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**Culture Studies of *Symphyocladia latiuscula*  
(Rhodophyceae: Rhodomelaceae)\***

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**Introduction**

*Symphyocladia latiuscula* (Harvey) Yamada is a member of the family Rhodomelaceae, belonging to the order Ceramiales. This plant is found on the Pacific and Japan Sea coasts of middle and northern Honshu and also grows commonly along the coast of Hokkaido. Okamura<sup>1)</sup> described the present species as *Symphyocladia gracilis* in 1912. Yamada<sup>2)</sup>, after comparing the type of *Rytiophloea latiuscula* Harvey<sup>3)</sup>, which had been collected at Hakodate, decided that the two names applied to the same species and accordingly made the combination *Symphyocladia latiuscula* (Harvey) Yamada. Mature tetrasporophytes of this species, 5-15 cm high, are found from late August to early December in the southern part of Hokkaido. However, sexual plants have not been previously reported. According to his unpublished observations, Dr. Tazawa found the tiny male plants, 15-35 mm high, at Charatsunai, Muroran, on July 20, 1954 and at Cape Erimo, Hidaka Prov., on August 3, 1955. The spermatangial clusters were lanceolate or cylindrical in shape and 180-200  $\mu\text{m}$  long and 50-60  $\mu\text{m}$  diam. Fertilized female plants, about the same in height as the male plants, were also found in the same collection at Muroran. Dr. Tazawa's thesis<sup>4)</sup>, including detailed developmental stages in the formation of the male organ of *S. latiuscula*, is now in preparation for publication (Tazawa, *per. comm.*, March 4, 1974). The present study has been carried out for the purpose of obtaining more information on the life cycle. Tetraspores from nature were germinated in the laboratory, the sporeling developing into dwarf male and female plants.

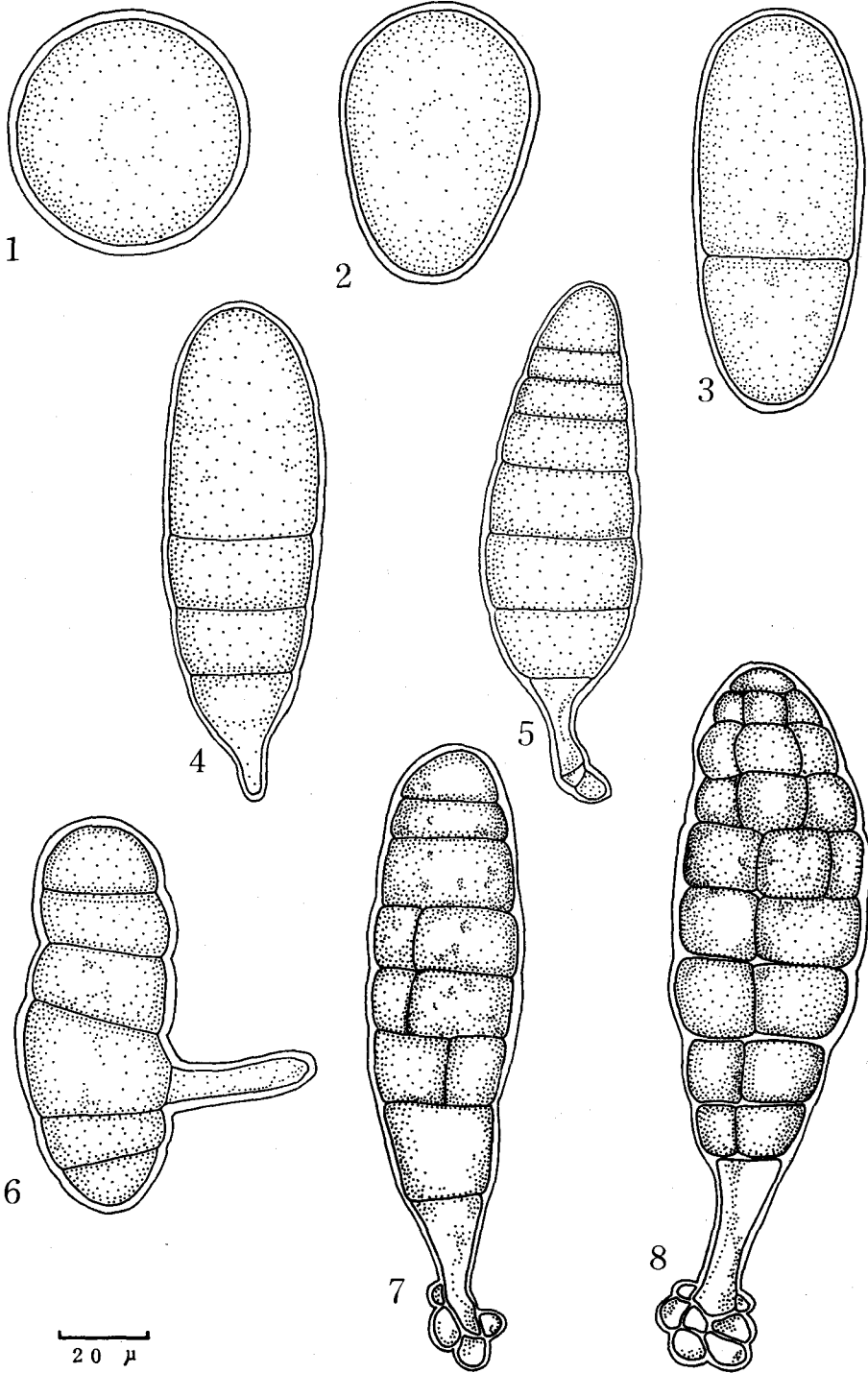
**Materials and Methods**

Plants with mature tetraspores were collected on October 13, 1972, at Usujiri, located on the Pacific coast near Hakodate in the southern part of Hokkaido.

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They were brought back to the laboratory of the Faculty of Fisheries, Hokkaido University in Hakodate immediately after collection, and were kept in a dish containing filtered sea water over night at 15°C. The following morning, released tetraspores were obtained from plants which had been washed in filtered sea water to remove as many epiphytes as possible. The free-floating spores were isolated by using a glass capillary tube under a dissecting microscope. Two types of enriched sea water, SWM-3 (Chen *et al.*<sup>5)</sup> and PES medium (Provasoli<sup>6)</sup>), were used for cultures. The sea water for the culture media was obtained off the coast outside of Hakodate port and was filtered with a membrane filter (Toyo TM-4 and GA-200). The filtered sea water was then steam sterilized for 30 minutes and the nutrients added. Cultures were maintained in petri dishes (5 cm diam.) with 20 ml of the medium during the early stages of spore germination. The temperature was kept at 15±1°C and light intensity held at 1200–1500 lux. After 30 days the germlings were each moved to separate cabinets in which various temperature and light intensities were employed to determine the influence of these factors on growth and reproduction. Throughout the experiment, illumination periods were 12 hr/day. Illumination values were determined with a Toshiba No. 5 Illumination Meter. 40 watt cool white florescent tubes (Toshiba DL) were used as light sources. The thallus length was measured every 7 or 10 days, and at the same time, the cultures were transferred to fresh media.

### Results and Discussion

Released tetraspores are spherical and vary from 73.4  $\mu\text{m}$  to 42.8  $\mu\text{m}$  (sample mean 57.6  $\mu\text{m}$ ) diam.; they have thin transparent walls and contain deep red pigments (Fig. 1). Spores which have made a firm attachment to glass slides begin to swell into oval shape within about six hours (Fig. 2). The elongation continues until the first division occurs, and the resulting septum forms perpendicular to the long axis in twenty-four hours, resulting in two unequal cells (Fig. 3). A second division results in a row of four cells, followed by the development of a short protuberance on one end of the germling (Fig. 4). It took four days to yield a cell filament consisting of seven or eight cells and a rhizoid as shown in Fig. 5. In

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Figures 1–8. Successive stages in the germination of tetraspores of *Symphycloadia latiuscula*.

Fig. 1. Liberated tetraspore.

Fig. 2. 6-hour old germling elongating into an oval shape.

Fig. 3. 24-hour old germling consisting of 2 unequal cells divided by a transverse cell wall.

Fig. 4. 3-day old germling consisting of 4 cells divided zonately, showing the formation of the primary rhizoid.

Figs. 5 and 6. 4-day old germlings, showing 7 celled stage with a little elongation (Fig. 5) and a 6 celled stage with a malformed rhizoid (Fig. 6).

Fig. 7. 6-day old germling.

Fig. 8. 10-day old germling, showing a well-developed rhizoid.

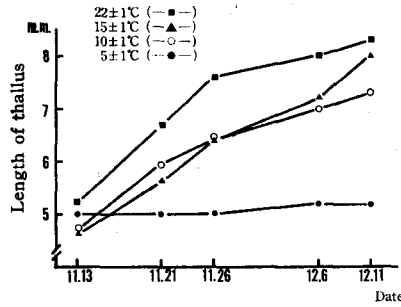


Fig. 9. Growth of *Symphyocladia latiuscula* with a photoperiod of 12: 12 and 1200 lux, fluorescent light, with four different temperatures, 22±1°C, 15±1°C, 10±1°C and 5±1°C.

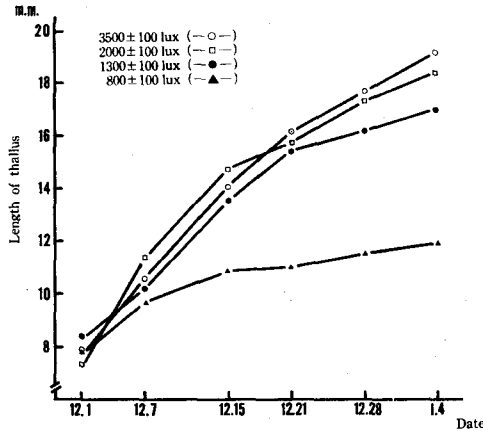


Fig. 10. Growth of *Symphyocladia latiuscula* with a photoperiod of 12:12 and a temperature of 15±1°C and four different light intensities, 3500±100 lux, 2000±100 lux, 1300±100 lux and 800±100 lux.

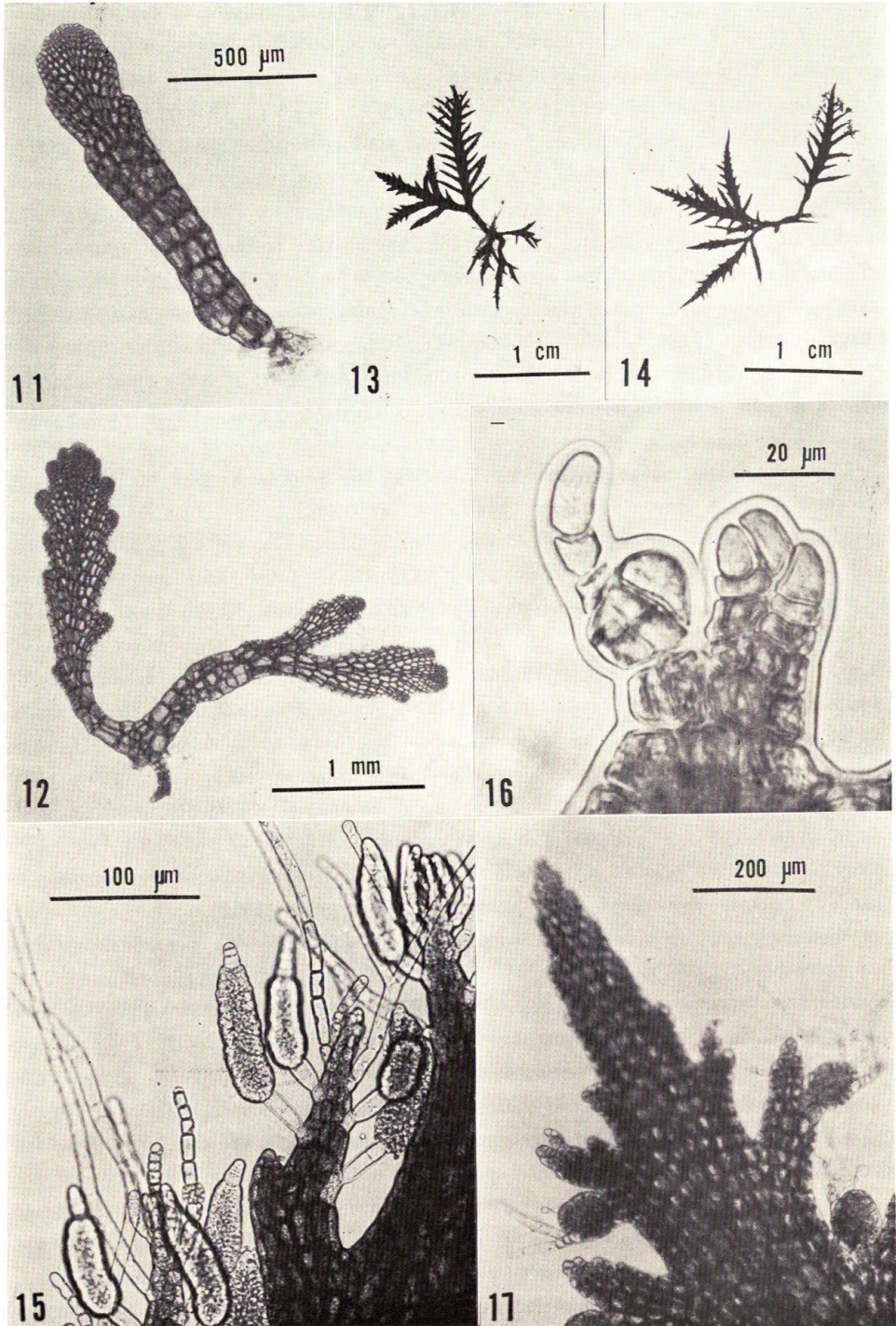
a few cases, a malformed rhizoid was produced laterally on the cell filament (Fig. 6). After six days the first division parallel to the long axis took place (Fig. 7). In ten days, subsequent cell divisions resulted in a multicellular germling with a holdfast, but no difference was observed in outline compared with the previous stage (Fig. 8). The mode of spore germination in the early stages of development is of the discal erect type and agrees well with the description of germination in the present species by Inoh.<sup>7)</sup>

At the end of 30 days, the sporelings had attained a length of about 4.2–5.5 mm. (Figs. 11, 12). Five individuals were then transferred to several cabinets under various temperature conditions and 1200 lux in order to determine the effects of temperature on growth and reproduction. The results for about one month are shown in Fig. 9. The sporelings which were cultured at temperatures of 10±1°C, 15±1°C and 22±1°C grew well, but at 5±1°C no growth occurred.

As there were no signs of reproductive structures when the plants had attained a length of 7 or 8 mm, experiments were also conducted under various light intensities. Three germlings were cultured in each cabinet under four different light intensities from  $800 \pm 100$  to  $3500 \pm 100$  lux at  $15 \pm 1^\circ\text{C}$ . After an additional month, the germlings which had been placed under illumination levels of  $3500 \pm 100$ ,  $2000 \pm 100$  and  $1300 \pm 100$  lux had grown considerably more than those under  $800 \pm 100$  lux, the former three being almost the same length (Fig. 10). On Dec. 28, the 76th day after initiation of the first culture from a single spore, male and female reproductive organs and young cystocarps were produced on the plants which had been kept under the highest two illuminations. These had attained 17 mm in length (Figs. 13, 14). Fertile plants were also obtained under  $2000 \pm 100$  lux. Furthermore, some plants, about 7 mm long, possessed mature spermatangial clusters on Jan. 4 in the culture maintained at  $1300 \pm 100$  lux. These plants were all dioecious.

Clusters of spermatangia are developed on the pinnae formed on the upper part of the main axis. They are produced on trichoblasts (colorless uniseriate filaments) by repeated cell division and become lanceolate-oblong when mature. Fully mature spermatangial clusters are 70–110  $\mu\text{m}$  long and 30–40  $\mu\text{m}$  diam. and are provided with one- or two-celled stalks which are about 15  $\mu\text{m}$  diam. (Fig. 15). Spermatangia are oval, about 7  $\mu\text{m}$  long and about 5.5  $\mu\text{m}$  diam., when attached. After liberation they become spherical bodies, 6–7  $\mu\text{m}$  diam. The dimensions of spermatangia in culture are smaller than those observed in nature by Dr. Tazawa. The procarys are also born on trichoblasts on the pinnae. A carpogonial branch consists of four cells surrounded by the developing cystocarp-wall (Fig. 16). After fertilization the auxiliary cell is cut off apically from the supporting cell. Young cystocarps are globose, 120–160  $\mu\text{m}$  long and 100–145  $\mu\text{m}$  diam. (Fig. 17). These reproductive organs are similar to those of *Symphyocladia marchantioides* (Harvey) Falk. as described and illustrated by Okamura (1912).

Masuda<sup>8)</sup>, on the basis of culture study of *Pterosiphonia pennata*, reported that tetraspores gave rise to sexual plants and that carpospores germinated to produce the tetrasporophyte again. Four other species of Rhodomelaceae have been studied in culture: *Pterosiphonia gracilis* (West & Norris<sup>9)</sup>); *Polysiphonia denudata* (Edwards<sup>10,11)</sup>); *P. echinata* and *P. gorgoniae* (Edwards<sup>12)</sup>). These five species all have a life history in which gametophytes alternate with sporophytes and all three phases are of the same general form and size. We did not pursue the complete life cycle of the present species in culture, but instead obtained the sexual plants from tetraspores taken from nature. The sexual plants obtained in culture are more or less the same length as specimens collected in nature by Dr. Tazawa, smaller than the sporophytes. Unlike other rhodomelaceous plants (including other species of *Symphyocladia*), *Symphyocladia latiuscula* has a dimorphic



life cycle.

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### Summary

1. The life history of *Symphyocladia latiuscula* has been studied in unialgal culture, beginning with tetraspores from a plant in nature.

2. The mode of germination of the tetraspores is that of the discal erect type, and the results obtained in the early development of spore germination coincide with those described by Inoh (1947).

3. On the 76th day after the commencement of germination, mature, dioecious, dwarf sexual plants about 17 mm long, were obtained at  $15 \pm 1^\circ\text{C}$  under light intensities of  $3500 \pm 100$  and  $2000 \pm 100$  lux from fluorescent lighting under a 12 h photoperiod. The life cycle in culture thus involves a dimorphic alternation of somatic phases.

4. The sexual reproductive organs of *S. latiuscula* are described for the first time.

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Figures 11-17. Photomicrographs of sexual plants of *Symphyocladia latiuscula* in culture.

Fig. 11. 21-day old plant.

Fig. 12. 32-day old plant.

Fig. 13. 84-day old dwarf female plant.

Fig. 14. 84-day old dwarf male plant.

Fig. 15. Spermatangial clusters; this is an enlargement of a part of the male plant shown in Fig. 14.

Figs. 16 and 17. Enlargement of a part of the fertilized female plant shown in Fig. 13; young procarp (Fig. 16) and young cystocarp (Fig. 17).

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