Bot. Mag. Tokyo **93**: 195–208, 1980

Laboratory Culture and Taxonomy of *Hymenomonas coronata* and *Ochrosphaera verrucosa* (Class Prymnesiophyceae) from the Northwest Pacific

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Hymenomonas coronata and *Ochrosphaera verrucosa*, both members of the coccolithophorids, Class Prymnesiophyceae, have been studied by means of electron microscopy and with the aid of laboratory culture. Living specimens of these two species were collected in temperate and subtropical regions of Japan, including the Kii Peninsula and the Ryukyu Islands, and unialgal cultures were established in the laboratory. Their life histories are fundamentally identical, and consist of a non-motile vegetative stage that produces motile cells. The vegetative stage is either unicellular, or a packet consisting of a few cells. Both the non-motile cells and the motile cells are covered with two kinds of scales: these are thin scales of unmineralized nature and coccoliths. These two species differ from each other in the shape of the coccoliths and in the presence or absence of visible rudimentary haptonema, and they have been in separate families. The present study reveals that both species are fundamentally identical in the structure and the distribution of major organelles, especially with respect to two opposed pyrenoids which bulge from chloroplasts, each being traversed by two thylakoid bands, and a group of microtubules forming a flagellar root. On the basis of these characteristics, it would appear more logical to place these two species in the same family, namely the Hymenomonadaceae.

Key words: *Hymenomonas* — *Ochrosphaera* — Prymnesiophyceae — Taxonomy — Ultrastructure.

In a previous paper, the authors described the results of a culture study of *Cricosphaera roscoffensis* var. *haptonemofera*, a new taxon of the coccolithophorids from the Pacific (Inouye and Chihara, 1979). This paper is the second of a series on the life history and taxonomy of the coccolithophorids in the waters of Japan and adjacent waters, and deals with two taxa assignable to *Hymenomonas coronata* and *Ochrosphaera verrucosa*, respectively. The present study was chiefly made by means of laboratory culture and electron microscope techniques.

Materials and Methods

In order to obtain living specimens of marine coccolithophorids, various kinds of materials, such as sea water, seaweed, sand, mud, pieces of wood and shells were collected in 1978 and 1979 from the coastal regions of Japan, including the Kii Peninsula and the Ryukyu Islands. Shortly after the collections were made, Erd-Schreiber

medium (Føyn. 1934; Stein, 1973) containing 5–10 mg/*l* GeO_2 for preventing diatom growth (Lewin, 1966; Stein. 1973) was inoculated with these samples. Inoculations were performed at 15–20 C. From one week to several months after inoculation, brown colonies often appeared on the culture vessels or floating on the water surface. They were picked up by micropipettes or fine needles and placed on Erd-Schreiber medium containing 0.4% agar, for establishment of unialgal cultures. Table 1 shows data for collections of *Hymenomonas coronata* Mills and *Ochrosphaera verrucosa* Schussnig. Source localities of collections are mapped in Fig. 1. The culture strain of *Ochrosphaera verrucosa*, no. LB–1722, isolated by Dr. J. A. West, was purchased from the Culture Collection of Algae at the University of Texas at Austin. These strains have been maintained in unialgal cultures in Erd-Schreiber medium or Provasoli's ES medium (Provasoli, 1968) and used for the studies of the life cycle and the ultrastructural features of the cell and cell covering.

The following procedures were used for electron microscopy. Cells concentrated by centrifugation were fixed by 5% glutaraldehyde in 0.1 M sodium cacodylate buffer or 0.1 M phosphate buffer at pH 7.0–8.2 containing 0.25 M sucrose for 1 to 2 hr. After several washes in buffer, with a gradual reduction of sucrose concentration, the post-fixation was done with 2% osmium tetroxide in 0.1 M buffer containing no sucrose for 1 to 24 hr. All procedures were performed at 4 C. Fixed materials were dehydrated by the usual ethanol series and embedded in Epon or Spur's resin. Sections were cut by glass knives and double stained with lead citrate and uranyl acetate (Reynolds, 1963). Observations were made with a JEOL JEM–100C electron microscope. For scanning electron microscopy, materials were fixed by the same procedure as above, and after

Table 1. Collection data of *Ochrosphaera verrucosa* and *Hymenomonas coronata*

Date of collections	Original samples	Locality	Strain no.
Ochrosphaera verrucosa			
May 29, 1979	sea water	Ago Bay	HOV–90514
May 29, 1979	sand	Kii-Nagashima	HOV–90515
May 28, 1979	sand	Shiono-misaki	HOV–90517
May 22, 1979	sand	Kawachi-gawa	HOV–90516
March 29, 1978	seaweed	Ayamaru-misaki	HOV–80302
March 27, 1978	sand	Setta	HOV–80303
March 24, 1978	sand	Henza	HOV–80304
March 12, 1979	mud	Awase	HOV–90305
March 29, 1978	tide pool water	Yo	HOV–80301
March 12, 1979	sand	Azama	HOV–90306
March 10, 1979	sand	Manzamo	HOV–90307
March 6, 1979	sea water	Kabira	HOV–90308
March 7, 1979	sand	Kondoi-saki	HOV–90309
March 3, 1979	estuarial water	Nakamagawa	HOV–90311
March 3, 1979	sand	Toyohara	HOV–90310
March 4, 1979	sea water	Shirahama	HOV–90312
March 4, 1979	seaweed	Sotobanare-jima	HOV–90313
July 23, 1979	sand	Minamitori-shima (Marcus)	HOV–90718
Hymenomonas coronata			
March 28, 1978	mud	Ura	HHC–80301
March 12, 1979	mud	Awase	HHC–90302

dehydration in the ethanol series, cells were put into amyl alcohol and dried by the critical technique. Observations were made by means of a JEOL JSM-25.

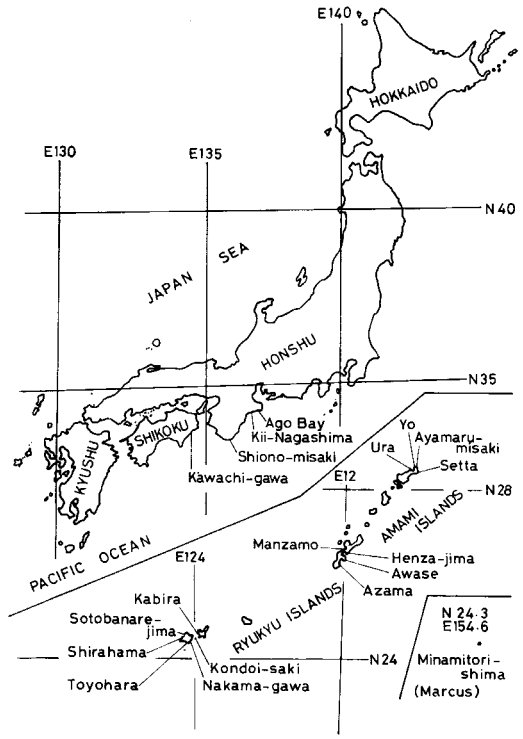
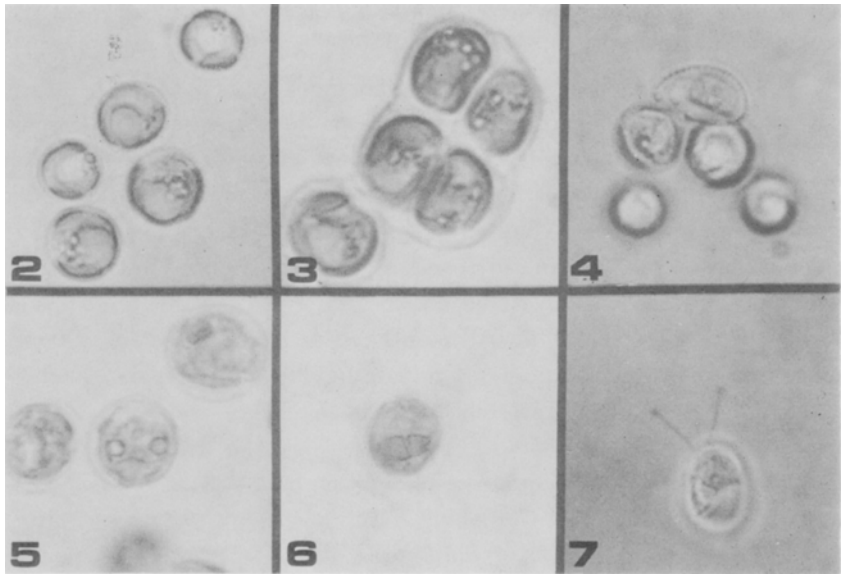


Fig. 1. Map showing localities where *Hymenomonas coronata* and *Ochrosphaera verrucosa* were isolated.

Results

Hymenomonas coronata

Light microscopy. Vegetative cells are usually solitary, spheroidal and non-motile (Fig. 2), measuring 6.5–11 μm in diameter. They sometimes form a small packet, consisting of two to four cells within a common envelope (Fig. 3). Cell divisions result in the formation of two or four swarmers per cell, or by desmoschisis (Groover and Bold, 1969) formation of packet cells. The cells in the packet are usually spheroidal but are sometimes ovoidal to hemispheroidal. The surface of solitary cells is often indented (Figs. 2 and 4). The cells usually contain one to two parietal chloroplasts with two bulging pyrenoids, but sometimes, probably during an early stage of cell fission, there are four chloroplasts, each possessing a pyrenoid. There are two types of pyrenoids: the first, termed "the separate type", consists of two opposed pyrenoids (Fig. 5), whereas the second, which is termed "the contact type", consists of two pyrenoids which are not completely separated (Fig. 6). The cells bear coccoliths on the surface. Coccoliths are too small and thin to be observable by means of a light microscope. However, when the cells are gradually dried on a glass slide, ellipsoidal coccoliths measuring less than 1 μm in diameter become barely visible. Cells lacking coccoliths often occur in culture.

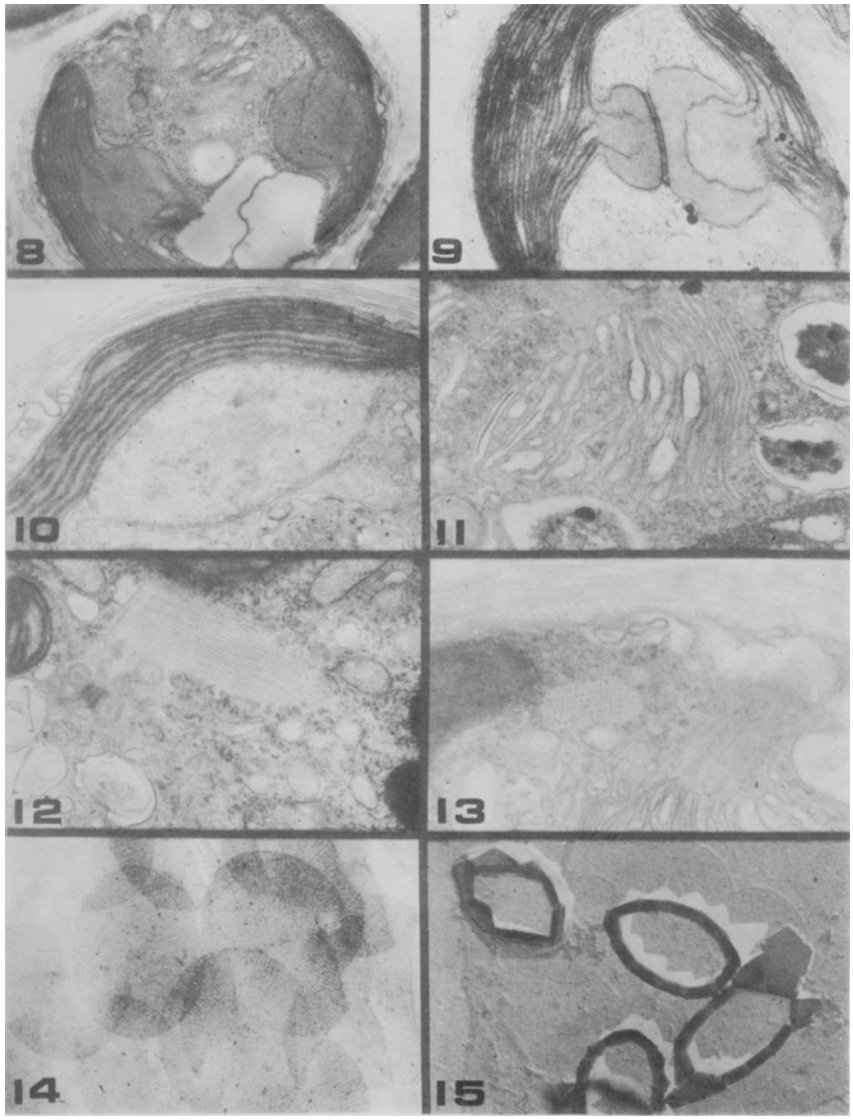


Figs. 2–7. *Hymenomonas coronata*. 2: Vegetative cells, each having an indented wall. 3: Colonial cells. 4: Empty cell having an indented cell wall. 5: Cell having the 'separate type' bulging pyrenoid. 6: Cell having the 'contact type' bulging pyrenoid. 7: Motile cell bearing two flagella. 2–4, 7, ×1240; 5, 6, ×1575.

Motile cells are produced more or less synchronously when the non-motile cells are inoculated into fresh medium. They are spheroidal to ovoidal in shape, measuring 6–10 μm, and contain one or two chloroplasts, each with a bulging pyrenoid. The motile cell bears two subequal flagella at the apical end of the cell (Fig. 7). A number of coccoliths present on the cell surface can be recognized under the light microscope, as in the case of the non-motile cells, after drying on a glass slide. Motile cells exhibit negative phototaxis for a short time, and settle on the wall of a glass vessel near the surface of the culture medium. Motile cells do not divide. Non-motile cells divide and form two or four swarmers, or sometimes divide by desmoschisis, resulting in a packet of cells.

Electron microscopy. The general features of motile cells, as shown in Figs. 8 and 16A, are very similar to those described previously for the species of *Cricosphaera* and *Hymenomonas* (Manton and Peterfi, 1969; Leadbeater, 1971; Pienaar, 1976). Chloroplasts are parietal, and possess a bulging pyrenoid at the inner face (Figs. 8 and 9). Pyrenoids are traversed by two thylakoid layers (Fig. 9). The nucleus is usually located in a central to posterior part of the cell, but sometimes occurs at the anterior end near the flagellar bases. The outer membrane of the nucleus is continuous, with endoplasmic reticulum around the chloroplast, in such a manner that these two organelles are attached to each other (Fig. 10). The Golgi body is located between the nucleus and the flagellar bases. In the center of the Golgi cisternae, there are often dilations (Fig. 11) similar to those referred to as "peculiar dilatations" by Manton (1967) in the case of *Chrysochromulina chiton*. Associated with the flagellar bases, there are flagellar roots

composed of a number of microtubules (Fig. 12), which are hexagonal in cross section (Fig. 13). Flagellar bases are interconnected by a striated dense band (Fig. 12). In Fig. 12, three flagellar bases are visible, one of them probably being the result of



Figs. 8–15. *Hymenomonas coronata*. 8: Longitudinal section of a motile cell, showing distribution of organelles. Two pyrenoids, one Golgi body, one flagellar root, one flagellum and unmineralized body scales are shown. ×10125. 9: The 'contact type' pyrenoid traversed by two thylakoids. ×9975. 10: Chloroplast and nucleus enclosed by common ER. × 15525. 11: Golgi body, showing dilations in the cisternae. ×18000. 12: Microtubular flagellar root and flagellar bases interconnected by a striated band. ×14775. 13: Cross section of the microtubular flagellar root. ×21000. 14: Unmineralized body scales showing radial ornamentation. ×37500. 15: Coccoliths composed of pentagonal rim elements (shadowcast preparation). ×23400.

duplication before cell division. The number of microtubules comprising a flagellar root may be as many as 240. This kind of flagellar root was observed first in *Hymenomonas roseola* and *Cricosphaera carterae* (Manton and Peterfi, 1969) and is now known in all species of these genera which have been examined by electron microscopy (Mills, 1975; Pienaar, 1976; Gayral and Fresnel, 1976). Neither the presence of haptonema nor of its microtubular root has been confirmed in the present specimens, though Mills (1975) has suggested the presence of a rudimentary structure in the material he observed. Two kinds of scales cover the cell surface. These are coccoliths, and thin scales of unmineralized nature. Coccolith morphology was examined by transmission electron microscopy. The coccoliths are very small and almost uniform in size in each strain, measuring 0.5–0.6 μm$\times$0.3–0.4 μm in strain no. HHC–80401 and 0.6–0.75 μm$\times$0.45–0.5 μm in no. HHC–90302. These are smaller than those described by Mills (1975) (0.9–1.0 μm$\times$0.5–0.6 μm), but their appearance is fundamentally the same. Each coccolith consists of 10–12 rim elements arranged in a crown shaped circle (Figs. 15 and 16B) as in other *Hymenomonas* species, although the height is lower than for other species (Manton and Peterfi, 1969; Gayral and Fresnel, 1976; Pienaar, 1976). The unmineralized scales are thin and flat, with a radial ornamentation on the surface (Fig. 14).

Note. The observations mentioned above show that the specimens used in the present study are virtually identical with *H. coronata*, although there are two minor differences. One is the presence of the contact type in addition to the separate type of pyrenoid. This contact type of pyrenoid was found in *H. lacuna* for the first time by Pienaar (1976). He used this characteristic as a diagnostic criterion for the species. As will be discussed later, both types of pyrenoid are also present in *Ochrosphaera verrucosa*. In these two algae, the two pyrenoid types were observed even in the cultures started

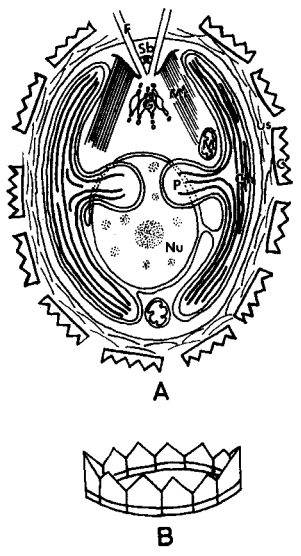


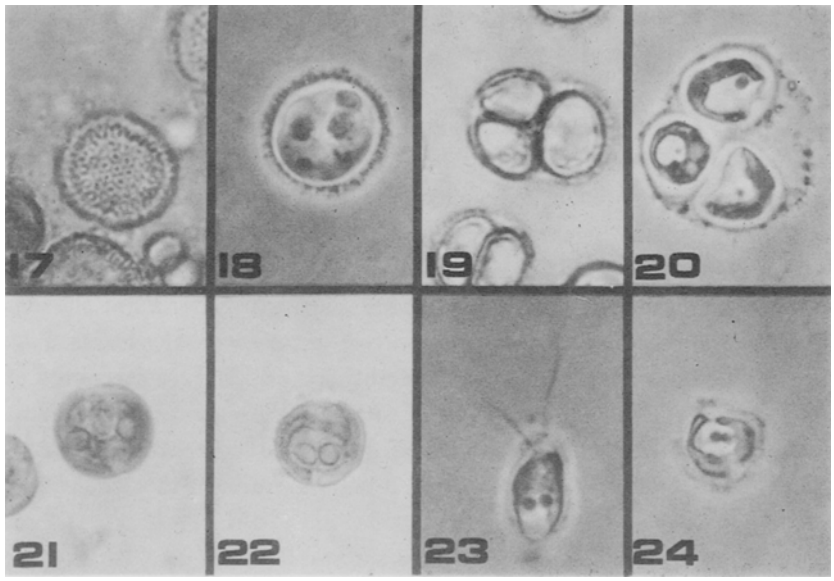
Fig. 16. Diagramatic illustration of *Hymenomonas coronata*. A: distribution of major organelles in the motile cell. B: coccoliths. C, coccolith; Ch, chloroplast; F, flagellum; G, Golgi body; Mr, microtubular flagellar root; Nu, nucleus; P, pyrenoid; Sb, striated band; Us, unmineralized scale.

from a single cell. Based on this evidence, the presence of two such pyrenoid types seems to be characteristic of *Hymenomonas* and *Ochrosphaera*. Another difference is the size of the coccoliths. The present specimens bear coccoliths fundamentally similar in shape and disposition to those of Mills' alga, but in strain no. HHC–80301, the coccoliths are half the size of those described by Mills. Since the coccolith size is very uniform in the present specimens and in the cultures of Mills' alga, the question arises as to whether or not these two algae should be treated as different taxa. In general, the coccolith form is stable, and characteristic of each taxon, and therefore this characteristic is used by the majority of phycologists as a diagnostic criterion at the generic and specific rank in the coccolithophorids. However, using coccolith size as a taxonomic criterion is unwise because, according to the authors' experience, coccoliths of certain species show a relatively wide variation in size. Taking this into consideration, the authors are inclined to conclude that the difference in coccolith size between Mills' alga and the present specimens indicates that different strains of this alga exist having a variation in coccolith size.

Ochrosphaera verrucosa

Light microscopy. Non-motile vegetative cells are solitary or in packets (Figs. 17–20). Both solitary and packet cells are surrounded by a transparent wall which is rather thick in old culture, deeply stainable by toluidin blue. Coccoliths are present on the surface of both solitary and colonial cells (Figs. 17–20), but they are not as densely distributed in the latter cells. Cells of smaller packets tend to bear more coccoliths. Solitary cells are spheroidal, measuring 7–12 μm in size, but cells in packets are hemispheroidal or ovoidal, each possessing one to two parietal chloroplasts with two bulging pyrenoids facing each other. As in *Hymenomonas coronata*, there are two types of pyrenoids with regard to localization and orientation. In the first type, termed "the separate type", a broad canal exists between the two pyrenoids (Fig. 21). This feature is known in certain species of *Cricosphaera* and *Hymenomonas*. In the other type, termed "the contact type", pyrenoids are in contact with each other, having formed a bridge-like connection between the two (Fig. 22), corresponding to that detected previously in *Hymenomonas lacuna* by Pienaar (1976). In the case of the present alga, both types are observed even in cultures derived from a single cell. The smaller cells tend to have the contact type while the larger cells tend to have the separate type of pyrenoids. Coccoliths are so small that it is difficult to confirm their shape and ornamentation in detail by light microscopy. However, dried material placed on a glass slide permits recognition of at least their basic shape. They are long and vase-shaped (Fig. 20), about 0.8–1.2 μm in diameter at the upper margin. Their height varies from 0.2 to 0.7 μm.

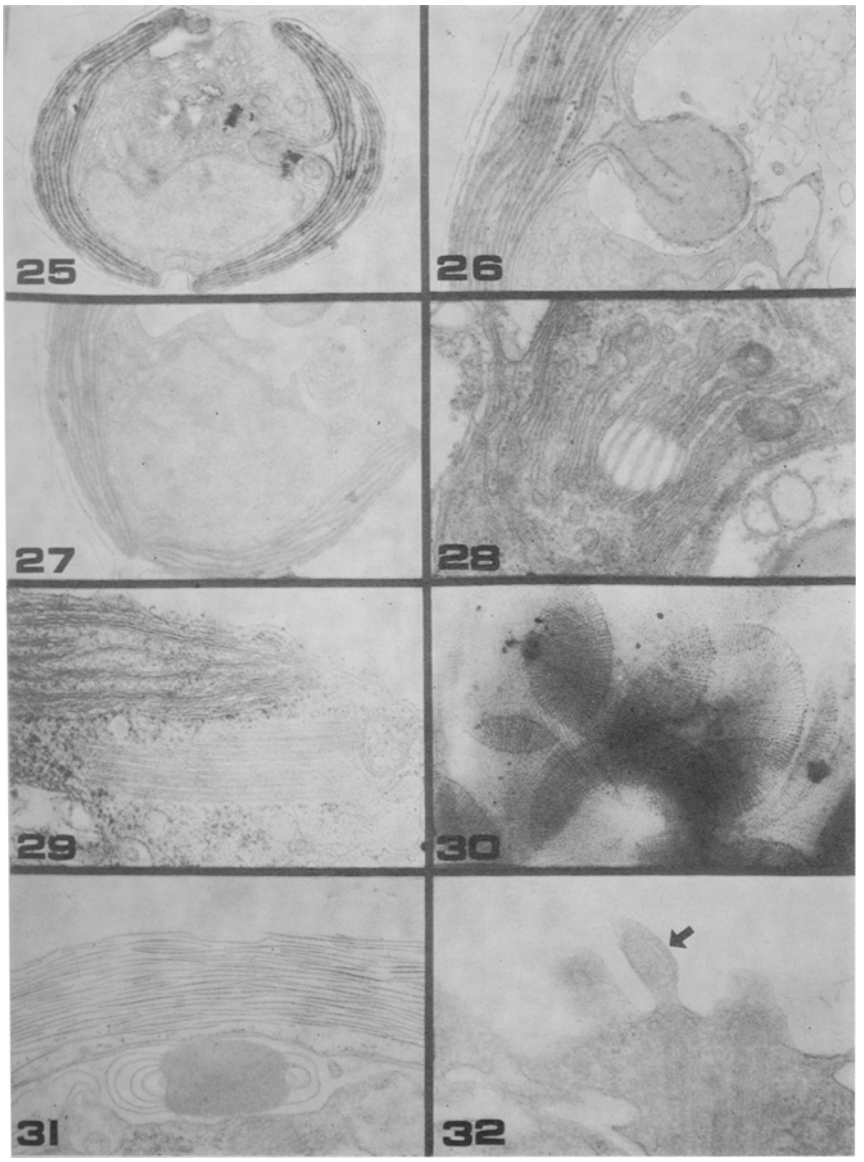
Motile cells were usually observed one to three days after inoculation of non-motile vegetative cells into fresh media. They are usually ellipsoidal, but often become ovoidal to pyriform by amoeboid movement. They measure 6.5–15 μm $\times$ 6.5–14 μm and bear two flagella, almost equal in length, arising from the apical to



Figs. 17–24. *Ochrosphaera verrucosa*. 17: Non-motile solitary cell, showing ring-shaped coccoliths. 18: Vegetative cell focused at median plane of the cell. 19, 20: Packets of cells derived from a single cell, bearing coccoliths on the surface. 21, 22: A vegetative cell fixed with Lugol solution, showing two kinds of pyrenoids: the separate type (Fig. 21) and the contact type (Fig. 22). 23, 24: Motile cell, showing two flagella, pyrenoids and coccoliths. 17–20, 23, 24, ×1240; 21, 22, ×1650.

subapical part of the cell (Fig. 23). A haptonema has not been detected in the motile cells at any stage. Coccoliths identical with those of vegetative non-motile cells are also present on the motile cells (Fig. 24). The cell contains one to two chloroplasts, with two bulging pyrenoids (Figs. 21 and 22). These features are in agreement with those illustrated by Lefort (1971) for *Ochrosphaera verrucosa* from Roscoff, France. Schwarz (1932) reported the occurrence of *Ochromonas*-like motile cells bearing two conspicuously unequal flagella in *O. neapolitana*. Such motile cells have not been observed in the present cultures, and conjugation of these cells, reported by Schwarz (1932) in *O. neapolitana*, has not been observed in the present alga. The motile cells swim for several hours, or sometimes longer, but they are rarely seen on the second day after liberation. Soon after settling, they undergo cell divisions, which are a kind of desmoschisis (Groover and Bold, 1969), resulting in a packet surrounded with a rather thick wall on which new coccoliths are soon produced.

Electron microscopy. Distribution of major organelles in the cells is shown in Figs. 25 and 36A. Chloroplasts are located peripherally. Pyrenoids bulge at the inner face of the chloroplasts, and are traversed by two thylakoids (Fig. 26). The nucleus is located in the posterior to central portion of cell, closely attached to the inner surface of the chloroplast. The nucleus and chloroplast are enclosed by common ER (Fig. 27). There is one Golgi body per cell and it usually is present between the nucleus and the flagellar base. Its cisternae have dilations (Fig. 28), found in all the species of the haptophycean algae examined except in species of the Pavlovales (Green, 1976). The

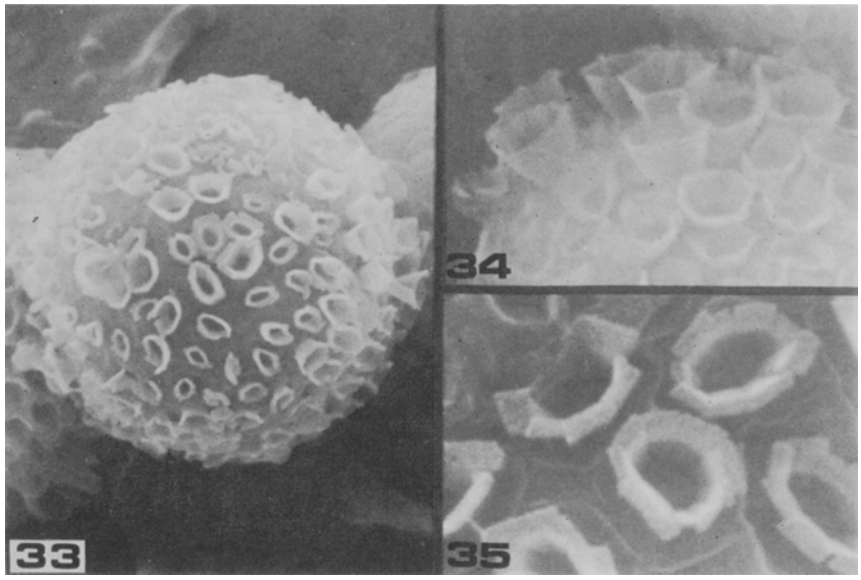


Figs. 25–32. *Ochrosphaera verrucosa*. 25: Section of vegetative cell, showing major organelles. Chloroplast, nucleus, Golgi body and pyrenoid are visible. ×9225. 26: A pyrenoid bulging from the inner face of the chloroplast and traversed by two thylakoids. ×19050. 27: Chloroplast and nucleus enclosed by common ER. ×14250. 28: Golgi body showing dilations in its cisternae. ×28350. 29: Longitudinal section of a flagellar root composed of numerous microtubules ×28350. 30, 31: Unmineralized scales, showing radial ornamentation (Fig. 30, ×33450) and distribution on the cell surface (Fig. 31, ×13125). 32: Longitudinal section of a vestigial haptonema (arrow). ×49500.

motile cell has flagellar roots composed of packed microtubules (Fig. 29) which are similar to, though not as developed as those in species of *Hymenomonas* and *Cricosphaera*. The flagellar roots can not always be observed, probably due to

unsolved problems of fixation. A cross section of a flagellar root has not been obtained, but judging from several photographs which show tangentially sectioned roots, the number of microtubules comprising a flagellar root is about 50–100, while in *Hymenomonas* and *Cricosphaera*, microtubules are more numerous (Manton and Peterfi, 1969). On the cell surface, there are two kinds of scales: coccoliths and thin scales of unmineralized nature. The general appearance of a cell bearing coccoliths on the body surface is shown in Fig. 33. The coccoliths consist of 6 to 10 (rarely 5) trapezoidal plates, forming a long vase-like structure (Figs. 34 and 37B). They are fundamentally identical with those of *Ochrosphaera neapolitana* and *O. verrucosa* (West, 1969; Lefort, 1971; Gayral and Fresnel-Morange, 1971). Lefort (1971) reported another type of coccolith similar to that of *Cricosphaera* in *Ochrosphaera verrucosa*, namely, a pulley-shaped cricolith. This type of coccolith has also been found in *O. neapolitana* (Gayral and Fresnel-Morange, 1971). In the present alga, there are two kinds of elements which make up a coccolith. Most of the elements are simple and rather flat, as shown in Fig. 34, but some of them bend outside at almost a right angle at the upper margin (Figs. 35 and 36C). The coccoliths made by the bent elements are pulley-like as a whole (Fig. 35), while those made by the flat elements are urn- or vase-shaped. The pulley-shaped coccolith is superficially similar to that of *Cricosphaera*, but structurally they are quite different from each other.

The size of the coccoliths varies greatly, measuring 0.6–2.0 μm in diameter of the upper margin and 0.2–1.2 μm in height. The variation is not always continuous; rather large, medium and small coccoliths are often intermixed on a single cell (Fig. 33). The unmineralized scales are present on the cell surface (Figs. 30 and 31).



Figs. 33–35. *Ochrosphaera verrucosa*. 33: General appearance of a whole cell covered with coccoliths. Note small and large coccoliths. ×10800. 34: Vase-shaped coccoliths. ×10800. 35: Surface view of the pulley-shaped coccoliths. ×21750.

Note. The results obtained from the present study are fundamentally in agreement with those for *Ochrosphaera verrucosa* studied by Lefort (1971, 1975), except with regard to a minor disagreement concerning the appearance of the coccoliths. Coccolith dimorphism was reported in *O. neapolitana* (Gayral and Fresnel-Morange, 1971) and *O. verrucosa* (Lefort, 1971). In both species, one of the coccoliths is vase-shaped while the other is pulley-shaped. Gayral and Fresnel-Morange (1971) compared the pulley-shaped coccolith with that of *Cricosphaera* in the sectioned material, and suggested the possibility that *Ochrosphaera* is a stage in the life-history of *Cricosphaera*. In the present alga, the pulley-shaped coccolith was observed in addition to the vase-shaped coccolith, but it is apparently different from that of *Cricosphaera*.

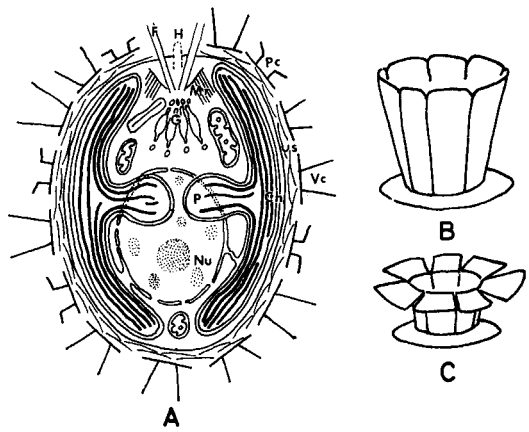


Fig. 36. Diagramatic illustration of *Ochrosphaera verrucosa*. A: distribution of major organelles in the motile cell. B: vase-shaped coccolith. C: pulley-shaped coccolith. H, haptonema; Pc, pulley-shaped coccolith; Vc, vase-shaped coccolith; others, see legend to Fig. 16.

It is worth noting that coccoliths of the present alga show a considerably wide variation in size, in contrast to those of the *Cricosphaera* species examined, but similar to those of *Hymenomonas coronata* discussed previously in this paper. Their fundamental structure, however, as in *H. coronata*, is not variable.

Since Schussnig (1940) established *Ochrosphaera verrucosa*, it has been reported twice in the Pacific, by Norris (1967) and West (1969),* in both cases in the eastern Pacific. This paper makes the third record of the species occurring in this basin.

Discussion

Cellular components of *Ochrosphaera verrucosa* as described in this paper are similar to those of the species of *Cricosphaera* and *Hymenomonas* so far examined. These three genera share two important features: 1) the size and shape of cells and 2) the internal structure and distribution pattern of major organelles, especially the two opposed pyrenoids which bulge from the chloroplasts, each being traversed by two thylakoid bands, and the flagellar root consisting of numerous microtubules. This

* According to Dr. J.A. West (personal communciation), this alga has been isolated by him several times, in tropical regions, including Guam, Australia, Samoa, Truk and Mexico. The alga seems to be widely distributed in warmer seas.

evidence suggests the probability of a close affinity of these three genera. According to Parke and Green (in Parke and Dixon, 1976), *Ochrosphaera* was placed in the Ochrosphaeraceae because there is no emergent haptonema, but a group of five microtubules are associated with the flagellar bases. On the other hand, *Hymenomonas* and *Cricosphaera* were placed in a separate family, the Hymenomonadaceae, because certain members of these genera (i.e. *H. roseola* and *C. carterae*) have a visible rudimentary haptonema. In the present work, haptonematal structure of *Ochrosphaera verrucosa* was not studied, but the presence of an emergent haptonema is indicated (Fig. 32). Therefore, despite the lack of clear information on haptonematal structure in *Ochrosphaera*, observations presented in this paper suggest that the presence or absence of a haptonema can not be used as a diagnostic criterion at the family rank. When Gayral and Fresnel (1976) studied *Hymenomonas globosa* and *Cricosphaera roscoffensis*, they also evaluated the taxonomic value of a bulbous haptonema and concluded that its presence or absence is a criterion of secondary importance to be used only at the rank of species or infraspecies. In a previous paper, the authors (Inouye and Chihara, 1979) concurred with Gayral and Fresnel (1976) in establishing a new taxon *Cricosphaera roscoffensis* var. *haptonemofera*. As described above, the results obtained in the present study reveal that *Ochrosphaera verrucosa* is similar to *Cricosphaera* and *Hymenomonas* species in all features other than the shape and structure of the coccolith. On this basis, it appears to be more logical to place *Ochrosphaera* together with *Cricosphaera* and *Hymenomonas* in the family Hymenomonadaceae. This family is characterized by having a characteristic structure and distribution pattern of major organelles, especially two opposed pyrenoids which bulge from chloroplasts, each being traversed by two thylakoid bands, and by having a rudimentary haptonema, or group of microtubules forming a flagellar root.

The following is a brief comparison of the life histories found in the Hymenomonadaceae. *Hymenomonas* and *Ochrosphaera* have a life history that is much the same in each genus, but the life history of *Cricosphaera* is unique. In most species of *Hymenomonas* and *Ochrosphaera*, the vegetative stage is one of non-motile coccolith-bearing cells that produce motile cells when injected into fresh media (Gayral and Fresnel-Morange, 1971; Lefort, 1971; Mills, 1975; Pienaar, 1976). By contrast, the vegetative stage of certain *Cricosphaera* species is composed of motile and coccolith-bearing cells (Leadbeater, 1971). Furthermore, these produce a filamentous benthic stage which is capable of production of *Prymnesium*-like zoospores (Stosch, 1967; Lefort, 1971; Inouye and Chihara, 1979). Schwarz (1932) studied *Ochrosphaera neapolitana* and reported sexual reproduction, but did not observe a filamentous benthic stage in the life history. No filamentous stage is reported for *Hymenomonas*. Probably *Hymenomonas* and *Ochrosphaera* have no such a stage in their life histories.

The authors wish to express their thanks to Dr. Richard E. Norris of the University of Washington and Dr. John A. West of the University of California, Berkeley, for their kind reading the manuscript. The authors' thanks are also due

to Dr. Takaaki Kobara, Mr. Minoru Saitow and Mr. Takeo Horiguchi of their laboratory, for their generously collecting some of the original samples.

This work constitutes the contributions from the Shimoda Marine Research Center, the University of Tsukuba, no. 366.

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Received February 29, 1980