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Egg release and germling development in *Myagropsis myagroides* (Mertens ex Turner) Fensholt

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The mature female conceptacle of *Myagropsis myagroides* (Mertens ex Turner) Fensholt has no ostioles. The oogonium formed in the conceptacle has a cell wall which can be distinguished into a dense outer and a less dense inner microfibrillar layer. Prior to egg release, a mucilaginous stalk is produced inside the inner layer and elongated paraphyses protrude onto the surface of receptacle from the conceptacles. Released eggs are retained on the receptacle by the stalk, which is anchored to the conceptacle. After fertilization, the eggs begin to form a primary wall. Subsequently, this primary wall develops into a polylamellated structure of microfibril layers. When germlings have differentiated into thallus and rhizoids, peripheral thallus cells form the cell wall, which consists of a microfibril layer under the primary wall, while the rhizoids, whose cell wall has an amorphous structure, break out of the primary wall. In both the peripheral thallus cells and the rhizoidal cells many osmiophilic materials accumulate and the latter also contain vesicles with fibrillar material. The germlings are then discharged from the stalk and become attached to the substratum by an adhesive substance secreted from rhizoidal cells; this substance is composed of fibrillar material.

Key Index Words: *attachment—egg release—embryogeny—germling*—Myagropsis myagroides.

Myagropsis myagroides (Mertens ex Turner) Fensholt is a common large brown alga in Japan and one of the main components of seaweed beds. Egg release and germling development are important events in the life history of seaweeds such as *M. myagroides*. These events were first observed by Tahara (1913 as *Cystophyllum sisymbrioides* (Turner) J. Agardh). He reported that the conceptacle has no ostioles and that released eggs and developing germlings are retained by paraphyses which break out of the conceptacle wall. On the other hand, Yoshida and Kawai (1987), who carried out a taxonomic study of the genus *Myagropsis*, reported that paraphyses protrude beyond the ostiole and that the eggs and germlings, which are surrounded by a gelationous stalk, are retained among paraphyses in *M. myagroides*. Furthermore, there have been no studies of attachment of germlings to the substratum in this species. The present paper describes details of egg release, embryogeny and attachment of germlings of *M. myagroides* using light and electron microscopy in order to elucidate the process of egg release and germling development.

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Materials and Methods

M. myagroides plants with mature receptacles were collected from Tsuyazaki, Northern Kyushu, Japan, in March 1991 and 1992. The plants were transported to the laboratory and male and female receptacles were excised. The receptacles were kept in 1 liter flasks filled with sterilized seawater, which was aerated, at 16–17°C and produced fertilized eggs and developing germlings, which were retained on the receptacles. The seawater was renewed daily. After the germlings had been discharged from the receptacles, they were transferred to 90 × 10 mm

Petri dishes with 40 ml of sterilized seawater, at the bottom of which was a layer of polymerized epoxy resin inserted as a settlement substratum. By the next day the germlings had settled onto the resin and small pieces of the substratum, with attached germlings, could be cut off.

For light and transmission electron microscopy, specimens were fixed in 5% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4) and 1% NaCl for 3 h on ice. They were washed with 0.1 M cacodylate buffer and post-fixed in 1% OsO_4 in 0.1 M cacodylate buffer for 3 h on ice, then dehydrated gradually with ethanol. Following dehydration, samples were transferred to methyl glycidyl ether and then embedded in Spurr's resin (Spurr 1969). Sections were cut on a Porter-Blum MT-1 ultramicrotome. For light microscopy, sections were stained with 1% toluidine blue O (pH 8.7). For transmission electron microscopy, sections were stained with lead citrate and observed with a JEM-200BS transmission electron microscope (at the Research Laboratory of High Voltage Electron Microscope, Kyushu University) at an accelerating voltage of 100 kV.

Specimens for scanning electron microscopy were prepared in the same way as for transmission electron microscopy, but without OsO_4. They were freeze-dried with *t*-butyl alcohol (Akahori *et al.* 1988, Inoue *et al.* 1989), sputter-coated with gold and observed with a JSM-T200A scanning electron microscope at an accelerating voltage of 10 kV.

Results

Egg release

At maturity, the male receptacle showed ostioles in conceptacles on its surface (Fig. 1A), but ostioles were absent from the surface of the female receptacle (Fig. 1B). In the female receptacle, the upper part of the conceptacle was occupied by a number of short paraphyses and the oogonium was situated in the lower part of the conceptacle (Fig. 1C). The oogonium had a cell wall which could be distinguished into a dense outer and a less dense inner microfibril layer (Figs. 1D, E). The outer layer of the oogonium was connected to the vegetative cells of the conceptacle (Fig. 1D).

It was observed that one day before egg release, the paraphyses elongated and broke out of the conceptacle wall (Fig. 2A) and a mucilaginous stalk was formed inside the inner layer of the oogonium (Figs. 2A, B). The inner part of the stalk was dense compared with the other parts (Figs. 2C-E). Subsequently, eggs were released from the conceptacles and retained close to the receptacle surface (Fig. 3A) by the stalk, which remained fastened to the interior of the conceptacle (Figs. 3B, 5D).

Embryogeny

Fertilization occurred immediately after egg release. The fertilized eggs began to form a primary wall (Fig. 3C) and then cell division commenced.

Two days after egg release, germlings had developed to a 3-celled stage. The primary wall of the germlings developed into a polylamellated structure of microfibril layers, and partitioning of wall material was observed beneath this primary wall (Fig. 3D). At this stage, the surface of the female receptacle was covered with elongated paraphyses (Fig. 3E).

After 4 days, the multicellular germlings had differentiated into thallus and rhizoids (Fig. 4A). Many osmiophilic inclusions, consisting of polyphenols, were observed in peripheral thallus cells and rhizoidal cells of the germlings (Figs. 4A, D). Each peripheral thallus cell formed a cell wall, which consisted of a microfibrillar layer, under the primary wall (Fig. 4B). Osmiophilic material accumulated between the primary wall and the cell wall of the peripheral thallus cells (Fig. 4B). In the rhizoidal cells, many vesicles containing fibrillar material aggregated around the nucleus (Figs. 4D, E). The rhizoidal cells were surrounded by numerous granular substances (Figs. 4C, D, F) and the rhizoid tips broke out of the primary wall (Fig. 4C). The cell wall of the rhizoid tip had an amorphous

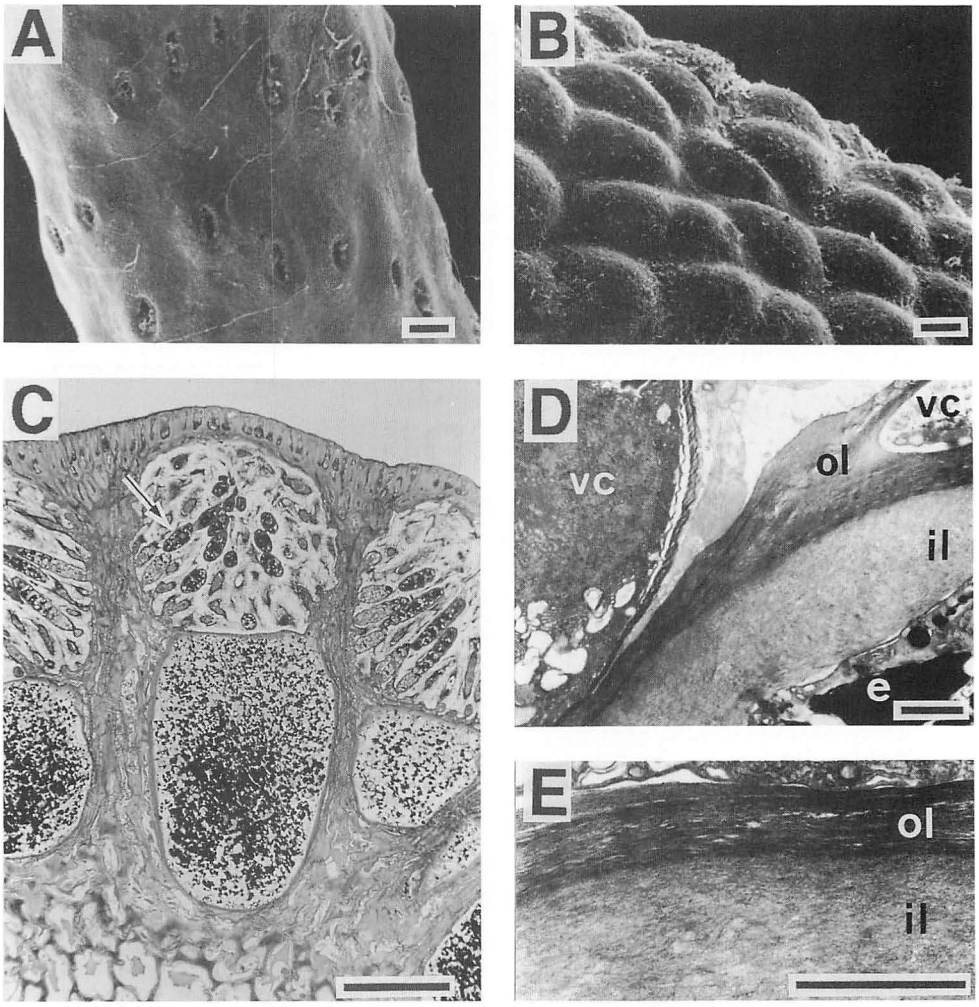


Fig. 1. Mature conceptacles. A: Surface of a male receptacle showing ostioles of conceptacles. B: Surface of a female receptacle showing lack of ostiole. C: Cross section of a female conceptacle. The conceptacle was filled with short paraphyses (arrow) and an oogonium. D: Peripheral region of an oogonium showing dense outer layer (ol) and less dense inner layer (il). The outer layer was connected to the vegetative cells (vc). e: egg in the oogonium. E: Outer layer (ol) and inner layer (il) in Fig. 1D. The layers were composed of microfibrils. Scales: 100 μm (A–C), 1 μm (D, E).

structure (Fig. 4F).

After 5 days, the paraphyses were shed from the surface of the female receptacle (Fig. 5A) and the multicellular germlings were discharged from the stalk (Figs. 5B–D). The primary wall surrounding the thallus (Fig. 5C) then began to break down (Fig. 5E). Rhizoidal cells, which broke out of the primary wall (Fig. 5C), were surrounded by a granular substance (Figs. 5F, G) and contained numerous osmiophilic bodies and vesicles containing fibrillar material (Fig. 5F).

Attachment of germlings

The germlings did not settle on to the substratum immediately after discharge from the stalk (Fig. 5C). However, by the next day, the thallus and rhizoids of the germlings had elongated and the germlings had become attached to the substratum by rhizoids (Fig. 6A). Moreover, an adhesive substance composed of fibrillar material was observed between the rhizoids and the substratum (Fig. 6C). Vesicles containing fibrillar material were observed in the rhizoidal cell (Fig. 6C).

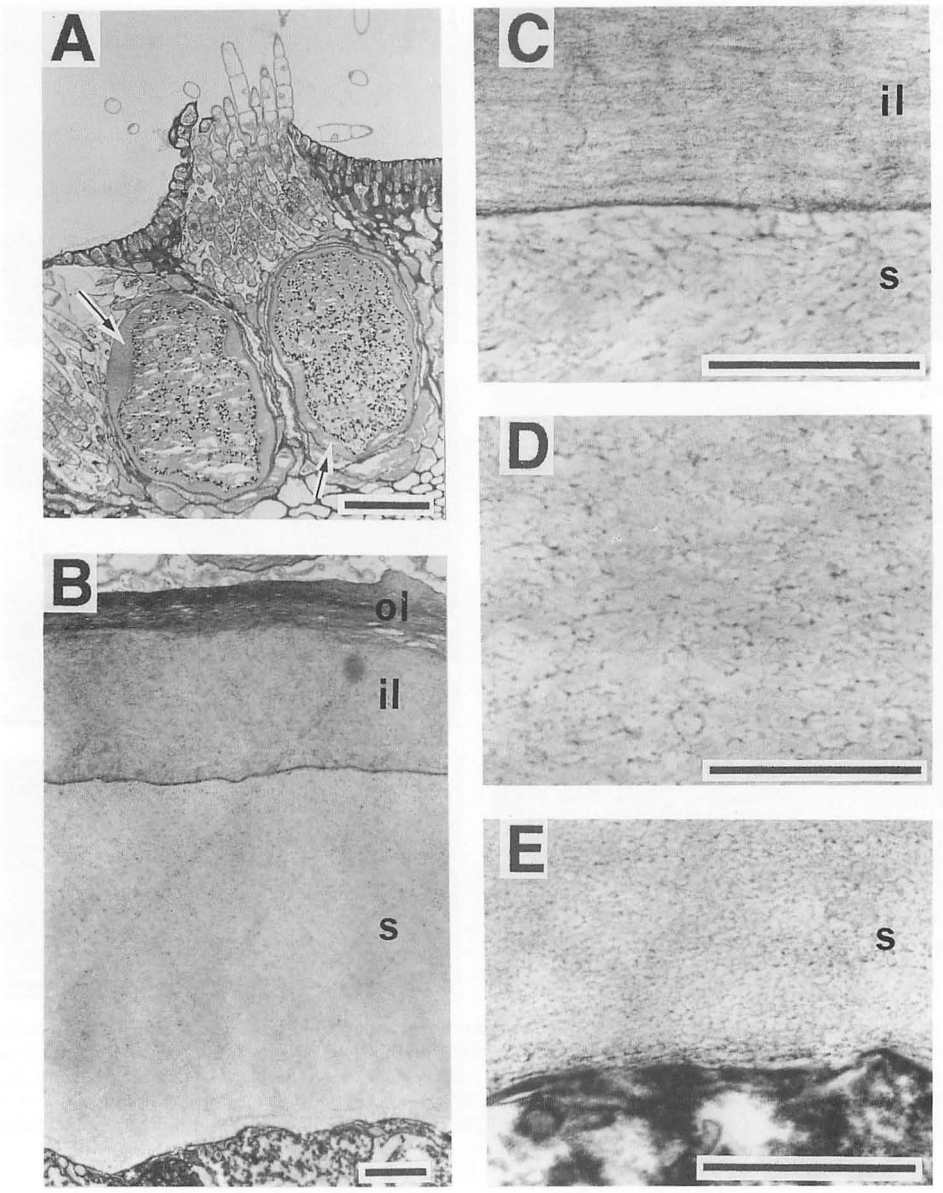


Fig. 2. Oogonia one day before egg release. A: Oogonia in female conceptacles showing the stalk formation (arrows). Paraphyses protrude on to the surface of the receptacle. B: Peripheral region of an oogonium. The stalk was formed (s) inside the inner layer (il). ol: outer layer. C–E: Outer (C), central (D) and inner (E) parts of the stalk (s) in Fig. 2B. The inner part was dense compared with the other parts. il: inner layer. Scales: 100 μm (A), 1 μm (B), 0.5 μm (C–E).

At the same time, the break down of the primary wall, which surrounded the thallus, was further advanced (Fig. 6B).

Discussion

The present study demonstrates the process of egg release and germling development in *M. myagroides*. The events reported here differ from those of the same species in previous papers (Tahara 1913, Yoshida and Kawai 1987) because the conceptacle was found to have no ostioles and the paraphyses broke out of the conceptacle; the released eggs and de-

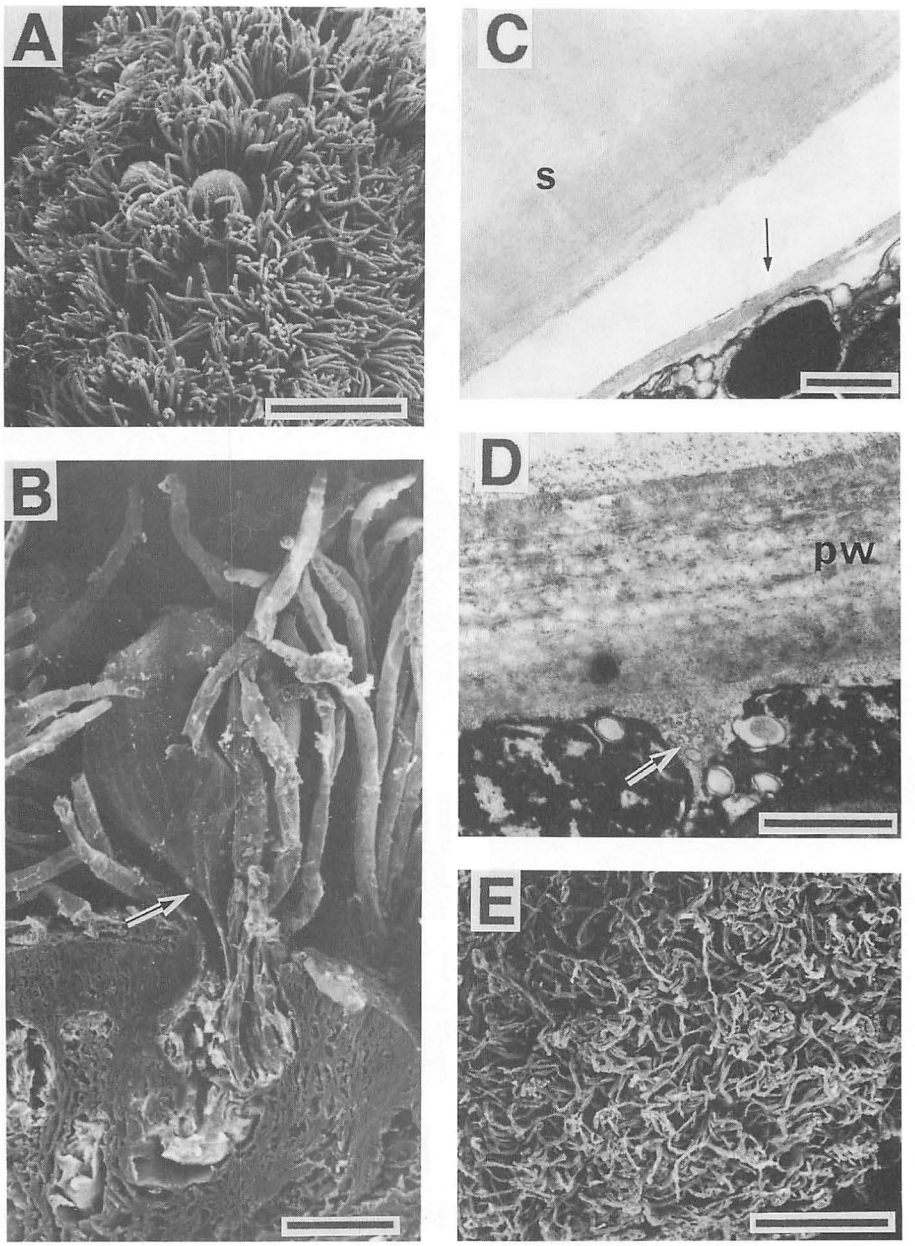
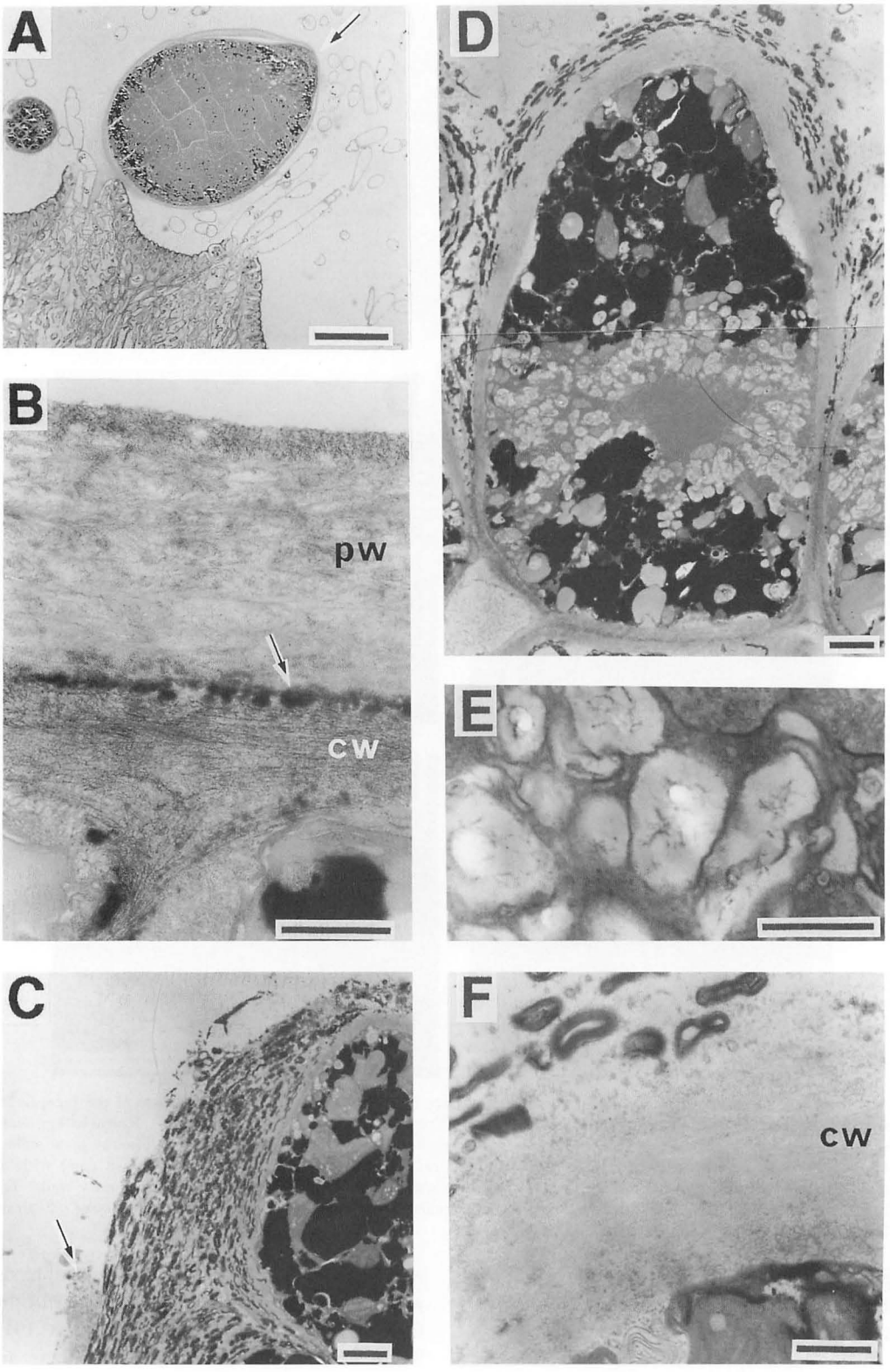


Fig. 3. Released eggs and germlings on the surface of a female receptacle. A: Surface of the receptacle showing released eggs and protruded paraphyses. B: Transverse section of the receptacle. Released egg was retained by a stalk (arrow). C: Peripheral region of a fertilized egg initiating the primary wall (arrow). s: stalk. D: Peripheral region of a 3-celled germling, 2 days after egg release, showing the primary wall (pw) with a polylamellated structure of microfibril layers. Partition wall (arrow)was observed under the primary wall. E: Surface of the receptacle with elongated paraphyses. 3-celled germlings were covered by the paraphyses. Scales: 500 μm (A, E), 100 μm (B), 1 μm (C, D).

veloping germlings were retained on the surface of the conceptacle by a stalk which was anchored to the conceptacle.

In this species, released eggs and germlings were covered with elongated paraphyses, whereas paraphyses do not elongate in other fucoids which form a stalk (Tahara 1913, Sokhi and Vijayaraghavan 1986, Kaur and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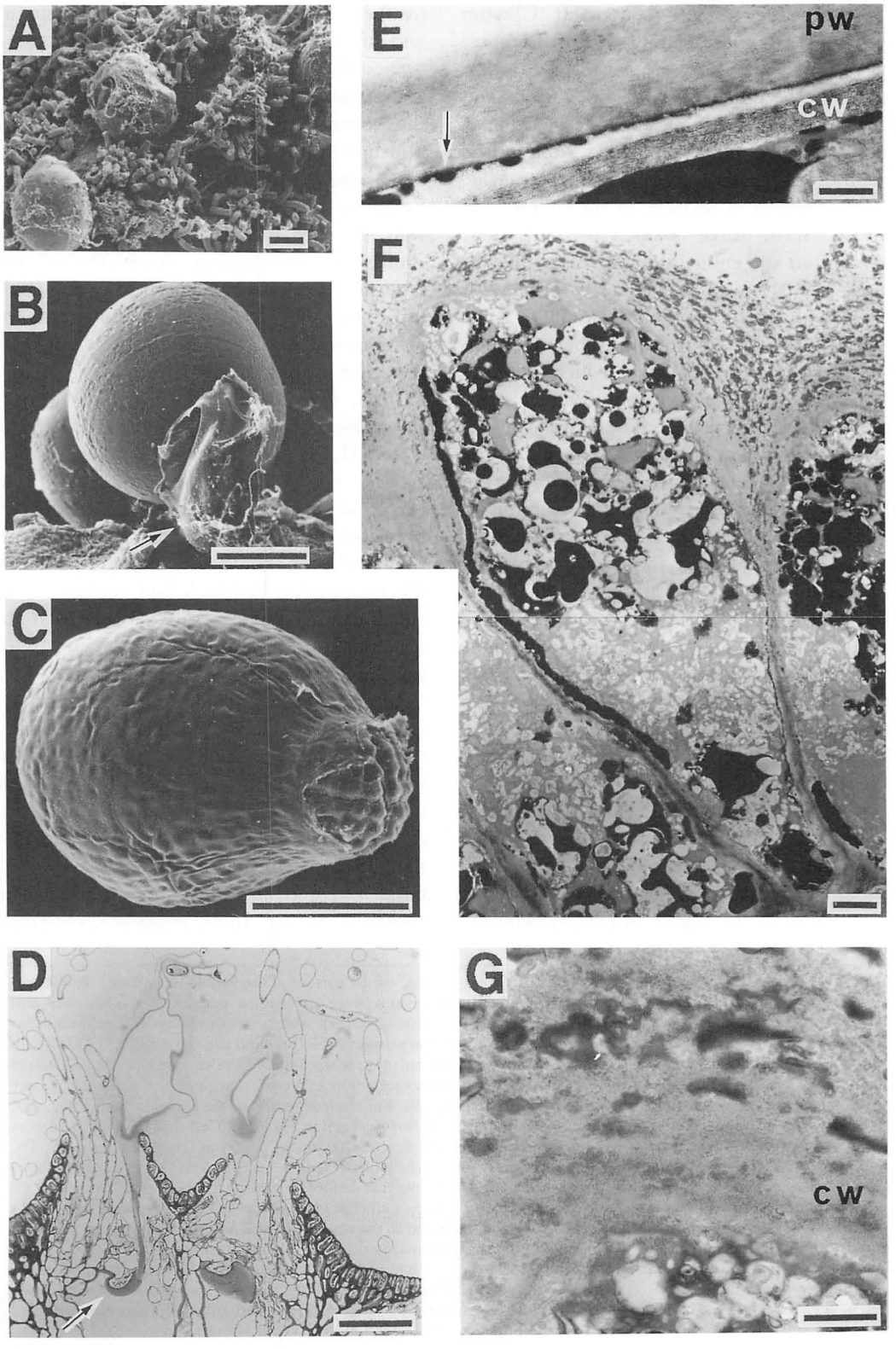
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Vijayaraghavan 1992, May and Clayton 1991, Burridge *et al.* 1993). Furthermore, eggs of *M. myagroides* are large when compared to other fucoids (Ino 1947, Yoshida and Kawai 1987). It is suggested that the elongated paraphyses of *M. myagroides* support the retention of the large eggs on the receptacle.

The oogonia of fucoids, at the time of egg release, have three walls, the exochiton, mesochiton and endochiton (Farmer and Williams 1898, Moss 1974, Hardy and Moss 1979, Sokhi and Vijayaraghavan 1986, Kaur and Vijayaraghavan 1992). The exochiton ruptures and releases eggs, which are surrounded by the mesochiton and endochiton. In *M. myagroides*, the oogonial cell wall consisting of 2 layers is applied to the exochiton, the outer and central parts of the stalk being the mesochiton, and the inner the endochiton.

In Fucales, the ultrastructure of the stalk has been investigated in *Sargassum vestitum* (May and Clayton 1991) and *Phyllospora comosa* (Burridge *et al.* 1993). In these species, the inner layer of the stalk immediately surrounding the egg has a fibrillar appearance. The structure of the stalk of *M. myagroides* differs from that of *S. vestitum* and *P. comosa* because the inner part of the stalk is composed of dense mucilaginous material.

After fertilization, *M. myagroides* first formed a primary wall and rhizoids, once they were developed, broke out of this wall. Hardy and Moss (1978) observed a similar type of wall in the zygote of *Halidrys siliquosa* (as the zygote wall). However, the structure and function of the primary wall in *M. myagroides* differ from those of the zygote wall in *H. siliquosa*. The primary wall did not have the external mucilage associated with initial attachment of the zygotes in *H. siliquosa*. In *M. myagroides*, prevention of polyspermy may be one of the main functions of the primary wall, because formation of the wall began shortly after fertilization, as with the cell wall of fertilized eggs of *S. vestitum* (May and Clayton 1991). However, it is not known why the primary wall develops into the polylamellated structure of microfibrillar layers.

In *H. siliquosa*, the zygote wall is ruptured mechanically by the elongation of primary rhizoids (Hardy and Moss 1978). In *M. myagroides*, however, many granular inclusions surround rhizoidal cells when the rhizoid tips break out of the primary wall. Subsequently, these inclusions could not be observed around the rhizoidal cells which had settled on the substratum. These results suggest that the granular substance may have a role in rupturing the primary wall.

In germlings of *M. myagroides*, the primary wall and the cell wall of peripheral thallus cells were composed of the microfibril layer, while the cell wall of the rhizoid tips had an amorphous structure. In the cell wall of *Fucus* (McCully 1965, 1966, Novotny and Forman 1975), a fibrillar material consists of alginic acid and an amorphous material consists of fucoidan. In *M. myagroides*, therefore,

Fig. 4. Multicellular germlings, 4 days after egg release, on the surface of the female receptacle. A: Germling with thallus and rhizoids (arrow). Osmiophilic inclusions were present in peripheral thallus cells and rhizoids. B: Peripheral region of thallus showing the formation of cell wall (cw) consisting of a microfibrillar layer. Osmiphilic material (arrow) accumulated between the primary wall (pw) and the cell wall. C: Rhizoid tip breaking out of the primary wall (arrow). Many granular substances surrounded the rhizoid tip. D: Rhizoidal cell showing abundance of osmiophilic material and the aggregation of vesicles around the nucleus. Many granular inclusions surrounded the tip of the rhizoidal cell. E: Perinuclear region of the rhizoidal cell in Fig. 4D. Vesicles with fibrillar material were observed. F: Tip of the rhizoidal cell in Fig. 4D. Cell wall (cw) had an amorphous structure and was surrounded by granular material. Scales: 100 μm (A), 0.5 μm (B, E, F), 2 μm (C, D).

Fig. 5. Discharge of multicellular germlings. A: Surface of the female receptacle with germlings prior to discharge. Elongated paraphyses were shed. B: Germling just before discharge from a stalk (arrow). C: Discharged germling. Thallus was surrounded by a primary wall, but rhizoids broke out of the wall. D: Stalks after germlings had been discharged. The stalk was anchored to the conceptacle (arrow). E: Peripheral region of thallus of discharged germling. Osmiophilic material (arrows) accumulated between the primary wall (pw) and the cell wall (cw). The primary wall began to collapse. F: Rhizoidal cell of the discharged germling showing an abundance of osmiophilic material and vesicles with fibillar material. Many granular inclusions surrounded the tip of the rhizoidal cell. G: Tip of the rhizoidal cell in Fig. 5F. Cell wall (cw) had an amorphous structure and was surrounded by granular material. Scales: 100 μm (A–D), 0.5 μm (E, G), 2 μm (F).

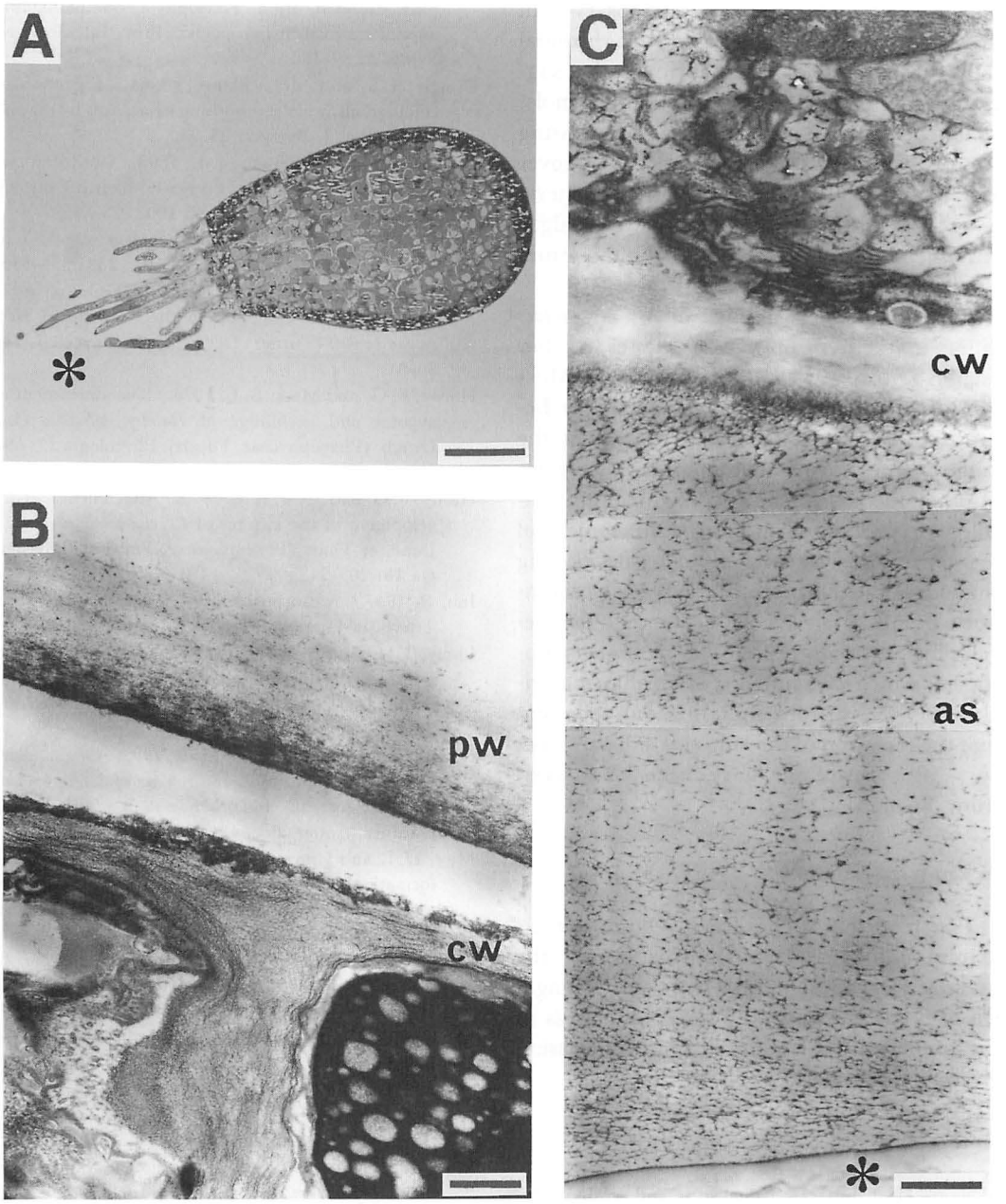

Fig. 6. Multicellular germlings one day after discharge from the stalk. A: Germling attached to the substratum (*) by rhizoids showing the elongation of thallus and rhizoids. B: Peripheral region of the thallus showing developed cell wall (cw) and degenerated primary wall (pw). C: Rhizoidal cell attached to the substratum (*) by adhesive substance (as) consisting of fibrillar material. Vesicles with fibrillar material were observed in the rhizoidal cell. cw: cell wall. Scales: 100 μm (A), 0.5 μm (B, C).

the alginic acid may be the main component of the primary wall and the cell wall of the peripheral thallus cells, and the fucoidan may be that of the cell wall of the rhizoid tips.

Forbes and Hallam (1979) reported that osmiophilic material consisting of polyphenols accumulates at the rhizoid tip in *Hormosira banksii*. In this study, a similar accumula-

tion of osmiophilic material was observed in the peripheral thallus cells and the rhizoidal cells of *M. myagroides*. This accumulation suggests that these cells secrete polyphenols, which have been implicated in suppressing barnacle and mussel colonization (Conover and Sieburth 1966) and growth of bacteria (Conover and Sieburth 1964), microalgae (Craigie and McLachlan 1964) and other marine benthic algae (Fletcher 1975).

Attachment of germlings in *M. myagroides* differs markedly from that of zygotes in other fucoids (Moss 1974, 1975, Hardy and Moss 1978, 1979, Forbes and Hallam 1979) because the germlings were not enclosed by the adhesive mucilage which anchors the zygotes of other fucoids to the substratum until rhizoids are produced, and initial attachment of the germlings was carried out by developing rhizoids. Furthermore, the present observations of *M. myagroides* indicate that the developing rhizoids secrete an adhesive substance, which is composed of fibrillar material, via vesicles from the rhizoidal cells and that the secretion of the adhesive substance occurs after germlings have been discharged from the stalk.

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References

Akahori, H., Ishii, H., Nonaka, I. and Yoshida, H. 1988. A simple freeze-drying device using *t*-butyl alcohol for SEM specimens. J. Electron Microsc. **37:** 351–352.

Burridge, T., Clayton, M. N. and Hallam, N. D. 1993. Oogenesis and stalk mediated fertilization in *Phyllospora comosa* (Labillardiere) C. Agardh (Seirococcaceae, Phaeophyta). Bot. Mar. **36:** 223–232.

Conover, J. T. and Sieburth, J. M. 1964. Effect of *Sargassum* distribution on its epibiota and antibacterial activity. Bot. Mar. **6:** 147–157.

Conover, J. T. and Sieburth, J. M. 1966. Effects of tannins excreted from Phaeophyta on planktonic animal survival in tide pools. Proc. Int. Seaweed Symp. **5:** 99–100.

Craigie, J. S. and McLachlan, J. 1964. Excretion of coloured ultra-violet absorbing substances by marine algae. Can. J. Bot. **42:** 23–33.

Farmer, J. B. and Williams, J. L. 1898. Contributions to our knowledge of the Fucaceae, their life history and cytology. Phi. Trans. B **190:** 623–645.

Fletcher, R. L. 1975. Heteroantagonism observed in mixed algal cultures. Nature, Lond. **253:** 534–535.

Ferbes, M. A. and Hallam, N. D. 1979. Embryogenesis and substratum adhesion in the brown alga *Hormosira banksii* (Turner) Decaisne. Br. Phycol. J. **14:** 69–81.

Hardy, F. G. and Moss, B. L. 1978. The attachment of zygotes and germlings of *Halidrys siliquosa* (L.) Lyngb. (Phaeophyceae, Fucals). Phycologia **17:** 69–78.

Hardy, F. G. and Moss, B. L. 1979. Attachment and development of the zygotes of *Pelvetia canaliculata* (L.) Dcne. et Thur. (Phaeophyceae, Fucales). Phycologia **18:** 203–212.

Ino, S. 1947. Kaiso no hassei. p. 1–93. Hokuryukan, Tokyo (in Japanese).

Inoue, T., Osatake, H. and Takahashi, H. 1989. A new freeze-drying instrument using *t*-butyl alcohol for scanning electron microscopy. J. Electron Microsc. **38:** 246–249.

Kaur, I. and Vijayaraghavan, M. R. 1992. Oogonial development, maturation and release in *Sargassum vulgare* C. Agardh and *S. johnstonii* Setchell and Gardner. Aquat. Bot. **42:** 173–185.

May, D. I. and Clayton, M. N. 1991. Oogenesis, the formation of oogonial stalks and fertilization in *Sargassum vestitum* (Fucales, Phaeophyta) from southern Australia. Phycologia **30:** 243–256.

McCully, M. E. 1965. A note on the structure of the cell wall of the brown alga *Fucus*. Can. J. Bot. **43:** 1001–1004.

McCully, M. E. 1966. Histological studies on the Genus *Fucus*. Protoplasma **62:** 287–305.

Moss, B. L. 1974. Attachment and germination of the zygotes of *Pelvetia canaliculata* (L.) Dcne. et Thur. (Phaeophyceae, Fucales). Phycologia **13:** 317–322.

Moss, B. L. 1975. Attachment of zygotes and germlings of *Ascophyllum nodosum* (L.) Le Jol. (Phaeophyceae, Fucales). Phycologia **14:** 75–80.

Novotny, A. M. and Forman, M. 1975. The composition and development of cell walls of *Fucus* embryos. Planta (Berl.) **122:** 67–78.

Sokhi, G. and Vijayaraghavan, M. R. 1986. Oogonial release in *Turbinaria conoides* (J. Agardh) Kutzing (Fucales, Sargassaceae). Aquat. Bot **24:** 321–334.

Spurr, A. R. 1969. A low-viscosity epoxy resin embedding medium for electron microscopy. J. Ultrastructure Res. **26:** 31–43.

Tahara, M. 1913. Oogonium liberation and the embry-

ogeny of some fucaceous algae. J. Coll. Sci. Tokyo Imp. Univ. **32:** 1–13.
Yoshida, T. and Kawai, H. 1987. Taxonomic study of the genus *Myagropsis*. Bot. Mag. Tokyo **100:** 165–173.

難波信由：ジョロモクの卵放出と幼胚の生長

成熟雌性生殖器巣に巣口は無く，巣内の生卵器壁は密度の異なる2つの微繊維層に区別された。卵放出前には生卵器壁の内側に膠質柄が形成され，側糸が生殖器床から突出した。放出卵は膠質柄により生殖器床上に保持され，受精後，微繊維層から成る一次壁を形成した。葉状部と仮根に分化した幼胚の葉状部周辺細胞は一次壁の内側に微繊維から成る細胞壁を形成したが，一次壁を破り突出した仮根先端の細胞壁は明確な構造を持たなかった。葉状部周辺と仮根にはオスミウム酸に良く染まる物質が多く，後者には繊維状物質を含む小胞も多く見られた。その後，幼胚は生殖器床から落下し，仮根細胞から分泌された繊維状物質から成る粘着物質で基盤に付着した。
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