

## DISTRIBUTION OF PYRENOIDS AMONG SOME BROWN ALGAE

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### SUMMARY

The distribution of pyrenoids among some orders of the brown algae has been investigated with the electron microscope and a report on their occurrence is given, with particular reference to the results obtained with the light microscope by Simon (1954). Some illustrated detail of the structure of the pyrenoids and of the chromatophores is included.

Pyrenoids were found to be present in the representatives examined of the Ectocarpales, Sphacelariales, Scytosiphonales and Dictyosiphonales. Excepting the Sphacelariales, this is in agreement with the results of Simon. Pyrenoids were found to be definitely absent in all members of the Dictyotales and Laminariales examined, and disagreement is expressed with Bouck (1965) who reported their presence in *Chorda filum*, a member of the latter order. The situation in the Fucales is the subject of another communication elsewhere.

The presence or absence of pyrenoids is regarded as an additional taxonomic character of possible phyletic use when more is known. The results are discussed from the standpoint of their possible value in assessing the relative position of the Phaeophyceae as a whole. Brown algal lamellations are composed of three, or occasionally four, parallel thylakoids which do not cohere and are not aggregated into stacks. This is thought to be more primitive than the condition in some of the other groups of the Chromophyta where there is adherence of thylakoids into stacks of two or three members as, for example, in the Haptophyceae, Xanthophyceae and Chrysophyceae. The Phaeophyceae are, however, regarded as much less primitive than the Rhodophyceae, where the widely spaced parallel thylakoids are arranged singly.

### INTRODUCTION

The conspicuously projecting pyrenoids of brown algae, common though not universally present in the group, have been subjected to microscopical study more than once. (For light-microscopical literature see Simon (1954), and for electron microscopy see Bouck (1965) and Manton (1966*a*.) In spite of this a good deal remains uncertain and even the distribution within the group is imperfectly and in some instances incorrectly recorded. My attention having been drawn to this in the course of a cytological study of the British Laminariales (Evans, 1965), an attempt was made to ascertain whether this type of pyrenoid was present or absent in British representatives of some of the more important brown algal orders, using electron microscopy.

As was to be expected, the scope of the inquiry soon outgrew the permissible limits of one communication and certain major groups, notably the Fucoids, must be reserved for separate treatment elsewhere. Enough will, however, be presented here to indicate that within the Phaeophyceae the distribution and character of pyrenoids is not random, and that differences, where they occur, are almost certainly phyletically significant. It is not possible to include a detailed developmental study of any one type

Table 1. *Details of specimens studied*

Species	Source	Collection	Material fixed
<i>Ectocarpus confervoides</i>	Menai Bridge	Sept. 1964	Motile zoospores
<i>Pylaiella littoralis</i>	Cullercoats	June 1964	
<i>Sphacelaria bipinnata</i>	Menai Bridge	Sept. 1964	
<i>Scytosiphon lomentarius</i>	Cable Bay, Anglesey	April 1964	
<i>Colpomenia peregrina</i>	Rhosneigr, Anglesey	April 1966	Motile zoospores and germlings
<i>Dictyota dichotoma</i>	Trearddur Bay, Anglesey	Sept. 1964	Thallus sections
<i>Padina pavonia</i>	Wied-iz-Zurrieq, Malta	March 1966	Eggs, thallus sections
<i>Dictyosiphon foeniculaceus</i>	Rhoscolyn, Anglesey	Sept. 1964	Thallus sections
<i>Laminaria digitata</i>	Cullercoats	June 1964	Motile zoospores
<i>Alaria esculenta</i>	Cullercoats	March 1965	
<i>Chorda filum</i>	Rhosneigr, Anglesey	Sept. 1964	Motile zoospores and germlings
<i>Saccorhiza polyschides</i>	Rhosneigr, Anglesey	Sept. 1964	Motile zoospores

on the lines recently carried out for a representative of a different group (Haptophyceae, *Chrysochromulina chiton*, by Manton (1966*b*)). Enough information will, however, be given to indicate the more obvious resemblances and differences among the various groups, and to suggest lines on which further work could usefully be carried out.

#### MATERIAL AND METHODS

The species studied in the present work are listed in Table 1, together with the places of origin and dates of collection. Wherever possible fixations were made on zoospores either freshly liberated or recently germinated (for experimental details see Evans (1965)). Where neither of these could be obtained, or occasionally in addition to these, thin slices of tissue cut with a razor were used. Fixations were carried out in 4% glutaraldehyde neutralized with excess barium carbonate or calcium carbonate, and made up in cacodylate buffer at pH 7, with 0.25 M sucrose added to the final mixture. (Troublesome crystals appeared in sections when material, in a small amount of sea water, had been fixed in glutaraldehyde treated with barium carbonate; these did not occur after fixation in glutaraldehyde neutralized with calcium carbonate.) The fixation time varied from 2 h to overnight. The glutaraldehyde was then washed out by 3 changes of cacodylate buffer (at pH 7) with or without gradual reduction of sugar content, each change being left for 30 min. Post-osmication in 2% osmium tetroxide made up to pH 7 with phosphate or acetate-veronal buffer was applied for periods ranging from 5 h to overnight. Dehydration and embedding in Epon 812 were standard. Fixation, washing, post-osmication and dehydration as far as 70% ethanol were carried out on ice. Treatment was completed at room temperature. Sections were cut with a Dupont diamond knife on an LKB microtome, stained with lead citrate (Reynolds, 1963), and examined in a Siemens Elmiskop I electron microscope.

## OBSERVATIONS

*General*

The general characteristics of the pyrenoids are illustrated at various magnifications in Figs. 1-7 in organisms representing the Ectocarpales, Sphacelariales and Scytosiphonales. The pyrenoids usually occur singly, but sometimes two widely spaced ones are seen. They project either from the inner face of the subtending chromatophore or terminally at one or both ends, as large bulges filled with amorphous, dense material, parts of which sometimes show a crystalline appearance. The thylakoids of the subtending chromatophore do not enter the pyrenoid. The distribution of this type of pyrenoid is shown in Table 2. The surface is complex, as described by Bouck (1965). There are two superposed pairs of membranes. The innermost of these continues around the chromatophore surface and the outermost is continuous with a layer of endoplasmic reticulum spread over the surface of the chromatophore (Figs. 1, 3, 4). On the cytoplasmic side of this latter there is commonly a large membrane-bounded cap of translucent material, thought to be a storage metabolite (Figs. 1, 2, 6). Although this cap usually appears empty it can sometimes be seen to contain material, probably in a precipitated state, as in Fig. 6. Occasionally, notably in *Sphacelaria bipinnata* (Fig. 5), the cap is lacking.

The pyrenoid in brown algae (unlike the equivalent in *Chrysochromulina*) appears not to divide with the chromatophore but a new pyrenoid is budded off from the point of attachment of the old one, the plane of fission then passing between the two. A stage in this process is illustrated in Fig. 4.

Simon (1954) recorded the presence of pyrenoids of varying sizes in 44 members of the orders Ectocarpales, Chordariales, Scytosiphonales and Dictyosiphonales and reproduced drawings of the representatives listed in the second column of Table 2, as seen with the light microscope. She was unable to investigate the Tilopteridales and Dictyosiphonaceae but quotes a drawing by Chadeffaud (1936) as evidence for their

Table 2. *Demonstration of the pyrenoid in brown algae*

Electron microscope	Light microscope (Simon, 1954)
Ectocarpales <i>Ectocarpus confervoides</i> (Fig. 4) <i>Pylaiella littoralis</i> (Figs. 1-3)	Ectocarpales, e.g. <i>Ectocarpus confervoides</i> <i>Giffordia mitchellae</i> <i>Pylaiella littoralis</i>
Sphacelariales <i>Sphacelaria bipinnata</i> (Fig. 5)	*Chordariales, e.g. † <i>Petrospongium berkeleyi</i>
Scytosiphonales <i>Scytosiphon lomentarius</i> (Fig. 6) <i>Colpomenia peregrina</i> (Fig. 7)	Scytosiphonales, e.g. <i>Petalonia fascia</i> <i>Colpomenia peregrina</i>
Dictyosiphonales <i>Dictyosiphon foeniculaceus</i> (not illustrated)	Dictyosiphonales <i>Giraudya sphacelarioides</i> <i>Punctaria latifolia</i>

\* Ectocarpales, Chordariaceae (Parke & Dixon, 1964)

† *Cylindrocarpus berkeleyi* (placed in the Ectocarpales, Corynophlaeaceae by Parke & Dixon, 1964)

absence in *Tilopteris mertensii* and illustrates their presence in families of the Dictyosiphonales other than the Dictyosiphonaceae. She failed to find evidence of pyrenoids in the Sphacelariales, Cutleriales, Dictyotales, Sporochnales, Desmarestiales, Laminariales and Fucales, but did not give details of the species investigated. The current investigation confirms the absence of pyrenoids in the Dictyotales and Laminariales and initial results point to their absence in the Cutleriales and Desmarestiales also. The present positive record for the presence of pyrenoids in *Sphacelaria bipinnata* is the only point of disagreement with Simon's findings so far discovered; some difference in optical properties conferred by the absence of capping material mentioned earlier might perhaps explain this.

### *Dictyotales*

In spite of the negative evidence quoted by Simon (1954) with regard to *Dictyota* special attention was paid to this plant because of its obvious phyletic importance. Sections of thalli and eggs were examined but only a chromatophore from the latter is illustrated here (Fig. 9). The chromatophore contains the usual thylakoids and dense spherical inclusions (lipid drops), but also shows patches of a less dense metabolite apparently arising in some relation to the thylakoids (Fig. 9, *l*), though of unknown chemical nature. These were not found in the related genus *Padina pavonia* (Fig. 8) and therefore no great phyletic significance need be attributed to them. In neither *Dictyota* nor *Padina* was any trace of a pyrenoid encountered. Preliminary observations on *Dictyopteris membranacea* also point to their absence in this plant.

### *Laminariales*

The negative evidence obtained by Simon (1954) on an unspecified number of species of Laminariales has been confirmed here for all the European genera listed in Table 1. In the swimming zoospores of *Alaria*, *Chorda*, *Laminaria* and *Saccorhiza*, the single chromatophore is strongly flexed (e.g. Fig. 12). In *Chorda* and *Saccorhiza* it carries a normal type of eyespot consisting of a single layer of large pigment chambers located on the chromatophore surface immediately posterior to the point of emergence of the hind flagellum (Fig. 12). Where the hind flagellum passes across the eyespot it is somewhat distended and closely pressed to it. In *Alaria* and *Laminaria* an eyespot is lacking, though the morphology of the chromatophore is otherwise similar. When the flagella are withdrawn and the zoospore rounds up preparatory to germination the chromatophore unfolds and proceeds to divide more or less in step with mitosis of the germinating spore. Figs. 10 and 11 illustrate the unfolding process in two representatives lacking eyespots (*Alaria esculenta* and *Laminaria digitata*), while Figs. 12 and 13 show the folded and unfolded state in *Chorda filum*, the latter figure being of a rounded, walled spore. In no case has any trace of a pyrenoid been seen in any member of the Laminariales. This is in disagreement with a recent communication by Bouck (1965), who states that pyrenoids occur in American representatives of *Chorda filum*. In addition to the observations illustrated here on sporelings, thin sections of tissue in the fertile region of adult sporophytes were also examined, with negative results. Bouck's findings are therefore most likely to be explained in terms of an unsuspected

epiphyte present on his material at the time of fixation, since a genuine difference in one species on the two sides of the Atlantic seems unlikely.

#### DISCUSSION

Until more is known no conclusions can be drawn regarding the meaning of the observations on *Dictyota* and this is an obvious place at which work should be resumed. On the other hand, the absence of pyrenoids from all the Laminariales, in contrast with the Ectocarpales, etc., in which they are consistently present, provides an additional taxonomic character for possible use in a phyletic context when more is known.

Another aspect of phylogeny on which additional information may have been contributed by the present findings is that of the relative position in the plant kingdom of the Phaeophyceae as a whole. In a recent discussion of this (Christensen, 1966), the brown algae are placed late in the phyletic system of algal groups lacking chlorophyll *b* (Chromophyta), but this conclusion can now perhaps be challenged from two points of view. Bulging pyrenoids comparable in general features with those of the brown algae are also known in certain flagellates, notably *Chrysochromulina* (Haptophyceae) (Manton, 1966*b*), and a number of euglenoids (Leedale, 1967). In all of these, however, the fine structure of the chromatophore lamellations seems to be more highly evolved than in the Phaeophyceae. The euglenoids possess chlorophyll *b* and *Chrysochromulina* lacks it. In both, however, there is a clearly developed capacity for the individual thylakoids to adhere together in stacks of two or three members (or sometimes more in the euglenoids) in a way which is lacking in the Phaeophyceae. From the enlarged views of thylakoid details contained in Figs. 3, 5 and 9, it is clear that the thylakoids within each unit lamellation do not mutually cohere in the brown algae; they run parallel but quite separately, usually in threes (though sometimes in fours), with occasional endings or changes of partner.

This character has become apparent only since glutaraldehyde fixation has been applied\* and its phyletic or physiological significance has scarcely been explored as yet. Recent studies in other groups have, however, already revealed resemblances to the Phaeophyceae in at least some centric diatoms (see Manton & von Stosch, 1966), but marked differences in members of the Xanthophyceae (Greenwood, 1959), Chrysophyceae (e.g. Manton & Harris, 1966) and the Haptophyceae (Manton, 1966*a*, *b*). The much more primitive red algae, as is well known, have their thylakoids arranged singly (Bouck, 1962; Evans, in Manton, 1966*a*), though in widely spaced parallel arrays. In comparison with all of these, the condition of the Phaeophyceae could perhaps be interpreted as more rather than less primitive than that of some of the other groups in the Chromophyta. To claim more than this in the present state of knowledge would be premature.

\* Observations on the electron microscopy of some of the algae quoted here made before the time of glutaraldehyde fixation include Berkaloff (1961) on *Laminaria saccharina*, with whom the present work is in substantial agreement, and Ueda (1961) whose results can only be explained in terms of inadequate fixation and the examination of too few representatives.

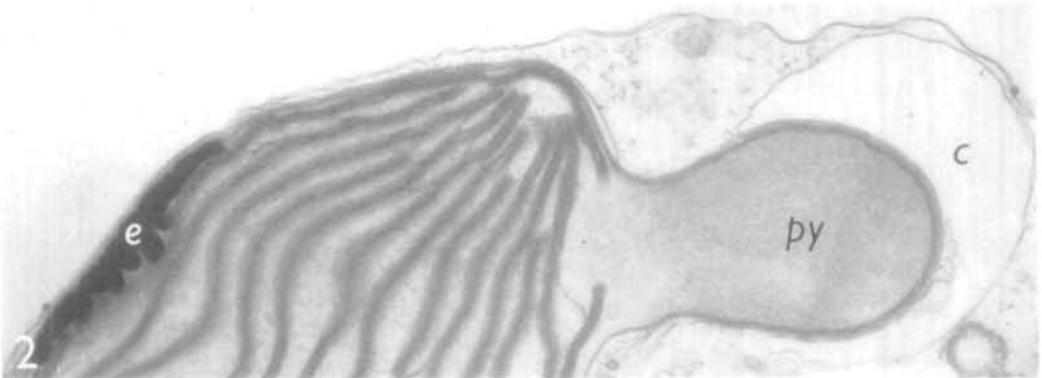
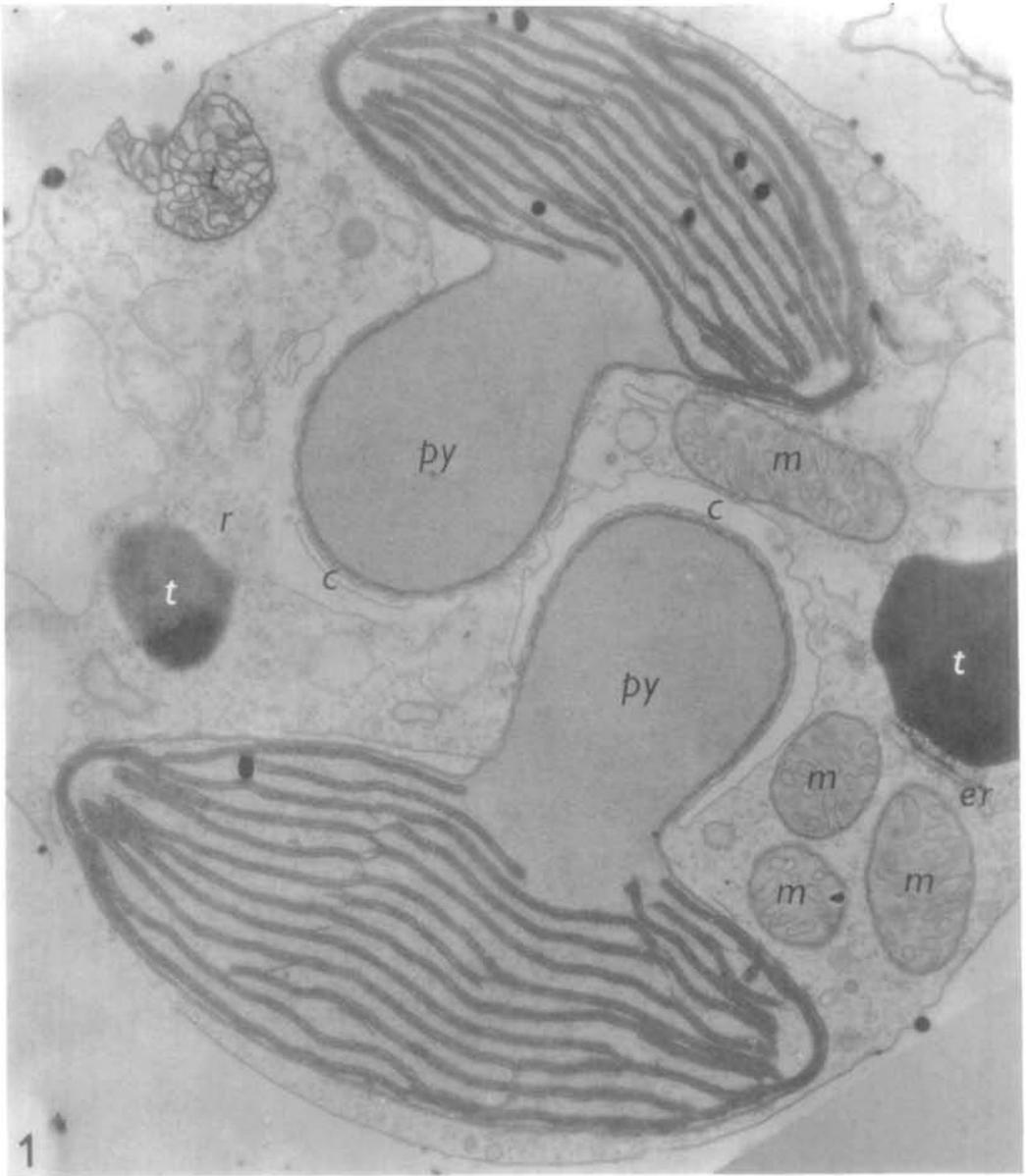
A feature of interest which is sometimes encountered in sections of surface cells from the thallus of *Colpomenia peregrina* is the presence of paired 'flagellar bases' made of nine skewed triplets, seen in transverse view lying near to the pyrenoid chromatophore complex in Fig. 7. These should presumably be interpreted as centrioles, since the tissues in which they are found are wholly vegetative or, at least, will not be involved in sporangial formation in the near future. Although they are not often seen, probably because of their small size in relation to the rest of the cell, they have been recorded previously in vegetative cells of *Fucus vesiculosus* (Bouck, 1965), and *Himanthalia lorea* (Berkaloff, 1963), and it is therefore quite probable that they are a constant feature of vegetative cells of brown algae.

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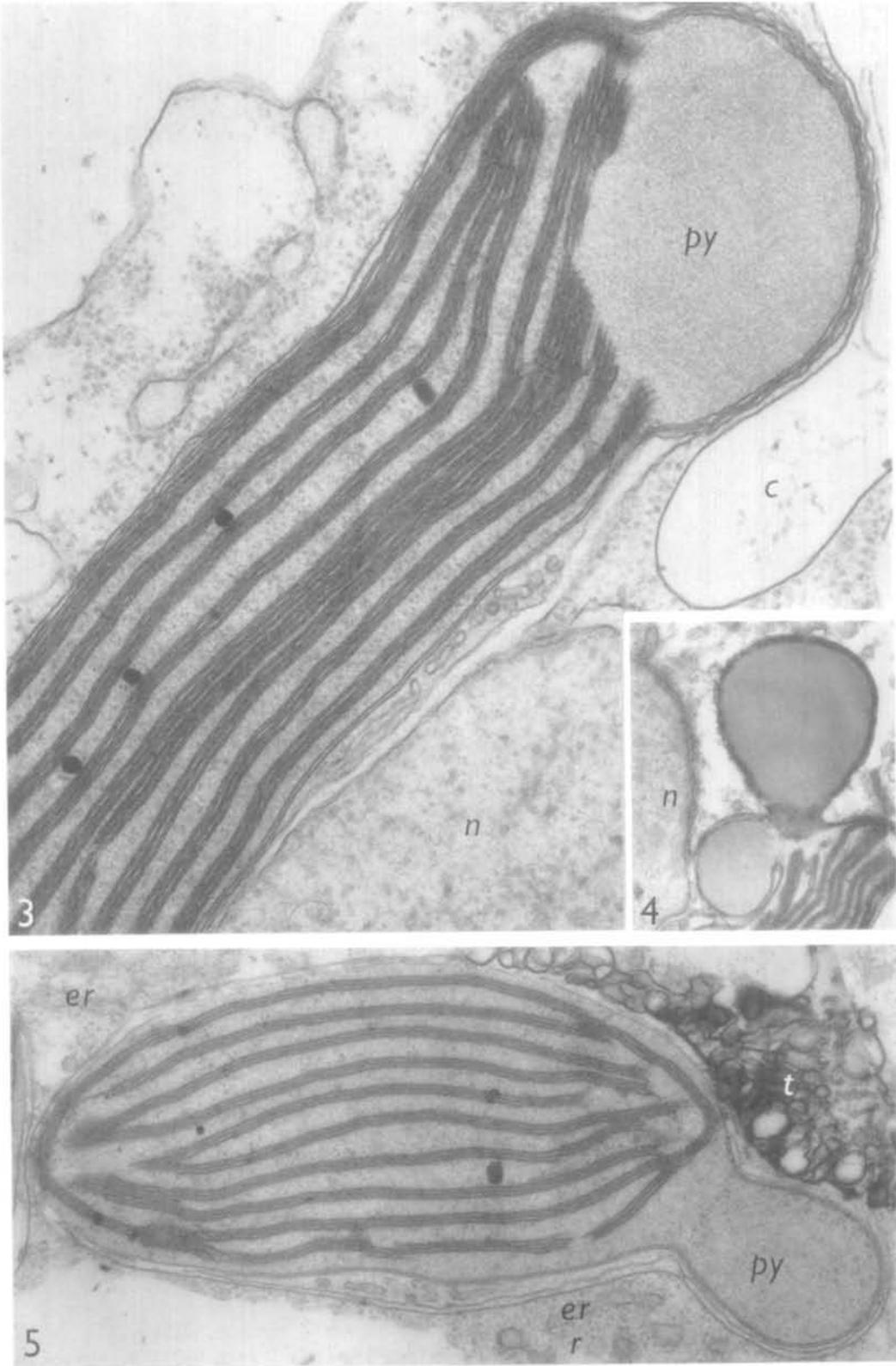
Fig. 1. *Pylaiella littoralis*. A section through a swimming zoospore showing two facing chromatophores with pyrenoids (*py*), the latter with caps (*c*) of transparent metabolite. Also visible are the two pairs of membranes covering the pyrenoid, the outermost of these being continuous with the layer of endoplasmic reticulum covering the chromatophore and the innermost continuing around the chromatophore surface. There are lipid droplets (dark bodies in the stroma of the chromatophores), mitochondria (*m*), ribosomes (*r*), endoplasmic reticulum (*er*) and metabolites (*t*) elsewhere in the field. Micrograph 1292,  $\times 20000$ .

Fig. 2. *Pylaiella littoralis*. A chromatophore in a swimming zoospore, with the pyrenoid (*py*) showing a distended cap (*c*) extending over its upper part. Also visible is part of the eyespot (*e*). Micrograph 1228,  $\times 20000$ .

Fig. 3. *Pylaiella littoralis*. Part of a chromatophore with lipid droplets (black globules) from a swimming cell to show the lamellations, each consisting of three non-cohering thylakoids, more clearly. The base of an end pyrenoid (*py*) can be seen and also part of the pyrenoid cap (*c*). The innermost pair of membranes covering the pyrenoid and chromatophore surfaces are visible, and also the outermost pair continuous with the endoplasmic reticulum over the chromatophore and with the envelope around the nucleus (*n*). Micrograph 352,  $\times 50000$ .

Fig. 4. *Ectocarpus confervoides*. Part of a chromatophore (in a swimming cell) lying close to the nucleus (*n*), with an older pyrenoid at the base of which a younger pyrenoid is developing. Micrograph 591,  $\times 15000$ .

Fig. 5. *Sphacelaria bipinnata*. A chromatophore with a pyrenoid (*py*) in a zoospore. The two pairs of membranes covering the pyrenoid and chromatophore are clearly visible but there is no cap of metabolite. Also in this figure are endoplasmic reticulum (*er*), ribosomes (*r*), and reserve metabolites (*t*) in the cytoplasm. Micrograph 1238,  $\times 30000$ .



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Fig. 6. *Scytosiphon lomentarius*. A settled walled spore showing two chromatophores, one with a pyrenoid (*py*). Its cap can be seen to contain the metabolite (*p*), probably in a precipitated state. Part of this is enlarged in the inset. In the speckled chromatophore stroma can be seen lipid drops (intense black), and in the cytoplasm mitochondria (*m*) and various metabolites (*t*). Micrograph 1378,  $\times 20000$ . Inset about  $\times 30000$ .

Fig. 7. *Colpomenia peregrina*. Part of a settled walled spore showing the two centrioles (*c*) in transverse section. In the complete one, nine triplets can be seen. Also visible are a little of the cell wall (*w*), parts of the chromatophore (*ch*) and pyrenoid (*py*), and other cytoplasmic inclusions. Micrograph 1510,  $\times 25000$ .

Fig. 8. *Padina pavonia*. A chromatophore from a cell at the thallus apex showing the absence of pyrenoids. *m*, mitochondria; *w*, part of cell wall. Micrograph 1541,  $\times 20000$ .

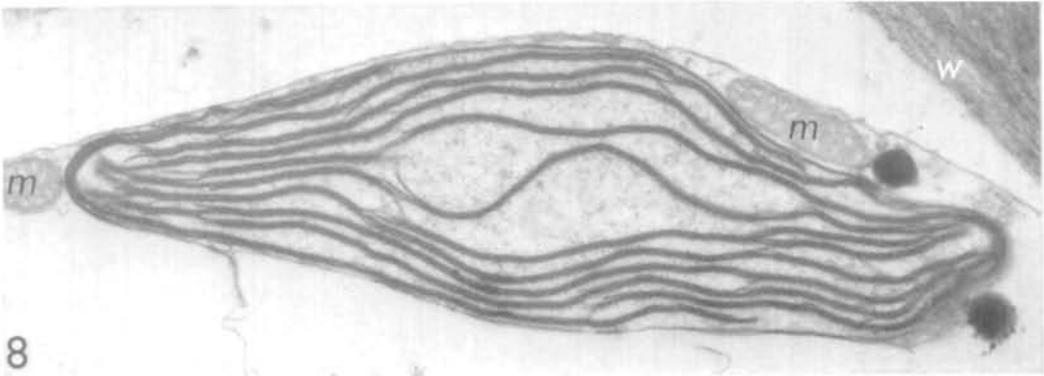
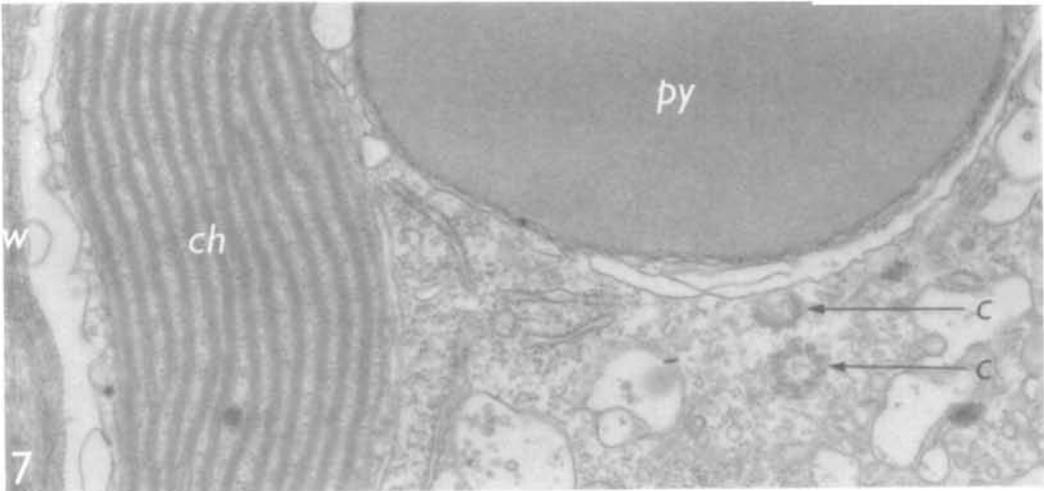
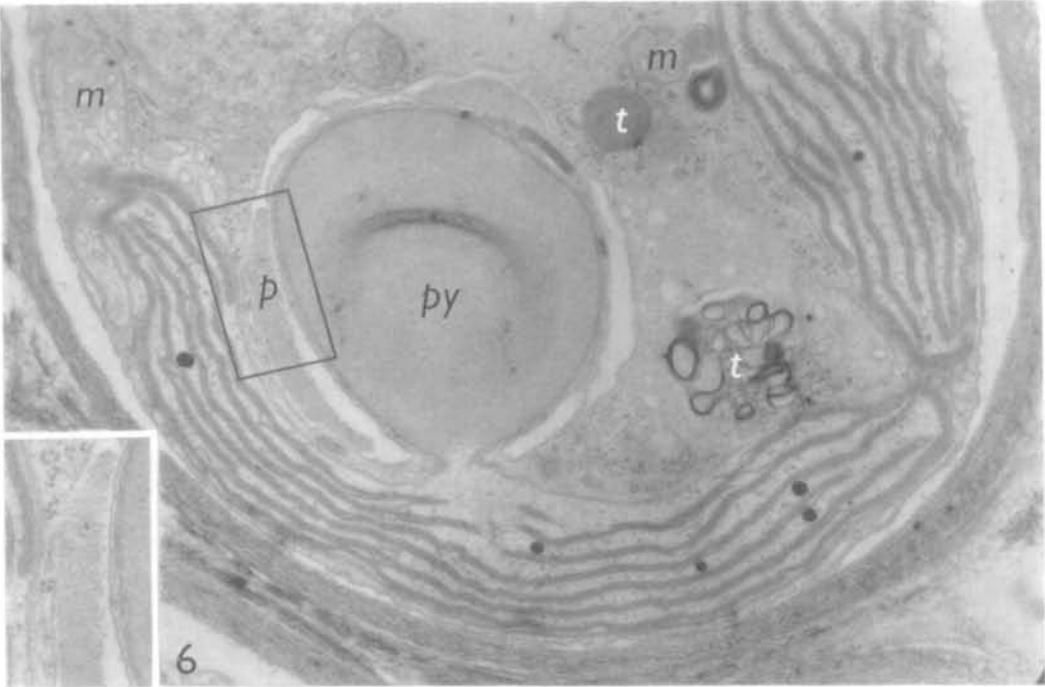


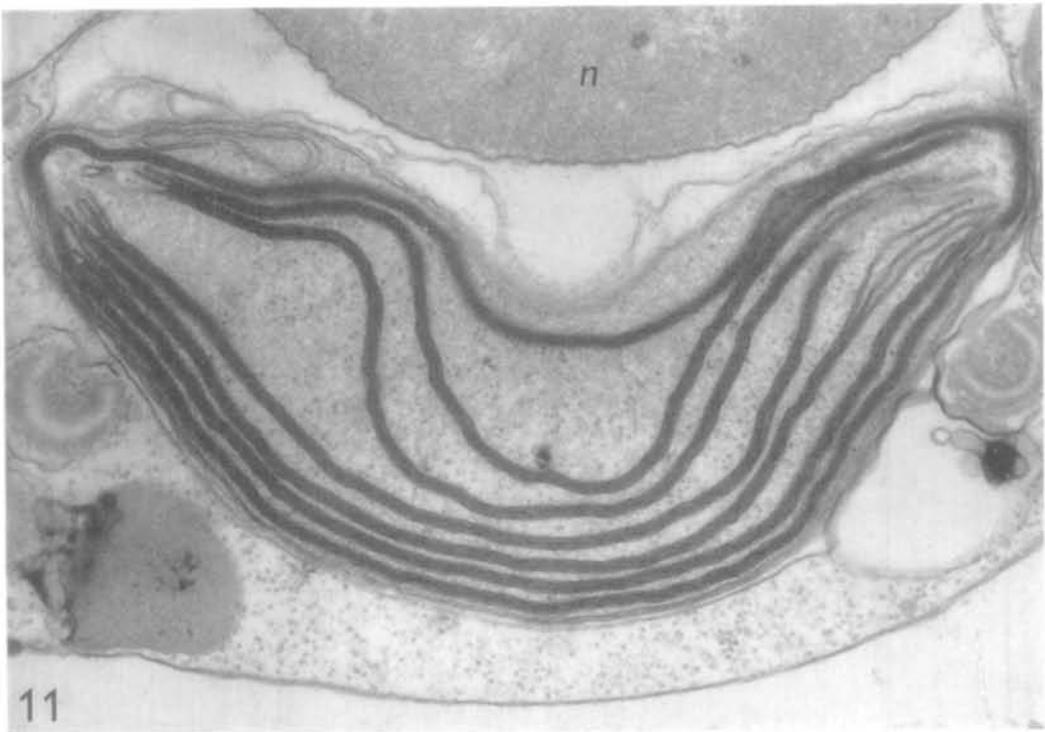
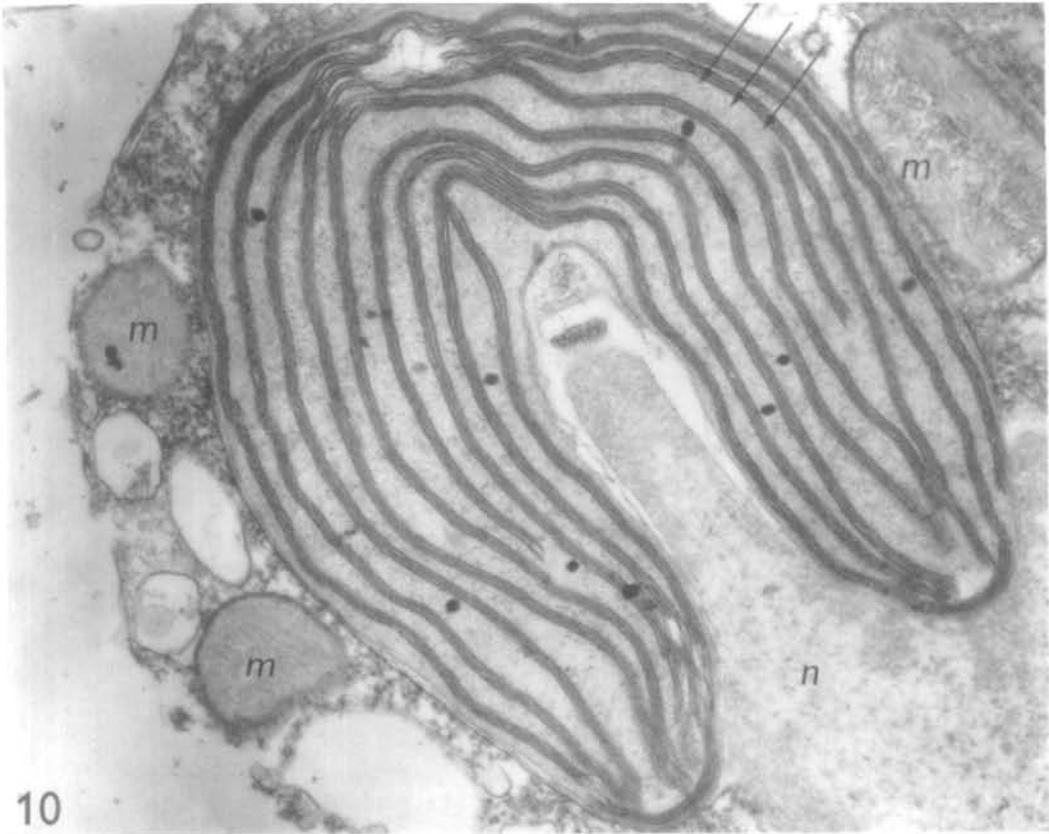
Fig. 9. *Dictyota dichotoma*. A chromatophore from an egg, showing the lamellations each consisting of three non-cohering thylakoids, lipid droplets (intense black bodies in the stroma), inclusions (*l*) of unknown nature, but no pyrenoid. Micrograph 554,  $\times 50000$ .



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Fig. 10. *Alaria esculenta*. A folded chromatophore with no pyrenoids from a swimming zoospore. The stroma shows a crystalline appearance in one region (arrowed), and contains lipid droplets (dark bodies). Also showing are mitochondria (*m*) and part of the nucleus (*n*). Micrograph 1036,  $\times 30000$ .

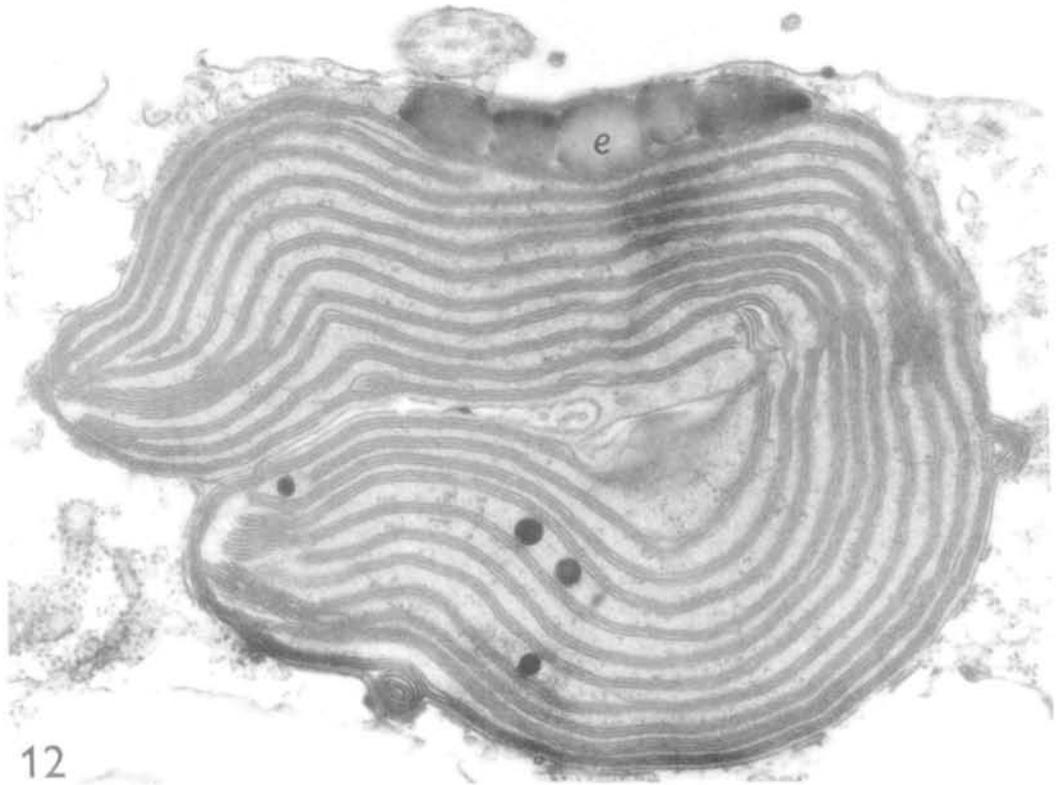
Fig. 11. *Laminaria digitata*. Part of a rounded-up spore showing the beginning of unfolding of the chromatophore, which is without a pyrenoid. Part of the nucleus (*n*) is also visible, together with other cytoplasmic inclusions. Micrograph 1219,  $\times 30000$ .



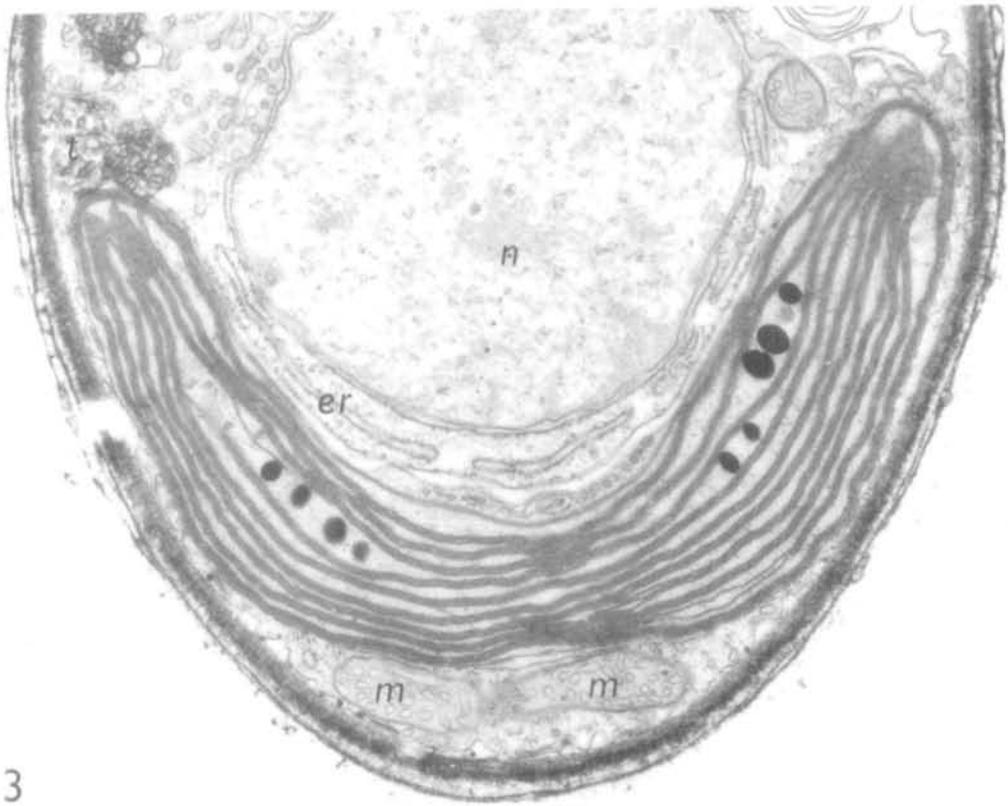
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Fig. 12. *Chorda filum*. Part of a swimming zoospore (posterior flagellum transected at the top) showing the strongly folded chromatophore with lipid droplets (intense black bodies) and a single layer of large pigment chambers (*e*) of the eyespot. There is no pyrenoid. Micrograph 1551,  $\times 30000$ .

Fig. 13. *Chorda filum*. Part of a walled settled spore with the chromatophore unfolded and containing lipid droplets (spherical black bodies). Also showing are part of the nucleus (*n*), rough endoplasmic reticulum (*er*), mitochondria (*m*), metabolite (*t*) and several wall layers. Micrograph 790,  $\times 20000$ .



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