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Author(s)	INOH, Shumpei
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Embryological Studies on *Turbinaria* and *Cystophyllum*

By

SHUMPEI INOH

(With 11 Text-figures)

Introduction

Embryological studies on the Fucaceous plants to be found in our country, namely *Fucus*, *Pelvetia*, *Cystophyllum*, *Hizikia*, *Coccophora* and *Sargassum*, have hitherto been carried out by TAHARA (12, 13), OKABE (9) INOH (2, 3, 5). The results obtained by these workers seem to suggest the fact that the number of primary rhizoids in each embryo is proportionate to the size of the egg, and species having larger eggs and more complex in their vegetative organization. It is regretful, however, that there are no detailed studies along the same line on *Turbinaria* which is found in the southern part of our country. The present writer, then, aims to describe two species in *Turbinaria*, *T. ornata* and *T. filiformis*, in respect to the mode of rhizoid formation, and to compare them with species hitherto investigated on this point. Also in addition to this report, the embryological study on *Cystophyllum crassipes* is dealt with in the present paper. From the results of the embryological studies on *Cystophyllum* by OKABE (9) and INOH (3), one knows that *C. hakodatense* (3) differs greatly from *C. sisymbrioides* and *C. Turneri* (9) as to the form and size of egg and the rhizoid formation, namely in the former the discharged egg has a single nucleus in the center of the body, it measures 120μ long and 80μ wide, and the embryonal development is carried out in the four cell type, whereas in the latter the discharged egg has eight nuclei, it measures 312μ long and 229μ wide, the egg is entangled by the paraphyses and the embryonal development takes place in the thirty-two cell type. This will be of interest when one considers the embryological difference between these species belonging to the same genus *Cystophyllum*. Therefore the present writer aims to describe and figure the embryo development in *C. crassipes*, with special reference to the mode of rhizoid formation, and to compare them with embryos in the above mentioned species in *Cystophyllum*, and

to consider the systematic position of these species. The expense incurred in collecting the materials used for the present study was covered by a grant from the Japan Society for the promotion of Scientific Research and from the Scientific Research Fund of the Department of Education, for which the writer here expresses his best thanks. The writer also wishes to express his gratitude to Prof. H. MATSUURA and Prof. Y. YAMADA of Hokkaido Imperial University for their valuable suggestions and assistance given him during the progress of this work, to Dr. A. KADONISHI, the director of the Koshun Zootechnical Experiment Station, Government Agricultural Research Institute, Taiwan, and to Mr. S. OHNO, the then president of the Shakotan primary school in the southern Kurile Islands, through whose kindness many facilities were afforded in the course of the investigation.

Materials and Methods

Turbinaria ornata and *T. filiformis* are found on the rocks between tide marks, mostly in tide-pools, in the vicinity of the Garanbi lighthouse in Formosa. They ripen there about March. In order to carry out embryological studies on these two species, the writer visited Konte (near the Garanbi lighthouse) in Formosa at the end of February in 1936 and stayed there for about one month.

The liberation of Sexual cells in these species occurs simultaneously and periodically as in *Sargassum*, *Coccophora* and *Cystophyllum*. The discharged eggs in both species were collected at high tide there on the 22nd and 23rd of March. *Cystophyllum crassipes* grows abundantly on rocks between tide marks at Chiboi in Shikotan Island. It ripens there in about June and July. The writer went there at the beginning of July in 1936 and stayed in Chiboi village for about one month. The liberation of sexual cells in the present species occurs also simultaneously and periodically. The discharged eggs in this species were collected at high tide on the 22nd of July. For the study of the embryonal development, in the first place some small branches of this alga which carried many discharged eggs on the surface of their receptacles were collected, and then favorable materials were cultured in small basins (mainly enamel-bowls) filled with natural sea water, partly replacing fresh sea water several times a day. The discharged eggs are usually covered with a thick layer of gelatinous substance and remain attached to the outer surface of the receptacle for several days and begin to develop there. Before the rhizoid formation at the lower extremity of the embryos took place, however, they were detached

from the outer surface of the receptacles, fell immediately to the bottom of the basins and attached themselves on the surface of slides which were previously prepared, and then the further development continued in this condition. The developing embryos were observed in natural sea water. For the histological observation, they were fixed exclusively by FLEMING'S weaker solution prepared with sea water. The microtome sections were cut at thicknesses varying between 5-10 μ (mainly 7 μ) and stained with safranin and light green.

Observation

1) *Turbinaria ornata* J. Ag. (Nom. Jap. Rappamoku). This species is one of the algae being found exclusively in the southern coast of our country and grows usually in abundance in tide-pools on rocks (Fig. 1),



Fig. 1. *Turbinaria ornata* J. Ag. growing in a tide-pool on rocks.

just below the highest level reached by the spring tide. This alga is about 15 cm high. It is strictly dioecious and receptacles of both sexes have a similar external appearance, namely, they are small and cylindrical, but the male receptacle is slightly longer and more slender than the female one. The discharged eggs are ovoid (diameter 140 μ) or ellipsoid (176 μ long and 124 μ wide) in shape and they are covered with a thick layer of gelatinous substance, and small eight nuclei, surrounding which a multitude

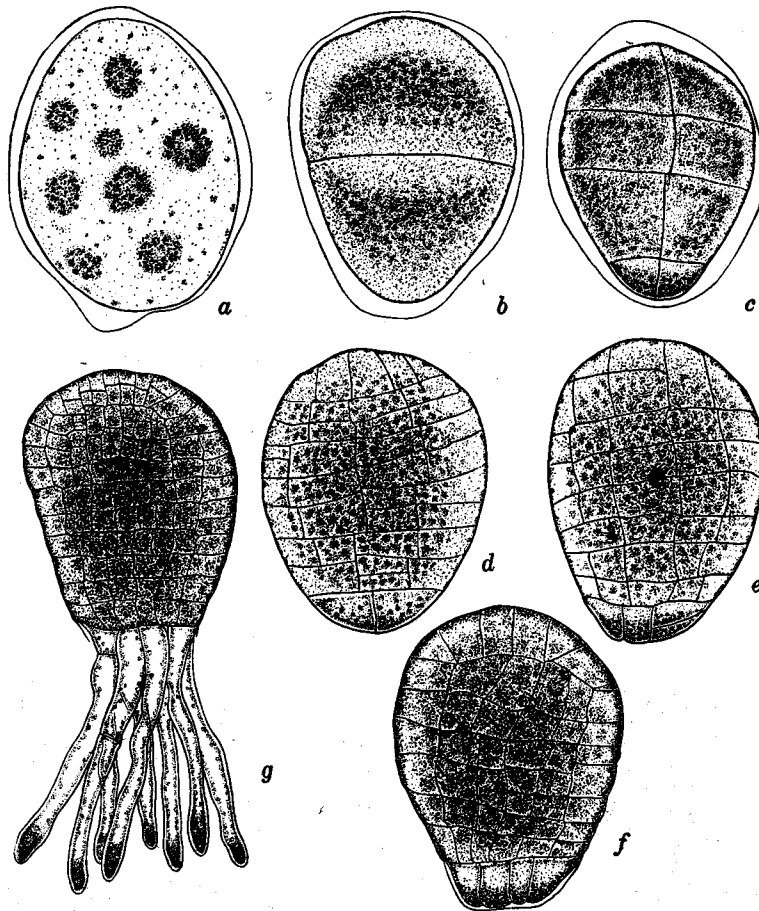


Fig. 2. *Turbinaria ornata* J. Ag. **a.** A discharged egg. **b.** First segmentation. **c, d, e, f.** Further segmentation of the embryo. The rhizoid cell becomes into eight small cells by the successive divisions. **g.** A young embryo having developed eight primary rhizoids. (3 days after oogonium liberation). All figures were drawn from fresh materials. $\times 225$.

of chromatophores are seen, lie scattered in the body of the egg (Fig. 2. a). After the fertilization, the first segmentation wall runs transversely, forming two cells (Fig. 2. b). The second one runs also parallel to the first one in the lower cell of these two and cuts a small lense-shaped rhizoid cell in the lower extremity of the embryo.

The first two segmentation-walls in the rhizoid cell run vertically

through the center of the cell and become perpendicular to each other. In the next stage, the third segmentation wall goes quite irregularly, some are parallel with the former ones, while some oblique. As the consequence, the rhizoid cell is divided into eight irregularly arranged small cells (Fig. 2. c, d, e, f. Fig. 3), just as described in *Hizikia* (*Turbinaria*?) *fusiformis*, *Coccophora Langsdorfi*, *Sargassum Thunbergii*, *S. hemiphyllum*, *S. Kjellmanianum* and *S. confusum* (2).

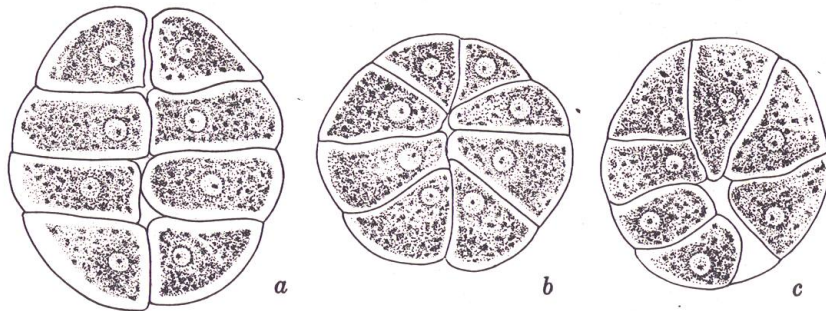


Fig. 3. *Turbinaria ornata* J. AG. a, b. Cross section of the rhizoid cell at eight cell stage. c. The same with one cell of the eight cells dropped out at the section. All figures were drawn from the microtome section being stained with HEIDENHAIN'S iron-alum haematoxylin. $\times 600$.



Fig. 4. *Turbinaria filiformis* YAMADA growing in a tidepool on rocks.

Later, one rhizoid is developed respectively in each small cell and, ultimately, a group of eight primary rhizoids develops at one extremity of the embryo (Fig. 2. g). Rhizoids which have no direct relation to the rhizoid cell cannot be seen, at least in these early stages of embryonal development.

2) *Turbinaria fliformis* YAMADA (Nom. Jap. Itorappamoku). This plant is found in the same place (Fig. 4), as that of the former species and ripens also there at the same season as the former species. The grown-up plant is 12 cm high and smaller and more slender in its vegetative

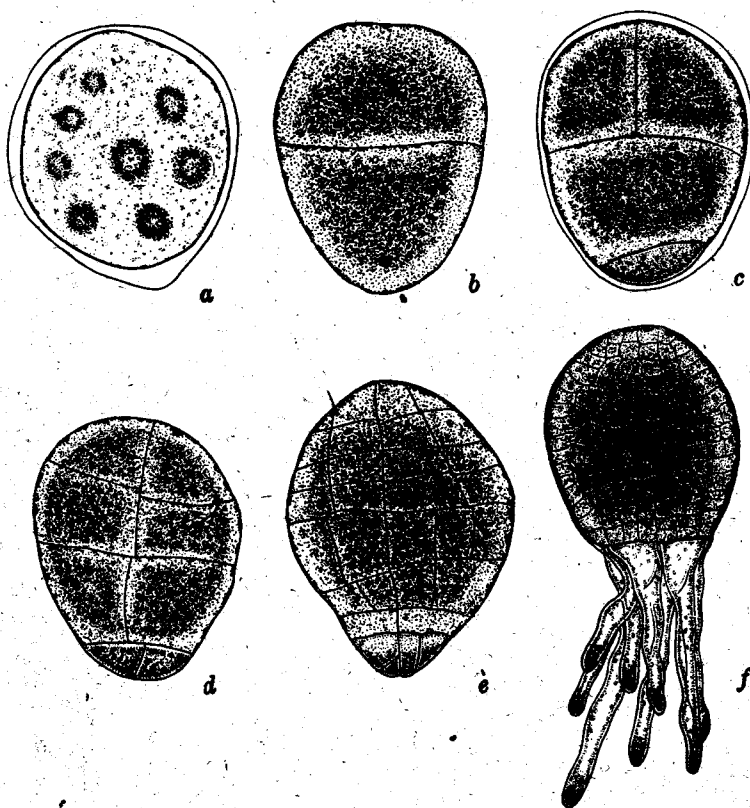


Fig. 5. *Turbinaria fliformis* YAMADA. a. A discharged egg, chromophores grouped around the nuclei. b. First segmentation. c. Second and third segmentation, forming the lense-shaped rhizoid cell at the distal end. d, e. The further segmentation of body cells. Rhizoid cell at the four cell stage and the eight cell stage. f. A young embryo having developed eight primary rhizoids (3 days after oogonium liberation). All figures were drawn from fresh materials. $\times 225$.

constitution than *T. ornata*. It is strictly dioecious. Receptacles are small and cylindrical and male receptacles are more slender than female ones. The discharged eggs in the present species are ovoid (diameter 130μ) or ellipsoid (152μ long and 108μ wide) and a little smaller than the eggs of the former species. They are usually covered with a thick layer of gelatinous substance and in the body of each egg, eight small nuclei, surrounding which many chromatophores are seen, lie just as in the egg of the former species (Fig. 5. a). In the present species, successive stages of the embryo development from the first segmentation to the complete formation of the rhizoid cell and the development of eight primary rhizoids at one extremity of the embryo, occur nearly in the same manner (Fig. 5. b-f) as those in the former species, *T. ornata*, with the exception of some slight difference in the size of eggs.

3) *Cystophyllum crassipes* J. Ag. (Nom. Jap. Nebutomoku). This species is quite similar in general appearance to *C. hakodatense* which was examined before by the present writer, it is distinguished only by a slight difference in the arrangement and constitution of vesicles and receptacles. This alga is found in abundance on the rocks between tide marks in the coast of Chiboi in Shikotan Island. It ripens there in June and July. This plant is strictly dioecious. Receptacles of both sexual plants are small and cylindrical in shape and similar in external and internal appearance, though the male one is more slender and longer than the female one (Fig. 6). The discharged eggs in *C. crassipes* are ovoid (diameter 92μ) and ellipsoid (108μ long, 80μ wide) in shape and they generally have only one central nucleus, surrounding which many chromatophores are seen (Fig. 7. 1), as in *C. hakodatense* (3) and *Coccophora Langsdorfi* (12). Therefore the eggs in the present species resemble so closely in shape and size to those in *C. hakodatense* (120μ long, 80μ wide). After the fertilization, the large nucleus commences to divide into 2 nuclei (Fig. 7.

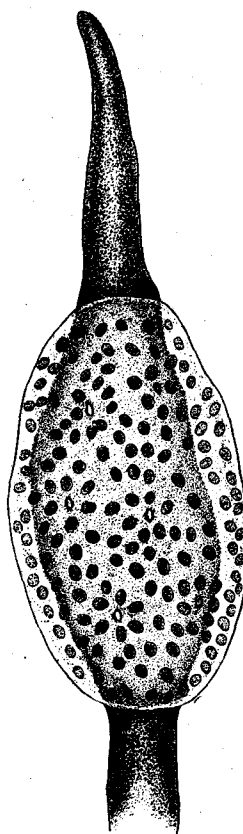


Fig. 6. The female receptacle of *Cystophyllum crassipes* J. Ag. Discharged eggs remain attached to the outer surface of the receptacle. $\times 12$.

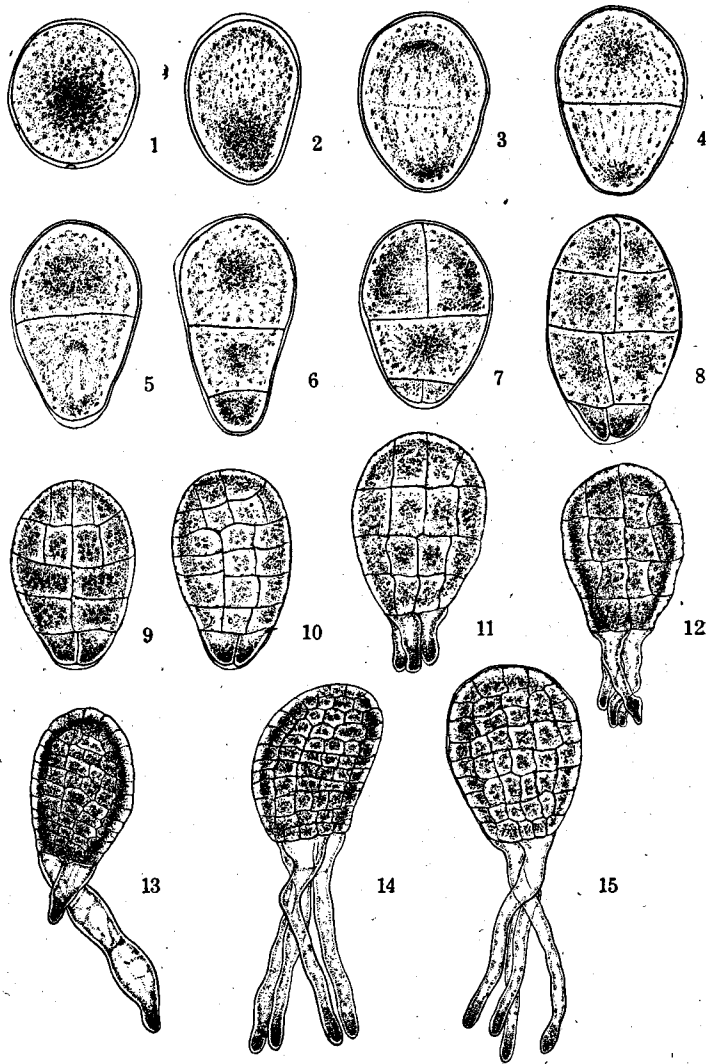


Fig. 7. *Cystophyllum crassipes* J. Ag. 1. a discharged egg with a central nucleus, its diameter 92μ . 2, 3, 4. First segmentation, forming two cells (1 day after the oogonium liberation). 5, 6. The second segmentation, forming the small lense-shaped rhizoid cell at one extremity. 7, 8, 9, 10. Further segmentations of the body cell, its rhizoid cell being divided into four small cells (2 days after the oogonium liberation). 11, 12. Four primary rhizoids begin to develop from the rhizoid cell. 13. An abnormal embryo having developed only two large rhizoids. 14, 15. An abnormal embryo having developed only two large rhizoids. 14, 15. Three days old embryos having developed four primary rhizoids at one extremity of the embryo. All figures were drawn from fresh materials. $\times 187$.

2-4). Of these two, one nucleus begins to move to the periphery, and at the same time, the body of embryo protrudes toward the same direction. The embryo becomes elongate in shape (Fig. 7. 4, 5). Then the first segmentation-wall runs transversely, forming two cells. The early successive stage of the embryo development in the present species is similar to those in *Pelvetia Wrightii* and *Fucus evanescens* (5) which are of a taxonomically lower class of the Fucaceae. In the lower one of these two cells, the second segmentation-wall runs also transversely and cuts a rhizoid cell in the lower extremity of the embryo (Fig. 7. 6). In the later stage, the two segmentation-walls in the rhizoid cell run vertically through the center of the cell and become perpendicular to each other, and the rhizoid cell is divided into four small initial cells of rhizoids (Fig. 7. 7-10 and Fig. 8. d).

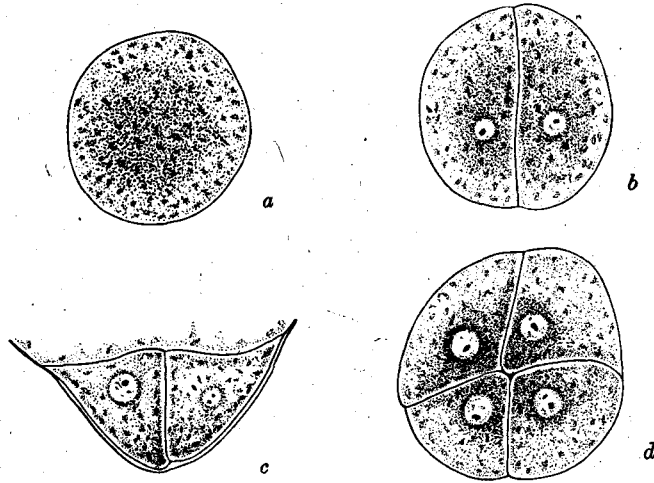


Fig. 8. *Cystophyllum crassipes* J. AG. a. Cross section of the rhizoid cell. b. The same at the two cell stage. c. Longitudinal section of the rhizoidal portion of an embryo at four cell stage. d. Cross section of the same. All figures were drawn from the microtome section which was stained with HEIDENHAIN'S iron-alum haematoxylin. $\times 675$.

when the embryo-development proceeds further, one rhizoid develops in each small initial cell of rhizoid, and young embryos in this stage have usually four primary rhizoids at the lower extremity of body (Fig. 7. 14-15). In later stages, rhizoids begin to develop, from the cells adjacent to the rhizoid cell, and at further development many secondary rhizoids become indistinguishable in appearance from the primary ones (Fig. 9. f). Though seldom, as is shown in Fig. 9, the cell divisions of the rhizoid cell in

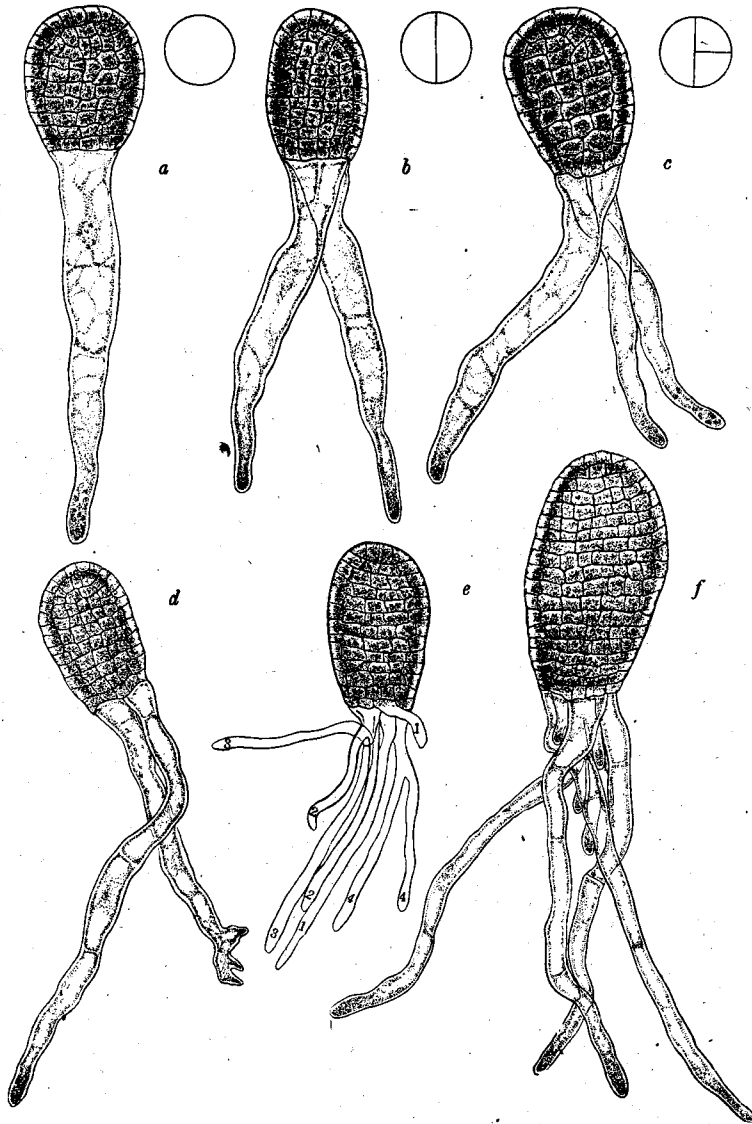


Fig. 9. *Cystophyllum crassipes* J. Ag. **a, b, c.** Three days old abnormal embryos after the oogonium liberation. **a.** Only one large rhizoid is developed from the rhizoid cell without the cell division. **b.** The rhizoid cell is divided into two cells, then two large rhizoids develop from those cells. **c.** As the result of the fact that the second division of the rhizoid cell is omitted in one of the two cells, the rhizoid cell consists of one large cell and two small cells. In the late stage, one large rhizoid develops from the large one and two normal rhizoids from two small ones. **d.** An abnormal embryo with a large rhizoid one of which is bifurcated at its terminal part (one week old after the oogonium liberation). **e.** An abnormal embryo having four forked rhizoids. (5 days old after the oogonium liberation). **f.** A normal young embryo having four primary rhizoids, being elongated and multicellular, and a few secondary rhizoids developing from the cells adjacent to the rhizoid cell. (one week old after the oogonium liberation). All figures were drawn from fresh materials. $\times 187$.

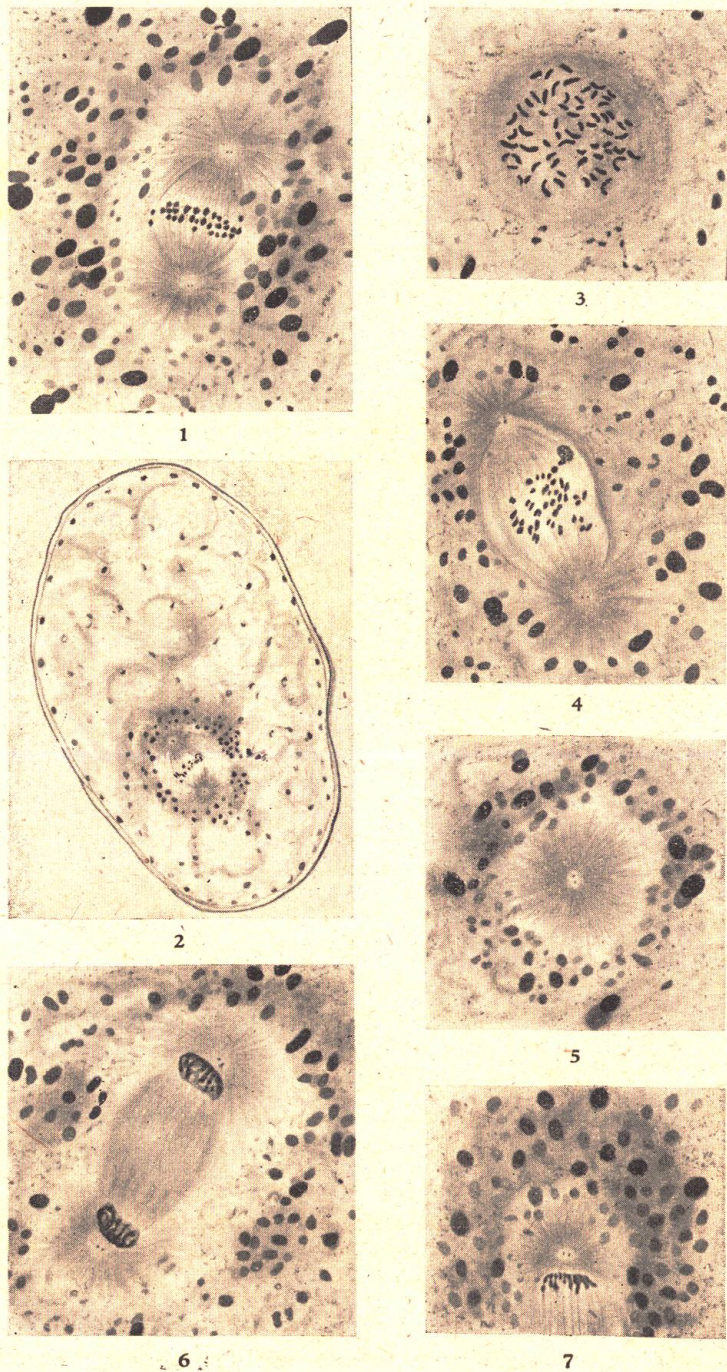


Fig. 10. *Cystophyllum crassipes* J. Ag. 1. Side view of the metaphase in the first mitosis of the embryo, after fertilization. 2. The complete view of the same embryo. 3. Polar view of the metaphase, showing 64 chromosome ($2n$). 4. Pro-metaphase, showing sister centrosomes. 5. Polar view of the sister centrosomes. 6, 7. Telophase of the same mitosis. All figures were drawn from the microtome sections which were stained with HEIDENHAIN'S iron-alum haematoxylin. 1, 4, 5, 6, 7. $\times 1560$. 2. $\times 624$. 3. $\times 2700$.

abnormal embryos are omitted, namely, in one embryo only one large abnormal primary rhizoid develops from the rhizoid cell without the cell division (Fig. 3. a). In another embryo the rhizoid cell is divided by the first segmentation-wall into the cells, but the next cell division does not occur and two large primary rhizoids develop at the lower extremity of the abnormal embryo: one large abnormal rhizoid is developed from each cell. (Fig. 7. 13 and Fig. 9. b, d). Also in another embryo the rhizoid cell is divided into two cells. Of these two, only one cell is divided into two small cells, but in another the cell division is omitted. As the result, the rhizoid cell is divided into three cells, namely two small cells and one large cell. Lately, two normal primary rhizoids develop from two small cells and one large abnormal rhizoid from the large cell respectively (Fig. 9. c). Though extremely seldom, all primary rhizoids of an embryo branch into two at their base (Fig. 9. e). In the metaphase in the first cell division of the fertilized egg of the present species, sixty-four chromosome ($2n$), which are rod-shaped or curved, are counted (Fig. 10. 3) and this number 64 is the same as in *Fucus vesiculosus* (15), *Coccophora Langsdorfi* (14) and *Sargassum Horneri* (9). The mantle fibres are very distinct, and centrosomes are clearly seen, surrounded with radiated plasma striation at both poles in this species. From the pro-metaphase to telophase the new daughter centrosomes become completely separated (Fig. 10. 1, 2, 4, 5, 6).

Comment

From the above observations, the manner of embryo development in *Turbinaria ornata* and *T. filiformis* is just the same as those of some species belonging to the irregular eight cell type, *Sargassum Thunbergii* (13), *S. hemiphyllum*, *S. Kjellmanianum* and *S. confusum* (2) in the following facts. i) The discharged eggs are ovoid or ellipsoid in shape, have eight nuclei and are covered with a thick layer of gelatinous substance. ii) The liberated eggs remain attached to the outer surface of the receptacles in each species for several days till the rhizoid formation commences. iii) 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. iv) The rhizoid cell is divided into eight irregularly arranged cells by three successive divisions. Later a group of eight primary rhizoids develops at one extremity of the young embryo.

Therefore it is clear that *T. ornata* and *T. filiformis* belong to the irregular eighth cell type (2) as *S. hemiphyllum* etc. As to the size of liberated eggs, these species differ slightly with each other, namely the egg

in *T. ornata* measure 174μ long and 124μ wide, in *T. filiformis* 152μ long and 103μ wide, in *S. Kjellmanianum* 139μ long and 97μ wide: the size of eggs increases in the following order, viz. *S. hemiphyllum*, *S. Kjellmanianum*, *T. filiformis*, *T. ornata* and *S. confusum*. In general, the sizes of eggs in these species are similar to each other and larger than those in species belonging to the four cell type as *C. crassipes* and *C. hakodatense*, and smaller than those in species belonging to the sixteen cell type as *S. enerve*, *S. patens*, *S. serratifolium* and *S. micracanthum*.

From the standpoint of the size of egg and the mode of embryonal development, it may be presumed that *T. ornata* and *T. filiformis* ought to be placed under the embryological order between *S. confusum* and *S. hemiphyllum*. Also these two species in genus *Turbinaria* are quite similar to the allied genus *Hizikia* (13) and to species belonging to the irregular eight cell type in *Sargassum* as to the embryonal development. Therefore, from the above mentioned facts, these species in *Hizikia*, *Turbinaria* and *Sargassum* should be placed under the same embryological order, namely the irregular eight cell type.

The other species dealt with in the present paper, *Cystophyllum crassipes*, resembles closely to *C. hakodatense* in the above mentioned facts, such as in the size and form of eggs, in the number (1) of the nucleus, the number (four) of primary rhizoids in young embryos and other features of the embryonal development. It may be presumed that these two species are very closely related. *C. crassipes*, however, differs greatly from *C. sisymbrioides* (10) as to the feature of the liberated egg, the manner of its development and especially the mode of rhizoid formation. The egg of the former measures 108μ long and 80μ wide and belongs embryologically to the four cell type (3), whereas the egg of the latter measures 321μ long and 229μ wide (the largest one in the Fucaceous plants which (9, 2) have hitherto been investigated) and belongs to the thirty-two type (9, 2). Also in *C. Turneri* which has been investigated by OKABE (9), though the egg is slightly smaller than *C. sisymbrioides*, its embryonal development occurs in the thirty-two cell type. Contrasted with this, in *C. hakodatense* which has been formerly investigated by the present writer, the egg is also slightly smaller than *C. hakodatense* and its embryonal development occurs in the four cell type.

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 applanated and sends out branchlets on both sides, whereas in the latter it is cylindrical

and sends out branchlets in all directions (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 (16). Therefore the above mentioned embryological differences appear to give some suggestions on the systematic consideration of these algae.

The data contained in *T. ornata*, *T. filiformis* and *C. crassipes* may be

Table 1.

Specific name	Number of nucleus in a discharged egg	Diameter or length and width of egg	Type of the rhizoid formation	Author
<i>Fucus evanescens</i> AG.	1	60 μ	one cell type	INOH (1935)
<i>Pelvetia Wrightii</i> YENDO	1	84 μ	one cell type or seldom two cell type	"
<i>Cystophyllum crassipes</i> J. AG.	1	108 μ \times 80 μ	four cell type	INOH (1937)
<i>C. hakodatense</i> YENDO	1	120 μ \times 80 μ	"	" (1932)
<i>C. sisymbrioides</i> J. AG.	8	321 μ \times 229 μ	thirty-two cell type	OKABE (1927), INOH (1930)
<i>C. Turneri</i> YENDO	8	—	"	OKABE (1929)
<i>Turbinaria ornata</i> J. AG.	8	176 μ \times 124 μ	irregular eight cell type	INOH (1937)
<i>T. filiformis</i> YAMADA	8	152 μ \times 108 μ	"	"
<i>Hizikia fusiformis</i> OKAMURA	8	—	"	TAHARA (1929)
<i>Cocophora Langsdorfi</i> GREV.	1	—	"	" (1928)
<i>Sargassum piluliferum</i> C. AG.	8	235 μ \times 110 μ	sixteen cell type	INOH (1930)
<i>S. tosaense</i> YENDO	8	220 μ + 180 μ	"	"
<i>S. patens</i> C. AG.	8	218 μ \times 177 μ	"	"
<i>S. Horneri</i> C. AG.	8	264 μ \times 198 μ	radial eight cell type	TAHARA (1913), INOH (1930)
<i>S. serratifolium</i> C. AG.	8	275 μ \times 202 μ	sixteen cell type	INOH (1930)
<i>S. tortile</i> AG.	8	333 μ \times 236 μ	"	"
<i>S. Ringgoldianum</i> HARV.	8	222 μ \times 153 μ	"	"
<i>S. sagamianum</i> YENDO	8	198 μ \times 147 μ	"	INOH (1941)
<i>S. confusum</i> AG.	8	210 μ \times 140 μ	irregular eight cell type	INOH (1930)
<i>S. enerve</i> C. AG.	8	250 μ \times 235 μ	sixteen cell type	"
<i>S. Thunbergii</i> O. KÜNTZE	8	—	irregular eight cell type	TAHARA (1929)
<i>S. Kjellmanianum</i> YENDO	8	139 μ \times 97 μ	"	INOH (1930)
<i>S. hemiphyllum</i> C. AG.	8	103 μ \times 125 μ	"	"
<i>S. nigrifolium</i> YENDO	8	236 μ \times 264 μ	"	" (1932)

taken as a further confirmation of the statement made in the previous paper that the size of eggs is proportionate to the size of the rhizoid cell, and the number of primary rhizoids is, accordingly, related to the size of the egg, and that the species having larger eggs are higher in systematic position and the size of the egg is related to the order of complexity of vegetative organization in Fucaceous algae.

The table 1 summarizes the writer's data concerning the size of liberated eggs and the number of primary rhizoids in these species hitherto investigated.

The comparison concerning the mode of division of the rhizoid cell in these species hitherto investigated, *Fucus evanescens*, *Pelvetia Wrightii*, *Cystophyllum crassipes*, *Turbinaria ornata*, *Sargassum enerve* and *Cystophyllum sisymbrioides*, is made as in the following diagram (Fig. 11). As is shown above, it seems to be safely said that the division of the rhizoid cell increases its complexity in the order of the following types: One cell type, two cell type, four cell type, irregular eight cell type, sixteen cell type and thirty-two cell type. Also, in rare cases, the rhizoid cell of

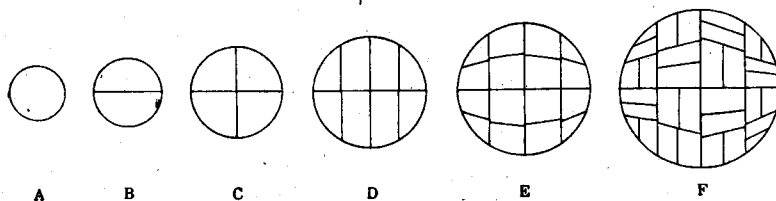


Fig. 11. Diagram of the division of rhizoid cells. A. One cell type (*Fucus evanescens*). B. Two cell type (?). C. four cell type (*Cystophyllum hakodatense*). D. Irregular eight cell type (*Turbinaria ornata*). E. Sixteen cell type (*Sargassum enerve*). F. Thirty-two cell type (*Cystophyllum sisymbrioides*).

abnormal embryos in *C. crassipes* is not divided at all, or divided into only two large cells, or into three cells, two small cells and one large cell. This is in connection with the following facts that the egg in *C. crassipes* is slightly smaller than that in *C. hakodatense* and it is an intermediate form between the one or two cell type and the four cell type as to the embryonal rhizoid formation.

The diploid chromosome number in *C. crassipes* is 64, the same as that in other species of *Fucaceae*, which were hitherto investigated by YAMANO-UCHI, OKABE and TOMITA, such as *Fucus vesiculosus*, *Sargassum Horneri* and *Coccophora Langsdorfi*. The centrosomes are visible in somatic mitosis

in the present species, as in *F. vesiculosus* (15) and *S. Horneri* (10). Contrasted with this, in *Coccophora Langsdorfi* (14) centrosomes are not visible.

Summary

- (1) In the following two species of *Turbinaria*, *T. ornata* and *T. filiformis*, the development of rhizoid cells was found to take place after the so-called "irregular eight cell type".
- (2) In *Cystophyllum crassipes*, the embryonic development occurs in the so-called "four cell type". Some abnormalities in early development of embryos were noted.

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