Jpn. J. Phycol. (Sôrui) 41: 131–135, June 20, 1993

In vitro life history and spermatangial type of *Gracilaria eucheumoides* (Gracilariaceae, Rhodophyta)*

Hirotoshi Yamamoto** and Tadahide Noro***

**Faculty of Fisheries, Hokkaido University, Minato-machi 3-1-1, Hakodate, Hokkaido, 041 Japan
***Faculty of Fisheries, Kagoshima University, Shimoarata 4-50-20, Kagoshima, 890 Japan

Yamamoto, H. and Noro, T. 1993. *In vitro* life history and spermatangial type of *Gracilaria eucheumoides* (Gracilariaceae, Rhodophyta). Jpn. J. Phycol. 41: 131–135.

The spermatangial arrangements of *Gracilaria eucheumoides* Harvey were found to be multicavitied (*Polycavernosa* type) for the first time in the completion of the life history *in vitro*. This species can therefore be placed in *Hydropuntia* group of the genus *Gracilaria* on the basis of its spermatangial type. The life history showed the possible *Polysiphonia* type. Cultured fronds were almost terete, differing from compressed axes of wild fronds, and the cause of the morphological variation was discussed. Extended cells penetrating into pericarp of cystocarps were confirmed to be present. The nature of monosporangium-like cells, which appear in carpospore-derived fronds, was discussed, comparing with the observation of other researchers.

Key Index Words: Gracilaria—Gracilaria eucheumoides—*life history*—Hydropuntia—Polycavernosa—*spermatangium*—*spermatangial type*—*Rhodophyta*.

Since Harvey (1859) established *Gracilaria eucheumoides* on the basis of Japanese materials, many researchers have reported that this species grows widely in the regions from the southern part of Japan to southeast Asia, including the Caroline Islands (Weber van-Bosse 1928, Trono 1969, Chang and Xia 1976, Yamamoto 1978, Trono *et al.* 1983, Meneses and Abbott 1987).

Chang and Xia (1976) described cystocarps and tetrasporangia on Chinese materials of this alga, and Trono *et al.* (1983) reported only tetrasporangia on Philippine collections. The features of the reproductive organs show that this species is a member of genus *Gracilaria*. However, the male structures which form the basis for grouping the species within the genus were not known. Additionally, although this alga is very abundant in the Philippines and also common in other regions, collected specimens that are fertile are very rare or are unknown in given regions, for example, in Japan.

* This work was supported by the International Scientific Research Program No. 01041067 of the Ministry of Education, Science and Culture in Japan.

A single mature cystocarpic frond was collected in the Philippines, and growth in culture conditions provided information on reproductive structures and the life history.

Materials and Methods

A mature cystocarpic frond (Fig. 1) was collected at Matabungkay Beach (Lian, Batangas, the Philippines) in November, 1989. At the Marine Station in Bolinao, the Marine Science Institute, the University of the Philippines (Diliman), the several cystocarps were cut off from the frond and shaken with sterile sand to remove the epiphytes and the other surface contaminations (*cf.* Yamamoto and Sasaki 1987). Each of these cystocarps was placed overnight in Petri dishes with sterile seawater for spore release. About ten spores released were transferred to each of 20 m*l* glass bottles by a pipette for establishing unialgal culture, and these bottles were brought back to Japan for culture. Sporelings of 2 mm length in about 60 days were detached from the bottom of 20 m*l* glass bottles and transferred into 500 m*l* flasks for free-living

culture.

Culture conditions were: a temperature of 28-29°C, *ca* 75 μE/m^2·sec of cool-white fluorescent lamps, a photoperiod of 14 h (light)-10 h (dark), and aeration (0.3 *l*/min) only in free-living culture. PES medium without vitamins was changed about once a week throughout the culture. Spores from cultured fronds were incubated under the same conditions.

Results

Carpospore-derived fronds grew up to a length of 7 cm to release spores* in about 19 months after carpospores were transferred to glass bottles (Fig. 2). Sporophytes* were almost terete or sometimes slightly compressed, irregularly branched and reddish brown to purplish red in color. Sporangia appeared on the fronds, undivided, up to 55 μm high, 20 μm wide, and brightly reddish. Each of them is connected with a mother cell by single pit connection and has single nucleus in it (Figs. 3, 4). The spores in turn gave rise to mature spermatangial and cystocarpic fronds of the length of 2-5 cm in about 6 months (Fig. 5). These gametophytes were almost terete and reddish brown to purplish red in color.

Male structures were formed only in the middle part of fronds and multicavitied (*Polycavernosa* type in Chang and Xia 1963; *Henriquesiana* type in Yamamoto 1984), showing a group of 5-6 conceptacles in the extention of up to 180 μm deep, up to 100 μm wide in longitudinal sectional view (Figs. 6, 7).

Cystocarps appeared all over the fronds except basal and apical portions, up to 1,000 μm high, up to 1,000 μm in diameter (Fig. 5). Of 20 spore-derived fronds, 4 developed into cystocarpic fronds and 5 into spermatangial, and the other remained sterile even in 5 months after the first cystocarp appeared. The released carpospores developed normally and completed the life history.

* We can not say at this time whether the released spores were tetraspores or not because divided sporangia have never been observed. Accordingly, "spore" or "sporophyte" are used in place of "tetraspore" or "tetrasporophyte" respectively in this paper.

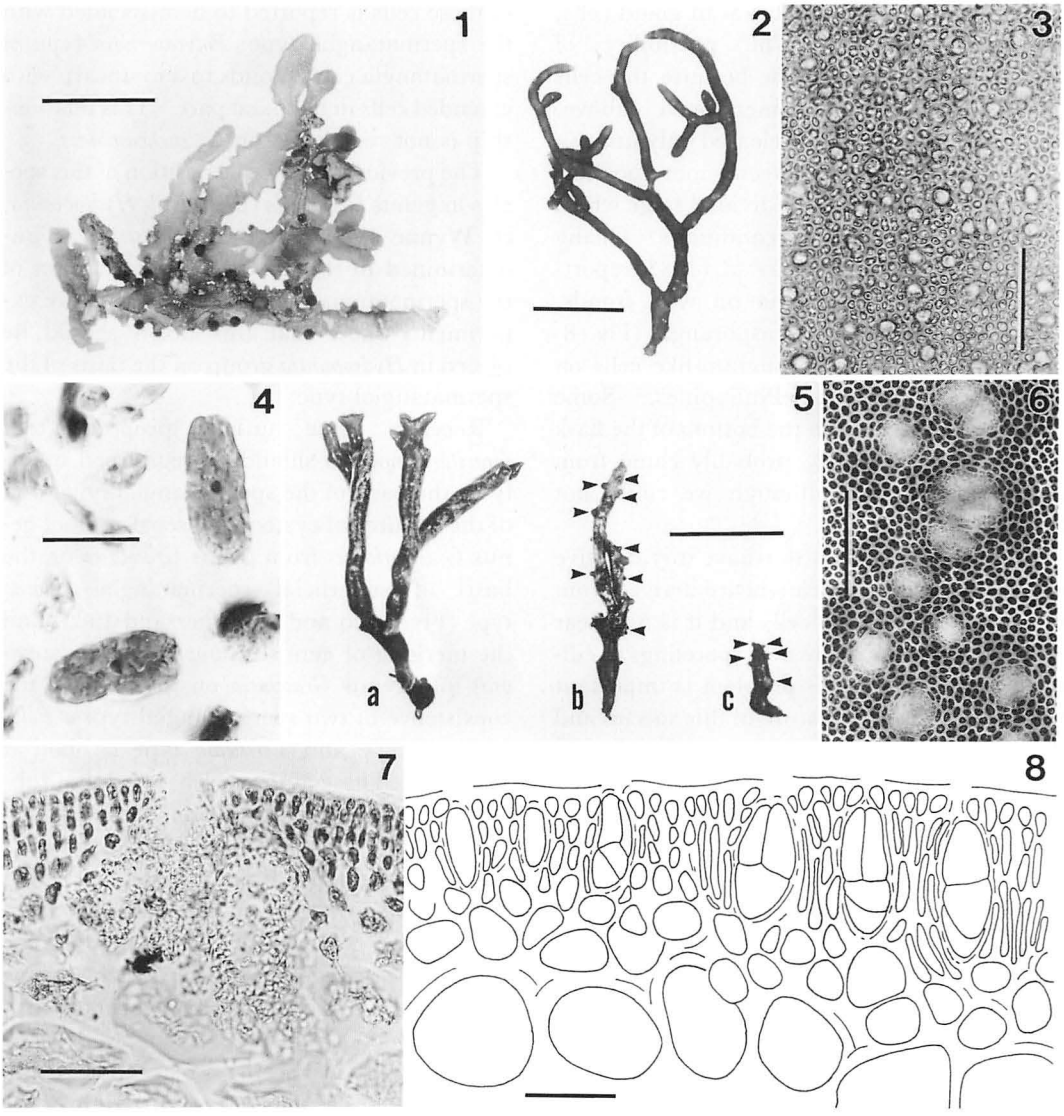
Discussion

Main axes and branches of wild fronds are almost always compressed and sometimes almost flat (Fig. 1). However, the fronds raised in free-living culture are only slightly compressed or terete (Figs. 2, 5). The wild fronds have both dorsal and ventral sides because of their prostrate habit. In free-living culture, on the other hand, the fronds do not have such a fixed dorso-ventral orientation. Accordingly, the condition in which they grow appears to cause this morphological variation. Branching mode of wild fronds is alternate or pinnate. However, that of the raised fronds is irregular in all directions. This irregularity also appears to be induced by the lack of fixed sides.

Both sporophytes and gametophytes matured into much smaller frond size than wild fronds, which are usually 10 cm or more. Generally, reproductive organs tend to be formed in smaller frond size under the optimum culture conditions. Accordingly, this difference in the size does not present a taxonomic problem.

It was confirmed that the life history is probably *Polysiphonia* type. This fact is not consistent with rare occurrence of gametophytes in the field. In this experiment, about half of possible gametophytes kept on remaining sterile. This result suggests that the life history may not always be *Polysiphonia* type in the field. It is possible that the life history repeats only sporophyte generations. Accordingly, it is required to ascertain whether or not reduction division occurres in the formation of spores.

We can often observe monosporangium-like cells which are crowded in the cortical layer almost only on ventral side of wild fronds. Those cells are similar to undivided tetrasporangia in their appearance. A structure corresponding to such cells was also formed on carpospore-derived fronds raised



Figs. 1–8. *Gracilaria eucheumoides* from the Philippines. Fig. 1. A cystocarpic frond collected at Matabungkay, showing prominant cystocarps formed on ventral side of almost flat frond. Fig. 2. A carpospore-derived frond (sporophyte) raised *in vitro*, showing almost cylindrical axes and branches. Fig. 3. Surface view of the frond with monosporangium-like cells, showing crowded formation. Fig. 4. Monosporangium-like cells, showing a nucleus in each. Fig. 5. Spermatangial and cystocarpic fronds. a: Spermatangial frond raised *in vitro*. b and c: Cystocarpic fronds raised *in vitro*, showing cystocarps (arrowheads) formed all over the fronds. Fig. 6. Surface view of the male frond with multicavitied spermatangial conceptacles. Fig. 7. Sectional view of multicavitied spermatangial conceptacles. Fig. 8. Diagram of wild tetrasporangial frond, showing cruciately but often irregularly divided tetrasporangia. Scale bars=1–2, 5: 2 cm, 3, 6–7: 100 μm, 4, 8: 50 μm.

in culture (Figs. 3, 4). We attempted to make spores release from both wild and raised fronds which have fully grown monosporangium-like cells but failed.

The nature of the monosporangium-like cells presents three possibilities: 1) gland cells, 2) monosporangia and 3) tetrasporangia in undivided stage.

Trono *et al.* (1983) described these cells as gland cells. However, we have doubt on their opinion because the cells are formed only on carpospore-derived fronds and the

color is not yellowish as usual in gland cells, but brightly red. The possibility of monosporangia seems low because the cells never discharge as mentioned above. However, they could be released only under a particular condition. It seems more possible to be tetrasporangia in undivided stage which can divide under certain conditions. Chang and Xia (1976) and Trono *et al.* (1983) reported cruciate tetrasporangia on wild fronds. We also found a few tetrasporangia (Fig. 8) together with monosporangium-like cells on wild fronds from the Philippines. Some sporelings appeared on the bottom of the flask during culture. They probably came from the tetrasporangia, although we could not find them.

At this time, we do not have any decisive evidence that tetrasporangia are derived from monosporangium-like cells and it is not clear what kinds of spores the new sporelings in culture came from. This problem is important for making clear the nature of this species and these three possibilities on the peculiar "cells" are needed to be solved.

Spermatangia are formed in multicavitied conceptacles. Although the conceptacles sometimes assume *Verrucosa* type, they must be in initial developmental stage because they are very small in size compared with multicavitied conceptacles. The single-cavitied conceptacles seem to develop finally into multicavitied ones (*Polycavernosa* type). Typical *Verrucosa* type never develops into *Polycavernosa* type. Consequently, the spermatangial type of *G. eucheumoides* was concluded to be not a mixture of *Verrucosa* type and *Polycavernosa* type but just the latter judging from the final form.

Cystocarps raised in culture are much smaller compared with those of the parent or of ones described by Chang and Xia (1976) but this small dimension seems only to depend on the small frond size. They were able to release normal carpospores. Cell extensions exist both in upper and lower parts of a cystocarp as the figure of Chang and Xia (1976) shows, and some of them penetrate into the basal tissue. Generally, the location of these cells is reported to be associated with the spermatangial type: *Polycavernosa* type of spermatangia corresponds to a cystocarp with extended cells in the basal part. This relationship is not recognized in *G. eucheumoides*.

The previous taxonomic position of this species in genus *Gracilaria* (including *Hydropuntia*, *cf.* Wynne 1989 and *Gracilariopsis*) was undetermined in the absence of information of the spermatangia. However, our culture experiments show that this taxon should be placed in *Hydropuntia* group on the basis of the spermatangial type.

Recently some authors proposed that *Gracilaria* species should be regrouped mainly on the basis of the spermatangial types and of the features of cystocarps: separation of genus *Gracilariopsis* from genus *Gracilaria* on the basis of superficial spermatangia—*Chorda* type (Fredericq and Hommersand 1989) and the merging of genus *Hydropuntia* (*Polycavernosa*) into genus *Gracilaria* on the basis of the coexistence of two spermatangial types: *Polycavernosa* type and *Verrucosa* type (Abbott *et al.* 1991). These authors also stated that subgenera *Textoriella* and *Gracilaria* (*cf.* Yamamoto 1975) might not be separatable because both spermatangial types (*Textorii* type and *Verrucosa* type) coexist on the same frond of *G. blodgettii*. However, the *Verrucosa* type which appears together with *Polycavernosa* type is almost restricted around branch tips (Abbott *et al.* 1991). As we observed on *G. eucheumoides*, it seems reasonable that *Verrucosa* type which is recognized in *Polycavernosa* type species is only on a developmental process to final stage (*cf.* Abbott *et al.* 1991, p. 20).

In spite of exceptional or indistinguishable cases in *G. blodgettii* and in *G. mixta*, spermatangial type is still a helpful criterion for grouping the species of *Gracilaria* because almost all the species have distinctive spermatangial types. *G. blodgettii* appears to be a species bridging two groups in the progress.

The relationship among genera (or groups) *Gracilariopsis*, *Gracilaria* and *Hydropuntia* (*Polycavernosa*) is important for systematics. Bird *et al.* (1992) studied the phylogenetic

relationships in the Gracilariales on the basis of DNA sequences, and suggested that *Hydropuntia* is a subgenus of *Gracilaria*. Their experiments could offer a new criterion for the systematics of *Gracilaria*. However, more detailed information on the developmental process of male and female organs of various species is required. Accordingly, at the present time, we place *G. eucheumoides* as a member of genus *Gracilaria*.

Acknowledgements

We sincerely thank Dr. I. A. Abbott, University of Hawaii, for critical reading of the manuscript.

References

Abbott, I. A., Zhang, J. F. and Xia, B. M. 1991. *Gracilaria mixta*, sp. nov. and other western Pacific species of the genus (Rhodophyta: Gracilariaceae). Pac. Sci. **45:** 12–27.

Bird, C. J., Rice, E. L., Murphy, C. A. and Ragan, M. A. 1992. Phylogenetic relationships in the Gracilariales (Rhodophyta) as determined by 18S rDNA sequences. Phycologia **31:** 510–522.

Chang, C. F. and Xia, B. M. 1963. *Polycavernosa*, a new genus of the Gracilariaceae. Stud. Mar. Sin. **3:** 119–126.

Chang, C. F. and Xia, B. M. 1976. Studies on Chinese species of *Gracilaria*. Stud. Mar. Sin. **11:** 91–163.

Fredericq, S. and Hommersand, M. H. 1989. Comparative morphology and taxonomic status of *Gracilariopsis* (Gracilariales, Rhodophyta). J. Phycol. **25:** 228–241.

Harvey, W. H. 1859. Characters of new algae, chiefly from Japan and adjacent regions, collected by Charles Wright in the north Pacific exploring expedition under Captain John Rodgers. Proc. Amer. Acad. Arts and Sci. **4:** 327–335.

Meneses, I. and Abbott, I. A. 1987. *Gracilaria* and *Polycavernosa* (Rhodophyta) from Micronesia. Micronesica **20:** 187–200.

Trono, G. C., Jr. 1969. The marine benthic algae of the Caroline Islands, II. Phaeophyta and Rhodophyta. Micronesica **5:** 25–119.

Trono, G. C. Jr., Azanza-Corrales, R. and Manuel, D. 1983. The genus *Gracilaria* (Gigartinales, Rhodophyta) in the Philippines. Kalikasan, Philipp. J. Biol. **12:** 15–41.

Weber van-Bosse, A. 1928. Liste des algues du Siboga IV, Rhodophyceae, troisieme partie, Gigartinales et Rhodymeniales. Siboga-Expeditie LIX d. 393–533.

Wynne, M. J. 1989. The re-instatement of *Hydropuntia* Montagne (Gracilariaceae, Rhodophyta). Taxon **38:** 476–479.

Yamamoto, H. 1975. The relationship between *Gracilariopsis* and *Gracilaria* from Japan. Bull. Fac. Fish. Hokkaido Univ. **26:** 217–222.

Yamamoto, H. 1978. Systematic and anatomical study of the genus *Gracilaria* in Japan. Mem. Fac. Fish. Hokkaido Univ. **25:** 97–152.

Yamamoto, H. 1984. An evaluation of some vegetative features and some interesting problems in Japanese populations of *Gracilaria*. Hydrobiologia **116/117:** 51–54.

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.

山本弘敏*・野呂忠秀**：培養によるリュウキュウオゴノリ (*Gracilaria eucheumoides*) の生活史と雄性生殖器官

リュウキュウオゴノリの属内での分類上の位置は，雄性生殖器官が不明なため確定されずにいた。1989年11月，フイリッピンで得た果胞子から培養することにより生活史はほぼイトグサ型であること，雄性生殖器官は多穴型 (*Polycavernosa* type) であることを確認し，本種をオゴノリ属の *Hydropuntia* グループに位置づけた。培養体は扁平な野生体とは異なり円柱状になったが，この原因について培養条件と関係づけて考察した。また，野生体，培養体双方に形成される単胞子状の細胞の本質について，1）腺細胞，2）単胞子嚢，3）分裂しない段階の四分胞子嚢の可能性を想定し検討した。多穴型の雄性生殖器官を持つ種の嚢果中の伸長細胞 (extended cell) は下方にのみ向うとされているが，本種では上方にも向い，果皮に侵入する程に伸長することを確認した。(*041 函館市港町3-1-1 北海道大学水産学部，**890 鹿児島市下荒田4-50-20 鹿児島大学水産学部)

(*Received September 14, 1992: Accepted March 24, 1993*)