Dictyocha speculum

(Silicoflagellata, Dictyochophyceae), studies on armoured and unarmoured stages

By ØJVIND MOESTRUP & HELGE A. THOMSEN

Biologiske Skrifter **37**

Det Kongelige Danske Videnskabernes Selskab
The Royal Danish Academy of Sciences and Letters

Kommissionær: Munksgaard · Copenhagen 1990

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Synopsis

Several different cell types believed to represent stages in the life cycle of the marine silicoflagellate *Dictyocha speculum* Ehrenberg have been investigated from Danish waters. The studies were initiated by a fish-kill in 1983, believed to be caused by a yellow flagellate, 10-20 µm in diameter and, as discussed in the present article, considered to represent a naked (skeleton lacking) form of *Dictyocha speculum*. Two further stages were found, a very large multinucleate stage (up to 500 µm long) and an amoeboid stage. They showed no trace of a skeleton. The different stages were examined by various techniques, including transmission and scanning electron microscopy, interference light microscopy and video recording.

The skeleton of *Dictyocha* is external, in contrast to what is often stated in the literature. The cell is biflagellate but the second flagellum is very short (the naked stage) or represented only by its basal body (the skeleton bearing stage).

The long flagellum is wing-like, and bears flagellar hairs. Cytoplasmic details include a unique type of pyrenoid in the chloroplasts, a complex internal canal system which is found only in the skeleton bearing stage, characteristic plate-like elements in the Golgi cisternae of all stages examined, and intracellular bacteria, also in all stages.

In Danish waters the skeleton bearing stage appears in maximum concentration in the autumn, with occasional small peaks in the spring. The skeleton lacking stage typically occurs in spring.

Findings of the naked stage are reported also from North America and Australia.

The data on the toxicological potential of silicoflagellates are inconclusive.

Some comments are given on the phylogenetic affinities of silicoflagellates. Based on pigments and type of flagellation clear affinities exist with other heterokont algae, and certain details of the flagellar apparatus indicate affinity to pedinellalean chrysophytes. In a natural classification these should probably be grouped together.

Our findings do not support the idea that *Dictyocha speculum* and *D. fibula* Ehrenberg are conspecific. They differ in skeleton morphology and a number of cytoplasmic details, and represent two of the three known extant species of a group of organisms much more diverse in previous geological periods.

The generic name *Dictyocha* is used, as the commonly used *Distephanus* Stöhr is a later homonym of *Distephanus* Cass. and thus illegitimate.

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Printed in Denmark by Special-Trykkeriet Viborg a-s
ISSN 0366-3612 ISBN 87-7304-207-2

Contents

Introduction

The results presented here have been obtained over a period of several years. The project began when, in early May 1983, a serious fish-kill took place in a mariculture farm in southwestern Denmark. The water in the area was coloured yellow brown, and a sample sent to Institut for Sporeplanter at University of Copenhagen contained large numbers of a distinct, but underscribed yellow flagellate. A large amoeboid organism, possibly representing another stage of the same species, was also present. Light microscopy gave few clues to its identity, and a sample was therefore fixed and examined using transmission electron microscopy. This showed a highly unusual cell structure, but certain features, notably pyrenoid structure, indicated that the organism was perhaps a silicoflagellate without its skeleton. Cells were isolated into pure culture, but they did not produce skeletons and this cast some doubt on whether they were indeed silicoflagellates.

The only common silicoflagellate in Danish waters is *Dictyocha speculum* Ehrenberg (= *Distephanus speculum* (Ehrenb.)Haeckel), a species which occurs worldwide, but has not been examined by high resolution electron microscopy. In October 1984 typical skeleton bearing cells occurred in relatively large numbers at the entrance to Isefjorden, Sealand, Denmark. The opportunity was taken to fix and section skeleton bearing cells for comparison with the naked cells from the fish-kill. Twelve cells were serial sectioned, and the pyrenoid structure typical of the naked flagellate was refound. Several surprising differences will be described below.

Finally in 1985 the plankton in Isefjorden contained large numbers of spherical brown bodies, some large enough to be visible with the naked eye and judged by their brown colour to be *Phaeocystis*. No flagella were observed, but transmission electorn microscopy showed that these cells almost certainly represented a third, multinucleate stage of *Dictyocha speculum*.

The only previous studies of silicoflagellates using transmission electron microscopy are on *Dictyocha fibula* from the Pacific west coast of U.S.A. (van Valkenburg 1971a, b). This author did not publish micrographs of the flagellar apparatus, and its structure is therefore unknown in silicoflagellates.

Since presenting some of our data at international meetings (e.g. Thomsen & Moestrup 1985) it has become clear from discussions with colleagues that the skeleton lacking stage of *Dictyocha* occurs worldwide. Details of the life cycle remain obscure, however.

The present paper includes observations on the ultrastructure of the 3 stages and what is possibly an intermediate stage. A separate chapter describes the seasonal occurrence of the different stages in Danish waters, and we include comments on the possible role of *Dictyocha* as a fish-killer. The phylogenetic relationship of silicoflagellates to other heterokont protists is discussed.

A note on the nomenclature used

The name *Distephanus speculum* (Ehrenberg) Haeckel was used in abstracts from meetings where some of our findings were reported (Thomsen and Moestrup 1985, Moestrup 1984). Like other authors working on silicoflagellates we overlooked that *Distephanus* Stöhr is an illegitimate name: *Distephanus* was used for a member of the Compositae by Cassini in 1817 while Stöhr's name was proposed in 1880. We have decided to follow, at least for the time being, Deflandre (1948, 1952) and others who included all extant silicoflagellates (3 species according to Deflandre) in the genus *Dictyocha* Ehrenberg 1837.

Material and methods

The uninucleate naked stage was obtained 2 May 1983 from the mariculture farm "Dan Marin" in Alssund, s.w. Denmark (temperature ~ 10°C, salinity ~ 15 ‰ S) (Text-fig. 1). The sample was first flown to the Marine Pollution Laboratory, Charlottenlund, and the following day brought to Institut for Sporeplanter. After light microscopical examination the cells were fixed for 45 min in cold 4 % glutaraldehyde in 0.1 M cacodylate buffer containing 0.15 M sucrose. They were then centrifuged into a pellet and all subsequent stages in the fixation/dehydration/embedding procedure were done in the centrifuge tube taking care not to break the pellet. The cells were rinsed in successive cold cacodylate buffers of decreasing sucrose content (30 min in 0.15 M sucrose, 20 min in 0.07 M sucrose and 10 min in sucrose-free cacodylate buffer). Cells were post-osmicated overnight in cold 2 % osmium tetroxide in 0.1 M cacodylate buffer. They were briefly rinsed in buffer before dehydration in an ethanol series: 25 min in each of cold 30 %, 50 %, 70 %, and 96 % ethanol. While in 96 % ethanol the material was brought to room temperature, and dehydration was completed in absolute alcohol (50 min, 2 changes), followed by propylene oxide (12 min, 2 changes). The propylene oxide was then replaced by a 1:1 mixture of propylene oxide and Spurr's resin and the centrifuge tube left open in a hood for 5 h to allow evaporation of the propylene oxide. The pellet was finally transferred to an embedding dish containing Spurr's resin, subdivided into smaller pieces and polymerized overnight at 70°C.

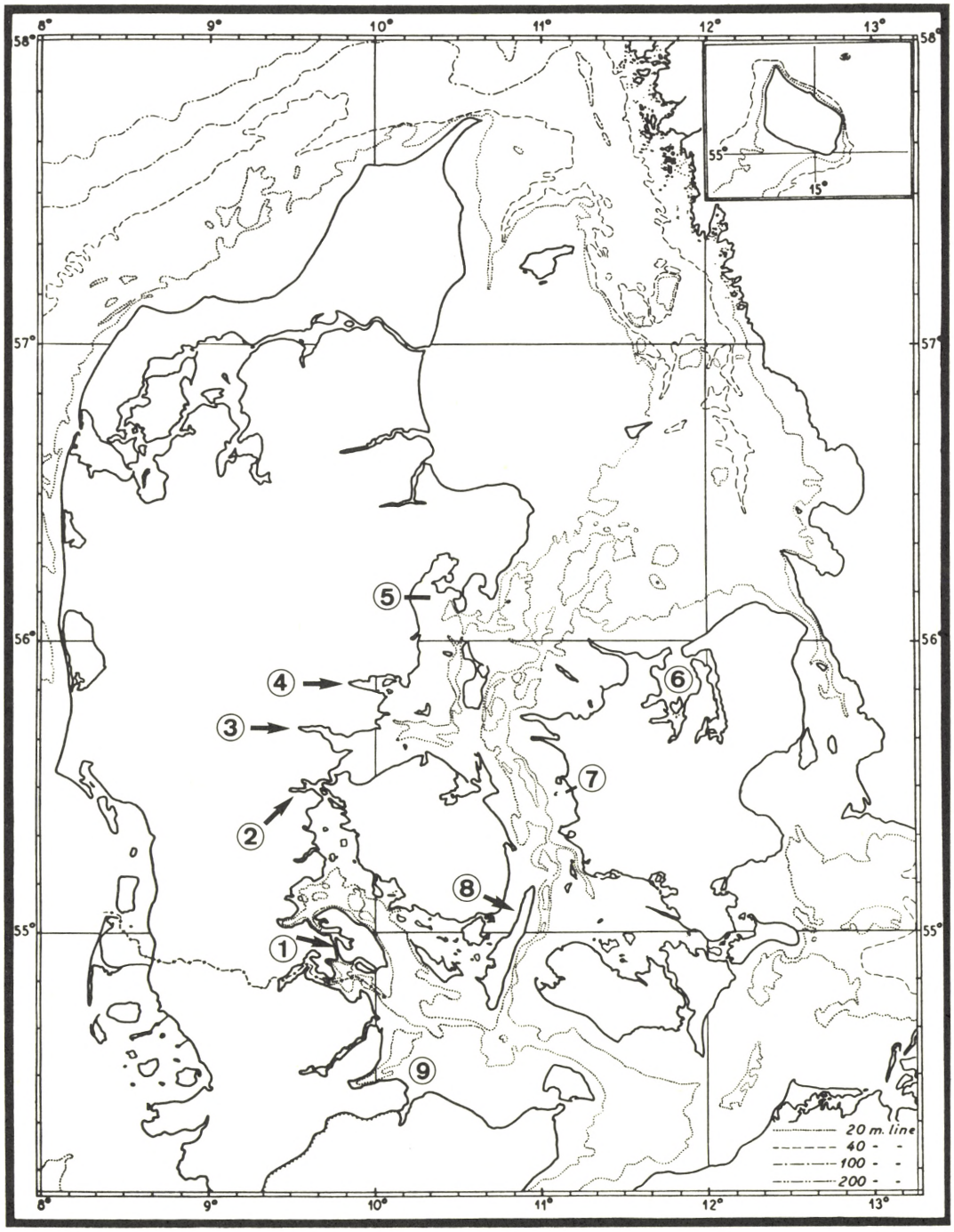
The cells were sectioned on Reichert OmU2 or Reichert-Jung Ultracut 4 ultramicrotomes, and the sections mounted on carbon or carbon/formvar films. Sections were stained for 20 min in saturated uranyl acetate in 50 % methanol, followed by 5 min in Reynold's lead citrate.

Most micrographs were taken on a JEM-T8 electron microscope, Figs 4, 19-20, 24 and 29 on a JEM 100CX microscope.

Cultures of the naked stage were started from water samples from the mariculture farm "Musholm Laks" in Musholm Bay near Reersø, western Sealand 15 June 1983 (temp. ~ 10°C, salinity ~ 20 ‰ S) (Text-fig. 1). The water sample was kept at 4°C, and growth medium was added daily, gradually replacing the natural seawater. The growth medium was made according to Throndsen (1969), using seawater from the Isefjord (salinity c. 20 ‰). After one week individual cells were isolated directly into culture medium, and rapid growth followed. The cultures were grown in tubes at 4°C, at a 16:8h light: dark regime.

The skeleton bearing stage of *Dictyocha* originated from a water sample taken in the southern part of Kattegat 30 October 1984 (sea temperature 10°C, salinity 21.9 ‰ S) (Text-fig. 1). The sample contained a bloom a *Dictyocha* and after being brought to Institut for Sporeplanter it was left in a 15°C culture room overnight. It was concentrated the following day by gentle filtration through a 20 µm plankton net. The cells, which were still swimming, were fixed in a 1:1 mixture of sample and the 4 % glutaraldehyde solution described above. The procedure followed that described for the naked stage except that post-osmication lasted c. 3 h rather than overnight. The sample was flat-embedded and 3 groups of cells, containing 3, 3 and 6 cells, isolated for thin sectioning. Sections were prepared on Reichert-Jung Ultracut 4 or LKB 2088 Ultratome V ultramicrotomes, and complete sets of serial sections were mounted on formvar-coated slot grids. They were stained as described above and examined in a JEM 100CX electron microscope.

The skeleton bearing cells from Kattegat were also used for SEM preparations. The organic mate-



Text-fig. 1. Map showing the localities mentioned in the text. 1: Alssund; 2: Kolding Fjord; 3: Vejle Fjord; 4: Horsens Fjord; 5: Århus Bugt; 6: Isefjorden; 7: Musholm Bugt; 8: Dageløkke Strand, Langeland, 9: Kieler Bucht. Salinity curves for the area are visible in Text-fig. 5.

rial was first oxidized (Simonsen 1974). Rinsed skeletons were then pipetted onto carbon/formvar coated grids and sputter coated with gold. The microscope used was a JEM 100CX electron microscope equipped with high resolution scanning attachment. In addition some of the very few skeletons in the Alssund material from May 1983 (uninucleate, naked stage bloom) were examined by SEM but without oxidation (Figs 39 and 40).

The multinucleate stage of *Dictyocha speculum* was collected from a station in the middle part of the Isefjord Outer Broad 29 April 1985 (sea temperature 5.7°C, salinity 19.1 ‰ S) (Text-fig. 1). The sample was left overnight in a 4°C culture room at Institut for Sporeplanter. The cells were concentrated the next day by gentle filtration through a 20 µm plankton net, and fixed according to the schedule used for the skeleton bearing sample. The cells were sectioned on a LKB 2088 Ultratome V, mounted on carbon/formvar films, stained as described above, and examined in a JEM 100CX or a JEM 100SX electron microscope.

All light microscopical work was carried out on a Leitz Dialux 20 microscope using interference contrast optics and either a WILD MPS 55 photoautomat or a SONY U-matic low-band videorecorder (VO-5800 PS) with a JVC KY-1900 colour video camera. The video technique proved particularly useful for studying the swimming behaviour and swimming speed of the uninucleate naked cells. The origin of the material selected for illustration was as follows (see also Text-fig. 1). Figs 1-3: Alssund, 2 May 1983; Figs 30-33: Isefjorden, 11 December 1984; (4.8°C, 22.2 % S); Fig. 75 Isefjorden, 29 April 1985.

Measurements of living cells were based on live cells in the microscope, or calculated from still-picture monitor images of videotaped material. The diameter of 50 uninucleate naked cells was measured on material fixed in Lugol's solution. The length of 100 skeletons was based on cleaned skeletons mounted in Naphrax. The measurements were obtained by computer-aided analysis of a microscopical video signal, using a Leitz ASM-68K system.

A videotape (PAL system; U-matic low band or VHS) showing the different stages of *Dictyocha speculum* (skeleton bearing form/uninucleate naked form/multinucleate naked form/amoeboid form) is available from the Institut for Sporeplanter at a small cost.

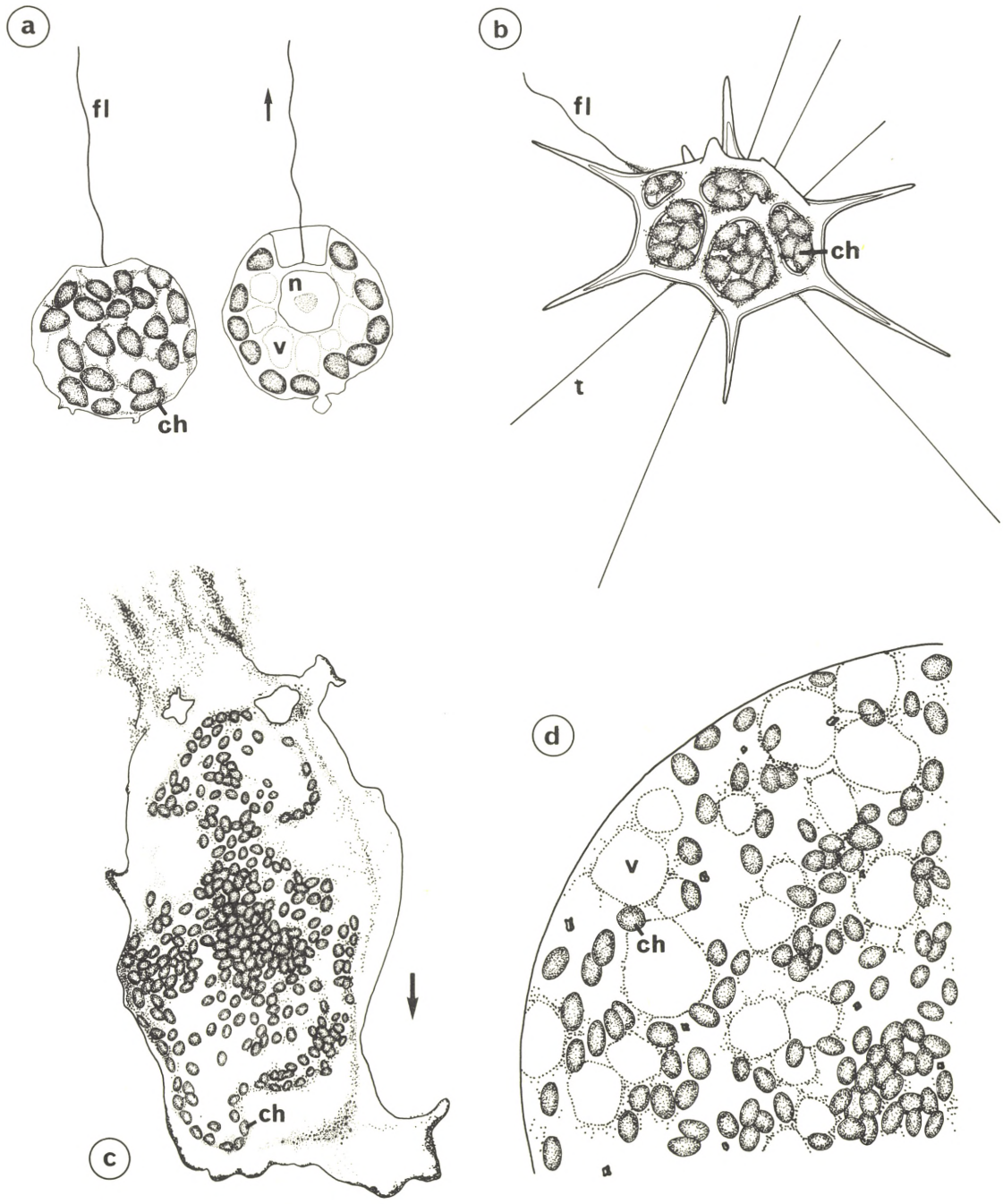
Observations

1. The uninucleate naked stage

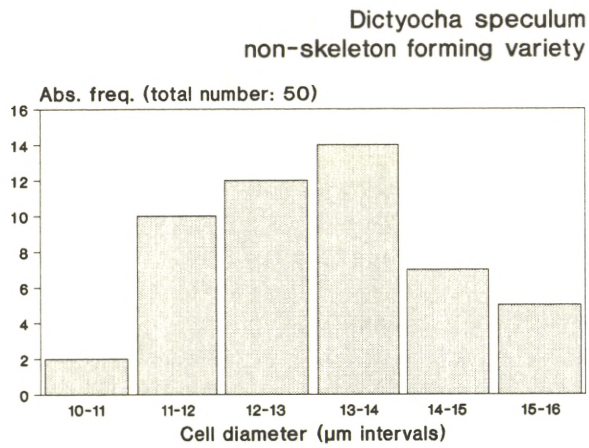
Light microscopy

The cells (Text-fig. 2a, Figs 1-3) are almost spherical, but slightly flattened at the anterior end. Live cells from Alssund measured 15-20 µm in diameter. Cultured cells from Musholm Bay fixed in Lugol's solution were slightly smaller (Text-fig. 3, 10.3-15.4 µm in diam; mean 12.7 µm; st. dev. 1.34 µm; n = 50).

The single, anteriorly directed flagellum is 20-30 µm long. In most cells it appears to arise from an apical depression (Figs 1,2). The flagellum beats with sinusoidal movements in one plane. When swimming, the flagellum is directed forwards, pulling the non-rotating cell body along straight, or very slightly curved tracks. The swimming speed is 20-25 µm/second (established from videotaped



Text-fig. 2. The different morphological stages of *Dictyocha speculum*. a: the naked form (the arrow indicates the direction of swimming); b: the skeleton bearing form; c: the amoeboid stage (the arrow indicates the direction of creeping); d: section of the cytoplasm in the multinucleate stage. ch = chloroplast; fl = flagellum; n = nucleus; t = tentacle; v = vacuole.



Text-fig. 3. Cell size (diameter) of the naked stage of *Dictyocha speculum*. Based on 50 cells from the Alssund cultures, fixed in Lugol.

cells). The posterior end of the cell may be smooth (Text-fig. 2), but cells with one or more trailing tentacles also occur. They give the cells an appearance very similar to that illustrated for *Pseudopedinella* (Zimmermann et al. 1984).

Small, oval chloroplasts (30-50 in typical cells) are scattered underneath the plasmalemma, often bulging out to give the cell a somewhat irregular appearance.

Neighbouring chloroplasts sometimes appear to be interconnected by cytoplasmic strands. Dividing chloroplasts are dumb-bell shaped. The nucleus is centrally located and stands out clearly in the light microscope. It is surrounded by dense cytoplasm, which is separated from the peripheral chloroplasts by a vacuolated region.

Electron microscopy

The general organization of the cell is very unusual for an algal flagellate (Figs 4-7). The central region contains the nucleus which is surrounded anteriorly by the Golgi apparatus. A depression with the flagellar groove extends from the anterior end to near the nucleus, whose position is slightly anterior to the centre of the cell. A very short second flagellum (Figs 8,9,12) is present in addition to the flagellum visible in the light microscope. Judging from micrographs such as Fig. 8, it measures c. 0.75 µm in length.

Mitochondria are found in both the outer and inner cell regions, and more rarely in the vacuolated middle layer (Figs 6,7). Endoplasmic reticulum and ribosomes occur throughout the central and the peripheral cell region, while the middle region appears conspicuously empty in the sections, apart from the large vesicles and the occasional mitochondrion.

A. *The flagellar apparatus*

This is characterized by several features that are unusual among algal flagellates. The short stubby flagellum often appears uniformly dense in the thin sections, in contrast to its longer neighbour (Fig. 9, also Fig. 8). Only the 9 peripheral doublets are present, the central pair lacks entirely. The long flagellum lacks the central pair of microtubules in its proximal part (Figs 9,10), but the exact length of this rather empty-looking part has not been determined (compare with the skeleton bearing stage below). Below the proximal end of the central pair the flagellar profile extends into a complex winglike structure (Figs 8,11,14,15,26). In exact transverse sections of the flagellum this sometimes appears as a swelling next to the axoneme (Fig. 11). Usually the flagellum contracts upon fixation, and its 3-dimensional structure is somewhat uncertain. Figs 14 and 15 both represent sections through contracted flagella, in Fig. 15 with the proximal part visible on the right, recognisable by the lack of the central pair of microtubules. Structural elements within the flagellar wing include a muscle-like fibre (Figs 8,14,15), with a periodicity of 0.2-0.3 µm in the cells examined. The dense material next to the axoneme in Fig. 11 may represent a segment of the "muscle". The wing also contains fields of tubules (about half a dozen is visible in the 2 profiles in Fig. 8 and Fig. 15), and more extensive opaque regions (Fig. 15, right) show indications of substructure. The flagellum is covered on the outside

by thick flagellar hairs, but the extreme sensitivity of the flagellum to chemical and physical changes has prevented a detailed study of the structure and arrangement of the hairs. We know that 2 of the 3 regions which characterize the tripartite hairs of heterokont organisms are present (the base and the shaft), but whether terminal filaments are present as singles or as a 2+1 system (see Moestrup 1982), or perhaps as a new type, is not known. Mommaerts (pers.comm., see chapter below on the occurrence of the different stages of *Dictyocha* in nature) in an organism which we believe is identical to the naked stage of *Dictyocha* drew a flagellum with a single row of flagellar hairs, and we hope to be able to return to this point using different preparation methods.

The transition region between the flagellum proper and the basal body contains the transverse partition characteristic of many heterokont algae, fungi and protozoa (Fig. 16). It has the position typical of these groups, slightly above the level of the cell body. Unlike typical chrysophytes the transition region lacks a transitional helix.

Also in contrast to most chrysophytes (and indeed most other heterokonts) the angle between the basal bodies is very slight (Figs 8,13,17). The basal bodies are anchored to the irregular anterior part of the nucleus (Figs 17,18, in the latter the basal bodies have duplicated). We have been unable to detect distinct fibres interconnecting the basal bodies, but there are indications of microtubular or banded structures: perhaps a 2-stranded microtubular root between the basal bodies in Fig. 17; perhaps banded structures in the opaque regions connecting the basal bodies to the nucleus in Fig. 18.

B. *The chloroplasts*

Externally each chloroplast is bounded by 4 membranes, and the outermost membrane carries ribosomes (Fig. 21). In contrast to most other heterokonts, the chloroplasts are widely separated from the nucleus, and there is no structural continuity between the outer nuclear membrane and the outer chloroplast membrane. The thylakoids are more or less parallel (Figs 19, 21, 23-24), with a girdle lamella of 3 thylakoids (Fig. 21).

The chloroplast contains an internal pyrenoid of a type which to our knowledge has not been found elsewhere. While most of the pyrenoid is uniformly grey in the micrographs, with indications of a surrounding membrane, each "pole" contains a group of 3 short thylakoids (Figs 19,21). The lumen of the 3 thylakoids is continuous (Fig. 21). A thin plate-like structure extends from each pole into the chloroplast proper (Fig. 21). Occasionally (Fig. 19, bottom) this space is occupied by a somewhat irregular cisterna, which is continuous with the triplet. The thylakoid triplet continues around the pyrenoid to form a more or less complete ring (Fig. 23).

The lumen between the 2 central membranes of each chloroplast contains vesicular material (Fig. 19, right) as seen in other heterokonts. This material, according to the endosymbiont theory on the origin of plastids, represents the remains of the symbiont cytoplasm. It is interesting, therefore, that during chloroplast division (Fig. 24) the vesicular material invades the fission furrow in a way indicating an active process.

C. *The mitochondria*

The long tubular cristae contain inclusions (Fig. 22), which in the micrographs appear as opaque spots within the lumen of each crista. Similar structures have now been found in many other heterokont organisms (see references in Zimmermann et al. 1984).

D. *The Golgi apparatus*

A crown of c. 5 Golgi bodies lines the anterior tip of the nucleus. There are no Golgi bodies around the posterior part of the nucleus (Figs 4,5), in contrast to what will be described below for the skeleton bearing stage. The Golgi bodies form a single anterior ring (Figs 6,7 and 12). Each Golgi body is composed of approx. 10 cisternae (Figs 28,29) and at least two structural elements are visible within

the cisternae. Hairlike structures are common, and usually located in the periphery (Figs 26,28). They occur 2-4 together and appear to be transported from the maturing face of each Golgi body (i.e. the side towards the flagellar pit), still within the cisternae (Fig. 26). Larger fields of similar hairlike structures occur in rough ER-cisternae on the opposite side of the Golgi body. We interpret such structures as immature flagellar hairs which – as in other heterokont organisms – are formed in the ER-system. In *Dictyocha* they appear to be transported from the ER to the Golgi apparatus. During passage through the latter system they become separated into smaller parallel bundles and carbohydrate elements may be added here (for a discussion of this phenomenon see Moestrup 1982). The flagellar hairs are released to the surface in the flagellar pit region, and a hair-containing cisterna fusing with the plasmalemma is shown in Fig. 27 (compare with van Valkenburg 1971b figs 10,11, which may illustrate the same process).

The second structural element visible in the Golgi apparatus is an extremely thin plate. In Fig. 29 two of the uppermost cisternae contain such plates, which in thickness appear to be thinner than the cisternal membrane itself. Fig. 25 shows another Golgi body with plates. Their significance is unknown, we have seen no such structures illustrated in other heterokonts. They occur also in the skeleton bearing stage (see below).

E. *Endocytoplasmic bacteria*

Bacteria were frequently seen in the cytoplasm of *Dictyocha* (Figs 20,24, bottom right). Each bacterium was surrounded by an electron translucent halo. They were never seen within vacuoles, nor did they occur in any of the organelles. Bacteria occurred also in the skeleton bearing stage (see below).

2. The skeleton bearing stage

Light microscopy and scanning electron microscopy

Healthy, living cells with intact flagella were rarely observed in natural samples. Most often even freshly collected *Dictyocha speculum* specimens showed stages of plasmolysis. In such cells the contracted cytoplasm only occupies a minor part of the skeleton chamber. In healthy, living cells (Figs 30-33; material from the Isefjord, October 1984) the protoplast fills the entire skeleton chamber. The numerous peripherally located chloroplasts sometimes bulge out of the skeleton windows (Fig. 31), but cytoplasmic details are otherwise difficult to observe. The single flagellum is most often in alignment with one of the radiating spines of the skeleton (Figs 30,33). In the light microscope the flagellum appears (as noted also by Hovasse 1932) to have a rather stiff basal part, almost as long as the skeleton spine. The distal part of the flagellum beats with rapid sinusoidal movements, the amplitude increasing towards the flagellar tip. As in the naked stage the flagellum of the skeleton bearing stage readily breaks up into a row of globules when examined under the light microscope (Fig. 33).

In some cells tentacles extend from the cell surface, in length often exceeding more than three cell diameters (Figs 30,33). Sometimes they appear as continuations of the spines of the skeleton, but more often they arise from other parts of the cell.

Twin cells with opposed (and partly interlocked) skeletons, representing dividing cells, were frequently observed in dense populations of *Dictyocha speculum*.

In its typical form the skeleton of *Dictyocha speculum* consists of two differently sized hexagonal rings interconnected by six bars (Figs 30,33 and 35-44). The bars attach to the corners of the small ring which may be almost circular. In the large ring the bars attach midway along the edges (Fig. 37). Differently sized spines (mostly 1-3 in number) project outwardly from the small ring (Figs 35,42). Six large spines typically radiate from the corners of the large ring, approximately in the plane of the large ring (Figs 40,43,44). A bilateral plane of symmetry is sometimes apparent, two oppositely positioned spines being slightly longer than the others (Fig. 41). The skeleton chamber (the space between the two hexagonal rings) holds the protoplast. This is

securely positioned by six small spines or "Stutzstacheln" (Lemmermann 1908) on the side of the large hexagonal ring (Fig. 38). Each small spine arises midway between a large spine and a bar interconnecting the two hexagonal rings (Figs 35,37). The small spines often hook outwards distally (Figs 38,40). The skeleton except for the pointed spine tips is hollow (Fig. 36). The external surface of the skeleton appears smooth, but the interior surface is more rugose (Fig. 36).

The terms apical ring and basal ring (Lemmermann 1908, Marshall 1934) have been omitted, because they suggest that the longitudinal axis of the cell runs through the centres of the hexagonal rings. The flagellum lies parallel to one of the large spines, and this suggests that the skeleton is lateral.

Figs 39 and 40 show skeletons from Alssund in which the organic material has not been oxidized. In such cells the plasmalemma curtains the skeleton windows on the inside.

The temporary interlocking of paired skeletons, representing pre-division stages (Fig. 39), is maintained by the inwardly bent hook-shaped spines (Fig. 39, arrows). See Boney (1976) for further discussion of this phenomenon.

A small percentage of skeletons display morphological anomalies. The most typical of these is a seven-fold rather than six-fold symmetry (Fig. 42, and less clear in Fig. 35). Although no attempt was made to register all anomalies, or to establish their relative numbers within a sample, the general picture was not unlike that reported by Boney (1973, Firth of Clyde).

Measuring of 100 skeletons (Text-fig. 4, material from the Isefjord; 21 May 1985) gave the following results: small hexagonal ring 6.8-10.6 µm in diam.; mean value 8.9 µm; st. dev. 0.7 µm; large hexagonal ring 17.1-22.5 µm in diam.; mean value 20.5 µm; st. dev. 1.0 µm; maximum distance between tips of large spines 37.5-56.0 µm; mean value 46.2 µm; st. dev. 3.8 µm.

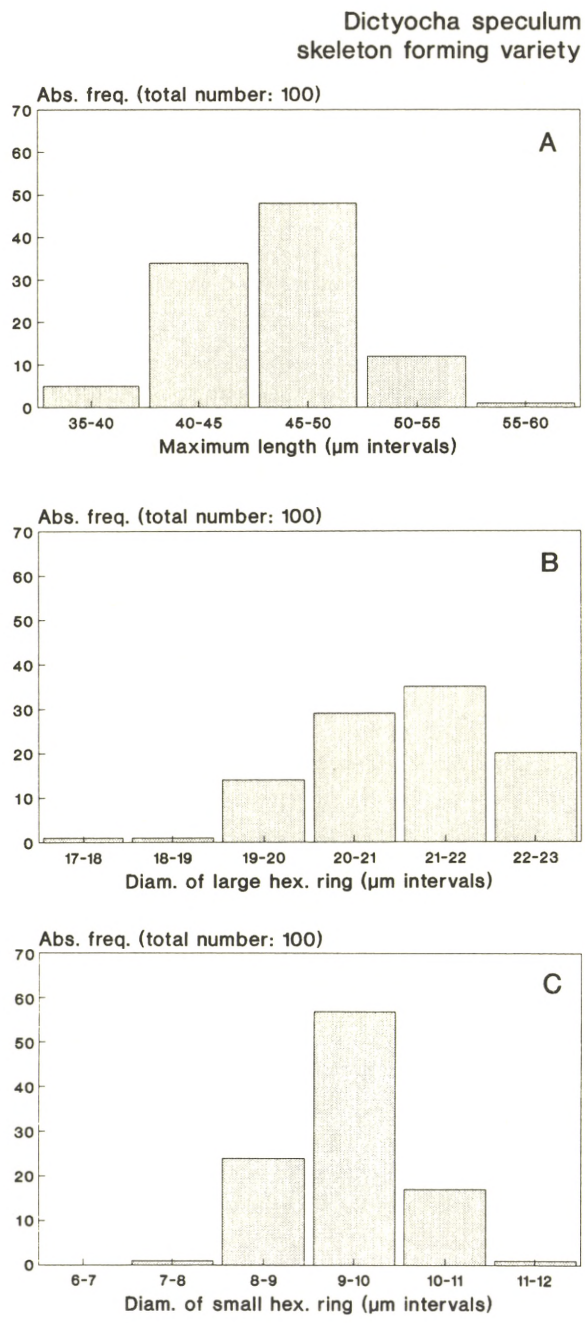
A rough calculation of the volume of the skeleton chamber, using the mean values above, and assuming that the distance between the two rings equals the diameter of the small ring, gives a figure of approximately 1200 μm^3. A similar rough calculation of skeleton lacking cells gives a figure of approximately 1150 μm^3 using the mean diameter of cells fixed with Lugol's solution (12.7 µm) and assuming that the cells are spheres. Naked and skeleton bearing cells are thus generally of equal volumes.

Transmission electron microscopy of the skeleton bearing cell

In contrast to what is often stated in the literature (e.g. Tappan 1980), the skeleton of *Dictyocha* is external rather than internal. The cell periphery is very irregular (Fig. 34), with numerous large indentations, and the skeletal elements are often present in these cavities. The cell lies in the cavity of the skeleton (compare also with the scanning electron microscopy, Figs 39,40) and is closely appressed to the skeletal elements (e.g. Fig. 34). Subdivision of the cytoplasm into three zones is not as distinct as in the naked stage. The inner region, with the nucleus and the Golgi bodies, resembles the naked stage, but the number of Golgi bodies is much higher and they surround the nucleus on all sides. The outer region contains the chloroplasts but many lie in the middle region as well. The wall-like structure described in some cells of *D. fibula* by van Valkenburg (1971b) was not observed in our material, but numerous tentacles emanate from all parts of the cell periphery (see also Text-fig. 2b). Sections through the skeleton give further information on its structure: the basal third or quarter of the radial spines and the basal ring are hollow (Fig. 46).

A. *The flagellar apparatus*

The flagellar pit has an irregular outline with numerous projections and indentations (Figs 45,48), unlike the regular outline of the naked cell. There are no microtubules lining the flagellar pit. The long flagellum is covered by flagellar hairs (Figs 48-52). As in the naked stage the flagellum carries a large unilateral wing (Figs 49-52,55). The wing was poorly preserved and the distinct cross-banded rod found in the naked stage was not seen.



Text-fig. 4. Measurements of one hundred skeletons from Isefjorden 21 May 1985. a: longest axis; b: diameter of large hexagonal ring; c: diameter of small hexagonal ring.

The presence of fibrillar material is an indication that the rod, if present, had disintegrated during the fixation procedure. A basal platform is present on the long flagellum and it carries a dense tuft of flagellar hairs (Fig. 47). This feature which recalls the situation in cryptomonad flagellates is seen again in the series of sections reproduced as Figs 49-52. The central pair of microtubules is absent in the proximal part of the axoneme (Figs 49-52). It first appears approx. 2 µm beyond the transverse partition of the flagellar transition region (Fig. 49). The transition region itself is similar to that of the naked stage and lacks a transitional helix (Figs 51,53). We have, however, occasionally seen (as illustrated in the cell in Figs 54,55, arrows) a ringlike structure in the transition region outside the axoneme, but below the transverse partition. We are grateful to Dr. J. Larsen, Copenhagen, for pointing this out, as it represents a feature seen so far only in a heterotrophic heterokont, *Actinomonas*, a member of the Pedinellales (Larsen 1985).

Unlike the naked stage, the second flagellum in the skeletal stage is represented only by its basal body, which measures c. 0.6 µm in length (Figs 53,54). The two basal bodies are inserted to each other at an angle of c. 30-35 degrees (Figs 53,54).

As in the naked stage, no well-developed flagellar roots were seen. A very thin rhizoplast is probably present, emanating from the basal bodies and ensheathing the anterior part of the nucleus (Fig. 57).

B. *Tentacles*

Silicoflagellate tentacles have been known for a long time and were illustrated beautifully by Marshall (1934). Considering the apparent similarity, at least in certain respects, between the naked form of *Dictyocha* and members of the Pedinellales, it was important to critically examine the tentacles of *Dictyocha*. As in the Pedinellales the tentacle microtubules of *Dictyocha* emanate from the surface of the nucleus (Fig. 56), from whence they proceed toward the cell periphery. Rather than taking a straight path the microtubules sometimes curve and the tentacles form branches (Figs 56,58), the microtubules

separating into smaller groups. Figs 59-69, representing 11 consecutive sections, illustrate this in more detail. Fig. 60 shows the proximal part of a tentacle, which contains 5 microtubules embedded in dense material on the nuclear surface. Three microtubules are close together and form a triad almost as in the Pedinellales. However, two additional microtubules are located nearby. Two sections later (Fig. 62) a sixth microtubule is added (arrow), and a seventh in the next section. Three sections later no. 8 and 9 are added (Fig. 66). The tentacle then extends from the cell surface (Fig. 69). The original triplet does not continue in its original triangle, but is displaced when the 8th and 9th microtubules are added. There is, however, no fixed number of microtubules in the tentacles. Next to the 9-microtubule tentacle in Fig. 69 is a slightly irregular tentacle, containing c. 5 microtubules and a piece of membrane. The previous six sections all show 5 microtubules in this tentacle. Fig. 69 also illustrates a tentacle with a single microtubule and, to the left and at the bottom, respectively, groups of 3 and 14 microtubules. The tentacles thus do not resemble tentacles of any other algal flagellates.

C. *The Golgi apparatus*

In *Dictyocha fibula* van Valkenburg (1971b) calculated that the cell contained a total of c. 72 Golgi bodies surrounding the centrally located nucleus. Our material shows a much lower number in *D. speculum*. Fig. 72 illustrates c. 8, Fig. 34 c. 6 Golgi bodies. Using the calculation of van Valkenburg (1971b) gives a total of c. 20 Golgi bodies per cell, compared to c. 5 in the skeleton lacking stage. Each Golgi body consists of c. 10 cisternae, and the two structural entities found in the naked stage occur also in the skeleton bearing cell. Thus Figs 70,71 show presumptive flagellar hairs in groups of three in the peripheral parts of several cisternae and the uppermost cisterna in Fig. 71 contains a mature flagellar hair. The second element found in the Golgi stack of the naked stage, the very thin plate-like structure, is visible in Fig. 71, cisterna no. 4 from the top (arrow).

D. *The chloroplasts, the mitochondria*

The chloroplasts and mitochondria of the skeleton bearing stage did not appear to differ from the naked cells. The same unusual type of pyrenoid was present (Fig. 73). Occasionally the central thylakoid of the pyrenoid triplet was seen to extend into the chloroplast proper, probably connecting to the thylakoid lamella outside the pyrenoid (Fig. 56).

E. *The internal canal system*

One of the most unusual features of the skeleton bearing stage is an extensive system of canals, not found in the naked stage and not known in any other alga. Part of the canal system is seen as a series of smooth cisternae outside but parallel to the Golgi bodies (Fig. 70, arrows). The cisternae surround the entire nucleus-Golgi apparatus complex (Fig. 72). It extends to the exterior through numerous radially oriented channels (Figs. 56,74).

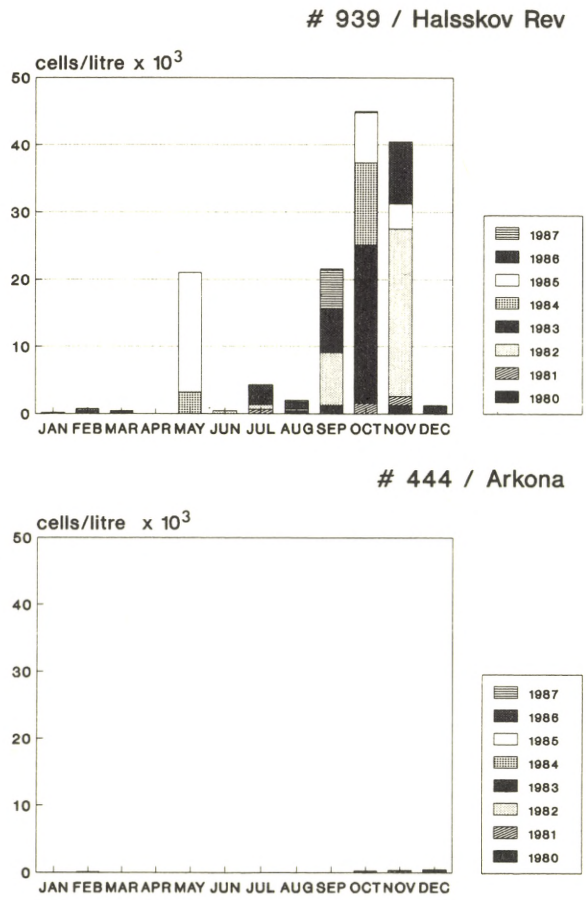
In the flagellar region the canal system widens into a little chamber (Fig. 53), connected to the exterior through the flagellar canal. The canal system separates the inner region of the cell from the intermediate and outer regions, and it is penetrated by tentacle microtubules (Figs 53,56,70) and other organelles. Coated vesicles apparently fuse with the canal system, which may contain flagellar hairs (Figs 56,70), bacteria (Fig. 53) or membranous aggregates. We interpret the canal system as an internal transport system through which nutrients may be transported into the cell, and debris discarded.

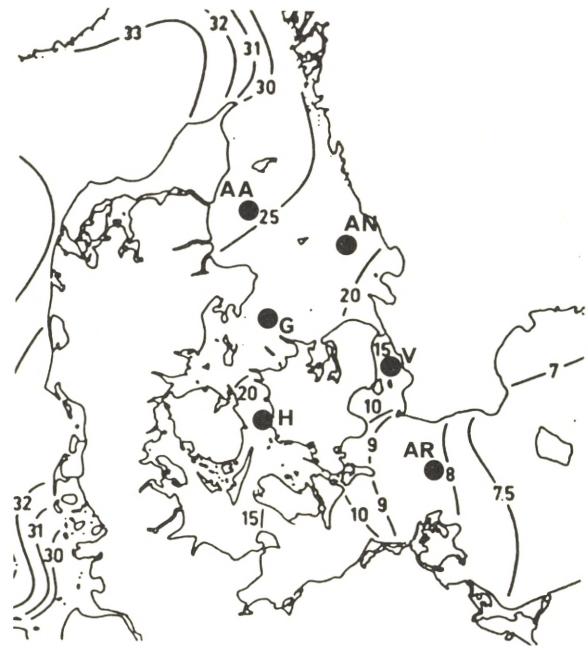
F. *Bacteria*

As in the naked stage cytoplasmic bacteria were commonly seen, both in the cytoplasm itself (Fig. 74) and in the canal system.

3. The multinucleate stage

Large, multinucleate cells were observed both in the cultures established from the Alssund material (May 1983), and in natural samples from the Isefjord (April 1985). They appear as large spheres





Text-figs 5 and 6. Occurrence of the skeleton bearing stage of *Dictyocha speculum* at 6 stations in Danish waters 1980-1987. The stations are indicated in the map, which also shows isohalines (highest monthly average, after Dietrich 1950). The station of highest salinity is Aalborg Bugt (AA), the lowest is Arkona (AR). The skeleton bearing stage usually occurs in highest numbers in Sept. – Nov. It occurs in all areas with a salinity above c. 10 ‰.

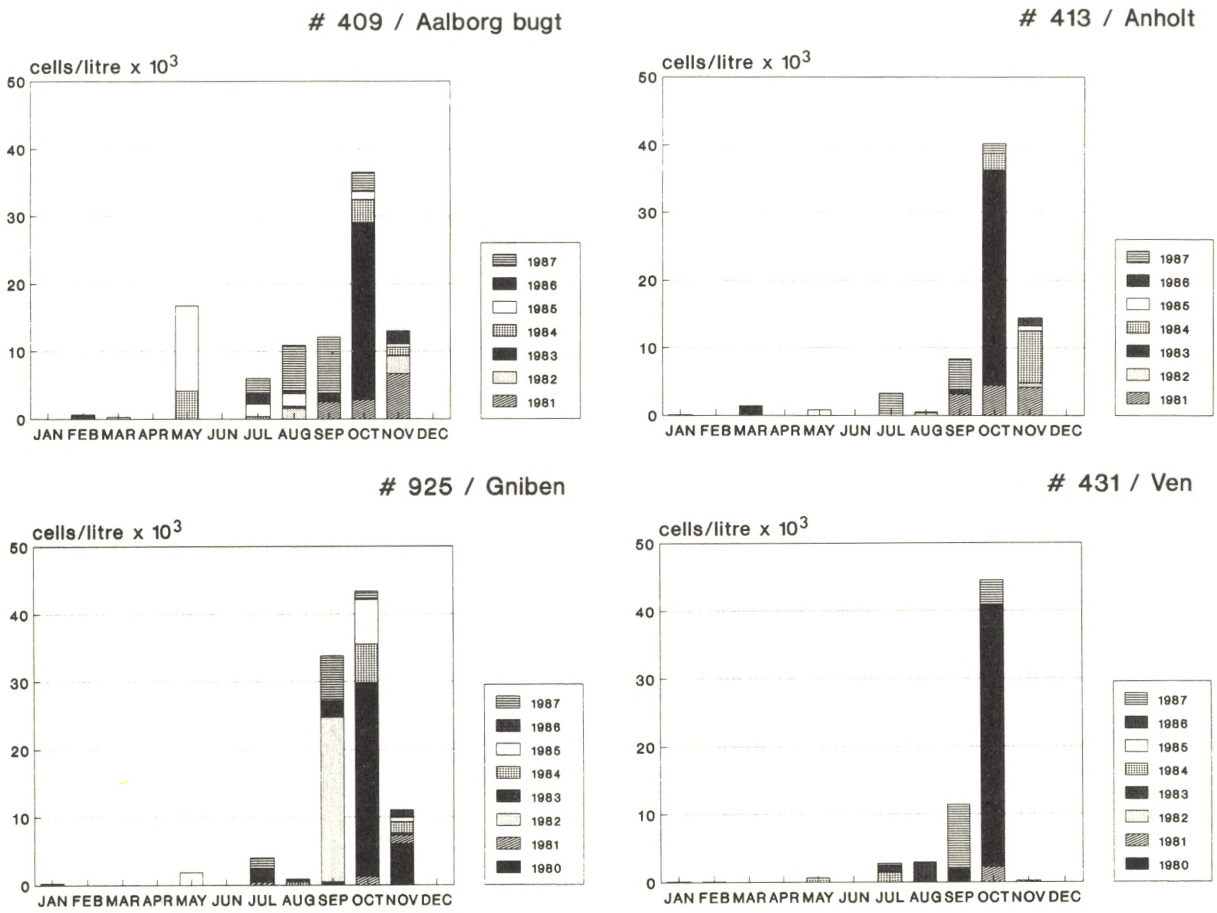
with numerous chloroplasts, often in clusters, interspersed among vacuolated cytoplasmic areas (Text-fig. 2d, Fig. 75). The nuclei are difficult to see under the light microscope. Small reddish spots of unknown origin and function are present throughout the cytoplasm. One or more flagella are occasionally seen. The multinucleate stages observed in culture measured 45-100 µm in diameter, while those from the Isefjord were up to 500 µm in diam. (volume: approx. 65×10^6 µm^3). Compared to the skeleton bearing cells of *Dictyocha speculum* this equals an increase in volume by a factor 50.000.

The chloroplasts, nuclei, mitochondria and Golgi bodies did not differ from the naked uninucleate cell. There was considerable cytoplasmic vacuolation (Fig. 76) but no clustering of the Golgi bodies around each nucleus. Golgi bodies were scattered in the cytoplasm with no fixed orientation relative to the nuclei. Each Golgi body contained c. 6 cisternae compared to 10-11 cisternae seen in the uninucleate stages. The thin plates seen in both the uninucleate and the skeleton bearing stages were also present (Fig. 78). Densely packed presumptive flagellar hairs were observed in cisternae of the endoplasmic reticulum (not shown).

Endocytoplasmic bacteria were also present (Fig. 77).

4. The amoeboid stage

Motile, amoeboid stages were seen in crude cultures of the Alssund material (May 1983). The cell



photographed measured 90 × 55 µm (Text-fig. 2c). Most chloroplasts were clustered in the middle of the cell, but some were located in a peripheral thin outer layer appressed to the substratum. The outer layer was in continuous movement in a characteristic rolling manner. While the borderline between the cell and the surroundings was distinct at the front end of the "amoeba", there was a more diffuse change from the cell to the exterior posteriorly (see Text-fig. 2c).

5. Occurrence of the different stages in nature

To visualize the occurrence of *Dictyocha speculum* in Danish waters, plankton monitoring data from an eight-year period (1980-1987) have been graphically assembled (Text-figs 5 and 6). The stations chosen are shown in Text-fig. 5 together with surface isohalines of Danish coastal waters (highest monthly average).

The skeleton bearing stage of *Dictyocha speculum* does not thrive in brackish water of less than c. 10 ‰ salinity (in all 8 years cells were practically absent at Arkona station). The preferred season is the early autumn, and the maximum cell densities (cells/litre) recorded from the various stations were: Aalborg Bugt 26.100 (Oct. 1983); Anholt 31.775 (Oct. 1983); Gniben 28.480 (Oct. 1983); Ven 38.400 (Oct. 1983); Halsskov 24.960 (Nov. 1982), and Arkona 435 (Nov. 1980). Considerably higher cell numbers have been observed in Norwegian

coastal waters (Tangen 1974: 303.000 cells/litre).

The geographical distribution and seasonal occurrence of the skeleton bearing stage in Danish waters has apparently not changed during this century. According to Hansen-Ostenfeld (1913) *Dictyocha speculum* is present in all Danish waters except the most brackish parts of the Baltic Sea, yet never in greater numbers (maximum September – December). Hansen-Ostenfeld (1913, p. 190) mentioned, however, that due to its size *Dictyocha speculum* may not be adequately represented in the plankton net tows on which he based his conclusions. He therefore quotes Lohmann (1908) who based on centrifuged water samples concluded that *Dictyocha speculum* was present in Kiel Bight throughout the year (most common in September; fewest cells in February).

In more recent studies from Kiel Bight large numbers of the skeleton bearing stage were seen also during spring. Smetacek (1975) found two maxima in 1973, one in March-April, and one of similar extent (c. 10^4 cells/litre) in September. Studies from the 1980s have given larger figures. Susanne Neuer (pers.comm.) reports 10^2 cells/litre in May 1985, but from 6×10^4 to 2.6×10^6 at the end of May 1986. In Danish waters skeleton bearing cells are often almost absent in spring, but spring-peaks occur sporadically (Text-figs 5 or 6), e.g. in May 1985 ($1\text{-}2 \times 10^4$ cells/litre), with lesser peaks at the same localities the previous year.

Seasonal observations on skeleton bearing silicoflagellates in other European coastal waters were reported in Margalef (1965), Nival (1965), Travers & Travers (1968) and Ignatiades (1970).

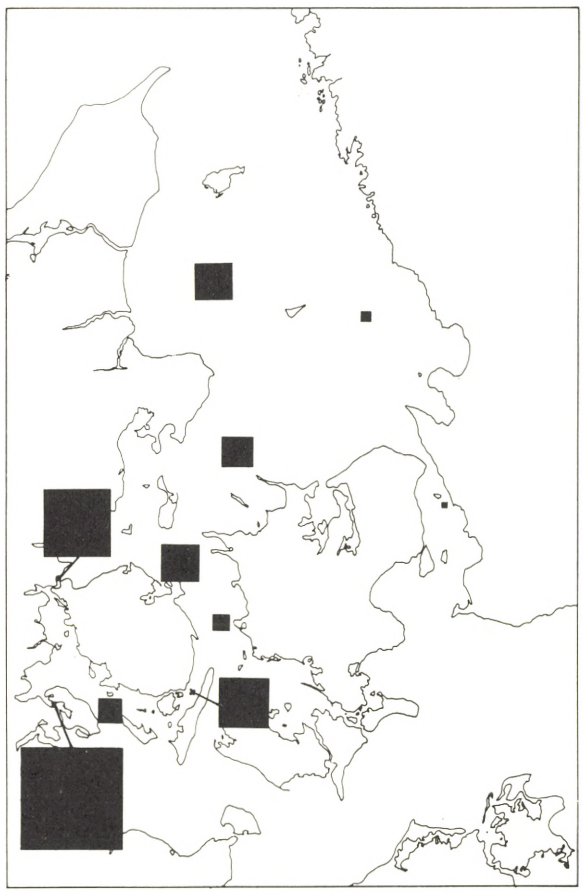
The first recorded appearance of the naked, uninucleate stage of *Dictyocha speculum* in Danish waters dates back to early May 1983. The centre of distribution (Text-fig. 7) was the western Baltic Sea, the Belts and the southern Kattegat area.

Maximum cell density was approximately 25×10^6 cells per litre. Typical skeleton bearing *Dictyocha speculum* cells were also present, and the ratio of skeleton bearing forms to naked cells was estimated to be roughly 1:100. Since 1983 the naked form of *Dictyocha speculum* has been recorded from Danish coastal waters every year, always in the beginning of May. Thus on 2 May 1984 2.5×10^6 cells per litre were found in the Aarhus Bugt halocline at 10 metres depth (Jytte Heslop Christensen, pers. comm.). A distinctive bloom similar to that from 1983 has yet not reappeared.

According to Susanne Neuer, Univ. of Kiel, (pers. comm.) the naked stage occurred in the Kieler Bight together with the skeleton bearing stage in both 1983, 1985 and 1986. The number of cells per litre was very high: 2×10^6 in May 1986, 7×10^6 in May 1985, but from 3×10^6 to 10×10^6 in May 1986, the last figure representing the highest number recorded anywhere (temp. 10-12°C). The skeleton bearing stage was less numerous, except in a sample from the pier at Institut für Meereskunde on 30.5.86, where the two phases co-occurred in similar high numbers (the naked 7×10^6 cells per litre, the skeleton bearing form 2.6×10^6 cells per litre).

Although the naked stage of *D. speculum* has not been mentioned in the literature before, it almost certainly occurs worldwide. According to Jahn Throndsen, Oslo (pers. comm.) correspondance from Dr. P. Mommaerts, Vrije Universiteit, Brussels, Belgium, dated 1969 and 1978, includes a drawing of a flagellate which almost certainly is identical to the naked stage of *D. speculum*. The organism was in culture in the Plymouth Culture Collection as no. 323, but had been lost by 1978. The cells were spherical with an apical depression, contained up to 20 chloroplasts and a central nucleus. The cells were biflagellate and the long hairy anterior flagellum possessed a wing while the second flagellum was very short. Golgi bodies were located on the anterior face of the nucleus. The only discrepancy between Mommaerts' drawing and our findings is cell size, given by Mommaerts as 6 µm, and in our cultures c. 10-15 µm. The same species occurs in Vancouver, Canada (pers. comm. to P. Mommaerts from Mrs. R. Waters, formerly R. Jowett).

Finally during the study leave of one of us (ØM)



Text-fig. 7. Occurrence of the naked stage of *Dictyocha speculum* in Danish waters 16-20 May 1983.

to University of Melbourne, Australia 1987-1988, cells indistinguishable from the naked stage of *D. speculum* were found on several occasions in Port Philip Bay, Victoria, Australia. Attempts to establish the cells in culture failed. The taxonomic affinity of these cells is not clear, as the plankton of Port Philip Bay contained both *D. speculum, D. fibula* and *D. octonaria*.

The amoeboid form and the multinucleate form are presently known from crude cultures established from the Alssund material (May 1983), the latter also from a natural sample (the Isefjord, April 1985).

6. Discussion

The present work raises several new questions, of which one is the life cycle of silicoflagellates. As mentioned above cultures of the naked form did not produce skeletons, even when extra silica was added to the culture medium. The only previous report on silicoflagellate culturing (van Valkenburg and Norris 1970) reported that the skeleton bearing cells of *D. fibula* lost their skeletons in dense cultures. By growing the cultures in continuous light and transferring to a 16h day cycle, they were, however, able to induce formation of new skeletons. A similar experiment with our cultures failed, no skeletons were produced (1 week continuous light, followed by a 16:8 light/darkness cycle). Van Valkenburg and Norris' cultures clearly belong to a different species, however, although skeletons from a single clonal culture gave rise to numerous morphological variants, which could be attributed to several taxa. We have found some variation also in Danish waters but there is rarely difficulty with the identification (see also Boney, 1976, material from Firth of Clyde, Scotland). *D. speculum* is the most common species in Danish waters, *D. fibula* is rarer, and *D. octonaria*, the third known extant species, has not been observed. Van Valkenburg and Norris' isolate of *D. fibula* (from the Northern Pacific) and our material of *D. speculum* from Danish waters differ also in the internal structure. In our material of *D. speculum* all 3 stages examined showed a triplet ring of short thylakoids and a thin plate along the periphery of the pyrenoid. In the American material of *D. fibula* the pyrenoid was without any photosynthetic lamellae (van Valkenburg 1971b). Also the Golgi apparatus is different. C. 20 Golgi bodies were present in *D. speculum*, c. 72 in *D. fibula* (van Valkenburg 1971b).

The ultrastructure of the skeleton in *D. fibula*, with small groups of "spinose structures" especially on the spine tips (van Valkenburg 1971a) is also very different from the smooth skeletons seen by us in *D. speculum*. The third species, *D. octonaria* is in this respect similar to *D. speculum* (Ø. Moestrup, unpubl. information on material from Port Philip

Bay, Vic. Australia, investigated by scanning electron microscopy).

The long standing discussion on whether the skeleton in silicoflagellates is external or internal has now hopefully reached a conclusion. Both our thin sections and the scanning electron microscopy show very clearly that the skeleton of *D. speculum* is external. Van Valkenburg (1971b) in *D. fibula* found a thick wall-like structure around both the cytoplasm and the skeleton, but such a structure has never been seen in any of the stages examined by us. The cells are located in the cavity formed by the skeleton and the latter may under certain circumstances be shed. The skeleton is almost certainly lateral relative to the flagellar insertion, and use of current terminology for the skeleton (Lemmermann 1908, Marshall 1934) (apical and basal ring) has therefore been discontinued. The long flagellum is located parallel to one of the longer spines, in Figs 30,33 the spine next to one of the 2 longest spines. The axis through the 2 longest spines is therefore not parallel to a longitudinal axis through the cell and the long flagellum.

One of our most surprising findings is the difference in ultrastructure between the different stages. The naked stage is the simplest. It possesses 2 flagella, of which one is very short, and the cells are regularly spherical, divided into 3 distinct zones. The skeleton bearing stage has only one flagellum, the short flagellum is represented by a second basal body only, the cytoplasm is irregular in outline and complex internally with no clear division into discrete zones.

The most unusual cytoplasmic features are the internal canal system and the tentacles of the skeleton bearing stage. The function of these structures remains unclear. The tentacles do not appear to function in food-uptake, because no food vacuoles with bacteria or other food particles were detected in the cell. Bacteria are present in the cytoplasm in all stages, but in the cytoplasm itself. Such bacteria are, however, common in wild populations of algae (pers. obs.). Hovasse (1932) suggested that the tentacles function during formation of new skeletons, while Deflandre speculated (1950) that they play a role in the vertical movement of the cells in the water column.

When the skeleton bearing stage sheds its skeleton, the canal system and the tentacles disappear, and the cells round up. For reasons unknown the extra basal body then elongates to form a very short reduced flagellum which lacks the central pair of microtubules.

Do these stages then belong to the same species? The evidence, in the lack of culture studies, is the identical chloroplasts with a type of pyrenoid which has never been seen in any other alga. Also the presence of very thin plates in the Golgi cisternae of all 3 stages examined ultrastructurally, a structure which has not been observed in other algae. Finally, a single cell sectioned from the Isefjord sample is probably intermediate between the naked and the skeleton bearing stage (Fig. 80). This cell from which we have only a single section (its significance was only realized during writing of this manuscript) shows a spherical cell with peripheral chloroplasts (as the naked stage), but the cell posesses a skeleton. The cell was in contrast to other cells in the fixation rather poorly preserved (this was the reason it was not photographed further). We believe that it represents a skeleton bearing cell which is shedding its skeleton and rounding off.

The phylogenetic relationship between silicoflagellates and other protists remains somewhat uncertain. Silicoflagellates are usually classified with the chrysophytes because of the similarity in chloroplast pigmentation (colour). They contain chlorophyll a and c and a number of carotenoids, including fucoxanthin (van Valbenburg 1980). There is no clear affinity to a chrysophycean type such as *Ochromonas*, however. The resting stages known as stomatocysts, and characteristic of many chrysophytes, have never been seen in silicoflagellates, although silicoflagellates are common and widely distributed. Silicoflagellates resemble the Mallomonadales in their ability to form silicified structures which are deposited outside the cell, but skeleton formation in silicoflagellates has not been

studied. The only group of chrysophytes to which there is similarity perhaps indicating phylogenetic relationship is the Pedinellales. Members of this group are uniflagellate, colourless or with 3-6 chloroplasts. The chloroplasts are firmly attached to the nucleus, with the outermost membrane continuous with the outer nuclear membrane, a characteristic feature of many heterokonts, but lacking in silicoflagellates. It also lacks in some other heterokonts with multiple chloroplasts, e.g. *Vaucheria* and may be a character of limited phylogenetic significance. Members of the Pedinellales are the only other algae known to form microtubule-supported tentacles but each tentacle is supported by a triad of microtubules, rather than the complex system seen in the silicoflagellates. In both groups, however, the microtubules terminate on the nuclear surface where the microtubule organizing centre is probably located. This feature and the presence in the skeleton bearing cells of *Dictyocha* of a ring-like structure below the transverse partition in the flagellar transition region are indications of a phylogenetic relationship between silicoflagellates and the Pedinellales. The ringlike structure occurs in the pedinellid *Actinomomas* (Larsen 1985), but is not known in other algal flagellates.

The heliozoans is another group of organisms which forms silicified skeletons and microtubule-supported tentacles produced by microtubule organizing centres on the nuclear surface. There is little similarity in the arrangement of the microtubules in the tentacles, however, and direct proof of a phylogenetic relationship between silicoflagellates and heliozoa/radiolaria is lacking. Studies in the poorly known desmothoracid heliozoan *Orbulinella smaragdea* Entz sen. and related genera would be of considerable interest in this respect. It contains a central nucleus and the cell is located in a siliceous capsule (Febvre-Chevalier 1985).

7. Is Dictyocha toxic to fish?

As mentioned above, a fish-kill in southwestern Denmark (Alssund) in May 1983 prompted this study. It hit the mariculture farm "DanMarin" and resulted in death of c. 7000 rainbow trout in the period 2-5 May, representing mortality rates of 13 % and 8 % in the 2 affected mariculture plants. The water was yellowish murky, the cell density up to c. 15 mill/litre and the Secchi depth in large areas reduced to 0.7-1.0 m. The dead fish showed bleedings from the gills and in the mouth, but the internal organs did not appear to be affected. The kidneys were normal. Fish from 2 May had an oily smell from the gills not noticed subsequently.

No other unusual effects were seen in the area, and no dead benthic animals were found.

A strong wind blew most of the cells away from the area after 5 May, but the naked stage of *Dictyocha* remained dominant in the plankton until the middle of May. By the beginning of June it had disappeared.

The mass occurrence of *Dictyocha* cells was explained by the heavy rains which preceded the blooms. Thus in the week before a total of 64 mm rain was registered at one station in the area (Rønhave). The *monthly* rainfall for a normal year is c. 45 mm. The runoff of fertilizers from the land probably triggered the blooms.

North of the area *Dictyocha* occurred in even larger numbers, but the effect on cultured fish was less serious. In Skærbæk Mariculture plant (Kolding fjord, see Text-fig. 1) the number of *Dictyocha* cells (naked form) was on 10 May 1983 c. 25 mill. at the surface (temp. 11°C), c. 12 mill. in 2 m depth, c. 8 mill. in 4 m depth (temp. 9.8°C). The water (salinity 15-15.2 ‰ S) was reddish or reddish yellow, but mortality rates among the fish only 1-2 %.

In the mariculture plant near Hjarnø in Horsens Fjord (Text-fig. 1), the number of *Dictyocha* cells in surface water was less than 3.3 mill. cells per litre, in Vejle Fjord c. 10 mill., but no effect on fish was detected.

Further fish mortality was observed in the Great Belt area near North Langeland (Dageløkke Strand) on 18 May. Water samples contained large numbers of the naked form of *Dictyocha*. About 10 %

of the garfish caught in pound nets were dead, a phenomenon which the local fishermen had not experienced before. Herring and flatfish in the nets appeared unaffected.

It was concluded from these observations that the fish mortalities were probably caused by *Dictyocha*, but conclusive evidence was lacking.

Accordingly G. Møller Christensen, the Marine Biological Laboratory, Helsingør, offered to grow our culture isolates into the larger quantities required for a mouse test. This failed, however, since the cells died when the cultures became dense, a phenomenon noticed also in *Dictyocha fibula* (van Valkenburg & Norris 1970).

Kodama & Ogata (1988) have suggested that endonuclear bacteria are responsible for the toxicity of the dinoflagellate *Alexandrium tamarense*. Saxitoxin-producing bacteria were isolated from the dinoflagellates by Kodoma & Ogata. It may therefore be significant that bacteria occurred in all stages of *Dictyocha*, though not in the nucleus. Whether these bacteria are capable of toxin production should be examined.

Dictyocha speculum has recently been associated with trout mortality in France (Erard-Le Denn & Ryckaert 1989). This was explained by the high densities of *D. speculum* whose skeletons irritated the gills of the trout and led to mucus secretion on the gills with subsequent asphyxia. No toxins were reported.

8. Classification

Many authors classify silicoflagellates as an order in the class Chrysophyceae. The correct name for the order is Dictyochales Haeckel 1894 (Silva 1980) rather than Dictyochales T. Christensen 1967 or Vallacertales Glezer 1966. The latter is illegitimate according to the Botanical Code of Nomenclature, because no Latin diagnosis was provided.

Alternatively silicoflagellates may be considered to represent a separate class, for which the descriptive name Silicoflagellata Borgert 1891 or the typified name Dictyochophyceae Silva 1982 may be used. A more radical approach in line with current trends is to include the Pedinellales together with the Dictyochales in a separate class, Silicoflagellata/Dictyochophyceae. At the present time any of these classifications are acceptable to us, the last the most challenging.

While the species concept in modern silicoflagellates now seems clearer, the generic concept is not. Considering that the much used name *Distephanus* is illegitimate (see p. 5) the most sensible solution is probably, at least for the time being, to include all 3 well-known extant species in the same genus, *Dictyocha* Ehrenberg 1837. Future studies on *D. fibula* and *D. octonaria* may show that separation into separate genera is warranted, but further studies are required on *D. fibula* before conclusions can be drawn. The ultrastructure of *D. octonaria* is unknown.

If future studies show that the 3 species belong to different genera the generic name *Octactis* Schiller 1925 is available for *D. octonaria* Ehrenberg. Schiller's *Octactis pulchra* is clearly identical to *D. octonaria*, a species characterized by an outer ring of thick skeletal elements and a very delicate inner ring of nearly the same diameter. Eight spines are present as in *D. speculum*, 2 usually slightly longer than the other.

We are not in a position to comment on the additional extant species mentioned by Glezer (1966), *Distephanus crux* (Ehr.) Haeckel and the 2 forms of *D. octonaria* sometimes raised to species level (Glezer 1966). Future studies on these taxa should include also cell structure.

Acknowledgements

The Danish Navy, and in particular the Torpedostation at Kongsøre, is acknowledged for providing boat facilities. The video system and the computer-aided picture analysis facilities (Leitz ASM 68K) were financed by grants from the Carlsberg Foundation to HAT. The Marine Pollution Laboratory, Charlottenlund, and Steffen Mariager Pedersen are acknowledged for placing the cell counts of *Dictyocha speculum* at our disposal. The following biologists supplied data: Peter Wiberg-Larsen from Fyns Amtskommune, Kim Forchhammer from Sønderjyllands Amtskommune, and Jens P. Thomsen from Vejle Amtskommune. Lene Christiansen, Lisbeth Haukrogh, Anne-Grete Kreiborg, Kirsten Pedersen and Niels-Henry Larsen are acknowledged for technical assistance and R.A. Andersen, Bigelow Laboratories, for scrutinizing the text. The electron and light microscopes used were provided by the Danish National Science Research Council.

References

Boney, A.D. 1973. Observations on the silicoflagellate *Dictyocha speculum* Ehrenb. from the Firth of Clyde. – J. mar. biol. Ass. U.K. 53: 263-268.

Boney, A.D. 1976. Observations on the silicoflagellate *Dictyocha speculum* Ehrenb.: double skeletons and mirror-images. – J. mar. biol. Ass. U.K. 56: 263-266.

Deflandre, G. 1948. Les silicoflagellidés. Morphologie-cytologie-evolution-systématique. – Rapport, XIII Congrès Int. Zool. Paris, sect. 3: 198-201.

Deflandre, G. 1952. Classe des silicoflagellidés. – *In* Traité de Zoologie (ed. P.P. Grassé), 1: 425-438. Masson et Cie, Paris.

Dietrich, G. 1950. Die natürlichen Regionen von Nord- und Ostsee auf hydrographischer Grundlage. – Kieler Meeresforsch. 7(2): 35-69.

Errard-Le Denn, E. & M. Ryckaert 1989. Trout mortality due to *Dictyocha speculum* Douarnenez-France, 1987. Fourth Int. Conf. toxic marine Phytoplankton, June 26-30, 1989, Lund, Sweden; abstracts, p. 60.

Febvre-Chevalier, C. 1985. IV. Class Heliozoea Haeckel 1866. *In* An illustrated guide to the Protozoa (ed. by J.J. Lee, S.H. Hutner & E.C. Bovee), 302-317, Society of Protozoologists, Kansas.

Glezer, Z.I. 1966. Silicoflagellatophyceae. Flora Plantarum Cryptogamarum URSS, vol. VII. Nauka, Moscow, Leningrad, pp. 1-330. (Also the English translation: Cryptogamic Plants of the USSR, vol. VII. Israel Program for Scientific Translations, Jerusalem 1970, pp. 1-363).

Hansen-Ostenfeld, C. 1913. De danske farvandes plankton i årene 1898-1901. Phytoplankton og protozoer. – Kgl. Danske Vidensk. Selsk. Skr. 7 Række, Afd. IX,2, 478 pp.

Hovasse, R. 1932. Contribution a l'étude des Silicoflagellés. Bull. Biol. Fr. et Belg. 66, 447-501.

Ignatiades, L. 1970. The relationship of the seasonality of silicoflagellates to certain environmental factors. – Bot. Mar. 13: 44-46.

Kodama, M. & T. Ogata 1988. New insights into shellfish toxins. – Marine Pollution Bulletin 19: 559-564.

Larsen, J. 1985. Ultrastructure and taxonomy op *Actinomonas pusilla*, a heterotrophic member of the Pedinellales (Chrysophyceae). – Br. phycol. J. 20: 341-355.

Lemmermann, C. 1908. Flagellatae, Chlorophyceae, Coccosphaerales und Silicoflagellatae. – Nordisches Plankton, Botanischer Teil, Part XXI: 25-32.

Lohmann, H. 1908. Untersuchungen zur Feststellung des vollständigen Gehaltes des Meeres an Plankton. – Wissensch. Meeresuntersuch. Neue Folge. Bd. 10. Abt. Kiel. 370 pp.

Margalef, R. 1965. Distribucion ecologica de las especies del fitoplancton marino en un area del Mediterraneo occidental. – Investigación pesquera 28: 117-131.

Marshall, S.M. 1934. The Silicoflagellata and Tintinnoinea. British Museum (Natural History) Great Barrier Reef Expedition, 1928-29. – Scientific Reports 4: 623-664.

Moestrup, Ø. 1982. Flagellar structure in algae: a review, with new observations particularly on the Chrysophyceae, Phaeophyceae (Fucophyceae), Euglenophyceae, and *Reckertia*. – Phycologia 21: 427-528.

Nival, P. 1965. Sur le cycle de *Dictyocha fibula* Ehrenberg dans les eaux de surface de la Rade de Villefranche-sur-Mer. – Cahiers de biologie marine 6: 67-82.

Simonsen, R. 1974. The diatom plankton of the Indian Ocean Expedition of R.V. "Meteor" 1964-65. – Meteor Forschungsergebn. (D.Biol.) 19: 1-66.

Tangen, K. 1974. Fytoplankton og planktoniske ciliater i en forurenset terskelfjord, Nordåsvatnet i Hordaland. – Hovedfagsopgave, Universitetet i Oslo. 449 pp.

Tappan, H. 1980. The palaeobiology of plant protists. – W.H. Freeman and Company, San Francisco, 1028 pp.

Thomsen, H.A. & Ø. Moestrup 1985. Is *Distephanus speculum* a fish-killer? A report of an unusual algal bloom from Danish coastal waters. – Bull. Mar. Sci. 37: 778.

Throndsen, J. 1969. Flagellates or Norwegian coastal waters. – Nytt Mag. Botanikk 16: 161-216.

Travers, A. & M. Travers 1968. Les silicoflagellés du Golfe de Marseille. – Marine Biology 1: 285-288.

van Valkenburg, S.D. 1971a. Observations on the fine structure of *Dictyocha fibula* Ehrenberg. I. The skeleton. – J. Phycol. 7: 113-118.

van Valkenburg, S.D. 1971b. Observations on the fine structure of *Dictyocha fibula* Ehrenberg. II. The protoplast. – J. Phycol. 7: 118-132.

van Valkenburg, S.D. 1980. Silicoflagellates. *In* Phytoflagellates (ed. by E.R. Cox), 335-350, Elsevier/North Holland.

van Valkenburg, S.D. & R.E. Norris 1970. The growth and morphology of the silicoflagellate *Dictyocha fibula* Ehrenberg in culture. – J. Phycol. 6: 48-54.

Zimmermann, B., Moestrup, Ø. & G. Hällfors 1984. Chrysophyte or heliozoon: Ultrastructural studies on a cultured species of *Pseudopedinella* (Pedinellales ord.nov.), with comments on species taxonomy. – Protistologica 20: 591-612.

Submitted to the Academy August 1989.
Published August 1990.

Plates 1-16

PLATE 1

Figs 1-3. Live cells of the naked form of *Dictyocha speculum*, collected Alssund 2 May 1983. The single long flagellum emerges from an apical depression in the cell. The centrally located nucleus and numerous chloroplasts are also visible. Nomarski interference contrast, × 1350. Scale bar = 10 μm.

Figs 4,5. Slightly oblique longitudinal (Fig. 4) and tranverse section (Fig. 5) through the naked stage, the latter at the level of the nucleus. Division of the cytoplasm into 3 regions is clearly visible: an outer containing mainly chloroplasts and mitochondria, an intermediate vacuolated region, and an inner region containing the nucleus, surrounded by a ring of Golgi bodies. The proximal part of the flagellum is visible in its cavity in Fig. 4. Fig. 4: × 5.000; Fig. 5: × 7.500. Scale bars = 5 μm.

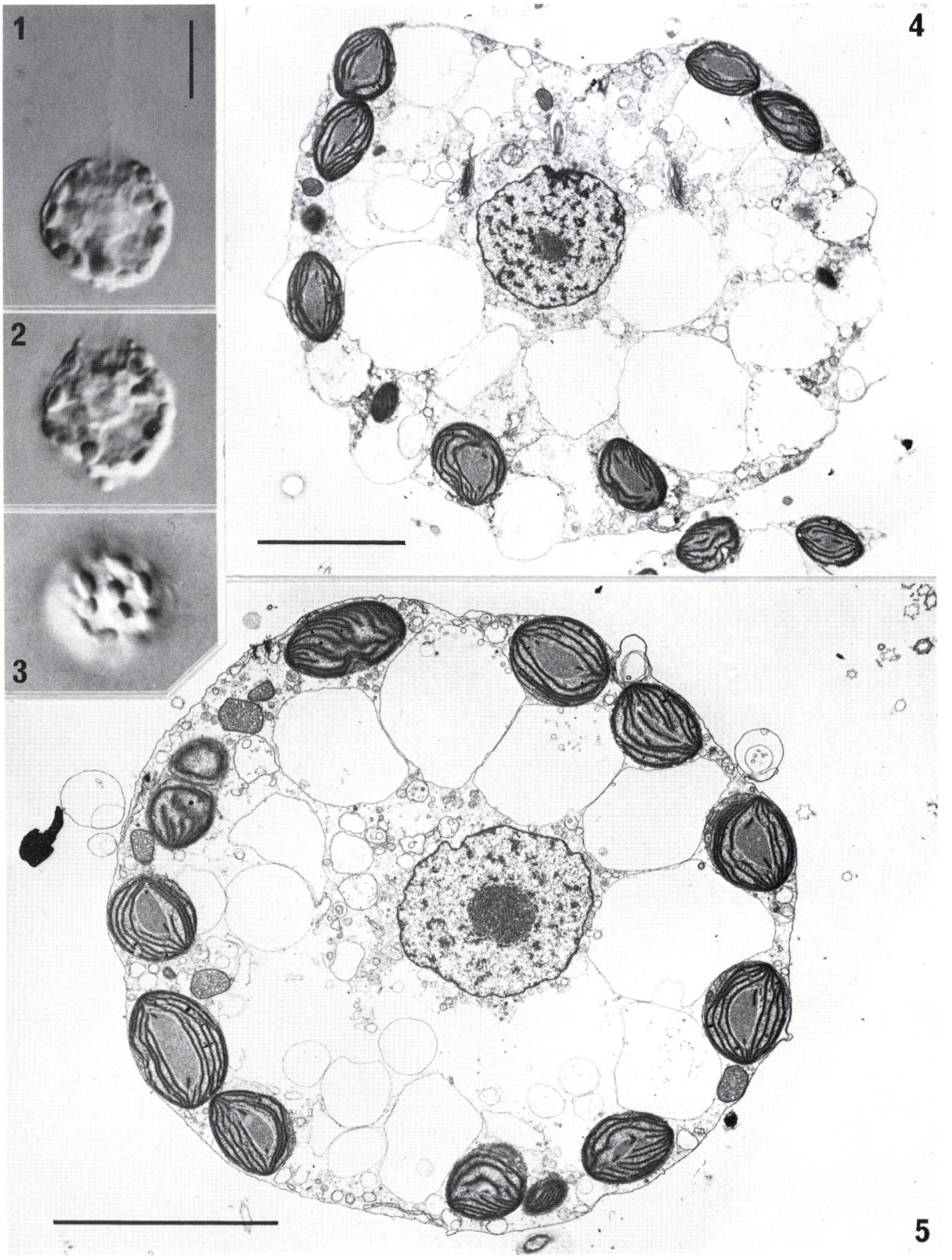

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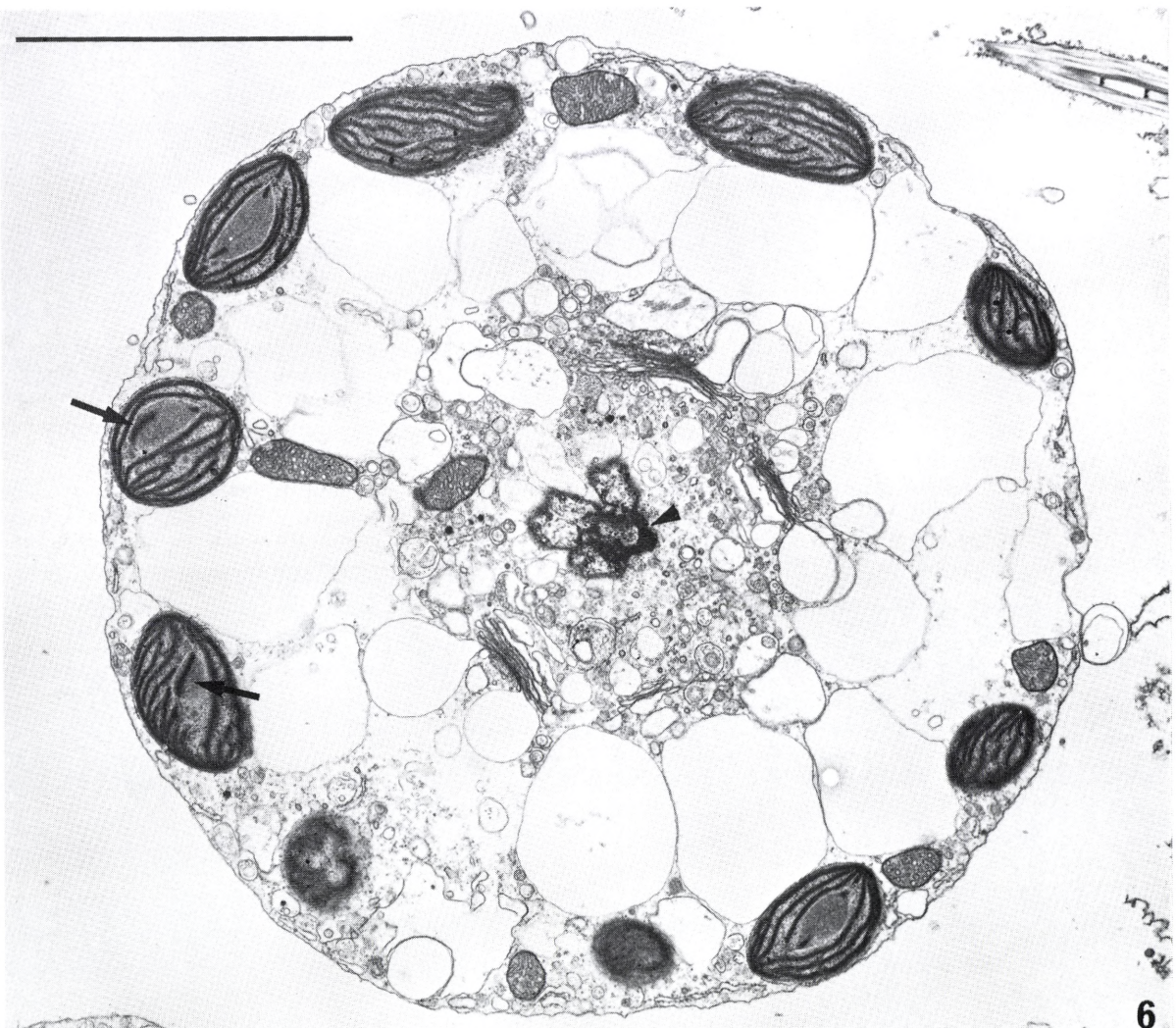
PLATE 2

Figs 6,7. Transverse sections of the naked stage of *Dictyocha speculum*, same cell as in Fig. 5, showing the anterior end of the nucleus. Four Golgi bodies are visible around the nucleus. One of the flagellar bases may be distinguished in Fig. 6 (arrowhead), embedded in the anterior end of the nucleus. Pyrenoids show the peripheral triplet lamella at various angles (arrows), compare with Figs 19-24. Both × 9.000. Scale bar = 5 μm.



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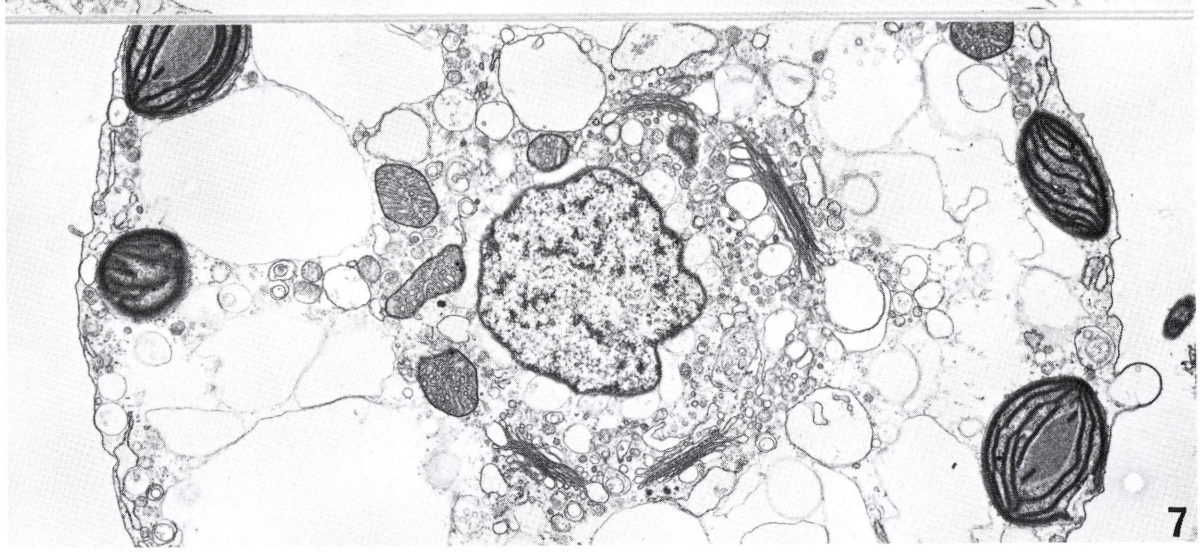

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PLATE 3

Fig. 8. Longitudinal section through the flagellar canal, showing both the short flagellum and the long emergent flagellum with its wing which contains a cross-banded muscle. × 30.000. Scale bar = 0.5 μm.

Figs 9,10. Transverse sections of the flagella, same cell as in Figs 5-7 and 12. The short flagellum (compare with Fig. 8) is uniformly grey without a central pair of microtubules. In Fig. 10 the short flagellum has terminated. The proximal part of the long flagellum also lacks the central pair of microtubules. × 48.000. Scale bar = 0.5 μm.

Fig. 11. Transverse section of the long flagellum, which at some distance from the cell shows the normal 9+2 configuration. The dense material on the left is probably part of the paraxial rod (muscle), compare with Figs 14-15. × 48.000. Scale bar = 0.5 μm.

Fig. 12. The cell from Figs 5-7, now sectioned at the level of the flagellar pit. Both flagella are visible (shown at higher magnification in Fig. 9). × 9.000. Scale bar = 5 μm.

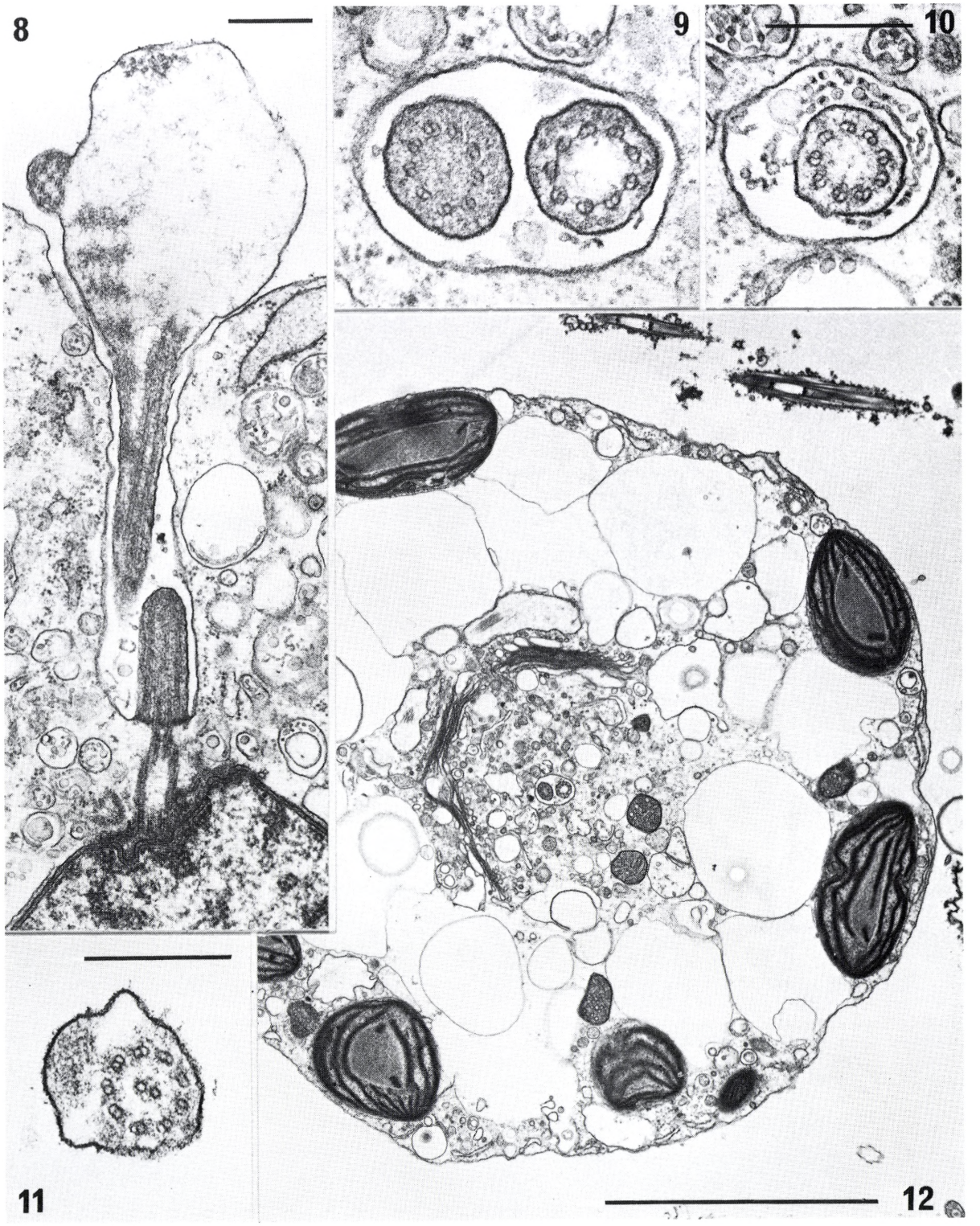
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PLATE 4

Fig. 13. The 2 basal bodies are almost parallel with identical orientation of the triplets. × 48.000. Scale bar = 0.5 μm.

Figs 14,15. Sections through the emergent flagellum, which has contracted as a response to the fixative. The flagellum carries a wing with a muscle-like component and groups of tubules. The proximal part of the flagellum with its 9+0 is visible on the far right (arrow). Both × 30.000. Scale bars = 0.5 μm.

Fig. 16. The transition region between the basal body and the flagellum proper contains a transverse partition located just above cell level. There is no sign of a transitional helix. × 48.000. Scale bar = 0.5 μm.

Figs 17,18. The basal bodies (which in Fig. 18 have duplicated) are firmly anchored to the anterior end of the nucleus, which in this region is very irregular. Profiles resembling microtubular roots are occationally visible (e.g. between the basal bodies in Fig. 17). Distinct rhizoplasts have not been detected, but may be part of the dense structures surrounding the basal bodies. Both × 39.000. Scale bars = 0.5 μm.

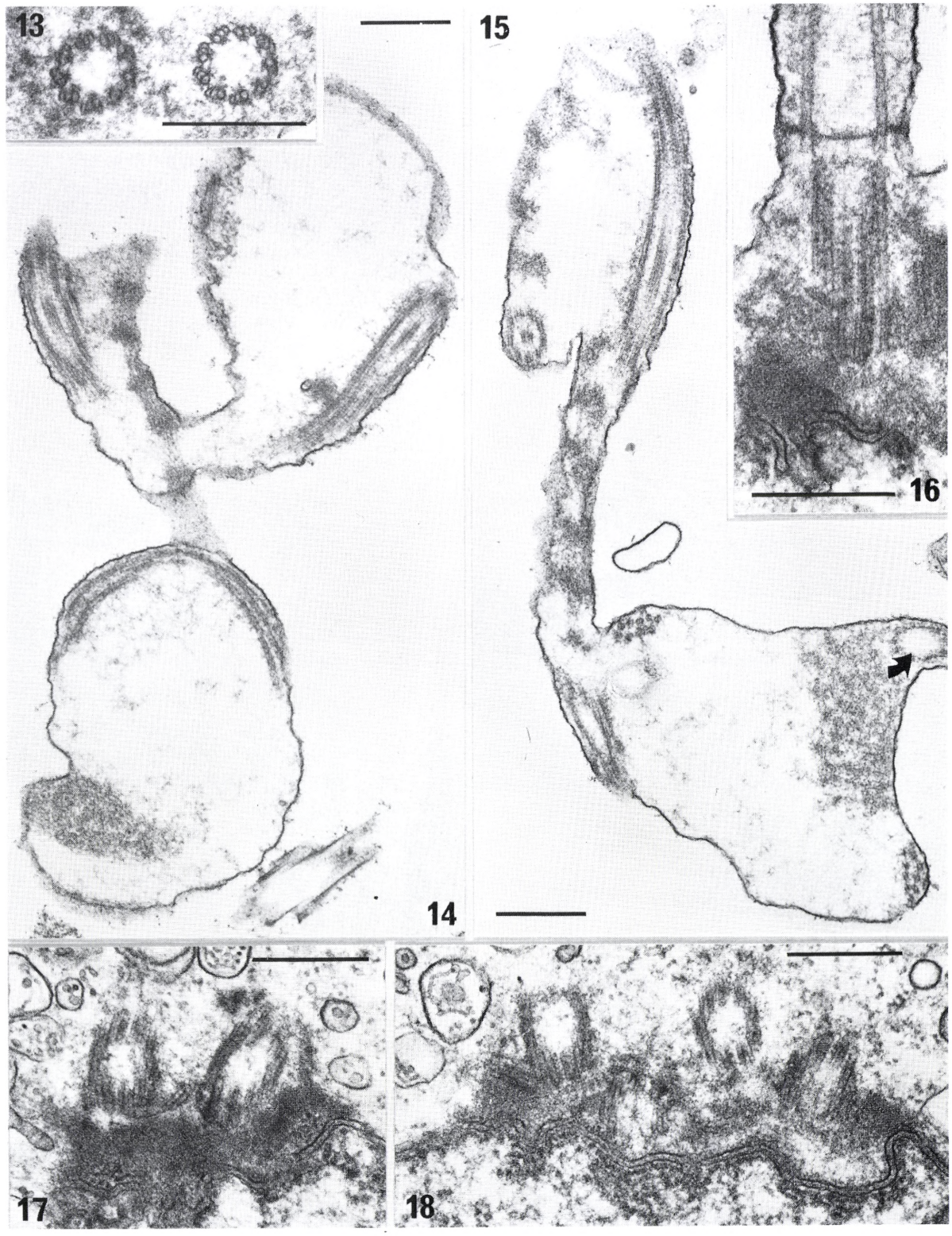
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PLATE 5

Figs 19-24. Chloroplast and mitochondrial structure. The disc-shaped chloroplasts each contain a usually lens-shaped internal pyrenoid, which at the circumference contains a short triplet of thylakoids (Fig. 19). These have fused at one end (Fig. 21), and at the other end is a thin dense plate, outside the pyrenoid (Fig. 21). In some cases this is replaced by a thylakoid (Fig. 19) which connects to the triplet. The triplet is not always continuous around the entire circumference of the chloroplast (Fig. 23). During division of the chloroplast (Fig. 24) membranous material is present in the invaginations, perhaps cytoplasmic remains of the organism believed to have become transformed into a chloroplast. Fig. 22 shows tubular mitochondrial cristae with dense inclusions in the cristae. The cytoplasm also contains bacteria (Fig. 20).

Figs 19,21,22: × 40.000; Figs 20,24: × 22.000; Fig. 23: × 30.000. Scale bars = 0.5 μm.

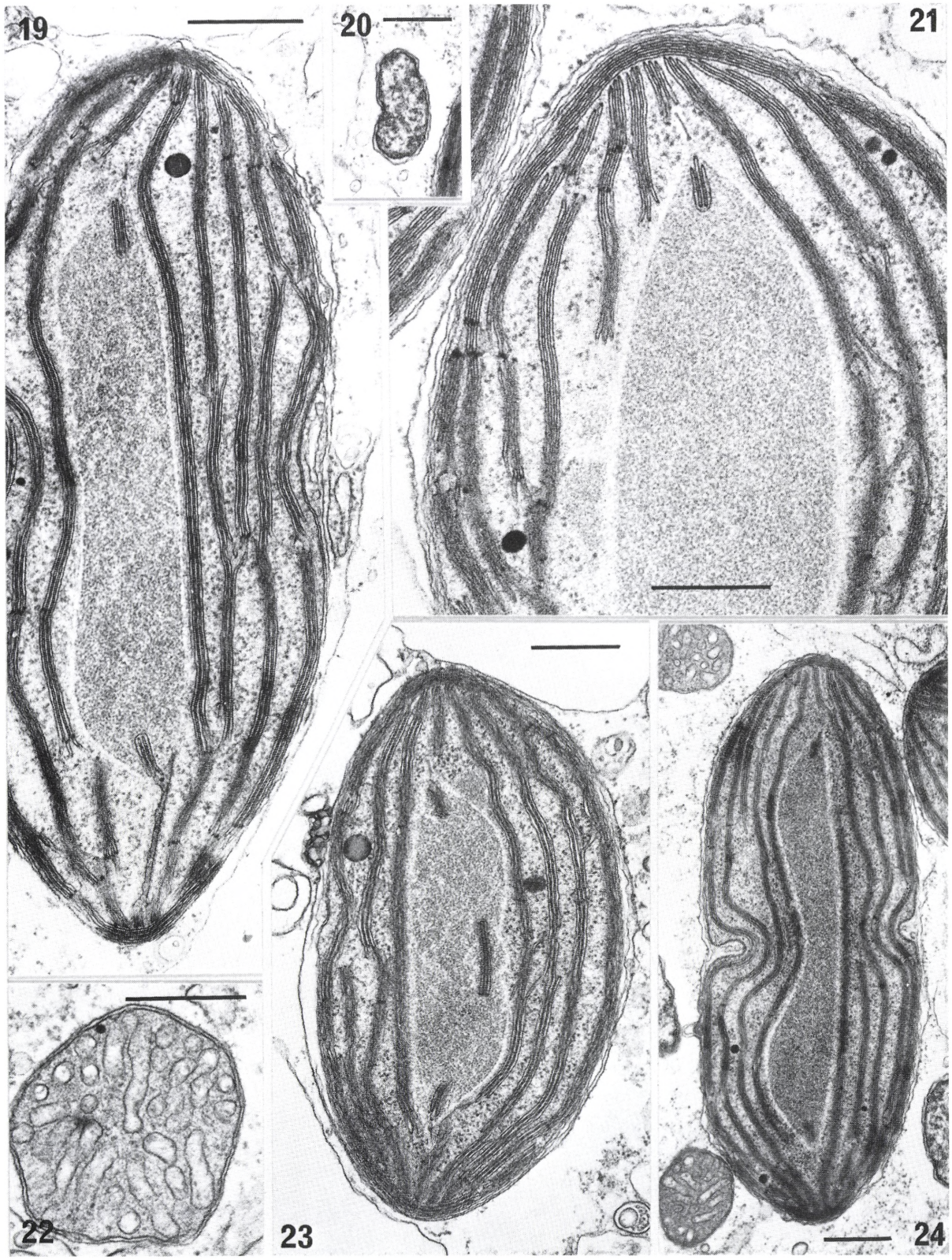
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PLATE 6

Figs 25-29. The Golgi apparatus. In the naked stage of *Dictyocha* the Golgi bodies consist of c. 10 cisternae. Two distinct elements are visible in the cisternae, a very thin plate-like structure visible only near the mature face (arrows), and so far not described from other algae (Figs 25,26,28,29). The cisternae also contain small groups of hair-like structures, probably immature flagellar hairs, which have been transferred from the adjacent endoplasmic reticulum (Fig. 26). After passing through the Golgi body the hairs are released to the surface (Fig. 27). The base of the emergent flagellum with its 9+0 structure and paraxial rod is visible on the left in Fig. 26. Figs 25,28,29: × 40.000; Fig. 26: × 35.000; Fig. 29: × 57.000. Scale bars = 0.5 μm.

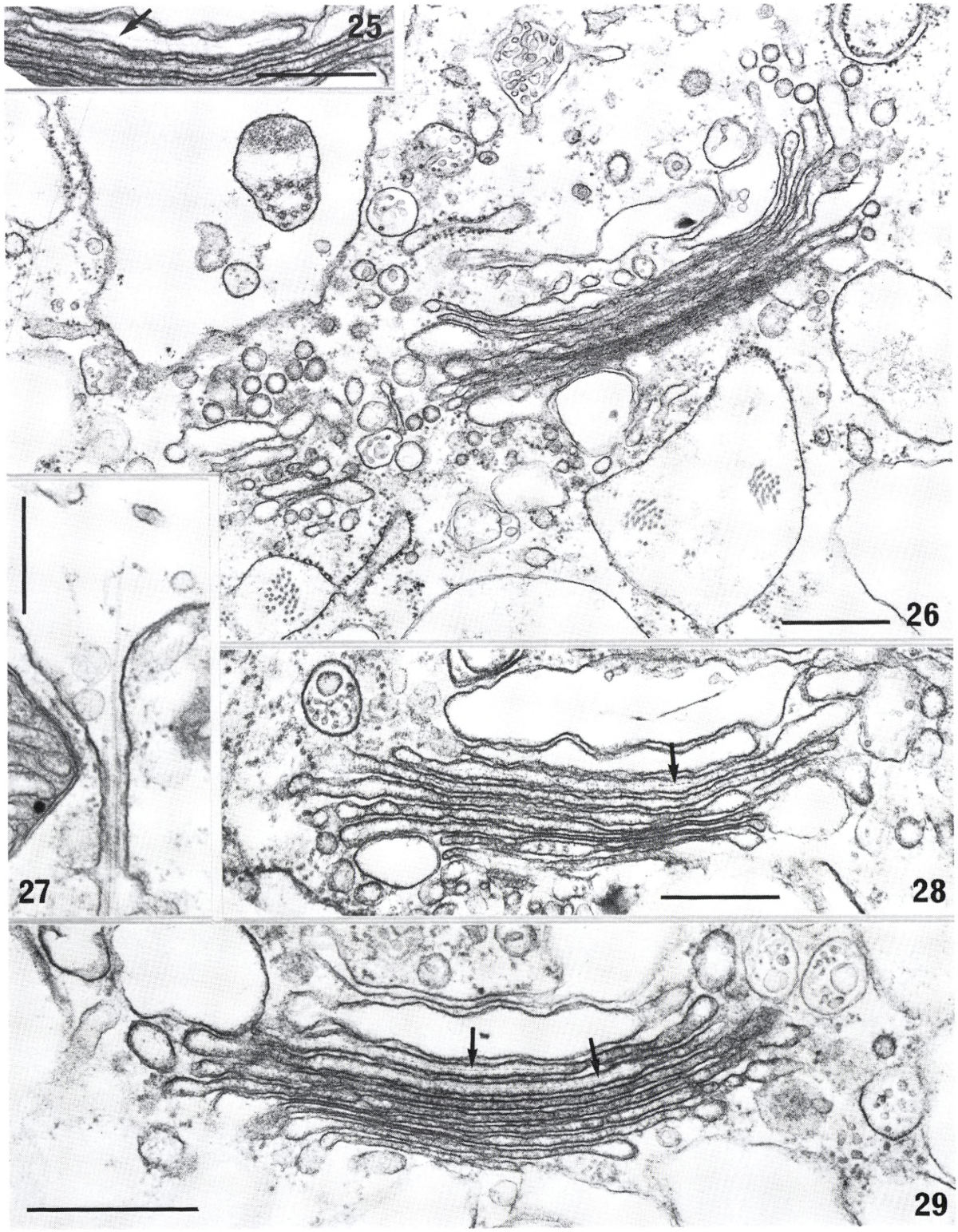
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PLATE 7

Figs 30-33. Nomarski Interference Contrast of two live cells of the skeleton bearing phase of *Dictyocha speculum*, material from Isefjorden 11 Dec. 1984. The flagellum is visible in Figs 30 and 33 (same cell), emerging along one of the long spines. The flagellum has a beaded appearance indicating that it will soon break up. Tentacles are visible in both figures. The cell in Figs 31,32 is slightly deviant in skeleton structure, as the small ring is absent (see text). The 2 longest spines are directed NE and SW. Some chloroplasts protrude from the central skeleton area, giving the (wrong) impression that the skeleton is internal. x 800. Scale bar = 10 μm.

Fig. 34. Transverse section through skeleton bearing cell at the level of the nucleus. Visible are the central area with the nucleus surrounded by Golgi bodies, the adjacent vacuolated layer, and the peripheral layer of chloroplasts. The skeleton which consists of hollow bars is external. × 57.000. Scale bar = 0.5 μm.

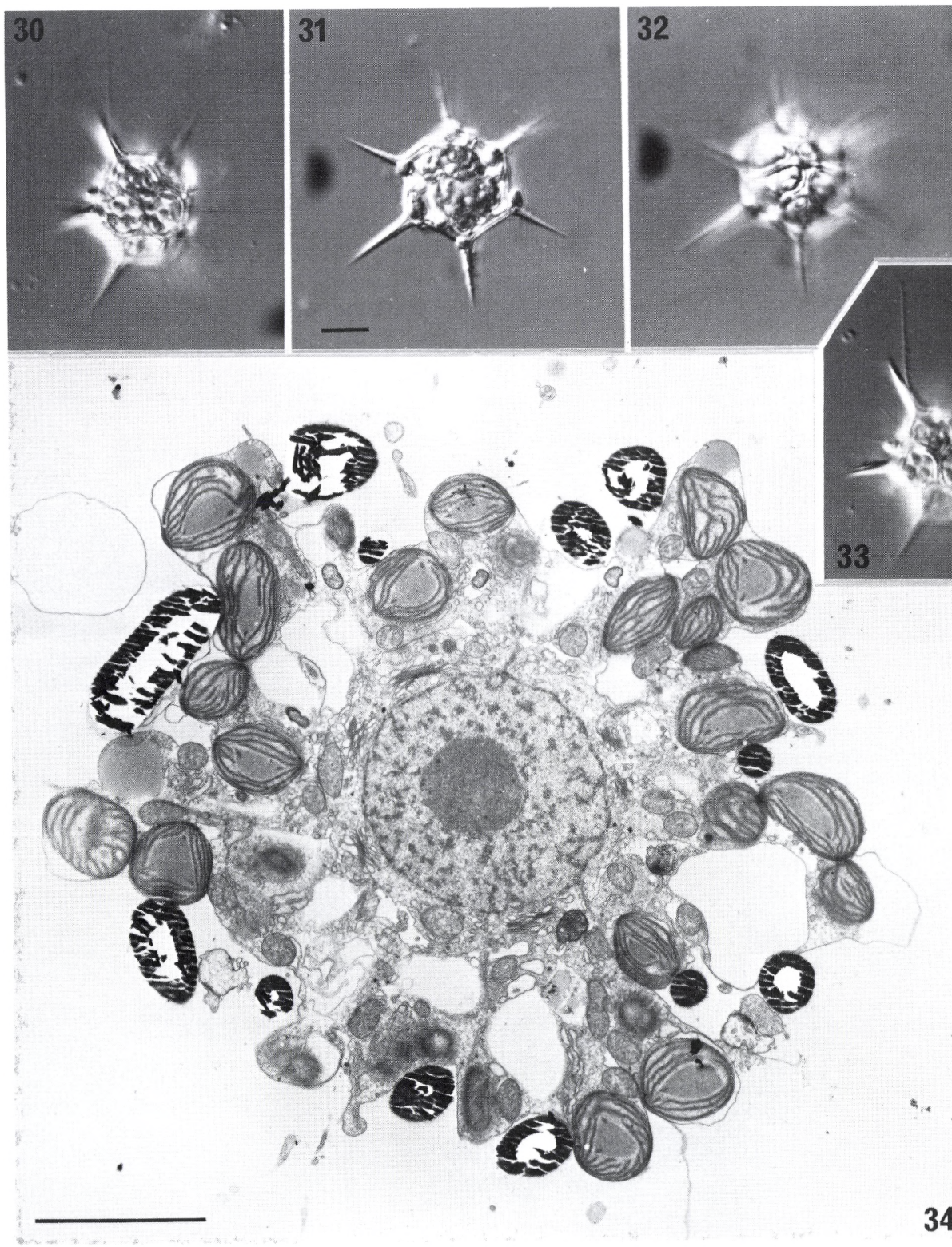
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PLATE 8

Figs 35-38. Skeletons of *Dictyocha*, which have been oxidized to remove the organic parts. From Isefjorden 30 October 1984. Scanning electron microscopy. The skeleton typically consists of 2 rings, a larger which is hexagonal and a smaller almost circular. The rings are connected through 6 bars, which alternate with 6 spines. Smaller, inwardly directed spines (Fig. 38) probably serve to keep the cell in the chamber formed by the skeleton (compare with Fig. 39). The small ring (Fig. 35) possesses only few spines. The bars of the skeleton are hollow as shown in the broken piece in Fig. 36. Figs 35,38: × 3.600; Fig. 36: × 12.000; Fig. 37: × 2.500. Scale bars = 5 μm.

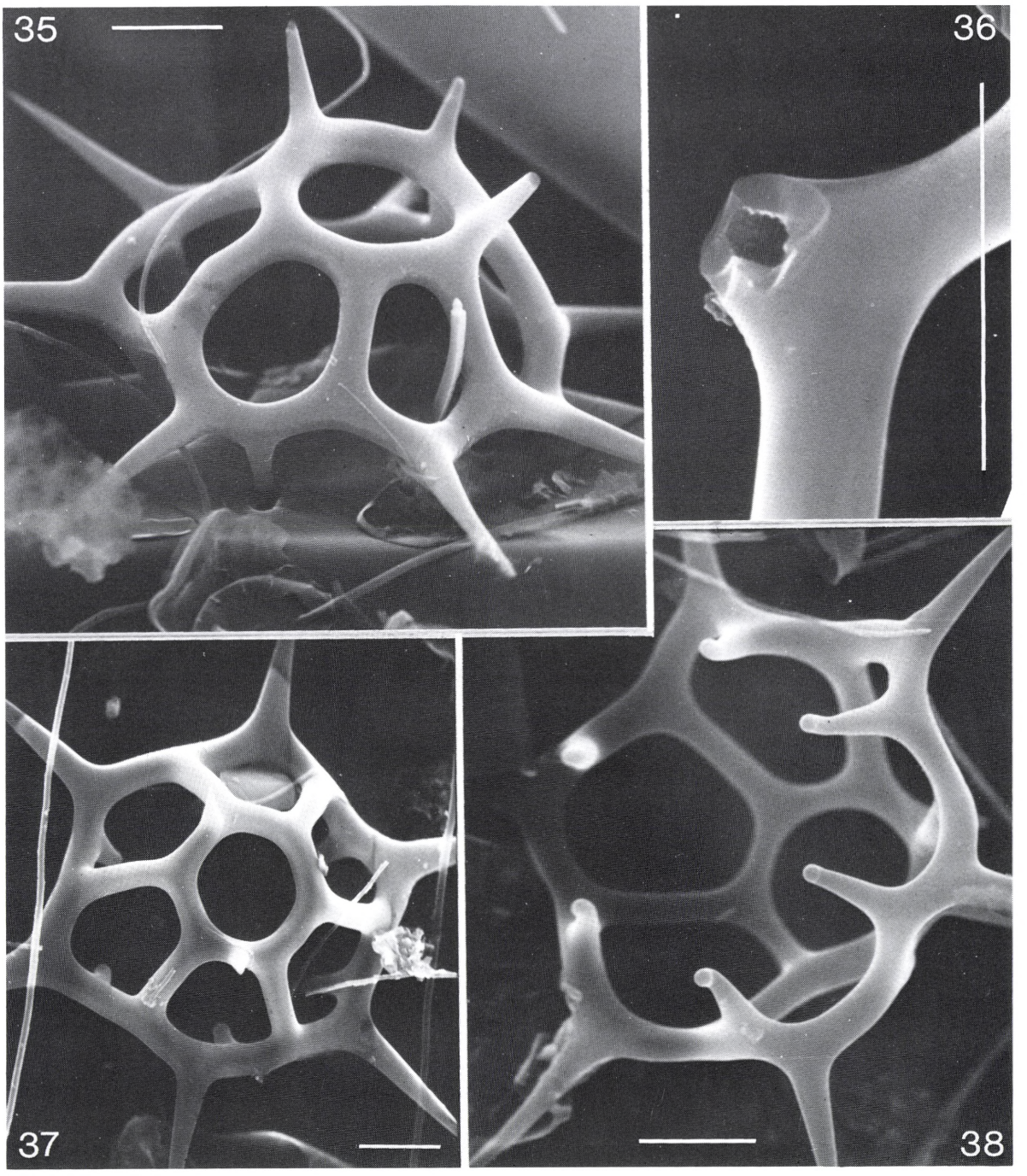
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PLATE 9

Figs 39,40. Material from Alssund May 1983. The skeletons have not been oxidized, and the cells are therefore still within the skeleton cavity, although they have broken in several places. Fig. 39 shows a cell prior to division, the new skeleton has been formed. The arrows indicate the small spines on the large skeleton ring. Fig. 39 is one of several indicating that these spines play a role in fixing the cytoplasm within the skeleton cavity. Both × 2.400. Scale bars = 5 µm.

Figs 41-44. Same material and treatment as Figs 35-38. The 2 long spines are clearly visible in Fig. 41. Fig. 42 shows a cell with 7 spines, these occur occasionally. The skeletons in Fig. 43 and 44 are seen from the side and clearly indicate the cavity in which the cells have been located, kept in position by the short spines which from the large ring point inwards. Fig. 41: × 1.800; Figs 42-44: × 2.500. Scale bars = 5 µm.

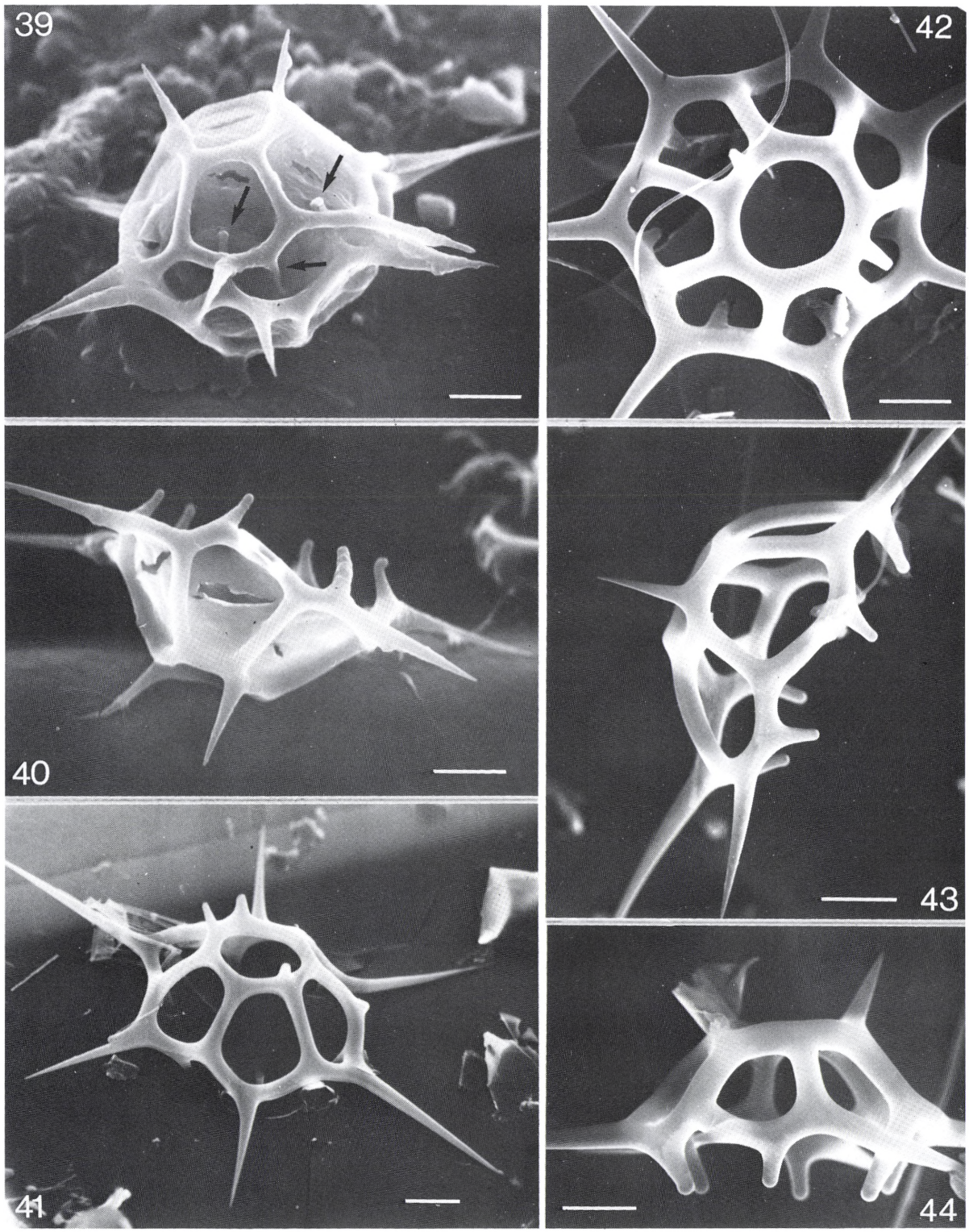
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PLATE 10

Fig. 45. Transverse section of a skeleton bearing cell at the level of the flagellar pit (flagellum indicated with arrow). All elements of the skeleton are located outside the plasmalemma, the skeleton being external rather than internal as sometimes claimed. × 5700. Scale bar = 1 μm.

Fig. 46. Longitudinal section through one of the six large spines. The skeleton is hollow except in the distal part of the large spines. × 5700. Scale bar = 1 μm.

Figs 47-48. The basal part of the flagellum, showing flagellar hairs and the presence of a platform, which carries a tuft of flagellar hairs. Fig. 48 is a higher magnification of the central region of Fig. 45. The second basal body (without emergent flagellum) is visible in Fig. 47. × 42.000 and 30.000, respectively. Scale bars = 0.5 μm.

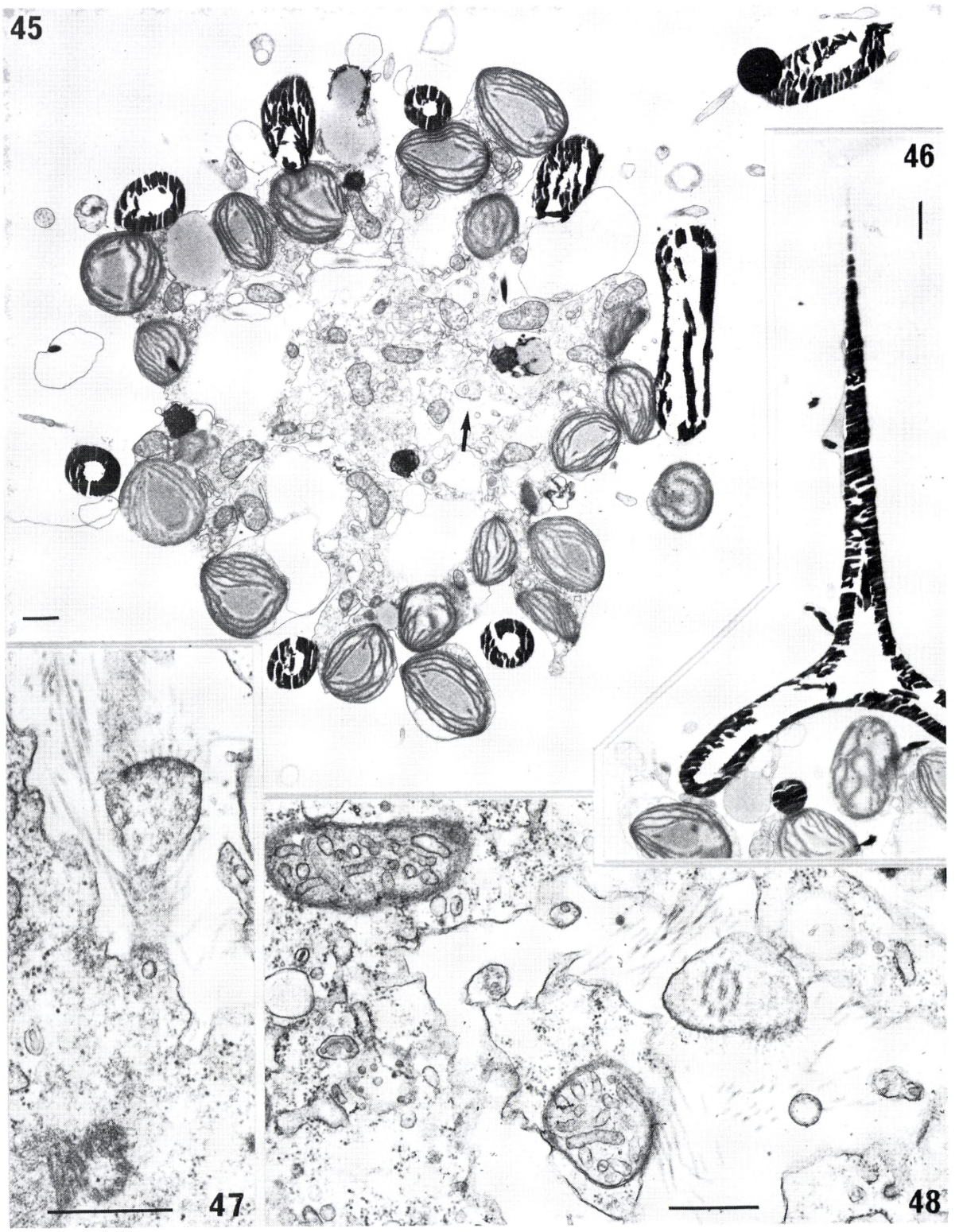
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PLATE 11

Figs 49-52. Consecutive serial sections through the emergent flagellum of a skeleton bearing cell. The lowermost arrow in Fig. 49 shows the transverse partition of the flagellar transition region, the uppermost shows the proximal end of the central pair of microtubules in the 9 + 2. The flagellum has been sectioned in the plane of the basal extension of the flagellum, with its tuft of flagellar hairs. × 42.000. Scale bar = 0.5 μm.

Figs 53-55. Sections of another cell, showing the emergent flagellum and the additional basal body, arranged at an acute angle to each other. Both are attached to the anterior part of the nucleus. The arrows indicate a dense and probably ringlike structure which is present below the transverse partition of the flagellar transition region, but outside the axoneme. A similar structure is known to occur in a member of the Pedinellales. The bundle of microtubules on the right hand side of Fig. 53 is part of a tentacle. Fig. 53: × 22.000; Figs 54 and 55 both × 57.000. Scale bars = 0.5 μm.

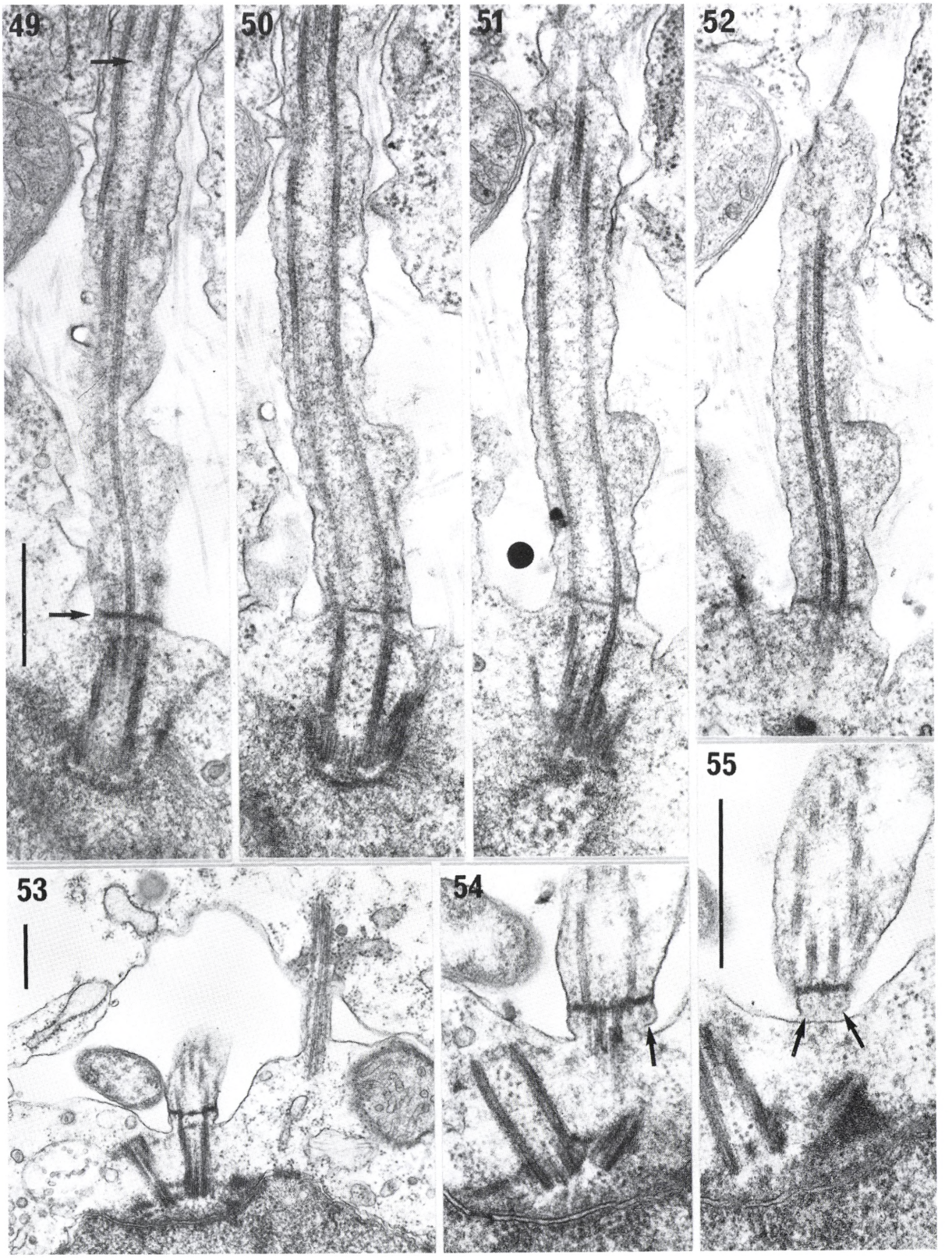
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PLATE 12

Figs 56 and 58. Section through the proximal end of a tentacle. It begins on the flagellar surface, passes through the canal system which separates the chloroplast containing region of the cell from the area near the nucleus, and the microtubules finally split into several groups, each extending as a tentacle from the cell. The tentacles are somewhat irregular in appearance and do not contain a fixed number of microtubules (compare with Figs 59-69). The arrow in Fig. 58 indicates the opening to the exterior of a canal from the internal canal system. Fig. 56: × 42.000; Fig. 58: × 57.000. Scale bars = 0.5 μm.

Fig. 57. Section through flagellar base and the anterior part of the nucleus, indicating the presence of a thin indistinct sheet of fibres (arrow) which probably represents a rhizoplast on the nuclear surface. × 57.000. Scale bar = 0.5 μm.

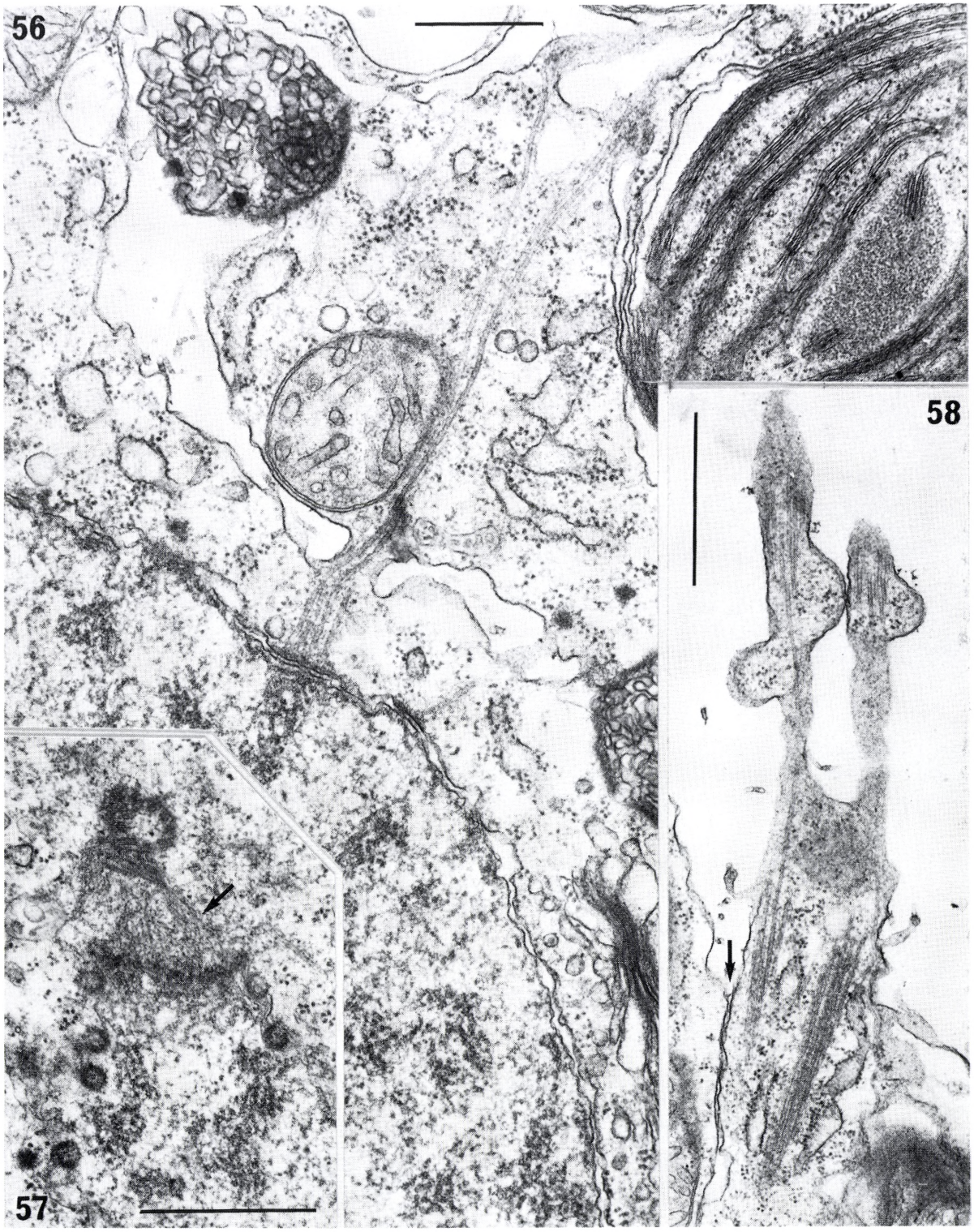
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PLATE 13

Figs 59-69. Consecutive serial sections (no sections missing) through a tentacle, from its appearance on the nuclear surface (Fig. 60) as a group of 5 microtubules to its emergent part (Figs 67-69), which contains 9 microtubules. The additional microtubules are added in Figs 62, 63 and 66 (arrows). Other tentacles are also visible, and contain different numbers of microtubules: parts of 5 tentacles are visible in Fig. 69, containing 3,5,9,1 and 14 microtubules, respectively. Figs 59,60: × 120.000; Figs 61,62 and 69: × 57.000; Figs 63-68: × 42.000. Scale bars = 0.1 μm.

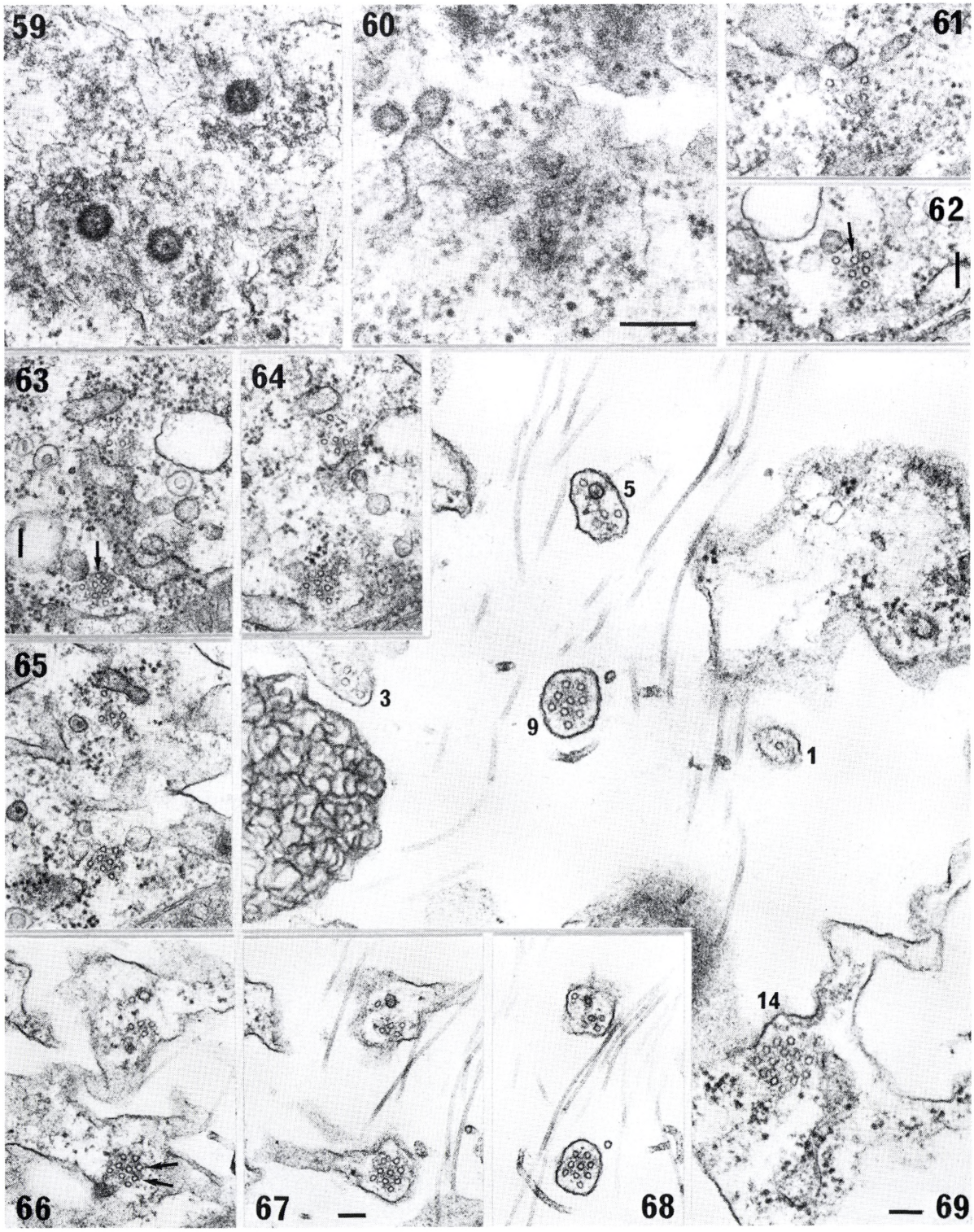
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PLATE 14

Figs 70-72. Details of the central cell area of the skeleton bearing stage of *Dictyocha speculum*. The centrally located nucleus is surrounded by Golgi bodies, whose cisternae contain very thin plate-like structures similar to those seen in the other stages of *Dictyocha* (arrowhead in Fig. 71). The arrows in Figs 70 and 72 indicate the internal canal system characteristic of the skeleton bearing stage (see further in the text). × 30.000, 50.000 and 11.000, respectively. Scale bars = 0.5 μm.

Fig. 73. Detail of pyrenoid showing the stalked triplet which is characteristic of all stages of *Dictyocha speculum*. × 42.000. Scale bar = 0.5 μm.

Fig. 74. The skeleton bearing stage contains endocytoplasmic bacteria, here a cell located near the internal canal system, which is indicated with arrows. The large dense structure at the top of the figure is a transverse section through one of the skeletal elements. × 16.000. Scale bar = 0.5 μm.

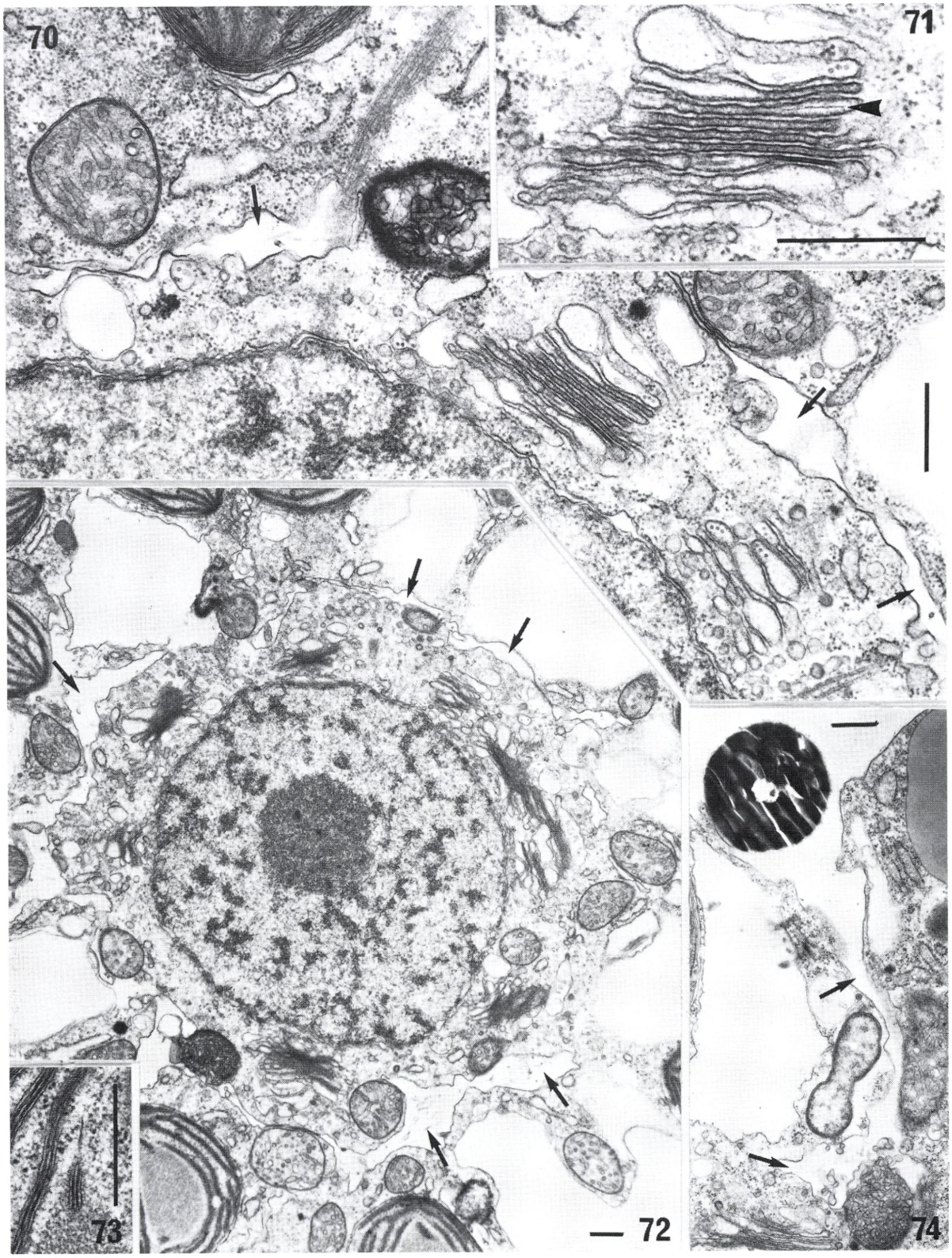
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PLATE 15

Figs 75-76. The large multinucleate stage of *Dictyocha speculum*, as seen in interference light microscopy and thin section electron microscopy. The cell shown in Fig. 75 is approx. 350 μm long. In the multinucleate stage nuclei, chloroplasts, vacuoles, and Golgi bodies are scattered in the cytoplasm, apparently without any fixed position relative to one another. Fig 75: × 250, scale bar = 40 μm. Fig. 76: × 4.000, scale bar = 5 μm.

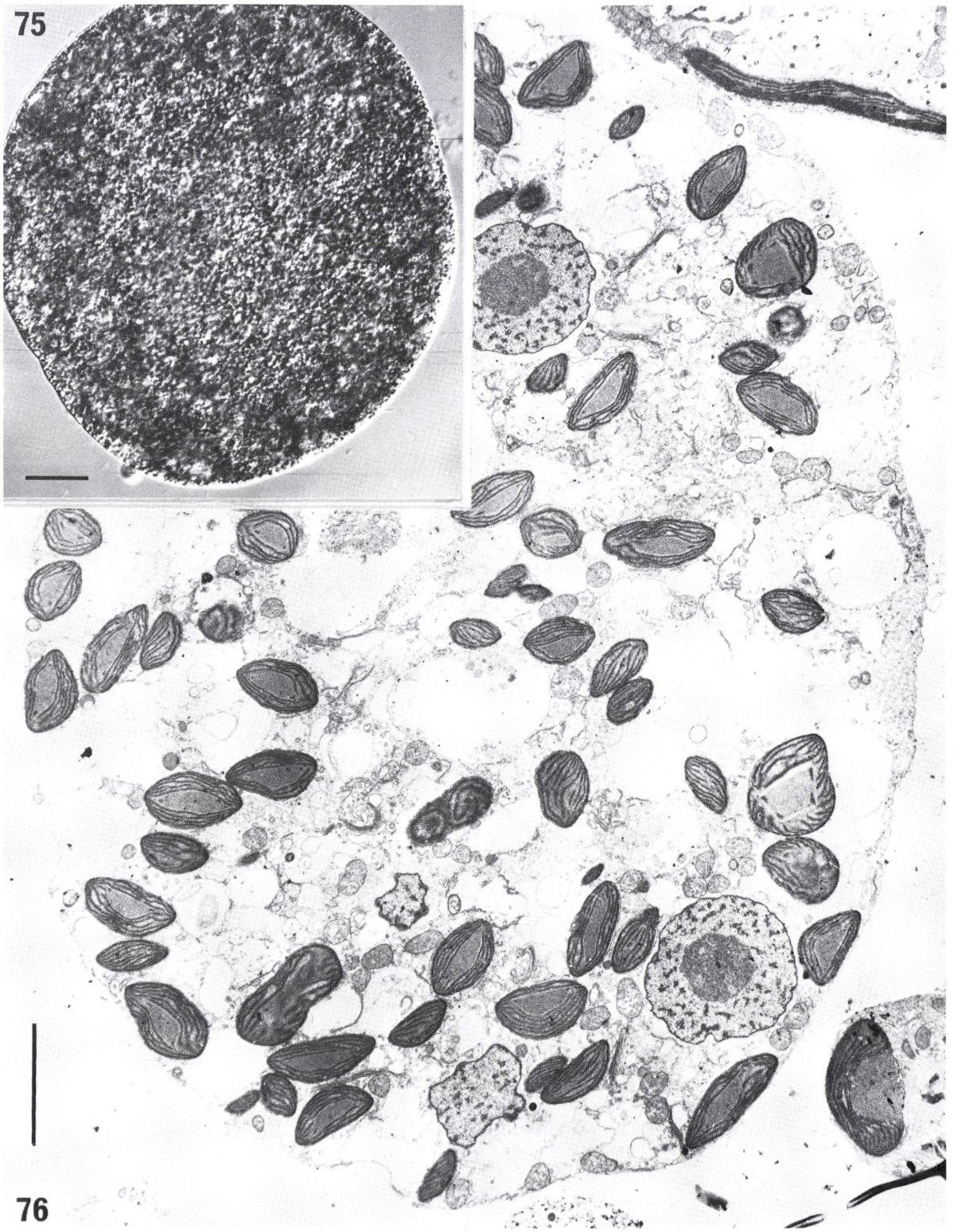
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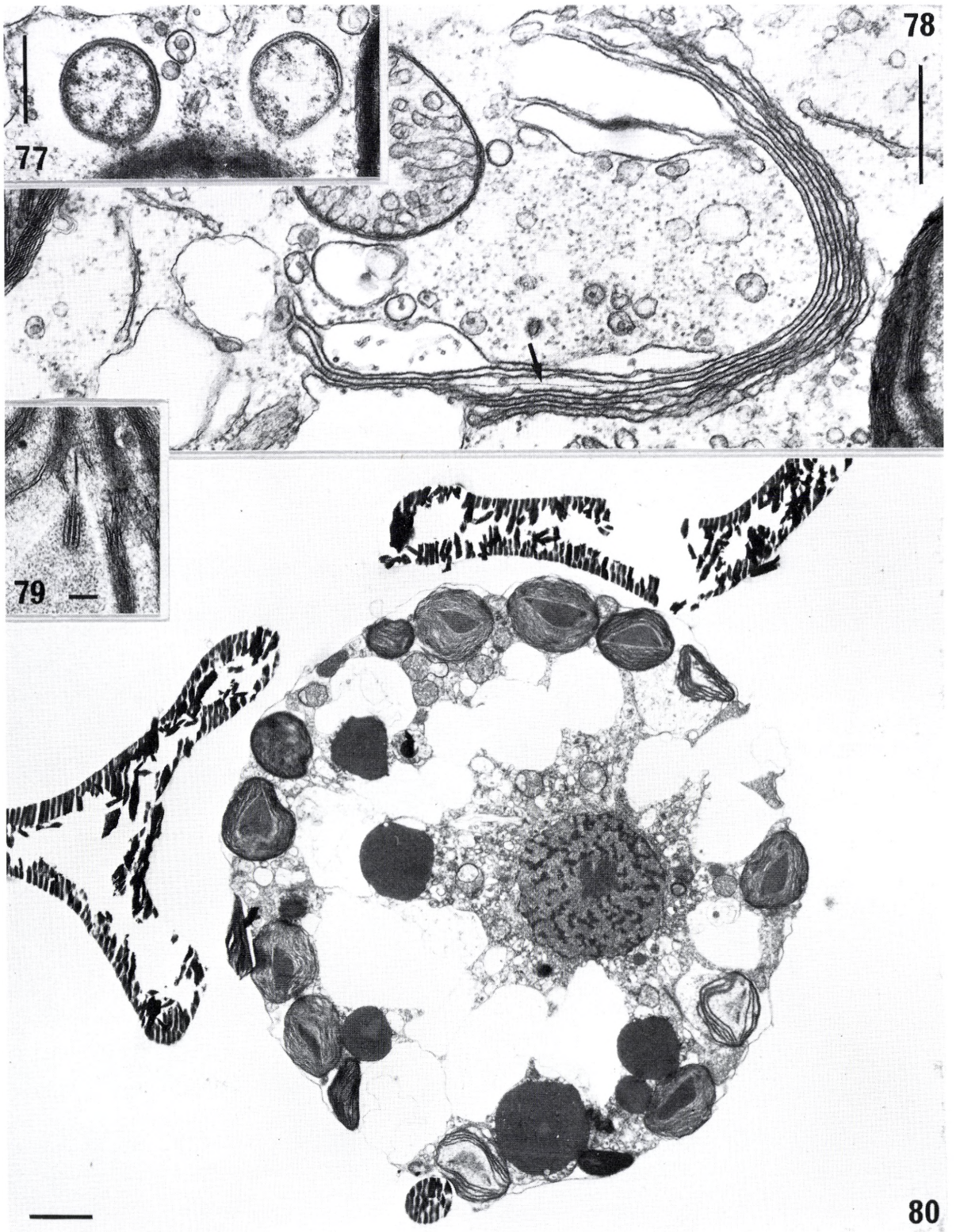
PLATE 16

Figs 77-79. Details of the multinucleate stage of *Dictyocha speculum* showing endocytoplasmic bacteria (Fig. 77), a Golgi body with thin plate-like elements seen also in the cisternae of the other stages of *Dictyocha* (arrow in Fig. 78), and the stalked triplet of the pyrenoid (Fig. 79). Fig. 77: × 30.000, scale bar = 0.5 μm. Fig. 78: × 40.000, scale bar = 0.5 μm. Fig. 79: × 45.000, scale bar = 0.1 μm.

Fig. 80. Cell believed to represent a stage intermediate between the naked and the skeleton bearing stages (see further in the text). × 5700, scale bar = 2 μm.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