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Ultrastructure and Taxonomy of *Jomonlithus littoralis* gen. et sp. nov. (Class Prymnesiophyceae), a Coccolithophorid from the Northwest Pacific

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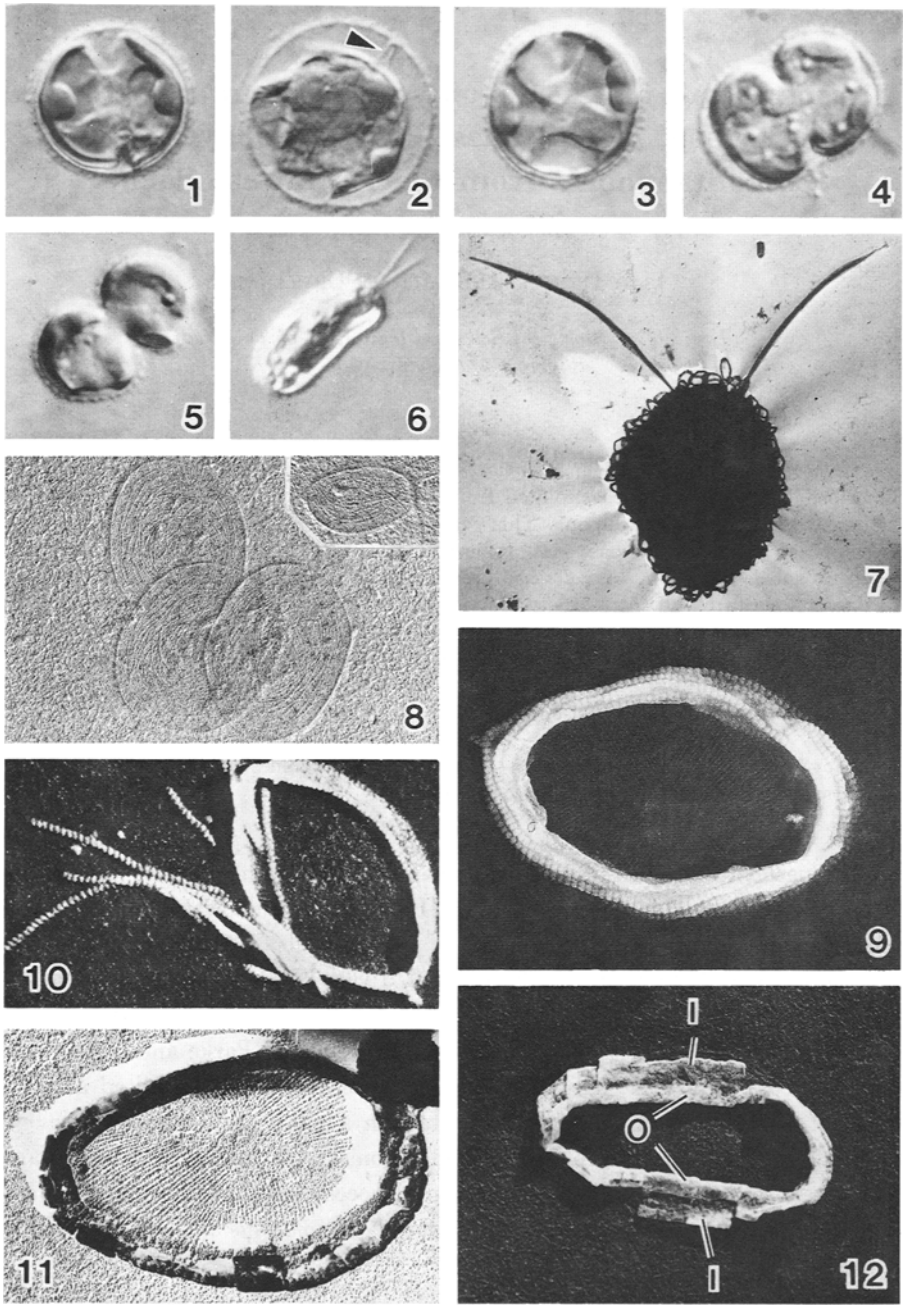
A new coccolithophorid genus *Jomonlithus* with *J. littoralis* as the type species, is described based on specimens isolated from a sand sample collected at the mouth of Nakagawa river, Ibaraki, Japan. This genus is characterized by the coccolith which is composed of an organic base-plate scale and calcified rim elements made up of two different subelements. *J. littoralis* has been found in several localities along the coast of Japan. Culture and ultrastructure studies gave special attention to the cell cycle, coccolith and scale morphology and the ultrastructure of the cellular organelles. It was confirmed that *Jomonlithus* is similar to *Wigwamma* Manton *et al.*, *Pappomonas* Manton et Oates on the basis of coccolith morphology and to *Cricosphaera* Braarud and *Hymenomonas* Stein in cellular structure.

Key words: Coccolithophorid — *Jomonlithus* — Prymnesiophyceae — Taxonomy of *Jomonlithus* — Ultrastructure of *Jomonlithus*.

For the past two decades life history and ultrastructure have been studied on the littoral coccolithophorids (e.g., Parke, 1961; Boney and Burrows, 1966; Von Stosch, 1967; Manton and Peterfi, 1969; Gayral *et al.*, 1972; Mills, 1975; Gayral and Fresnel, 1976; Pienaar, 1976; Inouye and Chihara, 1979, 1980; Hibberd, 1980 for review). As a result, the life history and ultrastructural features have been incorporated into the taxonomic criteria for the systematics of this group of algae (Parke and Green *in* Parke and Dixon, 1976). However, our knowledge of these characters is limited and a great deal of work is still necessary in order to establish a more natural taxonomic system. We have collected as many samples as possible from the littoral zone around Japan in order to obtain more information about littoral coccolithophorids from the Japanese coast and deal with the taxonomy of a new genus *Jomonlithus* with *J. littoralis* as the type species.

Materials and Methods

Sand samples collected from the mouth of Nakagawa river, Ibaraki-ken in May, 1979, Kujigawa river, Ibaraki-ken in May, 1979, Kidogawa river, Chiba-ken in August 1979 and sea water samples from Port Kashikojima, Mie-ken, in October 1979 and Port Naoetsu in October 1980, were brought back to the laboratory within one or two days



Figs. 1–12. *Jomonlithus littoralis*. 1–6: Light micrographs. 1: Note coccolith covering, two chloroplasts and two bulging pyrenoids. 2: Non-motile cell with one flagellum inside the coccosphere (arrowhead). 3–5: Undergoing partition. 6: Swimming cell. 1–3, ×1600; 4, 5, ×2000; 6, ×1500. 7–12: Electron micrographs. Shadowcast preparations. 7: Motile cell bearing two smooth flagella with tapering tips and numerous coccoliths on the cell surface (Holotype). ×2700. 8: Large and small (inlet) unmineralized scales with a concentric pattern on the surface. ×30000. 9: Calcified rim showing coiling rope

after collection and inoculated into GPM medium (Loeblich III, 1975). GeO_2 at a final concentration of 10–50 mg/*l* (Lewin, 1966; Stein, 1973) was added to the culture medium to prevent the growth of diatoms. Enrichment cultures were maintained at 15 C, 6000 lux and 12:12 LD cycles. Unialgal cultures were established by single cell isolation and dilution techniques. All cultures were grown at 18 C, 14:10 LD regime and exposed to a light intensity of 3000–6000 lux. For whole mount preparations, drops of medium containing cells were placed on colloidin-coated grids and fixed with osmium tetroxide vapour for 30 seconds. After drying at room temperature grids were gently rinsed with distilled water, dried again and shadowcasted with platinum palladium at an angle of about 45 degrees. For transmission electron microscopy cells were fixed for 1 hr in 3% glutaraldehyde in 0.2 M sodium cacodylate buffer (pH 8.1) containing 0.1 M $CaCl_2$ and 0.25 M sucrose (Klaveness, 1973). Specimens were washed several times with the same buffer containing decreasing concentrations of sucrose and then postfixed in 2% aqueous osmium tetroxide for 2 hr. These were dehydrated using a graded ethanol series and embedded in Epon or Spurr's low viscosity resin (Spurr, 1969). Sections were cut with glass and diamond knives and double stained with uranyl acetate and lead citrate (Reynolds, 1963). All materials were observed with a JEOL JEM 100C and JEM 100CX transmission electron microscope.

Results

Light microscope observations

Jomonlithus littoralis is a unicellular alga. Cultured cells are usually non-flagellate and do not exhibit motility. When cultured without shaking or aeration, they grow at the bottom of the culture vessel. They were, however, never observed to attach tightly to the surface of the vessel as do benthic coccolithophorids such as *Hymenomonas coronata* and *Ochrosphaera verrucosa*. Therefore the growth pattern of *J. littoralis* can be referred to as free living.

Flagellated cells were observed during two to three days after cells were transferred into fresh medium. Then they lost their functional flagella, reverted to the non-motile condition and replicated non-motile cells until they were inoculated once again into fresh medium. Except for the cell shape and presence or absence of flagella, the structure of the motile cells was identical to that of the non-motile cells.

Non-motile cells. Non-motile cells are usually flattened disc-shaped, 8–12 μm diam. and bilaterally symmetrical (Fig. 1). The entire surface of the cell is coated by small ring-shaped coccoliths (Figs. 1–3). Two brownish chloroplasts are located parietally and occupy the entire inner surface of the protoplast. They possess conspicuous pyrenoids which bulge toward the inner face of the cell at the equatorial region (Fig. 1). These two pyrenoids face each other as in *Hymenomonas*, *Cricosphaera* and *Ochrosphaera*.

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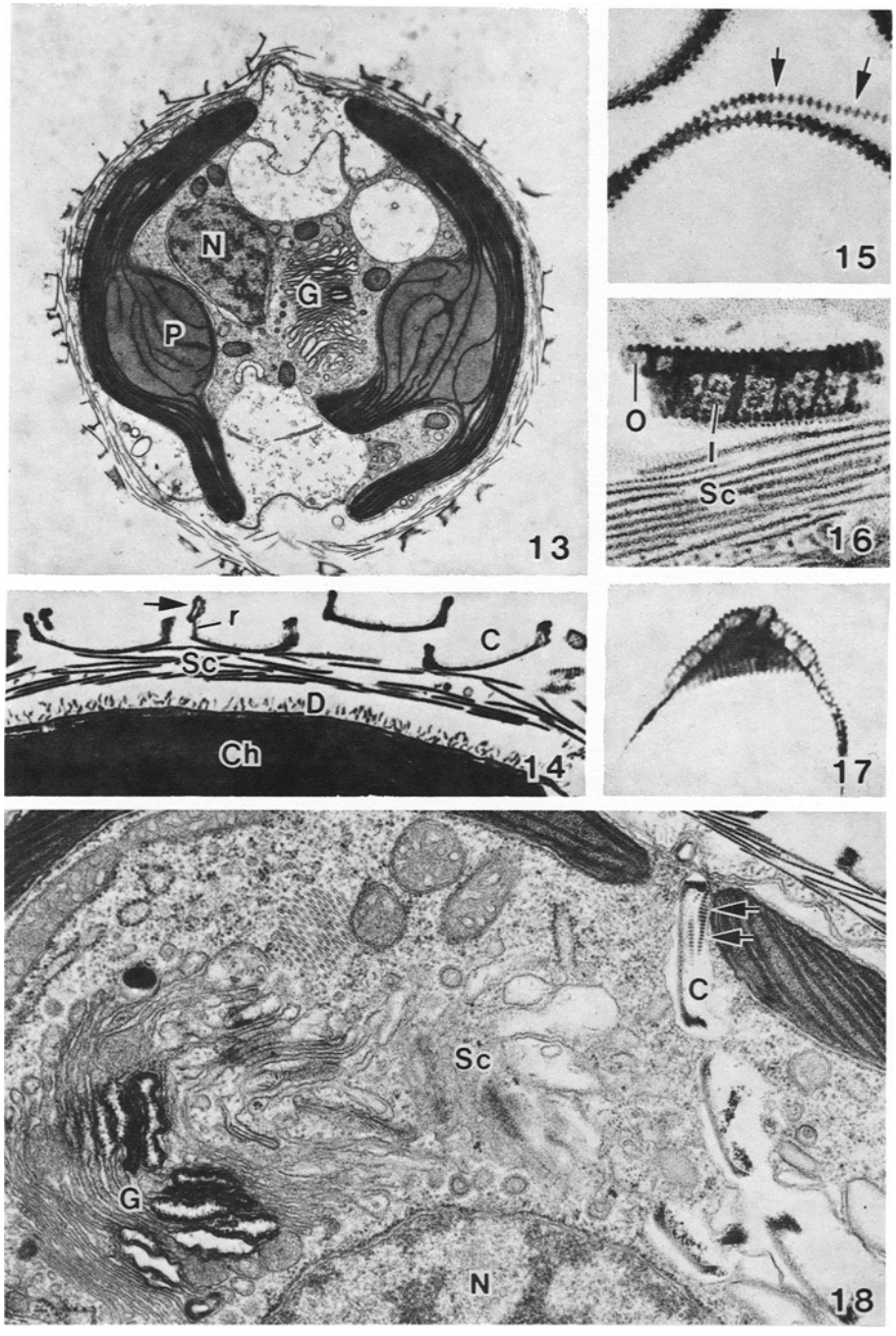
appearance. ×45000. 10: Rosary-like strands composed of small ovoidal to rod-shaped crystals. ×35000. 11: Base-plate scale with radiating pattern and calcified elements made up of small crystals. ×45000. 12: Calcified elements, each consisting of inner (I) and outer (O) subelements. ×37000. 9, 10, 12: reversed prints.



Figs. 13–18. *Jomonlithus littoralis*. Electron micrographs. 13: A section of a cell showing nucleus (N), Golgi apparatus (G), chloroplasts with bulging pyrenoids (P). ×6000. 14: Cell covering consisting of columnar deposits (D), unmineralized scales (Sc) and coccoliths (C). Raised rim of the base-plate (r) and calcified elements (arrow) are seen. ×20000. 15: Rosary-like strand. A thin strand linking small grains can be seen (arrows). ×70000. 16:

The nucleus is in the centre of the cell. A single leucosin vesicle is often seen in the periphery of the broad dimension of the cell. Occasionally flagella were observed in the narrow space between the protoplast and coccolith case (Fig. 2). Apparently even non-motile cells have short non-functional flagella.

The cell divides by binary fission in non-motile stage only. The process of cell division is similar to that of *Cricosphaera*. Each chloroplast divides synchronously, cleavage proceeding along the plane through the pyrenoid from the proximal to distal side (Fig. 3). Recently similar observations were made in *Cricosphaera carterae* (Stacy and Pienaar, 1980 as *Hymenomonas carterae*) and *C. roscoffensis* var. *haptonemofera* (Hori and Inouye, 1981). During chloroplast division the cell increases in size and changes from spheroid to ovoid shape. The cell then constricts in the median portion with a concomitant constriction of the coccolith covering (Figs. 4 and 5).

Motile cells. Motile cells are ellipsoidal to cylindrical (Fig. 6) and vary in size from $9 \times 4\ \mu m$ to $14 \times 7\ \mu m$. Each cell has two almost equal flagella, 1/2 to 1 times the cell length, which exhibit apical to subapical insertion. A visible haptonema has never been observed. The cell swims by beating flagella anteriorly and rotating around the long axis. It is positively phototactic. Eventually the organisms sink and grow at the bottom of the vessel as free living non-motile cells. Cells with short flagella were often seen a few days after inoculation. Apparently they then lose their flagella and become flattened non-motile cells.

Electron microscope observations

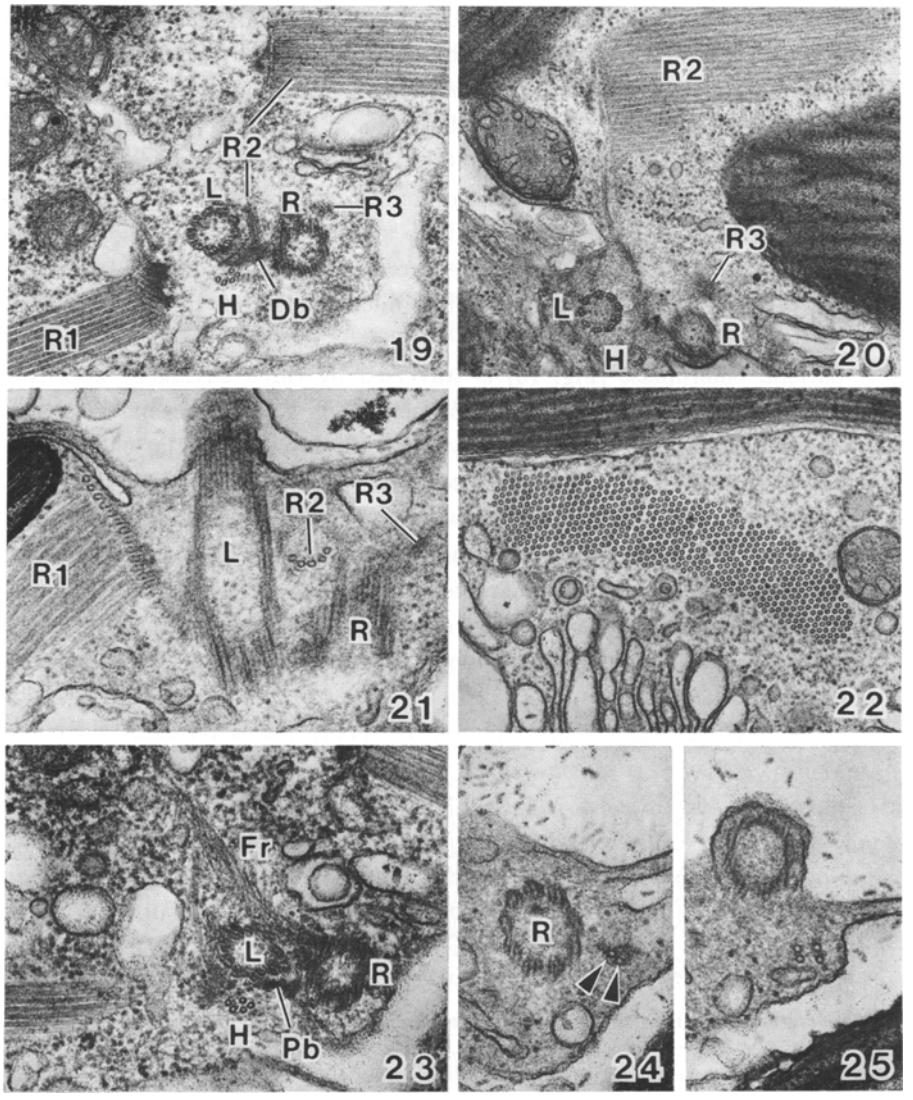
Cells are coated by two kinds of unmineralized organic scales as well as coccoliths. Unmineralized circular to oval scales, 750–800 nm × 650–850 nm in size (Fig. 8), are arranged in several layers adjacent to the columnar deposits which are situated immediately external to the plasmalemma (Fig. 14). Shadowed scales showed concentric fibrous patterns (Fig. 8), without exceptions, on both the proximal and distal surfaces. In sectioned material, however, radiating ridges are probably sandwiched between concentric fibriles so that they are obscured in the shadow preparations. In contrast, the base-plate scale of the coccolith shows only a radiating pattern on its distal surface (Fig. 11). Other unmineralized scales are occasionally encountered. They are elliptical, 500 × 200 nm. Both surfaces also seem to have a concentric fibrous pattern (Fig. 8 inlet). Similar small scales on the surface of the haptonema are known in other coccolithophorids (Manton and Peterfi, 1969; Leadbeater, 1970, 1971). Although in *J. littoralis* the emergent haptonema is absent, these same scales can be seen in the depression where flagella are inserted.

The coccolith is made up of an unmineralized base-plate scale which consists of

Longitudinal section of the rim of a coccolith, each consisting of outer (O) and inner (I) subelements; Unmineralized scale layers (Sc). ×70000. 17: Transverse section of the rim of a coccolith. Note that the broad side of each element is composed of rosary-like strands, while the narrow side is a straight line. ×50000. 18: Golgi apparatus and associated vesicles in which unmineralized scales (Sc) and coccoliths (C) are being produced. Note that rosary-like strands (arrows) can be seen on a coccolith. ×25000.

an elliptical disc-shaped base with a raised rim (Fig. 14) and calcium carbonate elements (Fig. 14). The base-plate measures 1.0–1.3 μm $\times$ 0.5–0.8 μm. Such a base-plate is not common in the coccolithophorids and has previously known in *Hymenomonas lacuna* (Pienaar, 1976). The morphology of the calcified rim varies widely and the coccoliths can be classified into three different types based on its characteristics. The rim of the Type 1 coccolith consists of characteristic rosary-like strands (Figs. 9 and 26A). These strands are arranged on top of each other at the surface of the raised rim of the base-plate scale, resulting in a coiled rope appearance (Figs. 9 and 10). They are easily loosened during the drying process for shdowcast preparation. Such loosened strands permit ready observations of their characteristic beaded appearance (Fig. 10). The grains or "beads" composing the rosary-like strands are ovoidal to rod-shaped and measure ca. 25 $\times$ 45 nm (Figs. 10 and 15). They are aligned in a row with their long sides facing each other and connected by a thin fibre to form a strand (Figs. 15 and 26A). Coccoliths of this type were commonly observed in all strains. The Type 2 coccolith, also common, is composed of rectangular elements, each of which consists of the same kinds of grains or "beads" as those strands of the Type 1 coccolith (Figs. 11, 16, 17 and 26B). Several strands are arranged on top of each other to make up the broad side of the rectangular element. The narrow sides (facing side) of the rectangular element do not show a beaded appearance. Instead, thin straight electron dense lines are aligned along the narrow sides of the elements (Figs. 16 and 17). In many cases the rectangular elements share narrow sides in common with neighboring elements so that the individuality of each element is not always clear. These rectangular elements are arranged in a circle at the margine of the rim of the base-plate (Fig. 14). The Type 3 coccolith was rarely encountered. It is similar to the Type 2 coccolith consisting of rectangular elements but it does not have a beaded appearance. The elements are smooth and more solid in appearance (Figs. 12 and 26c) and individual elements are easily recognized (Fig. 12). Approximately thirty to forty rectangular elements of Types 2 and 3 coccoliths are arranged in a circle. The rectangular elements of all Type 3 and some Type 2 coccoliths are made up of two kinds of subelements which differ in size and shape. A large rectangular to square shape subelement (the inner subelement) is arranged underneath a small rectangular one (the outer subelement). These subelements are very clear in the Type 3 (Fig. 12) but obscure in the Type 2 (Fig. 16). The calcified elements of the coccoliths are diagrammed in Fig. 26.

The two flagella are smooth, almost equal in length and taper towards their ends (Fig. 7). Only the haptonema base was detectable by electron microscopy. It consists of five microtubules (Figs. 19, 20 and 23). The flagellar apparatus is very similar to that of *Cricosphaera* (Inouye and Pienaar, in prep.). The two basal bodies and the haptonema base form the points of a triangle. One of the basal bodies (the left) is closer to the haptonema base than is the other (the right) (Figs. 19 and 23). They are linked by distal and proximal connecting bands. There are four types of flagellar roots. Three are the microtubular roots, 1, 2 and 3, and the 4th the fibrous root. Root 1 is compound consisting of two sets of closely aligned microtubules, one of which forms a sheet-like



Figs. 19–25. *Jomonlithus littoralis*. Electron micrographs. 19, 20: Sections cut through and above the distal band (Db). Basal bodies (L and R), haptonema base (H) and three microtubular roots (R1, 2 and 3) are seen. 19, ×30000; 20, ×25000. 21: Longitudinal section. Root 1 (R1) is compound and associated with the left basal body (L). Root 2 (R2) with 5 microtubules. Root 3 (R3) associated with the right basal body (R). ×50000. 22: Transverse section of secondary microtubules of compound root. ×25000. 23: Section through proximal band (Pb) linking basal bodies (L and R). Fibrous root (Fr) extends to the opposite direction to the haptonema base (H). ×40000. 24, 25: Sections of a single cell. Number of microtubules composing root 3 is two (24 arrows) near the right basal body (R) and increase to four (25) some distance away. ×60000.

structure associated with the left basal body (Fig. 21). A bundle of more than 200 microtubules arises from this sheet and extends into the cytoplasm along the inner surface of the chloroplast (Figs. 21 and 22). Root 2 is also compound and made up of sets of

microtubules similar to those of root 1. The sheet-like microtubules of root 2 consist of 5 microtubules (Fig. 21), while those of root 1 number about twenty (Fig. 21). This sheet-like structure arises from the space between two basal bodies and extends in the opposite direction toward the haptonema base (Figs. 19 and 20). Root 3 is a simple microtubular root associated with the right basal body. It is made up of two microtubules near the basal body (Fig. 24) and four some distance away (Fig. 25). In oblique sections this root appears to have two starting points, although this has not been confirmed in transverse sections. The fibrous root arises from the left basal body and extends towards and along the sheet-like microtubules of root 1 (Fig. 23). This root is not striated.

The parietal chloroplast (Fig. 13) is traversed by three thylakoid lamellae. The pyrenoid bulges from the inner face of chloroplast (Fig. 13) and its matrix is traversed by many paired thylakoids. The nucleus (Fig. 13) is close to the chloroplasts and their associated pyrenoids and its outer membrane is continuous with the chloroplast endoplasmic reticulum. The Golgi apparatus (Fig. 13) is composed of a single dictyosome and is situated between the nucleus and the basal bodies. The cisternae have the peculiar dilations (Fig. 18) characteristic of many species of the Prymnesiophyceae. Both the unmineralized scales and coccoliths are produced in the Golgi-derived vesicles (Fig. 18). The characteristic beaded appearance of the coccolith rim is present prior to release (Fig. 18).

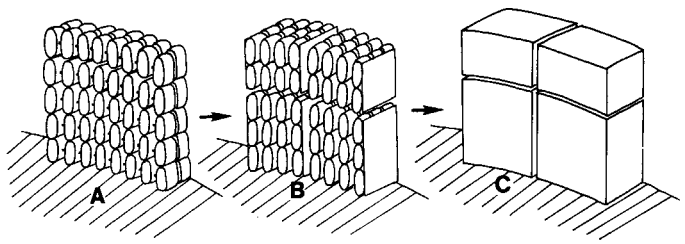


Fig. 26. Diagrammatic illustrations of possible structure of the calcified rim of three different coccoliths. A: Type 1 coccolith consisting of rosary-like strands. B: Type 2 coccolith with rectangular elements made up of rosary-like strands. C: Type 3 coccolith consisting of rectangular elements with solid appearance. Arrows indicate a possible calcification process. The raised rim of the base-plate scale is not drawn in the illustrations.

Discussion

The variety of coccoliths found in *J. littoralis* is very unusual and must be carefully interpreted since coccolith morphology is generally considered to be a stable trait on which to base the taxonomy of the coccolithophorids. In. *J. littoralis* there are three different types of coccoliths viz. Types 1, 2 and 3 coccoliths. The Type 1 coccolith and Type 3 coccolith are morphologically distinct from each other and no similarity is found between them. The Type 2 coccolith is, however, similar to both of them. The differences between the three coccolith types may be due to their being in different stages of calcification. It seems reasonable that the Type 3 coccolith

is fully calcified and the Types 1 and 2 are in the early stages of calcium carbonate deposition and later develop into the Type 3. Rectangular elements may be produced by fusion of the rosary-like strands. A possible calcification scheme is diagrammed in Fig. 26. If these interpretations are correct, it is of special interest that the incompletely calcified coccoliths (Types 1 and 2) occur frequently in cultured material of *Jomonlithus* while fully calcified coccoliths (Type 3) are rare. This fact raises the question as to whether such a frequent occurrence of the Types 1 and 2 are artifacts caused by culture conditions. However, Type 1 coccoliths were observed in natural samples collected from Ago Bay, Mie-ken, from which one of our strains was isolated. We are of the opinion therefore that *J. littoralis* is unusual in having both incompletely and fully calcified coccoliths and that this is a stable character because it is commonly observed in all of our strains.

Whether Types 1 and 2 coccoliths develop into fully calcified coccoliths outside the cell or calcification stops after they are released from the cell is not clear. However this is a central problem and should be the subject of further study.

No coccolithophorid has been described previously which has the rosary-like strands of Types 1 and 2 coccoliths. The Type 3 coccolith with its calcified rim made up of inner and outer subelements is reminiscent of the coccolith of *Wigwamma* (Manton *et al.*, 1977). In *W. arctica*, the type species, and *W. annlifera*, the coccolith rims have the inner and outer subelements arranged in two rows at the margin of the base-plate scale (Manton *et al.*, 1977). These subelements are similar to those of the coccolith of *Jomonlithus*. The arrangement of subelements is, however, different. The inner and outer subelements alternate in *Wigwamma* whereas they are coincident in *Jomonlithus* (Fig. 27). In addition, the coccolith of *Wigwamma* possesses a characteristic superstructure consisting of the rod-shaped crystallite "tent pole" architecture (Manton *et al.*, 1977). No such superstructure has been observed in *Jomonlithus*. By virtue of the coccolith morphology, therefore, *Jomonlithus* seems closely related to *Wigwamma*. A more detailed discussion of the relationship between the two genera is not possible because of the lack of information on the cellular morphology of *Wigwamma*. *Papposphaera* Tangen (1972) and *Pappomonas* Manton et Oates (1975) have coccoliths morphologically similar to those of *Jomonlithus*. They differ from *Jomonlithus* in the arrangement of the rim subelements and the presence of superstructures. The coccolith rim is made up of two types of subelements arranged in two alternating rows as in *Wigwamma* (Tangen, 1972; Manton and Oates, 1975; Manton *et al.*, 1976; Manton and Sutherland, 1975).

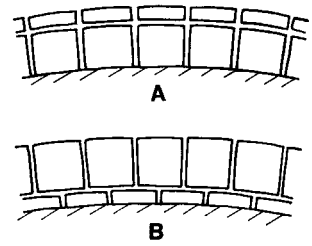


Fig. 27. Diagrammatic illustrations of the arrangement of rim subelements of *Jomonlithus* (A) and *Wigwamma* (B). The inner and outer subelements alternate in *Wigwamma* while they are coincident in *Jomonlithus*.

The ultrastructure of *Jomonlithus* is similar to *Cricosphaera* and *Hymenomonas* (Manton and Peterfi, 1969; Mills, 1975; Gayral and Fresnel, 1976; Pienaar, 1976; Inouye and Chihara, 1979, 1980). They all have two bulging pyrenoids which face each other and well developed flagellar root systems made up of two compound microtubular roots each of which is composed of two sets of microtubules, a simple microtubular root and a fibrous root (Inouye and Pienaar, in prep.). Although the flagellar apparatus of *Hymenomonas* has not been studied in detail preliminary observations suggest that it is almost the same as that of *Cricosphaera*. However in coccolith morphology *Jomonlithus* differs from all of these genera.

We find it difficult to assign the alga under consideration to any of the previously described genera. Therefore it seems appropriate to establish a new genus, *Jomonlithus*, for it and include one species, *J. littoralis* sp. nov. The generic name reflects the similarity of the ornamentation on the ancient Japanese earthenware called "Jomon [dʒɔ:mɔŋ]" to the stack of rosary-like strands on the coccolith.

Diagnosis

Jomonlithus Inouye et Chihara gen. nov.

Coccolithus ellipticus, ex squama laminae basis inmetallica et elementis orarum calcareis constans. Ora calcarea ex aut filmis cateniformis aut elementis rectangularibus constans. Elementum rectangularis ex subelementis typorum duorum, subelementis interioribus et exterioribus in duabus seriebus, dispositum.

Species typifica. J. littoralis.

Jomonlithus littoralis Inouye et Chihara sp. nov.

Cellula vegetativa solitaria, immobilis, libere viva, discoidia, 8–12 μm diametro. Cellula mobilis elliptica ad cylindrica, 9–14 μm $\times$ 4–7 μm, cum duobus flagellis aequalibus. Haptonema manifestum abest. Cellulae duarum typorum squamatis non-calcificatarum et coccolithis obtectae. Coccolithus ellipticus, annualis, 0.9–1.3 μm $\times$ 0.5–0.8 μm. Elementa interiora magna et elementa exteriora parva. Chloroplasti duo, parietales, cum pyrenoidibus protuberatiorum.

Holotypus. Figura 7.

Type locality. Nakagawa river, Ibaraki, Japan.

Habitat. Littoral region, especially estuary.

Distributions. Nakagawa river and Kujigawa river, Ibaraki Prefecture; Kidogawa river, Chiba Prefecture; Port Kashikojima, Mie Prefecture; Port Naoetsu, Niigata Prefecture; Iriomote-jima Island, Okinawa Prefecture, Japan.

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