Notes on *Gracilaria* (Gracilariales, Rhodophyta) from Tosa Bay and adjacent waters I: *Gracilaria chorda*, *Gracilaria gigas* and *Gracilaria incurvata*.

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Abstract: Three species of Gracilaria (Gracilariales, Rhodophyta): Gracilaria chorda Holmes, Gracilaria gigas Harvey, and Gracilaria incurvata Okamura growing at Tosa Bay and adjacent waters (Kochi Prefecture, Japan) were reported and their characteristic features described. G. chorda as well as G. gigas was founded at Uranouchi Inlet, Tosa City (central Tosa Bay). The morphology of G. chorda varied greatly among the different habitats and seasons. Some young plants collected from April to May had long chord-like appearances without branchlets, while the older plants collected from June to July showed numerous filiform branchlets. However, reproductive organs of these plants agree well with the descriptions by Ohmi (1958) and Yamamoto (1978). Although the old fronds of G. gigas were similar to in shape to the aged plant of G. chorda in terms of vegetative appearance, it differed from the latter in regard to its spermatangial features and the presence of traversing filaments in the cystocarp. G. incurvata was found at Uranouchi Inlet and was also found at Kashiwajima, Ohtsuki town, and at Oh-hama, Tosashimizu City (western Tosa Bay). This species closely resembled G. textorii in regard to its flattened appearance, while the reproductive characteristics of this species also resembled those of the latter. However, a curved or twisted shape was shown only in G. incurvata.

Key words: *Gracilaria chorda, Gracilaria gigas, Gracilaria incurvata*, Gracilariales, Kochi, morphology, Rhodophyta, seaweed, Tosa Bay

Introduction

Gracilaria (Greville 1830; Gracilariaceae, Rhodophyta) is well represented on the shores of Japan, with 21 species recognized for the region (Yoshida and Yamamoto, 1998; Terada and Yamamoto, 2000; Terada et al., 2000). On the species of Gracilaria at Tosa Bay, Kochi Prefecture, Okamura (1929) described Rhodymenia punctata Okamura (= Gracilaria punctata (Okamura) Yamada, Yamada, 1941) on the basis of the specimens from Okinoshima (east coast of Kochi Prefecture). Later, Yamamoto (1978) reported the monograph describing Japanese Gracilaria; the specimens of four species in his paper were collected from Kochi Prefecture (including other districts of Japan). Furthermore, Chirapart et al. (1994) reported the existence of an unidentified Gracilaria from Uranouchi Inlet. Later, this alga was described as the eastern Pacific species Gracilaria lemaneiformis (Bory) Greville by Yoshida and Yamamoto (1998).

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Although 21 species of *Gracilaria* are grown in Japan, it is not sufficiently known how many species of *Gracilaria* except for the above mentioned species, are grow at Tosa Bay and adjacent waters. Accordingly, we carried out taxonomic studies of *Gracilaria* growing at Tosa Bay and adjacent waters on the basis of the specimens collected at several localities of these regions. In this paper, we review the morphology of three species of *Gracilaria*: *Gracilaria chorda* Holmes, *Gracilaria gigas* Harvey, and *Gracilaria incurvata* Okamura.

Materials and Methods

The plants examined were collected at several localities at Tosa Bay and adjacent waters, Kochi Prefecture, Japan. Specimens, fixed in 5% formalin/seawater, were used for microscopic observations. Sections were made by freezing microtome and stained with 1% cotton blue in 50% glycerol/seawater. Voucher herbarium specimens are deposited at Kochi Prefectural Deep Seawater Research Institute. Herbarium specimens deposited in the Herbarium of Usa Marine Biological Institute, Kochi University were also reinvestigated for the distribution of each species.

Observations

Gracilaria chorda Holmes, 1896: 253.

Synonym: Gracilariopsis chorda (Holmes) Ohmi; Ohmi, 1958: 50, text fig. 24, pl. 10.

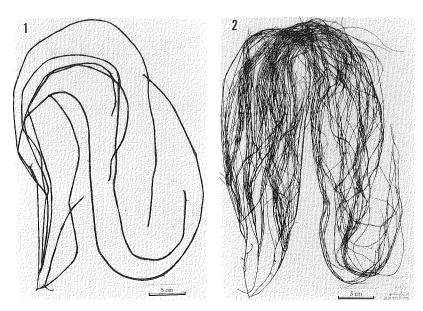
Type specimen: Saida 6, The Natural History Museum (BM), London.

Type locality: Enoura, Numazu City, Shizuoka Prefecture, Japan.

Distribution: Kyushu, Shikoku, Honshu and Hokkaido. Tosa Bay and adjacent waters:

Sukumo Inlet, Sukumo City; Uranouchi Inlet, Tosa City; Kannoura, Toyo Town.

Japanese name: Turu Shiramo (Yendo, 1911).



Figs 1, 2. Herbarium specimen of *Gracilaria chorda* from Uranouchi Inlet, Tosa City, Tosa Bay. 1. A specimen without branchlets (May 6, 2000, Tetrasporangial, *Terada 1270*). 2. A specimen with numerous filiform branchlets (June 18, 2000, Tetrasporangial, *Terada 1261*).

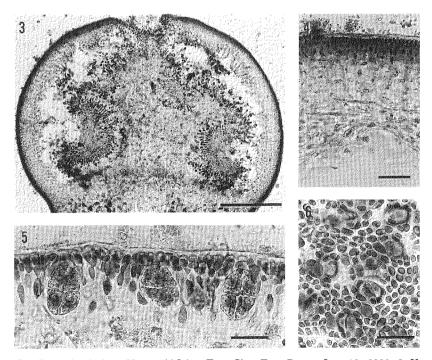
Specimens examined: *Terada* 1252-1255, 1260-1271, 1275, 1280-1284, 1315, Inoshiri (33°26'N, 133°26'E), Tosa City on March 23, May 6, June 18, July 2, 2000 (*leg.* R. Terada).

Habitat: Plants grow on pebbles, shells, and rocks on the sandy bottom of the lower intertidal zone. Mature plants appear in March-July at Uranouchi Inlet and Sukumo Inlet.

Plants are solitary or caespitose, generally up to 60 cm (sometimes up to 150 cm) long, reddish brown or somewhat purplish brown in color, not adhering to paper in drying; erect axes arising from a discoid holdfast (basal disc), terete throughout, branched alternately, sometimes secundly; branches and branchlets are sometimes slightly constricted at bases, tapering gradually toward the acute or obtuse apices (Figs 1, 2).

Thallus multiaxial; the cortex consists of globular cells with dense cytoplasm; the cortical cells are up to $10~\mu m$ high, up to $5~\mu m$ wide, and connected with only their parental cells by primary pit-connections; the medulla consists of 5-7 layers of unpigmented, spherical cells with vacuoles; the medullary cells increase abruptly in size toward the center, up to $1000~\mu m$ indiam., frequently connected with surrounding cells by secondary pit-connections.

Spermatangia form on whole surface except apical and basal parts, continuous superficial (*Chorda*-type: Yamamoto, 1978). Cystocarps form on the entire surface except for the basal and apical parts, prominently protruding from the surfaces, globose, up to 1.6 mm high and up to 3 mm wide, and are slightly constricted at bases, each with a slightly rostrate or not rostrate ostiole (Fig. 3); the gonimoblasts consist of small pesudoparenchymatous cells (up to 30



Figs 3-6. *Gracilaria chorda* from Uranouchi Inlet, Tosa City, Tosa Bay on June 18, 2000. 3. Vertical section of cystocarp showing gonimoblast consisting of small cells. 4. Vertical section of pericarp. 5. Vertical section of tetrasporophyte showing cruciately divided tetrasporangia. 6. Surface view of tetrasporohyte showing maturated tetrasporangia. Scales: 30 μm for Fig. 5, 50 μm for Figs 4, 6, 500 μm for Fig. 3.

 μ m in diam.); the traversing filaments are absent between gonimoblasts and pericarp; the pericarp is up to 120 μ m thick, consisting of 8-10 layers of cells (Fig. 4). Tetrasporangia form on the entire surface except the basal and apical portions, up to 50 μ m high, up to 30 μ m wide, and are cruciately divided (Figs 5, 6).

Remarks

Morphology of this species varies greatly among its different habitats and also with the seasons. It is known that some plants collected in April to May have long chord-like appearances without branchlets (Fig. 1), while the plants collected on June to July show numerous filiform branchlets (Fig. 2). The reproductive organs of this species agree well with the descriptions by Ohmi (1958) and Yamamoto (1978). Especially, absence of traversing filaments and gonimoblasts consisting of small cells was confirmed as diagnoses of this species.

Gracilaria gigas Harvey, 1859: 331.

Type specimen (lectotype): Herb. Harvey, Trinity College, Dublin (TCD, Masuda *et al.*, 1995).

Type locality: Shimoda, Shizuoka Prefecture, Japan.

Distribution: Kyushu, Shikoku, Pacific coasts of southern to middle Honshu. Tosa Bay and adjacent waters: Sukumo Inlet, Sukumo City; Uranouchi Inlet, Tosa City.

Japanese name: Oh Ogonori (Okamura, 1902).

Specimens examined: Terada 1257-1259, 1272-1274, 1316-1323, Inoshiri (33°26'N, 133°26'E), Tosa City on March 23, May 6, June 18, July 2, 2000 (leg. R. Terada).

Habitat: Plants grow on pebbles, shells and rocks on the sandy bottom in lower intertidal zones. Mature plants appear in March-July at Uranouchi Inlet and Sukumo Inlet.

Plants are solitary or caespitose, generally up to 30 cm long, pale green to purplish brown in color, cartilaginous, not adhering to paper in drying; erect axes arise from a discoid holdfast (basal disc), are terete throughout but sometimes slightly complanate in the middle portion, and branched alternately or secundly; branches and branchlets are sometimes slightly constricted at the bases, tapering gradually toward acute apices (Fig. 7).

Thallus multiaxial; the cortex consists of globular cells with dense cytoplasm; cortical cells are up to $10~\mu m$ high, up to $8~\mu m$ wide, and connected with only their parental cells by primary pit-connections; the medulla consists of 5-8 layers of unpigmented, spherical cells with vacuoles; the medullary cells increase abruptly in size toward the center, up to $600~\mu m$ in diam., frequently connected with surrounding cells by secondary pit-connections.

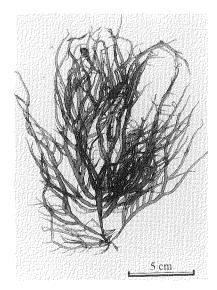
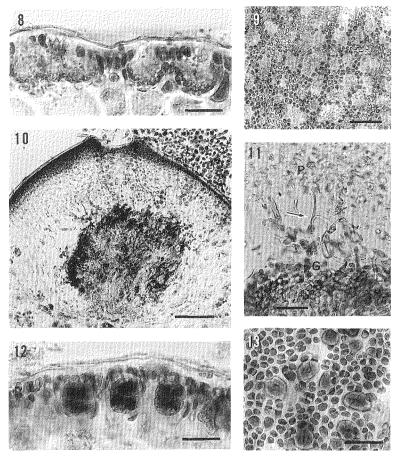


Fig. 7. Whole habit of *Gracilaria gigas* from Uranouchi Inlet, Tosa City, Tosa Bay (June 18, 2000, Tetrasporangial).



Figs 8-13. Gracilaria gigas from Uranouchi Inlet, Tosa City, Tosa Bay on June 18, 2000. 8. Transverse section of male gametophyte showing shallow spermatangial conceptacles (*Textorii*-type). 9. Surface view of male gametophyte showing spermatangial conceptacle. 10. Vertical section of cystocarp showing gonimoblast consisting of large cells. 11. Traversing filaments (arrow) connecting from gonimoblasts (G) to the pericarpic cells (P). 12. Transverse section of tetrasporophyte showing cruciately divided tetrasporangia. 13. Surface view of tetrasporophyte showing maturated tetrasporangia. Scales: 30 μm for Figs 8, 12, 13, 50 μm for Figs 9, 11, 200 μm for Fig. 10.

Spermatangia form on the whole surface except for the apical and basal parts, and in shallow conceptacles (Textorii-type, Yamamoto, 1978); spermatangial conceptacles are up to 30 μ m deep and 60 μ m wide, surrounded by elongated cortical cells often confluent with neighbor conceptacles (Figs 8, 9). Cystocarps form on the entire surface except for the basal and apical parts, prominently protruding from the surfaces, globose, up to 1.5 mm high and 2 mm wide, slightly constricted at bases, each with a slightly rostrate or not rostrate ostiole (Fig. 10); the gonimoblasts consist of large pseudoparenchymatous cells; traversing filaments are present between the gonimoblasts and pericarp (Fig. 11); the pericarp is up to 200 μ m thick, consisting of 10-14 layers of cells. Tetrasporangia form on the entire surface except for the basal and apical portions, up to 40 μ m high and 30 μ m wide, cruciately divided (Figs 12, 13).

Remarks

This species was originally described by Harvey (1859) on the basis of the specimens collected at Shimoda, Shizuoka Prefecture. As the frond becomes old, branches and branchlets are lost and sometimes the constrictions at branch bases become conspicuous. The old frond of this species is similar to in shape to the aged plant of *G. chorda* in terms of vegetative appearance (Yamamoto, 1978). However, it differs from *G. chorda* in regard to its spermatangial features and the presence of traversing filaments.

Gracilaria incurvata Okamura, 1931: 41.

Type specimen: Herb. Okamura, Faculty of Science, Hokkaido University (SAP).

Type locality: Misaki, Kanagawa Prefecture, Japan.

Distribution: Kyushu, Shikoku, Pacific coasts of southern to middle Honshu. Tosa Bay and adjacent waters: Kashiwajima, Ohtsuki Town; Oh-hama, Tosashimizu City; Nuno, Tosashimizu City; Hejima, Nomi Inlet, Susaki City, Ikenoura, Yokonami Peninsula, Susaki City; Uranouchi Inlet, Tosa City; Cape Muroto, Muroto City; Kannoura, Toyo Town.

Japanese name: Mizo Ogonori (Okamura, 1931).

Specimens examined: *Terada* 1256, 1324-1329, Inoshiri (33°26'N, 133°26'E), Tosa City on May 6, June 18, July 2, 2000 (*leg.* R. Terada); Kashiwajima (32°46'N, 132°37'E), Ohtsuki Town on March 22, May 4, 2000 (*leg.* R. Terada); Oh-hama (32°45'N, 132°58'E), Tosashimizu City on May 5, 2000 (*leg.* R. Terada);

Habitat: Plants grow on pebbles, shells, and rocks in lower intertidal zones in protected waters.

Plants are solitary or caespitose, generally up to 10 cm long, reddish brown in color, not adhering to paper in drying; foliose arises from a hold-fast (basal disc) with a short stalk, more or less curved and twisted, branching dichotomously or trichotomously up to 6 times, sometimes flabellately; the apices and margins of the entire blade are smooth (Fig. 14).

Thallus multiaxial; the cortex consists of globular cells with dense cytoplasm; the cortical cells are up to $12~\mu m$ high, up to $10~\mu m$ wide, and connected with only their parental cells by primary pit-connections (Fig. 15); the medulla consists of 3-6 layers of unpigmented, spherical cells with vacuoles; the medullary cells abruptly increase in size toward the center, up to $300~\mu m$ in diam., frequently connected with surrounding cells by secondary pit-connections.

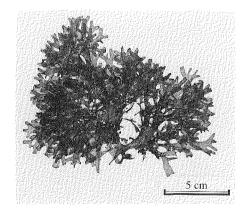
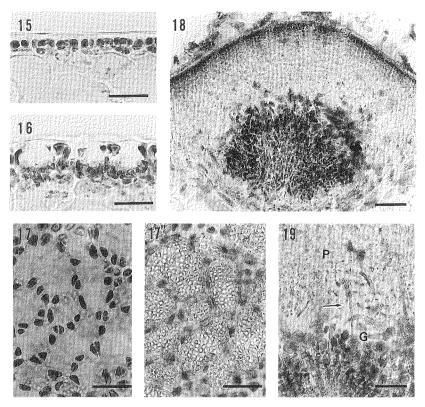


Fig. 14. Herbarium specimen of *Gracilaria* incurvata from Uranouchi Inlet,
Tosa City, Tosa Bay on June 18,
2000 (Tetrasporangial, *Terada 1256*).

Spermatangia form on whole surface except for the apical, basal and marginal parts, and in shallow conceptacles (Textorii-type, Yamamoto 1978); the spermatangial conceptacles are up to 25 μ m deep and 40 μ m wide, surrounded by elongated cortical cells often confluent with neighbor conceptacles (Figs 16, 17). Cystocarps form on the entire surface except for the basal, apical and marginal parts, prominently protruding from the surfaces, globose, up to 1.5 mm



Figs 15-19. *Gracilaria incurvata* from Uranouchi Inlet, Tosa City, Tosa Bay on June 18, 2000. 15. Transverse section of vegetative plants. 16 Transverse section of male gametophyte showing shallow spermatangial conceptacles (*Textorii*-type). 17. Surface view of male gametophyte showing spermatangial conceptacle. Surrounding cortical cells (Fig. 17) and spermatangia (Fig. 17"). 18. Vertical section of cystocarp showing gonimoblast consisting of large cells. 19. Traversing filaments (arrow) connecting from gonimoblasts (G) to the pericarpic cells (P). Scales: 30 μm for Figs 15-17, 50 μm for Fig. 19, 100 μm for Fig. 18.

high and 2 mm wide, sometimes slightly constricted at the bases, each with a slightly rostrate or not rostrate ostiole (Fig. 18); the gonimoblasts consist of large pseudoparenchymatous cells; traversing filaments are present between the gonimoblasts and pericarp (Fig. 19); the pericarp is up to 240 μ m thick, and consists of 10-12 layers of cells. Tetrasporangia form on the entire surface except for the basal, apical and marginal portions, up to 50 μ m high, up to 40 μ m wide, and are cruciately divided.

Remarks

This species was originally described by Okamura (1931) on the basis of the specimens collected at Kanagawa Prefecture. This species closely resembles *Gracilaria textorii* (Suringar) Hariot in it regard to its flattened appearance. Furthermore, the reproductive characteristics of *G. incurvata* also resemble those of *G. textorii* (Yamamoto, 1978). However, its curved or twisted shape is shown only in *G. incurvata*. Yamamoto (1978) reported an ecological difference between the two taxa in the field: *G. incurvata* generally grows in protected and calm waters, whereas *G. textorii* in places exposed to waves.

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