

## STUDIES ON MARINE FLAGELLATES

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(Plates I and II, and Text-figs. 1-73)

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## INTRODUCTION

During the years 1935 to 1940 experimental work was carried out at the Port Erin Marine Biological Station on the rearing of the larvae of the European flat oyster, *Ostrea edulis* L. At first, algal zooids and the naturally occurring microplankton in the sea water were used as sources of food for the larvae, but later the larvae were fed on pure cultures of flagellates with much more successful results (Bruce, Knight & Parke, 1940).

Six of the flagellates used in the experiments are here described. Three were isolated by Dr F. Gross at the Plymouth Laboratory (temporary labels 'B', 'C' and 'H', Gross) and three were isolated at Port Erin (temporary labels 'D', 'E' and 'I', Parke). Cultures of four of these flagellates are still maintained at the Plymouth Laboratory but the cultures of 'C' and 'H' were lost in 1941 when the laboratory was damaged.

## ACKNOWLEDGEMENTS

My most sincere thanks are due to Prof. F. E. Fritsch and Prof. E. G. Pringsheim for their helpful criticism of part of the work. I am most grateful also to Mr W. M. S. Russell, who translated my descriptions of the organisms into Latin and who suggested the name *Dicrateria* for one of the new genera.

## CHLOROPHYCEAE

## POLYBLEPHARIDACEAE

*Pyramimonas* Schmarda

*P. grossii* n.sp. (Flagellate 'H', Gross.)  
(Pl. II, figs. 17, 18; Text-figs. 1-12.)

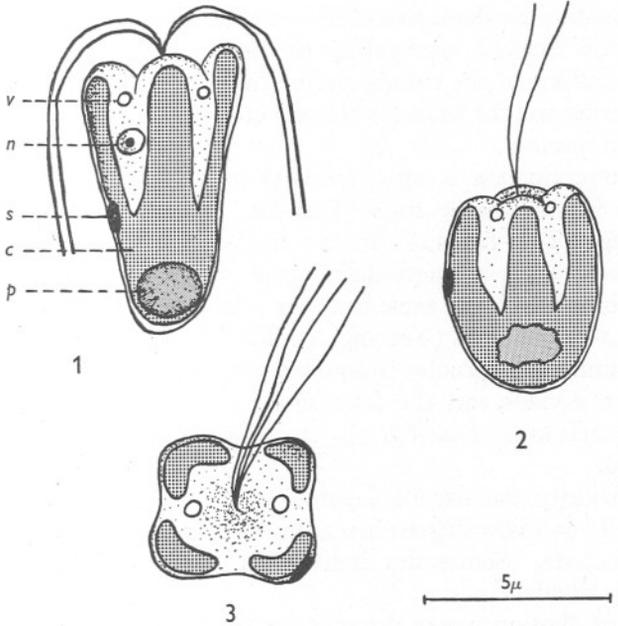
Cellula parva, pyramidiformi plerumque ac 4 lobis distinctis anterioribus praedita; quae transverse secta nisi ad apicem extremum formam praebet circularem; forma mutabili; 4 flagellis longitudine cellulae fere paribus in depressione mediocriter tenui ad apicem insertis; chromatophoro luteo viridi caliciformi cum 4 lobis anterioribus in margine dispositis et magno pyrenoide basali; amyli capsula pyrenoidi circumdata conspicua; stigmatate singulo, mediano plerumque, rarius anteriore; nucleo anteriore plerumque ac laterali; 2 vacuolis ad apicem sitis, haud contractiliis; cellula  $5.5-8\mu$  longa, lata  $4.5-5.5\mu$ . Ab nomine Doctoris F. Gross appellata, per quem segregata est.

This species was isolated by Dr F. Gross from a plankton sample taken from the sea water off Plymouth. In his records the first culture of 'H' was dated April 1936.

This species is normally inversely pyramidal, sharply truncate and four-lobed at the anterior end and somewhat tapering towards the posterior region (Text-fig. 1). As the periplast admits of extensive change of shape, transitions to shorter more or less cordate forms are frequent and longer, more subcylindrical forms also occur (Pl. II, figs. 17, 18). The lobes are confined to the anterior end of the cell and are continued backwards as ridges for only a short distance, so that an individual is circular in transverse section except at the extreme apex. Here the swelling of the lobes gives a transverse section the appearance of a square which has had the corners rounded and the sides indented (Text-fig. 3).

The anterior lobes surround a fairly shallow depression, from the centre of which arise four flagella, all originating from one point. The flagella are equal to, or slightly longer than, the length of the cell. No difference in the appearance of the four flagella could be detected when the staining methods of Loeffler (1889) and Fischer (1894) were used. The four are equal in length and thickness, and show no tapering towards the free end, but end quite bluntly. Movement of the organism is spasmodic and extremely rapid, but the organism can remain constant in direction for relatively long periods.

The chloroplast, bright yellowish green in colour, is cup-shaped with the sides deeply cleft into four lobes (Pl. II, figs. 17, 18). The thickened basal part of the chloroplast, behind the lobes, fills the posterior third of the body, occupying the whole periphery of the cell. It has a smooth outline since no posterior lobes or indentations are developed. Each lobe of the chloroplast passes forwards into one of the body lobes and is smooth at the apex, not indented. A large pyrenoid is situated in the thickened posterior portion of



Text-figs. 1-3. ( $\times 5000$ .) *Pyramimonas grossii* n.sp. 1. Normal shape of young motile stage, side view. c, chloroplast; n, nucleus; p, pyrenoid; s, stigma; v, vacuole. 2. Biflagellate stage, side view. 3. Young motile stage viewed from anterior end.

the chloroplast below the fusion of the lobes, usually at the extreme posterior end of the cell (Text-fig. 1). The pyrenoid is surrounded by a well-developed starch sheath.

A bright red stigma, projecting slightly above the general surface of the organism, is usually median in position (Text-fig. 1), but has frequently been observed at the extreme anterior end on a line with two non-contractile vacuoles which occur in the general cytoplasm within the chloroplast. The nucleus is visible in the living individual and is situated anteriorly in the body in a lateral position (Text-fig. 1).

Approximately 10% of the motile stages in a culture possess only two flagella. These individuals are smaller ( $4-5\mu$ ) than the individuals with four flagella and practically spheroidal in shape with the anterior lobes less well developed (Text-fig. 2). The nature and function of the biflagellate individuals

have not been determined but, as Pascher (1927) has suggested for other genera, they may possibly be the 'basic' form, and the more frequent individuals with four flagella can then be regarded as double cells resulting from the early multiplication of the flagella prior to division. Fusion of the bi-flagellate individuals was never observed.

The motile individuals are very strongly phototactic and therefore tend to collect on one side of the culture flask. Here they readily assume the 'palmella-state'; the flagella are lost and the cells become spheroidal, developing a thin hyaline gelatinous envelope measuring 6-8  $\mu$  in diameter (Text-fig. 11). When numerous cells become aggregated together they become very irregular in shape. The outline of the chloroplast becomes indistinct but the stigma and pyrenoid persist and the vacuoles become more apparent, the latter frequently increasing in number.

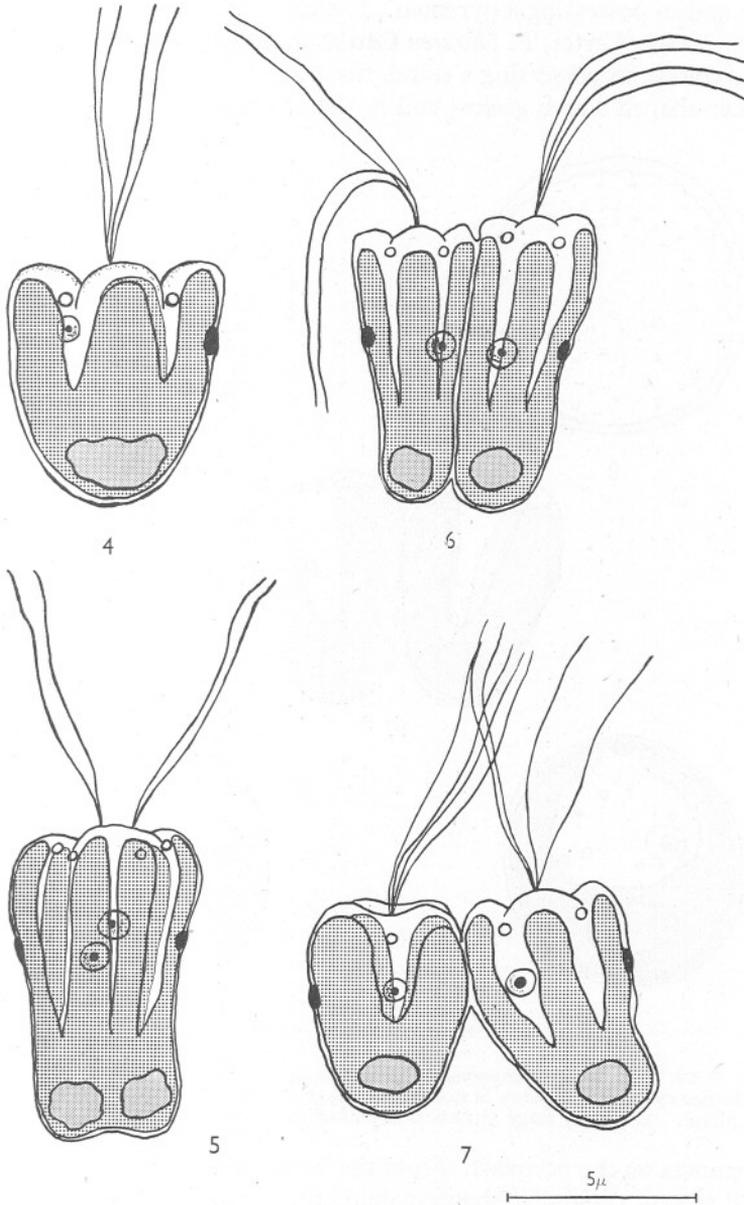
Asexual reproduction is most frequent in the motile individuals, but it occurs also in non-motile cells (Text-fig. 10), and in individuals in the palmella-stage (Text-fig. 12). In the motile individuals it takes place by simple division along a longitudinal plane. When about to divide the cells increase in breadth; at the same time the pyrenoid shows signs of elongation transverse to the cell axis (Text-fig. 4). The pyrenoid then divides into two parts, the number of vacuoles is doubled and a second stigma develops. The nucleus then divides and the four lobes of the chloroplast divide by the splitting of each lobe (Text-fig. 5). Occasionally the nucleus divides before the pyrenoid.

In the majority the flagella separate as the cell broadens, two to each daughter-cell; two new flagella are then developed by each daughter-cell as division proceeds. Sometimes individuals bear eight flagella before the pyrenoid has divided.

The line of division passes through the thickened posterior portion of the chloroplast so that each part has one complete, now divided into two, and two half lobes of the original chloroplast (Text-fig. 6). In *P. delicatulus* Griffiths two complete lobes pass to each daughter-cell. Separation of the two daughter-cells takes place by gradual constriction, starting simultaneously at both the anterior and posterior ends of the individual but advancing more rapidly from the posterior pole (Text-fig. 7). The periplast divides simultaneously with the rest of the protoplast and is gradually completed on adjacent surfaces of the new individuals. The daughter-cells are commonly of unequal size.

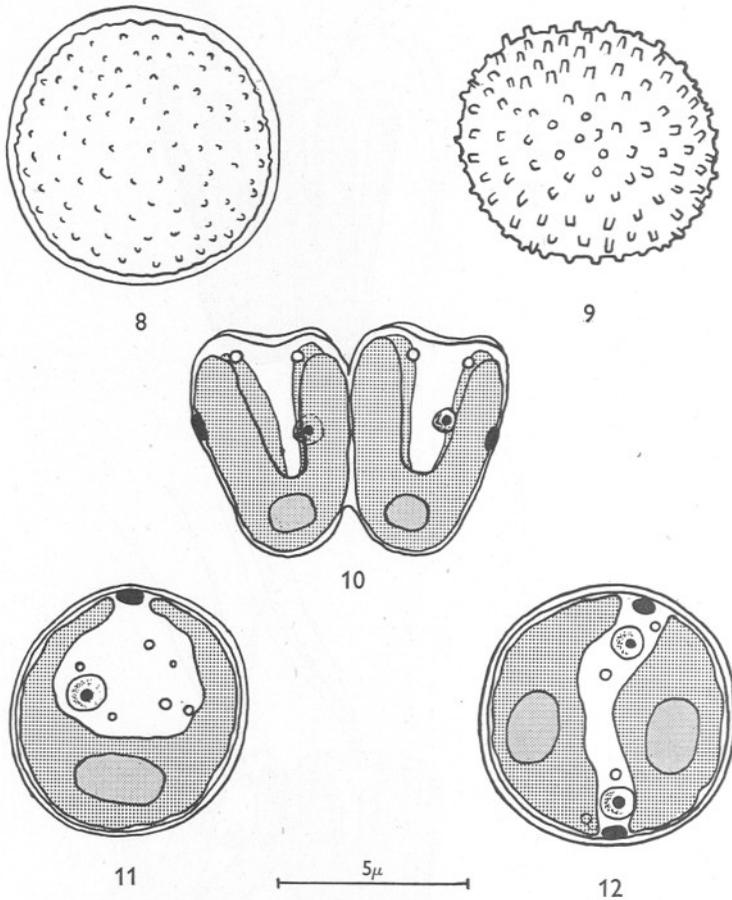
Hypnospores or cysts have been observed in the cultures (Text-figs. 8, 9). They are formed either from ordinary motile cells after the flagella have been withdrawn or from individuals already in the palmelloid phase. The cysts are usually spheroidal in shape, sculptured externally and measure 6-7  $\mu$  in diameter. The release of the contents of the cysts has not been observed.

From the marine species so far described, *P. adriaticus* Schiller (1913), *P. oltmannsi* Schiller and *P. impressus* Schiller (1925), *P. grossii* differs both



Text-figs. 4-7. ( $\times 5000$ .) *Pyramimonas grossii* n.sp. 4. Large motile stage just prior to division, side view. 5. Early division stage of motile individual, side view. 6. Later division stage of motile individual, side view. 7. Two daughter-cells just prior to separation, side view.

in shape and in possessing a pyrenoid. It differs also from the brackish-water forms *P. obovata* Carter, *P. olivacea* Carter and *P. angulata* Carter (1937) in shape, in colour, in possessing a starch sheath around the pyrenoid instead of two saucer-shaped starch grains, and in the absence of longitudinal series of



Text-figs. 8-12. ( $\times 5000$ .) *Pyramimonas grossii* n.sp. 8. Early stage in cyst formation. 9. Mature cyst. 10. Fission in non-motile stage, side view. 11. Individual in palmelloid phase. 12. Early stage in fission in palmelloid phase.

minute puncta on the periplast. From the brackish-water *P. octociliata* Carter (1937) *P. grossii* differs in shape, colour, number of flagella, in possessing a stigma and in lacking puncta on the periplast. *P. grossii* also differs in shape and size from the six species described by Pascher (1932).

It differs also from *P. utrajectina* Bretschneider (1925) and *P. ovalis* Conrad (1930a) in size, shape, development of the anterior lobes, and in the size, shape and position of the stigma. *P. botryodes* Jane (1944) is clearly distin-

guished from *P. grossii* by the rounded prominences on the surface of the cell and by the shape of the chloroplast.

*P. grossii* is somewhat similar in shape to *P. tetra-rhynchus* Schmarda (1850), *P. delicatulus* Griffiths (1909), *P. inconstans* Hodgetts (1920), and *P. montana* Geitler (1925). From all these species, however, it differs in its smaller size. In addition, it differs from *P. tetra-rhynchus* in the number of the anterior incisions in the chloroplast, in the position of the stigma and in the absence of stroma starch grains. It lacks also the hollowing at the posterior end that occurs in *P. delicatulus* and possesses a stigma and vacuoles which are lacking in that species. *P. grossii* differs from *P. inconstans* in having a lobed chloroplast and in the degree of development of the body lobes. From *P. montana* it differs in colour, in the absence of stroma starch and also in the development of the apical lobes.

### CHRYSOPHYCEAE

#### CHROMULINACEAE

##### *Chromulina* Cienk.

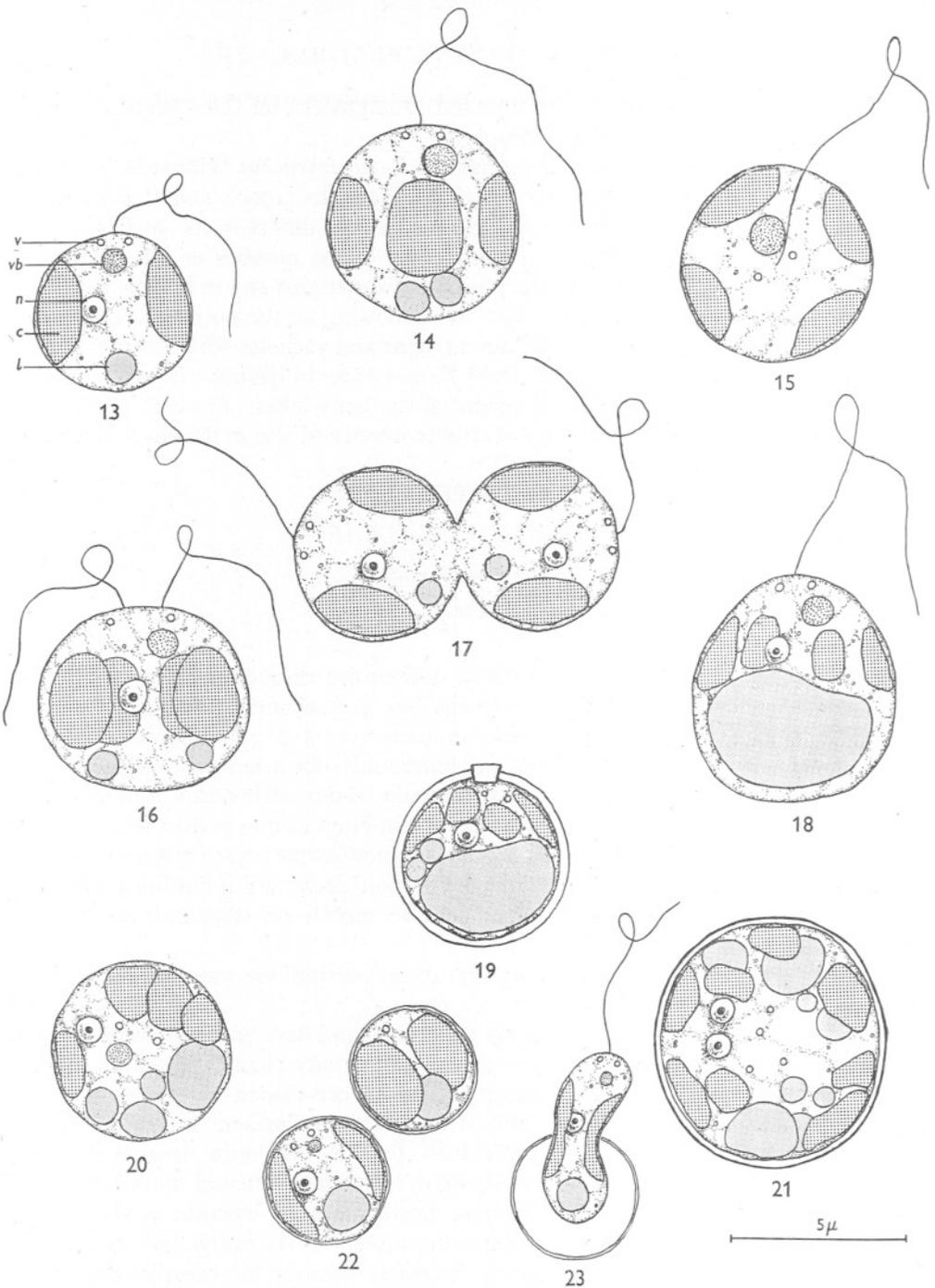
*C. pleiades* n.sp. (Flagellate 'E', Parke.)  
(Pl. I, fig. 2; Text-figs. 13-23.)

Cellula parva, sphaeroidi, periplasto differentiae carente praedita; 2 chromatophoros cellulis recentibus, vetustioribus 4 praebentibus fulvos pallidos crateriformes; flagello singulo cellulae diametro 1.5-2 plo longiore; nucleo fere centrali; 2 vacuolis parvis haud contractilibus ubi insertum est flagellum fere sitis; 'vacuolo' bene definito cui granula insunt refringentia vibrantia in cellula mobili plerumque visendo prope polum illum ex quo existit flagellum; sine sexu propagatur per gradus mobiles immobilesque; cystis sphaeroidibus subovatisve cum membrana externa laevi; non deest multiplicationis genus palmelloidem per gradum effectae; cellulae mobili est diametros 3.5-6 $\mu$ ; cysto diametros 5-6 $\mu$ .

This species was isolated at Port Erin from 'outside' sea water in February 1939.

The individuals of this species are spheroidal and have an undifferentiated, smooth, firm periplast, which does not admit of any change of shape; they measure from 3.5 to 6 $\mu$  in diameter. The saucer-shaped chromatophores, pale golden-brown in colour, are parietal; two are present in the smaller individuals, but in the larger individuals four is the more usual number (Text-figs. 13, 14). There is a medium-sized nucleus situated more or less centrally in the body and one or more small masses of leucosin at the pole opposite to that from which the flagellum arises. As the individuals increase in size the amount of leucosin also increases until it fills nearly half the volume of the cell (Text-figs. 13, 14 and 18). No extrusion of the leucosin masses has been observed.

The flagellum, one and a half to twice the cell diameter in length, is delicate



Text-figs. 13-23. ( $\times 5000$ .) *Chromulina pleiades* n.sp. 13. Young motile stage, equatorial view. *c*, chromatophore; *l*, leucosin; *n*, nucleus; *v*, vacuole; *vb*, vibrating body. 14. Older motile stage, equatorial view. 15. Older motile stage, viewed from pole at which flagellum is inserted. 16. Early division stage of motile individual. 17. Later division stage of motile individual. 18. Older motile stage containing large leucosin mass. 19. Mature cyst, equatorial view. 20. Reproduction in the palmelloid phase, stage 1. 21. Reproduction in the palmelloid phase, stage 2. 22. Reproduction in the palmelloid phase, stage 5. 23. Reproduction in the palmelloid phase, stage 6.

and difficult to see in the living individuals; stained preparations show that it does not arise from a basal granule and that it tapers very slightly towards the free end. No evidence was obtained of the presence of fine cilia on the flagellum although Vlk (1938) records them in an unnamed species of *Chromulina*. This species moves smoothly and fairly slowly; it swims for comparatively short distances, or round in circles, and then has long periods of quiescence. Strong illumination tends to stop movement in a fairly short time.

Two small non-contractile vacuoles are present near the point of insertion of the flagellum. Another larger 'vacuole', or clearly delimited spheroidal body, is usually developed in the motile individuals near to the two small vacuoles (Text-figs. 13-15, 18). This well-defined structure is sometimes quite large and contains shining vibrating bodies. The movement of these bodies is very similar to that found in Desmids in the terminal vacuoles containing minute crystals of calcium sulphate. In addition to the Brownian movement of crystals, Chifflet & Gautier (1905) have shown that Brownian movement of cytoplasmic granules frequently occurs. They observed its occurrence in many species of *Spirogyra*, in *Haematococcus pluvialis* and in the dense protoplasm at the tips of the root hairs of *Azolla caroliniana*. In the last genus they found Brownian movement also in the vacuoles in the root hairs, but in the vacuoles the motile bodies, more numerous than in the protoplasm, were found to be crystals of calcium oxalate.

Carter (1937) records the presence of large numbers of minute granules in active Brownian movement in individuals of *Prymnesium parvum* and *P. minutum* Carter. In these species, however, the granules are in 'an ill-defined region', not a clearly defined one as in *Chromulina pleiades*, and are therefore most probably cytoplasmic granules. The sharp outline of this body in *C. pleiades* suggests that it is a vacuole and that the 'bodies', showing Brownian movement, are probably of a crystalline nature.

There are records in the literature of the occurrence, in members of the Chrysophyceae, of collections of strongly refractive particles having a crystalline appearance (Scherffel, 1911; Doflein, 1922, 1923). Scherffel (1911) observed them in *C. nebulosa* Cienk., in addition to other genera, and Doflein (1923) records them as being a distinctive feature of *C. minima* Doflein. Neither author refers to movement of the particles nor to their enclosure in a clearly defined 'body'. Korshikov (1929) refers to leucosin masses becoming, under pressure, vacuoles containing suspended particles in Brownian movement; he records, however, that these vacuoles have lost the former refraction power of the leucosin. They are therefore different in nature from the 'vacuoles' in *C. pleiades*.

Asexual reproduction takes place by simple fission, usually of the motile individuals, but sometimes of individuals which have either cast or withdrawn their flagellum. Division of the cells takes place in both the light and the

dark, and occurs in the normal way. The individual first broadens and a second flagellum develops close to the original one, usually before the division of the nucleus. Four chromatophores are usually present in the cell before division starts so that two pass to each daughter-cell. One of the small vacuoles passes to each cell and the second arises anew as the two cells start to separate. The vibrating 'vacuole' can be seen in the early division stages but not in the late stages as the two daughter-cells draw apart (Text-figs. 16, 17).

Cysts are produced in cultures which have been left for at least 3 months without subculturing. They develop inside large individuals which are nearly half-filled by leucosin. No gelatinous investment forms round the individuals. The cysts are spheroidal to very slightly ovoid in shape and measure 5-6  $\mu$  in diameter (Text-fig. 19). The outer surface of the cyst wall is smooth and the plug, which protrudes only slightly above the surface, has the shape of an inverted truncated cone.

In this species (Text-figs. 20-23), and in the three following species belonging to the Chrysophyceae, a form of reproduction takes place in a palmelloid phase which has so far not been recorded in the literature, unless Lund's description of the production of sporangia in *C. sporangifera* is a somewhat similar process (Lund, 1942). The stages that have been obtained so far in this palmelloid form of reproduction are described and discussed under the next genus, *Isochrysis*.

*Chromulina pleiades* differs from *C. microplankton* Pascher (1913), *C. parvula* Conrad (1930b) and *C. sphaerica* Doflein (1923) in possessing two chromatophores; it differs further from the first two in size and from the last, which is about the same size, in lacking pyrenoids and also in the length of the flagellum. *C. pleiades* resembles most closely *C. sphaeridia* Schiller (1929), but it is smaller in size, has a shorter flagellum and its vacuoles are not contractile. It differs also from all other species of *Chromulina* in possessing a 'vacuole' containing vibrating bodies.

## ISOCHRYSIDACEAE

### *Isochrysis* n.gen.

Cellula solitaria, natante, nuda, periplasto differentiae carente praedita; ellipsoidi, ad anteriorem partem truncata, ad posteriorem rotundata; dorsiventraliter paulum depressa; quae transverse secta formam praebet ovatam; forma mutabili; 2 flagellis, longitudine paribus, ad anteriorem partem existentibus; 2 chromatophoris magnis lateralibus fulvis; ocello parvo; nucleo parvo mediano; desunt vacuola; cellula pabulum holophytica; nec oleo nec leucosino indigente; sine sexu propagatur fissione in longitudinem effecta per gradus mobiles immobilesque; cysto intra cellulam confecto; cystis sphaeroidibus cum membrana externa paulum sculpta; non deest multiplicationis genus palmelloidem per gradum effectae.

*I. galbana* n.sp. (Flagellate 'I', Parke.)

(Pl. I, figs. 4-10; Text-figs. 24-45.)

With the characters of the genus.

Motile cell: length, 5-6  $\mu$ ; breadth, 2-4  $\mu$ ; thickness, 2.5-3  $\mu$ .

Cyst diameter, 5-6  $\mu$ .

Isolated from sea water from one of the fish ponds at the Marine Biological Station, Port Erin, in January 1938.

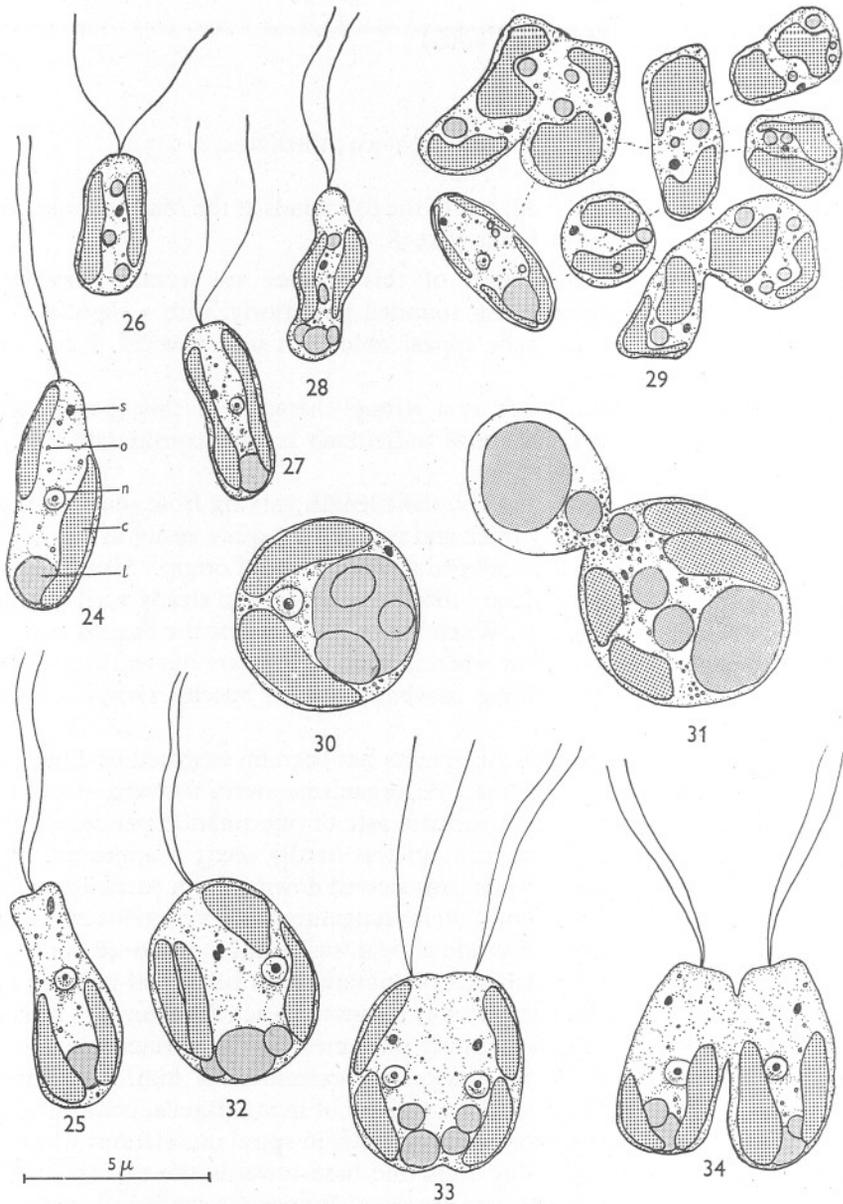
The actively motile individuals of this species are usually somewhat ellipsoidal, flattened anteriorly and rounded posteriorly, with a slight dorso-ventral flattening so that the cells appear oblong in side view (Pl. I, fig. 10), and ovoid in cross-section.

The capacity to change shape is a strong character of this species, and consequently variation in the shape of individuals is quite considerable (Pl. I, figs. 7, 10; Text-figs. 24-28).

Two equal flagella arise anteriorly, their length varying from once to twice that of the cell; they are fairly thick and can be seen quite easily in the living material; no basal granule is developed at their point of origin. Movement of the individuals, usually in a forward direction, is slow and steady with rotation of the body round the long axis. When the cells are at rest the flagella are stiff and lie away from the body, but when in motion they are out in front of the cell and carry out an undulating movement. This species shows a slight phototactic reaction.

The method of swimming of this species has been investigated by Lowndes (1943, pp. 101-2). He found that the organisms swam forward smoothly, rotating and gyrating at the approximate rate of one rotation per sec. When swimming, vibration of the anterior tip was hardly seen; it appeared quite definitely, however, when the organisms slowed down, and in particular when it suddenly changed its direction. 'On investigating these vibrations by means of the stroboscope, the maximum rate of beat was found to be 46-48 per sec., and since this somewhat regular beat or vibration can be looked upon as the rebound from each flagellum, it probably means that each flagellum was making about 24 beats per sec.' The swimming movement of this species was almost identical with that of a *Chlamydomonas* sp. examined by him. Lowndes's work (1936, 1941, 1943, 1947), on the activity of living flagella, confirmed by Brown (1945), has shown that a flagellum beats in spiral undulations with the waves of contraction progressing from the base towards the tip, the waves often increasing in amplitude as they progress. Brown also confirms Lowndes's theory that the rotation and gyration of the body alone may adequately account for the locomotion of many flagellates, without any forward component produced directly by the flagellum.

Fischer (1894), Loeffler (1889), Peterson (1918, 1929), Vlk (1938), and others, using drying and staining techniques, have described two types of



Text-figs. 24-34. ( $\times 5000$ .) *Isochrysis galbana* n.g., n.sp. 24-28. Young motile stage, illustrating the form variation and the variation in the shape and position of the chromatophores. 24. *c*, chromatophore; *l*, leucosin; *n*, nucleus; *o*, oil globule; *s*, stigma. 29. Fission in non-motile stage. 30. Reproduction in palmelloid phase; stage 1, spheroidal cell. 31. Reproduction in palmelloid phase; stage 1, apparent fusion of two of the spheroidal cells; nuclei could not be seen. 32. Early stage in fission of motile individual, front view. 33. Later stage in fission of motile individual, front view. 34. Separation of daughter-cells practically completed, front view.

flagellum: one which is whip-like, and one which bears fine cilia along its whole length. Brown (1945), by means of the electron microscope, investigated the structure of the flagellum of several species. He found that the flagella were of approximately uniform diameter throughout their entire length, and that each flagellum consisted of a denser axial core and a less dense sheath surrounding the core; some of the flagella also bore delicate filaments extending from the sheath. The two flagella of *Isochrysis*, studied from dried and stained preparations, are equal in length and uniform in diameter throughout their entire length; no evidence was obtained of the presence of fine cilia on either flagellum.

Two large, elongate, parietal chromatophores are present; they are usually placed laterally in the body, but their shape and position change with the change in shape of the body (Text-figs. 24-28). The chromatophores are golden-brown in colour; in actively motile individuals they appear to have a faint greenish tinge to the gold, but in older slow-moving individuals, palmelloid-phases and cysts the colour of the chromatophore lacks the slight tinge of green. A small dark red stigma, ovoid in shape, is most frequently in a median position, more rarely at the extreme anterior end. It does not project above the general surface of the cell. The nucleus, just visible in living individuals, is nearly always median; no mouth-band could be distinguished and no vacuoles are developed.

Oil and leucosin are developed as in other members of this class. Small oil drops are distributed through the general cytoplasm of the cell but the leucosin, in actively motile cells, occurs most frequently as a greyish, highly refractile, rounded mass at the extreme posterior end (Pl. I, fig. 7; Text-figs. 24, 25). In some of the smaller motile individuals from two to five small masses of leucosin may be present, and these are not always in a posterior position (Text-figs. 26, 28). The expulsion of leucosin from the body in the form of a droplet has never been observed in this species, although Carter (1937) records its occurrence in *Chromulina lunaris* Carter and *Prymnesium parvum* Carter; she concluded that the expulsion of leucosin was probably a process of excretion.

In this species the amount of leucosin present in the cells varies in different aged cultures; instead of expelling leucosin it seems to build up a large reserve. In actively dividing cultures, 2-4 weeks old, the quantity of leucosin in the cells is fairly small (Pl. I, figs. 7, 10; Text-figs. 24-28); as the cultures increase in age (over 6 weeks old), cell division is greatly reduced and the amount of leucosin in the cells increases. In still older cultures (3-4 months), the leucosin practically fills the posterior part of the cell so that the chromatophores, much reduced in size, and the nucleus, now occupy an anterior position (Pl. I, fig. 4; Text-fig. 35). The shape and size of an individual alters as the amount of leucosin increases; it becomes more pyriform, increasing very considerably in breadth and thickness, so that a cell can be 5-6  $\mu$  long, and 4-5  $\mu$  broad. These heavily laden individuals are extremely sluggish and exhibit a much

slower movement than the younger individuals. The cells packed with leucosin eventually become non-motile and either form cysts or pass into a palmelloid phase in which another form of reproduction occurs.

Asexual reproduction in this species occurs by the longitudinal division of both motile and non-motile individuals, and also by cyst formation. In the young motile stages division is most frequent in the early morning. Counts made of the numbers of individuals in both sterilized sea water and culture solution showed that individuals could divide more than once in 5 hr. When an individual is about to divide the body broadens, and division of the chromatophores occurs; one chromatophore, now divided into two, passes to each daughter-cell. A second stigma appears, frequently close to the one already present (Text-fig. 32). The two flagella of the parent pass to one daughter-cell, the other daughter-cell developing two anew; the new flagella arise frequently before the nucleus has divided but sometimes not until after separation of the two cells has already started.

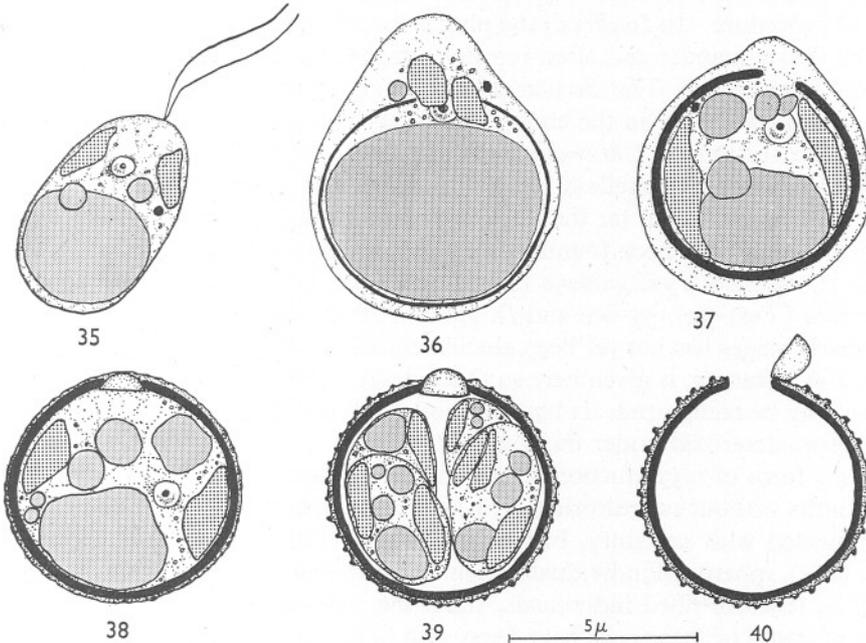
Before the separation of the two daughter-cells begins the leucosin appears to break up into a number of smaller masses, some passing to each daughter-cell (Text-fig. 33). Separation of the two cells is by constriction, mainly from the posterior end (Text-fig. 34). The products of division are, nearly always, of unequal size.

In cultures more than 4 weeks old some individuals lose their flagella before division takes place (Text-fig. 29). These non-motile individuals form quite a thick layer on the bottom of a flask; their shape becomes irregular but they are not surrounded by a mucilaginous investment. Fission takes place as in the motile stage, the products of division developing flagella when the separation of the individual is almost complete or after it has been completed.

Cyst formation in *Isochrysis* takes place in 3- to 6-months-old cultures. The cysts develop inside the large pyriform individuals mentioned earlier and their development follows very closely that already described for many Chryso-monadineae. When the large pyriform cells are about to develop into cysts they cast their flagella, but they do not form any gelatinous envelope around the protoplast; they can measure up to  $8\mu$  in length and  $7\mu$  in breadth. Each individual then secretes near the periphery a spheroidal cellulose membrane, measuring about  $6\mu$  in diameter. The membrane develops around the large posterior mass of leucosin leaving the anterior nucleus, stigma and small chromatophores external to it. It does not enclose the leucosin entirely however, since an opening remains in it; this faces the anterior end of the cell (Text-fig. 36). A great many very early cyst stages have been examined, but no evidence has been obtained to show that the opening in the membrane arises secondarily.

As the cellulose membrane of the cyst thickens and becomes impregnated with silica, the nucleus, stigma, small chromatophores and some of the cytoplasm pass into the cyst through the pore at the anterior end; a certain amount

of cytoplasm remains outside the cyst (Text-fig. 37). Once the chromatophores have passed into the cyst they become much larger in size (Text-fig. 37). The cytoplasm, external to the cyst, gradually shrinks, but it persists as a thin layer until after the anterior aperture of the cyst has been closed by a hemispherical plug measuring  $1.5\mu$  across the base (Text-fig. 38). The plug can be seen quite clearly when pressure is exerted on an early cyst stage. The outer surface of the cyst is covered by small protuberances which can be seen quite



Text-figs. 35-40. ( $\times 5000$ .) *Isochrysis galbana* n.g., n.sp. 35. Older motile stage containing large mass of leucosin, front view. 36. Start of cyst formation, development of cellulose membrane round leucosin mass, front view. 37. Cyst membrane thickened, cell contents passed into cyst, front view. 38. Fully formed cyst, equatorial view. 39. Early stage in the division of the contents of the cyst, equatorial view. 40. Cyst from which contents have been liberated, plug attached to one side of the pore, equatorial view.

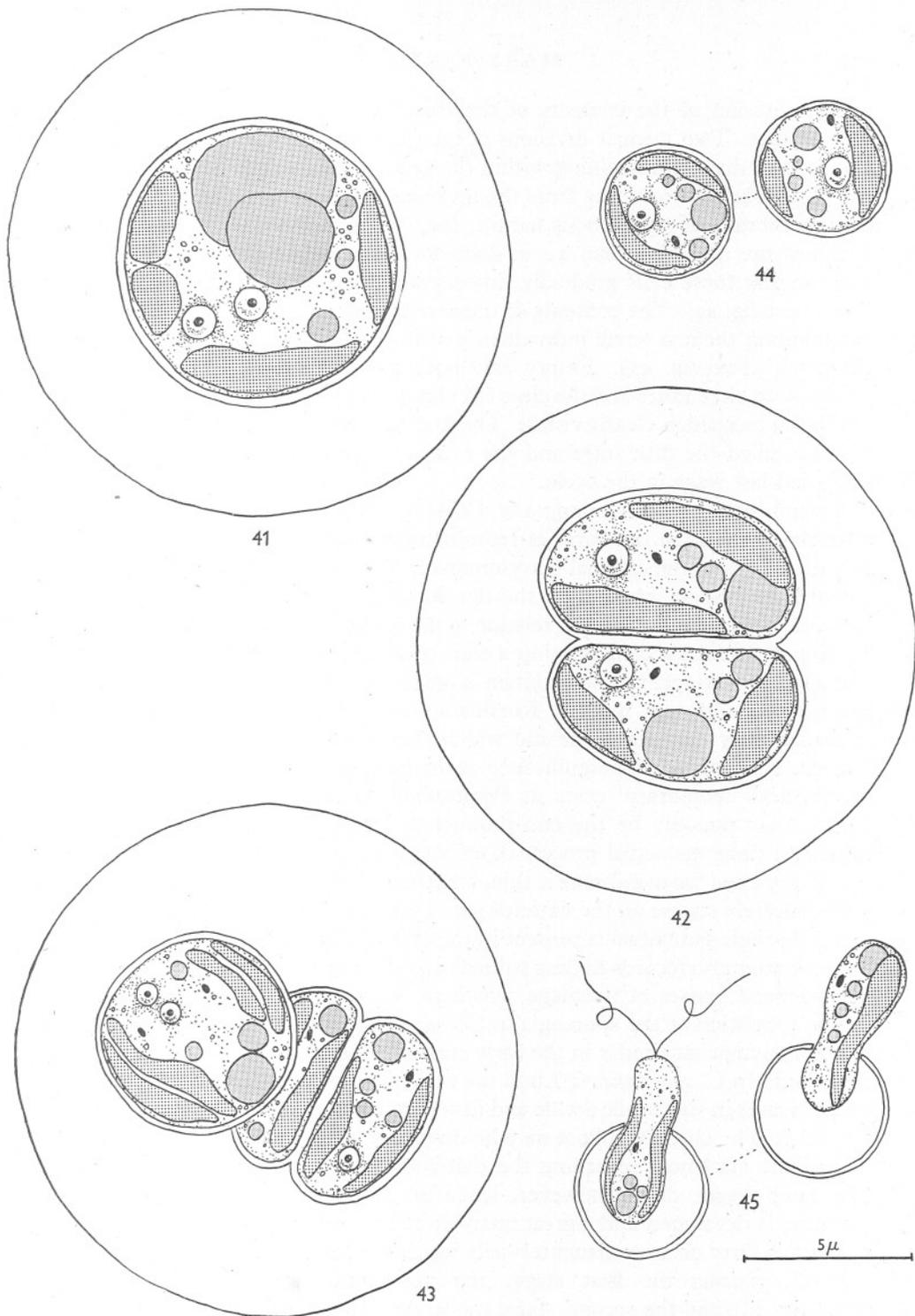
clearly after the remaining external cytoplasm has disintegrated (Pl. I, fig. 6; Text-figs. 39, 40). In this species, the spheroidal cysts,  $5-6\mu$  in diameter, are not as large as the individuals from which they were produced; the motile individuals themselves increased in size, mainly due to the increase in the amount of leucosin present, before the production of the cysts (Text-figs. 36, 39).

In old cultures, the cyst stage can persist indefinitely without the contents showing any division. If the deposit from the bottom of an old culture (containing many cysts) is added to fresh culture solution, however, the first division stage of the contents of a cyst can be observed within 24 hr. Four divisions take place in the cyst in a line at right angles to the bottom of the

plug. Text-fig. 39 shows a cyst in which the first two divisions have been completed. In a cyst in which the contents are fully divided (Pl. I, fig. 6) sixteen naked cells are present; these cells resemble the young motile stages described earlier, possessing two chromatophores, a stigma, a nucleus and one or more small masses of leucosin. The flagella may also be developed, but they could not be seen. The cells are released from the cyst as motile individuals measuring approximately  $3\mu$  in length. The release of the individuals from the cyst does not take place by the dissolving of the plug as seems to be the usual procedure. In *Isochrysis* the plug is forced out of the pore of the cyst in an explosive manner and often remains attached to the cyst at one side of the pore (Text-fig. 40). The circular pore through which the contents escape can be seen quite clearly in the empty cyst (Text-fig. 40).

In the section on *Chromulina pleiades* reference was made to a form of reproduction in a palmelloid phase which seems to differ from any form that has been described so far for the Chrysomonadineae. Stages in this form of reproduction have been found in the four species studied, *C. pleiades* (Text-figs. 20-23), *Isochrysis galbana* (Pl. I, figs. 8, 9; Text-figs. 41-45), *Dicrateria inornata* (Text-figs. 55-60) and *D. gilva*. Although the nuclear behaviour in the early stages has not yet been elucidated, a brief description of the process, as far as is known, is given here so that similar stages in other members of the class may be recognized. It has been followed most fully in *Isochrysis*, and is therefore described under this genus.

This form of reproduction occurs in cultures which have been left at least 2 months without subculturing. The very early stages in the cycle are still not established with certainty, but there is some evidence that fusion of non-motile(?), spheroidal individuals, about  $5\mu$  in diameter, derived from the large motile, leucosin-filled individuals, starts the process (Text-fig. 30). Possible fusion stages of these cells have been seen (Text-fig. 31). The next or second stage is a larger spheroidal individual, measuring  $8-9\mu$  in diameter; it is covered by a thin gelatinous envelope of firm consistency and it possesses two nuclei, two stigmata, four chromatophores and a number of large masses of leucosin (Pl. I, fig. 8). Stage 2 may be the product of the fusion of the large motile individuals which either cast or withdraw their flagella as soon as fusion starts; the non-motile spheroidal stage 1 may be individuals which have not fused. The third stage is figured on Pl. I, fig. 9, and shows that a larger secondary gelatinous envelope, of a less firm consistency than the first, has now developed; it measures about  $15\mu$  in diameter. In this stage one nucleus only is present so that the two nuclei, originally present, have either fused or one has degenerated. The third stage, therefore, may be a zygote. The fusion of motile gametes, and the subsequent reduction during the two nuclear divisions that follow the sexual fusion resulting in the formation of four daughter-cells, are recorded by Schwarz (1932) for *Ochrosphaera neapolitana* Schussnig.



Text-figs. 41-45. ( $\times 5000$ .) *Isochrysis galbana* n.g., n.sp. Stages in reproduction in the palmelloid phase. 41. Early stage 4, nucleus of stage 3 just divided. 42. Stage 4, first division. 43. Stage 4, second division. 44. Stage 5, products of division free from gelatinous envelope. 45. Stage 6, liberation of daughter-cells as young motile stage.

The division of the contents of the third stage may be regarded as the fourth stage. Two nuclear divisions occur (possible reduction division), the products of division remaining within the gelatinous envelope (Text-figs. 41-43). The four cells resulting from the divisions are each surrounded by a thin firm membrane of a gelatinous nature; they are spheroidal or ovoid in shape and measure not more than  $4\mu$  in diameter. The large gelatinous envelope surrounding these cells gradually disintegrates leaving the thin-walled cells free (Text-fig. 44). The contents of these cells escape from the firm envelope surrounding them as small individuals of the motile stage, measuring  $3.5-4\mu$  in length (Text-fig. 45). Empty envelopes are plentiful on the bottom of a flask of an old culture and the clear circular pore, through which the contents of the cell escaped, is clearly visible. The free cells covered by the firm envelope may be called the fifth stage and the escape of the contents of the cells the sixth and last stage in the cycle.

Several stages found among the Chrysophyceae by other workers may possibly be stages in this form of reproduction. Korshikov (1929) describes, in old cultures of the colonial Chryomonad, *Synochromonas pallida*, after the colonies had fallen into pieces, the development of solitary cells which are balled as if inflated so that their relation to the species is not readily established. He found that these cells develop a thin colourless membrane which is soft and slightly refringent. They contain large masses of leucosin, several chromatophores and two nuclei. Korshikov considers that these stages are probably early cyst stages, inside which the true cysts may eventually be formed, but says that the significance of the two nuclei is obscure. The ovoid or elliptical 'temporary' cysts in *Prymnesium parvum*, described by Carter (1937), may possibly be the end-product of the type of reproduction just described since the actual process of encystment was not observed. In these temporary cysts the membrane is thin, transparent, colourless or brownish and very delicately rugose on the exterior; the cyst lacks a plug as far as could be seen. A single individual is present in the cyst and it is released as the motile stage. Carter also records finding palmelloid groups of naked cells, two to four, in ill-defined masses of mucilage, which she believes belong to *P. parvum*.

The formation of the sporangia in *Chromulina sporangifera* given by Lund (1942) is somewhat similar in the early stages to the type of reproduction just described. In *C. sporangifera* Lund the protoplast assumes a spherical shape and increases in size, while a wide and firm mucilaginous membrane is secreted around it. The chromatophore may be divided into two or four portions, but there is no statement regarding the number of nuclei present at this stage. The later stages differ, however, since no large secondary mucilaginous envelope is developed and a great many more divisions occur so that the final product is forty or more daughter-cells instead of four.

In *C. pleiades* the first stage, the non-motile spheroidal individual (Text-fig. 20), and the second stage, the larger binucleate individual, covered

by a thin gelatinous envelope (Text-fig. 21), have been found, but neither the third stage, with the large secondary envelope, nor the fourth stage, the division of the contents of stage 3, has been seen. Although the fifth and sixth stages have been found (Text-figs. 22, 23) there is no proof at present that the large secondary envelope does develop and that two divisions only take place in *C. pleiades* during the fourth stage. The fifth stage does definitely possess, however, a very thin firm envelope from which the contents escape as motile individuals. Lund makes no reference to any thin-walled envelope around the daughter-cells from the sporangium of *C. sporangifera*; he says that when liberated they are spherical, but that they soon become actively motile and assume the ellipsoid shape.

In general form *Isochrysis* agrees rather closely with the two described species of the genus *Wyssotzкия* Lemm., but in the latter genus there appears to be considerable doubt concerning the number of flagella present; Büttner (1911, p. 126, fig. 5d, e) actually figures a third flagellum in *W. gladociliata* Lemm. Carter (1937) is of the opinion that *W. gladociliata* Lemm. is probably identical with *Prymnesium parvum* Carter and that *Wyssotzкия biciliata* (Wyss.) Lemm. belongs also to the genus *Prymnesium* and possibly also represents *P. parvum*. Conrad (1941), on the other hand, thinks that both species of *Wyssotzкия* belong to the Cryptophyceae.

#### *Dicrateria* n.gen.

Cellula natante, solitaria, nuda, periplasto differentiae carente praedita; protoplasto rigidiore, formam praebente immutabilem; cellula sphaeroidi subovatae; deest sulcus; 2 flagellis, longitudine paribus, ex granulo basali distincto existentibus in parte cytoplasmatis hyalina sito; 2 chromatophoris magnis parietalibus crateriformibus fulvis contraposis; nucleo mediocri propius polum illum sito ex quo existunt flagella; cellula pabulum holophytica; nec oleo nec leucosino indigente; sine sexu propagatur fissione per gradum mobilem; cysto intra cellulam confecto; cystis sphaeroidibus subovatisque cum membrana externa laevi; non deest multiplicationis genus palmelloidem per gradum effectae.

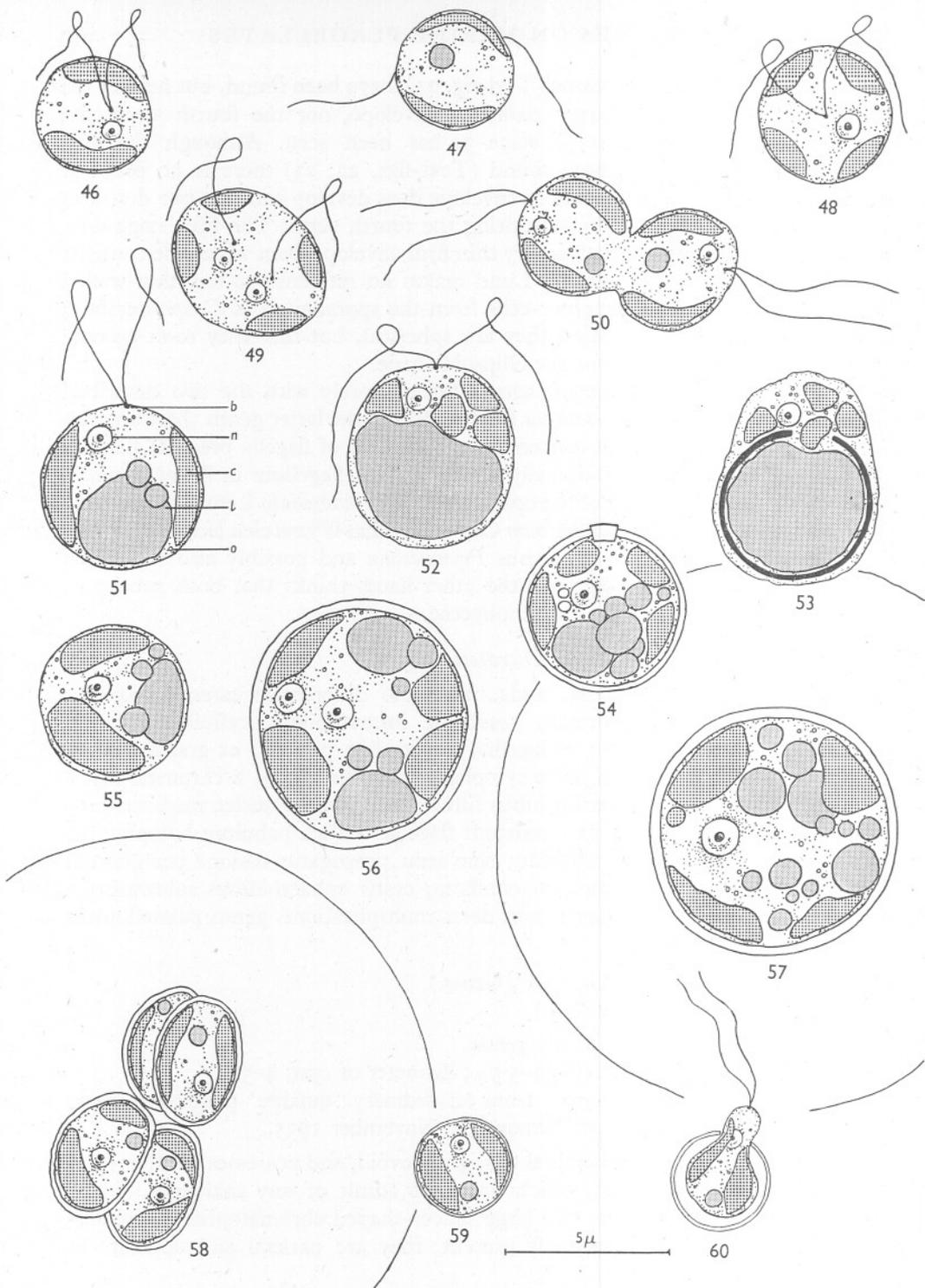
*D. inornata* n.sp. (Flagellate 'B', Gross.)  
(Pl. I, fig. 1; Text-figs. 46-60.)

With the characters of the genus.

Diameter of motile cells, 3-5.5  $\mu$ ; diameter of cyst, 4-5  $\mu$ .

Isolated by Dr F. Gross from an ordinary 'outside' plankton sample which was taken off Plymouth in November 1935.

The motile stage is spheroidal to slightly ovoid, and possesses a rather rigid undifferentiated periplast, which does not admit of any change of shape. In the young motile stage, two large saucer-shaped chromatophores, golden-brown in colour, are normally present; they are parietal and opposite in



Text-figs. 46-60.

position so that a clear zone of cytoplasm lies between them (Pl. I, fig. 1; Text-fig. 51). In the older motile stage four chromatophores are more frequently developed (Text-figs. 48, 52). The medium-sized nucleus is situated near to the pole from which the flagella arise and in the young stage a rather small mass of leucosin is present at the opposite pole (Pl. I, fig. 1). Small oil drops are distributed through the general cytoplasm, but the species lacks both stigmata and vacuoles.

Two equal flagella, one and a half to twice the diameter of the cell in length, arise from a distinct basal granule, or from two placed very close together, situated in the clear part of the cytoplasm (Text-figs 46, 48); they are very delicate, but can just be seen in the living cells. Stained preparations show that both flagella taper very slightly towards the free end, and neither are apparently plumate in structure. When in motion the cells rotate in a slow and steady manner and are constant in direction for relatively long periods. When swimming the flagella lie backwards down the sides of the body and carry out an undulating movement, but when at rest, they lie away from the body. Before the swimming individuals come to rest, however, they exhibit, for a short time, a peculiar dithering movement; they then remain motionless for a fairly long period. This species is slightly phototactic in reaction.

In this organism, as in *Isochrysis*, the quantity of leucosin in the cell varies in cultures of different ages (Pl. I, fig. 1; Text-figs. 51, 52). In the young actively dividing individuals, 3-4 $\mu$  in diameter, the quantity is very small (Pl. I, fig. 1), but as the culture increases in age so also does the quantity of leucosin in the cells (Text-fig. 51), until in 4- to 5-month-old cultures the leucosin may occupy from one-third to more than one-half the volume of the cell (Text-fig. 52). In the very slow-moving leucosin-filled cells, 4-5.5 $\mu$  in diameter, the chromatophores, usually four, may be so reduced in size that they appear as small disks, situated with the nucleus, at the pole from which the flagella arise (Text-fig. 52). These cells either form endogenous cysts or pass into the first stage of the reproductive cycle that takes place in the palmelloid phase.

Asexual reproduction by division of the motile cells takes place in both the light and the dark. Counts show that more than two divisions can take place within 6 hr. The procedure is similar to that described in *Isochrysis*. When

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Text-figs. 46-60. ( $\times 5000$ .) *Dicrateria inornata* n.g., n.sp. 46. Young motile stage, viewed from pole at which flagella are inserted. 47. Young motile stage, viewed from opposite pole to Fig. 46. 48. Older motile stage with four chromatophores, viewed from pole at which flagella are inserted. 49. Early division stage of motile individual. 50. Late division stage of motile individual. 51. Older motile stage with large leucosin mass, equatorial view. *b*, basal granule; *c*, chromatophore; *l*, leucosin; *n*, nucleus; *o*, oil-globule. 52. Older motile stage with four chromatophores and still larger mass of leucosin, equatorial view. 53. Contents of cell passing into cyst, membrane of cyst thickened. 54. Mature cyst. 55-60. Stages in reproduction in the palmelloid phase. 55. Stage 1. 56. Stage 2. 57. Stage 3. 58. Stage 4, second division. 59. Stage 5. 60. Stage 6.

about to divide the cell broadens, the two chromatophores divide, if they have not already divided, and the leucosin breaks up into two or more smaller masses. The nucleus then divides and the two flagella separate, each attached to its basal granule or part of the basal granule (Text-fig. 49). Fission of the cell starts at the pole from which the flagella arise and passes down through the individual until the two cells are connected only at the pole away from the flagella (Text-fig. 50). A second flagellum is developed by each daughter-cell before they finally separate; the cells are usually of equal size. No loss or withdrawal of the flagella before fission has been observed in this species.

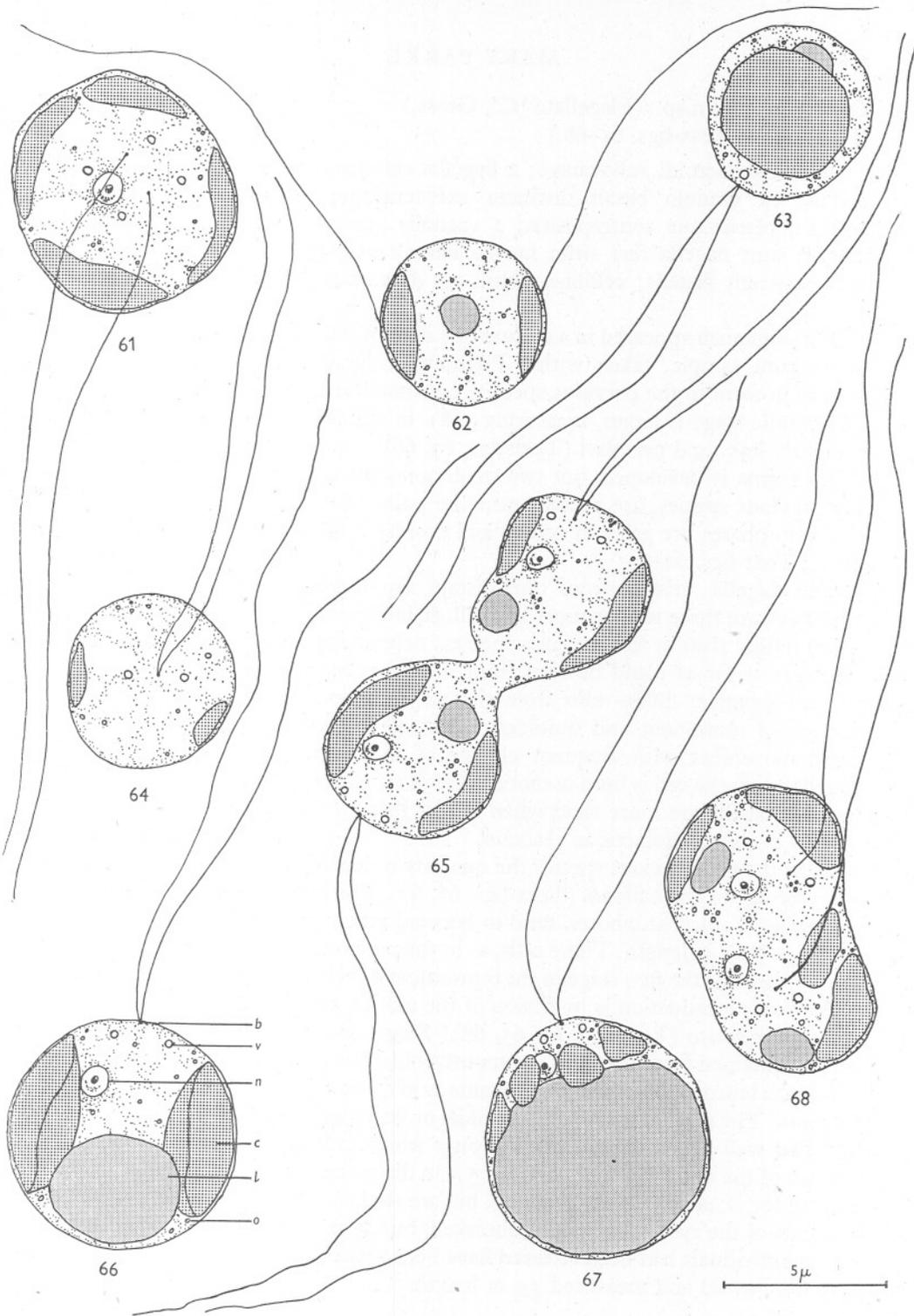
Cyst formation occurs in cultures left 5 months without subculturing. The cysts develop inside the leucosin-filled cells (Text-fig. 53) in a manner similar to that described for *Isochrysis*. The mature cysts are spheroidal with a smooth silicified wall and a plug, the shape of a truncated cone, that is raised slightly above the general surface of the cyst (Text-fig. 54).

The form of reproduction in the palmelloid phase found in the genus *Isochrysis* occurs also in this genus. In *Dicrateria inornata* the six stages in the cycle defined earlier have been obtained from old cultures (Text-figs. 55-60). The large spheroidal, leucosin-filled cells of stage 1 measure  $4.5-5\mu$  (Text-fig. 55); stages in the apparent fusion of pairs of these cells have been seen, but the actual fusion of the nuclei of the cells has not yet been observed. Stage 2, the larger binucleate cell covered by a thin gelatinous envelope, has been found frequently (Text-fig. 56); it measures  $7-8\mu$  in diameter. Stage 3, the uninucleate cell (Text-fig. 57), and stage 4, the division of this cell into four daughter-cells (Text-fig. 58), are not uncommon; the large secondary gelatinous envelope surrounding these stages measures  $15-17\mu$  in diameter. In stage 5 the four daughter-cells become free by the disintegration of the large gelatinous envelope: they are spheroidal and are covered by a very thin, smooth, firm wall, measuring about  $3\mu$  in diameter (Text-fig. 59). Stage 6, the liberation of the contents of these cells, has also been seen; the contents escape from a small pore as a single small individual of the motile stage, measuring approximately  $3\mu$  in diameter (Text-fig. 60).

*D. inornata* shows some resemblance to *Chrysidalis peritaphrena* Schiller, but it is smaller and lacks the decided groove, passing forwards and backwards from the base of the flagella, which is present in that species.

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Text-figs. 61-68. ( $\times 5000$ .) *Dicrateria gilva* n.g., n.sp. 61. Early stage in fission of motile individual, chromatophores and vacuoles doubled, viewed from pole at which flagella are inserted. 62. Young motile stage viewed from pole opposite to that at which flagella are inserted. 63. Young motile stage, equatorial view. 64. Young motile stage, viewed from pole at which flagella are inserted. 65. Late division stage, fission of cell nearly complete. 66. Older motile stage with two chromatophores and large mass of leucosin, equatorial view. *b*, basal granule; *c*, chromatophore; *l*, leucosin; *n*, nucleus; *o*, oil-globule; *v*, vacuole. 67. Older motile stage with four chromatophores, reduced in size, and very large leucosin mass, equatorial view. 68. Division stage of motile individual, nucleus and chromatophores divided, number of vacuoles doubled.



Text-figs. 61-68.

*Dicrateria gilva* n.sp. (Flagellate 'C', Gross.)  
(Pl. I, fig. 3; Text-figs. 61-68.)

Cellula sphaeroidi subovatave; 2 flagellis cellulae diametro 3-4 plo longioribus, ex granulo basali distincto existentibus; chromatophoris fulvis pallidis, plerumque contraposis; 2 vacuolis parvis non trepidantibus ubi inserta sunt flagella fere sitis; nucleo laterali propius polum illum sito ex quo existunt flagella; cellulae mobili est diametros 5-8 $\mu$ ; cysto diametros 7-7.5 $\mu$ .

This organism appeared in a culture of a diatom started by Dr F. Gross from a plankton sample, taken within Plymouth Sound in March 1936. It is similar in form to the previous species, but shows several points of difference. The motile stage is larger, measuring 5-8 $\mu$  in diameter, and appears to have a slightly less rigid periplast (Text-figs. 63, 66).

No stigma is developed, but two small non-contractile vacuoles, lacking in the previous species, are present near the point of origin of the flagella; the chromatophores are paler in colour and possess a faint greenish tinge (Pl. I, fig. 3; Text-figs. 62-64).

The flagella, arising from one distant basal granule (or two adjacent granules), are three to four times the cell diameter in length and are therefore much longer than in the previous species; neither flagellum shows a plumate structure as far as could be seen from stained preparations. The movement of this organism differs also from that of *D. inornata*; there are alternate periods of movement and quiescence, but when swimming it shows fairly rapid movement with frequent change of direction. The position of the flagella when the cell is both in motion and at rest is the same as in *D. inornata*, but the flagella are more rigid when at rest than in the former species. This species is also phototactic in reaction.

As in the two previous species the quantity of leucosin in the cells increases with the age of the cultures (Text-figs. 66, 67). The leucosin-filled cells, with the reduced chromatophores, tend to become rather pyriform in shape; they measure 7-8 $\mu$  in length. These cells, as in the previous species, give rise to the cysts or become the first stage in the reproductive cycle in the palmelloid phase.

Asexual reproduction is by fission of the motile individuals and takes place as in *D. inornata* (Text-figs. 61, 65, 68). Stages in the formation of the cyst can be obtained from cultures 4-5 months old. The development of the cyst, inside the leucosin-filled cells, is the same as in *Isochrysis galbana* and *Dicrateria inornata*. The mature cysts are spheroidal or very slightly ovoid with a smooth silicified wall and a conical hyaline plug which is raised slightly above the surface of the cyst; they measure 7-7.5 $\mu$  in diameter. They are similar to that figured for *D. inornata* (Text-fig. 54), but are slightly larger. The release of the contents of the cyst has not been observed, but cysts from which the majority of the individuals had been released have been seen; the cells remaining in the cyst were ovoid and measured 4 $\mu$  in length.

Five of the six stages in the palmelloid form of reproduction, described in and figured for the two previous species, have been seen in this species. The first stage, measuring  $6-7\mu$  in diameter, is spheroidal and shows two vacuoles and two or four chromatophores; in stage 2, the cells, covered by a thin gelatinous envelope, have four vacuoles, two nuclei and four or eight chromatophores; they measure  $9-10\mu$  in diameter. The large secondary envelope of stages 3 and 4 is not of such firm consistency as in the two previous species; it measures  $18-20\mu$  in diameter. The free cells of stage 5 measure  $4-5\mu$ , but stage 6, the escape of the contents of these cells, has not been observed.

## CRYPTOPHYCEAE

### NEPHROSELMIDACEAE

#### *Hemiselmis* n.gen.

Cellula solitaria, natante, nuda, phaseliformi, dorsiventraliter depressa; quae transverse secta formam praebet ellipsoidem; periplasto perspicuo differentiam praebente praedita; sulco bene notato a margine anteriore dorsuali trans latus laterale concavum oblique currente, in superficie ventrali evanescente plane mediana sub linea; deest gula; cellulis maioribus forma valde mutabilibus; 2 flagellis disparibus lateris concavi lateralem ad partem existentibus plane mediana sub linea; chromatophoro singulo magno, parietali; pyrenoide singulo, amyli capsula circumsaepito, in medio plerumque sito; vacuolo contractilio singulo ubi inserta sunt flagella fere sito; ocello singulo haud conspicuo ad posteriorem partem in superficie ventrali posito; nucleo parvo mediano; cellula pabulum holophytica; cellula amyli genus praebente; sine sexu propagatur fissione obliqua secundum sulcum effecta.

*H. rufescens* n.sp. (Flagellate 'D', Parke.)  
(Pl. II, figs. 11-16; Text-figs. 69-73.)

With the characters of the genus.

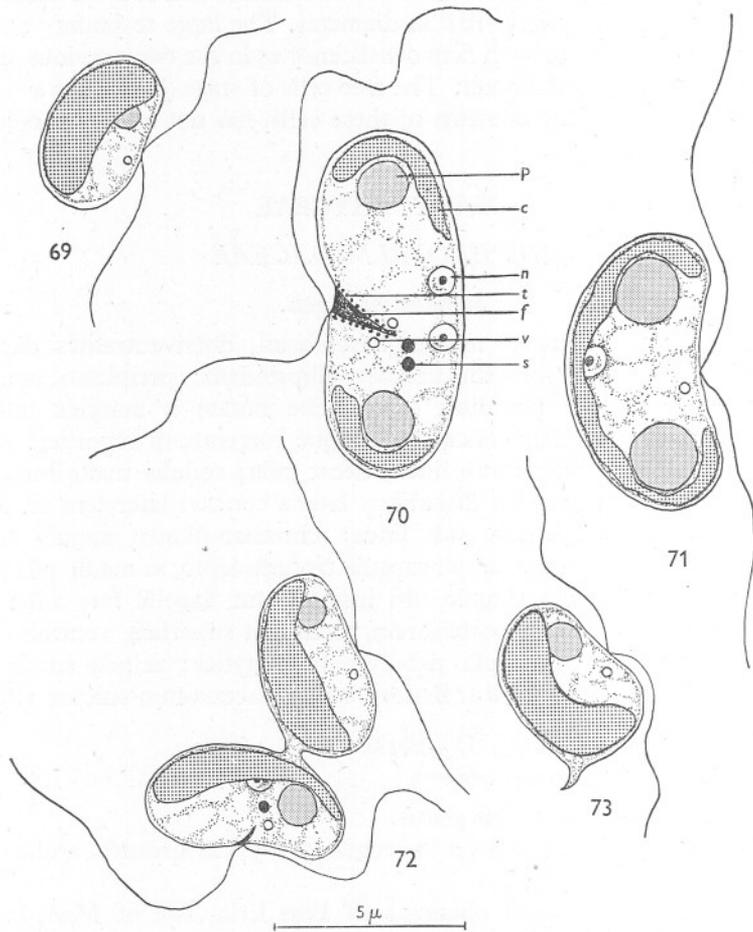
Motile cells measure  $4-8.5\mu$  in length,  $3.5-5\mu$  in breadth, and  $2-3\mu$  in thickness.

Isolated from sea water obtained off Port Erin, Isle of Man, in April 1937.

In this species the body is bean-shaped and in the larger individuals is very flattened in a dorsiventral plane (Pl. II, figs. 11-14; Text-figs. 69, 71); in the smaller stages, however, the body is not quite so flattened. A furrow, not deepened to form a gullet, arises near the dorsal anterior edge; it runs down obliquely over the lateral edge to die out on the ventral surface just below the median line (Pl. II, figs. 12-14; Text-fig. 70).

Two delicate flagella arise laterally just below the furrow, that is, a little below the middle of the concave surface. The flagella are unequal in length,

the one directed anteriorly is about one and a half times the length of the cell and the one directed posteriorly is slightly shorter (Pl. II, figs. 11-16). No difference in the structure of the two flagella could be detected from stained preparations; neither flagellum was plumate.



Text-figs. 69-73. ( $\times 5000$ .) *Hemiselmis rufescens* n.g., n.sp. 69. Young motile stage, dorsal view. 70. Early division stage of motile individual, ventral view. *c*, chromatophore; *f*, furrow; *n*, nucleus; *p*, pyrenoid; *s*, stigma; *t*, trichocysts; *v*, contractile vacuole. 71. Older motile individual, dorsal view. 72. Final stage in fission of motile individual, two daughter-cells just separating. 73. Daughter-cell immediately after fission has been completed, dorsal view.

The swimming of this organism has also been studied by Lowndes (1943, p. 102), and the following is his description of its movement. 'It swims rapidly, and with an enormous amount of gyration compared with the previous species (i.e. *Isochrysis galbana*). The individuals progressed in a very charac-

teristic manner traversing a spiral, the diameter of which is many times the length of the organism. It is obvious that the peculiar spiral swimming of the organism is not due to any kind of irregularity in the length or structure of the flagella, but it is dictated solely by the shape of the cell, with its strong dorsoventral flattening, and also by the fact that the flagella are attached laterally.' Movement of the cells, normally in a forward direction, is constant in direction for relatively long periods. All the cells show periods of quiescence, but these are of longer duration, and occur more frequently, in the larger individuals. This species is extremely phototactic in reaction.

The capacity to change shape is well marked in the large and in the dividing individuals, but it is not so obvious in the small stages. The periplast is clear and can be seen easily in all individuals (Pl. II, figs. 11-16). In the young stage there is a single, large, deep crimson parietal chromatophore covering about half the body surface (Pl. II, fig. 11; Text-fig. 69); it covers one lateral side and about half the surface of the ventral and dorsal sides of the body which are away from the lateral side on which the furrow arises. The edge of the chromatophore may be slightly lobed. A highly refractive, large, spheroidal pyrenoid, appearing greenish grey in colour, is present; it is normally surrounded by a sheath of solid material, which gives a blue coloration with iodine and appears therefore to be a form of starch. This organism is, therefore, undoubtedly a member of the Cryptophyceae. The pyrenoid is situated somewhat centrally in the body in most individuals and is frequently partly covered by the chromatophore (Text-fig. 69); it may be found towards either the anterior or posterior end of the body. The nucleus, round or ovoid in shape, is situated medianly or slightly posteriorly in the body, usually nearer to the ventral surface than to the dorsal. One contractile vacuole is developed immediately below the furrow in a median position in the body near the point of insertion of the flagella (Pl. II, figs. 11-13). A stigma is present on the ventral surface, placed usually near to where the furrow dies out; it can, however, be found nearer to the lateral side from which the flagella arise. In some individuals the stigma is dull in colour and difficult to detect, but its presence in this species has been confirmed for the writer by Prof. E. G. Pringsheim.

In nearly all the larger individuals two pyrenoids are present, one situated anteriorly and the other posteriorly (Pl. II, figs. 12, 14; Text-fig. 71). The quantity of starch around them has also increased so that they form large conspicuous bodies in the anterior and posterior regions of the cell. The chromatophore, now covering very little of the dorsal or ventral surface of the body (Pl. II, fig. 12; Text-fig. 71), becomes less intense in colour than in the smaller individuals, usually showing only a faint pink coloration, but occasionally becoming greenish; it may divide into two (Pl. II, fig. 14). The furrow is also more clearly defined in the large individuals. In these cells two rows of minute, highly refractive granules, probably trichocysts, can be seen, one row on either side of the furrow (Pl. II, figs. 12-14; Text-fig. 70).

Asexual reproduction in this species is by division of the cell in the motile stage (Pl. II, figs. 15, 16; Text-figs. 70, 72). Multiplication is very rapid, fission of individuals occurring in both the light and the dark. Division is oblique, the division line passing through the centre of the furrow. The large individuals, which contain two pyrenoids and two chromatophores (Pl. II, fig. 14), are probably early division stages, but the organism is more commonly found in this condition than in the small stage with one pyrenoid and one chromatophore.

When about to divide the cell becomes stretched out lengthwise, the breadth becoming slightly narrower. The nucleus then divides and a second contractile vacuole and second stigma make their appearance, usually close to the vacuole and stigma already present (Text-fig. 70). Fission starts at the anterior dorsal edge, passes down through the centre of the furrow (Pl. II, fig. 15) and continues obliquely, that is in the line of the furrow, across the cell until fission is nearly complete (Pl. II, fig. 16). The anterior chromatophore passes to the individual on the ventral side, and the posterior chromatophore to the one on the dorsal side (Pl. II, fig. 15). The two daughter-individuals, now separated except at a point below the mid-line on the convex lateral side, pull away from each other and are attached only by a very narrow connexion (Text-fig. 72). When the individuals separate, the remains of the connexion can be seen for a short while as a small papilla on the convex side of the cell (Text-fig. 73). The flagella of the dividing cell are retained by one of the daughter-cells (Pl. II, fig. 16), the other develops them anew before fission is completed (Text-fig. 72). Multiplication by fission of the motile stage is the only form of reproduction that has been found so far in this species.

This organism undoubtedly belongs to the Cryptophyceae, and adopting Pringsheim's (1944) suggested subdivision of the class, it belongs to family 5, Nephroselmidaceae Pascher, the characters of which are body bean-shaped and flagella laterally inserted. Pascher placed three genera in this family, *Nephroselmis* Stein (1878), *Protochrysis* Pascher (1911) and *Sennia* Pascher (1912), but their position in it does not yet seem to be definitely established; two species of *Nephroselmis* have been described, but the two other genera are monotypic.

*Hemiselmis rufescens* resembles *Nephroselmis olivacea* Stein (1878) in general shape, but appears to be more flattened. It differs from this species, however, in the absence of a gullet (as far as could be detected), in the presence of a stigma, and in the development of one contractile vacuole instead of two alternating contracting vacuoles. It is also much smaller in size and if Stein's figures (1878, Taf. XIX, figs. 36, 37) illustrate the method of fission in the motile stage of *N. olivacea*, the type of oblique fission found in *Hemiselmis* is again different. *Nephroselmis marina* Schiller (1925) is about the same size as *Hemiselmis*, but differs in shape, and lacks stigma, pyrenoid, vacuole and trichocysts. From Schiller's illustration (1925, Taf. 3, fig. 13), the flagella of

*Nephroselmis marina* also appear to be inserted on the convex side of the body.

*Hemiselmis* is very much more flattened than the genus *Protochrysis*; *Hemiselmis* differs also in its ability to change shape and in possessing a single vacuole. The lobing of the chromatophore is also very much more pronounced in the larger *Protochrysis*. A further difference is in the form of asexual reproduction; in *Hemiselmis* fission takes place in the motile stage, but in *Protochrysis phaeophycearum* Pascher it is recorded as occurring in the palmelloid phase.

The young motile stage of *Hemiselmis* is somewhat similar in shape to *Sennia commutata* Pascher, but it is more flattened. *Sennia*, however, has a fixed shape and it has only a slight indication of a furrow. The position of the nucleus in the two genera also differs.

*Hemiselmis* differs in colour from the three genera (golden-brown) already placed in the Nephroselmidaceae, but as Pringsheim (1944) points out, the custom of relying solely on colour as a means of generic distinction should be abandoned. There are, however, a sufficient number of differences, without the colour, to warrant the placing of this organism in a new genus.

#### SUMMARY

Six marine flagellates, used as food in the rearing of the larvae of *Ostrea edulis* L., are described.

One belongs to the Chlorophyceae, *Pyramimonas grossii* n.sp. (Polyblepharidaceae); four belong to the Chrysophyceae, *Chromulina pleiades* n.sp. (Chromulinaceae), *Isochrysis galbana* n.g., n.sp., *Dicrateria inornata* n.g., n.sp., *D. gilva* n.g., n.sp. (Isochrysidaceae); and one belongs to the Cryptophyceae, *Hemiselmis rufescens* n.g., n.sp. (Nephroselmidaceae).

A form of reproduction in a palmelloid phase, not previously described, is recorded for the Isochrysidaceae.

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## EXPLANATION OF PLATES I AND II

## PLATE I

(Figs. 1-10,  $\times 7000$ .)

- Fig. 1. *Dicrateria inornata* n.g., n.sp., young motile stage, equatorial view.  
 Fig. 2. *Chromulina pleiades* n.sp., young motile stage, from pole at which flagellum arises.  
 Fig. 3. *Dicrateria gilva* n.g., n.sp., young motile stage, equatorial view.  
 Figs. 4-10. *Isochrysis galbana* n.g., n.sp.  
 Fig. 4. Older motile stage containing large mass of leucosin, front view.  
 Fig. 5. Immature cyst, plug not yet formed, front view.  
 Fig. 6. Mature cyst with division of contents completed, front view.  
 Fig. 7. Young motile stage with lateral chromatophores, front view.  
 Fig. 8. Reproduction in the palmelloid phase; binucleate stage 2 with narrow gelatinous envelope.  
 Fig. 9. Reproduction in the palmelloid phase; uninucleate stage 3 with additional large gelatinous envelope, nucleus in early prophase.  
 Fig. 10. Young motile stage, lateral view.

## PLATE II

(Figs. 11-16,  $\times 7000$ ; figs. 17, 18,  $\times 4900$ .)

- Figs. 11-16. *Hemiselmis rufescens* n.g., n.sp.  
 Fig. 11. Young motile stage with vacuole, stigma and pyrenoid, ventral view.  
 Fig. 12. Older motile stage with two pyrenoids, ventral view.  
 Fig. 13. Older motile stage, lateral view.  
 Fig. 14. Older motile stage with two chromatophores, ventral view.  
 Fig. 15. Early division stage in motile individual showing the oblique line of fission, ventral view.  
 Fig. 16. Later division stage with fission nearly completed.  
 Figs. 17, 18. *Pyramimonas grossii* n.sp.  
 Fig. 17. Young motile stage, showing the position of the flagella when the organism is swimming, side view.  
 Fig. 18. Young motile stage, showing the position of the flagella when the organism is quiescent, side view.

