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Further observations on the life history of *Gymnogongrus flabelliformis* HARVEY (Rhodophyta) in culture[1)]

Michio MASUDA

The immature plants referable to *Gymnogongrus flabelliformis*, which were derived from the tetraspores of field-collected *Erythrodermis* sp. (MASUDA *et al.*, 1979), have become reproductive in laboratory culture. The plants grew into dioecious upright gametophytes. Female plants with procarps produced cystocarps only in the presence of male plants with spermatia. Liberated carpospores germinated and grew into crustose tetrasporophytes with seriate intercalary tetrasporangia in nemathecia. Cultured gametophytes derived from field-collected *G. flabelliformis* hybridized with *Gymnogongrus*-phase gametophytes cultured from tetraspores of *Erythrodermis* sp.

In the previous paper (MASUDA *et al.*, 1979), the culture of tetraspores of *Erythrodermis* sp. and carpospores of *Gymnogongrus flabelliformis* was reported to elucidate the life history relationship between both the species. However, the plants referable to *G. flabelliformis*, which were derived from tetraspores of *Erythrodermis* sp., did not become reproductive in laboratory culture at that time and have been maintained in culture for additional two years. The final results of the culture experiments started from *Erythrodermis* sp. are described here. Furthermore, the results of hybridization experiment between male and female *G. flabelliformis* and the *Gymnogongrus*-phase gametophytes cultured from tetraspores of *Erythrodermis* sp. are also reported. The same techniques of culture were employed.

Results and Discussion

Individual plants derived from single tetraspores of field-collected *Erythrodermis* sp. (MASUDA *et al.*, 1979) became either male or female gametangial plants. The male plants developed spermatangial sori covering middle to upper portions of the blade 12 months after inoculation at 20°C, 8 : 16 LD

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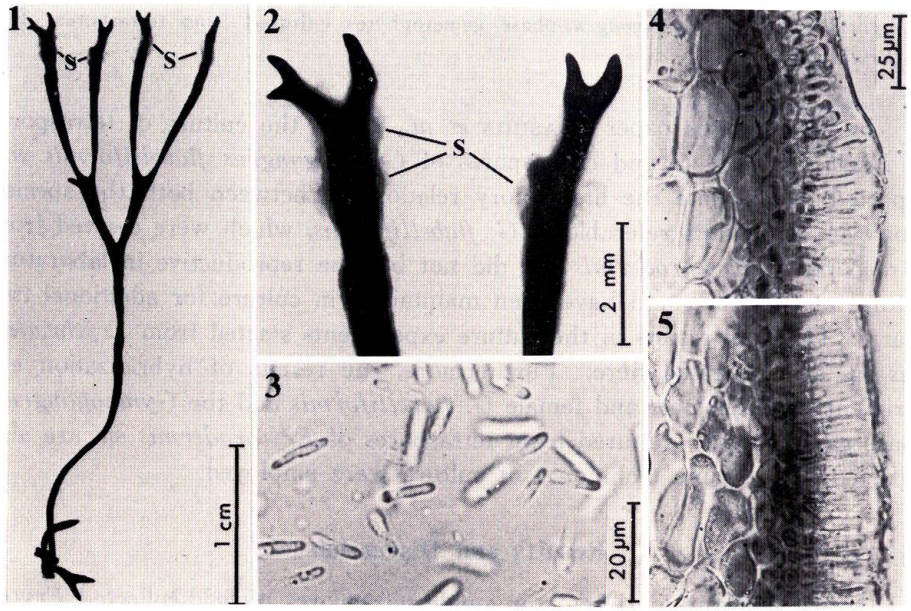
(Fig. 1). Numerous characteristic cylindrical spermatia were released forming an opaque, white film around the blade (Fig. 2). The spermatia (Fig. 3) were similar to those described previously for several species of the Phyllophoraceae (*Ahnfeltia concinna*, MAGRUDER, 1977) et the Gigartinaceae (*Chondrus crispus*, GRUBB, 1925; *Gigartina papillata*, POLANSHEK and WEST, 1975; *Gigartina stellata*, WEST *et al.*, 1977, DION and DELÉPINE, 1979; *Gigartina jardinii* = *G. agardhii*, WEST *et al.*, 1978). They are 7.5–10.0 μm in length and 2.0–2.5 μm in diameter. Spermatangial development was observed in sections of the blades (Figs. 4, 5). Two spermatangial parent cells[1] are cut off obliquely from the superficial cortical cells. Each parent cell bears terminally one or two elongated spermatangia.

Eighteen months later female gametangial plants with procarps were observed at 20°C, 8 : 16 LD. Trichogynes were visible projecting through the blade surface (Fig. 6). Procarps consist of a large supporting cell and a three-celled carpogonial branch (Fig. 7). One or two sterile cells issue from



Figs. 1, 2. Mature male gametangial plant releasing spermatia (s) cultured from tetraspore of field-collected *Erythrodermis* sp.

Fig. 3. Released spermatia.

Figs. 4, 5. Longitudinal section through male blade, showing spermatangial layer. Scale in Fig. 4 applies also to Fig. 5.

1) cf. SCHMID, 1977.

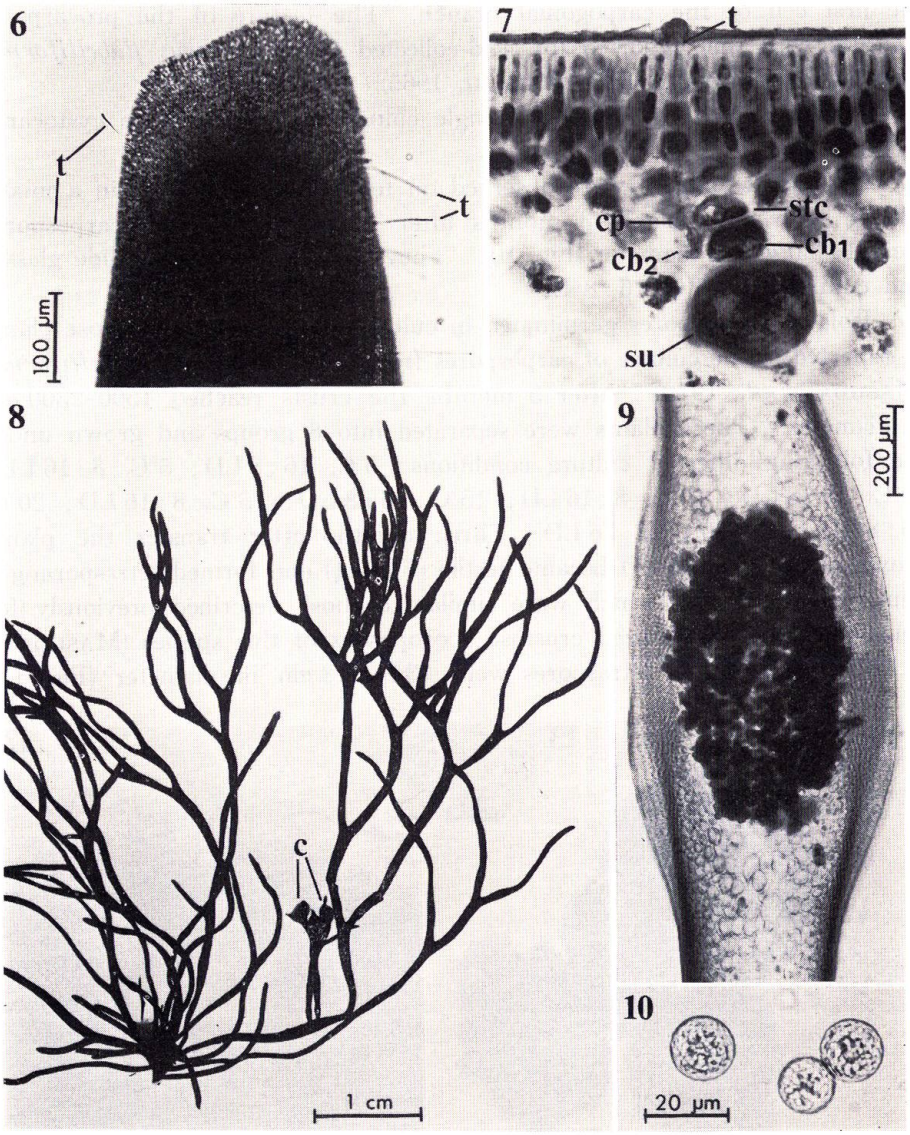


Fig. 6. Surface view of apical portion of female blade, showing trichogynes (t).

Fig. 7. Procarp. Supporting cell (su); carpogonial branch cells (cb_1, cb_2); carpogonium (cp); sterile cell (stc); trichogyne (t).

Fig. 8. Cystocarpic plant with ripe cystocarps (c) cultured from tetraspore of field-collected *Erythrodermis* sp.

Fig. 9. Longitudinal section of cystocarp.

Fig. 10. Liberated carpospores.

Scale in Fig. 10 applies also to Fig. 7.

the first cell of the carpogonial branch. The feature of the procarps is identical with that reported for field-collected *Gymnogongrus flabelliformis* (TOKIDA and MASAKI, 1959, MIKAMI, 1965).

Female plants established in single culture did not produce cystocarps while isolated.

Cystocarps (Fig. 9) were observed on female plants (Fig. 8) in a mixed culture with male plants 3 months after mixture. Liberated carpospores were 14–20 μm in diameter (Fig. 10). They were pipetted onto slide glasses and cultured at 15°C, 16 : 8 LD.

Isolated carpospores germinated in culture and grew into crustose plants as reported in the culture of carpospores from field-collected *G. flabelliformis* (MASUDA *et al.*, 1979). After 3 months the crusts reached 1500–2300 μm in diameter. These plants were separated into 8 groups and grown under the following different culture conditions: 5°C, 16 : 8 LD; 5°C, 8 : 16 LD; 10°C, 16 : 8 LD; 10°C, 8 : 16 LD; 15°C, 16 : 8 LD; 15°C, 8 : 16 LD; 20°C, 16 : 8 LD; and 20°C, 8 : 16 LD. Three months after transfer the plants grown at 15°C, 8 : 16 LD became fertile (Fig. 11) and formed tetrasporangial nemathecia (Fig. 12) which were similar to those described previously for field-collected and cultured crustose sporophytes of this species (MASUDA *et al.*, 1979). Liberated tetraspores were 13.8–18.8 μm in diameter (Fig. 13).

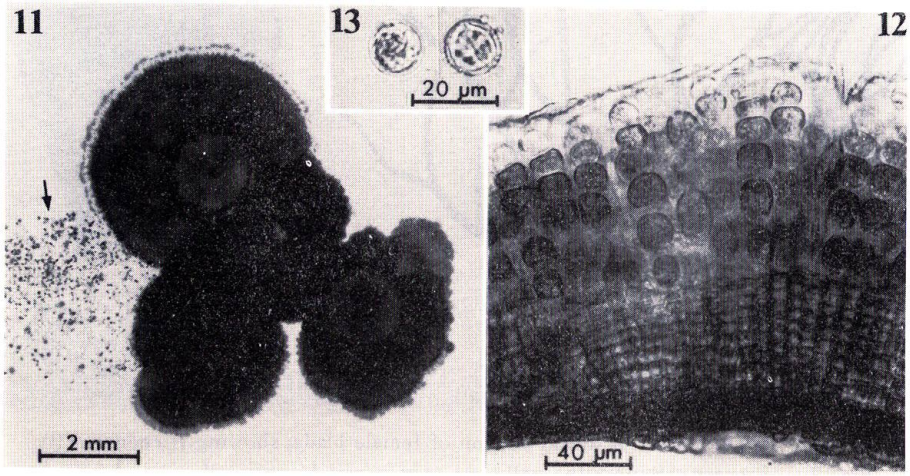


Fig. 11. Crustose tetrasporangial plants with nemathecia derived from carpospores of cultured cystocarpic plant. An arrow indicates the tetraspore germlings.

Fig. 12. Section through tetrasporangial nemathecium, showing young seriate intercalary tetrasporangia.

Fig. 13. Liberated tetraspores.

Thus, the life history of this alga, which was started from tetraspores derived from field-collected *Erythrodermis* sp. was completed in culture. Plants cultured for 15 months in the other seven conditions did not sporulate.

Carpospores from *G. flabelliformis* collected in Oshoro Bay on November 5, 1978 grew into *Erythrodermis* crusts and bore tetrasporangial nemathecia similar in every respect to those described above at 15°C, 8 : 16 LD and 20°C, 8 : 16 LD. The tetraspore germlings grew into dioecious gametophytes and reached reproductive maturity after 9 months at 20°C, 8 : 16 LD. Mature cystocarps appeared 2 months after starting mixed cultures of female and male plants. The female and male plants derived from cultured *Erythrodermis* were mixed with male and female plants derived from field-collected *Erythrodermis* sp., respectively. Mature cystocarps were observed about 3 months later and released carpospores. Thus, *Erythrodermis* sp. is the naturally occuring tetrasporophyte of *Gymnogongrus flabelliformis*.

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