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The Electron Microscopic Observation on the Zoospore of *Undaria pinnatifida* Sur.

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Abstract

In the performance of this study were adopted two experimental methods; first, we adopted the Cr-shadowing method considerably effective in observing the surface structure of the zoospore of *Undaria pinnatifida* Sur., with the comfirmation of the following facts; that the zoospore is usually pear-shaped; that there exist the front flagellum and the hind flagellum; that the fibrills called mastigonemes, about 1μ in length, are attached to the front flagellum. Secondly, we made minute observations of the ultrathin-sections. The following items were ascertained by the embedding; namely the aim of which was to make an investigation into the inner structure of the zoospore; the existence of nucleus, nucleolus, mitochondria, chloroplast as well as that of flagella, which run in parallel, forming two lines inside the zoospore; of mitochondoria which is of tubular structure; and of chloroplast which is of four lamellate layers.

Introduction

The electron microscopic observations of the reproductive cells of many kinds of species of Phaeophyceae were made by Manton and Clarke (1951a, 1951b 1956), Greenwood (1953), Petersen, Caram, and Hansen (1958), and others. In Japan, first electron microscopic study of the spermasozoid of *Sargassum horneri* Ag. was made by Ueda in 1953, in which he ascertained the existence of two kinds of front and hind flagella. And afterward, Ueda (1961) reported of the structure of chloroplast in the cell of the frond of Phaeophyceae.

Since 1965 the present writers have been studying electron microscopic observations of the zoospore of *Undaria pinnatifida* Sur. In the present paper, some investigations of outer and inner structure of zoospore were reported as a preliminary note.

Materials and methods

The materials of this investigation, *Undaria pinnatifida* Sur., were collected at an *Undaria* cultivating field in Kagoshima City in March and May, 1966. Out of the sporophyll half-dried for about 3 hours in the shade after the collection, the zoospores were made to liberate into the beaker filled with sea-water under the temperature kept at about 20°C; the solution containing the zoospores was used. Preparatory concentration of zoospores for electron microscope was carried out. The materials collected in March were chiefly used in Cr-shadowing. The suspension of zoospore was preliminarily filtered through double folded gauge to remove coarse dust and debris of *Undaria* in the medium. The filtered materials were then centrifuged at 500 r.p.m. for 20 minutes to remove heavy materials in

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suspension. Supernatant was centrifuged at 2500 r.p.m. for an hour or more, then the sediment of zoospore was taken up in about 10 m*l* of filtered seawater. It was recentrifuged in the same way and the pellet was brought to fixation. The materials removed to a small watch-glass were fixed by adding a few drops of 2% OsO_4, the fixed materials were mounted on the formvar coated copper grid. After their being dried, Cr-shadowing was made at an angle of 20–35 in the atmosphere of 10^{-5} Hg. m. m.

The materials collected in May were chiefly used for sectioning. The collected materials were fixed for 11 hours by 1% OsO_4 buffered by the 4 timed seawater. After being cleansed 2 times in the filtered seawater the materials were dehydrated by ethanol series. The materials were immersed successively in the following solutions for a fixed time as in the following: in the alcohol diluted to 50%, 70%, 95%, and 99% for one hour, respectively; in the absolute alcohol, for one hour and a half. Then the materials were immersed twice in the mixed solution of absolute alcohol and Epok-mixture (epoxy resin) with the ratio of 1:1 for 5 hours; and then they were immersed three times in another mixed solution with the ratio of 1:2 for the total of 11 hours. In the end, materials were immersed 2 times in Epok mixture for 5 hours and a half, and then were brought to the capsule embedding. In each procedure, centrifugation was used and in the capsule embedding, the methods fixed by Kondo and Takemura were adopted.

In dehydration, centrifugation was done under 2000–2500 r.p.m. and in embedding 3000 r.p.m. The embedded capsule was hardened by being left in the thermostat with the temperature of 50°C for more than 50 hours.

The hardened block was cut into ultrathin-section by Porter Blum MT–I ultramicrotome, stained in 3% aqueous uranyl acetate, post-stained with lead hydroxide, and observed with Hitachi HS–7 electron microscope.

Observation and Discussion

1) Observation by dint of shadowing method.

The zoospore of *Undaria pinnatifida* Sur. is generally pear-shaped, measuring about 8μ in length, 3–6μ in width, pointed at one end and rounded at the other; it has two laterally placed flagella, one pointing forward, as long as about 3 times the length of the body itself, and the other pointing backward, a little longer than the body itself. Only front flagellum has natural distinct hairy structures, mastigonemes. The length of each mastigonemes being about 1μ respectively.

2) Observation by dint of ultrathin section method.

In this study, the properties of the organs in the body of zoospore; namely nucleus, nucleolus, vacuole, mitochondria, chloroplast, and flagella etc. were observed.

Vacuole: it is clearly observable, however, the shape is not fixed, the size amounting to about 10–20% of the whole dimention.

Nucleus: almost all which are closely fused with chloroplast envelope, and usually shows bowl-shaped. Nuclear membrane is of the double membranes, and some chromatins are observable here and there in the nucleus.

Mitochondria: more or less than 10 pieces of mitochondria are seen in one body of zoospore, being spherical and covered in double membranes, and cristae of mitochondria shows somewhat tubular structure.

Chloroplast: it is of four lamellate layers, as already pointed out by Ueda (1961) in the cell of Phaeophyceae, and the lamellate adhering at its tip end is of the cylindrical structure. Osmiophilic granules can be seen in the stroma. Flagellum inside the zoospore is of the axial filamental structure, and two flagella, running somewhat parallel simultaneously, are to be seen, and this fact seems to be of much interest and further investigations are needed.

As to the transverse section of flagella outside the body of zoospore, the existence of rosette or corolla axonema, amounting to 9 + 2, is observable, and nine fibers surrounding the 2 axial fibers at the center are composed of 2 pieces of subfilament, respectively; and at the cross section of mastigonemes, it is only a bit of the root that can be discernible; there is no connection with axonema, this being a sort of protrusion of protoplasm.

What was most perplexing in the performance of this study was the establishment of the methods and technique of the series of fixation, dehydration and embedding, which will require further studies. Firstly, as the preliminary process of fixation, the fixative was made up out of the solution prepared by mixing about 12% seawater with 2% OsO_4 in the ratio of 1:2. While it seems to be more proper to add 0.25M s-collidine or 0.045 g sucrose per 1 m*l* at the final fixatives; but this will require further study, too.

The time necessary for the fixation was fixed to be 11 hours. This is considerably longer than the time necessary for the fixation of the animal tissue, but this will become less irrational when we consider that the cell walls of plants are less permeable than those of animals.

Dehydration was carried out for more than one hour in each series, but some shortening of this time may not be impossible in balance with the improve of the embedding medium.

As to the two flagella, it was ascertained that the front flagellum is of 'Tinsel type' while the hind flagellum is of 'Whiplash type'.

On the other hand, existence of the flagellum running parallel inside the body of the zoospore was ascertained, which was not a small interest to us.

In the type-figure drawn by Manton and Clarke on spermatozoid of *Fucus*, front and hind flagellum were connected with basal granule of blepharoplast, but in case of *Undaria* this is otherwise.

Rather the assumption of the existence of the two sorts of blepharoplast seems to be more reasonable.

As to mitochondria, the cristae shows the tubular structure which is discernible commonly through all the other ordinary algae.

As to chloroplast, this is not of grana structure but is of band shape and of four lamellate layers.

Acknowledgement

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Plate I

Electron microphotographs of zoospores of *Undaria pinnatifida* Sur.

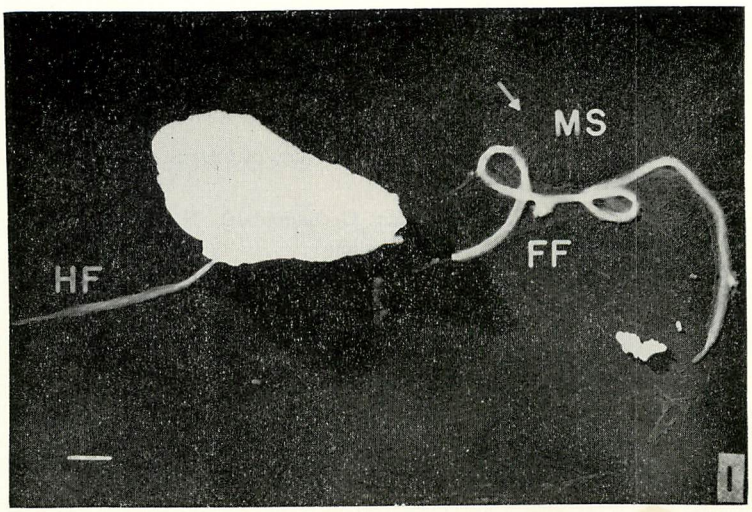
Explanation of Figures.

FF., front flagellum; HF., hind flagellum; MS. mastigonemes.

Fig. 1 The body of the zoospore with two flagella. ×5760

Fig. 2 Enlarged view of the middle part of the front flagellum of the zoospore. ×15200

Plate I



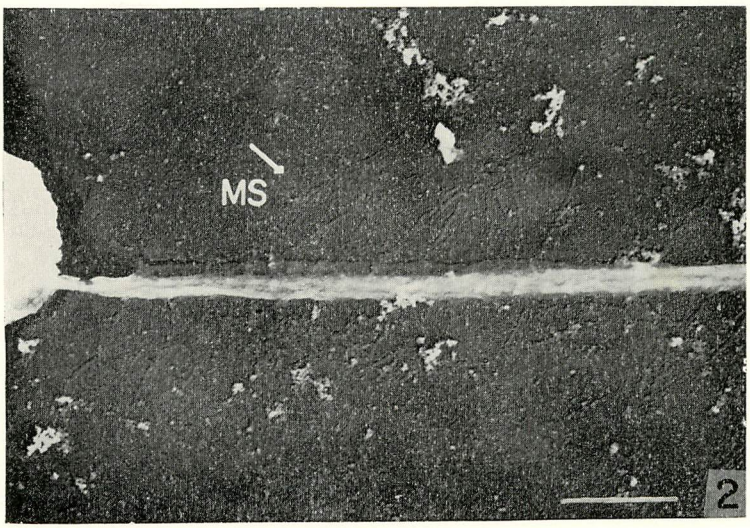


Plate II

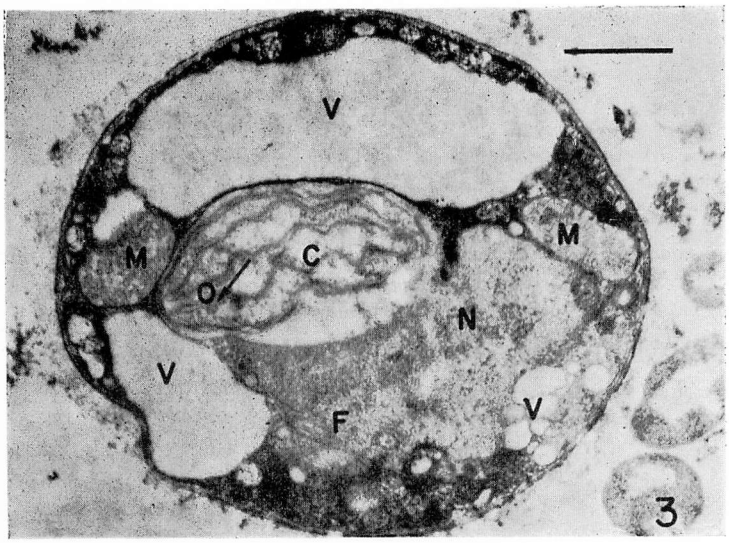
Electron microphotographs of the zoospores of *Undaria pinnatifida* Sur.
Explanation of figures.

V, vacuole; M, mitochondria; C, chloroplast; N, nucleus;
O, osmiophilic granule; F, flagellum.

Fig. 3 Transverse section of the body of zoospore showing the organs by the method of ultrathin section. ×14500

Fig. 4 Enlarged view of the portion in the nucleus. ×25200

Plate II



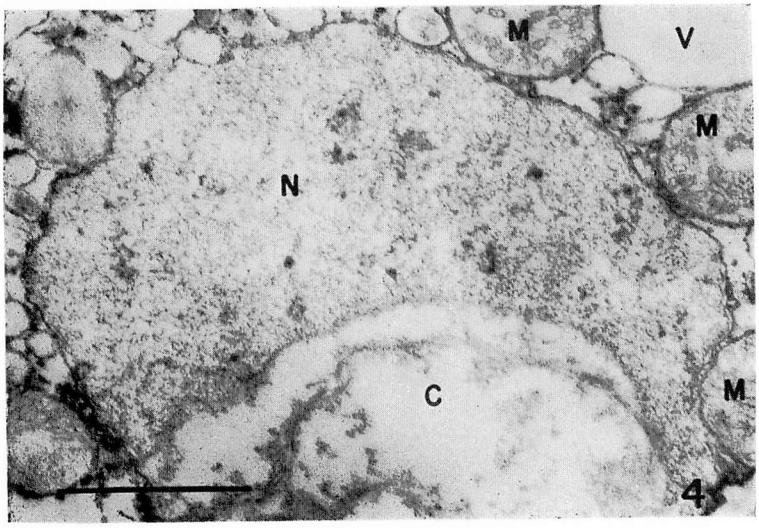


Plate III

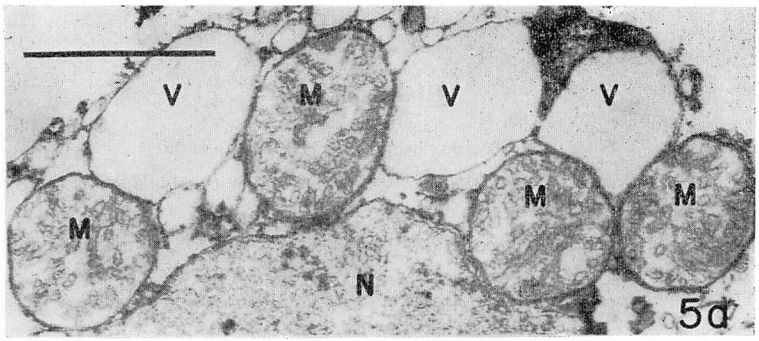
Inner structure of the zoospore of *Undaria pinnatifida* Sur.

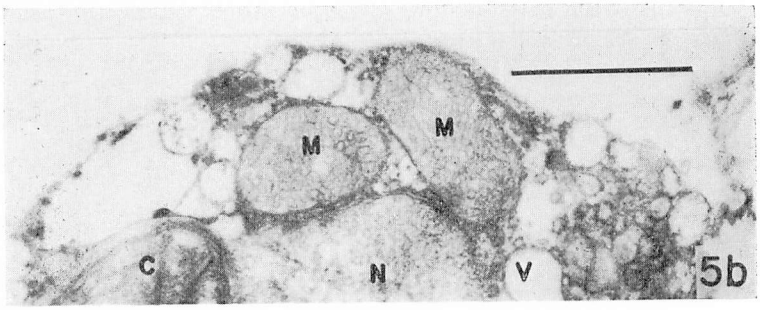
V, vacuole; M, mitochondria; C, chloroplast; N, nucleus
O, osmiophilic granule; L. lamella

Fig. 5a and 5b The portion of the mitochondria, showing the tubular structure.
5a ×25000, 5b×24000

Fig. 6a The tangential section of the chloroplast with four lamellate layers.
×30000

Plate III





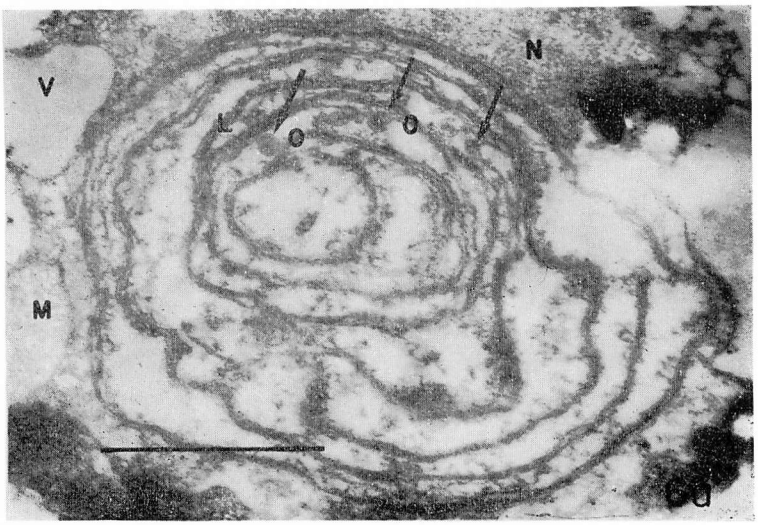


Plate IV

Inner structure of the zoospore of *Undaria pinnatifida* Sur.

V, vacuole; N, nucleus; O, osmiophilic granule; F, flagellum; L, lamella; MS, mastigonemes

Fig. 6b The longitudinal section of the chloroplast with four lamellate layers. ×28000

Fig. 7 The two flagella run through in the body of the zoospore. ×25200

Fig. 8 The tangential section of the flagellum, showing the mastigonemes. ×33600

Plate IV