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〈Article〉

Culture study of the red alga *Polysiphonia yendoi* Segi (Ceramiales, Rhodophyta)

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〈**Abstract**〉

Life history and morphological development of *Polysiphonia yendoi* Segi (Ceramiales, Rhodopbyta) were investigated by culture experiments. This species was shown to have the "Polysiphonia-type" life history. Morphological plasticity was assessed in several features which have been adopted as taxonomic criteria. The external appearance of individual plants, length/diameter ratios of segments, morphology of cystocarps showed phenotypic variation according to the growth stage, temperatures and photoregimes. On the other hand, some critical features of this species were discovered for the first time. Endogenous adventitious branches were formed from the basal portion of main axes, and cicatrigenous branchlets were exogenously produced from the basal cells of shed trichoblasts. It was confirmed that the secondary rhizoids were cut off from periaxial cells, which was different from the original description of *P. yendoi*.

Key Index Words: Life history; Ceramiales; *Polysiphonia* ; *Polysiphonia yendoi*; Rhodomelaceae; Rhodophyta; taxonomy.

*Dedicated to Professors Tadao YOSHIDA and Masakazu TATEWAKI on the occasion of their academic retirement.

〈Introduction〉

The red alga *Polysiphonia yendoi* Segi (1951) was described on the basis of material collected at Oshoro Bay in Hokkaido. This alga has been reported from various localities of Japan and adjacent waters (Segi 1951, Perestenko 1980, Yoon 1987). This species is characterized principally by the following features: thalli composed of a primary upright axis and several secondary, assurgent axes, developing from stolons; ecorticated thalli with four periaxial cells per segment; ordinary branches replacing trichoblasts; trichoblasts formed continuously in each segment. Segi (1951) reported the following several *Polysiphonia* species which share these features with *P. yendoi: Polysiphonia codiicola* Zanarrdini *P. obsoleta* Segi, *P. pulvinata* J. Agardh, *P. subtilissima* Montagne, *P. scopulorum* Harvey. These species have been reported from a few localities where they were included in the geographical range of *P. yendoi*, excepting *P. pulvinata*. *Polysiphonia pulvinata* was reported from more southerly areas. It is difficult to distinguish these five species from each other despite Segi's detailed descriptions. This strongly suggests that a critical study is needed to elucidate the taxonomic relationship between *P. yendoi* and related species.

Because most previous investigators have had little knowledge of morphological variation according to growth stages or environmental conditions, several species in this genus were described and identified with a few materials from a few localities. It is apparently necessary to assess the appropriateness of taxonomic criteria which have been adoped in each species of this genus. A comparative study through life history stages may be useful to evaluate taxonomic features as pointed out by Dixon (1963) and exemplified by Masuda (1982), Kudo and Masuda (1986 and 1992). This method may be effective for *P. yendoi* and related species, however, there has been no report on the variability of morphological features in this *Polysiphonia* group.

In this paper, morphological development, growth and reproductive responses in *Polysiphonia yendoi* at various temperatures and daylength are discussed. Furthermore, the morphological features which have been used as taxonomic criteria were assessed in their morphological variability to establish clear taxonomic features.

〈Materials and methods〉

Unialgal cultures were established from tetraspores and carpospores released

from tetrasporophytic and cystocarpic plants collected on 7, August and 7, November 1987 at Oshoro Bay (43°13'N, 140°52'W) in Hokkaido, according to the methods described earlier (Masuda 1982). Spores were cultured in plant growth chambers illuminated with cool white fluorescent lamps (2500-3000 lux). The temperatures and photoperiods were regulated in the following combinations: 5°C, 16:8 LD (light and dark cycle); 5°C, 8:16 LD; 10°C, 16:8 LD; 10°C, 8:16 LD; 15°C, 16:8 LD; 15°C, 8:16 LD; 20°C, 16:8 LD; 20°C, 8:16 LD (16:8 LD means the long day condition, 8:16 LD means the short day condition). Plants cultured at 15°C, 16:8 LD were chiefly used for description of morphological development. In order to investigate growth and reproductive responses to varying temperatures and photoregimes, germlings of both carpospores and tetraspores were cultured at the eight conditions mentioned above. These cultures were put into fresh medium every two weeks and maintained in glass dishes (71 x 61 mm or 65 x 80 mm) containing one-half strength Provasoli's Enriched Seawater (PES) (Provasoli 1968). When female and male reproductive organs were formed on individual plants, female and male plants were cultured in single dishes (71 x 61 mm) and placed on a Taiyo R-II Rotary Shaker at 90-100 rpm in 15°C, 16:8 LD to support the fertilization and development of cystocarps.

Morphological observations were carried out using living materials and specimens preserved in 10% formalin seawater. Microscope 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 account is based on cultured plants maintained at 15°C, 16:8 LD unless otherwise indicated. Liberated tetraspores were globular and brownish red or pale red in color. They averaged 42.3 μm (range 32.5-47.5 μm; 150 spores measured) in diameter (Fig. 1A). Isolated tetraspores soon attached to the substratum, elongated and stood upright. Within one day after inoculation, they grew into bipolar sporelings of 6-7 segments, which had differentiated into a colorless primary rhizoid and a pigmented upright axis (Figs. 1B, 2A). Excepting the apical, subapical and basal segments, each segment of the upright axis was composed of an axial cell and four periaxial cells. The basal segment was composed of a single cell. The suprabasal

segment usually had four periaxial cells but it sometimes was composed of a single cell like the basal segment. The apical cell cut off new segments somewhat obliquely under its own. The main axis became recurved (Figs. 1C, 2B) and began to form lateral initials exogenously on the upper segments other than the 7-8th. The first lateral initial was cut off from the dorsal side at the second or third segment from the apex (Figs. 1D, 2C). The second lateral initial was produced on the next upper segment. After formation of periaxial cells, it was situated in a position rotated a quarter cycle in a counterclockwise direction toward the apex of the upright axis. Subsequently, lateral initials were formed from each segment continuously in a spiral line as development of the main axis proceeded (Figs. 1E, 2D). With successive formation of these laterals the main axis gradually straightened (Fig. 1F). The first lateral initial grew into a vegetative trichoblast divided pseudodichotomously. Formation of ordinary branch was delayed: the first ordinary branch was formed after the production of 9-15 trichoblasts (Fig. 1G). In any case trichoblasts were formed again on 5-10 segments successively after initiation of the first ordinary branch and then they were replaced by a second ordinary branch (Fig. 2G). This process was repeated continuously, and thus ordinary branches of the first order were formed from every sixth to eleventh segment of the main axis. However, the ordinary branches near the apex of main axis were formed at intervals of 1-6 segments. All the ordinary branches grew indeterminately as did the main axis, forming vegetative trichoblasts (Fig. 1H) or ordinary branches of the second order. Growing apices of all branches were not surrounded by the young ordinary branches of the next order, but the vegetative trichoblasts grew over the apical cell of parent branches.

A primary rhizoid cut off from the basal segment became ramified at the growing tip (Fig. 2H), although it was not accompanied by the formation of a septum, and became an expanded disc-like holdfast (Figs. 1E, 3A). Primary rhizoids which were not ramified were also frequent and became elongated filamentous holdfasts (Figs. 1C, D, 2B). Some of the latter rhizoids later became ramified as was the former. Secondary rhizoids were cut off from the basal segment (Figs. 1F, 2E, H) and on rare occasions from basal portion of the primary rhizoid (Fig. 2F). They were also produced from the periaxial cells of the suprabasal segment (Figs. 1I, 26H) and several lower segments of the main axis (Figs. 1I, 2H, 3D). These secondary rhizoids became

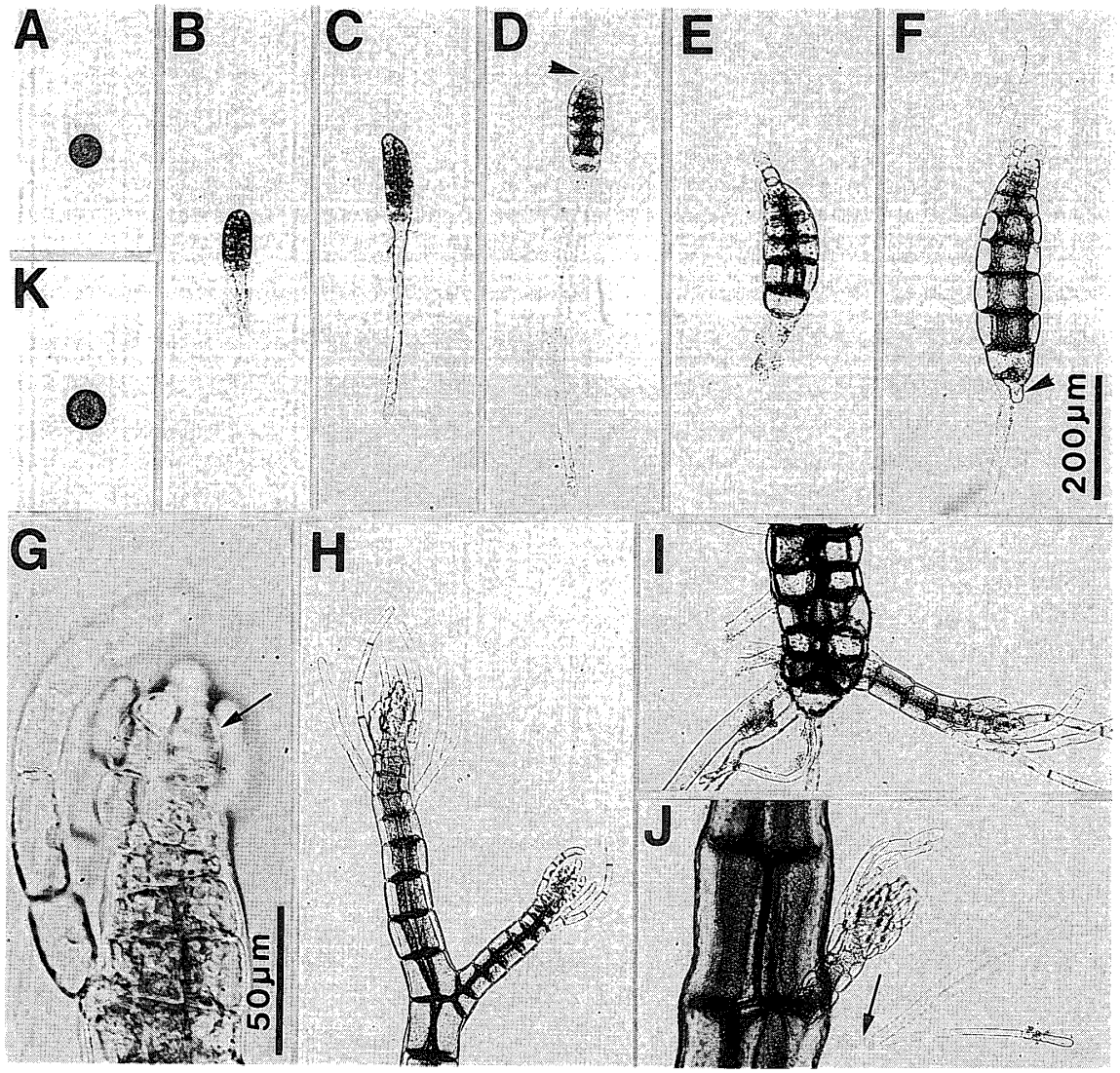
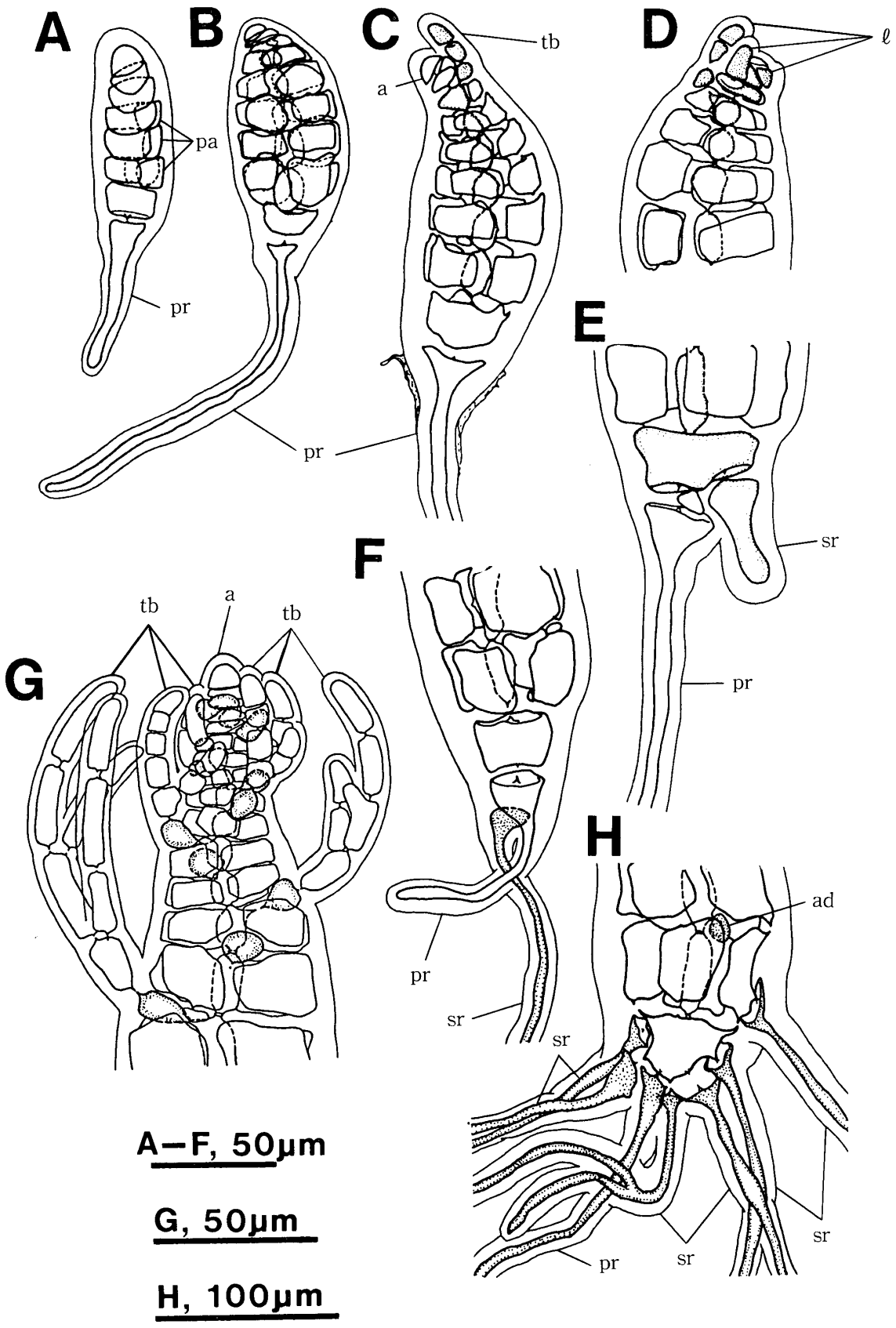


Fig. 1. Tetraspore and its development of *Polysiphonia yendoi* at 15°C, 16:8 LD and carpospore. All photographs from living material. A. Liberated tetraspore. B. One-day-old germling. C. Two-day-old germling; note the axis being recurved slightly. D. Three-day-old germling forming a lateral initial (arrowhead) on the dorsal side. E, Five-day-old germling with a digitate, primary rhizoid. F. Seven-day-old germling with an almost straight main axis forming a secondary rhizoid (arrowhead). G. Apical portion of a two-week-old plant forming vegetative trichoblasts and the first ordinary branch (arrow). H. Apical portion of a three-week-old plant of which the main axis and first order branch are forming vegetative trichoblasts. I. Basal portion of a 32-day-old plant forming an endogenous adventitious branch and eight secondary rhizoids. J. Cicatrigenous branch arising from the basal cell of a trichoblast (arrow) before its shedding (about four months old plant). K. Liberated carpospore. Scale in F applieds also to A-E, and H-K.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elongated and entangled loosely with each other. Some of them became disc-like holdfasts (Fig. 3A).

In this species two types of adventitious branches were observed. Three to five endogenous adventitious branches were formed from an axial cell of the suprabasal to several upper segments of the main axis 11 days after inoculation (Fig. 3A). The initial cell of an adventitious branch was cut off from lower half of the axial cell. These adventitious branches grew indeterminately. They developed first along the substratum (Fig. 1I), later became prostrate axes producing a number of secondary rhizoids from periaxial cells on their ventral side or stood up assurgently and became secondary upright axes (Fig. 3B). About one month after inoculation another type of adventitious branches (Fig. 3C) were also formed from the basal cells of trichoblasts after their shedding. They had exogenous origin because the trichoblasts were borne exogenously. These exogenous adventitious branches are known as cicatrigenous branches (Hollenberg 1942). Occasionally these branches were formed before trichoblast shedding (Figs. 1J, 3F). Their development was less vigorous than ordinary branches, but these adventitious branches later formed reproductive organs.

Plants reached reproductive maturity 21 days after inoculation. Spermatangial branchlets and procarps were produced on separate individuals. The spermatangial branchlets were formed as a first branch of the fertile trichoblasts (male trichoblasts) and borne on 4-6 segments successively at the uppermost of the main axis and the ordinary branches (Fig. 4B). These male trichoblasts were replaced by ordinary branches or vegetative trichoblasts. Then, male trichoblasts were formed again successively. This process was repeated continuously as long as the plants continued to grow well (Fig. 4A). Subsequently, male trichoblasts were also formed on the

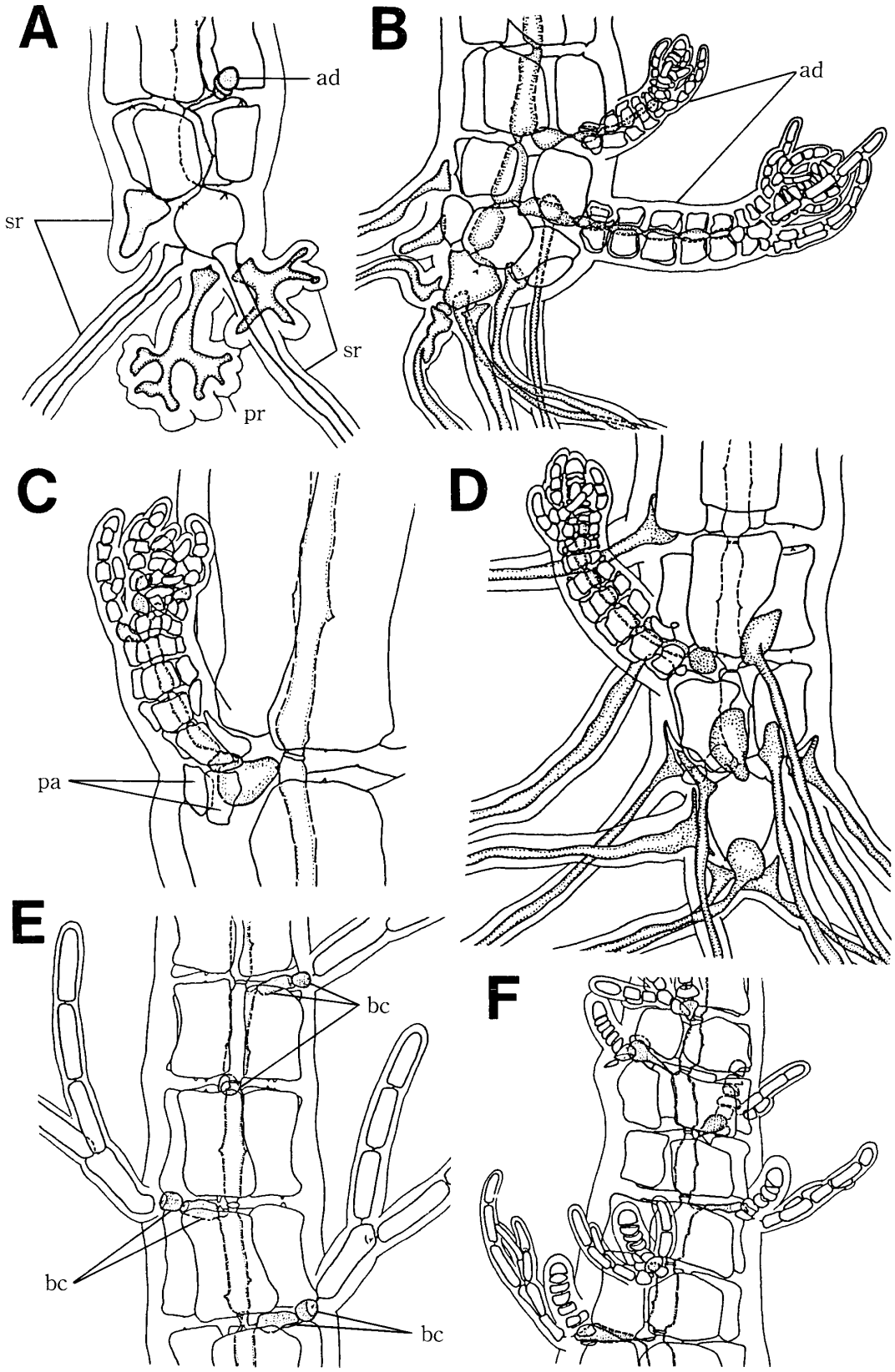
Fig. 2. Tetrasporelings of *Polysiphonia yendoi* grown at 15°C, 16:8 LD. A. One-day-old germling, bearing the first periaxial cells (pa) on the middle segments of the plant: pr, primary rhizoid. B. Two-day-old germling, producing four periaxial cells in several segments. C. Three-day-old germling forming one trichoblast (tb) from the dorsal side: a, apical cell. D-F. Five-day-old germling: D, apical portion forming spirally arranged three laterals (l); E, secondary rhizoid (sr) cut off from the basal segment; F, secondary rhizoid cut off from the basal portion of the primary rhizoid. G, H. Three-week-old plant: G, apical portion of the main axis forming 13 trichoblasts continuously; H, basal portion of the main axis issuing many rhizoids in which one forked rhizoid is included, and forming an initial cell of endogenous adventitious branch (ad) from the suprabasal segment.

cicatrigenous branches. Mature spermatangial branchlets were ellipsoid to conical-cylindrical, 150-275 μm long x 43-70 μm wide and had a blunt apex with one sterile cell. The procarps (Fig. 4D) were formed at a distance of one to four segments on the corresponding position to those of the male trichoblasts. These were produced from the suprabasal segments of fertile trichoblasts (female trichoblasts). The female trichoblasts were usually individually formed. They were repeatedly replaced by ordinary branches and/or vegetative trichoblasts.

Fertile female gametophytes were mixed in single dishes with male gemetophytes releasing spermatia and placed on a rotary shaker. All these female gametophytes (Fig. 4C) produced mature cystocarps which released viable carpospores 14 days after the initiation of mixed culture. The cystocarps were ovate to globular with wide ostiole, 450 μm long x 410 μm (Fig. 4E). Discharged carpospores (Fig. 4F) averaged 50.4 μm (range 47.5-52.5 μm; 17 spores measured) in diameter. They were slightly larger than the parent tetraspores. Isolated female gametophytes did not produce cystocarps but continued to form procarps.

Carpospores were inoculated onto glass slides. They germinated and grew into plants in a manner similar to that of the parent tetraspores. These plants reached reproductive maturity 22 days after inoculation and began to form tetrasporangia. The number of tetrasporangia increased over another week and many tetraspores were discharged. Tetrasporangia were formed in last two orders of branching and arranged spirally in 20-35 segments successively (Fig. 4H). The segments of mature tetrasprongia became slightly swollen and the tetrasporangial branchlets became distorted. The fertile tetrasporangial plants (Fig. 4G) produced many short cicatrigenous branches from the basal cell of trichoblasts after its shedding. The

Fig. 3. Vegetative structures of cultured plants (tetrasporophytes) of *Polysiphonia yendoi*. A. Basal portion of a 11-day-old plant issuing an initial cell of endogenous adventitious branches (ad) from the third segment and producing four secondary rhizoids (sr) on the basal and suprabasal segments. B. Basal portion of a three-week-old plant issuing two endogenous adventitious branches (ad) with four periaxial cells in its suprabasal segment. C. Cicatrigenous branch having two peraxial cells (pa) in its basal segment (about four months old plant). D. Endogenous adventitious branch having two periaxial cells in its suprabasal segment (three-week-old plant). E. Trichoblasts with two basal cells (bc) arranged in clockwise direction (41-day-old plant). F. Cicatrigenous branches formed exogenously from the basal cells of trichoblasts before their shedding (41-day-old plant).



A, C, 100μm B, 100μm D, 100μm E, F, 100μm

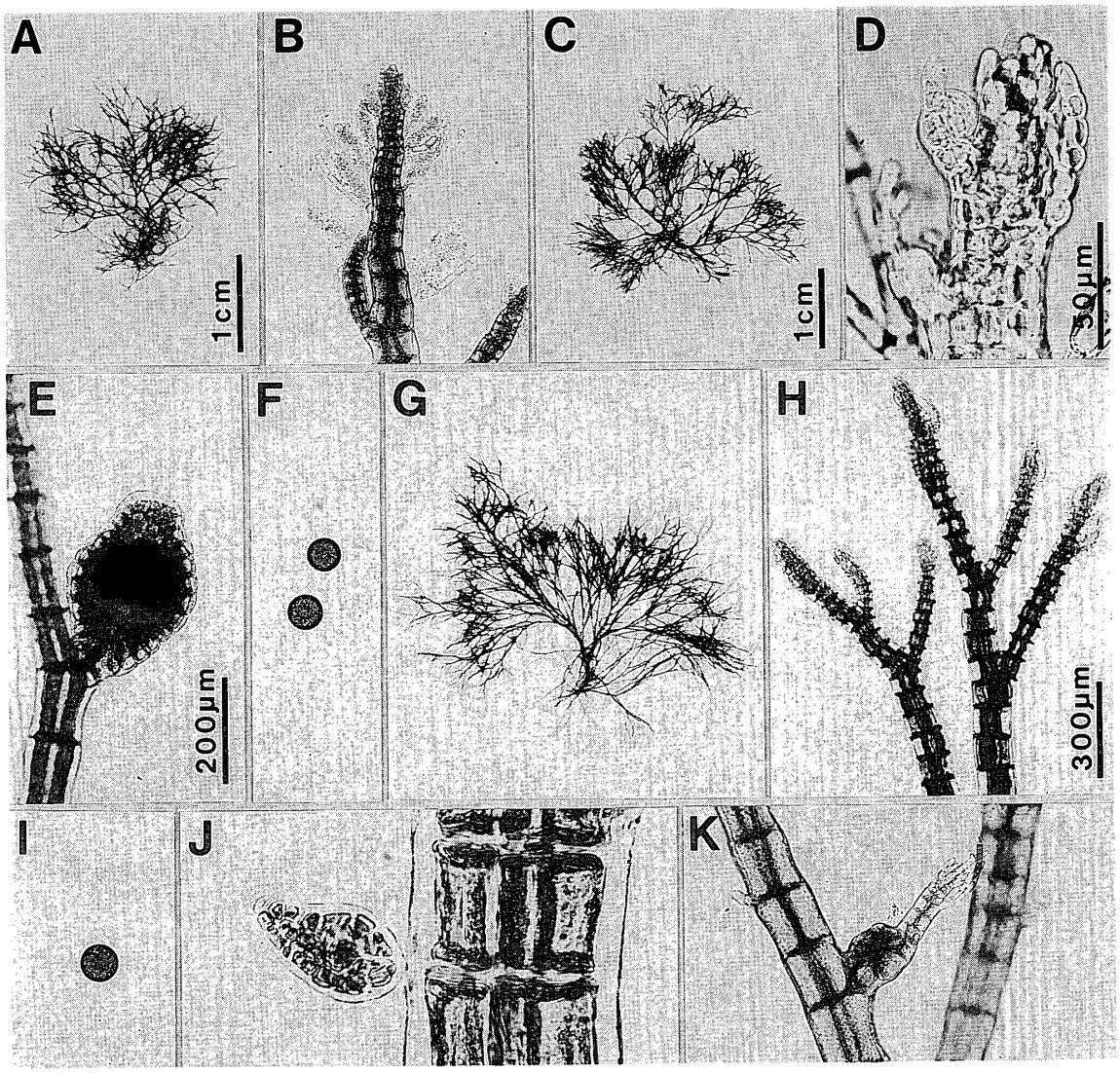


Fig. 4. Cultured plants of *Polysiphonia yendoi*. All photographs except for A, C and G from living material cultured at 15°C, 16:8 LD unless otherwise indicated. A. Fertile male gametophyte cultured for 83 days. B. Tufted spermatangial branchlets formed on the plant shown in A. C. Fertile female gametophyte cultured stationarily for about 6 months. D. Procarps borne at the uppermost portion of the main axis (41-day-old plant). E. Mature cystocarp formed on the plant which was mixed with spermatangial plants for two weeks at 20°C, 16:8 LD. F. Liberated carpospores. G. Fertile tetrasporophyte cultured stationarily for about 6 months. H. Tetrasporangia borne on the upper portion of the ordinary branches. I. Liberated tetraspore. J. Propagule formed on the upper portion of a male gametophyte. K. Adventitious branch borne on the female organ. Scale in C applies also to G; and scale in E applies also to B, F, and I-K.

tetrasporangia were first produced on the upper portion of ordinary branches and later on cicatrigenous branches. The resulting tetraspores were similar to those from the field except for their slightly larger size that averaged 48 μm in diameter (range 40-52. 5 μm; 77 spores measured) (Fig. 4I).

After formation of reproductive structures, both gametangial and tetrasporangial plants grew continuously and produced new branches on which reproductive structures were formed respectively. At one month after inoculation the fertile plants had reached 6.1-8.1 mm in length and 140-160 μm in diameter at the basal poriton, and formed branches up to the fourth order. The plants had 12-16 ordinary branches of which the lower one grew better, 3-5 endogenous adventitious branches which developed well like the ordinary branches, and 5-8 cicatrigenous branches which were clearly shorter than the ordinary branches. As the result of subsequent vegetative growth, branches which formed a large number of secondary rhizoids entangled with each other and the main axis of the plants became indistinct.

Propagules (Fig. 4J) were often formed on trichoblasts borne on male gametophytes. As the supporting trichoblasts were deciduous, these propagules became free from the parent plants, attached to the substrate, and bore rhizoidal filaments. They grew into fertile gametophytes bearing spermatangial branchlets. The upper, strile segments of female trichoblasts sometimes transformed into adventitious branches producing four pigmented periaxial cells in each segment.

Both vegetative and reproductive trichoblasts were usually formed in a counterclockwise spiral arrangement having one basal cell. However, trichoblasts with two basal cells arranged clockwise were observed on rare occasions (Fig. 3E).

In addition to analysis of morphological development, variations in growth and reproduction were assessed at 5°C, 16:8 LD; 5°C, 8:16 LD; 10°C, 16:8 LD; 10°C, 8:16 LD; 15°C, 16:8 LD; 15°C, 8:16 LD; 20°C, 16:8 LD; 20°C, 8:16 LD, using both tetraspores and carpospores released from field-collected plants respectively. Neither tetraspores and carpospores were germinated in 5°C, 16:8 LD 5°C, 8:16 LD, therefore the result of them were excluded from the following acount. The growth and reproductive responses of the gametophytic and tetrasporophytic phases to varying temperatures and photoregimes were similar. Plants grew most rapidly at 20°C, 16:8 LD and most slowly at 10°C, 8:16 LD. Spermatangial branchlets and procarps were formed first at 15°C, 16:8 LD

and 20°C, 16:8 LD 21 days after inoculation. Lastly at 10°C, 8:16 LD, spermatangial brachlets were produced 90 days after inoculation and procarps were formed after about half year. Tetrasporangia were produced first at 15°C, 16:8 LD 21 days after inoculation and last at 10°C, 16:8 LD after 35 days. However, development of tetrasporangia were not observed under other culture conditions. The diameters of main axes and length/diameter ratios of segments in cultured plants in general varied correlatively with thallus length.

〈Discussion〉

In this culture experiment *Polysiphonia yendoi* Segi were shown to have a typical "Polysiphonia-type" life history. The present study revealed that the following morphological features showed phenotypic plasticity according to the stage of growth, temperatures or photoregimes. (1) The external appearance of individuals varied according to the growth stage or culture conditions. Young plants had distinct main axes and showed penicillate appearance, because ordinary branches arising from the lower portion developed well and those from upper portion became progressively shorter. With age, reproductive plants formed large number of cicatrigenous branches and secondary rhizoids from all parts of branches. Consequently, these densely branched plants showed different external appearence from those of young plants. (2) Length/diameter ratios of segments were shown to vary irregularly. However the plants cultured at 20°C, 16:8 LD showed the most rapid growth and had the longest segments and showed the largest value of ratios. The ratios varied correlatively with length of segment. (3) Morphology of cystocarps varied acorrding to the stage of development. Developing young cystocarps showed an ovoid shape and mature cystocarps became globular. Consequently it is strongly suggested that these unstable morphological features are inappropriate for use as taxonomic criteria.

Some critical structural features of the speices under study should be added to Segi's description (1951). Two types of adventitious branches originate endogenously from axial cells of lower segments of the main axis and exogenously from scar cells (=basal cells of shed trichoblasts) as pointed out by Yoon (1986). The latter are referred to as cicatrigenous branches (Hollenberg 1942). In cultured plants this often occurs before the shedding of trichoblasts. The same phenomenon was reported for

field-collected plants of Danish *Polysiphonia elongata* (Hudson) Harvey, *P. nigrescens* (Hudson) Greville (Rosenbinge 1923-24), those of Hawaiian *P. tuberosa* (Hollenberg 1968) and Japanese *P. japonica* Harvey (Kudo and Masuda 1986).

Segi (1951) reported in his original description of *Polysiphonia yendoi* that the rhizoids were not cut off from periaxial cells. However, this characteristic feature was not always found in cultured plants nor the parent plants collected from field, the rhizoids were produced as independent cells which were accompanied by formation of a septum as pointed out by Yoon (1986).

Propagules were frequently found on trichoblasts of male plants in this culture experiment. They recycled the same phase that they themselves were producedby. In North Carolina *Polysiphonia ferulacea* (Kapraun 1977), Australian *P. propagulifera* Womersley (1979) and *P. mollis* Hooker et Harvey ex Harvey (Womersley 1979), cicatrigenously originated propagules were reported in field-collected plants. It can be speculated that propagules of these three species are really asexual reproductive organs in the field populations. Furthermore, similar structures were reported for cultured plants in Japanese *P. japonica* (Kudo and Masuda 1986) and European *P. fibrillosa* (Koch 1986). However propagules have not been confirmed in the field for the aforementioned two species and this species.

The transformation of sterile segments of female trichoblasts to a vegetative branch was also reported by Rosenvinge (1923-24) in *Polysiphonia urceolata* from field-collected plants. This phenomenon has not been confirmed in this species from field. The trichoblasts with two basal cells found in this culture study were also reported by Segi (1951) in the original description of *P. obsoleta* as a specific feature. However, this phenomenon has not been observed in the field-collected plants of *P. yendoi*. Further detailed studies are needed to evaluate the taxonomic relationship between *P. yendoi* and related species.

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