# Culture Study of the Red Alga *Polysiphonia senticulosa* Harvey (Ceramiales, Rhodophyta)

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<Abstract>

Life history and morphological development of *Polysiphonia senticulosa* Harvey (Ceramiales, Rhodophyta) were investigated by culture experiment. Morphological stability and plasticity were assessed in several features. The following features are confirmed as morphological stability of this species. (1) Thallus is composed of slender upright axes, and intricately entangled prostrate axes. (2) Thallus produces adventitious branches on the lowermost portion of the main axis. (3) Thallus is ecorticated with four periaxial cells. (4) Rhizoids are formed without septation. (5) Thallus is soft and flaccid in texture. (6) Determinate branches have sharply pointed apices. (7) One to three axillary branchlets are formed. (8) Tetrasporangia are formed in a straight line, on the last two orders of ordinary branchlets and on axillary branchlets. (9) Carpogonial branch is composed of four cells. (10) Cystocarps are urceolate in shape. (11) Spermatangial branchlets are formed replacing the whole trichoblasts and their shape is terete to cylindrical with sterile tips. These features are appropriate for the taxonomic criteria in *P. senticulosa*.

Key Index Words: *Polysiphonia senticulosa*, culture experiment, life history, *Polysiphonia morrowii*, *Polysiphonia urceolata*, taxonomy, Rhodomelaceae, Ceramiales.

## <Introduction>

The red alga *Polysiphonia senticulosa* Harvey (1862) is principally characterized by the following features. It has slender and profusely branched thallus, soft and flaccid texture, four periaxial cells, it lacks cortical cells, axillary branches are formed endogenously from the central axial cells, and ultimate branchlets are sharply pointed. This species was first described on the basis of material collected from Orcas Island, Washington, U. S. A. Since then, it has been reported from several localities in the higher latitude areas of eastern and western Pacific Coast (Kylin 1941, Yendo 1914, Segi 1951). Its occurrence reported from Japan was later discounted by Kudo and Masuda (1981), who concluded that the Japanese *P. senticulosa* was identical to the young plants of *Polysiphonia morrowii* Harvey (1857). However the name *P. senticulosa* was *de novo* restored by Kudo and Masuda (1991) on the Japanese algal list. In their brief report, it was shown that called *Polysiphonia urceolata* (Dillwyn) Greville (1824) from Japanese coast was identical to *P. senticulosa*. *P. urceolata* was originally described on the material collected from British Islands and has been reported from various localities over the world including Japanese coast and its vicinity (Harvey 1849-51, 1849, 1853, Rosenvinge 1923-24, Kapraun 1977, Yendo 1916, Yamada 1928, Inagaki 1933, Okamura 1936, Yamada and Tanaka 1944, Tokida 1954, Segi 1951, Kang 1966). It is known that this species has wide range of variation in the morphological features depending on its growth stages or environmental conditions (Rosenvinge 1923-24, Kapraun 1979). Maggs and Hommersand (1993) have considered that *P. urceolata* is composed of species complex in British Islands. Consequently they have dealt *P. urceolata* as a synonym of *Polysiphonia stricta* (Dyllwyn) Greville (1824) because that *P. stricta* is an oldest available name in the group.

Previous investigators have described several species in the genus *Polysiphonia* on the basis of a few materials collected from only several localities, using the taxonomic criteria without paying attention to the morphological variability. Now, however, it is necessary to examine the appropriateness of taxonomic criteria, which have been adopted to identify in each species of this genus. Comparative study through life history stages of these species seems to be useful to evaluate the taxonomic features first pointed out by Dixon (1963), then exemplified by Masuda (1982) and Kudo and Masuda (1986, 1992). This method seems to be effective to elucidate the relationship between *P. senticulosa* and its closely related species.

This paper will discuss the morphological development of *P. senticulosa*. Then, the present paper will report the growth and reproductive responses of this species at various combinations of temperatures and photoperiods. Furthermore, in order to establish more correct taxonomic criteria, the morphological stability and variability of *P. senticulosa* will be assessed.

## <Materials and Methods>

Materials used in this study was collected on 27, March 1986 at Matsukawa-ura (37° 48'N, 140° 58'W) in Fukushima Prefecture, Japan. Tetraspores released from tetrasporophytic plants were isolated for unialgal culture according to the methods described earlier (Masuda 1982). Liberated tetraspores were rinsed three times with autoclaved seawater and put into seawater droplet on the sterilized slide glass. One week after inoculation the germlings of tetraspores were transferred to the glass dishes (71 × 61 mm or 65 × 80 mm) containing Provasoli's Enriched Seawater (PES) (Provasoli 1968). The cultures were maintained in eight combinations of temperatures and photoperiods in order to investigate growth and reproductive

responses to varying temperature and photoregimes. The following combinations of temperatures and photoperiods were adopted: 5℃, 16:8 LD (Light and dark cycle); 5℃, 8:16 LD; 10℃, 16:8 LD; 10℃, 8:16 LD; 15℃, 16:8 LD; 15℃, 8:16 LD; 20℃, 16:8 LD; 20℃, 8:16 LD. They were cultured in plant growth chambers illuminated with cool-white fluorescent lamps (2500-3000 lux). All cultures were changed to fresh medium every two weeks. Plants cultured at 10℃, 16:8 LD were chiefly used for description of morphological development.

When female and male reproductive organs were formed on individual plants, female and male plants were cultured in single dishes (71 × 61 mm) and placed on a Taiyo-R-II Rotary Shaker at 90-100 rpm. The carpospores released from matured cystocarps were isolated and cultured in the same methods as tetraspores to investigate the morphological development, growth and reproductive responses in the tetrasporophytic generation.

Morphological observations were carried out using living materials and specimens preserved in 10% formalin seawater. Microscopic slides were made by mounting in a 50% glycerol-seawater mixture after staining with 0.5% (W/V) cotton blue in a lactic acid/phenol/glycerol/water (1:1:1:1) solution.

## <Results>

The following observation is based on cultured plants maintained at 10 ℃, 16:8 LD unless otherwise indicated. Liberated tetraspores were globular and brackish deep red in color. They averaged 48.6 μm (range 42.5-57.5 μm; 320 spores measured) in diameter (Fig. 2A). Isolated tetraspores soon attached to the substrate, elongated themselves and stood uprightly. They grew into bipolar sporelings of 4 segments that enveloped in mucilaginous pellucid matter. The sporelings were composed of one large upper cell and lower three

upper small cells (Figs. 1A, 2B). Two days after inoculation, the sporelings were differentiated into a colorless primary rhizoid and pigmented upright axis consisting of several segments (Figs. 1B, 2C). Besides the apical three or four segments and basal segments, each segment of the upright main axis consisted of an axial cell and four periaxial cells (Figs. 1C, 2D).

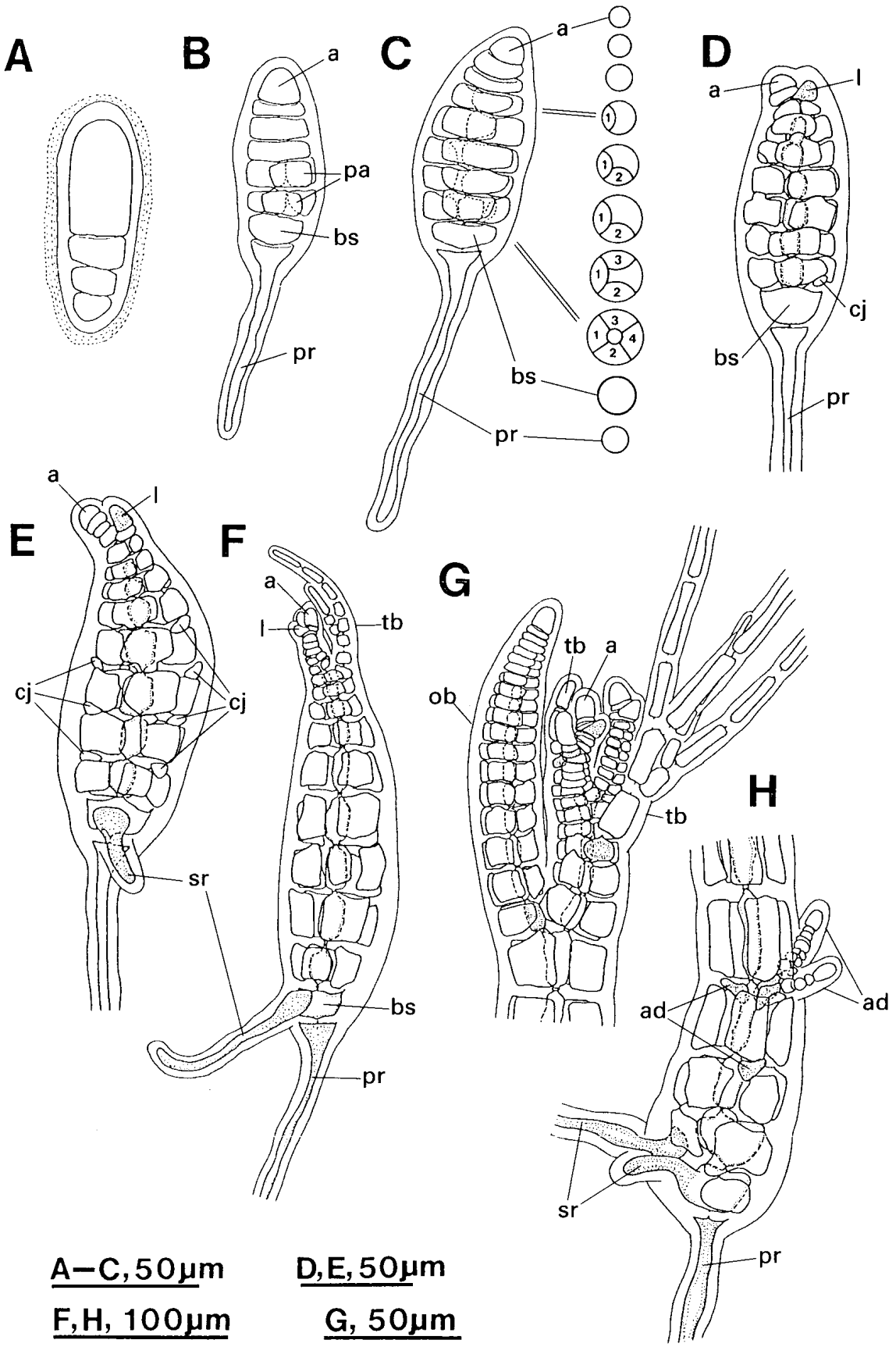
The formation of periaxial cell firstly began at the suprabasal segment and expanded into upper segments successively. The first periaxial cell of the suprabasal segment was divided from the suprabasal axial cell in random direction (Fig. 1B). The second periaxial cell was produced on counterclockwise side of the first one. The third one was produced on opposite side of the second one. Finally the fourth periaxial cell was formed between the second and third periaxial cells. The first periaxial cell of the third basal segment was formed on the position just above the suprabasal segment. Subsequently the second to fourth periaxial cells were formed in the same manner with those of the suprabasal segments. The periaxial cells of other upper segments were formed in the same way repeatedly. Consequently, the first and other three periaxial cells of each segment were arranged in a straight line (Fig. 1C). A small conjunctor cell was divided from the bottom of each periaxial cell after accomplishment of four periaxial cells. The conjunctor cell fused with the periaxial cell lying just below itself and made a secondary pit connection between neighboring segments (Fig. 1E). The basal segment was composed of a single cell, but sometimes it produced one to three periaxial cells or secondary rhizoid later (Figs. 1E, F, H and 2F, G, I).

Five days after inoculation, the main axis became recurved and began to form lateral initials from the dorsal side of apical third segment (Figs. 1D, 2E). As the development of the main axis proceeded, the lateral initials were formed with irregular intervals of

three or four segments in 1/4 divergence spiral line, running in a counterclockwise direction toward the apex of the main axis. With successive formation of these laterals the main axis gradually became straight. In many cases the first to third laterals grew into vegetative trichoblasts divided pseudodichotomously (Fig. 1F), and the fourth lateral initial developed into an ordinary branch. However, the first one infrequently gave rise to an ordinary branch. After the first ordinary branch was formed, majority of lateral initials grew into the ordinary branches with intervention of trichoblasts in the spiral line. The ordinary branches grew indeterminately or determinately. The indeterminate ordinary branches formed the second order of branches or a few vegetative trichoblasts as the main axis did (Fig. 1G). Thus process was repeated continuously, and the plants formed three to four orders of ordinary branches. The final order of ordinary branches, namely ultimate branchlets, were terminated as determinate branchlets with sharply pointed apices.

The first periaxial cell at the basal segment of ordinary branches was produced on abaxial side. The first periaxial cells of suprabasal and other upper segments were formed on the position just above the basal segment. The formation of the first periaxial cell of suprabasal

Fig. 1 Tetrasporelings of *Polysiphonia senticulosa* grown at 10℃, 16:8 LD. **A**. One-day-old germling. **B**. Two-day-old germling. **C**. Three-day-old germling with the slightly recurved apical portion, and its diagrams for formation of the periaxial cells. **D**. Five-day-old germling forming the first lateral at the upper portion. **E**. Seven-day-old germling forming a lateral on the dorsal side and a secondary rhizoid arising from the basal segment; note the axis being recurved slightly. **F**. Ten-day-old germling forming three laterals at the apical portion and a secondary rhizoid at the basal segment. **G**. Apical portion of a two-week-old plant forming ordinary branches and vegetative trichoblasts. **H**. Basal portion of a two-week-old plant with two endogenous adventitious branches, two initial cells of an endogenous branch, and two secondary rhizoids. a, apical cell; ad, adventitious branch; bs, basal segment; l, lateral initial; cj, conjunctor cell; ob, ordinary branch; pa, periaxial cell; pr, primary rhizoid; sr, secondary rhizoid; tb, trichoblast, 1, first periaxial cell; 2, second periaxial cell; 3, third periaxial cell; 4, fourth periaxial cell; 5, fifth periaxial cell.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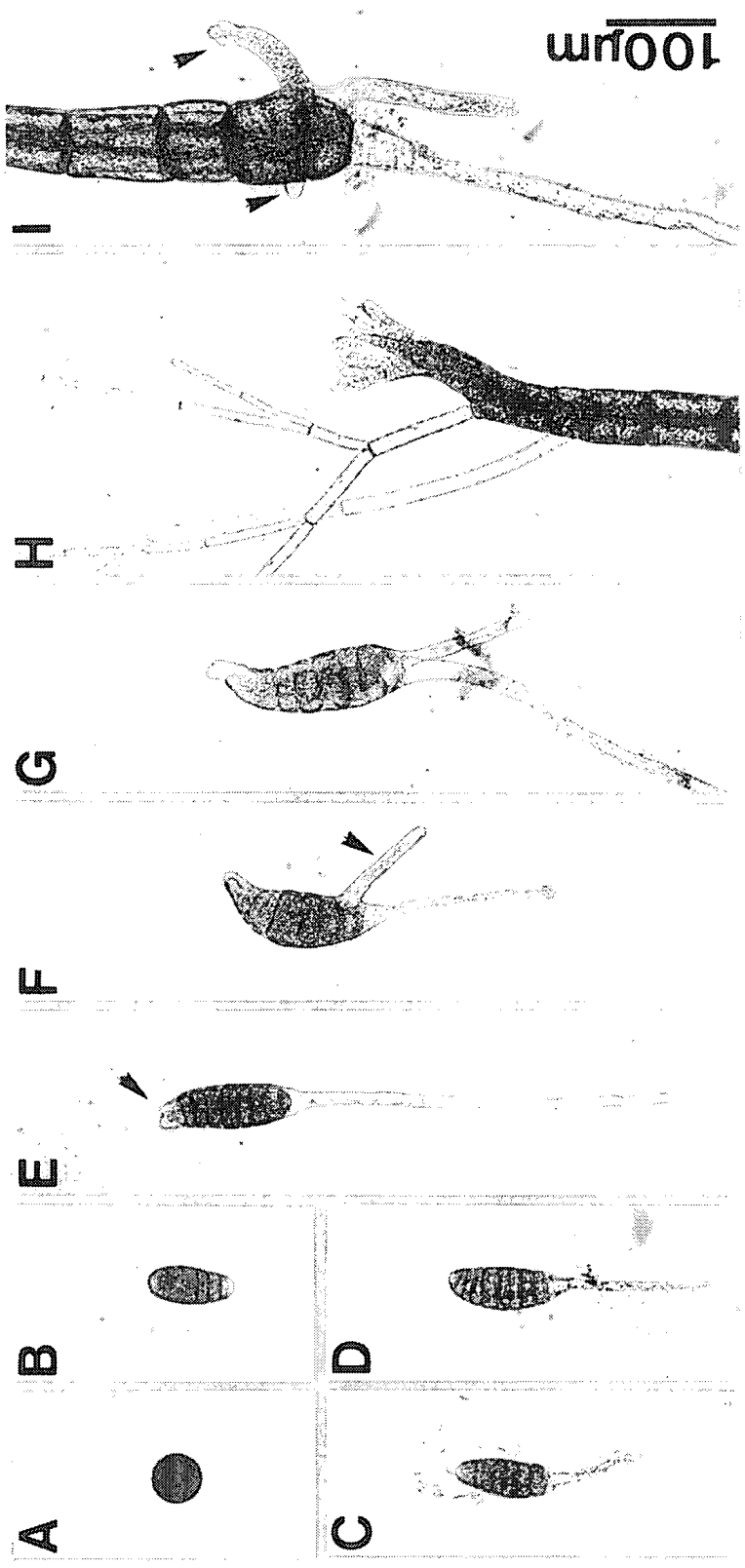


Fig. 2 Tetraspore and its development of *Polysiphonia senticulosa* at 10°C, 16:8 LD. All photographs from living material. **A**. Liberated tetraspore. **B**. One-day-old germling. **C**. Two-day-old germling. **D**. Three-day-old germling; note the axis beginning to incline slightly. **E**. Five-day-old germling forming a lateral initial (arrowhead) on the dorsal side. **F**. Seven-day-old germling with a primary and a secondary rhizoids (arrowhead). **G**. Ten-day-old germling. **H**. Apical portion of a two-week-old plant forming vegetative trichoblasts and ordinary branches. **I**. Basal portion of a two-old-plant forming one secondary rhizoid and two endogenous adventitious branches (arrowhead). Scale in I applies also to all of others.

and third basal segment predated the second periaxial cell of basal cell. Formation of the second to fourth periaxial cells in each segment of ordinary branches progressed in a manner similar to the main axis. Consequently the first and other three periaxial cells in each segment of ordinary branches were arranged in a straight line like the main axis did (Fig. 3A). Only two periaxial cells were usually formed on the basal segment of ordinary branches. However, four periaxial cells were formed on each of other segments except apical portion.

Primary rhizoids were developed from the lowermost cell of the ellipsoidal sporeling. They became elongated into simple filamentous holdfasts (Figs. 1B, C and 2C-F) or frequently became expanded into disc-like holdfasts by ramification at the growing tips. Secondary rhizoids were divided directly from the basal segment (Figs. 1E, F and 2F). They were also formed from periaxial cells, which remained open connection with them, on the basal or suprabasal segments of the upright main axis and variable portions of branches attaching to the substratum (Figs. 1H, 2I).

Two weeks after inoculation, the axial cell of the suprabasal and several upper segments of the main axis began to form one to three adventitious branches (Figs. 1H, 2I). The branches showed endogenous origin, because they were produced from distal end of axial cells after the accomplishment of four periaxial cells. Finally two to five adventitious branches were formed on the main axis. In many cases the second or further adventitious branches arose from lower portion of the main axis rather than the position of the first one. Two or three adventitious branches were sometimes formed from one segment to different directions (Fig. 1H). Thus the branches were irregularly formed in the sequence, number and direction. While these branches bore no periaxial cell on the basal segment, they bore three or four small periaxial cells on the suprabasal segment. The adventitious

branches grew indeterminately along the substratum. The majority of these branches produced the secondary rhizoids from the periaxial cells on their ventral side, but some of them became erect and grew into secondary upright axes.

Plants reached reproductive maturity and began to form procarps and spermatangial branchlets on separate individuals 59 days and 42 days after inoculation respectively (Figs. 4A, D). The procarps were formed from the suprabasal segments of female trichoblasts borne at the uppermost portion of indeterminate branches (Fig. 4B). They were formed in 1/4 divergence spiral line with intervals of three to five segments, replacing ordinary branches or vegetative trichoblasts. Carpogonial branches in the procarps were composed of four cells as those of other members of Rhodomelaceae. After the plants reached reproductive maturity, the axillary endogenous branches were produced singly on each axil of ordinary branches. These branches also formed procarps later like the ordinary branches (Fig. 4C). More than ten spermatangial branchlets were formed successively on each segment near the apical portion of indeterminate ordinary branches. The mature spermatangial branchlets were terete to cylindrical in shape and possessed 2-5 celled sterile tips at the apex. They were deciduous leaving their basal cells (i. e. scar cells) as vegetative trichoblasts were. Also in male plants the axillary branches were formed singly, which contributed to reproductive activity later.

Fertile female gametophytes mixed with male gametophytes releasing spermatia in single dish were placed on a rotary shaker to assist their fertilization. All these female gametophytes began to produce cystocarps after two weeks from the initiation of mixed inoculation. Matured cystocarps released viable carpospores 55 days after the initiation of mixed inoculation (Fig. 4F). The cystocarps were urceolate in shape, and when fully matured they clearly constricted at

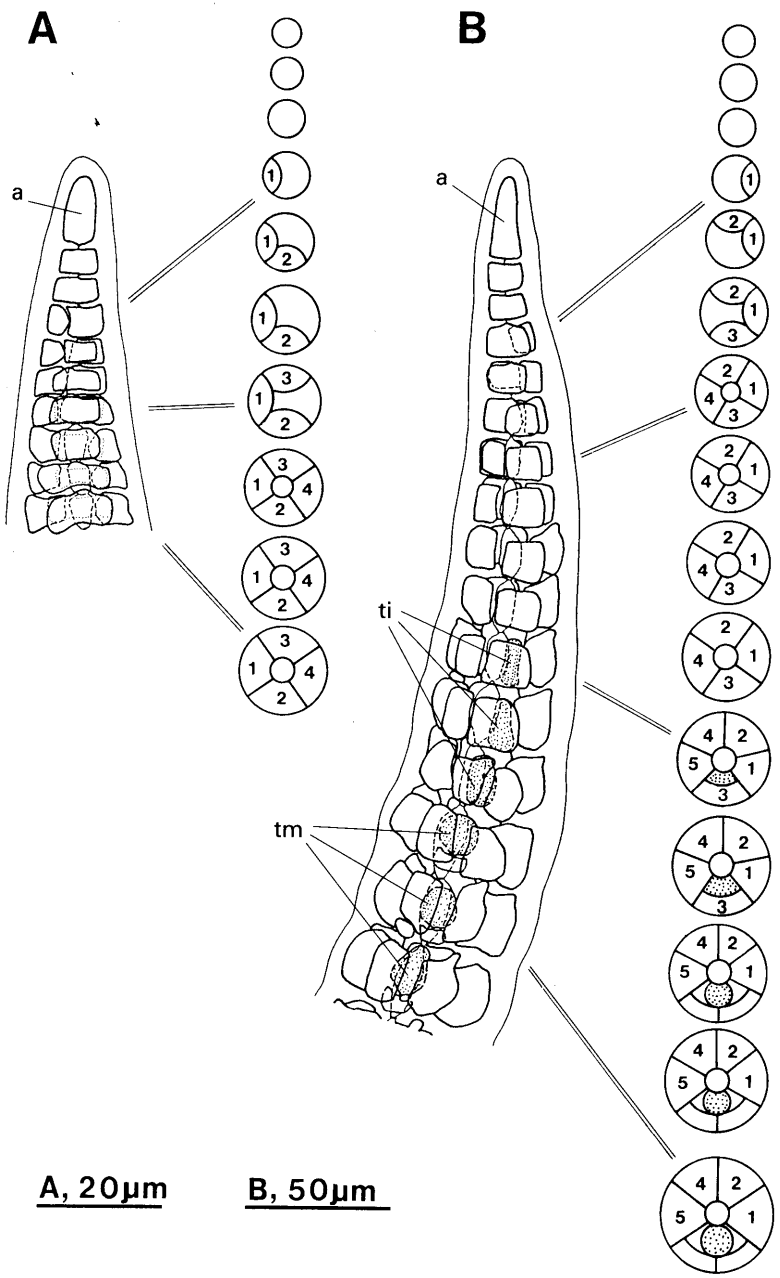


Fig. 3 Formation of periaxial cells and arrangement of tetrasporangia. **A**. Apical portion of a vegetative branch (three-week-old plant in 10 ℃, 16:8 LD), and its diagrams for formation of the periaxial cells. **B**. Tetrasporangia borne on an ordinary branch (62-day-old plant in 10 ℃, 16:8 LD), and its diagrams for arrangement of the tetrasporangia. The number indicates sequences of periaxial cells, and the dotted areas indicate the tetrasporangial initial cells or tetrasporangial mother cells. a, apical cell; ti, tetrasporangial initial cell; tm, tetrasporangial mother cell; 1, first periaxial cell; 2, second periaxial cell; 3, third periaxial cell; 4, fourth periaxial cell; 5, fifth periaxial cell.

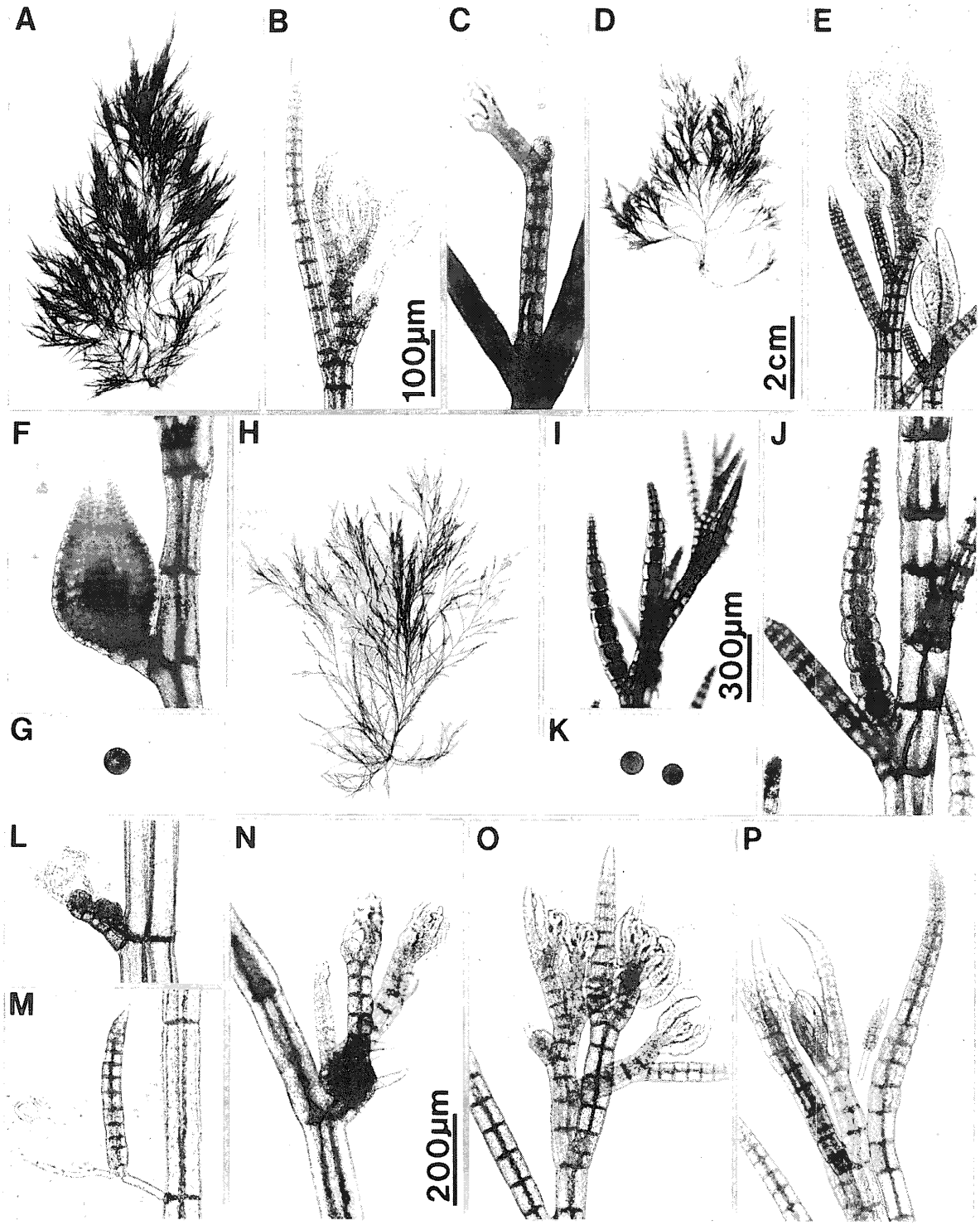


Fig. 4 Cultured plants of *Polysiphonia senticulosa*. All photographs from living materials except for **A**, **D** and **H**. **A**. Fertile female gametophyte cultured stationarily for about one and half year in 10℃, 16:8 LD. **B**, **C**. Female reproductive organs: **B**, procarps borne at the uppermost portion of the main axis (about two months old plant cultured in 10℃, 16:8 LD); **C**, Procarps borne on the axillary endogenous branch (about three months old plant cultured in 5℃, 8:16 LD). **D**. Fertile male gametophyte cultured stationarily for about 16 months in 10℃, 8:16 LD. **E**. Spermatangial branches borne on the upper portion of an ordinary branch (seven months old plant cultured in 5℃, 8:16 LD). **F**. Developing cystocarp formed on the plant which was mixed with spermatangial plants for 42 days in 10℃, 16:8 LD. **G**. Liberated carpospore. **H**. Fertile tetrasporophyte cultured stationarily for about four months in 10℃, 16:8 LD. **I**. Tetrasporangia borne on the upper portion of the ordinary branches (about six months old plant cultured in 5℃, 16:8 LD). **J**. Tetrasporangial branchlet borne singly at the axil. **K**. Liberated tetraspores. **L**. Two developing cystocarps arising from the same female trichoblast. **M-P**. Abnormal forms: **M**, propagule formed on the upper portion of a male gametophyte; **N**, adventitious branches bearing procarps, which produced from the pericarp of an aged procarp; **O**, procarps and mature spermatangial branches arising on the same plant; **P**, spermatangial branches formed on the tetrasporangial plant which bears mature tetrasporangia. Scale in **B** applies also to **G**, **K-M**; scale in **D** applies also to **A** and **H**; scale in **N** applies also to **C**, **F**, **J**, **O** and **P**.

their necks with wide ostiole rims. Isolated female gametophytes did not produce cystocarps but continued to form procarps.

Liberated carpospores (Fig. 4G) averaged 52.2 μm (range 42.5-60.0 μm; 36 spores measured) in diameter and were slightly larger than the parent tetraspores. They were inoculated onto glass slide. The carpospores germinated and grew into plants in a manner similar to that of tetraspores. These plants were reached reproductive maturity and began to form tetrasporangia on the last two orders of ordinary branches 51 days after inoculation (Figs. 4H, I). After two weeks of further inoculation, the tetraspores were released (Fig. 4K). Discharged tetraspores averaged 51.4 μm (range 45.0-60.0 μm; 21 spores measured) and repeated the same developmental process mentioned above. Also the tetrasporophytic plants formed the axillary endogenous branches. After the development of tetrasporangia on the ordinary branches was accomplished, the endogenous branches began to arise from the axil of ordinary branches. The number of these branches subsequently increased to three on each axil as the developmental process progressed. Each of the axillary branches began to form tetrasporangia. However, in many cases only one or rarely two branches on each axil reached full-grown sporangia (Fig. 4J) and contributed to the reproductive activity, as were those on male and female plants.

Tetrasporangia were formed from the third periaxial cell of fertile segments that produced five periaxial cells (Fig. 3B). The third periaxial cell (i.e. the fertile periaxial cell) divided into a tetrasporangial initial cell on adaxial side (i.e. inner side) and cover cell on abaxial side (i.e. outer side) by longitudinal cell division. The tetrasporangial initial cell subsequently divided into upper tetrasporangial mother cell and lower tetrasporangial stalk cell by horizontal cell division. The tetrasporangial mother cell was cylindrical

at first but later became spherical and enlarged. Finally the cell divided tetrahedrally to form a tetrasporangium. The cover cell divided again into a set of two cover cells. The fertile segment became swollen as the process of tetrasporangia development advanced. The fertile periaxial cells of each segment on the ordinary tetrasporangial branchlets usually located on the adaxial side to the main axis. Then, the same side with the direction of fertile periaxial cells swelled in the ordinary tetrasporangial branchlets. Four tetrads were discharged by extension of the cleavage furrow between the two cover cells.

In addition to the analysis of morphological development, the variation in growth and reproduction were observed at 5℃, 16:8 LD; 5℃, 8:16 LD; 10℃, 16:8 LD; 10℃, 8:16 LD; 15℃, 16:8 LD; 15℃, 8:16 LD; 20℃, 16:8 LD; 20℃, 8:16 LD, using the gametophytic generation derived from tetraspores and the tetrasporophytic generation derived from carpospores. The growth and reproductive responses of the gametophytic and tetrasporophytic plants to varying temperatures and photoregimes were similar. The plants grew most rapidly at 20℃, 16:8 LD and 8:16 LD. In 20℃, 16:8 LD upright axes grew vigorously at first issuing ordinary branches. As the growth progressed, they were entangled each other by adventitious rhizoids that were profusely formed from various part of the upright and prostrate axes. Consequently, their main axes became indistinct. In 15℃, 8:16 LD and 20℃, 8:16 LD prostrate axes were well developed compared with the upright axes. Adventitious branches formed from lower part of the main axis grew prostrately producing adventitious rhizoids and the plants showed prostrate appearance. All of prostrate axes were terminated in dome shaped apical cells differently from those of the ordinary branches on upright axes.

On the other hand, at 5℃, 16:8 LD and 8:16 LD the plants grew slowly. However, the plants showed normal morphology as field plants

with the percurrent upright main axes issuing ordinary branches or vegetative trichoblasts. In all cases of culture combinations the diameter of the upright axes remained slender similarly to those of field plants. The length/diameter ratios of segments in cultured plants in general varied correlatively with thallus length. When the plants showed prostrate feature issuing many adventitious branches and secondary rhizoids irregularly such cases as in 20℃, 16:8 LD and 8:16 LD, the relativity of its ratios and thallus length became failed. Procarps and spermatangia were formed first at 5℃, 16:8 LD 45 days after inoculation and lastly in 119 days at 5℃, 8:16. Neither male nor female organ was formed at 15℃, 8:16 LD; 20℃, 16:8 LD.

Several abnormal phenomena were observed in this culture study. Two developing cystocarps arisen from one female trichoblast were frequently observed when the female plants matured (Fig. 4L). Propagules were often formed on the upper portion of ordinary branches in male gametophytic and tetrasporophytic plants (Fig. 4M). They were shed from the parent thallus later and then developed into fertile male gametophytes or tetrasporophytes bearing reproductive organs respectively. Adventitious branches were infrequently formed from pericarp of aged procarps that produced another new procarps (Fig. 4N). Procarps and spermatangial branchlets were formed on the same gametophytic plant (Fig. 4O). However, in this culture experiment it was not confirmed whether they were functional reproductive organs. Furthermore a few spermatangial branchlets were formed on the tetrasporophytic plants in which tetrasporangia reached maturity (Fig. 4P). In this case tetraspores were normally discharged and grew into normal gametophytic plants, but reproductive function of the spermatangial branchlets was not confirmed.

## <Discussion>

This analysis has revealed that the life cycle of *Polysiphonia senticulosa* is characterized by alternation of isomorphic gametophytes and sporophytes, what is called "*Polysiphonia* type" life history. This culture experiment has confirmed the following features of this species. (1) The thallus is composed of slender upright axes and prostrate axes. The upright axes consist of the main axis and many secondary axes. They grow indeterminately producing the next order of ordinary branches profusely. (2) The upright main axes form several adventitious branches endogenously from their lower portion. Some of adventitious branches grow indeterminately as secondary upright axes. The rest of them extend along the substratum as the prostrate axes issuing many secondary rhizoids. (3) The thallus has four periaxial cells and lacks cortical cells. (4) The adventitious rhizoids are formed from periaxial cells as protrusions without septation. (5) The thallus has soft and flaccid texture during the matured stage. (6) The determinate branchlets have sharply pointed apices, while those of prostrate axes have dome shaped apical cells. (7) Axillary endogenous branchlets are formed on ordinary branches of matured plants, and they also contribute the reproductive activity. Only one branchlet is formed in gametophytic plants and develop reproductive organs near the apex later than those on the ordinary branchlets do. One to three or rarely four axillary branchlets on each axil are formed in matured tetrasporophytic plants. They begin to develop tetrasporangia later than those on the ordinary branchlets do, and only one of arisen axillary branchlets attains to the maturity and then performs its reproductive function. (8) On the ordinary branchlets tetrasporangia are formed on ultimate and penultimate branchlets arranging in a straight line because they are developed from the third periaxial cells located linearly adaxial side to the main axis. (9) Procarps are formed

from fertile trichoblasts near the apices of ordinary and endogenous axillary branchlets in the spiral line with intervals of three or four segments. Carpogonial branches are composed of four cells. (10) Cystocarps are ovate shape in course of development. They become urceolate shapes in maturity with clear constricted necks and wide ostioles. (11) Spermatangial branchlets are formed replacing the whole trichoblasts. They are terete to cylindrical in shape with two to five celled sterile tips on the apices. They arise continuously on each segment in 1/4 divergence spiral line near the apical portion of indeterminate ordinary and endogenous axillary branchlets. Several features of mentioned above had been already reported by Kudo and Masuda (1988), who examined the holotype specimens of *P. senticulosa*, and *Polysiphonia pungens* Hollenberg (1942). At the same time, they recognized these two species were identical and reduced the latter to the former.

However, it had been suggested that the length/diameter ratios of the segment on the upright main axes were inadequate feature for taxonomic criterion because the value varied according to the thallus length or growth stages.

In this culture experiment, *P. senticulosa* grew well morphologically and reproductively in lower temperature conditions, i.e. 5℃, 16:8 LD; 5℃, 8:16LD; 10℃, 8:16LD. On the other hand in higher temperature conditions, i.e. 15℃, 8:16 LD; 20℃, 16:8 LD; 20℃, 8:16 LD, plants arose the adventitious rhizoids profusely and irregularly. The upright axes were entangled each other and finally showed prostrate appearance. These results agree with the phenological feature of this species in the field, that is, *P. senticulosa* appears in winter and disappears by the end of spring in the coasts of Japan. It is considered that the washout of field plants is caused by the increase of resistance to the wave action brought by entanglement of branches each other

with profuse adventitious rhizoids.

*Polysiphonia morrowii* Harvey is similar to *P. senticulosa*, sharing several taxonomic features mentioned above (Kudo and Masuda 1992). Therefore young plants of *P. morrowii* have been misidentified as *P. senticulosa* in Japan until Kudo and Masuda (1981) pointed out. *P. morrowii* was investigated by Kudo and Masuda (1981, 1992) and furthermore its holotype specimen was examined (Masuda, *et al.* 1995). The species is characterized following features. It has thick main axis and setaceous texture, diameter of upright axis is up to 550 μm at the lowermost part of upright axes, tetrasporangia are formed on the ultimate branches and axillary branchlets, tetrasporangial axillary branchlets are formed seven to eight and majority of them become functional. It was confirmed in this study that both species are different from each other in several features, i.e. texture of thallus, diameter of upright axis, location of tetrasporangia on the ordinary branches, and number of tetrasporangial axillary branchlets formed in each axil.

Yoon (1986) has reduced *P. urceolata* auct. japon. to a synonym of *P. morrowii* by the reason of the existence of endogenous axillary branchlets in both of the species. However, he did not examined the respective holotype specimens or specimens from their type locality. Indeed when Kudo and Masuda (1988) examined the holotype specimens of *P. senticulosa*, the result of their examination has revealed that *P. urceolata* auct. japon. is identical with *P. senticulosa*. Accordingly, Yoon's suggestion is not acceptable.

Kylin (1941) described new genus, *Orcasia*, based on *P. senticulosa* together with *P. morrowii*. He regarded that the occurrence of endogenous axillary branches growing indeterminately was the significant taxonomic feature to divide the new genus from the genus *Polysiphonia*. However, in both *P. senticulosa* and *P.*

*morrowii*, it is now known that the axillary branches usually show the determinate growth or terminate as short branchlets. Although an elongated axillary branch was observed as an abnormal case in aged plant of *P. morrowii* (Kudo and Masuda 1981), a series of investigations conducted by Kudo and Masuda have shown that the establishment of the genus *Orcasia* is not acceptable.

In this culture study the propagules were formed on both gametophytic and sporophytic plants. They recycled the respective phases that produced themselves. Kapraun (1977) and Womersley (1979) have reported that several species of this genus bear propagules. According to them, these structures function for reproduction. Further investigation is needed to clarify whether the propagules of *P. senticulosa* contribute the reproductive activity in the field.

This study has shown male and female organs on the same plant. It has not been confirmed if they were functional reproductively. This observation has taxonomically significant implication. Segi (1949) has established a new genus *Enellitosiphonia* for one of the reasons that the genus has monoecious gametophyte. If further study reveals that *P. senticulosa* is also monoeciously reproductive, Segi's *Enellitosiphonia* must be reconsidered.

Finally, in the future, in order to elucidate the entity of *P. senticulosa*, it is necessary to conduct new approaches in addition to morphological analysis. For example, using DNA analysis, the taxonomic and phylogenic relationships between this species and other in this genus must be examined.

<Acknowledgment>

I am deeply indebted of Professor Michio Masuda, Hokkaido University, for his kind guidance. I am also grateful to Dr. Hiroto

Takamiya, Sapporo University, for his reading of English manuscript. This study was supported in part by Research Grant in 1997 from Sapporo University.

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