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A Report on the Cytology of Rhodymenia palmata, Rh. pertusa and Halosaccion saccatum (Rhodophyta)

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Abstract

Rhodymenia palmata, Rh. pertusa and Halosaccion saccatum had been studied cytologically. In these three species, it was ascertained that the nuclear division in the tetrasporangium is meiosis and the diffuse stage apparently exists before diakinesis. In the first meiotic division in Rhodymenia palamata and Halosaccion saccatum, a peculiar small rod-shaped body appears in rare occasions. In the tetrasporophytes, the chromosome count was given as follows: n=21 or 26 and 2n=ca 40-50 in Rhodymenia palmata, n=28 in Rh. pertusa, and n=17 and 2n=ca 30 in Halosaccion saccatum. In the meiosis of Rhodymenia palmata and Halosaccion saccatum, an extra large chromosome was found among the bivalents. large chromosome usually took a characteristic O-shape during diakinesis in the first division. The tetraspores of Rhodymenia palmata and the tetraspores and carpospores of Rh. pertusa had been cultured to observe the mitosis in the spore germlings. The chromosome number in the germlings indicated that the tetraspore germlings were haploid in Rhodymenia palmata, and the germlings of tetraspores and carpospores were haploid and diploid, respectively, in Rh. pertusa. In the tetraspore germlings of Rhodymenia palmata one of the chromosomes was somewhat longer than the others. The spore germlings of Rhodymenia palmata and Rh. pertusa were usually composed of uninucleate cells, but they occasionally contained multinucleate cells as a result of several successive synchronous mitotic divisions within the cells. In the meiosis of Rhodymenia palmata, Rh. pertusa and Halosaccion saccatum, a substance just like the centrosome was occasionally found in the polar position. From the unusual side views, with the precocious chromosomes which were frequently observed in the meiosis within the tetrasporangia of these three species, this substance was suspected to be nothing but such a precocious chromosome.

Introduction

Rhodymenia palmta (L.) Grev., Rh. pertusa (Post. et Rupr.) J. Ag. and Halosaccion saccatum Kütz., are the algae classified as the members of Rhodymeniales in Rhodophyta. Up to the present time, the cytological evidence on Rhodymenia palmata has been given by several investigators such as Westbrook¹, Austin², Magne³, Sparling⁵ and Yabu⁶. In my previous work⁶ I confirmed the presence of an extra large chromosome among the vivalents in the tetrasporangia and two long chromosomes among the univalents in the cortical cells which are dividing into the sporangial initial and its stalk cell.

Since then, I have continued to study the cytology of this alga; moreover fresh attempts have been made to study the cytology of *Rhodymenia pertusa* and

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Halosaccion saccatum because the study of these two species has not been reported so far.

Materials and Methods

Materials of the thalli used here were fixed at night after they had been kept alive in the vats containing sea water up to the time of fixing. The fixing was done mostly in acetic alcohol (1:3) and sometimes in Bouin's solution. The materials fixed in the former solution were stained with Wittmann's smear method 7), while those in the latter were preserved in the fixing fluid for 6–24 hrs, cut in 8 μ thickness after embedding in paraffin and stained usually with Heidenhain's haematoxylin and sometimes with Feulgen's tachnique. Of these experiments, Wittmann's method was most effective for the chromosome study of these three algae.

The mitotic divisions in spore germination were observed in the tetraspores of *Rhodymenia palmata* and the tetraspores and carpospores of *Rh. pertusa*. The spores were released on the slides in the vats containing sea water and the slides on which the spores had adhered were transfered into Schreiber solution. The culture dishes were preserved under the fluorescent light of ca 800 lux in the air-conditioned room maintained at ca 15°C temperature. The slides were taken out from the culture solution at various times of the day to be transferred into the fixing fluid of acetic alcohol. The fixing of the spore germlings lasted only from 3 to 5 minutes because a longer duration caused the germlings to fall from the slides. After fixing, the fluid recommended by Wittmann was poured on the slide and a cover slip was put on it. Then it was heated two or three times and squashed strongly.

Results

1. Rhodymenia palmata (L.) Grev.

The brief account of the cytology of *Rhodymenia palmata* collected between the year 1967 and 1969 from various places in Hokkaido, viz., Akkeshi, Oshoro, Muroran, Usujiri, Moheji and Hakodate, was reported in the previous paper⁶). The results described here are based on the materials newly collected in Hakodate and its vicinity from January to June from 1971 to 1973. During these periods, the numerous tetrasporophytes of young to mature had been fixed. The dividing nuclei had met frequently in the tetrasporangia and sometimes in the tetrasporangial mother cells of the maturing fronds.

Tetraspore formation: As it was stated by Westbrook in 1928 (p. 158)¹⁾, the tetrasporangia arise from small, superficial, cortical cells which early cut off a uninucleate stalk cell. This cortical cell, the so-called tetrasporangial mother cell, or the tetrasporangial rudiment, could be differenciated from the other cortical cells by the increase in size of the cell and from the tetrasporangial initials by the lack of stalk cell. As in the case of my previous study⁶, the dividing nuclei have been found only in the materials collected in the relatively defined short periods from Feburary to early March. In my previous study, the chromosome count was given as 2n=52 (including 2 long chromosomes) in the tetrasporangial mother

cells. In the present study, the chromosome number could not be estimated for certain, but it ranged between ca 40–50 and 2 long chromosomes were also easily recognized among them (Pl. I, Figs. D-G).

The process of the nuclear division in the tetrasporangium of *Rhodymenia* palmata was already well described in the earlier study by Westbrook¹⁾, and more minutely in the recent study by Magne³⁾⁴⁾. In the present study, the successive movement of nuclear stages within the tetrasporangium of this alga proved to be nearly similar to Magne's description, and moreover the occurrence of an extra large chromosome reported in my previous study was reaffirmed. The supplemental events found in the present case in the microtome sections and in the smeared preparations are as follows:

In the microtome section: Materials collected at Nanaehama near Hakodate in March and May from 1971 to 1973, and at Tachimachi-misaki and Shinori in Hakodate in April 1971 were used for this method. Among these materials some thalli collected at Nanaehama in March 1971 draw my attention because at the onset of prophase I, the sporangial nucleus with two nucleoli met at a considerably higher frequency for about 8% of the nuclei, and in such plants a small rod-shaped body just like the special chromosome which appears in the phenomenon of heteropycnosis was occasionally visible at early prophase I (Pl. II, Figs. A-B). In the side view of the first and second sporangial divisions at metaphase and anaphase, the centrosome-like body was occasionally observed (Pl. II, Figs. C-E), and it appeared positive to the Feulgen's nuclear reaction. In the materials from Nanaehama, some nuclei at very early stages of prophase II were found to have a peculiar body besides a nucleolus, which resembled a nucleolus in outer appearance, but somewhat differed from it in the points that it was smaller in size and showed a slightly deeper staining than the nucleolus. When this body is observable, it always appears in both daughter nuclei (Pl. II, Fig. F).

In the smear preparation: In smear preparations, of course, more extensive observations were possible than in sectioned ones. The early diplotene could be seen to have the chromatin threads which were obviously composed of the double strands (Pl. III, Figs. A-C). The evident twists occurred upon the threads, and the twisted threads which became discontinuous were frequently seen as if they were the distinct individual of chromosomes (Pl. III, Fig. C). Then, the nucleus increased in size, and the twists on the threads gradually decreased in number, becomming loose and translocated to their terminal portion (Pl. III, Fig. D). Hence, at late diplotene, some threads came to take shape as figures of large 8 or 0 (Pl. III, Fig. D). Next was the diffuse stage, which was characterized by only a faintly stained delicate network structure (Pl, III, Fig. E). In the course of the nuclear division in the tetrasporangium, the nucleus reached its maximum size at this stage. Magne⁴⁾ described the occurrence of a small cubic heterochromatic body which was closely associated with the nucleolus in the nucleus at the diffuse stage of Rhodymenia palmata. However, such a peculiar body could not be detected in my preparations. At early diakinesis, chromosomes emerge as weakly stained, long or short, complicated, intertwined heterochromatic threads and then they soon become shortened gemini (Pl. III, Figs. F-I; Pl. IV, Figs. A-I). Magne^{3,4}) gave the count of 14 chromosomes from the dividing figures in tetrasporangia.

In my preparations, the determination of the accurate chromosome count was not so easy, although late diakinesis and early metaphase usually ranged between ca 20-28 chromosomes. The careful observations in the good figures of these stages revealed that the materials treated here have at least two races, with 21 and 26 chromosomes respectively. The presence of an extra O-shaped, large chromosome is usually obvious during diakinesis to metaphase (Pl. IV, Figs. A-G). metaphase I, this chromosome was occasionally seen somewhat apart from the mass of the other chromosomes (Pl. V. Figs. A-B). In mid-metaphase I, chromosome gathering in the equatorial plate sometimes form a ring (Pl. V. Figs. D-E). metaphase and anaphase in the first division, various abnormalities, with precocious or lagging chromosomes, chromatin bridge or centrosome-like body, had been encountered occasionally (Pl. V. Figs. C-I; Pl. VI. Figs. A-H). In the second division, chromosomes appeared at first as short and thin twisted strands, but soon changed into pairs of glanular individuals. The size of each chromosome was far smaller than in the first division, however one chromosome could be easily distinguished as larger than the others (Pl. VII, Figs. D-I). With the exception of this large chromosome, the morphological distinctions are not enough to permit recognition of any chromosome as being from the first to the second division. late prophase and early metaphase in the second division the chromatids of each chromosome were in loose association (Pl. VIII, Figs. C-D). If the pressure is made strongly on the cover slip in making the smear preparation, the chromosome may break (Pl. VIII, Figs. D-E). As well as in the first division, 21 or 26 chromosomes were counted at late prophase and early metaphase and the irregular side views of metaphase and anaphase were occasionally visible, many of which were seen to have the dyads of the prematurely dissociated chromosome anticipating anaphase too (Pl. VIII, Figs. G-I).

Tetraspore germling: The early development of the tetraspore germling of Rhodymenia palmata has been reported by Killian⁸⁾, Ino⁹⁾¹⁰⁾ and Sparling⁵⁾. Magne³⁾ counted 14 chromosomes at the first mitosis in the tetraspore germlings of Rhodymenia palmata, while Sparling⁵⁾ gave the chromosome number as less than 14 from the mitotic division in the fixed germinating tetraspore of Rhodymenia palmata f. mollis. The culture for the investigation on the mitosis in the tetraspore germlings was started with the materials collected at Tachimachi-misaki in Hakodate on May 12th 1973, and it was continued for 2 weeks. Twelve thalli were used for this culture. The dividing nuclei in the tetraspore germlings could be obtained easily within the cells at their early development and were fixed at any time during the day or the night irrespectively. The spore which was attached on the slide soon started an active nuclear division. In the mid-prophase of the one-celled stage of its development, the chromosomes appeared as thin threads of about 6 μ in length, of which one was somewhat longer (Pl. IX, Figs. A-B). As the stages advanced further, the chromosomes became shorter and at last they changed into glanular form, even then one long chromosome was still easily distinguished (Pl. IX, Fig. D). Sometimes at metaphase, one or two chromosomes took an odd movement apart from the mass of the chromosomes gathering in the center of the nucleus, and at the side view of metaphase these chromosomes were occasionally seen like the centrosome (Pl. IX, Fig. F; Pl. X, Fig. B). At anaphase one chromosome usually left a long or short trail behind it. At the end of the nuclear division, the cell was divided by cleavage. Each cell consisting of a germling contained one nucleus, but occasionally multinuclei, as a result of the successive mitotic divisions within a cell; in the latter case the nuclear division in the cell occurred synchronously. In the germling not later than the four-celled stage, the chromosome count was possible at late prophase and early metaphase, and all the examined germlings had 21 chromosomes.

2. Rhodymenia pertusa (Post. et Rupr.) J. Ag.

The materials were obtained in May, 1971 and 1972 at Mori in Oshima Province, Hokkaido and in May, 1972 and 1973 at Tachimachi-misaki in Hakodate. The dividing nuclei had been observed in the tetrasporangia and in the germlings of tetraspores and carpospores. The fixed materials of *Rhodymenia palmata* were squashed easily after Wittmann's method; however, those of *Rh. pertusa* were hard to squash because of their stiff cell walls, even though they had been immersed in the solution for as long as two or three days. To become softer, the fixed materials had been immersed for a duration of 1-24 hrs in the solution of 2% lithium chloride recommended by Evans¹¹⁾ for the softening of *Fucus* in cytology. This solution produced a better effect, but not sufficient yet for the species. Thus in many cases, observations had to be made from the surface view of the fronds which could not be squashed well; nevertheless, the staining solution penetrated well into the inner portion of the frond and the stage of the nucleus within the sporangial cell was frequently clearly visible.

Tetrasporangium: The tetrasporangium of Rhodymenia pertusa was formed in both sides of the frond from the cortical cell in the same way as in Rh. pertusa. The sequence of the nuclear division in the tetrasporangium of this alga was identical with that of Rh. palmata described above, and the diffuse stage also existed before diakinesis. In the present species, the chromatin threads at diplotene were slightly more slender than those of Rh. palmata. At early diakinesis, the chromosomes began to appear as short tortuous threads which were scattered widely within the cavity of the nucleus, and passing through dumbell, triangle or O-shaped bodies, they became the clear glanule of 0.9–1.3 μ size at late diakinesis (Pl. XII, Figs. A-B). The chromosome count was possible during the stages from diakinesis to early metaphase and it gave 28–30 in number, of which 28 is thought to be correct judging from its appearance in frequency.

The number of the nuclei at mid-metaphase I, encountered in the sporangia was 58 in total, of which 21 were in polar view and 37 were in side view. Among those side views, 25 nuclei did not have a body at the pole, 6 nuclei had a precocious chromosome to one pole, 2 nuclei had each one precocious chromosome toward both poles, 2 nuclei had small centrosome-like bodies at both poles, and 2 nuclei had such bodies at one of the poles. The occurrence of such centrosome-like bodies was also noticed in metaphase II.

Spore germling: The spores were released in April 1972 from the tetrasporophytes and carposporophytes collected at Tachimachi-misaki, and they had been cultured for 15 days. The diameter of the spores was 25–38 μ in the tetraspores and 32–50 μ in the carpospores. The developmental process of the germlings of

both tetraspores and carpospores was the same and it was identical with that of the tetraspore germling of Rhodymenia palmata. In the germlings of the samecelled stage, the carpospore germlings were usually larger in size than the tetraspore germlings; however, at the same nuclear phases in the same-celled stages, the nucleus did not differ in size between the germlings of the tetraspores and those of the carpospores. The interphase nucleus in the one-celled stage is ca 15 μ in diameter and contains a nucleolus of ca 2.5μ in diameter. The chromosomes which appeared as the thin threads in mid-prophase became granular in form as the stages progressed (Pl. XIII, Figs. B-C; Pl. XV, Figs. A-B). In the late prophase, the spore as well as the nucleus took an elongated form towards its longitudinal direction (Pl. XIII, Fig. C; Pl. XIV, Fig. B), and soon the cleavage furrow made its appearance in the horizontal plane of the cell (Pl. XIV, Fig. B). About this stage, the nucleolus came to be stained weakly and it disappeared completely in the early metaphase. At telophase, the spindles became more evident and were seen to be curved (Pl. XIII, Fig. F). None of the substance could be detected at the pole (Pl. XIII, Fig. D). In the end of the nuclear division, the spore was cut into two cells by the cleavage. Near the end of the culture, the germlings had grown into disks consisting of 15-25 uninucleate cells; however, some cells of the germlings were found to have multinuclei. Although the accurate chromosome number was hard to count in the cell of the germlings, the approximate number was 20-28 in the cells of the tetraspore germlings and 30-40 in those of the carpospore germlings. Such a long chromosome which was detected in the tetraspore germlings of Rhodymenia palmata was not observed in the spore germlings of this alga.

3. Halosaccion saccatum Kütz.

Similarly to *Rhodymenia palmata*, the female plants of the present species are unknown. The cytological treaty in the genus *Halosaccion* was once attempted by Sparling⁵) in the tetrasporangium of *H. glandiforme*, but she could get no further than to obseve only the very early prophase stage of the nucleus. For the present study, tetrasporic and male plants were obstained at Akkeshi in May 1969 and Kushiro in July 1971 and they were examined in both the smeared and sectioned preparations. Between these two methods no marked difference could be noticed in the stage of the nucleus except that it became smaller in size with the sectioned method due to shrinkage.

Hair cell formation in tetrasporophyte: At the formation of the hair cell, the epidermal cell somewhat increased in size and elongated towards the direction of the thallus surface, taking a trigonal or an ellipsoidal form. At this stage, the nucleus set out the division. When the nucleus attained late prophase or early metaphase, the cell stretched the origin of the hair until about 60–80 μ long. Then the nucleus was divided completely, and one of the nuclei entered the original part of the hair, and soon the cell was divided into the rudiment of the hair cell and its stalk cell. In the rudiment of the hair cell, the nucleus was situated at its base and the nuclear division occurred at that place. With the growth of the hair, the nucleus migrated into the central place in the cell and took the aspect of the midprophase in the elongated form. In such hair cell formation in the tetrasporo-

phyte, the dividing nuclei with chromosomes were sometimes observed in the materials obtained from Kushiro (Pl. XVIII, Figs. A-C). The chromosome number counted in those nuclei was ca 30.

Tetraspore formation: The formula of the tetraspore formation in Halosaccion saccatum agreed with the one described and figured by Sparling⁵⁾ in H. glandiforme. The tetrasporangial mother cell which was enlarged from the cortical cell was found sometimes to possess a dividing nucleus (Pl. XVIII, Figs. D-I). At late prophase and early metaphase, some of the nuclei had ca 30 chromosomes, of which 2 were somewhat long.

The tetrasporangium of this alga, together with the nucleus in it, is much smaller in size than that of Rhodymenia palmata and Rh. pertusa, and the progress of the nuclear phases within the tetrasporangium of Halosaccion saccatum was quite similar to that of Rhodymenia palmata and Rh. pertusa. In both sectioned and smeared preparations, the nucleus at early prophase I in the tetrasporangium was rarely found to possess a rod-shaped body which seemed to be the same substance as of the nucleus found occasionsly in the sectioned material of Rhodymenia palmata obtained from Nanehama (Pl. XVIII, Fig. A). After passing through the diffuse stage, bivalent gemini appeared in the nucleus (Pl. XIII, Figs. D-I). Although, the chromosome count was not so easy, 17 chromosomes were occasionally counted in the good figures of the late prophase and early metaphase. those chromosomes an extra large chromosome, the shape of which was quite similar to the one found in the first meiosis in the tetrasporangium of Rhodymenia This chromosome sometimes left a palmata was visible (Pl. XIII, Figs. D-H). trail behind at anaphase and telophase. A centrosome-like body or odd chromosome going precociously towards the pole was occasionally visible in the side view of metaphase I & II (Pl. XIX, Fig. D).

Spermatium formation: Spermatium formation in Halosaccion saccatum was reported in considerable detail by Lee and Kurogi¹¹). In the present study, the dividing nuclei in the spermatium forming cells were seen in both sectioned and smeared preparations. Chromosomes were observed in the late prophase and early metaphase nuclei in the elongated epiderm cells which were leading to spermatium formation. Owing to the minute size of the nuclei, the chromosome count was mostly impossible; however, rarely, 10–15 chromosomes were noticed. A large chromosome like the one found in the tetrasporangium was not detected among them.

Discussion

Westbrook¹⁾, who observed the nuclear division in the tetrasporangium of *Rhodymenia palmata*, could not bring up sufficient results as to whether it was the real meiosis or not. Magne^{3,4)} is of the opinion that it is apomeiosis, because even if the nuclear behaviour within the sporangium displayed the aspect of meiosis, yet, the chromosome number had not been reduced. However, in my previous study⁶⁾, I gave the conclusion that it was the normal meiosis. Based on the chromosome numbers in tetrasporangia and others, viz., in the tetrasporangial mother cells in *Rhodymenia palmata* and *Halosaccion saccatum* and the spore germlings of *Rhodymenia palmata* and *Rh. pertusa*, the present study revealed that

the meiosis occurred undoubtedly in the tetrasporangium of Rhodymenia palmata, Rh. pertusa and Halosaccion saccatum.

In all of the three species studied here, it is evident that the nucleus passes though the distinct diffuse stage before diakinesis. At early prophase in the first tetrasporangial division, a peculiar, small, rod-shaped body was rarely found in the sectioned preparations of *Rhodymenia palmata*, and very rarely in both the sectioned and smeared preparations of *Halosaccion saccatum*. The characteristic found in the tetrasporangial division of *Rhodymenia palmata*, *Halosaccion saccatum* and *Rh. pertusa* is that an extra large chromosome was obvious among the bivalents in the former two species, and lacked in the latter.

As it was known, the life cycle of *Rhodymenia palmata* and *Halosaccion saccatum* is questionable for the uncertainty of the existence of female plants in nature. I feel that the presence of the extra large chromosome in these two peculiar species may play an important role in their life history.

At the side view of the metaphase in the tetrasporangial nuclear division of Rhodymenia palmata, the anomalous appearance of the body in the direction of the axis of the nucleus had been observed by Westbrook¹⁾ and Yabu⁶⁾. Westbrook1) said that it was justified to consider it as centrosome. In some other species of Rhodophyta, such a kind of body was mentioned by Westbrook¹³⁾ and Magne⁴). In his comments of this body in Rhodophyta, Dixon¹⁴) states; "Until the reasons for their irregular appearance and ephermal nature are understood it would be best simply to refer to these structure as 'polar body'". This body was occasionally seen in the side view of metaphase I & II in the tetrasporangium of all three species studied here. The test with Feulgen's technique for nuclear reaction in the sectioned material of Rhodymenia palmata were positive to such a centrosome-like body so that it is not supposed to be such a centrosome as was considered by Westbrook¹⁾. In the side view of the tetrasporangial metaphase I & II, the precocious chromosome was occasionally visible in these three species, and it was frequently hard to distinguish whether the centrosome-like body was a real chromosome or not. The result of the Feulgen's nuclear reaction in Rhodymenia palmata and of the observations on the odd movement of the chromosome at tetrasporagnial metaphase I & II, in each species, induces me at present to believe that the centrosome-like body seen in these three species should be a mere odd chromosome which travels hastily to the pole.

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Pxplanation of Plates

Plate I

Nuclear divisions in the tetrasporangial mother cells of *Rhodymenia palmata*. Fixed with aceto-alcohol, and stained with Wittmann's solution. A-F & H, \times 1,200; G, \times 2,600

A-B. Mid-prophase

C-G. Late prophase

H. Early metaphase

Plate II

Nuclear divisions in the tetrasporangia of *Rhodymenia palmata*. Fixed with Bouin's solution, and stained with Heidenhain's haematoxylin. All figures, \times 1,600

A-B. Early prophase

C-E. Early anaphase

F. Early prophase in the second division

Plate III

The first nuclear divisions in the tetrasporangia of *Rhodymenia palmata*. Fixed with aceto-alcohol, and stained with Wittmann's solution. All figures, \times 1,200

A-C. Diplotene

D. Late diplotene

E. Diffuse stage

F-G. Early diakinesis

H-I. Diakinesis

Plate IV

The first nuclear divisions in the tetrasporangia of *Rhodymenia palmata*. Fixed with aceto-alcohol, and stained with Wittmann's solution. All figures, \times 1,200

A-H. Diakinesis

I. Early metaphase. The chromosome count was given to be 26 from the nuclei shown in Figs. A-E, and 21 in Figs. F-I. One extra large chromosome is visible in Figs. A-G; it is out of the focus in Fig. H, and this chromosome changes into somewhat larger granular chromosome in Fig. I.

Plate V

The first nuclear divisions in the tetrasporangia of *Rhodymenia palmata*. Fixed with aceto-alcohol, and stained with Wittmann's solution. All figures, × 1,200

A-I. Metaphase. A-B. Showing an O-shaped large chromosome which is apart from the group of chromosomes. C-E. Showing the centrosome-like body which was suspected to be an odd chromosome at or near the pole.

Plate VI

The first nuclear divisions in the tetrasporangia of *Rhodymenia palmata*. Fixed with aceto-alcohol, and stained with Wittmann's solution. All figures, \times 1,200

A-I. Metaphase (A-B) and anaphase (C-I), with the centrosome-like body, precocious or lagging chromosome or chromatin bridge.

Plate VII

The second nuclear divisions in the tetrasporangia of Rhodymenia palmata. Fixed with aceto-alcohol, and stained with Wittmann's solution. All figures, × 1,200

A-H. Late prophase

I. Early metaphase

Plate VIII

The second nuclear divisions in the tetrasporangia of *Rhodymenia palmata*. Fixed with aceto-alcohol, and stained with Wittmann's solution. All figures, \times 1,200

- A.F. Metaphase. In Figs. D-E, most of the the chromosomes appear as paired bivalents but several as dissociated chromosomes.
- G-I. Side view of metaphase, showing the centrosome-like body, which is suspected to be the precocious chromosome moving to the polar position.

Plate IX

Nuclear divisions in the one-cell stage of the tetraspore germling of *Rhodymenia palmata*. Fixed with aceto-alcohol, and stained with Wittmann's solution. All figures, \times 1.200

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- A. Mid-prophase
- B. The same nucleus as shown in Fig. A, in the different focus level
- C-E. Late prophase
- F. Metaphase

Plate X

Nuclear divisions in the tetraspore germlings of *Rhodymenia palmata*. Fixed with aceto-alcohol, and stained with Wittmann's solution. All figures, \times 1,200

- A-B. Side view of metaphase in the one-cell stage
- C. Late prophase in the two-cell stage
- D. Late anaphase (in the left cell) and telophase (in the right cell) in the two-cell stage
- E. Anaphase nuclei in one of the cells in the four-cell stage
- F. Metaphase in the more advanced celled stage than Fig. E

Plate XI

The first nuclear divisions in the tetrasporangia of Rhodymenia pertusa. Fixed with aceto-alcohol, and stained with Wittmann's solution. All figures, \times 1,200

A-F. Successive nuclear stages from early prophase to early diakinesis

Plate XII

The first nuclear divisions in the tetrasporangia of *Rhodymenia pertusa*. Fixed with aceto-alcohol, and stained with Wittmann's solution. All figures, \times 1,200

- A-B. Diakinesis
- C-D. Metaphase
- E-G. Side view of metaphase

Plate XIII

Nuclear divisions in the tetraspore germlings of *Rhodymenia pertusa*. Fixed with aceto-alcohol, and stained with Wittmann's solution. All figures, \times 1,200

- A. Early prophase in the one-cell stage
- B. Mid-prophase in the one-cell stage
- C. Late prophase in the one-cell stage
- D. Side view of metaphase in the one-cell stage
- E. Anaphase in the one-cell stage
- F. Telophase in the one-cell stage
- G. Metaphase in the two-cell stage
- H. Early anaphase (in the upper cell) and metaphase (in the lower cell) in the two-cell stage
- I. Metaphase in the four-cell stage

Plate XIV

Nuclear divisions in the tetraspore germlings of Rhodymenia pertusa. Fixed with aceto-alcohol, and stained with Wittmann's solution. All figures, \times 12,00

- A-B. Metaphase in the two-cell stage. A. Showing two metaphase nuclei in the upper cell (one nucleus is seen in the figure and the other is out of focus) and four metaphase nuclei in the lower cell. B. Showing four metaphase nuclei in the large cell to the right of the figure
- C-F. Metaphase or anaphase in the more advanced celled stage than Fig. B

Plate XV

Nuclear divisions in the carpospore germlings of *Rhodymenia pertusa*. Fixed with aceto-alcohol, and stained with Wittmann's solution. All figures, \times 1,200

- A. Mid-prophase in the one-cell stage
- B. Late prophase in the one-cell stage. Cleavage furrow begins to appear.
- C. Four anaphase nuclei in the one-cell stage
- D. Four metaphase nuclei in the large cell in the two-cell stage. Two nuclei are in the focus and the other two are seen dimly in the lower portion of the cell.
- E-F. Metaphase nuclei in the two-cell stage

Plate XVI

Nuclear divisions in the carpospore germlings of *Rhodymenia pertusa*. Fixed with aceto-alcohol, and stained with Wittmann's solution. All figures, \times 1,200.

- A. Metaphase nuclei in the two-cell stage
- B-F. Metaphase and anaphase nuclei in the more advanced celled stage than Fig. A

Plate XVII

Nuclear divisions in the hair cell formation and in the tetrasporangia of *Halosaccion saccatum*. Fixed with aceto-alcohol, and stained with Wittmann's solution. A-C & E-I, \times 1,200; D, \times 2,600

- A. Early metaphase in the epiderm cell leading to the hair formation
- B. Metaphase in the rudiment of the hair cell, leading to the formation of hair and its stalk cell
- C. Metaphase in the hair cell
- D. Mid-prophase in the tetrasporangial mother cell
- E-I. Late prophase in the tetrasporangial mother cell. Among the chromosomes, two long chromosomes are visible in Figs. G-I.

Plate XVIII

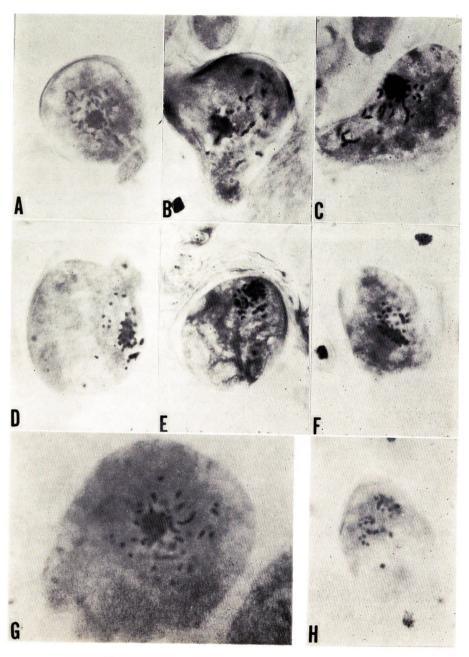
Nuclear divisions in the tetrasporangia of *Halosaccion saccatum*. Fixed with aceto-alcohol, and stained with Wittmann's solution. A-B & G, \times 1,400; C, \times 800; D-G & H-I, \times 1,200

- A. Early prophase, showing the rod-shaped body in the nucleus
- B. Early prophase (in the right side of the figure) and diffuse stage (in the left side of the figure)
- C. Early diplotene
- D-H. Diakinesis. Note, U- or O-shaped extra large chromosome in each nucleus

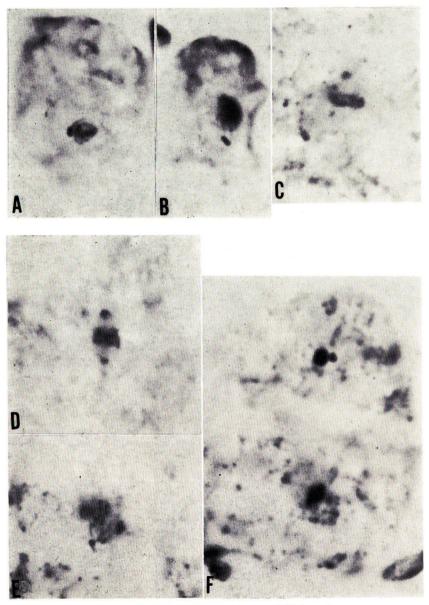
Plate XIX

Nuclear divisions in the tetrasporangia and in the antheridia of *Halosaccion saccatum*. Fixed with aceto-alcohol, and stained with Wittmann's solution. A & C-I, \times 1,200; B, \times 1,400

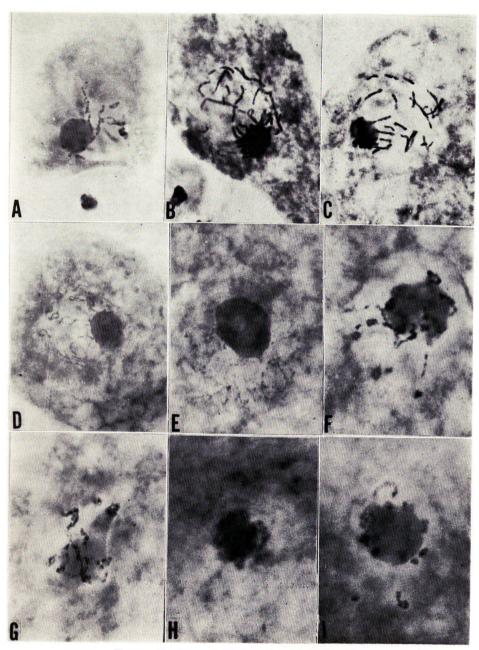
- A. Diakinesis
- B-C. Metaphase
- D. Side view of metaphase
- E. Anaphase
- F. Late prophase of the second division
- G. Anaphase of the second division
- H-I. Early metaphase in the antheridia



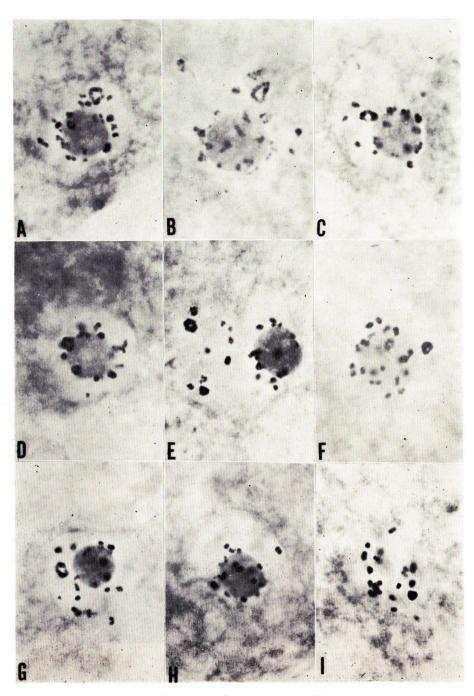
Yabu: Cytology of Rhodymenia and Halosaccion



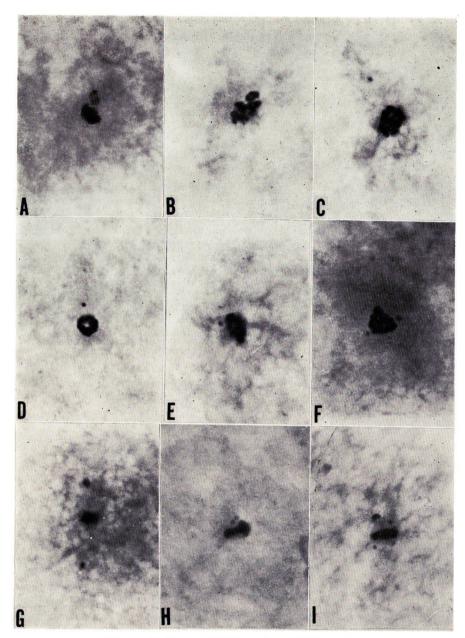
Yabu: Cytology of Rhodymenia and Halosaccion



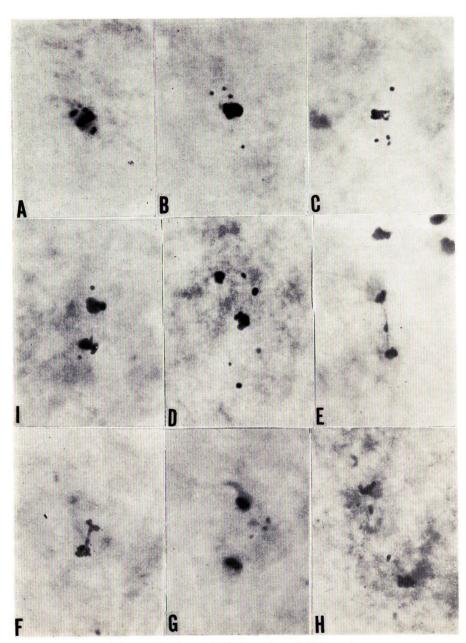
Yabu: Cytology of Rhodymenia and Halosaccion



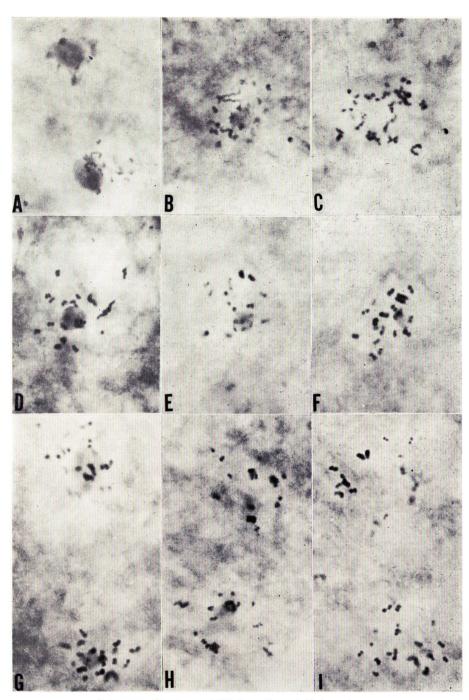
Yabu: Cytology of Rhodymenia and Halosaccion



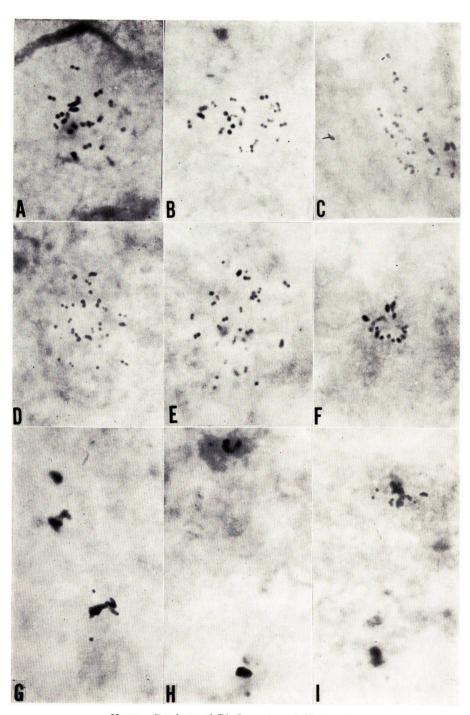
Yabu: Cytology of Rhodymenia and Halosaccion



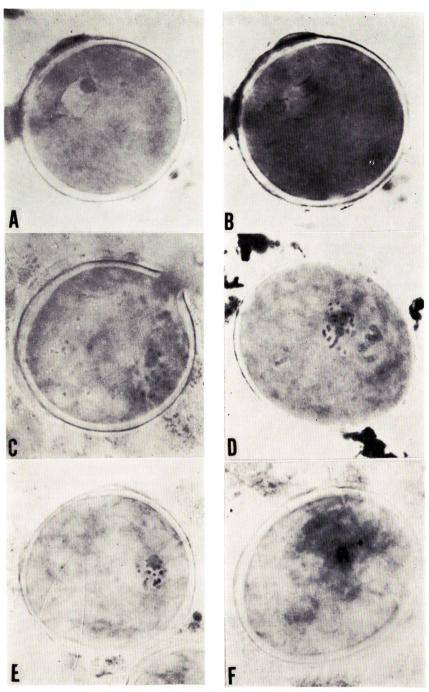
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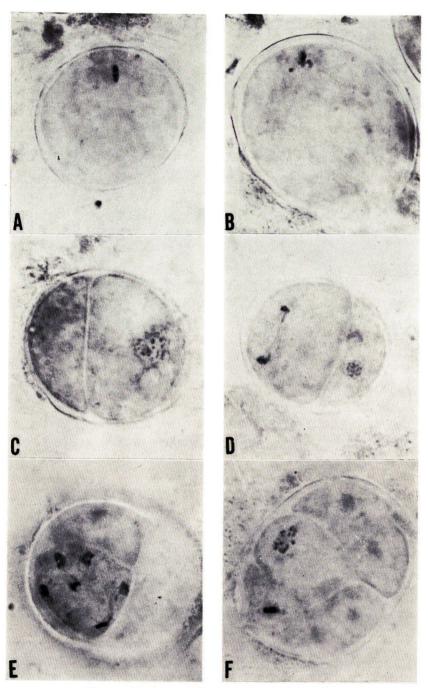
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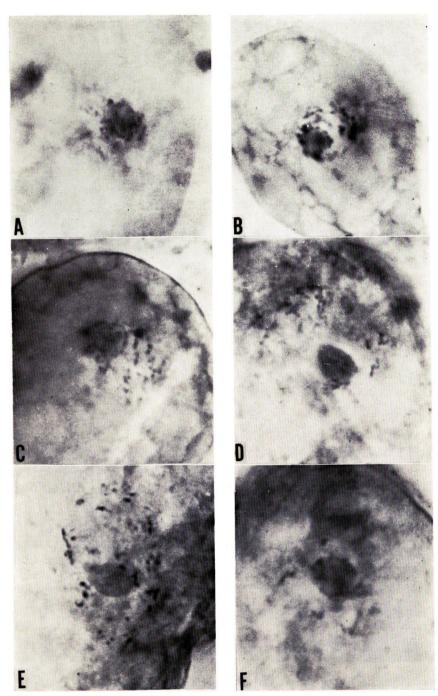
Yabu: Cytology of Rhodymenia and Halosaccion



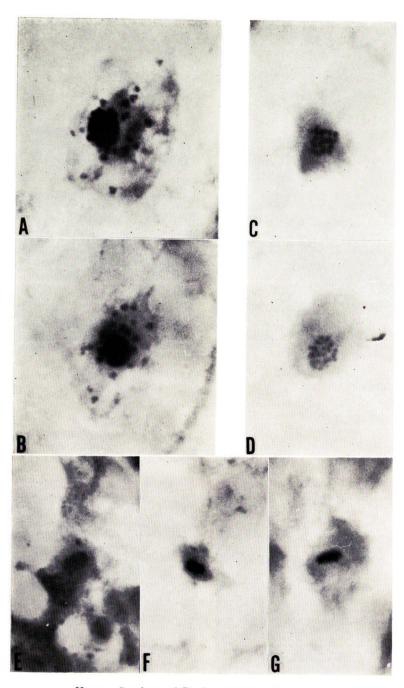
Yabu: Cytology of Rhodymenia and Halosaccion



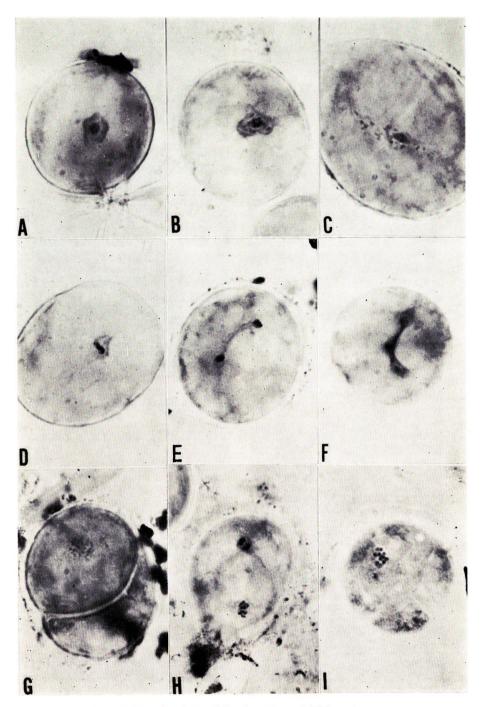
Yabu: Cytology of Rhodymenia and Halosaccion



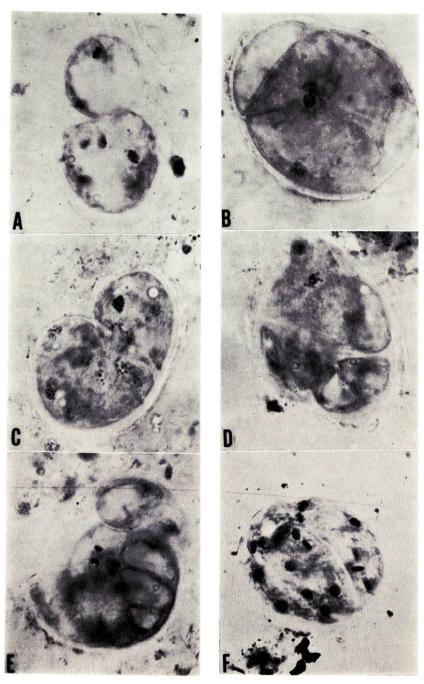
Yabu: Cytology of Rhodymenia and Halosaccion



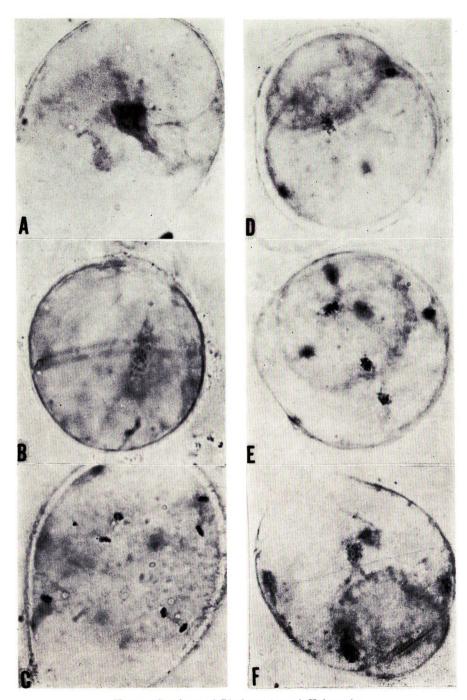
Yabu: Cytology of Rhodymenia and Halosaccion



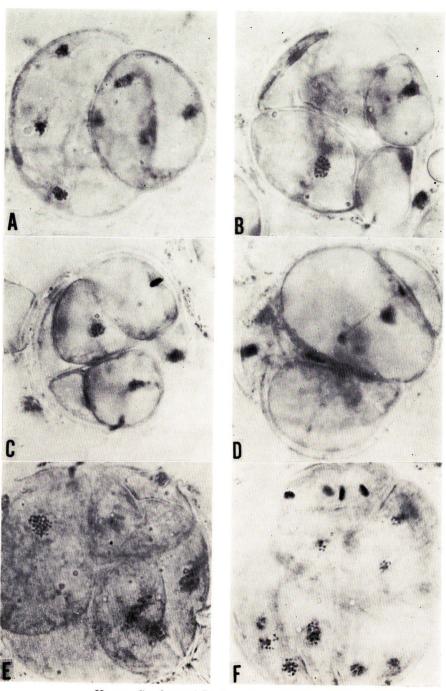
Yabu: Cytology of Rhodymenia and Halosaccion



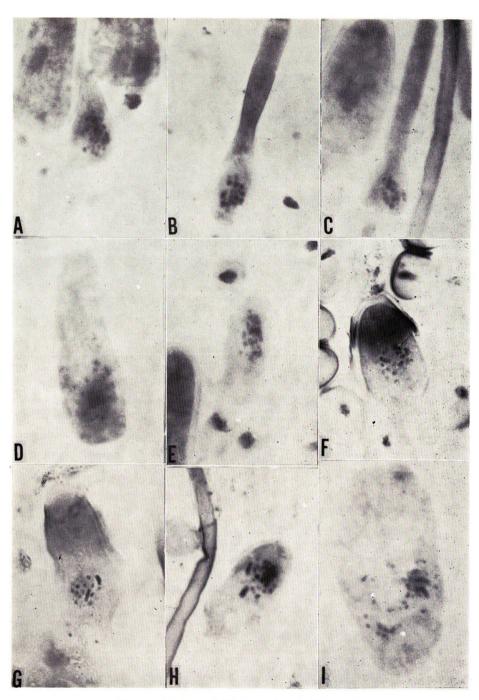
Yabu: Cytology of Rhodymenia and Halosaccion



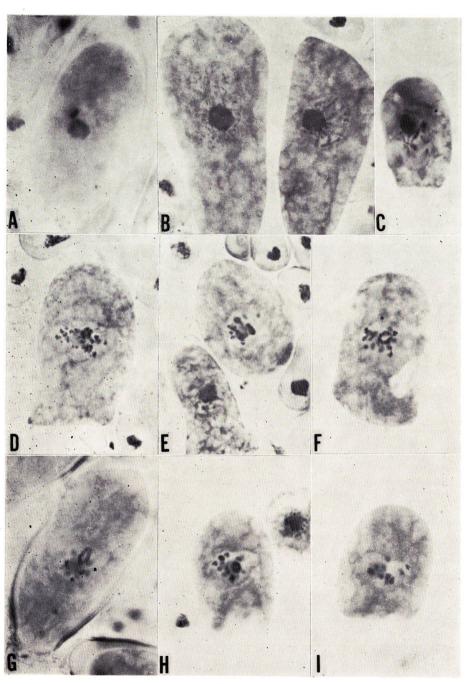
Yabu: Cytology of Rhodymenia and Halosaccion



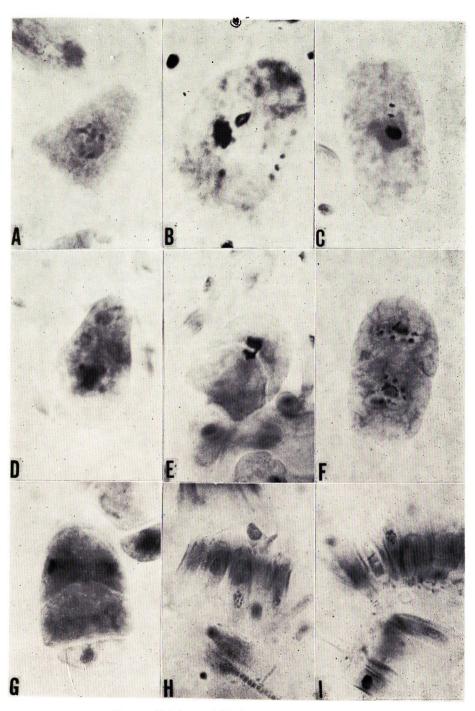
Yabu: Cytology of Rhodymenia and Halosaccion



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