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Relationship between *Gracilaria* "verrucosa"¹ and *Gracilaria* sp. (Gracilariaceae) of Manila Bay, Philippines²

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Abstract

Gracilaria "verrucosa" and *Gracilaria* sp. were cultured *in vitro* to verify these characteristics and to review the relationship between the two taxa.

Life histories of both algae were *Polysiphonia* type but spermatangial pattern was different: *Gracilaria* "verrucosa" is of *verrucosa* type while *Gracilaria* sp. is of *chorda* type, differing from *textorii* type described by other authors. Their morphological features were stable and were retained from generation to generation in culture conditions. Thus, the two taxa were demonstrated to be distinct species.

Introduction

Gracilaria "verrucosa" is widely distributed in the Philippines. However, because of its wide range of the morphology it seems possible that the taxon commonly called *G.* "verrucosa" is a mixture of several species.

Trono et al. (1983) segregated *Gracilaria* sp. from *G.* "verrucosa" on the basis of 1) spinous branchlets and non-constriction at branch bases, 2) absence of tubular cells in cystocarps and 3) shallow saucer-shaped spermatangial conceptacle. They recognized the need for the examination of more male materials of *Gracilaria* sp. to clarify its status in the genus *Gracilaria*. The type of spermatangial pattern is an important attribute in the grouping of *Gracilaria* species and a basic taxonomical criterion for them (Yamamoto, 1975). Thus, the type of the spermatangial apparatus and variability of their morphologies should be confirmed and be made as bases for the separation of these taxa.

A trip to Manila Bay in November-December 1989 to collect male materials of *Gracilaria* sp. was unsuccessful so that the author carried out *in vitro* culture of the two taxa to produce male fronds for the critical examination of their spermatangial pattern and morphological variability. A crossing experiment between the two taxa was also conducted to verify more critically their relationship.

Materials and Methods

Fertile tetrasporophytes of the two taxa were collected in Parañaque, Manila

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- 1) Dr. G. C. Trono, Jr., University of the Philippines, suggested to the author that quotation marks be placed on *G. verrucosa* of the Philippines, because Philippine taxon should be reviewed taxonomically.
 - 2) Contribution No. 69 from the Usujiri Fisheries Laboratory, Faculty of Fisheries, Hokkaido University.
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Bay on December 10, 1989. Spore release was carried out using methods described by Yamamoto and Sasaki (1987). Collected spores were placed in 20 ml glass tubes with culture solution and brought back to Japan for culture. Spores were allowed to grow up to 1 cm long and then transferred to 1,000 ml flasks for free-living culture. Six sporelings were cultured in each flask. Sterile portions (branch tips) of female and male fronds of the two species were used for reciprocal crosses. Self-crosses were used as controls.

Culture was conducted under the following conditions: 27–28°C temperature; 14 h (light)-10 h (dark) photoperiod with white fluorescent lamp and aeration in free-living culture. PES medium without vitamins was used throughout the culture. Medium was changed once a week. The spores released by the cultured fronds were transferred to glass tubes using a pipette.

Results

Gracilaria "verrucosa": Life history of this species was of the *Polysiphonia* type and completed in about 140 days. Cystocarps, spermatangia and tetrasporangia were formed only along the middle portions of main axes and long lateral branches of the female, male and tetrasporangial fronds. Tubular cells reaching the pericarp were present. The spermatangial pattern was of the *verrucosa* type (Fig. 7; Yamamoto, 1975) and tetrasporangia were cruciate. The highly constricted bases of the branches which are distinctive features of this taxon were still retained in the second gametophytic generation under culture (Fig. 6). The color of the fronds was similar to those of the original tetrasporophytes.

Gracilaria sp.: Life history of this taxon was also of the *Polysiphonia* type and was completed in about 150 days. The cystocarps, spermatangia and tetrasporangia were developed only at the middle portions of the main axes and long lateral branches. Tubular cells were absent while spermatangia which were formed superficially were of the *chorda* type (Fig. 4; Yamamoto, 1975). Tetrasporangia were cruciate. The distinctive morphologies characterized by the spinous branchlets and the non-constricted bases of the branches (Figs. 2-3) were transmitted to the second gametophytic generation.

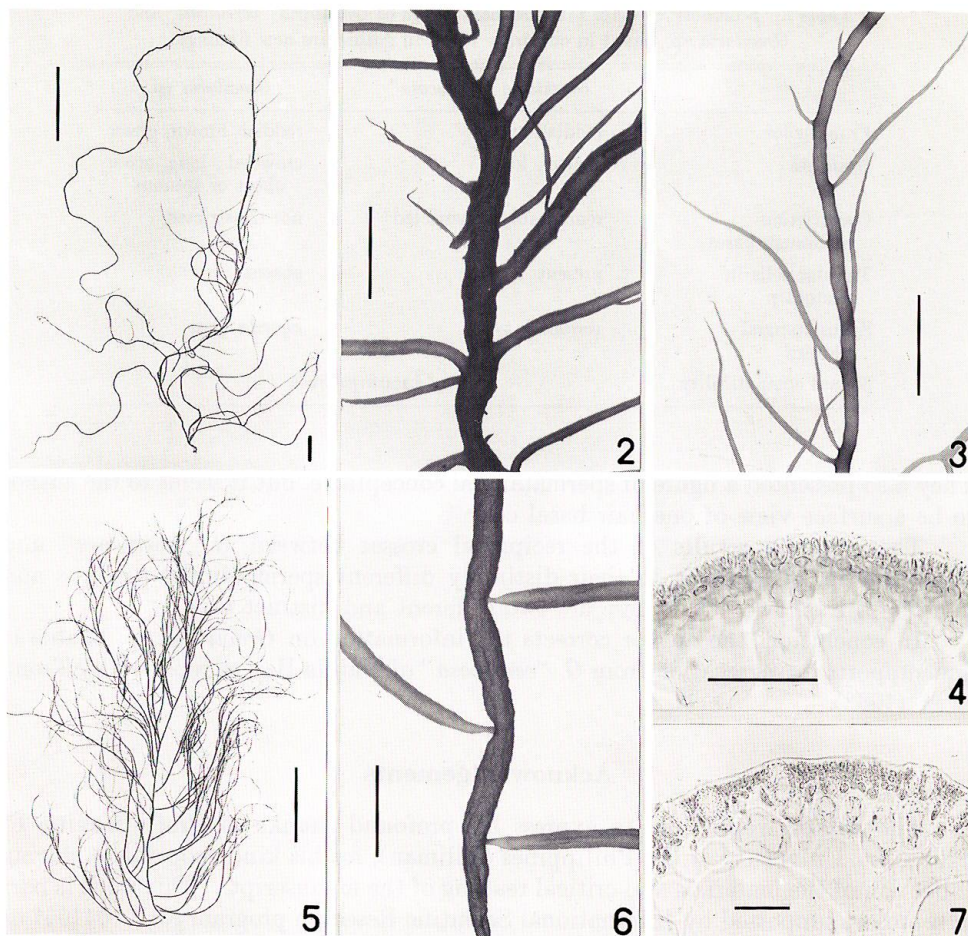
The two taxa were sexually incompatible in reciprocal crosses.

Discussion

Life histories of both taxa were typical *Polysiphonia* type which had been confirmed in the other *Gracilaria* species. The 140–150 days, which were required to complete one cycle of their life histories, are almost equal to those of the other *Gracilaria* species.

The distinctive morphologies of the two taxa were transmitted to next generations, thus demonstrating that their morphologies are very stable even in conditions different from the original habitats and can be used as taxonomical criteria.

Cystocarps of *Gracilaria* sp. were not different from those of *G. "verrucosa"* in basic structures except the absence of tubular cells as Trono et al. (1983) have already reported. The presence or absence of the tubular cells is said to be unreliable as a taxonomical character (Papenfuss, 1966; Yamamoto, 1975). Tubular cells



Figs. 1-4. *Gracilaria* sp. (male frond) raised in culture. Fig. 1. Habit of a fully grown frond. Fig. 2. Close-up of branch bases, showing non-constriction. Fig. 3. Close-up of main axis, showing short blanchlets. Fig. 4. Cross-sectional view, showing superficial spermatangia (*chorda* type).
 Figs. 5-7. *Gracilaria* "*verrucosa*" (male frond) raised in culture. Fig. 5. Habit of a fully grown frond. Fig. 6. Close-up of branch bases, showing marked constriction. Fig. 7. Cross-sectional view, showing deeply pot-shaped spermatangial conceptacles (*verrucosa* type). Scale: 5 cm for Figs. 1 and 5; 5 mm for Figs. 2, 3 and 6; 100 μ m for Figs. 4 and 7.

are variable in number even in the same species, and short tubular cells which exist among carpospores in chains are often difficult to be recognized and can be overlooked. However, the absence of tubular cells in *Gracilaria* sp. could be used when the other criteria are not found, because even the short tubular cells which do not reach the pericarp are not recognizable.

Spermatangia of *Gracilaria* sp. were formed in superficial layer to indicate the *chorda* type. This type is distinctly different from "shallow and saucer-shaped conceptacle" by Trono et al. (1983) which means the *textorii* type (Yamamoto, 1975).

Table 1. Summary of main taxonomical criteria of *Gracilaria* "verrucosa" and *Gracilaria* sp. raised in culture. Items in gothic are new findings.

	<i>Gracilaria</i> "verrucosa"	<i>Gracilaria</i> sp.
Fronde color	reddish brown	reddish brown, green
Branches	sparse; long	crowded; long, often short or spinous
Constriction at branch bases	remarkably constricted	not constricted
Tubular cells in cystocarp	present	absent
Spermatangial pattern	<i>verrucosa</i> type	<i>chorda</i> type
Sexual compatibility	incompatible	

They also presented a figure of spermatangial conceptacle, but it seems to the author to be a surface view of one hair basal cell.

The negative results of the reciprocal crosses between *G. "verrucosa"* and *Gracilaria* sp. in addition to their distinctly different spermatangial patterns and morphologies showed that these are two different and distinct taxa.

In conclusion, the author corrects the information on *Gracilaria* sp. (Table 1) and supports its segregation from *G. "verrucosa"* of Manila Bay as reported by Trono et al. (1983).

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