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Morphology and Life History of *Stylonema cornu-cervi* Reinsch (Goniotrichales, Rhodophyta) from Japan

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The morhology and life history of *Stylonema cornu-cervi* Reinsch from Japan were investigated. The species had multiseriate erect thalli from a basal cell. The thalli usually branched dichotomously, occasionally trichotomously near the base, and non-branched thalli were sometimes observed. A dichotomous branch on the upper portion near the base occurred only one time on each erect branch. Cells contained a stellate chloroplast, which was composed of a central rounded part with an obscure pyrenoid and 5-8 cup-like lobes connected to the central part by a small thin stipe. The biseriate part was observed on the six-celled stage in culture, and the grown thalli were multiseriate except for base and apices. Monospores forming from the immediate transformation of vegetative cells were observed. Thalli grew at 15-25°C and died at 10 and 30°C. The fastest growth and maturation were observed under 25°C and 14L:10D. Although *S. alsidii* (Zanardini) Drew usually had uniseriate thalli, irregularly branched multiseriate thalli had been reported in cultures. It is possible that in the previous report the thalli were confused with *S. cornu-cervi*. In this report, *S. cornu-cervi* were distinguished from *S. alsidii* in that the branches were few, the multiseriate portions were observed on the early stage (six-celled stage), and the grown thalli were multiseriate except at the base and apices.

Key Words: Goniotrichales, life history, morphology, Rhodophyta, *Stylonema cornu-cervi*

INTRODUCTION

Goniotrichales (Bangiophycidae, Rhodophyta) was established by Skuja (1939). Goniotrichaceae and Phragmonemataceae are included in this taxon, and the former was reported from Japan (Yoshida *et al*. 2000; Kumano 2000). Goniotrichaceae has 4 genera (*Bangiopsis, Chroodactylon, Colacodictyon* and *Stylonema*) in Japan (Yoshida *et al*. 2000), and their taxonomical and morphological observations were reported (Segawa 1941; Tanaka 1952; Kajimura 1992). Of them, the life history of *Stylonema alsidii* (Zanardini) Drew was the only one reported by Notoya *et al*. (1993). They observed the irregularly branched multiseriate thalli and irregularly grown clumpy thalli distinct from field-collected ones when the alga was cultured under various conditions and discussed that it was possible that the irregularly branched multiseriate thalli were *Stylonema cornu-cervi* Reinsch, although they did not observe *S. cornu-cervi* in culture.

Stylonema cornu-cervi, the type species of the genus *Stylonema*, is common in warm temperate seas around the world and epiphytic on subtidal macroalgae (Yoshida 1998; Abbott 1999). The morphology and distribution of the species were reported by Reinsch (1875), Howe (1914), Segawa (1941), Tanaka (1952), Abbott and Hollenberg (1976), Garbary *et al*. (1980), Bolton and Stegenga (1987), Lee *et al*. (1992), Womersley (1994), Abbott (1999), Sansón *et al*. (2002) and Brodie and Irvine (2003). The life history and morphological variation in culture, however, have not yet been reported. We studied the culture experiments and morphological observations on *S. cornu-cervi* from Japan in order to clarify the life history and the taxonomical relation with *S. alsidii*.

MATERIALS AND METHODS

Stylonema cornu-cervi specimens were collected at two localities from the southern parts of Chiba Prefecture, Japan by SCUBA diving: a depth of 20 m off Hasama

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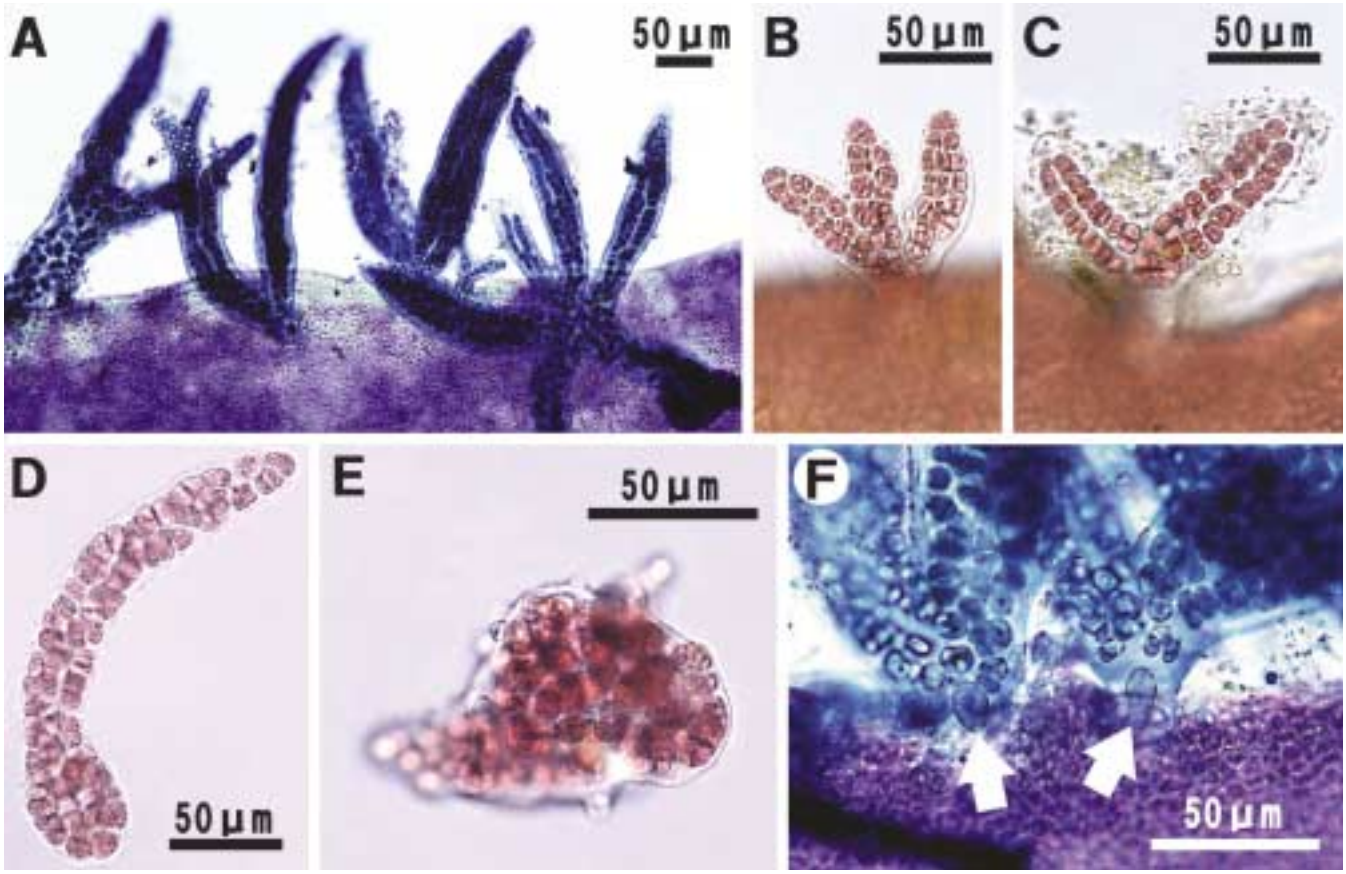

Fig. 1. *Stylonema cornu-cervi.* A. Slide specimen of field-collected thalli attached on *Callophyllis* sp. collected on 11 April 1998 from a depth of 20m off Hasama, Tateyama-city, Chiba Prefecture, Japan. B. Fresh trichotomously branched thallus collected on the same date from the same locality. C. Fresh dichotomously branched thallus collected on the same date from the same locality. D. Cultured non-branched thallus at 20°C under a long-day photoperiod (40 μmol m^{-2} s^{-1}). E. Cultured irregularly grown clumpy thallus at 25°C under a long-day photoperiod (40 μmol m^{-2} s^{-1}). F. Basal cells (arrows) of field-collected thalli attached to *Callophyllis* sp. collected on 11 April 1998 from a depth of 20 m off Hasama, Tateyama-city, Chiba Prefecture, Japan. Slide specimen.

(34°58'N, 139°46'E), Tateyama-city (15 March 1997 and 11 April 1998) and depths of 12-20 m off Ubara (35°07'N, 140°16'E), Katsuura-city (19 July 2000 and 4 April 2003). All specimens were epiphytic on red alga, *Callophyllis* spp. Fresh materials and some thalli stained with 0.4-4% (w/v) aniline blue in glycerol/seawater (1:1) solution were observed with a light microscope.

Unialgal cultures were obtained from isolated monospores of plants collected from Ubara, Katsuura-city on 4 April 2003. The released monospores were rinsed once in autoclaved seawater and attached to slide glasses using glass capillary pipettes and cultured in a glass tube containing about 50 ml of a modified Grund medium (Brown *et al.* 1977). The cultures were placed in plant growth chambers illuminated with cool white fluorescent lamps (40 μmol m^{-2} s^{-1}). The temperatures were 10, 15, 20, 25 and 30°C, and photoperiods of long- (14L:10D) and short-day (10L:14D), each at 40 μmol m^{-2} s^{-1}, were used. In addition, the fresh medium was replaced every three days throughout the experiment. Cultured thalli were observed with a light microscope without staining.

The cells of some cultured fresh materials were observed with a confocal laser scanning microscope (FMV500, Olympus, Japan) for the observation of the shape of chloroplast. The 488 nm line of an argon ion laser was used as the excitation wavelength, and the emission was recorded at 515-565 nm. The digital images of autofluorescence of chloroplasts were stored on an MO disk.

RESULTS

Morphology of field-collected and cultured thalli

Thalli are erect, multiseriate and usually dichotomously branched (Fig. 1A, C), occasionally

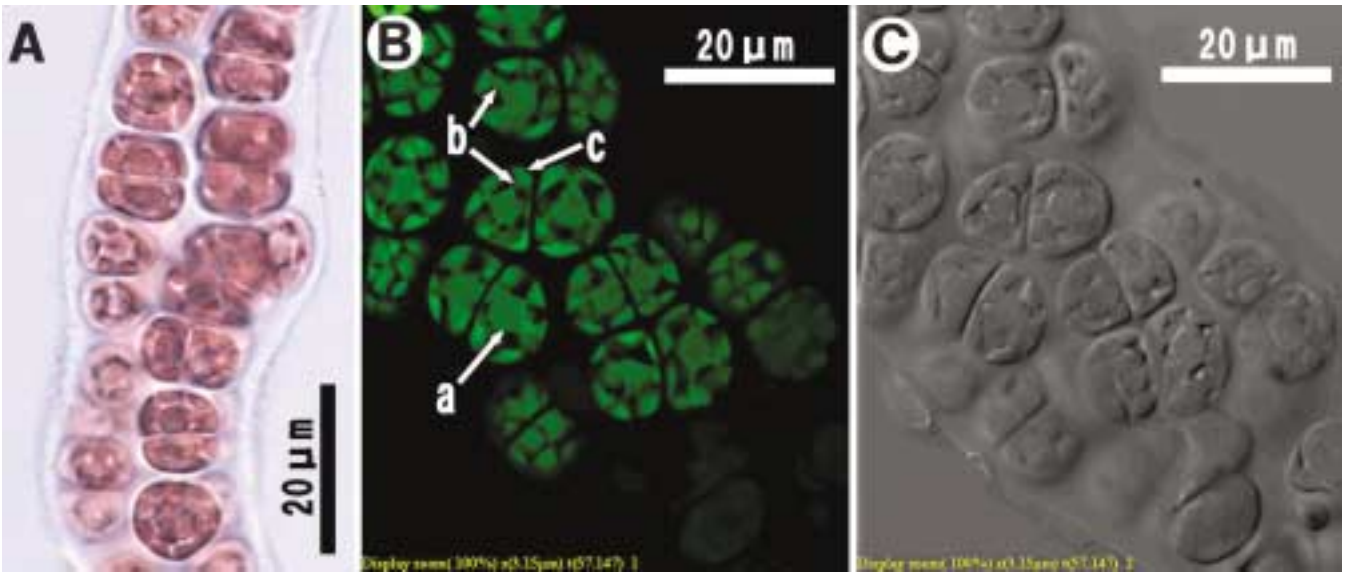

Fig. 2. The shape of chloroplasts of cultured thalli from *Stylonema cornu-cervi*. A stellate chloroplast presents in each cell. A. The image from a light microscopy. B. The image from a confocal laser scanning microscopy; a, central rounded part of a stellate chloroplast; b, small thin stipes connect a cup-like lobe with central rounded part; c, a cup-like lobe. C. The image from Nomarski interference microscopy of the same part of the thallus shown in B.

trichotomously (Fig. 1B) near the base; non-branched thalli sometimes are present (Fig. 1D), and only one time dichotomous branch pattern on each erect branch at the upper portion off the base is present. In culture, occasionally irregularly grown clumpy thalli are present (Fig. 1E). Thalli up to 500 μm in height, branches 27-52 μm in width, and 54-75 μm at the dichotomous part are observed. Grown thalli are multiseriate except at the base and apices. Cell arrangement is initially biseriate and subsequently irregularly multiseriate in well grown thalli. Cells are embedded in a mucilaginous matrix, rectangular with rounded angles, rounded and polygonal, dark brownish red in color, 3.2-9.0 μm in height, 4.9-11.4 μm in width on rectangular cells; the base consists of a longitudinal elliptical cell (Fig. 1F), 12.2-19.6 μm in height, 6.4-11.4 μm in width; a chloroplast (Fig. 2A-C) is present in each cell, stellate which is composed of a central rounded part with an obscure pyrenoid (Fig. 2B-a), and 5-8 cup-like lobes (Fig. 2B-c) connected on the central part by a small thin stipe (Fig. 2B-b). Pit connection is absent; monospores (Fig. 3A) formed from immediate transformation of vegetative cells (Fig. 3L-M), rounded, 10.5-12.0 μm and an average 10.8 μm in diameter, brownish red in color, grow into thalli similar with their parent.

Life history in culture

Released monospores (Fig. 3A) attached to slide glasses and divided into two cells after one day in culture at 25°C under long-day condition (Fig. 3B). The one cell became a basal cell, and the other divided into two cells after 3 days (Fig. 3C). Subsequently, cells divided in the direction of length and became erect thalli. The biseriate portion due to the sideways cell division of the lower part of the body were observed on the 6-8 cell-stage after 6 days (Fig. 3D). Uniseriate thalli composed of more than 8 cells were not observed. Monospores were released from the middle portion of a thallus after 6 days, and the released monospores attached to slide glasses and grew into thalli similar to their parents. A new branch was commonly produced from near the base due to the cell division of the basal cell when the erect thalli were at the 3 cell-stage after 6 days (Fig. 3E), and the thalli became dichotomous (Fig. 3F). Many dichotomously branched thalli became multiseriate on the lower portion after 9 days (Fig. 3G), and a few trichotomously branched thalli near the base were also observed (Fig. 3H). Some non-branched multiseriate thalli were also observed (Fig. 1D). A new dichotomous branching at the middle portion occurred on a few thalli (Fig. 3I). Branches at the upper portion of the thalli were rarely observed. Thalli reached to 200 μm high in maximum and branches 25 μm wide after 9 days. The sideways cell division at the middle portion occurred at random, cells irregularly arranged, and almost all thalli became multiseriate except at the base and apices after 12

Fig. 3. Cultured thalli of *Stylonema cornu-cervi* at 25°C under a long-day photoperiod (40 μmol m^{-2} s^{-1}). A. Monospore. B. 2-celled thallus (one-day-old). C. 3-celled thallus (3-day-old). D. Biseriate thallus (6-day-old). E-F. Dichotomously branched thalli from a basal cell (6-day-old). G. Dichotomously branched multiseriate thallus (9-day-old). H. Trichotomously branched thallus (9-day-old). I. New dichotomous branching had occurred on a branch (9-day-old). J. Well matured, two times dichotomously branched multiseriate thallus (12-day-old). K. Trichotomously branched multiseriate thallus (12-day-old). L. Monosporangium (arrow)(12-day-old). M. Released monospore (arrow) from the monosporangia of L. (12-day-old). N. Germlings from released monospores attached to the parental thallus. They were not branches since the basal cells of germlings were apart from the cells of parental thallus (arrows) (12-day-old).

days (Fig. 3J-K). Monospores were formed by the immediate transformation of vegetative cells (Fig 3L-M), and the released monospores grew into thalli similar to their parents. A few monospores germinated like parental branches attaching to the parental or adjacent thalli, but the cells of the germlings presented somewhat apart from the cells of the substrata branched differently (Fig. 3N). After 15 days, thalli were composed of more than 800 cells and reached to 500 μm high in maximum and branches of 40 μm wide. The number of branches did not increase. Sexual reproduction and asexual reproduction, except for monospores, were not observed.

Influence of temperature and photoperiod on growth and maturation

The growth and maturation at various temperatures

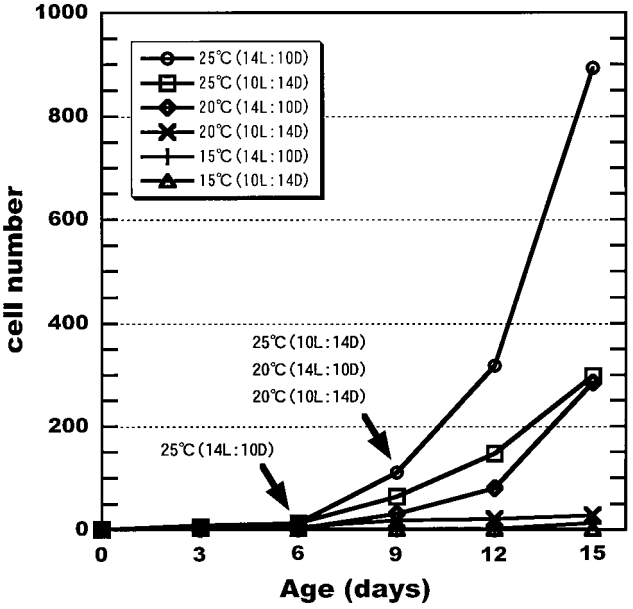

Fig. 4. The influence of temperature and photoperiod on the growth and maturation of *Stylonema cornu-cervi* under 40 μmol. Arrows indicate when the release of monospores was observed. At 10 and 30°C, monospores attached to slide glasses did not grow and died within 15 days.

under long-and short-day are shown in Fig. 4. Monospores attached on slide glasses died within 3 days at 30°C and 15 days at 10°C without division. At 15-25°C, the growth of thalli tended to be faster at high temperature than those at low temperature, and at the same temperature faster under long-day than those under short-day. At 15°C under short-day, monospores remained a cell after 15 days in culture, but they did not die. First release of monospores was observed at 25°C under long-day after 6 days. At 20-25°C, monospores were released under both photoperiods after 9 days, but at 15°C, monospores were not released within 15 days. Dichotomously (Fig. 3G, I, J) or trichotomously (Fig. 3H, K) branched thalli were observed at 20°C but only under the long-day photoperiod, while at 25°C, both kinds of thalli were recorded under both photoperiods. Non-branched thalli (Fig. 1D) were observed at 15-25°C under both photoperiods within 15 days. Moreover, a few irregularly grown clumpy thalli (Fig. 1E) were observed at 20-25°C.

DISCUSSION

In this study, morphology of field-collected and cultured thalli of *Stylonema cornu-cervi* largely agreed with previous reported plants (Segawa 1941, Tanaka 1952, Abbott and Hollenberg 1976, Lee *et al.* 1992, Abbott 1999, Sansón *et al.* 2002): multiseriate erect thalli branched dichotomously or trichotomously, or thalli were non-branched from a basal cell. Thalli became biseriate in part on the 6-8 cell-stage, and subsequently grew multiseriate except at the base and apices. Branching was mostly limited near the base and a few thalli branched at the middle portion. The number of branching were few. *S. alsidii* grew uniseriate thalli with many branches, and subsequently became multiseriate in culture (Notoya *et al.* 1993). Since the process of becoming multiseriate distinguished *S. cornu-cervi* from *S. alsidii*, it is concluded that *S. cornu-cervi* is a distinct species from *S. alsidii*. There is a possibility that *S. cornu-cervi* and the multiseriate thalli of *S. alsidii* were confused in nature; however, *S. cornu-cervi* is distinguished from *S. alsidii* in that there are very few branches in *S. cornu-cervi*.

Garbary *et al.* (1980) reported *S. cornu-cervi* from British Columbia and Northern Washington. They showed the illustration (p. 145, Fig. 1c) of *S. cornu-cervi* with somewhat long uniseriate parts in the upper portion of the thalli. We have never observed such a thallus in field-collected or cultured Japanese plants. Lee *et al.* (1992) reported the same results as ours on the materials from Korea. There is the possibility that the plant illustrated by Garbary *et al.* (1980) as *S. cornu-cervi* is multiseriate *S. alsidii*.

The shape of chloroplast was stellate with an obscure pyrenoid: it agreed with previous reported plants. However, the shape has never been reported in detail. We observed the detailed shape of chloroplast with a confocal laser scanning microscope for the first time, and we confirmed that the stellate chloroplast of *S. cornu-cervi* was composed of a central rounded part with an obscure pyrenoid and 5-8 cup-like lobes connected on the central part by a small thin stipe.

Only asexual reproduction by monospores formed by the immediate transformation of vegetative cells was observed. Sexual reproduction was not observed in field-collected or cultured thalli. Therefore, the species probably has only an asexual life history.

In this study, *S. cornu-cervi* died at low (10°C) and high temperatures (30°C). The results were different from *S. alsidii*, in which thalli grew and matured at 30°C and survived at 10°C, although they did not grow (Notoya *et al.* 1993). *S. cornu-cervi* grows in the subtidal zone (10m deep or more) of Susaki, Shizuoka Prefecture and Hayama and Misaki, Kanagawa Prefecture in Japan (Tanaka 1952), while *S. alsidii* grows in the intertaidal or

upper subtidal zone of wide reqions from Hokkaido to Ryuku Islands in Japan (Tanaka 1952). The difference in growing zones of the two species may cause the difference in optimum growth temperature. At Katsuura, the monthly average water temperature at 12m in depth was 23.6°C, the maximum in summer and 14.5°C, the minimum in winter in 2003. At Katsuura, the growing zone of *S. cornu-cervi* did not reach at 10 and 30°C which *S. cornu-cervi* died in this culture. *S. cornu-cervi* may grow under a more changeless environment than *S. alsidii*.

The growth and maturation of *S. cornu-cervi* was faster at 25°C from 15 to 25°C. The phenology of *S. cornu-cervi* was never been studied. The species may grow fast from May to December around the southern part of Chiba Prefecture, in which the monthly average water temperature is more than 15°C.

A few irregularly grown clumpy thalli, distinct from field-collected thalli, were observed at 20-25°C. For *S. alsidii*, many similar thalli were observed at 30°C (Notoya *et al.* 1993). The morphological variations of *S. cornu-cervi* were less than that of *S. alsidii*. Since the irregularly grown clumpy thalli have never been observed in the field, the thalli may appear only under culture conditions.

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