# Four new species of Chattonella (Raphidophyceae, Chromophyta) from Japan

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Four new species of *Chattonella* (Raphidophyceae, Chromophyta) were described in the status of "nomen nudum" in the book entitled "Red tide organisms in Japan", edited by Fukuyo *et al.* (1990). They were *Chattonella globosa* Y. Hara et Chihara, *C. minima* Y. Hara et Chihara, *C. ovata* Y. Hara et Chihara and *C. verruculosa* Y. Hara et Chihara. In this paper, the full descriptions are given for these four species to be validly published. A key to all species of *Chattonella* is also presented.

Key Index Words: Chattonella—C. globosa—C. minima—C. ovata—C. verruculosa—Raphidophyceae—red tide organisms—taxonomy

On Japanerse coasts, especially those of the inland sea, red tide blooms occur frequently from spring to autumn. Many kinds of flagellates have been recognized in these red tides and one of the dominant representatives among them is *Chattonella* species.

We have isolated and obtained many strains of Chattonella, some from local fisheries experimental stations and other research institutes, requesting us for the identification of them. Detailed examination of cultured specimens using electron microscopy showed that there were six taxa, only two of which were described species: Chattonella antiqua (Hada) Ono (Ono and Takano 1980) and C. marina (Subrahmanyan) Y. Hara et Chihara (Hara and Chihara 1982). The four novel species were colloquially referred to as "Globular Chattonella" (Akizuki et al. 1981, Takayama 1981, 1983), "Small Chattonella" (Yoshida personal communication, Imai and Itoh 1985) and "Burr-shaped Chattonella" (Yoshimatsu et al. 1990, Yamamoto and Tanaka 1990), on the basis of their appearance. All possess two subequal or distinctly unequal flagella inserted into the shallow depression near the anterior end of the cell,

and the cytoplasm is divided into two parts: a cytoplasmic endoplasm and a vacuolated ectoplasm. They all lack contractile vacuoles and eyespots. In all these characteristics the four taxa fit well with the type species of *Chattonella, C. subsalsa*, described by Biecheler (1936) and with more recent observations on the ultrastructure by Mignot (1976).

In the book "Red tide organisms in Japan" (Fukuyo et al. 1990), the names Chattonella globosa, C. minima, C. ovata and C. verruculosa were published for the four species but in that publication they have the status of "nomen nudum". In the present paper, formal descriptions are given for valid publication of the species.

#### Materials and Methods

The cultures examined are listed in Table 1, along with the localities from which they were isolated, the persons who made the isolation and the institutions in which they are deposited.

All of the strains were grown in ESM medium (Okaichi et al. 1982) and maintained at a temperature of 20°C under a 14 : 10 LD

Scientific names	Colloquial names	Collecting localities	Institutions of original isolates	Isolators
Chattonella globosa	Globular Chattonella	Kii Channel, Tokushima	Hiwasa Branch of Tokushima Pref. Fish. Exp. St.	M. Yoshida
C. minima	Small Chattonella	Kii Channel, Tokushima	Hiwasa Branch of Tokushima Pref. Fish. Exp. St.	M. Yoshida
C. ovata	Straw sandal-shaped Chattonella	Hiroshima Bay Hiroshima	Hiroshima Pref. Fish. Exp. St.	H. Takayama
C. verruculosa	Burr-shaped Chattonella	Harima-nada Kagawa	Akashiwo Research Inst. of Kagawa Pref.	S. Yoshimatsu

Table 1. List of cultures examined in this study.

cycle. Light was provided by cool white fluorescent lamps at 10-30  $\mu$ mol·m<sup>-2</sup>·s<sup>-1</sup>.

Light microscope studies were made on cultured and natural specimens of living cells or materials fixed by 1-2% osmium tetroxide solution.

Determination of chromosome numbers were made on cultured cells harvested 1 hr before onset of the light regime under 12L-12D cycle. The specimens were fixed with acetic alcohol solution (ethanol: acetic acid=3:1 or 2:1) for 2 hr prior to fixation and colcemid (ca. 0.2  $\mu$ g/ml at the final concentration) was then added to the specimens. Cells were harvested by gentle centrifugation and treated by KCl solution (0.75 M) for 30 min just before the fixation. Fixed specimens were transferred to 5% ferric alum solution for 2-3 min and stained by aceto-carmine. Chromosome numbers were counted under light microscopy using the squash method. For reference, strains of Chattonella antiqua (NIES-161, Watanabe and Satake, 1991) and C. marina (NIES-118, Watanabe and Satake 1991) were also examined.

Cultured specimens for scanning electron microscopy were fixed with 2% osmium tetroxide solution for 5 min at room temperature. After dehydration, drying by the critical point method and coating with gold were performed. Specimens were observed with an S-430 scanning electron microscope (Hitachi).

Cells prepared for transmission electron microscopy were concentrated by centrifugation, fixed for 20 min in a mixture of 2.5% glutaraldehyde (buffered with cacodylate buffer at pH 7.2-7.4 and added 0.6 M sorbitol) and 2% osmium tetroxide in the same buffer without sorbitol at same volume, and post-fixed with 2%  $OsO_4$  for 1 h at 4°C. After dehydration and embedding in Spurr's resin (Spurr 1969), the material was sectioned on an LKB Ultrotome 2088 using diamond knives, and stained with uranyl acetate for 30 min followed by lead citrate for 5-10 min. The sections were viewed with a JEM 100C or a JEM 100CXII electron microscope (JEOL) at 80 kV.

#### **Results and Discussion**

Results are presented as full descriptions of the four species of *Chattonella*, and associated micrographs and drawings.

## (1) Chattonella globosa Y. Hara et Chihara sp. nov. (Figs. 1-12)

Chattonella globosa Y. Hara et Chihara, in Fukuyo et al. (1990), p. 324, nomen nudum.

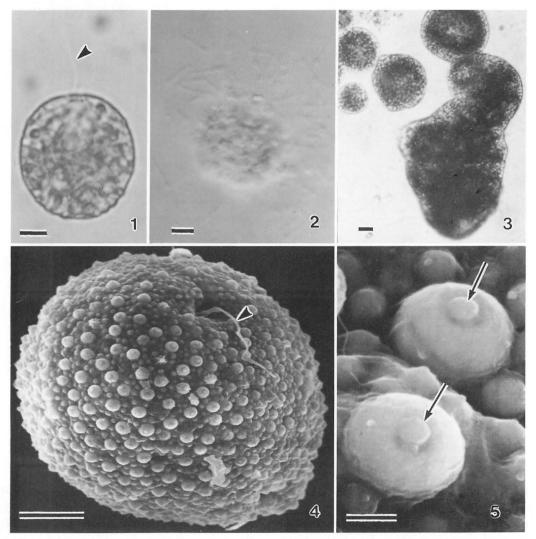
Cellulae luteolae vel brunneae, fere globosae, 40-55  $\mu$ m diam.; chloroplasti discoidei, comparate parvi, 1-2  $\mu$ m longi, 0.5  $\mu$ m lati, multi, in ectoplasmatibus et endoplasmatibus locati, pyrenoide destituti; nucleus sphaericus; mucocystes magnae, corpusculum unguiculatum capientes, multae, secus peripheriam cellulae dispositae; vacuolae contractiles et stigma absentes.

Holotypus: Figura 11.

Type locality: Kii Channel, Tokushima, Japan.

Geographical distribution: Known to occur from central and western Japan and southeastern Asia. Probably widely distributed in temperate to tropical neritic waters.

Cell are yellowish brown, nearly globose without protrusion at the posterior end, 40-55  $\mu$ m in diameter (Figs. 1, 4, 6). Two unequal flagella emerge from a shallow depression at the anterior end of the cell (Figs. 11, 12). The longer anteriorly directed flagellum is undurating during swimming, while the other, which is very short and often invisible under the light microscope, is trailing (Figs. 1, 4). Numerous small particles, which are well stained with  $OsO_4$ , are located in the cytoplasm beneath the cell surface (Figs. 6, 10). Large mucocysts containing nail-



Figs. 1-5. Chattonella globosa. 1: Photomicrograph of a living motile cell, showing anteriorly directed flagellum (arrowhead). 2: Photomicrograph of a punctured cell ejecting nail-shaped inclusions from mucocysts. 3: Photomicrograph of non-motile cells forming a plasmodial aggregation. 4: Scanning electron micrograph of a motile cell, showing numerous protrusions of mucocysts and fatty particles on the cell surface and the longer, anteriorly directed flagellum (arrowhead) from a funnel-shaped depression at top of the cell. 5: Scanning electron micrograph of a cell showing protrusion nail-shaped inclusion of the mucocysts (arrows). Scale bars=10  $\mu$ m in Figs. 1-4; scale bar=1  $\mu$ m in Fig. 5.

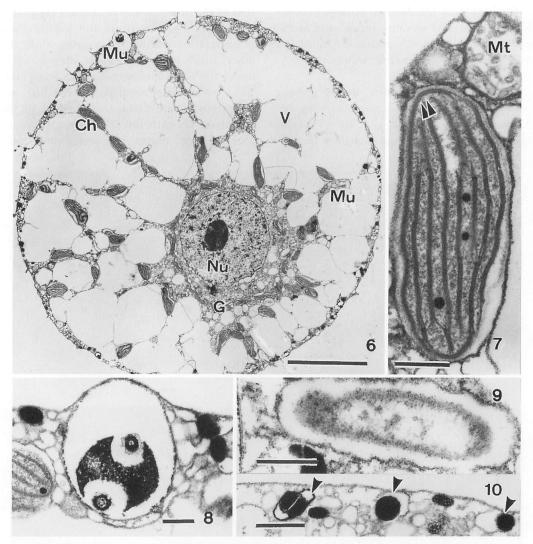
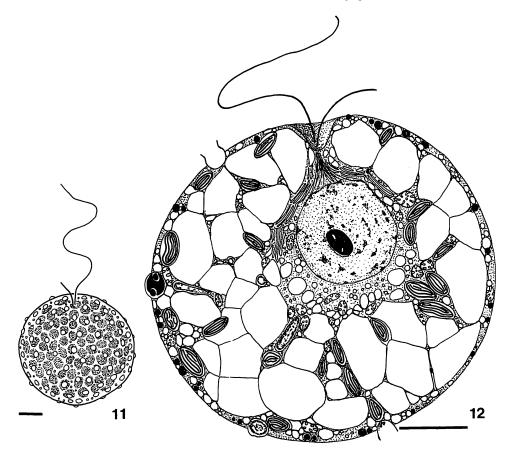


Fig. 6-10. *Chattonella globosa*. 6: Section of a motile cell, showing major cellular components and their arrangement. Ch: chloroplast, G: Golgi body, Mu: mucocyst, Nu: nucleus, V: vacuole. 7: Electron micrograph of a chloroplast, showing lamellae composed of two or three stacked thylakoids and a girdle thylakoid (double arrowheads). No pyrenoid is present. Mt: mitochondorion. 8: Electron micrograph of a part of the mucocyst, showing the head of a nail-shaped inclusion. 9: Electron micrograph of a part of mucocyst, showing the body of a nail-shaped inclusion. 10: Section of the outermost part of a cell, showing the distribution of fatty particles (arrowhead). Scale bar=10  $\mu$ m in Fig. 6; scale bars=0.5  $\mu$ m in Figs. 7–10.

shaped bodies are present around the cell periphery (Figs. 4-6, 8-10), and react discharge following change in the physical or chemical conditions (Fig. 2). Chloroplasts are discoidal and numerous, and are situated in both ectoplasm and endoplasm (Fig. 6). They are relatively small, 1-2  $\mu$ m in length, about 0.5  $\mu$ m in width, and lack pyrenoids (Fig. 7). The chloroplast is furnished with lamellae composed of two or three stacked thylakoids and a girdle thylakoid (Fig. 7). The spherical nucleus is situated in the middle of the cell (Fig. 6). Mitochondria with tubular cristae are randomly distributed in the cytoplasm. Contractile vacuoles and eye-spots are absent. Typical morphological and ultrastructural features of this species are illustrated in Figs. 11 and 12.



Figs. 11-12. Chattonella globosa. 11: Drawing of a motile cell. 12: Drawing showing the distribution of organelles in a motile cell. Scale bars=10  $\mu$ m in Figs. 11, 12.

Asexual reproduction takes place by binary fission while cells are swimming. Cyst formation and sexual reproduction are unknwon. Plasmodial aggregations are often formed under unfavorable growth conditon (Fig. 3).

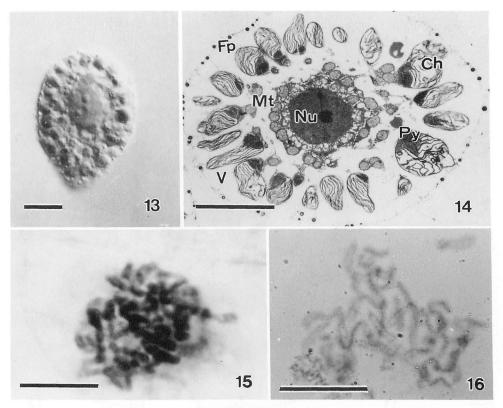
This alga is abundant in early spring and late autumn in several coastal localities in the Seto Inland Sea (Akizuki et al. 1981, where it has been referred to as the "Globular form of *Hornellia*") and in Tokyo Bay (Hosaka *et al.* 1991).

This species resembles *Chattonella subsalsa* Biecheler in the possession of a vacuolated ectoplasm, large mucocysts with nail-shaped inclusions and in the lack of a ring-shaped genophore, contractile vacuoles and eye-spots. It differs from *C. subsalsa* and other species of *Chattonella* in having a spherical shape, unequal flagella, and relatively small chloroplasts without pyrenoids. On the basis of the fact that C. globosa causes respiratory damages to cultivated and natural fish as do other species of Chattonella when the cultured cells of C. globosa at high concentration were added to the experimental pool of yellow tail (Akizuki et al. 1981), it seems appropriate to include it in this genus.

# (2) Chattlnella minima Y. Hara et Chihara sp. nov. (Figs. 13-15, 17, 18)

Chattonella minima Y. Hara et Chihara, in Fukuyo et al. (1990), p. 338, nomen nudum.

Cellulae luteolae vel flavo-brunneae, leviter depressae, obovatae, acutae ad extremum posticum cellulosae, 20-45  $\mu$ m longae, 20-30  $\mu$ m latae, particulae parvae coloratae cum osmio tetroxidi, in cytoplasmatibus infra paginam cellulae locatae; mucocystes



Figs. 13-16. Chattonella minima and C. marina. 13: Photomicrograph of a motile cell of C. minima. 14: Section of a motile cell of C. minima, showing major cellular components. The boundary between the cytoplasmic endoplasm and vacuolated ectoplasm is clearly visible. Fp: fatty particle, Py: pyrenoid. 15: Photomicrograph of chromosomes at the metaphase of C. minima. 16: Photomicrograph of chromosomes at the metaphase of C. minima. 13: Photomicrograph of chromosomes at the metaphase of C. marina. Scale bars= $10 \ \mu m$  in Figs. 13, 14; scale bars= $5 \ \mu m$  in Figs. 15, 16.

carentes; chloroplasti obvati, multi in ectoplasmate locati; pyrenoides ad extremum interaneum chloroplasti locata, invasa in matrice a aliquot thylakoidibus; nucleus guttiformis; vacuolae contractiles et stigma absentes.

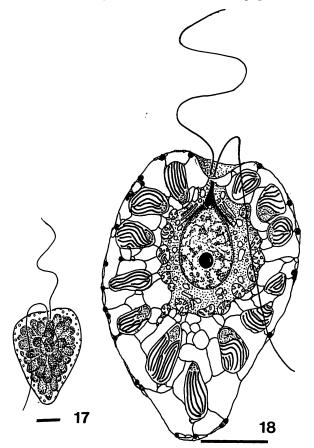
Holotypus: Figura 17.

Type locality: Kii Channel, Tokushima, Japan.

Geographical distribution: distributed in the Seto Inland Sea, Japan.

Cells are pale yellow or yellowish brown, slightly flattened, cordiform,  $20-45 \ \mu m$  long and  $20-30 \ \mu m$  wide, with a shallow depression at the anterior end and a tiny protrusion at the posterior end (Figs. 13, 14). Two heterodynamic flagella, subequal in length, emerge from the bottom of the depression (Figs. 17, 18). Many small particles which stain well with  $OsO_4$  are located in the cytoplasm beneath the cell surface (Fig. 14). Chloroplasts are ellipsoid and numerous, and are arranged radially in the ectoplasm (Fig. 14). Pyrenoids are located at the inner end of each chloroplast, are scarcely visible under the light microscope. A teardrop-shaped nucleus is situated in the middle of the endoplasm (Fig. 14). Globular mitochondria with tubular cristae are located in the endoplasm. Contractile vacuoles, eye-spots and mucocysts are lacking. Diagrammatic illustrations of the morphologial are ultrastructural features are given in Figs. 17 and 18.

Asexual reproduction occurs by binary fission while cells are swimming. Cyst formation and sexual reproduction are unknown. This alga inhabits coastal waters and is known only from Japan in the Seto Inland



Figs. 17-18. Chattonella minima. 17: Drawing of a whole motile cell. 18: Drawing showing the features of a motile cell based on observations using electron microscopy. Scale bars=10  $\mu$ m in Figs. 17, 18.

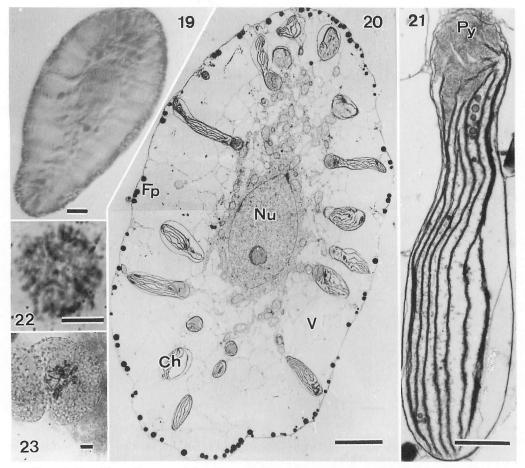
Sea along the coast of Tokushima Prefecture. It was so-called the "Small form of *Hornellia*". The species is abundant in autumn following red tides of C. antiqua and/or C. marina.

This is one of the smallest marine raphidophycean algae. It is similar in cell size to that of *Chattonella verruculosa*, but differs from it in lacking large mucocysts. The flagellation and subcellular organization are similar to those of *C. antiqua* and *C. marina*. During the preliminary survey of chromosome counts, the chromosomes of this species could be roughly recognized more than 90 (Fig. 15), while those in *C. antiqua* and *C. marina* were ca. 29 (Fig. 23) and ca. 50 (Fig. 16), respectively. Although it is earlier to discuss with their karyological properties before showing the precise chromosome number of each species, these disregarded differences are valuable to support the idea that this is a distinct species.

# (3) Chattonella ovata Y. Hara et Chihara sp. nov. (Figs. 19-22, 24, 25)

Chattonella ovata Y. Hara et Chihara, in Fukuyo et al. (1990), p. 340, nomen nudum.

Cellulae flavo-brunneae, ellipticae vel obovatae, satis depressae, 50-70  $\mu$ m longae, 30-45  $\mu$ m latae; particulae parvae coloratae cum osmio tetroxidi, in cytoplasmatibus infra paginam cellulae locatae; chloroplasti fusiformes elongati, multi, inter ectoplasma et ectoplasma locati; pyrenoides ad extremum interaneum chloroplasti locati, invasa in matrice a aliquot thylakoidibus; vacuolae bene evolutae, inter chloroplasto locatae; nucleus guttiformis; mucocystes, vacuolae contrac-



Figs. 19–23. Chattonella ovata and C. antiqua. 19: Photomicrograph of a motile cell of C. ovata. 20: Section of a motile cell of C. minima, showing conspicuous vacuoles between the elongated chloroplasts, and the cytoplasmic endoplasm with a teardrop-shaped nucleus and mitochondria. 21: Electron micrograph of an elongated chloroplast with a naked pyrenoid invaded by thylakoids at the inner polar region. 22: Photomicrograph of chromosomes at the metaphase of C. ovata. 23: Photomicrograph of chromosomes at the metaphase of C. ovata. 23: Scale bars=1  $\mu$ m in Figs. 19, 20 and Figs. 22, 23; scale bars=1  $\mu$ m in Fig. 21.

tiles et stigma absentes.

Holotypus: Figura 24.

Type locality: Ondo, Hiroshima Bay, Hiroshima, Japan.

Geographical distribution: in western Japan, including the Seto Inland Sea, and Kagoshima Bay in Kyushu. Probably widely distributed in eutrophic coastal waters from temperate to tropical regions.

Cell are yellowish brown, naked but not exhibiting metaboly, somewhat flattened, ovoid or obovoid, 50-70  $\mu$ m long and 30-45  $\mu$ m wide, with a shallow depression at the an-

terior end, but no protrusion at the posterior end (Fig. 19). The two heterodynamic flagella are subequal in length, and emerge from the base of a depression (Figs. 24, 25). Many small particles stainable with  $OsO_4$  occur in the cell periphery just beneath the cell surface (Fig. 20). Chloroplasts are elongated fusiform (Fig. 19) and numerous. They are distributed between the ectoplasm and the endoplasm, and are arranged radially (Figs. 19, 20). Vacuoles are well developed and occupy the spece between chloroplasts (Fig. 19, 20). A single pyrenoid, the matrix of which is invaded by a few thylakoids, is located at the

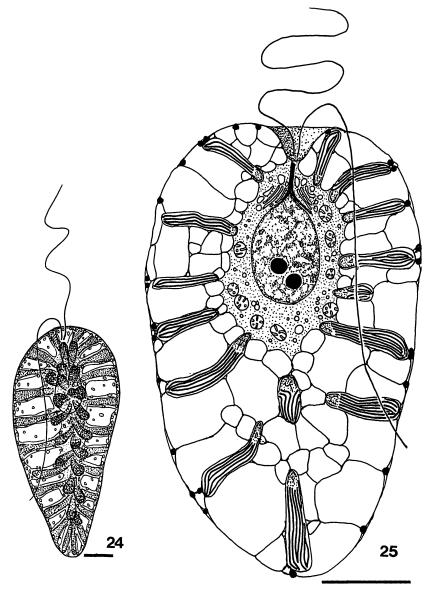


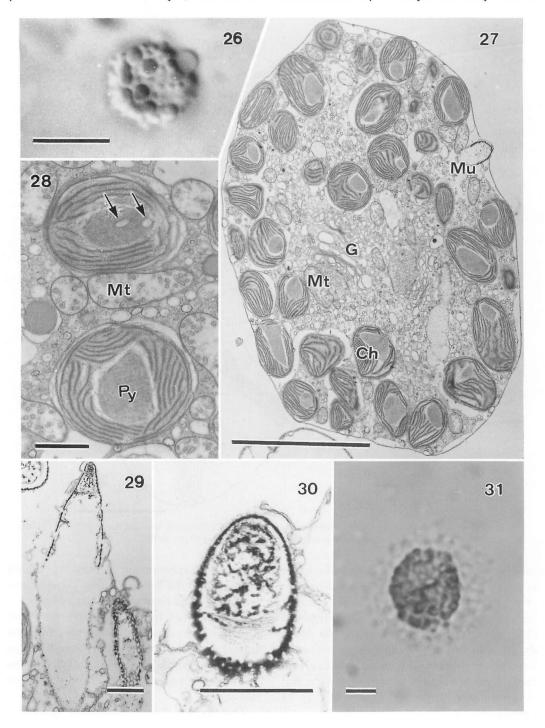
Fig. 24-25. Chattonella ovata. 24: Drawing of a motile cell. 25: Drawing showing the features of a motile cell based on the observations using electron microscopy. Scale bars=10  $\mu$ m in Figs. 24, 25.

inner end of each chloroplast (Fig. 21), but it is scarcely visible under the light microscope (Fig. 19). The nucleus is teardrop-shaped and is situated in the middle of the endoplasm (Fig. 20). Many small mitochondria abundantly appear in the endoplasm. Contractile vacuoles, eye-spots and mucocysts are absent. Typical features as seen with light and electron microscopy are illustrated in Figs. 24 and 25. Asexual reproduction occurs by binary fission while cells are swimming. Cyst formation and sexual reproduction are unknown.

This alga has been recognized to inhabit Japanese coastal waters, mainly in the Seto Inland Sea (Yoshimatsu and Ono 1986, as the "Straw sandal-shaped *Chattonella*"). No red tides are known to have been caused by this alga.

This species differs from other species of

*Chattonella* in the well developed vacuoles and the elongated fusiform chloroplasts. The ultrastructural chloroplast features are basically similar to those of *C. antiqua*, and this led Imai and Itoh (1985) to the conclusion that the two taxa are conspecific. However, we consider this taxon to be a distinct species characterized by a unique cell shape and a



characteristic subcellular organization related to well developed vacuolation that is maintained under various culture conditions. The difference of the chromosome numbers between them also supports our opinion (Figs. 22, 23). The chromosome number of this species was 90-110 while that of *C. antiqua* was less than 55, although they could not be determined precisely, because the squash method was not entirely successful in spreading the chromosomes (Fig. 22).

## (4) Chattonella verruculosa Y. Hara et Chihara sp. nov. (Figs. 26-33)

Chattonella verruculosa Y. Hara et Chihara, in Fukuyo et al., (1990), p. 342, nomen nudum.

Cellulae luteolae vel flavo-brunneae, fere globosae, 12-45  $\mu$ m diam.; aliquot verrucula conspicua circa paginam cellulae habentes; non particulae parvae coloratae cum osmio tetroxidi, in cytoplasmatibus infra paginam cellulae locatae; mucocystes magnae, corpusculum navicularem capientes, aliquot, secus peripheriam cellulae dispostae; chloroplasti disciformes, parvi comparate, 2-3  $\mu$ m longae, 1-2.5  $\mu$ m latae, multi, in ectoplasmate locati; pyrenoides in chloroplastis inclusa, invasa in matrice a uno vel duobus canalibus; nucleus sphaericus; vacuolae contractiles et stigma absentes.

Holotypus: Figura 32.

Type locality: Harima-nada, Tokushima, Japan.

Geographical distribution: western Japan in the Seto Inland Sea and in Hakata Bay in Kyushu. Probably widely distributed in temperate regions.

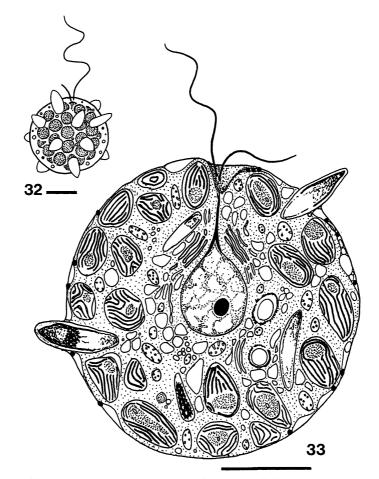
Cells are pale yellow or yellowish brown, nearly globose, with conspicuous warts 12-45  $\mu$ m in diameter on the surface (Fig. 26). The two unequal flagella emerge from the an-

terior end of the cell, the longer one being directed anteriorly and undulating during swimming, while the shorter one, which is often invisible under the light microscope, is trailing (Figs. 32, 33). No osmiophilic particles are located in the cytoplasm beneath the cell surface (Fig. 27). Several warts of large mucocysts, containing bullet-shaped inclusions in the outer half (Figs. 26, 29, 30), are distributed randomly around the cell periphery and these eject in response to slight changes in environmental conditions (Fig. 31). The discoid chloroplasts are numerous, and are situated mainly in the ectoplasm (Fig. 27). They are relatively small, 2-3  $\mu$ m long and 1-2.5  $\mu$ m wide, each possessing a single, embedded pyrenoid (Figs. 27, 28). The pyrenoid cannot be seen under the light microscope (Fig. 26). The pyrenoid matrix is invaded by one or two canals derived from chloroplast stroma (Fig. 28). The spherical nucleus is situated in the centre of the cell (Fig. 33). Comparatively large mitochondria are located in the endoplasm and smaller ones are in the ectoplasm. Neither contractile vacuoles nor eyespots are present.

Asexual reproduction taked place by binary fission while cells are swimming. Cyst formation and sexual reproduction are unknown. This alga inhabits coastal waters and is known to occur in Japan from Harima-nada (Akizuki et al. 1987, as the "Burr-shaped *Chattonella*") and other localities in the Seto Inland Sea.

This species is distinguished from all the known species of *Chattonella* by its verrucose cells possessing mucocysts with bullet-shaped inclusions and by the discoid chloroplasts with embedded pyrenoids invaded by one or two cytoplasmic canals. The absence of small osmiophilic particles in the peripheral cytoplasm is also characteristic. This alga is

Fig. 26-31. Chattonella vertuculosa. 26: Photomicrograph of a motile cell, showing the protrusion of a mucocyst, chloroplasts and fatty particles located just beneath the cell surface. 27: Section of a motile cell, showing major cellular components and their arrangement, exclusive of nucleus. There is no clear boundary between ectoplasm and endoplasm. 28: Electron micrograph of chloroplasts with embedded pyrenoids possessing one or two canals (arrows). 29: Longitudinal section of a mucocyst with a bullet-shaped inclusion. 30: Transverse section of a mucocyst passing through the upper part, showing the fibrous content in the bullet shaped inclusion. 31: Light micrograph of a punctured cell, ejecting mucocysts. Scale bars = 10  $\mu$ m in Figs. 26, 27 and Fig. 31; scale bars = 1  $\mu$ m in Figs. 28-30.



Figs. 32-33. Chattonella verruculosa. 32: Drawing of a motile cell from living specimens. 33: Drawing showing the features of a motile cell based on observations using electron microscopy. Scale bars = 10  $\mu$ m in Figs. 32, 33.

highly vacuolated like as other species of *Chattonella*, but less clear boundary between ectoplasm and endoplasm was recognized. This alga may be retained in the genus *Chattonella*, until further examination to establish the generic delimitation of marine raphidophycean algae will be done systematically.

Biecheler (1936), in the original description of *Chattonella*, adopted as basic generic criteria the absence of contractile vacuoles and the presence of what he termed a "capsule", the clear boundary between the cytoplasmic endoplasm and the vacuolated ectoplasm. The latter character is adopted here as a main generic criterion.

#### Key to species of Chattonella

Currently, seven species including the four new species described in this paper are known to belong to *Chattonella*. A key to the species of *Chattonella* is provided as follows.

- 1. Mucocysts present .....2
- 1. Mucocysts absent ······4
- 2. Cells elongated, with pointed posterior end .....C. subsalsa
- 2. Cells globose ······3
- 3. Mucocysts with nail-shaped inclusions.....C. globosa
- 4. Cells ovate  $\cdots C$ . ovata
- 4. Cells not ovate, with pointed posterior

end ......5

- 5. Cells less than 50  $\mu$ m long  $\cdots C$ . minima
- 5. Cells more than 50  $\mu$ m long  $\cdots 6$
- 6. Cells 50-70 μm long .....C. marina
- 6. Cells 70-130  $\mu$ m long ······C. antiqua

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# 原 慶明\*・土井考爾\*・千原光雄\*\*:日本産シャットネラ属4新種の記載

「日本の赤潮生物」(福代ら編, 1990) に裸名で記載した日本産シャットネラ属(ラフィド藻綱, 黄色植物門) の4新種, キュウケイシャットネラ(Chattonella globosa), コガタシャットネラ(C. minima), ワラジガタシャットネ ラ(C. ovata) およびイガグリシャットネラ(C. verruculosa) にラテン語記載文を付し, 正式発表した。同時にそれら の形態的特徴, 生育状況, 地理的分布およびシャットネラ属の全種の検索表を提示した。(\*305 つくば市天王台 1-1-1 筑波大学生物科学系, \*\*150 渋谷区広尾4-1-3 日本赤十字看護大学)

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