ANKYLONOTON LUTEUM (CHRYSOPHYTA), A NEW SPECIES FROM THE TAMAR ESTUARY, CORNWALL.

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Ankylonoton luteum Van der Veer, *sp. nov.*

Cells solitary, motile, bean-shaped in dorsal view, not metabolic, (6–) 7 (–10,5) μ long, (3–) 4 (–6) μ wide, dorsal side often convex. Two heterokont flagella inserted ventrally close together near the edge of the single chromatophore, at the edge of a furrow, 1/3–1/2 of the body length from the anterior end. Furrow on the ventral side, partially between keels, running from the flagella insertion obliquely forwards. One flagellum 7–9 μ long, 0,20 μ thick, with a blunt conical tip and two rows of hairlike appendages. Each appendage composed of a hollow shaft 0,9 μ long, 24 mμ thick, attached to the flagellum with a swollen base and ending in a very thin thread 1 μ long. The other flagellum 5 (–8) μ long, 0,16 μ thick, with a narrowed tip 1 μ long. Nucleus one, more or less irregularly conical, its apex pointing to the flagella bases. Chromatophore one, luteous, parietal, lobed, enclosing most of the cell, without eyespot; with a girdle lamella enclosing a number of parallel lamellae; with a large pyrenoid without covering of reserve material, traversed by one or two lamellae. Lamellae composed of three thylakoids. A chromatophore envelope of endoplasmic reticulum continuous with the nuclear envelope and containing parallel fibres. A periplastidial reticulum is present between the chromatophore and the chromatophore envelope. A large vacuole containing a granule. Synthemata with lipids and leucosin. Mitochondria numerous, with the inner membrane forming sacculate invaginations. Muciferous bodies numerous, small, adjacent to the plasmalemma. Outside the plasmalemma three parallel electrodense layers; the outer layer composed of tiny bodies. The other two smooth.

Type collected 20th May 1967, in a salt marsh alongside a tributary of the Lynher River, east of the village Polbathick, and south of the village St. Germans, Cornwall, England, cultured under no 6756 (Van der Veer), deposited in the Cambridge living collection.

Ankylonoton luteum

Cellulae solitariae, mobiles, in aspectu dorsali fabiformes, non metabolicae, (6–) 7 (–10,5) μ longae, (3–) 4 (–6) μ latae, saepe dorso convexo. Flagella duo heteroconta, in facie ventrali conferte inserta prope marginem chromatophoris unici, ad marginem sulci, pro 1/3–1/2 cellulae longitudine a parte apicali. Sulcus in facie ventrali partim inter carinas, currens ab insertione flagellorum oblique prorsum. Unum flagellum 7–9 μ longum, 0,20 μ latum, apice obtuso conico, appendicibus capilliformibus in seriebus duabus positis; appendices capilliformes compositae e scavo capo 0,9 μ longo, 24 mμ lato, ad flagellum affixae per basim
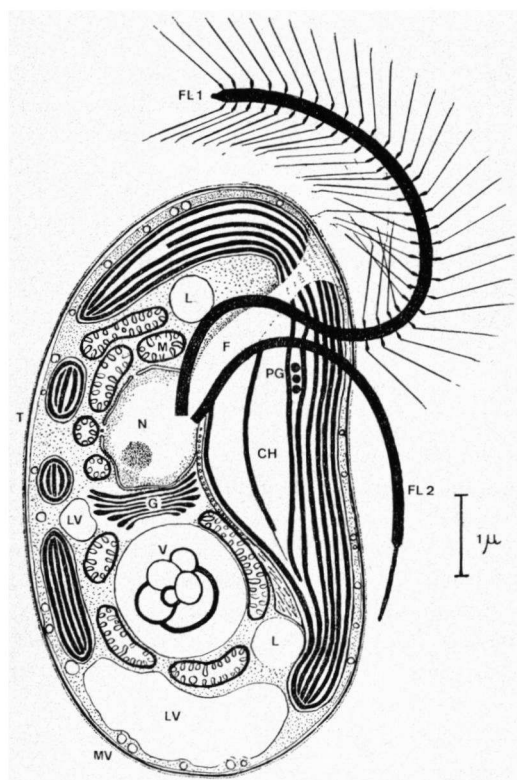
Fig. 1. Semidiagrammatic drawing of *Ankylonoton luteum*, based on living cells, direct preparations and sections. The hairs on the long flagellum are omitted where they would obscure the cellbody. (FL1) pleuronematic flagellum, (FL2) smooth flagellum, (N) nucleus, (CH) chromatophore, (M) mitochondrium, (G) golgi body, (V) large vacuole containing composite body, (L) lipid, (LV) leucosin vesicle, (PG) plastoglobuli, (F) furrow, (T) theca, (MV) muciferous vesicle.

Fig. 2. Three projections of the cell to planes perpendicular to each other. The planes of section of the *figs. 5a, 5b* and *6* are indicated respectively with 5a–5a, 5b–5b and 6–6.
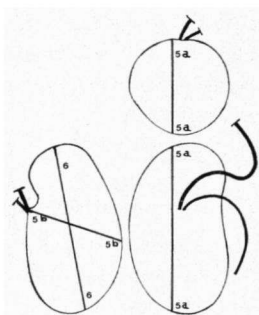
tumidam atque filo tenuissimo, 1 μ longo, terminatae. Flagellum alterum 5(–8) μ longum, 0,16 μ latum, parte apicali angustata 1 μ longa. Nucleus unicus, plus minusve irregulariter conicus, parte apicali versus flagellorum basin converta. Chromatophorum unicum luteum, parietale lobatum, maximum partem cellulae includens sine stigmate; lamella cingit lamellas parallelas plures; pyrenoides magna sine tegumento e materia penaria constituto, lamella vel lamellis duabus persecta. Lamellae e tribus thylacoidis compositae. Involucrum chromatophoricum reticuli endoplasmatici cohaerens cum involucro nuclei et fibras parallelas capiens. Reticulum periplastidiale positum inter chromatophorum atque involucrum chromatophoricum. Corpus golgii unicum. Vacuola unica grandis granum continens. Synthemata lipoida et leucosinea. Mitochondria numerosa, eorum membrana interior invaginationes saccatas formans. Corpora mucifera numerosa, parva, prope plasmalemmam posita. Extra plasmalemmam tria strata parallela radiis electronicis impervia; stratum exterius compositum e corpusculis minutis. Strata cetera laevia.

Typus collectus 20.V.1967, in palude salsa juxta tributarium fluminis Lynher, orientali ex loco Polbathick, australi ex loco St. Germans, Cornubia, Angliae, sub numero 6756 (Van der Veer) cultus, in vivario Cantabrigiense depositus.

1. INTRODUCTION

The genus *Ankylonoton* was described by PASCHER (1932), who isolated the single species, *A. pyreniger* from brackish water on the Frisian island Sylt, Germany. The species has one parietal chromatophore with a pyrenoid. Two very unequal flagella are inserted in a ventral depression. This species is also found in England (PARKE & DIXON 1968).

In this paper a yellow flagellate is described that may be related to it. It was collected from a saltmarsh near Plymouth during the author's stay at the laboratory of the Marine Biological Association of the United Kingdom. It was studied with light microscope and electron microscope.

2. ISOLATION AND CULTURING

The organism was isolated from a sample of water collected on the 20th May 1967 from a tide pool. Isolation was facilitated by the ability of the species to survive in very old cultures. The only other organism which could also endure this was a colourless flagellate, *Pteridomonas* (Penard). This organism was still sparsely intermingled in the cultures used for electron microscopy. The isolated organism thrived on the leaf mould extract-seawater medium, also used for *Pavlova mesolychnon* (GREEN & JENNINGS 1967, VAN DER VEER 1969). It could also grow on Parke's modification of the ASP2 medium of PROVASOLI *et al.* (1957).

3. LIGHT MICROSCOPY

The material was examined alive and fixed with the phase contrast and clear field illumination of a Carl Zeiss microscope.

3.1. Swimming
There are two types of movement. A creeping one with little jerky steps along the substrate, the long flagellum directed forwards. In this type of movement the dorsal side is kept upwards. Free swimming cells move smoothly in straight lines, towed by the long flagellum. The long axis of the cell body makes an angle with the direction of the movement. The magnitude of this angle depends on the distance of the flagella insertion from the apex of the cell. Superimposed on this movement the cell makes a sideways rocking movement, which seems to be caused by the short flagellum waggling in the same rhythm. In resting cells the small flagellum is often directed sideways, running close to the surface of the cell, and is sometimes attached to the substrate by its tip. The long flagellum is rather stiff and in rest has a posture of an S or a question mark.

In cultures of some months old, most cells become enlarged, sometimes circular, and sink to the bottom of the culture flask between the numerous empty thecae and dead individuals. These enlarged cells are frequently division stages with two or more pairs of sometimes moving flagella.

3.2. Colour

The colour of the chromatophore was observed with clear field illumination and fully opened condensor diaphragm. The tinge was yolk yellow, but not saturated and not homogenous. The peripheral layer of the chromatophore appeared to be honey-coloured. In phase contrast optics the correction of the colour shift is less than in clear field optics. This results in a change of the colour, from honey through greenish yellow to lemon yellow, when observed while going through focus.

3.3. Internal structure

3.3.1. *Flattening*

The internal structure of the cell is difficult to observe, even if the cell is at rest. This is due to an extensive system of peripheral leucosin vesicles, the high optical density of which masks the more centrally positioned organelles.

The method of flattening the cells by pressure under the coverslip, as described earlier for *Pavlova* (VAN DER VEER 1969), is not useful in this case. On pressure the theca disrupts at small spots through which the cell contents are extruded. The resulting preparations are difficult to interpret. Sometimes however the chromatophore is pushed through the theca. In this case the other organelles are able to expand inside the theca and a more informative preparation is obtained. The nucleus and leucosin vesicles remain difficult to distinguish, but a large circular vacuole with a prominent tonoplast becomes clearly visible. It contains an irregularly shaped grain or, in cells from old cultures, a large number of very fine grains in rapid Brownian movement. This vacuole is so stable that it remains intact even if it is pushed through the theca and floats free in the medium.

A better method for observation is to avoid the disruption of the theca by a high osmotic pressure of the medium. This was accomplished by sucking away less of the culture medium from under the coverslip and then allowing most of the water in the medium to evaporate. In cells so treated the chromatophore can be studied. The chromatophore has a broad, triangular lobe and a number of narrow lobes reaching to the opposite of the cell. In suitably orientated chromatophores the parallel lamellae can be distinguished. The pyrenoid can be traced easily in a bulge on the inner face of the chromatophore. Often a curved lamella can be seen traversing the pyrenoid. The outline of the pyrenoid is not sharp. A number of small lipid globules (plastoglobuli, LICHTENTHALER 1968) can be seen in the chromatophore.

3.3.2. *Omitting the coverslip*

A still more satisfying method of studying the internal structure of living cells is to omit the coverslip, put the immersion oil directly upon the sample of cells and drain the culture medium. A simple procedure to achieve this is illustrated in *fig. 3*. A number of cells stick to the slide in a little drop of culture medium. The masking effect of the leucosin vesicles is abolished. The whole length of the flagella can be observed. The proximal parts of the flagella stand out against the
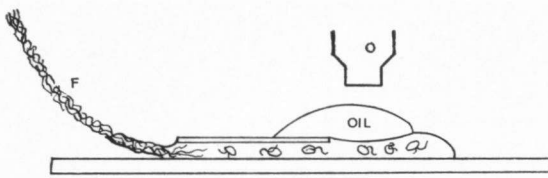
Fig. 3. Method to make a preparation in which living cells can be observed with the oil imersion objective, while avoiding the pressure of the coverslip. (F) filterpaper, (O) objective.

clear, colourless background of the nucleus. In cells lying on their lateral side the nucleus is visible as a long triangular or pear-shaped body orientated crosswise to the long axis of the cell in the equatorial region. Leucosin vesicles of varying size occupy most of the posterior part of the cell. They are somewhat less bright than the nucleus. In some cells the mitochondria are visible as grey bodies of different sizes and outlines, mostly oval or rod-shaped. A few are dumbbell-shaped or irregular. A cluster of little vesicles with very bright, colourless contents are to be found between the nucleus and a large circular vacuole. In some cases these vesicles are arranged in a stack. From this, together with their form, it can be inferred that they are golgi vesicles. The chromatophore looks brown. The shape of the chromatophore can be seen in great detail, the lamellae, however, do not become visible and the pyrenoid remains difficult to see. By focussing on the cell surface the numerous muciferous bodies become visible as reddish brown dots. In a few cases the contents of them were ejaculated. An inconvenience of this method is that during observation the cells disintegrate, probably because of the depletion of the oxygen in the very small drop of culture medium.

3.3.3. Extraction

To enhance the visibility of the internal structure, cells previously fixed in osmic vapour were extracted in either sucrose or glycerin. The sucrose was used as a 0.25 M. solution buffered with 0.1 M. sodium cacodylate to pH 7. In this fluid the masking effect of the leucosin disappeared at once and the organelles became visible. The nucleus and the root system of the flagella remained too vague to draw any conclusions as to their structure. The internal structure of the chromatophore became progressively clearer as the pigments were extracted. The lamellae became visible as dark lines in the focussing plane. Peripheral to the pyrenoid there were five or more parallel lamellae concentric with the convex outer face of the pyrenoid. In the lobes there were fewer lamellae. The lamellae overlying the pyrenoid and those traversing it were very prominent. It was impossible to see the girdle lamella. For extraction in glycerol the commercial product was applied without buffering or diluting. At first the cells shrunk considerably, but soon they regained their normal shape. The extraction went more rapidly than in sucrose. The results were the same.

3.3.4. Staining

Brilliant cresyl blue was used to indicate the leucosin by a pink colouration. The heterochromatic stain had a much deeper hue in cells of aged cultures than in

cells of cultures only a fortnight old, in which the vesicles appear more bluish pink. This is partly due to orthochromatic staining of the layer which immediately surrounds the vesicle. In young cultures there are one or a few leucosin vesicles in the basal part of the cell. In extremely long cells there is also a leucosin vesicle in the apical part of the cell. No staining of reserve material occured with diluted iodine-potassium iodate solution.

Sudan III was used to stain lipid deposits. In cells of young cultures a lipid globule in the anterior part as well as in the posterior part of the cell could be stained. A number of smaller ones became visible scattered in the peripheral layer of some cells.

Neutral red was accumulated in some vesicles in the most posterior part, indicating acid contents.

Vital staining with Janus green made a varying number of mitochondria visible, most of them showed as short rods, but a few were dumbbell-shaped. In a few cells the chondriome appeared as a reticulate complex.

When diluted hydrochloric acid was applied, most cell constituents dissolved. The chromatophore became pale blue due to a reaction of a carotenoid pigment. This reaction should not occur in *Chrysomonadina*, according to HOLLANDE (1952).

The proteinaceous nature of the pyrenoid was demonstrated by a specific staining with prussian blue according to GURR (1965). A drop of an aged culture was soaked in potassium ferrocyanide in acetic acid for an hour. The acid gave the blue colouration of the chromatophore, like hydrochloric acid. The absence of this blue colour in the pyrenoid was checked before the addition of ferric chloride. A blue colouring of proteins results, notably in the cytoplasm and also in the pyrenoid. The latter was visible as a homogeneous sphere with a more saturated blue colour than the rest of the chromatophore.

4. ELECTRON MICROSCOPY

4.1. Material and methods

For direct preparations 12-day old cultures were used. The cells were fixed in glutaraldehyde. The method of DESJARDINS *et al.* (1969) had to be adapted to the seawater medium. Four volumes of the culture were mixed with one volume of 25% glutaraldehyde solution of 0 °C. The cells were allowed to sediment overnight in a refrigerator. The supernatant liquid was decanted and the cells were washed several times by resuspending them in sterile, demineralized water and concentrating them with the centrifuge at low speed. To prevent osmotic shocks the water was added and mixed drop by drop during the first wash. Finally the cells were resuspended in a small volume of water to get a convenient number of cells on the grid. Drops of appropriate size were put on a piece of clean dental wax. The size of these drops could be carefully adjusted. The drops were lifted from the dental wax by touching the top of them with the grids. A drop containing cells stuck to the grid and was dried overnight. Finally the preparations were shadowed with platinum under an angle of 8° while rotating. In these preparations the flagellar hairs were well preserved although sometimes separated from the flagella.

For sections two cultures were used, one 39 days old, the other 144 days old. The cells were fixed for 2 hrs 20 minutes in ice-cold glutaraldehyde, postfixed in osmium tetroxide overnight, and embedded in epon. A 4% glutaraldehyde solution was used, buffered with 0.1 M. sodium
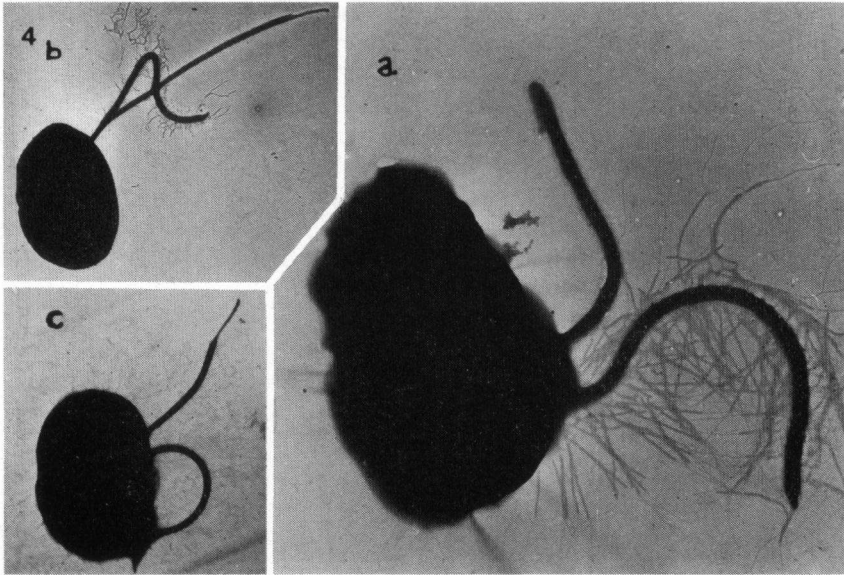
Fig. 4. Direct preparations of whole cells.
   a × 13,000
   b The smooth flagellum streched out during drying, × 4,000
   c × 6,500

cacodylate pH 7 and made isotonic to the culture medium by addition of 0.25 M sucrose (MANTON & PARKE 1965). The material was washed in a series of cacodylate buffer with decreasing sucrose concentrations. The osmium tetroxide was buffered with 0.1 M sodium cacodylate. Sections, cut on a Porter-Blum MT-1 microtome with glass knives and on a LKB microtome with a diamond knife, were either stained with diluted lead citrate (REYNOLDS 1963, SJÖSTRAND 1968) or double stained with uranyl acetate and lead citrate. Observations were made with Philips EM 100 B and Philips EM 300 microscopes.

4.2. General topography

The general topography of the internal structure of cells of young cultures is illustrated in *figs. 1, 5, 6* and *7*. The orientation of these sections is indicated in *fig. 3. Fig. 5* shows a section cut more or less longitudinally through the pear-shaped nucleus. The chromatophore encircles the internal organelles with its lobes. Profiles of the golgi apparatus, mitochondria and the large vacuole, hit tangentially, are also to be seen. A number of muciferous vesicles are lying between chromatophore and plasmalemma. In *fig. 6* the nucleus is cut transversely. In its vicinity the profiles of five narrow lobes are to be seen. The irregular vesicles surrounding the circular vacuole may be leucosin vesicles. On the left a lipid globule is hit. The plane of this section is perpendicular to that of the section of *fig. 5a*. A cross-section through the cell shows the insertion of both flagella (*fig. 5b*). The flagella protrude through an opening in the theca. The plane of this section is also perpendicular to the plane of the section of *fig. 5a*.
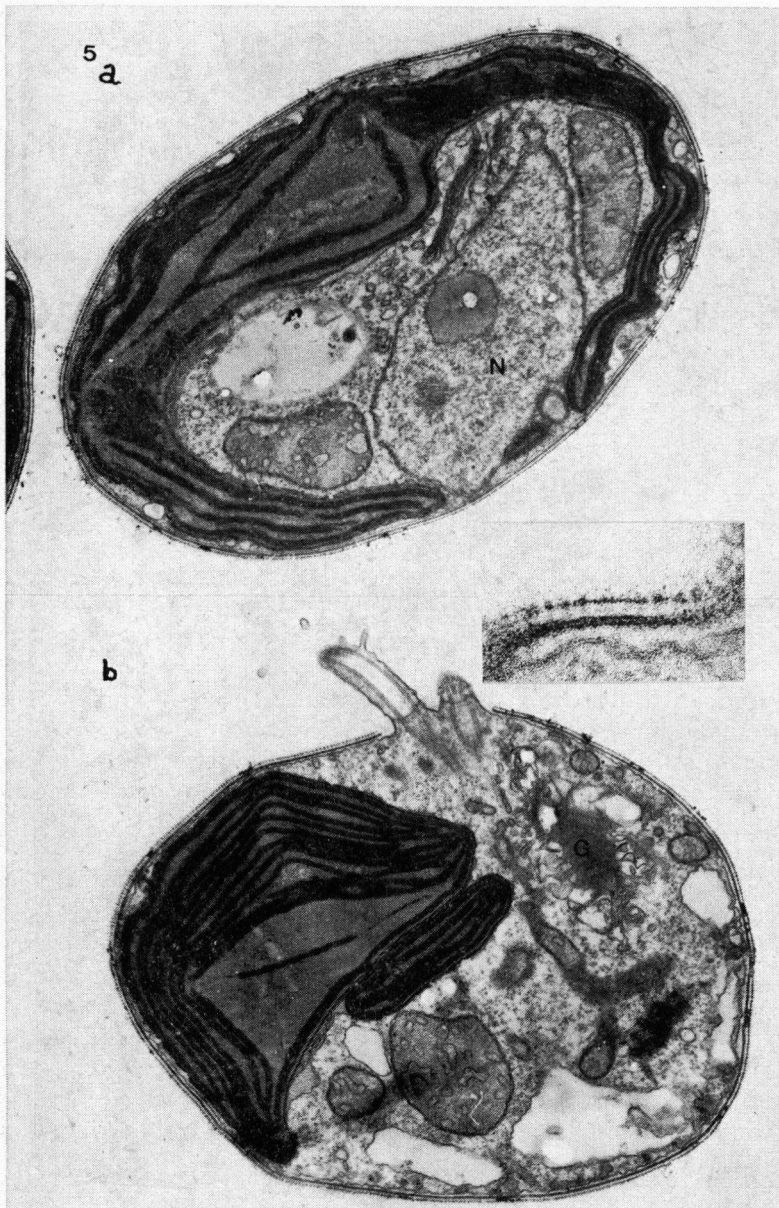
Fig. 5. General topography of the cell.
a Section about median longitudinal, the lobes of the chromatophore cut lengthwise, like the nucleus. × 22,000
b Transverse section, showing the proximal parts of the flagella. The lateral chromatophore is cut between the lobes. The golgi apparatus near the flagella bases is cut tangentially. A number of leucosin vesicles lies in the peripheral part of the cell. × 24,000. Inset: Theca. × 112,000.

Fig. 6. Section latero-laterad, showing the lobes of the chromatophore cut transversely, like the nucleus. One large vacuole and numerous small ones are situated in the posterior half of the cell. × 21,000.

The central part of the chromatophore is parietal. There are no lobes encircling the internal organelles, but one lobe of the chromatophore seems to be folded back against the pyrenoid region. It is enclosed in the same chromatophore envelope. Profiles of mitochondria and leucosin vesicles and the tangentially cut golgi body are also to be seen.

4.3. Flagella

The flagella bases touch each other at the proximal ends and diverge at the distal ends. The flagella thus arise about 0.1 μ from each other. A bundle of microtubuli is running longitudinally alongside the nucleus, in the same direction as the basal body from which it originates and penetrates deeply into the cell. A number of rows of microtubuli project from the proximal part of the flagella bases obliquely upwards to the plasmalemma. Other rows of microtubuli seem to run in various directions. A distinct, cross-banded root could not be traced.

The tip of the long flagellum is conical. The small flagellum is of the whip-lash type, ending in a prolonged narrowed tip.

The flagellar appendages are illustrated in *fig. 8*. There are two types of appendages on the long flagellum. A very thin one measuring up to 1,5 μ in length and about 5 mμ thick is comparable to the evanescent threads found by BROWN e.a. (1968) on *Chlamydomonas* flagella during mating. These very thin appendages are intermingled between the much more conspicuous filiform appendages of the chrysophycean type, with a hollow shaft 0,9 μ long and 24 mμ thick, and decorated with a double helix as described by BRADLEY (1965),
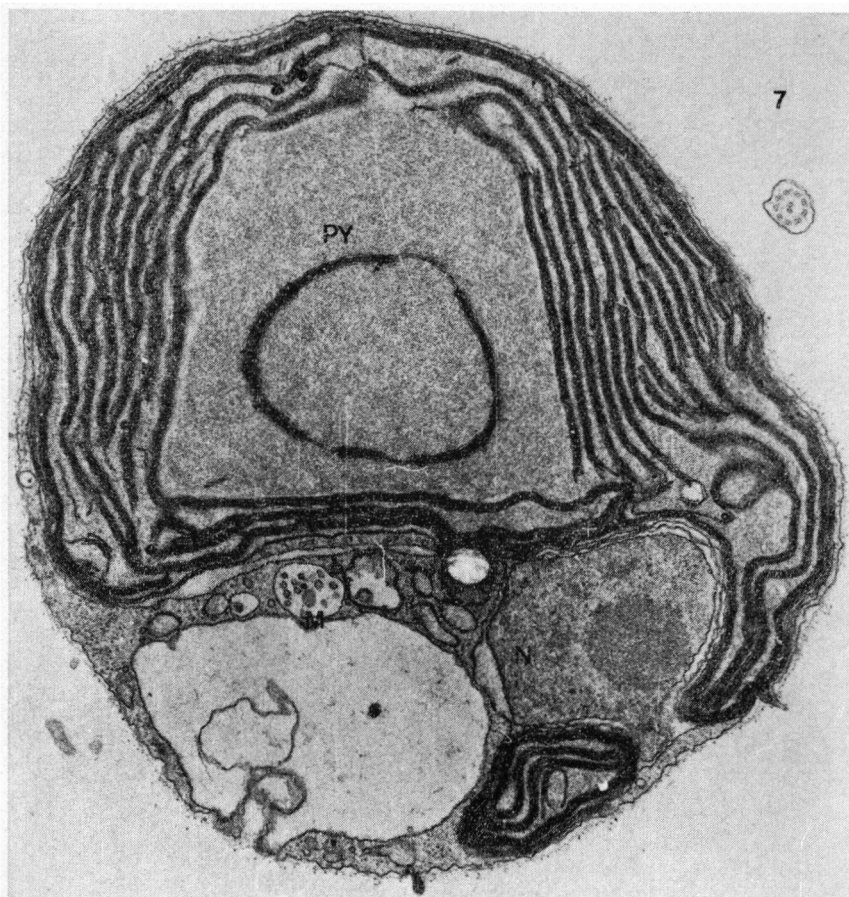
Fig. 7. Transverse section of an old cell, without a theca but having a large pyrenoid (PY). (N) nucleus, (M) multi-vesicular body. × 22,000.

SCHNEPF & DEICHGRÄBER (1969), LEADBEATER (1969) and MASSALSKI (thesis quoted by Leadbeater). The shaft ends abruptly and bears a very thin hair excentrically inserted on the tip. The hair is composed of a proximal part 0,7 μ long and 10 mμ thick, and a distal part still thinner and slack, 0,3 μ long, 6 mμ thick. The shaft itself is inserted on a swollen base that may be an evagination of the plasmalemma of the flagellum.

4.4. Chromatophore

Fig. 9 demonstrates the membranous structure of the chromatophore. The outer two membranes belong to the chromatophore envelope, a fold of endoplasmic reticulum completely enclosing the chromatophore. The two membranes immediately below this envelope form the double membrane limiting the chromatophore itself. Then come six membranes belonging to a girdle lamella enclosing
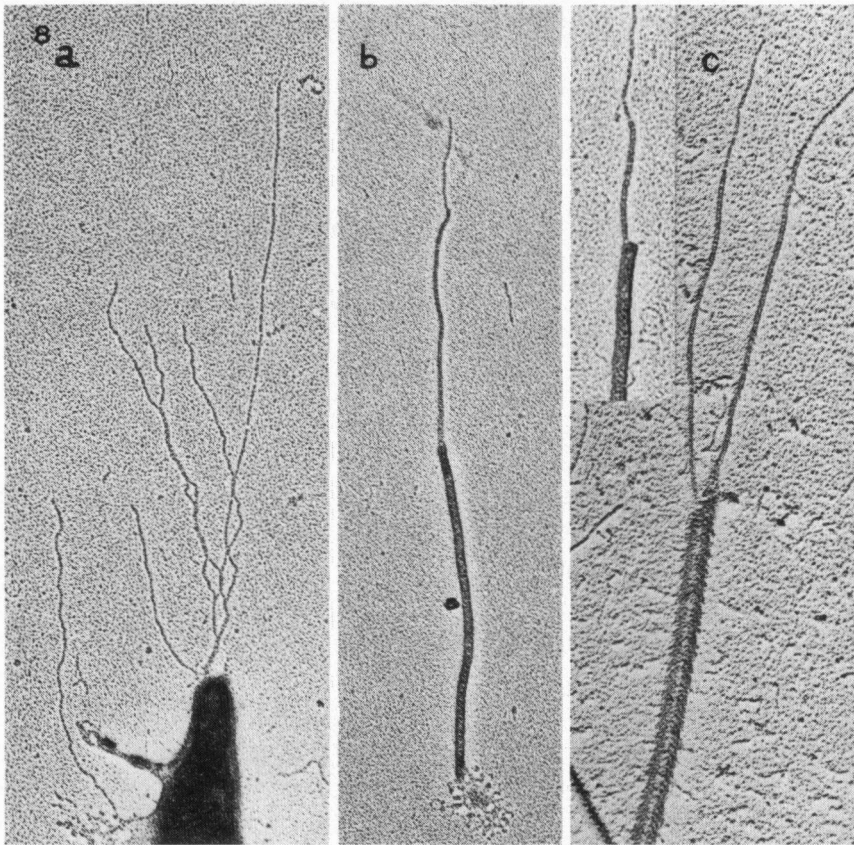
Fig. 8. Appendages of the pleuronematic flagellum.
   a Tip of the flagellum with very thin hairs. × 55,000
   b Loosened chrysophycean type of appendage, the swollen basement exploided. Three parts can be distinguished by their diameter. × 50,000.
   c Two hairs lying with the shafts side by side. The shafts show a spiral decoration. × 61,000
   Inset. Tip of a shaft on which the thinner ending is attached excentrically. × 64,000.
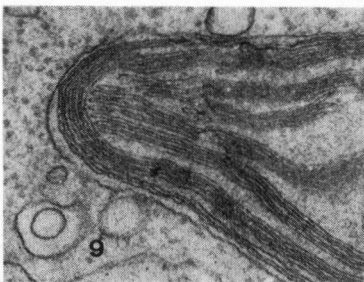

Fig. 9. Membraneous structure of the chromatophore. × 55,000

all other lamellae. The lamellae are composed of three thylakoids. There are relatively large distances between the thylakoids of one lamella, a condition that is known to occur in several algae. It may however depend on fixation and physiological differences between individual cells (MASSALSKI & LEEDALE 1969). Ribosome-like granules are found in the stroma.

In the cells of the aged culture the lamellae tend to break up into short pieces which aggregate into bundles. These alterations of the chloroplast structure were also found by BROWN, JOHNSON & BOLD (1968) in the hypnozygote of *Chlamydomonas moewesii*. Perhaps these alterations are due to the aging of the cells in both cases.

There is a system of interconnected tubules, the periplastidial reticulum, lying between the concave side of the chromatophore and the chromatophore envelope. It is of limited extent and only in a few sites more than one layer thick. The same structure occurs in *Chrysophyceae* as well as in *Xanthophyceae* (BELCHER 1967, MASSALSKI & LEEDALE 1969). It is not the same as the system of vesicles described by SHUMWAY & WEYER (1967) in the maize chloroplast. The latter system of vesicles lies within the chloroplast itself.

On the concave side of the chromatophore the chromatophore envelope is widened and contains a large number of parallel fibres. These fibres are 12 mμ thick and 5–15 mμ apart. Fibres in the chromatophore envelope are found in a number of *Chrysophyceae*. BELCHER & SWALE (1967) found them in *Chromulina placentula*, BELCHER (1969) in *Chrysococcus rufescens*, and LEADBEATER (1969) in *Olisthodiscus luteus*. They are, however, not restricted to this class of organisms. MANTON *et al.* (1965) found a ring-shaped bundle of fibres round the nucleus of *Heteromastix*, where the nuclear envelope and the chromatophore envelope coalesce.

In our material the continuity of the nuclear envelope and the chromatophore envelope exists everywhere the nucleus and the chromatophore meet, even if only a lobe of the latter is involved. The pyrenoid is traversed by one or two, mostly curved, lamellae that consist of three thylakoids each. The pyrenoids of most algae that were hitherto studied in this respect contain lamellae with a reduced number of thylakoids, or no lamellae at all. The occurrence of non-reduced lamellae in the pyrenoid was described by GIBBS (1962a) in *Olisthodiscus* and MASSALSKI & LEEDALE (1969) in *Bumilleria*, to mention only *Chrysophytes*.

In the cells from an aged culture the pyrenoids may contain a tube-shaped lamella, appearing as a ring in cross sections. The pyrenoid of these cells are much thicker as in the cells of the young culture. This may be due to the accumulation of storage material. The function of storing is also attributed by MANTON & PARKE (1959) to the pyrenoid of *Chrysochromulina strobilis*, which they describe in the diagnosis as "an evident internal storage region". The possibility of storage or synthesis of proteins in the pyrenoid core is expounded comparatively by MANTON in 1966. If the pyrenoid of *Ankylonoton luteum* is an internal storage region, the chromatophore synthesizes at least two storage products, a proteinaceous one that accumulates in the pyrenoid, and leucosin or its precursor that is accumulated outside the chromatophore in the leucosin vesicles.

Fig. 10. Chromatophore of old cell.
a The lamellae broken up and aggregated. The plastidial membrane becomes thus more conspicuous as a double line. × 34,000
b Detail enlarged to show the cross-sectioned fibres inside the chromatophore envelope. × 96,000

4.5. Nucleus and golgi apparatus

The nucleus is conical or pear-shaped and often somewhat irregular, in its broadest part half as wide as long. The apex is deformed to give room for the flagella bases. Tubules of smooth endoplasmic reticulum are connected with the nuclear envelope. The nucleolus seems to be bound by a membrane. The nucleus of *Olisthodiscus luteus* is also pear-shaped with its apex pointing to the flagella bases, but in that organism the apex of the nucleus is completely surrounded by a cone of microtubuli and a ring-shaped golgi complex (LEAD-BEATER 1969). This is not the case in *Ankylonoton luteum*.

The dictyosome, likewise situated next to the apex of the nucleus, is of the classical type in cells of young cultures and more horseshoe-shaped in sections of cells of aged cultures, its shape being modified by the room available for it. It consists of a stack of 5 to 9 cysternae (*fig. 11*). The leucosin, or its precursor, originating in the chromatophore, has to pass through the chromatophore envelope in its way to the leucosin vesicles. There is a faint indication that the regenerating cysternae of the dictyosome orginate from vesicles which are pinched off from the chromatophore envelope (*fig. 11b*). Perhaps these vesicles are "sluicing" leucosin or its precursor from the chromatophore envelope to the golgi apparatus, from which it then goes to the leucosin vesicles. There is no contradiction between this and the current theory about the relations of the golgi apparatus and the endoplasmic reticulum, for the chromatophore envelope belongs to the endoplasmic reticulum, just as the nuclear envelope with which it is continuous.

4.6. Vacuoles

Several leucosin vesicles may be found, mostly in the peripheral layer of the cytoplasm between the much smaller muciferous vesicles. The leucosin vesicles are empty, unlike the muciferous vesicles, because leucosin dissolves during the preparative process. The leucosin vesicles may coalesce and form a large, flattened, peripheral vesicle, the outline of which is influenced by the surrounding organelles. In the cells of old cultures the leucosin vesicles become distended.

The large spherical vacuole has a prominent membrane in accordance with its stability under mechanic stress. In the cells of the young culture it contains an electrodense body with vague outlines. This body can be very complex (*fig. 12*). Part of it seems to be of a lipid nature.

In the cells of the aged cultures this body had disappeared, and the vacuole was crowded with various types of granules, which could be classified in three groups (*fig. 10*). The first group is formed by a large number of small vesicles filled with a substance that appears grey on the photographs. These vesicles closely resemble those in the multivesicular bodies shown in *fig. 7*. A second type is formed by multi-membraned vesicles. These are of larger dimensions. They appear empty in the photographs. The membranes are not concentric, the inner one may form a polygon. The third group is formed by vesicles with clusters of electrodense globules enclosed in one or more membranes. Besides these groups there are sometimes larger membranous inclusions.
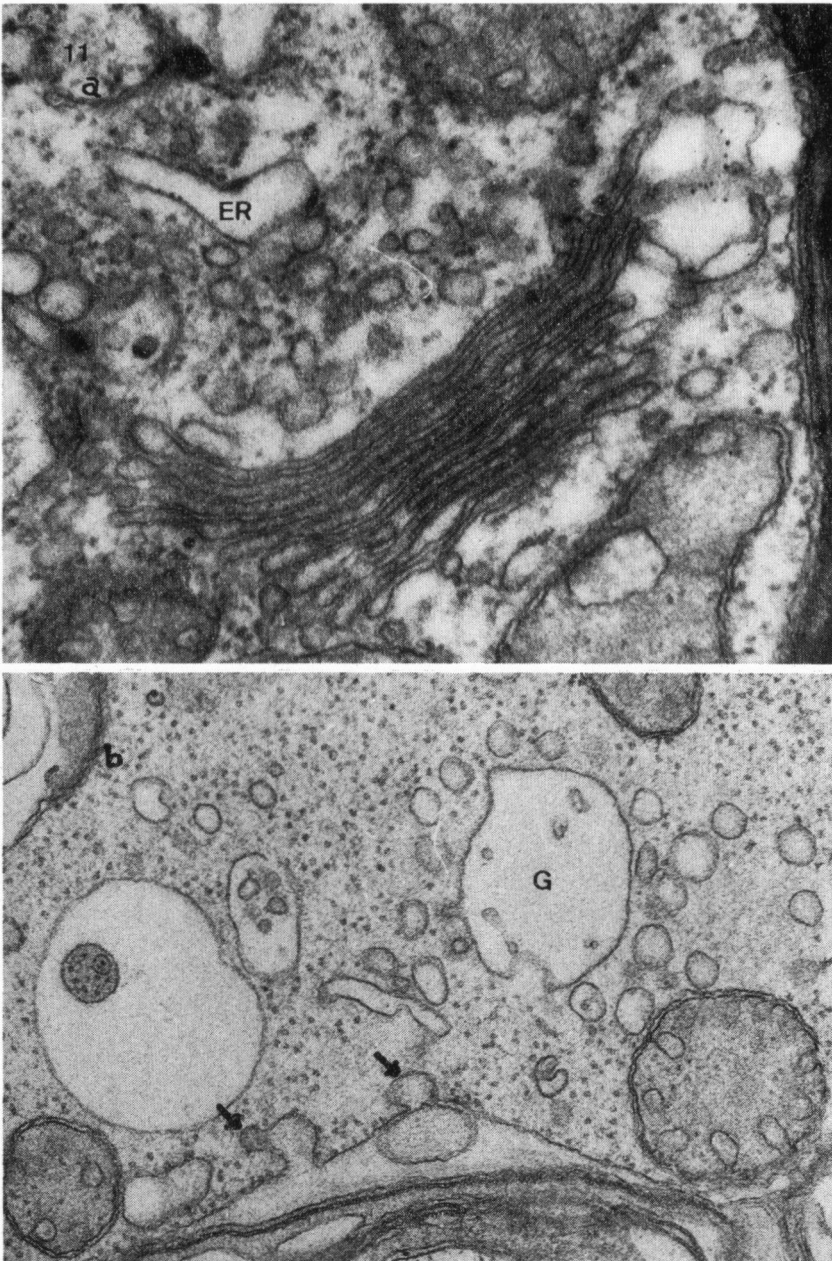
Fig. 11. Golgi apparatus.
    a Golgi body cut transversely. There is very little endoplasmic reticulum in its vicinity
    except that of the chromatophore envelope. × 62,000
    b Microvesicles seem to become pinched off from the chromatophore envelope
    (arrows), and are clustering around what may be a golgi cistern (G). × 50.000

Fig. 12. Large vacuole containing composite electron dense body. × 30,000
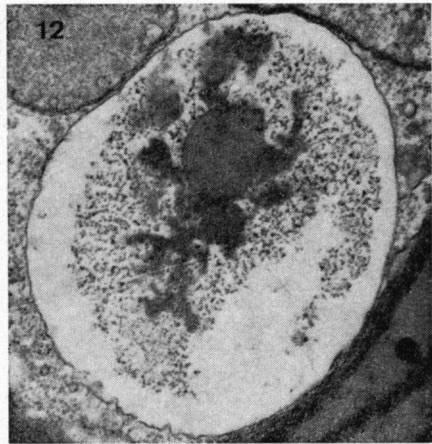
With the exception of the first group the contents of the vacuole may be the remains of autodigested cell components. Thus this vacuole seems to function in young cells as a storage of lipid and other substances, and in old senescent cells as a lysosome. The same phenomenon was observed by SPICHIGER (1969) in the sphaerosomes of the endosperm of germinating tobacco seeds.

4.7. Mitochondria

The mitochondria have bag-shaped invaginations of the inner membrane. These invaginations are relatively short tubules, the central part of the mitochondria sometimes being void of them. They are somewhat constricted at the junctia with the inner mitochondrial membrane. Some mitochondria at least are rod-shaped and can be several μ long. The chondriome may form an irregular reticulate complex. Vital staining with Janus green suggests that this condition occurs in a minority of cells.

4.8. Theca

The cells of the young culture are surrounded by a theca, composed of two electron opaque uninterrupted smooth layers, and on the outside a third layer consisting of tiny bodies, arranged in a more or less orthogonal pattern. In some places they can be seen to be grown together sidewise. The middles of the uninterrupted layers are 6–10 mμ apart, the distance between the middle layer and half way through the outmost layer being 15–21 mμ. The theca is mostly closely fitting, being only 20–60 mμ from the plasmalemma, but in a few cells the protoplast seems to be retracted from it. There is no continuity of the theca with the plasmalemma as in *Pavlova mesolychnon* (VAN DER VEER 1969). The two smooth layers appear much more heavily stained in the photographs than the plasmalemma. The layers are so strikingly equidistant that it is suggested that they are held together by definite layers of an electron pellicular substance.

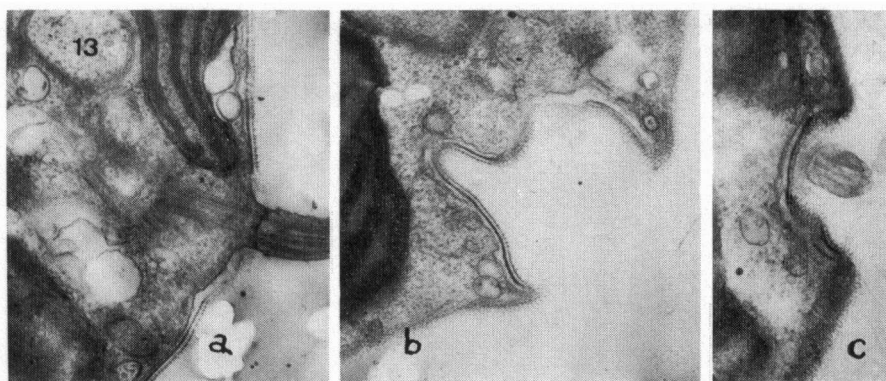The chemical composition, mode of origin, and fate during cell division of the theca are still completely unknown.

Fig. 13. Transverse sections through the furrow, all × 20,000
   a Section of the flagella bases, just outside the furrow.
   b Part of the furrow that runs between keels.
   c Section farther away from the flagella insertion.

5. TAXONOMIC POSITION

The new species does not fit readily into any of the genera encountered in the literature. But there are a number of organisms with the same general appearance, two ventrally inserted flagella and a single chromatophore.

The taxonomic position of some of them, *Heteromastix* Korshikov 1923 and *Nephroselmis* Stein 1878, has recently been clarified (MANTON *et al.* 1965; PARKE & RAYNS 1964). They belong to the *Prasinophyceae* sensu Parke. Our material cannot be related to them. The flagella of the *Prasinophyceae* are not heterokont and their reserve material is a starch.

In the class of the *Cryptophyceae* there are two genera described with an even stronger resemblance to the new species. *Protochrysis* PASCHER 1911 without a gullet is one of them. Nothing is known about the fine structure of the two species of *Protochrysis*, the type species *P. pheophycearum* PASCHER 1911 and *P. vinosa* CONRAD 1939, because the cultures of them did not survive into the era of electron microscopy. The two rows of refringent bodies lining the furrow, interpreted as trichocysts by HUBER-PESTALOZZI (1962) and BUTCHER (1967), and the starch-like reserve material round the pyrenoid seems to warrant their place among the *Cryptophyceae* and to rule out the affinity of the new species to them.

The other member of the *Cryptophyceae* which has to be taken into account is *Sennia parvula* SKUJA 1948, now named *Hemiselmis parvula* (Skuja) Butcher. There exists a careful drawing of it by ETTL (1960) to which our species shows a striking resemblance. Skuja described however a furrow with a gullet – 'sulcus' respectively 'fauces'. The new species lacks a gullet.

Among the *Chrysophyceae* there are a number of genera described which have two flagella of about equal length and as far as known both smooth. These genera are grouped together in the *Isochrysidaceae*. Nowadays they are provisionally transferred to the class of the *Haptophyceae*. One of them, *Erkennia*

SKUJA 1948, was placed among the *Ochromonadaceae* by BOURELLY (1966) solely on account of the heterodynamic movements of the flagella, the fine structure being still unknown. Heterodynamic movement of the flagella is however not enough to infer a heterokont structure. *Prymnesium* for instance, a *Haptophycea*, with two smooth flagella can also move heterodynamically. *Erkennia* Skuja has two widely spaced chromatophores in contrast to the new species, which has one.

A related organism, *Diacronema vlkianum* Prauser 1911, with two unequal ventrally inserted flagella and a single chromatophore, thus even more like our material, has recently been studied with the electron microscope by FOURNIER (1969). It proved to be a member of the *Haptophyceae*.

It seems to be fruitless to try and incorporate the new organism in an already described genus outside the heterokont classes. Within these heterokont classes, characterized by a pantonematic flagellum accompanied by a smooth flagellum, we have the choice between the *Chrysophyceae* sensu Christensen and the *Xanthophyceae*. The motile cells in the *Bacillariophyceae* have, as far as known, only one flagellum (MANTON & VON STOSCH 1966). The *Phaeophyceae* have pyrenoids which are not penetrated by lamellae (EVANS 1966). For this reasons these two classes, in which no flagellate cells are described as the dominant phase in a cycle, are excluded.

A prolonged narrowed tip of the smooth flagellum is encountered in the zoids of different *Xanthophyceae*. It is depicted in the drawings of VLK (1931, 1938) and in the electronmicroscopic photographs of MASSALSKI & LEEDALE (1969). The smooth flagellum of the *Chrysophyceae* should be truncated, lacking the hairpoint according to VLK (1938) and this is confirmed by the electron microscopic observations of PITELKA & SCHOOLEY (1955) on *Ochromonas malhamensis*, and MANTON & LEEDALE (1961) and LUCAS (1968) on members of the genus *Paraphysomonas*. The smooth flagellum of *Synura caroliniana* Whitford has a gradually tapering tip (MANTON 1955).

The yellow chromatophore suggests a taxonomic position within the *Chrysophyceae*. That would be within, or in the vicinity of, the genus *Ochromonas*. The few species of *Ochromonas*, that have been studied with the electron microscope, have a naked plasmalemma in contrast to the three-layered theca in our material (JOYON 1963, GIBBS 1962b). The blue colouration under influence of hydrochloric acid, in combination with the heterokont flagella, is supposed to be characteristic for the *Xanthophyceae*, being absent in *Chrysophyceae* (HOLLANDE 1952).

In the general features of the ultrastructure of the *Chrysophyceae* and the zoids of the *Xanthophyceae* are small differences which enable us to separate these classes. MASSALSKI & LEEDALE (1969) described a relation between the nuclear envelope and the golgi apparatus in the zoids of *Tribonema* and *Bumeriella*, FALK (1967) in *Botrydium granulatum*. Small vesicles are pinched off from the nuclear envelope in the direction of the golgi body. In our organism this type of vesicle seems to be pinched off from the chromatophore envelope, part of the endoplasmatic reticulum. SCHNEPF & DEICHGRÄBER (1969) could only

rarely find micro-vesicles between nuclear envelope and golgi apparatus in the Chrysophycean species *Synura petersenii.*

It appears that the knowledge of the fine structure of the *Chrysophyceae* and the *Xanthophyceae* is still too fragmentary to be helpful for the incorporation of the new species into one of these classes. Comparing the new species with the flagellate members of the *Chrysophyceae* and *Xanthophyceae* lumped together, the closest resemblance is found to exsist with the species *Ankylonoton pyreniger* PASCHER 1932 from which it differs in the preponderance of the yellow pigments, the relative lengths of the flagella that are less unequal, and the stronger lobation of the chromatophore. The furrow of the new species is more like that described by Pascher in *Botrochloris longeciliata,* but this organism lacks a pyrenoid (PASCHER 1939). For these reasons the new species is placed in the genus *Ankylonoton.*

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