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OBSERVATION ON CHROMOSOMES IN SOME SPECIES OF *PORPHYRA* II

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In a previous paper (Yabu, 1969), some Japanese species of *Porphyra* have been examined with respect to the number and length of the chromosomes in the nucleus at prophase. The present paper describes the results of my later observations on eight species of *Porphyra*, viz., *P. crispata*, *P. dentata*, *P. kumiedai*, whose chromosomes are first reported here, and *P. suborbiculata*, whose chromosomes have already been reported in the former paper, yet the present study is different as to number, and moreover four species with an interesting number of chromosomes, whose specific name could not be determined yet. Fixing and staining were made in the same way as reported already (Yabu, 1969). I wish here to acknowledge my indebtedness to Mr. I. Shinmura of the Kagoshima Prefectural Fisheries Experimental Station, to Mr. M. Tajima of the Nanao Branch of the Ishikawa Prefectural Fisheries Experimental Station, to Mr. M. Shiota of the Ōshima Branch of the Kagoshima Prefectural Fisheries Experimental Station and to Professor J.W. Kang of Pusan Fisheries College in Korea, in sending me the fixed materials. My thanks are also due to Mr. J.H. Branson of the Bureau of Commercial Fisheries, United States Department of the Interior Fish and Wildlife Service at Kodiak, Alaska, U.S.A. for his kindness in giving me facilities for the collection of materials.

Results

The results of observations are summarized in Table 1.

Porphyra crispata Kjellm. (Pl. I, fig. 1). - Fifteen thalli collected from Setouchi-cho, Ōshimagun in Kagoshima Pref. and fixed there, were obtained. Their dividing nuclei were observed in nearly matured male portions of the fronds, and the metaphase nuclei showed to have 3 chromosomes.

P. dentata Kjellm. (Pl. I, figs. 2-14). - About twenty thalli fixed could be obtained as materials. Chromosome counts were 3 in the male portion and 6 in the female portion of the frond. At prophase of these dividing nuclei, usually one out of three in haploid and occasionally two out of six in diploid were observed to be somewhat longer than the others. In this species, the first division

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Table 1. Chromosome number examined in eight species of leafy thalli of *Porphyra* and length of chromosome in prophase nucleus

Species (Collecting place and date)	Chromosome number	Chromosome length	
		long	short
<i>P. crispata</i> (Setouchi-cho, Ôshima-gun in Kagoshima Pref. Japan; March 1969)	spermatium formation 3		
<i>P. dentata</i> (Bonotsu-cho, in Kagoshima Pref. Japan; March 1969)	spermatium formation 3	1	2
	carpospore formation 6	2	4
<i>P. kuniedai</i> (taken from culture net at Taniyama in Kagoshima City Japan; March 1969)	spermatium formation 2	1	1
	carpospore formation 4	2	2
<i>P. suborbiculata</i> (Bonotsu-cho, in Kagoshima Pref. Japan; March 1969)	spermatium formation 3		
<i>P. sp. no. 1</i> (Kitamura in Shimane Pref. Japan; January 1970)	spermatium formation 7	2	5
	carpospore formation 14		
<i>P. sp. no. 2</i> (Uwano, Noto Peninsula in Ishikawa Pref. Japan; January 1969)	somatic division 5		
	spermatium formation 5	1	4
	carpospore formation 10		
<i>P. sp. no. 3</i> (Yosu, Ton-to in Challanam-Do, Korea; March 1968)	spermatium formation 6	1	5
<i>P. sp. no. 4</i> (Kodiak in Alaska, U.S.A. July 1970)	spermatium formation 2		

of diploid nucleus leading to carpospore formation is made parallel to the direction of the surface of the frond, so that the carpospore mother cell is divided vertically, as it can be seen in fig. 7.

P. kuniedai Kurogi (Pl. II, figs. 15-29). - *P. kuniedai* is a species mentioned by Kunieda (1934) and Tanaka (1952) as *Porphyra tenera* to be round-type in form. This alga was described as a new species by Kurogi in 1957, and was observed in detail by him in 1961 with respect to morphology, ecology and life-history. The materials of this species were obtained by Mr. Shinmura from the culture nets settled at the sea frond at Taniyama in Kagoshima City on March 16, 1969. According to the communication made by Mr. Shinmura, the leafy thalli of the culture net were derived from the carpospores of the thalli collected at Kiire-cho,

Ibusuki-gun in Kagoshima Prefecture. The dividing nuclei at metaphase showed to have 2 chromosomes in the male portion and 4 in the female portion of the fronds. In the nuclei at prophase and early metaphase, one chromosome in haploid and two in diploid were usually somewhat longer than the others. In spermatium forming cells, the chromosomes bridge was rarely found at anaphase, as is shown in fig. 17. In this species the nucleolus often appeared already in late anaphase nucleus (fig. 15).

P. suborbiculata Kjellm. (Pl. II, fig. 30). - I reported already the chromosome number of the present species in 1969, but the observations here do not agree with the former report of $n=2$. The fixed materials which could be obtained were few in number and the nuclear divisions were observable only in the nearly matured male portion of the fronds. The dividing figures of nuclei showed to have 3 chromosomes.

P. sp. no. 1 (Pl. III, figs. 31-43). - The present species was occasionally found among the fixed thalli of *Porphyra pseudolinearis* collected by Mr. Kajimura at Kitamura in Shimane Prefecture on January 16th in 1970 from the rocky shore about 1.5 meters above the low tide level. The frond is monoecious, without microscopic spine at its marginal portion, lanceolate or elliptical in shape and could hardly be distinguished from *P. pseudolinearis*. The dividing formula of the reproductive cells is $a/2$, $b/2$, $c/2$ in females and $a/4$, $b/4$, $c/4$ in males. This formula is identical with that of *P. tenera* (cf. Ueda, 1930, p. 20; Tanaka, 1952, p. 52) among the monoecious species of *Porphyra* in Japan. Chromosome counts were 7 in the male portion and 14 in the female portion of the fronds and in the former portion it was occasionally observed at prophase nuclei that two chromosomes were somewhat longer than the other five (fig. 32).

P. sp. no. 2 (Pl. IV, figs. 44-52; Pl. V, figs. 53-62; Pl. VI, figs. 63-68; Pl. VII, VII, figs. 69-76). - Numerous thalli of this species collected by Mr. Tajima at Uwano located on the west side of Noto Peninsula in Ishikawa Prefecture in January 1969 were obtained as materials. From Toyama Bay facing the east side of Noto Peninsula, two specimens of *Porphyra* viz., *P. okamurai* and *P. pseudolinearis*, have been known to occur (Oshima, 1952). The present species clearly differs from these two species. The frond is elliptical in shape, without microscopic spine at its marginal portion, being about 5 cm. long and about 2 cm. broad in the medium size and its external appearance resembles much that of *P. pseudolinearis*, but monoecious and dividing formula is $a/2$, $b/2$, $c/2$ in females and $a/4$, $b/4$, $c/4$ in males, that is, identical with *P. tenera*. The dividing figures of nuclei were observed in somatic and reproductive cells both of males and females. Photo-micrographs in figs. 46 and 47 show surface views of immature young frond in the preparations which could not be squashed well. As is seen

in these figures, 5 chromosomes were counted in somatic cells. Prior to the formation of the spermatium mother cell a somatic cell is divided transversely into two. The early prophase nucleus in this division is characteristic; at this stage, besides the nucleolus, one peculiar small body, rarely two, is occasionally found in its nuclear cavity. This body also occasionally appears in the early prophase nucleus of mother cells of spermatium and carpospore. When two bodies are found in the same nuclear cavity, each of these usually takes the position at the pole of the nucleus. The somatic cells in the process of division prior to the formation of the spermatium mother cell are shown in figs. 46-48. In this somatic division, the cell is usually divided by the slanting plane as is seen at the left side in fig. 47. The spermatium mother cell thus produced soon grows rapidly in size up to about $90\ \mu$ long and $30\ \mu$ broad, and takes an elongated ellipsoid form. Many figures of nuclei at metaphase could be obtained in spermatium mother cells, and 5 chromosomes were easily counted in them. The later successive nuclear divisions in spermatium formation are given in figs. 53-61. In the prophase nuclei of these spermatium forming cells, one chromosome was usually found to be somewhat longer than the other four. Carpospore mother cells with fertilized nucleus in various stages were often found in my preparations. With the advance of the stage of nuclear division from prophase to metaphase, the carpospore mother cell increases its size rapidly and at metaphase it becomes about $100\ \mu$ long and $50\ \mu$ broad. The nuclear division in the carpospore mother cell is made parallel to the surface of the fronds, so that the cell is divided eventually by the longitudinal plane (figs. 71-74). In the carpospore formation, all of the fine figures of dividing nuclei at late prophase and early metaphase showed to have 10 chromosomes.

P. sp. no. 3 (Pl. VIII, figs. 77-88). - The present species have met among the fixed thalli of *P. seriata* collected at Yosu, Tong-to in Challanam-Do in Korea in March 1969, which were sent to me through the kindness of Dr. Kang. The frond is ovovate or elliptical in shape, with microscopic spine at its marginal portion, and could not be distinguished from *P. seriata* until their chromosomes were ascertained under the microscope. In this species dividing nuclei were observed only in the cells leading to spermatium formation and chromosome counts were 6 in those cells, of which one was found to be somewhat longer than the other five at prophase. My materials were not sufficient to examine the formula for the formation of spermatium cells, but some spermatangia showed to have the dividing formula of $a/2$, $b/4$, $c/4$. Carpospore formation had not been found in any frond, so that the character of monoecious or dioecious could not be ascertained yet.

P. sp. no. 4 (Pl. IX, figs. 89-92). - The present species is found among the fixed materials of *P. amplissima* collected in July 1970 at Kodiak, Alaska, U.S.A.

The frond is monostromatic, thus it distinctly differs from *P. amplissima*. Nuclear divisions were found only in spermatium forming cells and chromosomes were ascertained to be $n=2$. Only a few pieces of the fronds could be observed so that the morphological features found were not enough to determine the name of the species.

Until now, in *Porphyra*, chromosome counts of $n=2$ have been reported in *P. linearis* (Dangeard, 1927), *P. suborbiculata* (Yabu, 1969) and *P. kuniedai* (Yabu, in the present paper). It is thought that the present species differs from those three species, because they had not been listed among the species of *Porphyra* from Alaska and its vicinity studied in detail by Hus (1902).

Porphyra pseudolinearis from Kitamura in Shimane Prefecture, *P. seriata* from Yosu in Korea and *P. amplissima* from Kodiak, U.S.A. which could be obtained for this time together with the materials treated here were, of course, examined again, although their chromosome number had been reported already (Kito, Yabu & Tokida, 1967; Yabu, 1969; Yabu, 1970). The number of chromosomes in these species was the same as in the former reported and was ascertained to be $n=4$ in *P. pseudolinearis*, $n=3$ and $2n=6$ in *P. seriata* and in *P. amplissima* in the nuclear division leading to spermatium or carpospore formation.

Summary

Chromosomes in eight species of *Porphyra*, viz., *P. crispata*, *P. dentata*, *P. suborbiculata*, *P. kuniedai* and four species whose specimen names could not be determined were reported. Among those species, *P. crispata*, *P. dentata*, *P. kuniedai* were first reported cytologically. Chromosome counts were as follows: $n=3$ in *P. crispata* and in *P. suborbiculata*; $n=3$, $2n=6$ in *P. dentata*; $n=2$, $2n=4$ in *P. kuniedai*; $n=7$, $2n=14$ in *P. sp. no. 1*; $n=5$, $2n=10$ in *P. sp. no. 2*; $n=6$ in *P. sp. 3*; $n=2$ in *P. sp. no. 4*. Photomicrographic evidences were presented in each species.

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Explanation of Plates

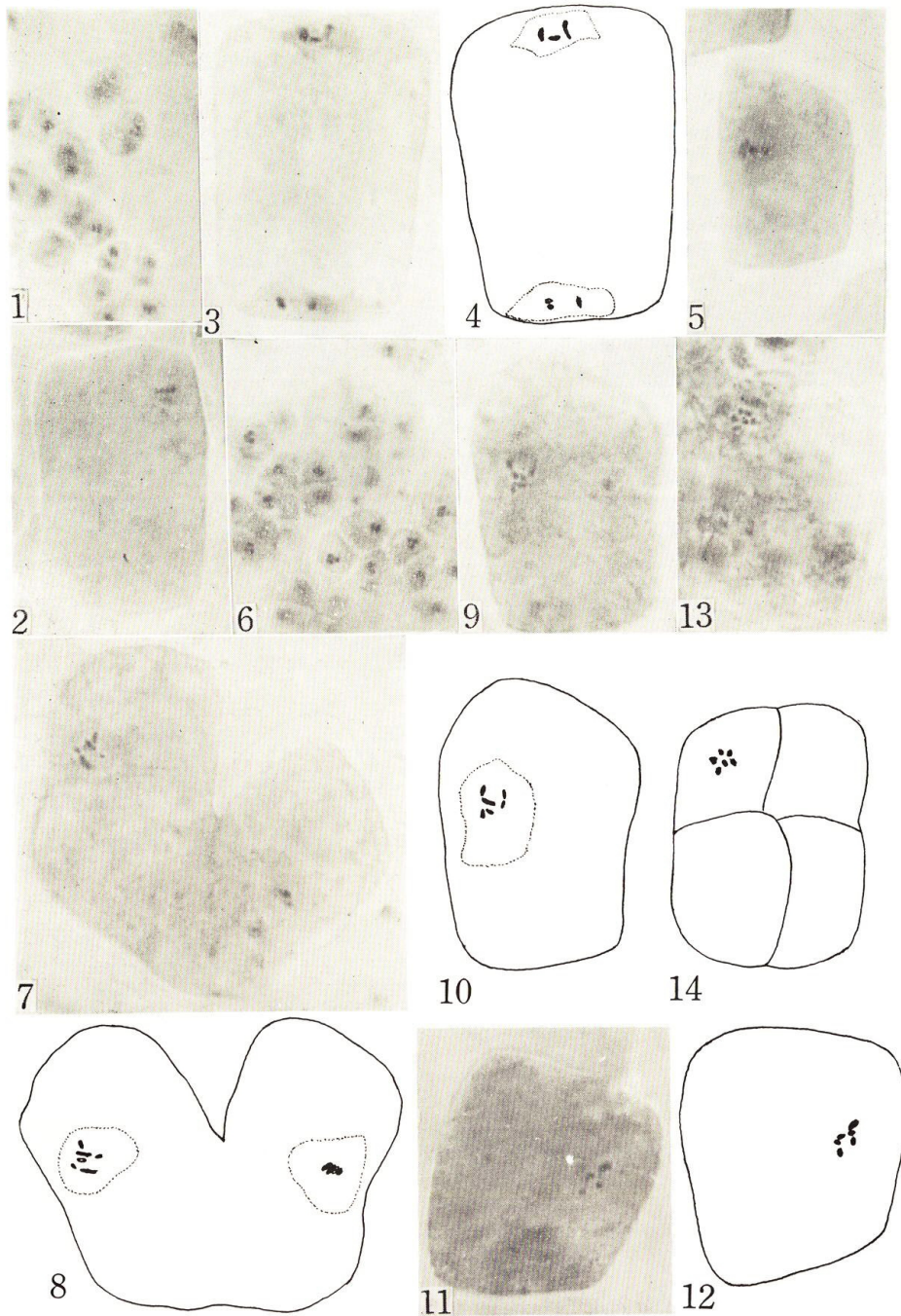
PLATE I

Porphyra crispata Kjellm.

1. Part of spermatium forming region. Three chromosomes are seen in some nuclei.

Porphyra dentata Kjellm.

- 2-6. Nuclear and cell divisions in spermatium formation
 2. Spermatium mother cell with metaphase nucleus
 3. Spermatium mother cell with late anaphase nucleus
 4. Drawing of fig. 3
 5. Cell with metaphase nucleus from two-cell stage
 6. Cell with metaphase nucleus in more advanced cell stage than above
 - 7-14. Nuclear and cell divisions in carpospore formation
 7. Carpogonium with late anaphase nucleus. Formation of cleavage furrow is seen at longitudinal plane of the cell.
 8. Drawing of fig. 7
 - 9 & 11. Cells with metaphase nucleus from two-cell stage
 - 10 & 12. Drawings of figs 9 & 11, respectively
 13. Four-cell stage. Metaphase nucleus within one of the cells is seen in the figure.
 14. Drawing of fig. 13
- (All figures, $\times 1130$)



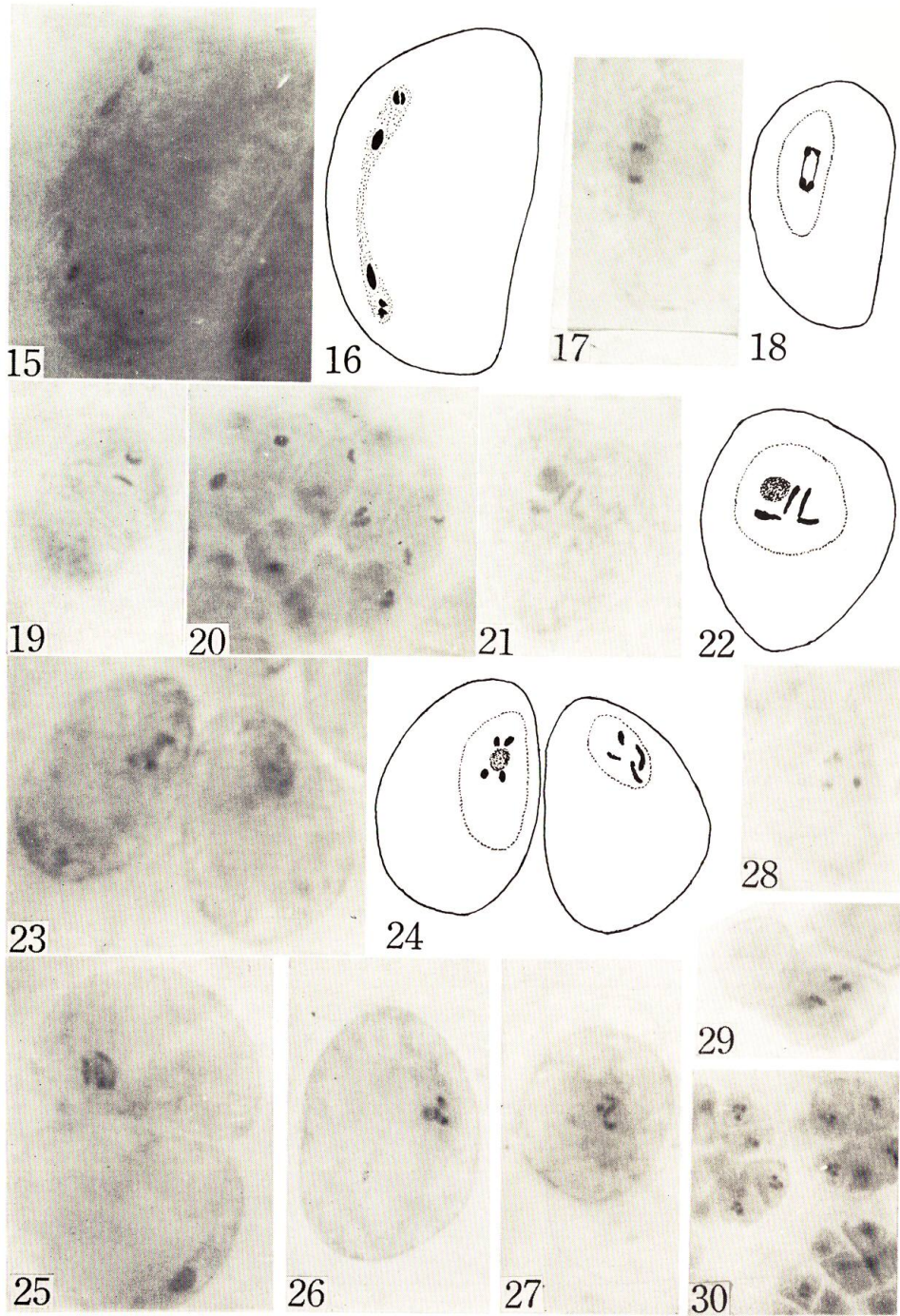


PLATE II

Porphyra kuniedai Kurogi

- 15-20. Nuclear and cell divisions in spermatium formation
15. Spermatium mother cell with anaphase nucleus
16. Drawing of fig. 15
17. Cell with anaphase nucleus from two-cell stage. Chromosome bridges are seen between the group of chromosomes.
18. Drawing of fig. 17
19. Cell with metaphase nucleus from four-cell stage. Figure is focussed to metaphase nucleus in the upper cell.
20. Cells with metaphase nucleus from sixteen-cell stage
- 21-29. Nuclear and cell divisions in carpospore formation
21. Cell with late prophase nucleus from two-cell stage
22. Drawing of fig. 21
23. Cells from two-cell stage. Nucleus in the cell seen in the left side of the figure is at late prophase and that in the right side is at early metaphase.
24. Drawing of fig. 23
25. Two-cell stage. Nucleus in the upper cell is at late prophase.
26. Cell with metaphase nucleus from two-cell stage
- 27-28. Cell with metaphase nucleus from four-cell stage
29. Cells with metaphase nucleus from eight-cell stage

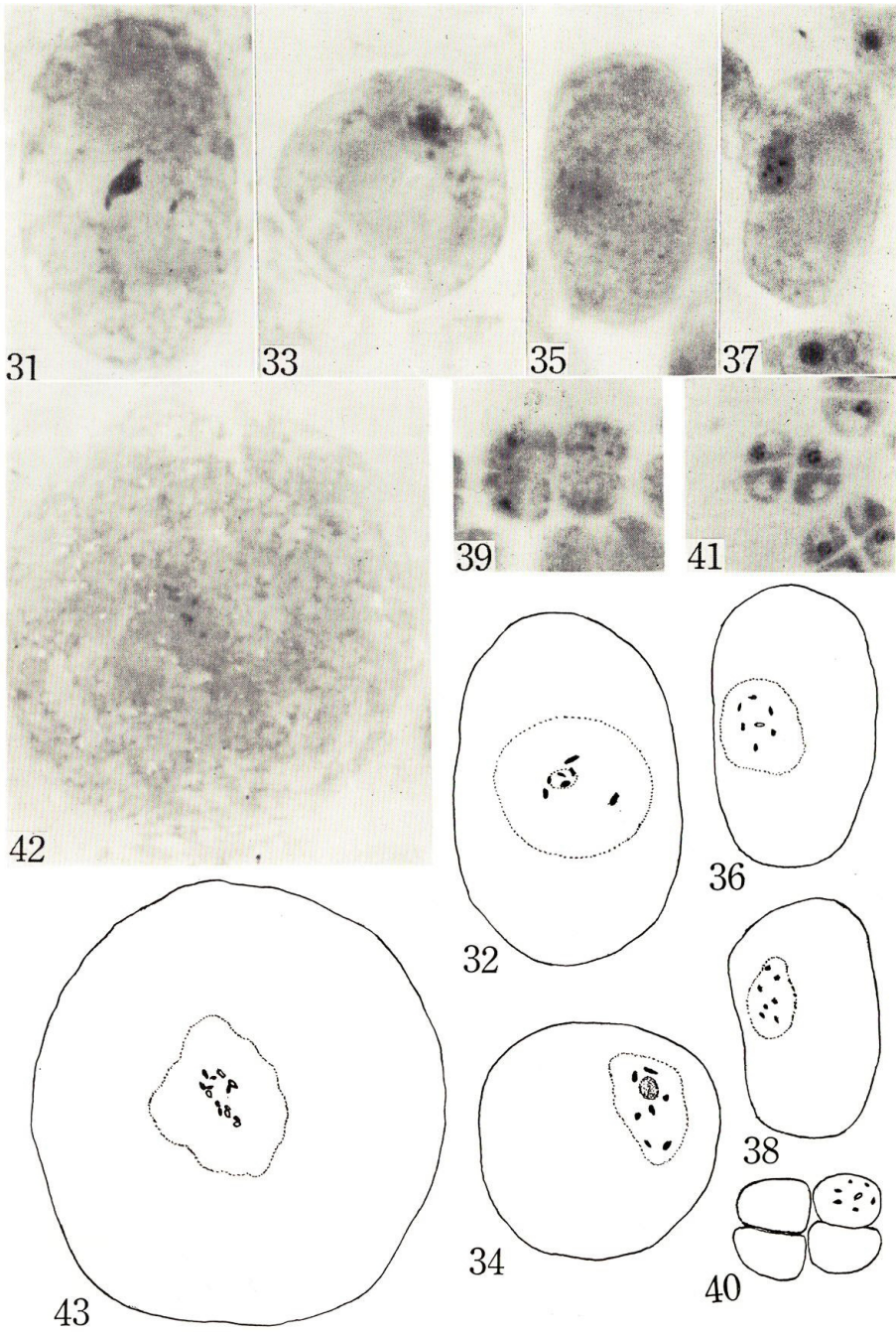
Porphyra suborbiculata Kjellm.

30. Part of spermatium forming region. Three chromosomes are seen in several nuclei.
- (All figures, $\times 1280$)

PLATE III

Porphyra sp. no. 1

- 31-41. Nuclear and cell divisions in spermatium formation
 - 31. Spermatium mother cell with late prophase nucleus
 - 32. Drawing of fig. 31
 - 33. Cell with late prophase nucleus from two-cell stage
 - 34. Drawing of fig. 33
 - 35 & 37. Cells with metaphase nucleus from two-cell stage
 - 36 & 38. Drawings of figs. 35 & 37, respectively
 - 39. Cells with metaphase nucleus from thirty-two-cell stage
 - 40. Drawing of fig. 39
 - 41. Cells with metaphase nucleus from sixty-four-cell stage
 - 42-43. Nuclear division in carpospore formation
 - 42. Carpogonium with metaphase nucleus
 - 43. Drawing of fig. 42
- (All figures, $\times 1260$)



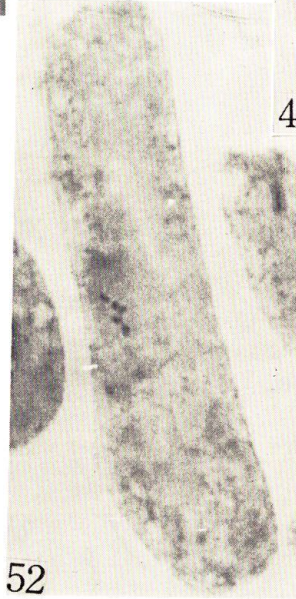
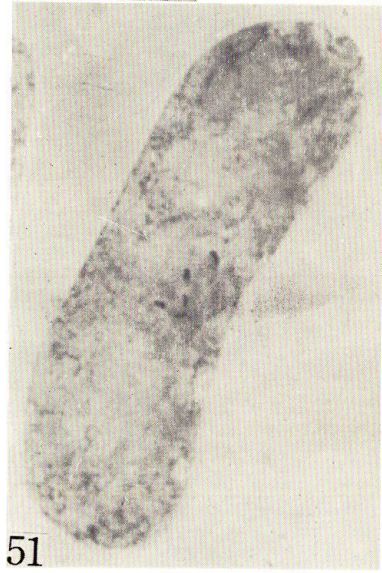
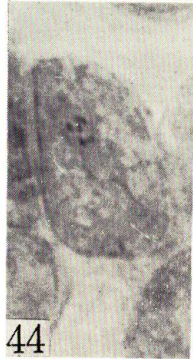
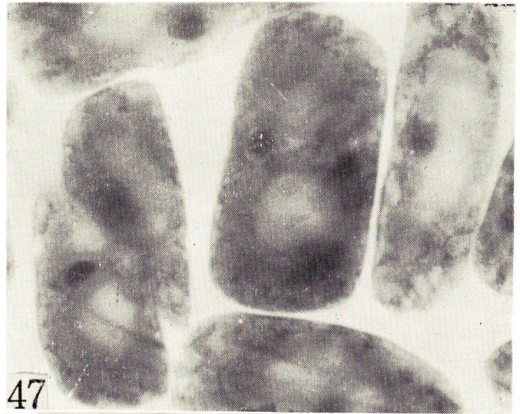


PLATE IV

Porphyra sp. no. 2

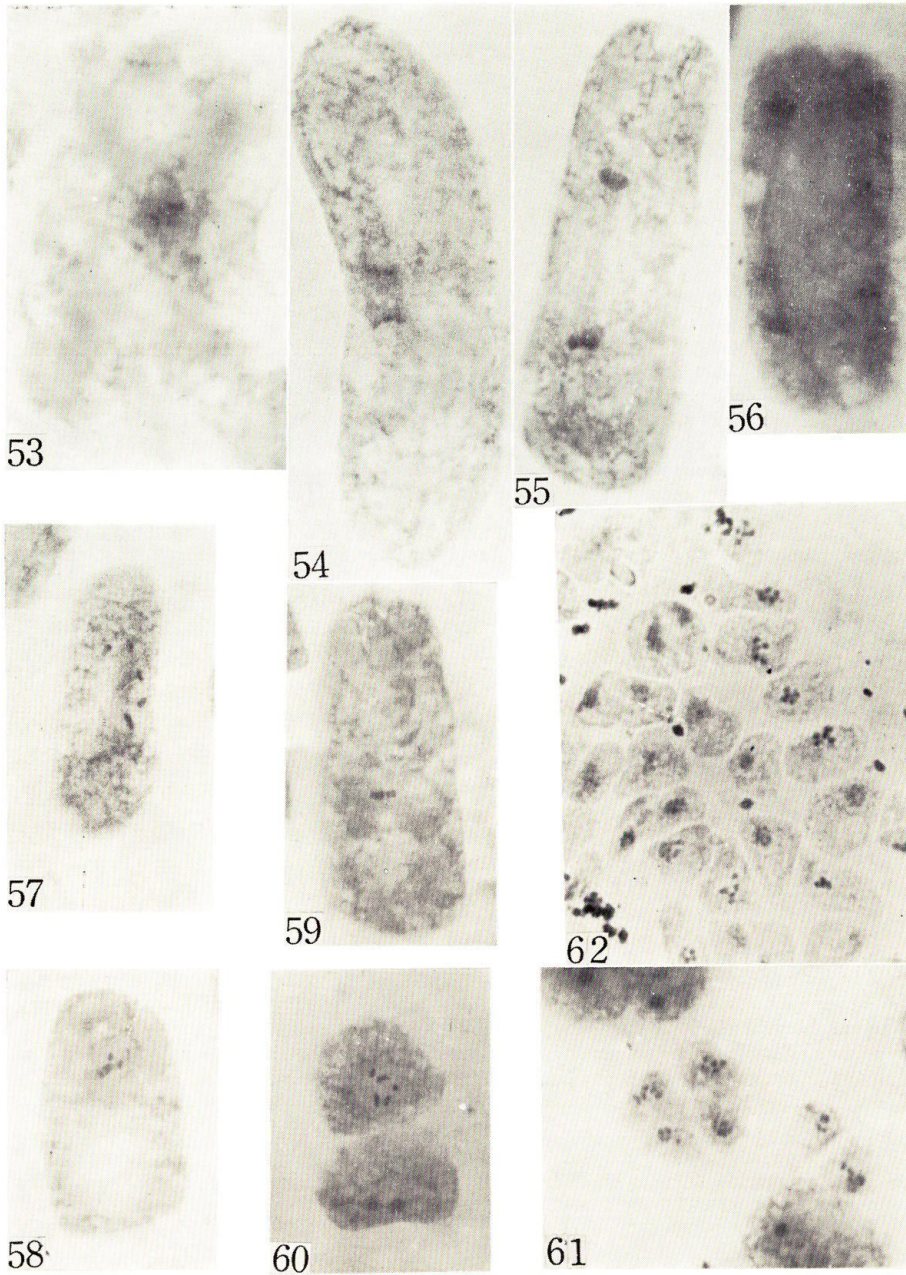
- 44-45. Polar views of metaphase of the somatic nuclear division in young frond
46-48. Somatic nuclear and cell divisions just before the formatiin of spermatium
mother cells
46. Eearly prophase nucleus. A peculiar small body is seen near nucleolus.
47. Telophase nucleus
48. Metaphase nucleus
49-52. Nuclear divisions in spermatium formation
49-50. Spermatium mother cells with early prophase nucleus. Besides nucleolus,
one or two peculiar small bodies are seen in the nucleus.
51-52. Spermatium mother cells with metaphase nucleus
(All figures, $\times 1280$)

PLATE V

Porphyra sp. no. 2

Nuclear and cell divisions in spermatium formation

- 53. Spermatium mother cell with metaphase nucleus
 - 54-56. Spermatium mother cells with anaphase nucleus
 - 57-59. Cells with metaphase nucleus from four-cell stage.
 - 60. Cell with metaphase nucleus from eight-cell stage. Figure is focussed to the metaphase nucleus in the upper cell.
 - 61-62. Cells with metaphase nucleus in more advanced cell stage than above
- (All figures, $\times 1260$)



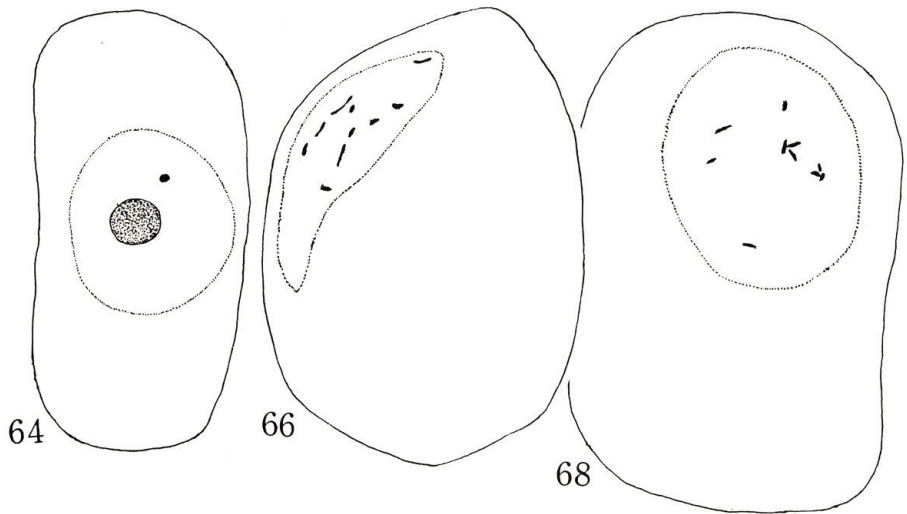
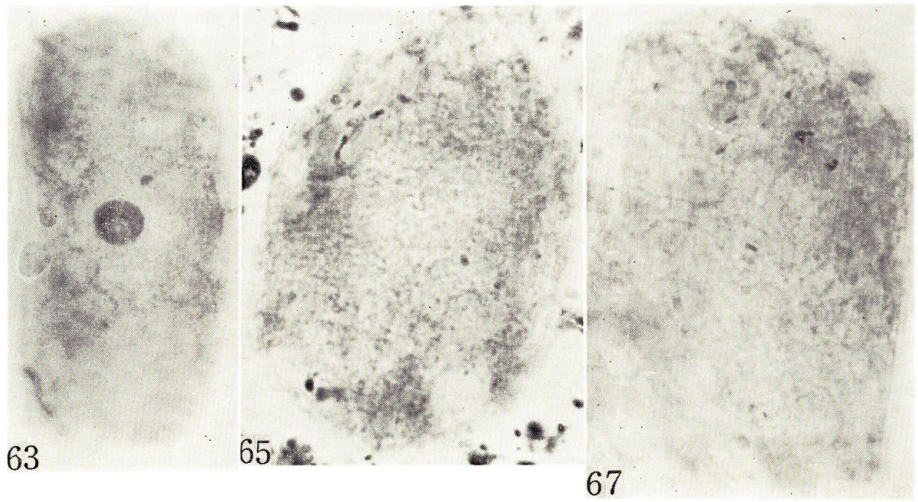


PLATE VI

Porphyra sp. no. 2

Nuclear divisions in carpogonia

63. Prophase nucleus. Besides nucleolus a peculiar small body is seen within the nuclear cavity.

65 & 67. Metaphase nuclei

64, 66 & 68. Drawings of figs. 63, 65 & 67, respectively
(All figures, $\times 1010$)

PLATE VII

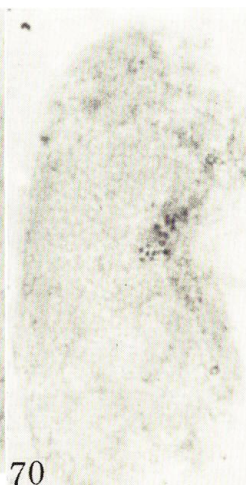
Porphyra sp. no. 2

Nuclear and cell divisions in carpospore formation

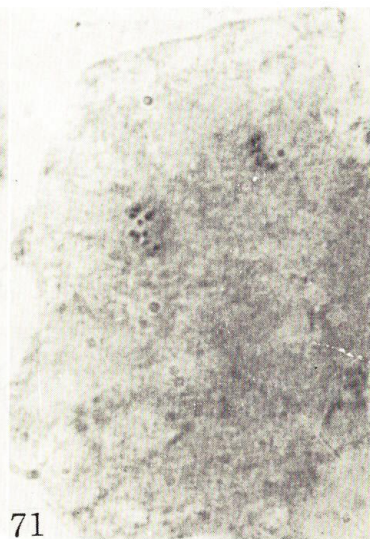
69. Carpogonium with metaphase nucleus
 70. Carpogonium with early anaphase nucleus
 71. Carpogonium with anaphase nucleus
 72. Carpogonium with late anaphase nucleus. Formation of cleavage furrow is seen at longitudinal plane of the cell.
 73. Drawing of fig. 72
 74. Two-cell stage. Metaphase nucleus is seen in the cell at the right side in the figure.
 75. Cell with metaphase nucleus from two-cell stage
 76. Cell with metaphase nucleus from four-cell stage
- (All figures, $\times 1070$)



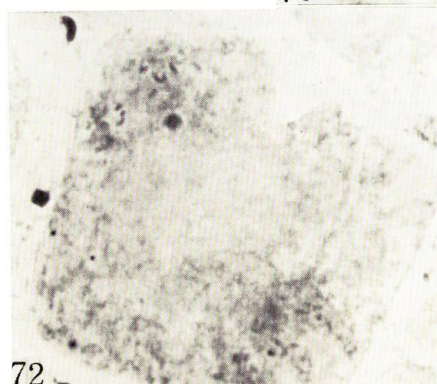
69



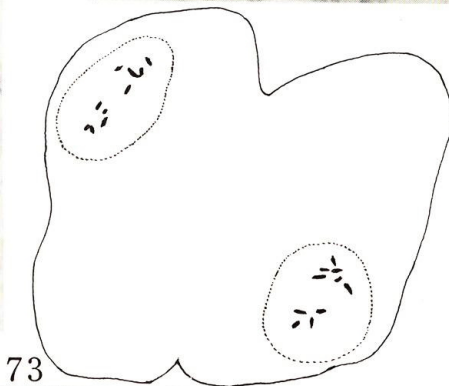
70



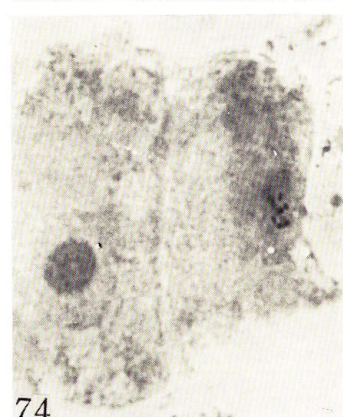
71



72



73



74



75



76

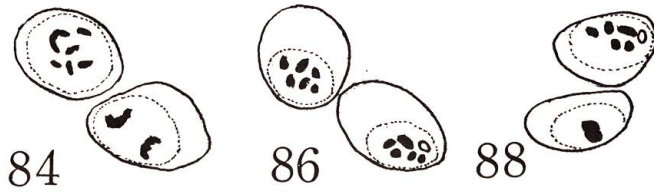
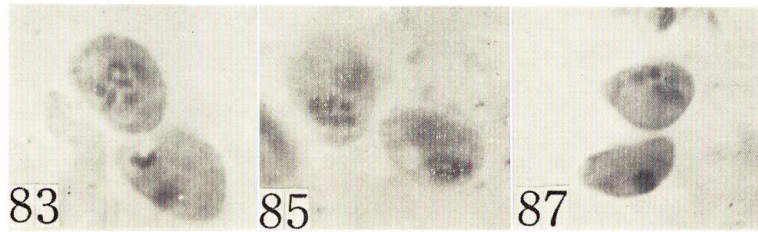
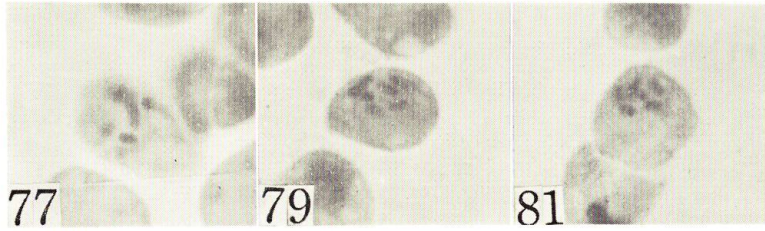


PLATE VIII

Porphyra sp. no. 3

Nuclear divisions in spermatium formation

77, 79, 81, 83, 85 & 87. Metaphase

78, 80, 82, 84, 86 & 88. Drawings of the figs. 77, 79, 81, 83, 85 & 87, respectively
(All figures, $\times 1600$)

PLATE IX

Porphyra sp. no. 4

Nuclear and cell divisions in spermatium formation

- 89. Spermatium mother cell with late anaphase nucleus
 - 90. Drawing of fig. 89
 - 91-92. Cells with metaphase nucleus from thirty-two-cell stage
- (All figures, $\times 1600$)

