On the Gametophytes of Some Japanese Species of Laminariales IV.

By

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With Plate LIX.

In the present paper there are described the results of culture experiments with the gametophytes and the young sporophytes of two species of Japanese Laminariales, viz., *Laminaria angustata* KJELLM. and *Agarum cribrosum* BORY. Cultures were made at the sea-side laboratory of the Institute of Algological Research at Muroran, by the same methods as those described in the previous work. The materials used in the present work were obtained at the coast of Muroran. The culture solution was made of natural sea-water, filtered twice through a layer of absorbent cotton. Small quantities of sodium nitrate and natrium phosphate were added at the ratio of the following table which has been recommended by SCHREIBER (1932).

$NaNO_3$	0.1 gr	distilled water	50 cc
Na_2HPO_4	0.02 gr	sea-water	1,000 cc

Materials bearing well matured sori were collected at the sea-shore, brought to the laboratory, washed carefully in sterilized sea-water. Then the blades were put into a glass vessel containing about eight litres of twice filtered sea-water and the vessel was illuminated with an electric lamp, that exerted a good effect for the liberation of zoospores. The zoospores were settled on the slide-glasses, and cultured in a culture vessel containing about 250 cc of medium. The culture vessels were placed in a room 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 best thanks to Professor Y. YAMADA for his kind guidance and criticisms in the course of the present study.

XVI. **Laminaria angustata** KJELLM.

The present species is one of the most common algae on the coast of

Muroran. It grows on rocks in the lower littoral and sublittoral belt, associated with *Alaria crassifolia* KJELLM. The zoosporangia appear in autumn and winter on the coast of Muroran. After the zoospores have been liberated, the greater part of the blade is gradually worn away, and only the stipe bearing a certain portion of the blade remains attached to the substratum. The blade practically ceases to develop further during autumn and early winter; then in the early months of the next year, the second year blade begins its sudden growth at the transition region, pushing up the first year blade. The second year blade increases in length and

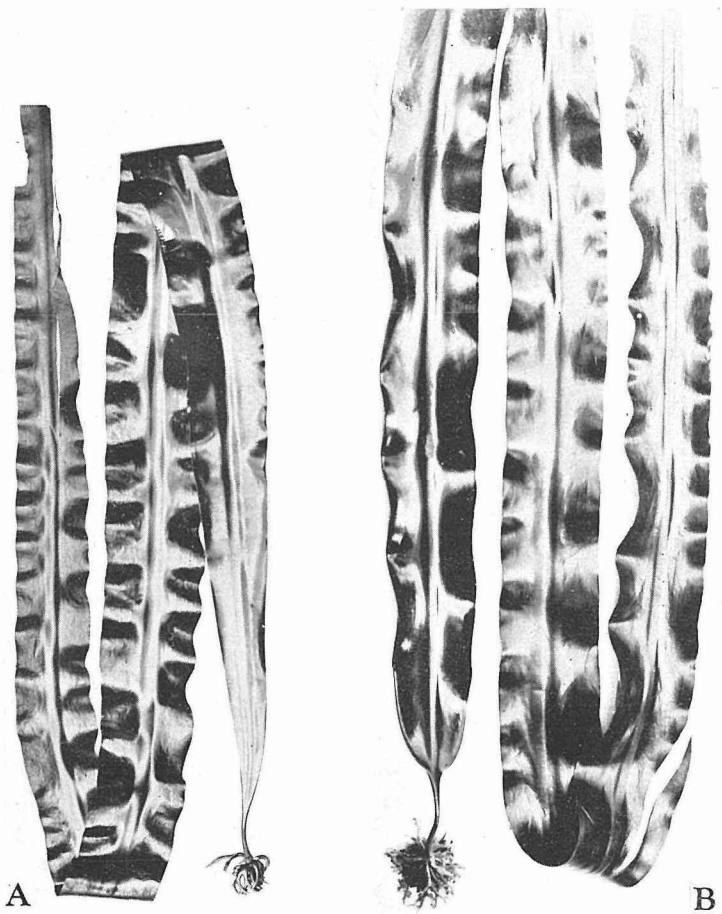


Fig. 1. *Laminaria angustata* KJELLM. A. Habit of young plant, collected at Muroran in May. B. Habit of an adult plant; the base of the blade becomes gradually cordate; collected in August.

thickness to attain its full growth in August and September and then becomes soriferous. On the coast of Muroran, the embryonal fronds appear from January to March and attain their adult stage in August.

The zoosporangia are produced at first on one side of the blade, the fascia on that side being projected, in two parallel rows, leaving the fascia and both margins sterile. Later, however, these two parallel rows join into one in several places, especially in the lower portion of the blade. Fully matured sori are dark olive in color.

Cultures have been made three times, hitherto, and the same results were obtained in every case.

Zoospores and their Germination

The zoospores are pear-shaped when they are active, pointed at one end and rounded at the other, measuring about 8–9.2μ in length, 4–4.5μ in breadth; they have two laterally placed cilia, one pointing forward, as

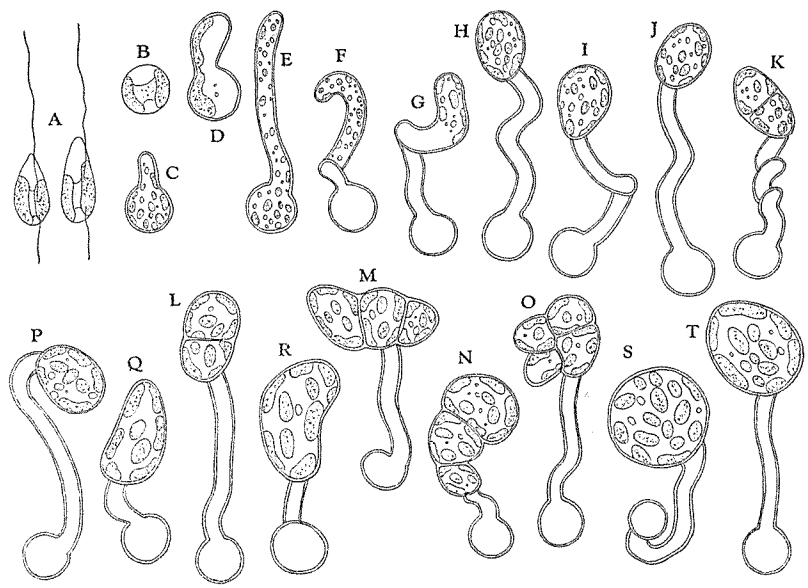


Fig. 2. *Laminaria angustata* KJELLM. Zoospores and their germination. ×1250. A. Zoospores in motile state. B. Embryospore. C–D. Germination of the embryospores. E–G. Development of the germination tube; the contents of the embryospore migrate into this tube; from 2 day culture. H–K. Sporelings from 4 day culture, showing the first transverse wall. L–O. Early stages of the male gametophytes, from 7–9 day culture. P–T. Early stages of the female gametophytes, consisting of only one cell; from 7–9 day culture.

long as 18–20μ, and the other pointing backwords, as long as about 6–8μ. The zoospores have one curved chromatophore which occupies the posterior part, one nucleus and some granular substances, but always lack 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). A germination tube grows out of the embryospore (Fig. 2, C–D). The germination tube increases its length more and more, as the culture progresses, and within 2 days it reaches about 20μ in length (Fig. 2, E). The distal end increases in diameter, and a wall is formed so as to separate the distal end from the tube. When the plants are about 7–9 days old, two types of gametophytes may be distinguished. Those belonging to one type consist of a number of cells as figured in Fig. 2, L–O. They are male gametophytes. The others consist of only one cell at this stage, the diameter of the cell, however, being much larger than that of the former; they are female gametophytes (Fig. 2, P–T).

Male Gametophytes and Male Gametes

The male gametophytes of the present species consist of a fairly large number of cells, the diameter of each cell measuring always less than 9μ. When the male gametophytes approach to maturity the antheridial cells are produced at the tips of the branchlets. The chromatophores of these cells become pale in color. The contents of the antheridium 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 matured antherozoid swims out (Fig. 3). The male gamete is ovate or sometimes rather round in shape, measuring about 6μ in length, and 2.5–3.5μ in breadth. Two cilia of almost equal length measuring about 20μ 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 60 day culture the greater part of them have disappeared.

Female Gametophytes and Eggs

The shape and size of the female gametophytes of this plant show some resemblance to those of *Laminaria Yendoana* MIYABE (Fig. 4). Some

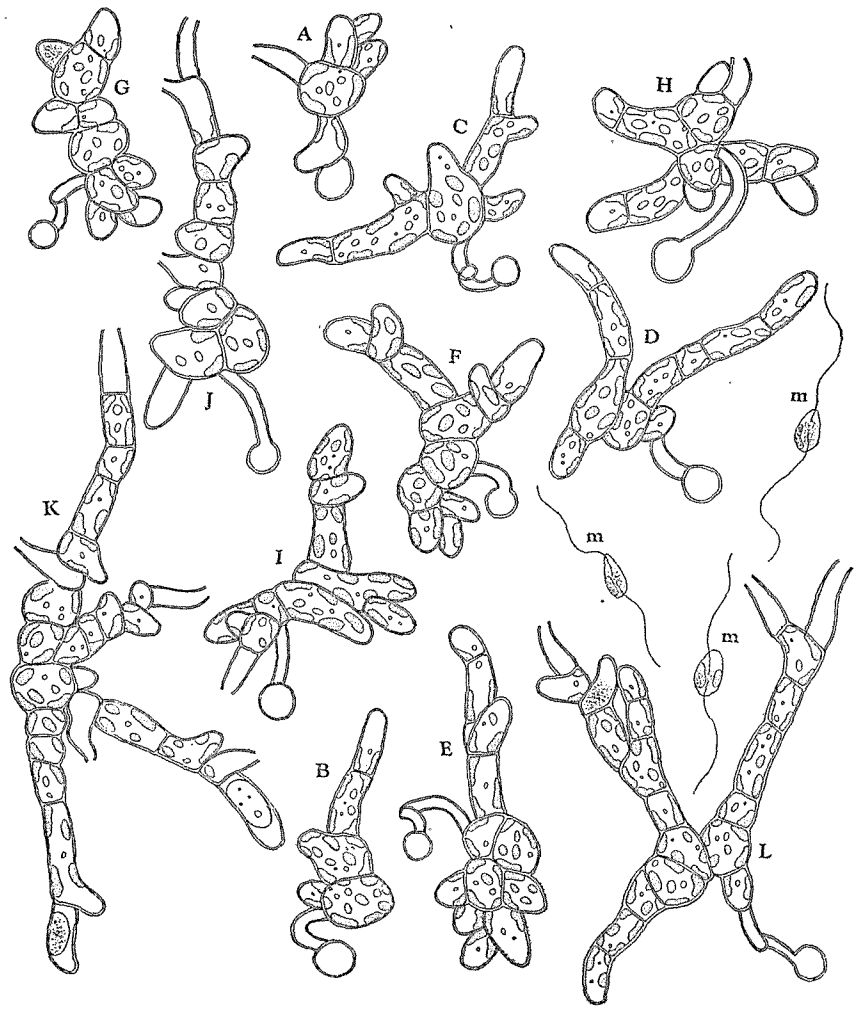


Fig. 3. *Laminaria angustata* KJELLM. Various forms of the male gametophytes and male gametes, from 15–20 day culture. ×980. m. Male gametes in motile state, bearing two cilia of almost equal length, measured up to 18μ in length.

consist of only one cell (Fig. 4, A–I), others consist of two or more cells (Fig. 4, J–N). In some cases, a unicellular female gametophyte showing curious appearance was observed (Fig. 4, F–I). When they come to maturity the whole vegetative cell changes into an oogonium (Fig. 4, O–G). At maturity the chromatophores in the oogonium become crowded together

with their long axes parallel to that of the oogonium (Fig. 4, D–G). The contents of the oogonium is then pressed out through the opening at the apex, where it remains attached (Fig. 5, A).

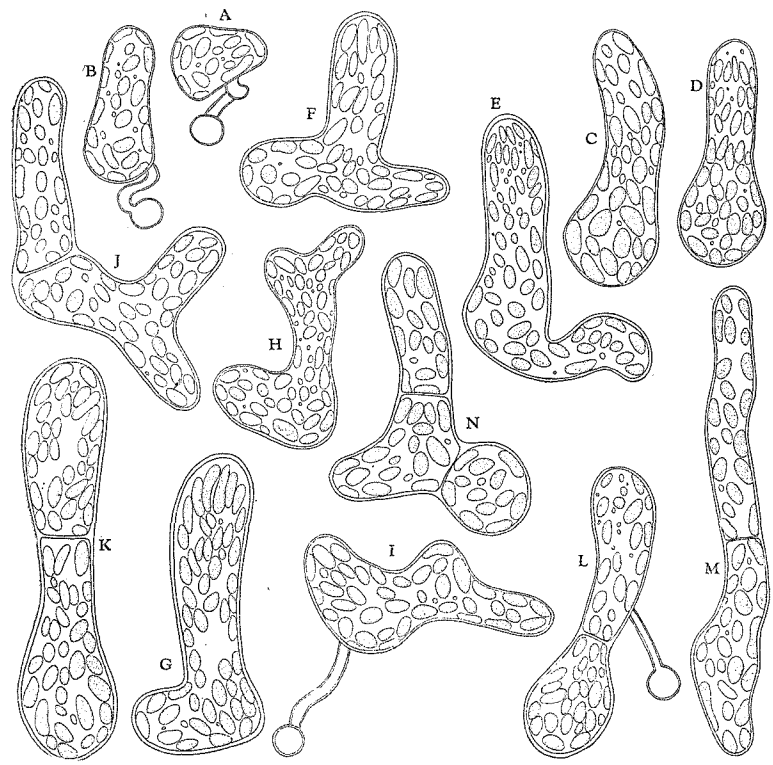


Fig. 4. *Laminaria angustata* KJELLM. Various forms of the female gametophytes, from 15–20 day culture. × 867. A–I. One celled female gametophytes. D–G. One celled female gametophytes with mature oogonia; the cell is decidedly elongated and the chromatophores in the oogonium cell are crowded together at the apex. J–M. Two celled female gametophytes. N. Three celled female gametophytes.

Development of young Sporophytes

When the egg is fertilized the cell division takes place (Fig. 5, B). The new sporophytes grow rapidly, and in the 30–50 day culture many of them develop one or more rhizoids (Fig. 5, C–D). Further development is entirely similar to that of other Laminariaceous plants, and there are no special remarks to be offered.

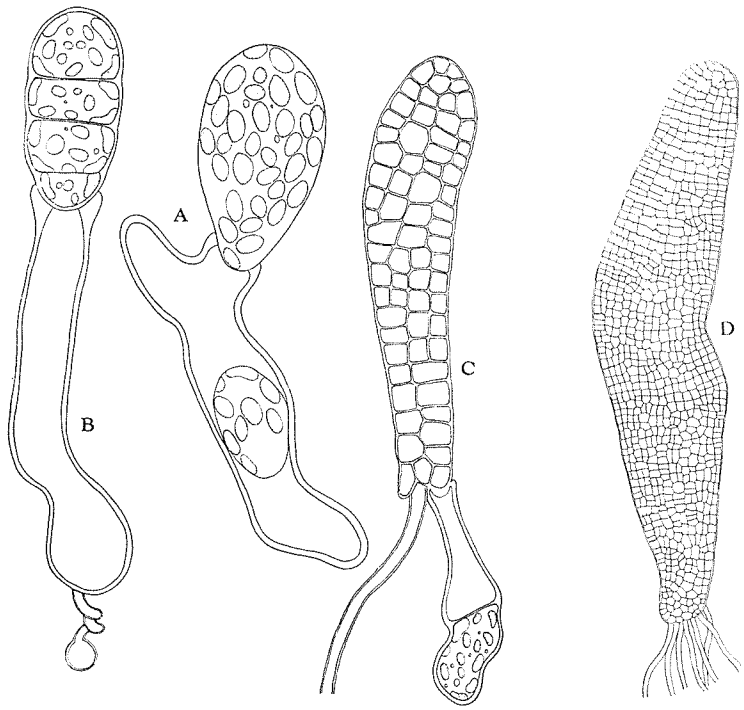


Fig. 5. *Laminaria angustata* KJELLM. A. An egg just discharged out of the oogonium, from 20 day culture. Small amount of plasma and chromatophores are left in the oogonium cell. ×622. B. Early stage of the sporophyte, from 20 day culture. ×622. C. Young sporophyte developing a rhizoid at the base, from 50 day culture. ×250. D. Further development of the sporophyte, from 2 month culture. ×160.

XVII. **Agarum cribrosum** BORY

Agarum cribrosum BORY is rather widely distributed in this country; it is found growing from the Kuriles to as far south as Ōma, Aomori Prefecture, along the Pacific coast of Hokkaidō, and on the Japan Sea side it is found from Saghalien to as far south as the north-eastern coast of Korea. It grows on rocks in the sublittoral belt, associated with *Phyllospadix*, a kind of sea grass. On the coast of Muroran its growing zone is limited only in the sublittoral belt at a depth of several fathoms where the water is rather quiet. An ecological habit of this plant is very characteristic when compared with other Laminariaceous plants in this district.

These plants become soriferous from late in winter to early in spring.

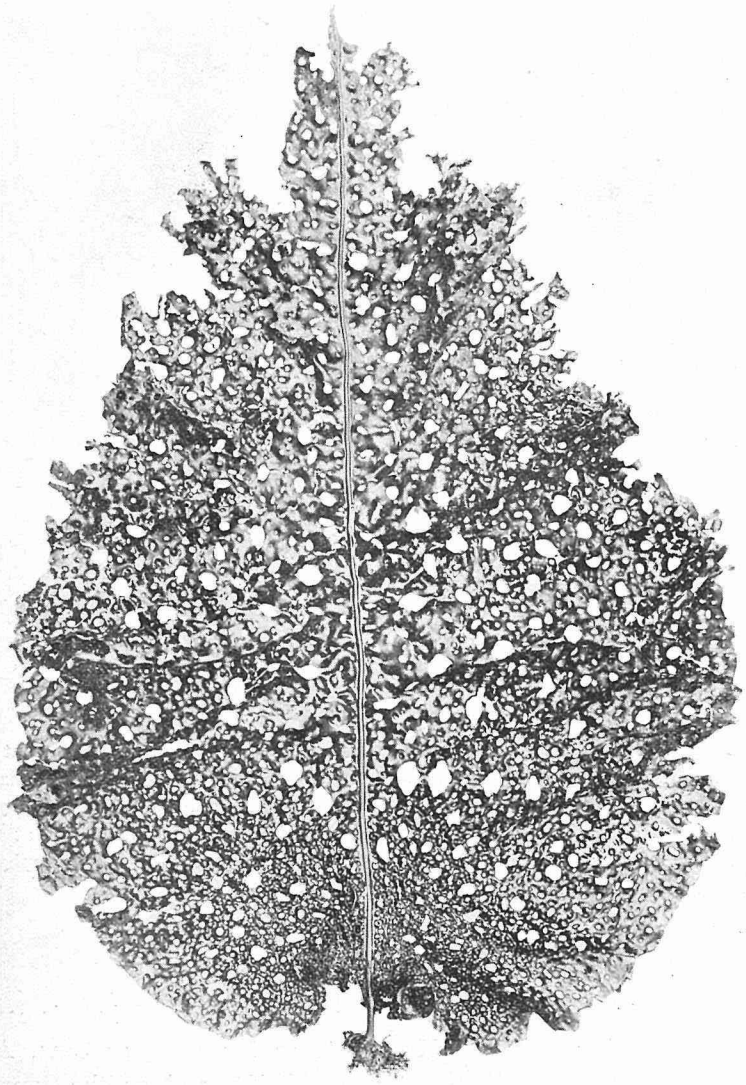


Fig. 6. *Agarum cribrosum* BORY. Habit of an old plant bearing zoosporangia; collected at Muroran in May.

The zoosporangia are produced at first in the hollows surrounding the perforations, or in the depressions on both surfaces of the blade, always leaving narrow borders along the perforations and both margins sterile. The sporophyte of *Agarum cribrosum* BORY is perennial, but the length

of time that it may live has not been observed exactly. A definite seasonal variation of the vegetative frond may not be recognized in this species. In early spring, from February to March, however, the second year blade begins its growth at the transition region between blade and stipe. The zoosporangia are produced on this newly developed part, while the old part remains sterile.

The material for the present investigation was collected on the coast of Muroran. Cultures were made twice, in February 1935 and in April 1939.

If the material is fully matured, the zoospores are liberated within an hour, but the liberation of zoospores is very poor when compared with

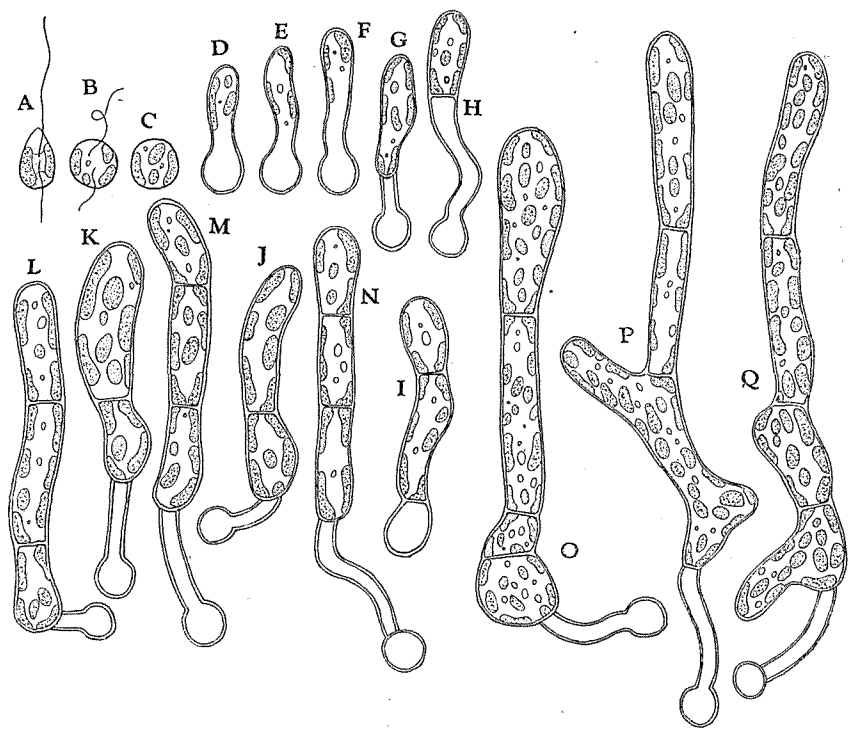


Fig. 7. *Agarum cribrosum* Bory. Zoospore and its germination. ×970. A. Zoospore in motile state. B. Resting Zoospore. C. Embryospore. D–F. Germination of the zoospore, from 6 day culture. G–H. Sporelings from 12 day culture; the contents of the original cell migrate into distal part of the tube. I–K. Sporelings from 24 day culture, showing the first cell division. L–N. Three celled gametophytes from 24 day culture. O–Q. Gametophytes from 30 day culture .

that of other Laminariaceous plants. 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 brown juice oozed out of the thalli. As was observed in *Eisenia* and *Ecklonia* the zoospores of *Agarum*

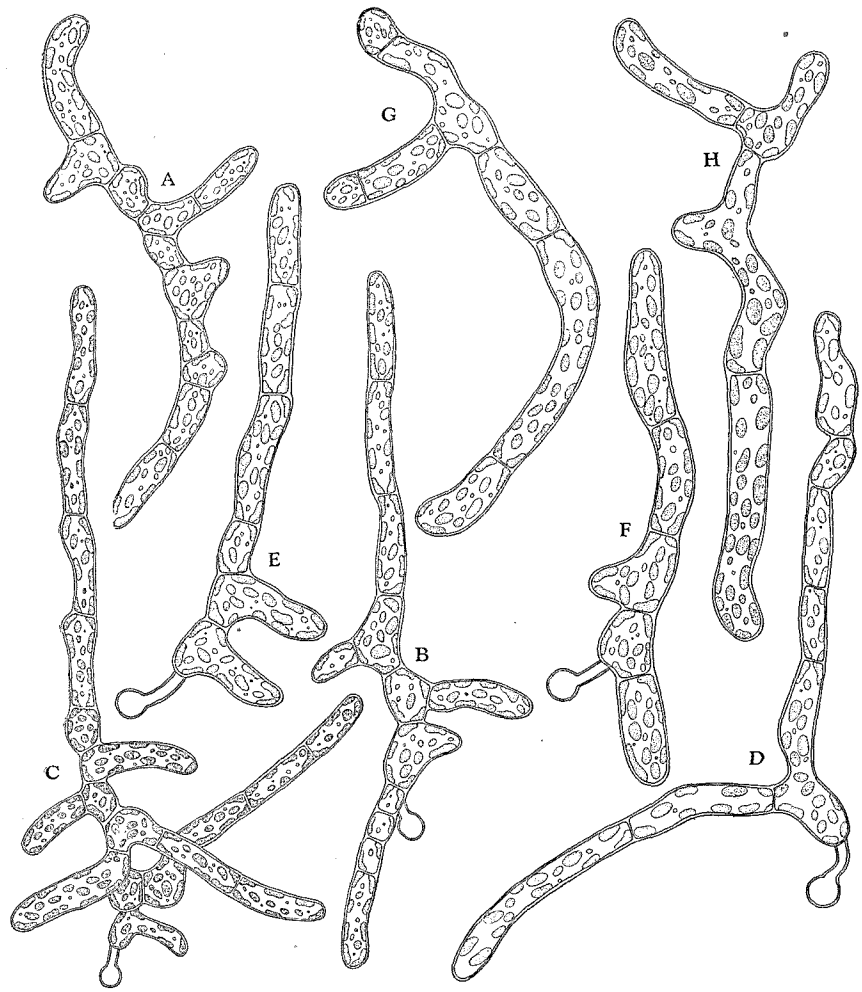


Fig. 8. *Agarum cribrosum* BORY. Development of the gametophytes from 20–42 day culture. A. Filamentous gametophyte with irregular branchlets. ×440. B. Irregularly branched gametophyte from 30 day culture. ×665. C. Ditto, from 42 day culture. ×440. D. Filamentous gametophyte from 20 day culture. ×665. E–F. Ditto, from 30 day culture. ×665. G–H. Ditto, from 20 day culture. ×665.

cribrosum Bory are easily poisoned by this brown juice, so that the treatment ought to be carried out as quickly as possible.

So far as the writer is aware, no cultural experiments of the zoospores of the genus *Agarum* have been made hitherto.

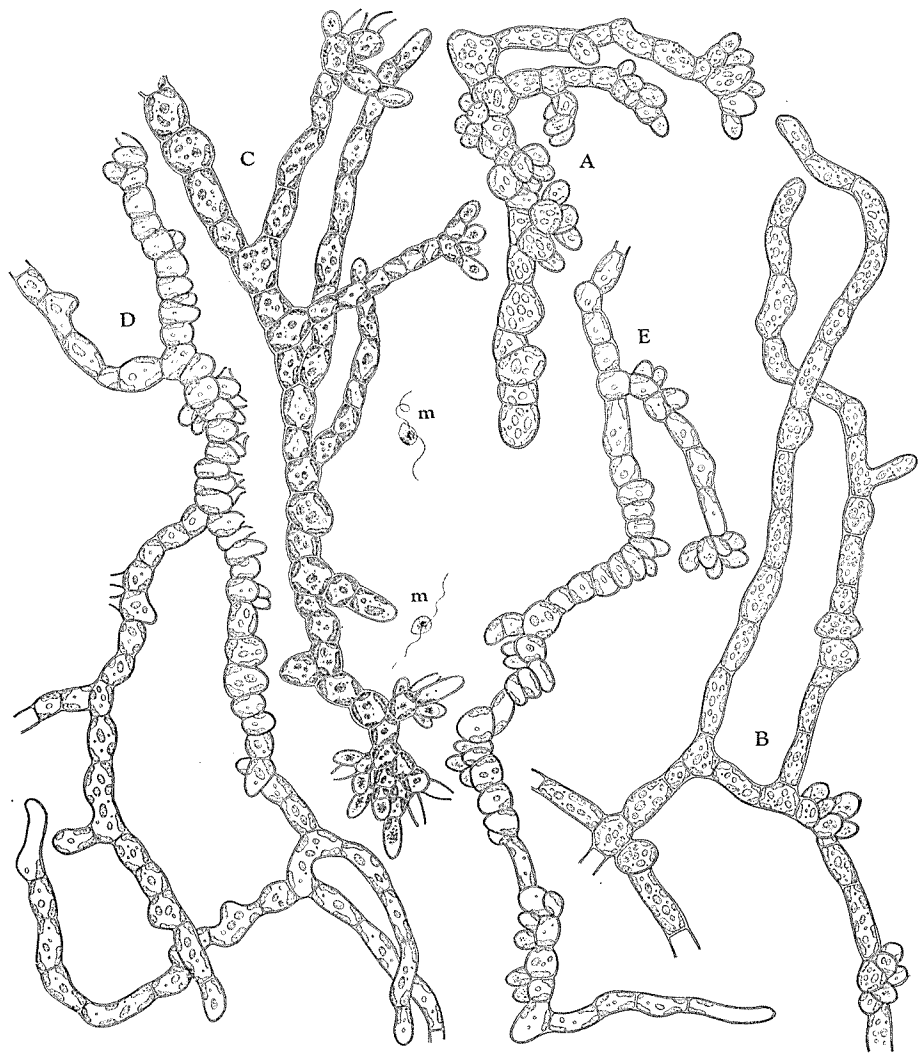


Fig. 9. *Agarum cribrosum* Bory. Various forms of the male gametophytes, with clusters of mature antheridia and empty beaks at the tips of the branches, from 30–40 day culture. m. Male gamete in motile state. ×544.

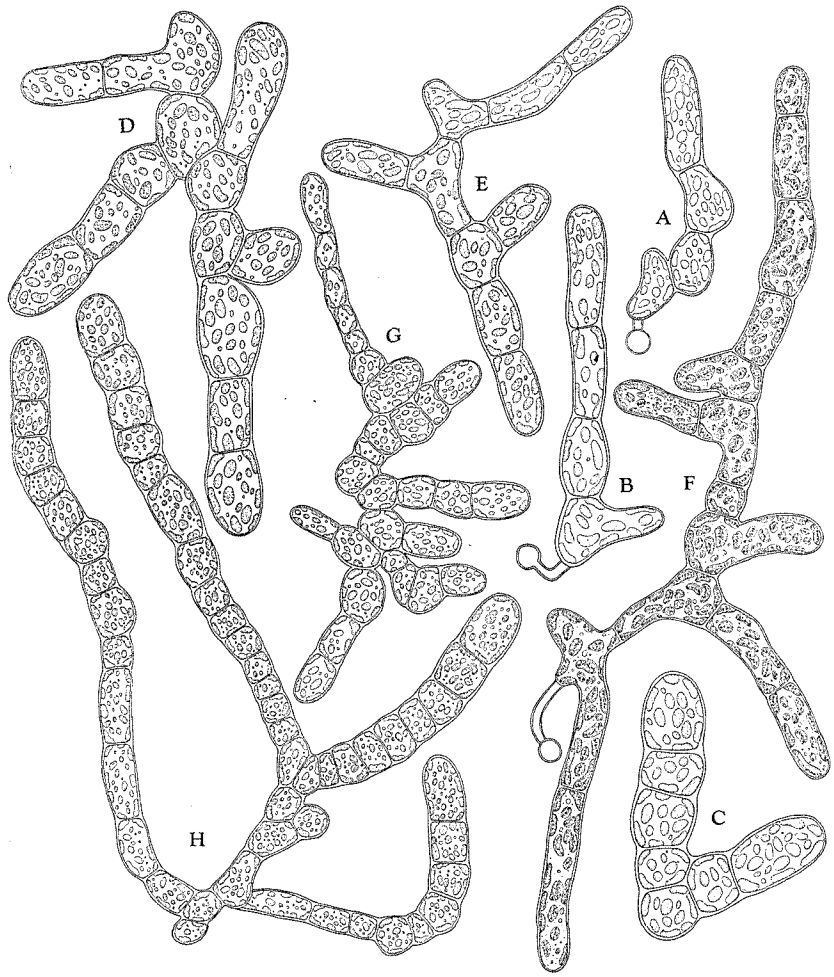


Fig. 10. *Agarum cribrosum* BORY. Various forms of the female gametophytes. A–B. Four celled stage of the female gametophytes, from 28 day culture. ×554. C–D. Well nourished female gametophytes consisting of many cells, from 40 day culture. ×554. E. Irregularly branched female gametophyte consisting of nine cells, from 34 day culture. ×554. F. Filamentous female gametophyte with branches. ×554. G–H. Well developed female gametophytes from 65 day culture. ×334.

Zoospores and their Germination

The zoospores are pear-shaped, about 8–9μ in length, have two laterally placed cilia, one curved chromatophore, one nucleus and some granular substances, but always lack an eye-spot (Fig. 7, 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. 7, B–C). Within 6 days after they have settled, a germination tube grows out from the spore (Fig. 7, D–F). The distal end of the tube swells up, and a transverse wall is formed so as to separate it from the tube (Fig. 7, G–H). As the culture progresses, this enlarged distal end gradually increases its length; within

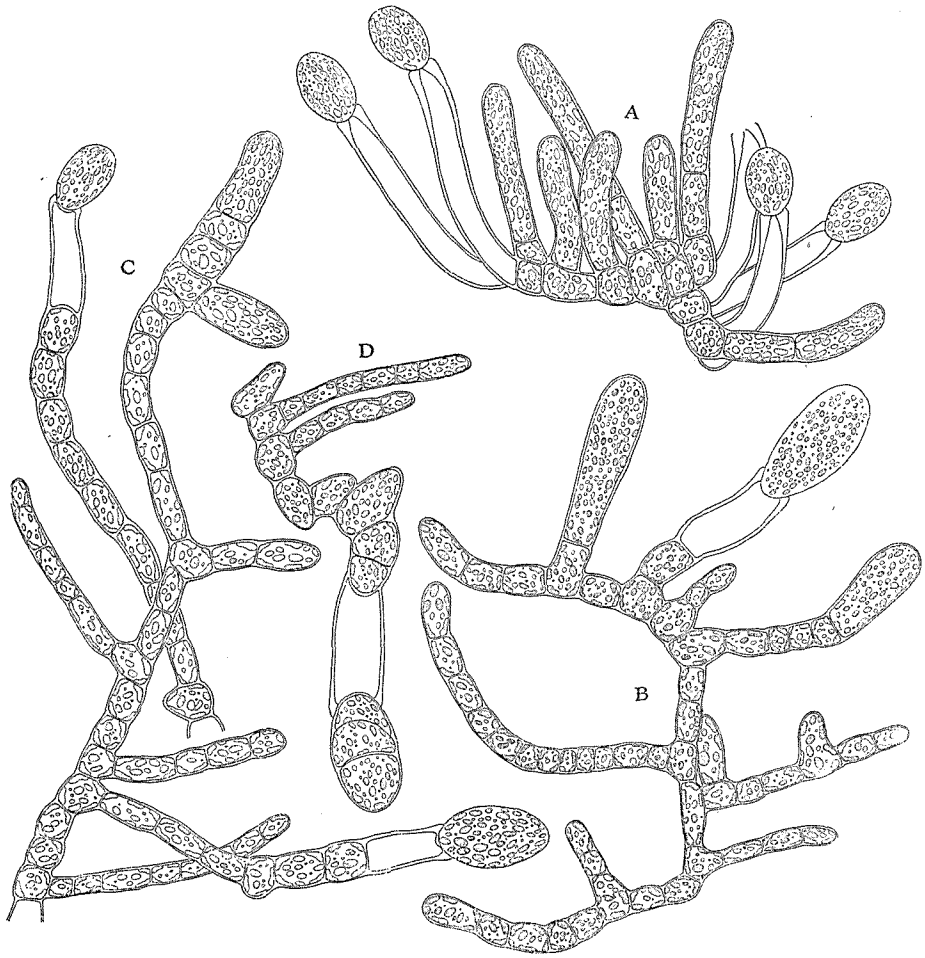


Fig. 11. *Agarum cribrosum* Bory. Female gametophytes, showing the formation of oogonia and egg-cells, and the development of the young sporophytes. ×312. A. Well developed female gametophytes with oogonia and egg-cells, from 40 day culture. B–C. Female gametophytes consisting of irregularly branched filament. D. Female gametophyte with young sporophyte.

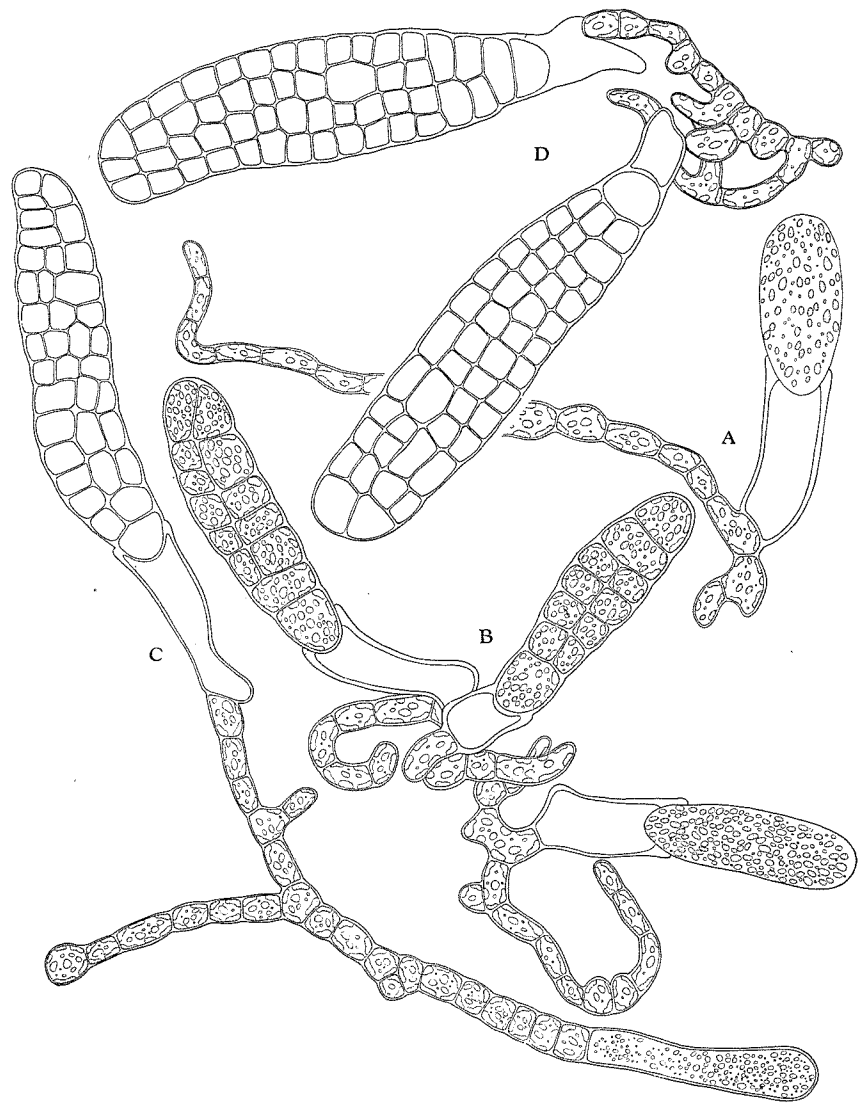


Fig. 12. *Agarum cribrosum* BORY. Female gametophytes and young sporophytes. ×320. A. An egg resting at the opening of the oogonium. The shape of the female gametophyte is very characteristic. B. Two young sporophytes and an egg cell developed from single female gamotophyte. C–D. Further development of young sporophytes, from 40 day culture.

24 days, the plants are divided into two or more cells by transverse walls, to give a filamentous appearance, the length of the single cell measuring up to 22μ, while the breadth of a cell does not exceed 8μ (Fig. 7, I–N). The general appearance of the gametophytes at this stage is very characteristic, and shows a strong resemblance to that of *Costaria costata* (TURN.) SAUND.

After this, the breadth of each cell gradually increases, and in the 30 day culture, the plants show loosely branched filaments consisting of several cells (Fig. 7, O–Q, Fig. 8). The differentiation of the two types of gametophytes may be recognized at this stage.

Male Gametophytes and Male Gametes

As the accompanying figures show, the shape and general appearance of the male gametophytes of this plant are very characteristic, and exceedingly different from those of *Laminaria, Eisenia, Ecklonia,* etc. They form an irregularly branched filament consisting of a large number of cells (Fig. 9). When the male gametophytes approach to maturity small cells are cut of at the apex of the branches, forming a cluster of antheridia (Fig. 9). At maturity the antherozoids are pressed out from the opening at the tips of the antheridia. They are pear-shaped, measuring about 4–5μ in length, have two laterally placed cilia of almost equal length, but neither chromatophore nor eye-spot is observed.

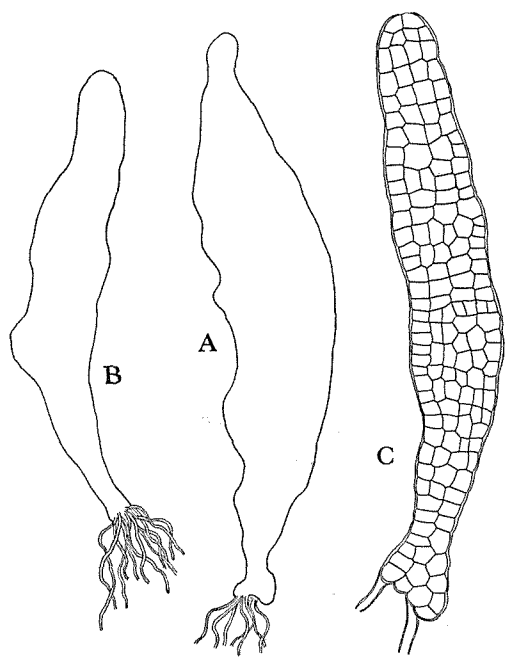


Fig. 13. *Agarum cribrosum* BORY. Development of the young sporophytes. A–B. Young sporophytes with numerous rhizoids. ×120. C. Ditto, from 40 day culture. ×230.

Female Gametophytes and Eggs

The shape and size of the female gametophytes as well as the diameter

of each cell of this plant is widely variable. Some consist of a rather small number of cells (Fig. 10, F–H, Fig. 11). When the female gametophyte is between 40–50 days old, some cells of the gametophyte metamorphose into an oogonium (Fig. 11). The chromatophores of the oogonium cell become small in size, and the contents become more liquefied, exerting strong internal pressure. At maturity the egg is pressed out through the opening, and remains attached there (Fig. 11).

Development of young Sporophyte

The first cell division of the fertilized egg-cell may be observed in the 40 days culture. (Fig. 11, D). The rhizoids make their appearance at the base of the sporophyte. A disc-shaped expansion develops at the base of the blade as in other cases. There are no special remarks to be offered on the development of the young sporophytes (Fig. 13).

Discussion and Summary

1. Most of the female gametophytes of *Laminaria angustata* KJELLM. are unicellular, and show some resemblance to those of *Laminaria Yendoana* MIYABE.

2. There are no special characteristics to be remarked on the shape and habit of the male gametophytes of *Laminaria angustata* KJELLM., when compared with those of other Laminariaceous plants.

3. In *Laminaria angustata* KJELLM. the length of time necessary for the maturation of both types of gametophytes is rather short; within 7–9 days after the liberation of zoospores, two types of gametophytes are distinguished, and within 14 days they reach maturity.

4. So far as the process of germination of zoospore, the shape and general appearance of the gametophytes are concerned, there exists a remarkable resemblance between *Agarum cribrosum* BORY and *Costaria costata* (TURN.) SAUND., especially the shape of the male gametophytes, having loosely branched filamentous structure gives a striking characteristic.

5. In *Agarum cribrosum* BORY, the differentiation of two types of gametophytes was observed in plants of 28 day culture.

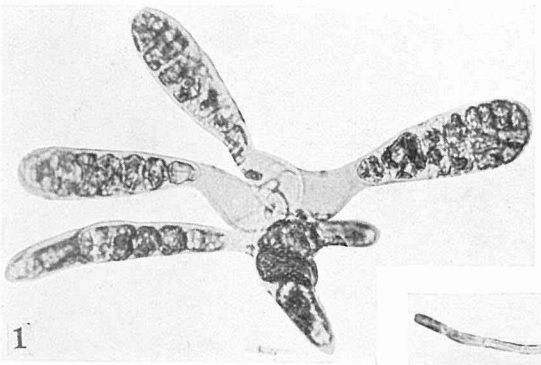
6. The zoospores, as well as the male gametes of both species studied in the present work always lack an eye-spot.

PLATE LIX

PLATE 59

Agarum cribrosum BORY.

1. Four young sporophytes and two oogonia, developed from single female gametophyte.
2. Female gametophytes with filamentous appearance, bearing oogonia.
3. Young sporophytes and matured oogonia.
4. Well developed female gametophyte with mature oogonia, and an egg, just discharged out of the oogonium.
5. Well developed male gametophyte with profusely branched filament. The habit and general aspect of the male gametophytes are very characteristic.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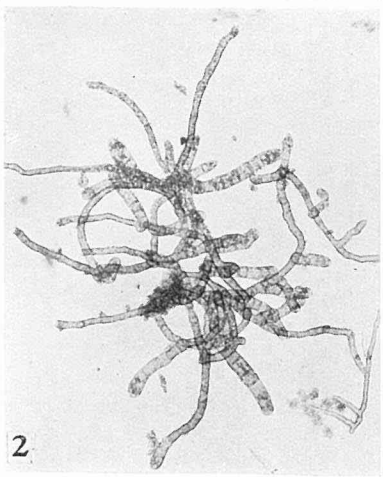
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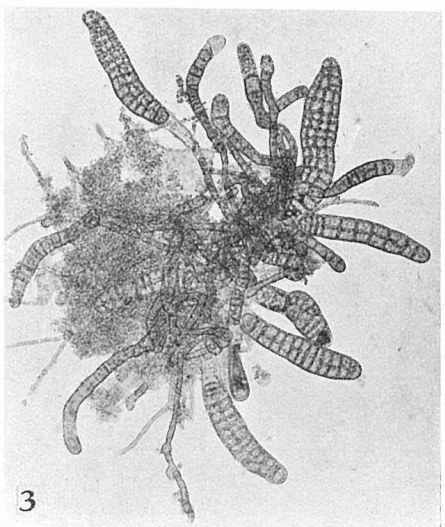
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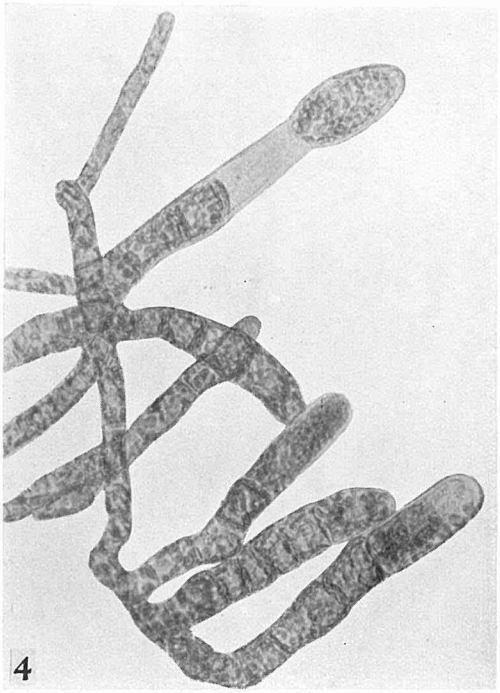
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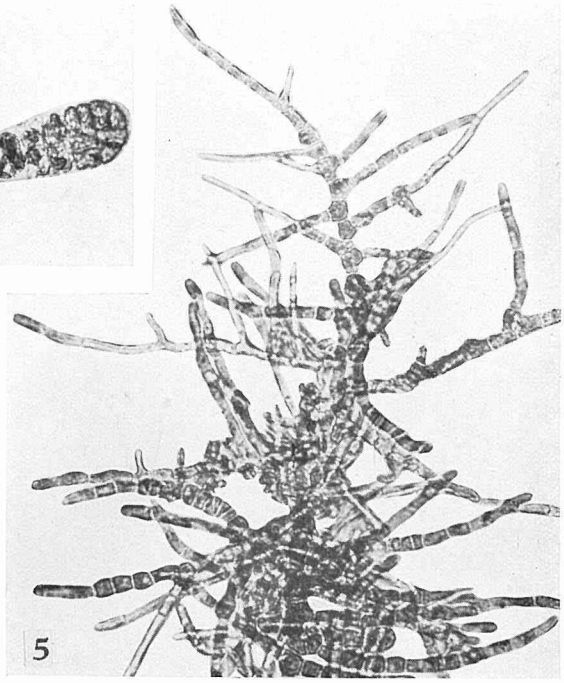
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