MITOSIS IN THE SPORANGIUM OF *AGARUM CRIBROSUM* BORY AND *ALARIA PLAELONGA* KJELLMAN

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Since 1939, when the first cytological study of the Japanese species belonging to the Laminariaceae was reported by Abe, five authors have published nine papers dealing with the cytological aspects of eight species as shown in Table I.

Table I.

Species	Chromosome number (n)	Author
Laminaria japonica	22	Abe, 1939
L. angustata	ca. 30	Nishibayashi & Inoh, 1956
L. diabolica	22	Yabu, 1958
Undaria pinnatifida	22	Inoh & Nishibayashi, 1954
U. pinnatifida	ca. 30	Inoh & Nishibayashi, 1960
U. undarioides	ca. 30	Nishibayashi & Inoh, 1960
Costaria costata	ca. 30	Nishibayashi & Inoh, 1957
Alaria crassifolia	22	Yabu, 1957
Arthrothamnus bifidus	22	Yabu & Tokida, 1963

In the present paper the writer wishes to add to the above list by reporting the results of his observations on the mitosis in the sporangium of *Agarum cribrosum* and *Alaria praelonga* collected in Hokkaido.

Materials and Methods

The materials of *Agarum cribrosum* and *Alaria praelonga* were collected near Tokotan, Akkeshi, Hokkaido, on June 13th, 1963, on an islet, Daikoku-jima, and on a reef, respectively. The materials were fixed at midnight of the collection day after having been kept in vats filled with sea water at the Akkeshi Marine Biological Station of Hokkaido University. Two kinds of fixatives were applied for 8–10 hours with satisfactory results. Sections were cut 4–5 μ thick by paraffin method and were stained with Heidenhain's haematoxylin.

The formulas of the fixatives employed are as follows:

1. 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 gr

These three solutions were mixed just before use in the ratio, a:b:c=2:1:1

2. Mixture of Tahara's fluid (Tahara, 1929) and formalin

(a)	2% osmic acid	5.0 cc
	2% chromic acid	70.0 cc
	Glacial acetic acid	2.5 cc
	Sea-water	30.0 cc
(b)	40% formalin	

These two solutions were mixed just before use in the ratio, a:b=2:1

Results Obtained

1. *Agarum cribrosum* Bory

The zoosporangial sori are formed in the depressions on both surfaces of the blade (cf. Kanda, 1941, p. 300). The development of the sporangium follows just the same course as described in *Arthrothamnus bifidus* (Yabu & Tokida, 1963).

The resting nucleus in a young sporangium contains usually one nucleolus, rarely two. The nucleus grows larger and comes to contain in its cavity a small number of chromatin granules. The granules soon become filamentous and they are well stained with haematoxylin. These chromatin threads form a loop in a corner of the nuclear cavity (Pl. I, Figs. 4 & 5, Pl. III, Fig. 45), and then gradually spread out within the cavity (Pl. I, Figs. 6–7, Pl. III, Fig. 46). The young sporangia with the nucleus in the diakinesis stage are shown in Pl. I, Figs. 8–12 and in Pl. III, Figs. 48 & 53. In metaphase the nuclear membrane and the nucleolus become obscure, and 22 chromosomes are clearly observed (Pl. I, Figs. 13–15, Pl. III, Figs. 49–51). The centrosomes are occasionally visible at the pole of the spindle from the side view in metaphase and sometimes also in anaphase (Pl. I, Figs. 19 & 20). The nuclear membrane and the nucleolus reappear in telophase. After the first nuclear division, four divisions follow successively. Twenty-two chromosomes are clearly counted in metaphase of those divisions. At the end of the fifth division, each chromatophore becomes closely associated with each

nucleus, and the apical wall of the sporangium is markedly thickened and stained faintly with haematoxylin (Pl. II, Fig. 30). Eventually 32 zoospores containing a single nucleus and a single chromatophore are produced in each sporangium.

2. *Alaria praelonga* Kjellman

The zoosporangial sori are borne on both surfaces of the sporophyll. The sporangium develops from the meristoderm just in the same way as described in *Arthrothamnus bifidus* (Yabu & Tokida, 1963). The protuberance destined to be a sporangium in the present species grows out from the lower cell only after the paraphyses are much elongated leaving wide intercellular spaces between themselves, while it grows out in *Agarum cribrosum* when the paraphyses are still very young. The mature sporangia of *Alaria praelonga* are usually longer and slenderer than those of *Agarum cribrosum* (cf. Pls. I & II). The resting nucleus in a young sporangium contains usually a single nucleolus, rarely two. The major stages of the meiotic division could be traced in the first nuclear division of the sporangium as shown in Pl. II, Figs. 33–40. Twenty-two chromosomes were clearly counted in metaphase of the first nuclear division (Pl. II, Fig. 38). The centrosomes are often visible at the pole of the spindle in the side view of metaphase (Pl. II, Figs. 39–41). After the fifth nuclear division is completed, 32 zoospores are formed in each sporangium.

Summary

In *Agarum cribrosum* and *Alaria praelonga* it has been established that 32 zoospores were formed in each sporangium after meiosis and four successive mitoses and that the haploid chromosome number was 22.

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Explanation of Plates

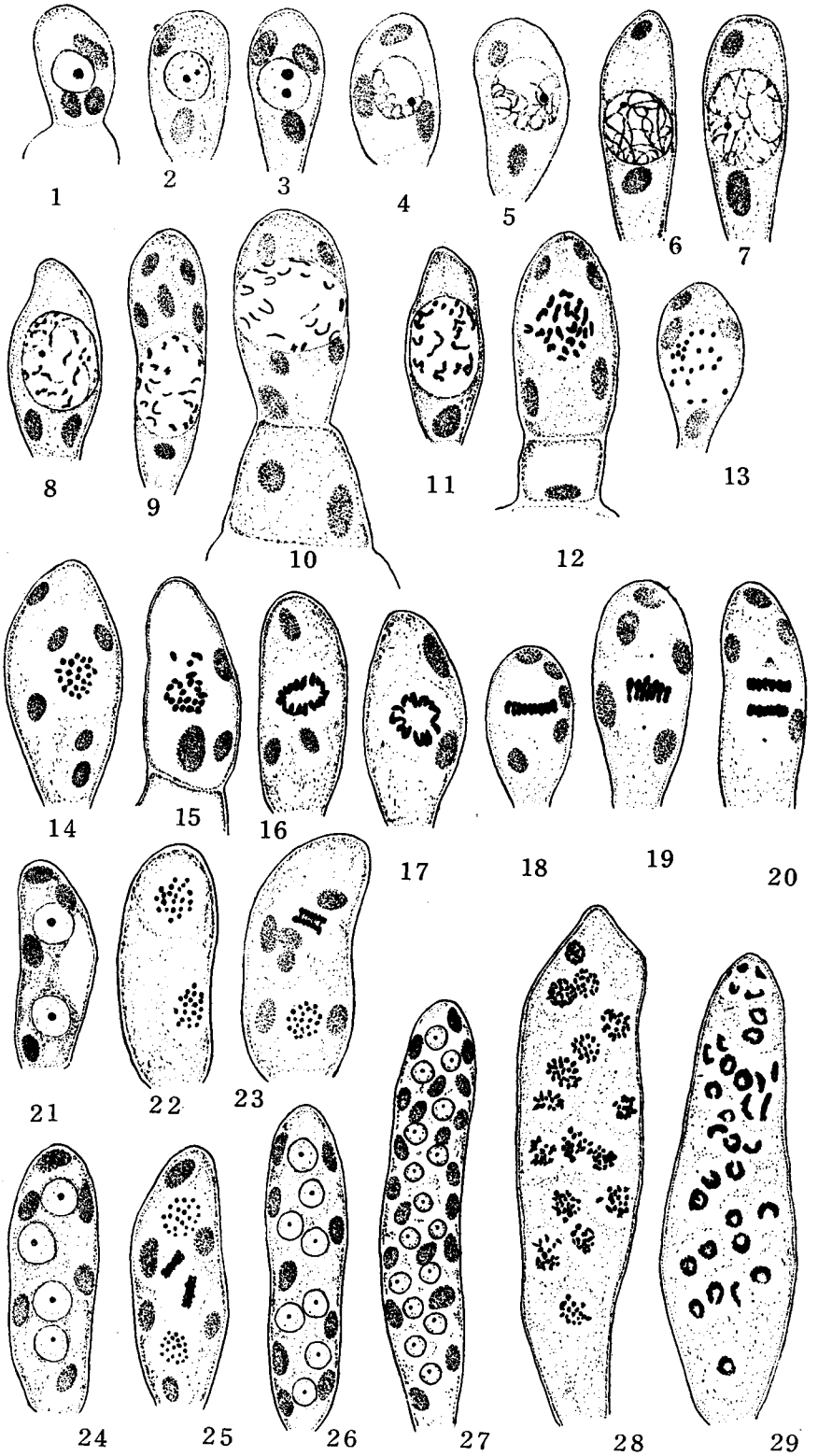
PLATE I

All the figures in Pls. I & II and the photomicrographs in Pls. III & IV are based on the preparations from the materials fixed with a mixture of Tahara's fluid and formalin.

(Figs. 1-44, X 1840; Figs. 45-66, X 1240)

Agarum cribrosum Bory

Figs. 1-3.	Resting stage.
Figs. 4-5.	Synapsis.
Figs. 6-7.	Spireme.
Figs. 8-12.	Diakinesis.
Figs. 13-19.	Metaphase.
Fig. 20.	Anaphase.
Fig. 21.	Two-nucleus stage.
Figs. 22-23.	Metaphase of the second division.
Fig. 24.	Four-nucleus stage.
Fig. 25.	Metaphase of the third division.
Fig. 26.	Eight-nucleus stage.
Fig. 27.	Sixteen-necleus stage.
Fig. 28.	Metaphase of the fifth division.
Fig. 29.	Late anaphase of the fifth division.



H. Yabu: Mitosis in the sporangium of *Agarum* and *Alaria*

PLATE II

Agarum cribrosum Bory

Figs. 30–31. Thirty-two nucleus stage following the end of the fifth division.

Fig. 32. Nearly matured zoosporangium.

Alaria praelonga Kjellm.

Fig. 33. Resting stage.

Fig. 34. Synapais.

Figs. 35–36. Spireme.

Fig. 37. Diakinesis.

Figs. 38–40. Metaphase.

Fig. 41. Metaphase of the second division.

Fig. 42. Metaphase of the fourth division.

Fig. 43. Metaphase of the fifth division.

Fig. 44. Thirty-two nucleus stage following the end of the fifth division.

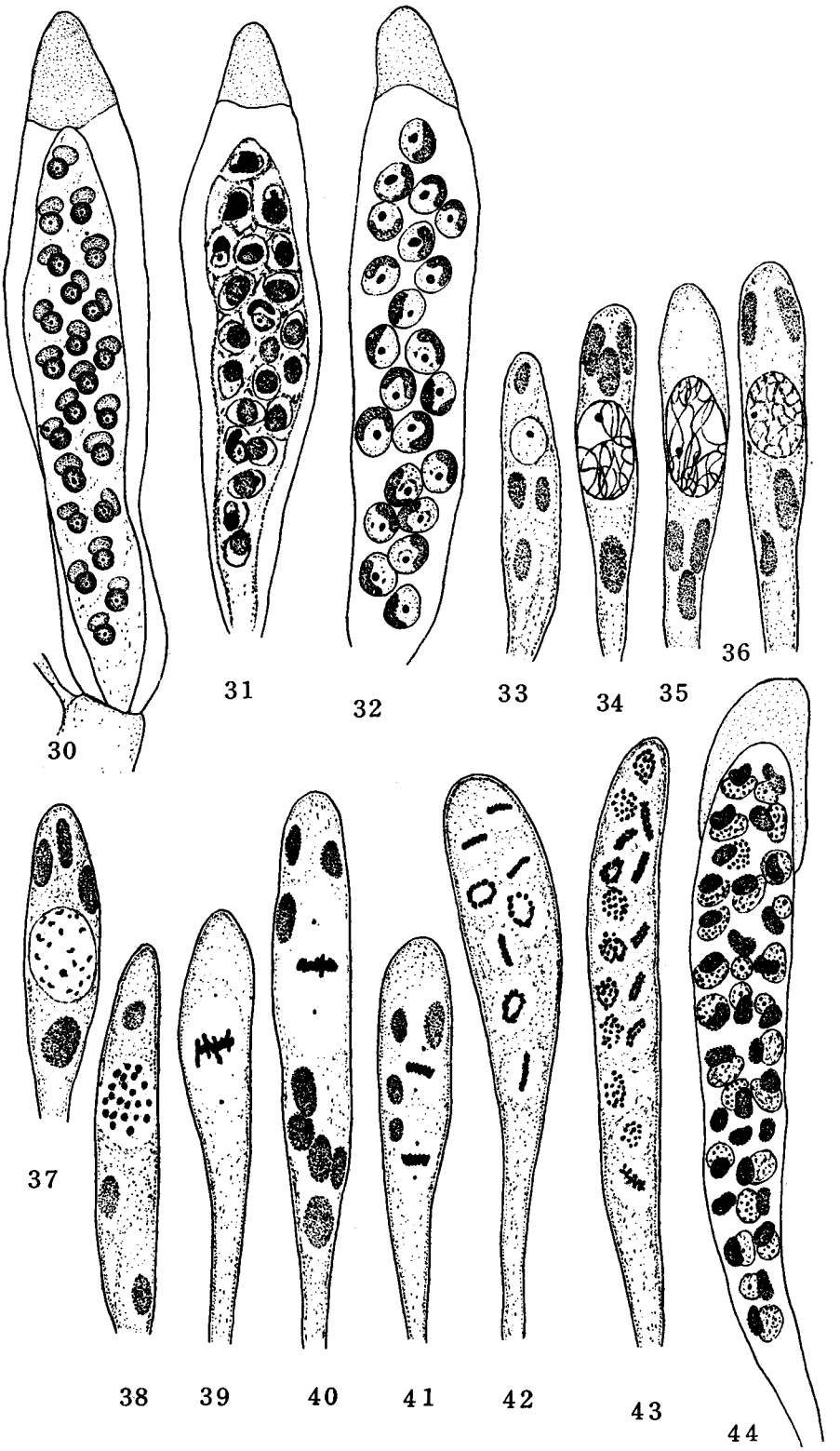


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PLATE III

Agarum cribrosum Bory

Fig. 45.	Synapsis.
Fig. 46.	Spireme.
Fig. 47.	Diakinesis.
Fig. 48.	Late diakinesis.
Figs. 49–52.	Metaphase.
Fig. 53.	Metaphase (to the left side) and diakinesis (to the right side).
Fig. 54.	Anaphase.
Figs. 55–56.	Metaphase of the second division.
Fig. 57.	Anaphase of the second division; one of the nuclei is seen in the figure.
Figs. 58–59.	Metaphase of the fifth division.
Fig. 60.	Anaphase of the fifth division.

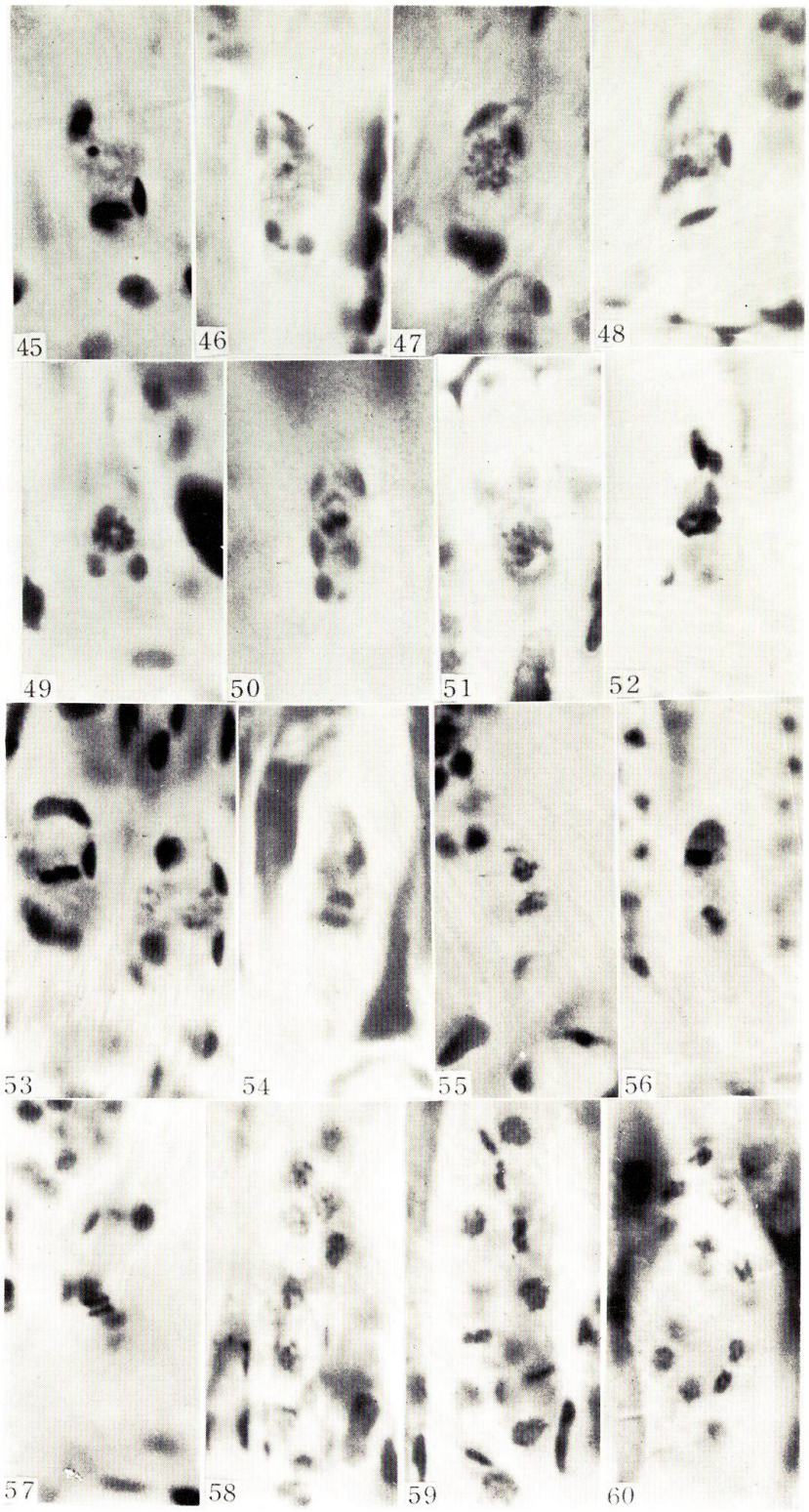

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PLATE IV

Alaria praelonga Kjellm.

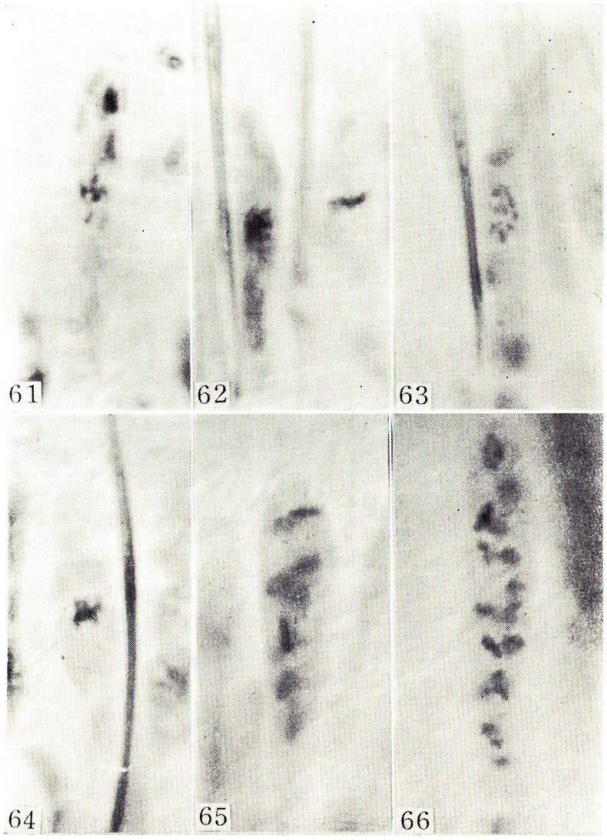
Fig. 61. Diakinesis.

Fig. 62. Diakinesis (to the left side) and metaphase (to the right side).

Figs. 63–64. Metaphase.

Fig. 65. Metaphase of the second division.

Fig. 66. Metaphase of the fifth division.



H. Yabu: Mitosis in the sporangium of *Agarum* and *Alaria*