Korean Journal of Botany
Vol. 27, No. 3, 163~171(1984)

Morphology and Reproduction of Some Species of *Ceramium* (Rhodophyta) in Culture

Suh, Youngbae and In Kyu Lee
(Department of Botany, Seoul National University, Seoul)

紅藻 비단풀屬 植物의 室內培養에 따른 形態變化와 生殖에 關하여

徐 榮 倍·李 仁 圭
(서울大學校 自然科學大學 植物學科)

ABSTRACT

Ceramium kondoi Yendo demonstrates a *Polysiphonia* type of life history without deviation in unialgal culture. However, cultures of *C. paniculatum* Okamura and *C. aduncum* Nakamura from Kangneung shows considerable phenotypic variations in laboratory. In *C. paniculatum*, the subulate spines disappear, the transverse cell-rows of corticating bands decrease in number, and the growing direction of corticating cells is changed during the culture. In *C. aduncum*, a species new to Korea, frond apices become slightly incurved in contrast to strongly rolled ones in the field, and the rows of corticating cells decrease in number and height during the culture. These results suggest that some significant taxonomic characters currently used for identification of *Ceramium* species are reconsidered.

INTRODUCTION

Since the genus *Ceramium* was first recognized in Europe, over a hundred species and variations have been described widely in the world (Kylin, 1925, 1956; Feldmann-Mazoyer, 1940; Hommersand, 1963; Itono, 1977). They are distinguished mainly by the morphological characters, especially such as the cortication of thallus, curvature of branch apices, occurrence of gland cells, shape of spines, and so on, of which validity is discussed by Nakamura (1954) and Dixon (1960). Especially, Dixon mentioned the taxonomy of *Ceramium* was chaotic due to a failure of referring to the original and authentic materials as well as to recognize the seasonal and environmental modification of the thallus.

Several species of *Ceramium* have been observed in culture and a *Polysiphonia*-type life history, a typical pattern of the order, has been demonstrated (Edwards, 1973; Rueness, 1973). Edwards (1973) has observed that in the northern part of the distribution range of *Ceramium suttleworthianum* (Kutz.) Silva, *C. rubrum* (Huds.) Ag. and *C. pedicellatum*

DC., the gametophytes are not fertile probably due to unfavorable environmental conditions. In addition to a normal life history, some plants of *C. strictum* Harv. in culture produce parasporangia that have the same nuclear phase as parent plants (Rueness, 1973). The parasporangia, which superficially resemble to gonimoblast but lack subtending adventitious lateral branches, give rise to new generations of parasporangium-bearing plants. On the other hand, according to laboratory culture, Garbary *et al.* (1978) suggest that *C. rubrum* and *C. rubriforme* Kylin cast doubt on the validity of the structure and development of cortical bands as taxonomic characters. Notoya and Yabu (1979) have carried out culture and cytological investigations of *C. kondoi* Yendo and *C. japonicum* Okamura. The chromosome number counted in tetraspore germlings and spermatia shows n=ca. 30 in *C. japonicum*, whereas n=12~15 in *C. kondoi*.

The present study was carried out with three species, *Ceramium kondoi* Yendo, *C. paniculatum* Okamura and *C. aduncum* Nakamura, in order to clarify the growth of thalli and the development of reproductive structures in laboratory culture, comparing with those in natural habitats.

MATERIALS AND METHODS

The materials were collected and isolated into laboratory culture as follows:

C. kondoi; tetrasporophytes epilithic in lower littoral zone near Sokcho City, eastern coast of Korea, 26 September, 1979 (Fig. 1).

C. paniculatum; (1) tetrasporophytes and carposporophytes epilithic in lower littoral zone of Anin near Kangneung City, 29 July, 1980; (2) tetrasporophytes at the same site, 9 September, 1980 (Fig. 1).

C. aduncum; tetrasporophytes epilithic in lower littoral zone of Anin, 9 September, 1980 (Fig. 1).

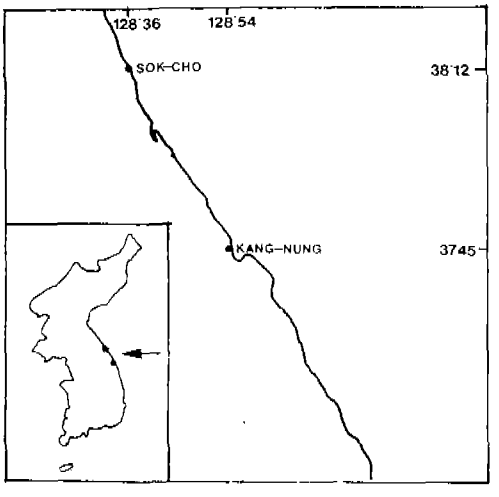


Fig. 1. A map showing the sampling sites.

The materials collected from the rock surface were put in the white plastic bottle with sea water and kept cool in the ice box during transport. They were placed in Petri dishes containing 1/2 strength PES medium under cool white fluorescence light below 300 lux (Lee and West, 1980) and left overnight. The released spores and the branch tips of vegetative thallus were isolated into unialgal culture. Spores were removed onto coverslips and their development was detected in every 1~3 days. Both the germlings and the excised vegetative apices were maintained

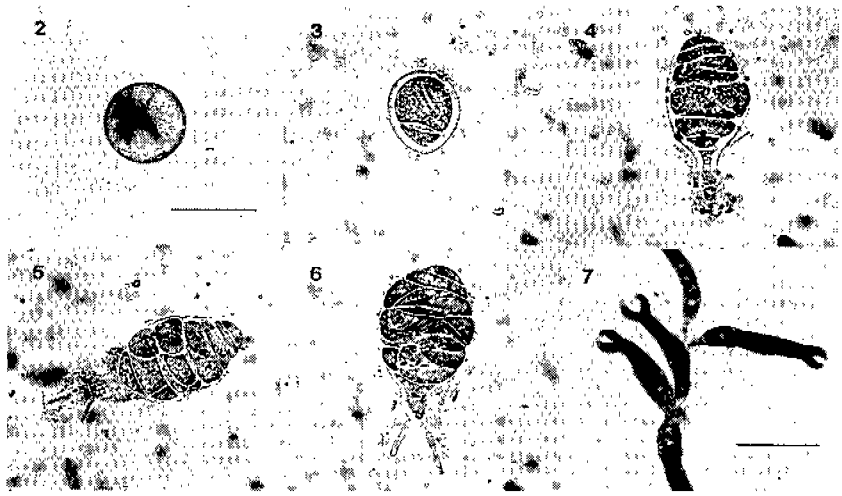
in the covered dishes (70×70 mm) containing about 150 ml PES medium (McLachlan, 1973). The medium was changed in every 10~14 days. In order to eliminate diatoms GeO_2 solution (6 ml/l) was added to the culture medium for a while (Lewin, 1966). Cultures were maintained at 16~19°C, under the cool white fluorescence illumination of 800~1,500 lux with 16 : $\overline{8}$ LD photoperiod.

RESULTS

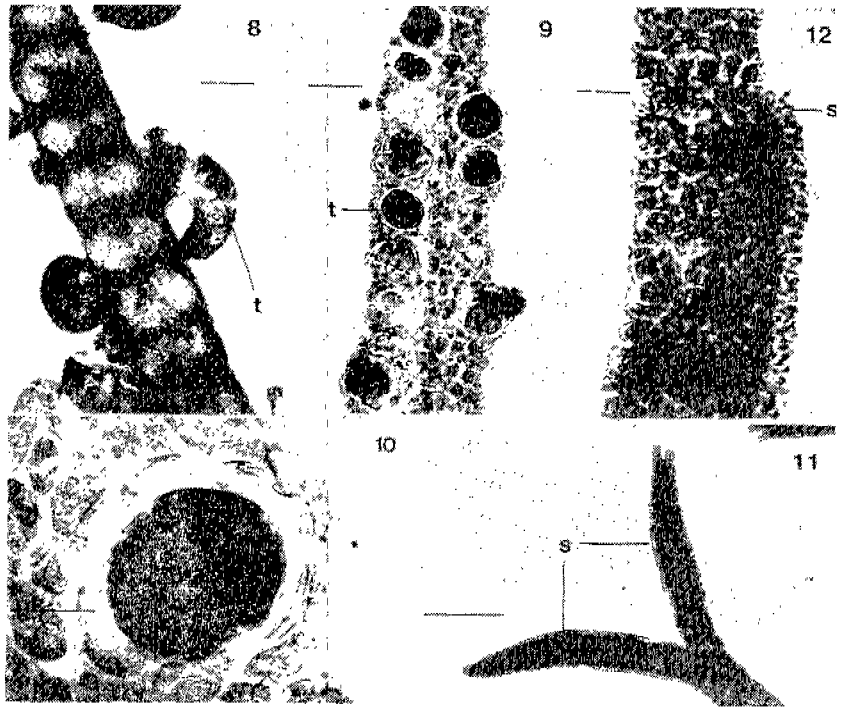
Ceramiun kondoi. From released tetraspores of field-collected plants, fertile gametophytes were obtained; male gametophytes produced spermatangia after 2~4 weeks and the female plants produced procarps after 3~4 weeks. Often spermatangia appeared on the dwarf thalli (500 μm long). Cystocarps released the carpospores in 3 weeks after fertilization. Carpospores germinated to form the mature tetrasporophytes that produced tetrasporangia in 4 weeks.

Thus, *C. kondoi* from Sokcho shows a typical *Polysiphonia*-type life history with isomorphic generations of tetrasporic and gametophytic phases. Both tetraspore and carpospore are uniform in diameter, 40~50 μm (Fig. 2), each germinating to form an erect axis and filamentous rhizoidal base (Figs. 3-7). This species in culture shows vegetative characters basically similar to those from the field (Nakamura, 1965). The main branch divides usually dichotomously or trichotomously, and tips of thalli are forcipated. The axes become completely corticated by the corticating cells.

Tetrasporangia immersed in the cortex of axial branches and somewhat scattered in the middle portion of the frond, divided cruciately (Figs. 9 and 10). In field-collected materials,



Figs. 2~7. The carpospore germination of *Ceramium kondoi* Yendo.
2. Carpospore. 3~6. Carpospore germination. 7. Ten days old carpospore germlings (Scale: 2~6; 50μm, 7; 500μm).

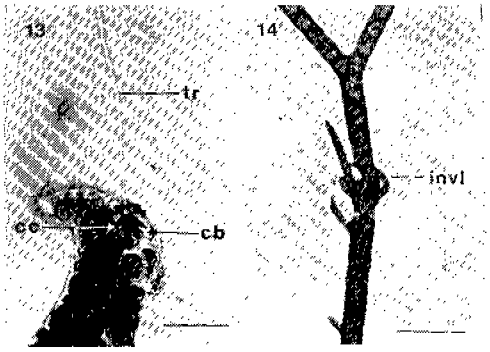


Figs. 8~12. Terasporangia and spermatangia of *Ceramium kondoi* Yendo.
8. Development of tetrasporangia in the field. 9~10. The same in culture. 11~12. Development of spermatangia in culture (s: spermatangium, t: tetrasporangium. Scale: 8; 250 μm, 9; 50 μm, 10, 12; 25 μm, 11; 500 μm).

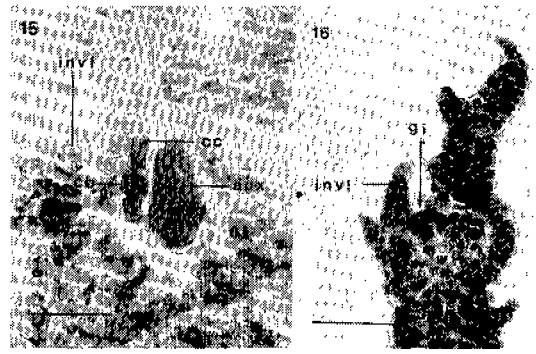
however, tetrasporangia commonly are produced in lateral ramuli, provided with thick pericarp (Fig. 8). Spermatangia are usually produced from the outermost cortical cells on upper portions of branches, forming sessile patches. They develop primarily on adaxial side of branches, spreading afterward over the branches entirely (Figs. 11 and 12). Procarps are common in the upper to apical portions of the thallus on abaxial side. The pericentral cell becomes of the supporting cell developing a four-celled single carpogonial branch (Fig. 13).

After fertilization, the supporting cell enlarges, and cuts off an auxiliary cell. The carpogonium cuts off the trichogyne and produces laterally a connecting cell that fuses to the auxiliary cell (Figs. 13 and 16). The auxiliary cell divides into the foot cell, central cell and gonimoblast initial. At the same time, involucral ramuli develop (Fig. 17). A few secondary gonimoblasts are developed commonly from the gonimoblast initial. Almost all the gonimoblast cells are converted into carposporangia. A mature cystocarp is spherical, 750~900 μm in diameter, and provided with the pericarp and 4~6 involucral ramuli. It develops on the branchlets in the apical portions or on the laterals of the main axis (Fig. 14).

Ceramium paniculatum. In field-collected materials, the thallus is coated with the

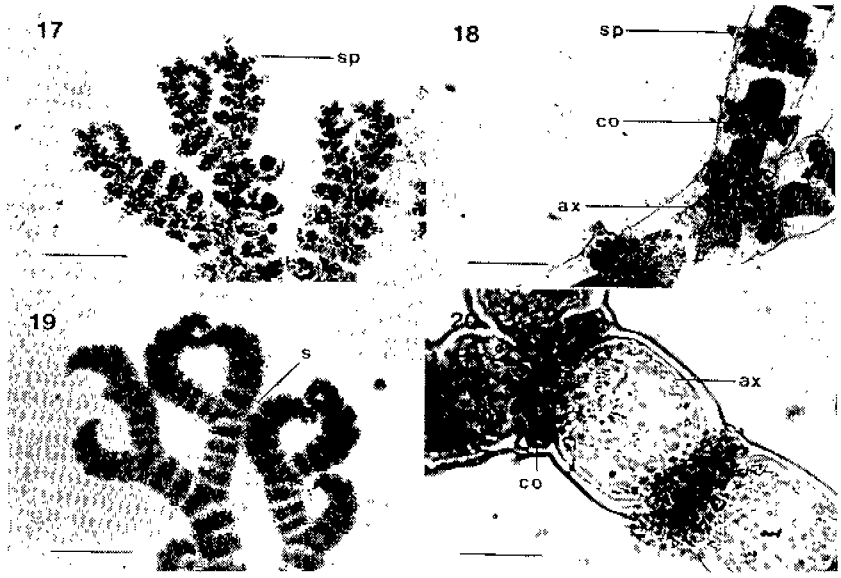


Figs. 13~14. The development of cystocarps in *Ceramium kondoi* Yendo.
13. Development of the procarp.
14. Development of cystocarp, involucres being developed (cb: carpogonial branch, cc: connecting cell, cy: cystocarp, invl: involucral process, p: pericarp, tr: trichogyne. Scale: 13; 50 μm, 14; 900 μm).



Figs. 15~16. Development of female reproductive structure in *Ceramium kondoi* Yendo, stained with cotton blue (aux: auxiliary cell, cb: carpogonial branch, cc: connecting cell, gi: gonimoblast initial, invl: involucral process. Scale: 15; 50 μm, 16; 500 μm).

colored cells forming a distinct band. Each corticating band consists of 5~6 transverse rows of cells and 80~110 μm high. The cortical cells in the upper and the lower borders are smaller than those in the middle portion of the band. This means that the cortical cell



Figs. 17~20. Tetrasporangia and spermatangia of *Ceramium paniculatum* Okamura.
17~18. Development of tetrasporangia in the field, stained with cotton blue. 19~20. Development of spermatangia in culture (ax: axial cell, co: corticating band, s: spermatangium, sp: spine, t: tetrasporangium. Scale: 17, 19: 500 μm, 18; 250 μm, 20: 50 μm).

filaments eventually grow both acropetally and basipetally (Itono, 1977). Besides, this plant is armed with 3~4 celled subulate spines longitudinally seriated on the abaxial side of each node in upper branches (Figs. 18 and 19). Tetrasporangia are seriated in a longitudinal row on the adaxial side of branches, bracteated by the cortical cells slightly bulging out from the cortical layers (Fig. 18).

The attempted establishment of cultures from the carpospores was unsuccessful since no carpospores were released from the field cystocarps. On the other hand, in unialgal culture of tetrasporophytes, tetraspores are released and germinated. After one month the male gametophyte forms spermatangia which are sessile patches on each node of the upper branches. The spermatangia are produced primarily from the cortical cells of the adaxial side of branchlets and gradually over entire area of corticating bands (Figs. 20 and 21). However, the female plants produce no procarps until five month culture after germination of tetraspores.

Tetraspores germinate to form a filamentous rhizoidal base and the erect axis, as in *C. kondoi* (Figs. 4~6). They show dichotomous ramification, same as the field-collected materials, and possess the distinct cortical bands. However, in contrast to 5~6 transverse

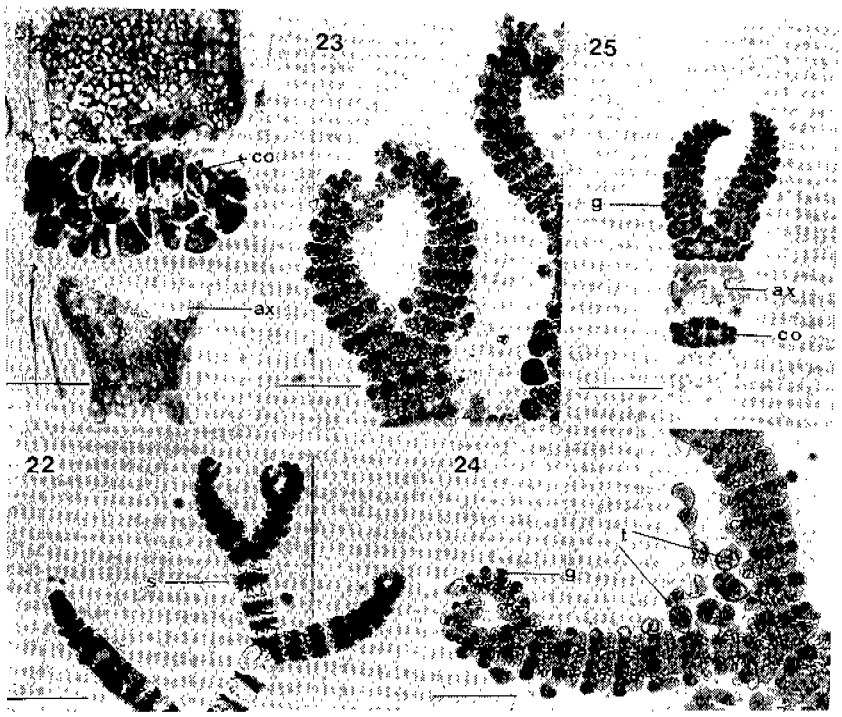


Fig. 21. Corticating band growing acropetally of *Ceramium paniculatum* Okamura in culture.
Fig. 22. Male gametophyte of *C. paniculatum*, without spines in culture.
Figs. 23~24. Tetrasporophyte of *Ceramium aduncum* Nakamura in the field, stained with cotton blue.
Fig. 25. Apex of *C. aduncum* in culture (ax: axial cell, co: corticating band, g: gland cell, s: spermatangium, t: tetrasporangium. Scale: 21~24; 500 μm, 25; 250 μm).

rows of cortical cells and 80~100 μm high corticating bands in the field-collected materials, the bands become of 2~4 rows and 20~50 μm high in laboratory culture. The cortical cells in the upper portion of the bands are smaller than those in the lower. Thus, the cortical cells seem to grow only acropetally in culture (Figs. 21 and 22). Especially, the subulate spines which are most commonly used as a diagnostic character for *C. paniculatum* disappear throughout the whole thallus during the culture (Fig. 23).

Ceramium aduncum. In field-collected materials the frond shows a smooth surface without spines or thumb-like processed on the node of branches. The cortication is interrupted, forming a zonate band at each node throughout the entire thallus. Frond-apices are strongly rolled inwards and the outer edge of the apex is dentate (Fig. 24). Corticating bands are 100~120 μm high with 7~9 transverse rows, which are characteristic of this species. Tetrasporangia are seriated in one longitudinal row on adaxial side of branches, and divided tetrahedrally (Fig. 25). This plant is recorded for the first time in Korea.

The attempted establishment of cultures from the tetraspores has failed upto now since no spores are released from the sporangia in the field materials. In experimental culture of the excised apices, the frond apex varies from strongly rolled inwards to slightly curved or almost straight ones, and the corticating bands become of 3~4 transverse rows of cells and 50~80 μm high in contrast to 7~9 rows and 100~120 μm high in the field (Fig. 26).

DISCUSSION

C. kondoi grows very well in the laboratory culture and shows vegetative characters basically same as those plants described from the natural habitats (Nakamura, 1965). This species has repeated the normal life histories four times during the culture. Repeating the life cycles, the dense cortication of whole thalli has become sparse so that carpogonial branches immersed in cortical cells can be seen directly out of the cortical cells. According to Okamura (1936), tetrasporangia are produced on the lateral fertile branchlets, or immersed in the cortex in his field-collected materials. However, in the field-collected materials for this culture, tetrasporangia were produced on the lateral fertile branchlets (Fig. 8). In repeated culture, all plants have produced tetrasporangia to be immersed in the cortex (Fig. 9).

In culture of *C. paniculatum* and *C. aduncum*, a considerable variation of the corticating bands occurs (Table 1). The most commonly adopted character for diagnosis of the *Ceramium* species is the morphology of cortical bands (Nakamura, 1954; Dixon, 1960). These may be confluent throughout the thallus, or in only a part, or in definite bands. In addition, characters related to the size and the shape of cortical cells, whether they are formed acropetally or basipetally (or both) from the node, the number of cortical cell layers, and the presence or absence of hairs and gland cells as well as size and position of sporangia are also considered.

Table 1. A variation of taxonomically significant characters in culture of *C. paniculatum* and *C. aduncum*

Character \ Species / Condition	*C. paniculatum*		*C. aduncum*	
	Field-collected	Cultured	Field-collected	Cultured
Subulate spine	Present	Absent	Absent	Absent
Height of cortical bands	80~110μm	20~50μm	100~120μm	50~80μm
Cortical cell layers	5~6	2~4	7~9	3~4
Direction of cortical cell growth	Basi-, and acropetal	Acropetal	Basi-, and acropetal	Basi-, and acropetal
Frond apex	—	—	Strongly rolled incurved	Slightly rolled inwards
Number of gland cells	Absent	Absent	Abundant	Decreased

Garbary *et al.* (1978) reported that during the culture of *C. rubrum* and *C. rubriforme* the structure and development of cortical bands were variable according to various conditions of light and temperature. Rueness (1978) reported that the delimitation of morphologically defined species did not coincide with the interbreeding unit in culture and artificial hybridization between *C. tenuicorne* and *C. strictum.*

All of these characters, however, have been used in the species typification for systematic treatments of the genus *Ceramium.* According to the present study, however, some of these characters is doubtful to adopt them as criteria for distinguishment of the species. Thus, the current criteria of these taxa must be reconsidered in this point of view.

摘 要

실내 배양을 통하여 *Ceramium kondoi* Yendo는 전형적인 *Polysiphonia*형 생활사를 거치는 것으로 밝혀졌고, *C. paniculatum* Okamura와 *C. aduncum* Nakamura는 피층 세포열에서 심한 형태적 변화가 나타났다. *C. paniculatum*의 가지 바깥쪽에 존재하는 가시는 없어졌으며 피층의 가로 세포열 수가 줄어들었고, 피층세포의 생장 방향도 변하였다. *C. aduncum*은 안쪽으로 심하게 굽은 정단부가 완만하게 되었으며, 피층의 가로 세포열의 수가 역시 감소되었다. 이 결과는 *Ceramium*속의 종을 구분짓는 중요한 현행 식별 형질이 불확실한 것을 암시하는 사실로서, 이에 대한 새로운 검토가 불가피함을 입증하고 있다.

REFERENCES

Dixon, P. S. 1960. Studies on the marine algae of the British Isles: the genus *Ceramium. J. mar. biol. Ass.* U.K. **39** : 331~374.

Edwards, P. 1973. Life history studies of selected British *Ceramium* species. *J. Phycol.* **9** : 181~184.

Feldmann-Mazoyer, G. 1940. Recherches sur les Céramiacées de la Méditerranée occidentale. Algiers. 510 pp. +erreta (4), 191 figs., 4 pls.

Garbary, D. J., D. Grund, and J. McLachlan. 1978. The taxonomic status of *Ceramium rubrum* (Huds.) C. Ag. (Ceramiales, Rhodophyceae) based on culture experiments. *Phycologia* 17 : 85~94.

Hommersand, M. H. 1963. The morphology and classification of some Ceramiaceae and Rhodomelaceae. *Univ. Calif. Publ., Botany* 35 : 165~366.

Itono, H. 1977. Studies on the Ceramiaceous algae (Rhodophyta) from southern parts of Japan. *Bibl. Phycol.* 35 : 1~499.

Kylin, H. 1925. The marine red algae in the vicinity of the biological station at Friday Harbor, Wash. *Lunds Univ. Arsskr., N.F.*, Adv. 2, 21(9)., 87 pp., 47 figs.

Kylin, H. 1956. Die Gattungen der Rhodophyceen. Gleerup, Lund. xv +673 pp., 458 figs.

Lee, I. K. and J. A. West. 1980. *Antithamnion nipponicum* Yamada et Inagaki (Rhodophyta, Ceramiales) in culture. *Jap. J. Phycol.* (Sōrui), 28 : 19~27.

Lewin, J. 1966. Silicon metabolism in diatom V. Germanium dioxide, a specific inhibitor of diatom growth. *Phycologia* 6 : 1~12.

McLachlan, J. 1973. Growth media-marine. *In* Handbook of Phycological Methods. Culture methods and growth measuments. J. R. Stein (ed.), pp. 25~57. Cambridge Univ. Press, London.

Nakamura, Y. 1954. The structure and reproduction of the genus *Ceramium* and *Campylaephora* in Japan, with special reference to criteria of classification. *Sci. Pap. Inst. Alg. Res., Fac. Sci. Hokkaido Univ.* 4 : 15~62.

Nakamura, Y. 1965. Species of the genera *Ceramium* and *Campylaephora*, especially those of northern Japan. *Ibid.* 5 : 119~180.

Notoya, M. and H. Yabu. 1979. Culture and cytology of *Ceramium japonicum* Okamura and *C. kondoi* Yendo (Ceramiales, Rhodophyta). *Bull. Fac. Fish. Hokkaido Univ.* 30 : 129~132.

Okamura, K. 1936. Nippon Kaisoshi, Tokyo. 964 pp.,

Rueness, J. 1973. Culture and field observations on growth and reproduction of *Ceramium strictum* Harv. from the Oslofjord, Norway. *Norw. J. Bot.* 20 : 61~65.

Rueness, J. 1978. Hybridization in red algae. *In* Modern Approaches to the Taxonomy of Red and Brown Algae. D. E. G. Irvine and J. H. Price (ed.), pp. 247~262. Academic Press, London.

(Received August 24, 1984)