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Goniotrichopsis reniformis (Kajimura) Kikuchi comb. nov. (Stylonematales, Rhodophyta) from Japan

Norio Kikuchi[1]*, John A. West[2], Mitsuo Kajimura[3] and Jong-Ahm Shin[4]

[1]*Coastal Branch of Natural History Museum and Institute, Chiba, Katsuura 299-5242, Japan*
[2]*School of Botany, University of Melbourne, Parkville 3010, Australia*
[3]*2589 Tsuma, Okinoshima-cho, Oki-gun, Shimane 685-0104, Japan*
[4]*Aquaculture Program, College of Fisheries and Ocean Sciences, Chonnam National University, Yeosu 550-749, Korea*

The morphology and life history of *Stylonema reniforme* Kajimura (Stylonematales, Rhodophyta) from Japan were investigated and its taxonomic placement was discussed. This species has 6-30 discoid chloroplasts devoid of a pyrenoid in each cell. This is a typical feature of the genus *Goniotrichopsis*. The species reproduced only by monospores, which were formed by the direct transformation of the vegetative cells similar to the type species *Goniotrichopsis sublittoralis* Smith. *Goniotrichopsis reniformis* (Kajimura) Kikuchi comb. nov. was proposed. The asexual life history of the present species was completed in 3-10 weeks at 15-20°C in culture.

Key Words: *Goniotrichopsis reniformis*, life history, Rhodophyta, *Stylonema reniforme*, Stylonematales, taxonomy

INTRODUCTION

Stylonema reniforme Kajimura (Stylonematales, Rhodophyta) is a foliaceous monostromatic species described by Kajimura (1992) based on the materials collected from deep-water in the vicinity of the Oki Islands in the Sea of Japan. Kajimura (1992) assigned this species to the genus *Stylonema* according to the Kornmann and Sahling's concept of the genus (Kornmann and Sahling 1977); namely, thalli are attached to the substratum by an unmodified basal cell, vegetative cells are embedded in a mucilaginous matrix, and reproduction occurs by transformation of vegetative cells into monosporangia. A single stellate chloroplast with a pyrenoid in each cell is one of the generic character of this genus (Tanaka 1952, Abbott and Hollenberg 1976). Kajimura (1992) described that each cell of the species had a stellate chloroplast and a central pyrenoid that was distinct in monosporangia but not as evident in vegetative cells. However, during course of our study to clarify the life history of this species, we found that the species has 6-30 discoid chloroplasts devoid of a pyrenoid in each cell. In the character of chloroplasts the present species accords well with the type species of the genus, *Goniotrichopsis sublittoralis* Smith. In this paper, we propose a new combination, *Goniotrichopsis reniformis*, for the species, and clarify the life history in culture.

*Corresponding author (kikuchin@chiba-muse.or.jp)

MATERIALS AND METHODS

Field materials were collected with a dredge from a depth of 25 m off Tsudo (36°09'N, 133°14'E), the Oki Islands in the Sea of Japan (27 April 1992) (see Kajimura 1981, 1987, 1992) and at two localities from the southern parts of Chiba Prefecture, Japan by SCUBA diving: a depth of 20 m off Hasama (34°58'N, 139°46'E), Tateyama (15 March 1997 and 11 April 1998) and a depth of 12 m off Ubara (35°07'N, 140°16'E), Katsuura (4 April 2003). Specimens from Tsudo were epiphytic on *Stenogramma interrupta* (C. Agardh) Montagne, those from Hasama on *Callophyllis* sp. and those from Ubara on *Callophyllis adhaerens* Yamada. 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 (E600, Nikon, Japan). Herbarium specimens deposited in National Science Museum, Tokyo (TNS) and Coastal Branch of Natural History Museum and Institute, Chiba (CMNH) were also observed.

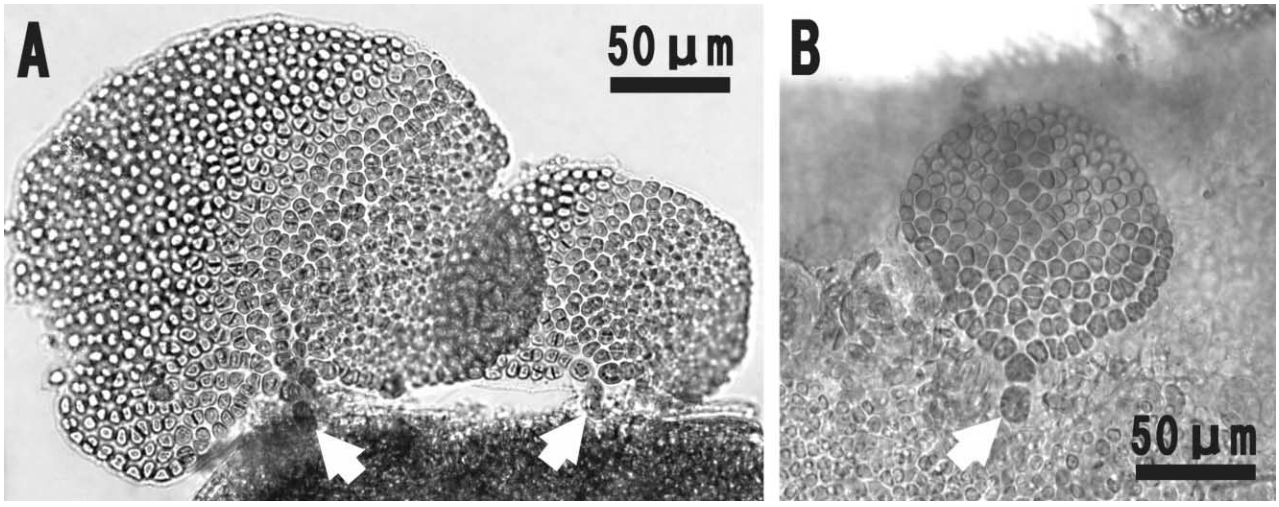

Fig. 1. Field-collected thalli of *Goniotrichopsis reniformis* comb. nov. Arrows indicate basal cells. A. Fresh thalli attached on *Stenogramma interrupta* collected on 27 April 1992 from depth of 25 m off Tsudo, Oki Islands, Shimane Prefecture, Japan. B. Fresh thallus attached on *Callophyllis* sp. collected on 11 April 1998 from depth of 20 m off Hasama, Tateyama, Chiba Prefecture, Japan.

Unialgal cultures were obtained from isolated monospores of thalli collected from Tsudo and Ubara. 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. The temperatures were 10, 15, 20, 25 and 30°C, and photoperiods of long-day (14L:10D) and short-day (10L:14D) at 10, 20, 40 and 80 μmol m^{-2} s^{-1} were used. The medium was replaced every three days throughout the experiment. Cultured thalli were observed with the light microscope without staining.

For observations of chloroplast autofluorescence, cultured fresh materials were viewed with a confocal laser scanning microscope (FMV500, Olympus, Japan) in which the 488 nm line of an argon ion laser was used as the excitation wavelength, and the emission was recorded at 515-565 nm, and with an epifluorescence microscope (E800, Nikon, Japan) under UV excitation. For observations of nucleus, cells were fixed with 1% glutaraldehyde in 50 mmol L^{-1} Tris-HCl buffer (pH 7.6) for 10 min at 4°C. They were stained with a mixture of 1 μg mL^{-1} 4′, 6-diamidino-2-phenylindole (DAPI) dissolved in the same buffer for 16 h at 4°C, and viewed with the epifluorescence microscope under UV excitation and with E800 fitted with the interference differential contrast objectives.

RESULTS

***Goniotrichopsis reniformis* (Kajimura) Kikuchi comb. nov.**

The thalli are microscopic, erect, foliaceous, reniform, monostromatic, epiphytic, and 100-1800 μm wide, 100-1200 μm high; margin entire, unbranched and occasionally lobed; base consists of a short stipe of one to three cells; vegetative cells polyhedral, round, dark brownish-red, 4-10 μm long, 3-8 μm wide and embedded in a mucilaginous matrix; chloroplasts discoid, without a pyrenoid, 6-30 in each cell; monosporangia formed by the direct transformation of vegetative cells.

Basionym: *Stylonema reniforme* Kajimura 1992, p. 415. figs. 1-12.

Holotype: TNS-AL-39664 (OS10085-A), collected at 40 m depth off Tsudo, Oki Islands (36°09′N, 133°14′E) on 27 April 1990, by M. Kajimura.

Isotype: TNS-AL-39665 (OS10085-B)

Japanese name: Nisebeniuchiwa

Other specimens examined: Hasama, Tateyama, Chiba (15 March 1997, leg. N. Kikuchi, CMNH-BA-5863-5868), Ubara, Katsuura, Chiba (4 April 2003, leg. N. Kikuchi, CMNH-BA-5869), Mimai-zaki, Misaki, Ehime (20 April 1988, leg. S. Ninomiya, CMNH-BA-5861), Ikata, Ehime (27 May 1988, leg. S. Ninomiya, CMNH-BA-5862).

Morphology

The mature thalli are entire, reniform, 100-1800 μm

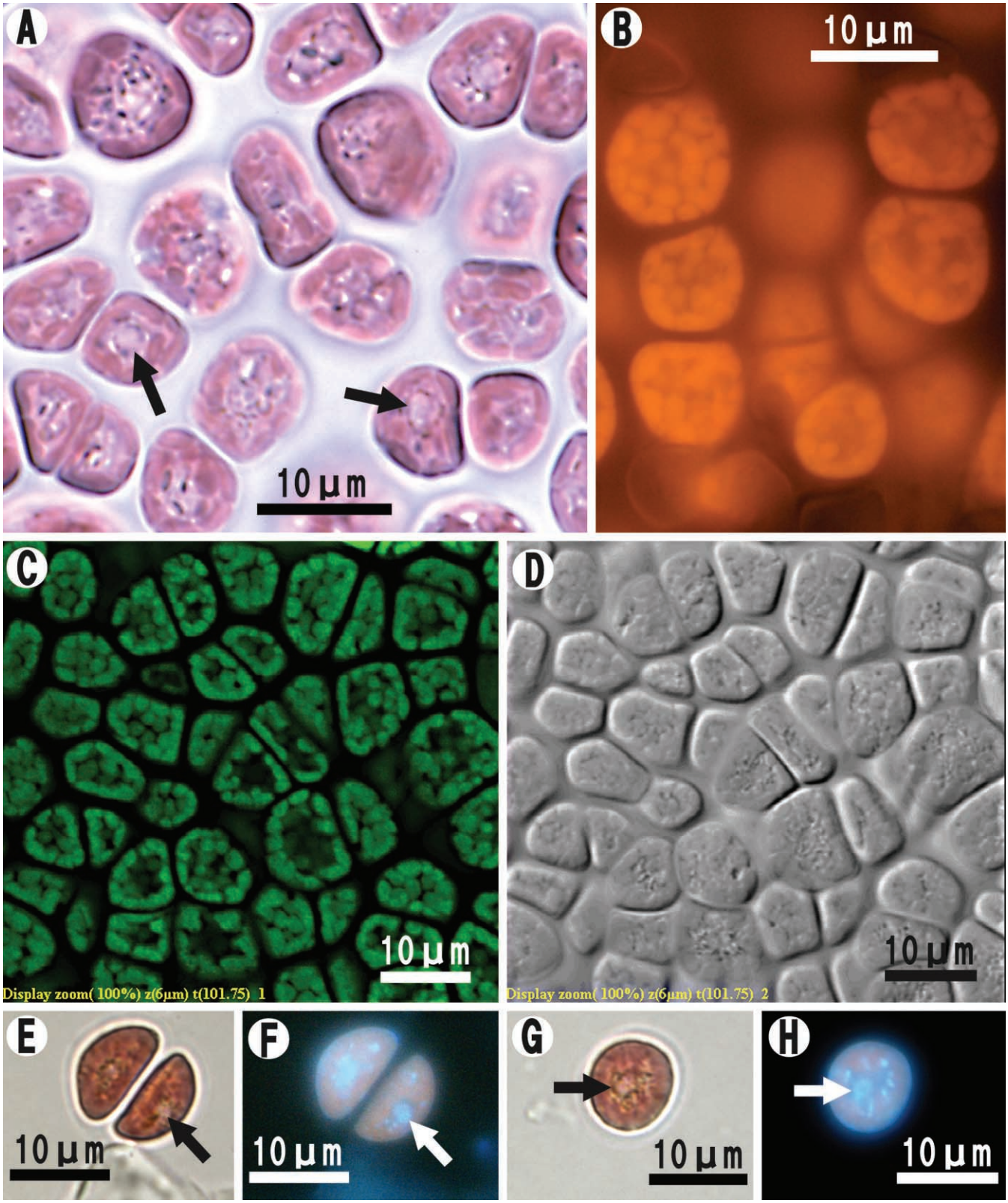

Fig. 2. The cells of cultured thalli of *Goniotrichopsis reniformis* comb. nov. Each cell has 6-30 discoid chloroplasts devoid of pyrenoid. A. The image of a light microscopy. Arrows indicate the central nuclei. B. The image of an epifluorescence microscopy. C. The image of a confocal laser scanning microscopy. D. The image of Nomarski interference microscopy of the same part of the thallus of C. E-H. Vegetative cells stained with DAPI. Arrows indicate central nuclei. E and G. The image of a differential interference contrast microscopy. F and H. The image of an epifluorescence microscopy of the same cells of E and G, respectively.

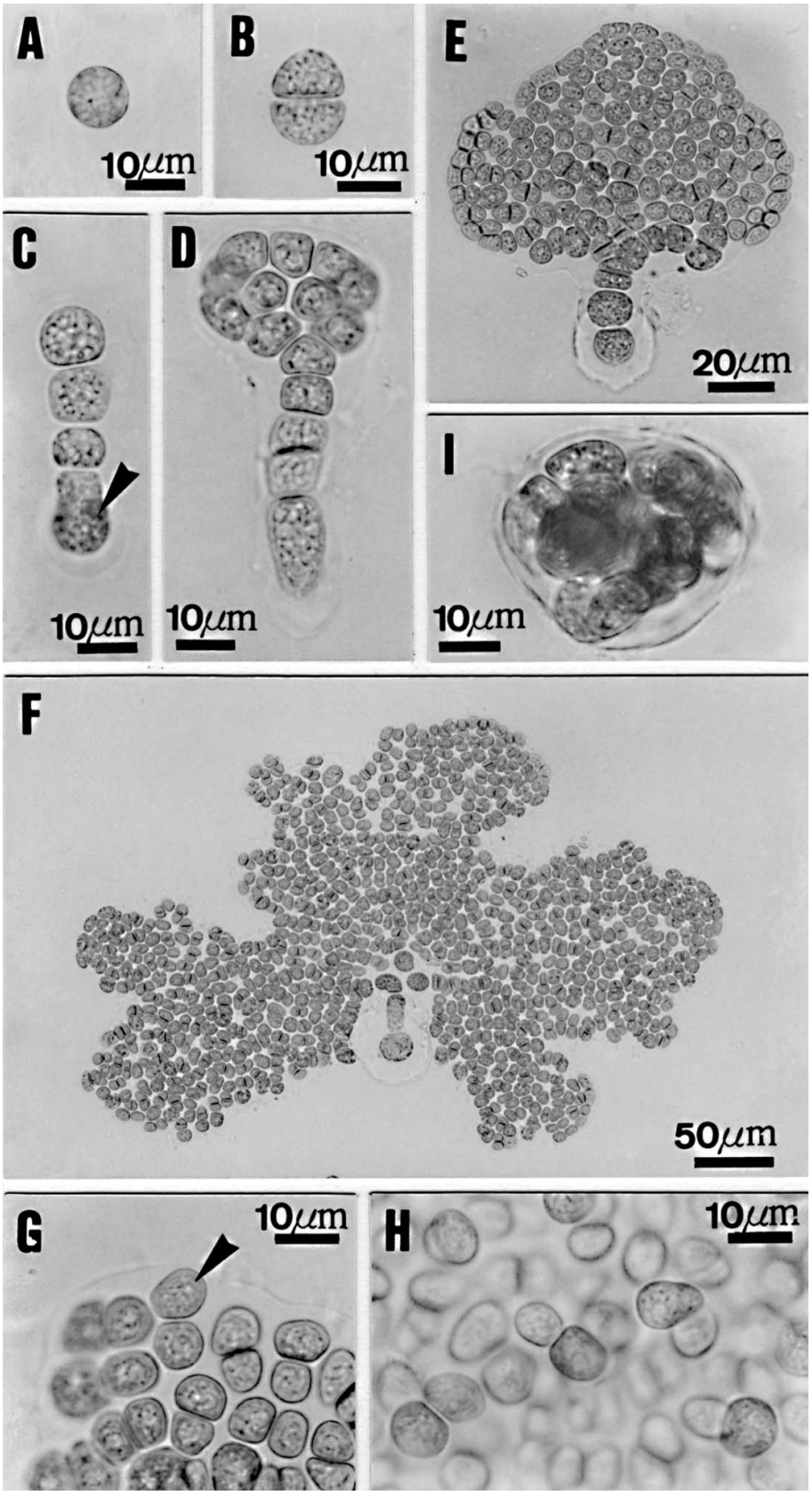

Fig. 3. *Goniotrichopsis reniformis* comb. nov. cultured at 20°C and 40 μmol m^{-2} s^{-1} under long-day (14L:10D). A. Monospore. B. Divided monospore after 3 days. C. Monospore germling after 9 days. Arrowhead indicates a basal cell. D. Monospore germling after 12 days. E. Monospore germling similar to field-collected thalli after 18 days. F. Monospore germling lobed after 2 months. G. Monosporangium (arrowhead). H. Distromatic region. I. Clumpy thallus after 18 days.

wide, 100-1200 μm high, and with a short stipe consisting of one to three cells (Fig. 1A, B). Basal cells are larger than the others (Fig. 1A, B, arrows). Vegetative cells are polyhedral to round, dark brownish-red in color, 4-10 μm long, 3-8 μm wide and embedded in a mucilaginous matrix. Pit connections are absent. Each cell contains 6-30 discoid chloroplasts devoid of a pyrenoid (Fig. 2A-D). A central nucleus is observed in each cell (Fig. 2A, E-H, arrows). Monosporangia (Fig. 3A) are formed by the direct transformation of vegetative cells. The released monospores are 8.5-13.6 μm (mean 11.4 ± 1.9 μm) in diameter.

Habitat and phenology

The species grows in the subtidal zones at depths of 10-40 m, epiphytic on other algae, such as *Dilophus okamurae* Dawson, *Callophyllis* spp. and *Stenogramma interrupta*. The thalli were observed growing from winter to spring.

Geographical distribution

The species is reported only from Japan: Katsuura and Tateyama, Chiba Prefecture, the Oki Islands, Shimane Prefecture and Misaki and Ikata, Ehime Prefecture.

Life history in culture

A released monospore (Fig. 3A) attached on a glass slide and divided into two cells after 3 days at 20°C and 40 μmol m^{-2} s^{-1} under long-day (Fig. 3B). Either cell divided to form a three-celled uniseriate filament in about 9 days (Fig. 3C). The undivided cell became a holdfast (Fig. 3C, arrowhead). The upper cells divided repeatedly in two dimensions, and the upper portion of the thallus became more or less elliptic in 12 days (Fig. 3D). The germlings became 100 to 180-celled, matured in 18 days and were very similar to the natural thalli (Fig. 3E). Some vegetative cells were released as monospores (Fig. 3G, arrowhead). Sometimes cells divided in three dimensions, and formed partial two-cell layers (Fig. 3H). A few thalli did not become foliaceous, but remained clumpy (Fig. 3I), and released monospores. Several foliose thalli continued to grow even after releasing monospores and formed several lobes, reaching a width of 400-500 μm in 2 months (Fig. 3F). We have studied the life history of this species repeatedly, and have determined that reproduction is asexual only by means of monospores.

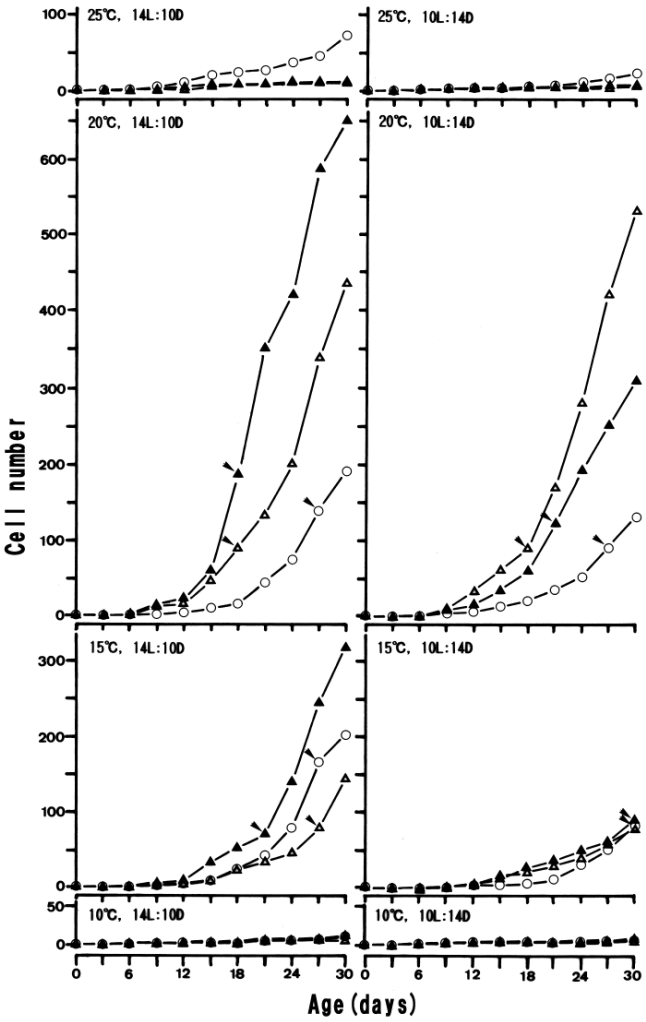

Fig. 4. The influence of temperature, photon flux density and photoperiod on the growth and reproduction of *Goniotrichopsis reniformis* comb. nov. Arrowheads indicate when the release of monospores was observed. At 30°C and at 80 μmol m^{-2} s^{-1}, monospores attached on slide glasses did not grow and died. △, 40 μmol m^{-2} s^{-1}; ▲, 20 μmol m^{-2} s^{-1}; ◯, 10 μmol m^{-2} s^{-1}.

Influence of temperature, photon flux density and photoperiod on growth and reproduction

Growth rates were followed at 10, 15, 20 and 25°C (Fig. 4). At 10°C sporelings reached a maximum of 10 cells at 30 days. Growth was optimum at 20°C with less growth at 15 and 25°C. Monospores died in 6 days at 30°C, so the growth curve was not shown in Fig. 4. At 20°C in a long-day photoperiod the optimum photon flux density was 20 μmol m^{-2} s^{-1}. However in the short-day photoperiod at 20°C optimum growth occurred at 40 μmol m^{-2} s^{-1}. At all temperatures with 80 μmol m^{-2} s^{-1} all thalli died. At 10, 15 and 25°C growth was optimum at 10-20 μmol m^{-2} s^{-1}. Monospore release occurred at 15 and 20°C within 30

days. The generation time usually was shorter under optimum growth conditions. Some irregular clumpy thalli were evident in most conditions.

DISCUSSION

Kajimura (1992) originally assigned this species to the genus *Stylonema* according to the Kornmann and Sahling's concept (Kornmann and Sahling 1977); attaching to the substratum by an unmodified basal cell, thallus consisting of the cells embedded in a mucilaginous matrix and reproduction by the transformation of entire vegetative cells into monosporangia. We could reconfirm these features in the present study. These characteristics in the present species accord with the recent circumscription of the order Stylonematales (as Porphyridiales) (Garbary *et al.* 1980a).

A single stellate chloroplast with a pyrenoid is characteristic of the genus *Stylonema* (Tanaka 1952, Abbott and Hollenberg 1976, Garbary *et al.* 1980b). Kajimura (1992) originally mentioned that this species has a single stellate chloroplast with a distinct or indistinct pyrenoid. However, we have shown that this species has 6-30 discoid chloroplasts devoid of a pyrenoid in each cell. Kajimura (1992) misinterpreted a group of the multiple discoid chloroplasts as a stellate chloroplast. Kajimura (1992) illustrated the central pyrenoid in a monosporangium (p. 417, Fig. 12), but in this study we have shown that it is the central nucleus. The genus *Goniotrichopsis* is known to have several to many discoid chloroplasts devoid of a pyrenoid in each cell (Smith and Hollenberg 1943, Garbary *et al.* 1980b). As a result, *Stylonema reniforme* is transferred to the genus *Goniotrichopsis* and new combination, *G. reniformis*, is therefore necessary.

Goniotrichopsis is monotypic. Thalli of the type species *Goniotrichopsis sublittoralis* Smith are microscopic and attain a height of 1 mm consisting of uniseriate to multiseriate pseudofilaments, branched three to four times (Smith and Hollenberg 1943, Garbary *et al.* 1980b). The present species differs from *G. sublittoralis* in that the thalli are foliaceous, monostromatic reniform blades that are not branched. *G. sublittoralis* has been reported from the northeastern Pacific (Smith and Hollenberg 1943, Norris and West 1967, Garbary *et al.* 1980b), the English Channel and the Mediterranean (Magne 1992) to date and the second species *G. reniformis* is still known to only exist in Japan.

The reproduction by monospores of this species is consistent with Kajimura's observations (Kajimura 1992). Therefore, the species probably has only an asexual life history (Fig. 5). This is consistent with observations on all other genera of the Stylonematales (Garbary *et al.* 1980b).

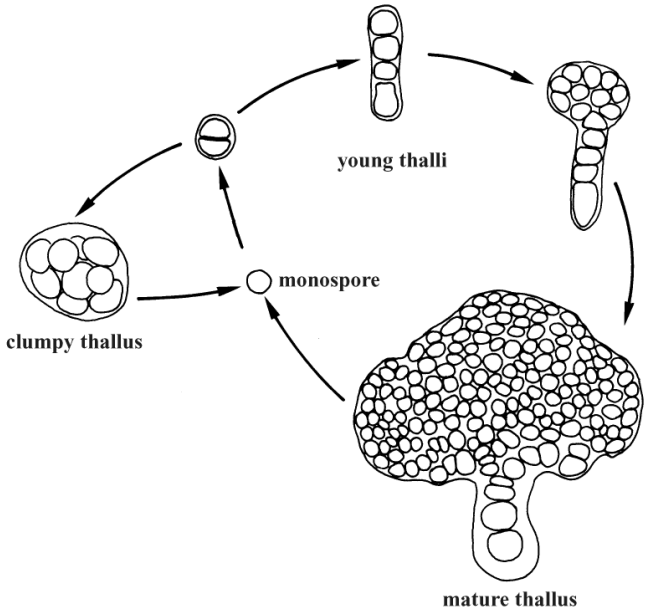

Fig. 5. The life history of *Goniotrichopsis reniformis* comb. nov.

The optimum temperature range for the growth of the species support its specific habitat at a depth of between 10 and 40 m where the seasonal temperature remains in the optimum range, *i.e.*, 12-13°C in winter and 18-19°C in summer in the Oki Islands (Kajimura 1987), and the monthly average water temperature at 12 m in depth was 23.6°C, the maximum in summer and 14.5°C, the minimum in winter in 2003 at Katsuura (Kikuchi and Shin 2005).

For *Stylonema alsidii*, many irregular clumpy thalli were observed at 30°C (Notoya *et al.* 1993), and for *S. cornu-cervi*, a few ones were observed at 20-25°C (Kikuchi and Shin 2005). *G. reniformis* has the similar morphological variations with *S. alsidii* and *S. cornu-cervi* in most conditions. Since the thalli have never been observed in the field, the thalli may appear only under culture conditions.

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REFERENCES

Abbott I. A. and Hollenberg G. J. 1976. *Marine Algae of California*. Stanford Univ. Press, Stanford.

Brown V., Ducker S. C. and Rowan K. S. 1977. The effect of orthophosphate concentration on the growth of articulated coralline algae (Rhodophyta). *Phycologia* **16:** 125-131.

Garbary D. J., Hansen G. I. and Scagel R. F. 1980a. Revised classification of the Bangiophyceae (Rhodophyta). *Nova Hedwigia* **33:** 145-166.

Garbary D. J., Hansen G. I. and Scagel R. F. 1980b. The marine algae of British Columbia and Northern Washington: Division Rhodophyta (red algae), Class Bangiophyceae. *Syesis* **13:** 137-195.

Kajimura M. 1981. *Streptophyllopsis* a new genus of Laminariaceae, Phaeophyta, from Japan. *Mem. Fac. Sci., Shimane Univ*. **15:** 75-87.

Kajimura M. 1987. Deep-water flora of benthic marine algae in the Oki Islands, Sea of Japan. *Bot. Mar*. **30:** 373-385.

Kajimura M. 1992. A new deep-water species of *Stylonema* (Stylonemataceae, Rhodophyta) from the Sea of Japan. *Bot. Mar*. **35:** 415-418.

Kikuchi N. and Shin J.-A. 2005. Morphology and life history of *Stylonema cornu-cervi* Reinsch (Goniotrichales, Rhodophyta) from Japan. *Algae* **20:** 37-42.

Kornmann P. and Sahling P.-H. 1977. Meeresalgen von Helgoland. Benthische Grün-, Braun- und Rotalgen. *Helgoländer Wiss. Meeresunters*. **29:** 1-289.

Magne F. 1992. *Goniotrichopsis* (Rhodophycees, Porphyridiales) en Europe. *Cryptogamie, Algol*. **13:** 109-112.

Norris R. E. and West J. A. 1967. Notes on marine algae of Washington and southern British Columbia. II. *Madroño* **19:** 111-116.

Notoya M., Kikuchi N. and Aruga Y. 1993. Life history of *Stylonema alsidii* (Rhodophyta) in culture. *Jpn. J. Phycol*. **41:** 43-46. (in Japanese)

Smith G. M. and Hollenberg G. J. 1943. On some Rhodophyceae from the Monterey Peninsula, California. *Amer. J. Bot*. **30:** 211-222.

Tanaka T. 1952. The systematic study of the Japanese Protoflorideae. *Mem. Fac. Fish., Kagoshima Univ*. **2:** 1-92.

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