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STUDIES ON MARINE FLAGELLATES

II. THREE NEW SPECIES OF *CHRYSOCHROMULINA*

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(With total of 81 Figures in text and on Plates I–IX)

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INTRODUCTION

The three new species to be described below are the first of a considerable series of related forms, recently isolated from marine plankton off the British coasts and possessing in common a number of rather unusual characters which make their classification difficult. They are all very small and extremely fragile unicellular flagellates, with the characteristic golden brown plastid colour and metabolic products found in the Chrysophyceae to which they undoubtedly belong. Within this group their placing is more difficult and it cannot even be adequately discussed until the whole range of new forms is available for comparison. The most that we can do on this occasion is to make some provisional generic comparisons in order to establish a system of nomenclature. Unfortunately, existing descriptions of relevant genera are both scanty and incomplete, a defect which is easily explained by the nature of the organisms and the need for special facilities for their adequate study. The generic identification which we propose to adopt is therefore provisional to the extent that the little known genus *Chrysochromulina*, into which we believe our organisms can be placed, is founded on one freshwater species which is described in terms which will need substantial revision if this identification is to be accepted. Generic revision must, however, wait until the whole range

of forms has been described, and we must therefore limit the present discussion to our reasons for the choice of the name.

These reasons are essentially that our forms and *Chrysochromulina* agree in possessing three rather long filiform appendages, two of which are flagella. In the only other Chrysophycean genera for which three appendages have been recorded, namely *Prymnesium* and *Platychrysis*, the third appendage is so short as scarcely to justify the epithet filiform, and it differs in other ways (to be discussed on a later occasion) which do not suggest identity. The third appendage in the only species of *Chrysochromulina* (*C. parva* Lackey), on the other hand, was described as a third flagellum (Lackey, 1939). This attribution is not borne out either by the description of its behaviour or by the figure which accompanies the description. Both resemble those of our organism sufficiently to make it virtually certain that the third organ in *C. parva*, as in our organism, is not a flagellum but an organ of very different properties and use.

In our organisms the third appendage is a retractile organ with an adhesive tip, in some species (though not in those to be described here) extensible to many times the length of the flagella and in all used for temporary anchorage of the cell to a solid surface, though this may perhaps not be its sole function. This power of anchorage is apparently correlated (though in ways which are not yet fully understood) with the practice of phagotrophic feeding, floating particles of various sizes being engulfed through the end of the cell away from the attachment of the appendages. This notwithstanding, the organisms are capable of photosynthesis and under normal conditions require light; their dependence on solid food for certain nutrients, however, explains the difficulty encountered in making unialgal cultures bacteria-free.

In addition to the various characteristics associated with the third appendage, all our species show a covering of scales when seen under the electron microscope; such scales being apparently of an organic nature since we can find no sign of mineral impregnation either calcareous or siliceous. They are of very varied sizes and shapes and in themselves they would provide most excellent diagnostic characters not only for the delimitation of species but also in all probability for the separation of several genera each with several species in our range of material alone. Unfortunately their small size, which makes the scales invisible with the light microscope in almost every species, would lead to great difficulties of identification of such genera by the ordinary student of plankton. While therefore we cannot avoid mentioning such characters—even if this introduces some doubt about specific identifications by a light microscopist—we do not propose to found genera on characters which cannot be seen with the light microscope. We therefore propose to describe most of our species under the one genus *Chrysochromulina*, and to amend the generic diagnosis to incorporate the new knowledge at the end of the series.

Since, however, it is obvious that an essential part of any generic diagnosis must involve a description of the third filiform appendage formerly referred to

as a third flagellum, it will be convenient if we give it at once some name less misleading than flagellum and less cumbersome than 'third filiform appendage'. We therefore propose to call it a *haptonema* (Gr. ἅπτω, to attach, and νῆμα, pl. νῆματα, a thread) to give it a term of its own without reference to other morphological categories. The full description of its structure and properties will then emerge as the species are themselves described and further investigated.

Materials and Methods

The establishment of unialgal cultures has been an essential pre-requisite to the effective study of the structure and behaviour of these organisms. The methods of isolation have been in no way peculiar and the cultures so established have been successfully maintained for several years at Plymouth in Erdschreiber culture solution in a culture room kept at about 15° C. and illuminated with fluorescent lighting to supplement limited daylight from a north window.

For studying the behaviour of the living cells, especially the motion of their various appendages, the use of the modified dark ground condenser of Cooke, Troughton and Simms, illuminated by a Pointolite lamp, has been of the very greatest help. Unfortunately photography at high magnifications is not possible with this system without a more powerful source of light than has been available to us. It is, however, the best means of demonstrating the haptonema to a visual observer.

Without dark ground it is possible, if the cells are not moving rapidly, to see the appendages quite well under an oil immersion objective or a good dry lens without special lighting, but recording them photographically by this means is extremely difficult (see, however, Fig. 24, Pl. I; Fig. 55, Pl. V; and Fig. 77, Pl. VIII). They can also be picked out by phase-contrast or ultraviolet photography, but in the smallest organisms such as those to be described here the optical interference caused by the body may be a serious difficulty. It was therefore found by experiment that the best way of recording these structures is to dry the cells down on to a glass slide after preliminary fixation with either osmic vapour or other simple killing agent such as iodine in KI. It is sometimes an advantage to precede killing by narcotizing with magnesium chloride to prevent retraction of the haptonema. After the preparation has been dried, it must be freed from the salt crystals by immersion in distilled water, followed by a second drying. This part of the process does not appear to disturb the attached cells which, after removal of the salt which otherwise will deliquesce, can be kept indefinitely in the dry condition. The appendages can then be seen even with a relatively low-power dry lens and for photographic purposes the fact that they are spread on a flat surface is a great advantage. They must, however, be examined dry, and so the use of high resolution lenses is precluded.

Formalin fixation, as commonly used for plankton studies, is quite inappropriate for these organisms. The appendages are invariably lost, the cell bodies become bloated, collapsed or otherwise misshapen and the chromatophores are indistinguishable. The organisms are then unrecognizable.

For investigating the feeding habits, and in particular for demonstrating the ingestion of solid food, it is sufficient to supply the cultures with suitable coloured particles and to leave them overnight undisturbed. By next morning the ingested particles can be detected inside the cells and recorded photographically. Various pigments such as carmine, or cultures of small coloured micro-organisms, have been used for this purpose successfully, but the most satisfactory both for its apparent palatability to almost all the organisms and also for purposes of photographic record have been the densely black particles of fine graphite sold under the name of 'Aquadag'. It is desirable to wash this several times in culture fluid before use to avoid risk of chemical contamination, after which it is readily ingested and very clearly visible.

The application of intravitam stains, notably cresyl blue, is a help for demonstrating the presence, though not the details, of the surface covering of scales and of the ejectile organelles which will be mentioned in the descriptions. The scales appear as a pale rose-violet line which could not be distinguished optically from a simple membrane.

For the electron microscopy the methods used have been those previously applied to other types of motile cells (see Manton & Clarke, 1951, *et seq.*) with the sole modification that the present material is considerably more sensitive than is usual and fixation times have to be shorter. It is sufficient to expose the live cells to the vapour from 2% osmium tetroxide solution for 5 sec to cause death and this is sufficient treatment for direct preparations killed on a formvar-coated carrier. After killing they are dried, and eventually shadowcast with gold-palladium.

Direct preparations of this kind are usually to be preferred for revealing the details of scales, but for the general form of the cell with its various appendages suitably spread it is often better to strip from glass as described in Manton, Clarke, Greenwood & Flint, 1952. This procedure makes possible a more extensive examination of alternative specimens before selecting a favourable cell for detailed study. Each of these methods will be represented in the plates.

In assembling our observations for publication we have attempted to obtain pictorial demonstration of all features which lend themselves to this treatment using photography where possible. For each species we have included photographs and drawings at a standard magnification with the light microscope, since such views are indispensable for the ordinary purposes of identification. For the relative lengths of the appendages with respect to the body size some verification is possible on the photographs, more especially of undried material,

though the figures quoted were actually obtained from living material and from cells recently killed with osmic vapour, in each species more than 100 individuals having been measured. For the type of detail which can only be seen with the electron microscope some adjustment of magnifications is necessary in relation to the actual size of the organisms in order to fit the micrographs on to a limited page area. This may obscure the relative differences of size which exist between the species, though these can at once be apprehended from the visual and low-power views assembled on the first plate of each species. It should also perhaps be pointed out that at high magnifications the performance of the electron microscope is liable to slight fluctuations and the values given on the remaining plates are therefore only approximate.

The electron microscope used has been the Philips instrument in the Leeds University Botany Department, except for Fig. 61, Pl. VI, which was taken on a similar instrument in the research laboratory of Messrs Tootal Broadhurst Lee Co. Ltd of Manchester, whom we wish to thank for giving one of us temporary facilities for working there as a guest.

In the descriptive part of the text, for convenience, the detailed account of each species is preceded by a formal diagnosis in English and Latin. The subsequent descriptions can then be abbreviated to comparative notes to incorporate the Figure and Plate references. The first species will be described the most fully and reference to it must be made for certain details common to all three species.

We wish to thank the following for special help: Dr J. E. Morton of Queen Mary College for translating the English diagnoses into Latin, Dr L. H. N. Cooper of the Plymouth Laboratory and Messrs D. Vaux and A. C. Burd of the Ministry of Agriculture and Fisheries Laboratory, Lowestoft, for bringing in the samples of sea water from which these organisms have been isolated, and Miss D. Ballantine for testing their possible toxicity to fish.

Specific Descriptions

Chrysochromulina kappa n.sp., Parke & Manton

Diagnosis

Motile cells sphaeroidal, showing some metaboly, 4·5–6·5 (exceptionally 4–10·5) μ in diameter. Two flagella and one haptonema[1] arising close together at one pole; the flagella equal, homodynamic, $1\frac{1}{2}$–$2\frac{1}{2}$ times the cell diameter in length, smooth, shortly mucronate at the tip under the electron microscope; the haptonema a little longer than the flagella when fully extended, with a basal swelling, a club-shaped tip and a translucent sheath visible only with the electron microscope. The periplast covered by very thin transparent sculptured scales visible only with the electron microscope, on any one cell ranging

[1] For derivation and use of this term see p. 581.

in shape from oval to polygonal and in size from $0{\cdot}3 \times 0{\cdot}4\mu$ to $0{\cdot}5 \times 0{\cdot}8\mu$ with, at the flagellar pole, a few scales bearing a soft central spine.

Cells uninucleate, no stigma. Chromatophores usually 2 or 4, sometimes 1 or none, golden brown; in cells of the motile phase usually saucer-shaped, parietal, with a single globular body (pyrenoid?) placed centrally on the internal face of each; in cells of the non-motile phase deeply lobed or stellate. Oil and leucosin produced. Ejectile muciferous bodies localized in the cytoplasm mainly at the non-flagellar pole. Nutrition holophytic and/or phagotrophic. Not toxic to fish.

In the motile phase asexual reproduction of pigmented and non-pigmented cells by fission into 2, 3 or 4 daughter-cells. In the non-motile phase reproduction (asexual?) by successive fission of amoeboid cells to produce 4 daughter-cells with walls; motile phase probably liberated from walled daughter-cells through a pore.

Habitat: the sea off Port Erin, Isle of Man (15 June 1939, type culture) and off the south coast of England at position Lat. N. 49° 51′, Long. W. 04° 00′ (8 April 1950) at 10 m. and Lat. N. 49° 21′, Long. W. 04° 54′ (9 May 1950) at surface. Type culture (Plymouth collection 'K') deposited with the Type Culture Collection, Cambridge, and the Marine Biological Association; preserved material and photographs lodged with the Marine Biological Association, Plymouth, England.

Cellula motili sphaeroidali, paululum formam mutanti, $4{\cdot}5\mu$ ad $6{\cdot}5\mu$ (rare 4 ad $10{\cdot}5\mu$) latitudine; duobus flagellis et unico haptonemate (vide p. 581) conjunctim exorientibus altero axi; flagellis similibus, homodynamicis, longioribus $1\frac{1}{2}$ ad $2\frac{1}{2}$

Legends to Text-figs. 1–12

Chrysochromulina kappa n.sp. (× 5000)

Fig. 1. Individual swimming with flagella and haptonema behind body in the position characteristic for the species during rapid swimming. *c*, chromatophore; *f*, flagellum; *h*, haptonema; *l*, leucosin vesicle; *m*, muciferous body; *n*, nucleus; *p*, pyrenoid-like body; *s*, scales; *ss*, spined scales.

Fig. 2. Individual anchored by haptonema which is scarcely extended; cell containing recently ingested graphite particles (*g*).

Fig. 3. Anchored cell with haptonema partly extended; cell ejecting large mass of graphite.

Fig. 4. Swimming cell containing large mass of graphite.

Fig. 5. Individual swimming with flagella and haptonema in front of body, haptonema fully extended; graphite particles being ingested.

Fig. 6. Swimming individual lacking chromatophores; an ingested bacterium at non-flagellar pole.

Fig. 7. Early fission stage: 4 flagella, 1 haptonema, 4 pyrenoid-like bodies, chromatophores dividing.

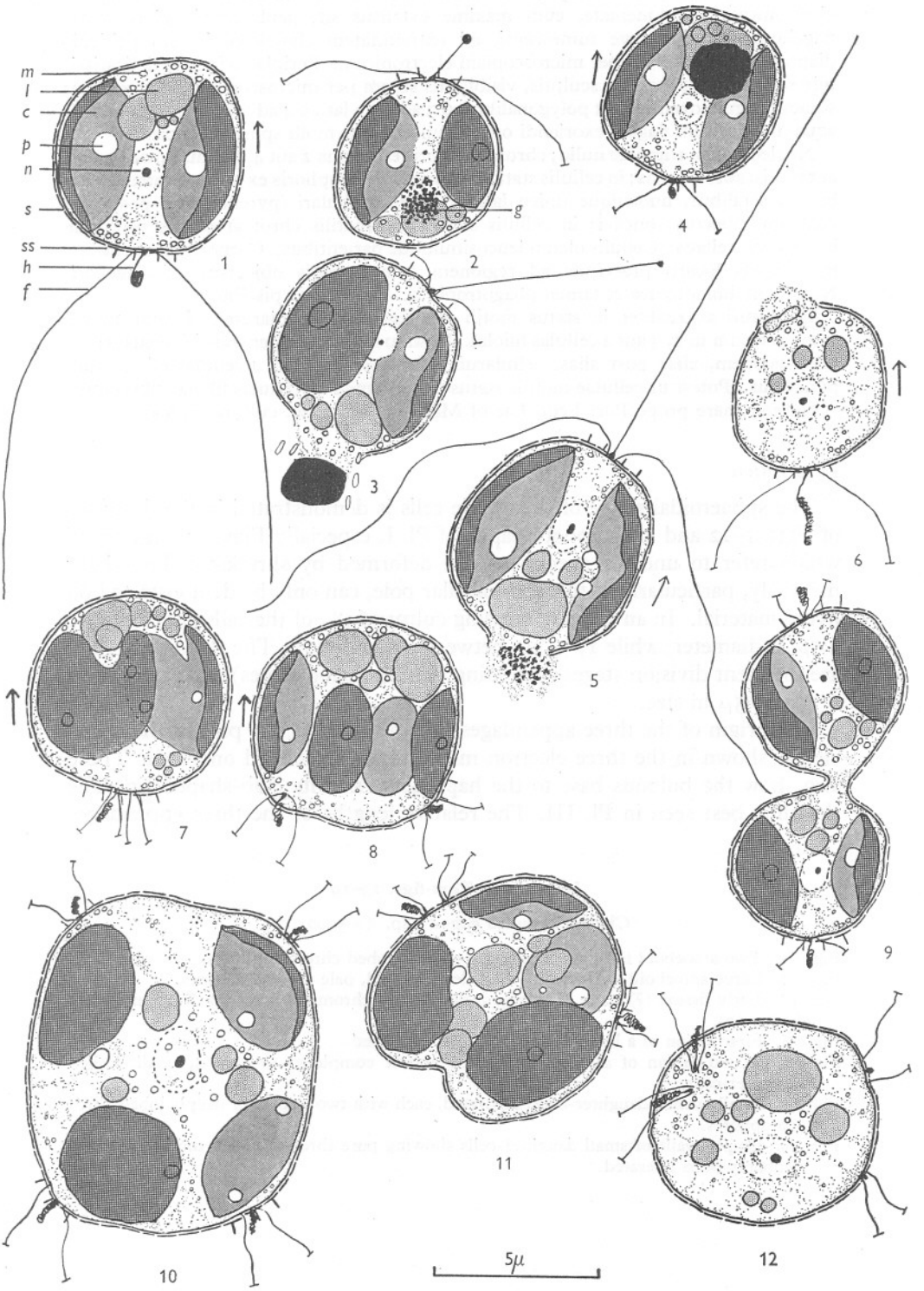
Fig. 8. Fission stage: flagella separating, 2nd haptonema formed, 4 chromatophores.

Fig. 9. Late fission stage just before separation of daughter-cells.

Fig. 10. Stage with 4 pairs of flagella and 4 haptonemata before double-fission to give 4 daughter-cells.

Fig. 11. Early fission stage to give 3 daughter-cells.

Fig. 12. Colourless individual with 4 pairs of flagella and 4 haptonemata prior to double-fission.



Text-figs. 1–12.

cellulae latitudine, teretibus, breve mucronatis ut videantur per microscopiam electronicam; haptonemate, cum maxime extensus sit, paululum longiore quam flagellum, basaliter leve tumescenti, ad extremitatem clavuloso, induto pelliculo diaphano visibili solum per microscopiam electronicam. Periplasto induto delicatissimis squamis diaphanis et sculptis, visibilibus solum per microscopiam electronicam; squamis figura ovalibus aut polygonalibus, 0·3 ad 0·5 μ lat., 0·4 ad 0·8 μ long.; nonnullis squamis ad situm flagelli exoriendi ornatis unaecuique molli spinulo centrali.

Nucleo unico, stigmate nullo; chromatophoris ex norma 2 aut 4, nonnunquam unicis aut nullis, aureo-fulvis; in cellulis status motilis chromatophoris ex norma crateriformibis, parietalibus, unocuique unico largi corpore globulari (pyrenoidali?) posito ad centrum aspectus concavi; in cellulis status non-motilis chromatophoris profunde lobatis vel stellatis. Cellulis oleum leucosinumque parientibus. Corporibus ejectilibus muciferosis locatis praecipue ad regionem cytoplasmatis objectam axi flagellari. Nutritione holophytica et tamen phagotrophica. Non toxica piscibus.

Generanti asexualiter in status motili per fissionem cellularum coloratarum vel incoloratarum in 2, 3 aut 4 cellulas filiolas. In statu non-motili generanti (asexualiter ?) per fissionem, aliae post alias, cellularum amoeboidalium ad 4 cellulas filiolas cum parietibus. Potest ut cellulae motilis status per foramen ex cellulas filiolas tiberentur.

Habitat mare prope Port Erin, Isle of Man (15 Jun. 1939, cultura typica).

Description

The sphaeroidal shape of the motile cells is demonstrated in the drawings of Figs. 1–12 and in the photographs of Pl. I, especially Figs. 22, and 24–26 which refer to undried specimens not deformed by shrinkage. The slight metaboly, particularly at the non-flagellar pole, can only be demonstrated on living material. In an actively growing culture 65% of the cells are from 5 to 6·5μ in diameter, while 17% are between 4·0 and 5·0μ. The remaining 18% are incipient division stages (including double-fission stages) and range from 6·5 to 10·5μ in size.

The origin of the three appendages near together at one pole of the cell is clearly shown in the three electron micrographs assembled on Pl. II. These also show the bulbous base to the haptonema and its club-shaped tip. The sheath is best seen in Pl. III. The relative lengths of the three appendages

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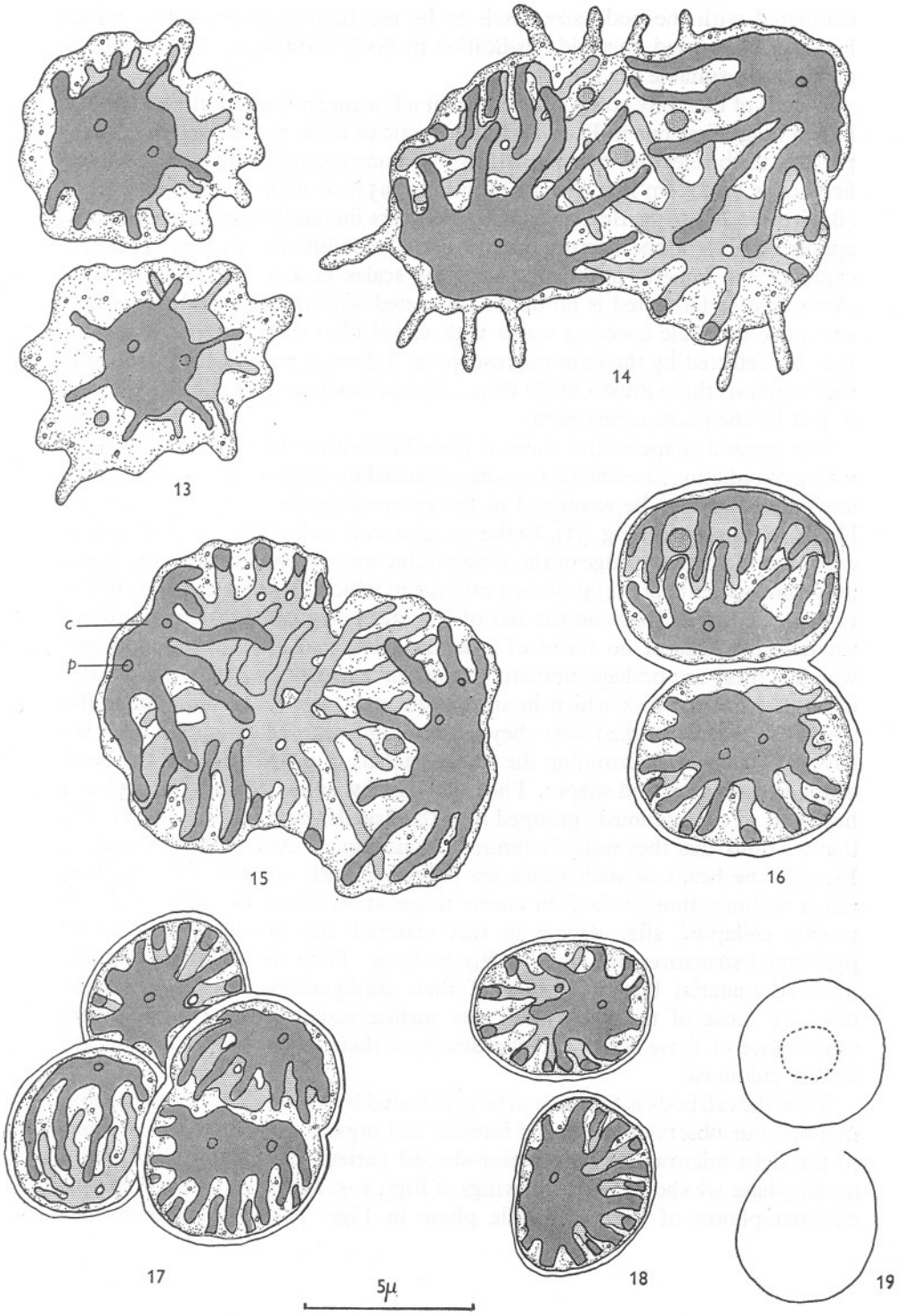
Legends to Text-figs. 13–19

Chrysochromulina kappa n.sp. (× 5000)

Fig. 13. Two amoeboid individuals showing slightly lobed chromatophores.
Fig. 14. Large amoeboid individual with 4 deeply lobed, pale chromatophores.
Fig. 15. Early fission (?) stage of a large individual: 4 chromatophores (*c*), 8 pyrenoid-like bodies (*p*).
Fig. 16. First fission of a large, walled cell just completed.
Fig. 17. Second fission of a large, walled cell almost completed to give 4, small, walled daughter-cells.
Fig. 18. Small, walled daughter-cells, separated, each with two stellate or deeply lobed chromatophores.
Fig. 19. Empty walls of small daughter-cells showing pore through which motile stage has probably been liberated.



Text-figs. 13–19.

compared with the body size needs to be ascertained on specimens which have not been dried, to avoid falsification by body shrinkage. Figs. 22 and 24 on Pl. I are suitable for this purpose.

Details of the body scales can be seen at a low magnification in Pl. II and at higher magnifications in Pl. IV. Characteristic of them is their extreme transparency to the electron beam in which they contrast markedly with the silicified scales of a form like *Synura* (Manton, 1955) or with calcified coccoliths (Braarud *et. al.*, 1952; Braarud, 1954), which are intensely opaque to electrons, appearing black in the electron microscope. Our failure to detect any chemical evidence for mineral impregnation of the scales in any species of *Chrysochromulina* so far tested is no doubt correlated with these optical properties, and since the scale covering stains with cresyl blue sufficiently strongly for it to be detected by the light microscope as a distinct rose-violet line outside the periplast, there must clearly be an organic basis to the scales which may in fact be their sole component.

The general shape of the scales is plate-like with a delicate surface sculpturing of radiating lines which become enhanced by shadow-casting and which may therefore best be examined in the reversed prints of Figs. 34 and 35, Pl. IV (compare with Fig. 33). In the smallest oval scales (Fig. 35) these ridges extend to the extreme edge of the scale producing a crenate margin; the larger polygonal scales (Fig. 34) possess a raised rim which obscures the ends of the ridges. Occasionally, as on the left of Fig. 34, polygonal rims can be found surrounding little or no traces of a central disk; it is not, however, known whether these are perhaps immature scales or whether the rim is in fact detachable from the disk which in such cases may have fallen out. Since the scales can only be studied after they have become detached from the body we have no means of determining the precise details of distribution on the body of the various sizes and shapes. The relatively few spined scales (Fig. 35) are, however, so conspicuously grouped near the flagellar pole (cf. Fig. 33, Pl. IV) that we think that they must be limited to this region. As may best be seen in Fig. 35, the heads of such scales are polygonal with a raised rim and faint radiating lines, though the four coarse ridges subtending the spine (which is usually collapsed after drying in this material) are possibly not entirely preformed structures but could partly be folds. They are invariably present in dried material but the details of their configuration are less constant than are those of the pattern of true surface sculpturing. The biological significance of these scales and the details of their modes of production are wholly unknown.

Since the cell body is too large to be penetrated by the electron beam in whole mounts, our observations on the internal cell organs are at present confined to the light microscope. The saucer-shaped parietal chromatophores of the motile phase are shown in the drawings of Figs. 1–5, and 7–11, with the stellate chromatophores of the non-motile phase in Figs. 13–18. Sometimes the

chromatophores of the motile stage appear striated, their number is usually two and we have never found more than four, the latter being in most cases early division stages. Cells with one chromatophore and division stages with three chromatophores are occasionally seen. Individuals which lack chromatophores are also occasionally seen, though they are always of small size, 4–4·5μ in diameter (Fig. 6).

A conspicuous refringent body, perhaps equivalent to the so-called pyrenoids of some other authors (see below), is situated near the centre of the inner face of each chromatophore (Figs. 1–5, 7–11). These bodies vary in size and sometimes appear to be surrounded by non-refringent material. They appear to divide at the same time as the chromatophores (Figs. 5, 7), but it is not known whether they are actually attached to the inner face of the chromatophore since they have sometimes been observed apparently lying away from it. They do not stain with acid fuchsin, iodine, neutral red or cresyl blue, but with Millon's reagent they become gold, pink or rose-red. The 'pyrenoids' of certain naviculoid diatoms were found to become orange-gold after treatment with Millon's reagent (Tschermak-Woess, 1953), and those of the coccolithophorid *Pontosphaera roscoffensis* showed a comparable negative reaction with other common reagents (Chadefaud & Feldmann, 1949). After osmic fixation these bodies in *Chrysochromulina kappa* become very conspicuous, sometimes appearing a vivid green. A similar behaviour has been found (Dr J. W. G. Lund personal communication) in the 'pyrenoids' of the chrysophycean *Apistonema pyrenigerum*.

The nucleus is sometimes visible in the living cells; it is of medium size and is placed centrally in the body, usually slightly nearer to the flagellar pole (Fig. 1). Vesicles of leucosin, varying greatly in number and size in different individuals, are present, usually towards the non-flagellar pole, but occasionally small leucosin vesicles are also present near the flagellar pole (Fig. 7). Small oil globules are distributed throughout the cytoplasm.

Very small refringent organelles, possibly corresponding to the muciferous bodies of some workers (Chadefaud, 1936), can be seen in the peripheral cytoplasm. No great number are present in cells of this species (Figs. 1–12), and they seem to be localized mainly near the non-flagellar pole, but small groups are also present round the flagellar pole (Figs. 1–6). Under bright illumination, or when very dilute cresyl blue is added to the live cells, these organelles shoot out their contents as small rods or batons if extruded quickly (Fig. 3) or, alternatively, their contents may ooze out as small balloons which can enlarge to equal the cell in diameter. Immediately before the rods are shot out, very small disks appear to be shot off in a manner reminiscent of the behaviour described by Hovasse (1949) for the discobolocysts of *Cyclonexis annularis*. In *Chrysochromulina*, however, it seems probable that these disks are surface scales which are removed as the organelles discharge their contents. The biological significance of these discharges is unknown.

Explanation of Plates I–IV

Chrysochromulina kappa n.sp.

I

Fig. 20. A cell killed with osmic vapour and dried on a glass slide. Photographed dry, without a coverslip. Magnification × 1000.

Fig. 21. Another cell of the same.

Fig. 22. A cell killed with osmic vapour and photographed in liquid culture medium under a sealed coverslip; the two flagella only but not the haptonema visible in the plane of focus chosen. Visual light, oil-immersion objective, magnification × 1000.

Fig. 23. A cell treated as for Fig. 20 but killed and dried on quartz before mounting in water with a trace of iodine in KI under a sealed quartz coverslip. High power U.V. photograph, glycerine-immersion lens, magnification × 3000.

Fig. 24. Parts of two undried cells from the preparation of Fig. 22 photographed with visual light 24 h after the preparation was set up. The protoplasm has darkened slightly in the dilute osmic solution resulting from vapour killing. The coiled haptonema and two flagella are visible for both cells though the body of the left-hand cell has broken away. Visual light, oil-immersion objective, magnification × 2000.

Fig. 25. An undried cell showing the effects of graphite feeding, otherwise like Fig. 22. A pellet of ingested graphite is in focus between the two plastids at the pole away from the flagella. Visual light, oil-immersion objective, magnification × 2000.

Fig. 26. Another cell of the same showing a larger pellet of ingested graphite.

Fig. 27. Low-power electron micrograph of a cell killed with osmic vapour and dried directly on the formvar film. Electron micrograph M. 113, 27 gold palladium shadowing, 60 kV, magnification × 3000.

II

Fig. 28. A cell killed with osmic vapour directly on the formvar film. The body, scales, coiled haptonema and the two flagella, are visible, with a bacterium and a *Caulobacter* type of organism touching the tip of the right-hand flagellum. Electron micrograph M 120·26, 60 kV, magnification × *c.* 6000.

Fig. 29. Another cell of the same showing the uncoiled haptonema and its bulbous base. Electron micrograph M 121·1, 60 kV, magnification × *c.* 6000.

Fig. 30. Parts of two other cells of the same: the right-hand cell showing the tightly coiled haptonema breaking away just above its bulbous base, the left-hand cell disrupted, the bulbous base and the two flagella alone remaining. Electron micrograph M. 122·14, 40 kV, magnification × *c.* 6000.

III

Fig. 31. Part of a cell similar in treatment to those on Pl. II, showing the coiled haptonema with its sheath. (For a better view of the scales see Pl. IV.) Electron micrograph M 114·6, 60 kV, magnification × 20,000.

Fig. 32. Part of a similar cell with the haptonema partially extended, and fibrillar components visible in the flagella. Electron micrograph M. 114·4, 60 kV, magnification × 20,000.

IV

Fig. 33. Part of the right-hand cell of Pl. II, Fig. 30, to show the bulbous base of the haptonema and body scales more clearly. Electron micrograph M. 122·18, 40 kV, magnification × 20,000.

Fig. 34. Reversed print of part of the preceding to show sculpturing of scales more clearly Electron micrograph M. 122·18, magnification × 30,000.

Fig. 35. Similar details from another cell to show some small rimless scales and a collapsed spined scale, from the region of the flagellar bases. Electron micrograph M.182·19, 40 kV, magnification × 30,000.

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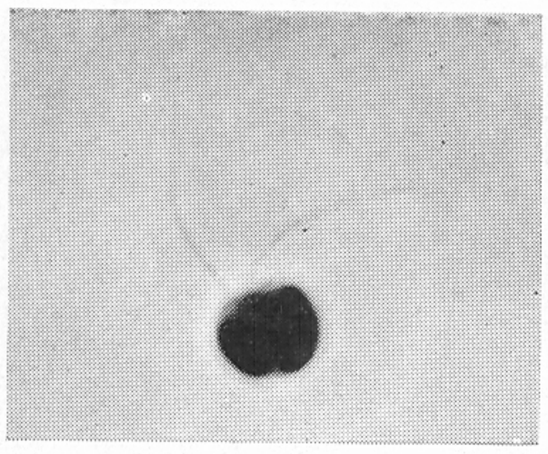

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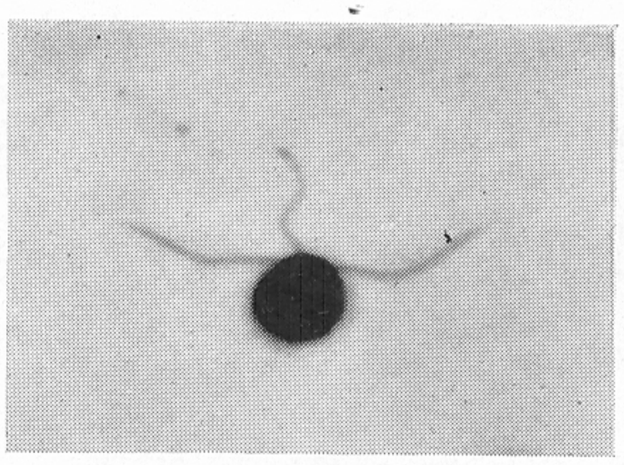

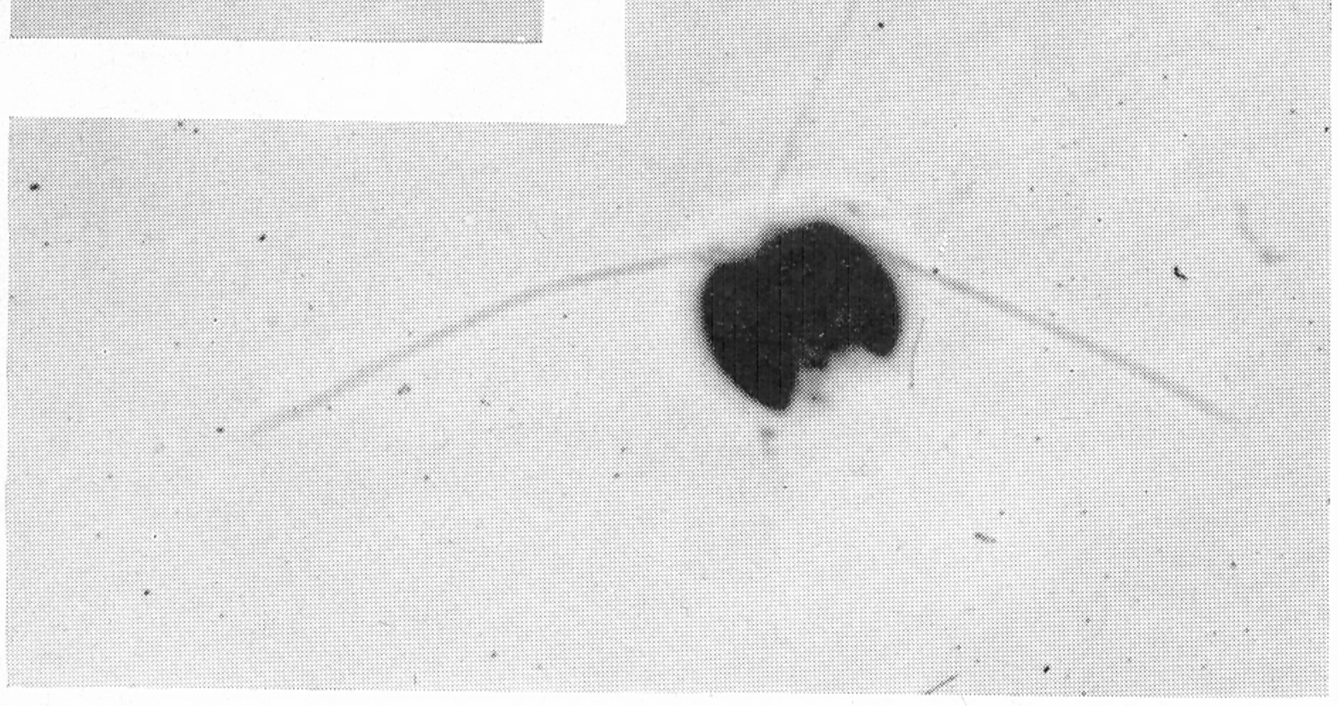

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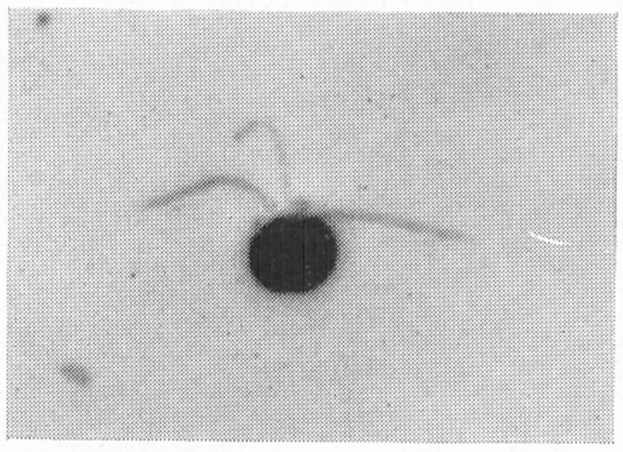

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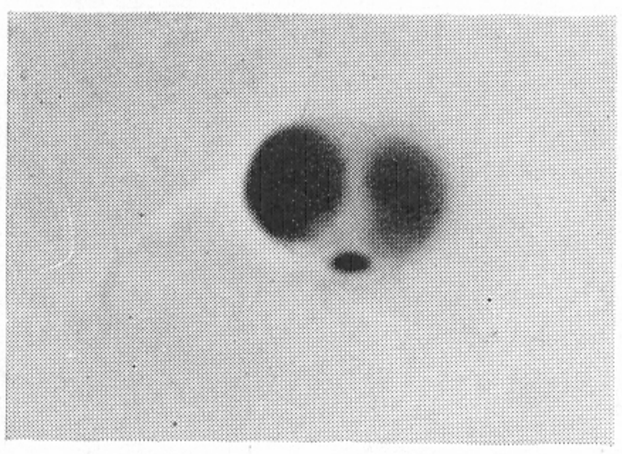

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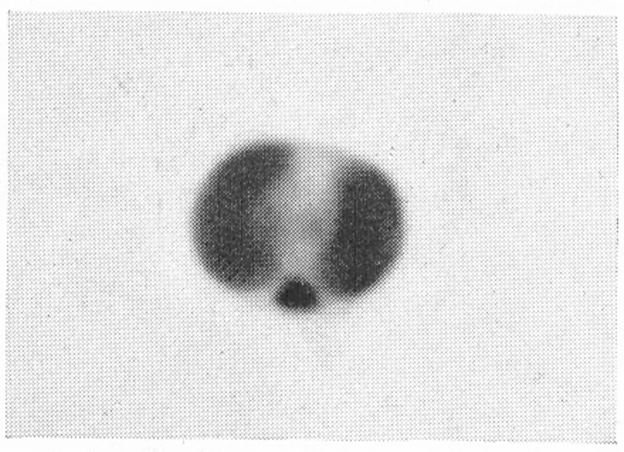

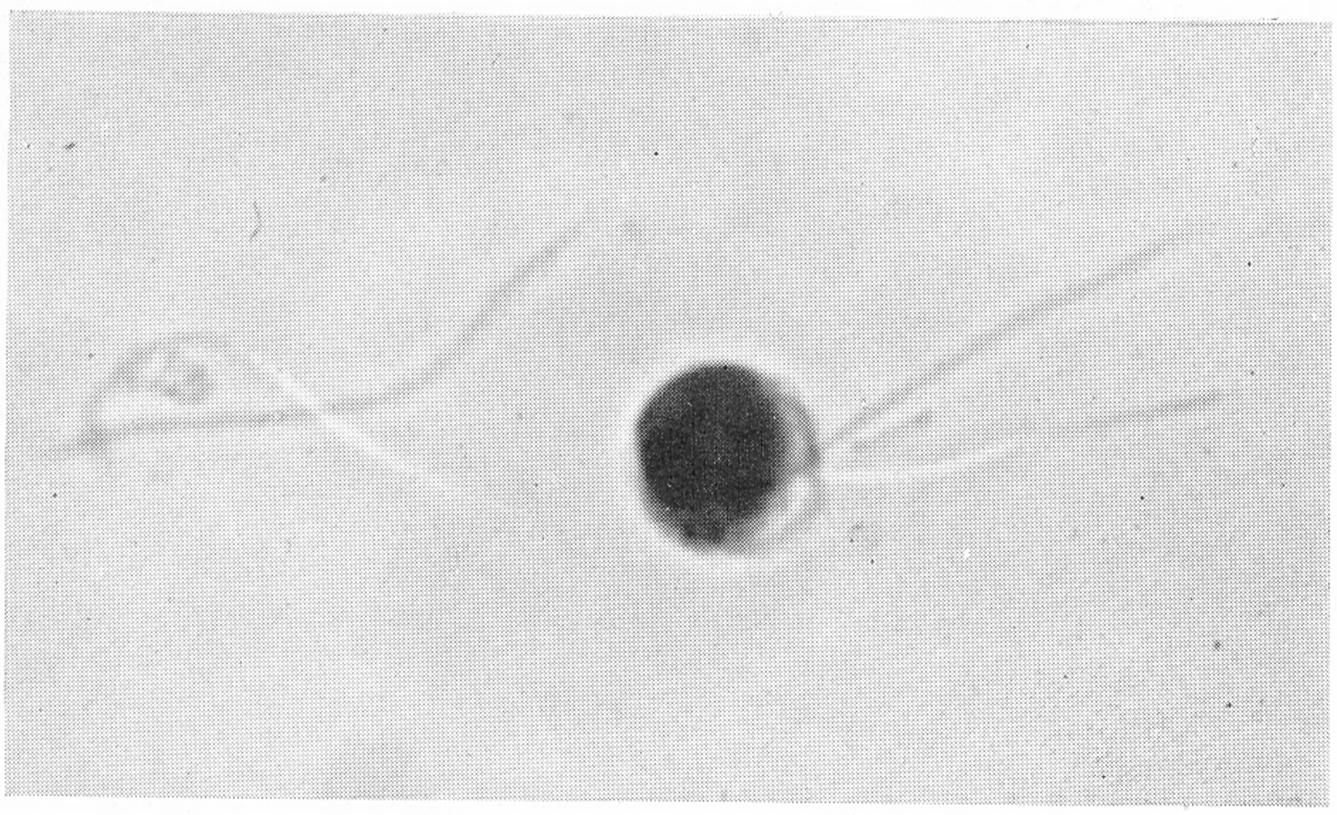

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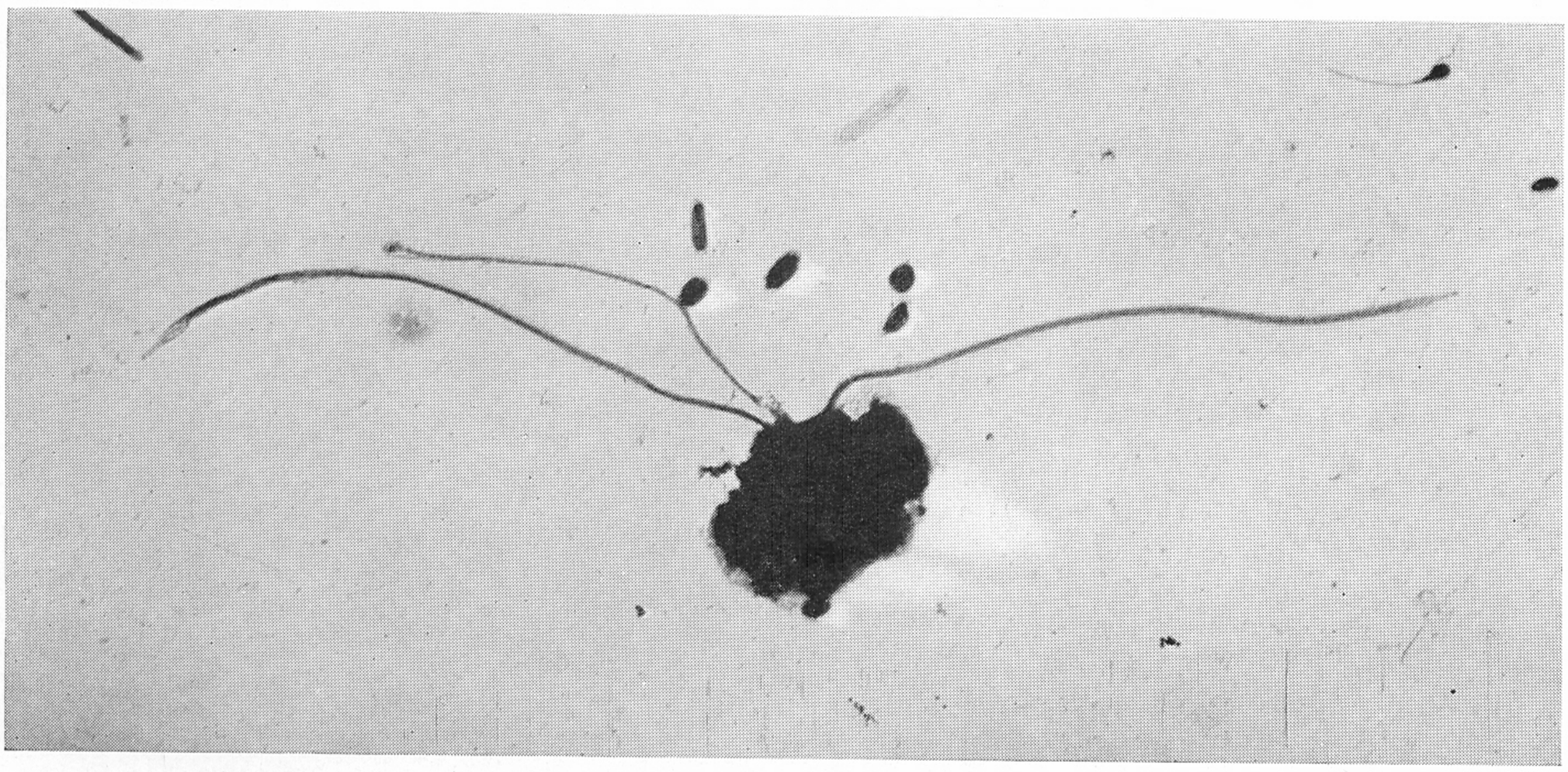

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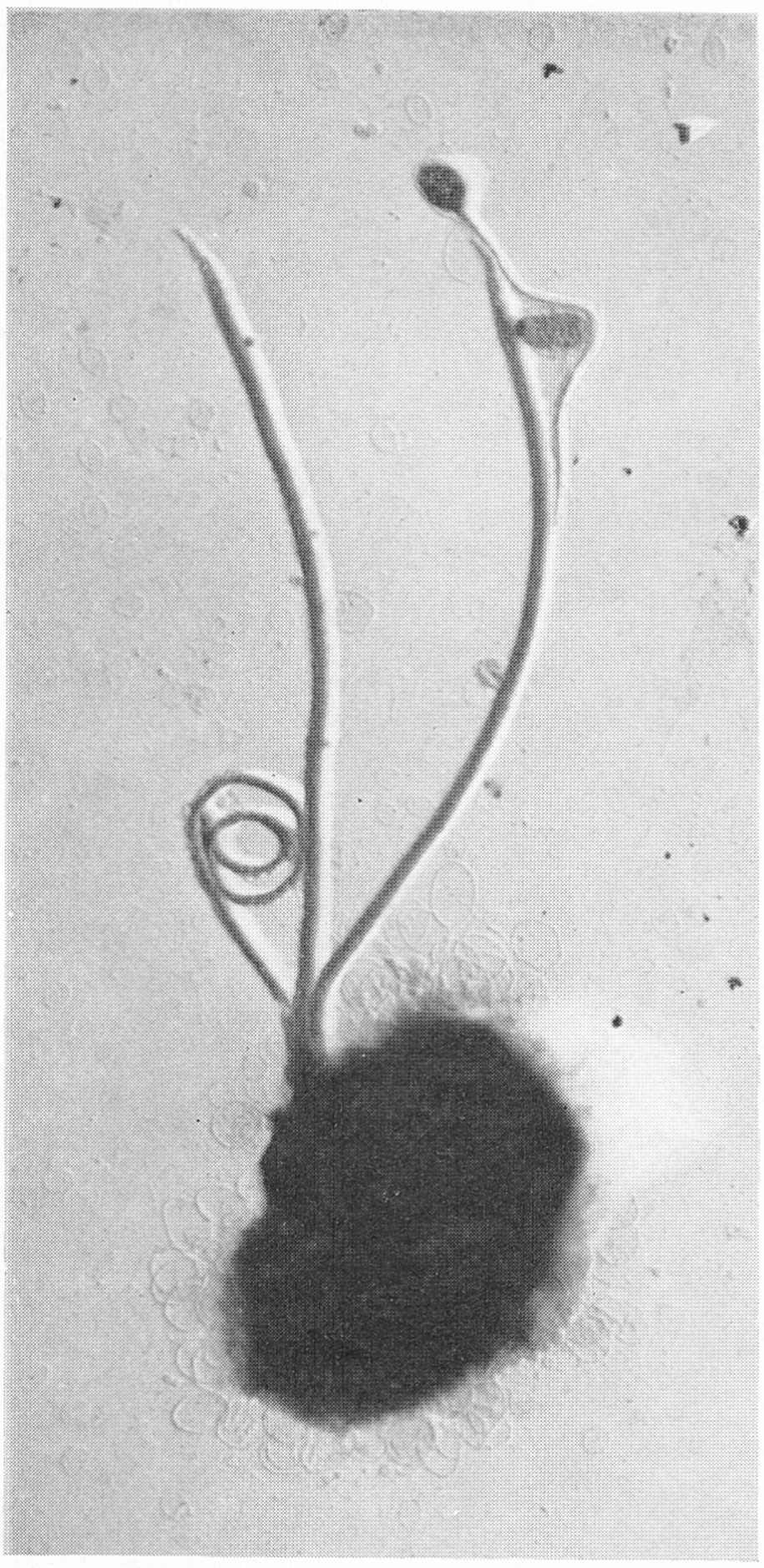

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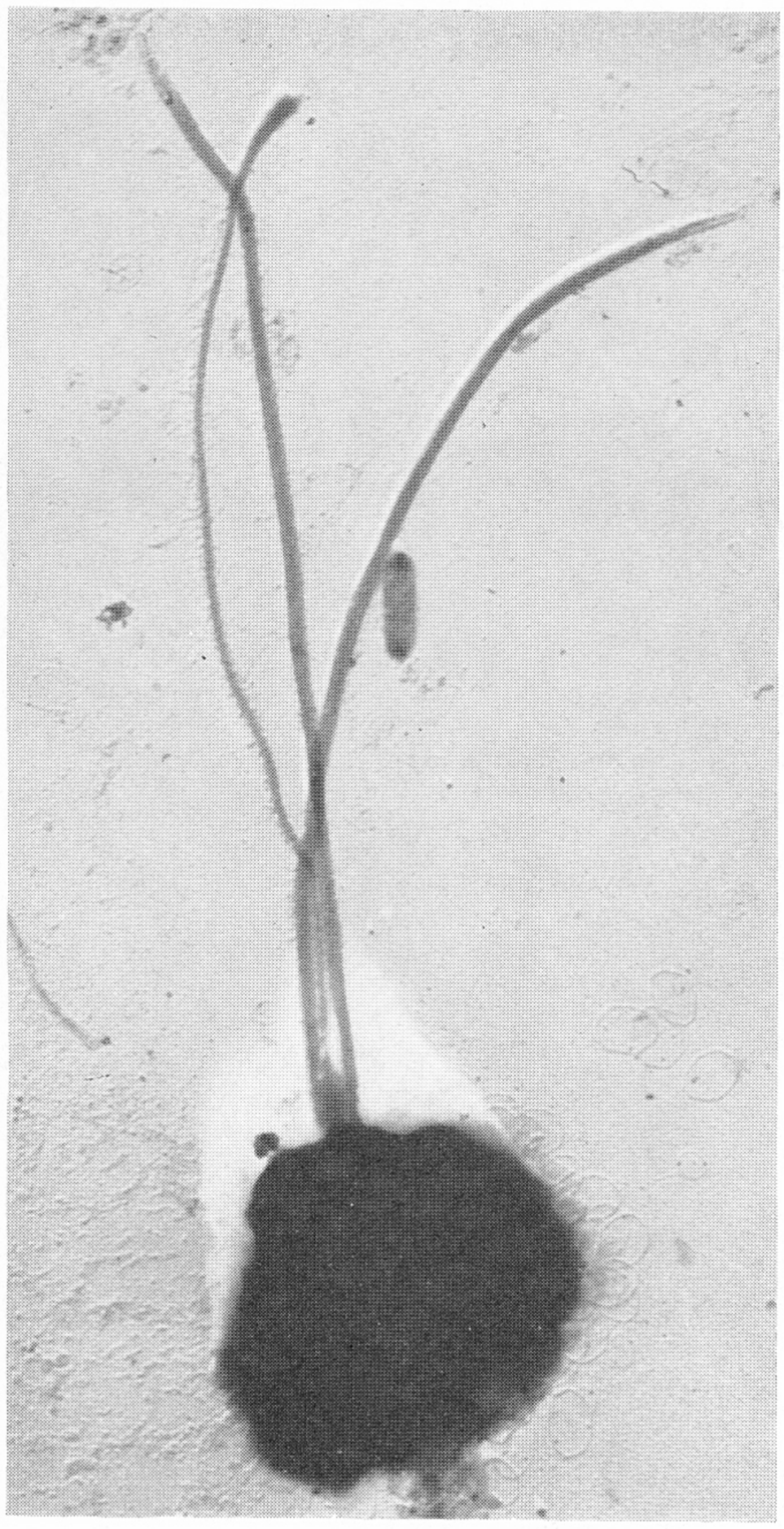

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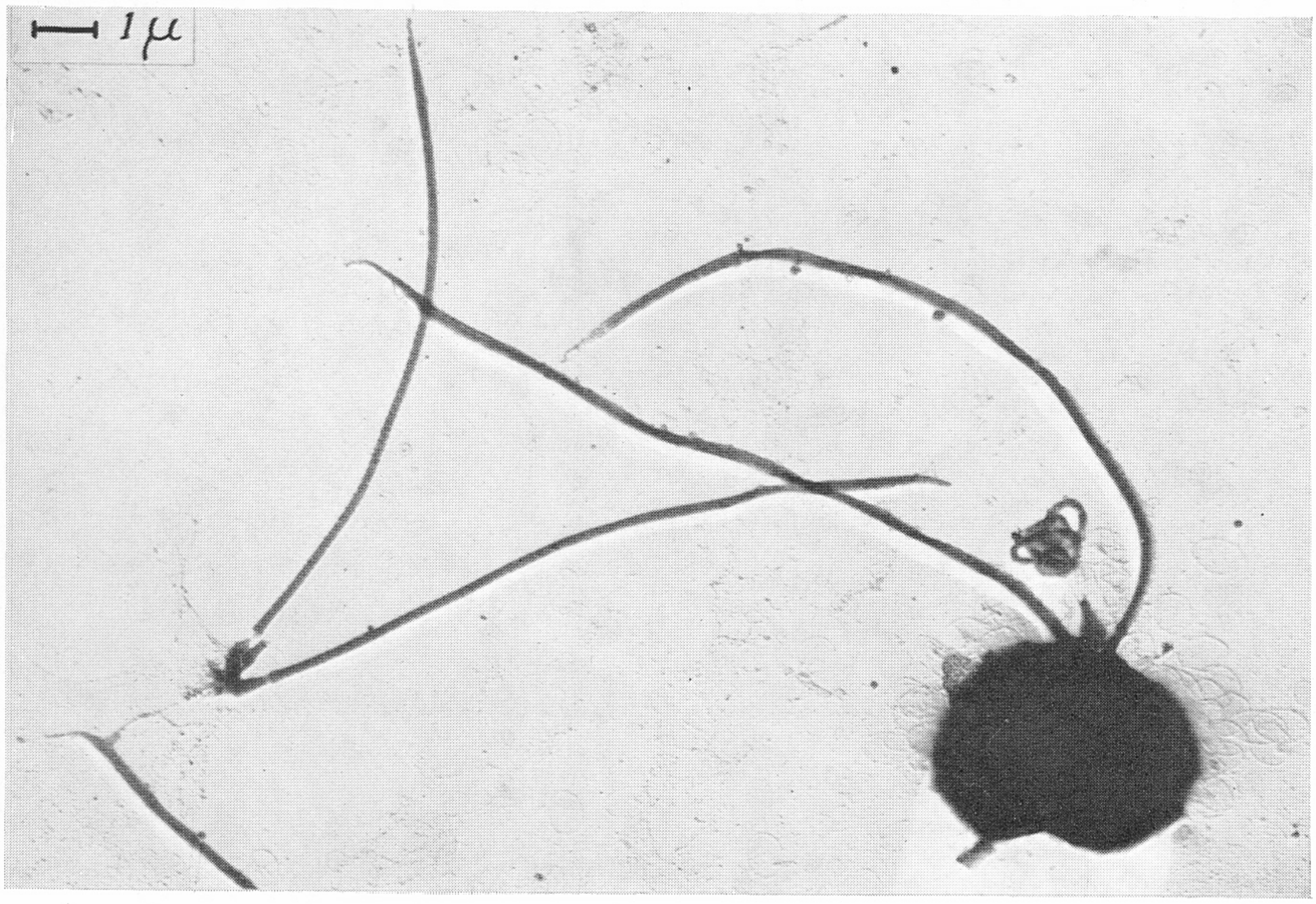

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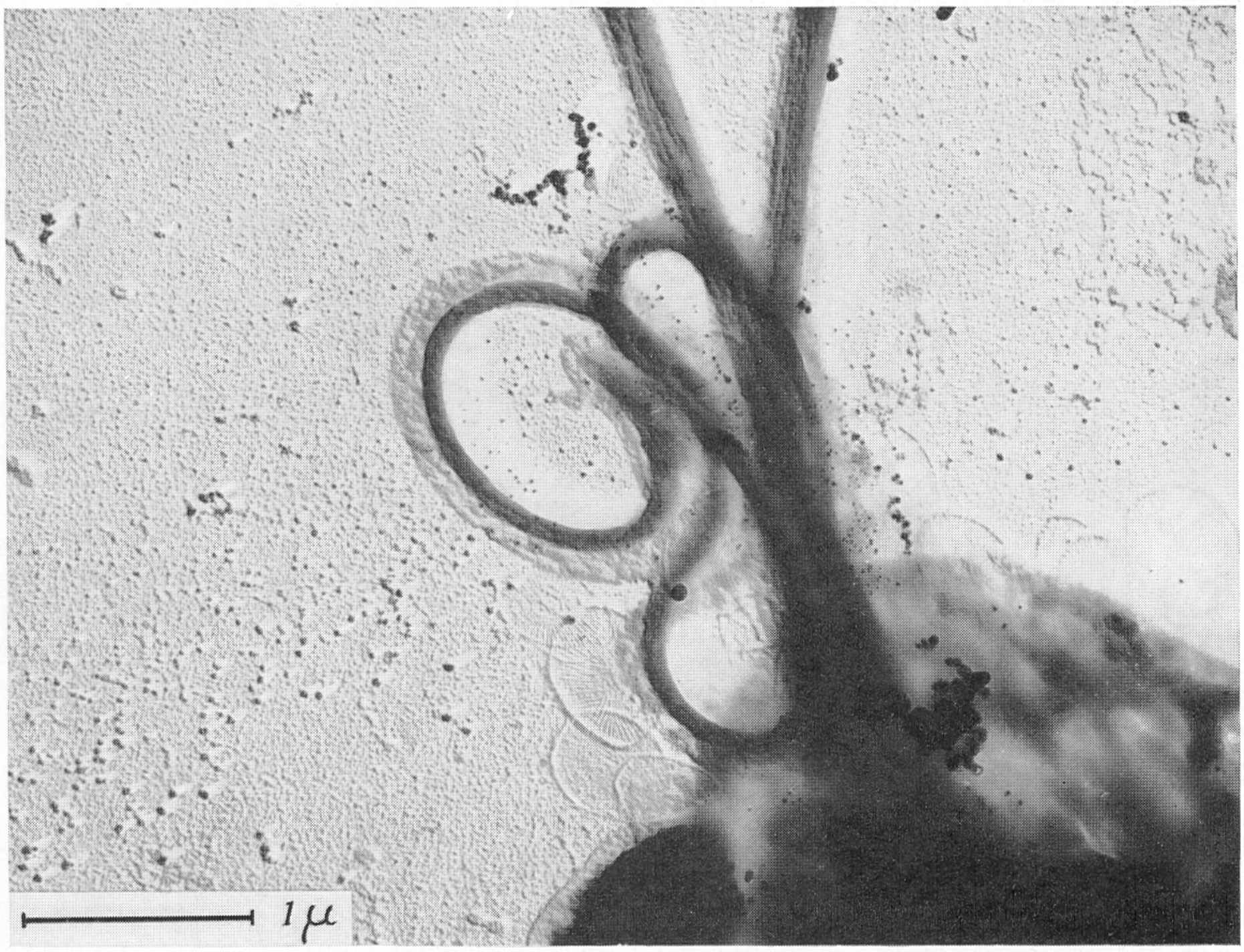

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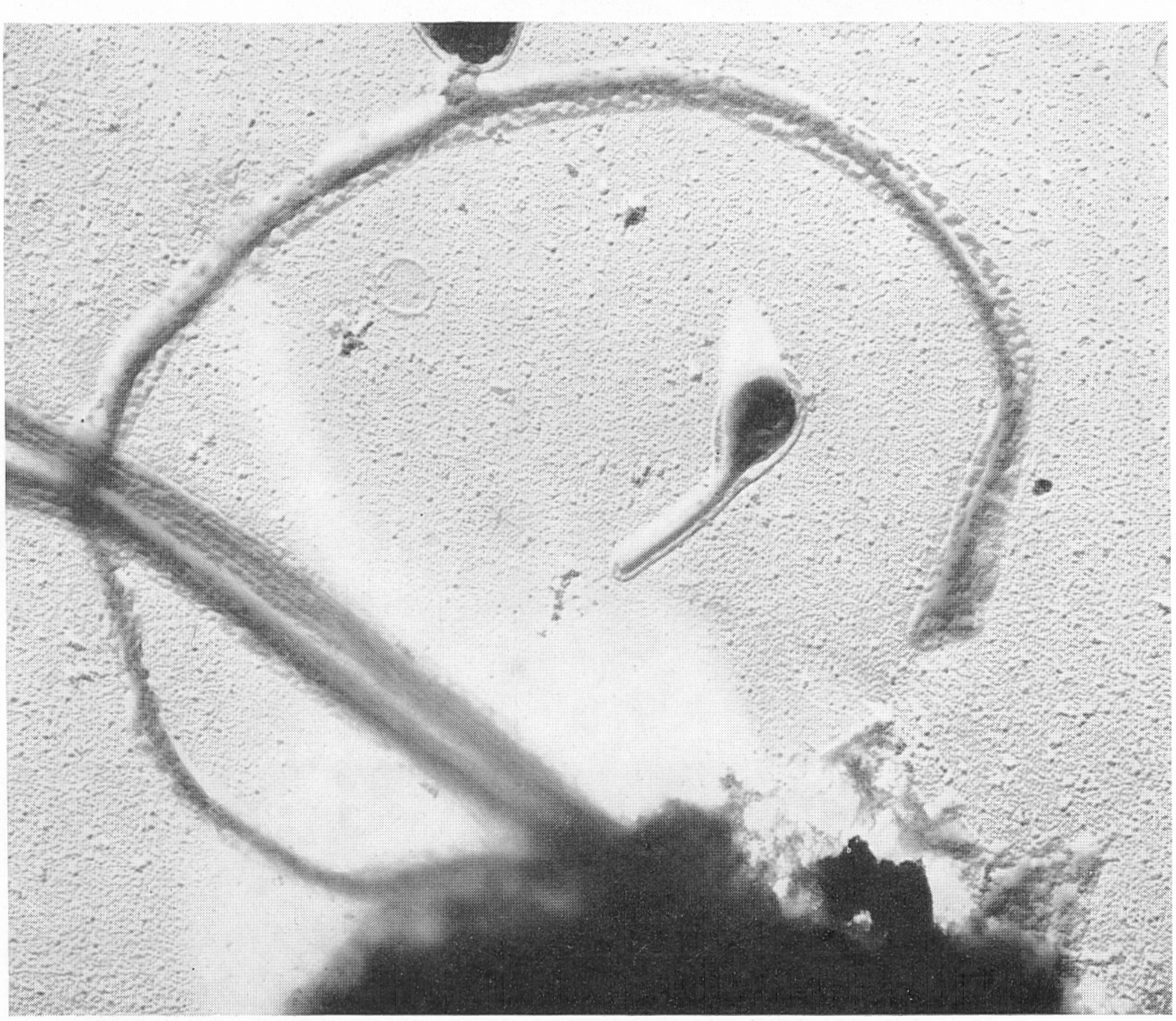

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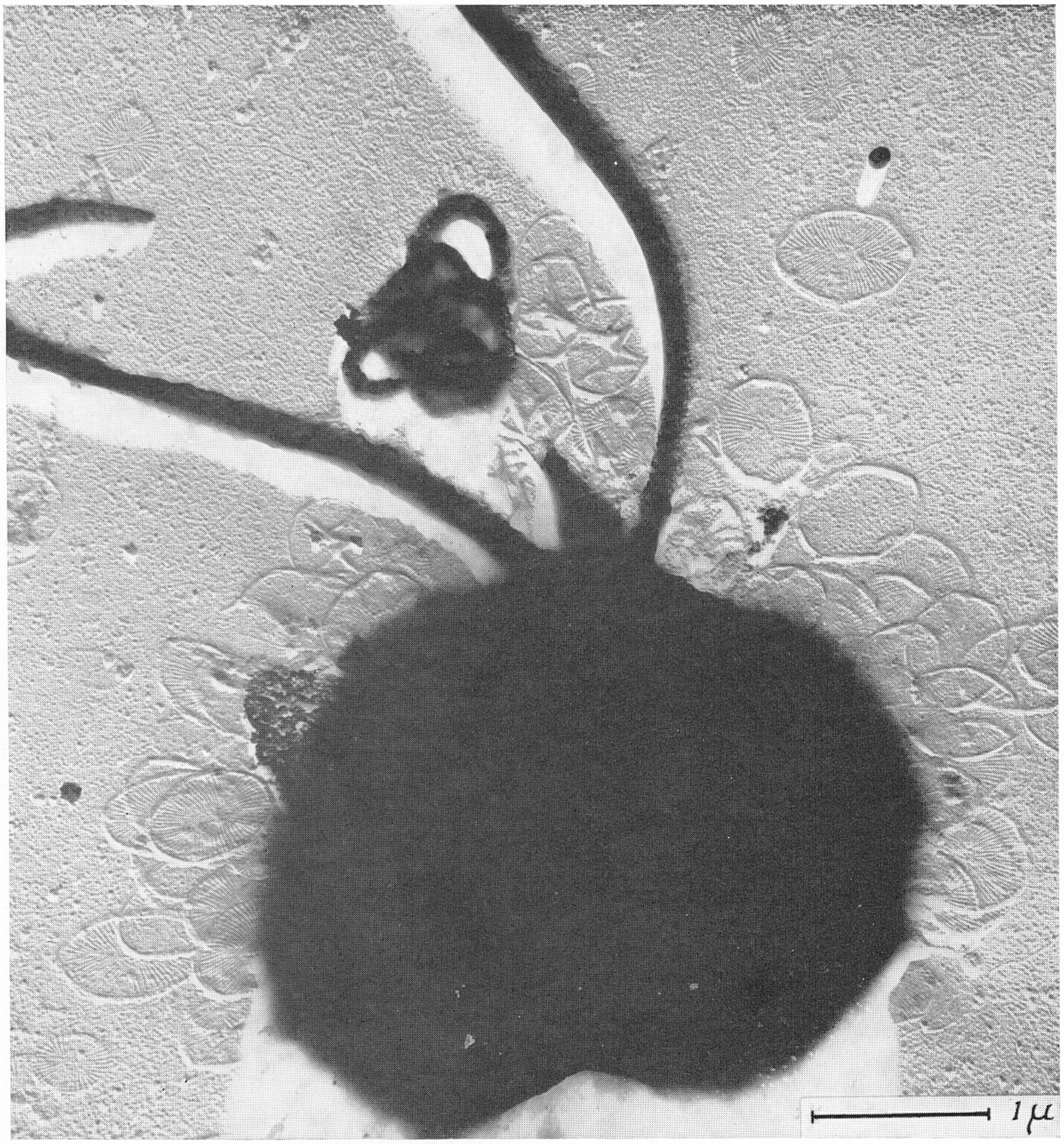

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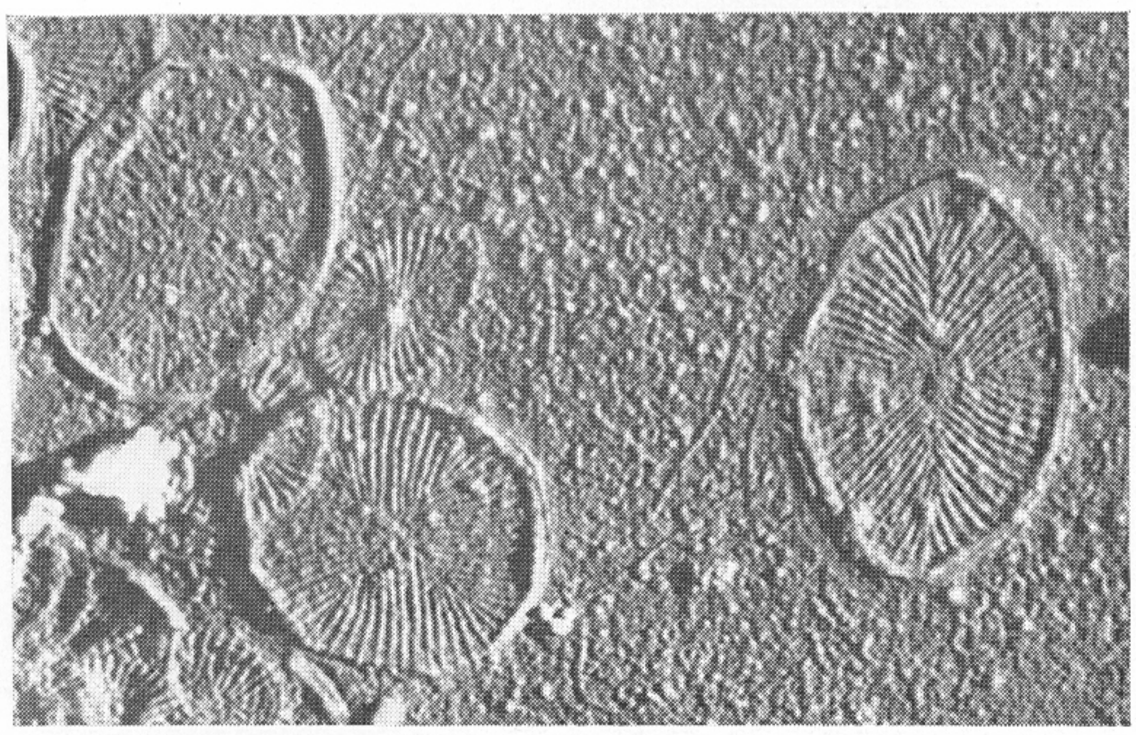

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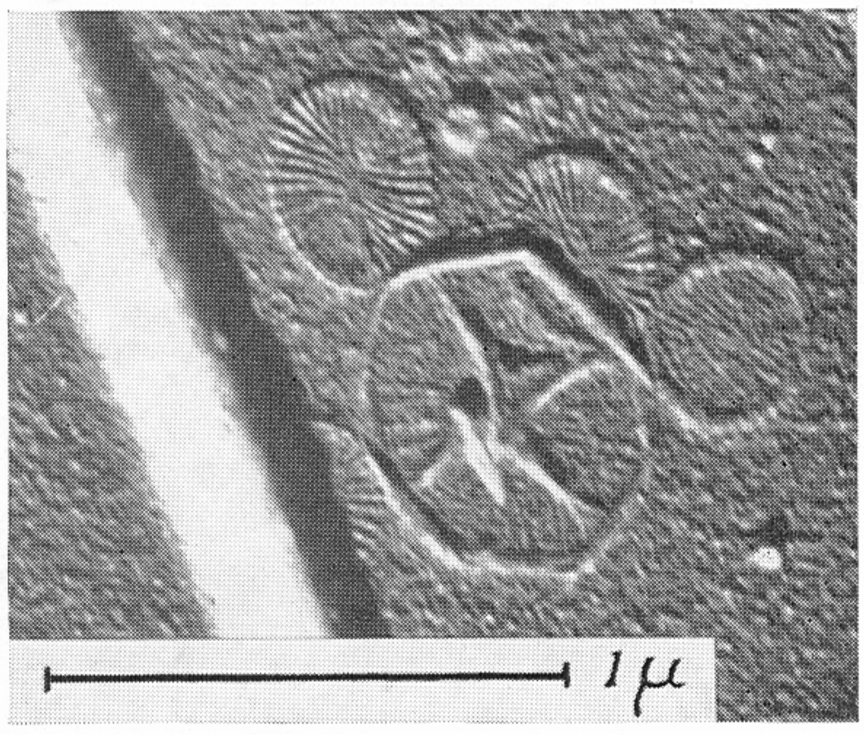

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In the localized peripheral areas in which the muciferous organelles occur, a few granules stain bright blue with cresyl blue and are perhaps mitochondria. Two or three slightly larger masses stain a blue-green with this reagent; these are perhaps physodes (cf. Bourrelly & Magne, 1953; Magne, 1954).

The locomotory movements, which are some of the most significant attributes of these organisms, are unfortunately unsuited to diagrammatic demonstration, and drawings of a few characteristic attitudes to accompany a brief verbal description must suffice. In spite of the absence of a stigma, the motile cells show a marked phototactic reaction when exposed to uneven illumination in a Petri dish, accumulating preferentially towards, or away from, the light according to the intensity. When mounted under the microscope the cells move rapidly in rather large circles for a limited period, after which they slow down and either attach themselves temporarily to the surface of the slide or else move off again in a different direction. The cell body rotates and gyrates during swimming. When viewed under dark ground illumination the appendages are held as in Fig. 1 during periods of most rapid swimming, that is directed *backwards* with the haptonema almost or completely coiled up and the two vibrating flagella diverging at a wide angle so that their free ends are further apart than the width of the cell body. In this position the haptonema has never been seen fully uncoiled, and it is generally coiled so closely to the body that it may be difficult to see. When slightly uncoiled at the base (Fig. 6) the cells appears to move more slowly. Sometimes, however, individuals are seen moving with the flagella and haptonema directed *forwards*, when the haptonema is commonly extended to its full length (slightly longer than the flagella) and is held stiffly out in front of the body while the flagella splay out laterally (Fig. 5) showing an undulating movement.

At any moment after a period of active swimming the cells may slow down and anchor themselves by the tip of the haptonema to any sufficiently firm surface which under the conditions of viewing is commonly the surface of the glass slide. The flagella may continue to vibrate with undiminished vigour and the cell body then carries out peculiar shaking or dancing movements, or it may occasionally move slowly in a circle round the point of anchorage. These movements are characteristic of most of the forms examined by us with short haptonemata and they are an extremely useful character for quickly recognizing these organisms in a mixed sample. When attached by its distal extremity the haptonema can be fully extended, but usually it is partly or even almost wholly coiled up except at the base (Figs. 2, 3). When tightly coiled it is liable to be hidden beneath the body, but the characteristic movements will show that it is attached. When only a short amount is uncoiled at the basal end the remainder is coiled in a flat spiral appearing to the eye as a disk with the point of attachment in the centre. This disk-like appearance, however,

can only be seen under an oil-immersion objective without which such an individual could momentarily be mistaken for a species of *Prymnesium*.

Phagotrophy is of common occurrence (Figs. 2–6), suspended particles, bacteria and small plant forms up to $2{\cdot}5\mu$ in diameter being ingested. The actual process of ingestment is difficult to analyse since the act itself is very rapid. Recently ingested cells of other organisms can sometimes be seen in Brownian movement within a vacuole. By the use of graphite it has been possible to ascertain with certainty that the site of ingestion is at the non-flagellar pole of the cell. A colourless, slightly granular, substance seems to flow out from this region to surround the particles (or cells) being ingested (Figs. 5, 6), which are then quickly drawn within the body (Fig. 2). Ingested particles at first seem to be moved about within the body but they are gradually compacted together, apparently within a vacuole, until they form a rounded pellet (Fig. 4; and Figs. 25 and 26, Pl. I). Material can be ingested, moved about in the cell, rounded off and then thrown out again (Fig. 3) all in the space of 5 min. In a very short time after graphite has been added to a culture individuals can be seen swimming quite normally with large masses of graphite inside them (Fig. 4). A cell 5μ in diameter is able to accommodate a mass as large as $2{\cdot}5 \times 2\mu$. This species tends to keep ingested material in the body fairly close to the pole at which it entered. When the graphite masses are ejected from the body some of the muciferous organelles (see p. 589) are commonly seen to discharge their contents as rods at the same time (Fig. 3). It is probable that all these processes can take place both when the cell is attached and when it is swimming.

Reproduction of the motile stage is by fission to produce usually two equal daughter-cells, each $4–5\mu$ in diameter, but fission can give daughter-cells of unequal size. When fission is unequal the smaller daughter-cell occasionally lacks chromatophores. The two new flagella generally develop as the chromatophores are dividing (Fig. 7). The second haptonema then appears as the cell broadens to separate the two pairs of flagella prior to the actual fission (Fig. 8). But the chromatophores can divide, giving four in an individual, before the new flagella are produced, or the cell can divide without concurrent chromatophore division, in which case one chromatophore passes to each daughter-cell or one has two and the other none.

Occasionally in this species large motile cells, $9–10{\cdot}5 \times 7–9\mu$, are found (Fig. 10) which bear four pairs of flagella and four haptonemata and contain four chromatophores. These cells divide into four daughter-cells, each of which usually contains one chromatophore but occasionally one of the daughter-cells may contain two chromatophores or none. Such cells are probably caused by a delay in the first cleavage until preparations for the next have already begun. Fig. 11 shows an early stage of fission in a cell $8 \times 7\mu$ in which one of the daughter cells has two flagella and a haptonema, while the other has already formed two new flagella ready for the next fission. Such double-

fission stages have been seen infrequently in individuals lacking chromatophores (Fig. 12), though such specimens are always smaller than those containing chromatophores, measuring 6–6·5 × 6·5–7 μ.

A culture of this species when grown in Erdschreiber culture solution produces from 2 to 3 million motile cells per ml. at the peak of growth. After the peak of growth has been passed, for reasons which have not been analysed, the motile cells migrate to the bottom of the flask forming there a dark olive-green skin. The word migrate is used to express the fact that cells collect preferentially on one side of the flask showing a phototactic response. If this skin is scraped from the bottom and examined the non-motile stages illustrated in Figs. 13–18 are found. When the cells lose motility they become amoeboid, measuring about 7 μ in diameter and, as far as can be checked by the use of cresyl blue, appear to discard their scale covering. At the same time small lobes appear at the periphery of the chromatophores (Fig. 13). Large naked (?) amoeboid stages up to 13 × 9 μ are also found in which the chromatophores are so very finely lobed, and so pale in colour, that they are very difficult to distinguish (Figs. 14, 15). The pyrenoid-like bodies could be seen in some of these amoeboid stages, but they are smaller than in the motile phase. A few very small leucosin vesicles are also present in some of the cells. Ingested bacteria could be detected inside these cells and graphite particles were ingested.

The origin of the large amoeboid cells (Figs. 14, 15) has not been traced. They may be either the product of fusion of the smaller amoeboid forms (Figs. 13) or they may be double-fission stages in the non-motile phase. Stages, which are apparently early division stages of these large naked amoeboid forms (Fig. 15), show four chromatophores and eight pyrenoids. From the sizes of the different stages the large amoeboid cells must either contract considerably in size or else divide before they round off and develop a thin smooth wall. No large secondary mucilaginous envelope, as was found in the genera *Prymnesium* by Carter (1937) and Conrad (1941), and in *Isochrysis* and *Dicrateria* by Parke (1949), has been found surrounding the walled stages.

The smooth-walled cells with two or four pale chromatophores, stellate or finely lobed, divide to produce four walled daughter-cells (Figs. 16, 17). These daughter-cells become free from the parent membrane and usually have two chromatophores, but sometimes there appears to be only one; the chromatophores are still stellate or deeply lobed in form (Fig. 18). These daughter-cells could quite easily be mistaken for the free, non-motile, walled cells belonging to either the genus *Phaeaster* Scherffel (1927) or *Sarcinochrysis* Geitler (1930). The genus *Pleurochrysis*, recently described by Pringsheim (1955), differs from the non-motile phase of *Chrysochromulina* in not possessing finely lobed chromatophores, and in showing longitudinal divisions in the cells, to give short filaments which grow out to produce irregularly ramified colonies.

The swarmer of *Pleurochrysis* differs also in lacking a haptonema, and has either fine granules on the external membrane or is surrounded by a solid granulated capsule.

The reconstitution of the motile phase from the small, walled, Phaeaster-like cells (Fig. 18), which are ovoid to slightly bean-shaped and measure from $3{\cdot}5 \times 2{\cdot}5$ to $6 \times 4\mu$, has not yet been observed, but empty walls (Fig. 19) with a circular pore about $1{\cdot}5\mu$ in diameter have been found, suggesting that their contents are so liberated. It would be particularly interesting to watch the behaviour of the chromatophores immediately prior to and immediately after the liberation. As was stated earlier the chromatophores of the motile stage sometimes appear striated, when seen in face view, as if there might be lobes lying flat against the inner face.

In this species, if the olive brown skin on the bottom of a flask is washed well a number of times to remove all free cells and all motile cells and then fresh culture solution added, in 7–10 days the skin has gone from the bottom of the flask and a thick culture of the motile phase is then present in the medium.

Chrysochromulina minor n.sp., Parke & Manton

Diagnosis

Motile cells sphaeroidal to ovoid with a flattened flagellar pole and the opposite pole slightly tapering, some metaboly, 3–5 (exceptionally $2{\cdot}5$–$7{\cdot}5)\mu$ in diameter. Two equal homodynamic flagella, 2–3 times the cell diameter in length, with a distinct knobbed mucronate tip more than twice the flagellar diameter in length visible with the electron microscope. One haptonema[1] slightly shorter than the flagella when fully extended, arising with them at the flattened cell pole. Periplast covered by very thin sculptured scales, nearly circular to ellipsoid, sometimes hexagonal to octagonal, $0{\cdot}2 \times 0{\cdot}3$ to $0{\cdot}5 \times 0{\cdot}7\mu$ in size.

Cells uninucleate, no stigma. Chromatophores usually 1 or 2, sometimes 4, 8 or none, deep golden-brown, in motile phase saucer-shaped, parietal, with medium sized globular body (pyrenoid ?) on inner face placed near the margin nearest the flagellar pole; in non-motile phase deeply lobed or stellate. Oil and leucosin produced. Muciferous bodies localized near the non-flagellar pole. Nutrition holophytic and/or phagotrophic. Non-toxic to fish.

Asexual reproduction of pigmented and non-pigmented forms in motile phase by fission into 2 or 4 daughter-cells; in non-motile phase by fission of amoeboid cells to produce 4 walled daughter-cells which probably liberate motile phase.

Habitat: the sea at position Lat. N. 49° 19′, Long. W. 7° 26′ at 5 m. depth on 14 June 1950. Type culture (Plymouth No. 52) deposited with the Type Culture Collection, Cambridge, and the Marine Biological Association;

[1] For derivation and use of this term see p. 581.

preserved material and photographs lodged with the Marine Biological Association, Plymouth, England.

Cellula motili sphaeroidali aut ovoidali, aliquanto planato ad axem qua inserta sint flagella, paululum subulata ad axem objectum flagellis, forma mutabili, 3 μ ad 5 μ (rare 2·5 μ ad 7·5 μ) latitudine. Duobus similibus flagellis homodynamicis, longioribus 2 aut 3 cellulae latitudine, utriusque ad extremitatem distincta regione nodosa et mucronata, longa plus bis obesitate flagelli, solum per microscopiam electronicam visibili; unico haptonemate, conjunctim flagellis exorienti, paulum breviore, cum maxime extensus sit, quam longitudo flagelli. Periplasto induto delicatissimis squamis sculptis, prope circularibus aut ellipsoidalibus, nonnunquam hexagonis aut octagonis, 0·2 μ ad 0·5 μ lat., 0·3 μ ad 0·7 μ long., visibilibus solum per microscopiam electronicam.

Nucleo unico, nullo stigmate; chromatophoris ex norma unico aut duobus, nonnunquam 4, 8 vel nullis, profunde aureofulvis, in cellulis status motilis crateriformibus, parietalibus, unocuique corpore globulari medio magnitudine (pyrenoidali?) in aspectu concavo locato ad marginem juxta axem qua oriantur flagella; in cellulis status non-motilis chromatophoris profunde lobatis aut stellatis; cellulis oleum leucosinumque parientibus. Corporibus muciferosis locatis ad regionem objectam axi flagellari. Nutritione holophytica et tamen phagotrophica. Non toxica piscibus.

Generanti asexualiter in statu motili per fissionem cellularum coloratarum vel incoloratarum in 2 aut 4 cellulas filiolas. In statu non-motili generanti per fissionem amoeboidalium cellularum ad 4 cellulas filiolas, parietibus praeditas, generandas, quibus potest ut cellulae status motilis liberentur.

Habitat mare ad locationem Lat. N. 49° 19′, Long. W. 7° 26′, profund. 5 m. (14 Jun. 1950).

Description

This species is smaller and usually slightly more ovoid in shape than *C. kappa* when alive (Fig. 36), though this difference is obscured after fixation. In an actively growing culture 75% of the cells are from 3 to 5 μ in length or diameter, 5% are 2–3 μ and the remaining 20% are early fission stages (including double-fission stages) from 5 to 7·5 μ. The flagella, 2–3 times the length of the cell, are relatively slightly longer than in *C. kappa* and are more easily seen, though they are more readily thrown off when the species is examined under the light microscope. No bulbous swelling can be detected at the base of the haptonema with the light microscope in this species though a comparable structure can be demonstrated with some difficulty with the electron microscope. The length of the haptonema when fully extended is slightly less than that of the flagella, thereby differing from the situation in *C. kappa* in which it is slightly more. The club-shaped extremity is similar in the two species but the translucent haptonemal sheath is slightly less conspicuous in *C. minor* (Fig. 60, Pl. VI).

The scales (Figs. 62–4, Pl. VII) are very similar in the two species, although we have so far failed to find any trace of spined scales in *C. minor*. The chromatophores of the motile phase are very similar to those of *C. kappa*, but are more deeply pigmented and more frequently show striations; they are

usually 1 or 2 but sometimes 4, 8 or none. Individuals lacking chromatophores, seen frequently in this species, measure usually 2·5–3μ (Fig. 40). When one chromatophore is present it lies laterally (Fig. 36); when about to divide it moves to the non-flagellar pole (Fig. 39) and after division the two chromatophores return to the lateral position (Fig. 38).

In this species the pyrenoid-like bodies (p. 589), reacting in the same way to stains and Millon's reagent as in *C. kappa*, are smaller and are excentrically placed near to the periphery of a chromatophore on the side towards the flagellar pole (Figs. 36–38), not centrally on its inner face as in that species. The position of the nucleus, leucosin vesicles, oil globules and muciferous bodies are as in *C. kappa* except that the muciferous bodies are fewer and none seem to be present at the flagellar pole. Their position at the opposite pole changes, as in *C. kappa*, with the metaboly of the body. The behaviour of the muciferous bodies is similar in both species.

Swimming is more rapid than in *C. kappa* and tends to be in straight lines over considerable distances rather than moving in circles. As in *C. kappa* the most usual attitude, and the only one associated with rapid swimming is with the flagellar pole and appendages directed backwards, but the flagella lie closer together so that their ends are separated by less than the width of the cell (Fig. 36). The attitudes of the haptonema are the same as those described for *C. kappa*, both when projecting backwards and when projecting forwards, but in the latter position the flagella lie more nearly in front of the body than in

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Legends to Text-figs. 36–44

Chrysochromulina minor n.sp. (× 5000).

Fig. 36. Individual swimming with flagella and haptonema behind the body, in the position characteristic for the species during rapid swimming. *c*, chromatophore; *f*, flagellum; *h*, haptonema; *l*, leucosin vesicle; *m*, muciferous body; *n*, nucleus; *p*, pyrenoid-like body; *s*, scales.

Fig. 37. Anchored individual with 2 chromatophores and very small masses of ingested graphite near flagellar pole.

Fig. 38. Anchored individual ingesting minute particles of graphite at non-flagellar pole; cell body swaying round in circles in a clockwise direction from anchoring point.

Fig. 39. Individual swimming with flagella and haptonema in front of the body, haptonema fully extended; ingested graphite (*g*) towards flagellar pole; chromatophore moved from lateral position to pole prior to division.

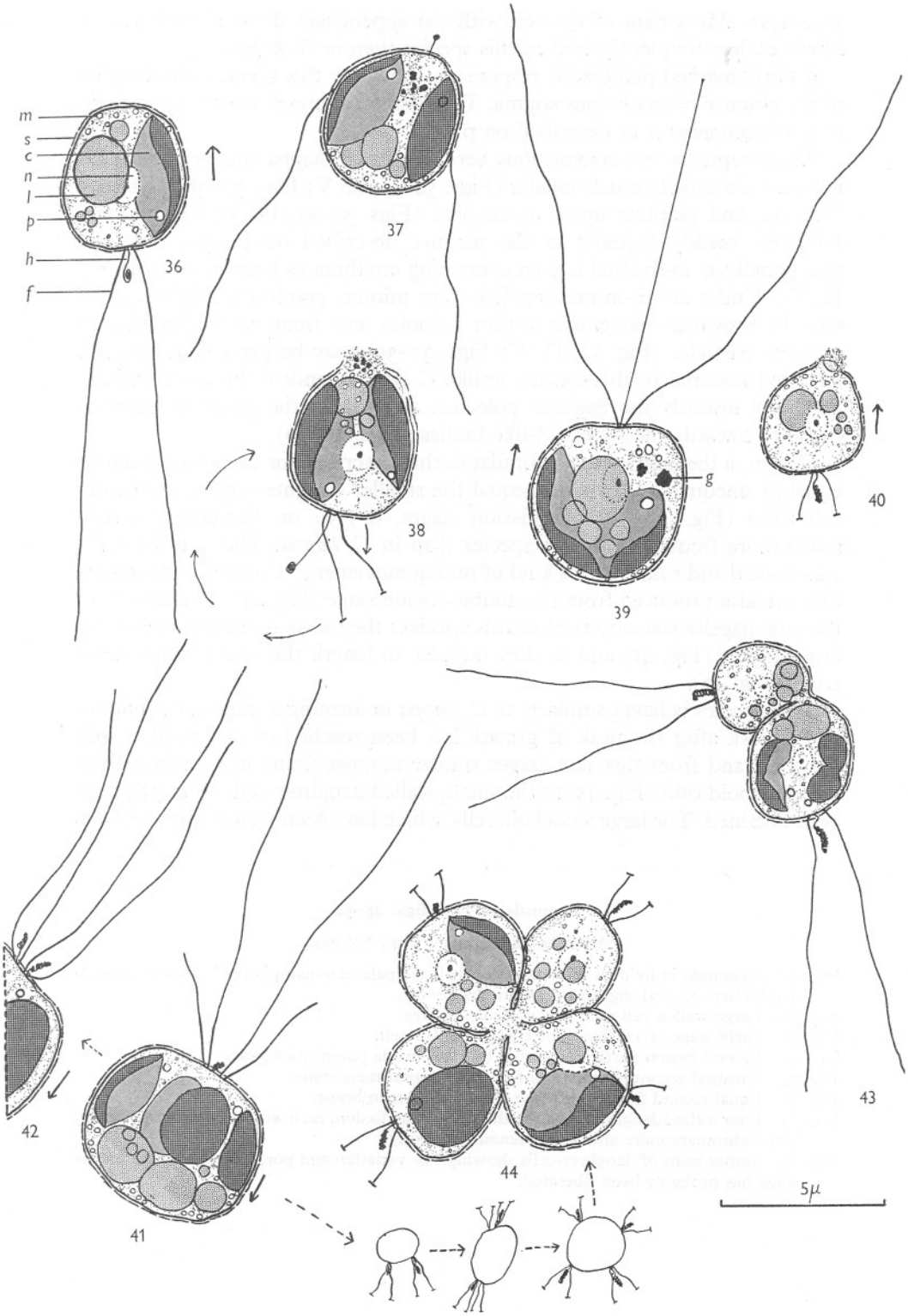
Fig. 40. Swimming individual lacking chromatophores, ingesting a bacterium at non-flagellar pole.

Fig. 41. Early fission stage with 4 chromatophores and two new flagella just developing; second haptonema not yet formed.

Fig. 42. Flagellar pole of individual in early fission stage; two new flagella nearly as long as original flagella and second haptonema developed.

Fig. 43. Two motile daughter-cells just before separation after unequal fission; larger daughter-cell with two dividing chromatophores and smaller without chromatophores.

Fig. 44. Double-fission practically completed giving 4 daughter-cells, one of which lacks a chromatophore; earlier stages shown diagrammatically below.



Text-figs. 36–44.

C. kappa. Movement of the cell with the appendages directed forwards is, however, less frequently seen in this species than in *C. kappa*.

A fairly marked phototactic response is shown by this species also, in spite of the absence of an obvious stigma. The method of attachment (Figs. 37–38) of the haptonema is as described on p. 591.

Phagotrophy is less conspicuous here than in *C. kappa* since the particles ingested are usually much smaller (Figs. 54–55, Pl. V; Figs. 37–38). Bacteria (Fig. 40) and graphite up to about 0·5μ (Figs. 54–55, Pl. V; Fig. 39) are, however, readily ingested in the manner described on p. 592, but only occasionally an individual is seen containing anything as large as 1μ (Fig. 53, Pl. V). Under an oil-immersion lens very minute graphite particles can be seen in Brownian movement within vacuoles and frequently a number of separate particles (Fig. 54, Pl. V; Figs. 37–39) may be present in one cell. Ingested material in this species, unlike *C. kappa*, tends to be moved within the body towards the flagellar pole, i.e. away from the point of entry or possibly towards the pyrenoid-like bodies (Figs. 37, 39).

Fission in the motile stage is similar to that described for *C. kappa*; it can be equal or unequal, and when unequal the smaller daughter-cell is frequently colourless (Fig. 43). Double-fission stages, 6–7·5μ in diameter, are seen much more frequently in this species than in *C. kappa*. They can be quite sphaeroidal and rotate with a kind of rolling movement. Colourless daughter-cells are also produced from the double-fission stage (Fig. 44). The growth of the new flagella was observed in this species; they arise on either side of the original pair (Fig. 41) and as they increase in length the second haptonema arises (Fig. 42).

This species behaves similarly to *C. kappa* in forming a skin on the bottom of the flask after the peak of growth has been reached (6 to 7 million cells per ml.), and from this skin stages similar to those found in *C. kappa*, from the amoeboid cells (Fig. 45) to the small, walled daughter-cells (Fig. 51), have been obtained. The large amoeboid cells, which have been seen to ingest masses

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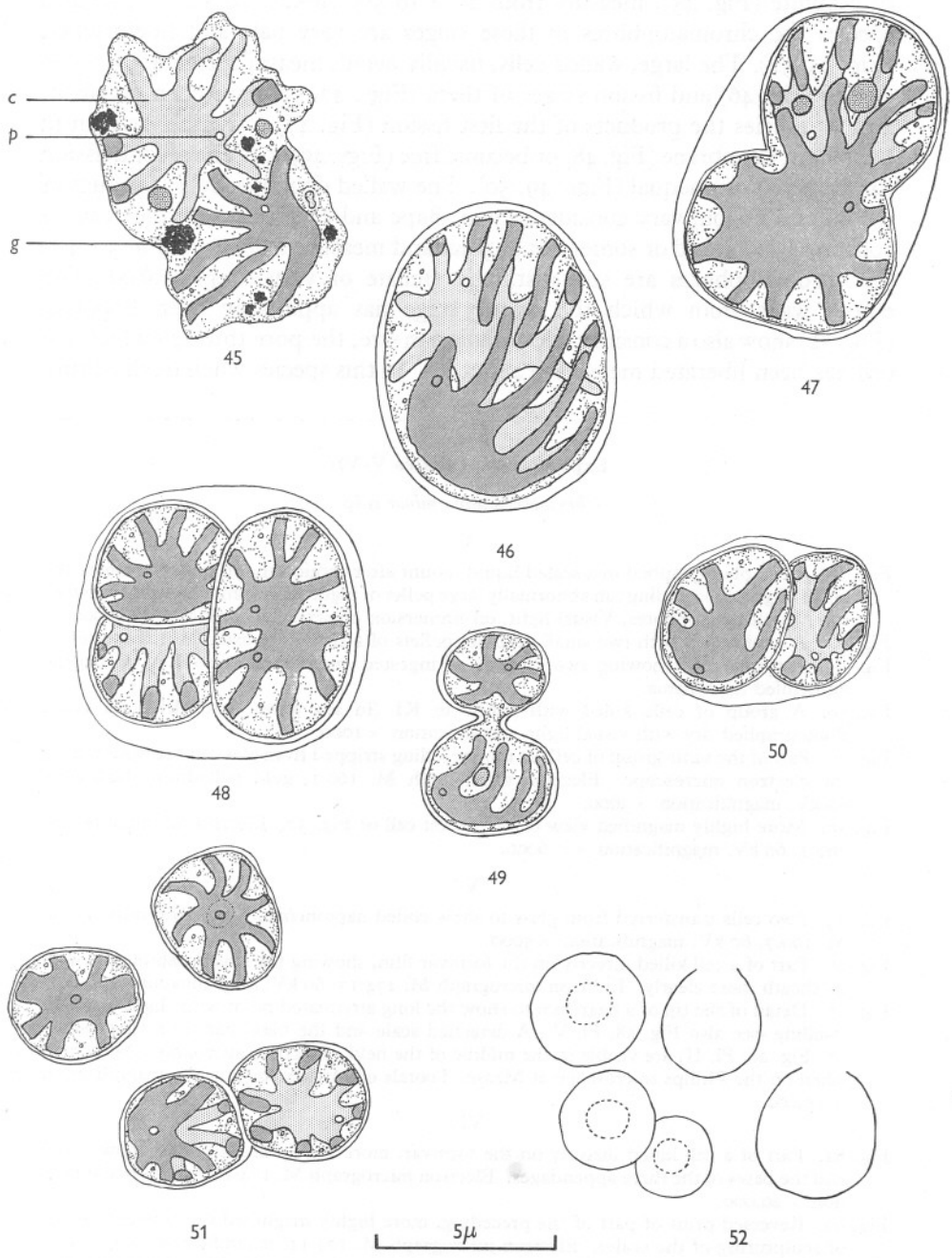
Legends to Text-figs. 45–52

Chrysochromulina minor n.sp. (× 5000).

Fig. 45. Amoeboid individual showing 2 deeply lobed pale chromatophores (*c*), two pyrenoid-like bodies (*p*), and ingested graphite masses (*g*).
Fig. 46. Large walled cell with two chromatophores.
Fig. 47. Early stage of 1st fission of a large, walled cell.
Fig. 48. Second fission partly completed but still inside parent membrane.
Fig. 49. Unequal second fission; cell free from parent membrane.
Fig. 50. Equal second fission; cell free from parent membrane.
Fig. 51. Four walled daughter-cells, product of second fission, each with one stellate or deeply lobed chromatophore and one pyrenoid-like body.
Fig. 52. Empty walls of daughter-cells showing size variation and pore through which motile stage has probably been liberated.



Text-figs. 45–52.

of graphite (Fig. 45), measure from 4×8 to $5{\cdot}5 \times 8{\cdot}5 \mu$. As in the previous species the chromatophores in these stages are very pale and finely lobed (Fig. 45–51). The large, walled cells, usually ovoid, measure from $4{\cdot}5 \times 7{\cdot}5$ to $6 \times 8 \mu$ (Fig. 46) and fission stages of them (Figs. 47–51) have been obtained. In this species the products of the first fission (Fig. 47) can either remain in the parent membrane (Fig. 48) or become free (Figs. 49, 50). The second fission can be equal or unequal (Figs. 49, 50). The walled daughter-cells, product of the second fission, vary considerably in shape and size (Fig. 51); they can be sphaeroidal to ovoid or somewhat angular and measure from $2 \times 2{\cdot}5$ to $3 \times 4 \mu$; the chromatophores are still distinctly stellate or very deeply lobed. The empty walls from which the motile stage has apparently been liberated (Fig. 52) show also a considerable variation in size, the pore through which the cell has been liberated measuring about 1μ. In this species when fresh culture

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Explanation of Plates V–VII

Chrysochromulina minor n.sp.

V

Fig. 53. A cell photographed in a sealed liquid mount after osmic vapour killing, to show the effect of graphite feeding; an abnormally large pellet of ingested graphite is visible between the two chromatophores. Visual light, oil-immersion objective, magnification $\times 2000$.

Fig. 54. A similar cell with two small graphite pellets of a size more usual in this species.

Fig. 55. A similar cell showing two patches of ingested graphite and also the two flagella and coiled haptonema.

Fig. 56. A group of cells killed with iodine in KI (in sea water) and dried on glass. Photographed dry with visual light, magnification $\times 1000$.

Fig. 57. Part of the same group of cells as the preceding stripped from glass and remounted for the electron microscope. Electron micrograph M. 160·1, gold palladium shadowing 60 kV, magnification $\times 3000$.

Fig. 58. More highly magnified view of the lowest cell of Fig. 57. Electron micrograph M. 160·2, 60 kV. magnification $\times c.$ 6000.

VI

Fig. 59. Two cells transferred from glass to show coiled haptonemata. Electron micrograph M. 160.7, 60 kV, magnification $\times 5000$.

Fig. 60. Part of a cell killed directly on the formvar film, showing the coiled haptonema with its sheath more clearly. Electron micrograph M. 174·17, 60 kV, magnification $\times 20{,}000$.

Fig. 61. Detail of the tip of a flagellum to show the long attenuated point with slight terminal swelling (see also Fig. 58, Pl. V). A detached scale and the basal part of a *Caulobacter* (cf. Fig. 28, Pl. II) are visible in the middle of the field. Electron micrograph M. 203·26 taken on the Philips microscope at Messrs Tootals of Manchester, 60 kV, magnification $\times 15{,}000$.

VII

Fig. 62. Part of a cell killed directly on the formvar, more highly magnified to show scales and the bases of the three appendages. Electron micrograph M. 174·11, 60 kV, magnification $\times 20{,}000$.

Fig. 63. Reversed print of part of the preceding, more highly magnified to show the details of sculpturing of the scales. Electron micrograph M. 174·11, magnification $\times 30{,}000$.

Fig. 64. Scales from another specimen showing raised rims more clearly. Electron micrograph M. 174·5, 60 kV, magnification $\times$ 30,000.

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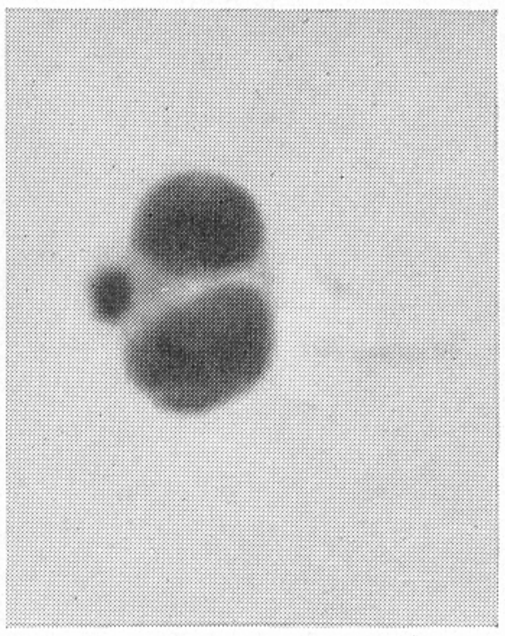

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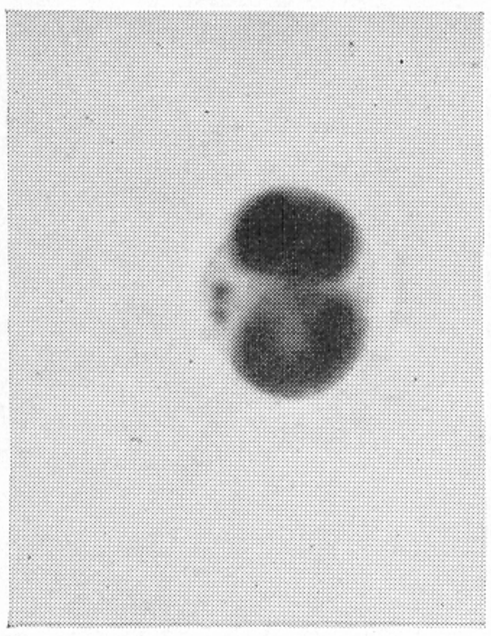

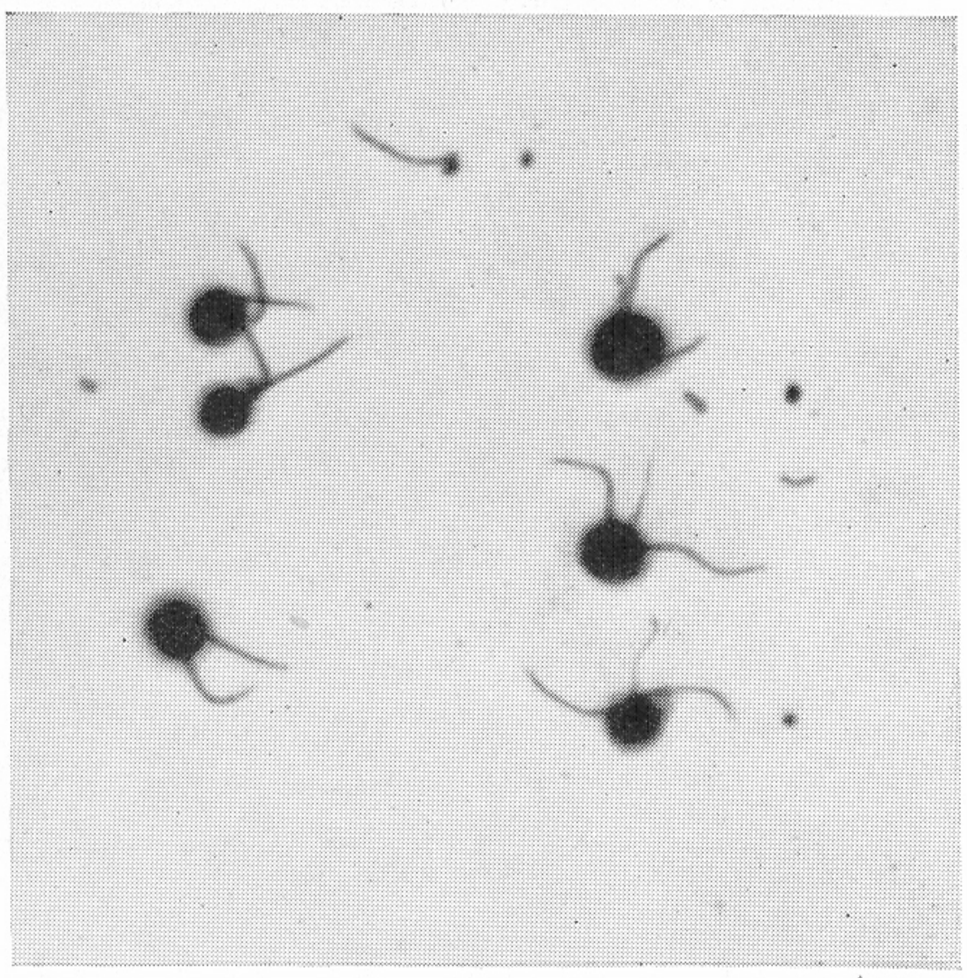

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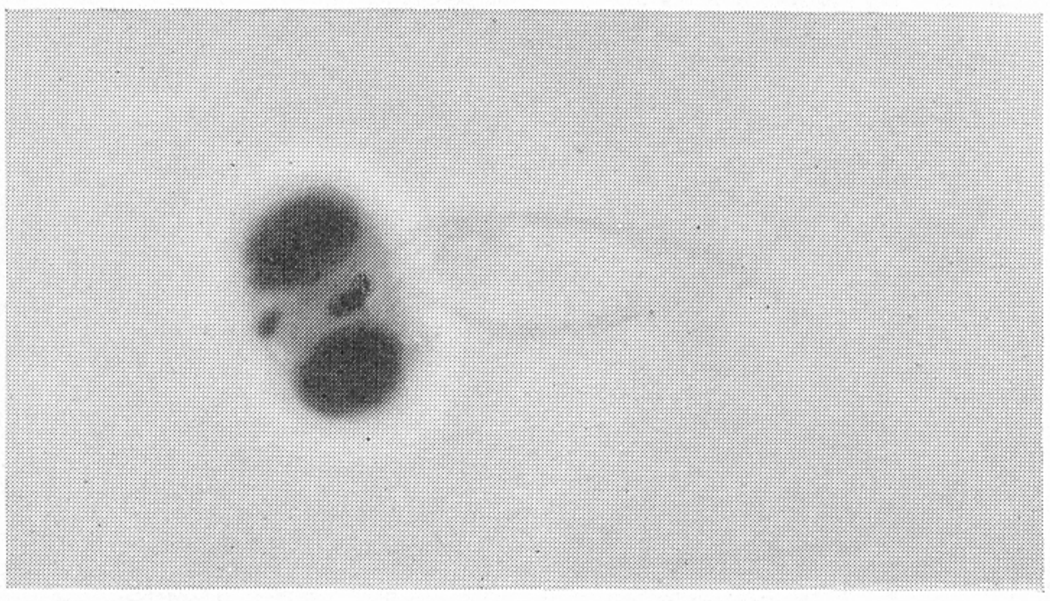

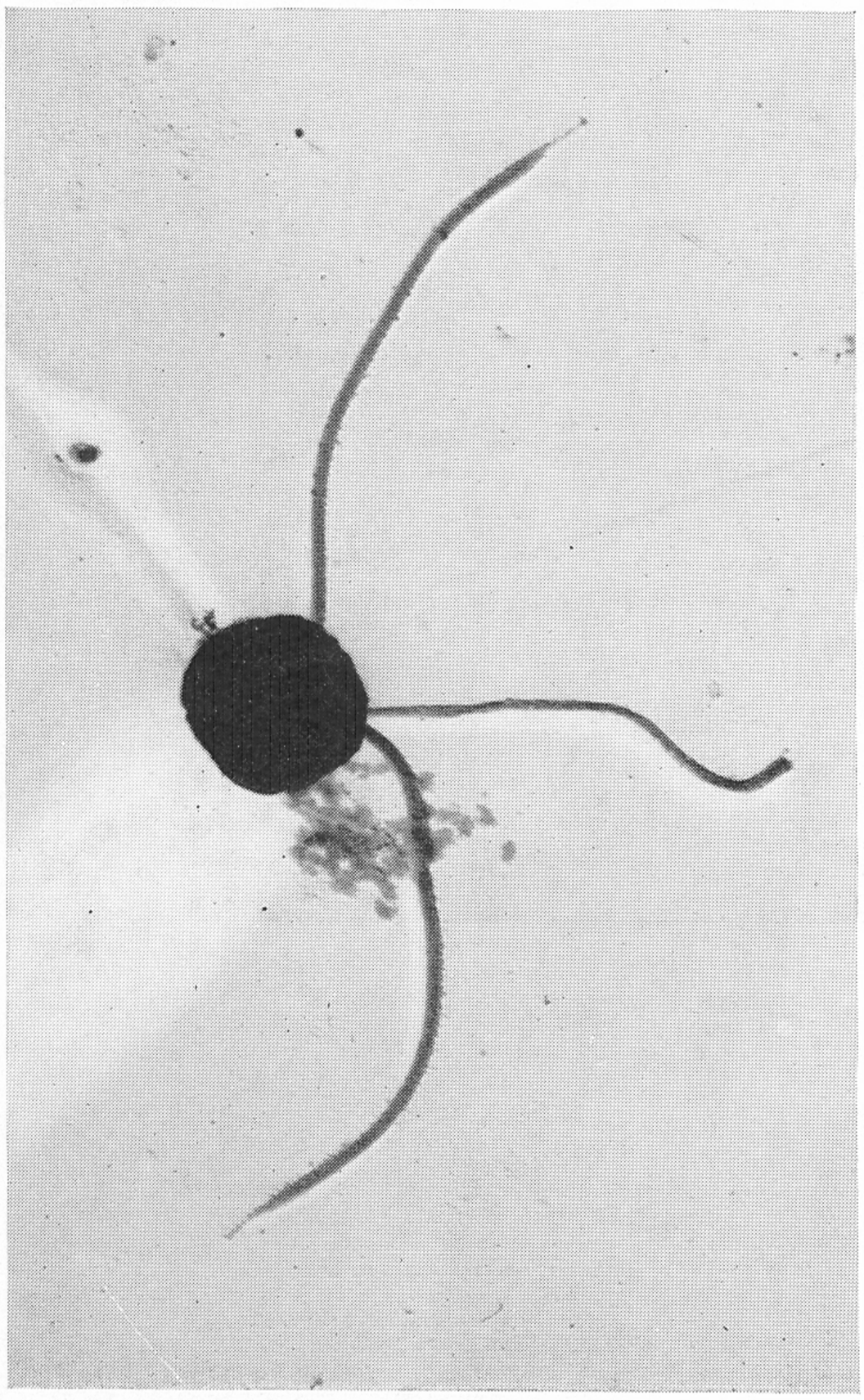

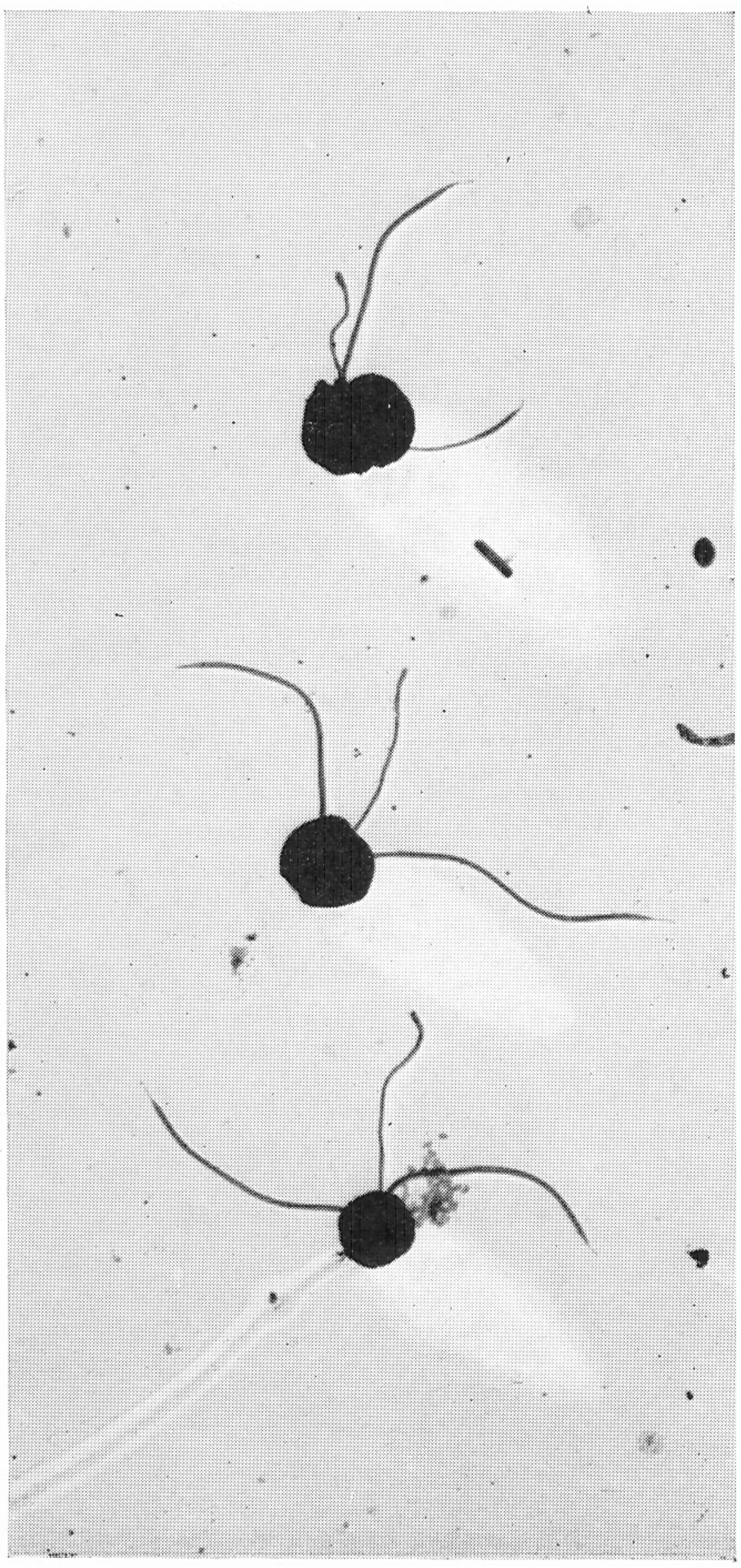

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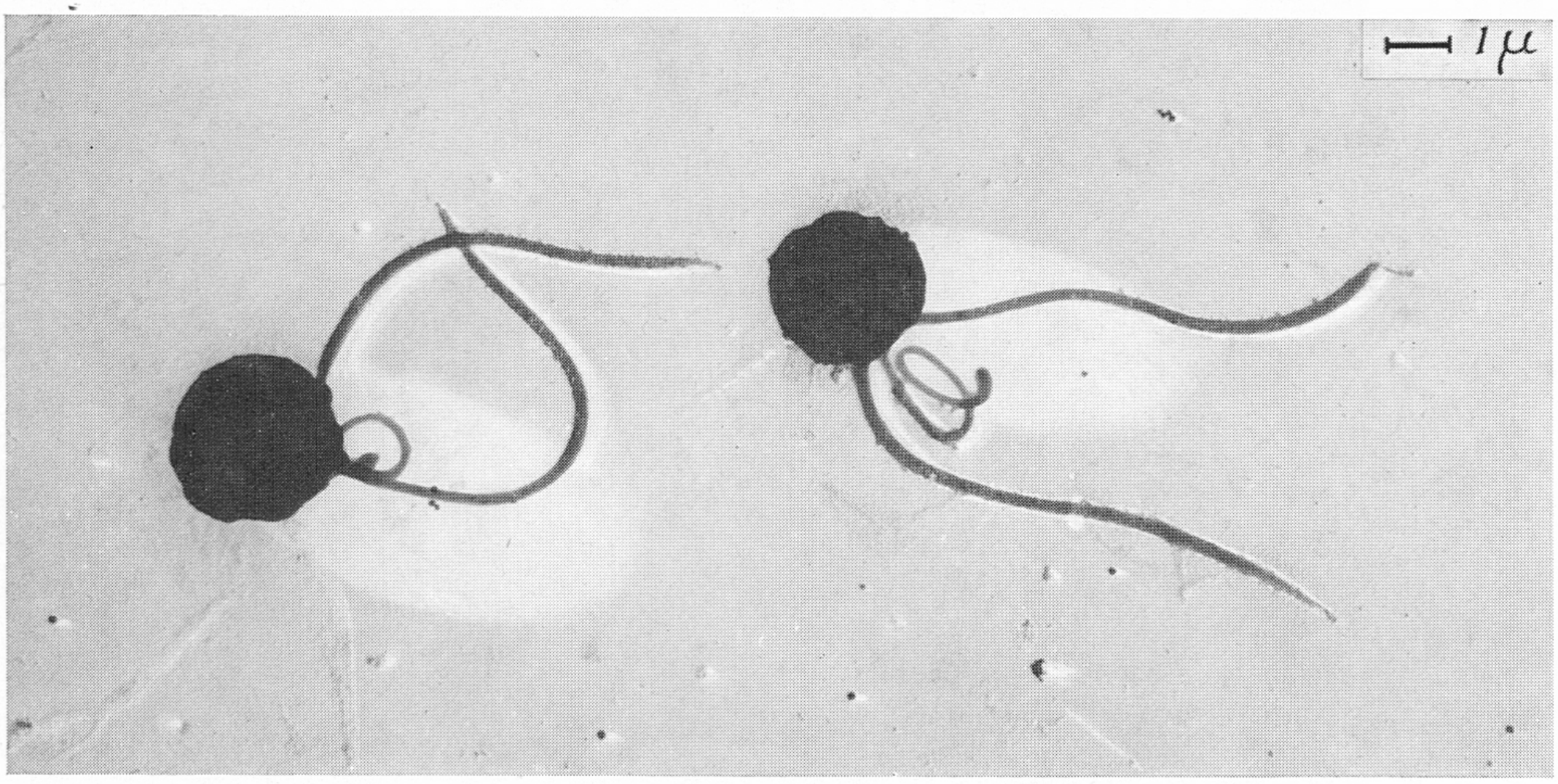

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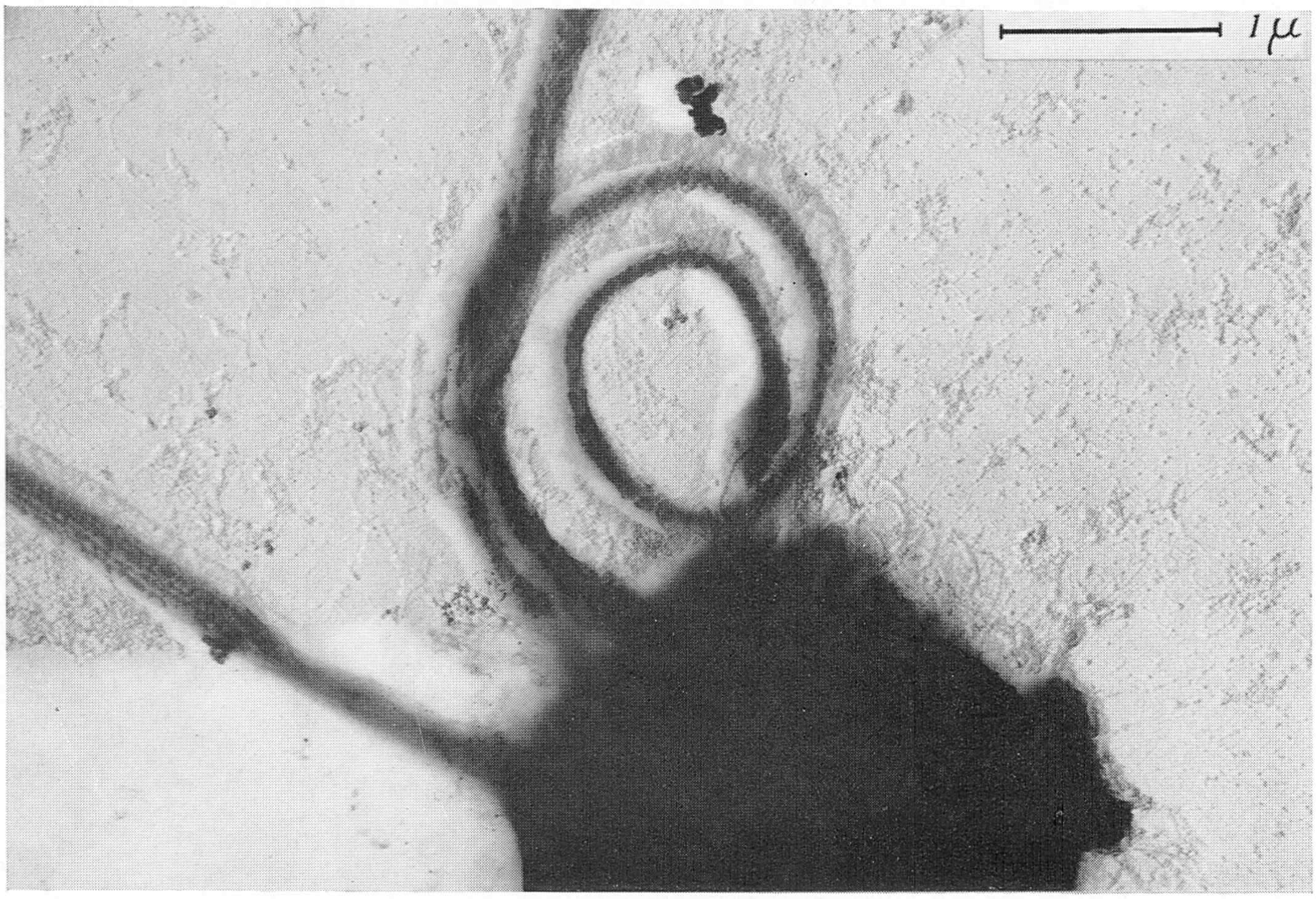

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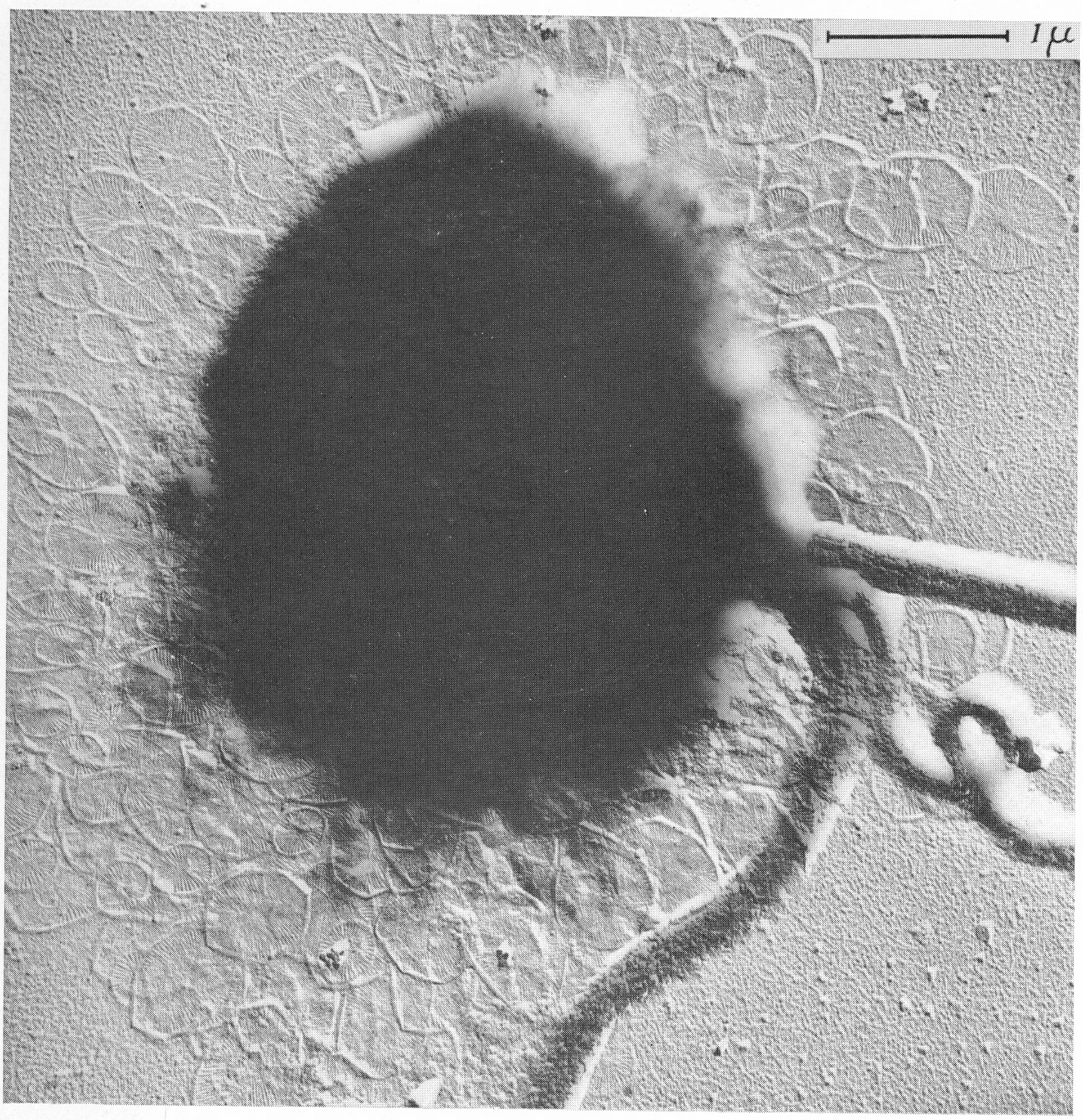

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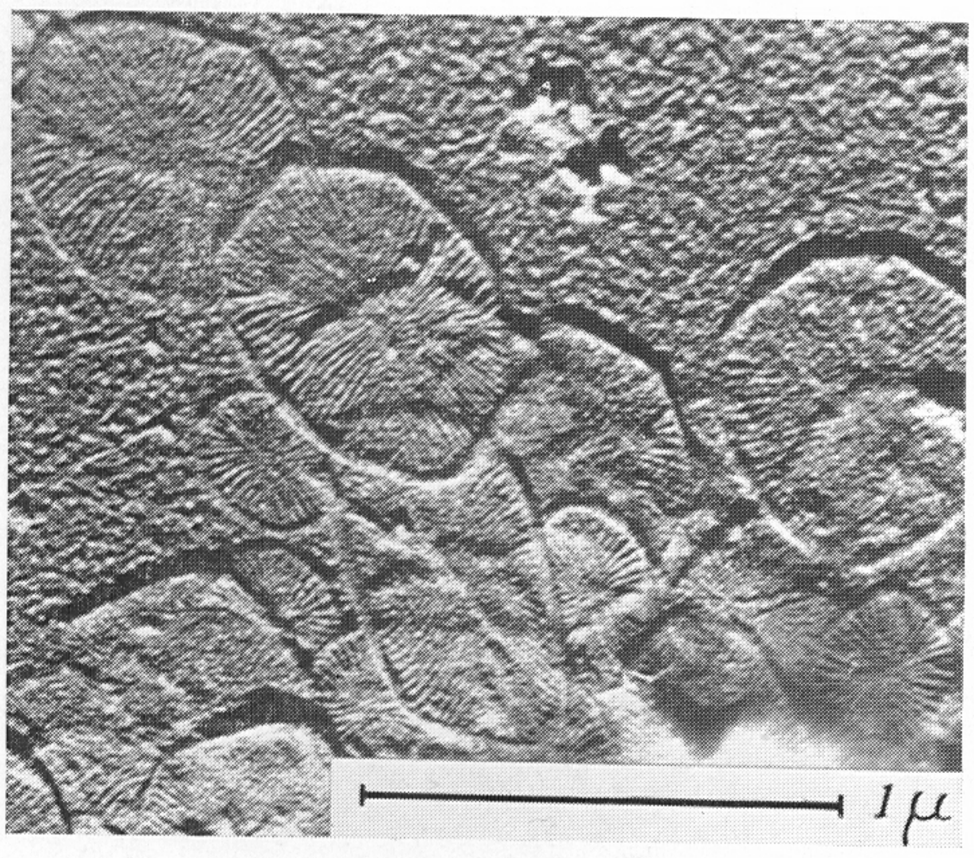

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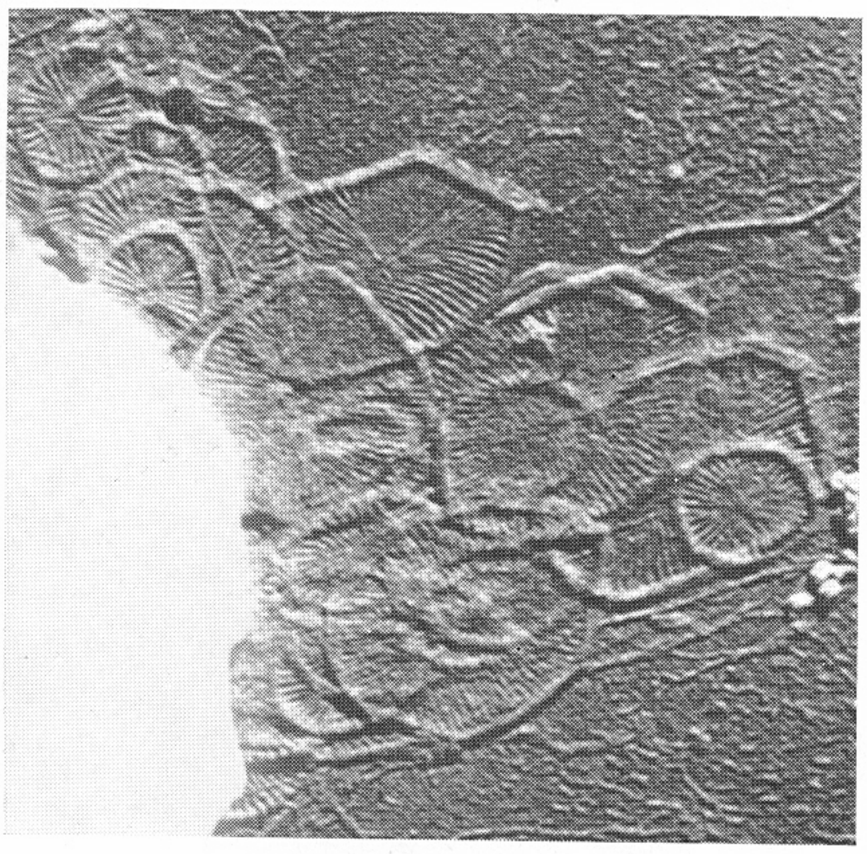

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solution is added to the skin on the bottom of a flask, after it has been washed, the motile phase is quickly produced, much more rapidly than in *C. kappa*, so that in 3–4 days a fairly thick culture of the motile stage is obtained.

Chrysochromulina brevifilum n.sp., Parke & Manton

Diagnosis

Motile cells sphaeroidal to pyriform with flagellar pole usually flattened; metaboly of body well-marked particularly at non-flagellar pole; cells 4–7μ (exceptionally 3·5–11·5μ) in length or diameter. Two equal, homodynamic, shortly mucronate flagella, $2\frac{1}{2}$–$3\frac{1}{2}$ times cell diameter in length and one haptonema,[1] 2–$2\frac{1}{2}$ times cell diameter in length when fully extended, arising close together at flattened pole. Periplast covered by very thin sculptured ellipsoid scales, approximately 0·7μ across, each with a central spine attached by four decurrent ridges, spine length approximately equal to scale diameter.

Cells uninucleate, no stigma. Chromatophores usually 2 or 4, occasionally 1 or 8, golden brown; in motile phase saucer-shaped, ellipsoid or oblong, parietal with small globular body (pyrenoid ?) on inner face of each near the margin towards the non-flagellar pole; in non-motile phase deeply lobed or stellate. Oil and leucosin produced. Muciferous bodies conspicuous, usually in rows, but their position changing with the metaboly of the body. Nutrition holophytic and/or phagotrophic. Not toxic to fish.

In motile phase asexual reproduction by fission into 2 or 4 daughter-cells: in non-motile phase by fission of amoeboid cells to produce 4 walled daughter-cells which probably liberate motile phase.

Habitat: the sea at position Lat. N. 49° 21′, Long. W. 04° 54′ at surface on 9 May 1950.

Type culture (Plymouth No. 39) deposited with the Type Culture Collection, Cambridge, and the Marine Biological Association: preserved material and photographs lodged with the Marine Biological Association, Plymouth, England.

Cellula motili, sphaeroidali vel pyriformi, ex norma planata ad axem qua inserta sint flagella; forma manifeste mutabili imprimis ad axem objectum flagellis; cellula 4μ ad 7μ (rare 3·5μ ad 11·5μ) latitudine. Duobus similibus flagellis homodynamicis, breve mucronatis, longioribus $2\frac{1}{2}$ ad $3\frac{1}{2}$ cellulae latitudine; unico haptonemate conjunctim flagellis exorienti, longiore, cum maxime extensus sit, 2–$2\frac{1}{2}$ cellulae latitudine. Periplasto induto delicatissimis squamis sculptis ellipsoidalibus, prope 0·7μ latitudine, unaecuique squamae spinulo centrali unico per quattuor juga decurrentia affixa; spinuli longitudine subaequali squamae latitudini, visibilibus solum per microscopiam electronicam.

Nucleo unico, nullo stigmate; chromatophoris ex norma 2 aut 4, nonnunquam unico aut 8, aureo-fulvis; in cellulis status motilis chromatophoris crateriformibus,

[1] For derivation and use of this term see p. 581.

ellipsoidalibus aut oblongis, parietalibus, unocuique chromatophoro parvo corpore globulari (pyrenoidali?) locato in aspecto interiore ad marginem remotam ab axi qua inserta sint flagella; chromatophoris profunde lobatis aut stellatis in cellulis status non-motilis. Cellulis oleum leucosinumque parientibus. Corporibus muciferosis ex norma conspicuosis, plerumque in ordinibus instructis, sed locatione variosa secundum mutationem formae cellulae. Nutritione holophytica et tamen phagotrophica. Non toxica piscibus.

Generanti asexualiter in statu motili per fissionem in 2 aut 4 cellulas filiolas; in statu non-motili generanti per fissionem cellularum amoeboidalium ad cellulas filiolas, parietibus praeditis, producendas, quibus potest ut cellulae status motilis liberentur.

Habitat mare ad locationem Lat. N. 49° 21′, Long. W. 04° 54′ ad summum mare (9 Mai 1950).

Description

In the motile phase this species is larger than *C. minor* (Figs. 73, 75, 77, Pl. VIII) and shows greater variation in cell-shape caused by the more pronounced metaboly of the body (Figs. 65–69) than either *C. kappa* or *C. minor*. In an actively growing culture, excluding cells in which fission has already started, 77% of the cells are from 4–7μ in diameter or length, 7% are from 3·5 to 4·0μ and the remaining 16% are early fission stages (including double-fission stages) from 7 to 11·5μ.

The flagella, $2\frac{1}{2}$–$3\frac{1}{2}$ times the body diameter in length, are fairly robust and can be seen quite clearly under the light field, although they tend to be thrown off from the body under light and dark ground fields even more rapidly than those of *C. minor*. The haptonema also shows up very clearly under the light

Legends to Text-figs. 65–72

Chrysochromulina brevifilum n.sp. (× 5000).

Fig. 65. Individual swimming with flagella and haptonema behind body, in the position characteristic for the species during rapid swimming; chromatophores dividing. *c*, chromatophore; *f*, flagellum; *h*, haptonema; *l*, leucosin vesicle; *m*, muciferous body; *n*, nucleus; *p*, pyrenoid-like body; *ss*, spined scales.

Fig. 66. Anchored cell swaying round in circles on nearly fully extended haptonema; chromatophores broadening preparatory to division, 4 pyrenoid-like bodies; small mass of graphite just ingested at non-flagellar pole.

Fig. 67. Individual anchored by haptonema which is hardly extended; cell elongated by metaboly: large mass of ingested graphite (*g*) at non-flagellar pole.

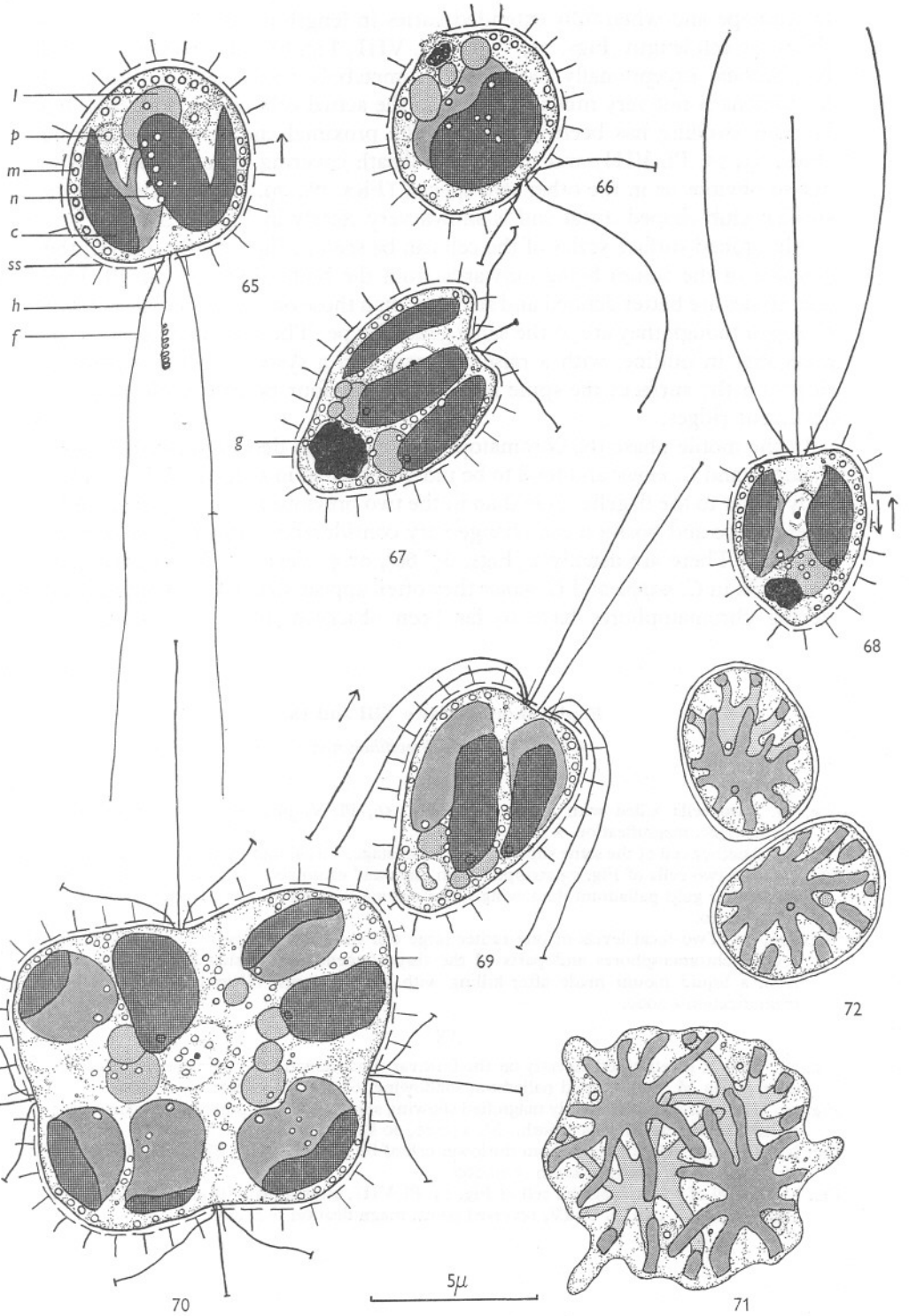
Fig. 68. Individual swimming with flagella and haptonema in front of body, haptonema fully extended; ingested graphite at non-flagellar pole.

Fig. 69. Early fission stage: 4 flagella, 2 haptonemata fully extended, body elongated and chromatophores displaced by metaboly; an ingested bacterium in a vacuole at non-flagellar pole.

Fig. 70. Stage with four pairs of flagella and four fully extended haptonemata developed prior to double-fission.

Fig. 71. Large amoeboid individual with four deeply lobed pale chromatophores and four pyrenoid-like bodies.

Fig. 72. Two walled daughter-cells from which motile stage is probably liberated; each with two pale stellate chromatophores.



Text-figs. 65–72.

microscope and when fully extended varies in length generally from $1\frac{1}{2}$ to 2 times the cell length (Figs. 73, 75, 77, Pl. VIII; Fig. 68); if, however, the cell has become exceptionally elongated by metaboly then the fully extended haptomena is not very much longer than the actual cell length (Fig. 69). No bulbous swelling has been detected at the proximal end of the haptonema (Figs. 73, 75, Pl. VIII) and its delicate sheath covering appears not so wide nor so obvious as in the other two species (Figs. 78, 79, Pl. IX); the slightly swollen club-shaped distal end is shown very clearly in Fig. 78, Pl. IX.

The spined surface scales of the cell can be seen in Figs. 78–81, Pl. IX, the position of the spines being outwards from the body (Figs. 78–79, Pl. IX). The spines are better defined and stronger than those on the few polar scales of *C. kappa* though they are of the same general type. The scale itself is ovoid to polygonal in outline, with a raised margin and a system of fine sculptural ridges on the surface; the spine is subtended by four powerful, buttress-like decurrent ridges.

In the motile phase the chromatophores are not so deeply pigmented as in *C. kappa* and *C. minor* and tend to be more elongated in shape and to lie in the body closer to the flagellar pole than in the two previous species (Figs. 65, 67). Their shape and position can change very considerably with the metaboly of the body. There are usually 2 (Figs. 65, 66), or 4 (Figs. 67, 69), occasionally 1 or 8. As in *C. kappa* and *C. minor* they often appear striated. No individuals lacking chromatophores have so far been observed in *C. brevifilum*. The

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Explanation of Plates VIII and IX

Chrysochromulina brevifilum n.sp.

VIII

Fig. 73. Two cells killed with iodine as in Fig. 56, Pl. V, photographed dry on glass. Visual light, magnification × 1000.

Fig. 74. Another cell of the same showing a division stage. Visual light, magnification × 1000.

Fig. 75. The two cells of Fig. 73 stripped from glass and examined with the electron microscope after gold palladium shadowing. Electron micrograph M. 185·1, 60 kV, magnification × 3000.

Figs. 76, 77. Two focal levels of one rather large cell showing an ingested graphite pellet, several chromatophores and parts of the three appendages. Visual light photograph from a liquid mount made after killing with osmic vapour, oil-immersion objective, magnification × 2000.

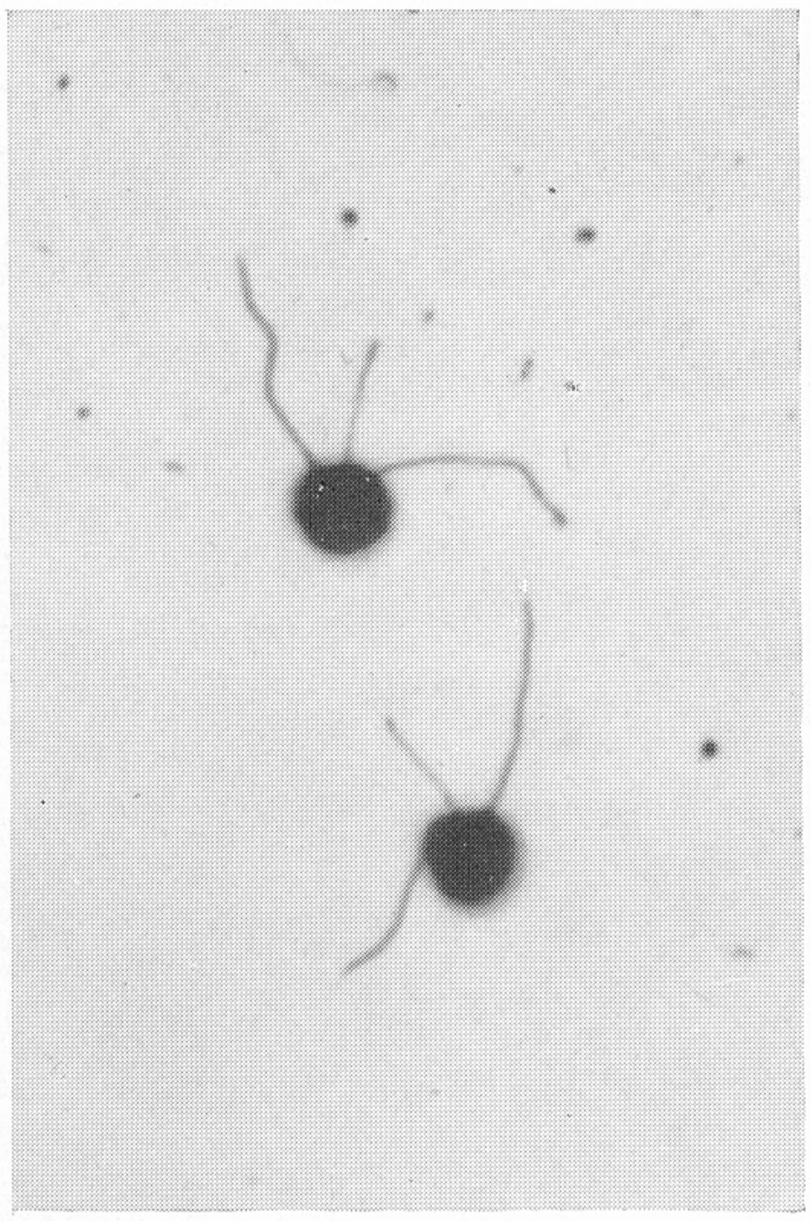
IX

Fig. 78. Part of a cell killed directly on the formvar film showing the haptonema. Electron micrograph M. 150·17, gold palladium shadowing, 60 kV, magnification × 5000.

Fig. 79. A similar cell more highly magnified showing spined scales in position and part of the coiled haptonema with its sheath. M. 150·20, 60 kV, magnification × 10,000.

Fig. 80. Reversed print of scales from the lower cell of Fig. 75, Pl. VIII. Electron micrograph M. 183·4, 60 kV, magnification × 20,000.

Fig. 81. Detail of scales from the cell of Fig. 74, Pl. VIII, after transfer from glass. Electron micrograph M. 183·10, 60 kV, reversed print, magnification × 20,000.



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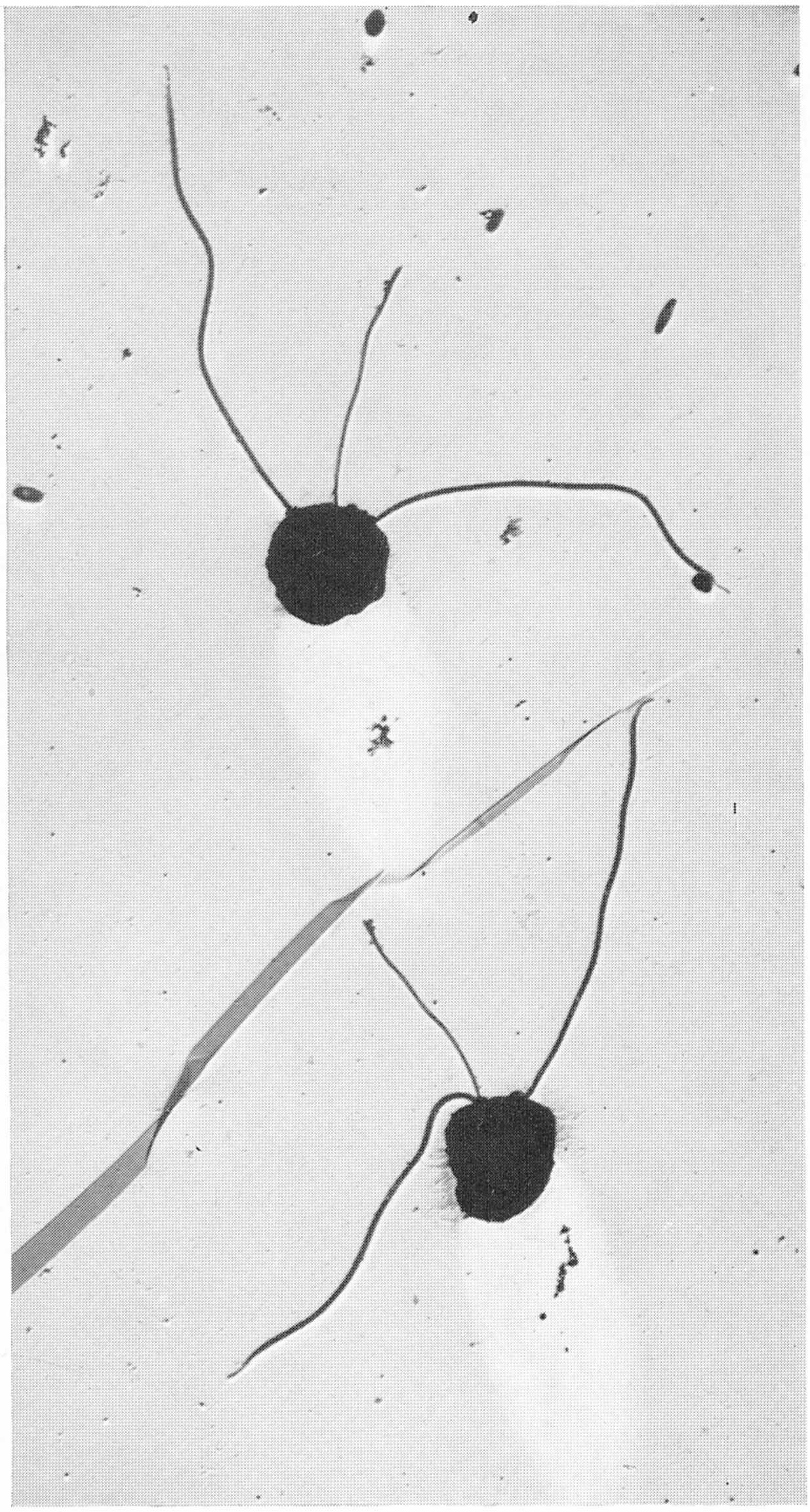

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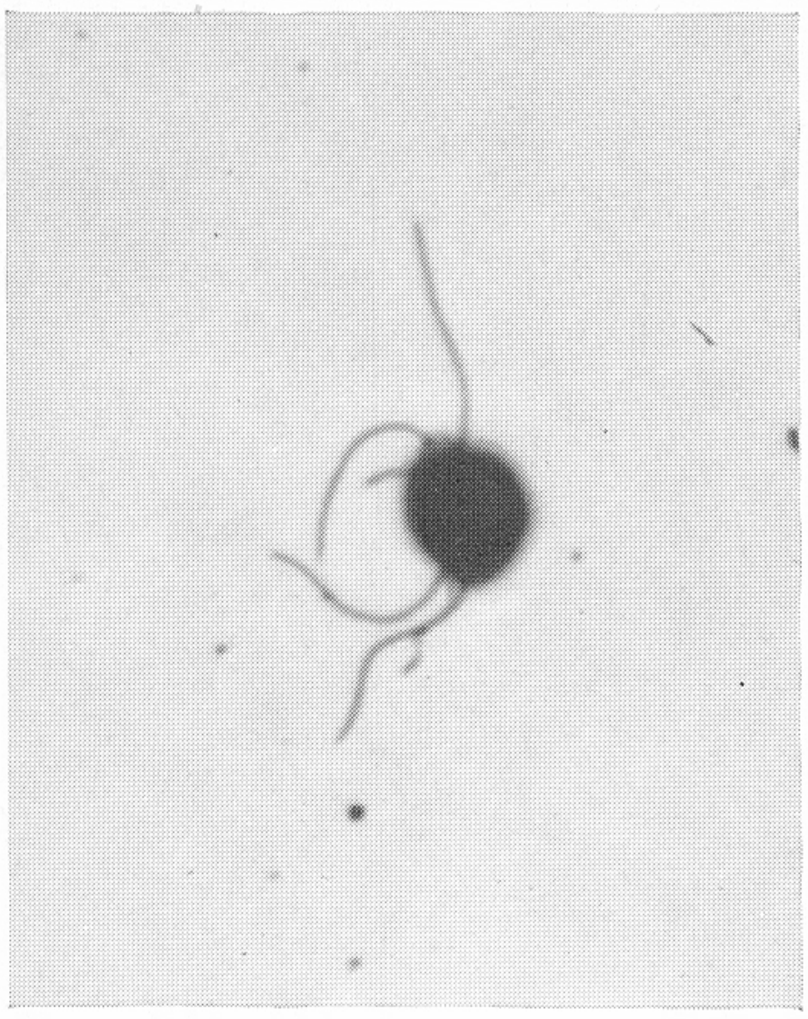

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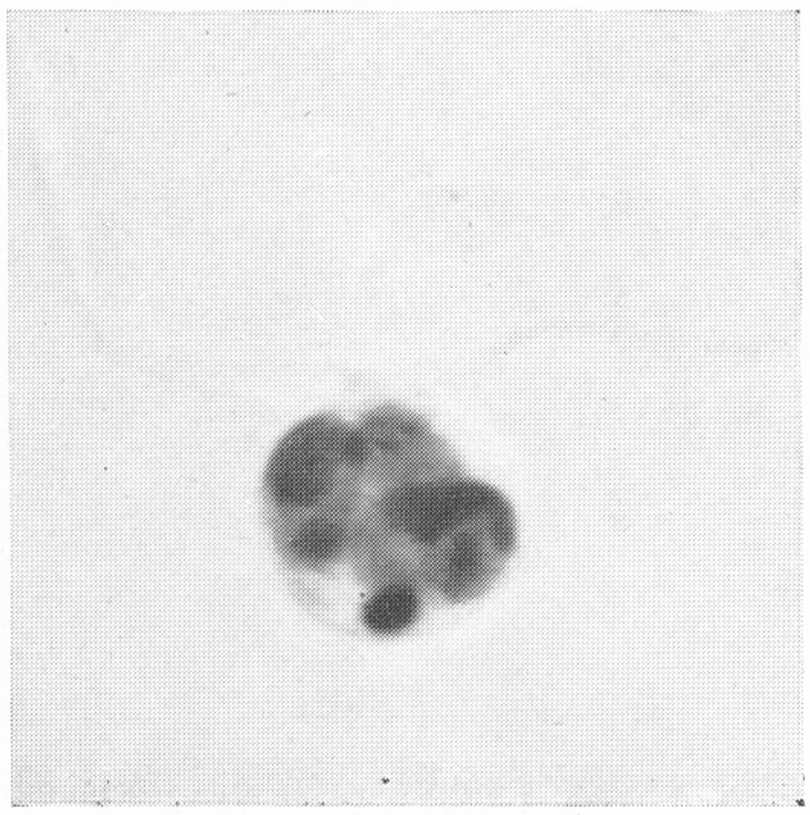

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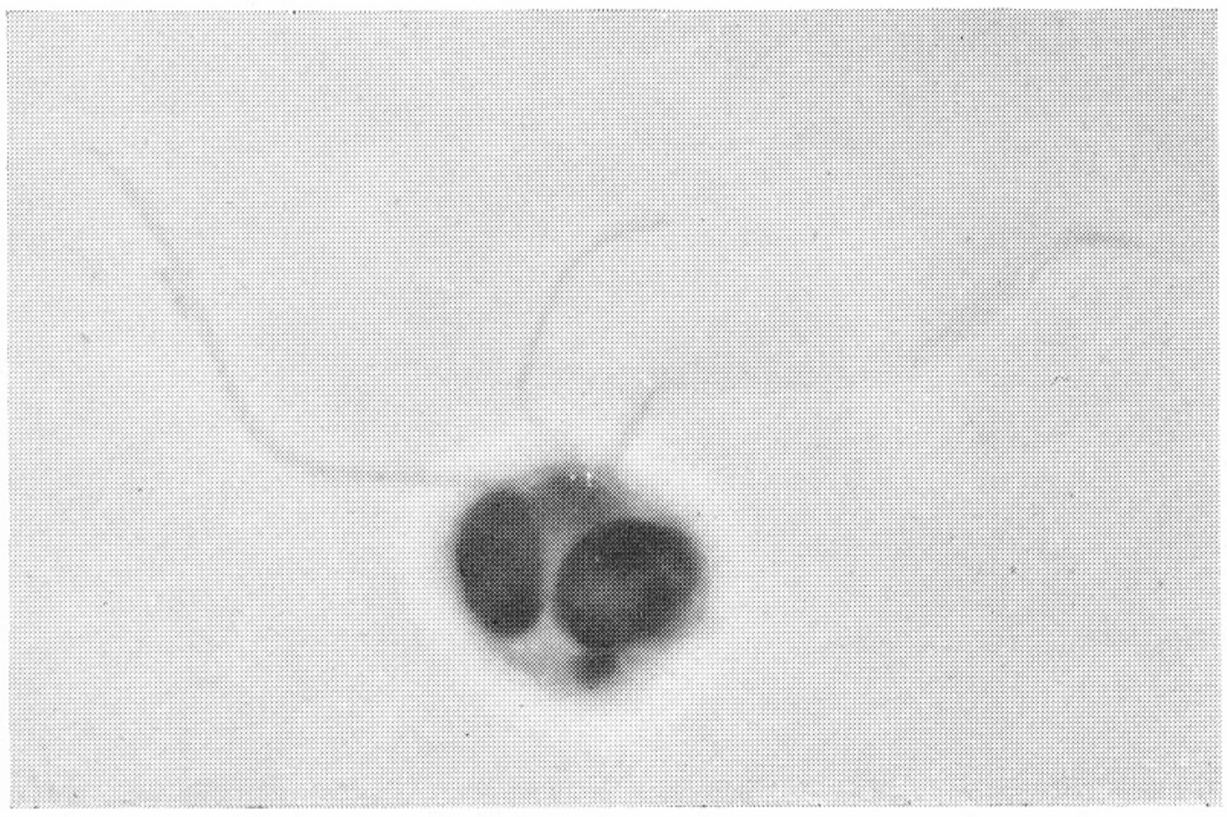

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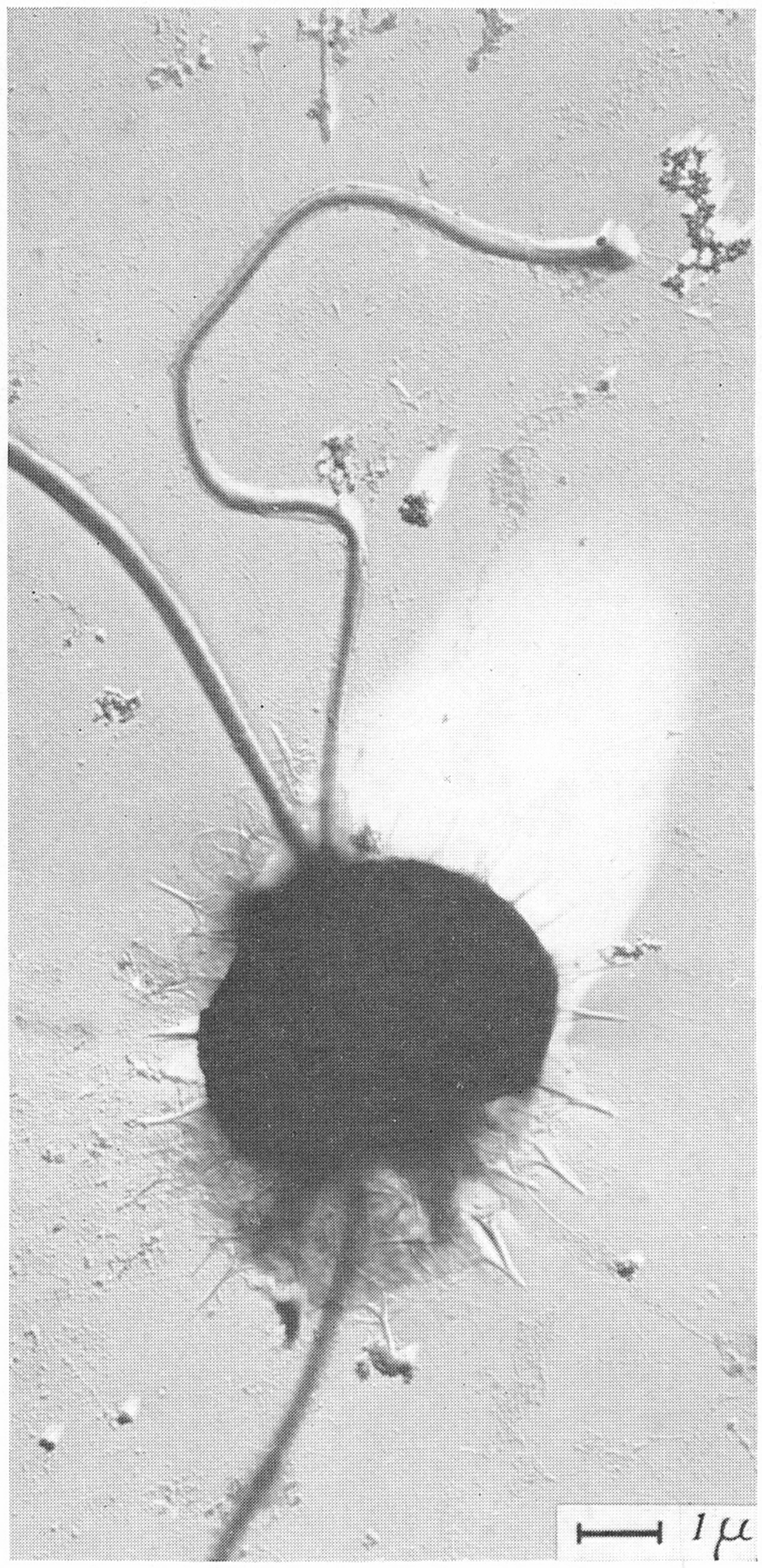

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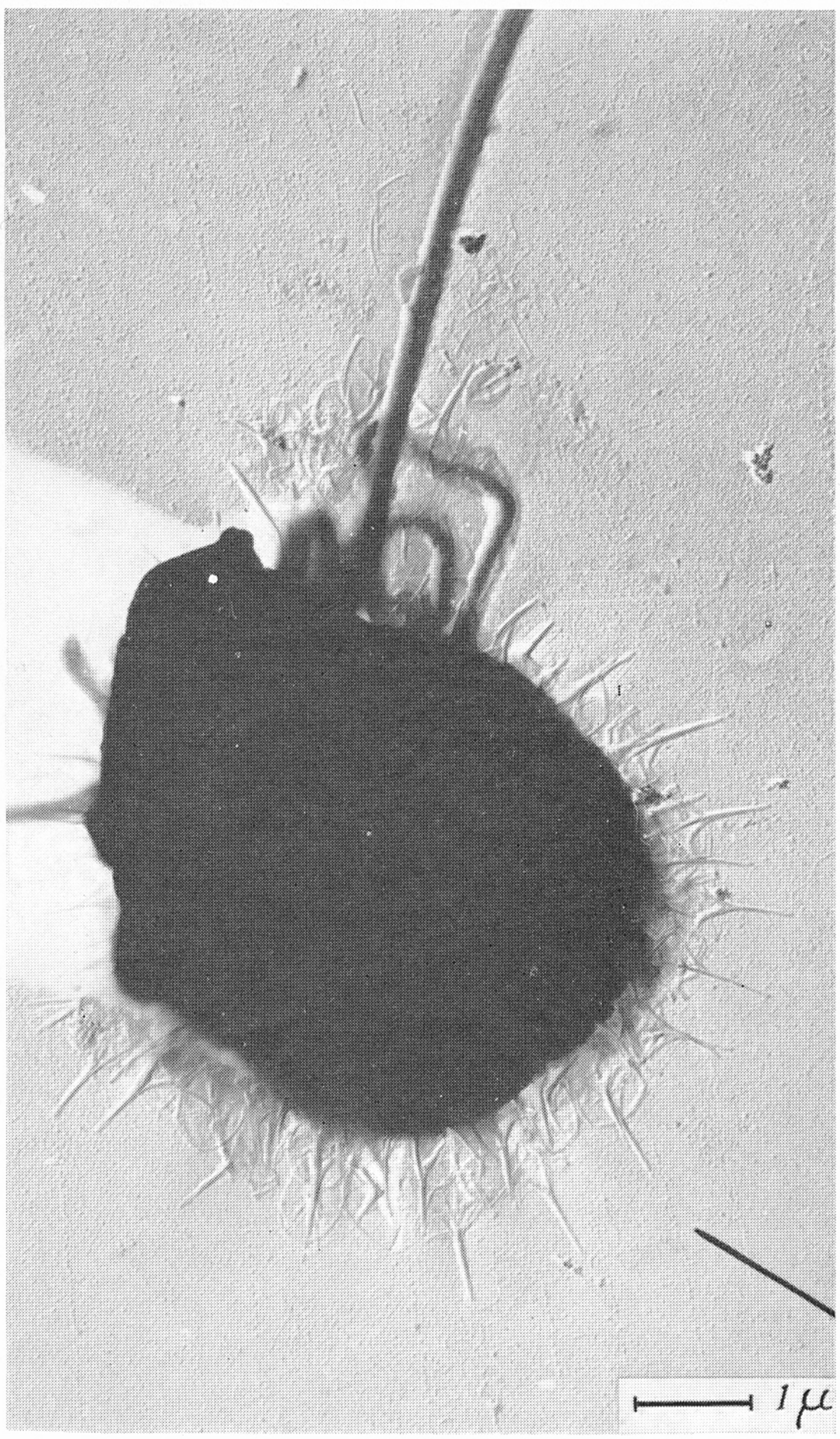

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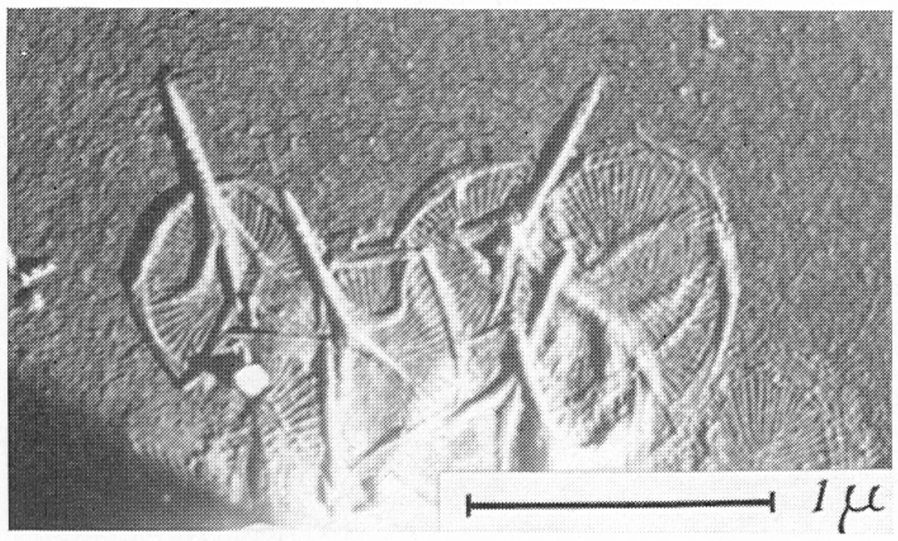

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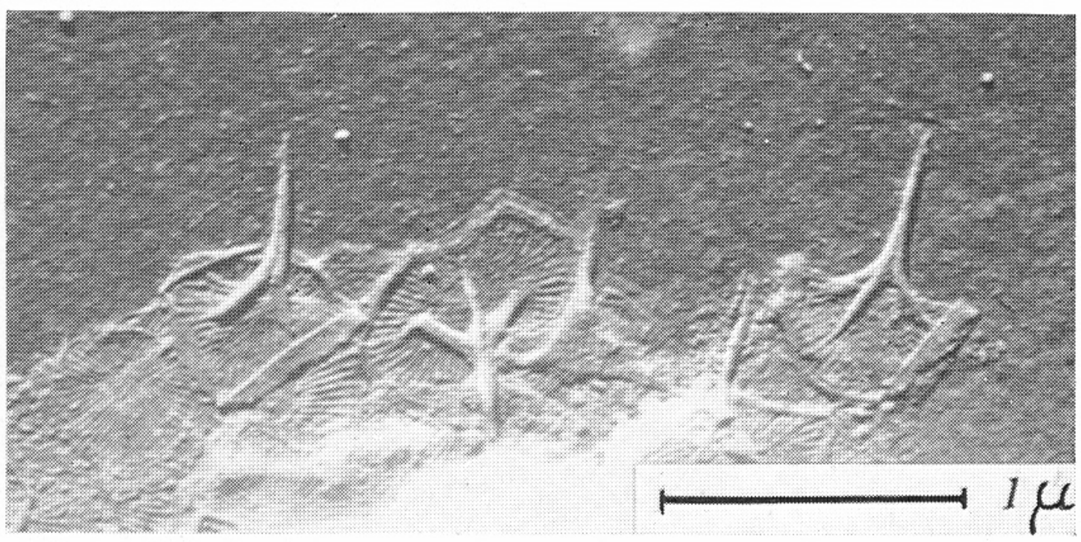

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pyrenoid-like bodies also react in the same way to stains and Millon's reagent as do those of *C. kappa* and *C. minor*. They are small and are excentrically placed near to the periphery of a chromatophore, as in *C. minor*, but in *C. brevifilum* they lie on the side away from the flagellar pole (Figs. 65–69). They are frequently surrounded by non-refringent material (Fig. 65).

The position of the nucleus, leucosin vesicles and oil globules is as in *C. kappa* and *C. minor*. The muciferous bodies are much larger and more conspicuous in *C. brevifilum* than in the two previous species. When the cell body is more or less sphaeroidal they appear to lie in the peripheral cytoplasm in four rows, running from the flagellar pole to the opposite pole, with additional ones at the non-flagellar pole (Fig. 65). Their position, however, does not remain constant but changes with the metaboly of the body (Figs. 66–69). When they discharge their contents rapidly, short rods are ejected, but if discharge is slow the contents ooze out to form a balloon which eventually becomes detached from the cell as a sphere which can remain visible for some considerable time.

In this species granules, possibly mitochondria, lying in the same position as the muciferous bodies, again stain bright blue with cresyl blue.

As in the two previous species, the flagella and haptonema are usually directed backwards when the cells are moving most rapidly; the flagella are not splayed out from the body as in *C. kappa*, but lie rather close together (Fig. 65) as in *C. minor*; the haptonema can be coiled up close to the body, partly extended, or sometimes, when movement slows down, even wholly extended straight out behind the body.

This species does not swim very rapidly. It has a slowish rolling movement rotating and gyrating, moving only for short distances; it then slows down, almost stops and in a very short time shoots off again with almost a jump usually in a different direction. This jumping type of movement is seen frequently: a cell jumps or jerks a short distance, stops, then jumps again and stops and can continue doing this for several minutes. This type of action is probably connected with a sweeping movement of the flagella characteristic of this species. The flagella lying either out from the body, or close to the body (Fig. 69), appear to stiffen suddenly and they are then brought close together into the position shown in Fig. 68 by a quick sweeping movement. This quick movement causes the cell to jump. Swimming with the flagella and haptonema in front of the body (Figs. 68–69) is seen more often than in *C. kappa* and *C. minor*. As in the two previous species, a marked phototactic response is shown although again there is no obvious stigma.

Phagotrophy occurs commonly in *C. brevifilum*. The cells readily ingest bacteria or graphite up to a size of 2μ, occasionally up to $2{\cdot}5\mu$, in the same manner (Figs. 76, 77, Pl. VIII; Figs. 66–69) as described for *C. kappa*; small cells $4{\cdot}5\mu$ in length have been found to contain graphite masses as large as 2μ in diameter. In this species the ingested material always remains very close to

the non-flagellar pole (Figs. 66–69) and apparently is not moved about inside the body as in *C. minor*.

Temporary attachment of the cells by means of the haptonema (Figs. 66, 67) takes place in a similar manner to that described for *C. kappa*. The usual dancing of the body occurs when the attached haptonema is very little extended (Fig. 67) and the cell, as in the previous species, could then be mistaken for a *Prymnesium*. The exact details of configuration of the coiled part of an attached haptonema are more difficult to determine than in the two previous species as the body usually obscures the whole organ; when seen, however, it appears to be coiled in a flat spiral as described on p. 591. When the attached haptonema is fully extended (Fig. 66) the cell can frequently be seen to swing round in a circle from the central anchoring point.

Fission in the motile stage (Fig. 74, Pl. VIII) can be equal or slightly unequal and is similar to that described for the two previous species, except that here the two new flagella and the second haptonema tend to be formed before there is any broadening of the cell preparatory to the actual fission (Fig. 69). Double-fission stages (Fig. 70), $8{\cdot}5 \times 10{\cdot}5$ to $9{\cdot}0 \times 11{\cdot}5\mu$, are seen much less frequently in this species than in *C. kappa* and *C. minor*. When seen, however, the four haptonemata are sometimes fully extended as shown in Fig. 70.

As in the two previous species the motile phase, after the peak of growth has been reached (1 to 2 million cells per ml.), eventually settles on the bottom of the flask and passes into the amoeboid phase, the cells of which develop finely lobed, very pale chromatophores (Fig. 71). In this species, as in *C. minor*, the products of the first fission can either remain in the parent membrane or become free. The second fission is usually equal, but can be slightly unequal. The walled daughter-cells (Fig. 72), produced by this fission, are usually ovoid with one side rather flattened; they range in size from $2{\cdot}5 \times 3$ to $4 \times 5\mu$. Empty walls from which the motile stage probably has been liberated have so far not been seen in this species. As in *C. kappa* and *C. minor*, if fresh culture-solution is added to a flask containing only the non-motile stage, the motile stage is produced and the skin disappears at approximately the same rate as in *C. kappa*.

Discussion

It is obvious that many details of structure, behaviour and life history are still uncertain in all of these species, some of which can be clarified only by much further work. A conspicuous omission, which we hope shortly to remedy, is a report on the micro-anatomy of the cells and their appendages by means of thin sections and the electron microscope. Had we delayed the present description until this side of the programme could be completed, however, it would have held up publication of existing facts inconveniently long, at

the risk also of producing a communication too large for publication in one piece.

On the experimental side we have not so far succeeded in observing the actual process of production of the motile phase from the dormant non-motile phase, nor have we demonstrated any process of sexual reproduction in these forms though negative evidence does not exclude it. There is indeed suggestive evidence, from the apparently invariable production of the small, walled daughter-cells in fours that here, if anywhere, is the place to look for some more direct indication of either a sexual fusion or a meiotic process without which these stages cannot be further interpreted.

With regard to that most characteristic organ, the *haptonema*, we can by no means yet specify its full biological significance in spite of the care with which its behaviour has been examined. That the detection of phagotrophic feeding was an immediate consequence of this examination can perhaps too easily suggest that there is a causal connexion. Such a suggestion admittedly is strengthened by finding that phagotrophic feeding is in fact practised by all our new organisms in which a haptonema has been demonstrated, and it was also reported in the analogous case of *Prymnesium saltans* by Conrad (1941). We cannot, however, state that a haptonema or analogous organ is essential to such feeding since this is also practised by the non-motile amoeboid stages and by other flagellates lacking the organ, notably some freshwater species of *Ochromonas* (Lund, 1942; Pringsheim, 1952) and *Chromulina* (Hutner & Provasoli, 1951). The reasons for the apparent association of a haptonema with phagotrophic feeding in the marine plankton are therefore not as self-evident as might have been expected and such description as we have so far been able to give of its behaviour has not yet provided the complete answer.

The formidable task of a biochemical analysis of the nutritional needs with respect to which phagotrophy is practised has been outside our scope of inquiry, though it would obviously be a matter of the very greatest interest if it could be undertaken as successfully as in recent work on *Ochromonas* (Pringsheim, 1952; Hutner, Provasoli & Filfus, 1953; Hutner & Provasoli, 1955). Another field in which we still lack information concerns the relative frequency of these organisms in the sea. We hope at a later stage, when a greater number of the commonest flagellates have been isolated and described, to attempt to obtain some quantitative figures for their frequency. At present, however, the undescribed species are so numerous that a quantitative estimate of individual species is scarcely obtainable. The most that we can state is that this type of organism is abundant both in species and in individuals, previous failure to detect them being solely due to their fragility and not at all to their rarity. In the total metabolic turnover in the plankton as a whole, therefore, they cannot fail to be playing an integral and perhaps an important part.

SUMMARY

Diagnoses and descriptions are given of three new species of marine plankton flagellates in the class Chrysophyceae. All possess two equal, homodynamic flagella and a third filiform appendage to which the name *haptonema* has been given. Phagotrophic feeding has been demonstrated in all. The descriptions include structural details visible only with the electron microscope as well as observations on behaviour and life-history visible only in the living material. The reasons for the use of the generic name *Chrysochromulina* Lackey are discussed in a preliminary way.

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