On the Gametophytes of Some Japanese Species of Laminariales II.

By

Tiyoiti Kanda

With Plates XVII–XVIII.

In the present paper there are described the results of culture experiments with the gametophytes and the young sporophytes of five species of Japanese Laminariales, viz., *Laminaria Yendoana* Miyabe, *L. cichorioides* Miyabe, *L. yezoensis* Miyabe, *Kjellmaniella crassifolia* Miyabe and *Chorda Filum* (L.) Lamx. Cultures were made at the sea-side laboratory of the Institute of Algological Research at Muroran, by the same methods as those used in the previous work. Materials bearing well matured sori were collected at the sea-shore, brought to the laboratory, washed carefully in filtered sea-water, kept over night in a dark place being wrapped in paraffin-paper. The next morning they were put into a glass vessel filled with twice filtered sea-water. The zoospores were settled on slide glasses, and were cultured in glass vessels containing about 250 c.c. of medium. The culture vessels were placed in a room with northeast exposure where they were never exposed to direct sunlight. The cultures were subjected to the fluctuation of the temperature of the room.

Before going further the writer wishes to express his sincere thanks to Professor Y. Yamada for his kind guidance and criticisms in the course of the present study. Thanks are also due to the Hattori Hōkō Kai, pecuniary aid from which helped him in carrying out the present study.

VI. **Laminaria Yendoana** Miyabe

This plant is a distinct species having fibrilose rhizines and a slender stipe. It has a limited area of distribution, having been collected, hitherto, from only a few localities, that is, Muroran and its vicinity, Mori etc., on the coast of Hunkawan (Volcano Bay), Hokkaido. It grows on rocks and shells in the sublittoral belt. At Muroran it is abunduntly found on the bed of the harbour, where the water is quiet. It becomes soriferous in the winter season. The zoosporangia are produced as irregular patches

on both sides of the blade, covering the greater part of its surface. The material used for the present study was collected at Etomo on the coast of Muroran on January 18th 1937, and on the next day the culture of the zoospores was started.

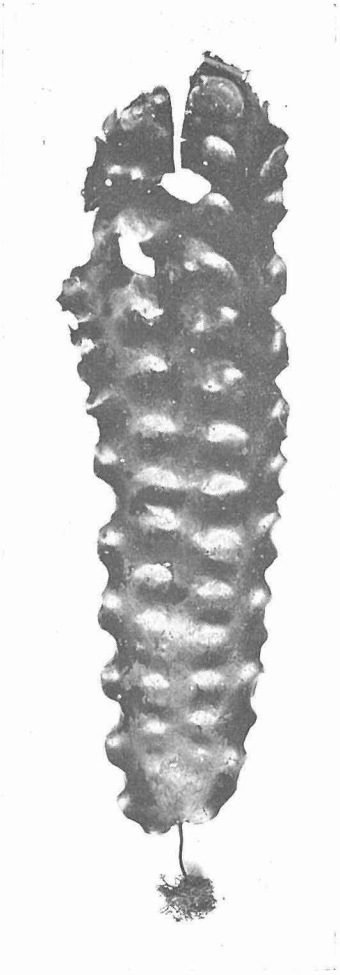

Fig. 1.
Laminaria Yendoana MIYABE.
The frond bearing zoosporangia.

Zoospores and their germination

The zoospores are pear-shaped, pointed at one end and rounded at the other, measuring about 9 μ in length, about 4 μ in breadth; they have two laterally placed cilia, one pointing in an anterior direction, measuring about 20 μ, and the other pointing backward, a little longer than the body itself. The zoospore has one chromatophore, one nucleus and some granular substances, but always lacks an eye spot (Fig. 2. A). The zoospores swim actively at first, but sooner or later become motionless. They lose their cilia before long, become spherical in shape, invested with a membrane and fasten themselves to the slide glass (Fig. 2. B–G). The germination tube increases its length more and more, as the culture progresses, and within 24 hours it reaches 25 μ in length. The distal end of the tube swells up and a transverse wall is formed so as to separate it from the tube (Fig. 2. H–I). This distal end increases its diameter, then the first cell division takes place in the 5 day old plant (Fig. 2. J). Within 12 days two types of plants may be distinguished. Those belonging to one type consist of a number of cells as figured in Fig. 3. A–C; they are male gametophytes. The others consist of only one cell at this stage, the diameter of the cell, however, being larger than that of male gametophytes; they are female gametophytes (Fig. 4. A–B).

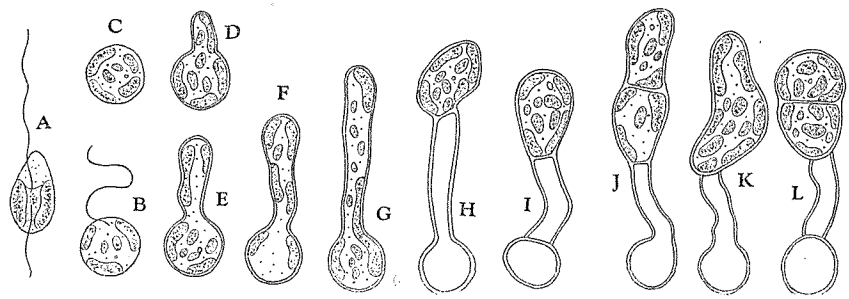

Fig. 2. *Laminaria Yendoana* MIYABE. Zoospore and its germination. ×1200. A. Zoospore in motile state. B. Zoospore in resting state. C. Embryospore. D. A spore from 12 hour culture, showing the development of a germination tube. E–G. Sporlings from 24 hour culture; the content of the original cell migrates into germination tube. H–I. Sporlings from 3 day culture, showing the first transverse wall. J–K. Sporlings from 5 day culture. L. A sporling from 7 day culture.

Male gametophytes and male gametes

Within 12 days the male gametophyte develops into a slightly branched filament consisting of more than 5 cells (Fig. 3. A–C). As the culture progresses, however, the number of cells of the plant is increased and the branching becomes denser (Fig. 3. D–H). When the male gametophytes approach to maturity, some of the apical cells of the branches become antheridia. The chromatophores of these cells disintegrate for the most part, and become pale in color. The contents of the antheridia is entirely used for the formation of a single antherozoid. At maturity the wall of the antheridium bursts open at the apex, forming a beak through which a mature antherozoid swims out (Fig. 3. D–H). The male gamete is ovate or sometimes rather round in shape, measuring approximately 6 to 8 μ in length. Two cilia of almost equal length measuring about 18 μ arise from the lateral side of the body. It has generally one or two pale colored chromatophores, but always lacks an eye spot (Fig. 3. m). The liberation of the male gamete from the opening of the antheridium was observed from time to time, but the actual process of fertilization with the egg cell has not been observed. The duration of life of the male gametophytes is generally short, and in the two month culture the greater part of them have disappeared.

Female gametophytes and eggs

The shape and size of the female gametophytes of this plant are very irregular, some consist of one cell (Fig. 4. A–E), others consist of two or

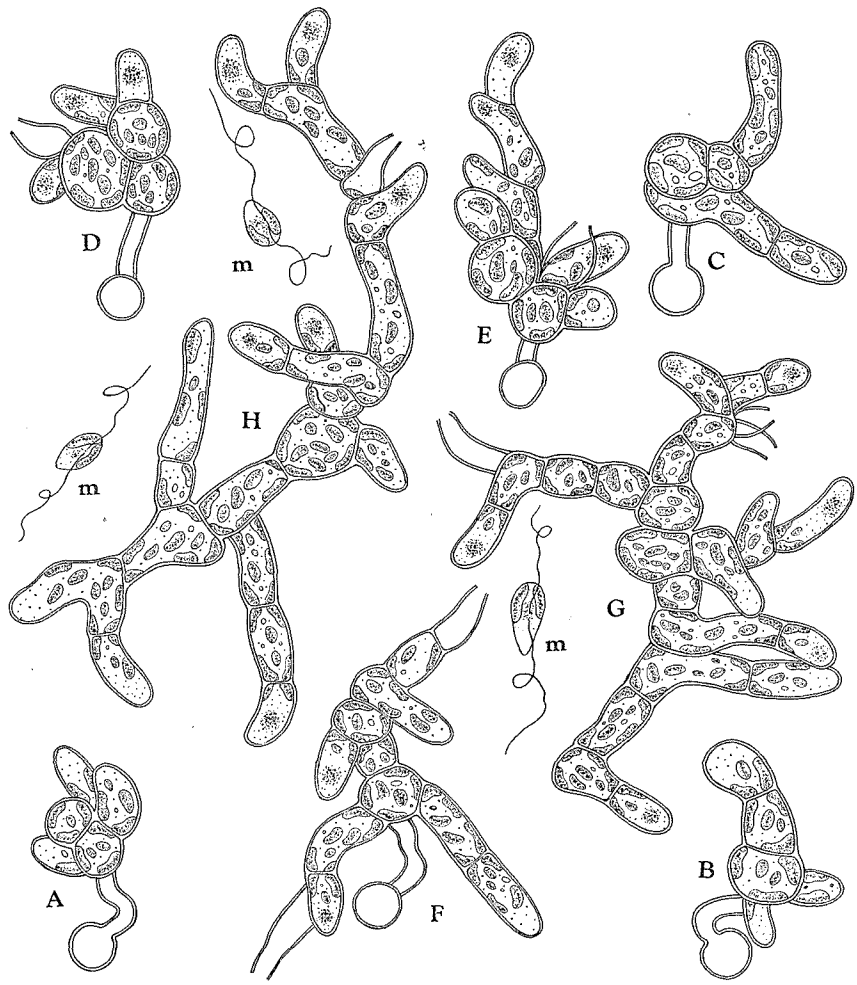

Fig. 3. *Laminaria Yendoana* MIYABE. Various forms of the male gemetophytes. × 1200. A. A male gametophyte from 12 day culture. B–C. Male gametophytes from 15 day culture. D–F. Mature male gametophytes with empty antheridia, from 18 day culture. G–H. Mature male gametophytes consisting of a fairly large number of cells, from 22 day culture. m. Male gamete in motile state.

more cells (Fig. 4. F–I). In some cases, a unicellular female gametophyte showing curious branching was seen (Fig. 4. D–E). When it comes to maturity the whole vegetative cell changes into an oogonium (Fig. 5. A). In case of two or more celled gametophytes, any cell, either terminal or intercalary may become an oogonium (Fig. 5. B). At maturity the chromatophores in the oogonium disintegrate and become crowded together with

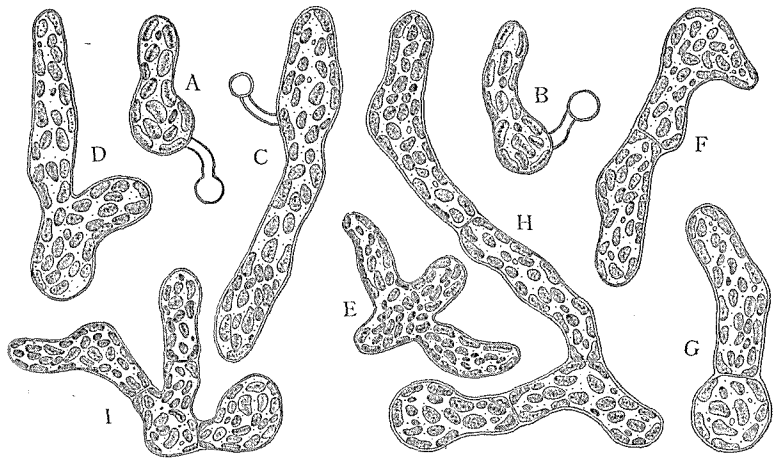

Fig. 4. *Laminaria Yendoana* MIYABE. Various forms of the female gametophytes. ×670. A–B. Female gametophytes from 12 day culture. C. Female gametophyte from 18 day culture; the cell of which is remarkably stretched out. D. Female gametophyte from 18 day culture. E. Unicellular female gametophyte from 22 day culture, showing a curious branching. F–I. Female gametophytes consisting of more than 2 cells.

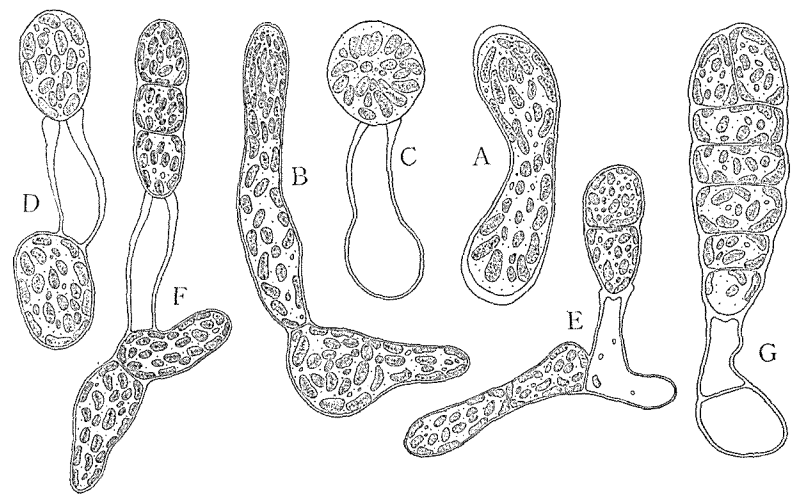

Fig. 5. *Laminaria Yendoana* MIYABE. Mature oogonia, eggs and young sporophytes. ×800. A. One-celled female gametophyte at maturity, from 21 day culture, the vegetative cell of which metamorphoses into an oogonium. B. Two-celled female gametophyte from 22 day culture. C. An egg discharged from one-celled female gametophyte from 18 day culture. D. An egg resting at the opening of the oogonium, from 22 day culture. E–G. Development of the young sporophyte.

their long axes parallel to that of the oogonium, while the contents of the oogonium become liquefied, and the oogonium shows the presence of strong internal pressure (Fig. 5. A–B). The contents of the oogonium is then pressed out through the opening at the apex where it remains attached (Fig. 5. C–D). The writer witnessed many times under microscope, how the egg is forced out of the oogonium.

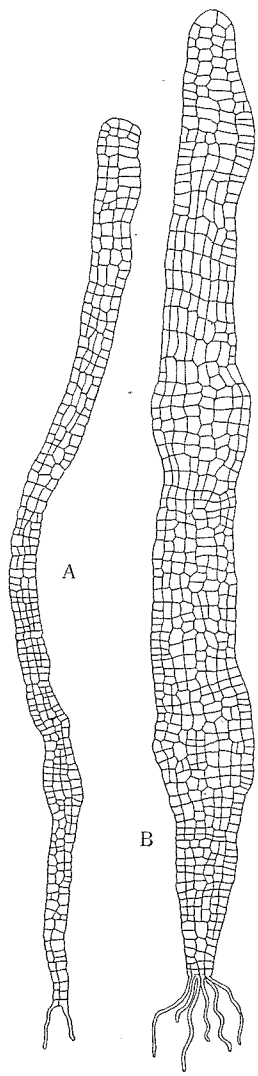

Fig. 6. *Laminaria Yendoana* MIYABE. Further development of the young sporophytes. × 55. A. A young sporophyte consisting of more than 300 cells, the rhizoid formation, however, is very poor. General appearance of the plant is slender in shape, from 60 day culture. B. More advanced stage of the young sporophyte, from 80 day culture.

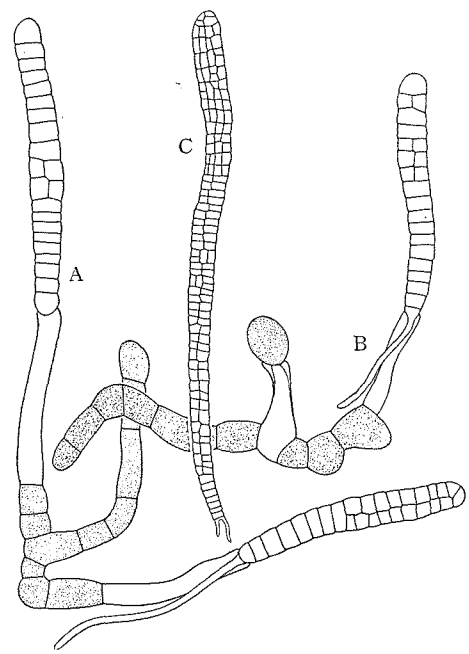

Fig. 7. *Laminaria Yendoana* MIYABE. Development of the young sporophytes. A. Two young sporophytes developed from a single female gametophyte. × 273. B. Female gametophyte bearing an egg and a young sporophyte. × 273. C. A young sporophyte protruding two rhizoids from its basal cell. × 73.

Development of sporophytes

When the egg is fertilized the membrane of it becomes thicker, and in the 22 day culture the first cell division takes place (Fig. 5. E). In the early stages of development the sporophyte is very slender in shape because the cell divisions are repeated only by transverse wall (Fig. 6–7). A rhizoid grows out of the basal cell of the filament in the plant of 40 day culture (Fig. 7. A–B), but its development is very poor when compared with that of other Laminariaceous plants (Fig. 6. A, 7. C).

VII. **Laminaria cichorioides** MIYABE

In habits, *Laminaria cichorioides* MIYABE shows some resemblance to *Laminaria Yendoana* MIYABE, which distributes only on the Pacific side of Hokkaido. But it is readily distinguished from the latter by the corrugations on both margins of the lower portions of the blade, moreover, the stipe and rhizines are not so slender as those of *L. Yendoana* MIYABE. It is found growing in Japan along the Hokkaido coast of the Japan Sea, as well as that of the Okhotsk Sea, and on the northern coast of Chosen on the Japan Sea side. It grows on rocks and shells in the sublittoral belt. At Osyoro (Siribesi Province, Hokkaido), where the material for the present investigation was collected, these plants are found on the bed of the harbour. This species become soriferous from late in autumn to winter. The zoosporangia are produced in the depressions of the bullae on one side at first, later on both sides of the blade covering the greater part of the surface.

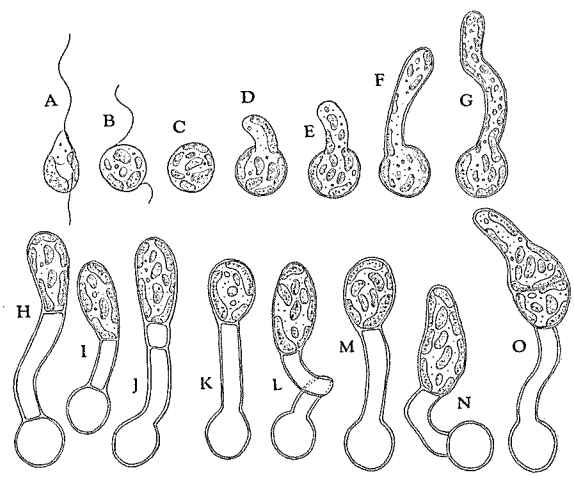

Fig. 8. *Laminaria cichorioides* MIYABE. Zoospore and its germination. ×1000. A. Zoospore in motile state. B. Resting zoospore. C. Embryospore. D. Germination of the embryospore. E–G. More advanced stages of the germination. H–J. Sporlings from 2 day culture; a transverse wall is formed at this stage. K–L. Sporlings from 3 day culture. M–O. Sporlings from 4 day culture; the first cell division takes place at this stage.

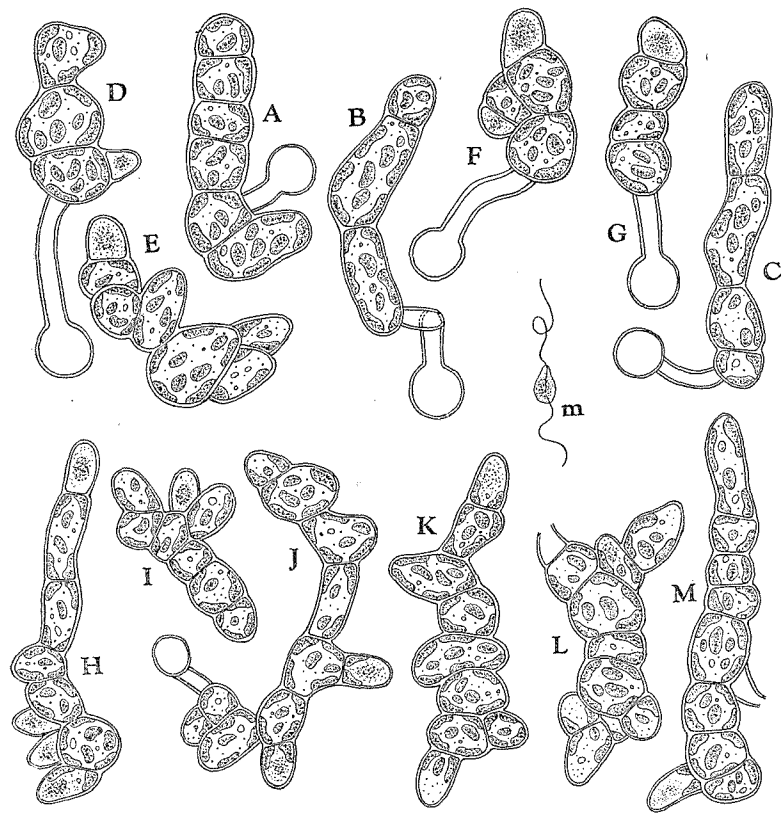

Fig. 9. *Laminaria cichorioides* MIYABE. Various forms of the male gametophytes. A–C. Male gametophytes from 8 day culture. ×1250. D–G. Mature male gametophytes from 13 day culture. ×1250. H–M. Mature male gametophytes from 13 day culture showing the empty antheridia at the tips of the branches. ×940. m. Male gamete in motile state.

Development of gametophytes

The shape and size of the zoospores and the process of their germination, are entirely similar to those of *Laminaria Yendoana* MIYABE (Fig. 8. A–O). When the plants are 8 days old, it is possible to distinguish two types of gametophytes. The male gametophytes reach maturity, then begin to produce gametes in 13 days after the germination of the zoospores. The male gametes were examined in fresh material; their liberation from the opening of the antheridia was also observed many times under microscope. They are pyriform in shape, measuring about 5μ in length, having two laterally placed cilia of almost equal length. In some cases there are

observed pale remnants of the chromatophores, but they always lack an eye spot (Fig. 9. m). In shape and size the female gametophytes are somewhat irregular, but the processes of the formation of oogonia and egg cell are entirely similar to those observed in other species (Fig. 10. A–D).

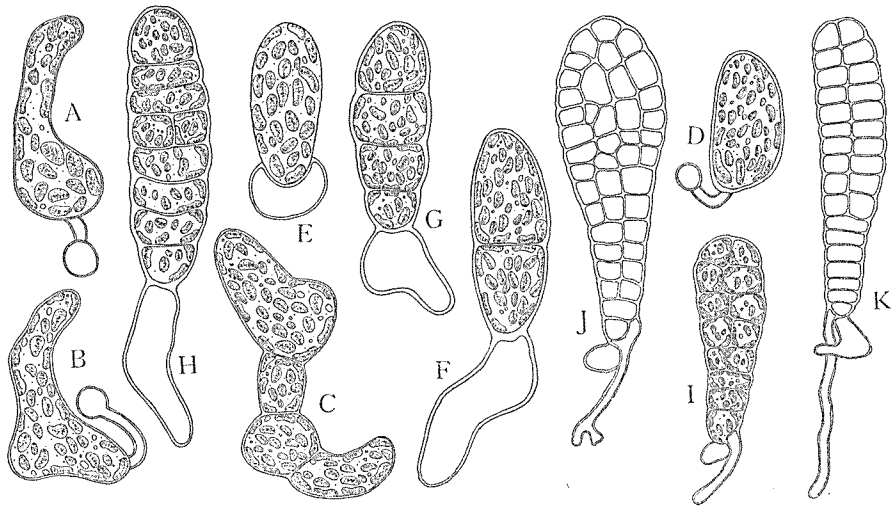

Fig. 10. *Laminaria cichorioides* MIYABE. Female gametophytes, the formation of egg-cells and the development of the young sporophytes. A. Female gametophyte from 8 day culture. ×856. B. Female gametophyte from 13 day culture. ×632. C. Female gametophyte consisting of 4 cells, from 13 day culture. ×632. D. Mature female gametophyte consisting of a single cell; the empty cell of the embryospore still attached; from 13 day culture. ×632. E. An egg discharged from unicellular female gametophyte, from 16 day culture. ×856. F. Showing the first cell division in a fertilized egg, from 13 day culture. ×632. G. A young sporophyte consisting of 4 cells. ×632. H. A. young sporophyte showing the first cell division by a longitudinal wall. ×559. I–K. Young sporophytes of various ages, showing the rhizoid. ×335.

Development of young sporophytes

The development of the sporophyte begins immediately after the egg is fertilized, and when the plants are 13 days old, the first cell division in the egg cell takes place. The new sporophytes grow rapidly, and in the 25 day culture many of them develop one or more rhizoids (Fig. 10 I–K). As the growth of the sporophyte progresses, the upper part of it increases rapidly forming a flat expanded blade (Fig. 11). So far as the shape and general appearance of the zoospores, gametophytes and sporophytes are

concerned no remarkable difference was observed compared with those of *Laminaria Yendoana* MIYABE.

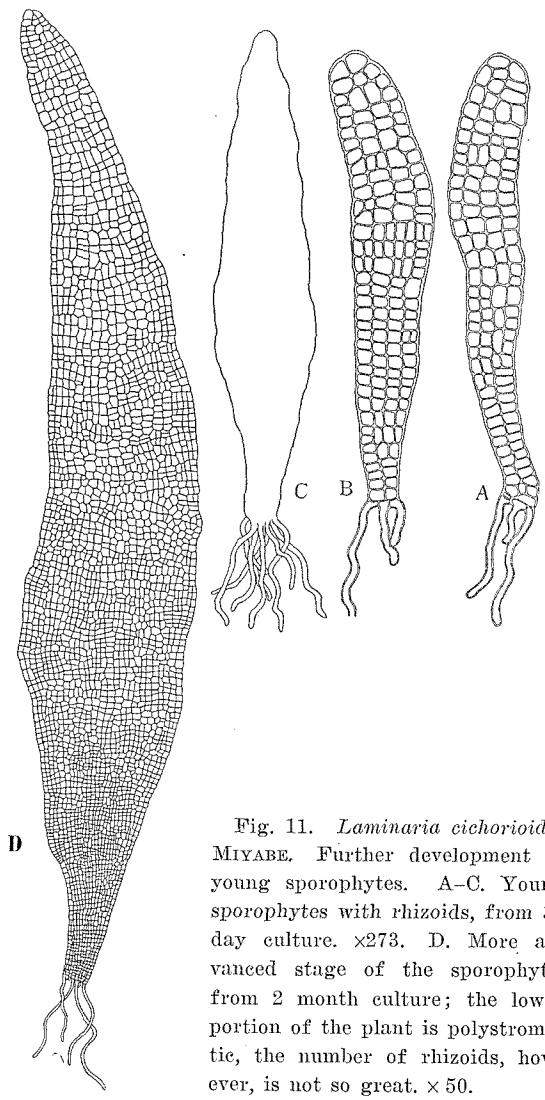

Fig. 11. *Laminaria cichorioides* MIYABE. Further development of young sporophytes. A–C. Young sporophytes with rhizoids, from 36 day culture. ×273. D. More advanced stage of the sporophyte, from 2 month culture; the lower portion of the plant is polystromatic, the number of rhizoids, however, is not so great. × 50.

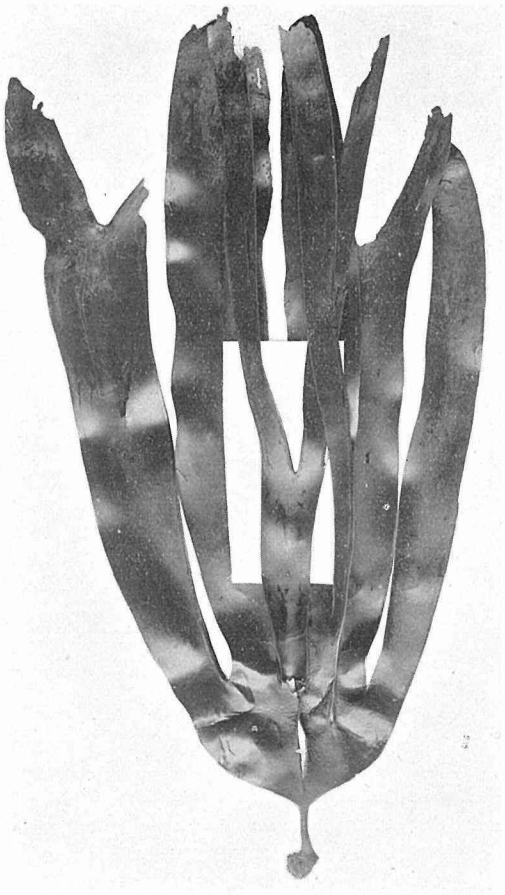

Fig. 12. *Laminaria yezoensis* MIYABE.
The frond bearing zoosporangia.

VIII. **Laminaria yezoensis** MIYABE

The present species is an inhabitant of the northern colder waters, distributing from Kusiro (Kusiro Province, on the Pacific side of Hokkaido) to the North Kuriles. It grows on rocks in the sublittoral belt. The zoosporangia are formed on both surfaces of the blades. They become soriferous in winter. Well matured materials were collected at Akkesi, near Kusiro, on February 24th, 1935, and transported by rail to the laboratory at Muroran. The culture of the zoospores was started on February 26th.

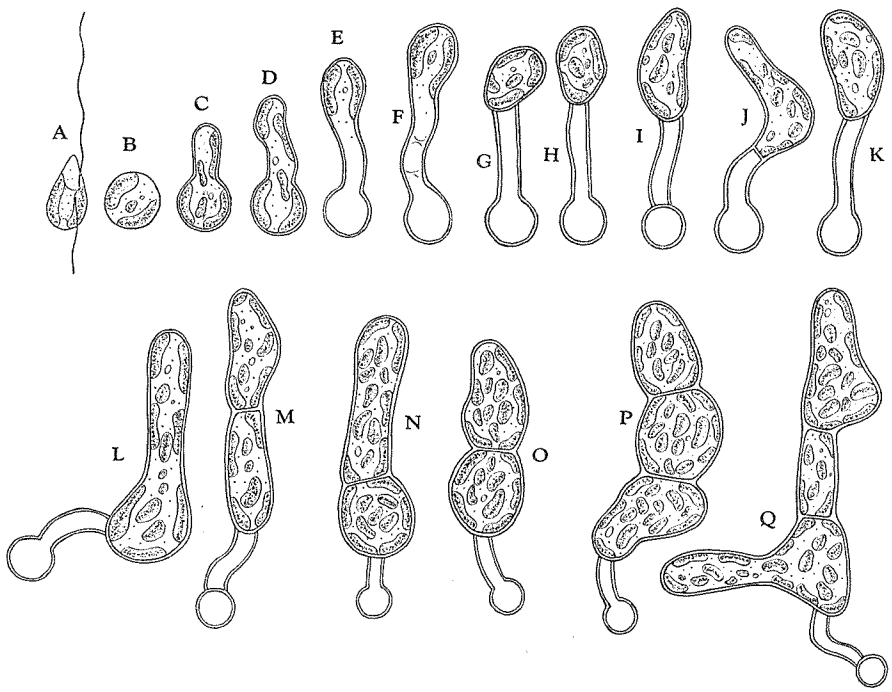

Fig. 13. *Laminaria yezoensis* MIYABE. Germination of the zoospore and early stages of the female gametophytes. ×1186. A. Zoospore in motile state. B. Embryospore. C–F. The development of the germination tube; the contents of the cell migrate into this part. G–H. Sporlings from 5 day culture. I–K. Sporlings from 8 day culture. L–M. Sporlings from 11 day culture; the first cell division may be observed at this stage. N–O. Early stages of the female gametophytes, from 15 day culture. P–Q. Female gametophytes consisting of 3 cells, from 20 day culture.

Development of gametophytes

The zoospores are pear-shaped, about 9 μ in length, have two laterally placed cilia, one curved chromatophore, one nucleus and some granular substances, but always lack an eye spot (Fig. 13. A). When the plants are 15 days old, two types of gametophytes may be distinguished (Fig. 13. N–O, 14. A). The outer appearances of the male gametophytes bear a close resemblance to those of other members of *Laminaria* studied. The female gametophytes of this species are widely variable in shape, some consist of a single cell, others consist of numerous cells forming an irregular filament (Fig. 15. A–C). At the time of fruiting, the oogonium is elongated, the contents become dense, and the chromatophores are crowded together at the apex (Fig. 15. B–C). At maturity the egg cell is forced out through

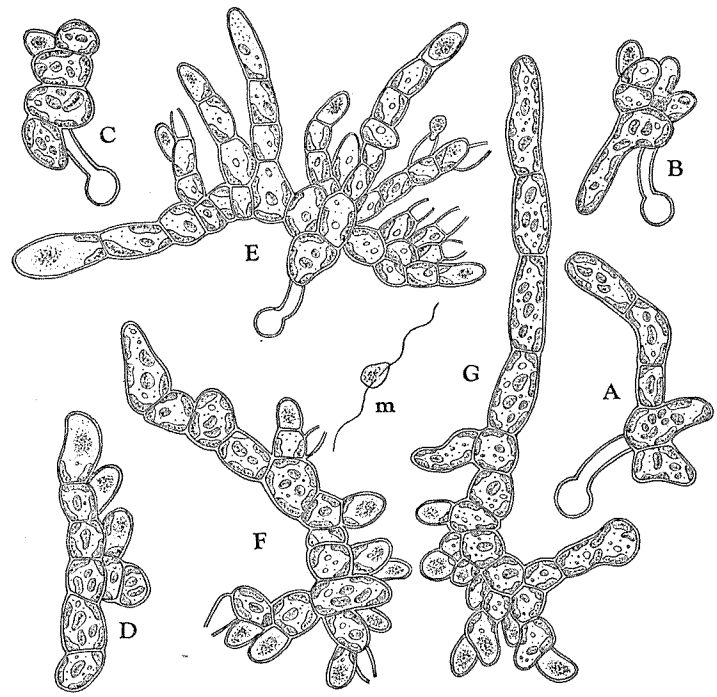

Fig. 14. *Laminaria yezoensis* MIYABE. Various forms of male gametophytes. ×1125. A. Male gametophyte from 15 day culture. B–D. Male gametophytes bearing antheridia. E. Mature male gametophyte from 28 day culture; the tips of the branchlets are divided forming the antheridial clusters. F–G. Mature male gametophytes, from 32 day culture. m. Male gamete in motile state; showing two cilia of almost equal length.

the opening at the apex of the oogonium and remains attached there (Fig. 15. D).

Development of young sporophytes

The fertilized egg begins germination soon. At first the cell divisions take place by a transverse wall, so that the sporophyte in the youngest stage is a row of cells. The rhizoids make their appearance in the 45 day culture (Fig. 15. H). Though the macroscopic generation of this species shows a remarkable difference in its outer appearance, having palmate blades and discoidal hapter, in comparison with those of other species of *Laminaria,* there is no great difference observed in the zoospores, gametophytes and sporophytes.

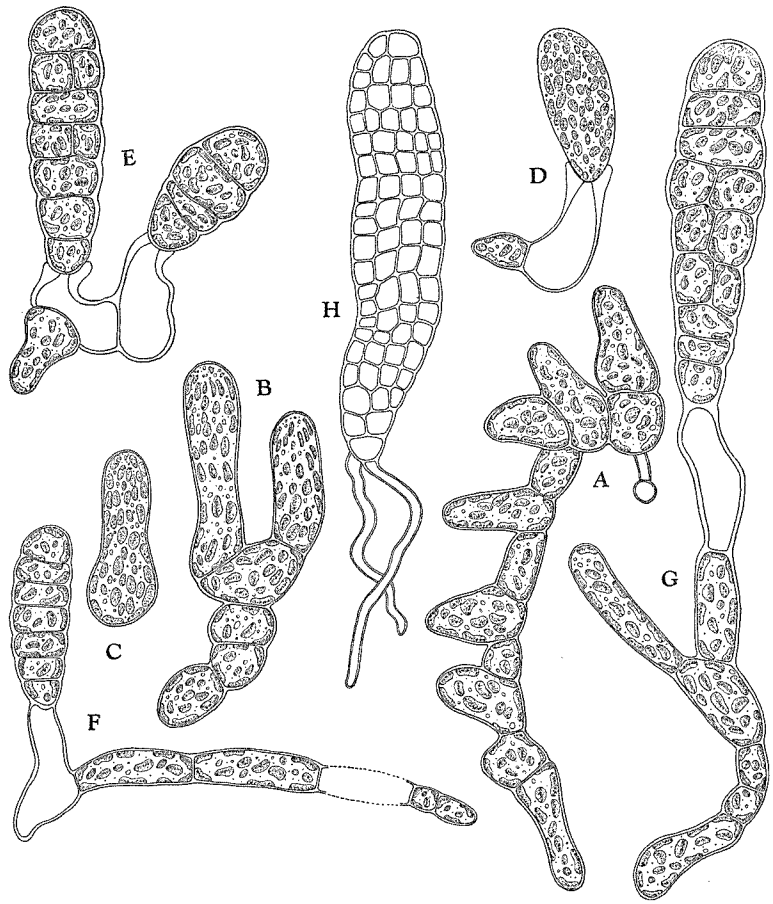

Fig. 15. *Laminaria yezoensis* MIYABE. Female gamotophytes, oogonia, eggs and young sporophytes. A. Female gametophyte consisting of numerous cells, from 31 day culture. ×566. B. Mature female gametophyte from 31 day culture, showing two oogonia at maturity; the chromatophores of the oogonial cell are divided into small pieces and crowded together at the apex. ×566. C. One-celled female gametophyte at maturity, from 24 day culture. ×566. D. An egg resting at the opening of the oogonium. ×566. E. Two young sporophytes developed from single female gametophyte, from 31 day culture. F. A female gametophyte and a young sporophyte, from 30 day culture. ×566. G. Young sporophyte resting on the tip of the oogonium, from 40 day culture. ×566. H. Young sporophyte with two rhizoids, from 45 day culture. ×342.

IX. **Kjellmaniella crassifolia** MIYABE

This plant is commonly found at Muroran. It grows on rocks in the sublittoral belts, associated with *Laminaria japonica* ARESCH., *Costaria*

costata (TURN.) SAUNDERS, *Undaria pinnatifida* SURING, etc. On the coast of Muroran the embryonal fronds of this plant appear late in December. It attains its full growth in the following autumn. After the zoospores have been liberated, the upper part of the blade is gradually worn away, and practically ceases to develop further. In November, however, the second year blade begins its sudden growth at the transition region, pushing up the first year blade. The second year blade grows so rapidly that the demarcation between the two parts is distinctly recognized. The zoosporangia are produced, at first, on one side of the blade, covering the greater part of it, later on both

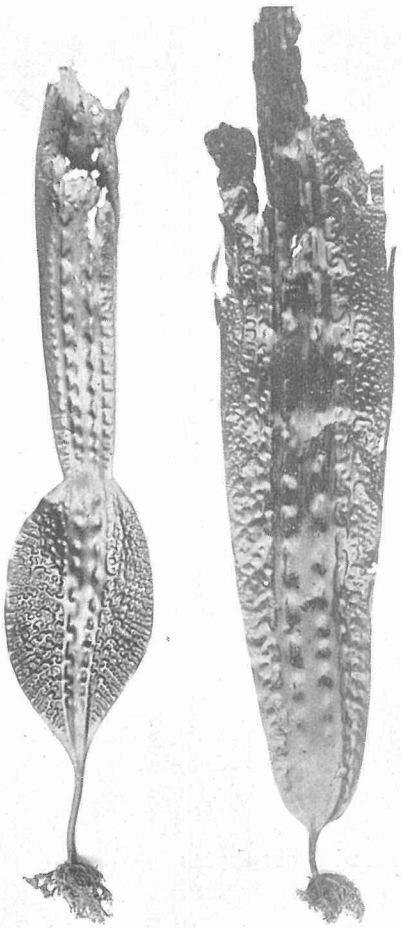

Fig. 16. *Kjellmaniella crassifolia* MIYABE. The plant (left) collected at Muroran in November, showing the demarcation between first and second year blades. The upper part of the old blade is gradually worn away. The zoosporangia are formed only in the first year blade. The bullae in the second year blade are very dense.

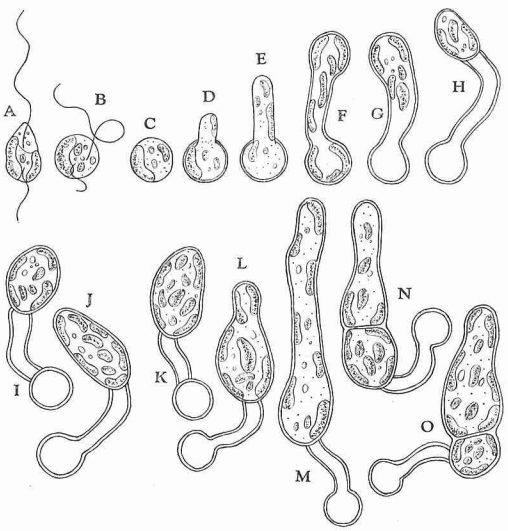

Fig. 17. *Kjellmaniella crassifolia* MIYABE. Zoospore and its germination. ×1000. A. Zoospore in motile state. B. Zoospore in resting state. C. Embryospore. D–E. Sporlings from 12 hour culture, showing the development of the germination tube. F–H. Sporling from 3 day culture, showing the first transverse wall. I–J. Sporlings from 5 day culture. K–L. Sporlings from 7 day culture. M–O. ditto K, from 9 day culture.

sides. The material for the present study was obtained at Masuiti on the coast of Muroran. The cultures of zoospores have been made three times, in October 1934, September 1935 and October 1936.

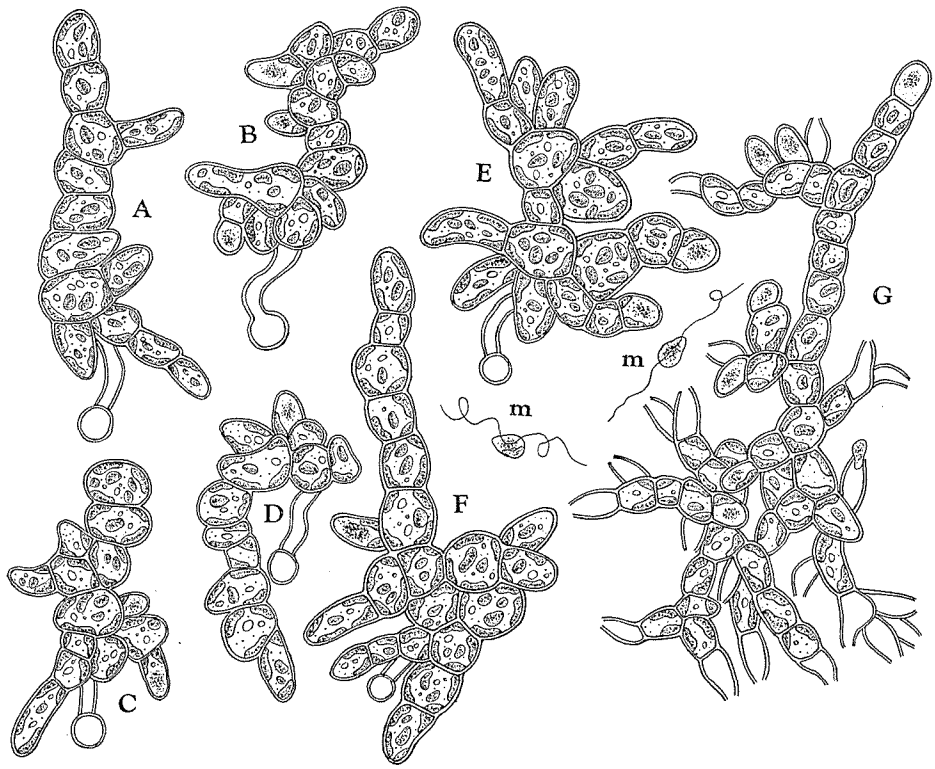

Fig. 18. *Kjellmaniella crassifolia* MIYABE. Various forms of the male gametophytes. ×868. A–F. Male gametophytes from 16 day culture. G. Mature male gametophyte consisting of numerous cells. m. Male gamete in motile state.

Development of gametophytes and sporophytes

As the accompanying figures show, the shape and size of the zoospores, the process of their germination, and the general aspects of both gametophytes and young sporophytes bear a strong resemblance to those of other Laminariaceous plants. As detailed descriptions of these characters have been given in other species described above, also as the course of the development, likewise the features of the zoospores, gametophytes and sporophytes may be traced in the following figures, mention is made here of only a few indispensable points regarding the present species.

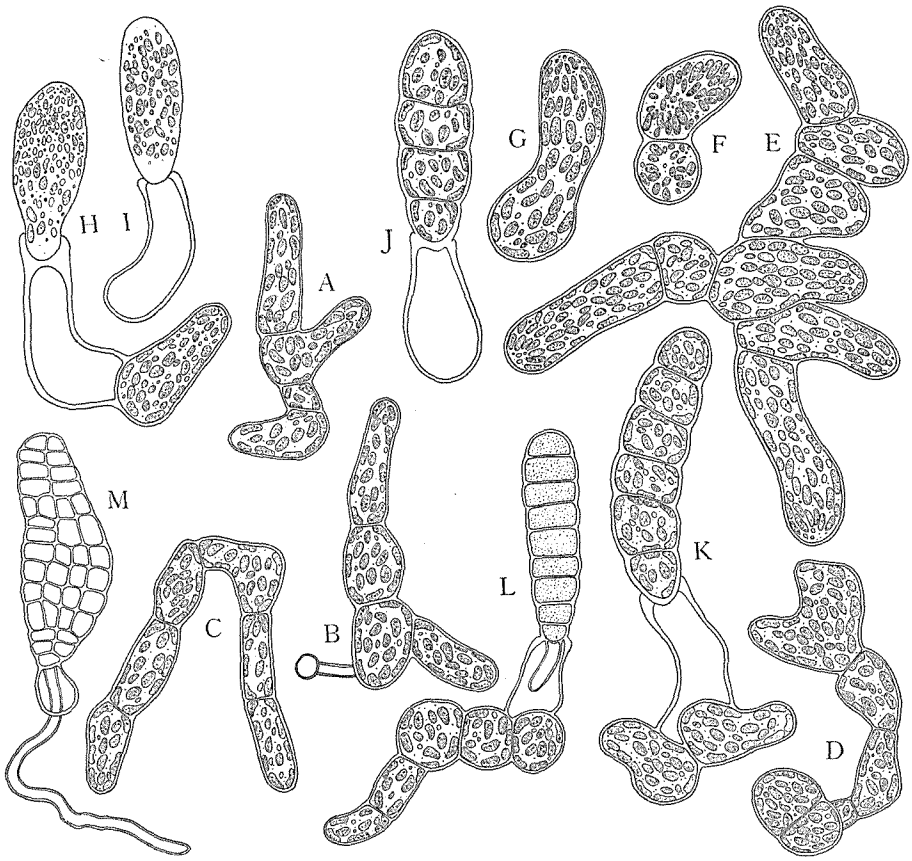

Fig. 19. *Kjellmaniella crassifolia* MIYABE. Female gametophytes, eggs and young sporophytes. A–C. Female gametophytes from 16 day culture. ×566. D. Female gametophyte consisting of 6 cells, from 25 day culture. ×566. E. Well developed female gametophyte consisting of 8 cells, from 38 day culture. ×566. F. Two-celled female gametophyte at maturity, from 25 day culture. ×566. G. One-celled female gametophyte at maturity, from 23 day culture. ×833. H. An egg discharged from two-celled female gametophyte, from 38 day culture. ×566. I. From 24 day culture. ×566. J. Young sporophyte from 23 day culture. ×833. K. From 38 day culture. ×833. L. Young sporophyte with rhizoid, from 25 day culture. ×566. M. Young sporophyte bearing rhizoid, from 30 day culture. ×340.

The zoospores have no eye spot; two types of gametophytes are distinguished in 9 day culture; each of the gametophytes attains its maturation within 16 days. The male gametes in this species were also observed many times; they have generally no chromatophore, but in some

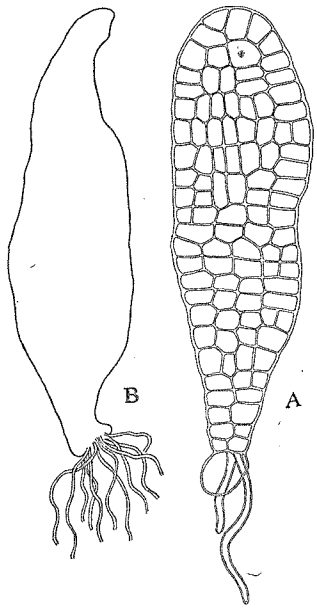

Fig. 20. *Kjellmaniella crassifolia* MIYABE. Development of the young sporophytes. A. Young sporophyte with two rhizoids, from 2 month culture. ×328. B. Young sporophyte with numerous rhizoids; a disc-shaped expansion developed at the base of the blade, from 3 month culture. ×88.

cases a pale remnant of chromatophore may be seen. They have two laterally placed cilia of almost equal length, but they always lack an eye spot. The cells composing the male and female gametophytes are filled with deep brown colored chromatophores, therefore the gametophytes of this species are distinguished without failure from those of others. There are no special remarks to be offered on the development of the young sporophytes. The rhizoids are formed as usual, and a disc-shaped expansion, the initial of the rhizines, by means of which the plant gains permanent attachment to the substratum is also produced when the plants are 3 months old (Fig. 20. B).

X. **Chorda Filum** (L.) LAMX.

Chorda Filum (L.) LAMX. is rather widely distributed in this country on the Pacific coasts, as well as on those of the Japan Sea. Its northern limit is near Muroran on the Pacific coast. It grows on rocks and shells in quiet waters in the sublittoral belt. It attains full maturity from late in summer to autumn. The materials for the present study were collected at Osyoro (Siribesi Province, Hokkaido) and Hukaura (Aomori Prefecture), then transported by rail to the laboratory at Muroran. The cultures of the zoospores have been made three times, viz., in July 1932, October 1934 and November 1937.

The present species has already been cultured by KYLIN (1918, 1933) and WILLIAMS (1921). The systematic position of *Chorda* had been left undecided for a long time, until KYLIN published his notable discovery revealing its life-cycle. Culturing the zoospores, KYLIN proved that they gave rise to the male and female gametophytes, and that new sporophytes started soon after the fertilization of two gametes took place. According to these evidences he proposed that the genus *Chorda* should be placed systematically in the same order as the Laminariaceae. With regard to the development of the gametophytes, sufficient descriptions have been given

in KYLIN's paper, but the writer intended in this study, to confirm KYLIN's results with the Japanese material. In addition to this an attempt was made to discuss the differences in the characteristics of the zoospores, gametophytes, gametes and sporophytes between *Chorda* and other Laminariaceous plants.

Zoospores and their germination

The zoospores are pear-shaped, measuring about 9 μ in length, 4.5 μ in breadth; they have two laterally placed cilia, one curved chromatophore, one nucleus and a distinct eye spot, which characterizes the present species (Fig. 21. A). They are very active at first, but sooner or later their movement becomes slower; meanwhile they lose their cilia, assuming a spherical form, are invested with a membrane, and become fastened to the slide glass (Fig. 21. B). Then the tube grows out from a spore (Fig. 21. C), the chromatophores and granular substances together with the eye spot migrate into the distal end of the tube (Fig. 21. D). By the end of 24 hours the distal end of the tube swells up, a cross wall is formed separating this part from the germination tube (Fig. 21. F). In *Chorda* the germination tube grows long as shown in Fig. 21. G. When the sporlings are 3 days old, the first cell division takes place in a direction perpendicular to the long axis of the cell (Fig. 21. K).

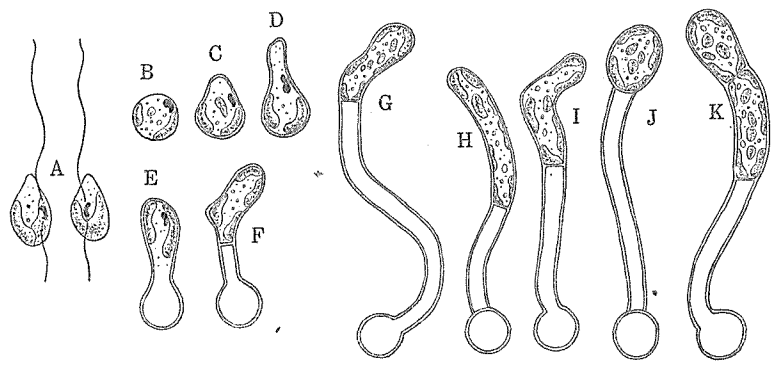

Fig. 21. *Chorda Filum* (L.) LAMX. Zoospores and their germination. × 836. A. Zoospores in motile state; showing one curved chromatophore and an eye spot. B–E. Germination of the zoospore. F. Sporling from 24 hour culture, the first transverse wall is formed. G–I. Sporlings from 2 day culture. J–K. Sporlings from 3 day culture; the first cell division takes place at this stage.

Male gametophytes and male gametes

The development of the gametophytes is rather rapid compared with that of other species of *Laminaria*; two types of gametophytes may be

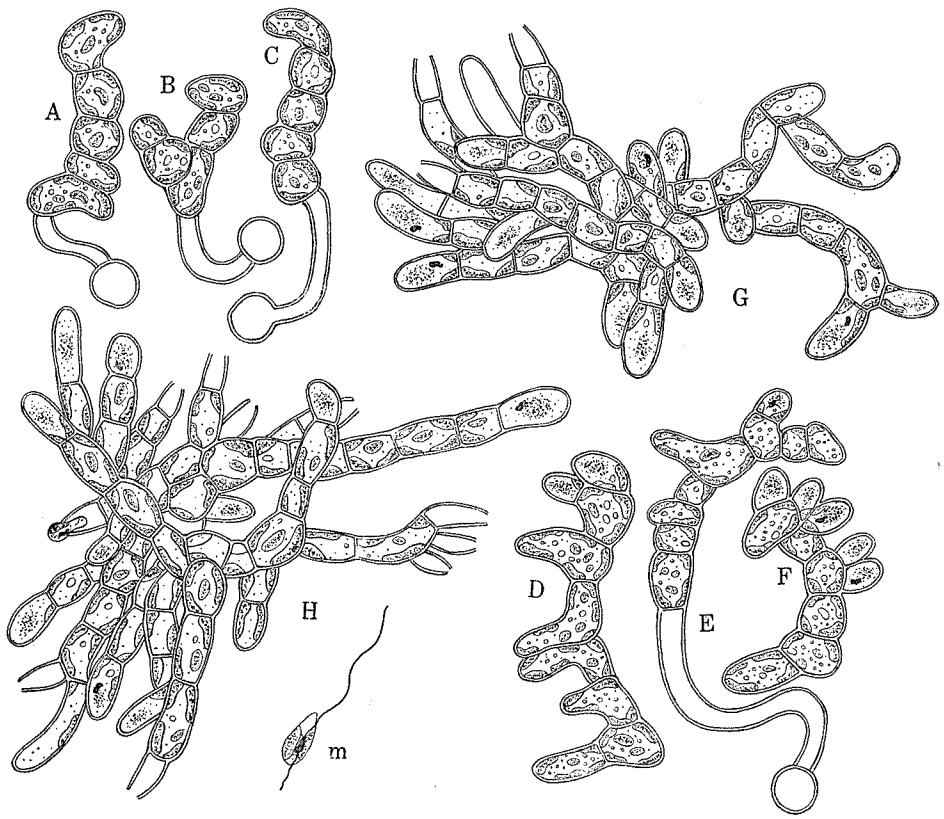

Fig. 22. *Chorda Filum* (L.) LAMX. Development of the male gametophytes. × 960. A–C. Male gametophytes from 6 day culture. D–F. Mature male gametophytes; antheridia are produced at the tips of the branches. G–H. Well developed male gametophytes. m. Male gamete in motile state, showing two laterally placed cilia of unequal length, one curved chromatophore associated with an eye spot.

distinguished in the 6 day old culture. At this time the male gametophytes, consisting of 5 cells or so, form a somewhat branched filament (Fig. 22. A–C). When the plants are 10 days old, they reach maturation (Fig. 24. D–F), and further development gives profusely branched filaments with clusters of antheridia at the tips of the branches (Fig. 24. G–H). The outer appearance of the plants, at this stage, shows some resemblance to that of *Laminaria* and *Kjellmaniella*. When they approach to maturity, some of the apical cells become antheridia. The content of these cells becomes paler, most of the chromatophores disintegrate, then the wall bursts open at the apex of the antheridium forming a beak through which the mature antherozoid swims away (Fig. 22. H). The male gamete of

Chorda has a distinct eye spot, one or sometimes two chromatophores, and two laterally placed cilia of unequal length. It resembles in general appearance, a minute zoospore (Fig. 22. m.). These characters in the male gemetes should be considered as the distinguishing characteristics for the present species.

Female gametophytes and eggs

When the female gametophyte approaches to maturity, the terminal cell, as well as the intercalary, forms an oogonium. The chromatophores in the oogonium disintegrate, and are crowded together at the upper part of the cell. As noted by KYLIN (1933), the egg cell does not detach from the oogonium, but only swells up at the distal end of the oogonium, leaving

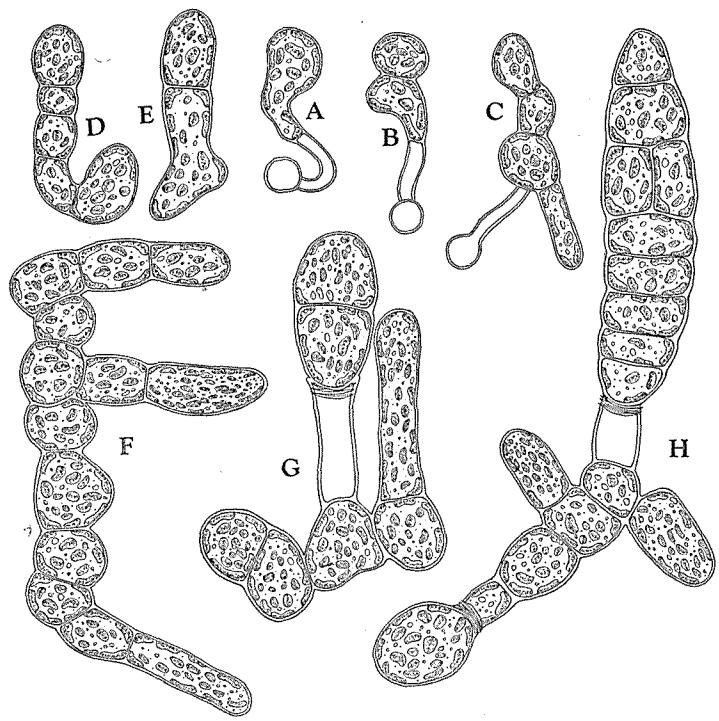

Fig. 23. *Chorda Filum* (L.) LAMX. Development of the female gametophytes, oogonia and eggs. × 644. A–C. Early stages of the female gametophytes, from 6 day culture. D–E. More advanced stage of the female gametophytes, from 10 day culture. F. A female gametophyte consisting of numerous cells, from 45 day culture. G. A female gametophyte bearing a young sporophyte and mature oogonium; from 45 day culture. H. A young sporophyte consisting of 9 cells, and an egg developed from a single female gametophyte.

some chromatophores and cytoplasma in the lower portion of it (Fig. 23. H). Moreover, several wrinkles are formed transversely outside of the oogonium between the enlarged end and the lower part. It is difficult to explain, for the present, the nature of these wrinkles, and how they were formed, though this phenomenon is worth noting as a characteristic of the present species (Fig. 23. G–H).

Development of young sporophytes

The youngest stage of the sporophyte is similar in shape to that of *Laminaria* and *Kjellmaniella* (Fig. 23. H), while as the growth advances

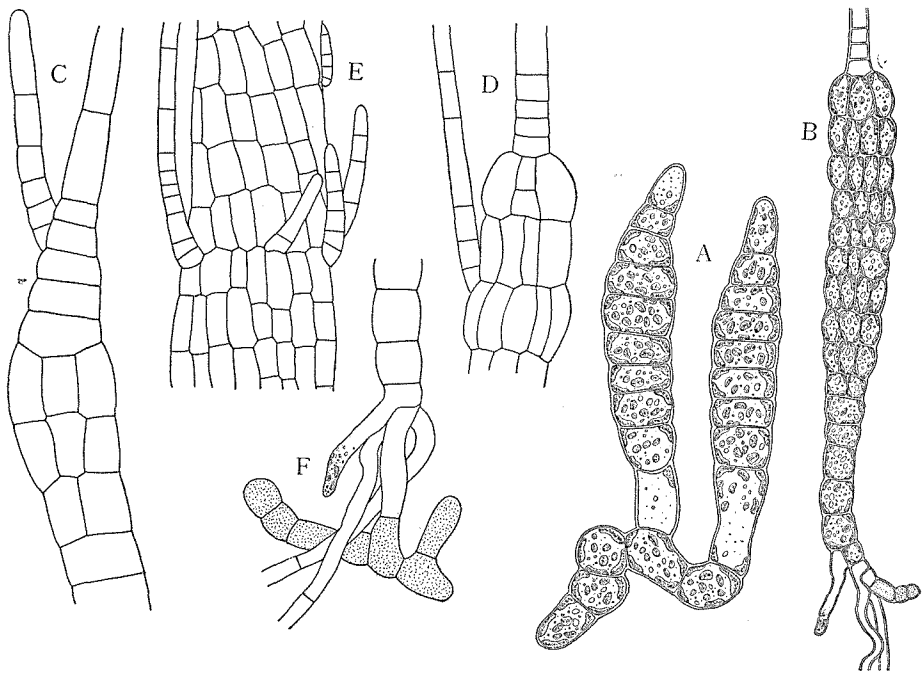

Fig. 24. *Chorda Filum* (L.) LAMX. Further development of the young sporophyte. A. Two sporophytes developed from a single female gametophyte; the apical cell of the sporophyte metamorphoses into a hair disintegrating the chromatophores. ×528. B. A young sporophyte from 3 month culture, with a hair emitted from the apical cell and 3 rhizoids developed from the basal cells; the terminal end of the rhizoid is filled with some chromatophores and granular substances; the female gametophyte, from which this sporophyte had developed is still alive at the base (dotted portion). ×197. C. A young sporophyte with two hairs, the one is terminal, the other lateral; from 45 day culture. ×528. D. ditto C, from 6 month culture. ×317. E. Basal part of the sporophyte with rhizoids and female gametophyte (dotted portion); the chromatophores fill the end of the rhizoid; the rhizoid is separated by septa; from 6 month culture. ×317. F. A part of the frond, showing the arrangement of the cells, and the development of the hairs; from 6 month culture. ×317.

the apical cell metamorphoses to a hair, disintegrating the chromatophores in it (Fig. 24. A–D). When the terminal hair attains to a certain length, the lateral one is emitted (Fig. 24. C–D). At last the frond becomes surrounded by numerous hairs when the plant is about 6 months old (Fig. 24. F). Many rhizoids are produced from the basal cells of the sporophyte in the one or two month old plant. The distal end of the rhizoid is always filled with some chromatophores and granular substances, and many septa are formed in a single rhizoid as the length of it increases (Fig. 24. E). When the plants are about 5 c.m. high, hundreds of rhizoids are produced from the basal part of the frond, entangling with each other. The small disc-shaped hapter, by means of which the macroscopic generation of *Chorda* gains a permanent attachment to the substratum, is constructed of a clump of these entangled rhizoids. The mode of the formation of the hapter differs fundamentally from that of other Laminariaceous plants.

The present culture was continued as long as 6 months, from October to May; at the end of the culture some of the sporophytes measured more than 6.5 c.m. in height.

Discussion and Summary

1. The presence of an eye spot in the zoospores of Laminariaceae has been reported, hitherto, by three authors, namely, (a) IKARI (1921) on *Laminaria religiosa* MIYABE, (b) MYERS (1928) on *Egregia Menziesii* (TURN.) ARESCH., (c) MCKAY (1933) on *Pterigophora californica* RUPR., but as repeatedly mentioned above, the writer has never met with the zoospores bearing an eye spot in all species of Japanese Laminariales studied, with the exception of *Chorda Filum* (L.) LAMX.

2. As previously reported by HARRIES (1932), the shape and size of the gametophytes and the period of time taken for their full growth vary according to several conditions of the culture, such as temperature, intensity of light, amount of nutrient salts etc. Though an exact physiological examination has not been carried out, the facts above mentioned were also recognized in the present study.

3. Though the outer appearances of the sporophyte of the three species of *Laminaria* and *Kjellmaniella* differ to a certain extent from each other, no remarkable differences were observed in the gametophyte of these species.

4. So far as the writer is aware, an exact explanation on the male gamete had never been made, until MCKAY (1933) published her study on *Pterigophora californica* RUPR., noting that "The male gametes are

measured 2.2 by 3.4μ, and are pyriform in shape, having the general appearance of minute zoospores. There are two laterally placed cilia of unequal length, the longer one, which points in an anterior direction is 6–7 μ long. The shorter cilium, which usually points in a posterior direction, is evident only when the antherozoid is in motion. It is approximately equal in length to the diameter of the antherozoid at the point of attachment, i.e., 2μ. The distinct red eye spot is associated with the pale remnant of the chromatophore and is situated close to the base of the cilia." As repeatedly mentioned above, although hundreds of male gametes in each species of *Laminaria* and *Kjellmaniella* have been carefully examined in motile state with the aid of an oil immersion object lens, the writer was unable to confirm the presence of an eye spot. Moreover, both of the cilia were almost equal in length, measuring about 18 μ.

5. Differences in habits and features of the zoospores, gametophytes, gametes and young sporophytes between *Chorda* and other species of Laminariaceae are noted as follows:

a. The zoospores, as well as the male gametes of *Chorda* always have an eye spot.

b. In the male gametes of *Chorda* one of the cilia, pointing in a posterior direction is short, measuring about 8 μ or so.

c. The egg cell in *Chorda* by no means detaches from the oogonium; therefore, the young sporophytes always make their development as they are attached to the gametophytes.

d. In *Chorda* a hair is emitted from the apical cell of the sporophyte at first, and later, as the development of the sporophyte advances many hairs are produced laterally.

e. The distal end of the rhizoid of *Chorda* is filled with some chromatophores and granular substances; the rhizoids are divided into several parts by transverse septa.

f. When the young sporophytes of *Laminaria* and *Kjellmaniella* become several centimeters high, a disc-shaped expansion is formed at the base of the stipe so as to cover the primary rhizoids. From this disc are produced the hapters by means of which the macroscopic plant gains permanent attachment to the substratum. In other words, rhizines of Laminariaceous plants are formed secondarily, while in *Chorda*, a small disc-shaped hapter is constructed of a clump of numerous rhizoids, developing out of the cells at the basal part of the frond.

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PLATE XVII

PLATE 17

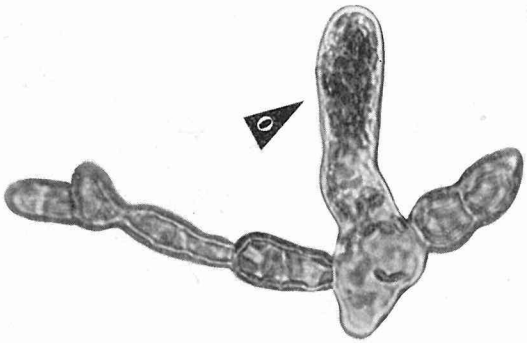
Laminaria yezoensis MIYABE.

1. Female gametophyte with mature oogonium (O), from 30 day culture. ×600.
2. Female gametophyte at maturity, from 40 day culture. The chromatophores in the oogonium are crowded together with their long axes parallel to that of the oogonium. ×400.
3. Female gametophyte with egg (E), mature oogonium (O) and young sporophyte (S), from 30 day culture. ×400.
4. An egg just extruded from the oogonium, from 30 day culture. ×400.

5, 6. Mature male gametophytes with antheridia at the tips of the branches. From 30 day culture. ×400.

Kjellmaniella crassifolia MIYABE.

7. Young sporophyte developed from a unicellular female gametophyte. From 20 day culture. ×600.



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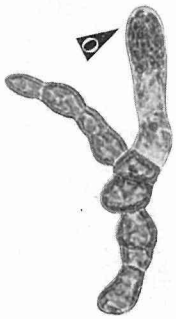

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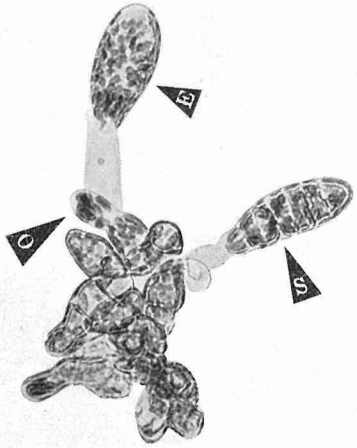

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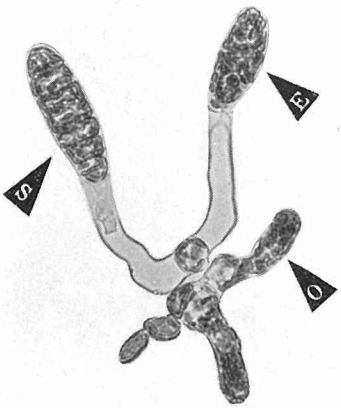

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PLATE XVIII

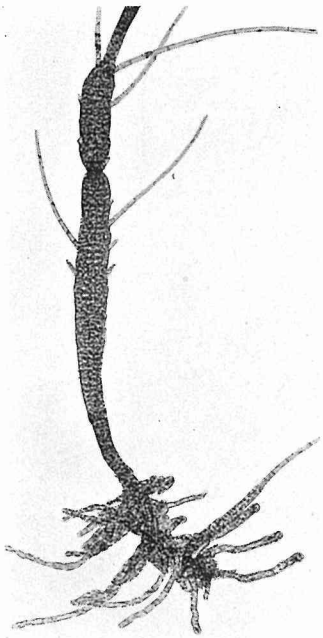
PLATE 18

Laminaria cichorioides MIYABE.

1. Young sporophytes, from 85 day culture. ×400.

Chorda Filum (L.) LAMX.

2. Young sporophytes consisting of three or four cells. From 26 day culture. ×400.
3. Young sporophyte producing a hair and two rhizoids. ×600.
4. Young sporophytes, from 32 day culture. ×400.
5. Young sporophyte producing lateral hairs and rhizoids. The rhizoids are separated by septa. ×250.



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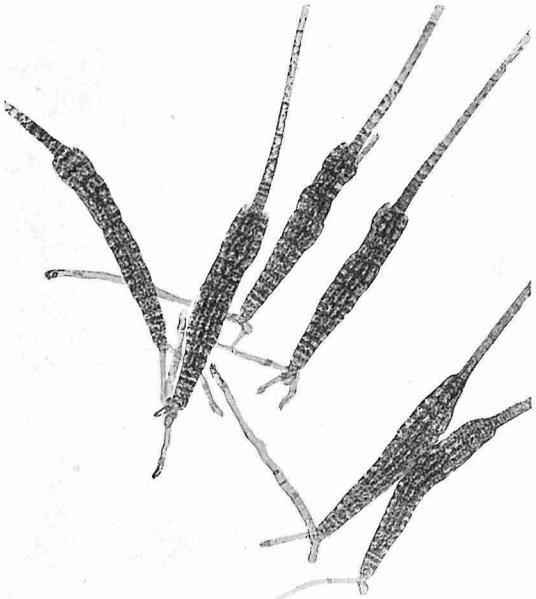

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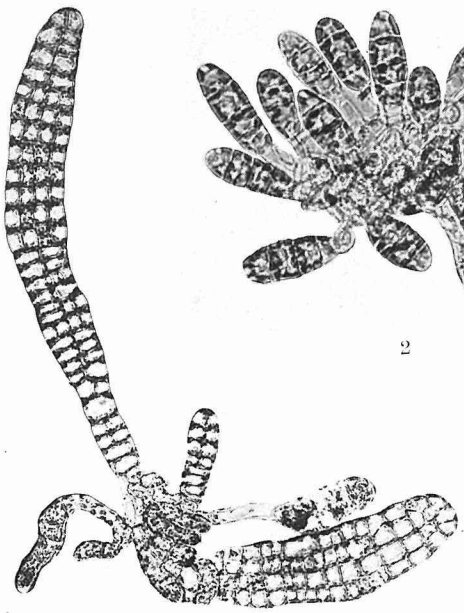

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