EARLY DEVELOPMENT OF SEVERAL SPECIES OF LAMINARIALES IN HOKKAIDO

By

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CONTENTS

I. Introduction

It was in 1915 that discovery of the microscopic gametophytes in the order Laminariales was first reported by Sauvageau in his study on the development of *Saccorhiza bulbosa*. Since that time, numerous culture studies of Laminariales have been published by many investigators.

Culture studies of the Japanese species of Laminariales were attempted in the early days by Yendo (1919), Ikari (1921, 1927), and Ueda (1929), and in more recent years by Kanda (1936-1946), Kinoshita (1947), Saito (1956, 1958, 1960), Kurogi and Akiyama (1957), Segi and Kida (1957, 1958), and Hasegawa (1962).

With the object of obtaining the fundamental knowledge essential for efficient measure to induce propagation of the useful species of Laminariales, the present writer has been engaged for several years in the study of the development of the gametophytes and young sporophytes of these plants in Hokkaido. In the present paper are reported the results of the writer's investigations on the interrelation between some culture factors and the development of gametophytes and young sporophytes of *Laminaria japonica* and *L. religiosa*, and also on parthenogenesis and crossing of several species of Laminariales. Some observations on the gametophytes of *Laminaria religiosa* are also dealt with.

The present study was carried out under the guidance of the writer's teacher, Professor Jun Tokida. The writer wishes to express here his heartiest thanks to Dr. Tokida for his guidance and advice, and his kindness in reading and correcting the manuscript of the present thesis. To Dr. Yoshiteru Nakamura of the Institute of Algological Research, Faculty of Science, Hokkaido University, and to Mr. Tomitaro Masaki, of the Faculty of Fisheries, Hokkaido University, the writer is much indebted for their kind help in affording him many facilities. The writer's thanks are also due to Mr. Tamezo Yamazaki of the Oshoro Marine Biological Laboratory and to Mr. Mamoru Ueda of the Akkeshi Marine Biological Laboratory, for supplying the writer with some materials used in the present study.

II. Development of gametophytes and young sporophytes in *Laminaria japonica* and *L. religiosa* under various conditions of culture

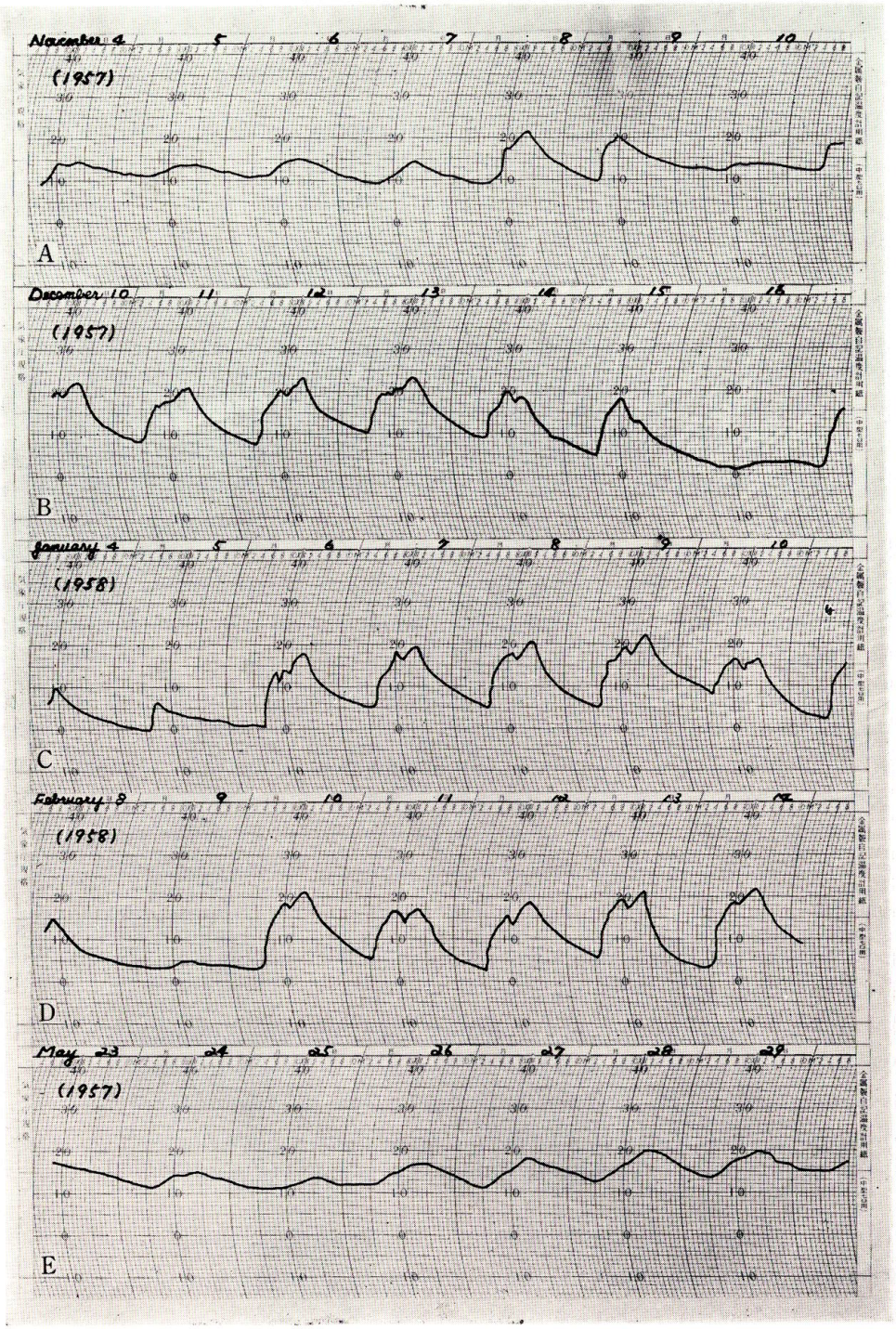
A. Interrelation between the growth of gametophytes and their population density per unit area

That the early development of Laminariaceous plants in a vessel is affected by the capacity of the vessel has been noted already by Drew (1910). He observed that the development of germlings of the zoospores, or zoosporelings, of *Laminaria digitata* and *L. saccharina* was much influenced by the culture vessel's capacity, and that culture experiments conducted in glass jars containing about 600 ml of medium were quite successful while those in Petri dishes were not. However, other later investigators have proved that the culture of Laminariaceous species in smaller quantities of culture solution gives good results: for example, *Laminaria digitata* was cultured in vessels containing 30–40 ml of medium by Kylin (1916), *Alaria esculenta* and *A. pylaii* in vessels containing 30–250 ml of sea-water by Prints (1922), *L. digitata*, *L. saccharina* and *L. cloustoni* in vessels containing 250 ml of sea-water by Harries (1932), and various Japanese species of Laminariaceae in vessels containing about 250 ml or even about 100 ml of medium by Kanda (1936–1946).

However, none of the previous workers has ever investigated the interrelation between the growth of gametophytes and their population densities per unit area. So the writer carried out culture experiments with *Laminaria japonica* from October 2, 1955 to September 1956, in the following two ways; (1) with various population densities per unit area in a fixed volume of culture solution, and (2) with a fixed population density per unit area in various volumes of culture solution.

1. Growth of gametophytes with various population densities per unit area in a fixed volume of culture solution

The material used for this experiment was *Laminaria japonica* collected at Nanaehama on September 30, 1955. A few drops of zoospore-solution, or filtered sea-water loaded with liberated swarming zoospores, were pipetted into Petri dishes containing 50 ml of Schreiber's culture solution (Schreiber, 1930). These dishes were placed on tables in the laboratory and in the corridor. For an outdoor culture, glass beakers, 9 cm in height and 5 cm in diameter, containing 100 ml of Schreiber's solution were used. In each of these beakers was placed a slide-glass which had been immersed previously in zoospore-solution and made covered with attached embryospores all over its surface. Culture vessels were


November 4 5 6 7 8 9 10
(1957)
A
December 10 11 12 13 14 15 16
(1957)
B
January 4 5 6 7 8 9 10
(1958)
C
February 8 9 10 11 12 13 14
(1958)
D
May 23 24 25 26 27 28 29
(1957)
E

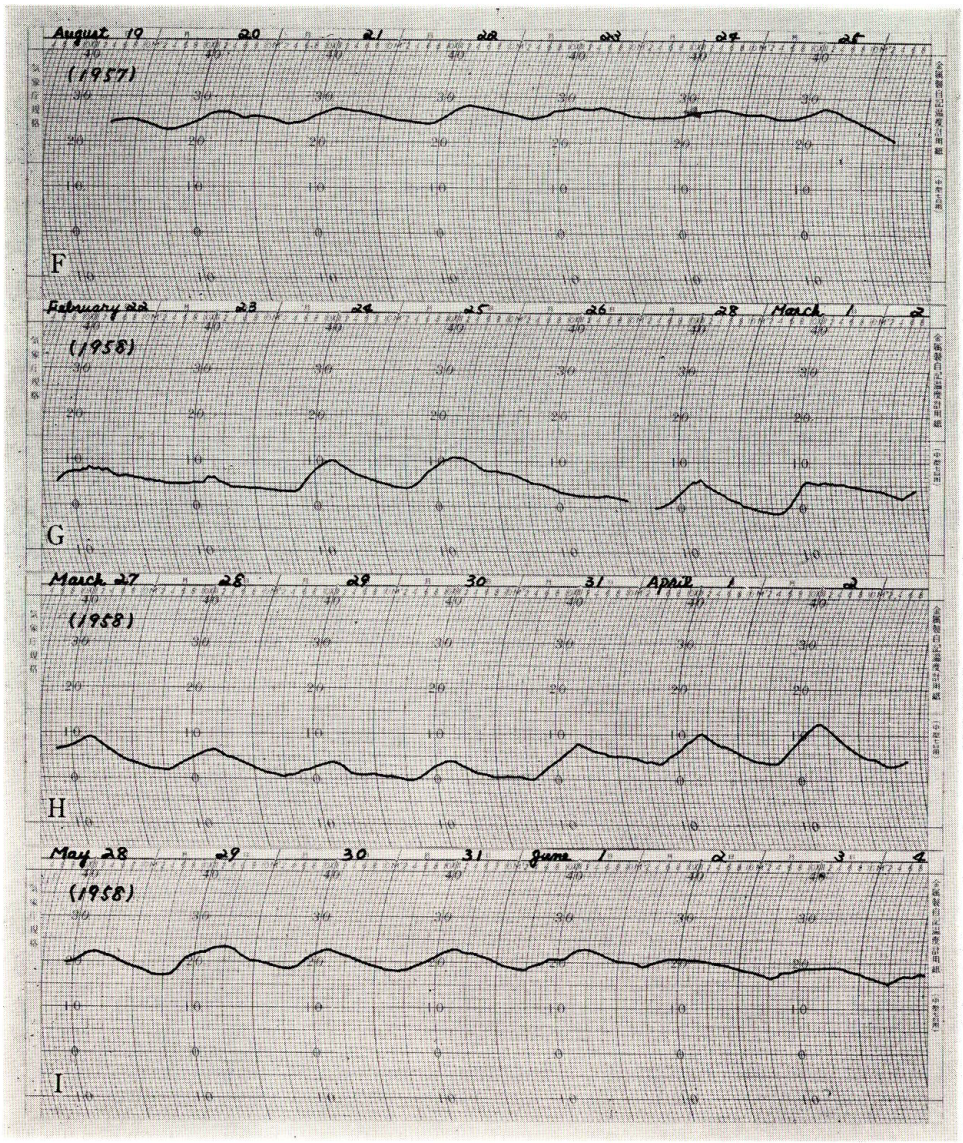


Fig. 1. Thermograph records of air temperature in the laboratory (A–D) and in the corridor (E–I)

placed on tables in three different places, viz., in the laboratory room, in the corridor in front of the laboratory room and in the open air. Text-figure 1 (A–I) shows the thermograph records of the air temperature taken in the laboratory and in the corridor. In Fig. 1 A, the air temperature curve rises suddenly on November 8, 1957, from about 10°C to about 20°C. This shows that the steam-

Table I. Average maximum, mean and minimum air temperature in Hakodate (outdoors), in the laboratory, and in the corridor

	*Average air temp. in Hakodate (1941–1955)				Average air temp. in the laboratory (1958)				Average air temp. in the corridor (1960)			
	Date	Max.	Mean	Min.	Date	Max.	Mean	Min.	Date	Max.	Mean	Min.
Jan.	1—10	−4	−4.6	−8.5	4—10	22.5	9.8	−1.0				
	11—20	−0.5	−3.8	−7.8	11—12	20.5	9.5	−1.5				
	21—31	−0.7	−4.1	−8.0	21—30	23.0	9.6	1.0	20—31	6.0	−1.0	−5.0
Feb.	1—10	−0.5	−3.9	−7.6	1—10	22.0	8.5	0.0	(1958)			
	11—20	−1.0	−4.7	−8.6	11—14	21.5	10.5	2.5				
	21—29	−0.9	−2.9	−7.0					23—27	10.5	5.2	2.5
Mar.	1—10	1.8	−2.0	−5.6					1—10	9.5	3.5	−1.8
	11—20	3.6	−0.2	−4.0					11—20	13.0	6.0	1.0
	21—31	5.4	1.4	−2.1					24—30	11.4	6.0	2.9
Apr.	1—10	9.2	4.7	0.8					1—10	14.0	8.0	3.0
	11—20	10.7	6.0	1.8					11—20	15.5	8.0	2.8
	21—30	12.3	7.3	2.8					21—23	15.3	12.0	8.5
					(1960)							
May	1—10	14.8	9.7	4.8					1—10	15.5	12.0	7.1
	11—20	15.1	10.4	5.9	8—20	21.5	17.0	10.1	11—20	19.0	12.0	6.0
	21—31	16.3	11.8	7.5	21—30	23.0	18.0	15.0	21—23	20.0	14.0	11.0
Jun.	1—10	17.0	13.1	9.5	1—10	21.0	16.0	12.0	1—10	21.1	17.0	11.8
	11—20	18.9	14.9	11.4	11—20	25.0	19.0	13.5	11—20	21.0	16.0	13.0
	21—30	20.4	16.4	12.8	21—30	24.5	19.5	17.0				
									(1958)			
Jul.	1—10	21.5	17.9	14.9	4—10	24.0	22.0	17.5	1—10	24.5	21.0	15.0
	11—20	23.0	19.4	16.2					11—20	25.5	22.0	16.5
	21—31	25.0	21.2	18.2					21—29	26.5	23.0	17.5
Aug.	1—10	25.8	21.9	19.2					1—10	26.0	23.5	20.0
	11—20	26.3	22.3	18.9					11—20	26.0	22.4	20.0
	21—31	25.3	21.3	17.9					21—30	27.0	24.1	21.2
Sept.	1—10	23.5	18.9	15.1					2—10	22.5	20.1	17.0
	11—20	22.1	17.6	13.8					11—20	22.5	18.5	15.8
	21—30	20.1	15.1	10.8					21—30	19.1	17.0	13.0
Oct.	1—10	18.4	12.6	8.6					1—10	18.0	16.0	13.0
	11—20	16.6	11.5	7.2					11—17	19.5	17.0	14.0
	21—31	14.0	8.9	4.5					19—30	18.0	15.0	11.0
					(1957)							
Nov.	1—10	11.2	6.7	2.5	4—10	21.0	12.4	8.5				
	11—20	8.0	3.8	−0.1	11—20	25.0	16.5	7.5				
	21—30	6.8	2.6	−1.4	21—29	24.0	13.8	5.0				
Dec.	1—10	3.5	0.1	−3.3	3—10	25.0	14.5	7.0				
	11—20	2.1	−1.2	−5.0	11—20	23.0	11.4	2.0				
	21—31	0.1	−3.2	−6.7	21—31	23.0	11.2	−0.1				

* According to the data given in the "Calendar and Tide Table of Hakodate for the year 1960" issued by the Hakodate Marine Meteorological Observatory

heating of the room began on that day. The room was heated in the daytime through the winter every day except Sundays and holidays from the beginning of November 1957 to the beginning of April 1958, and the room temperature in the daytime on week-days was kept on an average, at a range between 9°C and 14°C, the maximum between 20°C–25°C. The general aspect of the outdoor air temperature in Hokodate was known from the data given in the "Calendar and Tide Table of Hakodate for the year 1960" issued by the Hakodate Marine Meteorological Observatory (cf. Table I).

The population density of the gametophytes to be grown in these vessels was controlled to some extent by changing the density of the zoospore-solution used and the volume of the solution to be pipetted into the dishes. Actual population densities of the vessels (A–K) used for the present experiments were known by counting the number of zoosporelings, or gametophytes, growing in a unit area of 2 mm^2 on the bottom of the dishes and on the slide-glasses under a lower magnifications of a microscope. The culture in the laboratory and the corridor were started on October 2, 1955, and continued till early September in 1956. Outdoor cultures were started on December 3, 1955, and when the culture-solution froze the culture-vessels were placed inside the laboratory for a while to thaw the ice. However, the gametophytes did not show any sign of further growth or maturity under such low temperature conditions, so the outdoor cultures in winter were discontinued on February 2, 1956, by moving the vessels from outdoors to the corridor. Outdoor cultures were newly started again on February 27, 1956, and were continued till early June of that year. The results of this culture experiment are shown in Tables II–V. The growth rates of the gametophytes were compared with each other by counting the number of cells consituting a medium-sized female, or large-celled, gametophyte.

Table II. Number of cells constituting a medium-sized female gametophyte of *Laminaria japonica* in culture dishes (A–E) with various population densities, placed in the corridor for the period October 2, 1955 to April 21, 1956

Date of counting \ Population density	A 4	B 25	C 110	D 800	E 1200
Nov. 8, 1955	19	9	6	2	1
Dec. 4, 〃	28	15	11	2	1
Jan. 11, 1956	40	18	17	3	1
Feb. 2, 〃	71	25	17	3	2
Mar. 7, 〃	94	36	21	5	2
Apr. 21, 〃	130	36	21	5	2

Table III. Number of cells constituting a medium-sized female gametophyte of *Laminaria japonica* in culture dishes (A–E) with various population densities, placed in the laboratory for the period October 2, 1955 to April 21, 1956

Date of counting \ Population density	A 4	B 25	C 110	D 800	E 1200
Nov. 8, 1955	72	34	16	2	1
Dec. 4, //	340	53	22	2	1
Jan. 11, 1956	960	80	36	5	2
Feb. 2, //			48	5	2
Mar. 7, //			52	5	2
Apr. 21, //			71	8	2

Table IV. Number of cells constituting a medium-sized female gametophyte of *Laminaria japonica* in culture beakers (F–H) with various population densities, placed outdoors for the period December 26, 1955 to February 2, 1956

Date of counting \ Population density	F 5	G 250	H 740
Jan. 28, 1956	1	1	1
Feb. 2, //	2	1	1

Table V. Number of cells constituting a medium-sized female gametophyte of *Laminaria japonica* in the culture beakers (I–K) with various population densities, placed outdoors for the period February 27, to June 1, 1956

Date of counting \ Population density	I 4	J 380	K 830
Mar. 7, 1956	1	1	1
Apr. 21, //	2	2	1
May 30, //	6	2	1
Jun. 1, //	29	13	4

It was learnt from those results that the gametophytes grow better in the vessels with smaller population densities, and the best growth was observed in the dish (A) with the smallest population density which had been placed in the laboratory.

2. Growth of gametophytes with a fixed population density per unit area in various volumes of culture solution

In this culture experiment, Petri dishes and beakers were employed in the same way as in the preceding experiment, but the volume of the culture solution

in each vessel was made different, viz., 20 ml, 40 ml and 60 ml in Petri dishes, and 100 ml, 200 ml and 300 ml in beakers. Two sets of these vessels were prepared, of which one was loaded with gametophytes of lower density and the other with those of higher density. The cultures were started on October 13, 1955, and were continued until May 7, 1956. Twenty days after the start of the cultures, the gametophytes grown in the former set of vessels were counted to be 4 in 2 mm^2 and those in the latter set of vessels to be 470 in 2 mm^2. The culture solution was changed once in two months. The results of this culture experiment

Table VI. Number of cells constituting a medium-sized female gametophyte of *Laminaria japonica* in culture dishes (A–C) and beakers (D–F), with a population density of four gametophytes in 2 mm^2 and containing various quantities of culture solution

Quantity of culture solution in ml. / Date of counting	A 20	B 40	C 60	D 100	E 200	F 300
Oct. 29, 1955	4	5	4	5	5	5
Dec. 24, 〃	19	23	21	25	27	24
Jan. 28, 1956	48	51	46	53	59	58
May 7, 〃	131	142	145	152	149	161

Table VII. Number of cells constituting a medium-sized female gametophyte of *Laminaria japonica* in culture dishes (A–C) and beakers (D–F), with a population density of four hundred and seventy gametophytes in 2 mm^2 and containing various quantities of culture solution

Quantity of culture solution in ml. / Date of counting	A 20	B 40	C 60	D 100	E 200	F 300
Oct. 29, 1955	2	2	2	2	2	2
Dec. 24, 〃	3	3	3	3	4	4
Jan. 28, 1956	5	5	5	5	6	7
May 7, 〃	5	8	9	9	11	11

are shown in Tables VI & VII, from which it is learnt that the growth of gametophytes is affected by population density, but not by volume of culture solution.

In the writer's culture experiments relating to parthenogenesis, which will be treated in the next chapter, the growth was far better in the gametophytes that had been detached from the substratum and were floating in the culture solution than in those attached crowdedly to the substratum. Population density seems to have been a controlling factor of growth in those experiments too.

B. Effect of temperature

In a culture experiment with *Laminaria religiosa* Miyabe, from Hokkaido, Ueda (1929) showed that young sporophytes were produced in vessels kept between 5.5°C and 11.3°C. Schreiber (1930) reported that the gametophytes of *Laminaria* became fertile when they were placed in a lower temperature than 10°C, and that he could induce the production of young sporophytes even in summer by lowering the water temperature. Kinoshita (1947) studied the optimum temperature for the development of gametophytes and sporophytes in five species of *Laminaria*, viz., *Laminaria japonica* Aresch., *L. ochotensis* Miyabe, *L. religiosa* Miyabe, *L. fragilis* Miyabe and *L. chichorioides* Miyabe; and Saito (1956) reported recently on the influence of water temperature upon the development of *Undaria pinnatifida* Sur. In the present study, *Laminaria religiosa* Miyabe collected in Oshoro Bay was employed as material for the following two experiments to investigate the effect of temperature upon the growth of gametophytes in two ways.

1. Development of gametophytes under various constant temperatures

Zoospores were liberated in a dish containing filtered sea-water and slide glasses. After two days, each of the slide glasses was put into a test tube containing Schreiber's solution. These test tubes were placed in five wooden tanks which were filled up with tap water and placed in the corridor. The water temperature of four of these tanks was regulated with Chino's thermostat, and was maintained constantly at about 8°C, 12°C, 16°C and 20°C, respectively. In the remaining tank the water temperature was regulated at 20°C in the daytime, from about 9 a.m. to 6 p.m., but the water was exposed to the air temperature in the corridor (cf. Table I) for the rest of the day. This experiment was continued for two months. The results of these culture experiments are shown in Text-figure 2.

It was observed that gametophytes grew best in the tank regulated at 20°C in the daytime only. Female gametophytes cultured for 30 days in this tank were found to consist of nearly one hundred cells. Cultures in other tanks which were regulated to maintain a constant water temperature gave the following results: the growth of gametophytes was good at 16°C, while it was not good at 12°C and 8°C, and was worst at 20°C. Female gametophytes cultured for 30 days at a constant 20°C were mostly found to be still single-celled. When cultured at a constant 20°C for about 60 days, both female and male gametophytes, 1-2-celled and 3-4-celled respectively, became pale in color, and at the same time their cells were often swollen more or less markedly. Maturation of both female and male gametophytes was observed in the cultures kept constantly at 8°C or 12°C. The male attained maturity a few days earlier than the female.

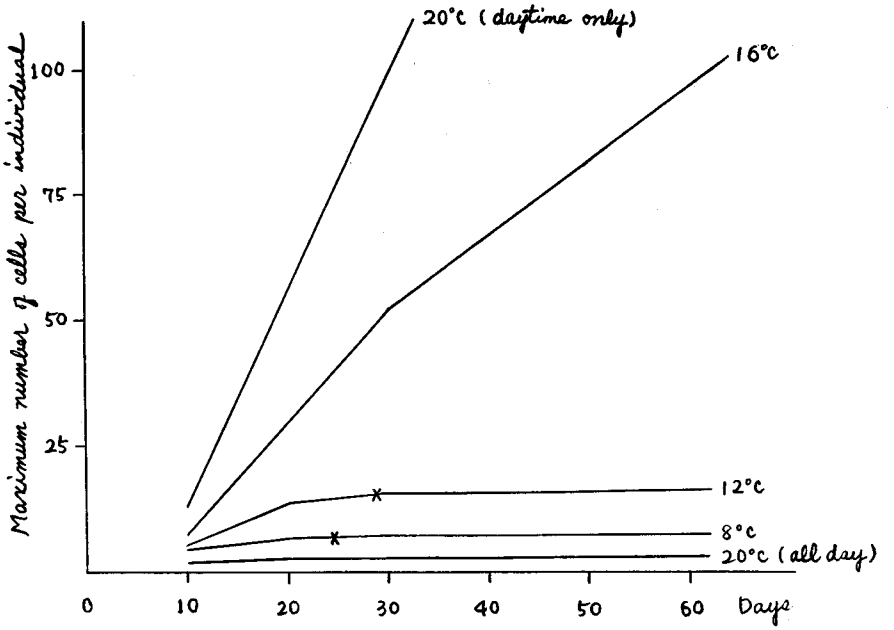


Fig. 2. Effects of temperature on the growth rate of the female gametophytes of *Laminaria religiosa* in term of the number of body cells. "20°C (daytime only)" means the culture in which water temperature was maintained constantly at 20°C in the daytime only. X denotes the time of the first appearence of reproductive organs

2. Development of gametophytes cultured in cold and warm places alternately

Zoospores were liberated in a dish containing filtered sea-water and slide glasses. About two weeks later, when the sex of a gametophyte was already easily known by the size of its cells, each of the slide glasses was transferred into one of six dishes, three containing Erd-Schreiber and the other three Schreiber's solution. The six dishes were divided into three pairs (I, II & III in Text-fig. 3), and each pair was placed alternately in the warm, heated laboratory and in the cold, unheated corridor with an interval of 5 days, 10 days and 15 days, respectively.

In the first pair of dishes, with the interval of 5 days (I in Text-fig. 3), oogonia began to appear when the culture was 42–45 days old, but no egg was produced within the 75 day period of the present culture experiment. Then the dishes were exposed to room temperature in the laboratory, and in the meantime all of the oogonia reassumed the features of vegetative cells. In the second pair of dishes, with the interval of 10 days (II in Text-fig. 3), oogonium and antheridium formation was first observed on December 25, 1953, when the pro-

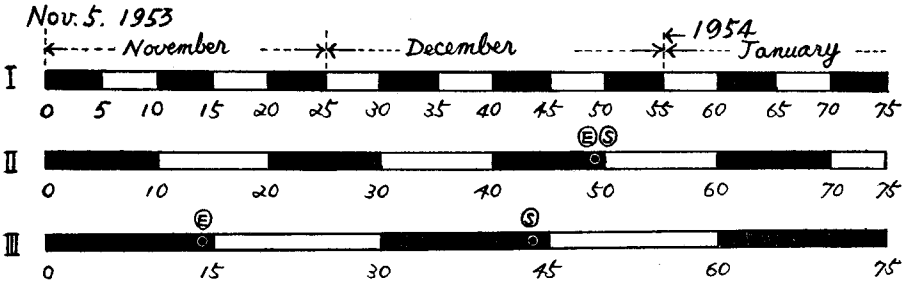


Fig. 3. Diagram showing the data and intervals in the culture experiment of *Laminaria religiosa* which was started on November 5, 1953, and performed to know the effect of alternate change of temperature upon the growth of gametophytes

■···a period in which the dishes were placed in the corridor, or in a cold place
□···a period in which the dishes were placed in the laboratory, or in a warm place
Ⓢ····the time when oogonium formation was observed in Schreiber's solution
Ⓔ····the time when oogonium formation was observed in Erd-Schreiber

portion of mature females to immature females was about 1 : 9 in the dish containing Erd-Schreiber, and about 1 : 15 in the dish containing Schreiber's solution. In the third pair of dishes, with the interval of 15 days (III in Text-fig. 3), oogonium formation was first observed in Erd-Schreiber on the 15th day of culture, but in Schreiber's solution on the 42nd day of culture. As far as the number of cells constituting the thallus of a gametophyte is concerned, there was no marked difference between the two culture solutions. Female gametophytes consisted of 20–30 cells when this culture came to an end. The culture in Erd-Schreiber, as compared with that in Schreiber's solution, was of course better in keeping darker color of chromatophores, and in inducing faster maturity of female gametophytes and more abundant production of sporophytes.

C. Effect of light intensity

The effect of light intensity upon the development of gametophytes and sporophytes of Laminariaceous plants has been studied by a number of investigators, such as Harries (1932), Segi & Kida (1957, 1958), and Saito (1956). To investigate this problem the writer carried out experiments with the gametophytes of *Laminaria religiosa* Miyabe, from Oshoro Bay, in the following two ways: (1) under various light intensities, and (2) in a long glass tube covered with black paper except at the mouth.

1. Development of gametophytes under various light intensities

Zoospores were liberated on September 9, 1954. The culture was carried out until May, 1955, in Petri dishes containing Schreiber's solution. The dishes

were placed at five spots in the laboratory and five in the corridor, (A–E and F–J in Table VIII) exposed to different light intensity in the daytime. The darkest spots (E and J in Table VIII) were arranged by screening with black paper, and the brightest spots (A and F in Table VIII) by selecting a place near windows, but not exposed to direct sun-shine. For the air temperature in the laboratory and the corridor, Table I is to be referred to. Culture solution was changed once a month. The results of the culture experiment are shown in Table VIII.

Table VIII. Number of cells constituting medium-sized female gametophytes of *Laminaria religiosa* cultured at ten spots (A–J) under various light intensities. The light intensity in lux at each spot here given is that which was measured at 2 p.m., May 13, 1955

Results from the culture in the laboratory					
Lux / Date	A 4100	B 2500	C 1700	D 400	E 50
Jan. 18, 1955	2	20	18	6	1
May 2, 〃	3	90	95	26	2
Results from the culture in the corridor					
Lux / Date	F 4400	G 2200	H 1900	I 400	J 50
Jan. 18, 1955	2	4	3	2	2
May 2, 〃	2	15	12	4	2

As seen from these results, the female gametophytes grew well under medium light intensities, 400–2500 Lux, but not well under higher intensities, 4400–4100 Lux, or a lower one, 50 Lux. Sporophytes were formed only in the dishes placed in the corridor. On May 2, 1955, it was observed that the ratio of mature female gametophytes bearing sporophytes to immature female gametophytes was about 4 : 1 at F (4400 Lux) and G (2200 Lux), 5 : 1 at H (1900 Lux), 1 : 1 at I (500 Lux), and 1 : 16 at J (50 Lux).

In the culture dishes placed at I & J (in Table VIII), that is, in lower light intensity and lower temperature, the germination tube attached to a gametophyte had not disappeared about two months after the start of the culture, while the germination tube was lost when the culture was about 25 days old in culture dishes placed at A (in Table VIII), that is, in higher light intensity and higher temperature. The gametophytes in culture dishes placed at C, D, H & I (lower light intensities) were composed of longer and more slender cells than those at A, B, F and G (higher light intensities). In culture dishes placed at D, E, I & J (lower light intensities), the chromatophores of the gametophytes were dark in color and

the sexual organs were easily distinguishable from vegetative cells, while in culture dishes placed at A and F (higher light intensities), the chromatophores were light in color and the sexual organs were apt to be confused with vegetative cells.

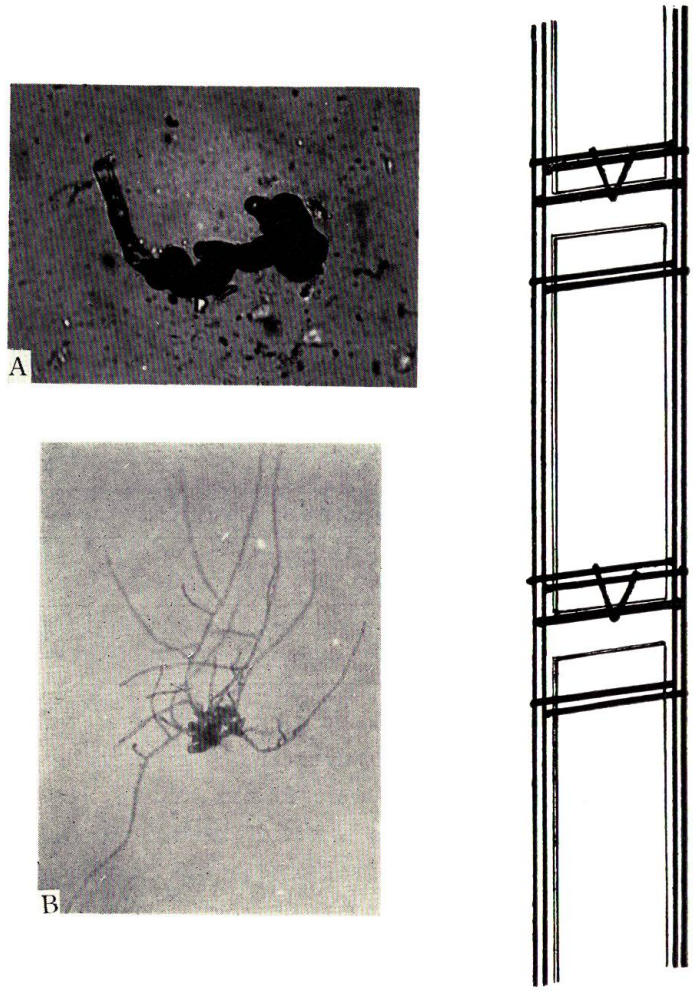


Fig. 4.

A. A female gametophyte, 40 days old on the slide settled 10 days before at 80 cm below the water surface in the tube covered with black paper all over except at the top, showing three slender and faintly colored branches newly developed in those 10 days ×256

B. A female gametophyte, observed on April 2, on the same slide as above at 80 cm ×64

C. Part of the slide holder with three slide glasses to show how they are settled in the holder

2. Development of gametophytes in a long glass tube covered with black paper except at the top

In this experiment, a glass tube ca. 150 cm long by 5 cm diam. was used. The tube was covered with black paper all over except at the mouth, so as to let the light come in from above.

On Otober 7, 1953, zoospores were liberated in a vat containing sea-water and slides. After culturing for about one month, when the female gametophytes on the slides consisted of about 8–10 cells, fifteen of these slides were placed in a longitudinal row on a slide holder made of brass wire as shown in Text-fig. 4 C. Then the holder was inserted into the above-mentioned tube containing Schreiber's solution. On December 15, 1953, when the culture was about 10 weeks old, the gametophytes were observed to show no sign of maturation. The growth rate of gametophytes showed some difference according to the depth in the tube at which a slide was placed; the female gametophytes on the slides near the top of the tube consisted of 13–14 cells, while those on slides 50–80 cm below the surface were of about 40–50 cells. On February 2, a few of the female gametophytes were observed to have become mature, and the sporophytes produced were still in their 5 or less celled stages. When observed on April 2, the number of mature female gametophytes had increased, of course, but many of the gametophytes were still immature. Text-fig. 4 B is a photomicrograph, taken on April 2, of an immature female gametophyte on a slide 80 cm below the surface. It shows a number of long, slender, divided, faintly colored branches which developed upward from the dark-colored initial thallus after the slide was placed in the tube. The ratio of mature female gametophytes to immature ones on April 2 was about 1 : 10 at the top of the tube, and from 1 : 15 to 1 : 20 near the bottom. It was also noticed that the sporophytes found on the slides near the top of the tube were fan-shaped broadening upwards, while those found near the bottom were rather slender.

D. Effect of salinity

As to the effect of salinity of the culture medium upon the growth of Laminariaceous plants, Schreiber (1930) showed that male gametophytes were stronger than female ones against the increase of osmotic pressure, so that in a long-term culture, which had become dense in salinity by evaporation, the females were extinguished leaving only the males surviving. Recently Saito (1956) reported the results of his culture experiments with *Undaria pinnatifida* in several kinds of salinity. Here the result is given of the writer's experiments with the gametophytes of *Laminaria religiosa* Miyabe, from Oshoro Bay, cultured during the

period from October 1955 to March 1956 in media of various kinds of salinity. Sea-water samples of high salinity used for the experiment were obtained by evaporating either one-half or one-third of the original volume of natural sea-water (32.087% salinity) obtained from Hakodate Harbor on October 21, 1955, and those of lower salinity by diluting the natural sea-water with filtered river-water, added in the following amounts; 5%, 10%, 20%, 30%, 40% and 50%. River-water was used instead of re-distilled water because the latter caused the death of gametophytes in preliminary cultures. To these diluted sea-water samples were added nutrient salts according to the formula of Schreiber's solution. Zoospores were liberated on October 23, 1955. On November 20, a slide with attached gametophytes was put into each Petri dish, which contained exactly 30 ml of one of the sea-water samples prepared as above. Most of the female gametophytes at that time were 1-2-celled and no reproductive organs could be observed. The culture dishes were placed in the corridor. The sea-water in the culture dishes was changed once a month. The results of this experiment are as follows. In the sea-water sample whose salinity was twice that of natural sea-water the gametophytes were alive throughout the period of the experiment and oogonia were found to have been formed on some of the female gametophytes and to have given rise to sporophytes. However, in the sample with salinity thrice that of natural sea-water about half the gametophytes were dead within one month. The surviving ones were alive throughout the period of the experiment, but neither growth nor formation of reproductive organs was observed in them. In the samples diluted with 5%, 10% and 20% river-water, the growth of gametophytes and the development of sporophytes was good as in natural sea-water. In the sample containing as much as 30% river-water, the gametophytes were found to be alive, but no sporophyte developed; in the sample containing 40% river-water, the gametophytes were mostly alive throughout the period of the experiment without showing any sign of growth, though some of them were found dead within 15 days; and in the sample containing 50% river-water, the gametophytes were dead within 10 days, but they were occasionally found alive after as long as 20 days.

E. Effect of culture solution

So far as the writer is aware, Drew (1910) was the first to discuss the relation between the development of gametophytes and sporophytes of Laminariaceous algae and the nutrient substances in the culture solution. He stated that the growth of gametophytes and sporophytes was promoted by adding $NaNO_3$, KNO_3, NH_4NO_3 and Na_2HPO_4 to sea-water. Kylin (1916) obtained good results in his

culture of *Laminaria digitata* by adding traces of calcium phosphate and sodium nitrate to filtered sea-water. At the same time he tried a culture in filtered sea-water containing 0.2 percent sodium nitrate and found that the addition of nitrate favoured the development of sporophytes and gave a dark color to the chromatophores. Schreiber (1930) was successful in culturing three species of Laminariaceae with a medium which he himself had recommended in 1927 for the culture of phytoplankton. This medium, known as Schreiber's solution, is composed of 0.1 gr of $NaNO_3$, 0.02 gr of Na_2HPO_4, and 50 ml of distilled water in one litre of sea-water. In 1932, Schreiber reported that he had gained better results by adding 2 ml of soil extract to the above-mentioned medium. This modified medium is known as Erd-Schreiber. Hollenberg (1933) reported that the gametophytes of *Eisenia arborea*, after they had once grown to an elongated thallus, could not be induced to produce reproductive organs by changing the temperature, light intensity, and concentration of nutrient matter. Papenfuss (1942) cultured *Laminaria pallida* and *Macrocystis pyrifera* in sea-water alone and in Schreiber's solution, and found after 25 days that the sporophytes were formed only in the former culture (sea-water alone). He stated that the earlier development of reproductive organs was attributable to the effect of starvation. Kinoshita (1947) studied the effect of $CaHPO_4$ in several concentrations in the culture of *Laminaria fragilis* and *L. ochotensis*, and stated that 0.002 percent $CaHPO_4$ was efficient in the development of sporophytes. Recently, Kurogi and Akiyama (1957) reported that the gametophytes of *Undaria pinnatifida* derived from healthy zoospores liberated on April 17, 1952, and cultured in Schreiber's solution, produced sporophytes in 20 days, while the gametophytes derived from mostly immotile zoospores liberated on July 31 and cultured in sea-water alone, without changing the medium, remained sterile for one year, only showing remarkable growth lengthwise. Transferring those sterile gametophytes into Schreiber's solution on June 29, the authors could observe the formation of reproductive organs on them after 7 days, and the production of sporophytes on the female gametophytes after 11 days.

Since the quantity of inorganic salts in natural sea-water is thought to differ according to season, it was suspected that gametophytes cultured in sea-water samples obtained in different months would show some difference in growth. To make sure of this point, the following experiments were carried out with *Laminaria japonica* Aresch. collected at Nanaehama near Hakodate.

1. Development of gametophytes in sea-water samples obtained in defferent months

The sea-water samples used in this study were obtained in October and November 1954, and in March, July and September 1955, from near the distal

end of the third break-water in Hakodate Harbor. The sea-water was filtered twice through cotton, and was kept in the dark. Zoospores were liberated on October 2, 1955. Three days later, slide glasses, on which zoospores had been allowed to settle were placed in Petri dishes containing exactly 50 ml of each sea-water sample. On an average, each slide glass had about 180 gametophytes per 2 mm^2. During the period of the present experiment, the culture solution was only once changed, on January 25, 1956, when the solution was completely renewed. The results of the present experiment are shown in Table IX.

Table IX. Number of cells constituting a medium-sized female gametophyte of *Laminaria japonica* cultured from October 2, 1955 to March 21, 1956 in dishes containing sea-water samples obtained in October and November from Hakodate Harbor

Date when sea water samples were obtained / Date	Oct. 5, 1954	Nov. 21, 1954	Mar. 6, 1955	Jul. 2, 1955	Sept. 8, 1955
Nov. 4, 1955	3	3	3	2	3
Dec. 5, //	6	7	7	5	6
Jan. 17, 1956	8	8	9	7	8
Mar. 21, //	14	15	15	13	13

In this culture, the gametophytes did not grow well in any of the sea-water samples, and no remarkable difference was observed in their growth. The only differences observed were as follows. The sea-water sample obtained in July was different from the others in the period of oogonium formation and in the ratio of mature gametophytes bearing sporophytes to immature ones. In other words, the oogonium formation was first observed on November 8, 1955, in the sea-water samples obtained in October and November, while it was observed on January 27, 1956, in the sea-water sample obtained in July. The ratio of mature female gametophytes producing sporophytes to immature females was observed on March 16, 1956, to be 3 : 2 in the sea-water samples obtained in October, November, and March, 2 : 2 in that obtained in September, and 1 : 10 in that obtained in July. From these results it may be concluded that sea-water samples obtained in winter, but not in summer, have a promoting effect upon the development of the reproductive organs of *Laminaria* gametophytes.

2. Development of gametophytes in the sea-water sample obtained in July 1956 to which some nutrient substances were added

The sea-water sample used in this experiment was obtained on July 2, 1956, from near the distal end of the third break-water in Hakodate Harbor and the following five culture media were prepared.

1. Sea-water alone
2. Schreiber's solution
   $NaNO_3$ ..... 0.1 gr
   Na_2HPO_4 ..... 0.02 gr
   Distilled water ..... 50 ml
   Sea-water ..... 1000 ml
3. Culture solution consisting of
   $NaNO_3$ ..... 0.1 gr
   Distilled water ..... 50 ml
   Sea-water ..... 1000 ml
4. Culture solution consisting of
   Na_2HPO_4 ..... 0.02 gr
   Distilled water ..... 50 ml
   Sea-water ..... 1000 ml
5. Erd-Schreiber
   $NaNO_3$ ..... 0.1 gr
   Na_2HPO_4 ..... 0.02 gr
   Distilled water ..... 50 ml
   Soil extract ..... 50 ml
   Sea-water ..... 1000 ml

Zoospores were liberated on October 21, 1956. Six days later, each slide glass on which zoospores were settled was placed in a Petri dish containing one of the above-mentioned media. The average number of gametophytes growing on each slide glass was about 140 per 2 mm^2. The dishes were placed in the corridor

Table X. Development of the gametophytes of *Laminaria japonica* cultured in five media (1-5) during the period from October 21, 1956, to the end of May 1957

Culture medium*		1	2	3	4	5
Number of cells constituting a medium-sized female gametophyte	Nov. 15, 1956	1	2	2	2	2
	Dec. 15, //	2	5	4	2	2
	Jan. 15, 1957	2	14	9	3	3
	Feb. 15, //	4	26	24	9	8
	Mar. 14, //	6	43	40	13	16
	Apr. 18, //	9	65	60	17	25
	May 16, //	15	83	70	24	34
Date when oogonia were first formed		Jan. 15, 1957	Dec. 26, 1956	Jan. 17, 1957	Dec. 26, 1956	Dec. 15, 1956
Ratio of mature female gametophytes to immature ones, on Feb. 24		1:8	4:1	1:5	4:1	6:1

* For the constitutents of the five culture media, 1-5, see text

and the culture was continued till the end of May 1957. Culture solutions were changed once every two months. The results obtained are shown in Table X.

As learnt from the data shown in Table X, the growth of gametophytes understood from the number of cells was better in the media to which sodium nitrate had been added (2, 3 & 5) than in those without it (1 & 4). Sporophytes were produced in all of the culture media, but the date when oogonia were first observed to have been formed as well as the ratio of mature female gametophytes to immature ones differed according to the nature of the medium. The oogonia were formed earlier in the media to which sodium phosphate had been added (5, 2 & 4) and the ratio of mature female gametophytes to immature ones was observed on February 24, 1957, to be greater in the same media. These results seem to suggest that sodium nitrate is more effective for the growth of gametophytes than sodium phosphate, while sodium phosphate is more effective for the formation of oogonia than sodium nitrate. A preliminary culture experiment carried out by the writer from November 1957, to May 1958, to study the development of gametophytes of *Alaria crassifolia* has shown that the sporophytes were produced in cultures of sea-water alone and in Schreiber's solution, but not in a medium containing ten times the quantity of nutreint salts to be found in Schreiber's. Too great a supply of nutrient salts is thus considered to have a negative effect upon the formation of oogonia.

III. Parthenogenesis and crossing experiments

A. Previous investigations of parthenogenesis and crossing in Laminariales

Schreiber (1930) studied pathenogenesis and crossing in three species of *Laminaria*, viz., *L. saccharina*, *L. digitata* and *L. hyperborea*. He observed that the eggs from the isolated female gametophytes developed parthenogenetically into embryonic plantlets which differed in shape from the normally developed embryonic sporophytes. His crossing experiments between these species were concluded to have had a negative result from the fact that the obtained plantlets were abnormal in shape. On the other hand, from the result of crossing experiments between the forms of *Laminaria digitata*, Sundene (1958) concluded that they were interfertile. Recently, Segi and Kida (1957, 1958) reported in their culture study of *Undaria undarioides* that the terminal cell of some immature female gametophytes underwent repeated cross and longitudinal divisions resulting in an embryonic plantlet of abnormal shape. They interpreted such abnormal plantlets as having developed parthenogenetically. However, the development of such a plantlet from a terminal cell is described as having taken place prior to the formation of oogonia, so it cannot strictly be called parthenogenesis.

B. Parthenogenesis

a) Materials and methods

In the present study, the following nine species were examined; *Laminaria religiosa* Miyabe, *L. japonica* Areschoug, *L. angustata* Kjellman, *L. angustata* var. *longissima* Miyabe, *L. ochotensis* Miyabe, *L. diabolica* Miyabe, *Alaria crassifolia* Kjellman, *Undaria pinnatifida* (Harv.) Suringar f. *distans* Miyabe et Okamura, and *Arthrothamnus bifidus* (Gmel.) Ruprecht.

A piece of the mature frond was thoroughly washed in filtered sea-water and placed in a vat containing filtered sea-water. A small amount of water containing released zoospores was pipetted into Petri dishes filled with sea-water, some containing Schreiber's solution and some without. The solution was stirred to make the zoospores adhere evenly to the slides placed on the bottom of the dish. Isolation of gametophytes was carried out as follows. For a gametophyte consisting of few cells, a blood corpuscle counter was used. A drop of water containing a gametophyte was sucked up into the centre of a hollow slide. After making sure of the presence of a single gametophyte in the hollow under the microscope, a small quantity of filtered sea-water was pipetted into the hollow to fill it up, and then a cover glass was placed on the slide. The hollow slide thus loaded with a single gametophyte was placed, to prevent evaporation, in a Petri dish containing just enough culture solution to almost cover the surface of the slide. A few days later, the culture solution was gently poured into the dish so as to float away the cover glass. In case the gametophyte happened to attach itself to the under side of the cover glass, the latter was of course left in the dish. The culture solution was changed usually once every two or three weeks. The culture dishes were placed in the corridor, not inside the laboratory room, in view of the results of the culture experiment described in Chapter II, Section A.

b) Results

1. *Laminaria religiosa* Miyabe

The materials used were collected at Oshoro Bay in 1952, 1953 and 1954 (Table XI). The culture of the material collected in 1952 was undertaken in Sapporo, at the laboratory of the Hokkaido University Fisheries Department, Faculty of Agriculture, and that of the other materials in Hakodate, at the Hokkaido University Faculty of Fischeries' laboratory of Phycology. The results obtained will be described for each material as follows. Table XII shows the number of parthenosporophytes produced in each isolated female and their thallus shape.

Table XI. Data of the materials of *Laminaria religiosa* Miyabe from Oshoro Bay used for the experiment of parthenogenesis

Date of collection	Nov. 25, 1952	Oct. 12, 1952		Oct. 15, 1953	Oct. 22, 1954	
Date of zoospore liberation	Nov. 26, 1952	Oct. 13, 1952		Oct. 19, 1953	Oct. 25, 1954	
Date of gametophyte isolation	Dec. 7–13, 1952	Jan. 20, 1953		Jan. 12–13, 1954	Dec. 10–15, 1954	Sept. 12, 1955
Number of isolated females	15	20	ca. 60*	20	25	30
Number of females observed	12	20	60	20	23	30
Number of mature females: Date	(a)** 8 Mar. 20, 1953	(b) 7 Mar. 20, 1953	(c) 3 Mar. 20, 1953	(d) 11 Mar. 18, 1954	(e) 13 Mar. 27, 1955	(f) 12 Mar. 22, 1956

* Isolated females were increased in number by cutting the filamentous thallus of the mother gametophytes

** (a)–(f) denote each group of the material analysed in Table XII

Table XII. Number of parthenosporophytes produced in each group (a–f) of isolated female gametophytes (cf. Table XI), and number of malformed sporophytes

Group of isolated females	Individual females in each group	Number of sporophytes produced	Number of malformed sporophytes	Date of counting
a	1	60	53	Mar. 21, 1953
	2	45	41	//
	3	40	36	//
	4	38	35	//
	5	36	32	//
	6	21	20	//
	7	12	3	//
	8	5	2	//
b	1	151	108	May 2, 1953
	2	132	101	//
	3	21	18	//
	4	18	18	May 3, 1953
	5	17	16	//
	6	6	5	//
	7	5	3	//
c	1	3	3	May 18, 1953
	2	2	2	//
	3	2	2	//
d	1	74	65	May 7, 1954
	2	68	51	//
	3	60	58	//
	4	42	39	//
	5	41	35	May 8, 1954
	6	30	26	May 7, 1954
	7	27	24	May 8, 1954
	8	25	21	//
	9	11	11	//
	10	6	6	May 6, 1954
	11	3	2	//

Table XII. (Continued)

Group of isolated females	Individual females in each group	Number of sporophytes produced	Number of malformed sporophytes	Date of counting
e	1	64	54	Apr. 4, 1955
	2	59	52	//
	3	43	40	Apr. 5, 1955
	4	41	36	//
	5	37	36	//
	6	33	29	//
	7	21	15	Apr. 4, 1955
	8	20	20	//
	9	18	15	Apr. 6, 1955
	10	15	14	Apr. 4, 1955
	11	12	10	Apr. 6, 1955
	12	3	1	//
	13	1	1	//
f	1	84	72	Apr. 21–23, 1956
	2	73	65	//
	3	71	53	//
	4	64	62	//
	5	61	58	//
	6	42	40	//
	7	40	34	//
	8	37	35	//
	9	35	30	//
	10	18	5	//
	11	12	6	//
	12	2	2	//

Material collected on November 25, 1952: Isolation of gametophytes in the culture of the present material was carried out with the aid of a blood corpuscle counter on December 7–13, 1952 (cf. Table XI). Fifteen female and fifteen male gametophytes were isolated. The females mostly consisted of two to three cells when isolated. When observed on December 20, 1952, only four of the slides were found to bear in the central hollow a filamentous gametophyte composed of about 20–30 cells. Early in January of the following year, these four gametophytes were found to have grown to a profusely branched thallus consisting of more than one hundred cells, and were visible to the naked eye as a minute brown spot when the culture dish was placed on white paper. By that time, other isolated female gametophytes which had become detached from the slide and attached to some other place in the dish were also visible to the naked eye. However, by the middle of May, 1953, when the present culture was ended, the number of living isolated gametophytes was reduced to twelve, of which eight individuals attained maturity. The oogonia were formed in January and February. The eggs liberated from those oogonia germinated parthenogenetically, either while they were attached to the opening of the oogonium or after becoming detached from the oogonium. Pl. I, Fig. C shows an egg just liberated and

detached from an oogonium. The parthenosporophytes thus developed were mostly malformed when observed on March 21, 1953 (cf. Table XII, column (a); Text-fig.

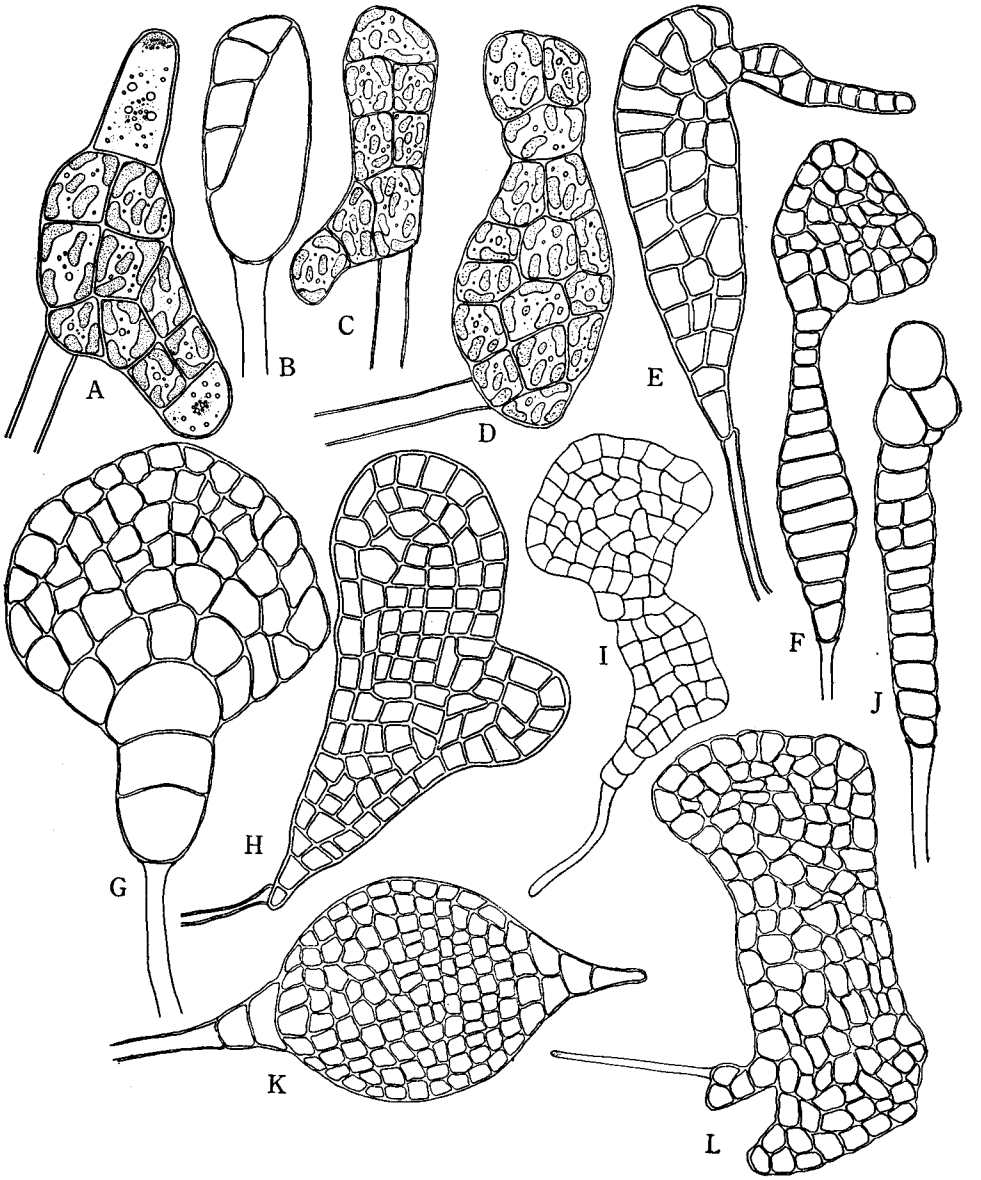


Fig. 5. *Laminaria religiosa* Miyabe

Parthenosporophytes developed on the female gametophytes which had been isolated in December 1952 from the material collected at Oshoro Bay on November 25, 1952. From a culture 50 days old after isolation

A-D, ×200; E-L, ×170

5, A-L; Text-fig. 6, A-E; Pl. I, Fig. B). Photomicrographs (Figs. A-G) in Pl. II show the progress of the development of parthenosporophytes on the gametophyte No. 7 in column (a) of Table XII. Some of them were provided with rhizoids at the tip or on the margin of their frond. In the middle of May, the largest parthenosporophyte produced in the culture of the present material was about 3 mm in length.

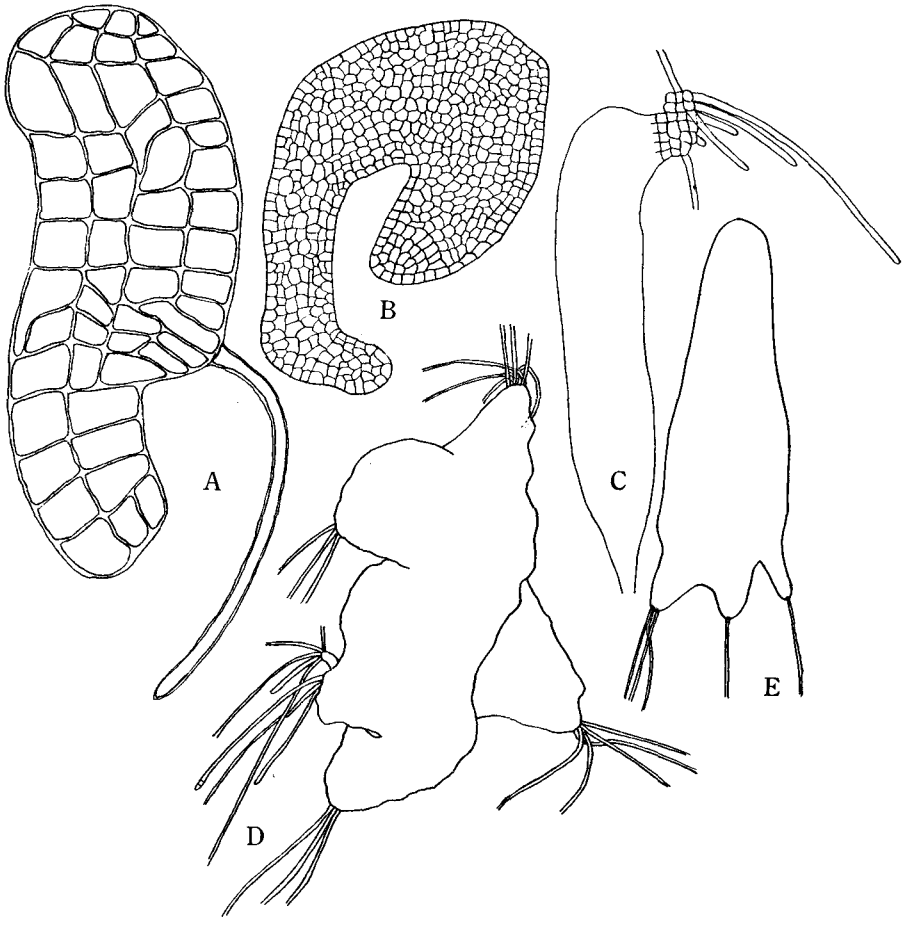


Fig. 6. *Laminaria religiosa* Miyabe

Parthenosporophytes developed on the female gametophytes which had been isolated in December 1952 from the material collected at Oshoro Bay on November 25, 1952. From a culture three months old after isolation

A, ×200; B-C, ×95; D-E, ×20

The isolated male gametophytes developed into profusely branched filamentous thalli consisting of numerous cells, and eventually became visible to the naked eye.

However, they did not produce any antheridia until the end of May, 1953, when the present culture was abandoned. One of those male gametophytes, 164 days after isolation, is shown in Pl. II, Fig. H.

Material collected on October 12, 1952: In the present culture, reproductive organs of the gametophytes were formed earlier when cultured in sea-water alone than in Schreiber's solution; the oogonia were formed within about one month in sea-water alone while they were formed within about two to three months in Schreiber's solution. In some dishes containing sea-water alone, there were found besides the small gametophytes attached densely to the bottom, a few well-developed gametophytes floating sparsely on or beneath the surface of the solution. The floating gametophytes, especially those on the surface, had much better developed thalli than the attached ones. In the middle of January 1953, most of the attached female gametophytes were found to consist of about 20 cells, and to have produced small sporophytes, while those floating on the surface of the solution consisted of hundreds of cells, but were mostly still immature. Fig. A of Pl. III shows an attached female gametophyte in a less dense area, and Fig. B of the same plate a floating female gametophyte. The floating female gametophytes were found to bear only one to three malformed parthenosporophytes which were in a much more advanced stage of growth than those borne on the attached gametophytes. Young parthenosporophytes were often found floating solitarily in the culture solution or on its surface. They were mostly abnormal in shape. Fig. C of Pl. III shows a malformed sporophyte floating on the surface of the culture solution, and in the upper corner of Fig. A of the same plate is shown part of a malformed sporophyte floating in the solution.

In late January 1953, twenty immature female gametophytes floating on the surface of the culture solution

Fig. 7. *Laminaria religiosa* Miyabe

A malformed sporophyte developed on a female gametophyte floating on the surface of a mixed culture four months old derived from the material collected at Oshoro Bay ×70

were isolated, each being picked up with a platinum needle. At the same time one immature female gametophyte was cut into small pieces consisting of 20–30 cells (Pl. III, Fig. E), and these pieces were placed either singly in each of 10 Petri dishes or in a group of about five pieces in each of 10 other Petri dishes. These isolated gametophytes and cut pieces were all cultured in filtered sea-water. Of the above-mentioned 20 isolated female gametophytes, seven became fertile 15 to 30 days after isolation; two of them produced numerous oogonia (Pl. I, Fig. F) and the remaining ones only a few oogonia (cf. Table XII (b)). Some of the eggs thus formed were found to be moribund but many of them soon developed into parthenosporophytes consisting of several cells arranged in a row. These parthenosporophytes could not be distinguished from diploid sporophytes at a similar embryonal stage except for their rather reduced vitality. The surviving individuals usually became malformed in the more advanced stages of development.

The cut pieces of an immature female gametophyte, regardless as to whether they were placed singly or in a group, grew so rapidly that about one month and a half after isolation all of them developed into profusely branched filaments as large as the mother plant. One month later, one or two parthenosprophytes were produced per one thallus, in several dishes. They were all malformed, sometimes being provided with rhizoids at their frond apices.

Material collected on October 15, 1953: Zoospores from this material were cultured in Petri dishes containing Schreiber's solution, placed in the corridor of the laboratory. As in the culture from the preceding material, many gametophytes were found floating on the surface of the culture solution, and a marked difference in growth was noticed between the gametophytes attached to the bottom of the dish and those floating on or in the solution. For example, in early January 1953, the female gametophytes attached to the bottom consisted of only 20–35 cells and were mostly fertile, while the floating ones consisted of many cells, up to 120–180, and were mostly still sterile.

Twenty of the floating immature female gametophytes were isolated on January 12 and 13, 1954 and cultured till May. Eleven of them became fertile during this time. The eggs were formed in the period from late January to early March. The developmental processes of parthenosporophytes were identical to those observed in the females isolated from the material collected on November 25, 1952. Most of the parthenosporophytes produced on the isolated females were malformed, as shown in Table XII (d).

Material collected on October 22, 1954: Zoospores from this material were cultured in Petri dishes containing Schreiber's solution, placed in the corridor and in the laboratory. Immature gametophytes were isolated from among the gameto-

phytes cultured in the corridor on several days between December 10 and 15, 1954, by means of a blood corpuscle counter; and on September 12, 1955, by means of a platinum needle from among the gametophytes cultured in the laboratory. Each female gametophyte thus isolated was cultured in a Petri dish containing Schreiber's solution.

The female gametophytes isolated in December 1954, twenty five in number, consisted of about 1–3 cells when isolated. They were cultured until the end of May, 1955. About one month after isolation, they became visible to the naked eye. Oogonium formation was observed to begin in eleven of them during the period ranging from the middle of January to the middle of February, and the eggs developed parthenogenetically into malformed sporophytes (cf. Table XII (e)). It was sometimes observed that an empty oogonium cavity was filled up by the outgrowth of the adjoining cell that had intruded into the cavity after egg discharge.

On September 12, 1955, thirty female gametophytes were isolated, when their fronds consisted of more than one thousand cells. They were cultured till June 1956. Twelve individuals became fertile and the eggs were found to be formed in the period from late December 1956 to early February 1957. The parthenosporophytes developed from those eggs were generally found to be already abnormal in shape in their early stages of growth.

2. *Laminaria japonica* Areschoug

Materials were collected near Hakodate, namely at Shirikishinai, Zenikamezawa and Nanaehama. Date of collection and other data concerning these materials are summarized in Table XIII.

(1) Material collected at Shirikishinai (cf. Table XIII, (a) & (b))

The gametophytes were cultured in the laboratory and in the corridor with either Schreiber's solution or Erd-Schreiber. The cultures in the laboratory were carried out until the end of May, 1956. In the corridor cultures, gamete-formation in both female and male gametophytes began earlier in Erd-Schreiber than in Schreiber's solution. The gametophytes cultured in Schreiber's solution under room temperature are shown in Pl. V. During this culture, the diatoms grew abundantly in some dishes seven to ten months old which have been placed under room temperature, so that the gametophytes became partly colorless and decayed. The survived cell rows of the gametophytes (Pl. V, Fig. B) began later to grow into independent gametophytes after the culture solution had been frequently changed, about twice a week. In August and early September, 1955, some dishes were found to contain gametophytes provided with remarkably swollen cells at their apices and intercalary portions (Pl. V, Fig. E; Text-fig. 8). From some of those cells arose normal branches after the cool season had set in. The reason why

Table XIII. Data of the materials of *Laminaria japonica* used for parthenogenesis experiment

Collection place	Shirikishinai		Zeni-kamezawa	Nanaehama					
Date of collection	Nov. 23, 1953		Oct. 27, 1954	Sept. 24, 1955					
Date of zoospore liberation	Nov. 25, '53		Oct. 28, '54	Sept. 24, '55					
Date of gametophyte isolation	Dec. 14 '53	Jul. 15 '54	Nov. 18, '54	Nov. 18 '55	Jul. 10 '56	Sept. '56	Jul. '57	Sept. '57	Sept. '58
Number of isolated females	20	20	20	30	20	20	20	20	40
Number of females survived	18	20	12	23	20	20	20	20	40
Number of matured females	(a)* 7	(b) 2	(c) 4	(d) 8	(e) 5	(f) 4	(g) 2	(h) 8	(i) 18
Culture was finished in	May '54	Sept. '54	Apr. '55	Apr. '56	May '57	May '57	May '58	May '58	May '59

* (a), (b),····(i) denote respectively each group of mature female gametophytes analyzed in Table XIV

such swollen cells were formed on them was that the gametophytes in those dishes had been cultured for more than one year under somewhat weak light intensity and in the unchanged solution. In mid-December, 1955, some of the dishes 25 months old containing such gametophytes with swollen cells were removed from the laboratory to the corridor. They became fertile in mid-January, 1956. In some dishes cultured for 10 to 15 months under room temperature, many cells of the female gametophytes became slender, as thin as those of the male. Those dishes were removed to the corridor in mid-October, 1955, and about two months later, they produced a few young sporophytes which were abnormal in shape (Pl. VI, Figs. E & F). In some other dishes containing Schreiber's solution and placed in the corridor, many sperms were found sometimes liberated simultaneously from many of the antheridia and swarmed around the eggs.

(a) Gametophytes isolated in December 1953 (Table XIII, (a))

Gametophytes were isolated by means of a blood corpuscle counter from a dense culture. Twenty females consisting mostly of one cell or rarely two were isolated and each of them was placed in a Petri dish containing Schreiber's solution. Of these females seven became fertile and oogonia were formed in the period between mid-January and early February, 1954. The egg discharged from the apical opening of the oogonium usually remains attached there for a while, but soon it

becomes easily detached and free. Most of the eggs produced from each isolated female germinated parthenogenetically but some of them lost vitality. The partheno-sporophytes produced from each mature female were only one to eight in number and all of them were found to be malformed already in the early stages of growth, often producing rhizoids from the apical or marginal portion of their fronds (Pl. IV, Figs. C & D; Text-fig. 9).

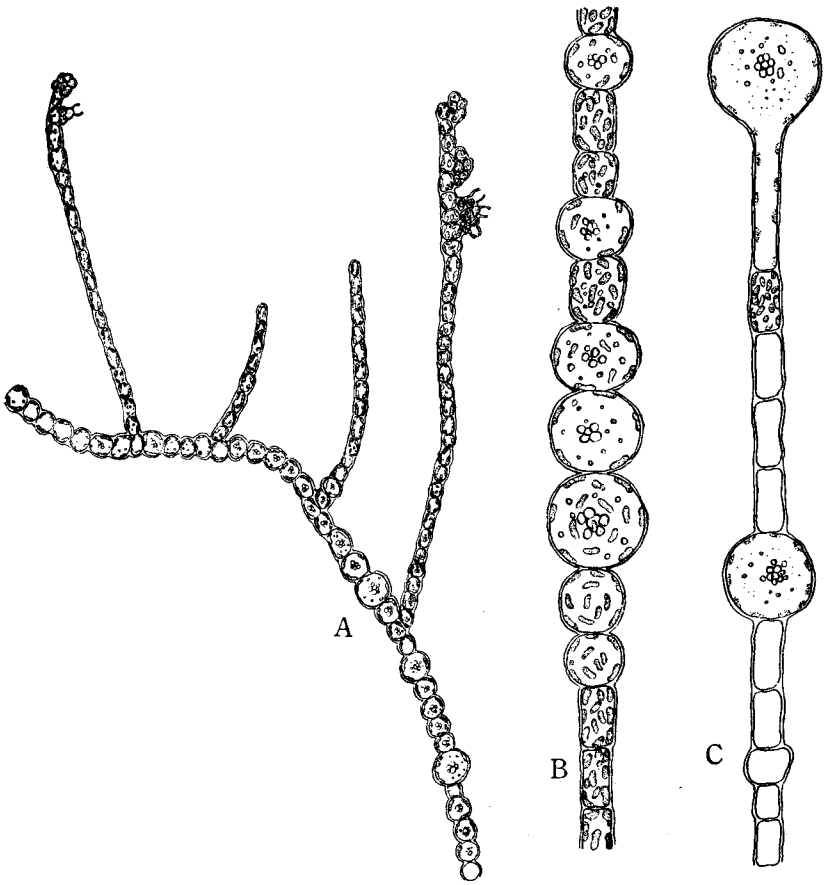


Fig. 8. *Laminaria japonica* Areschoug

Male gametophytes with swollen cells from culture vessels placed for two months in the corridor after having been kept for two years in the laboratory
A, ×315; B-C, ×620

(b) Gametophytes isolated in July 1954 (Table XIII, (b))

Twenty female gametophytes and fifteen male ones were isolated by means of a platinum needle from a sparse culture and they were placed in small glass tubes containing Schreiber's solution in a vat filled with ice water. They were cultured

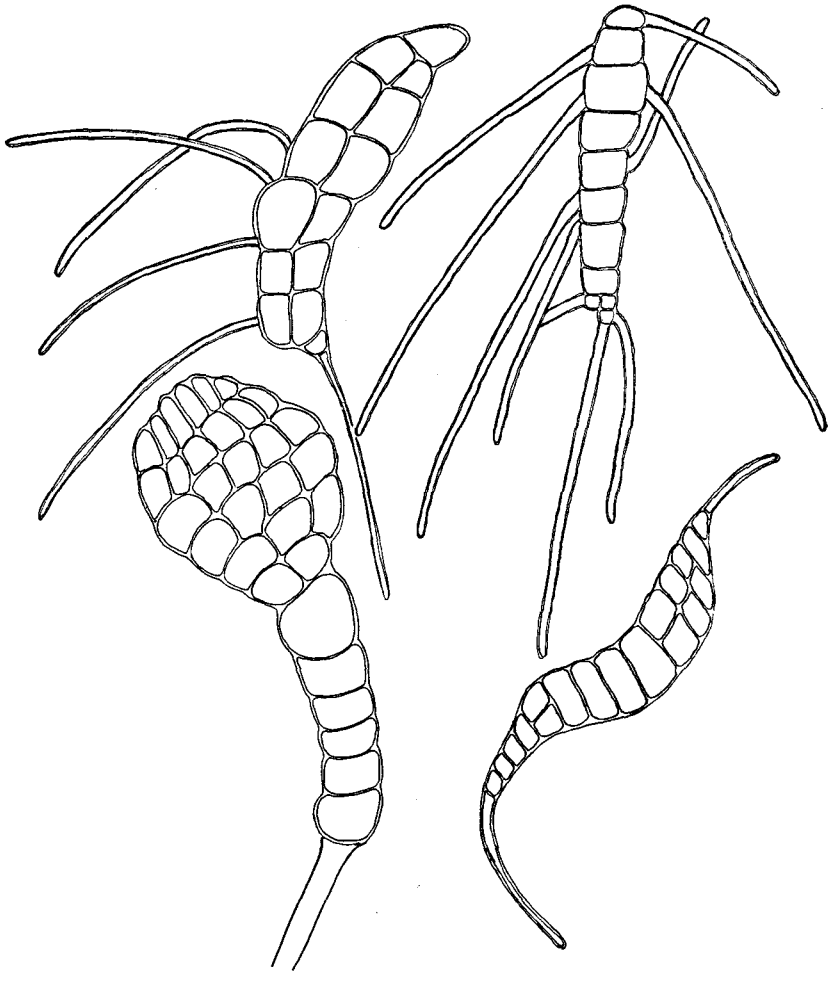


Fig. 9. *Laminaria japonica* Areschoug

Parthenosporophytes developed on the female gametophytes which had been isolated in December 1953 from the material collected at Shirikishinai in November 1953. From a culture one month old after isolation ×200

until early September 1954, and only two females became fertile producing only one and two parthenosporophytes respectively. These sporophytes were first observed on August 8, when they were composed of two or three cells and already assuming an abnormal shape (Pl. IV, Fig. F). On the other hand, the isolated male gametophytes did not become fertile in this culture.

(2) Material collected at Zenikamezawa (cf. Table XIII, (c))

The liberated zoospores were cultured in Petri dishes with sea-water under

Table XIV. Number of parthenosporophytes produced on each of the female gametophytes (1, 2, ···) which are grouped into nine (a–i) according to their isolation date (cf. Table XIII) and number of malformed sporophytes

Group of isolated females	Individual females numbered in each group	Number of sporophytes produced	Number of malformed sporophytes	Date of counting
a	1	8	8	April 22–25, 1954
	2	6	6	
	3	5	5	
	4	4	4	
	5	3	3	
	6	3	3	
	7	1	1	
b	1	2	2	Sept. 3, 1954
	2	1	1	
c	1	2	2	April 28, 1955
	2	1	1	
	3	1	1	
	4	1	1	
d	1–8 (cf. Table XVII)			
e	1	34	29	May 9–5, 1957
	2	27	26	
	3	21	18	
	4	19	16	
	5	9	9	
f	1	21	20	May 1–3, 1957
	2	13	12	
	3	2	2	
	4	1	1	
g	1	2	2	May 6, 1958
	2	1	1	
h	1	56	55	April 16–18, 1958
	2	52	50	
	3	38	38	
	4	31	28	
	5	17	15	
	6	5	5	
	7	5	4	
	8	2	2	
i	1	44	41	May 14–16, 1959
	2	41	38	
	3	39	27	
	4	37	35	
	5	26	25	
	6	24	24	
	7	24	23	
	8	22	20	
	9	21	21	
	10	20	19	
	11	10	9	
	12	9	9	
	13	9	8	
	14	8	8	
	15	7	7	
	16	7	7	
	17	4	4	
	18	3	3	

room temperature. Twenty female gametophytes, one- or two-celled, were isolated by means of blood corpuscle counter on November 18, 1954 (Pl. IV, Fig. E). They were cultured in Petri dishes containing sea-water until April 1955, but they did not grow well, consisting of only three to five cells even at the end of the culture. By the end of the culture, they were reduced to twelve in number, of which only four became fertile in late December, 1954, or in early January, 1955. Each mature female produced only one to two eggs. All of these eggs developed into parthenosporophytes of abnormal shape (Pl. IV, Figs. A & B) which also did not grow well consisting of six to eight cells when observed on April 28, 1955.

(3) **Material collected at Nanaehama (cf. Table XIII, (d)-(i))**

Gametophytes were cultured in the laboratory and in the corridor with either Schreiber's solution or Erd-Schreiber (cf. Pl. VII–XII). Well developed female gametophytes cultured for nine months in Schreiber's solution under room temperature consisted of more than one hundred cells (Pl. VII, Fig. A) and they consisted of several thousand cells after being cultured for two years (Pl. VII, Figs. B–D; Pl. IX). In the culture vessels containing both female and male gametophytes of nine Laminariales plants used for the experiment of parthenogenesis, it was found that the reproductive organs were usually formed earlier in males than in females. When isolated, however, the male gametophytes did not become fertile, as already described in *Laminaria religiosa* and *L. japonica* and as described below in *L. angustata* and *Alaria crassifolia*. This fact seems to suggest that the female gametophyte might secrete into the culture solution a certain substance that urges the male to be fertile. To make a research on this problem, cultures of the male gametophytes of the present material were carried out from January 9 through March 7, 1958, with the following four kinds of culture solution; (1) filtered Schreiber's solution in which numerous mature female and male gametophytes had been cultured, (2) filtered sea-water in which the fertile female gametophytes consisting of numerous cells provided with eggs and sporophytes had been placed for some time, (3) pure filtered sea-water, and (4) pure Schreiber's solution. Twenty sterile male gametophytes were isolated from culture dishes placed under room temperature and they were separately put into twenty Petri dishes grouped into four according to the above-mentioned four culture solutions poured into them. When observed on January 28, the male plants in two of the five dishes of both Group-1 and Group-2 containing the culture solution (1) and (2) respectively were found to be fertile (Pl. XII), while the males in the remaining dishes as well as in the dishes of Group-3 and Group-4 containing the solution (3) and (4) respectively were all sterile yet. From this result, though it is not so convincing, the female gametophytes are supposed to

Table XV. Number of eggs, sporophytes, and malformed sporophytes produced on each of eight female gametophytes belongining to the group "d" in Table XIII and XIV, as counted on January 24 (A), February 18 (B), and on April 29 (C) in 1956

Individual female numbered in group "d"	Number of eggs discharged	Number of sporophytes	Number of malformed sporophytes	Date of counting
1	3	5	5	A
	5	13	11	B
	2	14	13	C
2	11	6	4	A
	10	18	10	B
	3	18	16	C
3	6	3	2	A
	8	7	6	B
	1	8	6	C
4	7	3	3	A
	4	7	7	B
	1	7	7	C
5	2	4	4	A
	2	5	5	B
	7	6	5	C
6	18	4	2	A
	19	5	4	B
	8	5	5	C
7	24	0	0	A
	35	0	0	B
	31	0	0	C
8	19	0	0	A
	25	0	0	B
	12	0	0	C

secrete into the culture solution a certain substance that urges the male gametophytes to be fertile.

The female gametophytes were isolated in November 1955, in July and September 1956, in July and September 1957, and in September 1958, by means of blood corpuscle counter in the first case and by means of platinum needle in other cases. They were all cultured in Schreiber's solution. The oogonium was found to be formed during a period from late December to mid-March. Contents of the oogonium were sometimes observed to partly remain within the cavity after discharge of the egg (Pl. XVIII, Figs. D-E). The discharged eggs which have detached from the oogonia were often observed to be connected by colorless mucilage with the empty cavity of the oogonium.

(a) Gametophytes isolated in November 1955 (Table XIII (d))

When isolated, the female gametophytes were 2-4-celled. Thirty individuals were isolated, of which twenty-three survived and only eight became fertile. In Table XV are shown the results of the culture. The females No. 7 and No. 8 in the Table are different from others in having many eggs but no parthenosporophytes produced from them. No. 7 is illustrated in Pl. IV, Fig. H. The remaining females, No. 1-6, were all found to have produced parthenosporophytes which were mostly malformed in their early stages of development. Six months after the isolation, that is in May, most of the isolated females were found to have attained a vigorous growth of the frond consisting of hundreds of cells.

(b) Gametophytes isolated in July and September 1956 (Table XIII, (e)-(f))

Twenty female gametophytes were isolated in July and the same number of them again in September. Most of the eggs produced from them developed parthenogenetically. Rarely some eggs were found to have swollen up markedly. The parthenosporophytes in early stages of growth did not differ in shape from the diploid sporophytes of similar stages, but they usually became malformed when their fronds consisted of more than 20 cells. In two of the females isolated in July, No. 1 and No. 2 in "e" group of Table XIV, the writer could find four partially extruded eggs, so he made observations to learn how long it took for their completion of extrusion. The results are shown in Table XVI.

Table XVI. Data on the time taken for the complete extrusion of four partially extruded eggs of *Laminaria japonica*

Individual female number in group "e" of Table XIV	Length of oogonium (μ)	Length of empty space left in oogonium by partially extruded egg when observation started (μ)	Date and hour when observation started (A)	Date and hour when extrusion of egg completed (B)	Lapse of time (minutes) (B-A)
No. 1	68	11	9.00 hr., Jan. 16	9.02 hr., Jan. 16	2
No. 1	82	8	9.12 hr., Jan. 17	9.36 hr., Jan. 17	24
No. 1	74	5	10.10 hr., Feb. 4	10.31 hr., Feb. 4	21
No. 2	86	16	10.05 hr., Feb. 5	10.09 hr., Feb. 5	4

Hollenberg (1933) states in his paper on *Eisenia arborea*, "I am of the opinion that extrusion of the egg takes place in a relatively short period of time. Partially extruded eggs are very rarely found in stained preparation showing

numerous seemingly mature cogonia and numerous extruded eggs." In the writer's observations, as shown above, it took 2–24 minutes for the partially extruded eggs to complete their extrusion. The passive movement of the eggs was not smooth,

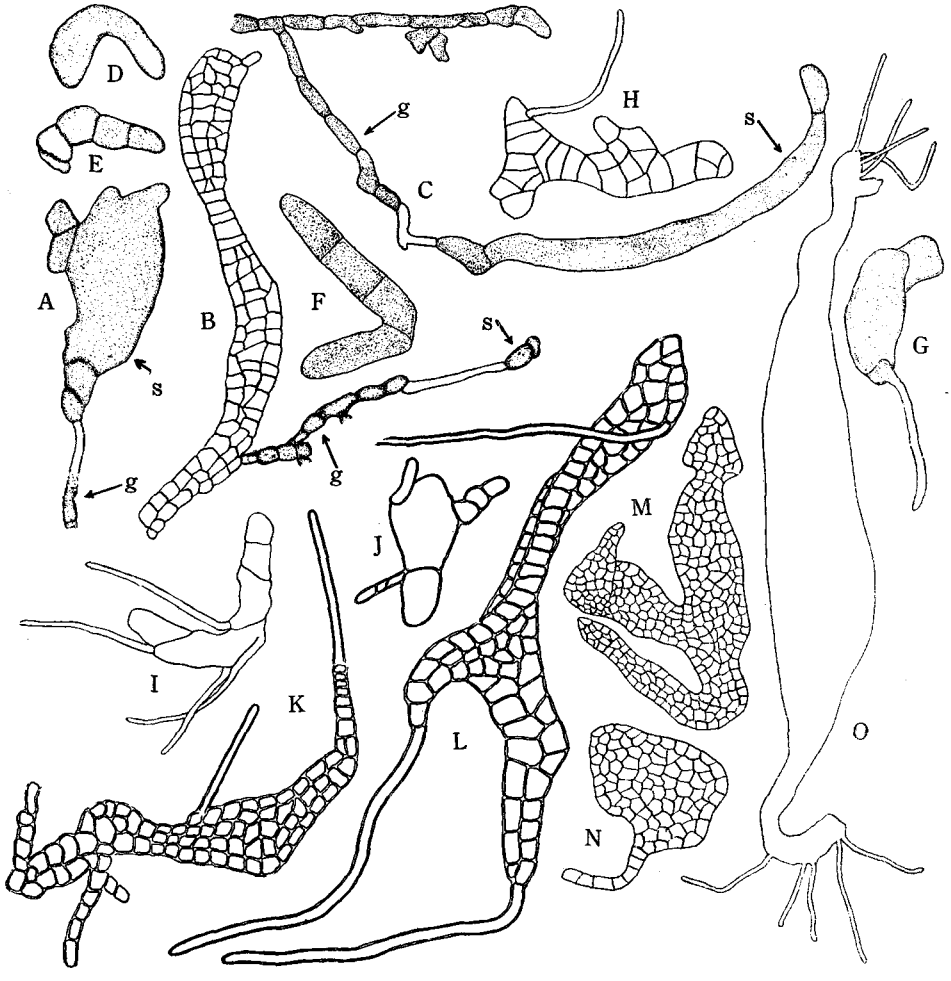


Fig. 10. *Laminaria japonica* Areschoug

A–C. Parthenosporophytes (s) developed on the female gametophytes (g) isolated in September 1957 from the material collected at Nanaehama in September 1955. From a culture four months old after isolation (A–B) and a culture six months old after isolation (C)

D–O. Parthenosporophytes developed on the female gametophytes isolated in September 1957 from the material collected at Nanaehama in September 1955. From a culture four and half months old after isolation (D–G), and cultures five months old (H–J), six months old (K–L), and seven months old (M–O) after isolation

A–N, ×162; O, ×94

but it stopped for a while after a little advance. This was repeated several times before the egg is liberated from the oogonium.

(c) Gametophytes isolated in July and September 1957, and in September 1958 (Table XIII, (g)-(i))

In July 1957, twenty female gametophytes were isolated, of which two indivi-

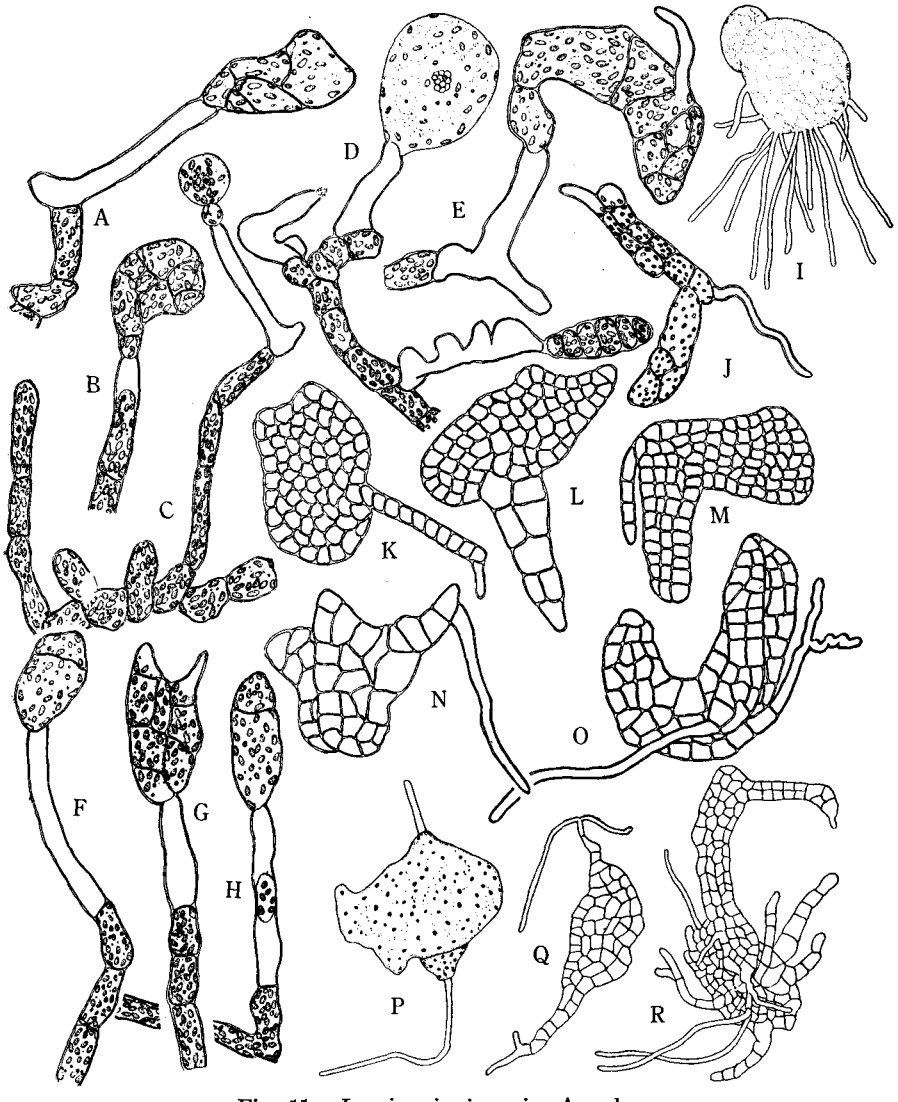


Fig. 11. *Laminaria japonica* Areschoug

Parthenosporophytes developed on the female gametophytes isolated in September 1958 from the material collected at Nanaehama in September 1955. From a culture five months old after isolation

A–H & J–O, ×186; I & P–R, ×133

duals became fertile and produced one and two parthenosporophytes respectively (Pl. VIII, Fig. D). They were abnormal in shape when observed on May 6, 1958. In September of 1957 and 1958, the female gametophytes were isolated from the culture dishes containing many of them which had been developed from the cut pieces of five well grown sterile female thalli obtained from amongst the gameto-

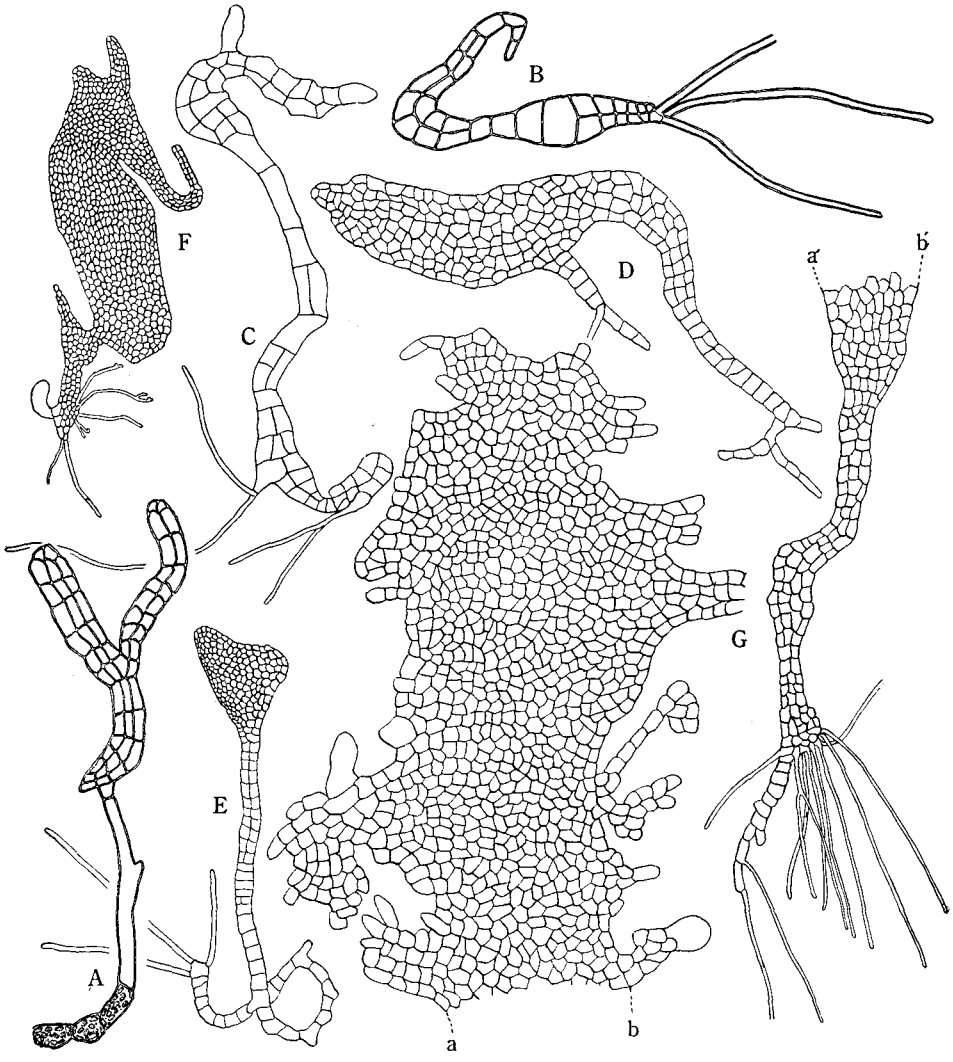


Fig. 12. *Laminaria japonica* Areschoug

Parthenosporophytes developed on the female gametophytes isolated in September 1958 from the material collected at Nanaehama in September 1955. From a culture six months old (A–D) and a culture seven and half months old (E–G) after isolation

A, ×186; B–G, ×133

phytes cultured in Schreiber's solution under room temperature. The number of isolated females was twenty in 1957 and forty in 1958, and eight and eighteen of them attained maturity respectively. Most of the eggs produced from them

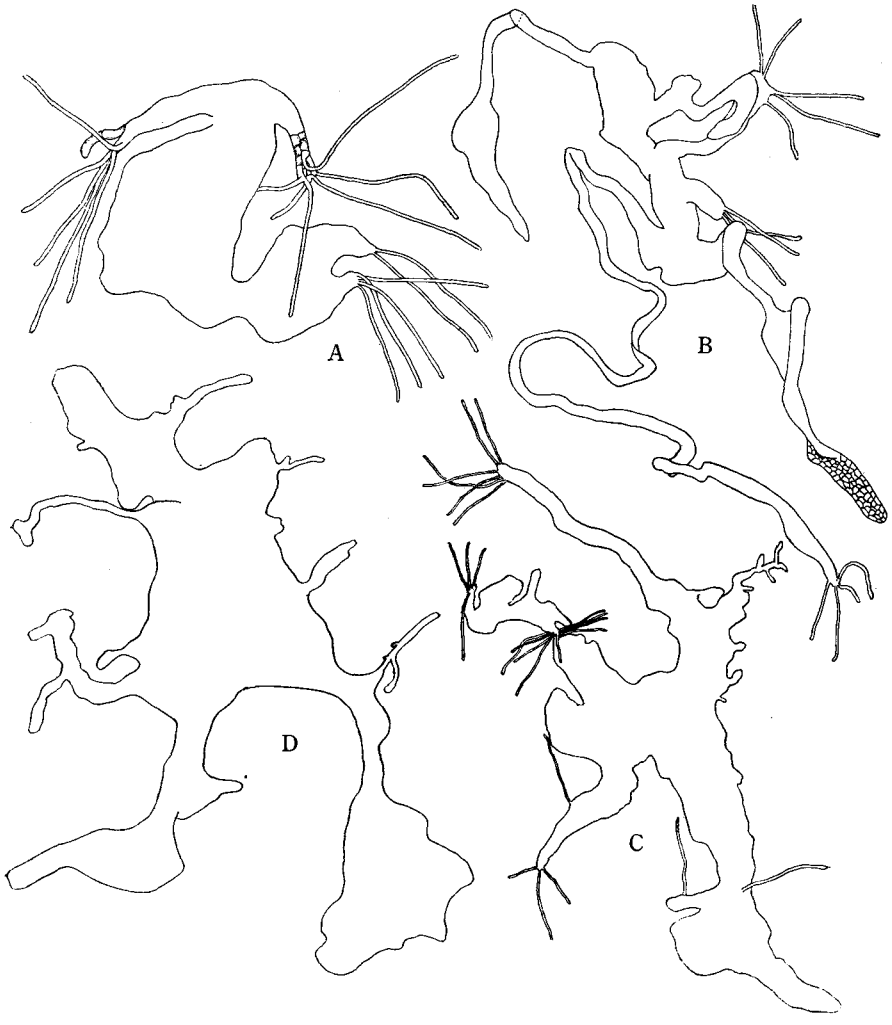


Fig. 13. *Laminaria japonica* Areschoug

Parthenosporophytes developed on the female gametophytes isolated in September 1958 from the material collected at Nanaehama in September 1955. From a culture nine months old after isolation

A–B, ×71; C, ×47; D, ×31

developed into parthenosporophytes which were abnormal in shape even in early stages of their growth (Text-figs. 10–13; Pls. XIII–XVII).

3. *Laminaria angustata* Kjellman

The materials of this species were collected at Shizunai in November 1952 and at Muroran in November 1953. They were cultured in Sapporo and in Hakodate respectively (Table XVII). Table XVIII shows the number of parthenosporophytes produced in each isolated female and the number of malformed sporophytes developed.

Table XVII. Data of the materials of *Laminaria angustata* Kjellm. used for parthenogenesis experiment

Collected place	Shizunai	Muroran
Date of zoospore liberation	Nov. 26, 1952	Nov. 27, 1953
Date of gametophyte isolation	Dec. 5–10, 1952	Dec. 16–20, 1953
Number of isolated females	15	20
Number of females observed	7	18
Number of mature females	(a)* 1	(b)* 7
Culture was finished in	May 1953	May 1954

* Cf. Table XVIII

Table XVIII. Number of parthenosporophytes produced in each group (a & b) of mature female gametophytes (cf. Table XVII) and number of the malformed among them, counted on March 4, 1953 (a) and on April 2–3, 1954 (b)

Group of isolated females	Individual female numbered in each group	Number of sporophyte produced	Number of malformed sporophyte
a	1	2	2
b	1	8	8
	2	7	7
	3	7	6
	4	6	5
	5	4	4
	6	3	3
	7	2	2

Material collected at Shizunai: Zoospores were liberated on November 26, 1952. About two weeks later, fifteen female gametophytes and fifteen males were isolated by means of a platinum needle, and cultured in Petri dish containing Schreiber's solution until May 1953. Pl. XIX, Fig. A shows a photomicrograph of the female and male gametophytes just after isolation. In late February and early March, 1953, the isolated females were visible to the naked eye as minute brown spots on the bottom of the culture dish. By the end of this culture, the number of

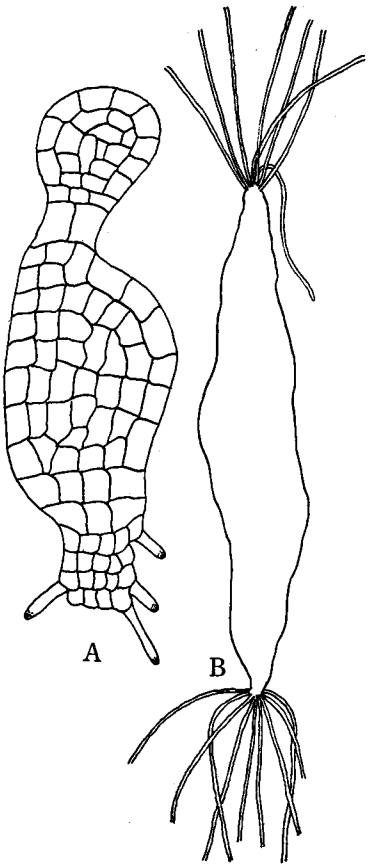


Fig. 14. *Laminaria angustata* Kjellman

Parthenosporophytes developed on the female gametophytes isolated in December 1952 from the material collected at Shizunai in November 1952. From a culture four and half months old after isolation

A, ×95; B, ×20

surviving females was reduced to seven and only one of them attained maturity. On the other hand, the number of surviving male gametophytes was nine at the end of the culture. Their thalli were also found to consist of a globular mass of filamentous thallus, but none of them became fertile during the period of this culture.

Material collected at Muroran: Liberation of the zoospores was performed on November 25, 1953. Three weeks later, twenty females were isolated by means of a platinum needle, and they were cultured in Petri dishes containing Schreiber's solution. By the middle of May 1954, the surviving females were reduced to eighteen, of which seven individuals attained maturity. Their thalli were much smaller than those cultured from the material collected at Shizunai mentioned above, consisting of about 40 to 50 cells even at the end of the culture. About one month after isolation, some of the females were found to have enlarged, pale colored cells, about 18–24 μ in diameter, whereas the normal cells of the females

in a mixed culture were about 11–13 μ in diameter. The isolated females began to produce oogonia in late January 1954. When observed in early April 1954, the parthenosporophytes produced on each female were only two to eight in number and all of them were malformed (Text-fig. 15; Pl. XIX, Fig. D). They were mostly connected directly with a vegetative cell of the mother thallus (Pl. XIX, Fig. D), but not with an empty oogonium. Such a sporophyte is considered to have developed

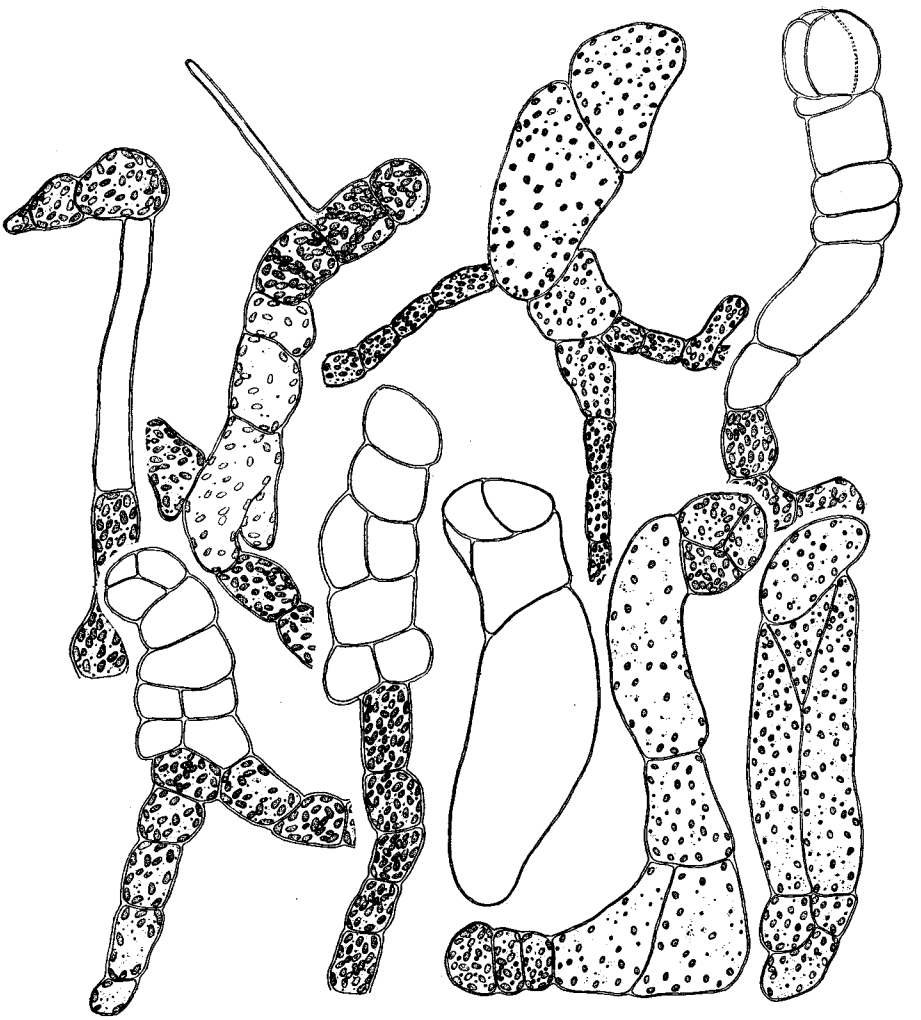


Fig. 15. *Laminaria angustata* Kjellman

Parthenosporophytes developed on the female gametophytes isolated from the material collected at Muroran in November 1953. From a culture two and half months old after liberation of zoospores. ×390

in either of the following two ways: (1) the sporophyte is normally developed from an egg which was discharged from an oogonium and attached to its apex, and the empty cavity of the oogonium is then filled up by the outgrowth of the adjoining cell, or (2) the sporophyte is developed from an egg which remained within the oogonium. On January 25, 1954, the writer happened to observe a two-celled parthenosporophyte partially extruded from an oogonium. It took about twenty minutes for this sporophyte to accomplish its complete extrusion from the oogonium, after repeating a little forward movement and a short stop.

4. *Laminaria angustata* var. *longissima* Miyabe

The material was collected at Muroran. Zoospores were liberated on November 26, 1954. Twenty days later, fifteen female gametophytes were isolated by means of a blood corpuscle counter and they were cultured in Petri dishes containing Schreiber's solution until May 1955. By the end of this culture the number of

Table XIX. Number of parthenosporophytes produced on each female of *Laminaria angustata* var. *longissima* Miyabe and number of the malformed among them, counted on May 4, 1955

Individual female gametophyte	Number of sporophyte produced	Number of malformed sporophyte
1	15	13
2	13	10
3	8	8
4	5	5

surviving individuals was reduced to thirteen. Their thalli consisted of about 80–140 cells at the end of the culture. Only four of those females became fertile and began to produce oogonia in early March. Most of the eggs developed into parthenosporophytes. When observed on May 4, 1955, the parthenosporophytes produced on each of the four females were from five to fifteen in number and most of them were found to be abnormal in shape (cf. Table XIX: Text-fig. 16).

5. *Laminaria ochotensis* Miyabe

The material was collected on October 26, 1953 at Kutsugata in Rishiri Island. Zoospores were liberated on October 30, 1953. About twenty days later, twenty-five female gametophytes were isolated with the aid of a blood corpuscle counter and they were cultured in Petri dishes containing Schreiber's solution until mid-May 1954. By the end of this culture the number of surviving females was reduced to twenty-one, of which nine individuals attained maturity and began to form oogonia during a period ranging from late December 1953 to early February 1954. Most of the eggs produced on them developed parthenogenetically. When observed on April 7–9, 1954, the maximum number of the parthenosporophytes

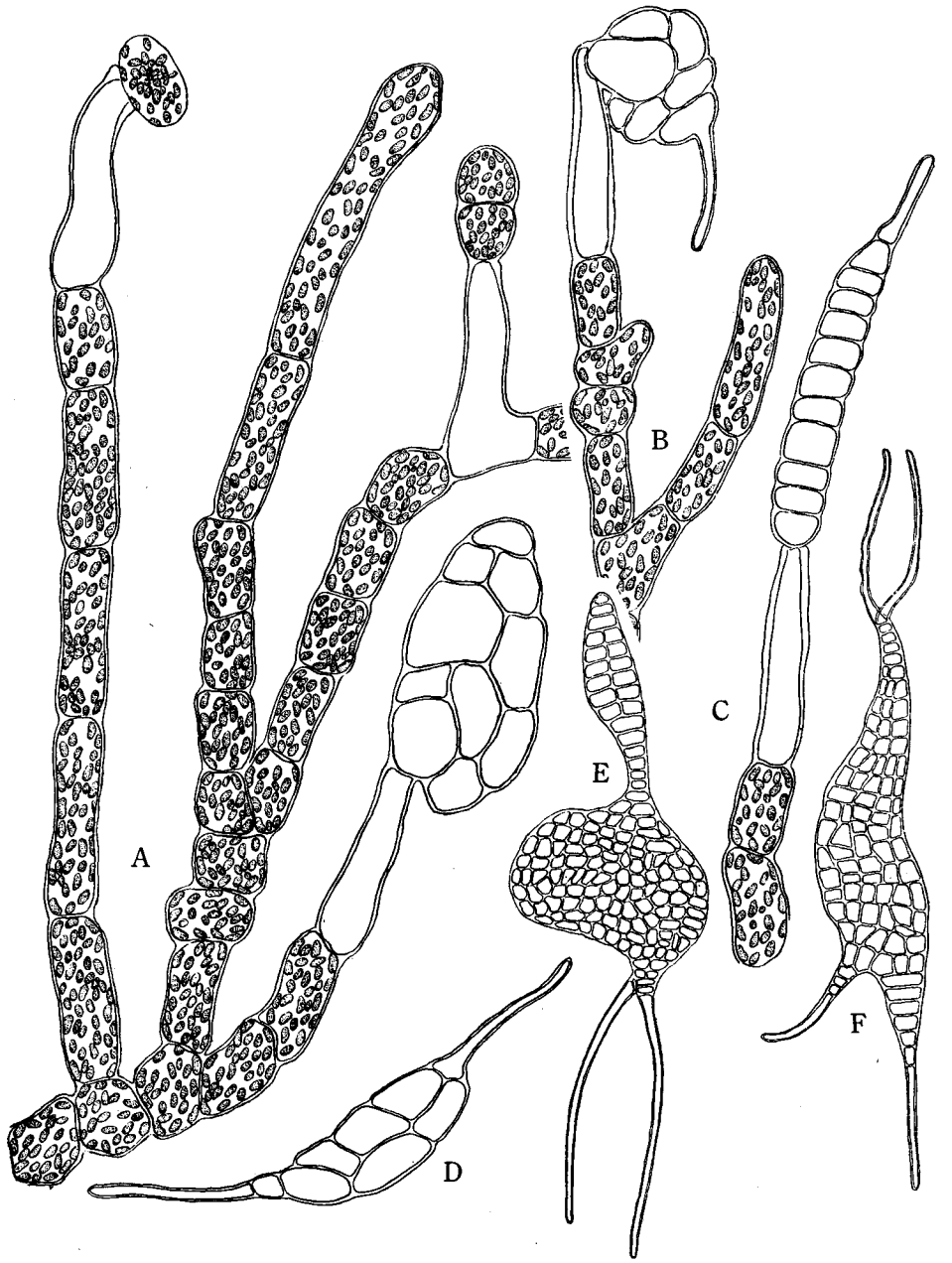


Fig. 16. *Laminaria angustata* Kjellm. var. *longissima* Miyabe

Development of parthenosporophytes on the female gametophytes isolated in December 1954 from the material collected at Muroran in November 1954. From a culture 50 days old (A–C), and a culture two months old after isolation (D–F)

A–C, ×507; D–F, ×225

Table XX. Number of parthenosporophytes produced on each female of *Laminaria ochotensis* Miyabe and number of the malformed among them, counted on April 7–9, 1954

Individual female gametophyte	Number of sporophyte produced	Number of malformed sporophyte
1	numerous*	numerous
2	numerous	numerous
3	ca. 150	over 100
4	ca. 100	ca. 100
5	32	30
6	24	21
7	20	18
8	15	12
9	9	9

* About 300–400 in number

produced on a single female gametophyte was nearly 400 (Pl. XIX, Fig. E), while the minimum number was nine, and most of them were found to be malformed in the early stages of their development (cf. Table XX). Kanda (1946) observed many malformed sporophytes in his culture of *Laminaria ochotensis* Miyabe and *L. chicorioides* Miyabe (Kanda, 1946, p. 13, text-fig. IV, 9–10 & p. 16, text-fig. VII, 2–11). They bear a close resemblance to the malformed parthenosporophytes in the writer's culture of *L. ochotensis*.

6. *Laminaria diabolica* Miyabe

The material was collected at Akkeshi in September 1953. The culture of zoospores was started on September 15, 1953. About one month later, twenty-five female gametophytes were isolated with the aid of a blood corpuscle counter

Table XXI. Number of parthenosporophytes produced on each female of *Laminaria diabolica* Miyabe and number of the malformed among them, counted on April 12–13, 1954

Individual female gametophyte	Number of sporophyte produced	Number of malformed sporophyte
1	11	10
2	9	9
3	6	6
4	6	6
5	5	5
6	4	4
7	4	4
8	3	3

and they were cultured in Petri dishes containing Schreiber's solution until mid-May 1954. By the end of culture, the number of surviving females was reduced to eighteen, of which eight individuals became fertile and began to form oogonia in early January 1954. Near the end of the culture the females were visible to

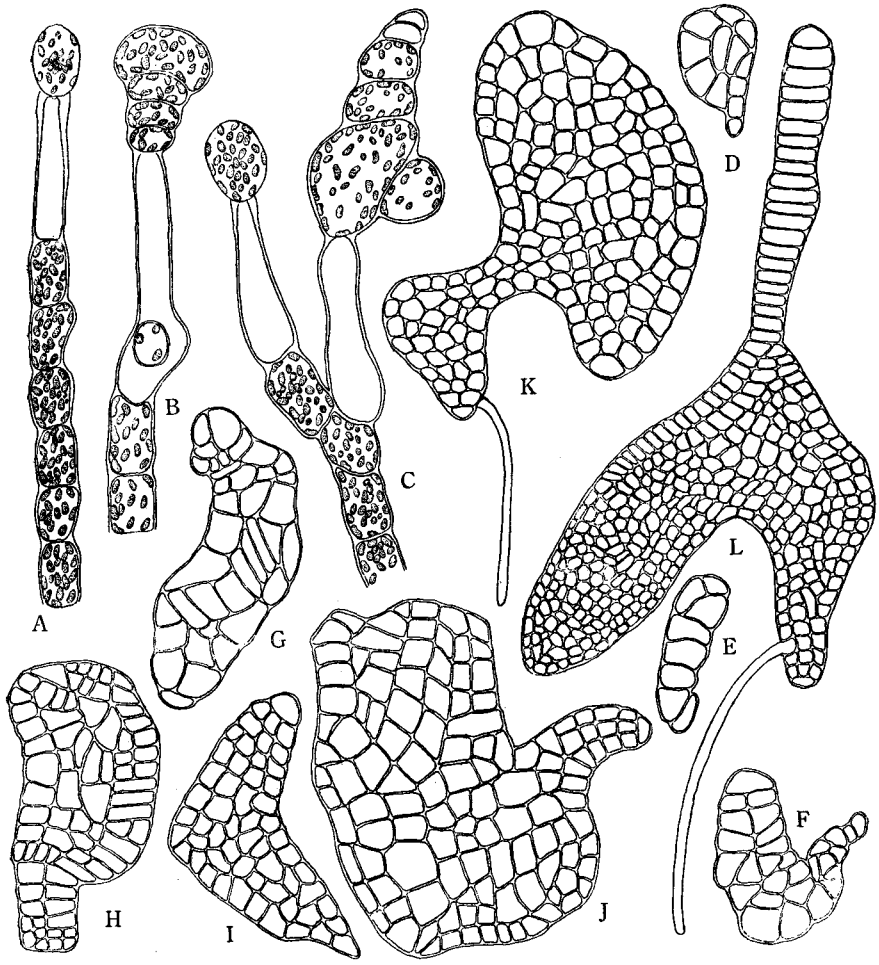


Fig. 17. *Laminaria ochotensis* Miyabe

Development of parthenosporophytes on the female gametophytes isolated in November 1954 from the material collected at Kutsugata in October 1953. From a culture three and half months old after isolation

A-C, ×422; D-L, ×188

the naked eye as brown spots consisting of profusely branched filamentous thalli. In the present culture it was often noticed that the contents of an oogonium did not wholly contribute to the egg formation but remained in part within the empty oogonium after the egg had been discharged. Most of the eggs developed parthenogenetically. Sometimes the eggs were found to have swollen remarkably (Text-fig. 18, A, B, & E). Most of parthenosporophytes produced were abnormal in shape in the early stages of their development (cf. Table XXI: Text-fig. 18; Pl. XX, Figs. C-E).

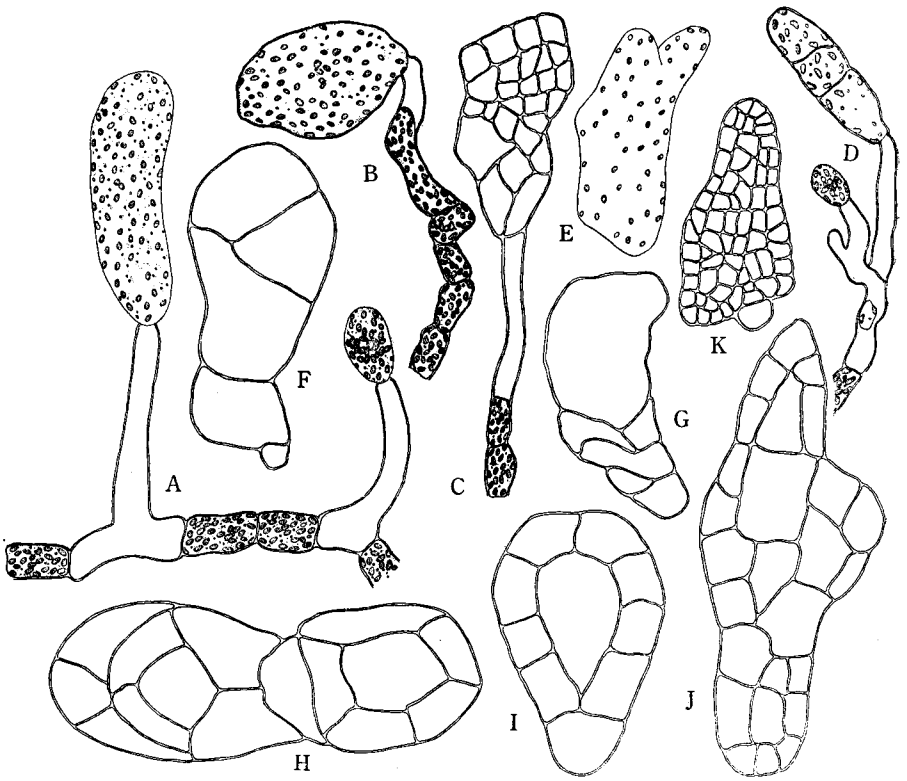


Fig. 18. *Laminaria diabolica* Miyabe

Development of parthenosporophytes on the female gametophytes isolated in October 1953 from the material collected at Akkeshi in September 1953. From a culture four months old after isolation

A–J, ×347; K, ×142

7. *Alaria crassifolia* Kjellman

In his description of *Alaria crassifolia* Kjellm., Yendo (1916) stated that the smallest specimen in which the gland cells were found was 2 mm in total height and 0.6 mm at the broadest part of the blade. However, the writer could detect the occurrence of the gland cells in much younger sporophytes of this species as mentioned below. The material was collected at Tachimachi-misaki in late December 1954. Young sporophytes were produced in the mixed culture about one month after the liberation of the zoospores. When the sporophytes attained about 2.0–3.0 mm in total length, it was possible to tell the gland cells from other cells by their yellowish contents. Not only those yellowish gland cells but also the colorless ones were stained well with the solutions of various kinds of pigments such as methylen-blue, methyl-green, neutral-red, and toluidin-blue (Pl. XXIII, Figs. A–F).

The methyl-green solution prepared by dissolving 1 gr of methyl-green and 1 ml of gracial acetic acid in 100 ml of distilled water, was especially good for staining the gland cells in young sporophytes. The smallest sporophyte in which the writer could detect the presence of the gland cells with this solution was 0.1 mm in total height, consisting of seventy-six cells. By staining the colorless upper portion of some weakened sporophytes with methyl-green solution, the gland cells in the colorless portion could be made conspicuously outstanding (Pl. XXIII, Figs. E & F).

Parthenogenesis in the present species was studied with the material collected at Shirikishinai near Hakodate on November 23, 1954. Two days later, the zoospores were liberated, and they were cultured in Schreiber's solution in the laboratory until September 1955. Many gametophytes were found floating on or beneath the surface of the culture solution (Pl. XXI, Figs. A, B & D). They were somewhat better in growth, though paler in color, than those attached to the bottom of the dish. About three months later after the start of the culture, some female gametophytes floating in the solution began to produce slender branches (Pl. XXI, Fig. D), and in September 1955, or at the end of this culture, they consisted mostly of numerous slender branches bearing a close resemblance to the branches of male gametophytes. From mid-August to early September 1955, many of the male and female gametophytes were found to have many swollen cells (Pl. XXII, Fig. A). Isolation of the gametophytes in the present culture was performed in December 1954 and in July 1955.

Gametophytes isolated in December 1954: Twenty female and fifteen male gametophytes were isolated in mid-December from the culture dishes in which the gametophytes were densely attached to the bottom of the dish. For isolation, a blood corpuscle counter was used. The isolated gametophytes were cultured in Petri dishes containing sea-water, the females until May 1955, and the males until August 1955. The female gametophytes, when isolated, were composed of three to

Table XXII. Number of parthenosporophytes produced on each female of *Alaria crassifolia* and number of the malformed among them, counted on April 24-25, 1955

Individual female gametophyte	Number of sporophyte produced	Number of malformed sporophyte
1	4	4
2	3	3
3	2	2
4	2	2
5	2	2
6	2	2
7	1	1

four cells. Two months later they were found to have developed into filamentous thalli consisting of about 60–80 cells. The oogonia began to be formed in late

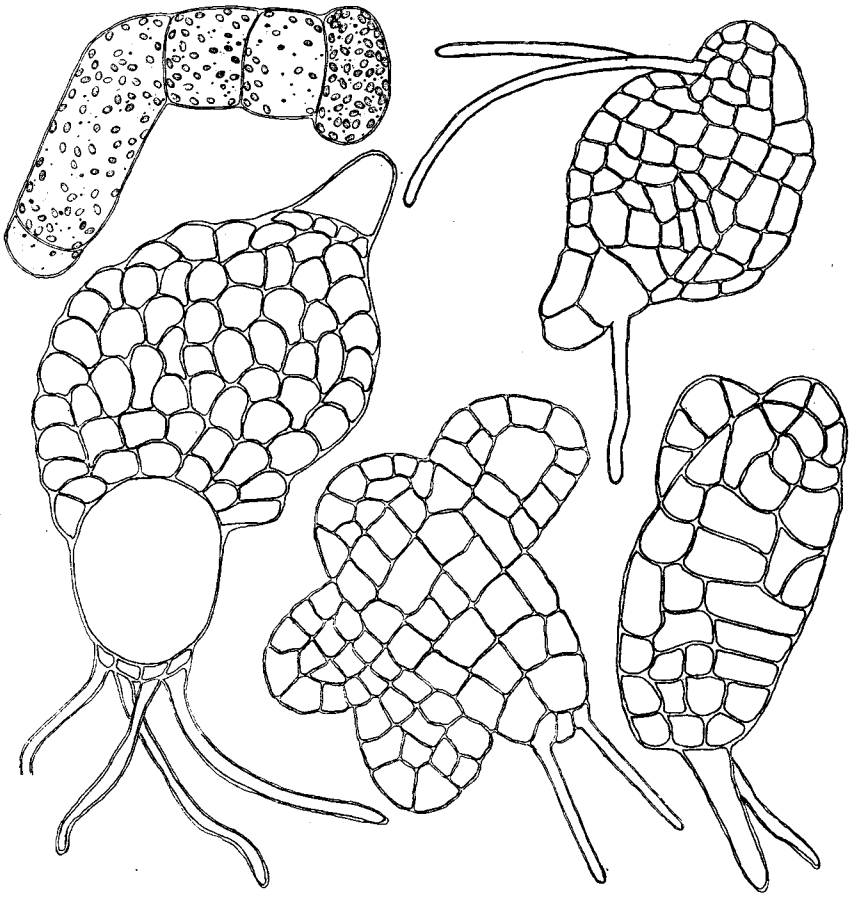


Fig. 19. *Alaria crassifolia* Kjellman

Parthenosporophytes developed on the female gametophytes isolated in December 1954 from the material collected at Shirikishinai in November 1954. From a culture two months old after isolation. ×400

January or in early February, 1955. The number of surviving females was reduced to seventeen, of which seven individuals attained maturity. Most of the eggs developed parthenogenetically. All of the parthenosporophytes produced were abnormal in shape (cf. Table XXII: Pl. XXII, Fig. C; Text-fig. 19).

On the other hand, the number of surviving male gametophytes was twelve at the end of the present culture. They were visible to the naked eye as minute brown spots but none of them were fertile yet.

Gametophytes isolated in July 1955: Fifteen female gametophytes were isolated on July 14 by means of a platinum needle from amongst the well developed female gametophytes floating in the culture solution, and they were cultured until late September 1955 in small glass tubes placed in a vat containing ice water. At the end of this culture, the females were all still living, but only one of them was found to have attained maturity. On September 8, the only egg that had been formed on the single fertile female was found to have grown into a three-celled parthenosporophyte (Pl. XXII, Fig. D).

8. *Undaria pinnatifida* (Harv.) Suringar f. *distans* Miyabe et Okamura

The materials of this species were collected at Nanaehama near Hakodate City on July 30, 1954. The zoospores were liberated on the next day and cultured in Petri dishes containing Schreiber's solution or sea-water alone placed in the laboratory. Globular masses of filamentous thalli of the female gametophytes cultured for two years were nearly 8 mm in diameter. In the dishes which contained sea-water alone and were left standing with no change of the solution,

Table XXIII. Number of parthenosporophytes produced on each female of *Undaria pinnatifida* (Harv.) Suringar f. *distans* Miyabe et Okamura and number of the malformed among them, counted on April 16, 1955

Individual female gametophyte	Number of sporophyte produced	Number of malformed sporophyte
1	2	2
2	2	2
3	2	2
4	1	1
5	1	1

the gametophytes began to die when the culture was ten to thirteen months old. In some of those dishes, the female gametophytes consisted partly of slender filaments as thin as the males (Pl. XXIV, Fig. B). In other dishes, the females were found to have swollen cells here and there (Pl. XXIV, Fig. C). One month after the liberation of zoospores, twenty-five female gametophytes were isolated with the aid of a blood corpuscle counter, and they were cultured in Petri dishes containing sea-water until the end of May, 1955, when the number of surviving individuals was reduced to nineteen, of which only five attained maturity, and the oogonium began to be formed in the period ranging from early January to early February 1955. The eggs produced on them developed parthenogenetically. When observed on April 14, 1955, the parthenosporophytes produced on each female were one to three in number and were abnormal in shape (cf. Table XXIII: Pl. XXIV, Fig. D; Text-fig. 20).

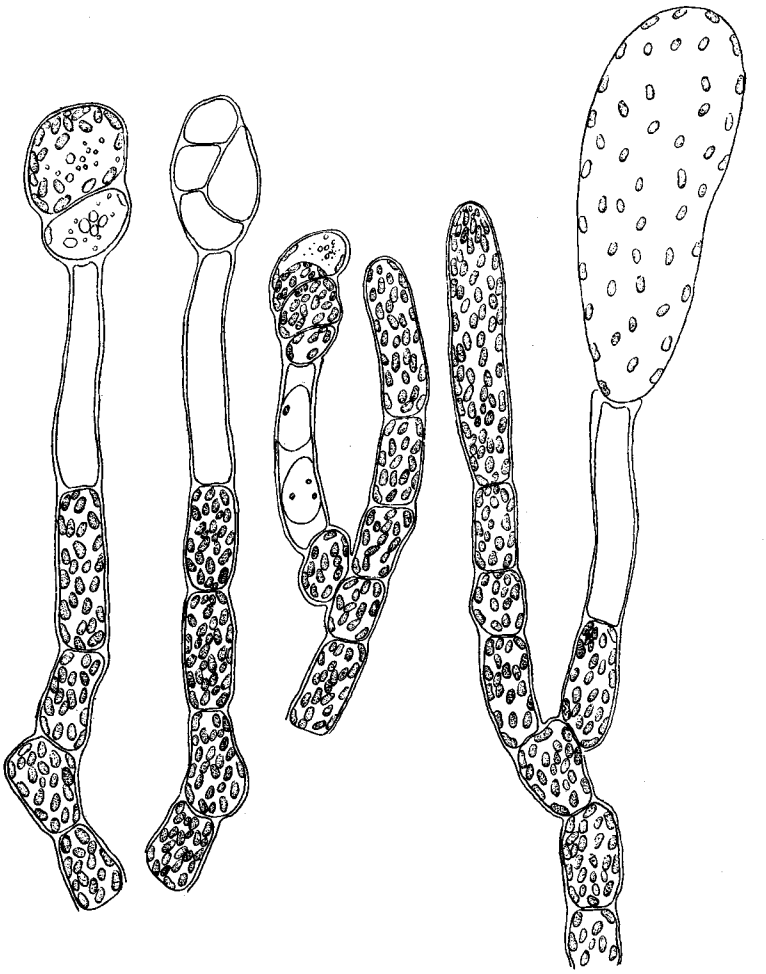


Fig. 20. *Undaria pinnatifida* (Harv.) Suringar f. *distans* Miyabe et Okamura

Development of parthenosporophytes on the female gametophytes isolated in August 1954 from the material collected at Nanaehama in July, 1954. From a culture five months old after isolation. ×507

9. *Arthrothamnus bifidus* (Gmel.) Ruprecht

The materials of this species were collected at Nemuro in mid-March 1955. Zoospores were liberated on March 17, 1955 and cultured in Petri dishes containing Schreiber's solution placed in the laboratory. Eight months later, twenty female gametophytes consisting of profusely branched filaments were isolated by means of a platinum needle, and cultured in small glass tubes containing Schreiber's solution until the end of May 1956. Seven of them became fertile and began to

Table XXIV. Number of parthenosporophytes produced on each female of *Arthrothamnus bifidus* (Gmel.) Rupr. and number of the malformed among them, counted on March 22–25, 1956

Individual female gametophyte	Number of sporophyte produced	Number of malformed sporophyte
1	over 300	ca. 300
2	ca. 80	ca. 80
3	24	23
4	14	14
5	6	6
6	6	6
7	4	3

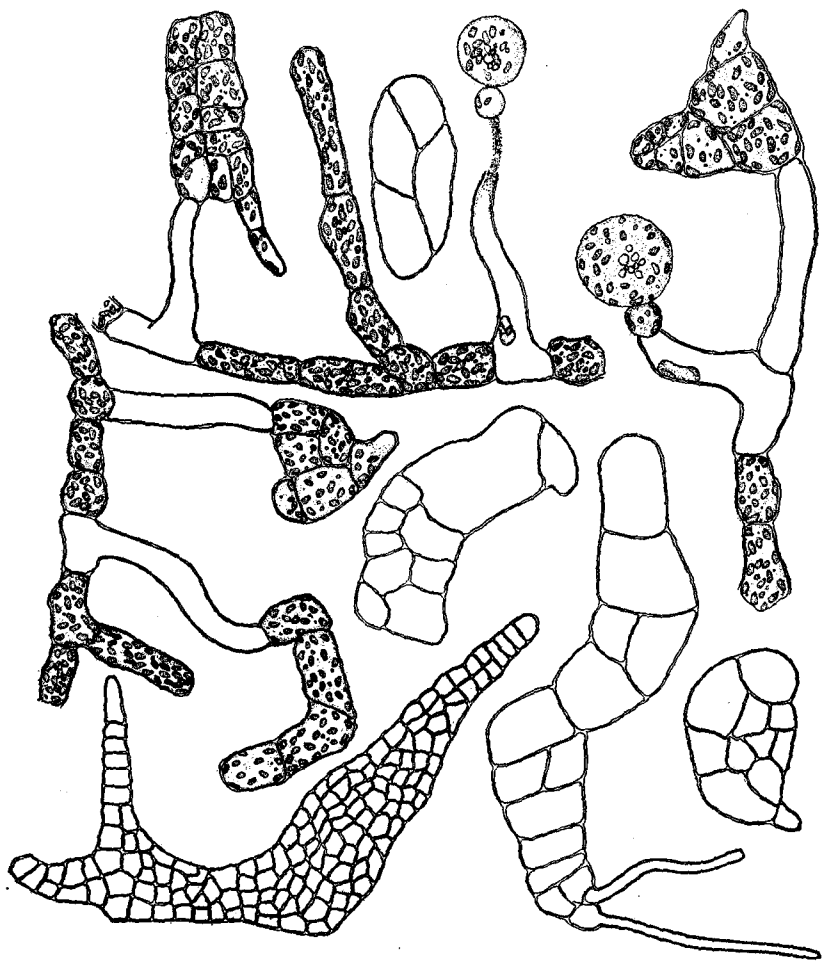


Fig. 21. *Arthrothamnus bifidus* (Gmel.) Ruprecht

Development of parthenosporophytes on the female gametophytes isolated in November 1955 from the material collected at Nemuro in March 1955. From a culture three months old after isolation. ×400

form oogonia during the period ranging from late January to early February. Most of the eggs produced on them developed parthenogenetically. The partheno-sporophytes in the early stages of their development were usually abnormal in shape (cf. Table XXIV: Pl. XXIV, Figs. E & F; Text-fig. 21). They were usually formed to be attached singly to the apical edge of an empty oogonium, but rarely they were connected directly with a vegetative cell of the mother thallus (Pl. XXIV, Fig. E).

C. Crossing experiments

a) Materials and methods

The crossing experiments were undertaken during the periods from October 1955 to May 1956 and from October 1957 to May 1958, with five species in the former period andwith another three species in the latter period as shown in Table XXV.

Table XXV. Materials used for the crossing experiments

Species	Collected at	Collected in	Period of experiment
L. japonica	Shirikishinai	October 1953	From October 1955 to May 1956
L. religiosa	Oshoro	October 1953	
L. angustata	Muroran	November 1953	
L. ochotensis	Kutsugata	September 1953	
A. crassifolia	Shirikishinai	October 1953	
L. japonica	Nanaehama	October 1956	From October 1957 to May 1958
L. religiosa	Oshoro	October 1956	
L. diabolica	Akkeshi	June 1956	

The zoospores of those materials were cultured in Petri dishes containing Schreiber's solution or sea-water alone, and were placed in the laboratory. Isolation of the gametophytes was performed mostly by means of a platinum needle when they became visible to the naked eye consisting of numerous cells, and partly by means of a blood corpuscle counter while they were still very young consisting of three to five cells. The isolated gametophytes were cultured in glass tubes or Petri dishes containing Schreiber's solution or sea-water alone, and were placed in the laboratory. A couple of female and male gametophytes belonging respectively to a single or to either of two different species was put in a tube or dish containing Schreiber's solution placed in the corridor.

b) Results

1. Experiments during the period from October 1955 to May 1956

The results of the peresent experiments are shown in Tables XXVI and XXVII.

In the culture vessels containing a couple of male and female gametophytes of a single species, nearly seventy per cent of the females produced the sporophytes and most of them were normal in shape, while in the vessels containing an isolated female alone, only twenty to thirty per cent of the females produced the sporophytes and most of them were abnormal in shape (Table XXVI, I-M).

Table XXVI. Data on crossing (A-H) and parthenogenesis (I-M) experiments among four species of *Laminaria* and one species of *Alaria*, performed during the period from October 1955 to May 1956, including some of the results observed during the period from February 20 to March 16 in 1956

Species and sex of gametophytes	Number of couples & females gametophytes			
	Couples, females†	Fertile female gametophytes		
		Total	Sporophytes* Norm.>Abnorm.	Sporophytes* Norm.<Abnorm.
L. religiosa ♂×*L. japonica* ♀	20	A 16	14	2
L. religiosa ♀×*L. japonica* ♂	21	12	11	1
L. religiosa ♂×*L. ochotensis* ♀	20	B 15	13	2
L. religiosa ♀×*L. ochotensis* ♂	20	16	14	2
L. religiosa ♂×*L. angustata* ♀	21	C 8	0	8
L. religiosa ♀×*L. angustata* ♂	22	10	0	10
L. japonica ♂×*L. ochotensis* ♀	20	D 12	10	2
L. japonica ♀×*L. ochotensis* ♂	21	13	12	1
L. ochotensis ♂×*L. angustata* ♀	20	E 6	1	5
L. ochotensis ♀×*L. angustata* ♂	21	6	0	6
L. religiosa ♂×*A. crassifolia* ♀	20	F 3	0	3
L. religiosa ♀×*A. crassifolia* ♂	21	4	0	4
L. japonica ♂×*A. crassifolia* ♀	20	G 11	0	11
L. japonica ♀×*A. crassifolia* ♂	20	4	0	4
L. angustata ♂×*A. crassifolia* ♀	20	H 8	0	8
L. angustata ♀×*A. crassifolia* ♂	21	4	0	4
L. religiosa ♂×♀	20	I 14	11	3
L. religiosa ♀	20†	7	0	7
L. japonica ♂×♀	15	J 12	10	2
L. japonica ♀	20†	4	0	4
L. ochotensis ♂×♀	17	K 14	11	3
L. ochotensis ♀	20†	7	0	7
L. angustata ♂×♀	20	L 15	13	2
L. angustata ♀	20†	4	0	4
A. crassifolia ♂×♀	18	M 13	12	1
A. crassifolia ♀	20†	5	0	5

* Number of female gametophytes producing a greater (smaller) number of normal sporophytes than the abnormal ones

Table XXVII. Details of the results of crossing (A–H) and parthenogenesis (I–M) experiments as shown in Table XXVI

Crossing No.	Number of sporophytes produced	Number of abnormal sporophytes
A♂×♀		
1	85	6
2	72	3
3	54	9
4	43	2
5	40	0
6	38	1
7	21	3
8	18	2
9	17	0
10	15	1
11	14	1
12	12	0
13	12	1
14	8	2
15	5	4
16	3	3
A♀×♂		
1	130	4
2	84	4
3	62	2
4	51	1
5	50	0
6	47	0
7	45	0
8	32	1
9	30	0
10	15	1
11	4	1
12	2	2
B♂×♀		
1	56	3
2	43	2
3	40	1
4	37	0
5	29	2
6	28	1
7	25	1
8	25	3
9	20	2
10	17	1
11	16	1
12	9	0
13	8	0
14	7	6
15	6	4

Crossing No.	Number of sporophytes produced	Number of abnormal sporophytes
B♀×♂		
1	46	2
2	45	1
3	37	0
4	34	0
5	34	1
6	31	1
7	30	2
8	27	0
9	25	1
10	21	1
11	21	0
12	17	2
13	15	2
14	6	1
15	3	3
16	3	3
C♂×♀		
1	21	21
2	18	17
3	17	17
4	17	15
5	9	8
6	8	8
7	5	3
8	4	3
C♀×♂		
1	15	14
2	13	13
3	13	12
4	10	8
5	8	5
6	6	4
7	6	4
8	4	3
9	4	4
10	3	3
D♂×♀		
1	34	1
2	33	1
3	27	2
4	26	1
5	18	0
6	17	1
7	16	0
8	16	1
9	8	0
10	4	1
11	3	3
12	3	2

(Continue)

Table XXVII. (Continued)

Crossing No.	Number of sporophytes produced	Number of abnormal sporophytes
D♀×♂		
1	51	1
2	47	2
3	31	0
4	30	1
5	15	3
6	14	1
7	14	1
8	12	0
9	9	0
10	6	0
11	6	1
12	5	0
13	3	3
E♂×♀		
1	18	18
2	15	14
3	15	15
4	8	8
5	8	8
6	4	1
E♀×♂		
1	15	14
2	14	13
3	11	11
4	11	10
5	5	5
6	4	4
F♂×♀		
1	15	15
2	8	7
3	5	5
F♀×♂		
1	12	11
2	4	4
3	4	4
4	2	2
G♂×♀		
1	25	24
2	16	16
3	14	14
4	13	13
5	13	12
6	7	7
7	7	6
8	5	5
9	3	3
10	3	3
11	1	1

Crossing No.	Number of sporophytes produced	Number of abnormal sporophytes
G♀×♂		
1	8	8
2	8	8
3	7	6
4	5	5
5	4	4
6	2	2
H♂×♀		
1	18	17
2	17	15
3	12	12
4	12	12
5	8	8
6	8	7
7	3	3
8	3	3
H♀×♂		
1	15	14
2	11	11
3	6	6
4	5	5

Couple or female No.	Number of sporophytes produced	Number of abnormal sporophytes
I♂×♀		
1	44	3
2	39	4
3	39	2
4	37	2
5	32	0
6	28	2
7	28	3
8	24	3
9	23	2
10	15	3
11	13	0
12	5	3
13	5	4
14	1	1
I♀		
1	21	18
2	15	14
3	15	13
4	13	13
5	8	8
6	8	7
7	4	3

(Continue)

Table XXVII. (Continued)

Couple or female No.	Number of sporophytes produced	Number of abnormal sporophytes
J ♂×♀		
1	43	1
2	40	2
3	36	0
4	34	0
5	31	0
6	21	1
7	19	0
8	18	0
9	18	0
10	9	1
11	8	7
12	5	4
J ♀		
1	15	15
2	12	11
3	10	10
4	9	9
K ♂×♀		
1	52	3
2	48	2
3	47	4
4	39	4
5	38	2
6	38	1
7	32	0
8	30	0
9	28	2
10	25	1
11	18	1
12	5	4
13	3	2
14	2	2
K ♀		
1	18	17
2	7	7
3	7	7
4	5	5
5	2	2

Couple or female No.	Number of sporophytes produced	Number of abnormal sporophytes
L ♂×♀		
1	32	2
2	32	1
3	28	0
4	27	0
5	27	2
6	25	1
7	21	0
8	18	0
9	17	1
10	17	1
11	15	0
12	14	0
13	10	1
14	10	7
15	5	4
L ♀		
1	8	8
2	7	7
3	4	4
4	4	3
M ♂×♀		
1	48	2
2	40	1
3	39	0
4	35	0
5	35	0
6	32	0
7	28	0
8	28	0
9	25	0
10	21	0
11	20	0
12	12	2
13	7	4
M ♀		
1	8	8
2	7	7
3	5	5
4	5	4
5	2	2

On the other hand, the results of the crossing experiments among *Laminaria religiosa*, *L. japonica* and *L. ochotensis* agreed quite well with the former results of the above-mentioned single-species experiments, while the results of the crossing experiments between one of the afore-mentioned three species of *Laminaria* and *L. angustata* or *Alaria crassifolia* and between *L. angustata* and *A. crassifolia* agreed quite well with the results of the above-mentioned parthenogenesis experiments. From these results it can be concluded that the interspecific crossing was successful among the three closely allied species of *Laminaria*, viz., *L. religiosa*,

Table XXVIII. Details of the results of crossing experiments (N & O) between *Laminaria japonica* and *L. religiosa* (N), and between *L. japonica* and *L. diabolica* (O), observed during the period from February 26 to March 14 in 1958

Crossing No.	Number of sporophytes produced	Number of abnormal sporophytes
N♂×♀		
1	68	4
2	52	2
3	50	3
4	48	4
5	48	1
6	46	0
7	43	0
8	43	0
9	42	2
10	42	1
11	38	2
12	37	0
13	36	0
14	36	1
15	35	0
19	33	0
17	33	0
18	32	1
19	30	1
20	29	0
21	18	1
22	16	1
23	13	2
N♀×♂		
1	58	4
2	56	6
3	54	1
4	49	1
5	48	0
6	48	0
7	45	0
8	45	1
9	36	2
10	36	0
11	34	3
12	32	0
13	32	1
N♀×♂		
14	28	1
15	28	1
16	26	0
17	24	0
18	18	0
19	15	0
20	15	1
21	12	1
22	11	0
23	11	6
24	8	6
O♂×♀		
1	62	0
2	60	0
3	54	1
4	54	0
5	51	0
6	50	1
7	43	2
8	43	1
9	41	0
10	38	1
11	38	2
12	36	3
13	35	5
14	25	0
O♀×♂		
1	24	0
2	21	0
3	21	0
4	20	0
5	20	2
6	15	0
7	14	1
8	12	1
9	11	0
10	11	1
11	9	1

L. japonica and *L. ochotensis*, but it failed to take place between one of the afore-mentioned three species of *Laminaria* and *L. angustata* or *Alaria crassifolia* and between *L. angustata* and *A. crassifolia*. The male gametophyte placed together with a female in one and the same vessel usually became fertile when observed during the period from February 20 to March 16, 1956, but rarely remained sterile leaving the eggs on the female gametophyte unfertilized and often developing into parthenosporophytes. Such exceptional cases in the crossing experiments are seen in the crossing No. 15 of the couple A ♂×♀, No.

14 of B ♂×♀, and No. 15 of B ♀×♂, in Table XXVII.

2. Experiments during the period from October 1957 to May 1958

The results of the present experiments are shown in Table XXVIII. The crossing results of the couples N ♀×♂ and N ♂×♀ are naturally identical with those of the couples A ♂×♀ and A ♀×♂ shown in Table XXVII. In the crossing experiments of the couples O ♂×♀ and O ♀×♂, 98 per cent of the female gametophytes produced sporophytes and most of them were normal in shape. Thus, the interspecific crossing is considered to have been proved to be successful between *L. japonica* and *L. diabolica*.

IV. Development of gametophytes of *Laminaria religiosa* in the sea observed at Oshoro

1. Development on the slide glasses settled in Oshoro Bay

Twelve concrete blocks, each holding five slide glasses, were settled in Oshoro Bay on October 13, 1953, in the midst of the *Laminaria religiosa* beds at a depth of 1.5 m or less below the low water mark. Five days later, eight slides were taken out from the blocks and examined under the microscope. They were densely covered with microscopic germling of Phaeophyceae and Chlorophyceae, but it was impossible to detect *Laminaria* gametophytes among them. These eight slides were put into the glass tubes containing sea-water and brought to Hakodate for further observations.

On October 13, 1953, one more concrete block was settled in Oshoro Bay. This block held five slide glasses on which the zoospores of *L. religiosa* had been sown on the previous day. All of these five slides were taken out from block on October 18, when the gametophytes on them were still all sterile and composed of two to three cells in the females and five to nine cells in the males. A photomicrograph of the gametophytes on one of these slides which had been placed in Schreiber's solution for twenty-five days after taking it out from the sea is reproduced in Pl. XXXII, Fig. F.

From the remaining blocks which had been settled in the Bay since October 13, eleven slide glasses were taken out on November 8, of which nine slides from the blocks settled near the low water mark, one from a block at the depth of about 50 cm, and one from a block at the depth of about 1.5 m below the low water mark. Four of the afore-mentioned nine slide are shown in Pl. XXVIII, Figs. A–C. They were found to bear a number of *Laminaria* sporophytes visible to the naked eye, up to about 3 mm in length, and many other microscopic *Laminaria* sporophytes growing upon the thalli of the mature gametophytes (Pl. XXIX,

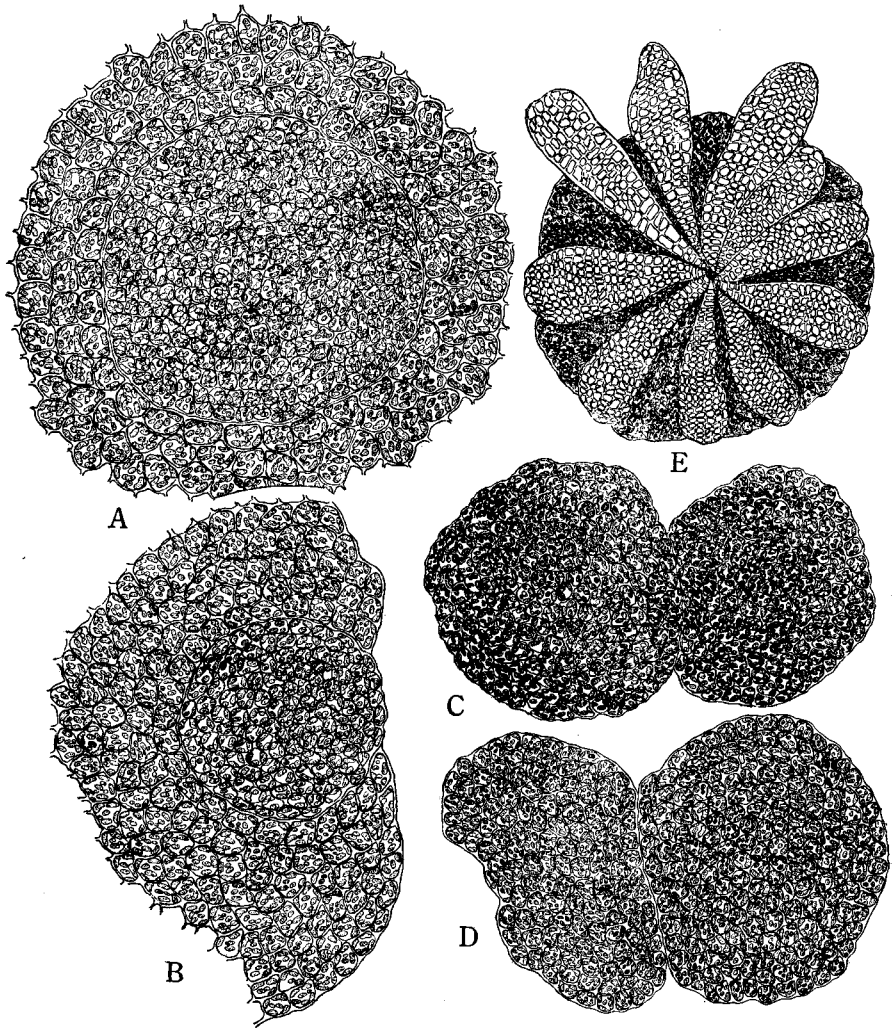


Fig. 22. *Laminaria religiosa* Miyabe (?)

Disk-shaped gametophytes developed in nature on the slide glasses settled in Oshoro Bay during the period from October 13 to November 8 in 1953

A & B. Male gametophytes surrounded by the cells of the female disks

C & D. Male disks

E. A mature disk-shaped female gametophyte on which ten young sporophytes are grown radiately

A–D, ×517; E, ×172

Figs. E & F; Pl. XXX, Figs. A–D; Text-fig. 22, E). Many other germlings, probably immature gametophytes of *Laminaria*, were also found attached to the slide (Pl. XXXI, Figs. A–F; Text-fig. 22, A–D). Most of these gametophytes were disk-shaped, consisting of numerous cells but sometimes their thalli consisted of long

uniseriate filaments (Pl. XXXII, Figs. A–C). The gametophytes on the slide from the depth of 1.5 m below the low water mark were also disk-shaped but their disks were smaller in size than those from near the low water mark, and were provided with many erect uniseriate, rarely branched, filaments. The gametophytes on the slide from the depth of about 50 cm below the low water mark were also disk-shaped, either provided with erect uniseriate filaments or not, while those from the depth of about 1.5 m below the low water mark were provided on their disks with longer and more numerous filaments.

Of the eight slides that have been brought to Hakodate and kept in Petri dishes containing Schreiber's solution, four were cultured in the corridor and the remaining four in the laboratory. When observed on November 10, the filamentous thalli of the Phaeophycean germlings on these slides consisted of about forty to fifty cells and a few young sporophytes of *Laminaria* were found to grow on the germlings in the dishes placed in the corridor.

On December 2, five concrete blocks were placed in Oshoro Bay. After 18 days, nine slide glasses were taken out from these blocks and kept in vats filled with sea-water at Oshoro Marine Biological Laboratory. On January 5, 1954, these slides were observed to bear young sporophytes of *Laminaria*, up to about 7 mm long, growing upon them. At the base of these sporophytes were observed disk-shaped gametophytes consisting of numerous cells and producing occasionally erect uniseriate filaments (Pl. XXXIII, Fig. A). Some of the slides from a block settled near the low water mark were found to bear young fronds of a brown alga which closely resembled the young sporophytes of *Laminaria* but differed from them in having colorless hairs. They are considered to be germlings of *Heterochordaria abietina* (Pl. XXXIII, Figs. C & D).

On February 21, 1954, three blocks were settled in the Bay and left there until March 8, when only one slide was recovered from these blocks. On this slide, the writer could detect two young sporophytes of *Laminaria* with the naked eye, but no other sporophytes were found in spite of carefull observation under the microscope. The growth of the *Laminaria* gametophytes on this slide was also far less abundant than that on the slides settled in autumn and recovered in November or December in 1953. In early March 1954, a male gametophyte of *Laminaria* was found growing beneath a disk-shaped germling of a Corallinaceous alga on a slide recovered from a block on November 8 and placed under room temperature in a vessel containing Schreiber's solution unchanged (Pl. XXXII, Fig. E). The gametophyte was in a normal condition while the germling was partly colorless in its marginal portion.

Some of the filaments developed from the *Laminaria* gametophytes were

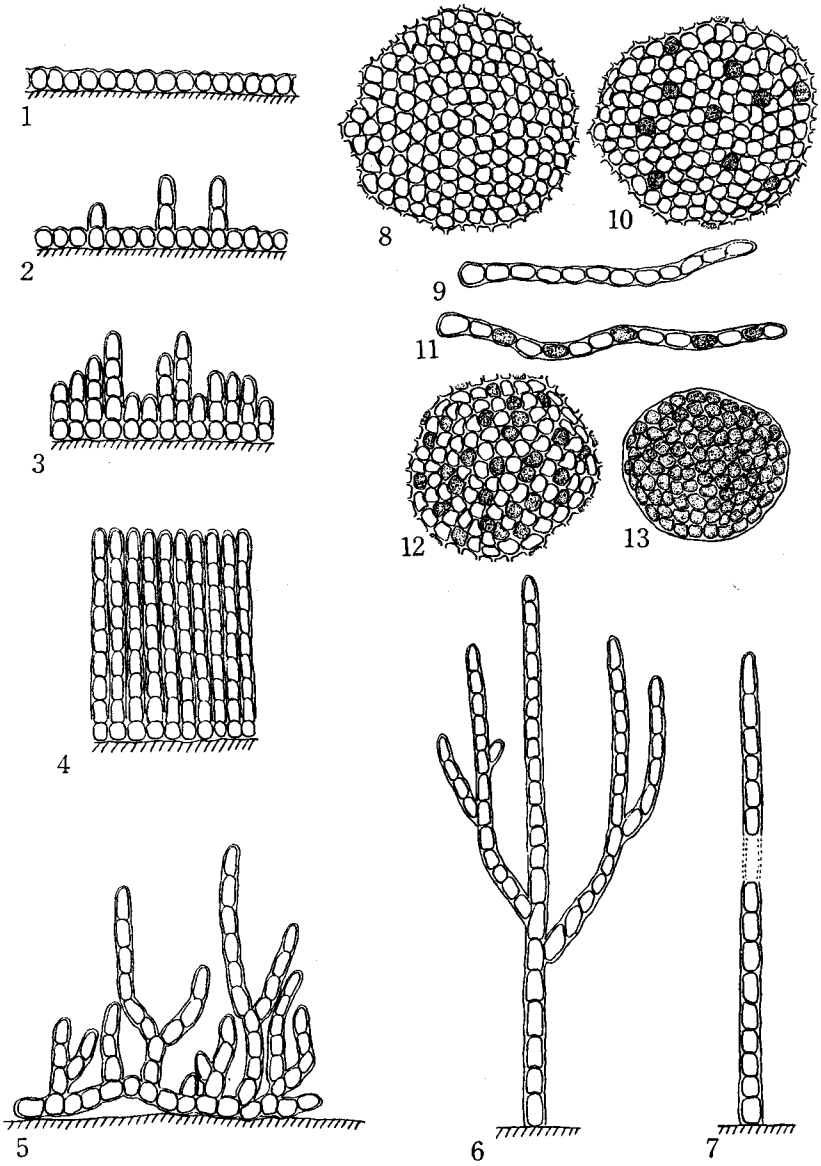


Fig. 23. Diagrammatical figures showing the shape of the gametophytes of *Laminaria religiosa* Miyabe growing at various depths of water in Oshoro Bay. Shaded cells in 10–13 are those bearing an erect uniseriate filament

1, Side view of gametophyte growing near the low water mark; 2–4, Side view of gametophytes growing at a depth of about 50–150 cm below the low water mark; 5–7, Side view of gametophytes growing at deeper levels than above; 8–9, Surface view of gametophytes growing near the low water mark; 10–13, Surface view of gametophytes growing at deeper levels than above

isolated by means of a pointed pincette in mid-November 1953 from the slide which had been settled in the Bay from October 13 to November 8 in 1953. They were cultured in the glass vessels containing Schreiber's solution placed either in the laboratory or in the corridor. When observed in late March 1954, they were found to have grown to profusely branched filaments consisting of numerous cells and to have become fertile in the vessels placed in the corridor.

From one of the slide glasses which had been placed in Oshoro Bay during the period from the 2nd to the 21st of December, 1953, the writer tried to remove carefully all the mature male and female gametophytes of *Laminaria* and other germlings by means of a pincette in late December 1953, so as to load the slide only with the sterile gametophytes of *Laminaria*. This slide was kept in Schreiber's solution under room temperature until September 1954, and then removed to the corridor. In late December 1954, the gametophytes became fertile, and eventually produced young sporophytes of *Laminaria* (Pl. XXXIV, Figs. A-E).

From the results of the present investigation on the development of the

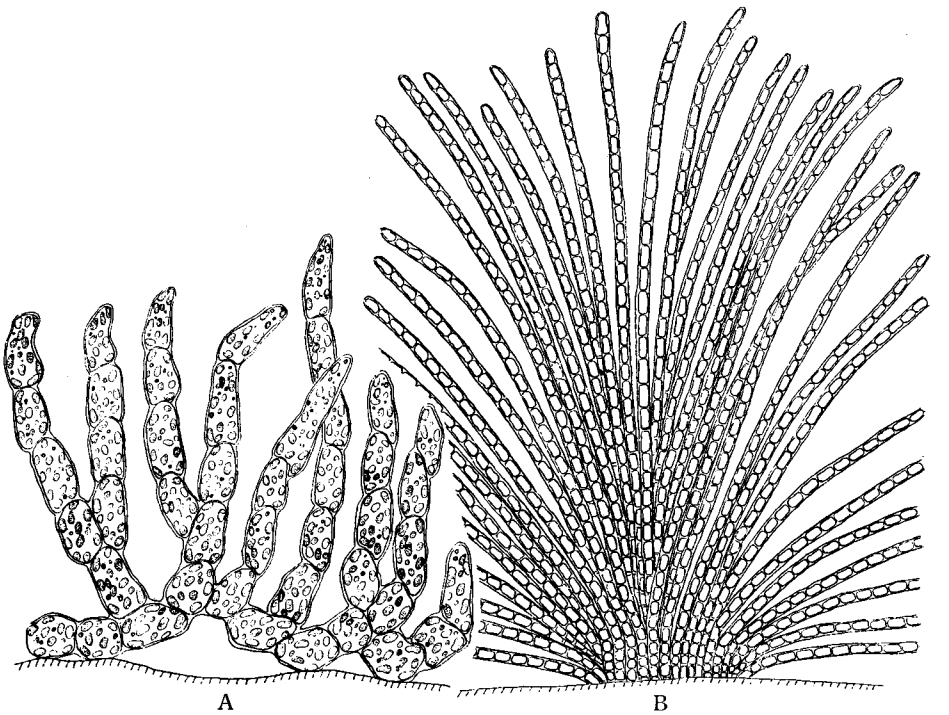


Fig. 24. *Laminaria religiosa* Miyabe (?)

Side view of immature female gametophytes attached in nature to stones collected in Oshoro Bay in November 1953

A, ×517; B, ×172

gametophytes of *Laminaria* on the slide glasses settled in Oshoro Bay and of the culture experiment of the gametophytes in a long tube covered with black paper, it is concluded that there is a close relation between the shape of the gametophytes and the depth of their habitat in the sea, as shown in Text-fig. 23.

2. Development on rocks in Oshoro Bay

On December 2, 1954, the writer collected several young sporophytes of *Laminaria*, about 1.5 cm in length, growing in nature on a few pieces of rocks taken from among an exclusively pure vegetation of *L. religiosa* at a depth of about 1 m below the low water mark in Oshoro Bay. From the base of those young sporophytes, the writer could separate by means of a pincette a number of filamentous thalli of the female gametophyte (Pl. XXXIII, Fig. B). The thinner filaments which were found growing on those pieces of rocks are considered to be the male gametophytes though no fertile branches could be observed on them. In Pl. XXXV, Figs. A–F are shown similar gametophytes collected in nature from the base of young sporophytes of *L. japonica*, about 1–2 cm in length, growing on rock and on the thalli of *Chondrus ocellatus* which attached to rocks at a depth of about 1 m below the low water mark at Nanaehama, in mid-February, 1956.

V. Discussion

In many studies on the embryonal development of the Laminariaceous plants that have been reported to date, the female gametophytes are often described as usually consisting of only one to several cells. Schreiber (1930) was the first to report that the isolately cultured gametophytes, either female or male, have grown to large thalli consisting of numerous cells in his culture studies of three species of *Laminaria*, viz., *L. saccharina*, *L. digitata*, and *L. hyperborea*. Hollenberg (1933) also described well developed gametophytes in *Eisenia arborea*. Recently, Sundene (1952) obtained well developed gametophytes in his crossing culture experiments with three varieties of *L. digitata*. In Japan, Kurogi & Akiyama (1957) and Segi & Kida (1958) observed large gametophytes consisting of numerous cells in their culture studies of *Undaria pinnatifida* and *U. undarioides* respectively. In the writer's culture studies on the parthenogenesis of nine species of Laminariaceae reported in the present paper, the isolately cultured gametophytes were all well developed consisting of numerous cells, from several dozens to several hundreds in number, with the exception of the female gametophytes obtained from the samples of *L. japonica* collected at Zenikamezawa on October 27, 1954. Effects of inorganic nutrient salts and light intensity on the development of the Lami-

nariaceous gametophytes had already been studied in detail by Harries (1932). In the present investigations, the writer could prove that the gametophytes' growth is greatly affected not only by light intensity and water temperature but also by the population density in the unit area. The above-mentioned well developed gametophytes obtained by Schreiber (1930) in his culture were those growing in the least density per unit area. Ikari (1921) cultured *L. religiosa* by pouring a zoospore solution into the culture vessels in which the slide glasses were laid on the bottom, and reported that the growth of the gametophytes was much better on the side walls of the vessels than on the slide glasses. In such a culture method, the zoospore attachment is much more abundant on the slide glasses than on the side walls of the vessels. In the writer's culture of the zoospores from *L. religiosa* collected at Oshoro Bay on October 12, 1952, many gametophytes were observed floating on the surface of the culture solution. Their growth was much better than the growth of those attached to the bottom of vessels. In this case too, the population density of the floating gametophytes was much smaller than that of those attached. Thus the population density per unit area seems to have an effect on the growth of the gametophytes.

The relation between the temperature and the maturity of the Laminariaceous gametophytes was investigated by Ueda (1929), Schreiber (1930), Kinoshita (1949), and Saito (1956). They all reported that the gametophytes attained maturity under lower temperature. In *L. religiosa*, it was observed by Ueda (1929) and the writer that the sporophytes were produced under temperature ranging between 5.5°C and 11.3°C or below 12°C, respectively. Schreiber (1930), cooling the culture solution in mid-June as low as 2° to 4°C, succeeded in inducing nine days later the egg formation on three isolately cultured female gametophytes of *L. saccharina*. The writer was also able to induce the production of the sporophytes in mid-July on the isolately cultured female gametophytes of *L. japonica* and *A. crassifolia* by cooling the culture solution. In his culture experiment of *L. religiosa* carried out by placing the culture vessels alternately under higher and lower temperature at constant intervals of five, ten or fifteen days, the gametophytes did not become fertile at an interval of five days. From this result it can be concluded that a certain period of low temperature is necessary for the maturation of the gametophytes.

Growth and maturation of the gametophytes of *L. japonica* in the writer's culture showed some variation according to the sea-water samples which were taken from the sea to be used as a culture solution in five different months, viz., May, July, September, October, and November. Such a variation in the growth of gametophytes is supposed to have occurred in the cultures by Kanda (1936–1946)

who obtained unparallel results in his repeated culture experiments with *L. religiosa*, *L. diabolica*, and *L. cichorioides*.

Maturation of the gametophytes is supposed to be hindered when an excess amount of nutrient salts is contained in the culture solution since the writer could observe that the gametophytes of *A. crassifolia* became fertile when cultured in sea-water alone but not when cultured in enriched Schreiber's solution containing ten times the ordinary amount of its ingredients. Papenfuss (1942) observed that the gametophytes of *L. pallida* and *Macrocystis pyrifera* attained maturation when cultured in sea-water alone but not in Schreiber's solution, so he assumed that the lack of nutritive salts in the solution has induced their maturation. However, in view of the result of the writer's above-mentioned culture experiment, it is supposed that the nutrients in the sea-water sample used by Papenfuss in preparing the Schreiber's solution was rich enough to make it contain an excessive amount of nutrient salts.

The relation between the light intensity and the growth of gametophytes was studied by Harries (1932) who reported that the growth was better under somewhat weaker light as a result of his cultures exposed for 10 hours per day to constant light of various intensities. Segi and Kida (1957, 1958) also observed a better growth of the gametophytes under weaker light in their culture of *U. undarioides* exposed to light controlled with semi-transparent vinyl cloth. On the other hand, Saito (1956) reported that the gametophytes of *U. pinnatifida* attained normal growth and produced many sporophytes when cultured under average daytime light intensity of about 2000 Lux, but their growth stopped when cultured under weaker light intensities, viz., 500 Lux, 200 Lux, or 50 Lux. In the writer's culture experiment with *L. religiosa*, the gametophytes grew well under somewhat dim light but they attained maturity under bright light.

The result of the writer's experiment of parthenogenesis with the nine species of Laminariaceae in Hokkaido agrees well with the report of similar experiment given by Schreiber (1930) in that most of the parthenosporophytes produced were abnormal in shape even in their juvenile stages consisting of a few cells.

The crossing experiment carried out during the period ranging from October 1955 to May 1956 with *L. japonica*, *L. religiosa*, *L. ochotensis*, *L. angustata* and *A. crassifolia* gave the result that the interspecific fertilization was supposed to have taken place among the former three species, as reported in a preliminary paper presented to the Ninth Pacific Science Congress, 1957, held in Bangkok through the kindness of Professor G. F. Papenfuss (Tokida & Yabu, 1962). The crossing between *L. japonica* and *L. diabolica* was tried late, from October 1957 to May 1958, and a positive result was again obtained. These results seem to

suggest a possibility of the specific identity of those four species of *Laminaria*. Okamura (1936, p. 248) already treated *L. ochotensis* and *L. fragilis* Miyabe as synonyms of *L. japonica* and expressed his view that *L. fragilis* is considered to be nothing but a local form growing in calm water in bays and *L. ochotensis* to be another local form or variety growing vigorously around the two islets, Rishiri and Rebun, which are situated at a junction of distal reaches of warm and cold ocean currents that wash the coast of Hokkaido, just as *L. japonica* grows vigorously in the region near Hakodate which is situated at another junction of distal reaches of those currents. He also stated that the broad thick specimens of *L. ochotensis* collected by Dr. K. Okada at Otomari in Aniwa Bay, Saghalien, were quite identical with *L. japonica* from Hakodate. Recently, Hasegawa (1959) gave a review on the concepts of the previous investigators about the specific correlations among the above mentioned allied species of *Laminaria*, and supported Okamura's view in mentioning *L. ochotensis* and *L. fragilis* as synonyms under *L. japonica*. He committed an error, however, in stating that Okamura had treated *L. diabolica* as a variety of *L. japonica*. Okamura (1936, p. 250) placed var. *diabolica* Miyabe under *L. longipedalis* Okamura on the authority of Miyabe (1926, 1933). Close affinities which were suggested to exist between *L. japonica* and *L. diabolica* by Yamada (1949) and between *L. ochotensis* and *L. diabolica* by Kanda (1946) find now a strong support in the results of the writer's crossing experiments reported in the present paper. In case the transplanting experiments of the species concerned, which are now under way, bring a decisive result in support of their suggested affinities, the varietal names of *L. japonica* will be as follows: *Laminaria japonica* Areschoug var. *japonica*, *L. japonica* var. *religiosa* (Miyabe) Tokida et Yabu, *L. japonica* var. *ochotensis* (Miyabe) Okamura, and *L. japonica* var. *diabolica* (Miyabe) Tokida et Yabu.

Observations of the gametophytes developed in nature were made with *L. religiosa* in Oshoro Bay and with *L. japonica* at Hakodate. The shape of their thalli was markedly different from that of the cultured ones. The gametophytes of *L. religiosa* which attached in nature to the slide glasses settled with concrete blocks in Oshoro Bay varied in shape with the level of their settlement in the sea, taking a disk form near the low water mark but becoming smaller disks provided with erect filaments in lower levels. These naturally developed gametophytes in the sea consisted of more numerous cells than those cultured in the vessels. This fact is supposed to be attributable to their paucity in number of individuals per unit area and to the abundance of nutrients in the coastal water.

The disk-shaped thalli of the gametophytes growing near the low water mark

are probably due to the wave action that prevails there, and the formation of erect filaments on the disks at lower levels in the sea is supposed to be a response to the diminished light intensities.

L. religiosa in Oshoro Bay sometimes begins to discharge zoospores as early as mid-August. The germlings from those zoospores continue their vegetative growth until the water temperature goes down as low as 11°C in mid-October and they attain a fairly large thallus consisting of numerous cells before maturation is attained. As a matter of fact, many sterile gametophytes consisting of numerous cells were observed in nature even in winter. So, it may be assumed that some female gametophytes in the sea would produce sporophytes after spending a number of years in a sterile state.

VI. Summary

1. In the present paper are reported the results of investigations on the interrelation between some cultural factors and the development of the gametophytes of *Laminaria japonica* and *L. religiosa*, and on the parthenogenesis and the crossing of several species of Laminariales in Hokkaido.

2. Gametophytes of *Laminaria religiosa* were cultured in two ways: (1) with various population densities per unit area in a fixed volume of culture solution, and (2) with a fixed population density per unit area in various volumes of culture solution. It was observed that the growth of gametophytes has been affected by the population density, but not by the volume of the culture solution. The gametophytes grew better in the vessels with a small population density.

3. Gametophytes of *L. japonica* were cultured in the vessels placed in four tanks which were regulated to maintain water temperature constantly at about 20°C, 12°C and 8°C, respectively, and in other vessels which were placed in the daytime, from 9 to 18 h, in a tank regulated to maintain water temperature at 20°C, but placed in the night, from 18 to 9 h, in the corridor to be exposed to the air temperature. The growth of gametophytes was observed to be best in the vessels placed in the tank regulated to maintain water temperature at 20°C in the daytime only, while it was good in the vessels kept at constant 16°C, not good at constant 12°C and 8°C, and worst at constant 20°C. Maturation of both female and male gametophytes was observed in the cultures kept constantly at 8°C and 12°C.

4. Gametophytes of *L. japonica* were cultured in the vessels placed alternately in the heated laboratory and in the unheated corridor at intervals of 5 days, 10 days or 15 days. Sporophytes were produced in the vessels at intervals of 10 days and 15 days, but not in those at intervals of 5 days.

5. Gametophytes of *L. japonica* were cultured under various light intensities. They grew well under the medium light intensities, 400–2500 Lux, but not well under the higher, 4400–4100 Lux, nor under the lower intensities, 50 Lux. When cultured in a tube, about 1.5 m long, covered with black paper, except its top, the growth and maturation of gametophytes on a slide glass were observed to differ with the depth of the settlement of the slide in the tube or the change of light intensities.

6. Gametophytes of *L. japonica* were cultured in sea-water mixed with various proportions of fresh water, viz., 5%, 10%, 20%, 30%, 40% and 50%, and also in sea-water evaporated to one-half or one-third of its original volume. It was observed that the gametophytes have grown normally and become fertile in sea-water evaporated to one-half of its original volume and in sea-water mixed with 5%, 10%, and 20% of fresh water.

7. Gametophytes of *L. japonica* were cultured in sea-water samples obtained in five different months, viz., March, July, September, October, and November, with no remarkable difference observed in their vegetative growth. However, the development of their reproductive organs seemed to have been promoted by the sea-water samples obtained in winter months.

8. Gametophytes of *L. japonica* were cultured in the sea-water sample obtained in July 1956 and enriched with some amounts of sodium nitrate, sodium phosphate or soil extract. The results obtained seem to suggest that the sodium nitrate is more effective for the growth of gametophytes than sodium phosphate, while sodium phosphate is more effective for the formation of oogonia than sodium nitrate.

9. Female gametophytes of the following nine species were cultured in isolation: *Laminaria religiosa, L. japonica, L. angustata, L. angustata* var. *longissima, L. ochotensis, L, diabolica, Alaria crassifolia, Undaria pinnatifida* f. *distans* and *Arthrothamnus bifidus*. It was ascertained that the eggs produced from the isolated female gametophytes of these species usually developed parthenogenetically but rarely lost their vitality. Most of those parthenosporophytes were observed to be abnormal in shape even in early stages of their development.

10. Crossing experiments were tried among five species, viz., *Laminaria religiosa, L. japonica, L. ochotensis, L. angustata* and *Alaria crassifolia*, and between *L. japonica* and *L. diabolica*. Considering the results obtained, it was concluded that the interspecific fertilization had taken place among three species, viz., *L. religiosa, L. japonica*, and *L. ochotensis*, and between *L. japonica* and *L. diabolica*.

11. Gametophytes of *L. religiosa* which have grown on the slide glasses

settled in Oshoro Bay near the low water mark were mostly disk-shaped consisting of numerous cells. On the glasses placed at about 1.5 m below the low water mark, the gametophytes were mostly disk-shaped too, but they were smaller in size than those from near the low water mark and were provided with many erect filaments consisting of uniseriate cells. From the results of this experiment and of the culture in a long tube covered with black paper mentioned above, it is supposed that there is a close interrelation between the shape of the gametophytes and the depth of their habitat in the sea as shown diagrammatically in Text-fig. 23.

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Explanation of Plates

PLATE I

Laminaria religiosa Miyabe

A & B. Parthenosporophytes developed on the female gametophytes isolated in December 1952 from the material collected at Oshoro Bay on November 25, 1952. From a culture 49 days old after isolation

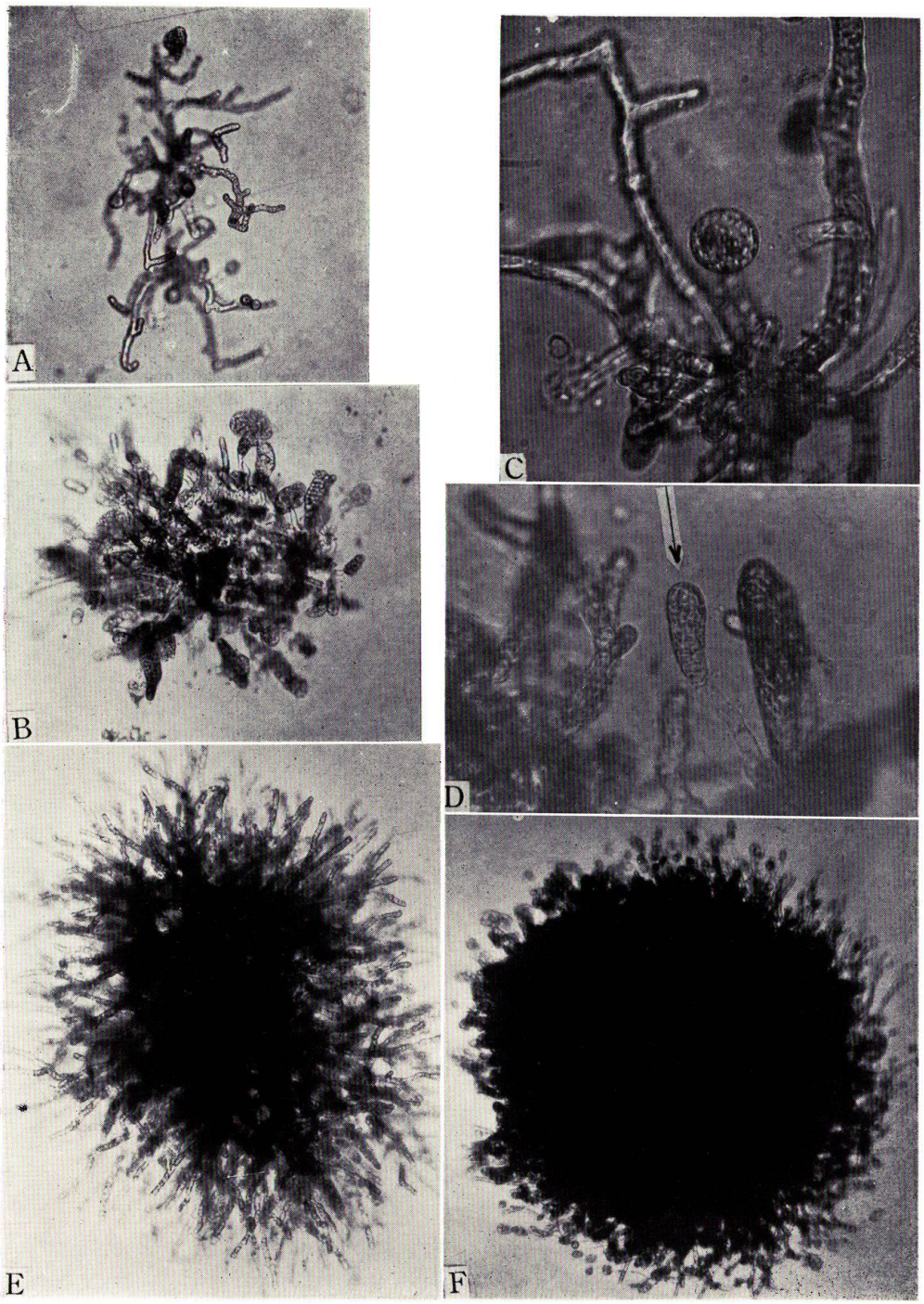
C. Part of gametophyte shown in Fig. A, showing an egg just discharged

D. Part of a female gametophyte isolated in December 1952 from the material collected at Oshoro Bay on November 25, 1952. From a culture 49 days old after isolation. A young parthenosporophyte (pointed by an arrow), composed of seven uniseriate cells, is normal in shape

E. A sterile female gametophyte, 3.7 months old, isolated from the material collected at Oshoro Bay on October 12, 1952

F. A mature female gametophyte which has grown from a state as shown in Fig. E within three weeks after transferred to a newly prepared Schreiber's solution

A, B, E & F, ×80; C & D, ×320



H. Yabu: Early Development of Laminariales

PLATE II

Laminaria religiosa Miyabe

A–G. A female gametophyte isolated in December 1952 from the material collected at Oshoro Bay on November 25, 1952, showing various stages of its development

A & B. Two months old stage, photographed by focussing at different levels to show a malformed parthenosporophyte in B

C & D. Two months and half old stage, photographed by foccusing at different levels

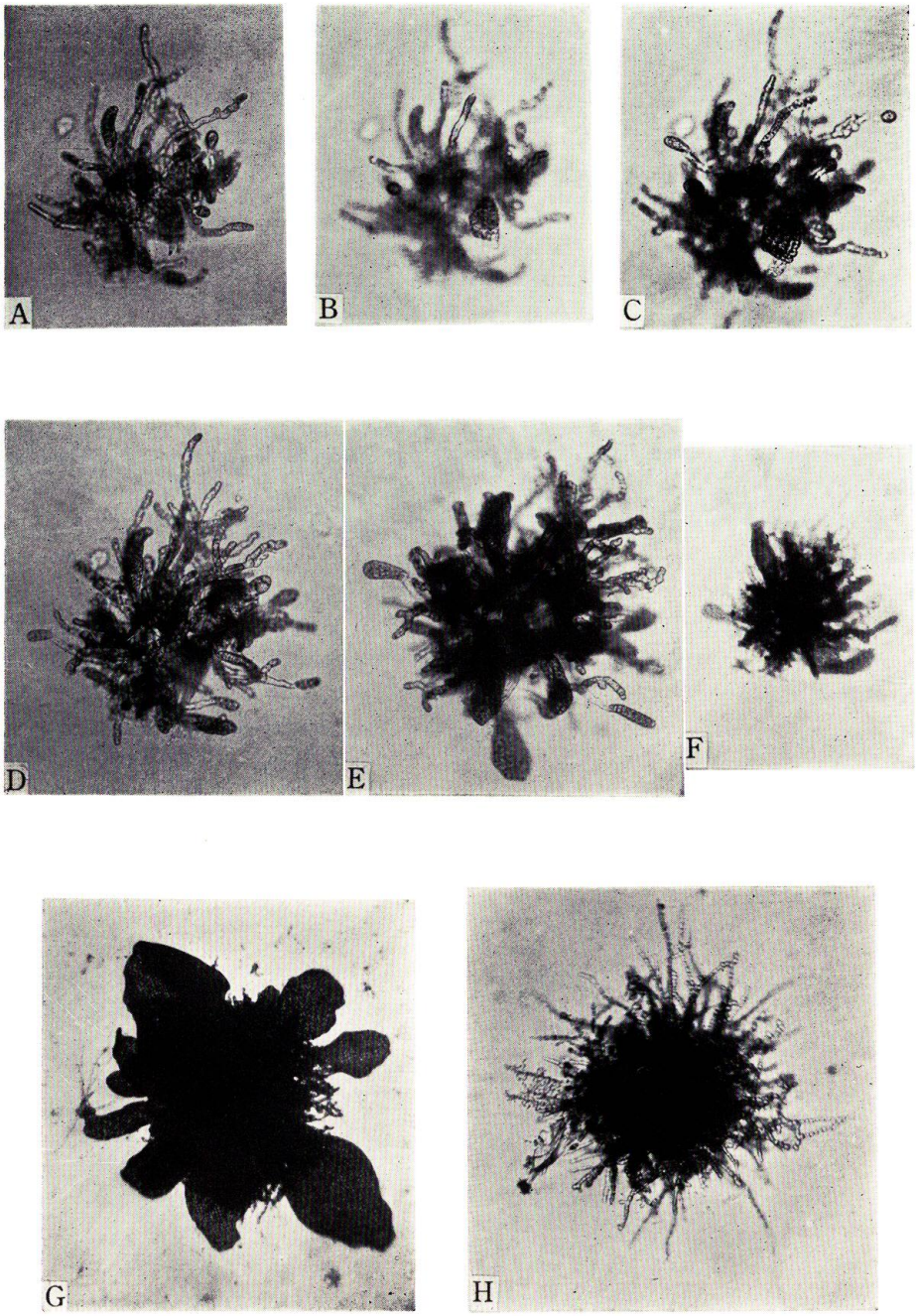
E. Three months old stage

F. Four months and half old stage

G. Five months old stage

H. A male gametophyte isolated in December 1952 from the material collected at Oshoro Bay on November 25, 1952, at six months old stage of its development

A–E & H, G, ×80; F & G, ×50



H. Yabu: Early Development of Laminariales

PLATE III

Laminaria religiosa Miyabe

Gametophytes and sporophytes developed from the material collected at Oshoro Bay on October 12, 1952

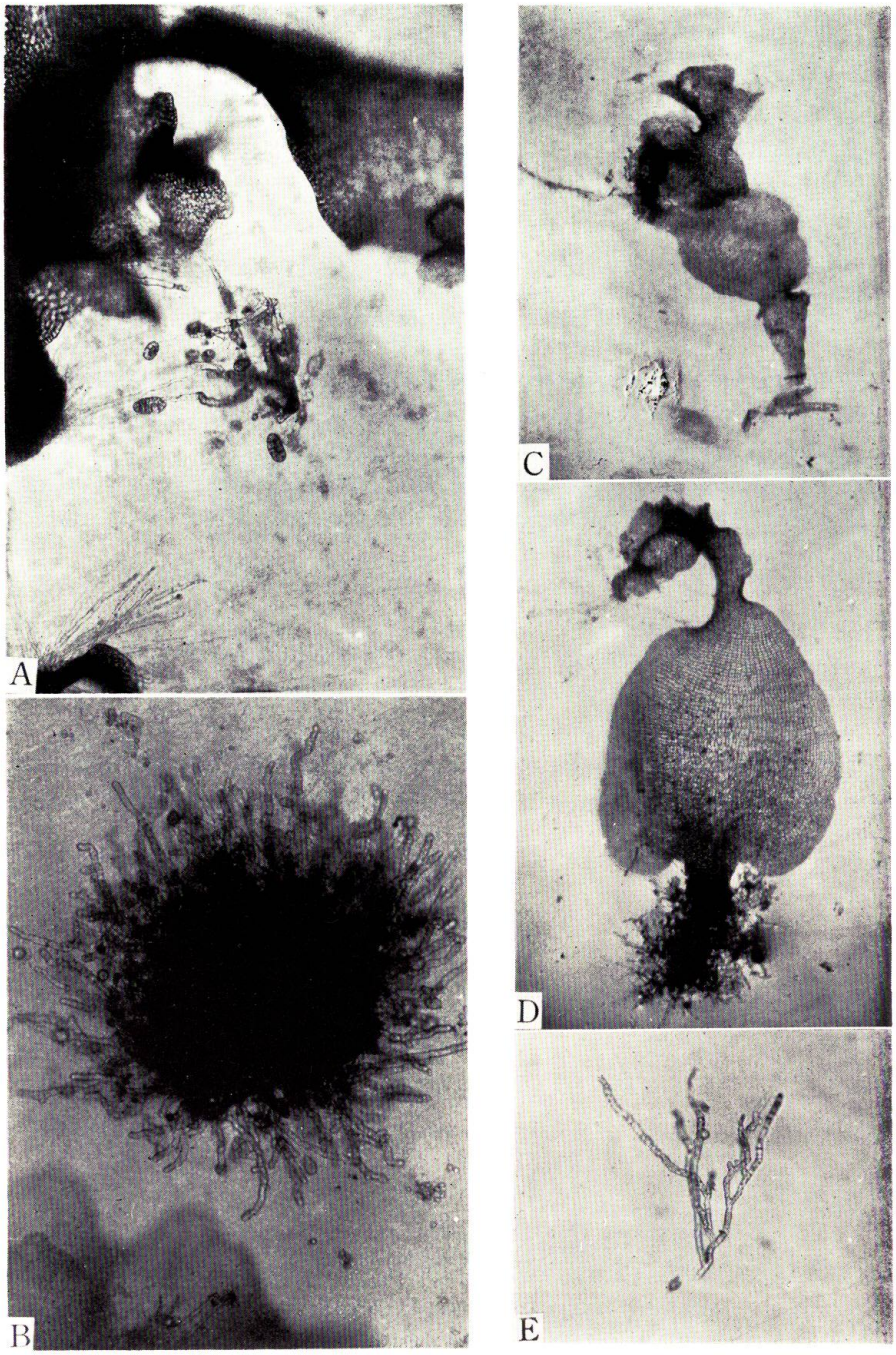
A. Seventy-seven days old female gametophyte attached to the bottom of the culture dish

B. Seventy-seven days old female gametophyte floating on the surface of the culture solution

C & D. Malformed sporophytes developed on the female gametophytes floating on the surface of the culture solution

E. A piece of filament taken from a floating three months old female gametophyte with the purpose of studying its subsequent development; it has no direct connection with the female gametophyte illustrated in Pl. II as elucidated by mistake in Tokida & Yabu (1957), Fig. 4, J)

A, B & E, ×80; C & D, ×50



H. Yabu: Early Development of Laminariales

PLATE IV

Laminaria japonica Areschoug

A & B. Female gametophytes isolated in November 1954 from the material collected at Zenikamezawa in October 1954. From a culture one month old after isolation to show a two-celled parthenosporophyte in A and a five-celled sporophyte in B

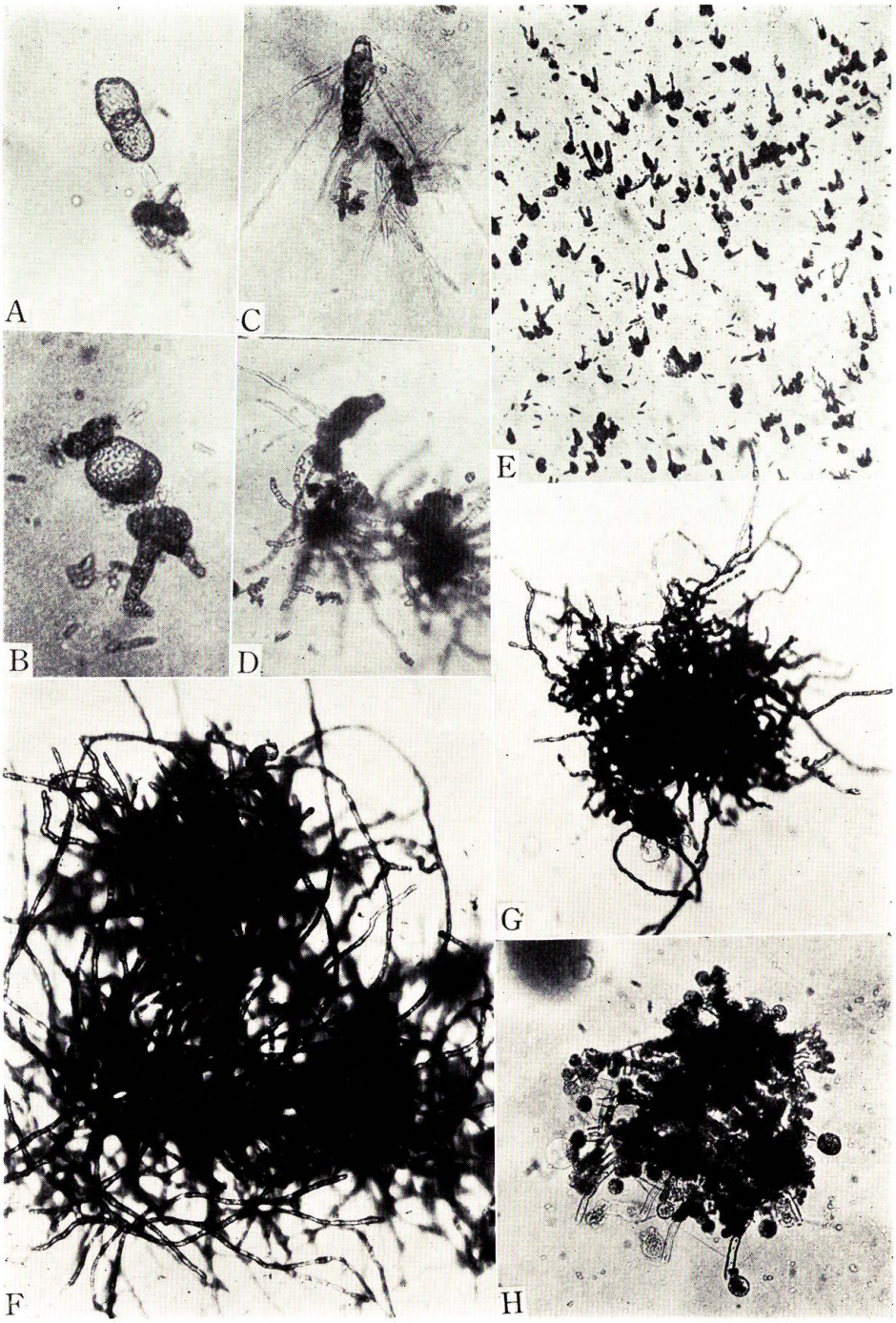
C & D. Parthenosporophytes developed on the female gametophytes which had been isolated in December 1953 from the material collected at Shirikishinai in November 1953. From a culture two months old after isolation

E. A group of the female and male gametophytes from which the female gametophytes shown in Figs. A & B were isolated

F & G. A female gametophyte with young parthenosporophytes (F) and a male gametophyte (G) isolated in mid-July 1954 from the material collected at Shirikishinai in November 1953. From a culture 45 days old after isolation

H. A female gametophyte with a number of eggs isolated in November 1955 from the material collected at Nanaehama in September 1955. From a culture two months old after isolation

A & B, ×120; C–H, ×80



H. Yabu: Early Development of Laminariales

PLATE V

Laminaria japonica Areschoug

Gametophytes developed from the material collected at Shirikishinai in November 1953 and cultured in Schreibers's solution. ×80

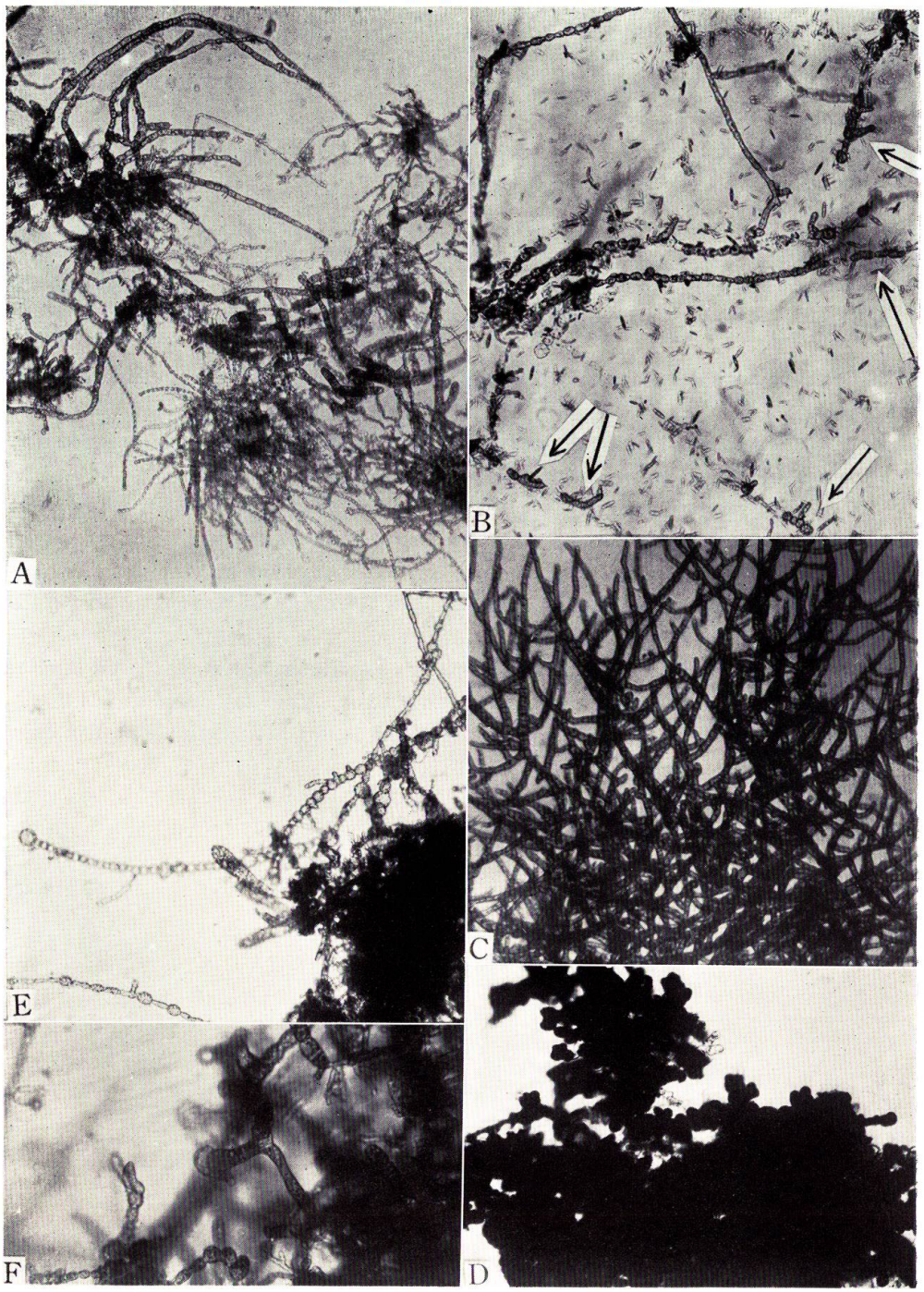
A. Female and male gametophytes cultured for three months in the laboratory

B. Part of a female gametophyte cultured for 10 months in the laboratory. Many cells are decaying but there are some cell rows that maintain vitality as indicated by arrows

C. Part of a large sterile female gametophyte cultured for two years in the laboratory

D. A female gametophyte cultured for 25 months in the laboratory under a dim light and with a solution which had been allowed to evaporate to about one-half of the original volume

E & F. Part of the female and male gametophytes cultured for three months in the corridor after having been kept for 25 months in the laboratory



H. Yabu: Early Development of Laminariales

PLATE VI

Laminaria japonica Areschoug

Gametophytes and sporophytes developed from the material collected at Shirikishinai in November 1953

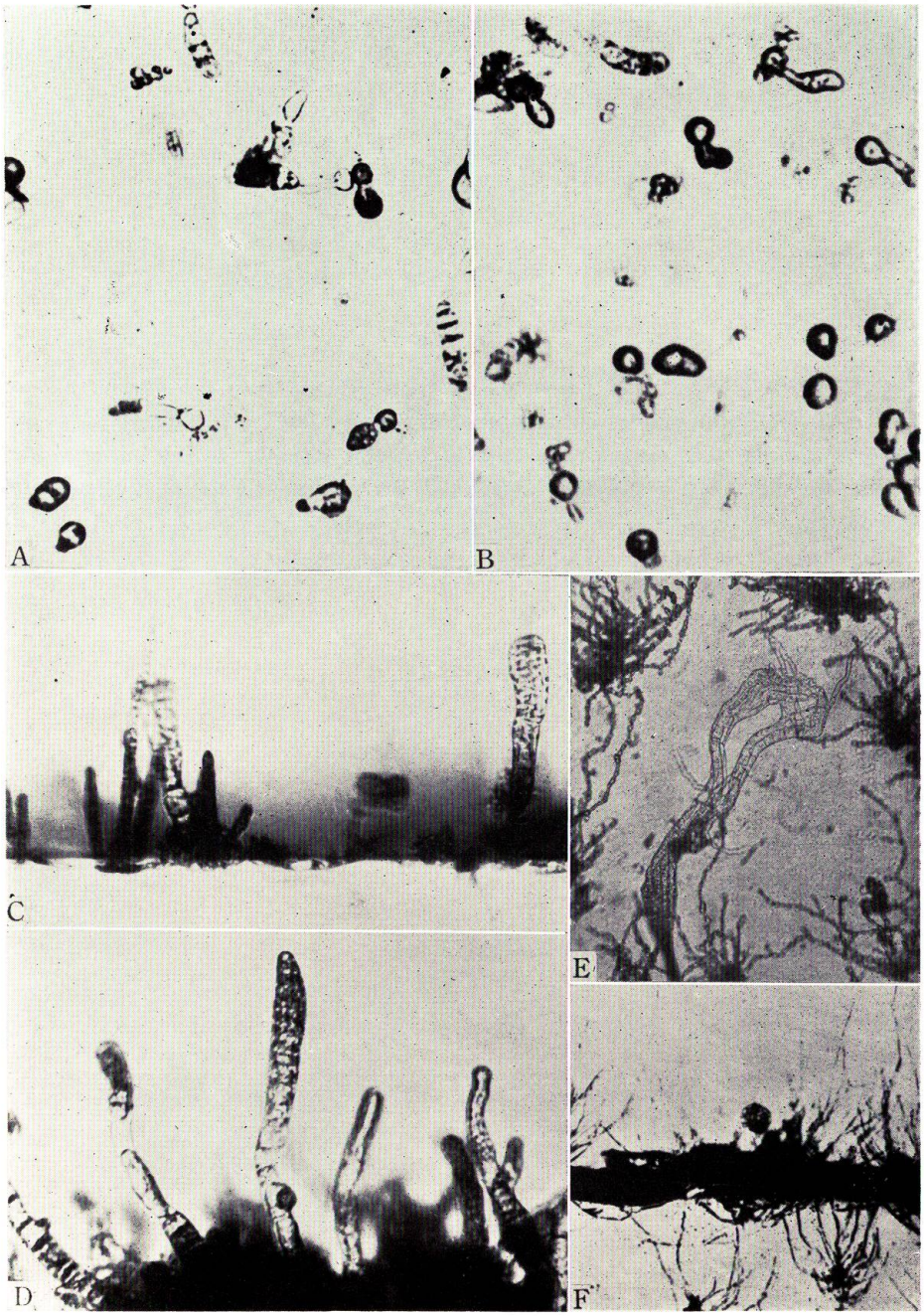
A & B. Female and male gametophytes cultured for 35 days with Erd-Schreiber solution in the corridor; female gametophytes with young sporophytes

C. A side view of gametophytes and sporophytes growing on a slide glass with a dense population of gametophytes cultured with Schreiber's solution for 110 days in the corridor

D. Part of a female gametophyte bearing eggs and sporophytes growing on a slide glass with a thin population of gametophytes cultured with Schreiber's solution for 110 days in the corridor

E & F. Gametophytes cultured with Schreiber's solution for three months in the corridor after having been kept for 10 months in the laboratory. Their sex cannot be told by the size of their cells. A few young sporophytes developed in this culture were abnormal in shape in their early development

A & B, ×240; C, ×160; D, ×290; E & F, ×80



H. Yabu: Early Development of Laminariales

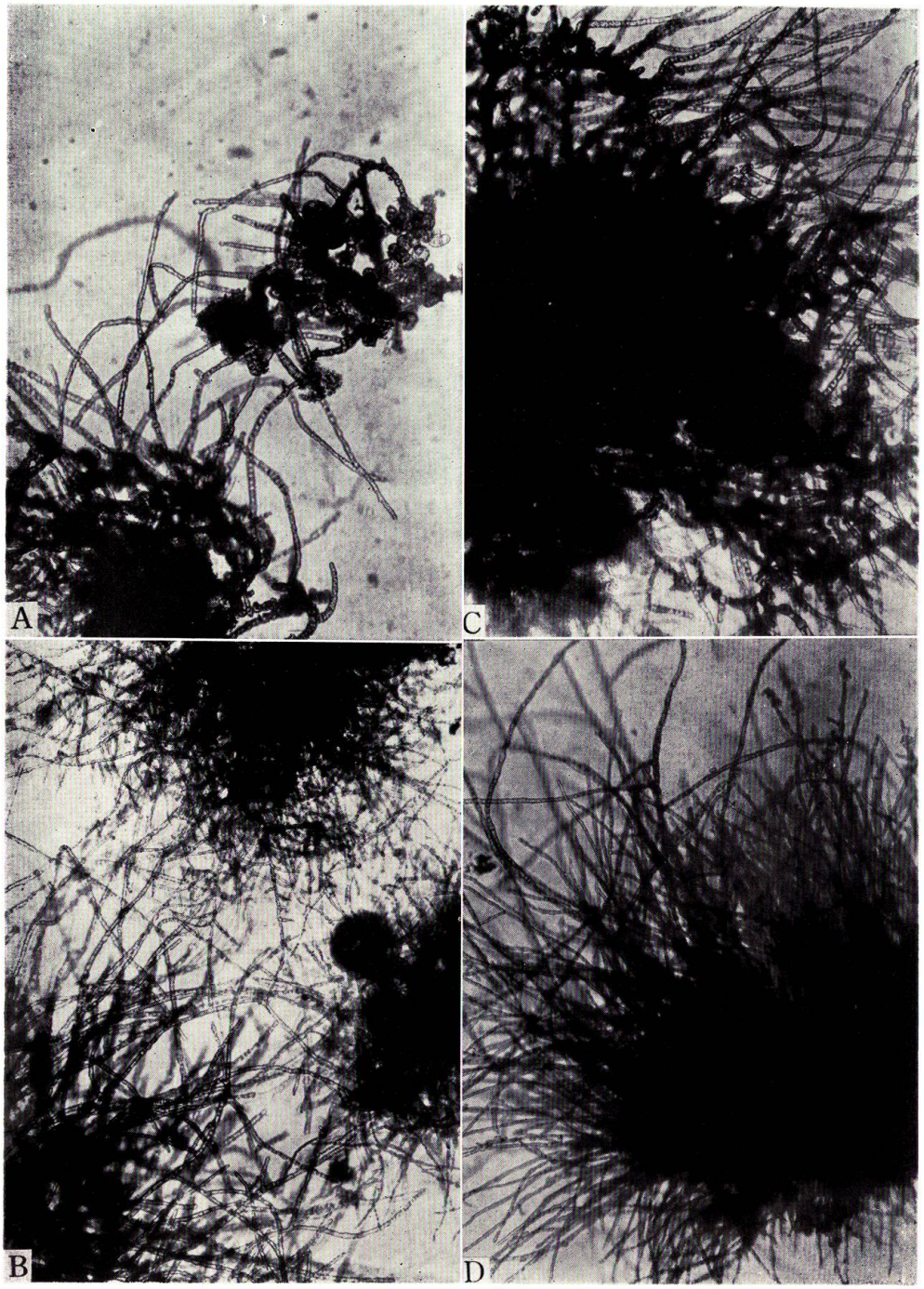
PLATE VII

Laminaria japonica Areschoug

Gametophytes developed from the material collected at Nanaehama in September 1955 and cultured with Schreiber's solution in the laboratory. ×80

A. Female and male gametophytes cultured for nine months. A male gametophyte is seen as a filamentous thallus composed of slender cells in the lower left hand corner of the photograph

B–D. Female and male gametophytes cultured for two years



H. Yabu: Early Development of Laminariales

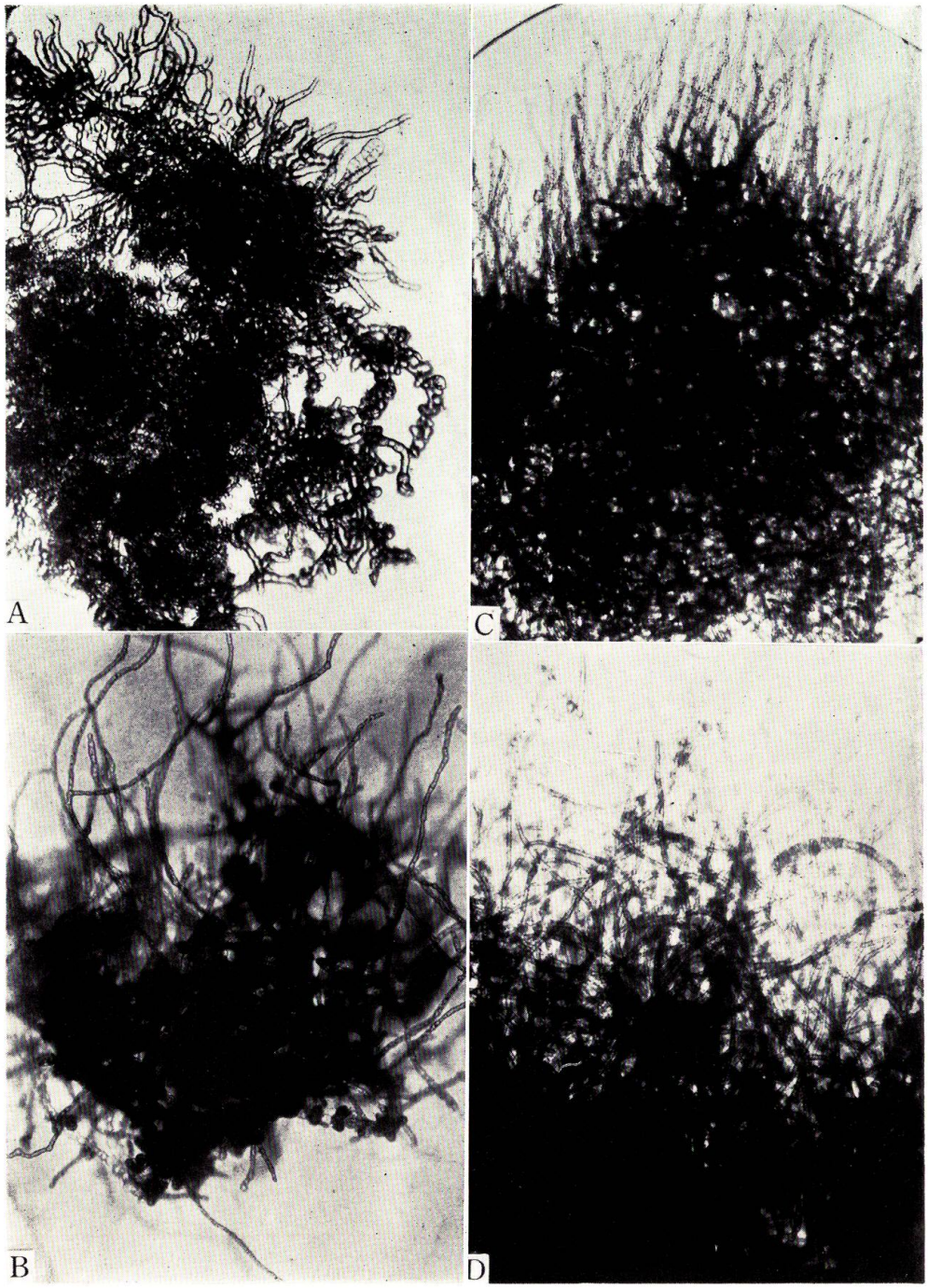
PLATE VIII

Laminaria japonica Areschoug

Gametophytes developed from the material collected at Nanaehama in September 1955 and cultured with Schreiber's solution. ×80

A. Female and male gametophytes cultured for eight months in the corridor

B–D. Female gametophytes isolated in July 1957. Fig. B, five days after isolation; Figs. C & D, eight months after isolation

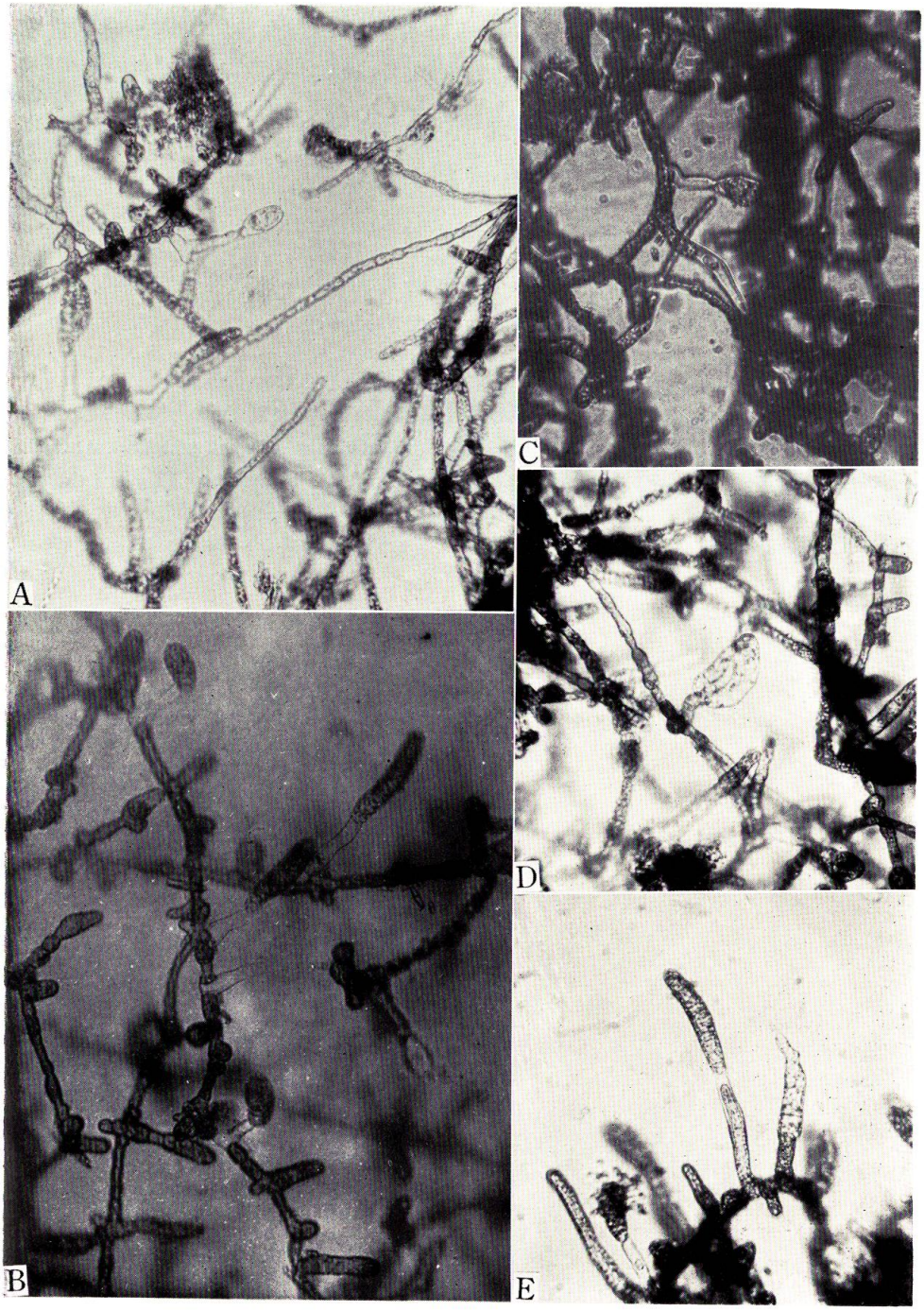


H. Yabu: Early Development of Laminariales

PLATE IX

Laminaria japonica Areschoug

A–E. Female gametophytes in a mixed culture developed from the material collected at Nanaehama in September 1955 and cultured for five months in the corridor, after having been kept for two years in the laboratory. A malformed sporophyte is seen in Fig. D. In Fig. E, a number of male gametes or sperms are seen swarming around an egg. ×110

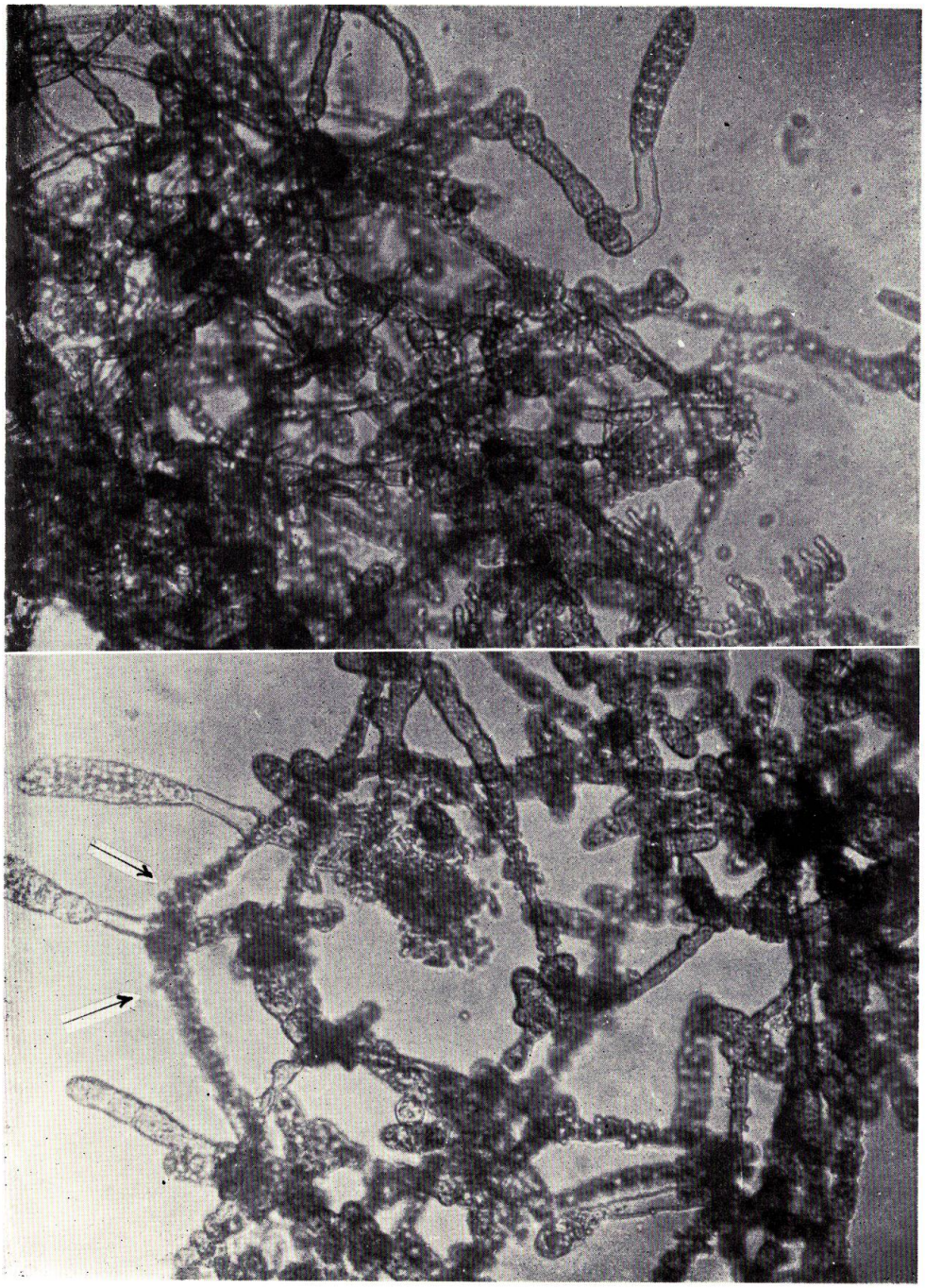


H. Yabu: Early Development of Laminariales

PLATE X

Laminaria japonica Areschoug

A & B. Mature female and male gametophytes developed from the material collected at Nanaehama in September 1955 and cultured for three months in the corridor after having been kept for two years in the laboratory. In Fig. B, a number of small granules are attached to the filamentous thallus of the female at the places indicated by arrows. They are the male gametes. ×320

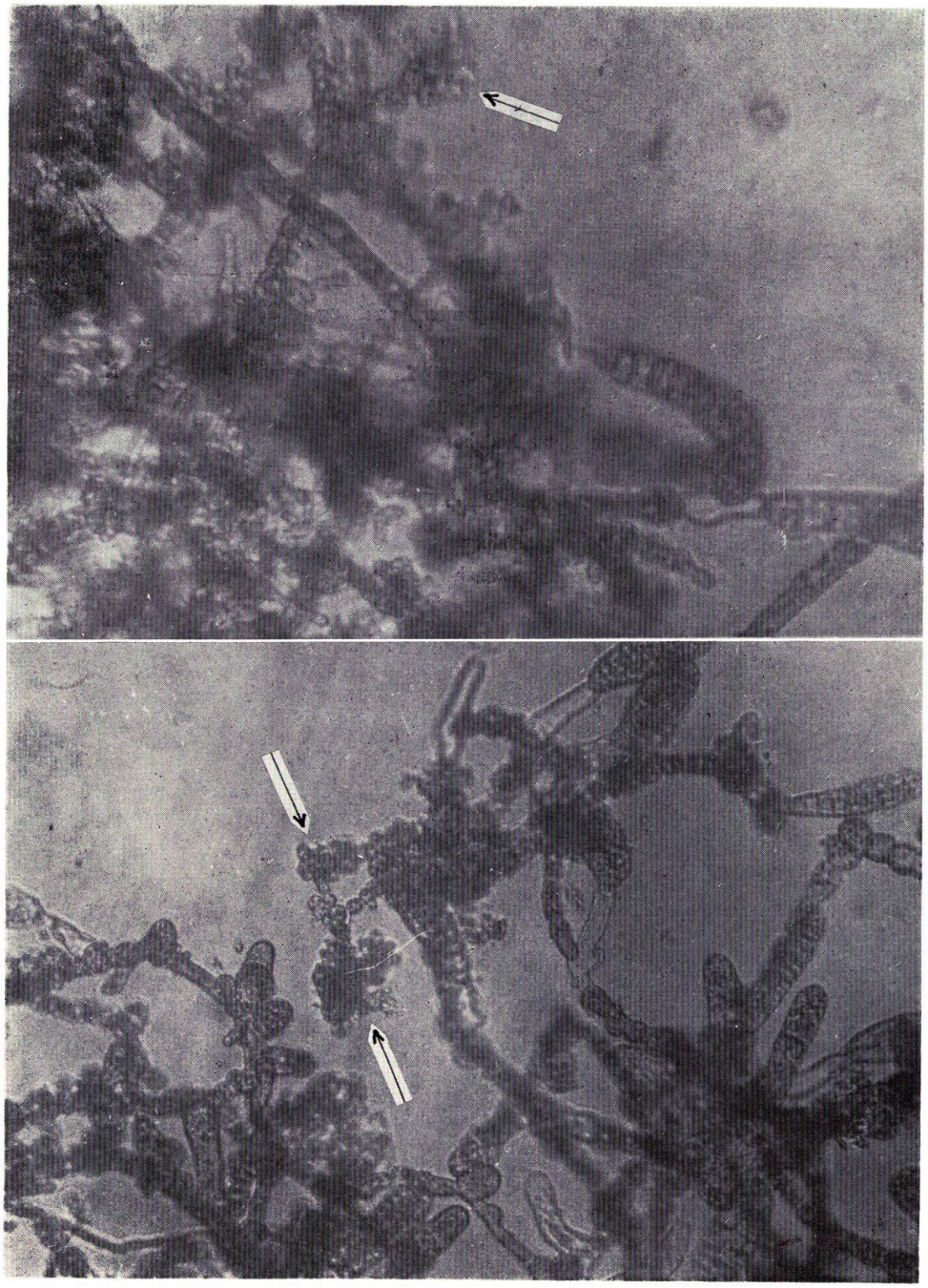


H. Yabu: Early Development of Laminariales

PLATE XI

Laminaria japonica Areschoug

A & B. Mature female and male gametophytes developed from the material collected at Nanaehama in September 1955 and cultured for three months in the corridor after having been kept for two years in the laboratory. Antheridia are indicated by arrows. ×320



H. Yabu: Early Development of Laminariales

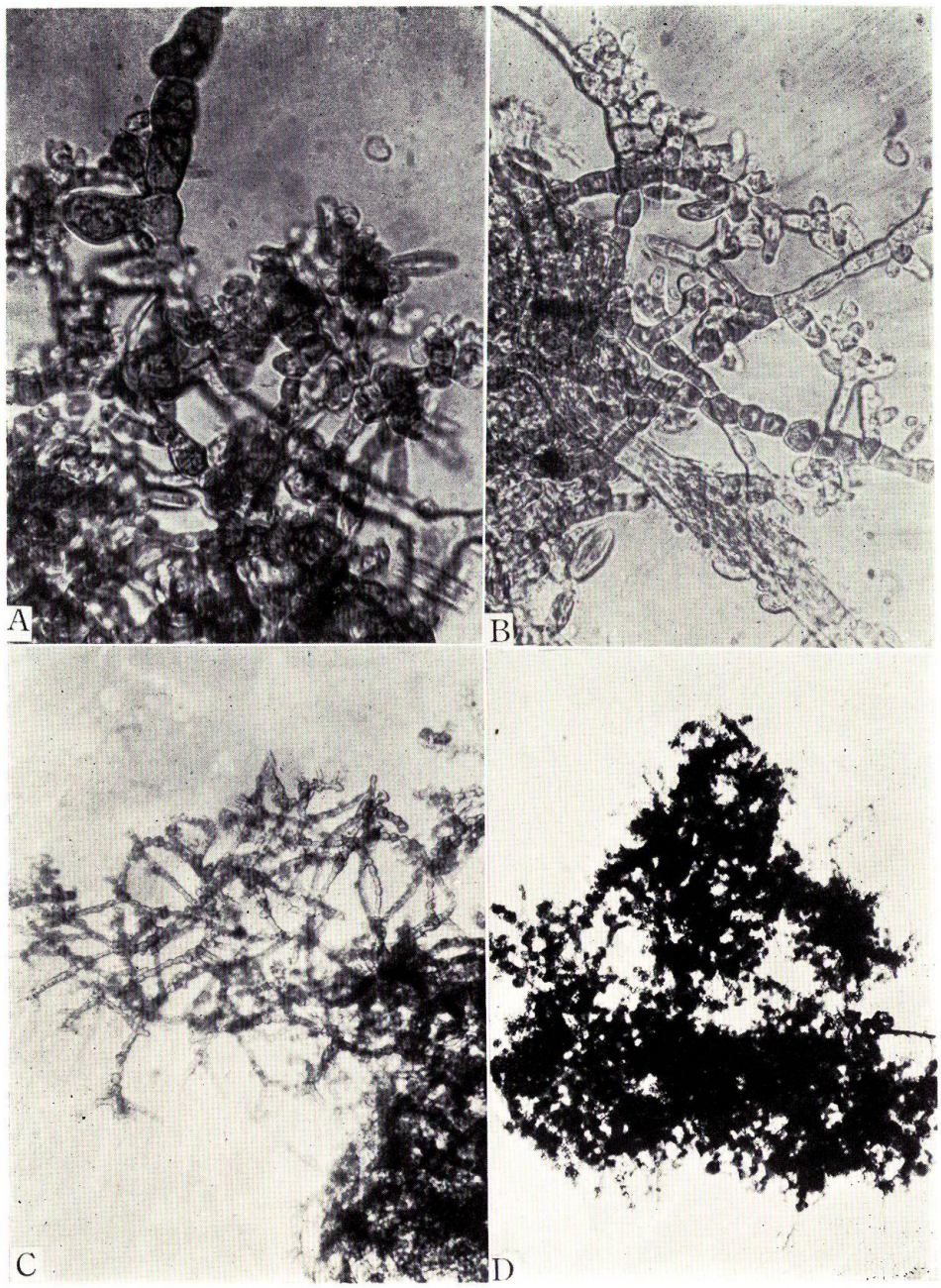
PLATE XII

Laminaria japonica Areschoug

A & B. Isolated male gametophytes developed from the material collected at Nanaehama in September 1955 and cultured for 25 days with the filtered Schreiber's solution in which numerous female and male gametophytes had been cultured. Numerous antheridia are formed on them

C & D. Isolated male gametophytes developed from the same material as above and cultured for 25 days with the filtered Schreiber's solution in which fertile female gametophytes had been placed for some time. Numerous antheridia are formed on them

A & B, ×320; C, ×120; D, ×80



H. Yabu: Early Development of Laminariales

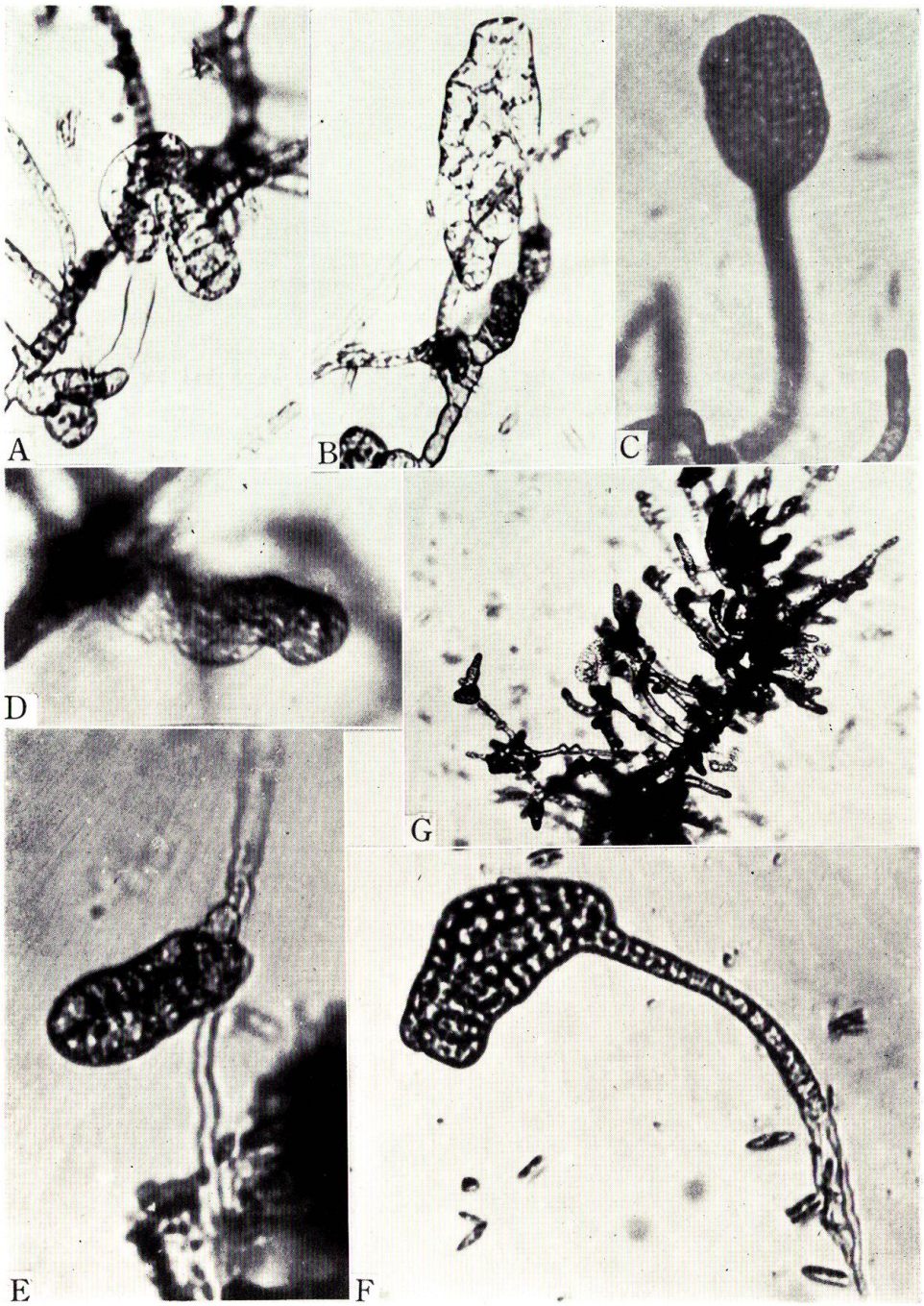
PLATE XIII

Laminaria japonica Areschoug

A–F. Parthenosporophytes on the female gametophytes which had been developed from the material collected at Nanaehama in September 1955, isolated in September 1957, and cultured for five months after isolation

G. Parthenosporophytes on a female gametophyte which had been developed from the material collected at Nanaehama in September 1955, isolated in September 1957, and cultured for five months after isolation

A–F, ×280; G, ×80

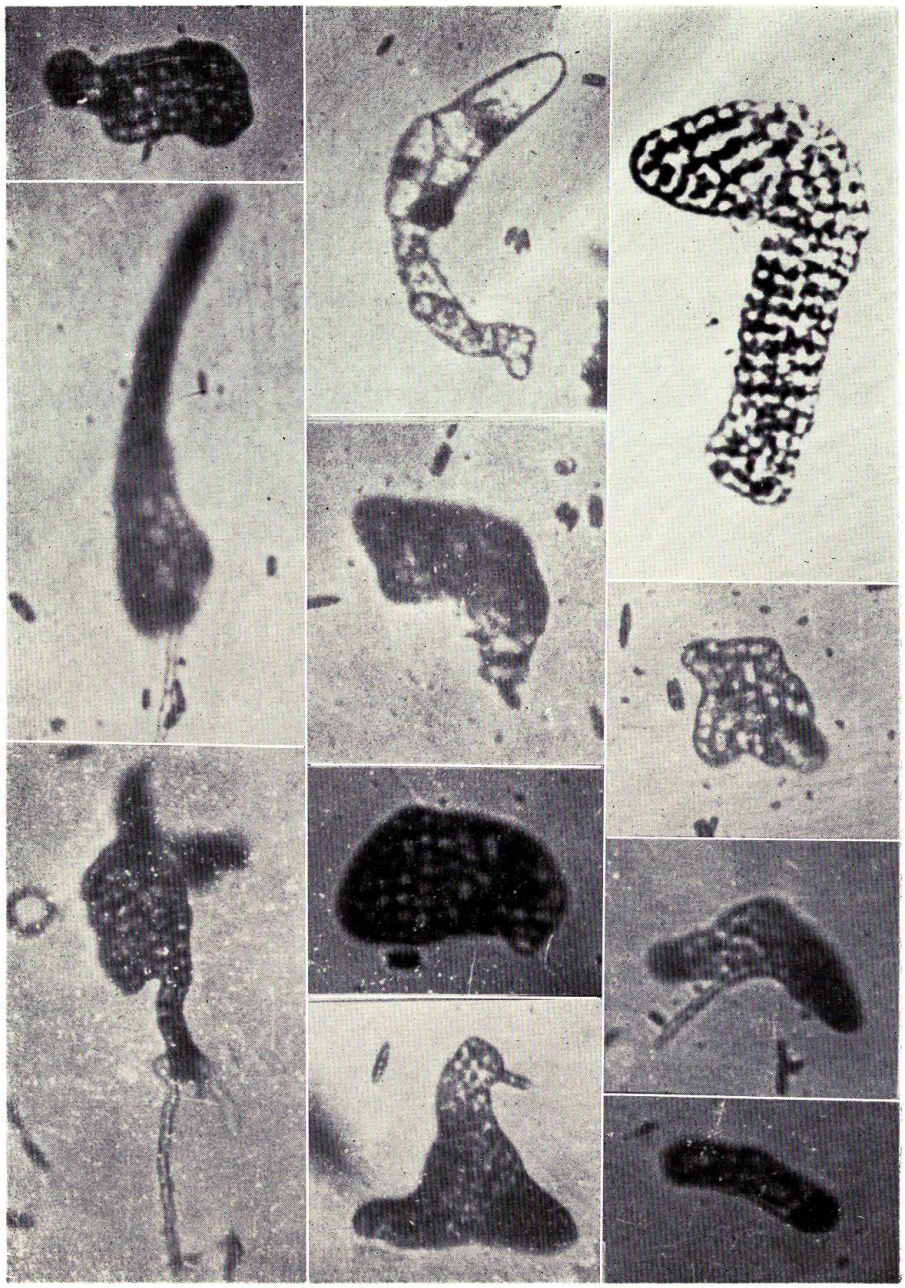


H. Yabu: Early Development of Laminariales

PLATE XIV

Laminaria japonica Areschoug

Eleven parthenosporophytes from the female gametophytes which had been developed from the material collected at Nanaehama in September 1955, isolated in September 1957, and cultured for seven months after isolation. ×280



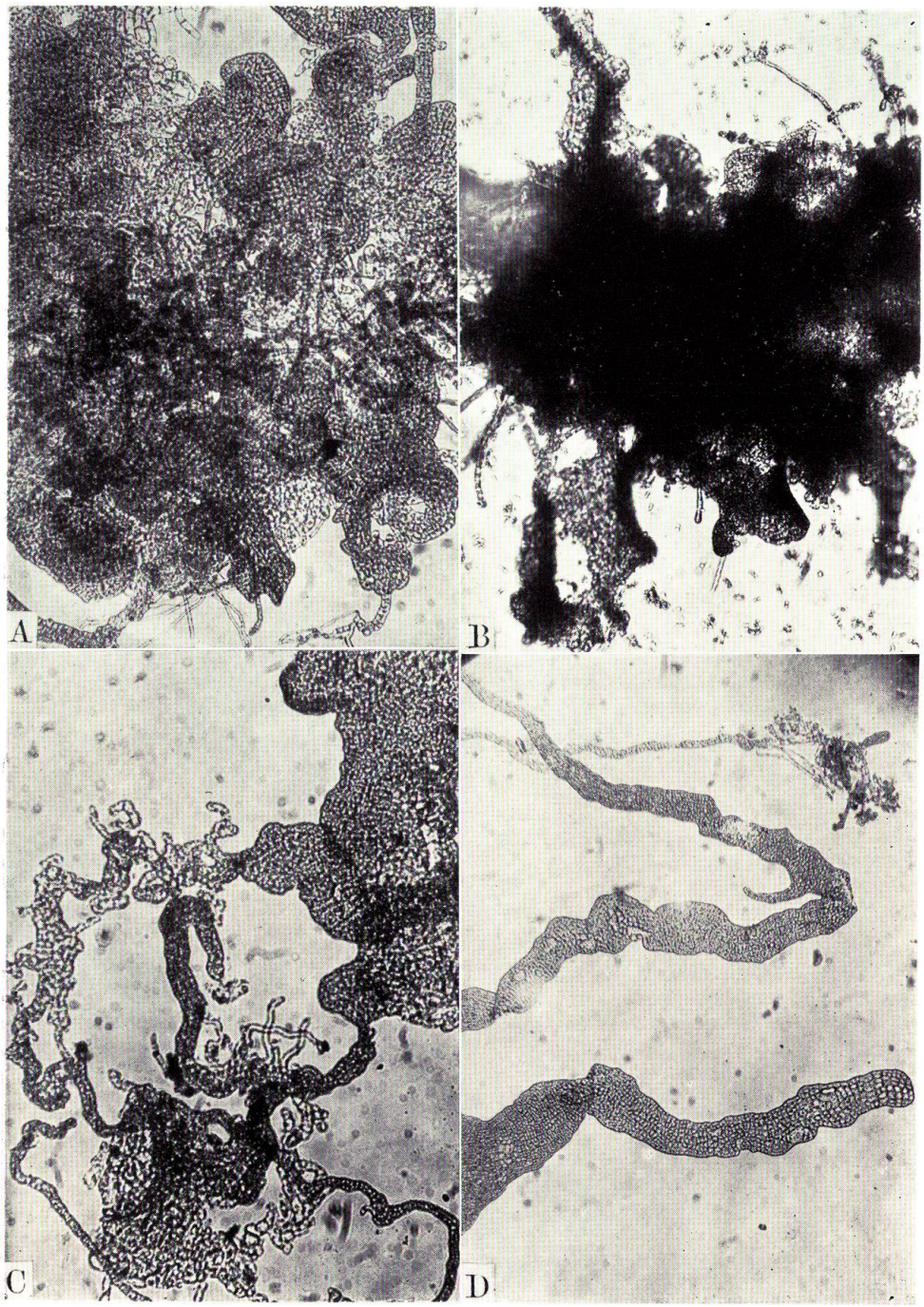
H. Yabu: Early Development of Laminariales

PLATE XV

Laminaria japonica Areschoug

Parthenosporophytes from the female gametophytes which had been developed from the material collected at Nanaehama in September 1955, isolated in September 1958, and cultured for seven months after isolation

A–C, ×80; D, ×15

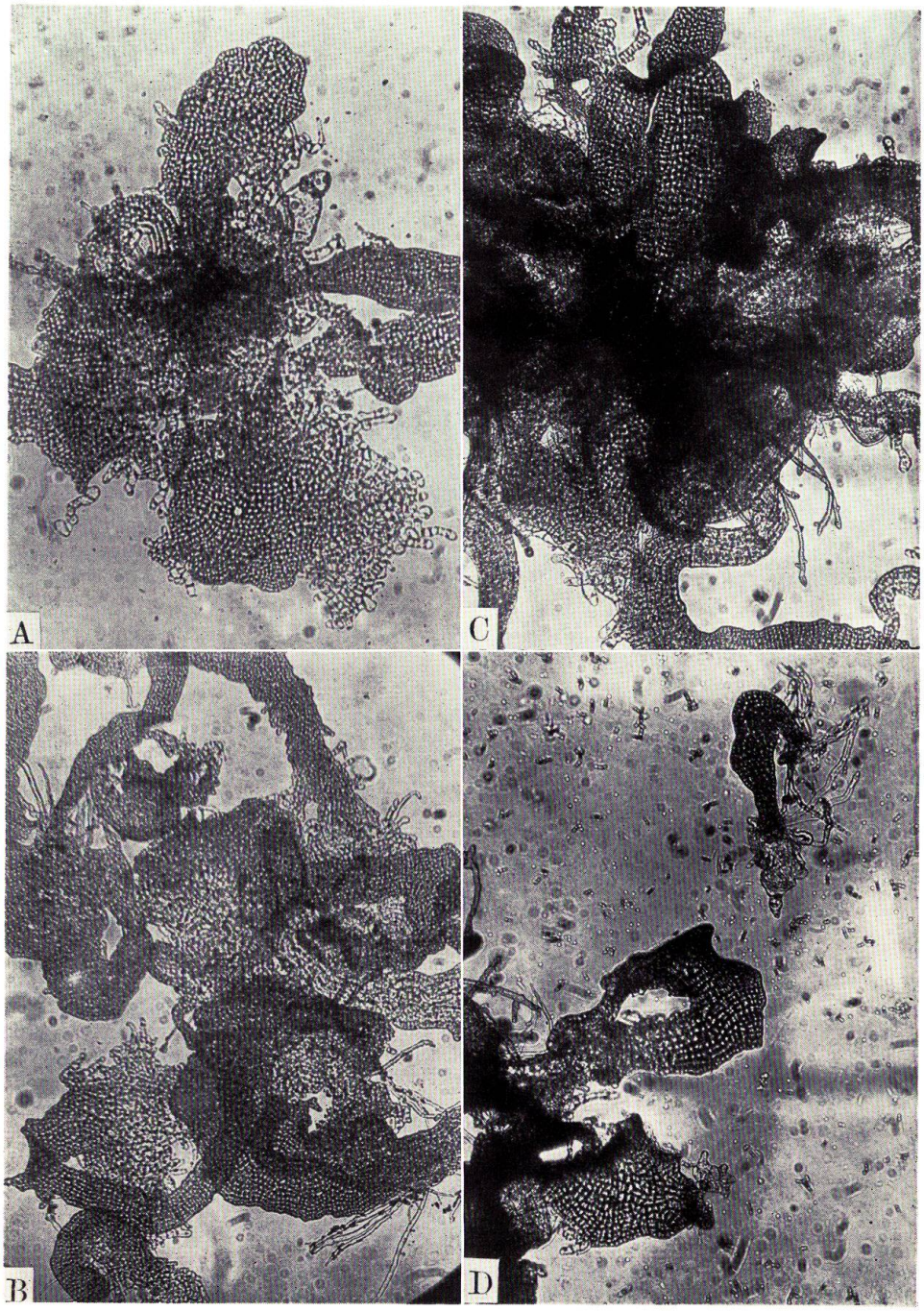


H. Yabu: Early Development of Laminariales

PLATE XVI

Laminaria japonica Areschoug

A–D. Parthenosporophytes from the female gametophytes which had been developed from the material collected at Nanaehama in September 1955, isolated in September 1958, and cultured for seven months after isolation. ×80

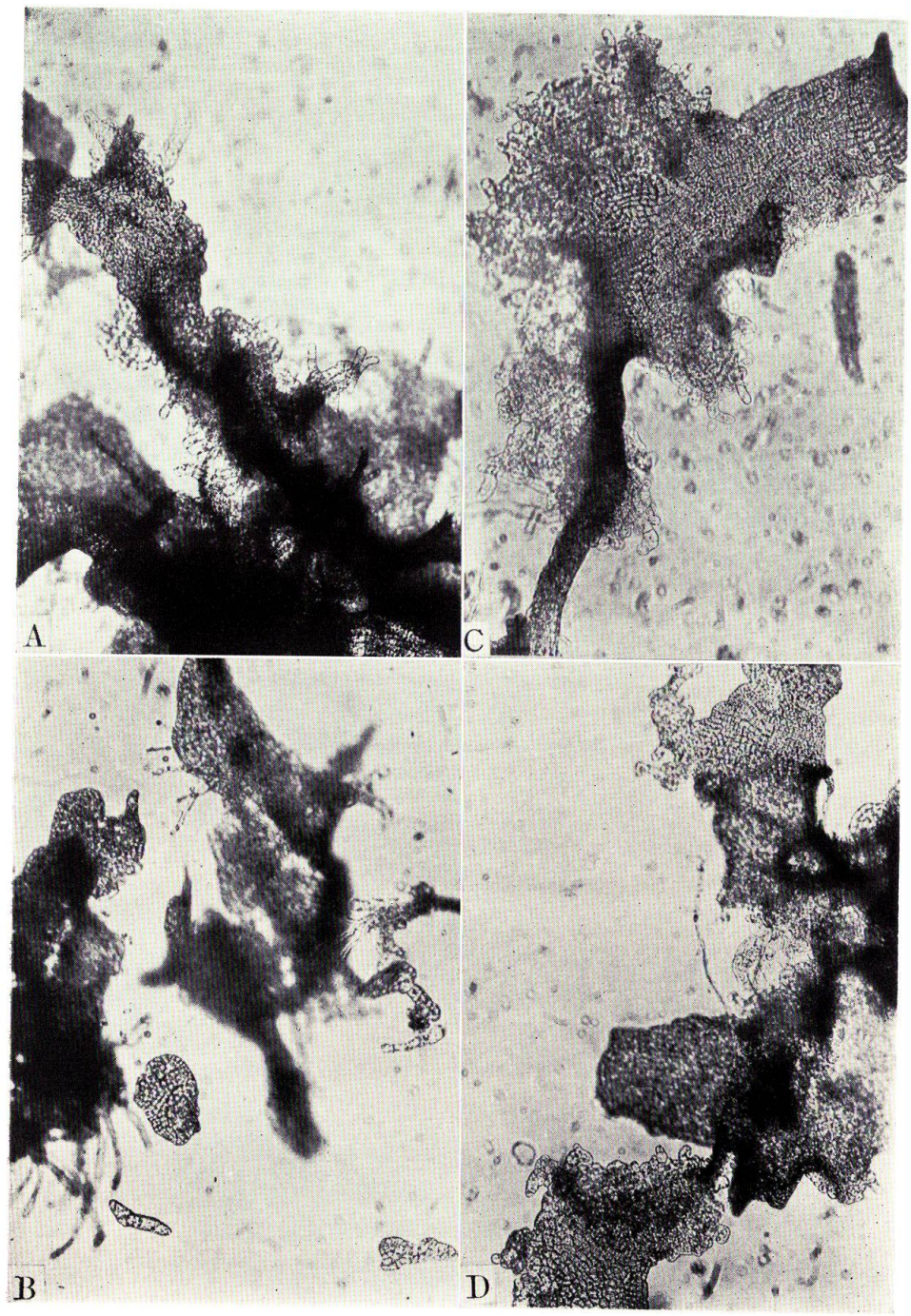


H. Yabu: Early Development of Laminariales

PLATE XVII

Laminaria japonica Areschoug

A–D. Parthenosporophytes from the female gametophytes which had been developed from the material collected at Nanaehama in September 1955, isolated in September 1958, and cultured for seven months after isolation. ×80

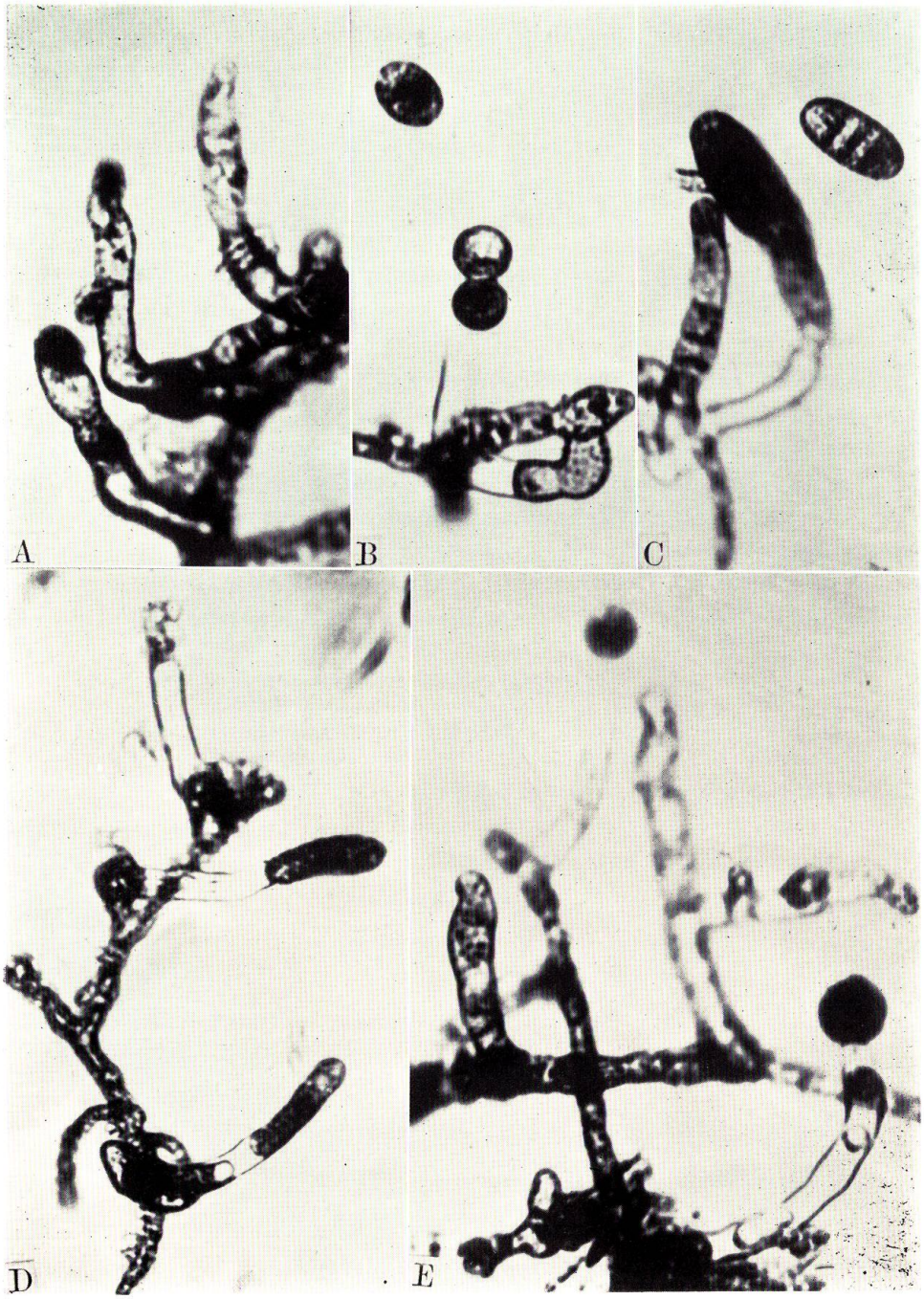


H. Yabu: Early Development of Laminariales

PLATE XVIII

Laminaria japonica Areschoug

A–E. Female gametophytes developed from the material collected at Nanaehama in September 1955, isolated in September 1957, and cultured for six months after isolation showing mature oogonia (A), extruded eggs, one being dumbbell-shaped, detached from the oogonia (B), young sporophytes, one being detached and five-celled (C), and extruded eggs attached to the opening of the emptied oogonia (D & E). ×420



H. Yabu: Early Development of Laminariales

PLATE XIX

Laminaria angustata Kjellman

A. Female and male gametophytes developed from the material collected at Shizunai in November 1952, and cultured for nine days

B. A female gametophyte developed from the material collected at Shizunai in November 1952, isolated in December 1952, and cultured for six months after isolation

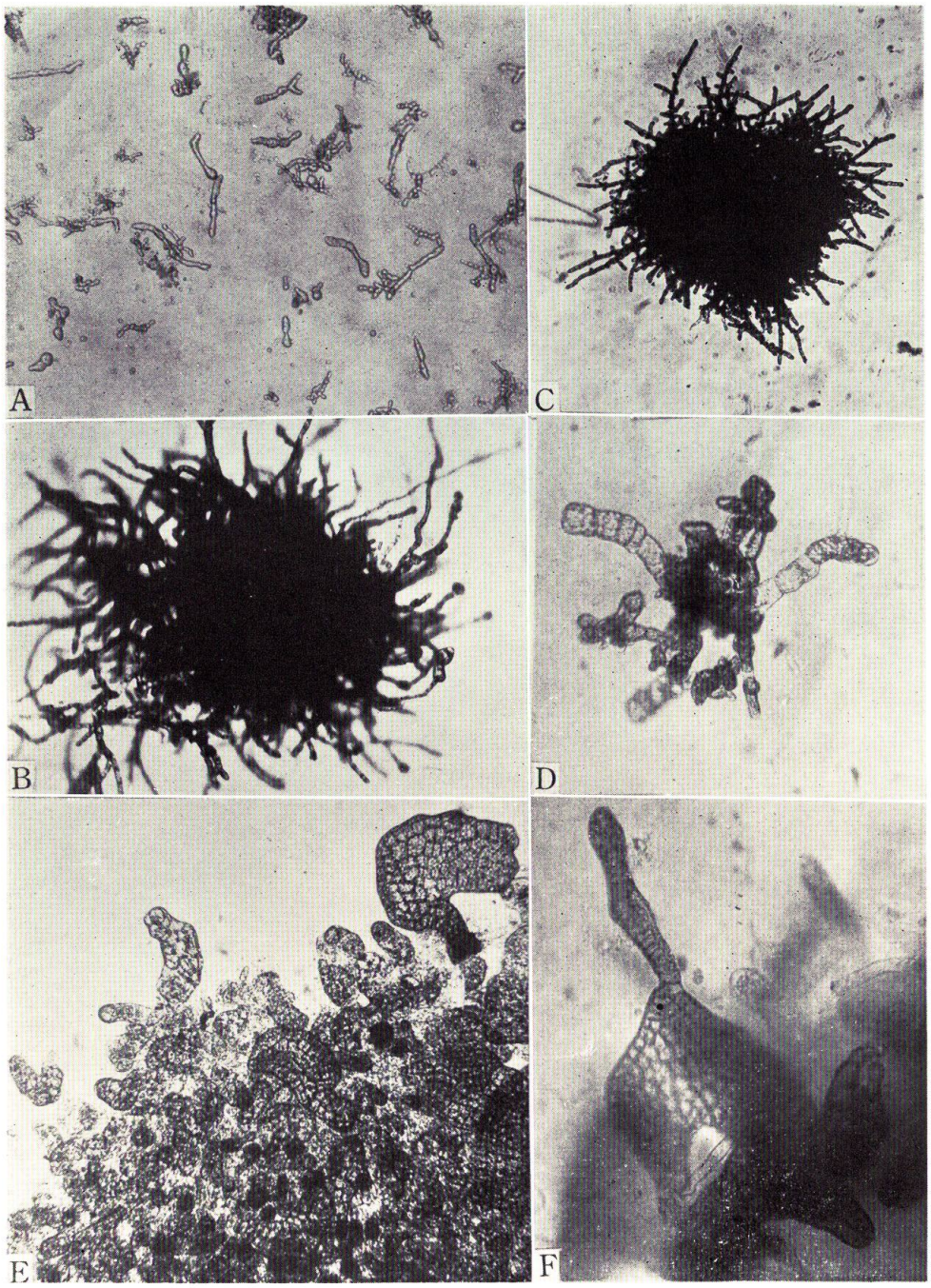
C. A male gametophyte developed from the material collected at Shizunai in November 1952, isolated in December 1952, and cultured for six months after isolation

D. A female gametophyte developed from the material collected at Muroran in November 1953, isolated two weeks later, and cultured for two months after isolation

Laminaria ochotensis Miyabe

E & F. Parthenosporophytes from the female gametophytes which had been developed from the material collected at Kutsugata in October 1953, isolated two weeks later, and cultured for three and half months after isolation

A-C, ×80; D & E, ×120; F, ×160



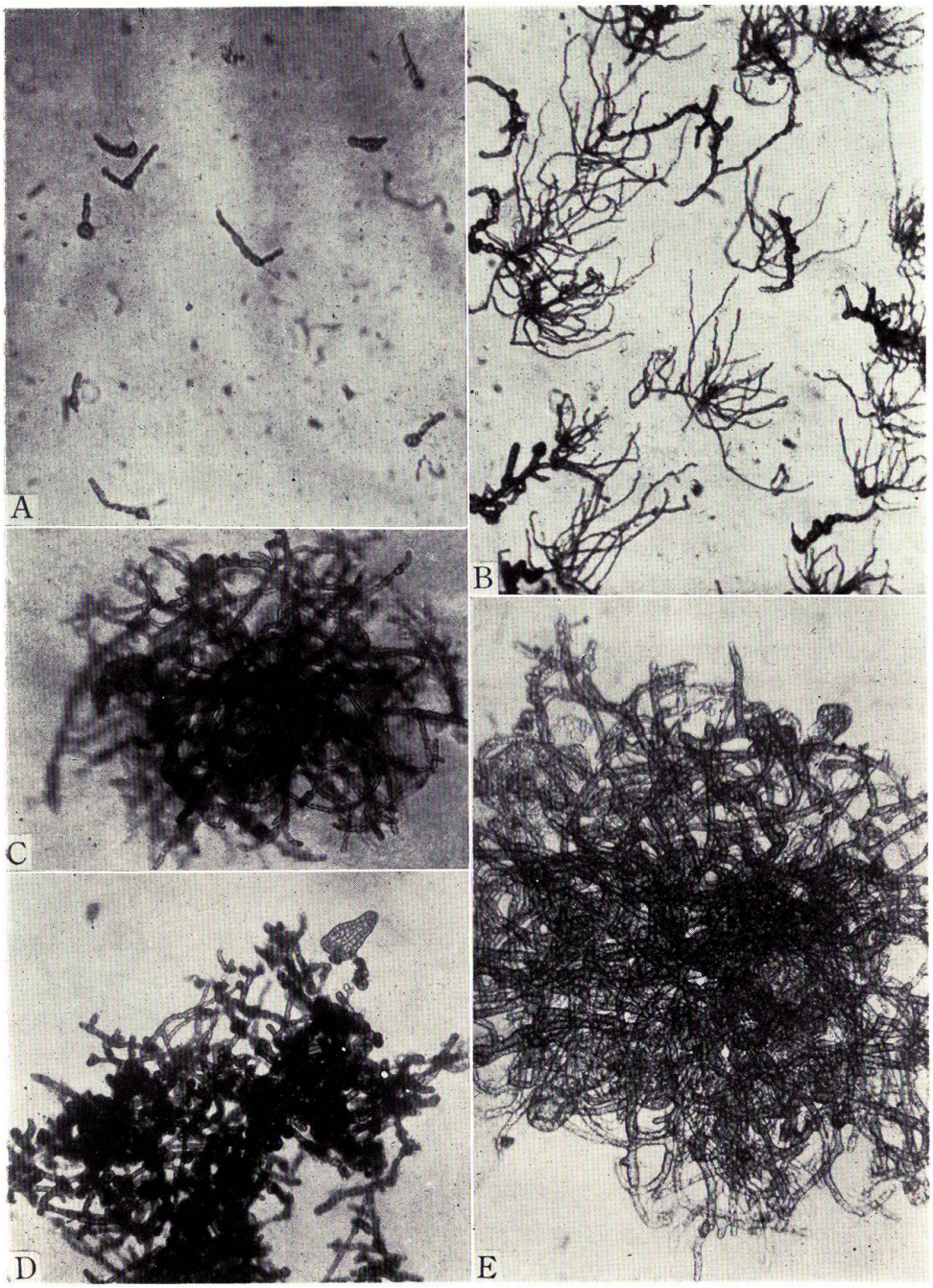
H. Yabu: Early Development of Laminariales

PLATE XX

Laminaria diabolica Miyabe

A & B. Gametophytes developed from the material collected at Akkeshi in September 1953, cultured for 21 days (A) and for three months (B) after the start of culture. ×80

C–E. Parthenosporophytes on the female gametophytes which had been isolated in October 1953, and cultured for 78 days after isolation. ×80



H. Yabu: Early Development of Laminariales

PLATE XXI

Alaria crassifolia Kjellman

Female and male gametophytes, which had been developed from the material collected at Shirikishinai in November 1954

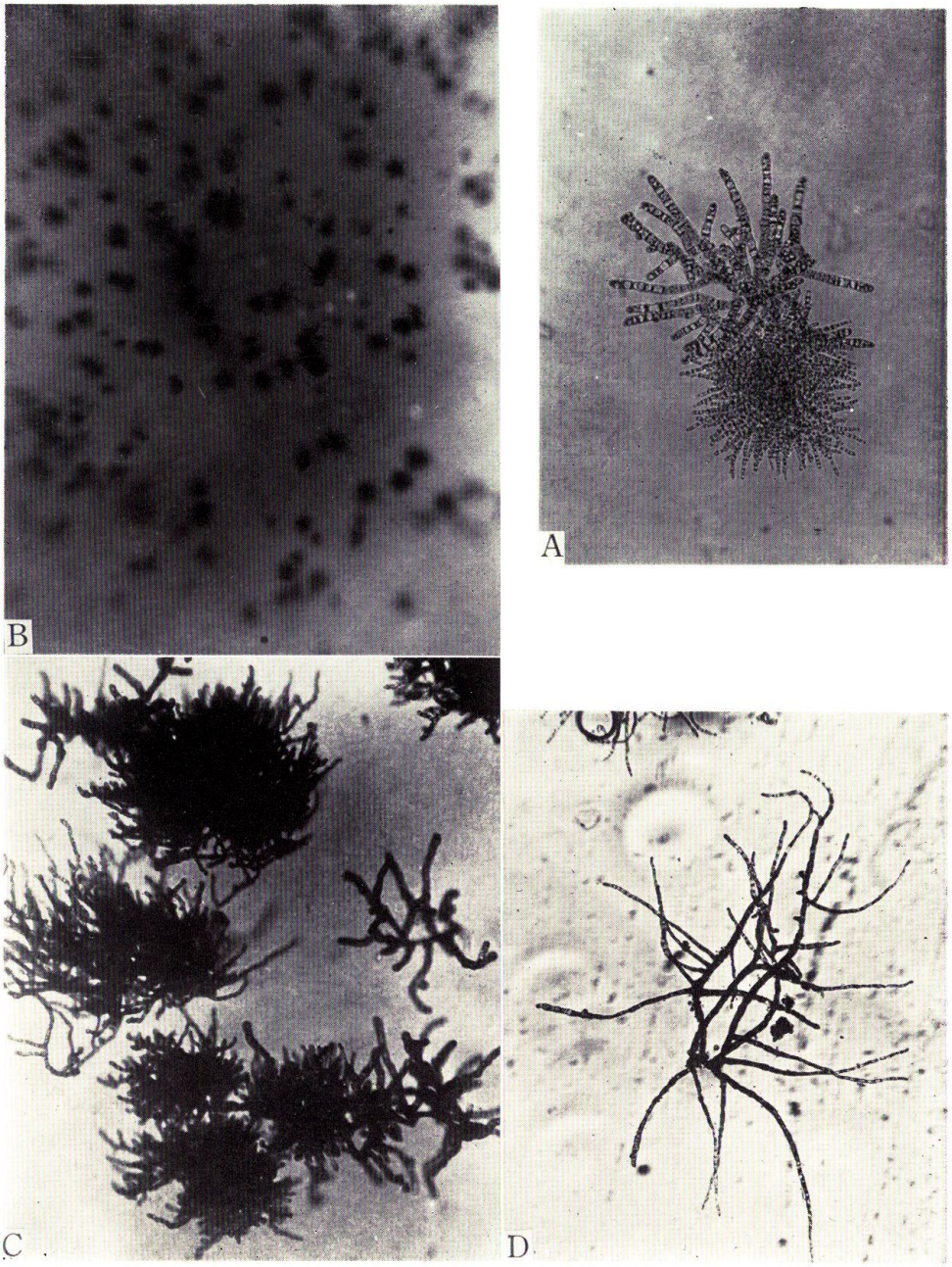
A. Female and male gametophytes floating on the surface of the culture solution, from a culture three months old since the start of culture

B. Gametophytes floating in the upper layer of the culture solution, from a culture five months old since the start of culture

C. Gametophytes growing on the bottom of the culture dish, from a culture five months old since the start of culture

D. A female gametophyte floating in the middle layer of the culture solution, from a culture three months old since the start of culture

A, C & D, ×80; B, ×5



H. Yabu: Early Development of Laminariales

PLATE XXII

Alaria crassifolia Kjellman

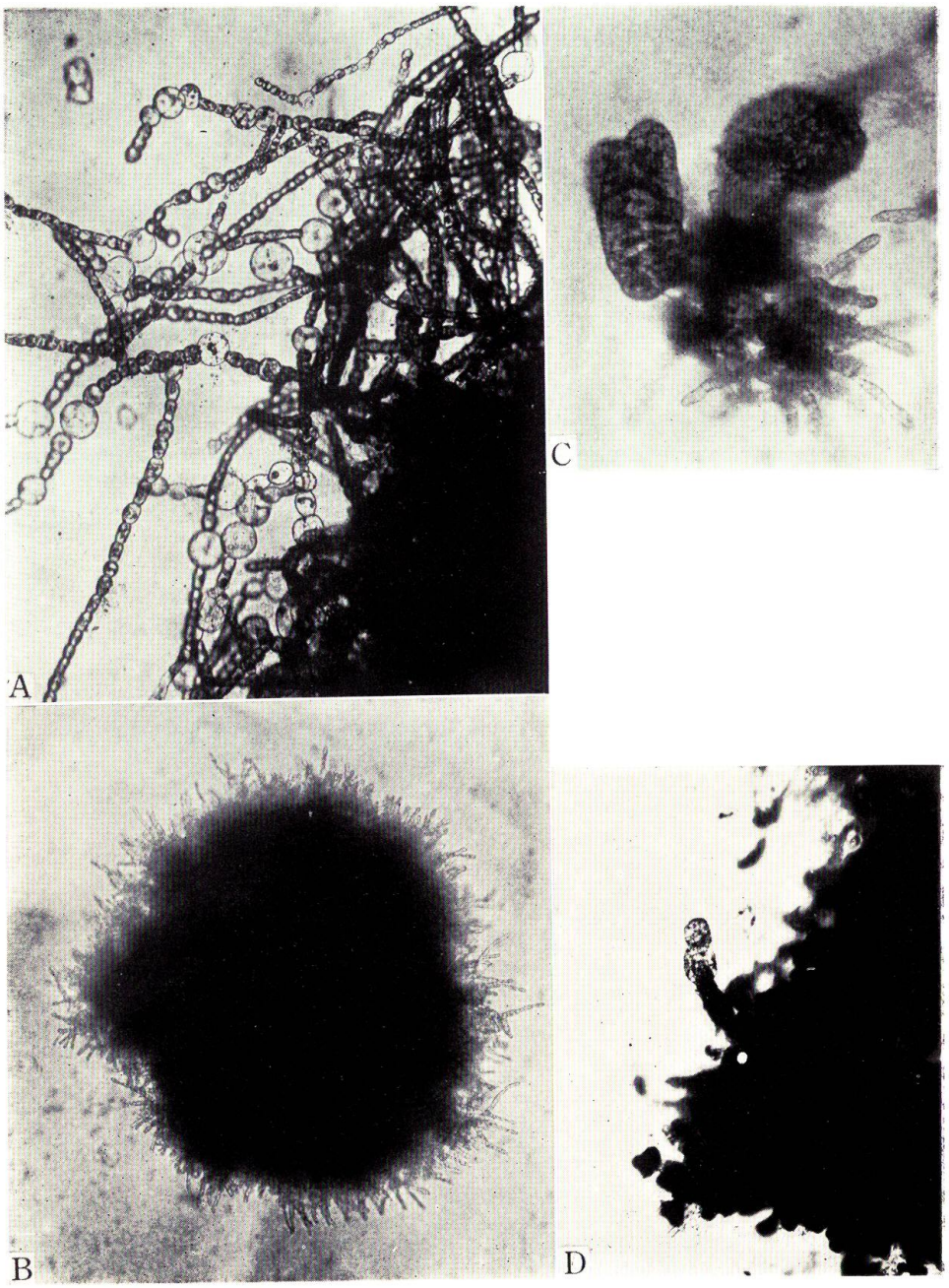
Female and male gametophytes developed from the material collected at Shirikishinai in November 1954. ×80

A. Part of a well developed female thallus with numerous swollen cells, from a culture ten months old since the start of culture

B. Male gametophyte cultured for eight months after isolation in December 1954

C. Two young parthenosporophytes on a female gametophyte cultured for one month after isolation in December 1954

D. A parthenosporophyte on a female gametophyte cultured for 45 days after isolation in July 1955



H. Yabu: Early Development of Laminariales

PLATE XXIII

Alaria crassifolia Kjellman

A. Part of a surface view of a young sporophyte showing mucilage cell (or fucosan-receptacles) stained with 1% aqueous solution of methyl-green

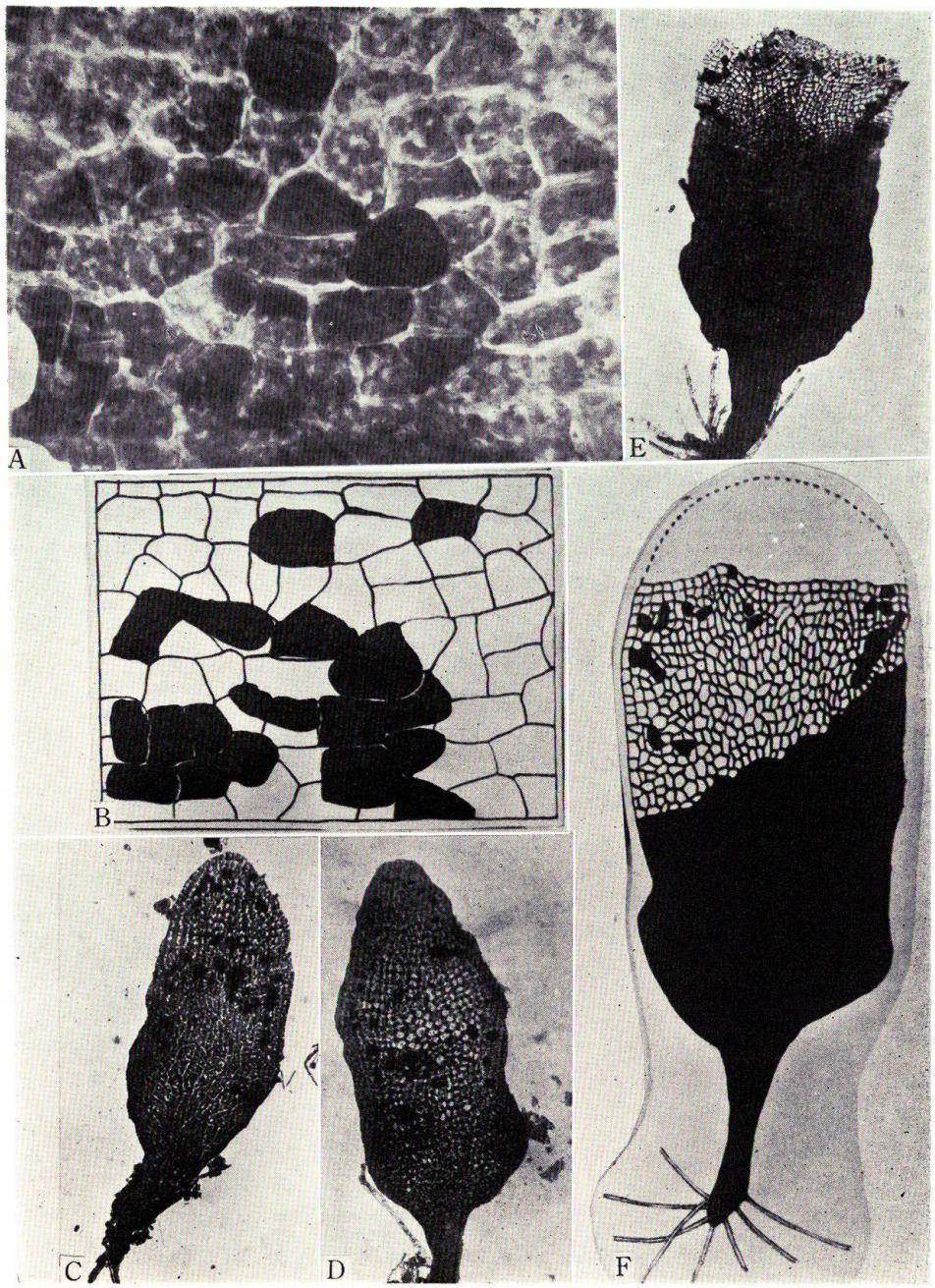
B. Camera lucida outline sketch of the same cells as shown in Fig. A; the cells painted dark are the mucilage cells stained with methyl-green

C & D. Young sporophytes spotted with mucilage cells stained dark either with 1% aqueous solution of methyl-green (C) or with 1% aqueous solution of toluidin-blue (D)

E. A further developed young sporophyte, polystromatic in the lower portion, showing mucilage cells stained with 1% aqueous solution of methyl-green in the upper thin-layered portion

F. Camera lucida sketch of the same sporophyte as shown in Fig. E; the cells painted dark in the upper portion of the thallus are the mucilage cells stained with methyl-green

A, ×720; C–E, ×50; F, ×75



H. Yabu: Early Development of Laminariales

PLATE XXIV

Undaria pinnatifida (Harv.) Suringar f. *distans* Miyabe et Okamura

A–D. Gametophytes developed from the material collected at Nanaehama in July 1954. ×80

A. Female and male gametophytes, cultured for five months

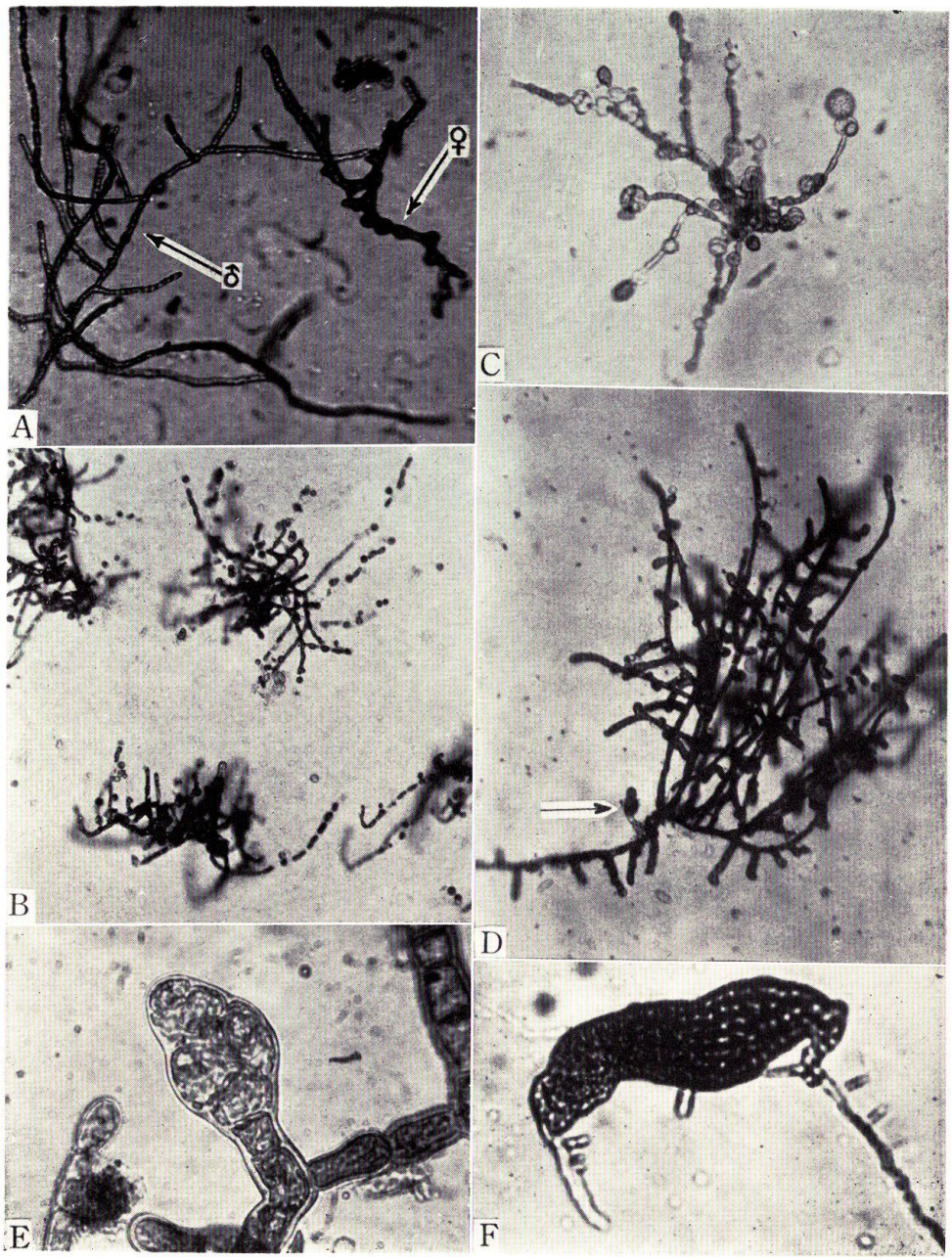
B. From a culture thirteen months old

C. A female gametophyte with many swollen cells, from a culture ten months old

D. A female gametophyte isolated in August 1954 and cultured for six monts after isolation, showing a young parthenosporophyte (pointed by an arrow)

Arthrothamnus bifidus (Gmelin) Ruprecht

E & F. Parthenosporophyte on a female gametophyte developed from the material collected at Nemuro in March 1955, isolated eight months later, and cultured for three months after isolation. E, ×420; F, ×150



H. Yabu: Early Development of Laminariales

PLATE XXV

Crossing experiments (cf. Table XXIV); in A–C are shown the young sporophytes of normal shape, and in D the young parthenosporophytes of abnormal shape. Photographed in March 1956, five months after the start of experiment. ×80

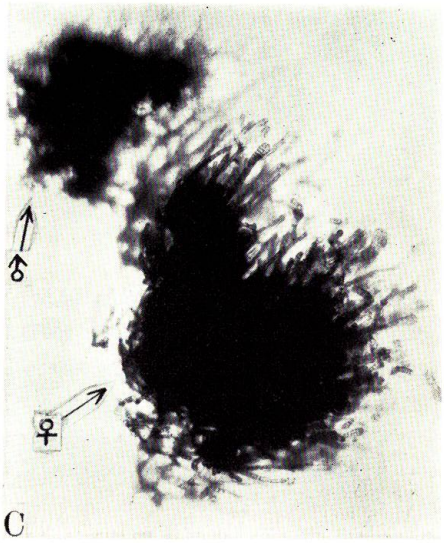
A. *Laminaria ochotensis* ♀×*L. religiosa* ♂

B. *Laminaria religiosa* ♀×*L. ochotensis* ♂

C. *Laminaria japonica* ♀×*L. religiosa* ♂

D. *Laminaria angustata* ♀×*Alaria crassifolia* ♂


A


♂
♀
C


B

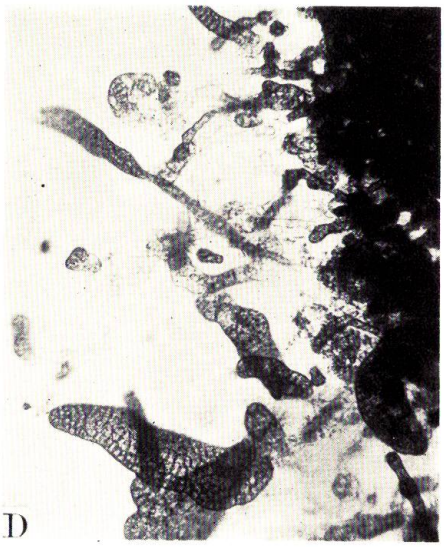

D

PLATE XXVI

Crossing experiments (cf. Table XXIV); in A and B are shown the young sporophytes of normal shape, and in C–E the young parthenosporophytes of abnormal shape. Photographed in April 1956, six months after the start of experiment. ×80

A. *Laminaria religiosa* ♀×*L. japonica* ♂

B. *Laminaria japonica* ♀×*L. ochotensis* ♂

C. *Laminaria ochotensis* ♀×*L. angustata* ♂

D. *Alaria crassifolia* ♀×*L. religiosa* ♂

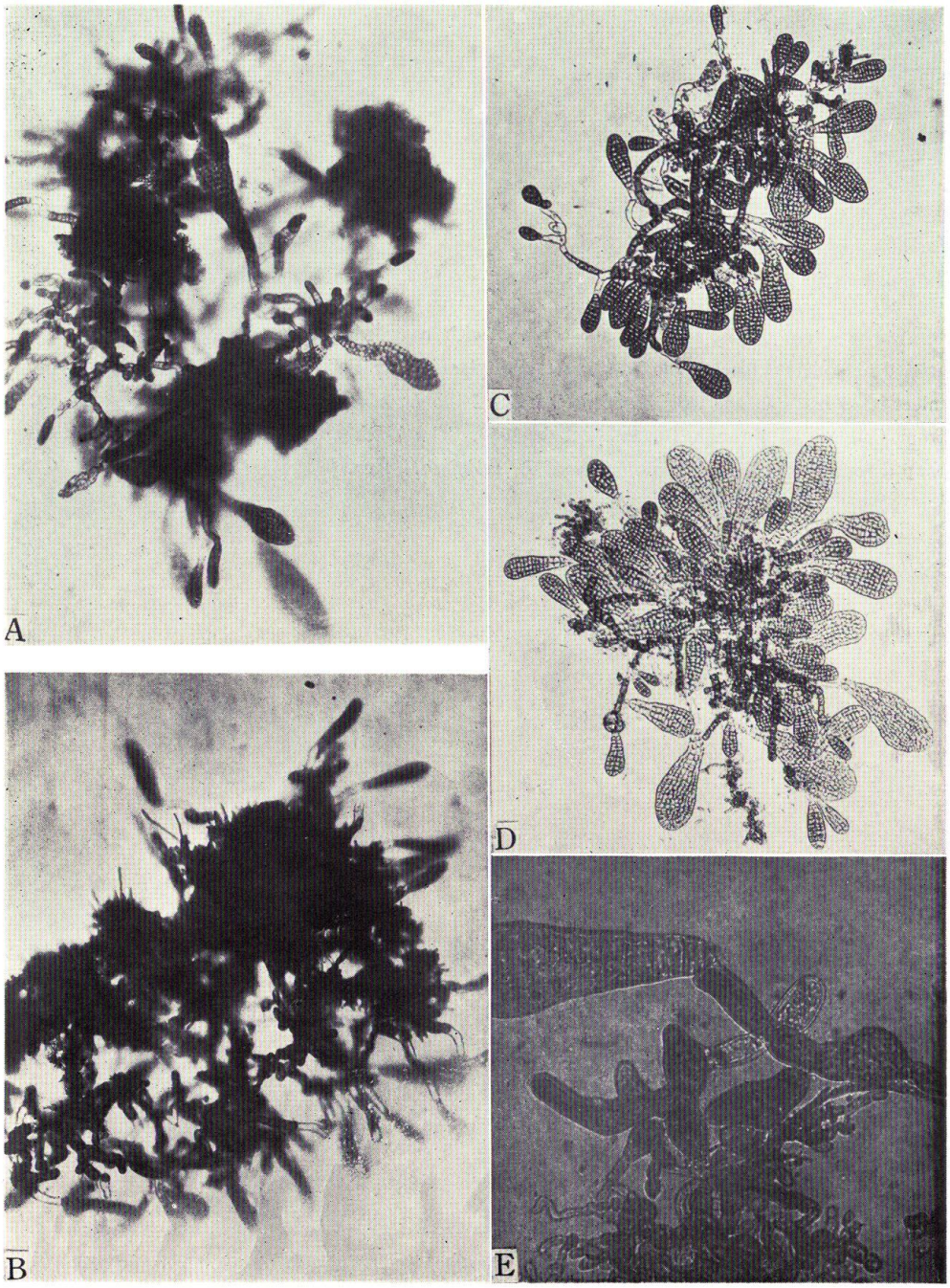
E. *Laminaria angustata* ♀×*L. religiosa* ♂

H. Yabu: Early Development of Laminariales

PLATE XXVII

Crossing experiments (cf. Table XXIV); in A–E are shown the young sporophytes of normal shape. Photographed in April 1956, six months after the start of experiment. A–D, ×80; E, ×320

A–E. *Laminaria diabolica* ♀×*L. japonica* ♂



H. Yabu: Early Development of Laminariales

PLATE XXVIII

Surface view of the slide glasses covered with seaweed germlings after they had been settled on concrete blocks (Pl. XXIX, A) and placed near the low water mark, either in the midst (A & B) or in the neighborhood (C & D) of the *Laminaria religiosa* bed in Oshoro Bay, from October 13 through November 8 in 1953. The black spots in B are mostly the disk-shaped female and male gametophytes of *L. religiosa*. ×ca. 3

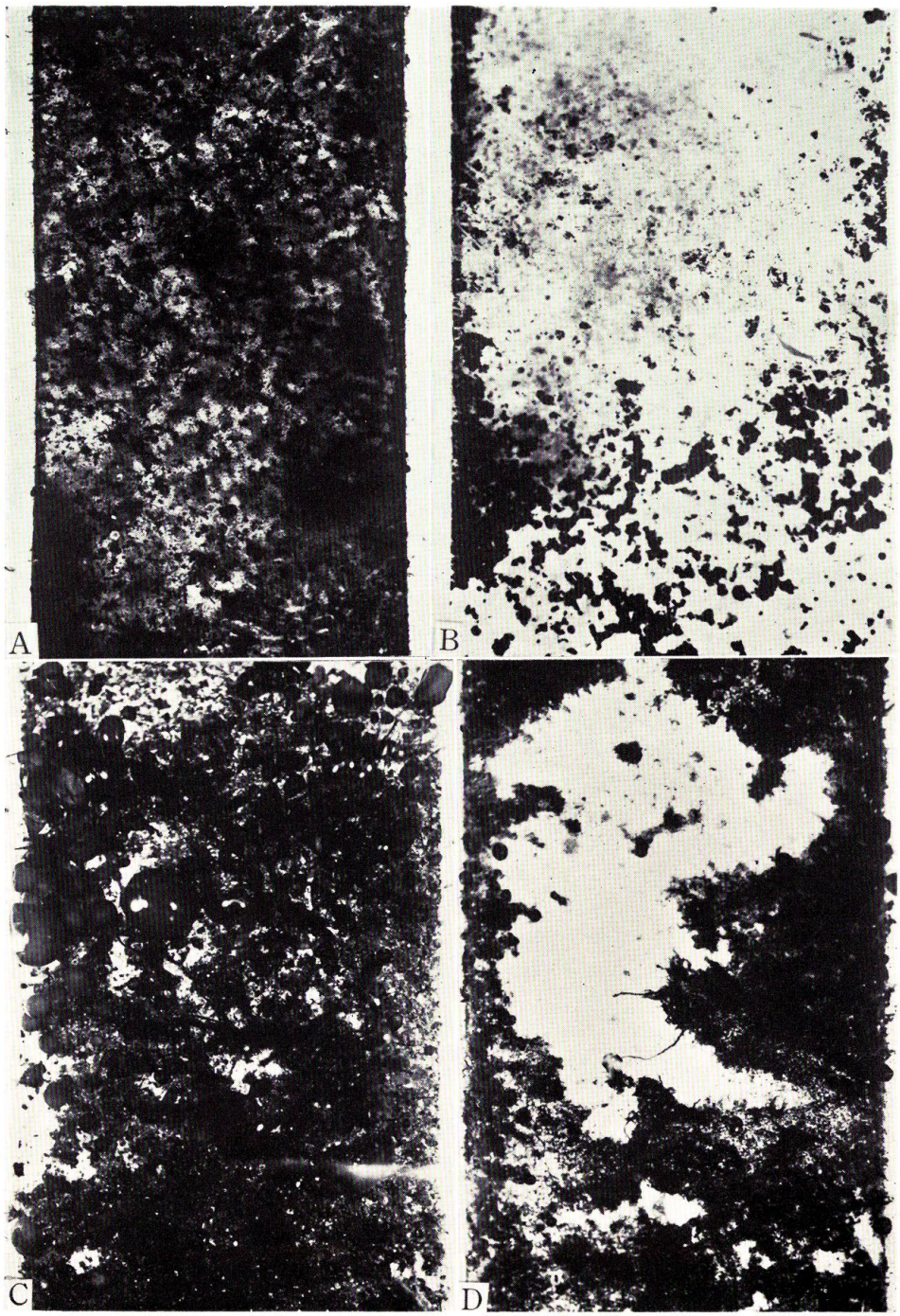


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PLATE XXIX

A. Slide glasses settled on a concrete block and placed in the neighborhood of the *Laminaria religiosa* bed in Oshoro Bay on October 13, 1953

B & C. *Laminaria religiosa* beds near the low water mark in Oshoro Bay on October 13, 1953

D. A depression on the rocky reef near the *Laminaria religiosa* bed in Oshoro Bay, into which a concrete block was placed

E. A disk-shaped female gametophyte (pointed by an arrow) of *Laminaria religiosa* attached to a slide glass which had been settled in Oshoro Bay from October 13 through November 8 in 1953. Several young sporophytes were found developed on this female

F. Young sporophytes (pointed by an arrow) of *Laminaria religiosa* attached to a slide glass which had been settled in Oshoro Bay from October 13 through November 8 in 1953. A disk-shaped female gametophyte was seen at each base of those sporophytes

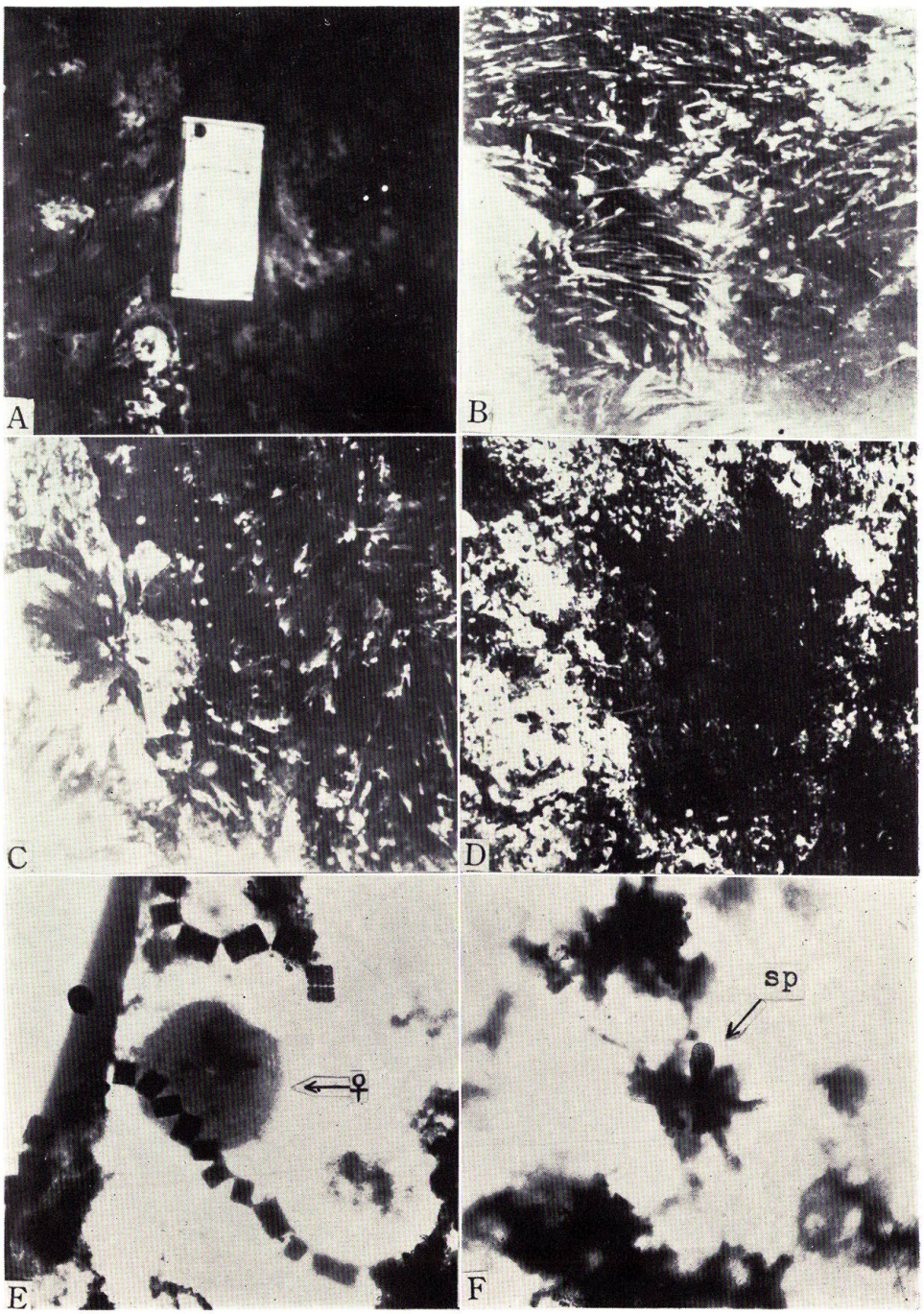

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PLATE XXX

A–D. Young sporophytes of *Laminaria religiosa* attached to the slide glasses which had been settled in Oshoro Bay from October 13 through November 8 in 1953

A disk-shaped female gametophyte was seen at each base of those sporophytes. In Fig. B, many erect uniseriate filaments are shown to have grown from the disk-shaped female gametophytes

E. Seven young sporophytes of *Laminaria religiosa* collected on November 8, 1953, from among the *Laminaria* bed in Oshoro Bay. Six of them are connected at their bases with a mass of uniseriate filamentous thalli of the female gametophytes (pointed by arrows)

A & B, ×80; C, ×ca. 9; D, ×120; E, ×50

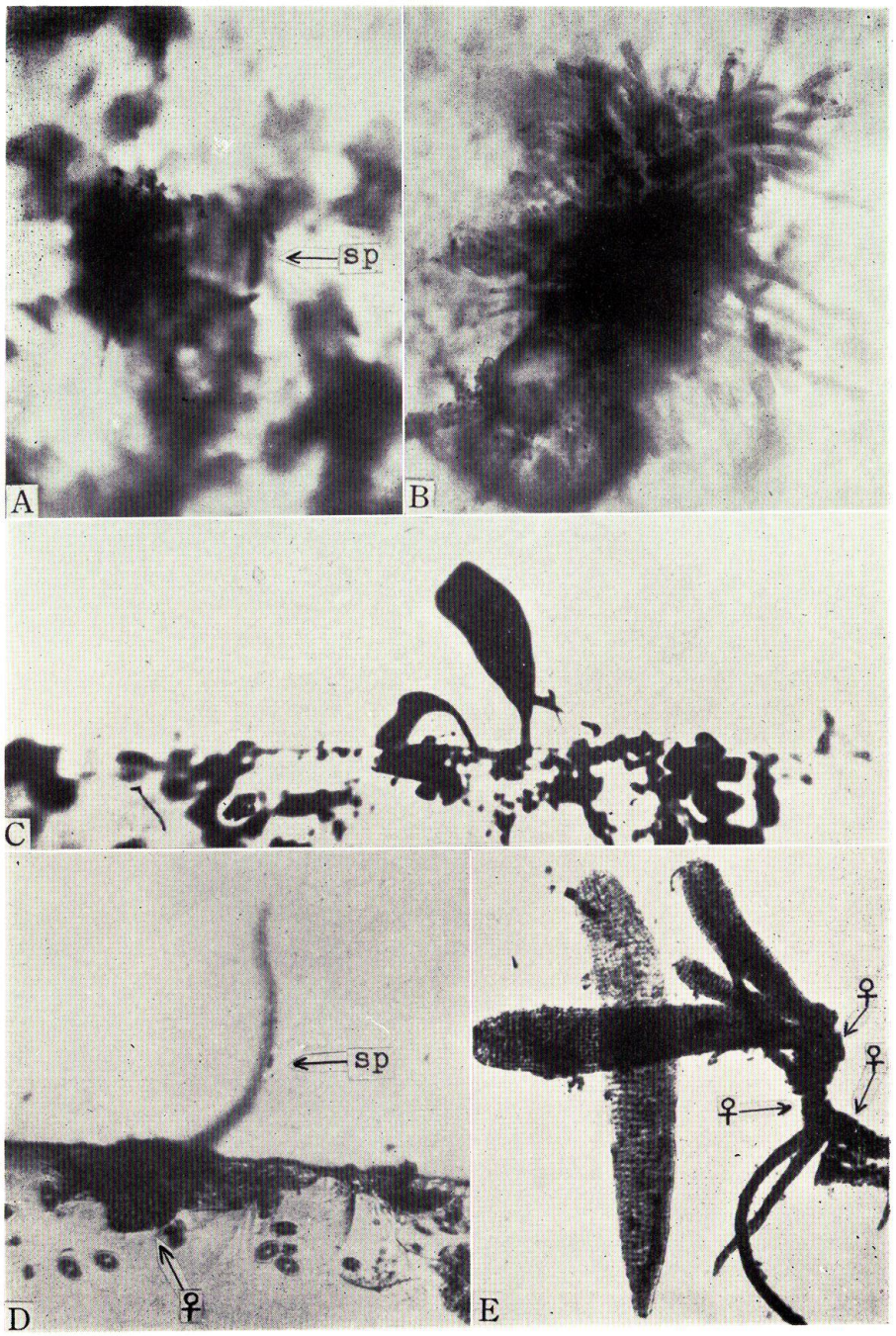

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PLATE XXXI

Immature female and male gametophytes of *Laminaria religiosa* (?) attached to the slide glasses (A, in the central portion of a glass; B–D, at the edge of a glass) and to the frosted glass plates (E and F). ×80

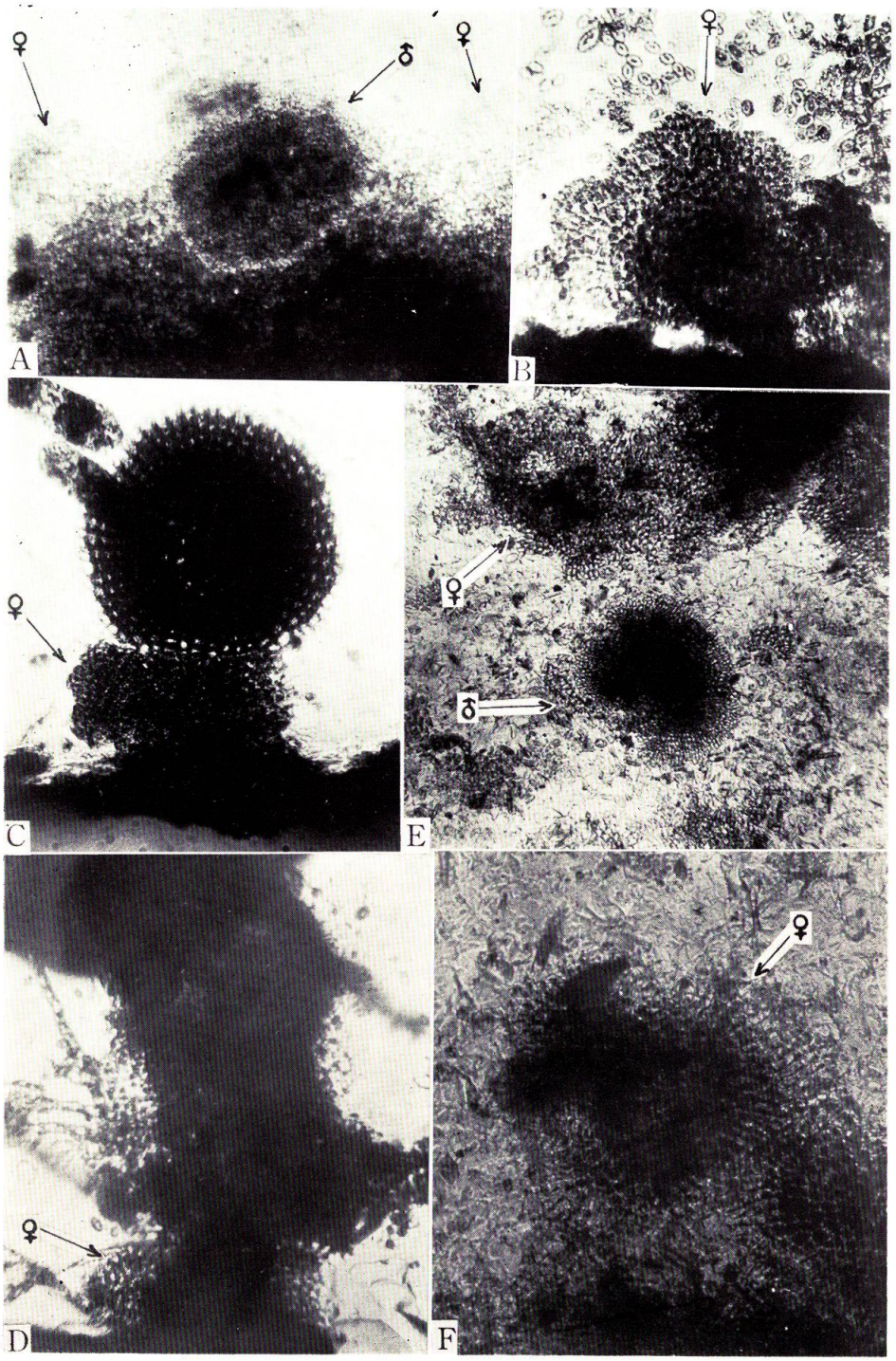

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PLATE XXXII

A–C. Immature female gametophytes (pointed by arrows) of *Laminaria religiosa* (?) attached to the slide glasses which had been settled among the *Laminaria* bed in Oshoro Bay from October 13 through November 8 in 1953

D. A germling of a calcareous red alga (c) growing upon a female gametophyte of *Laminaria religiosa* (?) attached to the same slide glass as shown in Fig. B

E. A male gametophyte (pointed by an arrow) of *Laminaria religiosa* (?) growing beneath a partly faded young crust of a calcareous red alga attached to the same slide glass as shown in Fig. B

F. Gametophytes of *Laminaria religiosa* attached to a slide glass which was immersed in sea-water containing the zoospores on October 12, 1952; from a culture in Schreiber's solution in the laboratory, twenty-five days old after removed from Oshoro Bay wherein the slide glass had been placed from 13 to 18 of October in 1953

A–F, ×80

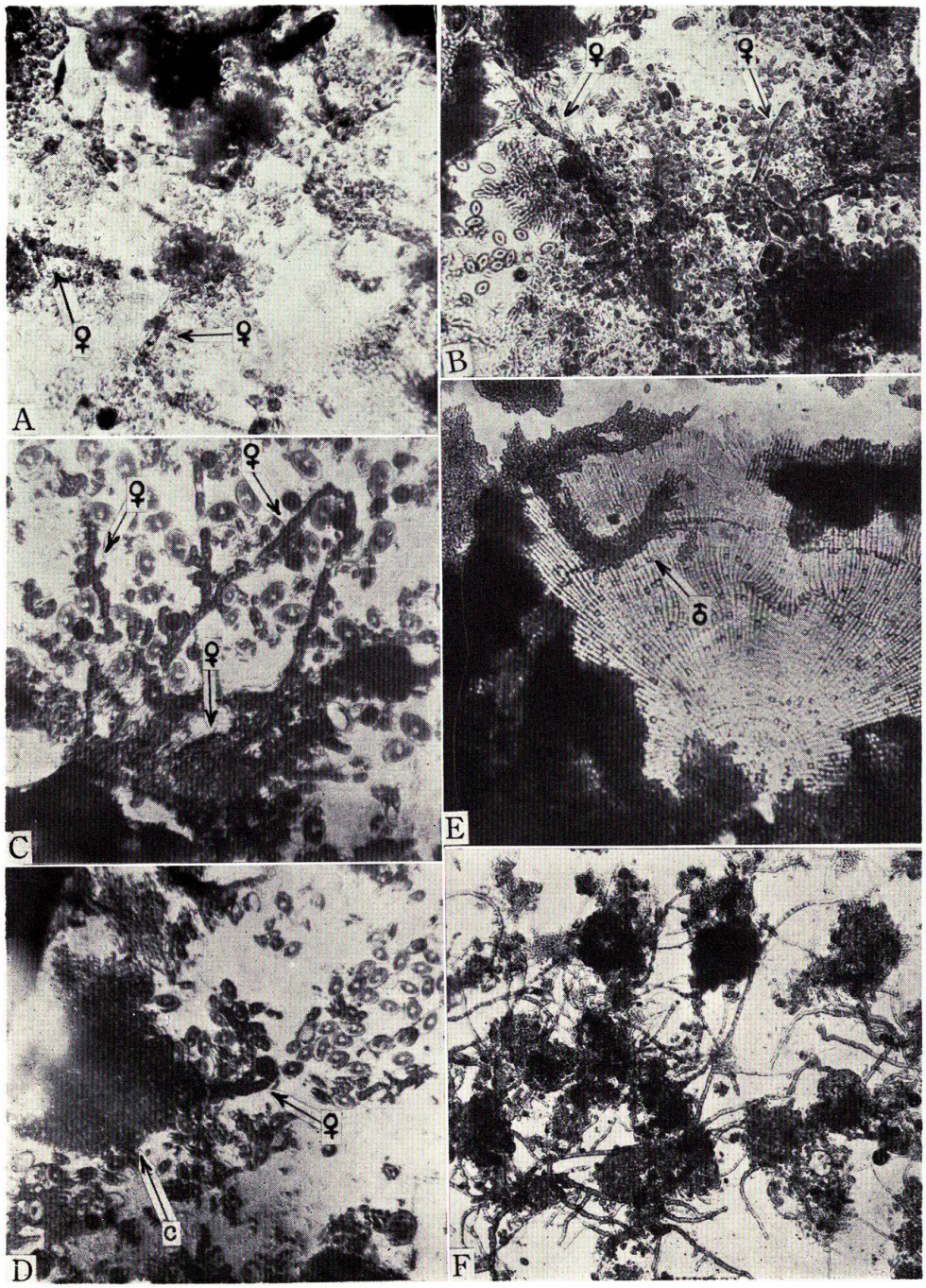

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PLATE XXXIII

A. Disk-shaped gametophytes of *Laminaria religiosa* (?) attached to a slide glass which had been placed in Oshoro Bay from 2 to 21 of December in 1953, and the sporophytes (pointed by arrows) developed upon them

B. A mass of filamentous female gametophytes of *Laminaria religiosa* (?) taken from the base of young sporophytes of *Laminaria*, which were collected in Oshoro Bay in December 1953

C & D. Young thalli of *Heterochordaria abietina* (?), which are apt to be confused with the sporophytes of *Laminaria*, attached to the slide glasses which had been placed in Oshoro Bay from 2 to 21 of December in 1953

A, ×50; B-D, ×80

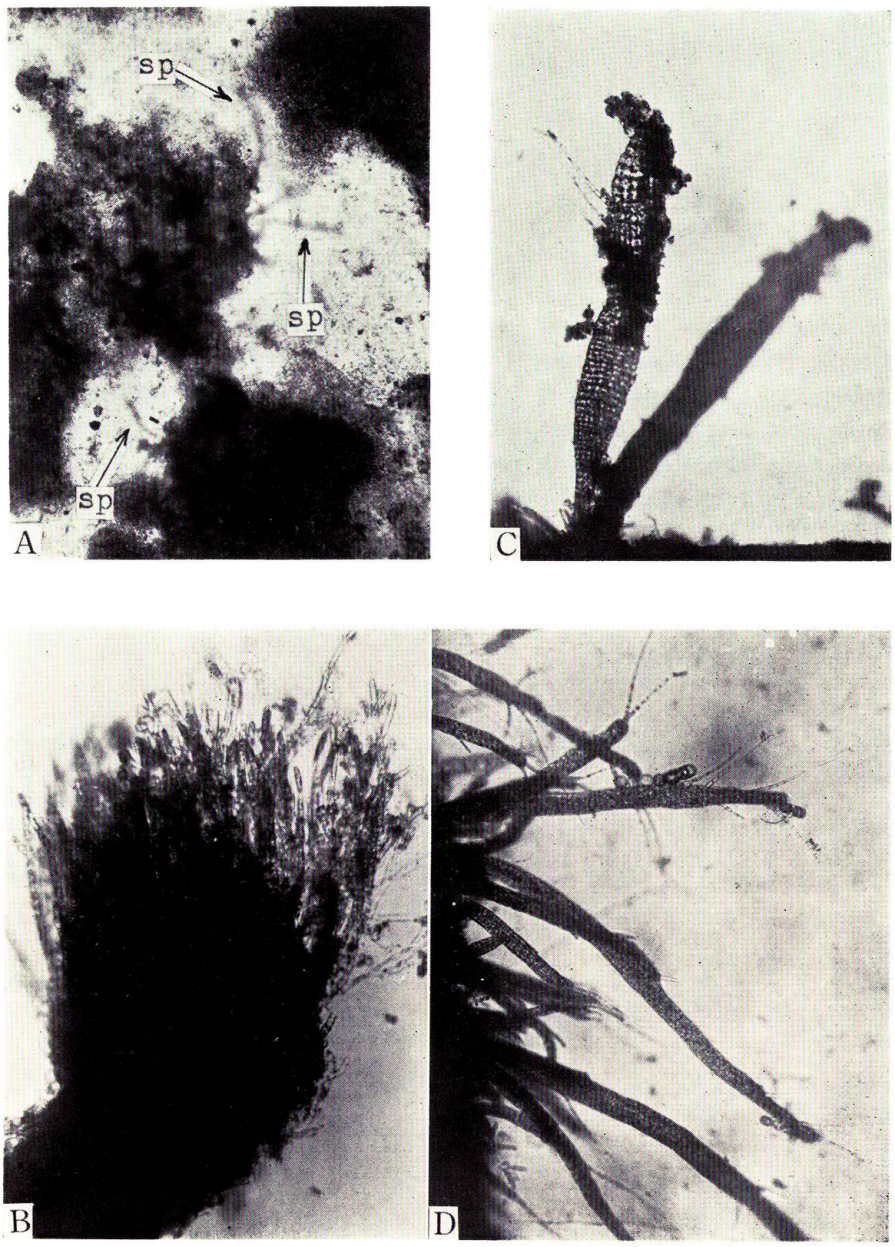

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PLATE XXXIV

Fertile gametophytes of *Laminaria religiosa* attached to the slide glass which had been made partly clear by means of a pincett to be loaded with sterile gametophytes only and cultured in Schreiber's solution for one year in the laboratory after having been placed in Oshoro Bay from 2 to 21 of December in 1953 (cf. text in p. 63)

A–C. Female gametophytes with young sporophytes. In A & B are seen the male gametophytes with antheridia too

D & E. Male gametophytes with antheridia

A–C, ×80; D, ×210; E, ×540

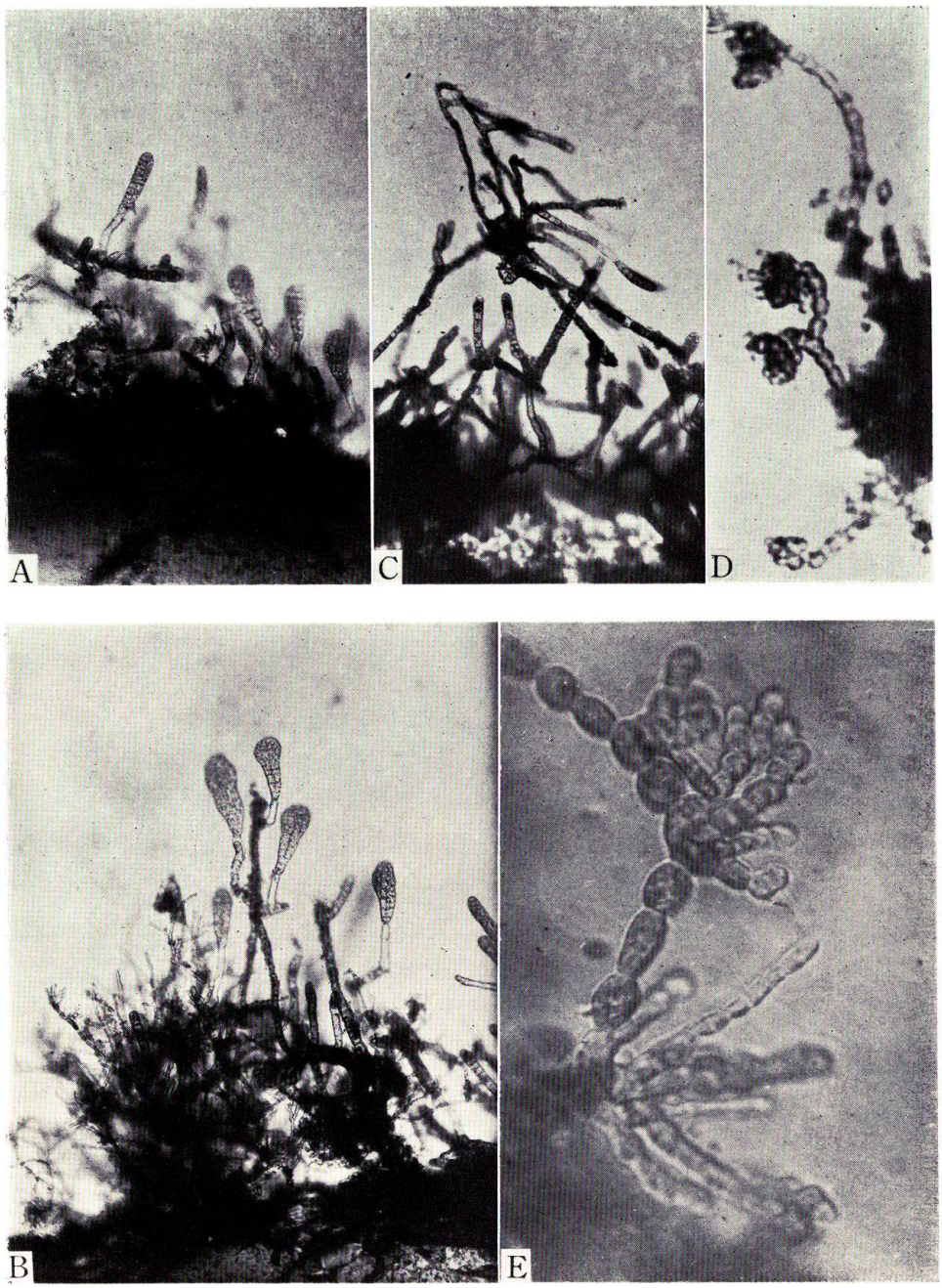

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PLATE XXXV

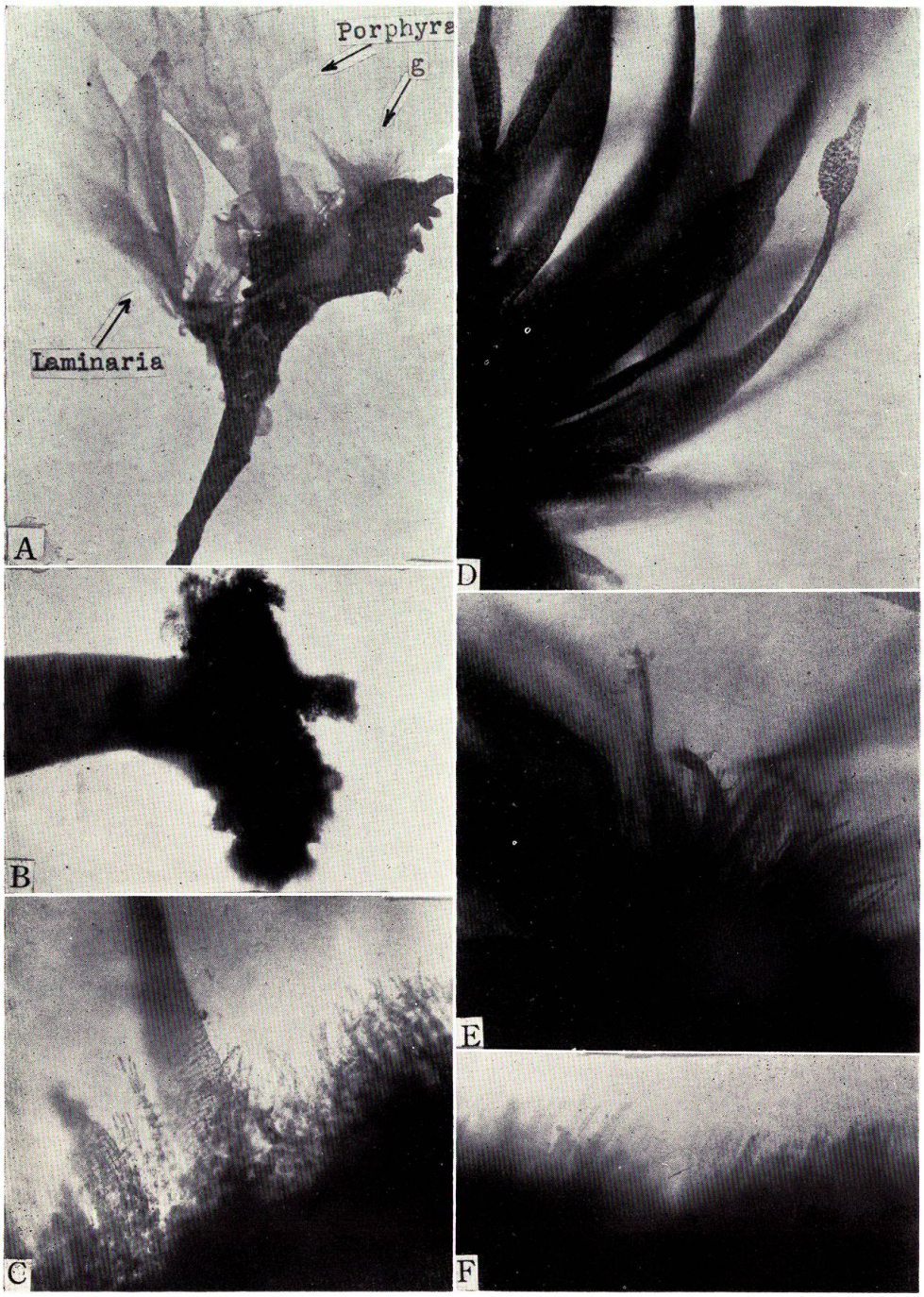
A. Young fronds and the gametophytes (g) of *Laminaria japonica* attached to a thallus of *Chondrus ocellatus* Holmes, collected at Nanaehama in February 1956

B. A mass of filamentous thalli of the gametophytes at the base of a young frond of *Laminaria japonica* collected at Nanaehama in February 1956

C-E. Gametophytes and young sporophytes of *Laminaria japonica* on a piece of stone, collected at Nanaehama in February 1956

F. Gametophytes of *Laminaria japonica* on a piece of stone, collected at Nanaehama in February 1956

A, ×ca. 2; B, ×40; C-F, ×80



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