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Life history and taxonomy of *Callithamnion callophyllidicola* YAMADA (Ceramiaceae, Rhodophyta)

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We report a life history of *Callithamnion callophyllidicola* YAMADA from Korean water and discuss its taxonomic problems in species limit. The plants grow well in most coasts of Korea and all phases of *Polysiphonia*-type of life history are usually collected at one sampling. Though dioecism is a general form of the gametophytes, monoecious plants in culture produce dioecious gametophytes in next generation. Of factors tested in culture, growth rate depends much on light period than on light intensity, and much on light intensity than on temperature. All tetrasporelings are synchronously fertile under long-day condition with high light intensity, but male gametophytes are first fertile under short-day or low light condition. Induction of tetrasporangia also depends much on light period. In morphology, *C. callophyllidicola* resembles *C. minutissima* YAMADA and *Aglaothamnion oosumiense* ITONO, and their taxonomic relationship should be critically reassessed with thorough examination of their type specimens.

Key Index Words: Callithamnion callophyllidicola—*Ceramiaceae—growth rate—life history—reproductive organs' induction—taxonomy.*

Modern concept of species needs the full interpretation of life history of organisms because they are quite variable, and some species may vary by altering their pattern of growth in response to environmental differences (JONES and LUCHSINGER 1986). Furthermore it is a life history which maximizes the potential for genetic recombination and genetic diversity from the union of one pair of gametes (SEARLES 1980). Therefore the developmental and environmental differences at all stages of the life history should be observed on a taxonomic view in field as well as in laboratory.

Recent taxonomic studies of the red algal genus *Callithamnion* have clearly demonstrated that much of confusion in species delimitation of the genus is due to failure in previous works to recognize the diverse form range of the taxa (RUENESS and RUENESS 1980). Furthermore PRICE *et al.* (1986) pointed out that *Callithamnion* and its allied genera, *Aglaothamnion, Pleonosporium* and *Seirospora*, should critically be reexamined about their taxonomic status based on herbarium, laboratory and field studies in the various locations concerned.

In Korea just three species of *Callithamnion* have been described in flora (Boo 1985; LEE and KANG 1986) and until now there has not been any report on its experimental taxonomy. *Callithamnion callophyllidicola* YAMADA grows well in most coasts of Korea and is subject to be collected year round, even though its small size and delicateness. It was first described by YAMADA for the plants epiphytic on *Callophyllis crispata* OKAMURA and *C. japonica* OKAMURA at Enoshima, Sagami Province, Japan (YAMADA 1932). The long specific epithet was derived from the generic name of its host plants. Afterwards it has been known at most coasts of Japan and Korea, but is still restricted to the Japanese and Korean coasts (YOSHIDA *et al.* 1985; LEE and KANG 1986). In this paper we report its life history in the various laboratory conditions and discuss taxonomic problems in species delimitation.

Materials and Methods

Many field samplings were haphazardly carried out at the intertidal zones of coasts of Korea (Fig. 1). All plants collected were stored in 4% formalin-seawater and transported to the laboratory, where the morphological features were observed as thoroughly as possible.

Cultures for life history were established from excised apices from tetrasporophytes collected at the intertidal zone of Dokdo, East Sea of Korea, in September 1981. The other cultivation techniques followed BOO and LEE (1983). The experiments on growth rate and induction of reproductive organs were carried out with the plants collected at a small tidepool in Sungsan, Chejudo, in August 1986. The isolates were maintained in IMR 1/2 medium (EPPLY *et al.* 1967) under cool white fluorescent lighting. The growth rate was measured at varied light intensities, photoperiods, and temperatures with the tetrasporelings. The tetraspores were allowed to settle on coverslips at the bottom of Petri dishes for one day under 17±1°C, 16 : 8 LD and 50 μmol $m^{-2}s^{-1}$ and then transferred into each experimental conditions on a temperature-gradient table. The cell number of the tetrasporelings was counted at the seventh day after their settlement on coverglasses for comparison of growth rate. Eight tetraspores from two tetrasporangia were used for induction of sexual reproductive organs and five carpospores for induction of tetrasporangia. All reproductive organs were checked out every three days, when medium was exchanged to avoid nutrient limitation.

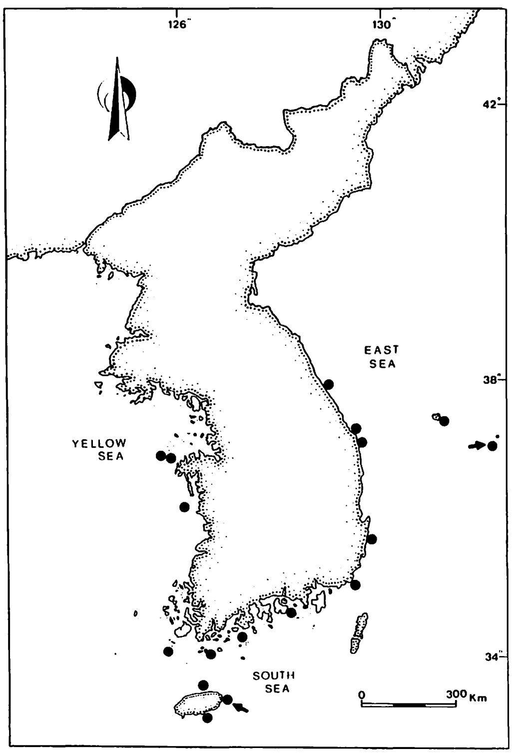

Fig. 1. Geographical distribution of *Callithamnion callophyllidicola* YAMADA along the coasts of Korea. Arrow represents sampling site for culturing plants.

Results

PLANTS IN FIELD: *Callithamnion callophyllidicola* grows well in the intertidal and subtidal zones of most coasts of Korea (Fig. 1), where the plants are epizoic on sponges and *Hydrozoa* spp. or epiphytic on various seaweeds including *Callophyllis* spp. They are small, delicate and beautiful.

The plants were ca. 2 cm high and attached to substrata with their digitate rhizoids. The axial cells were formed from apical cells by oblique division and were, in the middle portion of plants, 70–90 μm broad and 200–220 μm long, thus the L/B being 2–3 : 1. The primary branches were derived alternately from every axial cells except lower ones. The third to fourth branches were formed in series similarly to the primary branches. All branches were usually distichous and could grow ultimately. The gland cells were not observed in Korean plants.

The gametophytes were dioecious. The spermatangia were formed on the adaxial portion of branches of male plants. The carpogonial branches were observed on the 5th to 6th axial cells from apex and arranged in zig-zag form in female plants. After fertilization young gonimolobes were formed, which became lobed cystocarps and 300–500 μm long when mature. The tetrasporophytes were isomorphic with gametophytes. Tetrasporangia were formed on the adaxial portion of branches. They were divided tetrahedrally, and 40–55 μm × 60–70 μm in size. Phenologically all phases of *Polysiphonia*-type of life history were usually collected at one sampling. In January, April and August gametophytes and tetrasporophytes were observed at Sungsan, Chejudo. In February they were also collected at Imwon, Hupo and Gampo, eastern coast of Korea, and in April at Whanggumdo, western coast of Korea. In September and October, they occurred at Dokdo and Ulreungdo.

Life History in Culture: Germination of tetraspores in culture resulted in the typical bipolar or irregular growth within two to three days after shedding on coverglasses. At sixth to seventh day the tetrasporelings produced some primary branches, which were first irregular but later regularly alternate. Filamentous rhizoids became digitate at this time. At fifteenth day spermatangia were observed on the adaxial portion of branches of male plants and at eighteenth day carpogonial branches began to occur on the 5th to 6th axial cells from the apex of female plants. At twenty-fourth day young cystocarps were observed on the upper portion of female plants. At 30th day carpospores were shed from the mature cystocarps and began to germinate bipolarly as tetraspores. After two weeks the carposporelings became tetrasporophytes which were isomorphic with the gametophytes. Thus Korean plants of *Callithamnion callophyllidicola* in culture completed the typical *Polysiphonia*-type of life history during 44 days. Several successive life history cycles of two isolates were achieved. The cultured plants were basically same to plants in field.

On the other hand, some monoecious plants (below 1% of frequency) were observed. They were suspected to be derived from male plants because most branches were covered with spermatangia while just one to three carpogonial branches were observed. When isolated and cultured, they produced mature cystocarps of which carpospores were functional as those of dioecious plants. The carposporelings grew mature tetrasporophytes which produced tetraspores, and the

Table 1. Influence of photoperiod and light intensity on growth rate of *Callithamnion callophllidicola.* *

Photoperiod (hr)	Photon flux density (μmol m^{-2} s^{-1})		
	199	122	73
$16:\overline{8}$	77.1±6.72	50.1±6.27	42.4±5.36
$8:\overline{16}$	24.4±2.39	19.0±2.39	12.1±1.39

* Growth rate is expressed as mean value of cell yield with standard deviation. Fifteen to twenty tetrasporelings were investigated under each condition.

Table 2. Influence of temperature and light intensity on growth rate of *Callithamnion callophyllidicola.* *

Temperature (°C)	Photon flux density (μmol m^{-2} s^{-1})				
	269	132	83	43	23
27	dead	dead	dead	7.6±1.17	4.6±1.11
22	35.2±6.69	80.2±16.58	39.9±6.61	17.8±3.06	10.6±0.80
17	23.8±5.01	57.5±12.19	61.8±7.68	20.1±2.02	10.7±1.42
12	6.0±0.80	23.1± 5.99	25.9±4.11	13.2±2.60	8.7±0.75

* Growth rate is expressed as mean value of cell yield with standard deviation. Fifteen to twenty tetrasporelings were investigated under each condition.

Table 3. Time course on induction of sexual reproductive organs from tetraspores of *Callithamnion callophyllidicola*.

Photoperiod (hr)	Light intensity (μmol m^{-2} s^{-1})	Number of days from tetraspores					
		12	15	18	21	24	28
16:$\overline{8}$	199	—	4M4F*				
	122	—	4M1F	2F	4F		
	73	—	1M	4M2F	4F		
8:$\overline{16}$	199	—	1M	4M3F	4F		
	122	—	—	2M	3M	3F	
	73	—	—	1M	3M	1F	3F

* M, male plant with spermatangia; F, female plant with carpogonial branches.

Table 4. Time course on induction of tetrasporangia from carpospores of *Callithamnion callophyllidicola*.

Photoperiod (hr)	Light intensity (μmol m^{-2} s^{-1})	Number of days from carpospores					
		11	14	17	20	23	27
16:$\overline{8}$	199	—	5T*	shedding			
	122	—	5T	shedding			
	73	—	5T	shedding			
8:$\overline{16}$	199	—	—	—	—	2T	5T
	122	—	—	—	—	1T	5T
	73	—	—	—	—	1T	5T

* T, plant with tetrasporangia.

tetrasporelings grew to mature dioecious gemetophytes. Thus the monoecious plants became normal dioecious gametophytes.

GROWTH RATE ON VARIED LABORATORY CONDITIONS: Tetrasporelings which received much light usually grew much fast (Table 1). However tetrasporelings under short-day condition with high light intensity grew much slowly than those under long-day condition with low light intensity, even though the former received 1692 μmol m^{-2}s^{-1} per day and the latter 1168 μmol m^{-2}s^{-1} per day. On temperature gradiant table tetrasporelings showed various growth rate. They were dead or grew slowly under high temperature (27°C). It is interesting that their growth rate at 22°C became maximum under 132 μmol m^{-2}s^{-1}, while at 17 and 12°C it became maximum under 83 μmol m^{-2}s^{-1}. In all conditions tested, the thallus form was most typical under 17°C and 83 μmol m^{-2}s^{-1} (Table 2).

INDUCTION OF REPRODUCTIVE ORGANS: The time course on induction of sexual reproductive organs was very similar both under long-day condition with low light intensity and under short-day condition with high light intensity even though the growth rate was not. In long-day condition all gametophytes became synchronously fertile under 199 μmol m^{-2}s^{-1}, but male plants became first fertile under 73 μmol m^{-2}s^{-1} (Table 3). Time course on induction of tetrasporangia was same under three different light intensities tested in long-day condition. Carposporelings produced tetrasporangia at the 14th day. In short-day condition, they also produced tetrasporangia at nearly the same time (Table 4).

Discussion

Callithamnion callophyllidicola was characterized by its corymbose to dichotomous ramification, distichous branches, and gland cells commonly in tetrasporophytes (Yamada 1932). Its Japanese plants were investigated morphologically by Segawa (1942, 1949) and Kawashima (1960). In general, the vegetative and reproductive features of Korean plants were concordant with original description and figure of Yamada (1932). The gland cells which were reported on Japanese tetrasporophytes (Yamada 1932; Kawashima 1960) were never observed in Korean plants. Judging from Yamada's figure, we suppose that he would mistake small protuberances on branches for gland cells.

C. callophyllidicola has a relatively rapid life history in culture. Though dioecism is a general form of gametophytes in *Callithamnion*, monoecism often occurs in field (Knaggs 1969) and laboratory (Whittick and West 1979; Rueness and Rueness 1980). Monoecious plants of *C. bayleyi* Harvey and *C. bipinnatum* Crouan isolated from field produced monoecious gametophytes in next generation in culture (Whittick and West 1979; Rueness and Rueness 1980). It is interesting that our result contrasts with monoecism of *C. bayleyi* and *C. bipinnatum*. Abnormalities in expression of sex or phases of life history have been reported to occur often in field and cultured plants, Rhodophyta (Knaggs 1969; Rueness and Rueness 1980). Until now it is also very difficult to understand how abnormalities in sex and phases of life history might occur in field or cultured plants of marine red algae.

The growth rate can be directly expressed as the number of cells in a filament, since the plants of *Callithamnion* basically consist of an uniseriate filament and intercalary transverse divisions are absent (Dixon 1973). Of factors tested in culture, growth rate of tetrasporelings in our plants depends much on light period than light intensity and much on light intensity than on temperature. Thus our data are in agreement with the findings of Kain (1987), while contrast with those of Edwards (1977, 1979) and Whittick (1981).

The induction time required for sporelings to become fertile can be correlated with growth rate and photosynthesis (Whittick 1981). All tetrasporelings are synchronously fertile under long-day condition with high light intensity. The less tetrasporelings receive light, the more slowly the female gametophytes are fertile. This result suggests that induction of sexual reproductive organs may be directly correlated with photosynthesis and much energy is required for the formation of female reproductive organs. Induction of tetrasporangia also depends much on light period because they are produced at nearly the same time under varied light intensities. In *C. hookeri*, daylength has no effct on fertility (Edwards 1979), while Newfoundland materials require long daylength (Whittick 1981). Thus it is also difficult for us to explain environmental factors controlling induction of reproductive organs. It is also interesting that induction time is similar in conditions tested while growth rate is not similar even though the former may be directly correlated with the latter.

According to Yamada (1944), *C. callophyllidicola* resembles *C. minutissima* Yamada but can be easily distinguished by its more slender frond, longer cells and ultimate ramuli which do not taper conspicuously. As Harris (1962) pointed out, the quantitative characters noted by Yamada (1944) are usually not stable taxonomically and are subject to change in varied environments. Therefore *C. minutissima* may be a same species with *C. callophyllidicola*, but rejection of the species should be possible after thorough examination of type specimens of two species. In addition Dawson (1962) pointed that *C. minutissima* seems to be closely related to *C. paschale* Börgesen, but the latter may be distinguished in cortication of lower part of frond and multifarious branch (Table 5).

On the other hand, *C. callophyllidicola* also resembles *Aglaothamnion oosumiense* Itono in having uninucleus in axial cells and carpogonial branches being a zig-zag form (Segawa 1949), of which characters established the genus *Aglaothamnion* (Feldmann-Mazoyer 1940). Even so, Boddecke (1958) and Harris (1962) rejected identity of

Table 5. A comparison of some taxonomic characters among *Callithamion callophyllidicola* and its related species.

Characters	*C. paschale*	*C. minutissima*	*Aglaothamnion oosumiense*	*C. callophyllidicola*	*C. callophyllidicola*
Type locality	Easter Island, Chile	Hayama, Sagami Province, Japan	Tajiri, Oosumi Peninsula, Japan	Enoshima, Sagami Province, Japan	—
Thallus	erect (2.5cm)	erect (0.2–0.5cm)	erect (2.5cm)	erect (0.2–0.8cm)	erect (0.5–2cm)
Axis	naked	naked	naked	naked	naked
Lower axial cell	1.5–2.5:1 L/B	70–100μm in dia. 1–1.5:1 L/B	45μm in dia. 2.5:1 L/B	160μm in dia. 1.5–2:1 L/B	70–90μm × 200–220μm 2.4–2.8:1 L/B
Branching pattern	alternate distichous multifarious in part	dichotomous in general	alternate to pinnate distichous	alternate to dichotomous-pinnate distichous	alternate to subdichotomous distichous
Lateral branch	4–5 order	—	3 order	3 order	3–4 order
Apex	—	obtuse	blunt	obtuse	blunt
Gland cell	absent	absent	absent	present*	absent
Spermatangia	seriate	seriate	seriate	seriate	seriate
Cystocarp	nearly spherical	lobed	lobed irregular	nearly spherical subterminal	lobed
Tetrasporangia	45–55μm in dia. solitary sessile	70μm × 45μm tetrahedral sessile	70μm × 27μm tetrahedral sessile	70μm × 60μm tetrahedral sessile	40–55μm × 60–70μm tetrahedral sessile
References	BÖRGESEN (1924)	YAMADA (1944)	ITONO (1971)	YAMADA (1932)	This paper

* We suppose that YAMADA (1932) would mistake small protuberances for gland cells.

Aglaothamnion because the diagnostic characters of the genus overlapped those of *Callithamnion*. In recent PRICE *et al.* (1986) also pointed out that *Callithamnion* and its allied genera including *Aglaothamnion* should critically be reexamined about their taxonomic status based on herbarium, laboratory and field studies in the various locations concerned. However, if the genus *Aglaothamnion* is considered as a legitimate taxonomic category, *C. callophyllidicola* should be recombined into *Aglaothamnion callophyllidicola.* We will discuss the taxonomic relationship between *Callithamnion* and *Aglaothamnion* in a next chance.

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Sung-Min Boo*· Jan Rueness**· In Kyu Lee***: キヌイトグサ（紅藻，イギス科）の生活史と分類

韓国沿岸産の紅藻キヌイトグサ (*Callithamnion callophyllidicola* Yamada) の生活史を報告し，その分類学的諸問題を論じた。本種は韓国のほとんどの沿岸でよく生育し，通常 *Polysiphonia* 型生活史のすべての段階のものが同時に採集される。配偶体は一般に雌雄異体であるが，培養では雌雄同体の藻体から次の世代には雌雄異体の配偶体がつくられる。成長速度は，培養で調べた要因の中では，光強度より光周期に，また温度より光強度に，より依存していた。四分胞子発芽体は，強光長日条件下ではすべて同調的に成熟したが，短日または弱光条件下では雄性配偶体がまず成熟した。四分胞子嚢の形成も，より光周期に依存していた。本種は，形態的にはヒナノキヌイトグサ (*C. minutissima* Yamada) および *Aglaothamnion oosumiense* Itono に似ており，これらの分類学的相互関係はタイプ標本の比較によって検討されなければならない。(*Department of Biology, Chungnam National University, Daejeon 301–764, Korea; **Department of Biology, Marine Botany, Oslo University, Oslo 3, Norway; ***Department of Botany, Seoul National University, Seoul 151–742, Korea)