A NEW MEMBER OF THE EUGLENINEÆ, *PROTOEUGLENA NOCTILUCÆ* GEN. ET SP. NOV., OCCURRING IN *NOCTILUCA MILIARIS* SURIRAY, CAUSING GREEN DISCOLORATION OF THE SEA OFF CALICUT*

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Introduction

The presence of small green flagellates inside *Noctiluca* appears to have been first recorded by Weber and Weber-van Bosse (1890) who, however, did not observe the living material.

Delsman (1939–40) observed small green flagellates in large numbers swimming inside *Noctiluca*. He records them as a peculiarity of the tropical *Noctiluca* and that an accumulation of this *Noctiluca* at the surface imparts a green colour to the water. Further, according to him, this great quantity of *Noctiluca* is present in more or less brackish-water, quite near the coast.†

There appears to be no other record of this phenomenon nor any account dealing with these flagellates and their identity.

Occurrence

In recent months, the sea off Calicut was found coloured bright green in patches at several places. This occurred in December 1952 (10th to 13th), and again in March 1953, from the 12th onwards when it persisted for a longer period (several weeks). In December, the patches were seen extending from about three miles from the shore to about ten miles out; in March, the patches occurred nearer and extended from about a half mile to four miles from the shore. At night, it was reported, the sea glowed with a phosphorescent light in long patches extending North-South. The green discoloration of the sea water was found to be due to *Noctiluca* harbouring

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† Recently Prasad (1953) has recorded *Noctiluca* with a green flagellate inside it.

numerous green flagellates. It is well known that *Noctiluca* emits a phosphorescent light.

The patches of *Noctiluca* were also found to extend up to a depth of 36 metres; but, the individuals below the surface were noticed to be unhealthy unlike those occurring at the surface.

It may be interesting to mention here that, in the earlier years the plankton has been under study by the writer (May 1949 onwards), there was no bloom at all of *Noctiluca* (either with or without the green flagellates) during March as during this year. No *Noctiluca* was present in February and March 1950, November 1950 to April 1951 and February to April 1952. This year 1953, since the second week of March, *Noctiluca* with the green flagellates was the dominant form in the plankton eclipsing even the Diatomaceæ on several occasions, a very unusual feature. In December 1952, the bloom of ' green *Noctiluca* ' was poor as compared with that of March 1953.

During the occurrence of this green *Noctiluca* bloom, the salinity† of the surface water varied from 33‰ to 35·4‰. These values are more like those for normal sea-water than those for brackish-water and are comparable with the values for salinity recorded at this time of the year (which are among the highest values recorded here) during the earlier years. This is mentioned because of the fact that Delsman (1939–40) appears to emphasize the occurrence of *Noctiluca* in great quantity in more or less brackish-water near the coast.

An estimation of the quantity of phytoplankton at this time of the maximum occurrence of green *Noctiluca* by means of acetone extract (Harvey, 1934) gave a value of 5,200 to 6,800 Harvey units of pigment for approximately one metre-cube of sea-water. This value bears a close approximation to the average monthly values for phytoplankton bloom at its peak during the south-west monsoon period when the plankton consisted almost purely of Diatoms (Subrahmanyan, MSS.). It may be mentioned that the plankton now consisted almost entirely of this green *Noctiluca*.

Another interesting fact observed was that during the peak in the occurrence of this *Noctiluca*, high values were recorded for the silicates-content of the sea water, which bears a close inverse relationship to the absence of Diatoms in the catches. It has been observed (Subrahmanyan, MSS.) that a steep rise in the values for silicates, excepting the monsoon period, is more or less correlated with a fall in the quantity of the Diatom flora. For the corresponding period in the earlier years, the silicates values

† The data for salinity are from the records kept at the Sub-Station.

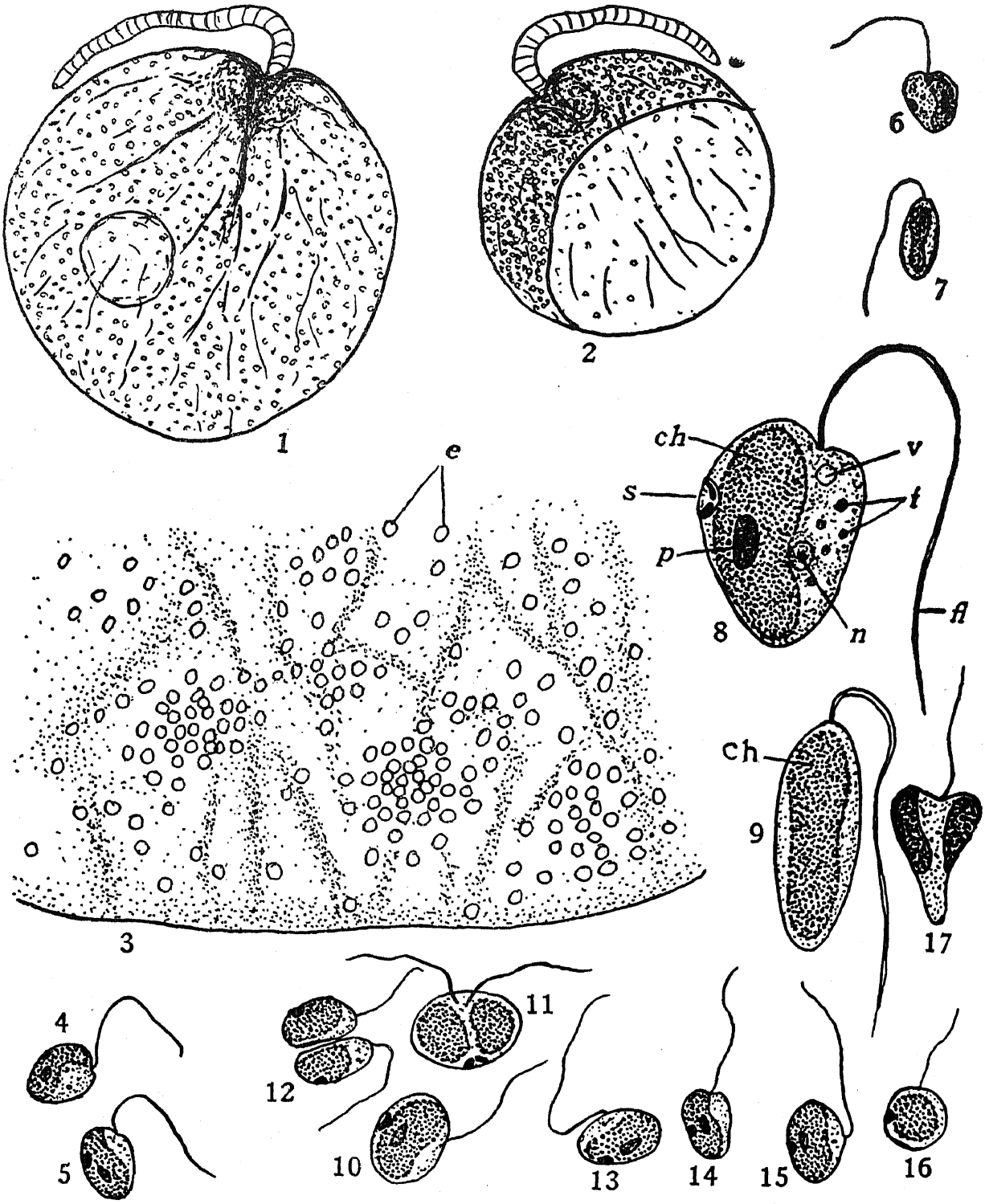
have never been so high as during this year when green *Noctiluca* occurred (recorded for the first time) dominating the plankton flora.

DESCRIPTION

The individuals of the *Noctiluca* in the plankton samples examined, showed hundreds of a tiny green flagellate swimming inside them. The flagellate was found normally uniformly distributed inside the *Noctiluca* (Figs. 1 and 3) which was alive and active as noticed by the waving of its flagellum; and, in other instances, they formed a crescent-shaped patch at the anterior end of the *Noctiluca*-cell (Fig. 2). The latter condition was more common when the plankton was crowded with *Noctiluca*, and in such instances, the *Noctiluca* also was less active or completely quiescent. The crowded samples emit an unpleasant odour when kept for some time. This leads one to infer that the conditions are not quite favourable for the flagellate. The individuals of the flagellate aggregate in large numbers near the anterior end of the *Noctiluca*-cell and then escape out and swim about in the water. Bottom samples of the plankton were invariably overcrowded and contained *Noctiluca* in the condition just described. Addition of a fixing fluid also (*e.g.*, formaldehyde) sometimes brings about this condition. Nevertheless, if a small quantity of the *Noctiluca* floating at the surface of the sample is taken and kept in a large volume of sea water, the material remains in a healthy condition for some hours. Attempts at keeping the green *Noctiluca* as such or the flagellate alone in cultures in the laboratory were not successful. At best they could be kept alive only for about six days.

When fixing agents are used, the flagellate shrinks and becomes rounded. Bouin's fluid (Gatenby and Painter, 1946), however, brings out most of its features if the fixing fluid is not allowed to act for long. The details regarding the structure of the flagellate are best studied in living individuals which have just come to rest and are quiescent.

The flagellate (Figs. 4 to 9) is pear-shaped, and is somewhat laterally compressed, the broader end being its anterior end. There is a slight invagination at the anterior end which bears a single long flagellum, which is two to two and a half times as long as the body (Fig. 8, *fl*). The narrow side-view of the flagellate is more or less elongate-elliptic (Fig. 9). The flagellate is 5 to 6 μ long, 4 to 4·5 μ broad and about 3 μ thick. It is actively motile and executes forward and generally rapid backward movements. It also often moves in a circle around a small space, rotating on its axis all the time irrespective of the nature of the movement. While moving forward, the flagellum is lashed more towards one side and while



(Figs. 1 and 2, ×80; Fig. 3, ×800; Figs. 4 to 7 and 10 to 16, ×1,700; Figs. 8 and 9, ×*c* 5,000).

moving backward, the flagellum is held more or less trailing, its distal end vibrating very actively.

The flagellate has no firm cell-wall. Its periplast is smooth. It exhibits slight metaboly while not actively swimming (Figs. 13 to 16). A bright green plate-like chloroplast is laterally disposed and, when viewed from the narrow side, it is rectangular in shape and is slightly drawn in at the middle (Figs. 8 and 9, *ch*). A conspicuous, more or less ellipsoid, body is found apposed to the chloroplast (Fig. 8, *p*). A non-contractile vacuole and a few refractile granules are seen in the hyaline portion of the flagellate (Fig. 8, *v* and *f*). A bright red eye-spot or stigma is present on one side, about the median region of the cell (Fig. 8, *s*). The stigma consists of two bright red, tiny, rod-like granules embedded as it were in a lens-shaped hyaline matrix, the granules being on the inner concave side of the lens. A tiny nucleus could be made out on careful examination (Fig. 8, *n*).

The flagellate reproduces by longitudinal division and forms two daughter cells (Figs. 10 to 12). Dividing cells are slightly larger than the active vegetative cells (Fig. 10). During division, the flagellate becomes somewhat quiescent. The chloroplast divides first and soon a second flagellum is formed close to the original flagellum (Fig. 11) and then cytokinesis is completed giving rise to two daughter-cells (Fig. 12). In the enlarged cell which is about to divide, the two granules of the stigma are seen standing out more apart than in the ordinary cells. Presumably one granule goes to each daughter-cell. Nuclear division could not be observed due to the smallness of the individual.

Microchemical tests (Johansen, 1940; Gatenby and Painter, 1946; Fritsch, 1935; Jahn, 1951) showed no presence of a cellulose wall. The conspicuous ellipsoid body lying apposed to the chloroplast (Fig. 8, *p*) showed paramylon qualities in that it did not take iodine stain, dissolved in sulphuric acid and potash and disintegrated and disappeared in formaldehyde when kept for long. The nature of the refractile globules (Fig. 8, *f*) could not be determined.

The characteristic features of the flagellate here described agree more or less closely with those of the Euglenineæ. But, the flagellate is, however, remarkably simple in its structural features as compared with the other members of the Class. The flagellate has not got a well-defined "gullet" as in the genus *Euglena*; but, the slight depression at its anterior end evidently represents the beginnings of a primitive gullet. Its chloroplast is a very simple plate. Its periplast is smooth and is not striated. The stigma is far away from the flagellum. The flagellate is clearly a primitive member

of the group, and may be placed in a new genus by name *Protoeuglena* inside the Euglenaceæ; and the flagellate itself may be named *Protoeuglena noctilucæ* gen. et sp. nov.

DIAGNOSES

Protoeuglena gen. nov.

Pertinet ad Euglenoideas, flagellatas. Cellulæ pyriformes, lateraliter compressæ; latior anterior apex unico flagello ornatus atque invaginatione ad medium. Corpus metabolicum. Periplastum leve. Chloroplastum lucide viride, unicum, laterale. Paramylum, stigma atque unicum vacuolum non-contractile adsunt.

Protoeuglena noctilucæ sp. nov.

Pertinet ad Euglenoideas, flagellatas. Cellulæ pyriformes, corpore parduranteve vita activa lateraliter compresso, 5–6 μ longitud., 4–4·5 μ latit. atque 3 μ crassit. Latior apex anterior ornatus invaginatione definita in medio, ornatus etiam unico flagello duplo vel duple et dimidio longiore quam individuum. Aspectu laterali flagellata, elongato-elliptica. Periplastum leve. Organismus tenuiter metabolicus, præsertim in statu quiescenti vel cum sub microscopio observatur per spatium longum. Chloroplastum lucide viride, unicum atque laterale; lamina oblonga tenuiter inducta ad medium complens latus aspectu laterali. Paramylum unicum, minutum atque ellipsoideum, chloroplasto appositum. Stigma lucide rubra, constans duplici virgato granulo in lenticula hyalina, granulo utroque posito in interiore latere concavo lenticulæ. Nucleus parvus. Nonnulli globuli refractiles atque vacuolum non contractile adsunt. Reproductio per divisionem longitudinalem in duas cellulas filiales.

Typus lectus in interiore parte *Noctiluca miliaris* Suriray in oris maris ad Calicut, in littore occidentali Indiæ.

Protoeuglena gen. nov.

Euglenoid flagellate. Cells pyriform, compressed laterally, broader end anterior, with a depression (invagination) in the middle and bearing a single flagellum. Body metabolic. Periplast smooth. Chloroplast bright green, single and lateral. Paramylon, stigma and a single non-contractile vacuole present.

Protoeuglena noctilucæ sp. nov.

Euglenoid flagellate. Cells pyriform, body in active life compressed laterally, measuring 5 to 6 μ long, 4 to 4·5 μ broad and 3 μ thick. Broader end anterior, with a definite depression (invagination) in the middle and

bearing a single flagellum two to two and a half times as long as the body. In side-view, flagellate elongate-elliptic. Periplast smooth. Organism slightly metabolic, particularly when stationary or when kept under observation for long under the microscope. Chloroplast bright green, single and lateral; an oblong plate slightly drawn in at the middle, filling the side when viewed laterally. Paramylon single, tiny and ellipsoid, apposed to the chloroplast. Stigma bright red, consisting of two tiny, rod-shaped granules inside a hyaline " lens " the granules being on the inner concave side of the " lens ". Nucleus small. A few refractile globules and a non-contractile vacuole present. Reproduction by longitudinal division into two daughter-cells.

Occurrence.—Recorded inside *Noctiluca miliaris* Suriray in the sea off Calicut, on the west coast of India.

DISCUSSION

It may be of interest to mention here that a few Euglenineæ have been described from the sea and they have been found to be very simple in their structure (Schiller, 1925; Fritsch, 1935). Fritsch (*op. cit.*, p. 732) states that these simple types " may well afford data as to the affinities of the whole Class ".

The present flagellate (*Protoeuglena noctilucæ*) shows a certain amount of resemblance to the marine member *Ottonia caudata* Schiller (Fig. 17) in having a slight depression (invagination) at its anterior end. It is much more primitive than *Ottonia* in having only a single lateral chloroplast whereas *Ottonia* has two lateral chloroplasts.

Pringsheim (1948, p. 52) states that " Judged by other primitive algal types, the primitive *Euglena* should have a single, more or less cup-shaped, chromatophore ". And further, he is of the view that a really primitive form has yet to be described though, *Euglena archæplastidiata* Chadefaud shows certain primitive characteristics. It would appear that the present flagellate (*Protceuglena noctilucæ*) supplies the missing link or one of the missing links. The chloroplast, in this instance, though not cup-shaped is simple enough. In fact, it may be considered more primitive to a cup-shaped one. The absence of a gullet is another feature worth noting. The small depression (invagination) at the anterior end may be considered as the beginning of a gullet. The present euglenoid appears to be the simplest member of the Euglenineæ so far known.

A few words may be added here on the relationship between the euglenoid and *Noctiluca*. It is not clear how this association is brought about.

The *Noctiluca*-cells concerned, as already mentioned, appear to be quite healthy and active. The association appears to be more of a symbiotic nature rather than of parasitism or saprophytism. The euglenoid, however, does not appear to have a free existence, for, they did not survive outside the *Noctiluca* for any length of time as became evident by keeping them in cultures. Saprophytism is ruled out; if it were so, one should expect an increase in their abundance after the " host " *Noctiluca* dies; the observations are to the contrary. How they happened to be inside *Noctiluca* could not be decided; but, they were seen to multiply by division inside them. Cases of symbiotic relation between two algæ of two different Classes have been recorded previously. For instance, the alga known as *Glaucocystis* is now considered to be a case of symbiosis between a colourless species of *Oocystis* (a green alga) and an unicellular member of the blue green algæ (*cf.* Fritsch, 1935, p. 186). The present case may, therefore, be considered a case of symbiotic relationship between a member of the Dinophyceæ and a member of the Euglenineæ.

Some experiments could have been carried out, but no *Noctiluca* free from the euglenoid was available during the corresponding period when the green *Noctiluca* occurred. Samples of plankton towards the fag-end of the green *Noctiluca* occurrence and at other times also were crowded and contained dead *Noctiluca*, and the euglenoid was found both inside the dead *Noctiluca* and also outside it in the water. But the euglenoid was pale green or yellowish in colour and presented a very unhealthy appearance, and the majority of them were non-motile and appeared to be quite dead. It was mentioned earlier that overcrowding leads to an unhealthy condition and the plankton sample emits an unpleasant odour. It would appear that some such cause or other external factors have hastened the death of the *Noctiluca* and when the *Noctiluca* becomes unhealthy and dies, the euglenoid inside it also naturally becomes unhealthy and dies. Observations are being continued for obtaining further information on these obscure factors.

Summary

A new marine member of the Euglenineæ, *Protoeuglena noctilucæ* gen. et sp. nov., is described in detail. The euglenoid occurs in very large numbers inside healthy and active *Noctiluca miliaris* Sur. individuals which consequently appear green. Such *Noctiluca* individuals occur in swarms frequently colouring the sea green in patches. The euglenoid is very simple in its structure. It is pear-shaped with a broad anterior end and a narrow posterior end and possesses a single flagellum, a single green plate-like chloroplast, a paramylon granule in close apposition to the chloroplast. And it

shows a slight depression at the middle of its anterior end. This depression (invagination) very probably represents the beginnings of a gullet. It reproduces by longitudinal division. It appears to be a very primitive member of the class. Its association with *Noctiluca* appears to be symbiotic. It is suggested that this euglenoid is a most primitive member of the Euglenineæ coming very close to the ancestral type from which the rest of the Euglenineæ have been derived.

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N.B.—A list of the papers consulted in connexion with this account will be too long for inclusion here; only those directly used in the preparation of this paper are cited here.

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Explanation of Text-Figures

Protoeuglena noctilucæ gen. sp. nov.

Text-Figs. 1–17. Fig. 1. *Noctiluca* with the euglenoid distributed uniformly inside; Fig. 2. *Noctiluca* with the euglenoid aggregated into a crescent-shaped band inside; Fig. 3. Portion of *Noctiluca* magnified to show the distribution of the euglenoid, *e*, inside; Figs. 4–9. The euglenoid showing structure; Figs. 8 & 9. freehand drawing to scale to show details: *v*—vacuole; *f*—refractile bodies; *ch*—chloroplast; *s*—stigma; *p*—paramylon; *n*—nucleus; *fl*—flagellum. Figs. 10–11. Division stages; in 11 the chloroplast has already divided and two flagella are also seen; cytokinesis has not taken place. Fig. 12. Daughter cells. Figs. 13–16. Euglenoid showing changes of body shape—metaboly. Fig. 17. *Ottonia caudata* Schiller (after Schiller).