PAVLOVA MESOLYCHNON (CHRYSOPHYTA) A NEW SPECIES FROM THE TAMAR ESTUARY, CORNWALL

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Pavlova mesolychnon Van der Veer, *sp. nov.*

Cells solitary, motile, dorsoventrally compressed, not metabolic, of variable shape, often oval, obovate, ovate or pyriform, often slightly asymmetric in dorsal view, (5½–)6–9(–13) × 4–4½ × 2½ μ. Two unequal, heterodynamic flagella and a contractile filament arising ventrally, close together, $\frac{1}{5}-\frac{1}{3}$ of the body length from the anterior end. The long flagellum thick (about 0,4 μ) convoluted, 14–17 μ long, covered with tiny cylindrical to clubshaped scales, the basal part with very delicate hairs. The short flagellum 3–4½ μ long, about 0,2 μ thick. Contractile filament up to four times the length of the cellbody when fully extended, thickness variable, 0,1–0,2 μ, basal part thicker, 0,3 μ, covered with very delicate hairs. The contractile filament displays in transverse section a cavity surrounding partially or complete about 8 fibres arranged in a ring.

Chromatophores two, large, parietal and lateral, yellow-green, extended over $\frac{7}{8}$ of the sides of the cell, oval. In each chromatophore a pyrenoid central on the inner side. Pyrenoids without apparent reserve material.

Two to four bright refractive bodies between the chromatophores. Nucleus anterior, next to the insertion of the flagella and contractile filament. One central golgi apparatus, composed of many cisternae. Cells surrounded by a theca composed of two unit membranes, continuous with the plasmalemma. Theca covered with tiny clubshaped scales.

Type collected 20th May 1967 in a saltmarsh alongside a tributary of the Lynher River, East of the village Polbathick, and South of the village St. Germans, Cornwall, cultured under no. 6755 (Van der Veer), deposited in the Cambridge living collection.

Pavlova mesolychnon

Cellulae solitariae, mobiles, dorsiventraliter compressae, non metabolicae, forma variabiles-saepe ovales, obovatae, ovatae vel pyriformes, in aspectu dorsali saepe plusminusve asymmetricae, (5½–)6–9(–13) × 4–4½ × 2½μ.

Flagella bina inaequalia heterodynamica et filamentum contractile in mutua propinquitate ventraliter 1/5–1/3 corporis ab apice anteriore exoriuntur. Flagellum quidem longum crassum circum 0,4μ diam., convolutum, 14–17μ longum, squamis minutis cylindricis-clavatis, in parte basali pilis tenellulis velatum; flagellum breve tantum minus crassum, 0,2μ diam., 3–4½μ longum. Filamentum contractile statu maxime extenso usque ad quatuor longitudinem corporis attingens, crassitudine variabili, 0,1–0,2μ, parte basali incrassata 0,3μ, pilis tenellulis velata. Filamentum contractile in sectione traversa cavum cingentum pro parte vel in toto fibras

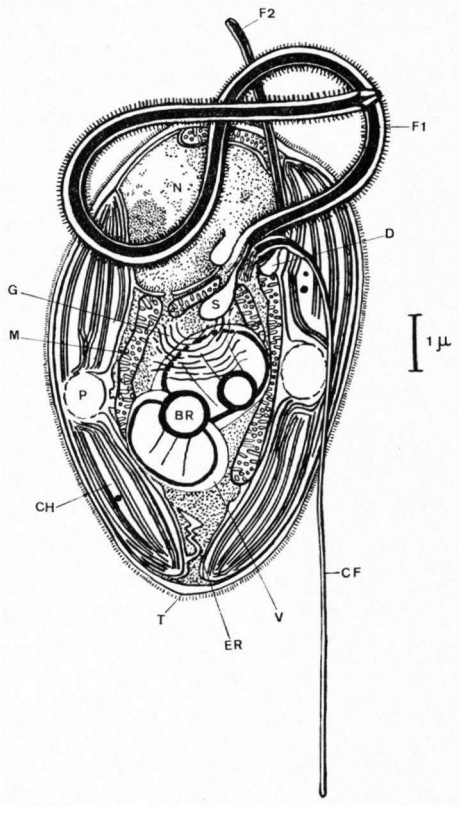


Fig. 1. Semidiagrammatic drawing of *Pavlova mesolychnon* based on living cells, direct preparations and sections. (F1) long flagellum, (F2) short flagellum, (CF) contractile filament, (N) nucleus, (M) mitochondrium, (CH) chromatophore with (P) pyrenoid, (T)theca, (ER) endoplasmic reticulum, (BR) bright refractive body, (V) vacuole containing BR, (S) sac between flagellar roots, (D) depression surrounding the papilla.

circa octo in orbem dispositas praebens. Chromatophora dua, magna, parietalia et lateralia, flavovirentia, super 7/8 lateris corporis extensa, ovalia. In quoque chromatophoro pyrenoides unica in centro lateris interioris invenitur. Pyrenoides sine materia penaria manifesta. Corpora lucida refractiva 2–4 inter chromatophora extant. Nucleus ante juxta insertionem flagellorum et filamenti contractilis situs. Apparatus golgii unicus in centro corporis numerosis cisternis compositus. Cellulae theca composita duabus membranis in plasmalemmam transeuntibus involutae. Theca squamis minutis claviformibus tecta.

Typus collectus 20.V.1967, in palude salsa juxta flumen influens in flumen Lynher, orientali ex loco Polbathick, australi ex loco St. Germans, Cornubia, sub numero 6755 (Van der Veer) cultus, in vivario Cantabrigiense depositus.

1. INTRODUCTION

The Chrysophycean genus *Pavlova* can easily be recognized because it has two brigth refractive bodies and two unequal flagella, of which the long one is typically convoluted, and a contractile filament attached near the point of insertion of the flagella (BUTCHER 1952; BOURELLY 1957; GREEN 1967).

Of this genus two species are known: *Pavlova gyrans*, isolated and described by BUTCHER (1952) from the Helford River, Cornwall, and *Pavlova pinguis*, isolated and described by GREEN (1967) from the North Atlantic Ocean near

Madeira. The contractile filament was first discovered by Parke in *Pavlova gyrans* (BOURELLY 1957). It is called a "haptothrix" by GREEN (1967), who amplified the diagnosis of the genus to include this character. He also gives an electron microscopical photograph of the shadowed flagella and haptothrix, and this seems to be the only observation published on the fine structure of these organisms.

A third species of the genus *Pavlova* was collected from a saltmarsh near Plymouth during the author's stay at the Laboratory of the Marine Biological Association of the United Kingdom.

In this paper a preliminary account of the investigation of its ultrastructure is given.

2. ISOLATION AND CULTURING

The organism was isolated from a sandy mud sample collected on 20th May 1967. In the laboratory subsamples were put into a culture medium. The resulting mixed population was freed from Crustaceans by straining and from Ciliates by pipetting out the flagellates under a stereoloupe, avoiding to suck up the predators. After that the culture was gradually purified by picking up single cells with the use of the inverted microscope.

Study with light microscope and electron microscope was started before a unialgal culture had been obtained. Other organisms living in the same culture were: a *Heteromastix*, a *Paraphysomonas*, a *Choanoflagellate* and a colourless *Dinoflagellate*.

Parke's modification of the Erd-Schreiber medium was used (PARKE, roneotyped prescription; GREEN & JENNINGS 1967) until October 1967, when the culture was taken to Groningen. By then the soil extract was replaced by a decoction of decayed leaves from a deciduous wood (the "Noordlaarder Bos"), a nature reserve, this being virtually the only source free from fertilizers, herbi-, fungi-, and insecticides, in contrast to the availlable garden soil.

3. LIGHT MICROSCOPY

Living cells were studied with phase contrast optics. The following description can be given for healthy motile cells. Usually the long flagellum moves in front, dragging the cellbody. Sometimes, in escape reactions for instance, the cell can swim backwards for a few moments, being pushed by the flagellum. In rapid swimming the cell rotates, slowly swimming cells do not rotate. In this type of movement the ventral side is kept downwards. The long flagellum is thick and has a blunt tip. In rest it has two or three turning points, and often forms a cricoid, almost in a flat plane. The short flagellum is thinner. In rest it is straight or forms a single arc. The contractile filament is mostly shorter than the cell, and rarely longer than $1\frac{1}{2}$ times the length of the cell, pointing backwards and running close to the surface of the cell. It can however be extended up to four times the length of the cellbody, trailing behind in slowly swimming individuals.

It can attach the cell to the substrate. In this condition the insertion near the flagella may become apparent when the cell changes its orientation before swimming away.

The contractile filament is often straight or slightly curved, but in a few instances it was observed to be irregularly undulated, with irregular movements suggesting its flexibility. It was never seen to coil up. During prolonged observation all filaments became invisible, apparently by their contraction.

Two bright refractive bodies are mostly present. Sometimes there are two big and two small ones. These bodies are supposed to be food reserves. They are stained pink with brilliant cresyl blue. Fractures can be seen in the greater ones, even if the cell is not put under pressure. The chromatophores have about the same shape as a splitted peanut. The little pyrenoids are just discernable in flattened or burst cells.

The cells can be flattened under the coverslip by sucking up as much medium as possible with filterpaper, applied to opposite edges of the coverslip. Evaporation of the medium will lessen the space between object glass and coverslip still more. *Pavlova mesolychnon* flattens under pressure without forming pseudopodia. Even in thus flattened cells the nucleus is not easy to find, because its perimeter is obscured by other organelles. It is situated at the anterior end of the cell, between and in front of the chromatophores.

In flattened cells some granules become visible in various positions. Using knowledge of electron microscopical origin, they can be interpreted as mitochondria or fat globules. Once a dark circular spot was observed, surrounded by small dots with a size near the limit of visibility in the microscope. This could be a golgi body.

Neither an eyespot nor a contractile vacuole could be detected.

4. ELECTRON MICROSCOPY

For direct preparations the cells were fixed in an isotonic solution of buffered osmium tetroxide. After they had been allowed to settle down the cells were washed in distilled water to get rid of the salt from the culture medium. Drops of resuspended cells were put on grids with carbon-coated formvar films. The dried cells were shadowed with gold-paladium.

For sections the material was fixed and embedded according to two different procedures. I. Fixation in glutaraldehyde and postfixation in osmium tetroxide, and embedding in Epon. II. Fixation in osmium tetroxide and after that in potassium permanganate, and embedding in styrene-butylmethacrylate.

Contrast was heightened with uranyl acetate and either lead citrate or potassium permanganate.

The electron microscope revealed the existence of a "theca", composed of two unit membranes, one continuous with the plasmalemma of the flagella and the other one with the plasmalemma of the cellbody. The theca is perforated (*fig. 4* and *11*). It can be smooth or undulated. It is covered with tiny clubshaped scales, 800 Å long and 200 Å wide, attached to the theca by very thin hairs about 1200 Å long (*fig. 2, 10* and *18*). The flagella and contractile filament are inserted in a papilla surrounded by a depression in the cell surface. This papilla

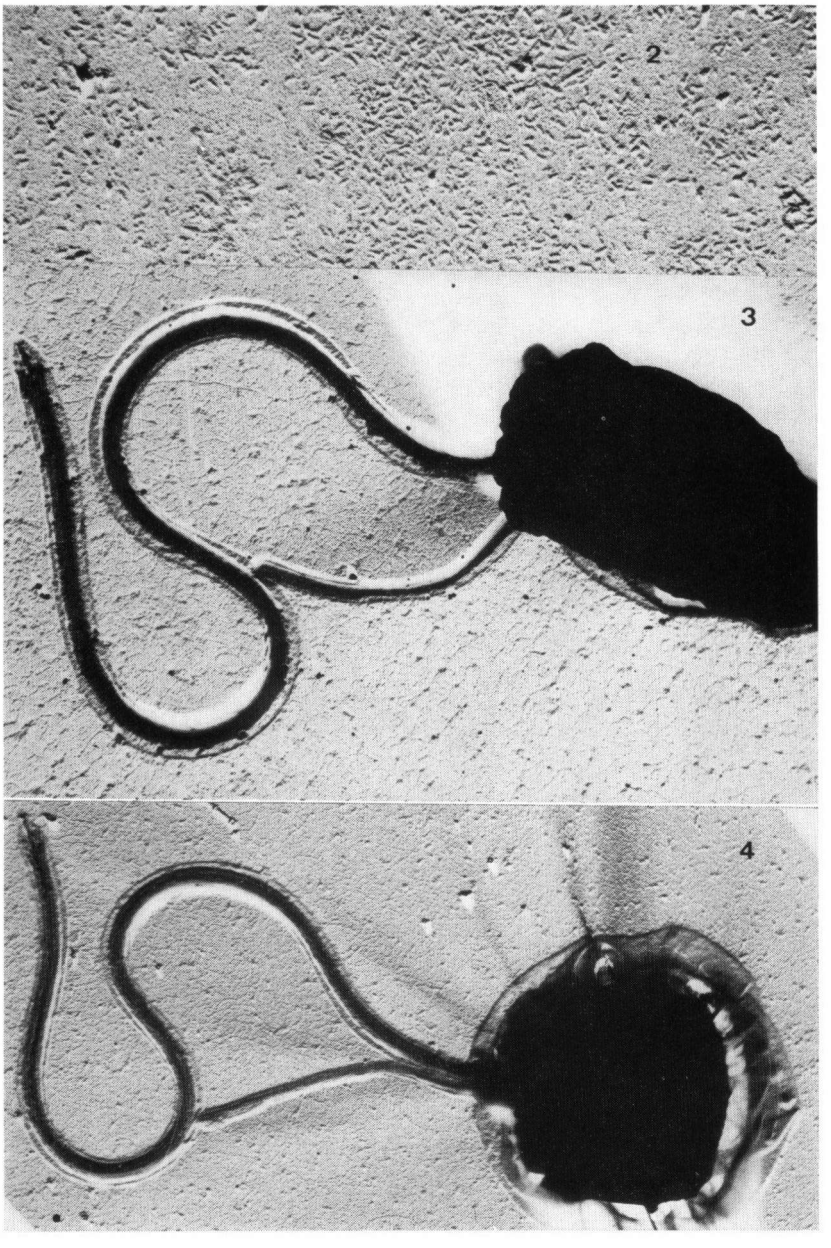


Fig. 2. Direct preparation of clubshaped scales. 21000x
Fig. 3. Direct preparation of whole cell. 10000x
Fig. 4. Direct preparation of whole cell, showing the theca with perforation. 7500x

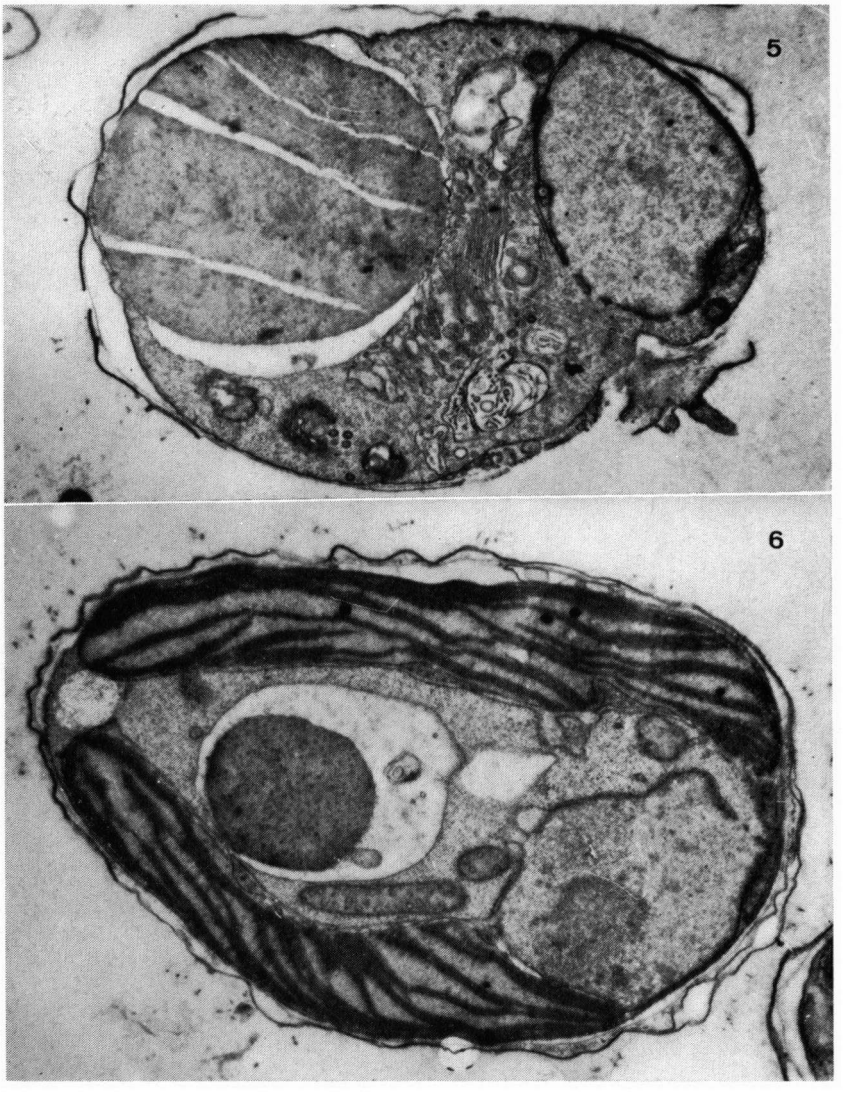


Fig. 5. Median longitudinal section, showing nucleus, a large bright refractive body and golgi apparatus in between, papilla and disrupted theca. 19000×.

Fig. 6. Section latero-laterad, showing the nucleus, lateral chromatophores lined by E.R., a vacuole containing a bright refractive body, another vacuole hit tangentially, and mitochondria. 19000×.

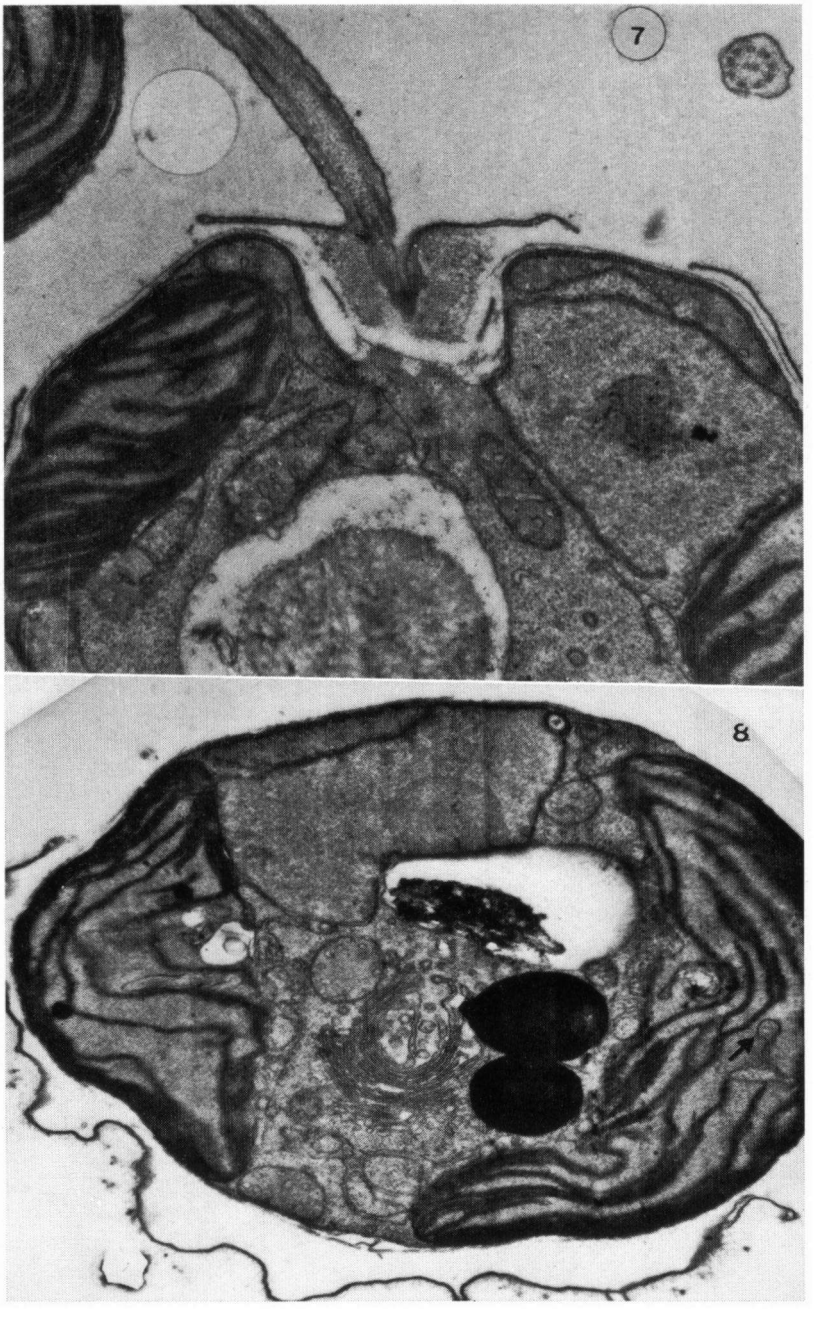
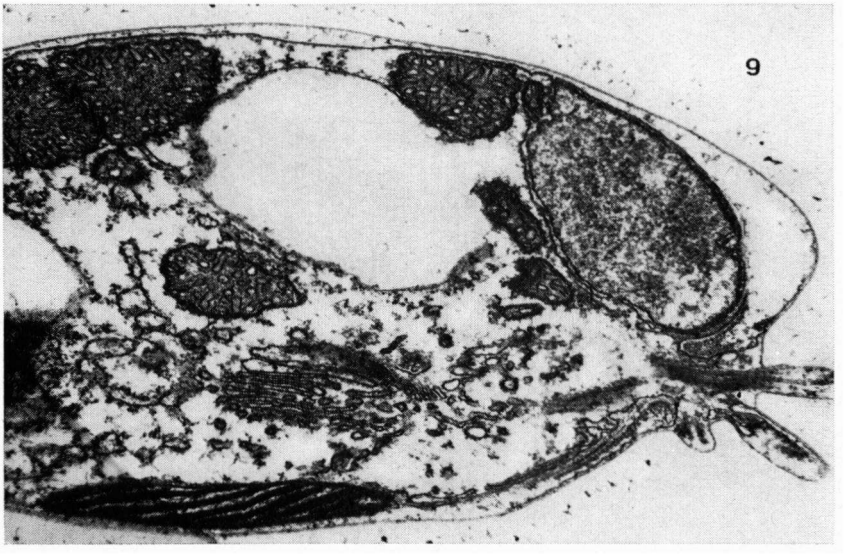


Fig. 7. Oblique section going through the long flagellum longitudinally near the insertion and transversely in the upper right of the figure. The canal of the sac is to be seen at the point of insertion of the flagellum. 25000 ×.

Fig. 8. Oblique section through the nucleus and chromatophores in the region of the pyrenoids. Between the chromatophores the golgi apparatus, two globules of fat, mitochondria and E.R. A piece of cytoplasm inside the righthand chromatophore. (arrow). 19000 ×.



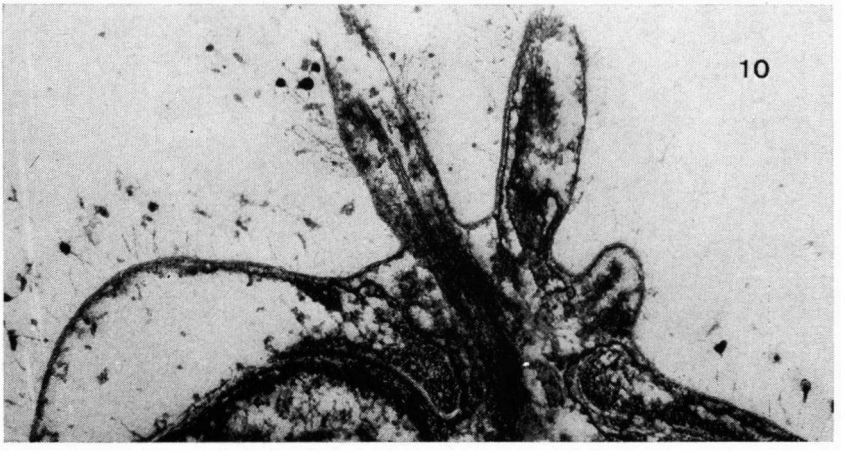
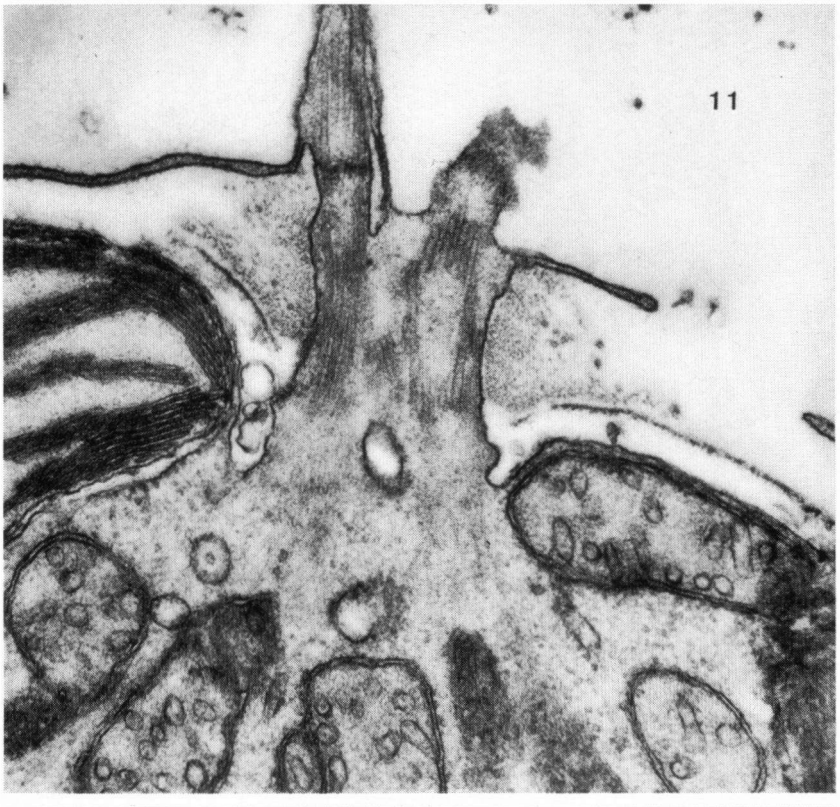


Fig. 9. Longitudinal section showing a smooth theca and a number of well developed mitochondria. 19000×.

Fig. 10. Detail of fig. 9 to show the clubshaped scales attached to the theca with very thin hairs; also very delicate hairs on the basal part of the long flagellum and cavity system in the contractile filament. 40000×.



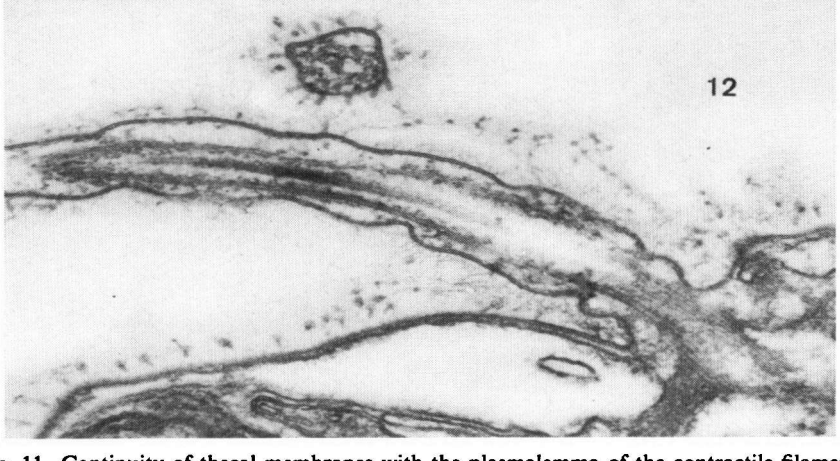


Fig. 11. Continuity of thecal membranes with the plasmalemma of the contractile filament, the long flagellum and the papilla. Perforation of the theca on the right. The canal of the sac is cut obliquelly at the base of the papilla. Vesicles traverse the plasmalemma between chromatophore and papilla. 50000 ×.

Fig. 12. Long flagellum longitudinally and transverse, showing the continuity of the flagellar plasmalemma with the outer thecal membrane, and scales and hairs on the theca and flagellum. 28000 ×.

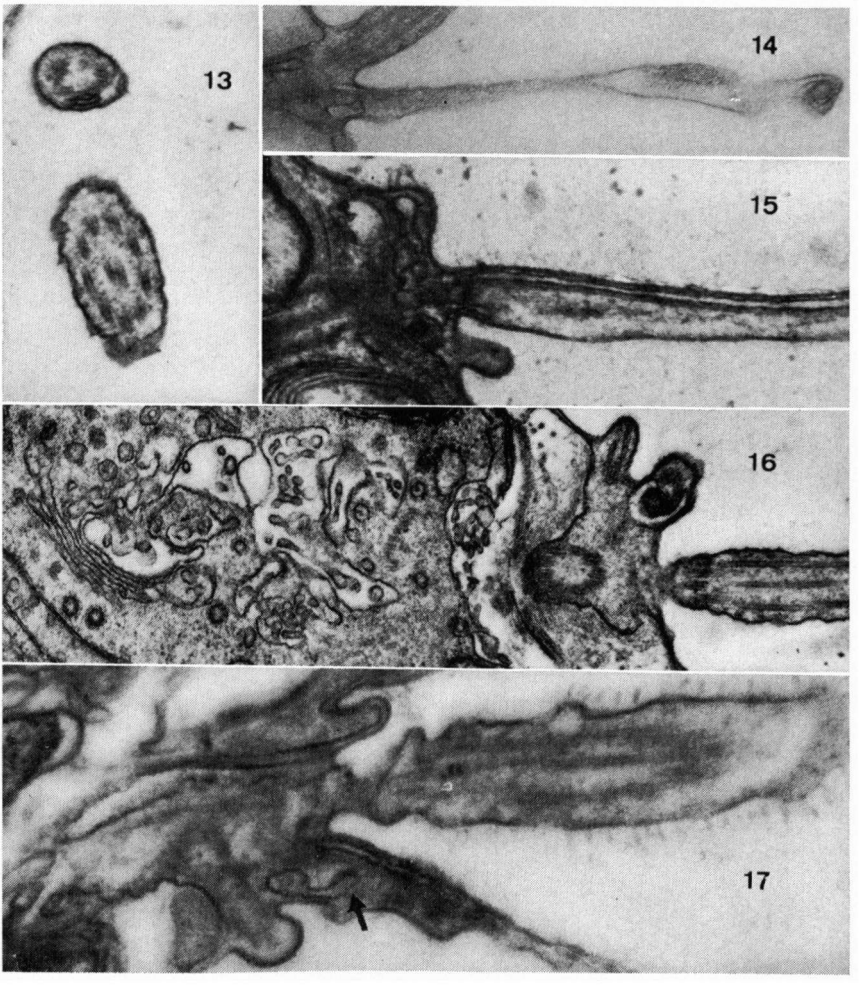
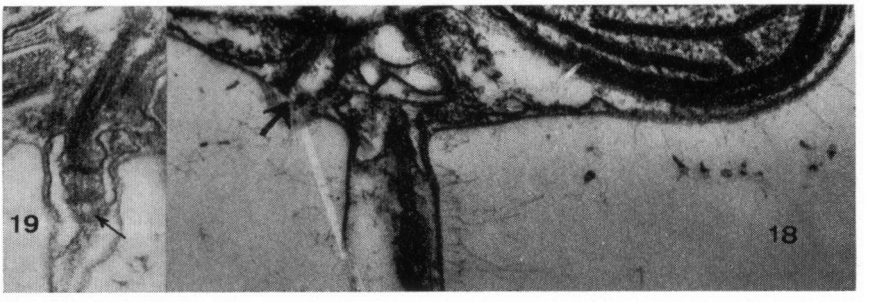


Fig. 13. Long flagellum and contractile filament, the latter with a ring of microtubuli and a crescent shaped cavity. A clubshaped scale at right. 28000 ×.
Fig. 14. Contractile filament cut longitudinally in the basal part but more or less transversely where it bends at right, showing a ringshaped cavity. Unstained section. 21000 ×.
Fig. 15. Basal part of contractile filament containing a lateral cavity. 42000 ×.
Fig. 16. Basal part of contractile filament cut obliquely. The cavity system C-shaped and penetrating the core of microtubuli. Under the theca a number of vesicles, perhaps originated in the golgi apparatus. A row of microtubuli belonging to a root system in the middle of the figure 40000 ×.
Fig. 17. Long flagellum with scales. Basal part of contractile filament with a process of the cavity system penetrating the core of microtubuli, cut longitudinally (arrow). 50000 ×.



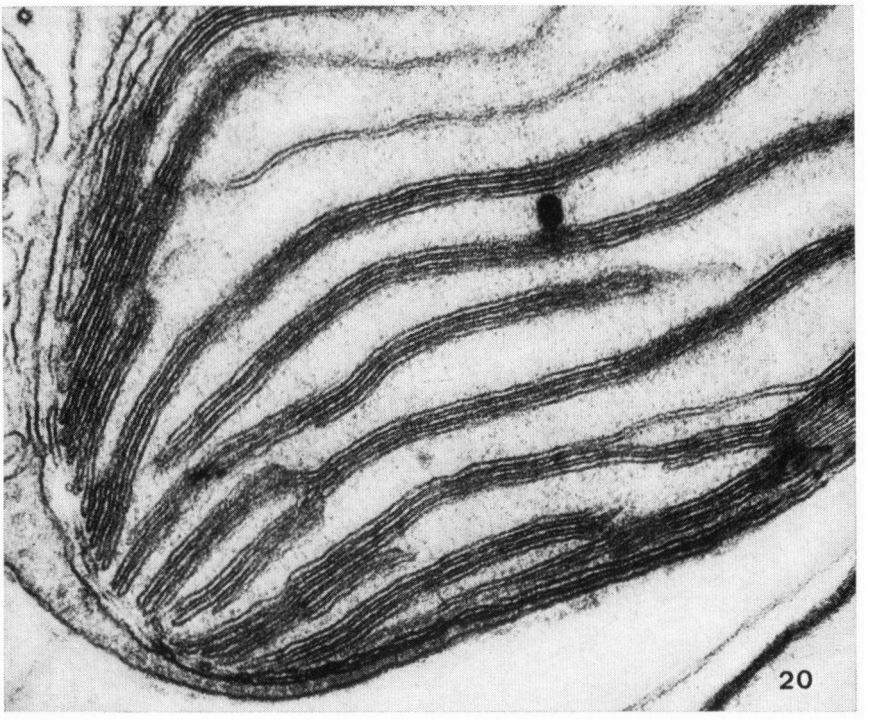


Fig. 18. Very delicate hairs on the basal part of the contractile filament. Clubshaped scales on very thin hairs. Base of short flagellum at left (arrow). 38000×.

Fig. 19. Basal part of contractile filament with the process of the cavity system penetrating the core of microtubuli, cut transversely (arrow). 38000×.

Fig. 20. Detail of chromatophore. Lamellae mostly formed by three apposed thylakoids. Envelope adjacent to plasmalemma composed of three unit membranes. 68000×.

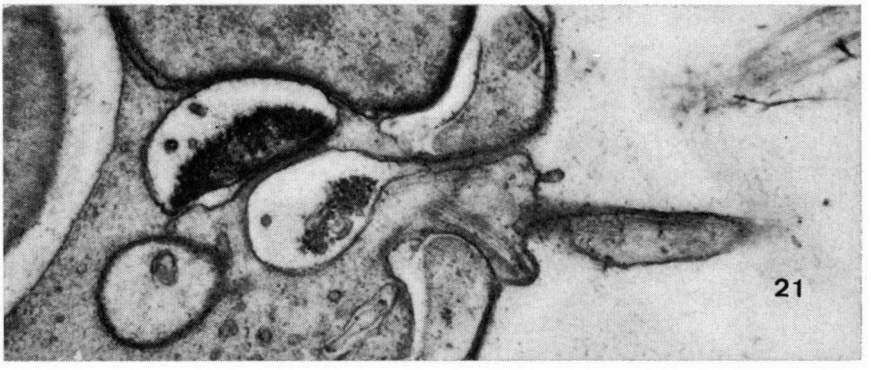


Fig. 21. Sac underneath the papilla with the canal. 35000×.

forms a bridge between the main body of the cell and the theca (*fig. 10* and *11*).

The long flagellum is covered by small cylindrical to clubshaped, hollow scales, 700 Å long and 200 Å wide. Its basal part is covered with very thin hairs, 2500 Å long (*fig. 10* and *12*). Its relative thickness is caused by the distance between the outer membrane and the core of microtubuli, which is greater than usual. The short flagellum seems to be smooth. There are roots running from the flagella bases to various regions of the cell, but the root system could not be analysed completely until now. Between the roots lies a sac which opens to the outer world through a canal traversing the papilla. The significance of this sac is not clear. it may be a reservoir of scales. as in *Pyramimonas*, or something like the Dinphycean pulsule.

The structure of the contractile filament is illustrated in *fig. 13* to *19*. The contractile filament contains a core of 7–8 microtubuli arranged in a ring. The core penetrates deep into the cellbody. An electrodense "septum" traverses the contractile filament just outside the papilla. This septum is penetrated by a cavity system, which below the septum has developed into a cavity surrounding most of the core of microtubuli. A fingerlike process intrudes into the ring of microtubuli in this region. In more distal parts of the contractile filament the cavity system is represented by a tube which is crescent shaped in transverse section. About 3 μ from the insertion a transverse section shows a cavity completely surrounding the axial core of microtubuli. The basal part of the contractile filament is covered with very thin hairs as is the long flagellum.

The nucleus lies adjacent to the plasmalemma and the chromatophores. The nuclear membrane has only a few pores, which are about 700 Å in diameter. A fold of endoplasmic reticulum continuous with the nuclear membrane lines the chromatophores (*fig. 6*).

The structure of the chromatophores is illustrated in *fig. 20*. There is very little cytoplasm between the chromatophore and the plasmalemma. The lamellae usually consist of three thylakoids. On the inner surface of each chromatophore is a depression, occupied by a number of vesicles, which are probably an

elaboration of the endoplasmic reticulum. Next to this depression lies the pyrenoid inside the chromatophore. The pyrenoid is not very conspicuous, but canals penetrate deep into this region of the chromatophore. In *fig. 8* intrusions of cytoplasm inside the chromatophore can be seen.

The mitochondria are of the tubular type. They are oval, rodshaped, up to a few μ, or fused into irregular complexes. The golgi apparatus consists of a stack of 8–15 cisternae (*fig. 8*). The relations of the golgi apparatus to other structures need further investigation. The bright refractive bodies are confined each in a separate vacuole. They are crystallized, as a fine striation reveals (*fig. 5*). Droplets of lipids also occur in the cytoplasm. A group of small vesicles, perhaps paramural bodies (MARCHANT & ROBARDS 1968), and a thin electrodense layer in the cavity between theca and cellbody are still forming a problem.

5. DISCUSSION

GREEN (1967) gives the following diagnosis of the genus *Pavlova*: "Cells solitary, motile naked with undifferentiated periplast. Two unequal flagella and a haptothrix (attaching organ) arising laterally, close together from the anterior end; the longer flagellum with hairs, the shorter smooth; haptothrix contractile. Asexual reproduction by division in the motile or non-motile phase".

The new species fits well into this description. Only the theca is a deviation. For exactness we have to go back to the text of BUTCHER (1952), which in English is the same, and in Latin; "Cellula solitaria, natante, nuda periplasto differentia carente", which means "Cell solitary, swimming, naked with a periplast which lacks differentiation".

The theca is very difficult to see (round the papilla) in living cells, but it is left behind if the cell contents are pressed out when the cell bursts by pressure under the coverslip, or by a mild osmotic shock. The same holds true for a periplast. Only an inspection with the electron microscope can reveal the difference. Clearly electron microscopic observation of the surface structures of the two species, *P. gyrans* and *P. pinguis* are required for exact evaluation.

For the moment it seems not justified to create a new genus to contain the new species. Since in all other respects the new species conforms to Green's generic description of *Pavlova* it has been placed in this genus.

For convenience of comparison the following table is given. It contains the most important characters to distinguish the three species.

The theca of *Pavlova mesolychnon* is not homologous with the theca of *Platymonas* (*Prasinophyceae*) (MANTON & PARKE 1965). This latter genus constructs its theca with scales formed in the golgi-apparatus. Something like the structure in *Pavlova* is described for the Haptophycean species *Prymnesium parvum* by MANTON (1964). There a subcutaneous space underlies the plasmalemma. The plasmalemma and adjacent membrane delimiting the subcutaneous space, together, could be homologous with the theca of *Pavlova mesolychnon*. It is, however, not known to be perforated, as is the latter.

The pore in the theca in *Pavlova* may be secondary, but it is also possible that

Pavlova gyrans	*Pavlova pinguis*	*Pavlova mesolychnon*
Cells naked with undifferentiated periplast.	Cells naked with undifferentiated periplast.	Cells with theca covered with scales.
Stigma present.	Stigma present.	Stigma absent.
Bright refractive bodies posterior.	Bright refractive bodies posterior.	Bright refractive bodies between chromatophores.
Cells metabolic.	Cells slightly metabolic.	Cells not metabolic.
Cells compressed.	Cells not compressed.	Cells compressed.

the theca is formed by a folding of the plasmalemma, beginning around the point of insertion of the flagella. Another possibility is formation of the space below the theca by invagination, beginning at the site of the pore. The ontogeny of the theca remains to be cleared.

The contractile filament is comparable to the haptonema of *Prymnesium* (MANTON 1964, 1968). There may be minor differences, because in *Pavlova* the organelle is not analysed to the same extent as in *Prymnesium*, but this does not exclude the use of the same word to designate both organelles. The word "haptonema" should, in the author's opinion, denote an "attaching thread, arising near the flagella", irrespective of the movability of the organelle. It may be coilable as in *Chrysochromulina*, only movable as in *Prymnesium*, or contractile as in *Pavlova*.

The long flagellum of *Pavlova mesolychnon* is not pleuronematic as to be expected in a member of the *Chrysophyceae*. *P. gyrans* is said to be heterokont by BOURELLY (1957), who based this statement upon unpublished photographs by Manton. *Pavlova pinguis* has hairs on its long flagellum. These are nearly invisible in the photograph published by GREEN (1967), who describes them as being very fine, and not easy to demonstrate in direct preparations. So it is not obvious that they are the characteristic stiff hairs of *Ochromonas* and other *Chrysophyceae* (BUTCHER 1965; HAPPEY & MOSS 1967; BELCHER & SWALE 1967). The chromatophores of *Pavlova mesolychnon* closely resemble those of *Prymnesium* (MANTON 1968).

It is suggested that *Pavlova mesolychnon* may be conceived as a very asymmetric Haptophycean rather than as a member of the *Chrysophyceae*, but more information is needed to verify this. An examination of the fine structure of *Pavlova gyrans* and *Pavlova pinguis* will be required to ascertain the coherence of the genus.

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