ON THE LIFE-HISTORY AND ECOLOGY OF *HORNELLIA MARINA* GEN. ET SP. NOV., (CHLOROMONADINEAE), CAUSING GREEN DISCOLORATION OF THE SEA AND MORTALITY AMONG MARINE ORGANISMS OFF THE MALABAR COAST

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I. Introduction

There are many published accounts dealing with "red", "yellow" and "pink" discoloration of the sea water, caused by the occurrence in swarms of micro-organisms, particularly members of the Dinophyceae (*cf.* Hayes and Austin, 1951). Some of these organisms are known to affect the fishery adversely. Green discoloration of the sea, however, has not been recorded frequently. Swarms of *Noctiluca* harbouring a green flagellate have been recorded as having rendered the sea green by Weber and Weber van-Bosse (1890), Delsman (1939–40), Prasad (1953) and recently by Subrahmanyan (1953).

While engaged on an investigation of the marine phytoplankton of the west coast of India near Kozhikode, Malabar, the writer observed a green discoloration of the sea on certain occasions. The organism causing this phenomenon occurred at other times also, but not in such quantity as to discolour the sea water.

II. Occurrence

A discoloration of the sea at West Hill, near Kozhikode, was noted in August, 1949, and collections of plankton on the date when this occurred were sent to the writer at Madras. From this sample which was preserved in formaldehyde no detail could be made out as the material was of an amorphous jelly-like mass. Detailed study of the organism concerned was taken up from September, 1949, when the author was posted to Kozhikode.

The sample of plankton taken from the 4–6 fathoms area on November 1st, 1949, was of a pea-soup consistency and brownish-green in colour, the green colour predominating. Examination of this plankton showed the presence of millions of a green flagellate, all of the same species, actively swimming about to the almost total exclusion of other planktonic elements. The writer had observed earlier (14th October, 1949), in the plankton from the 8 fathoms area, a few cells of the same flagellate occurring along with the usual members of the plankton flora (Diatomaceae and Dinophyceae) and zooplankton elements. It has been confirmed by my colleagues that this was the same form which caused discoloration of the water a few months earlier.

Observations on the plankton during the last 4 years indicate that the occurrence of this flagellate is sporadic and only of short duration. Table I

Table I. *The Days on which* Hornellia marina *gen. et sp. nov. Occurred in the Sea off West Hill, Kozhikode*

1949	1950	1951	1952	1953
Aug. ?	Aug. 14 to 21	April 14	Sept. 6 to *8 to 22	Sept. 21
Oct. 14	Sept. 4 to 25	Aug. 20 to 27		26
*Nov. 1 to 3	Oct. 30	Sept. 10 to 24		*28
				*29
				*30
Dec. 7	Nov. 6 to 27	Oct. 15		Oct. *1
		Dec. 10		*2
				*3
				*4
				*5 to 10

* Dates on which the sea was discoloured due to high concentration of the flagellate.

shows the dates on which it has been recorded. Only on two other occasions since November, 1949, did the flagellate occur, during this period of 4 years, in such numbers as to discolour the sea water, *i.e.*, on the 8th of September, 1952, and from 28th of September to 5th of October, 1953.

In September, 1952, it made its appearance on the 6th in very few numbers and a peak in its concentration, causing green discoloration of the water, was attained on the 8th, after which there was a decline in its quantity and no more were seen after the 22nd. In the earlier years, between November, 1949 and September, 1952, though the flagellate appeared roughly at about the same time each year (*vide* Table I), its number was not appreciable enough to cause any discoloration of the water. But for the close examination of the tow-net collections the flagellate might have been overlooked.

On the 21st September, 1953, a few stray ones of the flagellate appeared in the plankton samples from areas of 8- and 10-fathoms depth. There was none in the plankton obtained farther out from the shore (16 fathoms-depth area) on the 23rd*. On the 26th, the flagellate was pretty common in the 4 fathoms area. During the following days (28th September to 10th of October, 1953) there was a bloom of the organism very near the shore, and the water was discoloured green on several of these days and found teeming with millions of the organism to the almost total exclusion of the other usual planktonic elements. The maximum concentration of the flagellate in the water was reached on the 3rd of October when the water was opaque and brownish green in colour as it was in November, 1949. On this day 4 litres of sea water brought from the shore and left standing for about 3 hours showed the organisms settled down to a volume of 1½ litres. Since the 6th of October, the concentration of the flagellate in the water declined. The other particulars relating to the organism and its occurrence are discussed elsewhere.

It may be mentioned here that the water in which the flagellate occurred, has at times an amber tint due to the suspension of minute particles of mud in it. It has been noticed that the flagellate generally occurs following the stormy south-west monsoon season when, due to agitation of the water, the sea bottom which consists of mud on this coast (Hornell, 1910, pp. 72–73) is stirred up and the fine suspension of mud particles imparts an amber or ochreous colour to the water, and probably also to the flagellate sometimes;

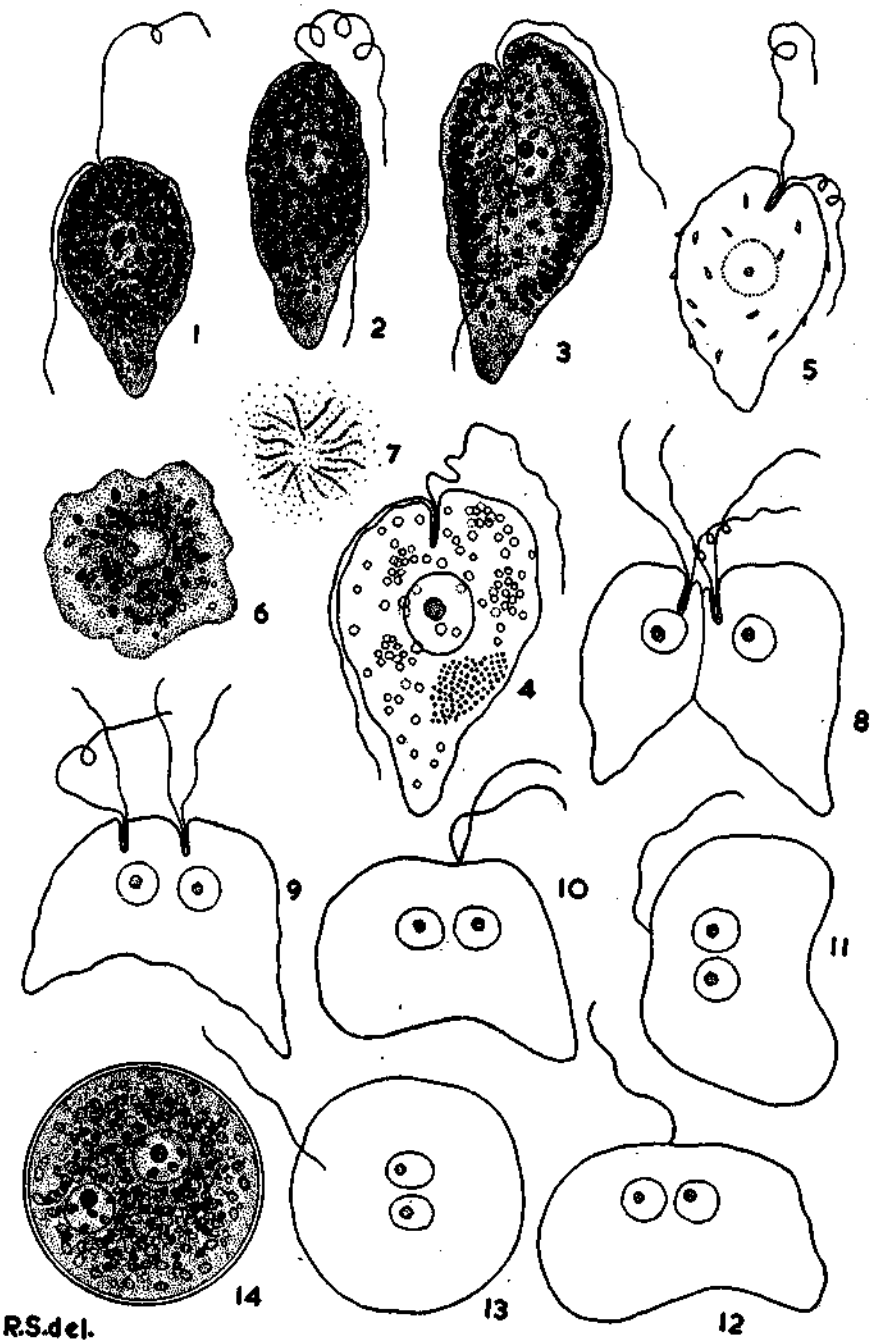
* *Particulars of the hauls:*—8 fathoms and 16 fathoms areas: vertical hauls, from bottom to surface; surface, horizontal hauls; bottom, horizontal hauls at a depth of 15 and 25 metres respectively; and in the 10 fathoms area vertical haul from bottom to surface.

but the green colour of the individuals is unmistakable and when occurring in millions the water also is distinctly green.

III. Description of the Flagellate

Structure.—The flagellate (Figs. 1–3) has a compressed pear-shaped body, with the anterior end broad, and the posterior drawn out into a short stumpy tail. The periplast is delicate and slightly undulated. The surface of the periplast, under high magnification, shows tiny glistening punctæ-like dots (Fig. 4), perhaps homologous with the striations or sculpturing seen on the periplast of the Euglenineae. At the anterior end, there is a narrow canal-like vacuolar reservoir or " gullet " situated a little to one side. Two flagella, more or less of the same length, arise close to each other from the base of the vacuolar reservoir; one is directed forwards and the other backwards trailing closely on one side of the organism (Figs. 1–4). The latter is seen only on careful examination and is likely to be overlooked when the flagellate is swimming about. The anteriorly directed flagellum is more active than the other. The flagellate is very active and rotates on its own axis while swimming about.

Numerous bright green, disc-shaped chromatophores are distributed all over the body of the flagellate at the peripheral region leaving a central space in which is seen a fairly large nucleus with a large nucleolus. The tail end of the flagellate is without any chromatophores, and is colourless. The individual chromatophores which are normally uniformly distributed (Figs. 1 and 2), appear at certain times to present their narrow edge towards the peripheral region tending to aggregate at the two sides (Fig. 3). This arrangement is invariably found in samples examined during the middle of the day. The orientation of the chromatophores in this manner is probably due to the influence of strong light. Similar orientation of the chromatophores in relation to light conditions is known to occur in some of the other algae also (*e.g.*, *Mougeotia,* Fritsch, 1935, p. 318) and in several groups of higher plants. In one instance, the flagellate kept in a glass trough settled down to the bottom at noon remaining there the whole day, but uniformly distributed in the medium in the trough the next morning. This is perhaps a reaction of the flagellate to the intensity of the light. In overcrowded samples of plankton, soupy in appearance, the chromatophores tend to become yellowish indicating an unhealthy condition. Moreover, after the concentration of the flagellate had attained its peak, the green colour of the water, which is obviously due to the colour of the chromatophores of the flagellate, tends to become yellowish green and then yellow as the phenomenon wanes, the changes corresponding to those of the chromatophores from a healthy to an unhealthy state.



FIGS. 1–14. *Hornellia marina* gen. et sp. nov.— Figs. 1 & 2. Flagellate, flat view and side view respectively, shown with contents. Fig. 3. Flat view of flagellate showing chromatophores oriented more towards the narrow sides of the flagellate under the influence of strong

light. Fig. 4. Flagellate shown with fat globules (?) and the "punctæ" (black dots) on the periplast (Chromatophores not shown). Fig. 5. Flagellate with wart-like protuberances (Contents of cell not shown). Fig. 6. Aspect of flagellate on bursting. Fig. 7. Thread-like substance ejected by the flagellate. Figs. 8–16. Sexual reproduction ; fusion of two individuals observed on 28–9–1953. Fig. 8. at 1–25 p.m. ; Fig. 9. at 1–30 p.m. ; Fig. 10. at 1–43 p.m. ; Fig. 11. at 1–45 p.m. ; Fig. 12. at 1–57 p.m. ; Fig. 13. at 2–15 p.m. ; and Fig. 14. at 3–40 p.m., the zygote surrounded by wall. Note flagella dropped off as fusion of individuals advances. (Figs. 2, 3 & 4, × 850; rest, × 700.)

The flagellate shows rich cytoplasm content. Numerous glistening globules (fats or oils ?) are distributed inside the cell (Fig. 4), which sometimes show passive movement due to the streaming of the cytoplasm in the cell. Neither starch nor any other recognisable inclusions are present.

The size of the flagellate varies from 26–73 μ in length, 16–35 μ in breadth and 16–26 μ in thickness.

Reproduction.—The flagellate reproduces both vegetatively and sexually. During vegetative reproduction, the individual undergoes longitudinal division while still swimming about and forms two daughter cells. The division appears to commence from the anterior end. A closer examination was not possible owing to the motility of the organism and its extreme sensitivity which forbids the use of fixing fluids.

During sexual reproduction two ordinary individuals attach themselves by their anterior region and become stationary. Gradually the cytoplasm of both merge into one. During the last stages of this process, the mass exhibits metaboly which appears to hasten the union of the two protoplasts. The stages in the fusion of the two individuals are represented in Figs. 8 to 14. In the course of the process, the flagella, of the two individuals, are dropped off one by one as the union of the protoplasts progresses. The zygote becomes rounded with a wall surrounding it (Fig. 14). Two nuclei are seen in the zygote, one contributed by each of the partners. After a couple of days the zygote was noticed to be brownish-orange in colour and, from its nature, would appear to have a period of " rest " before undergoing germination. Observations are being continued on them. Fusion of the two nuclei in the zygote may occur only after sometime as in some of the Volvocales, Zygnemoideae (Fritsch, 1935, pp. 116 and 333) and Diatomaceae (*Navicula halophila*, Subrahmanyan, 1946, p. 259).

The flagellate resembles in its vegetative reproduction several of the unicellular motile members of the other classes of Algae and, in its sexual reproduction, *Dunaliella* and *Polytomella* of the family Polyblepharidaceae (Chlorophyceae). This subject is further discussed in another section.

Behaviour of the Flagellate.—All attempts at fixing and preserving the flagellate proved unsuccessful. The addition of a fixing fluid leads immediately to its disintegration into an indefinable, hyaline speck of matter shaped like an amœba, in which the chromatophores, several refractile glistening globules and a nucleus stand out (Fig. 6). A drop of water containing the organisms kept under observation for some time becomes an amorphous mass due to the bursting up and coalescence of the individuals. Before breaking up wart-like protuberances become visible on the body of the flagellate (Fig. 5) and it is almost an indication that the flagellate would disintegrate shortly. Sometimes, the moving flagellate stops and ejects several specks of a hyaline refractile substance, and soon after moves away and disintegrates, with the substance ejected also dissolving. The trailing flagellum comes into full view as the flagellate is tending to break up (Fig. 5) and both the flagella drop off almost immediately. The same effect ensues on keeping a rich collection of the organism in a glass trough, often resulting in the death of the individuals. The dead clump of organisms thus formed sinks to the bottom and appears as a highly slippery mucilaginous mass, bulky, having the consistency of a thick syrup and coloured more yellowish than green. Sometimes, on careful examination, the outline of several individuals in their burst condition could be made out (Fig. 6). The mucilaginous mass before long, on certain occasions, emitted an unbearable odour. Very rarely, in one or two instances, when the flagellate in few numbers was kept in the laboratory in troughs, a few showed a tendency to lose the pear-shaped appearance, become rounded, the tail being not evident. In this instance also the flagellate died out ultimately. It was possible to keep the flagellate alive in the laboratory for about a week only. Whatever be the concentration of the flagellate in the water, as long as it is alive, no putrid smell ensues from the sample. The sample sent to the writer in August, 1949, from Kozhikode was of the amorphous nature described above. Because of the extreme sensitivity of the flagellate, it could be studied only by observing the living material and by exposing it to osmic acid vapour for a moment, which alone kept the flagellate quiescent for a short while enabling sketches to be made. A very dilute aqueous solution of iodine also proved useful at certain times to study the flagellate.

IV. Discussion

Identity of the Flagellate.—Hornell (1917) and Hornell and Nayudu (1923, pp. 141–42, Pl. III, Figs. B, B^1 and B^2) have described an organism almost similar to the present one occurring in amber coloured sea water on the Malabar Coast, which they designate as an "Euglenid", "Flagellate B", "Infusorian", "Protozoan", etc. This organism, it is reported, when

occurring in high concentration, has a deleterious and fatal effect on all the other marine organisms; when living, it causes asphyxiation and when dead, liberates toxins during its decay. They have observed the beach strewn with dead fishes, crustaceans, burrowing molluscs and the sand-rooted alcyonarian, *Cavernularia* (*op. cit.*, 1917, pp. 54–55; 1923, p. 142). However, Hornell (1917, p. 65) states that, "The masses of jelly-cased resting euglenids which accumulate on the bottom form an important food source of the oil sardine (*C. longiceps*)† and hence possess an important economic value as an offset to the mortality they occasionally entail among fishes." But elsewhere (*op. cit.*, 1917, p. 54) and in the later account (*op. cit.*, 1923, p. 142) it is observed that mortality of sardines as well as other marine organisms also occurs when a swarm of the "Euglenid", "Euglenoid" or "Flagellate B" occurs in the water. Jacob and Menon (1948) also recorded mortality of fishes on the west coast in 1944 and 1946 and attributed it to the occurrence of "Euglenoids" similar to those observed by Hornell and they support the latter's observations in this regard.

In the other instances where mortality of fishes has been reported to occur in Indian waters owing to the superabundance of a micro-organism, the concerned forms were Peridinians (Hornell, 1917, p. 63), *Trichodesmium erythraeum* (Chacko, 1942; Chidambaram and Unny, 1944) and *Noctiluca* (Aiyar, 1936; Bhimachar and George, 1950).

According to Aiyar (1936, p. 488) it would appear that an oxygen deficiency results during the swarming of *Noctiluca*, caused by several factors leading to the mortality of marine fauna. Bhimachar and George (1950, pp. 348–49) state that the water rendered slimy by decaying *Noctiluca* causes mechanical obstruction to the movement of fish while its foulness is toxic to fish life, and the fish either die or pass out of such waters. There is also, according to these authors, considerable destruction of fish food, fish eggs and larvae; moreover, the avoidance of such waters swarming with *Noctiluca* by the majority of fish population is of greater consequence to the fishery production rather than mortality of a few fishes. Hornell (1917, p. 65), however, did not observe mortality among fishes during abundant occurrence of *Noctiluca* on the west coast and held that *Noctiluca* is not an active agent in causing fish mortality and is innocuous. Prasad (1953, p. 43) did not notice any mortality of marine life during the swarming of *Noctiluca* in the Palk Bay nor any appreciable reduction in the oxygen saturation during the period. He, however, believes (*op. cit.*, p. 46) that pelagic fishes avoid such areas. Oxygen data collected at Kozhikode for over four years show that there is no correlation between abundant occurrence of *Noctiluca* and oxygen saturation of the water.

It may be mentioned here that in September, 1952 and 1953, a mortality of marine organisms (similar to that observed by Hornell, Hornell and Nayudu; and Jocob and Menon during the occurrence of the organism described by them as "Euglenoid" and so on), was noticed in and near about Kozhikode during the

† = *Clupea longiceps* = *Sardinella longiceps* (Cuv. et Val.)

bloom of the flagellate described in this paper. In September, 1952, dead fishes were observed on the shore on the 7th and 8th.

During September, 1953, on the 21st a few numbers of the flagellate here described appeared in the plankton in the 8- and 10-fathoms areas along with some of the usual Ciliates, Diatomaceae and Dinophyceae including *Noctiluca*. The fishermen of the Laboratory were asked to keep a watch for dead fishes or other marine organisms floating in the sea. On the 26th they were sent for an examination of the surface of the sea and for plankton collection. They reported dead stinking fishes in considerable numbers floating on the surface of the sea beyond the 3 fathom line. The following fishes were found dead and floating in the sea: *Otolithes argenteus, Kowala coval, Anchoviella heterolobus, Nemipterus japonicus* and *Mugil speigleri*.

Sample of the plankton from the area showed the flagellate here mentioned in considerable numbers, but not in such concentration as to discolour the water. No *Noctiluca* was present in the sample, but a few Diatomaceae, mostly unhealthy, and a few of the naked Dinophyceae occurred along with some Ciliates. It would appear that a bloom of the flagellate had occurred in some other locality bringing about a mortality of the fishes and the fishes so dead were being washed ashore. From the stinking condition of the fishes it may be surmised that death had occurred a considerable time earlier. During the following days (28th September to 9th October) the flagellate appears to have "moved" nearer to the shore and the water was discoloured green on some of the days (*vide* Table I). There were practically no other plant or animal organisms present in the water except empty shells of some of the Diatomaceae and a stray specimen of a species of *Navicula* and *Gyrosigma* which were alive, as also some stray Ciliates. Even these were absent on the 3rd when the flagellate occurred in a high concentration (*cf.* p. 184). The fishermen are positive in their assertion that fishes will not enter *this* green zone of discoloured water and if they do they die soon.

Hornell (1917, p. 56) describes the flagellate observed by him thus: "I did however examine the water microscopically and to my surprise found it to be full of myriads of brownish yellow Euglenoids to the virtual exclusion of all other organisms. The Euglenoids were filled with very granular protoplasm; had a large colourless nucleus and contained many minute dirty yellow chloroplasts and usually several fairly large oil globules. Unlike the typical Euglenoid of fresh-water, this specimen had no red eye-spot. A long flagellum emerged from a well marked pit at the blunt end of the body."

In a later account it is stated about "Flagellate B" (Hornell and Nayudu, 1923, p. 141): "It is a pear-shaped flagellate infusorian, dirty brownish in colour. The protoplasm is much granulated. A distinct 'gullet' is present at the wide anterior end, from which a long and stout locomotor flagellum issues. While the organism is in motion the flagellum is directed forwards. When watched upon a slide under the microscope, the animal after a little while becomes less active and

the body deformed while wart-like protuberances appear on the surface; the long flagellum becomes entirely retracted and a short slender vibratile one comes into view, which appears to be such an accessory flagellum as has been noted to occur in *Trentonia flagellata* Stokes, an infusorian probably closely related. Finally the deformed rounded mass which the organism now becomes, bursts with explosive suddenness, and is resolved into a granular film in which minute oil globules and yellowish chromatophores are scattered. It is noticeable that this infusorian is particularly rich in oil-globules." Further they (*op. cit.*, 1923, pp. 141–42; *cf.* also Hornell, 1917, p. 56) observe that, "When a sample of the amber brown water containing Flagellate B in abundance is allowed to stand for a while the organisms settle rapidly to the bottom where they form a gelatinous deposit of considerable bulk. In this jelly-like mass derived from the swelling up and coalescence of the colourless limiting membrane or cortex of each individual, they are seen to be embedded, more or less rounded in form, inactive and without a trace of a flagellum. The colour of the mass as a whole is dirty yellowish." Jacob and Menon (1948, p. 457) also observed a similar settling down of the "Euglenoid" observed by them to form a light yellow jelly. They believe the organism to be the same as the one observed by Hornell.

These remarks of Hornell and Hornell & Nayudu quoted here are borne out by the writer's observations on the flagellate described in this paper except their reference to the "short vibratile flagellum" and the reported colour of the chromatophores.

Both the flagella in the present organism are more or less of the same length. The writer has observed that the flagella have a tendency to drop off wholly or portions get detached when fixing fluids are added or even when the individuals look apparently normal; in instances such as the latter, the flagellate soon disintegrates (Fig. 5). It is not unlikely that Hornell and Nauydu are referring to such a flagellum when they state that one is short and vibratile. A short flagellum has always a tendency to vibrate while a long one lashes about. It may be noted that the "short" one seen by these authors was at a stage when the flagellate was tending to break up. Jacob and Menon (1948, p. 457) who centrifuged their material prior to examination failed to observe any flagella on the organism. The writer has noticed in other instances such treatment of certain flagellates often leading to the dropping off of the flagella and sometimes even bringing about a change in the orientation of the cell contents. This would explain the failure of Jacob and Menon to establish the flagella on the "Euglenoids" recorded by them.

Hornell (1917, p. 56) and Hornell and Nayudu (1923, p. 141) state that the chromatophores are coloured "dirty yellow" or "yellowish". Jacob and Menon (1948, p. 457) observe that the "Euglenoids" were brownish-yellow with plenty of chloroplasts. It has already been pointed out that the chromatophores which in the healthy individuals are bright green, tend to be coloured yellowish when the concentration of the flagellate in the water is rather high and it was observed that that appeared to be an unhealthy state. Plankton samples of such a nature very

soon settle down to form a jelly-like mass already referred to. The accounts of these authors cited above deal with the organism at a time when it either occurred in such a quantity as to discolour the water and/or caused fish mortality thus attracting their attention to the phenomenon, and it would appear that they have been able to visit the affected areas where this occurred only after the phenomenon had already come into existence or begun to wane; in other words, they have not been able to follow the sequence of occurrence of this organism by a continuity of observations, which alone could have explained the change in the colour of the chromatophores from green to yellow. It is not improbable that the suspension of fine mud particles colouring the water amber or amber brown in which the flagellate occurs sometimes may also have hindered a correct assessment of the colour of the flagellate and of the chromatophores.

In this connexion, it is interesting to note that while recording a calamitous mortality among sardines during his cruise along the west coast between Cannanore and Mangalore in 1908, Hornell (1910, pp. 101–05) found in places where the dead sardines were fewer, the water was coloured *greenish-grey*; as he steamed into areas showing greater number of dead sardines, the colour of the water was *yellowish* and then *dark-yellow*; and where the water was *darkest yellow* or *ochreous yellow* dead sardines appeared most numerous. A haul of the plankton consisted of (*op. cit.*, p. 103) "obscure organic and unrecognisable debris, so fine that the meshes of tow-net became clogged almost immediately, rendering it difficult to collect a satisfactory sample". He was unable at that time to establish the presence of any organism in the plankton as being responsible for this phenomenon except bacteria in quantity in the water (Hornell, 1917, p. 55) which apparently is a reference to the crust of bacterial scum covering and connecting the bodies of fishes which in thousands were found by him in one continuous sheet of putrefaction (Hornell, 1910, p. 104). He also quotes (*op. cit.*, p. 104) a somewhat similar strange mortality among fishes noticed by Dr. Thurston in 1899. Though Hornell, at that early time had pointed out a probable source for this foul water to lie in the contamination of the backwaters and rivers wherein the process of steeping coconuts is carried on, on an extensive scale, the change in the colour of the water mentioned by him which resembles somewhat the change in the colour of the chromatophores from a healthy to an unhealthy state, referred to earlier, is worth noting. The discoloration of the sea then was, very probably, due to the same organism recorded in later years by him (1917), and himself and Nayudu (1923) and designated "Euglenoid", "Flagellate B", "Protozoan", etc.

The same authors (Hornell and Nayudu, 1923, p. 141) appear to believe that the jelly-like mass resulting from the settling down of the numerous individuals of the flagellate is a kind of a resting stage when the sea is very calm. They recorded the presence of such a jelly at the bottom of the sea to a depth of 2–3 feet and believed that the range of the organism seaward is greater. They presume that the flagellate assumes "the motile stage only so long as the water is in gentle agitation; in very calm weather this area is quite narrow and it is probable that for some

distance seawards from the limit of breaking or disturbed water the organism remains in the quiescent condition but ready to throw off new swarms of the motile form when the proper excitation recurs." In the earlier account Hornell (1917, p. 66) claims that a bottle containing the "Euglenid jelly" kept by him for nearly six months did not putrefy but showed the same appearance it did when first it was formed as such by the free-swimming "Euglenoids". The only difference noticed by him was that the chloroplasts were distinctly more green than "when the jelly stage was entered upon". No further details are available as to the ultimate fate of this jelly. As already mentioned the flagellate described here also forms an amorphous jelly-like mass at certain times which tends to putrefy and emit an unpleasant odour as was also noticed sometimes by Hornell and Nayudu (1923, p. 142) in the case of their "Euglenoid" cited above; at other times it appears to remain healthy as recorded by Hornell (1917, p. 66). This point which appears to have a bearing on the life-history of the organism is treated separately in another section dealing with the reproduction of the organism.

Nevertheless, the structure and behaviour of the flagellate and its more or less seasonal occurrence about the same time during successive years (*vide* Table I; also more or less noticed by the earlier workers cited above), its effects on the marine fauna when occurring in high concentration, and the absence of any other similar flagellate organism in the plankton answering to the same description in spite of careful and intensive search during the past four years leads to the inescapable conclusion that the organism described by Hornell (1917), Hornell and Nayudu (1923) and Jacob and Menon (1948) under various names such as "Euglenoid", "Flagellate B" and "Protozoan", etc., and alleged to cause mortality among marine fauna, particularly the calamitous mortality of sardines in 1908 recorded by Hornell (1910), and the flagellate here described are all one and the same.

In connexion with the identity of the flagellate concerned here, a reference may be made to the remarks of Bhimachar and George (1950, p. 348) who differ from Hornell (1917, p. 65) and hold that abundance of *Noctiluca* is followed by their death and decay and formation of a slimy substance which causes fish mortality at times and a definite set-back in the fisheries and go even further and state, "On the other hand we are inclined to think that the only case of severe mortality of fish recorded by Hornell (1910) off Mangalore was due to the abundance and subsequent death and decay of *Noctiluca*". They quote in support Hornell's (1910, p. 103) statement, "A haul of plankton where dead sardines were plentiful almost entirely consisted of obscure organic and unrecognisable debris, so fine that the meshes of the tow-nets became clogged almost immediately rendering it difficult to collect a satisfactory sample," and say that this agrees well with their observations during occurrence of "red water" in October 1948. It is difficult to conceive that Hornell, who cruised over a very vast area of sea showing dead sardines floating in large numbers, would have failed to observe *Noctiluca* either in its decaying or putrefying condition if this was the organism responsible for the mortality of sardines reported by him. Hornell's (1910, p. 101) description of the colour

of the water at the place of occurrence of the phenomenal mortality of sardines, already referred to earlier—a change from greenish-grey to yellow and darkest yellow—may be recalled here. The colour could not have been the same if *Noctiluca* were the organism involved; the observation agrees more with the state of changes had the organism been the flagellate recorded here. The jelly-like, amorphous, yellowish mass to which state the swarm of the flagellate observed settles could not have been described in any other manner than as "obscure organic and unrecognisable debris", for it certainly is so in appearance. It is all the more likely to be so if bacterial action had set in which, evidently, appears to be the case for, Hornell mentions an unbearably foul smell emanating from the area concerned.

It may be interesting to mention here that in September, 1952, during the bloom of the flagellate described here, *Noctiluca* in normal healthy condition was also present in all the plankton hauls (surface and bottom horizontal, and vertical). This feature was again seen in September, 1953, in the beginning of its occurrence. Subsequently when the concentration of the flagellate increased, *Noctiluca* was absent in the region concerned, but was present in the collections from other areas where the flagellate was absent, *i.e.*, 4 to 16 fathoms area, and the plankton in these regions besides showed several of the usual phyto- and zoo-plankton elements in their healthy state and normal fish collections had also been made from these areas including the 4-fathom area where *Noctiluca* was abundant. In other words, sometimes, the flagellate gains the upper hand and blooms to the total exclusion of the usual phyto- and zoo-plankton organisms as it did in November, 1949 and September, 1953. Hornell (1917, p. 61) has also noted that forms of plankton abundant in neighbouring uncontaminated water were practically absent from the water discoloured by his "Euglenid". It cannot be stated with certainty whether this condition is brought about by any deleterious effect the flagellate exercises on the other organisms or owing to currents, etc.

Prasad (1953, p. 43) observed that the swarming of *Noctiluca* is not accompanied by their death and decay and subsequent formation of a slimy substance as noticed in 1948 by Bhimachar and George (1950, p. 347). The writer also, during the course of his study extending over 4 years in the sea off West Hill where occurrence of *Noctiluca* frequently in a swarm condition is a regular feature every year from June to September and even October, did not come across any such slimy decaying matter following an abundant occurrence of this Dinoflagellate. In the light of the observations of Hornell (1910, 1917), Hornell and Nayudu (1923), Prasad (1953) and of the writer, and also in view of their statement (Bhimachar and George, 1950, p. 347) that "It must, however, be admitted that even in the present instance, to begin with, large pink patches of *Noctiluca* were noticed on the surface waters at several places in the coast, which soon gave place to slimy masses", it would appear that, presumably, there has been a mistake in the identity of the organism giving rise to the "slimy" matter; and, it is likely that the organism concerned is the flagellate described here. It has been noticed that sudden striking changes in the constituents of the plankton occur sometimes

in the sea off West Hill (Subrahmanyan, unpublished observations) and in this particular instance, it would appear that the "succession" of *Noctiluca* by the flagellate may have been overlooked by these authors; and, the recent observation of the writer made in September, 1953, when a similar change occurred lends additional evidence in support of the interpretation put forward here. It may also be recalled here that the appearance of the flagellate is sporadic and only of short duration.

Bhimachar and George (*op. cit.*, p. 348) further observe that " mere abundance of *Noctiluca* unaccompanied by their death and decomposition is harmless to the fishery" and state that "The contention of Hornell that abundance of *Noctiluca* is innocuous to fish life while the dominance of euglenoids is the prime cause of mortality of marine animals, especially fishes, along this coast, appears to us to be untenable." From what has been given above it is probable that swarms of *Noctiluca* may not affect the fauna leading to their mortality; on the other hand, the present form, the individuals of which have been observed under certain conditions to coalesce to form a highly slimy and bulky matter, appears to be the organism concerned in the mortality of marine fauna on the west coast recorded by earlier workers.

Hornell's (1900, p. 103) description of the plankton from the place of the calamitous mortality among sardines, cited above, is favourably comparable with his later observations during the occurrence of his "Euglenoid" (Hornell, 1917; Hornell and Nayudu, 1923), those of Jacob and Menon (1948) and those of the author on the flagellate described here rather than to an appearance presented by dead and decaying *Noctiluca*. The writer is inclined to the conclusion that the organism involved in the mortality of fishes recorded in 1908 by Hornell (1910) is not *Noctiluca* but the flagellate mentioned above.

Again, Bhimachar and George (1950, p. 348) state that on 29th August, 1949, "dense populations of Euglenoids mixed with a small number of Ciliates made their appearance in the shore waters near West Hill" and a bottom haul of the plankton taken at the time revealed the presence of a thick layer of putrefying *Noctiluca* and prior to this date there was an abundance of *Noctiluca* in this part of the sea.

It is well known that Ciliates tend to develop very fast when sea water is kept stagnant or when the several normal elements of the plankton are absent, or in water in which organic matter is decaying. De Morgan (1925, 1926) has described a number of species of Ciliates living in the Laboratory tanks at Plymouth. Many species of saprophytic Euglenineae also are known to multiply in such a medium (*cf.* Fritsch, 1935, pp. 734–35; Jahn, 1951, p. 69). The writer has observed Ciliates developing in abundance in the media kept in glass troughs in which the present flagellate placed for observation had died out whereas in the collection itself there was no decaying organic matter evident and Ciliates practically non-existent. Bhimachar and George (1950, p. 348) on the strength of such observations in the laboratory by keeping decaying *Noctiluca* in glass troughs, appear to

suggest that what occurred in the sea in August, 1949, was of a similar nature. However, on the basis of observations made by the writer on the sample sent to him in 1949 and on the basis of his subsequent study of the flagellate during its occurrence over a period of 4 years, to all of which earlier observations of Hornell (1910, 1917), Hornell and Nayudu (1923) and Jacob and Menon (1948) lend full support, he is convinced that the organism which causes a discoloration of the sea and mortality of fishes at times is not a member of the Euglenineae but the flagellate described here and termed by earlier workers as "Euglenid", "Euglenoid", "Flagellate B" and so on, owing to their inability to establish its correct identity. It does not appear to be the "Euglenoid" which is recorded by Bhimachar and George as developing in the laboratory cultures in a medium containing decaying *Noctiluca*, which is probably a genuine *Euglena* sp. The writer has recorded true *Euglena* sp. in the plankton on a number of occasions in the post-monsoon months along with Ciliates, Tintinnids and Gymnodineaceae. Hornell and Nayudu (1923, p. 147, Pl. III, Figs. E and E 1–4) also have recorded typical *Euglena* sp. sometimes occurring in profusion. This *Euglena* sp. which shows the characteristic structure (metaboly, green chromatophores, paramylon, flagellum, eye-spot and so on) could be kept under observation and preserved unlike the present flagellate which is highly sensitive. Moreover, the present flagellate appears to be an autotrophic one and not a saprophyte.

The flagellate at first glance is likely to be mistaken for an *Euglena* as it has been so done by the earlier workers; its shape, colour of the chromatophores, etc., are not unlike those of a species of the genus. But the appearance of wart-like protuberances under certain conditions, its bursting up to form an amorphous mass, its delicate periplast, and absence of metaboly, paramylon and eye-spot, all show a resemblance to the members of the Chloromonadineae, a Class of Algae of uncertain relationships (*cf.* Fritsch, 1935, p. 723). It is interesting to note that Hornell and Nayudu (1923, p. 141) came very near to identifying the flagellate recorded by them by drawing attention to its resemblance to *Trentonia flagellata* Stokes (Stokes, 1886; 1888; Penard, 1921–22, pp. 160–65, Pl. VIII, Figs. 64–67 *a*, *b*; *cf.* Fritsch, 1935, p. 721, Fig. 238 D), a member of the Chloromonadineae, and the only form showing some resemblance to the present flagellate also. It differs from *Trentonia flagellata* in having only a narrow canal-like vacuolar reservoir which is placed slightly to one side at the anterior end and absence of metaboly and contractile vacuole and in its marine habitat. In *Trentonia* the anterior end is obliquely truncated and the reservoir is triangular in shape in optical section. Further, structures which are regarded as trichocysts have been recorded in this form. In the presence of wart-like protuberances at certain stages, the present flagellate resembles somewhat *Reckertia sagittifera* Conrad (Conrad, 1922; Fritsch, 1935, p. 725, Fig. 238, H and I) and *Thaumatomastix setifera* Lauterborn (Lauterborn, 1899; Fritsch, 1935,

p. 723, Fig. 238, J and K), colourless members of the Chloromonadineae, which readily protrude " pseudopodia". Contractile vacuoles are not present in this flagellate unlike the fresh-water members of the Class. This is in line with the well-known view tnat they are not present as commonly in marine as in fresh-water forms (Fritsch, 1935, pp. 35–36; *cf.* Panikkar, 1948, pp. 7–8). The characteristics of the flagellate and their resemblance to the Chloromonadineae cited here permit its inclusion in this Class. The present form appears to be the first in the Class to be recorded from a marine habitat and occurring in quantity. As it is different from all other genera known so far, a new genus is created and named *Hornellia*‡ and the flagellate itself named *Hornellia marina* sp. nov.

Effect of the Bloom of the Flagellate on Marine Organisms.—How exactly the flagellate is involved in the causation of mortality of marine organisms, whether directly or indirectly, is not clear at present, for, the evidences so far sought out are circumstantial. Explanations have been offered that death is caused by asphyxiation due to lack of oxygen and/or due to toxic effects of the decaying matter of the flagellate (Hornell, 1917; Hornell and Nayudu, 1923; and Jacob and Menon, 1948).

The writer wishes to place for consideration here purely as hypotheses some points which might explain how the flagellate affects marine organisms fatally. It was mentioned earlier that the flagellate puts out wart-like protuberances under certain conditions. A closer examination of these structures in the flagellate could not be made owing to the extreme sensitivity of the organism. Nevertheless, their nature permits a comparison with the nematocysts or trichocysts occurring in the other members of the Class, *Gonyostomum semen* Dies, *Raphidomonas* sp. (*cf.* Fritsch, 1935, pp. 721-23) and *Trentonia flagellata* (Penard, 1921-22, p. 162), where they become protruded as long threads under the influence of stimuli. The resemblance of these organs to the nematocysts of Coelenterates is striking and it may be mentioned that almost similar structures are known to occur in some of the Dinophyceae also, *e.g.*, *Polykrikos, Gymnodinium, Gyrodinium* and *Nematodinium* (Kofoid and Swezy, 1921; Lebour, 1925; Schiller, 1933; Graham, 1951; and Fritsch, 1935). It is tempting to compare the wart-like protuberances seen here with the nematocysts and it is not improbable that these may prove to be similar structures and function in a similar manner as those of the Coelenterates and when the flagellate is in bloom its enormous number is capable of stinging and poisoning the fishes and other marine organisms to death.

It was also mentioned that sometimes the flagellate stops while in motion, ejects several of a colourless thread-like refractile substance, moves away and disintegrates; and the substance soon dissolves away. The nature of this substance

‡ Name after the late Dr. James Hornell, a former Director of Fisheries, Madras, who was the first to record and make a detailed study of the flagellate.

could not be determined; however, this behaviour of the flagellate is significant and the substance so ejected by millions of them may also be considered to have a deleterious effect on the marine organisms in the region or coming into the region where the flagellate is in bloom. In this respect the flagellate shows a resemblance to *Trentonia flagellata* in which the organism has been observed to eject a " white cloud " when bursting or when brought into contact with India ink; in the latter case the organism leaps forwards, backwards or to one side throwing out or discharging the " white clouds " (Penard, 1921-22, p. 164).

A third possibility may also be mentioned. It was stated earlier that when a rich collection of the flagellate is kept in a trough, the individuals settle down to form a highly slippery mucilaginous bulky mass having the consistency of a thick syrup. It is possible that fishes trapped in this mass or that happen to enter it meet with death as swimming out of such a syrupy mass will be impossible.

The hypotheses put forward here are tentative. Data from direct observations will be necessary to substantiate the rôle of this flagellate in causing mortality among marine organisms.

Reproduction and Affinity.—Very little is known about the reproduction of the members of this Class. It has been described only for *Vacuolaria virescens*, where the individuals divide longitudinally after they have come to rest and have become enveloped in mucilage. Spherical cysts with a thick mucilaginous envelope are also known in this form (Fritsch, 1935, p. 721). In the present flagellate, as already mentioned, division takes place while the individuals are actively swimming about and in this respect it resembles some of the Chlamydomonadineae (*Brachiomonas, Sphaerella, Polytoma*) the simpler motile Chrysomonadineae (*Microglena*), some Cryptomonadales and some of the Euglenineae (*Eutreptia, Astasia, Distigma*) (*cf.* Fritsch, 1935, pp. 92–93; 521; 659; 740). In some of the Dinophyceae also (Fritsch, 1935) vegetative reproduction takes place by longitudinal division but often the plane of division is oblique except probably in *Prorocentrum*; the individuals may be motile or stationary during the process (Kofoid and Swezy, 1921; Fritsch, 1935).

Mention has already been made of sexual reproduction in the present flagellate, in which two ordinary individuals somewhat different morphologically fuse forming a zygote. This fusion of two ordinary individuals of unequal size indicates a probable existence of two strains in the flagellate; it is likely that a detailed study of the germination stages might throw some light on this point. The zygote appears to be a " resting " type. All attempts at keeping the flagellate or the zygotes for any length of time in the laboratory have proved futile so far. However, observations are being continued.

Examination of the sticky mass to which state the flagellate settles down at times showed several zygotes amongst numerous disintegrated individuals. It is likely that after a period of vegetative activity, the flagellate undergoes sexual reproduction and settles down as zygotes and bursts into activity again when the zygotes undergo germination with the onset of favourable conditions. In this connexion, the observation of Hornell (1917, p. 66) on the "Euglenid jelly" remaining without undergoing putrefaction for nearly 6 months is significant; it is probable that *that* was a collection containing zygotes. It would appear that if the "jelly" is formed at an earlier stage in the vegetative phase of the flagellate, it undergoes putrefaction; if at a later stage as a result of sexual reproduction it remains healthy and represents a stage in the life-history.

The present record of sexual reproduction for the first time in a member of the Chloromonadineae indicates for this Class of flagellates an affinity with the Chlamydomonadineae, especially the family Polyblepharidaceae, where (*e.g.*, *Dunaliella*, *Polytomella*, Fritsch, 1935, p. 109) the individuals are without a firm cell wall and fusion takes place between two ordinary cells (hologamy) and the resulting zygote is motile. In the present instance, however, the individuals concerned in fusion are unequal in size, one being larger than the other; on being attached by their anterior end, they become quiescent and fusion is completed in this state; the flagella are dropped off one by one; and ultimately a "resting" zygote results.

In this connexion the observation of Penard (1921–22, p. 165) on *Trentonia flagellata* may be cited here. He recorded gelatinous cysts very similar to those of *Cryptomonas ovata* which contained either one single individual or very often two, longitudinally applied to each other; sometimes, instead of two, four individuals were found inside the cysts, all of which he regards as products of divisions. In view of the establishment of sexual reproduction in the present flagellate, it is not improbable that the cyst recorded in *Trentonia* represents a zygote and the products the result of the germination of the same. A similar sequence may occur in the present flagellate also on the germination of the zygote.

It may be interesting to mention here that this Class of flagellates is considered as a highly specialised group of organisms and is being treated by authorities as an isolated Class and of uncertain relationships (Fritsch, 1935, p. 723; Smith, 1951, p. 8). The results of the present investigation besides confirming the relationship of the Class to the Euglenineae in a few respects pointed out by Fritsch, *viz.*, the character of the chloroplasts and the vacuolar apparatus, also indicates an affinity for the Class with the family

Polyblepharidaceae in the Chlorophyceae. It is also probable that the vegetative phase in this flagellate is haploid as in the Chlorophyceae, Chrysophyceae and Xanthophyceae and the diploid phase resulting from the sexual fusion is confined to the zygote and the haploid phase is restored by meiosis during germination of the zygote.

V. Diagnoses

Hornellia gen. nov.

Flagellata chloromonadina, unicellularis, piro similis, corpore compresso; apex latus anterior ornatus canali vacuolari posito aliquantum lateraliter. Periplastum tenue. Flagella bina adsunt, quorum alterum protinus dirigitur, alterum vero trahitur corpori proximum. Chromatophori lucide virides, plurimi, discoidei. Nucleus atque nonnulli nitentes globuli conspicui.

Hornellia marina spec. nov.

Flagellata chloromonodina, unicellularis, piro similis, corpore compresso; apex latus anterior ornatus canali vacuolari angusto aliquantum lateraliter posito. Periplastum tenue, aliquantum undulatum. Ex basi canalis vacuolaris bina flagella emergunt, quorum alterum protinus dirigitur, alterum vero trahitur proximum corpori ad latus; utraque plus minusve eiusdem longitudinis. Flagellata active mobilis, rotans circum ipsius axem quandocumque movetur. Chromatophori lucide virides, plurimi, discoidei, distributi per totem cellulam prope peripheriam; apex posterior nullos habet chromatophoros. Nucleus prominens, positus in centro. Plures globuli nitentes (an lipoidei?) conspicui. Flagellata valde facile sentiens; cum per tempus aliquod sub observatione detinetur, vel stimulo quodam excitatur, emittit protuberantias verrucosas atque explodens degenerat. Reproductio vegetativ per divisionem longitudinalem. Reproductio sexualis per fusionem duorum corporum, quorum unum altero aliquantum maius est. Magnitudo variabilis; 26–73 μ longa, 16–35 μ lata, 16–25 μ crassa.

Typus lectus ad oras maritimas ad Malabar, in India, saepe valde prolifica aquas virides reddens, atque creditur mortem inferre piscibus marinis atque crustaceis.

Hornellia gen. nov.

Chloromonadine flagellate, unicellular, pear-shaped, body compressed; broad end anterior with a vacuolar canal situated a little to one side. Periplast delicate. Two flagella present, one directed forwards and the other trailing close to the body. Chromatophores bright green, numerous, discoid. Nucleus and a number of glistening globules present.

Hornellia marina sp. nov.

Chloromonadine flagellate, unicellular, pear-shaped, body compressed; broad end anterior with a narrow vacuolar canal situated a little to one side. Periplast delicate, somewhat undulated. Two flagella arise from the base of the vacuolar canal, one directed forwards and the other trailing close to the body of the flagellate on one side; both more or less of the same length. Flagellate actively motile, rotates on its own axis while in motion. Chromatophores bright green, numerous, discoid, distributed all over the cell nearer the periphery; posterior narrowed end contains no chromatophores. Nucleus prominent, situated at the centre. Many glistening globules (fats ?) conspicuous. Flagellate highly sensitive; when kept under observation for long or on some stimuli, puts out wart-like protuberances and it degenerates by bursting. Vegetative reproduction by longitudinal division. Sexual reproduction by fusion of two ordinary individuals, one of which is slightly larger than the other. Size of the flagellate variable; measures 26–73 μ in length, 16–35 μ in breadth and 16–26 μ in thickness.

Occurs in the sea off the Malabar coast, India, often in swarms colouring the water green and is alleged to cause mortality among marine fishes and crustaceans.

VI. Summary

The structure, life-history and ecology of *Hornellia marina* gen. et sp. nov., a member of the Chloromonadineae, are described in detail. The flagellate shows the following characteristics: body compressed, pear-shaped, broad end anterior; possesses two flagella arising from the base of a narrow vacuolar reservoir at the anterior end, one directed forwards and the other trailing close to the body; has a delicate undulated periplast; numerous bright green discoid chromatophores distributed all over the cell at the peripheral region except the posterior narrowed end; nucleus prominent at the centre; shows several glistening globules (fats ?); highly sensitive and puts out wart-like protuberances sometimes. Vegetative reproduction takes place by longitudinal division. Sexual reproduction takes place by the fusion of two ordinary individuals, one of which is larger than the opposite partner. This appears to be the first record of sexual reproduction in this Class and this indicates an affinity for the Class with the family Polyblepharidaceae of the Chlorophyceae. The vegetative phase appears to be haploid, the diploid phase being represented by the zygote as in many members of the Chlorophyceae, Chrysophyceae and Xanthophyceae. The form occurs in swarms at times causing a green discoloration of the sea off the Malabar coast of India and is associated with the mortality among marine organisms.

fishes, crustaceans, etc. Similar observations made earlier on this coast are referred to and discussed in connexion with the identity of the flagellate.

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