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Inderdeep Kaur and M. R. Vijayaraghavan: Histochemical studies on the mesochiton-stalk, egg and zygote of *Sargassum vulgare* C. Agardh (Fucales, Phaeophyta)

*Key Index Words: egg—germling—mesochiton-stalk—polysaccharides—**Sargassum vulgare**—zygote.*
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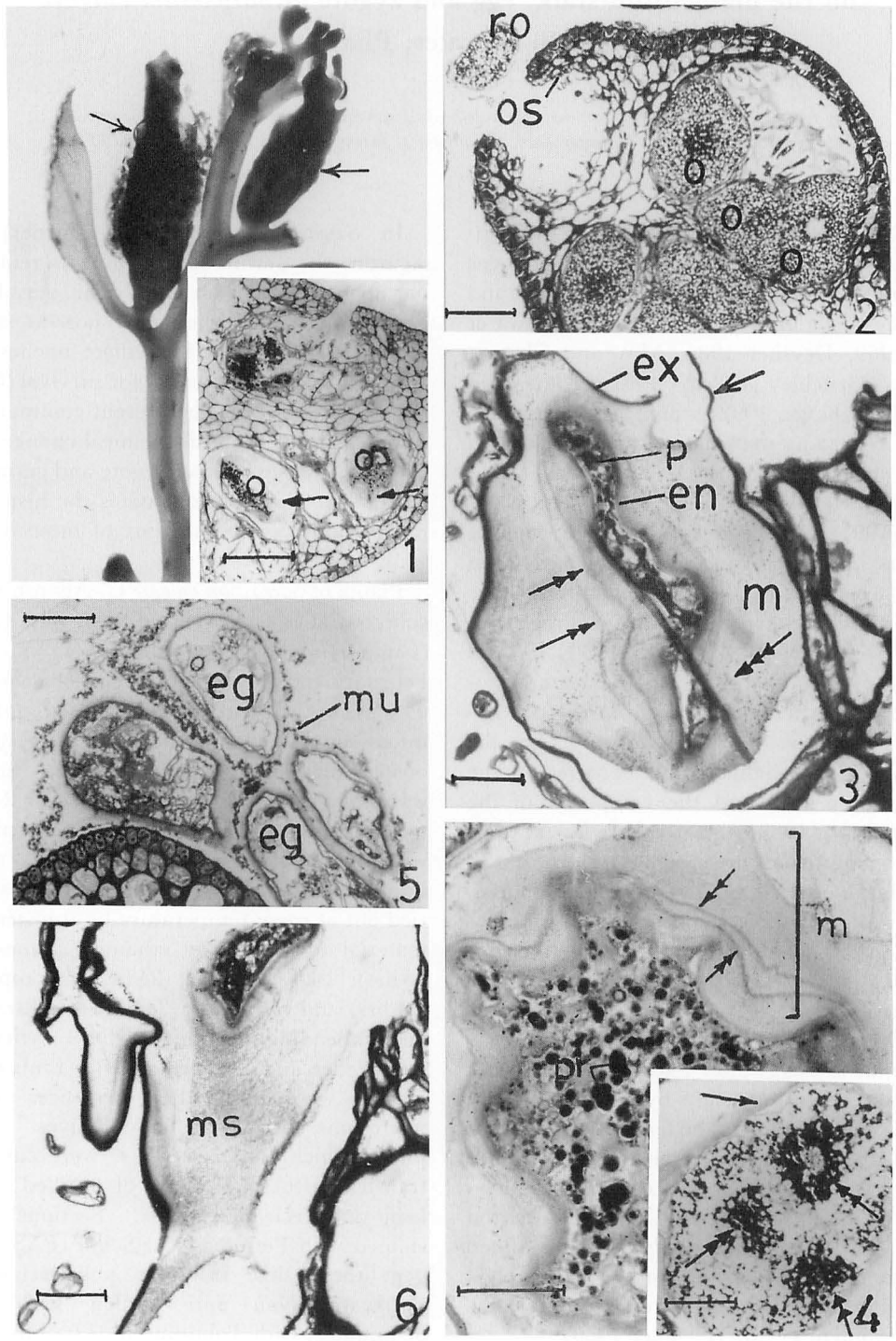
In various species of *Sargassum* the cytological, histochemical and ultrastructural changes accompanying oogenesis, fertilization and post-fertilization are documented (Ogawa et al. 1969, Deysher 1984, May and Clayton 1991; Critchley et al. 1991; Kaur and Vijayaraghavan 1992). Some confusion persists, regarding the usage of terms oogonium and egg in Fucales. Attempts have been made to clarify their usage. May and Clayton (1991) use the term oogonium when it occurs inside the conceptacle and egg when it is extruded to the exterior through the ostiole. However, Sokhi and Vijayaraghavan (1986), Critchley et al. (1991), Kaur and Vijayaraghavan (1992) use the term oogonium even after its release from the conceptacle. We use the term egg only after the histochemical changes have occurred in the mesochiton-stalk and the cytoplasm of the oogonium. In *Sargassum vulgare* C. Agardh, the oogonium has three wall layers—the outer, exochiton is rich in alginates, the middle, mesochiton consists mainly of sulphated polysaccharides and the inner, endochiton contains a mixture of alginates and sulphated polysaccharides (Kaur and Vijayaraghavan 1992). In *S. vestitum* (Brown ex Turner) C. Agardh, the eggs after release are held close to the receptacle surface by the mesochiton-stalk. This increases the fertilization efficiency. Upon fertilization, the zygote shows latent polarity which becomes apparent following the initial cell division (May and Clayton 1991). The eggs of *S. muticum*, are retained on the receptacle surface for approximately 2 or 3 days during which time early zygote development occurs (Fletcher 1980).

In *Sargassum heterophyllum* (Turner) C. Agardh, the germinating zygote is retained on the receptacle surface. The germlings are only released when they possess sticky rhizoids, which help immediate anchorage. This increases the potential for survival (Critchley et al. 1991). The present communication deals with the histochemical changes accompanying egg release, zygote and germling formation. It also emphasizes the histochemistry and varied functions of mesochiton-stalk.

Plants of *Sargassum vulgare* C. Agardh were collected at low tide period from Port Okha (Gujarat) during the months of January, February and November of 1987–89. Selected parts of the plants were processed for light microscopy, fixed on spot in 10% (v/v) aqueous acrolein for 24 hrs; washed in distilled water and post-fixed in 1% $HgCl_2$ for 24 hrs to stabilize the polyphenols. Later, the pieces were rinsed three times in distilled water at an interval of 15 minutes. Dehydration was carried out at room temperature by transferring material to 2-methoxy ethanol (2 times for 48 hrs); 100% ethanol (24 hrs); n-propanol (24 hrs) and n-butanol (24 hrs). Infiltration and embedding was done in glycol methacrylate (Feder and O'Brien 1968). Embedded samples were sectioned by a Spencer rotary microtome fitted with glass knives. Two micron thick, serial sections, were cut and transferred to small drops of distilled water kept on precleaned slides. Sections were stained with Periodic-Acid Schiff (PAS) reagent to localize insoluble polysaccharides (Vijayaraghavan and Shukla 1990) for 30 minutes; with Toluidine Blue 0 (TBO) for

sulphated and carboxylated polysaccharides (Mc Cully, 1966) for 5 minutes and with Coomassie Brilliant Blue (CBB) for proteins (Weber and Osbörn 1975) for 15 minutes. The photographs were taken using B/W, ORWO film on Reichert photomicroscope.



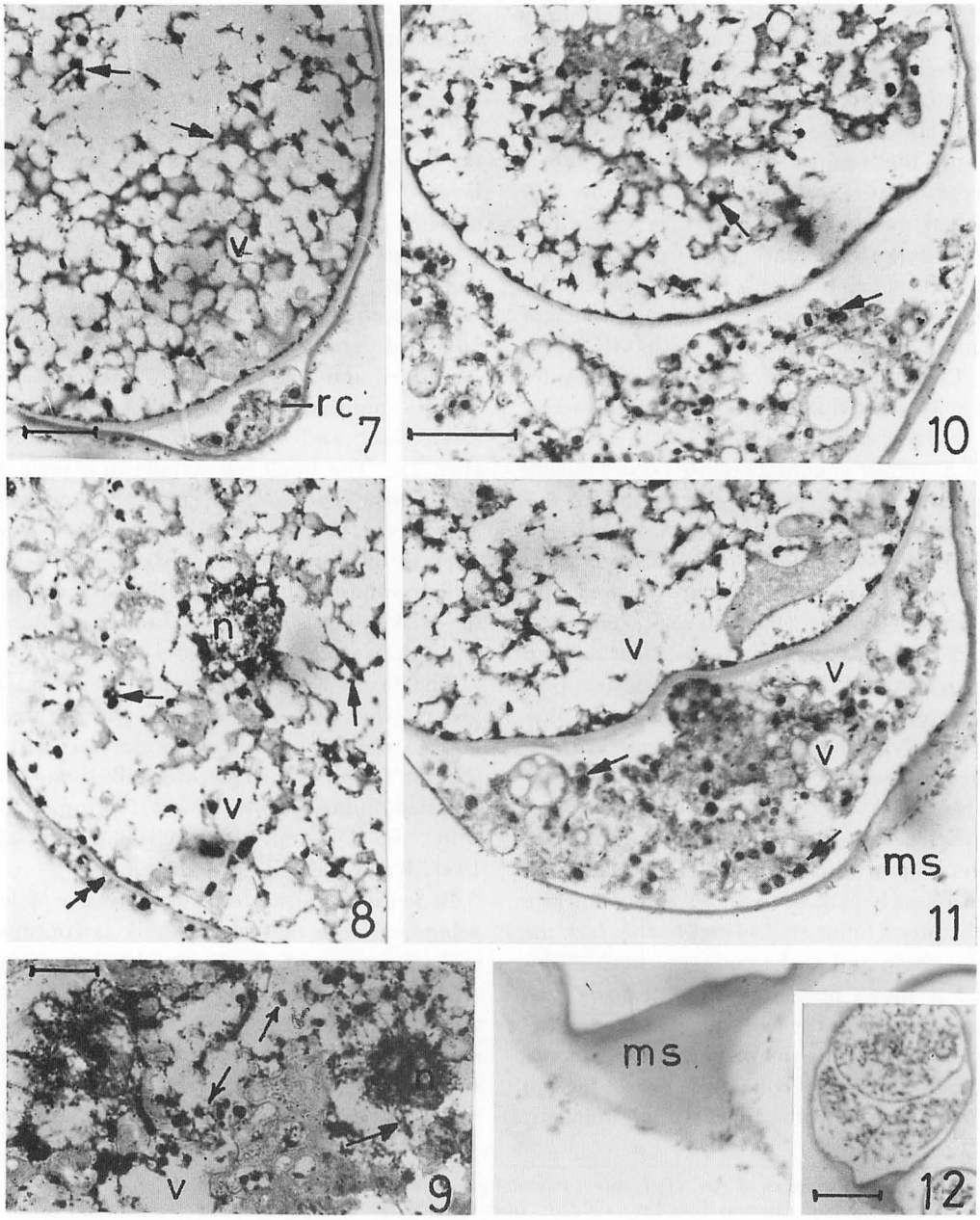
Sargassum vulgare occurs in the reproductive phase during October and January. The receptacles are axillary (Fig. 1) and contain unisexual conceptacles (Fig. 1 inset). The female conceptacle bears 3 or 4 oogonia (Fig. 2) and the mature oogonium wall shows three distinct layers—the exochiton, the mesochiton and the endochiton (Figs 3, 4). The mesochiton shows dark and light bands or zones and stains well for sulphated polysaccharides (Fig. 3). It also shows the presence of grains that stain bright blue with TBO (Fig. 3). Oogonial cytoplasm is rich in physodes and polysaccharides (Fig. 2). Total proteins are at a low ebb (Fig. 4).

In *S. vulgare* oogonial release occurs during January. Once released, the oogonium undergoes histochemical changes and is referred to as the egg. The egg cytoplasm shows eight darkly stained regions which are the presumptive sites of 8 nuclei (Fig. 4 inset). The eight nuclei of the egg appear to be identical and are surrounded by numerous physodes, protein aggregates and small vacuoles. The physodes, polysaccharides and proteins are dispersed in the egg cytoplasm. The mesochiton is present as a thin layer (Fig. 4 inset), but the mesochiton-stalk and the endochiton are prominent at this stage. The eggs, upon release, are held close to the receptacle surface by the mesochiton-stalks (Fig. 6) that lack the distinct light and dark bands seen in mesochiton. It shows polysaccharidic grains. The mucilage around the eggs helps in their cohesion (Fig. 5) and is rich in sulphated polysaccharides. During fertilization, one of the egg nucleus fuses with the male gamete nucleus, and the remaining 7 nuclei degenerate. The cytoplasm around the degenerating nuclei shows decrease in number of polysaccharides and proteins (Figs 8, 9) but there is however, an increase in the size of vacuoles. Around the zygote the mesochiton persists as a thin layer. The zygote cytoplasm shows a low amount of physodes and polysaccharides; its wall contains alginates and sulphated polysaccharides. The zygote undergoes an unequal transverse division, forming a two-celled, polarised germling (Figs 7, 12 inset), with a larger cell and a smaller cell. The large cell contains numerous small vacuoles, a few polysaccharides and many protein aggregates (Fig. 7). The small cell cytoplasm is poor in inclusions. Total proteins occur in moderate amounts in the cytoplasm of both the cells and often form aggregates (Figs 10, 11). The 2-celled germling shows a persisting mesochiton-stalk which shows a mixture of alginates and sulphated polysaccharides (Fig. 12).

The taxon *Sargassum vulgare* belongs to a group where a) the oogonia bear three-layered walls, b) the eggs are not very large (Nanba 1993), c) the eggs are stalked and are retained on the receptacle wall (Norton 1976; May and Clayton 1991, Critchley et al. 1991, Kaur and Vijayaraghavan 1992).

In *Sargassum vulgare*, during the course of development, the mesochiton-stalk is continuously present and shows variable functions. As seen in *Sargassum vestitum* (May and Clayton 1991), in *S. vulgare*, there is no collar and no apical pad to function as an attachment site. The mesochiton-stalk of *S. vulgare*, resembles that of *S. vestitum* in composition

Figs. 1–6. *Sargassum vulgare*, (1) External morphology. Portion of a reproductive plant to show fruiting bodies, the receptacles (arrows) Scale bar=100 μm. Inset shows female conceptacles containing mature oogonia (o) with thick mesochiton (arrows). TBO stained, scale bar=25 μm. (2) Transverse section of a receptacle to show female conceptacles, each one containing three oogonia (o). A released oogonium (ro) is also seen near the ostiole (os). TBO stained, scale bar=250 μm. (3, 4) Longitudinal sections of the conceptacles to show oogonia ready for release. In *3*, the exochiton (ex) is ruptured (arrow) but the mesochiton (m) and the endochiton (en) are firm. The oogonial cytoplasm is rich in physodes (p). The polysaccharide grains (triple arrows) in the mesochiton are distinct. In *4*, the cytoplasm shows protein aggregates (pr). The mesochiton shows dark and light bands (double arrows). The inset in *4* shows an egg with thin mesochiton (arrow) and three nuclear sites with perinuclear cytoplasm rich in physodes (double arrows). *3* and inset, TBO stained, *3*, scale bar=10 μm; inset, Scale bar=25 μm; *4*, CBB+PAS stained, scale bar=10 μm. (5) Longitudinal section of receptacles to show a group of four eggs (eg) settled on the receptacle wall. The mucilage (mu) that enmeshes the eggs is rich in sulphated polysaccharides. TBO stained, scale bar=250 μm. (6) Portion of the conceptacle to show mesochiton-stalk (ms) of the egg. The stalk is firm and has granular appearance. TBO stained, scale bar=10 μm.



Figs. 7–12. *Sargassum vulgare*, (7, 8, 10–12 CBB+PAS stained; 9. CBB stained). (7) The two-celled germling showing a larger cell and a smaller one, the rhizoidal cell (rc). The cells show protein aggregates (arrows) and vacuoles (v) in the cytoplasm. Scale bar=10 μm. (8, 9) The areas around degenerating nuclei (n) show decrease in number of protein aggregates (arrows) and increase in vacuoles (v). Persisting mesochiton (double arrow) is also seen. Scale bar=10 μm. (10–12) Two-celled germling (inset) to show an upper cell in *10* and a lower cell in *11* with a few protein grains (arrows) in the cytoplasm. The upper cell has a moderate amount of polysaccharides, whereas the lower cell has numerous small vacuoles (v). The mesochiton-stalk (ms) of the two-celled germling is quite prominent in *12*. (10–12, scale bar=10 μm; inset, scale bar=250 μm).

and in possessing bands (May and Clayton 1991). In the mature oogonium, the mesochiton is rich in sulphated polysaccharides, shows bands of light and dark regions and randomly distributed polysaccharide grains. The mesochiton acts as a blanket and protects oogonium against turbulent water action. It also helps to push the oogonium out of the conceptacle (Kaur and Vijayaraghavan 1992). In the released oogonium and the egg, the mesochiton remains as a thin, homogeneous layer rich in sulphated polysaccharides and gives protection against desiccation. The mesochiton also attenuates into a stalk which is homogenous and contains a mixture of alginic acids and sulphated polysaccharides (present work). The variable roles attributed to this mesochiton-stalk are: a) to anchor the released oogonium and egg to the parent thallus. This prevents the egg being lost in the vast sea and helps in its settlement and recruitment thus compensating for the low number of eggs produced in this genus (one egg per oogonium). The role of mesochiton-stalk in *S. vulgare* supports the contention of Norton (1976) in *S. muticum* (Yendo) Fensholt and Critchley et al. (1991), in *S. heterophyllum*, where the mesochiton-stalk is said to help to retain the egg close to parent body. b) To trap the spermatozoids in its mucilage ensuring fertilization.

During the post-fertilization events, the enveloping mucilaginous-stalk in zygote prevents it from damage (Fletcher 1980). The mesochiton-stalk acts merely as a 'holdfast' for the zygote and germling ensuring firm establishment (present work).

In *Sargassum vulgare* the presence of mucilage rich in sulphated polysaccharides is an adaptation for successful propagation. The mucilage helps to hold the eggs together, thereby preventing them from sinking and also make spermatozoid contact easy, ensuring successful fertilization.

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Inderdeep Kaur・Vijayaraghavan, M. R.：褐藻 *Sargassum vulgare* C. Agardh（ヒバマタ目）の中膜柄，卵および受精卵の組織化学的観察

褐藻 *Sargassum vulgare* C. Agardh の中膜柄，卵および受精卵につき組織化学的観察を行った。本種は Port Okha (Gujarat) では10月から1月にかけて成熟し，雌雄異巣である。雌性生殖器は3－4個の生卵器を生じ，成熟した生卵器の壁は顕著な3つの層，外膜，中膜，内膜からなる。中膜は硫酸多糖にとみ，同時に TBO でよく染まる顆粒も含んでいる。生卵器の細胞質はフィソードと多糖を多く含んでいる。2細胞の発芽体はアルギン酸と硫酸多糖からなる顕著な中膜柄を持つ。中膜柄は発達の過程において放出された生卵器や卵を基物に付着させたり，粘質の中に精子をとらえ受精がより効率的におこるようにするなどの様々な機能を担っていると考えられる。

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