On the Gametophytes of Some Japanese Species of Laminariales III.

By

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With Plates XXXVII–XXXIX.

In Japanese waters, there are fifteen genera and about fifty species of *Laminariales*. As to the distribution they are divided roughly into two series of forms. The one is a cold current form, to which belong the following ten genera; *Thalassiophyllum, Hedophyllum, Pleuropterum, Cymathaere, Arthrothamnus, Alaria, Kjellmaniella, Agarum, Costaria* and *Laminaria,* being inhabitants of the northern colder seas, such as the Pacific coast of Hokkaidō and the Kuriles, Okhotsk coast of the Kuriles and Saghalien, and the Japan Sea coast of Saghalien, Hokkaidō and northeastern parts of Korea. The other is a warm current form which includes the following five genera; *Eisenia, Ecklonia, Eckloniopsis, Undaria* and *Chorda,* being inhabitants of the southern warmer seas, such as the Pacific coast of Honsyū (the main Island of Japan), Sikoku and Kyūsyū, and the Japan Sea coast of Honsyū and Kyūsyū. All the species of the cold current forms are limited to the coasts of Hokkaidō, Saghalien and the Kuriles all to the northward of Tugaru Strait, which lies between Honsyū and Hokkaidō, with the exception of *Alaria crassifolia* KJELLM., *Laminaria angustata* KJELLM. and *L. japonica* ARESCH. which occur as far south as the coast of Miyagi Prefecture, and *Costaria costata* (TURN.) SAUND., *Agarum cribrosum* BORY., and *Kjellmaniella crassifolia* MIYABE which extend at least to the coast of Aomori Prefecture, and as far south as to the northeastern coast of Korea. On the other hand, all the species of the warm current forms have their area of distribution from Tugaru Strait to Kyūsyū, all round the coasts of Honsyū, Sikoku and Kyūsyū, with the exception of *Chorda Filum* (L.) LAMX. and *Undaria distance* MIYABE et OKAM. which occur as far north as the Pacific, as well as the Japan Sea coasts of Hokkaidō.

Since the writer published in 1938 his paper on the same line of research as this, the following papers on the study of *Laminariales* have been added by three authors; ABE, K. (1939), Mitosen im Sporangium von

Laminaria japonica; CLARE, T. S. and HERBST, C. C. (1938), The life history of *Eisenia arborea*; HOLLENBERG, J. G. (1939), Culture Studies of Marine Algae. I. *Eisenia arborea*.

In the present paper there are described the results of culture experiments with the gametophytes and the young sporophytes of five species of the warm current forms of Japanese *Laminariales*, viz., *Eisenia bicyclis* (KJELLM.) SETCH., *Ecklonia cava* KJELLM., *Ecklonia stolonifera* OKAM., *Eckloniopsis radicosa* (KJELLM.) OKAM. and *Undaria Peterseniana* (KJELLM.) OKAM.

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. The greater parts of these studies have been done at the Mitsui Institute of Marine Biology, Kominato Marine Biological Station of the College of Fisheries, and Hukaura Branch Fisheries Experiment Station of Aomori Prefecture. Here the writer wishes further to acknowledge his indebtedness to Mr. T. MITSUI and Professor I. AMEMIYA of the Mitsui Institute of Marine Biology, and Professor N. NAKAMURA, the chief of the Kominato Marine Biological Station of the College of Fisheries, and the Director of the Hukaura Branch Fisheries Experiment Station of Aomori Prefecture, for placing facilities at disposal at their laboratories. Thanks are also due to the members of these institutes and stations for their kindness in helping him in many ways.

XI. **Eisenia bicyclis** (KJELLM.) SETCH.

Eisenia bicyclis (KJELLM.) SETCH. is a representative species of the warm current form of the Japanese *Laminariales*, and is rather widely distributed along the coasts of the Pacific as well as the Japan Sea, from Kyūsyū to Aomori Prefecture, and its northern limit is near Tugaru Strait. At Susaki, Sizuoka Prefecture, where the material for the present investigation was collected, they are found growing on rocks in the sublittoral belt.

This plant becomes soriferous from autumn to winter, and the embryonal fronds appear in the following spring. As it has already been reported by SETCHELL, the embryonal fronds are not easily to be distinguished from young plants of *Laminaria* species. In plants of more advanced stages, however, the important differences appear on the margins of the blade, there may be seen several coarse, tooth-like outgrowths (Fig. 1, A). These outgrowths increase in size and number, until finally they are

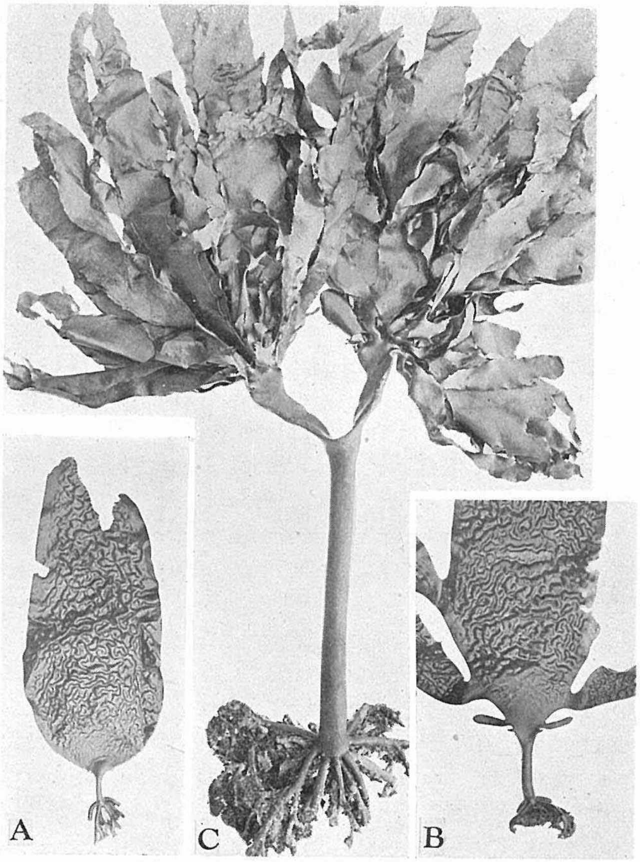

Fig. 1. *Eisenia bicyclis* (KJELLM.) SETCH. A. Habit of a young plant showing primary lamina with small teeth on the basal margins of the blade; collected at Misaki, Kanagawa Prefecture, in March. B. Habit of a young plant, showing the large blade with pinnules at the base, and the thickenings on the basal margins of the blade to form the initials of arms. C. Habit of an old full grown plant collected at Kominato, Tiba Prefecture, in November, showing the second year blade bearing zoosporangia, and two arms, long cylindrical stipe and hapters.

seen in the form of outgrowths so large that they give the blade a pinnate habit. The basal margins of the blade begin to thicken and to turn in one side of the frond (Fig. 1, B). The blade wears away gradually until it disappears almost entirely except for the small side pieces, ligules, each bearing a bunch of sporophylls. The base of the ligules posseses meristematic tissue, so that they are constantly renewed along the outer edge while they are still wearing away on the inner edge. This leads to the formation of two arms supporting the ligules and their bunches of sporophylls. As the arms increase in length they make a half twist (Fig. 1, C).

The zoosporangia are produced on both surfaces of the blade. The sori are small, elliptical in shape, and are produced usually at the middle part of the blade. As they continue to produce sporangia they become fairly large patches covering the greater part of the sporophylls, leaving narrow sterile strips along the margins. Fully matured sori are dark olive in color. HOLLENBERG (1939) has described the formation of the zoosporangia, thus: "The sori are frequently irregular in outline or even composed of discontinuous patches, but I am unable to agree with the statement of CLARE and HERBST (1938) that the sori first appear as irregular patches which later fuse. My observations indicate that the sori are typically entire and continuous from the beginning". The present writer's own observations confirm the descriptions of HOLLENBERG.

The present culture experiment was carried out at the sea-side laboratory of the Mitsui Institute of Marine Biology at Susaki near Simoda, from 27 September 1937 to 30 October 1937. Then some of these culture slides were brought by train, keeping them alive, to the laboratory of the Institute of Algological Research at Muroran, where they were cultured till March of the next year.

Materials bearing well matured sori were collected at the sea-shore; they were brought to the laboratory, washed carefully in filtered sea-water, and the whole sporophylls with matured sori were put into a glass vessel containing about eight litres of twice filtered sea-water, and the vessel was illuminated with an electric lump. If the material is fully matured, the zoospores are liberated within about a quarter of an hour. The liberation of zoospores is continued for a fairly long time, but the thalli were removed as quickly as possible, because the zoospores are liable to decay if they were left standing too long, on account of the mucilaginous substances and brown juice oozed out of the thalli. As the zoospores of *Eisenia* are easily poisoned by this brown juice, the treatment ought to be carried out as quickly as possible.

The zoospores were allowed to settle on slide-glasses which had previously placed in the vessel. When the zoospores have settled, the slide-glasses were transferred to the culture-vessels containing about 250 cc. of medium, and these vessels were placed on the table by the window. The cultures were subjected to the fluctuation of temperature of the room; much attention was paid, however, that they should not exposed to the direct sunlight.

Culture studies of the zoospores of the genus *Eisenia* have been made, hitherto, by three authors, namely IKARI (1926), CLARE and HERBST (1938) and HOLLENBERG (1939).

Zoospores and their Germination

The zoospores are pear-shaped when they are active, pointed at one end and rounded at the other, measuring about 8–9μ in length, 4–5μ in breadth; they have two laterally placed cilia, one pointing forward, as long as about four times the length of the body itself, and the other pointing backward, a little longer than the body. CLARE and HERBST (1938) have described the habits of zoospores of *Eisenia arborea* ARESCH., thus; "Contrary to the findings of other workers on the *Laminariaceae,* the spores do not seem to possess cilia or to be motile. Although several staining tecknigues were employed, no cilia were observed. When placed in the artificial culture solution, the spores float from 8–24 hours and then attach themselves to a surface". Their inability to detect motility of the zoospores or to see the flagella would appear to be due to faulty technique in starting their culture.

The zoospores has one chromatophore which occupies the posterior part, one nucleus and some granular substance, but always lacks an eye-spot (Fig. 2, A); the anterior end is always hyaline. HOLLENBERG (1939) reported that a stigma is definitely present in the form of a more or less elongated body associated with the chromatophore on one side of the cell. In spite of careful examinations the present writer could not ascertain the presence of an eye-spot in the zoospores of *E. bicyclis.* They are very active at first, but sooner or later their movement becomes slower; within 8 hours after the liberation of the zoospores the majority of them lose their cilia, assuming a spherical form; they are invested with a membrane and become fastened to the slide-glass (Fig. 2, B). Some embryospores, however, remain floating on the surface of the medium. Soon after they have fastened to the slide, a germination tube grows out from the embryospore (Fig. 2, C). As the germination tube grows longer, the contents of the original cell migrates into this part, and within 48 hours a cross wall is formed so as to separate the distal end from the tube (Fig. 2, D–F). The distal end increases its diameter, then the first cell division takes place in the 6 days old plants (Fig. 2, L–M). Under favorable conditions, the male and female gametophytes are distinguishable in cultures from five to six days after germination (Fig. 2, L–P). HOLLENBERG (1939) reported that in *Eisenia arborea* ARESCH. the male and female gametophytes are

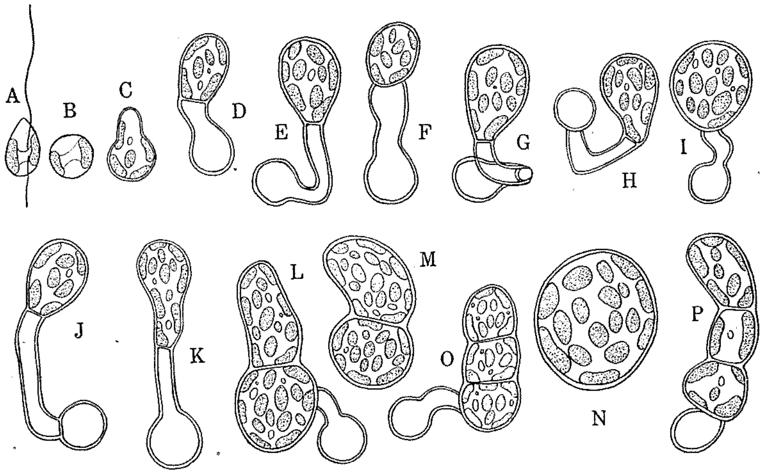

Fig. 2. *Eisenia bicyclis* (KJELLM.) SETCH. Zoospore and its germination. ×1250. A. Zoospore in motile state. B. Embryospore. C. Sporeling from 12 hour culture, showing the development of the germination tube. D–F. Sporelings from two day culture, showing the first transverse wall. G–K. Sporelings from 4 day culture. L–N. Early stages of the female gametophytes, from 6 day culture. O–P. Early stages of the male gametophytes consisting of 3 cells, from 6 day culture.

distinguishable in cultures about 15 days after germination; CLARE and HERBST (1938), on the other hand, observed the differentiation of two types of gametophytes in the 36 days old culture.

The female gametophyte consists of only one or two cells at this stage, the diameter of which measures about 15μ (Fig. 2, L–N). The other type, that is, the male gametophytes, however, consists of three or more cells in a row. The diameter of each cell is much smaller than the former, measuring about 8μ (Fig. 2, O–P).

Male Gametophytes and Male Gametes

As the culture progresses the male gametophytes increase in number of cells more and more, and in the 8 day culture they form irregularly branched filaments with clusters of antheridia at the tips of the branches (Fig. 3, A–I). The cells composing the male gametophytes are larger in size and filled with more deep brown colored chromatophores than those of *Laminaria, Alaria,* etc. When they came to maturity the cell at the apex divides several times, producing a cluster of antheridia at the tips of the branches (Fig. 3, H–I). In the other cases any cell of the male gametophyte may become an antheridium (Fig. 3, H–I).

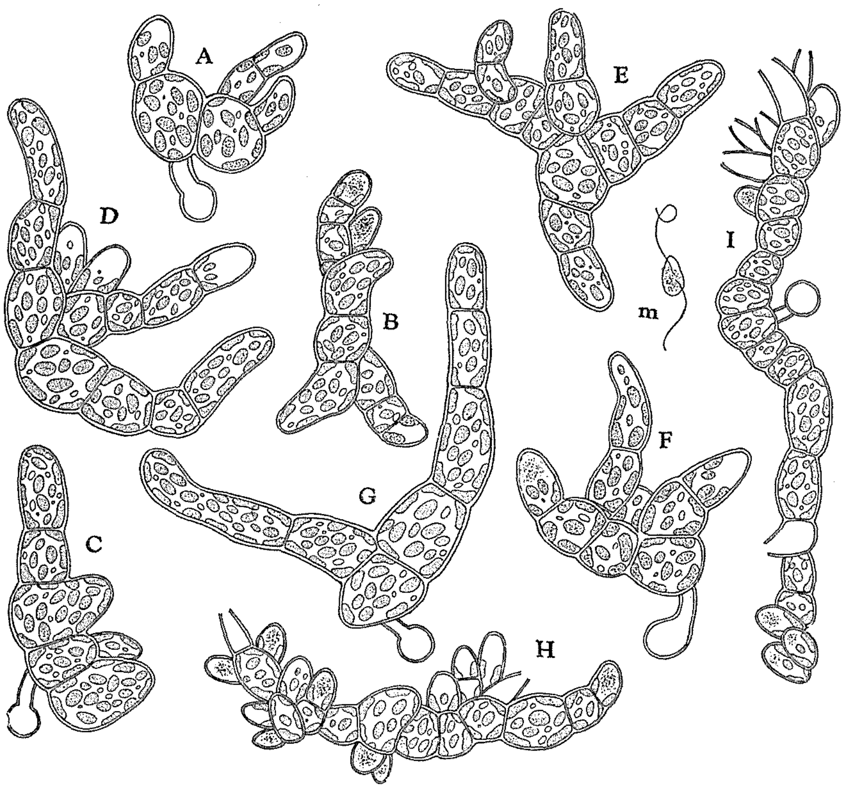

Fig. 3. *Eisenia bicyclis* (KJELLM.) SETCH. Various forms of the male gametophytes. ×1174. A–G. Male gametophytes from 8 day culture. H–I. Mature male gametophytes from 10 day culture. The tips of the branchlets are divided forming the antheridial clusters. m. Male gamete in motile state, showing two cilia of almost equal length.

Studying on *Pterygophora californica* McKAY (1933) states that the production of antheridia is limited largely to the apical region of the branches and the cells of the main axis remain entirely sterile. The writer, however, observed many times that the cell of the main axis transformed directly to the antheridia in *Eisenia bicyclis* (KJELLM.) SETCH. Working on *Eisenia arborea* ARESCH. HOLLENBERG (1939) reported; "No evidence was obtained which would indicate that the cells of the branches function as antheridia or are converted into antheridia. In the case of very short lateral branchlets a cell of the branchlet is occasionally found to be continuous with an empty antheridium, suggesting that a cross wall failed to form at the base of the antheridial outgrowth". The writer's own observation differs to some extent from HOLLENBERG's statements. The content of the antheridial cell grows paler and at last it assumes a spherical form. Entire contents of the antheridium are used for the formation of a single male gamete (Fig. 3, H). Then the wall bursts open at the apex of the antheridium, and the male gamete swims away leaving an empty beak (Fig. 3, H–I). The male gamete is paler in color, ovate in shape, sometimes rather round, measuring about 5μ in length. Two cilia of almost equal length, measuring about 10μ arise from the side of the body (Fig. 3, m). HOLLENBERG (1939) has reported that the male gamete of *Eisenia arborea* ARESCH. has a distinct red eye-spot, but in the present species the presence of it was not confirmed, in spite of careful examination.

McKAY (1933) reported that the male gametophytes of *Pterygophora californica* RUPR. reach maturity and begin to produce gametes in about 50 days after the germination of the zoospores; HOLLENBERG (1939) on the other hand, described how in vigorous cultures of *Eisenia arborea* ARESCH. antheridia first appear in about 40 days after germination of the spores. Contrary to the descriptions of these authors, the writer has observed that the length of time necessary for the maturation of male gametophytes of this species is very short, and in the present cultures mature antheridia were observed within 8 days. Not a few male gametes swimming actively in the medium were observed under microscope.

Female Gametophytes and Eggs

The shape and size of the female gametophyte of this plant are very irregular, some consist of one cell (Fig. 4, A–B), others consist of two or more cells (Fig. 4, C–H), measuring from 10μ to 30μ in diameter. Under favorable conditions they reach maturity and begin to produce oogonia in 8 days after the liberation of the zoospores (Fig. 4, I–J). When the female gametophyte consisting of only one cell comes to maturity, this vegetative cell changes into a single oogonium (Fig. 4, I–J, Plate 37, 1–2). In case of a two or more celled gametophyte, any cell either terminal or intercalary, may become an oogonium (Fig. 4, J, Plate 37, 3).

At maturity the oogonium cell is elongated, the contents become dense, the chromatophores are divided into small piece, and are crowded together at the apex (Fig. 4, I–J, Plate 37, 1–3). Then the egg cell is forced out through the opening at the apex of the oogonium and remains attached there (Fig. 4, K–L, Plate 37, 3–4). Though the actual fertilization of the egg was not observed under microscope, it is certain that the egg is fertilized

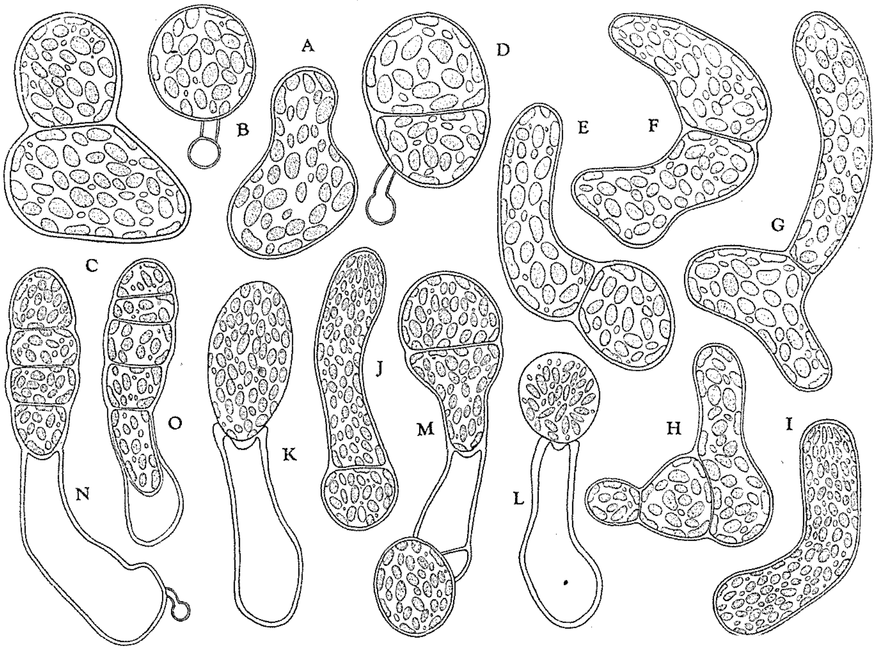

Fig. 4. *Eisenia bicyclis* (KJELLM.) SETCH. Female gametophytes, eggs and young sporophytes. A–H. Female gametophytes from 8 day culture. ×951. I. One celled female gametophyte at maturity, from 13 day culture. ×680. J. Two-celled female gametophyte at maturity, from 20 day culture. ×680. K–L. Eggs discharged from one celled female gametophytes, from 15 day culture. ×630. H–O. Young sporophytes, from 15 day culture. ×630. M. Showing the first cell division of the fertilized egg. ×630. O. Young sporophyte consisting of 5 cells, the basal cell of which grows downwards into the empty oogonium. ×630.

immediately after emergence, because the membrane of the egg is very thin when it was discharged from the oogonium (Fig. 4, K–L). The egg cell is spherical or ellipsoid in shape, measuring from 16μ to 38μ in diameter, and filled with comparatively small chromatophores.

Development of the young Sporophytes

Within 15 days after the liberation of zoospores the egg is fertilized, the membrane of it becomes thicker, and begins to germinate while attached to the apex of the oogonium, from which it was discharged (Fig. 4, M). At the youngest stages of the sporophyte, only transverse divisions take place, and the sporophyte forms a monosiphonous filament (Fig. 4, N–O, Plate 37, 5). It was observed that the female gametophytes consisting of

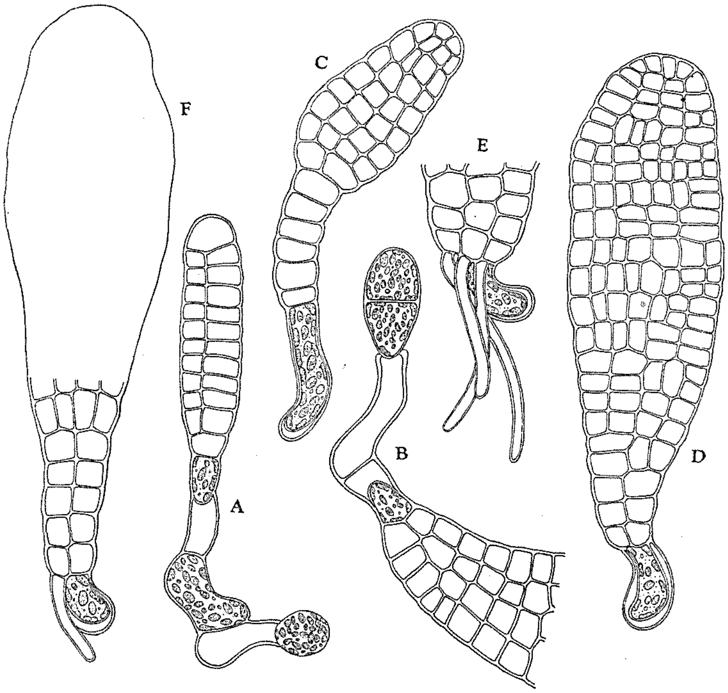

Fig. 5. *Eisenia bicyclis* (KJELLM.) SETCH. Development of the young sporophytes, from 20–23 day culture. ×620. A. Three celled female gametophyte bearing an egg and young sporophyte, from 20 day culture. B. Two young sporophytes developed from two celled female gametophyte, from 23 day culture. C–D. Young sporophytes from 23 day culture. The basal cell of the sporophyte grows downwards into the empty oogonium cell. E–F. Young sporophytes developing a rhizoids. The basal cell grows downwards into the empty oogonium; the rhizoids always develope from the cell next to the basal cell.

two or three cells develop two sporophytes (Fig. 5, A–B). The basal cell of the young sporophyte grows gradually downwards in the empty oogonium (Fig. 4, O, Fig. 5, A–F), and at last it fills the greater part of the empty oogonium (Fig. 5, C–F). This basal cell remains in position at the base of the blade (Fig. 5, C–F); it may be observed in later stages of development of the sporophyte consisting of as many as five hundred cells (Fig. 6, A). The new sporophytes grow rapidly, and in 23 day culture many of them develop rhizoids. In *Laminaria, Alaria, Kjellmaniella, Undaria, Arthrothamnus,* etc., the rhizoids are produced from the basal cell or cells, while in the present species this basal cell usually takes no part in the formation of the rhizoids (Fig. 5). It is characteristic of this species that the rhizoids are not produced from the basal cell, but from the cell or cells next to this (Fig. 5, E–F, Fig. 6, A). When the plants are about one mm in total length, the initial of the stipe is differentiated at the base of the frond (Fig. 6, B). The cells which compose the beginning of the stipe elongate, as the plant increases its height, to a length much exceeding the diameter (Fig. 6, B). They divide longitudinally and transversely, quite irregularly, to form a cylindrical stipe (Fig. 6, Plate 37, 7–10). The resulting cells are poor in chromatophores, and those in the lower part are almost entirely devoid of them. In the two month culture, the writer met with a young sporophyte measuring

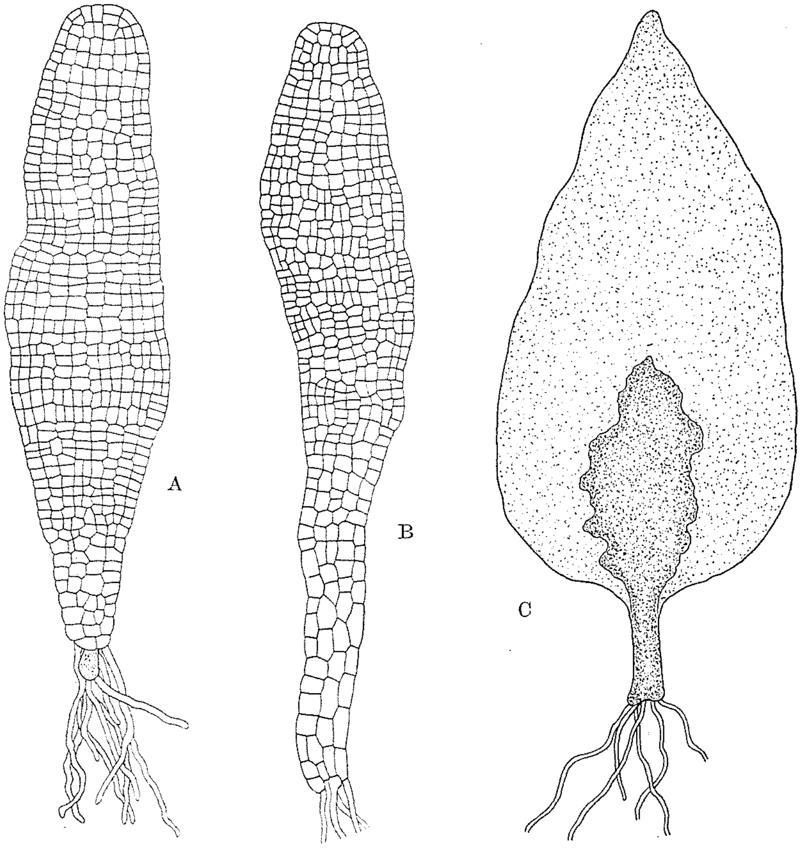

Fig. 6. *Eisenia bicyclis* (KJELLM.) SETCH. Further development of the sporophytes. A. Well developed sporophyte with numerous rhizoids, the basal cell remains undivided; from 23 day culture. ×140. B. Sporophyte with rhizoids; lower part of the blade changes into the initial of the stipe. ×140. C. Sporophyte from 40 day culture, showing the differenciation of the blade, stipe and rhizoids. The blade becomes distromatic at the base.

about 1 cm in height. In such a plant a disc-shaped expansion developes at the base of the stipe. From this expansion are produced the hapters, by means of which the plant gains permanent attachment to the substratum (Plate 37, 9–10). When the plants have attained some mm in length, the monostromatic lamina becomes distromatic. The process is first carried on at the point which corresponds to the transitional region in an adult frond (Fig. 6, C, Plate 37, 7–10).

XII. **Ecklonia cava** KJELLMANN

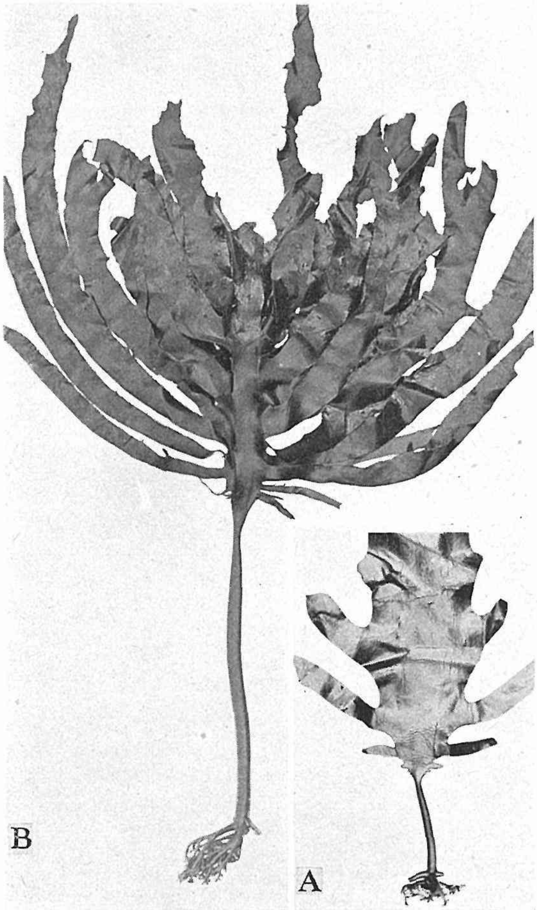

Fig. 7. *Ecklonia cava* KJELLM. A. A young sporophyte collected at Misaki, Kanagawa Prefecture in March. B. Habit of an adult plant collected at Misaki in March.

This species is an inhabitant of the southern warmer waters, having a rather limited area of distribution, ranging from Miye Prefecture to as far north as Ibaragi Prefecture, along the Pacific coast of Honsyū. It is one of the most common algae on the coast of Susaki, where associated with *Eisenia bicyclis* (KJELLM.) SETCH., it grows on rocks in the sublittoral belt. The present species is also widely variable in shape; in deeper waters the stipe becomes elongated, while in shallow places it remains short. In habit this species has a close resemblance with *Eisenia bicyclis* (KJELLM.) SETCH. This species becomes soriferous from autumn to winter, and the embryonal frond appears in the following spring. The zoosporangia are produced at first on both surfaces of the blade, as a small patch with either irregular or entire outline and later as the stages advance, they become a fairly large patch covering the greater part of the sporophyte, leaving a narrow sterile stripe along the margin.

Cultures were made in the laboratory of the Mitsui Institute of Marine Biology at Susaki, from September 1937 to the end of October together with *Eisenia bicyclis* (KJELLM.) SETCH. Then the culture slides were brought to Muroran, keeping them alive, where they were cultured till March of the next year.

Zoospores and their Germination

The zoospores are pear-shaped, measuring about 8–9μ in length, 4–5μ in breadth; they have two laterally placed cilia, one curved chromatophore, and some granular substances, the eye-spot, however, always lacking (Fig. 8, A). They swim actively at first, but sooner or later they become motionless. They lose their cilia before long, become spherical in shape, invested with a membrane and fasten themselves to the slide-glass (Fig. 8, B). Soon after they have fastened to the slide, a germination tube grows out

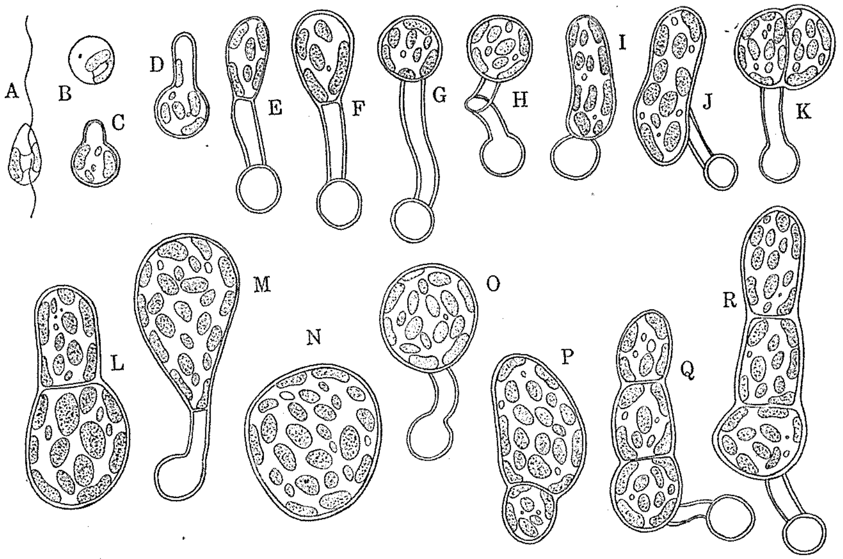

Fig. 8. *Ecklonia cava* KJELLM. Zoospore and its germination. ×1326. A. Zoospore in motile state. B. Embryospore. C–D. Germination of the embryospores, from 12 hour culture. E–K. Sporelings from 2–6 day culture. L–P. Early stages of the female gametophytes, from 6–8 day culture. Q–R. Early stages of the male gametophytes, from 6–8 day culture.

of the embryospore (Fig. 8, C). As the germination tube grows longer, the contents of the original cell migrate into this part, (Fig. 8, D), and within 24 hours a cross-wall is formed so as to separate the distal end from the empty tube (Fig. 8, E). The distal end increases its diameter and in 6–8 day culture two types of gametophytes are distinguished (Fig. 8, L-R).

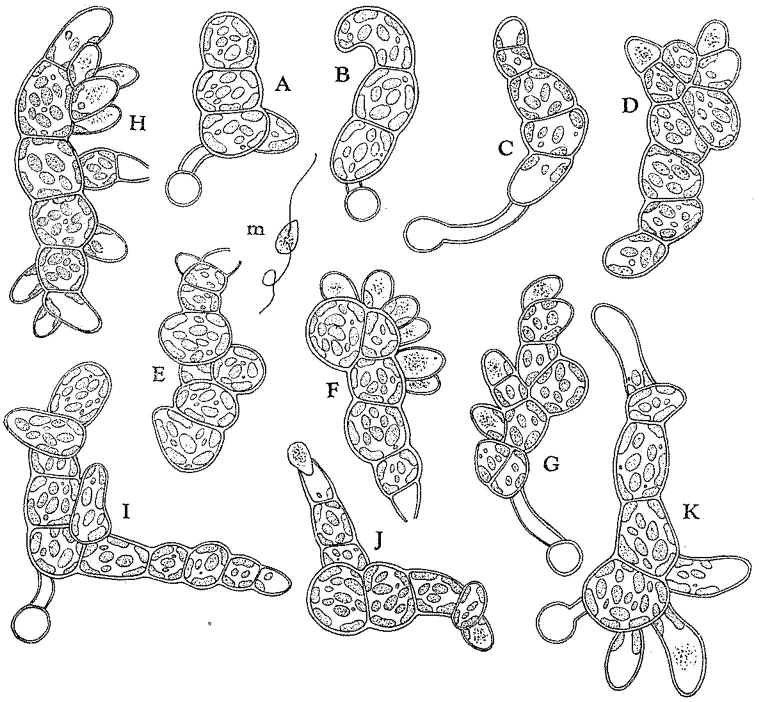

Fig. 9. *Ecklonia cava* KJELLM. Male gametophytes and male gemate, from 8–23 day culture. ×1220. A–C. Immature male gametophytes, from 8 day culture. D–K. Mature male gametophytes; the tips of the branches are divided forming the antheridial clusters. J. Mature antheridium; a male gamete is about to escape from the antheridium. m. A male gamete in motile state.

Development of Gametophytes

The male gametophytes of the present species show a close resemblance to those of *Eisenia bicyclis* (KJELLM.) SETCH. Within 9 days the male gametophytes develop into a slightly branched filament consisting of more than 3 cells (Fig. 9, A–C). As the development advances, the number of cells increases, and as the gametophytes approach to maturity, some of the apical cells, as well as intercalary, develop antheridia (Fig. 9, D–J). The entire contents of the antheridium are used for the formation of a single male gamete. Then the wall bursts open at the apex of the antheridium, forming a beak through which the mature antherozoid swims away (Fig. 9, J). The antherozoids are ovate in shape, sometimes rather round, measuring about 4μ in length. Two cilia of almost equal length, measuring about 10μ arise from the side of the body. Sometimes a pale remnant of chromatophores may be observed in them, but an eye-spot is always lacking (Fig. 9, m).

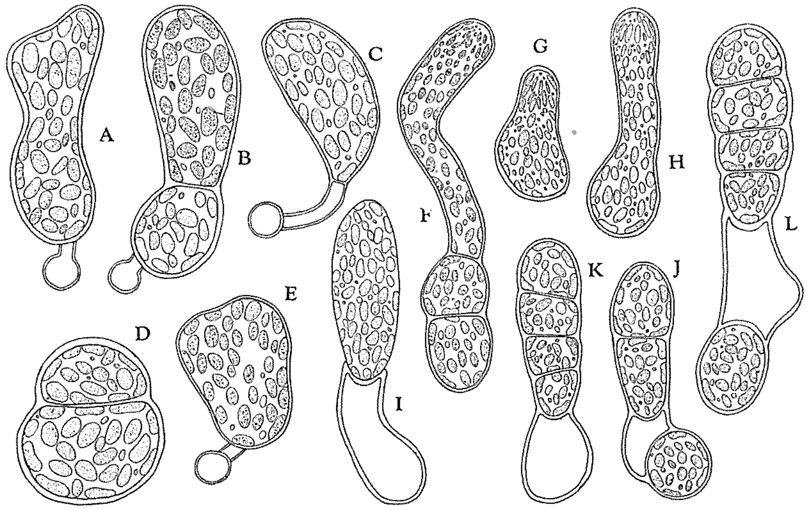

Fig. 10. *Ecklonia cava* KJELLM. Female gametophytes, eggs and young sporophytes, from 8–23 day culture. A–E. Immature female gametophytes from 8 day culture. ×1025. F–H. Female gametophytes, with mature oogonia, from 13 day culture. ×680. I. An egg recently discharged out of the oogonium. ×680. J–L. Young sporophytes from 23 day culture. ×680.

Most of the female gametophytes of this species consist of only one or two cells, which are somewhat isodiametric and measure from 10μ to 30μ (Fig. 10, A–F). When the female gametophytes are 12 days old, most of them reach maturity. One of the signs of approaching maturity is the elongation of the oogonial cell (Fig. 10, F–H, Plate 38, 1). The chromatophores at the apex of the oogonium are divided into small pieces, having a spindle-shaped appearance, and are crowded together with longitudinal arrangement (Fig. 10, F–H). At maturity the egg cell is forced out through the opening at the apex of the oogonium and remains attached there (Fig. 10, I). The egg-cell is spherical or elliptical in shape, invested with thin plasma-membrane.

As accompanying figures and plate show, the shape and size of the zoospores, the process of their germination, and general aspect of both male and female gametophytes bear a strong resemblance to those of *Eisenia bicyclis* (KJELLM.) SETCH.

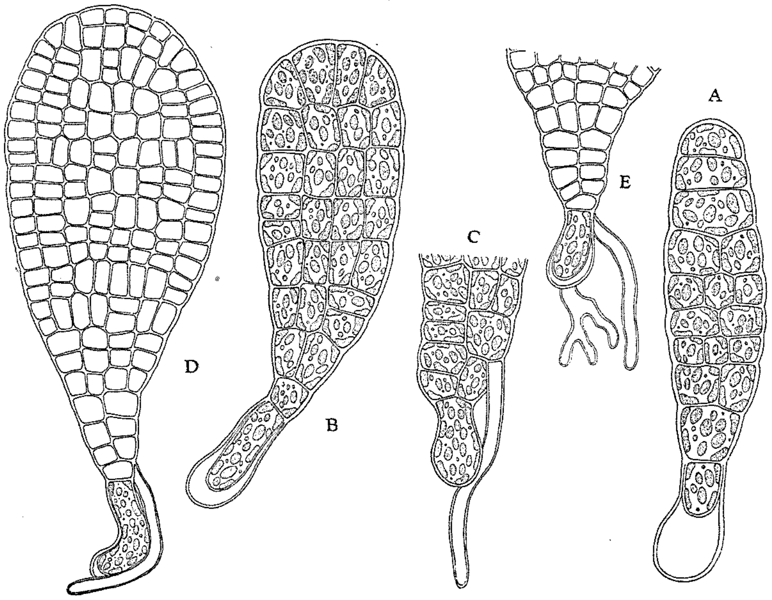

Fig. 11. *Ecklonia cava* KJELLM. Development of the young sporophytes. A–B. Early stages of the sporophytes. The basal cell of the sporophyte grows downwards into the empty oogonium cell; from 10 day culture. ×610. C–E. Showing a characteristic feature of the basal cell and the formation of rhizoids, from 24 day culture. ×370.

Development of young Sporophytes

The fertilized egg begins germination soon. At first the cell division takes place by a transverse wall (Fig. 10, J–L, Plate 38, 2–3). The new sporophytes grow rapidly, and in the 24 day culture many of them develop rhizoids (Fig. 11, C–E). As was observed in *Eisenia bicyclis* (KJELLM.) SETCH. the basal cell of the sporophyte grows downwards into the oogonium cell (Fig. 11, A–E), and no rhizoids are produced from this basal cell (Fig. 11, C–E, Fig. 12, A). As development of the sporophytes progresses the upper part grows rapidly forming a flat expanded blade, while the lower part becomes cylindrical to form a stipe (Fig. 12, A–B, Plate 38, 4–8). Thus the blade and stipe of the sporophyte are differentiated in early stages of the development.

Though the adult plant of this species shows some differences, in its outer appearance, from *Eisenia bicyclis,* there exists a striking resembrance in the shape and habits of the embryonal frond. As was described in *Eisenia bicyclis,* the growth of the basal cell of the young sporophyte into empty oogonium cell, the development of the rhizoid, the process of formation of the stipe, are considered as characteristic to these species.

XIII. **Ecklonia stolonifera** OKAM.

This plant has a limited area of distribution, having been found, hitherto, only on the coast of the Japan Sea, from Hirato Strait of Kyūsyū, to as far north as Tugaru Strait. It grows on rocks in the sublittoral belt. Examining the sample dredged by H. NIINO (1935) off the coast of Wakasa Bay, Hukui Prefecture (Long. 135°41′42″E., Lat. 36°11′6″ N.), UEDA and OKADA (1938) have reported that this species may thrive at a depth of 199 meters below the sea level, and this depth is perhaps the deepest record ever known of the growing zone of marine algae of the world. The holdfast of this species is at first composed of few hapters, arising from the basal portion of the stipe; afterwards some of these hapters turn to rhizomes, giving off lateral secondary hapters. New erect fronds are produced from these rhizomes. On the coast of Aomori Prefecture, the embryonal fronds of this plant appear late in November, and 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. In winter, however, the second year blade begins its sudden growth at the transition region, pushing up the first

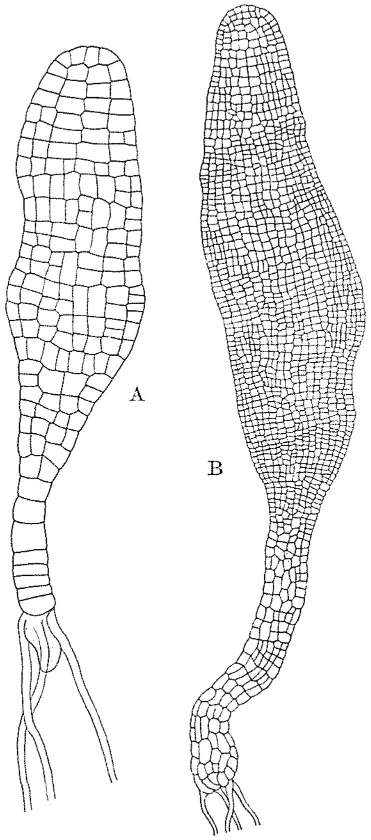

Fig. 12. *Ecklonia cava* KJELLM. Further development of the sporophytes. A. Well developed sporophyte with rhizoids. The basal cell remains undivided; from 47 day culture. ×220. B. Sporophyte from 40 day culture. Lower part of the blade becomes the initial of the stipe. ×66.

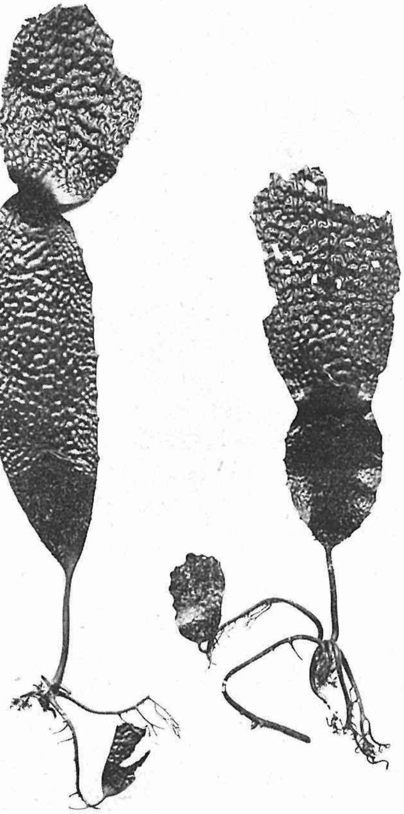

Fig. 13. *Ecklonia stolonifera* OKAM. Habit of the plant, collected at Hukaura, Aomori Prefecture, in November 1937. The root fibres are verticillately arising, some of which elongate into stolen, at whose apices young leaflets are formed and grow up into new fronds. The primary frond decays down in autumn, and new lamina begins to grow in the following winter, pussing up the first year frond; the demarcation between those young and old blades is clearly recognized.

year blade. The demarcation between the two parts is distinctly recognized (Fig. 13).

The zoosporangia are produced at first in the depression on both surfaces of the blade, and later may cover the greater parts of the blade always leaving a narrow border along the margin. The second year blade usually remains sterile. The material for the present investigation was collected at the coast of Hukaura, Aomori Prefecture, on 23 November 1937, and the culture was started on that day at the laboratory of Hukaura Branch Fisheries Experiment Station. The blades with matured sori were washed carefully, brought to the laboratory, kept in a dark place for several hours, then they were placed in a glass vessel containing about 8 liters of filtered sea-water. Soon the medium turned yellowish-brown on account of millions of zoospores which were liberated from the thallus. The treatment was carried out as quickly as possible, because the zoospores were apt to die on account of the brown juice which oozed out of the blade. The plants were cultured for 5 days at Hukaura, then they were brought to Muroran, keeping them alive, and cultured till January of the next year.

Development of Gametophytes

The zoospores are pear-shaped, about 8μ in length, have two laterally placed cilia, one curved chromatophore, one nucleus and a number of granular particles, but always lack an eye-spot (Fig. 14, A). In this culture, when the temperature of the culture medium was about 8°C, the greater part of the zoospores remained in active state for as long as 48 hours. Then they lose their cilia, become spherical in shape, invested with a membrane and fastened themselves to the slide-glass (Fig. 14, B). Within 2 or 3 days the germination tube grows out of the resting spore and the content of the cell begins to migrate into this tube (Fig. 14, C–F). Soon the whole contents of the original cell enters this part, then the cross wall is formed so as to separate it from the tube (Fig. 14, G–I). The germination tube of *Eisenia bicyclis* (KJELLM.) SETCH. and *Ecklonia cava* KJELLM. measured as long as about 10–20μ, while those of the present species were very short, measuring about 10μ in their maximum length, and in some extreme cases a sporeling destitute of the germination tube was observed (Fig. 14, G–K, M, O, R, T, V). The shape and appearance of the sporelings at this stage differ to some extent from those of *Eisenia bicyclis* (KJELLM.) SETCH. and *Ecklonia cava* KJELLM. In the plants of 7 day culture, the distal part of the tube swells until it becomes much

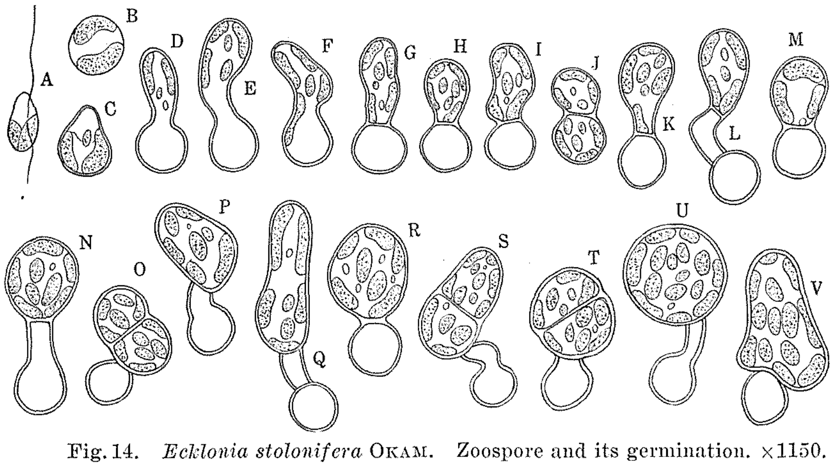

Fig. 14. *Ecklonia stolonifera* OKAM. Zoospore and its germination. ×1150. A. Zoospore in motile state. B. Embryospore. C. Germination of the embryospore. D–F. Sporelings from 24–48 hour culture, showing the development of germination tubes; The contents of the original cells migrate into germination tubes. G–J. Sporelings from 3–5 day culture, showing the first transverse wall. The germination tube is very short in length. K–V. Sporelings from 7–13 day culture.

larger than the original cell (Fig. 14, K–N), and when the plants are 10 days old, the first cell division takes place (Fig. 14, O). The gametophytes continue to grow and the differentiation of the male and female gametophytes may be recognized in the 13 day culture (Fig. 14, Q–V).

The male gametophytes consist of three or more cells, forming either a slightly branched filament or a cell-mass (Fig. 15, A–G). When they came to maturity, some of the apical cells produce antheridia. The content of the antheridium grows paler and more granular, and the entire content of it is used for the formation of a single male gamete (Fig. 15 G). The antherozoids are ovate or spherical in shape measuring about 4μ in diameter, with two cilia of almost equal length, measuring about 10μ which arise from the side of the body. Some times a pale remnant of the chromatophores may be observed in them, but an eye-spot is always lacking (Fig. 15, m).

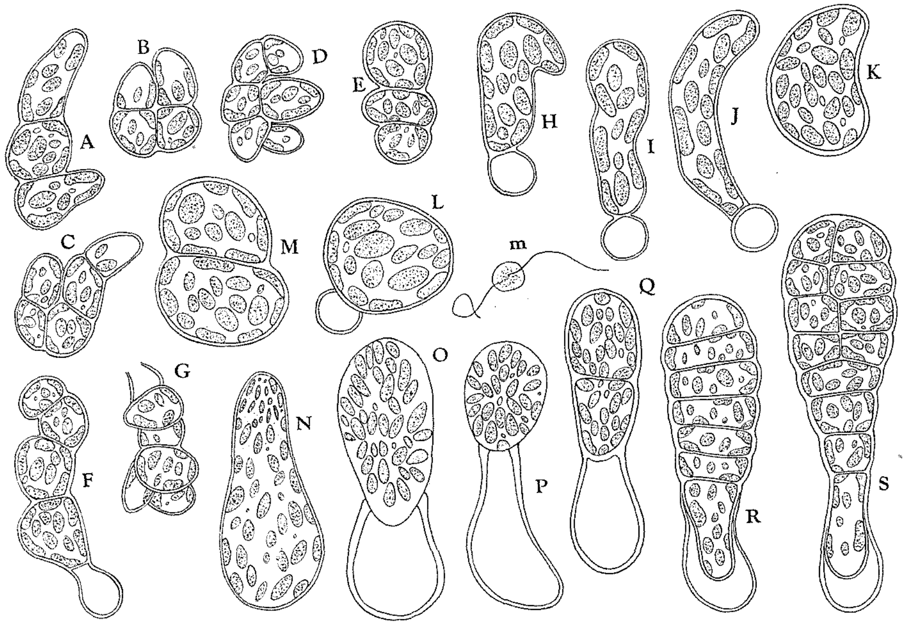

Fig. 15. *Ecklonia stolonifera* OKAM. Male and female gametophytes, eggs and young sporophytes. A–G. Male gametophytes from 15–23 day culture. ×995. H–J. Female gametophytes from 15 day culture. ×995. K–L. Female gametophytes from 23 day culture. ×995. M. Two celled female gametophytes. ×995. N. One celled female gametophyte at maturity, from 23 day culture. ×995. O. Egg cell discharged from one celled female gametophyte, from 23 day culture. ×995. P. Ditto. ×663. Q–S. Development of the young sporophytes. ×663. m. Male gamete in motile state.

In this species most of the female gametophytes are unicellular, and the whole contents of the vegetative cell metamorphoses into a single oogonium (Fig. 15, H–N). When the plants are about 23 days old, the female gametophytes reach maturity (Fig. 15, N); the oogonium cell is elongated, the chromatophores are divided into small pieces, and are crowded together at the apex; the contents of the oogonium become more and more liquefied, and it shows the presence of strong internal pressure (Fig. 15, N). Then the egg is discharged through the opening at the apex, remaining attached there (Fig. 15, O–P).

Development of young Sporophytes

When the egg cell is fertilized the development of the sporophyte starts. In the 25 day culture the first cell division takes place at a right angle to the longitudinal axis of the egg (Fig. 15, Q), and in the plants of 30 day culture a twelve-celled sporophyte was observed, the basal cell of which grows downwards into the empty tube of the oogonium. The formation of the rhizoids, however, has not been observed. It was impossible to trace the development beyond this stage, since the culture condition became more and more unfavorable for the plants.

In the present culture the growth of the gametophytes and the young sporophytes was very slow when compared with those of *Eisenia bicyclis* (KJELLM.) SETCH. and *Ecklonia cava* KJELLM.

XIV. **Eckloniopsis radicosa** (KJELLM.) OKAM.

Eckloniopsis radicosa (KJELLM.) OKAM. is a typical member of the warm current form of *Laminariales,* found growing on rocky shores in the sublittoral belt along the Pacific coast of Japan, washed by warm current, from Kyūsyū to as far north as Bōsyū, Tiba Prefecture, incuding the Seven Islands of Izu. The present species was first described by KJELLMANN, from specimens collected at Nomo, Nagasaki Prefecture, who referred it to the genus *Laminaria*. In 1927, however, OKAMURA established a new genus '*Eckloniopsis*' and transferred the present species into this genus. Since that time, no species of this genus have been described, and *Eckloniopsis* now stand as a monotypic genus in the order *Laminariales*.

The young sporophyte of *Eckloniopsis radicosa* (KJELLM.) OKAM. is a small Laminarioid plant, having short and flattened stipe, at the end of which numerous rhizoids are produced, while the blade is simple, elliptical lanceolate (Fig. 16, A). As they grow older, however, there begin to appear

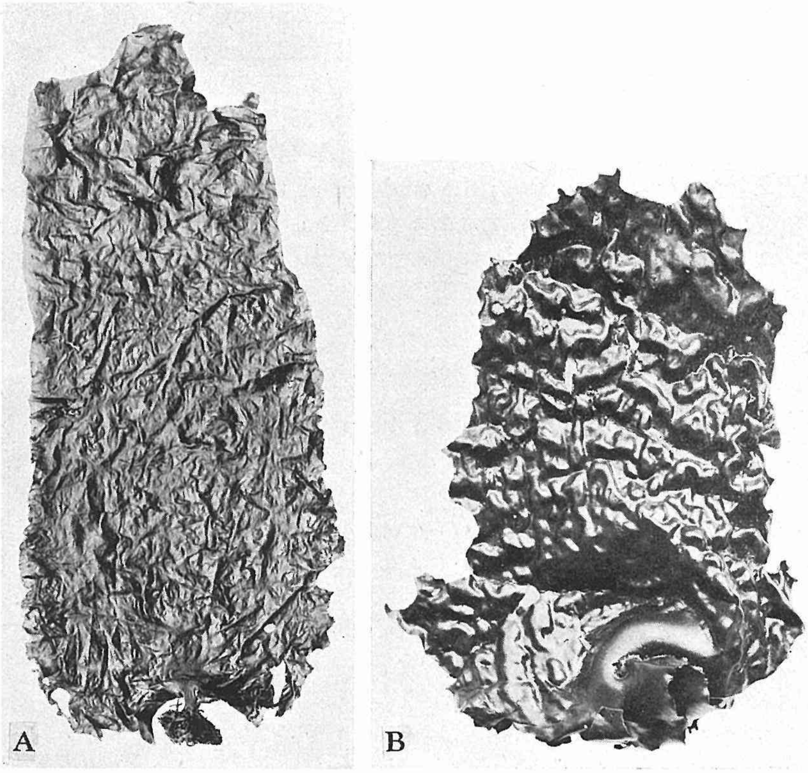

Fig. 16. *Eckloniopsis radicosa* (KJELLM.) OKAM. A. Habit of a plant, just entering the adult condition, showing short, flat stipe producing numerous rhizoids; collected at Mera, Tiba Prefecture, in June. B. Habit of an old plant, showing roughly bullated blade, the upper part of which becomes gradually worn away. A scrolled marginal lamina is produced from thickened and decumbent base of the blade; collected at Susaki, Sizuoka Prefecture, in October.

modifications which finally result in the characteristic frond of *Eckloniopsis*. One of the most noticeable modifications is a thickening of the blade and a development of marginal lamina at the base. As the development advances this marginal lamina becomes rolled in. The blade becomes bent at the transition region, until it forms an angle of nearly 90° to the stipe (Fig. 16, B, Plate 39, 5–6). In young plants the rhizoids develop only from the end of the stipe, forming dense clumps of slender fibres (Fig. 16, A). As the plants become older, numerous rhizoids develop secondarily from both surfaces of the flattened stipe, as well as the decumbent and thickened base of the blade. These secondarily produced rhizoids are larger than the primary ones, and they grow until they cover the latter

(Plate 39, 5–6). This process of the thickening of the base as it becomes more and more decumbent, the issue of more and more hapters from the surface of the stipe as well as the thickened base of the blade, go on until there results a stipe which is buried in a complex and bulky mass of hapters (Plate 39, 5–6).

This plant is very common along the coast of Izu, growing on rocks in the sublittoral belt; it attains full maturity from August to October, and before the end of autumn when the zoospores are liberated, the fronds are entirely washed away from the substratum. In the following winter, from December to February of the next year, the young sporophytes make their appearance associated with *Eisenia bicyclis* (KJELLM.) SETCH. and *Ecklonia cava* KJELLM. The sori appear at first in the depression on both surfaces of the blade. As the development progresses they become confluent into fairly large patches of irregular outline. After the zoospores have been liberated the blade begins to decay from the apex, and by early October when the writer collected the material for the present study, only a clump of hapters with small portions of the thickened and decumbent base of the blade was observed. In such an older plant as this the sori are produced not only on the surface of the blade but on those of the stipe and rhizoids (Plate 39, 1–2). It is a very characteristic habit to the present species that the rhizoids produce zoosporangia.

The present culture experiment was carried out at the Mitsui Institute of Marine Biology, from 5 October to 30 October 1937, together with the cultures of *Eisenia bicyclis* (KJELLM.) SETCH. and *Ecklonia cava* KJELLM.

Development of Gametophytes

The shape and size of the zoospores and the process of their germination are entirely similar to those of *Eisenia bicyclis* (KJELLM.) SETCH. and *Ecklonia cava* KJELLM. (Fig. 17, A). It swims 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. 17, B–C). Within 24 hours a germination tube grows out of the resting spore, and the contents of the original cell migrate into this tube (Fig. 17, D–F). The length of the germination tube of this plant is shorter than those of *Eisenia bicyclis* (KJELLM.) SETCH. and *Ecklonia cava* KJELLM. measuring less than 10μ (Fig. 17, H–L). Within 6–8 days two types of gametophytes may be distinguished (Fig. 17). One type consists of only one or two cells, rarely more than two cells, and later development shows that this type of plant is the female gametophyte (Fig.

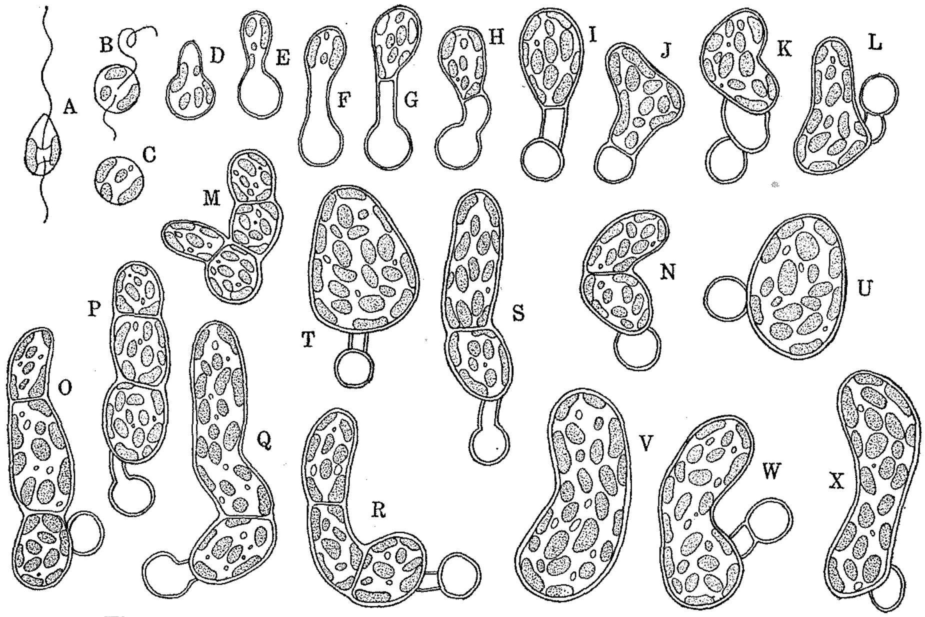

Fig. 17. *Eckloniopsis radicosa* (KJELLM.) SETCH. Zoospore and its germination. ×1175. A. Zoospore in motile state. B. Zoospore in resting state C. Embryospore. D–F. Germination of the embryospore, from 2 day culture. G–L. More advanced stages of the germination, from 3–5 day culture. M–S. Early stages of the male gametophytes, from 6–8 day culture. T–X. Early stages of the female gametophytes, from 6–8 day culture.

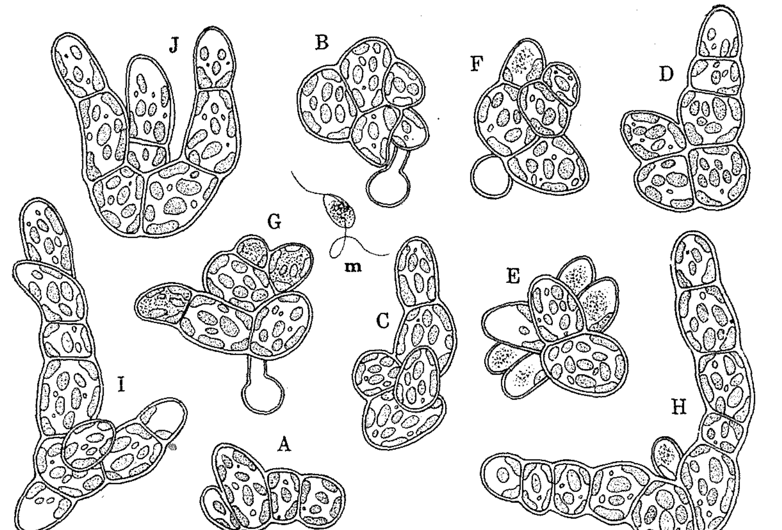

Fig. 18. *Eckloniopsis radicosa* (KJELLM.) OKAM. Various forms of the male gametophytes. ×1280. A–D. Male gametophytes from 8 day culture. E–H. Mature male gametophytes from 16 day culture. m. Male gamete in motile state.

17, T–X). The other type, however, consists of two or more cells. The diameter of each cell is smaller than that of the former, measuring about 8–10μ. This type of the plant is the male gametophyte (Fig. 17, M–S).

As the cultures progress the number of cells of the male gametophytes increases more and more and in the 8–16 day culture, they form a slightly branched filament, with matured antheridia at the tips of the branches (Fig. 18). The entire contents of the antheridia is used for the formation of a single male gamete. The shape and size of the male gamete is altogether similar to those of other Laminariaceous plants. They are about 5μ in length and lack an eye-spot.

Most of the female gametophytes consist of one or two cells (Fig. 19, A–F). Within 14 days the female gametophytes reach maturation (Fig. 19, G–H). At maturity the egg is pressed out through the opening and remains attached there (Fig. 19, J–K). The writer was fortunate enough to observe the egg under microscope just as it was being discharged from the oogonium (Fig. 19, I).

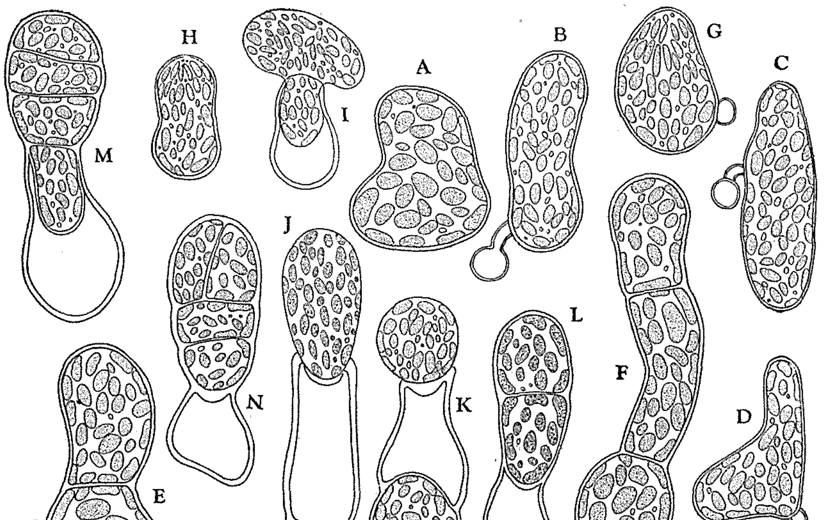

Fig. 19. *Ecklonìopsis radicosa* (KJELLM.) OKAM. Female gametophytes, oogonia, eggs and young sporophytes. ×1025. A–F. Female gametophytes from 8–12 day culture. G–H. Mature female gametophytes from 14 day culture. I. One celled female gametophyte at maturity, showing the actual liberation of egg. J–K. Egg cells. L–M. Young sporophytes from 14 day culture. N. Young sporophyte consisting of 4 cells; showing the first cell division by a longitudinal wall, from 14 day culture.

Development of young Sporophytes

The development of the young sporophyte begins immediately after the egg is fertilized, and when the plants are 14 days old, the first cell division in the egg cell takes place (Fig. 19, L). In other species of *Laminariales,* the cell divisions in the youngest stages of the sporophytes are made by a transverse wall, forming monosiphonous filament, while in the present species, the longitudinal cell division took place in earlier stages of the development, and in an extreme case the first longitudinal cell division was observed in the sporophyte consisting of only four cells (Fig. 19, N). As growth of the sporophytes progresses, their upper part grows rapidly forming a flat expanded blade. In this plant the longitudinal cell divisions are repeated so many times that the young sporophytes become more and more round in shape assuming an aspect much resembling a round fan (Fig. 20, 21, Plate 39, 3–4). The basal cell grows downwards into the the empty oogonium as was observed in *Eisenia bicyclis* (KJELLM.) SETCH. and *Ecklonia cava* KJELLM. (Fig. 20). Rhizoids make their appearance at the base of the sporophyte in plants of 25 day culture, but their development is poor when compared with those of other Laminariace-

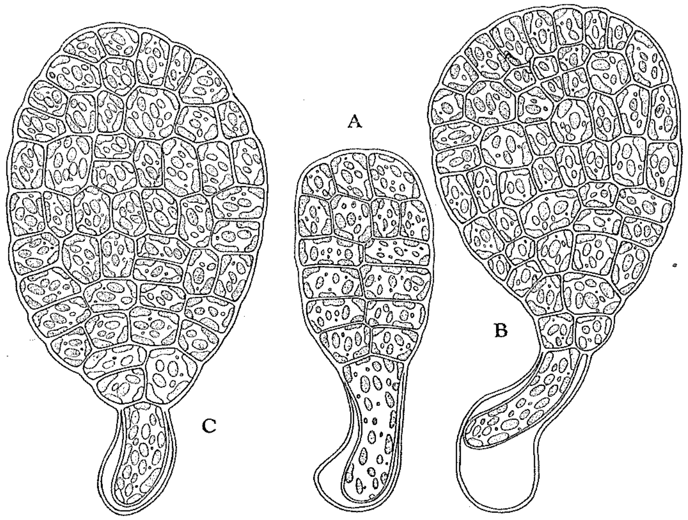

Fig. 20. *Eckloniopsis radicosa* (KJELLM.) OKAM. Early stages of the development of young sporophytes, from 18 day culture. ×680. The basal cell grows downwards into empty oogonium. The cell-divisions by a longitudinal wall are repeated so many times that the young sporophyte of this plant assumes an appearance of a round fan.

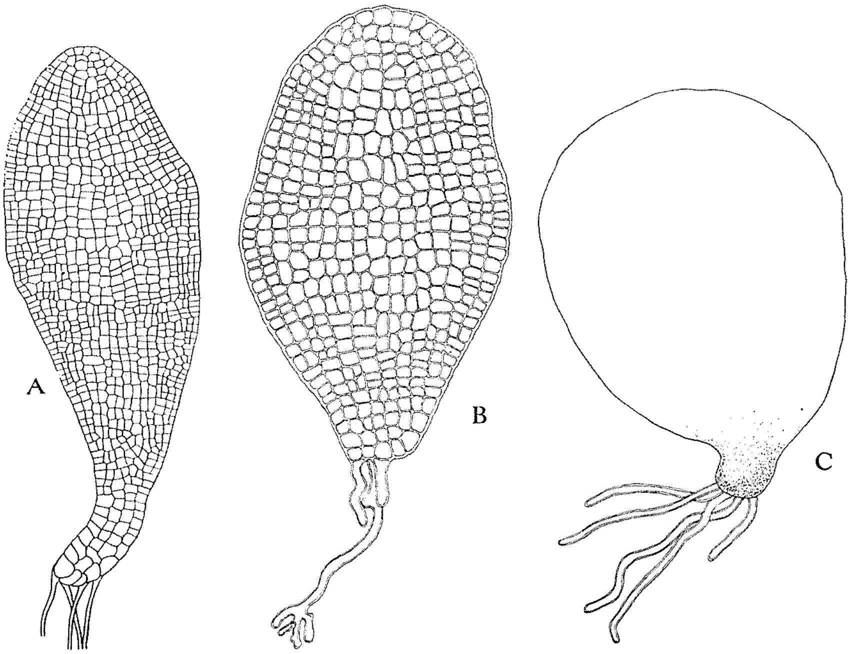

Fig. 21. *Eckloniopsis radicosa* (KJELLM.) OKAM. Further development of the sporophytes. A. Young sporophyte with rhizoid, from 25 day culture. ×300. B. Ditto, from 30 day culture. ×200. C. Ditto, from 25 day culture, showing an expanded blade as round as a round fan.

ous plants (Fig. 21, Plate 39, 3–4). So far as the shape and habit of the zoospores and gametophytes are concerned no remarkable differences were observed compared with those of *Eisenia* and *Ecklonia*, while the shape of the young sporophyte supplies the most noticeable features.

XV. **Undaria Peterseniana** (KJELLM.) OKAM.

This species is also one of the typical warm current forms of Japanese *Laminariales*. It distributes along the coasts of the Pacific as well as the Japan Sea; its northern limit on the Pacific side is near Bōsyū, Tiba Prefecture, and that on the Japan Sea side is near Etizen, Isikawa Prefecture. In habit this species has a certain resemblance to *U. pinnatifida* SURING., but it may easily be distinguished from the latter, by its usually broadly linear-lanceolate, entire margined frond having fascia, the breadth of which is very variable. The fascia of the present species differs histologically from the midrib of *U. pinnatifida* SURING.; the midrib of the

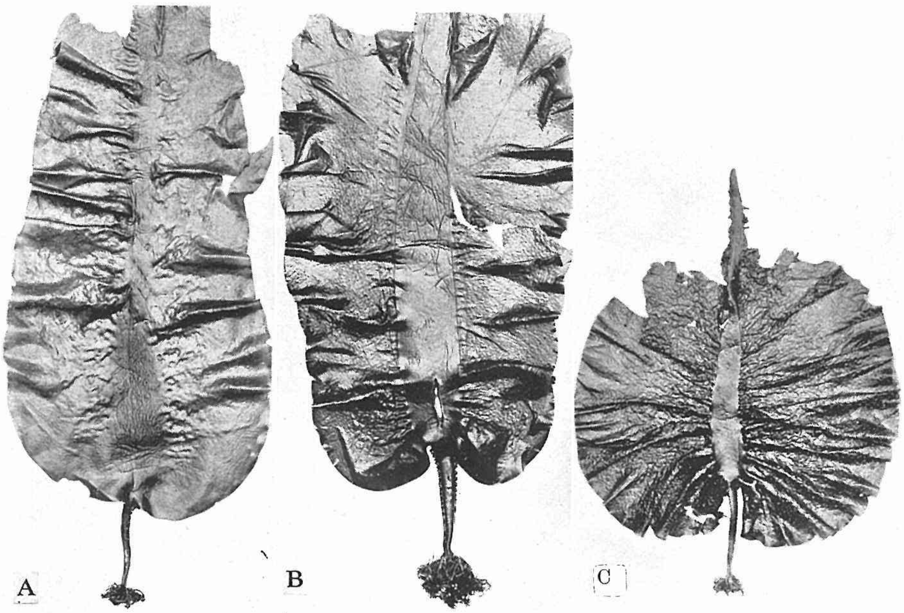

Fig. 22. *Undaria Peterseniana* (KJELLM.) OKAM. A. Habit of a young plant just entering an adult stage, showing smooth surface and entire margin of the blade, the middle portion of which gradually increases its thickness, forming the initial of a fascia; collected at Mera, Tiba Prefecture, in May. B. Habit of an adult plant, showing more or less broad fascia, bearing zoosporangia, cordate base of the blade, flattened stipe, on both margins of which developing sterile wings; collected at Mera, in June. C. Well developed plant growing in sheltered locality. The blade is round in shape, and is rugosely bullated. Rather narrow fascia bearing zoosporangia runs throughout the middle part of the blade; collected at Hunakata, Tiba Prefecture, in June.

latter is produced at the youngest stage of development of the sporophyte, when the length of the blade is only several centimeters. The breadth of the midrib is narrow and uniform, and it extends through the middle part of the blade from the base to apex, having direct connection with the stipe. On the other hand in *U. Peterseniana* (KJELLM.) OKAM. the blade of the young frond, measuring as long as nearly half a meter, possesses neither midrib nor fascia (Fig. 22, A). During May and June, however, when the plant attains its adult stage, the middle part of the blade grows thicker, which leads to the formation of a fascia. As the plants approach to maturation, the zoosporangia are produced on both surfaces of this thickened fascia. In some specimens the zoosporangia are produced not only on the surface of the fascia but also on the sporophylls which developed as undulato-folded wings from both margins of the stipe. The presence of these undulato-folded wings, either sterile or fertile, is very characteristic to the genus *Undaria*.

The size of the plant and the amount of bullations depend largely upon the habitat in which they grow. On exposed coasts, e. g., at Mera, Tiba Prefecture, on the Pacific side, they are long, linear-lanceolate, and may be entirely smooth or slightly bullate (Fig. 22, A–B). While at Hunakata, Tiba Prefecture, on the Tokyō Bay side, in a sheltered location, where they grow in great profusion, the blade becomes round in shape and the whole surface is much bullated (Fig. 22, C). A definite seasonal variation may be recognized in this species. In winter the young plants make their appearance and attain their adult stage in early summer, from May to June. When the zoospores are liberated, the blade begins to decay from the apex, and at last they are entirely washed away from the substratum before the end of August.

The material for the present investigation was collected at the coast of Mera, on 18 May 1938; brought to the laboratory at Kominato, Tiba Prefecture, by train, and the culture was started on that day. When the plants were about 30 days old, they were brought by train, keeping them alive, to the laboratory at Muroran, where the culture were continued for as long as about 40 days.

Development of Gametophytes

As the accompanying figures show, the shape and size of the zoospores, and the process of their germination are altogether similar to those of other species above described (Fig. 23, A–G). Within 24 hours the germination tube grows out of the resting spore (Fig. 23, C), and the contents of the cell begins to migrate into this tube (Fig. 23, D–G). The germination tubes are much longer than those of *Eisenia bicyclis* (KJELLM.) SETCH. and *Ecklonia cava* KJELLM., measuring about up to 16μ in length. Soon the distal end of the tube gradually swells up and the whole contents of the original cell enters this part, then the cross wall is formed so as to separate this part from the tube (Fig. 23, H). Within 5 days the first cell division takes place (Fig. 23, I). At the end of 10 days the two types of gametophytes may be distinguished. One type consists of more than three cells; the diameter of each cell is always less than 8μ. They are the male gametophytes, as later development shows (Fig. 23, U–W). The other type consists at this time, of only one or several cells which measure up to 10–15μ in diameter; they are the female gametophytes (Fig. 23, O–T).

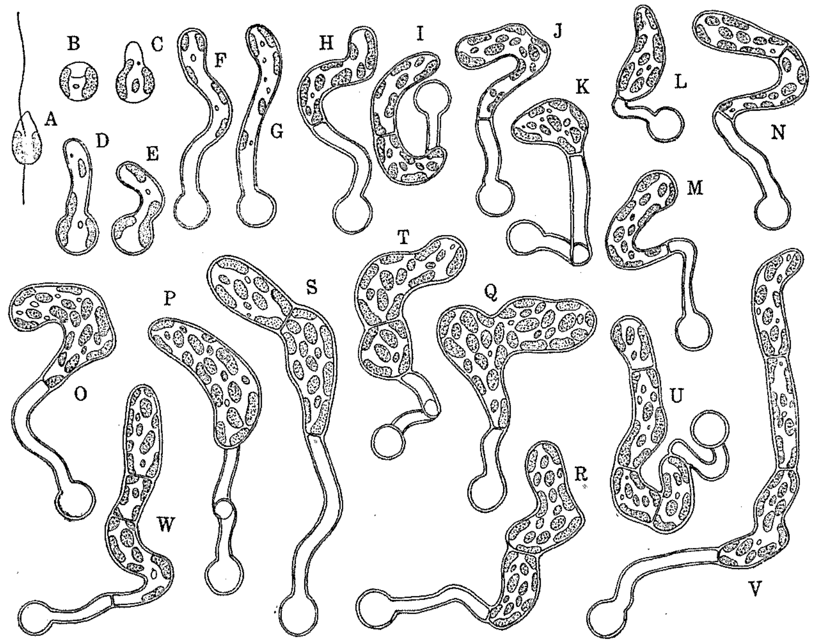

Fig. 23. *Undaria Peterseniana* (KJELLM.) OKAM. Zoospore and its germination. ×1240. A. Zoospore in motile state. B. Embryospore. C–G. Germination of the embryospore; the contents of the original cell migrate into this tube, from 24–48 hour culture. H–N. Sporlings from 2–7 day culture, showing the transverse wall between the tube and distal end. O–T. Early stages of the female gametophytes, from 5–10 day culture. U–W. Early stages of the male gametophytes, from 5–10 day culture.

In 14 day culture the male gametophyte forms a filament consisting of 5 or 6 cells (Fig. 24, A–B), and as the development advances the number of cells increases. In plants of 36 day culture the male gametophytes bear mature antheridia in dense clusters at the tips of the branches (Fig. 24, C–F). The male gamete measuring about 4.5μ, swims about rather slowly by means of two, laterally placed cilia. In some cases there are observed pale remnants of the chromatophores, but they always lack an eye-spot (Fig. 24, m).

Most of the female gametophytes of this plant consist of two or more cells. When they are about 20 days old, the cell at the apex metamorphoses into an oogonium (Fig. 23, K–M). At the time of fruiting, the oogonium is elongated, and the contents become denser, the chromatophores being crowded together at the apex (Fig. 24, L–M). At maturity the egg-cell is

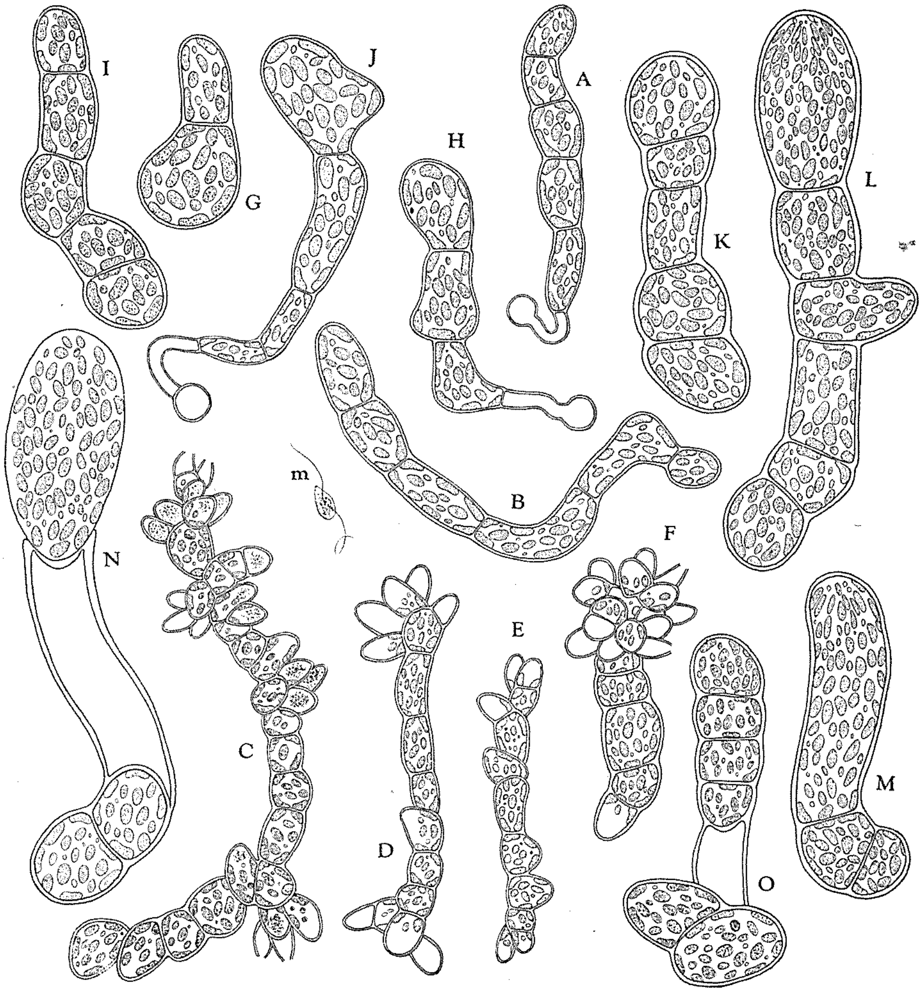

Fig. 24. *Undaria Peterseniana* (KJELLM.) OKAM. Male and female gametophytes, oogonia, egg and a young sporophyte. A–B. Male gametophytes, from 14 day culture. ×1080. C–F. Mature male gametophytes from 24–36 day culture, showing the antheridial clusters and mature antheridia. ×716. G–J. Female gametophytes from 24–36 day culture. ×1080. K. Ditto, from 36 day culture. ×716. L–M. More advanced stage of the female gametophytes, producing mature oogonia, from 36 day culture. ×716. N. An egg resting at the opening of the oogonium from 36 day culture. ×716. O. Young sporophyte resting at the tip of the oogonium, from 28 day culture. ×716. m. Male gamete in motile state. ×1080.

pressed out through the opening at the apex, and remains attached there (Fig. 24, N). In the early stages of development the gametophytes are very slender in appearance, and the length of time necessary for the maturation of the gemetophytes is much longer than in *Eisenia, Ecklonia* and *Eckloniopsis*.

Development of Sporophytes

The fertilized egg begins to germinate while attached to the apex of the oogonium (Fig. 24, O). In plants of 58 days old, young sporophytes consisting of more than 20 cells were observed (Fig. 25). Further development of the sporophyte is wholly similar to that of *Undaria pinnatifida* Suring. The rhizoid makes its appearance from the basel cell of the sporophyte in the 58 day culture (Fig. 25, B). As growth of the sporophyte progresses, its upper part grows rapidly forming a flat expanded blade (Fig. 26). As in *U. pinnatifida* Suring. the female gametophytes of this species retain the function of growth for a long time, and the formation of the new gametophytes may occur in the empty oogonium, at the apex of which the sporophyte is still attached (Fig. 25, B–C). As the gametophyte grows larger and larger the empty oogonium becomes filled with this newly formed gametophyte cell, and at last it breaks out the wall of the oogonium at the base of the sporophyte (Fig. 25, C). The formation of the rhizoids is rather poor when compared with *Eisenia* and *Ecklonia* (Fig. 26).

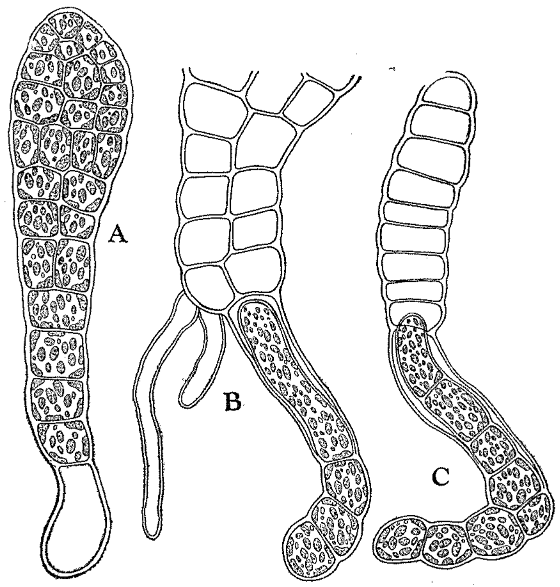

Fig. 25. *Undaria Peterseniana* (Kjellm.) Okam. Development of the sporophytes. ×400. A. Young sporophyte developed from one celled female gametophyte, from 58 day culture. B–C. Basal part of the sporophyte with rhizoids; the empty oogonium becomes filled with newly developed cells of the gametophyte.

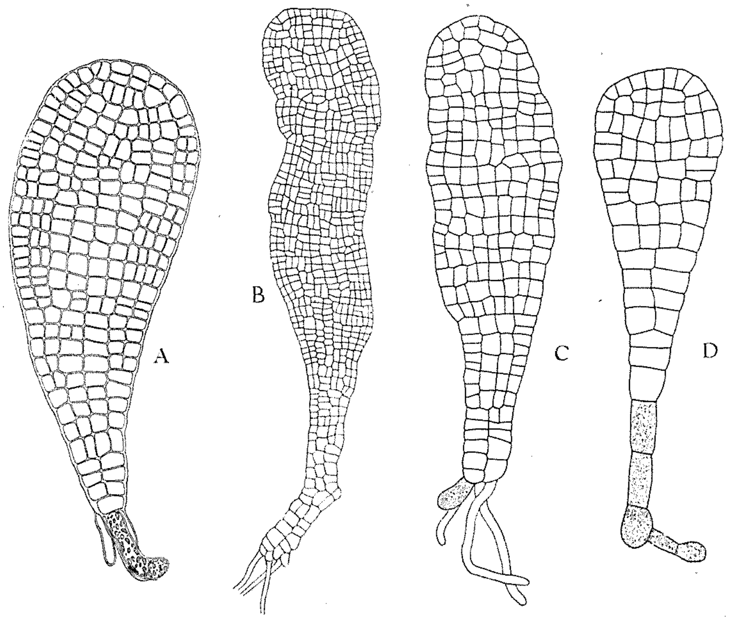

Fig. 26. *Undaria Peterseniana* (KJELLM.) OKAM. Further development of the sporophytes. A. Young sporophyte with rhizoid, from 36 day culture. ×234. B–C. Young sporophytes from 60 day culture. ×110. D. Ditto, from 40 day culture. ×142.

Discussion and Summary

1. In the present paper there are described the results of culture experiments with the gametophytes and the young sporophytes of five species of the warm current form of Japanese *Laminariales*, namely *Eisenia bicyclis* (KJELLM.) SETCH., *Ecklonia cava* KJELLM., *Ecklonia stolonifera* OKAM., *Eckloniopsis radicosa* (KJELLM.) OKAM., and *Undaria Peterseniana* (KJELLM.) OKAM.

2. Though *Eisenia bicyclis* (KJELLM.) SETCH. and *Ecklonia cava* KJELLM. are referred systematically to different genera respectively, so far as the shape of the zoospores, the process of their germination, the development of the male and female gametophytes, and the habit of earliest stages of the sporophytes are concerned, there exist close resemblances between them. As has already been reported by SETCHELL, the chief differences, and the only ones of any importance, are the formation of two arms at the transition region of the stipe in *Eisenia bicyclis* (KJELLM.) SETCH. In earliest stages of the development, the shape of the frond of *Eisenia bicyclis* (KJELLM.) SETCH. is exactly the same as that of a young *Ecklonia*

cava KJELLM. In *Eisenia,* as the development advances, the basal margins of the blade begin to thicken and to turn in on one side of the frond, the blade wears away until it disappears almost entirely except for the small side pieces, ligules. These small ligules grow gradually and lead to the formation of two arms. In *Ecklonia cava* KJELLM., the base of the blade does not thicken at all; the blade is constantly renewed at the transition region, pushing up the old one, the apex of which is worn away gradually.

3. Culturing the zoospores of *Ecklonia bicyclis* (Syn. *Eisenia bicyclis*), IKARI (1926) has published his paper with several accompanying figures. CLARE and HERBST (1938) made culture studies of *Eisenia arborea* Aresch. growing along the coasts of California. In the following year HOLLENBERG (1939) published his study on cultural and cytological experiments in the same species. In this study he counted the haploid number of chromosomes to be 15. The shape and habit of the zoospores, both of the gametophytes, and the young sporophytes described by those authors differ to some extent from the results obtained by the present writer. The chief differences are the number of cells composing the female gametophytes, and general aspects of both gametophytes. The female gametophytes figured by those authors have a slender aspect, consisting of a fairly large number of cells, and they bear one to several branches. The male gametophytes figured by IKARI (1926) show somewhat shrunken appearance, while those figured by HOLLENBERG (1939) are profusely branched ones consisting of hundreds of cells. Most of the female gametophytes observed by the present writer, however, are uni- or bi-cellular, and the female gametophytes are less branched. Furthermore, between the writer's experiments and those of others, there are striking differences in the length of time necessary for the gametophytes to reach sexual maturity. Working on *Eisenia arborea* ARESCH., CLARE and HERBST (1938) observed that two types of gametophytes are distinguished in 36 day culture, and they reach maturity in 60 days after the germination of zoospores. Working on the same species, HOLLENBERG (1939) has reported thus, "under favorable conditions, male and female plants are distinguishable about 15 days after germination, and in vigorous cultures the antheridia first appear about 40 days, and female gametophytes arrive at sexual maturity 40–50 days after germination". Contrary to the descriptions of these authors, the writer has observed that the length of time taken for arrival at sexual maturity is very short; for instance, in *Eisenia bicyclis* (KJELLM.) SETCH. and *Ecklonia cava* KJELLM. mature gametangia were observed within 8 days after the germination of zoospores. The writer is inclined to consider that these differences are

caused chiefly by the different conditions of the culture.

4. CLARE and HERBST (1938) reported that the zoospores of *Eisenia arborea* ARESCH. do not seem to possess cilia or to be motile. Similar results were reported by HERBST and JOHNSTONS (1937) on *Pelagophycus pora* (LEMAN) SETCH. Working on the same species as CLARE and HERBST, HOLLENBERG (1839) has differed with them thus, "Their inability to detect motility of the zoospores or to see the flagella would appear to be due to faulty tecknique in starting cultures. In this connection one is also inclined to question the findings of HERBST and JOHNSTONE (1937), who report their inability to find flagella or evidence of motility of zoospores in the case of *Pelagophycus pora* (LEMAN) SETCH." The writer entertains the same views as HOLLENBERG. In order to liberate the zoospores, CLARE and HERBST (1938) placed ten to twelve fragments of sori about one centimeter square in separate dishes and allowed them to stand from 12 to 24 hours before being removed. The zoospores are easily poisoned by the mucilaginous substances and especially by the brown juice oozing out of the thallus of *Eisenia, Ecklonia* and other Laminariaceous plants. When the zoospores were poisoned by these substances, their motility was decidedly weakened; they became spherical in shape, and floated on the liquid being deprived of their cilia.

5. As previously reported by the present writer, he has never met with zoospores, nor with male gametes bearing an eye-spot in all the species of Japanese *Laminariales* studied, with the exception of *Chorda Filum* (L.) LAMX. HOLLENBERG (1939) has reported the presence of an eye-spot in the zoospore, as well as the male gamete of *Eisenia arborea* ARESCH., saying, "A stigma is definitely present in the form of a more or less elongated body associated with the chromatophor on one side of the cell". Consequently one practical example showing the presence of an eye-spot in the zoospores and male gametes of *Laminariaceae* has been added hereby. It is worth noting that the zoospores and male gametes of the Laminariaceous plants from the Japan side of the Pacific are always lacking an eye-spot, while those from the American side are provided with it.

6. In *Eisenia bicyclis* (KJELLM.) SETCH., *Ecklonia cava* KJELLM., *Ecklonia stolonifera* OKAM., and *Eckloniopsis radicosa* (KJELLM.) OKAM. the basel cell of the young sporophyte grows downwards gradually within the empty oogonium cell, and at last it fills the greater part of that cell. This basal cell remains in position at the base of the blade without showing any cell division. In *Eisenia bicyclis* (KJELLM.) SETCH. and *Ecklonia cava* KJELLM., the rhizoids are not produced from this basal cell, but from the

cell or cells next to it. A very interesting character of these species is that the basal cell usually takes no part in the formation of rhizoids.

7. In *Eisenia bicyclis* (KJELLM.) SETCH. and *Ecklonia cava* KJELLM. the beginning of the stipe is differentiated at the base of the frond when the plants are as long as one mm or so. The cells which compose the origin of the stipe elongate, as the lamina increases in height, to a length much exceeding the diameter. They divide longitudinally and transversely, quite irregularly to form a cylindrical stipe. The resulting cells are poor in chromatophores, and those in the lower part are almost entirely devoid of them.

8. As accompanying figures show, the shape and habit of the gametophytes and young sporophytes of *Undaria Peterseniana* (KJELLM.) OKAM. bear a close resemblance to those of *Undaria pinnatifida* SURING., described in the previous paper by the present writer (1936); the germination tube is much longer than that of any other species of *Laminariales* studied, the gametophytes in early stages of development show a slender appearance, the female gametophytes remain alive, and retain the function of growth for a long time, and the formation of the new gametophytic cells may occur in the empty oogonium, at the apex of which the sporophyte is still attached. It takes a much longer time than other Laminariaceous plants studied by the writer, until the gametophytes reach maturity.

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PLATES XXXVII-XXXIX

PLATE 37

Eisenia bicyclis (KJELLM.) SETCH.

1–2. One celled female gametophytes with mature oogonia, from 13 day culture. The chromatophores are crowded at the tip of the oogonium.

3. Two celled female gametophyte with mature oogonium and an egg just discharged from the oogonium.

4. An egg just discharged from the oogonium, from 15 day culture.

5–6. Young sporophytes from 18 day culture.

7–10. Further development of the sporophytes, showing the differentiation of the blades, stipes and hapters. The blade becomes distromatic at the transition region.

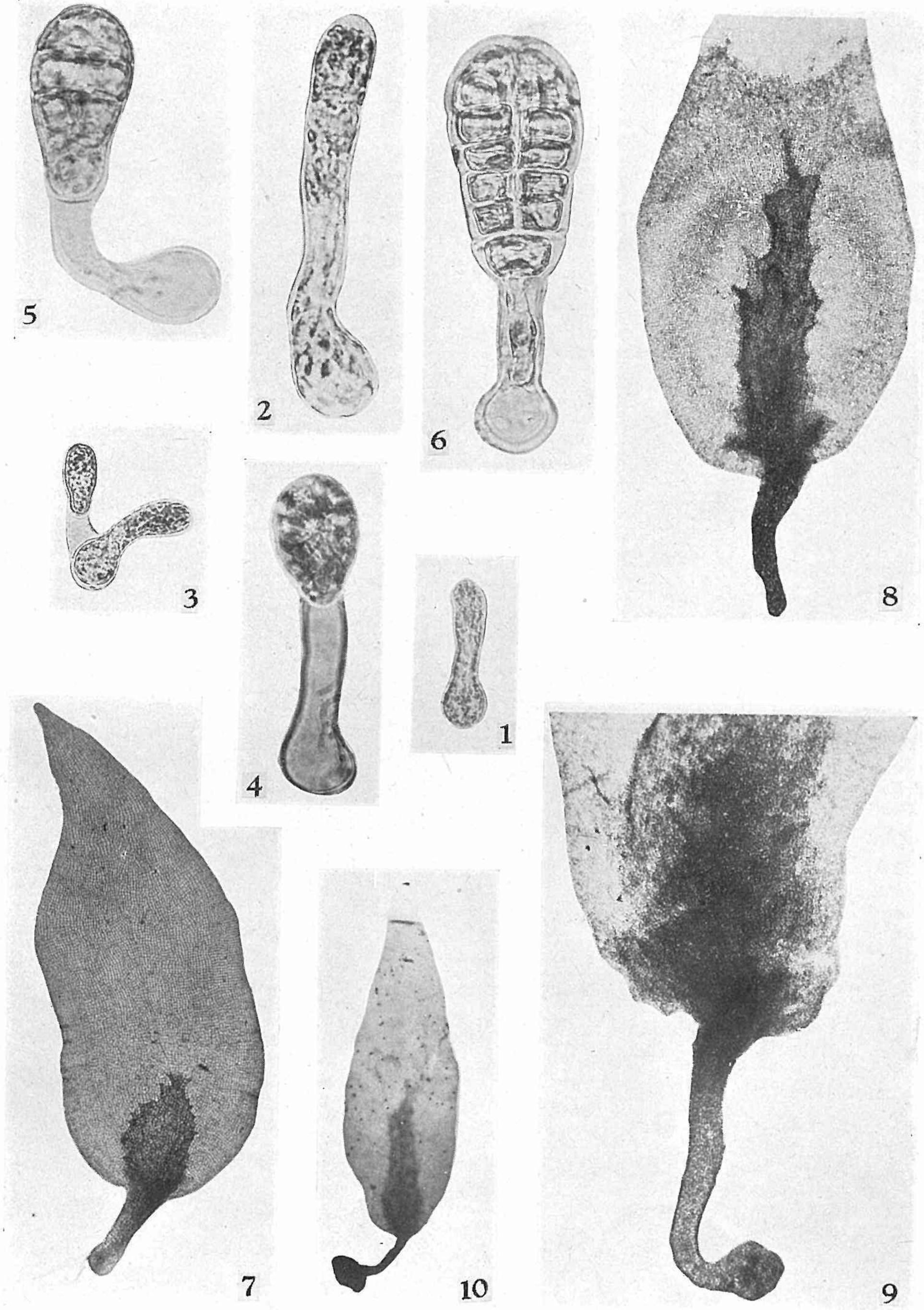
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PLATE 38

Ecklonia cava KJELLM.

1. Mature female gametophyte consisting of two cells, showing two male gametes (S), adhering on the surface of the mature oogonium.
2–3. Young sporophytes.
4–8. Further development of the sporophytes, showing the differentiation of the blades, stipes, and hapters. The blade becomes distromatic at the transition region (8).

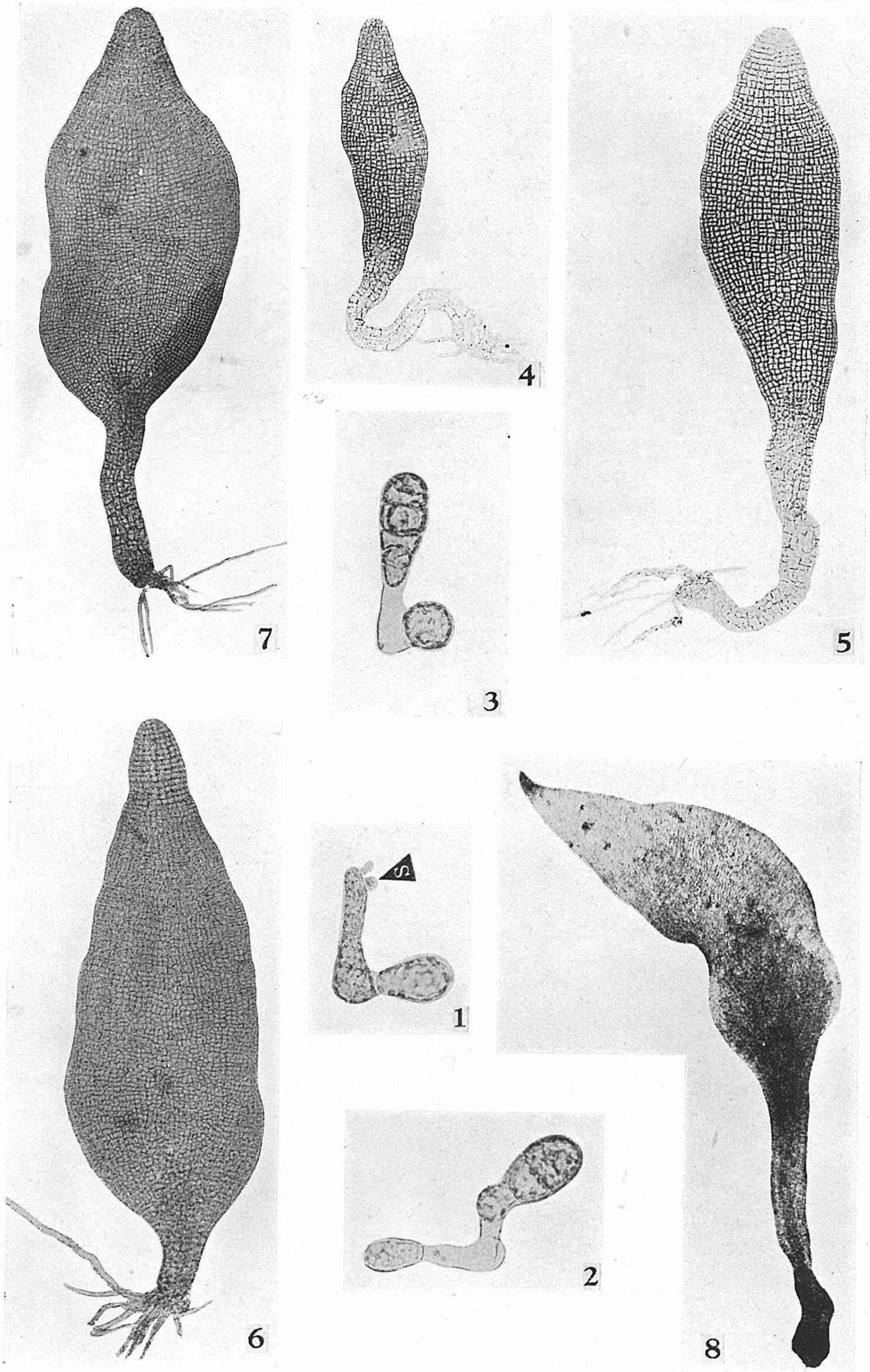
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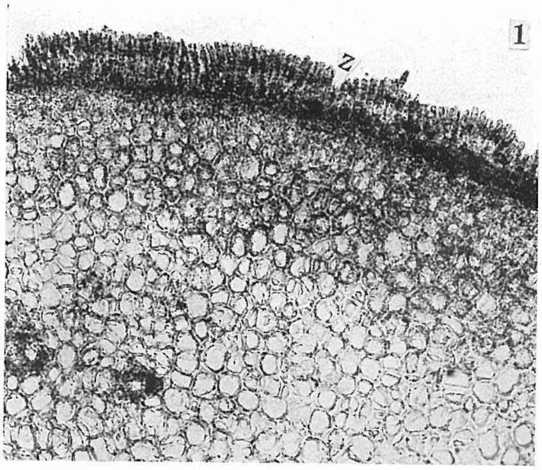
PLATE 39

Eckloniopsis radicosa (KJELLM.) OKAM.

1–2. Zoosporangia produced on the surface of the rhizoid.
3–4. Young sporophytes with rhizoids.
5. Base of an old plant cut through longitudinally to show the thickened and decumbent base of the blade; numerous rhizoids which are produced secondarily from both margins of the stipe, forming a complex and bulky mass of hapters.
6. Ditto, more advanced stage.



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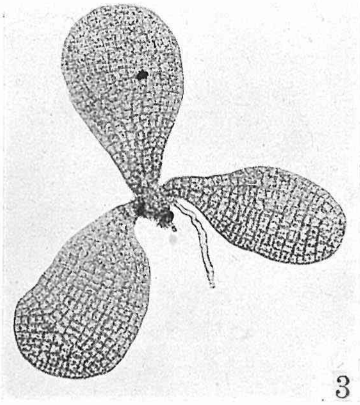
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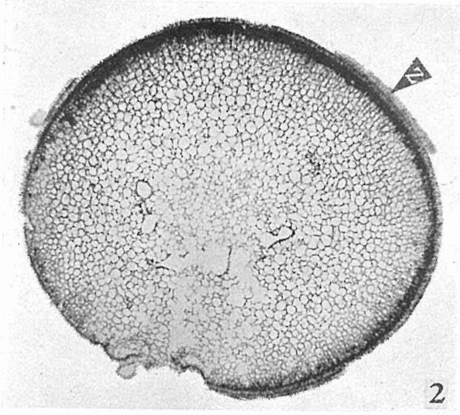

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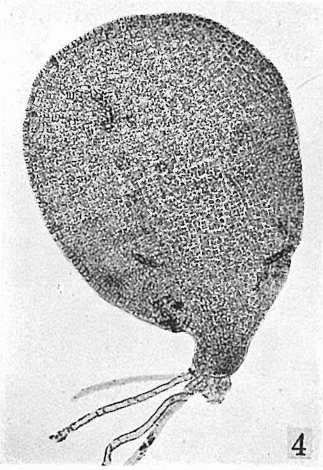
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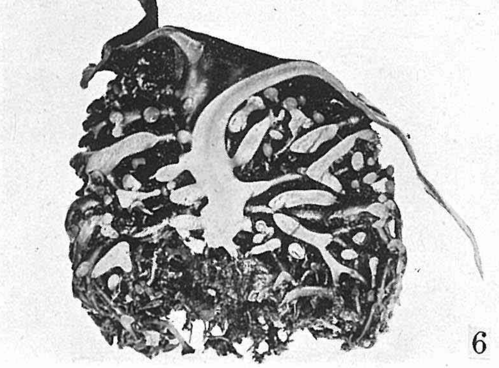
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