Jpn. J. Phycol. (Sôrui) **41**: 303–313, December 10, 1993

Taxonomic notes on the Halymeniaceae (Rhodophyta) from Japan. II. *Halymenia rotunda* Okamura.

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Kawaguchi, S. 1993. Taxonomic notes on the Halymeniaceae (Rhodophyta) from Japan. II. *Halymenia rotunda* Okamura. Jpn. J. Phycol. **41**: 303–313.

Morphological study of the type and recent collections of *Halymenia rotunda* has revealed that this alga has substantial stalks, firmly membranous in texture, periclinally aligned medullary filaments, containing highly refractive cells in the medulla and profusely branched auxiliary cell ampullae with a narrow neck. Distinctive anticlinal medullary filaments, a characteristic feature of *Halymenia*, are absent and the features agree with the current circumscription of *Cryptonemia*. Therefore the binomial ***Cryptonemia rotunda*** (Okamura) Kawaguchi, comb. nov. is proposed. Spore development of *C. rotunda* was studied in culture. Carpospores initially develop into branched uniseriate filaments, but the filaments soon coalesce laterally to form irregularly circular crusts without forming acrochaetioid plants.

Another poorly known *Halymenia* species described from the Seto Inland Sea, *H. iyoensis* Yagi, is compared and concluded to be synonymous with *C. rotunda*.

Key Index Words: Cryptonemia, Cryptonemia rotunda, Halymenia, *Halymeniaceae*, Halymenia iyoensis, Halymenia rotunda, *Rhodophyta, Spore development, Taxonomy.*

In 1930, Okamura described *Erythrymenia obovata* Schmitz (a species of the Rhodymeniaceae) from Enoshima on the central Pacific coast of Japan. On the basis of Okamura's (1930, pl. 266) illustrations, Kylin (1931, p. 31) suggested that the species belonged in the Grateloupiaceae (=Halymeniaceae) and was probably a species of *Halymenia*. Consequently, Okamura (1936, p. 536) described the species as *Halymenia rotunda* Okamura, but with the comment that further studies were needed to clarify its taxonomic status. Segawa (1938, p. 142–143) listed it as *Halymenia* (?) *rotunda* Okamura from Susaki, Shizuoka Pref. and remarked that it was only provisionally placed in *Halymenia* by Okamura. Segawa (1956, p. 76) again expressed his doubt about the generic position of the species.

In the course of my taxonomic studies on the Halymeniaceae from Japan, I have obtained many specimens of this alga from various localities. These specimens presented the opportunity for detailed study of their anatomy and spore development. In addition, consideration was given to the taxonomic relationship between *H. rotunda* and a poorly known Japanese species, *Halymenia iyoensis* Yagi (Yagi 1938), thought to be closely related to *H. rotunda*.

Materials and Methods

The plants studied were either fresh materials collected mainly by SCUBA diving, or dried herbarium specimens. Hand sections were made with a razor blade, mounted in glycerol-seawater mixture, and stained with 0.5% cotton blue solution (Kawaguchi and Masuda 1984), 1% aqueous toluidine blue or 1% aqueous light green. The microscope slides used in this study are help by the author.

Carpospores were obtained from the specimens collected from depths of 5–15 m at Takeno, Hyogo Prefecture on 1 August 1991. Liberated spores were inoculated into small Petri dishes containing full strength PES

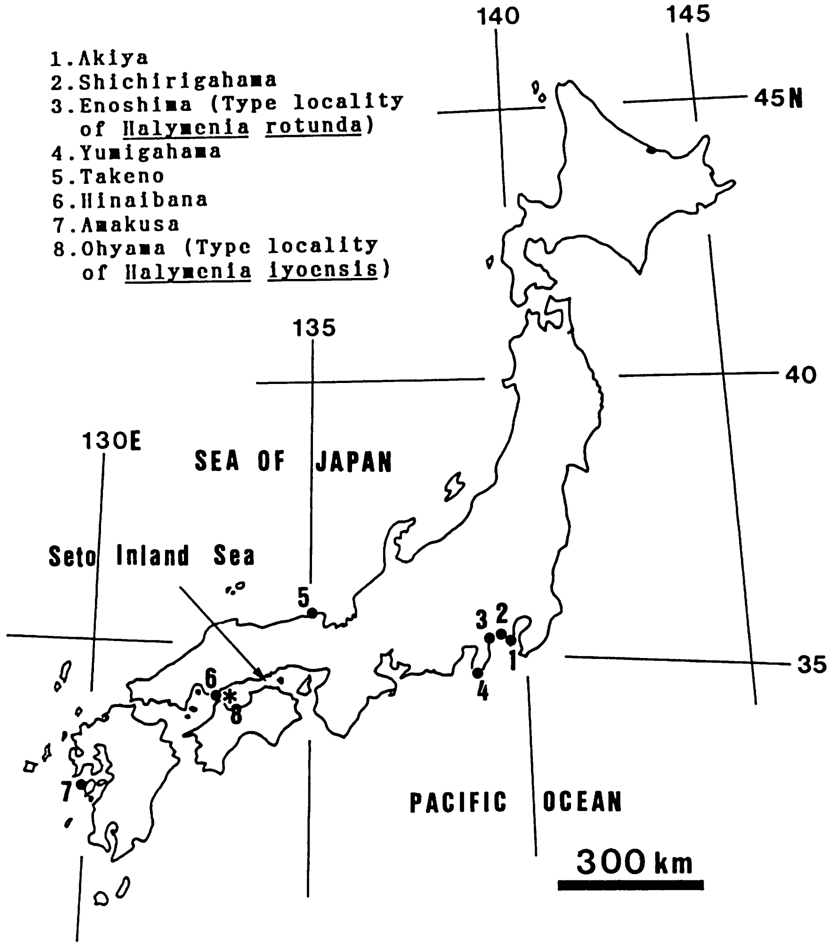

Fig. 1. Type locality and collection sites of *Halymenia rotunda* Okamura.

(Provasoli 1966). The Petri dishes were kept in the incubator under the condition of 18-20 °C and a 12 : 12 h LD (light and dark) photoregime illuminated by cool-white fluorescent lamps (30-40 μmol m^{-2} s^{-1}).

Specimens used for anatomical study: (1) sterile, cystocarpic, Akiya, Kanagawa Pref., 5. vii. 1990, leg. S. Arai, *Kawaguchi* 1027-36 (3-15 m deep). (2) sterile, tetrasporangial, Shichirigahama, Kanagawa Pref., 23, 30. vi. 1983, leg. S. Kawaguchi, SAP 047539, 045544 (drift). (3) sterile, cystocarpic, Yumigahama, Shizuoka Pref., 27. vi. 1983, leg. S. Kawaguchi, SAP 047540-3 (drift). (5) cystocarpic, Takeno, Hyogo Pref., 1. viii. 1990, leg. S. Enomoto *et al.*, *Kawaguchi* 1039-41 (5-15 m deep). (6) cystocarpic, sterile, Hinaibana (Ohshima island), Ochi Province, Ehime Pref., 10. v. 1990, leg. S. Enomoto *et al.*, *Kawaguchi* 1042-1045 (3-10 m deep). (7) tetrasporangial, Amakusa, Kumamoto Pref., 1. vii. 1957, leg. T. Yoshida, *Kawaguchi* 0893 (drift) (Fig. 1).

Abbreviation of herbarium follows Holmgren *et al.* (1990).

Observations

Vegetative structure: One to three (occasionally more) stalks to 1 cm long arise from a holdfast (Fig. 2D). A single circular or ellipsoid (rarely irregularly shaped) blade up to 13 cm in diameter expands from the top of each stalk (Figs. 2A-C). The stalk does not extend into the blade as a midrib. In older plants, the stalk is very stiff (up to 2-3 mm in diameter),

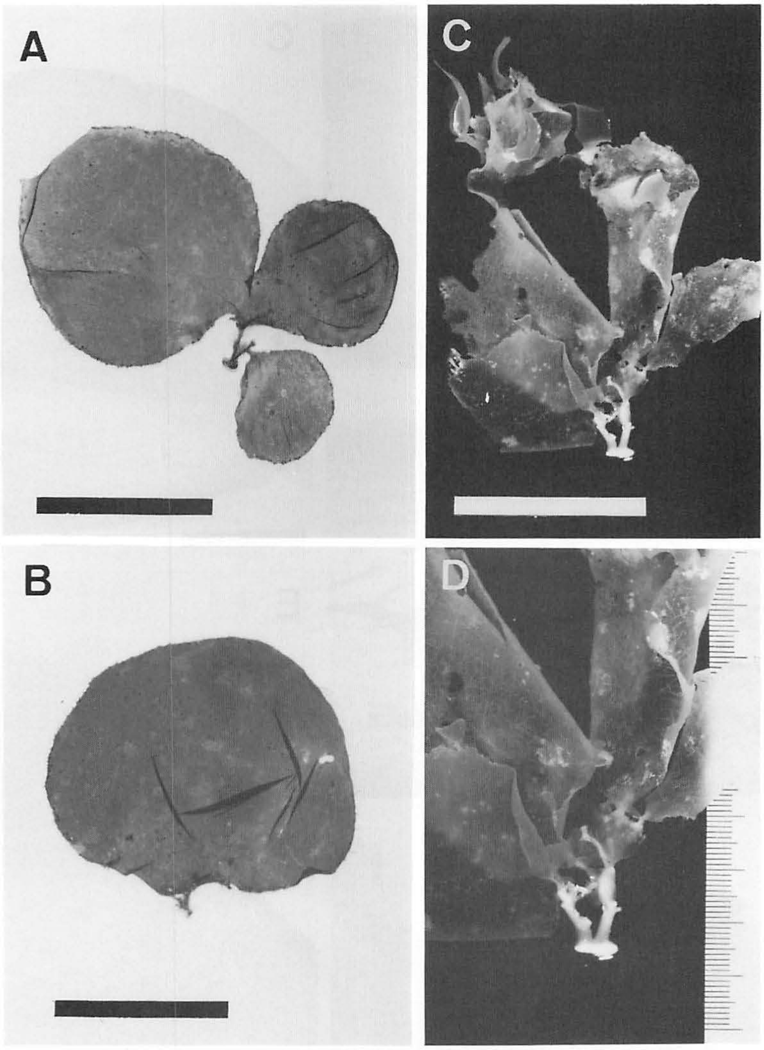

Fig. 2. *Halymenia rotunda* Okamura. A, B. Cystocarpic spceimens showing typical circular blades. Scale=5 cm. C. Cystocarpic specimen having irregularly shaped blades. Scale=5 cm. D. Magnification of Fig. 2C showing stout basal system.

branched (Fig. 2D), and has several growth rings in section (Fig. 3C). The blade margin is thickened and almost always beset with numerous, small protuberances that give the blade a toothed appearance (Figs. 3D, E). The texture is firmly membranous and plants scarcely adhere to paper on drying. The color is cerise to blood red, or purplish red.

Blades are up to 300 μm thick (to 500 μm or more at the margin), composed of cortical and medullary filaments (Fig. 3B). Cells of an outer cortex are ellipsoidal to rounded, arranged in anticlinal rows 3-4 cells deep. This layer connects to an inner cortex, 3-4 cells deep, of larger, irregularly shaped or stellate cells laterally attached by secondary pit connections. Medullary cells are generally elongate (rarely narrowly ellipsoidal) and form simple or branched filaments. These filaments are mainly periclinal in direction (Fig. 3B). Among these filaments are long-armed stellate cells containing a highly refractive substance which stains intensely with cotton blue or light green (Figs. 3A, B).

Reproductive structure: Female reproductive structures were scattered over the entire blade

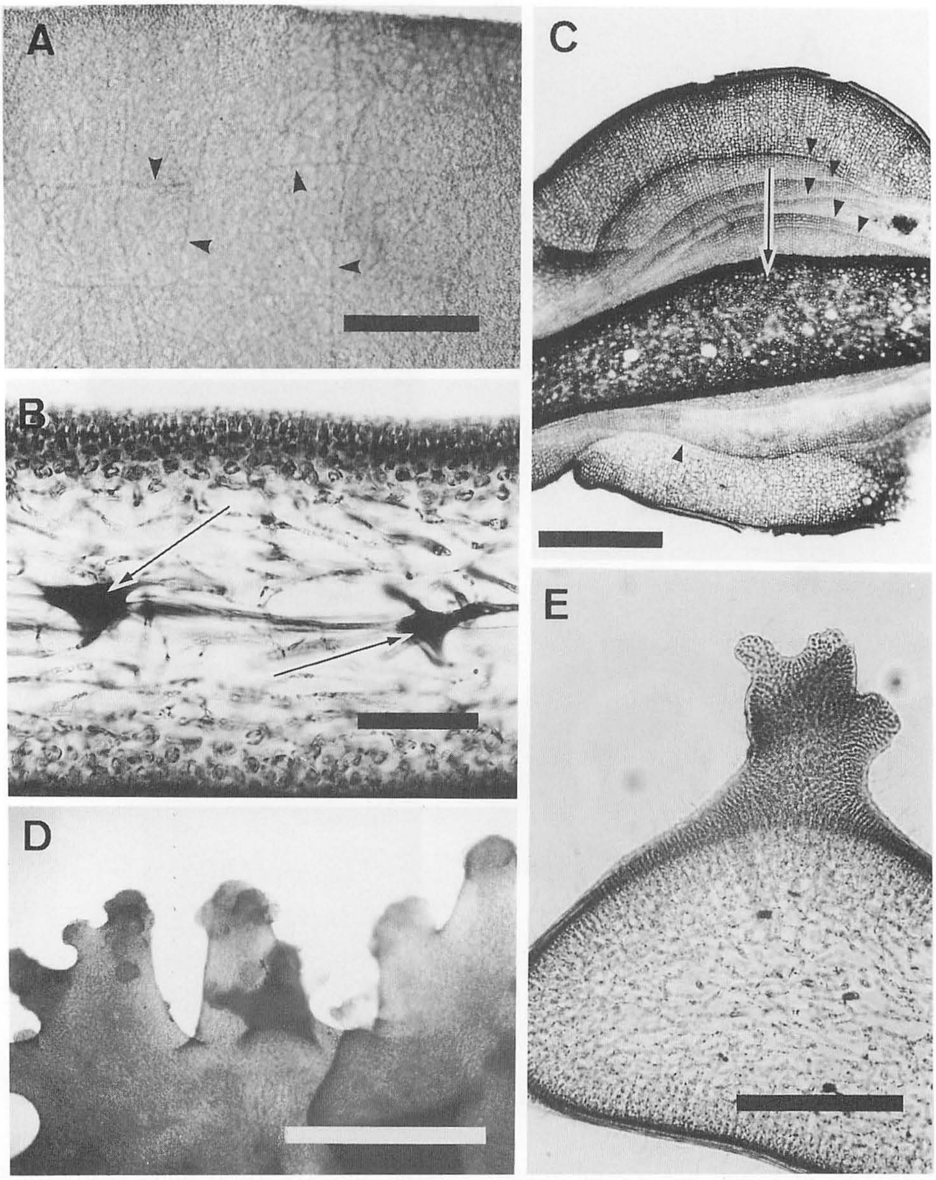

Fig. 3. *Halymenia rotunda* Okamura. A. Surface view of blade after staining with light green. Arrowhead shows highly refractive filament. Scale=250 μm. B. Transverse section of blade showing periclinal medullary filaments and highly refractive ganglionic cell (arrow). Scale=30 μm. C. Cross section of stipe. Note that several growth rings (arrowheads) are seen around the medulla (arrow). Scale=300 μm. D. Marginal portion of blade showing small protuberances. Scale=1 mm. E. Transverse section of thickened margin. Scale=200 μm.

except near the base. Carpogonial branches and auxiliary cells were formed in separate clusters of filaments (ampullae). Carpogonial ampullae were rare. The carpogonial branch in an ampulla consisted of a carpogonium that subtended a trichogyne, and a hypogynous cell (Figs. 4A, B, 5A). Auxiliary cell ampullae were profusely branched to the third or fourth order and were narrow at the top (Figs. 4C, D, 5B). The cells of primary filament became elongated when fully developed. Auxiliary cells were enlarged, oval to oblong in shape, and were located centrally toward the base of the ampulla (Figs. 4C, D,

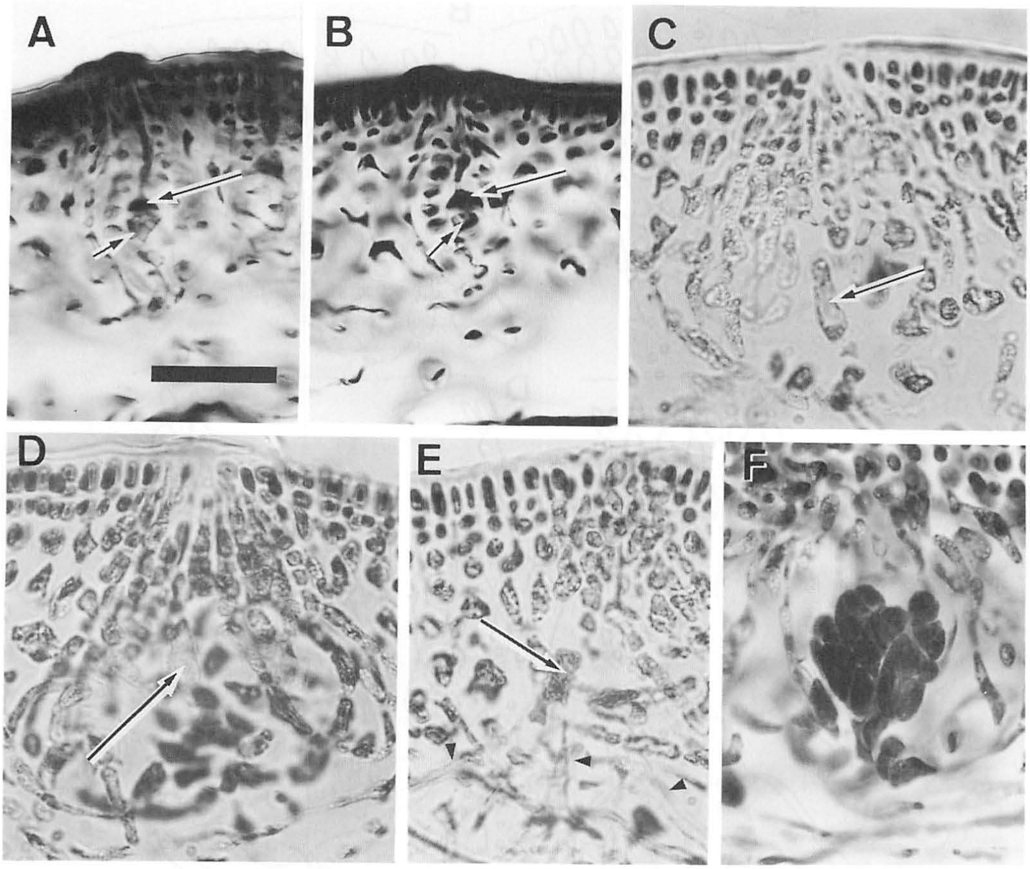

Fig. 4. *Halymenia rotunda* Okamura. A, B. Carpogonial ampulla. Long arrow shows carpogonium with trichogyne, and short arrow shows hypogynous cell. Scale=30 μm, applying also to B–F. C, D. Auxiliary cell ampulla. Arrow shows auxiliary cell. E. Initiation of connecting filaments (arrowheads) from irregularly shaped cell (arrow). F. Early stage of gonimoblast development.

5B).

Early stages of fertilization were not observed. Several aseptate filaments were produced from an irregularly shaped large cell or its derivative cells (Figs. 4E, 5C). These filaments are possibly connecting filaments, and the large cell is presumably a fertilized carpogonium or its fusion complex. The auxiliary cell cut off a gonimoblast initial toward the surface after presumed contact with a connecting filament (Figs. 4F, 5D). Whether the connecting filament grew on to affect further contacts or not was not ascertained. The gonimoblast initial in turn cuts off a few gonimoglast cells which devide to form a mass of carposporangia (Fig. 6B). During the development of gonimoblast cells, ampullary cells elongate to form an involucre (=pericarp). No supplementary vegetative cells seemed to be involved, and the pericarp around the carposporophyte was poorly developed (Fig. 6A). Fully developed cystocarps were 170–220 μm in diameter, deeply embedded within the blade, and ostiolate. Blades were usually swollen around the cystocarp (Fig. 6A).

Male reproductive structures were not observed.

Tetrasporangia were also scattered over the blade surface. Tetrasporangial initials were cut off from the third (rarely second) cortical cells from the surface. The tetrasporangial initials first elongate, then enlarge into narrowly ellipsoidal sporangia (Figs. 6C, D). Ma-

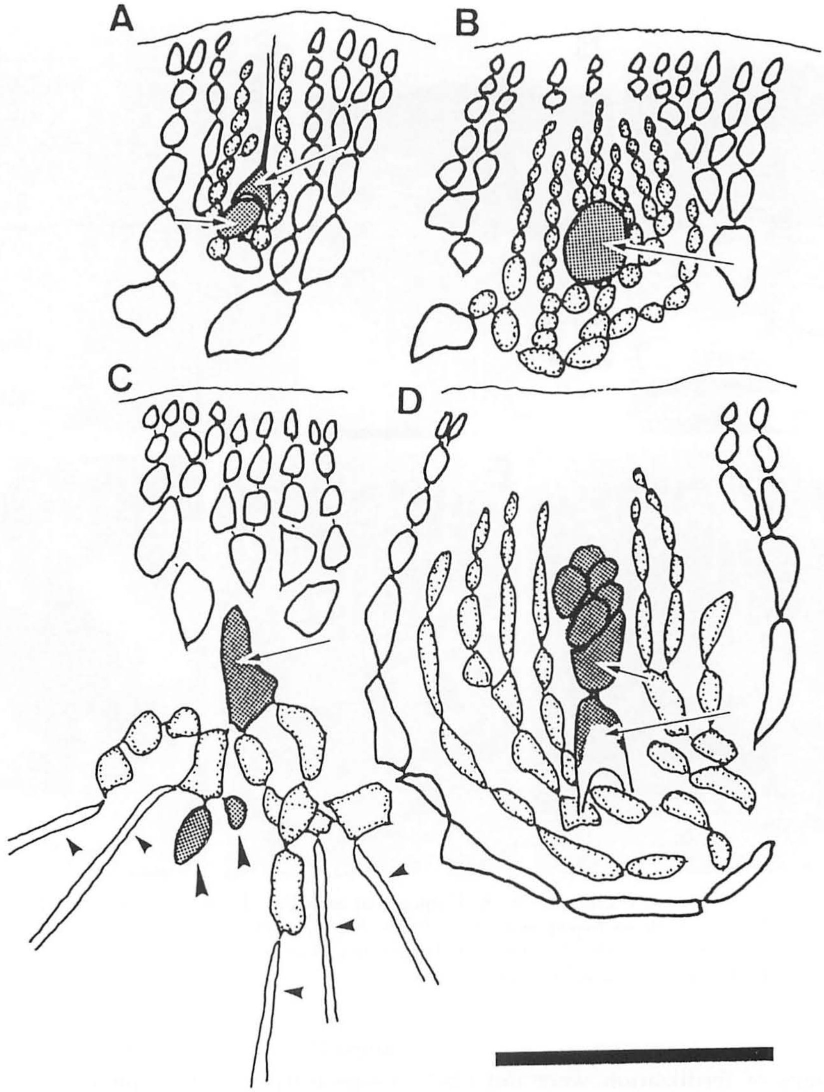

Fig. 5. *Halymenia rotunda* Okamura. A. Carpogonial ampulla. Long arrow shows carpogonium, and short arrow shows hypogynous cell. B. Profusely branched auxiliary cell ampulla. Arrow shows auxiliary cell. C. Initiation of connecting filaments (arrowheads) from cells derived from large irregularly shaped cell (arrow). Large arrowhead shows initiation of connecting filament. D. Early stage of gonimoblast development. Long arrow shows presumably fusion complex and short arrow shows gonimoblast initial. Scale=50 μm, applying to A–D.

ture sporangia were 7–10 μm wide by 20–25 μm long, submerged in the outer cortex, and cruciately or decussately divided (Fig. 6D).

Development of carpospores: Liberated carpospores are 8.3–13.3 μm (average 10.3 μm, N=75) in diameter (Fig. 7A). One or two days after settling, the spores produced a germ tube into which the spore content migrated (Fig. 7A). After further two or three days, the elongated tube developed into a 2–3 celled filament. The cells of the filament then produced lateral cells to form a branched uniseriate filament (Fig. 7B). As the sporeling developed further, branched filaments came in contact and coalesced with one another (Figs. 7C, D). After about 30 days, a sporeling had developed into an irregularly shaped crust with a marginal meristem (Fig. 7E). Erect axes grew from the center of the

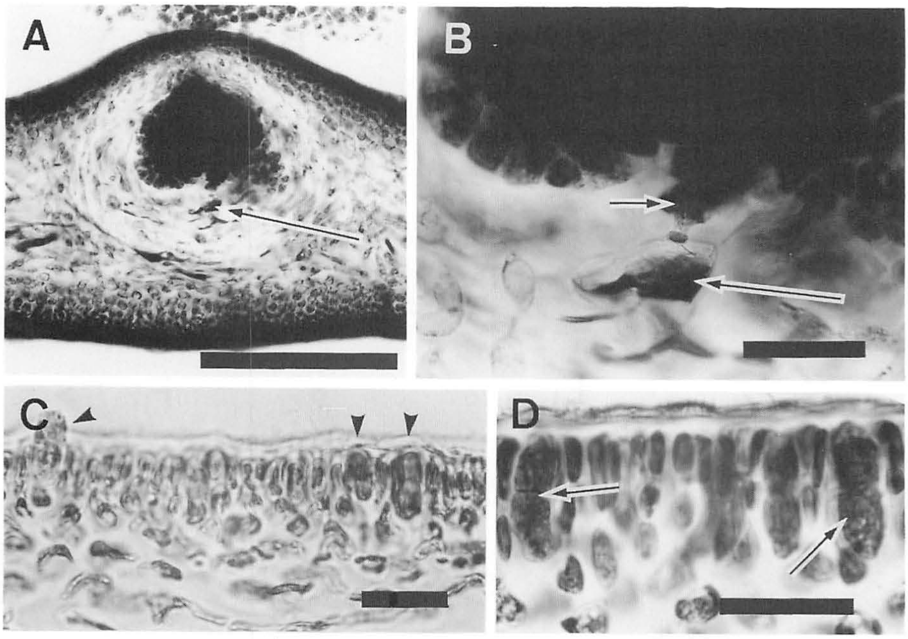

Fig. 6. *Halymenia rotunda* Okamura. A. Mature cystocarp. Note that thallus is swollen. Arrow shows fusion complex. Scale=100 μm. B. Magnification of Fig. 6A showing fusion complex (long arrow) and gonimoblast initial cell (short arrow). Scale=30 μm. C, D. Tetrasporangial formation. Arrowhead in C and arrow in D show tetrasporangium. Scale in C=30 μm and scale in D=30 μm.

crust after about two months (Fig. 7F).

Discussion

Taxonomic position of Halymenia rotunda *Okamura*

The genus *Halymenia* is characterized as having distinctive, anticlinally directed medullary filaments, a slippery texture and highly refractive cells in the medulla (Abbott 1967, Kraft 1977, Maggs and Guiry 1982). Of these the first, which is considered the most diagnostic of the genus, is lacking in *H. rotunda*. The genus *Cryptonemia* is characterised by its conspicuous stalks, occasionally midribbed blades, mostly periclinally aligned medullary filaments and highly refractive medullary cells (Abbott 1967, Scott *et al.* 1982). Gross morphological and vegetative features of *H. rotunda*, which include substantial stalks, periclinally aligned medullary filaments, highly refractive stellate cells in the medulla and firmly membraneous texture, are therefore more in accord with the current circumscription of *Cryptonemia*, rather than *Halymenia*.

In addition, the present alga has profusely branched auxiliary cell ampullae which converge to a narrow neck. This type of auxiliary cell ampullae is considered by Chiang (1970) to be typical of *Cryptonemia* and contrasts with those of *Halymenia* which are mostly wide across the top. Although Gargiulo *et al.* (1986) reported that *Halymenia assymetrica* from the Mediterranean Sea has narrow-necked auxiliary cell ampullae, *H. rotunda* is more typical of *Cryptonemia* in this aspect of morphology.

There therefore seems to be no justification for keeping this alga in the genus *Halymenia*. Vetetative and reproductive features suggest that it should be placed in the genus *Cryptonemia* as ***C. rotunda*** (Okamura) Kawaguchi comb. nov.

Spore germination pattern

To my knowledge, no information on the spore development of *Cryptonemia* species has been published. Carpospores of *C. rotunda* germinate by initially evacuating the spore

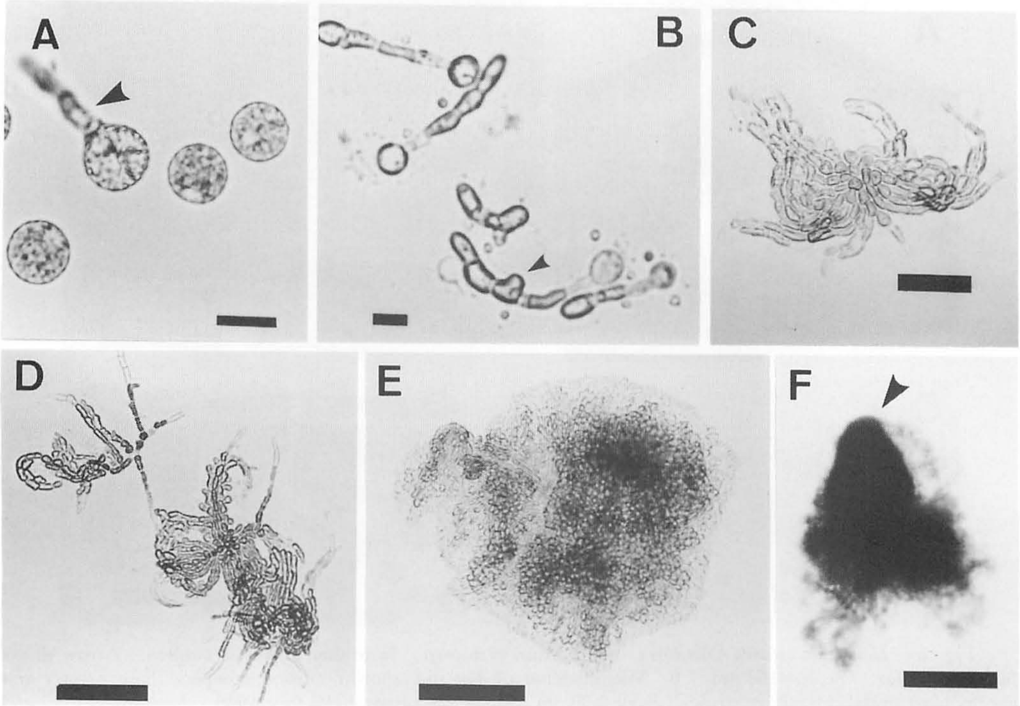

Fig. 7. *Halymenia rotunda* Okamura. A. Liberated carpospores and initiation of germ tube 1 day after liberation. Scale = 10 μm. B. 7-day sporeling developing into two- to five-celled filament with lateral protrusion (arrowhead). Scale = 10 μm. C. 20-day sporeling forming crust by lateral adhesion of filaments. Scale = 40 μm. D. 24-day sporeling transforming into disc. Scale = 80 μm. E. 1-month sporeling. Note that it has been transformed into irregularly shaped disc with marginal meristem. Scale = 100 μm. F. Initiation of erect thallus (arrowhead) from disc in 2 months. Scale = 200 μm.

content into a germ tube as commonly reported for other members of the family (Berthold 1884, Chemin 1937, Inoh 1947, Hayashida and Chihara 1967, Codomier 1974, Maggs and Guiry 1982). After developing into a 2–3 celled filament, the sporeling produced lateral

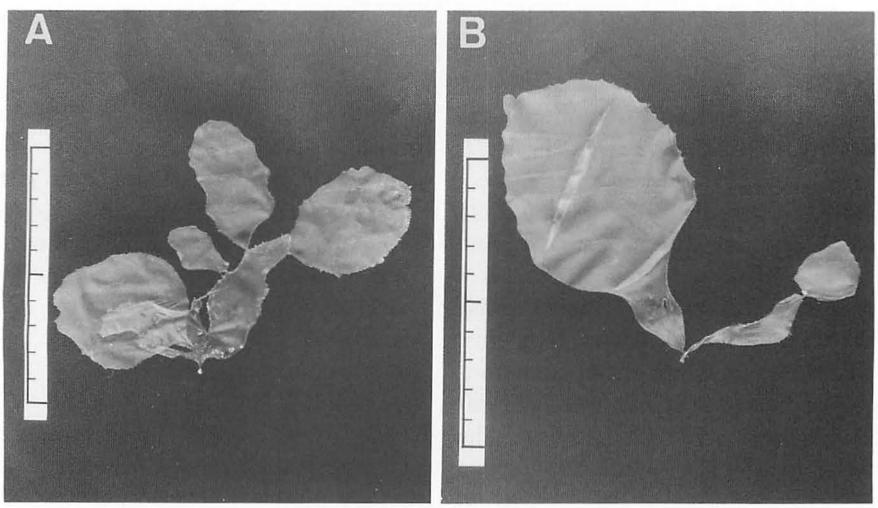

Fig. 8. *Halymenia rotunda* Okamura. A, B. Cystocarpic specimens having gourd-like blade (Ohshima collections).

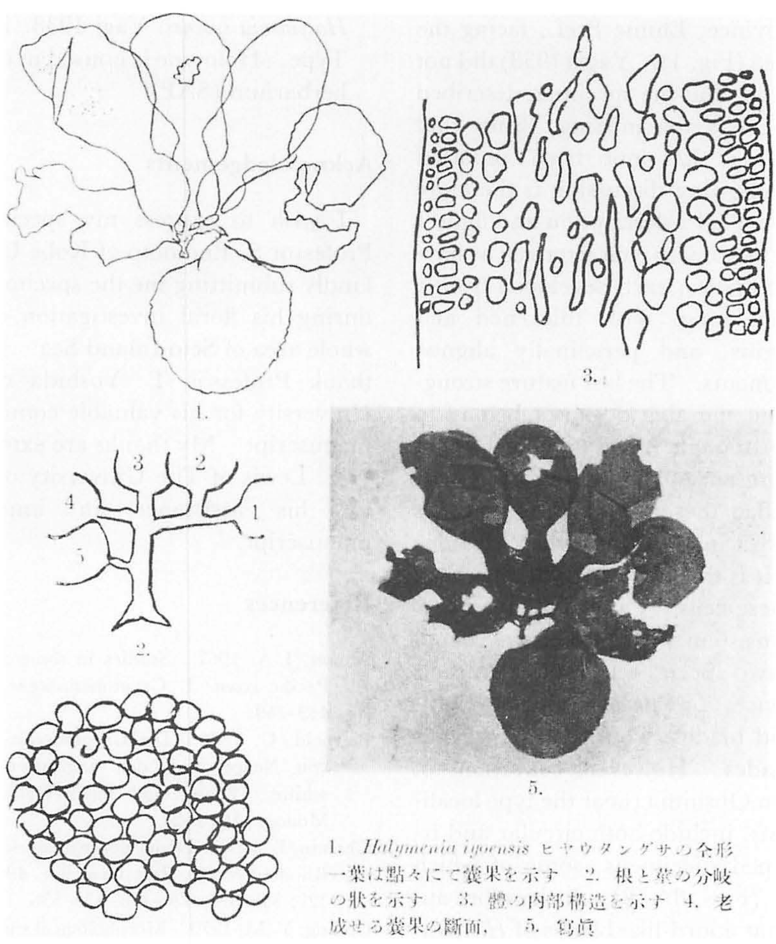

Fig. 9. *Halymenia iyoensis* Yagi. Original illustrations.

cells to form a branched filament, similar in pattern to that reported for *Halymenia* (van den Hoek and Cortel-Breeman 1970, Codomier 1974, Maggs and Guiry 1982). In other genera of the family, such as *Grateloupia* (Chemin 1937, Inoh 1947, Hayashida and Chihara 1967, Kawaguchi 1991), *Prionitis* (Hayashida 1965) and *Dermocorynus* (Guiry and Maggs 1982), after the spore contents are evacuated into the germ tube, the sporeling divides rapidly to form a radially expanded crust ("mediate discal" type; Inoh 1947). In *Halymenia* the sporeling grows further into a small acrochaetioid plant (van den Hoek and Cortel-Breeman 1970, Codomier 1974, Maggs and Guiry 1982). In *C. rotunda*, however, the sporeling does not develop into such a filamentous plant, but the filaments coalesce laterally and eventually transform into an irregularly shaped crust with a marginal meristem. This crust is similar to those of *Grateloupia* and other genera showing mediate discal type of spore development. The same pattern of spore development as *C. rotunda* is also observed in *C. luxurians* from Japan (Kawaguchi, unpublished data). Although known for only two species, this unique spore development pattern may be characteristic of *Cryptonemia*, and could suggest a possible ancestral link between *Cryptonemia* and *Halymenia* during evolution of the Halymeniaceae.

Comparison of Cryptonemia rotunda *and* Halymenia iyoensis

Yagi (1938, p. 1450) described *Halymenia iyoensis* based on a specimen collected at Ohyama, Ochi Province, Ehime Pref., facing the Seto Inland Sea (Fig. 1). Yagi (1938) did not designate a type, and the specimen described in the protologue is now missing. Since that time, *H. iyoensis* has not been reported again. The following discussion is therefore based solely on Yagi's description and figures (Fig. 9). *H. iyoensis* was characterized as having gourd-like blades, each developed from a stalk to 4 mm long, with thickened and toothed margins, and periclinally aligned medullary filaments. The last feature strongly suggests that the alga does not belong in *Halymenia*. Although Yagi (1938) did not mention the presence of highly refractive cells in the medulla, these cells are sometimes difficult to detect in the places where sections were made. It is therefore possible that Yagi overlooked these cells. Other features of *H. iyoensis* are consistent with *C. rotunda*. Separation of the two species relies solely on their outer appearances; *C. rotunda* has mostly circular or ellipsoid blades, whilst *H. iyoensis* has gourd-like blades. However, collections of *C. rotunda* from Ohshima (near the type locality of *H. iyoensis*) include both circular and irregularly shaped specimens, some of which are gourd-like (Figs. 8A, B). It therefore appears likely that gourd-like blades of *H. iyoensis* are morphological variants formed during regeneration of a new blade, or by environmental or some other cause in the course of blade development. Both taxa have basically circular blades and thickened, toothed margins. These features are very conspicuous and characteristic of *Cryptonemia rotunda*. *Halymenia iyoensis* is therefore considered to be a synonym of *Cryptonemia rotunda*.

In conclusion, the following new combination and synonymy is proposed:

Cryptonemia rotunda (Okamura) Kawaguchi comb. nov.

Basionym: *Halymenia rotunda* Okamura 1936: 536-537

Erythrymenia obovata sensu Okamura (non Schmitz) 1930: 22-23

Halymenia (?) *rotunda* Okamura; Segawa 1938: 142-143

Halymenia iyoensis Yagi 1938: 1450-1451

Type. Holotype is housed in the Okamura herbarium (SAP).

Acknowledgements

I wish to express my special thanks to Professor S. Enomoto of Kobe University for kindly submitting me the specimens collected during his floral investigation covering the whole area of Seto Inland Sea. I also wish to thank Professor T. Yoshida of Hokkaido University for his valuable comments on the manuscript. My thanks are extended to Mr. J. A. Lewis of The University of Melbourne for his assistance in improving the manuscript.

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川口栄男：日本産紅藻ムカデノリ科に関する分類ノート
II. *Halymenia rotunda* Okamura.

マルバグサ *Halymenia rotunda* Okamura のタイプ標本及び最近得られた材料の観察を行なった。本種は頑丈な茎状部，固い膜質の藻体，藻体に平行な髄糸及び髄中に屈折性の細胞を有する。又，助細胞 ampulla は密に分枝し開口部が狭い。イソノハナ属 *Halymenia* の特徴とされる藻体に垂直な髄糸は認められず，上記の特徴はカクレイト属 *Cryptonemia* に一致する。従って，*Cryptonemia rotunda* (Okamura) Kawaguchi の新組合せを提案した。

培養により胞子の発達を調べた。果胞子からは初期に分枝した単列糸状体を形成するが，その後盤状体に変化していく。アクロケチウム様の糸状藻体に発達することはなかった。

瀬戸内海から記載されたヒョウタングサ *H. iyoensis* Yagi をマルバグサと比較し，この種はマルバグサの synonym であると結論した。(812 福岡市東区箱崎6-10-1 九州大学農学部水産学第二教室)

(*Received July 12, 1993: Accepted September 24, 1993*)