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# The Japanese Journal of PHYCOLOGY

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THE JAPANESE SOCIETY OF PHYCOLOGY

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Jap. J. Phycol. (Sôrui) **35**: 155–166, September 20, 1987

# Loricate and scale-bearing protists from Lützow-Holm Bay, Antarctica II. Four marine species of *Paraphysomonas* (Chrysophyceae) including two new species from the fast-ice covered coastal area*

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TAKAHASHI, E. (1987) Loricate and scale-bearing protists from Lützow-Holm Bay, Antarctica II. Four marine species of *Paraphysomonas* (Chrysophyceae) including two new species from the fast-ice covered coastal area. Jap. J. Phycol. **35**: 155–166.

In a one year survey carried out from February 1983 to January 1984 at four sampling sites in the fast-ice covered coastal area north of East Ongul Island (69°00′S, 39°35′E), Antarctica, four marine species of *Paraphysomonas* (Paraphysomonadaceae, Chrysophyceae) were found. They were recorded for the first time in the Antarctic, and two of these species are new to science. One of these, *P. antarctica* n. sp., belongs to the *imperforata* group, and the other, *P. oligocycla* n. sp., to the *foraminifera* group. The two previously described species are *P. vestita* and *P. butcheri*.

*Key Index Word: Antarctic Ocean*; *Fast-ice covered coast*; *Chrysophyceae*; *East Ongul Island*; *New marine species*; Paraphysomonas; *Taxonomy*.

Since the time that two new marine species of the originally monospecific genus *Paraphysomonas* were described by LUCAS in 1967 (LUCAS 1967), many freshwater and marine species of this genus have been found in many parts of the world. Until now, 48 taxa in total have been described. Among them, 39 have been found in freshwater, 6 in seawater, and 3 in both fresh and sea-water (REES et al. 1974; PREISIG and HIBBERD 1982a, 1982b; WUJEK 1983). They were classified into 11 groups based on their scale structures (PREISIG and HIBBERD 1982b, 1983). One of the marine taxa, *P. imperforata* is widely distributed in brackish water and seawater from the coast of Norway in the Northern hemisphere (LEADBEATER 1972) to the coast of New Zealand in the Southern hemisphere (MOESTRUP 1979). This organism, showing little variation in scale structure, have been recorded from freshwater lakes and ponds in many countries between Sweden in the Northern hemisphere (CRONBERG and KRISTIANSEN 1980) and Chile in the Southern hemisphere (DÜRRSCHMIDT 1980). The type species of this genus, *P. vestita*, is also recorded from many freshwater and some seawater localities throughout the world (PREISIG and HIBBERD 1982a). Altogether, this genus is widely distributed in both freshwater and seawater all over the world from ca. 65°N to 40°S.

Furthermore, four marine species of this genus, two of them new to science, were found in the fast-ice covered coastal area north of East Ongul Island, Antarctic Ocean. One of the new species belongs

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to the *imperforata* group and other to the *foraminifera* group.

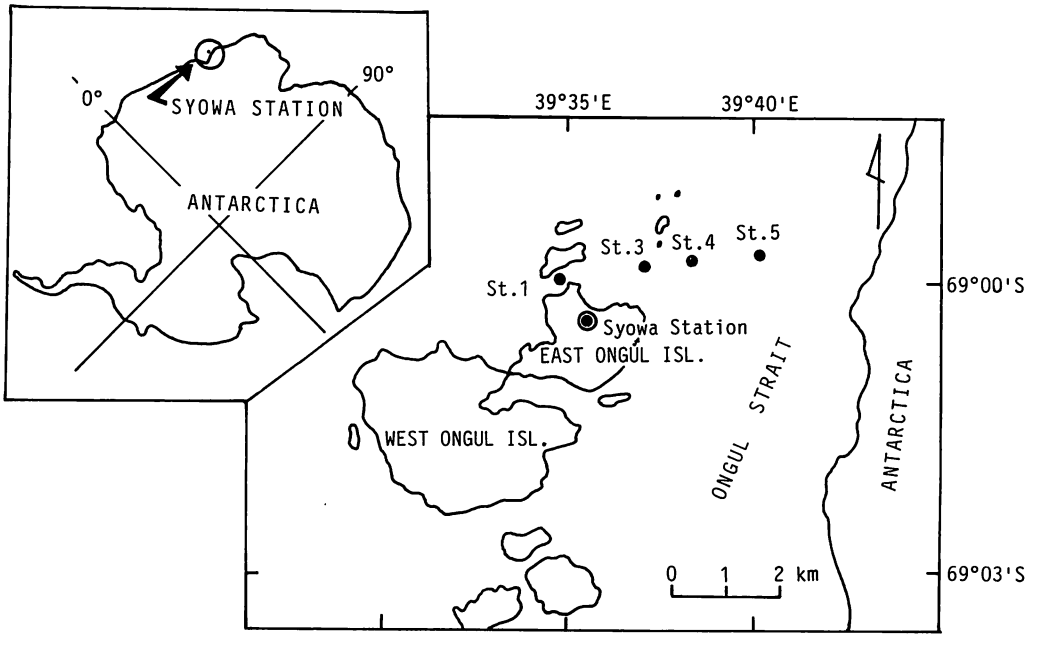
Their characteristics are described and the taxonomy of the *imperforata* group is discussed in this paper.

## Materials and Methods

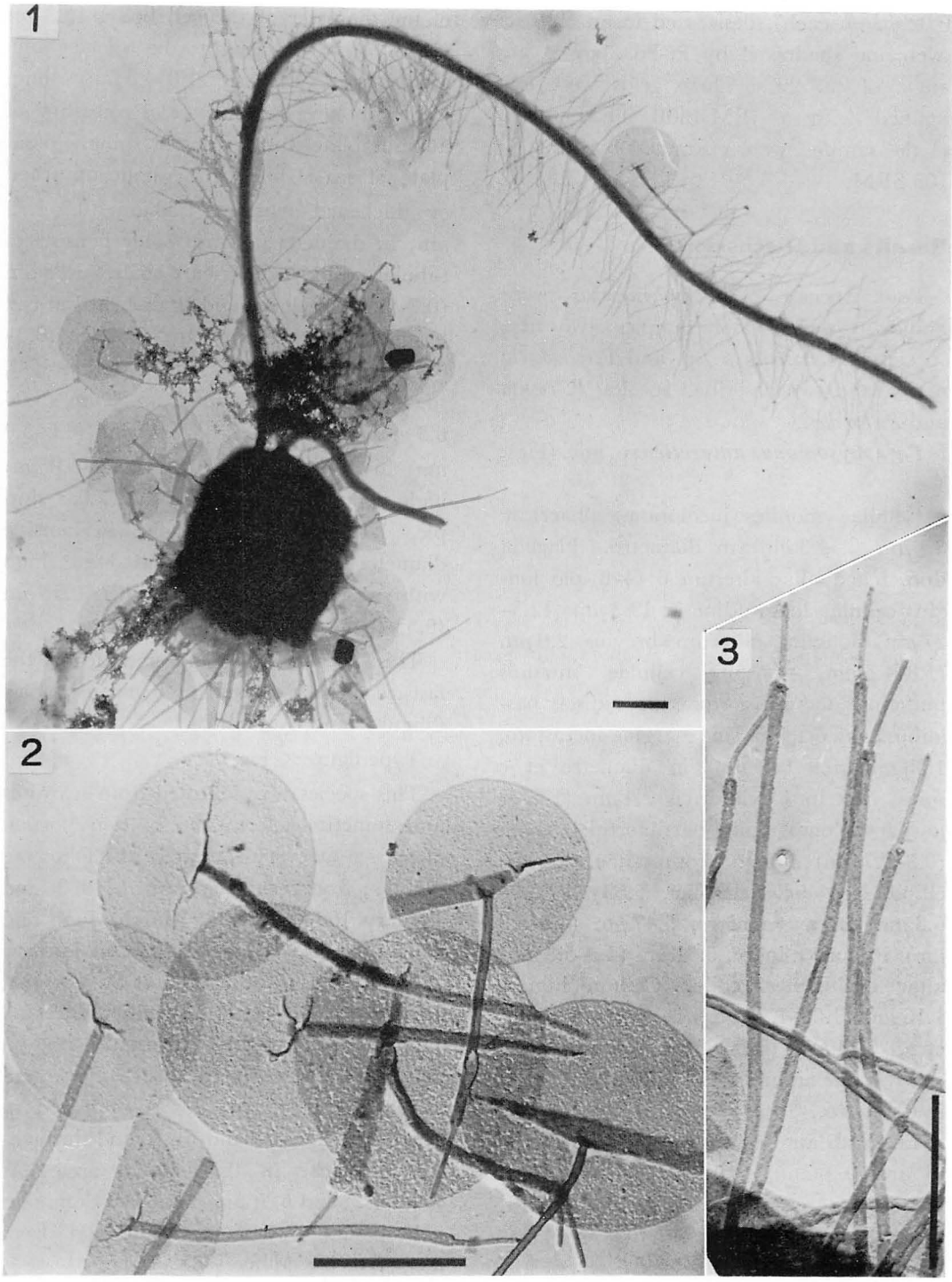
Seawater and sea ice core samples were collected at four sampling sites (Stations 1, 3, 4 and 5) on the fast-ice at the coastal area north of the Syowa Station, East Ongul Island (69°00'S, 39°35'E) in the Lützow-Holm Bay, from February 1983 to January 1984 (Map. 1). The depth to the sea bottom at Stations 1, 3, 4 and 5 were ca. 12, 38, 160, and more than 700 meters respectively. Seawater samples were collected with a Van Done bottle from the following depths at each station: 2, 5, 8 and 11 m at St. 1; 2, 5, 10, 15, 25 and 35 m at St. 3; 2, 5, 10, 25, 50, 75, 100 and 150 m at St. 4; 2, 5, 10, 25, 50, 75, 100, 150, 200, 400 and 600 m at St. 5. In addition to these samples, surface water samples were taken in 0.5 *l* polyethylene bottles from a depth of ca. 10 cm at the sampling hole at each station. Fast-ice cores were taken with a SIPRE electric ice core sampler from each sampling station. The thickness of sea ice varied from ca. 50 to 120 cm. Except for one day, May 3 1983, when the pack ice was blown offshore, the Ongul Islands remained icebound.

The climatic and oceanographic conditions in this area have been described in another paper (WATANABE et al. 1986).

The method used to examine the seawater and sea ice samples by use of the scanning electron microscope (SEM) is described in a previous paper (TAKAHASHI et al. 1986). For transmission electron microscopy (TEM), both live and fixed concentrated water samples were mounted on collodion carbon coated grids (ca.




Map. 1. Map showing four sampling sites on the fast-ice in the coastal area north of East Ongul Island, Antarctica.




Figs. 1–3. *Paraphysomonas antarctica* n. sp.; Fig. 1. an intact cell, Fig. 2. typical scales, Fig. 3. shafts and rod-like tips of spines. (all scale bar shows 1 $\mu$m)

0.02 m*l* on each), desiccated in an electric oven and shadowed by Pt-Pd alloy at an angle of ca. 20°. These grids were examined with a JEM-100B TEM. Part of the samples were examined by JSM-T-100 SEM.

## Results and Discussion

Four species of *Paraphysomonas* were found in these water samples: two new species, *P. antarctica* n. sp. and *P. oligocycla* n. sp., and two described species, *P. vestita* and *P. butcheri*.

***Paraphysomonas antarctica*** sp. nov. (Figs. 1–5)

Cellulae mobiles incolorata, sphaericae 3.1 $\mu$m (2–4.3 $\mu$m) in diametro. Flagella duo, inaequalia; alterum 6 (4–8)-plo longius cellulae longitudine et 19.3 $\mu$m (12.5–27 $\mu$m) longum, alterum brevius 2.9 $\mu$m (1.8–4.5 $\mu$m). Corpus cellulae squamis numerosis tectum. Squamae e discis basalibus orbicularibus sine margine incrassato, 1.38 $\mu$m (0.9–1.75 $\mu$m) in diametro et e spinis centribus cylindricis 2.4 $\mu$m (1–3.25 $\mu$m) totis longis apice bacilliformi 0.52 $\mu$m (0.3–0.7 $\mu$m) longo compositae. Cystae ellipsoideae collo simplici, 5.58 $\mu$m (5.0–6.3 $\mu$m) in axe maiore, 5.47 $\mu$m (4.6–5.9 $\mu$m) in axe minore, 5.31 $\mu$m (4.9–5.8 $\mu$m) altae excludentes collo. Collum humile 1.16 $\mu$m (1.06–1.27 $\mu$m) in externo diametro, 0.24 $\mu$m (0.2–0.3 $\mu$m) altum, poro centrali sine annulo 0.56 $\mu$m (0.55–0.65 $\mu$m) in diametro.

Lecta ab autore ipso in mari glaciolento in area septentrionali insulae Ongul orientalis, Antarctica: holotypus in herb. Inst. Biol. Kobe Univ. conservatus.

Iconotypus: Fig. 1.

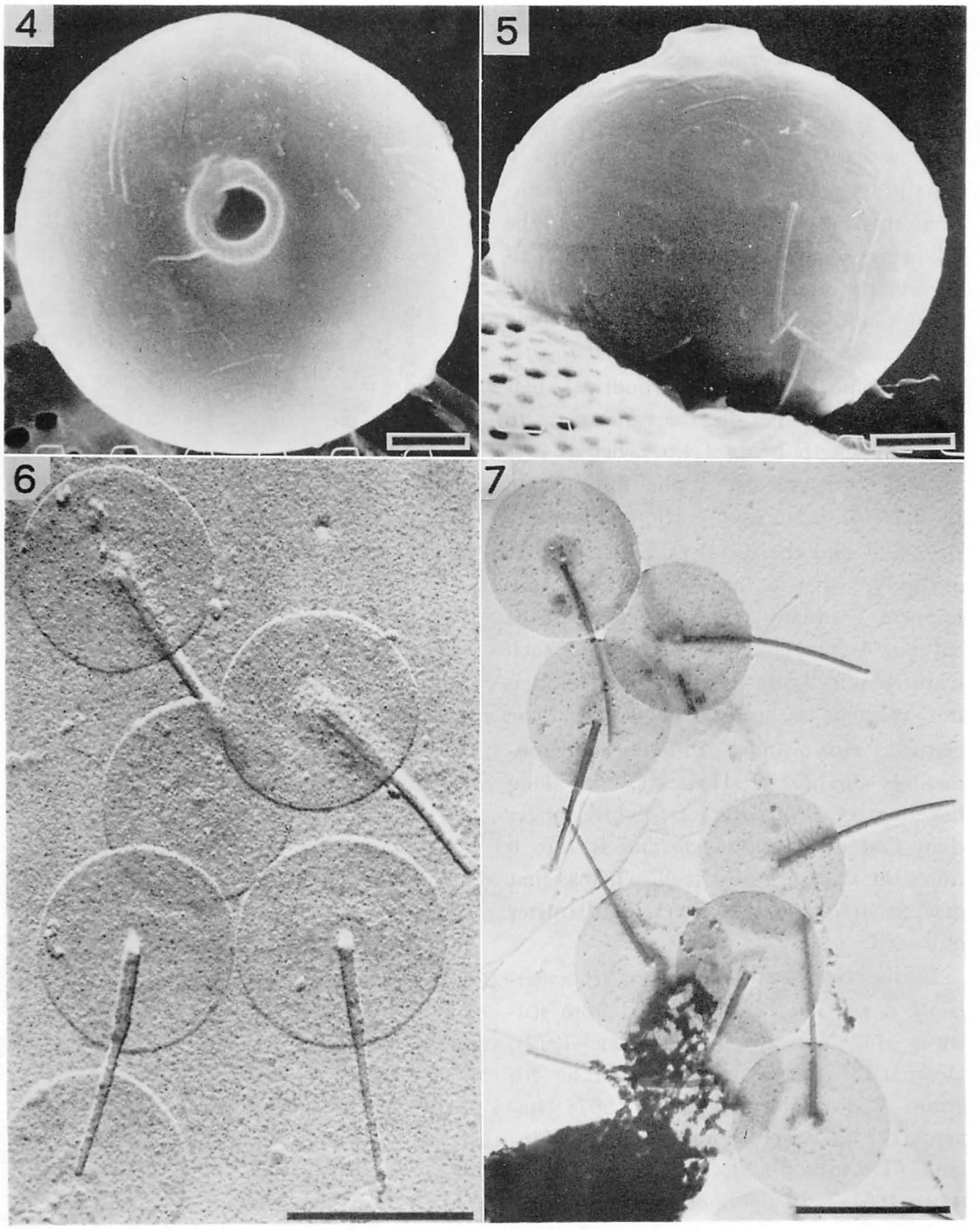
Cell motile, colourless, spherical, 3.1 $\mu$m (2–4.3 $\mu$m) in diameter. Flagella two, unequal; the longer flagellum pleuronematic, the length about 6 (4–8) times that of the diameter of the cell body, 19.3 $\mu$m (12.5–27 $\mu$m) in length; the shorter one acronematic, 2.9 $\mu$m (1.8–4.5 $\mu$m) long. Cell body covered with scales consisting of a basal plate and a central spine. Basal plate of scale spherical without upturned or thickened margin, 1.38 $\mu$m (0.9–1.75 $\mu$m) in diameter; central spine consists of tubular and cylindrical shaft, 1.88 $\mu$m (0.7–2.55 $\mu$m) long, and thinner apical rod terminating in a round tip, 0.52 $\mu$m (0.3–0.7 $\mu$m) long. Cyst slightly ellipsoidal, smooth, with a low collar, 5.58 $\mu$m (5.0–6.3 $\mu$m) in major axis, 5.47 $\mu$m (4.6–5.9 $\mu$m) in minor axis, 5.31 $\mu$m (4.9–5.8 $\mu$m) high excluding collar height. Collar simple, 1.16 $\mu$m (1.06–1.27 $\mu$m) in outside diameter and 0.24 $\mu$m (0.2–0.3 $\mu$m) high with a pore without annulus 0.56 $\mu$m (0.55–0.65 $\mu$m) in diameter.

This species was collected from the fast-ice covered coastal area north of East Ongul Island, Antarctica.

Type figure: Fig. 1.

This species was collected from seawater and sometimes from the bottom portion of sea ice at every station in the following months; from seawater, April 1983 and January 1984 at St. 1; March, June and September to December 1983 and January 1984 at St. 3; March 1983 at St. 4; June, July and September to December 1983 and January 1984 at St. 5; from the bottom portion of sea ice, April 1983 at St. 1, and January 1984 at St. 3. It appeared in every sampling site and was found throughout the year in this coastal area. At Stations 3 and 5, it appeared as a dominant phytoplankton during the period from August to December 1983.

As all the cells collected from every sampling site during the period surveyed were covered with monotypic scales of a homogeneous structure, this scale structure




Figs. 4, 5. a cyst of *P. antarctica* n. sp.; in upper view (Fig. 4, SEM) and in side view (Fig. 5, SEM).
Figs. 6, 7. *P. imperforata*; Fig. 6. scales from L. Saroma, Japan, Fig. 7. scales from Western Australia.
(all scale bar shows 1 $\mu$m)

can be considered stable.

*P. antarctica* is classified in the *imperforata* group based on a scale structure consisting of a basal disc plate without a thickened or upturned margin and a central spine with a basally thickened portion and a distally thinner portion. It can be distinguished from *P. imperforata* by the following characteristics: 1) in *P. antarctica*, the diameters of the cyst and the basal plate, and the length of the spine are twice as much as those in *P. imperforata*; 2) the spine consists of a cylindrical shaft and a thin rod-like tip, the ratio of tip to spine in length being 1/4.6, whereas it is 1/2 in *P. imperforata*; 3) the thickness of the spine at the median portion, in between the basal and the distal portions, changes abruptly, whereas in *P. imperforata* this happens gradually within a length of ca 0.1 $\mu$m; 4) taxonomical weight of such features as cell size and flagellar length is not as great as scale structure, as these features vary under different environmental conditions. However, the long flagellum of *P. antarctica* is slightly longer than that of *P. imperforata* and is 4 to 8 times the diametre of its cell, whereas that of *P. imperforata* 3 to 4 times the diameter of its cell.

In the Northern hemisphere, *P. imperforata* s. str. has been recorded from seawater in Norway (LEADBEATER 1972), Denmark (THOMSEN 1975, except for his group 2), England (LUCAS 1967; HIBBERD 1979), Israel (THOMSEN 1978), Finland (THOMSEN 1979), and Japan (TAKAHASHI 1981). In the Southern hemisphere, it has been recorded in New Zealand (MOESTRUP 1979) and Western Australia (this paper). Among cells and scales found in Denmark and designated as *P. imperforata* by THOMSEN (1975), a cell (his figure 11) and a scale (his figure 8) differ

Table 1. Dimensional characteristics of *P. antarctica* n. sp. and of the *P. imperforata* complex.

| Species | Cell diam. ($\mu$m) | Scale: Form*1 | Base plate diam. ($\mu$m) | Spine length (total l.) ($\mu$m) | Spine Tip length ($\mu$m) | Spine l./Base plate diam. | Tip l./ Spine l. | Locality*2 | References |
|---|---|---|---|---|---|---|---|---|---|
| *antarctica* | 3.1 (2–4.3) | C | 1.38 (0.9–1.75) | 2.4 (1.0–3.25) | 0.52 (0.3–0.7) | 1.73 (0.9–2.5) | 1/4.6 | M. | coastal area, Antarctic |
| *imperforata* | 4.5 (3.8–5.1) | C | 0.77 (0.7–0.85) | 1.0 (0.88–1.13) | ca. 0.5 | 1.25 (5:4) | 1/2 | M. | LUCAS (1967) |
| do. | 3.5 (1.7–4.3) | C | 0.7 (0.64–0.76) | 0.76 (0.7–0.82) | 0.45 (0.4–0.52) | 1.1 (0.99–1.22) | 1/1.7 | M. | Japan (Lake Saroma) |
| do. | — | C | 0.86 (0.72–0.97) | 1.34 (1.0–1.68) | 0.8 (0.67–1.03) | 1.56 (1.17–2.15) | 1/1.7 | M. | W-Australia (TAKAHASHI) |
| do. (group 1) | — | C | 0.7 (0.5–0.9) | 1.17 (0.8–1.6) | 0.43 (0.4–0.6) | 1.9 (1.1–2.7) | 1/2.7 | M. | THOMSEN (1975) |
| do. (group 2) | — | C | 1.08 (0.7–1.5) | 3.18 (2.6–4.2) | 0.38 (0.2–0.6) | 3.2 (1.9–4.1) | 1/8 | M. | THOMSEN (1975) |
| do. (fo. no. 1) | — | C | 1.4–1.5 | 3.9 (2.8–4.2) | 0 | ca. 2.7 | 0 | F. | TAKAHASHI (1976) |
| do. (fo. no. 2) | — | E | 2.2–2.5×1.9–2.0 | 7.0 (5.1–8.5) | 0.2–0.25 | 3–3.6 | 1/30 | F. | TAKAHASHI (1976) |
| do. | (1.7–5.1) | E | — | (0.8–4.2) | — | — | — | F. | PREISIG & HIBBERD (1982) |
| do. (fo. no. 3) | — | E | 2.1–3.0×1.6–1.9 | 4.1–5.1 | — | — | — | F. | KLING & KRISTIANSEN (1983) |

(*1, C=circular, E=elliptical; *2, M=marine, F=freshwater)

from other scales described as *P. imperforata* and resemble more those of *P. antarctica* than those of *P. imperforata*. His scales have the same three dimensional characteristics as scales of *P. antarctica* (Table 1). This suggests the existence of an *antarctica*-like taxon in the Northern polar region.

Besides the above seawater localities, scales and cells of *P. imperforata* have been recorded from many freshwater localities (ANDERSEN 1978; CRONBERG and KRISTIANSEN 1980; DÜRRSCHMIDT 1980; HIBBERD 1979; JACOBSEN 1985; KLING and KRISTIANSEN 1983; KRISTIANSEN 1976, 1978, 1980, 1983, 1985, 1986; NICHOLLS 1981a; PREISIG and HIBBERD 1982a; ROIJACKERS 1981; ROIJACKERS and KESSELS 1981; SKOGSTAD 1982; TAKAHASHI 1976, 1978; WAWRZYNIAK and ANDERSEN 1985; WEE 1982; WUJEK 1983, 1984). These freshwater specimens designated as *P. imperforata* deviate from the type by distinctive differences in scale structure. They are divided into three groups based on scale structure; Forma no. 1 contains scales consisting of a circular plate and a long curved spine terminating in a round tip; Forma no. 2 contains scales consisting of an elliptical plate and a long, straight, cylindrical spine terminating in an abruptly and acutely pointed minute tip (TAKAHASHI 1976, 1978); and Forma no. 3, which consists of an elliptical base plate and a curved long spine terminating in a round tip, has been found in Canada by KLING and KRISTIANSEN (1983). PREISIG and HIBBERD (1982a), the first to examine cells of Forma no. 2, considered that this organism might be a separate taxon. Whereas, THOMSEN (1975) and LEE (1978) questioned the separation of species of *P. vestita* and *P. imperforata* because scale and spine structure shows a gradual transition from one species to the other. In another study many cells of *P. imperforata* Forma no. 2 which were collected from lakes and ponds in Alaska were examined. It is concluded that Forma no. 2 should be considered a separate species because all the scales of examined cells were homogeneous and stable in structure. A taxonomic treatment of Forma no. 2 will be published in a subsequent paper. As for Forma no. 1 and Forma no. 3, two different kinds of scales may characterize two separate species. Further investigation is necessary.

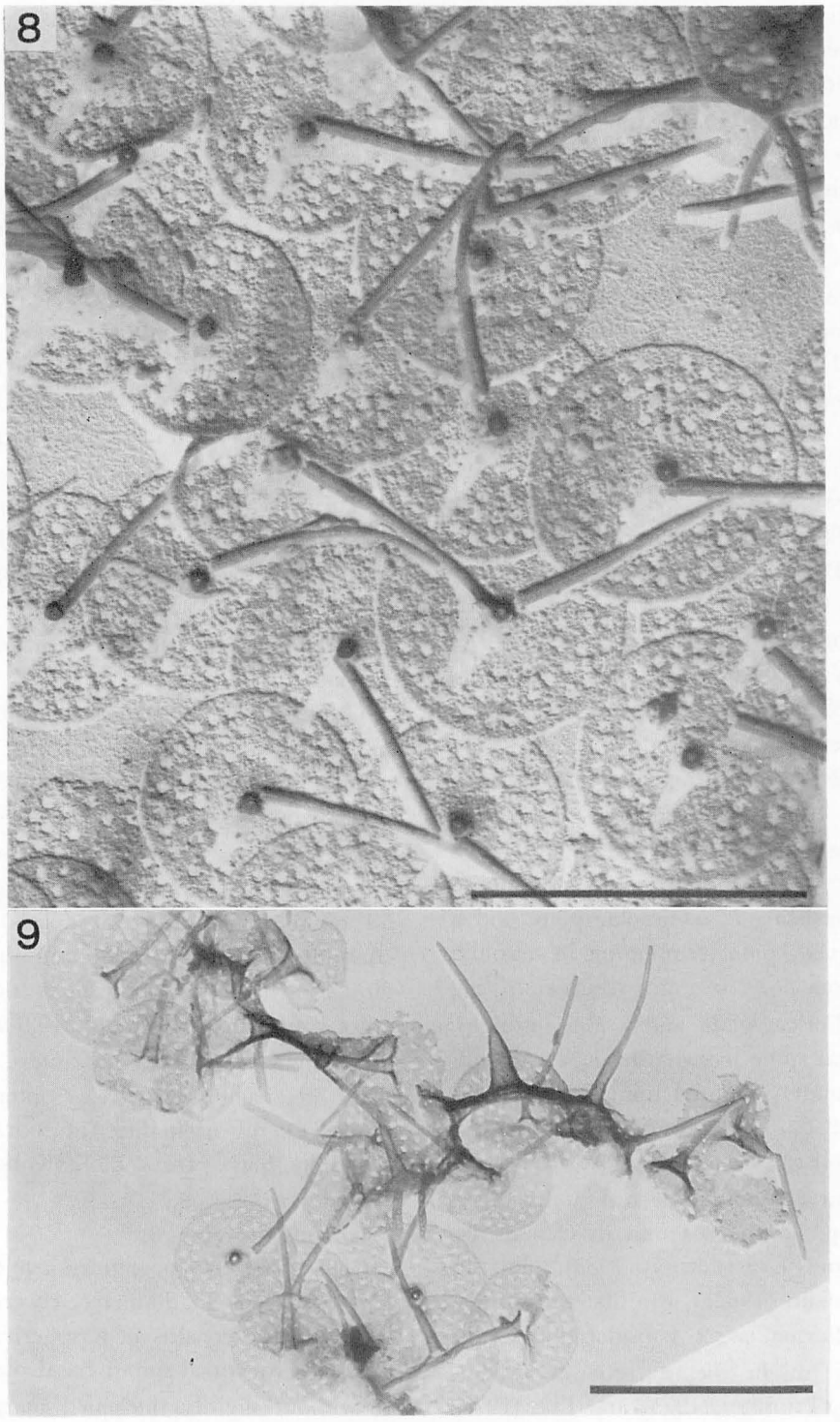
The cyst shown in a previous paper (TAKAHASHI et al. 1986, figure 24) is not that of *P. imperforata* but belongs to *P. antarctica*.

***Paraphysomonas oligocycla*** sp. nov. (Figs. 8, 9)

Cellulae mobiles incoloratae, sphaericae, 6.2 $\mu$m (6–6.5 $\mu$m) in diametro. Corpus cellulae squamis numerosis tectum. Squamae e discis basalibus orbicularibus margine complanato vel leviter incrassato 0.6 $\mu$m (0.47–0.8 $\mu$m) in diametro a duobus ad quattuor annulis concentricis paribus constantibus numerosis foraminibus perforatis et e spinis centralibus deminutis apice rotundato 0.78 $\mu$m (0.5–0.9 $\mu$m) longis compositae. Lecta ab autore ipso in mari glaciolento in area septentrionali insulae Ongul orientalis, Antarctica: holotypus in herb. Inst. Biol. Kobe Univ. conservatus.

Iconotypus: Fig. 8.

Cells spherical to slightly ovoidal, 6.2 $\mu$m (6–6.5 $\mu$m) in diameter, covered with scales. Scale consists of a spherical basal plate and a central spine; basal plate with or without slightly thickened margin, 0.6 $\mu$m (0.47–0.8 $\mu$m) in diameter, ornamented with two to four concentric rings of irregularly shaped perforations; spine slight-




Figs. 8, 9. *P. oligocycla* n. sp.; Fig. 8. typical scales, Fig. 9. scales with spine having swollen basal portion. (all scale bar shows 1 $\mu$m)

ly tapering, with or without swollen basal part, terminating in a round tip, 0.78 μm (0.5–0.9 μm) in length. Cyst unknown.

It was collected from the fast-ice covered coastal area north of East Ongul Island, Antarctica.

Type figure: Fig. 8.

This species was collected from seawater in January 1984 at St. 1 and St. 5, and in March 1983 at St. 4, and also from the bottom portion of sea ice in April 1983 at St. 1.

*P. oligocycla* is placed in the *foraminifera* group together with *P. foraminifera* LUCAS 1967, *P. circumforaminifera* WUJEK 1983, and *P. takahashii* CRONBERG et KRISTIANSEN emend. THOMSEN et al. 1981. It is distinguished from the others by structural differences of the scales: three outer and five inner rings of perforations in the basal plate in *P. foraminifera*; only one ring of perforations just inside the upturned margin in *P. circumforaminifera*; and evenly and closely distributed perforations in the basal plate and three-forked spine base in *P. takahashii*.

Between two to four rings of perforations were found in the basal plate of *P. oligocycla*. A basal plate with three rings of perforations was 54.3% of all scales examined, that with two rings 34.3%, and that with four rings 11.3%. The number of perforations in the outer ring of the basal plate with three rings of perforations was 27 (20–30), that in the middle ring 18 (6–22), and that in the innermost one 13 (4–16). The perforations in the middle and innermost rings were arranged irregularly and varied in number. Scales of some cells possessed a central spine with a swollen basal portion as observed in *Spiniferomonas bourrellyi* (NICHOLLS 1981b). The swollen portion varied from 8 to 41% (mean 24%) of the whole spine length.

***Paraphysomonas vestita*** (STOKES) DE SAEDELEER 1929

(Figs. 10, 11)

This species, one of the most widely distributed species of this genus, has been recorded mainly in freshwater. At Station 1, many scales were collected from the middle and bottom parts of sea ice in April 1983, and from seawater in April 1983 and January 1984. Cysts were collected from the middle part of sea ice in April 1983.

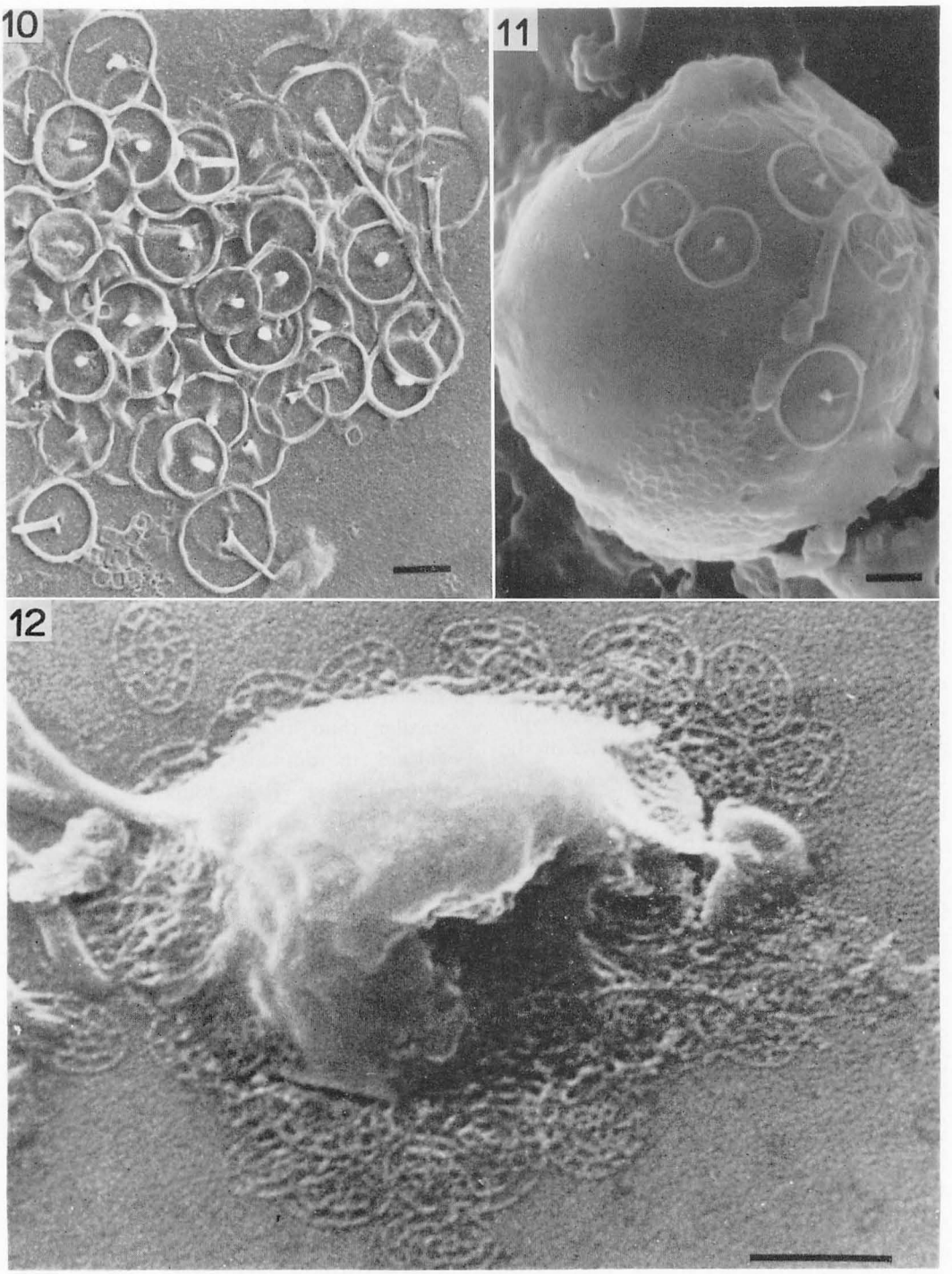
The scale consists of a circular basal plate with a wide upturned margin and a tapering central spine terminating in a pointed tip. Spines of Antarctic specimens varied from 0.1 μm to 4.2 μm in length, which was shorter than those in other localities; e.g. Japanese specimens varied from 1.3 μm to 7.0 μm in length (TAKAHASHI 1978). The cyst, 8.6 μm in diameter in the Antarctic material, was also smaller than that in Japanese material (11 μm in diameter, TAKAHASHI 1978). Although LEE (1978) found that *P. vestita* is capable of euryhaline growth in a wide range of salinities, records for this species in seawater are very few (LEADBEATER 1972; THOMSEN 1975, 1978; LEE 1978; TAKAHASHI 1981). The salinity of seawater at Station 1 was 3.296–3.407‰ throughout the year.

This is the first record of *P. vestita* in the Antarctic.

***Paraphysomonas butcheri*** PENNICK et CLARKE 1972

(Fig. 12)

A few cells of this species were collected from seawater in March 1983 at Stations 1 and 4. All of them were covered with plate scales only. Cells were spherical and 2.85 μm (2.4–3.2 μm) in diameter, and plate scales were elliptical and 0.79 μm (0.7–0.82 μm) × 0.55 μm (0.5–0.63 μm).




Figs. 10, 11. *P. vestita*; scales (Fig. 10, TEM) and a side view of cyst (Fig. 11, SEM). Fig. 12. SEM image of *P. butcheri*. (all scale bar shows 1 $\mu$m)

The scales were slightly smaller than those in the type material (PENNICK and CLARKE 1972).

This is the first record of *P. butcheri* in the Antarctic.

The distribution of marine species of *Paraphysomonas* in the Southern hemisphere has not been fully described. Prior to 1984, only two records of this genus had been made in this area. *P. vestita* was discovered at Durban, Natal, RSA (LEE 1978), and *P. imperforata* s. str. and *P. butcheri* in the coast of New Zealand by MOESTRUP in 1979 (MOESTRUP 1979). The four species recorded in this paper and collected from the Antarctic Ocean, and *P. imperforata* s. str. collected from the Mundura Estuary near Bunbury, Western Australia, comprise the third and most recent record of this genus in the Southern hemisphere. These collections suggest a distribution of this genus as wide in the Southern hemisphere as in the Northern hemisphere. If adaptation to severe environmental conditions promotes a wide distribution, then the four marine species, reported in this paper as living under low water temperature and low light intensity beneath the Antarctic fast-ice, must indeed be widely distributed.

In the past, they were overlooked because they are easily destroyed or transformed by fixatives, and too small to be examined alive under a light microscope.

## Acknowledgement

The author wishes to express his gratitude to Dr. J. KRISTIANSEN (University of Copenhagen) for his helpful advice and reading of the manuscript, and to Dr. S. MAE (Leader of JARE 24) and Dr. T. HOSHIAI (Natl. Inst. Polar Res. Tokyo) for their encouragement. He wishes also to thank Dr. H. KANDA and Mr. K. WATANABE (Natl. Inst. Polar Res. Tokyo) and Dr. H. SATOH (Tokyo Univ. of Fisheries) for their assistance in sampling.

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## 高橋永治：南極，リュツオ・ホルム湾産の有殻・有鱗片原生生物 II。2新種を含む4種の氷海産パラピソモナス

オングル島，昭和基地北方の氷海産の黄金藻綱パラピソモナス科，パラピソモナス属の2新種を含む4種を記載し，インペルフォラータ群の分類について論議した。本報告は本属の海産種についての南半球から3番目の記録であり，南極海域からの最初の記録である。新種の一つ，パラピソモナス　アンタルクチカはほぼ周年にわたって出現した。(657 神戸市灘区六甲台1-1　神戸大学理学部生物学科)

Jap. J. Phycol. (Sôrui) **35**: 167–177, September 20, 1987

# Culture studies on *Caulerpa* (Caulerpales, Chlorophyceae) I. Reproduction and development of *C. racemosa* var. *laetevirens*

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ENOMOTO, S. and OHBA, H. 1987. Culture studies on *Caulerpa* (Caulerpales, Chlorophyceae) I. Reproduction and development of *C. racemosa* var. *laetevirens*. Jap. J. Phycol. **35**: 167–177.

Reproduction and development of the marine green alga *Caulerpa racemosa* var. *laetevirens* from two localities of the southern part of Japan were studied in laboratory culture experiments.

Wild mature plants collected during May and July and kept in autoclaved seawater produced gametes within about one month in culture under the following conditions: 25°C, 1.0–3.0 klux, and 14L/10D cycle.

Both sexes of biflagellate gametes were produced in the same plant. Gametes are anisogamous. A stigma was found in the relatively large female gametes, but not in the relatively small male gametes. Copulation was observed between gametes from the same plant. Settled zygotes became spherical and increased their volume for five weeks while retaining their spherical shape.

After five weeks, each spherical germling attained a diameter of about 120 $\mu$m and formed two germ tubes bipolarly. A fine primary germ tube was formed on the side away from the light. After about a week a thick secondary tube was formed on the side facing the light. Both tubes elongated and branched, resulting in creeping, filamentous, protonema-like plants. These creeping plants formed thick primary shoots which differentiated into creeping rhizomes and upright shoots.

The upright shoot formed ramuli and developed into an assimilator. Three types of assimilators were produced under different culture conditions —*laetevirens*-type under 20.0°C, 5.0 klux, *peltata*-type under 25.0°C, 1.5 klux, and intermediate-type under 20.0°C, 1.5 klux or 25.0°C, 5.0 klux. After 4–5 months, germlings developed into mature plants. After 5–6 months, they became fertile and produced both male and female gametes on the same plant. No quadriflagellate or stephanokontic zooids were observed.

*Key Index Words:* Caulerpa, Caulerpa racemosa *var.* laetevirens, *Caulerpales, Chlorophyceae, culture, development, life-history, reproduction.*

The coenocytic marine green alga *Caulerpa* is widely distributed in the littoral and sublittoral waters of tropical and subtropical seas. Several species are utilized as food, and mariculture has been started in some Asian countries.

Much information concerning the structure of the gametangium and the copulation of gametes in *Caulerpa* has accumulated from the work of many investigators (MONTAGNE 1838, DERBÈS & SOLIER 1850, WEBER-VAN BOSSE 1898, DOSTÁL 1928a, 1928b, 1929, SCHUSSNIG 1929a, 1929b, 1939, SCHWARTZ & SCHWARTZ 1930, ARWIDSSON 1930, ERNST 1931, IYENGAR 1933, 1940, YAMADA 1934, MIYAKE & KUNIEDA 1937, HAGIHARA & HIROSE 1969, KAJIMURA 1969, 1970, 1976, 1977, and GOLDSTEIN & MORRALL 1970). GOLDSTEIN & MORRALL (1970) gave a historical review of these results. However, the development of the thallus of *Caulerpa* has been reported preliminarily in only two species, *C.*

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*serrulata* from Australia (PRICE 1972) and *C. okamurae* from Japan (ISHIWARA *et al.* 1981).

Our research concerns the reproduction and development of several species of *Caulerpa* in laboratory culture experiments. The present paper presents the results of our studies of *C. racemosa* var. *laetevirens* from two localities of the southern part of Japan.

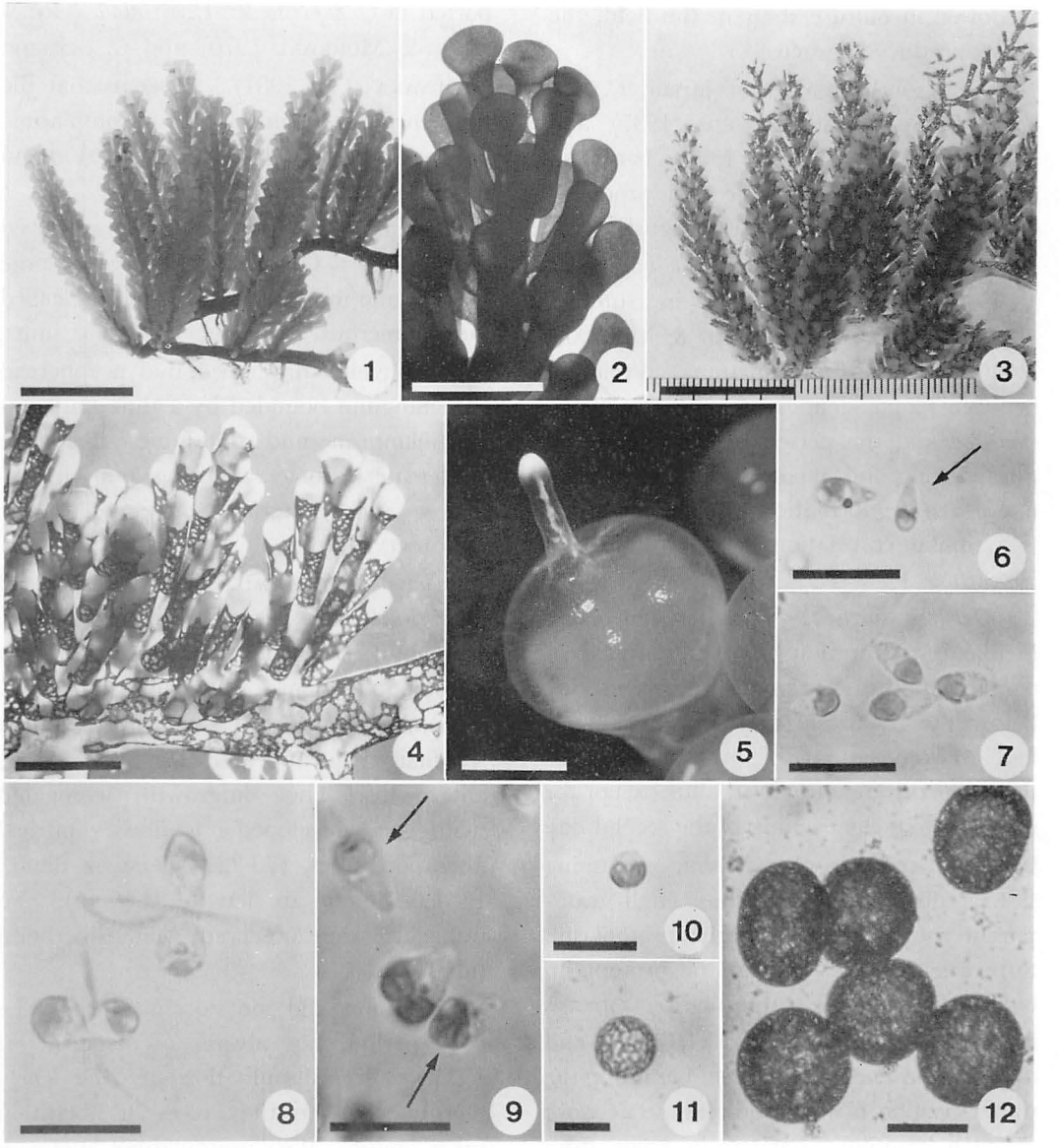
## Materials and Methods

Plants of *Caulerpa racemosa* var. *laetevirens* were collected from the lower part of the littoral zone at Muroto-misaki (33°16'N, 134°14'E) in Shikoku and the upper sublittoral zone in the outer fringe of coral reef flats at Ayamaru-misaki (28°28'N, 129°43'E) in Amami-ôshima. The collections were made from May to July of 1981–1985. Materials were kept at 13–15°C and immediately brought to the laboratory. After the fronds were freed from epiphytes and small animals, they were rinsed with filtered (Toyo filter paper No. 4A and No. 5C) and autoclaved (125°C, 20 min) seawater. Each frond was placed in a separate glass vessel containing 350 m*l* of sterilized seawater. For prevention of the luxuriant growth of algal epiphytes, no nutrients were added. The seawater was changed every 5 days. The plants were kept under 25°C, 1.0–3.0 klux, 14L/10D (06:00–20:00L/20:00–06:00 D) cycle. Gametes were discharged about one month after the beginning of preculture, in the early morning within 1–2 hr after illumination. They were discharged through liberation tubes as a highly viscous, dark green material, which precipitated onto the bottom of the vessel. With a slight agitation of the medium, male and female gametes swarmed out from the viscous material and immediately copulated. About 0.5 m*l* of suspension of zygotes was diluted with 300 m*l* of sterilized seawater. One or two drops of diluted suspension were inoculated into screw-capped glass tubes containing 15 m*l* of PROVASOLI's ES medium (prepared according to MCLACHLAN, 1973) with a micropipette. In another series of experiments, one or two drops of the suspension were inoculated onto glass coverslips (20 $\times 20 \times 1$ mm) covered with 1 m*l* of seawater, and placed in petri dishes. The zygotes attached themselves to the coverslips within 30 min, whereas uncopulated gametes continued swimming. An hour after being placed in petri dishes, the coverslips were rinsed with running seawater to remove the uncopulated gametes and then transferred into glass vessels (60 mm diam., 90 mm high) containing 150 m*l* of the same medium. These coverslips were used for observation of zygote development. Zygotes were cultured at first under the above-mentioned conditions. When germlings grew to 1–2 mm in length, they were isolated and transplanted into separate glass tubes. Two months after inoculation, germlings had grown to 10 mm in length and then were transferred into glass vessels (90 mm diam., 90 mm high) containing 350 m*l* of the same medium. These vessels were placed under the following four conditions: 1) 20.0°C, 1.5 klux; 2) 20.0°C, 5.0 klux; 3) 25.0°C, 1.5 klux; and 4) 25.0°C, 5.0 klux. A daylength of 14 hr (06:00–20:00) was employed. In the field, the present alga appeared luxuriantly in April-May and gradually disappeared in July-August. The water temperature of the habitats in April was about 20°C and that of July about 25°C. The culture medium was changed every 2 weeks. Cultures were not axenic, but they were strictly unialgal.

For light microscopy, gametes were fixed in 4% seawater glutaraldehyde.

## Results and Discussion

1. *Maturation of plants*: Within about




Figs. 1–12. Reproduction of *C. racemosa* var. *laetevirens*. 1. Mature vegetative plant from Murotomisaki. 2. A part of a vegetative assimilator. 3. Fertile plant with protoplasmic networks, one day before liberation. 4. Protoplasmic network in fertile assimilator. 5. Liberation tube on a ramulus. 6. Biflagellate male gamete (arrow). 7. Biflagellate female gametes. 8. Copulation of gametes. 9. Heart-shaped planozygote and completely copulated quadriflagellate planozygotes (arrows). 10. Settled zygote, after 3 hr copulation. 11. Spherical body, after 15 days. 12. Spherical bodies increasing cell volume, after 24 days. Scale: (Figs. 1, 3)=20 mm, (Figs. 2, 4)=5 mm, (Fig. 5)=1 mm, (Figs. 6–10)=10 $\mu$m, (Fig. 11)=20 $\mu$m, (Fig. 12)=50 $\mu$m.

one month after the beginning of the laboratory culture, vegetative plants from the field (Figs. 1 and 2) became fertile under 25.0°C, 1.0–3.0 klux, 14L/10D conditions. Although the light intensity was far lower in culture than in the field, the plants produced gametes.

In the southern part of Japan, *C. brachypus* (MIYAKE & KUNIEDA 1937) and *C. okamurae* (KAJIMURA 1969, ISHIWARA *et al.* 1981) became fertile and produced gametes during July and August. Our results suggest that *C. racemosa* var. *laetevirens* also becomes fertile in summer (June and July). GOLDSTEIN & MORRALL (1970) observed an apparent correlation between gametogenesis in some Caribbean *Caulerpa* and the period of extreme spring tides during full moon. Our study does not provide information on whether there is a similar correlation in *C. racemosa* var. *laetevirens*.

2. *Gamete formation*: The first sign of the incipient maturation of plants was recognized in the evening of the third day before the liberation of the gametes as a loss of homogeneity of protoplasmic distribution throughout the thallus except for rhizoids. In the evening of the second day before liberation, protoplasmic streaming slowed down and numerous small transparent spots appeared in the protoplasm. Subsequently, the spots in the protoplasm enlarged and the protoplasmic masses formed an irregular network (Figs. 3 and 4). In the evening before liberation, the upper portion of the protoplasmic network of each ramulus changed in color from green to dark yellowish-green, while the networks of lower portions of ramuli, upright shoots and rhizomes remained green. Just before liberation, the contrast between the colors of the network increased. By dissection, it was confirmed that the male gametes were formed in the green portion, while the female gametes, each of which had a reddish stigma, were formed in the dark yellowish-green portion. Such a sexual localization in a frond has been reported in *C. cupressoides*, *C. serrulata* (GOLDSTEIN & MORRALL 1970) and *C. okamurae* (ISHIWARA *et al.* 1981). It seems that the difference in color of the protoplasmic networks is caused by the reddish stigmata in female gametes.

GOLDSTEIN & MORRALL (1970) observed that in some Caribbean *Caulerpa* a large cytoplasmic mass of the erect frond cleaved into numerous smaller cytoplasmic units, each of which developed into a spherical gametangium bounded by a thin gametangial membrane and containing either male or female gametes. During our investigation, such spherical gametangia were not observed.

3. *Gamete liberation*: The formation of liberation tubes (papillae) started in the evening of the second day before liberation at the apical portions of upright shoots, the upper surfaces of ramuli and rhizomes. At first they appeared as tiny whitish outgrowths. The outgrowths elongated swiftly and developed into fine cylindrical liberation tubes, 170-220-250 $\mu$m in diam., 1.0-1.5-1.8 mm in length (Fig. 5). No trabeculae were observed in any liberation tube.

Liberation did not occur during the dark period, but always occurred about 1–2 hr after illumination in the early morning. When the apices of liberation tubes burst, the protoplasmic network broke down rapidly into dark green viscous material. The viscous material liberated through the liberation tubes and precipitated on the bottom of the vessel. Liberation continued for about 15 min, the mother plant losing its contents and fading. Nu-

merous gametes swam out from the viscous material with a slight agitation of the medium.

Liberation in the early morning was reported also in *C. brachypus* (MIYAKE & KUNIEDA 1937) and *C. okamurae* (ISHIWARA *et al.* 1981). It was possible to postpone liberation two or three hr by extension of the dark period. These facts suggest that illumination probably triggers liberation.

4. *Male and female gametes*: Two types of gametes were recognized. One (male) was relatively small, 4.5–6.0 $\mu$m in length, and 2.0–2.5 $\mu$m in breadth, and lacked a stigma (Fig. 6). The other (female) was larger, 5.5–7.5 $\mu$m long and 2.5–3.0 $\mu$m broad, and contained a reddish stigma (Fig. 7). Both sexes of gametes were biflagellate, 7.5–10.0 $\mu$m long, and tear-drop-shaped or slender pear-shaped, being pointed at the anterior and rounded at the posterior. They showed a weak positive phototactic response. The motion of the female gamete was rather slow, while the male gamete was more active. The swimming period of female gametes was shorter than that of male gametes.

The present alga always produced both sexes of gametes on the same frond and therefore is considered to be monoecious. Other monoecious taxa of *Caulerpa* that have been reported are *C. racemosa* var. *uvifera* (IYENGAR 1940), *C. mexicana*, *C. racemosa*, *C. serrulata*, *C. sertularioides*, and *C. taxifolia* (GOLDSTEIN & MORRALL 1970), and *C. okamurae* (ISHIWARA *et al.* 1981), while dioecious taxa include *C. clavifera* (ERNST 1931) and *C. brachypus* (MIYAKE & KUNIEDA 1937). Concerning *C. prolifera*, SCHUSSNIG (1939) reported that the Mediterranean plants were dioecious, while GOLDSTEIN & MORRALL (1970) described the Caribbean plants as monoecious.

5. *Copulation and zygotes*: When the gametes swam out from the viscous mass, males copulated with females and became quadriflagellate planozygotes, each with two chloroplasts and a stigma (Figs. 8 and 9). The planozygotes had a weak negative phototactic response and swam vivaciously at first, then gradually slowed down and came to rest on the substratum, becoming spherical (Fig. 10). Within a few hours after settling, zygotes had become completely spherical and their flagella had disappeared. Twenty-four hr after settling, zygotes had developed into spherical bodies, 3.5–4.5 $\mu$m diam., surrounded by thin cell walls and containing two chloroplasts and a stigma. The swimming period of planozygotes was shorter than that of uncopulated gametes, which continued to swim ten hr after liberation. Twenty-four hr after liberation, uncopulated gametes followed the pattern of zygotes in settling and becoming spherical on the substratum, but faded away within a few days. No parthenogenetic reproduction was observed.

6. *Germination and development of zygotes*: The spherical bodies did not immediately produce germ tubes, but continued to enlarge for about one month. Five days after settling, the number of chloroplasts

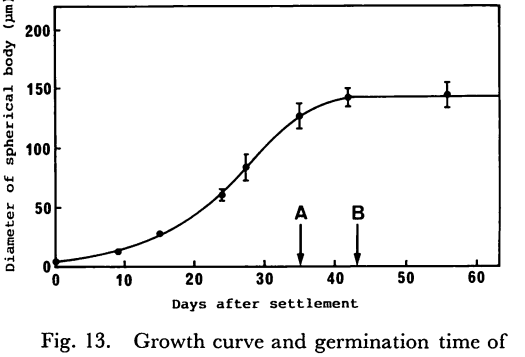
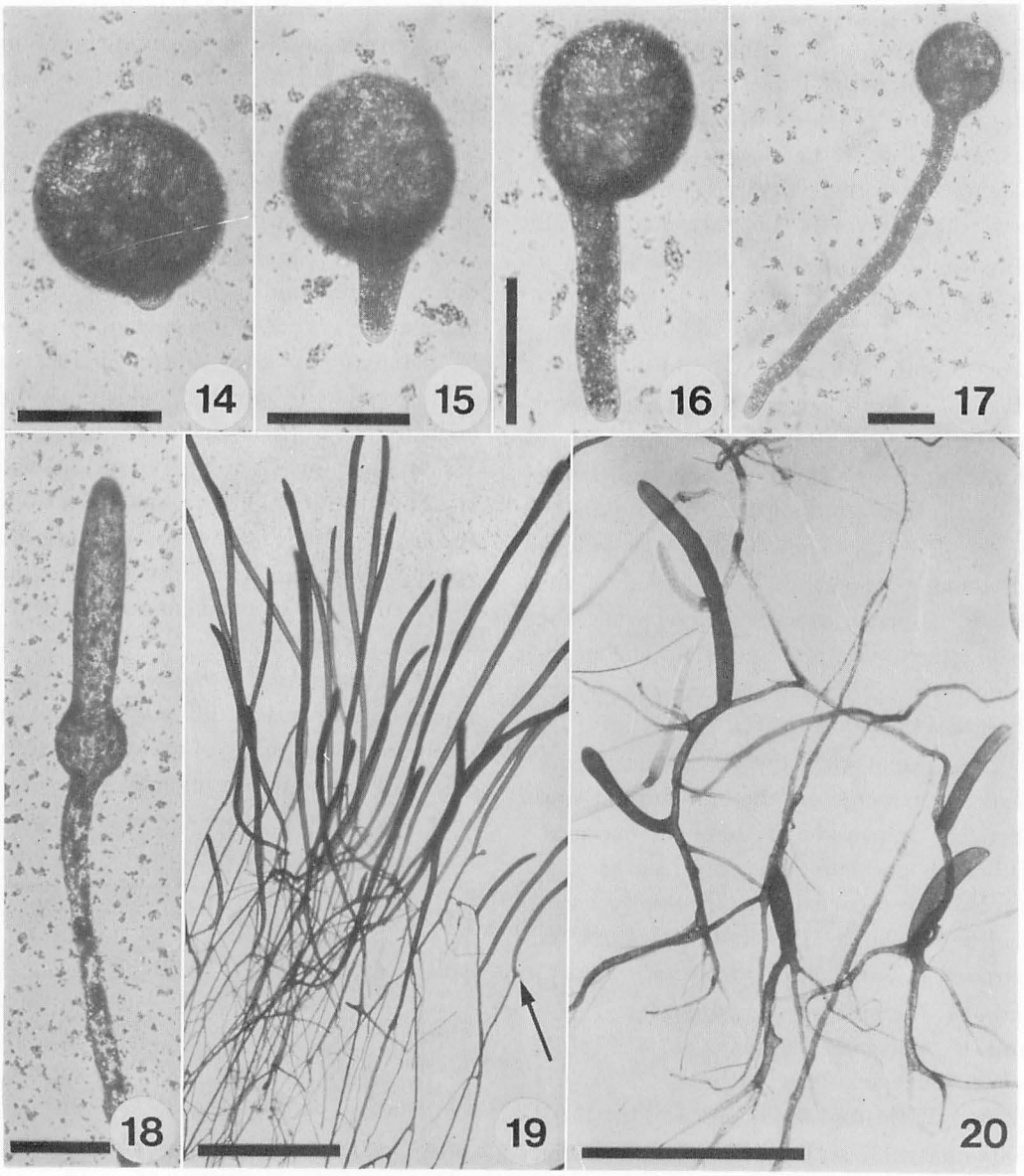



Fig. 13. Growth curve and germination time of spherical bodies of *C. racemosa* var. *laetevirens* under 25.0°C, 3.0 klux, 14L/10D hr cycle. Bars represent standard deviations. A. Beginning of germination. B. Beginning of secondary germ tube formation.

had increased to 3–6, and the stigma had disappeared. After 15 days, the spherical bodies had enlarged further, contained numerous chloroplasts, and appeared to have a distinctive cell wall (Fig. 11). After 24 days, they attained a diameter of 55–65 $\mu$m (Fig. 12). The chloroplasts and other organelles were distributed along the entire inner surface of the spherical bodies.

After about 35 days, the spherical bod-




Figs. 14–20. Germination and development of *C. racemosa* var. *laetevirens*. 14. Primary germ tube formation, 35 days after settling. 15. Primary germ tube elongation, after 37 days. 16. After 39 days. 17. After 42 days. 18. Germling with a fine primary germ tube and a thick secondary one, after 47 days. 19. Protonema-like plants with thin and thick filaments, original cell shown with an arrow, after 2 months. 20. Erect shoots from creeping filaments, after 3 months. Scale: (Figs. 14–17) = 100 $\mu$m, (Fig. 18) = 200 $\mu$m, (Figs. 19, 20) = 5 mm.

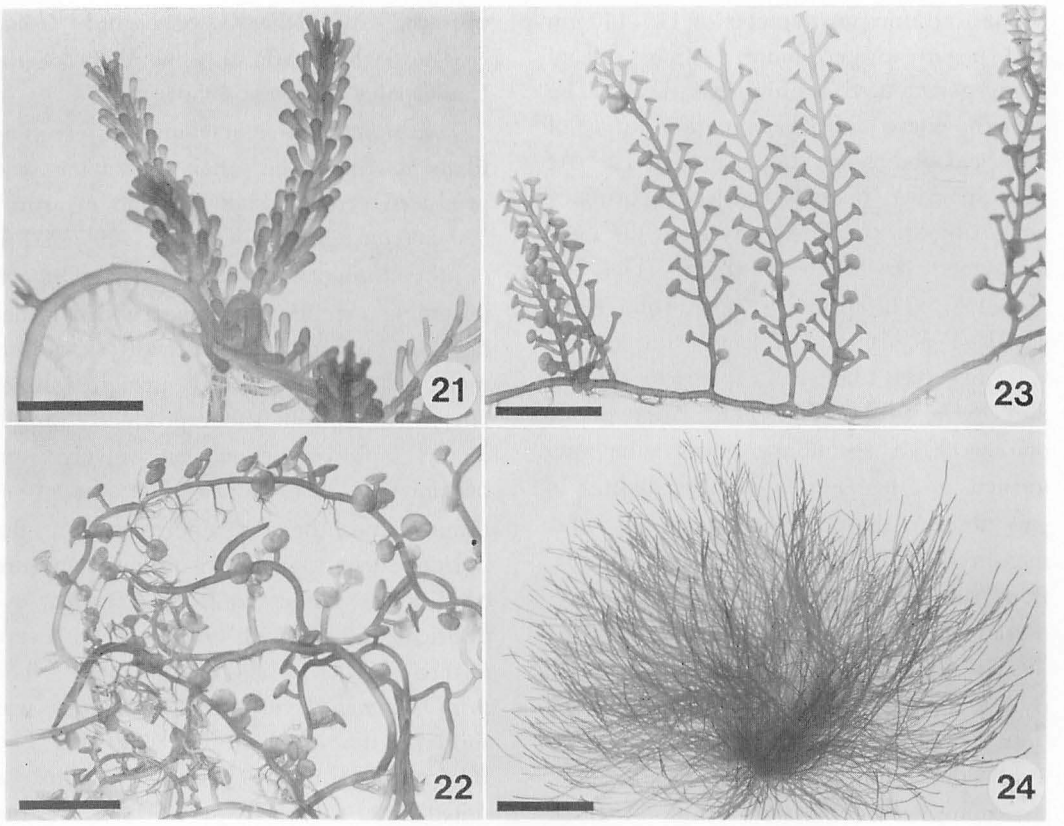
ies had attained a diameter of 115–140 μm and began to germinate. Almost all of them germinated within five days. The growth curve and germination time of spherical bodies are shown in Fig. 13. At first, spherical bodies produced a primary germ tube on the side away from the light measuring 30–40 μm in diam. (Figs. 14 and 15). The primary germ tube elongated (Figs. 16 and 17) and no septum was observed between spherical bodies and germ tubes (Fig. 17). After about one week, a secondary germ tube was formed on the side facing the light. It was thicker than the primary one and measured 90–130 μm in diameter (Fig. 18). Both tubes elongated and branched. About one month after germination, germlings developed into creeping, filamentous, protonema-like plants which consisted of branched thick and thin filaments (Fig. 19). Trabeculae and vigorous protoplasmic streaming were observed inside the thick filaments.

In spite of many culture studies of the spherical stage of *Caulerpa* zygotes, their germination was not reported until recently. In *C. serrulata* (PRICE 1972) and *C. okamurae* (ISHIWARA *et al.* 1981), zygotes germinated bipolarly about seven weeks after settling. The zygotes of the present alga also germinated bipolarly, five weeks after settling. Because the spherical bodies continued to increase their cell volume prior to germination, this period is not considered as dormancy, but as a preparatory step for germ tube formation. In the present alga, a fine primary germ tube was formed on the side away from the light, whereas a thick secondary one was formed on the side facing the light. The developmental sequence, as in the present alga, has not been clarified in other species of *Caulerpa*, but has been observed in *Halimeda tuna* and *Udotea petiolata*, which are also members of the Caulerpales (MEINESZ 1980).

Two months after germination, creeping filaments increased their diameter and produced primary shoots which measured 300–500 μm in diam. (Fig. 20). Most of these shoots continued to elongate, becoming creeping rhizomes which either developed directly into upright shoots or, more usually, produced upright shoots later. An upright shoot produced ramuli at its apical portion successively and became an assimilator. The shapes of ramuli and their arrangement on the upright shoot varied with different culture conditions. Under 20.0°C, 5.0 klux, cylindrical ramuli with obtuse heads were formed in a radial arrangement. The form of well-developed assimilators was similar to that of the mother plant (Fig. 21). By contrast, under 25.0°C, 1.5 klux, a shield-form ramulus was formed at the tip of each erect shoot, the fronds being similar to those of *C. racemosa* var. *peltata* (Fig. 22). Moreover, under 20.0°C, 1.5 klux or 25.0°C, 5.0 klux trumpet-form ramuli were formed alternately on an upright shoot. The well-developed assimilators were not similar to those of the mother plant, but were intermediate between the *laetevirens*-type and the *peltata*-type (Fig. 23).

When zygotes were inoculated in high density, they did not differentiate into thick rhizomes, upright shoots and ramuli, but developed into tufty, sometimes branched, filamentous plants which were similar to *Derbesia* or *Chlorodesmis* (Fig. 24).

PRICE (1972) pointed out that a germling of *C. serrulata* produced a pinnate branch (assimilator) which was very different from the mother plant. ISHIWARA *et al.* (1981), on the other hand, reported that




Figs. 21–24. Plants of *C. racemosa* var. *laetevirens* cultured under different conditions. 21. *Laetevirens*-type plant, cultured under 20.0°C and 5.0 klux, after 6 months. 22. *Peltata*-type plant, cultured under 25.0°C and 1.5 klux, after 6 months. 23. Intermediate-type plant, cultured under 20.0°C and 1.5 klux, after 6 months. 24. Tufty filamentous plant, cultured under 25.0°C and 5.0 klux, after 3 months. Scale: (Figs. 21, 23)=10 mm, (Figs. 22, 24)=20 mm.

the new plants derived from zygotes of *C. okamurae* were similar to the mother plant. In the present alga, germlings derived from the same mother plant produced three types of assimilators according to different culture conditions. It seems that these morphological variations are not caused by genetic polymorphism, but depend on culture conditions. The whole process of assimilator formation and analysis of the morphological variations under various conditions will be detailed in a subsequent article.

7. *Reproduction of cultured plants*: About six months after inoculation, germlings developed into mature plants and became fertile. They produced biflagellate male and female gametes on the same plant, which copulated with each other. The process of gamete formation, gamete liberation, and developmental sequences were similar to those of the mother plant. Quadriflagellate or stephanokontic zooids, which have been reported in the sporophytes of some members of the Bryopsidales (HUSTEDE 1964, RIETEMA 1972, VAN DEN HOEK *et al.* 1972, TATEWAKI 1973, 1977, KOBARA & CHIHARA 1978a, 1978b, 1984, and OKUDA *et al.* 1979), were not observed.

8. *Life history*: In the present alga, the zygotes derived from wild plants developed

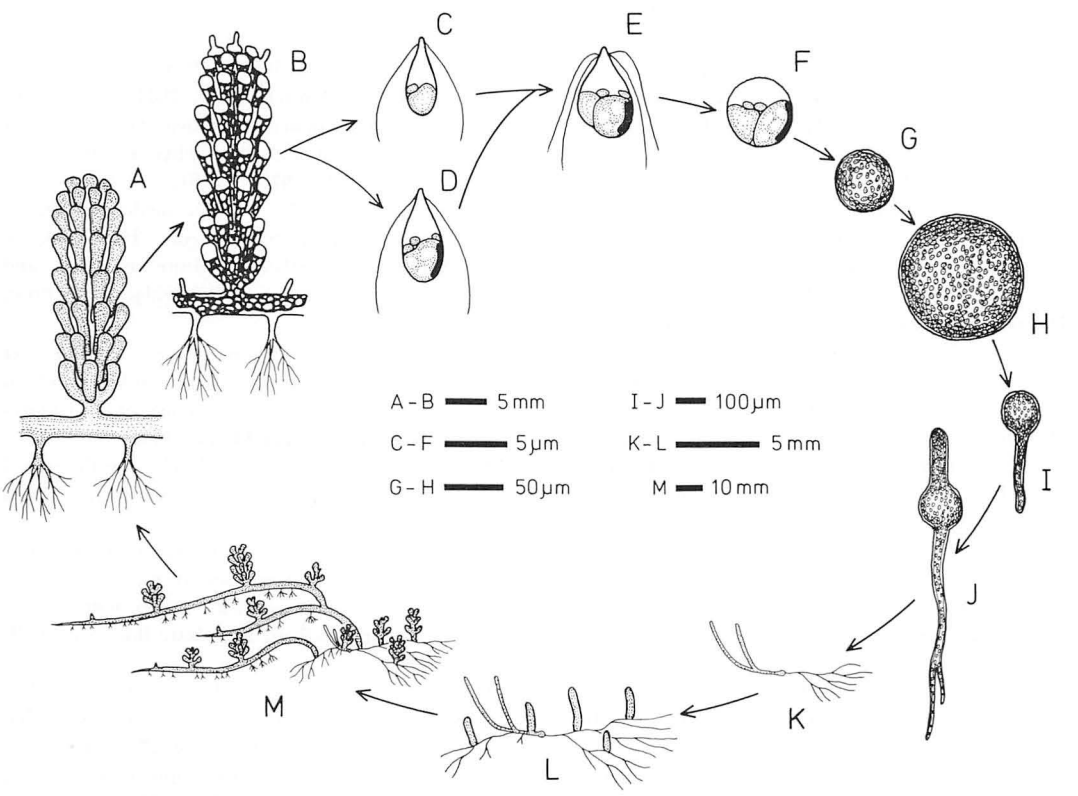



Fig. 25. Life history of *C. racemosa* var. *laetevirens*. A. Vegetative plant. B. Fertile plant with protoplasmic networks. C. Male gamete. D. Female gamete. E. Planozygote. F. Settled zygote. G. Spherical body, 3 weeks after settling. H. Enlarged spherical body, after 5 weeks. I. Germination, germling with a primary germ tube, after 6 weeks. J. Germling with a primary and a secondary germ tube. K. Protonema-like plant. L. Creeping filament with erect shoots. M. Juvenile plant with assimilators.

directly into macroscopic plants which also produced both male and female gametes. The resulting zygotes also developed directly into macroscopic plants. SCHUSSNIG (1939) demonstrated meiosis in gametogenesis of *C. prolifera*. Although the present study lacks cytological observations, a scheme showing the succession of somatic stages in the life history of *C. racemosa* var. *laetevirens* can be drawn (Fig. 25). GOLDSTEIN and MORRALL (1970) suggested the possibility of an alternation of heteromorphic generations in *Caulerpa*, but the present study does not support this idea.

**Acknowledgement**

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**榎本幸人・大葉英雄*：緑藻スリコギヅタの生殖，発生，体形成について**

室戸岬および奄美大島産のスリコギヅタの生殖，発生，体形成を単藻培養により観察した。5～7月に採集した野生体は両産地のものとも25℃，1.0～3.0 klux，14 L/10 D の条件下で1カ月以内に成熟し，同一藻体に2鞭毛の雌雄配偶子を形成する。雌性配偶子は大型で1個の眼点をもち，雄性配偶子は小型で眼点を欠く。雌雄配偶子は接合して接合子となり，基物に定着し球形化する。その後の生育も両産地のものの間で差はなく，球形体は球状のまま肥大生長し，約5週間後に二極的に発芽する。細胞の反光源側に細い第一次発芽管を，次いで光源側に太い第二次発芽管を形成する。発芽管は伸長，分岐し糸状の protonema 様体となり，匍匐茎および直立茎を形成する。直立茎は小枝を形成し直立部に発達する。20℃，5.0 klux の培養条件下で直立部はスリコギヅタ状，25℃，1.5 klux ではタカツキヅタ状，20℃，1.5 klux あるいは25℃，5.0 klux では両者の中間型を示す。4～6ケ月後，藻体は成熟し同一藻体上に2鞭毛の雌雄配偶子を形成する。多鞭毛性の遊走細胞は観察されなかった。(656-24 兵庫県津名郡淡路町　神戸大学理学部臨海実験所。*現住所：108 東京都港区港南4-5-7　東京水産大学植物学教室)

Jap. J. Phycol. (Sôrui) **35**: 178–188, September 20, 1987

# Culture studies on *Caulerpa* (Caulerpales, Chlorophyceae) II. Morphological variation of *C. racemosa* var. *laetevirens* under various culture conditions

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The effects of temperature and light intensity on the morphogenesis of *Caulerpa racemosa* var. *laetevirens* from southern Japan were investigated in unialgal culture under 25 combinations of 5 temperatures (20.0°–30.0°C) and 5 light intensities (0.5–8.0 klux). The morphological variation of the present alga is correlated with environmental factors. The *laetevirens*-type assimilator observed on the mother plants was formed under combinations of low temperatures (20.0°, 22.5°C) and high light intensities (5.0, 8.0 klux). By contrast, under combinations of low and high temperatures (20.0°–30.0°C) and low light intensities (0.5, 1.5 klux) the *peltata*-type assimilator was formed. The intermediate-type between these two types of assimilators was formed under the remaining combinations. The results suggest that *C. racemosa* var. *laetevirens* and *C. racemosa* var. *peltata* are morphological variations (ecophenes or ecads) of a single species.

*Key Index Words*: Caulerpa, Caulerpa racemosa *var.* laetevirens, *Caulerpales, Chlorophyceae, culture, morphogenesis, morphological variation, plasticity, southern Japan alga.*

The genus *Caulerpa* LAMOUROUX is a large group of coenocytic siphonous marine green algae exhibiting a remarkably high degree of morphological variation (plasticity). The thallus is characterized by having a prostrate cylindrical rhizome with branched filamentous attachment rhizoids and assimilators. The assimilator consists of an upright shoot bearing numerous ramuli (branchlets).

The occurrence of intermediate or transitional growth forms between various taxa of *Caulerpa* has often been reported. Moreover, different parts of the same thallus sometimes have features characteristic of two or more taxa. Many investigators have suggested that this morphological variation may be strongly affected by environmental factors (WEBER-VAN BOSSE 1898, SVEDELIUS 1906, BOERGESEN 1907, GILBERT 1942, EUBANK 1946, TAYLOR 1950, 1960, NIZAMUDDIN 1964, REHM and ALMODOVAR 1971).

TANDY (1934) made field transplant experiments on several taxa of *Caulerpa* and offered evidence that *C. peltata* and *C. fastigiata* were only forms of *C. racemosa*. GILBERT (1942) and EGEROD (1975) emphasized that the use of cultures should prove a most valuable aid to systematic studies in the genus. However, there have been few culture studies on the relationship between morphological variation and environmental factors.

PETERSON (1972) transplanted and cul-

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tured wild plants of several taxa of *C. racemosa* in a light-controlled aquarium. He demonstrated that when a single specimen of *C. racemosa* var. *uvifera* was exposed to different light intensities, it formed new assimilators that were characteristic of other taxa. He stated that the ability of *C. racemosa* to change growth form in altered light environments provides evidence for an environmental control of this kind of morphological variability. Moreover, he suggested that the varieties of *C. racemosa*, such as var. *peltata* and var. *lamourouxii*, should be considered ecological phenotypes (ecophenes or ecads). CALVERT (1976) confirmed these results, showing that when five taxa of *Caulerpa* were transferred into aquaria and cultured with a light intensity lower than that typical of their natural habitat, their newly formed ramuli changed in arrangement from radial to bilateral.

As mentioned above, *Caulerpa racemosa* shows an extreme degree of morphological variability, which has led to taxonomic confusion. In a previous paper on culture studies of reproduction and development in *C. racemosa* var. *laetevirens* (ENOMOTO and OHBA 1987), the authors briefly mentioned the morphological variation of this alga. The present paper provides details of assimilator formation under 25 combinations of temperature and light intensity regimes and offers some taxonomic discussion.

## Materials and Methods

Plants of *Caulerpa racemosa* (FORSK.) J. AG. var. *laetevirens* (MONT.) WEBER-VAN BOSSE (Figs. 1A, 1B) were collected at Muroto-misaki (33°16′N, 134°14′E), Shikoku Island and Ayamaru-misaki (28° 28′N, 129°43′E), Amami-ôshima Island in the southern part of Japan. Collections

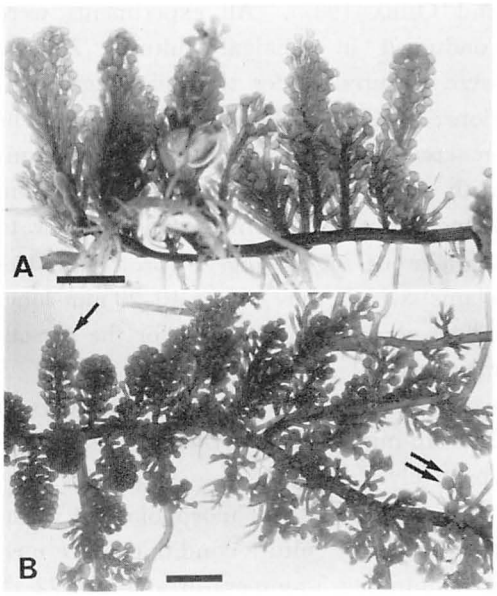


Fig. 1. Wild plants of *Caulerpa raeemosa* var. *laetevirens*. A. Typical plant from low tidal mark at Muroto-misaki; B. Morphologically complex plant from 3.0 m depth at Ayamaru-misaki, with *laetevirens*-type assimilators (single arrow) and *peltata*-type assimilators (double arrow). Scale: 10 mm.

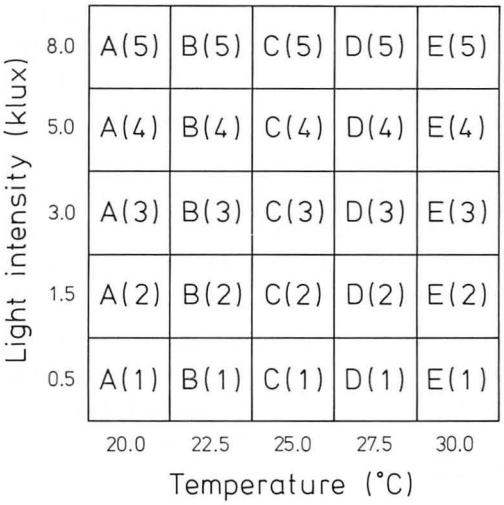


Fig. 2. Schematic representation of the temperature and light intensity gradients used in the present culture experiments. A symbol is given to each combination.

were made during May and July of 1981–1985. The procedures of pre-culture for wild plants and isolation of zygotes are detailed in a previous paper (ENOMOTO

and OHBA 1987). All experiments were conducted in unialgal culture. Zygotes were cultured under the following conditions: 25°C, 1.0–3.0 klux, L:D=14:10 hr in screw-capped glass tubes (18×130 mm) with 15 m*l* of PROVASOLI's ES medium. After about two months, zygotes grew to filamentous germlings (protonema-like plants) which were about 10–20 mm long. These germlings were used for the present experiments. Culture strains A and B were isolated from thalli collected at Muroto-misaki and Ayamaru-misaki, respectively.

For the analysis of morphological variation 25 sets of culture conditions were used by combining 5 temperatures (20.0°, 22.5°, 25.0°, 27.5°, and 30.0°C) with 5 light intensities (0.5, 1.5, 3.0, 5.0 and 8.0 klux). These combinations are represented schematically and each is given a symbol in Fig. 2. A daylength of 14 hr (06:00–20:00) was employed. Toshiba daylight fluorescent lamps (6100 K; FL40SD-SDL) were used as the light source. Light intensity was measured at the beginning and the end of an experiment. When germlings reached about 10 mm in length, each was transferred to a separate glass vessel (90×90 mm) containing 350 m*l* of the same medium (PES). Three vessels were placed under each set of conditions mentioned above. The medium was changed every two weeks. After 4 months, the germlings were analyzed as to assimilator morphology.

The wild plants of *C. racemosa* var. *laetevirens* from Muroto-misaki (Fig. 1A) and Ayamaru-misaki were found to agree well with the description given by OKAMURA (1913, 1936). The rhizome is cylindrical, branched, and intricate, 1.0–3.0 mm in diameter, 10–30 (–50) cm in length. The upright shoots are cylindrical, simple or rarely branched distally, 20–60 (–100) mm in height, bearing 20–70 (–100 or more) imbricate ramuli. The ramulus is cylindrical with an obtuse or somewhat swollen head, 1.2–6.0 (–8.5) mm in length, 0.5–2.0 mm in diameter at its head, 0.3–0.9 mm in diameter at its base.

On the Pacific coast of Japan, this alga is common between Bôsô Peninsula (35°N, 140°E) and Amami-ôshima Island (28°N, 129°E), but is rare on Okinawa Island (26°N, 128°E) (SEGAWA 1935, OKAMURA 1936, YAMADA and TANAKA 1938). It is usually found on sunny rocks in the lower intertidal zone, but sometimes it grows at a depth of 4–5 m on the waveswept outer margin of the reef where light intensity is relatively high.

Sometimes morphologically complex plants were found (Fig. 1B) in which *laetevirens*-type assimilators were produced by rhizomes exposed to the sun while *peltata*-type assimilators were produced in the shade. Such plants grow at a sun/shade interface, as in hollows and on the undersurface of overhanging rocks in shallow water.

## Results

*Experiments on strain A*

1. Cross-gradient experiments:

The germlings that had been cultured for two months under 25 different sets of conditions began to form assimilators. After four months, the assimilators were sufficiently mature to allow a morphological analysis to be carried out. The morphology of assimilators varied with the culture conditions (Figs. 3 and 5A).

Three aspects of the assimilators were considered for morphological analyses: shape of the ramuli, arrangement of the ramuli on the upright shoot, and overall appearance. In all instances the rhizomes

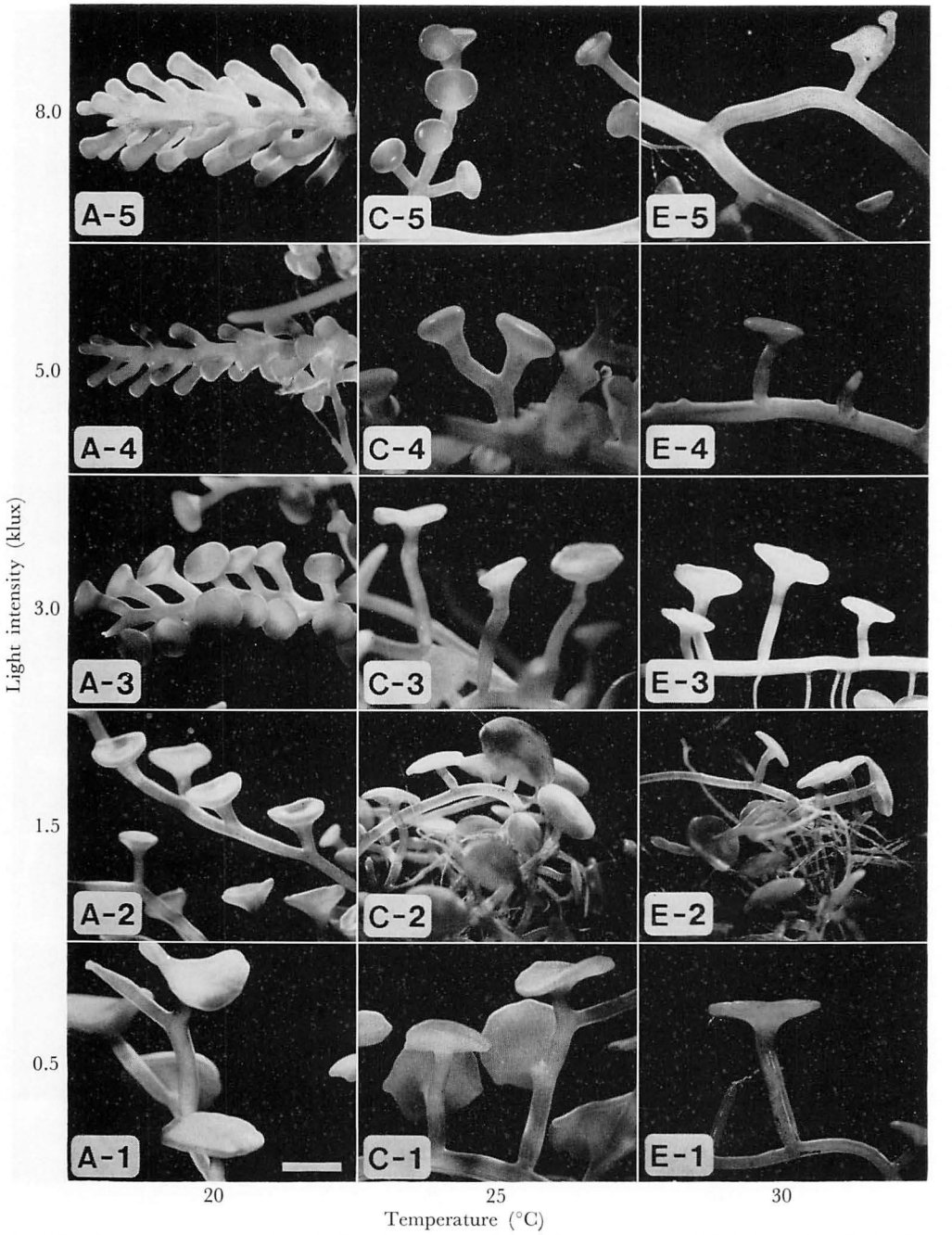


Fig. 3. Morphogenetic response of strain A to the cross-gradients of temperature and light intensity. Scale: 2 mm.

and upright shoots were cylindrical.

Three types of assimilators were recognized:

1) *Laetevirens*-type: The ramuli are cylindrical with obtuse heads and are arranged in a polystichous pattern (tristichous, decussate, or spiroscalate phyllotaxis) on the upright shoot. These two characters appear to be linked. The overall appearance of the assimilators is similar to that of the mother plants. This type developed under 3 sets of conditions, A(4,5) and B(5), i.e.,

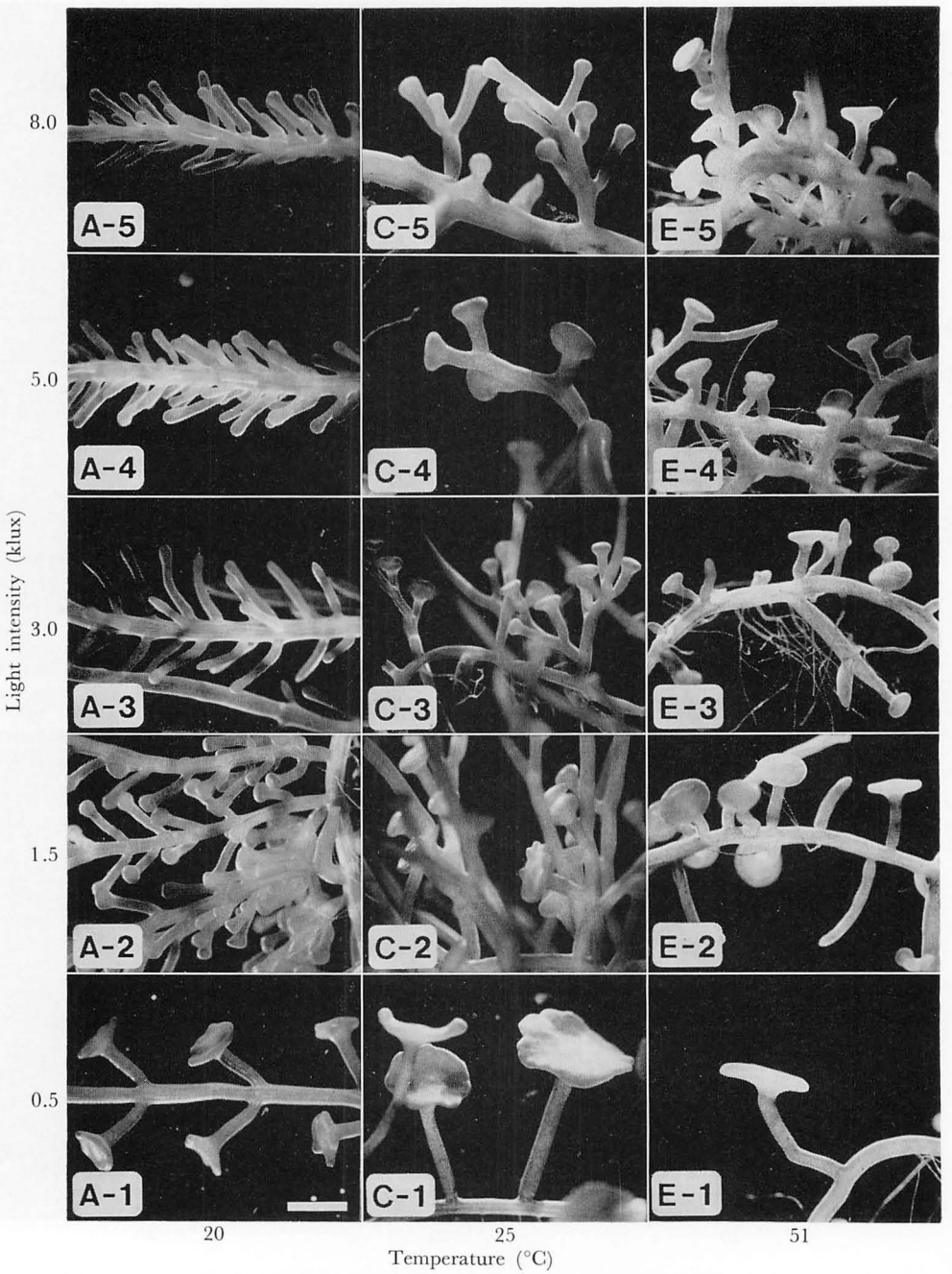


Fig. 4. Morphogenetic response of strain B to the cross-gradients of temperature and light intensity. Scale: 2 mm.

low temperature and high light intensity. This type is shown in Fig. 3 and is represented by the symbol L (C P) in Fig. 5A.

2) *Peltata*-type: The ramuli have the form of a shield, with flat discoidal heads, and are arranged in a monostichous pattern (solitary or secund) on the upright shoot or the rhizome. The shape of the ramuli was linked to their arrangement on the upright shoot. The overall appearance of the assimilators was similar to that of

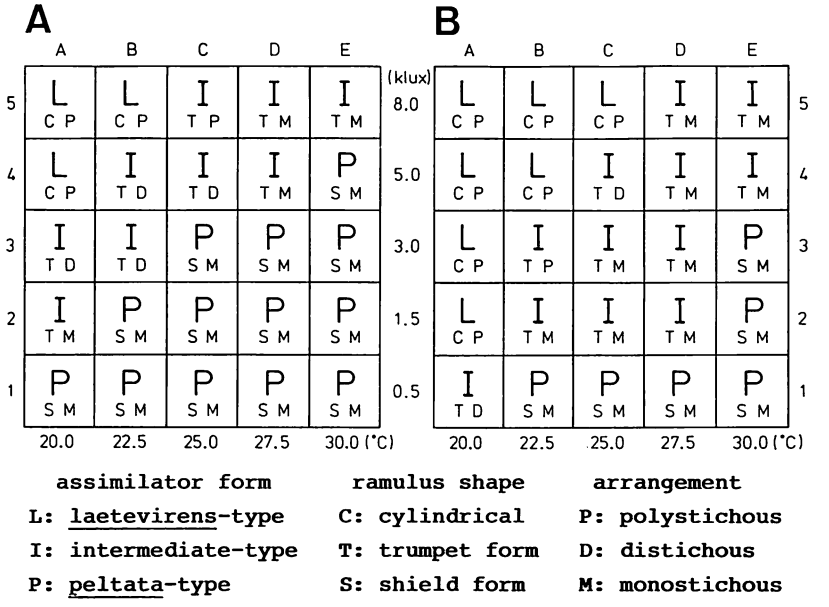


Fig. 5. Diagrammatic representation of the morphogenetic response to the cross-gradients of temperature and light intensity. A. Strain A, B. Strain B.

*C. racemosa* var. *peltata*. This type developed under 13 sets of conditions, A(1), B(1,2), C(1,2,3), D(1,2,3) and E(1,2,3,4), i.e., from low to high temperature and low light intensity. This type is shown in Fig. 3 and is represented by the symbol P (S M) in Fig. 5A.

3) Intermediate-type: The ramuli are trumpet-form with obconical heads. The arrangement of the ramuli on the upright shoots or the rhizomes varied with the culture conditions, allowing the recognition of three kinds of intermediate-type assimilators, as follows:

(a) Intermediate-type assimilator with polystichous arrangement of ramuli. This kind developed under only one set of conditions: C(5), i.e., moderate temperature and high light intensity. It is shown in Fig. 3 and is represented in Fig. 5A by the symbol I (T P).

(b) Intermediate-type assimilator with distichous (opposite or alternate) arrangement of ramuli. This kind developed under 4 sets of conditions: A(3), B(3,4) and C(4), i.e., low temperature and moderate light intensity. It is shown in Fig. 3 and is represented in Fig. 5A by the symbol I (T D).

(c) Intermediate-type assimilator with monostichous arrangement of ramuli. This kind developed under 4 sets: A(2), D(4,5) and E(5), i.e., low temperature and low light intensity or high temperature and high light intensity. It is shown in Fig. 3 and is represented by the symbol I (T M) in Fig. 5A.

The formation of assimilators was inhibited at 30.0°C, the assimilators being dwarfed.

2. Transplant experiment:

When a thallus that had formed *laetevirens*-type (polystichous arrangement) assimilators under 22.5°C, 5.0 klux was transferred and cultured under 20.0°C, 0.5 klux for a month, it formed intermediate-type assimilators on its rhizomes and upright shoots (Fig. 6A).

*Experiments on strain B*

1. Cross-gradient experiments:

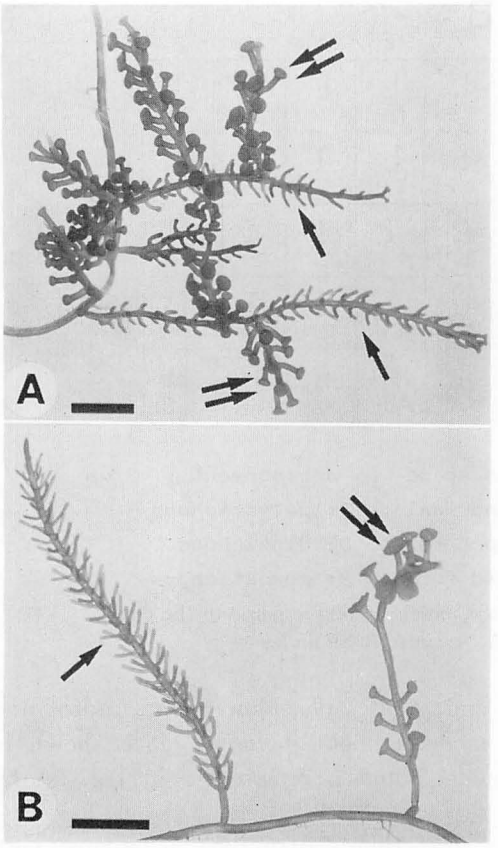


Fig. 6. Transplant experiments. A. Plant (strain A) with *laetevirens*-type assimilators (single arrows) produced in culture under 22.5°C, 5.0 klux after being transplanted to 20.0°C, 0.5 klux. Note newly formed intermediate-type assimilators with trumpet-form ramuli (double arrows). B. Plant (strain B) with a *laetevirens*-type assimilator (single arrow) produced in culture under 20.0°C, 3.0 klux after being transplanted to 22.5°C, 0.5 klux. Note newly formed *peltata*-type assimilators (double arrow). Scale: 10 mm.

The form and arrangement of ramuli and the overall appearance of assimilators produced in strain B under each of the 25 sets of conditions corresponded essentially with those of strain A, but with several minor exceptions. *Laetevirens*-type assimilators occurred under six rather than three sets of conditions and typical *peltata*-type assimilators appeared in only three rather than eight sets of conditions. Results are shown in Figs. 4 and 5B.

2. Transplant experiment:

When a thallus that had formed *laetevirens*-type assimilators under 20.0°C, 3.0 klux was transferred and cultured under 22.5°C, 0.5 klux for a month, it formed *peltata*-type assimilators on the original rhizome (Fig. 6B).

*Process of assimilator formation*

The development of assimilators of *laetevirens*-type, intermediate-type (with distichous arrangement), and *peltata*-type is shown in Figs. 7 and 8.

1) *Laetevirens*-type: A conical erect shoot was formed on a creeping rhizome (Fig. 8A-a). After 5–7 hr, a pair of tiny whitish outgrowths were formed opposite one another a little below the apex (growing point) of the shoot (Figs. 7A, 8A-b). After about 10 hr, each outgrowth elongated and became an acute, cylindrical, lateral branchlet which curved upward slightly (Figs. 7B, 8A-c,d). Subsequently, it continued to elongate, attaining a size of 0.5 mm diam. × 2.0–4.0 mm long (Figs. 7C, D, E, 8A-e, f). After about 60 hr, its tip enlarged and developed into a cylindrical ramulus with an obtuse head (Figs. 7F, 8A-g). About 4–5 hr after the appearance of the primary pair of outgrowths, a secondary pair was produced a little below the apex of the upright shoot, i.e., a little above the primary pair. It was formed at an angle of 60°–90° to the primary pair in a horizontal plane (Figs. 7B, 8A-d, e). After about 60–70 hr, the secondary pair of outgrowths developed into ramuli (Figs. 7F, 8A-h). The formation of successive ramuli proceeded in the same sequence (Figs. 7G, 8A-i). About one week after the appearance of the erect shoot, the assimilator had grown to a height of 10–20 mm (Fig. 7H). After 2 weeks, it had grown to 25–30 mm.

2) Intermediate-type with trumpet-form

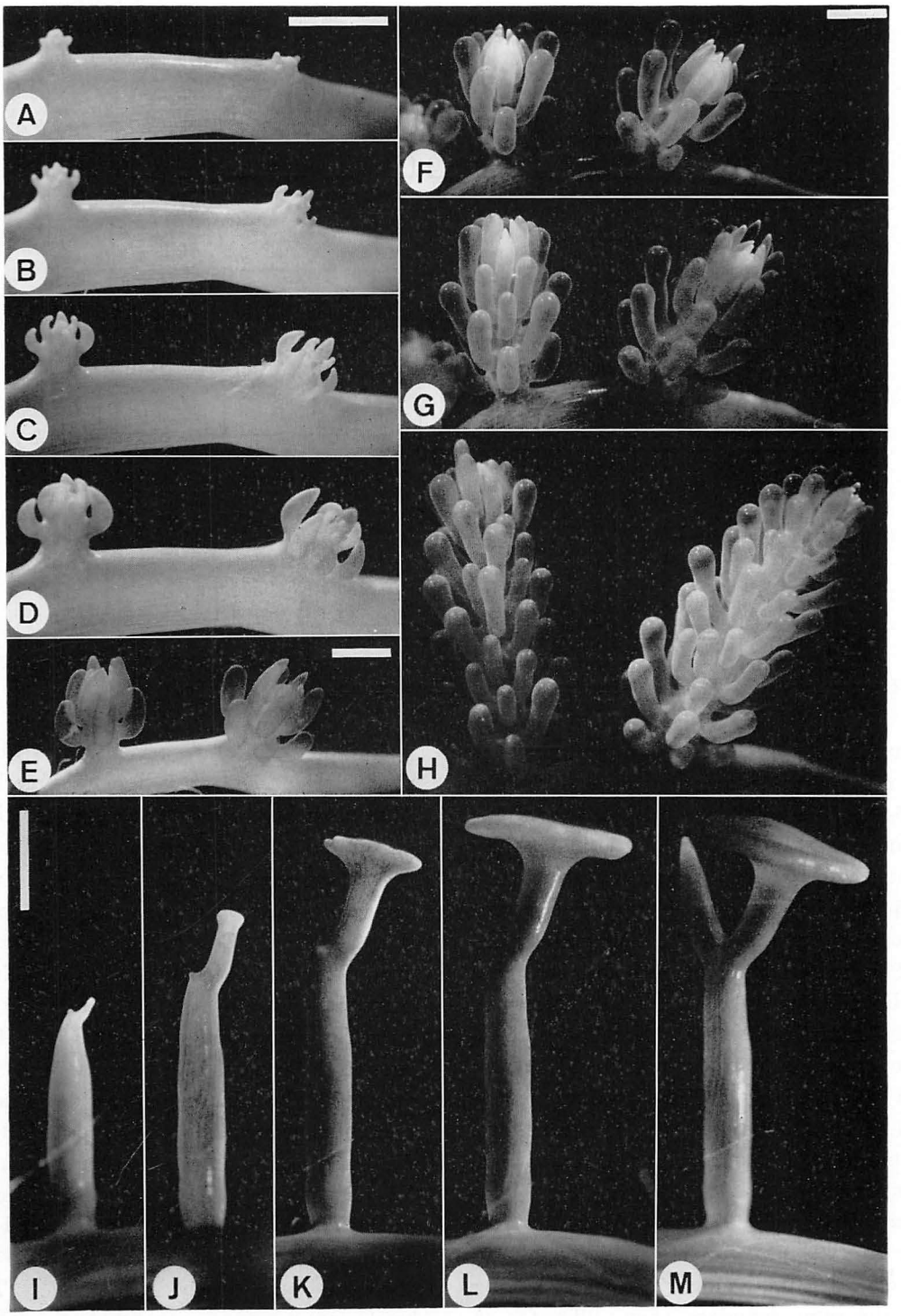


Fig. 7. The process of assimilator formation. A–H. *Laetevirens*-type, I–M. *Peltata*-type. Scale: 2 mm.

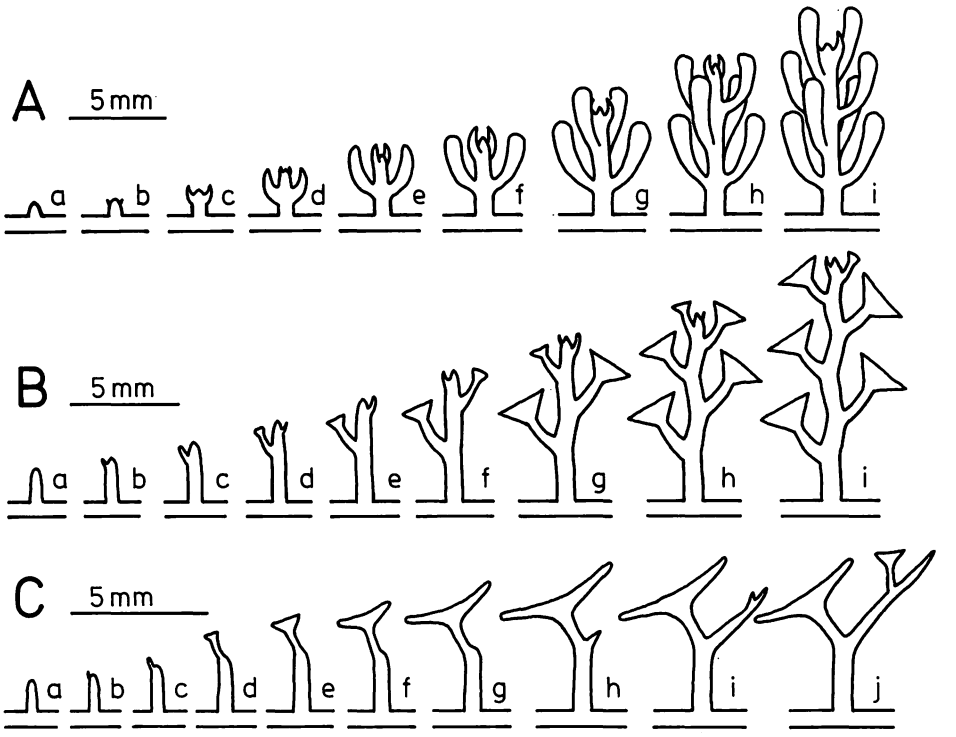


Fig. 8. Schematic representation of the process of assimilator formation. A. *Laetevirens*-type, B. Intermediate-type with trumpet-form ramuli arranged distichously, C. *Peltata*-type.

ramuli: An erect shoot was formed on a creeping rhizome and elongated (Fig. 8B-a). When it grew to a length of 2–3 mm, it produced laterally a tiny whitish outgrowth a little below its apex (Fig. 8B-b). The outgrowth appeared in the morning (around 08:00). After about 10–12 hr, it elongated upward and became a cylindrical protuberance which measured 0.5–1.0 mm in length (Fig. 8B-c). Subsequently, its apex expanded and became an obconical head (Figs. 8B-d, e). After 36–48 hr, it developed into a trumpet-form ramulus which measured 2–3 mm in diameter (Figs. 8B-f, g). During the formation of the primary ramulus, the apex of the erect shoot (growing point) continued to elongate, producing a secondary ramulus in the same sequence (Figs. 8B-e, f). A tertiary ramulus and successive ramuli were formed in the same way (Figs. 8B-g, h, i). The ramuli are arranged alternately or nearly opposite one another. After about two weeks, the assimilator had grown to 20–25 mm in height.

3) *Peltata*-type: First an erect shoot was formed on the upper surface of a creeping rhizome (Fig. 8C-a). When it grew to about 2 mm in length, a tiny whitish outgrowth was formed a little below its apex (growing point) (Fig. 8C-b). This outgrowth appeared in the morning (around 08:00), after which the erect shoot ceased to elongate. After about 7–8 hr, the outgrowth had attained a length of 0.5–1.0 mm (Figs. 7I, 8C-c). Subsequently, it thickened (Figs. 7J, 8C-d) and its apical portion began to expand into an obconical head (Figs. 7K, 8C-e). The head continued to expand horizontally, becoming a disk measuring 2.0–3.0 mm in diameter (Figs. 8C-f, g). After about

36 hr, the outgrowth had developed into a primary peltate or shield-form ramulus and its head measured 4.0–5.0 mm in diameter (Figs. 7L, 8C-h). Sometimes, when a primary ramulus was completed, the apex of the erect shoot began to elongate again (Figs. 7M, 8C-h, i), producing a secondary and a tertiary ramulus in the same sequence (Figs. 8C-i, j).

## Discussion

The present experiments reveal that the form and arrangement of ramuli in *Caulerpa* may vary considerably with temperature and light intensity. They prove that the morphological plasticity of the present alga is correlated with environmental factors rather than with genetic polymorphism. All cross-gradient experiments were carried out using thalli derived from the same plant at the same time. As shown in Figs. 6A and 6B, when a thallus was transferred into a different set of conditions it developed assimilators of a different shape. The fact that the results of the cross-gradient experiments in strain A are almost identical to those in strain B suggests that morphological plasticity is not the expression of a single genotype and that similar results could be expected if other populations of *C. racemosa* var. *laetevirens* were investigated.

Peterson (1972) reported that when wild plants of several taxa in *C. racemosa* were transferred into an aquarium, they changed their form in response to different light conditions, and that *C. racemosa* var. *uvifera* f. *intermedia*, after being transferred to high light (21.0 klux), produced *laetevirens*-type assimilators, which were not found on the original plants. Calvert (1976) also showed that when wild plants of five taxa in *Caulerpa* were kept under low light, the arrangement of their ramuli varied from radial to bilateral. In the present study, at 20.0° and 22.5°C, the assimilators varied in form with increasing light intensity from *peltata*-type with shield form ramuli through intermediate-type to *laetevirens*-type with cylindrical ramuli. These results suggest that the *laetevirens*-type assimilator is adapted to high light intensity and that the *peltata*-type is adapted to a lower light intensity. Svedelius (1906) thought that radial arrangement was adapted to high light and bilateral arrangement to low light. In the present study, a polystichous arrangement was favored by low temperature and high light intensity.

In the field, *laetevirens*-type assimilators were observed on typical plants of *C. racemosa* var. *laetevirens* growing on sunny rocks in shallow water and on morphologically complex plants growing on sunny rocks in hollows. *Peltata*-type assimilators, on the other hand, were found on typical plants of *C. racemosa* var. *peltata* in deep water and on morphologically complex plants growing on shady rocks in hollows and on the undersurface of overhanging rocks. Light intensity is probably a factor affecting vertical distribution of the three types of assimilators.

In the present experiments, the *laetevirens*-type assimilator appeared at relatively low temperatures (20.0°, 22.5°C), but did not appear at relatively high temperatures, whereas the *peltata*-type appeared at low and high temperatures (20.0°–30.0°C). These results correspond with field observations that plants with typical *laetevirens*-type assimilators are common in relatively cold water at Murotomisaki and Ayamaru-misaki, but were not observed in warm water at Okinawa Island. Seawater temperature is probably a factor in the geographical distribution of these two types of assimilators. The exist-

ence of morphologically complex plants and the results of the present experiments suggest that *C. racemosa* var. *laetevirens* and *C. racemosa* var. *peltata* are probably ecophenes (ecads) of a single species.

## Acknowledgement

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**大葉英雄*・榎本幸人：緑藻スリコギヅタの各種設定条件下での発現形態について**

室戸岬および奄美大島産のスリコギヅタの単藻培養体を用い，温度および光照度が形態発現に及ぼす影響を調べた。20℃～30℃の5温度と 0.5～8.0 klux の5照度を組み合わせ，25の実験区を設定した。低温・高照度（20℃，8.0 klux）では母藻体と同様のスリコギヅタ型直立部が，高温・低照度（27.5℃，0.5 klux）では母藻体とは全く異なるタカツキヅタ型直立部が形成される。他の実験区では両者の中間的あるいは移行的な形態を示す直立部が発現した。本藻の著しい形態的変異は遺伝的多型性によるものではなく，生育環境条件によるものと考えられる。またスリコギヅタ，タカツキヅタは生育環境によって生じる同種のエコフェーン（エケード）である可能性が示唆される。（656-24 兵庫県津名郡淡路町　神戸大学理学部臨海実験所。*現住所：108　東京都港区港南4-5-7　東京水産大学植物学教室）

Jap. J. Phycol. (Sôrui) **35**: 189–200. September 20, 1987

# Developmental process of the gametangium in *Pseudobryopsis hainanensis* TSENG (Codiales, Chlorophyceae)

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The developmental process of the gametangium in *Pseudobryopsis hainanensis* was investigated by synchronized culture using light- and electron-microscope. Cytoplasm in ramelli periodically migrates upward and downward in the vegetative stage, accumulating at ramellus bases during dark periods. In the reproductive stage, the cytoplasm aggregates by upward streaming at the site where gametangium formation is expected. Gametangium formation starts by protrusion of the ramellus wall near the aggregated cytoplasm. The gametangium continues to expand for 12 hrs after protrusion. During this period many interphase nuclei migrate from a ramellus into the developing gametangium. A papilla is formed at the distal end of the gametangium. The wall around the papilla is stained by dyes identifying proteins. A plug which isolates the cytoplasm of the gametangium from that of the ramellus is formed in the orifice present in the basal constriction of the gametangium. When a large vacuole occupies the basal portion of the gametangium, nuclei begin to divide for gamete formation.

*Key Index Words*: *coenocytic green alga*; *gametangium development*; Pseudobryopsis hainanensis; *synchronized culture.*

Ultrastructural investigations have contributed to our understanding of reproductive differentiation in coenocytic green algae (BURR and WEST 1970, MARCHANT and PICKETT-HEAPS 1970, 1971, WHEELER and PAGE 1974, HORI and ENOMOTO 1978). These investigations deal only with those species in which the vegetative cells directly differentiate into the reproductive organ. For instance, when the thallus of *Bryopsis hypnoides* attains reproductive maturity, the side branch is converted into a gametangium by itself (BURR and WEST 1970). The ramellus in *Pseudobryopsis* is morphologically similar to the side branch in *Bryopsis* but the former produces a gametangium on its outer surface. However the ultrastructure in developmental stages of the aforementioned gametangium remains obscure.

In *Pseudobryopsis hainanensis* the main axis is clavate-cylindrical in shape and is surrounded by dense radially arranged ramelli. It originates from rhizome-like creeping filaments at the basal portion. The gametangium born near the proximal portion of the ramellus is provided with a papilla at the distal end and a plug at the base (OKUDA *et al.* 1979). Therefore it is assumed that several morphogenetic regulatories such as positional control of gametangium, local differentiation for papilla and plug formation proceed. The present study clarifies the developmental

process of the gametangium in *P. hainanensis* through light and electron microscopic observations.

## Materials and Methods

*Experimental organism*

Gametophytic plants of *Pseudobryopsis* (mainly clone MK-065*) were used in the present study. This strain, clone MK-065, was obtained at Ayamaru Point, Amami Oshima, Japan on June 2, 1977 and then maintained as unialgal stock culture grown in artificial medium $ASP_{12}$ (PROVASOLI, 1963) at 22°C and 14:10 hr L:D cycle at the Institute of Algological Research, Hokkaido University at Muroran. The gametophytic plants in some experiments were newly collected at the same habitat on May 22–23, 1985 (Fig. 1a). The thalli from these cultured plants were morphologically quite similar to those of the clone MK-065 grown in culture (Fig. 1b).

*Culture*

Methods of culture and induction of gametangium formation were essentially the same as those described by OKUDA and TATEWAKI (1982). Briefly, plants, precultured at 22°C and 14:10 hr L:D cycle for 3–4 weeks, were re-cultured in a fresh modified medium at 24°C and continuous light. Groups of plants under the same preculture condition synchronously formed gametangia (OKUDA and TATEWAKI 1982). Developmental stages of the gametangia were defined by hours after the beginning of light regime independent of the time when the plants were induced. In the present study, plants were fixed and observed at 3 hr intervals, commencing at the beginning of continuous light regime.

*Specimen preparation*

Plants were fixed in 0.5% glutaraldehyde

* Although this strain has been reported as *Pseudobryopsis* sp. in our previous papers (OKUDA *et al.* 1979, OKUDA and TATEWAKI 1982), we describe here our strain as a synonym of *Pseudobryopsis hainanensis* TSENG (1936) in accordance with the opinion of KOBARA and CHIHARA (1978), working on the specimen from the same habitat where we collected the material.

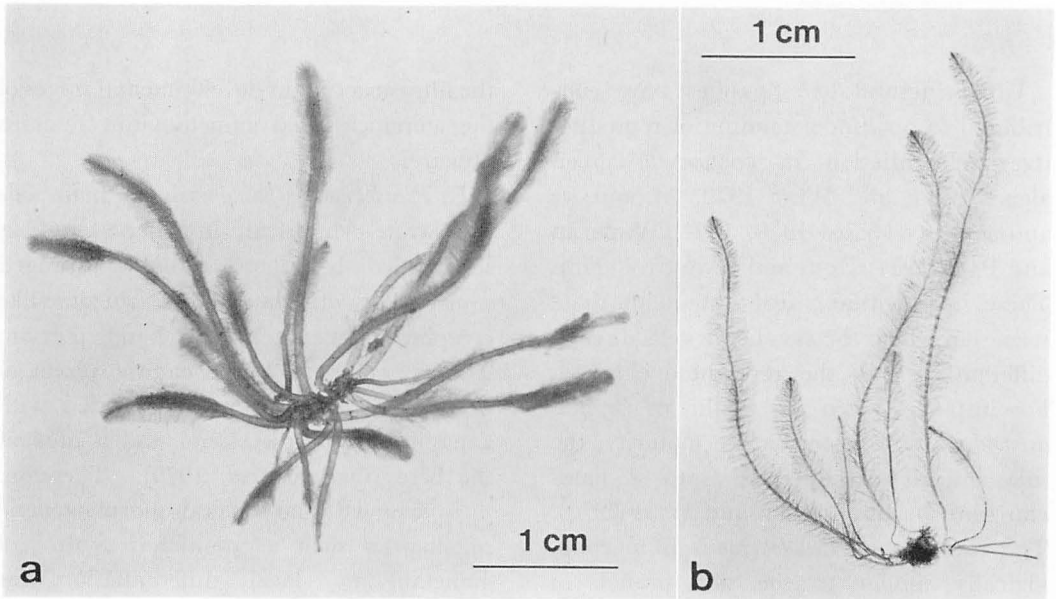



Fig. 1. *Pseudobryopsis hainanensis* TSENG. collected at Amami Oshima Island in 1985 (**a**) and some of them cultured for a month (**b**).

in 10 m*l* culture medium for 5 min. This was followed by 5% glutaraldehyde treatment in 0.1 M sodium cacodylate at pH 7.2 with 50% major salt solution (0.451 M NaCl, 0.052 M $MgSO_4$, 0.018 M $MgCl_2$, 0.008 M KCl and 0.009 M $CaCl_2$) for 25 min at room temperature, and subsequently for 1.5 hr at 5°C. After rinsing briefly in 50% major salt solution, the specimen was washed in a series of 50, 40, 30, 20, 10% major salt concentrations. Then the plants were postfixed with 2% $OsO_4$ in 0.05 M sodium cacodylate buffer at pH 7.2, for 2 hr. The lateral ramelli were cut off at the base with dissection scissors in cold pure water. These ramelli were dehydrated slowly with an acetone series by 10% increments until 80% at 5°C, then placed at room temperature which followed complete dehydration with absolute acetone. The material dehydrated in fresh absolute acetone was first added dropwise to 1/10 resin diluted with acetone for 2 hr to prevent collapse of cells. Then it was put in a small vial (15 mm × 40 mm) containing 3 m*l* of 1/5 concentration of resin and placed in a sealed box containing 3–4 g well dried Silica gel on a rotator (Penetron Mark IV, Sunkay Laboratories Inc.). After complete evaporation of acetone from this resin mixture, several changes of 100% resin were followed to obtain proper penetration. Finally the resin was polymerized at 70°C for 1 day.

Thin sections were cut with a diamond knife on a Solvall Porter-Blum MT-1, mounted on formvar-coated slot mesh grids, stained with saturated uranyl acetate solution in 50% ethanol for 5 min, and then with lead citrate (Reynolds 1963) for 7 min, and examined in a Hitachi H-300 electron microscope.

Some materials were preserved in 50% ethanol solution at 5°C for several days after fixation with glutaraldehyde and subsequent brief rinsing. These were then stained by either 1% acid fuchsin, 1% amidoblack or 0.5% fast green FCF on a glass slide. Some were treated with 1% pepsin or 1% protease for 30 min before staining.

## Result

*Gametangium formation*

The first visual indication of gametangial differentiation in *Pseudobryopsis hainanensis* is a local accumulation of the cytoplasm around the central vacuole below the apex of a ramellus at 0–9 hr (Figs. 2c, 4a and 4b). Many chloroplasts are seen in this area,

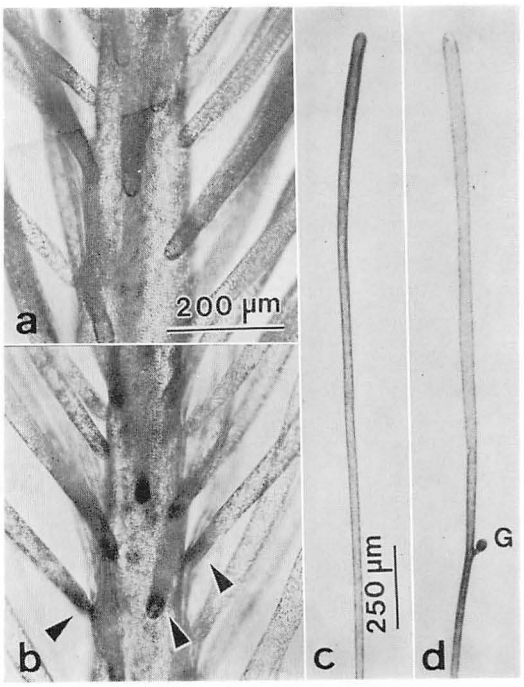



Fig. 2. Changes of cytoplasmic distribution in ramellus. Ramellus bases during light (**a**) and dark period (**b**) in vegetative stage. Arrowheads indicate accumulation of cytoplasm during dark period. Ramelli at 9 hr (**c**) and 18 hr (**d**) of reproductive stage. G, gametangium protruding at the lower part of ramellus. Scale bars in **a** and **c** apply to **b** and **d** respectively.

but the number decreases toward the base. They contain no starch or small grains, if present, until 3 hr (Fig. 4a), but large ones at about 6 hr (Fig. 4b). Then, at 12–15 hr the aggregated cytoplasm gradually migrates downward and accumulates at ramellus bases as that during the dark period in the vegetative stage (Fig. 2b). At 18–21 hr, the cytoplasm (Fig. 3a) migrates a little above the ramellus bases where gametangium formation initiates (Fig. 2d).

The surface view of the initiation of gametangium looks like a hyaloid circle (Fig. 3b), and its lateral view like a convex lense (Fig. 3c). The cytoplasm of this portion is fenestrated due to ER (endoplasmic reticulum) and vacuolar evagination (Fig. 4d) and contains many nuclei (Fig. 4c). Most of the chloroplasts are limited to the regions adjacent to a central vacuole. At about 21 hr the gametangium initial begins to protrude upward (Figs. 3d and 5a), many small vesicles are produced in the cytoplasm just below the wall of the gametangium (Fig. 5d). At 24 hr, vacuoles in the cytoplasm gradually enlarge (Fig. 5d), concomitant with the expansion of a gametangium initial (Fig. 3e) which develops a stalk (Figs. 3f and 3g). At 27 hr, a large vacuole occupies the center of the gametangium and its ramifications intrudes the peripheral cytoplasm (Fig. 5b). At 30 hr, the gametangium completely expands (Fig. 3g) and a gametangial central vacuole shifts towards the basal part (Fig. 5c). The cytoplasm is divided

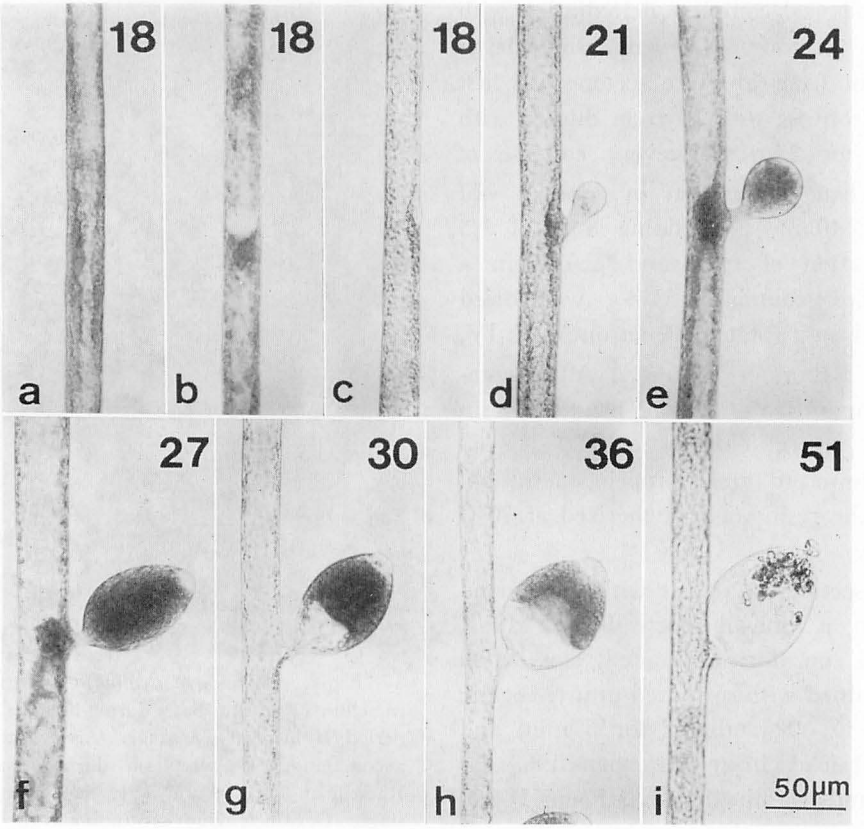



Fig. 3. Light micrographs showing developmental stages of a gametangium. Numerals at the upper right side show the time of the development in hr. Scale bar in **i** applies to all figures.

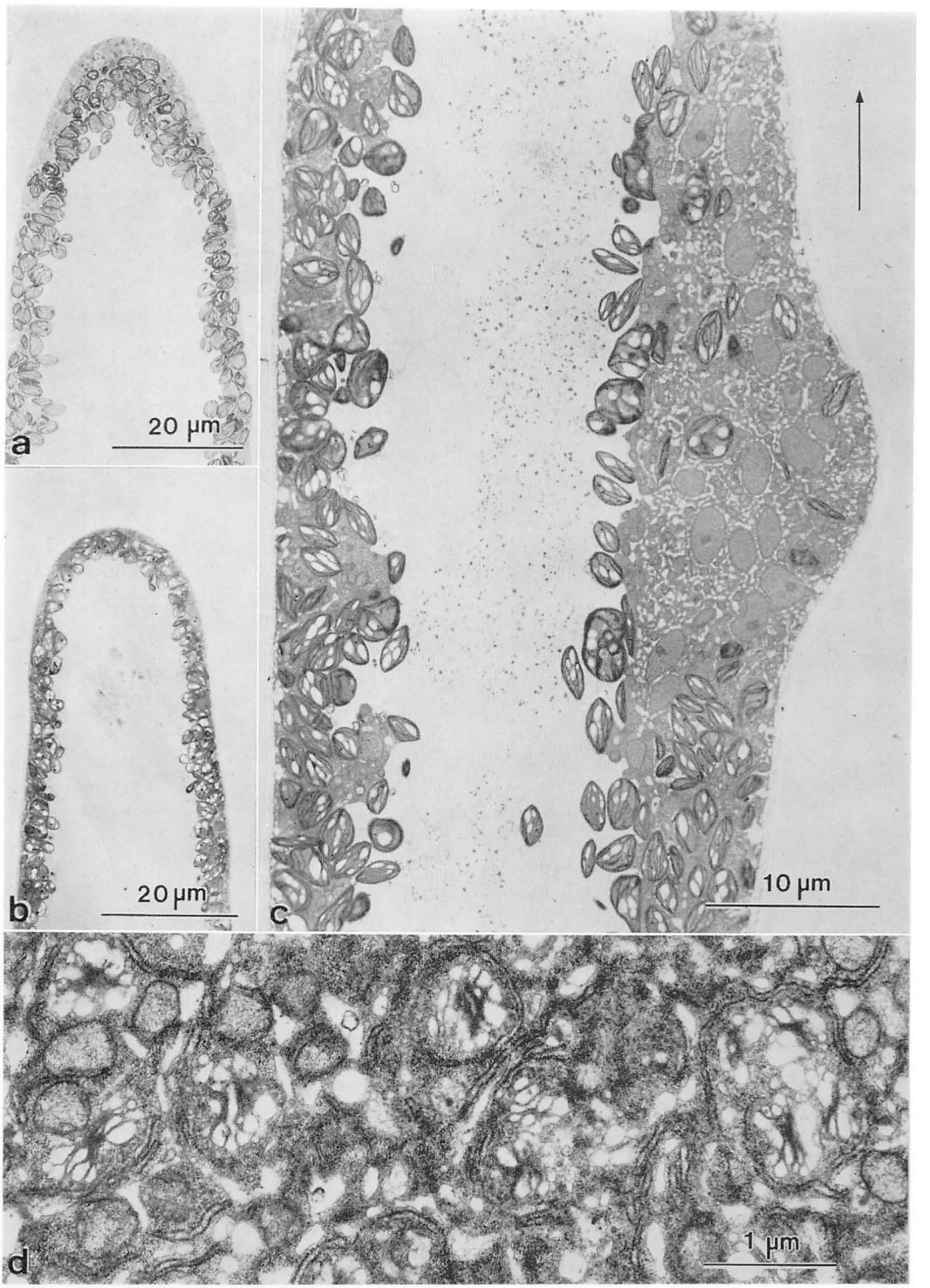



Fig. 4. Electron micrographs of longitudinal sections of ramellus. Apical portion at 03 hr (**a**) and 06 hr (**b**), cytoplasmic aggregation (**c**) and reticulated cytoplasm (**d**) at the site where gametangium formation is expected at 18 hr. Arrow indicates direction of ramellus apex.

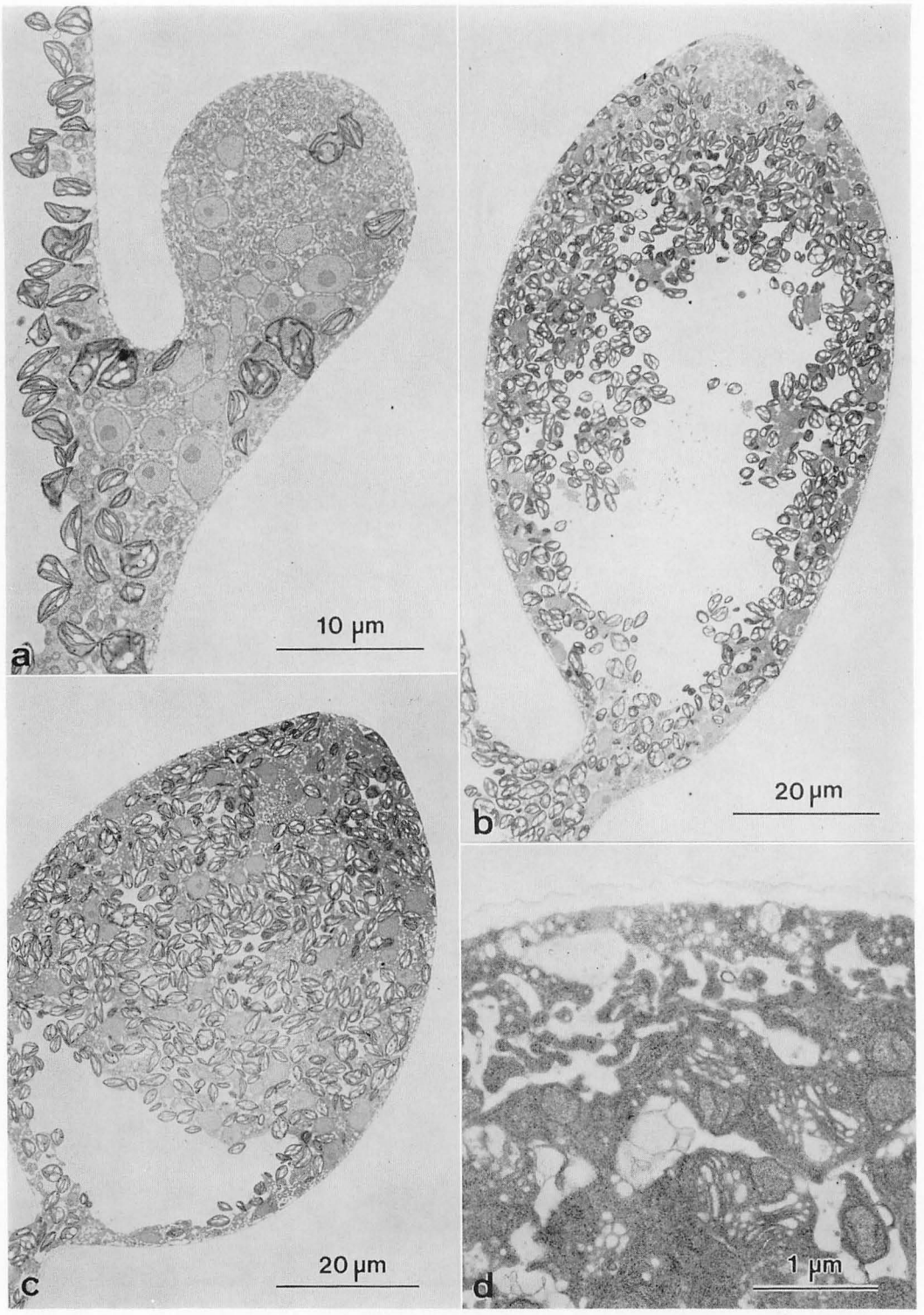



Fig. 5. Electron micrographs showing the developmental stages of a gametangium. **a**, early stage of gametangium formation at 21 hr, showing reticulated cytoplasm containing many nuclei. **b**, gametangium at 27 hr. Note the vesiculate cytoplasm located at the distal portion, from where chloroplasts are excluded. **c**, gametangium at 30 hr. **d**, many small vesicles and dictyosomes in peripheral cytoplasm of gametangium at 24 hr.

into two parts by the gametangial vacuole; a large portion which is later differentiated into gametes and a small one which is later involved in plug formation.

*Plug formation*

By 30 hr the cytoplasm migrates into the gametangium through the orifice which is plugged at 33–36 hr (Fig. 6e). At 30 hr, many electron dense, fine granules appear in vacuoles present in the stalk (Fig. 6b). At 33 hr, the plug material is deposited centripetally on the inner side of the stalk (Fig. 6a). It is successively deposited on both gametangium and ramellus side of a plug (Figs. 6c and 6d) and eventually results in separating the gametangial cytoplasm from that of the ramellus at 36–39 hr (Fig. 6e).

The forming plug contains electron dense granules and bubble-like globules (Figs. 6c and 6d) which overlie successively and form some ordered arrays (Fig. 6a). Dense vesicles are present in the cytoplasm near the plug (Figs. 6c and 6d), but their role in plug formation is unknown.

*Papilla formation*

At 27–30 hr, a papilla differentiates at the distal end of a mature gametangium (Figs. 3f and 3g). Sooner or later it becomes more translucent, because the inner side of the cell wall of the papilla is dissolved into a loosely disorganized layer of granules and the ramifying cytoplasm containing many vesicles and multivesicular bodies intrude it (Figs. 7a and 7b). At 33–36 hr, the cytoplasm retracts from the wall of the papilla (Fig. 7c) and the apex becomes somewhat flat (Fig. 3h). Finally the papilla changes to a pore for gamete liberation (Fig. 3i).

The papilla wall is stained by either acid fuchsin, fast green or amido black (Fig. 7d), but not when treated with pepsin or protease before staining (Fig. 7e).

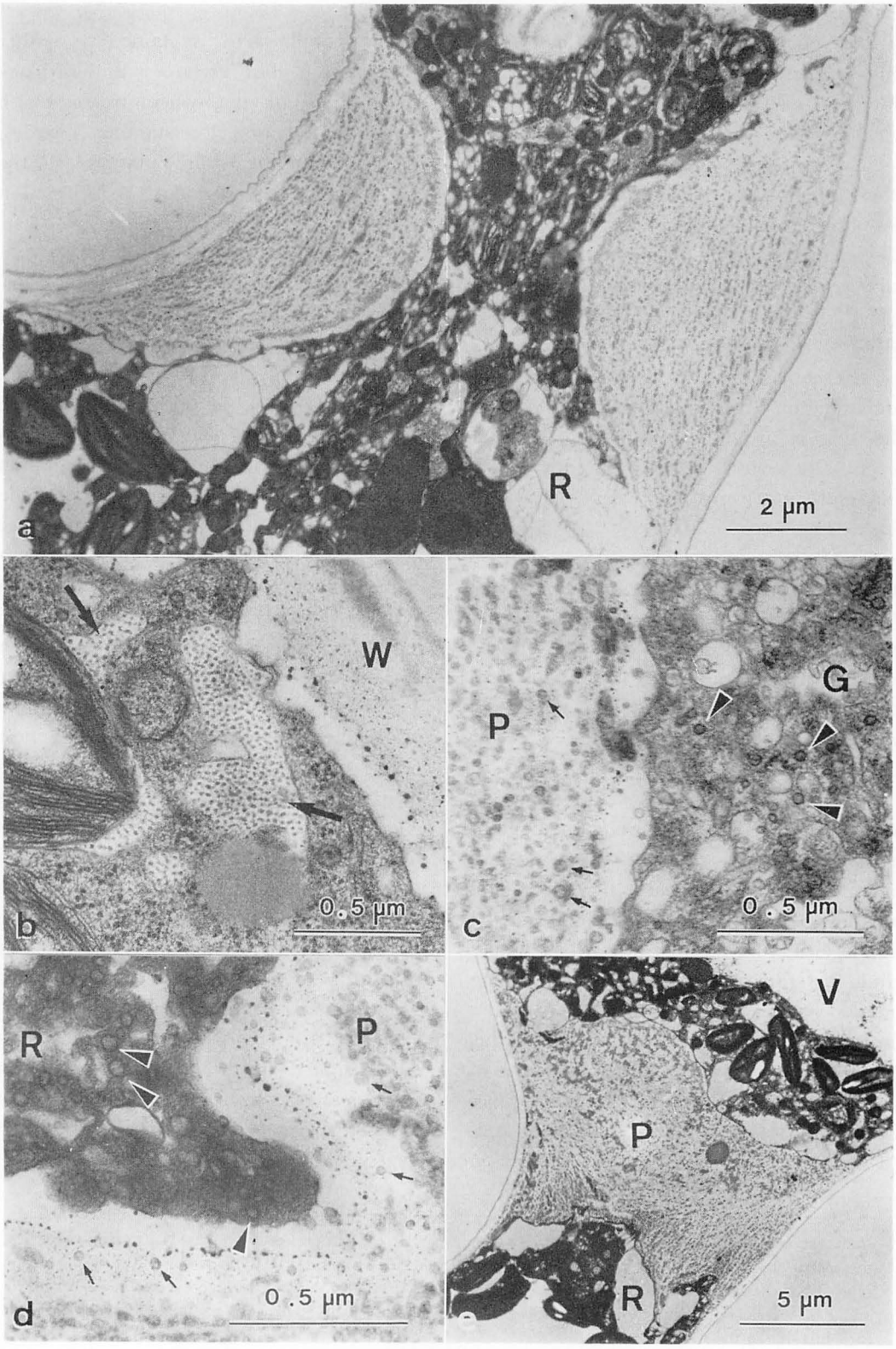
A diagramatic representation of the gametangial development is given in Fig. 8.

## Discussion

It has been suggested that the photosynthesis-dependent accumulation of a large amount of starch is prerequisite for the induction of sexual cell division in *Closterium* (ICHIMURA 1971). The accumulation of starch grains in chloroplasts of the ramellus seems to be essential for the cytoplasmic aggregation and subsequent induction of gametangium formation in *Pseudobryopsis hainanensis*.

In the vegetative stage of *P. hainanensis* and *Bryopsis hypnoides* (BURR and WEST 1970) the following periodical changes occur due to cytoplasmic streaming: most of the cytoplasm accumulates at ramellus bases during the dark period, while it is widely distributed in ramelli during the light period, though being more aggregated at ramellus apices. In the reproductive stage of *P. hainanensis* the cytoplasm at the ramellus bases gradually migrates towards the site where the gametangium is to be formed, possibly by upward cytoplasmic streaming. After that, the change in cytoplasmic distribution differs from that in the vegetative stage, because the cytoplasm seems to be trapped at the site where the gametangium is formed so that this cyto-

Fig. 6. Formation of a plug. **a**–**e** show electron micrographs of longitudinal sections of gametangial stalk. **a**, centripetal growth of plug resulting in constriction of cytoplasm at 33 hr. R, ramellus side of the stalk. **b**, dense granules (arrows) in cytoplasmic vacuole at 30 hr. W, stalk wall. **c** and **d**, growing plug (P) at the side of gametangium (G) and ramellus (R) at 36 hr, showing bubble-like globules (arrows) and dense vesicles (arrowheads). **e**, a plug formed completely at 36 hr. V, gametangial vacuole; R, ramellus side of the stalk.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plasm may be subsequently supplied to the developing gametangium. In *Acetabularia* the cytoplasm in the stalk migrates into cap rays when the cap attains a maximum diameter (SCHULZE 1939). This is similar to the migration of the cytoplasm into gametangia in *Pseudobryopsis*, but the gametangial development in *Pseudobryopsis* is accompanied by the cytoplasmic migration, whereas the cap is already formed prior to the migration.

The role assigned to the cytoplasm varies depending on the developmental stage of gametangium and the position of cytoplasm. Thus for non-septate cell differentiation, like gametangium formation in *Pseudobryopsis*, time- and position-dependent functional change of the cytoplasm seems to be important.

In *P. hainanensis* the gametangium can be produced only on the ramellus but not on the main axis. The ramellus hardly produces branches, so that it is functionally different from the main axis. Further, both the main axis and the ramellus never transform into the gametangium. Two steps of differentiation, ramellus formation and gametangium formation, are necessary in reproduction stage of *P. hainanensis*.

*Caulerpa okamurae* is an example of zero step differentiation. In this alga the entire protoplasmic content of the highly differentiated vegetative thallus is converted into gametes (ISHIWARA *et al.* 1981).

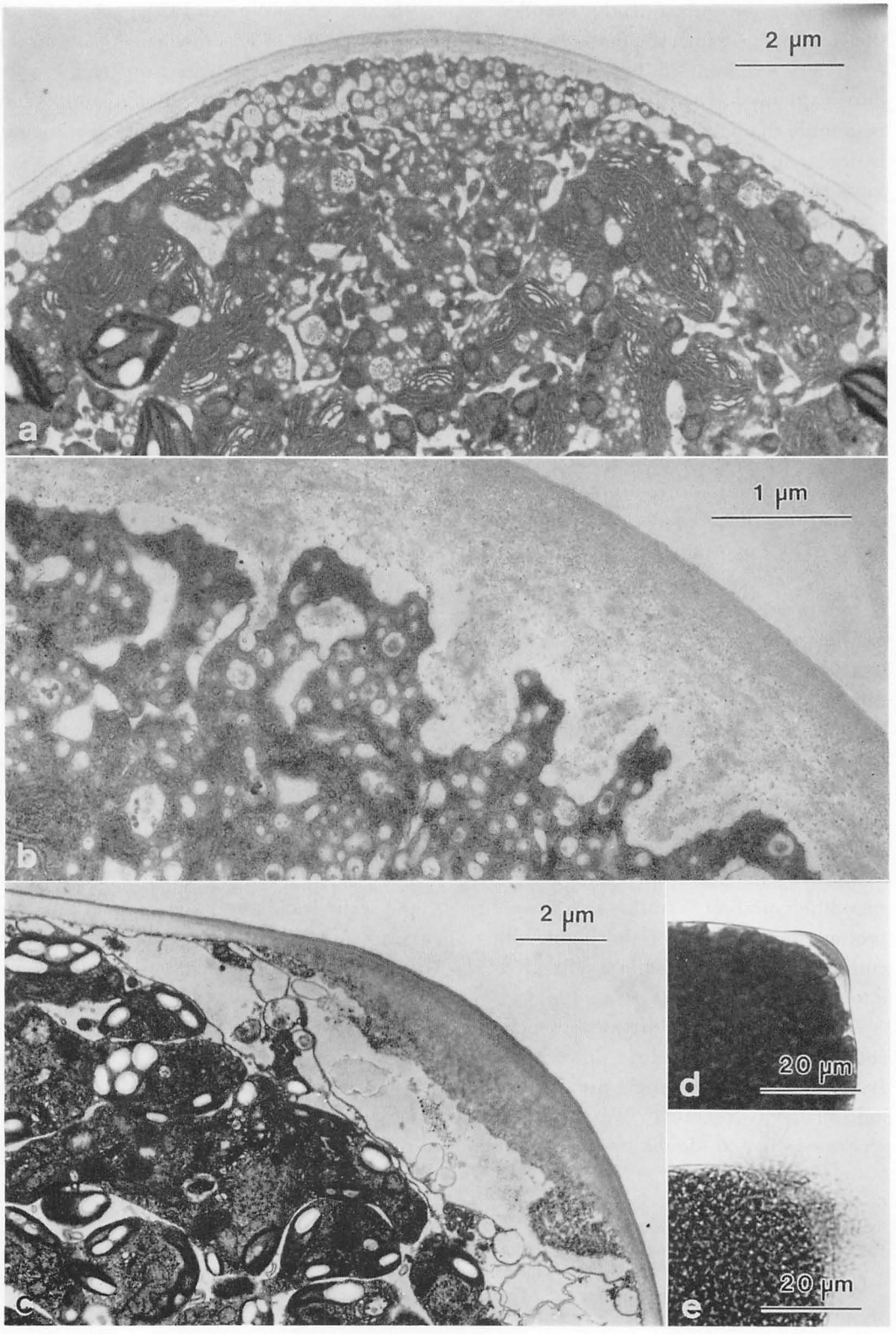
*Derbesia* and *Bryopsis* are algae which are endowed with one step differentiation. In *D. marina* the sporangia are formed on indistinctive, vegetative filaments, being separated by a double walled septum (SEARS and WILCE 1970); while in *B. hypnoides* the side branches separated by a plug from a main axis are transformed into the gametangia (BURR and WEST 1970). In these species the sporangium formation or the side branch formation is a differentiation step. However such sporangia and side branches often revert to a vegetative stage (SEARS and WILCE 1970, BURR and WEST 1970). Therefore, the differentiation of the reproductive organs of these two algae does not seem to be an elaborate differentiation. In *Codium* the gametangia are formed on an utricle which is produced on the tip of medullary interwoven siphons through two-step differentiation, though the gametangia often revert to vegetative daughter utricles in *C. giraffa* (SILVA 1979).

WERZ (1970) noticed the appearance of ER and small vesicles in the early stage of the development of whorls or cap rays in *Acetabularia*. According to him, ER is specifically associated with the region of the cell wall where hydrolysis takes place. In *P. hainanensis* ER is well developed in the aggregated cytoplasm when the gametangium formation starts. Subsequently, the gametangium protrudes and many small vesicles which seem to contribute to the cell wall formation appear in the peripheral cytoplasm. This suggests that expansion of the gametangial wall follows hydrolysis of the ramellus wall.

When expansion of the gametangium is almost complete, two kinds of local differentiation occur in the gametangium: papilla and plug formations.

WHEELER and PAGE (1974) reported

Fig. 7. Formation of papilla. **a–c** show electron micrographs of papilla at the distal portion of gametangium. **a**, many vesicles and dictyosomes in cytoplasm at 27 hr. **b**, dissolved papilla wall at 30 hr. **c**, swelled papilla wall at 39 hr, showing change in wall structure from homogenous transparent material to granular nature. Gametangium stained with acid fuchsin solution after treatment with protease (**e**) and without protease (**d**).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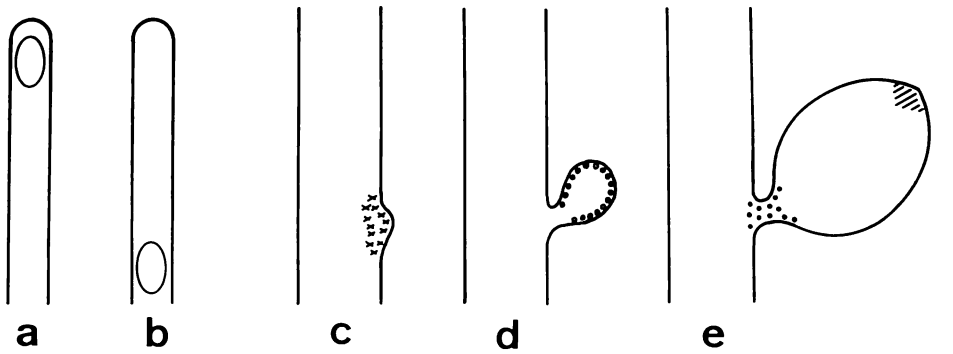


Fig. 8. Diagram showing changes in the distribution and function of cytoplasm during gametangium formation. Cytoplasm absorbing light energy for gametangium formation at upper part of ramellus (**a**) migrates downward and accumulates in ramellus base (**b**). Then the cytoplasm migrates upward to the site expected for gametangium formation. **c–e**. Distribution and function of local cytoplasm. **c**, cytoplasm softening ramellus wall at definite point (xed area). **d**, peripheral cytoplasm (small circles) synthesizing wall of developing gametangium. **e**, apical cytoplasm (oblique lines) degenerating papilla wall and basal cytoplasm (dotted area) secreting plug material.

that in *Derbesia* gametophytes, the degradation of the papilla wall takes place prior to gamete discharge and that many clustered small mitochondria and ER appear in the cytoplasm adjacent to this portion. They have considered that some hydrolytic enzyme is produced in the region and contributes to the wall degradation. In *P. hainanensis* the papilla wall is disorganized and can easily break down at gamete discharge. The papilla wall stained with dyes identifying protein, suggests that the wall contains such protein enzymes.

The development and fine structure of the plug have been described in detail in *Bryopsis hypnoides* (Burr and West 1971) and *Acetabularia acetabulum* (Menzel 1981). The developmental pattern of plug formation and the ultimate fine structure of plugs differ from species to species, although the plug of siphonous green algae may be homologous in structure on the basis of the occurrence of peroxidase (Menzel 1980, 1981). The branch plug in *Bryopsis* is usually composed of three layers: a middle proteinous layer derived from vacuolar protein bodies and two polysaccharide layers, which are structurally similar to the thallus wall (Burr and West 1971). The cap ray plug in *Acetabularia* consists of a voluminous, sponge-like structure, gaps between which are filled with condensed material (Menzel 1981). Unlike *Bryopsis* and *Acetabularia* the gametangial plug in *Pseudobryopsis* consists of electron transparent material with many dense, bubble-like globules. The cytoplasm involved in the plug formation remains near the plug and is never converted into gametes.

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**奥田一雄*・榎本幸人**・舘脇正和***：多核緑藻ハネモモドキの配偶子嚢の発達過程**

ハネモモドキの配偶子嚢の発達過程を同調培養と光顕及び電顕観察によって解析した。

栄養期の側枝の細胞質の大部分は，明期に頂端部，暗期に基部に集積する。配偶子嚢形成が誘起されると，側枝基部に集積していた細胞質が基部より少し上方の位置に移動する。集合した細胞質は，多数の核と大量の ER を含んでいる。細胞質が集合してくる位置で側枝の細胞壁が突出し，配偶子嚢に発達する。配偶子嚢の成長拡大に伴って側枝にある細胞質が配偶子嚢へ流入する。配偶子嚢の細胞壁に接する細胞質に多数の小胞が出現する。配偶子嚢の頂端部の細胞壁は電子密度が増加し，小突起となる。隔壁（plug）は，配偶子嚢と側枝の間の細胞壁に壁物質が蓄積することによって形成され，配偶子嚢の細胞質を側枝から分離する。配偶子嚢の細胞質の大部分が上方に集合し，下方部に大きな液胞が占めるようになった時，配偶子形成のための核分裂が始まる。

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# Fusion of protoplasts from thalli of two different color types in *Porphyra yezoensis* UEDA and development of fusion products*

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Protoplasts isolated enzymatically from thalli of wild type and green type mutant in *Porphyra yezoensis* were fused by polyethylene glycol (PEG) treatment. Many heterokaryocytes (up to 12.0%) were formed soon after the addition of washing medium to the protoplast-PEG preparation. Fusion products involving one wild type and one green type protoplast grew to form a mass of cells after regenerating cell walls. When cultured in aerated medium, the masses of cells grew as a callus-like cell aggregate. Cell aggregates differentiated into a number of full-grown thalli. Those thalli were microscopically chimeral thalli irregularly variegated with greenish cell and reddish purple cell groups. Carpospores released from mature chimeral thalli grew into greenish conchocelis, and the greater part of $F_1$ thalli produced from the conchocelis mostly consisted of greenish cells.

*Key Index Words: Genetic engineering*; *polyethylene glycol*; Porphyra yezoensis; *protoplast fusion*; *Rhodophyceae.*

Protoplasts produced from different tissues in higher plants and the resulting induced callus have been used as tools in physiological and cytological studies as well as in the research of protoplast fusion to produce somatic hybrids in the field of plant genetic engineering (GALUN 1981, AHUIA 1982, POTRYKUS *et al.* 1983). Somatic hybridization by protoplast fusion offers great promise for achieving wide crosses between species that are difficult or impossible to hybridize conventionally, with the hope to produce new crop varieties. There have been a large number of successful intraspecific and interspecific somatic hybrid experiments in higher plants reported to date (EVANS 1983, GLEBA and SYTNIC 1984).

Protoplast researches in algae have been limited so far to those of fresh water and marine unicellular algae (ADAMICH and HEMMINGSEN 1980, BERLINER 1981, BRADLY 1983), and only a very few dealing with marine multicellular algae (so-called "seaweeds") (CHENEY 1986). In the last few years, however, the isolation of protoplasts from seaweeds has been increasingly reported, and CHENEY (1986) listed a total of 15 species (nine genera) from which protoplasts have been isolated. The number of algae in which successful protoplast fusion has been reported is very limited; *Polyphysa* (=*Acetabularia*) *cliftonii* (PRIMKE *et al.* 1978), *Zygnema extenue* and *Spirogyra gracilis* (OHIWA 1978), *Chlamydomonas reinhardi* (MATAGNE *et al.* 1979), *Ulva linza* and *Monostroma angicava* (ZHANG 1983), and

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*Porphyra yezoensis* and *Enteromorpha intestinalis* (SAGA *et al.* 1986).

The red seaweed *Porphyra* "Nori" is being extensively cultured in Japan as one of the important edible seaweeds. Recently, however, *Porphyra* cultivation has frequently suffered from damage due to pathogenic microorganisms and unusual weather. It is hoped that strains of cultivated *Porphyra* can be improved by means of genetic engineering. We have already reported on the isolation and culture of the protoplasts in *Porphyra yezoensis* (FUJITA and MIGITA 1985). In this paper, we will further report on the fusion of protoplasts isolated from wild type and green type thalli in *P. yezoensis*, and on the development of fusion products cultured *in vitro*.

## Materials and Methods

*Vegetative thallus* of Porphyra yezoensis UEDA

Two kinds of specimens were used in the present study, one of wild type (normal color) and the other green type (color mutant). They were both collected from "Nori-nets" experimentally set at the culture farm in Kashima City, Saga Prefecture, Western Kyushu, Japan. Two kinds of shell-living conchocelis filaments for making nursery nets were prepared by using free-living conchocelis filaments kept in our laboratory. The thalli, about 10 cm long, were collected from the "Nori-nets", washed with sterile sea water, drained to semi-dry condition (30–40% moisture content) and stored at −20°C until used. After thawing, the thalli were used for isolation of protoplasts.

*Preparation of bacterial crude enzyme solution*

Protoplasts from the thalli of the two different types were produced by using a crude enzyme prepared from the cultured supernatant of *Pseudomonas* sp. strain P-1 which causes "Green spot rotting" on *Porphyra* thalli. The crude enzyme solution was prepared by a slightly modified procedures reported by FUJITA and MIGITA (1985). The strain was inoculated in a 200 m*l* Erlenmeyer flask containing 50 m*l* of ZOBELL 2216E broth and precultured for 3 days at 20°C. All of the preculture was inoculated in 1 *l* of medium which was composed of 0.1% potassium nitrate, 0.01% yeast extract, 0.01% potassium phosphate (dibasic), 0.001% ferric chloride, 0.1% tris(hydroxymethyl)aminomethane and 0.2% "Nori" powder in 75% sea water (pH 7.5) in 2 *l* Erlenmeyer flask and incubated with stirring for a week at 20°C. The culture was centrifuged at 12,000×g for 30 min. Ammonium sulfate (80% saturation) was added to the supernatant. The precipitate was collected and dissolved in 50 m*l* sea water containing 0.01% tris(hydroxymethyl)aminomethane (pH 7.2). The enzyme solution was dialyzed with the same sea water for 12 hr.

*Protoplast isolation*

Protoplasts from the vegetative thalli of the two kinds were produced separately. Basal parts of the thalli were removed in advance and the remaining parts washed several times by shaking in a semi-solid agar layer, then in sterile sea water. After washing three times in sea water, thalli were cut into small pieces (1–2 $mm^2$) with a razor blade. About 0.2 g (fresh weight) of the small pieces was incubated in a test tube (10×150 mm) containing 10 m*l* of the bacterial crude enzyme solution containing 0.8 M mannitol, 6,000 IU penicillin G potassium and 10 mg streptomycin sulfate. The incubation was carried out at 18–20°C on a reciprocating shaker, shaking it at about 120 cycles per min. After 3–4 hr incubation, the enzyme-protoplast mixture was passed through a

nylon mesh (25 $\mu$m opening) to remove undigested cell wall clumps and cell wall debris. The pellet was washed three times with sterile sea water containing 0.8 M mannitol. The washed cells were re-suspended at the concentration of $10^{5-6}$ cells per m*l* of the mannitol/sea water.

*Protoplast fusion and culture of fusion products*

To induce protoplast fusion the modified method of KAO and MICHAYLUK (1974) was used. Approximately 100 $\mu l$ of each protoplast suspension was placed on a cover slip (24×24 mm) in a Petri dish (9×2 cm) and allowed to settle for 10 min then 200 $\mu l$ of polyethylene glycol (PEG) solution [30% PEG 4000 or PEG 6000 (Wako Pure Chem. Indust. Ltd., Osaka, Japan) 0.2 M mannitol in sea water] was slowly added. After the protoplasts were incubated in PEG solution for 20 min, 10 m*l* of washing medium (KAMEYA *et al.* 1981) was added to the protoplast-PEG preparation. The mixture was then diluted with 10 m*l* of modified PROVASOLI's enriched sea water (PES) medium (PROVASOLI 1968) containing 0.8 M mannitol, 1,200 IU penicillin G potassium and 2 mg streptomycin sulfate. The Petri dishes containing protoplasts were maintained under continuous light (1,000 lux) at 15°C for a period of 24–48 hr. The frequency of protoplast fusion was examined microscopically. Identification of heteroplasmic fusion was easily possible, since the heterokaryocytes contained both reddish purple chloroplast(s) from the wild type protoplast(s) and green chloroplast(s) from the green type protoplast(s). Fusion products were transferred by using a fine pipet to Petri dish (6×2 cm) containing 12 m*l* of PES medium, and the cultures were maintained at 4,000 lux, 12:12 hr LD, 18°C. After 1–2 months plants developed from fusion products were transferred to flat bottom flasks containing 200 m*l* or 1 *l* of PES medium, and the cultures were maintained at 6,000 lux, 12:12 hr LD, 18°C and aerated. All cultures were changed to fresh medium weekly and placed under the "daylight" fluorescent lamps.

## Results and Discussion

Immediately after the addition of PEG 4000 or PEG 6000 solution to the suspension of protoplasts of two types, adjacent protoplasts began to aggregate and the number of aggregates increased with time (Fig. 1A, B). Aggregates tightly adhered to the surface of the cover slip. As long as protoplasts were being incubated in PEG solution, however, very few fusion occurred. The greatest number of fusion occurred as the aggregates began to leave the surface of the cover slip soon after the addition of washing medium to the protoplast-PEG preparation. The cells that fused had initially oval or irregular shape (Fig. 1C, E), but gradually became spherical (Fig. 1D). The constituents of the chloroplast mixture intermixed very slowly even 48 hr after the addition of washing medium, so there was little difficulty in identifying the fusion products which held two different chloroplasts. The frequency of heterokaryocyte formation due to PEG 4000 and PEG 6000 were 10.4% and 12.0% respectively. The fusion products involved two to four or more protoplasts. Seven fusion products which involved only one wild type and one green type protoplast were selected and transferred to PES medium in separate Petri dishes for cultivation. The two different chloroplasts in the fusion products became yellowish brown through the gradual mixing. Four of seven fusion products perished after a few days. The reason for the death of four

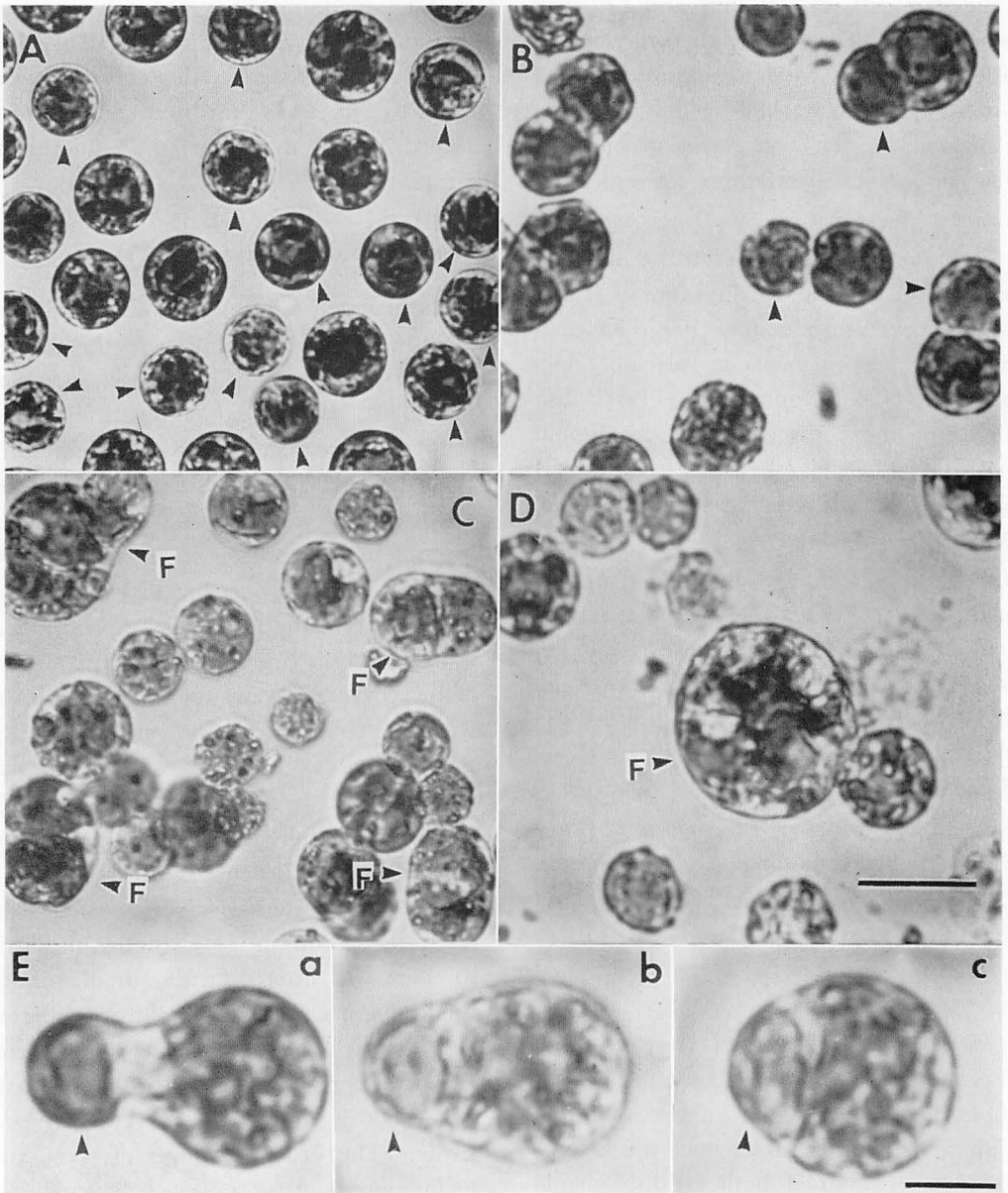


Fig. 1. Freshly isolated protoplasts and fusion events.

A. Mixture of wild type and green type protoplasts.

B. Aggregated protoplasts 15 min after the addition of the PEG solution to protoplast mixture.

C. Fusion cells (F) 20 min after the addition of the washing medium to protoplast-PEG preparation.

D. Nearly spherical fusion product (F) involving four or more protoplasts 12 hr after stage C. Scale bar= 20 $\mu$m.

E. Progressive stages of fusion between one wild type and one green type protoplast: a–c; 10, 25, 45 min after the addition of the washing medium to protoplast-PEG preparation. Scale bar=10 $\mu$m.

Arrows in A, B and E indicate wild type protoplasts. Scale bar in D applies also to A, B and C.

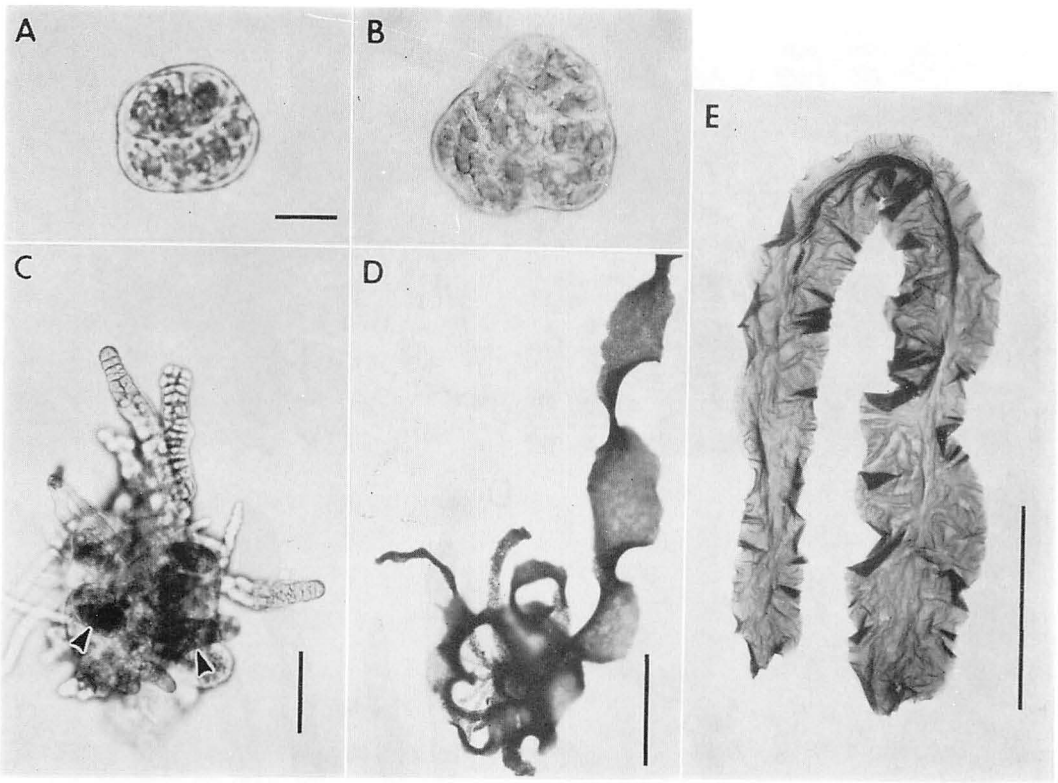


Fig. 2. Various stages in development of fusion product involving one wild type and one green type protoplast.
A and B. 20-day old and 30-day old germlings "masses of cells" from fusion product cultured in Petri dish.
C–E. Thalli differentiated from "callus-like cell aggregate" (arrows) induced after transfer "mass of cells" stage in B to aerated culture. C; 25 days, D; 65 days, E; 125 days after transfer to aerated culture.
Scale bar: 20 $\mu$m for A; 150 $\mu$m for C; 1 mm for D; 10 cm for E. Scale bar in A applies also to B.

fusion products may be the increase of bacteria in the culture medium. The remaining three fusion products divided very slowly after regenerating a cell wall and grew to form a mass of 10–30 cells after 20–30 days (Fig. 2A, B). The masses were composed of greenish and reddish purple cells of various diameters. The masses grew as callus-like cell aggregates in medium with continuous aeration. The callus-like cell aggregates are similar to the callus-like clumps of cells induced from the isolated cells of *Porphyra perforata* by POLNE-FULLER *et al.* (1984).

By maintaining the aerated cultures, a large number of young thalli differentiated from the callus-like cell aggregates (Fig. 2C). Those young thalli grew well in aerated culture to the length of 2–5 mm after two months (Fig. 2D) and the largest of those attained to about 60 cm in length after four months (Fig. 2E). They were microscopically chimeral thalli irregularly variegated with greenish and reddish purple cell groups.

Four months after the differentiation of young thalli, some chimeral thalli matured, forming carpogonia and spermatangia (Fig. 3A). Carpospores released from mature thalli grew into greenish conchocelis-phase filaments which formed conchosporangial branches (Fig. 3B). The conchospores

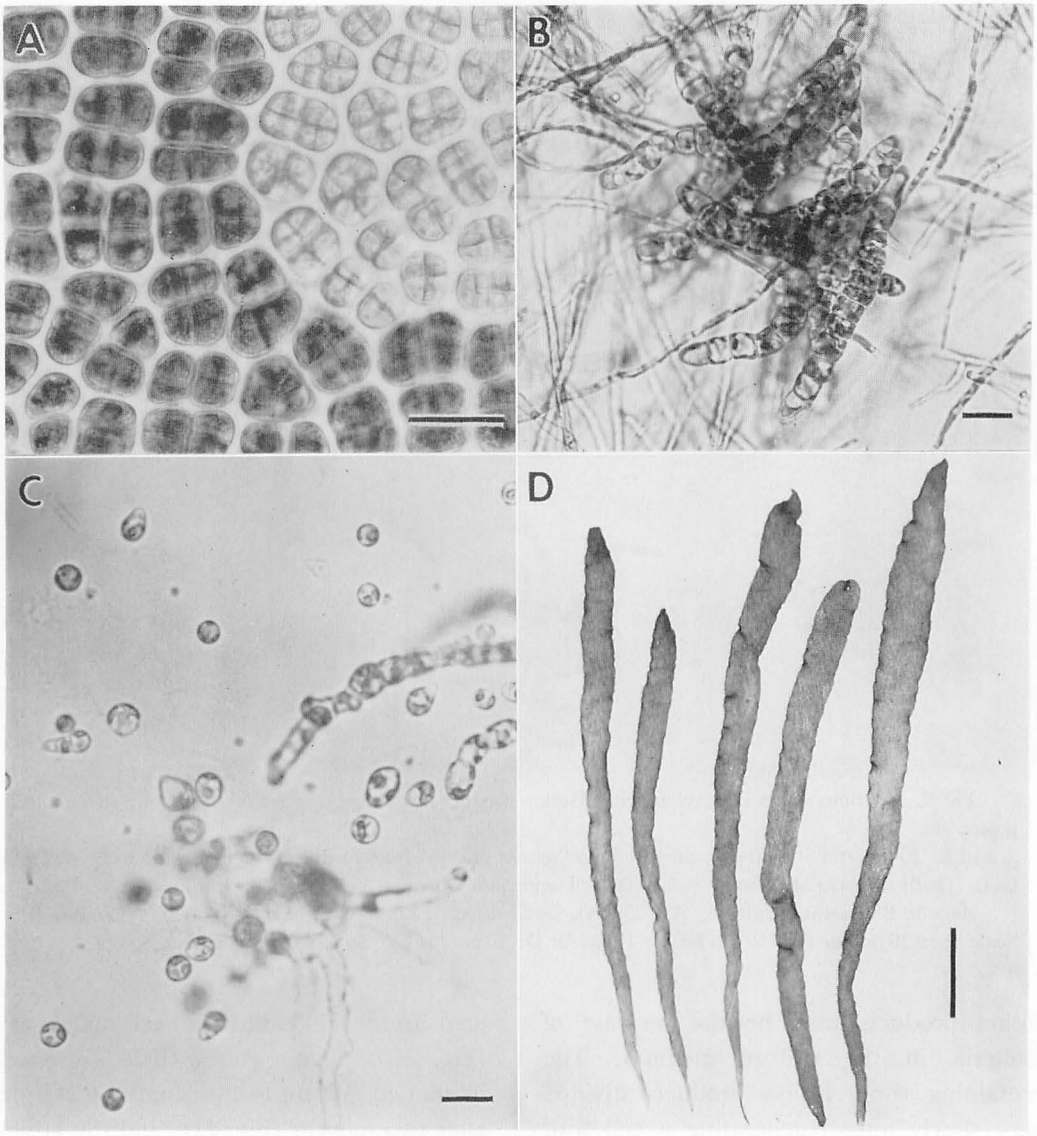


Fig. 3. Further development of thallus differentiated from "callus-like cell aggregates".
A. A portion of mature thallus in Fig. 2E showing carpogonia and spermatangia.
B. Conchosporangial branches formed on the conchocelis filaments derived from the carpospores.
C. Conchospores released from the conchosporangia.
D. $F_1$ thalli arisen from the germinated conchospores.
Scale bar: 20 $\mu$m for A–C; 1 cm for D.

(Fig. 3C) released from the conchosporangia of wild-like type and that of the greenish type could be distinguished by the difference in their color. Conchospores attached to synthetic fibers ("Cremona" monofilaments) of about 4 cm length grew into thalli ($F_1$) of oblanceolate or linear oblanceolate shape in aerated culture (Fig. 3D), similar to germlings from conchospores of original wild and green types. The greater part of those thalli was mostly composed of greenish

cells, while in thalli derived from the conchospores of wild-like type only a few cells at the basic part appeared reddish purple. There was no appreciable difference in color and morphology between the plants arisen from the three fusion products.

The phenomenon of chimeral thalli regenerated from the protoplast fusion products is regarded as a result of the occurrence of two type of cells with parental colors. This may be traced back to the gradual process of segregation of two different colored chloroplasts that had once undergone fusion. It is hypothesized that during the cell-divisions in the thalli and the growth of the conchocelis-phase the green type chloroplasts gained predominance over the wild type ones. As a result, in the $F_1$ thalli the greater part was replaced by cells of green type. The segregation to one or the other parental type of chloroplast has been often observed in regenerated hybrid plants and in the progeny of somatic hybrids of higher plants (cf., GLEBA and EVANS 1983, COCKING 1983).

OHME *et al.* (1986) reported that the heterozygous conchocelis from the sexual cross of the green and wild types in *P. yezoensis* were all the wild type, and frequently produced sectorially variegated chimeral thalli composed of parental color sectors. The difference between their results and ours suggests that the mechanisms of the formation of chimeral thalli and the presentation of colors in the progeny are different in the protoplast fusion and in the sexual cross between the green type and the wild type.

In the present study, only distinct green plants were obtained. If many more fusion products had been successfully regenerated, it might have been possible to find some that gave the intensive wild type color. We plan to engage in continuous research on further details of new fusion products and their regenerated plants with respect to the behavior of chloroplasts, nuclei and chromosomes and to the biochemical characters (e.g., constituents of pigments).

The PEG method has been used principally for the fusion of protoplasts to produce somatic hybrids, especially in higher plants. In recent years, attempts to accomplish algal protoplast fusion based on PEG treatment have been successful in *Zygnema extenue* and *Spirogyra gracilis* (OHIWA 1978), *Chlamydomonas reinhardi* (MATAGNE *et al.* 1979), *Ulva linza* and *Monostroma angicava* (ZHANG 1983), and *Porphyra yezoensis* and *Enteromorpha intestinalis* (SAGA *et al.* 1986). We have shown that the PEG method is also suitable for producing intraspecific parasexual hybrids of *Porphyra* by means of protoplast fusion.

## Acknowledgements

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**藤田雄二・右田清治：スサビノリの二つの色彩型葉体から分離されたプロトプラストの融合ならびに融合細胞の発生**

スサビノリ *Porphyra yezoensis* UEDA の野生型と緑色型葉体から酵素法によって分離されたプロトプラストは，ポリエチレングリコール処理によって融合した。野生型と緑色型の各1個のプロトプラストからなる融合細胞は，細胞壁を再生した後，分裂し，1か月後に約30細胞の細胞小塊となった。その細胞小塊から誘導されたカルス状の細胞塊からは，よく生長する多数の葉体が発生した。それらの葉体は，顕微鏡的に赤紫色の細胞群と緑色がかった細胞群からなるキメラ葉体であった。成熟キメラ葉体からの果胞子は緑色を帯びた糸状体に生長した。その糸状体からの殻胞子は赤紫色あるいは緑色とみなされたが，いずれも発芽するとほとんど緑色を帯びた細胞からなる葉体となった。（852 長崎市文教町1-14　長崎大学水産学部藻類増殖学研究室）

Jap. J. Phycol. (Sôrui) **35**: 209–213, September 20, 1987

# Attachment of the tetraspores of *Padina dubia* HAUCK (Phaeophyta, Dictyotales)

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AMPILI, P., PANIKKAR, M.V.N. and CHAUHAN, V.D. 1987. Attachment of the tetraspores of *Padina dubia* HAUCK (Phaeophyta, Dictyotales). Jap. J. Phycol. **35**: 209-213.

After liberation, the tetraspores of *Padina dubia* secreted an adhesive which firmly attaches them to suitable substratum. The secretion started immediately after their liberation around the spores and the rhizoids. This secretory substance is of mucopolysaccharide type. It is suggested that the synthesis of the bioadhesive has some ecological and physiological importance. Histochemical studies were carried out to examine the nature of the extracellular bioadhesive by using specific stains like alcian dyes, periodic acid Schiff's (PAS) and toludine blue (TB). From the staining reactions it was found that the spores contain large amounts of both acidic and sulfated polysaccharides, while the extracellular bioadhesive contains only a sulfated polysaccharide, probably fucoidin.

*Key Index Words: Bioadhesive; Dictyotales; histochemistry;* Padina dubia; *Phaeophyta; polysaccharide; tetraspores.*

*Padina dubia* HAUCK is a common intertidal alga belonging to the family Dictyotaceae of the order Dictyotales. It is a tropical alginophyte found plenty in the intertidal regions of the Gujarat coast of India. The tetrasporic plants produce enormous number of spores and only a very few of them may get a chance to settle and adhere to suitable substratum. The attachment of these non-motile spores to the substratum against the tidal current is one of the most important events in the life history of an intertidal alga (HARDY and Moss 1979). Only a very little information is available on the nature of the extracellular bioadhesive secreted by the spores at the time of germination.

HARDY and Moss (1978, 1979) studied the attachment of the zygotes and germlings of *Halidrys siliquosa* and *Pelvetia canaliculata* by using specific stains. FORBES and HALLAM (1979) investigated the nature of the bioadhesive secreted by the zygotes of *Hormosira banksii* at the time of germination. The histochemical studies on the extracellular substance in brown algae have been carried out by a few researchers (MCCULLY 1965, 1966, 1970; FULCHER and MCCULLY 1969) and these substances were identified as non-sulfated acidic polysaccharide (alginic acid) and sulfated polysaccharide (fucoidin).

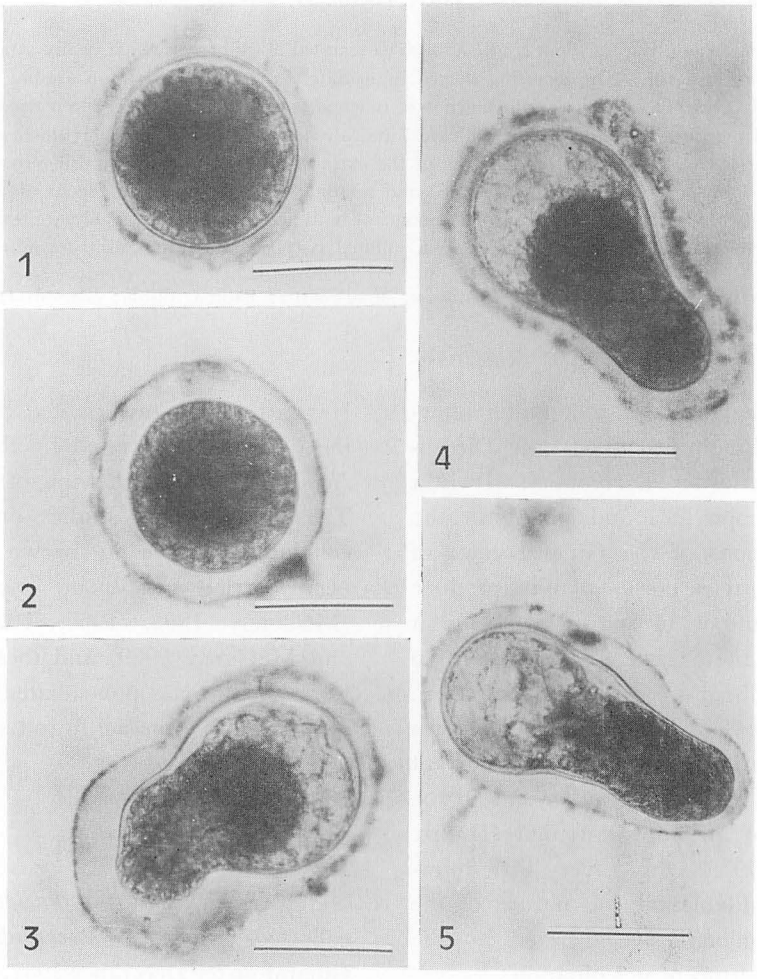
## Materials and Methods

Tetrasporic plants of *Padina dubia* were collected from the Porbander coast of Gujarat on 21 December 1982. The plants were transported to the laboratory in plastic buckets containing seawater, and kept in the culture room at 20°C. The next day the fertile portions were removed

and rinsed with sterilised seawater several times. The upper portions of the thallus with mature bands were cut into pieces and they were placed on glass slides in petri dishes containing sterilised seawater. The slides with the spores were fixed in 5% formaldehyde at 10 minute intervals for two days. The spores were stained by using different stains for histochemical studies. Alcian dyes were prepared according to PARKER and DIBOLL (1966). Periodic acid Schiff's (PAS) stain was used after the method of MCMANUS (1948). The spores were also stained with toluidine blue (MCCULLY 1970). Cultures were maintained at 20°C, 14:10 hr LD cycle and 1500 lux. The stained spores and the germlings were observed under a light microscope.

## Results

The spores started secreting an extracellular substance after 1–2 hrs of libera-




Figs. 1–5. *Padina dubia* tetraspores stained in toluidine blue.
Fig. 1. One hr old spore. Fig. 2. Three hrs old spore with bioadhesive of extracellular polysaccharide. Fig. 3. Five hrs old germinating spore. Fig. 4. Six hrs old germinating spore. Fig. 5. Eight hrs old germinating spore. All with extracellular polysaccharide around the spore and the rhizoid. Bar=25 $\mu$m.

Table 1. Different stains, their staining reactions on the tetraspores of *Padina dubia* and the polysaccharides identified.

| Stains | Regions stained | Reaction colour | Staining reaction | Polysaccharides identified |
|---|---|---|---|---|
| Alcian Yellow | SP | Yellow | + | Non-sulfated |
| (AY) pH 0.5 | EM | — | − | ———— |
| Alcian blue | SP | Blue | + | Sulfated |
| (AB) pH 2.5 | EM | Blue | + | Sulfated |
| AY and AB | SP | Green | + | Carboxylated and sulfated |
| | EM | Blue | + | Sulfated (PARKER and DIBOLL 1966) |
| Peridic acid | SP | Red | + | With hydroxyl groups |
| Schiff's (PAS) | | | | (MCCULLY 1965) |
| | EM | — | − | ———— |
| Toludine blue | SP | Red | + | Carboxylated and sulfated |
| | EM | Pink | + | Sulfated (MCCULLY 1965) |

SP=spore, EM=extracellular material, +=positive, −=negative.

tion (Fig. 1). They adhere to the slide firmly after 3–4 hrs and the secretory substance was about 2 $\mu$m in thickness (Fig. 2). Their attachment was so firm that it was difficult to remove them even by applying a jet of water. After 5–6 hrs, the spores started producing a lateral rhizoid (Fig. 3). Gradually the rhizoid elongated and firmly attached on the slides (Figs. 4 & 5) by secreting an adhesive around it. The results of the staining reactions of different stains are presented in Table 1. The accumulation of alcian yellow (AY) was found only in the spore. The blue colour of alcian blue (AB) was found both in the extracellular bioadhesive and the spore. When stained with AY and AB the spores remained greenish, while the bioadhesive was bluish in colour. When the spores were treated with 1% $NaCO_3$ to remove the carboxylated alginic acid, prior to staining AB accumulation was found in the extracellular bioadhesive and the spore. Metachromasia with TB occurred both in the spore and the bioadhesive, the former being reddish and the latter pinkish in colour. An intense PAS positive reaction was found only in the spore, while the bioadhesive remained PAS negative.

## Discussion

The spore alone was stained bright yellow with AY. This is due to the presence of a non-sulfated acidic polysaccharide (alginic acid). The AY at pH 2.5 readily complexes only with a non-sulfated polysaccharide. The AB at pH 0.5 complexes only with sulfated polysaccharide, its accumulation and blue staining both the spore and extracellular substance indicating the presence of polysaccharides with sulfate groups. Histochemical studies have already shown that sulfated polysaccharide in the brown algal tissue is fucoidin (PARKER and DIBOLL 1966, MCCULLY 1970, FORBES and HALLAM 1979). When the stains AY and AB were used simultaneously the spore was stained green, showing the occurrence of both sulfated and carboxylated polysaccharides, and the blue colour of the extracellular substance further confirms that it is a sulfated polysaccharide. When the germinating spores were treated with $NaCO_3$ solution to remove the non-

sulfated polysaccharide, the spore and the adhesive were stained blue, indicating the presence of sulfated polysaccharides. The AY staining substance was removed from the spore, which is a carboxylated polysaccharide (alginic acid). The PAS positive reaction occurred in the spore, while the bioadhesive remained PAS negative. The PAS positive reaction is quite specific for polysaccharide having free hydroxyl groups on 2 vicinal carbon atoms (JENSEN 1962). The alginic acid has free hydroxyl groups and therefore PAS positive, however fucoidin lacks hydroxyl groups and it is PAS negative (MCCULLY 1966). From these staining reactions it is clear that the bioadhesive may be a sulfated ester of fucose. The bioadhesive stained with TB was intense pink, while the spore was reddish. The metachromatic pink colour of TB is very characteristic of sulfated polysaccharide (LISON 1936), while the reddish and blue colour are generally produced by reaction with carboxyl groups (MCCULLY 1965). This staining reaction further confirms that the bioadhesive is a sulfated polysaccharide (fucoidin). The spore contains both sulfated and non-sulfated polysaccharides.

At the time of germination the zygotes of brown algae secrete a sulfated polysaccharide for firm attachment (Moss 1974, HARDY and Moss 1978, FORBES and HALLAM 1979). This bioadhesive is secreted by the spores and the rhizoids. FORBES and HALLAM (1979) observed the presence of polyphenols during the growth of the rhizoids of *Hormosira* zygotes. Such a secretion during germination may have some ecological and physiological significance in the establishment of benthic marine algae. The extracellular bioadhesive probably suppresses the growth of bacteria and other microorganisms (CRAIGIE and MCLACHLAN 1964). From the histochemical studies it is clear that the bioadhesive may be fucoidin and it may possibly contain some polyphenolic materials having antibacterial activities. Thus the bioadhesive is meant for adhesion and protection of the developing germling.

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**Ampili, P. · Panikker, M.V.N. · Chauhan, V.D.: *Padina dubia*（褐藻植物門　アミジグサ目）の四分胞子の付着**

*Padina dubia* の四分胞子は放出された後，粘着性の物質を分泌し，それによって基質にしっかり付着する。この分泌は四分胞子放出直後から胞子と仮根のまわりで始まる。この分泌物はムコ多糖類型のものである。この生物粘着物質(bioadhesive)の合成は何等かの生態的生理的重要性をもつことが示唆される。この細胞外に産生される生物粘着物質の性質を調べるため，アルシアン・イエロー，アルシアン・ブルー，PAS，トルイジン・ブルーなどを用いて組織化学的研究を行なった。染色反応から，四分胞子には酸性多糖類と硫酸含有多糖類とが共に多量に含まれるが，細胞外産生の生物粘着物質には硫酸根をもつ多糖類（恐らくフコイジン）のみが含まれることが判明した。

## 新　刊　紹　介

**徳田　廣・大野正夫・小河久朗著　海藻資源養殖学。**水産養殖学講座第10巻。緑書房，東京，v＋2＋354 pp.（定価 5,500円）

本書は9章からなる。第Ⅰ章は地球の生態系としての海藻；第Ⅱ章は光・温度・塩分等の環境要因と生長・垂直及び地理的分布；第Ⅲ章は食用・飼料・肥料・医薬用・化学工業用となる海藻の種類・利用法，その特性；第Ⅳ章は漁業区別にみた世界の海藻の資源と生産量，我が国への原藻の輸入とその製品の輸出量；第Ⅴ章は種毎にその生活史及びその生態特性を解説して，採苗から収穫までの養殖の理論と技術，その製品の品質と出荷；第Ⅵ章は種毎の藻場特性に基づいた藻場造成法；第Ⅶ章は世界の主要地区別または国別にみた養殖の現状；第Ⅷ章は主要海藻類の養殖への問題点と展望；第Ⅸ章は品種改良・品種保存等の将来配慮すべき問題点。

本書は日本及び世界の海藻資源と養殖の現状を系統的且つ理論的に纏めた世界に類のない専門書であり，大学の教科書として，また養殖に携わる漁業者や水産行政担当者の必携の良書である。海外の最近の多くの文献及び統計資料が引用され，また多くの写真と図表があるし，巻末には専門術語を解説した用語集があって読者の理解を援けている。

梅崎　勇（京大・農・熱農）

Jap. J. Phycol. (Sôrui) **35**: 214–217, September 20, 1987

# Scanning electron microscopic studies on *Cyclotella obliquata* Qi et Yang[1)]

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Qi, Y.Z. and Yang, J.R. 1987. Scanning electron microscopic studies on *Cyclotella obliquata* Qi et Yang. Jap. J. Phycol. **34**: 214–217.

Scanning electron microscopic studies were carried out on *Cyclotella obliquata* Qi et Yang. The species occurs in early Pleistocene deposits of Miyi, Sichuan Province, southwest of China. It is similar to the *Cyclotella bodanical comta* complex, but best distinguished from this by having only strutted processes scattered in the central part and the marginal zone of the valve. The striae of the marginal zone are divided into 5–7 fascicles by the broader hyaline areas.

*Key Index Words:* Cyclotella obliquata; *fine structure; morphology; taxonomy.*

In a previous paper (Qi and Yang 1985) the authors reported a new species, *Cyclotella obliquata* Qi et Yang, and gave just a short diagnosis on its characteristics. In the present paper we will focus on the scanning electron microscopic structure of this species in detail.

## Materials and Methods

The fossil materials were collected from fluviolacustrious facies diatomaceous earth of the Xigida Group, Miyi, Sichuan province of China and the period is considered as in early pleistocene.

For scanning electron microscopy, specimens were either cleaned of organic matter by oxidation or simply washed free of preservative and then mounted on stubs. The preparation was coated with gold to suppress charging, and observed in different ways and magnitudes.

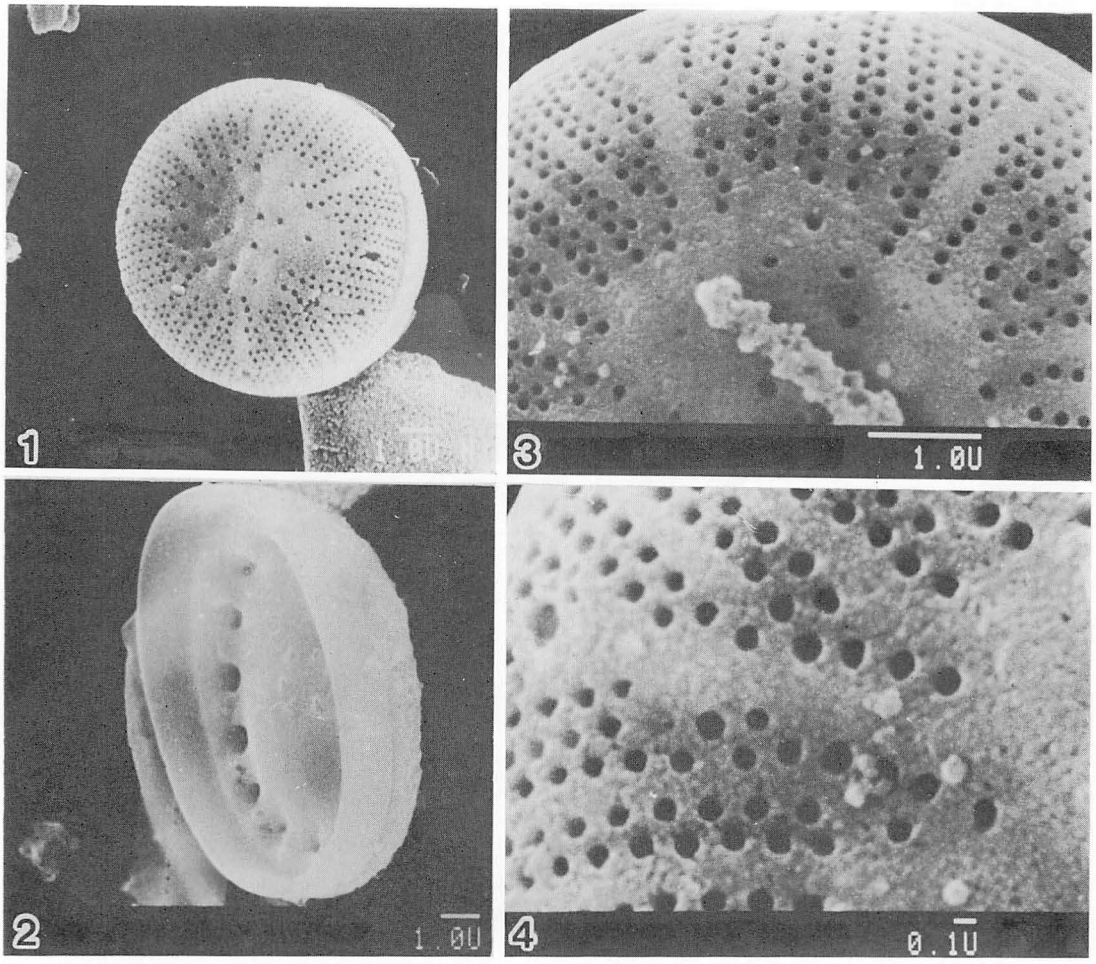
1) This work was partly supported by the Science Fund of the Chinese Academy of Science.

## Results

*External valve structure*: The cells of *Cyclotella obliquata* are solitary and drum-shaped (Figs. 1, 2). The marginal zone of the valve is divided into five to seven areas by radial broader hyaline areas (interfascicles). Striae are fasciclate, of unequal length, parallel to each other and composed of two or rarely three rows of poroid areolae (Figs. 1, 3, 4). The areolae are round, simple openings on the surface but not of the same size (Fig. 4).

The central area is smooth or verrucose and tangentially waved. In the central part of the central area, several outer openings of the strutted processes are scattered (Figs. 1, 3, 5). A ring of the openings of the marginal strutted processes is located along the margin of the valve mantle. These are quite apparently seen on the end of every radial interfascicle (Figs. 1, 3, 5, 8).

*Internal valve structure*: Figure 6 shows the whole outline of the internal valve



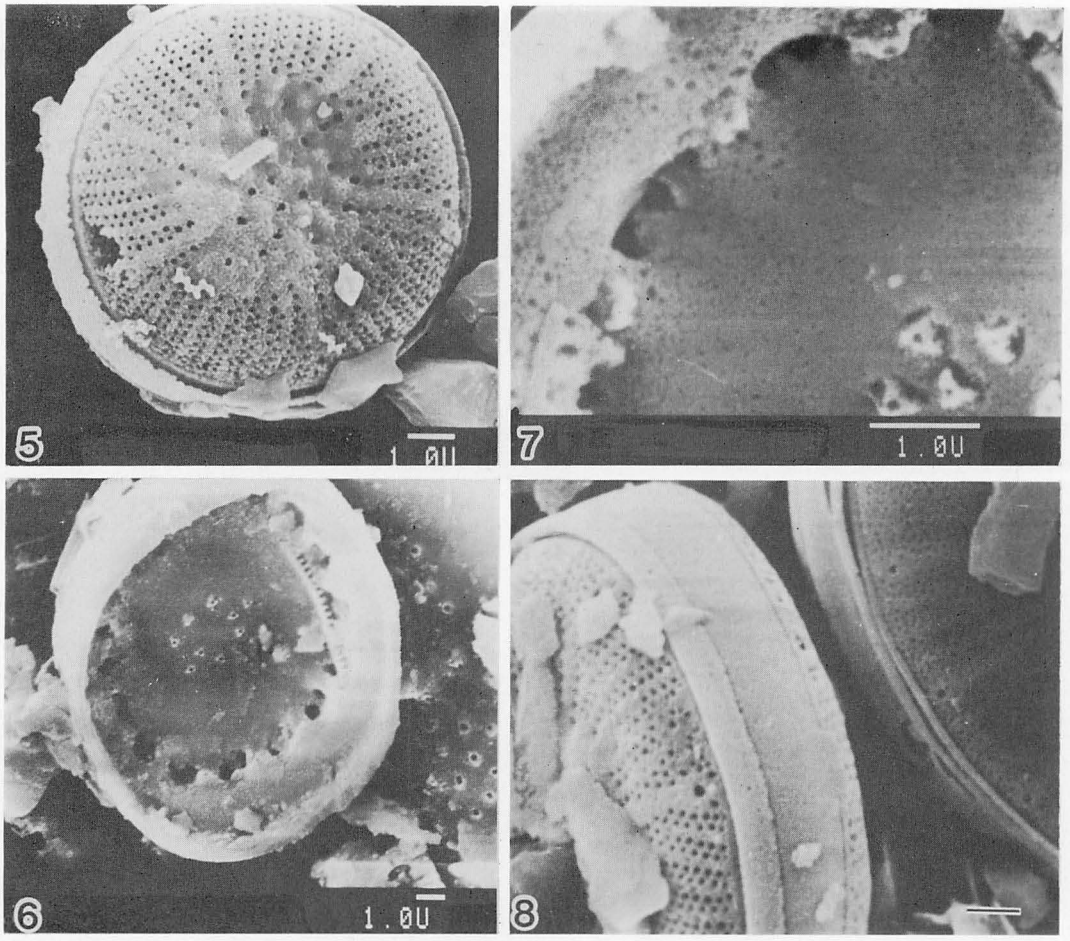

Figs. 1–4. *Cyclotella obliquata* QI et YANG
Fig. 1. External valve with tangentially waved central area. ×5500. Fig. 2. Internal valve showing the strutted process on every costa. ×5000. Fig. 3. Valve margin showing the fasciclate striae and radial interfascicles with an outer opening of the strutted process near the marginal end. ×15000. Fig. 4. Enlargement of the outer valve margin. ×30000.

view. A ring of strutted processes is located along the mantle on every costa. All valves observed were eroded, so that the exact structure of the marginal and central strutted processes was inconspiquous. However, the internal tube of a marginal strutted process is short and with three struts. The number of these processes per valve varies from 14 to 16. The internal tube of a central strutted process is very short but has clearly three struts (Figs. 2, 6, 7). Unfortunately we have unable to find a labiate process, however, finding this can strongly expected.

*Girdle*: As seen in Fig. 8, the cingulum appears to be composed of two bands, valvocopula and pleura both without ornamentation, though complete frustules are very scarce in the materials.

## Discussion

We placed this species in the genus *Cyclotella*, because it shares the basic diag-




Figs. 5–8. *Cyclotella obliquata* QI et YANG

Fig. 5. External valve with slightly waved and verrucose central area. ×6000. Fig. 6. Internal valve showing the central area with scattered strutted processes. ×4000. Fig. 7. Enlargement of the internal valve margin showing the marginal and central strutted processes. ×15000. Fig. 8. Girdle view showing the valve copula and pleura. ×7000.

nostic characters of the genus (LOWE 1975, HÅKANSSON 1986, XIE and QI 1984). The difference between our species and those of the *Cyclotella bodanical comta* complex lies in; (1) central zone of our species has only strutted processes and no areolae with domed cribrum internally. (2) the marginal zone of the valve is divided into five to seven fascicles by the broader hyaline areas (interfascicles). However, *Cyclotella curvistriata* CHEN et ZHU (CHEN and ZHU 1985) has curved striae but arranged in different fashion. Meanwhile, *Cyclotella kuetzingiana* THWAITES has shorter and longer striae in the marginal zone of the valve but not the same structure as in *Cyclotella obliquata*. Moreover, SERIEYSSOL (1984) described *Cyclotella iris* complex with many pictures showing a lot of striae structure of unequal length and curved, but it still differs from *Cyclotella obliquata* in the number and arrangement of striae. Such a striae arrangement has never been observed before in the genus *Cyclotella*.

## Acknowledgements

The authors would like to thank Dr. HANNELORE HÅKANSSON for her critically reviewing of the manuscript. Thanks are also due to Prof. Dr. H. KOBAYASI who provided much interesting discussion and encouragement. We thank Miss WANG FAN as well, the senior author's student, for typing the manuscript.

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**斉雨藻*・楊景栄**：*Cyclotella obliquata* QI et YANG の走査型電子顕微鏡による研究**

南西中国，四川省，米易の更新世初期の堆積物中から見出された *Cyclotella obliquata* QI et YANG の走査型電子顕微鏡による研究を行った。本種は *Cyclotella bodanical* comta complex に似るが，殻面中心部と縁辺部とに散在する有基突起を持つ点で後者と区別出来る。縁辺部の条線は広いハイアリン領域によって 5 — 7 個の胞紋に分画される。(*中国広州暨南大学生物学系，**中国　南京　中国科学院南京地質古生物学研究所)

Jap. J. Phycol. (Sôrui) **35**: 218–220, September 20, 1987

# P. Ampili, M. V. N. Panikkar and V. D. Chauhan: Male organs of *Sarconema filiforme* (Sonder) Kylin (Rhodophyta)

*Key Index Words: Gigartinales; male organs; Rhodophyta;* Sarconema filiforme.

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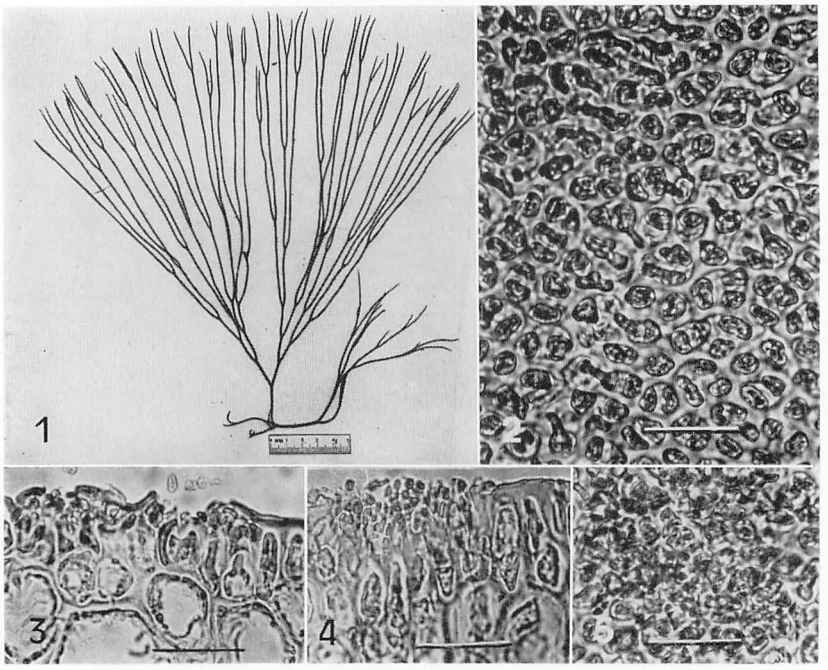
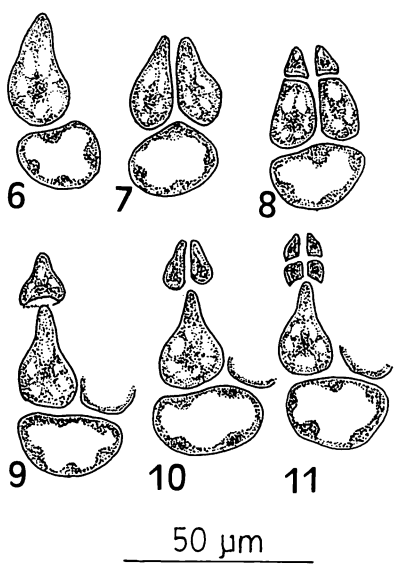
The male reproductive organs of Gigartinales have been studied by a number of investigators (Kylin 1932, Hewitt 1960, Min-Thein and Womersley 1976, Gabrielson and Hommersand 1982). The detailed pattern of development of the male organs in most of the species is still unknown (Min-Thein and Womersley 1976). Often it seems to be difficult to separate male plants from female and tetrasporic ones. In general, spermatangial sori are scattered on the surface of the male plants. The peripheral cells are cut off two or more ovoid or spherical spermatangial




Figs. 1–5. *Sarconema filiforme.*
Fig. 1. A male plant. Fig. 2. Surface cells showing early stages of spermatangial development. Figs. 3 & 4. Sections of the thallus with early division stages and the liberation of the spermatangia. Fig. 5. Surface view of the spermatangial sorus. Bar=50 μm.

mother cells, each of which cuts off two or more spermatangia (MIN-THEIN and WOMERSLEY 1976). However the actual pattern of the spermatangial development may slightly vary in different species.

*Sarconema filiforme* taxonomically belongs to the family Solieriaceae in Gigartinales. It is a tropical carrageenophyte, which contains higher percentage of phycocolloids (HOPPE 1979). The taxonomy and reproductive morphology of female and tetrasporic plants have been studied by PAPENFUSS and EDELSTEIN (1974). The male plants and the development of the male organs have not been described so far. Considering its economic importance, as a part of thorough investigation of the species, the male plants and the spermatangial development were studied separately.




Figs. 6–11. Stages of the spermatangial development in *Sarconema filiforme*.
Fig. 6. A peripheral elongated cell. Fig. 7. Initials of the primary spermatangial cells. Fig. 8. Upper small primary spermatangial mother cells and lower large basal cells. Fig. 9. Cap like primary spermatangial mother cell and the basal cell with elongated upper region. Fig. 10. Two spermatangial mother cells. Fig. 11. Four separated spermatangia.

Male plants of *Sarconema filiforme* were collected in September 1982 from the Porbander coast of Gujarat. They were 18–25 cm in height and branched dichotomously as the tetrasporangial and female plants (Fig. 1). However, they were slender with a pale yellow colour. The spermatangial sori were found as patches on the branchlets and the main axis. By close observation rough dot like appearance of the sori could be very easily recognisable with the naked eyes. The sori were localised irregularly on the surface. The observed pattern of spermatangial development is summarised below.

The peripheral cells are elongated and they measure $25.5–29.5 \times 14.5–17.5\ \mu m$ (Fig. 6). Each of these divides longitudinally to form two cells (Figs. 4 & 7), and they function as the initials of the primary spermatangial cells (IPSC). Each divided cell cuts off a small upper primary spermatangial mother cell (PSMC). The lower cell elongates and presses the upper PSMC towards outside, and often comes out of the cuticle (Fig. 8). The basal cell has a swollen base and an elongated upper region, while the PSMC remains as a cap-like structure (Figs. 2, 3 & 9). The latter divides longitudinally to produce two cells (Fig. 10). Each of these functions as the spermatangial mother cell (SMC), and cuts off 2–3 spermatangia which liberate outside (Figs. 4, 5 & 11). Each spermatangium measures $2.5–3.5\ \mu m$ in diameter.

The developmental pattern of male reproductive organ in *Sarconema* is slightly different from the typical pattern as described in *Hypnea* (HEWITT 1960), in *Callophycus* (MIN-THEIN and WOMERSLEY 1976) and in *Solieria* (GABRIELSON and HOMMERSAND 1982). However, the general pattern of development and the size of

the spermatangia are almost similar in all the members of Gigartinales.

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## 新 刊 紹 介

**西澤一俊（1986）ワカメが高血圧も成人病もハネ返す** 主婦の友社，191頁，690円

健康食品海藻，ミネラルとビタミンに富むシーヴェジタブルなどのキャッチフレーズとともに海藻の食品化が喧伝され，それに伴って最近一般家庭向に書かれた海藻食品の小冊子の幾つかが店頭を飾るようになった。しかし，題名や宣伝文ほどに内容が満足出来るものは少なく，このところ健康食品としてこの海藻の優秀性をわかりやすく書いた本の出現が待ち望まれていた。今回出版された本学会元会長西澤一俊博士の「ワカメが高血圧も成人病もハネ返す」は一見奇をてらった題名と言えなくとないが，内容はそうではなく，実に科学的に人体生理と薬理の立場から海藻の効能を誰にもわかるように解説している。一例として，コンブやワカメのヌメリの記述を紹介しよう。“コンブを一片コップの水につけておくとトロッとした成分がとけ出します。この上澄みを毎日飯んでいると動脈硬化や高血圧，脳卒中が防げるといわれています。この上澄みに出てきたのは「ヌメリ」の成分で，ヌメリはアルギン酸とフコイダンという多糖類にわずかにタンパク質がまじったものです。この多糖類は①消化吸収されにくい，②酸性で種々のミネラルと結合しやすいことが大きな特徴です。コンブやワカメに含まれるアルギン酸はカリウムやカルシウムなどと結合した形で存在し，強い酸性状態の胃の中ではこれらのミネラルを分離します。腸の中は胃と反対にアルカリ性です。そこで改めてミネラルと結合しようとするのですが，このとき一番手近にあって量の多いのは食塩が分解してできたナトリウムイオンです。アルギン酸は体内にカリウムやカルシウムを残し，血圧を上げるナトリウムを道づれにした形で便の中に出てくるのです。こうして血圧を下げる働きをすることになるのです。

文章は多少かえてあるが，ほぼこういった調子で，時に成分表や臨床実験例などをあげ，ワカメを主体として海藻と動脈硬化，脳卒中，制ガン，ミネラル，美容との関係などを5章にわたってわかりやすく解説している。さらに各章には料理研究家，木村民恵氏によるワカメ料理が紹介され，その数は計50に及ぶ。栄養面から海藻を活用したいと考える人々に広く推奨したい本であり，また藻類の専門家にも教えられるところが多く，充分読みごたえがある。

（筑波大学生物科学系　千原光雄）

藻類 Jap. J. Phycol. **35**: 221–225, September 20, 1987

# アカモクにおける雌雄同株個体と秋季の成熟

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OKUDA, T. 1987. Monoecism and autumn-fruiting of *Sargassum horneri*. Jap. J. Phycol. **35**: 221–225.

Although *Sargassum horneri* was reported to be strictly dioecious, the author chanced to collect on the coast of northern Kyushu a few fragments of this alga with androgynous receptacles. The receptacle is lance-shaped at its base and male conceptacles are exclusively found here, and the rest of it is predominantly occupied with female conceptacles. The proportion of male to female part in a receptacle is variable even in a plant. Hermaphrodite conceptacles occur in rare cases. Some plants have male receptacles along with androgynous ones.

Though *S. horneri* generally liberates eggs in April and May around Fukuoka, northern Kyushu, a population was confirmed in the western Seto Inland Sea to become fertile around November. Same was the case in Yanai, Yamaguchi Pref. where the population flourished in autumn instead of spring. Receptacles of this population are much more slender, and some of them produce leaves, vesicles, and vegetative areas with no conceptacles.

*Key Index Words: fruiting; monoecism; Phaeophyceae; Sargassaceae;* Sargassum horneri; *sexuality.*

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褐藻ホンダワラ科のアカモクはわが国の周辺でごく普通にみられる種類で，従来いろいろな分野での研究に用いられてきた。そのため多くの性質が明らかにされており，雌雄性については厳密に雌雄異株と考えられている。しかし筆者は1979年の春以来，若干の機会に少量ながら雌雄同株の個体を採集してきた。筆者はすでに4回にわたってアカモクと外観は非常によく似ているが雌雄同株であるシダモクについて報告し，両者の類似点，相違点を明らかにしている。今回得られたアカモクには雌雄性に関してシダモクと非常によく似た性質がみられたので比較して述べたい。

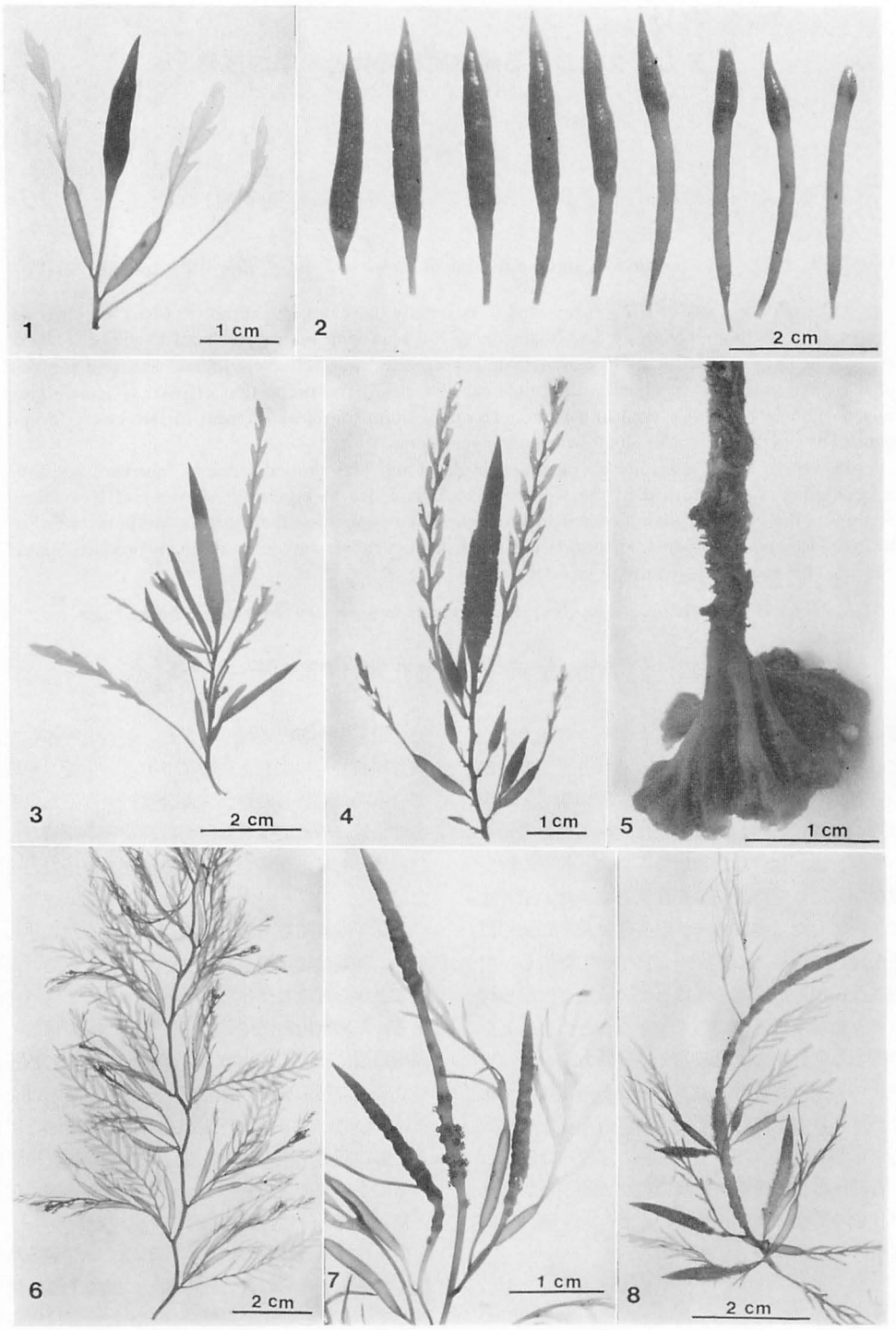
一方北部九州におけるアカモクの成熟時期は4～5月頃であり，日本全体としてもほぼ春から夏の間に限られているが（丸伊ら1981），11月頃が成熟の盛期と思われる群落の存在を瀬戸内海で確かめることができたので，この時期の個体が示すいくつかの性質についても春季のものと比較して報告する。

## 雌 雄 性

材料：雌雄同株の個体を得た場所は全て福岡市外の津屋崎である。このような個体にはじめて気付いた1979年3月25日以来現在までに17回採集した。時期として最も早かったのは2月7日，最もおそかったのは5月20日で，いずれも1982年である。岸近くを漂流している場合もあったが，大部分は打上げられたものであり，量は極めて少なかった。1979年5月12日に得た材料はひもで舟からつり下げ，幼胚の形成経過を調べた。

外形：附着器をもった完全な個体はまだ入手していない。外観は通常のアカモクと同じで，気胞，冠葉，葉などから雌雄異株のものと区別することは不可能である。気胞は円筒形で，だ円形に近いものはなく，津屋崎周辺に生育しているものに比べるとむしろ長めでアカモクの特徴をより強く備え，形態上からはシダモクに近いことはない。外観から雌性生殖器床と思われるものは基部が楔形に細くなっている（Fig. 1）。通常のものやシダモクのものでは，基部が多少細くなることはあっても一般に鈍円形である。

雌雄性：外観が雌の生殖器床に似ていながら基部が細くなっているものは，その部分に雄の生殖器巣を内蔵している。この部分以外のほとんど全ては雌の生殖器巣で占められている。まだ卵を放出していないものでは基部以外は濃褐色を示すのに対し，雄の部分は色がうすく，黄色味をおびているので，外形と合わせてこのような生殖器床の識別は容易である。このような




Figs. 1–8. *Sargassum horneri*. 1–4, androgynous; 5–8, autumnal.
1, androgynous receptacle with cuneate base; 2, variable proportion of male to female part; 3, male and androgynous receptacles; 4, egg liberation from an androgynous receptacle; 5, holdfast; 6, a part of branch; 7, female receptacles with verrucous appearance; 8, female receptacle with leaves, vesicles, liberated eggs, and vegetative areas.

生殖器床をもつ個体には，完全な雌性生殖器床はみられなかった。

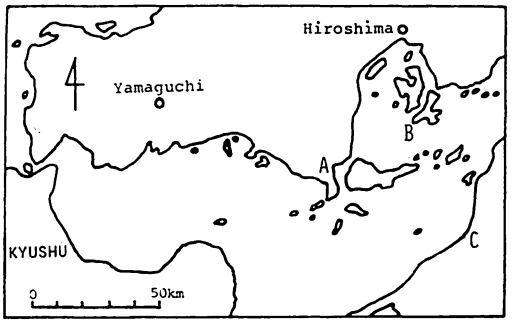
それぞれの生殖器床内で雄性生殖器巣が占める割合は一定せず，基部で一部を占めるもの，下半分を占めるもの，先端近くまでを占めるものなどいろいろな例がみられた（Fig. 2)。雄の部分は細く，雌の部分は太い。最も普通なものは Fig. 1 に示す外形のものである。切片を作って調べたところ，細い部分に雌性生殖器巣はみられなかったが，太い部分には雄性生殖器巣が少数ながら散在していた。また非常に少数ではあるが雌雄同巣（hermaphrodite）のものも太い部分に観察された。この場合，生卵器，造精器は巣内に相対して形成され，混在したり，放出口近くと奥とに分かれることはなかった。1生殖器床の全体をハンドセクションによって連続切片とし，雌雄同巣の生殖器巣がどの程度あるかを調べたところ，全く観察できないことも多かったが，存在する場合は普通1個，多くても2個であった。

採集した雌雄同株個体のうち少数のものは前記雌雄同床（androgynous）の生殖器床の他に雄性生殖器床も同時に付けていた（Fig. 3)。このような外観は全く雄性である 22 mm の生殖器床をハンドセクションで約 0.3 mm の厚さに切って調べたところ，雌性生殖器巣を1個だけ先端部で観察した。すなわちそれらは完全に雄性である場合が多いが，少数の雌性生殖器巣が含まれている場合もある。

幼胚形成：卵放出から仮根形成までの経過は，すでに報告のあるアカモク，シダモクの場合と同様であった。すなわち卵放出は1生殖器床の基部から先端部に向かい，帯状に何回かに分けて行われる。最初の放出では，最下部は雄性生殖器巣で占められているので，すこし底上げされた外観となる（Fig. 4)。放出卵が8核をもつこと，精子の侵入部位と考えられている帯状の膨潤部があること，仮根細胞の分裂は放射八細胞型であること，第二次仮根が遅れて出現し，急速に伸長して第一次仮根と区別できなくなることなどが観察された。

### 秋に成熟する個体群

調査場所及び消長の概要：秋に成熟するアカモクの群落をみたのは瀬戸内海の2か所で，山口県柳井及び広島県黒島である（Map 1)。柳井（A）の生育地は波の穏やかな場所で，秋の大潮の低潮時に水深は 1〜2 m 程度である。砂泥質の海底に散在する大小の石に着生し，波立つと海が濁る場所にも多数生育する。黒島（B）は安芸灘西部，倉橋島の南西端近くに位置する周囲 2.1 km の無人島で，柳井からは直線距離で約 35 km の北東にある。波当たりはよく，生育水深は柳井のものより深くて 3 m，あるいはそれ以上と思われる。




Map 1. Locations of autumnal *Sargassum horneri* in the western Seto Inland Sea. A, Yanai, habitat; B, Kuroshima (uninhabited islet), habitat; C, Iyo, beached fragments.

藻体と生殖器官の消長の概要を Table 1 に示す。柳井での観察によると，3〜4月には藻体はみられず，7月には 5〜6 cm の幼体となり，9月下旬には 1 m 以下のものも 2 m 以上のものも混在しているがまだ生殖器床はない。卵放出は10月下旬〜11月上旬に始まり，11月中旬〜下旬が盛期と思われる。年によって差があり，10月下旬には卵放出のみられる年も，まだみられない年もある。黒島の観察は12月2日の1回だけであるが，外観では成熟の盛期は過ぎているものの，まだ末期ではないように思われた。

外形：どの面からみてもアカモクの特徴を備えているが，全般的に細づくりである。附着器は仮盤状であるが（Fig. 5)，指状の隆起は目立たぬこともあり，うすくて盤状とまぎらわしいものもある。藻体は長くても附着器は直径 7.5 mm，立上がる単条の茎は太さ 1.6 mm 程度と細いものが多い。気胞は円柱状で典型的なアカモクといえる。葉も同様であるが一般に羽状裂片は細く，裂け目は深い。裂片の先端が叉状あるいは掌状に分裂する場合もあり（Fig. 6)，幼体や基部においては目立つが必ずしも一般的ではない。

生殖器床は春季に成熟する個体群のものと同様円柱状であるが，雌，雄ともに細い。とくに雌のもので著しく，すでに卵を放出して褐色味のうすくなったものでは雄とまぎらわしいほどである。生殖器床内での生殖器巣の形成は一様でなく，一部に生殖器巣のない部

Table 1. Occurrence of thalli and of reproductive organs in autumnal *Sargassum horneri*.

| Date | Locality | Thallus | Receptacle | Discharged eggs |
|---|---|---|---|---|
| 1982 | | | | |
| Nov. 6 | Yanai | ++ | ++ | ++ |
| Dec. 2 | Kuroshima | ++ | ++ | ++ |
| 1983 | | | | |
| Mar. 19 | Yanai | − | − | − |
| Sept. 22 | Yanai | ++ | − | − |
| Oct. 21 | Yanai | ++ | + | − |
| Nov. 9 | Yanai | ++ | ++ | + |
| 1984 | | | | |
| Apr. 4 | Yanai | − | − | − |
| Oct. 22 | Yanai | ++ | + | + |
| Nov. 5 | Yanai | ++ | ++ | ++ |
| 1985 | | | | |
| Jul. 12 | Yanai | + | − | − |
| Oct. 25 | Yanai | ++ | + | − |

++ present in common, + present a few, − non.

分が介在することも珍しくない。このような部分は1か所がくびれたように細くなることも，2〜3 mm にわたることもある。生殖器床が細いため，生殖器巣のある部分が紅藻オゴノリの嚢果を思わせる外観となることもある（Fig. 7）。

生殖器床から葉や気胞を生ずる例がしばしばみられる。またそれらは1個とは限らず，むしろ数個あるいは数組生じ，さらに小型の生殖器床を含むこともある。図に示したのは 6 cm の生殖器床で（Fig. 8），葉，気胞，生殖器床，放出卵，中間の無性の部分などのあることがわかる。雄性生殖器床にも同様な例がみられたが，頻度は雌性の方が高いように思われた。

雌雄性については同床も同巣もまだ観察していない。

## 考　　察

雌雄同株のアカモクは，どの時も大量の材料の内から選び出したもので，アカモク全体としては極めて少量と思われる。比較的打上げ藻が少ない年には採集できなかったこともある。現在のところ筆者が採集したのは津屋崎だけであるが，筆者以外の採集が1例ある。すなわち九州大学の標本庫に保管されている1枚のおし葉で，山本虎夫氏が1957年3月28日，京大瀬戸臨海実験所前で採集されたものである。代表的な生殖器床は Figs. 1, 4 に示すように基部が細くなっており，放出卵を付けている。またこの個体には上半分が雌と下半分が雄で上部に放出卵があるという生殖器床もみられる。これらのことは津屋崎のものと一致する性質であり，雌雄同株個体の分布はさらに広範囲にわたることが予想される。

雌雄同株のアカモクについては KÜTZING（1860）の報告がある。図示された個体は4個の生殖器床を付けているが，そのうち2個は雌性である。しかし他の2個は上半分が太くて下半分が細く，説明文にも上部に生卵器，下部に造精器を含むとしている。おし葉を作る際，材料における卵の放出状態によっては完全な雌性生殖器床であってもこのような外観になることがあり，また図では基部が今回の Fig. 1 のものより鈍円形である点で KÜTZING の材料については検討を要すると思われる。

本観察では，雌雄同株個体の多くは1生殖器床内に雌と雄の生殖器巣をもち，雄のものは生殖器床の基部のみにみられたが，シダモクの場合と同様に（奥田 1977）雄の部分の割合には変化があること，かつ少数ながら雌雄同巣の生殖器巣も存在することを明らかにした。日本産ホンダワラ属の種類が示す雌雄性で雌雄同巣が観察された例は *Bactrophycus* 亜属ではアカモク以外に3種で報告されている。すなわちシダモク（沢田1956），タマハハキモク（OGAWA 1976），エゾノ

ネジモク(小河1977)である。これらの他に小河(1977)が *Eusargassum* 亜属の5種にも同巣のものがあると考察の項で記しているのは山田（1942）の報告に基づく。山田は南日本産ホンダワラ属の18種を3報にわたって報告し，雌雄性にもふれている。小河が同巣と考えた5種はヒラエモク，ヒュウガモクが其一に，フクレミモク，キレバモク，カタワモクが其三にそれぞれ記述され,「同一器托内ニ雌雄両生殖窠ヲ生ズル」と表現されている。しかしウスバモクなど其二に記述されたものは「同一器托内ニ雌性並ニ雄性ノ生殖窠ヲ生ズル」とされている。後者が同床異巣 androgynous を示すことは明らかであるが，前者は後者と表現が異なるため雌雄同巣 hermaphrodite とも考えられる一方，異巣と解釈することも可能である。従来の報告のように androgynous の生殖器床内には hermaphrodite の生殖器巣が含まれる可能性が高いとはいえ，確認を要することと思われる。

秋に成熟する個体群がみられた柳井での3，4月の調査では，秋季に成熟するものの幼体も，北部九州で4～5月頃成熟する外観のものもみられなかった。したがって今回報告したアカモクは1年生であり，11月頃に年1回だけ成熟する個体群であることは明らかである。新井ら（1985）がウミトラノオで明らかにした春秋2回の成熟は，多年生であるその種の同一個体が示すものであり，一方アカモクにみられる4月頃と11月頃の2回の成熟は異なった個体群が示すものであり，異質である。

秋に成熟する個体群の分布についてはさらに広い範囲での調査を考えているが，筆者は1984年1月10日，愛媛県伊予市（C）で少量の秋に成熟する個体を打上げで得ている。瀬戸内海では中国側，四国側ともにもっと広範囲で生育しているものと予想される。秋に形成される生殖器床が示す多様な不規則な例は，春のものでも皆無ではないが，出現の頻度は著しく低い。春秋の別の時期に成熟する両個体群については今後も採集につとめて多くの点で比較し，両者の関係を追究したい。

## 謝　辞

雌雄同株のアカモク標本を寄贈していただいた山本虎夫氏にあつく御礼申し上げる。秋に成熟するアカモクの採集に当たっては水産大学校の方々に便宜をいただいた。すなわち同校田名臨海実験実習場長松井敏夫氏，同校大貝政治氏，同実習場滝沢敬氏，三木浩一氏である。また吉岡俊夫氏にも同実習場在職中に有益な教示をいただくとともに材料の採集に協力していただいた。また広島県水産試験場高場稔氏には黒島の調査について便宜を図っていただいた。感謝する次第である。この報告に用いた写真は当水産学科木村清朗氏の手を煩わしたものであり，記して深謝する。

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# 緑藻リボンアオサの培養における生活史

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MIGITA, S. and FUJITA, Y. 1987. The life history of *Ulva fasciata* DELILE (Chlorophyceae, Ulvales) in culture. Jap. J. Phycol. **35**: 226–230.

The life history of *Ulva fasciata*, collected from Nomozaki, western Kyushu, was studied in laboratory culture. The formation of gametes and zoospores was observed almost throughout the year at Nomozaki. The gametes are pear-shaped, with two flagella, one eyespot and showing positive phototaxis. The gametes from different sex conjugated anisogamously. The zygotes began to germinate and developed into lanceolate thalli, having single and lobed blades. Parthenogenetic germination of female and male gametes was also observed. The dentate cells began to form along the marginal parts after the thalli attained to length of 1–2 cm. They were abundant on young thalli, but scarce on fully grown or adult thalli. The zoospores were produced on the thalli developed from zygotes. The zoospores are bigger than the gametes and have four flagella, but their shape and behavior closely resemble those of the gametes. The chromosome number of this alga was counted to be nine in haploid phase and eighteen in diploid phase. The results show that the life history of *Ulva fasciata* consists of an alternation of isomorphic generations.

*Key Index Words:* Ulva fasciata; *Chlorophyceae*; *Life history*; *Chromosome number.*
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緑藻リボンアオサ *Ulva fasciata* は，体がリボン状の裂葉に分かれ，縁辺に顕微鏡的鋸歯を持つアオサ属の1種で，世界各地の暖海域に生育する。日本では YAMADA (1935) によって沖縄でその生育が報ぜられ，その後日本の中南部に広く分布することが知られている。本種の生殖については配偶子や遊走子が知られているが (GAYRAL 1963, SUBBARAMAIAH 1970, MSHIGENI and KAJUMULO 1979)，生活史の一循環は明らかにされていない。また，日本では梶村 (1973) が島根県産のリボンアオサで配偶子の単為生殖を繰返していると報告している。

筆者らは，長崎市付近に生育するリボンアオサで，配偶子と遊走子の形成，放出を以前から観察していたが，1984年に室内培養でその生活史を完結し，葉体一葉体のアオサ・アオノリ型（シオグサ型）をとることを明らかにしたので，生育生態や染色体数の観察と合せて報告する。

## 材料と方法

培養実験に用いたリボンアオサは，長崎県野母崎町の野母港内に設置してある長崎大学水産実験所の養殖筏より1984年6月に採集した。それらを1個体毎に管瓶に入れ，まず2鞭毛の配偶子を放出したものを選び，さらに接合を調べて雌雄の組合せで配偶子液を等量同一シャーレに入れ，負の走光性で暗所に集まった接合子をピペットアップして培養を開始した。接合子の発芽体は約 1 mm の長さまではシャーレの止水中で培養し，その後は枝付平底フラスコで通気培養した。また，それらの生長した胞子体が成熟し，放出した遊走子を接合子の場合と同様にして培養した。培養は20～22℃，12：$\overline{12}$の光周期，白色蛍光灯光 2,500 lux のもとで行ない，培養液には PES 液を用い，止水では1週間，通気では2日毎に換水した。

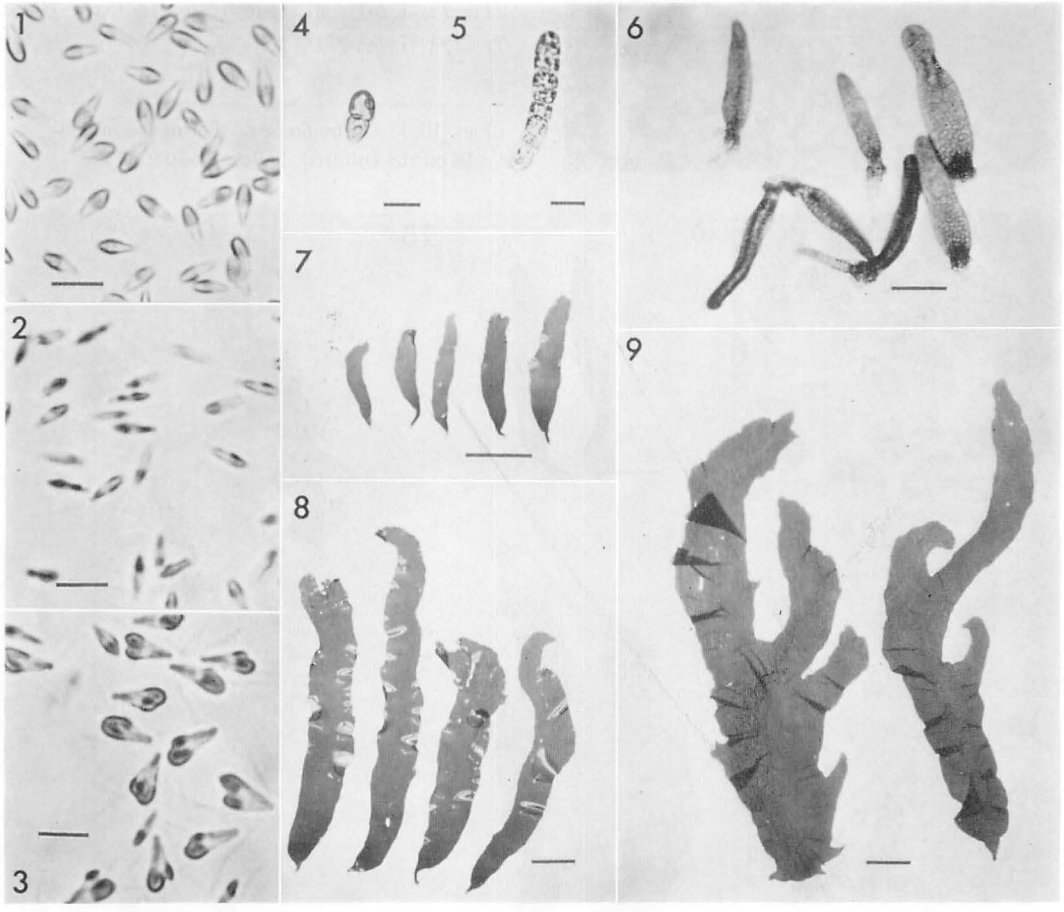
天然の生育生態は，前記水産実験所の筏および長崎港内の浮桟橋に付着生育するリボンアオサ群落から，1984年より2年間機会ある毎に20～30個体を採集し，形態，生殖，繁茂状態などの概略を調査した。また，染色体数を観察した核染色には，酢酸アルコール固定，WITTMANN 法を用いた。

## 結　　果

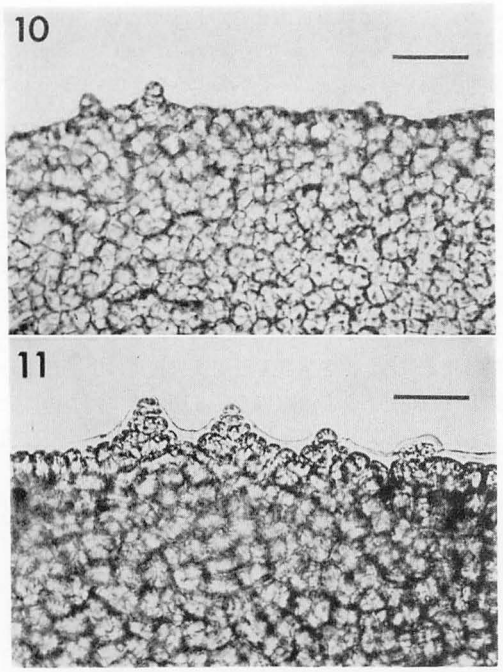
リボンアオサは，長崎市周辺では潮間帯下部の岩上

にもまれにみられるが，常時海中にある筏や桟橋の側壁で多く生育している。本種の葉体は周年みられ，一般に春より初夏にかけてよく繁茂し，初秋の一時期衰微する。これらの場所ではアナアオサと混生するが，本種はアナアオサのように穴があかず色がややうすく，とくにリボン状の裂葉を持つなど，両種の区別は容易である。生育期間中は何時でも成熟葉体がみられ，それらの放出胞子は時期や場所により配偶子が多い場合，遊走子が多い場合があるが，常に両者がみられた。また，1年間を通じてみると配偶体の雌雄比はほぼ1：1であった。

リボンアオサの配偶子は，長い西洋梨型で1個の色素体と眼点を有し，等長の約 11 $\mu$m の2鞭毛を頂生する。配偶子は雌雄で大きさが若干相違し，雌性配偶子は長さ 6.5～8.0 $\mu$m 太さ 3.0～4.2 $\mu$m で (Fig. 1)，雄性配偶子は長さ 5.5～7.0 $\mu$m 太さ 2.0～2.5 $\mu$m であった (Fig. 2)。なお，雌性配偶子嚢内では8，16まれに32個，雄性配偶子嚢内では16，32きわめてまれに64個の配偶子がつくられた。雌雄の配偶子は1，2分以内の短時間でよく接合し (Fig. 3)，配偶子が正の走光性を示すのに，接合子は負の走光性でシャーレの暗い方に集るので，ピペットアップで容易に接合子のみを分離培養できた。接合子は付着後径 4.2～5.0 $\mu$m の球状となり，直ちに発芽して3日後には上下に2分裂し (Fig. 4)，7日後には6～8細胞の1列の体に生長した (Fig. 5)。発芽体はやがて下部の細胞から糸状



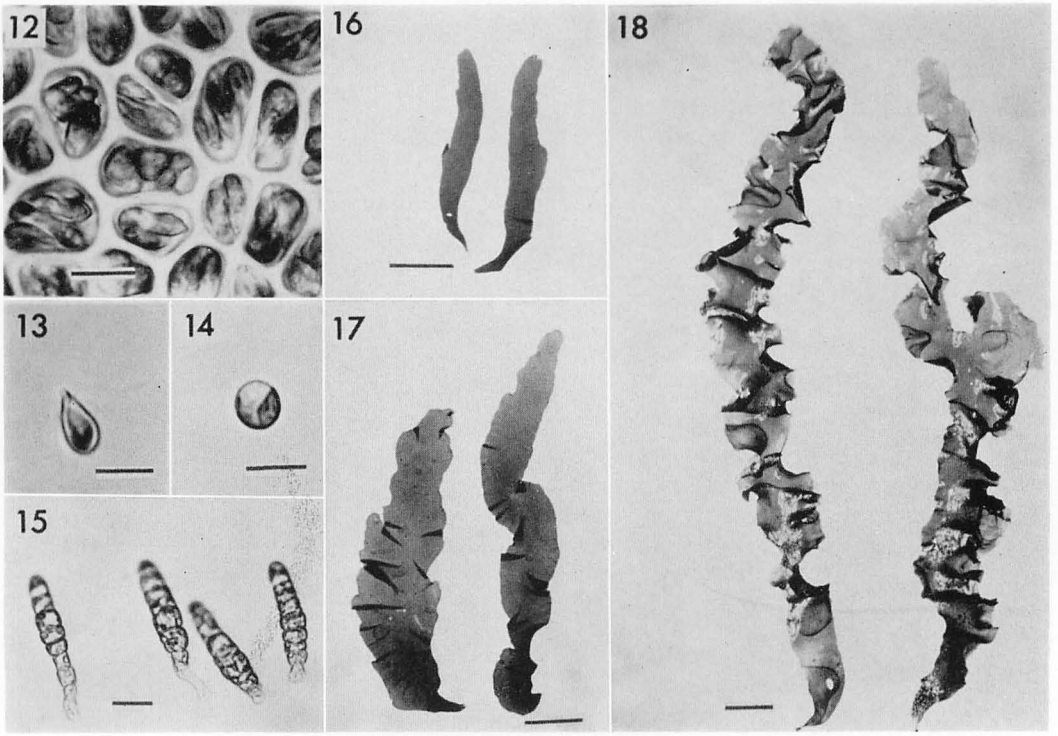

Figs. 1–9. *Ulva fasciata*. Gametes and development of zygotes.
1. Female gametes. 2. Male gametes. 3. Fusion of gametes. 4. 3-day-old germling from the zygote. 5. 7-day-old germling. 6. 25-day-old germlings. 7. Young sporophytes from the zygotes after 35 days culture. 8. 45-day-old sporophytes, forming lobes. 9. 2-month-old sporophytes, having deeply lobed blades.
Scale: Figs. 1–5 10 $\mu$m; Fig. 6 100 $\mu$m; Figs. 7–9 1 cm.



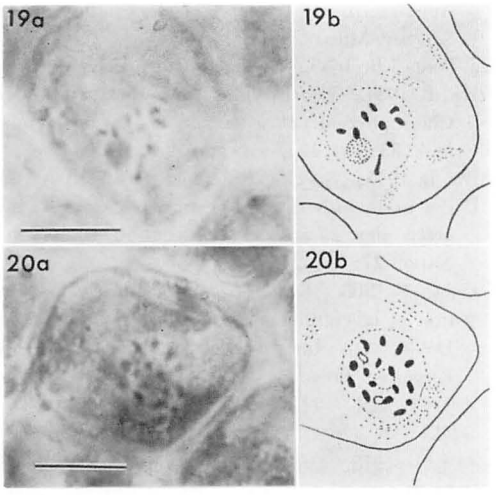

の仮根を出し20日後には長さ0.7～0.9 mm 幅 250 μm に生長し（Fig. 6），その後枝付フラスコで通気培養したところ，25日後には 1.8～2.2 mm，35日後には 2～3 cm（Fig. 7），さらに45日後には 5～6 cm に達した（Fig. 8）。

この間，1～2 cm に伸長した葉体の縁辺に，顕微鏡的鋸歯が形成されるようになった（Fig. 10, 11）。通気と止水培養では，前者で鋸歯が早く多く形成され，止水では鋸歯形成が遅れる傾向がみられた。また，5 cm 以上の葉体になって縁辺部で分裂するものがみられるようになったが，裂葉の初期のもので鋸歯が起原となって分れたような部分も観察された。培養2カ月後になると，葉体は 12～15 cm に達し，裂葉もリボン状に細長くなり，天然の葉体に類似した体に生育した（Fig. 9）。しかし，培養葉体のなかには 15 cm 以上になっても分裂せず単葉のものも少なくなかった。な

Figs. 10, 11. *Ulva fasciata*. Formation of dentate cells on the cultured thallus. Scale: 50 μm.




Figs. 12–18. *Ulva fasciata*. Zoospore formation and development of zoospores.
12. Zoospore formation on the cultured sporophytes. 13. Zoospore.. 14. Settled zoospore. 15. 7-day-old germlings from zoospores. 16, 17. 30-day-old (16) and 45-day-old (17) gametophytes. 18. 2-month-old mature gametophytes.
Scale: Figs. 12–15 10 μm; Figs. 16–18 1 cm.




Figs. 19, 20. *Ulva fasciata*. Chromosomes in somatic cell division.
19a. Chromosomes at late prophase in the gametophyte. 19b. Drawing of Fig. 19a (n=9). 20a. Chromosomes at late prophase in the sporophyte. 20b. Drawing of Fig. 20a (2n=18).
Scale: 10 μm.

お，接合しなかった雌雄の配偶子は両者ともに単為生殖で成体まで生長した。

遊走子嚢は，接合子の培養2カ月後の葉体で縁辺部から形成され，その形成部は黄褐色を呈し肉眼でも識別できた。遊走子は，胞子嚢内で4，8個まれに16個つくられ（Fig. 12），配偶子と同じ外観を呈し，約11 μmの4鞭毛を頂生し，長さ10.0～11.5 μm太さ5.5～7.0 μmで，正の走光性を示した（Fig. 13）。遊走子は付着後径7.0～7.5 μmの球状になり（Fig. 14），直ちに発芽し7日後には数細胞に生長した（Fig. 15）。約1 mmになったものを通気培養に移したところ，発芽30日後には2～3 cm（Fig. 16），40日後には7～8 cmになり（Fig. 17），2カ月後には12～15 cmに達し，一部の葉体は成熟して配偶子嚢を形成した（Fig. 18）。遊走子の発芽体でも1，2 cm以上で鋸歯の形成がみられ，10～15 cmの体で裂葉を出すものもあったがその個体数は少なかった。

本種の染色体数をWittmannの核染色法で調べてみたところ，体細胞の核分裂前期の終りの像で，配偶子を放出した葉体でn=9の染色体がみられ（Fig. 19 a，b），一方遊走子を放出した胞子体では2n=18の染色体が観察された（Fig. 20 a，b）。

## 考　　察

アオサ属の生活史については，多くの種で葉体—葉体の同型世代交代をすることがよく知られている（Föyn 1934, Yamada and Saito 1938, Smith 1947, Bliding 1968, Chihara 1968）。リボンアオサにおいても，配偶子や遊走子の形成が報告されているが（Gayral 1963, Subbaramaiah *et al.* 1966, Subbaramaiah 1970, Mshigeni and Kajumulo 1978），梶村（1973）は島根県産の本種で配偶子の単為生殖を繰返していると述べている。この研究で，長崎市周辺に産するリボンアオサは，アオサ属の他の多くの種と同様に，配偶子と遊走子を形成し，単相の配偶体と複相の胞子体の両世代が交代することが明らかになった。

アオサ属では，配偶子の接合で異型または同型配偶が報ぜられているが，異型配偶を行なう種が多いようである。すなわち，外国産の種では *Ulva lobata*, *U. angusta*, *U. stenophylla*, *U. linza* (Smith 1947), *U. lactuca* (Föyn 1934, 1955, Bliding 1968), *U. rigida*, *U. gigantea*, *U. rotundata*, *U. curvata* (Bliding 1968) などで，日本産ではアナアオサ，ナガアオサ（Chihara 1969）などで異型配偶とされている。リボンアオサでもそれらの種と同じく，雌雄配偶子の大きさが異なり，異型配偶を行なうことが判明した。接合子および遊走子の発生形式は，梶村（1973）が配偶子の単為生殖で述べているように直接型を示し，新崎（1946）のアオサ・アオノリ型，Föyn（1934, 1955）の *Ulva lactuca* type であった。

ところで，リボンアオサは縁辺に顕微鏡的鋸歯を有することが種の一つの特徴として知られている（Agardh 1883, De Toni 1889, Collins 1909）。この培養実験でも1～2 cm以上の葉体で鋸歯形成がみられた。しかし，天然では若い葉体には鋸歯があるが，成体ではほとんどみられなくなる。これは，大型葉体では一般に鋸歯の形成が少ないこと，また縁辺部が胞子放出，流失を繰返すことなどのためと考えられる。Segawa（1936）は三宅島産の鋸歯のあるアオサを *Ulva spinulosa*（新種）として記載している。その後，一木（1956）は同種が長崎県女島に分布するが，リボンアオサなど他の鋸歯のある種と比較検討する必要があると述べている。著者らは，三宅島，女島産のアオサ属を調べていないので即断はできないが，両産地がリボンアオサの分布範囲の温暖な海域であること，裂葉を有すること，また葉長が2～3 cmでリボンアオサとすれば鋸歯の多い長さであることなどから，*U. spin-*

*ulosa* はリボンアオサの幼体ではないかと考える。また，アミアオサ（岡村 1936），ボタンアオサ（一木 1956）に鋸歯があるような記述もあるが，アミアオサでは確認できず（右田・藤田 1984），長崎産のボタンアオサの培養でも著者らのこれまでの観察では鋸歯はみていない。

なお，本種は熱帯地方で肥料，飼料藻として利用され，海での移植，養殖試験が試みられており（SUBBARAMAIAH 1970, MSHIGENI and KAJUMULO 1979），波静かな場所で生長が良好であるとされているが（MSHIGENI and KAJUMULO 1979），長崎市付近でもやや内湾で生育が多いようである。

アオサ属の染色体数については，*U. lactuca* で n=10（CARTER 1926），n=13，2n=26（FÖYN 1934），アナアオサで n=9，2n=18（YABE and PARK 1968）とされている。この研究でリボンアオサの染色体数は n=9，2n=18 で，上記のアナアオサの数と一致した。

終りに，この研究を進めるにあたり，文献などのご教示をいただいた北海道大学吉田忠生，九州大学奥田武男の両教授に心から感謝の意を表する。

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# 川嶋昭二：外国産コンブ目植物の漂着記録（3）エナガオニコンブについて

Shoji KAWASHIMA: Drifting records of alien species of the Laminariales (3). *Laminaria diabolica* MIYABE f. *longipes* MIYABE et TOKIDA

**(3) *Laminaria diabolica* MIYABE f. *longipes* MIYABE et TOKIDA エナガオニコンブ**

オニコンブは北海道東部の厚岸附近から根室半島周辺を経て知床半島東岸一帯に生育し，また南千島のクナシリ，エトロフ両島や，サハリン南端の亜庭湾や西能登呂岬周辺および沿海州にも分布する（宮部1936）。

宮部（1902）が初めて霧多布産のオニコンブに基づいて記載したと思われる性質は，茎が短く，葉は基部が円く，非常に幅広く，かつ中帯部の厚さにくらべて縁辺部が薄く，著しく波縮する特徴をもっている。一方，永井（1936）は潮流の静穏な所では茎が短く，葉は基部が円く，かつ幅広くなるが，潮流の烈しい所では茎が長く，葉は基部くさび形で幅もせまくなるという一連の傾向があることを示している。著者の経験でも本種の形態的特徴は生育地によって大きく変化し，中でも茎の長さと葉の基部の形の違いが注目される。

ところで，サハリン南端の西能登呂岬やクナシリ島の根室海峡に面する地方には茎長が 30〜70 cm に達するものがあって，このような葉体は葉長 7〜13 m，葉幅 20〜40 cm と非常に大きくなるが，その基部はせまいくさび状で，中帯部の厚さは 2〜3.5 mm しかなく乾燥すると破れやすい（NAGAI 1940, TOKIDA 1954）。宮部（1936）はこのような特徴をもつオニコンブを f. *longipes* MIYABE et TOKIDA エナガオニコンブとし，北海道沿岸に多い基本型と区別した。この品種にあたるコンブは北海道では利尻島のコンブ養殖用ロープに着生したものが発見されただけで（山本・鳥居1983），天然の岩礁などに生育しているという記録はない。

さて，第1図に示したコンブは1971年10月（発見日不明）に知床半島東岸の羅臼町沖合約 5.5 浬の海上を漂流しているのを漁船により発見されたものである。葉体は少し破れているが，全長 6 m，大きな円錐状の附着器を持ち，茎は長さ 28 cm，基部ほぼ円柱状で径 2 cm ほどあるが，上部は扁圧している。葉は長さ5.7 m，最大幅 38 cm，披針状を呈し，基部くさび状，中帯部は幅の約5分の3を占め，その下部で厚さ 3 mm ほど，縁辺部は大きく波縮して 1〜1.5 mm とかなり薄い。茎と葉に円形の粘液腔道がある。子嚢斑は形成されていない。

ここには漂流物の性質を簡単に述べたが，多くの点で宮部（1936）のエナガオニコンブの記載に良く適合し，また永井（永井1936, NAGAI 1940）がクナシリ島南部のハッチヤス岬附近，ポンコタンから報じたものに酷似する。漂流物の発見地点がこの岬のごく近くであることからみて，対岸の生育地から流れてきたと考えてまちがいないだろう。

次に，第2図に掲げたコンブは1984年8月27日，北海道オホーツク海沿岸北部の枝幸（えさし）町沖合約8浬，水深 110 m 地点で，ケガニかご漁のロープに

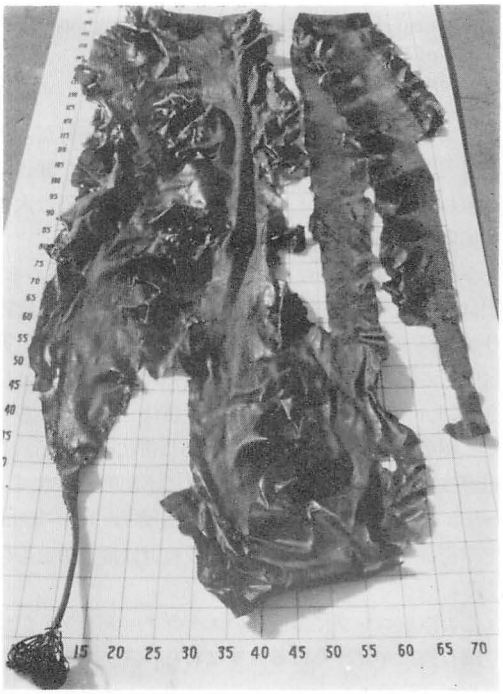


Fig. 1. *Laminaria diabolica* f. *longipes*, drifted on the sea of 5.5 nautical miles off Rausu (羅臼), Shiretoko Peninsula, Hokkaido. October, 1971. (6 m in length)

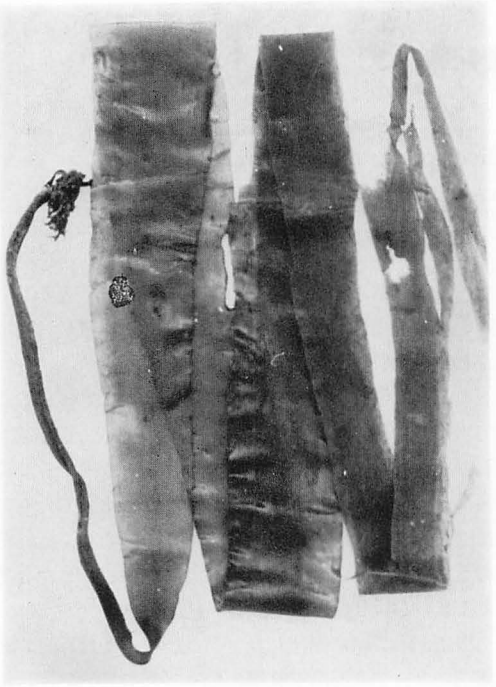

Fig. 2. *Laminaria diabolica* f. *longipes* prox. The driftage was entangled on rope of crab fishing cages set on the sea bottom at a depth of 110 m at about 8 nautical miles off Esashi (枝幸), the Okhotsk Sea coast of Hokkaido.
August 27, 1984. (6.3 m in length)

からまり引き上げられた。発見時の葉体は新鮮だったが附着器の大部分は失われ，また葉の上半分の縁辺部が破れたり葉面に少しすり傷が認められた以外は大きな損傷はなかった。

葉体は全長 6.3 m，そのうち茎が 90 cm を占める。根は 7 ～ 8 回分岐し，3～5 mm 太い。茎は基部ほぼ円柱状，径 2.3 cm あり，上部に次第に扁平になり，質は堅くて特に下部はほとんど木質化している。葉は長さ 5.4 m，基部やや円味あるくさび状で上部にゆるやかに幅広くなり，最大幅 21 cm あり帯状を呈する。中帯部は葉幅の 2 分の 1 ほどで非常に不明瞭，厚さ 2.5～3 mm，縁辺部は大きくうねり，厚さは 1.5～2 mm で中帯部とあまり変らない。粘液腔道は茎，葉とも存する。

この漂着コンブには前述のエナガオニコンブとかなり違う特徴が認められる。すなわち，茎が非常に長く全長の 7 分の 1 を占め，太く，ほとんど木質化して堅い。特に葉の基部がやや幅広いくさび状で全体に帯状を呈し，中帯部や縁辺の様子もオニコンブ本来の特徴と違う点が見られる。

このコンブの漂着地，枝幸町はサハリン南西端の西能登呂岬からわずか 110 km ほどの近距離にあり，また葉体の新鮮さから考えても同岬周辺か亜庭湾東部沿岸から比較的短時間で流れ着いた可能性が強い。しかし，著者の知る限りではこれらの地方を含めてサハリン全島やその周辺の北方海域からこのようなコンブはまだ報告されていない。また，サハリン南部や千島列島産標本を多数所蔵する北海道大学農学部標本室にもこの漂流物に相当する標本は見あたらない。

ここに紹介した二つの漂着コンブのうち，前者は典型的なエナガオニコンブと判断できるが，後者は上に述べたように多少の疑問が残っている。しかし，他の種類にする根拠が明確になるまでは茎の特徴から仮にエナガオニコンブとし，その 3 年目またはそれ以上の古い葉体として取り扱い，将来さらに正確を期したい。また，前報（川嶋1986）に述べた茎にオニワカメ幼体が着生していた“*Laminaria* sp.”とはこのコンブであることを附記する。

漂着コンブを提供下さった坂本富蔵氏と四ツ屋義則氏に，また標本調査に多大の便宜をいただいた北海道大学農学部四方英四郎教授に心からお礼申し上げる。

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（042 函館市湯川町 1 丁目　北海道立函館水産試験場）

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# 池原宏二：日本海沿岸における食用としてのホンダワラとアカモク

Koji IKEHARA: *Sargassum* (*Sargassum fulvellum* and *S. horneri*) as a food in the coast of Japan Sea

ホンダワラ *Sargassum fulvellum* は本州の日本海及び太平洋の両沿岸及び四国，九州の全沿岸に，アカモク *S. horneri* は北海道西岸から本州の日本海側及び太平洋側及び四国，九州の沿岸に広く分布する (YOSHIDA 1983)。両種とも岩礁上に生育する1年生のホンダワラ類である。両種は冬の佐渡海峡の代表的な流れ藻で，1月から2月にかけて急速に生長する。新潟市では2月頃にホンダワラは 1〜4 m の長さに，アカモクは 2〜10 m の長さになったものが打ち上げられる（池原・佐野 1986)。

ホンダワラとアカモクは古くから食用に供された。

ホンダワラは秋田県男鹿半島では「じばさ」，佐渡島では「ぎんばそう」，「人馬藻」，鳥取県東部と中部及び隠岐島では「じんばそう」と呼んでいる。ホンダワラについては良寛和尚が1812年頃に新潟県分水町で「ちむばそに　さけにわさびに　たまはるは　はるはさびしく　あらせじとなり」と歌っている。

現在，男鹿半島では3〜4月にホンダワラを刈り取り，地元では生で販売している（秋田県水産振興センター　山田潤一氏による)。佐渡島や隠岐島では乾燥品（Fig. 1　right）や塩蔵品として販売している。また，鳥取県東部及び中部では11〜2月頃に海岸に打ち上げられたホンダワラを選別し，乾燥して保存する。


図 1. ホンダワラ（右）とアカモク（左）の乾燥品

Fig. 1. Commercial dried specimens of *Sargassum fulvellum* (right) and *S. horneri* (left).

食用の際はその都度水に戻してから佃煮にしている（鳥取県栽培漁業試験場　古田普平氏による)。

アカモクは男鹿半島では「ずばさ」，「しばさ」，秋田県八森地方では「ぎばさ」と呼んで食用にしている（黒木　1962)。同県では一般にアカモクを「ぎばさ」と呼び，特に男鹿半島では「じばさ」（ホンダワラ）と区別している（山田潤一氏による)。秋田県のアカモクは粘り気が少ないために県外から生で購入し，冬に県内全域で食用にしている。

新潟県柏崎市ではホンダワラ類を「じばさ」や「ぎばさ」と呼び，このうちアカモクを食用にしている（海洋生物環境研究所実証試験場　山本正之氏による)。柏崎おけさに「吹けよ　西風　あがれよ　じばさ　可愛い殿さの　磯まわり」と歌われている。この作詩の年代に2説がある。1185年頃であるという説と，1666年頃という説がある（宮川　1978)。

佐渡島ではアカモクを「ながも」と呼んでいる。佐渡島前浜海岸では冬から春にかけてアカモクを刈り取り，俵につめて新潟県や福島県に出荷している。同地方の山間部では春の山菜の出まわるまでのつなぎの野菜として珍重した（浜口　1979)。この出荷は1958年頃に一度終った。

日本海沿岸の冬季の気象は厳しい。過去30年間（1951〜1980年まで，新潟地方気象台　1981）の統計によれば，12〜2月の新潟市は降雪日が44%，降雨日は22%である。海岸地方は北西の季節風が強く，陸地内では積雪があって，新鮮な野菜の入手は困難であった。このため冬に採取できるホンダワラ類（ホンダワラ・アカモク）は野菜の代用として利用されていたと考えられる。

現在，佐渡島前浜海岸や相川町二見ではアカモクを刈りとり，生のまま，あるいは乾燥品（Fig. 1 left）や塩蔵品として島内や新潟市，秋田県に出荷している。両津市東浜のアカモクの盛漁期は3〜4月である。1986年3月にはトロ箱一杯の価格が2,000〜3,000円，終漁期の4月には約400円であった。同期間に約1,000箱が出荷された（東浜漁業協同組合　川口徳一氏による)。

新潟市漁業協同組合では1985年にアカモクを漁獲物して扱った。2～4月に市内の鮮魚店やスーパーマーケットで生のまま販売された。1987年は暖冬のため漁期が1カ月早まり，1月から店先にならんだ。

また，新潟県山北町，聖籠町及び柏崎市では2～3月に打ち上げられたアカモクを塩蔵や冷凍して保存し食用にしている。

永見市ではアカモクを「ながらも」と呼んでいる。同市では1972年頃から，1月にアカモクを刈り取って秋田県の業者に販売している。また，1976年頃から地元の民宿でも利用するようになった。1982年には生で39トン，800万円の水揚げがあった（川崎　1982）。

能登半島ではホンダワラ類を食べる習慣はないが，最近，永見市の影響を受けて七尾市の店先にアカモクが生で販売されている（金沢水族館　佐野　修氏による）。

鳥取県東部ではアカモクを1907年頃には三杯酢として，または大根の細切りと混ぜて煮て食用とした（遠藤　1909）。現在は同地方では食用に供していない（古田普平氏による）。

新潟県ではホンダワラは酢のもの，おひたし，みその一夜漬，または大根の千切りと油いためにするし，アカモクはこの他にサラダ，みそ汁，雑炊などとして食用にしている。

北海道大学理学部植物分類学教室吉田忠生博士から投稿をお奨めいただき，また，ご校閲をいただきました。茲に厚くお礼申し上げます。

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（951 新潟市水道町1丁目　日本海区水産研究所）

# ニュース

## 1. 環境科学シンポジウム1987

主　催：環境科学シンポジウム実行委員会

日　時：昭和62年11月25日（水）～27日（金）

会　場：東京虎ノ門パストラル　〒105 東京都港区虎ノ門4-1-1　Tel 03（432）7261〈大代表〉

〔交通〕地下鉄銀座線虎ノ門駅下車徒歩8分，地下鉄日比谷線神谷町駅下車徒歩2分

一般講演時間：発表15分（予定）

一般講演申込締切：9月30日（水）必着のこと。

講演申込方法：B5 版400字詰原稿用紙1枚に(1)講演題目，(2)研究者名および所属機関（略名カッコを附す）（講演者に○印），(3)発表希望分野（下記の分類を参照）(4)連絡先，郵便番号，住所，所属機関，部局，電話番号を明記の上，実行委員会までお申し込み下さい。

講演の採否，順序，講演時間の調整等は実行委員会にご一任下さい。

講演要旨原稿：講演申込者にはシンポジウム所定の原稿用紙をお送り致します。（図表を含めて1枚）

参加費：要，当日渡しの講演要旨集代を含む。会場にて徴収し，事前の参加申込みは不要です。

懇親会：11月26日（木）18時より同パストラル宴会場にて。

講演分類：（2ケタの数字を記入）

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 環境動態 | 10 | 全般 | 11 | 気圏 | 12 | 陸域 | 13 | 海域 |
| | 14 | 水域地下水 | 15 | 物質循環 | 16 | 富栄養化 | 17 | 赤潮 |
| | 18 | 都市域 | 19 | その他 | | | | |
| 人体影響 | 20 | 全般 | 21 | 変異原性 | 22 | 遺伝 | 23 | 毒性・重金属 |
| | 24 | 呼吸器障害 | 25 | 地域生態 | 26 | その他 | | |
| 改善技術 | 30 | 全般 | 31 | 水処理・水圏 | 32 | 脱窒・脱リン | 33 | 固体廃棄物 |
| | 34 | 重金属 | 35 | 難分解性 | 36 | ガス・気圏 | 37 | 分解技術 |
| | 38 | その他 | | | | | | |
| 環境理念 | 40 | 全般 | 41 | 理念 | 42 | データベース | 43 | 計画 |
| | 44 | 環境教育 | 45 | 住民意識 | 46 | その他 | | |
| 環境情報 | 50 | 全般 | 51 | レーザー・レーダー | 52 | 計測 | 53 | 自動化 |
| | 54 | 計測法の開発 | 55 | その他 | | | | |

申込および連絡先：〒305 茨城県新治郡桜村　筑波大学大学院環境科学研究科内

環境科学シンポジウム実行委員会　実行委員長　山　中　　啓

（Tel 0298（53）4752，6598 何れも直通）

## 2. "The Chromophyte Algae" 国際シンポジウム

英国 Plumouth にて1988年4月5日から9日まで，The Systematics Assn. 主催で開催されます。詳細については下記のいづれか1名に問合せて下さい。

Dr. J.C. Green, The Laboratory, Citadel Hill, Plymouth PL1 2PB, U.K.; Dr. B.S.C. Leadbeater, Dept. of Plant Biology, Univ. of Birmingham, PO Box 363, Birmingham B15 2TT, U.K.

# ―学　会　録　事―

## 日本藻類学会主催海藻採集会（第2回ワークショップ）参加記

昨年，筑波大学での第1回ワークショップ（淡水藻類の採集・分類）に引続き，今年も日本藻類学会第11回大会終了後の3月31日から4月2日にかけて，第2回ワークショップが開催された。今回は「若狭湾の海藻採集会」というテーマで，京都大学農学部附属水産実験所を会場として行われた。参加者は総勢30名で大会終了後，路線バスにて舞鶴へ向った。

翌4月1日，梅崎勇講師により，本日の海藻採集地，日本海の潮汐及び海藻植生の特徴等について簡単な講義を受けた後，用意して戴いた自家用車に分乗して，採集地の福井県大飯郡高浜へ向う。天候は薄曇りでやや肌寒さを感じたが，雨に見舞われることもなく，まずまずの採集日和であった。採集は，湾口幅20～30m，奥行200m程の波静かな小湾，及び東に隣接する磯で行われた。各自思い思いの採集姿に身を固めて浜に集合し，梅崎講師から採集についての説明を受けた後，めいめい海藻採集に散る。湾奥には砂浜が拡がり，アマモ，ホンダワラ類，ハバモドキ類，その他の微小藻が見られた。湾口に向うに従い，転石帯や小岩盤が拡がり始め，フクロノリ，ワタモ，ハバノリ，マメタワラ，ヤツマタモク，アキヨレモク，アカモク，ウミトラノオ，ミヤベモク，マクサ，オバクサ，ムカデノリ，カバノリ等が観察できた。さらに湾口付近の岩上では，ツヤナシシオグサ，ハンモンソウ，アミジグサ，シワノカワ，ヒライボ，ピリヒバ，ウスカワカニノテ，カイノリ等が観察できた。湾内漸深帯にはガラモ場がよく発達しており，上記のホンダワラ類に加えてノコギリモク，フシスジモク等が観察できた（漸深帯の海藻採集は，京都大学の学生諸氏の潜水による）。東隣りの磯に出ると，アナアオサ，ボウアオノリ，ウスバアオノリ，ヒメアオノリ，ツヤナシシオグサ，カイゴロモ，ハネモ，ミル，シオミドロ，ウミウチワ，カヤモノリ，ハバノリ，アカモク，イソモク，ムカデノリ，キョウノヒモ，マツノリ，オキツノリ，カイノリ，コスジフシツナギ，ワツナギソウ，ケイギス，ユナ，クロソゾ，ミツデソゾ等が採集できた。

午後はまず若狭湾海藻リストが配布され，本日，観察・採集できた海藻種のチェックが行われた。続いて梅崎，川井浩史講師による海藻標本作製の実演があった後，各自，採集品の整理，種検索，標本作製を行った。ある程度，標本整理が片付いた頃，梅崎講師による「海産ラン藻類の観察」に関する講義が行われた。*Kyrtuthrix*, *Calothrix*, *Nostoc* を材料として，顕微鏡観察を交えた講義は大変興味深く聴かせて戴いた。晩には懇親会が催され，皆賑やかに酒を酌み交わし，楽しい一時を過ごした。

翌4月2日は，午前中に中原紘之講師による「海藻類に付着する珪藻の処理方法」の実演があり，ウミトラノオに付着している珪藻類の観察を行った。最後に標本館内を案内して戴き，昼食後解散となった。

日本海の海藻及びその植生を見る機会が少ない私にとりましては，今回新たにいくつかの発見をし，とても有意義な採集会でした。また，私のみならず，新たなる感動を得た方々も多かったことと思います。これも一重に，いろいろとお世話下さった梅崎・中原両氏をはじめとする京都大学の職員，学生諸氏のお蔭だと思います。この場を借りて，お世話戴いた皆様にお礼申し上げます。採集会の写真は梅崎，川井両氏の撮影によるものです。今後も引続き，藻類に関したあらゆるテーマのもとに，多くのワークショップが開催されることを強く希望して参加記を終えます。

（大葉英雄：東水大・植物）

## —会員移動—

### 新　入　会

### 住所変更

## 退　　会

サンドラ・フォトス，保坂信仁（東京都），浜　　篤（長野県），西沢順子（岡山県），木村純子（山口県），秋山商店（賛助会員）

## 訃　　報

本会会員，糸野　洋氏は去る昭和62年5月17日逝去されました。謹んで哀悼の意を表します。

日本藻類学会

**賛助会員**

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理研食品株式会社　985 宮城県多賀城市宮内2丁目5番60号

日本学術会議だより　No. 6

# マン・システム・インターフェース(人間と高度技術化社会)特別委員会設置さる

昭和62年８月　日本学術会議広報委員会

日本学術会議では，特別委員会が追加設置され，活動を開始しました。また，現在第14期（昭和63年７月22日より３年間）会員の選出手続きが進められています。今回の「日本学術会議だより」では，これらの概要に加えて，来年度に開催される共同主催国際会議及び研究連絡委員会報告等についてお知らせします。

## マン・システム・インターフェース（人間と高度技術化社会）特別委員会

日本学術会議は，昭和62年４月の第102回総会において新たに「マン・システム・インターフェース（人間と高度技術化社会）特別委員会」を設置した。

高度な技術革新とその急速な浸透により，現代の社会はいわゆる「高度技術化社会」ということができる。すなわち，今日社会の各分野で，化学プラントや原子力発電所等に見られるごとく「システムの巨大化」が進むとともに，OA 機器などのように「高度技術の大衆化」等も起こってきている。

「高度技術化社会」においては，機械システム又はソフトシステムに対する人間の役割が，従来のものと大幅に変化しており，人間は新たに重要な役割を担うようになってきている。これらの人間の役割を軽減したり代替するために各種のインターフェースが設計され，装備されている。

これらのインターフェースは，人間―システム系の信頼性・安全性を高める上で極めて重要である。従って「高度技術化社会」を維持・発展させるためには，この方面の研究，開発が今後ますます重点的に行われなければならない。

しかし，現実には「高度技術化社会」における「システムの巨大化」や「高度技術の大衆化」に対して，人間は個人としても，社会としても，必ずしも十分な対応・受容ができているとは言えない。人間の能力を超えるシステムが技術的に実現したことによって，かえって人間としての生甲斐を喪失する人も一部に生じている。その結果，いわゆるテクノストレスの状態に陥ったり，人間味の喪失による不適応状況に悩む者が増加している。これはまた，人間―システム系のヒューマン・エラーによる大事故の一因ともなっている。また「高度技術化社会」から取り残されたと感じる人々の中には，種々の回避的ないし攻撃的な不適応行動を呈する者もみられ，今後，大きな社会問題となることが予想される。

「高度技術化社会」では，以上のような諸問題に対する対処策ないしは予防策のみでなく，人間性の回復・維持の問題を含めて，十分な対応が講ぜられる必要がある。

以上の観点に立って，このような問題を学際的かつ総合的に検討するために特別委員会を設置することとした。

日本学術会議第13期は，その活動期間を１年余残すのみになっているが，この問題の重要性に鑑み，期の途中であるが着手することとした。

## 日本学術会議会員選出制度

日本学術会議は，210人の会員をもって組織されているが，その会員は次の手続きにより選出（推薦）される。現在第14期会員（任期：昭和63年７月22日から３年間）を選出(推薦)するための手続きが進められているところである。

〔手続概略〕

1　会員の候補者を選定し，及び推薦人（会員の推薦に当たる者）を指名することを希望する学術研究団体は，日本学術会議に登録を申請する(昭和62年６月30日締切り)。

申請する場合には，その学術研究団体の目的とする学術研究の領域と関連する研究連絡委員会を届け出なければならない。届け出られた研究連絡委員会が『関連研究連絡委員会』（３参照）である。

関連研究連絡委員会により区分された学術研究の領域（以下「学術研究領域」という。）ごとに，会員の候補者及び推薦人を届け出ることになる。

2　日本学術会議会員推薦管理会は，この申請を審査し，その学術研究団体が所定の要件を満たすものであるときは，関連研究連絡委員会その他の事項を登録する。

登録された学術研究団体が「登録学術研究団体」である。

3　登録学術研究団体が届け出た関連研究連絡委員会が複数あるときは，日本学術会議会長は，登録学術研究団体の意見を聴いて関連研究連絡委員会を限定（指定）する（11月30日までに指定）。

4　登録学術研究団体は，その構成員である科学者のうちから，会員の候補者を「学術研究領域」ごとに選定し，日本学術会議に届け出る（昭和63年２月１日締切り）。

5　日本学術会議会員推薦管理会は，届け出られた会員の候補者が会員の資格を有する者であるかどうか認定する。

6　登録学術研究団体は，その構成員である科学者のうちから，推薦人を「学術研究領域」ごとに指名し，日本学術会議に届け出る（２月20日締切り）。

7　推薦人は，「学術研究領域」ごとに，日本学術会議会員推薦管理会が会員となる資格を有すると認定した会員の候補者のうちから，会員として推薦すべき者及び補欠の会員として推薦すべき者を選考・決定する（５月中旬～６月上旬）。

8　推薦人は，会員として推薦すべき者及び補欠の会員として推薦すべき者を，日本学術会議を経由して，内閣総理大臣に推薦する（６月中旬）。

9　内閣総理大臣は，その推薦に基づいて，会員を任命する（７月22日）。

## 昭和63年度共同主催国際会議

本会議は，昭和28年以降毎年おおむね4件の学術関係国際会議を関係学術研究団体と共同主催しているが，昭和63年度は次の4国際会議を我が国において開催することとした。(昭和62年6月16日(火)閣議了解)

**国際家族法学会第6回世界会議**

開催期間：昭和63年4月6日～12日
開催場所：日本大学会館（東京都）
共催団体：日本家族〈社会と法〉学会

**第9回世界地震工学会議**

開催期間：昭和63年8月2日～9日
開催場所：ホテルニューオオタニ(東京都)，国立京都国際会館（京都市）
共催団体：土木学会，日本建築学会，土質工学会，日本機械学会，地震学会，震災予防協会

**第8回国際内分泌学会議**

開催期間：昭和63年7月17日～23日
開催場所：国立京都国際会館（京都市）
共催団体：日本内分泌学会

**第5回国際植物病理学会議**

開催期間：昭和63年8月20日～27日
開催場所：国立京都国際会館（京都市）
共催団体：日本植物病理学会，日本植物防疫協会

## 我が国の理科教育について（意見）

―日本学術会議科学教育研究連絡委員会報告―

本研究連絡委員会は，かねて我が国と世界各国との学校における理科教育の実態について関心を持ち比較を行ってきたが，昨年教育課程審議会の発表した教育課程改定の大綱に関する中間報告と各教科の時間数に関する試案は，我が国の理科教育の世界の動向からの逸脱をはっきりさせたものとして，深い憂慮の念を示すものである。

**意見（要旨）**

第2次大戦後，科学技術立国は我が国の国是であった。この方向に資するため，我が国は学校における理科教育の振興に努め，大学における科学・技術の教育・研究にも多大の力を注いできた。しかるに，現今の国の施策を見ると，上述の方向とは逆行するものが増えていると言わねばならない。今回の中間報告に見られる小学校低学年理科の廃止，小学校から中学校まで9年間の理科の時間数は昭和43年に比べて6～7時間の減，高等学校においては，昭和35年に6単位（4科目必修）が昭和53年に4単位（理科Ⅰのみ必修）となり今回もそれが引き継がれようとしている。

学校教育における時間数の削減は必ずしも他の教科になかった現象ではないが，理科においてその減少が特に顕著であった。我々はこの点について強い危機感を抱くものであるが，その理由は理科に関する教育は児童・生徒の心身の発達に見合って，その内容を設定していく必要があるからで，時間数の削減がその適期を逸する恐れが強くなったからである。我々は，今後の理科教育において次の手当がなされるべきであると考える。

1　小学校においては，健全な自然観の育成を目標とし，低学年の理科も存続させる。
2　中学校・高等学校においては，科学技術に生きる人間としての能力を育成するため充分の時間を確保する。

## 地区会議活動について

日本学術会議は，全国を，北海道，東北，関東，中部，近畿，中国・四国，九州・沖縄の7ブロックに分け，「地区会議」を組織している。

これらの地区会議は，運営審議会附置広報委員会の下に置かれ，学術会議の各部・委員会等の活動状況を各地区内の科学者等に周知し，また，学術会議に対する意見，要望を汲み上げて，学術会議と科学者との意志疎通を図るとともに，地域社会の学術の振興に寄与することを目的としている。

各地区会議は，原則として，当該地区に居住，あるいは勤務している学術会議会員の中から各部(第1部～第7部)1人ずつ計7人をもって構成することとされているが，該当する会員全員を構成員としている地区も多い。また，部によっては，該当する会員のいない地区があり，その場合には研究連絡委員会委員を構成員としている。

各地区会議は，構成員である会員の中から代表幹事1人（関東地区のみ2人）を選び，その主宰者としている。

さらに，各地区会議には，その活動に関する事務を処理するために，「地方連絡委員」を置いている。この地方連絡委員には，北海道地区会議は北海道大学，東北地区会議は東北大学，中部地区会議は名古屋大学，近畿地区会議は京都大学，中国・四国地区会議は広島大学，九州・沖縄地区会議は九州大学の事務局長以下6～10人の職員が委嘱されている。各地区会議は，これらの各大学事務局職員の多大な協力の下に運営されているのである。

各地区会議は，前述の目的を果たすために，科学者との懇談会・学術講演会等の開催，地区会議ニュースの発行等の事業を活発に行っている。先般，運営審議会で決定された今年度の各地区会議事業計画によると，全国各地で，科学者との懇談会は12回，学術講演会は14回それぞれ開催される予定である。

## 日本学術会議主催公開講演会

本会議は，学術の成果を広く国民生活に反映浸透させるという日本学術会議法の主旨に沿うため，公開講演会を主催していますが，昭和62年度には，本会議会員（演者）による公開講演会を次のとおり3回企画しています。

開催日・演者等詳細は決定次第新聞広告等でお知らせする予定ですが，多数の方々のご来場をお願いします。

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多数の学術研究団体の御協力により，「日本学術会議だより」を掲載していただくことができ，ありがとうございます。

なお，御意見・お問い合わせ等がありましたら下記までお寄せください。

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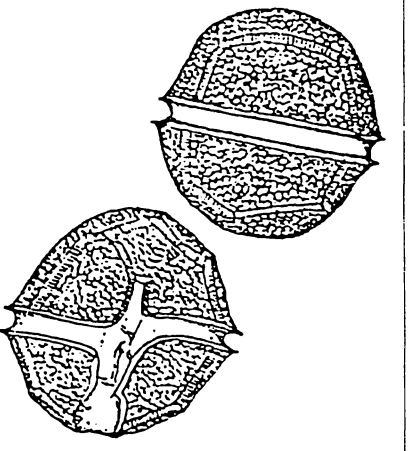
新刊！

# 淡水赤潮



## 門田　元編

取水上の衛生問題，不快臭の発生，養殖淡水魚のへい死原因などに関連し，湖沼・人工湖における“水の華”発生が社会問題化している。

本書は1979年９月，環境庁水質保全局の肝煎りで組織された淡水赤潮研究会（座長　門田　元博士）の研究成果を広く関係者に利用していただくために公刊するもので，淡水赤潮に関する生物学的知見を網羅し，その発生機構の解明と対策も論究される。また琵琶湖におけるウログレナ *Uroglena* 及び永瀬ダム湖におけるペリディニウム *Peridinium* の調査研究をケーススタディに，我が国各地で頻発する淡水赤潮問題解決の資料を直接に提供するものである。

**主な内容と執筆者**　①淡水赤潮をひき起こすプランクトン（根来健一郎）　②淡水植物プランクトンの生活史（中原紘之・左子芳彦）　③淡水赤潮プランクトンをめぐる生物間相互関係（安野正之・花里孝幸・深見公雄・門田　元・石田祐三郎・内田有恒）　④湖沼の富栄養化と植物プランクトンの異常増殖（坂本　充）　⑤赤潮による被害（岡市友利・門田　元）　⑥わが国各地における淡水赤潮の発生状況（山中芳夫）　⑦琵琶湖における淡水赤潮の発生（門田　元・中西正己・吉田陽一・石田祐三郎）　⑧ダム湖における淡水赤潮の発生事例（畑　幸彦）

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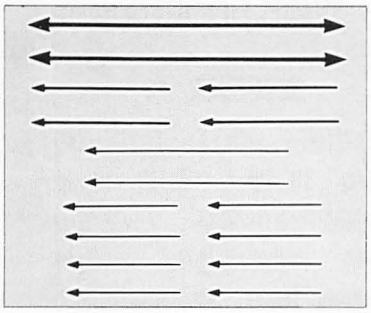

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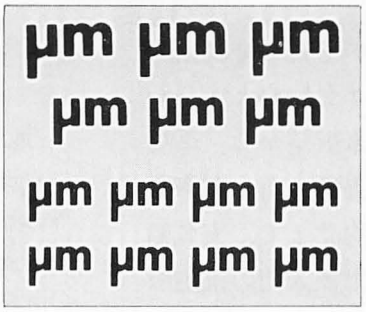


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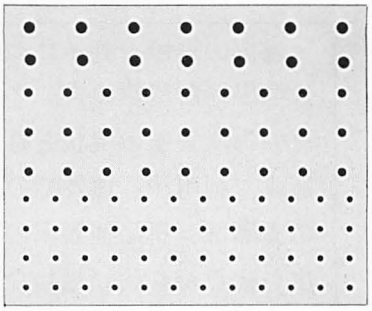

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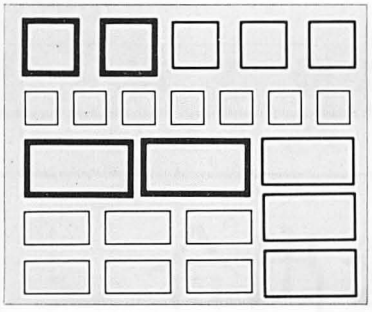

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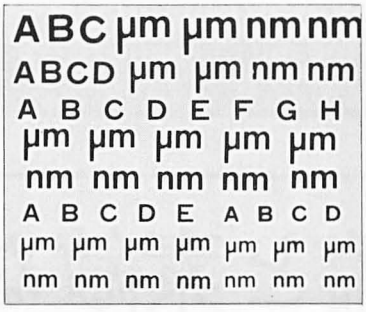


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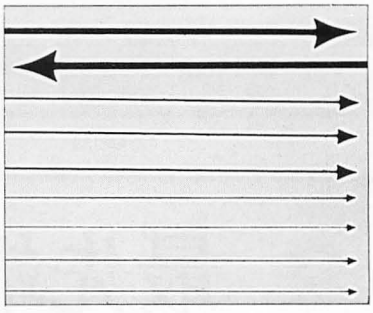

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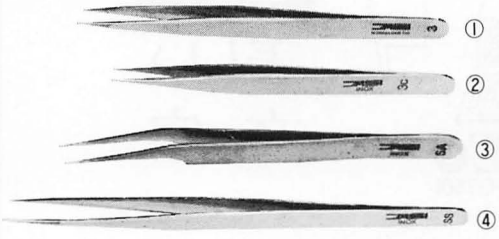


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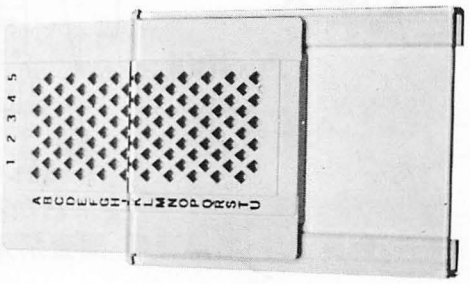

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