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Ultrastructure of a red tide chloromonadophycean alga, *Chattonella* sp., from Kagoshima Bay, Japan

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A chloromonad associated with red tides in Kagoshima Bay, Japan was isolated. Cells measure 47.0±4.0 μm long and 24.8±1.2 μm wide ($\bar{x}$±95% C.I.). A gullet is seen to be present at the ultrastructural level. Two flagella, one of which possesses hairs, are attached at the base of the gullet and are associated with the nucleus by a rhizoplast. The cell lacks a wall. A large number of vacuoles, lipid bodies, and chloroplasts occupy the peripheral layer of the cytoplasm. The chloroplasts contain lamellae of 3 thylakoids, lipid droplets, and pyrenoids. The nucleus contains 1-4 nucleoli and scattered chromatin. Golgi bodies and mitochondria occur arcund the nucleus, but no contractile vacuoles, eyespots, trichocysts or mucocysts are present. It is suggested that this organism is a speries of *Chattonella*.

Key Index Words: Chattonella; *Chloromonadophyceae*; *chloroplast*; *flagellum*; *Kagoshima Bay*; *lipid body*; *red tide*; *rhizoplast*; *ultrastructure*.

From June to August, 1977, and again during the same months in 1978, blooms of a chloromonadophycean alga were observed in Kagoshima Bay. This marine chloromonad is the most common red tide alga causing mortality of yellow tail fish in the coastal areas of Japan. ADACHI (1974) identified it as *Hornellia* SUBRAHMANYAN (1954). HOLLANDE *et al.* (1956) considered *Hornellia marina* SUBRAHMANYAN to be the synonym of *Chattonella subsalsa* BIECHELER (1936) and this opinion seems to be accepted by most taxonomists.

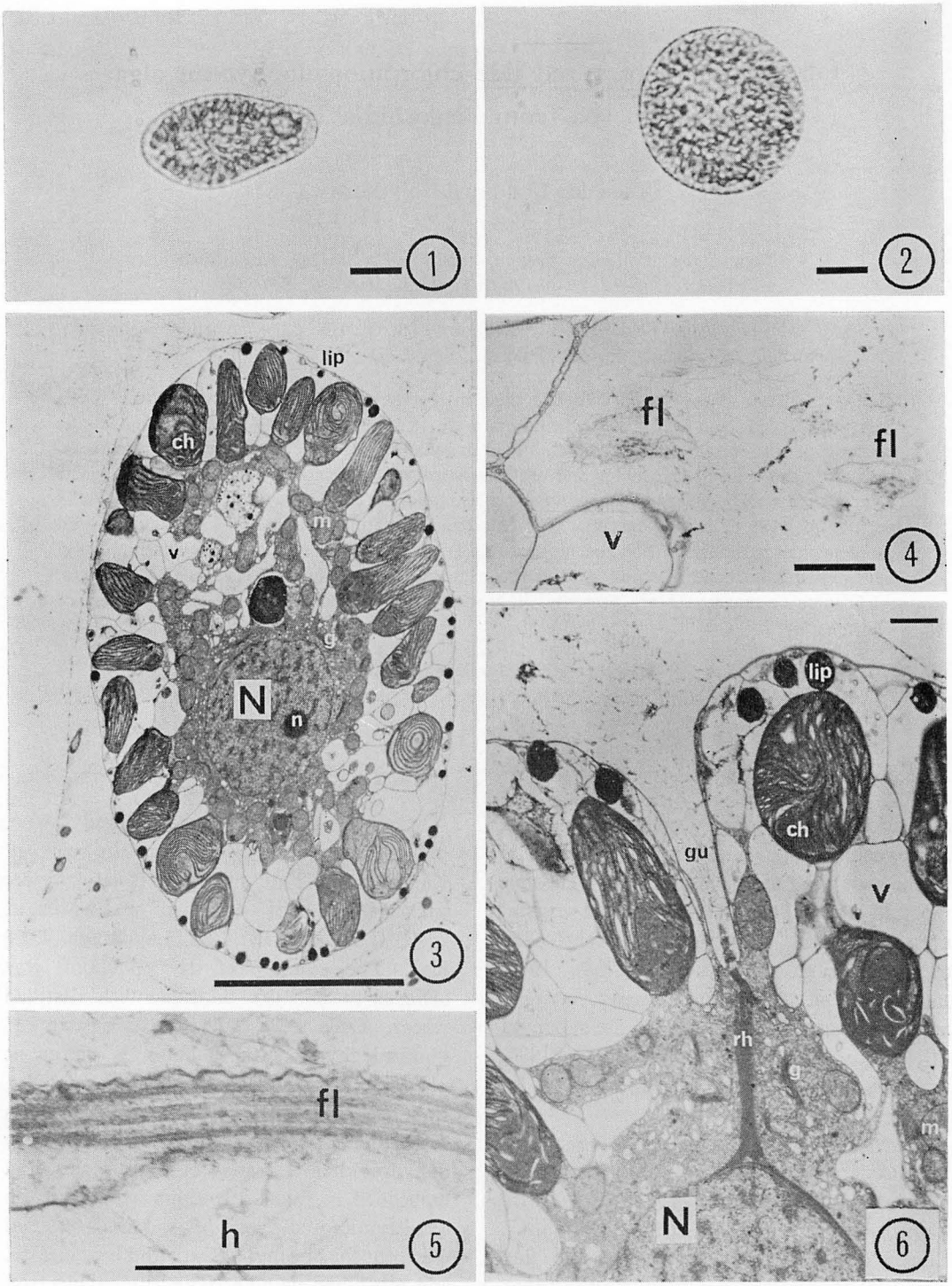
Chattonella ultrastructure has been studied by MIGNOT (1976) (on *C. subsalsa* BIECHELER) and LOEBLICH *et al.* (1977) [on *C. japonica* (=*Fibrocapsa japonica*) LOEBLICH *et al.*]. LEADBEATER (1969) also studied the fine structure of *Olisthodiscus luteus* CARTER that was subsequently transfered to *Chattonella* by LOEBLICH *et al.* (1977). The purpose of this paper is to present a general description of *Chattonella* sp. from Kagoshima Bay.

Materials and Methods

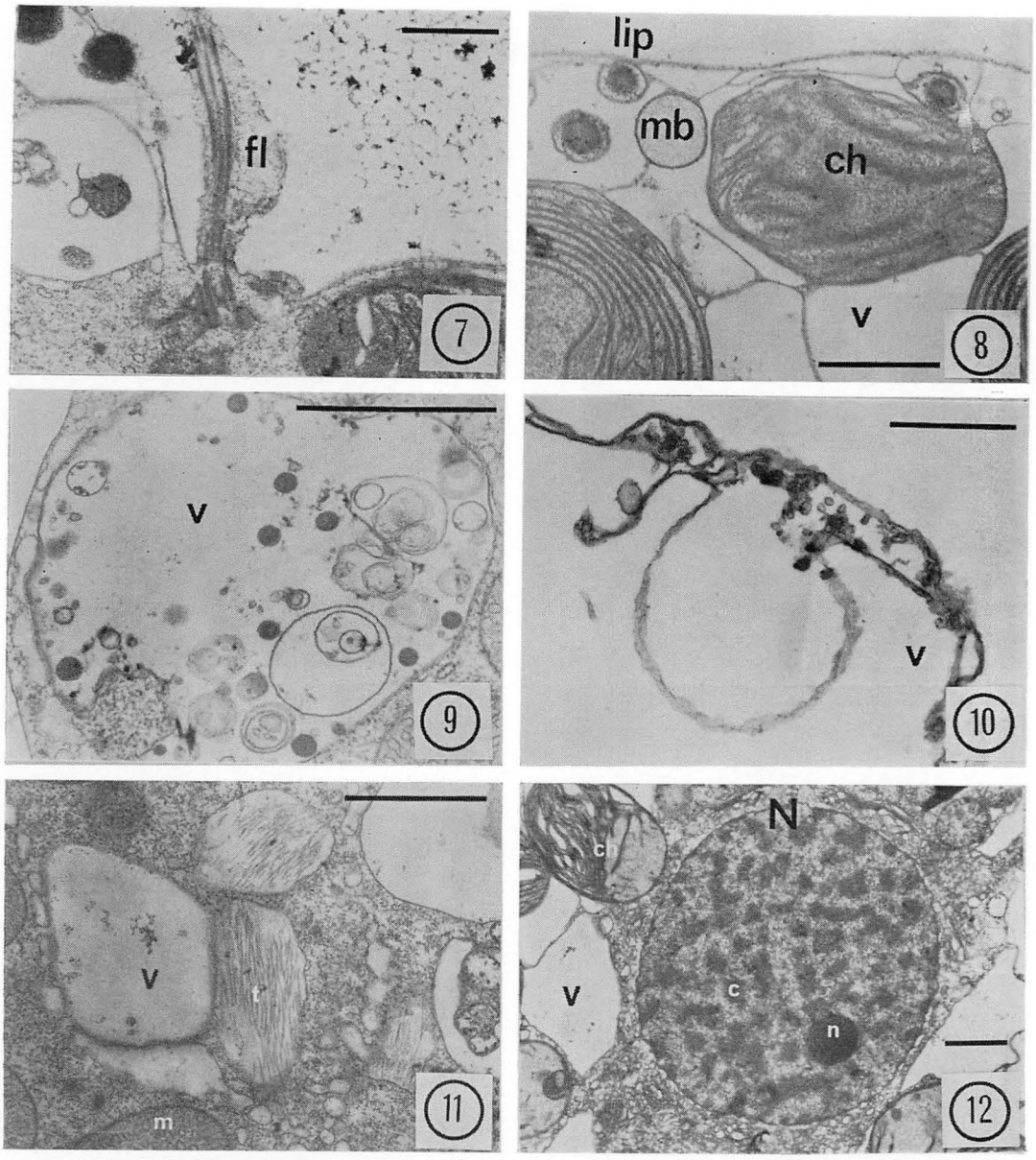
Specimens utilized for this study were collected in July, 1978, from the port of Kagoshima. The unialgal isolates were grown in 2% ESP enriched sea water at 20-23°C under a 12: 12 LD cycle (ca. 4,000 lux). The salinity of the medium was lowered to 25‰ by dilution with distilled water. Cells were fixed for 1.5 hrs. in 2% glutaraldehyde with Milloning's buffer at pH 7.5. After several washes in the buffer, they were postfixed in buffered 1% osmium tetroxide. All cells were subsequently dehydrated in a graded ethanol series and embedded in Epon. Sections were cut on an ultramicrotome using glass knives, stained with aqueous uranyl acetate and lead citrate, and examined using a H-300 Hitachi electron microscope.

Observations

Light microscopy. Motile cells of the

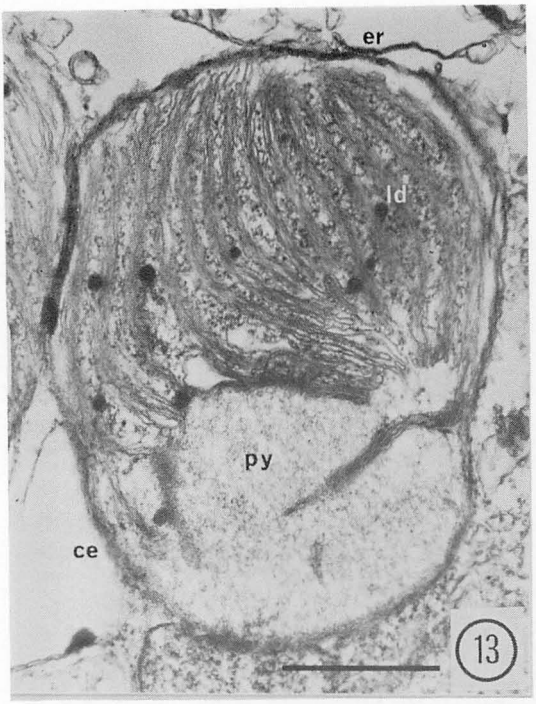


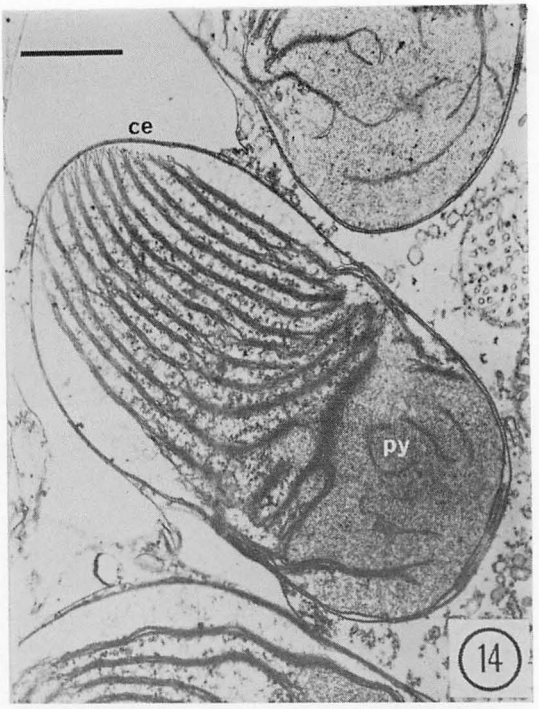
Figs. 1-2. Light micrographs of living cells of *Chattonella* sp. 1. Lateral view of elongated cell showing chloroplasts. No trichocyst rods are seen. Scale=10 μm; 2. Round cell produced under unfavorable conditions as viewed with the light microscope. The flagella are detached from the cell. Scale=10 μm.

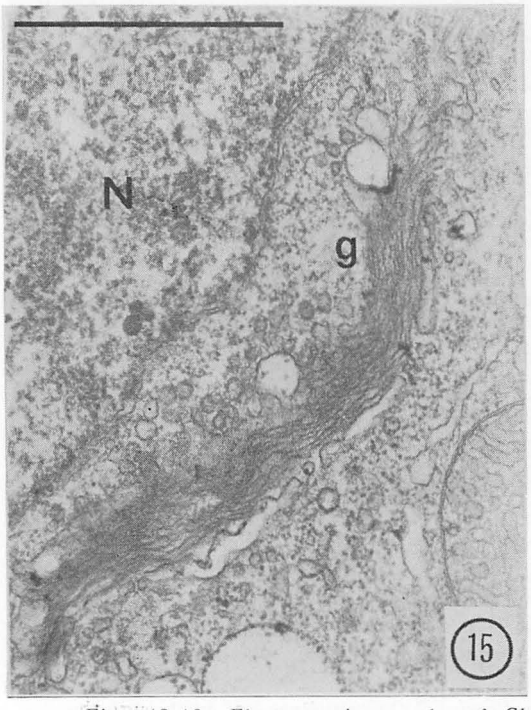


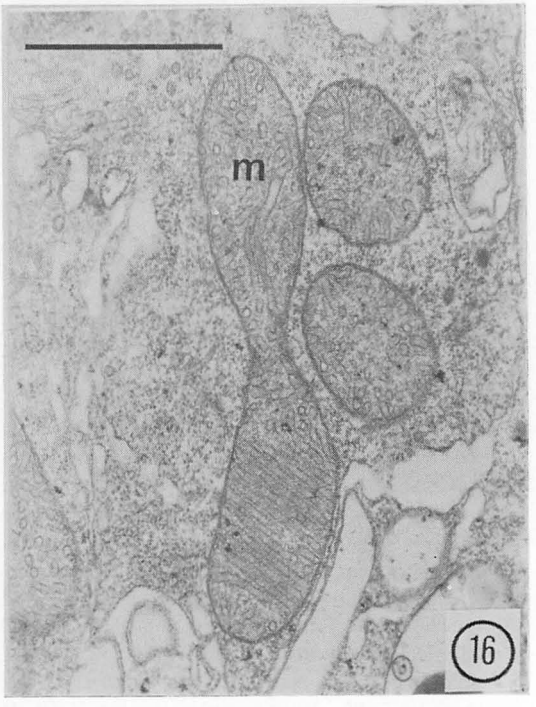
Figs. 3-6. Electron micrographs of *Chattonella* sp. Scale=1 μm. 3. Longitudinal section of *Chattonella* sp. showing chloroplasts (ch), vacuoles (v), and lipid bodies (lip) in large numbers, and dense cytoplasmic layer surrounding nucleus (N). Also note nucleolus (n), mitochondria (m), and Golgi bodies (g). The flagellar apparatus is not present in this section; 4. Transverse section of two flagella (fl); 5. Longitudinal section of posterior flagellum showing the hairs (h); 6. Gullet (gu) and rhizoplast (rh) which runs directly from the basal bodies of flagella to the nucleus.

Figs. 7-12. Electron micrographs of *Chattonella* sp. 7. Longitudinal section through the base and transition zone of flagellum; 8. The vesiclar cortex containing chloroplast (ch), lipid bodies (lip), and microbody (mb); 9. Thin section of vacuole containing material; 10. Envelope of lipid body after discharge of material; 11. Cytoplasmic region where numerous vesicles occur with tubular contents (t). The tubules are thought to be stored flagellar hairs; 12. A typical interphase nucleus. The nucleus is bound by a double nuclear envelope and contains a nucleolus (n) and scattered chromatin (c).









Figs. 13-16. Electron micrographs of *Chattonella* sp. 13. Chloroplast fixed in glutaraldehyde and osmium tetroxide. External to girdle band is the chloroplast envelope (ce) and endoplasmic reticulum (er). Chloroplast stroma contains large numbers of lipid droplets (ld). Simple internal pyrenoids show a lamella passing into the pyrenoid (py); 14. Chloroplast with 3 thylakoid lamellae fixed in osmium tetroxide; 15. Vertical section through the Golgi body. Note the swollen ends of the cisternae and the adjacent Golgi vesicles. The Golgi body is situated above the nucleus; 16. Mitochondria showing a two-membrane envelope and tubular cristae. The elongated mitochondrion is dividing.

organism are pear-shaped with two flagella arising in a gullet. The cells are 47.0±4.0 μm long and 24.8±1.2 μm wide ($\bar{x}$±95% C. I.) (Fig. 1). Under unfavorable conditions, these cells altered to a spherical shape (Fig. 2). The periplast is thin and flexible, and in some instances numerous discoid chloroplasts and cytoplasmic refractive bodies, assumed to be lipid globules, are located just beneath the cell membrane. The species lacks an eyespot. Although chloromonadophycean algae are generally bright green in color, this organism forms golden yellow colored blooms.

Electron microscopy. Fig. 3. is a longitudinal section of the organism showing profiles of most of the organelles except the flagella. The cell is bounded only by a cytoplasmic membrane, without a cell wall or scales. Two flagella extend from the base of a gullet (Fig. 4). The axonemes appear to have the typical 9+2 microtubular arrangement (Figs. 4, 5, 7). One of the flagella has a row of fine hairs (Fig. 5). There is a broad, striated flagellar root which runs directly from the basal bodies to the nucleus where it appears to be attached to the nuclear envelope (Fig. 6). Many earlier workers termed this type of root a rhizoplast, and it has been found in *Chattonella subsalsa* (MIGNOT 1976) as well as in *Olisthodiscus luteus* (LEADBEATER 1969). Vesicles containing fibrous material occur in the protoplast, and this type of vesicle has been previously reported in *C. subsalsa* (MIGNOT 1976) and *C. japonica* (LOEBLICH and FINE 1977). The fibrous material appears to be an early stage in development of the flagellar hairs. Large numbers of vacuoles and chloroplasts occupy most of the region between the cell membrane and the layer of cytoplasm surrounding the nucleus (Fig. 3, 8). The vacuoles are easily broken in the fixative solution, especially under low osmotic conditions. Sometimes the vacuoles contain digested materials or lipid bodies (Fig. 9). The arrangement of lipid bodies beneath the surface of the cell membrane can be seen in Fig. 8. Each sphere is enclosed in a vesicle surrounded by a single membrane (Fig. 10). Chloroplasts are 2.4-2.5 μm long× 1.5-3.3 μm wide and generally disc-shaped (Figs. 13, 14). The lamellae are arranged approximately parallel to the longitudinal axis of the chloroplasts, each band consisting of 3 thylakoids (Fig. 14). The chloroplast stroma contains large amounts of small electron dense materials which are assumed to be lipid droplets (Fig. 13). Large pyrenoids penetrated by lamellae are present in the chloroplast. External to the girdle band is the chlorplast envelope which is bound by endoplasmic reticulum. This endoplasmic reticulum may be involved in maintaining the chloroplast in position, but a direct continuity between the nuclear envelope and the endoplasmic reticulum is not present. The nucleus is also surrounded by a two-membrane envelope (Fig. 12). The nuclei possess 1-4 nucleoli and distinct chromatin which is evenly distributed within the nucleoplasm. This type of densely granular chromatin is called heterochromatin by DODGE (1973 p. 143). Several Golgi bodies are situated around the nucleus (Fig. 15). Each of the Golgi bodies consists of a stack of 4 or 5 cisternae. Numerous mitochondria are also found around the nucleus (Fig. 16). They are elongate and ovoid in shape. The inner mitochondrial membrane forms tubular cristae which have slightly constricted bases.

Discussion

Our observations on this chloromonad are partly in agreement with those on *C. subsalsa* (MIGNOT 1976) in the following ways: 1) at the anterior end of the cell there is a narrow canal-like gullet; 2) two flagella, nearly the same in length, arise close to each other from the base of a gullet; 3) only the anterior flagellum bears hairs or mastigonemes; 4) there is a rhizoplast; 5) the nucleus is filled with dense chromatin; 6) the vesicular cortex contains many chloroplasts with basal pyrenoids and lipid bodies. We noted, however, that the color

of the cells differed in our chloromonad from that of *C. subsalsa*. Cells of *Chattonella* sp. were golden yellow, whereas those of *C. subsalsa* were green. Little variation in cell dimensions occurred within these species. We have never observed mucocysts in this chloromonad, but they have been seen in *C. subsalsa*. In *C. subsalsa* no lamellae enter the pyrenoid, while several lamellae pass through the pyrenoid in *Chattonella* sp.. This suggests that this chloromonad and *C. subsalsa* are not the same species, but they have many similarities and may be closely related.

Detailed ultrastructural descriptions were given by LEADBEATER (1969) for *C. luteus* (as *Olisthodiscus luteus*) and LOEBLICH *et al.* (1977) for *C. japonica*. There are no detailed observations on *C. akashiwo* (=*Heterosigma akashiwo*, see LOEBLICH *et al.* 1977) using the electron microscope. Nevertheless, all of these organisms greatly differ from *Chattonella* sp. in cell dimensions, plastid number, situation of the groove, and presence of mucocysts.

Some morphological differences, especially in cell length and width, were observed between the specimens of *Chattonella* collected from Maizuru Bay, Seto Inland Sea and Yatsushiro Bay (ONO *et al.* 1979). One of these three strains may be *Chattonella subsalsa* BIECHELER, but additional studies are needed to answer this question. In this paper we apply the name *Chattonella* sp. to the chloromonad from Kagoshima Bay.

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野呂忠秀・野沢治治：鹿児島湾産緑色鞭毛藻 *Chattonella* sp. の微細構造について

鹿児島湾で1978年に発生した赤潮鞭毛藻 *Chattonella* sp. は長さ 47.0±4.0 μm, 幅 24.8±1.2 μm で, 前方の溝から二本の鞭毛を前後に伸出しており, 内一本は羽型であった。この鞭毛の基部は, リゾプラストによって核膜と結ばれていた。さらに本藻は細胞壁を欠き, 多数の液胞, 脂肪球, 葉緑体が細胞の表層を占めていた。葉緑体内部には, 三層からなる多数のチラコイドバンド, 油滴やチラコイドの陥入したピレノイドがみられた。核の中には1〜4個の核小体と多数のクロマチンがあり, ゴルジ体やミトコンドリアが核周辺に散在していた。しかし収縮胞, 眼点, 刺胞, 粘液胞は観察されなかった。以上の結果から本種は緑色鞭毛藻 *Chattonella* 属の一種であることが確かめられた。(890 鹿児島市下荒田 4-50-20, 鹿児島大学水産学部)