

## Reproductive Structures of Three *Padina* Species (*Dictyotales*, *Phaeophyceae*) from Japan

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*Padina crassa*, *P. japonica* and *P. minor* were collected from the central Pacific Ocean and the Japan Sea coasts, near their northern distributional limits on both coasts. Their asexual sporophyte generations were dominant, and their sexual gametophyte generations rarely occurred. A morphological and anatomical comparison of the three species was carried out. Thallus of *P. crassa* consists of 4–8 cell layers, and only 2 cell layers are present among the thalli of *P. japonica* and *P. minor*. The sporangia, oogonia and antheridia of the three species occur along concentric hairlines on the dorsal surface of a thallus. This is the first report for the female gametophyte of *P. crassa*, and for the male and female gametophytes of *P. minor*. The reproductive structures of *P. crassa* and *P. japonica* are distributed on both sides of the hairline and covered with indusia, whereas those of *P. minor* are distributed on the upper side of the hairline without indusia. The positional relation between reproductive structures and hairlines can be taken as an important taxonomic characteristic at the rank of species of *Padina*.

**Key words:** *Dictyotales*, hairline, *Padina*, *Phaeophyceae*, reproductive structures.

The genus *Padina* comprises about 30 taxa and most taxa occur widely in tropical to warm temperate regions (Guiry and Guiry 2007). The members can be easily distinguished from the other dictyotalean algae on the basis of three conspicuous characteristics: 1) fan-shaped thallus, 2) deposition of calcium carbonate on surfaces of the thallus, and 3) rolled apical margin. Among the species of *Padina*, a number of names are regarded as synonyms. On the other hand, some independent species are sometimes described under the same scientific name. Therefore, it is important to make detailed descriptive observations on the species recorded so far.

Yamada (1925, 1931) reported three new species of *Padina* from Japan, *P. crassa*,

*P. japonica* and *P. minor*. But he only described their sporophytes, and did not observe their gametophytes. Umezaki and Yoneda (1962) reported gametophytes of *P. japonica*, but they did not describe the morphological differences between female gametophyte and sporophyte. Kawaguchi et al. (1992) reported male gametophytes of *P. crassa*, but they did not find female gametophytes in nature. Unfortunately, gametophytes of *P. minor* have never been reported. In the present study, we report not only tetrasporophytes but also gametophytes of the three above-mentioned species, and the morphology of reproductive structures is described in detail. We also try to revalue their distinguishing characteristics at the rank of species.



Fig. 1. Map of collection sites. Banjin, Kashiwazaki City, Niigata Pref. Nago, Tsuruga City, Fukui Pref. Banda, Tateyama City, Chiba Pref.

We use the term “ventral surface” in this paper to the surface where margin is rolled in, “dorsal surface” to the other surface, and the term “hairline” to be a line with phaeophycean hairs arranged in a concentric row.

### Materials and Methods

The collection sites were Banjin, Kashiwazaki City, Niigata Pref.; Nago, Tsuruga City, Fukui Pref.; Banda, Tateyama City, Chiba Pref. (Fig. 1). The collections were made by snorkelling. *Padina crassa* and *P. japonica* were collected on the Japan Sea coast, and *P. minor* was collected in a tide pool on the Pacific coast. The specimens were fixed with 10% formalin seawater solution soon after collection. In order to dissolve calcium carbonate the surfaces, some specimens were dipped in 10% hydrochloric

acid solution before observation. Sections were made from liquid-preserved specimens by a freezing microtome. They were stained with 1% aqueous cotton blue solution, and mounted in 20–30% corn sugar syrup solution prior to microscope examination. Voucher herbarium specimens were lodged in the Tokyo University of Marine Science and Technology, Phycology Laboratory (MTUF-AL).

### Results

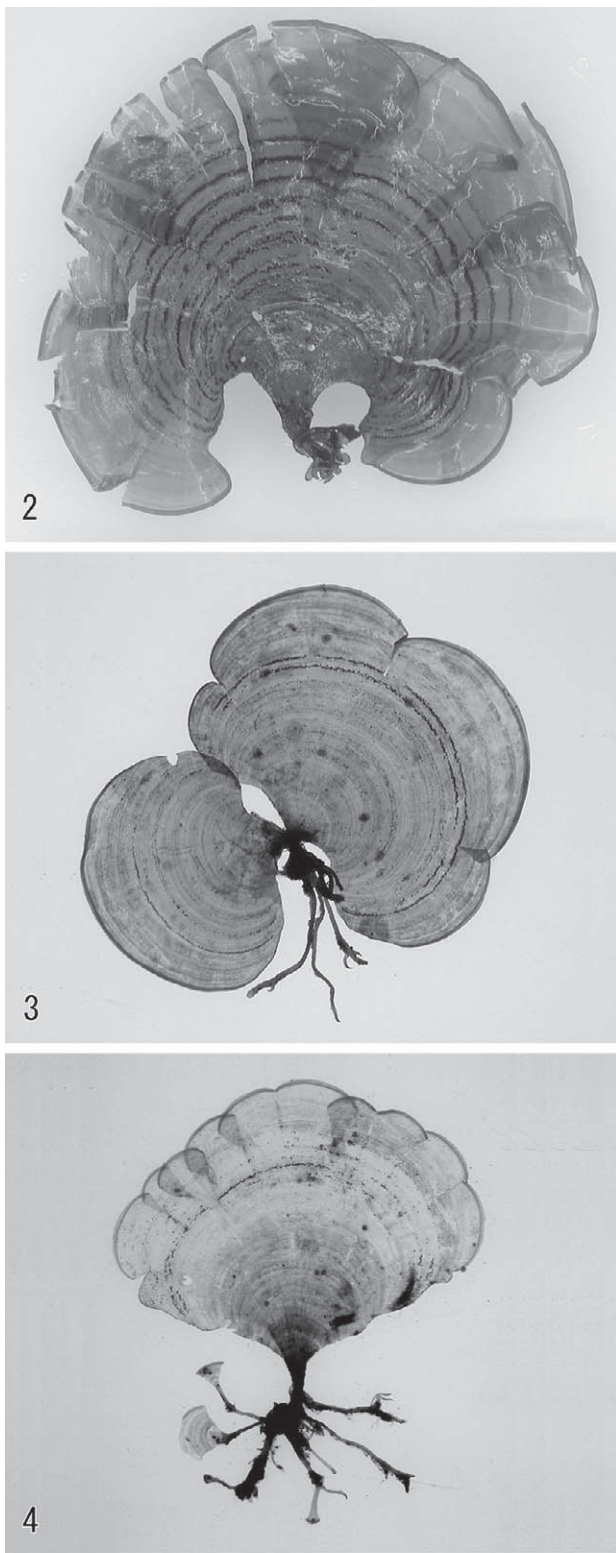
***Padina crassa*** Yamada in J. Fac. Sci., Hokkaido Imp. Univ., Ser. V. 1: 67, pl. 17, fig. 2 (1931); Okamura, Icon. Jap. Algae 6: 83, pl. 294, figs. 5-11 (1932); Segawa, Col. Illust. Seaweeds Jap. 30, pl. 16, fig. 137 (1956); Tseng, Common Seaweeds China 199, pl. 101, fig. 1 (1984); Silva & al., Cat. Benthic Mar. Algae Philippines 78 (1987); Kawaguchi in Hori (ed.), Illust. Atlas Life Hist. Algae 2: 98 (1993); Silva & al., Cat. Benthic Mar. Algae Ind. Ocean 603 (1996); Yoshida, Mar. Algae Japan 226 (1998).

Type locality: Shibagaki, Ishikawa Pref.

Distribution: Common in tropic and warm temperate waters of North Pacific Ocean. Japan (the Japan Sea coast of Honshu, Seto Inland Sea, Kyushu, Okinawa), Korean Peninsula, China (Huanghai Sea, East China Sea).

Specimens examined: Banjin, Kashiwazaki City, Niigata Pref., 9 August 2005, Z. Sun, MTUF-AL-30726, 30728; Nago, Tsuruga City, Fukui Pref., 5 August 2005, Z. Sun, MTUF-AL-30741, 30742, 30743.

Plant consists of a fan-shaped thallus and a holdfast, 9–13 cm high, up to 15 cm (Fig. 2). Holdfast is often planiform or stick-like. The lower to middle part of the thallus is lightly calcified, and the upper part looks somewhat transparent. In cross section, thallus consists of one cell layer of cortex and several cell layers of medulla. The medulla near the apex has two cell layers and the layers increase to 6–8 cells toward the



Figs. 2–4. Mature thalli of three *Padina* species. Fig. 2. *P. crassa* (sporophyte, MTUF-AL-30726). Fig. 3. *P. japonica* (sporophyte, MTUF-AL-30744). Fig. 4. *P. minor* (sporophyte, MTUF-AL-23138). Scale bar = 1 cm.

lower part. The thickness of the upper to middle part of a thallus is 120–160  $\mu\text{m}$ . Reproductive structures are distributed mainly on the dorsal surface and arranged in concentric rows.

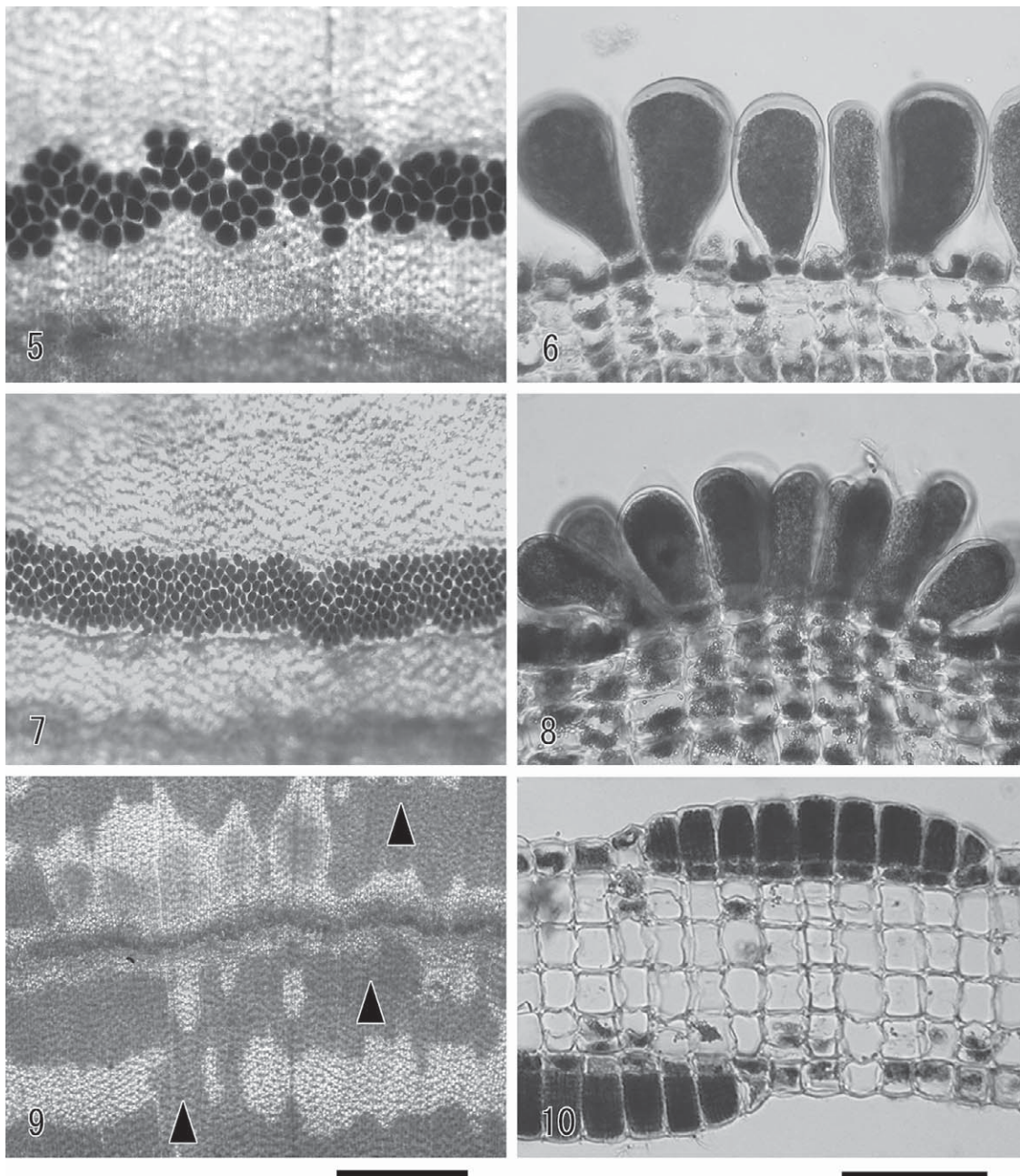
Sporangia develop along both sides of a hairline, and then two dark concentric sporangial rows are noticeable to naked eye. More than eight sporangial rows exist on a mature thallus, and each row consists of 4–5 sporangia in longitudinal direction. Young sporangia are covered with thin indusia that break when sporangia mature. Sporangia are 90–130  $\mu\text{m}$  high and 80–100  $\mu\text{m}$  in diameter (Figs. 5, 6).

Oogonia also develop along both sides of a hairline, and up to eight concentric oogonial rows can be observed on a thallus. Oogonia are more abundant, smaller in size and lighter in color than sporangia. Most of them are covered with indusia. At the middle-lower part of a thallus, oogonia form big sori instead of lines, where empty oogonia can be observed in order along lines. Well-developed oogonia are 50–80  $\mu\text{m}$  high and 45–60  $\mu\text{m}$  in diameter (Figs. 7, 8).

The distribution of antheridia is similar to that of oogonia, but antheridial sori form a broader line than oogonial ones. Some sori between two hairlines are associated to become a larger antheridial zone (Fig. 9). Antheridial sorus consists of many rectangular cells in a surface view. Well-developed antheridia are 40–55  $\mu\text{m}$  high (Fig. 10).

***Padina japonica*** Yamada in J. Fac. Sci., Hokkaido Imp. Univ., Ser. V. 1: 69, pl. 19, fig. 2 (1931); Okamura, Icon. Jap. Algae 6: 82, pl. 294, figs. 1–4 (1932); Segawa, Col. Illust. Seaweeds Jap. 30, pl. 16, fig. 138 (1956); Umezaki & Yoneda in Acta Phytotax. Geobot. 19: 79, figs. 1–7 (1962); Silva & al., Cat. Benthic Mar. Algae Philippines 78 (1987); Yoshida, Mar. Algae Jap. 226 (1998).

Type locality: Ehime Pref.



Figs. 5–10. Reproductive structures of *Padina crassa*. Fig. 5. Surface view of a sporangial sorus in a line. Fig. 6. Transverse section of a sporangial sorus. Fig. 7. Surface view of an oogonial sorus in a line. Fig. 8. Transverse section of an oogonial sorus. Fig. 9. Surface view of antheridial sori (darker regions, arrowheads). Fig. 10. Transverse section of an antheridial sorus. Scale bars = 500  $\mu\text{m}$  (Figs. 5, 7, 9), 100  $\mu\text{m}$  (Figs. 6, 8, 10).

**Distribution:** Common in warm temperate waters of North Pacific Ocean. Japan (the Japan Sea coast of Honshu, Seto Inland Sea, Kyushu), Korean Peninsula.

**Specimens examined:** Banjin, Kashiwazaki City, Niigata Pref., 9 August 2005, Z. Sun, MTUF-AL-

30727, 30729; Nago, Tsuruga City, Fukui Pref., 5 August 2005, Z. Sun, MTUF-AL-30744, 30745, 30746.

Fan-shaped thallus is 3–8 cm high, not more than 10 cm (Fig. 3). Plant growing on intertidal rock has a hairy holdfast instead of

stick-like ones, while that growing on pebbles in shallow water tend to possess a long stick-like holdfasts. Thallus is opaque, dorsal surface looks yellow brown and ventral surface looks whitish in seawater. Both surfaces of thallus are deeply covered with calcium carbonate. Two cell layers are present throughout a thallus. The thickness of the middle to upper part of a thallus is 70–80  $\mu\text{m}$ . Reproductive structures occur mainly on dorsal surface and arranged in concentric rows.

Sporangia are distributed along the upper side of a hairline, and one darker conspicuous sporangial row can be observed (Fig. 11). But sometimes an additional sporangial row is born along the lower side of a hairline. On the opposite (ventral) surface, one hairline occurs between two hairlines of the dorsal surface that makes the distance between hairlines narrower. The sporangia observed are mostly immature and covered with indusia. However, many empty sporangia also exist on the middle-lower part. Sporangia are 90–130  $\mu\text{m}$  high, and 85–100  $\mu\text{m}$  in diameter (Fig. 12).

On dorsal surface, oogonia grow along both sides of a hairline, forming two oogonial rows in a dark concentric zone (Fig. 13). However, skipping down one hairline of dorsal surface, oogonia are fragmentarily distributed along another hairline, and form another reproductive zone. Between the two reproductive zones, empty oogonia remain along the hairline, following oogonia release. Immature oogonia are covered with indusia (Fig. 14). Oogonia are smaller in size and lighter in color than sporangia, 50–70  $\mu\text{m}$  high, 45–60 in diameter.

Antheridial sori are obscured by calcium carbonate deposits and can not be observed easily with the naked eye. Antheridia are distributed like oogonia, but they are always closer to the apex than oogonia. Antheridial sori do not tend to join into a line (Fig. 15). Well-developed antheridia are 40–50  $\mu\text{m}$

high (Fig. 16).

**Padina minor** Yamada in Bot. Mag. (Tokyo) **39**: 251, fig. 1 (1925); Okamura, Icon. Jap. Algae **6**: 56, pl. 279, figs. 6–9 (1931); Segawa, Col. Illust. Seaweeds Jap. **30**, pl. 16, fig. 136 (1956); Tseng, Common Seaweeds China **200**, pl. 101, fig. 1 (1984); Silva & al., Cat. Benthic Mar. Algae Philippines **78** (1987); Verheij & al. in Blumea **37**: 429, pl. 10, fig. 7 (1993); Silva & al., Cat. Benthic Mar. Algae Ind. Ocean **605** (1996); Yoshida, Mar. Algae Jap. **226** (1998).

Type locality: Garan-bi, Taiwan.

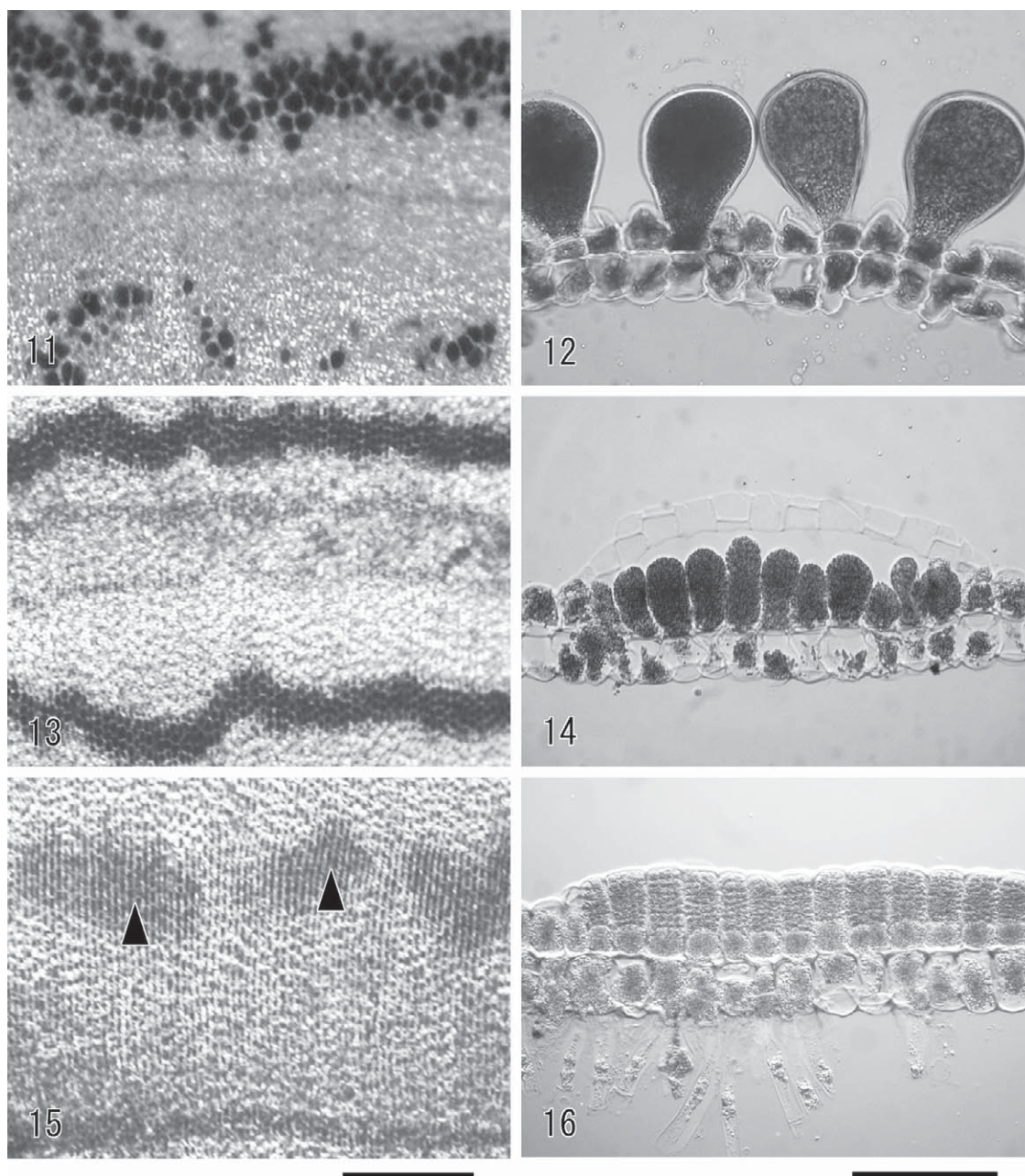
Distribution: Common in tropic temperature waters of North Pacific Ocean. Japan (Pacific coast of the southern part of Honshu, Kyushu, Okinawa), China (East China Sea, South China Sea), Philippines, Indonesia.

Specimens examined: Banda, Tateyama City, Chiba Pref., 11 July 2004, Z. Sun, MTUF-AL-23138, 23139, 23140.

Plant consists of a fan-shaped thallus and a holdfast, 2–5 cm high, often divided into some small cuneate segments at the upper part (Fig. 4). Holdfast is obvious, slender and stick-like, from which new foliose thalli develop. Thallus consists of two cell layers throughout. The thickness of a thallus is 60–90  $\mu\text{m}$  at the middle to upper part. The ventral surface is heavily calcified and bright white in color. Many long hairs are born near the apex of dorsal surface, while hairs become fewer toward the base. When calcium carbonate is dissolved by hydrochloric acid, the thallus looks transparent. Reproductive structures are distributed on dorsal surface. Indusium is breakable and not noticeable because of early loss before maturation.

Sporangia grow in a row along the upper side of a hairline. The number of sporangial rows is not more than three on a thallus. Indusia can be observed over young sporangia. Sporangia are 90–120  $\mu\text{m}$  high, and 85–100  $\mu\text{m}$  in diameter (Figs. 17, 18).

Oogonia grow along the upper side of



Figs. 11–16. Reproductive structures of *Padina japonica*. Fig. 11. Surface view of sporangial sori, showing an additional sorus under a hairline. Fig. 12. Transverse section of a sporangial sorus. Fig. 13. Surface view of two oogonial sori up and down along a hairline. Fig. 14. Transverse section of a young oogonial sorus, covered with an indusium. Fig. 15. Surface view of antheridial sori (arrowheads). Fig. 16. Transverse section of an antheridial sorus. Scale bars = 500  $\mu\text{m}$  (Figs. 11, 13, 15), 100  $\mu\text{m}$  (Figs. 12, 14, 16).

hairlines like sporangia but they are more uniformly arranged, smaller in size and lighter in color than sporangia. Oogonia are 50–70  $\mu\text{m}$  high and 40–60  $\mu\text{m}$  in diameter (Figs. 19, 20). Oogonial rows are not more than three lines on a thallus.

Being covered by calcium carbonate, antheridia are not obvious to naked eyes. When calcium carbonate is dissolved, the antheridial sori are clearly visible (Fig. 21). They are distributed along hairlines but with not more than three lines per thallus. Young

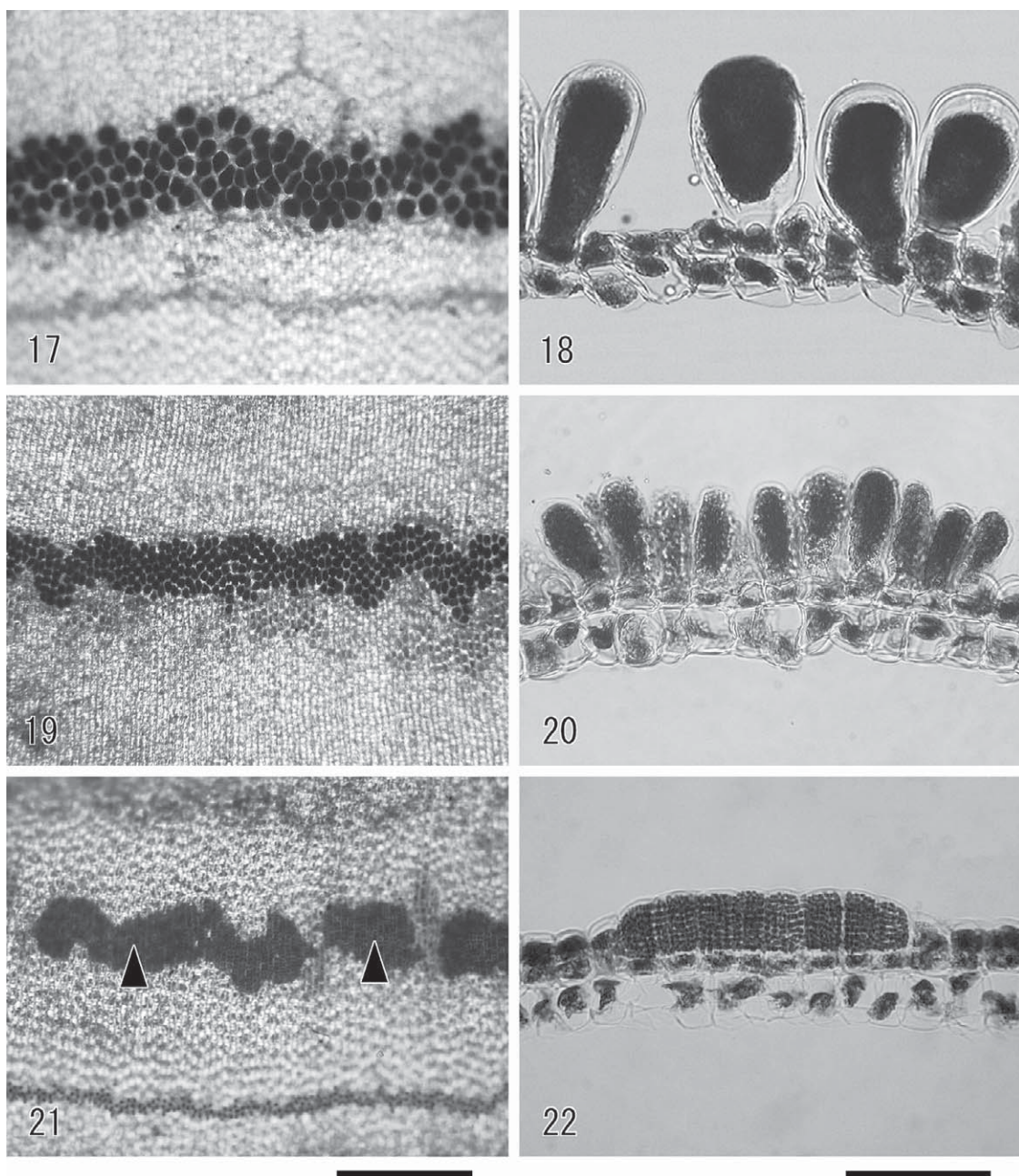


Fig. 17–22. Reproductive structures of *Padina minor*. Fig. 17. Surface view of sporangial sorus in a line. Fig. 18. Transverse section of a sporangial sorus. Fig. 19. Surface view of oogonial sorus in a line. Fig. 20. Transverse section of an oogonial sorus without indusium. Fig. 21. Surface view of antheridial sori which are darker in color (arrowheads). Fig. 22. Transverse section of an antheridial sorus. Scale bars = 500  $\mu\text{m}$  (Figs. 17, 19, 21), 100  $\mu\text{m}$  (Figs. 18, 20, 22).

antheridia are initiated from dark-brown cortical cells, and mature ones are arranged in darkly colored sori. Well-developed antheridia are 40–50  $\mu\text{m}$  high (Fig. 22).

The morphological characteristics of *Padina crassa*, *P. japonica* and *P. minor* are

generalized in Fig. 23. All the three species possess hairs on their surfaces, especially near apices, a lot of long hairs form the first hairline. Each cortical cell can form hair only once, after the old hair falls, a new one grows nearby. Reproductive structures occur

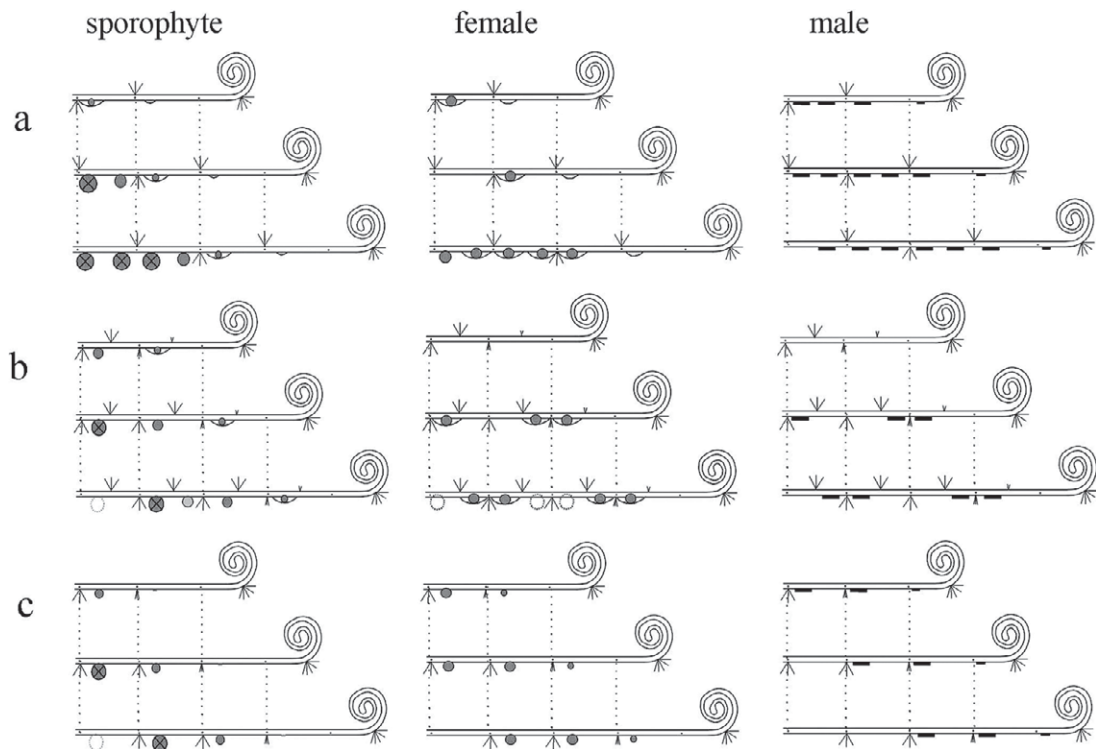


Fig. 23. Generalization and comparison of the morphological characteristics of the three *Padina* species, showing three developmental stages of sporangia (●), oogonia (◐) and antheridia (—) on the dorsal surface. (a) *P. crassa*: sporangia along both sides of hairlines, indusia breaking down as sporangia grow; young oogonia along both sides of hairlines covered with indusia; antheridia along both sides of hairlines, closer to the apex than oogonia. (b) *P. japonica*: sporangia mainly along the upper side of hairlines, young sporangia covered with indusia; empty oogonia (○) remaining between two oogonial zones, young oogonia along both sides of hairlines with indusia; development of antheridia in similar manner like oogonia. (c) *P. minor*: few hairs on ventral surface; reproductive sori only along the upper side of hairlines without indusia.

along hairlines as follows: (1) The second hairline of *P. crassa* grows on ventral surface, and the third hairline grows along the line where the first elder one fell. Thus hairlines grow on the both surfaces alternately. Normally, rows of reproductive sori grow along the both sides of each hairline on dorsal surface. Immature sporangia and oogonia are covered with indusia. (2) After the first hairline of *P. japonica* fell, the new hairline arises again near the old line. Then a hairline appears on the opposite (ventral) surface between the two hairlines of dorsal surface, that makes the distance of hairlines become narrow. Indusia over younger sporangia and

oogonia are noticeable. (3) Because the ventral surface of *P. minor* is heavily deposited with calcium carbonate, few hairs can be observed. Reproductive structures are only distributed on the upper side of each hairline of dorsal surface. Indusia are not obvious.

### Discussion

*Padina crassa* can be distinguished from the other two species by the thicker thallus with more than four cell layers in its section, on the other hand, *P. japonica* and *P. minor* possess two cell layers throughout a thallus. Yamada (1931) and Noda (1987) noticed that the distance between hairlines of



*P. japonica* is narrower than other species occurring in Japan. That is a very useful distinguishing characteristic between *P. japonica* and *P. minor* by naked eyes.

Fully grown plant of *Padina crassa* was regarded as a synonym of *P. gymnospora* (Kützing) Sonder (Abbott and Huisman 2004), however, Wormersley (1987) pointed out that *P. gymnospora* has sporangial sori mainly on ventral surface instead of dorsal surface as *P. crassa*. *Padina gymnospora* has only one sporangial row between two hairlines, while *P. crassa* has normally two sporangial rows. The size of sporangia of the former species is smaller than the latter. We propose *P. crassa* should not be the synonym of *P. gymnospora* for the present.

Gaillard (1975) noted the distinguishing characteristics between *Padina japonica* and *P. sanctae-crucis* are ambiguous, and that was also noted by Verheij et al. (1993) and Abbott and Huisman (2004). When Yamada (1931) described the new species *P. japonica*, he had critically compared with the type specimen of *P. sanctae-crucis*. Although he agreed to the resemblance of the two species, he emphasized that an additional sorus exists along the lower side of a hairline on *P. japonica*. In the present study, we also recognized this characteristic. Furthermore, the plant of *P. japonica* is no more than 10 cm high, it is smaller than the West Indian species, *P. sanctae-crucis*. We suggest that *P. japonica* and *P. sanctae-crucis* are independent species one another.

*Padina minor* is a most common *Padina* species occurring in the tropic of Japan. It is till accepted as a current name by Verheij et al. (1993), and our observation agrees well with that. The foliose thallus is so durable that it can survive for a long time when out of seawater, and that may be an important characteristic to distinguish it from the related species. It is mostly regarded as the smallest species among *Padina*, but sometimes it can reach a large size. *Padina*

*sanctae-crucis* described by Abbott and Huisman (2004) in Hawaiian Island is more similar to this species than *P. japonica*.

The arrangement of reproductive structures, especially, the positional relation of reproductive structures, hairlines, and rolling direction of apex is a useful taxonomic characteristic. This characteristic was also used to identify *Padina* species by other authors (Yamada 1931, Okamura 1932, Gaillard 1975, Wormersley 1987, Lee and Kamura 1991, Verheij 1993, Yoshida 1998, Abbott and Huisman 2004, Geraldino et al. 2005). On the other hand, the presence or absence of indusia is not as good for distinguishing the species among *Padina*, because indusia can be observed only on much younger sporangia and oogonia. It suggests that the stick-like holdfast does not seem to be a reliable characteristic, because its presence or absence in *Padina japonica* is influenced by the growing environment.

In the present study, we collected samples near the distributional limits of the three species, especially Banda, Tateyama City, could be regarded as the northern limit of the distribution of *P. minor*. *Padina crassa* and *P. japonica* are abundant from July to September, and *P. minor* is abundant from June to October. Occurrence of fan-shaped thallus of *Padina* species in these areas owes to the higher water temperature in summer to early autumn. In higher water temperature season, all the three species produce reproductive structures.

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孫 忠民, 長谷川和清, 田中次郎: 日本産ウミウチワ3種(褐藻, アミジグサ目)の生殖器官の形態

コナウミウチワ, オキナウチワ, ウスユキウチワの胞子体および雌雄配偶体が採集され, この3種のいずれでも胞子体が優占していることが明らかになった. コナウミウチワの藻体は4~8細胞層からなるが, オキナウチワとウスユキウチワの藻体はすべて2細胞層からなる. これら3種の四分胞子嚢, 生卵器, 造精器は藻体の背面に同心円状の毛線に沿って形成される. コナウミウチワの

雌性配偶体, ウスユキウチワの雌雄配偶体は初めての記録である. 生殖器官の藻体上での形成部位を比較すると, コナウミウチワとオキナウチワの生殖器官は毛線の両側に生じ, 若い生殖器官を保護する包膜は明瞭である. この3種を含むウミウチワ属の種を認識する形質として, 生殖器官の形成場所が毛線とどのような位置関係にあるかが重要であることを明らかにした.

(東京海洋大学大学院海洋科学技術研究科)