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Embryological Studies on *Sargassum* and *Cystophyllum*

By

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(With 7 Text-figures)

INTRODUCTION

In the author's previous paper (4), he described three types of rhizoid formation in ten species of *Sargassum*. It will be of interest to determine whether or not other species of *Sargassum* can be divided into these three types concerning the rhizoid formation.

The present paper deals with *S. nigrifolium* and *S. micracanthum*, the rhizoid formation of which have remained undecided in the foregoing paper and also here with *S. tosaense*.

A species of the allied genus, *Cystophyllum hakodatense* was also studied for the comparison with *Sargassum*.

MATERIALS AND METHODS

Seeking the adequate time of ripening of sexual cells in each species, the author visited the Misaki Marine Biological Station in May 1930, the Seto Marine Biological Laboratory in June of the same year and the Oshoro Marine Laboratory in June and July 1931.

The discharged eggs of each species were collected on the days shown in the following Table I and left to develop in glass basins containing natural sea-water, the method of culture being the same as stated in the previous paper (4).

TABLE I.

Species	Nom. Jap.	Date of Oogonium liberation	Locality
<i>S. nigrifolium</i> Yendo	Narasa-mo	May 12th 1930	Misaki
<i>S. micracanthum</i> (Kütz.) Yendo	Toge-moku	May 13th 1930	„
<i>S. tosaense</i> Yendo	Tatsukuri	June 11th 1930	Seto
<i>C. hakodatense</i> Yendo	Ugano-moku	June 22nd and July 12th 1931	Oshoro

For the fixation of the embryo in which the rhizoid-cell division was accomplished, Flemming's weaker solution prepared with sea water was exclusively used, and the microtome sections were generally cut 5-10 μ in thickness and stained with safranin and light green.

OBSERVATIONS

The discharged eggs have eight nuclei and are usually covered with a thick layer of gelatinous substance.

But in *Cystophyllum hakodatense* the liberated eggs have only a central nucleus similar to *Coccophora Langsdorfi* (8), and are covered simply by a layer of gelatinous substance, not at all entangled by the paraphyses protruded from conceptacle as in *C. sisymbrioides* (7).

In *Sargassum* and *Cystophyllum* the first segmentation-wall runs transversely. The second one is also transverse and cuts a rhizoid cell in the lower extremity of the embryo.

The author's intention is particularly devoted to giving full information on the further segmentation of the rhizoid cell in each species.

Plants Investigated

I) *Sargassum nigrifolium* Yendo

Dioecious. It ripens at Misaki about May. The discharged eggs measure 265 μ long and 236 μ wide. The rhizoid cell is divided into sixteen cells and measures, at this stage, about 78 μ in diameter. (Fig. 1).

Later a group of sixteen rhizoids is developed at one extremity of the embryo.

II) *Sargassum micracanthum* (Kütz.) Yendo

Dioecious. It ripens at Misaki from May to July. The discharged eggs measure 384 μ long and 275 μ wide. The rhizoid cell is divided into sixteen cells and measures, at this stage, about 84 μ in diameter (Fig. 2).

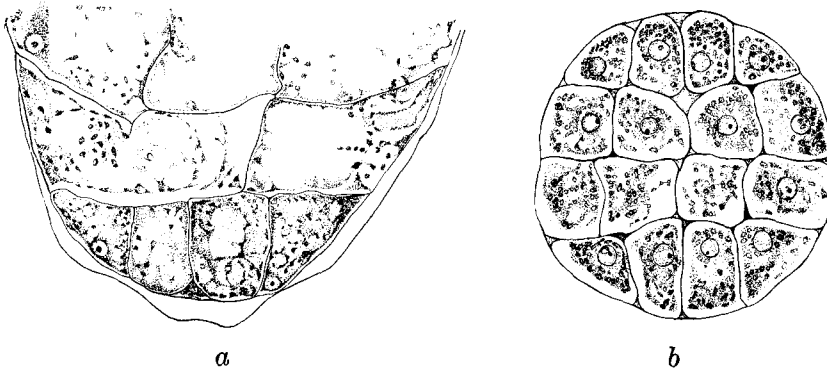


Fig. 1.

Text-fig. 1. *Sargassum nigrifolium*. *a*. Longitudinal section of the rhizoidal portion at the sixteen cell stage. *b*. Cross section of the same. ($\times 512$)

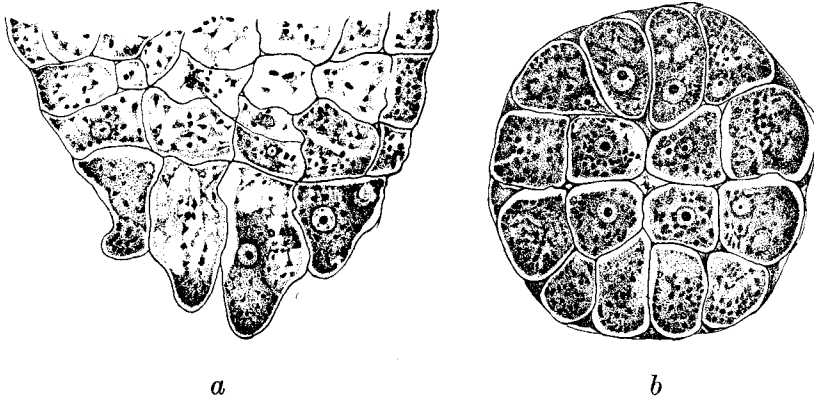


Fig. 2.

Text-fig. 2. *Sargassum micracanthum*. *a*. Longitudinal section of the rhizoidal portion at the sixteen cell stage. *b*. Cross section of the same. ($\times 512$)

Later a group of sixteen rhizoids is also developed at one extremity of the embryo (Fig. 3).

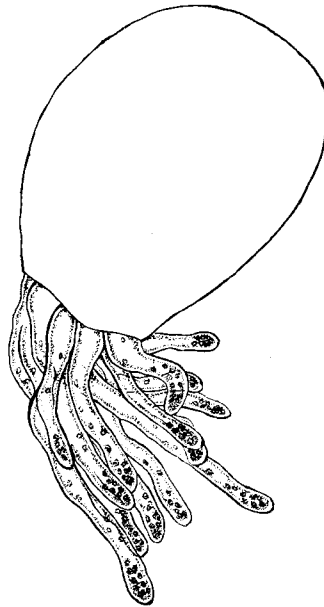


Fig. 3.

Text-fig. 3. Young embryo having developed primary rhizoids of *Sargassum micracanthum*. ($\times 190$)

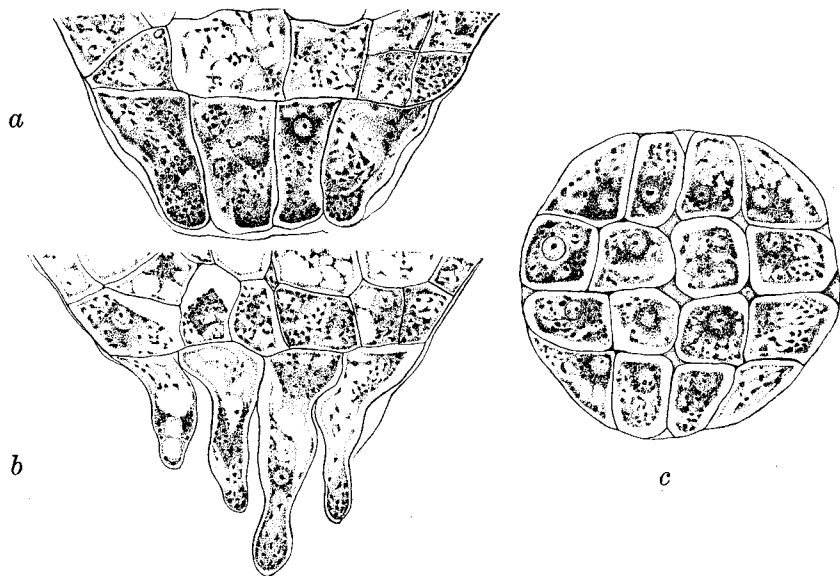


Fig. 4.

Text-fig. 4. *Sargassum tosaense*. *a*. Longitudinal section of the rhizoidal portion at the sixteen cell stage. *b*. The same at a still later stage. *c*. Cross section of the rhizoidal portion at the sixteen cell stage. ($\times 512$)

III) *Sargassum tosaense* Yendo

Dioecious. Receptacles small cylindrical, each with a short cylindrical stem below.

It ripens at Seto about June. The discharged eggs measure $220\ \mu$ long, by $180\ \mu$ wide. The rhizoid cell is divided into sixteen cells and measures, at this stage, $70\ \mu$ in diameter (Fig. 4).

Later a group of sixteen rhizoids is developed (Fig. 5).

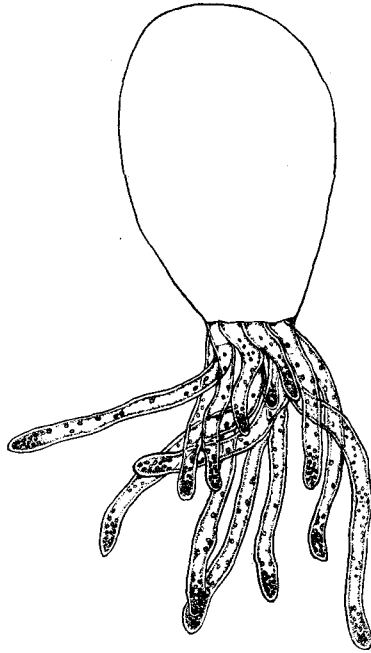


Fig. 5.

Text-fig. 5. Young embryo having developed primary rhizoids of *Sargassum tosaense*. ($\times 213$)

IV) *Cystophyllum hakodatense* Yendo

Dioecious. Receptacles lanceoid-cylindrical, acuminate above, with a filiform stipe below. It ripens at Oshoro about June. The discharged eggs have a single nucleus in the center and measure $120\ \mu$ long and $80\ \mu$ wide (Fig. 6. b). The first two segmentation-walls in the

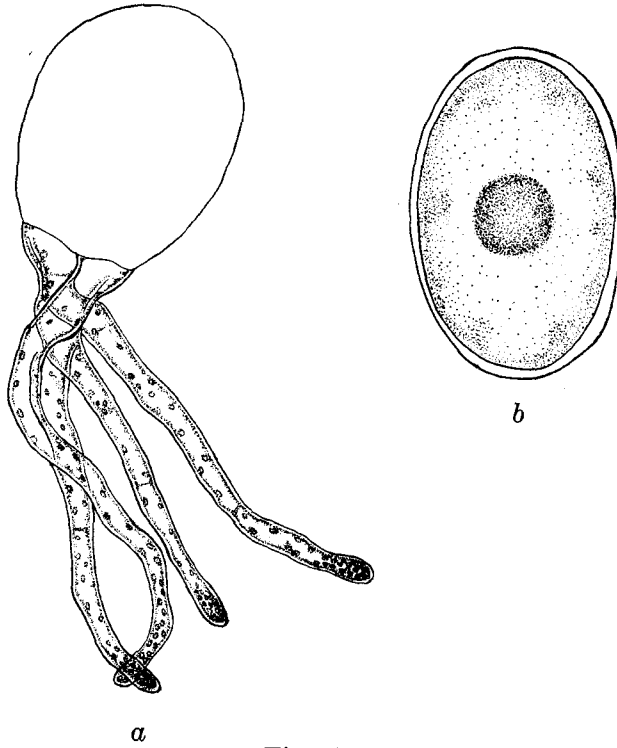


Fig. 6.

Text-fig. 6. *Cystophyllum hakodatense*. a. Young embryo having developed four primary rhizoids. b. Discharged egg. ($\times 320$)

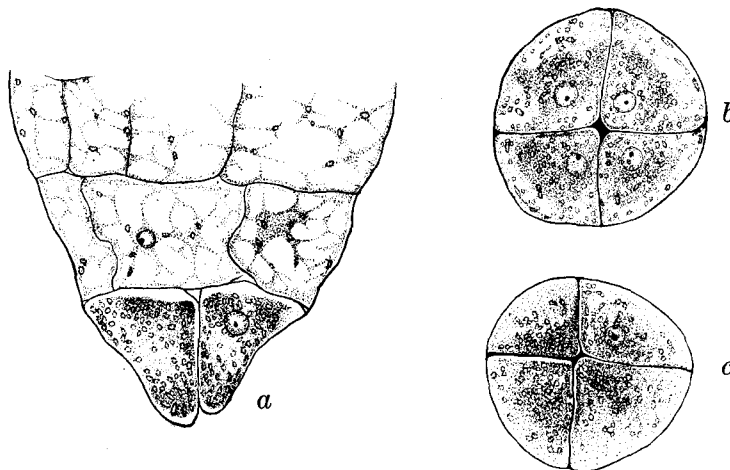


Fig. 7.

Text-fig. 7. *Cystophyllum hakodatense*. a. Longitudinal section of the rhizoidal portion at a stage still later than the four cell stage. b, c. Cross section of the rhizoidal portion at the four cell stage. ($\times 577$)

rhizoid cell run vertically through the center of the cell and are perpendicular to each other.

The rhizoid cell in the four cell stage is about $40\ \mu$ in diameter (Fig. 7).

Later four rhizoids are developed, one rhizoid being developed in each cell (Fig. 6. a).

DISCUSSION AND CONCLUSION

From the observations above-mentioned, it can be ascertained that *S. nigrifolium*, *S. micracanthum* and *S. tosaense* belong to the sixteen cell type (4) in respect to the rhizoid formation, and *Cystophyllum hakodatense* to the four cell type (new type).

Since *S. tosaense* and *S. patens* (4) resemble so closely with each other in several points, such as the size of the egg, the diameter of the rhizoid cell and the features of the rhizoid formation, it may be presumed that these two species are related very closely (9).

The average area of the rhizoid cell of *C. hakodatense* belonging to the four cell type is smaller than that of *Sargassum* belonging to the irregular eight cell type. This fact may be therefore clearly justified by the statement made in the previous paper that the primary rhizoid number in the early stage of the embryo development has a definite relation to the size of the rhizoid cell.

The discharged eggs attached to the outer surface of the receptacle of *C. hakodatense* are not entangled by paraphyses as those in *C. sisymbrioides* (7).

C. hakodatense differs also greatly from *C. sisymbrioides* and *C. Turneri* as to the rhizoid formation, the former belonging to the 4-cell type and the latter to the 32-cell type (4, 5.).

AGARDH classified *C. sisymbrioides* in one group and *C. crassipes* in another. These two groups are easily distinguished from each other by the difference in the upper part of the stem; in the former it is complanated and sends out branchlets in both side, whereas in the latter it is cylindrical and sends out branchlets in all direction (1).

Later, YENDO adopted his classification and considered that *C. Turneri* is a member of the former group and *C. hakodatense* belongs to the latter group.

In each group, the two species, *C. sisymbrioides* and *C. Turneri* on one hand and *C. crassipes* and *C. hakodatense* on the other hand are quite similar in general appearance, being distinguished only by the difference of vesicles and receptacles (10).

This will be of interest when we consider the embryological difference between these two types.

That the young embryo of *Cystoseira barbata* in the early stage has four rhizoids, has been reported by A. DODEL-PORT (2). From this embryological similarity between *Cystophyllum hakodatense* and *Cystoseira barbata*, these two species seem to be closely related systematically.

The remarkable character of *C. hakodatense* in which the eggs have generally only one central nucleus as in *Coccophora Langsdorfi*, seems to give some suggestion of a relationship between these species.

Summarising the author's observation, the comparison data of the size of eggs and the area of rhizoid cells in 15 species hitherto examined, are given below (Table II).

TABLE II.

Species	Long axis	Short axis	Diameter of rhizoid-cell	Type
<i>Cystophyllum hakodatense</i> Yendo	120	80	40	4-cell type
<i>Sargassum hemiphyllum</i> Ag.	125	103	43	irreg. 8-cell type
„ <i>Kjellmanianum</i> Yendo	139	97	63	„
„ <i>confusum</i> Ag.	210	140	64	„
„ <i>patens</i> Ag.	218	177	67	16-cell type
„ <i>tosaense</i> Yendo	220	180	70	„
„ <i>piluliferum</i> Ag.	235	110	72	„
„ <i>enerve</i> Ag.	250	235	75	„
„ <i>Ringgoldianum</i> Harv.	222	153	75	„
„ <i>nigrifolium</i> Yendo	264	236	78	„
„ <i>serratifolium</i> Ag.	275	202	90	„
„ <i>tortile</i> Ag.	333	236	100	„
„ <i>micracanthum</i> (Kütz.) Yendo	384	275	84	„
„ <i>Horneri</i> Ag.	264	198	83	Radial 8-cell type
<i>Cystophyllum sisymbrioides</i> Ag.	312	229	120	32-cell type

SUMMARY

i. *S. nigrifolium*, *S. micracanthum* and *S. tosaense* belong to the sixteen cell type.

ii. *C. hakodatense* belongs to the four cell type. In the genus *Cystophyllum*, therefore, two types (4-cell type and 32-cell type) are distinguished.

In conclusion, the writer wishes to express his thanks to Prof. Y. YAMADA for his help in the identification of the materials, and to Dr. M. TAHARA, Professor of Tōhoku Imperial University, and Prof. T. KOMAI, Director of Seto Marine Biological Laboratory, through whose kindness many facilities were afforded in the course of the investigation.

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