

**ANTITHAMNION SPARSUM,
ITS LIFE HISTORY AND HYBRIDIZATION WITH
A. DEFECTUM IN CULTURE**

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ABSTRACT

Antithamnion sparsum Tokida isolated from the southern and western coasts of Korea showed a basically *Polysiphonia*-type life history. However, it sometimes exhibited a monoecious reproduction and the carpospores released from the cystocarp by self-fertilization unexpectedly developed into plants bearing spermatangia alone. These male plants were not functional up to 60 days in culture.

The results of intraspecific crosses between populations of *A. sparsum* were successful and the hybrid carpospores gave rise to normal tetrasporophytes. On the other hand, the interspecific crosses between *A. sparsum* and *A. defectum* were only partly successful, as evidenced by gonimoblast development and the release of carpospores in case of *A. sparsum* (male) × *A. defectum* (female), but not in *A. sparsum* (female) × *A. defectum* (male). These results seem to suggest that both species are still undergoing speciation.

INTRODUCTION

Several species of *Antithamnion* have been cultured in the laboratory. These are considered to have a regular life history characterized by a tetrasporophyte, dioecious gametophyte, and carposporophyte (Drew 1955, Sundene 1959, Lee and West 1980). Some of them, however, show irregular reproductive cycles in addition to a typical *Polysiphonia*-type of life history (Sundene 1964, West and Norris 1966, Rueness and Rueness 1973). Still,

others in culture repeat the tetrasporic generation (Sundene 1962) or exhibit vegetative growth alone (Whittick and Hooper 1976).

The life history of *A. sparsum* has not been confirmed in laboratory culture. The species is expected to show a typical *Polysiphonia*-type although cystocarpic plants have not been reported in the field (Tokida 1932, 1954, Kang 1966, Noda 1970, Lee and Kim 1977). The plants are distributed from Saghalien to Korea (Tokida 1932, Kang 1966) and are closely related to *A. defectum* occurring in the Pacific North America (Tokida 1932). The two species have been distinguished by their cell dimensions, position of tetrasporangia, and gross morphology (Tokida 1932) which, however, can be modified by environmental factors (Sundene 1962). Thus, Wollaston (1971) suggested that *A. sparsum* may be con specific with *A. defectum*, and Yoshida (1981) recently proposed the former as a synonym of the latter.

In this paper the life history and reproduction of *A. sparsum* from Korea were investigated in laboratory culture, and the assessment of the species was considered by interspecific cross with *A. defectum* from the Pacific North America.

MATERIALS AND METHODS

Two isolates of *A. sparsum* were used in the study. One (#138 was obtained from the southern coast of Korea at the intertidal zone of Jamdo, Jinhae Bay (35°03'N, 128°40'E) on November 17, 1979, and the other (#238) from the western coast of Korea at Gopado, Garolim Bay (36°24'N, 126°21'E) on May 15, 1980. They were placed in cooler and transferred to the laboratory for culture. On the other hand, for interspecific cross experiments, culture strain of *A. defectum* (JAW #240, 241: Lee and West 1980) from California coast was obtained through the courtesy of Dr. J.A. West, University of California, Berkeley on October 8, 1980.

Unialgal cultures were set up using the methods of Lee and West (1980). Preculture of all isolates was maintained in 1/2 PES medium under cool white fluorescent light at an intensity below 300 lux. After 3-7 days, they were transferred to the incubation condition in full strength PES media, under 16-19°C, 800-1300 lux, 16:8 LD, using 7 x 7 cm glasswares. In order to eliminate diatoms, GeO₂ solution was added to the culture medium for a while (West 1970). The medium was usually changed every fortnight.

Tetraspores were obtained from fully mature tetrasporangia. After 24 h, the sporelings were transferred to a culture dish. Carpospores were cultured in the same manner as the tetraspores. To observe fertilization, the plants bearing cystocarps were isolated individually and cultured for a while to make sure that unfertilized young branches were newly grown. In

addition, a few excised apices of a female plant were kept singly in a glass container for observation of possible parthenogenesis.

RESULTS

The vegetative development of all isolates of *A. sparsum* was identical in the laboratory. There was also no detectable difference in the vegetative morphology of the tetrasporophyte from plants described from the field (Tokida 1932, 1954).

Vegetative Morphology

Erect thallus with prostrate base is 3-5 cm high and is attached to the glassware by means of rhizoidal filaments arising from the spherical basal cells of the determinate branchlets. The rhizoidal filaments with blunt tips are 4-8-celled. These also arise from the upper portion of the thallus. The cells of the main axis are 59 μ m broad and 348 μ m long, about six times as long as broad at maximum compared to 2-5.5 times in the field (Tokida 1932). Determinate branchlets on the main axis are opposite, usually 12-16-celled and semi-pinnately pectinate on the upper side. Indeterminate branches arise from every 3-7 segments of main branch and basically produce no branchlets at the opposite side.

Adventitious indeterminate branches sometimes arise from the basal cell of determinate branchlets. Hairs, which have not been recorded in the field, occur frequently on the terminal cell of determinate branchlets in the apical portion of the thallus associated with sexual reproductive structures. Gland cells are usually located on 2-3 cells of a pinnule and on the average measure 24 μ m long and 19 μ m broad.

Reproduction in Culture

The germination pattern of both tetraspores and carpospores isolated from the field for laboratory culture is basically identical. After attachment, the spores synchronously develop two opposite primordia. One later forms a rhizoid and the other develops into an apical cell from which the erect frond appears (Figs. 1-5). However, there is no definite sequence in the appearance of the rhizoid or the frond. In some sporelings, the rhizoidal cell, and in others, the frond, develops much later. Determinate branchlets at first arise alternately (Figs. 6, 8), or sometimes secondly (Fig. 7), with 16-18 days after germination. Later, after full growth, they are situated opposite one another.

The tetraspores grow into gametophytes within 20-30 days after germination. Spermatangial ramuli are observed early, and carposporangial plants appear about 10 days later. Gametophytes are basically dioecious in culture. Spermatangia develop in all parts of the pinnules in determinate branchlets. Each cell of a spermatangial ramulus cuts off a few spermatangial parent cells, which divide once or a few times, forming 2 to 4 spermatangia (Fig. 9).

Carpogonial branches are common in the upper to apical portion of the thallus, occurring singly or very rarely in pairs successively on the basal cell of determinate branchlets along the main axis and laterals. The small basal cell bearing the carpogonial branch grows larger than others and becomes the supporting cell. A mature carpogonial branch develops a long trichogyne (Figs. 11, 17).

It is known that a single carpogonial branch on each fertile apex usually matures into the cystocarp while the rest of the carpogonial branches degenerate (Wollaston 1968). However, in our culture, two to three carpogonial branches were not fertilized and grew into mature cystocarps at the same time. Such an occurrence has not been observed previously among the species of *Antithamnion*.

The development of the carposporophyte is basically similar to that preciously described by Wollaston (1968) and Lee and West (1980). The enlarged supporting cell, after fertilization, cuts off a characteristic dome-shaped auxiliary cell and becomes acetabuliform. The carpogonium, cutting off the trichogyne and leaving a cap cell at the top, produces a connecting cell that fuses with the auxiliary cell (Fig. 12) and through which the presumed diploid nucleus is moved into the auxiliary cell. After fusion, the auxiliary cell divides transversely to form the lower foot cell and upper central cell, which gives rise to the gonimoblast initials (Fig. 15). The gonimoblast cell is produced terminally on the auxiliary cell and at this stage the axial, supporting, and the foot cells are fused (Fig. 14). No special involucre is formed but the pinnae of axial cells below the cystocarp grow upwards, partially surrounding the mature cystocarp. The carpospores are released in thirty days after the formation of the auxiliary cell.

Carpospores grow to tetrasporophytes that produce tetrasporangia in thirty days after germination. Mature tetrasporangia are ovoid to ellipsoidal and measure 41 x 59 μ m on the average. Cruciate tetrasporangia are pedicellate in one or two cells or sessile on the upper part of the pinnae of determinate branchlets (Fig. 10) in contrast to the description by Tokida (1954) that they are pedicellate or sessile. Tetrasporangia release tetraspores in two weeks.

Thus, *A. sparsum* in culture completes its life cycle in four months.

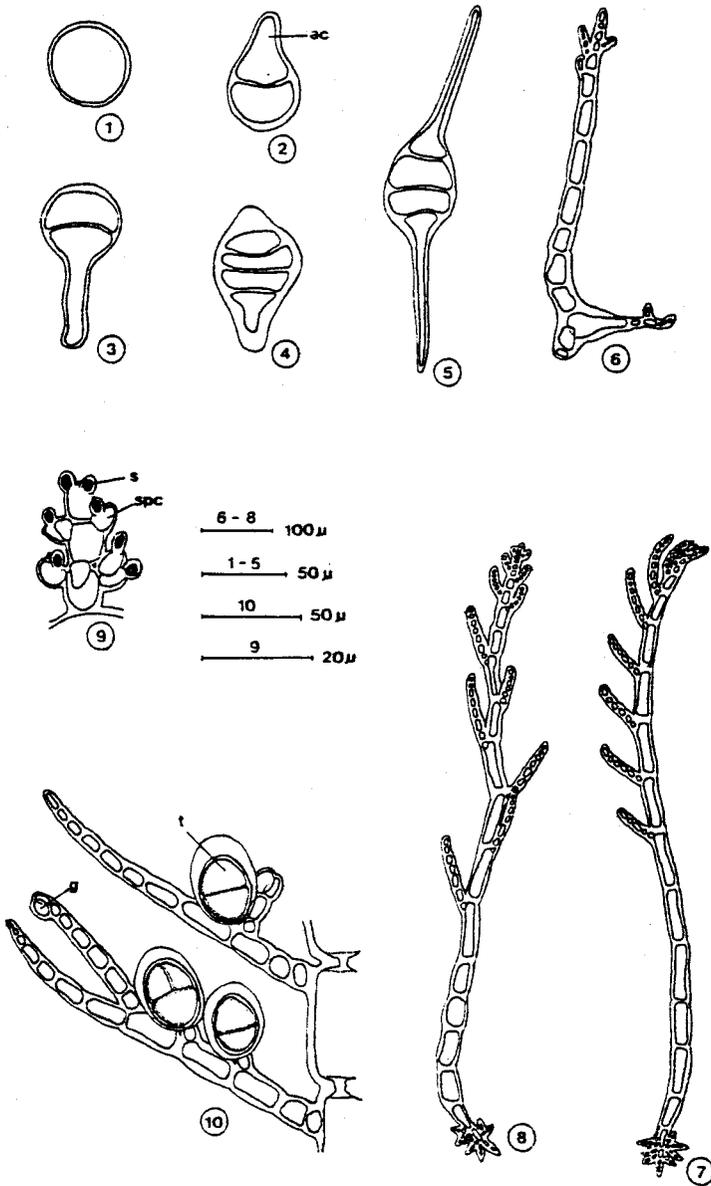


Fig. 1-10. Development of vegetative thallus, tetrasporangia and spermatangia of *Antithamnion sparsum* in culture. Fig.1. Released tetraspore. Figs. 2-3. One-day tetrasporelings. Figs. 4-5. 4-celled stage with bipolar apices. Figs. 6-8. Branching types of young plant. Fig. 9. Development of spermatangia. Fig. 10. Development of tetrasporangia (ac: apical cell, g: gland cell, r: rhizoidal cell, s: spermatangial parent cell, t: tetrasporangium).

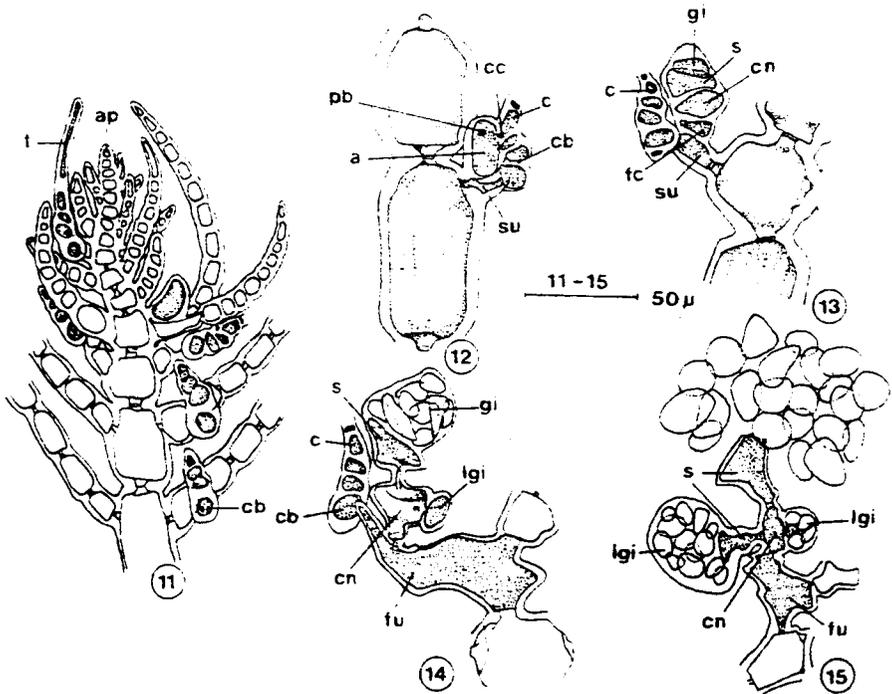


Fig. 11-15. Development of female reproductive structure of *Antithamnion spars urn Tokida* in culture. Fig. 11. Procarpus in apical portion of main axis. Fig. 12. Auxiliary cell and connecting cell. Fig. 13. Development of early gonimoblast cells. Fig. 14. A young cystocarp with secondary gonimoblast initial. Fig. 15. A mature cystocarp (ac: auxiliary cell, ap: branch apex, c: carpogonium, cc: connecting cell, cn: central cell, fc: foot cell, fu: fusion cell, gi: gonimoblast initial, pb: protein body, s: sterile cell, su: supporting cell, t: trichogyne).

A typical *Polysiphonia*-type life history is repeated three times during the culture period.

Unusual Life Histories

On the other hand, several monoecious gametophytes (#138-522) derived from male gametophytes were observed in Jamdo isolates during culture. Each monoecious gametophyte, isolated individually, developed cystocarps, indicating self-fertilization. However, all the released carpospores germinated and unexpectedly grew into plants bearing spermatangia quite similar to a common male gametophyte. The fertility of the spermatangia was, however, not confirmed completely. Even though these carpospore-derived spermatangial plants were placed in culture together with normal female plants for sixty days, no cystocarps were developed.

Intra- and Interspecific Crosses

The cross between the Jamdo (# 138) and the Garolim Bay (#238) isolates of *A. sparsum* produced viable carpospores. Crosses between male *A. sparsum* and female *A. defectum* were also successful; normal cytocarps and viable carpospores were formed (Table 1). However, crosses between female *A. sparsum* and male *A. defectum* produced no mature cytocarps and viable carpospores. The gonimoblast stopped growing during early development (Figs. 16-17).

Table 1. Cross experiments among populations of *Antithamnion sparsum* from Korea and *A. defectum* from Pacific North America

Female		Male	Fertilization	Carpospore release
<i>A. sparsum</i> #138	×	<i>A. sparsum</i> #138	+	+
<i>A. defectum</i> #240	×	<i>A. defectum</i> #241*	+	+
<i>A. sparsum</i> #138	×	<i>A. sparsum</i> #238	+	+
<i>A. defectum</i> #240	×	<i>A. sparsum</i> #138	+	+
<i>A. sparsum</i> #138	×	<i>A. defectum</i> #241	+	-

* After Lee and West (1980).

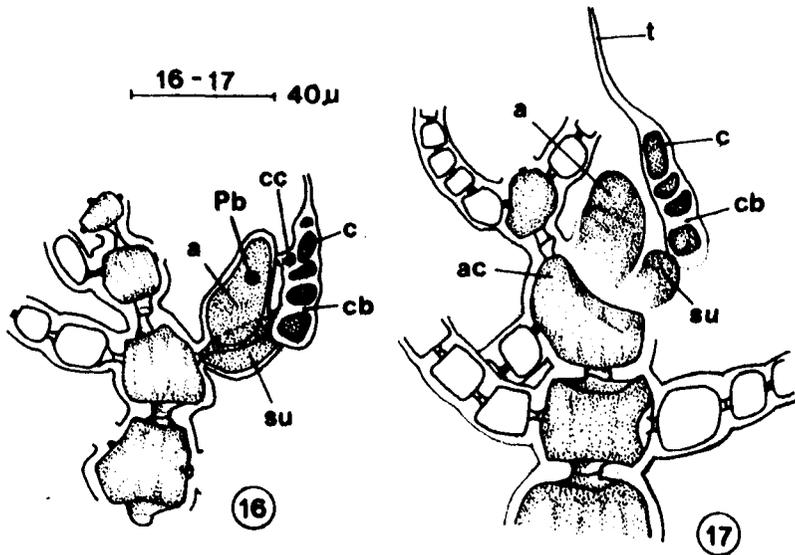


Fig. 16-17. Unsuccessful development of gonimoblast in interspecific cross of *Antithamnion sparsum* (female) and *A. defectum* (male) in culture. Fig. 16. Development of connecting cell after fertilization. Fig. 17. Development of early gonimoblast cell (a: auxiliary cell, ac: axial cell, c: carpegonium, cb: carpegonial branch, cc: connecting cell, pb: protein body, su: supporting cell, t: trichogyne).

DISCUSSION

As summarized in Fig. 18, *A. sparsum* basically shows a typical *Polysiphonia*-type of life history (Drew 1955, Sundene 1959, Lee and West 1980). Some unusual appearances of reproductive structures were also reported among *Antithamnion* species not only in culture (Sundene 1962, 1964, West and Norris 1966, Rueness and Rueness 1973), but also in the field (L'Hardy-Halos 1968, Knaggs 1969).

Monoecious reproduction is another unusual phenomenon in the life history of *A. sparsum*. Hence, the female reproductive structures were developed on the male gametophyte, and the resulting carpospores, missing the tetrasporic phase, developed exclusively male gametophytes whose spermatia were not functional. On the contrary, no female plant developed monoecious male branches in culture.

There are two previous reports on the monoecism of *Antithamnion* in culture (Drew 1955, West and Norris 1966). Thus, monoecism may not be a rare occurrence in this genus as it is also apparently common in *Callithamnion*, a related genus. However, it is peculiar that such monoecious plants in the present experiment miss the tetrasporophyte. Whittick and West (1979) demonstrated in the life history of a monoecious species of *Callithamnion* that the carpospores from the cystocarp developed into tetrasporophytes as seen in regular dioecious plants. Polanshek and West (1977) also reported the repetition of cystocarpic generations in the life history of *Gigartina papillata*. However, the lack of tetrasporic generation would differentiate *A. sparsum* from these species.

While Sundene (1962, 1964) and West and Norris (1966) reported that apomeiotic tetraspores in the gametophyte of *Antithamnion* developed only gametophytes of the same sex as the parent, Rueness and Rueness (1973) noted that the tetraspores in the male gametophytes developed into both male and female plants. A similar phenomenon also occurs in *Dasysiphonia chejuensis* (Lee and West, unpublished data). Light conditions apparently play an important role in inducing sexual reproductive structures in *Antithamnion* (Rueness and Rueness 1973).

Van de Meer and Todd (1977) reported mixed phase reproduction in the life history of *Gracilaria* sp., and suggested that the sexuality is controlled by the genetic recombination of a pair of alleles rather than a pair of chromosomes. But this was in case of diploid tetrasporophytes. They did not explain the mixed reproduction in the gametophytes observed by West and Norris (1966) and Rueness and Rueness (1973).

The monoecism of *A. sparsum* seems to be genetically stable since the female branches that develop on the male thallus are sexually irreversible and produce cystocarps successively, as in the case of tetrasporangium

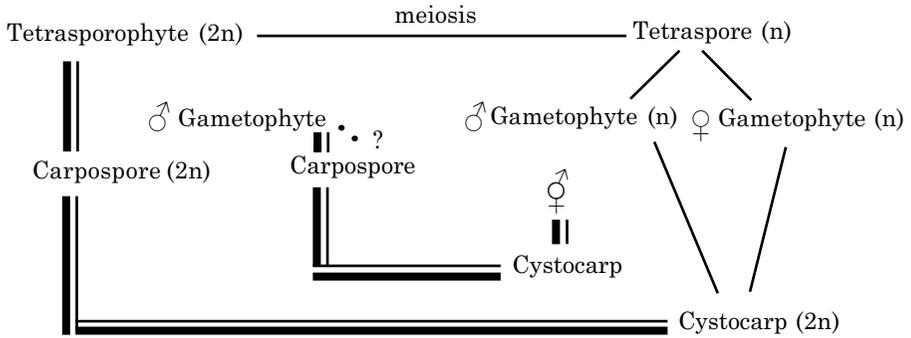


Fig. 18. Life history of *Antithamnion sparsum* in culture.

formation on cultured gametophytes of *Symphyocladia pennata* and *D. chejuensis* (Lee and West 1979). However, the presence of non-functional spermatia from such male thalli suggests that these gametophytes would be diploid rather than haploid.

In reporting *A. sparsum* as a new species, Tokida (1932) mentioned that this species showed more affinity to *A. defectum* Kylin and that both species were basically distinguished by differences in cell dimension. Wollaston (1971), therefore, doubted that both species might be conspecific and Yoshida (1981) treated the former as a synonym of the latter. In fact, such morphological characters used in separating the two species are easily subject to environmental influence (Sundene 1962, Norris and West 1967), considered vague (Wollaston 1968), and are also of little value in this study. A comparison of some significant taxonomic characters between *A. sparsum* according to Tokida and *A. defectum* Kylin is shown in Table 2.

Table 2. A comparison of some significant taxonomic characters between *Antithamnion sparsum* and *A. defectum* Kylin

Characters	<i>A. defectum</i> *	<i>A. sparsum</i> **	<i>A. sparsum</i> ***
Attachment	rhizoidal	rhizoidal	rhizoidal
Branching pattern	opposite	opposite	opposite
Cell dimension	2-3 times	2-5.5 times	5-6 times
Cell tip	tapering	blunt	blunt & tapering
Gland cell	on 2-5 cells	on 2-3 cells	on 2-3 cells
Tetrasporangia	1-2-pedicellate	1-pedicellate	1-2-pedicellate
	ovoid /80 μ m	sessile /ovoid	sessile / ovoid
	long	59 \times 78 μ m	41 \times 59 μ m
Spermatangia	adaxial	adaxial	adaxial

*After Kylin (1925), Wollaston (1971).

**After Tokida (1932, 1954), Lee and Kim (1977).

***After present study.

The formation of carposporophyte and production of viable carpospores in the cross between *A. sparsum* (male) \times *A. defectum* (female) could indicate that both species are partially interfertile. However, the reciprocal cross between the female *A. sparsum* and the male *A. defectum* was not successful. They produced an auxiliary cell after fertilization, but failed to develop gonimoblast cells (Figs. 16,17) which could suggest that both species are still undergoing the speciation process.

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