I. MANTON AND B. S. C. LEADBEATER

Fine-structural Observations on six Species of *Chrysochromulina* from Wild Danish Marine Nanoplankton, including a Description of *C. campanulifera* sp. nov. and a Preliminary Summary of the Nanoplankton as a Whole

Det Kongelige Danske Videnskabernes Selskab
Biologiske Skrifter **20, 5**

Kommissionær: Munksgaard
København 1974

DET KONGELIGE DANSKE VIDENSKABERNES SELSKAB **udgiver følgende publikationsrækker:**

THE ROYAL DANISH ACADEMY OF SCIENCES AND LETTERS *issues the following series of publications:*

	Bibliographical Abbreviation
Oversigt over Selskabets Virksomhed (8°) *(Annual in Danish)*	Overs. Dan. Vid. Selsk.
Historisk-filosofiske Meddelelser (8°) Historisk-filosofiske Skrifter (4°) *(History, Philology, Philosophy, Archeology, Art History)*	Hist. Filos. Medd. Dan. Vid. Selsk. Hist. Filos. Skr. Dan. Vid. Selsk.
Matematisk-fysiske Meddelelser (8°) Matematisk-fysiske Skrifter (4°) *(Mathematics, Physics, Chemistry, Astronomy, Geology)*	Mat. Fys. Medd. Dan. Vid. Selsk. Mat. Fys. Skr. Dan. Vid. Selsk.
Biologiske Meddelelser (8°) Biologiske Skrifter (4°) *(Botany, Zoology, General Biology)*	Biol. Medd. Dan. Vid. Selsk. Biol. Skr. Dan. Vid. Selsk.

Selskabets sekretariat og postadresse: Dantes Plads 5, 1556 København V.

The address of the secretariate of the Academy is:

Det Kongelige Danske Videnskabernes Selskab,
Dantes Plads 5, 1556 København V, Denmark.

Selskabets kommissionær: MUNKSGAARD's Forlag, Nørre Søgade 35,
1370 København K.

The publications are sold by the agent of the Academy:

MUNKSGAARD, *Publishers,*
35 Nörre Sögade, 1370 København K, Denmark.

I. MANTON AND B. S. C. LEADBEATER

Fine-structural Observations on six Species of *Chrysochromulina* from Wild Danish Marine Nanoplankton, including a Description of *C. campanulifera* sp. nov. and a Preliminary Summary of the Nanoplankton as a Whole

Det Kongelige Danske Videnskabernes Selskab
Biologiske Skrifter **20**, 5

Kommissionær: Munksgaard
København 1974

CONTENTS

Page

Introduction 3
Material and methods 4

Microanatomy of the selected *Chrysochromulina* species:
1. *C. ephippium* Parke and Manton 5
2. *C. herdlensis* Leadbeater 7
3. *C. aff. fragilis* Leadbeater 8
4. *C. bergenensis* Leadbeater 9
5. *C. mantoniae* Leadbeater 11
6. *C. campanulifera* sp. nov. 14

Discussion 16
Summary of observations amplifying or emending type descriptions previously published 21
Summary of identified taxa of nanoplankton from the sea near Frederikshavn in the month of July (1971, 1972) 22
Bibliography 25

Synopsis

A list of 60 odd species of small flagellates, most of them not previously recognised in Danish waters, has been compiled from marine nanoplankton* collected near Frederikshavn, Jutland. Excluding peridinians and cryptomonads which have been ignored, the main phyletic groups represented are: Haptophyceae (brown), Prasinophyceae (green) and Choanoflagellatae (colourless), as in other European seas.

Five of the six species of *Chrysochromulina* dealt with in greater detail have been selected because they contribute important new information on microanatomy not available in the type descriptions based on whole mounts; the sixth species (*C. campanulifera* sp. nov.) is described for the first time mainly on the basis of sections. Among the six taxa, three different kinds of pyrenoid have been recognised, one of which (in *C. herdlensis* and C. *aff. fragilis*) is new for the genus. New information on scale structure and arrangement based on sections has amplified the taxonomic descriptions of all six species with respect to the degree of concavity or convexity of plate scales and the presence or otherwise of additional periplast components, notably a skin beyond the scales on *C. bergenensis* and columnar material under or upon the scales in *C. herdlensis*, *C. aff. fragilis*, and *C. bergenensis*. In the light of fuller knowledge of the complex morphology of the large spines of *C. mantoniae* in different parts of the northern and southern hemispheres, the diagnosis of this species has been emended with respect to the shape of the spine tips. Emendations have also been shown to be needed for measurements of protoplast sizes previously given for four species and a procedure is recommended for estimating body sizes more accurately if these have to be based on whole mounts in the absence of fresh or embedded material. Information on haptonema substructure and on phagotrophic feeding is provided for *C. ephippium*, *C. herdlensis*, *C. bergenensis*, *C. mantoniae* and *C. campanulifera*, food vacuoles being described for four species and demonstrated in two (*C. bergenensis* and *C. campanulifera*). Virus infection is demonstrated in *C. mantoniae* and compared with the only other known susceptible species present in the samples *Coccolithus huxleyi*. These findings are discussed in a preliminary way both taxonomically and biologically with respect to the nanoplankton as a whole.

* This spelling has been adopted instead of the equally correct and more usual double *n*, in order to conform to the recently established usage in the physical sciences for standard units of measurement such as *nanometre*.

BIANCO LUNOS BOGTRYKKERI A/S
PRINTED IN DENMARK
ISBN 87 7304 0274

Introduction

This paper is based on wild Danish nanoplankton processed directly from the sea during one week in early July in two successive years. Most of the six species considered in detail are common near Frederikshavn, Jutland, at least in summer. Several, if not all, are also widespread having been recorded elsewhere as noted individually under each. Many other species of *Chrysochromulina* are of course associated with these (see list pp 22–3) in the natural populations sampled, but some are so well known that it is sufficient merely to record their presence while others require further study, preferably with the aid of cultures, before they can be adequately understood. Yet others will be described elsewhere since it will be necessary to draw on material from different sources including, but not confined to, Denmark. Those that remain share the condition that their microanatomy has not previously been studied although external morphology as ascertainable from whole mounts may already have been recorded for purposes of taxonomic diagnosis and naming.

Wherever possible, the new observations include information on haptonema microanatomy, more especially with respect to the number of component microtubules, on pyrenoid substructure and on scale structure and arrangement. In addition we have information on phagotrophic feeding (found in four of our six species) and on virus infection (one species) under natural conditions in wild material.

In recording our results, we would have liked to adopt a uniform system of magnifications applicable to all taxa but this has proved incompatible with the range of sizes involved. We have nevertheless been at pains to provide at least one micrograph of a cross section of each species, supplemented by details of its periplast, at standard magnifications of 10,000 and 30,000 respectively, to assist direct comparisons, no matter how many other magnifications may also have been introduced.

Finally, in a concluding section, we have listed our main taxonomic findings for the nanoplankton samples involved in the investigation taken as a whole, omitting for this purpose the better known groups (peridinians, cryptomonads and diatoms) some of which will always slip through the finest nylon mesh intended to remove them. Since the list necessarily includes the authorities responsible for specific names we have not felt it necessary to introduce them elsewhere in the text except in major headings or in the actual citation of literature. That relatively few of the 60 odd taxa contained in the list have previously been recorded from Denmark is a measure of their predominantly small size and the difficulty in many cases of attaining exact identifications without the aid of electron microscopy.

Material and Methods

The material has mainly been derived from freshly gathered seawater samples, concentrated either centrifugally or by means of a filter before being processed in the usual way for whole mounts or sections. The initial processing was carried out in the Marine Laboratories of the University of Copenhagen at Frederikshavn, Jutland, where facilities were kindly made available to us for a week in July 1971 and for 3 days in July 1972. All the samples were obtained within a few kilometres of the laboratory, in most cases with the aid of a boat and a small hand sampler adjusted to collect water from a depth of 10 metres.

Fixation was by means of osmium tetroxide either used as vapour for some of the whole mounts or as a 2% solution in 0.1 molar cacodylate buffer at pH 7 applied for $^1/_2$ hour or less for material intended for embedding. The latter was into epon by standard procedures.

Further processing was completed in England. The whole mounts which had been made directly onto carbon coated grids were merely rinsed and shadowcast before being examined. In two cases (*C. ephippium* and *C. campanulifera*) material that had been dried onto glass slides for light microscopy was stripped and transferred to electron microscope carriers. The method of doing this involves a stripping film derived from a 2% solution of cellulose nitrate in amyl acetate. A drop of this is put onto the surface to be stripped which will already have been moistened with the pure solvent. When the film has dried it can be strengthened if necessary by adding a second drop. When finally dry, the chosen area is circumscribed with the point of a needle and allowed to float off by immersion in water. It can then be manoeuvred onto any other desired surface such as that of a coated grid to which it will adhere on drying. The stripping film is then dissolved off in amyl acetate leaving the specimen attached to the new surface. When again dry it can be shadowcast and examined.

Sections of embedded material were cut in the usual way with a diamond knife on an LKB microtome, mainly in Leeds. They were mounted on carbon films and double stained with uranyl acetate (4% applied for 20 min) followed by lead citrate (10 min).

Cultured material was available for only one species, *C. ephippium* Parke & Manton. This had been described in 1956 (Parke, Manton & Clarke) before the advent of thin sectioning and only embedded subsequently using methods that are now obsolete. The type culture has since died out but is still represented by this embedded material. Fixation had been for 1 h in 2% osmium tetroxide dissolved in acetate veronal buffer at pH 7 followed by methacrylate embedding. New sections were cut from the old blocks and stained for 5 min with lead citrate. They proved adequate for determining the number of microtubules in the haptonema (Fig. 8) which was confirmed by some better fixed material embedded in epon, from a more recent isolate from the English Channel (not reproduced).

Several different microscopes have been used for the observations as indicated in the legends. Two micrographs from the earlier work on cultures of *C. ephippium* had been taken before Sept. 1969 with the Siemens Elmiskop I and the A.E.I. EM6B microscopes in the Botany Department of Leeds University. Subsequent work on the shadowcast whole mounts from Denmark mainly involved an EM6 microscope in the Botany Department of Birmingham University. Stripped material was processed and examined in Lancaster University using an A.E.I. 801 microscope. For sections, the microscopes used included a Siemens Elmiskop IA at Carlton University, Ottawa, two A.E.I. 801 microscopes, respectively located in the University of Lancaster and the Culture Collection of Algae and Protozoa, Cambridge, three A.E.I. EM6B microscopes respectively in the Zoology Department of Leeds University, the Botany Department at Imperial College, London, and the Cell Biology Unit in the University of Nottingham. Finally one critical but oblique section (the haptonema of *C. herdlensis*) was successfully analysed with the aid of a tilting stage on a Philips EM 300 in the Biophysics Department of Leeds University.

Acknowledgements

Grateful thanks are due to the owners and other users of all the electron microscopes listed above. We are also grateful to the Universities of Birmingham and Leeds for ancillary laboratory facilities and to the Science Research Council for a small grant. Without the hospitality of and help from the University of Copenhagen, including Dr. T. Christensen and his staff, the initial collecting could not have been carried out. Finally we are indebted personally to K. Oates for technical help, more especially in the stripping of dried specimens from glass, and to Dr. T. Christensen for help with the Latin description of *C. campanulifera*. To all these persons and institutions we offer our best thanks.

Microanatomy of the selected *Chrysochromulina* species

1. *C. ephippium* Parke & Manton. Figs. 1–8 on Plate I.

This very common species, originally described from an isolate from the English Channel before the advent of thin sectioning (Parke, Manton & Clarke 1956) is characterized by a cell of moderate size for the genus, associated with an extremely long haptonema and a characteristic combination of spiny and spineless scales. As encountered by us in the Kattegat (Deget and Jerup) and North Sea (Hirtshals) the cell size, as measured in sections ranged, from 8×8 μm to 6×10 μm, the shape of such sections thus varying from approximately isodiametric (cell C in Fig. 1) to extremely anisodiametric, all features in close agreement with the type description. The very long haptonema is sufficiently demonstrated by Fig. 5 which represents one of several cells stripped from glass, each with substancially more than 20 gyres in the helix,

obtained from a gathering near Deget, Frederikshavn in July 1972. The scales illustrated from a whole mount in Fig. 4 obtained from the same locality in July 1971 compare closely with those of the type culture (loc. cit. 1956).

Further details about these and other features are best ascertained from sections. The microanatomy of the haptonema in the type culture (Fig. 8) is that normal for the genus, namely, a ring of 7 microtubules surrounded by three concentric membranes, the two inner membranes enclosing a space, here dilated as a fixation artifact. The apparent emptiness of the centre within the ring of microtubules is also an artifact denoting loss of material following imperfect fixation as explained on p. 4. It is perhaps important to draw attention explicitly to this fact since a central cavity (sometimes postulated in review articles) if present in life would appear membrane bounded. This is never the case, the true haptonema cavity being peripheral with respect to the microtubules.

The nature of the pyrenoid is indicated in Figs. 6 & 7. Each of the two plastids possesses a conspicuous pyrenoid sunk in the substance of the plastid and with the storage area traversed by a single paired thylakoid, as recently described for *C. megacylindra* Leadbeater (Manton 1972b).

The scales were described accurately and in some detail by Parke, Manton & Clarke 1956, though their relative positions on the cell could not at that time be directly observed. On the other hand, the presence of spines which could scarcely be other than outwardly directed, permitted recognition of the separate identities of dorsal and ventral surfaces of the subtending plates, expressed in terms of their characteristically different patterning (radiating ridges on one face and concentric lines on the other), to be deduced in this species for the first time. Sections nevertheless add more than the details that were known to be undetermined. A vertical section such as that of Fig. 2 shows that, while spined and spineless scales are separately arranged in two close-set superposed layers, the plates themselves differ in unexpected ways. Those subtending spines appear flat or slightly convex, each being adnate to the four struts and with no more than a narrow erect rim at the margin. The spineless plates on the other hand are not only more broadly rimmed and larger but are deeply concave with the rims strongly inflexed, features which could not have been accurately inferred from dried specimens (Fig. 4). That both scale layers are close-packed laterally can be seen in a tangential section such as that of Fig. 3, as well as in the vertical section of Fig. 2.

Finally phagotrophic feeding, known to occur in culture (loc. cit. 1956) was confirmed by many examples of food vacuoles containing pellets of detritus up to 2 μm across, in wild material.

This species was common on both sides of the tip of Jutland and was also met with in Norway (Leadbeater 1972b) and in the English Channel. Outside these waters it occurs in the Adriatic and Mediterranean Seas (Leadbeater unpub.), South Africa and West Greenland (Manton unpub.), the latter with a sea temperature of 4°C as opposed to 17°C in Denmark in July 1972.

2. *C. herdlensis* Leadbeater Figs. 9–16 on Plates II and III.

This rather small-celled species, originally described from Norway (Leadbeater 1972a), was represented in Denmark by two types of cell, differing in the presence or absence of columnar material upon and beneath the scales. We have not given taxonomic recognition to this difference since both types agree in other respects; we have nevertheless thought it wise to separate the micrographs of the two types onto different plates.

When seen in section, the body sizes of both cell types appear somewhat larger than when measured on dried material. Thus instead of 4×3 μm (Leadbeater 1972a) we have sections ranging from 6×5 μm to 7×7 μm. In other respects, notably in the scale and haptonema characters (Figs. 9, 10, 15) agreement with the type description is close.

The short haptonema with only a limited capacity for coiling (Fig. 9) makes sections of it infrequent even through the organelle is not usually shed. The best section that we have been able to secure (Fig. 13) is oblique and only analysable by means of a tilting stage. There are 7 microtubules arranged in a compact arc with suggestions of an 8th somewhat out of line and perhaps about to terminate. The haptonema cavity (endoplasmic reticulum) is represented by two separate profiles (c) on opposite sides of the organelle. These features recall those of a normal haptonema cut near the base as in *C. chiton* or *Prymnesium parvum* (Manton 1968), though whether, in this species, a level can be reached at which the microtubules form a ring surrounded by a continuous endoplasmic reticular cavity is uncertain since we failed to find it in a series of several sections through this specimen.

The plastid in this species appears to be single, on the evidence of sections (e.g. Figs. 11 and 14). The pyrenoid is immersed, as in many other species, but in this case the storage area is traversed by several thylakoidal layers, each layer being either single or partitioned i.e. composed of one or two thylakoids (Fig. 12). We have not previously encountered a pyrenoid with this substructure in the genus *Chrysochromulina* but another example (*C. aff. fragilis*) will be found in the next section (Pl. IV).

The scales of *C. herdlensis* are characteristically oval and of three sizes namely: i) large flat or slightly concave plates with thickened margins but without upstanding rims, ii) somewhat smaller, strongly concave, plates with conspicuous inflexed rims and iii) tiny flat plates with inflexed rims. All show the usual patterns of radiating ridges on one face (proximal as in Fig. 10) and mottled or with concentric lines on the other face (distal as in Fig. 15). Sections are necessary to reveal the degree of concavity (Figs. 12 and 16) of the different types of scale as well as the details of their arrangement on the cell. The smallest scales (type iii) occupy the interstices between the next larger plates (type ii); this arrangement can also to some extent be retained in a fortunate whole mount (Fig. 10). The largest scales cover the others though perhaps discontinuously, forming a separate layer on the outer side of the periplast.

The ancillary material on and beneath the scales is very striking when present in a section (Fig. 16) though less so in a whole mount where nevertheless some of it

can often be seen (Fig. 15). The deposits are of two kinds namely, i) long wormlike columns of dense material often arranged perpendicularly to the outer surface of a subtending scale (Fig. 15) and ii) serried ranks of shorter columns of somewhat similar dense material located upon the plasmalemma and arranged with their long axes perpendicular to it. Within the cell, traces of the second type of deposit have been found within the Golgi cisternae (Fig. 16). In addition some solid masses of dense material are regularly present (arrow in Fig. 14) beyond the periplast or still contained in vesicles beneath the cell surface. The nature and mode of production of all these components of the periplast need further investigation.

Phagotrophic feeding certainly occurs in this species since both types of cell have been seen to contain food vacuoles occupied by detritus compacted into pellets of up to 3 μm in diameter.

This species was fairly common on both sides of the tip of Jutland as well as in Norway (LEADBEATER 1972a). It is even more abundant in the Mediterranean and Adriatic Seas (LEADBEATER unpub.) but has not so far been recognised elsewhere.

3. *C. aff. fragilis* Leadbeater Figs. 17–23 on Plate IV.

This relatively uncommon species is inserted here because of the close resemblance to the preceding shown by its plastid. In other respects the species is still incompletely known since, although encountered in several of our samples, it was never abundant and we have no new information on the haptonema which is at present represented only by the illustration in LEADBEATER 1972a where it was shown to be short.

Fortunately the scales usually remain firmly attached to intact protoplasts permitting recognition, while sheets of scales from dead or broken cells can also be found in whole mounts (Fig. 17) and in sections (Fig. 20). The scales are characteristically of two types, namely exceptionally large, rounded or slightly oval, very thin, rimless, plates with a pattern of ridges peripherally but patternless in the centre (Fig. 17) together with numerous small compact oval plates, each with a conspicuous central thickening and an inflexed rim. Scale distribution on the cell is such that the large plates cling closely to the cell surface becoming slightly convex (Fig. 21) and sometimes overlapping laterally while the small scales are clustered under the edges of the large scales or in the interstices between them. This arrangement is often retained after separation from the cell (Fig. 20).

There are three discrepancies from the Norwegian material on which the specific description was based (LEADBEATER 1972a). The ends of the small oval plates are more pointed and the peripheral striations on the large plates are more numerous and curved (Figs. 17 and 20). We do not propose at present to give taxonomic recognition to these details though this might eventually be needed. We have as yet no information on the haptonema in Danish material from which confirmatory evidence might perhaps have been obtained.

A discrepancy of another kind is provided by the dimensions of the cell body

since, as in *C. herdlensis*, measurements on sections, though few, are consistently higher than those based on whole mounts. Thus we have more than one set of measurements exceeding 4.5×5.5 μm (cf. Fig. 23) as compared with 3.5×3.5 μm quoted in the type description. Shrinkage on drying is the most probable reason for this difference which will be further discussed on p. 18 below.

The plastid appears to be single on the evidence of sections (confirmation from living cells is of course desirable). The pyrenoid is immersed and multilayered as in *C. herdlensis*, the layers being composed of both single and double thylakoids (Fig. 22). In one fortunate section (Fig. 19) we were able to demonstrate a nascent large scale in a cisterna of Golgi origin. Though no food vacuoles were encountered, the evidence is insufficient to rule out the possibility of phagotrophic feeding.

C. fragilis has been sparingly recorded in the Mediterranean and Adriatic Seas (LEADBEATER unpub.) as well as in Norway (LEADBEATER 1972a) and *C. aff. fragilis* was found on both sides of the tip of Jutland but it never seems to be abundant and has not, so far, been recognised elsewhere.

4. *C. bergenensis* Leadbeater Figs. 24–42 on Plates V–VII.

This very elegant small-celled species is conspicuous both in whole mounts and in sections by virtue of its smoothly rounded contour and characteristic closely fitting periplast. The haptonema (Fig. 24) is short[1] (LEADBEATER 1972a).

The scales are of two main types (Fig. 28), namely, oval rimless plates appearing flat in section and at a deeper level, a layer of smaller, strongly concave plates with inflexed rims, occupying the interstices between the larger plates (Fig. 29). Locally, some of the large rimless plates can occasionally be found to be replaced by rimmed equivalents (Fig. 28 extreme left) while close to the flagellar insertion all the rimmed plates become more crowded and smaller (Fig. 33). All the scales possess the customary differences in surface patterning, namely, radiating ridges on the proximal face (Fig. 28 extreme left and right) and relatively patternless on the distal face. A granular deposit can often be found peripherally on the rimless plates both in whole mounts (Fig. 28) and in sections (Figs. 29 etc.). In addition, sections reveal two other components of the periplast, namely a peripheral skin (see further on p. 10) and an underlying deposit of columnar bodies and subspherical dense particles or droplets located between the lower scales and the plasmalemma (Figs. 29 and Plate VII).

The haptonema, though apparently straight, or almost so in Fig. 24 and as illustrated in LEADBEATER 1972a, nevertheless almost certainly possesses some, if a limited, capacity for coiling (see Figs. 30, 32). The abrupt flexure of the tip in Fig. 24, which is also seen in the flagella is of course not coiling in this sense, but expresses a well known fixation artifact caused by local (probably post-mortem), osmotic swelling. When an example of this artifact is encountered in a section (Fig. 30, top right) it is

[1] Owing to an undetected typing error the numerals 30 instead of 8 μm appear in the type description of this species on p. 75 of that paper, although the accompanying illustration (loc. cit Fig. 47) shows clearly the true position.

dominated by the dilated haptonema cavity surrounded by the distended outermost membrane and often with several profiles of the contorted haptonema core displaced to the periphery. One such profile, from the specimen of Figs. 30 and 32 gave the micrograph of Fig. 31 in which a somewhat flattened ring of exactly 6 microtubules is demonstrated.

An entirely different kind of localized swelling has been encountered twice (Figs. 25 and 26–27). In both cases it consisted of a compact intercalary bulge filled with structures giving little or no indication of disturbance. We have unfortunately no transverse sections to compare with the longitudinal views illustrated and beyond the general statement that microtubules are present, together with profiles of apparently undistorted endoplasmic reticulum, we cannot analyse them further. There is nevertheless a suggestive resemblance to the subterminal swellings commonly found on the haptonemata of *Phaeocystis* zoids (PARKE, GREEN and MANTON 1971) and which are also of unknown functional significance. Further investigation is thus necessary in both cases before this feature can be understood.

One of the more perplexing components of the periplast is the skin. In sections it appears to be not only continuous but to possess a fairly complex substructure (see especially Fig. 36) which is quite different from that of scales; yet it seems never to be retained in whole mounts. When scale production is taking place (Figs. 37–41), fragments with identical substructure to that found in the skin outside the cell can be recognised in cisternae of Golgi origin (see especially Fig. 40), together with scales and dense materials not yet liberated to the underlayer (Figs. 37, 40 etc.). All these components of the periplast are thus of Golgi origin though they are not necessarily identical chemically. Indeed a chemical difference between skin and scales correlated with different solubilities in distilled water, unless hardened by the kind of preparative treatment involved in embedding, is the most likely explanation for the difficulty in detecting the skin directly in whole mounts. When preserved, as in sections, the skin is apparently continuous, but overlapping free edges can sometimes be detected (Fig. 34, left), while at other times (Fig. 36) an edge to edge union of plate-like subunits is sometimes also suggested.

Somewhat unexpectedly, the skin seems to be no barrier to phagotrophic feeding and an example of a cell containing detritus within a large food vacuole, or perhaps two fused vacuoles, is illustrated in Fig. 35. The skin is missing on the adjacent surface though present elsewhere on the cell. Unfortunately it is impossible in such a case to distinguish accidental surface damage from that incurred naturally during ingestion.

Apart from food vacuoles, the other cell contents are relatively compact though, as in the two preceding species, there is a difference between measurements of cell size based on sections and on whole mounts. Thus we have sectional profiles ranging from $7\times4-5.5$ μm as opposed to 4×3.4 μm cited in the type description. Even with this degree of emendation, *C. bergenensis* remains one of the smaller members of the genus *Chrysochromulina*.

The two plastids are clearly visible in a transverse section such as that of Fig. 34,

the pyrenoids being immersed and otherwise simple. Thus the storage area is only penetrated by a few unpartitioned tubes as in *C. acantha* (LEADBEATER & MANTON 1971), and other species.

C. bergenensis was present on both sides of the tip of Jutland, sometimes being very abundant locally. In addition to Norway (LEADBEATER 1972a) it has been sparingly recorded in the Mediterranean and Adriatic Seas (LEADBEATER unpub.) but has not, so far, been recognised elsewhere.

5. *C. mantoniae* Leadbeater Figs. 43–66 on Plates VIII–X and Text – fig. 1

There are several unusual features about this species, not all of them easily elucidated. The exceptionally massive scales (Fig. 48) cling tenaciously to the cell surface (Fig. 52 and Plate IX), no matter what its configuration may be (see for example the scale-lined concavity at the non-flagellar pole in Fig. 58b), a circumstance greatly assisting taxonomic recognition of protoplasts. The very large spines (Figs. 43–47) are unmistakable, whether associated with cells or seen isolated in sections though elucidation of their morphology is, if anything, impeded by their large size. Fortunately they are commonly retained in their characteristic positions near the ends of the cell, doubtless aided in this by the considerable convexity of their bases (Figs. 52 atc.) which can cling so closely to the underlying cytoplasm that the latter becomes deformed into a bulge which can still persist (Fig. 54) after a spine has been knocked off. In contrast, the flagella break easily and both have been shed from the specimen of Fig. 43 which nevertheless still retains its relatively short haptonema. There is little, if any, direct evidence of coiling in the latter.

Before considering the periplast further, it will be convenient in this case to deal first with the cell interior which is fortunately straight forward. The haptonema microanatomy is illustrated in Figs. 59 and 60; there are the usual 7 microtubules surrounded by three membranes and the relevant space. The two plastids are separately detectable in Fig. 57, possessing an immersed pyrenoid traversed only by a few simple tubes (Fig. 55) as in *C. acantha* (LEADBEATER & MANTON 1971) and *C. bergenensis*. The Golgi system (Fig. 57) shows the normal haptophycean construction though we have not, as yet, encountered scale production in it. We have likewise failed to find direct evidence of phagotrophic feeding although indirect evidence is suggested by the concavities referred to above (Fig. 58b) which are not uncommon. These recall somewhat similar appearances interpreted in *C. megacylindra* (MANTON 1972b) as temporary deformations of the surface following recent discharge of food vacuoles. In that species however, very clear evidence of detritus ingestion was also provided which is as yet absent from *C. mantoniae*. Further information on feeding mechanisms in the latter is thus essential before conclusions can safely be drawn.

Sections of the periplast reveal a number of new features, less easily, or not ascertainable from dried specimens. All the scales are rimmed and exceptionally massively constructed though they must be more pliable than their appearance in section at first seems to imply since the base plates of spines are commonly collapsed in

whole mounts (Fig. 47). The largest of the body plates are flat, oval and with blunt erect rims (Fig. 52 etc.). Smaller, slightly concave, round to oval, plates with strongly inflexed sharp-edged rims are arranged under the interstices between the larger plates. The bluntly rimmed base-plates of spines, in contrast, are not only larger than either but are also convex in all planes of vertical section. In addition, they carry four adnate ridge-like struts arranged diagonally to the main axes of the subtending plate; one strut is transected in Fig. 52 and two in Fig. 53. These struts not only support the spine but are continued up it for varying distances (Fig. 44–45) giving a winged appearance when transected at appropriate levels. Each strut seems to represent a fold in the upper layer of material forming the base-plate, the centre of the fold being clearly distinguishable within the strut when transected from almost any direction (Figs. 50 and 52). The surface patterning, visible after shadowing on all types of scale, consists of the usual radiating ridges proximally and concentric lines distally (Fig. 48). On the other hand when seen in face view in a stained section, a condition in which both surfaces become concurrently visible, a pattern of tiny perforations between the radiating ridges is visible on the larger scales, including the base plates of spines (Fig. 62–64). This appearance recalls the large plate scales of *C. polylepis* (MANTON & PARKE 1962b), though confusion with that species (which is also sparingly present in our samples) is ruled out by the very different smaller scales and absence of spines in *C. polylepis*.

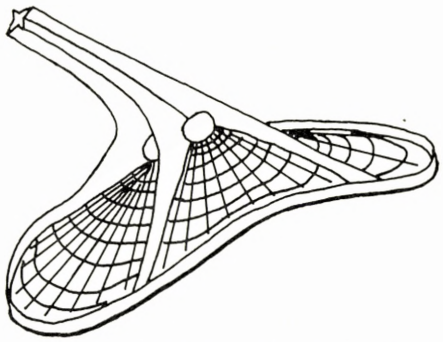
The spines of *C. mantoniae* are almost certainly tilted with respect to their own base plates since both can be concurrently transected to an extent that seems impossible otherwise (see for example Figs. 49a–c). This fact increases the difficulty of interpreting 3-dimensionally an extended series such as that in Figs. 61a–g for which however comparison with the reconstruction in Text-fig. 1 may perhaps provide clarification. As may be seen in Fig. 61g, adjacent struts on one and the same specimen can be very different in size and shape at the base, although a symmetrically cruciform profile is encountered in the spine itself (Fig. 61a). Apparent perforations, located near the point of union of the struts with the crown of the base-plate (Fig. 61e), shown more highly magnified beside another specimen in Figs. 62 and 63, seem to represent vestiges of the arch above the base plate found in other species in which the struts are not adnate (e.g. *C. pringsheimii*, PARKE & MANTON 1962); in our present species there are exactly four such channels, corresponding to one for each strut. The spines of *C. mantoniae* are hollow proximally (Figs. 49a–c), but solid distally (Figs. 51a, b). The tip is smoothly tapered to a point.

This description conflicts in several respects with expectation based on LEADBEATER (1972a), In particular the spine base, described here for the first time, is quite unlike that illustrated in Fig. 40 of that paper which also includes the conspicuously apiculate tip attributed to the spines of *C. mantoniae* in Norway but not found in Denmark. These discrepancies can now in part be explained by the recent recognition that the spine in Fig. 40 (loc. cit.) is an alien intruder derived from an as yet undescribed freshwater Chrysophyte, almost certainly of riverine origin. In con-

trast, the authenticity of the new features described above has recently been confirmed on additional wild material from the Mediterranean and Adriatic Seas (LEADBEATER unpub.) and from South Africa (MANTON unpub.). They can therefore now safely be incorporated into the taxonomic description though reference to apiculate spine tips should be delected.

There is no ancillary material between the periplast and underlying plasmalemma, contrary to the situation in each of the three preceding species. In *C. mantoniae* the fit between scales and cytoplasm is so close that the latter is distorted into depressions by concave scales or mounds under convex ones (Fig. 52 etc.).

As in the previous three species, there is a discrepancy between the cell size as registered on dried material and that ascertainable from sections. The latter works out



Text-fig. 1. Reconstruction of the basal part of a spine and subtending plate of *C. mantoniae*.

in this case as 10 μm long $\times$4–5 μm wide instead of 6 μm long $\times$3 μm wide. However both estimates agree in recognising a substantially greater degree of elongation relative to width compared with the other species discussed here.

C. mantoniae, though not detectably practising phagotrophic feeding, has given clear evidence of susceptibility to virus infection (Figs. 65, 66). The virus appears hexagonal in section, a well known sign that the shape is that of a regular 20 sided polyhedron. The size is approximately 22 nm (220 Å) but the number of capsomeres is not known. Infection with an apparently similar virus was not uncommon in cells of *Coccolithus huxleyi*, in all cases as a pathological condition indicating a moribund or dead cell. We did not encounter it in other species.

C. mantoniae was locally abundant, notably at Jerup near Frederikshavn, but was not ubiquitous in our other Danish samples. Specimens apparently identical with these have subsequently been met with in the Mediterranean and Adriatic Seas (LEADBEATER unpub.) and at Cape Town (MANTON unpub.) while body type scales (though as yet no spines) from broken cells have been seen in material from West Greenland. There is no doubt therefore that the species is widely distributed in both northern and southern hemispheres.

6. *C. campanulifera* sp. nov. (from Latin *campanula* a little bell) Figs. 67–81 on plates XI and XII and text – fig. 2.

DIAGNOSIS:

Cells c. 10×10 μm or larger, saddle-shaped with two incurved flanges covering the haptonema when helically coiled and with two equal flagella c. 25 μm long. The haptonema substantially longer than the flagella and with a substructure of 6 microtubules as in other members of the *C. strobilus* group. Two lateral plastids, each with an immersed pyrenoid penetrated by a single contracted thylakoid pair. Periplast of two scale-layers covered with copious mucilage; the outer scales bell-shaped with an erect forward edge, the sides gently curving outwards and carrying two (rarely three or one) rows of translucent "windows" collectively appearing as transparent transverse lines, the base rounded or flat but not conical, stalkless but with a central thickening projecting into the scale concavity, the commonest size 0.18×0.18 μm but exceptionally larger (up to 0.22 μm wide ×0.18 μm high) or smaller (0.18 μm wide ×0.12 μm high); the under scales flat oval plates 0.28×0.22 μm with a slightly thickened raised marginal ridge delimiting a peripheral thin border with radial striations and with a central cushion marked by a conspicuous cruciform thickening. Food vacuoles common, usually containing detritus.

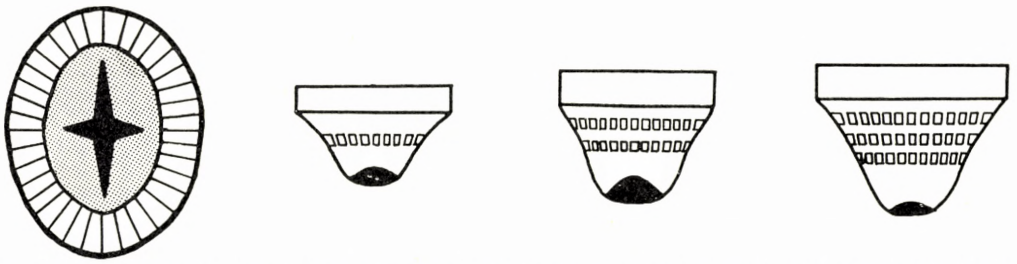
Abundant in the sea on both sides of the northern tip of Jutland in summer (July 1971, July 1972). Not as yet recorded elsewhere. The type: the specimen in Figs. 70–73.

C. campanulifera sp. nov.

Cellula circiter 10 μm diam. vel ultra, ephippiomorpha, haptonemate in statu retracto inter lobos apicales incurvos abscondito. Flagella aequalia, circiter 25 μm longa. Haptonema multo longuis, ut in *C. strobilo* aliisque speciebus eae affinibus 6 microtubulis percursum. Chloroplasti duo laterales, quisque pyrenoides internum binis thylacoidibus contractis transectum fovens. Periplastus duo strata squamarum mucilagine copiose induta praebens: squama strati exterioris campanuliformis, margine erecto, lateribus praeterea subcurve divergentibus fenestris translucidis in duas (raro tres vel unam) series dispositis una lineas transversas pellucidas efficientibus ornatis, basi plana vel rotundata non conica nullum stipitem praebente sed medio incrassata intra protuberante, plerumque 0.18×0.18 μm magna, interdum major (ad 0.22 μm lata, 0.18 μm alta) vel minor (0.18 μm lata, 0.12 μm alta); squama strati interioris plana, ovalis, 0.28×0.22 μm magna, carinula paulum incrassato-elevata taeniam tenuem marginalem radiatim striatam limitante cincta, medio nota cruciformi incrassata manifesta ornata. Vacuola alimentaria frequentia, plerumque materiem detritam continentia.

Species mense Julio anni 1971 ut anni 1972 in utroque mari ultimam paeninsulam Jutlandicam alluente crebra (praeterea adhuc nusquam inventa), figuris hic appositis 70–73 omnibus eandem cellulam monstrantibus typificata.

This description is incomplete in certain details. Thus the exact length of the haptonema can only be specified in general terms. A section such as that in Fig. 72 includes no more than part of the helix and though the whole mount in Figs. 68, 69 is complete, the preservation is not good enough to permit definitive counting of the number of gyres though a rough estimate of "not less than 20" can be made. This is nevertheless sufficient to indicate a haptonema substantially longer than the flagella, as in the closely related *C. strobilus*, *C. cymbium* and *C. camella* (LEADBEATER & MANTON 1969a, 1969b), though further observations, preferably on living cells, are still needed to confirm and establish this more accurately.



Text-fig. 2. *C. campanulifera*. Drawing of a plate scale and three bell-shaped outer scales carrying one, two or three rows of translucent "windows" respectively.

Some uncertainty also pertains to the cell size since there is the usual discrepancy between measurements on dried cells (8×8 μm in Fig. 67 and 7×7 μm in Fig. 68) compared with those on sections. Even the latter could be too small since it is rarely possible to know whether a given section is exactly median and therefore registering the widest possible dimensions or not. The measurements actually included in the diagnosis (10×10 μm) may therefore eventually need to be emended in the upward direction. On the other hand, the range of sizes among scales, as illustrated in Figs. 79–81, represent genuine differences between individual specimens of a kind that can sometimes be encountered in adjacent cells in one preparation (Fig. 76). Our reason for including them all within one taxonomic diagnosis is that in spite of these differences they share the characteristic shape distinguishing them from other members of the group present in the same blocks, as for example *C. strobilus* illustrated in Figs. 82–84. Moreover in spite of their observed differences of size all the scales attributed here to *C. campanulifera* fall within a size range not previously represented in the group all being smaller than the scales of *C. camella* but larger than those of *C. cymbium* or *C. strobilus*. This suggests that in spite of clonal differences in surface patterning (summarised in text-fig. 2), which may or may not prove to be genetically stable, there is no reason to attribute the taxonomically significant characters to the action of transient environmental factors.

The relatively large cell size is in this case perhaps associated with active predation since traces of relatively intact alien protoplasts, that seem likely to have been alive before ingestion, can sometimes be found in food vacuoles. Detritus is never-

theless more usual and pellets up to 0.4 μm in diameter have been sectioned. The particular food vacuole included in Fig. 71 on the other hand contains a partly decomposed alien haptonema, doubtless ingested as organic detritus.

The very copious mucilage present all over the cell surface is a serious impediment to observation of scale patterning in whole mounts. These usually appear as in Fig. 75 in which only the outer edges of cups can be discerned through the mucilage, without any sign of the presence of underlying plates. To remove the mucilage requires more vigorous washing than was applied when we were concerned to retain intact cells still showing their appendages, a task which for some reason proved difficult. A renewed search for this species in the type locality with this need in mind might therefore prove rewarding.

Discussion

Before giving further consideration to these findings, it will perhaps be helpful to recall the nature of the enquiry and its cognate limitations and advantages. The latter include the insight gained into cell structure and behaviour of selected taxa under natural conditions and regardless of their amenability or otherwise to life in culture. The limitations include the inherent technical difficulties, notably that of obtaining satisfactory sections in sufficient numbers from blocks inevitably loaded with detritus of various kinds, to which must be added the further requirement that sections must be taxonomically identifiable and should represent species for which microanatomical information is needed. It is therefore no accident that five of the species discussed above have already been recorded in other ways elsewhere and only one (*C. campanulifera*) is new, though with some well known close relatives.

It is likewise no accident that each of the species discussed has possessed an exceptional capacity to retain the periplast in position during the processing to which they have all been subjected, and the apparent absence of certain desired species, notably *C. parkeae*, well represented by scattered scales in whole mounts prepared from the same water samples, almost certainly reflects an inability to do this. A protoplast without its scales either on the outside or within (as in *C. mactra* and *C. megacylindra*, Manton 1972a, 1972b) is all too often unrecognisable, no matter how well fixed. It is therefore in a sense a measure of the success of this particular undertaking that not more of the desired taxa have become lost to view in this way.

A capacity to retain scales is almost certainly determined in part by their physical shape. Small scales are always more easily retained than large ones, as for example in *C. campanulifera* but also in many other genera such as *Phaeocystis* (Fig. 1) or in *Scourfieldia marina*, introduced here incidentally in Fig. 29. The rank and file of other *Chrysochromulina* species, some 20 in all, have scales larger than these. In all those previously sectioned, the scale surfaces in closest contact with the plasmalemma have been flat; many examples could be quoted, including *C. ericina* (Manton & Leedale 1961) *C. chiton* (Manton 1967), *C. acantha* (Leadbeater & Manton 1971)

etc., in addition to literature already cited. This is however not so in any of the species recorded here, apart from *C. campanulifera*. In *C. ephippium*, *C. herdlensis* and *C. bergenensis* the scales of the underlayer are concave. In *C. aff. fragilis* the exceptionally wide but very thin plates are convex, while in *C. mantoniae* both concave small scales and convex large ones (base-plates of spines) were encountered. That so many examples of a new phenomenon in a large genus should have been met with together in Denmark might, at first sight, have been interpreted in terms of some local adaptive response to previously unknown ecological conditions. As it is we must recognise that the sample is not a random one but heavily biassed by the method of selection towards these particular features. The question as to whether there is or is not a natural advantage, under specifiable ecological conditions, in a capacity to retain scales in situ on a cell in spite of relatively rough handling is one which is raised but not solved by our findings and only a further study explicitly directed towards it is likely to find the answer.

The close attention to pyrenoids, for which information has been supplied for each of our 6 taxa, has considerably extended previous concepts for this genus. It has hitherto been customary to recognise only two main categories namely projecting pyrenoids recorded in four species (*C. chiton*, *C. kappa*, *C. minor* and *C. mactra*) and immersed pyrenoids possessed, on present knowledge, by all the others. Among the latter however we have had occasion here to distinguish no less than three different sorts of immersed pyrenoid according to the nature of the thylakoidal endings penetrating the storage region. These were simple tubes in *C. bergenensis* and *C. mantoniae*, a condition already known in *C. acantha* (Leadbeater & Manton 1971) in which each tube doubtless represents a single contracted thylakoid. In *C. ephippium* and C. *campanulifera*, each apparent tube is longitudinally partitioned indicating equivalence to a thylakoidal pair as in *C. megacylindra* (Manton 1972b). Finally, the multilayered condition in *C. herdlensis* and *C. fragilis* introduces a type of pyrenoidal substructure not previously known in the genus. A semblance of a functional interpretation could only be suggested for the last type which seemed, in these two species, to be correlated with possession of one instead of the more usual two plastids per cell. Even this is however uncertain unless validated by further observations, preferably on living cells. Without this, pyrenoids can usefully be recorded as additional critera for identification of species but not as the basis for other conclusions. This, it may be said in passing, is a very usual finding within very diverse groups of flagellates (e.g. Dinoflagellates, Dodge & Crawford 1971 etc.).

Relative sizes have come up for special mention in more than one context. A discrepancy between measurements of body size made respectively on whole mounts and on sections is demonstrable for each of the six species under discussion. Only in one case (*C. ephippium*), for which the published estimates of size had been based on light microscopy of living cells (Parke, Manton & Clarke 1956), are our sections in close agreement with these though the whole mounts are less so. There can be no doubt therefore that sections are more trustworthy than whole mounts as evidence of

TABLE I.

Comparison of protoplast dimensions as indicated by sections and by air dried whole mounts. Figures for the living cells of *C. ephippium* from PARKE, MANTON & CLARKE (1956), and the rest from the text or illustrations to this paper.

Name	Material	wet dimensions (μm)	dry dimensions (μm)	Approximate % dry/wet
C. ephippium	sections	8×8, 10×6	5×4	50–70
	living	10×6		
C. herdlensis	sections	7×7, 6×5	4×3	50–60
C. fragilis	sections	6×5, 5.5×4.5	4.5×3.5	70–75
C. bergenensis	sections	7×4 – 5.5	4×3.4	60–80
C. mantoniae	sections	10×4–5	6×3	60–75
C. campanulifera	sections	10×10	7×7, 8×8	70–80

cell size and that the latter, if used alone, will tend to produce values which are too low by an amount which varies (Table I) but which can be roughly assessed as of the order of 50% of the true values. Shrinkage on drying, unless special precautions have been taken, is undoubtedly the main factor involved and an instructive comparison can perhaps usefully be made between some early micrographs assembled to test this in MANTON & CLARKE 1951. In Figs. 1–5 of that paper, on the spermatozoid of *Fucus*, some photographs taken with ultra-violet light, of freshly killed cells floating in seawater, are set beside an electron micrograph of a similar cell in an air-dried, shadowcast, whole mount; this comparison shows plainly that flagellar length and body shape are essentially unchanged in the latter though body size is greatly reduced. This is therefore a matter for which an allowance or corrections ought explicitly to be introduced when species have to be described on the basis of air-dried material only. We recommend for this purpose that not less than 50% of the apparent body size of a dry cell should be added to the observed measurements to obtain a rough estimate of the true dimensions.

Though measurements of length of the appendages can be made without ambiguity if these adhere thoroughly to a support film or slide this condition is by no means invariably met. The haptonema, in particular, does not remain fully extended after death of the cell and its length can often be determined only by direct observation of living cells or by calculation from the dimensions of the helix, more especially the number of gyres, into which it becomes coiled. With a long haptonema such as that of *C. ephippium* or *C. campanulifera* the number of gyres can equal or exceed 20. A longitudinal section through the helix can then provide numerous transverse views at different levels through a haptonema permitting evidence on its substructure to be multiplied. In contrast, the short haptonemata of species such as *C. herdlensis*, *C. bergenensis* or *C. mantoniae* provide so few sections that we have in each case no more than one clearly analysable specimen. This evidence is sufficient to indicate a substantial difference between *C. bergenensis* with 6 microtubules and the other two species

with 7 but supplementation would be essential if a fuller description of the whole organelle were required.

Study of haptonema movement, which can only be based on observation of living cells, is more important, in the present state of knowledge, for short haptonemata than for long ones. The latter have recently been investigated in some detail by LEADBEATER (1971) who has shown that, in an array of species of *Chrysochromulina* with long haptonemata, the helical coiling of the organelle is not only the condition at rest but is one immediately brought about by almost any chemical or mechanical stimulation, being completed in $^1/_{50}$th sec. or less. Any fixed cell will therefore necessarily have its haptonema coiled unless it has either been shed or in other ways disturbed. In the special case of *C. mantoniae* however, where the haptonema is short but clear evidence of coiling absent (see p. 11 above), it is not possible to know, without supplementary observations on living cells, whether such absence of evidence denotes post mortem disturbance of a limited number of susceptible specimens or is a positive sign of a difference in the attributes of the organelle. Moreover since a capacity to coil is at present regarded as a significant taxonomic criterion for separating species of Chrysochromulina from those of at least three other genera (*Phaeocystis* Lagerheim, *Prymnesium* Conrad and *Chrysocampanula* Fournier) a negative decision with respect to this character for *C. mantoniae* might inevitably lead to a change of its generic name. This matter is therefore more important than might otherwise have been thought.

Phagotrophic feeding, though practiced in most if not all of the species under discussion (*C. mantoniae* being the most probable exception), is also, almost certainly, influenced to some extent by cell size. Thus only in *C. campanulifera*, with relatively large cells, have we encountered occasional food vacuoles suggesting active predation. Even in this species however most food vacuoles contain detritus, as illustrated here in *C. bergenensis*. More recent observations on *C. mactra* (MANTON unpub.) added to those already published on *C. megacylindra* (MANTON 1972b) and the initial descriptions of other species by PARKE et al 1955, 1956 et seq., indicate that most members of the genus, as at present construed, are contributing to the metabolic turn-over in the sea, not only by photosynthesizing but also by ingesting detritus. Somewhat paradoxically however, the type species, *C. parva* Lackey from freshwater, does not ingest, while bacteria, the favoured food of some other members of the marine nanoplankton, notably collared flagellates (Fig. 67), are not normally found in the food vacuoles of species of *Chrysochromulina*. Some degree of selectivity in food must therefore be attributed to these various organisms.

Problems which have necessarily to be left aside unless and until they can be studied by means of cultures are those of life histories. It has been assumed that all of the taxa dealt with here are good species of *Chrysochromulina* unless proved to be otherwise. Some nevertheless have already been shown to possess characters better known among coccolithophorids. Thus the columnar deposits on the plasmalemma beneath the scales noted in *C. herdlensis*, *C. fragilis* and *C. bergenensis* but quite absent

from other known species of *Chrysochromulina* are already familiar in two species of *Hymenomonas* (MANTON & PETERFI 1969) as well as in the motile and non motile phases of *Coccolithus pelagicus* (MANTON & LEEDALE 1963, 1969). The motile stage of the latter, formerly designated *Crystallolithus hyalinus* Gaarder & Markali before the life history had been worked out (PARKE & ADAMS 1960), also possesses a skin reminiscent of that in *C. bergenensis* as well as a relatively short haptonema with 6 microtubules (MANTON & LEEDALE 1963). The possibility that some of the relatively new taxa may in future reveal themselves as uncalcified motile stages of as yet unrecognised coccolithophorids is thus a real one and the prime suspect in such a context is *C. bergenensis.*

Finally, a wider sampling in different geographical areas, with or without concurrent study of clonal cultures, is still needed to clarify topics such as temperature tolerance and range of intraspecific variability. The former has been introduced here with exceptional force by *C. ephippium* which in one month (July 1972) was encountered both in West Greenland, with a prevailing water temperature of 4°C and in the sea near Frederikshavn where the equivalent figure was 17°C. This topic is one which we hope to pursue elsewhere.

On the other hand, genetically based variations within species collectively represent a very large topic which is only beginning to be approachable. Apparently stable deviations from the normal have hitherto tended to appear sporadically in cultures, notably in *C. chiton* (MANTON 1967a and b, GREEN & JENNINGS 1967) and *Coccolithus huxleyi* (PAASCHE & KLAVENESS 1970, KLAVENESS & PAASCHE 1971). Wild populations of these and other species are nevertheless by no means uniform. Thus a substantial difference in scale sizes was encountered in 1970 in the English Channel versus the sea near Bergen, Norway, with respect to *C. parkeae,* at that time (GREEN & LEADBEATER 1972) a new species so peculiar in several respects that confusion with anything else seemed impossible. More recently LEADBEATER 1972b has placed on record micrographs of an array of wild specimens collected near Bergen in 1970 and attributable in a general way to such distinctive species as *C. chiton, C. ericina* and *C. megacylindra* but differing in details from the type descriptions. In the present communication we also have had occasion to note divergences among specimens attributable on other grounds to *C. herdlensis, C. fragilis,* and *C. campanulifera.*

Some of these divergent types may eventually prove to belong to separate taxa resembling but not con-specific with those to which they have at first been referred. Others may prove to represent local and perhaps temporary deviations from the normal, not recognisable as such when a species was first described; the apiculate versus smoothly tapered spine tips in *C. mantoniae* (p. 12 above) might have been cited as an example of this. The order of discovery of representatives of a new taxon can materially affect the treatment it receives as we have ourselves shown. Thus if the various scale types illustrated in Figs. 79–81 had turned up first in unialgal cultures as true breeding clones, one or more species might have been described, each more narrowly based than is now the case.

It must be recognised that even in non-sexual organisms, which we believe these to be, variability is a fact of nature for which some, but not too much, provision must be incorporated into the taxonomic system if specific concepts are to be useful. The problem of how best to do this in any one case can only be decided empirically. Study of unialgal cultures and of wild populations are both needed since the one type of approach can act as an essential corrective for the shortcomings and weaknesses of the other. In the light of all the evidence, descriptions can then be adjusted to fit the facts that can be ascertained.

Summary of Observations Amplifying or Emending Type Descriptions Previously Published

1. *C. ephippium* (Parke, Manton & Clarke, 1956).

7 microtubules in haptonema; immersed pyrenoid penetrated by a single paired thylakoidal ending; scale types segregated into superposed layers, the plate scales of the underlayer strongly concave with wide inflexed rims, the spined scales of the outer layer flat or slightly convex with short erect rims; ingested detritus pellets common, up to 2 μm in diameter; present distribution records include Europe, West Greenland and South Africa with an attested temperature range from 4° C to 17° C.

2. *C. herdlensis* (Leadbeater 1972 a).

Two cell types, respectively with and without a columnar deposit on the outer surface of the larger scales, both types with another columnar deposit between the scales and the plasmalemma; cell size larger than in the original diagnosis (see Table I); the 3 scale types in a regularly layered arrangement, the largest and smallest scales flat, the middle sized scales concave with inflexed rims; plastid apparently single with an immersed, multilayered pyrenoid; 7 microtubules in the haptonema; ingested detritus pellets up to 3 μm in size.

3. *C. aff. fragilis* (Leadbeater 1972 a).

Cell size larger than in the type description (see Table 1), plastid single with an immersed multilayered pyrenoid; columnar material between the plasmalemma and the exceptionally thin scales; large rimless plate scales slightly convex with the small rimmed plates clustered under their edges; minor discrepancies from the type noted but not recognised taxonomically; no information as yet on phagotrophic feeding or haptonema substructure.

4. *C. bergenensis* (Leadbeater 1972 a).

Cell size larger than in the type description (see Table 1) but still one of the smallest in the genus; haptonema with 6 microtubules; plastids with pyrenoids immersed and penetrated by simple thylakoid endings; periplast bounded externally by a skin; the scales layered according to size, all somewhat concave but the larger rim-

less plates less so than the smaller rimmed plates beneath them; some ancillary dense material among and beneath the scales; origin of the various materials present in the periplast traced to the Golgi cisternae; detritus ingestion demonstrated.

5. *C. mantoniae* (LEADBEATER 1972a).

Cell size larger than in the type description (see Table 1); 7 microtubules in the haptonema; pyrenoids immersed and penetrated by simple thylakoid endings; outer scales plane, under scales concave, base plates of spines convex; scale rims either erect or inflexed according to scale type; spine shape more fully elucidated than in the type description especially with respect to the base and to the tip which is smoothly tapered in all known localities outside Norway; the word 'apiculate', should be therefore deleted from the diagnosis.

Summary of Identified Taxa of Nanoplankton from the Sea near Frederikshavn in the Month of July (1971 and 1972)

The commonest individual organisms in the waters sampled are not those discussed above but rather the still smaller cells of *Phaeocystis pouchetii* (zoids) and *Micromonas pusilla* (the smallest known eucaryote) both of which have appeared incidentally in the field of Fig. 1. The nanoplankton in the sense used here (p. 3) is dominated by no more than three major phyletic groups, namely, Haptophyceae (brown), Prasinophyceae (green) and Choanoflagellatae (colourless), with a much smaller representation of other affinities. This is exactly as already demonstrated in Norway (THRONDSEN 1969, LEADBEATER 1972b) and shortly to be demonstrated in other seas (LEADBEATER unpub., MANTON unpub.).

List of Species Recorded in Whole Mounts and in Sections Except where otherwise Stated

HAPTOPHYCEAE

Chrysochromulina acantha LEADBEATER & MANTON
C. alifera PARKE & MANTON
C. bergenensis LEADBEATER
C. brevifilum PARKE & MANTON
C. campanulifera sp. nov.
C. chiton PARKE & MANTON
C. cymbium LEADBEATER & MANTON
C. ephippium PARKE & MANTON
C. ericina PARKE & MANTON
C. aff. fragilis LEADBEATER
C. herdlensis LEADBEATER

′ *C. kappa* or *C. minor* Parke & Manton
C. mantoniae Leadbeater
C. megacylindra Leadbeater
C. microcylindra Leadbeater
° *C. parkeae* Green & Leadbeater
C. polylepis Manton & Parke
C. pringsheimii Parke & Manton
C. strobilus Parke & Manton
°° *Acanthoica quattrospina* Lohm.
°° *Calyptrosphaera* sp. Lohm.
Coccolithus huxleyi (Lohm.) Kampt.
Phaeocystis pouchetii (Hariot) Lagerheim (zoids + and – threads)
′ *Pavlova* sp.

CHRYSOPHYCEAE
Dinobryon petiolatum Willén
D. balticum (Schütt) Lemm.
° *Paraphysomonas butcheri* Pennick & Clarke
P. imperforata Lucas
P. foraminifera Lucas
Kephyrion sp.
Chrysosphaerella sp.
Apedinella spinifera Throndsen
Meringosphaera mediterranea Lohm.

SILICOFLAGELLATA
′ *Dictyocha* sp.

PRASINOPHYCEAE
′ *Heteromastix* 3 spp., probably:
H. rotunda (Carter) Manton
′ *H. minuta* Carter
′ *H. pyriformis* (Carter) Manton in Parke 1964
Micromonas pusilla (Butcher) Parke & Manton
M. squamata Parke & Manton
Nephroselmis gilva Parke & Rayns
′ *Platymonas* aff. *tetrathele* West
′ *P. convolutae* Parke & Manton
Pyramimonas orientalis Butcher sensu Throndsen 1969
P. grossi Parke
P. aff. obovata Carter sensu Manton, Oates & Parke 1963
′′ *Pyramimonas* 2 other species not yet named
Scourfieldia marina Throndsen

EUGLENOPHYCEAE

′ *Eutreptiella* sp.

°°° CHOANOFLAGELLATA (Craspedophyceae)

° *Acanthoeca brevipoda* ELLIS
° *Diaphanoeca grandis* ELLIS
° *D. pedicellata* LEADBEATER
° *Diplotheca costata* VALKANOV
° *Parvicorbicula quadricostata* THRONDSEN
° *Pleurasiga cupula* LEADBEATER
° *P. minima* THRONDSEN var. *minuta* LEADBEATER
° *P. aff. reynoldsii* THRONDSEN
° *Salpingoeca natans* GRØNTVED
° *S. spinifera* THRONDSEN
° *Savillea parva* (ELLIS) LOEBLICH
° *Stephanoeca diplocostata* ELLIS
° *S. pedicellata* LEADBEATER

INCERTAE SEDIS

′ *Luffisphaera* sp. BELCHER & SWALE

′ Only identified in sections.
° Only identified in whole mounts.
°° Only seen in 1972 in whole mounts stripped from glass. Identification kindly supplied by Mrs. Ringdal Gaarder.
°°° For this group a list has already been published in LEADBEATER (1972c) on identifications based on whole mounts. Use of sections for this purpose is virtually ruled out by the difficulty of adequately reconstructing the lorica, without which naming is impossible, though the group as such can be recognised.

I. MANTON: The University, Leeds, England.
B. S. C. LEADBEATER: Botany Department, The University, Birmingham, England.

Bibliography

Dodge, J. D. & Crawford, R. M. (1971): A fine structural study of dinoflagellate pyrenoids and food-reserves. Bot. J. Linn. Soc. 64: 105–115.

Green, J. C. & Jennings, D. H. (1967): A physical and chemical investigation of the scales produced by the Golgi apparatus within and found on the surface of cells of *Chrysochromulina chiton* Parke & Manton. J. exp. Bot. 18: 359–370.

Green, J. C. & Leadbeater, B. S. C. (1972): *Chrysochromulina parkeae* sp. nov., (Haptophyceae) a new species recorded from S.W. England and Norway. J. mar. biol. Ass. U.K. 52: 469–475.

Klaveness, D. & Paasche, E. (1971): Two different *Coccolithus huxleyi* cell types incapable of coccolith formation. Arch. Mikrobiol. 75: 382–385.

Leadbeater, B. S. C. (1971): Observations by means of ciné photography on the behaviour of the haptonema in plankton flagellates of the class Haptophyceae. J. mar. biol. Ass. U.K. 51: 207–217.

— (1972a): Fine structural observations on six new species of *Chrysochromulina* (Haptophyceae) from Norway. Sarsia 49: 65–80.

— (1972b): Identification, by means of electron microscopy, of nanoplankton flagellates from the coast of Norway. Sarsia 49: 105–132.

— (1972c): Ultrastructural observations on some marine Choanoflagellates from the coast of Denmark. Br. phycol. J. 7: 195–211.

Leadbeater, B. S. C. & Manton, I. (1969a): New observations on the fine structure of *Chrysochromulina strobilus* Parke & Manton with special reference to some unusual features of the haptonema and scales. Arch. Mikrobiol. 66: 105–120.

— & — (1969b): *Chrysochromulina camella* sp. nov., and *C. cymbium* sp. nov., two new relatives of *C. strobilus* Parke & Manton. Arch. Mikrobiol. 68: 116–132.

— & — (1971): Fine structure and light microscopy of a new species of *Chrysochromulina* (*C. acantha*). Arch. Mikrobiol. 78: 58–69.

Manton, I. (1967a): Further observations on the fine structure of *Chrysochromulina chiton*, with special reference to the haptonema, 'peculiar' Golgi structure and scale production. J. Cell Sci. 2: 265–272.

— (1967b): Further observations on scale formation in *Chrysochromulina chiton*. J. Cell Sci. 2: 411–418.

— (1968): Further observations on the microanatomy of the haptonema in *Chrysochromulina chiton* and *Prymnesium parvum*. Protoplasma 66: 35–53.

— (1972a): Preliminary observations on *Chrysochromulina mactra* sp. nov. Br. phycol. J. 7: 21–35.

— (1972b): Observations on the biology and micro-anatomy of *Chrysochromulina megacylindra* Leadbeater. Br. phycol. J. 7: 235–248.

Manton, I. & Clarke, B. (1951): An electron microscope study of the spermatozoid of *Fucus serratus*. Ann. Bot. N.S. 15: pl. XX.

MANTON, I. & LEEDALE, G. F. (1961): Further observations on the fine structure of *Chrysochromulina ericina* Parke & Manton. J. mar. biol. Ass. U.K. 41: 145–155.

— & — (1963): Observations on the microanatomy of *Crystallolithus hyalinus* Gaarder & Markali. Arch. Mikrobiol. 47: 115–136.

— & — (1969): Observations on the microanatomy of *Coccolithus pelagicus* and *Cricosphaera carterae*, with special reference to the origin and nature of coccoliths and scales. J. mar. biol. Ass. U.K. 49: 1–16.

MANTON, I. & PARKE, M. (1962): Preliminary observations on scales and their mode of origin in *Chrysochromulina polylepis* sp. nov. J. mar. biol. Ass. U.K. 42: 565–578.

MANTON, I. & PETERFI, L. S. (1969): Observations on the fine structure of coccoliths, scales and the protoplast of a freshwater coccolithophorid, *Hymenomonas roseola* Stein, with supplementary observations on the protoplast of *Cricosphaera carterae*. Proc. roy. Soc. B. 172: 1–15.

MANTON, I., OATES, K. & PARKE, M. (1963): Observations on the fine structure of the Pyramimonas stage of *Halosphaera* and preliminary observations on three species of *Pyramimonas*. J. mar. biol. Ass. U.K. 43: p. 236 and pl. XIV.

PAASCHE, E. & KLAVENESS, D. (1970): A physiological comparison between coccolith forming and naked cells of *Coccolithus huxleyi*. Arch. Mikrobiol. 73: 143–152.

PARKE, M. (1964): A revised check-list of British marine algae. J. mar. biol. Ass. U.K. 44: 499–542.

PARKE, M. & ADAMS, I. (1960): The motile (*Crystallolithus hyalinus* Gaarder & Markali) and non-motile phases in the life history of *Coccolithus pelagicus* (Wallich) Schiller. J. mar. biol. Ass. U.K. 39: 263–264.

PARKE, M., GREEN, J. C. & MANTON, I. (1971): Observations on the fine structure of zoids of the genus *Phaeocystis* (Haptophyceae). J. mar. biol. Ass. U.K. 51: 927–941.

PARKE, M. & MANTON, I. (1962): Studies on marine flagellates. VI *Chrysochromulina pringsheimii* sp. nov. J. mar. biol. Ass. U.K. 42: 391–404.

PARKE, M., MANTON, I. & CLARKE, B. (1955): Studies on marine flagellates. II (*Chrysochromulina kappa*, *C. minor* & *C. brevifilum*). J. mar. biol. Ass. U.K. 34: 579–609.

— — & — (1956): Studies on marine flagellates. III (*Chrysochromulina ephippium*, *C. alifera* & *C. ericina*). J. mar. biol. Ass. U.K. 35: 387–414.

— — & — (1958): Studies on marine flagellates. IV (*Chrysochromulina chiton*). J. mar. biol. Ass. U.K. 37: 209–228.

— — & — (1959): Studies on marine flagellates. V (*Chrysochromulina strobilus*). J. mar. biol. Ass. U.K. 38: 169–188.

THRONDSEN, J. (1969): Flagellates of Norwegian coastal waters. Nytt Mag. Bot. 16: 161–216.

Indleveret til Selskabet den 17. juli 1973.
Færdig fra trykkeriet den 15. marts 1974.

PLATES

PLATE I

C. ephippium PARKE and MANTON, from Danish wild material except where otherwise stated.

Fig. 1 A field of mixed nanoplankton, micrograph Y 4253 (Siemens Elmiskop 1A Ottawa) × 7,500 showing bacteria, detritus of various kinds, transected flagella of organisms not otherwise included in the field, sections of three flagellates: a) *Micromonas pusilla* (green) the smallest known eucaryotic cell, b) *Phaeocystis pouchetii* (Haptophyceae) zoid and c) *Chrysochromulina ephippium*.

Fig. 2 Section of the periplast cut perpendicularly to the subtending surface showing spined scales and concave, rimmed, plate scales arranged in superposed layers. Micrograph Y 2350 (EM6B, Nottingham) × 30,000.

Fig. 3 Section of a periplast cut tangentially to the subtending surface showing the two types of scale; for further description see text. Micrograph Y 3855 (EM6B Leeds Zoology Dept.) × 30,000.

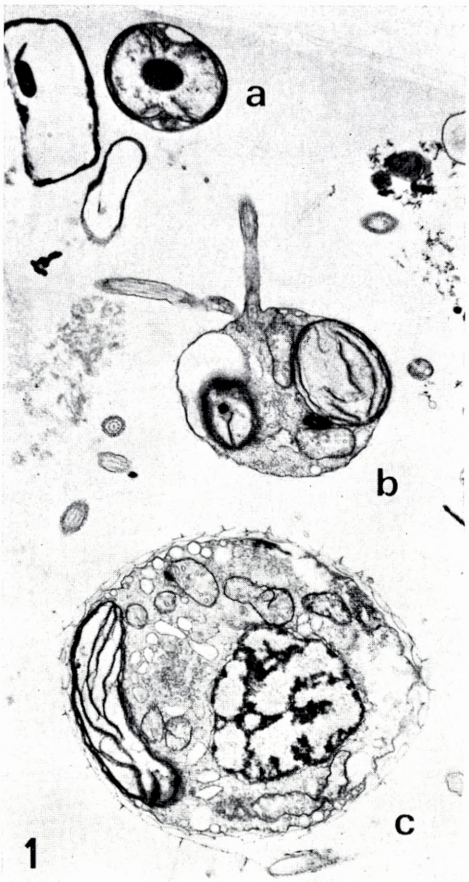
Fig. 4 Scales of the two types in a shadowcast whole mount. Micrograph L 3261 (EM6 Birmingham) × 30,000.

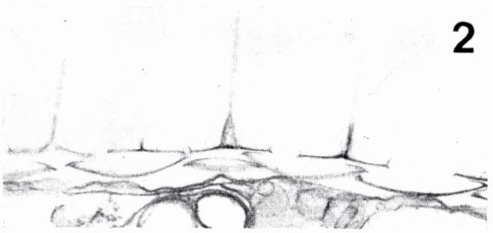
Fig. 5 Part of a complete cell stripped from glass, showing one complete flagellum and the very long helically coiled haptonema. Micrograph Y 4396 (801, Lancaster) × 5,000.

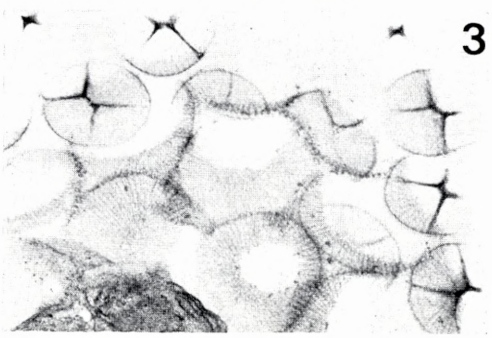
Fig. 6 Section of a large cell showing authenticating scales on the surface, the nucleus, part of the Golgi system, mitochondria and one of the two plastids with a pyrenoid. Micrograph Y 3741 (EM6B Leeds Zoology Dept) × 10,000.

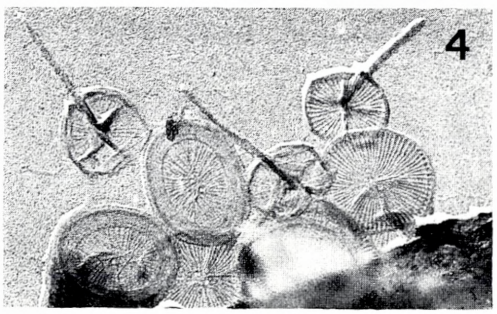
Fig. 7 Part of a pyrenoid showing a single paired thylakoid penetrating the storage area, from a culture (not the type culture) originating from the English Channel. Micrograph Z 2144 (EM6B Leeds Botany Dept) × 50,000.

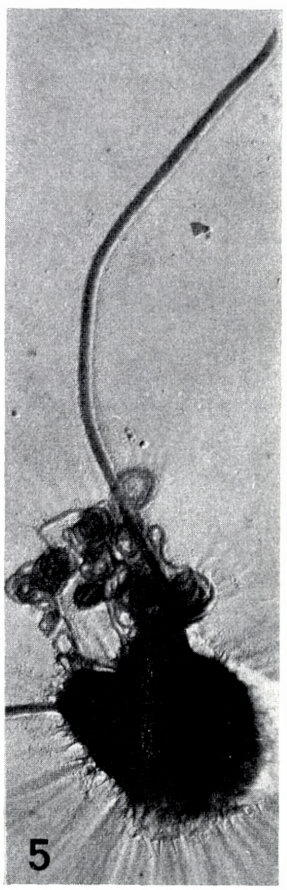
Fig. 8 Section through a haptonema in the type culture (osmic fixation methacrylate embedding) showing a ring of 7 microtubules surrounded by the customary membranes; for further description see text. Micrograph D 7506 (Siemens Elmiskop I, Leeds Botany Dept) × 100,000.

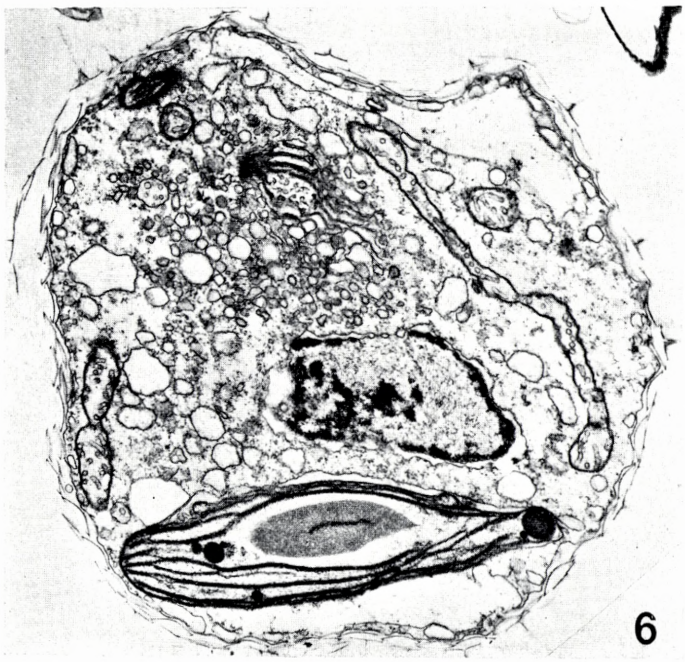

a
b
c
1

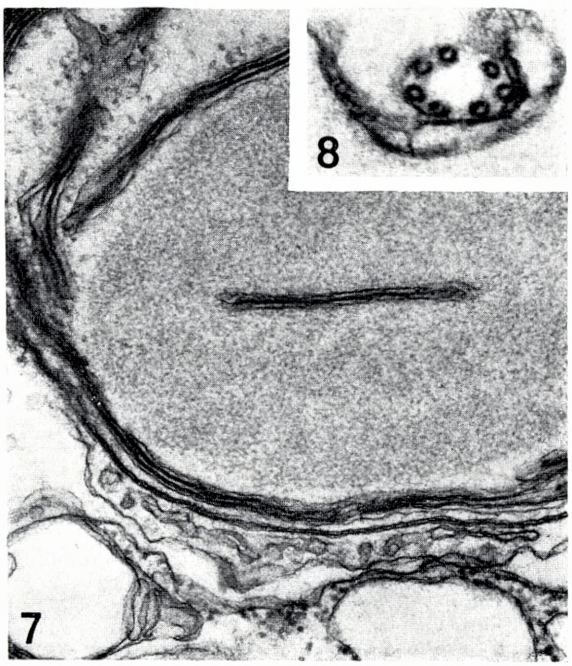

2


3


4


5


6


7

8

PLATE II

C. herdlensis Leadbeater from wild Danish material.

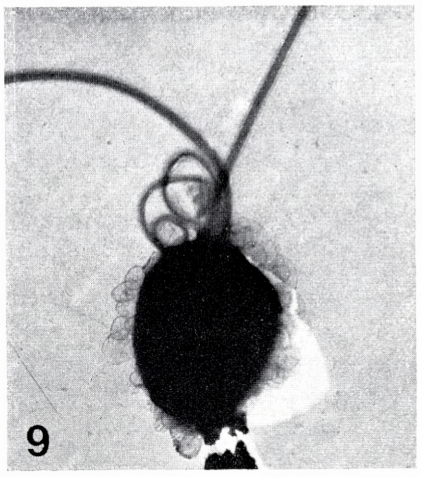
Fig. 9 A complete cell from Deget showing the short, coiled, haptonema and the bases of the two flagella. Micrograph L 3359 (Birmingham EM6) × 5,000.

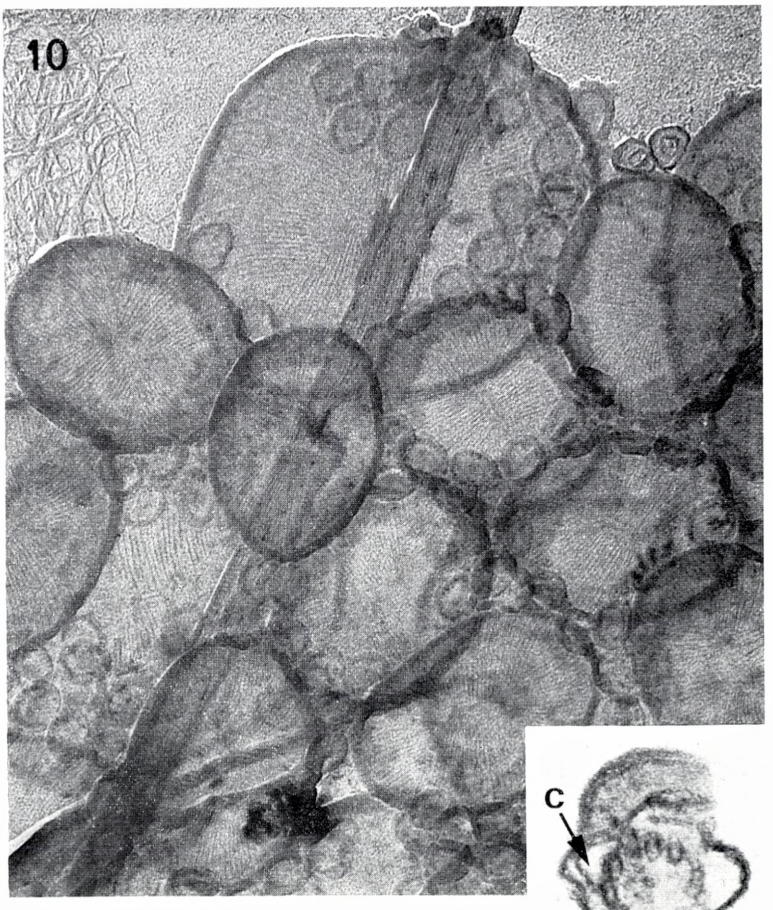
Fig. 10 Scales of three sizes showing the proximal (ridged) surfaces uppermost; note the arrangement of the smallest rimmed scales in the interstices between the other scales. Micrograph L 3263 (Birmingham EM6) × 30,000.

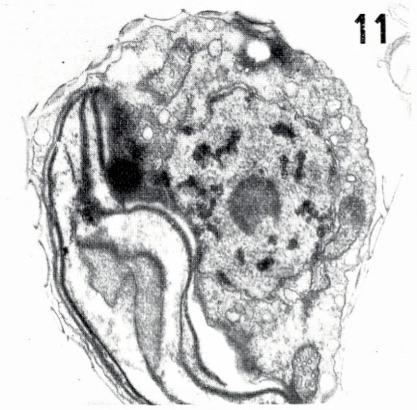
Fig. 11 Section of a cell with authenticating scales on the surface, showing the nucleus and other organelles including a multilayered pyrenoid in a plastid (for further details see fig. 12). Micrograph Y 3529 (Nottingham EM6B) × 10,000.

Fig. 12 Pyrenoid of another specimen showing the layering of double and single thylakoids in the storage area. Micrograph Y 3529 (Nottingham EM6B) × 30,000.

Fig. 13 Oblique transverse section through a haptonema showing an arc of 7 microtubules beside a possible 8th and with the haptonema cavity respresented by two separate profiles on opposite sides (arrows, c) Micrograph Y 4385 (Phillips 300 Biophysics, Leeds) × 100,000.


9


10


11

C
C
13

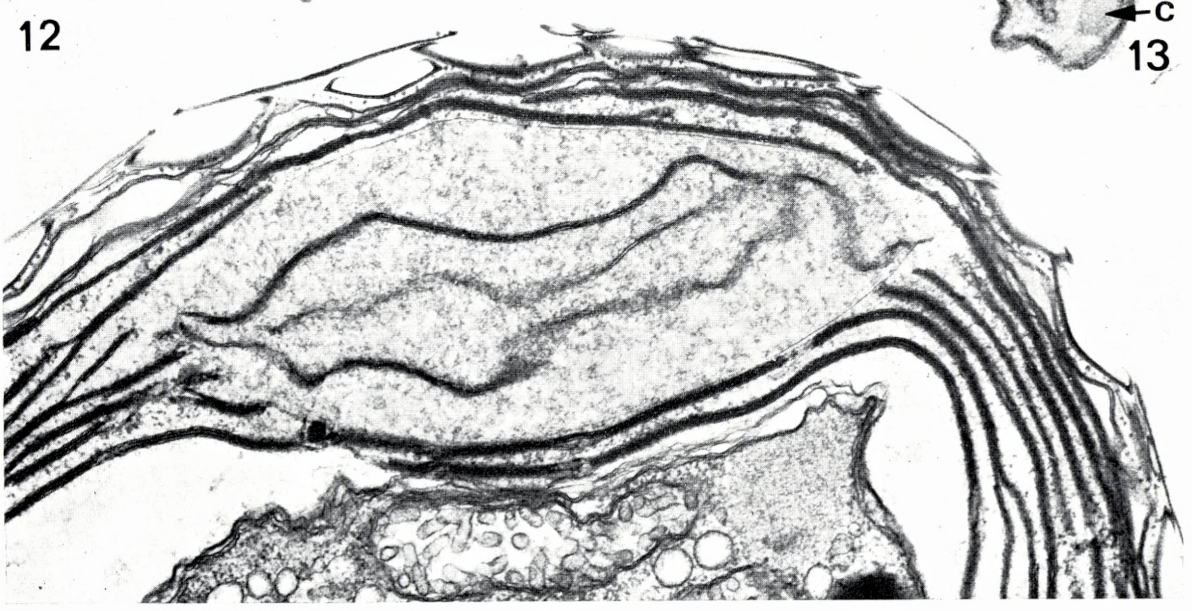

12

Plate III

C. herdlensis cont.

Fig. 14 Section of a cell of the "hairy" form of the species, with a layered pyrenoid as in fig. 11, and with authenticating scales loaded with filamentous deposits (for further details see fig. 16) Micrograph Y 2868 (EM6B Leeds Zoology Department) × 10,000.

Fig. 15 Scales of three sizes showing the outwardly directed distal faces and with many examples of the filamentous deposit (see especially centre left). Micrograph L 3238 (Birmingham EM6) × 30,000.

Fig. 16 Part of a section showing the columnar deposits on and beneath the scales and with traces of the latter within some of the Golgi cisternae. Micrograph Y 2444 (EM6B Leeds Zoology Dept.) × 30,000.

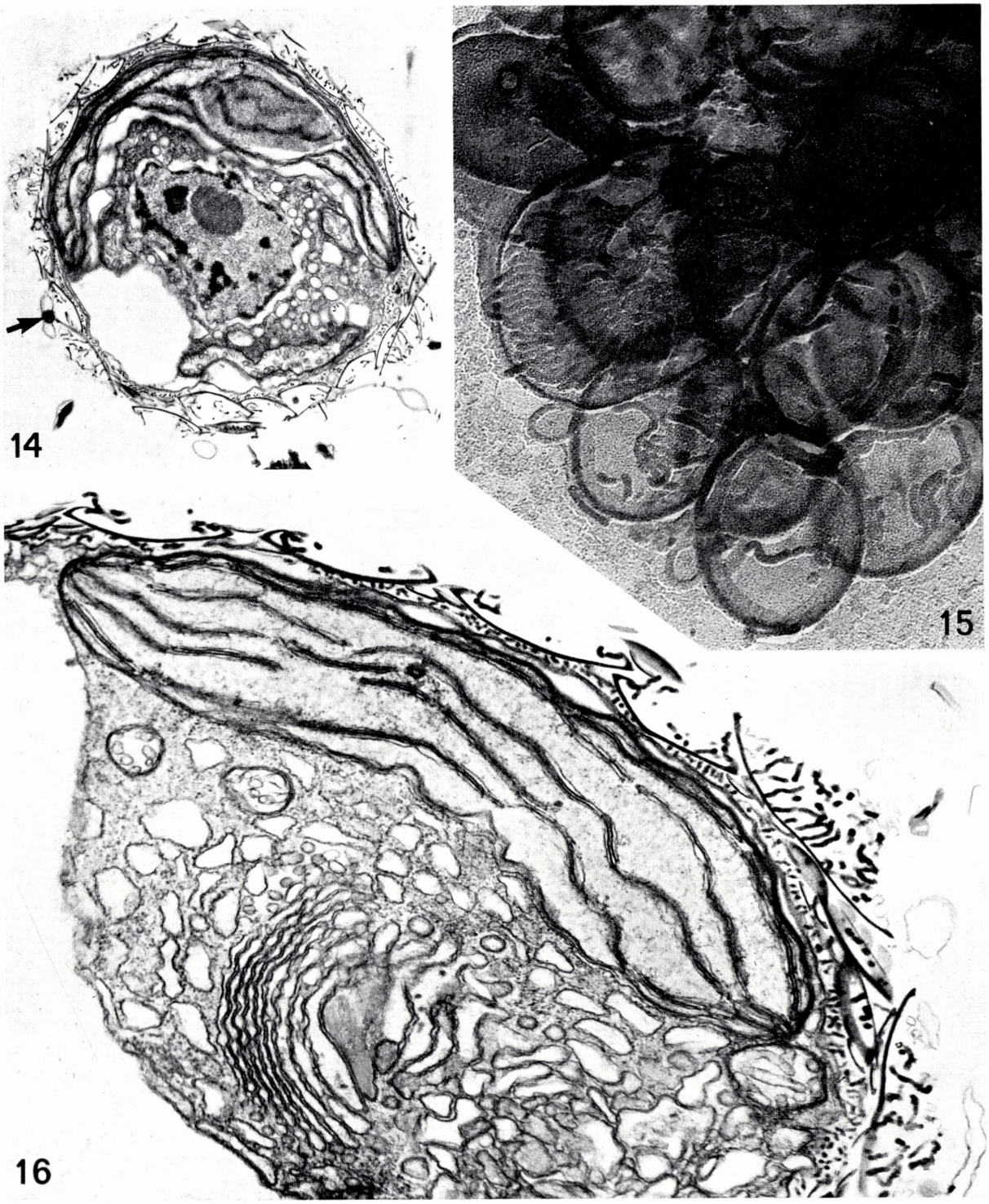

14
15
16

Plate IV

C. aff. fragilis Leadbeater

Fig. 17 Scales and debris, the former consisting of many small rimmed ovals each with a central streak superposed on some extremely thin large rimless plates, patternless centrally but with a pattern of slightly curved ridges peripherally but no rim. Micrograph L 3631 (Birmingham EM6) reversed print × 30,000.

Fig. 18 A cell with flagellar bases, a plastid (not transecting the pyrenoid), mitochondria, superficial scales and the Golgi system centrally (more highly magnified in fig. 19). Micrograph Y 3601 (Nottingham EM6B) × 10,000.

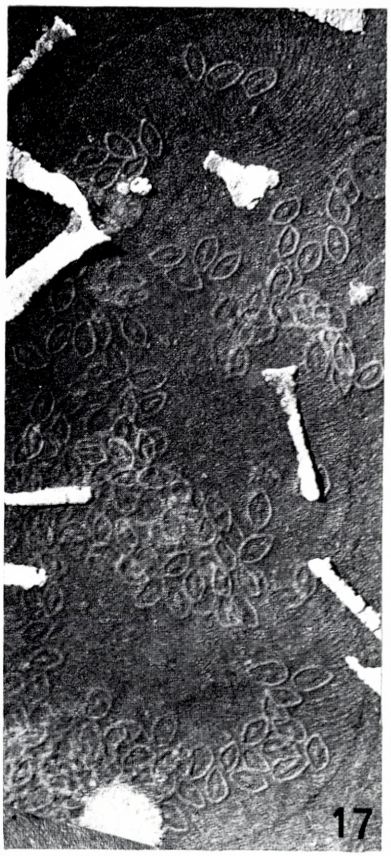
Fig. 19 Part of the Golgi system with adjacent flagellar bases from the section of fig. 18 showing part of a large scale inside a cisterna. Micrograph Y 3601 (Nottingham EM6B) × 30,000.

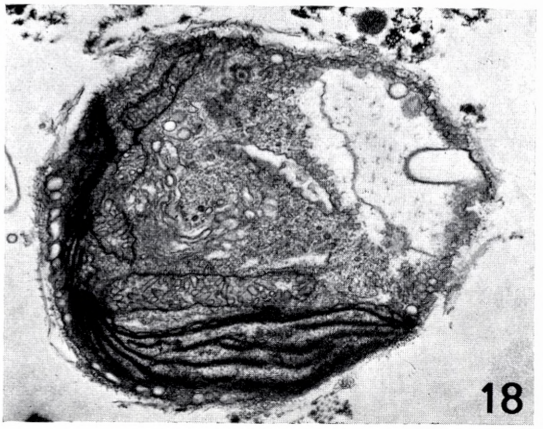
Fig. 20 Field of detached scales in a section, showing many small oval scales and curved ridges from the edges of large scales. Micrograph Y 3438 (Nottingham EM6B) × 50,000.

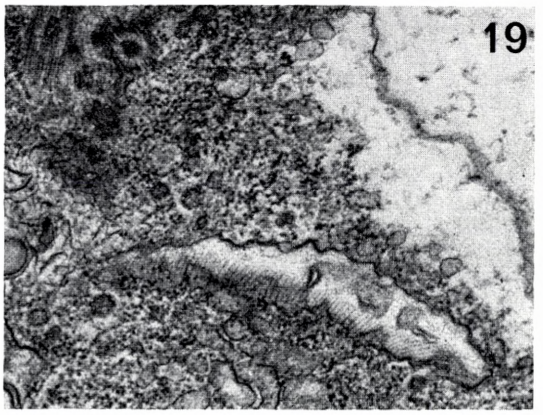
Fig. 21 Scales on a surface (cell of Fig. 23) showing a large scale overlying small scales (left) and overlapping another large scale (right), a deposit of small columnar dense particles between the scale and the plasmalemma. Micrograph Y 2547 (Cambridge 801) × 50,000.

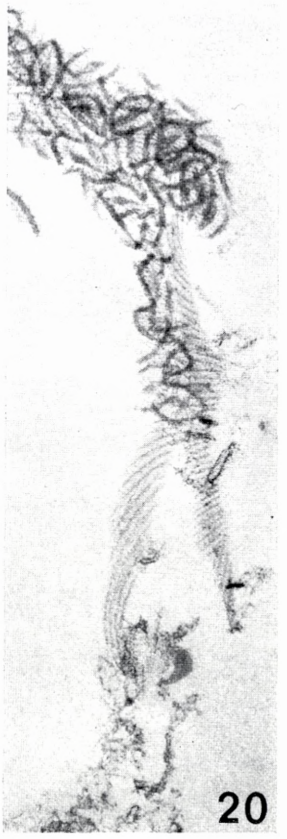
Fig. 22 Plastid from the cell of Fig. 23 showing the layered structure of the pyrenoid as in *C. herdlensis*. Micrograph Y 2546 (Cambridge 801) × 25,000.

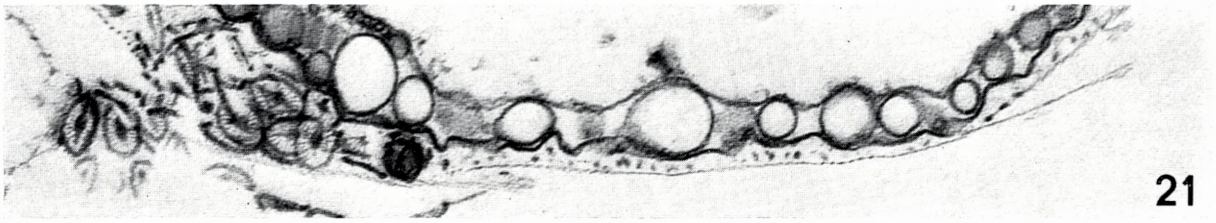
Fig. 23 The cell of Figs. 21 and 22 showing the single plastid, part of the nucleus, Golgi system and a flagellar base. Micrograph Y 2544 (Cambridge 801)× 10,000.

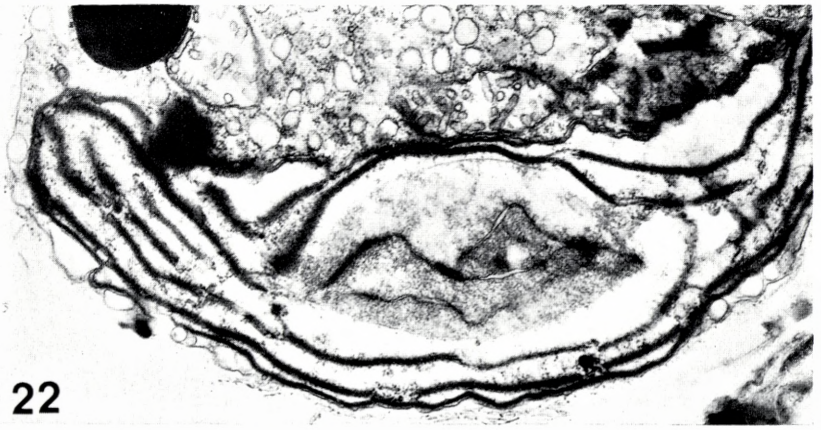

17


18


19


20


21


22

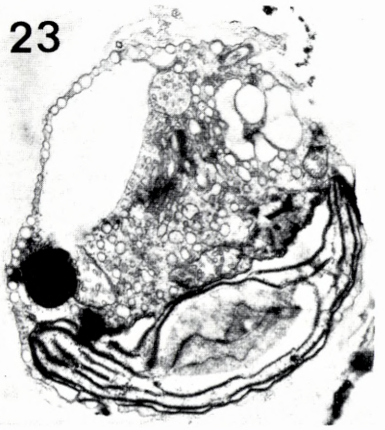

23

Plate V

C. bergenensis Leadbeater

Fig. 24 A complete cell in a shadowed whole mount. Micrograph L 3458 (Birmingham EM6) × 5,000.

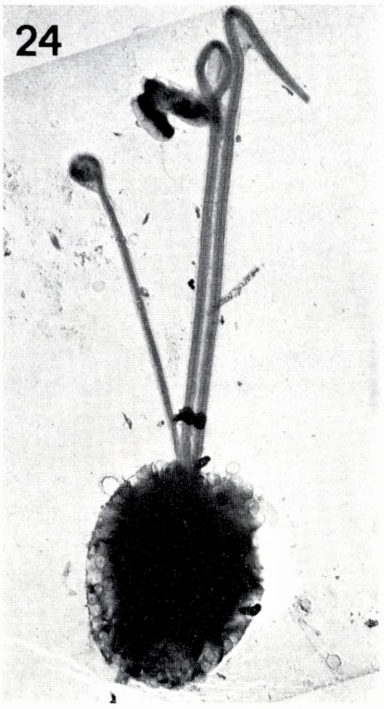
Fig. 25 Part of a longitudinally cut haptonema with authenticating scales at the bottom and an intercalary swelling (for further description see text). Part of a cell of *Micromonas pusilla* near the haptonema. Micrograph Y 4207 (Ottawa Siemens Elmiskop 1A) × 20,000.

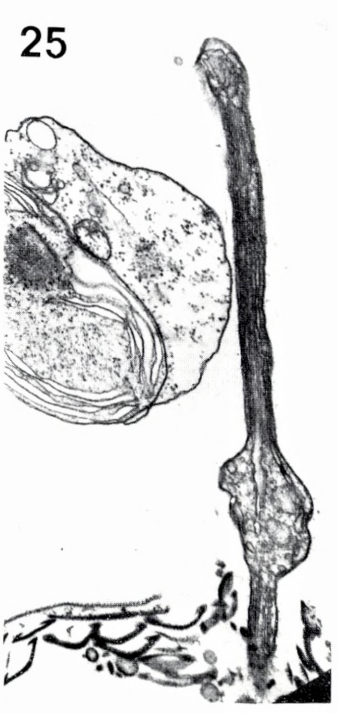
Fig. 26 Another specimen showing an intercalary swelling on the haptonema and with part of an obliquely cut flagellum at the top of the field. Micrograph Y 3618 (Nottingham EM6B) × 20,000.

Fig. 27 Another section through the specimen of Fig. 26 showing the densely compacted interior of the intercalary swelling with traces of longitudinally running microtubules centrally and lateral arcs of endoplasmic reticulum (for further description see text). Micrograph Y 3620 (Nottingham EM6B) × 30,000.

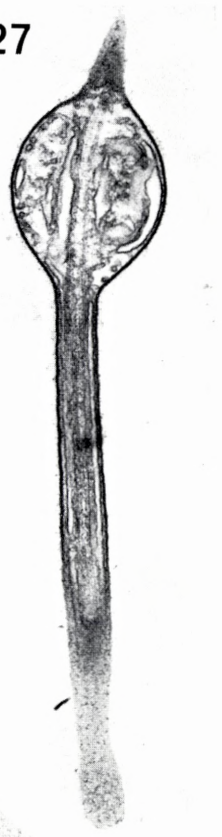
Fig. 28 Part of the edge of a scale case showing folded plate scales, some with a rough peripheral deposit and a few with rims (left), all underlain by smaller rimmed plates; part of the ridged proximal surface of both sizes of scales visible here and there (see especially top left and bottom right). Micrograph L 3139 (Birmingham EM6) × 30,000.

Fig. 29 Part of a section cut perpendicularly to the plasmalemma showing flat rimless and concave rimmed scales, collectively covered by a skin and underlain by columnar and globular dense material; part of a cell of *Scourfieldia marina* Throndsen entering field at left and of *Phaeocystis pouchetii* (Hariot) Lagerheim at right. Micrograph Y 3157 (Nottingham EM6B) × 30,000.


24


25


26


27


28

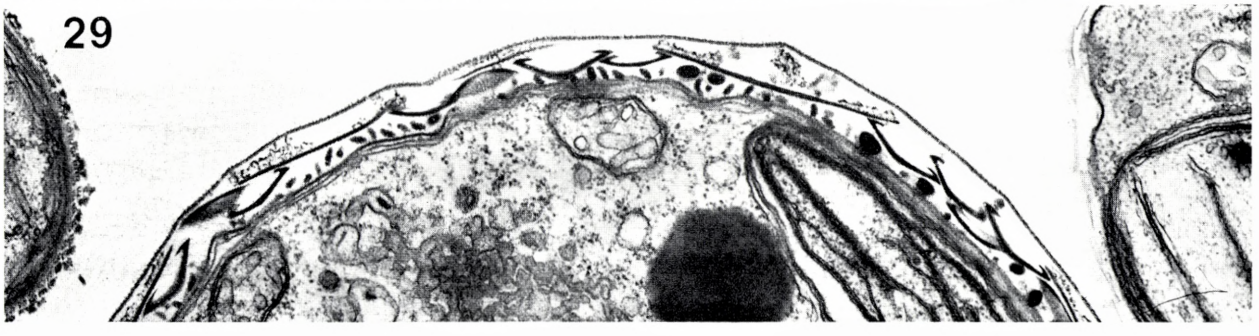

29

Plate VI

C. bergenensis contd.

Fig. 30 Part of a cell with authenticating scales, an obliquely cut flagellum (right) and several profiles from a coiled haptonema, "balooned" in one place (top right) as a fixation artifact quite distinct from the intercalary swellings illustrated in Figs. 25–27. A detached stellate scale from a species of *Heteromastix* beside the haptonema at top centre; for further details of the haptonema see Fig. 31. Micrograph Y3760 (Leeds Zoology, EM6B) × 20,000.

Fig. 31 Detail from another section through the "balooned" region of the haptonema at top right in Fig. 30, showing a flattened ring of 6 microtubules. Micrograph Y 3755 (Leeds Zoology EM6B) × c. 100,000.

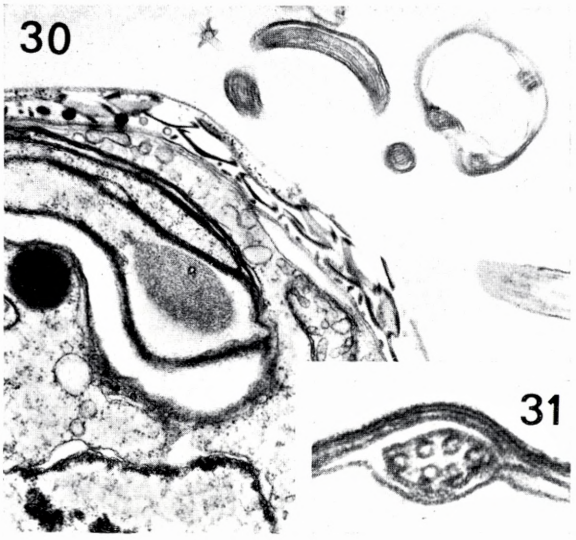
Fig. 32 Another section through the specimen of Figs. 30 and 31 showing the whole cell with additional evidence of coiling of the haptonema. Micrograph Y 3764 (Leeds Zoology EM6B) × 10,000.

Fig. 33 Oblique section of a cell, showing part of an emerging flagellum, authenticating scales on the cell surface, a plastid with immersed pyrenoid of simple structure, the Golgi system etc. from the specimen which also gave Figs. 26 and 27. Micrograph Y 4614 (Nottingham EM6B) × 15,000.

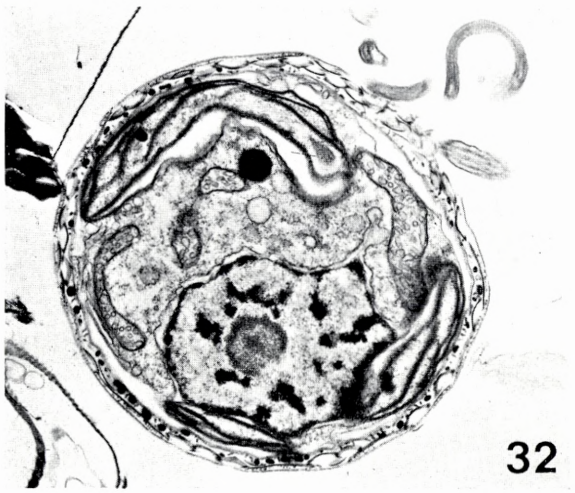
Fig. 34 Transverse section of a cell with authenticating scales, showing the nucleus and two plastids, each with a pyrenoid; for further description see text. Micrograph Y 2353 (Nottingham EM6B) × 15,000.

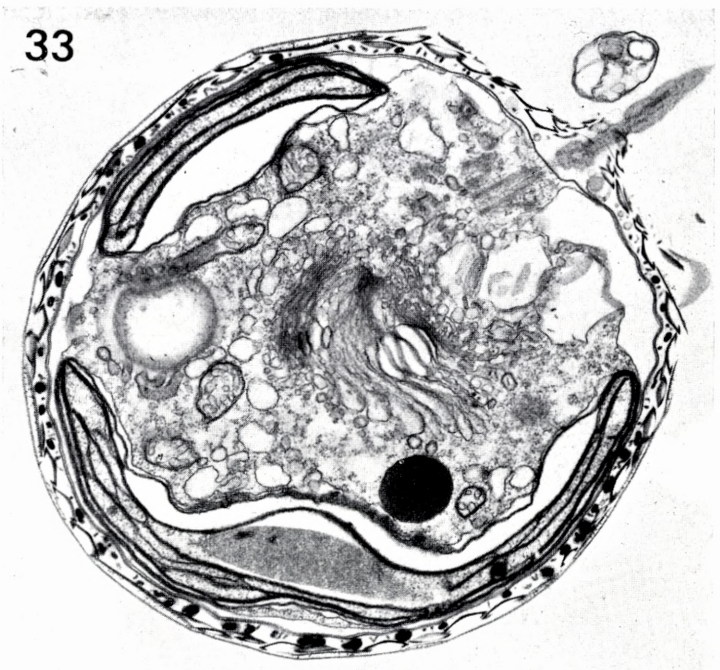
Fig. 35 A cell with authenticating scales and with a very large food vacuole containing detritus at the end remote from the flagella. For further details of this specimen see Plate VII. Micrograph Y 2401 (Nottingham EM6B) × 10,000.

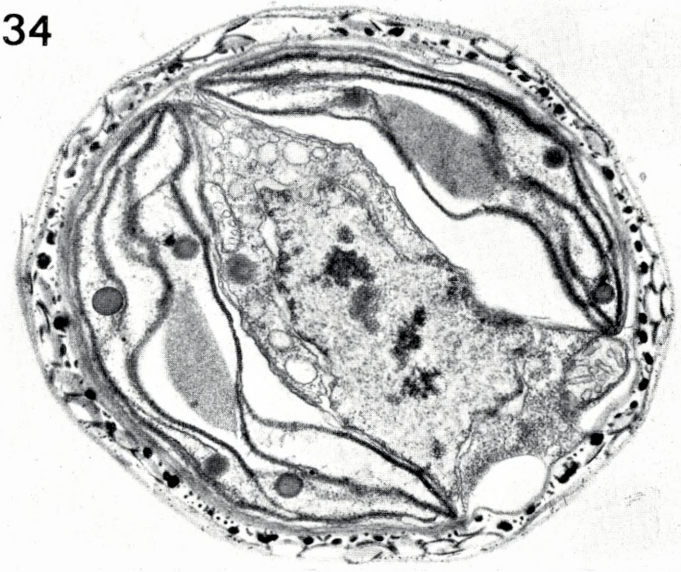
Fig. 36 Section of a periplast showing the conspicuously different texture of the skin versus both types of scale. Micrograph Y 4386. 18 (Philips 300, Leeds, Biophysics) × c. 60,000.

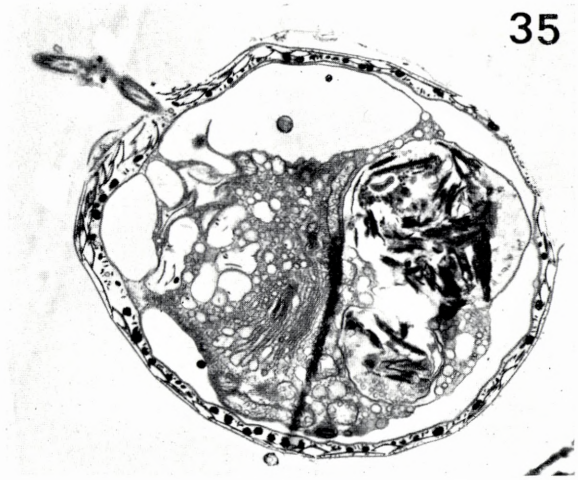

30

31


32


33


34


35

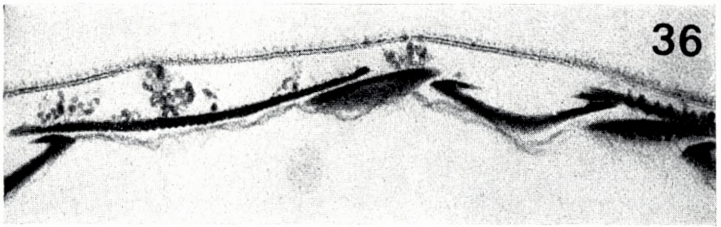

36

Plate VII

C. bergenensis contd.

Figs. 37 and 38 — Two successive sections through the flagellar end of the specimen of Fig. 35 to show the periplast, Golgi system and derived vesicles more clearly; for more highly magnified details see Figs. 39–41. Micrographs Y 2402 and 2403 (Nottingham EM6B) × 25,000.

Figs. 39–41 — More highly magnified details from the sections of Figs. 37 and 38 showing the various periplast components outside the cell (fig. 39) and inside the cell in Golgi derived cisternae (Figs. 40, 41). Micrographs Y 2402 and 2403 (Nottingham EM6B) × 50,000.

Fig. 42 — Periplast from another specimen showing a granular deposit on and above the large rimless scales and with coarse columnar material beneath. Micrograph Y 3157 (Nottingham EM6B) × 50,000.

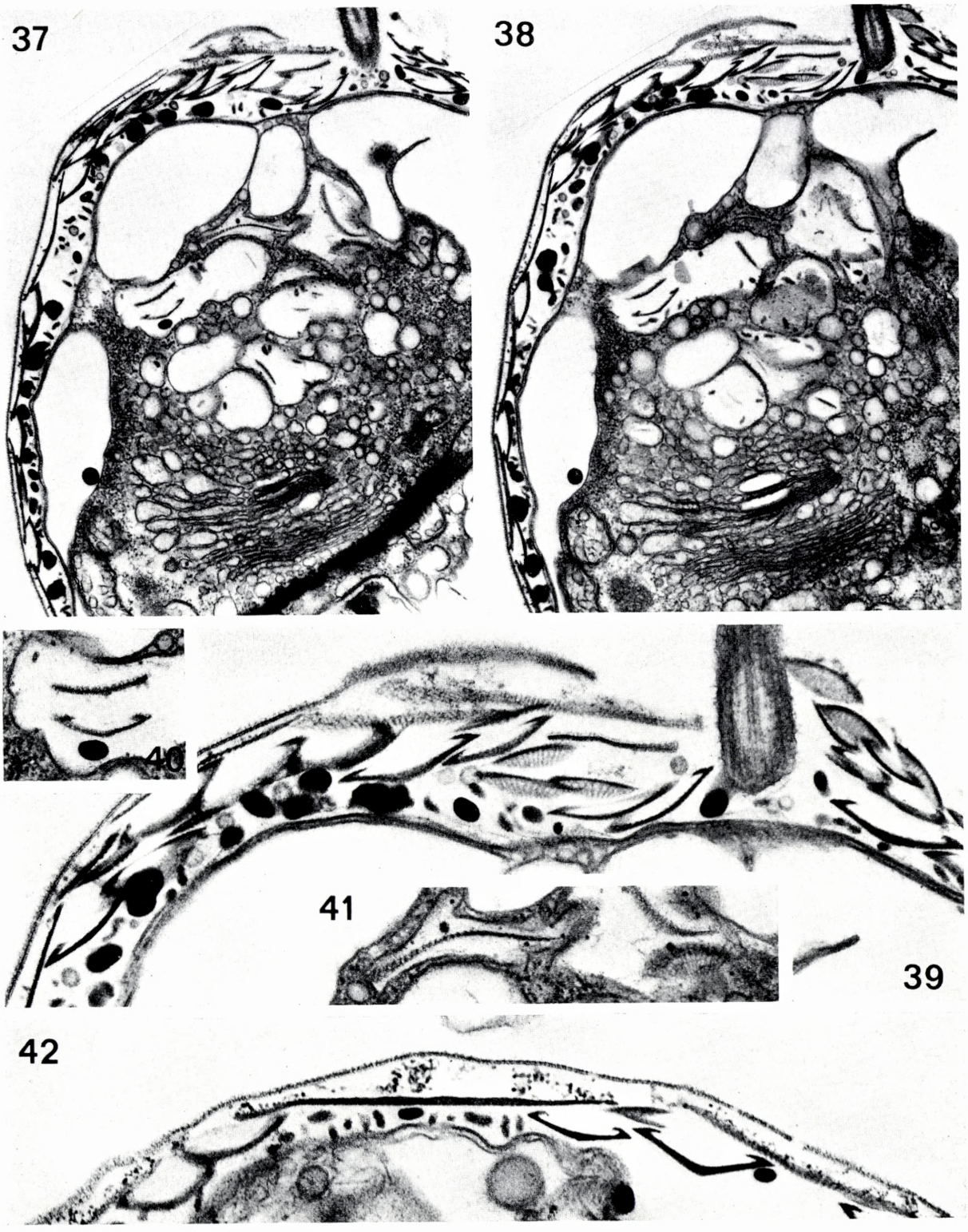

37
38
40
41
39
42

Plate VIII

C. mantoniae Leadbeater

Fig. 43 A dried cell of *C. mantoniae* showing a flexed haptonema, plate scales and two large spines; an alien "tintack" spine from *Paraphysomonas imperforata* at right, part of a small diatom frustule at bottom. Micrograph L 3697 (Birmingham EM6) × 5,000.

Fig. 44 A spine and associated scales; further details of this specimen in Fig. 47. Micrograph L 3635 (Birmingham EM6) × c. 9,000.

Fig. 45 Shaft of another spine showing spiral twist; further details of the tip in Fig. 46. Micrograph L 3681 (Birmingham EM6) × 15,000.

Fig. 46 Tip of the spine from Fig. 45 showing the smoothly tapered point. Micrograph L 3681 (Birmingham EM6) × 30,000.

Fig. 47 Base of the spine and accompanying scales of the specimen in Fig. 44. Micrograph L 3636 (Birmingham EM6) × 20,000.

Fig. 48 Plate scales of two sizes showing the rims and superficial patterning. Micrograph L 3452 (Birmingham EM6) × 30,000.

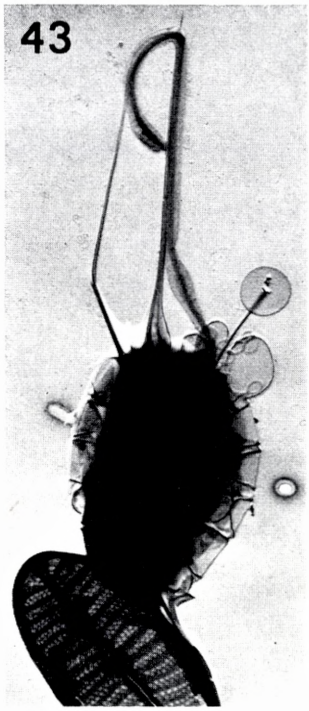
Fig. 49a–c Series of three sections through a small spine beside a piece of diatom frustule, the base plate visible in Fig. 49c(bottom) and the hollow condition of the spine itself in Figs. 49a and b. Micrographs Y 4234, 4224, 4225 (Siemens Elmiskop 1A, Ottawa) × 30,000.

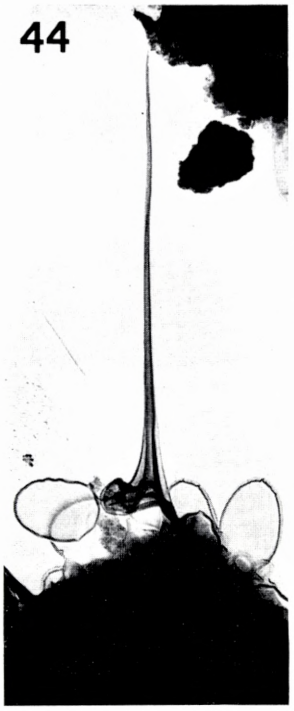
Fig. 50 Oblique longitudinal section through a spine, mainly passing through a strut attached to the base plate (below) with the spine itself above. Micrograph Y 4148 (Siemens Elmiskop 1A, Ottawa) × 30,000.

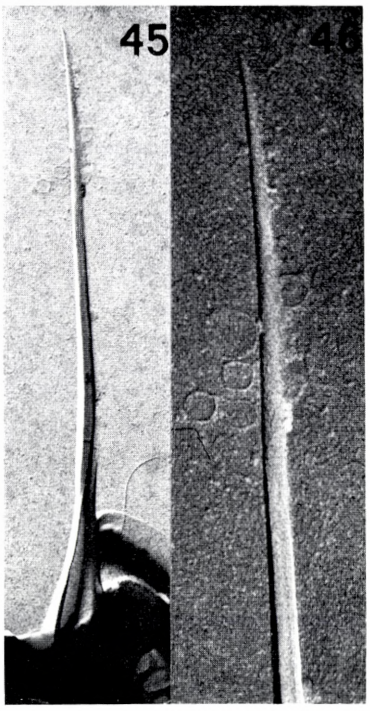
Figs. 51a and b Successive longitudinal sections through the distal end of a spine showing the solid condition (in contrast to the hollow condition lower down as seen in Figs. 49a and b), the apex itself (Fig. 51a) slightly bent during sectioning but otherwise smoothly tapered. Micrographs Y 3211 and 3210 (Nottingham EM6B) × 30,000.

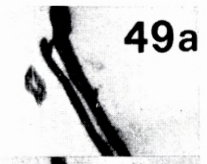
Fig. 52 Vertical section of scales on a surface including the base of a spine at right. Micrograph Y 3896 (Nottingham EM6B) × 30,000.

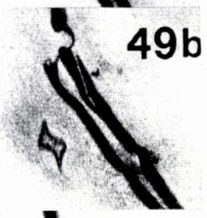
Fig. 53 Vertical section of a spine base and other scales in position on a cell surface, for further description see text. Micrograph Y 4331 (Leeds Zoology Dept. EM6B) × 30,000.


43

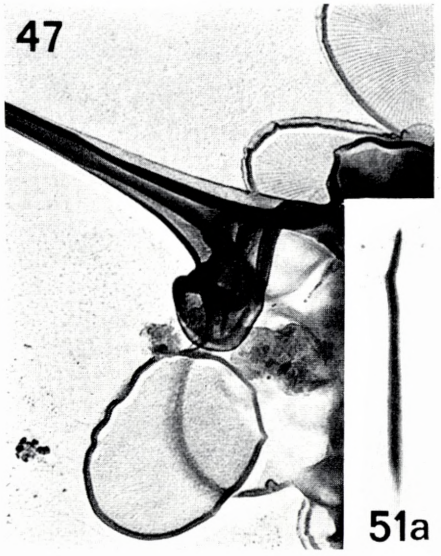

44


45

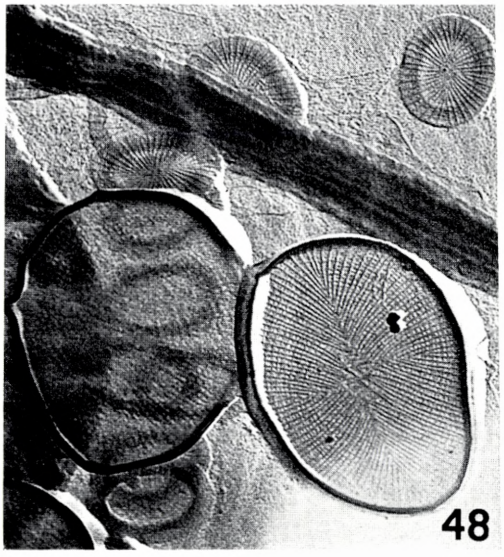

49a

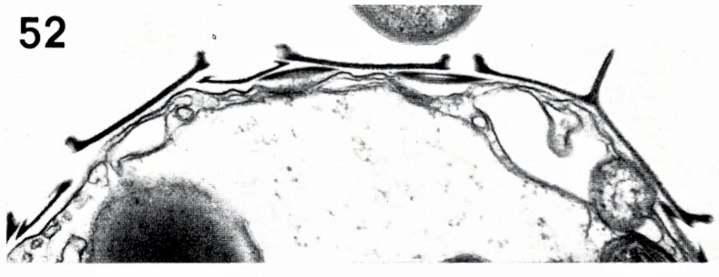

49b


49c


47
51a


50
51b


48


52

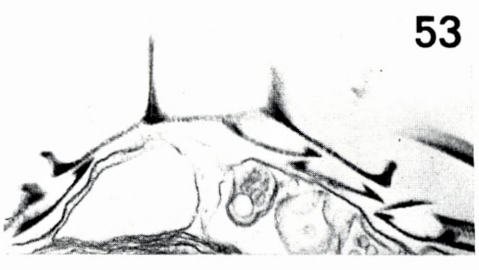

53

Plate IX

C. mantoniae contd.

Fig. 54 Oblique longitudinal view of a cell showing the nucleus and other organelles, including a laterally attached flagellum and a superficial protuberance resulting from recent detachment of a spined scale; profiles of two spines from the same section in upper inset after deletion of 10 mm of ground on the print between these and the cell. Micrograph Y 3051 (Leeds Zoology Dept. EM6B) × 10,000.

Fig. 55 Part of a transversely cut cell showing the superficial scales and a plastid with an immersed pyrenoid penetrated by three unpartitioned tubular extensions of thylakoids. Micrograph Y 3905 Nottingham EM6B) × 20,000.

Fig. 56 Section of a cell exposing a pyrenoid cut longitudinally. Micrograph Y 3892 (Siemens, Ottawa) × 10,000.

Fig. 57 Part of an oblique longitudinal section, near but not including the flagellar pole, showing authenticating scales, the Golgi system and the two lateral plastids each with an immersed pyrenoid; for other views from the same specimen see Figs. 58a and b. Micrograph Y 3305 (Leeds Zoology Dept. EM6B) × 20,000.

Figs. 58a and b Parts of other sections through the specimen of Fig. 57, one (Fig. 58a) showing the flagellar attachment beside a spine base still in situ, the other (Fig. 58b) showing the complete section with a scale-lined depression at the non-flagellar pole and another view of the attached spine beside the flagellar pole. Micrographs Y 3823 and Y 3816 (Leeds Zoology Dept. EM6B) × 10,000.

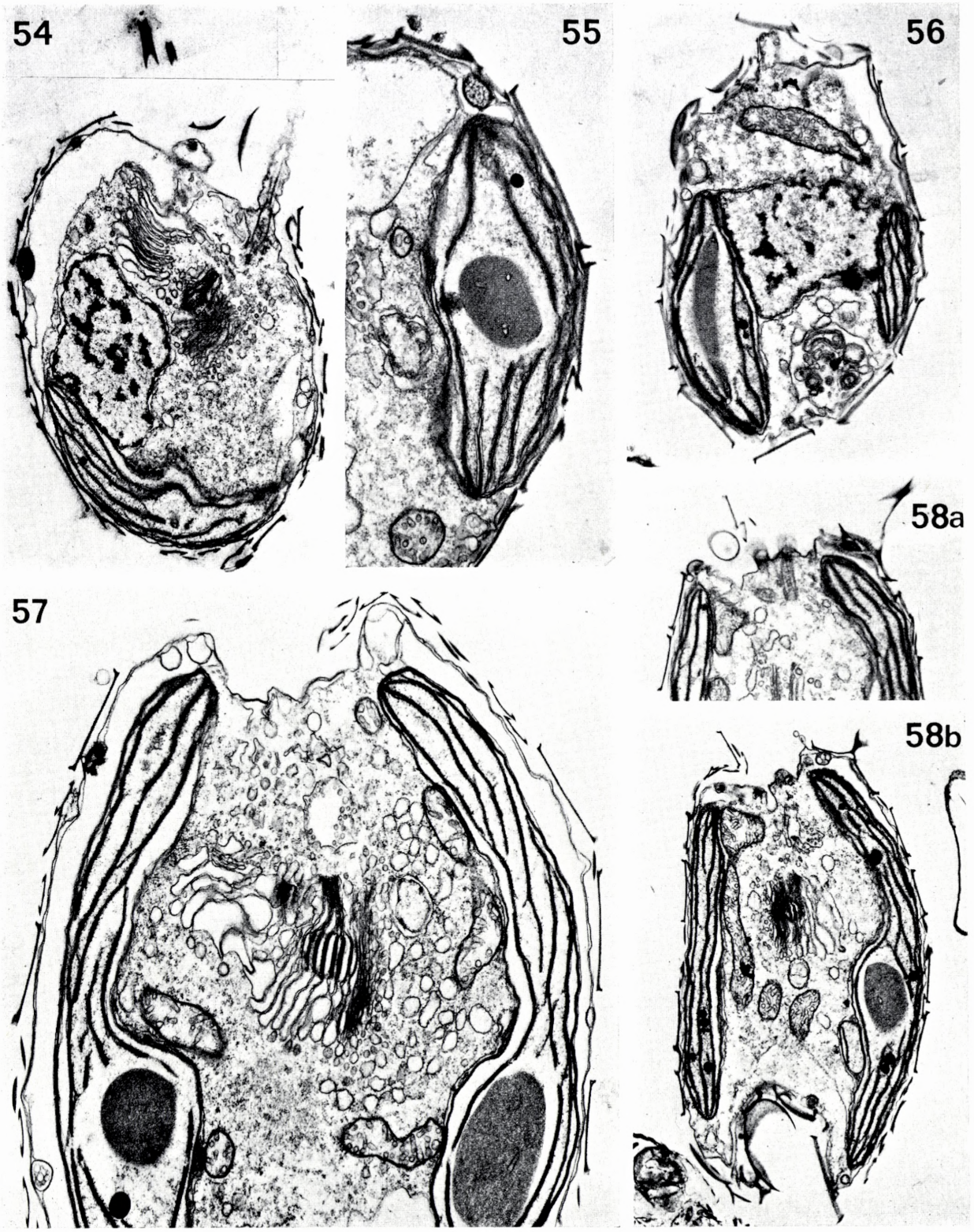

54
55
56
58a
57
58b

Plate X

C. mantoniae contd

Fig. 59 A field with two sections of a haptonema (arrows) separated by an oblique section of a spine, the subtending cell below; for further detail of upper haptonema profile see Fig. 60. Micrograph Y 2637 (Cambridge 801) × c. 20,000.

Fig. 60 The uppermost section of the haptonema in Fig. 59 more highly magnified to show the 7 component microtubules plus investing membranes and cavity. Micrograph Y 2638 (Cambridge 801) × 100,000.

Fig. 61a–g Successive sections through a spine from above downwards all somewhat oblique and with a considerable gap between f and g. For further description see text. Micrographs Y 3930, 3931, 3927, 3929, 3923, 3924 and 3934 (Nottingham EM6B) × 30,000.

Fig. 62 Oblique section through a spine near the point of union of the four struts showing a perforation between a strut and the subtending base plate (arrow) for further description see text. Micrograph Y 3196 (Nottingham EM6B) × 40,000.

Fig. 63 Part of the specimen of Fig. 61e showing the perforation and the patterning on the various surfaces more clearly. Micrograph Y 3923 (Nottingham EM6B) × 40,000.

Fig. 64 Part of a large plate scale to show surface patterning, for comparison with that on the base plate of the spine. Micrograph Y 2982 (Leeds Zoology Dept. EM6B) × 40,000.

Fig. 65 Part of a dead or moribund cell, authenticated by scales, showing infection with a virus, giving hexagonal cross sections (for further description see text and Fig. 66). Micrograph Y 3153 (Nottingham EM6B) × 30,000.

Fig. 66 Some of the infecting virions from Fig. 65 showing contents and hexagonal shape, indicating a 20 sided three dimensional condition. Micrograph Y 3153 (Nottingham EM6B) × 100,000.

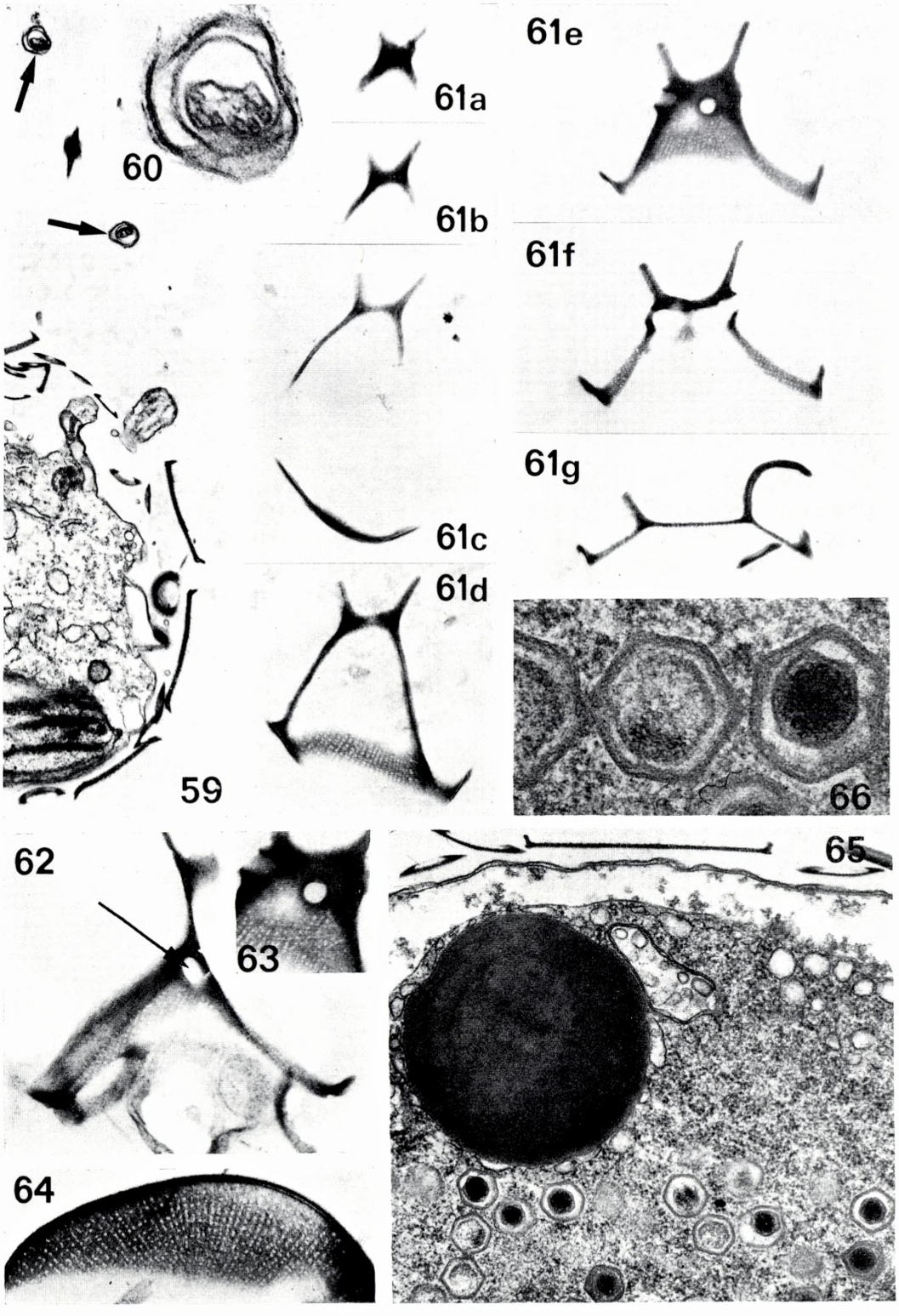

60
61a
61b
61c
61d
61e
61f
61g
59
66
62
63
65
64

Plate XI

C. campanulifera sp. nov

Fig. 67 Field from a shadowcast whole mount stripped from glass, showing a cell of *C. campanulifera* (left) with flagella, but no haptonema (probably superposed upon or beneath the body); for further details of the periplast see Fig. 75. A collared flagellate (*Salpingoeca natans*) and part of a broken diatom frustule included in the field at right. Micrograph Y 4391.4 (Lancaster 801) × 2,500.

Fig. 68 Cell body with a coiled haptonema and the bases of the two flagella from a direct preparation not stripped from glass. Micrograph Y 4394.42 (Lancaster 801) × 5,000.

Fig. 69 Haptonema on the specimen of Fig. 68 more highly magnified showing many incompletely resolvable gyres, estimated as not less than 20. Micrograph Y 4394.39 (Lancaster 801) × 10,000.

Figs. 70 and 71 Two sections through one specimen, collectively showing the periplast, nucleus, mitochondria, parts of the Golgi system and a somewhat broken food vacuole containing the partly decomposed remains of an alien haptonema. Microgrphs Y 4260 and 4282.
(Siemens Elmiskop 1A, Ottawa) × 10,000.

Fig. 72 Part of a cell with a coiled haptonema covered by an extension from the periplast (compare with Fig. 70). Micrograph Y 3114 (Nottingham EM6B) × 30,000.

Fig. 73 A more highly magnified view of one of the adjacent haptonema sections, showing the 6 microtubules and two profiles of the haptonema cavity, one crescentic and the other circular in outline. 100,000.

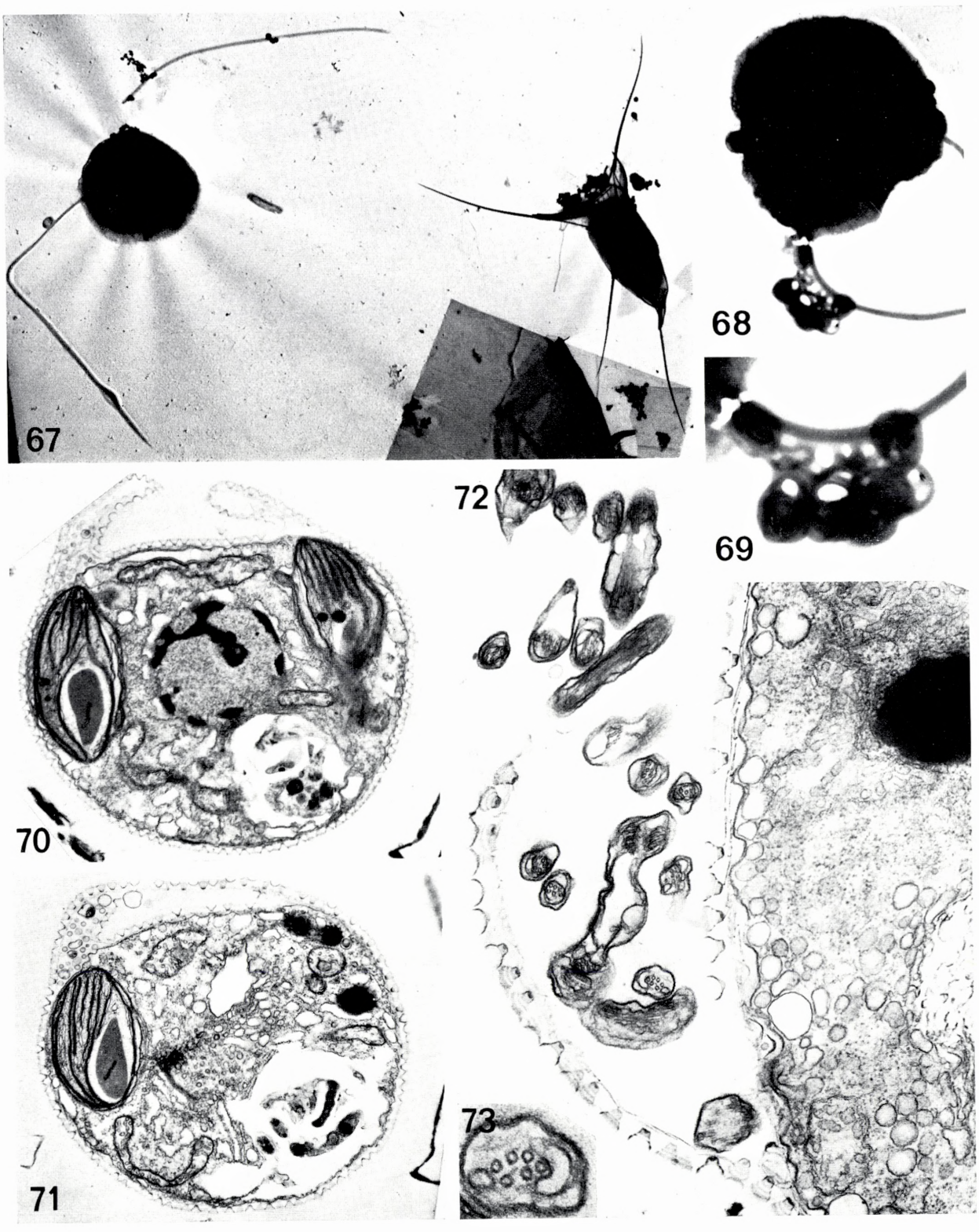

67
68
69
70
71
72
73

Plate XII

C. campanulfera cont. and C. strobilus Parke & Manton (Figs. 81–83)

Fig. 74 Pyrenoid of *C. campanulifera* showing authenticating scales on the cell and parts of a septate tube (paired thylakoid) penetrating each end of the storage area. Micrograph Y 3632 (Nottingham EM6B) × 20,000.

Fig. 75 Periplast from the specimen of Fig. 67 showing cup scales embedded in copious mucilage. Micrograph Y 4391.47 (Lancaster 801) × 30,000.

Fig. 76 Periplasts of two adjacent individuals showing different scale sizes (compare Figs. 79–81). Micrograph Y 3589 (Nottingham EM6B) × 30,000.

Fig. 77 Tangential section through the periplast of a cell with scales as in (Fig. 80) showing hexagonal close packing of the cup scales and somewhat imbricated arrangement of the underlying plate scales. Micrograph Y 3710 (Leeds Zoology Dept. EM6B) × 30,000.

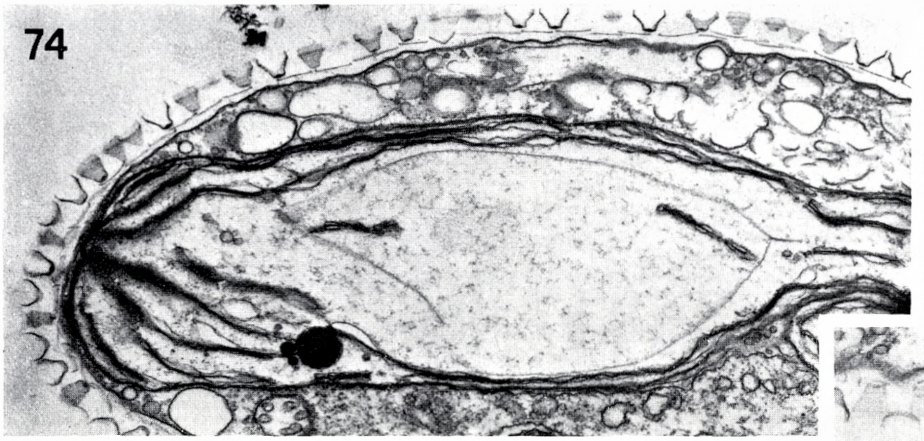
Fig. 78 Tangential section otherwise comparable to that of Fig. 77 showing the shape and patterning of plate scales more clearly. Micrograph Y 3712 (Leeds Zoology Dept. EM6B) × 60,000.

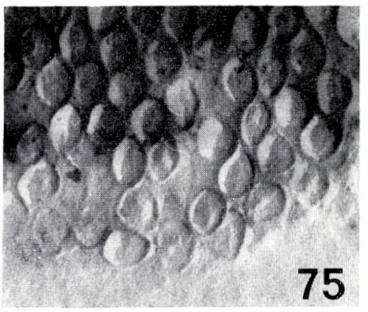
Fig. 79–81 Three variant types of cup scale of supposedly the same species (*C. campanulifera*) respectively with 3, 2, and 1 horizontal bands of patterning (for further description see text), the middle condition (Fig. 80) the commonest; for the equivalent in *C. strobilus* see Fig. 83. Micrographs Y 3715, Y 4001.5 and Y 3020 (Leeds Zoology Dept. EM6B) × 60,000.

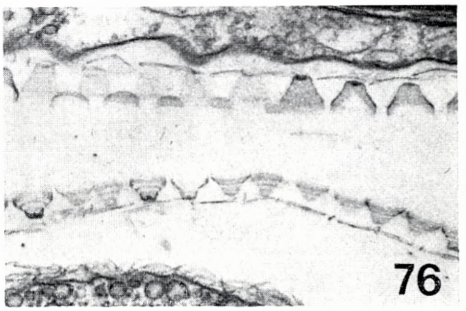
Fig. 82 A cell of *C. strobilus* in the same block as preceding micrographs of *C. campanulifera*. Micrographs Y 3629 (Nottingham EM6B) × 10,000.

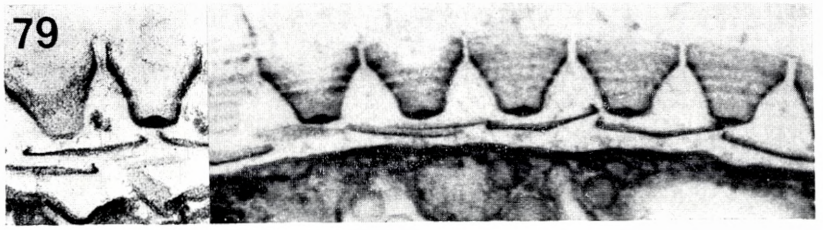
Fig. 83 Vertical section of scales of *C. strobilus* from another section of the specimen of Fig. 82 for comparison and contrast with those of *C. campanulifera* (Figs. 79–81); for further description see text. Micrograph Y 4656 (Imperial College, London EM6B) × 60,000.

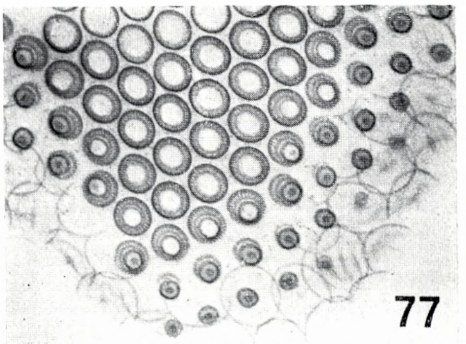
Fig. 84 Tangential section through the scales of *C. strobilus* to show the relatively patternless small oval scales of the underlayer for comparison with those of *C. companulifera*. Micrograph Y 4669 (Imperial College, London EM6B) × 30,000.

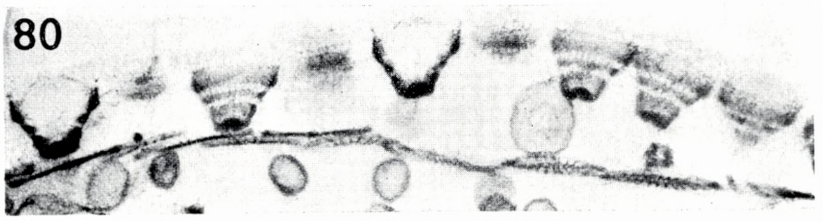

74

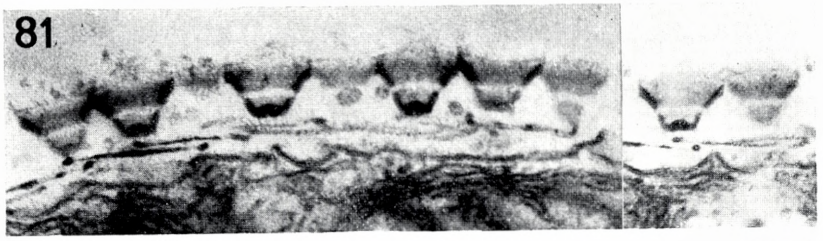

75

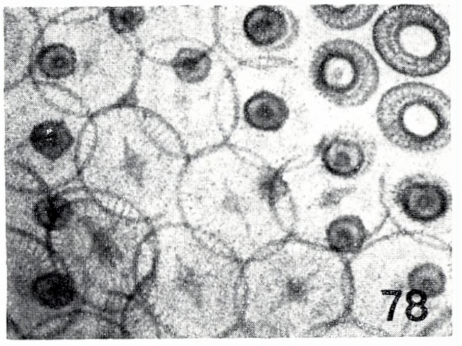

76

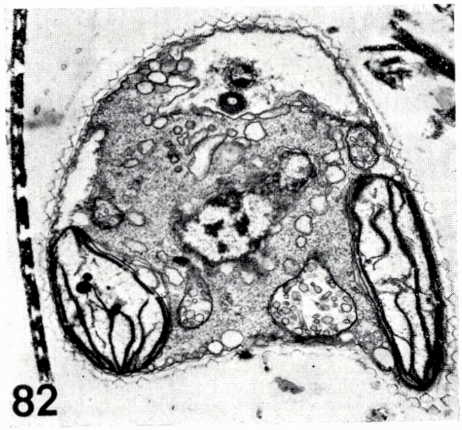

79

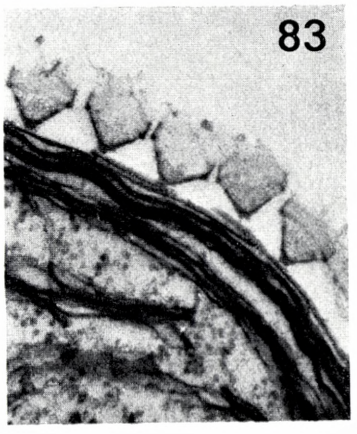

77

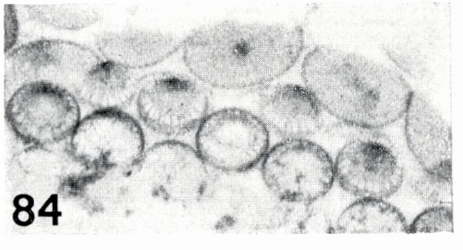

80


81


78


82


83


84