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Embryological Studies on Sargassum.

By

SHUMPEI INOH.
(Biological Institute, Tōhoku Imperial University, Sendai.)
(With 13 Text-figures)

INTRODUCTION.

Embryological studies on Sargassum, Turbinaria (?), Cystophyllum and Coccophora, all members of Fucaceae, have recently been carried out by TAHARA (1928, '29) and OKABE (1929). The results of these authors appear to throw some light on the systematic position of these algae.

Among the genera above mentioned, Sargassum is the largest one. According to YENDO'S monograph on Japanese Fucaceae (1907) we have 41 species of Sargassum on our coast. To our regret, however, hitherto only two species, S. Horneri and S. Thunbergii have been investigated embryologically. So, at the suggestion of Prof. TAHARA further studies in this line on a fairly large number of species of Sargassum have been undertaken since the spring of last year.

MATERIALS AND METHOD.

As is well known, the liberation of sexual cells in Sargassum occurs simultaneously and periodically and, in most species, usually in the spring tide. The season of the ripening of sexual cells is different in each species, so I visited the Marine Biological Stations at Misaki and Asamushi several times from March to July to collect the materials for the study. The dates of oogonium liberations in the different species are shown in the following table:
TABLE I.

<table>
<thead>
<tr>
<th>Specific name</th>
<th>Nom. Jap.</th>
<th>Date of Oogonium liberation</th>
<th>Locality</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sargassum Horneri AG.</td>
<td>Aka-moku</td>
<td>March 16th</td>
<td>Misaki</td>
</tr>
<tr>
<td>... enerse AG.</td>
<td>Hon-dawara</td>
<td>March 16th and May 15th</td>
<td>&quot;</td>
</tr>
<tr>
<td>... Kjellmanianum YENDO</td>
<td>Hahaki-moku</td>
<td>March 18th and May 10th</td>
<td>&quot;</td>
</tr>
<tr>
<td>... tortile AG.</td>
<td>Yore-moku</td>
<td>May 9th</td>
<td>&quot;</td>
</tr>
<tr>
<td>... piluliferum AG.</td>
<td>Mame-tawara</td>
<td>May 12th</td>
<td>&quot;</td>
</tr>
<tr>
<td>... hemiphyllum AG.</td>
<td>Iso-moku</td>
<td>May 13th</td>
<td>&quot;</td>
</tr>
<tr>
<td>... nigrofolium YENDO</td>
<td>Narasa-mo</td>
<td>May 13th</td>
<td>&quot;</td>
</tr>
<tr>
<td>... confusum AG.</td>
<td>Fushisuzi-moku</td>
<td>May 30th</td>
<td>Asamushi</td>
</tr>
<tr>
<td>... patens AG.</td>
<td>Yatsumata-moku</td>
<td>May 20th</td>
<td>Misaki</td>
</tr>
<tr>
<td>... Ringgoldianum HARV.</td>
<td>Ooba-moku</td>
<td>June 22th</td>
<td>&quot;</td>
</tr>
<tr>
<td>... serratfolium AG.</td>
<td>Nokogiri-moku</td>
<td>June 24th</td>
<td>&quot;</td>
</tr>
<tr>
<td>... micracanthum (KÜTL.) YENDO</td>
<td>Toge-moku</td>
<td>July 7th</td>
<td>&quot;</td>
</tr>
</tbody>
</table>

The days of new and full moon of last year are shown in Table II.

TABLE II.

<table>
<thead>
<tr>
<th>Month</th>
<th>March</th>
<th>April</th>
<th>May</th>
<th>June</th>
<th>July</th>
</tr>
</thead>
<tbody>
<tr>
<td>New moon</td>
<td>11</td>
<td>10</td>
<td>9</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>Full moon</td>
<td>25</td>
<td>24</td>
<td>23</td>
<td>22</td>
<td>22</td>
</tr>
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</table>

From these tables, it will be seen that in each species the liberation of sexual cells occurs generally in the spring tide.

To make a study of the embryonal development, in the first place some small branches of those algae which carried many discharged eggs on the surface of their receptacles were collected and cultured in small glass basins filled with natural sea water.

The discharged eggs remain attached to the outer surface of the receptacle for several days and begin to develop there. But before the rhizoid formation begins in the lower extremity of the embryos,
they are detached from the receptacles and fall to the bottom of the glass basin and further development continues in that condition.

For the fixation of the embryos, Flemming's weaker solution prepared with sea water was used exclusively, and the microtome sections, generally cut in 5–10 μ, were stained with safranin and light-green.

OBSERVATION.

Eggs discharged from the conceptacle are generally ovoid or ellipsoid in shape and are covered with a thick layer of gelatinous substance. The first segmentation-wall runs transversely. The second one is also transverse and cuts a small lense-shaped rhizoid cell in the lower extremity of the embryo.

The further segmentations of this rhizoid-cell are quite interesting and make a significant characteristic of each species. In the present paper particular attention is paid to this point. Detailed description will be given below.

PLANTS INVESTIGATED.

1. Sargassum hemiphyllum Ag.

This plant is strictly dioecious and receptacles of both sexes have a similar external appearance. They are small and cylindrical, generally measuring about 2 mm. long, and ripen at Misaki about the middle of May. The discharged eggs measure 125 μ long and 105 μ wide.

The first two segmentation walls in the rhizoid cell run vertically through the center of the cell and are perpendicular to each other. But the third goes quite irregularly. Some are parallel with the former ones, while some are oblique but without passing through the center, just as described in S. Thunbergii by TAHARA (1929) (Text-fig. 1). The rhizoid cell in the eight-cell stage is about 43 μ in diameter. Later a group of eight rhizoids is developed, one rhizoid being developed in each cell.
Text-fig. 1. *Sargassum hemiphyllum.* a, Four cell stage of the rhizoid cell. b, Eight cell stage of the same. c, Longitudinal section of the rhizoidal portion of an embryo. d, The same in a still later stage. ×420.

Text-fig. 2. Cross section of a receptacle of *Sargassum Kjellmanianum.* ×72.

II. *Sargassum Kjellmanianum* YENDO.

This plant is typically monoecious. The male and female conceptacles are contained in the same cylindrical receptacle, which measures about 10–12 mm. long (Text-fig. 2). The receptacles ripen at Misaki generally from the middle of March to the latter part of April. The discharged eggs mea-
sure 139 μ long and 97 μ wide.

The manner of embryonal development of this species is just the same as that of *S. hemiphyllum*. The rhizoid cell in the eight-cell stage is about 63 μ in diameter, that is, slightly larger than that of the former species. Rarely, the rhizoid cell of this plant is divided into nine or ten cells (Text-fig 3. d), a feature perhaps suggesting that this species is higher in systematic position than the former one.

### III. *Sargassum confusum* AG.

Dioecceous. Receptacles cylindrical, its entire length being 10–15 mm. in the male, and 7–10 mm. in the female. Germ-cell liberations occur at Asamushi from the latter part of May to the middle of June. The discharged eggs measure 210 μ long and 140 μ wide.

The rhizoid cell is divided into eight (rarely nine or ten) cells, as in *S. Kjellmanianum* (Text-fig. 4), and measures, in this stage, about 60 μ in diameter, that is, about the same as that of the former species.

### IV. *Sargassum enerve* AG.

Dioecceous. The male receptacles are slender, often measuring 18 mm. long, while the female ones are fatty, 13–15 mm. long. They
Text-fig. 4. *Sargassum confusum.* a, Longitudinal section of the rhizoidal portion of an embryo. b₁, b₂, b₃, Eight cell stage of the rhizoid cell. c, Rhizoid cell divided into nine cells. ×420.

Text-fig. 5. *Sargassum enerve.* a, Longitudinal section of the rhizoidal portion in the sixteen cell stage. b, The same in a still later stage. c, Eight cell stage of the rhizoid cell. d, Sixteen cell stage of the same. ×420.
ripen at Misaki from the beginning of January to the latter part of April. The discharged eggs measure 250 $\mu$ long and 235 $\mu$ wide, about twice as large as the ones of *S. hemiphyllum*.

The rhizoid cell division of this species differs from that in the foregoing ones. At first it divides into eight cells, nothing unusual up to this stage. But then each of these eight cells divides once more and sixteen cells are produced, as shown in Fig. 5, and the rhizoid formation begins in this stage. The rhizoid cell of the 16-cell stage measures about 75 $\mu$ in diameter.

Later, a group of sixteen rhizoids is developed at one extremity of the embryo; rhizoids which have no direct relation to the rhizoid cell can not be seen, at least in the early stage of development.

V. *Sargassum piluliferum* Ag.

Dioeceous. Receptacles small, cylindrical, measuring 3–8 mm. long; the male ones slightly longer than the female. Ripen at Misaki about May. The rhizoid cell is divided into sixteen cells as in the former one and measures, in this stage, about 72 $\mu$ in diameter.

The fourth division of the rhizoid cell of this species is sometimes omitted in 3 or 4 cells, thus producing only 12 or 13 cells in the final stage (Text-fig. 6. b, c).

VI. *Sargassum patens* Ag.

Dioeceous. Receptacles linear, simple or sometimes divided, measuring 5–7 mm. long. Ripen at Misaki about June. The discharged eggs measure 218 $\mu$ long, 177 $\mu$ wide. The rhizoid cell is divided into sixteen cells and measures, in this stage, 67 $\mu$ in diameter.
The fourth division of the rhizoid cell is omitted sometimes in 3 or 4 cells, as in the former species (Text-fig. 7. d₁, d₂). The fourth

Text-fig. 7. *Sargassum patens*. a, Longitudinal section of an embryo. b, The rhizoidal portion in a still later stage. c, Sixteen cell stage of the rhizoid cell. d₁, d₂, Rhizoid cell divided into only twelve cells. e, Two-storied cell arrangement in the rhizoidal portion. f, The same in a still later stage. ×420.
division is, however, occasionally carried out parallel to the outer surface, forming, as a result, a two-storied cell arrangement in the rhizoidal portion, as seen in *Cystophyllum sisymbrioides* (Okabe 1929) (Text-fig. 7. e).

VII. *Sargassum Ringgoldianum* Harv.

Dioecious. Receptacles differ greatly in external shape according to sex. The male receptacles are linear-spathulate, measuring 5 cm. long and 7 mm. wide, while the female ones are compressed siliquaeform, measuring 11 mm. long and 3 mm. wide. Germ-cell liberations occur at Misaki from the latter part of June to the middle of July. The discharged eggs measure about 222 \( \mu \) long, 135 \( \mu \) wide. The

![Text-fig. 8. *Sargassum Ringgoldianum*. a, e, Longitudinal section of the rhizoidal portion. b, The same in a still later stage. c, Sixteen cell stage of the rhizoid cell. d, Rhizoid cell divided into 17 cells. \( \times 420 \).](image)
rhizoid cell is divided into sixteen cells and measures, in this stage, about 75 μ in diameter (Text-fig. 8).

It is noteworthy that in this species one or two of the sixteen cells are rarely divided once more and 17 or 18 cells are produced, as shown in Fig. 8. d.

VIII. *Sargassum nigrifolium* YENDO.

Dioecious. Receptacles spathulate or subcuneate, with a few dentations at the apex, often measuring 7 mm. long and 5 mm. wide. They ripen at Misaki about May. The discharged eggs measure 264 μ long and 236 μ wide.

Unfortunately, owing to the failure of the culture, only a very few embryos were available and the exact manner of cell division of the rhizoid cell could not be ascertained in this plant. But I suppose it may be the same as found in *S. enerve*, for about 16 rhizoids were counted in the lower extremity of the embryo.

IX. *Sargassum serratifolium* AG.

Dioecious. Receptacles complanated, spathulate-clavate, often measuring 10–15 mm. long. They ripen at Misaki in June. The discharged eggs measure 275 μ long and 202 μ wide. The rhizoid cell is divided into sixteen cells and measures, in this stage, about

Text-fig. 9. *Sargassum serratifolium*. a, Longitudinal section of the rhizoidal portion. b₁, b₂, Sixteen cell stage of the rhizoid cell. ×420.
90 $\mu$ in diameter (Text-fig. 9).

X. *Sargassum tortile* Ag.

Dioecious. Receptacles linear-spathulate, often measuring 10-15 mm. long and ripening at Misaki from April to May. The discharged eggs measure 333 $\mu$ long and 236 $\mu$ wide. The rhizoid cell is divided also into 16 cells and measures, in this stage, 100 $\mu$ in diameter (Text-fig. 10).

XI. *Sargassum micracanthum* (Kütz.) Yendo.

Dioecious. Receptacles obovate, the apical margin minutely toothed. They ripen at Misaki from June to July. The egg of this plant is the largest in *Sargassum*, often measuring 384 $\mu$ long and 275 $\mu$ wide.

When I went to Misaki, it was too late to collect sufficient material for observation and I could not examine the manner of the rhizoid cell division or the number of rhizoids produced in young embryos.

XII. *Sargassum Horneri* Ag.

The embryological study of this plant has been carried out already by Tahara. My own observation at Misaki entirely coincides with his.

The root of this plant, differing from that of other species, is scutellate, irregularly lobed on the margin. Also dioecious. The large female receptacles are cylindrical, often measuring 20-30 mm. and the male ones are two or three times longer than the female. They ripen at Misaki from January to April. The discharged eggs measure 264 $\mu$ long and 198 $\mu$ wide.
As Tahara describes, through three successive divisions the rhizoid cell is divided into eight radially arranged cells and at this stage rhizoid formation begins. At first, eight rhizoids originating from the rhizoid cell are observed, but soon another group of rhizoids developed from the cells adjacent to the rhizoid cells are seen. This group of rhizoid protrudes outward through the central crevice among the group of primary rhizoids (Text-fig. 11).

**DISCUSSION AND CONCLUSION.**

From the results above described, the rhizoid formation in *Sargassum* will be divided into three types; namely irregular eight-cell type, sixteen-cell type, and radial eight-cell type.  

i) Irregular eight-cell type.  

In this type the rhizoid cell is divided into eight irregularly arranged cells. Later a group of eight rhizoids is developed at one extremity of the young embryo. *S. Thunbergii, S. hemiphyllum, S. Kjellmanianum* and *S. confusum* belong to this type.

ii) Sixteen-cell type.  

In this type the rhizoid cell is divided into sixteen irregularly arranged cells. Later a group of sixteen rhizoids is developed at the lower extremity of the young embryo. *S. piluliferum, S. patens, S. enerve, S. Ringgoldianum, S. tortile* and *S. serratifolium* belong to this type.

iii) Radial eight-cell type.  

In this type the rhizoid cell is divided into eight radially arranged cells. In addition to the eight rhizoids originating from the rhizoid
Text-fig. 12. Young embryos having developed their primary rhizoids: 1, Sargassum hemiphyllum. 2, Sargassum confusum (×196). 3, Sargassum enerve (×170). 4, Sargassum patens (×252).
cell, another group of rhizoids which are produced from the cells situated just behind the rhizoid cell are seen in the early stage of embryonal development. Only *S. Horneri* belong to this type.

The plants belonging to the first type, for example *S. hemiphyllum*, *S. Thunbergii* and *S. Kjellmanianum*, generally have a filiform leaf attached to the base of the stipe of the receptacle. It seems to me that this is a feature common to all species of the first type. Further, it may be mentioned that in general the species belonging to the irregular 8-cell type are much smaller in stature and grow on rocks near to the low-tide mark or in slightly deeper places.

*S. patens* and *S. piluliferum*, which in embryonal rhizoid formation show an intermediate form between the first and the second type, have in the upper portion also linear or filiform receptacular leaves.

*S. Horneri*, which belongs to the third type, has a number of peculiar characteristics. The peculiar scutellate root, irregularly lobed on the margin (*Yendo, Okamura 1907, '23*), is quite unique. This kind of root can not be found in any other species examined. Furthermore, *S. Horneri* has exceptionally large receptacles, as already mentioned. By some algologists this species is placed under the subgen. *Bacterophycus*, with *S. enerve*, *S. serratifolium* and *S. tortile*. But it seems to me that this alga should be displaced to the other systematic position.

The size of the discharged eggs of each species is different. It seems to me that the algae having larger eggs are higher in systematic position. Data concerning this point are given in the Table III.

As we see from the table, there is a remarkable difference in the size of the eggs. Generally speaking, the eggs of the 16-cell type are much larger than those of the irregular 8-cell type. The average area of the primary rhizoid cell in the former is about twice that of the latter. It may be worth mentioning here that the primary rhizoid cell of the eggs of *Cystophyllum sisymbrioides*, which, as *Okabe*’s recent studies have shown, can be called a 32-cell type, is twice as large as that of the average eggs of the 16-cell type. Thus it is apparent that the number of rhizoids to be developed in the early stage of embryo-formation has a definite relation to the size of the primary rhizoid cell.
In my opinion, the species, eggs of which are relatively larger, should be placed in a higher systematic position. Thus the most primitive species in *Sargassum* is to be found in the group of the irregular eight-cell type. *Cystophyllum*, which perhaps may have descended from a *Sargassum* of the 16-cell type has, so far as examined, still larger eggs than any species of *Sargassum* of the 16-cell type.
and should rightly be placed in a higher systematic position than *Sargassum*.

In *Turbinaria (?) fusiformis*, which some algologists think should be placed under *Sargassum*, the rhizoid formation (TAHARA '29) resembles closely that of the irregular 8-cell type. So this opinion appears to receive an affirmation from the embryological studies.

**SUMMARY.**

i) The manner of the rhizoid formation in *Sargassum* is divided into three types; irregular 8-cell type, 16-cell type, and radial 8-cell type.

ii) The rhizoid number in *Sargassum* and *Cystophyllum* has a definite relation to the size of the primary rhizoid cell.

iii) The species having larger eggs are higher in systematic position.

In conclusion, I wish to express my hearty thanks to Professor M. TAHARA for his valuable suggestions and assistance given me during the progress of this work, and to Dr. K. OKAMURA, Director of the Imperial Fisheries Institute, for his help in the identification of my materials, and to Prof. YATSU, Director of the Misaki Marine Biological Station, through whose kindness many facilities were afforded in the course of my investigations.

**LITERATURE CITED.**