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Taxonomic studies on *Ulva pertusa* (Ulvophyceae). I. Morphological study

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Ulva pertusa Kjellman (Ulvales, Ulvophyceae) is abundant at Ebisujima (=Ebisu Island), Shimoda, Shizuoka Prefecture, and at this locality plants with a distinct stalked appearance, but otherwise similar to *U. pertusa*, occur with a limited distribution on the southern side of the island. The gross morphology and the distribution of rhizoidal cells between cell layers differ at extremes, but intermediate forms exist and neither of these characters proved to be taxonomically useful. In addition, test crosses showed that there was no reproductive isolation among any combinations of *U. pertusa* and "stalked-*Ulva*". It is concluded that *U. pertusa* is morphologically variable species and forms with a petiolate morphology are included within this taxon.

Key Index Words: *crossing experiment, morphological variation, stalked*-Ulva, *taxonomy*, Ulva pertusa.

Ulva species are common components of the marine intertidal flora of Japan and at least 10 species are recognized in Japan (Yoshida *et al.* 1990). Among them, *U. pertusa* is abundant throughout the year and widely distributed. The gross morphology of the thallus can be highly variable, making specific assignment of individual specimens difficult.

Blades of *Ulva pertusa* found at Ebisujima, Shimoda, Izu Peninsula, are light to dark green, irregularly orbicular or broad, and often perforated. The blades are flat and sheet-like with a short stipe and small discoid holdfast.

They are seasonally abundant from early spring through summer. However, some of the plants have narrow, extended stipes, which branch one to several times and are sometimes twisted; the terminal blades are fan-shaped. Such plants are referred to here as "stalked-*Ulva*".

Plants with this morphology have been placed in the genus *Letterstedtia* (Areschoug 1851). He distinguished *Letterstedtia* from *Ulva* on the basis of the gross morphology and the existence of the so-called hyphae forming a medulla-like layer in the stipe, similar to the rhizoidal cells at the base of the thallus in *Ulva*. Papenfuss (1960) considered that the gross morphology was too variable for such a distinction to be valid and further noted that the "hyphae" could also be seen in some *Ulva* species. He therefore transferred the 3 species formerly ascribed to *Letterstedtia* to *Ulva*.

The aim of the present study was to compare the morphology of "stalked-*Ulva*" with typical *U. pertusa* and to clarify their relationships. Test crosses were also performed to investigate potential reproductive isolation between the two morphological entities.

Materials and Methods

Several plants were intermittently collected from each of 8 sites at Ebisujima (Fig. 1) from 1989 to 1992 in order to observe their morphology. The size of vegetative and reproductive cells of *Ulva pertusa* and "stalked-*Ulva*"

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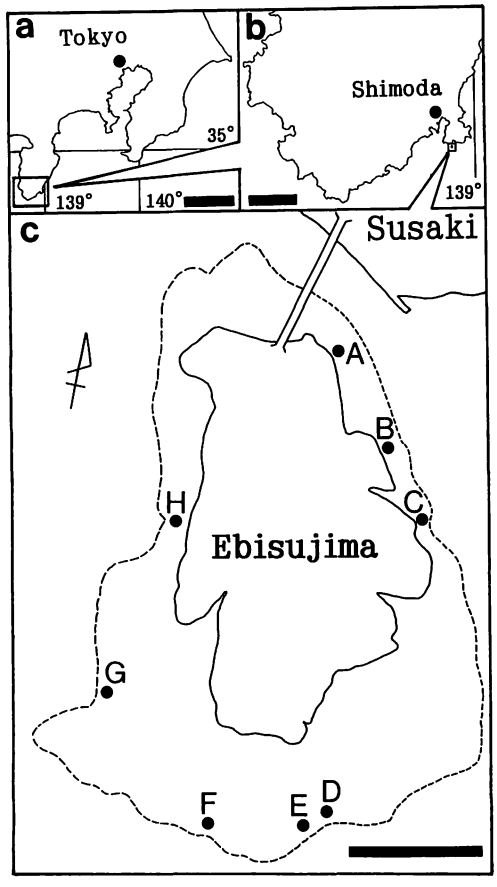

Fig. 1. The location of Ebisujima (a; scale bar=50 km, b; scale bar=5 km) and the sampling sites (c; scale bar=50 m). A broken line indicates 0 meter sea level.

were measured in the specimens collected in Ebisujima on August 7, 1992 and October 21, 1992. Ten plants were randomly collected from each site for measurement of thallus thickness during April, May, July, November and December, 1989. The thickness of thallus was measured in the center of the expanded thallus, using freezing microtome sections and light microscopy.

To test for reproductive isolation several fertile thalli of *Ulva pertusa* from site B and of "stalked-*Ulva*" from site D were collected (see Fig. 1c), and placed in filtered sea water in the laboratory. A pair of male and female gametes of "stalked-*Ulva*" was selected by preliminary crosses between biflagellate gametes released from each fertile thallus, because it was difficult to distinguish definitely male and female gametes from their size and color. Five individuals of *U. pertusa* and "stalked-*Ulva*" were examined to cross with each mating type of "stalked-*Ulva*" on sterilized glass slides. When positive crossing was judged by observing aggregation and conjugation, the zygotes were isolated by using their negative photoaccumulation and cultured in small plastic vessels containing PES medium (Starr and Zeikus 1993).

Results

Morphology

The following morphological characters of *Ulva pertusa* and "stalked-*Ulva*" were considered to have taxonomic significance and recorded: (1) gross morphology, (2) shape and size of cells in surface and sectional view and the number of pyrenoid in a vegetative cell, (3) thallus thickness, (4) the internal structure of the stipe and the basal region, (5) shape and size of reproductive cells.

Gross morphology. "Stalked-*Ulva*" specimens were typically medium green, perforated, 3-15 cm in height and with long and narrow stipes (5-25 mm long and 0.5-3.5 mm wide) branching one to several times (Fig. 2f). Plants with a morphology intermediate between *U. pertusa* and "stalked-*Ulva*" were collected at the south of the island where both forms existed together (Fig. 1c, sites D, E and F). Figure 2 shows a range of plants of the intermediate type. The thalli of these plants were similar in height to those of "stalked-*Ulva*". The stipes of the plants were relatively wide and scarcely differentiated from upper blades. The expanded fronds of the plants were partially interconnected.

Shape and size of cells in surface and sectional view and the number of pyrenoid in a vegetative cell. Cells in middle and apical regions in surface view were irregularly polygonal, with 3-5 corners that were a little rounded. These cells were arranged in indistinct groups, or formed short straight to curved rows with diverse orientations. Ordinary cells in the basal regions were relatively round, arranged in short curved rows. The abundant dark

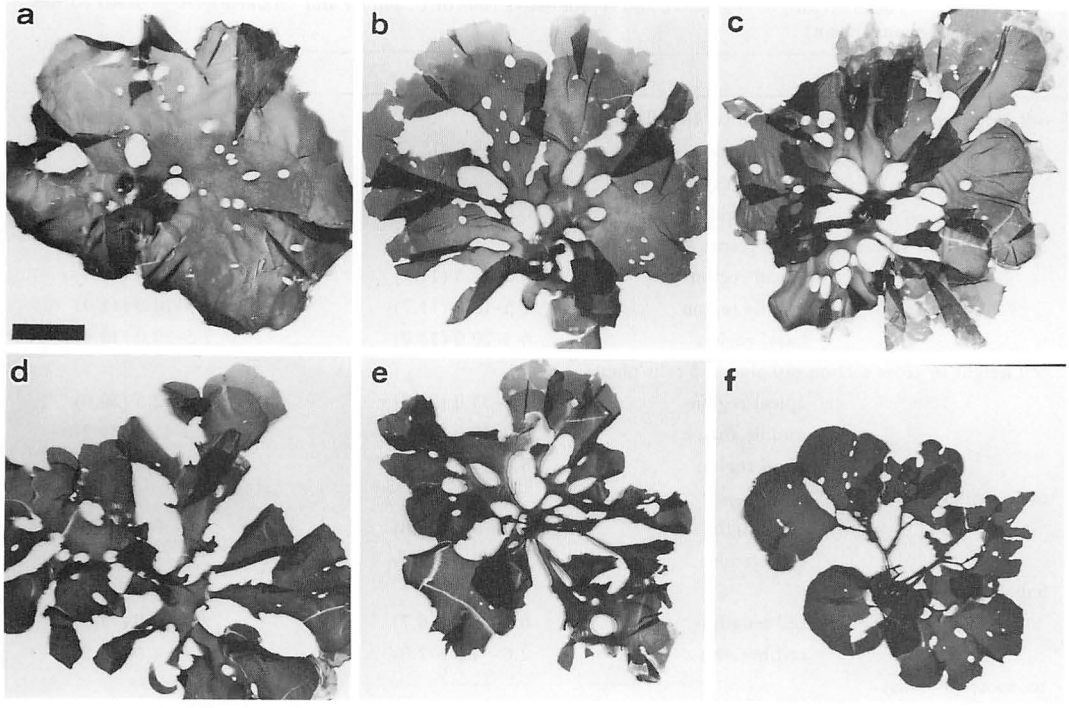

Fig. 2. Typical and intermediate morphologies of *U. pertusa* and "stalked-*Ulva*" with various forms from sites D-F in Fig. 1. Scale bar (2 cm) in Fig. 2a applies to all the figures. a. Typical *U. pertusa*. b–e. Intermediate form between *U. pertusa* and "stalked-*Ulva*". f. Typical "stalked-*Ulva*".

colored or colorless rhizoidal cells were slightly larger than the ordinary cells. In cross-section, cells were subrectangular to cylindrical in shape with rounded corners. The number of pyrenoids was mostly 1 or 2 in a vegetative cell and rarely more than 3. These characteristics of "stalked-*Ulva*" were quite similar to those of *U. pertusa*, and the cell size in the middle region showed no significant difference ($P>0.5$) between them (Table 1).

Thallus thickness. The thallus thickness in the middle region of blades was compared throughout the year (Table 2), but there was no significant difference between specimens assigned to *Ulva pertusa* and "stalked-*Ulva*". The only significant difference ($P<0.01$) among specimens from different sites was that the plants at site H were thinner than elsewhere.

The internal structure of the stipe and the basal region. Algae in the genus *Ulva* are composed of two layers of the cells, with numerous cells having rhizoidal extensions between the cell layers in the basal region that are directed towards the base of the plant. Figure 3 shows the distribution of rhizoidal cells of *U. pertusa* (a) and "stalked-*Ulva*" (b). In typical *U. pertusa* several chlorophyllous rhizoidal cells (Fig. 4a-c) appeared at the position 1 in Fig. 3a, and the abundance of rhizoidal cells increased toward the holdfast (position 2 in Fig. 3a, Fig. 4d-f). Numerous colorless rhizoidal cells (Fig. 4g-i) were seen only near the holdfast (position 3 in Fig. 3a). In "stalked-*Ulva*" several chlorophyllous rhizoidal cells (Fig. 5a-c) appeared in the thallus near the stipe (position 1 in Fig. 3b). Many rhizoidal cells (Fig. 5d-i) were seen throughout the stipes (position 2 and 3 in Fig. 3b). Rhizoidal cells often occurred in narrowed parts of the upper thallus of the "stalked-*Ulva*" as indicated in Fig. 3b. The distribution of the rhizoidal cells of typical "stalked-*Ulva*" differed from typical *U. pertusa*. However, this character could not be used to separate clearly "stalked-*Ulva*" from

Table 1. The size (μm) of vegetative and reproductive cells of *U. pertusa* and "stalked-*Ulva*". Each datum indicates range and (mean).

		U. pertusa	stalked-*Ulva*
cell length in surface view (20 plants, 10 cells/plant)			
	apical region	8.8–23.8 (15.6)	10.0–20.0 (14.2)
	middle region	10.0–22.5 (15.9)	10.0–22.5 (15.7)
	basal region	10.0–27.5 (18.3)	12.5–27.5 (19.4)
cell breadth in surface view (20 plants, 10 cells/plant)			
	apical region	6.3–16.3 (11.1)	6.3–15.0 (10.4)
	middle region	7.5–18.8 (11.7)	7.5–18.8 (11.4)
	basal region	6.3–20.0 (13.0)	7.5–20.0 (12.8)
cell height in cross section (20 plants, 5 cells/plant)			
	apical region	22.5–35.0 (27.3)	22.5–42.5 (30.0)
	middle region	25.0–45.0 (33.9)	27.5–45.0 (34.2)
	basal region	18.8–47.5 (31.2)	17.5–47.5 (27.7)
male gamete (50 cells)			
	cell length	6.0– 8.0 (6.6)	6.0– 8.0 (7.0)
	cell breadth	2.0– 3.0 (2.4)	2.0– 3.7 (3.1)
female gamete (50 cells)			
	cell length	6.0– 8.2 (6.7)	7.0– 9.0 (7.5)
	cell breadth	2.0– 4.0 (2.9)	2.3– 4.0 (3.2)
zoospore (50 cells)			
	cell length	6.8–10.8 (9.4)	7.5–12.0 (9.6)
	cell breadth	3.3– 6.8 (4.8)	4.0– 7.0 (5.5)

U. pertusa because specimens were often observed with intermediate morphologies.

Shape and size of reproductive cells. In all seasons, most plants in the collected materials of both *Ulva pertusa* and "stalked-*Ulva*" were gametophytes, which produced slightly narrower swarmers of male gamete or slightly broader swarmers of female. These differences could be found by careful observations after crossing tests. Sporophytes produced larger quadriflagellated zoospores (Table 1).

Reproductive isolation

Different mating types of "stalked-*Ulva*" (D1 and D2 in Table 3) confirmed by preliminary crosses were selected. This pair was used for test crosses with gametes from each of 5 individuals of *U. pertusa* and "stalked-*Ulva*", including D1 and D2. Almost all gametic swarmers crossed between different mating types showed aggregation and conjuga-

Table 2. Mean thallus thickness (μm) in the middle region of blade and standard deviation of 10 plants from each site. Bars indicate that plants were missed the chance to be collected from the sites.

Sites	April 5	May 5	July 1	November 11	December 26
A	87.08± 9.38	90.30±11.5	102.9 ±11.4	81.15± 7.57	68.95± 5.80
B	70.01± 6.28	75.10± 9.20	—	85.15±11.6	94.06±11.4
C	93.20±23.0	61.45±11.3	100.5 ±10.1	81.10±17.1	104.5 ±12.2
D	61.88± 4.35	72.35± 3.93	70.30±10.5	83.30± 9.35	84.45±14.0
E	64.40± 5.40	81.28± 5.04	67.15±12.7	80.90±11.6	88.95± 1.20
F	79.45± 9.50	61.38±10.4	57.25± 4.40	77.25± 7.70	91.50± 7.47
G	78.23±12.9	71.30± 6.18	—	78.65± 4.18	84.05±20.2
H	62.68±16.7	—	47.90± 7.80	63.30± 8.48	62.20±15.7

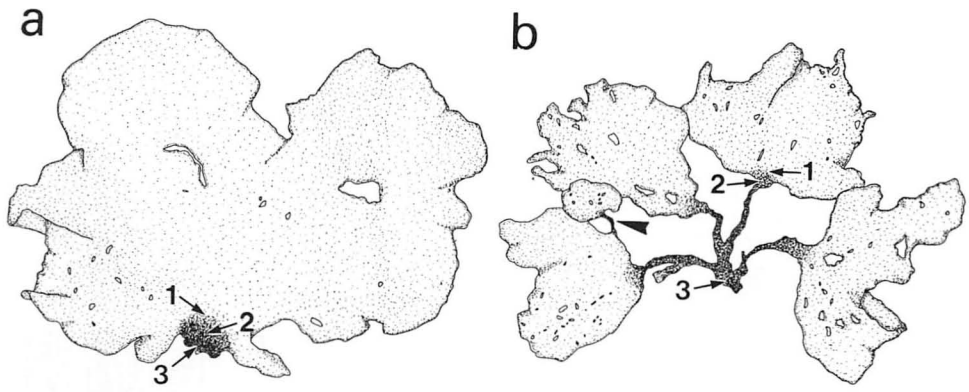

Fig. 3. The distribution of rhizoidal cells of *U. pertusa* (a) and "stalked-*Ulva* (b) is indicated by higher dense dots. Rhizoidal cells are also seen in a narrowed part of the upper thallus (arrowhead).

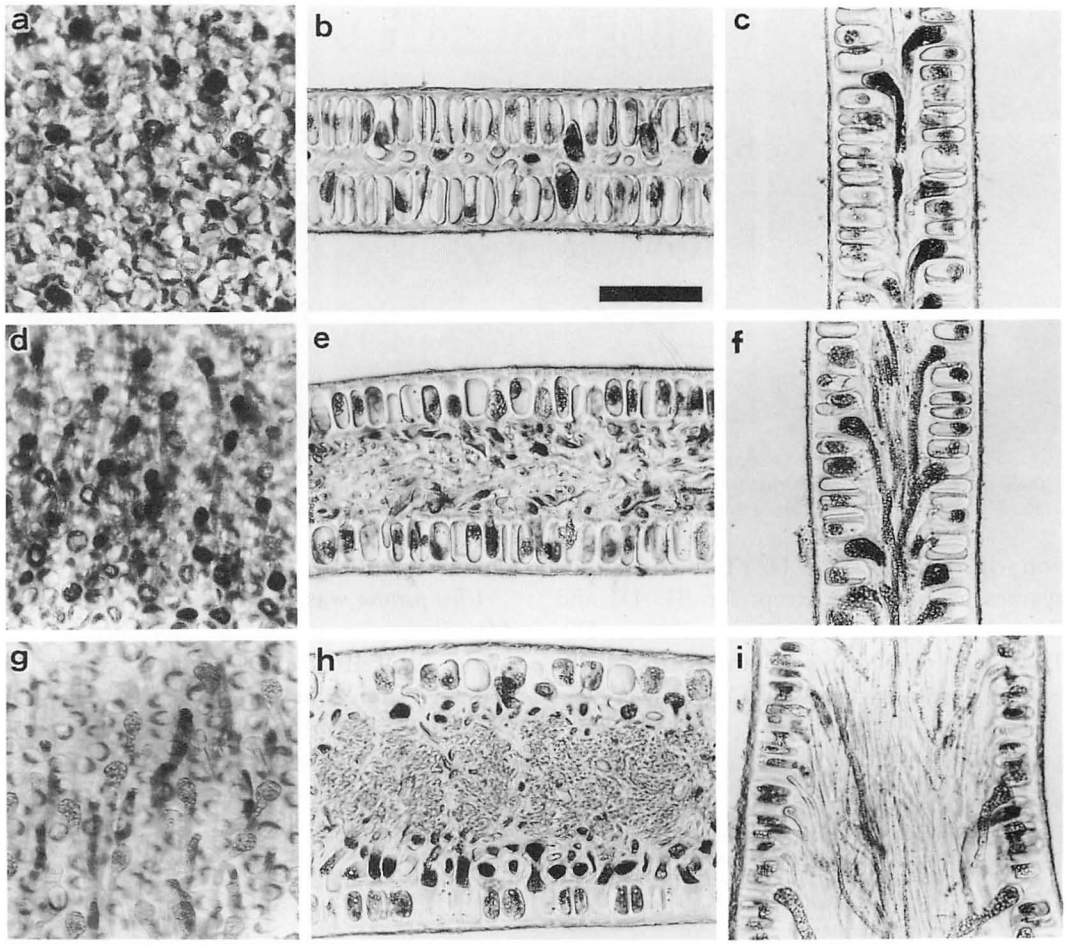

Fig. 4. Surface view (a, d, g), transversal section (b, e, h) and longitudinal section (c, f, i) of vegetative thalli of *U. pertusa* with rhizoidal cells that are directed towards the base of the plant. a–c, position 1 in Fig. 3a; d–f, position 2 in Fig. 3a; g–i, position 3 in Fig. 3a. Scale bar (50 μm) in Fig. 4b applies to all the figures.

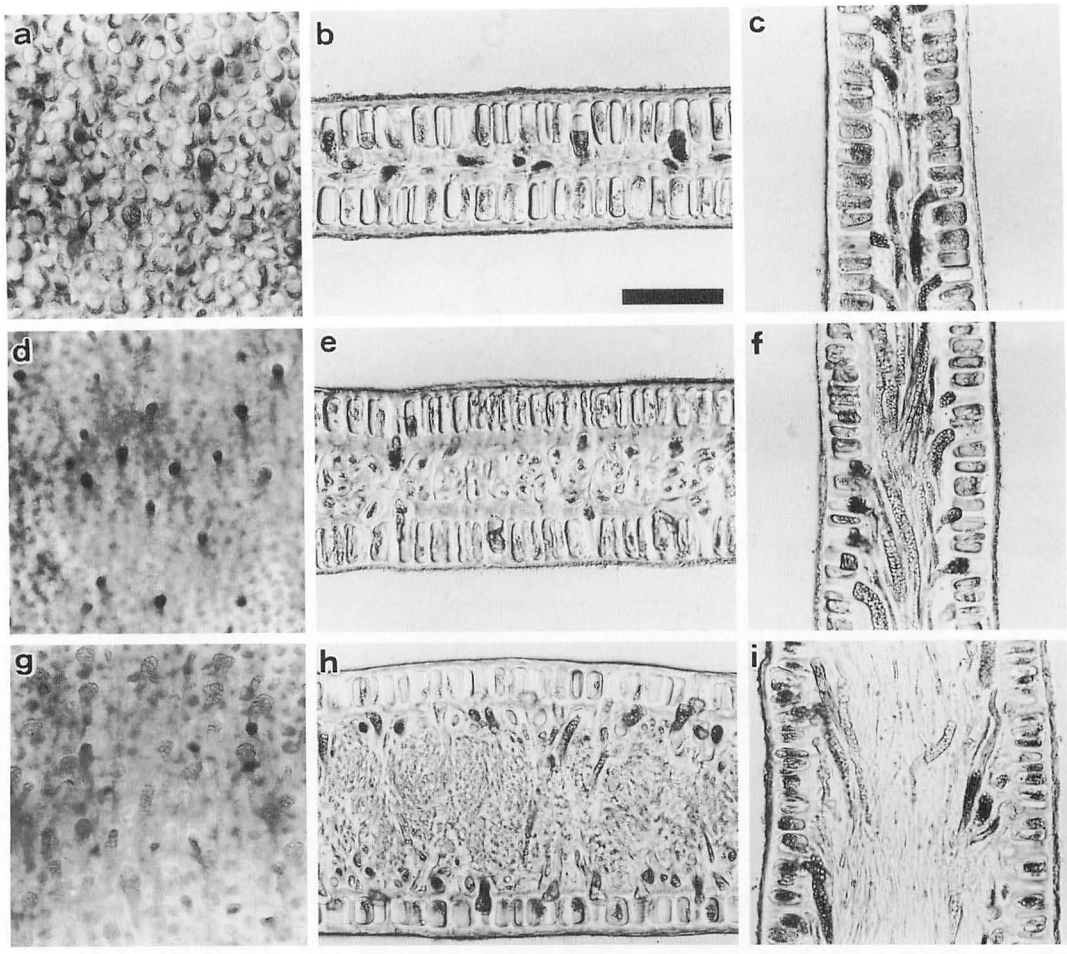

Fig. 5. Surface view (a, d, g), transversal section (b, e, h) and longitudinal section (c, f, i) of vegetative thalli of "stalked-*Ulva*" with rhizoidal cells that are directed towards tha base of the plant. a–c, position 1 in Fig. 3b; d–f, position 2 in Fig. 3b; g–i, position 3 in Fig. 3b. Scale bar (50 μm) in Fig. 5b applies to all the figures.

tion with those of D1 or D2 (Table 3) and the zygotes germinated, except for B4, D3 and D4, which crossed with neither D1 nor D2. The germlings are developing to the thalli, however, releasing zoospores have never been observed.

Table 3. The result of test crosses between *U. pertusa* and "stalked-*Ulva*". Each number designates one plant, and B and D indicate *U. pertusa* from site B and "stalked-*Ulva*" from site D, respectively. + shows that gametes crossed and the zygotes germinated, and − shows not crossed.

	B1	B2	B3	B4	B5	D1	D2	D3	D4	D5
D1	+	+	+	−	−	−	+	−	−	−
D2	−	−	−	−	+	+	−	−	−	+

Habitat

Ulva pertusa was widely distributed around Ebisujima except on the northern shore, while "stalked-*Ulva*" was restricted to the southern shore which consists of a broad flat rock platform with strong wave action. *U. pertusa* grew inshore of "stalked-*Ulva*" at the southern shore, but there was no clear boundary between the populations.

Discussion

Ulva pertusa was described by Kjellman (1897) on the basis of specimens from Japan. According to the description, thalli are 15–20 cm high, perforated by many roundish or

irregularly shaped foramina, at least 125 μm thick in the lower part and about 40 μm thick in the marginal region. Kjellman's description was augmented with habit photos of five specimens and illustrations of thallus structure. He cited Hakodate, Enoshima and Yokohama as their localities, with a comment that the species seemed to be widely distributed. In the present study, the *Ulva* species growing abundantly at Ebisujima had thalli up to 17 cm high, and a thickness of middle and basal regions of 40-130 μm and 80-400 μm, respectively. These plants agree well with the description and illustrations of *U. pertusa* given by Kjellman. Incidentally, Shimoda is near the TYPE localities of Enoshima and Yokohama.

When typical *Ulva pertusa* and "stalked-*Ulva*" are compared, their gross morphology is quite distinct. However, the existence of plants with morphology intermediate between *U. pertusa* and "stalked-*Ulva*" makes it difficult to distinguish them. Similar difficulties are reported in distinguishing Australian plants of *U. rigida* C. Agardh, which is a most common species in southern Australia (Phillips 1988). *U. spatulata* Papenfuss (previously referred to *Letterstedtia*) has long stipes like those of "stalked-*Ulva*"; and *U. australis* Areschoug shows transitional stages of the gross morphology between *U. spatulata* and *U. rigida*. Phillips (1988) regarded *U. australis* and *U. spatulata* as synonyms of *U. rigida* because the two taxa were identical to *U. rigida* in diagnostic characters and developmental patterns.

Rhizoidal cells in "stalked-*Ulva*" were more highly developed than those in *U. pertusa*, but the distinction of rhizoidal cell development was not clear. The rhizoidal cells were found near the base of the plants as well as in narrow parts of the upper thallus (Fig. 3b). So the development of rhizoidal cells may simply relate to the width of the thallus.

The process of the formation of the stipes has not been observed, but it is possible to infer the process by comparing plants with intermediate morphologies between *Ulva pertusa* and "stalked-*Ulva*". The holes of the thalli become larger and join with one another near the holdfast (Fig. 2b, c). The thalli divide into several main portions by the further enlargement of the perforations (Fig. 2d, e) and the upper expanded thalli are then attached by narrow stipes (Fig. 2f). This process may occur throughout the thallus so that "stipes" can be observed in upper thallus parts (Fig. 3b).

Some workers (Bliding 1968; Koeman and van den Hoek 1981; Hoeksema and van den Hoek 1983) considered pyrenoid number, thallus thickness, and size and arrangement of vegetative cells to be useful taxonomic characters, whereas others (Titlyanov *et al.* 1975; Steffensen 1976; Phillips 1988) reported that, in some *Ulva* species, thallus thickness, cell size and pyrenoid number were too variable for taxonomic use. In this study the cell size of middle and apical region, measured in surface view and cross-section, exhibited considerable variations and overlapped between *U. pertusa* and "stalked-*Ulva*" (Table 1). It was obvious that there was no critical difference between them in the shape and arrangement of their vegetative cells and the pyrenoid number in a cell. The thallus thickness was also highly variable in the individual plants and there was no significant difference between those of *U. pertusa* and "stalked-*Ulva*". It may be attributed to thallus size, season and/or habitat in *U. pertusa*.

The crossing of gametes between *Ulva pertusa* and "stalked-*Ulva*" and germination of the zygotes were observed, but the progeny development has not been observed yet. Now zygotes, gametes and zoospores of "stalked-*Ulva*" and zygotes between *U. pertusa* and "stalked-*Ulva*" are continued to culture, so it will become clarified in the near future whether the gross morphology of "stalked-*Ulva*" is genetically stable or not.

Gametes of several plants (B4, D3 and D4 in Table 3) crossed with neither D1 nor D2, but it is not clear whether the results were caused by the absence of their crossing ability or some difference of the experimental conditions.

Plants of the stalked form were also seen with *Ulva pertusa* on relatively flat rock plat-

forms with strong wave action in Arashima, Miyagi Prefecture, Tanesashi, Aomori Pref. and Ooarai, Ibaraki Pref. The formative process of the morphology of "stalked-*Ulva*" has not been observed, but it may be related to environmental factors. However, the possibility remains that genetic differences exist between the ecological forms. Consequently, a molecular taxonomic analysis using isozymes was initiated. Results of this investigation will be reported separately.

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神谷充伸*・土井考爾*・原　慶明*・千原光雄**：アナアオサ（アオサ藻綱）の分類学的研究．I．形態学的研究

恵比須島（静岡県下田市）の周囲の潮間帯にはアナアオサが繁茂するが，この島の南側のアナアオサの集団に，柄を有するアオサ（エツキアオサと仮称）がみられる。本研究では，形態学的研究および交配実験を行い，両藻の分類学的な関係を検討した。エツキアオサは外部形態や基部付近の仮根細胞の分布においてアナアオサと明らかに異なるが，南側の集団の個体を詳細に調査すると，両者の中間の形態を示す個体も存在し，形態的特徴からは両者を区別できないことが判明した。交配実験ではアナアオサとエツキアオサの配偶子は交雑し，その接合子は正常に発芽することが確認された。これらの結果から，アナアオサは形態的に変異に富んだ種であり，エツキアオサはこの分類群に含まれると考えられる。（*305 つくば市天王台1-1-1　筑波大学生物科学系，**150 渋谷区広尾4-1-3　日本赤十字看護大学）

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