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The life history of *Gigartina ochotensis* (RUPRECHT) RUPRECHT (Rhodophyta) in culture[1)]

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The life history of the red alga *Gigartina ochotensis* (RUPRECHT) RUPRECHT collected at Utoro, north coast of Hokkaido in Japan was completed in laboratory culture. Carpospores germinated to form crustose thalli similar in anatomy to *Petrocelis*. The crustose thalli formed tetrasporangia and released viable spores at 5°C, 8 : 16 LD. The tetraspores developed in a manner similar to that of carpospores, but all of the germlings issued upright thalli. The upright thalli grew into dioecious *Gigartina* blades which produced procarps and spermatangia at 15°C, 16 : 8 LD and 10°C, 16 : 8 LD. Then, cystocarps developed and released carpospores which grew into crustose thalli.

The occurrence of both sexual and apomictic life histories has been reported in *Gigartina* subgenus *Mastocarpus* for *G. papillata* (POLANSHEK and WEST, 1977), *G. stellata* (CHEN *et al.*, 1974, WEST *et al.*, 1977, RUENESS, 1978, DION et DELÉPINE, 1979) and *G. jardinii* (=*G. agardhii*, WEST *et al.*, 1978). In the sexual life history, the foliose male and female gametophytes alternate with the crustose tetrasporophytes, but in the apomictic life history the female gametophytes form carposporophytes in the absence of male gametophytes and are reproduced by only carpospores. In an earlier paper apomictic life history of *Gigartina ochotensis* was reported (MASUDA and UCHIDA, 1976). This paper presents the sexual life history of the species.

Materials and Methods

Fertile cystocarpic plants[2)] were collected at Utoro, north coast of Hokkaido in Japan, on August 24, 1976 by M. MASUDA and A. M. NONOMURA. The plants were transported to the laboratory in a plastic chest on ice and stored in an icebox at about 5°C. Culture experiments were started on

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2) The material collected is identical with *Gigartina ochotensis* circumscribed by MIKAMI (1965). This species can hardly be distinguishable from *G. pacifica* KJELLMAN. The taxonomic relationship between both the algae will be discussed elsewhere.

September 1, 1976. Liberated carpospores were pipetted onto several drops of PES medium on slide glasses (76×26 mm) or 18 mm cover glasses placed on the bottom of Petri dishes (90 mm×20 mm). The carpospores attached to the substrate 24–48 hr after inoculation and then about 50 m*l* of PES medium were introduced into the Petri dishes. After 7 days the slide or cover glasses were transferred to culture vessels (65 mm×80 mm) containing 200 m*l* of medium.

Cultures were maintained in freezer-incubators illuminated with cool-white fluorescent lamps (2000–3000 lux). The temperatures and photoperiods were regulated in the following combinations: 5°C, 16 : 8 LD (light-dark cycle); 5°C, 8 : 16 LD; 10°C, 16 : 8 LD; 10°C, 8 : 16 LD; 15°C, 16 : 8 LD; and 15°C, 8 : 16 LD. The carpospore cultures were maintained at 15°C, 16 : 8 LD during first 5 months and then, divided into 6 groups. Five of these groups were shifted to the five other conditions. The tetraspore cultures were maintained at 15°C, 16 : 8 LD during first 2 months, then, divided into 6 groups and five of them were also shifted to the five other conditions. The culture medium was changed monthly. Mixed cultures of mature female and male plants were rotated by a magnetic stirrer for first one week.

Results and Discussion

Liberated carpospores are light red in color and 17.5–25.0 μm in diameter (Fig. 2). The spores germinated and grew into crustose discs in a manner similar to that previously reported for this species (MASUDA and UCHIDA, 1976) and shown in Figs. 3–6. Five-month-old crusts reached about 4 mm in diameter (Fig. 7). All of the carpospore germlings grew into *Petrocelis*-like crusts (Fig. 8) and did not issue upright blades, differing from those of Muroran isolate reported by MASUDA and UCHIDA (1976). However, no reproductive structures were observed in the crusts under all culture conditions tested until June, 1979.

The crusts shifted to 5°C, 8 : 16 LD reached approximately 15 mm in diameter and became reproductive after about 3 years from inoculation (Fig. 9). Intercalary tetrasporangia were formed solitarily on erect filaments of the perithallus (Fig. 10). This feature is similar to *Petrocelis cruenta* (WEST *et al.*, 1977) and *P. middendorfii* (WEST, 1972, POLANSHEK and WEST, 1975), both of which are tetrasporangial plants of *Gigartina* subgenus *Mastocarpus*. The crusts are 300–500 μm thick at the fertile portion. The observed tetrasporangia are 30.0–37.5 μm in length and 22.5–32.5 μm in diameter, dividing regularly or irregularly cruciately. Plants cultured in the other five

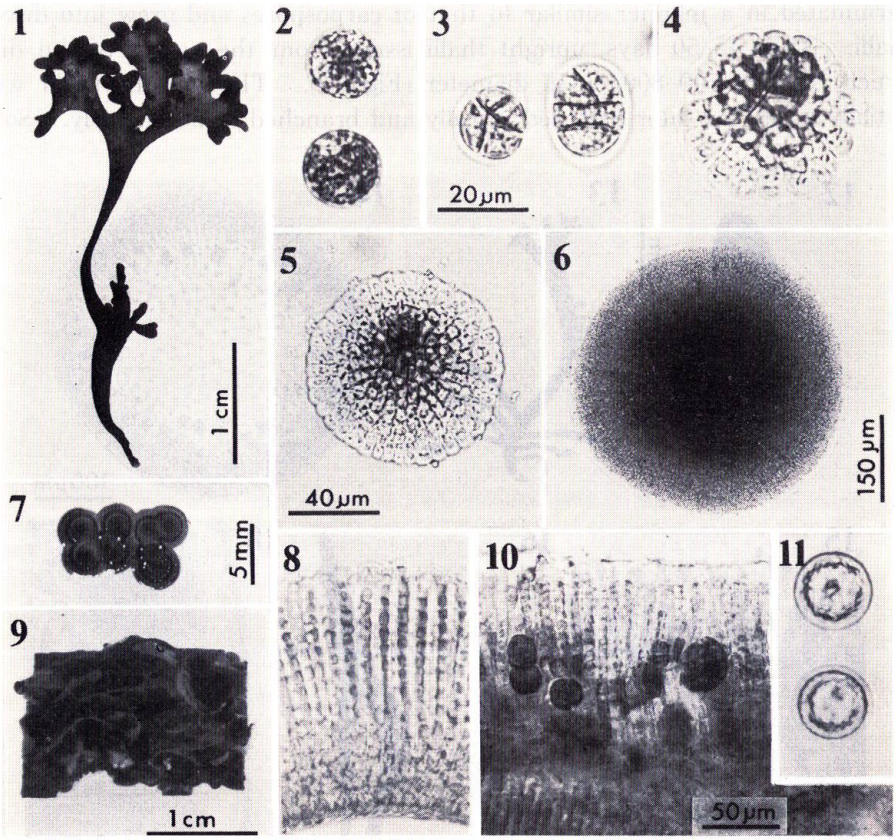


Fig. 1. Parent cystocarpic plant of culture experiments collected at Utoro, north coast of Hokkaido on August 24, 1976.

Fig. 2. Carpospores.

Figs. 3–7. Carpospore germlings grown at 15°C, 16 : 8 LD : 3, two-day old ; 4, seven-day old ; 5, fourteen-day old ; 6, one-month old ; 7, five-month old.

Fig. 8. Section through the 14-month-old crust (grown at 15°C, 16 : 8 LD for 5 months and then transferred to 5°C, 16 : 8 LD).

Fig. 9. Thirty five-month-old crusts reaching reproductive maturity (grown at 15°C, 16 : 8 LD for 5 months and then shifted to 5°C, 8 : 16 LD).

Fig. 10. Section through the fertile crust.

Fig. 11. Tetraspores.

Scale in Fig. 3 applies also to Figs. 2, 4 and 11 ; scale in Fig. 5 applies also to Fig. 8.

conditions did not reach reproductive maturity even after 4 years, and the cultures were then terminated.

Liberated tetraspores are 15.0–22.5 μm in diameter (Fig. 11). They

germinated in a manner similar to that of carpospores and grew into discoid thalli. After 45–50 days, upright thalli issued from the center of the discs which reached 700–800 μm in diameter (Fig. 12). The upright thalli were initially terete but later flattened apically and branched dichotomously. Some

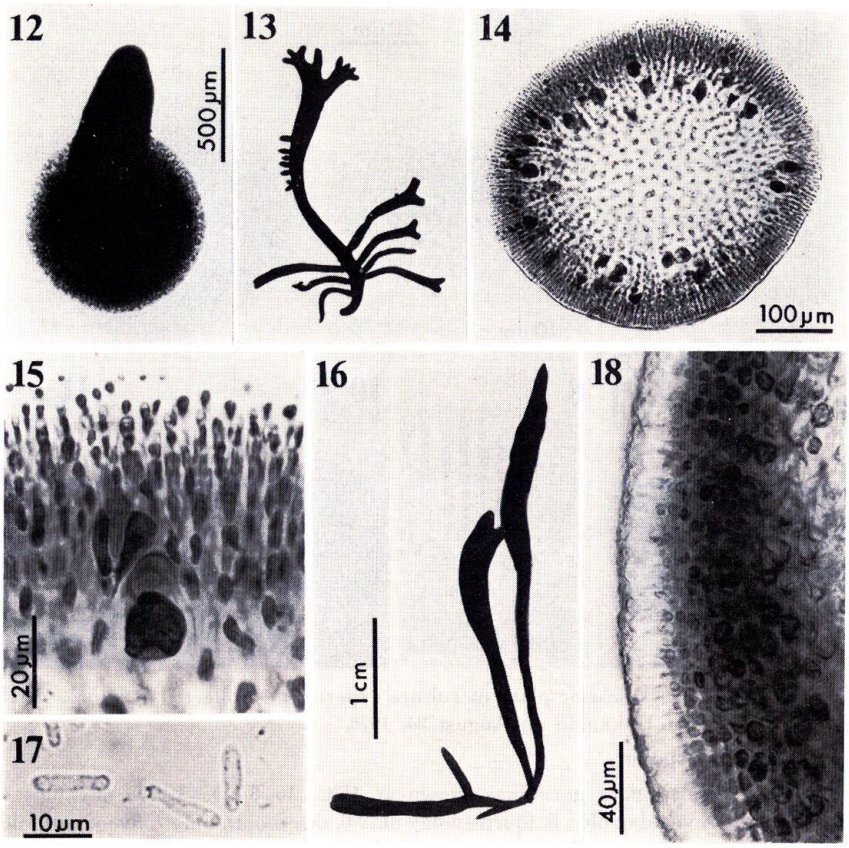


Fig. 12. One and a half-month-old tetraspore germling with an upright thallus grown at 15°C, 16 : 8 LD.

Fig. 13. Fertile female gametophyte with papillae (7-month old) grown at 15°C, 16 : 8 LD.

Fig. 14. Cross section of a papilla, showing many procarps stained with cotton blue.

Fig. 15. Procarp (trichogyne being out of focus).

Fig. 16. Fertile male gametophyte (7-month old) grown at 15°C, 16 : 8 LD.

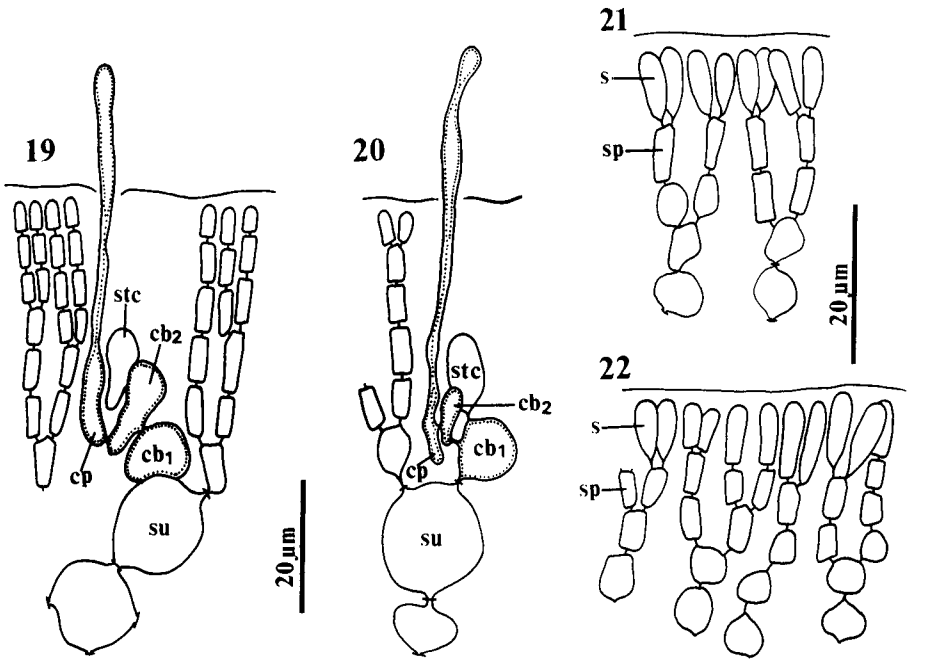
Fig. 17. Spermatia.

Fig. 18. Section through the spermatangial sorus. Note spermatangia and their parent cells borne at the outermost pale portion.

Scale in Fig. 16 applies also to Fig. 13.

plants bore adventitious branches from the blade margin (Fig. 13). The upright thalli reached 22-36 mm in height after 7 months and became reproductive at 15°C, 16 : 8 LD and 10°C, 16 : 8 LD. Procarps and spermatangia were formed on separate individuals (Figs. 13, 16). Female gametangial plants formed several papillate outgrowths from blade margins (Fig. 13). Many procarps were borne within inner cortex of the papillae (Fig. 14), although in the Muroran isolate reported earlier no procarps were observed (MASUDA and UCHIDA, 1976). The procarps consisted of a large supporting cell and a three-celled carpogonial branch. A single sterile cell was borne on the first or second cell of the carpogonial branch (Figs. 15, 19, 20). The feature of the procarp is similar to that of other species of *Gigartina* subgenus *Mastocarpus* (WEST, 1972, KIM, 1976, POLANSHEK and WEST, 1977, WEST *et al.*, 1977, 1978).

In male gametangial plants a continuous spermatangial sorus (Fig. 18) covered the surface of the blade except near the lower portion and the



Figs. 19, 20. Procarps showing the supporting cell (su) and the carpogonial branch with the carpogonium (cp), two branch cells (cb_1, cb_2) and sterile cell (stc).

Figs. 21, 22. Section through the spermatangial sorus, showing spermatangial parent cells (sp) and spermatangia (s).

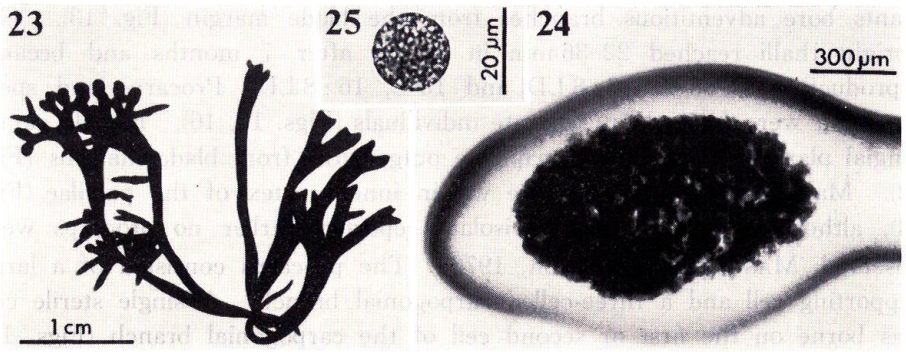


Fig. 23. Fertile cystocarpic plant grown at 15°C, 16 : 8 LD (eight and a half-month old).
Fig. 24. Longitudinal section of a mature papilla.
Fig. 25. Liberated carpospore.

growing tips. One or two spermatangial parent cells are produced from the superficial cortical cells. Each parent cell bears terminally one or two elongated spermatangia (Figs. 21, 22). Numerous colorless cylindrical spermatia which are 9–15 μm in length and 2.5–3.0 μm in diameter (Fig. 17) were released forming an opaque, white film around the blases as in the case of male gametophytes of *Petrocelis cruenta* (WEST *et al.*, 1977).

Of 26 plants cultured from single tetraspores and maintained at 15°C, 16 : 8 LD and 10°C, 16 : 8 LD, 14 were female and 12 were male. Female plants established in single culture did not produce cystocarps. Mature cystocarps were observed on six female gametophytes 45 days after starting two mixed cultures of male and female plants (each including three females and two males) at 15°C, 16 : 8 LD (Fig. 23). Two months after starting two mixed cultures (each with two females and two males) at 10°C, 16 : 8 LD mature cystocarps appeared on all the females. The mature cystocarps lacked a specialized inner pericarp (Fig. 24). They released numerous carpospores (Fig. 25) which gave rise to crustose thalli. Other plants cultured at 5°C, 16 : 8 LD, 5°C, 8 : 16 LD, 10°C, 8 : 16 LD and 15°C, 8 : 16 LD did not bear reproductive structures.

Our culture experiments show that *Gigartina ochotensis* has also sexual life history in addition to apomictic life history as do *G. papillata* (POLANSHEK and WEST, 1977), *G. stellata* (CHEN *et al.*, 1974, WEST *et al.*, 1977, RUENESS, 1978, DION te DELÉPINE, 1979) and *G. jardinii* (WEST *et al.*, 1978).

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