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MITOSIS IN PORPHYRA

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Since the publication in 1921 of Ishikawa's classical work on the cytology of *Porphyra tenera* Kjellm., cytological studies in the geus *Porphyra* have been

Table I. Chromosome number and meiosis in *Porphyra* species which have ever been studies cytologically

Species	Chromosome number		Meiosis	Author
	<i>n</i>	<i>2n</i>		
<i>Porphyra tenera</i>	3 (in vegetative cells of leafy thallus, and in spermatium parent-cells and spermatia)	—	+ (primitive type, in carpospore formation)	Ishikawa, 1961
<i>P. umbilicalis</i> <i>f. linearis</i>	2 (in veg. cells of leafy thallus)		+ (in fertilized carpogonia)	Dangeard, 1927*
<i>P. linearis</i>	4 (in veg. cells of leafy thallus & in spermatia)	8 (in fertilized carpogonia & carpospores)	?	Magne, 1952
<i>P. tenera</i>	5 (in spermatium parent-cells & in carpospore formation)	10 (in fertilized carpogonia)	+ (in fertilized carpogonia)	Tseng & Chang, 1955
<i>P. tenera</i>	4 (in veg. cells of leafy thallus & in some Concho-celis filaments)	8 (in carpospores & in some Concho-celis filaments)	?	Fujiyama <i>et al.</i> , 1955 (I & II) Fujiyama, 1957
<i>P. sp.</i>	3	?	?	Fujiyama <i>et al.</i> , 1955 (III)
<i>P. umbilicalis</i> <i>var. laciniata</i>	5 (in all stages of life-history)	none	none	Krishnamurthy, 1959
<i>P. yezeensis</i>	3 (in spermatium parent-cells & spermatia)	6 (in fertilized carpogonia & their daughter cells)	?	Yabu & Tokida, in the present paper
<i>P. onoi</i>	3 (in veg. cells of leafy thallus & in spermatium parent-cells)	—	?	Yabu & Tokida, in the present paper

* Not consulted, so the entries are incomplete.

reported to date by several authors such as Dangeard (1927), Magne (1952), Tseng and Chang (1955), Fujiyama *et al.* (1955), Fujiyama (1957), and Krishnamurthy (1959). The species and their chromosome numbers as investigated by these authors as well as the results of the present study are summarized in Table I.

As may be seen from Table I, the chromosome number of *Porphyra tenera* was reported to be 3 by Ishikawa (1921), 4 by Fujiyama and collaborators (1955, 1957), and 5 by Tseng and Chang (1955). The meiosis in the same species was described to take place immediately after fertilization by Ishikawa, though as a primitive type, and by Tseng and Chang. The same was the case with *P. umbilicalis* f. *linearis* reported by Dangeard (1927). On the other hand, Fujiyama and collaborators reported that their material of *P. tenera* produced diploid carpospores without meiosis, just in accordance with the results of Magne's observations on *P. linearis* (1952). Krishnamurthy (1959) is unique among these previous investigators in his conclusion that *P. umbilicalis* var. *laciniata* is haploid in all stages of its life-history with neither fertilization nor meiosis.

From February 1961 through May 1962 the writers carried out cytological studies on *Porphyra yezoensis* and *P. onoi* collected in and near Hakodate. The results of their observations on the mitosis in spermatium- and carpospore-formation of *P. yezoensis* and in somatic cells of leafy thallus and antheridium of *P. onoi* are reported in this paper.

Material and Methods

Porphyra yezoensis was collected at Nanaehama, Hakodate, in February and March 1961, and in March 1962, while *P. onoi* was gathered at Shinori, near Hakodate, in May 1962. Fixation of *P. yezoensis* in 1961 was done at various times in both the daytime and the night, partly at each place of collection, partly at the laboratory after keeping the materials for some time in vats filled with filtered seawater. The following fixatives were applied for various length of time ranging from 1 to 24 hours.

(1) A mixture of equal parts of two percent solution of osmic acid and two percent aqueous solution of acetic acid, which was employed by Ishikawa for fixing his materials of *Porphyra tenera*;

(2) Navashin's fluid; and

(3) Bouin's fluid.

Sections were cut 4-5 μ in thickness by paraffin method and stained with Heidenhein's iron-alum haematoxylin. Among these fixatives, Navashin's fluid applied for 30-60 minutes and Bouin's fluid applied for 1-2 hours gave good results. On the other hand, the fixative used by Ishikawa proved ineffective for fixing both

the resting and dividing nuclei in the writers' materials.

In view of the results of these preliminary experiments, the fixation of *Porphyra yezoensis* and *P. onoi* collected in 1962 was done in the laboratory by applying Bouin's fluid for one hour to the materials kept alive in vats filled with filtered seawater.

Results

Porphyra yezoensis Ueda

Nuclear division in spermatium formation. Antheridia originate from some vegetative cells in the marginal portion of thallus. They are pale yellow in color or nearly colorless and can be easily distinguished from the vegetative cells. The nucleus in the vegetative cell usually occupies the central portion of the cell, but sometimes it is situated near the thallus surface (Pl. I, Fig. 1), while the nucleus in the antheridium is always in the center of the cell. Stages of nuclear divisions in the antheridia are illustrated in Plate I, Figs. 2-34 and Plate IV, Figs. 1-11. In prophase the nucleus gradually increases in size and a vacuolated nucleolus becomes visible in the center of the nucleus (Pl. I, Figs. 3 & 4). In late prophase the nucleolus disappears and three chromosomes make their appearance in the nuclear cavity (Fig. 5). In metaphase the nuclear membrane totally disappears (Fig. 6). In anaphase spindle fibers are observed between the groups of daughter chromosomes migrating to the poles (Fig. 7). Each chromosome group migrated further and finally occupied the polar position of the longitudinal axis of the cell. About this stage the chromatophore is divided crosswise into two. Then the chromosomes disappear, and the nuclear membrane and nucleolus make their appearance again to complete the formation of two daughter nuclei. Meanwhile a cleavage is formed in cytoplasm from periphery inwardly along the equatorial plate of the cell with the result that the cell is eventually divided into two (Fig. 9). Then follow five nuclear and cell divisions successively and 64 small cells or spermatia are produced within an antheridium (cf. Ueda, 1932, p. 23-24; Tanaka, 1952, p. 39). In metaphase of those divisions the chromosomes were always counted to be three; in anaphase the spindle fibers were often clearly observed (Figs. 23, 24 & 28). In telophase the chromatophore divided into two in the same way as in the first division so that each daughter cell contained one nucleus and one chromatophore. In telophase of the sixth division the nucleus did not enter a resting stage but three chromosomes were clearly visible in the ultimate daughter cells and even in spermatia (Fig. 36; cf. Ishikawa, 1921, Pl. IV, Fig. 8, 10 & 11).

Nuclear division in carpospore formation. The present species is monoecious.

Carpogonia are also converted from some vegetative cells in the marginal portion of thallus. They are ellipsoid or ovoid, often slightly pointed at one or both ends of their longitudinal axis to form a little projection on the thallus surface as a temporary trichogyne-like structure, which is called prototrichogyne by Tseng and Chang (1955). A spermatium attached to the prototrichogyne is illustrated in Fig. 36. After the spermatium had entered the carpogonium, the male nucleus rapidly increased its size and a nucleolus and chromatin granules made their appearance in the nuclear cavity (Figs. 37-39). Fertilized nuclei just after the nuclear fusion are shown in Figs. 40 & 41 as containing two nucleoli. The nucleoli soon disappeared, and chromatin granules appeared in the fertilized nucleus which entered the prophase stage of nuclear division (Pl. V, Fig. 5). Then well stained chromatin threads were found spreading in the nuclear cavity (Fig. 42), and they gradually grew shorter to become chromosomes (Figs. 43-45). Six chromosomes were counted in late prophase and metaphase (Figs. 43-48). Nuclear and cell divisions of the fertilized carpogonium generally follow the same course as in the divisions of the antheridium. As a result of four successive divisions, 16 carpospores are produced in a fertilized carpogonium (cf. Ueda, 1932, p. 23; Tanaka, 1952, p. 39). In metaphase of those divisions 6 chromosomes were always counted (Figs. 57-59 & 62), and in anaphase the spindle fibers were often clearly observed (Figs. 54 & 55).

Porphyra onoi Ueda

Somatic division. Vegetative cells of the present species are larger than those of *Porphyra yezoensis*. They often contain two chromatophores in the monostromatic portion. The chromatophore stains with haematoxylin better than that of *P. yezoensis*. A large nucleus, ca. 3μ in diam., occupies the center of the cell and it contains a nucleolus. The nucleolus usually stains well with haematoxylin but sometimes it is found vacuolated (Figs. 64 & 65, Pl. VI, Fig. 1). In late prophase the nucleolus disappears and three chromosomes make their appearance in the nuclear cavity (Figs. 66 & 67). In metaphase the nuclear membrane disappears (Fig. 68). The chromosomes are somewhat larger than those of *P. yezoensis* (compare Pl. I, Figs. 5 & 6 with Pl. III, Figs. 66-68). Spindle fibers were clearly observed in anaphase (Fig. 69; Pl. VI, Fig. 4). In telophase the chromosomes disappear and nuclear membrane and nucleolus make their appearance in newly formed two daughter nuclei which are shown in Figs. 70-73 as represented by their respective nucleoli only because the nuclear membrane often becomes colorless and obscure when surplus stain of sections is extracted with iron-alum solution. Meanwhile a cleavage is formed aslant in

cytoplasm from periphery inwardly and the cell is divided into two cells each of which contains one nucleus and one chromatophore. These two cells contribute either to the growth of distromatic part of thallus (Figs. 73 & 74) or to the expansion of the original monostromatic layer (Figs. 75 & 76).

Nuclear division in spermatium formation. Development of spermatia from their mother-cells or antheridia was observed in the marginal monostromatic portion of thallus (Figs. 77-82; cf. Ueda, 1932, Pl. VIII, fig. 8; Tanaka, 1952, text-fig. 28 c). Spermatium mother-cells contain one or two chromatophores and one central nucleus. The nucleus increases its size in the prophase stage of division. In metaphase, nuclear membrane and nucleolus disappear and three chromosomes make their appearance (Figs. 77 & 78). Each of the daughter cells of the first and subsequent five divisions always contains one nucleus and one chromatophore (Figs. 80-82). As a result of these divisions 64 spermatia, divided according to the formula $64 \left(\frac{a}{4}, \frac{b}{4}, \frac{c}{4} \right)$, are formed in an antheridium (cf. Ueda, 1932, p. 35; Tanaka, 1952, p. 62). Sometimes one more division was observed to occur in the *c* axis resulting in the production of 128 spermatia. The spermatium contains three chromosomes and one faintly stained chromatophore; the nucleus is usually not in the resting stage. In the third nuclear division of an antheridium shown in Fig. 81, three chromosomes can be counted in two cells while a spindle is clearly observed in one cell.

Summary

Nuclear divisions in spermatium- and carpospore-formation of *Porphyra yezoensis* Ueda and in somatic cells and spermatium-formation of *Porphyra onoi* Ueda were established to be mitotic. In all of those divisions three chromosomes were counted. In *P. yezoensis*, fusion of male and female nuclei in fertilized carpogonia was clearly observed; the carpospores were established to be formed without meiosis and to have six chromosomes. Information relating to meiosis in these two species awaits future studies.

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Explanation of Plates

PLATE I

Porphyra yezoensis Ueda

Fig. 1. Five vegetative cells in cross section of thallus showing nuclei as black dots situated near thallus surface

Figs. 2-34. Nuclear and cell divisions in antheridia

Fig. 2. Nucelus in resting stage

Figs. 3 & 4. Nucleus in prophase

Fig. 5. Late prophase

Fig. 6. Metaphase

Fig. 7. Anaphase

Fig. 8. Telophase

Figs. 9-15. Two-cell stage, nucleus in prophase of the second division

Fig. 9. Prophase

Figs. 10-13. Metaphase

Fig. 14. Anaphase

Fig. 15. Telophase

Figs. 16-28. Four-cell stage or cells from four-cell stage in the third division

Figs. 16 & 17. Prophase

Figs. 18-20. Prophase and metaphase

Fig. 21. Prophase and anaphase

Fig. 22. Metaphase

Fig. 23. Side view of metaphase

Fig. 24. Metaphase

Figs. 25-26. Prophase and metaphase

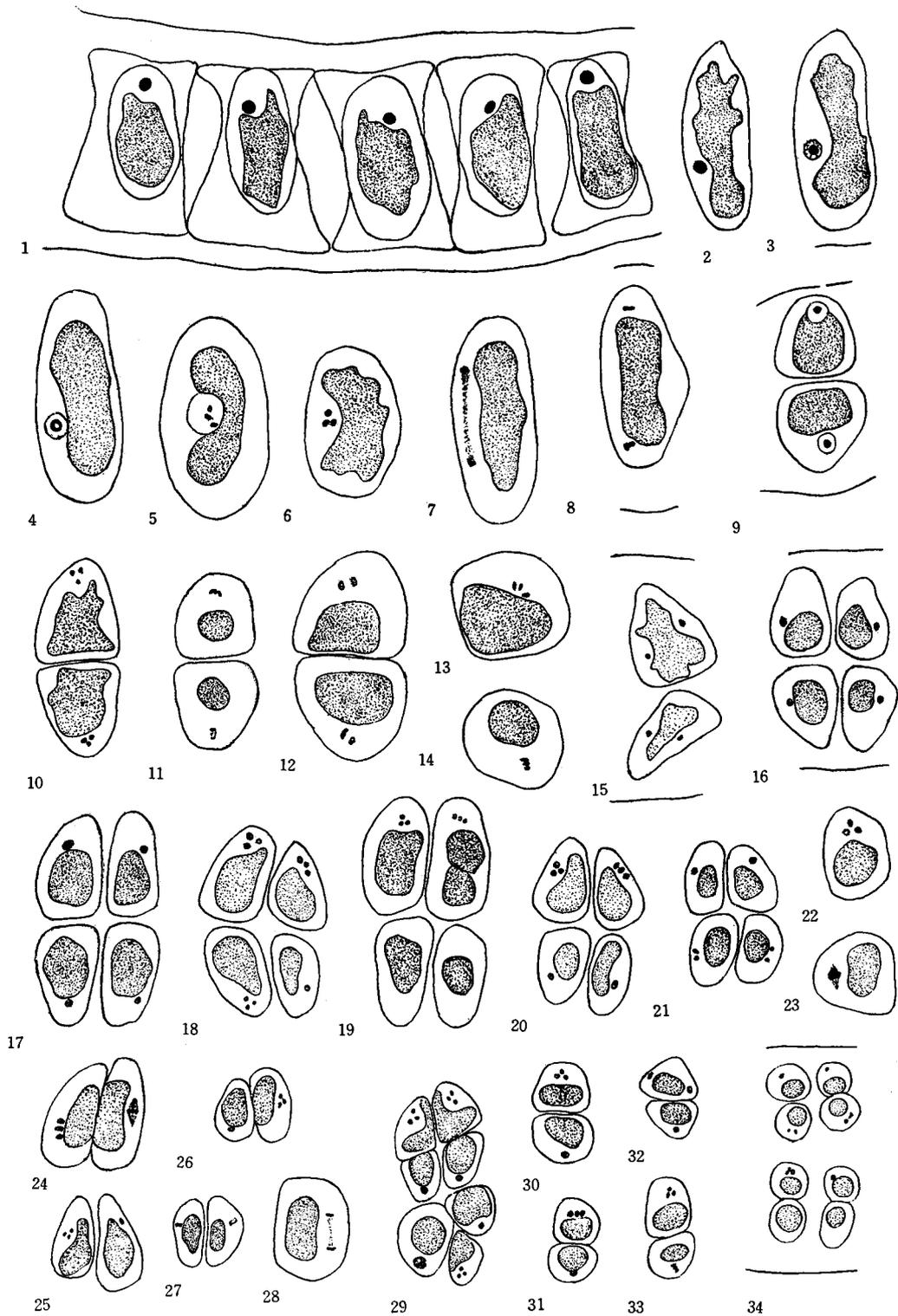
Fig. 27. Metaphase

Fig. 28. Anaphase

Figs. 29-33. Cells from sixteen-cell stage; nuclei in prophase and metaphase

Fig. 34. Part of side view of antheridium in thirty-two cell stage; nuclei in prophase, metaphase, and in anaphase of the sixth division

(Figs. 1-34, $\times 1610$)

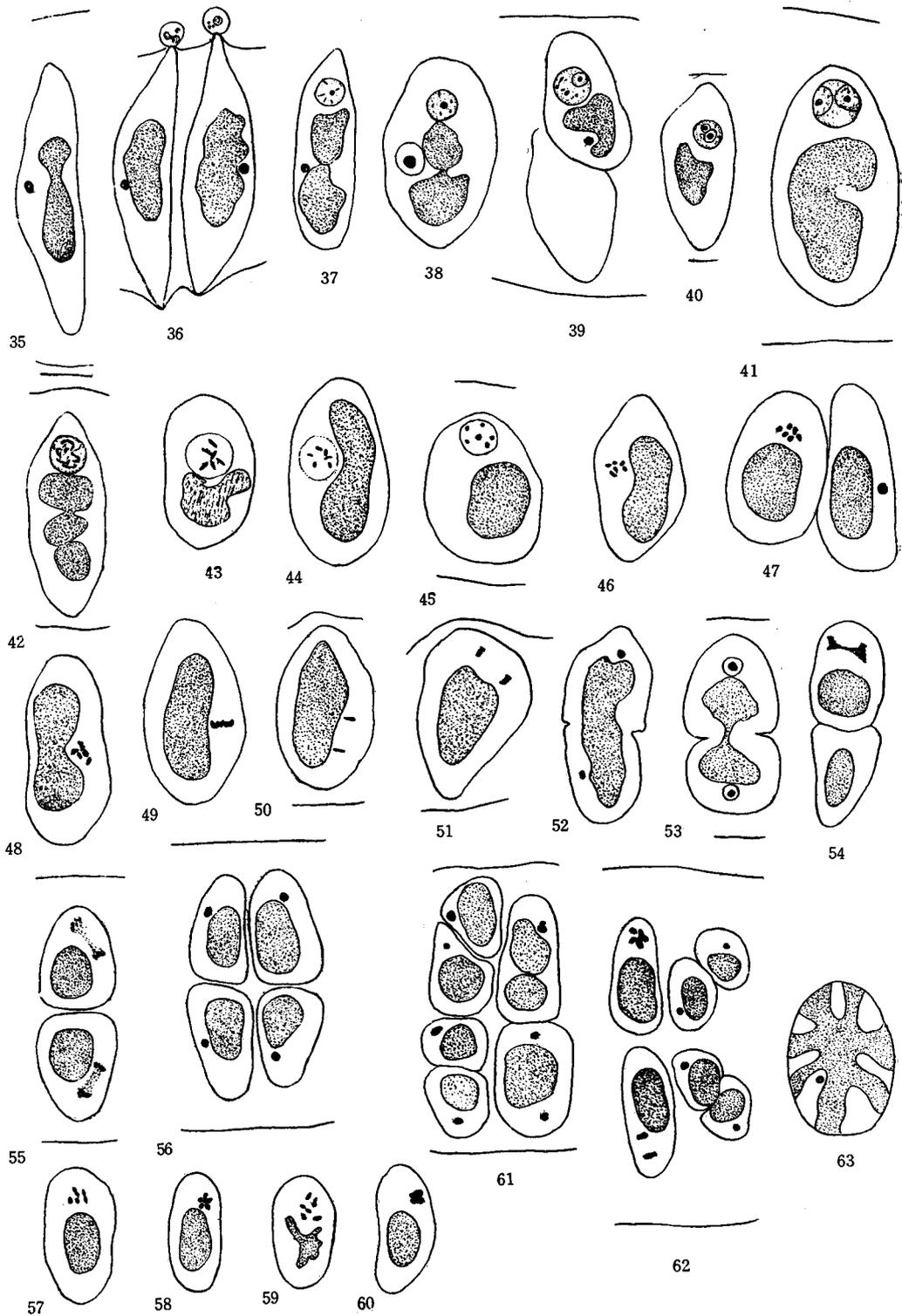


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PLATE II

Porphyra yezoensis Ueda

- Fig. 35-63. Nuclear and cell divisions in carpospore-formation
- Fig. 35. Carpogonium
- Fig. 36. Two carpogonia, each with a spermatium attached on prototrichogyne
- Figs. 37-39. Carpogonium with male nucleus entered within
- Figs. 40-41. Carpogonium with fertilized nucleus
- Fig. 42. Fertilized nucleus in prophase
- Figs. 43-45. Late prophase
- Figs. 46-48. Metaphase
- Fig. 49. Side view of metaphase
- Figs. 50 & 51. Anaphase
- Figs. 52 & 53. Telophase
- Figs. 54 & 55. Two-cell stage; nuclei in anaphase of the second division
- Fig. 56. Four-cell stage; nuclei in prophase of the third division
- Figs. 57-59. Metaphase of the third division
- Fig. 60. Anaphase of the third division
- Figs. 61 & 62. Part of side view of cystocary in the course of the fourth division; nuclei in metaphase, anaphase, telophase, and resting stage
- Fig. 63. Carpospore fully matured
(Figs. 35-63, $\times 1610$)

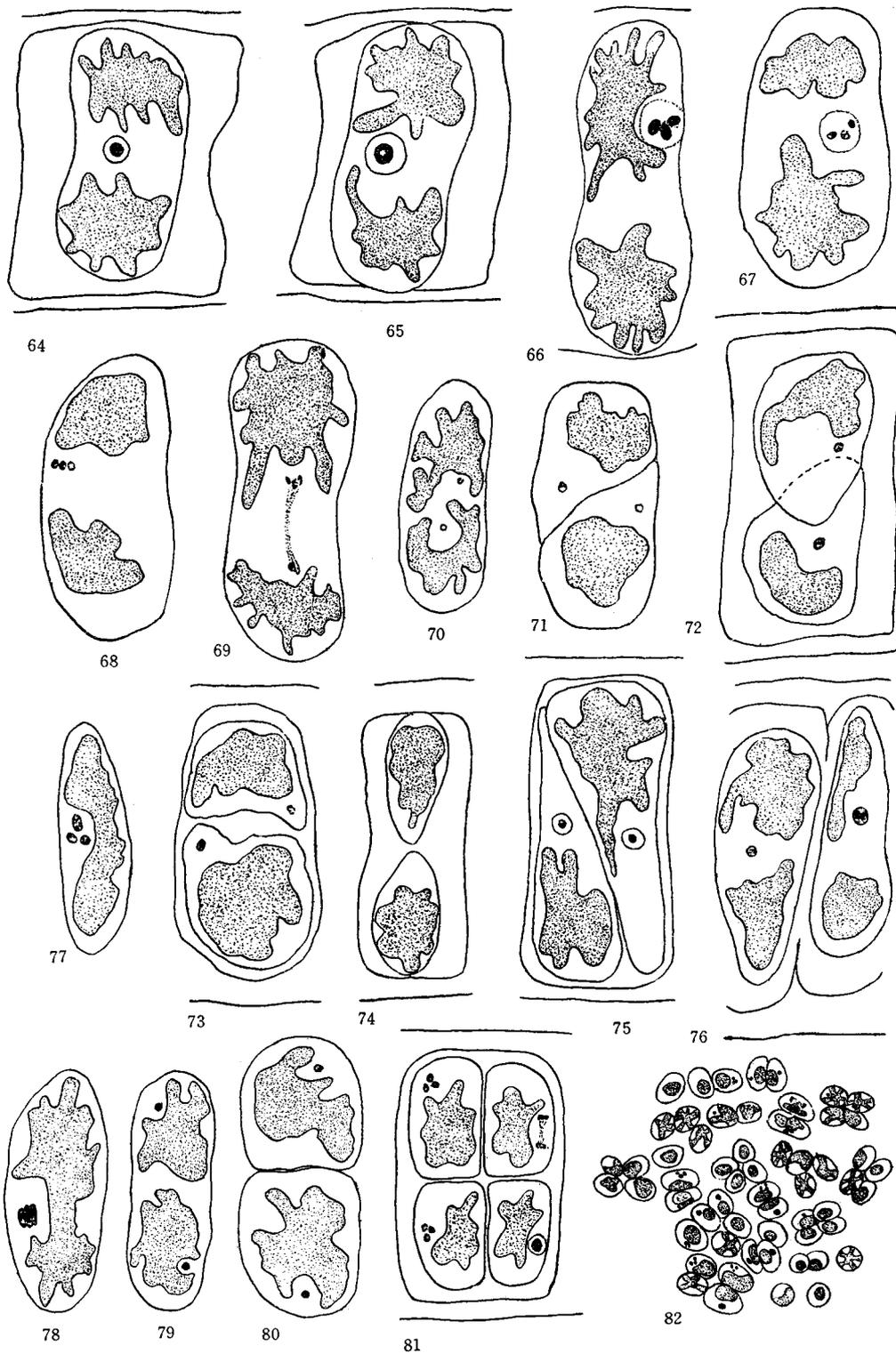


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PLATE III

Porphyra onoi Ueda

- Fig. 64. Cell in the monostromatic part of thallus, containing a central nucleus in resting stage and two chromatophores
- Fig. 65. Early prophase in vegetative nuclear division
- Figs. 66-67. Late prophase
- Fig. 68. Side view of metaphase
- Fig. 69. Anaphase
- Fig. 70. Telophase
- Figs. 71 & 72. Two daughter cells produced in vegetative division
- Figs. 73 & 74. Two daughter cells in distromatic distromatic disposition
- Figs. 75 & 76. Two daughter cells in transition stage to a monostromatic disposition
- Figs. 77-82. Nuclear and cell divisions in spermatium-formation
- Figs. 77 & 78. Metaphase of the first division
- Fig. 79. Telophase
- Fig. 80. Two-cell stage
- Fig. 81. Four-cell stage; nuclei in prophase, metaphase, and anaphase
- Fig. 82. Group of cells from collapsed antheridial area of thallus, showing a few cells in the sixth division and spermatia
- (Figs. 64-82, $\times 1840$)



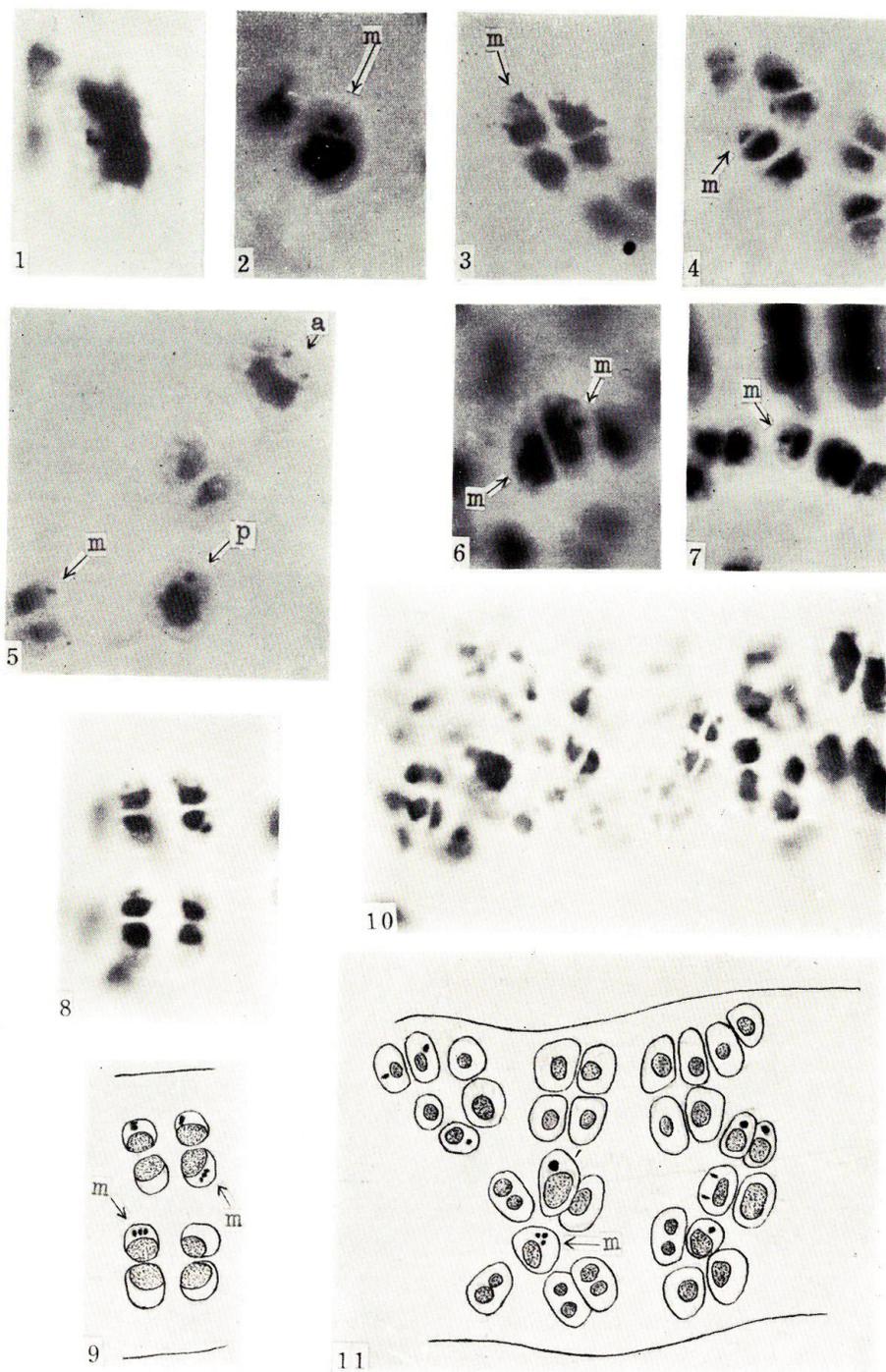
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PLATE IV

Porphyra yezoensis Ueda

Photomicrographs showing nuclear divisions in spermatium-formation

- Fig. 1. Metaphase of the first division
 - Fig. 2. Metaphase of the second division
 - Figs. 3 & 4. Metaphase of the third division
 - Fig. 5. Prophase (p), Metaphase (m), and anaphase (a) of the fourth division
 - Fig. 6. Metaphase of the fourth division
 - Fig. 7. Metaphase (m) of the sixth division
 - Fig. 8. Metaphase of the sixth division
 - Fig. 9. Camera lucida drawing of the same cells as shown in Fig. 8
 - Fig. 10. Group of cells in a cross section of antheridial part of thallus
 - Fig. 11. Camera lucida drawing of the same section as shown in Fig. 10; nucleus in prophase, metaphase, and telophase
- (Figs. 1-10, $\times 1600$; Fig. 11, $\times 2080$)

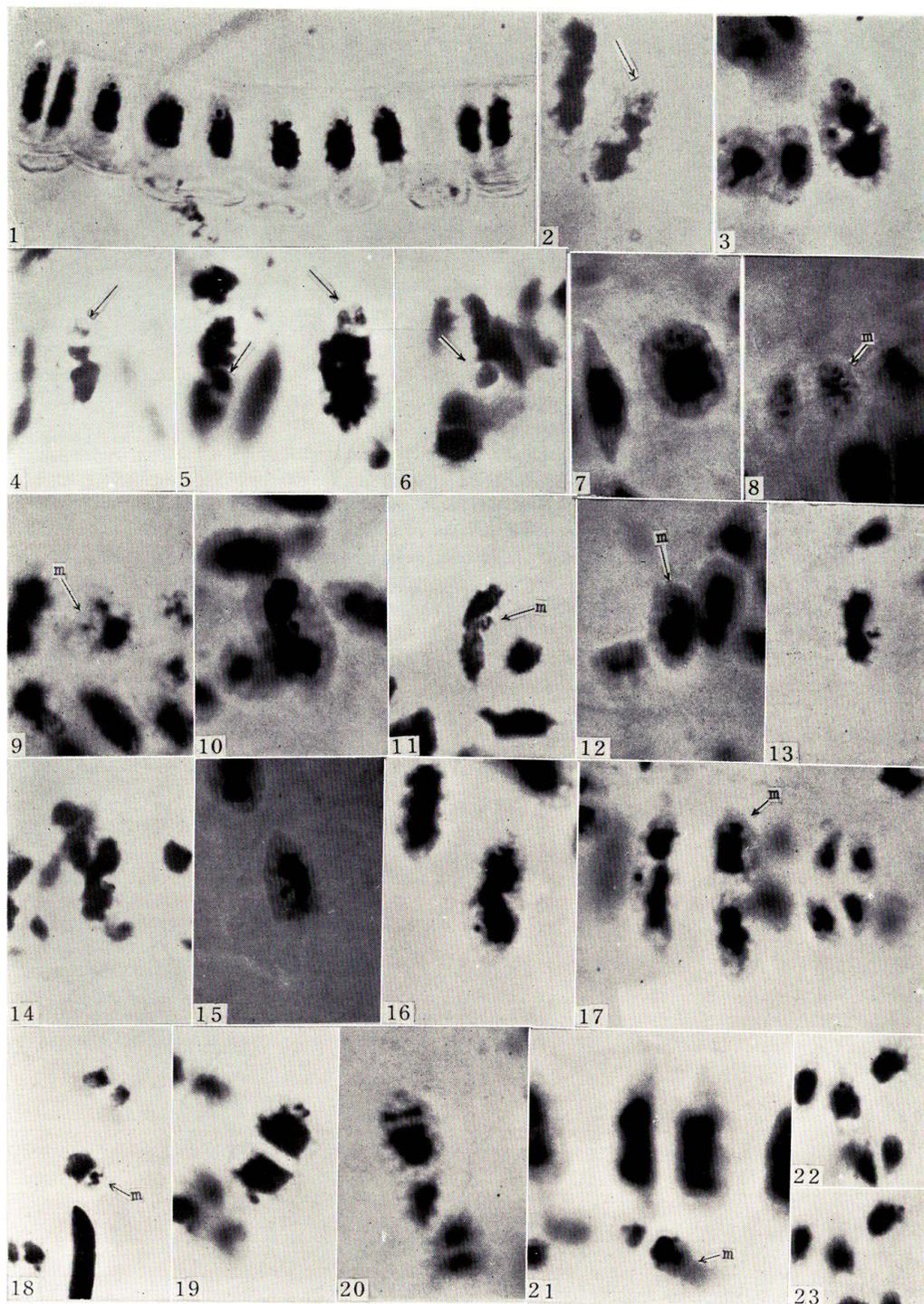


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PLATE V

Porphyra yeozensis Ueda

- Photomicrographs showing vegetative cells and carpospore-formation
- Fig. 1. Vegetative cells with nucleus in resting stage
 - Figs. 2 & 3. Fertilized carpogonium containing spermatium nucleus
 - Figs. 4-6. Carpogonium with fertilized nucleus
 - Figs. 7-12. Fertilized nucleus in metaphase of the first division
 - Figs. 13-15. Side view of metaphase in the first division
 - Fig. 16. Telophase of the first division
 - Fig. 17. Carpogonium before fertilization (to the left), two-cell stage with nucleus in metaphase of the second division (in the center), and four-cell stage with nucleus in prophase of the third division (to the right)
 - Figs. 18 & 19. Metaphase of the second division
 - Fig. 20. Anaphase of the second division with clearly visible spindle. The same cells as shown in Pl. II, Fig. 54
 - Fig. 21. Metaphase of the third division
 - Fig. 22. Two daughter nuclei in telophase of the third division (in the center)
 - Fig. 23. The same cells as shown in Fig. 22 focused at a different level, showing two daughter nuclei in telophase of the third division (upper right)
- (Fig. 1, $\times 560$; Figs. 2-23, $\times 1120$)



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PLATE VI

Porphyra onoi Ueda

Photomicrographs showing nuclear division in somatic cell and in spermatium-formation

Fig. 1. Early prophase of somatic division; vacuolated nucleolus is seen near the center of the cell

Fig. 2. Metaphase of somatic division

Fig. 3. Side view of metaphase in somatic division

Fig. 4. Anaphase

Fig. 5. Two daughter cells produced

Fig. 6. One of the daughter cells produced

Figs. 7-15. Divisions in spermatium formation

Fig. 7. Metaphase of the first division

Fig. 8. Side view of metaphase of the first division

Fig. 9. Two spermatium mother-cells, one with resting nucleus (to the left), the other with two daughter nuclei in telophase (to the right)

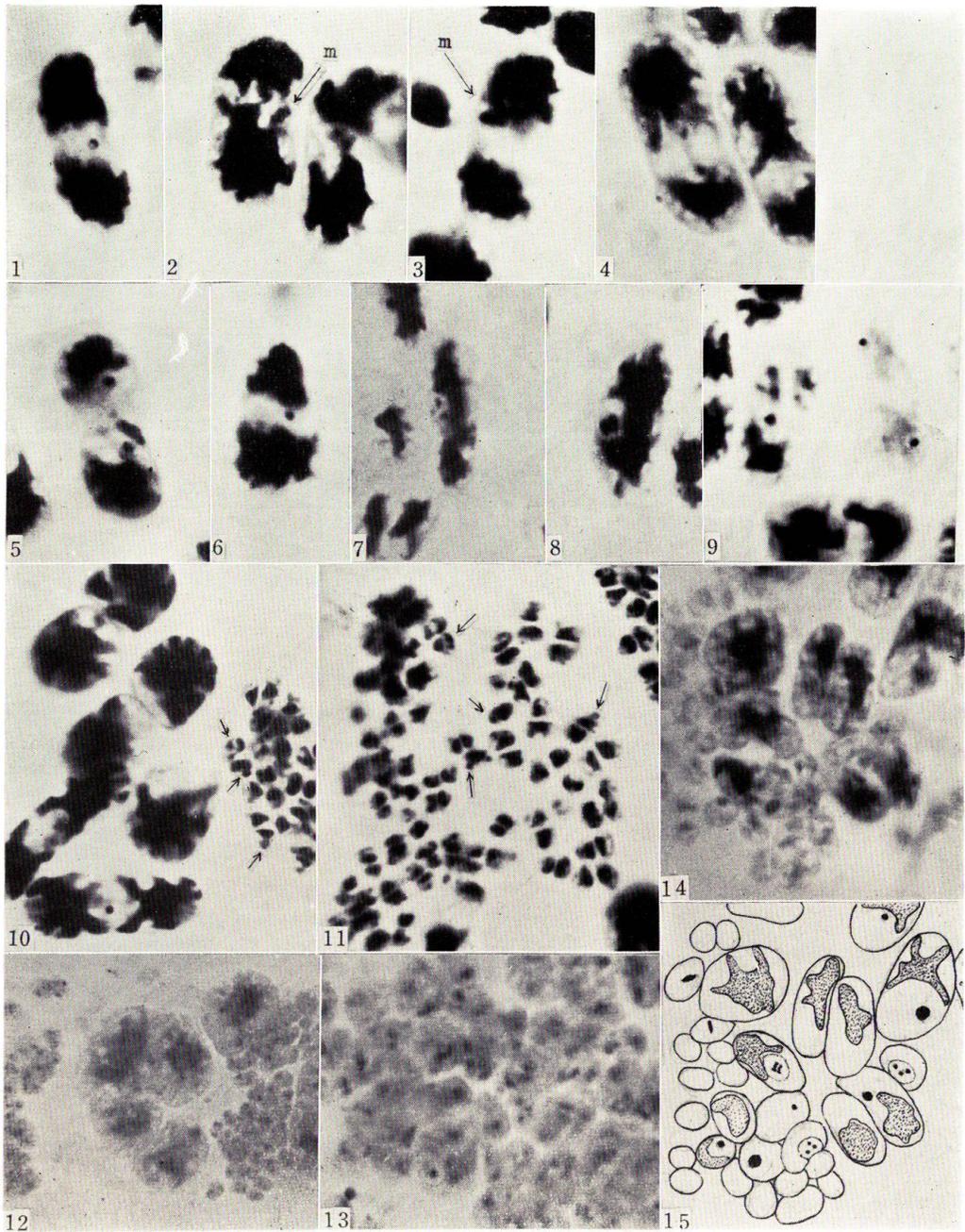
Figs. 10, 11 & 14. Group of cells from collapsed antheridial area of thallus, nucleus indicated by arrow in divided cells

Fig. 12. Collapsed section of fertile area of thallus showing dividing cells in spermatium- and carpospore-formation

Fig. 13. Collapsed section of antheridial area of thallus showing dividing cells in spermatium-formation

Fig. 15. Camera lucida drawing of the same cells as shown in Fig. 14 showing nuclei in prophase, metaphase, and early anaphase

(Figs. 1-11, $\times 1280$; Fig. 12, $\times 1008$; Fig. 13, $\times 1920$; Figs. 14-15, $\times 1280$)



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