

Mayumi ISHIMITSU* & Mitsuo CHIHARA*: **Four species of
Cryptomonas (Class Cryptophyceae) in Japan****

石光真由美*・千原光雄*: 日本産クリプトモナス属の4種
(クリプト藻綱)

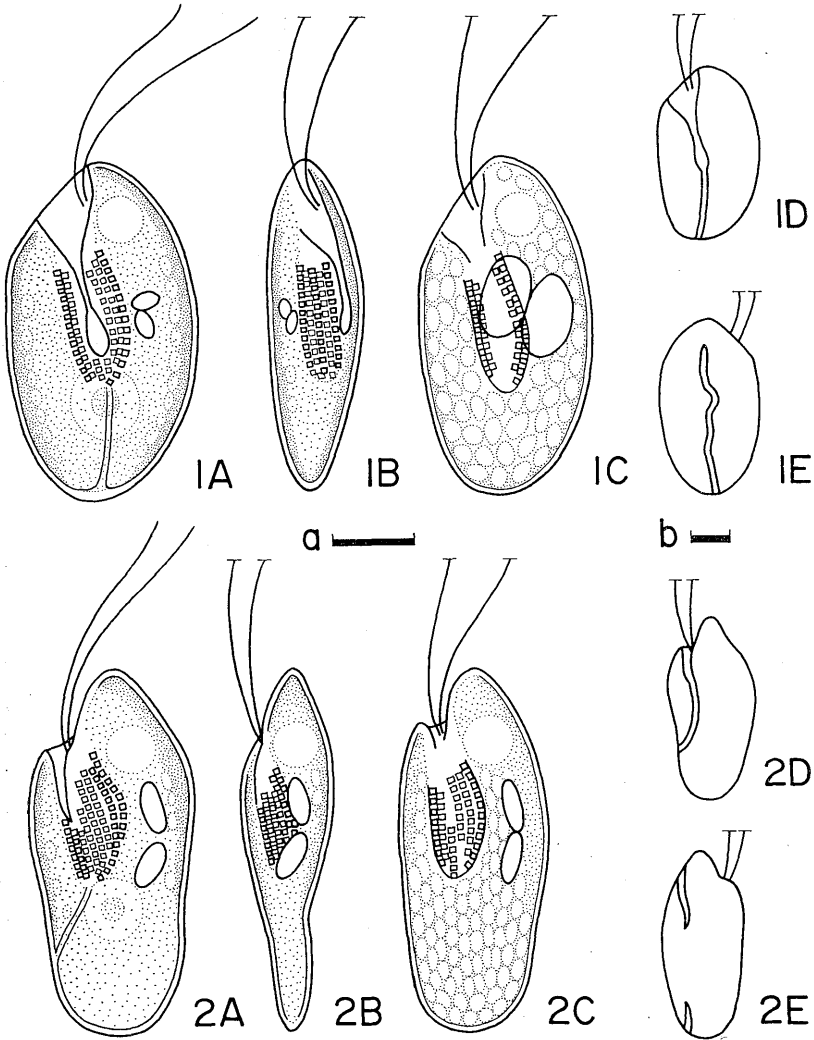
The Cryptophyceae comprise a group of biflagellate unicellular organisms that are called "cryptomonads". They inhabit both fresh-water and marine habitats. There are more than 200 species described so far in this algal group, but almost nothing is known of the Japanese representatives. For accumulating a basic knowledge of the cryptomonads in Japan, we have made the collections in various places and then carried out their laboratory cultures to find characteristics more reliable in separation of species.

This and the succeeding papers will give accounts of the cryptomonads of Japan, based on the results that we have obtained. In this way, it is hoped that the accounts will contribute to a future flora of the Japanese cryptomonads.

Materials and methods The specimens were isolated from samples of waters, sediment or detritus collected from various places, which will be indicated in the description of species examined. The samples were isolated directly by means of glass micropipette method. Each isolate was inoculated into a 30 ml test tube, as culture container, containing 10 ml of sterile VT medium (Provasoli & Pintner 1959). The component of VT medium is as follows: $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$, 11.78 mg; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 4 mg; $\beta\text{-Na}_2$ glycerol $\text{PO}_4 \cdot 5\text{H}_2\text{O}$, 5 mg; KCl, 5 mg; Vitamin B₁, 1 μg ; Vitamin B₁₂, 0.01 μg ; Biotin, 0.01 μg ; Glycylglycine, 50 mg; P IV metals, 0.3 ml; H₂O, 99.7 ml; pH 6.0. The culture containers were placed in a culture room under the following conditions: light intensity, ca 2,000 lux; photoperiod, 14 hrs light/10 hrs dark; temperature, 20°C. In the most cases after about two or three weeks, growth became visible in all the culture tubes and those cultures were observed with light microscopy. For the observations, living specimens were used as possible. A drop of glutaraldehyde or Lugol solution was used for fixation or for the detection of pyrenoids.

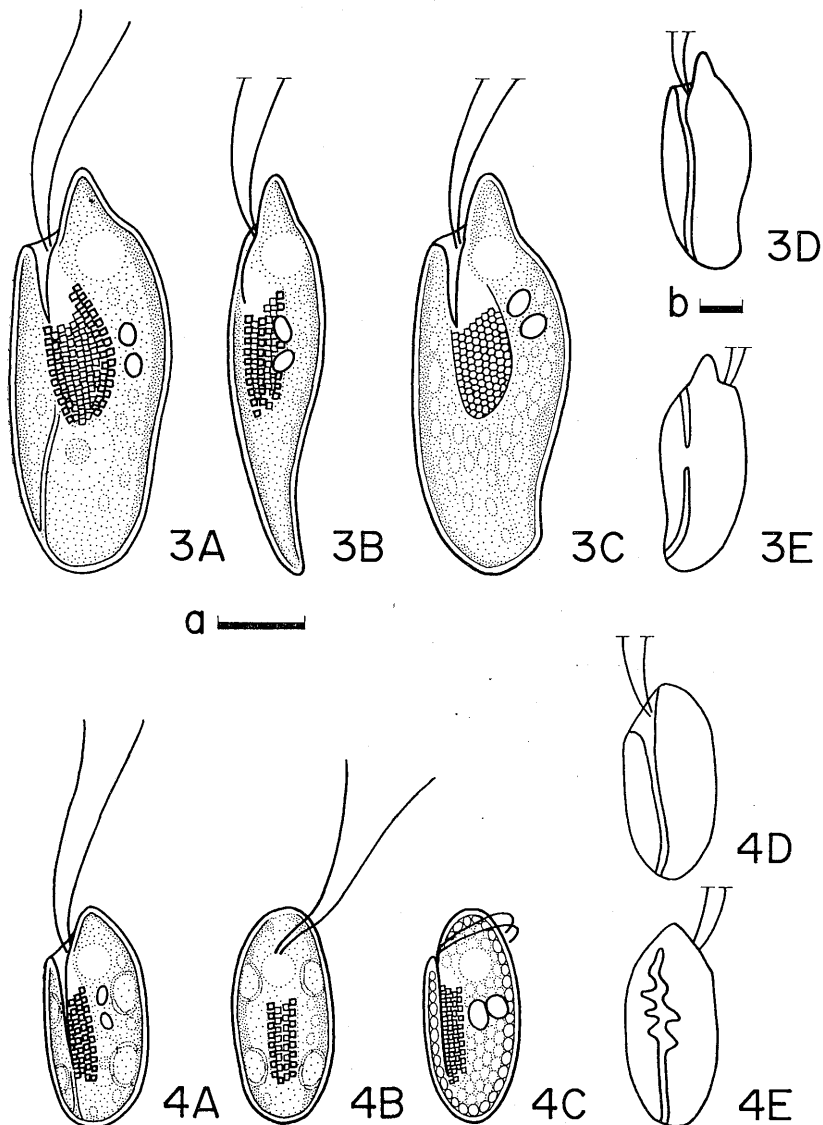
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Figs. 1A-E. *Cryptomonas ovata* Ehrenberg. A-B. Forms in culture; A. lateral view, B. ventral view. C. Form in nature (Shishizuka, Ibaraki). D-E. Forms showing the outline of chloroplast; D. left-lateral view, E. right-lateral view.

Figs. 2A-E. *Cryptomonas platyris* Skuja. A-B. Forms in culture; A. lateral view, B. dorsal view. C. Form in nature (Shishizuka, Ibaraki). D-E. Forms showing the outline of chloroplast; D. left-lateral view, E. right-lateral view. Scales (10 μ m): (a) 1A-C, 2A-C; (b) others.



Figs. 3A-E. *Cryptomonas rostratiformis* Skuja. A-B. Forms in culture; A. lateral view, B. dorsal view. C. Form in nature (Zennyuji, Hiroshima). D-E. Forms showing the outline of chloroplast; D. left-lateral view, E. right-lateral view.

Figs. 4A-E. *Cryptomonas tetrapyrenoidosa* Skuja. A-B. Forms in culture; A. lateral view, B. ventral view. C. Form in nature (Minami-izu, Shizuoka). D-E. Forms showing the outline of chloroplast; D. left-lateral view, E. right-lateral view. Scales (10 μ m): (a) 3A-C, 4A-E; (b) others.

- 1) **Cryptomonas ovata** Ehrenberg, Abh. K. Acad. Wiss. Berlin. 1831. Phys. 57, 1832. Huber-Pestalozzi, Phytoplank. Süßwas. 3: 46, pl. 4, f. 24, 1950. (Figs. 1A-E)

Cells ovate or ellipsoidal, laterally compressed, with broadly rounded posterior and obliquely truncate anterior, 26-40 μm in length, 15-23 μm in width, 8-10 μm in thickness; ventral side almost straight, dorsal side convex; furrow evident, vertical, reaching a point just below the center of the cell, with rather broad funnel-like opening and a little wider ending; gullet present under the furrow, extending beyond it, about 3/5 times the cell length, lined with 5-8 rows of trichocysts, almost unique in width, about 1/3 times the cell width; periplast smooth; chloroplast one, olive-green to brown, divided into two parts, covering the inside of the cell entirely; pyrenoid absent; eyespot absent; contractile vacuole situated anteriorly, near the opening of furrow; nucleus large, situated near the base of the cell; starch grains 2-3 μm in diameter, often distributed densely inside the chloroplast; refractive bodies sometimes present, two in number, oval, 3-10 μm in diameter, distributed near the center in dorsal side; flagella two, subequal in length, about 3/4 to 4/5 times the cell length; cell division occurring in gelatinous matrix.

Habitat: Occurring in ponds.

Type locality: Berlin, Germany.

Geographical distribution: Europe, North America and Japan (Ibaraki).

Specimens examined: Shishizuka, Tsuchiura-shi, Ibaraki-ken, Sept. 10, 1982 (M. Ishimitsu, #00042, 45-47, 51).

Our alga agrees very well with the description by Pascher (1913) and Huber-Pestalozzi (1950) except for the feature of chloroplast. According to the description, the alga has two chloroplasts, whereas our specimen has not two but only one chloroplast, which is "bilobed", i. e. it consists of two large parietal parts joined by a narrow bridge located at almost anterior end (Fig. 1E) and each of the parts covers half of the cell respectively (Fig. 1D). This condition of chloroplast was first revealed by Ettl (1980), with certain species of *Cryptomonas*, including *C. curvata* Ehrenberg, *C. ozolinii* Skuja, *C. rostratiformis* Skuja and *C. tetrapyrenoidosa* Skuja. It appears to be considerably common in this group of algae. As will be presented later, we have also found such a "bilobed" chloroplast in many specimens of other *Cryptomonas* species from Japan.

This species is very similar to *C. erosa* Ehrenberg in general morphology

but the latter species differs in its smaller size and uncompressed shape and in having shorter gullet.

In the traditional classification, species of the cryptomonads have been distinguished mainly on the basis of shape, size and color of cell body, number and size of starch grains, and presence or absence of refractive body. Pringsheim (1944, 1968) and Butcher (1967) made taxonomic studies of this group of algae and considered none of these characteristics to be very reliable in separation of species. For obtaining further knowledge of these features as diagnostic criteria, we have compared them between specimens from nature and cultured ones for each species. In this species, the cultured specimens (Fig. 1A) became more compressed in shape and more brownish in color and accumulated smaller starch grains less in number than natural ones (Fig. 1C). The two refractive bodies also less developed in cultured ones.

2) **Cryptomonas platyuris** Skuja, Symb. Bot. Upsal. 9 (3) : 358, *pl.* 37, *f.* 37, 38, 1948. Huber-Pestalozzi, Phytoplank. Süßwas. 3 : 56, *pl.* 6, *f.* 34, 1950. (Figs. 2A-E)

Cells narrowly oblong, laterally strongly compressed, particularly in a posterior-half, with truncate and rostrate anterior and broadly rounded posterior, 34-46 μm in length, 14-21 μm in width, 7-10 μm in thickness; ventral side slightly concave, dorsal side convex; furrow short, about 1/3 times the cell length, slightly curved, tapering off to the point; gullet somewhat obliquely developed, about a half of cell length, becoming wider in the middle, 1/3 times the cell width, lined with several rows of trichocysts which appear at times to be a reticulum; periplast smooth; chloroplast one, brownish-green to brown, divided into two parts, covering the inside of the cell entirely; pyrenoid absent; eyespot absent; contractile vacuole situated anteriorly, just beside the gullet; nucleus large, situated just below the center of the cell; starch grains 1-2.5 μm in diameter, scattering inside the chloroplast, sometimes densely distributed; refractive bodies sometimes present, two in number, oval or rod-shaped, 4-8 μm in length, distributed near the center in dorsal side; flagella two, subequal in length, about 2/3 to 3/4 times the cell length.

Habitat: Occurring in marsh and ponds.

Type locality: Säbysjön, Uppland, Sweden.

Geographical distribution: Europe and Japan (Ibaraki, Nagano and Hiroshima).

Specimens examined: Shishizuka, Tsuchiura-shi, Ibaraki-ken, Nov. 9 and 18.

1982 (M. Ishimitsu); Sugadaira, Sanada-cho, Nagano-ken, July 31, 1982 (M. Ishimitsu, # 00034); Saijo, Higashihiroshima-shi, Hiroshima-ken, Aug. 23, 1983 (M. Ishimitsu, # 00096, 103).

Our alga mostly coincides with the original description (Skuja 1948). Although Ettl (1980) reported that it has always two chloroplasts, our result partially supports Skuja's view that it has one or rarely two chloroplasts. In our observation the alga has always one bilobed chloroplast with the bridge much wider and situated in the more posterior part than that of *C. ovata* (Figs. 2D and E).

This species is similar to *C. ovata* and *C. rostratiformis* in cell size, but it differs from these two in the shape of the anterior part of cell body and the form or the length of the furrow.

As in *C. ovata*, specimens in natural waters (Fig. 2C) have more and larger starch grains and more or less well-developed refractive bodies than cultured ones (Fig. 2A).

3) **Cryptomonas rostratiformis** Skuja in Huber-Pestalozzi, *Phytoplank. Süßwas.* 3: 55, pl. 6, f. 33, 1950. (Figs. 3A-E)

Syn: *Cryptomonas rostrata* Skuja, *Symb. Bot. Upsal.* 9(3): 359, pl. 37, f. 39, 40 (1948) non Troitskaya (1922).

Cells narrowly oblong or ellipsoidal, remarkably compressed, particularly in posterior half, with evidently rostrate (beak-shaped) anterior and broadly rounded posterior, which sometimes recurves, 32-57 μm in length, 14-22 μm in width, 8-15 μm in thickness; ventral side almost straight, dorsal side convex or sigmoid; furrow slightly curved, about 1/4 times the cell length, tapering off to the point; gullet vertically or somewhat obliquely developed, extending beyond the center of the cell, becoming slightly wider in the middle, with 8-10 rows of trichocysts which are sometimes densely packed; periplast smooth; chloroplast one, light vermilion or orange-brown, divided into two parts, occupying most of the cell, sometimes with folds on periphery; pyrenoid absent; eyespot absent; contractile vacuole situated anteriorly, just below the rostrate part; nucleus large and evident, situated near the base of the cell; starch grains 2-2.5 μm in diameter, scattering inside the chloroplast; refractive bodies often present, two in number, spheroidal, 3-4 μm in diameter, distributed in the upper-dorsal part of the cell; flagella two, subequal in length, about 1/2 to 2/3 times the cell length; cell division occurring while swimming.

Habitat: Occurring in marsh and ponds.

Type locality: Säbysjön and Bredsjön, Uppland, Sweden.

Geographical distribution: Europe and Japan (Nagano and Hiroshima).

Specimens examined: Sugadaira, Sanada-cho, Nagano-ken, July 31, 1982 (M. Ishimitsu, # 00003, 6, 11, 20); Zennyuji, Hongo-cho, Hiroshima-ken, Oct. 19, 1983 (M. Ishimitsu, # 00148, 154).

Our alga agrees well with the original description except the number and the color of chloroplast. The chloroplast of the specimens, as reported in other Japanese representatives, is only one and bilobed with the narrow bridge part in the middle of the cell (Figs. 3D and E). On the other hand, the color of chloroplast is olive-green, or grayish- or brownish-green, according to Skuja (1948), but that of our specimens from both collecting sites is somewhat reddish orange-brown.

This species is rather similar to *C. platyuris*. However, it can be readily identified by its beak-shaped anterior and slightly recurved posterior parts of cell body. This species is also distinct from *C. platyuris* by its curved cell body towards the opposite direction of the furrow when seen from dorsal side.

4) **Cryptomonas tetrapyrenoidosa** Skuja, Symb. Bot. Upsal. 9(3): 352, *pl.* 37, *f.* 32-36, 1948. Huber-Pestalozzi, Phytoplank. Süßwas. 3: 62, *pl.* 7, *f.* 45, 1950. (Figs. 4A-E)

Cells cylindrical or obovate, scarcely compressed, with obliquely truncate anterior and round to somewhat subacute posterior, 16-25 μm in length, 8-13 μm in width, 8-12 μm in thickness; both ventral and dorsal sides slightly convex; furrow evident, vertical, reaching almost the base of the cell, with apparently concave opening; gullet underlying the furrow, straight and unique in width, about 1/3 times the cell, extending to the base, with 4-5 rows of trichocysts; periplast sometimes striate; chloroplast one, yellowish brown, divided into two parts, occupying the most of the cell; pyrenoids present, ordinarily four, rarely six or seven, sometimes very hard to find, embedded in the periphery of the cell; eyespot absent; contractile vacuole situated anteriorly, just above the beginning of the gullet; nucleus situated near the base of the cell; starch grains small, scattering inside the chloroplast; refractive bodies two, small, spheroidal or rod-shaped, distributed in the center of dorsal part; flagella two, subequal in length, almost the same length of the cell, sometimes coiling around the cell; cell division occurring in gelatinous matrix.

Habitat: Occurring in marsh and ponds.

Type locality: Lakes in Uppland, Sweden.

Geographical distribution: Europe and Japan (Ibaraki, Nagano, Shizuoka and Hiroshima).

Specimens examined: Sugadaira, Sanada-cho, Nagano-ken, July 31, 1982 (M. Ishimitsu, # 00009, 14); Shishizuka, Tsuchiura-shi, Ibaraki-ken, Sept. 10, 1982 (M. Ishimitsu, # 00056); Minamiizu-cho, Shizuoka-ken, May 13, 1983 (M. Ishimitsu, # 00072, 73); Saijo, Higashihiroshima-shi, Hiroshima-ken, Aug. 23, 1983 (M. Ishimitsu, # 00099, 109).

Our alga is in well accordance with Skuja's description (1948), although it has only one chloroplast as other *Cryptomonas* species treated in this paper (cf. Ettl, 1980). It is somewhat smaller in cell size than described (20-49 μm in length).

The presence of pyrenoids is one of the most remarkable characters of this species. Among the species which have pyrenoids, *C. parapyrenoidifera* Skuja seems to be the most similar to this species, but the former is conical in shape rather than cylindrical and smaller in size. It is often difficult to recognize the presence of pyrenoids in specimens from nature because they have many and large starch grains (Fig. 4C).

The specimens in culture, as in other species, possessed less and smaller starch grains and they became brownish in color. Those cultured cells were often embedded in a gelatinous matrix floating on the surface of medium. They were in non-swimming state but with flagella. Cell division was often observed under such conditions.

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日本産クリptomonナス属の淡水産種4種について、形態、他種との区別点および天然細胞と培養細胞との差異等について報告した。本属を含むクリプト藻植物は主として単細胞性鞭毛藻の一群であり、淡水および海水に広く分布する。本植物群については現在全世界で200種以上が記載されているが、日本産のものに関してはこれまでほとんど報告がない。今回報告したのは以下の4種である。1) *Cryptomonas ovata*: 細胞は扁平な楕円形で、特徴的な長いたて溝をもつ(産地: 土浦)。2) *C. platyuris*: 細胞はやや細長く、後部が著しく扁平で幅広く丸い。たて溝は短い(産地: 土浦, 菅平, 東広島)。3) *C. rostratiformis*: 細胞はやや細長く、前端がくちばし状にとがる。後部は扁平でわずかに背側へわん曲する。たて溝は短い(産地: 菅平, 広島県本郷)。4) *C. tetrapirenoidosa*: 細胞は円筒形で前3種よりも小形。たて溝は長い。ふつう4個のピレノイドをもつ(産地: 土浦, 菅平, 南伊豆, 東広島)。これらの種では、葉緑体の色調やデンプン粒の大きさと分布状態、および彩光体の発達程度に関して天然細胞と培養細胞とのあいだで差異がみられた。