Chrysocystis fragilis gen. nov., sp. nov. (Chrysophyceae, Sarcinochrysidales), with Notes on Other Macroscopic Chrysophytes (Golden Algae) on Guam Reefs

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Abstract—Golden algae up to several centimeters long occur on the reefs of Guam and other western Pacific islands. In particular, a sometimes-abundant saccate chrysophyte occurs that does not fit any of the known genera. This alga forms fragile colonies with the cells dispersed in a common matrix, and forms motile cells by two successive divisions of the vegetative cells. The motile cell possesses two unequal, lateral flagella. These features suggest that this alga is a new member of the order Sarcinochrysidales in the class Chrysophyceae. Another macroscopic member of the Sarcinochrysidales, the filamentous Chrysonephos lewisi (Taylor) Taylor, also occurs on Guam, together with some stiff gelatinous colonies and the enigmatic alga Phaeocystis taylori Lewis et Bryan.

Introduction

The Chrysophyceae comprises golden-brown microscopic algae (containing chlorophylls a and c, and fucoxanthin as a major accessory pigment) and their colorless relatives. They are classified in the Division (Phylum) Chrysophyta, which is part of the new Kingdom Chromista (Cavalier-Smith 1986, 1989). The
Chrysophyceae were relatively poorly studied until recently, perhaps because
many are small or rare phytoplankton (Andersen 1987). Studies in the last twenty
years or so have led to the separation of several new classes, most recently the
Pelagophyceae (named after the nannoplankter *Pelagomonas*, not the giant kelp
*Pelagophycus*) (Andersen et al. 1993). Thus the presence of macroscopic species
or large colonies of chrysophytes is of interest for extending our knowledge of
this class, and potentially for better understanding its relationships with other
Chromista (such as the brown algae) (O’Kelly & Floyd 1985, O’Kelly 1989, Gayral

Among the red, green, and brown seaweeds on the reefs of Guam and other
islands in the western Pacific Ocean are some golden-yellow algae, most of which
proved to be in the Sarcinochrysidales—an order of Chrysophyceae which An­
dersen et al. (1993, p. 713) characterized as “enigmatic.” One of these, which can
be abundant and conspicuous, is in a new genus. We also report the presence of
several other macroscopic chrysophytes.

**Materials and Methods**

Field observations were made by snorkeling or diving on the reef platforms
and fore-reef slopes of Guam, Iriomote (Ryukyus), Palau, Pohnpei, and Majuro.
The fragile colonies of *Chrysocystis* were collected by floating them into plastic
bags or sucking them off the algal turf with large pipettes. Other species were
coherent enough to pick by hand.

Cultures of *Chrysocystis* were established from single cells isolated from
samples collected from Guam and Iriomote. Single cells were isolated by capillary
pipette and transferred into ESM medium (cf. Watanabe & Kasai 1985). Cultures
were maintained at 20°C and 25°C on a 12:12h light-dark cycle.

For light microscopy, an equal volume of cell suspension and 2% glutara­
raldehyde solution (prepared in cacodylate buffer [pH 7.2] containing 0.5M su­
crose) were mixed and observed with a Nikon Optiphot ZF and XF-NT fitted
with a Nomarski interference differential contrast objective.

For observation of chloroplast DNA, a drop of cell suspension was mixed
with 2% glutaraldehyde solution and 1 µg mL⁻¹ 4',6-diamidino-2-phenylindole
(DAPI) solution. Both solutions were prepared in TAN buffer consisting of 0.25M
sucrose, 20 mM Tris-HCl (pH 7.6), 0.5 mM EDTA, 1.2 mM spermidine, 7 mM 2-
mercaptoethanol and 0.4 mM PMSF (Nemoto et al. 1988). Cells were then viewed

Figures 1–2 (color plate).  *Chrysocystis fragilis* colonial morphology. Figure 1. Nat­
ural habitat at a depth of 5 m, Anae Island, Guam. Figure 2. The fragile, saccate
colonies attached to dead coral ca. × 4.
with an epifluorescence microscope (Olympus BHS-RFK) under ultra-violet excitation.

For observations of non-fixed zoospores, we used a VHS high-speed video of NTSC format (NAC MHS-200) mounted on a Nikon Optiphot bright field microscope at 200 frames per second.

*Chrysocystis fragilis*

**HABITAT**

Colonies are present year round on the reefs of Guam, from lower water to at least 20m deep. Water temperatures are 28–30°C. Colonies are extremely abundant in shallow water in April–June, when they form extensive epiphytic masses on algal turf over rocks and dead coral (Fig. 1). Scattered colonies of a filamentous chrysophyte, *Chrysonephos lewisi* (Taylor) Taylor (Taylor 1951, 1952), are often intermixed with *Chrysocystis fragilis*. Colonies have been rarely found on the windward side of the island except in flowing seawater tanks. On the leeward side, many sites have significant wave action during the wet season when tropical storms and typhoons are common, and at these sites we have only found colonies during the “spring bloom”. These observations suggest that *C. fragilis* can exist in the microbenthos and is probably very widespread. Other factors besides wave action are expected to be involved in the abundant development of saccate colonies, but we have not attempted to define these.

*Records* (partial list)—Orote Pt., 6/10/89 (GU38A), 10/3/89 (GU38F, G); Glass Breakwater/Luminao Reef, 7/1/89, 9/5/89 (GU41C), and many subsequent occasions; Anae I., 4/10/89 (GU35A); Hilaan Reef, 11/11/89 (GU6H); Manoan Channel, Merizo, 9/15/89 (GU14K). Chance observations on other islands indicate that *C. fragilis* is also abundant on Tinian, Saipan and probably other northern Mariana Islands in April–June. In Japan, *C. fragilis* was observed on Iriomote Island (Ryukyu Islands), 3/91.

**MORPHOLOGY**

Colonies are cylindrical or irregular sacs in which cells distribute in a layer of very fragile, amorphous mucilage (Fig. 2). The interior of the colony contains very watery mucilage (Fig. 3). Colonies are too fragile to pick up by hand and are easily dislodged by water currents such as from a diver’s fins. However, they grow in areas where there is some water exchange or slow currents. The colonies are usually 20–50mm long and about 5mm wide in size, but may be much wider or longer because we observed 0.3m long specimens in calm places. Probably the colony size is affected by the extent of water motion or other disturbance. In culture condition this alga never formed cylindrical narrow colonies. It formed irregular, gelatinous, heaped colonies on the bottom of the culture dish. There is no distinct holdfast or point of attachment; rather, the lower part of the colony engulfs the turf. Colonies often harbor other algae, especially diatoms, benthic dinoflagellates and cyanophytes. Older colonies are invaded by small crustaceans, other animals and protists.
Cells are irregularly spherical, 8–14 µm in diameter, and disposed mainly at the periphery of the colony (Figs. 3, 4). There are neither boundary lines nor layered structures in the gelatinous material surrounding the cells. They contain four to eight light-brownish chloroplasts which are slightly constricted disk-shaped. Each of them possesses one pyrenoid (Figs. 4–7). Under the cell membrane there are noticeable refractive muciferous bodies which give the cell outline a faintly beaded appearance, especially in optical section (Figs. 4, 5).

Reproduction by motile cells has been infrequently observed; the abundance and fragility of colonies suggest that fragmentation may be an important means of dispersal. When freshly collected colonies were put in an air-conditioned room (22–24°C) overnight, motile cell formation sometimes occurred. Motile cell production involves two successive cell divisions of vegetative cells, resulting in formation of four biflagellate cells (Figs. 5–8). Although this is suggestive of meiosis, we could not count chromosome number and have no evidence of cell fusion. Cytokinesis takes place by furrowing of plasmalemma from one side of the cell (Figs. 5, 8). While chloroplast division occurs at vegetative cell division, at the cell divisions of the motile cell formation it does not occur and the chloroplasts are distributed among the daughter cells.

Each motile cell is oval or somewhat pyriform, measuring typically 4–5 µm long and 2–3 µm diam. (Figs. 9, 10). One light-brownish chloroplast is present at the posterior part of the cell. Two unequal flagella are inserted laterally near the edge of the chloroplast. The anterior locomotive flagellum, 9–13 µm long, displays rapid sinusoidal quivering. The posterior flagellum, 5–6 µm long, exhibits...
active lashing or undulating. The cell swims with a rotary motion. Although the eyespot is absent, the basal portion of the posterior flagellum faces a part of the chloroplast. The cell has muciferous bodies and the electron dense cell covering as do vegetative cells (Figs. 13–14). Under the electron microscope the pyrenoids are seen to be stalked (Fig. 16). Scale-like structures have been noticed in a vacuole (Fig. 15).

Unlike typical chrysophytes, the chloroplast DNA is not ring-shaped. Many small DNA-containing areas are scattered throughout the chloroplast (Figs. 11, 12). Their distribution pattern is stable throughout the life history of this alga.
Figures 8–12. *Chrysocystis* fragilis. Figure 8. Cytokinesis of a vegetative cell showing the cleavage furrow from one side (open arrow). Figures 9 and 10. Motile cells, showing the anterior flagellum (arrow) and the posterior flagellum (arrow head); Figure 9. Fixed motile cell. Fig. 10. Swimming motile cell (high-speed video image). Figures 11 and 12. Crushed vegetative cell fixed and stained for DNA with DAPI in phase contrast (Figure 11) showing the chloroplasts and the same cell under ultraviolet excitation (Figure 12) showing a number of separate small fluorescences of chloroplast DNA in the chloroplasts. Scale bars = 5 µm.

**DESCRIPTION**

*Chrysocystis* Lobban, Honda et Chihara gen. nov.

*Planta colonialis, gelatinosa, solida, pallide aurea, cellulis periphericis locatis constans; cellulae chloroplastos aureos habentes.*

*Reproductio asexualis a fragmento coloniae vel forma zoosporarum effecta; reproductio sexualis ignota; zoosporae per duas successivas divisiones cellularum vegetativarum, factae pyriformes vel ovatae, unum aut aliquot chloroplastos et flagella bina inaequalia inserta lateraliter habentes, sine stigmate.*

*Typus generis: Chrysocystis fragilis sp. nov.*

Plant colonial, gelatinous, solid, pale golden yellow, consisting of peripherally located cells; cells having golden yellow chloroplasts.

Asexual reproduction by means of fragmentation of colony or formation of zoospores; sexual reproduction unknown; zoospores formed by two successive
cell divisions of vegetative cells, pyriform or ovoid, with one or several chloroplasts and two laterally inserted unequal flagella; stigma absent.

Type of the genus: *Chrysocystis fragilis* sp. nov.


**Chrysocystis fragilis** Lobban, Honda et Chihara sp. nov.

*Colonia cylindrica*, *gelatinosa*, *fragilis*, 30–50 mm longa, ca. 5 mm diam.; *cellulae sphaericae*, 8–14 µm diam., *pariete tenui obtectae*, *corporibus mucilaginis parvis parietalibus*; 4–8 *chloroplasti parietales*, aurei, *elliptici*, *acetabuliformes*.
saepe ad centra constricti, cum una vel duabus pyrenoidibus protuberantibus; zoo­sporae pyriformes vel ovatae, 4–5 µm longae, 2–3 µm latae, chloroplasto unico, postice; flagellum anticum 9–13 µm longum, flagellum posticum 5–6 µm longum. 


Colony cylindrical, gelatinous, fragile, 30–50 mm long, about 5 mm in de­iameter; cells spherical, 8–14 µm in diameter, covered with a thin thecate wall, with small muciferous bodies peripherally located; four to eight peripheral chloroplasts, elliptical, saucer-shaped, often constricted at the center; possessing one or two projecting pyrenoids; zoospores pyriform or ovoid, 4–5 µm long, 2–3 µm wide, with single posterior chloroplast; anterior flagellum 9–13 µm long, posterior flagellum 5–6 µm long.


Paper mounts of colonies: TNS-AL39674-896777; UC1612796; and at UMICH.

Etymology: fragilis, Latin: fragile.

*Chrysonephos lewisi* (Taylor) Taylor

Dichotomously branched filaments forming yellow tufts (Taylor 1951; Taylor 1960, p. 195 and pl. 28). Tufts as seen on Guam are generally short (10–20 mm) in the field, but plants found in seawater tank were very much longer (Figs. 17, 18). Although filaments resemble an ectocarpoid brown alga, they are not truly multici­ellular but comprise mucilaginous tubes into which individual cells are packed. This structure becomes evident during reproduction, when cells in the filaments round up and become motile swar­mers that are released from the end of the tube (Figs. 19, 20). Zoospore release may often be induced by keeping filaments in a dish at room temperature (20–22 C—cool compared to field conditions). Zoospores are released after about 24 h and showed photoaccumulation (Fig. 21).


Other Sarcinochrysidales

Sturdy gelatinous colonies, resembling *C. fragilis* except for being firm and stiff have been occasionally found on Guam. We have tenta­tively identified these

Figures 17–20 (color plate). *Chrysonephos lewisi*. Figure 17. Unusually long col­onies in seawater tank, rising ca. 30 cm from bottom and forming floating mats. Figure 18. Normal colony in seawater tank, approx. 2× life size. Figure 19. Zoospore release. Scale = 50 µm. Figure 20. Detail of zoospore in mucilage tube. Scale = 10 µm.
as including *Sarcinochrysis* sp. and *Pulvinaria* sp. (Figs. 22, 23). *S. marina* has been reported from enrichment cultures from a Hawaiian reef (West 1969).

**Chrysophaeum taylori** Lewis et Bryan

Clumps of gelatinous streamers which have the texture of large blue-green algal colonies, but with yellow cells powdering the tips (Fig. 24). Cells are on the ends of delicate, intertwined stalks but the stalks cohere in the copious mucilage. The cells are very unusual, with a tubular invagination at the anterior end (Figs. 25, 26). While Lewis & Bryan (1941) described this as a "putative Cryptophyte", no work has been done on the cytology or pigmentation of this organism. See also Taylor (1960, p. 194). This species has sometimes been abundant on Rizal
Beach, Guam, and blooms at Townsville, Australia were extensive enough to be considered a public nuisance (I. R. Price, pers. communication).

Discussion

The structures of vegetative and motile cells of *Chrysocystis* are similar to those of *Pulvinaria* (Billard & Fresnel 1980) but *Chrysocystis* has a distinctive

| Table 1. Comparison of *Chrysocystis* with related Sarcinochrysidaceae |
|-------------------------------------------------|-------------------------------------------------|-------------------------------------------------|
| Colony type | Pulvine colonies a few mm in diameter | Unicellular & planktonic or small palmelloid colonies |
| Habitat | Sublittoral, epiphytic, stenohaline? | Upper littoral, saltmarsh; sublittoral; epiphytic; euryhaline |
| Arrangement of cells | Irregularly dispersed in a common matrix | Irregular packets surrounded by thick matrix; layers of matrix around older cells | Cube; short filament; irregular packet |
| Muciferous bodies | Yes | Yes | Yes |
| Plastid: number | 4–8 | *P. feldmannii*: 2 (more in mature cells) | 1–2 |
| Pyrenoid genome | Type III | *P. algicola*: 4 | Type III |
| Motile cell formation | Two successive divisions of vegetative cell | *P. feldmannii*: “emission” of single flagellated cells | “Emission” of single flagellated cells |
| Motile cells | 4–5 × 2–3 µm; 1 plastid; no eyespot | 2.5–3 × 6–7.5 µm; 1 plastid; no eyespot | No eyespot |
| Flagella | Ant: 9–13 µm; Post: 5–6 µm | Ant: 7–8 µm; Post: 5–6 µm (for genus) | Ant: 10–12 µm; Post: 6–7 µm |

* Data on *Pulvinaria* and *Sarcinochrysis* from Geitler (1930), Gayral (1972); and see Billard & Fresnel (1980) and Billard (1984).

Figures 22–24 (color plate). Figures 22–23. Stiff gelatinous colony, possibly *Pulvinaria*. Fig. 22. Whole colony. Fig. 23. Detail of cells showing evidence of layered mucilage (arrows). Scale = 10 µm. Figure 24. *Chrysophaeum taylori* colonies, approx. × 3.
macroscopic morphology and formation of motile cells (Table 1). *Chrysocystis* should thus be placed in the Sarcinochrysidales, Sarcinochrysidaceae. The cell covering, projecting pyrenoid, scattered chloroplast DNA, and scale-like structures suggest that *Chrysocystis* belongs to the Sarcinochrysidales *sensu stricto* (O'Kelly 1989, Honda & Inouye 1995).

The mode of thallus formation suggests an extension of the series identified by Billard & Fresnel (1980, p. 291), “Dans cette famille [Sarcinochrysidaceae] une série évolutive serait esquissée qui, partant de formes planctoniques telles *Ankylonyton luteum*, en passant par un genre intermédiaire, le *Sarcinochrysis*, aboutirait à une organisation strictement benthique et palmelloïde chez *Pulvinaria*.” The tendency of *Chrysocystis* to form heaped colonies in culture rather than the typical field morphology emphasizes the basically palmelloid type of colony, and distinguishes *Chrysocystis* from more-organized genera in the Sarcinochrysidalean family Phaeosaccionaceae, such as *Phaeosaccion* and *Antarctosiphon*.

These little-known algae are common and very visible on the reef platforms of Guam, and (as suggested by the scattered records) probably much more widely on tropical Pacific reefs. Part of the reason for their being little-known is that they are not “typical” seaweeds (i.e., Chlorophyta, Phaeophyta and Rhodophyta) but belong to Protistan/Chromistan Divisions that have mostly microscopic members. Yet, their unusual taxonomic position makes them fascinating and important for further study.

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**References**


