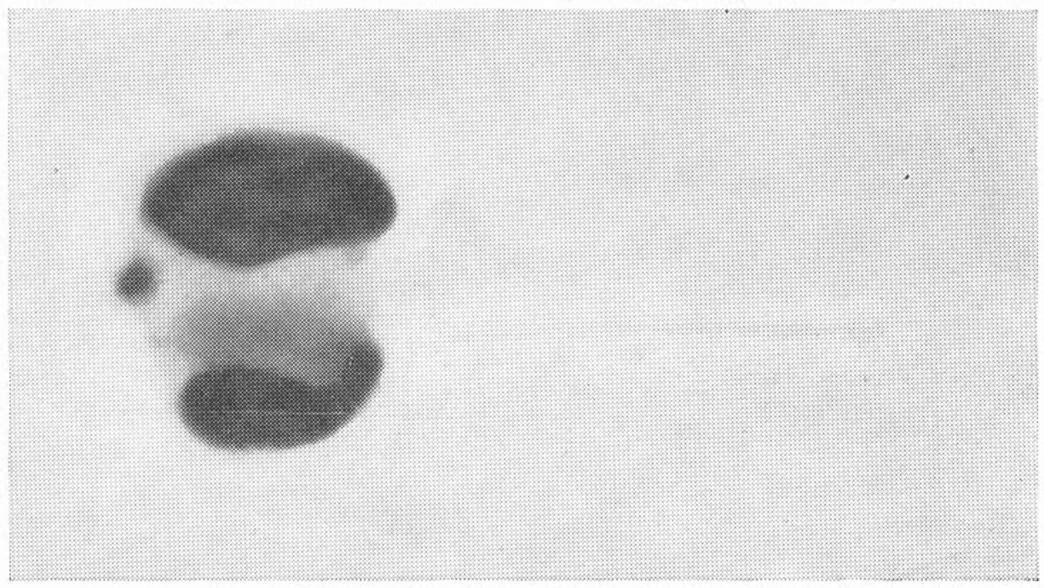

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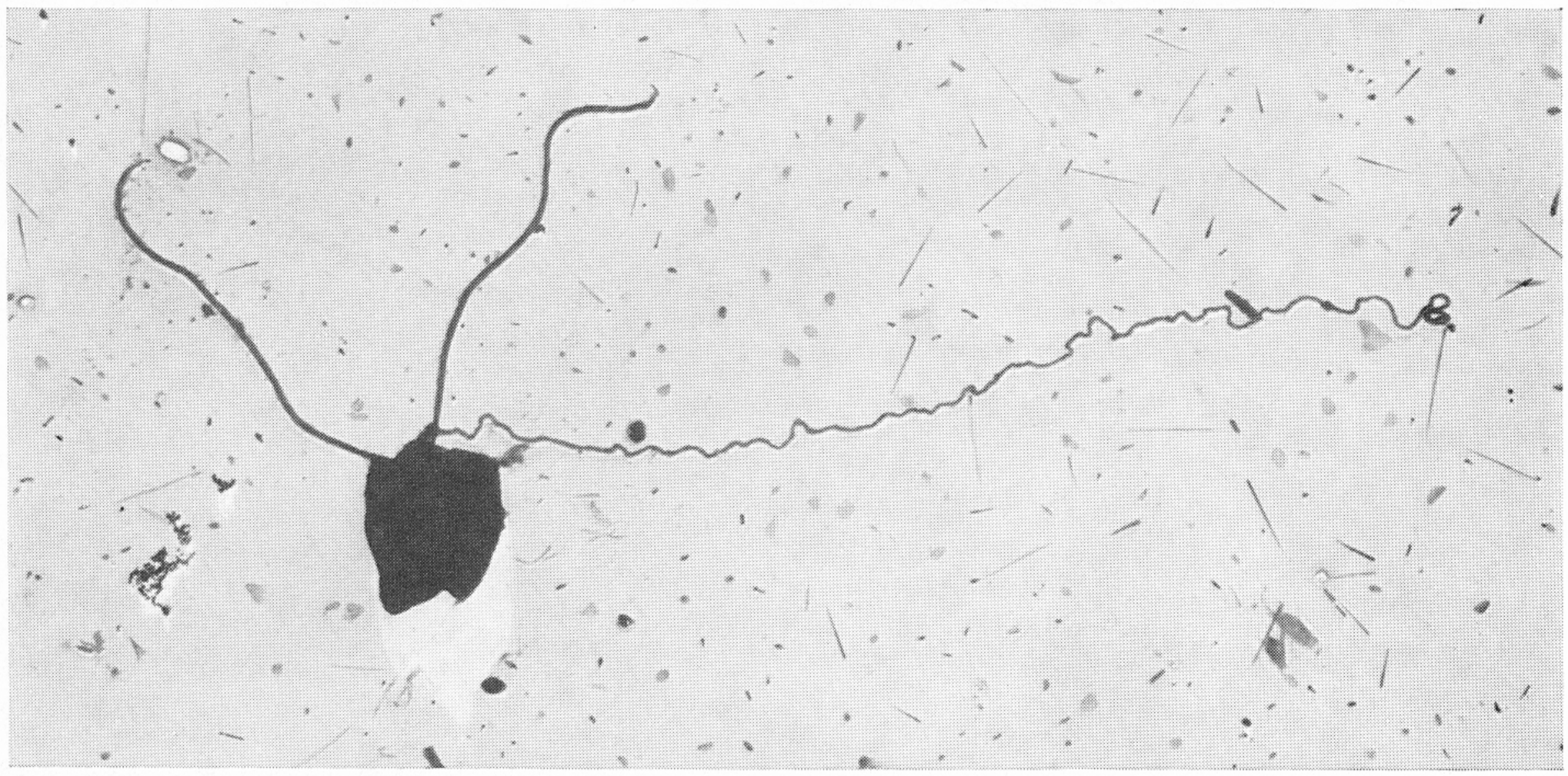

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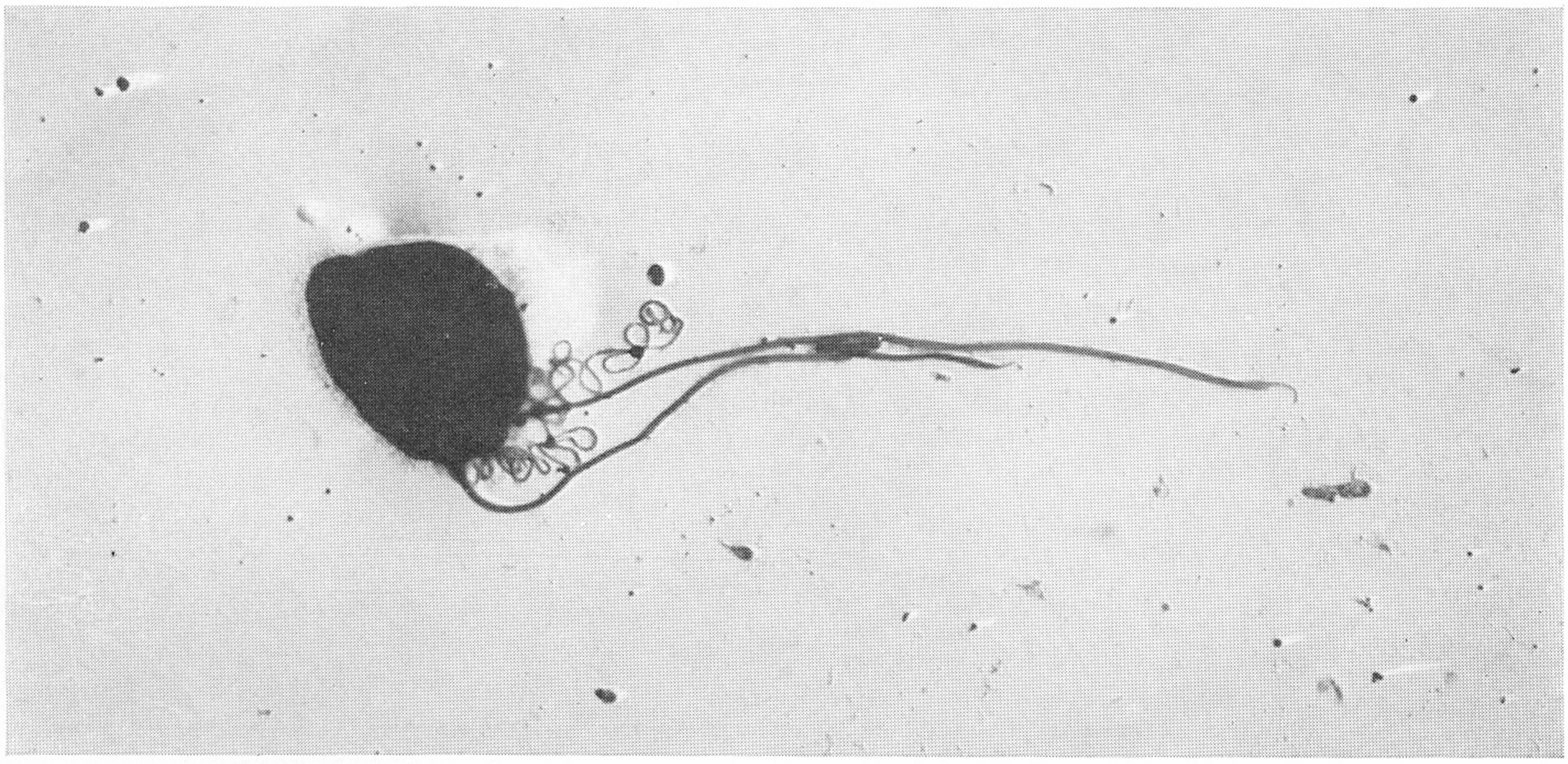

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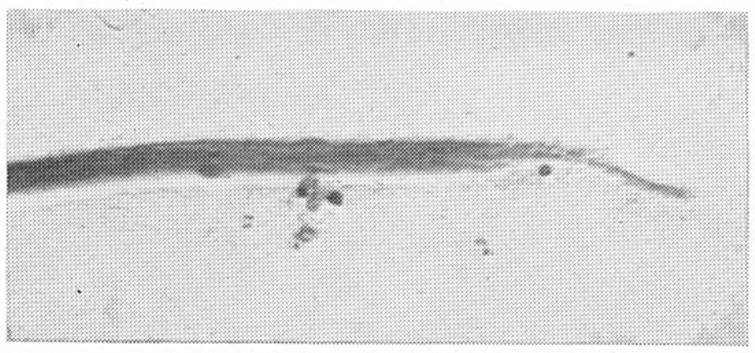

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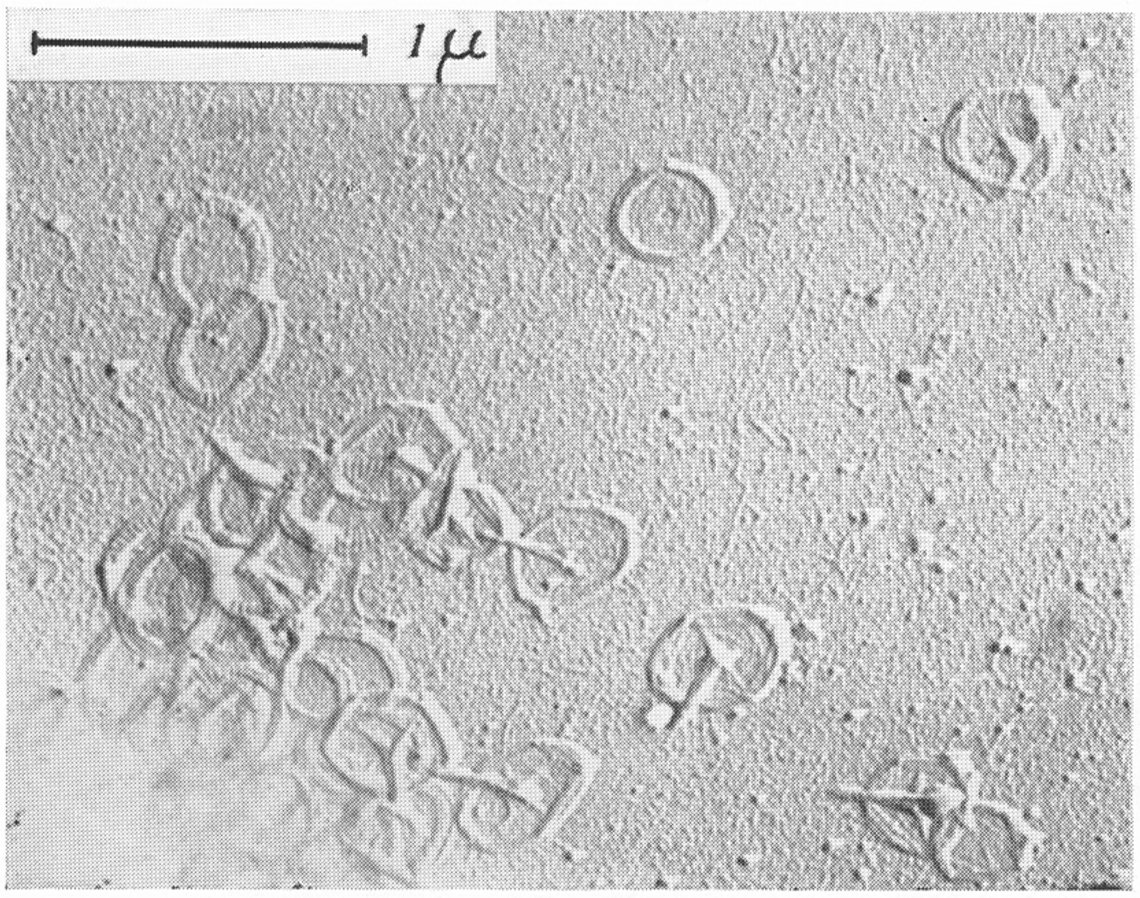

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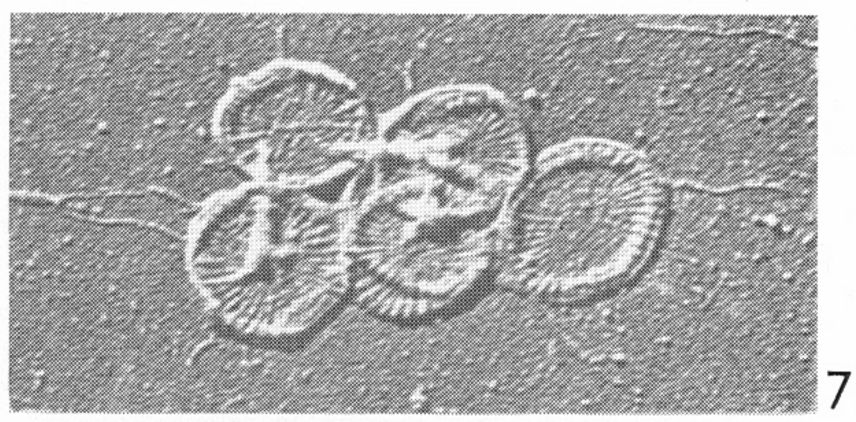

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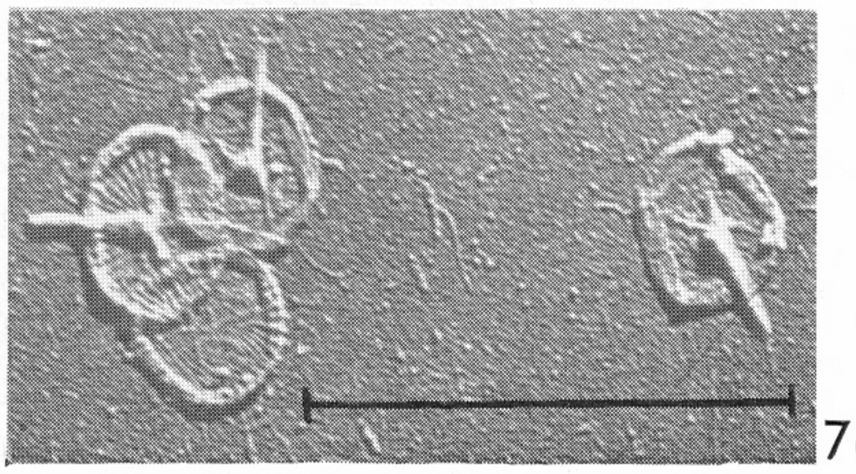

76

periods as in *C. ephippium*. Cells are commonly seen stationary, or gliding through the water with their haptonemata extended for longer periods than in *C. ephippium*, and, as in that species, the cells always give a sudden backward jerk when the haptonemata are coiled up rapidly.

Phagotrophy has been demonstrated (Fig. 69, Pl. VIII; Figs. 60, 63, 66). Cells containing ingested material are frequently but not commonly seen, the maximum size of ingested material being $2 \times 1\,\mu$. Ingestion of material takes place at the back end of the saddle (Fig. 60), that is the part of the body foremost (Fig. 63) when the cell is swimming rapidly with the flagella and haptonema behind. No vacuoles containing granules in Brownian movement have so far been seen.

Before fission the back of the saddle widens (Fig. 63), not lengthens, and cells showing two very short flagella and two long flagella (Fig. 45), are sometimes seen. The second haptonema, as well as the two new flagella, are formed before the actual fission starts (Figs. 52, 53, 63). In this species incipient fission stages are frequently seen anchored by one haptonema whilst the second remains coiled (Fig. 53). Fission down through the back of the saddle starting at the non-flagellar edge gives two daughter-cells (Fig. 64), which can be from equal to very unequal in size.

In culture from $\frac{1}{2}$ to 1 million cells per ml. are produced at the peak of growth. Non-motile stages similar to those already described for the other species are then produced. The large amoeboid and walled cells, with chromatophores more finely lobed than in *C. ephippium*, measure from 14×10 to $16 \times 12\,\mu$. The four ovate daughter-cells produced by the large walled cell have exceptionally thin walls and fairly finely lobed or stellate chromatophores and measure from 4×3 to $7 \times 5\,\mu$ in size (Fig. 67). The shrinkage of the contents of these cells away from the walls has not so far been seen in old cultures, nor has the liberation of the contents been observed.

Discussion

The only point which at this stage perhaps merits further discussion is our treatment of the facts for heterodynamic flagellar motion in *C. ephippium* and *C. alifera*. We are well aware that, on some systems of classification, this character would at once remove these species not only from the same genus but even from the same order as that containing the other species with which we have been concerned. That we have not, at this stage, chosen to do this is partly due to the striking similarity of all these species in other respects, which in this particular group are perhaps as important taxonomically, but in part also to our inability to find any structural differences between the two flagella of the kind which normally accompanies the truly heterokont condition, cf. *Synura* (Manton, 1955); when fixed therefore they cannot be distinguished from isokonts. There is the further difficulty caused by our present ignorance

of the relevant facts for the type species *C. parva* Lackey. We do not yet know whether the apparent resemblance between this species and our *C. ephippium* extends to the motion of their flagella, and without this knowledge we should be in grave danger of misapplying the generic name were we to attempt to split the assemblage of species at present included under *Chrysochromulina* on a character as elusive as flagellar motion.

This negative attitude does not, however, preclude the possibility that subdivision may have to be carried out at a later stage. The six species which we have now described fall into three or perhaps four distinct assemblages, namely *C. ericina*; *C. ephippium* and *C. alifera*; *C. kappa* and *C. minor*; and *C. brevifilum*. We are not, however, yet prepared to say whether these ought to be thought of as subgenera or as genera, and since we have additional groups still undescribed, further discussion of the larger topic must necessarily be deferred.

Summary

Diagnoses and descriptions are given of three new species of marine plankton flagellates in the class Chrysophyceae: *Chrysochromulina ericina*, *C. ephippium* and *C. alifera*. All possess two equal or subequal flagella and a long haptonema. In two of the species flagellar movement is heterodynamic and in one homodynamic. Phagotrophic feeding has been demonstrated in all. The descriptions include structural details of scale characters visible only with the electron microscope as well as observations on behaviour and life-history visible only in living material. The reasons for temporarily placing the three organisms in the genus *Chrysochromulina* Lackey are given.

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