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Published in:
Acta Botanica Neerlandica

Acta Bot. Neerl. 26(2), April 1977, p. 159–176.

PAVLOVA ENNOREA SP. NOV., A HAPTOPHYCEAN ALGA WITH A DOMINANT PALMELLOID PHASE, FROM ENGLAND

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SUMMARY

Pavlova ennorea sp. nov. is described at both the light and electron microscope level with emphasis on the palmelloid stage. Characteristic features of the genus *Pavlova* observed in our material include the reserve material paramylon, the system of flagellar roots, and the pit entering the cell at the flagellar pole. The flagellar apparatus and the pit are not continually present in the encapsulated condition. An investment of minute dense particles on the plasmalemma, as known from other *Pavlova* species, is completely absent. In *Pavlova ennorea*, ecdysis of the periplast is a natural phenomenon, in which the plasmalemma is replaced by a membrane of the endoplasmic reticulum. The original plasmalemma and the substituting E.R. membrane have dissimilar garnitures of internal particles, which suggest that periplast ecdysis effectuates a change in the protoplast's boundary conditions. The mucilage envelope is composed of long microfibrils. Staining experiments indicate an anionic nature of the mucilage. However, the permeability for anions seems not impaired to a biologically signifidegree.

1. INTRODUCTION

Palmelloid stages have been mentioned for most species of the genus *Pavlova* Butcher (BUTCHER 1952), and it was realised (TSCHERMAK-WOESS 1972) that close relatives can be expected in taxa with a palmelloid organization of the dominating life stage. GEITLER's discovery (1969) of a slender haptonema in the anisokontic zoids of *Chrysocapsella granifera* (Mack) Bourrelly caused GREEN (1973) to investigate the ultrastructure of the organism, which proved to be a genuine *Pavlova* species. Another palmelloid representative of the genus is the recently described *P. virescens* Billard (BILLARD 1976).

The present paper devotes attention to a palmelloid alga of similar light microscopic appearance. Investigation of its fine structure was undertaken to determine the taxonomic position. Moreover the material provided an opportunity to collect information on the palmelloid condition as a general topic.

The water in contact to a swimming flagellate is constantly refreshed, but once the cell has become encapsulated, the flow of nutrients depends on diffusion through the mucilage envelope. Some authors claim that electrically charged cell walls create a microsphere that is more favourable than the external environment. Among the authors holding this view are CUMMINS et al. (1966), HELLER et al. (1974), who worked on *Ulva*, and *Acer*, respectively.

We examined the structure of the mucilage envelope, and tested it for the presence of electrically charged groups. Apart from the encapsulation, a palmelloid cell probably differs from a free swimming one in the morphology of the protoplast. Differences can be expected in the peripheral layers, because these too make the boundary conditions for the life-processes. We investigated such cytological pecularities.

2. MATERIAL AND METHODS

2.1. Origin and culture conditions

The organism was collected on 20–5–1967 from the bottom of a small puddle in a saltmarsh near the village of Polbathick, Cornwall, England. It was maintained in a seawater medium enriched with leaf-mould extract and nutrients (VAN DER VEER 1969) in a cabinet with a 16 hours' diurnal light period at 17°C.

2.2. Light microscopy

The nature of the reserve materials was tested by iodine-potassium iodide, brilliant cresyl blue, sudan III, and toluidine blue 0 in combination with extractability by cold trichloro acetic acid, and with the light-refractory properties.

The presence of anionic groups in the mucilage envelope was traced by the cationic dyes methyl green and ruthenium red. For cationic groups in the mucilage envelope the anionic dyes erythrosin and rose bengale were applied. In general, the penetration of dyes into mucilage envelopes, even if the latter are not stained themselves, can be checked by staining of the protoplasts. However, living *Pavlova* protoplasts are not readily stainable, and must be killed or weakened for the purpose without interfering with the chemistry of the mucilage. This was achieved by presoaking the cells in demineralized water, and after that again in filtered seawater.

Iodine-potassium iodide was dissolved in demineralized water, and sudan III in 96% ethanol. The other dyes were dissolved in filtered seawater or in the culture solution.

2.3. Electron microscopy

For sections, material was fixed for 28 minutes in 4% glutaraldehyde buffered by cacodylate, and made isotonic with the culture solution by the addition of sucrose. The sucrose content was lowered to 1/4 of the original percentage during washing with buffer prior to postfixation in osmiumtetroxide. Dehydration took six ethanol steps and the material was embedded in Epon. Sections were stained with diluted Reinold's lead citrate, sometimes directly and sometimes preceded by uranyl acetate.

Freeze etch preparations were made in a Balzers Freeze Etch Unit. The platinum-carbon shadowed replicas were cleaned on sulphuric acid and sodium hydroxide.

For whole mounts, zoids were harvested three days after refreshing of the

culture medium. The cells were fixed by osmic vapour and shadowed with gold-palladium.

3. OBSERVATIONS

3.1. Light microscopy

3.1.1. Living cells

Palmelloid cells are predominantly hemispherical and cup-shaped. The cells are often laterally flattened against each other when tightly clustered. Each cell contains a parietal greenish yellow plastid which is deeply bifid *(fig. 1)*, the lobes having irregular outlines with small lobules frequently inflected into the central cytoplasm. Neither an eyespot, nor a pyrenoid could be detected with the light microscope. The nucleus is positioned at the edge of the cell between the lobes of the plastid. Contractile vacuoles were not observed.

Motile cells *(fig. 1)* appeared within a few days after the start of a subculture, but were rarely observed during the growth phase of the culture. The swarmers are typically *Pavlova*-like, with a convoluted propulsive flagellum inserted some distance behind the front end. They resemble those of *P. calceolata* Veer (VAN DER VEER 1976) in particular, because of the stiff backwards pointing secondary flagellum.

3.1.2. Staining

Staining experiments to detect starch or leucosin had negative results. A varying number of lipid globules was demonstrated by sudan III, though not in every cell. Treatment with hot acetic acid destroyed the protoplasts and set free two or more refractive bodies of varying shape and dimensions. A few excessively large ones were circular discs with a perforated centre. This shape is typical for the reserve material paramylon and has been observed in *Pavlova granifera* also (TSCHERMAK-WOESS 1972). Two or three strongly refractive bodies stained red with toluidine blue O. This metachromatic reaction did not occur in cells previously extracted with cold trichloro acetic acid, and therefore was due to the presence of polyphosphate.

The colony matrix could be dissolved completely in hot, demineralized water. The mucilage stained with the cationic dyes. A lamellation became visible after prolonged ruthenium red staining, but this might be a precipitation artefact. The mucilage did not react to the anionic dyes. The dye certainly penetrated the mucilage envelopes, for the protoplasts inside stained within a couple of minutes. Occasionally the mucilage contained stainable bacteria and other inclusions: vaguely outlined objects and prominent shells parallel to the protoplast surface.

3.2. Electron microscopy

3.2.1. Observations on whole mounts of zoids

The features of the flagellar apparatus (*figs. 1, 6* and *7*) are best observed in shadowed whole mounts. The long flagellum has a blunt tip, and the ending of

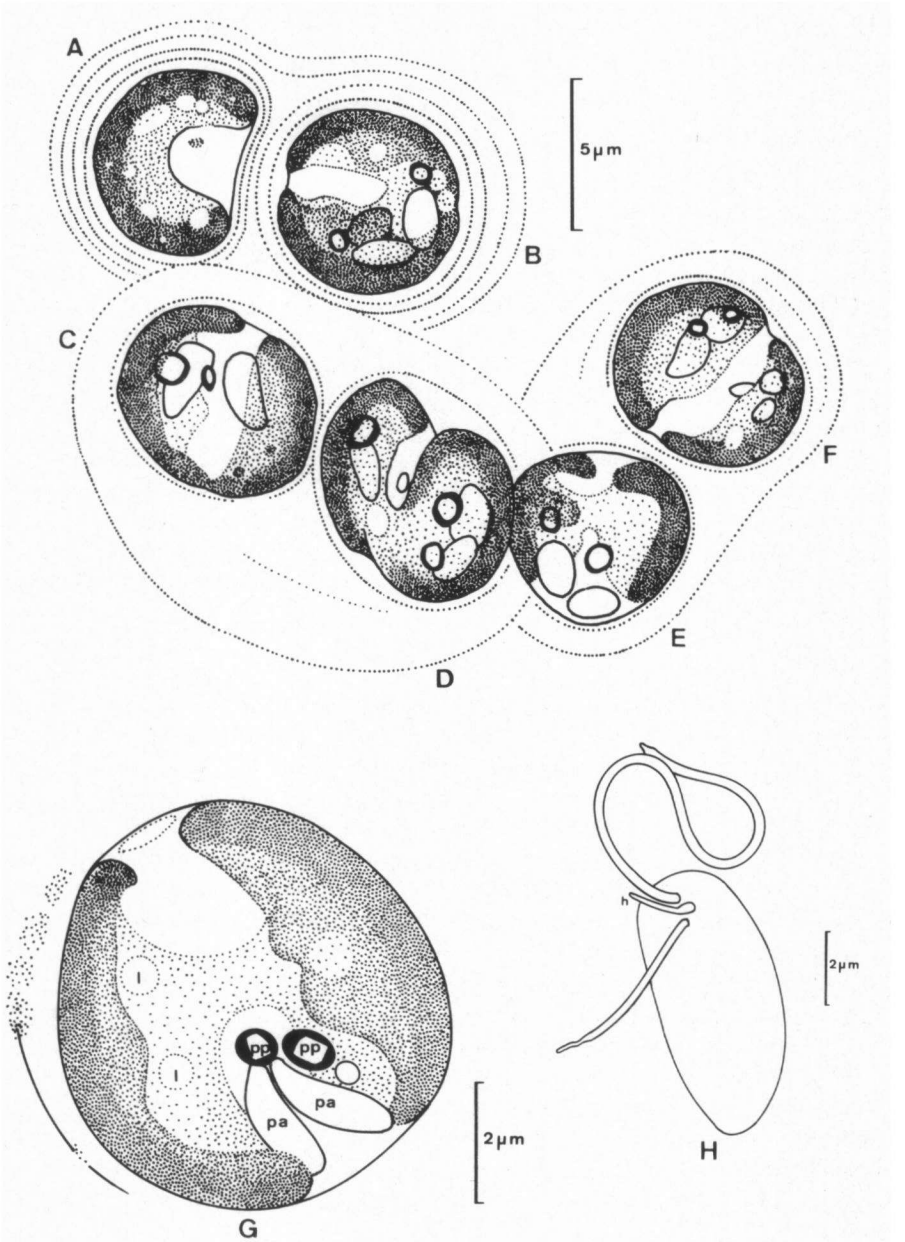


Fig. 1. *Pavlova ennorea;* A-F. a selection of palmelloid cells, mucilage envelopes of A and B after staining with ruthenium red, mucilage envelopes of the others observed under phase-contrast conditions, D. division stage, G. individual cell somewhat flattened under the coverslip to show bilobed plastid, remains of former periplast, and deposits of lipid(l), polyphosphate(pp) and paramylon(pa), H. motile cell, the body from life, flagella and haptonema from an electron micrograph.

the short flagellum is gradually tapering. The slender haptonema ends in a thin thread.

Fig. 3 shows a tomentum of fine hairs on the long flagellum. The short flagellum is likewise covered by fine hairs. Dense particles were not observed between the flagellar hairs, nor on the cell body.

3.2.2. Observations on sections

3.2.2.1. Mucilage envelope and inclusions

In sections, the structure of the mucilage envelope shows up as a random reticulum of dark lines *(fig. 13)*. The inclusions of the mucilage other than bacteria (see *fig. 11*), are membrane structures: neat double membranes parallel to the cell surface, tortuous membranes, and hosts of small vesicles *(fig. 12)*. Occasionally such extra-cellular membrane structures form layers alternated with layers of mucilage.

3.2.2.2. Periphery of the protoplast

Many cells have a superficial cisterna which separates a periplast from the main part of the cell body. *Fig. 8* illustrates this periplast, which is constituted by the plasmalemma, the outer membrane of the superficial cisterna, and a thin layer of very dense material in between. The periplast contains several osmiophilic blisters, a pair being visible in the micrograph. Some of the blisters are alveolate at their margins, like shown in *fig. 10*.

The superficial cisterna is curved inwards at the flagellar pole where it may contain some cytoplasmic sheets *(fig. 15)*. One or two very thin layers of electron dense material, apparently not membranes, are present inside the superficial cisterna *(fig. 8)* all along the perimeter of the cell. Sections of the superficial cisterna may show foamy objects, in which the peripheral alveolae are markedly larger than the central ones *(fig. 9)*.

A number of cells do not have a superficial cisterna, and these cells also lack the thin layer of dense cytoplasm adjacent to the plasmalemma; in other words, they have no periplast either. In these cases, extra-cellular membrane structures as described in section 3.2.2.1 are usually present close to the cell surface.

3.2.2.3. Flagella and associated structures

In the encapsulated stage, the external flagellar apparatus is often incomplete. *Fig. 15* for instance, shows a single flagellum cross-cut just before entry into the protoplast. The root system includes microtubules and non-microtubular bands of dense material. A microtubular root, shown lengthwise in *fig. 16*, has the proximal end between the basal bodies, and points away from the nucleus. It consists of circa eight microtubules mutually arranged as in *Pavlova gyrans* (GREEN & MANTON 1970). Several bands of dense material radiate from the basal bodies along the surface of the nucleus. Some of these bands are visible in *fig. 14*, together with dense material interconnecting the basal bodies themselves.

A vesicle-like profile is found between the components of the internal flagel-

lar apparatus and a concavity in the nuclear surface (*figs. 14* and *16*). A coating with dense material on the cytoplasm side makes it similar to the pit or sac in flagellate representatives of the genus *Pavlova* (GREEN & MANTON 1970, GREEN 1973, VAN DER VEER 1969, 1972, 1976).

3.2.2.4. Other cell constituents

Figs. 2, 4 and *5* provide a survey of the main cell constituents. The plastid is surrounded by a layer of endoplasmic reticulum that is directly continuous with the nuclear envelope (*figs. 2, 4, 5*). The thylakoids occur in stacks of three or four (*figs. 17, 18*). The thylakoid stacks approach each other very closely at the plastidial edge, and in the micrograph of *fig. 18* an actual thylakoidal continuity is to be noted between a central and a peripheral stack. The electron micro-

Legends to the figures in plates I-IV

Key to labelling

cer, plastid (chloroplast) associated endoplasmic reticulum
dl, electron dense layer
f, flagellum
fb, flagellar base
g, Golgi body
h, haptonema
isc, inner membrane of superficial cisterna
l, lipid
m, mitochondrium
mc, membrane of cytoplasmic sheet
n, nucleus
nr, non-microtubular root
ob, osmiophilic blister
osc, outer membrane of superficial cisterna
pa, paramylon
pe, periplast
pi, pit
pl, plasmalemma
pp, polyphosphate
py, pyrenoid
r, microtubular root
sc, superficial cisterna
th, thylakoid
v, vacuole

Plate I

Fig. 2. Two sister cells lying close together, and presenting a synopsis of the main cell constituents: superficial cisternae separating periplasts from the interior of the cells, lobes of the parietal plastids, mitochondria, and vacuolar deposists of paramylon and polyphosphate; a flagellar base, the obliquely sectioned pit, and continuity of the plastid associated E.R. with nuclear envelope (arrow) in the upper cell; the Golgi body and droplets of lipid in the lower cell; two cross-sectioned flagella and several extracellular vesicles near the cleavage plane. × 15000

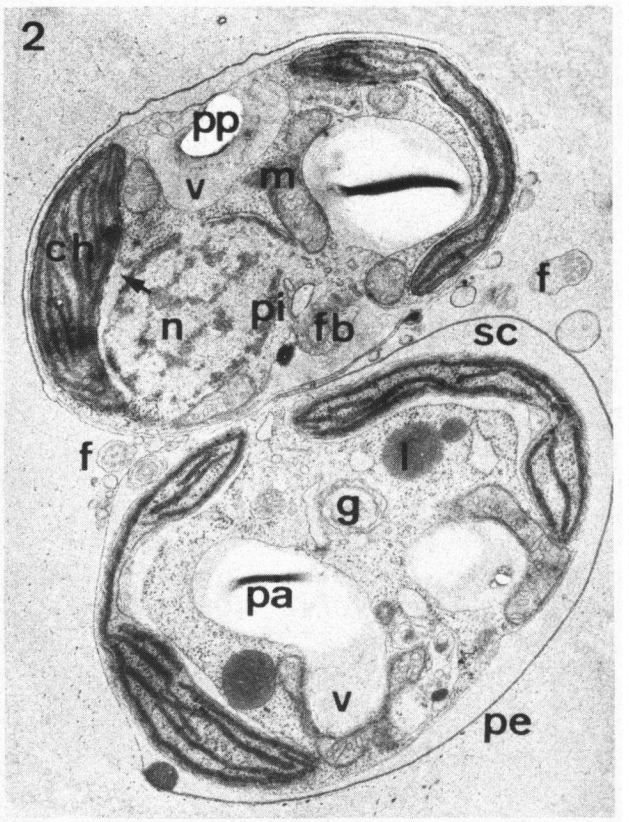
Fig. 3. Detail of the long flagellum with investment of fine hairs, shadowed preparation. × 20000

Fig. 4. A section which sliced a crescent shaped profile from the cup-shaped cell body. × 10000

Fig. 5. A cell sectioned so as to show its almost circular outline; continuity of the plastid associated E.R. and nuclear envelope (arrow), Golgi Body, droplets of lipid, and a granule of paramylon. × 8500

Fig. 6. Shadowed preparation of the flagellar apparatus including the haptonema, and posteriorly directed short flagellum. From the long flagellum, only the proximal part is shown. × 8000

Fig. 7. Shadowed complete cell, arrow indicates tip of haptonema. × 4500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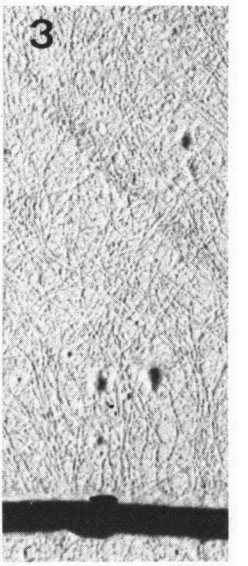
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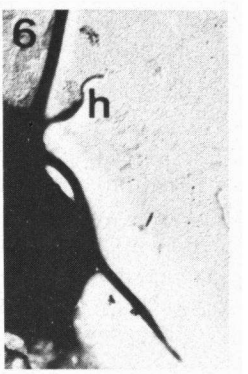
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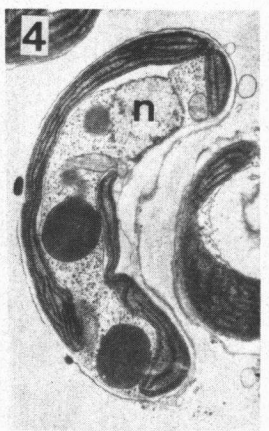
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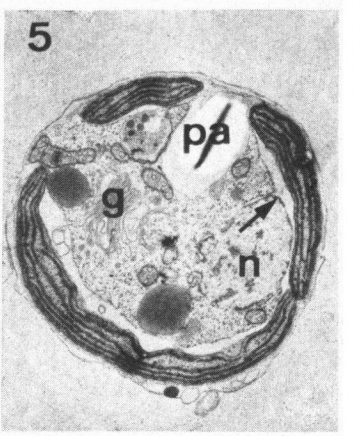
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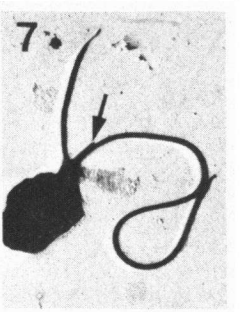
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scope revealed that the plastid has a protruding pyrenoid, which is not penetrated by thylakoids *(fig. 17)*. Globules of lipid *(figs. 2, 4, 5)* in the central cytoplasm are frequently seen in contact with endoplasmic reticulum.

Vacuolar inclusion bodies can be recognized as polyphosphate deposits and grains of paramylon. Polyphosphate manifests itself either similarly as in *Trebouxia* (FISHER 1971), or it is dissolved from the sections, and then is represented by holes in the Epon, as in *fig. 2*. Paramylon, shown at low magnification in *figs. 2, 5*, was recognizable as such by the fingerprint-like striation at high magnification (VAN DER VEER 1976). Other features are the side position of the nucleus, the unfixed position of the single dictyosome, and the presence of several scattered mitochondria.

3.2.3. Observations on freeze-etch replicas

3.2.3.1. Mucilage envelope

Fractures grazing the protoplast and inner layers of the mucilage revealed numerous microfibrils arranged grosso modo parallel, deviations from the main direction being slight *(fig. 21)*. Other fractions, as shown in *fig. 19* for example, reveal a random arrangement of the microfibrils. Such random arrangement is predominantly found at larger distances from the protoplast surface. The diameter of the individual microfibrils varies up to circa 30 nm; their length possibly is indeterminate. The inclusions of the mucilage envelope described from sections were also encountered in freeze-etch replicas.

3.2.3.2. Periphery of the protoplast

Observations on freeze-etch replicas confirm and supplement those on sections, this fact being especially of interest with respect to the surface morphology of the protoplast. When present, the superficial cisterna is very narrow; only the part curved inward at the flagellar pole is somewhat wider *(fig. 25)*.

Observation of the internal particles of two membranes are of special bearing in the context of our investigation. The inner membrane of the superficial cisterna has a characteristic particle garniture illustrated in *figs. 20, 22* and *23*. The

Plate II

Fig. 8. Periphery of the protoplast. Periplast constituted by the plasmalemma and the outer membrane of the superficial cisterna, with dense material between them, and with two osmiophilic blisters at left. Two thin electron-dense layers inside the superficial cisterna. Nucleus and plastid close to the inner membrane of the superficial cisterna. × 50000

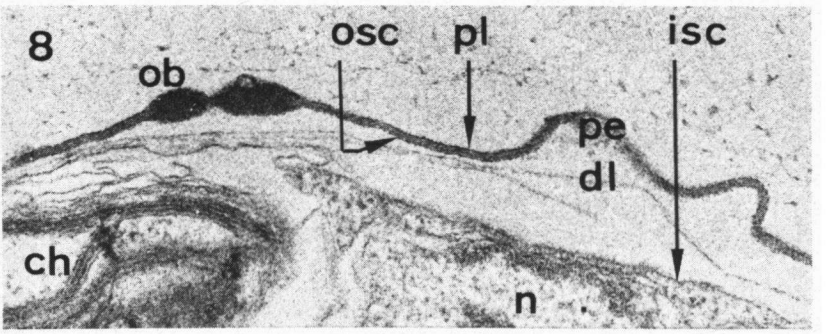
Fig. 9. Foam-like material inside the superficial cisterna. Foam bubbles at the periphery markedly larger than in the centre. × 50000

Fig. 10. Osmiophilic blister in the periplast, with small alveoles at its underside (arrows). Section about perpendicular to the cell surface. × 50000

Fig. 11. Longitudinal section through a bacterium in the algal mucilage envelope. × 40000

Fig. 12. Tortuous membranes and associated vesicles inside the mucilage envelope, but outside the intact periplast. × 30000

Fig. 13. Fine structure of the mucilage envelope appearing as an irregular reticulum in section. × 55000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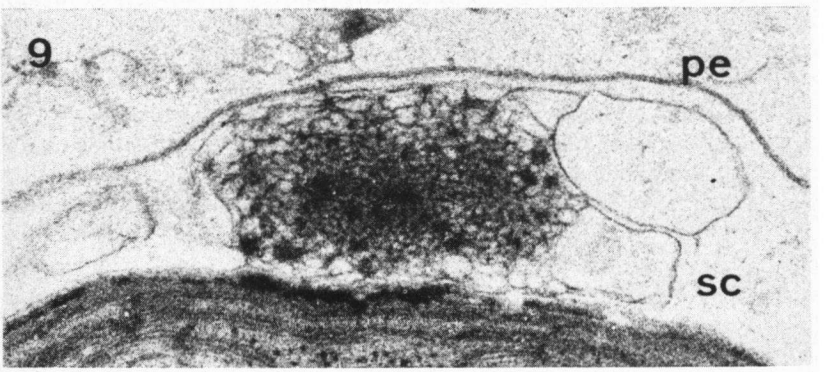
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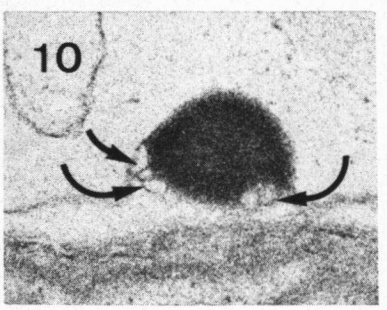
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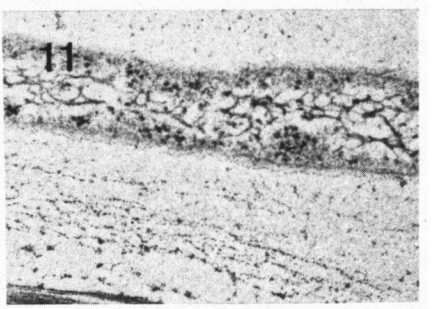
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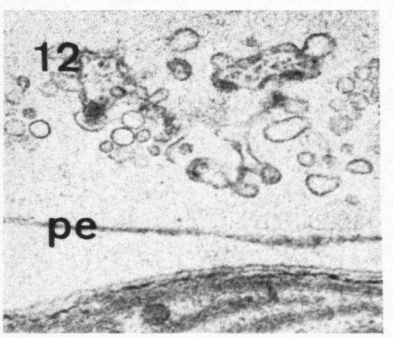
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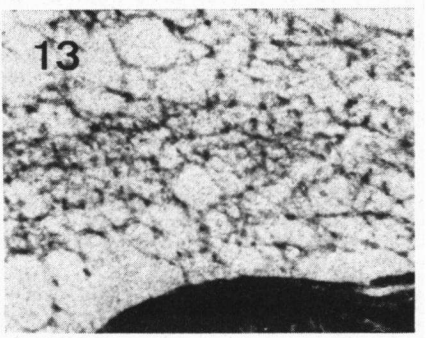
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large particles are evenly distributed and protrude little from the surface of the membrane leaflet. Both leaflets of the membrane have the same texture. The plasmalemma either has an internal particle garniture with irregularly distributed particles of varying sizes *(figs. 20, 24)* – as costumary for this type of membrane – or it has a particle garniture resembling that of the inner membrane of the superficial cisterna.

Appropriate fractures had to be selected carefully to check that the membrane in fact formed the outermost boundary of the protoplast, that of *fig. 21* being an example.

3.2.3.3. Flagellar apparatus and pit

Incomplete external flagellar apparati were encountered more often than complete ones. *Fig. 22* shows three separate buds on the bottom of an oblong concavity which are the first manifestation of the external flagella and haptonema. Another example of an immature flagellar apparatus is illustrated by *fig. 23*. The fracture opened the superficial cisterna, allowing observation of the flagellar pole from within the cell. One flagellum and the haptonema are represented by short buds; the other flagellum is broken off, and may have been longer.

The complete external flagellar apparatus was present in the cell from which the micrograph represented in *fig. 24* was taken. The flagella and haptonema are inserted some distance apart, at the corners of a triangle. *Fig. 25* shows a wide pit, with lateral inflations, entering the cell at the flagellar pole. In *fig. 24* however, where the orifice of the pit could be expected by comparison with *fig. 25*, it is not present.

Plate III

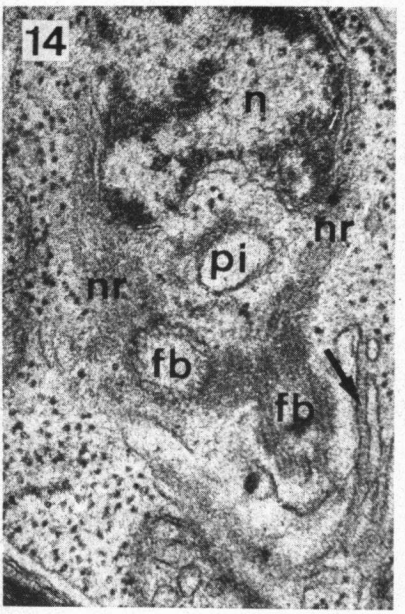
Fig. 14. Details of the internal flagellar apparatus: flagellar bases interconnected by dense material, one sectioned transversely, the other obliquely. Profile of the pit coated by dense material, between nucleus and flagellar bases. Cytoplasmic sheets inside superficial cisterna (arrow). × 60000

Fig. 15. Single flagellum sectioned just outside the cell, surrounded, in sequel, by the narrow space of a depression in the cell surface, periplast, superficial cisterna with cytoplasmic sheets, and finally mitochondria and nucleus. × 60000

Fig. 16. Details of the internal flagellar apparatus: one flagellar base cut obliquely, the microtubular root shown lengthwise. Profile of the pit coated by dense material, vacuole of reserve materials, mitochondrium, nucleus and Golgi body. × 60000

Fig. 17. Detail of plastid: triplets of thylakoids, and pyrenoid protruding between nucleus and mitochondria. Plastid associated E.R. directly continuous with the nucelar envelope. × 45000

Fig. 18. Detail of plastid showing thylakoidal arrangement. Thylakoid stacks closely approaching each other at the edge. Actual thylakoidal continuity between a central and a peripheral stack (arrow). Plastid associated E.R. and superficial cisterna. × 90000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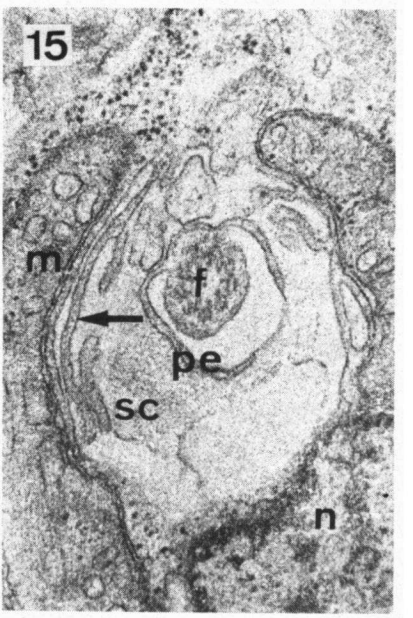
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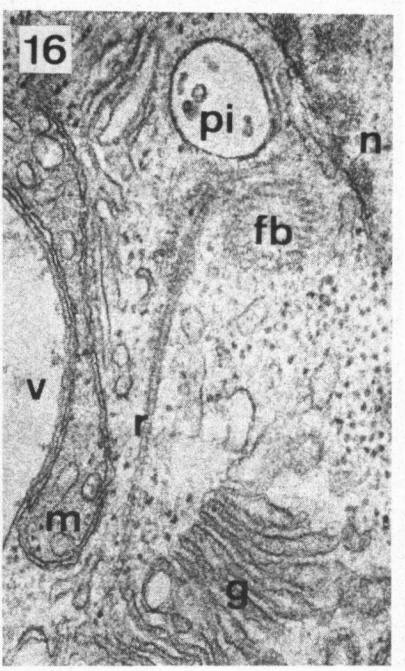
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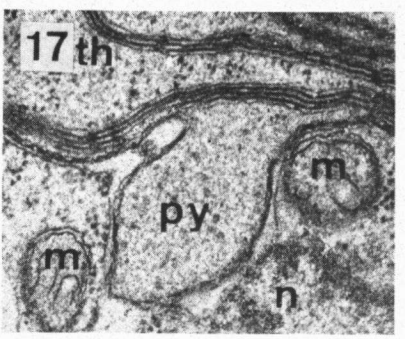
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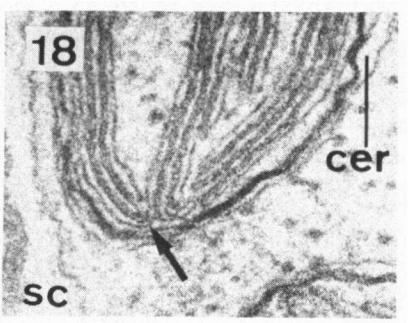
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4. DISCUSSION

4.1. Mucilage envelope

Freeze-etch replicas demonstrate that the reticulum in sections is sliced from a spatial wicker-work of long microfibrils. In all likelihood, the mucilage envelope grows in thickness by apposition on the inside. Adjacent to the protoplast the fibres are directed about parallel. This suggests that the fibres are laid down in parallel bundles, and are shuffled in two or three directions to produce the final random orientation. The suggested course of events is reminiscent of ROELOFSEN & HOUWINK's (1953) multi-net growth of primary cell walls. In *Nitella* internodal cells (PROBINE & PRESTON 1961) and other instances, the rearrangement of the fibres is brought on by cell elongation. In *Pavlova* it is related to the swelling of the mucilage itself, but similarly as in genuine multi-net growth, initially present deviations from the main fibre-direction are needed for the mechanism to work.

The stainability with methyl green and ruthenium red indicates that the mucilage has a strongly anionic character. As far as analogy exists with the cationic dyes, metal-ions will be adsorbed on the fibres, and seep through the mucilage envelopes only slowly.

CUMMINS et al. (1966) supposed that adsorption of sodium ions on the material of the cell wall of *Ulva* could extract enough salinity from the water between the fibres to create a more favourable environment for the protoplast. It can be easily understood that this would require an incessant and copious secretion of cell wall material. A high rate of secretion is present in *Fucus* epidermis cells

Plate IV

Fig. 19. Freeze etch preparation showing randomly arranged microfibrils in the mucilage envelope some distance from the protoplast. × 80000

Fig. 20. Fracture exposing three layers at the cell periphery: plasmalemma with irregularly distributed, non-uniformly sized particles; intra-cisternal electron dense layer, inner membrane of superficial cisterna with large, widely spaced particles. × 95000

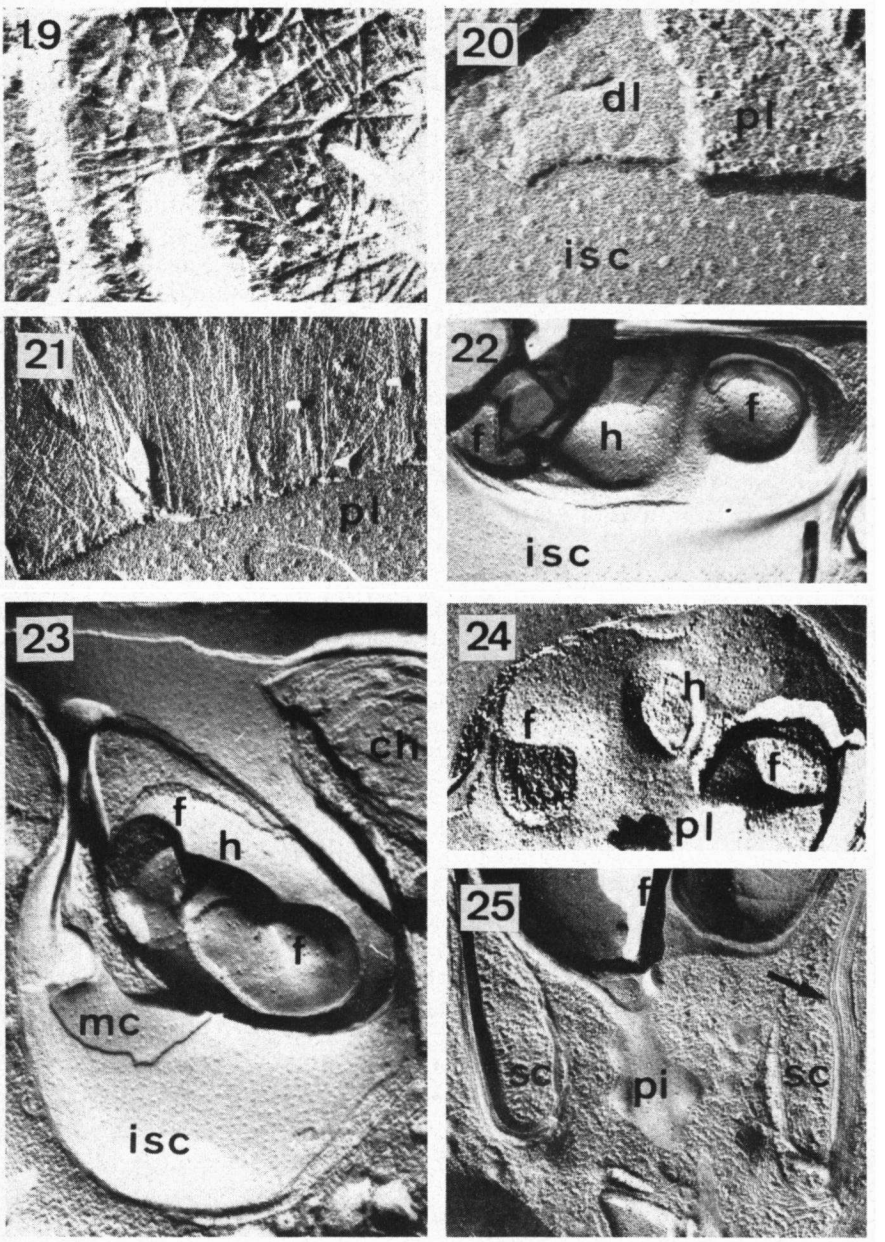
Fig. 21. Freeze etch preparation showing strongly preferred direction of microfibrils in the mucilage envelope close to the protoplast. Internal particles of plasmalemma similar in size and distribution to those of inner membrane of superficial cisterna (see fig. 20). × 65000

Fig. 22. Immature flagellar pole seen from outside the cell: two flagellar buds and a haptonemal bud on the bottom of an oblong concavity in the cell surface, inner membrane of superficial cisterna. × 30000

Fig. 23. Immature flagellar pole exposed by breaking away part of the superficial cisterna. Seen from within the cell, and hence, represented in inverse relief: haptonemal and flagellar bud, the other flagellum fractured across. Characteristic garniture of internal particles of inner membrane of superficial cisterna, and a piece of membrane of a cytoplasmic sheet inside this cisterna, fractured plastid. × 40000

Fig. 24. Mature flagellar pole seen from outside the cell. External parts of flagella and haptonema severed at their junction to the cell body. Irregular distribution of internal particles of the plasmalemma. × 40000

Fig. 25. Fracture through the flagellar pole, partially exposing the pit with its lateral inflations. Proximal part of a flagellum next to entrance of pit. Profiles of the superficial cisterna with sets of parallel membranes (arrow) of cytoplasmic sheets inside. × 40000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(McCULLY 1968). However, McCully related this secretion to an intracellular osmoregulation. We did not observe signs of a high secretory activity in *Pavlova*, and we do not attribute an osmoregulatory function by either of these mechanisms to its mucilage envelope.

For free cells of *Acer*, HELLER et al. (1974) found a distinct influence of the cell wall on the electrical potential of the cytoplasm, They explain this phenomenon by the anionic character of the cell wall, which should lower the permeability for anions. It can be envisaged that naturally occurring anions behave in the same way as the anionic dyes used by us. The rapid accumulation of the anionic dyes erythrosin and rose bengal in dead or moribund *Pavlova* cells, suggests that the mucilage envelopes are permeated almost freely. From this it can be inferred that the permeability for the much smaller inorganic anions which naturally occur in the environment of the alga will not be affected to a biologically significant extent.

One function to be attributed without hesitation to the mucilage envelope is to damp occasional extraneous fluctuations in the chemical environment of the protoplast by constituting a turbulence-free zone.

4.2. Periphery of the protoplast

The variation in the morphology of the peripheral layers of the protoplast can be regarded as a secondary development of the type common in Haptophycean algae and among them in *Pavlova*. In that basic type, the endoplasmic reticulum separates the periplast from the main body of the cell. According to this interpretation a number of cells in our material have lost their periplast, including the outer membrane of the superficial E.R. cisterna. Noxious circumstances are known to provoke this reaction in flagellate *Pavlova* cells (VAN DER VEER 1969). However, agreement between observations on freeze-etch preparations and on sections demonstrates that in palmelloid *P. ennorea*, ecdysis of the periplast is a natural phenomenon, not a fixation artefact.

The fate of the slough will be disintegration. Tortuosely shaped membrane structures outside the protoplast, but within the mucilage envelope, represent periplast fragments involved in a vesiculating process. The observed groups of small vesicles are the end products of this process. An intact superficial cisterna, when associated with such debris, indicates that the first can be regenerated. The remains of multiple periplasts testify of repeated regenerations.

After loss of the periplast, the boundary of the protoplast is formed by the inner membrane of the superficial cisterna. Apparently, a plasmalemma with an unusual internal structure being similar to that of the inner cisternal membrane acquired the position at the protoplast's boundary secondarily, and has retained the internal structure from its former status.

The great difference in the garnitures of the intramembranous particles suggests that a functional change-over accompagnies the substitution of the inner cisternal membrane for the plasmalemma. Especially an effect on transport functions can be expected. However, the overall effect of such an alteration of the protoplast's permeability characteristics would be smoothened by the

presence of the mucilage envelope, whose own permeability is limited to diffusion. In the centre of the colonies, this may be a factor to concern.

According to Green's observations the external flagella are perpetually present in the palmelloid cells of *P. granifera* (GREEN 1973). We found strong indications that this is not the case in *P. ennorea*. Nevertheless, it would be uncautious to distinguish a separate immobile life-stage in addition to the palmelloid condition, since it is not ruled out that the flagella disappear and return in each mitotic cycle.

4.3. Taxonomic position

The peripheral thylakoid stacks approach so closely to each other at the plastidial edge that it may be pondered if their individuality could not be the result of fragmentation of a girdle lamella. The thylakoid continuity found between a central and a peripheral lamella tells against this possibility, and hence, the lamella configuration unequivocally is of the parallel type. This, in combination with a layer of E.R. around the plastid and the occasionally present haptonema, warrants the position of the investigated alga in the class of Haptophyceae.

It is possible to designate the genus to which the species belongs. The microtubular root is similar to the root described for *Pavlova gyrans* (GREEN & MANTON 1970) and other *Pavlova* species (VAN DER VEER 1969, 1972, 1976; GREEN 1973). The non-microtubular roots resemble those in *P. calceolata* (VAN DER VEER 1976). The position of the organism in the genus *Pavlova* is confirmed by the pit entering the cell at the flagellar pole. The coating with dense material of the inflated termination of this pit is very characteristic. Another valuable feature is the presence of the reserve material paramylon, which outside the Euglenophyceae only has been demonstrated in the genus *Pavlova* (KREGER & VAN DER VEER 1970; TSCHERMAK-WOESS 1972; VAN DER VEER 1976).

The organism differs from all *Pavlova* species that are examined in the electron microscope, in lacking the characteristic dense particles on the long flagellum. In other respects the external flagellar apparatus is typically *Pavlova*-like (GREEN & MANTON 1970, GREEN 1973, BILLARD 1975, VAN DER VEER 1976). The absence of minute dense particles on the surface of the cell body is shared with *P. virescens* (BILLARD 1975) and *P. calceolata* (VAN DER VEER 1976).

Thin electron dense layers are observed in the superficial cisterna of most *Pavlova* species, but not in the other Haptophycean genera that have so far been examined. Osmiophilic blisters in the periplast were also described by GREEN & MANTON (1970) in *P. gyrans*. These authors suggested that the blisters could be the points of origin of filopodia. In our material the blisters seem to develop into intra-cisternal foams, perhaps as artefacts.

There are two algae described under other generic names to which our material shows resemblances; *Corcontochrysis noctivaga* (KALINA 1970, 1975) and *Exanthemachrysis gayralii* (LEPAILLEUR 1970). The absence of a stigma in *Pavlova ennorea* excludes specific identity with *E. gayralii*, which moreover has two plastids as opposed to the single one in *P. ennorea*. *C. nocti-*

TABLE 1. Characteristics of *Corcontochrysis noctivaga*, *Pavlova ennorea* and *Pavlova calceolata*

Corcontochrysis noctivaga	*Pavlova ennorea*	*Pavlova calceolata*
Plastid: one parietal, clefted, light to dark brown, without stigma, without pyrenoid. Pit a narrow tube.	Plastid: one parietal, clefted, greenish yellow, without stigma, with a pyrenoid. Pit with lateral inflations.	Plastid: one parietal, tetra-lobed yellow-green with a stigma, without pyrenoid. Pit a narrow tube with globose ending.
One or two pulsating vacuoles.	No pulsating vacuoles.	No pulsating vacuoles.
Presumably filopodia: 'Scharfe Ausläufer und Ausstülpungen' KALINA (1975).	Osmiophilic blisters in periplast.	Vesicles in periplast.
Two to three conspicuous vacuolar inclusion bodies.	Two paramylon granules in vacuoles.	Two paramylon granules in vacuoles.
No external particles anywhere.	No external particles on the plasmalemma anywhere.	External particles on the long flagellum only.
Two unequal flagella covered with fine hairs, and a short haptonema.	Two unequal flagella covered with fine hairs, and a short haptonema.	Two unequal flagella covered with fine hairs, and a short haptonema.

vaga and *P. ennorea* are very similar indeed, as shown by *table 1*, in which the main characteristics of the two species are summarized together with those of *P. calceolata*.

Some words have to be devoted to the peripheral morphology in special. KALINA (1975) describes a three-layered envelope surrounding the whole cell body, and a superficial cisterna which does not do so. This seems at variance with the structure found in *P. ennorea* and other *Pavlova* species. However, possibly Kalina's three-layered envelope may be the same thing we described as the periplast plus the electron dense, non-membraneous layer inside the superficial cisterna, with the same finely granular material we found only around the flagellar insertion (VAN DER VEER 1969, 1976) present everywhere between these layers. Although *P. ennorea* and *C. noctivaga* differ in a number of features, the close affinities of both of them to known *Pavlova* species are undeniable. KALINA (1975) points to the variation within the genus *Pavlova* for maintaining *Corcontochrysis* as a separate genus. As long as a full account of variability is not within reach, a broad genus concept is to be favoured, and therefore we propose to include both of the two species in the genus *Pavlova*, as *Pavlova noctivaga* comb. nov. and *P. ennorea* respectively.

DIAGNOSIS

Pavlova ennorea *sp. nov.*

Cellulae sedentariae colonias palmelloides formantes, patelliformes, 6–9 μm diam., saepe compressae si contingentes inter se.

Chloroplastus unus, parietalis, flavovirens, sine stigma, lobis duobus magnis et pyrenoide protuberante. Nucleus excentricus. Corpus Golgii unicum. Deposita lipida, paramylica et polyphosphatica. Cellulae mobiles elongatae, 6–9 × 3–4½ μm. Flagella duo inaequalia, 13 μm et 5 μm, et haptonema brevis tenuis distantia parva a polo anteriore cellulae. Systema radicum flagellorum radicibus pluribus juxta nucleum et microtubulis parallelis octo ab nucleo currentibus. Puteus inflationibus lateralibus in interiorem cellulae prope insertionem flagellorum intrans.

Typus lectus 20 V 1976, in palude salsa prope Polbathick, Cornubia, Angliae; sub numero 6758 (Van der Veer) cultus.

Sedentary cells forming palmelloid colonies, kneecap-shaped, 6–9 μm diam., often flattened if touching each other. Chloroplast single, parietal, yellow-green, without stigma, with two large lobes and a protruding pyrenoid. Nucleus excentrically positioned. Golgi body single. Deposits of lipid, paramylon and polyphosphate. Motile cells elongate, 6–9 × 3–4½ μm. Two unequal flagella, 13 μm and 5 μm, and a short slender haptonema inserted at some distance from the anterior end of the cell body. System of flagellar roots with several roots near the nucleus and eight parallel microtubules running away from the nucleus. Pit with lateral inflations entering the interior of the cell near the flagellar insertion.

Type collected the 20th of May 1967, from a saltmarsh near Polbathick, Cornwall, England; culture nr 6758 (Van der Veer).

ACKNOWLEDGEMENTS

Thanks are due for the hospitality of the Laboratory of the Marine Biological Association U.K., and for the valuable advice of Dr. Parke during the visit of the first author to Plymouth, on which occasion the organism was collected. The Department of Ultra Structure Biology, Biological Centre, Haren, is thanked for providing instruments and technical aid during the investigation.

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