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CYTOLOGICAL STUDY ON SOME JAPANESE SPECIES OF RHODOMELACEAE

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INTRODUCTION

The first cytological study in the species belonging to the Family Rhodomelaceae is that which was made by Yamanouchi (1906) with *Polysiphonia violacea*. After that there have been published four papers dealing with the same subject by three other investigators, *viz.*, Kylin (1914, 1923), Westbrook (1928), and Austin (1956).

The following table shows the chromosome numbers of nine Rhodomelaceous species counted by these workers.

Table I

Species	Chromosome number		Author
	n	2n	
Polysiphonia violacea	20	40	Yamanouchi, 1906
P. nigrescence	20		Kylin, 1923
〃 〃	30	60	Austin, 1956
P. elongata	37		Austin, 1956
P. lanuosa	27		Austin, 1956
Chondria dasyphylla	18-19	40	Westbrook, 1928
Laurencia hybrida	31		Austin, 1956
L. pinnatifida	29	40	Kylin, 1923
〃 〃			Austin, 1956
Rhodomela virgata	20		Kylin, 1914
Rh. confervoides		40	Kylin, 1914
〃 〃	32		Austin, 1956

Since none of the Japanese species of Rhodomelaceae has ever been studied cytologically, the writers undertook the present study in 1957 with several species, and fortunately could obtain satisfactory results in the following four species: *Polysiphonia japonica* HARV., *Chondria crassicaulis* HARV., *Laurencia papillosa* HARV. and *L. obtusa* LAMOUR. var. *majuscula* HARV. So the writers wish to report here the results of their observations. On this opportunity the writers acknowledge their indebtedness to Professor J. Tokida for his kindness in reading the manuscript, and in giving valuable advices.

MATERIAL AND METHOD

Of the four species studied, *Polysiphonia japonica* and *Chondria crassicaulis* were collected several times in 1957 at Nanaehama near Hakodate, the former in the period ranging from late September to the middle of December and the latter from early September to the middle of December. The collected materials were brought to the laboratory and kept alive for some time in glass vessels containing sea water. On the other hand, the remaining two species belonging to the genus *Laurencia* were collected by the junior writer on the 2nd and the 3rd of August 1957 at Okinoshima, Sukumo City in Kôchi prefecture, and fixed on the spot. The fixed materials kept in 75% alcohol were brought to the

laboratory.

Fixation of the materials was done with the following seven fixatives applied for various lengths of time respectively (Table II). Sections were cut 4–7μ in thickness by paraffin method and stained with Heidenhein's haematoxylin.

Table II

Material	Fixing fluid	Fixing hours
Polysiphonia japonica	Yamanouchi's fluid	3
	Abe's fluid	3
	Modified Yamanouchi's fluid	5
	Modified Abe's fluid	5
Chondria crassicaulis	Abe's fluid	3
	Tahara's fluid	3-4
	Modified Abe's fluid	3
	Modified Yamanouchi's fluid	3
Laurencia papillosa	Fluid consisting of chromic, picric, and acetic acid and formalin	10
Laurencia obtusa	Fluid consisting of chromic and acetic acid and formalin	1
	Modified Allen's fluid	10

The formulas of the fixing fluids employed are as follows :

1) Yamanouchi's fluid (Yamanouchi, 1909)

2% osmic acid	1.0 cc
1% chromic acid	5.0 cc
Glacial acetic acid	2.0 cc
Sea-water	11.0 cc

(Yamanouchi used 1% solution of osmic acid in various volumes)

2) Modified Yamanouchi's fluid

2% osmic acid	1.0 cc
1% chromic acid	5.0 cc
Glacial acetic acid	1.0 cc
Sea-water	11.0 cc

3) Abe's fluid (Abe, 1933)

A) Stock solution of chromic acid (sea-water 98 cc, saturated water solution of chromic acid 2 cc) 50.0 cc

Sea-water	50.0 cc
2% osmic acid	5.0 cc
Glacial acetic acid	2.5 cc

B) Saturated solution of picric acid 50.0 cc

	Glacial acetic acid	5.0 cc
	Chromic acid	1.0 cc
C)	Saturated solution of picric acid	25.0 cc
	40% formalin	25.0 cc
	Urea	0.5 cc

These three solutions were mixed just before use at the ratio A : B : C = 2 : 1 : 1.

4) Modified Abe's fluid

Each of the solutions (A) and (B) of Abe's fluid is adjusted to contain 1.0 cc of glacial acetic acid in this modification.

5) Tahara's fluid (Tahara, 1929)

2% osmic acid	5.0 cc
2% chromic acid	70.0 cc
Glacial acetic acid	2.5 cc
Sea-water	30.0 cc

6) Fluid consisting of chromic, picric and acetic acid and formalin

Stock solution of chromic acid (2 cc of saturated water solution of chromic acid in 98 cc of sea-water)	5.0 cc
Saturated picric acid	50.0 cc
Formalin	5.0 cc
Glacial acetic acid	2.0 cc

7) Fluid consisting of chromic and acetic acid and formalin

Stock solution of chromic acid (2 cc of saturated water solution of chromic acid in 98 cc of sea-water)	5.0 cc
Glacial acetic acid	5.0 cc
Formalin	10.0 cc
Sea-water	50.0 cc

DESCRIPTION OF THE RESULTS OBSERVED

Polysiphonia japonica HARV.

In this species, the nuclear divisions in the somatic and reproductive cells of the tetrasporophyte and the gametophytes, female and male, were observed. The materials were fixed at various times between 7 p.m. and 4 a.m., and the nuclei were found dividing in those fixed between 9 p.m. and 3 a.m. Three hours in the middle of the night between 11 p.m. and 2 a.m. were best for fixing to obtain a good number of dividing nuclei. The fluids recommended by Abe (1933) and Yamanouchi (1909), *viz.*, Nos. 1 and 3 of the above listed formulas, gave satisfactory results in this species, but the writers' modifications, *viz.*,

Nos. 2 and 4, which contain a smaller amount of glacial acetic acid than the former were much better in bringing forth good nuclear figures in both somatic and reproductive cells.

a) Somatic division. All stages of nuclear divisions in somatic cells of both tetrasporic and sexual plants were observed at the terminal portions of the thallus. Colourless hair cells on upper branches are always uninucleate and good for counting the chromosome number in metaphase. Somatic cells in other parts of the thallus are usually uninucleate but sometimes multinucleate. The hair cells and the multinucleate cells were generally not good for the present purposes on account of small size of their nuclei. So the following descriptions are based on larger nuclei in the cells at or near the thallus apices.

Nucleus in early prophase shows a network structure; soon it begins to produce small chromatin granules on the net. These granules become larger in size, while chromatin threads become more slender and gradually disappear. Nucleus in later prophase contains single nucleolus together with well-stained chromatin granules which were called prochromosomes by Yamanouchi (1906). In early metaphase the nuclear membrane and the nucleolus disappear. The number of chromosomes was counted at this state. It was twenty in both female and male plants and forty in tetrasporic plants. In *Polysiphonia violacea*, Yamanouchi (1906, p. 408) observed at the metaphase of somatic division deeply stained centrosome-like bodies which were described as "conspicuous at the poles just before the spindle is formed." Such bodies could not be found in *P. japonica* by the present writers in spite of careful observations. The spindle fibers were not clearly seen in most cases, but sometimes they were visible in a faintly stained material. In anaphase, long or short spindle fibers attached to or detached from chromosomes were found between two groups of daughter chromosomes. After the chromosomes disappeared and the daughter nuclei were produced in telophase, a somewhat vacuolated structure appeared in the equatorial plate of the cell and the cell membrane was formed there perpendicularly to the direction of nuclear division. In Pl. I, Figs. 3–9 are shown the nuclear divisions in the hair cells of sexual and asexual plants.

b) Nuclear division in tetrasporangia. Tetrasporangia make their appearance first near the top of branches and then gradually in lower portions of the thallus. They are cut off from the pericentral cells as follows. A pericentral cell is divided vertically into two cells, of which the inner one is then divided transversely into two *i.e.*, into tetrasporangium mother cell and its stalk cell. The sporangium mother cell soon increases in size and becomes oval in shape. Its nucleus in early prohase has usually one, but rarely two, nucleoli. The nucleus sometimes looks vacuolated or reticulated in structure because of unequal staining of its content. Then, slender chromatin threads appear at one side of the nuclear cavity, and next the nuclear cavity becomes filled with chromatin threads. Existence of chromatin threads in early prohase was clearly recognized in the materials fixed with Abe's fluid. In diakinesis stage of a nucleus, in which the nucleolus had already disappeared, the writers could count 20 gemini which were V-, O-, or rod-shaped. These chromosomes gradually became spherical in shape and somewhat more deeply stained. In metaphase, the nuclear

membrane disappeared completely, and the nuclear cavity which had usually been stained light blue in prophase became colourless and indistinguishable from the surrounding cytoplasm of the sporangium, so that the boundary of the nucleus was quite indistinct. Well-stained small granules, which made their appearance in the cytoplasm of a sporangium when the latter's nucleus was in the prophase of the first division, do not disappear even in metaphase. Hence the chromosomes in metaphase were sometimes apt to be confounded with these granules. Spindle fibers were visible in the materials fixed with Abe's fluid, but centrosome-like bodies at the spindle poles could not be observed in any material. As soon as the chromosomes are drawn to each pole, the second nuclear division sets in as shown in Pl. II, Figs. 11–13. The chromosomes which appeared in the second division are much smaller as compared with those in the first division, but in some cases 20 chromosomes are clearly counted in metaphase. The axes of the second nuclear division are perpendicular to each other. In anaphase of the second division, the outline of the original nuclear membrane comes into view again. The four groups of grand-daughter chromosomes reach the periphery of the original nuclear cavity and remain there for a while as small granules. Then the chromosomes lose their individual outlines and the chromatin network makes its appearance. At this stage a large nucleolus-like body suddenly appears in the center of the nucleus. The nucelous-like body is occasionally broken into small pieces. The cytoplasmic portion of the tetrasporangium shows an alveolar structure in late anaphase of the second division. Prior to this, cleavage furrows are fomred at the periphery of the sporangium. The furrows sometimes make their appearance as early as in the metaphase of the first nuclear division. They gradually grow inward and eventually divide the cytoplasm of the sporangium into four equal parts or young tetraspores, each of which contain one of the four daughter nuclei produced as a result of the nuclear division described above. The alveolar structure of the young tetraspores disappears, and numerous well-stained granular chromatophores are produced at the periphery of the spore. The nucleus in the center of each tetraspore becomes much smaller in size and its chromatin networks fade away. In the meanwhile, the chromatophores change their form and become rod-like or elliptical in shape and are somewhat scattered in the inner cytoplasmic portion of the mature spores.

c) Nuclear division in spermatogenesis. Antheridia are formed on short branches at or near the tips of the main filaments. The antheridium is composed while young of a few monosiphonous cells, but later it becomes polysiphonous, oval in outline, being composed of a central siphon surrounded by numerous small cells which have been formed in the following manner. The central siphon gives rise laterally to two-celled short branches, the basal cell of which is larger than the apical. The latter cell is the spermatangium mother cell and it cuts off two spermatangia by oblique walls. The antheridia were easily fixed well for the observation of nuclear divisions with all the above described fixing solutions. The nuclei were much smaller than those in the somatic divisions, but it was possible to count clearly 20 chromosomes in metaphase. Each group of the chromosomes which was drawn to one pole in telophase of the spermatangium mother cell becomes the nucleus

of the spermatangium without losing the outlines of the chromosomes, and thus one spermatium containing 20 chromosomes is produced in each spermatangium.

d) Nuclear division in the development of procarp and gonimoblast. The procarp initial cell is born on the subapical segment of the short apical hairy portion of a branch. The hair is converted from one of several cells cut off from the apical cell or it arises directly from the undivided apical cell itself. The subapical segment of the hair produces five pericentral cells, of which the innermost one becomes the initial of the procarp. As shown in Plate IX, Figs. A and C, the procarp initial cell can be recognized from early by its larger size as compared with nearby vegetative cells. It cuts off a mother cell of the carpogonial branch, and finally a four-celled carpogonial branch is produced. The supporting cell of this branch, which is no other than the initial cell of the procarp, cuts off two neutral cells, one near its base and the other at its side. This latter cell divides into two cells, each of which usually remains simple but sometimes abnormally develops into a row of several neutral cells. Pl. III, Fig. 6 shows a procarp just before fertilization. The trichogyne was always found to be destitute of a nucleus, though Yamanouchi (1906, p. 413) mentioned the existence of the trichogyne nucleus in *Polysiphonia violacea*. Pl. III, Fig. 3 shows the nuclear division in metaphase in an initial cell of the procarp. The number of chromosomes counted in the cells was exactly or approximately 20. The nucleus of a spermatium which has entered into the trichogyne is deeply stained with haematoxylin and is occasionally seen as a mass of small chromatin granules. However, during the period from the attachment of a spermatium to a trichogyne till the appearance of a fertilized nucleus in prophase in the carpogonium, the cells of a carpogonial branch are usually stained so dark that it is difficult to observe their nuclei. Therefore the process of fusion between the female and male gamete nuclei in a carpogonium could not be observed. On the other hand, the diploid nuclei in later prophase and early metaphase were frequently observed in the carpogonium (Pl. X, Figs. C–F). The diploid nucleus in prophase was already found to have chromatin granules on the reticulum ; those granules in later prophase were about 40 in number. Then the nucleus increased its size and passed into early metaphase. At this time the nuclear membrane was invisible and the chromosomes, so far as the writers have observed, attained their maximum size as they did also in the division of somatic cells, tetrasporangia, and of spermatangia. In the first mitosis of the diploid nuclei, Yamanouchi (1906, p. 417) observed in his plant two kinds of chromosomes differing from each other in size ; he believed that some of the smaller ones might have come certainly from the male nucleus. However, the writers could not recognize in their material any difference in size among the chromosomes in metaphase of the diploid nucleus. The chromosomes in early metaphase of the fertilized nucleus were 40 in number. After fertilization, the supporting cell of the carpogonial branch increases rapidly in size and soon becomes pear-shaped containing two nuclei (Pl. X, Fig. A). When the diploid nucleus in the carpogonium enters into prophase, the supporting cell divides into two, the upper one of which becomes an auxiliary cell (Pl. X, Fig. D). As soon as the diploid nucleus

enters into early metaphase, the connection between the auxiliary cell and the carpogonium is accomplished (Pl. X, Fig. F). The nucleus of the carpogonium in resting stage is small and well stained ; the auxiliary cell contains one large faintly stained nucleus in the center, which is no doubt one of the daughter nuclei of the fertilized carpogonium nucleus, and one small rod-shaped nucleus, which must be haploid, in the periphery. The auxiliary cell and the supporting cell soon fuse with each other, and the connecting pit between the supporting cell and the central siphon becomes somewhat broadened. The nucleus of the supporting cell occupies the basal portion of the fusion cell. The nucleolus of the resting diploid nucleus is attached to a large globular body which contains minute granules (Pl. IV, Figs. 2 & 3). These granules soon form a reticulum (Pl. IV, Fig. 4). Then the globular body disappears and the nuclear cavity becomes filled with the reticulum (Pl. IV, Fig. 5). The neighboring neutral cells fuse with each other and with the large fusion cell which was formed by the coalescence of the auxiliary cell and the supporting cell. Several haploid nuclei derived from the fusion of the neighboring cells occupy the basal portion of the central cell. The diploid nucleus soon begins its first division, and 40 chromosomes are easily counted in its metaphase. Spindles and centrosome-like bodies were not observed. The daughter nuclei derived from the diploid nucleus undergo successive divisions simultaneously. The gonimoblast-initials arise from the central cell as stated by Yamanouchi (1906) in *Polysiphonia violacea* but not directly from the auxiliary cell as stated by Kylin (1923) in *P. nigrescens*. As Yamanouchi says, the nucleus in each lobe of a gonimoblast usually divides once more and a carpospore is cut off terminally from each lobe, but sometimes the nucleus divides several times in each lobe as shown in Pl. IV, Figs. 11–14 before the carpospores are produced. The nucleus in the spore usually contains one or two nucleoli but rarely several nucleolus-like bodies (Pl. IV, Figs. 13–15). After the spores have been discharged, the walls of the emptied sporangia are seen remaining in the cystocarpic cavity (Pl. IV, Fig. 15).

Chondria crassicaulis HARV.

The tetrasporangial specimens of the present species were fixed in the middle of the night to obtain good figures of dividing nuclei. For the somatic nuclear division, the modified Abe's fluid gave the best result; for the maturation division of the tetrasporangia, Tahara's, Yamanouchi's and Abe's fluids gave good results. On the other hand, Allen's modification of Bouin's fluid and the fluid composed of formalin, glacial acetic acid and alcohol in a formula recommended by Westbrook (1928) were also tried for fixing the present species, but they proved unsuitable for the writer's purpose because the nuclei fixed with these fluids were stained too dark.

a) Somatic division. Somatic nuclear divisions can be observed in the cortical cells of the terminal portion of the branches and branchlets, especially in those at the growing apex. A resting nucleus usually has a single central nucleolus, but rarely two nucleoli, and a few chromatin granules. Westbrook (1928) stated that in *Chondria dasy-*

phylla the fragmentation of nucleoli was commonly observed in resting somatic nuclei and it was very marked in the axial cell. However, such a phenomenon could not be observed in the writers' material. The nucleolus disappears in later prophase. In metaphase, the chromosomes can be counted as 40 in number. In the material fixed with Abe's fluid, side views of the dividing nuclei in metaphase and anaphase could be observed freqeuntly. The spindle fibers were distinctly visible in anaphase (Pl. V, Figs. 5 & 6), but centrosome-like bodies were not observed. After the nuclear division was completed, the cell division generally followed, but, rarely, it did not. Multinucleate cells containing 2 to 5 nuclei were found in cortical cells. The inner larger cells had small nuclei which were nearly always in the resting stage or were remaining quite unstained. Those cells also contained several large well-stained ellipsoidal or spherical granules and one or two somewhat faintly stained hexagonal bodies (Pl. V, Fig. 2).

b) Nuclear division in tetrasporangia. Tetrasporangia are cut off from the pericentral cells in the upper part of the branches. The connecting pit between the sporangium and the pericentral cells is usually stained dark. The sporangia are usually spherical or ovate but, rarely, elongated toward the base. The nucleus of young sporangia usually has a deeply stained nucleolus but, rarely, two. The successive stages of the first nuclear division observed are shown in Pl. V, Figs. 7–21. The spireme and synapsis stages could not be observed. In metaphase, nearly 20 gemini were counted (Figs. 13 & 14). Centrosome-like granules were rarely observed (Figs. 19 & 20), but no spindle was visible. After the second nuclear division was completed, the tetraporangium increased in size rapidly, cleavage furrows appeared at the periphery of the cell and gradually grew inward to divide the sporangium into four tetraspores. Then the chromatophores made their appearance in the periphery of each spore (Pl. VI, Figs. 11 & 12).

Laurencia papillosa HARV. and *Laurencia obtusa* var. *majuscula* HARV.

In the present two species of *Laurencia*, the tetrasporic mateirals were fixed with two fixing fluids in the above list, Nos. 6 & 7, containing stock solution of chromic acid. These fluids gave excellent results in bringing forth good nuclear figures in both somatic cells and tetrasporangia.

a) Somatic division. Somatic nuclear divisions were observed in the cells of the trichoblasts at the apical depression of the thallus. The resting nucleus had a reticulum and a single, or rarely two, nucleoli in the center. In metaphase, the chromosomes were counted to be less than 40 in number. Spindle fibers could not be observed in either species, while the centrosome-like granules were sometimes clearly present in *L. papillosa* but not at all in *L. obtusa* var. *majuscula*.

b) Nuclear division in tetrasporangia. Young tetrasporangia sometimes contained several granules besides a central nucleus. These granules made it difficult to observe the

nucleus in the prophase stage of division but usually they disappeared by the time of metaphase. The resting nucleus of the sporangium usually contained one nucleolus, but sometimes two, or, rarely, in *L. papillosa* even three. The chromatin threads in early prophase very thin, faintly stained, and gathered at one side of the nuclear cavity. In *L. papillosa*, the nucleus containing two nucleoli was sometimes observed in synapsis to have two groups of chromatin threads, each of which was in contact with one of the nucleoli respectively (Pl. VII, Fig. 14). Nuclei in diakinesis were occasionally met with in *L. papillosa* (Pl. VII, Figs. 16 & 17); they were revealed by a careful observation to contain V- or dumbbell-shaped chromosomes. In metaphase of the first and the second nuclear divisions, a large centrosome-like body was always present at each pole in *L. papillosa* (Pl. VII, Figs. 18 & 19; Pl. XII, Figs. E-J) but not in *L. obtusa* var. *majuscula* (Pl. VII, Figs. 24–26). Spindle fibers were observed in the latter species (Fig. 26) but not in *L. papillosa*. Later stages in the development of tetrasporangia in the present two species are quite similar to those observed in the other two species described above.

SUMMARY

1. The present study deals with the mitosis in somatic cells and the meiosis in tetrasporangia of the following four species : *Polysiphonia japonica, Chondria crassicaulis, Laurencia paipllosa*, and *Laurencia obtusa* var. *majuscula*. The mitosis in spermatogenesis as well as in the development of procarps and gonimoblasts was also examined in *Polysiphonia japonica*.

2. The chromosome numbers of the four species here investigated have been established to be forty in the diploid nculeus and twenty in the haploid. These numbers are in accordance with those reported by Yamanouchi (1906), Kylin (1914, 1923) and Westbrook (1928) in other species of *Polysiphonia, Chondria*, and *Laurencia*, and also in two species of *Rhodomela*, but not with those reported by Austin (1956). (Cf. Table I).

3. In *Polysiphonia japonica* it was established that the nucleus of a spermatium goes down through the trichogyne and fuses in the carpogonium with the female gamete nucleus. It was also found that the gonimoblast initials do not come out directly from the auxiliary cell itself but from the large central cell formed by coalescence of the auxiliary cell with supporting cell, and also with the netural cells in the neighborhood of the procarp.

4. Centrosome-like bodies were observed in the nuclear division of the somatic cells in *Laurencia papillosa* and in the meiotic division of tetrasporangia in *Chondria crassicaulis* and *Laurencia papillosa*. However, they were absent in the division of the somatic cells in *Chondria crassicualis* and in the division of both somatic cells and reproductive cells in *Polysiphonia japonica* and *Laurenica obtusa* var. *majuscula*.

5. Spindle fibers were visible in the mitosis of the somatic cells in *Chondria crassicaulis*. They were also visible in the meiotic divisions of *Polysiphonia japonica* fixed with Abe's fluid and of *Laurencia obtsua* var. *majuscula*.

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EXPLANATION OF PLATES

PLATE I

Polysiphonia japonica HARV.

Figs. 1 & 2. Vertical sections through branch apices of a tetrasporophyte showing nuclear divisions in somatic cells and young tetrasporangia. × 480

Fig. 3. Part of a hair on a female gametophyte showing somatic nuclear divisions. × 1440

Fig. 4. Part of a hair on a male gametophyte showing somatic nuclear divisions. × 1440

Figs. 5–9. Various stages of somatic nuclear division in cells of hairs on a tetrasporophyte. × 1440

Figs. 10–14. Various stages of somatic nuclear division in cells of a male gametophyte. × 1440

Figs. 15–23. Various stages of somatic nuclear and cell divisions in a tetrasporophyte. × 1440

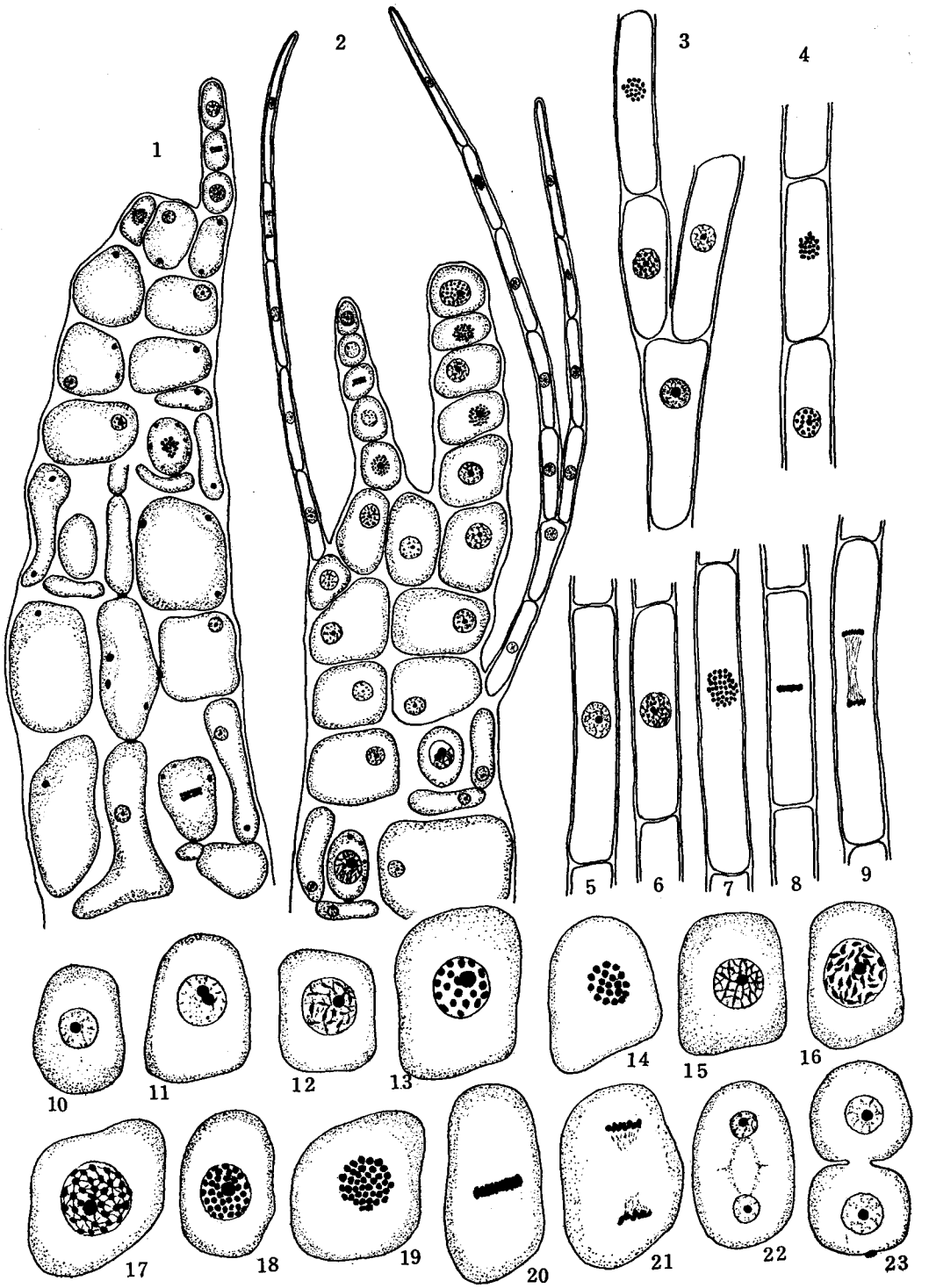

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PLATE II

Polysiphonia japonica HARV.

Figs. 1–10. Various stages of the first nuclear division in tetrasporangia; 1 & 2, early prophase; 3 & 4, diakinesis stage; 5–8, metaphase ; 9 & 10, anaphase. × 1260

Figs. 11–16. Various stages of the second nuclear division in tetrasporangia : 11 & 12, metaphase; 13, anaphase; 14–16, telophase. × 1260

Figs. 17–19. Various stages of cell division of tetrasporangia. × 1260

Fig. 20. Mature tetrasporangium. × 1260

Fig. 21. Part of terminal portion of a male gametophyte showing young antheridia. × 840

Fig. 22. Part of thallus of a male gametophyte showing initial stage of development of an antheridium. × 840

Fig. 23. Vertical section of an antheridium. × 210

Fig. 24. Part of matured antheridium showing spermatangia and their mother cells. × 1260

Fig. 25. Various stages of nuclear division in spermatangium mother cells and in development of first and second spermatangia. × 1470

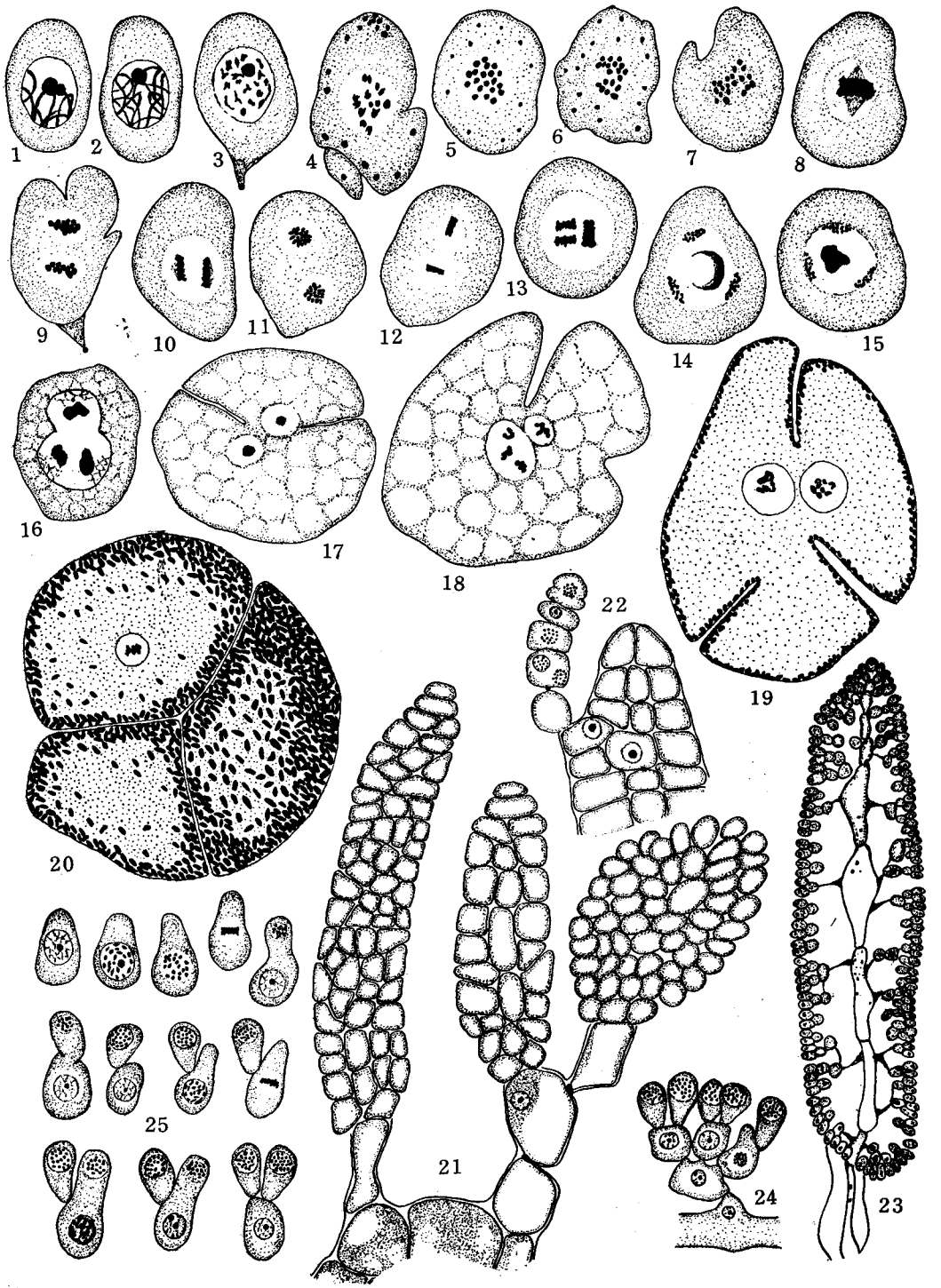

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PLATE III

Polysiphonia japonica HARV.

Earlier stages of development of female reproductive organs.

Fig. 1. Fertile trichoblalst showing fertile pericentral cell, which later gives rise to procarp, on suprabasal segment.

Fig. 2. Abnormality in the development of original cell of procarp.

Fig. 3. Metaphase of mitosis in a fertile pericentral cell on suprabasal segment of a trichoblast, showing 20 chromosomes.

Fig. 4. Young procarp.

Fig. 5. Young procarp with a sterile cell at the base of supporting cell.

Fig. 6. Young procarp with fully constructed carpogonial branch.

Figs. 7–9. Procarps showing passage of spermatium nucleus through trichogyne.

Fig. 10. Procarp at the time of fertilization and the formation of auxiliary cell.

Fig. 11. Procarp showing prophase of mitosis of fertilized nucleus in carpogonium.

Fig. 12. Procarp showing metaphase of mitosis of fertilized nucleus in carpogonium.

(Figs. 1–7, × 720; Figs. 8–12, × 960)

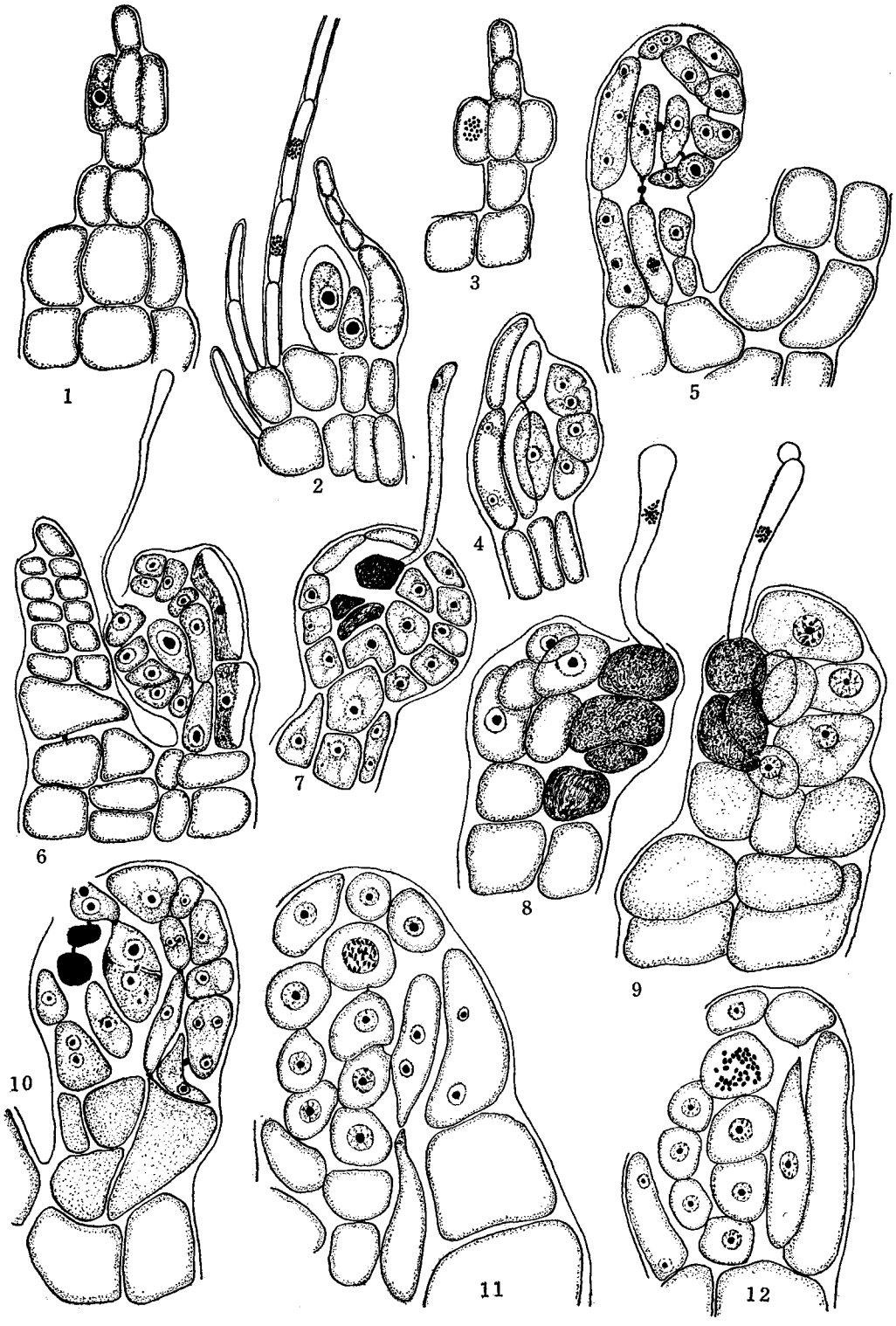

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PLATE IV

Polysiphonia japonica HARV.

Later stages of development of cystocarp.

Fig. 1. Procarp showing metaphase of mitosis of fertilized nucleus in carpogonium.

Fig. 2. Young cystocarp showing an auxiliary cell containing a diploid nucleus and showing fusion between the auxiliary cell and the supporting cell.

Fig. 3. Part of Fig. 2. enlarged to show details of a diploid nucleus.

Figs. 4 & 5. Resting diploid nucleus in the auxiliary cell which has fused with the supporting cell (Fig. 4) and also with adjacent cells (Fig. 5).

Figs. 6–10. Mitosis in fusion-cells derived from the fusion between the auxiliary cell and other cells.

Fig. 11. Young cystocarp showing gonimoblast cells developed from the fusion cell.

Fig. 12. Young cystocarp.

Figs. 11–15. Successive stages of development of the cystocarp. In Fig. 15 there are shown walls of empty carposporangia remaining after discharge of carpospores.

(Figs. 1, 2 &, 4, ×600; Fig. 3, × ca. 2000; Figs. 5–10, × 750; Figs. 11–15, × 600)

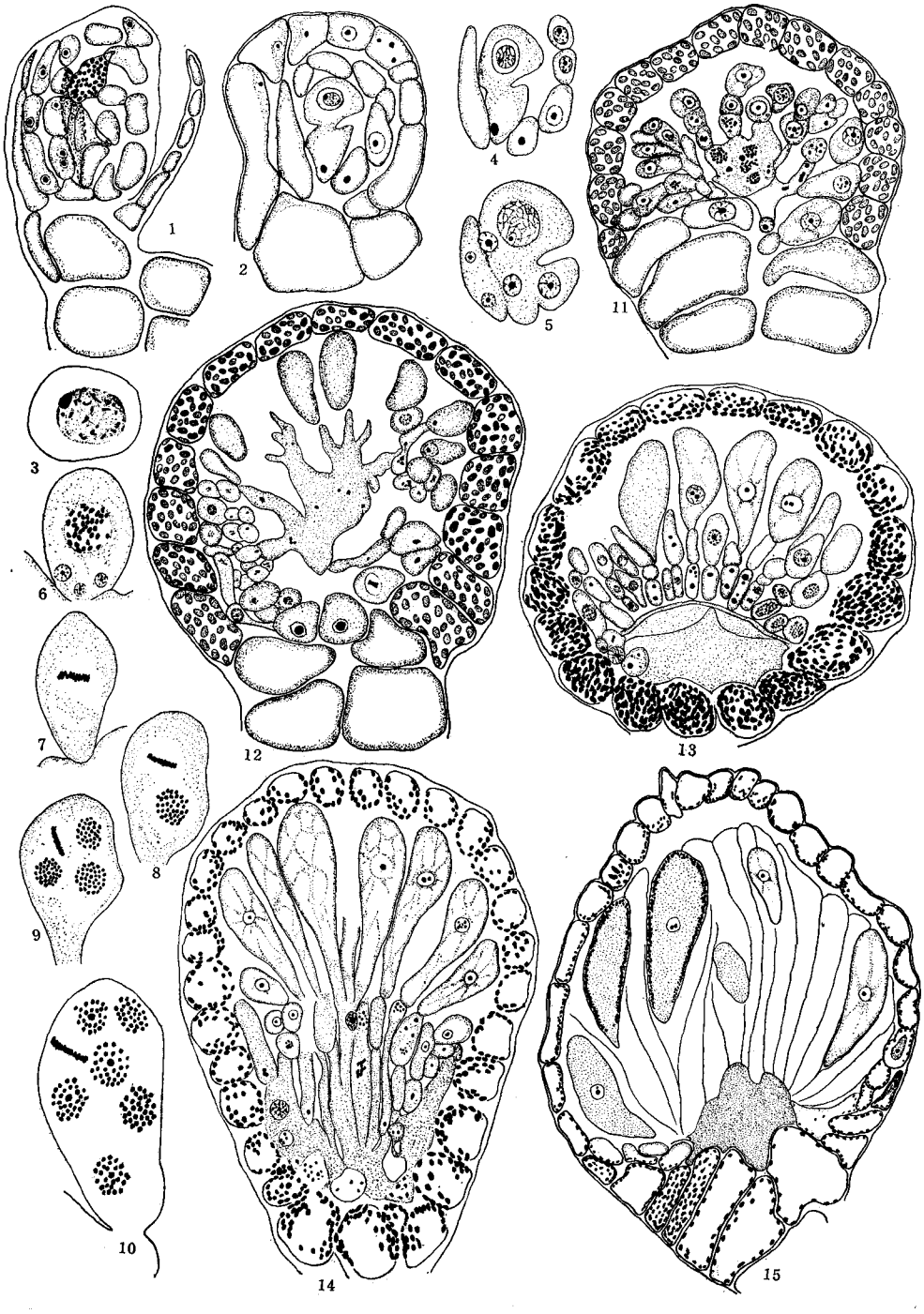

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PLATE V

Chondria crassicaulis HARV.

Fig. 1. Part of cross section through the upper part of a branch showing somatic cells with nuclei in various stages of mitosis and two tetrasporangia, each with a resting nucleus.

Fig. 2. Part of vertical section through the lower part of a branch showing nuclear divisions in the superficial multinucleate cells.

Figs. 3 & 4, Metaphase in somatic cells.

Figs. 5 & 6. Anaphase in somatic cells.

Figs. 7–12. Prophase of the first nuclear division in tetrasporangia.

Figs. 13–18. Metaphase of the first nuclear division in tetrasporangia.

Figs. 19 & 20. Side view of metaphase of the first nuclear division in tetrasporangia.

Fig. 21. Telophase of the first nuclear division in tetrasporangia.

Figs. 22 & 23. Metaphase of the second nuclear division in tetrasporangia.

Fig. 24. Telophase of the second nuclear division in a tetrasporangium.

Fig. 25. Later stage of the above, showing nucleolus-like granules.

(Fig. 1, × 1200; Fig. 2, × 640; Figs. 3–6, × 1680; Figs. 7–25, × 1200)

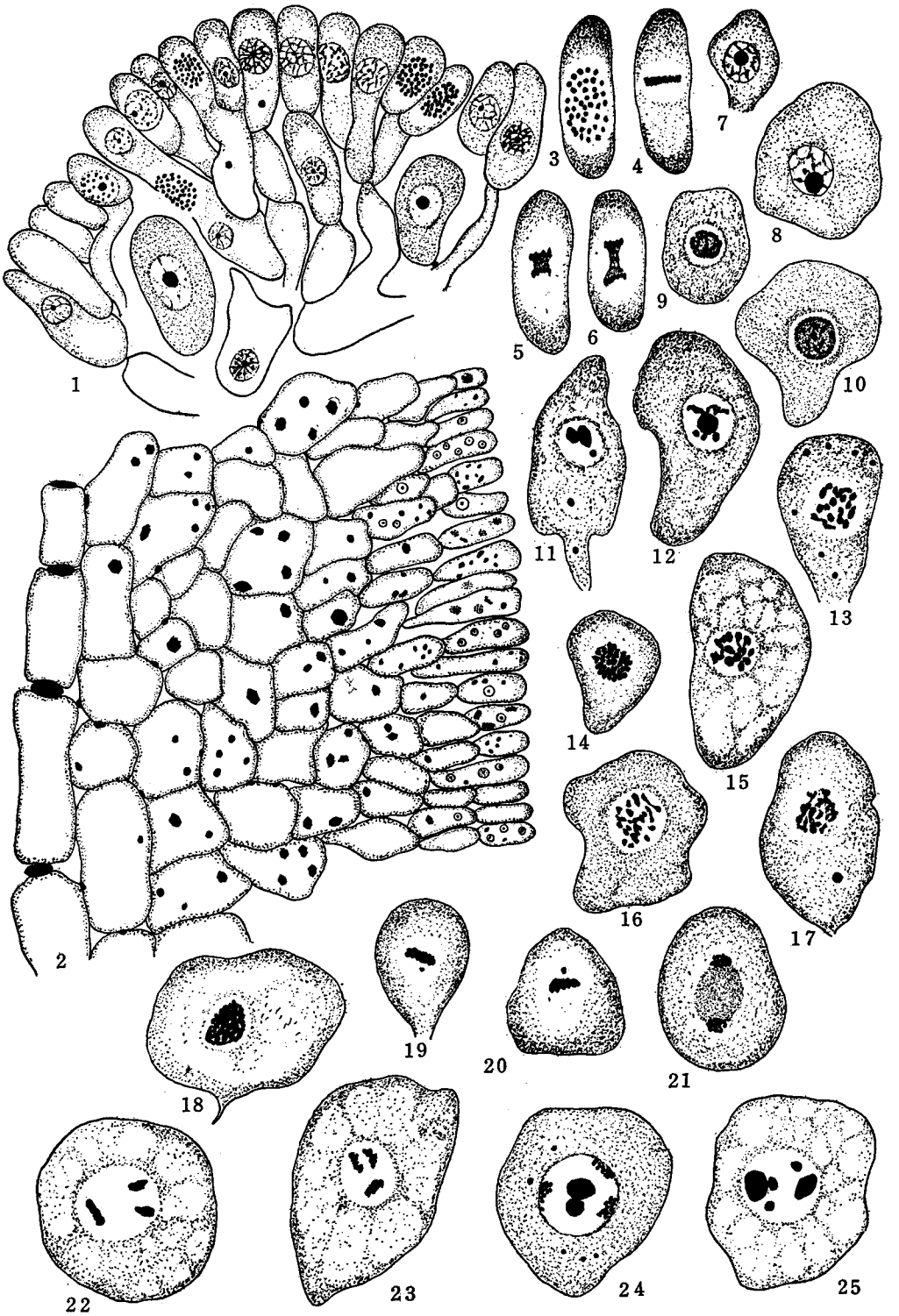

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PLATE VI

Chondria crassicaulis HARV.

Figs. 1–3. Tetrasporangia after meiosis of their nuclei has finished, showing nucleolus-like granules in the nuclear cavity.

Figs. 4–8. Later stage than the above, showing four daughter nuclei.

Figs. 9 & 10. Tetrasporangia with four daughter nuclei; cleavage furrows appearing at periphery.

Fig. 11. Young tetraspores just separated by cleavage furrows.

Fig. 12. Mature tetraspores.

(Figs. 1–12, ×1200)

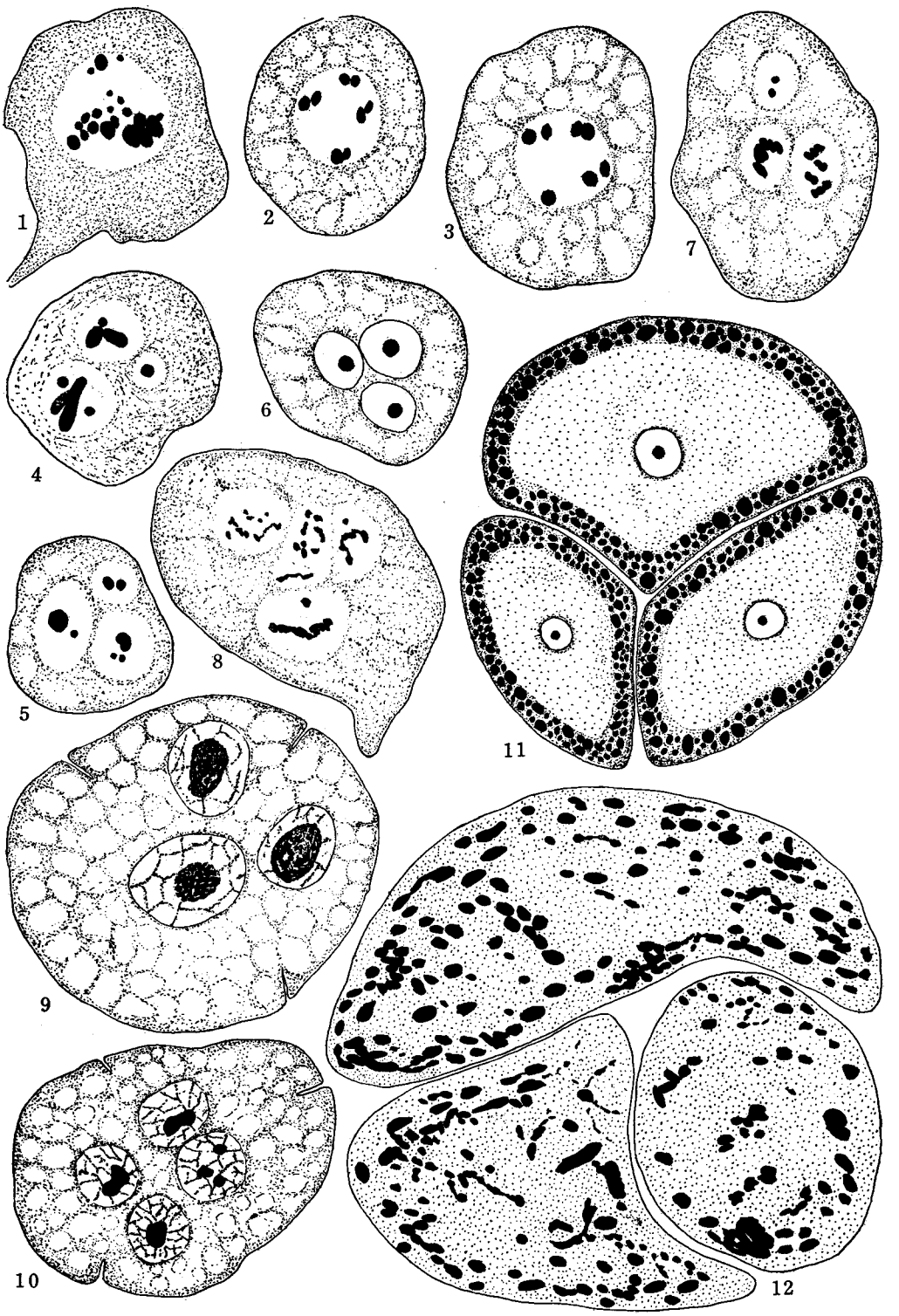

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PLATE VII

Laurenica papillosa HARV.

Fig. 1. Longitudinal section through apical depression of thallus in tetrasporophyte, to show trichoblasts.

Figs. 2–6. Young trichoblasts showing mitotic figures in some of their cells : 2, apical cell containing two nucleoli; 3, late prophase; 4 & 5, metaphase; 6, side view of metaphase.

Figs. 11–22. Tetrasporangia, showing various stages of nuclear division and development of cleavage furrows : 14, synapsis, with two nucleoi, each of which is attached to chromatin threads; 16 & 17, diakinesis; 18, side view of metaphase in the first nuclear division; 19, side view of metaphase in the second nuclear division; 20–22, nucleolus-like granules in the nuclear cavity and cleavage furrows growing towards the center.

Laurencia obtusa var. *majuscula* HARV.

Figs. 7 & 10. Young trichoblasts showing late prophase of nucleus (Fig. 7) and side view of metaphase (Fig. 10).

Figs. 8 & 9. Metaphase in cells of a young trichoblast.

Figs. 23–30. Tetrasporangia showing various stages of nuclear division : 24, side view of metaphase in the first nuclear division; 26, anaphase in the second nuclear division; 27–30, later stages.

(Fig. 1, × 480; Figs. 2–10, × 1120; Figs. 11–13, 15–22 & 24–30, × 960; Figs. 14 & 23, × 1200)

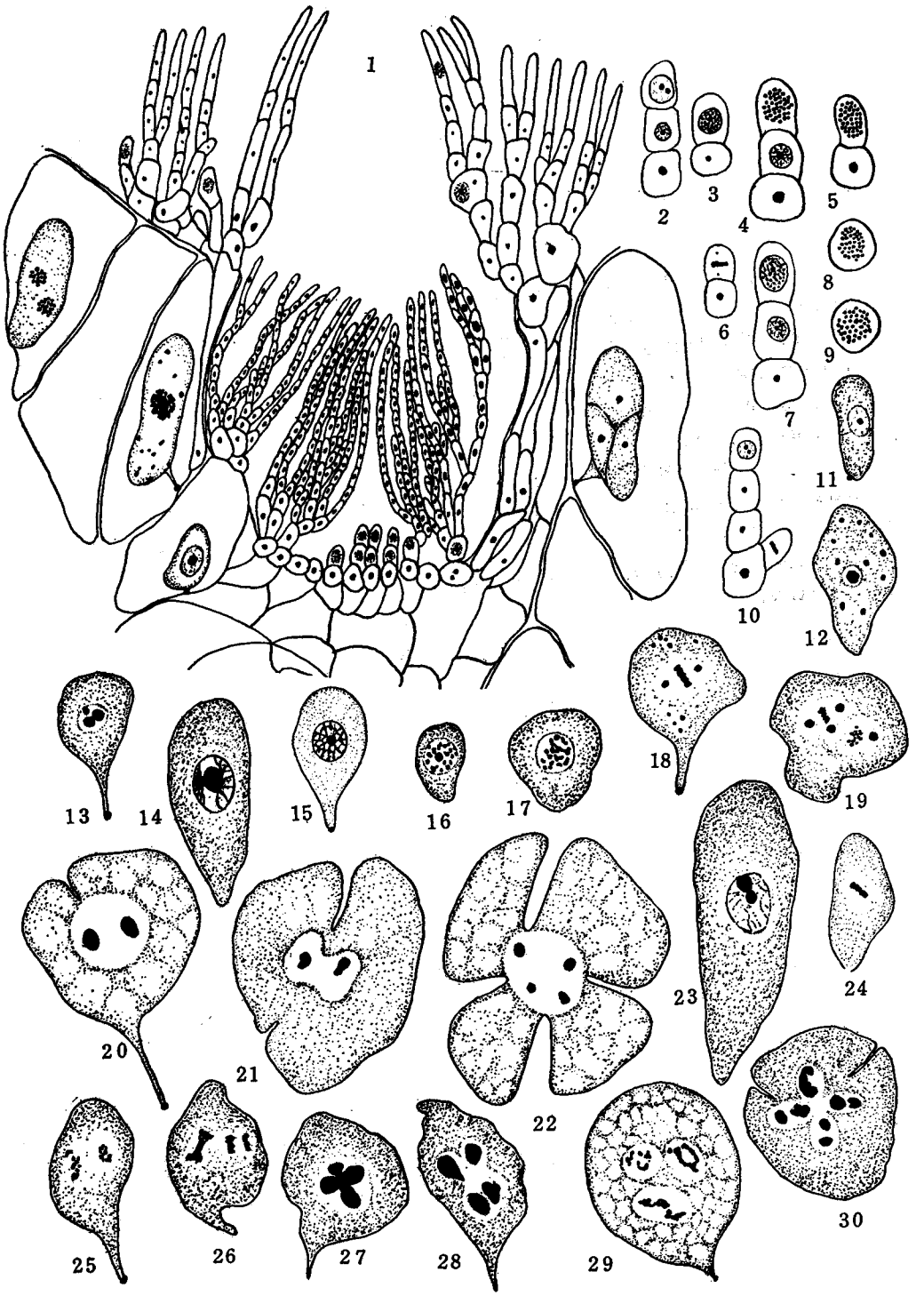

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PLATE VIII

Polysiphonia japonica HARV.

Figs. A-C. Phototmicrographs of cross sections of tetrasporophyte, showing tetrasporangia each with dividing nucleus.

Fig. D. Part of Fig. A, enalrged, showing side view of metaphase of the first nuclear division in a sporangium.

Fig. E. Part of Fig. B, enlarged, showing anaphase of the first nuclear division in a sporangium.

Figs. F-H. Photomicrographs of cross sections of a tetrasporophyte, showing tetrasporangia with nuclei in metaphase of the first division (Figs. F & G) and in diakinesis (Fig. H).

Figs. I-K. Camera lucida drawings of the same sections as shown in Figs. F-H respectively.

(Figs. A-C, F, G, I & J, × 480,; Figs. H & K, × 960)

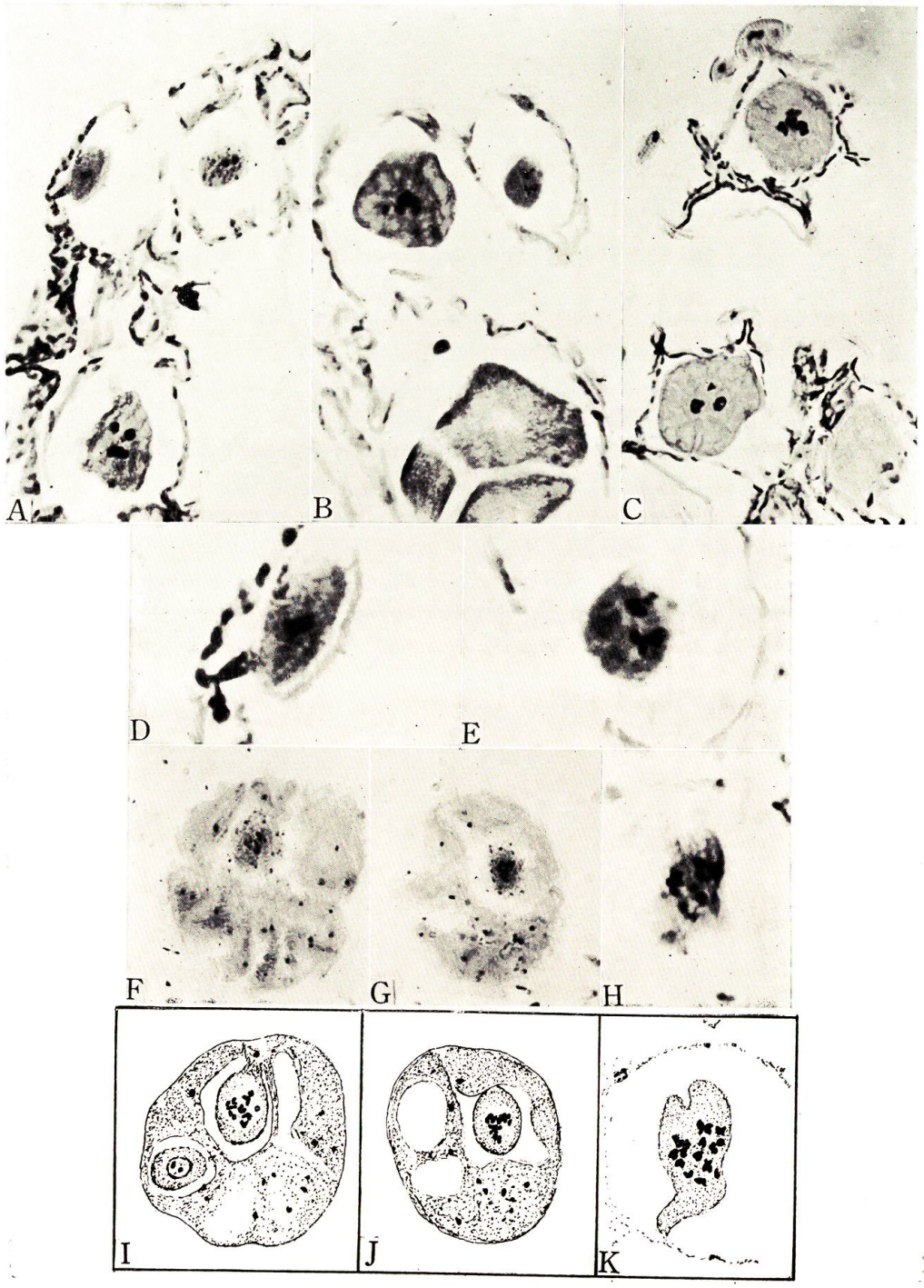

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PLATE IX

Polysiphonia japonica HARV.

Figs. A-C. Photomicrographs of feritle trichoblasts showing fertile pericentral cells (pc) and chromosomes (ch) at metaphase in cells of hairs.

Figs. D & E. Photomicrographs of young carpogonial branches consisting of four cells (1–4). (*Cf.* Plate III, Fig. 4 and Fig. 5).

Fig. F. Photomicrograph of carpogonial branch consisting of four cells (1–4), of which the fourth cell (4) bears a trichogyne (tr). (*Cf.* Plate III, Fig. 6).

Figs. G & H. Photomicrographs of procarps showing passage of a spermatium nucleus (s) through the trichogyne (tr). (*Cf.* Plate III, Figs. 7–9).

Fig. I. Photomicrograph of vertical section of procarp, showing carpogonial branch (1–4), of which the fourth cell (4) has diploid nucleus in metaphase.

(Figs. A–G, × 720; Fig. H, × ca. 480; Fig. I, × ca. 960)

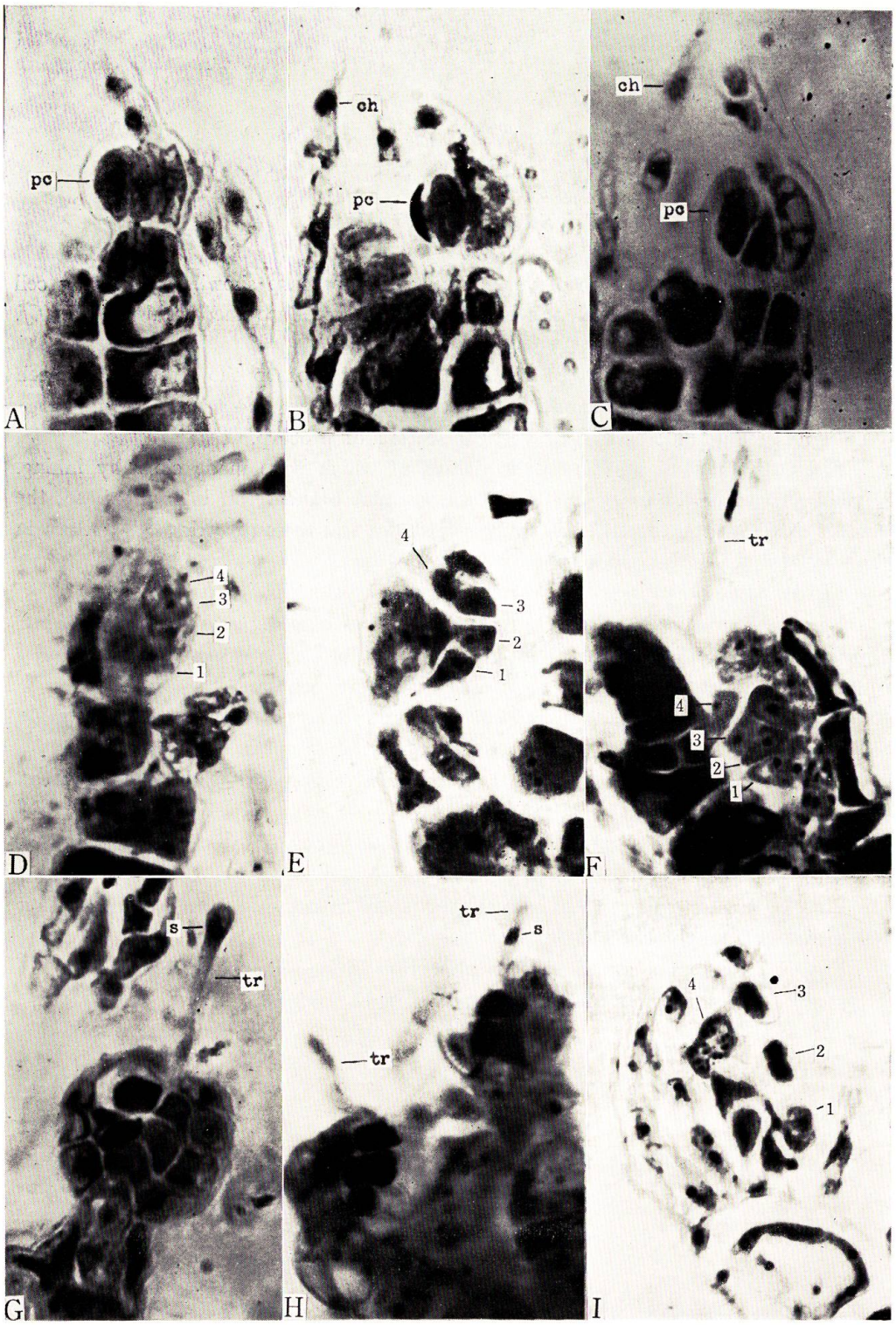

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PLATE X

Polysiphonia japonica HARV.

Fig. A. Photomicrograph of vertical section of procarp showing supproting cell (sc) which contains two nuclei and is just about to produce the auxiliary cell. (*Cf.* Plate III, Fig. 10).

Fig. B. Photomicrograph of vertical section of procarp showing auxiliary cell (ac) cut off upwardly from supporting cell. .

Fig. C. Photomicrograph of vertical section of procarp.

Fig. D. The same as Fig. C, the details of which were made clear by inking. Diploid nucleus in the fourth cell (4) of carpogonial branch is in later prophase; the nculeus of the supporting cell has divided into two and the supporting cell itself is in the way of division to produce the auxiliary cell (ac).

Fig. E. Photomicrograph of vertical section of procarp.

Fig. F. The same as Fig. E, the details of which were made clear by inking. Diploid nucleus in the fourth cell (4) of carpogonial branch is in metaphase; auxiliary cell (ac) is completely formed and is fused with carpogonium.

Fig. G. Photomicrograph of vertical section of young cystocarp with large fusion cell at the center in which is seen a side view of dividing nucleus (n) in metaphase.

Fig. H. Photomicrograph of vertical section of young cystocarp, showing fusion cell with two daughter nuclei in metaphase (n).

Fig. I. Photomicrograph of vertical section of young cystocarp, showing fusion cell with four daughter nuclei in metaphase (n).

Fig. J. Photomicrograph of vertical section of young cystocarp, showing fusion cell with eight daughter nuclei (n).

Fig. K. Photomicrograph of vertical section of two young cystocarps.

(Figs. A-F, × 960; Figs. G-K, × 384).

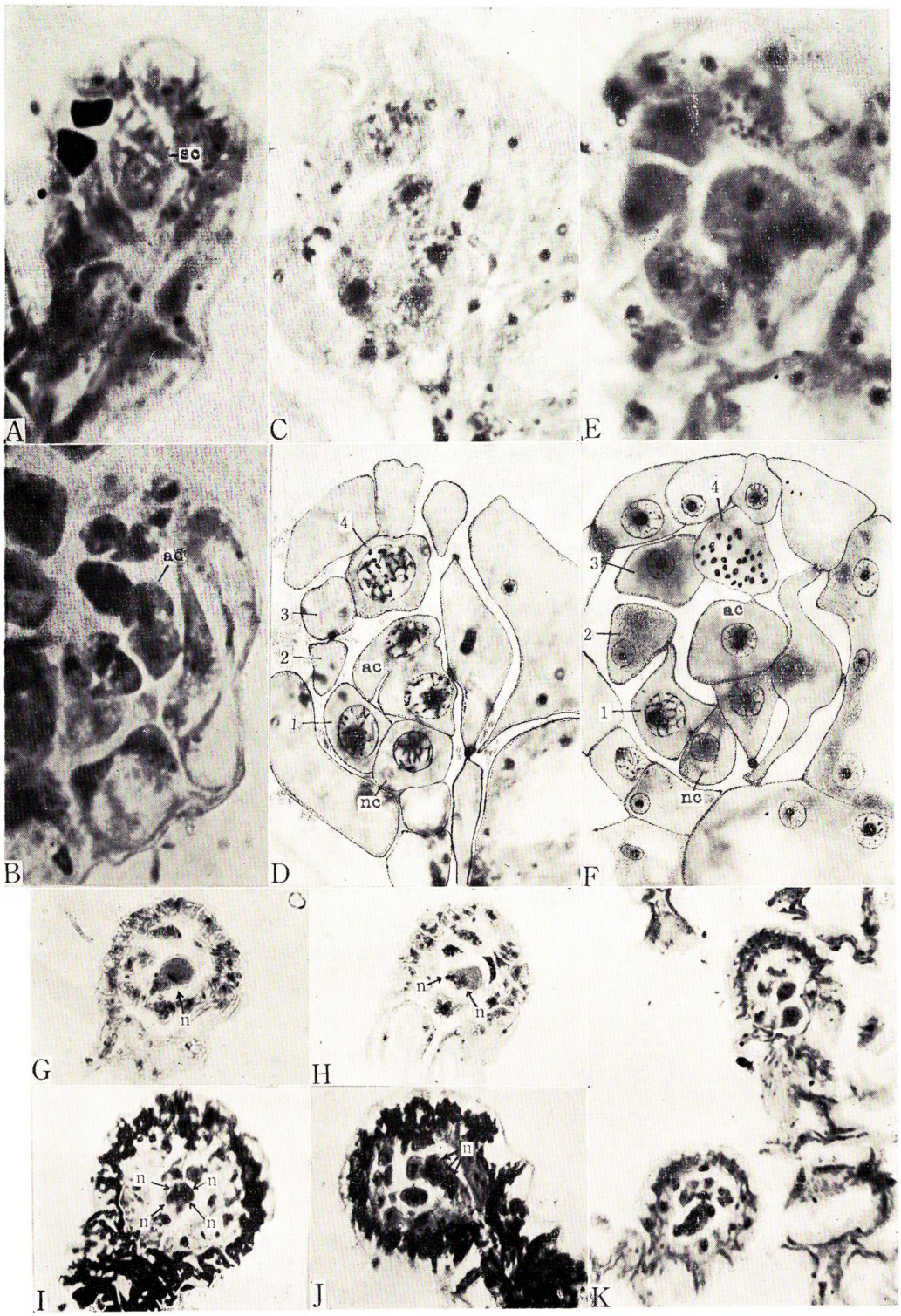

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PLATE XI

Chondria crassicualis HARV.

Fig. A. Photmicrograph of cross section through tapical portion of thallus bearing hairs and branchlets.

Fig. B. Central portion of Fig. A, enlarged, showing a cell with two nuclei in metaphase.

Fig. C. Camera lucida drawing of the same cells as shown in Fig. B.

Fig. D. Photomicrograph of vertical section of a branchlet.

Fig. E. Part of Fig. D, enlarged, showing nucleus in metaphase.

Fig. F. Photomicrograph of cross section of thallus showing an abnormal tetrasporangium with elongated slender base.

Fig. G. Photomicrograph of cross section of thallus showing tetrasporangia.

Fig. H. Photomicrograph of cross section of thallus showing two tetrasporangia containing nucleus in metaphase of the first division.

Fig. I. Photomicrograph of a sporangium with nucleus in metaphase of the first division.

Fig. J. Photmicrograph of a sporangium just after the meiosis was finished.

Figs. K-L. Photomicrographs of sporangia with separated daughter nuclei.

Fig. M. Photomicrograph of a sporangium with cleavage furrows growing towards its center.

(Figs, A, D, F, H, & M, × 3 4; Fig. G, × 64; Fig. I, × ca. 960; Figs. J, K, & L, × 720)

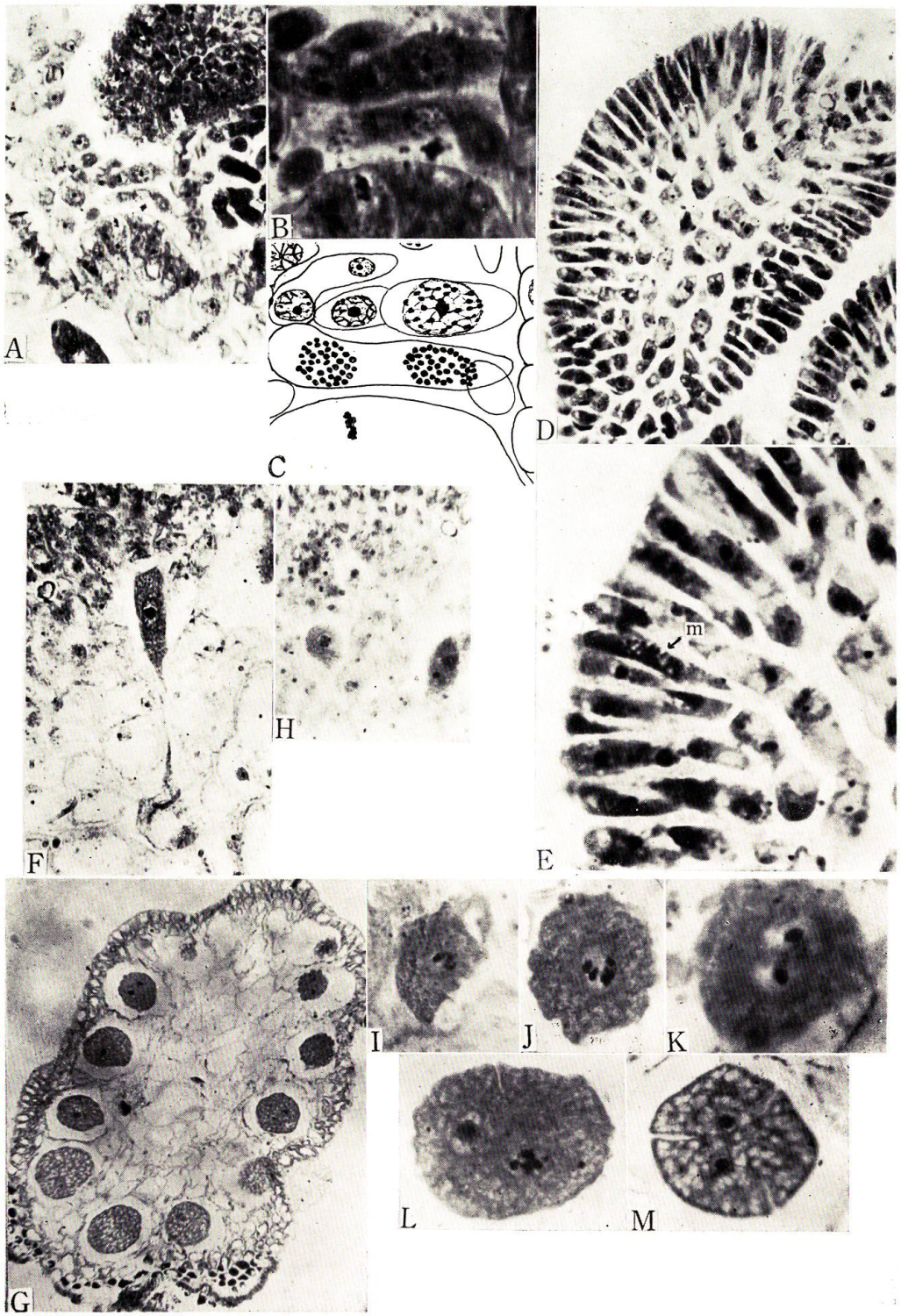

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PLATE XII

Laurenia papillosa HARV.

Fig. A. Photomicrograph of vertical section through apical portion of a stichidium showing young tetrasporangia and a cell with a side view of the dividing nucleus in metaphase.

Fig. B. Camera lucida drawing of the same section as shown in Fig. A.

Figs. E, G & I. Photomicrographs of sporangia whose nuclei are in metaphase of the first division.

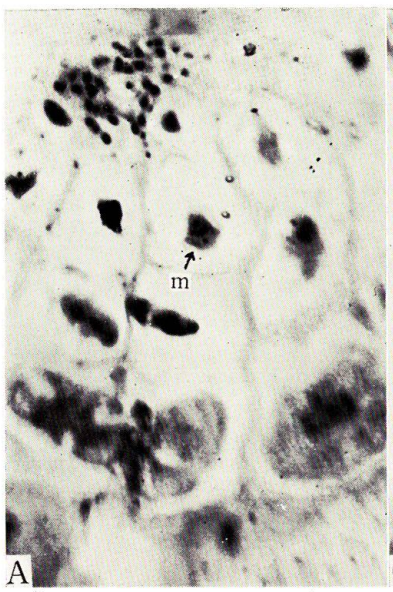
Figs. F, H & J. Camera lucida drawings of the same sporangia as above respectively.

Laurencia obtusa var. *majuscula* HARV.

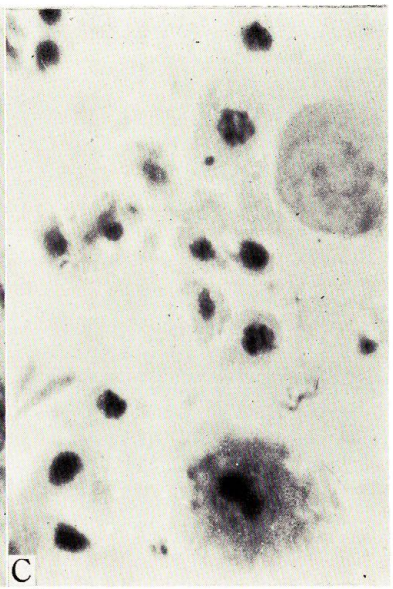
Fig. C. Photomicrograph of collapsed section of thallus showing somatic cells with nuclei in porophase and a young tetrasporangium with two nucleoli in its nucleus.

Fig. D. Camera lucida drawing of the same section as shown in Fig. C.

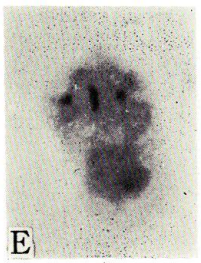
(Figs. A & B, × 74; Figs. C, D, E-J, × ca. 1200).



A

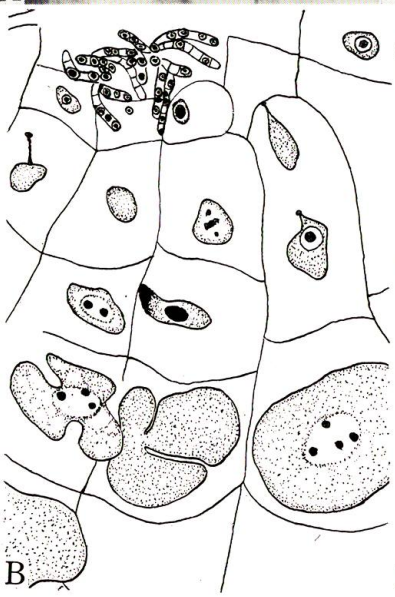


C



E

F



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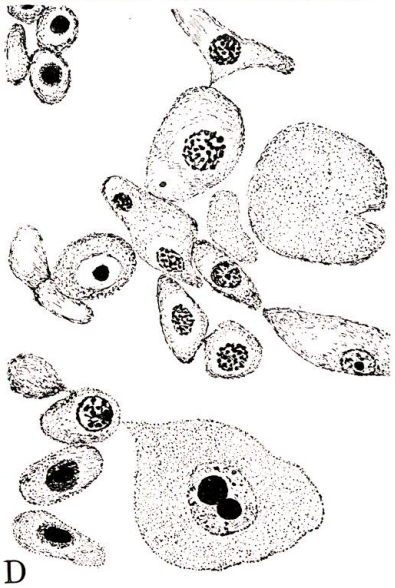
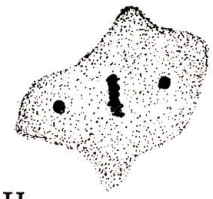
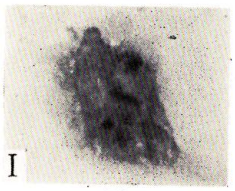


D

G



H



I

J