

Growth of excised pieces containing elongated denticles from the lower marginal parts of *Porphyra tanegashimensis* and *P. haitanensis* gametophytes

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Abstract *Porphyra tanegashimensis* and *Porphyra haitanensis* gametophytes have elongated denticles that take the form of small lobes on their lower marginal parts. We investigated the ability of these elongated denticles to form gametophyte lobes, using excised pieces that were freed from the influence of other parts. Gametophytes of the two species were cultured from conchospores for a period of 2 months. Small pieces containing elongated denticles were excised from the lower marginal parts of the gametophytes and cultured at 20 °C and 40 μ mol photons $m^{-2} s^{-1}$ under a photoperiod regime of 10L : 14D (light : dark). After about 1 month, some of the elongated denticles further developed, and the excised pieces came to resemble lacerated thalli. These results suggest that the elongated denticles possess the ability to grow into lobes under natural conditions.

Key words: *Porphyra*, denticle, thallus, culture, gametophyte

Some species of the genus *Porphyra* possess small denticles along the margins of their gametophytes (foliose thalli). The role of the denticles has not yet been revealed. *Porphyra tanegashimensis* Shinmura gametophytes are lacerated and have specific elongated denticles that take the form of small lobes on their lower marginal parts. Although the elongated denticles of *P. tanegashimensis* which characterize this species are expected to grow into lobes, they reached only a few mm in intact thalli in an earlier culture study (Shinmura, 1974). However, this result does not preclude the possibility that the growth of elongated denticles is limited in intact thalli, as is lateral bud inhibition in higher plants. *Porphyra haitanensis* Chang et Zheng gametophytes which are not lacerated also have elongated denticles (Chang, T. and B. Zheng, 1960).

In this study, we investigated the ability of the elongated denticles to form gametophyte lobes in

these two species, one with lacerated gametophytes and the other with unlacerated gametophytes, using excised pieces that were freed from the influence of other parts in reference to the experiments of Notoya (1995 ; 1999).

Gametophytes used in the present study were initiated from conchocelis of *P. tanegashimensis* and *P. haitanensis*. The former originated from a gametophyte collected at Ginowan, Okinawa Prefecture, Japan, on 26 February, 1998 and the latter was a cultivar provided by the Saga Prefectural Ariake Fisheries Research and Development Center. Culture methods were conducted as described previously (Shinmura, 1974 ; Migita and Ito, 1987 ; Aramaki *et al.*, 2001).

Gametophytes were cultured from conchospores for a period of 2 months at 20°C and 40 μ mol photons $m^{-2} s^{-1}$ under a photoperiod regime of 10L : 14D (light : dark). Although enriched

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seawater SWM III (Ogata, 1970) is recommended (Kito, 1985) for *Porphyra* gametophyte culture, the enrichments in SWM III are considered to be too concentrate, especially for *P.tanegashimensis* gametophyte culture (Fujiyoshi, unpublished). Therefore 1/10 modified SWM III medium and 1/2 SWM III medium (Table 1) were used for gametophyte culture of *P.tanegashimensis* and *P.haitanensis*, respectively. Gametophytes of *P.tanegashimensis* and *P.haitanensis* grew to reach 7.0–9.7cm and 17.2–36.8cm, respectively (Fig. 1A, 2A). As in the previous culture experiment (Shinmura, 1974), our cultured *P.tanegashimensis* gametophytes were not lacerated (Fig. 1A). Small pieces containing elongated denticles (Fig. 1B–C, 2B–C) were excised from the lower marginal parts of the gametophytes and cultured under the same conditions. For *P.haitanensis*, a dioecious species, a male gametophyte was used.

The elongated denticles on the margin of an excised piece of *P.tanegashimensis* were smaller than 1mm in length (Fig. 1D). Ten days later, one of the cut edges became umbilicate, with the appearance of rhizoids, and the elongated denticles resembled lobes (Fig. 1E). After 15 days, the lobes that had developed from elongated denticles reached 3mm in length (Fig. 1F). After 30 days, the marginal parts of the lobes had matured (Fig. 1H). However, lobes grew larger, and one of the lobes reached nearly 3cm in length after 38 days (Fig. 1I).

The elongated denticles on the margin of an excised piece of *P.haitanensis* were smaller than 0.5mm in length (Fig. 2D). Eleven days later,

one of the elongated denticles reached 3mm (Fig. 2E). After 24 days, the elongated denticles grew significantly and transformed into lobes (Fig. 2F). After 32 days, the marginal parts of the lobes had matured and one of them reached nearly 8cm in length (Fig. 2G).

Although the elongated denticles of *P.tanegashimensis* were hypothesized to grow into lobes, they reached only a few mm in length in intact thalli (Shinmura, 1974). Migita and Ito (1987) reported that the gametophytes of *P.tanegashimensis* were torn into many lobes along straight slits in culture. In this study, the gametophytes from which the pieces were excised were not lacerated (Fig. 1A), and elongated denticles on the margin of the piece grew and transformed into lobes (Fig. 1D–I). These results suggest that the elongated denticles possess the ability to grow into lobes under natural conditions as detailed in Shinmura's hypothesis. When most of the thallus is cut off, for example, by natural wave action, it seems that elongated denticles on the margin of the remaining lower part freed from the influence of the upper part grow significantly. However, torn-like wild thalli as reported by Migita and Ito (1987) have been observed at Ginowan. Further study is needed to reveal the mechanism by which lobes are formed in nature.

P.haitanensis gametophytes, which are not considered to be lacerated, have characteristic elongated denticles (Chang, T. and B. Zheng, 1960). In this study the elongated denticles on the margin of the excised piece grew and transformed

Table 1. Contents of 1/2 and 1/10 modified SWM III media.

	1/2 modified SWM III	1/10 modified SWM III
NaNO ₃	1.0 mM	0.2 mM
Na ₂ HPO ₄	50 μM	10 μM
Na ₂ EDTA	15 μM	3 μM
FeCl ₃	1.0 μM	0.2 μM
P I -metal*	1 ml	0.2 ml
Sea water	1000 ml	1000 ml

*Composition of P I -metal (Ogata, 1970) in mg/liter: H₃BO₃, 6184; MnCl₂, 692.5; ZnCl₂, 54.5; CoCl₂·6H₂O, 2.3795; CuCl₂·2H₂O, 0.017.

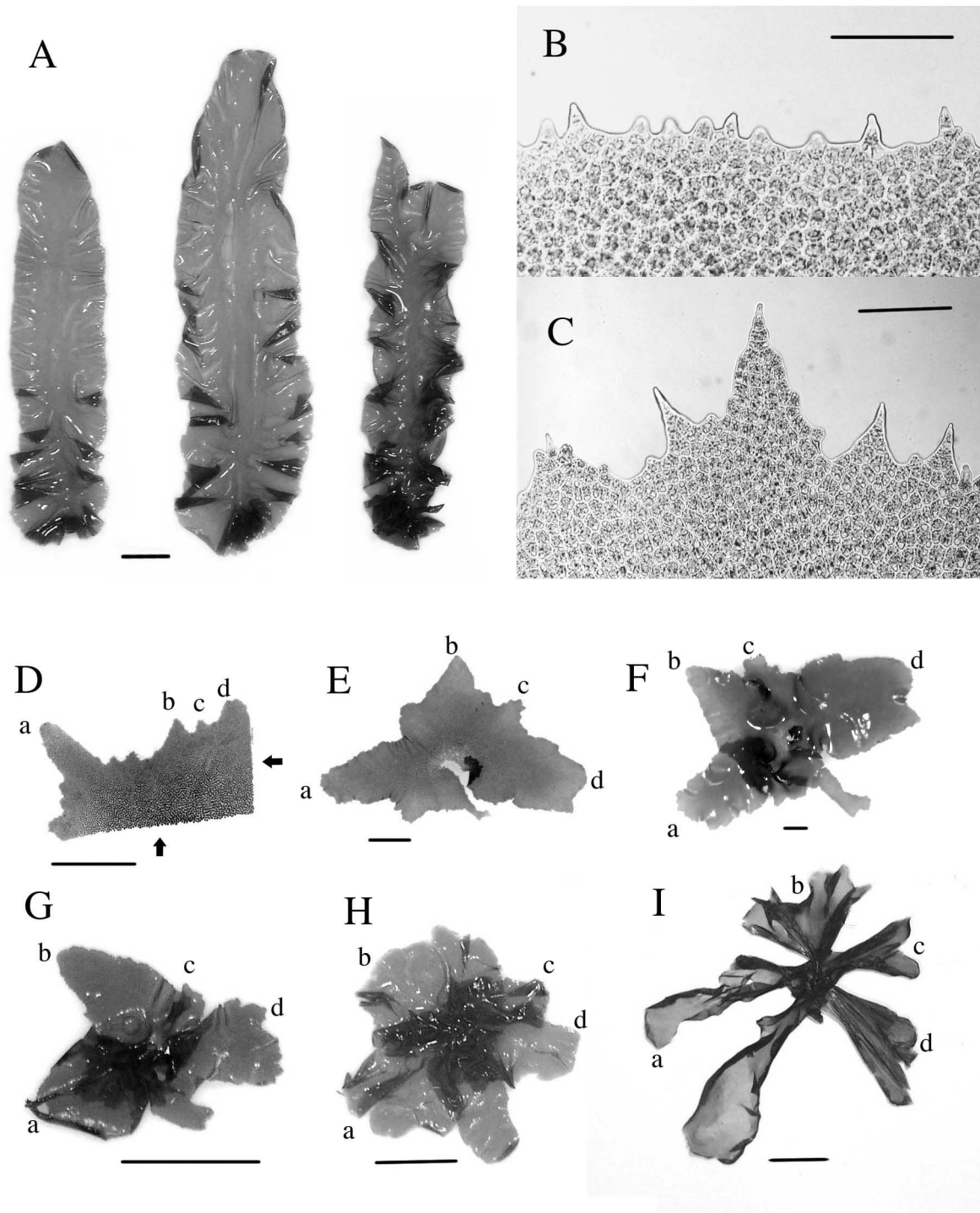


Fig. 1. Gametophytes of *Porphyra tanegashimensis*, and growth of a piece containing elongated denticles that was excised from the lower marginal part. (A) Intact thalli grown from conchospores after 2 months in culture. (B) Microscopic denticles on the middle marginal part. (C) Elongated denticles on the lower marginal part. (D) Excised piece containing elongated denticles (a-d). Arrows indicate cut edges. (E-I) Successive stages of the excised piece (D) in culture, showing development of the denticles (a-d). (E) 10 days later. (F) 15 days later. (G) 20 days later. (H) 30 days later. (I) 38 days later. Scale bars: A, 1 cm; B-C, 100 μ m; D-F, 1 mm; G-I, 1 cm.

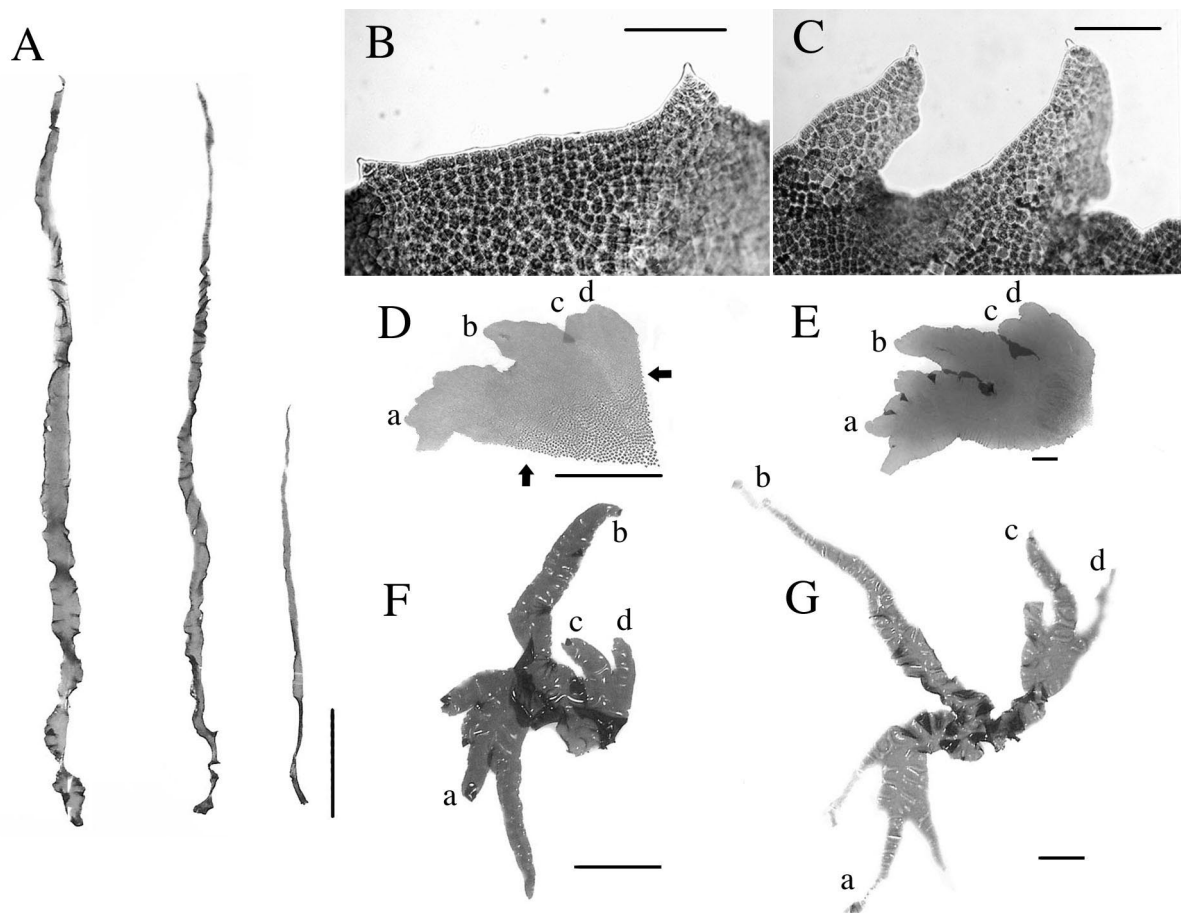


Fig. 2. Gametophytes of *Porphyra haitanensis*, and growth of a piece containing elongated denticles that was excised from the lower marginal part. (A) Intact thalli grown from conchospores after 2 months in culture. (B) Microscopic denticles on the middle marginal part. (C) Elongated denticles on the lower marginal part. (D) Excised piece containing elongated denticles (a-d). Arrows indicate cut edges. (E-G) Successive stages of the excised piece (D) in culture, showing development of the denticles (a-d). (E) 11 days later. (F) 24 days later. (G) 32 days later. Scale bars : A, 5 cm ; B-C, 100 μ m ; D-E, 1 mm ; F-G, 1 cm.

into lobes (Fig. 2D-G) in the same way as did *P. tanegashimensis*. This result suggests there may exist lacerated thalli of *P. haitanensis* in nature; however, we do not have any clear information in this regard.

Notoya (1995, 1999) cultured small excised pieces from *Porphyra dentata* gametophytes, and reported regeneration of bladelets on the cut edge of the upper side, but did not report the growth of denticles. Denticles growth does not seem to be typical for the *Porphyra* in which gametophytes possess denticles.

Elongated denticles on the margin of the excised pieces from *P. tanegashimensis*. and *P. haitanensis* gametophytes developed and transformed into

lobes in culture, and the pieces came to resemble lacerated thalli. These results suggest that the elongated denticles possess the ability to grow into lobes under natural conditions.

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