Developmental morphology of a *Polysiphonia* species (Ceramiales, Rhodophyta) in a culture experiment

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<Abstract>

The morphological development and life history of a *Polysiphonia* species (Ceramiales, Rhodophyta) were investigated by culture experiment. This alga was reported as *Polysiphonia urceolata f. lepadicola* by Segi in 1951. Morphological stability and plasticity were assessed in several features, which have been adopted as taxonomic criteria for the genus *Polysiphonia*. The following features are confirmed as morphological stability in this alga: (1) Thallus is composed of slender upright axes, and intricately entangled prostrate axes; (2) Thallus is soft and flaccid in texture; (3) Thallus produces adventitious branches on the lowermost portion of the main axis in the early stage, and also on the upper portion of upright axes in the late stage; (4) Thallus is ecorticated with four periaxial cells; (5) Rhizoids are formed without septation; (6) Determinate branches have sharply pointed apices; (7) Endogenous axillary branchlets are formed; (8) Vegetative trichoblasts are rarely formed; (9) Carpogonial branch is composed of four cells; (10) Spermatangial branchlets are formed replacing the whole trichoblasts, they are terete to cylindrical in shape with sterile tips; (11) Tetrasporangia are formed in a straight line, on the last two orders of ordinary branchlets and on axillary branchlets. These features are appropriate taxonomic criteria.

The external appearance of individual plants, length/diameter ratios of segments showed phenotypic variation according to the growth stage, temperatures and photoregimes.

Key Index Words: developmental morphology, *Polysiphonia urceolata f. repadicola*, culture experiment, *Polysiphonia morrowii*, *Polysiphonia senticulosa*, taxonomy, Rhodomelaceae, Ceramiales.

<Introduction>

The red algal genus *Polysiphonia* Greville distributed widely throughout the world is a large and common genus including more than 200 species (Wormersley 1979, Stegenga et al 1997). About 40 species in this genus have been reported on the Japanese coast (Yoshida, et al 1985). This genus is also well known as a group which shows wide range of morphological variation. Kim & Lee (1999) established a new genus *Neosiphonia* and proposed to transfer several species from the genus *Polysiphonia* to the new genus.

Consequently, the *Polysiphonia* species listed for the Japanese coast was reduced to 18 species including *Polysiphonia morrowii* Harvey (1857), *Polysiphonia senticulosa* Harvey (1862), etc (Yoshida, et al 2005). *Polysiphonia* species might now be characterized by (1) rhizoids formed from periaxial cells without septation, (2) ecorticated thalli, (3) four periaxial cells in each segment, (4) spermatangial branches formed by replacing the whole of a trichoblast, (5) tetrasporangia arranged in straight line, (6) carpogonial branches constructed from four cells.

Segi (1951) reported this alga as *Polysiphonia urceolata f. lepadicola* (Lingbye) Segi comb. nov. based on a specimen from Rausu in Hokkaido, Japan. The geographic distribution of this alga has been confirmed from Utoro in Hokkaido which faces the Sea of Okhotsk, through the Nemuro Strait, to the Pacific coast of Hokkaido and the Sanriku coast of northern Honshu. This alga resembles *Polysiphonia senticulosa* Harvey or *Polysiphonia morrowii* Harvey in several features involving the formation of endogenous axillary

branches, but it is distinguished from the latter two species in the following points. The plants of this alga are smaller, and upright axes are slenderer than in the latter two species. The prostrate axes of this alga are entangled in a relaxed manner with other algae, however, those of the other two are tightly and intricately entangled with each other making a discoidal holdfast system. The habitat of this alga is the upper intertidal zone of wave-exposed areas, while that of *P. senticulosa* is the middle intertidal zone in calm areas or in tide pools, and that of *P. morrowii* is the sublittoral to middle intertidal zone of wave-exposed areas.

Polysiphonia urceolata f. lepadicola was reported as a recombination of *Polysiphonia lepadicola* (Lingbye) Kuetzing (1849). However, the description of axillary branches was not confirmed in reports about *Polysiphonia lepadicola.* In addition, the number of periaxial cells was described as being four or five at the basal portion of *P. lepadicola.* The features of *P. lepadicola* mentioned above differ distinctly from this alga. On the other hand it has not been confirmed that *Polysiphonia urceolata* (Dillwyn) Greville (1824) forms axillary branches. There is a strong possibility that this alga is not conspecific with *P. lepadicola or P. urceolata,* but that this alga might be an independent species.

The investigation of morphology, analysis of life history and/or ecological study using field plants or its population are basically needed to clarify the taxonomic status of this alga. All characteristics shown throughout its whole life history must be recognized to determine the appropriate taxonomic features (Dixon 1963). From this point of view, an investigation employing a culture experiment would be effective for the observation of the features shown in early developmental stage and the analysis of morphological stability or variability.

This paper will report the morphological development of this alga in a culture experiment, and will report the growth and reproductive responses of this alga to various combinations of temperatures and photoperiods. Furthermore, the stability and variability of morphological features will be assessed in order to establish more accurate taxonomic criteria of this alga.

<Materials and Methods>

Materials used in this study were collected in October 1986 at Erimo-misaki (44° 55'N, 143° 14'W), Hokkaido and in June 1987 at Shiriya-zaki ((41° 25'N, 141° 27'W), Aomori Prefecture, Japan. Plants for culture study were transported to the laboratory in sterile seawater in an insulated chest on ice. Unialgal cultures were established from carpospores, tetraspores or excised indeterminate branchlet apices. According to the methods described by Masuda (1982), liberated tetraspores and carpospores or excised apices were rinsed three times with autoclaved seawater and put into Provazoli's Enriched Seawater (PES) (Provasoli 1968) droplets on sterilized slide glass. Spores or apices from individual plants were cultured separately from each other. Thus each strain represents a single individual plant. These were cultured in plant growth chambers illuminated with cool-white fluorescent lamps (2500-3000 lux). The cultures were chiefly maintained at 10℃, 16:8 LD and transferred to other conditions in order to investigate the 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. All cultures were changed to fresh medium every two weeks and maintained in glass dishes (71 x 61 mm or 65 x 80 mm) containing

PES. When female reproductive organs were formed on individual plants, female and male plants were cultured in single dishes (71x61 mm) and placed on a Taiyo-R-II Rotary Shaker at 90-100 rpm.

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.

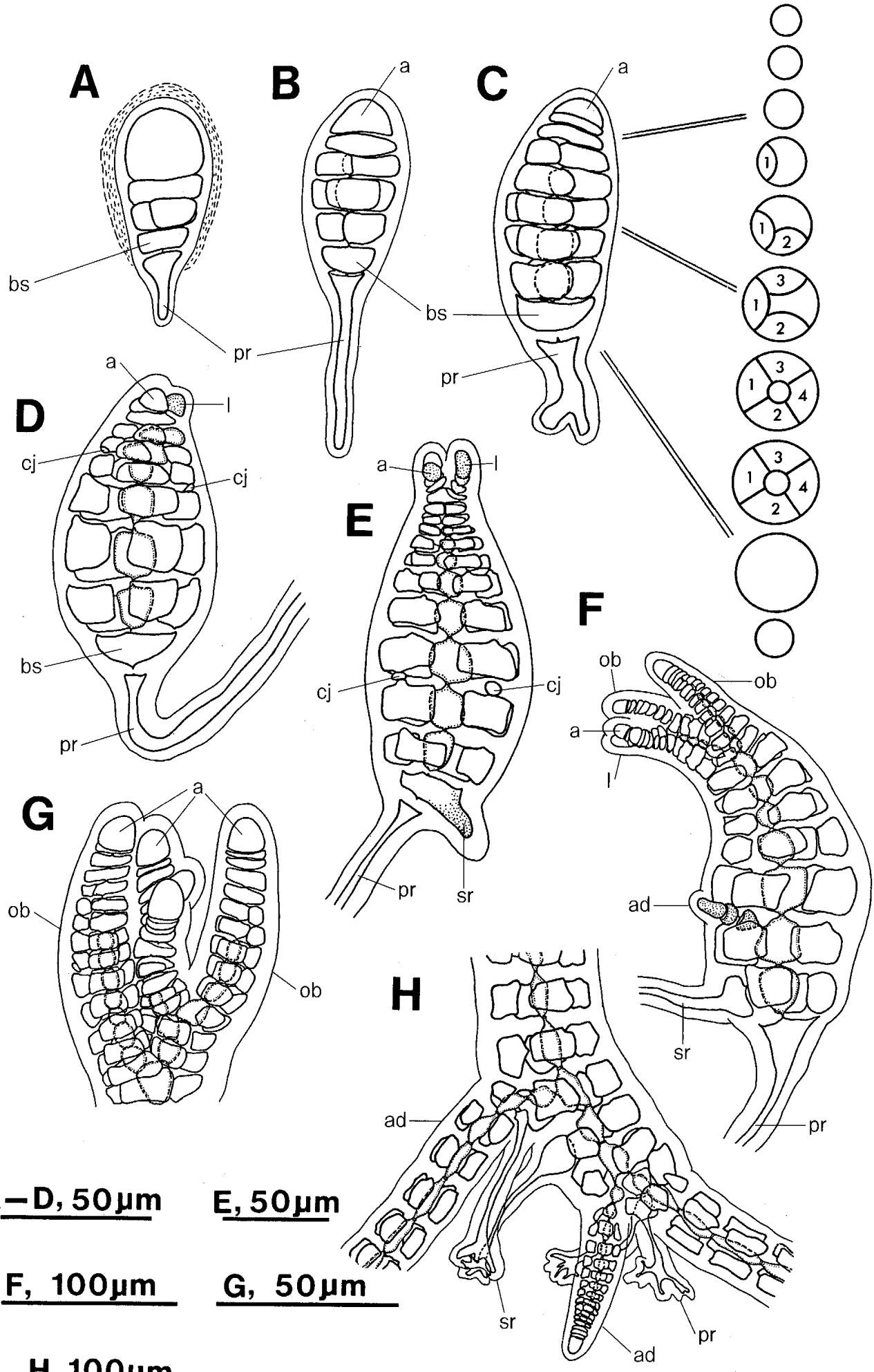
<Results>

The following observation is based on cultured plants, which were collected from Erimo-misaki, maintained at 10℃, 16:8 LD unless otherwise indicated. Liberated tetraspores were globular and blackish deep red in color. They averaged 45.0 μm (range 40.0-51.3 μm; 166 spores measured) in diameter (Fig. 2A). Isolated tetraspores soon attached to the substratum, elongated themselves, and became ellipsoid standing upright. One day after inoculation, they grew into bipolar sporelings, which had differentiated into a colorless primary rhizoid and a pigmented upright axis composed of 4 segments. Mucilaginous pellucid matter enveloped the sporeling except for the portion of primary rhizoid and a basal segment. The apical segment was dome shaped and larger than the lower three segments. The second segment began to form the first cell of periaxial cell (Figs. 1A, 2B). Two days after inoculation, the upright axis of the sporeling was composed of 6 segments and an elongated colorless primary rhizoid. Two to three periaxial cells on each segment were formed at the second to fourth segments of the upright axis (Figs 1B, 2C). Three days after inoculation, the number of segments increased to 8 or 9 and the upper part of the upright axis began to curve slightly by the oblique cell division of the

apical cell. Except for the apical three or four segments and basal segments, each segment of the upright axis was made up of an axial cell and four periaxial cells (Figs 1C, 2D).

The formation of periaxial cells began at the suprabasal segment and expanded successively into the upper segments. 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 the 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 in the position just above the first periaxial cell of the suprabasal segment. Subsequently the second to fourth periaxial cells were formed in the same manner as those of the suprabasal segments. The periaxial cells of the other upper segments were formed in the same way. Consequently, the first and other three periaxial cells in each segment were approximately arranged in a straight line (Figs 1C, 2D). A small conjunctor cell was divided from the bottom of each periaxial cell after the accomplishment of four periaxial cells. The conjunctor cell subsequently fused with

Figure 1.
Tetrasporelings grown at 10℃, 16:8LD. **A**. One-day-old germling. **B**. Two-day-old germling. **C**. Three-day-old germling with the slightly curved apical portion and its diagram showing the process of periaxial cell formation. **D**. Five-day-old germling forming the first lateral at the upper portion. **E**. Seven-day-old germling with two laterals and a secondary rhizoid beginning to elongate from the basal segment in open connection with the segment cell. **F**. Ten-day-old germling with four laterals at the upper portion and an endogenous adventitious branch arising from the central axial cell of the suprabasal segment. **G**. Apical portion of a three-week-old plant forming ordinary branches. **H**. Basal portion of a three-week-old plant with three endogenous adventitious branches, and three secondary rhizoids. a, apical cell; ad, adventitious branch; bs, basal segment; l, initial of lateral; cj, conjunctor cell; ob, ordinary branch; pa, periaxial cell; pr, primary rhizoid; sr, secondary rhizoid; 1, first periaxial cell; 2, second periaxial cell; 3, third periaxial cell; 4, fourth periaxial cell.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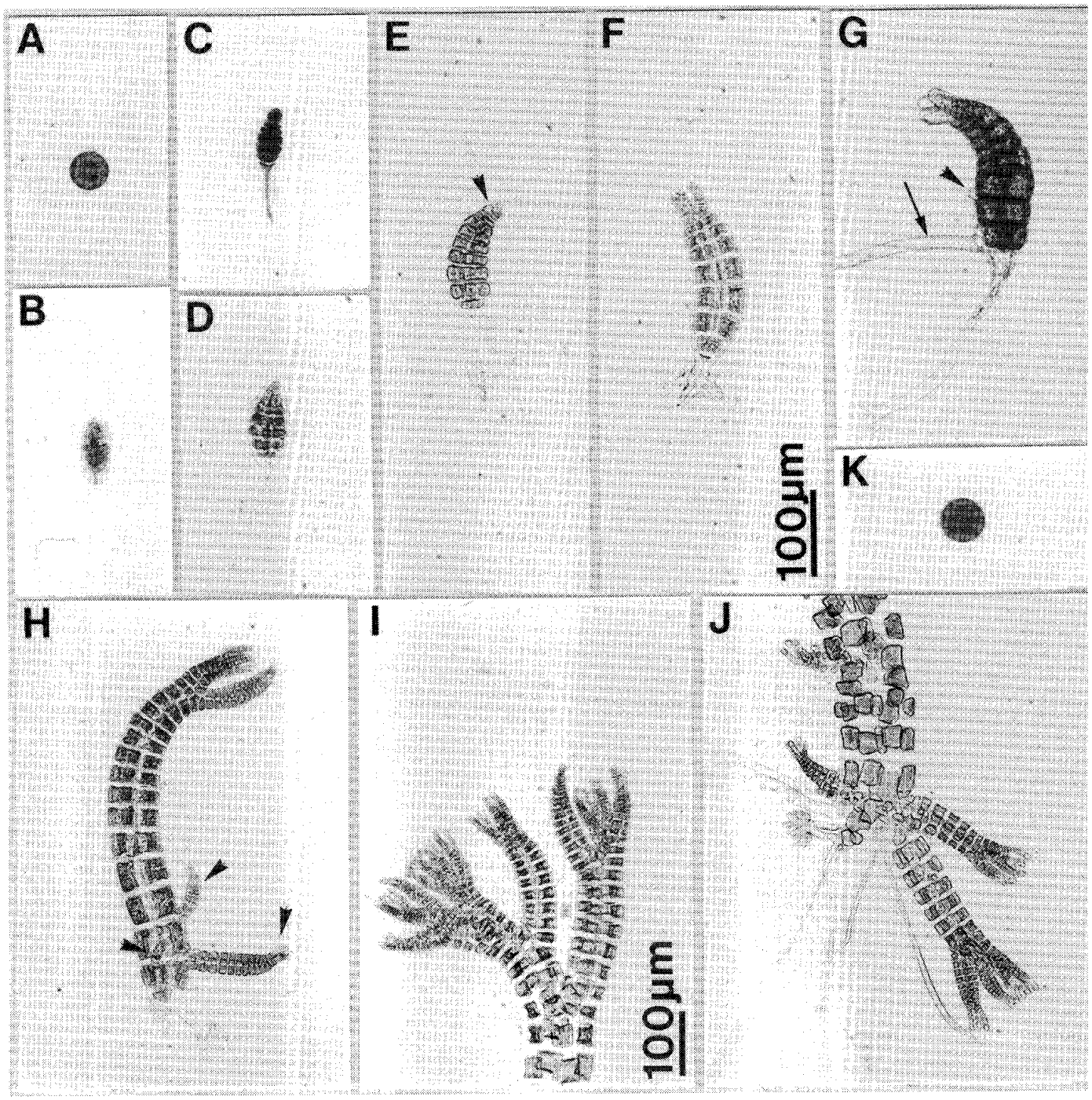


Figure 2.
Tetraspore and its development at 10℃, 16:8LD and carpospore. **A**. Liberated tetraspore. **B**. One-day-old germling. **C**. Two-day-old germling. **D**. Three-day-old germling; note the axis beginning to incline slightly. **E**. Five-day-old germling forming a lateral initial (arrowhead) on the dorsal side of slightly curved axis. **F**. Seven-day-old germling with the first ordinary branch (arrowhead) and a primary rhizoid. **G**. Ten-day-old germling with an adventitious endogenous branchlet (arrowhead) and a secondary rhizoid (arrow). **H**. Two-week-old plant with a recurved axis forming four ordinary branches at the upper portion and three endogenous adventitious branches (arrowhead) at the lower portion. **I**. Apical portion of a three-week-old plant with several ordinary branches. **J**. Basal portion of a three-week-old plant forming four endogenous adventitious branches and six secondary rhizoids. Scale in F applies also to A-E, G and K; scale in I applied also to H and J.

the periaxial cell located just below. As the result of division and fusion of this conjunctor cell, the secondary pit connections between neighboring segments above and below were formed (Fig 1D).

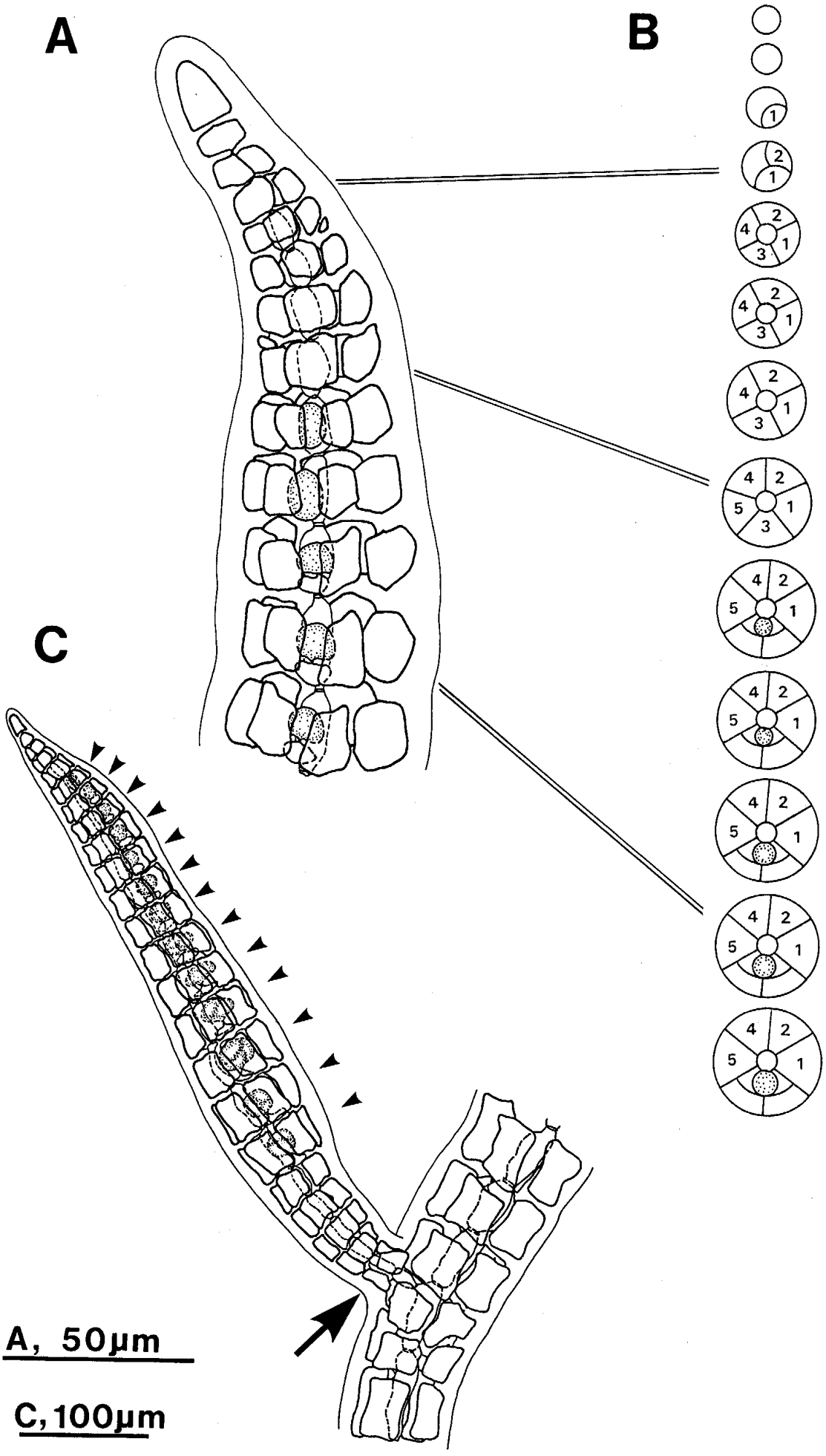
Five days after inoculation, the upright axis was composed of 10-12 segments and its apical portion was slightly curved. The first lateral initial was divided from the dorsal side of the apical second or third segment (Figs 1D, 2E). Seven days after inoculation, the sporelings were composed of 12-16 segments and grew to 125-175 μm in length with 50.0-70.0 μm in diameter at the lowermost portion of the upright axis. The one to two laterals were formed at the eighth to eleventh segment (Figs 1E, 2F). Ten days after inoculation, the plants were composed of 18-20 segments, the length of the upright axis became 250-260 μm and the diameter at the lowermost portion thickened to 78-90 μm. Two to four lateral initials were formed on the upper part of the upright axis, and grew into ordinary branches. An adventitious lateral initial was divided from the central axis of the second segment that had accomplished four periaxial cells (Figs 1F, 2G).

Primary rhizoids were developed from the lowermost cell of the ellipsoidal sporeling. They became filamentous simple holdfasts (Figs 1B, and 2C-E, 2G) or frequently became expanded into disc-like holdfasts by ramification at the growing tips (Fig 2F). The basal segment was composed of a single cell in the early stage, but sometimes it later produced secondary rhizoid (Fig 1E), or one to three periaxial cells (Figs 1F, 2G). Secondary rhizoids were directly formed as protrusion without septum from the basal segment or periaxial cells. They were also formed from periaxial cells of various portions of upright and prostrate branches (Figs 1H, 2J). Numerous secondary rhizoids from prostrate axes attached the plants to the substratum.

Two weeks after inoculation, the upright axes of plants grew to 400-460 μm in length and 80-100 μm in diameter, and were composed of 18-31 segments. Two to six laterals were formed on the upright axis; they almost all grew into ordinary branches. In addition to the ordinary branches, one to three adventitious laterals were formed from the second to fourth, rarely seventh, segment of the upright axis (Fig 2H). Three weeks after inoculation, the plants grew to 1.3-1.8 mm in length and 90.0-105.0 μm in diameter. The upright main axis was distinct and held 7-9 ordinary laterals that developed into third order branches (Figs 1G, 2I). Besides ordinary laterals, three to four adventitious laterals were cut off at right angles from the lower portion of the upright axis (Figs 1H, 2J).

The ordinary laterals were formed with irregular intervals of two to four segments in 1/4 divergence spiral line, running in a counterclockwise direction toward the apex of the main axis (Fig 2I). The ordinary laterals were of exogenous origin, because they were formed before the formation of periaxial cells. These laterals almost all developed into ordinary branches, except a few cases in which the laterals became the vegetative trichoblasts. They grew indeterminately forming next order laterals as the indeterminate branches (Figs 4A, 4D, 4F), or grew determinately as the determinate branchlets with sharply pointed apices (Figs 3C, 4B, 4E,

Figure 3.
Tetrasporangial branches and arrangement of tetrasporangia. **A**. Tetrasporangia borne on an ordinary branch (about ten-month-old plant in 5℃, 18:6LD). **B**. Diagrams for arrangement of the tetrasporangia of the tetrasporangial branch showing in A. The number indicates sequences of periaxial cells, and the dotted areas indicate the tetrasporangial initials or tetrasporangial mother cells. **C**. Endogenous adventitious branch forming tetrasporangia (arrow heads) with long longitudinal series (about ten-month-old plant in 5℃, 16:8LD). The second segment is consisted of four periaxial cells and central axial cell (arrow). 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.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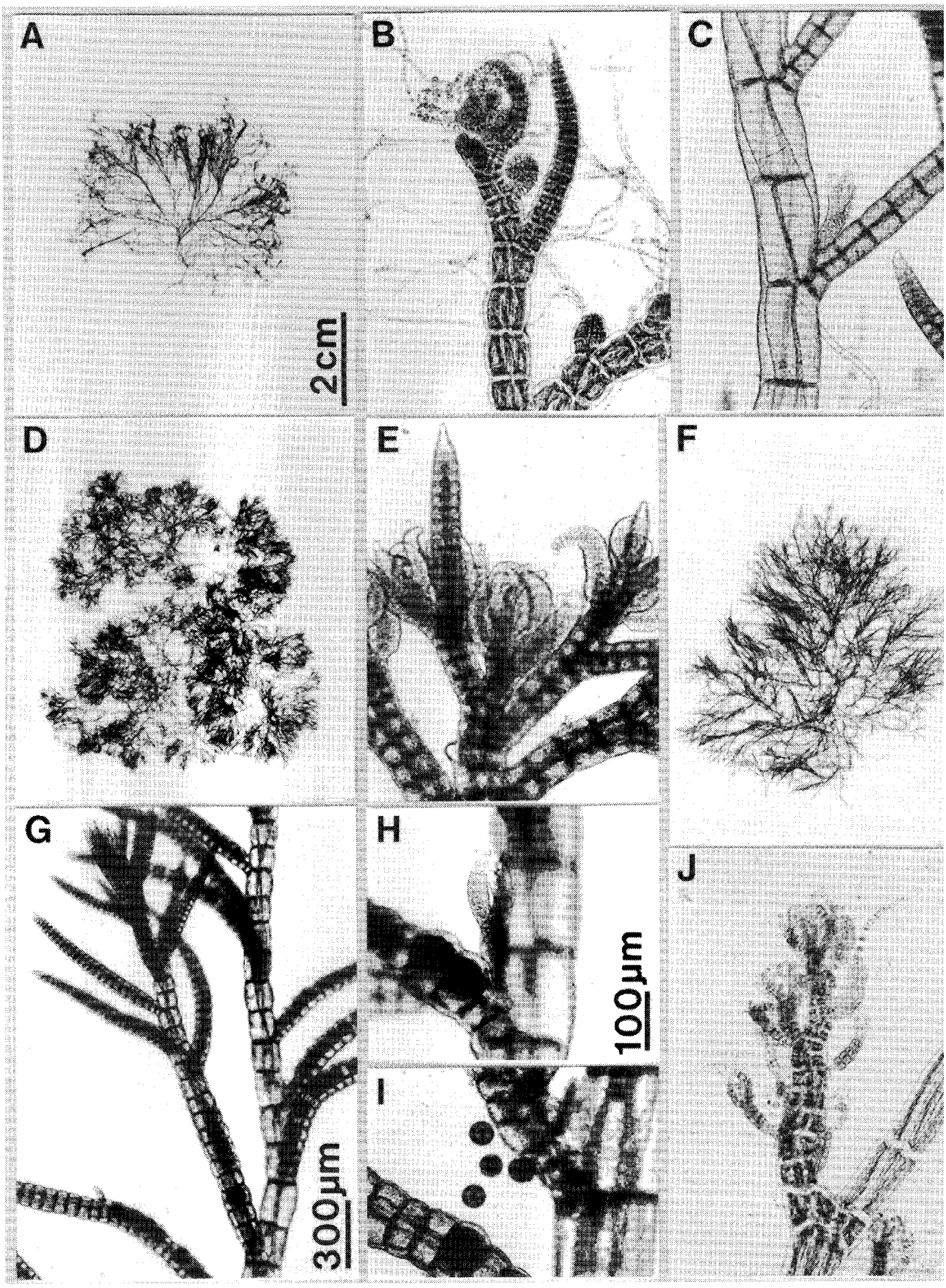
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4G). With the successive formation of these laterals the main axis gradually became straight. Only two periaxial cells were usually formed on the basal segment of ordinary branches. However, four periaxial cells were formed on each of the other segments except the apical portion. The first periaxial cell at the basal segment of ordinary branches was produced on the abaxial side. The first periaxial cells of the suprabasal and other upper segments were formed on the position just above the basal segment. The second periaxial cell of the basal segment was divided into two periaxial cells behind the formation of the first periaxial cell of the suprabasal and third segment. 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 the same as the main axis (Fig 1G). The vegetative trichoblasts showing exogenous origin rarely arose from the apical portion of indeterminate axes, however, the formation of vegetative trichoblast was observed in a few cases.

On the other hand, the adventitious laterals showed endogenous origin, because they were produced from the upper distal end of

Figure 4.
Cultured plants. All photographs from living material except for A, D, and F. **A**. Fertile female gametophyte cultured stationary for about one and a half years in 5℃, 16:8LD. **B, C**. Female reproductive organs: B, apocarps borne at the uppermost portion of the main axis (about two months old plant cultured in 5℃ , 16:8LD); C, borne on the axillary endogenous branch (about three months old cultured in 10℃ , 8:16LD). **D**. Fertile male gametophyte cultured stationary for about one year in 5 ℃ , 8:16LD. **E**. Spermatangial branches borne on the upper portion of an ordinary branch (about three months old plant cultured in 5℃ , 8:16LD). **F**. Fertile tetrasporophyte cultured stationary for about ten months in 5 ℃ , 16:8LD. **G**. Tetrasporangia borne on the ultimate and penultimate ordinary branches (about ten months old plant cultured in 5 ℃ , 16:8LD). **H**. Young axillary tetrasporangial branchlet. **I**. Tetrasporangia and just released tetraspores. **J**. Propagules formed on the upper portion of a male gametophyte. Scale in A applies also to D and E; scale in H applies also to B, C, E, I and J.

the axial cell after the completion of four periaxial cells. Their basal segment was only composed of a central axial cell sinking in the articulation of main axis (Figs 1F, 2H, 2J). The second segment and its upper segments in adventitious branches produced periaxial cells. The first periaxial cell of the second segment was formed at the abaxial side in this type of lateral. The first periaxial cell of the third segment was formed continuously just above the one in the second segment. The second to fourth periaxial cells in the third segment were formed in the same manner in the ordinary laterals. Three or four small periaxial cells in the second segment were produced behind those of upper segments. The adventitious branches were produced in irregular number and directions. In some cases two or three branches were formed from one segment. They also developed determinately or indeterminately the same as ordinary branches. The indeterminate adventitious laterals elongated and crept along the substratum forming several secondary rhizoids from its ventral side (Figs 1H, 2J). The prostrate indeterminate branches formed the next order of branches which from them grew erectly as the secondary upright axes.

Plants reached reproductive maturity and began to form procarps and spermatangial branchlets on separate individuals 61 days after inoculation (Figs 4A, D). The procarps were formed from the suprabasal segments of female trichoblasts borne at the uppermost portion of ordinary indeterminate branches (Fig 4B). They were formed in 1/4 divergence spiral line with intervals of two to four segments, sometimes on continuous segments, replacing ordinary branches. Carpogonial branches in the procarps were composed of four cells the same as those of related members in *Polysiphonia* species. At the stage that the plants reached reproductive maturity, another type of adventitious laterals, namely the axillary branches,

were produced on each axil of ordinary branches. The axillary branches were endogenous in origin. The axillary branches were produced singly on each axil of ordinary branches. These branches also formed procarps later the same as the ordinary branches (Fig 4C).

More than ten spermatangial branchlets were successively formed from the firtile trichoblasts 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 (Fig 4E). They were deciduous leaving their basal cells (i. e. scar cells) the same as the vegetative trichoblasts. Any formation of axillary branches was not confirmed in male gametophytic plants, and accordingly spermatangial branchlet on the axil of branches was not observed.

Fertile female gametophytes mixed with mature male gametophytes in a single dish were placed on a rotary shaker to assist their fertilization. However the occurrence of fertilization was not confirmed for about two months of mixed inoculation. Consequently the post fertilization phenomena and development of cystocarp could not be observed. Isolated female gametophytes did not produce cystocarps but continued to form procarps.

The formation of sexual reproductive organ was confirmed at 5℃, 16:8LD (light and dark cycle); 5℃, 8:16LD; 10℃, 16:8LD; 10℃, 8:16LD. Plants cultured at 10℃, 16:8LD, which were mentioned above, matured fastest and plants cultured at 5℃, 8:16LD matured latest at four months after inoculation. No reproductive organ was formed in the combination of 15℃, 16:8LD or 8:16LD and 20℃, 16:8LD or 8:16LD.

Carpospores liberated from field materials (Fig 2K) averaged 55.4 μm (range 45.0-70.0 μm; 152 spores measured) in diameter and

were slightly larger than the tetraspores. They were inoculated onto glass slide. The carpospores germinated and grew into plants in a manner similar to those of tetraspores. No plant derived from liberated carpospores reached reproductive maturity in eight combinations of temperatures and photoperiods.

The culture plants from the Shiriya-zaki material, which were started from excised indeterminate branchlet apices of young sterile plants, reached reproductive maturity about ten months after inoculation at 5℃, 16:8LD. These plants began to form tetrasporangia on the last two orders of ordinary branches, adventitious branches and axillary branchlets (Figs 3C, 4G, 4H). After the development of tetrasporangia on the ordinary branches advanced enough, axillary branches began to form on branch axils (Fig 4H). A single branch began to arise endogenously on each axil of ordinary branches. Each of the axillary branches began to form tetrasporangia, however, they did not achieve maturity, because the development of tetrasporangia was extremely delayed.

As a result of further inoculation, the tetraspores were released (Fig 4I). Discharged tetraspores averaged 43.2 μm (range 42.5–50.0 μm; 19 spores measured) and repeated the same developmental process mentioned above.

Tetrasporangia were formed from the third periaxial cell of fertile segments that produced five periaxial cells (Figs 3A, B). The third periaxial cell (i. e. the fertile periaxial cell) divided into a tetrasporangial initial cell on the adaxial side (i. e. inner side) and a cover cell (i.e. the pre-sporangial cover cells) on the abaxial side (i. e. outer side) by longitudinal cell division. The tetrasporangial initial cell subsequently divided into an upper tetrasporangial mother cell and lower tetrasporangial stalk cells by horizontal cell division. The tetrasporangial mother cell was cylindrical at first but became

spherical later 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 began to swell as the development process of tetrasporangia advanced. The fertile periaxial cells on each segment of the ordinary tetrasporangial branchlets usually located on the adaxial side to the main axis. Thus an ordinary tetrasporangial branch was swollen on the abaxial side, because the fertile periaxial cell of each segment was located at the same abaxial side. Four tetrads were discharged by extension of the cleavage furrow between the two cover cells. In this study, the formation of secondary cover cell (i.e. the post-sporangial cover cell), was not observed.

As the growth of plants advanced, the thallus length and diameter of upright axes further increased and reached about 30-40 mm long and 120 μm in diameter after three months of inoculation. Full development of ordinary branches resulted in the fourth or fifth order of branching. The plants began to show a fan shape in appearance, because numerous branches were produced with a wide axil resembling a dichotomous branching manner. The main axes of plants gradually became indistinct. Adventitious branches arisen from the lowermost portion of upright axes also developed forming the next order of prostrate branches and became entangled with each other. Especially the plants cultured in the combinations of 15℃ and 20℃, 16:8LD or 8:16LD grew rapidly and elongated forming determinate branchlets, but they did not produce indeterminate branches. 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. In all cases of culture combinations the diameter of the upright axes remained slender and soft and flaccid in texture similar

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 a prostrate feature issuing many adventitious branches and secondary rhizoids irregularly, in such cases as in 15℃ and 20℃, 16:8 LD or 8:16 LD, the relativity of its ratios and thallus length failed.

Two abnormal phenomena were observed in this culture study. Several adventitious branches were developed from a vegetative cell in the aged procarp in 5℃, 16:8LD, and they newly produced some procarps on its apical portion.

Propagules were often formed in aged male gametophytic plants (Fig 4J). They arose from spermatangial trichoblasts, in which the basal segment was single cell and the upper part from basal segments became vegetative branchlets forming pigmented periaxial cells. They were shed from the parent thallus later and then developed into fertile male gametophytes reproductive organs.

<Discussion>

The present study by culture experiment reveals that this alga has the following morphological features. (1) Plants are composed of slender upright axes branching profusely and prostrate axes arising numerous secondary rhizoids. (2) The secondary rhizoids, which attach the plants to the substratum, are formed from periaxial cells as cytoplasmic protrusion not cut off from periaxial cells by cell division. (3) Thallus are ecorticate overall without basal segment in early development or apical portion of branches. Each segment of the plants is consisted of four periaxial cells and central axial cell. (4) Plants produce ordinary branches exogenously on the upper portion of upright axes and adventitious branches endogenously at the lowermost portion. (5) Some ordinary laterals

become determinate branchlets with sharply pointed apices and others develop into indeterminate branches the forming next order of branches. (6) Adventitious prostrate branches are formed at the lowermost portion of main axes in the early developmental stage. They are endogenous in origin and develop indeterminately forming the next order of ordinary branches which from them develop into the secondary upright axes. Adventitious branches are sometimes formed from the upper part of upright axes, which also develop indeterminately or contribute to the reproductive activities. (7) Vegetative trichoblasts are rarely formed at the apical portion of indeterminate branches. (8) Axillary endogenous branchlets are formed on the axils of ordinary branches in mature plants, on which reproductive organs are formed behind those on ordinary branches. (9) Procarps are formed from fertile trichoblasts arising near the apices of ordinary branches and endogenous axillary branchlets in 1/4 divergence spiral line with intervals of two to four segments. Carpogonial branches are composed of four cells. (10) Spermatangial branchlets formed replacing the whole fertile trichoblasts are terete to cylindrical in shape with two to five celled sterile tips at the apex. Fertile trichoblasts arise on each segment successively, and they are deciduous leaving its basal cell. (11) Tetrasporangia are formed on the ultimate and penultimate branchlets of ordinary branches, and also on the axillary branchlets and adventitious branchlets at late stage of maturity. Tetrasporangia are arranged in a straight line at the adaxial side of the branch, because the development of tetrasporangia in each segment begins from the third periaxial cells that are arranged linearly on the adaxial side to the main axis of the branch according to the manner of periaxial cell formation.

These morphological features above mentioned were confirmed as stable in this study. These are accordingly considered to be

appropriate as taxonomic criteria for this alga. However, the taxonomic validity of several features shown in field plants, for example the development of prostrate axes, was not confirmed in this culture experiment. It needs to be confirmed in further study whether they are actually stable or variable. In this study the prostrate axes developed moderately, but essential differences between cultured plants and those of related species were not found. The morphological character of cystocarps is one important taxonomic criterion in *Polysiphonia* species. As the occurrence of fertilization was not confirmed in this study, the post fertilization process, development of cystocarps and discharges of carposores from cultured plants were not observed. Data about these characteristics need to be collected de novo in another experiment. The length/diameter ratios of segments on upright main axes, which has been adopted as a taxonomic criterion, was confirmed to vary according to the length of thallus. It is considered that this feature is inadequate for use as a taxonomic criterion.

Totally morphological features confirmed in this study inform us that this alga is a member of the genus *Polysiphonia*. It was inferred from this culture experiment that the life cycle of this alga was a "*Polysiphonia* type" life history, characterized by the alternation of isomorphic gametophytes and sporophytes, although the life cycle was not completed in any of the culture conditions and strains used.

Segi (1951) reported this alga as *Polysiphonia urceolata f. lepadicola*, as he considered that *Polysiphonia lepadicola* (Lyngbye) Kuetzing (1849) was included in *Polysiphonia urceolata* as one of forma. Originally *Polysiphonia urceolata* (Dillwyn) Greville (1824) was the type species of genus *Polysiphonia*, and it has been reported from the British Isles and various localities in the world, in European coasts (Harvey 1846-51, 1849,1853, Rosenvinge 1923-24)

or the Atlantic coast of North America (Kapraun 1979). Maggs and Hommersand (1993) considered *P. urceolata* to be conspecific with *P. stricta* (Dillwyn) Greville (1824) and consequently adopted the older name *P. stricta* having priority in nomenclature. They considered that *P. urecolata*, equal to *P. stricta*, composed a species complex, because the morphological variation of the species showed an extremely wide range.

Kylin (1941) described new genus *Orcasia*, based on *P. senticulosa* Harvey (1862) together with *P. morrowii* Harvey (1857). He regarded the occurrence of endogenous axillary branches growing indeterminately to be 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 determinate growth or terminate as short branchlets. Although an elongated axillary branch was observed as an abnormal case in an 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. Kylin (1937) reported the development of reproductive organs in *P. urceolata*, but he did not report the occurrence of axillary branches in the species. No investigator has reported the formation of axillary branches in *P. urceolata* except for the reports from the coasts of the Far East. Consequently it was concluded that *P. urceolata* never formed axillary branches. In addition, the description of the axillary endogenous branches was also never confirmed in *P. lepadicola* to be the same as those of *P.urceolata*.

Polysiphonia urecolata from various localities on the Japanese coast has also been reported (Yamada 1928, Inagaki 1933, Okamura 1936, Yamada and Tanaka 1944, Tokida 1954), since Yendo (1916) firstly reported its existence. Yoon (1986) reported that the name

P. urceolata from Japan and adjacent coasts was a synonym of *P. morrowii* based on the occurrence of axillary branchlets. However, he did not examine the holotype specimens of these species or specimens from their type localities. Kudo and Masuda (1988) examined the holotype specimens of *P. senticulosa* collected from Orcas Island, Washington, U.S.A. They reduced the name *P. urceolata* from Japan as a synonym of *P. senticulosa* according to the results of their examination with holotype specimens, and rejected the conclusion of Yoon (Kudo and Masuda 1991). Later Masuda et al (1995) also examined the lectotype specimen of *P. morrowii.*

This alga has several features in common with *P. morrowii* (Kudo and Masuda 1992) and *P. senticulosa* (Kudo 2000). The occurrence of axillary branchlets is one typical characteristic in these three species. The features confirmed in this study reveal that there is a taxonomic affinity between *P. morrowii, P. senticulosa* and this alga. It was confirmed in this alga that the formation of axillary branchlets was a stable feature in the cultured plants the same as in the field plants. One to three axillary branchlets were formed on each branch axil in the field plants of this alga, but only one was formed on an axil in this culture study. In terms of the number of axillary branchlets formed on each axil, this alga is clearly different from *P. morrowii* which bears seven to eight branchlets, but is not different from *P. senticulosa*, which produces one to three. At any rate, according to this point of view, it was clarified that this alga was not conspecific with *Polysiphonia urceolata* or *Polysiphonia lepadicola* .

The slender upright axis in this alga is one of its taxonomic feature. Plants in this culture experiment reached only 120 μm in diameter even after ten months of inoculation. The value of the diameter of this alga is clearly smaller than those of *P. senticulosa*

and *P. morrowii*, this thinness of the upright axes is accordingly considered to be a genetically stable feature in this alga.

Another typical feature of this alga is the formation of adventitious branches on the upper portion of upright axes. This feature was observed in both field plants and cultured plants. Adventitious branches arising from the upper portion of upright axes in *P. morrowii* or *P. senticulosa* are generally observed in aged plants. However, in this alga the adventitious branches are formed in plants that are not so aged, and they contributed the reproductive function. It is considered that the adventitious branches arising at the upper portion of upright axes are one of the general taxonomic features in this alga.

The formation of vegetative trichoblasts is an usual phenomenon in the mature plants of *P. morrowii* or *P. senticulosa*, though the number of trichoblasts formed on a plant is not so abundant. In contrast it was confirmed in this alga that vegetative trichoblasts are rarely formed in either field or cultured plants. This is the remarkable feature differentiating *P. morrowii* from *P. senticulosa*.

Plants in this culture experiment 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 produced adventitious rhizoids profusely and irregularly. The upright axes were entangled with each other and finally showed prostrate appearance. It would appear that these results agree with the geographical feature of this alga, that is, this alga is distributed in the northern part of Japan subject to a cold current, the Kurile Current.

The propagules were formed on male gametophytic plants in this culture study. The plants recycled the respective phases that

produced themselves. Kapraun (1977) and Womersley (1979) have reported that several species of this genus bear propagules, which function as one of its reproductive structures. Further investigation is needed to clarify whether the propagules of this alga contribute to reproductive activity in the field.

Finally, in the future, in order to elucidate the entity of this alga, it is necessary to use approaches that employ new technology in addition to the analysis of morphology or life history. The phylogenic relationships between this alga and related species must be examined using culture experiments on hybridization such as those done on *Neosiphonia* japonica (as P. japonica) (Kudo and Masuda 1986), or using DNA analysis, currently the most popular method in the phylogenic investigation.

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