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Reproductive structure of *Caloglossa ogasawaraensis* Okamura (Ceramiales, Rhodophyceae) in nature and culture

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Reproductive structures of *Caloglossa ogasawaraensis* Okamura are described based on the field and cultured plants from brackish areas in the Pacific coast of Japan. Tetrasporangia, carpogonia and cystocarps, and spermatangia develop on separate thalli in wild populations. Reproductive characteristics of cystocarpic and tetrasporic plants agree well with those given by Okamura. The spermatangium and carpogonial branch of this taxon are described for the first time. A carpogonial branch is composed of a four-celled filament arising on a supporting cell, which is a common feature in the Ceramiales. Tetrasporic, female and male plants obtained from culture produced tetrasporangia, many pseudocystocarps and abundant spermatangia respectively.

Key Index Words: Caloglossa ogasawaraensis—*Ceramiales—Delesseriaceae—Morphology—red algae—Reproduction—Rhodophyceae—Taxonomy.*

The genus *Caloglossa*, which comprises at least 6 species, is widely distributed not only in many marine or brackish habitats but also in the fresh-waters all over the world. Okamura (1897) established a new species, *Caloglossa ogasawaraensis*, based on the sterile plant from the fresh-waters in Ogasawara Islands, which could be distinguished from *Caloglossa leprieurii* by its narrower blades. It grows in brackish and fresh-waters in the temperate and tropical regions of the Indo-Pacific oceans and the southern Atlantic, such as South Africa (Goebel 1898 as *C. zanzibariensis*), Malaysia (Ratnasabapathy and Kumano 1982), India (Jose and Patel 1990), Perú (West 1991) and West Africa (Post 1966). There are several studies on taxonomical and morphological respects on *C. ogasawaraensis* (Post 1936, 1943, Fan 1952). This species is widely distributed along the south and middle parts of Japanese coasts, growing especially in brackish waters of estuaries (Umezaki 1962; Yoshizaki *et al.* 1985; Kamiya 1992).

In spite of the wide distribution of *Caloglossa*, reproductive plants, especially gametophytes have rarely been observed. During the course of taxonomical study on Japanese *Caloglossa*, senior author (J. T.) collected reproductive plants of some species, and described female and male gametophytes in *Caloglossa leprieurii* f. *continua* (Tanaka 1992). In the present paper, the reproductive structure of *Caloglossa ogasawaraensis* obtained in nature and culture is described in detail.

Materials and Methods

The materials were collected and soon after they were fixed and preserved with 1 : 2 : 2 : 5 formalin-alcohol-glycerol-seawater solution, and deposited at the National Science Museum, Tokyo (TNS-AL). Photographs were taken from the slide preparations which were mounted with 50% sugar syrup after being stained with 1% aqueous cotton blue. A Nikon photomicroscope fitted with differential interference-contrast optic was used for some photomicrographs. A freezing Microtome (American Optical Co.) was used for sectioning the materials.

Sexual plants of *Caloglossa ogasawaraensis* were also obtained by culturing tetraspores released from the materials collected in Wakayama and Chiba Prefecture. Mature tetrasporic plants were obtained by 1 year culture of vegetative plants from Miyazaki Prefecture. Cultures were incubated in a photoperiod of 14 L : 10 D under 30 μmol $m^{-2} s^{-1}$ cool-white fluorescent lamps at 20°C. Cultures were grown in plastic cups with 20-30 m*l* of 40% Provasoli's Enriched Seawater (PES: Starr and Zeikus, 1987), and media were changed once a month.

Description

Caloglossa ogasawaraensis Okamura, Bot. Mag. Tokyo, **11:** 13, figs. A-D, 1897; Icon. Jpn. Alg. **1:** 183, pl. 37: figs. 1-11, 1908.

C. zanzibariensis Goebel, Flora **85:** 65, figs. 1-6, 1898.—Okamura, Icon. Jpn. Alg. **1:** 183, 1908.

Habitat: Growing on rocks and stems of a grass *Phragmites australis* and some other grasses growing in the river mouth, often mixed with *Caloglossa leprieurii* f. *continua* and *Bostrychia moritziana* in the upper to lower parts of the intertidal zone where are just above and overlapping with the growing zone of *C. leprieurii* f. *continua.*

Representative specimens examined: TNS-AL-6294 (tetrasporic plant) and TNS-AL-6295 (female and male), the mouth of Aono River, Minamiizu-machi, Shizuoka Pref., coll. J. Tanaka, Aug. 12, 1992; TNS-AL-6300 (male plant after 5 months culture), the mouth of Hiki River, Miyazaki Pref., coll. D. Honda, Apr. 14, 1992; TNS-AL-6301 (female plant after 10 months culture), the mouth of Kido River, Chiba Pref., coll. M. Kamiya, Nov. 22, 1991; TNS-AL-6309 (tetrasporic plant after 1 year culture), Aoshima, Miyazaki Pref., coll. M. Kawachi, May 31, 1991.

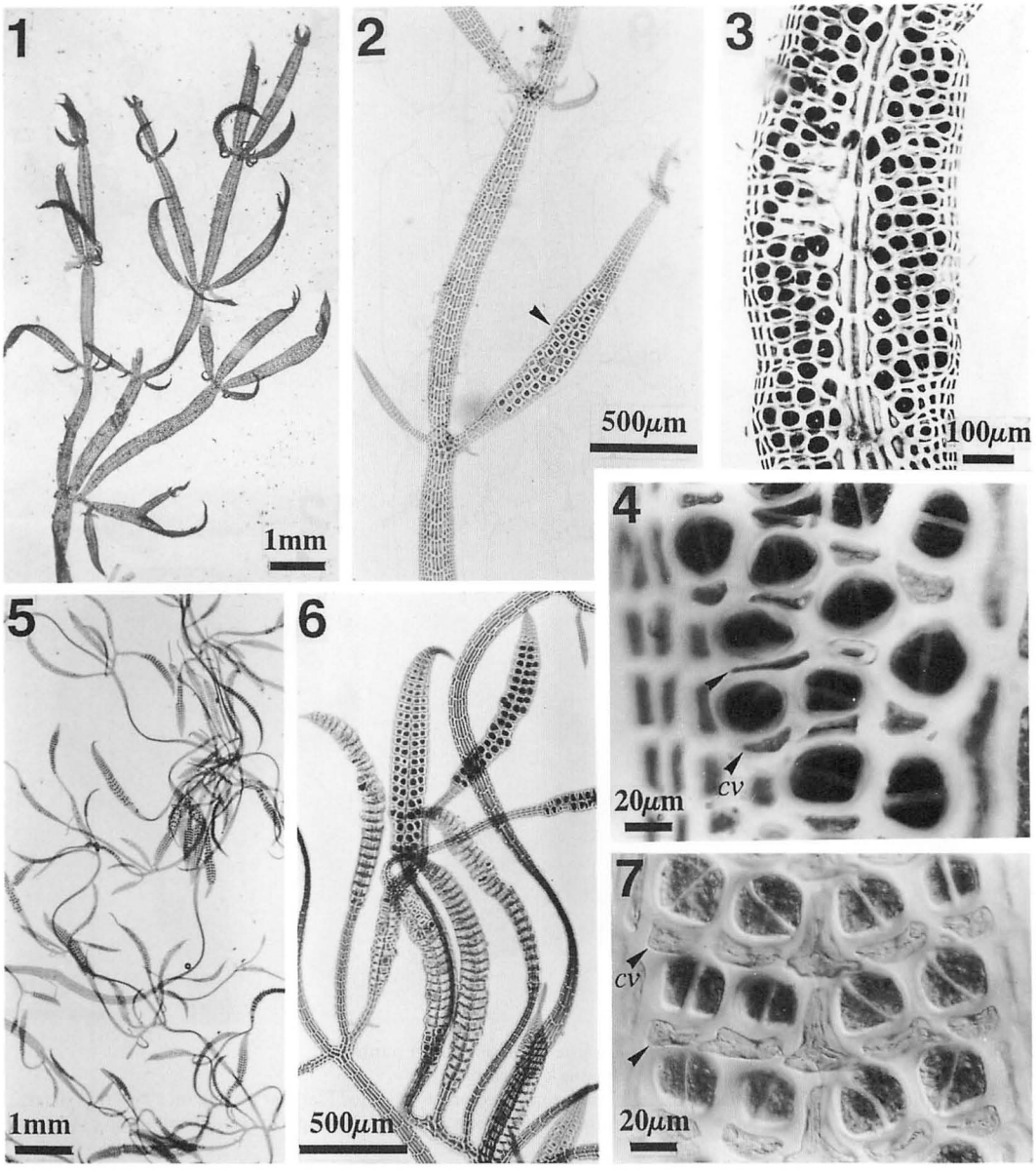
Vegetative field plant:

Thallus is spreading in one plane, sometimes forming a dense tuft, creeping at the basal part with rhizoids, dark violet, subdichotomously divided at 5-10 times, sometimes tri- or tetrachotomously divided, 0.5-3 cm high, composed of many blades (Fig. 1). Each blade, lanceolate, 2-3 mm long in the lower part of a thallus and becomes shorter toward the apex, 200-350 μm wide at the middle part, constricted at the node. Secondary branches and rhizoids are often arising from the central cell of the nodes. Blade is composed of a central axis and one cell layer of wing cells which are regularly arranged in both sides of an axis. Central axis is composed of a longitudinal line of central cells and four pericentral cells around a central cell. Wing cells are composed of five or six cell rows of the second and third order arising from lateral pericentral cells. No intercalary cell divisions occur in the wing cells.

Three kinds of reproductive plants, sporophyte, male and female gametophytes occur simultaneously in wild population in Shizuoka Prefecture, but gametophytes were collected only in August. They are slightly different in general appearance, and can be distinguished from each other by their size of thalli and the shape of reproductive structures (Figs. 2, 8, 16).

Tetrasporophyte:

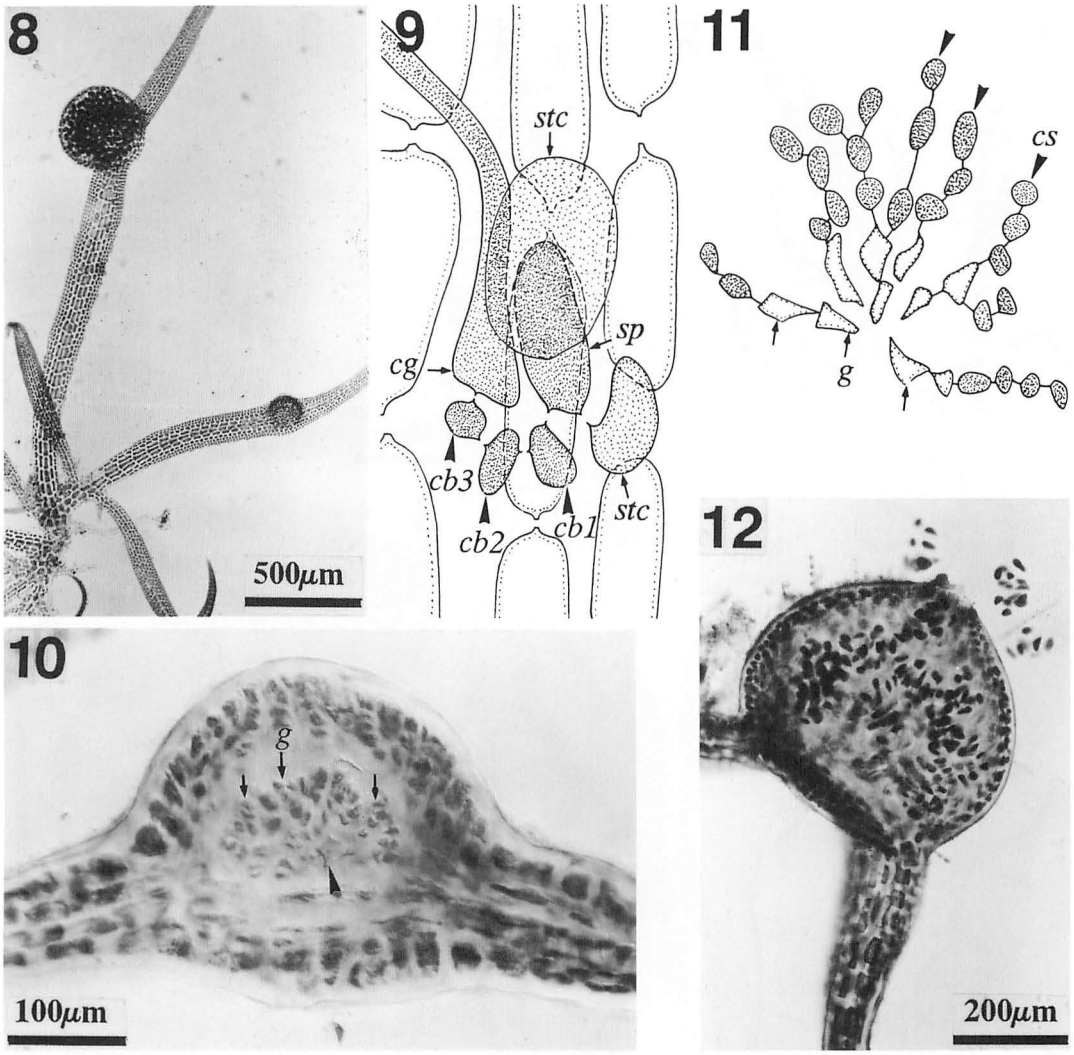
Field plant: Tetrasporophytes are bigger than gametophytes at least in natural populations, branches out up to ten times and becomes 3 cm high. Tetrasporangial sori are produced on both sides of a central axis in the upper and middle parts of a thallus, linear in shape, 400-900 μm long and 150-300 μm wide (Fig. 2). Tetrasporangia are cut off from the lateral pericentral cells (Fig. 2) and the cells of the second, and sometimes from the cells of third order (Figs. 3, 4) except 2-3 marginal cells. They are arranged in 2-5 rows from center to margin (Figs. 2, 3, 6). At first a lateral pericentral cell or a wing cell divides transversely to form a tetrasporangial mother cell on the upper side and a residual stalk cell. A stalk cell cuts out two cover cells towards the both surfaces. Tetrasporangial mother cells become larger in diameter, and when matured, tetrahedrally or cruciately



Figs. 1–7. Field and cultured plants of *Caloglossa ogasawaraensis*.
Figs. 1–4. Field plants.
Fig. 1. Habit of a field plant.
Fig. 2. A branch with a tetrasporangial sorus (arrowhead).
Fig. 3. Surface view of a tetrasporangial sorus formed on the both sides of a central axis.
Fig. 4. Divided tetrasporangia with cover cells (*cv*, arrowhead).
Figs. 5–7. Cultured plants.
Fig. 5. Habit of a tetrasporophyte with matured tetrasporangial stichidia.
Fig. 6. Mature and emptied tetrasporangial sori in stichidia.
Fig. 7. Divided tetrasporangia with cover cells (*cv*, arrowhead).

divided to produce four tetraspores. Tetrasporangia are spherical, 35–40 μm in diameter (Fig. 4). Cover cells do not fully cover the tetrasporangia (Fig. 4).

Cultured plant: Branches are much narrower than those of natural plants as shown in

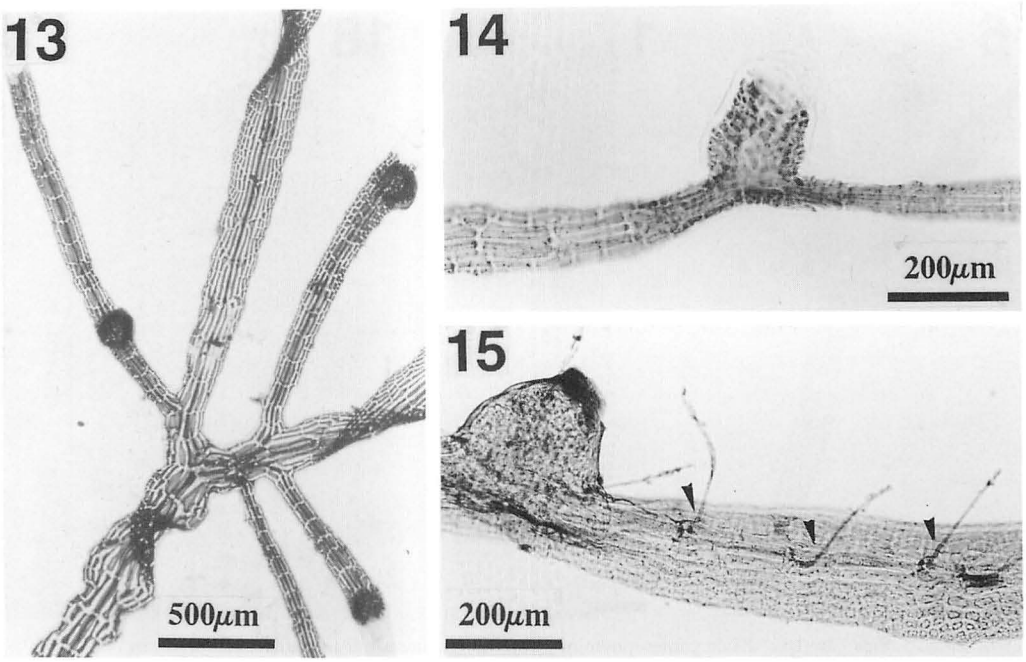


Figs. 8–12. Female gametophyte of *Caloglossa ogasawaraensis* in nature.
Fig. 8. Branches with a mature and a young cystocarps.
Fig. 9. Surface view of a carpogonial branch composed of a four-celled filament arising on a central cell (*cg*: carpogonium; *cb1*, *cb2*, *cb3*: first, second and third cell of carpogonial branch, respectively; *sp*: supporting cell; *stc*: sterile cell).
Fig. 10. An immature cystocarp, showing a fusion cell (arrowhead) and gonimoblast cells (*g*, arrows).
Fig. 11. Gonimoblast cells (*g*, arrows) and immature carposporangia (*cs*, arrowheads).
Fig. 12. A Mature cystocarp, showing carpospores discharging from a terminal ostiole.

Fig. 5. Tetrasporangial branches are abundantly formed, and tetrasporangia and cover cells are formed by the similar manner in natural plants (Figs. 6, 7).

Female gametophyte:

Field plant: Female plants are 5–8 mm high and 150–250 μm wide, smaller than tetrasporophytes, divide at 2–3 times, often occurring with male plants, and distinguished from the male plant by possessing globular cystocarps (Fig. 8). Several procarps are produced at the upper part of a blade along a central axis. Before the formation of a carpogonial branch, a pericentral cell cuts on a sterile cell initial. A carpogonial branch initial is produced from a pericentral cell, which is called a supporting cell from then on. An



Figs. 13–15. Female gametophyte of *Caloglossa ogasawaraensis* in culture.
Fig. 13. Branches with pseudocystocarps.
Fig. 14. A pseudocystocarp. No gonimoblast cell is seen.
Fig. 15. A pseudocystocarp and several unfertilized carpogonial branches (arrowheads).

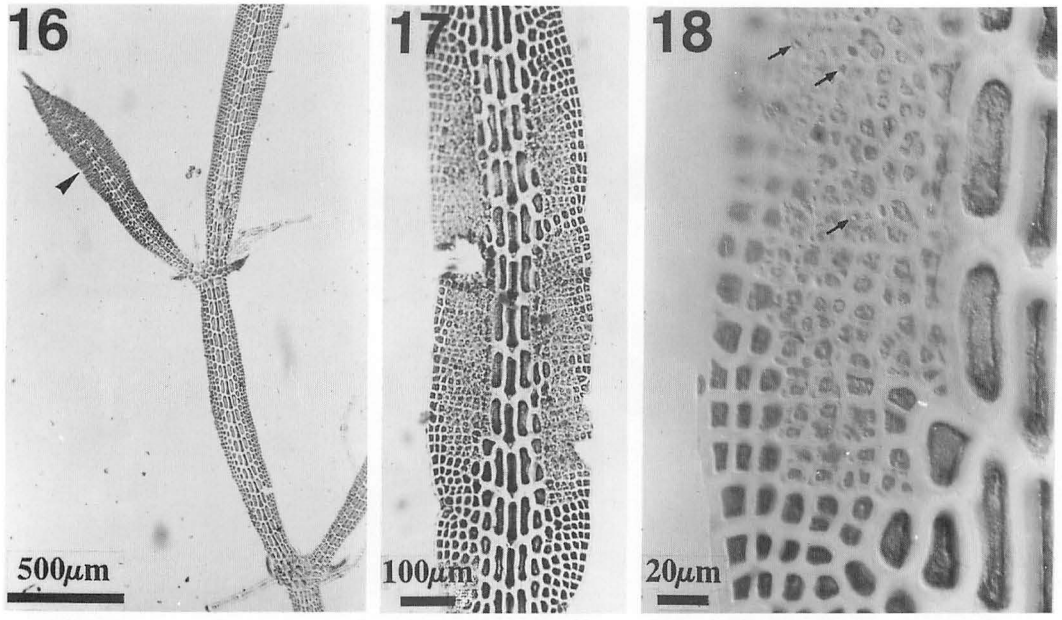
initial cell divided into 4 segments including a carpogonium with a long (about 100 μm) trichogyne. A procarp is finally composed of a four-celled carpogonial branch, a supporting cell and two mother cells of sterile groups (Fig. 9). One of these procarps develops to form a fusion cell giving rise to many elongated gonimoblast cells (Fig. 10). Many carposporangia are formed on gonimoblast cells in chains (Fig. 11). A cystocarp is subspherical, forming a conspicuous projection upon the central axis near the apex of a female blade, 500–800 μm in diameter (Fig. 12). Many carpospores are produced in a cystocarp and they are teardrop-shaped and 28–40 μm long and 15–25 μm in diameter. Mature plants with cystocarps can be easily recognized in the field by the unaided eyes.

Cultured plant: Thalli grow up to 2 cm high and 200–400 μm wide, longer and wider than field plants (Fig. 13). Many carpogonial branches were produced in a line along a central axis (Fig. 15). The trichogynes of a cultured plant are longer (100 μm or more) than those of the field ones. One, sometimes two, of the carpogonial branches in a blade became pseudocystocarps parthenogenetically (Fig. 13). They could not function as the true cystocarps and not produce any gonimoblast cells or carpospores (Fig. 4).

Male gametophyte:

Field plant: Male plants are smaller than tetrasporophytes, 5–9 mm long. Spermatangial sori are produced on both sides of a central axis of at the upper to middle parts of a thallus, 300–900 μm long and 100–220 μm wide (Fig. 16). Spermatangia are cut off from almost of all wing cells except a few marginal cells (Figs. 17–18).

Cultured plant: Thalli more frequently branch than the field plants. Branches grow up to 3 mm long and 300 μm wide, longer and wider than field plants (Fig. 19). After six months culture, most branches of a male plant were transformed into spermatangial sori on the whole surfaces except the central cells, pericentral cells and marginal cells (Fig.



Figs. 16–18. Male gametophyte of *Caloglossa ogasawaraensis* in nature.
Fig. 16. A branch with a spermatangial sorus (arrowhead).
Fig. 17. Spermatangial sori formed on the both sides of a central axis.
Fig. 18. Surface view of a spermatangial sorus, showing spermatangia (arrows).

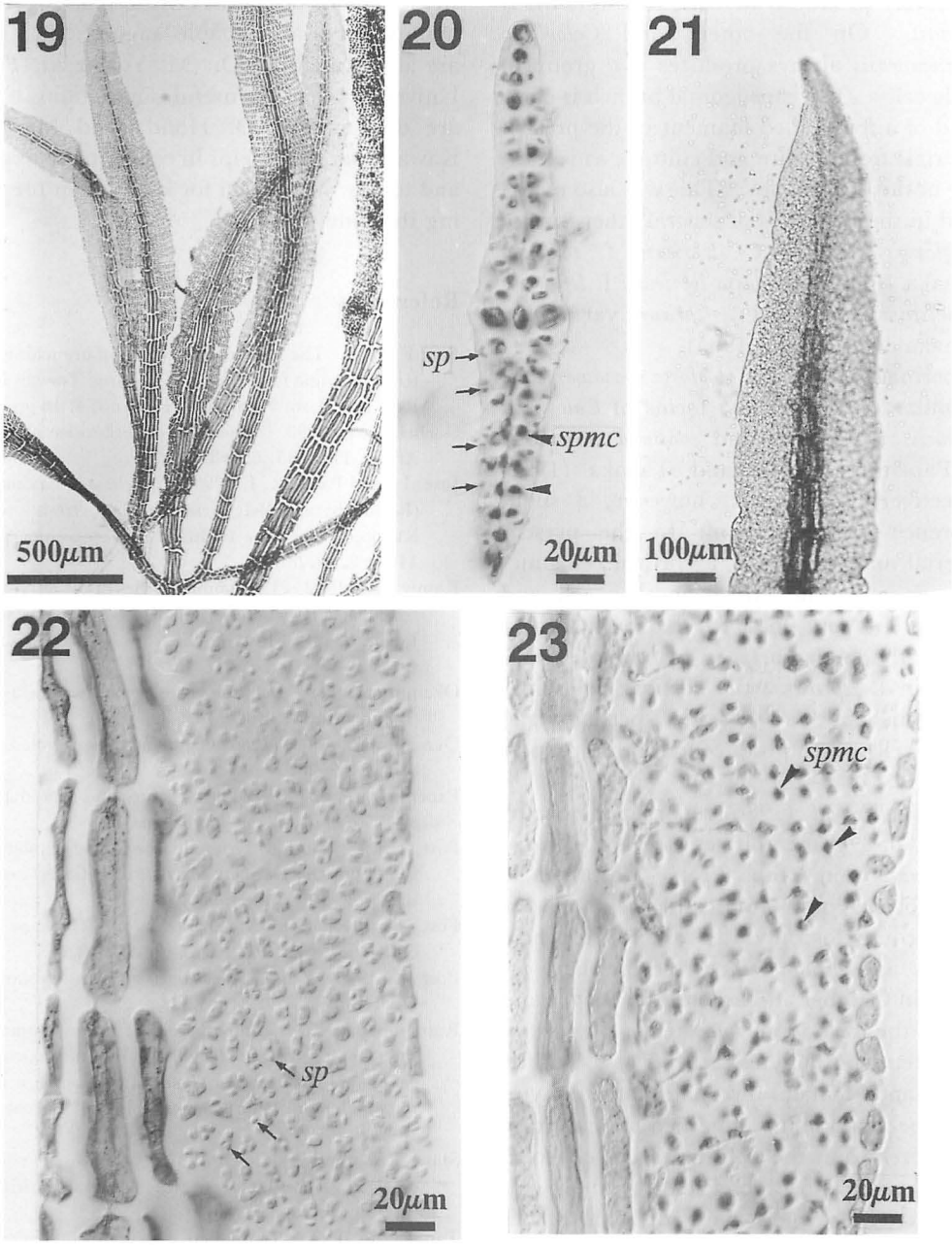
22). At first step in the formation of spermatangia, a wing cell cuts out four or more cortical cells on both surfaces. Then a cortical cell which functions as a spermatangial mother cell cuts out four to six spermatangia by oblique walls (Fig. 20). Each spermatangium differentiates a single spermatium. After the release of spermatia (Fig. 21), wing cells and spermatangial mother cells remained (Fig. 23), and soon these collapsed and disintegrated.

Discussion

Okamura (1897) newly described *Caloglossa ogasawaraensis* based on the sterile specimens from fresh water of the Ogasawara Islands, distinguished from the commonest species in Japan, *Caloglossa leprieurii* f. *continua* by its narrower (up to 1 mm wide) blade with clear constrictions at the nodes. Later Okamura (1908) described and figured the cystocarpic and tetrasporic plants of this species in detail. The present observation on female gametophytes and tetrasporophytes agree well with the descriptions and figures given by Okamura. However, he did not described the male plant and the procarps of female plant, which are described for the first time here.

As to the formation of tetrasporangia, the cultured plants irregularly branched and formed abundant terminal ultimate branches which transformed into the tetrasporangial stichidia. The formation of stichidia have been observed only under culture. While the field plants formed tetrasporangial sori on the middle part of the internodes of branches. Cover cells never completely cover the enlarging tetrasporangia which remain partially exposed on the surfaces in mature stage on both of the culture and field plants. This type of cover cell formation corresponds with the other *Caloglossa* species.

Carpogonial branch formation, the arrangement and number of carpogonial branch cells, the supporting cell and sterile cells agrees with that of *Caloglossa leprieurii* f. *continua* given by Tanaka (1992). According to Papenfuss (1961) a second sterile cell group



Figs. 19–23. Male gametophyte of *Caloglossa ogasawaraensis* in culture.

Fig. 19. Branches with spermatangial sori.

Fig. 20. Cross section of a spermatangial sorus, showing spermatangial mother cells (*spmc*, arrowhead) and spermatangia (*sp*, arrows).

Fig. 21. Release of spermatia from a sorus.

Fig. 22. Surface view of a spermatangial sorus, showing matured spermatangia (*sp*, arrows) developed on spermatangial mother cells.

Fig. 23. Surface view of an empty spermatangial sorus, showing spermatangial mother cells (*spmc*, arrowheads).

was rarely produced in *Caloglossa leprieurii* f. *leprieurii*. On the other hand *Caloglossa ogasawaraensis* always produces two group of sterile cells. The carpogonial branch is composed of a four-celled filament in the present materials from nature and culture, as is ordinary in the Ceramiales. This was also recognized in the materials of several other taxa of *Caloglossa*, such as *C. leprieurii* f. *continua* (Tanaka 1992), *Caloglossa leprieurii* f. *leprieurii* (Papenfuss 1961), and *C. leprieurii* var. *hookeri* (Tanaka and Chihara 1985).

Spermatogenesis of *Caloglossa ogasawaraensis* is similar to that in two forms of *Caloglossa leprieurii*, f. *leprieurii* and *continua* described by Papenfuss (1961) and Tanaka (1992) respectively. There is, however, a slight difference between them: In the present material and f. *continua*, a cortical cell functions as a spermatangial mother cell and directly cuts out four to six spermatangia toward the both surfaces by oblique wall. While in f. *leprieurii*, the cortical cell sometimes cuts out two to seven spermatangial mother cells which produce four or more spermatangia.

Yoshizaki *et al.* (1986) reported the occurrence of gametophytes of *Caloglossa ogasawaraensis* in Chiba Pref. Mature male plants occurred in June and October, but they account for only 1% among the whole samples, and female and cystocarpic plants occurred in October and account for 0.5%, and among the rest materials sterile plants are 30%, tetrasporophytes are 68%. On the other hand, female and male gametophytes could be abundantly obtained in culturing tetraspores from the field materials collected in Wakayama and Chiba Prefectures. In cultural materials, most parts of male blades were transformed into spermatangial sori. Soon after the release of matured spermatia, the blades disintegrated. The rare occurrence of the fertile male plants in nature is caused by their caducous blades.

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田中次郎*・神谷充伸**：日本産ホソアヤギヌの生殖体の構造

ホソアヤギヌ (*Caloglossa ogasawaraensis*) は，岡村（1897)により小笠原島の淡水域から記載されたが，その後全世界の亜熱帯を中心に広く分布していることが知られてきた。これまで雄や雌の生殖器官についての詳しい記載は行われていない。そこで日本産の天然，及び培養で得られた生殖藻体（四分胞子体，雄と雌の配偶体，果胞子体）の形態を詳しく記載した。

本藻は，天然では有性の生殖藻体は四分胞子体の１％以下しか見つからない。一方，天然で得た四分胞子を培養すると，簡単に雄と雌の配偶体に生長する。天然藻体の生殖器官の形成方法およびその形態は，Tanaka (1992) が記載した日本産アヤギヌ (*Caloglossa leprieurii* f. *continua*) のものと基本的には同じである。しかし培養下では，以下のように天然のものと形態の異なる藻体が得られた。1）四分胞子嚢は最末枝に形成される。2）雌には擬似嚢果 (pseudocystocarp) が形成される。これには果胞子は形成されない。3）精子嚢は最末枝のほぼ全面に形成される。(*169 東京都新宿区百人町3-23-1　国立科学博物館，**305 茨城県つくば市天王台1-1-1　筑波大学生物科学系)

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