



Gametangial Ontogeny in Intertidal Green Alga: *Monostroma latissimum* (Kützinger) Wittrock

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ABSTRACT

The gametangial ontogeny in dioecious green alga *Monostroma latissimum* as studied by light-microscopy has been described for the first time. Mature gametophytes were collected from the Pacific coast of Japan. Two morphotypes were observed and both were confirmed to be of one panmictic population. Gametangial maturation occurred in discontinuous patches along the frondal apex. During maturation, each gametangial mother cell (GMC) was transformed into one gametangium. The gametangial plastids divided and cell volume increased. Fully mature GMCs were large, loosely arranged and numerous gametes with chromatic eyespots present in them. Gametes were released by the dehiscence of gametangial sheath in a posterior faced linear fashion by disintegration of thalli. This was brought about by providing an intense illumination at the end of dark period. The release was synchronous within each mature patch.

Keywords : “Gametangial dehiscence”; Gametophyte; GMC; Morphotype; Plastid; Seaweed

INTRODUCTION

The genus *Monostroma* (Ulotrichales, Chlorophyta) consists of green unistratose foliose species having a basic life history consisting of an alternation of heteromorphic generations; a haploid foliose thallus alternating with a diploid single codioid/cyst cell. Various life forms are known; sexual forms (dioecious/monoecious, isogamous/anisogamous), and asexual forms without a codiolum/cyst stage or with a codiolum/cyst stage (produced via parthenogenetic female pseudogametes). This genus has recently attracted attention from the molecular systematists who argue for a synthesis of new phylogenetic classification due to apparent polyphyly (van Oppen 1995, Bast *et al.* 2009b). The species, *Monostroma latissimum* has a considerable economic value in Japan and is commercially cultivated by either natural (Ise Bay, Mie Prefecture) or artificial (Shimanto estuary, Kochi Prefecture) seeding methods.

Systematic studies on this genus are mainly based on life cycle and thallus ontogeny (Tatewaki 1972, Bast *et al.* 2009c). However, gametophytic characters have been used in some cases and can contribute to a better understanding of the evolutionary lineages within this

genus (Dube 1967, Golden & Garbary 1984). Gayral (1964) has found two types of flagellate release mechanisms in this genus, *viz.*, those in which release occurs through a small pore in gametangial cell wall with prolonged survival of the parent thalli and those in which release occurs by dehiscence with destruction of the mother thalli. Gayral (1964) has argued that the characteristic of flagellate release by dehiscence, as in the case of *Gayralia oxysperma* (synonymous to *Monostroma oxyspermum*; Bast *et al.* 2009b), should be considered as a key taxonomic feature for this genus and supported Papenfuss' (1960) argument that the lectotype of this genus should be *M. oxyspermum*. Zoid release mechanisms hitherto known in monostromatic green algae are summarized in Table 1.

Monostroma latissimum (Wittrock 1866) is a dioecious species of this genus that has both sexual (heteromorphic haplo-diplontic) and asexual life cycle (Bast *et al.* 2009b & 2009c). Reproduction in the sexual plants of this species is reported to be slightly anisogamous (Kida 1990, Bast *et al.* 2009a), thereby defining the sex of gametes (larger gamete is considered female) and secondary sex ratio is 1:1 (Bast *et al.* 2009a). Despite life cycle and thallus ontogeny of *M. latissimum*

Table 1 — Zoid release mechanisms hitherto known in monostromatic green algae.

Genus	Flagellate release
<i>Monostroma</i>	Dehiscent (This study)
<i>Gayralia</i> ¹	Dehiscent (Gayral 1964, Vinogradova 1969)
<i>Protomonostroma</i>	Dehiscent (Vinogradova 1969)
<i>Ulvopsis</i>	Pored (Gayral 1964)
<i>Kornmannia</i>	Pored (Tatewaki 1972)
<i>Ulvaria</i>	Pored (Gayral 1964)
<i>Capsosiphon</i>	Pored (Tatewaki 1972)

¹A synonymy of *Gayralia* with *Monostroma* had been proposed by Bast *et al.* 2009b and adopted in this report.

had been studied extensively (Yoshida 1967, Kida 1990, Bast *et al.* 2009c), the reports on its gametangial development or gamete release mechanisms are not available. Objectives of the present study are to understand ontogenetic patterns during the gametogenesis and gamete release, as well as to find in which group of Gayral's classification this alga belongs. Based on the findings of the present study, an evaluation of the taxonomical implications of gamete release mechanisms in this and related species is discussed.

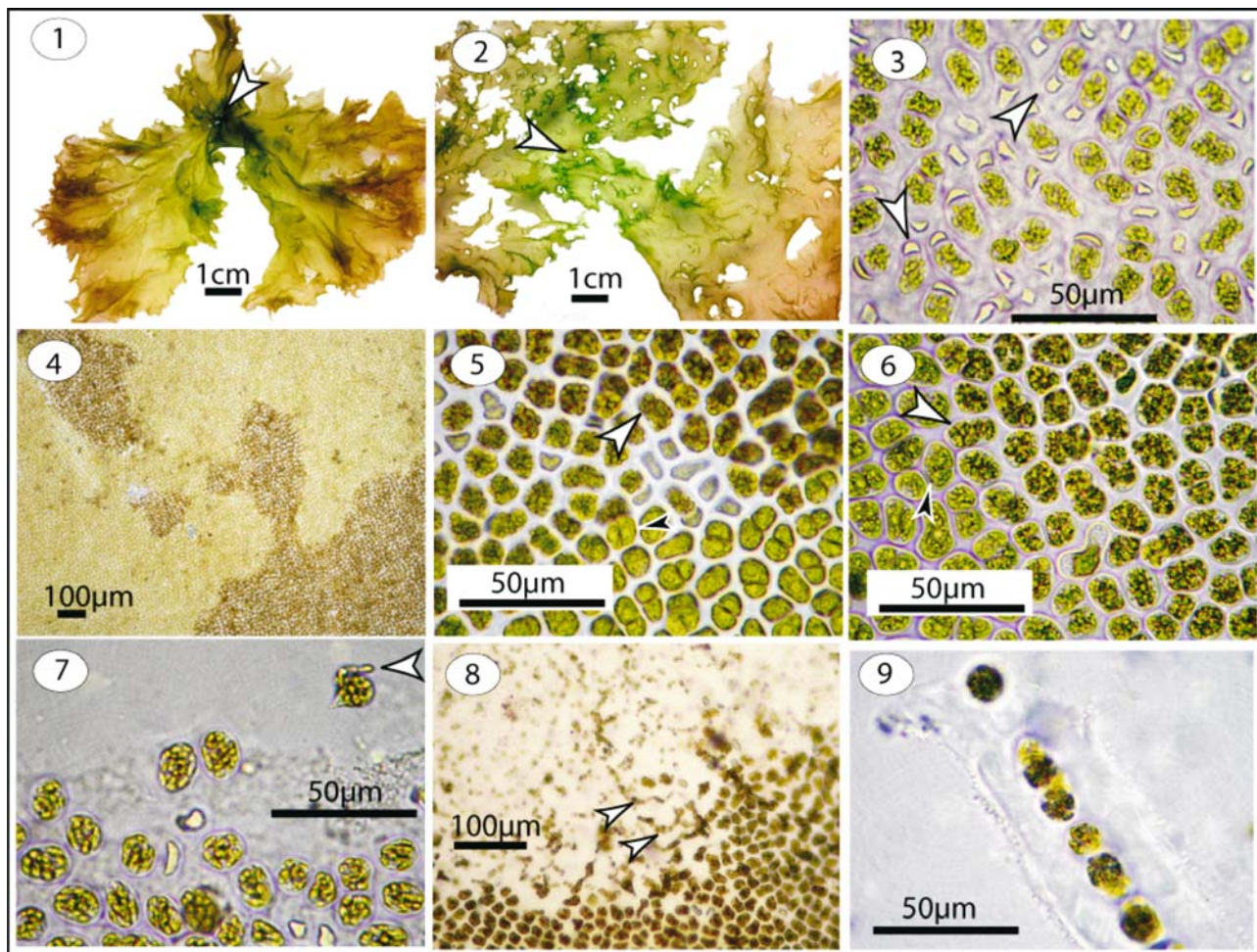
MATERIALS AND METHODS

Mature gametophytes of the algae identified as *Monostroma latissimum* were collected from a sheltered habitat at Uranouchi Inlet, Kochi Prefecture, Japan (33°43'48.77"N, 133°44'33.99"E) shortly before sunset (18:30 h) on 29th April, 2009 and samples were transported to the laboratory in sealable polyethylene bags. Maturation is clearly discernable by the yellow-green coloration of the apical regions of the fronds (Figs. 3.1 and 3.2). Thalli were washed in fresh water, carefully not to remove mature regions. Each thallus was separately incubated in a glass dish containing untreated seawater at room temperature (20–25° C) under dark conditions. At the sunrise of the following day (06:00 h), dishes were illuminated from the side by placing within 30 cm from two 15W cool white fluorescent lamps (actual luminous intensity was not measured) to induce gametangial dehiscence. The sex of plants was determined by the size of gametes and crossing tests against known types. Mating within and

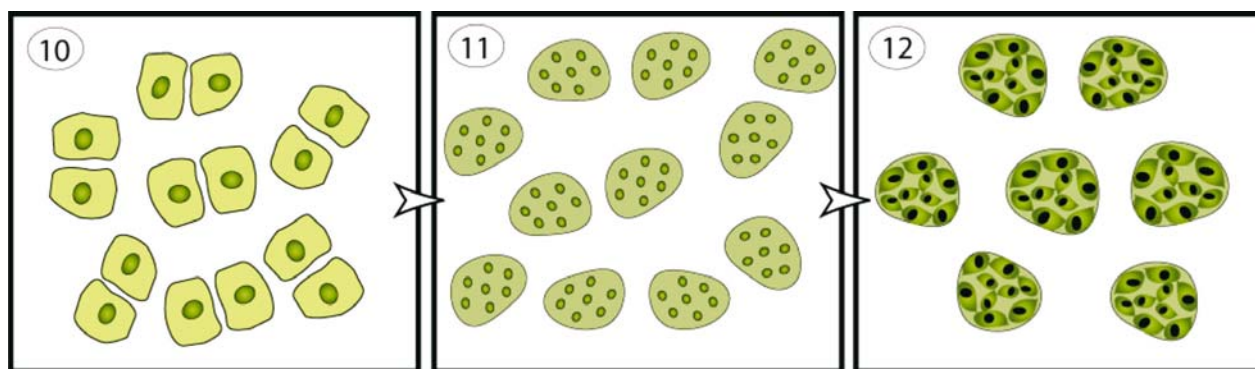
between the two morphotypes were conducted. Different parts of the thalli were dissected along the maturing mid region towards the mature thallus apex, unstained sections were observed under a microscope and photographs were taken using a digital camera. The experiments were repeated 6 times on different days.

RESULTS

Two kinds of morphotypes were observed, viz., 'smooth type' in which fronds have a smooth consistency and devoid of any pores (Fig. 1), and 'rough type' in which fronds have a rough consistency with numerous pores (Fig. 2). We did not observe any herbivores attached on the rough morph and thus the pores are unlikely be due to herbivory. Both male and female gametes of the two morphotypes cross-fertilized. Subsequent observations on developmental patterns were based on female gametophytes of rough morph, however, no conspicuous differences were observed between male and female gametangia, or between the two morphs. Intercalating crescent-shaped colorless islets were observed in the apical regions of the thalli, sometimes attached with the cells (Fig. 3). Gametangial formation and maturation occurred in discontinuous patches (Fig. 4) along apical regions of the fronds in which only parts of the blade mature at a time. Division between reproductive and vegetative regions of the blade is usually sharp when observed under low magnification (40X). Mature patches of the thalli appeared dark (Figs. 5 & 6) and bordered either with light green vegetative regions (Fig. 5) or with dark green maturing regions (Fig. 6). Cells in vegetative, maturing and matured regions have diameters, $7.23 \pm 1.35 \mu\text{m}$, $12.61 \pm 1.62 \mu\text{m}$ and $15.94 \pm 1.89 \mu\text{m}$, respectively (mean \pm SD; n=30). Vegetative regions have one, rarely two, prominent plastid inside each cell and maturing regions have divided plastids (>5 per cell). Mature regions have a discernable shape- with clusters of gametes, noticeable by its chromatic eyespots, arranged in a circular fashion that protrudes from the gametangia. Prior to gamete release, gametangial mother cells (GMC) disintegrated from the mother thalli (Fig. 7). Gametes liberated in a posterior faced (Fig. 7) linear fashion (Fig. 8), resembling swimming backwards in a row. All the fertile parts of the blade released gametes synchronously. Once gametes were liberated, no cytoplasmic material or the cell wall was visible on the completely disintegrated gametangia (analogous to holocarp). Cross section of the mature patches showed a lateral view of the gametangium (Fig. 9) in which no release pore was observed. Mature frondal parts have a thickness of $44.06 \pm 3.56 \mu\text{m}$ (mean \pm SD; n=30).



Figs. 1-9 — Habit of *Monostroma latissimum*. Relative position of rhizoid is indicated with *arrows*. **1.** Smooth morphotype. **2.** Rough morphotype. **Figs. 3-9.** Different stages in gametangial ontogeny of *M. latissimum*. **3.** Colorless crescent-shaped islets. **4.** Patches of mature gametangia in the peripheral regions. **5.** Mature regions (white arrow) bordering with vegetative regions (black arrow). **6.** Mature regions (white arrow) bordering with maturing regions (black arrow). **Figs. 7 and 8.** Gamete liberation. **9.** Cross section of mature thalli.



Figs. 10-12 — Schematic illustration of a group of cells during gametogenesis. **10.** Immature GMCs are tightly paired, smallest, quadrilateral and appears lightest with one distinguishable plastid. **11.** Semi-mature GMCs, loosely paired, intermediate in size, spherical and dark in appearance. Plastids are divided and distributed in the cytoplasm. **12.** Mature, largest, spherical and darkest appearing and loosely arranged GMCs. Each of them contains numerous gametes with an eye spot. They are arranged in a typically circular fashion.

DISCUSSION

Two morphotypes of *M. latissimum* were found but present observations are insufficient to raise each type to species level. Our observations of positive crossing between them and similar pattern of gametangial ontogeny suggest that these two are conspecific morphotypes. In order to determine whether these two morphs are genetic variants, further molecular studies (screening for single nucleotide polymorphisms) are necessary.

The present study has established for the first time that the gamete release in *M. latissimum* is brought about by the dehiscence of gametangial sheath, leading to the destruction of the mother thalli. The self-destructive reproductive investment of the thalli might be an adaptive strategy to increase the quality or quantity of the gametes thereby to increase the chance of successful fertilization. Present light microscopic observation revealed the basic gamete release mechanism. Thus, there is an ample scope for further studies in order to understand this process and its functional significance. For further analysis of gametangial ontogeny, histochemical and electron microscopic investigations of the gametangial sheath before and during the gamete release should also be performed. Based upon the findings of the present study, it can be presumed that the gametangial maturation in *M. latissimum* reflect *a priori* expectation i.e. the plastid division precedes gamete formation in plant cells. A group of cells undergoing gametogenesis are schematically illustrated in Figs. 10-12 to compliment our explanations on the change in shape and arrangement of gametangia and plastids upon maturation.

Our observations indicate that the gametangial maturation occurs in patches. This suggests the presence of a synchronous gametangial maturation induction mechanism in the group of cells of each patch. It is generally known that the gametogenesis in algal thalli takes place in response to a different cue from the one that confers an overall seasonality to its life cycle. It has been known and utilized for years that tearing of algal fronds, and subsequently transferring to fresh media, facilitates gametogenesis in a related genus *Ulva* (Nordby & Hoxmark 1972), although such a phenomenon was not observed in presently studied algae. In the present study, we were successful in inducing the gamete release in matured gametophytes of *M. latissimum* by providing an intense illumination at the end of dark period. However, the mechanism of light-strongest known zeitgeber in any biological system which might have triggered gametangial dehiscence remains unanswered. Because crescent-shaped cells are

observed only in the apical parts, it is presumable that these cells might have some functional role in either induction of gametangial maturation or the liberation of gametes. Similar islets of crescent-shaped cells have also been reported in the apical regions of a related alga *M. oxyspermum* (Brodie *et al.* 2008, as *Gayralia oxysperma*). In the green algae *Chlamydomonas*, blue light responsive gene products have been discovered to be involved in the induction of gametogenesis (von Gromoff & Beck 1993, Pan *et al.* 1996). Detailed molecular studies are needed to investigate gametogenesis/gametangial dehiscence induction mechanisms in *Monostroma*.

By deciphering developmental processes in the gametangial formation and maturation, we have also attempted to explain why the mature parts of the thalli appear yellowish. It can be observed that chlorophyll-containing plastids of the GMCs divide to distribute it to the daughter gametes, which has been reported to contain an eyespot (Bast *et al.* 2009a). The yellowish coloration of matured parts might be attributed to the production of these photopigment-containing eyespots during the gametogenesis. Vacuoles within the gametangia of *Ulva* (Tatewaki 1979) and *Bryopsis* (Okada *et al.* 1987) are reported to contain several water-soluble yellow-red pigments and an existence of such a pigment system might also be present in our algae. Chromatological studies on the eyespots are warranted to address this phenomenon in greater detail.

As expected from the similarity in morphology and thallus ontogeny, cytology of gamete formation and liberation in *M. latissimum* closely resembles that of *M. oxyspermum* and *Protomonostroma undulatum* rather than that of the other members in this genus. Further, zooids of *M. oxyspermum* are biflagellate and shares similar pyrenoid ultrastructure (Hori 1973) as that of *M. latissimum*. On the other hand, zooids of *P. undulatum* are quadriflagellate and pattern of its pyrenoids are distinctly different from that of *M. latissimum* (Hori 1973). Results of a phylogenetic analysis based on nuclear DNA internal transcribed spacer sequences conducted in Monostromataceae also suggest that *M. latissimum* and *M. oxyspermum*, along with a related species *M. nitidum*, form a distinct taxonomical clade (Bast *et al.* 2009b). By following Gayral's taxonomical revisions based on gamete releasing mechanisms, it can be deduced from our findings that *M. latissimum* ought to be placed within this genus even though Gayral intended *Monostroma* only for the asexual members. Given the shared ontogeny, life cycle and pyrenoid structure, *M. nitidum* might also undergo similar events in gametogenesis as described here. We have not attempted to examine the nuclear degree of ploidy in this study. It is expected that the gametogenesis

described here is mitotic, as in the case of other sexually reproducing monostromatic green algae reported elsewhere (Tatewaki 1972).

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