Short communication

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Abnormal asexual reproduction of thalli of *Ecklonia stolonifera* (Laminariales, Phaeophyceae) off the coast of Nakanoshima in the Oki Islands, Japan

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Abstract: Multiple abnormal individuals of *Ecklonia stolonifera* with thalli that developed numerous shoot-like structures on the lateral blades were found in two littoral regions of Nakanoshima in the Oki Islands, Shimane Prefecture, Japan. Except for the presence of these diagnostic structures, no other morphological differences were observed between the abnormal and normal individuals. The development of the shoot-like structures isolated from the lateral blade was the same as that of typical vegetative reproductive sporophytes that formed on stolons. In the abnormal individuals, when cultivation was performed using zoospores, the shoot-like structures were observed on the progeny after 18 months of culture and 20.0% of individuals exhibited these structures.

Keywords: abnormal asexual reproduction; *Ecklonia stolonifera*; Oki Islands; shoot-like structure.

Ecklonia stolonifera is a species of kelp belonging to the genus *Ecklonia* that forms seaweed forests in coastal areas of the Japan Sea in Japan and Korea and supports regional marine ecosystems (Okamura 1936, Ito and Ezaki 1991, Kawashima 1993a, Notoya 2003, Trawaki and Arai 2004). New thalli of this species are generated through sexual reproduction, as in other *Ecklonia* spp.; however, thalli are also generated through a vegetative reproductive process not observed in other species. Vegetative reproduction in this species typically involves the generation of sporophytes from shoots that form at the tips of stolons (Notoya and Aruga 1990). However, Notoya and Aruga (1992) reported observing abnormal thalli that exhibited differentiation of the tips of serrations along blade margins into stem and leaf structures in kelp off the coast of Aomori Prefecture. Unfortunately, the authors of the study collected only one such sample and did not provide a detailed discussion of the structures.

In this study, we were able to find multiple individuals off the coast of Nakanoshima in the Oki Islands, Shimane Prefecture, Japan, with thalli that developed numerous shoot-like structures on the lateral blades. We investigated the development and detailed morphology of individuals exhibiting such abnormal structures. Furthermore, we cultivated these shoot-like structures and progeny generated through sexual reproduction via mariculture, investigated their growth and the ability of each sporophyte type to attach to surfaces.

Kelp individuals exhibiting the abnormal morphology were found along the Hobomi and Toyoda coasts of Nakanoshima Island (Figure 1). We investigated the occurrence (bottom material, coverage, interplant spacing) of target individuals by swimming using scuba gear along an underwater straight-line transect with a length of 100 m and a width of approximately 1–2 m at a depth of 10 m along the Hobomi coast (36° 5.15′ N, 133° 8.20′ E – 36° 5.11′ N, 133° 8.24′ E) on December 5, 2006, and a straight-line transect with a length of 50 m and a width of approximately 1 m at a depth of 7–10 m along the Toyoda coast (36° 6.56′ N, 133° 7.99′ E – 36° 6.55′ N, 133° 6.02′ E) on June 17, 2007. We collected samples of both abnormal and normal individuals at each of the sites and measured 11 parameters (Figure 2). In addition, we investigated and measured various characteristics [location, number, organs (blade, stem, root)] of the shoot-like structures on the lateral blades. Differences in morphological characters between abnormal and normal individuals were analyzed by Tukey's test.

In addition, on February 1, 2010, we isolated shoot-like structures growing on old lateral blades of abnormal individuals found along the Toyoda coast. These structures were inserted between the strands of a polypropylene rope (diameter 8 mm, length 3000 mm) at 12 positions

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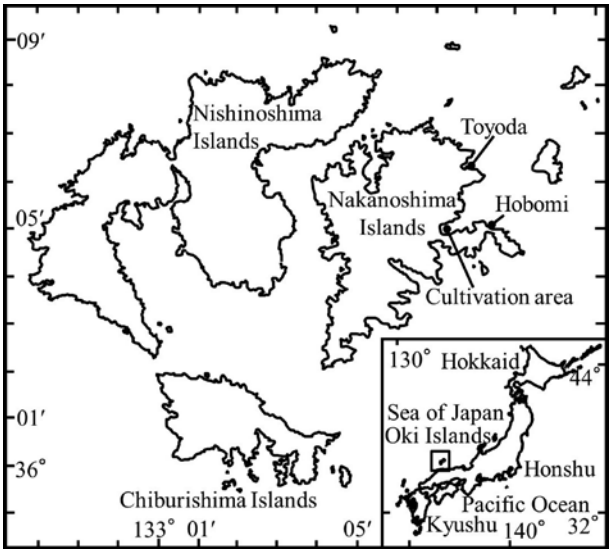

Figure 1: Locations of field investigations and mariculture sites in the Oki Islands, Japan.
The inset map shows the location on a larger scale map.

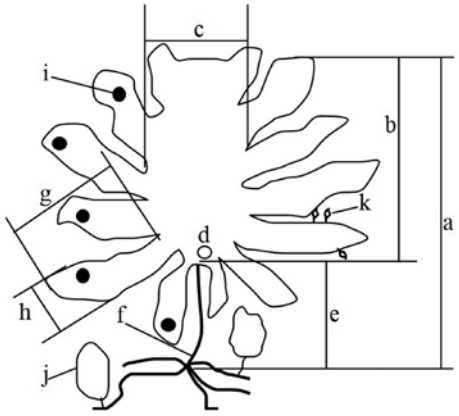

Figure 2: Morphological parameters of thallus of *Ecklonia stolonifera* investigated in this study.
(a) Thallus length, (b) lamina length, (c) lamina width, (d) lamina thickness at meristem, (e) stipe length, (f) stipe diameter, (g) lateral length, (h) lateral width, (i) number of laterals, (j) number of shoots (asexual reproduction from stolon), (k) number of shoots (asexual reproduction from lateral blade).

spaced 20 cm apart (sample nos. 1–12) and the ropes were suspended at a depth of 2–5 m in Hobomi Bay (Figure 1). The cultivated sporophytes were brought to the surface on February 1, April 4, and June 6 of 2010 and six parameters (lamina length, lamina width, lamina thickness, stipe length, stipe diameter, and shoot number) were measured (Figure 2).

On December 6, 2006, zoospores obtained from mature parts of abnormal individuals were attached to Cremona threads and cultured in a 100-I water tank until January 15, 2007. The zoospores were cultured under the following conditions (Kawashima 1993b, Ohno and Matsuoka 1993): water temperature, 13–17°C; irradiance, approximately 60 μmol m^{-2} s^{-1}; photoperiod regime, 12 h light, 12 h dark; and Provasoli's enriched sea water (PESI) medium (using seawater passed through a 1-μm filter; Nishizawa and Chihara 1979) changed every 7 days. On day 25 of culture, irradiance was increased to 100 μmol m^{-2} s^{-1}, and culture medium was replaced with running seawater. On day 42 of culture, the Cremona threads were wound around 200 polypropylene ropes (diameter 16 mm, length 500 mm) which were then suspended at a depth of 2–7 m in Hobomi Bay (Figure 1). The cultivated sporophytes were brought to the surface a total of eight times; five times in 2007 (January 30, March 30, June 20, September 14, December 29) and three times in 2008 (January 28, March 30, June 23). On these occasions, the serrated blade margins of 60–100 individuals were examined for the occurrence of shoot-like structures. In addition, we measured 11 parameters of 10 large individuals (Figure 2). We analyzed the morphological differences between individuals with differing blade margin characteristics using Tukey's test.

Luxuriant growth of *Ecklonia stolonifera* was observed off the Hobomi coast at a depth of 10 m in an area with rock as the dominant substratum (100%). Abnormal individuals occurred in seven locations along the survey transect and the distance between abnormal individuals ranged from 5.8 to 23.7 m. Normal individuals were widely distributed with distances between individuals ranging from 0.1 to 0.3 m. The substratum off the Toyoda coast at a depth of 7–10 m consisted entirely of concrete blocks. Among the individuals observed, abnormal individuals occurred in five locations along the survey transect, with the distance between abnormal individuals ranging from 1.6 to 3.8 m.

Regarding the abnormal individuals collected (Table 1), those collected off the Hobomi coast developed pinnate lateral blades and generated shoots from stolons (Figure 3A). A clear boundary could be discerned between new and old blades of sporophytes. Shoot-like structures were formed along the margins of the lowermost blades. The number of shoot-like structures ranged from 1 to 25 per lateral blade and from 1 to 80 per sporophyte. Serrations along lateral blade margins were spiny and formed shoot- and stolon-like structures at their tips (Figure 3B, C). Abnormal individuals collected off the Toyoda coast also developed pinnate lateral blades (Figure 3D–G) and generated shoots from stolons. In one individual, clearly formed stem-, blade-, and stolon-like structures were developed at the tips of serrations (Figure 3H). As shown in Table 1, the sizes

Table 1: Measurement values of morphological characteristics of wild *Ecklonia stolonifera* at two locations.

Location, depth (data)	Hobomi, 10 m (December 5, 2006)		Toyoda, 7–10 m (June 17, 2007)
Sporophyte type (age)	Normal (3 years)	Abnormal (3 years)	Abnormal (3 years)
(a) Thallus length (cm)	40.60 ± 4.93	40.29 ± 7.17	42.57 ± 4.47
(b) Lamina length (cm)	20.80 ± 3.61	19.86 ± 4.91	21.86 ± 5.46
(c) Lamina width (cm)	9.80 ± 0.24	9.57 ± 2.65	13.29 ± 2.31
(d) Lamina thickness (mm)	1.90 ± 0.20	02.90 ± 0.30[a]	2.31 ± 0.80[b]
(e) Stipe length (cm)	19.80 ± 2.94	20.43 ± 5.06	20.71 ± 3.33
(f) Stipe diameter (cm)	0.73 ± 0.07	00.78 ± 0.06	0.68 ± 0.05
(g) Lateral length (cm)	15.60 ± 1.24	17.71 ± 3.25	28.71 ± 6.06[b]
(h) Lateral width (cm)	06.80 ± 1.17	06.29 ± 1.19	7.79 ± 0.75
(i) Lateral numbers (individuals)	6.60 ± 1.02	4.57 ± 1.92	4.71 ± 1.03
(j) Number of shoots (asexual reproduction from stolon)	7.20 ± 1.72	7.71 ± 5.00	9.43 ± 4.24
Range (individuals)	5–10	5–13	5–18
(k) Number of shoots (asexual reproduction from lateral blade)	None	25.00 ± 28.77	05.29 ± 2.91
Range (individuals)	–	1–80	2–10
Sample numbers	7	7	7

For definition of characters a–k, see Figure 2. Means marked by asterisks are significantly different from the values for normal blades at Hobomi (Tukey's-test; [a]$p < 0.05$ or [b]$p < 0.01$).

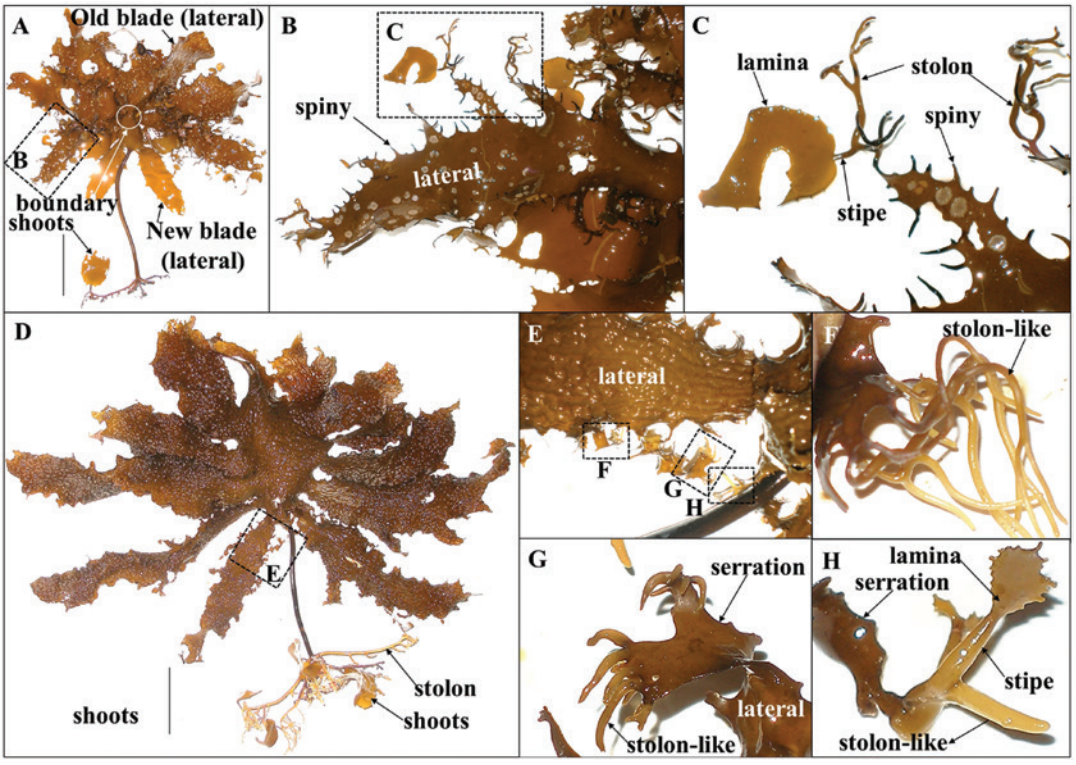

Figure 3: Morphology of an abnormal individual of *Ecklonia stolonifera*.
(A) Thallus collected at a depth of 10 m at Hobomi on December 15, 2006. (B) Magnification of one of laterals from (A). (C) Magnification of rectangle in (B). (D) Thallus collected at a depth of 7 m at Toyoda on June 17, 2007. (E) Magnification of rectangle in (D). (F–H) Magnification of rectangles in (E).

of different parts of normal and abnormal individuals were approximately the same except for lamina thickness and lateral length.

Shoot-like plants that formed on the lateral blades of abnormal individuals (Figure 4A, B) were separated, and were fixed onto ropes (Figure 4C) for further

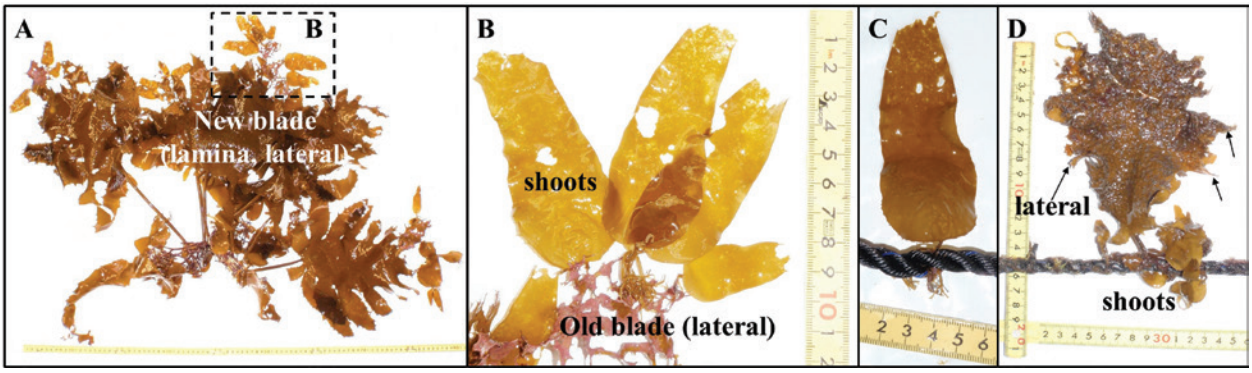

Figure 4: Morphology of cultivated thalli of abnormal individuals of *Ecklonia stolonifera* originating from shoot-like structures.
(A) Thalli collected at a depth of 10 m at Toyoda on February 1, 2010. (B) Magnification of part of rectangle in (A). (C) One of the shoots from (B) fixed onto a rope for further cultivation. (D) Sporophyte after 4 months of mariculture.

mariculture. After 126 days of culture, they were observed to grow and differentiate into blades, stems, and root-like structures (Figure 4D).

The results of zoospore culture of abnormal plants are shown in Figure 5A, B and C. Compared to reported normal (parent-strain of Hobomi) individuals (Hayashi et al. 2017), measurements of abnormal individuals and their offspring showed similar growth to normal individuals in terms of the length and width of the lamina (normal plants: 10–30 cm long, 6–12 cm wide), length and width of lateral blade (normal plants: 5–25 cm long, 2–10 cm wide), and number of lateral blades (normal plants: 1–7 individuals), with these characters increasing and decreasing repeatedly over a 6-month cycle. Such seasonal change (Park et al. 1994, Kim and Yoon 2003) was a characteristic of this species. However, as mariculture progressed, shoot-like structures were observed to form on the sporophytes. Specifically, after 3 months of mariculture, minute serrations were observed along short and small lateral blade margins. These serrations became spiny after 6 months and biserrate after 12 months. After 18 months, shoot-like structures were observed forming at the tips of serrations (Figure 6A–C). Based on a sample of 50 or more individuals, the percentage of individuals exhibiting these shoot-like structures was 20.0% (Table 2). Comparing the morphological characters of abnormal and normal individuals after 18 months showed a significant difference in 3 characters ($p < 0.05$) (Table 3). The abnormal individuals investigated in this study were observed to spread laterally at the two locations off the coast of Nakanoshima in the Oki Islands, Shimane Prefecture, suggesting that they may be also be distributed in other areas. In addition to exhibiting the typical vegetative reproduction in which sporophytes are generated from shoots developing from stolons, the abnormal individuals collected at these two sites developed stolon- and shoot-like structures on lateral blades that developed into sporophytes of the same type. In this study, approximately 20.0% of the progeny exhibited similar characteristics, suggesting that the trait has a genetic basis.

We hypothesize that the incidence of abnormal individuals is a result of crossing with normal individuals. In the area in and around the study site, hybridization between *Ecklonia kurome* and *Ecklonia stolonifera* is

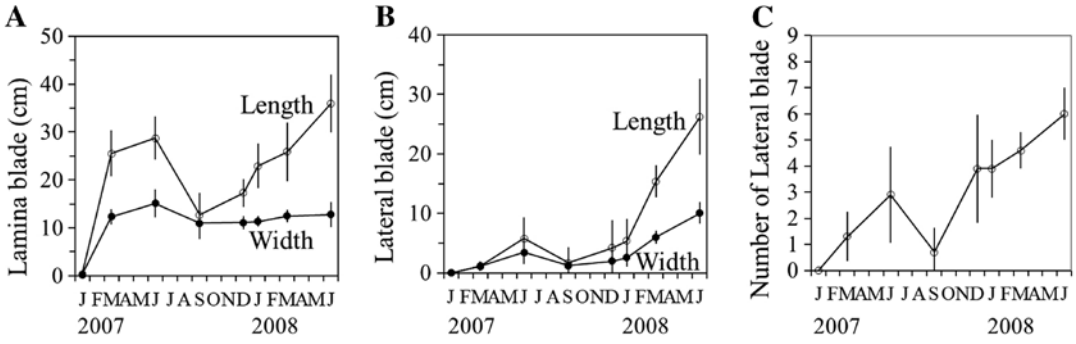

Figure 5: Monthly changes in different parts of cultivated thalli of abnormal individuals of *Ecklonia stolonifera* originating from shoot-like structures.
(A) Length and width of lamina. (B) Length and width of lateral. (C) Number of lateral blades.

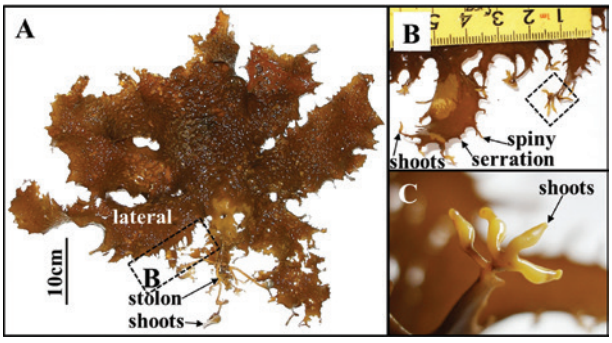

Figure 6: Morphology of a cultivated thallus that was produced by mariculture of zoospores from abnormal individuals of *Ecklonia stolonifera*.
(A) 18-month-old sporophyte (June 23, 2008). (B) Lateral blade, serration and spiny serration. (C) Shoot-like structures.

Table 2: Percentage of abnormal thalli of *Ecklonia stolonifera* cultivated from Toyoda.

Culture months	Abnormal thalli (%)	Number of sporophytes	
		Normal thalli	Abnormal thalli
1	0	100	0
3	0	100	0
6	0	100	0
9	0	100	0
12	0	100	0
13	1.6	60	1
15	2.9	70	2
18	20.0	56	14

known to occur frequently (Hayashi et al. 2017). Leaving the classification of the abnormal individuals aside, it is possible that genetic exchange is occurring between the abnormal and normal individuals. Accordingly, the abnormal trait may be expressed at a lower frequency in the progeny of abnormal individuals generated through sexual reproduction due to backcrossing with normal individuals. Such backcrossing may also contribute to the morphological differences in the shoot-like structures observed among the abnormal individuals. Upon isolation and culture, the sporophytes from abnormal individuals developed stolons, attached to the substratum, and developed sporophytes that did not differ from those generated by typical vegetative reproduction. Although we did not observe subsequent propagation, we believe the sporophytes that develop on lateral blades survive at the same rate as sporophytes that develop on stolons.

In the study area, abnormal individuals were found in locations beyond the area in which asexual reproduction occurs. It is unlikely that the stolons of the shoot-like plants formed on the lateral blades of these abnormal individuals attach to the natural rock substratum. This is because the blades are unstable and break off seasonally. The holdfasts spread horizontally from a single sporophyte to a maximum distance of 80 cm through asexual reproduction (Notoya and Aruga 1990). The fact that the abnormal individuals are distributed at intervals of several meters to several tens of meters on natural rock substratum suggests that they are spread via sexual reproduction. The results of the mariculture trial indicate that the traits are inheritable.

However, genetic analysis is required to clarify relationship between abnormal and normal individuals.

Table 3: Morphological characters (means ± standard deviation) of cultivated *Ecklonia stolonifera* at two types (normal and abnormal).

Sporophyte type	Normal	Abnormal
(a) Thallus length (cm)	43.35 ± 14.67	43.74 ± 5.43
(b) Lamina length (cm)	36.60 ± 14.24	36.00 ± 6.02
(c) Lamina width (cm)	13.65 ± 2.58	12.78 ± 2.61[a]
(d) Lamina thickness (mm)	00.30 ± 0.05	00.29 ± 1.00[a]
(e) Stipe length (cm)	06.75 ± 1.37	7.74 ± 1.37
(f) Stipe diameter (cm)	00.72 ± 0.06	0.70 ± 0.11
(g) Lateral length (cm)	025.00 ± 9.82	26.24 ± 6.40
(h) Lateral width (cm)	8.45 ± 0.53	10.08 ± 1.81
(i) Lateral numbers (individuals)	05.50 ± 1.73	06.00 ± 1.00[a]
(j) Number of shoots (Asexual reproduction from stolon), Range (individuals)	6.50 ± 4.36 3–12	10.40 ± 6.19 2–17
(k) Number of shoots (Asexual reproduction from lateral blade), Range (individuals)	Non.	22.0 ± 28.31 3–68
Sample numbers	5	5

Measurements were taken 18 months after the beginning of mariculture. Means marked by the some superscript letter are significantly different from the values of Normal plants by Tukey's-test ([a]$p < 0.05$).

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