

Hirotohi Yamamoto, Masao Ohno and Nguyen Huu Dinh: *In vitro* life histories and spermatangial types of two *Gracilaria* species from Vietnam, *G. heteroclada* and *G. firma* (Gracilariaceae, Rhodophyta)

Key Index Words: Gracilaria—*G. firma*—*G. heteroclada*—life history—spermatangium—spermatangial type—Rhodophyta.

Hirotohi Yamamoto, Faculty of Fisheries, Hokkaido University, Hakodate, Hokkaido, 041 Japan

Masao Ohno, Usa Marine Biological Institute, Kochi University, Tosa, Kochi, 781–11 Japan

Nguyen Huu Dinh, National Center for Scientific Research of Vietnam, Union for Science, Nhatrang, Vietnam

On Vietnamese species of the genus *Gracilaria*, several researchers conducted taxonomic studies (Dawson 1954, Pham 1969, Nguyen 1969, 1972). Recently Nguyen (1991) reexamined their herbaria and listed 15 species. However, more information is needed for decisive identification of some taxa. The information on male structures is indispensable for basically grouping *Gracilaria* species.

A taxon (Taxon A) called tentatively *Gracilaria bursa-pastoris* (Gmelin) Silva or *G. heteroclada* Zhang and Xia in Vietnam has been increasingly used in pond culture for agar materials of good quality. However, since the male structures were not known, identification remained provisional. Another taxon (Taxon B), which is mixed up with *G. salicornia* (C. Agardh) Dawson in Vietnam, seemed close to *G. changii* (Xia and Abbott) Abbott, Zhang and Xia or *G. firma* Chang and Xia in morphology but the accurate identification was also suspended owing to the lack of information on the males. Accordingly, we attempted to complete the life histories of the two taxa to confirm their male structures and to finalize their names.

Ohno and Nguyen collected several mature female fronds of both Taxon A (Ha Tien, Kien Giang Prov.) and Taxon B (Dong Xauan, Phu Yen Prov.) in Vietnam in Febru-

ary, 1993 and Ohno brought them back to Japan for *in vitro* culture. The culture experiments were conducted by the first author of this paper at the laboratory in Faculty of Fisheries, Hokkaido University.

Several cystocarps were cut off from the fronds and shaken with sterile sand to remove surface contamination (*cf.* Yamamoto and Sasaki 1987). Each of these cystocarps was placed overnight in Petri dish with sterile seawater. Fifteen to twenty spores released were transferred to each of 20 ml glass bottles by pipette for establishing unialgal culture. Six sporelings of *ca* 5 mm in length (*ca* 40 days culture) were detached from the bottom of the glass bottles and transferred into each of 500 ml flasks for free-living culture.

Culture conditions: Temperature of 26–27°C, light of *ca* 75 $\mu\text{E m}^{-2}\text{s}^{-1}$ of cool white fluorescent lamps, a photoperiod of 14 h (light)–10 h (dark) and aeration (*ca* 0.2 l/min) only in free-living culture. PES medium (1/2 concentration of original prescription) without vitamins was changed about once a week throughout free-living culture. Spores from raised fronds were cultured under the same conditions.

Taxon A: Carpospore-derived fronds grew up to 15–20 cm in length and released tetraspores in about 70 days after carpospores were transferred to glass bottles. Tetrasporophytes were terete and irregularly branched. Branches were long, but often spinous in upper parts of axes. Color was pale green. Tetraspore-derived fronds gave rise to male

This study was supported by a Grant-in-Aid for Science Research (International Science Research Program-Field Research No. 04041015) from the Ministry of Education, Science and Culture, Japan.

(10–15 cm long) and female (*ca* 5 cm long) fronds in 60–70 days (Figs. 1 and 2). These gametophytes were the same as wild tetrasporophytes in general appearance and color.

Spermatangia were formed superficially (*Chorda* type, *cf.* Yamamoto 1975) all over the fronds except basal and apical portions. Cystocarps appeared all over the fronds except apical portions, up to 1 mm high and 1 mm in diameter. Traversing filaments in cystocarps are absent. Released carpospores developed normally and completed the life history of typical *Polysiphonia* type in about 140 days from the initial culture.

Taxon B: Carpospore-derived fronds grew up to 5–7 cm and released tetraspores in about 120 days after carpospores were transferred to glass bottles. Tetraspores released developed into male and female fronds in about 100 days (Figs. 3 and 4). Both tetrasporophytes and gametophytes were terete, irregularly branched and dark to blackish brown in color. Branches were more or less inflated in middle portion and branch bases were markedly constricted and sometimes stipitate (Figs. 3 and 4). The fronds were small but similar to wild ones in morphology. Tetrasporangia, spermatangia and cystocarps were formed all over the fronds except basal and apical portions.



Figs. 1 and 2. Habit of *Gracilaria heteroclada* raised in culture, showing characteristic spinous branchlets. Fig. 1. Female. Fig. 2. Male. Scale bars: 2 cm for 1 and 3 cm for 2.

Figs. 3 and 4. Habit of *Gracilaria firma* raised in culture, showing distinctive constriction (rather stipitate) of basal part of branches. Fig. 3. Male. Fig. 4. Female. Scale bars: 2 cm for 3 and 4.

Male structure is *Verrucosa* type (*cf.* Yamamoto 1975). Life history was completed in about 250 days and showed typical *Polysiphonia* type.

Taxonomy

Taxon A: Nguyen (1991) thought that *G. heteroclada* was similar to plants he had earlier identified as *G. bursa-pastoris* (Nguyen 1969, 1972), but he did not have spermatangial material.

Our culture experiment showed that male type of *Taxon A* coincided with that of *G. heteroclada* and not *G. bursa-pastoris* (*Textorii* type, *cf.* Yamamoto 1975). Raised *Taxon A* was almost the same morphologically as the former in having spinous branchlets (Zhang and Xia 1988, Fig. 2) and having pale green color. *G. heteroclada* was identified by Zhang and Xia as having *Chorda* type spermatangia.

The distribution of *G. heteroclada* was confirmed to range as far west as Vietnam from Hainan island, China (type locality).

Taxon B: *Taxon B* was sometimes included in *G. salicornia* or *G. firma* owing to not understanding the morphological variation, and also sometimes considered as *G. changii*. Morphology of raised fronds almost coincides with that of *G. firma* with few second order branches and *Verrucosa* type male structure. The plants never showed characteristic branch constrictions of *G. salicornia*. *Taxon B* is basically different from *G. changii* which has male structures of *Polycavernosa* type (Xia

and Abbott 1987).

Acknowledgements

We are grateful to Dr. I. A. Abbott, University of Hawaii, for advice and correcting English.

References

- Dawson, E. Y. 1954. Marine plants in the vicinity of the Institut Oceanographique de Nha Trang, Vietnam. *Pac. Sci.* 8: 373-481.
- Nguyen, H. D. 1969. Raucau. *Gracilaria* of North Vietnam. 66 pp. Hanoi.
- Nguyen, H. D. 1972. Marine algae of North Vietnam, Thesis, 208 pp. Leningrad.
- Nguyen, H. D. 1991. Vietnamese species of *Gracilaria* and *Gracilariopsis*. In Abbott I. A., ed. Taxonomy of economic seaweeds 3: 207-210. California Sea Grant College Program, La Jolla.
- Pham, H. H. 1969. Marine algae of South Vietnam, 559 pp. Ministry of Education and Youth, Trung-Tam Hoc-Lieu Xuat-Btan, Saigon.
- Xia, B. and Abbott, I. A. 1987. New species of *Polycavernosa* Chang & Xia (*Gracilariaceae*, *Rhodophyta*) from the western Pacific. *Phycologia* 26: 405-418.
- Yamamoto, H. 1975. The relationship between *Gracilariopsis* and *Gracilaria* from Japan. *Bull. Fac. Fish. Hokkaido Univ.* 26: 217-222.
- Yamamoto, H. and Sasaki, J. 1987. Crossing experiments between populations of so-called *Gracilaria verrucosa* (Huds.) Papenfuss from two localities, Shinori and Kikonai in Hokkaido. *Bull. Fac. Fish. Hokkaido Univ.* 38: 335-338.
- Zhang, J. and Xia, B. 1988. On two new *Gracilaria* (*Gigartinales*, *Rhodophyta*) from south China. In Abbott I. A., ed. Taxonomy of economic seaweeds 2: 131-136. California Sea Grant College Program, La Jolla.

山本弘敏*・大野正夫**・Nguyen Huu Dinh***:ベトナム産オゴノリ属植物2種

Gracilaria heteroclada, *G. firma* の生活史と雄性生殖器官の型

ベトナム産オゴノリ属2種の種名は雄性生殖器官が不明なため確定していなかった。この2種について、室内培養により果胞子からイトグサ型の生活史を完結させると共にそれぞれの雄性生殖器官の型を確認した。その結果、表層型(*Chorda* type)を示す種を *Gracilaria heteroclada*, 深いツボ型の種を *G. firma* と同定した。(*041 函館市港町3-1-1 北海道大学水産学部, **781-11 土佐市宇佐町 高知大学海洋生物教育研究センター, *** National Center for Scientific Research of Vietnam, Union for Science, Nhatrang, Vietnam)

(Received May 10, 1994. Accepted July 12, 1994)

