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A taxonomic study of *Polysiphonia japonica* HARVEY and *P. akkeshiensis* SEGI (Rhodophyta)

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Polysiphonia japonica HARVEY and *P. akkeshiensis* SEGI were studied through their life history stages in laboratory culture. These two species are similar in morphology, growth and reproductive responses to varying temperatures (10-20°C) and photoregimes (16:8 LD and 8:16 LD). Cultured plants of the two species possessed segments similar in length, which strongly suggests that the taxonomic criteria show phenotypic plasticity; alternative criteria have not been found. It may be concluded that employing morphological features, these two algae are referable to the same taxonomic species. However, artificial hybridization experiments reveal that two virtually non-interbreeding groups separated by various isolating mechanisms exist among ten local populations studied. *Polysiphonia novae-angliae* auct. japon. is included in the species complex studied.

Key Index Words: *Life history*; Polysiphonia akkeshiensis; Polysiphonia japonica; Polysiphonia novae-angliae; *reproductive isolation*; *Rhodomelaceae*; *Rhodophyta*; *taxonomy*.

Polysiphonia japonica HARVEY (1856) is one of common species of the genus in Japan (SEGI 1951). This alga is characterized principally by having four pericentral cells, trichoblast-unconnecting branches and cortical cells (SEGI 1951). In a laborious monograph SEGI (1951) reported the following six species sharing these features with *P. japonica*: *Polysiphonia spinosa* (C. AGARDH) J. AGARDH (1842), *P. harlandii* HARVEY (1859), *P. decumbens* SEGI (1951), *P. nipponica* SEGI (1951), *P. novae-angliae* W.R. TAYLOR (1937) and *P. akkeshiensis* SEGI (1951). Except for *P. harlandii* these species have been reported from either a single locality or only a few localities included in the geographical range of *P. japonica*. *P. harlandii* has a more southerly distribution: Taiwan, Hong Kong and Hainan Island (SEGI 1951, TSENG 1983). It is difficult to distinguish these species from each other despite SEGI's (1951) detailed descriptions. Later, NODA described *Polysiphonia grateloupeoides* NODA (1970) and *P. echigoensis* NODA (1975) from single localities, Iwagasaki and Kakumi-hama respectively. These localities are also included in the range of *P. japonica*. These two species are distinguished from *P. decumbens* and *P. harlandii* by only a few features (NODA 1970, 1975). This may require a critical study to elucidate the taxonomic relationship between *P. japonica* and related species.

Polysiphonia japonica and related species have densely ramified, numerous branches and give a bushlike habit. For the *P. japonica* species complex a comparative study through life history stages may be useful to evaluate their taxonomic features as pointed out by DIXON (1963) and exemplified for *Neorhodomela* (Rhodomelaceae) by MASUDA (1982). Little information is

Abbreviations used in the figures. a, apical cell; ab, adventitious branch; cc, cortical cell; ob, ordinary branch; pr, primary rhizoid; sr, secondary rhizoid; tb, trichoblast.

available concerning the vegetative ontogeny of the genus, although life history studies have been reported for several taxa: *Polysiphonia hemisphaerica* var. *boldii* (WYNNE et EDWARDS) RUENESS (as *P. denudata*) (EDWARDS 1968), *P. denudata* (DILLWYN) KÜTZING (EDWARDS 1970, KAPRAUN 1978a), *P. hemisphaerica* ARESCHOUG (RUENESS 1971), *P. ferulacea* SUHR (KAPRAUN 1977), and *P. urceolata* (DILLWYN) GREVILLE (KAPRAUN 1978a). In this paper we present the pattern of morphological development for *Polysiphonia japonica* and *P. akkeshiensis* and the results of hybridization experiments among their cultured strains.

Materials and Methods

Plants were collected at the localities shown in Fig. 1. Date of collection and culture numbers for cultured plants are given in Table 1, although additional samples for morphological study were obtained in each collection, which were fixed and preserved in 10% formalin in seawater. 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 branch apices according to the methods described earlier (MASUDA 1982). 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 temperatures and photoperiods were regulated in the following combinations: 10°C, 16:8 LD (light and dark cycle); 10°C, 8:16 LD; 15°C, 16:8 LD; 15°C, 8:16 LD; 20°C, 16:8 LD and 20°C, 8:16 LD. The cultures were chiefly maintained at 15°C, 16:8 LD and transferred to other conditions in order to test growth and reproductive responses to varying temperatures and photoregimes.

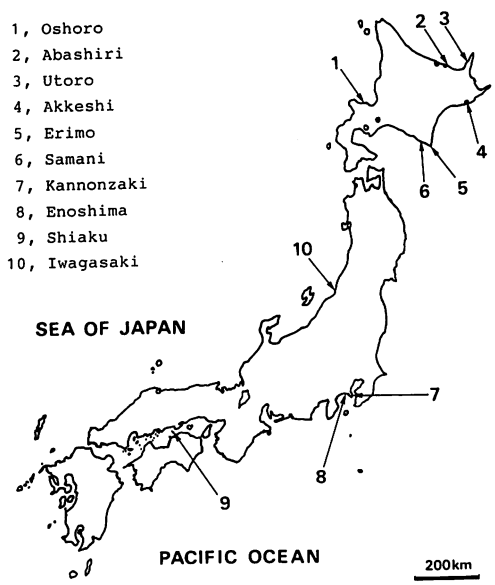


Fig. 1. Outline map of Japan indicating collection sites.

All cultures were changed to fresh medium every 2 weeks and maintained in glass dishes (71×61 mm or 65×80 mm) containing one-half strength PES. They were not agitated except when female and male crosses were being attempted; these were placed on a Taiyo R-II Rotary Shaker at 90-100 rpm. Clonally cultured fertile female and male plants were crossed to determine interfertility among the strains. Excised small pieces (2-3 cm long) of fertile female and male branches were introduced into single dishes (71×61 mm) and placed on a Taiyo R-II Rotary Shaker at 90-100 rpm in 15°C, 16:8 LD. Crosses were considered positive if cystocarps developed and carpospores were released from the cystocarps. Plants from resulting carpospores were cultured to test their viability and fertility. Crosses in which no carpospores were released were treated as negative and negative crosses were repeated once.

Microscopic examination was carried out on living and liquid-preserved materials. Sections were made by hand using a razor blade and pith stick. Voucher specimens are deposited in the Harbarium of Faculty of Science, Hokkaido University, Sapporo (SAP 047547-047568).

Table 1. Collection data and culture numbers.

Species	Locality	Date	Plants sampled	Reproductive state	Isolation material	Culture number
P. japonica	Oshoro	4 Feb 84	2	⊕	B A	2373, 2375
		5 Nov 84	2	⊕	T S	2711, 2712
			2	♀	C S	2713, 2714
	Abashiri	2 Jul 83	1	♂	B A	1995
	Erimo	19 Sep 83	2	♀	B A	2144, 2145
			2	♂	B A	2146, 2147
	Samani	30 Jul 84	1	⊕	T S	2590
	Kannonzaki	9 Mar 84	2	⊕	B A	2425, 2426
			2	♀	B A	2423, 2424
			2	♂	B A	2422, 2427
	Enoshima	30 May 84	1	♂	B A	2499
	Shiaku	27 Sep 84	2	⊕	B A	2627, 2633
			1	♀	B A	2628
			2	♂	B A	2631, 2632
	Iwagasaki	8 Oct 83	1	⊕	B A	2203
			2	♀	B A	2204, 2206
			1	♂	B A	2201
P. akkeshiensis	Utoro	1 Jul 83	1	⊕	B A	1993
		1 Jul 84	3	♀	B A	2571-2573
			1	♂	B A	2574
	Akkeshi	28 Jun 83	2	⊕	B A	1978, 1979
			2	♀	B A	1980, 1981
			2	♂	B A	1976, 1977
		30 Jun 84	3	♀	B A	2567-2569
			1	♂	B A	2570
		6 Jul 85	1	⊕	T S	2990
			1	♀	C S	2991

⊕, plants with tetrasporangia; ♀, plants with cystocarps; ♂, plants with spermatangia; BA, branch apices; CS, carpospores; TS, tetraspores.

Results

Examination of voucher specimens

The specimens identified as *Polysiphonia japonica* and those as *P. akkeshiensis* fit the descriptions for these species given by SEGI (1951). The following features are common to all the specimens examined. The thallus arises from a tuft of densely aggregated rhizoids which are cut off from pericentral cells and cortical cells as separate cells. It has a main axis which bears deciduous trichoblasts or ordinary branches* of the first order in a spiral manner running in a counterclockwise direction toward the apex from every segment except the lower ones. The ordinary branches are exogenous, replacing the trichoblasts in development, and grow in a manner similar to the main axis. They are divided into progressively

* We use the term 'ordinary branch' for a branch arising from a subapical segment and the term 'adventitious branch' for a branch arising secondarily from another position.

shorter branches and so give a bushlike habit. Each segment has four pericentral cells and is corticated in the older parts. In addition to ordinary branches, two kinds of adventitious branches arise either from axial cells of lower segments of the main axis or from scar cells (basal cells of fallen trichoblasts). The latter branches are known as cicatrigenous branches (HOLLENBERG 1942). Tetrasporangia are spirally arranged in swollen segments of branches. Cystocarps are ovate and have a stalk consisting of a segment. Spermatangial branchlets arise from a primary branch of the trichoblasts, and are nearly cylindrical and have one or two-celled sterile tips. The dimensions of some vegetative and reproductive structures of the fertile specimens examined are given in Table 2.

The diameters of main axes and length/diameter ratios of segments vary correlatively with thallus length within each species: smaller specimens have more slender axes with shorter segments and larger specimens possess thicker axes with longer segments. Utoro and Akkeshi populations belonging to *Polysiphonia akkeshiensis*, however, have much longer segments than the others: the length/diameter ratios of Utoro and Akkeshi populations range between 2.6 and 4.7, whereas those of other populations vary between 0.4 and 2.6 (Table 2). This difference does not correlate with thallus length: smaller plants of these two populations have

Table 2. The dimensions of vegetative and reproductive structures of ten local populations examined.

Locality	Number of specimens	Thallus length (mm)	Diameter of main axes (μm)[1]	Length/diameter of segment[2]	Diameter of tetrasporangia (μm)	Cystocarps Length × Diam. (μm) (μm)	Spermatangial branchlets Length × Diam. (μm) (μm)
Oshoro	14	21-57	350-825	1.1-2.3	70-93	300-530 × 230-460	165-250 × 50-83
	41	7-25	180-450	1.1-3.1	58-88	240-390 × 250-490	125-300 × 53-98
Abashiri	5	60-91	750-1125	1.4-2.1	70-90	280-490 × 350-540	93-153 × 40-68
	1	—	—	—	—	—	118-163 × 53-78
Erimo	14	21-54	375-750	1.1-2.6	73-100	370-700 × 420-630	115-195 × 45-68
	1	—	—	—	—	—	115-165 × 55-80
Samani	9	18-31	550-925	1.2-2.2	60-83	290-560 × 300-510	90-160 × 33-53
	5	11-16	460-600	0.9-1.3	55-73	250-360 × 290-430	145-185 × 70-90
Kannonzaki	17	7-28	250-775	0.7-1.1	68-93	330-500 × 390-580	—
	4	7-12	330-600	0.7-1.1	70-103	250-500 × 260-550	95-253 × 38-110
Enoshima	8	6-9	220-320	0.8-1.1	60-68	260-410 × 260-400	—
	1	—	—	—	—	—	125-180 × 68-90
Shiaku	9	3-15	150-360	0.6-1.2	63-75	—	80-160 × 30-58
	5	15-19	350-460	1.0-1.2	65-88	250-420 × 280-480	155-240 × 63-75
Iwagasaki	28	4-12	170-430	0.4-1.4	65-103	210-430 × 200-420	103-183 × 30-63
	7	9-17	170-330	1.3-2.4	65-88	190-410 × 230-470	123-283 × 53-95
Utoro	5	40-56	600-700	2.7-4.6	75-100	270-510 × 300-570	108-200 × 50-83
	2	—	—	—	—	300-450 × 300-430	145-253 × 73-105
Akkeshi	8	25-107	500-750	2.6-4.7	70-105	310-520 × 300-510	140-268 × 50-75
	22	11-29	260-390	1.1-3.2	63-100	260-430 × 250-440	165-283 × 53-70

The data in the upper half are for field-collected plants and those in the lower half for cultured plants. Dashed line indicates no information. 1) The lowermost portions of main axes were measured. 2) Middle portions of main axes were measured.

longer segments than those of other populations. Two groups thus can be recognized by the length of segments among the local populations studied. The segment length affects gross morphological features. The specimens with longer segments are laxly expansive and give an impression that they are sparsely and distantly branched and have a flaccid texture. *P. akkeshiensis* is vegetatively characterized by these features (SEGI 1951). However, no significant differences in dimensions of reproductive organs were found among the populations studied.

Culture experiments

Polysiphonia japonica

The following account is based on observations of Oshoro 2713 and 2714 strains. Cultures were maintained at 15°C, 16:8 LD unless otherwise indicated. Liberated carpospores were globular and deep red in color. They averaged 60.3 μm (range 57.5–65.0 μm; 120 spores measured) in diameter (Fig. 2A). Isolated carpospores soon attached to the substrate and grew into bipolar sporelings of 6–7 segments, which, one day after inoculation, had differentiated into a

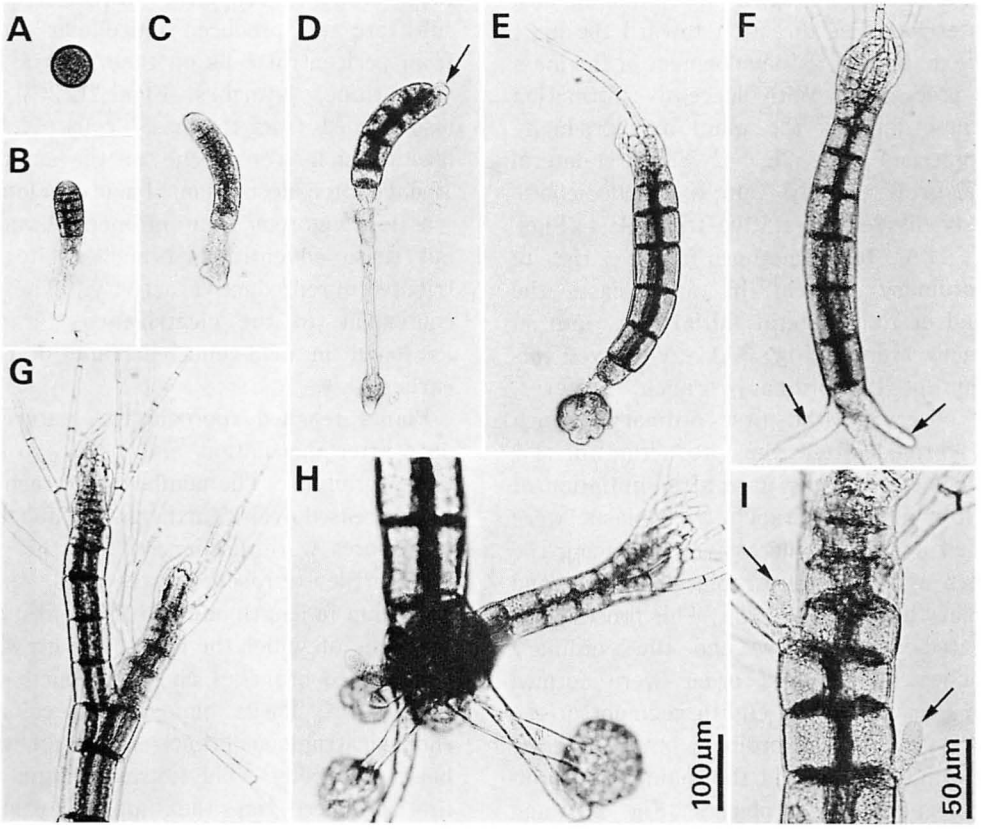


Fig. 2. Carpospore and its development of *Polysiphonia japonica* at 15°C, 16:8 LD (Oshoro 2713 and 2714 strains). All photographs from living material. A. Liberated carpospore. B. One-day-old germling. C. Two-day-old germling; note the axis being recurved. D. Three-day-old germling forming a lateral initial (arrow) on the dorsal side. E. Five-day-old germling with spirally arranged laterals and an expanded disc-like rhizoid; note the main axis becoming straight. F. Seven-day-old germling with an almost straight main axis and two secondary rhizoids (arrows). G. Apical portion of a 10-day-old plant of which main axis and first order branch are forming vegetative trichoblasts. H. Basal portion of a 14-day-old plant forming an adventitious branch and five secondary rhizoids. I. Two adventitious branches (arrows) arising from the basal cells of trichoblasts before their shedding (17-day old). Scale in H applies also to A–G.

colorless rhizoid and a pigmented main axis (Fig. 2B). Each segment of the main axis was composed of an axial cell and four pericentral cells except apical, subapical, suprabasal and basal segments. The suprabasal segment had usually four pericentral cells but it sometimes possessed three or five pericentral cells. The basal segment was composed of a single cell. The main axis became recurved (Figs. 2C, D) and began to form lateral initials from a subapical segment, first on the dorsal side and second on the flank (Figs. 2D, 3A). Subsequently, lateral initials were formed from each segment in a spiral line running in a counterclockwise direction toward the apex of the main axis as development of the main axis proceeded. With successive formation of these laterals the main axis gradually straightened (Figs. 2E, F). The first lateral initial grew usually into a pseudodichotomously divided vegetative trichoblast (Figs. 2E, F, 3A), but sometimes it gave rise to an ordinary branch. In many cases the second or third lateral initial grew into an ordinary branch (Fig. 3A). A delayed formation of the ordinary branch, however, was observed: the first ordinary branch was formed after the production of 4-7 trichoblasts. In any case after initiation of the first ordinary branch trichoblasts were formed again, 2-7 successively replacing the branch and they were replaced by a second ordinary branch (Fig. 3C). This process was repeated continuously, and thus ordinary branches of the first order were formed from every third to eighth segment of the main axis. All the ordinary branches grew indeterminately as did the main axis, forming vegetative trichoblasts (Fig. 2G) and ordinary branches of the second order.

A primary rhizoid cut off from the basal segment became ramified at the growing tip (Fig. 3B), although it was not accompanied by the formation of a septum, and became an expanded disc-like holdfast (Fig. 2E). Primary rhizoids which were not ramified were also frequent and became elongated filamentous holdfasts (Figs. 2F, 3D). Some of the latter rhizoids later became ramified as was the former. Secondary rhizoids were cut off from the basal segment (Figs. 3D, E) and from the pericentral cells of the suprabasal segment (Fig. 3F). Some of these secondary rhizoids became disc-like holdfasts (Fig. 2H).

One to three adventitious branches were formed from an axial cell of the suprabasal or third segment of the main axis 10 days after inoculation (Fig. 3E). These branches grew indeterminately (Fig. 3F). They developed first along the substrate (Fig. 2H) and later became upright. These adventitious branches sometimes attached to the substrate and produced unicellular rhizoids from pericentral cells on their ventral side. Adventitious branches (Figs. 2I, 3G) were also formed from the basal cells of trichoblasts which were borne on the main axis 17 days after inoculation. Their development was less vigorous than ordinary branches, but these adventitious branches later contributed to reproductive activity. These are equivalent to the cicatrigenous branches as found in field-collected plants described earlier.

Plants reached reproductive maturity 28 days after inoculation and began to form tetrasporangia. The number of tetrasporangia increased over a further week and many tetraspores were discharged. At this stage the fertile tetrasporophytes had reached 14-25 mm in length and had 14-26 first order branches of which the lowest one grew best and formed branches up to the fourth order (Fig. 4A). These plants produced many short cicatrigenous branches from the trichoblast scar cells. The tetrasporangia were first produced on the upper portion of ordinary branches (Fig. 4B) and later on cicatrigenous branches. Liberated tetraspores were slightly smaller than the parent carpospores and averaged 51.0 μm (range 42.5-60.0 μm ; 120 spores measured) in diameter (Fig. 4C). The fertile plants produced a few cortical cells from pericentral cells of the lower segments (Fig. 5A). Cortical cells developed acropetally to the middle

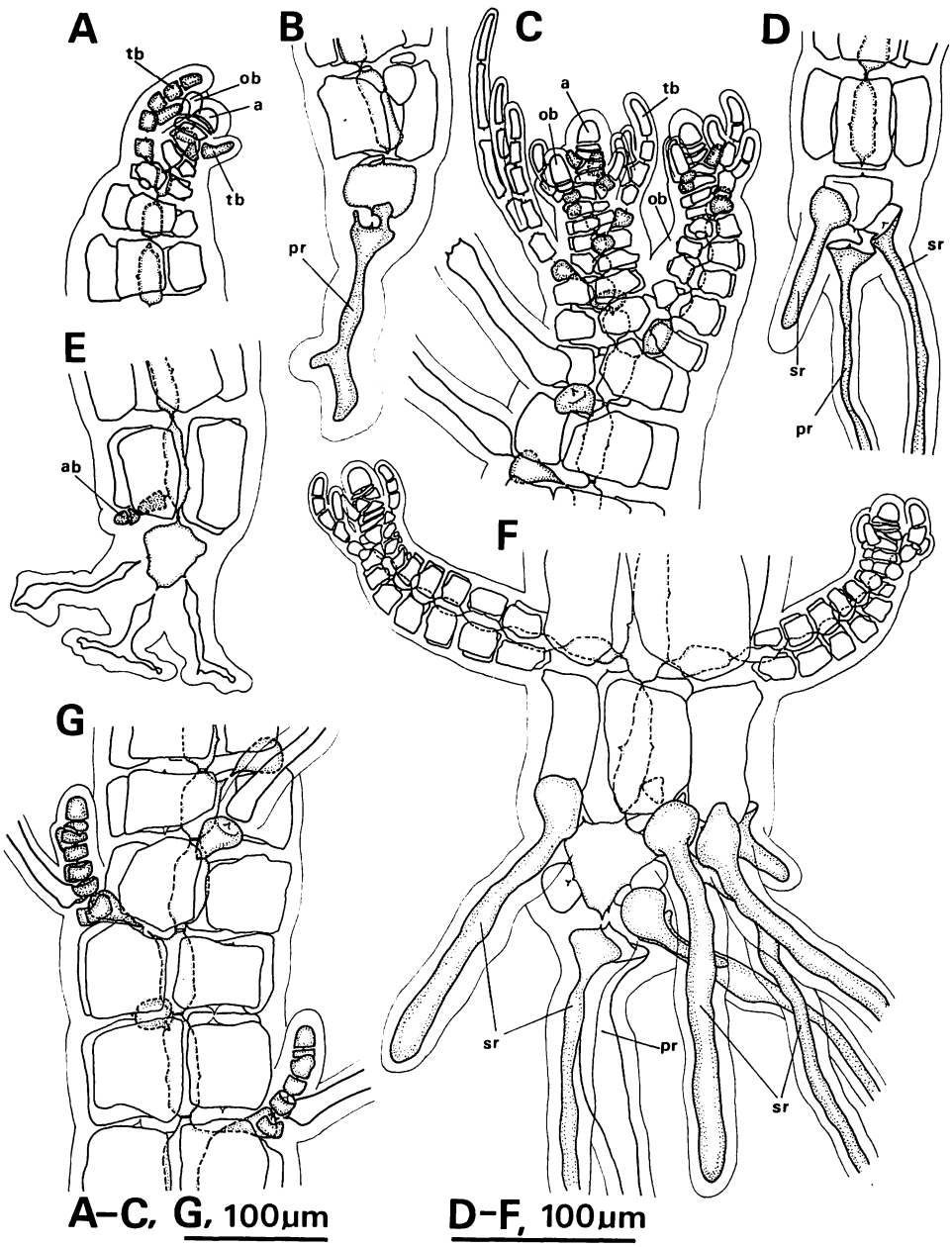


Fig. 3. Carposporelings of *Polysiphonia japonica* grown at 15°C, 16 : 8 LD (Oshoro 2713 and 2714 strains). A, B. Three-day-old germling (A, apical portion forming two trichoblasts and one ordinary branch; B, basal portion forming a primary rhizoid). C, D. Seven-day-old germling (C, apical portion forming spirally arranged trichoblasts and ordinary branches; D, basal portion with two secondary rhizoids cut off from the basal cell). E. Adventitious branch initial arising from an axial cell of the suprabasal segment of a 10-day-old germling. F. Basal porion of a 14-day-old sporeling issuing two adventitious branches and several secondary rhizoids (dotted) cut off from the pericentral cells of the suprabasal segment and from the basal cell. G. Adventitious branches formed from the basal cells of trichoblasts before their shedding (17-day old).

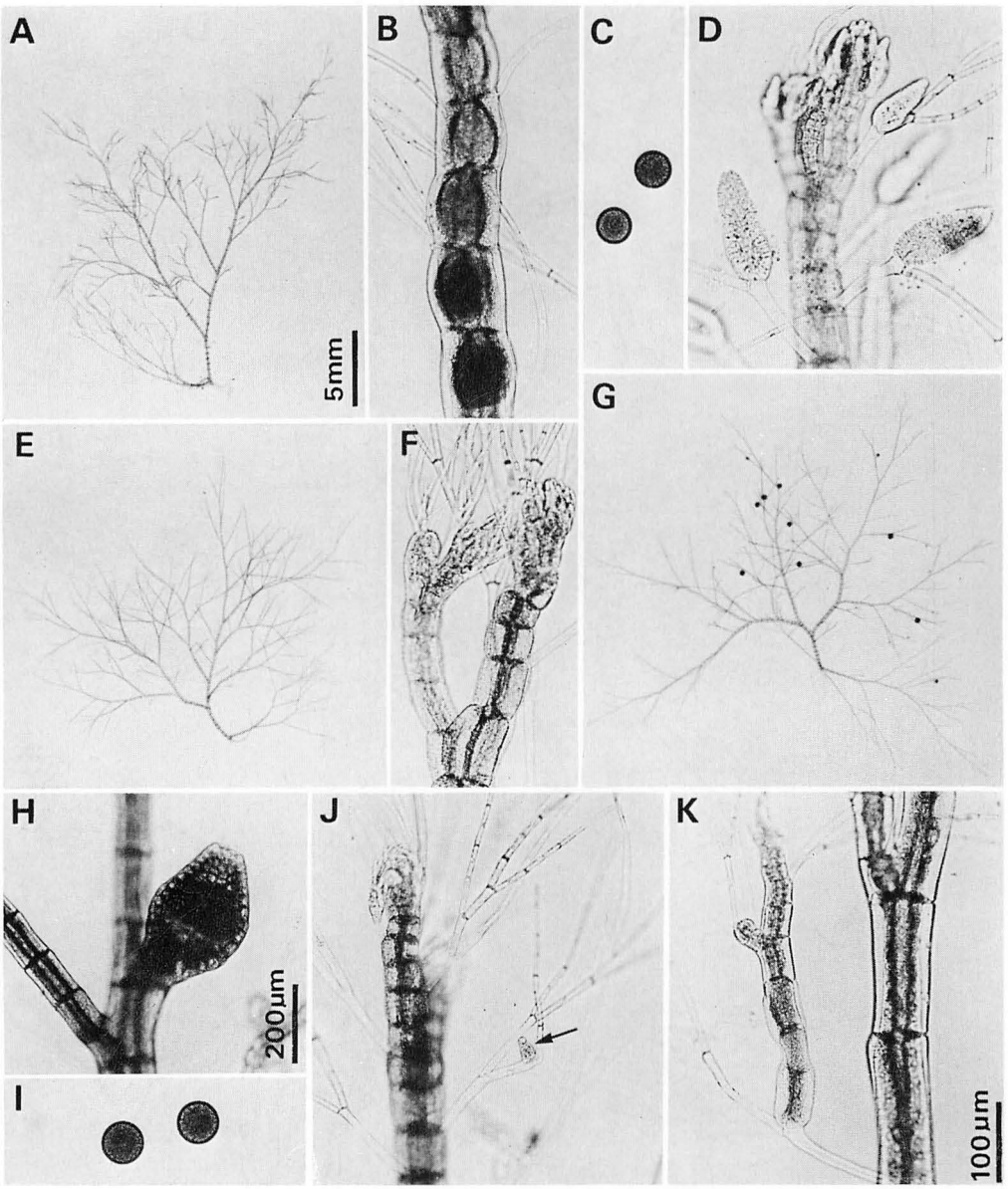


Fig. 4. *Polysiphonia japonica* cultured at 15°C, 16 : 8 LD (Oshoro 2713 and 2714 strains). All photographs from living material. A. Fertile tetrasporophyte (35-day old). B. Tetrasporangia formed on the upper portion of an ordinary branch. C. Liberated tetraspores. D. Spermatangial branchlets formed on the uppermost portion of the main axis (21-day old). E. Fertile male gametophyte cultured for 42 days. F. Procarps borne at the uppermost portion of the main axis and branch (21-day old). G. Fertile female gametophyte cultured stationarily for 24 days and then mixed with a male gametophyte for 18 days on a shaker. H. Mature cystocarp formed on the plant shown in G. I. Liberated carpospores. J. Propagule (arrow) formed on a trichoblast borne on a 28-day-old female gametophyte. K. Developing propagule on a supporting trichoblast before its shedding. Scale in A applies also to E and G; scale in K applies also to B–D, F, I and J.

portion of the main axis from pericentral cells (Fig. 5B) and from scar cells, but their increase was less vigorous than field-collected plants. The cortical cells produced on the lower segments cut off secondary rhizoids (Fig. 5A).

Tetraspores were inoculated onto glass slides. They germinated and grew into plants in a manner similar to that of the parent carpospores. These plants began to form spermatangial branchlets and procarps on separate individuals 14 days after inoculation. The spermatangial branchlets were formed as a first branch of the fertile trichoblasts (male trichoblasts) borne 2-6 successively at the uppermost portion of the main axis and branches (Fig. 4D). These male trichoblasts were replaced by ordinary branches or vegetative trichoblasts. Then, male trichoblasts were formed again, 2-6 successively. This process was repeated continuously as long as the plants continued to grow well (Fig. 4E). Male trichoblasts were also formed on short cicatrigenous branches. Mature spermatangial branchlets were nearly cylindrical and 125-300 μm long $\times$53-98 μm wide. The procarps (Fig. 4F) were formed on the suprabasal segments of fertile trichoblasts (female trichoblasts) borne on parts similar to those of male trichoblasts. The female trichoblasts were usually individually formed and rarely two successively. They were repeatedly replaced by ordinary branches and/or vegetative trichoblasts.

Fertile female gametophytes were mixed in single dishes with male gametophytes releasing spermatia and placed on a rotary shaker. All these female gametophytes (Fig. 4G) produced mature cystocarps which released viable carpospores 18 days after the initiation of mixed culture. The cystocarps were ovate and 240-390 μm long $\times$ 250-490 μm wide (Fig. 4H). The resulting carpospores (Fig. 4I) were similar in every respect to those from field. Isolated female gametophytes did not produce cystocarps but continued to form procarps.

Propagules were often formed on trichoblasts borne on both female (Fig. 4J) and 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 reproductive structures of their respective parents. The propagules sometimes grew rapidly on their supporting trichoblasts before release and formed gametangia (Fig. 4K).

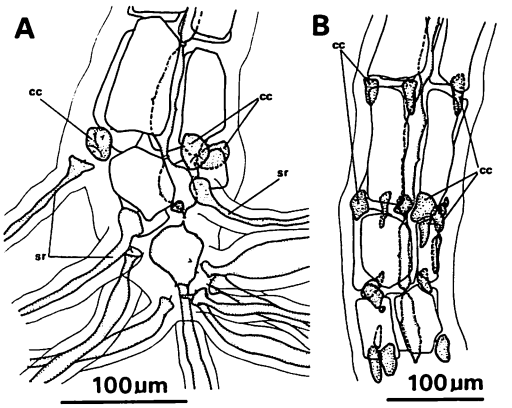


Fig. 5. Development of cortical cells of *Polysiphonia japonica* grown at 15°C, 16:8 LD (tetrasporophytes of Oshoro 2713 strain). A. Cortical cells cut off from pericentral cells of the lower portion of a 35-day-old plant; note two cortical cells issuing secondary rhizoids. B. Cortical cells cut off from pericentral cells of the middle portion of the main axis (3-month old).

Oshoro 2711 and 2712 strains and Samani 2590 strain, all of which were initiated from tetraspores of field-collected plants (Table 1), showed the same developmental pattern as did the above-described 2713 and 2714 strains.

All strains initiated from branch apices were cultured at 15°C, 16:8 LD and grew well, as did the sporelings of Oshoro and Samani strains. The apices grew into upright shoots and produced rhizoidal filaments from their lower segments. Secondary shoots sometimes developed from these rhizoidal filaments for Erimo 2147 male and Iwagasaki 2206 female strains. They reached reproductive maturity 1-2 months after inoculation. The gametophytic strains formed

the same gametangia as their parents. The morphological features of these strains were similar to those of their parent plants. Some of the strains were used for hybridization studies (Fig. 9). Tetraspores of the tetrasporophytic strains (Oshoro 2373, 2375; Utoro 1993; Kannonzaki 2425, 2426; Shiaku 2627, 2633 and Iwagasaki 2203) were cultured and their developmental patterns from sporelings to mature plants were traced at 15°C, 16:8 LD. These sporelings developed in a manner similar to that described for Oshoro 2713 and 2714 strains. No significant differences among the strains were observed. All the tetrasporelings gave rise to dioecious gametophytes at a ratio of 1:1 and formed procarps and spermatangia on separate individuals. Mature cystocarps were formed in mixed cultures of female and male plants of each strain. Some of these strains were clonally cultured from branch apices and used for hybridization experiments (Fig. 9).

In addition to analysis of morphological development, variations in growth and reproduction were observed at 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 and 20°C, 8:16 LD, using the second generations of Oshoro 2711 and 2714 strains (2711 tetrasporophytes and 2714 gametophytes) and the first (tetrasporophytes) and second (gametophytes) generations of Iwagasaki 2203 strain. The growth and reproductive responses of the tetrasporophytic and gametophytic phases to varying temperatures and photoregimes were similar. The plants grew most rapidly at 15°C, 16:8 LD and most slowly at 10°C, 8:16 LD. Tetrasporangia were formed first at 20°C, 16:8 LD and 20°C, 8:16 LD 21 days after inoculation and lastly at 10°C, 8:16 LD 77 days after inoculation. Spermatangia and procarps were formed first at 15°C, 16:8 LD, 20°C, 16:8 LD and 20°C, 8:16 LD 14 days after inoculation and lastly at 10°C, 8:16 LD 63 days after inoculation.

The dimensions of some vegetative and reproductive features of cultured fertile plants are given in Table 2. The diameters of main axes and length/diameter ratios of segments in cultured plants in general varied correlatively with thallus length as in field-collected plants. Some plants of Oshoro strains possessed slightly longer segments than field-collected plants. No

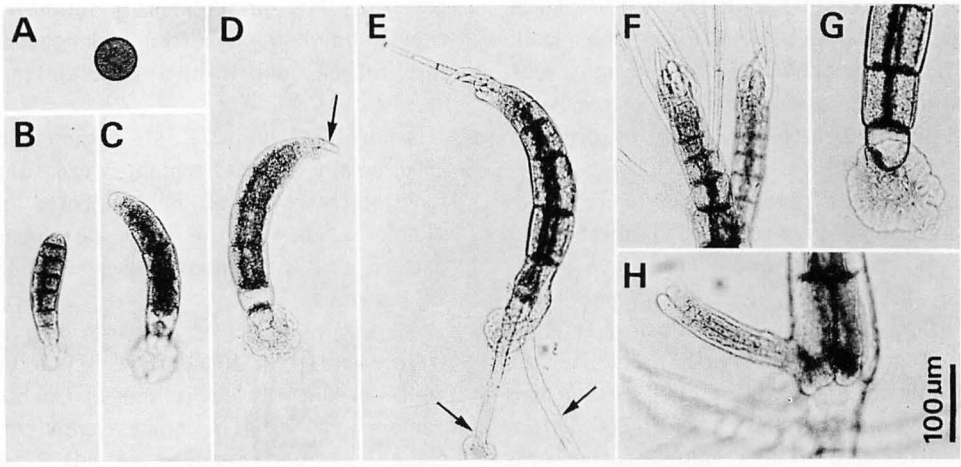


Fig. 6. Carpospore and its development of *Polysiphonia akkeshiensis* at 15°C, 16:8 LD (Akkeshi 2991 strain). All photographs from living material. A. Liberated carpospore. B. One-day-old germling. C. Two-day-old germling; note the axis being recurved. D. Three-day-old germling forming a vegetative trichoblast (arrow) on the dorsal side. E. Four-day-old germling with spirally arranged laterals and primary and secondary (arrows) rhizoids. F, G. Seven-day-old germling (F, apical portion; G, basal portion). H. Adventitious branch originated from the suprabasal segment of the main axis (14-day old). Scale in H applies to all of A–H.

significant differences in the segment length among plants cultured at different six conditions mentioned above were observed.

Polysiphonia akkeshiensis

The following observations are based on Akkeshi 2991 strain which was cultured at 15°C, 16:8 LD. Liberated carpospores were deep red in color and averaged 57.6 μm (range 52.5-65.0 μm; 140 spores measured) in diameter (Fig. 6A). Isolated carpospores germinated and grew into plants (Figs. 6B-H, 7A-H) in a manner similar to that of *Polysiphonia japonica* described above. The following features were observed for growing tetrasporophytes of the 2991 strain: recurved sporelings at a very young stage (Figs. 6C-E), irregular number of pericentral cells on suprabasal segments of the main axis, repeating replacement between ordinary branches and vegetative trichoblasts, adventitious branches originating from an axial cell of the lower segments of main axes (Figs. 6H, 7F) and from a basal cell of vegetative trichoblasts before (Fig. 7G) or after (Fig. 7H) the shedding of trichoblasts.

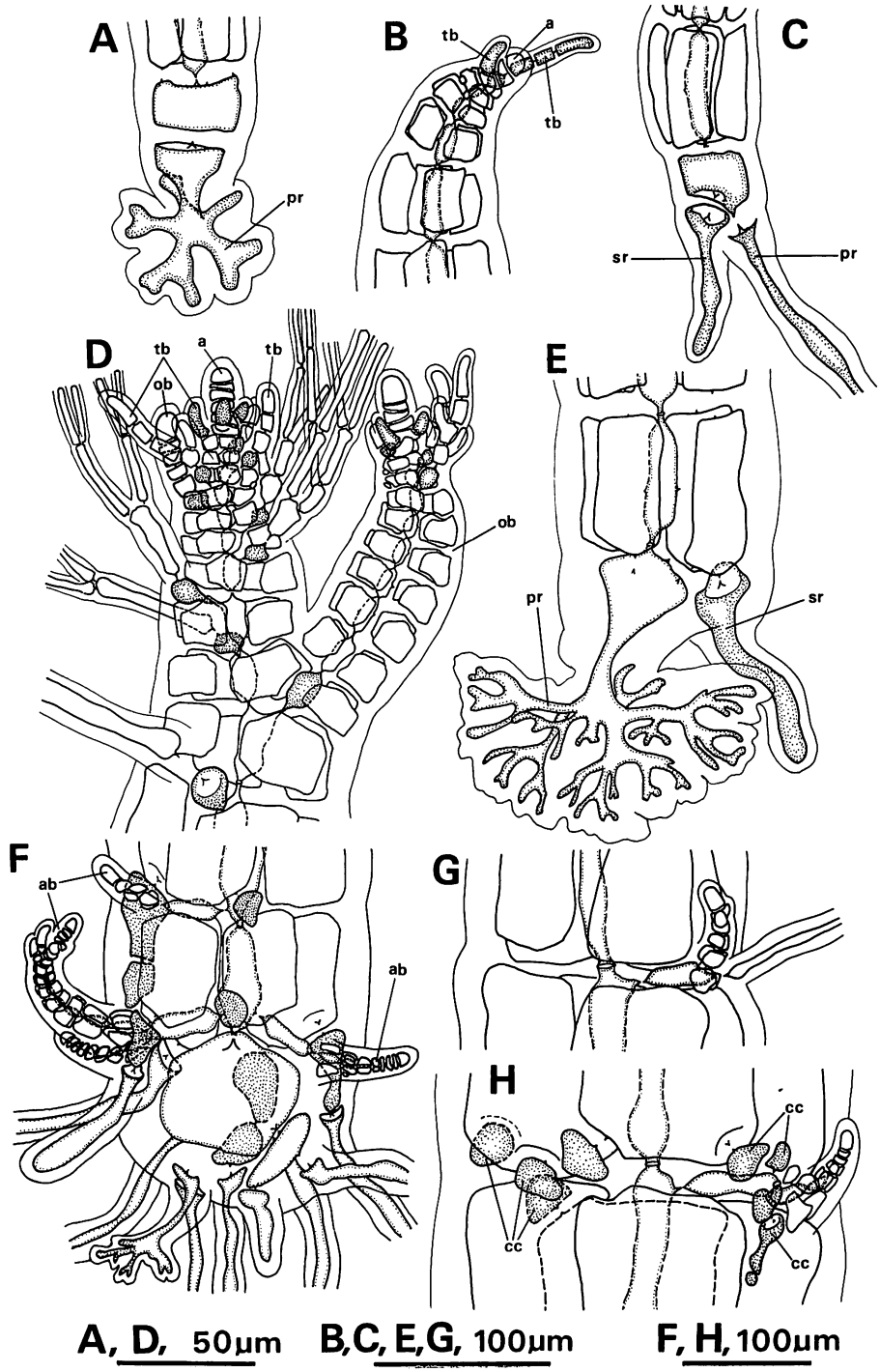
Plants began to form tetrasporangia 28 days after inoculation. They formed tetrasporangia more abundantly within a further week and released many tetraspores. At this stage the fertile plants had reached 15-23 mm in length (Fig. 8A) and possessed 18-27 branches of the first order and many short cicatrigenous branches. The tetrasporangia were first produced on the upper portion of ordinary branches (Fig. 8B) and later on the short cicatrigenous branches. Liberated tetraspores were slightly smaller than the parent carpospores and averaged 49.3 μm (range 37.5-57.5 μm; 80 spores measured) in diameter (Fig. 8C). These tetrasporophytes formed cortical cells, which cut off rhizoidal cells, from pericentral cells of the lower segments of the main axis (Fig. 7F). They also formed cortical cells from pericentral cells and scar cells of the middle segments of the main axes (Fig. 7H). More developed cortical cells are shown in Figs. 8D, E. No propagules were observed on the tetrasporophytes of the 2991 strain.

Tetraspores inoculated onto glass slides germinated and grew into gametophytes in a pattern similar to that of the parent tetrasporophytes. The gametophytes began to produce procarps and spermatangial branchlets on separate plants 14 days after inoculation. The spermatangial branchlets were borne on male trichoblasts replacing ordinary branches or vegetative trichoblasts (Fig. 8F). Two to seven male trichoblasts were formed successively, then replaced by ordinary branches or vegetative trichoblasts, after which they were formed successively again. This process was repeated continuously as long as the plants grew well (Fig. 8G). Mature spermatangial branchlets were nearly cylindrical and 165-283 μm long×53-70 μm wide. The procarps were borne on the suprabasal segments of female trichoblasts (Fig. 8H) which arose from the growing apex of main axes and branches. The female trichoblasts were repeatedly replaced by ordinary branches and/or vegetative trichoblasts and were usually formed individually.

When female gametophytes bearing procarps were placed in dishes with male gametophytes releasing numerous spermatia and shaken they formed cystocarps (Fig. 8I). Viable carpospores were released 16 days after the initiation of mixed cultures. Mature cystocarps were ovate and 260-430 μm long×250-440 μm wide (Fig. 8J). Isolated female gametophytes did not produce cystocarps.

Propagules were often formed both on female and male gametophytes; they grew into fertile gametophytes bearing reproductive organs of their respective parents. The propagules formed on male gametophytes are shown in Figs. 8K, L.

Akkeshi 2990 strain initiated from tetraspores showed the same developmental pattern as did the aforementioned 2991 strain. All strains derived from branch apices grew well and reached reproductive maturity 1-2 months after inoculation at 15°C, 16:8 LD. Some of these strains were clonally cultured and used for hybridization experiments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A, D, 50μm B,C, E,G, 100μm F, H, 100μm

(Fig. 9).

The growth and reproductive responses of the tetrasporophytic (the second generation of 2990 strain) and gametophytic (the second generation of 2991 strain) phases to varying temperatures and photoregimes were examined as has been described earlier for *Polysiphonia japonica*. The responses of both phases were similar. The plants grew most rapidly at 15°C, 16:8 LD and most slowly at 10°C, 8:16 LD. Tetrasporangia were formed first at 15°C, 16:8 LD, 20°C, 16:8 LD and 20°C, 8:16 LD, 28 days after inoculation and lastly at 10°C, 8:16 LD, 77 days after inoculation. Spermatangia and procarps were formed first at 15°C, 16:8 LD, 20°C, 16:8 LD and 20°C, 8:16 LD, 14 days after inoculation and lastly at 10°C, 8:16 LD, 63 days after inoculation.

Hybridization experiments

Our results from crosses between 11 female and 12 male clones derived from 8 local populations of *Polysiphonia japonica* and *P. akkeshiensis* are shown in Fig. 9. Cystocarp development was evident on female plants in compatible crosses after 7 days at 15°C, 16:8 LD and carpospores were released 7-14 days afterward. Cystocarps, however, did not develop on female plants in other crosses even 2 months after the initiation and these crosses were then terminated. Since no cystocarps were observed in isolated female controls, the results indicate that cross-fertilization occurred in the compatible crosses and did not occur in the incompatible crosses. In crosses between female plants from Oshoro, Kannonzaki, Shiaku and Iwagasaki (the southern group) and male plants from Abashiri, Utoro, Akkeshi and Erimo (the northern group) were completely negative. About half of their reciprocal crosses between female plants of the northern group and male plants of the southern group, however, was positive and viable carpospores were released. In negative crosses between these groups pericarp development was observed on the female plants in 7-14 days, but their gonimoblasts did not become mature. Any of these crosses repeated showed the same results. These breeding groups did not correspond to taxonomic groups defined by field-collected plants (Tables 1, 2). Abashiri and Erimo populations, which can be identified as *P. japonica*, belong to the northern group including *P. akkeshiensis*.

Carpospores resulting from compatible crosses were cultured at 15°C, 16:8 LD. No significant differences in developmental pattern and morphology among the sporelings (=F_1 tetrasporophytes) were observed. All F_1 tetrasporophytes obtained formed tetrasporangia and released viable spores within 30-40 days. Tetrasporelings were grown at the same culture condition. The tetrasporelings (=F_1 gametophytes) derived from many compatible crosses grew rapidly and formed procarps and spermatangia on separate plants within 14-21 days after germination, whereas those derived from the northern females×southern males (except for Akkeshi females×Iwagasaki males) were less vigorous. F_1 gametophytes of Akkeshi 1979 female×Kannonzaki 2422 and 2427 males grew slowly and eventually died

Fig. 7. Carposporelings of *Polysiphonia akkeshiensis* grown at 15°C, 16:8 LD (Akkeshi 2991 strain). A. Basal portion of a 2-day-old sporeling. B. Apical portion of a 3-day-old sporeling which issues two trichoblasts. C. Basal portion of a 5-day-old sporeling which produces a secondary rhizoid from the basal cell. D. Apical portion of a 7-day-old sporeling which forms spirally arranged trichoblasts and ordinary branches. E. Basal portion of a 10-day-old sporeling with a primary expanded disc-like rhizoid and a secondary rhizoid produced from the pericentral cell. F. Basal portion of a 17-day-old sporeling which forms four adventitious branches and cortical cells; note secondary rhizoids cut off from the pericentral cell, cortical cells and basal cell. G. Middle portion of a main axis issuing an adventitious branch from the basal cell of a trichoblast before its shedding (20-day old). H. Middle portion of a main axis issuing an adventitious branch from a scar cell (the basal cell of a trichoblast after its shedding) and cortical cells from the pericentral cells and from the scar cell (21-day old).

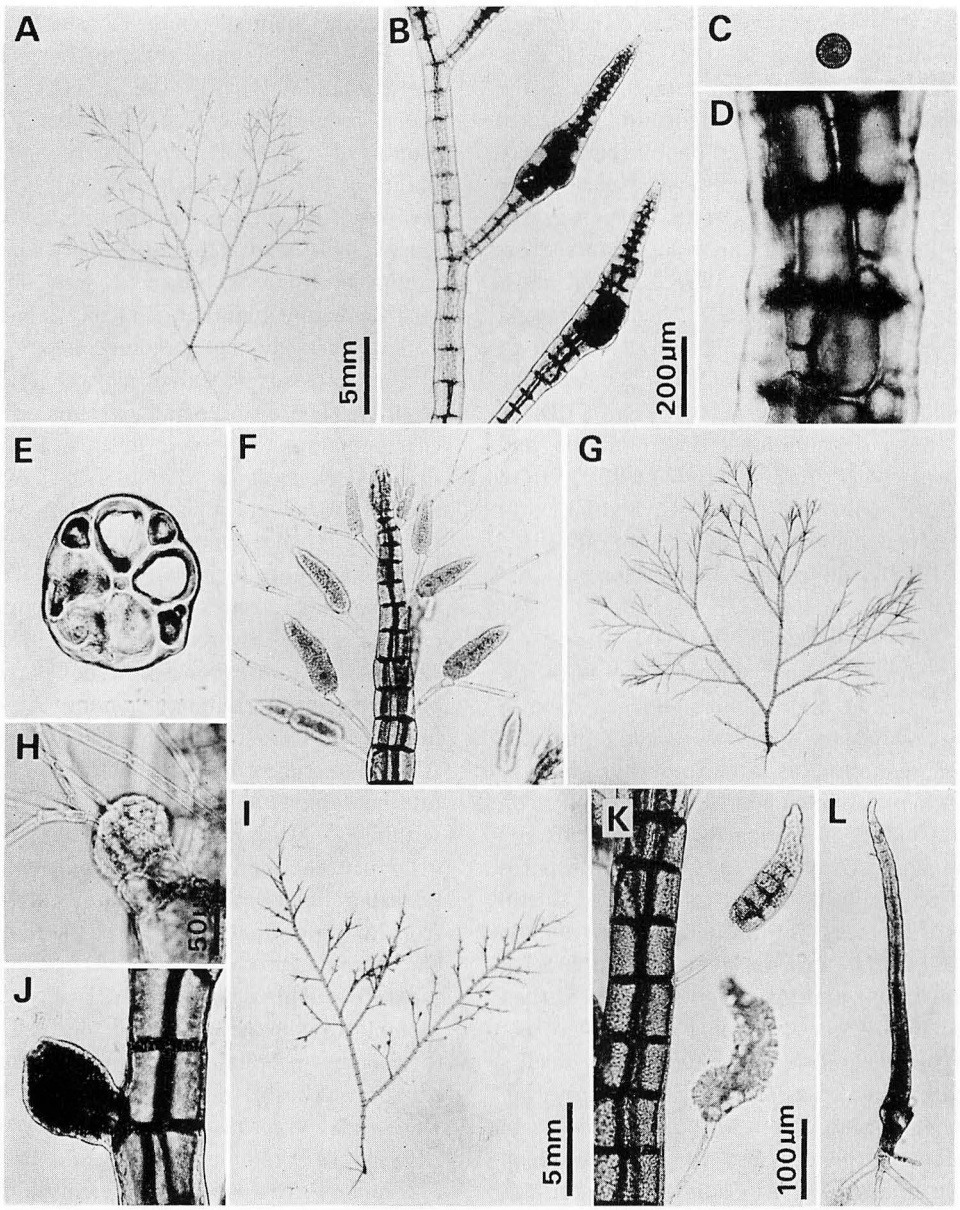

Fig. 8. *Polysiphonia akkeshiensis* cultured at 15°C, 16 : 8 LD (Akkeshi 2991 strain). All photographs from living material. A. Fertile tetrasporophyte cultured for 34 days. B. Tetrasporangia formed on the ordinary branches. C. Liberated tetraspore. D, E. Cortication of the main axis of a 53-day-old tetrasporophyte (D, surface view; E, cross section). F. Spermatangial branchlets formed on the uppermost portion of the main axis (21 day old). G. Fertile male gametophyte cultured for 34 days. H. Procarp borne at the upper portion of an ordinary branch. I. Fertile female gametophyte cultured stationarily for 14 days and then mixed with a male gametophyte for 16 days on a shaker. J. Mature cystocarp. K. Propagules formed on the upper portion of a male gametophyte. L. Germinating propagule. Scale in A applies also to G; scale in B applies also to D-F, J and L; scale in K applies also to C.

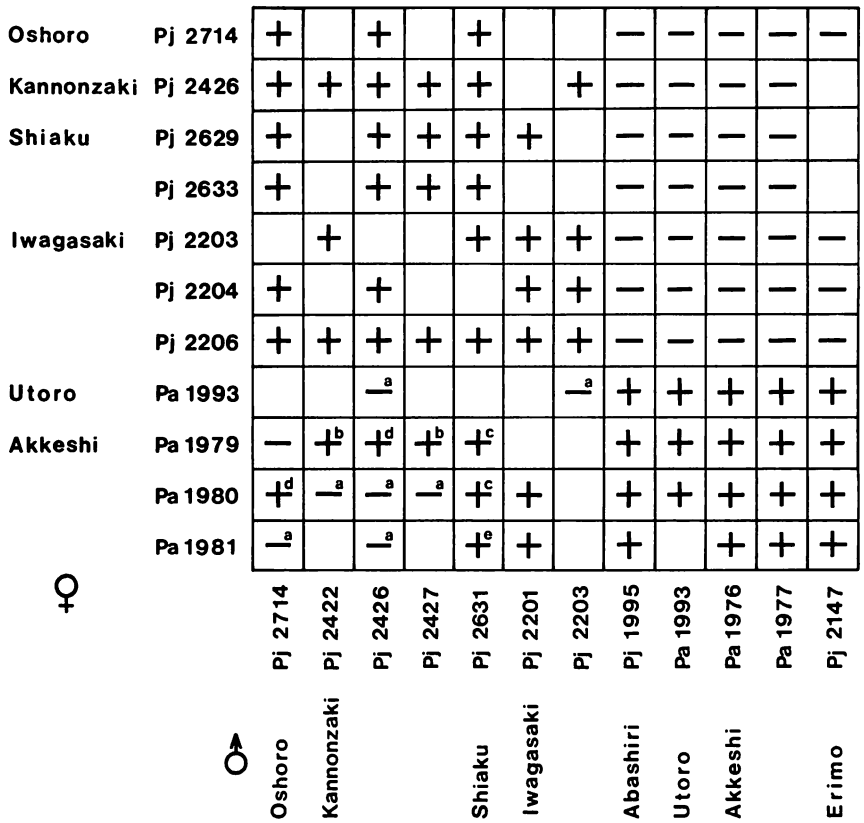

Fig. 9. Attempted crosses between strains of *Polysiphonia japonica* (Pj) and *P. akkeshiensis* (Pa). Each number designates the individual culture number. −, No cystocarps developed; $-^{a}$, abortive cystocarps developed; $+^{b}$, F_1 tetrasporophytes sporulated, but F_1 gametophytes died before reproductive maturity; $+^{c}$, F_1 gametophytes did not become reproductive; $+^{d}$, abortive cystocarps developed in self-crosses of F_1 gametophytes; $+^{e}$, F_2 tetrasporophytes did not become reproductive; +, F_2 tetrasporophytes sporulated; blank, cross was not attempted.

before reproductive maturity. F_1 gametophytes of Akkeshi 1979 female×Shiaku 2631 male and of Akkeshi 1980 female×Shiaku 2631 male did not form reproductive organs even 4 months after germination and then the cultures were terminated. These suggest that hybrid inviability and hybrid sterility occurred in their F_1 gametophytic generation.

In self-crosses of dioecious F_1 gametophytes cystocarps developed and carpospores were discharged except those of Akkeshi 1979 female×Kannonzaki 2426 male and Akkeshi 1980 female×Oshoro 2714 male, which formed abortive cystocarps, although in isolated cultures of female gametophytes cystocarps did not develop. The vast majority of F_2 tetrasporophytes became reproductive within 30-40 days, but those derived from a cross between Akkeshi 1981 female and Shiaku 2631 male grew slowly and did not become reproductive even 4 months after germination. The latter suggests that hybrid breakdown occurred in the F_2 tetrasporophytic generation. Sporelings (=F_3 gametophytes) from F_2 tetrasporophytes of Akkeshi females×Iwagasaki males grew normally and formed cystocarps when dioecious gametophytes were mixed in single dishes. Thus, their progeny did not show

hybrid breakdown up to F_3 gametophytic generation.

Propagules were frequently formed on female and male gametophytes of interpopulation hybrids. Propagules on tetrasporophytes were observed in a single case: F_1 tetrasporophytes of Akkeshi 1980 female × Oshoro 2714 male. The propagules were less abundant than those of gametophytes. Released propagules gave rise to fertile tetrasporophytes.

Discussion

Our morphological study of the life history stages of *Polysiphonia japonica* and *P. akkeshiensis* reveals that these two species are similar at every stage to each other. The two species are also similar in growth and reproductive responses to varying temperatures and photoregimes. *P. akkeshiensis* has been characterized by having laxly expanded to subflabellate thalli and a flaccid texture (SEGI 1951). These characters are brought about long segments of which length/diameter ratios range between 2.6 and 4.7 at the middle portion of the main axes. Cultured plants of this alga possessed shorter segments than did field-collected plants and could not be distinguished from *P. japonica*. This strongly suggests that these features show phenotypic plasticity and that such unstable characters are inappropriate for use as taxonomic criteria. Alternative criteria, however, have not been found. It may be concluded that employing morphological features, the two algae refer to the same taxonomic species. Our artificial hybridization experiments, however, show that incompletely isolated northern and southern breeding groups are present among the local populations studied. Samani 2590 and Enoshima 2499 strains, which are not shown in Fig. 9, probably belong to the northern group and southern group respectively according to a few crosses attempted. The two breeding groups are entirely allopatric in the range of our collections (Fig. 1). Their geographical patterns should be confirmed by more extensive sampling and hybridization experiments. Isolating mechanisms are diverse: incompatibility, hybrid inviability, hybrid sterility and hybrid breakdown. However, no reproductive isolation exists between Akkeshi and Iwagasaki populations, which are geographically distant (Fig. 1). It can be speculated that natural hybridization between these local populations does not occur. These results suggest the possibility that two virtually non-interbreeding groups separated by various isolating mechanisms exist in Japan, but the local populations sampled are small and hybridization experiments are as yet incomplete. These groups may have reached a certain stage of gradual speciation before morphological differentiation. This situation is similar to that of the red algal species *Gymnogongrus flabelliformis* HARVEY of Phyllophoraceae (MASUDA, unpubl.). Based on the subtle morphological differences and reduced viability of F_1 gametophytes, RUENESS (1973) concluded that Texas *Polysiphonia boldii* WYNNE et EDWARDS was reduced to varietal status of Scandinavian *P. hemisphaerica*. On the basis of morphological, cytological and hybridization studies, KAPRAUN (1978b) reported that *P. ferulacea* and *P. harveyi* BAILEY consist of reproductively isolated sibling species groups respectively. It is premature to decide the formal taxonomic status of the groups of *Polysiphonia japonica* complex until a biosystematic investigation of the species complex throughout the whole coasts of Japan can be undertaken.

Some critical structural features of the species 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 (1984). The latter is referred to as cicatrigenous branches (HOLLENBERG 1942). In cultured plants it occurs often before the shedding of trichoblasts. The same phenomenon was reported for field-

collected plants of Danish *Polysiphonia elongata* (HUDSON) HARVEY and *P. nigrescens* (HUDSON) GREVILLE (ROSENVINGE 1923-24) and those of Hawaiian *P. tuberosa* HOLLENBERG (1968). Dichotomous branching, which was described by SEGI (1951) and adopted as a characteristic feature of *P. japonica* by YOON (1984), is entirely absent, but the main axis and any of the branches grow monopodially. Their descriptions are probably based on specimens one of whose lower branches grow conspicuously. Propagules were frequently found on trichoblasts of cultured male and female plants and rarely on tetrasporangial plants and recycled the respective phase that produced them. A similar structure was reported for cultured and field-collected plants of North Carolina *P. ferulacea* (KAPRAUN 1977). Cicatrigenously originated propagules were described for field-collected plants of Australian *P. propagulifera* WOMERSLEY and *P. mollis* HOOKER et HARVEY ex HARVEY (WOMERSLEY 1979). It can be speculated that propagules of these three species are really asexual reproductive organs in the field populations, but this has not been confirmed for the species complex under study in the field.

It is noteworthy that cortical cells of cultured plants developed slowly. Cultured plants, which began to form reproductive organs, had weakly developed cortical cells from the lower segments of the main axis. Similar fertile plants are also found in nature. This suggests a close relationship between *Polysiphonia japonica* complex and *P. savatieri* HARIOT. The latter is characterized by the absence of cortical cells (HARIOT 1891), although its gross morphology seems to be similar to that of *Polysiphonia decumbens* SEGI and young plants of *P. japonica* as pointed out by SEGI (1951) and YOON (1984). The existence of cortical cells in the Korean *P. savatieri* varies according to the habitat (YOON 1984); specimens growing on *Chondria* sometimes have a slight cortication near the base of older axes, although those growing on *Codium* do not produce cortical cells. YOON (1984) proposed that *P. savatieri* should be reduced to varietal status of *P. japonica*, although his paper, which has not been printed and has been distributed by photostatic copies, is not an effective publication according to Article 29. 1 of the International Code of Botanical Nomenclature (ICBN, VOSS *et al.* 1983). Our data obtained in the laboratory and field support his opinion. More detailed studies, however, are needed to evaluate their genetic affinities.

SEGI (1951) reported *Polysiphonia novae-angliae* W.R. TAYLOR from a single locality, Okushiri Isl., Hokkaido. His identification is based on the similarity in texture (spongy rather than slippery) and cystocarp shape (elongato-urceolate) (SEGI 1951). TAZAWA (1975) described spermatangial branchlets of this alga on the basis of specimens collected at Otaru, Hokkaido. Judging from TAZAWA's voucher specimens preserved in SAP (028547), his identification may be based on the spongy texture. Older specimens of *P. japonica*, however, always have a spongy texture. The shape and dimension of cystocarps of *P. novae-angliae* given by SEGI (1951) can be frequently found in our collections of *P. japonica* (Table 2). Thus, *P. novae-angliae* auct. japon. is included in the circumscription of *P. japonica* complex.

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工藤利彦・増田道夫：紅藻キブリイトグサとアッケシイトグサの分類学的研究

キブリイトグサとアッケシイトグサの生活史を培養実験によって比較した結果，両者は異なる培養条件（温度，10-20°C；日長，16:8 LD と 8:16 LD）で，形態，生長及び成熟の反応においてよく似ていた。後者の特徴とされている形質は，節間が前者よりも長いことに起因することがフィールド個体で確認された。しかし，培養個体ではその差異は認められなかった。これは両者の識別に用いられてきた基準形質が不安定であることを示す。代わりうる形質もみつからなかったので両者は同一の分類学的種として扱いうる。一方，様々な機構によって生殖的に融離されている異所的な2つの交配群が存在することが交雑実験で明らかになった。またナガツボイトグサも今回調べられた種群に含められうる。(060 札幌市北区北10条西8丁目　北海道大学理学部植物学教室)