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Chrysochromulina quadrikonta sp. nov., a quadriflagellate member of the genus *Chrysochromulina* (Prymnesiophyceae=Haptophyceae)

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An unusual quadriflagellate species of *Chrysochromulina, Chrysochromulina quadrikonta* sp. nov. (Prymnesiophyceae=Haptophyceae) is described based on observations of cultured material isolated from sea-water samples collected from Tokyo Bay. The four flagella are equal and homodynamic and only one flagellum shows a green autofluorescence. The cells were covered with two types of scales, a plate-like scale and a spiny scale, which are morphologically similar to those of a previously described species, *Chrysochromulina ericina*. This indicates the close affinity of these two species. However, *C. quadrikonta* is distinguished from *C. ericina* in various respects, including scale sizes, cell shape and the peculiar distribution of the spiny scales.

Key index words: Chrysochromulina—*flagellum—haptonema—Haptophyceae—Prymnesiophyceae—quadriflagellate.*

The genus *Chrysochromulina* is a member of the Prymnesiophyceae, and is characterized by both a well developed haptonema that emerges between the two flagella and unmineralized organic scales that cover the cell surface (Green *et al.* 1989). Species of *Chrysochromulina* are widely distributed in the oceans, and the genus contains ca. 50 species (Estep *et al.* 1984; Estep and MacIntyre 1989).

Recently, we discovered a quadriflagellate species that exhibits a prymnesiophycean nature, including a long haptonema and many spiny scales that cover the cell surface. The organism was observed in Tokyo Bay, Japan, August to October 1988, September 1989 and September and October 1990. This alga was also observed in oyster farms in Kesen-numa, Miyagi Prefecture (the northern part of Honshu Island), Japan, in October and November 1991, where it formed a bloom and caused a brown coloration of oyster gills, although no toxin was detected (M. Fujita, pers. comm.). The same species was recently observed in Melbourne, Australia (D. Hill pers. comm.) and in Nelson, New Zealand (L. Rhodes, pers. comm.). Obviously, this unusual prymnesiophyte species is widely distributed in the Western Pacific.

In this paper, light microscopy and external cell morphology are presented, and based on these results we discuss the taxonomy of this organism and we propose a new name, *Chrysochromulina quadrikonta* sp. nov. for it.

Materials and Methods

Chrysochromulina quadrikonta occurred in enriched seawater cultures of water samples collected at the surface in the port of Yokohama (34°28′N; 139°09′E), Tokyo Bay, Kanagawa Prefecture, Japan, on 11 September 1988. Some other species of *Chrysochromulina*, *C. hirta* Manton (Manton 1978), *C. spinifera* (Fournier) Pienaar et Norris (Pienaar and Norris 1979), *C. pringsheimii* Parke et Manton (Parke and Manton 1962), *C. alifera* Parke et Manton. (Parke *et al.* 1956) and several undescribed species, were also present in the same samples. The unialgal culture was established by single cell isolations using micropipettes and dilution techniques. For

both enrichment and unialgal cultures, an ESM medium (Okaichi *et al.* 1982) was used and cultures were grown at 20°C, and about 46 μmol m^{-2} s^{-1} were provided from cool-white fluorescent tubes for 14 : $\overline{10}$h. 1 mg l^{-1} GeO_2 was added to the enrichment cultures to inhibit diatom growth.

To observe the living and fixed specimens, we used a Nikon Optiphoto microscope with differential interference contrast (DIC) optics as well as an Olympus BH-2 epifluorescence microscope. A high-speed (200 frames s^{-1}) video (NAC MHS-200, NAC Inc., Tokyo 106, Japan) mounted on a Nikon Optiphoto bright-field microscope was used to observe the cell behaviour, especially the flagellar movement. The video system was carried out in the negative mode.

Whole mount specimens (Moestrup and Thomsen 1980) were prepared for observations of the cell coverings. A drop of the cell suspension was placed on formvar-coated grids and exposed for 30 s to OsO_4 vapour provided from drops of a 4% solution. The grids were then dried, rinsed in distilled water, and dried again prior to shadowcasting with platinum/palladium at an angle of about 30° or staining with 2% uranyl acetate for 15 min (McFadden *et al.* 1986). The uranyl acetate-stained specimens were rinsed in distilled water so that the positively stained specimens could be obtained. The specimens were examined with a JEOL 100 CX II transmission electron microscope.

The following two prymnesiophytes were examined to compare scale morphology with the quadriflagellate alga. A biflagellate organism that resembles *C. quadrikonta* in cell shape and scale morphology produced a bloom on 20 July 1986 at the same location in Tokyo Bay, where *C. quadrikonta* had been collected in 1988. A unialgal culture of this organism was established in the same way as indicated above. *Chrysochromulina ericina* Parke et Manton (Parke *et al.* 1956) was present in enriched cultures of the water samples collected at the surface in the port of Nagoya (35°02′N; 136°48′E), Aichi Prefecture, Japan, on 3 April 1989. These were also used to observe the scale morphology.

Results

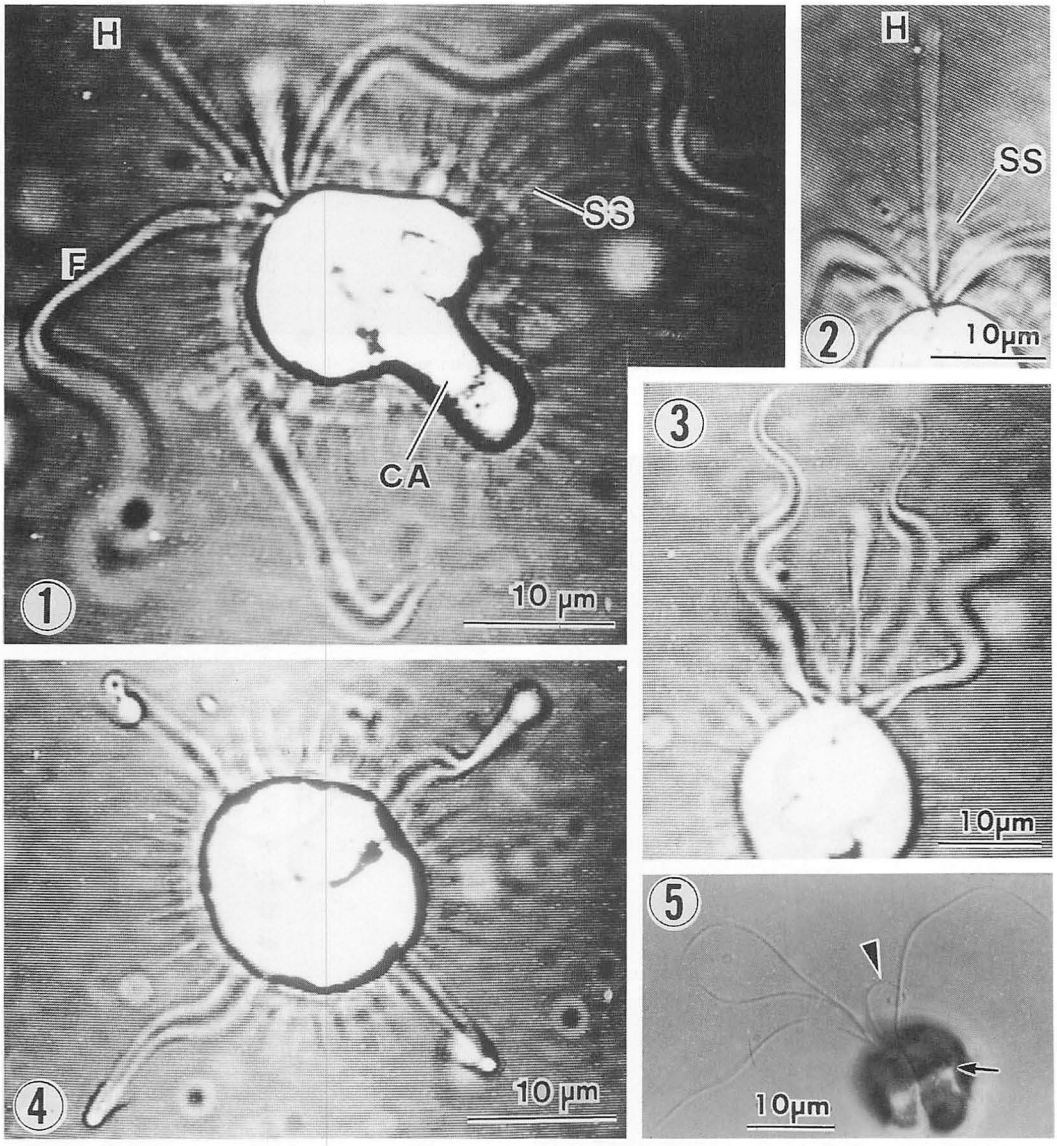
Chrysochromulina quadrikonta sp. nov. (Figs. 1-17)

DIAGNOSIS: Cellula subsphaerica, 10-25 μm longa, 10-18 μm lata, appendicem caudata, 2-5 μm longa; flagella 4, aequalia, 30-40 μm longa. Haptonema, 25-30 μm longa. Chloroplasti, 2 vel 4, parietales, lobis pofundes, quisque pyrenoides unicum immersum fovens. Periplastus et basis haptonemae squamais dimorphis obtectus. Squama strati exteriori cylindrica, 4-6 μm longa, 0.35 μm lata, basis conico, 0.8-1.2 μm lata. Squama strati interioris laminaris, suborbicularis, 1.2-1.6 μm × 1.4-2.0 μm, margine paulum incrassato, ora inconspicua 0.1 μm lata, cristis radiantibus et fibris inconspicuis. Affinis *Chrysochromulinae ericinae*, sed numero flagelli, forma cellulae, magnitudine squamae et distributione squamae diversa.

HOLOTYPUS: Fig. 1

Cell subspherical, 10-25 μm long and 10-18 μm wide, with a caudate appendage, 2-5 μm long; flagella 4, equal, 30-40 μm long. Haptonema, 25-30 μm long. Chloroplasts, 2 or 4, parietal, deeply lobed, each with an immersed pyrenoid. Periplast and haptonematal base coated with two different types of scales. Outer scales, cylindrical, 4-6 μm long and 0.35 μm wide, with a conical base, 0.8-1.2 μm wide. Inner scales plate-like and subcircular, 1.2-1.6 μm × 1.4-2.0 μm, with a slightly thickened margin and an inconspicuous rim, 0.1 μm wide, with a pattern of radiating ridges and inconspicuous fibrils. *Chrysochromulina quadrikonta* is a close relative of *Chrysochromulina ericina*, however, there are differences in the number of flagella, cell shape, scale sizes and distribution pattern of scales.

HOLOTYPE: Fig. 1, from the culture established from a water sample collected on 11 September 1988 at the port of Yokohama, Tokyo Bay, Kanagawa Prefecture, Japan (34°28′N; 139°09′E).

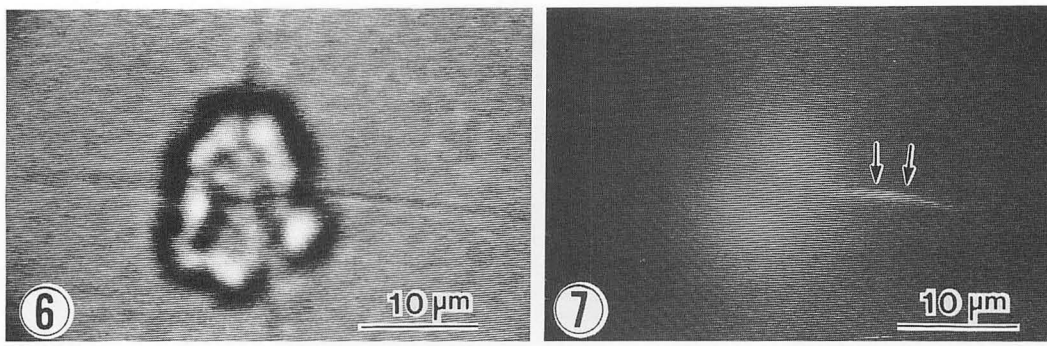


Figs. 1–5. *Chrysochromulina quadrikonta* sp. nov. Figs. 1–4. High-speed video images of the cells. Fig. 1. A typical cell during forward swimming, showing four flagella (F), a haptonema (H), spiny scales (SS) and a caudate appendage (CA). Fig. 2. Anterior part of the cell. Note the distribution of the spiny scales (SS) around the base of the haptonema. Fig. 3. Backward swimming of the cell. Fig. 4. Proximal view of the cell, showing flagella radiating in a cruciform pattern. Fig. 5. Light micrograph of a fixed cell, showing the four flagella, a coiled haptonema (arrowhead) and a deeply lobed chloroplast (arrow).

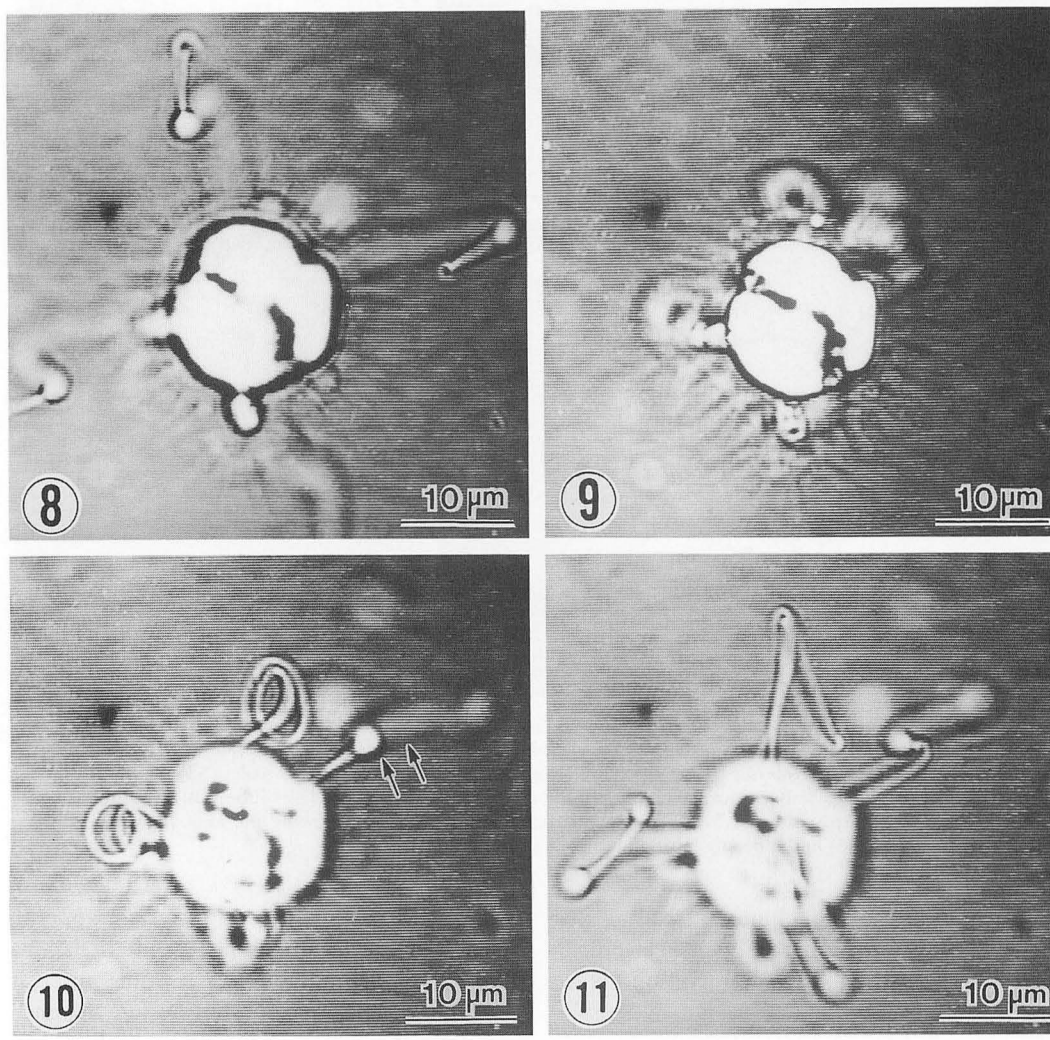
DISTRIBUTION: Yokohama, Kanagawa Prefecture, Kesen-numa, Miyagi Prefecture, Japan; Melbourne, Australia; Nelson, New Zealand.

ETYMOLOGY: quadrikonta (Latin), meaning four-flagella.

The cells are subspherical and usually possess a posteriorly projecting caudate appendage (Figs. 1, 17). Each cell has four flagella and a haptonema that arise from the anterior side of the cell, opposite the caudate appendage, and the entire surface of the cell is covered by spiny scales (Figs. 1, 17). The caudate appendage is distinctive in actively grow-



Figs. 6 and 7. Video images of *Chrysochromulina quadrikonta* sp. nov., showing flagellar autofluorescence. Fig. 6. Phase contrast image. Fig. 7. Fluorescent image. Note autofluorescence in one flagellum (arrows).



Figs. 8–11. High-speed video images of *Chrysochromulina quadrikonta* sp. nov., showing the successive processes of flagellar coiling (Fig. 8, 9) and uncoiling (Fig. 10, 11). Arrows in Fig. 10 show the first uncoiling flagellum. Time elapsed (milli-second): Fig. 8 (0), Fig. 9 (20), Fig. 10 (160), Fig. 11 (260).

ing cells, however, it is lost or disappears in both the fixed cells (Fig. 5) and the living cells in the old cultures.

Internally, two or four lateral chloroplasts are visible under the light microscope. Each chloroplast is deeply lobed into two sections and possesses an embedded pyrenoid (Figs. 5, 17).

Prior to cell division, the flagella and haptonema duplicate and then segregate to opposite sides.

The four flagella are equal in length, and they radiate forming a cruciform pattern when viewed from above the cell (Fig. 4). All the flagella are homodynamic. There is a green autofluorescence in only one of the four flagella (Figs. 6, 7).

The haptonema usually extends straight (Figs. 1, 2, 17). It is capable of coiling (Fig. 5), although coiling occurs only occasionally. Cells usually swim extending the haptonema ahead, with the flagella beating alongside the cell body, extending their distal ends backward (Fig. 1). Sometimes flagellar coiling was also observed. It coils into a helix with two or three gyres (Figs. 8–11). Coiling occurs simultaneously in all four flagella when the cells cease swimming and stop flagellar beating (Fig. 7). In a few seconds after coiling, one flagellum uncoils (Fig. 10), and then other three flagella follow (Fig. 11). Then the cells start to swim again. The haptonema is extended and never coils during the time period that flagellar coiling and uncoiling occur. Backward swimming was also observed. The cells extend all the flagella forward and generate enough propulsive force to swim backward (Fig. 3). The haptonema was usually kept stretched during backward swimming.

Haptonematal activities such as prey capturing, transportation and aggregation of prey particles that occurs in the mixotrophic species, *Chrysochromulina hirta* Manton (Kawachi et. al. 1991), have never been observed in *C. quadrikonta*. *C. quadrikonta* does not take up any food particles, and food vacuole have not been detected.

Individual spiny scales can be easily observed with the light microscope. They are distributed not only on the cell body but also around the proximal part of the haptonema, having a dome-like appearance (Fig. 2). Shadowcast whole mounts revealed that *C. quadrikonta* has two types of unmineralized scales, spiny scales and plate-like scales (Figs. 12–14). The spiny scale consists of a cylindrical upper part and a conical base (Fig. 14). The conical base bears a pattern of concentric and radiating ridges (Fig. 14). The proximal surface of the conical base has obvious radiating ridges at the edge (Fig. 14). The inner plate-like scale is subcircular and the margin is slightly thickened (Fig. 13). Each surface of the plate-like scale shows different patterns (Fig. 13). The distal surface (Fig. 13, A) bears a pattern of fine fibrils, and there is a rim at the edge (Fig. 13, arrows). The proximal surface (Fig. 13, B) is characterized by a pattern of radiating ridges.

Discussion

Regardless of possessing four flagella, it is obvious based on the presence of the haptonema and the characteristics of the scales that *C. quadrikonta* belongs to the genus *Chrysochromulina* (Prymnesiophyceae), i.e. the haptonema is well developed and the scales are different types. Ultrastructural observations of the scales indicate that this organism is closely related to the previously described species, *Chrysochromulina ericina* Parke et Manton (Parke *et al.* 1956). *C. ericina* has two different types of scales, spiny scales and plate-like scales (Fig. 16) (Parke *et al.* 1956; Manton and Leedale 1961) that resemble those of *C. quadrikonta*, i.e. the shape and surface patterns are almost identical. However, there are obvious size differences (Table 1). The plate-like scale of *C. quadrikonta* is considerably larger than that of *C. ericina*. The cylindrical part of the spiny scale of *C. quadrikonta* is wider than that of *C. ericina*, while both the length of the spiny scale and the width of the conical base of *C. ericina* are much longer than those of *C. quadrikonta*. These differences are so clear that *C. quadrikonta* is distinguishable

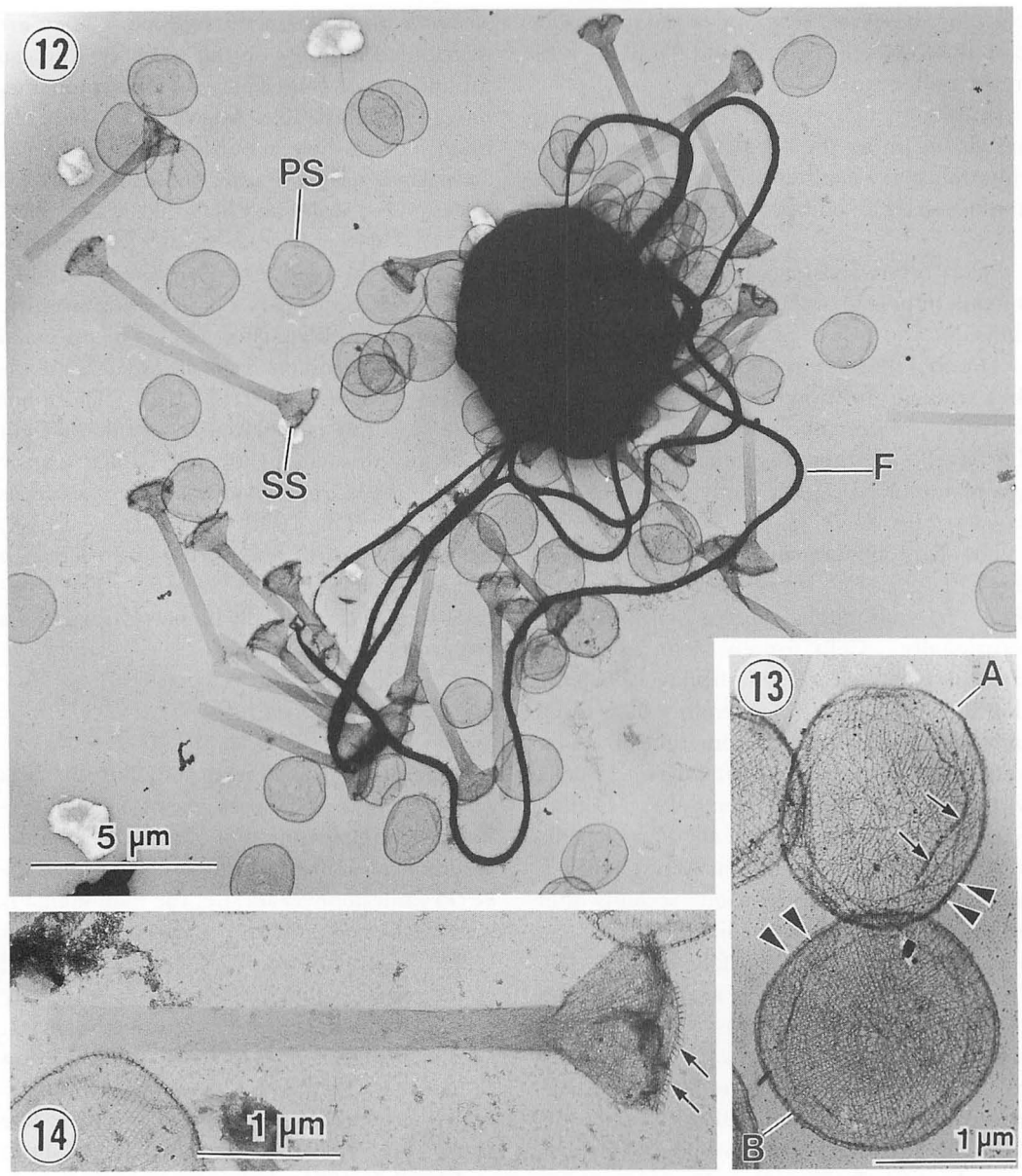


Fig. 12–14. Uranyl acetate-stained whole mount specimens of *Chrysochromulina quadrikonta* sp. nov. Fig. 12. A cell showing flagellum (F), spiny scales (SS) and plate-like scales (PS). Fig. 13. Two plate-like scales bearing different patterns. The upper plate-like scale (A) shows the pattern of the distal surface. Fine fibrils and a rim (arrows) are visible. The lower plate-like scale (B) shows the pattern of the proximal surface. The margin of plate-like scales is slightly thickened (arrowheads). Fig. 14. A spiny scale. The pattern of the conical base is visible. Note the proximal surface of the conical base bearing radiating ridges (arrows).

from *C. ericina* based only on the morphology of the scales. The dome-like distribution of spiny scales is also a characteristic feature of *C. quadrikonta* which has never been observed in other *Chrysochromulina*. Moreover, there are many distinctive differences between *C. quadrikonta* and *C. ericina* such as the shape and size of the cell, and the length of both the flagella and haptonema (Table 2). The coiling ability of the haptonema and prey capture also appears to be a difference between these two species. Parke *et al.* (1956) reported a

Table 1. Comparisons of scale size between *Chrysochromulina quadrikonta* sp. nov. and *C. ericina*.

	C. quadrikonta	*C. ericina**
plate-like scale		
width	1.2–1.6 μm	0.5–0.8 μm
length	1.4–2.0 μm	0.6–1.0 μm
spiny scale		
length	4–6 μm	8–15 μm
width of cylinder	0.35 μm	0.2–0.3 μm
width of conical base	0.8–1.2 μm	1.0–1.4 μm

* Parke et. al. (1956) and present study.

phagotrophic ability in *C. ericina*; whereas, no food capture and uptake was even observed in *C. quadrikonta*. However, *C. quadrikonta* is a close relative of *C. ericina*; therefore, based on these differences, we can conclude that the quadriflagellate prymnesiophyte is a natural taxon and should be recognized as an independent taxonomic entity.

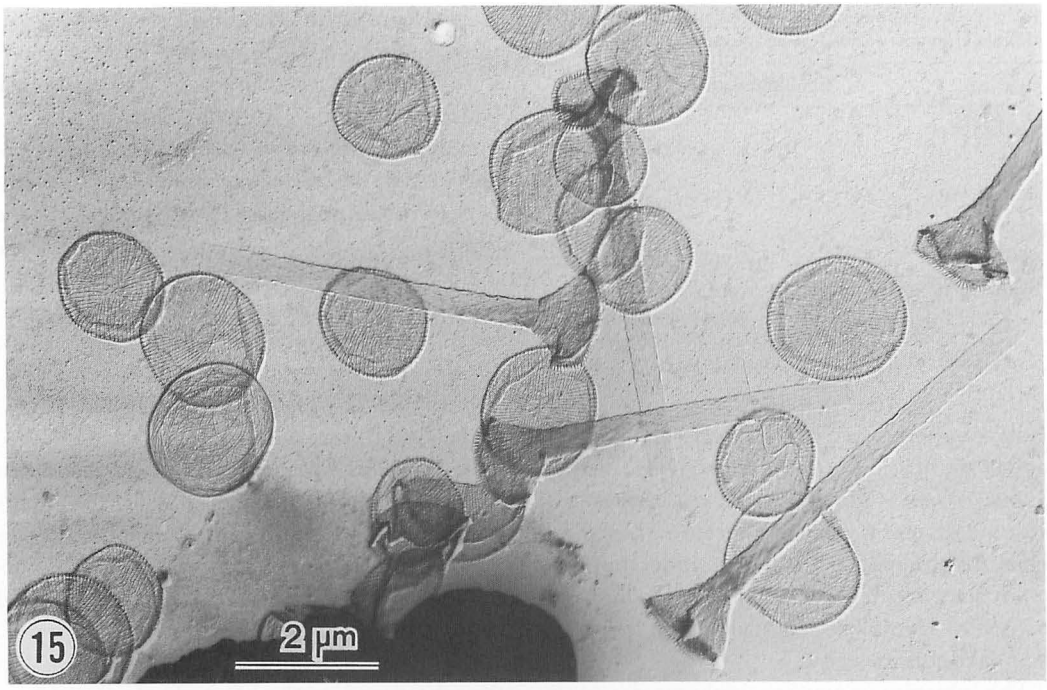
The majority of prymnesiophytes possesses two flagella, and only *Chrysochromulina birgeri* Hallfors et Niemi (Hallfors and Niemi 1974) has been described as a species possessing four flagella. However, it should be noted that the population of *C. birgeri* in the natural sea water sample consisted of not only quadriflagellate but also biflagellate forms, i.e. the number of flagella of this species are not so strict. In contrast, in the culture of *C. quadrikonta*, biflagellate cells have never been observed. Therefore, the presence of four flagella is one of the most characteristic and stable features of *C. quadrikonta*, hence the species epithet.

In green algae, the number of flagella is often regarded as a diagnostic feature of generic rank (e.g. *Chlamydomonas* vs. *Carteria* in the Chlorophyceae). However, there are examples of algae that possess different numbers of flagella that are taxonomically treated as members of the same genus. For example, in the genus *Pyramimonas* (Prasinophyceae), the majority of the species have four flagella; however, species possessing eight (e.g. *Pyramimonas octopus* Moestrup, Hori et Christeusen, Moestrup *et al.* 1987) or 16 flagella (e.g. *Pyramimonas cyrtoptera* Daugbjerg et Moestrup, Daugbjerg and Moestrup 1992) were also found in *Pyramimonas*. It is postulated that these species originated from the quadriflagellate species in the subgenus *Pyramimonas*, because of the similarities in the morphological characteristics such as scales and intracellular ultrastructures, (Moestrup *et al.* 1987; Daugbjerg and Moestrup 1992). On the other hand, in the life cycle of various species of the Ulvophyceae, quadriflagellates often occur as zoospores, while gametes are biflagellates. As mentioned above, the phenomenon of duplication of the number of flagella is not very unusual in green algal lineages. However, it is most unusual and has never been recorded in chlorophyll *c*-containing algae. Of these, only prymnesiophytes have nearly equal and homodynamic flagella. Other chlorophyll *c*-containing algae have heterokont and heterodynamic flagella so that functional differentiation of flagella may be much larger than that of prymnesiophytes. Therefore, the situation of prym-

Table 2. Comparisons of cellular characters between *Chrysochromulina quadrikonta* sp. nov. and *C. ericina*.

	C. quadrikonta	*C. ericina**
cell size	10–25 μm	5–12 μm
flagellar length	30–40 μm	20–30 μm
haptonemal length	25–30 μm	40–50 μm
cell shape	subspherical and caudate	spherical to oblong
distribution of spiny scales	periplast, haptonemal base	periplast only
coiling ability of haptonema	yes (but rare)	yes
phagotrophy	probably no	yes
cell form	motile	motile, amoeboid, walled

* Parke et. al. (1956).



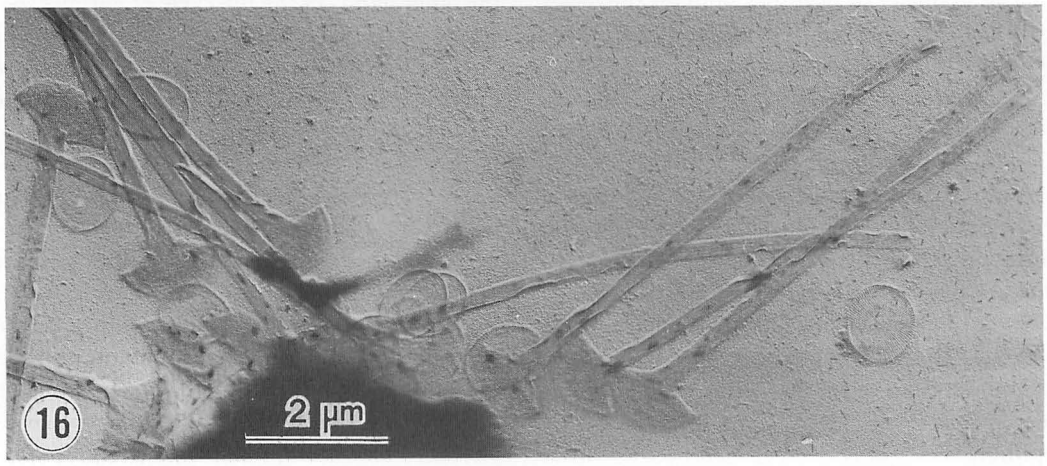


Fig. 15 and 16. Shadowed whole mount specimens allied algae. Fig. 15. Scales of a biflagellte organism collected in 1986 from Tokyo Bay. Fig. 16. Scales of *Chrysochromulina ericina*.

nesiophytes is almost the same as in green algae. Hence, it tempts us to speculate that the doubling of flagella is apt to happen in the evolution of organisms that possess equal and homodynamic flagella and whose functions are more or less the same.

Despite its distinct morphology and its ability of forming a bloom, it is strange that *C. quadrikonta* has not been described before. It may have been overlooked in previous floristic studies; however, its rather sudden emergence in various parts of the Western Pacific during the last several years may require other explanations. One possible postulation is that *C. quadrikonta* may be a species recently established from a biflagellate species of *Chrysochromulina* by the "doubling" of the flagella. It should be noted in relationship to this interpretation that a biflagellate organism exists which has a cell form and scales (Fig.

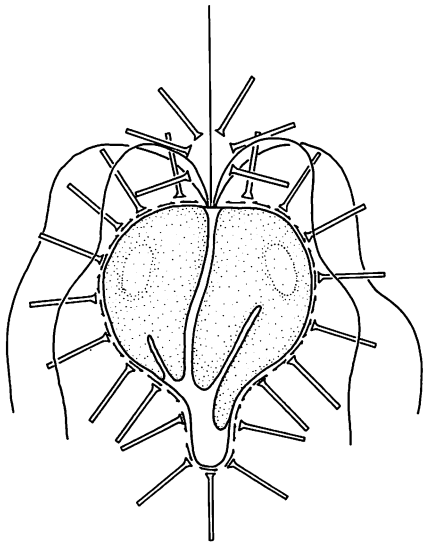


Fig. 17. Diagram of *Chrysochromulina quadrikonta* sp. nov.

15) almost the same as *C. quadrikonta*. We collected this alga from Tokyo Bay in July 1986, two years before the first record of *C. quadrikonta*. In the natural sea water samples and a culture of the biflagellate, we did not notice a quadriflagellate form. At that time we identified the biflagellate alga as *Chrysochromulina ericina* and provided it as a material for a previous work on flagellar autofluorescence of chlorophyll *c*-containing algae (Kawai and Inouye 1989, Figs. 16, 17). Since 1988, however, only the quadriflagellate form has been collected from this location. It is interesting to know how the quadriflagellate species was established, and what type of cytological changes made such a drastic transfiguration possible. Careful investigations on the flagellar-haptonematal apparatus architecture and morphological changes during cytokinesis may provide clues to answer these questions.

Acknowledgments

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河地正伸・井上　勲：4本鞭毛をもつハプト藻，*Chrysochromulina quadrikonta* の新種記載

東京湾から分離，培養した，4本鞭毛をもつハプト藻を記載した。本種の鞭毛は等長で，同調した運動を示し，1本のみが自家蛍光をもつ。2種類の鱗片形態は *Chrysochromulina ericina* のそれに類似するが，サイズは異なり，容易に区別される。更に，本種と *C. ericina* は細胞形態や刺状鱗片の分布様式も異なる。これらの差異に基づいて，本種を *Chrysochromulina quadrikonta* と命名した。(305 茨城県つくば市天王台1-1-1　筑波大学生物科学系)

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