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Oogonium Liberation
and
the Embryogeny of Some Fucaceous Algae.

By

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With 3 Plates and 5 Text Figures.

It had long been my wish to make some biological as well as ytological observations on the representative members of Japanese ucaceae, as our knowledge of this family especially in respect of iese points is still very imperfect.

Taking the opportunity of a short stay at the Misaki Marine iological Station of the Tokyo Imperial University in the winter 1908–1909, I made some observations on *Sargassum*, especially n the liberation phenomena of oogonia. The results were ublished in a preliminary note in the Botanical Magazine, Tokyo, ol. XXIII. 1909.

To carry out more extensive studies, I made a second visit to ie station at the end of December, 1909 and stayed there for bout three months. The present paper presents the results of iat visit. The substance of it was reported on the 28th of April, 910, before a meeting of the Tokyo Botanical Society[1]; and riefer accounts were given in Japanese in the Bot. Mag. Tokyo. ol. XXV. 1911.

According to YENDO's well known monograph on Japanese ucaceae[2] there are eight genera of this family in Japan, namely, ucus, *Pelvetia**, *Ishige**, *Cystoseira*, *Cystophyllum*, *Coccophora*, *Turbi-*

1) Proceedings of the Tokyo Bot. Society. Bot. Mag. Vol. XXIV., P. (246).
2) YENDO, The Fucaceae of Japan. Journ. Coll. Sci., Imp. Univ. Tokyo. Vol. XXI., Art. 12.

*naria** and *Sargassum**; but only the four with asterisk are found at Misaki. Of these genera only a few species were available for my studies, as the other species did not come to maturity during my stay there. *Sargassum enerve*, *Sargassum Horneri* and *Cystophyllum sisymbrioides* furnished the principal materials of my investigation. All these three species are dioeceous. At Misaki *Sargassum enerve* grows in such profusion as often to present a serious obstacle to the navigation of smaller craft. The liberation of oogonia begins usually at the end of December.

Sargassum Horneri is also common at Misaki. The receptacle of this species are very large and well-suited for investigation. The liberation of oogonia begins at the end of December.

Cystophyllum sisymbrioides is not so common at Misaki as the former two, but it is by no means rare. The liberation of oogonia begins at about the middle of February.

First let me give my observations on oogonium liberation.

I. Oogonium Liberation.

In the preliminary paper mentioned above, I gave the following acount of my observations on the oogonium liberiation of *Sargassum*.—At that time I used the term 'Oosphere liberation' intead of 'Oogonium liberation.' But strictly speaking, the products liberated from the female receptacle on the day of the so-called 'Oosphere liberation' are not oospheres but oogonia containing in their bodies one oosphere initial.

"On the 24th of December 1908, the next day after the full moon, almost all individuals of *Sargassum enerve* of the coast discharged their oospheres simultaneously. The discharged oospheres stayed on the receptacle for about three days and then dropped off also simultaneously, so that on the 28th there was not a single stock that bore the sporelings on the receptacle.

"After a fortnight, i. e. on the day of new moon, the next general liberation of oospheres occurred."

These facts reminded me of the periodical liberation of sexual products in *Dictyota dichotoma* and led me to the conclusion that, "The liberation of oospheres in *Sargassum* takes place simultane-

ously, not only for a given plant, but also for all plants of the same locality. This simultaneous liberation proceeds in fortnightly crops on a particular day with a fixed interval after the highest spring tide, the interval varing however in different species."

As this conclusion was based on observations made during a relatively short time, naturally entire confidence could not be put in its validity. My second visit to Misaki was made mainly to determine this point.

I arrived at Misaki on the 27th of December, 1909, which was just the day before the highest spring tide. To my disappointment I found that most of the *Sargassum* growing in that locality had not yet attained their maturity and for some time no general oogonium liberation could be observed.

The first general oogonium liberation of *Sargassum enerve* occurred in fact on the 12th of January, the next day after the highest spring tide. Three days later, on the 15th of January, the first general oogonium liberation of *Sargassum Horneri* also took place.

So far these observations confirmed in the main the record of the preceding year. But the liberation went on thereafter quite irregularly, without showing any fixed relation to the highest spring tide. The actual state of things is shown in the following table.

Species	Jan.	Feb.
S. enerve	12* 21 31	11* 16
S. Horneri	15 23	2 14 21

The numbers in the table denote the dates of general oogonium liberations. The intervals between two successsive liberations is quite irregular, for example, the intervals in *Sargassum enerve* are 5, 6, 9, 10 or 11 days. But the intervals between the two corresponding liberations in *Sargassum enerve* and *Sargassum Horneri* are, as is seen from the table, tolerably constant; namely,

two or three days after the liberation of *Sargassum enerve* occurs almost always the liberation of *Sargassum Horneri*. The same relation is given in my record of the previous year.

The highest spring tides occurred on the 11th and 25th of January and on the 10th and 24th of February. The dates with asterisk in the table correspond to the days next after the highest spring tides.

I have also observed such oogonium liberation in *Sargassum Kjellmanianum*, *Sargassum tortil*, and *Cystophyllum sisymbrioides*. In these algae too, the successive liberations do not seem to show a fixed relation to the highest spring tide; for example, in *S. Kjellmanianum* the liberations took place on the 7th, 15th and 23rd of February and in *Cyst. sisymbrioides* on the 17th of February and the 3rd and 20th of March.

All these facts led me to the conclusion that the liberation of oogonia in *Sargassum* and *Cystophyllum* takes place periodically and simultaneously among individuals of the same species growing in the same locality; but the intervals between two successive liberation vary in an irregular manner, without having at least any fixed relation to the highest spring tide. Thus the liberation phenomena in our plants are somewhat different from those in *Dictyota dichotoma*, the periodicity of which was studied first by WILLIAMS[1] and recently by HOYT[2] and LEWIS.[3] Probably owing to differences in local conditions, the result of the observations of these authors do not agree in details, but all of them agree that the liberation phenomena of this alga have a certain relation to the highest spring tide.

To ascertain how the liberation of oogonia proceeds under artificial conditions, I kept some branches of *S. Horneri* and *Cyst. sisymbrioides* in a glass vessel filled with sea-water. To prevent contamination the culture water was renewed almost every day. The liberation did not occur as in nature at all; but after about a

1) WILLIAMS, Studies in the Dictyotaceae. Ann. Bot., XIX. 1905.
2) HOYT, Periodicity in the production of the sexual cells of *Dictyota dichotoma*. Bot. Gaz., XLIII. 1907.
3) LEWIS, Periodicity in *Dictyota* at Naples. Bot. Gaz. L. 1910.

month's culture the branches liberated the oogonia in a very feeble manner.

I often observed the actual mode of oogonium liberation in *Sargassum* and *Cystophyllum* in common sea-water under the microscope. But when I collected some small branches on the day before the day of an oogonium liberation and put them into a mixture of sea-water and fresh water (one volume of sea-water to four of fresh water proved to be the best proportion). I could quite easily observe the mode of the oogonium liberation. I studied the mode of oogonium liberation in *S. Horneri* more minutely than in other species, and the following description refer to this plant.

Generally speaking, the paraphyses of *Sargassum* do not protrude from the conceptacle as they do in *Fucus*. In *S. Horneri* they compose a disklike plug at the opening of the conceptacle. In a few seconds after immersion in the mixture medium above mentioned, the plug comes out slowly, with some broken pieces of paraphyses on its inner surface (Text. Fig. 1); and then the conceptacle commences to discharge its oogonia one after another. The discharged oogonium has a thick outer layer of gelatinons substance, which trails out a tail fastened to the interior of the conceptacle (Text Fig. 1). In one or two days the gelatinous substance becomes less rigid and the tail can no more be recognized with certainty, but still for a time the discharged oogonium remains attached to the receptacle.

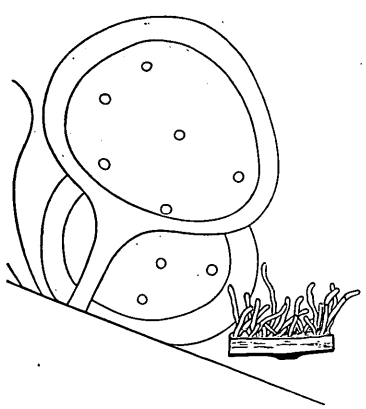

Fig. I. ×140

As already stated in my preliminary note, the oogonia in one receptacle are not liberated at one time, but in succession, one zone after another in acropetal order. Fig. 1 of Plate I shows the

receptacle in its first oogonium liberation: the black spots on the receptacle are oogonia. The area in which the discharged oogonia have already dropped off shows a remarkable contrast to the area in which the oogonia are in situ in the conceptacle (Fig. 2, PL. I). Fig. 3, PL. I shows the second oogonium liberation. The older part of the receptacle becomes more slender and the surface has the appearance of a young pine cone (Figs. 2, 3, 4, PL. I).

The mode of oogonium liberation in *Cystophyllum sisymbrioides* differs considerably from that of *S. Horneri* and deserves special mention. The receptacle of *Cyst. sisymbrioides* is rather small and slender and what is remarkable is that it shows no trace of the conceptacle opening on its outer surface. As above stated *S. Horneri* has something like a plug at the conceptacle opening, but the outline of the conceptacle opening on its outer surface can be seen under the microscope without any difficulty; this however, is not the case with *Cyst. sisymbrioides*.

On the day before the oogonium liberation, paraphyses in this alga begin to grow very rapidly. As there is no opening, paraphyses must make their way through the outer wall of the conceptacle. The tips of paraphyses peeping out through the cracks on the outer surface of the receptacle are disposed quite regularly in a line parallel to the main axis of the receptacle. And in the mean time, the oogonium liberation takes place in the same way as in *Sargassum*; paraphyses continue to grow and entangle with one another about the oogonia, giving an appearance of a mycelium growing on a nutritive substratum (Fig. 1, PL. III). As in the case of *Sargassum*, the liberation at one time is always restricted to a zone of receptacle, the liberation proceeding acropetally. The discharged oogonia remain attached to the surface of the receptacle for about five days and then drop off, when the paraphyses protruded from the conceptacle and entangled about the oogonia are also cleared away from the surface of the receptacle.*

* The protruded paraphyses are at first relatively stiff, but become gradually slushy.

I often came across some detached branches of *Cyst. Turneri*, floating on the sea near the Marine Biological Station; and the receptacle on the branches bore many discharged oogonia within the mass of entangled paraphyses. Thus the rapid growth of paraphyses at the time of the oogonium liberation appears to be a characteristic of the genus *Cystophyllum*.

II. Early Stages of Embryogeny.

Since the appearance of OLTMANNS' classical work on Fucaceae[1], it has been generally believed that in the Fucaceae in general the three successive nuclear divisions in the oogonium take place before the formation of oospheres, no matter how many oospheres come to function in one oogonium.

At this stage of the discussion Miss E. B. SIMONS' paper on *Sargassum filipendula*[2] attracted the attetion of plant morphologists. According to her, in this alga the three successive nuclear divisions before the oosphere formation are entirely suppressed and the one nucleus of the oogonium initial remains in a resting condition throughout the entire period of growth of the oogonium and becomes directly the nucleus of the single oosphere,

During my stay at Misaki, I paid special attention to this point and was so fortunate as to be able to observe the successive developmental stages of the oosphere in the oogonium of *Sargassum* and *Cystophyllum*. The result of my observations differs, however, remarkabley from that of Miss SIMONS; namely the oogonium development in these algae is quite normal, showing the usual three successive nuclear divisions in the oogonium.

As a matter of course, the periodical oogonium liberation is accompanied by the periodical development of the oogonium. All the oogonia in one conceptacle are liberated at one time, so the developmental stage of all the oogonia in one conceptacle is always the same. In other words, differing from the other cases

1) OLTMANNS, Beiträge zur Kenntnis der Fucaceen. Bibl. Bot. Cassel. 1898.
2) SIMONS, A morphological study of *Sargassum filipendula*. Bot. Gaz. XVIX. 1906.

in Fucaceae[1], in *Sargassum* and *Cystophyllum* one can not observe several developmental stages of the oogonium in the same conceptacle. Not only the same conceptacle, but also all the materials collected on the same place, on the same day, do not show in general the several developmental stages of the oogonium.

After the occurrence of an oogonium liberation, the single nucleus of the oogonium, which is to be liberated for the next period, remains in a resting condition for a time and for the first time on the day before the day of the next oogonium liberation, the nucleus begins to divide to form the nuclei of oospheres, and the oogonium attains the di- or tetra-nucleate condition. On the day of the liberation, the oogonium contains eight nuclei evenly distributed in its substance; the dense mass of chromatophores assembled around each nucleus facilitates the counting of the number of the nuclei. Fig. 1, PL. II, Fig. 3, PL. III show this condition of the oogonium in *S. Horneri* and *Cyst. sisymbrioides* respectively. NIENBURG's recently published paper on *Cystoseira* and *Sargassum*[2] states also that three successive nuclear divisions take place in the oogonium before the formation of oospheres. While the result of my observations is based on living materials, NIENBURG made his study on the microtome-sections of fixed materials. At any rate the occurrence of the three successive nuclear divisions in the oogonium development seems to be general in Fucaceae. The eight nuclei formed in one oogonium have at first the same appearance. But *Sargassum* and *Cystophyllum* develop only one egg in one oogonium, so only one of the eight nuclei becomes the functional nucleus of the oospheres and the others are destined to degenerate in the course of future development. Fig. 4, PL III shows a stage in which some of the eight nuclei are about to degenerate. The degeneration of the seven nuclei does not proceed simultaneouly.

In other Fucaceae the superfluous nuclei are regularly thrown out into the space between the oogonium wall and the oosphere. But it seems to me that this is not the case in *Sargassum* and

1) OLTMANNS, Beiträge zur Kenntnis der Fucaceae, Bibl, Bot. 1889. p. 84.
2) NIENBURG, Die Oogonentwicklung bei *Cystoseira* und *Sargassum*. Flora Bd. I. 1910.

Cystophyllum, for in living materials I have very often had the chances to observe the stage of oogonium development, in which such cast off plasma-masses would likely be found, had they ever been present, but I failed to find any trace of such a body.

It is rather a curious fact that none of the foregoing observers have succeeded in observing not only the fertilization but even the moving spermatozoids in *Sargassum* and *Cystophyllum*. Miss SIMONS writes in her paper already cited that, "A study of fertilization in *Sargassum* is surrounded by serious technical difficulties, because both eggs and sperms develop upon the same plant, thus making it difficult to isolate the sexual cells." As above described, the common species of *Sargassum* and *Cystophyllum* in our coast are all dioeceous, so the difficulty pointed out by Miss SIMONS does not occur in our materials. Nevertheless the study of the fertilization of these algae, both in living and fixed materials, is not an easy task. I have never succeeded in observing even the spermatozoid itself. From the phenomena of the periodical development of the oospheres, one naturally presupposes the periodical development of spermatozoids, but I could find no sign of such a phenomena in the antheridia of these plants.

Now let me give my observations on the development of the sporelings of *Sargassum* and *Cystophyllum*. The early development of these algae goes on within the oogonia which after having been discharged from the conceptacle, are attached to the surface of the receptacle: this condition fascilitates the investigation in no small degree. The later development may also be studied easily in materials cultured in a glass basin. The method is very simple. I collected some small branches of these algae which carried many hundreds of attached sporelings on their receptacles and cultured them in natural sea-water, some of the sporelings may in the course of development fall to the bottom of the glass basin but many remain attached to surface of the receptacle for a long time and still persue the normal course of development. The detached

sporelings become fastened to the bottom of glass basin and may also be used for investigation.

Generally speaking, the oospheres of *S. Horneri*, *S. enerve* and *Cyst. sisymbrioides* are equally oval or elliptical and common to all of them the first segmentation wall runs perpendicular and about midway to the long axis of the oosphere (Fig. 2, PL. III; Fig. 5, PL. III): the second wall runs parallel to the first, cutting off a small lens-shaped cell at one end of the sporeling. NIENBURG states in his paper, "Die erste Wand steht senkrecht zur Längsachse. Die zweite steht senkrecht auf der ersten und teilt das Vorderende in zwei gleiche Hälften. Darauf wird von der unteren Spitze durch eine Wand, die der ersten parallel ist, eine schmale Rhizoidzelle abgeschnitten." Thus his observations do not agree with mine.

Further development differs in *Sargassum* and *Cystophyllum*, and would better be described separately.

Sargassum.

The lens-shaped cell, cut off by the cecond segmentation wall will hereafter be called for the sake of convenience the 'Rhizoid cell.' This cell divides simultaneously with the segmentations of the other cells, until the eight-celled stage is reached. The segmentations of the rhizoid cell are quite regular and the segmentation wall are all perpendicular to the outer surface of the sporelings. (Figs. 5, 8, 9, PL. II) clearly show this regularity. While the segmentation of the other cells proceeds further, the rhizoidal portion remains in the eight-celled stage, and in the mean time the rhizoid formation begins. At the outset, we see the papilla-like protuberances, eight in number (Fig. 11, PL. II). These protuberances grow gradually and become a group of rhizoids arranged in a circle. But later there arises in the central region another group of rhizoids which elongate with greater rapidity so that they become longer than those of the first and outer group (Figs. 13 and 14, PL. II). This difference in length becomes, however, obscure in the further development of the rhizoids, the outer group of rhizoids also growing rapidly (Fig. 15, PL. II).

To ascertain, if possible, the origin of this central group of rhizoid I made some microtome-sections of the sporelings in this stage. Text-Fig. 2 was drawn from one of such sections. The two rhizoids in shade are the descendants of the rhizoid cell formed by the second segmentation wall. Other rhizoids situated in the central portion seem to have been derived from the body cells, without having any direct relation to the above mentioned rhizoid cell. In the later development the number of rhizoids gradually increases. The rhizoids are at first unicellular but later we find several partitions here and there (Fig. 16, PL. II). Under a strong magnification we see peculiar thickenings on the cell walls of the rhizoids, which remind us of the spiral thickenings of tracheids of higher plants (Fig. 16 b, PL. II). The cells contain small granules of what seems to be a fat-like substance, so far as can be seen by the reactions of osmic acid and Sudan III. The same substance is found not only in the rhizoids but also very abundantly in the body cells in general.

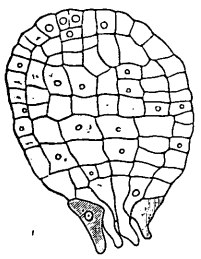

Fig. 2. ×140

For a long time, the shape of the sporelings remains oval or elliptical, but later becomes like a flask and at last at the boundary between the slender and the swollen part of the sporeling, there grows a new branch (Figs. 17–20, PL. II). This is the last stage that I observed at Misaki.

Cystophyllum.

The segmentation process in the rhizoid cell of this plant differs considerably from that above described; that is, in this plant before the rhizoid formation begins, the rhizoid cell is already divided into about 30 small cells. Text-fig. 3 is the surface view of the rhizoidal potion of the sporeling and text-fig. 4 presents a median longitudinal section of the same. In the latter figure, we can see the two-storied arrangement, a

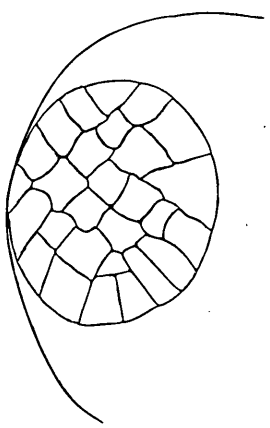

Fig. 3. ×240

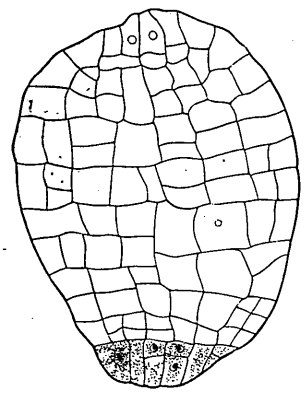

Fig. 4. ×140

state which is never found in *Sargassum*. The rhizoid cells elongate to form a numebr of rhizoids; similar to what has been described in the case of *Sargassum*, the rhizoids originating from the central region grow more rapidly than those originating from the outer region (Fig. 8, PL. III). But in this case the central group of rhizoids are also derived from the rhizoid cell formed by the second segmentation wall, although in later development there may be some rhizoids derived from body cells as in *Sargassum*.

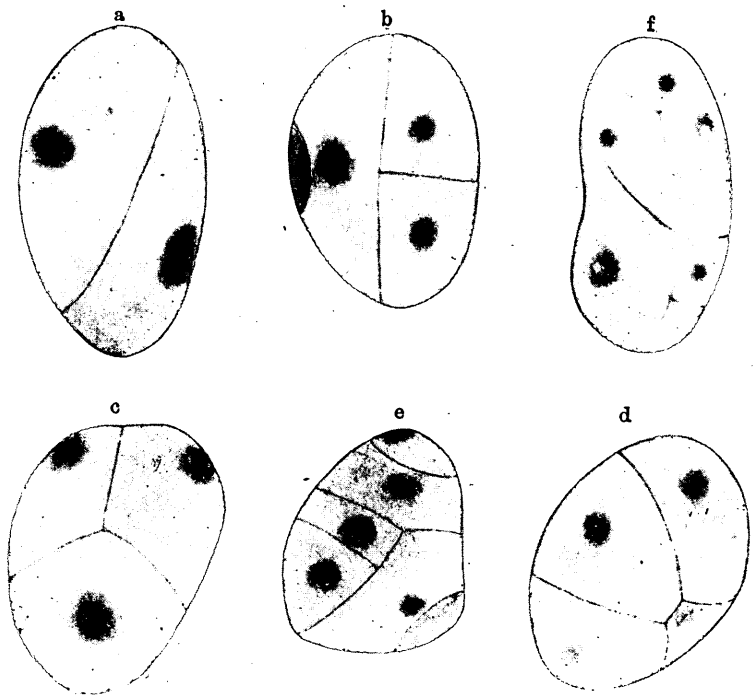

Fig. 5. ×175

As already stated. the early development of sporelings, both in *Sargassum* and *Cystophyllum*, is carried out while they are enveloped in the oogonium wall. But in the course of develop-

ment the wall ruptures at one end by the pressure of the growing rhizoids (Fig. 14, PL. II) and the sporeling becomes free thereafter.

During the study of the sporeling-development I often met with different abnormities and some which are interesing are shown in text-fig. 5. These abnormities are often found, especially in *S. enerve*, and all these figures were sketched from the sporelings of this alga. Fig. 5,*a* represents an abnormity in which the first segmentation wall is oblique to the long axis, fig. 5,*b* is the later stage of this abnormity, *c* and *d* of the same figure show something like 'Simultan-dreier' and 'Simultan-vierer.' Superfluous nuclei in the oogonium often fail to degenerate, and this results in the formation of a curious abnormity in the later development (Fig. 5,*f*).

In conclusion I wish to express my hearty thanks to Professor K. Fujii for his valuable suggestions and assistance given me during the progress of this work and to Professor Iijima, the director of the Misaki Marine Biological Station through whose kindness many facilities were afforded me in the course of my investigations.

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OOGONIUM LIBERATION AND THE EMBRYOGENY OF SOME FUCACEOUS ALGAE.

Plate I.

Explanation of Pl. I.

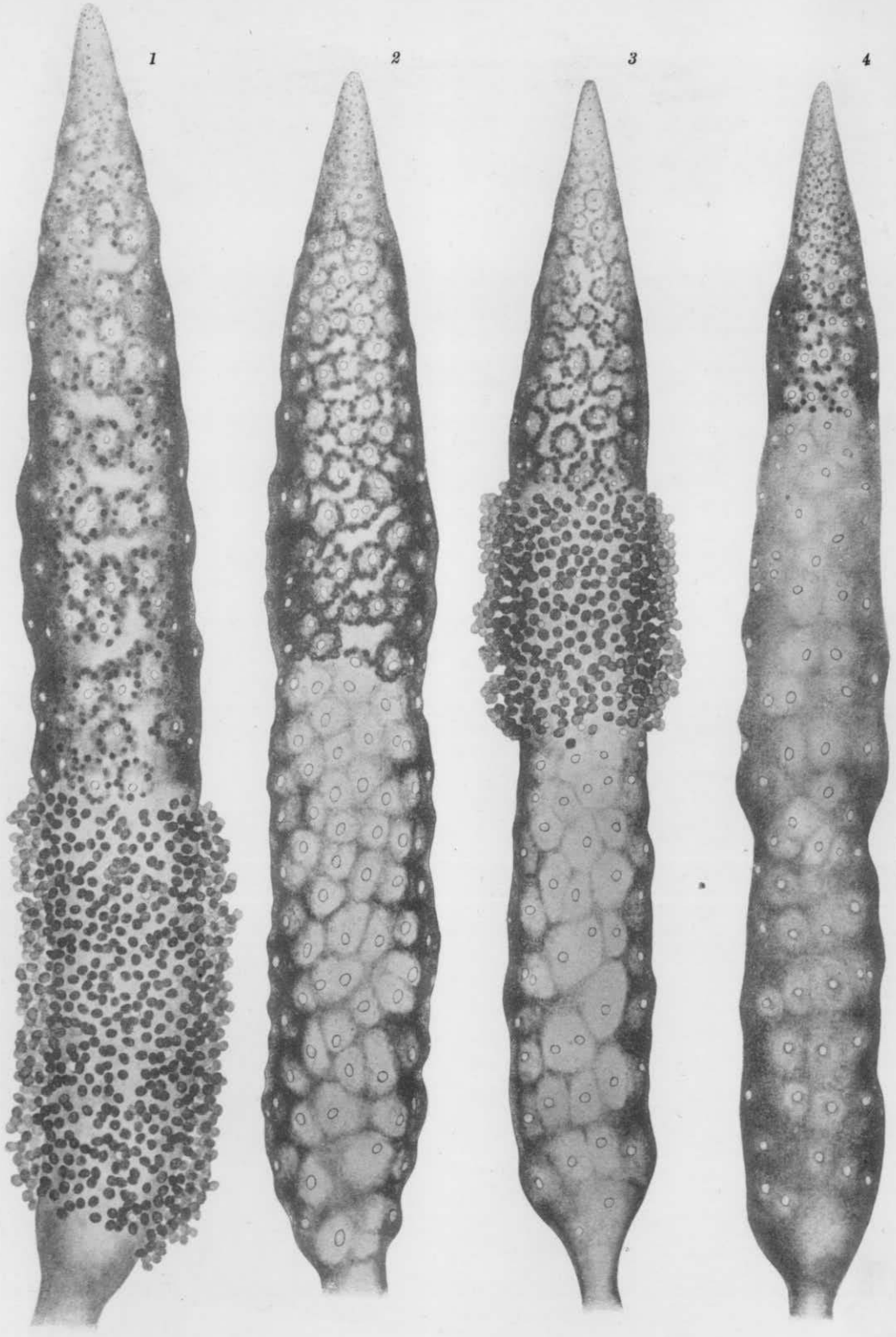
Female receptacle of *Sargassum Horneri*. All figures were drawn with the aid of camera lucida from living materials. Magnification: ca. 10 times.

Fig. 1. First oogonium liberation.

Fig. 2. After the oogonia discharged in the first liberation had dropped off.

Fig. 3. The second oogonium liberation.

Fig. 4. After the oogonia discharged in the first and second liberations had dropped off.



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OOGONIUM LIBERATION AND THE EMBRYOGENY OF SOME FUCACEOUS ALGAE.

Plate II.

Explanation of Pl. II.

Sporeling-development of *Sargassum Horneri*. All Figures were drawn with the aid of camera lucida from living materials. Magnifications: figs. 1–16a ca. 140 times; figs. 17–20 ca. 50 times; fig. 16b ca. 500 times.

Fig. 1. Oogonium with eight nuclei, chromatophores grouped around the nuclei.

Fig. 2. First segmentation.

Fig. 3. Second segmentation, forming the rhizoid cell at one end.

Fig. 4. Beginning of the third segmentation.

Fig. 5. Completion of the third segmentation.

Fig. 6, a. Side view at the beginning of the 4th segmentation.

Fig. 6, b. Polar view of the same.

Fig. 7. First segmentation of the rhizoid cell.

Fig. 8. Second segmentation of the same.

Fig. 9. Rhizoid cell in the eight-celled stage.

Fig. 10. Further segmentation of body cells. Rhizoid cell remains in the eight-celled stage, a, side view; b, polar view.

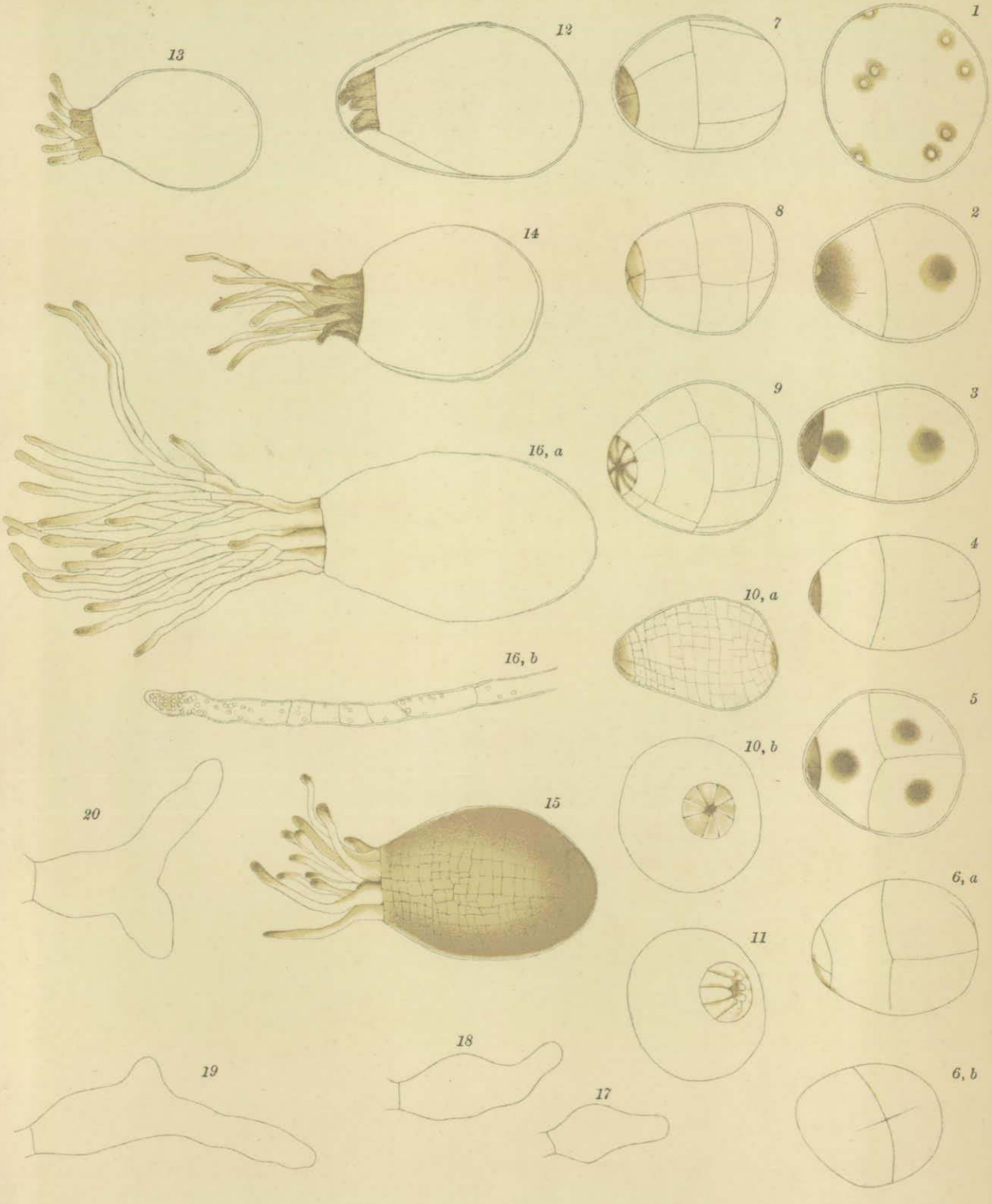
Fig. 11. Beginning of rhizoid-formation.

Fig. 12. Rhizoids somewhat elongated.

Figs. 13–16 a. Further development of rhizoids.

Fig. 16 b. The terminal portion of a rhizoid.

Figs. 17–20. Development of the body of a sporeling.



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OOGONIUM LIBERATION AND THE EMBRYOGENY OF SOME FUCACEOUS ALGAE.

Plate III.

Explanation of Pl. III.

Female receptacles and sporeling development of *Cystophyllum sisymbrioides*. All figures were drawn with the aid of camera lucida from living materials. Magnifications: figs. 1 and 2. ca. 10 times; figs. 2–9 ca. 170 times.

Fig. 1. Female receptacle in the first oogonium liberations. Paraphyses protruding from the conceptacle and forming a mass resembling a mycelium.

Fig. 2. After the oogonia discharged in the first liberation had dropped off.

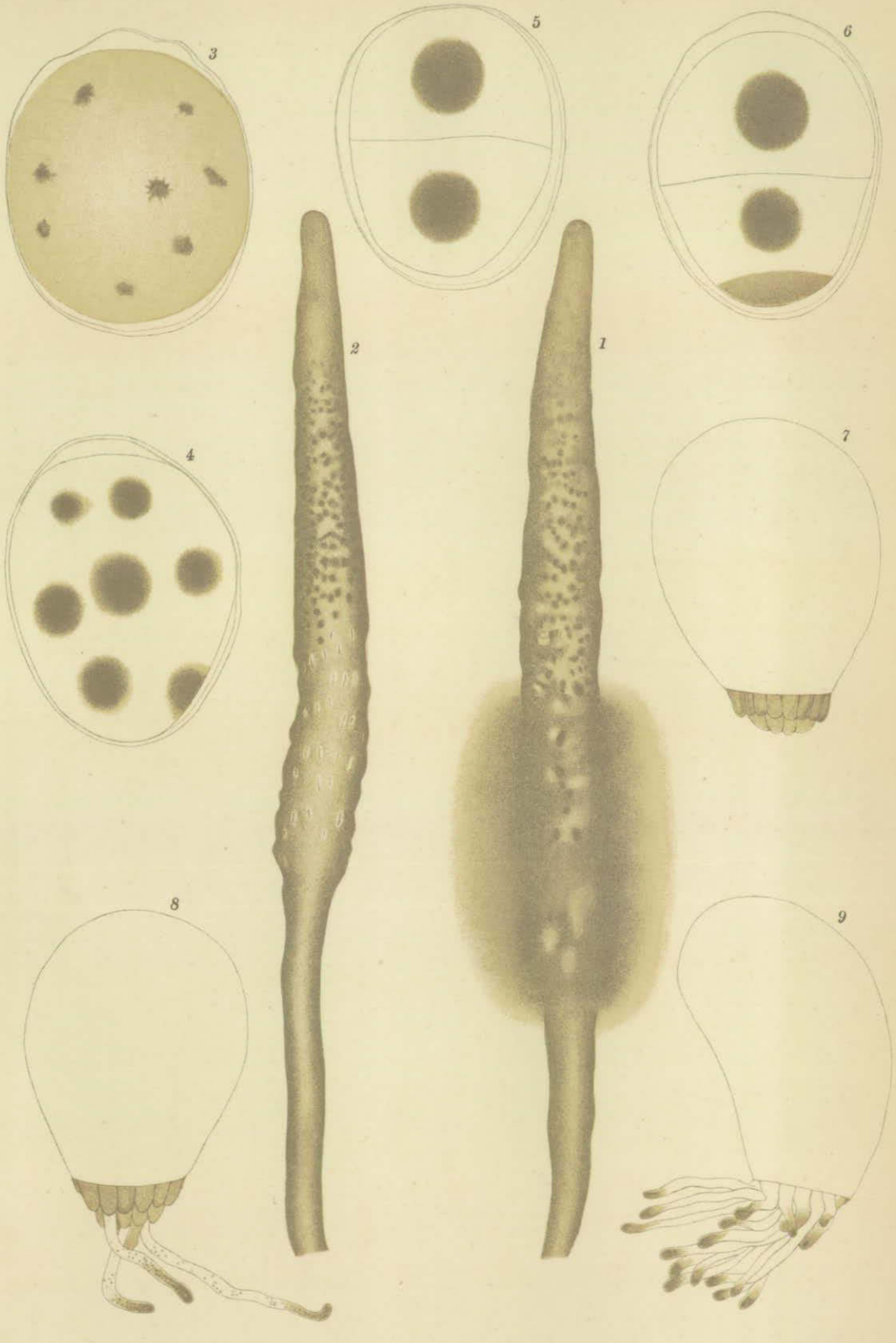
Fig. 3. Oogonium with eight nuclei.

Fig. 4. Some of the eight nuclei in the oogonium about to degenerate.

Fig. 5. The first segmentation.

Fig. 6. The second segmentation.

Figs. 7–9. Later stages of the sporeling development.



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