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A Study on Cultivation of *Petalonia fascia* (Scytosiphonales, Phaeophyta) by Vegetative Regeneration

Kang-Hwa Lee*, Jae-Hyun Cho[1] and Jong-Ahm Shin[2]

Jindo Fisheries Management Division, Mokpo Regional Maritime Affairs and Fisheries office, Jindo 530-892,
[1]Fisheries Policy Bureau, Ministry of Maritime Affairs and Fisheries, Seoul 120-725 and
[2]Division of Aqualife Science, Yosu National University, Yeosu 550-749, Korea

To establish a cultivation method of *Petalonia fascia*, seeds and seedlings cultures and growth tests were performed at the Daeri aquafarm in Haeui, Shinan, Jeollanamdo, Korea. Gametes were easily released from the mature plurilocular sporangia. They developed to crustose discoidal stolons and grew to filamentous and discoidal stolons. The indoor seeding was performed by using the 100-150 μm long fragments of stolons on *Porphyra* nets and the erect thalli developed from the cuttings when the seawater temperatures were 10-15°C. In the experimental cultivation in the sea, 1-2 mm long plantlets were found after 15 days of cultivation; after two months thalli grew to their maximal size of 215-355 mm long blades; after three months the length of thalli began to decrease due to distal disintegration and the plant color changed to yellow and epiphytic diatoms were attached on the thalli, which deteriorated the quality of products. The cultivation of *P. fascia* by the regeneration of filamentous-discoidal complexes was carried out successfully for the first time in Korea

Key Words: cultivation, *Petalonia fascia*, Scytosiphonales, vegetative regeneration

INTRODUCTION

There are not many species grown in the seaweed cultivation. The number of seaweed cultivar was also limited to a few species such as *Porphyra, Undaria, Laminaria* and *Hizikia*, etc. in Korea. Consequently the seaweed market has been largely dependent on the production of these species only and the market price has been easily dropped due to the overproduction. Seaweed farmers have frequently experienced such economic loss due to overproduction. Therefore, the diversification of local varieties with the introduction of new economic species is required to reduce the risk of unstable seaweed market and to maximize the utilization of seaweed resources with an additional income.

Petalonia is a good candidate for the seaweed cultivation in Korea. Several species have been described in the genus *Petalonia* (Scytosiphonales, Phaeophyta); *P. binghamiae, P. fascia* and *P. zosterifolia* in Japan (Yoshida 1998); and *P. binghamiae, P. fascia* and *P. zosterifolia* in Korea (Cho *et al.* 2002; Lee and Kang 2002). Plants of *Petalonia* are distributed in Korea, China, Taiwan, Japan, the North Pacific coast, the west coast of America and the Atlantic, along the temperate to cold temperate coasts worldwide. In Korea, they are mainly distributed in the coast of South Korea. They grow on rocks in the intertidal zone, on other algae and on seagrasses of *Zostera marina* and *Phyllospadix iwatensis* (Kang 1968; Kogame 1993; Yoshida 1998; Lee and Kang 2002; Cho *et al.* 2002)

Cultivation of *Petalonia* has not been cultivated yet, but the experimental cultivation was conducted in Japan (Migita 1992). Limited amount of wild plants have been marketed as raw and dried goods in Chiba, Kanagawa, Tottori and Shimane Prefecture (Ueda *et al.* 1978), but they have been decreased. In Korea, this plant has not been cultivated. Usually plants are found growing with *Hizikia fusiformis* on the cultivation rope of *H. fusiformis* in the southern part of the Yellow Sea in Korea and their dried goods have been exported to Japan in high price.

Now it is strongly recommended to cultivate *Petalonia* as a new economic species in Korea. It could be easily cultured on ropes as found in the *H. fusiformis* cultivation facilities and its market value is promising. This study was conducted to develop a new *Petalonia* cultivation

*Corresponding author (Kanghwa05@hanmail.net)

method. Several methods including the regeneration of filamentous-discoidal stolon complexes, seeds and seedlings cultures and growing tests were initiated for the commercial cultivation.

MATERIALS AND METHODS

The plants grew together with *Hizikia fusiformis* on cultivation ropes of *H. fusiformis* at the Daeri aquafarm, Haeui, Shinan, Jeollanamdo, Korea were collected at March 25, 1998 and used as materials in this study (Fig.1 and Pl. A). The plurilocular sporangial sori were formed on the surface of the plants. Plants were cleaned, dehydrated at about 50% of moisture content and transferred to the laboratory. They were washed again and dried in the shade for 2-3 hours as a pre-treatment procedure.

A 5 g of plants was placed and stirred in 500 m*l* of the sterilized seawater. They began to release gametes after about 10 minutes. After stirring for about 20 min, 50-80 gametes were recognized under a view of 10 x 10-field of a light microscope and then plants were removed. The gamete solution was filtered with a gauze. A 100 m*l* of the filtered gamete solution was diluted with 500 m*l* of sterilized seawater in a 1,000 m*l* Erlenmeyer flask.

The culture medium was made as follows: the offshore seawater (specific gravity; around 1.020) was filtered by a carbon filter (retention size, 1 μm) and a U·F filter (retention size, 0.01 μm); and filtered seawater was sterilized at 60-70°C for 20-30 minutes and passed through a UV treatment chamber; then finally 0.1 g sodium nitrate and 0.02 g sodium phosphate were added to 1 *l* of sterilized seawater.

Gametes were cultured under water temperature at 15-16°C, a specific gravity of 1.0225, illumination at 1,000-2,000 lux and 14L:10D photoperiod. After about one month gametes germinated and developed to blackish purple or blackish brown filaments on the bottom. These filaments were carefully collected with a sterile rubber stick and inoculated into a 500 m*l* Erlenmeyer flask with 300 m*l* of culture medium. They were cultured in free-living state.

The cultures of seedlings were performed in a light-temperature controlled culture room and in a culture room with ambient temperature. Various culture vessels were used depending on the scale of cultures; 300, 500 and 1,000 m*l* Erlenmeyer flasks, 5 *l* round flasks and 500 *l* cylindrical plastic vessels.

The cultures of discoidal and filamentous stolons were

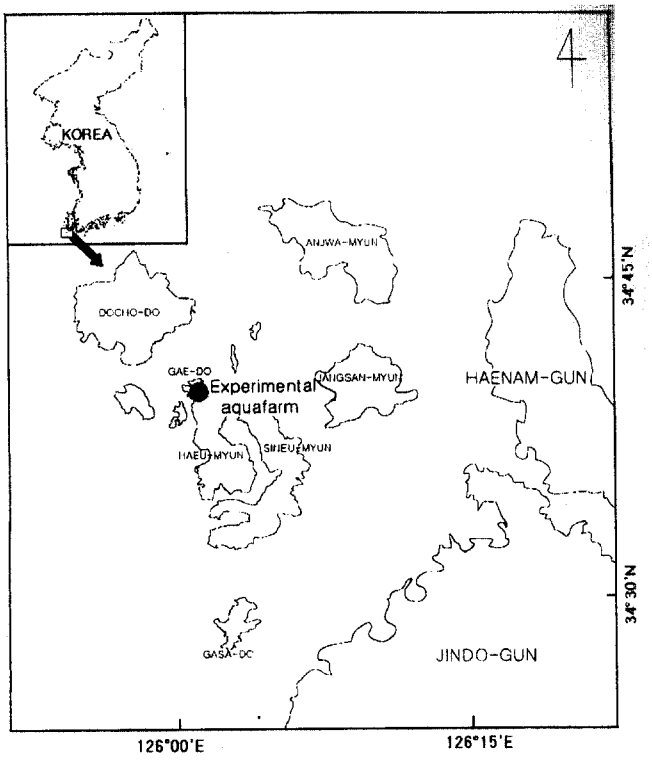

Fig. 1. A map showing the experimental aquafarm, Daeri, Haeui-myeon, Shinan-gun, Jeollanamdo, Korea.

maintained without aeration from late March to late June 1998 and aerated from early July to late October. Water temperatures were maintained at 14.8-23.6°C until late May by the window operation and at 20.8-27.5°C from early June to late October by the forced ventilation in the indoor culture (Fig. 2). The illumination was maintained at 2,000 lux level in early culture period and down to 500 lux level using a screen shade when the water temperatures went up.

In the light-temperature controlled culture room, water temperature in the early culture period was maintained at 18.5-19.5°C and at 21.2-23.4°C after mid May (Fig. 2). The illumination was maintained at 500-600 lux. The culture media were exchanged in every month.

The water temperature and illumination level were measured weekly and specimens were observed once a week under a light microscope.

Ten *Porphyra* nets (cremona No. 5, 1.8 × 8 m) were used as seeding substrate. The nets was immersed in the freshwater for 12 hours and dried in the shade before seeding. Two 500 *l* cylindrical plastic vessels were used for seeding operation. The seeding nets were submerged in the vessel by adding 200-250 *l* of filtered seawater. Air was introduced in the vessel from blowers.

The indoor seeding was performed in November 5,

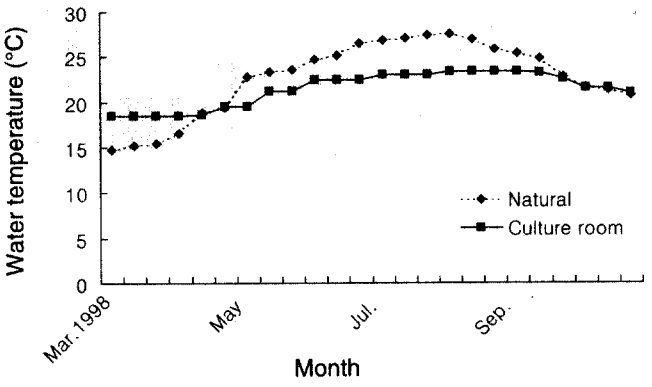

Fig. 2. Changes of water temperature in the light-temperature controlled culture room, and in the indoor culture of ambient water temperature during the culture period of filamentous-discoidal complexes.

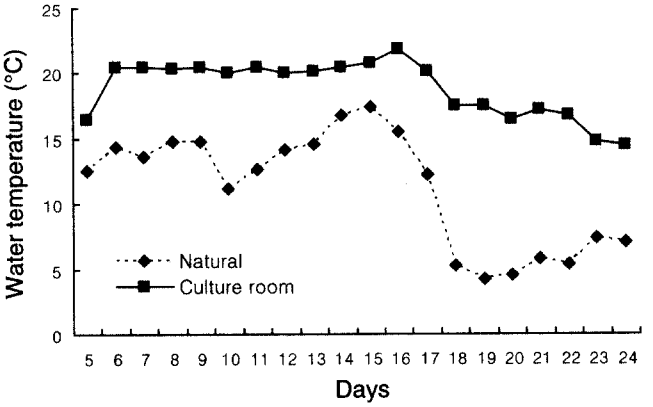

Fig. 3. Changes of water temperature in the indoor and the outdoor places during the growth process of filamentous-discoidal complexes after seeding before the experimental field cultivation started in the sea.

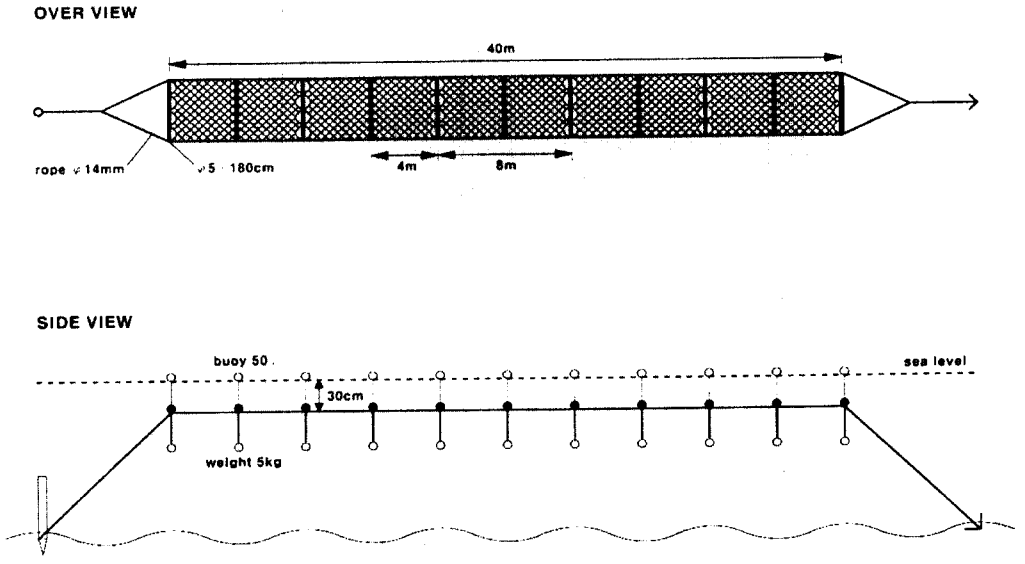

Fig. 4. A diagram of the cultivation facility for the growing test of *Petalonia fascia*.

1998. Previously cultured filamentous and discoidal stolons were cut to make fragments of 100-150 μm in length using a blender for 10-20 sec; the seeding was carried out with placing 4 nets in a 500 *l* vessel at the seeding density of 1-2 fragment per a view of 10 × 10 field in light microscope and applying 6 nets in another vessel at the density of 3-4 fragments. The seawater was stirred by aeration using blowers to help the attachment of fragments onto the nets for 4 days after seeding and seed nets were overturned twice or three times a day.

The attached fragments on nets were cultured in the indoor and the outdoor places for 20-25 days to observe their growth features. The indoor water temperature varied between 14.5°C and 20.5°C and the outdoor temperature fluctuated between 4.2°C and 17.4°C depending on the weather conditions (Fig. 3).

The experimental cultivation facilities were set up at Daeri, Haeui, Shianan, Jeollanamdo in November 24 for the first experiment and December 1, 1998 for the second one. A floating system was applied for cultivation; the main line (6 mm in diam.) was maintained at 30-40 cm depth with buoys and sinkers (Fig. 4); miscellaneous things attached on cultivation ropes were removed for release, attachment and germination of zoospores from the seed.

The ambient surface water temperatures were 14.5°C in Nov. 24 and 12.2°C in Dec. 1, 1998, when the first and second experiment started. Water temperature and

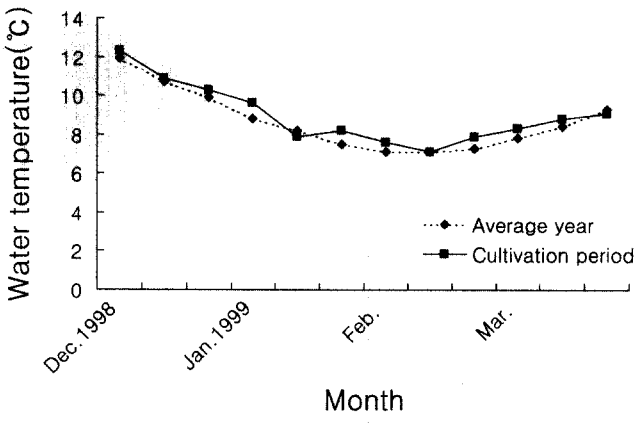

Fig. 5. Changes of water temperature of the year-average and cultivation year during the cultivation period in the sea.

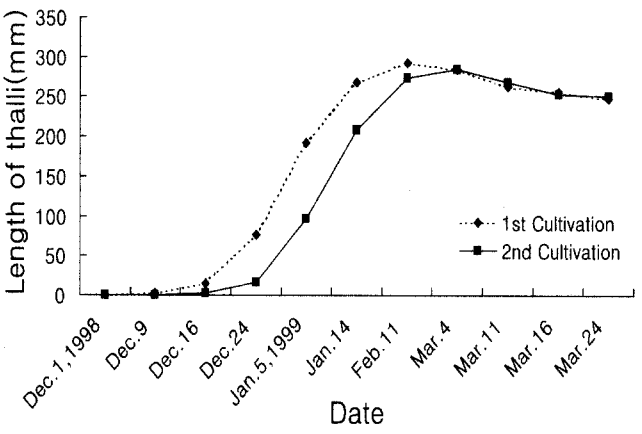

Fig. 6. Growth patterns of *Petalonia fascia* during the first and second cultivation periods.

growth (thallus length increase) were measured weekly and developments of plurilocular sporangial sori were monitored once a week for the entire period of cultivation.

RESULTS AND DISCUSSION

The plants used in the present study were *Petalonia fascia* based on the description of Nakamura and Tatewaki (1975), Fletcher (1987), Wynne (1969), Kogame (1993, 1994, 1997), Yoshida (1998) and Kang (1968). The macroscopic thallus was erect and lanceolated or long loceolated; the basal part was cuneate; this plant was tussock (Pl. A). The thallus was compressed and composed of cortex and medulla. The cortex was composed of small cells with chloroplasts of a few strata and the medulla was composed of hyaline, big cells (13-36 × 17-50 μm). The rhizoidal filament in the medulla was absent or few rhizoidal filaments were 7-15 μm thick.

Gametes were released easily from the fully matured plurilocular sporangia. The gametes after one month of the stationary culture were germinated and the blackish purple or blackish brown filaments began to appear in the bottoms of the Erlenmeyer flasks. The released gametes were germinated to filaments. When the filaments grew to filamentous and discoidal stolons, they seemed weak and disappearing (Pls B-E).

The number of released free gametes was about one individual per a view of 10 × 10 field of a light microscope during the stationary culture period. After application of aeration, non-conjugated free gametes were actively released. The released gametes were attached to inner side of the culture vessel and they germinated to develop filamentous or discoidal stolons. The erect thalli was found from the stolons in early May when seawater temperatures were below 20°C (mainly 10-15°C), but those were not found when temperatures were above 20°C (mainly 22-25°C) (Pl. F).

Female and male gametes could develop parthenogenetically and experience two different pathways; one may develop into erect thalli with plurilocular sporangia and the other may develop into tufts with unilocular organs. The expression of these two types of development was dependent on the culture conditions; under relatively cool and short-day conditions erect thalli were developed and under relatively warm and long-day conditions tufts were formed (Wynne 1969; Fletcher 1987; Kogame 1993, 1994, 1997); iodine was essential element for morphogenesis and growth of this alga (Hsiao 1969).

The filamentous and discoidal stolons could be easily maintained in the unialgal culture condition and their long-term storage was possible by repeated fine cutting, regeneration and inoculation like conchocelis of *Porphyra* (Migita 1992).

The fragments of filaments were easily attached on seeding nets in the indoor and outdoor culture conditions from November 5 to 30, 1998 (Pls G and H). Attachment and regeneration of the fragments were similar between indoor and outdoor cultures when they were observed after 12 days (Pls I and J). The erect thalli were not developed from zoospores during seeding and the subsequent cultures.

After 7days of the initiation of the standard field cultivation, 7-10 celled plantlets were recognized. After 15 days, the size of individuals were 1-2 mm long (Pl. K). After one month, the thalli grew up 130-275 mm (mean 200 mm) long blades (Pl. L) and the plurilocular

Table 1. Results of the first cultivation started from Nov. 24, 1998 by regeneration of filamentous discoidal stolon complexes

Mean thalli length (mm)											
Days	Dec. 1 (0)	Dec. 9 (8)	Dec. 16 (15)	Dec. 24 (23)	Jan. 5 (35)	Jan. 14 (44)	Feb. 11 (72)	May 4 (93)	May 11 (100)	May 16 (105)	May 24 (113)
	0.40	2.8	14.8	79.6	202.2	268.2	292.8	284.7	263.2	255.4	248.5
Relative growth rate											
Days	0-8	8-15	15-23	23-35	35-44	44-72	72-93	93-100	100-105	105-113	0-113
	0.245	0.236	0.211	0.078	0.031	0.003	-0.001	-0.011	-0.006	-0.003	0.057

Table 2. Result of the second cultivation started from Dec. 1, 1998 by regeneration of filamentous discoidal stolon complexes

Mean thalli length (mm)										
Days	Dec. 9 (0)	Dec. 16 (7)	Dec. 24 (15)	Jan. 5 (27)	Jan. 14 (36)	Feb. 11 (64)	May 4 (85)	May 11 (92)	May 16 (97)	May 24 (105)
	0.5	3.1	16.9	96.7	207.9	273.6	285.2	268.1	253.8	250.7
Relative growth rate										
Days	0-7	7-15	15-27	27-36	36-64	64-85	85-92	92-97	97-105	0-105
	0.259	0.213	0.145	0.085	0.010	0.002	-0.009	-0.011	-0.002	0.059

sporangial sori were found in January 1999. In early February 1999, thalli grew to the maximally sized blades (215-355 mm; mean 289 mm) (Pl. M). The non-conjugated zoospores (gametes) were released and grew to thalli on floating frames (Pl. N) during the cultivation period. When the length of a day increased and water temperature went up, the thalli began to be thicker; plurilocular sporangial sori were formed on the surface of thalli; length of thalli began to decrease due to distal disintegration. In March 1999, the color of thalli changed into yellow and epiphytic diatoms were attached on the thalli, which deteriorated the quality of products. The dried product was shown in Plate O.

The changes of water temperatures during the periods of culture experiment of *P. fascia* were similar to those of averaged temperatures (Fig. 5). The growth patterns of the first and the second cultivation year were shown in Fig. 6. The results of the two cultivation practices were similar (Tables 1 and 2).

The test cultivation of *P. fascia* by vegetative regeneration with fragments of filamentous and discoidal stolons was succeeded for the first time in Korea. Studies on other cultivation method through sexual reproduction of *P. fascia* are needed for developing cultivation of this plant.

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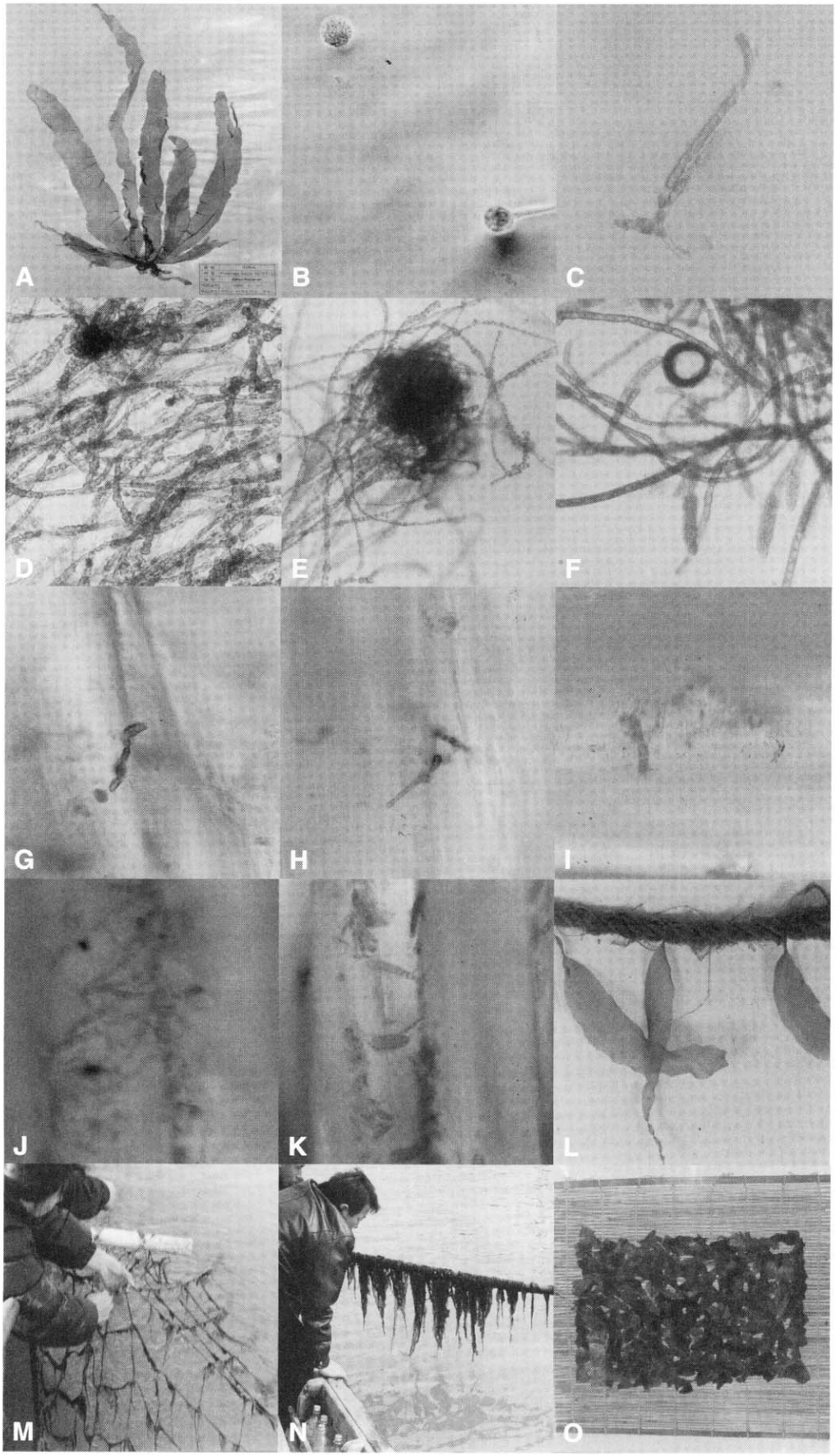
Explanation of Plates

Petalonia fascia (Müller) Kuntze

Plate A. Herbarium of thalli. B. Gametes. (× 200). C. Germination of a gamete. (× 200). D. Filamentous stolons. (× 200). E. Discoidal stolons. (× 200). F. Erect thalli below 20°C (× 200). G. Stolons attached on a *Porphyra* net after 5 days of seeding. (× 100). H. Stolons attached on a *Porphyra* net after 8 days of seeding. (× 200). I. Germinating stolons after 12 days of seeding. (× 200). J. Germinating stolons after 25 days of seeding. (× 200). K. Germlings. (× 200). L. Young thalli. (× 200). M. Thalli attached on *Porphyra* nets. N. Thalli attached on a floating frame. O. Adried product.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