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# *Antithamnion nipponicum* YAMADA et INAGAKI (Rhodophyta, Ceramiales) in culture

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*Antithamnion nipponicum* YAMADA et INAGAKI from Cheju Island, Korea was investigated in culture, comparing its growth and reproduction with *A. defectum* KYLIN and *A. kylinii* GARDNER from Pacific North America. *A. nipponicum* showed a typical *Polysiphonia*-type life history and exhibited neither mixed phase reproduction nor apomixis. In culture it developed hairs not observed in the field material, but produced no gland cells. Procarps occurred successively in pairs on upper segments of main axes and laterals. After fertilization a connecting cell was formed between the carpogonium and auxiliary cell. A *Polysiphonia*-type life history was usually exhibited by *A. defectum* isolates although tetrasporophytes lost the ability to form tetrasporangia after three years in culture and gametophytes showed mixed reproduction. *A. kylinii* initially produced tetrasporangia for one year and remained vegetative afterward (14 years). Hybridization attempts between *A. nipponicum* and *A. defectum* were negative.

*Key Index Words*: Antithamnion defectum, A. nipponicum, A. kylinii, *Ceramiales, hybridization, life history, Rhodophyta.*

Several laboratory culture studies on *Antithamnion* have been carried out (SUNDENE 1959, 1962, 1964, 1975, WEST & NORRIS 1966, RUENESS & RUENESS 1973, 1975). Their basic life histories were considered to be a *Polysiphonia*-type (SUNDENE 1959, 1964, RUENESS & RUENESS 1973). However, some species such as *A. boreale* (GOBI) KJELLMAN from the Northwestern Atlantic reproduced solely by apomeiotic tetrasporangia (SUNDENE 1962) and some others, *A. defectum* KYLIN (including *A. pygamaeum*) from Pacific North America (WEST & NORRIS 1966) and *A. tenuissimum* (HAUCK) SCHIFFNER from Northeastern Atlantic (SUNDENE 1964, RUENESS & RUENESS 1973) showed varied mixed phase reproduction in which tetrasporangia were borne on male and female plants or all three reproductive structures were present on one plant.

Even though about twenty species of *Antithamnion* are recorded from the Northwestern Pacific (ITONO 1969, 1961, 1977), no data on their life histories in culture are available at present, except some early development of the spores (TANIGUCHI 1972). In this study we describe the life history and development of reproductive structures in cultured isolates of *A. nipponicum* YAMADA et INAGAKI from Korea, comparing it with some laboratory cultures of *A. defectum* and *A. kylinii* GARDNER from Pacific North America.

*Antithamnion nipponicum* ranges from Saghalien to Korea (TOKIDA 1954, KANG 1966). It was first recorded as *Acrothamnion pulchellum* J. AGARDH by YENDO (1916) and YAMADA (1928) from Japan, and transferred later to *Antithamnion* as a new species by YAMADA and INAGAKI (1935). Both tetra-

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sporic and cystocarpic thalli are common in the field.

## Materials and Methods

Both female and tetrasporic plants of *Antithamnion nipponicum* (JAW #1865, #1852) were collected from Seongsan-po, Cheju Island, Korea (127°E, 33°N), 5 July 1978 in shady rock recesses of the upper subtidal zone. Surface water temperature at the time of collection was 20°C. The plants were placed in sea water, transferred to Seoul National University in a cooler, and held at 22–25°C for 7 days before air shipment to Berkeley, California. Unialgal cultures were obtained from excised vegetative apices at Berkeley, in 1/2 strength PES medium (McLACHLAN 1973) under 15–17°C, 300–800 lux, 14 : $\bar{1}\bar{0}$ LD, using 6.5×5 cm Pyrex dishes. Media for the cultures were changed every 2–3 weeks.

The other species, *A. defectum* from San Juan Island, San Juan County, Washington, January 1965 (JAW #230, #240, #241) and from Larrabee State Park, Whatcom County, Washington, October 1965 (JAW #477), and *A. kylinii* from Shilshole Bay Marina, King County, Washington, December 1965 (JAW #453) and from Berkeley Yacht Harbor, Alameda County, California, July 1975 (JAW #1662) were cultured under the same conditions with full strength PES medium. Procedures for hybridization experiments were as described in POLANSHEK and WEST (1975).

## Results

*Culture Experiments*: From the excised vegetative apices of field collected *A. nipponicum*, female gametophytes (#1865) developed procarps after two and a half months, and tetrasporophytes (#1852) produced sporangia after two months. Released tetraspores formed gametophytes; male plants produced spermatangia after one and a half months and the female plants produced procarps after two months. Often spermatangia appeared on dwarf (650 μm long) thalli which had only a few determinate branches (Fig. 20) whereas procarps developed only on well grown (2–3 cm long) thalli. Cystocarps released carpospores one month after fertilization. Carpospores germinated to form mature tetrasporophytes that produced tetrasporangia in two months.

Thus, *A. nipponicum* from Cheju Island showed a typical *Polysiphonia*-type life history with isomorphic generations of tetrasporic and gametophytic phases. Both tetraspores and carpospores were uniform in diameter, 20–25 μm, each germinating to form a filamentous rhizoidal base and erect axis (Figs. 2–6, 17). They showed the same branching pattern as the field material (YAMADA & INAGAKI 1935). Neither mixed phase reproduction seen in the other species of *Antithamnion* (SUNDENE 1964, WEST & NORRIS 1966, RUENESSS & RUENESS 1973), nor apomeiosis (SUNDENE 1962), were observed in our cultures.

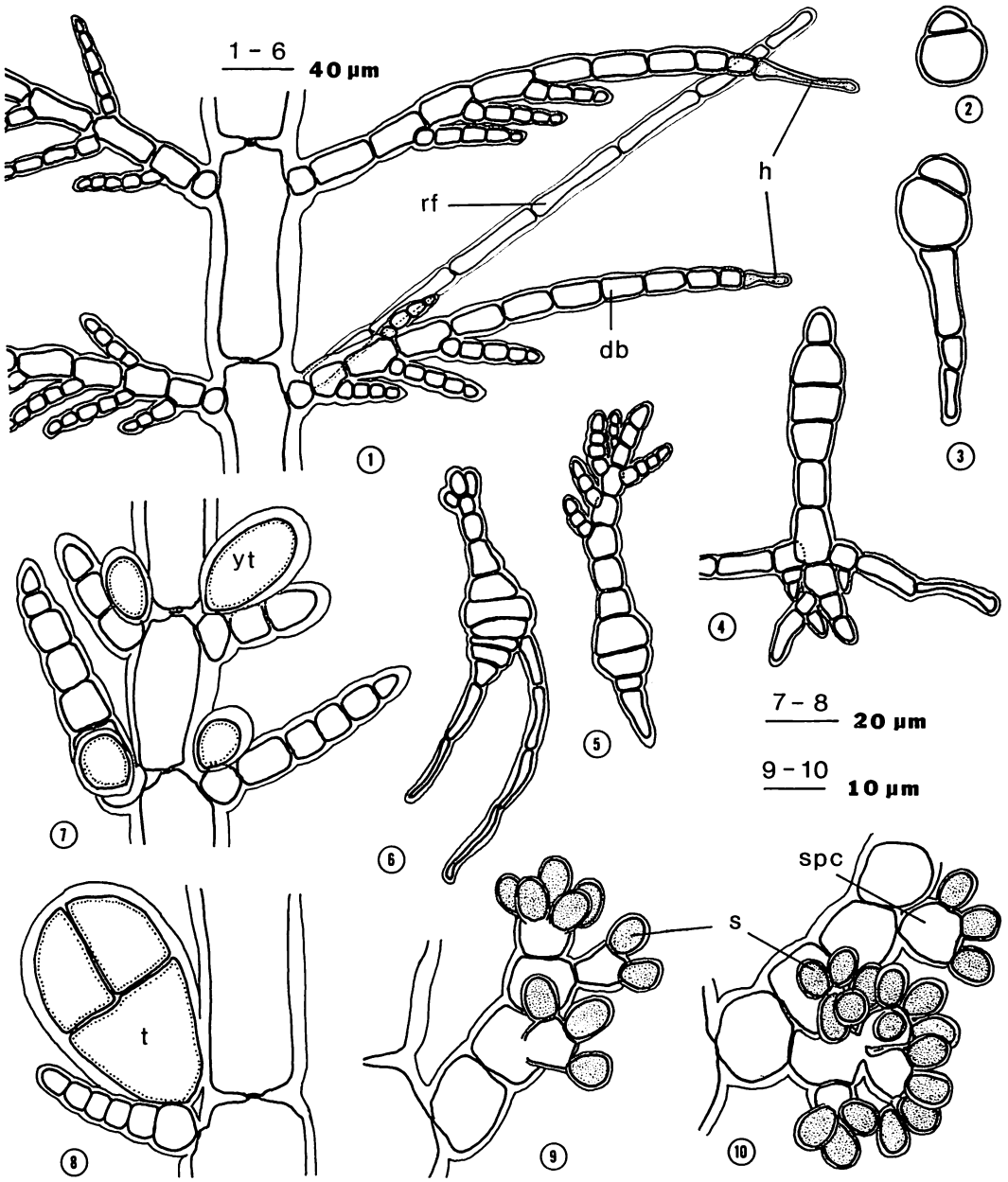
On the other hand, in *A. defectum* from San Juan Island, the original tetrasporophyte (#230) has produced no tetrasporangia in culture since 1965, and the original tetrasporophyte (#477) from Larrabee State Park produced viable tetraspores for three years until 1968, whereas the original female plant (#240) continued to produce procarps, and in May, 1979 one branch produced numerous spermatangia that released spermatia but showed no fertility with the procarps on the same plant. Moreover, the original male plant (#241) produced spermatangia continuously and also produced

Table 1. Intra- and interspecific crosses with *Antithamnion nipponicum* and *A. defectum*.

| Cross | Results |
|---|---|
| *A. nipponicum* 1852 ♂×1865 ♀ (control) | + |
| *A. nipponicum* 1852 ♂×*A. defectum* 240 ♀ | − |
| *A. nipponicum* 1865 ♀×*A. defectum* 241 ♂ | − |
| *A. nipponicum* 1852 ♀×*A. defectum* 241 ♂ | − |
| *A. defectum* 241 ♂×240 ♀ (control) | + |

procarps twice during culture. In one case (March 1966) procarps did not develop cystocarps, whereas in the second case (March 1969) they produced cystocarps and released carpospores, having been fertilized by spermatia from the same thallus.

*A. kylinii* from Shilshole Bay Marina, originally a tetrasporophyte (#453), produced

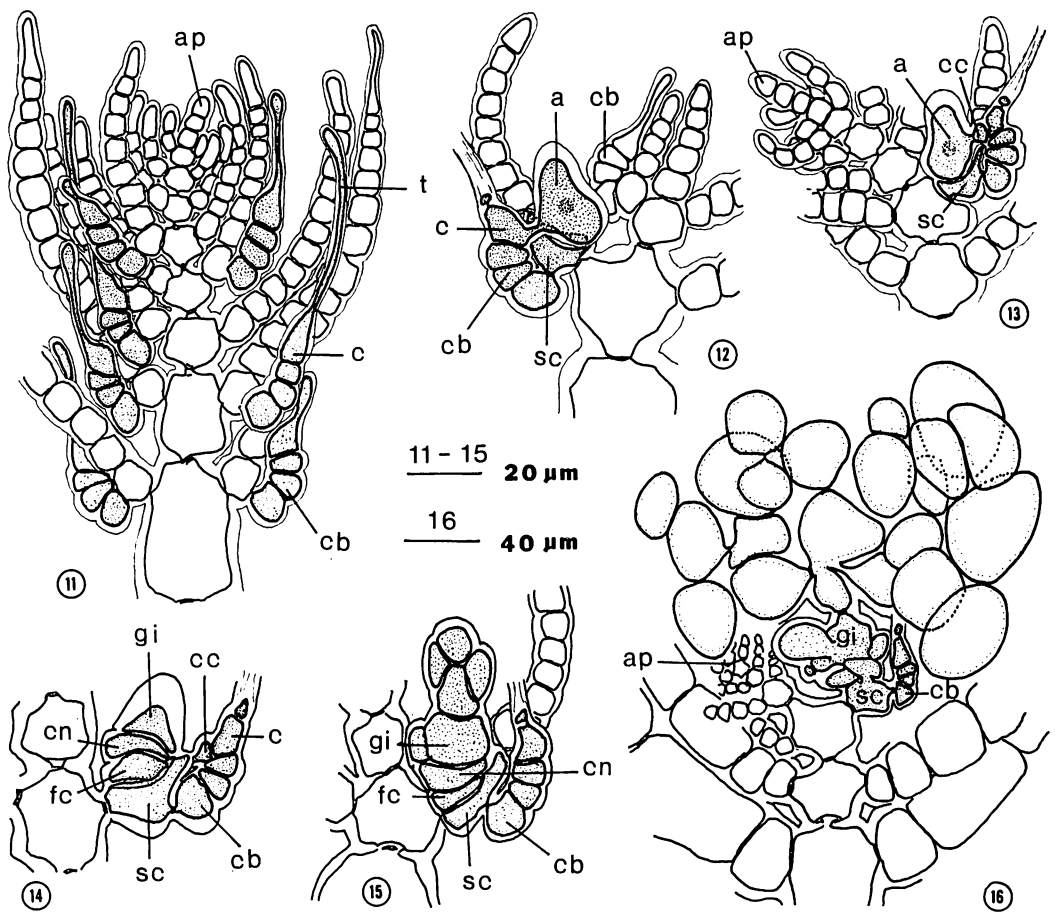



Figs. 1–10. Vegetative structure, tetrasporangia and spermatangia of *Antithamnion nipponicum* YAMADA et INAGAKI.

1. Part of vegetative thallus with hairs and rhizoidal filaments. 2–6. Tetraspore germination. 7–8. Development of tetrasporangia. 9–10. Development of spermatangia. (db: determinate branch, h: hair, rf: rhizoidal filament, s: spermatangium, spc: spermatangial parent cell, t: tetrasporangium, yt: young tetrasporangium).

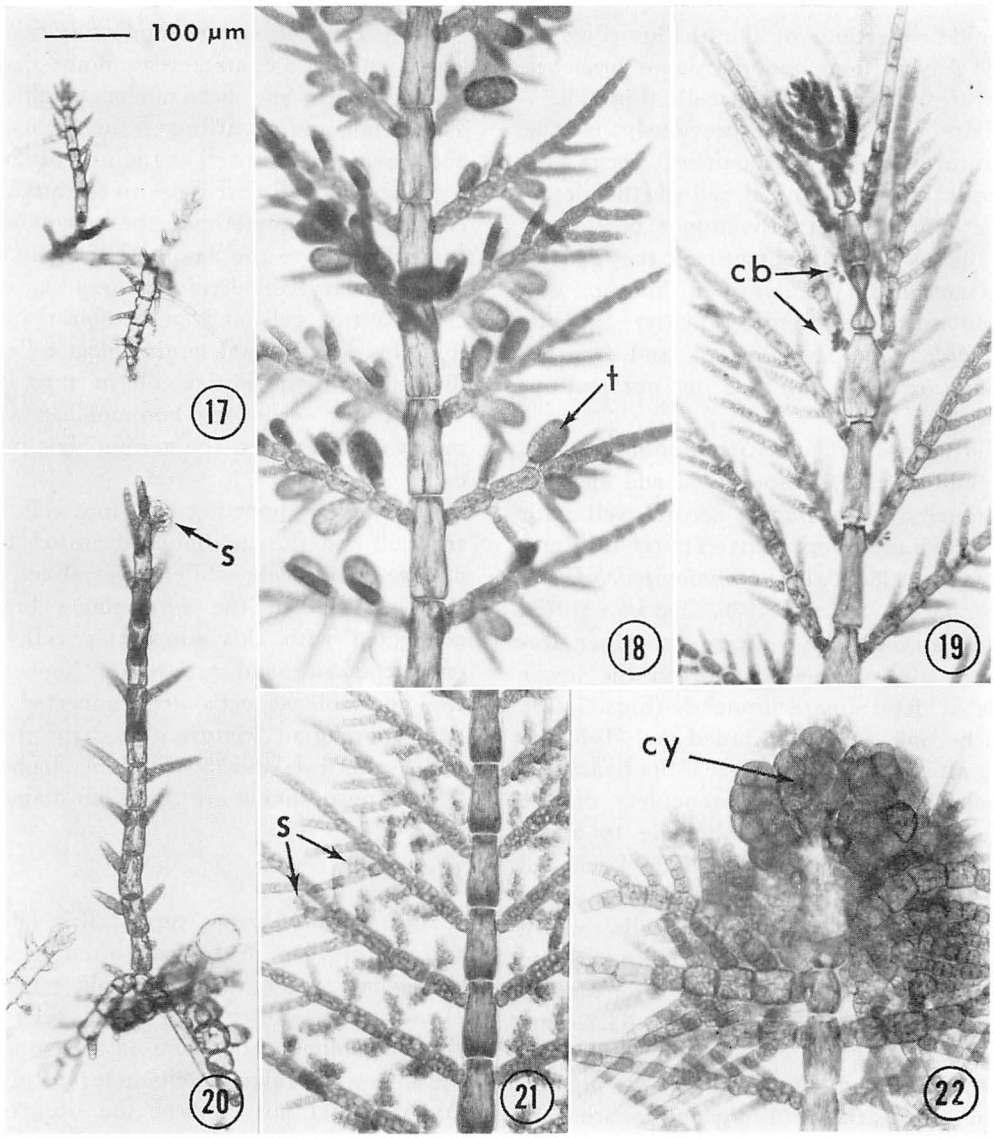
viable tetraspores until 1966, whereas the plant (#1662) from Berkeley Yacht Harbor formed no reproductive structures at all, although it continues to grow vigorously. Hybridization was attempted with reciprocal crosses between *A. nipponicum* and *A. defectum* but none were positive (Table 1) except intraspecific controls which released carpospores in four weeks.

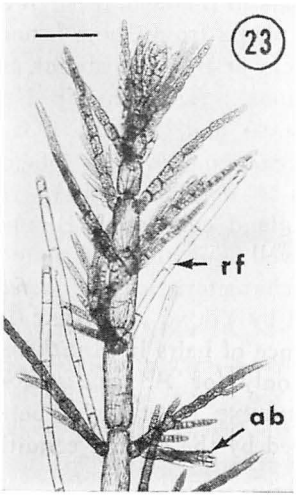
*Vegetative Structure of* A. nipponicum: This species in culture shows vegetative characters basically similar to those plants described from the field (YAMADA & INAGAKI 1935). The upright main axes arise from a rhizoidal base and develop repeatedly pinnate lateral branches. The cells of the main axes are 50–65 $\mu$m broad and 80–120 $\mu$m long. Determinate branches on the main axes and laterals are opposite and regularly 10–12 celled. The cells of determinate branches bear 5–6 celled short branchlets in opposite pairs or frequently in a secund manner, especially on the upper portion of the thallus (Fig. 1). The short basal cell of the determinate branch remains spherical without producing branchlets. Rhizoidal filaments developing from the basal cell of determinate branches are common in lower




Figs. 11–16. Development of female reproductive structures on *Antithamnion nipponicum* YAMADA et INAGAKI.

11. Development of procarps in apical portion of the main axis. 12. Fusion of carpogonium and auxiliary cell after fertilization. 13. The same as Fig. 12, developing a connecting cell from carpogonium. 14–16. Development of gonimoblast in young cystocarp. (a: auxiliary cell, ap: apex of the branch, c: carpogonium, cb: carpogonial branch, cc: connecting cell, cn: central cell, fc: foot cell, gi: gonimoblast initial, sc: supporting cell, t: trichogyne).




Figs. 17–23. *Antithamnion nipponicum* YAMADA et INAGAKI in culture.

17. Ten day old tetraspore germlings. 18. Part of mature tetrasporophyte. 19. Apical portion of female thallus with procarps, stained with 50% Karo aniline blue thymol solution. 20. Some early spermatangia on 650 μm long thallus, after one month culture. 21. Part of mature male plant. 22. A mature cystocarp. (cb: carpogonial branch, cy: cystocarp, s: spermatangial ramulus, t: tetrasporangium). 23. Rhizoidal filament (rf) and adventitious indeterminate branch (ab) arising from basal cell of determinate branch (scale: 100 μm).

to middle portions of the thallus (Figs. 1, 23). Adventitious indeterminate branches also arise from the basal cells (Fig. 23).

Hairs, not recorded previously in the literature for *A. nipponicum*, occur frequently on the terminal cell of the determinate branches in the upper portion of the thallus (Fig. 1). Sometimes, they remain as a cap on the branch tip without growth. Gland cells mentioned by YENDO (1917, as *A. applicatum*) and YAMADA and INAGAKI (1935), are not observed in our culture materials.

*Tetrasporangia and Spermatangia of* A. nipponicum: Tetrasporangia and spermatangia of *A. nipponicum* accord well with the previous descriptions (YENDO 1916 and YAMADA 1928 as *Acrothamnion pulchellum*; YAMADA & INAGAKI 1935, TAZAWA 1975). Tetrasporangia are solitary or rather frequently, in successive pairs on the lower cells of determinate branches (Figs. 7, 18), and become 50–55 $\mu$m broad and 70–80 $\mu$m long after maturation (Fig. 8). Spermatangia develop on the short branchlets of the determinate branches in middle to upper portion of the thallus (Fig. 21). Each cell of a spermatangial ramulus cuts off a few to ten spermatangial parent cells, which divide one or more times forming three to four spermatangia (Figs. 9, 10). Mature spermatangia are colorless and 5–6$\times$7–8 $\mu$m in size.

*Procarps and Cystocarps of* A. nipponicum: Procarps of *A. nipponicum* are common in the upper to apical portion of the thallus, being formed singly or in pairs successively on every segment of the main axes and indeterminate laterals (Figs. 11, 19). The basal cell of a determinate branch becomes the supporting cell, developing a single four-celled carpogonial branch. Not all the procarps on a branch in the apical portion show elongation of the trichogynes (Fig. 11). Usually only a single cystocarp matures on each branch apex (Fig. 22). Thus, apical growth of the thallus is suppressed by the cystocarp formation, as seen in the other species of *Antithamnion* (WOLLASTON 1968, 1971).

After fertilization, the supporting cell enlarges, cuts off a characteristic dome-shaped auxiliary cell, and becomes acetabuliform. The carpogonium, cutting off the trichogyne and leaving a small cell at the top, produces a connecting cell that fuses to the auxiliary cell (Fig. 13). Sometimes, the carpogonium fuses directly to the auxiliary cell (Fig. 12). The auxiliary cell divides into a flat foot cell, central cell and gonimoblast initial (Fig. 14). Additional gonimoblast cells are divided from the initial cell in turn (Fig. 15). A few secondary gonimoblasts commonly develop from the gonimoblast initial cell.

Later, the supporting cell, foot cell, central cell and the gonimoblast initial form a large fusion cell, while the axial cell and the first cell of the determinate branch connected with this supporting cell, also enlarge in size and pit-areas. Almost all the gonimoblast cells are converted into carposporangia. Mature cystocarps are naked, spherical and 500–650 $\mu$m diameter. The carposporangia are 25–30 $\mu$m diameter.

## Discussion

*Antithamnion* grows very well in laboratory culture. *A. nipponicum* often becomes somewhat larger than the field material. However, the absence of gland cells and the development of hairs in this species are somewhat unique in character in culture. As we could not confirm the occurrence of the gland cells in the field, it is not clear whether these plants from Cheju Island do not produce them or they are absent under culture conditions. According to YENDO (1917) and YAMADA and INAGAKI (1935) the gland cells occur on the cells of lower branchlets. In *A. defectum* and *A. kylinii*, however, the gland cells occur commonly in culture as well as in the field materials. The gland cell characteristics of *A. defectum* were described by YOUNG and WEST (1979).

The occurrence of hairs in *Antithamnion* was reported only for *A. tenuissimum* in culture by SUNDENE (1964), who believes they are induced by the culture conditions.

*A. defectum* as well as *A. nipponicum* also produced such hairs in laboratory culture, although they are not reported in the field materials. The presence of hairs in *Callithamnion* (PRICE 1978) and *Acrochaetium* (WEST 1972) generally relates to high light intensities. The occurrence of hairs in *Antithamnion* may be explained as an artificial result during culture.

TAZAWA (1975) described the spermatangial ramuli of *A. nipponicum* as consist ing of three to four cells. In our plants, however, they generally are five to six celled, and 450–600 μm long.

Although development of procarps and cystocarps in *A. nipponicum* were not recorded previously, they are basically similar in cell shape and development to those of other species in the genus (KYLIN 1923, L'HARDY-HALOS 1968, WOLLASTON 1968, 1971, ITONO 1977). The connecting cell, not common in *Antithamnion*, may be a valuable character of this species. They were reported only from *A. plumula* (ELLIS) THURET (KYLIN 1923), *A. divergens* (J. AG.) J. AGARDH and *A. hanowioides* (SONDER) DE TONI (WOLLASTON 1968). As mentioned by WOLLASTON, the carpogonium may fuse directly to the auxiliary cell, although they commonly produce the connecting cell for this function.

This isolate of *A. nipponicum* seems to be a genetically stable strain, because it does not show mixed phase reproduction or apomixis even though such unusual reproduction is reported in other species not only in culture (SUNDENE 1962, 1964, WEST & NORRIS 1966, RUENESS & RUENESS 1973), but also in the field (L'HARDY-HALOS 1968, KNAGGS 1969). As reported by WEST and NORRIS (1966) and by the present observations, *A. defectum* from Pacific North America is rather unstable although the basic life history is a *Polysiphonia*-type.

VAN DER MEER and TODD (1977) demonstrated that the formation of gametangia on the tetrasporophyte of *Gracilaria* sp. resulted from a mitotic recombination of the genes controlling sexuality. They did not offer an explanation for mixed phase reproduction of *Antithamnion*, in which gametophytes produced tetrasporangia. On the other hand, RUENESS and RUENESS (1973) mentioned that the light conditions appeared to be important for the induction of gametophytic reproductive structures. They did not discuss the inductive factors for mixed phase reproduction. These complicated mixed sexualities cannot be explained without cytogenetic information. However, we expect some environmental factors may strongly affect the shift of such reproduction. According to our observation, some culture materials of *Symphyocladia pennata* OKAMURA (LEE & WEST unpublished data) and *Dasysiphonia chejuensis* LEE et WEST (LEE & WEST 1979) from Korea show such possibilities. The latter species exhibits an almost complete reproductive shift from procarps to tetrasporangia on the female gametophytes under some culture conditions.

The two species, *A. defectum* and *A. nipponicum*, are different in vegetative morphology and evidently not closely related genetically because the attempted crosses were not successful.

## Acknowledgements

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## References

ITONO, H. 1969. The genus *Antithamnion* (Ceramiaceae) in southern Japan and adjacent waters I. Mem. Fac. Fish., Kagoshima Univ. **18**: 29–45.

ITONO, H. 1971. The genus *Antithamnion* (Cer-

amiaceae) in southern Japan and adjacent waters II. Mem. Fac. Fish., Kagoshima Univ. **20**: 209-216.

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

KANG, J. W. 1966. On the geographic distribution of marine algae in Korea. Bull. Pusan Fish. Coll. **7**: 1-125, 7 pl.

KNAGGS, F. W. 1969. A review of Florideophycidean life histories and of the culture techniques employed in their investigation. Nova Hedwigia **18**: 293-330.

KYLIN, H. 1923. Studien über die Entwicklungsgeschichte der Florideen. Kungl. Sv. Vet. Akad. Handl. **63** (11): 1-139.

LEE, I. K. and WEST, J. A. 1979. *Dasysiphonia chejuensis* gen. et sp. nov. (Rhodophyta, Dasyaceae) from Korea. Syst. Bot. **4**: 115-129.

L'HARDY-HALOS, M.-Th. 1968. Les Ceramiaceae (Rhodophyceae Florideae) des côtes Bretagne. 1. Le genre *Antithamnion* NÄGELI. Rev. Algol. **9**: 152-183.

MCLACHLAN, J. 1973. Growth media-marine. *In*, J. R. STEIN (ed.), Handbook of phycological methods. Culture methods and growth measurements. Cambridge Univ. Press. London.

POLANSHEK, A. and WEST, J. A. 1975. Culture and hybridization studies on *Petrocelis* (Rhodophyta) from Alaska and California. J. Phycol. **11**: 434-439.

PRICE, J. H. 1978. Ecological determination of adult form in *Callithamnion*: Its taxonomic implications. *In*, D. E. G. IRVINE and J. H. PRICE (ed.), Modern approaches to the taxonomy of red and brown algae. Academic Press, London and N. Y.

RUENESS, J. and RUENESS, M. 1973. Life history and nuclear phases of *Antithamnion tenuissimum*, with special reference to plants bearing tetrasporangia and spermatangia. Norw. J. Bot. **20**: 205-210.

RUENESS, J. and RUENESS, M. 1975. Genetic control of morphogenesis in two varieties of *Antithamnion plumula* (Rhodophyceae, Ceramiales). Phycologia **14**: 81-85.

SUNDENE, O. 1959. Form variation in *Antithamnion plumula*. Experiments on Plymouth and Oslofjord. Nytt Mag. Bot. **7**: 181-187.

SUNDENE, O. 1962. Reproduction and morphology in strains of *Antithamnion boreale* originating from Spitsbergen and Scandinavia. Skr. Norske Vidensk. A-kad. Oslo I. Mat.-Nat. Kl. N. S. **5**: 1-19, 2 pl.

SUNDENE, O. 1964. *Antithamnion tenuissimum* (HAUCK) SCHIFFNER in culture. Nytt Mag. Bot. **12**: 5-10, 3 pl.

SUNDENE, O. 1975. Experimental studies on form variation in *Antithamnion plumula* (Rhodophyceae). Norw. J. Bot. **22**: 35-42.

TANIGUCHI, K. 1972. Spore germination in three species of Ceramiales (Rhodophyta). Bull. Fac. Fish., Hokkaido Univ. **25**: 127-134.

TAZAWA, N. 1975. A study of the male reproductive organ of the Florideae from Japan and its vicinity. Sci. Pap. Inst. Alg. Res., Fac. Sci. Hokkaido Univ. **6**: 95-179, 10 pl.

TOKIDA, J. 1954. The marine algae of southern Saghalien. Mem. Fac. Fish. Hokkaido Univ. **2**: 1-264, 15 pl.

VAN DER MEER, J. P. and TODD, E. R. 1977. Genetics of *Gracilaria* sp. (Rhodophyceae, Gigartinales). IV. Mitotic recombination and its relationship to mixed phases in the life history. Can. J. Bot. **55**: 2810-2817.

WEST, J. A. 1972. Environmental control of hair and sporangium formation in the marine red alga *Acrochaetium proskaueri* sp. nov. Proc. Int. Seaweed Symp. **7**: 377-384.

WEST, J. A. and NORRIS, R. E. 1966. Unusual phenomena in life histories of Florideae in culture. J. Phycol. **2**: 54-57.

WOLLASTON, E. M. 1968. Morphology and taxonomy of southern Australian genera of Crouanieae SCHMITZ (Ceramiaceae, Rhodophyta). Aust. J. Bot. **16**: 217-417, 10 pl.

WOLLASTON, E. M. 1971. *Antithamnion* and related genera occurring on the Pacific coast of North America. Syesis **4**: 73-92.

YAMADA, Y. 1928. Report of the biological survey of Mutsu Bay. 9. Marine algae of Mutsu Bay and adjacent waters II. Sci. Rep. Tohoku Imp. Univ. 4th ser. (Biology) **3**: 481-534.

YAMADA, Y. and INAGAKI, K. 1935. On *Acrothamnion pulchellum* YAMADA (*non* J. AGARDH) from Japan. Sci. Pap. Inst. Algol. Res., Fac. Sci., Hokkaido Imp. Univ. **2**: 212-226, pl. 49-52.

YENDO, K. 1916. Notes on algae new to Japan

V. Bot. Mag., Tokyo **30**: 243–263.
YENDO, K. 1917. Notes on algae new to Japan VII. Bot. Mag., Tokyo **31**: 183–207.
YOUNG, D. N. and WEST, J. A. 1979. Fine structure and histochemistry of the vesicle cells of the red alga *Antithamnion defectum* (Ceramiaceae). J. Phycol. **15**: 49–57.

### 李 仁圭・J. A. ウエスト： 紅藻フタツガサネ *Antithamnion nipponicum* YAMADA et INAGAKI の培養研究

韓国済州島から採集したフタツガサネ *Antithamnion nipponicum* を主としてアメリカ太平洋岸産 *A. defectum* 及び *A. kylinii* との培養結果と比較した。*A. nipponicum* は典型的 *Polysiphonia* 型生活史を示し，無性生殖のみ，または雌雄両生殖器官の同時形成現象などがみられなかった。しかし，*A. defectum* は基本的に *A. nipponicum* と同じ生活史を示すが，培養中四分胞子体が胞子形成能力を失い，配偶体が雌雄両生殖器官を同時に作る事もあった。*A. kylinii* は四分胞子体が胞子形成能力を失い生殖器官をまったく作らなかった。又 *A. nipponicum* と *A. defectum* の交雑は出来なかった。今般の研究から *A. nipponicum* の procarp と嚢果発達過程が明らかになり，procarp は体上部の有限生長枝の基部細胞に連続して作られ，造果器は受精後 connecting cell を出す。尚，この種の培養材料には gland cell がなく，有限生長枝の頂端に hair が形成された。

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## CHINESE PHYCOLOGICAL SOCIETY の創立

The Chinese Phycological Society was established in October 1979, with the inaugural meeting being held at Wuhan, Hupeh Province. The Society has more than 200 members, and more than 70 attended the first meeting where 63 papers were presented. The Society intends to publish a phycological journal. The executive consists of: C. C. JAO, Honorary President; C. K. TSENG, President; H. Z. CHU, T. C. FANG, and S. N. LI, Vice-Presidents; C. Y. WU, Secretary; X. G. FEI and M. C. YU, Vice-Secretaries. (J. MCLACHLAN, Atlantic Regional Laboratory, N.R.C., Halifax, Canada)