Jap. J. Phycol. (Sôrui) 31 : 250-258. December 20, 1983

Phenological and morphological studies on *Punctaria flaccida* NAGAI (Dictyosiphonales, Phaeophyceae)*

Masataka OHTA

Central Laboratory, Marine Ecology Research Institute, 300 Iwawada, Onjuku-machi, Chiba, 299-51 Japan

OHTA, M. 1983. Phenological and morphological studies on *Punctaria flaccida* NAGAI (Dictyosiphonales, Phaeophyceae). Jap. J. Phycol. 31 : 250-258.

Punctaria flaccida NAGAI was investigated phenologically and morphologically using specimens collected at Muroran in Hokkaido, northern Japan. Small, barely visible plants appear in October. They grow slowly during the winter, and become gradually larger in spring, reaching a maximum of 5-7 cm long, 140-170 μm thick and 4-7 cells thick in April to May. From April some of them begin to be eroded in the upper portion of thallus. Plants are absent from July to September. From November to March only plurilocular sporangia are formed and from April unilocular sporangia are formed in the thalli with or without plurilocular sporangia. The similarities of the young fertile plants with only plurilocular sporangia to *Punctaria hiemalis* and of the old fertile plants with both plurilocular and unilocular sporangia to *Punctaria hesperia* are discussed.

Key Index Words: *Dictyosiphonales*; *morphology*; *Phaeophyceae*; *Phenology*; Punctaria; P. flaccida; P. hesperia; P. hiemalis; *taxonomy*.

Eight species of the genus *Punctaria* have been reported from Hokkaido. These are 1) *Punctaria chartacea* SETCHELL et GARDNER (UMEZAKI 1961), 2) *P. conglomerata* YAMADA et IWAMOTO (IWAMOTO 1960), 3) *P. flaccida* NAGAI (YAMADA and TANAKA 1944, UMEZAKI 1961, CHIHARA 1972), 4) *P. kinoshitae* YAMADA et IWAMOTO (IWAMOTO 1960), 5) *P. latifolia* GREVILLE (HASEGAWA 1949, KAWABATA 1959, TOKIDA and MASAKI 1959, CHIHARA 1972), 6) *P. plantaginea* (ROTH) GREVILLE (YAMADA and TANAKA 1944), 7) *P. rubescens* J. AGARDH (YENDO 1909) and 8) *P. tenuis* YAMADA et IWAMOTO (IWAMOTO 1960).

These species grow on rocks, other algae, *Phyllospadix* or *Zostera* in the lower intertidal to upper subtidal zones and are distinguished by the following taxonomic features: the thallus size, thallus form, the type of thallus margin, the number of cell layers, the shape and size of cells and the shape of the reproductive organs. In order to analyze the variability of these features I have been studying the species of *Punctaria* found in Hokkaido. The results of phenological and morphological observations of *Punctaria flaccida* are reported in this paper.

* Dedicated to Professor Munenao KUROGI on the occasion of his academic retirement.

Materials and Methods

Phenological and morphological observations on *Punctaria flaccida* were conducted monthly at Muroran (42°21'N, 140°59'E), on the south coast of Hokkaido from January to November 1978. The plants were sampled when the macroscopic thalli were present. The plants collected were fixed and preserved with 10% formalin in seawater for morphological observations.

Results

a) Phenological observations

Plants of *Punctaria flaccida* (Figs. 1, 2) attach to leaves of *Phyllospadix iwatensis* and thalli of *Palmaria palmata*, both of which grow in the lower intertidal zone exposed relatively to wave action. The plants are usually gregarious but rarely solitary when they are young. From data collected from January to November 1978 (Table 1, Fig. 3), small thalli (0.1–0.4 cm long) appeared in mid-October and increased in number from February. After that, thalli gradually grew larger and reached up to 7.0 cm long, 2.4 cm wide and 170 μm thick in May. The uppermost and marginal portion of thalli began to be eroded from April. All of the thalli collected in June were devoid of the upper portion. No thalli were found from July to September.

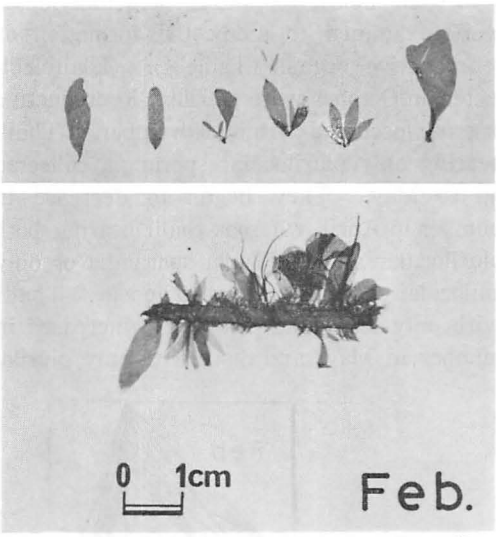

Fig. 1. Herbarium specimens collected on February 13, 1978 at Muroran.

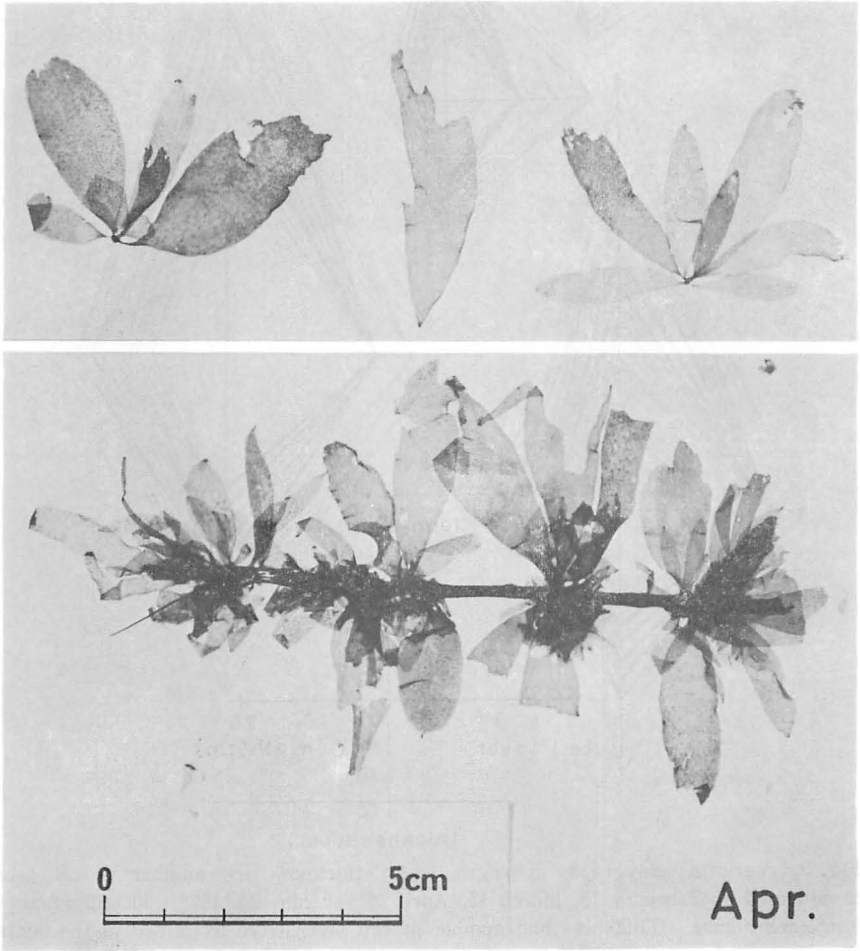

Fig. 2. Herbarium specimens collected on April 26, 1978 at Muroran.

In each month 100, 50, 20 or 4 individuals were examined to study the formation of reproductive organs (Table 1). Thalli collected in October were sterile. Reproductive organs occurred from November. Thalli bearing only plurilocular sporangia collected up to May. They began to decrease in number in April, whereas thalli bearing both plurilocular and unilocular sporangia or only unilocular sporangia began to appear. Thalli with only unilocular sporangia increased in number in May, and those with only plurilocular sporangia were reduced in number.

b) Morphological observations

External appearance: Thalli were comparatively small, oblanceolate and soft in texture. The habit in February and April is shown in Figs. 1 and 2. The maximum length was 7 cm and the maximum width 2.4 cm, the ratio of length to width was 1.5–10.0 (3.7 in average) except for October, when the thalli were very small and the ratio was 3.0–18.0 (7.0 in average) (Table

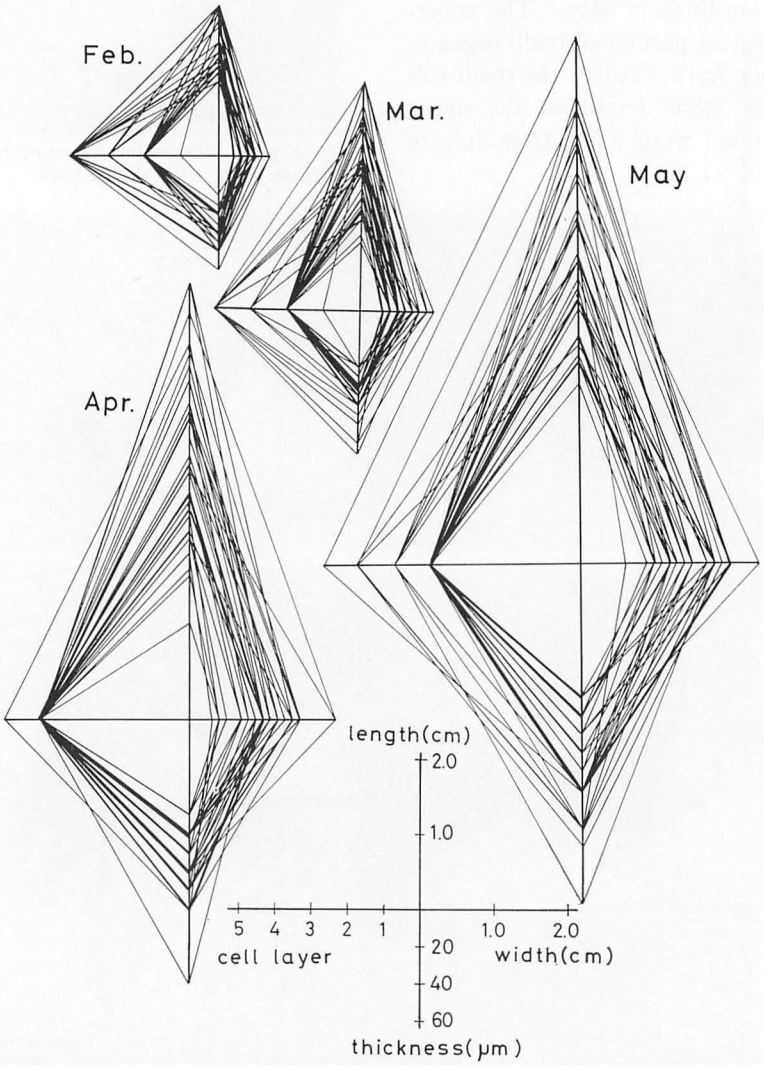

Fig. 3. Variation polygraphs in length, width, thickness and number of cell layer of plants collected on February 13, March 15, April 26 and May 26, 1978. 30 individuals were lined in each figure. Thickness and number of cell layer were examined in the center of thallus.

1, Fig. 3). They were pale greenish-brown in color from October to March and became darker as they grew older. They generally had entire margins throughout the growing season. They possessed a short complanate stipe which was 0.5-2.0 mm long and a discoid holdfast.

Vegetative structure: In surface view thalli were covered over with comparatively large 4-5 sided surface cells (Figs. 5A, 6A). Hairs were scattered all over the thallus. They were numerous when the thallus was young, and decreased as the thallus grew older. In cross section, thalli thicknesses varied according to the age (or growth season) and with the portion of the thallus examined.

The seasonal changes in thickness and cell layer of thallus are shown in Table 1 and Fig. 3. They were measured in the center of thallus. In October to November and February to March the thickness ranged from 20 to 70 μm and the number of cell layer from 1 to 4 (Fig. 5B-G). In this period one cell layered individuals in addition to multi-cell layered ones were found. In April to May the thickness reached 140 or 170 μm and the number of cell layers 4 or 7 (Fig. 6B-E). In this period one cell- or two cell-layered individuals were rarely encountered.

On the other hand, the cell layer in the lower portion of thallus was thicker than that in the central portion. For example, the fertile thalli in November and February to March were 1-4 cell-layered and 20-70 μm thick in the upper to middle portions, 4-7 cell-layered and 30-100 μm in the lower portion, and 8-13 cell-layered at the stipe. The fertile thalli in May were 4-7 cell-layered and 70-170 μm thick in the upper to middle portions, 8-9 cell-layered and 80-180 μm thick in the lower porition, and 10-18 cell-layered at the stipe.

In a cross section of a multi-cell layered thallus surface cells with many parietal and discoid chloroplasts were seen but the inner

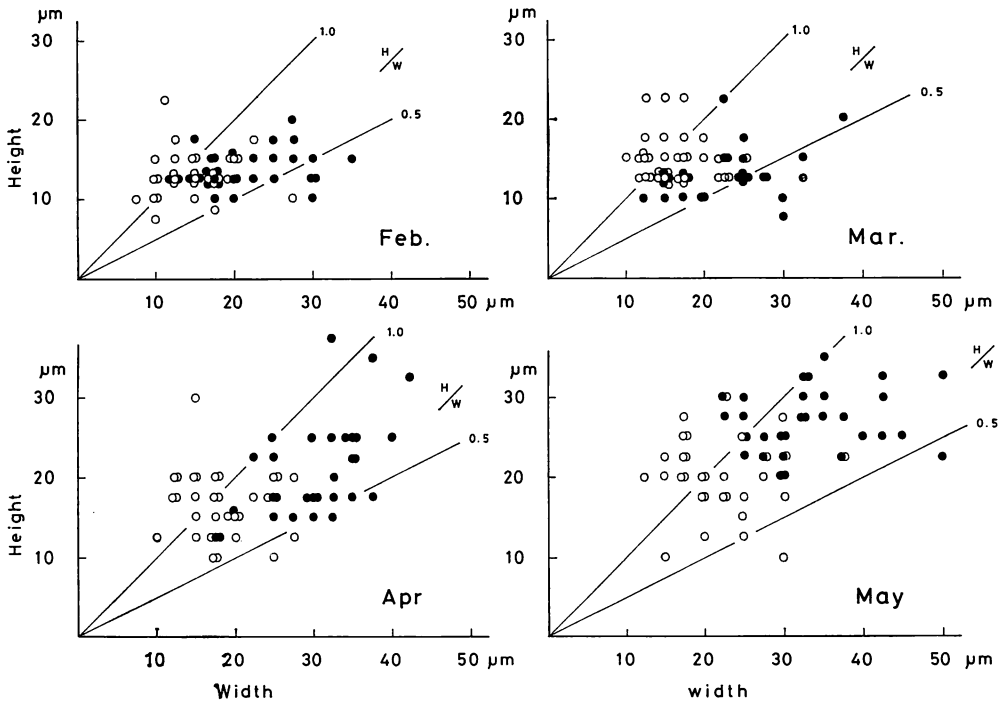

Fig. 4. Scattered diagrams in cell size in cross section of plants collected on February 13, March 15, April 26 and May 26, 1978, measured in the center of thallus. ○: surface cell, ●: inner cell.

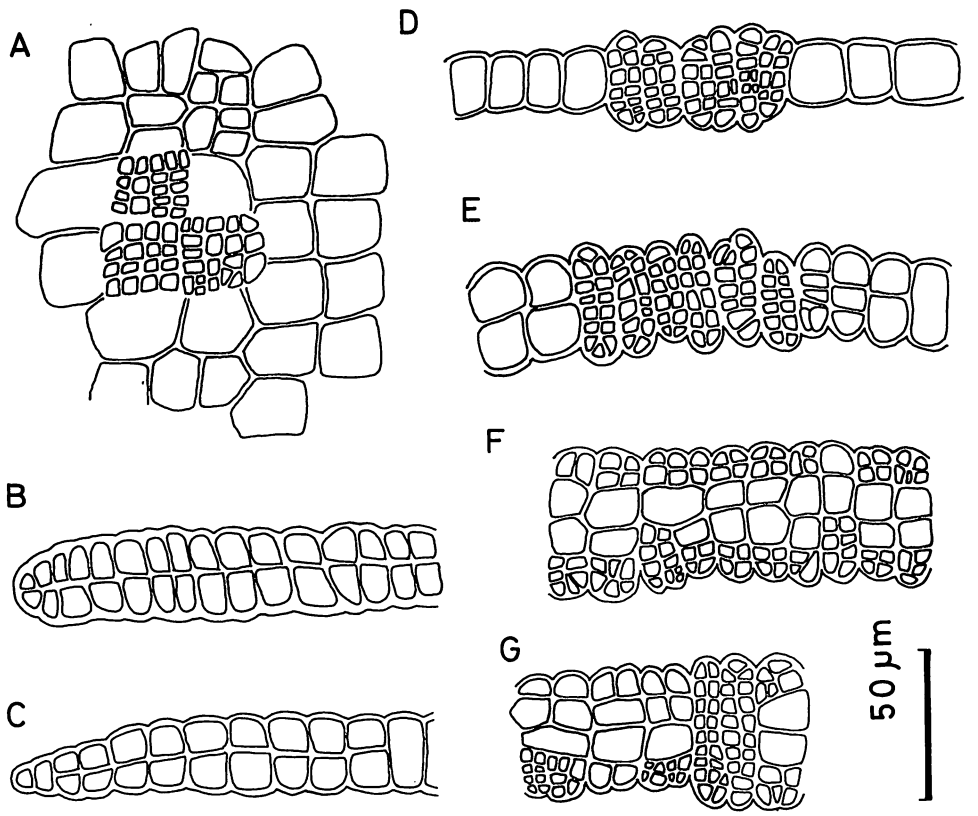

Fig. 5. Structure of thallus collected on February 13 (B-D, G) and March 15 (A, E, F), 1978. A, surface view; B-G, cross section. A, D-G, center of thallus bearing plurilocular sporangia; B, C, margin of the middle portion of thallus.

cells had very few. The cells were quadrate to rectangular, and 7.5-30.0 μm (height) $\times$ 7.5-37.5 μm (width) in surface cells and 7.5-35.0 μm (height) $\times$ 12.5-50.0 μm (width) in inner cells (Table 2, Figs. 5, 6). The size of cells did not show a remarkable difference between surface cells and inner cells in the early growing season of the year, but inner cells became generally larger and wider than surface cells as the thalli grew older (Fig. 4).

Rhizoidal filaments issued from the lower and basal portions of the stipe. They were 5.0-11.3 μm in diameter and entangled with each other to form a single discoid holdfast.

Reproductive organs: As mentioned before, this alga forms both plurilocular and unilocular sporangia. Up to March only thalli with plurilocular sporangia alone were collected (Fig. 5A, D-G), and after April thalli with both plurilocular and unilocular sporangia (Fig. 6A) and ones with only unilocular sporangia (Fig. 6E) as well as ones with only plurilocular sporangia (Fig. 6D) were found. Plurilocular sporangia were formed in one to two cell-layered small thalli (Fig. 5D, E) as well as in four or more cell-layered thalli (Fig. 6D). On the other hand, unilocular sporangia were formed only in four or more cell-layered thalli (Fig. 6E).

Plurilocular sporangia were formed by the repeated divisions of surface cells on both sides of thalli. They were first produced at the upper portion of thalli and formed a small patch. The subsequent formation of the plurilocular sporangia was basipetal.

In two-layered thalli collected from November to March, the two cells situated back to back were frequently almost simultaneously

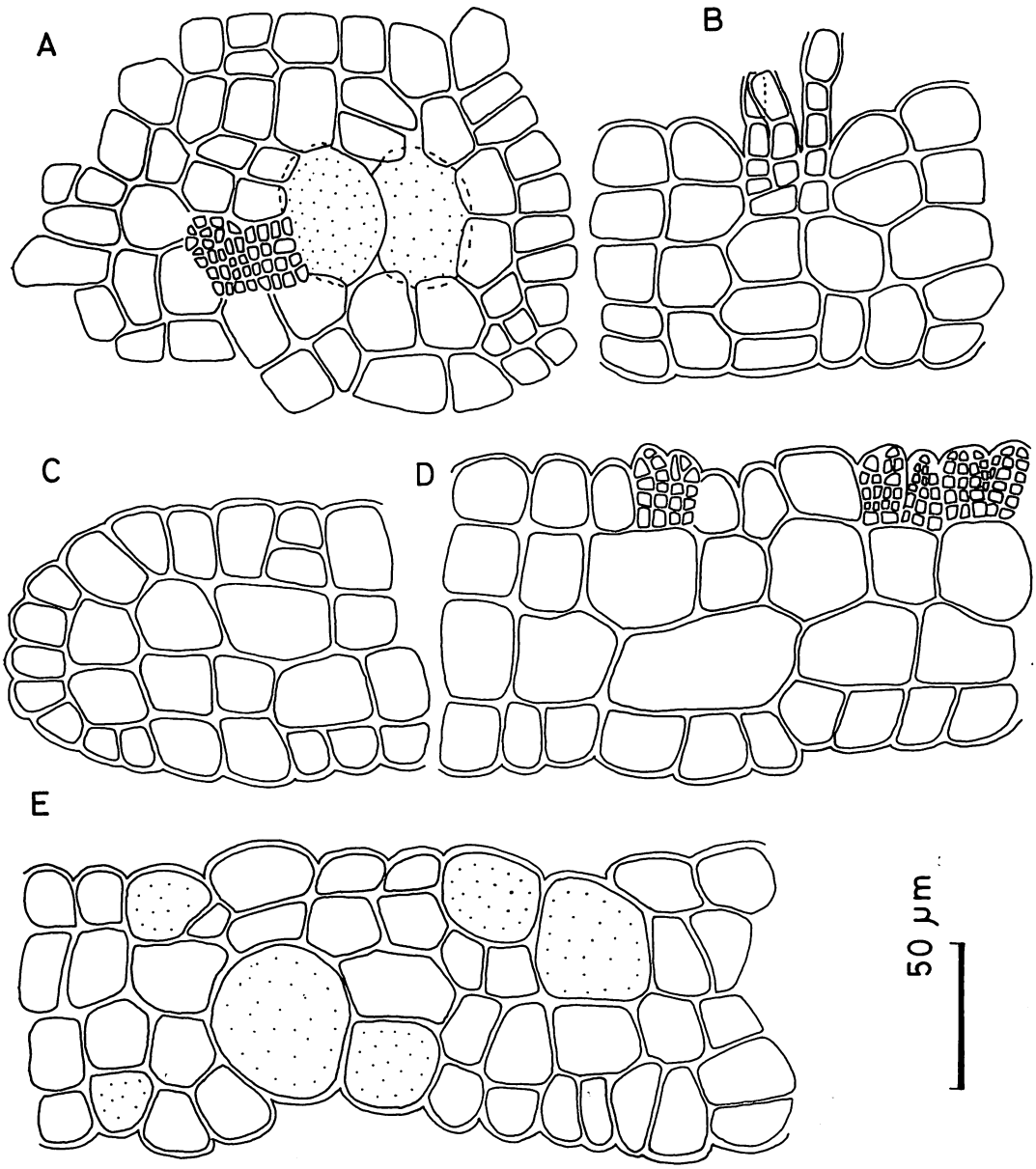

Fig. 6. Structure of thallus collected on May 26, 1978. A, surface view; B-E, cross section. A, center of thallus bearing both unilocular and plurilocular sporangia; B, central sterile portion with hair; C, margin of the middle portion; D, center of thallus with only plurilocular sporangia; E, center of thallus with only unilocular sporangia.

divided and formed two plurilocular sporangia (Fig. 5E, G). In four- to seven-layered thalli collected from April to May, the surface cells of each side were divided independently to form the sporangia. Mature plurilocular sporangia were cylindrical to conical with blunt apex. They were 17.5-25.0 μm high and 10.0-17.5 μm wide in cross section in February, then becoming slightly larger and 22.5-32.5 μm high and 12.5-20.5 μm wide in May (Table 2).

Unilocular sporangia were formed from

Table 1. Seasonal observations on the size and structure of plants and on the occurrence of plurilocular sporangia and unilocular sporangia

		Jan. 10	Feb. 13	Mar. 15	Apr. 26	May 26	June 25	Oct. 18	Nov. 15
Thallus	length (cm)	not measured	0.8-2.0 (1.4)	0.9-3.0 (2.0)	1.0-5.4 (3.4)	1.5-7.0 (3.9)	not measured	0.1-0.4 (0.2)	0.3-1.2 (0.6)
	width (cm)	〃	0.1-0.7 (0.4)	0.3-1.0 (0.5)	0.3-2.0 (1.0)	0.6-2.4 (1.2)	〃	0.01-0.10 (0.04)	0.1-0.4 (0.2)
	length/width	〃	1.6-10.0 (4.2)	1.8-6.8 (3.8)	1.7-9.0 (3.6)	1.6-6.8 (3.4)	〃	3.0-18.0 (7.0)	1.5-7.0 (3.2)
	thickness (μm)	〃	20-60 (38)	30-70 (42)	40-140 (74)	70-170 (114)	〃	20-70 (22)	20-63 (42)
	cell layer	〃	1-4	1-4	2-4	4-7	〃	1-2	1-4
Occurrence of reprod. organs (%)	with only pluril. sporang.	25	88	73	51	22	0	0	60
	with only unil. sporang.	0	0	0	10	48	61	0	0
	with both pluril. and unil. sporang.	0	0	0	19	12	3	0	0
	sterile	75	12	27	20	18	36	100	40
	examined	4	100	100	100	100	50	20	20

Thickness and cell layer: measured in the center of thalli, ( ) : showing the average.

Table 2. Seasonal observations on the size of vegetative cells, plurilocular sporangia and unilocular sporangia in cross section, showing height × width in μm

	Feb. 13	Mar. 15	Apr. 26	May 26
Surface cells	7.5-22.5 × 7.5-27.5	12.5-22.5 × 10.0-25.0	10.0-30.0 × 10.0-27.5	10.0-30.0 × 12.5-37.5
Inner cells	10.0-20.0 × 12.5-35.0	7.5-22.5 × 12.5-37.5	12.5-35.0 × 17.5-42.5	12.5-35.0 × 22.5-50.0
Pluril. sporang.	17.5-25.0 × 10.0-17.5	not measured	not measured	22.5-32.5 × 12.5-20.0
Unil. sporang.	absent	absent	not measured	17.5-55.0 × 22.5-45.0

surface cells without cell division. They were scattered on both sides of thalli. They were variable in shape, being ovoid or ellipsoidal in cross section (Fig. 6E), measuring 17.5-55.0 μm high and 22.5-45.0 μm wide (Table 2).

Discussion

From the present investigation it is clear that macroscopic thalli of this alga appear in October and continue vegetative growth in length, width, thickness and cell layer of thallus and in inner cell size to reach the maximum in May. Meanwhile, the formation of reproductve organs begins in the early developmental stage of thallus consisting of one to two cell layers in November and continues until reaching the maximum thallus size in May. That is, there occur young small fertile thalli and old large fertile ones. Plurilocular sporangia are formed throughout the growing season, and unilocular sporangia from April in the later season in the same thallus as plurilocular sporangia are borne or in the separate thallus without plurilocular sporangia.

The plants, which can be collected from April to June and have 4-7 layers of cells at the middle portion, are identical to *P.*

flaccida as reported from Kunashiri Island, southern Kuriles by NAGAI (1940) and from Akkeshi, eastern coast of Hokkaido by UMEZAKI (1961) in size, form, color of thallus and in cell size. The plurilocular sporangia of the Kunashiri plants project two-thirds or more of their length beyond the thallus surface (NAGAI 1940, pl. II, fig. 12), whereas those of the Muroran plants do not project conspicuously beyond the thallus surface as shown in Fig. 5. However, plurilocular sporangia similar to those of the Kunashiri plants were obtained in cultured plants in laboratory from Muroran (OHTA, unpublished observation), although I am unable to assess the influence of environmental factors on the development of protuberant plurilocular sporangia of *P. flaccida*.

The plants found from November to March resemble *Punctaria hiemalis* KYLIN (1907) with respect to bearing only plurilocular sporangia in 1-2 layered thalli (Fig. 5B-E). This species was described by KYLIN (1907) on the basis of material from Kristineberg, west coast of Sweden. It occurs in winter on leaves of *Zostera* and it is characterized by having small (2.5-6.0 cm long and 0.2-0.8 cm wide) and thin (1-2 layered) thalli and bearing only plurilocular sporangia (KYLIN 1907, 1947, KORNMANN and SAHLING 1977). *Punctaria hiemalis*, however, differs from *P. flaccida* in having 1-2 layered thalli reaching up to 6.0 cm long. Although *P. flaccida* has 1-2 layered thalli less than 3 cm long during winter and early spring, the thalli become thicker (4-7 layered) as they become older and reach up to 7.0 cm long in May. It is unknown whether *P. hiemalis* becomes thicker and produces unilocular sporangia as *P. flaccida* does.

The other species that resembles *P. flaccida* is *Punctaria hesperia* SETCHELL et GARDNER (1924) described on the basis of specimens collected at Pacific Grove, California. It is characterized by having small (1.5-2.5 cm long and 0.5-1.0 cm wide) and 4-6 layered thalli, and by having both plurilocular and unilocular sporangia on the same thallus. It grows on leaves of *Phyllospadix* and is distributed from Vancouver, British Columbia to San Pedro, California (SETCHELL and GARDNER, 1925, ABBOTT and HOLLENBERG 1979). It is similar to *P. flaccida* in the thallus form and the number of cell layers, but it has a smaller and thinner (35-50 up to 80 μm) thallus than the latter species. The morphology and reproduction of the early stages of *P. hesperia* are unknown.

In spite of the similarities of *P. flaccida* to *P. hiemalis* and *P. hesperia*, it is difficult at present to clarify the taxonomic relationship between the first and the latter two, because of a lack of information on *P. hiemalis* and *P. hesperia*, especially of their phenology of growth and reproduction.

Acknowledgements

I wish to express my gratitude to Professor Munenao KUROGI, Hokkaido University, for his criticism of the manuscript and helpful suggestion. I wish to acknowledge Dr. Michael D. GUIRY, University College, Galway, for his reading of the manuscript. I am also grateful to Dr. Michio MASUDA, Hokkaido University, for his help in preparing the manuscript.

References

ABBOT, I. A. and HOLLENBERG, G. J. 1976. Marine algae of California. Stanford Univ. Press.

CHIHARA, M. 1972. Marine flora communities along the coast of Hidaka, Hokkaido. Mem. Nat. Sci. Mus. Tokyo **5**: 151-162 (in Japanese).

HASEGAWA, Y. 1949. A list of the marine algae from Okushiri Island. Sci. Pap. Hokkaido Fish. Sci. Inst. **3**: 38-72.

IWAMOTO, K. 1960. Marine algae from Lake Saroma, Hokkaido. J. Tokyo Univ. Fish. **46**: 21-49, pls. 1-15.

KAWABATA, S. 1959. A list of marine algae in the vicinity of the Shirikishinai Marine Station. J. Hokkaido Gakugei Univ. **10**: 285-296 (in Japanese).

KORNMANN, P. and SAHLING, P.-H. 1977. Meeresalgen von Helgoland. Helgoländer wiss. Meeresunters. **29**: 1-289.

KYLIN, H. 1907. Studien über die Algenflora der schwedischen Westküste. Akad. Abhndl., Upsala.

KYLIN, H. 1947. Die Phaeophyceen der schwedischen Westküste. Lunds Univ. Årsskr. N. F. Avd. 2, **43**: 1-99, Taf. 1-18.

NAGAI, M. 1940. Marine algae of the Kurile Islands. I. J. Fac. Agr., Hokkaido Imp. Univ. **46**: 1-137, pls. 1-3.

SETCHELL, W. A. and GARDNER, N. L. 1924. Phycological contributions. VII. Univ. Calif. Publ. Bot. **13**: 1-13.

SETCHELL, W. A. and GARDNER, N. L. 1925. The marine algae of the Pacific coast of North America. III, Melanophyceae. Univ. Calif. Publ. Bot. **8**: 387-898.

TOKIDA, J. and MASAKI, T. 1959. A list of marine algae collected in the vicinity of Oshoro Marine Station, at Oshoro, Hokkaido, Japan. Bull. Fac. Fish., Hokkaido Univ. **10**: 173-195.

UMEZAKI, I. 1961. Some new and noteworthy species of genus *Punctaria* (brown algae) from Japan. J. Jap. Bot. **36**: 362-367.

YAMADA, Y. and TANAKA, T. 1944. Marine algae in the vicinity of Akkeshi Marine Biological Station. Sci. Pap. Inst. Alg. Res., Fac. Sci., Hokkaido Imp. Univ. **3**: 47-77. pl. 8.

YENDO, K. 1909. Note on algae new to Japan. Bot. Mag. Tokyo **23**: 117-133.

太田雅隆： 褐藻チシマハバモドキの生物季節学的および形態学的研究

北海道の室蘭に生育するチシマハバモドキ (*Punctaria flaccida* NAGAI) の生物季節学的および形態学的研究を行った。本種は10月中旬に肉眼的大きさとして現われ，冬期間の生長は遅いが，3月頃から次第に大きくなり，4～5月には 7 cm となる。それ以後，藻体は衰微して7～9月の間には見られなくなる。この生育期間のうち，11～3月の藻体には複子嚢だけが形成され，4月以降は複子嚢を形成する藻体のほかに，複子嚢と単子嚢の双方を持つ藻体，単子嚢だけを持つ藻体が現われる。3月以前の複子嚢だけを持つ藻体は *P. hiemalis* KYLIN に，4月以降の複子嚢と単子藻を持つ藻体は *P. hesperia* SETCHELL et GARDNER に類似するが，この2種との関係については両種の生物季節学的調査の資料が少なく，結論は出せない。(229-51 千葉県夷隅郡御宿町岩和田300番地 海洋生物環境研究所 中央研究所)