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Systematic Studies of *Ceramium kondoi* (Ceramiaceae, Rhodophyta) in the Field and in Culture

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Structure and reproduction of *Ceramium kondoi* Yendo was investigated in Oeyondo Island on the Yellow Sea of Korea and under laboratory conditions. Type materials in SAP were also examined. *C. kondoi* plants showed obvious seasonal differences in growth and reproduction in the study area. The mean length was a maximum of 14.6 ± 3.0 cm in May, when plants were mature with tetrasporangia or sexual reproductive organs, but without occurrence of plants between August and November. Cultured isolates from two different locations showed a *Polysiphonia*-type life history, similar morphologies and interfertility. Detailed examination of structure and reproduction of materials in the field and in herbarium showed variation within the range obtained in culture. However, one important distinguishing feature of *C. kondoi*, trichotomous ramification, occurred at a frequency of 23.6 ± 18. 8% in the field, but without trichotomy in culture. Two *Ceramium* species from France, which were tentatively referred to *C. nodulosum* (Lightfoot) Ducluzeau and *C. fruticulousm* Kützing, were grown through their life cycles. Attempted crosses between the two French were negative and neither were interfertile with *C. kondoi*.

Key Words: Ceramiaceae, *Ceramium kondoi*, crossability, life history, phenology, Rhodophyta, reproduction, structure, systematics.

INTRODUCTION

Ceramium is a well-known genus of common occurrence and morphological variation in the Rhodophyta. Literature analysis by Boo and Lee (1993) from many papers on *Ceramium* species shows important points as follows; First, a world monograph has never been published. Second, many species are unstable in species concept. Third, there have remained nomenclatural problems, and last, many varieties and forms have been known in a single species. According to Maggs and Hommersand (1993), the name of *Ceramium rubrum* (Hudson) C. Agardh, the type of the genus, is changed to *C. nodulosum* (Lightfoot) Ducluzeau, but the species concept is still unstable. *C. nodulosum* commonly occurs on most coasts of the world, but no reports in Korean and the surrounding waters. In this area, *C. kondoi*, being quite similar to *C. nodulosum*, has been reported.

Ceramium kondoi has been known by Yendo (1920), who described it based on materials collected in Hariusu in Hokkaido, Japan. It shows diverse morphologies and, according to Nakamura (1965), four forms are separated in the field; f. *kondoi, ambiguum, abbreviatum* and *trichotomum*. Important features dividing forms are branching pattern, tetrasporangial arrangement and occurrence of tri- and tetrachotomous branches. Although Nakamura (1950, 1954, 1965) gave descriptions on the structure and reproduction of the forms, there have been no comparative studies on the features of the species in different months and life history phases. In the Canadian *C. rubrum* plants, females had a large number of primary and secondary branches as

Table 1. Origin of *Ceramium* species cultures used for life history and crosses.

Species	Date	Phase	Location
Ceramium kondoi	I 19, 1992	Female	Oeyondo, Yellow Sea of Korea
C. kondoi	VII 24, 1992	Tetrasporic	Wido, Yellow Sea of Korea
C. nodulosum	VIII 21, 1992	Tetrasporic	St. Malo, northern part of France
C. fruticulosum	VIII 21, 1992	Tetrasporic	St. Malo, northern part of France

well as trichotomous ones than tetrasporic plants (Garbary *et al.*, 1980).

Culture studies of *C. kondoi* showed a *Polysiphonia*-type life history (Notoya and Yabu 1979; Suh and Lee, 1984). There has been no differences in morphology and life history between in culture and in the field. The chromosome number of haploid tetraspores of Japanese plants was n = 12-15 in the culture (Notoya and Yabu, 1979).

A few attempts of crossability tests have been given in assessment of reproductive affinity in the *Ceramium* species. Rueness (1978) reported that the Baltic strain, which differed in various morphological and physiological features from others of *C. strictum*, was interfertile, and treated it as a subspecies. Garbary (1988) also showed that two types of supposed *C. rubrum* could be identified by the interoceanic hybridization study. So, crossability test is required for a means of specific and subspecifc delimitation in variable species like *C. kondoi* and *C. rubrum*.

In this study structure and reproduction of the Korean *Ceramium kondoi* plants was investigated both in the field and in culture. Phenology and crossability tests between local plants and the related European species were for the first time studied in *C. kondoi*.

MATERIALS AND METHODS

Field investigations were carried out in Oeyondo (36° 14′ N, 126° 4′ E) on the Yellow Sea of Korea. The study site was selected because of the abundance of *Ceramium kondoi* plants. They occurred in the tide pools and on watery rock or small pebbles in littoral areas. To know phenology, they were at random collected at bimonthly intervals between January 1992 and June 1993. Each plant was examined for reproductive organs. The numbers of male, female, tetrasporic and vegetative plants were recorded as percentages of the total number of plants in each collection. Plant length was also measured and compared in different months and life history phases. Because tri- or tetrachotomy is one feature in separating *C. kondoi* from *C. nodulosum* (Nakamura, 1950, 1965), the occurrences in different months and life history phases were recorded as percentage of total number of branching nodes on a single axis.

Data for culture strains are shown in Table 1. For unialgal culture, healthy apices were isolated and cultures were maintained in an IMR/2 medium. Cultures were kept at 13 and 17°C under a 16:8 LD cycle at a photon fluence rate of about 20 μmol $m^{-2}s^{-1}$. Crossability tests were given based on cultured sexual plants. Three fertile female apex of 2 cm and one fertile male of 1 cm were cultured together in a glass dish with three replicates. As a control of parthenogenesis, a single fertile female apex was cultured in a dish in each cross.

Structure and reproduction was observed based on materials in the field and in culture. Herbarium specimens of SAP were also available. For microscopic examinations, materials were fixed in 4% neutral formalin-seawater and mounted in glycerine. Anatomical details were observed by gently squashing the specimens in 1 % HCl solution and mounted in glycerine. In most observations specimens were stained on the slide with phenol-aniline blue.

RESULTS

Type materials: *Ceramium kondoi* is separated into four forms, e.g. f. *kondoi, ambiguum, abbreviatum* and *trichotomum*, of which the types are housed in SAP, Hokkaido University (Fig. 1). The protologue of the species was at first given in 1920 by Yendo based on materials collected by K. Kondo in June 29, 1905 in Hariusu, Hokkaido,

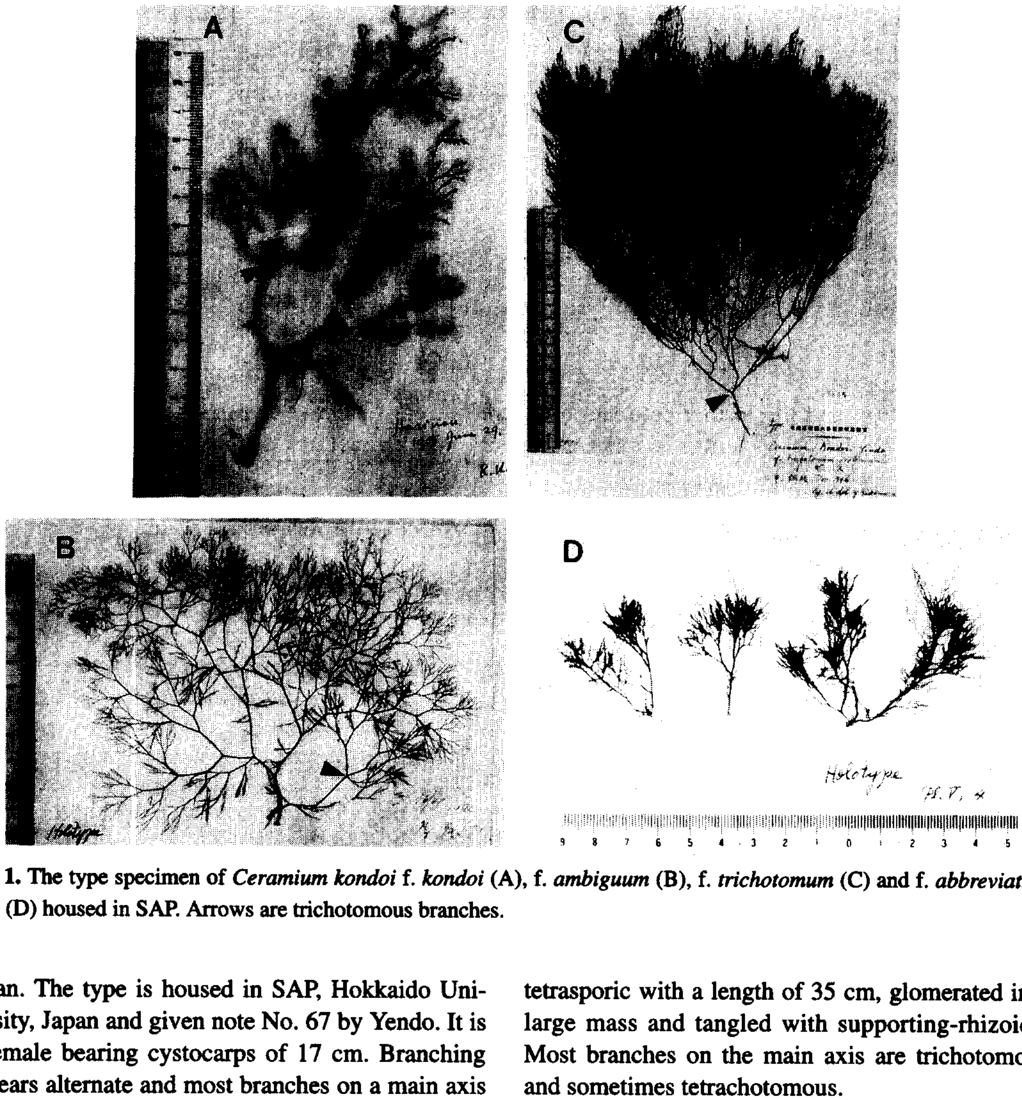

Fig. 1. The type specimen of *Ceramium kondoi* f. *kondoi* (A), f. *ambiguum* (B), f. *trichotomum* (C) and f. *abbreviatum* (D) housed in SAP. Arrows are trichotomous branches.

Japan. The type is housed in SAP, Hokkaido University, Japan and given note No. 67 by Yendo. It is a female bearing cystocarps of 17 cm. Branching appears alternate and most branches on a main axis are trichotomous.

The type of f. *abbreviatum* is on a sheet given SAP 25847, which is composed of three tetrasporic plants. The right plant is 5.5 cm high and has a percurrent axis with abbreviated branches on all sides, but with few trichotomous branches. The type of f. *ambiguum* was given SAP 25849 and collected at Akkeshi, Hokkaido. It is a tetrasporic plant of 12 cm, in which three trichotomous branches in the eighteen branches on a single axis are observed. The cortex is very thin and axis banded. The type of f. *trichotomum* was given SAP 25846 and collected in July 17, 1946 in Akkeshi, Hokkaido. It is tetrasporic with a length of 35 cm, glomerated in a large mass and tangled with supporting-rhizoids. Most branches on the main axis are trichotomous and sometimes tetrachotomous.

Each form was found by its characteristic features as well as the protologue, but in the herbarium of Hokkaido University, there have remained specimens as "intermediate" between forms, annotated by Nakamura himself.

Phenology and habit: *Ceramium kondoi* plants were vegetative in January 1992 in Oeyondo. In March a few males and tetrasporic plants began to occur, but most plants were still sterile and light red in colour. Plants were mature with tetrasporangia or sexual reproductive organs in May, when they appeared to be decolorized and were scarcely attached on herbarium sheets. Plants withered in

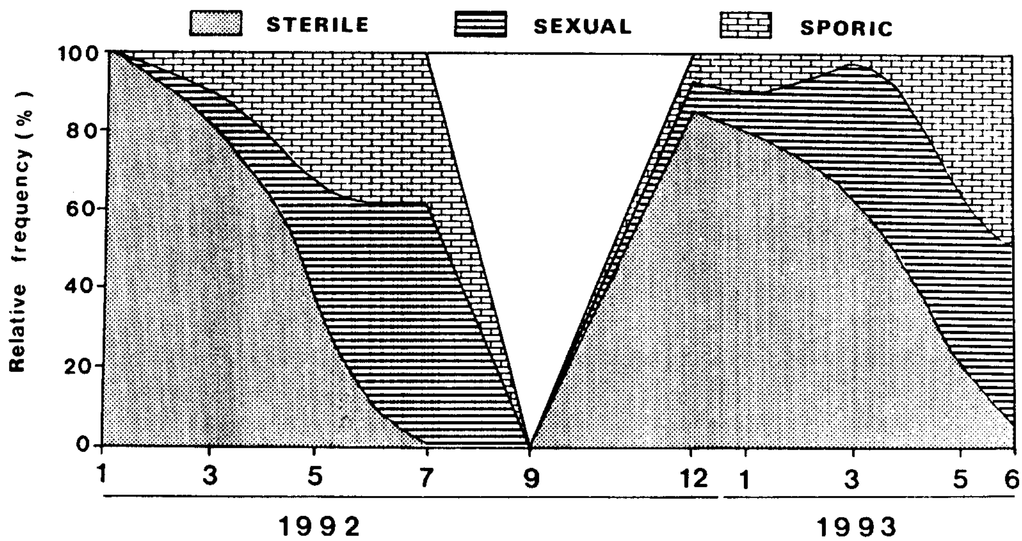

Fig. 2. Reproductive phenology of *Ceramium kondoi* plants in Oeyondo on the Yellow Sea of Korea.

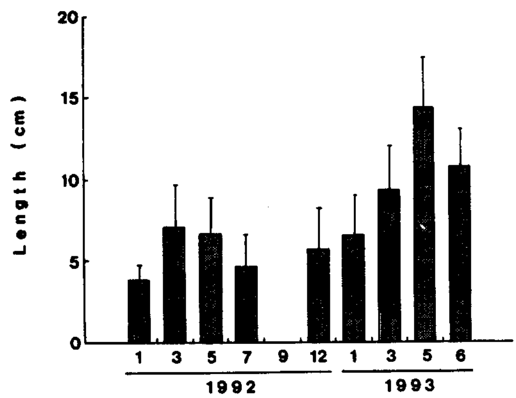

Fig. 3. Mean lengths of *Ceramium kondoi* plants in different months in Oeyondo.

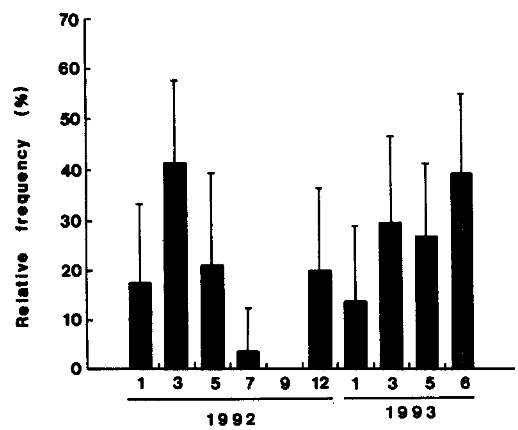

Fig. 4. Occurrence of trichotomous branches of *Ceramium kondoi* plants in different months in Oeyondo.

July and only two plants were collected in September. New young plants were found in December. The continued observations between March and July, 1993 showed a same phenology with the previous one, so *Ceramium kondoi* plants exhibited obvious seasonal differences in growth and reproduction on the central western coast of Korea (Fig. 2); in late spring most of plants were mature with reproductive organs, whereas in early winter most sterile.

Figure 3 shows mean lengths in different months in Oeyondo. In May, it was a maximum of 14.5 ± 3.0 cm (n = 66), whereas in January a minimum of 3.8 ± 0.9 cm (n = 32). The longest was a tetrasporic plant of 21 cm in May. Mean lengths in different life history phases showed that tetrasporic plants were a mean of 11.2 ± 4.0 cm, the longest 21 cm and larger than other phase ones.

Branching is alternate with 14.3 ± 3.1 segments between two successive dichotomies. The longer axis has 15.8 ± 3.4 segments while the shorter one 12.8 ± 2.0 segments. The number of di-, tri- and tetrachotomies also differed in different months in Oeyondo and trichotomous branches occurred in

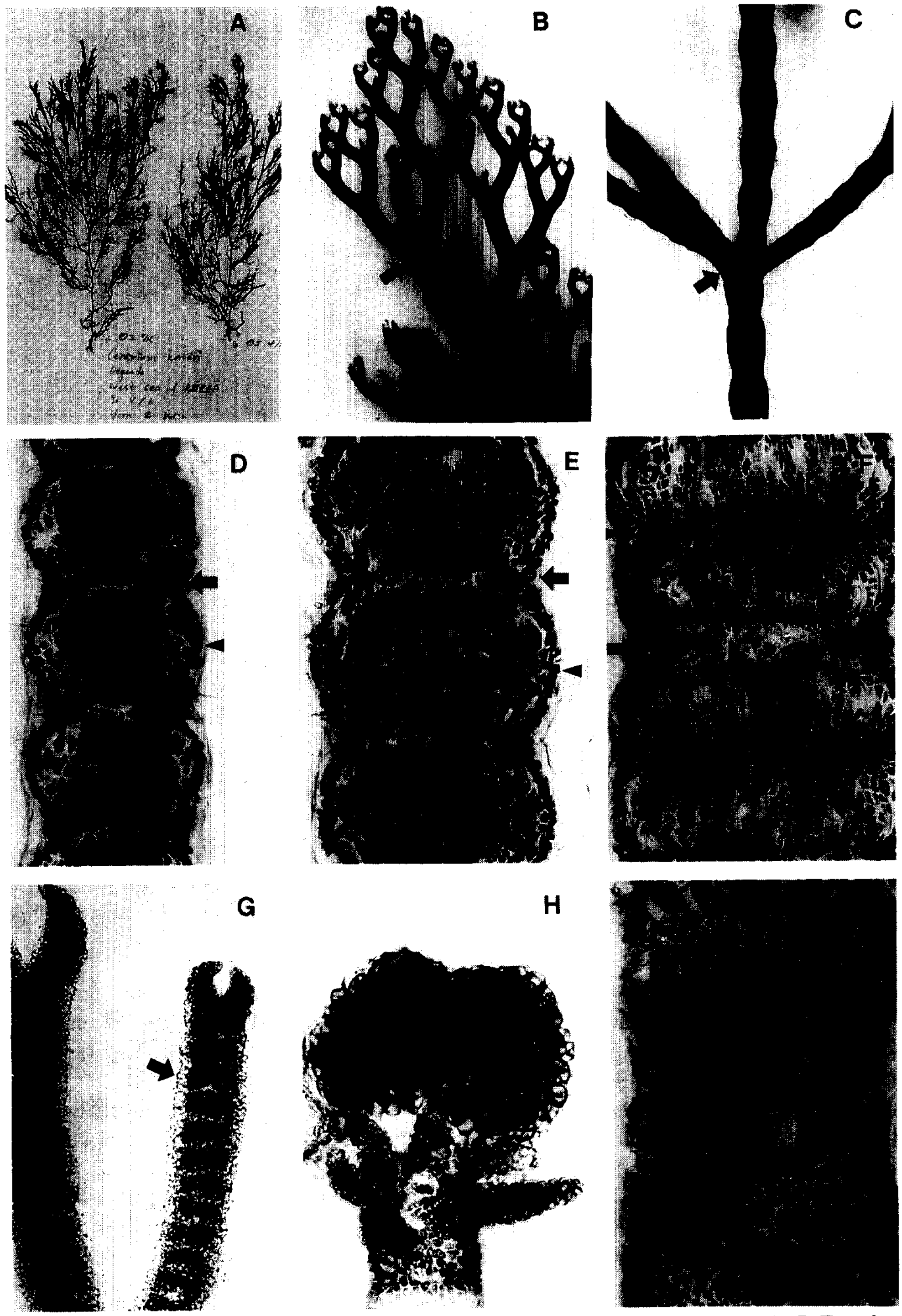

Fig. 5. *Ceamium kondoi* plants. A: Habit of tetrasporic plants. B: Trichotomous branch (arrow). C: Tetrachotomous branch (arrow). D - F: Cortical cells in the upper part (D), in the middle part (E) and in lower part (F). Tailed arrows are cortical node, arrows are internode. G: Spermatangial branch. H: Cystocarp and involucral branches. I: Tetrasporangial branch.

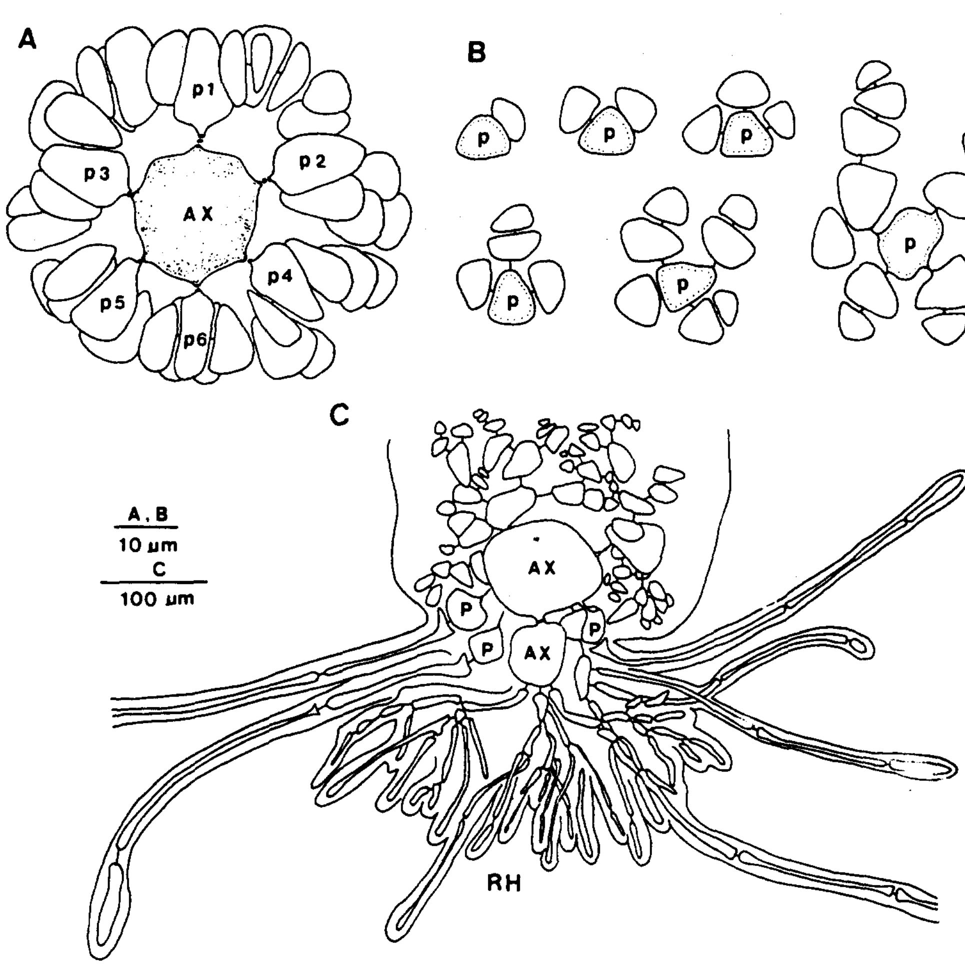

Fig. 6. Origin of periaxial (A), cortical cells (B) and rhizoids (C) in *Ceramium kondoi* plants (AX, axial cell; P, periaxial cell; RH, rhizoids).

every collection. The mean frequency was 23.6 ± 18.8% throughout the year. Trichotomy showed a similar tendency to plant length. Between March and June trichotomy were observed in most plants and a maximum trichotomy of 88% on a single sterile plant in March (Figs 4, 5). Trichotomy showed difference between life history phases and a frequency of 29% in male, 25% in females, whereas tetrasporic and vegetative ones 22%, respectively. Tetrachotomy was also found in Oeyondo plants and showed a mean occurrence of 1.2 ± 4.8%. Like trichotomy, a large number of tetrachotomies was observed in sexual plants (data not shown).

We observed Nakamura's separation in Oeyondo specimens, but it was difficult that most plants were identified under forms.

Vegetative development: Rhizoids are usually produced from axial and periaxial cells. Usually rhizoids from axial cells are branched and aggregate to disc-like appearance, while ones from periaxial cells become unbranched filaments. Axis is composed of axial, periaxial and cortical cells. Axial cell is produced by means of a transversley dividing apical cell. It is naked by the apical two to three segments and, below it, covered by periaxial and cortical cells. Periaxial cells are cut off in an alternating sequence to the abaxial side and the

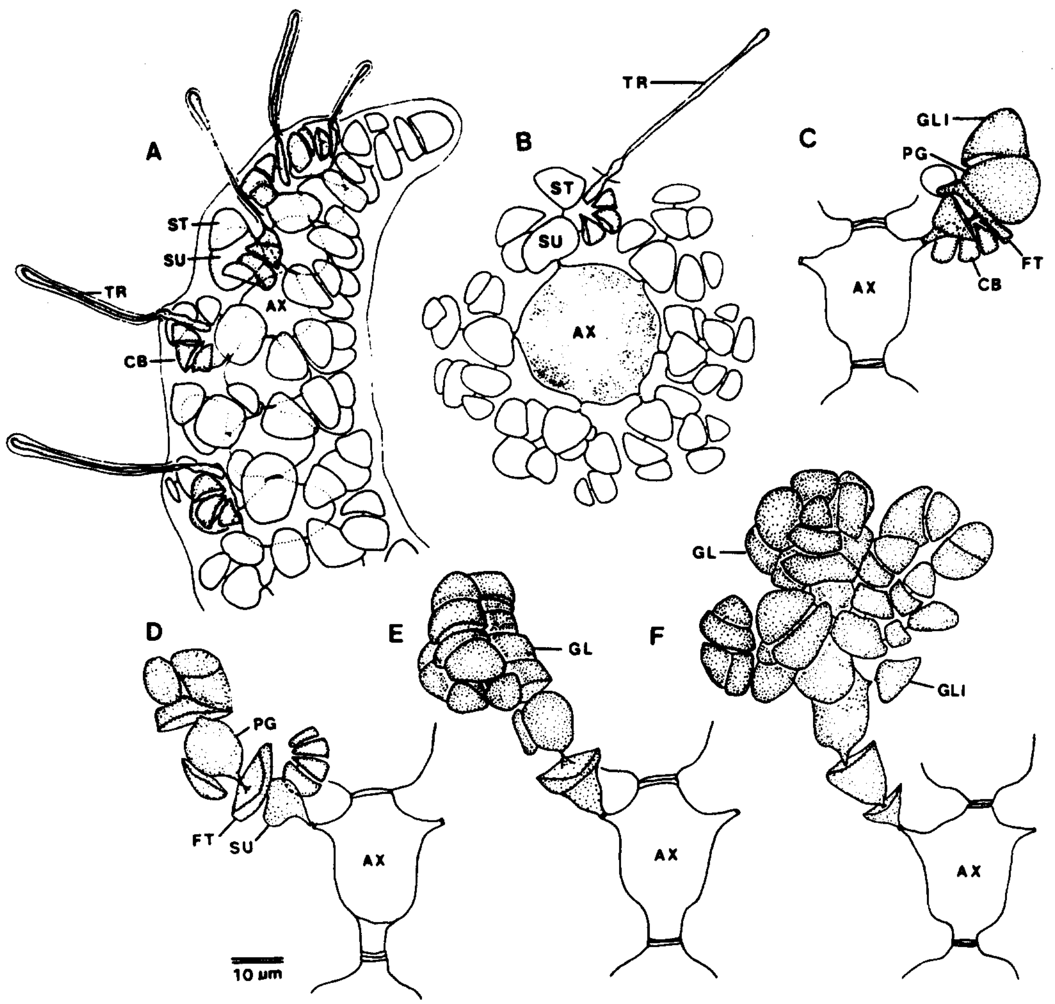

Fig. 7. Reproduction of *Ceramium kondoi* plants; postfertilization process. A: Female apex with carpogonial branches, B: Transverse view of node, C: Primary gonimoblast, D - F: Two to three gonimolobes (AX, axial cell; CB, carpogonial branch; FT, foot cell; GL, gonimolobe; GLI, gonimolobe initial; PG, primary gonimoblast; SU, supporting cell).

number is six to seven in each axial cell (Fig. 6). Four to five inner cortical cells are produced from each periaxial cell. The upper two inner cortical cells are acropetally divided two to three times, while the lower inner cortical cells basipetally in a same time. They are round to elongate. The outer cortical cells are cut off from inner cortical cells. They are rectangular to round and the number is several. Branches are pseudodichotomously produced from apical cell, which is obliquely divided. A cell in an adaxial side is first cut off and an abaxial cell later, each of which grow pseudodichotomous branch. Usually the adaxial branches grow faster than the abaxial ones, so branching form is alternate. Trichotomous branch is produced from an axial cell between pseudodichotomous branches. Tetrachotomous branch is also resulted in a same manner of the trichotomous branches. Adventitious branches are cut off from periaxial cells, but grow small.

Reproductive development: Tetrasporangia occur around the nodes and the internodes of the upper part of plants (Fig. 5). The first formed periaxial cell laterally cuts off a tetrasporangium,

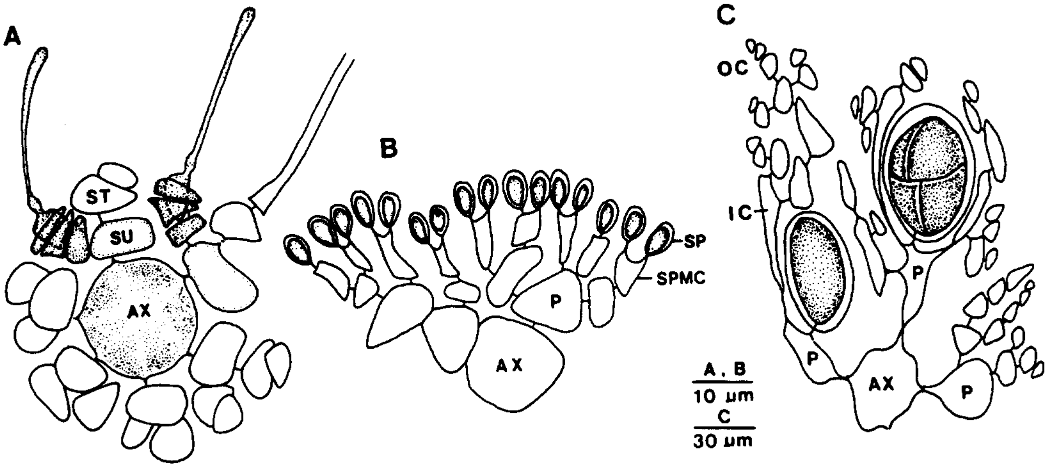

Fig. 8. Reproduction of *Ceramium kondoi* plants. A: Two carpogonial branches on a single axial cell, B: Spermatangial development and C: Tetrasporangial development.

which is formed from the second formed periaxial cell, while tetrasporangia are also produced from the inner cortical cells of different orders (Fig. 8). So, the tetrasporangial arrangement is first whorled around in a single transverse row and later dispersed to the internodes. Each tetrasporangium is covered by the inner to the outer cortical cells, and is immersed within the cortex. The mean size is about 55 x 73 μm and the division is irregularly cruciate.

Spermatangia become sessile patches covering the whole axis of the upper portion of plants (Fig. 5). They are first produced from the cortical cells in the adaxial side of the upper thallus and later in all over the axis. One to two spermatangia are formed from each spermatangial mother cell (Fig. 8). They are colourless and elliptical to spherical and measured about 3 μm x 6 μm.

Carpogonial branches occur in the abaxial side of the upper portion of plants (Fig. 5). The first-formed periaxial cell in each node produces a single carpogonial branch with one to two vegetative cell groups and turns a supporting cell. Carpogonial branch is four-celled with a carpogonium and a long trichogyne, and remain attached on the supporting cell after fertilization. When a spermatangium enter a trichogyne, fertilization is completed and the basal supporting cell produces an auxiliary cell in the upper side. The auxiliary cell is divided into the upper gonimoblast initial and the lower foot cell. The gonimoblast initial is repeatedly divided into two to three gonimolobes. Gonimolobes become a round cystocarp, measuring a mean size of about 285 x 326 μm. Each cystocarp is surrounded by five to seven involucral branchlets, which are produced from the periaxial cells below the axial cell bearing gonimolobes (Figs 7, 8).

Life history and morphology in culture: Three isolates of *Ceramium kondoi* in culture showed a *Polysiphonia*-type life history and no irregularities. Tetraspores bipolarly germinated after attachment on substratum. Sporelings were transversly divided by the four-celled stage while later transversly and longitudinally. Spermatangia were often produced on about fifteen-day old young plants. They at first occurred on the adaxial side of the upper branches and later whorled. Carpogonial branches were observed on the abaxial side of the upper branches in female plants of about one month culture. A single carpogonial branch was produced on each node, but sometimes two on a single node. Morphological features like nodal swelling and the initials of involucral branches showing fertilization were shortly observed. After 45 days mature cystocarps began to release carpospores. They germinated similarly to tetraspores and grew into tetrasporic plants within about one month. Regular life history cycle has been repeated until now.

Morphological features in culture also showed variation within the range obtained in the field for

Table 2. Attempted crosses between *Ceramium kondoi* and related species in culture

Male / Female	*C. kondoi* (Oeyondo)	*C. kondoi* (Wido)	*C. nodulosum* (St. Malo)	*C. fruticulosum* (St. Malo)
C. kondoi (Oeyondo)	+	+	–	NA
C. kondoi (Wido)	+	+	–	NA
C. nodulosum (St. Malo)	–	–	+	NA
C. fruticulosum (St. Malo)	–	–	–	+

NA: Not attempted

C. kondoi. Cortical cells were well developed from young stage. They were small, roundish and loosely arranged. In the lower part of mature plants they became elongate and much loosely arranged, but cortication was always continuous in every culture. Cortical cells were acropetally and basipetally produced from periaxial cells in each node, so acropetal cells from the lower axial cell met basipetal ones from the upper axial cell in the center of each axial cell, where the area was constricted and looked like cortical node (Fig. 5). Branching pattern seemed similar to field materials, but trichotomous or tetrachotomous ramification was never produced in our laboratory.

Crossability tests: The culture strains used for the crossability tests were the strictly unisexual gametophytes of the normal plants. All the females in crosses between strains of Oeyondo and Wido Island produced many cystocarps after about 15 days and these released carpospores. The developmental pattern of the offsprings was similar to that carpospores of within-strain crosses. A typical Ceramiacean life history was cycled and no irregularities were shown. European *Ceramium nodulosum* and *C. fruticulosum* plants from St. Malo, France were grown through their life cycles in laboratory. The two French species didn't exhibit interfertility in interspecific crosses and didn't hybridized with cultured strains of Korean *C. kondoi*.

DISCUSSION

According to Dixon (1960), the taxonomic chaos of the genus *Ceramium* is the result of the failure to refer back to original authentic materials and the failure to recognize and interpret seasonal and environmental modifications of the features. In order to overcome the taxonomic difficulty, detailed studies of type materials as well as of spatial and temporal variations are required for the *Ceramium* species.

There have been few reports on reproductive phenology of the *Ceramium* species. In *Campylaephora crassa*, a related species of *Ceramium kondoi*, reproductive organs occurred throughout the year (Boo *et al.*, 1991), but *C. kondoi* plants showed an obvious seasonality in reproduction and growth. The plant habits seems to be variable during this study. We observed Nakamura's separation in Oeyondo specimens, but it was difficult that most plants were identified under forms of Nakamura (1950, 1954). This problem has been studying in our laboratory.

Although *C. kondoi* is very closely related to *C. nodulosum*, it is characterized by trichotomous ramification and thick cortex. With detailed observations of Japanese *C. kondoi* plants, Nakamura (1950) reported that two European specimens, filed under the name *C. rubrum* in SAP, were rather considered as *C. kondoi* because of having trichotomous branches while that *C. kondoi* was probably merged into *C. rubrum* because of *C. kondoi* f. *ambiguum* being thin in cortex and rare in trichotomous branches. He (1965) proposed that trichotomous ramification was one of the most distinguishing features of *C. kondoi*. In this study, trichotomy occurred in a frequency of 20-30 % in every collection. Our plants agree with Canadian *C. rubrum* plants (Garbary *et al.*, 1980), in that sexual plants produced a large number of trichotomies than asexual ones. This results support taxonomic validity of the feature in the *C. kondoi* species in the field. However, further culture studies would be valuable because they occurred in the previous culture (Suh and Lee, 1984) and didn't in our culture.

Based on Figure 2 of Garbary *et al.* (1980), *C. kondoi* plants very resemble Canadian *C. rubrum* plants in trichotomous ramification. Judging from few trichotomous branches in European (Feldmann-Mazoyer, 1940) and Australian *C. rubrum* (Womersley, 1978), there is an urgent need of comparative morphology, hybridization and other experimental approaches for the correct conceptions of the *C. kondoi* and Canadian *C. rubrum* plants.

Tetrasporangial arrangement is one of the taxonomic features used in separating forms by Nakamura (1950, 1965). Tetrasporangia in *C. kondoi* as well as the fully corticated *Ceramium* species originate from the inner cortical cells as well as periaxial cells. When the inner cortical cell grow elongate or is divided in two to three orders, tetrasporangia become scattered on all over the axis. The division of tetrasporangia appears cruciate.

The female reproductive development agrees well with the previous observation (Nakamura, 1954) and the other *Ceramium* species (Hommersand, 1963). A single carpogonial branch is usually produced in each node, as is the case of most *Ceramium* species (Boo, 1993). Two carpogonial branches on two different periaxial cells, however, are observed on a single axial cell in culture strains of *C. kondoi*, that is a rare case in the genus *Ceramium*. Its taxonomic validity requires further observations on other regional plants. The spermatangial development also shows no differences with the previous observations (Nakamura, 1954, 1965)

Culture isolates of *C. kondoi* from Oeyondo and Wido on the Yellow Sea showed a *Polysiphonia*-type life history, similar morphologies and interfertilty. *C. nodulosum* and *C. fruticulosum* were grown through their life cycles in culture. Interspecific hybridization were negative and neither were interfertile with *C. kondoi*. This result also agrees with morphological differences of the species (Boo, unpublished data). *C. kondoi* has the more narrow biogeographic distribution than *C. nodulosum*, which has been known as a cosmopolitan species with Australian (Womsersley, 1978) and North Atlantic distribution (Wilce, 1990). In Korean and the surrounding waters, *C. kondoi* occurs, whereas *C. nodulosum* on most coasts of the world, so *C. kondoi* seems a vicariant species to *C. nodulosum* in the Northeast Pacific.

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