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Author(s)	YAMAMOTO, Hirotohi; 山本, 弘敏
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***In vitro* life history and spermatangial pattern of  
*Gracilaria arcuata* Zanardini (Gracilariaceae)  
from the Philippines<sup>1,2</sup>**

Hirotooshi YAMAMOTO<sup>3</sup>

**Abstract**

The writer revealed life history and spermatangial pattern of Philippine *Gracilaria arcuata* in culture. Life history was *Polysiphonia* type and spermatangial pattern was *Verrucosa* type. Cystocarps raised in culture were similar to those collected in the field. Morphological modification was seen and some green gametophytes appeared among the predominantly brownish fronds.

*Gracilaria arcuata* was established based on materials from the Red Sea by Zanardini (1858). Since then, this species has been known to grow widely in subtropical and tropical regions: southern part of Japan (see Yamamoto, 1978), southern China (Chang and Xia, 1976), Philippines (Trono et al. 1983), Indonesia (Weber-van Bosse, 1928), Ceylon (Durairatnam, 1961), India (Rao, 1972), Mauritius (Børgesen, 1943), etc.

Three phases of the reproductive organs of this species have already been known in Japanese and Indian materials, but only tetrasporophytes have been reported in Philippine ones. Spermatangial pattern is an indispensable basis for the taxonomical study of *Gracilaria* species. Accordingly the writer attempted to confirm the life history and to reveal the pattern of male reproductive organ in culture in order to review conspecificity between the taxa of Japan and the Philippines.

**Materials and methods**

Fertile carposporophytes were collected at Bolinao in northern Luzon, the Philippines on Nov. 30, 1989. Spore release was carried out using procedures described previously (Yamamoto and Sasaki, 1987). Carpospores were distributed into 20 ml glass tubes at the Marine Station, Marine Science Institute, University of the Philippines (Diliman).

The writer brought these glass tubes back to Japan for culture. The culture was carried out under these conditions: 3,500-4,000 lux of a white fluorescent lamp, a photoperiod of 14h (light)-10h (dark), a temperature of 26-27°C and aeration after detaching the sporelings from the substratum. PES medium without vitamins was changed once a week throughout the culture.

Carpospore-derived sporelings cultured up to 5 mm in length in about 40 days

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  - 3) Usujiri Fisheries Laboratory, Faculty of Fisheries, Hokkaido University

were detached from the substratum and 10 were transferred into each 1,000 ml flask for free-living culture.

### Results

Carpospore-derived sporelings grew up to 7 cm in length in about 70 days and released tetraspores. Tetraspores gave rise to male and female gametophytes which formed normal cystocarps in about 80 days (Fig. 1). The life history was completed in about 150 days from the initial incubation of carpospores and was of the typical *Polysiphonia* type. Spermatangia were formed over the entire frond surface except basal and apical portions. Spermatangial pattern was *Verrucosa* type (Yamamoto, 1975). Conceptacles were up to  $37.5\ \mu\text{m}$  wide and up to  $75\ \mu\text{m}$  deep and were isolated from each other by vegetative cells, but sometimes fused with each other at fully mature stage.

Both carpospore- and tetraspore-derived sporelings often developed one or two holdfast-like structures after growing over 2-3 cm in length under free-living condition (Fig. 2).

External appearance of the fronds cultured was polymorphic: from arcuated

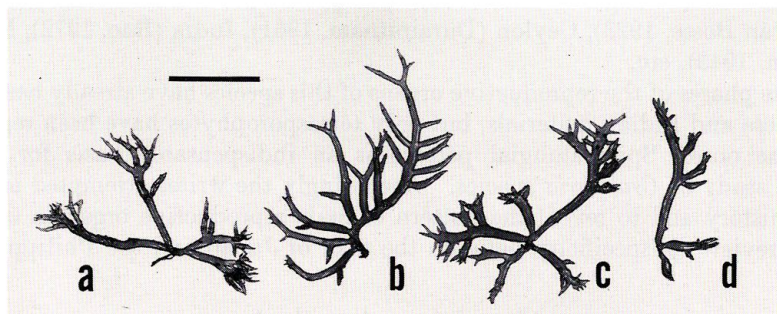


Fig. 1. Habits of *Gracilaria arcuata* cultured in flasks in free-living condition. a and b are brownish fronds: female (a) and male (b). c and d are green fronds: female (c) and male (d). Scale bar = 3 cm.

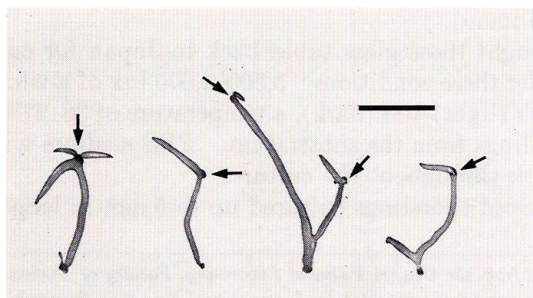


Fig. 2. Young fronds of *Gracilaria arcuata* cultured in flasks in free-living condition, showing holdfast-like structures (arrows). Scale bar = 1 cm.

main axes with secund branches to straight axes with irregular branching mode (Fig. 1). Some green fronds appeared among brownish tetraspore-derived sporelings. These green fronds were easily distinguishable from brownish ones and retained this coloring all throughout their lives. Cystocarps formed by crosses between green gametophytes released carpospores which germinated normally.

### Discussion

The taxon of the Philippines showed the typical *Polysiphonia* type of life history. This is the same as those of the other *Gracilaria* species which have been demonstrated until now.

Spermatangial pattern of *Verrucosa* type verified that this taxon was a member of subgenus *Gracilaria* (Yamamoto, 1975). The writer once was a little doubtful about whether Philippine taxon was the same as Japanese one owing to morphological difference and lack of the knowledge on gametophytes. However, the results obtained in this culture showed basic taxonomic grounds to be conspecific for the taxa of the two regions, because Japanese taxon has isomorphic tetrasporophytes and gametophytes and has spermatangial pattern of *Verrucosa* type. Size of the conceptacles was smaller than those of Japan (Yamamoto, 1978) and India (Rao, 1972). This difference in size may suggest its being a distinct species. However, reproductive organs of fronds cultured *in vitro* often show variation in size from those collected in the field. We need further studies on size of the reproductive organs.

The writer collected carposporophytes for the first time in the Philippines. Additionally, he was able to raise the carposporophytes in this culture. Cystocarps both in the field and in the culture have the absorbing filaments and showed no difference in basic structure between the two. These cystocarps were also similar to those of Japanese taxon.

Chang and Xia (1976) and Trono et al. (1983) described that *G. arcuata* var. *snackeyi* f. *rhizophora* could form secondary holdfast on branches in contact with the substrata. It is interesting that young fronds which have no continuous contact with the other branches or substrata in free-living condition developed such organs. The formation of secondary attachment organs to the substrata or to other branches is observed in *Gracilaria eucheumoides* and *G. coronopifolia*, but it is never observed in such species as *G. verrucosa*, thus this seems to be a specific characteristic of each taxon.

External appearance of the fronds cultured in flasks differed in form of axes and in branching mode. Morphological modification according to habitats is often observed in *Gracilaria* species. Trono et al. (1983) report occurrence of two morphological forms of this taxon according to habitats in the Philippines: *G. arcuata* var. *snackeyi* and var. *snackeyi* f. *rhizophora*. As some of *Gracilaria* species are said to be flexible in morphology, this species seems to be one of them.

Occurrence of green fronds in culture is interesting. They may be used for the studies on heredity, coalescence between tissues, etc., because they retained the coloring all their lives which is very clear and distinguishable from the other brownish fronds.

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