



Title	Culture Studies on the Life History of some Species of the Genus Monostroma
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Citation	北海道大學理學部海藻研究所歐文報告, 6(1), 1-56
Issue Date	1969-11
Doc URL	<a href="http://hdl.handle.net/2115/48095">http://hdl.handle.net/2115/48095</a>
Type	bulletin (article)
File Information	6(1)_1-56.pdf



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# Culture Studies on the Life History of some Species of the Genus *Monostroma*

By

MASAKAZU TATEWAKI

## Introduction

On the life history of *Monostroma* species, REINKE (1874), working on *M. bullosum*, first observed conjugation of biflagellate isogametes and development of zygotes. He reported that the zygote increased in size, developing into an enlarged spherical cell and after a certain resting period such a cell (Dauerspore) germinated by successive cell divisions, forming a hollow sphere which represented a young *Monostroma* plant. According to CHODAT (1894), however, the zygote of *M. bullosum* soon germinated without a resting period and developed into a prostrate system which gave rise to a *Monostroma* plant.

Since their observations, studies on the life history of *Monostroma* species have hitherto been carried out by many investigators. According to them, the life cycle of *Monostroma* species can be divided into the following five types.

1) Dimorphic alternation of a macroscopic gametophyte (*Monostroma* plant) and a microscopic unicellular sporophyte (cyst); the *Monostroma* plant produces gametes and the zygote develops into a unicellular cyst which forms a number of zoospores, each of which develops into the *Monostroma* plant (KUNIEDA, 1934; MOEWUS, 1938; YAMADA and SAITO, 1938; ARASAKI, 1946, 1949; KORNMAN, 1962; KIDA, 1966).

2) Dimorphic alternation of a *Monostroma* plant and a unicellular cyst, both of which are asexual; the *Monostroma* plant produces quadriflagellate swimmers which develop into unicellular cysts and the cyst forms quadriflagellate swimmers, each of which grows into the *Monostroma* plant. No sexual reproduction is found in this type of life cycle (YAMADA and SAITO, 1938; YOSHIDA, 1964; KORNMAN and SAHLING, 1962).

3) Dimorphic alternation of a macroscopic sporophyte (*Monostroma* plant) and a minute gametophyte (discoid thallus); the *Monostroma* plant produces zoospores which develop into minute discoid gametophytes. The gametophyte forms isogametes and the zygote grows into the *Monostroma* plant (YAMADA and TATEWAKI, 1959, 1965).

4) Monomorphic alternation of generations; the gametophyte and the sporo-

phyte are morphologically identical, and the zygote and the zoospore develop into the *Monostroma* plant (GAYRAL, 1962; DUBE, 1962; TATEWAKI, 1962, 1963).

5) No alternation of generations; the *Monostroma* plant produces bi- or quadriflagellate asexual swimmers and the swimmer develops directly into the *Monostroma* plant again. This type of life cycle by biflagellate swimmers has been reported by BLIDING (1935), IWAMOTO (1960), TATEWAKI (1962), KORNMAN and SAHLING (1962), KORNMAN (1964), GAYRAL (1964) and KIDA (1964), and by quadriflagellate swimmers by YAMADA and KANDA (1941), and KORNMAN and SAHLING (1962).

Regarding parthenogenesis, two types have been recorded as follows: a) the gamete of either sex develops into an enlarged cyst which forms a number of swimmers, each of which grows into the respective gametophyte (CARTER, 1926; ARASAKI, 1946 and 1949; KORNMAN, 1962), and b) the gamete of either sex develops directly into the respective gametophyte (MOEWUS, 1938; KORNMAN, 1962; GAYRAL, 1962; DUBE, 1962; TATEWAKI, 1962 and 1963).

Since 1957 the writer has been studying the life history of the Japanese species of the genus *Monostroma* under the guidance of Emer. Prof. Y. YAMADA and Prof. Y. NAKAMURA at the Institute of Algological Research, Faculty of Science, Hokkaido University at Muroran. The present work was carried out to complete the entire life cycle of the species of *Monostroma* by culturing them from generation to generation in the laboratory, and also to clarify in details the development and structure of the frond and reproductive organ. During the course of this work, the writer has reported new findings on the life histories of *M. fuscum* var. *splendens* (1962, 1963), *M. oxyspermum* (1962) and *M. zostericola* (1959, 1965).

This paper deals with the investigations on the life history of six species of *Monostroma* mainly from Hokkaido. It gives some observations on the growth and maturation of the plant in freezer-incubators permitting regulation of temperature and photoperiod, and also a discussion on the relationships between the patterns of life history and the characters of the frond structure in the materials examined.

### Acknowledgments

The writer wishes to express his heartfelt thanks to Emer. Prof. Y. YAMADA and Prof. Y. NAKAMURA who gave him very kind guidance and many facilities. The writer also expresses his gratitude to Dr. L. PROVASOLI, Haskins Laboratories, N. Y. for kind guidance and help in affording him many facilities when he was there from April 1961 to March 1963. The writer is indebted to the members of the Akkeshi Marine Biological Station, Faculty of Science, Hokkaido

University for help in the study of *M. groenlandicum*. The writer's thanks are also due to Drs. M. ANRAKU and M. ÔMORI for their kind help in collecting *M. oxyspermum*, while they were on the staff of the Woods Hole Oceanographic Institution, Woods Hole. A part of the expense of this work was borne by a grant in aid of Scientific Research from the Ministry of Education (no. 4084).

### Materials and Methods

The present investigation was carried out on the following six species of *Monostroma*; *M. groenlandicum* J. AGARDH, *M. undulatum* WITTROCK, *M. angicava* KJELLMAN, *M. zostericola* TILDEN, *M. oxyspermum* (KÜTZING) DOTY and *M. fuscum* (POSTELS and RUPRECHT) WITTROCK var. *splendens* (RUPRECHT) ROSENVINGE.

The collection data of fertile materials of these species are as follows:

Species	Locality	Date month/day
<i>M. groenlandicum</i>	Akkeshi, Hokkaido	6/6-6/8, 1959.
		6/6-6/10, 1963-1964.
<i>M. undulatum</i>	Muroran, Hokkaido	2/10-6/15, 1957-1960.
		3/2-3/24, 1964.
<i>M. angicava</i>	Muroran, Hokkaido	2/20-6/15, 1957-1960.
		3/2-4/13, 1964.
<i>M. zostericola</i>	Muroran, Hokkaido	1/8-6/20, 1957-1960.
		5/27-5/30, 1963.
		4/2-4/5, 1964.
<i>M. fuscum</i> var. <i>splendens</i>	Muroran, Hokkaido	5/14-6/5, 1959-1960.
		5/15-5/30, 1964.
<i>M. oxyspermum</i>	North Falmouth Massachusetts, U.S.A.	8/4-8/12, 12/3, 1961.
		4/22, 1962.

For the culture study, the collected fertile plants were rinsed with filtered seawater and placed in Petri dishes containing filtered or autoclaved seawater, each plant in a separate dish. Newly liberated swimmers showing a strong positive phototaxis were washed three or five times in filtered or autoclaved seawater by the micropipette method. After washing, the swimmers were placed on a glass slide with several drops of seawater and kept in a moist condition while swimming, usually for 30-60 minutes, often for about 1-3 hours. Then this slide was transferred to a glass vessel (6.5 cm × 8 cm.) containing 200 ml of medium. The swimmers which were apt to attach themselves to the substratum soon after liberation, were discharged from a piece of the mother plant directly on a glass slide. After rinsings with running seawater, this slide was transferred to the culture vessel.

Sexual reproduction was examined by conjugation tests. Small drops of seawater containing gametes from different individuals were placed on a glass slide with a micropipette and combined, examining the fusion of the gametes under a microscope. The zygotes could be easily detected by the two eyespots which were presented for a few days after conjugation. Most zygotes soon attached themselves to the glass slide, which was rinsed with running seawater to remove unfused gametes. After rinsings, the glass slide was re-examined microscopically and the remains of the gametes were removed with a fine glass needle or a micropipette.

To culture gametes parthenogenetically, they were usually introduced into a test tube (2 cm × 13 cm.) with a screw cap, containing 10 ml of medium after washing in serial baths with a micropipette.

For a unialgal culture, the 7–10-day old germlings attached to the glass slide were isolated with a micropipette and transferred to a test tube.

The following two media were employed in these studies; 1) SCHREIBER'S solution and 2) ESP medium.

1) SCHREIBER'S solution

Distilled water	50 ml
NaNO <sub>3</sub>	100 mg
Na <sub>2</sub> HPO <sub>4</sub>	20 mg
Filtered seawater	1000 ml

2) ESP medium (enriched seawater by PROVASOLI)

ES enrichment*	20 ml
Autoclaved seawater	1000 ml

\* 100 ml of ES enrichment contains

Distilled water	100 ml
NaNO <sub>3</sub>	350 mg
Na <sub>2</sub> glycerophosphate	50 mg
Fe (as EDTA, 1:1 molar)	2.5 mg
PII metals**	25 ml
Vitamin B <sub>12</sub>	10 μg
Thiamine HCl	0.5 mg
Biotin	5 μg
Tris buffer	500 mg
pH	7.8

\*\* One ml of PII metals contains: B (as H<sub>3</sub>BO<sub>3</sub>) 0.2 mg; Fe (as Cl) 0.01 mg; Mn (as Cl or SO<sub>4</sub>) 0.4 mg; Zn (as Cl or SO<sub>4</sub>) 0.005 mg; Co (as Cl or SO<sub>4</sub>) 0.001 mg; Na<sub>2</sub>-EDTA 1 mg.

ES enrichment in tubes (10 ml) or Erlenmeyer flasks (300–500 ml) was sterilized by the autoclave (*ca.* 120°C, 1 kg/cm<sup>2</sup>) for twenty minutes.

The culture media were replenished at intervals of 10–15 days in SCHREIBER's solution, and of 50–60 days in ESP medium.

Cultures were kept near the north-east window during 1957–1961 at room temperatures as shown in Figure 1. Since 1963 cultures were kept in freezer-incubators illuminated with cool white fluorescent lamps (*ca.* 1500–2500 lux), and

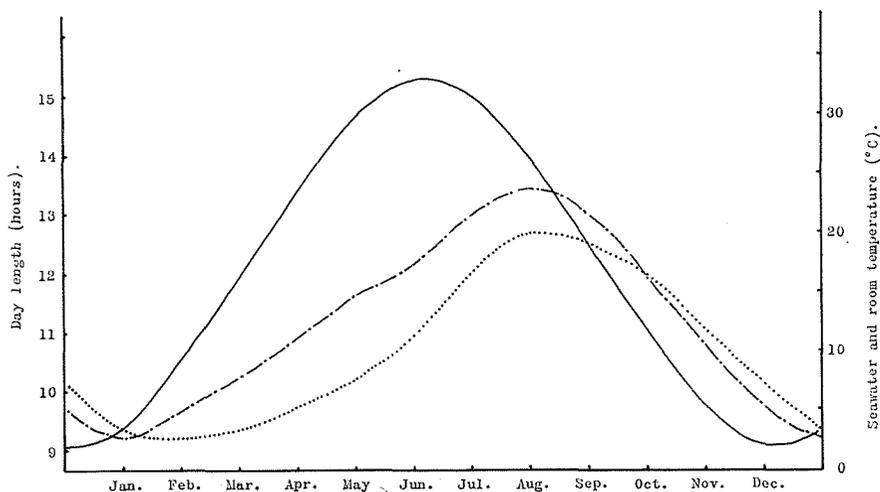


Fig. 1. Day length (—) at Muroran, surface seawater temperature (.....) at Charatsunai, Muroran and room temperature (— · — · —) in the laboratory where the cultures were grown. (Average of three years, 1958–1960)

the temperatures and photoperiods were regulated in the following combinations: 5°C. and 10 hours of light daily, 12°C. and 10 hours of light daily, 10°C. and 14 hours of light daily, and 14°C. and 14 hours of light daily.

Most figures and plates of the present paper were made from living materials. But those of the anatomical study of the thallus were made from materials preserved in formalin, when not otherwise notes. Several dyes, e. g., safranin, alum carmine, methyl green, methylene blue and others were used for staining of the cell wall to observe the liberation-pore of swarmers.

The observation and measurement of the swarmers were made on living materials as well as on materials fixed with formalin vapour for 5–10 seconds.

## Observations

*Monostroma groenlandicum* J. AGARDH

Figs. 2-5, Pls. I-III

## Habitat

*Monostroma groenlandicum* is found growing densely on rocks exposed to breakers in the upper littoral zone. The material was collected at Daikoku-jima and Aikkapu, Akkeshi, Hokkaido, in early June 1959, 1963 and 1964; it could not be found in summer, after late June.

## Frond structure

The frond is cylindrical or tubular, very slender at the base, slightly broadened upward up to about 1 mm. in diameter, attaining a height of 5-10 cm. (Pl. I, A). It is solid at first and later becomes hollow, but never opens out to form a monostromatic membrane as in other species of *Monostroma*. The frond often

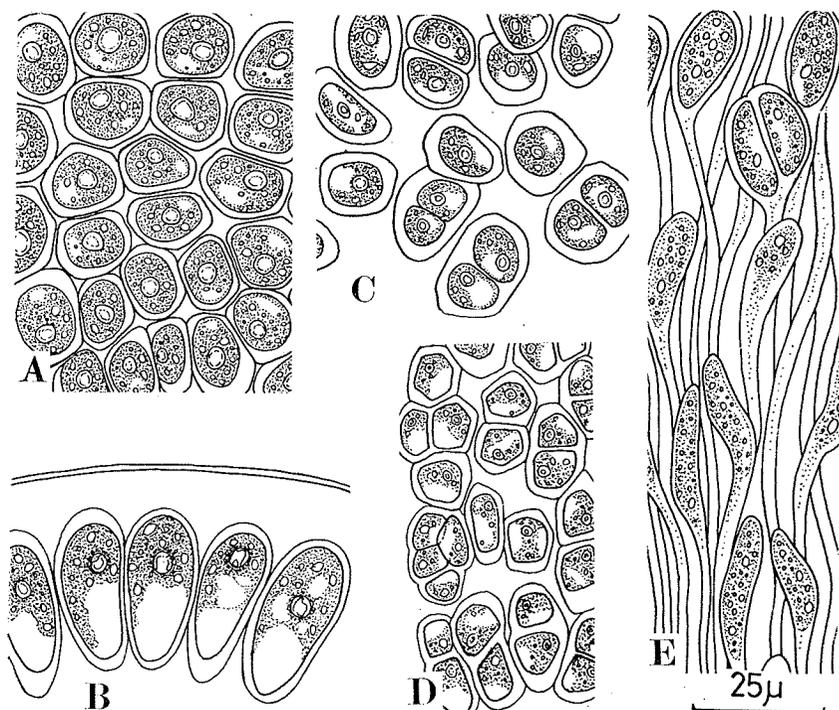


Fig. 2. *Monostroma groenlandicum* J. AGARDH

- A. Surface view of the middle part of a frond.
- B. Cross section of the middle part of a frond.
- C-D. Surface view of the lower part of a frond.
- E. Vertical section of the rhizoid-bearing part of a frond.

remains solid, even when it reaches maturity, being filled with a gelatinous substance in its central cavity (Pl. I, F-G).

The cells in surface view are round or roundish angular in shape, 10-18  $\mu$  in diameter in the upper and the middle part of the frond, and arranged loosely in groups of twos or fours in the material preserved in formalin (Fig. 2, A, C-D). The cells in cross section are obovate or elliptical, 24-37  $\mu$  high in the upper and the middle part of the frond, usually placed with their long axes at right angles to the surface of the frond, and enclosed within a gelatinous matrix (Pl. I, D-F). The cells contain a single parietal chloroplast with one pyrenoid.

### 1) **Cultures at room temperature**

Cultures of the zygotes were started on June 6-8, 1959, June 6, 1963 and June 10, 1964, at the Akkeshi Marine Biological Station, Faculty of Science, Hokkaido University, using SCHREIBER's solution and ESP medium as culture media. After a few days those grown on glass slides or in test tubes were carried back to the Institute of Algological Research at Muroran and were kept near the north-east window at room temperature.

#### Gametes and their movement

The cylindrical or tubular frond is a monoecious gametophyte. The gamete formation begins at the summit of the frond, extending gradually downwards. The fertile part of the frond can be easily distinguished by its yellowish-brown color.

The gametes are discharged from a gametangium as a mass enclosed within a hyaline sac (a thin gametangium membrane) through a pore formed on the surface wall of the frond (Fig. 3, C; Pl. II, C-D). After a few seconds outside of the pore, the gametes acquire motility inside the sac and swim away by breaking the membrane explosively. The liberation-pore is a slit which appears to be linear or somewhat crescent-shaped in surface view (Fig. 3, A; Pl. II, E). After liberation of the gametes the emptied colorless gametangia remains in situ.

The gametes are elongated pear-shaped or fusiform and provide with two flagella of equal length (11-13  $\mu$ ) at the anterior end. They contain a single chloroplast with one pyrenoid, and one eyespot (Fig. 3, D), showing a positive phototaxis. They vary widely in size, measuring 5.7-9.8  $\mu \times$  2.5-4.3  $\mu$  (average, 7.62  $\mu \times$  3.28  $\mu$ ). Conjugation may occur between a pair of the gametes of the same size or of different size and also between the gametes liberated from the same gametangium or from the same or different individuals. Consequently, it is difficult to determine the sex of the gametes. The gametes conjugate immediately after liberation side by side or end to end anteriorly and form zygotes (Fig. 3, E-F; Pl. II, F). After sexual fusion, the zygotes swim actively for about

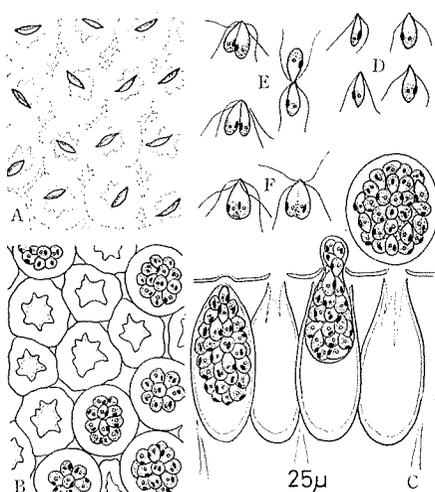


Fig. 3. *Monostroma groenlandicum*  
J. AGARDH

A. Surface view of emptied gametangia, showing liberation-pores on the surface wall of a frond. B. Surface view of the fertile part of a frond with emptied gametangia. C. Cross section of the fertile part of a frond, showing liberation of gametes. D. Gametes. E. Conjugation of gametes. F. Zygotes.

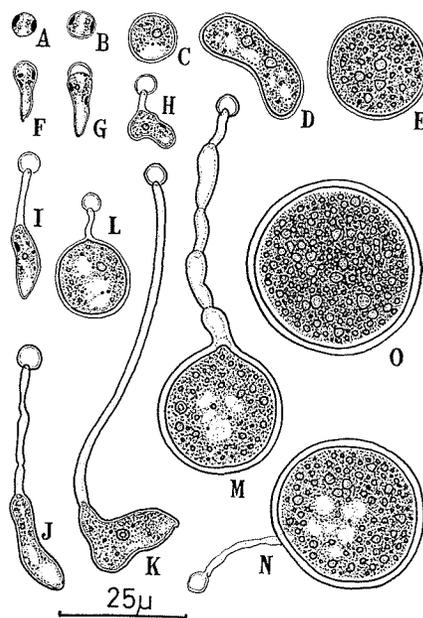


Fig. 4. *Monostroma groenlandicum*  
J. AGARDH

A-B. Settled zygotes. C-E. Further development of zygotes grown in SCHREIBER's solution. F-O. Further development of zygotes grown in ESP medium. F-K. Migration of cell contents. M-N. 20-day old cysts. O. 2-month old cyst.

10 minutes, showing a negative phototaxis and then settle down on the substratum, losing the flagella.

#### Zygotes and their development

The zygotes settled on the substratum are spherical, measuring  $4.5\text{--}6.1\ \mu$  in diameter, and soon form a wall (Fig. 4, A-B). They contain two chloroplasts with one pyrenoid in each and two eyespots. The eyespots disappear within 3-5 days.

The early development of the zygotes seems to be fairly changeable depending upon culture conditions, especially media. For example, in cultures grown in both seawater and SCHREIBER's solution, most zygotes immediately increase in size, taking a spherical or somewhat elongated elliptical shape and finally becoming spherical cysts (Fig. 4, C-E; Pl. II, C-H). In cultures grown in ESP medium, most of the zygotes produce a germination tube within 1-2 days after settlement

(Fig. 4, F-G), often attaining a length of 100-200  $\mu$  or more (Fig. 4, I-K) and the cell contents migrate completely into this tube. The distal end of this tube gradually becomes swollen and forms a new cell. The new cell increases in size, taking various shapes and finally develops into a spherical cyst (Fig. 4, L-M). The vestige of the germination tubes gradually degenerates but often remains in the 30-40-day old cysts.

From the end of July to September (room temperature ranging from 20° to 24°C.) the cysts did not show any remarkable change in size or contents. In October (15-18°C.) they began to enlarge and reached 30-40  $\mu$  in diameter at the end of November (5-7°C.). In December (3-5°C.) the contents of the cysts began to divide into 2-8 or more cells (Fig. 5, A-C; Pl. III, C) and eventually produced 4-16 or more aplanospores. It was expected that these daughter cells would become zoospores and would swim out of the cyst, but no zoospores could be found.

#### Aplanospores and their development

From January to February, each aplanospore germinated within the cyst, taking an elongated shape and grew into a germling, breaking through the cyst wall (Fig. 5, E-F). By successive transverse cell divisions, the germling developed into an erect uniseriate filament consisting of a number of cells, the lowest cell

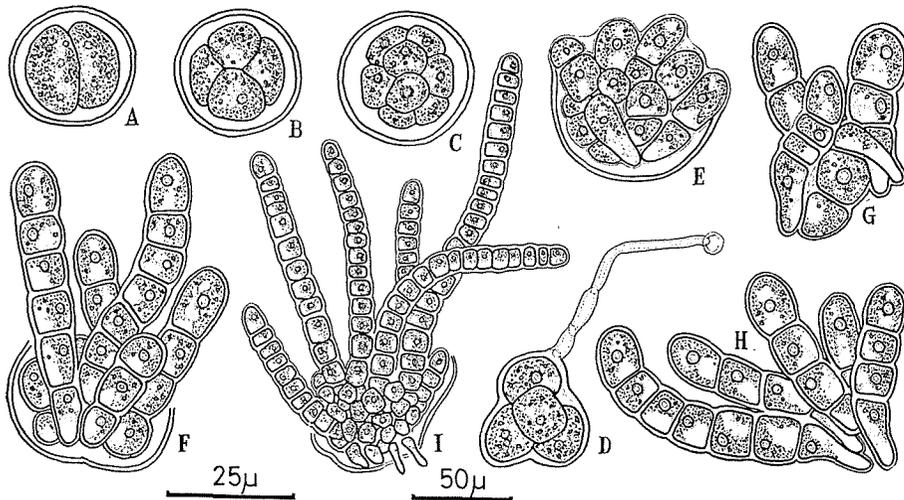


Fig. 5. *Monostroma groenlandicum* J. AGARDH

A-D. Formation of aplanospores. A-C. 6-month old cysts grown in SCHREIBER's solution at room temperature. D. 25-day old cyst grown in ESP medium at 5°C. in a 10-hr photoperiod. E-I. Germination and development of aplanospores. G-H. Germlings grown in ESP medium at 5°C. in a 10-hr photoperiod.

A-H drawn to 25  $\mu$  scale. I drawn to 50  $\mu$  scale.

of which elongated and formed a primary rhizoid. Thus 4-16 or more erect filaments are issued from a single cyst (Fig. 5, I).

In March (7-10°C. or more) these uniseriate filaments developed into cylindrical fronds by successive transverse and longitudinal cell divisions. At the beginning of April, the fronds reached maturity and produced biflagellate gametes.

As mentioned above, the plant of *M. groenlandicum* is a monoecious gametophyte which forms isogametes. After sexual fusion, the zygote develops into a unicellular sporophyte (cyst) which produces 4-16 or more aplanospores. The aplanospore develops into a cylindrical gametophyte. Thus, *M. groenlandicum* has a dimorphic alternation of generations between the macroscopic cylindrical gametophyte and the microscopic unicellular sporophyte. In this culture experiment, the life cycle completed in about 10 months.

## 2) Cultures in freezer-incubators

Since 1963, cultures grown in ESP medium were maintained in the freezer-incubators at the following temperatures and photoperiods: 5°C. and 10 hours daily, 10°C. and 14 hours daily, and 14 °C. and 14 hours daily, and they have been grown from generation to generation.

When maintained at 5°C. in a 10-hr photoperiod, the zygotes increased in size and became unicellular thin-walled cysts, attaining about 20  $\mu$  in diameter in 20 days. In 25-30 days the cysts divided into 4-(8) cells, each of which developed into an aplanospore (Fig. 5, D). In 35-day old cultures, most of the aplanospores germinated and developed into erect uniseriate filaments (Fig. 5, G-H), but these filamentous germlings did not grow well; in about 3 months, a few cylindrical immature fronds were seen in some cultures. However, the aplanospores formed at 5°C. in a 10-hr photoperiod, grew well into cylindrical fronds when they were transferred to conditions of 10°C. and a 14-hr photoperiod. In 20 days after transfer, these fronds attained a height of 1-3 cm. and reached maturity, producing gametes.

When the zygotes were grown at 10°C. or 14°C. in a 14-hr photoperiod, they increased enormously in size, measuring 20-25  $\mu$  in diameter in 15 days, 30-37  $\mu$  in 30 days, and 50-60  $\mu$  in 60 days. They finally formed thick-walled cysts, but their contents did not show any remarkable change. However, when the 15-day old cysts which attained 20-25  $\mu$  in diameter, were transferred to conditions of 5°C. and a 10-hr photoperiod, they divided into 4-8 cells within 7 days after transfer, each of which developed into an aplanospore. These aplanospores germinated and developed into cylindrical fronds when they transferred to conditions of 10°C. and a 14-hr photoperiod, as mentioned above.

The entire life cycle of *M. groenlandicum* can be completed in 40-50 days in

culture as follows. Under conditions of 14°C. and a 14-hr photoperiod, the zygote forms a unicellular cyst in about 15 days. At 5°C. in a 10-hr photoperiod, the cyst divides into 4-8 cells and produces aplanospores within 7 days after transfer. At 10°C. in a 14-hr photoperiod, these aplanospores soon germinate and develop into the fertile cylindrical frond within 20 days.

#### Parthenogenesis

It is difficult to isolate unfused gametes of this alga, because the sexual plant is monoecious and the conjugation occurs indiscriminately even between a pair of the gametes derived from one and the same gametangium. In order to obtain unfused gametes, small quantities of gametes were pipetted in a drop of seawater on a glass slide immediately after liberation and kept in a moist and dark condition for several hours. Though most of the gametes conjugated and formed zygotes, some unfused gametes were found attached to the glass slide. These unfused gametes could be easily distinguished from the zygotes by a single eyespot. The zygotes were removed with a fine glass needle under a microscope and only the gametes were left on the glass slide.

These gametes were at first cultured at 14°C. in a 14-hr photoperiod for one month, and then were transferred to conditions of 5°C. and a 10-hr photoperiod. They developed parthenogenetically into cysts in the same way as the zygotes and the cysts attained a diameter of 30-38  $\mu$  in 2 months after transfer, but most of them bleached.

### *Monostroma undulatum* WITTROCK

Figs. 6-7, Pls. IV-VI

#### Habitat

*Monostroma undulatum* is found very commonly in the vicinity of Muroran, growing on other algae, such as *Gloiopeltis furcata*, *Pelvetia wrightii*, *Sargassum thunbergii*, etc., in the littoral zone. This alga occurs at first in the middle of December and begins to become fertile in early February of the next year. It thrives most luxuriantly from March to May and almost disappears at the end of June.

#### Frond structure

The frond is lanceolate, linear or somewhat ovate with an elegantly ruffled margin, attaining a height of 10-20 cm., often up to 40 cm. (Pl. IV, A-C). It is flaccid and very delicate, and light green. The thickness of the frond measures 12-18  $\mu$  in the marginal and the upper part, 30-37  $\mu$  in the middle, 34-42  $\mu$  in the lower, and up to 56  $\mu$  in the rhizoid-bearing portion.

The cells in surface view are quadrate or roundish quadrate in shape,

measuring 7.6–15.2  $\mu$  in diameter in the marginal and the upper part of the frond, rectangular with rounded angles, measuring 15–40–(150)  $\mu \times$  12–18  $\mu$  in the middle and the lower part (Pl. IV, D–G). They are disposed in groups of twos or fours in the material preserved in formalin. The cells in cross section are roundish quadrate or roundish in shape (Pl. IV, I), measuring 10–15  $\mu$  high in the marginal and the upper part of the frond, 22–26  $\mu$  in the middle, and often broader than high in the lower. The cells contain a single chloroplast with one pyrenoid.

### 1) Cultures at room temperature

Cultures of the swimmers from the leafy frond were begun on April 30 and May 1, 1957, March 22 and 30, 1958, and March 2, 1964. Those of the swimmers from the cysts were started in December, 1957, January, 1958 and 1959. Cultures grown in SCHREIBER's solution were kept near the north-east window at room temperature.

#### Swimmers from the leafy frond and their movement

The formation of swimmers first occurs in the upper margin of the frond and extends gradually downwards. The fertile part of the frond changes to yellowish-green or yellowish-white and becomes gelatinous, gradually loosening the arrangement of sporangia (Pl. V, A–B). The fertile part disintegrates into pieces which usually consist of small groups of sporangia floating on the surface of water. Each detached sporangium breaks and swimmers are liberated in a group (Pl. V, C–E).

The swimmers are pear-shaped, elongated pear-shaped or fusiform, measuring 6.0–11.4  $\mu \times$  2.7–5.3  $\mu$ . They have four (rarely two to three) flagella of equal length (7.5–10.6  $\mu$ ) at the anterior end, and a single chloroplast with one pyrenoid (Fig. 6, A–B; Pl. V, G). They have no eyespot and show no phototaxis.

After liberation, the swimmers do not swim away freely, but they form an oscillating mass being attached to each other by their posterior ends (Fig. 6, A; Pl. V, F). However, no evidence of conjugation is found among the swimmers. After oscillating for 10–20 minutes, sometimes even for 2–4 hours, they swim away freely and soon settle down on the substratum, losing the flagella, as described by YAMADA and SAITO (under the name of *M. pulchrum*) and TOKIDA.

#### Development of swimmers from the leafy frond

The settled swimmers become spherical and soon form a wall, measuring 4.5–7.0  $\mu$  in diameter (Fig. 6, C–D). Within 2–3 days they form a germination tube (Fig. 6, E–G), into which the cell contents migrate completely (Fig. 6, H). The distal end of the germination tube becomes swollen to form a new cell (Fig. 6, I; Pl. V, H), in which a single pyrenoid appears clearly in a week. The vestige

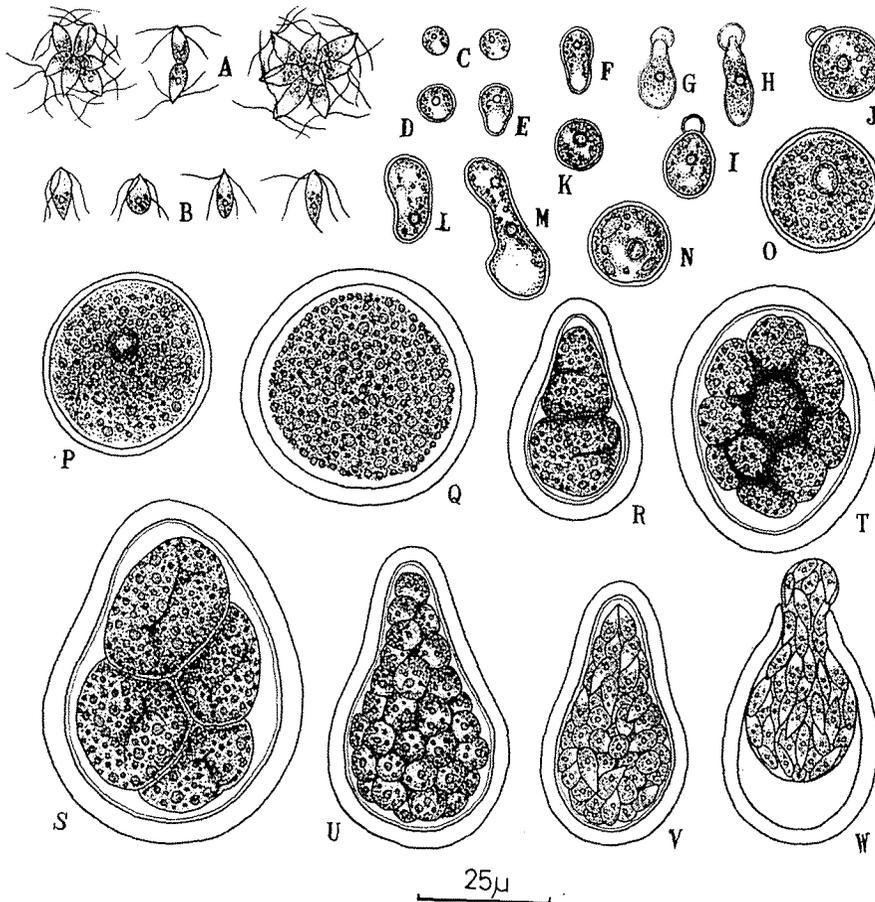


Fig. 6. *Monostroma undulatum* WITTROCK

A. Swarmer cells liberated from the frond. B. Swarmer cells from the same. C-D. Settled swarmer cells. E-F. Germination of swarmer cells. G-H. Migration of cell contents. I. 8-day old germling. J. 10-day old germling. K-M. 8-day old germlings without migration of cell contents, taking various shapes. N-W. Further development of swarmer cells from the frond grown in SCHREIBER'S solution at room temperature. N. 12-day old germling. O. 25-day old germling. P. One-month old cyst. Q. 3-month old cyst. R-V. Formation of swarmer cells. W. Liberation of swarmer cells from a cyst.

of the original cell gradually degenerates, though it often persists even in 10-15-day old cultures. In some cultures, however, the settled swarmer cells increase enormously in size without formation of the germination tube, and sometimes they take various shapes, becoming elliptical, gourd- or pear-shaped (Fig. 6, K-N;

Pl. V, I).

The further development of the two types of germlings is quite the same. In one month, most of them are spherical or somewhat elliptical in shape and measure 25–40  $\mu$  in diameter, forming cysts (Fig. 6, O–P; Pl. VI, A). These cysts are light green color, full of starch and have a large pyrenoid in the central part. In about 3 months they attain a diameter of 45–60  $\mu$  (Fig. 6, Q; Pl. VI, B). The size of cysts, however, seems to be fairly changeable according to culture conditions and also individuals.

From July to October (room temperature ranging from 18 to 24°C.), the cysts showed no remarkable change in size or contents. From November to December (5–10°C.) the contents of the cysts became somewhat yellowish-green in color and divided into 2–8 or more cells. In this stage most of the cysts are pear-shaped or obovoid in shape (Fig. 6, R–T). In the middle of December the cysts developed into sporangia, forming 32–64 or more swarmers (Fig. 6, U–V; Pl. VI, C). The wall of fertile cysts splits at the tip. The swarmers usually slip out through the split as a mass enclosed in a thin membrane (Fig. 6, W). Then the swarmers acquire motility and soon swim away, breaking the membrane. Sometimes they are liberated through the split as a group of cells without enclosing in the membrane. The formation of swarmers in the cyst occurs during the winter (3–5°C.).

#### Swarmers from the cyst and their development

After liberation from the cyst, the swarmers usually oscillate for a while, attaching to each other by their posterior ends in the same way as the swarmers liberated from the frond (Fig. 7, A). These swarmers soon become free from the group and swim away. They are more active than the swarmers from the frond in movement, but both are quite the same in structure. The swarmers from the cyst are elongated pear-shaped or fusiform, measuring 5.6–9.8  $\mu \times 2.8$ –5.6  $\mu$ . They have four flagella of equal length (10–16  $\mu$ ) at the anterior end, and a single chloroplast with one pyrenoid but no eyespot (Fig. 7, B). After swimming for 30–60 minutes, they settle down on the substratum, losing the flagella. Settled swarmers become spherical and soon form a wall, measuring 4.5–7.0  $\mu$  in diameter (Fig. 7, C–D).

In about 10-day old cultures, the settled swarmers begin to germinate by pushing out a protuberance and then they divide into two cells with a transverse wall, one of which elongates and forms a primary rhizoid (Fig. 7, G–J). The other cell becomes an erect uniseriate filament of 3–6 cells by successive transverse cell divisions (Fig. 7, K–M). In 20–25 days, longitudinal cell divisions begin to take place in the cells of the erect filament, and secondary rhizoids are given off from the basal cells (Fig. 7, O; Pl. VI, D–F). In 40 days, the germlings develop into monostromatci fronds (Fig. 7, Q), attaining a height of about 0.5 cm. in about 2

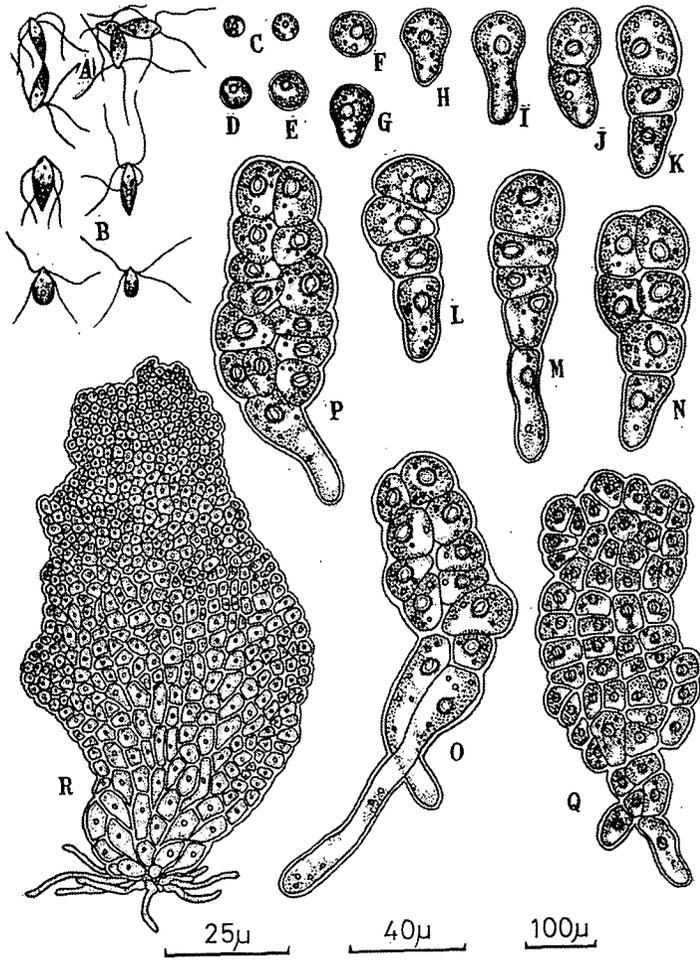


Fig. 7. *Monostroma undulatum* WITTRÖCK

A. Swarmer liberated from the cyst. B. Swarmer from the same. C-F. Settled swarmer. F. 7-day old culture. G-I. Germination of swarmer. J-R. Development of swarmer from the cyst grown in SCHREIBER's solution at room temperature. K-L. 18-day old germling. M-P. 25-day old germling. Q. 40-day old germling. R. Young monostromatic frond from 2-month old culture.

A-P drawn to 25  $\mu$  scale. Q drawn to 40  $\mu$  scale.  
R drawn to 100  $\mu$  scale.

months (Fig. 7, R; Pl. VI, I). The 3-month old plants reached maturity, producing swarmers.

As mentioned above, in *M. undulatum*, the quadriflagellate swarmers liberated from a leafy frond develop into unicellular cysts which produce quadriflagellate swarmers. The swarmer from the cyst germinates and develops into a monostromatic leafy frond which forms quadriflagellate swarmers. No sexual generation is found, but there are two alternating asexual generations which are quite different morphologically. In this culture experiment, the life cycle completed in 11–12 months.

## 2) Cultures in freezer-incubators

Since 1964, cultures grown in ESP medium were maintained in the freezer-incubators as the following temperatures and photoperiods: 5°C. and 10 hours daily, 10°C. and 14 hours daily, and 14°C. and 14 hours daily, and they have been grown from generation to generation.

When the swarmers from the leafy frond were cultivated at 5°C. in a 10-hr photoperiod, they increased in size and grew into thin-walled cysts, measuring 30–40  $\mu$  or more in diameter in 30 days. In 40 days the cysts divided into 2–8 or more cells and reached maturity, producing swarmers within 50 days. These swarmers from the cyst germinated and grew into monostromatic leafy fronds attaining a height of 0.5–1.0 cm. in 30 days (Pl. VI, D–H). The leafy fronds reached maturity and formed swarmers within 40–50 days after germination. Under these culture conditions the entire life cycle completed within 100 days.

When the swarmers from the leafy frond were cultivated at 10°C. or 14°C. in a 14-hr photoperiod, they increased enormously in size and became thick-walled cysts. In 30 days the cysts measured (45)–56–76  $\mu$  in diameter and in 60 days, 75–90  $\mu$ . But they did not become fertile under these culture conditions. These cysts, however, became fertile when they were placed at 5°C. in a 10-hr photoperiod. For example, the cysts which were grown at 14°C. in a 14-hr photoperiod for 20 days (about 40–56  $\mu$  in diameter), reached maturity and produced 32–64 swarmers within 20 days after transfer to conditions of 5°C. and a 10-hr photoperiod.

The swarmers from the cyst obtained at 5°C in a 10-hr photoperiod, did not grow into a normal leafy frond when cultured at 14°C. in a 14-hr photoperiod. However, when transferred to conditions of 10°C. and a 14-hr photoperiod, they developed into monostromatic leafy fronds, attaining a height of 0.5 cm. in 35–40 days, but they did not mature.

The results of this experiment show that both generations, cysts and leafy fronds, grow well in the freezer-incubator when the temperature is 5°C. and the

photoperiod is 10 hours.

***Monostroma oxyspermum* (KÜTZING) DOTY**

Fig. 8, Pls. VII-VIII

Habitat

*Monostroma oxyspermum* was collected at one of the marshes of Buzzards Bay, North Falmouth, Massachusetts, U.S.A. on August 4 and 15, December 3, 1961, and on April 22, 1962. This alga is found growing densely on stones and roots of grasses in a small brook which is running down from a freshwater pond but immersed in seawater at high tide.

FronD structure

The frond is saccate when young but soon opens intercellularly at the tip, gradually expanding into a monostromatic membrane. The expanded frond is somewhat fan-shaped or elongated with a flat or an irregularly ruffled margin, attaining a height of 2-15 cm. The frond measures 8-15 cm. high in April and 2-5 cm. in August and December. It is soft and light green. The thickness of the frond is 20-26  $\mu$  in the marginal and the middle part, and up to 40-56  $\mu$  in the lower and the rhizoid-bearing portion (Pl. VII, G-I).

The cells in surface view are 4-6-cornered or somewhat roundish in shape, 10-18  $\mu$  up to 25  $\mu$  diam., in the marginal and the middle part of the frond, and are disposed usually in groups of twos or fours, but without any definite order in the material preserved in formalin (Pl. VII, C-D). The cells in cross section are roundish rectangular or ovate, 12-15  $\mu$  high in the marginal and the middle part of the frond, and contain a single parietal chloroplast with one pyrenoid.

**Cultures in freezer-incubators**

Cultures were begun on December 4, 1961 and April 24, 1962, at the Haskins Laboratories, New York. The cultures grown in ESP medium were kept in a freezer-incubator illuminated with fluorescent lamps and regulated at 14-15°C. and a 14-hr photoperiod. Since 1963, the cultures have been kept in the freezer-incubators regulated at 10°C. or 14°C. and a 14-hr photoperiod at Muroran, and they have been grown from generation to generation.

Swarmers and their movement

The formation of swarmers begins in the upper margin of the frond and extends gradually downwards. The fertile part of the frond can be easily distinguishable by its yellowish-brown color. This part usually becomes gelatinous, consisting of loosely arranged cells, and finally disintegrates into pieces composed of a few sporangia. The swarmers come out in groups, rupturing the sporangia

and soon swim away (Pl. VIII, A). Each sporangium produces 16–32 biflagellate swimmers.

The swimmers are elongated pear-shaped, measuring  $5.4\text{--}8.3\ \mu \times 2.4\text{--}4.7\ \mu$  (average,  $7.3\ \mu \times 3.06\ \mu$ ). They have two flagella of equal length ( $10.8\text{--}13.5\ \mu$ ) at the anterior end, a single chloroplast with one pyrenoid, and one eyespot (Fig. 8, A; Pl. VIII, B). They show no sexual fusion nor strong positive phototaxis (slightly positive). After swimming for 30–(60) minutes, they show a negative phototaxis and settle down on the substratum, losing the flagella.

#### Development of swimmers

The settled swimmers become spherical and soon form a wall, measuring  $3.5\text{--}5.2\ \mu$  in diameter (Fig. 8, B–C). In 2–3 days they germinate by pushing out a protuberance (Fig. 8, D–F) and the eyespot becomes undetectable in most germ-

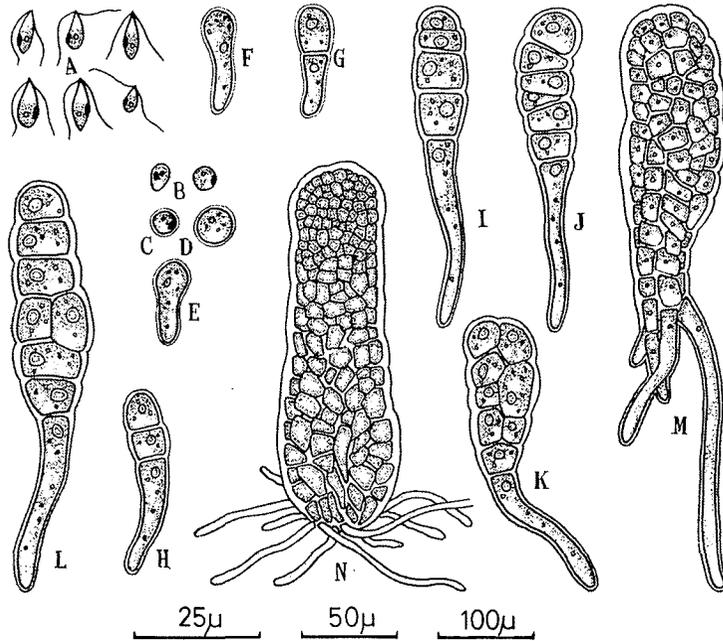


Fig. 8. *Monostroma oxyspermum* (KÜTZ.) DOTY

A. Swimmers. B–D. Settled swimmers. E–F. Germination of swimmers. G–N. Development of germlings grown in ESP medium at  $14^{\circ}\text{C}$ . in a 14-hr photoperiod. H. 7-day old germling. I–J. 10-day old germlings. K–L. 14-day old germlings. M–N. Young saccate fronds from 20-day old culture.

A–L drawn to  $25\ \mu$  scale. M drawn to  $50\ \mu$  scale.

N drawn to  $100\ \mu$  scale.

lings at this stage. In 5-6 days the germlings divide into two cells transversally, one of which forms a primary rhizoid (Fig. 8, G). The other cell develops into an erect uniseriate filament consisting of 3-7 cells by successive transverse divisions (Fig. 8, H-J). In 14-15 days longitudinal divisions occur in the cells of the erect filament (Fig. 8, K-L) and the secondary rhizoid is given off from the basal cells. The erect filaments become cylindrical and soon develop into saccate fronds (Fig. 8, M-N; Pl. VIII, C-D). In 25-30 days the saccate fronds disintegrate to open intercellularly at the tip, forming a funnel structure (Pl. VIII, E-G) and then develop into an expanded monostromatic membrane.

In 40-50 days the fronds attain a height of about 1-2 cm. and reach maturity (Pl. VIII, H), producing biflagellate swarmers. The swarmers never conjugate and soon germinate, developing into the *Monostroma* plants. No sexual reproduction is found in *M. oxyspermum*; the plant produces only biflagellate asexual swarmers which develop directly into monostromatic membranous fronds identical to the mother plant.

***Monostroma angicava* KJELLMAN**

Fig. 9-11, Pls. IX-XII

Habitat

In the vicinity of Muroran, *Monostroma angicava* is found growing on rocks and other algae in the middle littoral and the lower littoral zone. This alga appears in the middle of December and becomes fertile in February of the next year. It grows most abundantly from March to May and usually disappears in the middle of June.

Frond structure

The frond is saccate when young but later splits intercellularly at the tip, developing into an expanded monostromatic membrane, but often persists in a saccate habit. The expanded frond is usually segmented and it is plicate, fan-shaped or elongated with plane or lobed margins. The frond is 5-10 cm. or up to 20 cm. in height. It is not very soft and bright green. The thickness of the frond is 30-45  $\mu$  in the upper and the middle part, up to 55  $\mu$  in the lower and the rhizoid-bearing portion.

The cells in surface view are 4-6-cornered in shape and 11-17  $\mu$   $\times$  8-15  $\mu$  in the upper part of the frond, rectangular 15-34  $\mu$   $\times$  8-12  $\mu$  in the middle and the lower part. They are arranged without definite order in the upper part, but arranged in longitudinal series in the middle and the lower part (Pl. IX, D-G), often disposed in groups of twos (or fours) in the material preserved in formalin. The cells in cross section are roundish quadrate or rectangular in shape, 18-23  $\mu$

high in the upper and the middle part of the frond, higher than broad in the fertile part. The cells contain mostly a single parietal chloroplast with one pyrenoid, often 2-3 pyrenoids in the lower and the rhizoid-bearing part of the frond.

### 1) Cultures at room temperature

Cultures of the zygotes were begun on April 15 and 25, 1957, April 26 and May 3, 1958, and March 14 and April 13, 1964. Those of zoospores were started in December, 1957 and 1958. The cultures grown in SCHREIBER's solution were kept near the north-east window at room temperature.

#### Gametes and their movement

The leafy frond is a dioecious gametophyte. The formation of gametes begins in the upper margin of the frond and extends gradually downwards. Usually, the male frond is easily distinguished from the female by the color of the fertile part; the former is yellowish-brown and the latter is yellowish-green. The gametangia are somewhat loosely arranged in a gelatinous substance. The gametes are liberated from a gametangium simultaneously through a pore formed in the surface wall on one and the same side of the frond. The liberation-pore

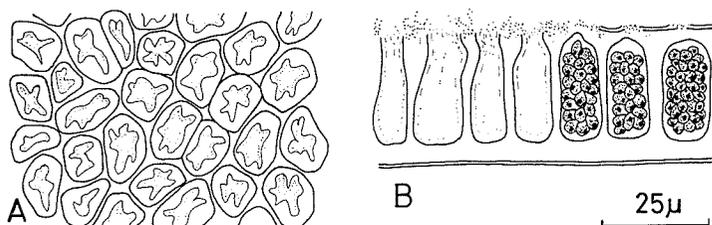


Fig. 9. *Monostroma angicava* KJELLMAN

A. Surface view of emptied gametangia, showing liberation-pores with irregular margins. B. Cross section of the fertile part of a frond with emptied gametangia.

is a rent with irregular margins in surface view (Fig. 9, A; Pl X, C-D). The surface wall of the frond on the side where the gamete-liberation takes place, disintegrates and is not seen in cross section (Fig. 9, B; Pl. X, F). After liberation of the gametes, emptied gametangia remain on the frond and usually shrink.

The gametes have two flagella of equal length ( $12-16.7 \mu$ ) at the anterior end, a single chloroplast with one pyrenoid, and one eyespot (Fig. 10, A-B; Pl. X, G-H). The female gametes are elongated pear-shaped or pear-shaped and measure  $5.6-10.6 \mu \times 2.7-5.3 \mu$  (average,  $8.04 \mu \times 4.12 \mu$ ). The male gametes are elongated pear-shaped or fusiform,  $3.7-7.6 \mu \times 1.5-3.3 \mu$  (average,  $6.04 \mu \times 2.06 \mu$ ) and pale green in color. There is a remarkable difference in size between the female and the male gametes. Sexual reproduction is apparently anisogamous.

After liberation, the gametes swim very vigorously for several hours or more, sometimes even for about 1-2 days, showing a strong positive phototaxis. But they become sluggish in their movement towards the end of the swimming period and finally settle down on the substratum. The gametes usually develop parthenogenetically.

When the female and the male gametes are mixed, conjugation occurs generally side by side, rarely end to end at their anterior ends, forming clumps of male and female gametes which result in zygotes (Fig. 10, C-D; Pl. X, I).

#### Zygotes and their development

After sexual fusion, the zygotes swim actively for 10-15 minutes and gradually become less active, showing a negative phototaxis. They finally settle down on the substratum, losing the flagella. Settled zygotes become spherical and soon form a wall, measuring 5.0-7.3  $\mu$  in diameter. They have two chloroplasts with one pyrenoid each, and two eyespots (Fig. 10, E-F; Pl. XI, A). Two eyespots are distinctly visible in each zygote for about 3 days after conjugation, but they disappear in 5-day old cultures.

The zygotes usually form a germination tube and within 1-2 days the cell contents migrate completely into this tube, forming a new cell (Fig. 10, G-J). The new cell gradually increases in size (Fig. 10, K-M; Pl. XI, B), and the vestige of the germination tube begins to degenerate, but often persists in the 10-15-day old cultures. In some cultures, however, the zygotes sometimes increase enormously in size without formation of the germination tube, taking various shapes; spherical, elliptical, pear-shaped or gourd-shaped, and 3-6 or more pyrenoids are seen in each zygote (Fig. 10, N-S; Pl. XI, C-E). In one month, the zygotes become spherical in shape, dark green in color, and full of starch (Fig. 10, T; Pl. XI, F). In 3-4 months they develop into thick-walled cysts, measuring 70-120  $\mu$  in diameter (Fig. 10, U; Pl. XI, G). The size of the cyst seems to be varied depending upon culture conditions, seasons and individuals.

From July to October (18-24°C.), the cysts did not show any remarkable change in size and contents. At the beginning of November (10-14°C.) the cysts became somewhat yellowish-green and formed a number of zoospores with an eyespot (Fig. 10, W; Pl. XII, A). Most of the cysts produced a long slender tube. At the end of November (about 10°C.) the slender tubes opened at the tip and zoospores were liberated one by one through the opening (Fig. 10, X-Z; Pl. XII, B). Formation and liberation of zoospores in the cysts continued until January, but some of the cysts remained sterile until November of the next year. The number of zoospores produced in each sporangium, is about 60-130 or more and seems to vary with the size of the sporangia.

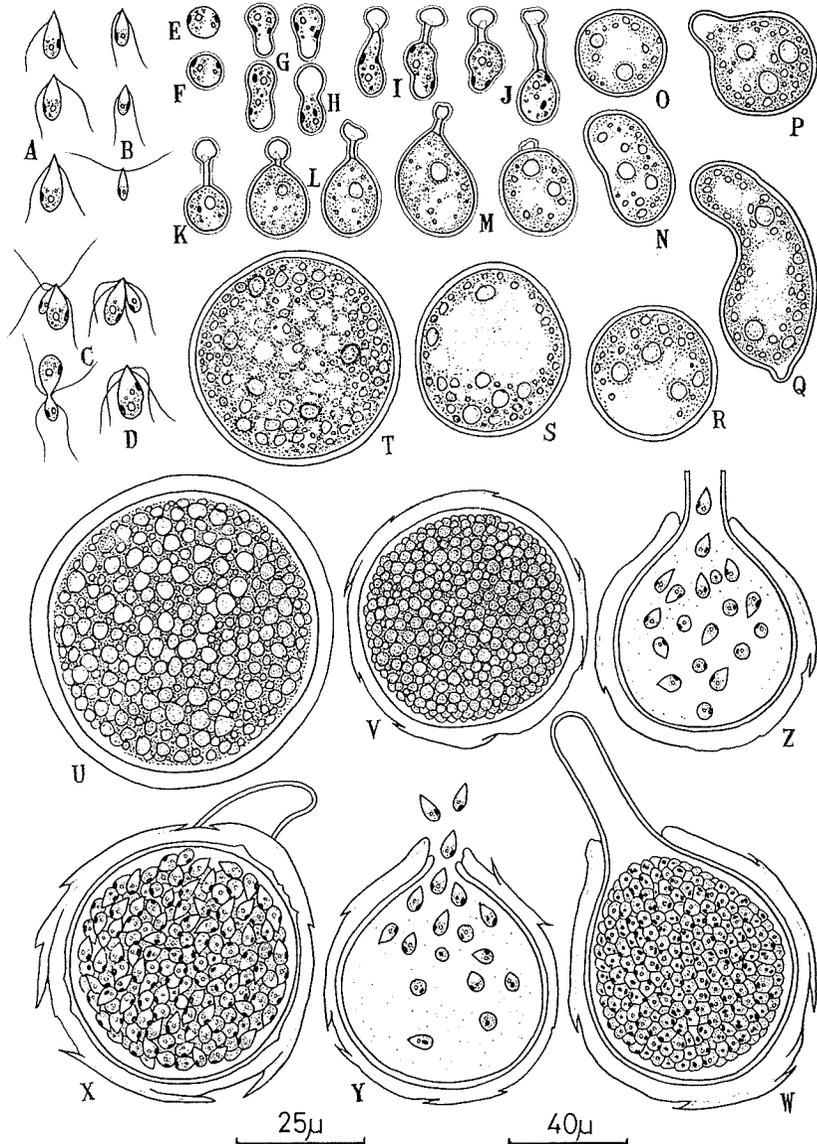


Fig. 10. *Monostroma angicava* KJELLMAN

A. Female gametes. B. Male gametes. C. Conjugation of gametes. D. Zygote. E-F. Settled zygotes. G. Germination of zygotes. H-J. Migration of cell contents. K-Z. Development of zygotes grown in SCHREIBER'S solution at room temperature. K. 4-day old zygote. L. 5-day old zygotes. M-O. 7-day old zygotes. P-R. 15-day old zygotes. S. 20-day old zygote. T. One-month old zygote. U. 4-month old cyst. V. 5-month old cyst. W-Z. Fertile cysts, showing formation and liberation of zoospores, from 6-month old culture.

A-U drawn to 25  $\mu$  scale. V-Z drawn to 40  $\mu$  scale.

### Zoospores and their development

The zoospores are pear-shaped or elongated pear-shaped, measuring 7.2–10.6  $\mu \times$  3.0–5.8  $\mu$  (average, 8.52  $\mu \times$  4.55  $\mu$ ). They have four flagella of equal length (12.0–15.2  $\mu$ ) at the anterior end, a single chloroplast with one pyrenoid, and one eyespot (Fig. 11, A; Pl. XII, C). They show a positive phototaxis, but become negatively phototactic after swimming for 10–20 minutes, and finally settle down on the substratum, losing the flagella. The settled zoospores become spherical and soon form a wall, measuring 4.8–6.9  $\mu$  in diameter (Fig. 11, B–C). Within 2–3 days they begin to germinate, taking an elongated shape and divide transversally into two cells (Fig. 11, E–G). In 10–15 days the germlings develop into creeping filaments, consisting of 3–5 cells and then each cell of the filament begins to branch off (Fig. 11, H–K; Pl. XII, D). By successive cell divisions and branchings the creeping filament develops into a small prostrate disc which consists of one layer of cells (Fig. 11, L–M). In 30 days the disc acquires two or more layers of cells except its periphery (Fig. 11, N; Pl. XII, E). At the central region of the disc, the surface layer gradually upheaves by active cell divisions, separating from the underlying layer fastened to the substratum. As the result of this, a saccate frond arises from the central part of the disc (Fig. 11, O; Pl. XII, G).

In March (7–10°C. or more) the saccate fronds attained a height of 0.5 cm. and reached maturity, producing biflagellate gametes, though such fronds did not develop into an expanded monostromatic condition. The male and the female gametes are produced on separate fronds.

As mentioned above, in *M. angicava* the sexual plant is dioecious and sexual reproduction is anisogamous. The zygote develops into a unicellular sporophyte which produces a number of quadriflagellate zoospores. The zoospore first develops into a creeping filament and finally forms a small disc, from the central part of which a saccate frond arises and it develops into a monostromatic gametophyte. *M. angicava* has a dimorphic alternation of the macroscopic gametophyte (leafy frond) and the microscopic unicellular sporophyte (cyst). In this culture experiment, the life cycle completed in about 10–12 months.

### 2) Cultures in freezer-incubators

Since 1964, cultures grown in ESP medium were kept in freezer-incubators at the following temperatures and photoperiods: 10°C. and 14 hours daily, 12°C. and 10 hours daily, and 14°C. and 14 hours daily, and they have been grown from generation to generation.

When the zygotes were grown at 14°C. in a 14-hr photoperiods, they increased enormously in size, measuring 50–70  $\mu$  in diameter in 20 days, 70–90  $\mu$  in 30 days, and 120–150  $\mu$  in 60 days. Most zygotes grew into unicellular spherical thick-

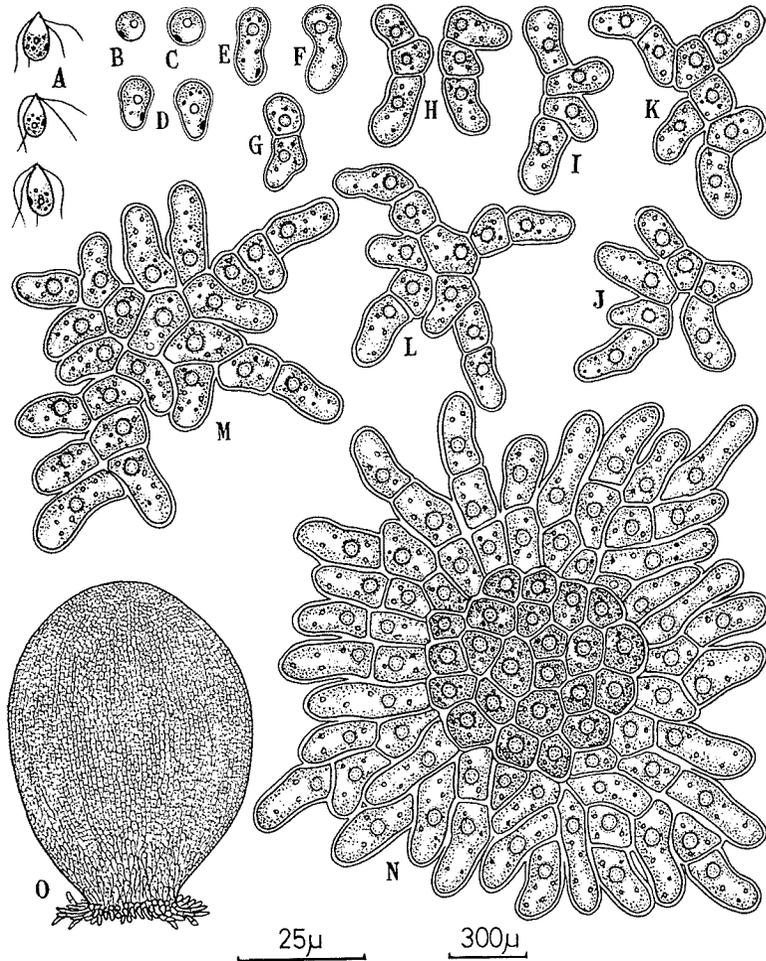


Fig. 11. *Monostroma angicava* KJELLMAN

A. Zoospores. B-C. Settled zoospores. D-F. Germination of zoospores. G-O. Development of zoospores grown in SCHREIBER's solution at room temperature. G. 2-celled stage. H-I. 10-day old germlings. K-M. 20-day old germlings. N. One-month old germling (small disc) upheaved at the central part. O. Young saccate frond.

A-N drawn to 25  $\mu$  scale. O drawn to 300  $\mu$  scale.

walled cysts, but some of them developed into *Codiolum*-like cysts which were provided with a striped rhizoid (Pl. XI, H). When retained at 14°C. in a 14-hr photoperiod, these cysts did not show any remarkable change in size and contents. They became fertile, however, when they were transferred to conditions of 12°C.

and a 10-hr photoperiod, and produced 64 or more quadriflagellate zoospores within 20-30 days after transfer. Under these culture conditions, the zoospores soon germinated and grew into discs which produced saccate fronds. In 60 days the fronds attained a height of 0.5 cm., but fertile fronds were not obtained.

Under conditions of 10°C. and a 14-hr photoperiod, the zoospores formed at 12°C. in a 10-hr photoperiod, developed into saccate fronds which attained a height of 1-1.2 cm. in 30 days. In 50-days the saccate fronds reached maturity and produced biflagellate gametes, though they did not develop into expanded membranous fronds. The male and the female gametes were formed on separate plants. When the zoospores formed at 12°C. in a 10-hr photoperiod were cultured at 14°C. in a 14-hr photoperiod, they developed into prostrate discs or tufts and saccate fronds were not formed.

The zygote grows into a spherical cyst within 20-30 days at 14°C. in a 14-hr photoperiods. The cyst reaches maturity and produces quadriflagellate zoospores within 20-30 days under conditions of 12°C. and a 10-hr photoperiod. The zoospore grows into a saccate fertile frond which produces biflagellate gametes within 50 days at 10°C. in a 14-hr photoperiod. Thus, the entire life cycle of *M. angicava* is completed within 100-110 days in culture.

#### Parthenogenesis

The gametes of both sexes develop parthenogenetically. After swimming vigorously for several hours or more (sometimes 24-48 hours), the gametes settle down on the substratum, losing the flagella. Settled gametes become spherical and soon form a wall. In this stage, the female gametes measure about 4.0-5.8  $\mu$  in diameter, while the male about 3.5-4.5  $\mu$ . Within 2-3 days most of the gametes form a germination tube into which the cell contents completely migrates. The distal end of the tube becomes swollen and forms a new cell which increases in size, developing into a cyst. Sometimes settled gametes develop into cysts without forming a germination tube.

The gametes cultured parthenogenetically at 14°C. in a 14-hr photoperiod increased enormously in size, forming unicellular cysts and within 30-50 days attained a diameter of 50-60  $\mu$ . When transferred to conditions of 12°C. and a 10-hr photoperiod, these cysts produced a long slender tube and reached maturity within 30 days after transfer, but some cysts bleached without forming swarms. The fertile cysts produced a number of bi- or quadriflagellate swarms. The biflagellate swarms are smaller than the quadriflagellate ones which are similar to the zoospores in size and structure. These two kinds of swarms liberated from the parthenogenetic cysts were cultured together, and both the two germinated and developed into saccate fronds at 10°C. in a 14-hr photoperiod. In 50-60 days these fronds attained a height of 1 cm. and reached maturity, pro-

ducing biflagellate gametes.

The fronds derived from female gametes produced larger gametes than those derived from male gametes. Conjugation of these gametes was not observed. However, when both kinds of the fertile fronds were cultured together in the same test tube, the zygotes with two eyespots were found on the wall of the tube in the 4-day old cultures.

In *M. angicava* the gamete develops parthenogenetically into a unicellular cyst which forms bi- or quadriflagellate swimmers developing into new gametophytes.

### *Monostroma zostericola* TILDEN

Figs. 13-14, Pls. XIII-XV

#### Habitat

In the vicinity of Muroran, *Monostroma zostericola* is always found growing densely on the leaves of *Phyllospadix* in the middle littoral and the lower littoral zone. This alga appears in early December and becomes fertile in early January of the next year. It grows abundantly from March to May and usually disappears at the end of June.

#### Frond structure

The frond is saccate when young but soon opens intercellularly at the tip, forming an expanded monostromatic membrane. The expanded frond is plicate or cuneate-obovate with a plane margin. The frond is 2-4 cm. or even to 7 cm. in height, soft and bright green. The thickness of the frond measures 9-14  $\mu$  in the marginal and the middle part and more than 14  $\mu$  at the rhizoid-bearing portion.

The cells in surface view are roundish angular and ovate, measuring 4-6  $\mu \times$  3-5  $\mu$  in the marginal and the middle part of the frond, and quadrate 5-9  $\mu \times$  4-7  $\mu$  in the lower. The cells are arranged in somewhat longitudinal or transversal series, assuming the appearance of a vein, in the material preserved in formalin (Pl. XIII, D-F). In cross section they are roundish quadrate, measuring 7-9  $\mu$  high. The cells contain a single parietal chloroplast.

#### 1) Cultures at room temperature

Cultures of zoospores were begun on May 10 and 25, 1957, January 8-11, March 10, and April 13, 1958, March 14, 1960, May 27-30, 1963, and April 2, 1964. Those of zygotes were started between December and March, 1958-1960 and December, 1963. Cultures grown in SCHREIBER's solution were kept near the north-east window at room temperature.

#### Zoospores and their movement

The leafy frond is a sporophyte and produces zoospores. The formation of zoospores begins to occur in the upper margin of the frond, extending gradually downwards. The fertile part changes slightly to yellowish-green, but it is not clearly distinguishable from the other part. The fertile sporangia produce a papillate protuberance with a pore on the surface wall at the same side of the

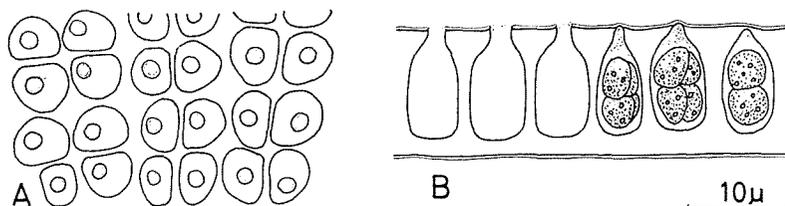


Fig. 12. *Monostroma zostericola* TILDEN

A. Surface view of emptied sporangia, showing liberation-pores. B. Cross section of the fertile part of a frond with emptied sporangia.

frond (Fig. 12, B; Pl. XIV, B). The zoospores are liberated one by one through the pore which is round in surface view. The liberation-pore is not seen in the living material, as far as the writer examined, but can be clearly detected in the material stained with safranin (Fig. 12, A; Pl. XIV, A). After liberation, emptied sporangia remain persistently on the frond. Each sporangium produces 4-8 quadriflagellate zoospores.

The zoospores are pear-shaped or ovate, measuring  $4.2-6.8 \mu \times 3.3-5.2 \mu$  (average,  $5.35 \mu \times 4.27 \mu$ ). They have four flagella of equal length ( $7.6-10.6 \mu$ ) at the anterior end and a single chloroplast with several granular substances (Pl. XIV, C). They have no eyespot and show no phototaxis.

#### Development of zoospores

The zoospores after swimming for 10-20 minutes settle down on the substratum, losing the flagella and soon form a wall. Within 2-3 days they germinate, taking an elongated shape and dividing transversally first into two cells, and form 3-4-celled germlings (Pl. XIV, D). Each cell of the germling then branches off (Pl. XIV, E). Sometimes the settled zoospores send out a germination tube into which all the cell contents migrate, leaving the original cell empty (Pl. XIV, D). Within 2-4 days such germlings divide into 2-4 cells with transverse walls.

In cultures started from March to May the germlings developed into small discs within 20 days by successive branchings and cell divisions. In one month the discs gave off prostrate branches radially. The central part of the discs became multiseriate and upheaved, taking an irregular shape (Pl. XIV, F). From July to September ( $20-24^{\circ}\text{C}.$ ) the discs grew very slowly and they did not show

any marked change. At the end of October (10-12°C.) the discs began to grow actively and attained about 500  $\mu$  in diameter. Sometimes the discs produced many erect and tangled branches besides prostrate branches, forming a green tuft. In November most surface cells of the disc began to enlarge and divided into 2-8 or more cells, producing 8-32 or more gametes (Fig. 13, A-B). Generally at

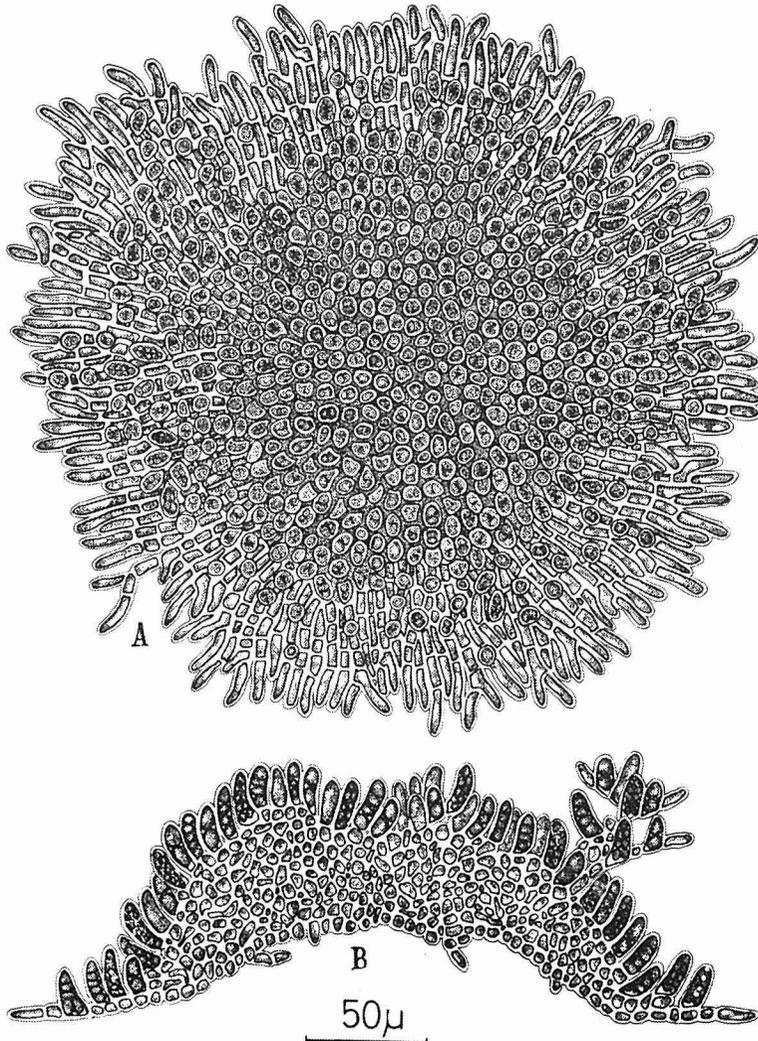


Fig. 13. *Monostroma zostericola* TILDEN

A. Surface view of a fertile disc (gametophyte), from 7-month old culture grown at room temperature. B. Cross section of the same.

room temperature ranging from 3 to 7°C. (winter) the gametangia reached maturity. In this culture experiment the discs became fertile in 6–10 months.

In cultures started on January 8–11, 1958, the settled zoospores developed within 50 days into flat discs (300–500  $\mu$  in diameter) composed of a single layer of cells. The discs became fertile and discharged gametes in 60 days (Pl. XIV, G).

#### Gametes and their movement

The gametangia are club-shaped in side view, measuring 15–35  $\mu$  in height and 4.5–6.1  $\mu$  in breadth (Fig. 14, A–D; Pl. XIV, H–I). The gametes are liberated one by one through a pore at the tip of each gametangium.

The gametes are pear-shaped, measuring 2.7–5.7  $\mu \times 1.8$ –4.5  $\mu$  (average, 4.12  $\mu \times 2.91 \mu$ ). They have two flagella of equal length (7.6–10.6  $\mu$ ) at the anterior end and a single chloroplast with several granular substances. They have no eyespot and show no phototaxis, as far as examined. After liberation from gametangia,

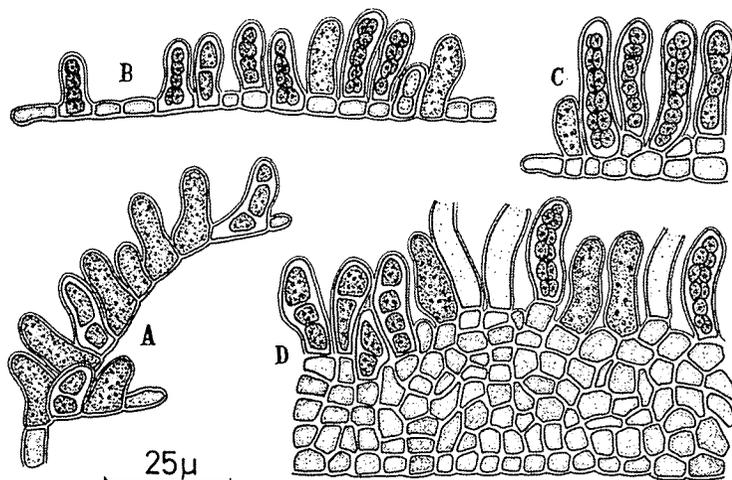


Fig. 14. *Monostroma zostericola* TILDEN

A–D. Formation of gametes. A. Branch with gametangia.

B–C. Cross section of the peripheral part of a fertile disc.

D. Cross section of the central part of a fertile disc.

the gametes swim rapidly and soon conjugate even between gametes derived from the same gametophytic disc. Under a microscope, no marked size difference is noted between the conjugating gametes of a pair. The gametes conjugate side by side or end to end anteriorly and form zygotes (Pl. XV, A).

#### Zygotes and their development

The zygotes after swimming for a while settle down on the substratum, losing the flagella and soon form a wall. Within 3-4 days the zygotes germinate, taking an elongated shape and divide transversally into two cells (Pl. XV, B) which develop into a creeping filament consisting of 4-5 cells. Some zygotes send out a germination tube into which the cell contents migrate, leaving the original cell empty. Then the creeping filaments develop into small prostrate discs. The discs consist of a single layer of cells, but they are composed of two or more layers of cells in the central region. The central region of discs gradually upheaves monostromatically from the prostrate part. Within 35-40 days a saccate or tubular frond arises from the prostrate system. Sometimes, the single layered prostrate discs become swollen at the central part, developing into a saccate frond (Pl. XV, D-E). The saccate frond soon opens intercellularly at the tip and finally forms an expanded monostromatic membrane (Pl. XV, F). The discs, however, often form monostromatic membranous fronds without passing through the saccate stage. In this case, one erect uniseriate filament from the disc develops directly into an expanded monostromatic frond (Pl. XV, G-I). In 50-60 days the monostromatic frond attains a height of 0.3-0.5 cm. and reach maturity, producing quadriflagellate zoospores.

As mentioned already, in cultures started on January 8-11, 1958, the discs derived from zoospores formed gametangia at the beginning of March of the same year, discharging gametes. The zygotes from these gametes cultured on March 9-11, developed into fertile sporophytes (*Monostroma* plants) at the beginning of May. Consequently, it took about 4 months to complete one life cycle. On the contrary, in the cultures started from March to May, the discs grew into fertile gametophytes from the middle of November to the middle of the next March (6-10 months). The zygotes developed into fertile sporophytes from the end of January to the beginning of May. In this case, it took about 8-12 months to complete one life cycle. Evidently the gametophyte and sporophyte did not grow well during summer to autumn at room temperature (18-24°C.). The gametophyte required a temperature of 3-7°C. for maturity. The sporophyte grew well during winter to spring at room temperature ranging from 3 to 10°C. or at least less than 14°C.

The culture study of this species has been reported by YAMADA and KANDA (1941). According to them, the quadriflagellate zoospores developed directly into new leafy sporophytes and the gametophytes were not found. In the succeeding culture studies (YAMADA and TATEWAKI, 1959, 1965) the zoospores developed into minute discoid monoecious gametophytes and they did not grow into leafy sporophytes. Thus in *M. zostericola*, there is a dimorphic alternation of a macroscopic leafy sporophyte and a minute discoid gametophyte.

## 2) Cultures in freezer-incubators

Since 1963, cultures grown in ESP medium were maintained in the freezer-incubators at the following temperatures and photoperiods: 5°C. and 10–(12) hours daily, and 14°C. and 14 hours daily, and they have been grown from generation to generation.

When the zoospores were cultured at 5°C. in a 10-hr photoperiod, they soon developed into prostrate discs. In 30–35 days the discs attained a diameter of 150–300  $\mu$  and reached maturity, producing gametes. After sexual fusion, the zygotes soon germinated and formed small discs which consisted of a single layer of cells. These discs became swollen at the central region and the swelling developed into a saccate frond (Pl. XV, C–E). Also they gave off very often an erect filament which developed directly into a monostromatic frond without passing through the saccate stage (Pl. XV, G–I). In 30–40 days the fronds attained a height of 0.3–0.5 cm. and reached maturity, producing zoospores. Under these culture conditions one life cycle was completed within 60–75 days.

Under culture conditions of 14°C. and a 14-hr photoperiod, the zoospores developed rapidly into prostrate discs, measuring 500–700  $\mu$  in diameter within 30–40 days. Then the discs increased in diameter and became thickened, but they did not develop into fertile gametophytes. These discs, however, became fertile when they were transferred to conditions of 5°C. and a 10-hr photoperiod. Also, the zygotes obtained at 5°C. in a 10-hr photoperiod did not grow into leafy fronds and remained prostrate discs, when they were transferred to conditions of 14°C. and a 14-hr photoperiod. Within 20 days after transfer, however, these discs became fertile and produced zoospores.

### Parthenogenesis

In *M. zostericola*, the sexual discoid plant is monoecious and the gametes conjugate with each other immediately after liberation. Further, the gametes have no eyespot. Therefore, it is very difficult to distinguish gametes from zygotes. The parthenogenetic development of gametes was not observed in this alga.

### *Monostroma fuscum* (POSTELS and RUPRECHT) WITTRÖCK

var. *splendens* (RUPRECHT) ROSENVINGE

Figs. 15–19, Pls. XVI–XVIII

### Habitat

*Monostroma fuscum* var. *splendens* is found growing in the grounds used for cultivating *Laminaria* at Muroran. This alga grows on stones, rocks and con-

crete blocks at a depth of 2-3 meters, and large quantities of the fronds are found washed ashore from May to August.

#### FronD structure

The frond is at first saccate but later opens at the tip, developing into an expanded monostromatic membrane with a short stipe at the base. The expanded frond is ovate or somewhat elongated with a more or less irregularly lobed margin, attaining a height of 30-50 cm. or up to 80 cm. and a breadth of 20-50 cm. or more. It is not so soft and is bright green but turns to brown or black in drying. The thickness of the frond measures 34-45  $\mu$  in the marginal part, 40-56  $\mu$  in the middle, 55-70  $\mu$ , up to 90  $\mu$  in the lower and up to 150  $\mu$  or more at the rhizoid-bearing portion (Pl. XVI, E-G).

The cells in surface view are 4-6-cornered or roundish angular in shape, measuring 10-25  $\mu \times 10-20 \mu$ , and are arranged without definite order. In cross section, they are rectangular or somewhat roundish rectangular, measuring 30-41  $\mu$  high in the marginal part of the frond, 36-50  $\mu$  in the middle and 50-60  $\mu$  in the lower. The cells contain a single parietal chloroplast with 2-4 or more pyrenoids (Pl. XVI, C, E).

#### 1) Cultures at room temperature

Cultures were begun on May 15 and 24, 1959 and 1960, and May 30, 1964. Cultures grown in SCHREIBER's solution were kept near the north-east window at room temperature.

#### Formation of swarmers

There are three kinds of plants; female and male gametophytes, and sporophytes, all of which are identical morphologically. The formation of swarmers begins in the upper marginal part of the frond, extending gradually downwards. A microscopical examination shows that the marginal cells of the frond remains sterile (Pl. XVII, E). The fertile part can be easily distinguished by its yellowish-green or yellowish-brown color. The fertile cells form a papillate protuberance with a pore on the surface wall at the same side of the frond (Fig. 15, B; Pl. XVII, D). The swarmers are liberated one by one through the pore which is round in surface view, as in *Ulva* (Fig. 15, C; Pl. XVII, C). After liberation the emptied cells remain on the frond.

#### Gametes and their movement

The gametes are elongated pear-shaped or fusiform and measure 4.5-7.6  $\mu \times 1.5-3.7 \mu$  (average, 6.06  $\mu \times 2.65 \mu$ ; one sex, 6.08  $\mu \times 2.85 \mu$  and opposite sex, 6.04  $\mu \times 2.55 \mu$ ). Sexual reproduction is isogamous. They have two flagella of equal

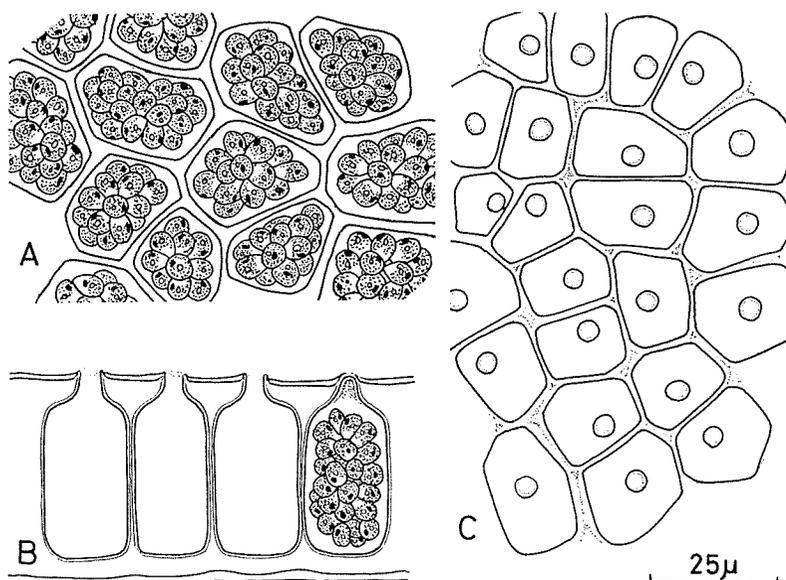


Fig. 15. *Monostroma fuscum* var. *splendens*  
(RUPR.) ROSENVINGE

A. Surface view of the fertile part (sporangia) of a frond. B. Cross section of the same with emptied sporangia. C. Surface view of emptied gametangia, showing liberation-pores.

length (12-13  $\mu$ ) at the anterior end, a single chloroplast with one pyrenoid, and one eyespot (Fig. 16, A-B; Pl. XVII, F-G). The gametes swim rapidly for 30-60 minutes after liberation and are positively phototactic. They gradually become sluggish in their movement and finally settle down on the substratum. Parthenogenesis is a common feature in the gametes of this alga. By mixing both kinds of gametes, two gametes generally conjugate side by side and form a zygote (Fig. 16, C-D).

#### Development of zygotes

After sexual fusion, the zygotes swim for 10-20 minutes and are negatively phototactic. After swimming, they settle down on the substratum, losing the flagella and soon form a wall (Fig. 16, E-F). Settled zygotes are spherical, measuring 4.2-5.0  $\mu$  in diameter. They have two chloroplasts with one pyrenoid each and two eyespots. Within 5 days, the zygotes measure 6.4-8.0  $\mu$  in diameter and the eyespots disappear (Fig. 16, H; Pl. XVII, I). Then they germinate by pushing out a protuberance (Fig. 16, I; Pl. XVII, J) and rapidly increase in size (Fig. 16, J). In 10 days the zygotes divide into two cells transversally, one of

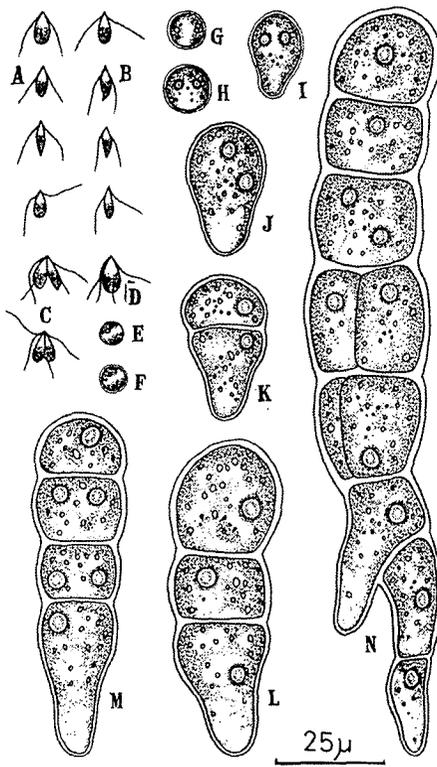


Fig. 16. *Monostroma fuscum* var. *splendens* (RUPR.) ROSENVINGE

A-B. Gametes. C. Conjugation of gametes. D. Zygote. E-H. Settled zygotes. I-N. Germination and development of zygotes grown in SCHREIBER's solution at room temperature. L-M. 14-day old germlings. N. 20-day old germling.

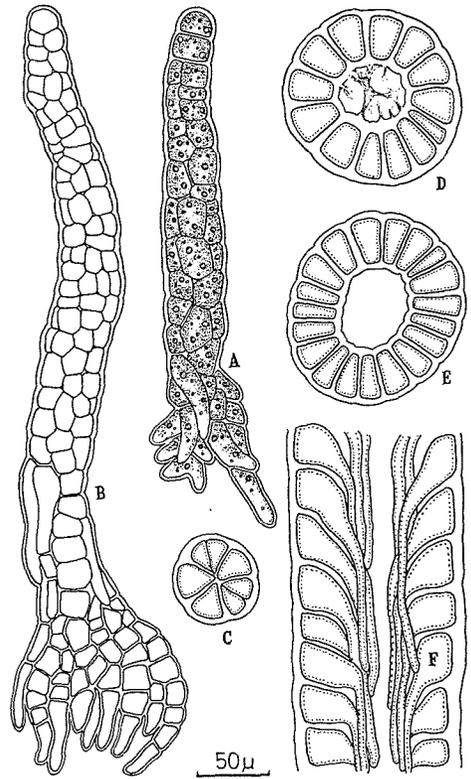


Fig. 17. *Monostroma fuscum* var. *splendens* (RUPR.) ROSENVINGE

A. 25-day old germling. B. One-month old germling. C-E. Cross section of a cylindrical or tubular frond. C. 30-day old culture. D. 40-day old culture. E. 50-day old culture. F. Vertical section of the basal part of a 50-day old frond.

which elongates and forms a primary rhizoid (Fig. 16, K; Pl. XVII, M). The other cell develops into an erect uniseriate filament by successive transverse cell divisions, consisting of 3-7 cells and in each cell 1-2 pyrenoids are clearly visible (Fig. 16, L-M; Pl. XVIII, B). In 20 days longitudinal cell divisions occur in the cells of the erect filament, and the secondary rhizoid is given off from the basal cell (Fig. 16, N). In 25-30 days the filamentous germlings develop into a cylindrical structure (Fig. 17, A-C; Pl. XVIII, D), and sometimes they attain a height of 1-3 mm. Within 2 months after culture, they form tubular or saccate fronds

with a central cavity (Fig. 17, D-E). The saccate frond opens at the tip by breaking of enlarged cells and develops into an expanded monostromatic condition except at the basal part (Pl. XVIII, E-G). In about 10 months the frond attains a height of 1-2 cm. and reaches maturity, producing quadriflagellate zoospores.

#### Zoospores and their development

The zoospores are elongated pear-shaped and measure  $8.3-12 \mu \times 3-4.8 \mu$  (average,  $10.15 \mu \times 4.07 \mu$ ). They have four flagella of equal length ( $12-13.5 \mu$ ) at the anterior end, a single chloroplast with one pyrenoid, and one eyespot (Fig. 18, A; Pl. XVII, K). They show a slightly positive phototaxis and are less active than the gametes.

After swimming for 10-20 minutes, the zoospores settle down on the substratum, losing the flagella and soon form a wall (Fig. 18, B-C). Settled zoospores are spherical and measure  $4.5-5.4 \mu$  in diameter. In 3 days they begin to germinate by pushing out a protuberance and at this stage the eyespot is not recognizable in most of them (Fig. 18, D-E; Pl. XVII, L). They rapidly increase in size and the 7-day old germlings divide into two cells transversally (Fig. 18, F-G). Then by successive transverse divisions they develop into erect uniseriate filaments consisting of 3-7 cells with a primary rhizoid at the base (Fig. 18, H-I; Pl. XVIII, A). In 15 days longitudinal divisions occur in the cells of the erect filament (Fig. 18, J), and the secondary rhizoid is given off from the basal part (Fig. 19, A). Further development of the germlings is quite similar to that of the zygotes (Fig. 19, B-C; Pl. XVIII, C). In about 10 months the monostromatic frond attains a height of about 1-2 cm. and reaches maturity, producing biflagellate gametes. Male and female gametes are produced on separate fronds.

#### Parthenogenesis

The gametes commonly develop parthenogenetically. After swimming for 30-60 minutes or more, they settle down on the substratum, losing the flagella and soon form a wall. Settled gametes are spherical and measure about  $3.2-4.0 \mu$  in diameter. Then they begin to germinate by forming a protuberance (usually in 7-10 days). Further development of the parthenogametes is identical with that of the zygotes and the zoospores, as mentioned above. In 10 months the fronds derived from female gametes produce female gametes, and the fronds derived from male gametes produce male ones again. When these female and male gametes were mixed, conjugation occurred, forming zygotes.

In *M. fuscum* var. *splendens* the sexual plant is dioecious and sexual reproduction is isogamous. The sexual and the asexual plants are morphologically identical. The development of zygotes, zoospores and parthenogametes is quite identical. Namely, they first develop into an erect uniseriate filament with a rhizoid

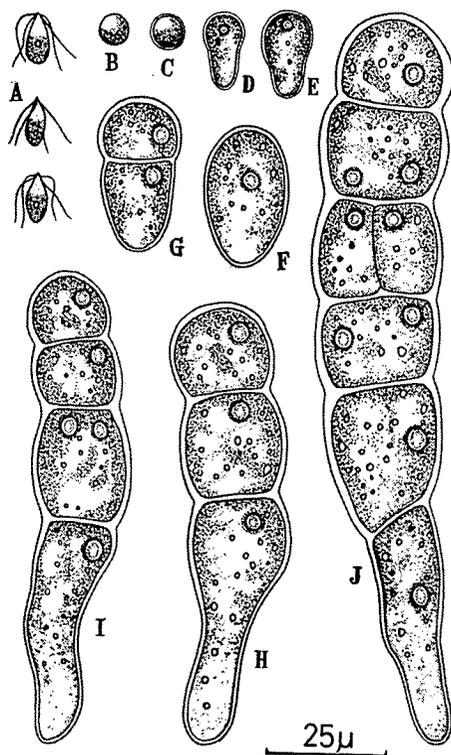


Fig. 18. *Monostroma fuscum* var. *splendens* (RUPR.) ROSENVINGE

A. Zoospores. B-C. Settled zoospores. D-J. Germination and development of zoospores grown in SCHREIBER's solution at room temperature. H. 7-day old germling. I. 10-day old germling. J. 14-day old germling.

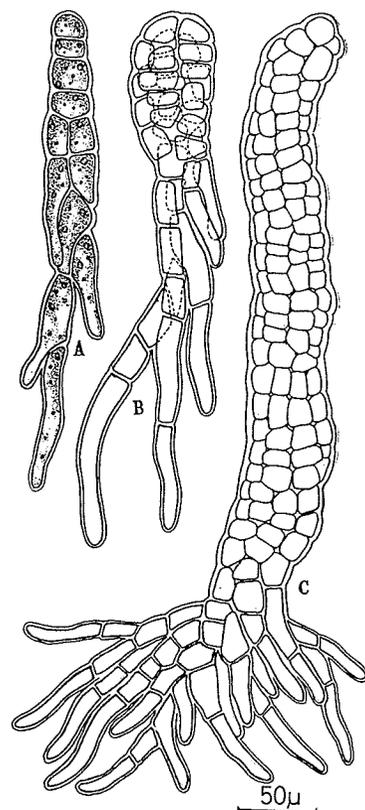


Fig. 19. *Monostroma fuscum* var. *splendens* (RUPR.) ROSENVINGE

A-C. Further development of germlings grown in SCHREIBER's solution at room temperature. A. 15-day old germling. B. 21-day old germling. C. One-month old germling.

at the base. Then the erect filament becomes cylindrical and grows into saccate fronds. The saccate frond opens at the tip and finally develops into a monostromatic membranous frond. The frond derived from the zygote produces zoospores, while the frond derived from the zoospores produces male and female gametes on separate plants. The parthenogamete gives rise to a plant of respective sex. The life cycle of this alga is a monomorphic alternation of generations as that of *Ulva* and most species of *Enteromorpha*. In this experiment, it took about 10 months to complete one generation (spore to spore).

## 2) Cultures in freezer-incubators

Cultures grown in SCHREIBER's solution and ESP medium were maintained in the freezer-incubators at the following temperatures and photoperiods: 10°C. and 14 hours daily, and 14°C. and 14 hours daily, and they have been grown from generation to generation.

In cultures grown in ESP medium and SCHREIBER's solution at 10° or 14°C. in a 14-hr photoperiod, the germlings derived from zygotes, zoospores and gametes of either sex grow into saccate fronds and in 30 days attain a height of 0.5–0.8 cm. The saccate frond develops into an expanded monostromatic membrane, attaining a height of 1.5–2 cm. in 60–70 days and reaches maturity in 90–100 days. However, it often maintains a saccate structure without developing into a monostromatic expanse and reaches maturity, attaining a height of 1.5–2 cm. Under these culture conditions the germlings derived from zygotes, zoospores and parthenogametes grow up into fertile plants within about 3 months, and the entire life cycle of this alga is completed within 6–7 months.

## Discussion

The results of the present study will be discussed here under the following eight topics.

### 1. Structure and development of the frond

The genus *Monostroma* is characterized by the monostromatic condition of the mature frond with the exception of *M. groenlandicum* which has a cylindrical or tubular habit throughout the life. In cross section, the frond is composed of a single layer of cells enclosed within a gelatinous matrix. In most species so far described, the intercellular substance of the frond is abundant and more or less gelatinous, adhering to paper in drying, but *M. fuscum* var. *splendens* does not adhere closely to paper. In *M. groenlandicum*, *M. oxyspermum* and *M. undulatum*, the cells in surface view are arranged in groups of twos or fours and in *M. zostericola* they are arranged in somewhat longitudinal or transversal series, assuming the appearance of a vein. In *M. angicava*, the cells are arranged in longitudinal series in the middle part and the lower part of the frond, and often in groups of twos (or fours).

The early development of the frond in the genus *Monostroma* can be broadly divided into two types; 1) an erect uniseriate filament-type and 2) a prostrate disc-type.

#### 1) Erect uniseriate filament-type

In the species belonging to this type, the germling develops at first into an erect uniseriate filament with a primary rhizoid at the base by successive trans-

verse cell divisions, and then longitudinal cell divisions occur in each cell of the erect part.

In *M. groenlandicum*, the longitudinal cell divisions occur perpendicularly to the surface of the filamentous germling at the 10-20-celled stage. The filament develops into a cylindrical structure and never grows into an expanded monostromatic membrane.

In *M. oxyspermum* and *M. fuscum* var. *splendens*, the longitudinal cell divisions occur at the 3-7-celled stage and the filament develops into a saccate plantlet. The plantlet opens at the tip, forming a funnel-shaped structure and then splits out into an expanded monostromatic membrane as in *M. nitidum* (ARASAKI, 1946), *M. tubiforme* (IWAMOTO, 1960), *M. obscurum* (GAYRAL, 1962) and *M. wittrockii* (KIDA, 1964). However, there is found a marked difference in the method of opening of the saccate frond between the two species; in *M. oxyspermum* opening occurs intercellularly at the tip of the saccate frond, while in *M. fuscum* var. *splendens* it occurs by breaking of enlarged cells.

In *M. undulatum*, the longitudinal cell divisions occur in one and the same direction in each cell of the erect filaments at the 3-6-celled stage. Accordingly, the erect filament develops directly into a monostromatic membrane, as described by ARASAKI (1946) and KIDA (1966) in *M. latissimum*.

## 2) Prostrate disc-type

In *M. angicava* and *M. zostericola*, the germling develops at first into a creeping uniseriate filament and then forms a prostrate disc by successive transverse cell divisions and branchings. The disc assumes two or more layers of cells in the central region, the surface layer of which upheaves to form an erect saccate frond. The saccate frond splits intercellularly at the tip, developing into an expanded monostromatic membrane. This type has been reported in *M. grevillei* (KORNMAN, 1962; BLIDING, 1963), and *M. arcticum* and *M. leptodermum* (KORNMAN and SAHLING, 1962).

In *M. zostericola*, however, two other developmental types of the frond were observed in culture; a) the one-layered prostrate disc upheaves in the central region, separating from the substratum and forms an erect saccate frond which soon opens at the tip, and b) the prostrate disc produces an erect uniseriate filament which develops directly into a monostromatic membrane without passing through a saccate stage. The latter type has been recorded by IWAMOTO (1960) in *M. latissimum* from Tokyo Bay, but according to ARASAKI (1946) and KIDA (1966), this alga from Ise Bay shows an erect uniseriate filament type without the prostrate disc stage.

The developmental types of the frond so far described in *Monostroma* are shown diagrammatically in Fig. 20 and are summarized as follows:

1) Erect uniseriate filament-type

The germling grows into an erect uniseriate filament

a) The erect filament develops into a cylindrical frond persistently (Fig. 20, A)

*M. groenlandicum*

b) The erect filament develops into an expanded monostromatic membrane,

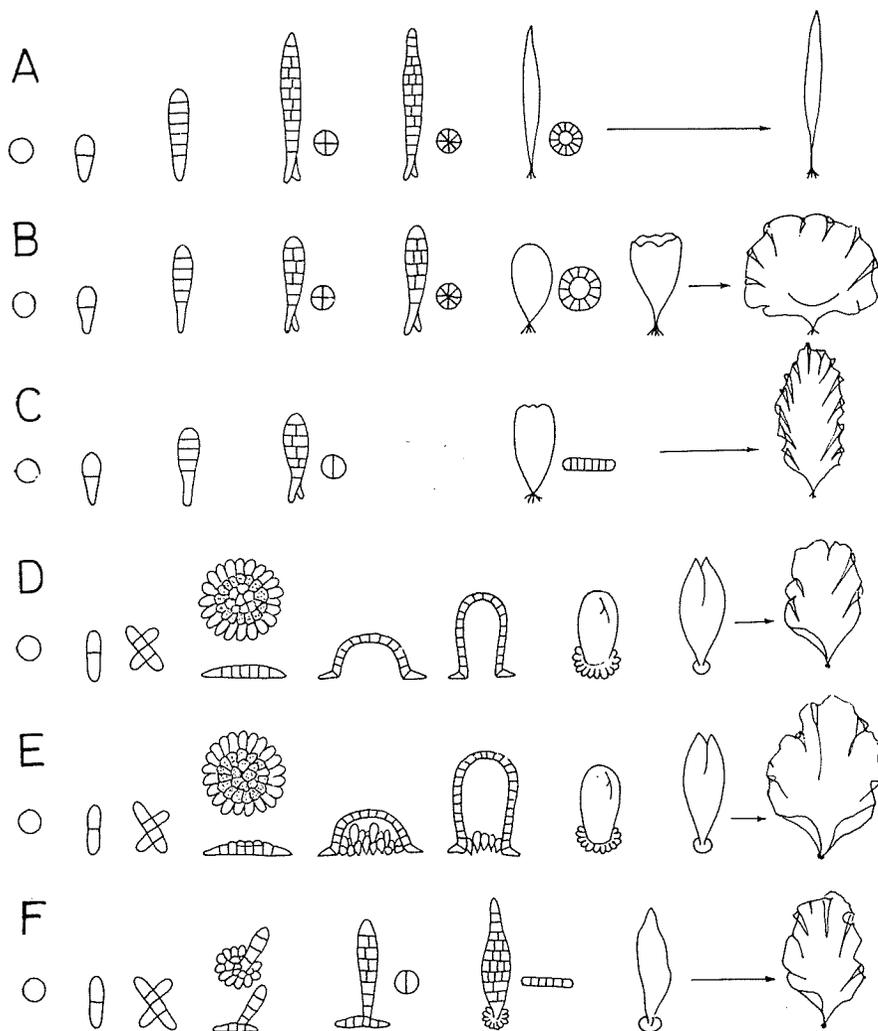


Fig. 20. Various types of development of the frond in the genus *Monostroma*

passing through a saccate stage (Fig. 20, B)

*M. oxyspermum*                    *M. wittrockii*  
*M. tubiforme*                    *M. nitidum*  
*M. fuscum* var. *splendens*      *M. obscurum*

- c) The erect filament develops directly into an expanded monostromatic membrane (Fig. 20, C)

*M. undulatum*                    *M. latissimum*

## 2) Prostrate disc-type

The germling grows into a creeping uniseriate filament, forming a prostrate disc by successive branchings

- a) The mono- or distromatic prostrate disc gives off an erect saccate frond which develops into an expanded monostromatic membrane (Fig. 20, D-E)

*M. zostericola*                    *M. leptodermum*  
*M. bullosum*                    *M. angicava*  
*M. grevillei*                    *M. arcticum*

- b) The prostrate disc gives off an erect uniseriate frond which develops directly into an expanded monostromatic membrane (Fig. 20, F)

*M. zostericola*

These developmental types of the frond are useful as a criterion for classification of the species of the genus *Monostroma*.

## 2. Method of swarmer-liberation

In *M. groenlandicum*, gametes leave the gametangium as a mass enclosed in a hyaline sac through a pore formed on the surface wall of the frond (Pl. II, C-D). A few seconds after liberation the gametes acquire motility in the sac outside the mouth of the pore and then swim away, rupturing the sac explosively. The emptied gametangium remains on the frond and the liberation-pore is seen as linear or somewhat crescent-shaped in surface view (Pl. II, E). This type of swarmer-liberation has been reported on the species of *Ulothrix*, *Percursaria* and *Capsosiphon*, but it has not been found in *Monostroma* species.

In *M. undulatum*, as reported by TOKIDA (1954) in *M. undulatum* var. *farlowii* from Hakodate, the fertile marginal part of the frond becomes gelatinous and is torn in pieces from the vegetative part, which floats on the water surface. The wall of each detached sporangium breaks and swarmers are liberated in groups, oscillating for a while (Pl. V, C-F).

In *M. oxyspermum*, the method of swarmer-liberation is almost similar to that of *M. undulatum*, but the swarmers swim away freely immediately after liberation (Pl. VIII, A), as described by GAYRAL (1964, 1965). This type of

swarmer-liberation has been recorded in *M. latissimum* and *M. wittrockii* by KIDA (1966).

In *M. angicava*, gametes are liberated simultaneously through a pore formed on the same side of the frond. In surface view, the pore is a rent with a irregular margin (Pl. X, C-D). But it can not be seen in cross section, because the surface wall of this side of the frond falls off after swarmer-liberation (Pl. X, F). The emptied cell remains on the frond and usually shrinks.

In *M. fuscum* var. *splendens*, swarmers (gametes and zoospores) are liberated one by one through a pore formed on the same side of the frond and the emptied cell remains on the frond. In surface view, the liberation-pore is round and it is clearly detected in the living material (Pl. XVII, C). This type of swarmer-liberation has been reported in *M. obscurum* (GAYRAL, 1962).

In *M. zostericola*, the method of liberation of zoospores is similar to that of *M. fuscum* var. *splendens*. In surface view, however, the liberation-pore of this alga could not be seen in the living material, as far as examined, but it could be detected in the material stained with safranin (Pl. XIV, A).

Regarding the method of swarmer-liberation as mentioned above, each of the species examined by the writer exhibits its own characteristics and this seems to be closely related to the constituent of cell membrane. This outstanding feature of swarmer-liberation is one of the most important criteria for classification of the *Monostroma* species.

### 3. Sexual reproduction

Sexual reproduction is by biflagellate isogametes or anisogametes. Of the species examined, four species have sexual plants. *M. angicava* and *M. fuscum* var. *splendens* are dioecious, and *M. groenlandicum* and *M. zostericola* are monoecious, though the species of *Monostroma* so far described are all dioecious except *M. bullosum* (KORNMAN, 1964).

In *M. fuscum* var. *splendens*, *M. groenlandicum* and *M. zostericola*, sexual reproduction is isogamous. Isogamy has been reported in *M. membranacea* (WEST and WEST, 1903), *M. sp* (MIYAKE and KUNIEDA, 1931), *M. wittrockii* (MOEWUS, 1938), *M. latissimum* (ARASAKI, 1946, 1949; KIDA, 1966), *M. nitidum* (ARASAKI, 1946, 1949), and *M. bullosum* (KORNMAN, 1964). According to CARTER (1926), however, in *M. latissimum* the female gametes are slightly larger than the male ones, though there is a considerable variation in size of the gametes.

*M. angicava* is anisogamous, as described by YAMADA (1932), and YAMADA and SAITO (1938). Anisogamy has been reported in *M. grevillei* (SUNESON, 1947; KORNMAN, 1962), and *M. obscurum* (GAYRAL, 1962). According to SCHREIBER (1942), *M. grevillei* from Helgoland is isogamous, but later on, it has been de-

scribed as anisogamous by KORNMANN (1962).

YAMADA, working on *M. angicava* from Oshoro and Muroran, reported that the female gametes measured  $6-8 \mu \times 4-5.5 \mu$  and the male ones  $5-6 \mu \times 2-3.5 \mu$ . According to the writer, the female gametes measure  $5.6-10.6 \mu \times 2.7-5.3 \mu$  (average  $8.04 \mu \times 4.12 \mu$ ), while the male ones  $3.7-7.6 \mu \times 1.5-3.3 \mu$  (average  $6.04 \mu \times 2.06 \mu$ ). In this species the gametes of both sexes vary widely in size and it may occur that some of larger male gametes are as large as some of smaller female gametes or even larger.

The variance in size of gametes seems to depend upon localities, seasons and individuals. The shape of gametes are also changeable from elongated pear-shape to rounded while swimming.

Usually the gametes have an eyespot and show a strong positive phototaxis. In *M. zostericola*, however, they have no eyespot and show no phototaxis, as far as examined.

The female gametes conjugate with the male gametes usually side by side or end to end anteriorly and form a zygote. After sexual fusion, the zygotes become negatively phototactic and soon become sluggish, settling down on the substratum. The gametes of either sex can develop parthenogenetically.

#### 4. Asexual reproduction

In the present culture studies, the writer observed three kinds of asexual reproduction. Namely, asexual reproduction by quadriflagellate swimmers occurs in *M. angicava*, *M. undulatum*, *M. fuscum* var. *splendens* and *M. zostericola*, by biflagellate swimmers in *M. oxyspermum* and by aplanospores in *M. groenlandicum*. In this paper, the writer used the term "zoospore" for quadriflagellate swimmer only in the species with alternation of sexual and asexual generations.

The quadriflagellate swimmers or zoospores of *M. undulatum* and *M. angicava* are formed in the unicellular cyst, as described in *M. wittrockii* (MOEWUS, 1938), *M. latissimum* and *M. nitidum* (ARASAKI, 1946, 1949), and *M. grevillei* (KORNMANN, 1962). Those of *M. fuscum* var. *splendens*, *M. zostericola* and *M. undulatum* are formed on the leafy frond as in *M. obscurum* (GAYRAL, 1961, 1962) and *M. leptodermum* (KORNMANN and SAHLING, 1962). *M. undulatum*, however, has two kinds of quadriflagellate swimmers derived from the leafy frond and the cyst. According to KORNMANN and SAHLING, in *M. leptodermum* the quadriflagellate swimmers are produced on the branched filament as well as on the leafy frond.

The quadriflagellate swimmers or zoospores have an eyespot in most species of *Monostroma* so far described, with the exception of *M. undulatum*, *M. zostericola* and *M. leptodermum*. These swimmers with the eyespot show a positive phototaxis, but those with no eyespot usually show no phototaxis. They are less

active than the gametes and also have a shorter period of motility.

The biflagellate asexual swimmers were observed by the writer in *M. oxyspermum* as described by KORNMAN (1964) and GAYRAL (1964). Asexual reproduction by biflagellate swimmers has been described in *M. wittrockii* (BLIDING, 1935; KIDA, 1964, 1966), *M. tubiforme* (IWAMOTO, 1960) and *M. arcticum* (KORNMAN and SAHLING, 1962). In *M. oxyspermum*, these swimmers have an eyespot and show a slightly positive phototaxis shortly after liberation, as described in *M. tubiforme* (IWAMOTO) and *M. wittrockii* (KIDA). In general, such swimmers soon change phototaxis from positive to negative before settling down on the substratum. According to KORNMAN and SAHLING, in *M. arcticum* some swimmers show no phototaxis and some are negatively phototactic. These biflagellate swimmers are quite similar to the gametes of a certain species (e.g. *M. angicava*) in structure but they are less sensitive to light and less active than the gametes.

The aplanospores of *M. groenlandicum* are formed in the unicellular cyst. They are usually spherical in shape and vary widely in size according to individuals and culture conditions. This kind of asexual reproduction has been observed by YOSHIDA (1967) in *M. latissimum* grown at a high temperature of 29.5°C. or more. This shows that the formation of aplanospores in the cyst depends upon culture conditions.

##### 5. Parthenogenesis

Since CARTER (1926) first reported that in *M. latissimum* the gamete of either sex developed parthenogenetically into an enlarged cyst, the parthenogenetic development of gametes in *Monostroma* has been reported by various investigators; by MOEWUS (1938) in *M. wittrockii*, by ARASAKI (1946, 1949) in *M. latissimum* and *M. nitidum*, by KORNMAN (1962) in *M. grevillei*, by GAYRAL (1962) in *M. obscurum*, and by TATEWAKI (1962, 1963) and DUBE (1962) in *M. fuscum* var. *splendens*.

In the present study the writer observed the development of parthenogametes in *M. angicava* and *M. fuscum* var. *splendens*. In *M. angicava* the parthenogamete developed into an enlarged unicellular cyst which produced a number of bi- or quadri-flagellate swimmers, each of which developed into the leafy gametophyte of the respective sex. In *M. fuscum* var. *splendens* the gamete of either sex germinated parthenogenetically and developed into the respective leafy gametophyte.

According to the previous records, the latter developmental type of parthenogametes has been observed not only in the species which have a monomorphic alternation of generations (*M. obscurum* and *M. fuscum* var. *splendens*), but also in the species with a dimorphic alternation as in *M. wittrockii* (MOEWUS, 1938).

Further, according to KORNMAN, in *M. grevillei* most of the parthenogametes develop into the cyst which forms a number of quadriflagellate swimmers, each of which develops into the respective gametophyte, but some of them develop directly into the gametophyte without passing through the cyst stage.

On the other hand, ARASAKI reported that in both *M. latissimum* and *M. nitidum* the parthenogamete developed into the cyst as described by CARTER, but the cyst produced 64–80 swimmers as large as the gametes of these species.

The parthenogenetic development of gametes is summarized as follows:

- 1) The gamete develops into a new gametophyte of the respective sex, passing through the cyst stage
  - a) The cyst produces bi- or quadriflagellate swimmers  
*M. angicava*
  - b) The cyst produces only quadriflagellate swimmers  
*M. grevillei*
  - c) The cyst produces biflagellate (?) swimmers as large as gametes  
*M. latissimum*                      *M. nitidum*
- 2) The gamete develops directly into a new gametophyte of the respective sex  
*M. wittrockii*                      *M. grevillei*  
*M. fuscum* var. *splendens*      *M. obscurum*

## 6. Types of life cycle

In the present culture experiment, the writer has succeeded to observe life histories of six species of *Monostroma* from generation to generation by a "spore to spore" culture method. These six species showed quite different types of life cycle respectively.

In *M. groenlandicum*, the gametophyte is a cylindrical or tubular frond and the sporophyte is a unicellular cyst which produces aplanospores. The aplanospore grows into a cylindrical gametophyte which produces gametes. Consequently, in *M. groenlandicum*, dimorphic generations alternate; one is a cylindrical gametophyte and the other is a unicellular sporophyte. This type of life cycle with a cyst which forms aplanospores has been found in *Ulothrix flexuosa* by KORNMAN (1964).

In *M. angicava*, the gametophyte is a leafy frond and the sporophyte is a unicellular cyst which produces zoospores. The zoospore grows into a new gametophyte which produces gametes. The male and the female gametes are produced on separate fronds. There is thus in *M. angicava* a dimorphic alternation of a macroscopic gametophyte (leafy frond) and a microscopic unicellular sporophyte (cyst), as reported by YAMADA and SAITO (1938). The same dimorphic type of life cycle has been reported by MOEWUS (1938) in *M. wittrockii*, by ARASAKI

(1946, 1949) in *M. latissimum* and *M. nitidum*, and by KORNMANN (1962) in *M. grevillei*.

According to KORNMANN (1964), however, in *M. bullosum* the zygote developed branched filaments as well as unicellular cysts in the same culture. The former produced zoospores which developed into new gametophytes, but the latter did not become fertile. There are two kinds of asexual phases in this alga, if the cyst produces zoospores. Namely, *M. bullosum* has trimorphic (?) generations; leafy sexual generation, filamentous and unicellular asexual generations.

In *M. undulatum*, two generations alternate and are dimorphic; one is a leafy frond and the other is a unicellular cyst, but both are non-sexual. The leafy frond produces quadriflagellate swarmers which develop into cysts. The cyst produces a number of quadriflagellate swarmers. The swarmer from the cyst develops into a leafy frond which forms quadriflagellate swarmers. The life history of this alga has been reported by YAMADA and SAITO (1938) and YOSHIDA (1964) under the name of *M. pulchrum*, and by KORNMANN and SAHLING (1962). The results of the writer agree well with theirs.

In *M. zostericola*, two generations alternate and are dimorphic; the sporophyte is a macroscopic leafy frond, while the gametophyte is a minute prostrate disc. The zoospore from the leafy frond develops into a minute discoid gametophyte which produces gametes. The zygote develops into a new leafy sporophyte which produces zoospores. This type of life cycle with discoid gametophytes has never been reported in *Monostroma* species. YAMADA and KANDA (1941), working on the same species, have reported only asexual reproduction by quadriflagellate swarmers. According to them, the leafy frond produces the quadriflagellate swarmers which develop directly into leafy fronds. However, the writer could not observe that the zoospores from the leafy frond developed directly into leafy fronds without passing through the discoid gametophytic generation.

In *M. oxyspermum*, the leafy frond produces biflagellate swarmers, but no sexual reproduction is found. The swarmer develops into a leafy frond which produces biflagellate asexual swarmers only. *M. oxyspermum* has no alternation of generations. The life history of this alga has been studied by KORNMANN (1964) and by GAYRAL (1964). The writer's result agrees quite well with theirs. The same type of life cycle has been reported by BLIDING (1935) and by KIDA (1964) in *M. wittrockii*, by IWAMOTO (1960) in *M. tubiforme*, and by KORNMANN and SAHLING (1962) in *M. arcticum*.

Another type of non-alternation of generations has been reported by KORNMANN and SAHLING (1962) in *M. leptodermum*. According to them, the leafy frond of this alga produces only quadriflagellate swarmers which develop directly into leafy fronds, when grown at 3–4°C. At 14–15°C. these swarmers do not grow

into new leafy fronds, but develop into creeping filaments which produce quadri-flagellate swarmers, although they grow into leafy fronds when transferred to 3-4°C. Consequently, this alga has two kinds of asexual phase by quadriflagellate swarmers according to culture conditions. In this case, the creeping filament grown at high temperature and produced swarmers, may be considered a stunted form of the leafy frond of this alga.

In *M. fuscum* var. *splendens*, two generations alternate and are monomorphic. Both sexual and asexual plants are leafy and quite identical morphologically. The sexual plant produces isogametes and the asexual plant produces zoospores. The zygote grows into an asexual plant and the zoospore grows into the male and the female plant. This type of life cycle has been reported by GAYRAL (1962) in *M. obscurum* and it is quite similar to that of *Ulva*.

From a view-point of life histories, the species of the genus *Monostroma* can be classified into the following groups, basing upon the results of the culture studies so far described.

- 1) Dimorphic alternation of generations
  - A) With sexual and asexual generations
    - a) The sexual generation is leafy and the asexual one is a unicellular cyst
      - M. groenlandicum* (Fig. 21, A)
      - M. latissimum* (Fig. 21, B)
      - M. nitidum* (Fig. 21, C)
      - M. wittrockii* (sensu MOEWUS, Fig. 21, D)
      - M. angicava* (Fig. 21, E)
      - M. grevillei* (Fig. 21, F)
      - (*M. bullosum* Fig. 22, A)
    - b) The sexual generation is a minute disc and the asexual one is leafy
      - M. zostericola* (Fig. 22, C)
  - B) With two asexual generations
    - One is leafy and the other is a unicellular cyst
      - M. undulatum* (Fig. 22, B)
- 2) Non-alternation of generations
  - A) By biflagellate asexual swarmers
    - M. oxyspermum* (Fig. 22, F)
    - M. wittrockii* (sensu BLIDING and KIDA)
    - M. tubiforme*
    - M. arcticum* (Fig. 22, E)
  - B) By quadriflagellate asexual swarmers

*M. leptodermum* (Fig. 22, D)

3) Monomorphic alternation of generations

*M. fuscum* var. *splendens* (Fig. 22, G)

*M. obscurum* (Fig. 22, H)

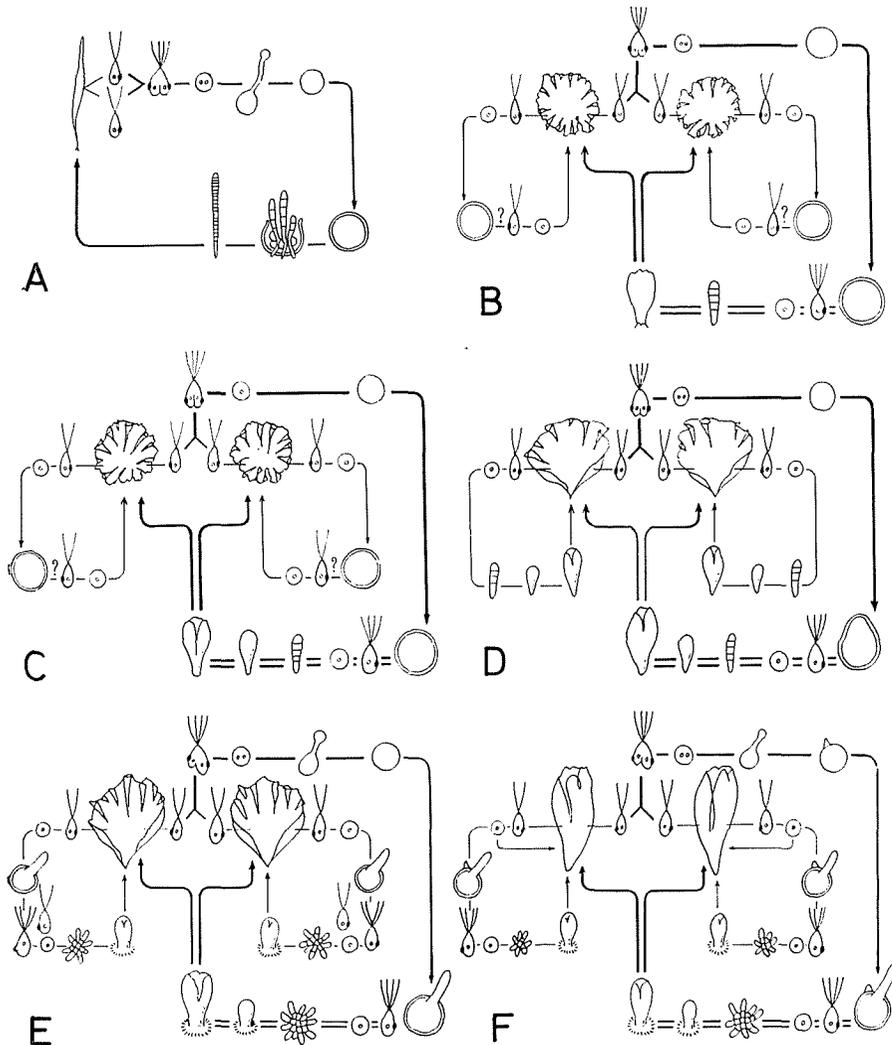


Fig. 21. The life cycles in the genus *Monostroma* hitherto known

- A. *M. groenlandicum*    B. *M. latissimum*    C. *M. nitidum*  
 D. *M. wittrockii*    E. *M. angicava*    F. *M. grevillei*

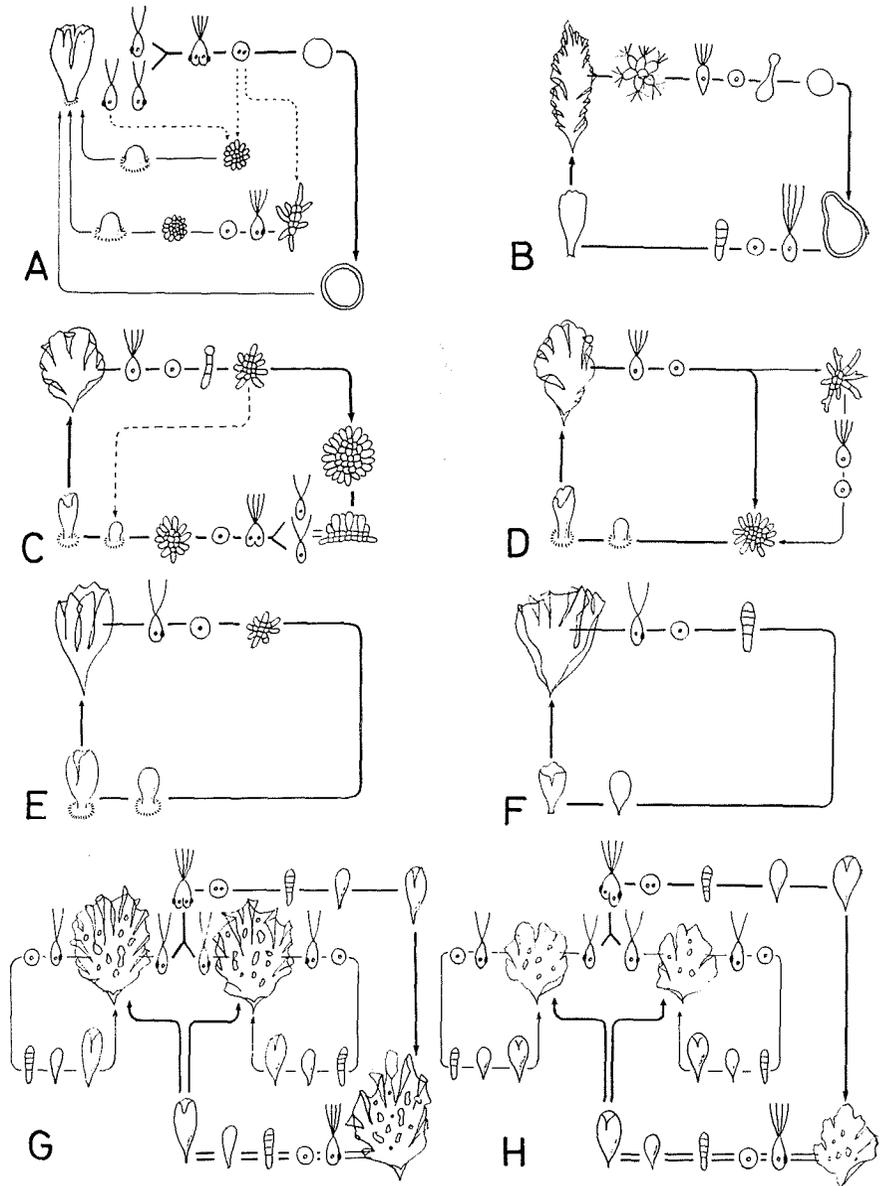


Fig. 22. The life cycles in the genus *Monostroma* hitherto known

- A. *M. bullosum*      B. *M. undulatum*      C. *M. zostericola*  
 D. *M. leptodermum*    E. *M. arcticum*      F. *M. oxyspermum*  
 G. *M. fuscum* var. *splendens*    H. *M. obscurum*

### 7. Effect of temperatures and photoperiods on the growth and maturation

At the beginning of this investigation (1957-1961), culture experiments were carried out near the north-east window of a room which the temperature and light conditions were subjected to seasonal changes. Under these conditions it was difficult to complete the life cycle of a certain species of *Monostroma* in culture and at least 10-12 months were needed when successful. However, it succeeded to complete the entire life cycle and to reduce its length by using freezer-incubators regulated temperatures and photoperiods. For example, one entire life cycle was completed within 40-45 days in *M. groenlandicum*, 60-75 days in *M. zostericola*, 3 months in *M. undulatum*, and 4 months in *M. angicava*.

#### 1) Culture experiments at 14°C. in a 14-hr photoperiod.

Under this culture condition the cysts of *M. groenlandicum*, *M. angicava* and *M. undulatum* increased enormously in size and attained a diameter of 60  $\mu$ , 150  $\mu$  and 90  $\mu$  in 60 days respectively, but they did not become fertile. The gametophytic disc of *M. zostericola* also increased in size rapidly and attained a diameter of 500  $\mu$  or more in 60 days, often forming a green tuft, but did not mature. On the other hand, in *M. groenlandicum*, the germling from the aplanospore developed into a somewhat dwarf cylindrical frond which soon matured to produce gametes. In *M. zostericola*, the germling from the zygote did not develop into the leafy frond, but remained as a prostrate disc which matured to produce zoospores. In both *M. angicava* and *M. undulatum*, the germlings did not form leafy fronds. However, the leafy fronds of *M. fuscum* var. *splendens* and *M. oxyspermum* showed the best growth and reached maturity.

#### 2) Culture experiments at 10°C. in a 14-hr photoperiod.

In *M. groenlandicum*, *M. angicava* and *M. undulatum*, the growth and maturation of their cysts were similar to those obtained in the cultures at 14°C. in a 14-hr photoperiod. The fronds of *M. groenlandicum* and *M. angicava*, however, grew very well and reached maturity normally.

#### 3) Culture experiments at 12°C. in a 10-hr photoperiod.

The cyst of *M. angicava* grown at 14°C. in a 14-hr photoperiod for 30 days, produced zoospores within 20-30 days after it was transferred to this culture condition. This shows that a short photoperiod favours the maturation of the cyst.

#### 4) Culture experiments at 5°C. in a 10-hr photoperiod.

In *M. groenlandicum*, the 25-30-day old cyst attained a diameter of 20  $\mu$  and reached maturity. The cyst grown at 14°C. in a 14-hr photoperiod for 15-30 days, produced aplanospores within 7-15 days after it was transferred to this culture condition. However, the frond of this alga did not grow well and remained unfertile for a long time. The cyst of *M. undulatum* attained a diameter of 30-40  $\mu$

in 30 days and reached maturity in 50 days. The leafy frond of this alga also grew well and became fertile. The cyst grown at 14°C. in a 14-hr photoperiod for 20 days, became fertile within 20 days after transfer to this culture condition. The same results were obtained in *M. zostericola*. The discoid gametophyte usually became fertile in 30–35 days. The leafy frond of this alga also grew well and reached maturity in 30–40 days.

The results obtained from the above-mentioned experiments under four kinds of culture conditions show that a short-day condition combined with comparatively low temperature favours the maturation of cysts (*M. groenlandicum*, *M. undulatum* and *M. angicava*) and of gametophytes (*M. zostericola*). This condition also seems to be suitable for the growth and maturation of leafy fronds of *M. undulatum* and *M. zostericola*. On the contrary, a long-day condition combined with high temperature seems to be unfavorable for the maturation of cysts (*M. groenlandicum* and others), and of gametophytes of *M. zostericola*, however, it favours the growth of them. This condition also favours the growth and maturation of the frond of *M. fuscum* var. *splendens* and *M. oxyspermum*. A long-day condition with relatively low temperature may bring about a good growth and maturation of the frond of *M. groenlandicum* and *M. angicava*, though it suppresses the maturation of their cysts. Namely, it shows that the species examined require different environmental factors, especially temperature and photoperiod for growth and maturation according to the respective phases in the life cycle.

#### 8. Systematic remarks

KUNIEDA (1934), working on *Monostroma* sp. from Misaki, proposed that on the basis of its life history with an alternation of a leafy gametophytic generation and a unicellular sporophytic generation, the genus *Monostroma* should be removed from the Ulvaceae to the new family Monostromaceae. Afterwards, the life histories of the species of *Monostroma* have been studied by several investigators, and types different from the typical type described by KUNIEDA were found.

BLIDING (1960) indicated that on the basis of life histories the genus *Monostroma* should be divided into two genera; a) the genus *Monostroma* having an alternation of sexual and asexual generations, and b) the genus *Ulvaria* with only asexual reproduction by biflagellate swarmers. Later he (1963) stated that the genus *Monostroma* sensu WITTRÖCK contains three types of life cycle; an alternation of heteromorphic generations, an alternation of isomorphic generations, and no alternation of generations. He suggested that one of these groups comprising *M. fuscum* ought to be transferred to a resurrected genus *Ulvaria* (RUPRECHT, 1951) and the remaining two groups of *Monostroma* should be

divided into two genera.

GAYRAL (1964, 1965) proposed that on the basis of her studies on *M. oxyspermum*, *M. obscurum* and *M. grevillei* regarding the frond structure, reproduction and life cycle, the genus *Monostroma* should be divided into three genera, *Monostroma*, *Ulvaria* and *Ulvopsis*. According to her, the genus *Monostroma* is based upon *M. oxyspermum* (KÜTZ.) DOTY as lectotype by the fact that the cell-arrangement of the frond is in groups of twos or fours, the swarmers are discharged destructively, and its life cycle is monogenetic. She also proposed to transfer *M. obscurum* to the genus *Ulvaria* because the life cycle is digenetic and isomorphic, and because of the peculiar swarmer-liberation and structure of chloroplast, and *M. grevillei* to the newly established genus *Ulvopsis* GAYRAL because the life cycle is digenetic and heteromorphic.

KORNMAN (1964), working on *M. bullosum* and *M. oxyspermum*, stated that the genus *Monostroma* THURET is characterized by a heteromorphous life cycle and special developmental features of the former species, and that the latter species differs from these characteristics and must be excluded from this genus. He indicated that *M. grevillei* resembles *M. bullosum* in the habit, life history and development of the frond, and also *M. arcticum* is included in this group because of similarity in these features except life history. But KORNMAN made no mention of the definite taxonomic position of *Monostroma*.

The results obtained from the present study regarding life history, method of swarmer-liberation, and development of the frond, indicate that the genus *Monostroma* contains a certain number of heterogeneous members.

*M. groenlandicum* has a cylindrical habit and never assumes the expanded monostromatic structure typical of the genus *Monostroma*. On account of this, SETCHELL and GARDNER (1920) transferred this species to the genus *Enteromorpha*. However, this alga is closely related to the species of the genus *Monostroma* in cell-arrangement, intercellular substance of the frond, and life history. Accordingly, the writer described this alga as a species of the genus *Monostroma* in this paper. On the other hand, this alga has a similarity to a certain species of the genus *Ulothrix* or *Capsosiphon* in the life history and method of swarmer-liberation. Therefore, this alga seems to be an intermediate species between *Monostroma* and *Ulothrix* or *Capsosiphon*.

Both *M. fuscum* var. *splendens* and *M. obscurum* have a great similarity to the species of the genus *Ulva* in the life history and method of swarmer-liberation. Judging from the results of the present study, the writer agrees with the proposal of BLIDING (1963) and GAYRAL (1964, 1965) that *M. fuscum* and *M. obscurum* should be transferred to the revived genus *Ulvaria*.

*M. angicava* closely resembles *M. grevillei* in the habit, life history and

development of the frond. It is reasonable that GAYRAL has placed *M. angicava* in the group of *M. grevillei*. However, the writer hesitates to follow GAYRAL who is of opinion that both the species should be removed from the Monostromaceae to the Ulvaceae as the genus *Ulvopsis*.

*M. zostericola* and *M. leptodermum* is alike in the habit, early development of the frond and structure of swarmers, though the latter is lacking sexuality in the life cycle. *M. zostericola* also resembles the species of the genus *Blidingia* in the structure of swarmers, cell-size and early development of the frond, while it is similar to *M. fuscum* and *Ulva* species in the method of swarmer-liberation. From this, *M. zostericola* seems to be an intermediate species between the genera *Monostroma* and *Blidingia* belonging to the Ulvaceae.

*M. oxyspermum* closely resembles *M. wittrockii* described by KIDA (1964, 1966) in the habit, development of the frond and method of swarmer-liberation. The life cycle of *M. oxyspermum* is identical with that of *M. wittrockii* sensu BLIDING (1935) and KIDA, though it differs from that of *M. wittrockii* sensu MOEWUS (1938) and it corresponds to that of parthenogenesis of MOEWUS' plant.

Both *M. undulatum* and *M. latissimum* are included in the same group because of similarity in the habit, development of the frond and method of swarmer-liberation. Both species are alike in the dimorphic life cycle with a cyst generation, but the former is lacking in sexual reproduction.

These four species, *M. oxyspermum*, *M. wittrockii*, *M. latissimum* and *M. undulatum* have a great similarity in the swarmer-liberation and structure of the mature frond, especially cell-arrangement in surface view.

As mentioned above, the genus *Monostroma* is demonstrated to include heterogeneous species as follows: 1) *groenlandicum*-group, 2) *undulatum*-group, 3) *oxyspermum*-group, 4) *angicava*-group, 5) *zostericola*-group and 6) *fuscum*-group.

The systematic status of these groups will be discussed in near future after further investigations regarding the constitution of cell walls and nuclear phase of the species concerned.

### Summary

Six species of the genus *Monostroma*, *M. groenlandicum*, *M. undulatum*, *M. oxyspermum*, *M. angicava*, *M. zostericola* and *M. fuscum* var. *splendens* were investigated regarding the structure and development of the frond, method of swarmer-liberation, reproduction, and life cycles.

1. The structure and development of the frond are divided in the following types. A) The erect filament type; the germling develops into an erect uniseriate filament, a) developing into a cylindrical frond (*M. groenlandicum*), b) developing

into an expanded monostromatic membrane, passing through the saccate stage (*M. oxyspermum* and *M. fuscum* var. *splendens*), and c) developing directly into an expanded monostromatic membrane without passing through the saccate stage (*M. undulatum*). B) The prostrate disc-type; the germling develops into a minute prostrate disc, a) giving rise to a saccate frond which develops into an expanded monostromatic membrane (*M. zostericola* and *M. angicava*) and b) giving rise to an erect filament which develops directly into an expanded monostromatic membrane (*M. zostericola*).

2. The method of swarmer-liberation are summarized as follows: A) The fertile marginal part of the frond disintegrates into pieces, and then detached cells break and liberate swarmers; liberated swarmers form a) an oscillating mass, remaining attached to each other before swimming away (*M. undulatum*), or b) soon swim away freely (*M. oxyspermum*). B) Swarmers are liberated a) as a mass enclosed within a sac through a linear-shaped pore (*M. groenlandicum*), b) simultaneously and freely through a pore with an irregular margin (*M. angicava*), or c) one by one through a round pore (*M. fuscum* var. *splendens* and *M. zostericola*).

3. The life cycle of the species examined are as follows: a) In *M. groenlandicum*, two generations alternate and are dimorphic; one is a monoecious cylindrical gametophyte and the other is a unicellular sporophyte which produces aplanospores. b) In *M. angicava*, two generations alternate and are dimorphic; one is a dioecious leafy gametophyte and the other is a unicellular sporophyte which produces zoospores. c) In *M. undulatum*, two generations alternate and are dimorphic; one is a leafy frond and the other is a unicellular cyst, but both are non-sexual, producing quadriflagellate swarmers. d) In *M. zostericola*, two generations alternate and are dimorphic; one is a leafy sporophyte and the other is a minute discoid gametophyte which is monoecious. e) In *M. oxyspermum*, there is no alternation of generations and only an asexual generation is repeated by biflagellate swarmers. f) In *M. fuscum* var. *splendens*, two generations alternate and are monomorphic; the dioecious gametophyte and the sporophyte are morphologically identical.

4. The genus *Monostroma* is demonstrated to include heterogeneous groups as follows: *groenlandicum*-, *undulatum*-, *oxyspermum*-, *angicava*-, *zostericola*-, and *fuscum*-group.

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PLATE I

*Monostroma groenlandicum* J. AGARDH

- A. Habit of fertile plants.
- B. Surface view of the middle part of a frond.
- C. Surface view of the lower part of a frond.
- D-E. Cross section of the middle part of a frond.
- F. Cross section of the middle part of a young frond.
- G. Cross section of the basal part of a frond.

A,  $\times 2/3$ . B-D,  $\times 1000$ . E-F,  $\times 400$ . G,  $\times 500$ .

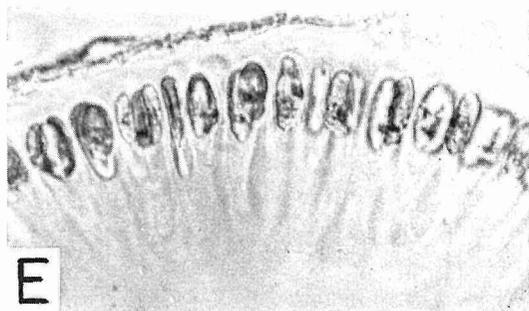
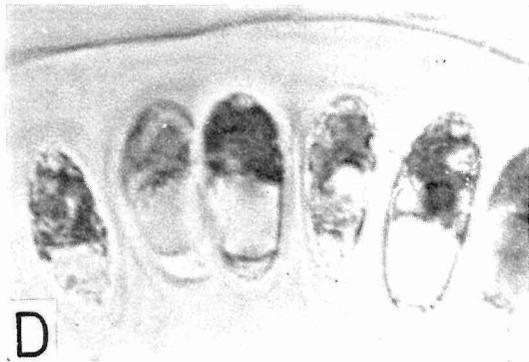
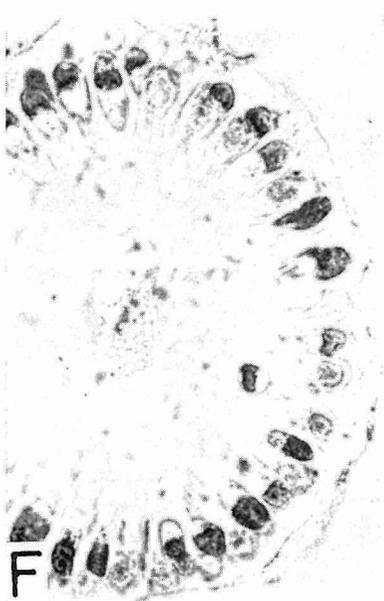
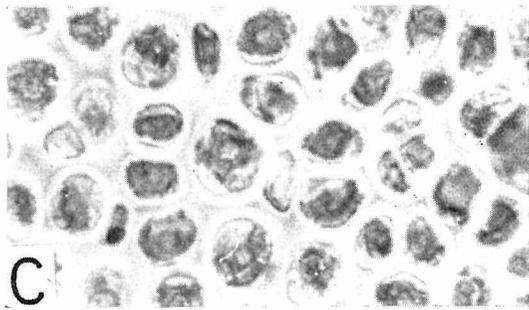
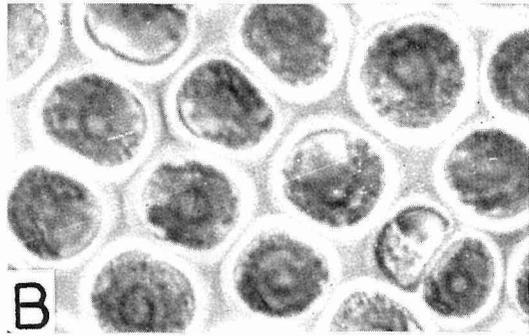


PLATE II

*Monostroma groenlandicum* J. AGARDH

- A. Surface view of the fertile part (gametangia) of a frond.
- B. Side view of the same.
- C. Liberation of gametes from a frond in nature.
- D. Liberation of gametes from a frond in culture.
- E. Surface view of emptied gametangia, showing liberation-pores on the surface wall of a frond.
- F. Conjugation of gametes.
- G. Zygotes from 10-day old culture grown in SCHREIBER's solution.
- H. Zygotes from 25-day old culture grown in SCHREIBER's solution.
- I. Zygotes from 5-day old culture grown in ESP medium.
- J. Zygotes from 15-day old culture grown in ESP medium at 14°C. in a 14-hr photoperiod.
- K. Cysts from 180-day old culture grown in SCHREIBER's solution at room temperature.

A-B & E-K, × 1000. C, × 100. D, × 500.

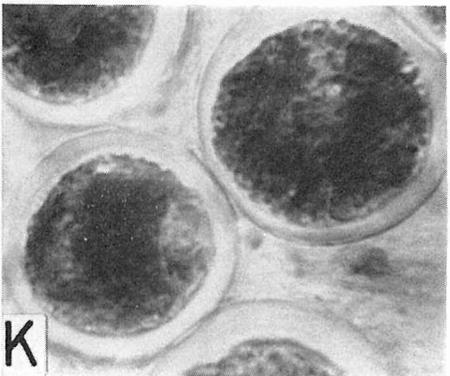
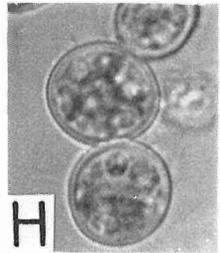
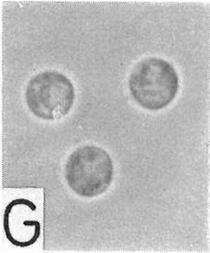
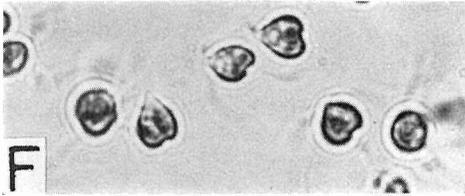
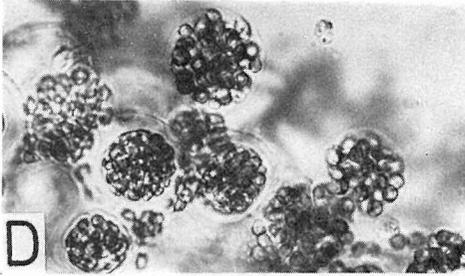
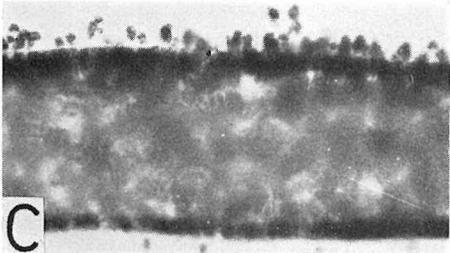
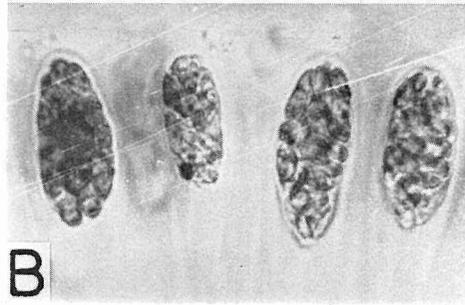
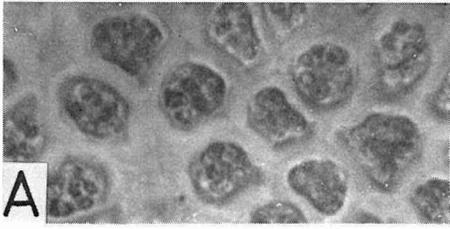


PLATE III

*Monostroma groenlandicum* J. AGARDH

- A. Zygotes with a long germination tube, from 7-day old culture grown in ESP medium.
- B. Cysts from 25-day old culture grown in ESP medium at 5°C. in a 10-hr photoperiod, showing the formation of aplanospores.
- C. Cysts from 220-day old culture grown in SCHREIBER's solution at room temperature, showing the formation of aplanospores.
- D-E. Young germlings grown at 5°C. in a 10-hr photoperiod.
- F-H. Development of cylindrical frond showing a progression from uniseriate, through biseriate, to multiseriate stages.
- I. Surface view of fertile gametangia of a 25-day old plant grown at 10°C. in a 14-hr photoperiod.

A & D, × 400. B & F-H, × 300. C, × 1000. E, × 600. I, × 500.

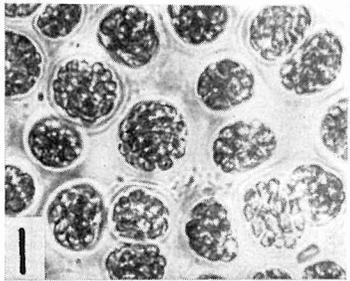
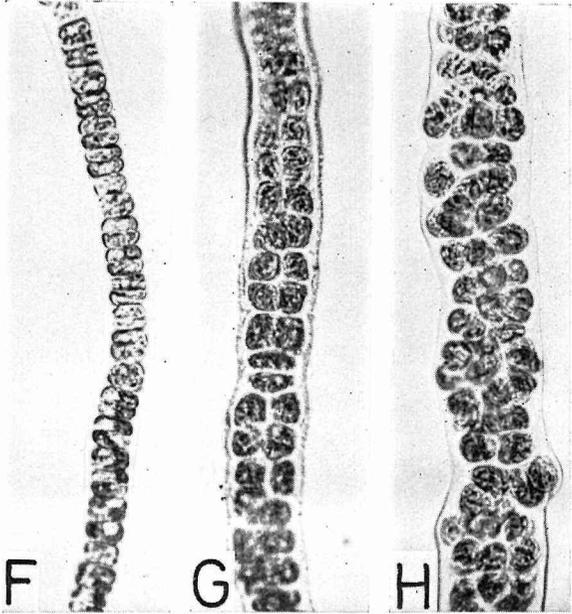
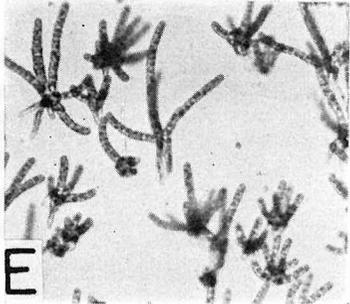
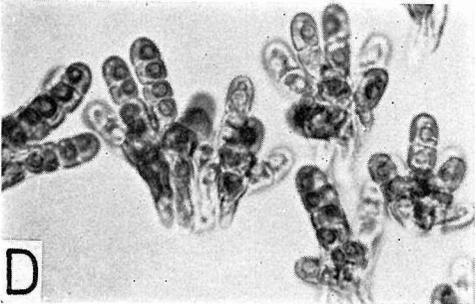
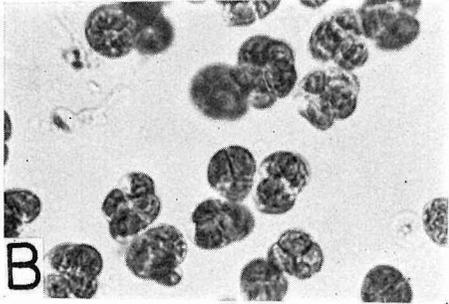
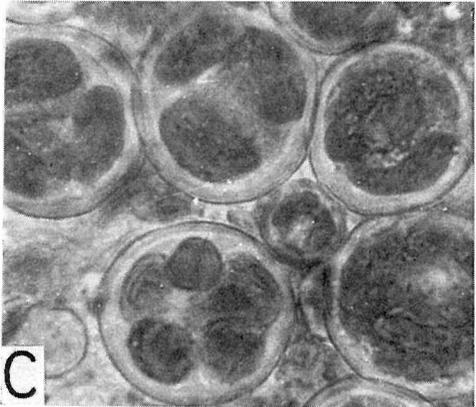
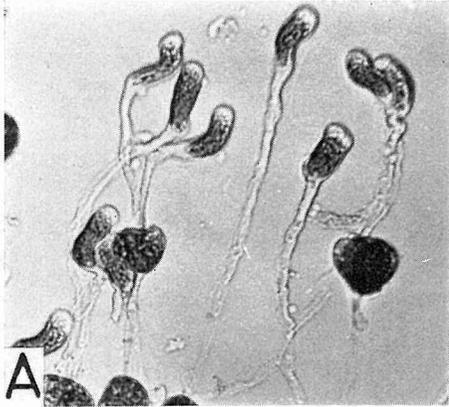


PLATE IV

*Monostroma undulatum* WITTROCK

- A-C. Fertile plants showing various shapes.
- D. Surface view of the upper part of a frond.
- E-F. Surface view of the middle part of a frond.
- G. Surface view of the lower part of a frond.
- H. Surface view of the rhizoid-bearing part of a frond.
- I. Cross section of the middle part of a frond.
- J. Cross section of the rhizoid-bearing part of a frond.

(D-H. Photographed from living material.)

A-C,  $\times 1/2$ . D-H,  $\times 300$ . I-J,  $\times 400$ .

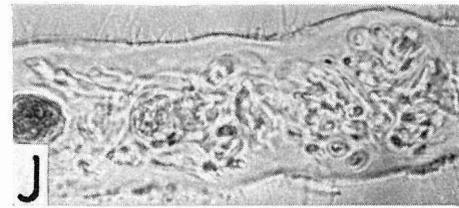
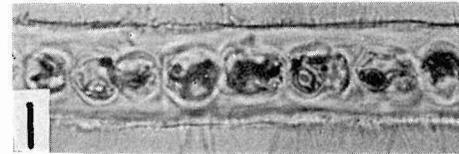
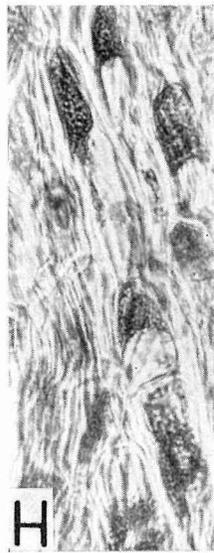
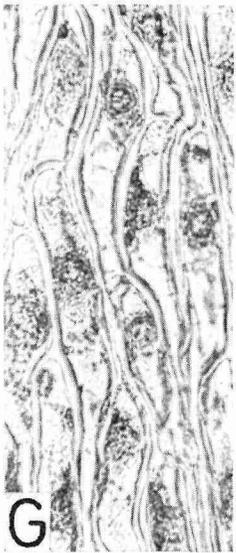
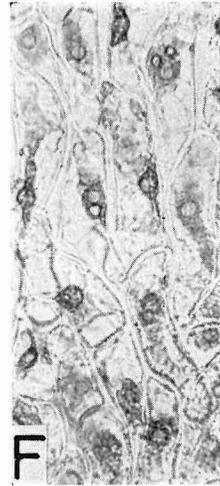
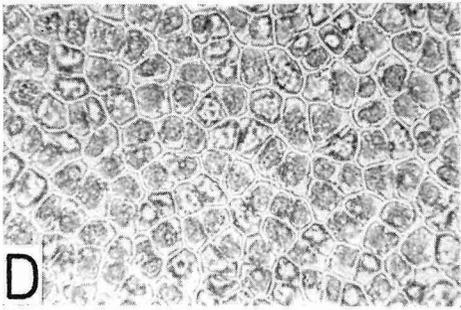
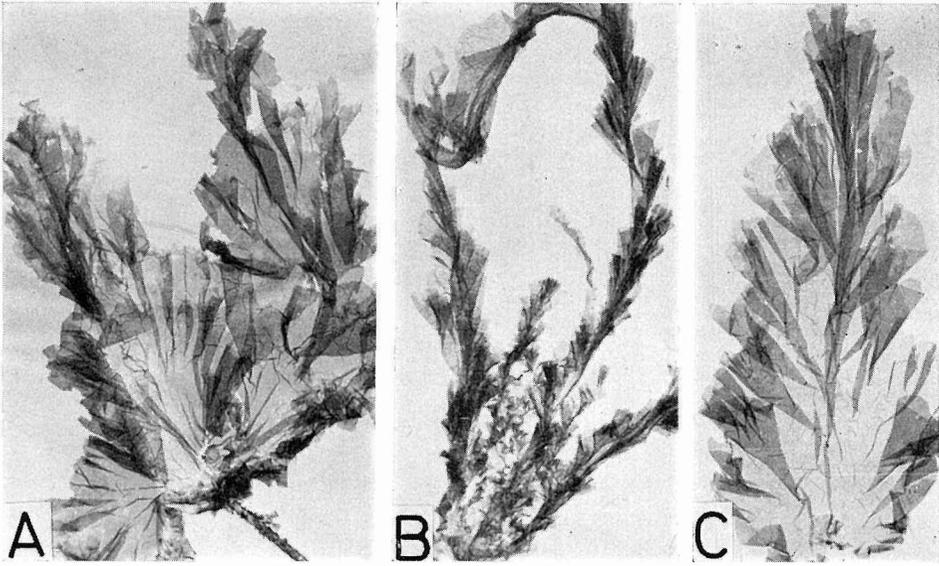


PLATE V

*Monostroma undulatum* WITTROCK

- A-B. Surface view of the fertile part of a frond, showing a loose arrangement of fertile sporangia.
- C-F. Liberation of swarmers from a leafy frond, showing a mass of oscillating swarmers.
- G. Swarmers from a leafy frond, fixed by formalin vapour.
- H. Germlings from 3-day old culture, showing migration of cell contents.
- I. Germlings from 8-day old culture, which develop without taking place migration of cell contents.

A, D, E, G & H, × 1000. B & I, × 665. C & F, × 400.

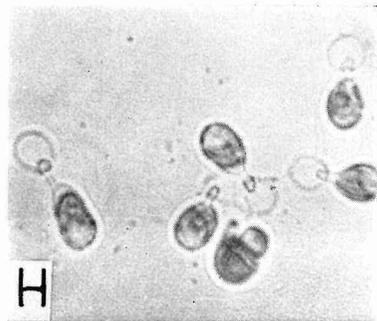
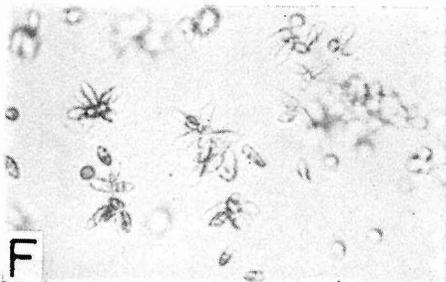
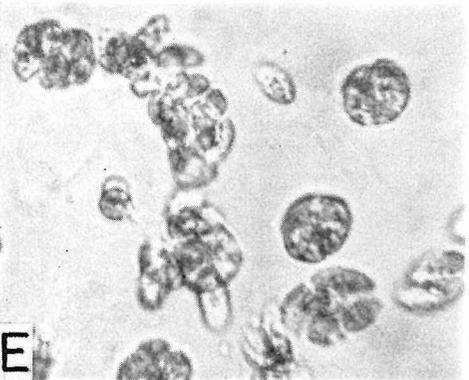
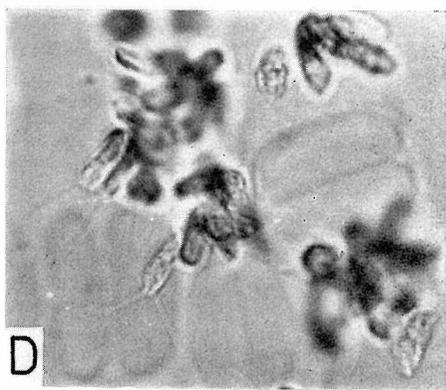
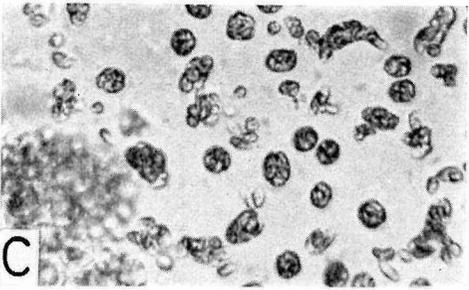
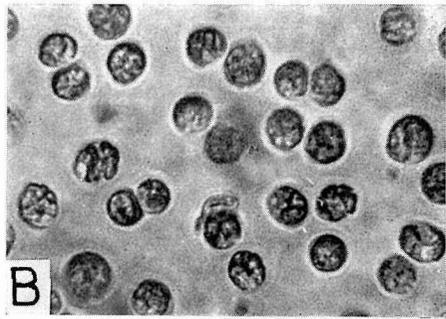
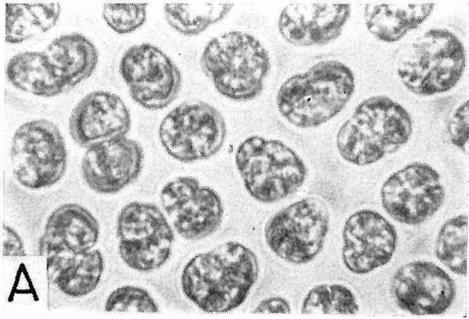


PLATE VI

*Monostroma undulatum* WITTROCK

- A. Cysts from 45-day old culture.
  - B. Thick-walled cysts from 3-month old culture.
  - C. Fertile cysts from 8-month old culture.  
(A-C. Cultures grown at room temperature).
  - D-H. Young fronds from 20-day old culture grown at 5°C. in a  
10-hr photoperiod, showing the monostromatic development.
  - I. Two-month old frond grown at room temperature.
- A, × 850.    B, × 560.    C, × 715.    D & G, × 500.    E, × 650.  
F, × 400.    H, × 250.    I, × 52.

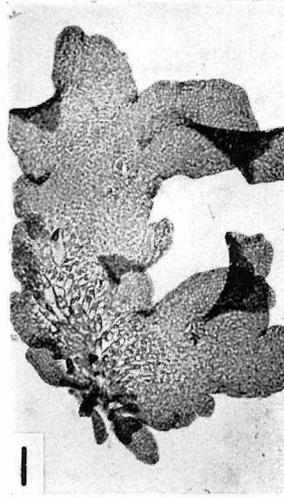
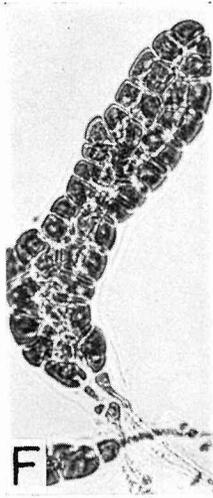
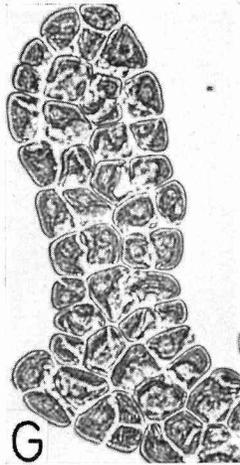
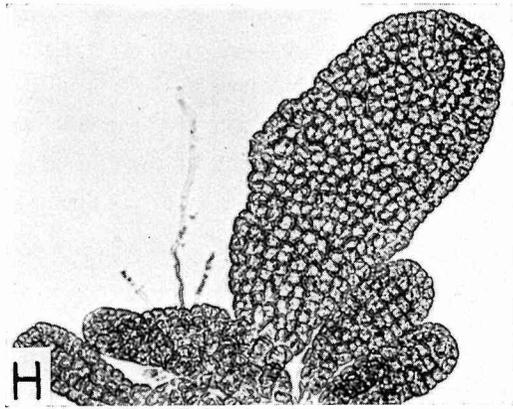
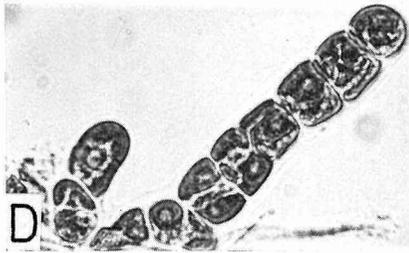
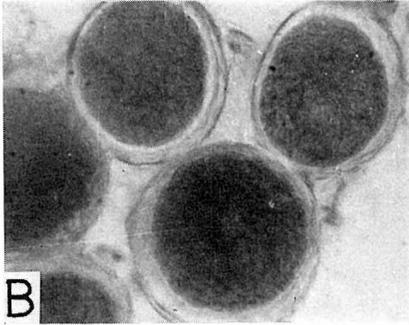
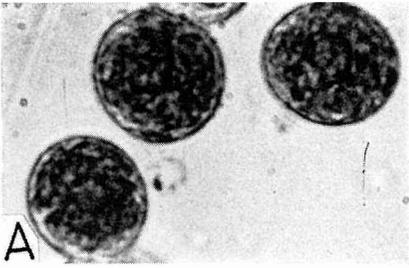
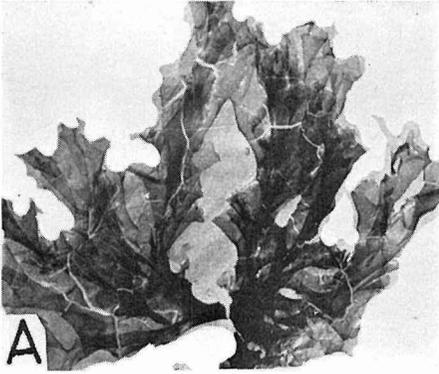


PLATE VII

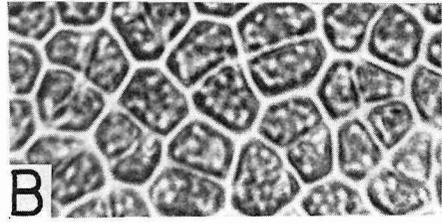
*Monostroma oxyspermum* (Kütz.) Doty

- A. Fertile plant.
- B-C. Surface view of the upper part of a frond.  
(B. Photographed from living material.)
- D. Surface view of the middle part of a frond.
- E. Surface view of the lower part of a frond.
- F. Surface view of the rhizoid-bearing part of a frond.
- G. Cross section of the middle part of a frond.
- H. Cross section of the rhizoid-bearing part of a frond.
- I. Vertical section of the lower part of a frond.

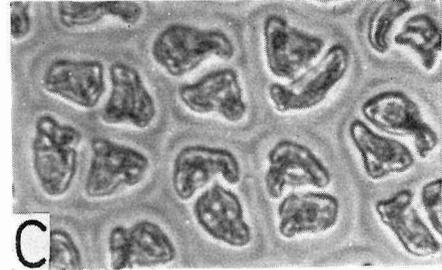
A,  $\times 1$ . B,  $\times 800$ . C,  $\times 1000$ . D-I,  $\times 400$ .



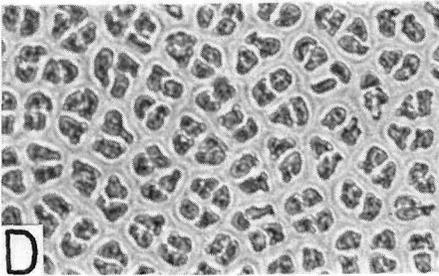
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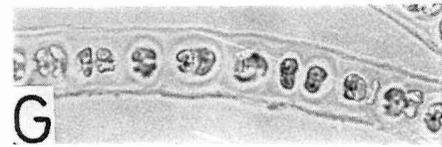
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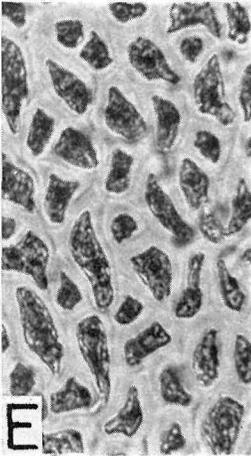
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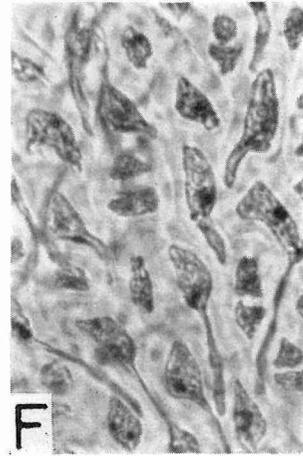
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G



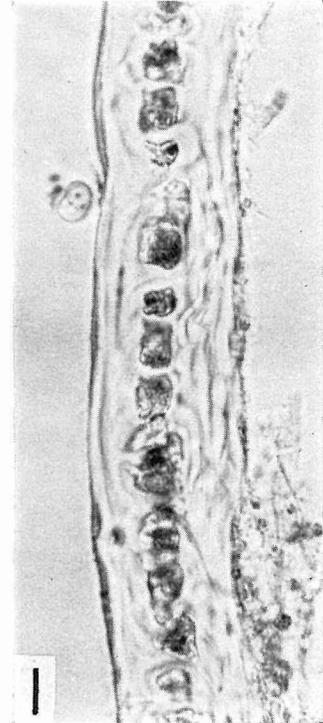
E



F



H



I

PLATE VIII

*Monostroma oxyspermum* (Kütz.) Doty

- A. Surface view of the fertile part of a frond, showing liberation of swarmers.
- B. Swarmers.
- C-D. Saccate frond from 20-day old culture.
- E. Opening of a saccate frond in 25-day old culture.
- F-G. Young funnel-shaped fronds from 30-day old culture.
- H. Liberation of swarmers in 50-day old culture.

A, × 800.    B, × 1000.    C, × 150.    D-E, × 200.  
F-G, × 50.    H, × 500.

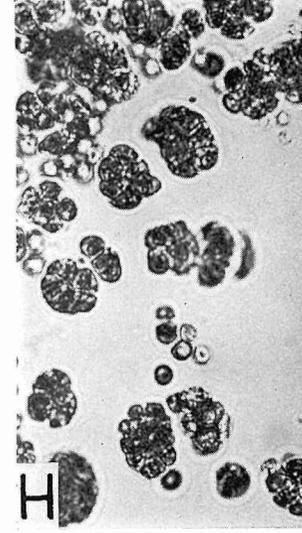
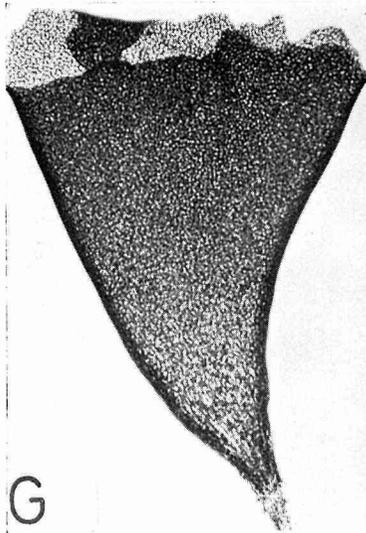
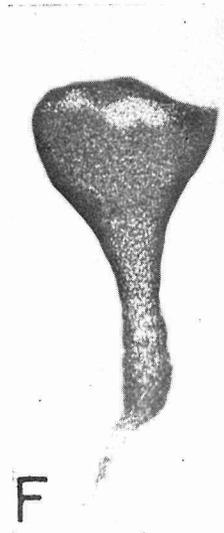
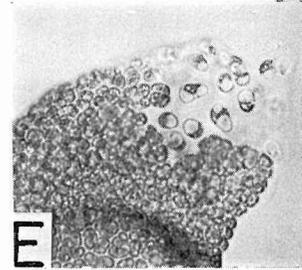
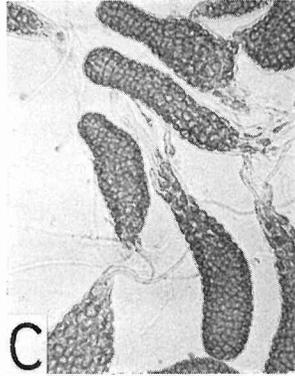
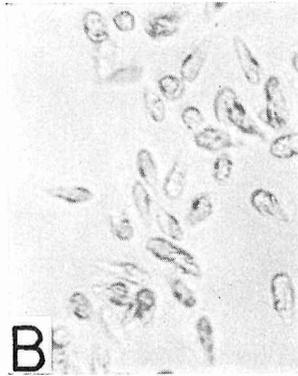
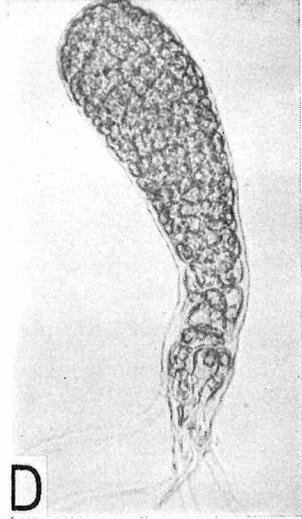
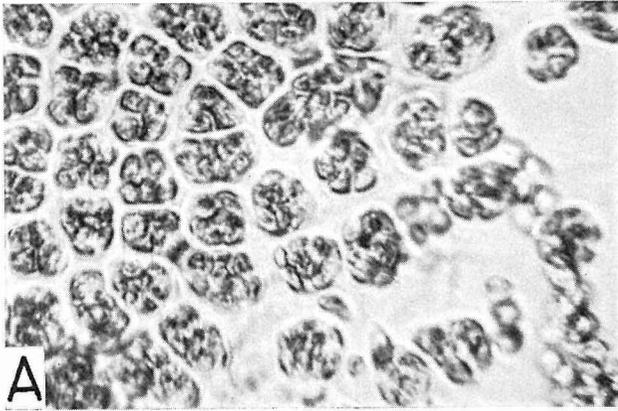


PLATE IX

*Monostroma angicava* KJELLMAN

- A-B. Fertile plants.
  - C. Young saccate plant.
  - D. Surface view of the upper marginal part of a frond.
  - E. Surface view of the middle part of a frond.
  - F-G. Surface view of the lower part of a frond.
  - H. Vertical section of the rhizoid-bearing part of a frond.
  - I. Cross section of the upper part of a frond.
  - J. Cross section of the middle part of a frond.
  - K. Cross section of the rhizoid-bearing part of a frond.
- (D-G. Photographed from living material.)

A-B,  $\times 2/3$ . C,  $\times 1$ . D & F-K,  $\times 400$ . E,  $\times 1000$ .

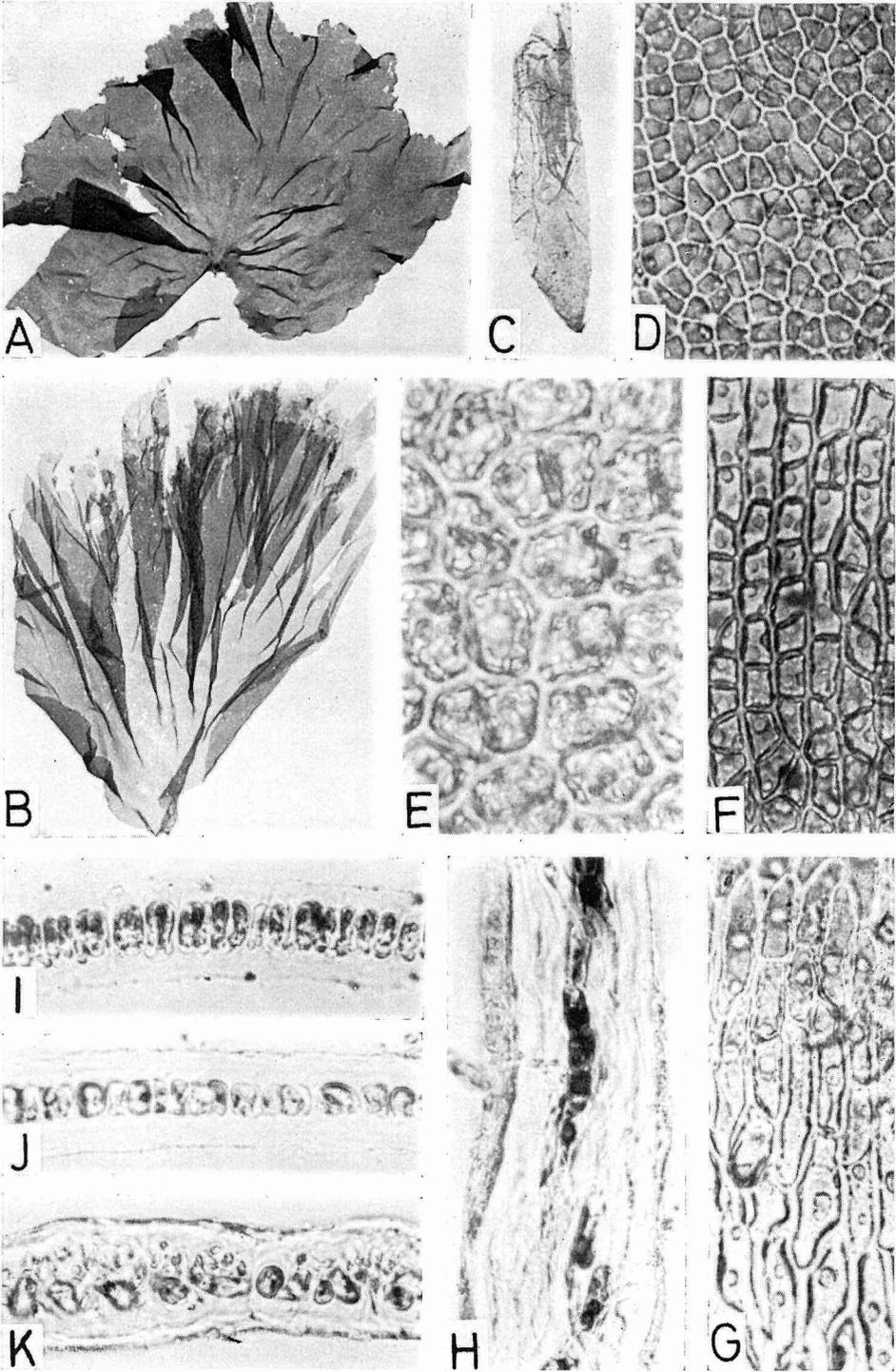


PLATE X

*Monostroma angicava* KJELLMAN

- A. Surface view of the fertile part (gametangia) of a female frond.
- B. Surface view of the fertile part (gametangia) of a male frond.
- C. Surface view of emptied gametangia stained with methyl green, showing liberation-pores.
- D. Surface view of emptied gametangia photographed from living material.
- E. Side view of fertile gametangia stained with methyl green.
- F. Side view of emptied gametangia stained with methyl green.
- G. Female gametes.
- H. Male gametes.
- I. Conjugation of gametes.

A-I, × 1000.

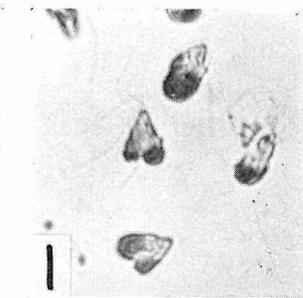
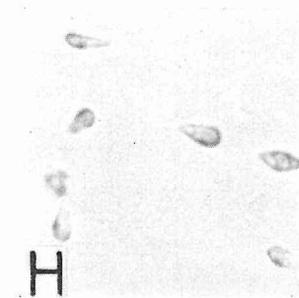
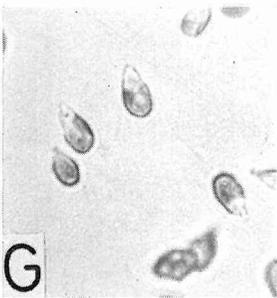
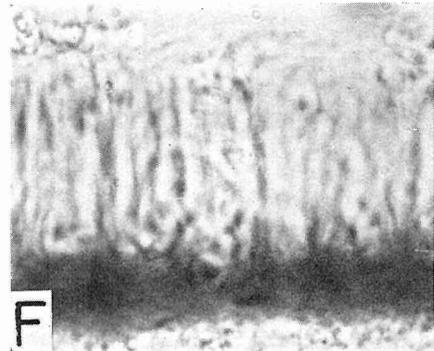
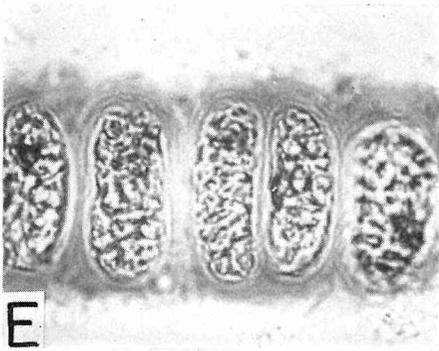
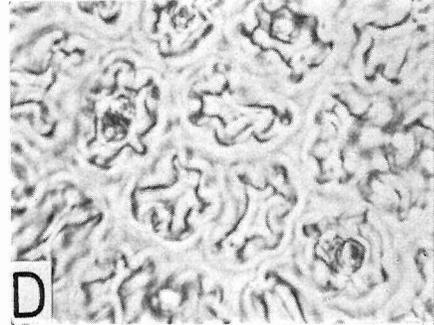
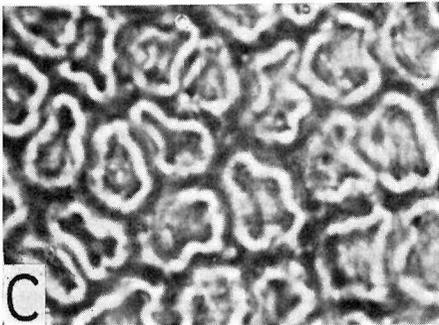
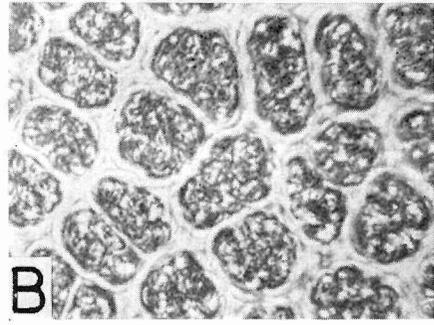
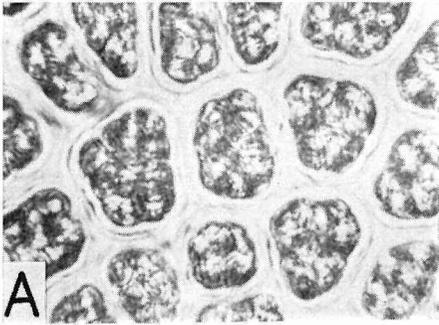


PLATE XI

*Monostroma angicava* KJELLMAN

- A. Zygotes.
  - B. Zygotes with a vestige of the original cell, from 5-day old culture.
  - C. Zygotes from 7-day old culture, taking various shapes.
  - D-E. Zygotes from 20-day old culture.
  - F. Cysts from 32-day old culture.
  - G. Thick-walled cysts from 5-month old culture grown at room temperature.
  - H. Cysts with a striped rhizoid (*Codiolum*-like cysts) from 50-day old culture grown at 12°C. in a 10-hr photoperiod.
- A-B, × 1000.    C-E, × 300.    F-G, × 500.    H, × 200.

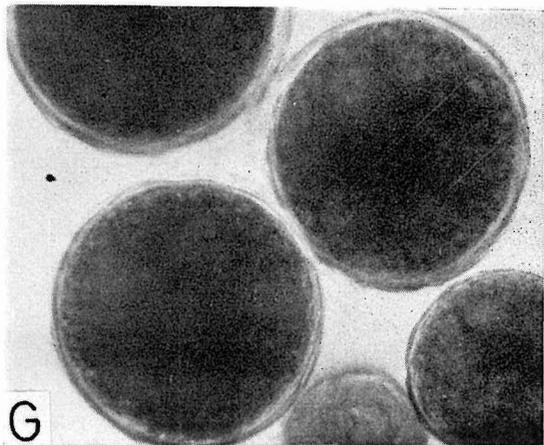
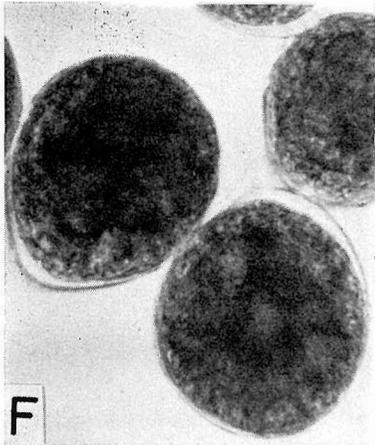
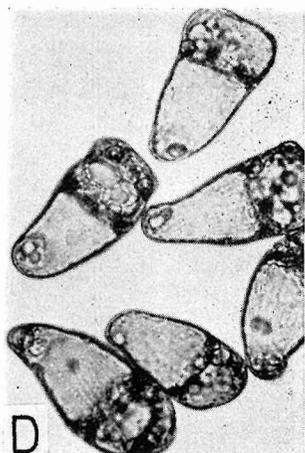
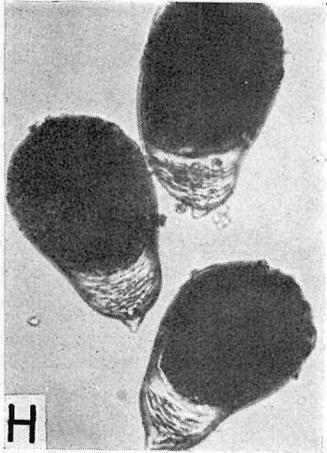
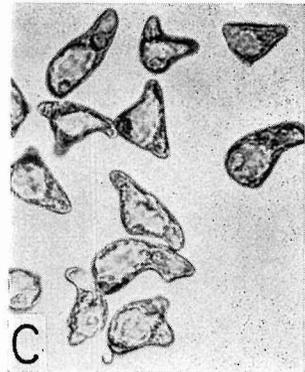
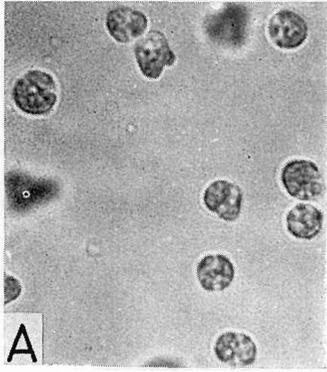


PLATE XII

*Monostroma angicava* KJELLMAN

- A. Fertile cyst grown at 12°C. in a 10-hr photoperiod.
- B. Liberation of zoospores.
- C. Zoospores stained with a weak solution of iodine.
- D. Creeping filamentous germling from 5-day old culture.
- E. Prostrate disc from 35-day old culture grown at room temperature.
- F. Prostrate disc from 30-day old culture grown at 12°C. in a 10-hr photoperiod, showing an upheaval.
- G. Surface view of a saccate frond arising from the disc, from 50-day old culture grown at room temperature.

A-B, × 300. C-D, × 1000. E, × 500. F, × 100. G, × 160.

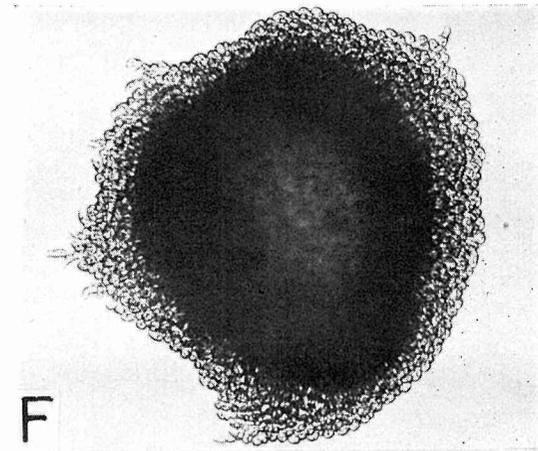
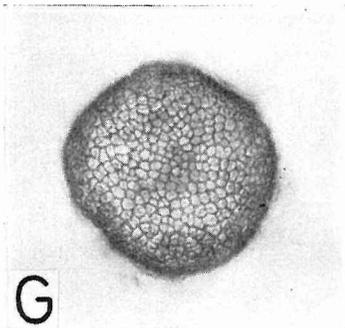
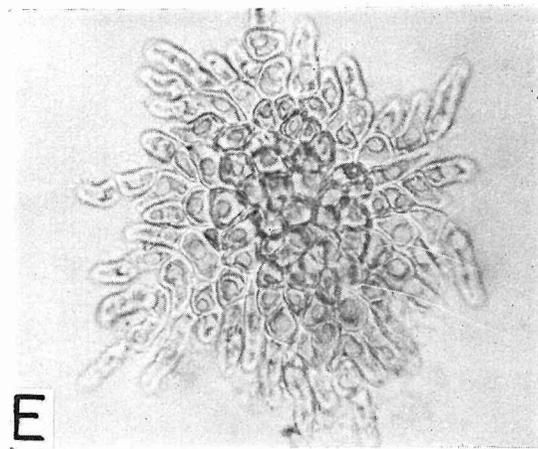
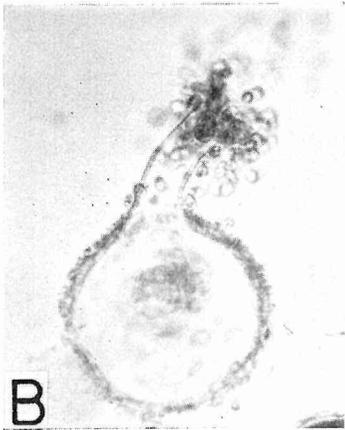
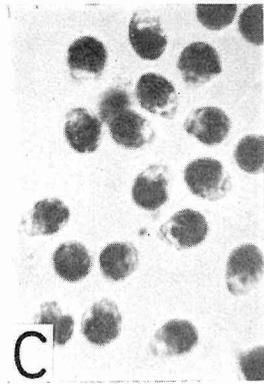
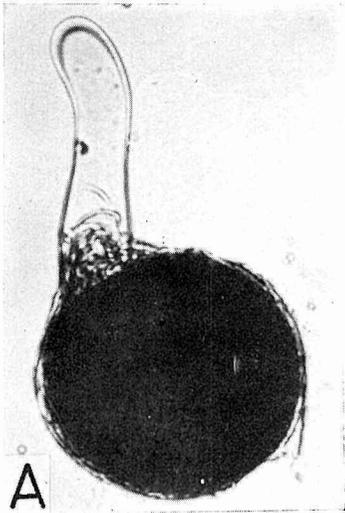


PLATE XIII

*Monostroma zostericola* TILDEN

- A. Habit of fertile plants growing on leaves of *Phyllospadix*.
  - B. Fertile plant.
  - C. Surface view of the fertile part (sporangia) of a frond photographed from living material.
  - D. Surface view of the upper part of a frond.
  - E. Surface view of the middle part of a frond.
  - F-G. Surface view of the lower part of a frond.
  - H. Surface view of the rhizoid-bearing part of a frond.
  - I. Cross section of the upper part of a frond.
  - J. Cross section of the lower part of a frond.
- A,  $\times 1/2$ .   B,  $\times 1$ .   C & I,  $\times 1000$ .   D-H & J,  $\times 400$ .

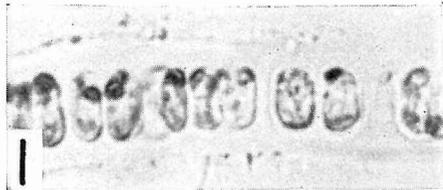
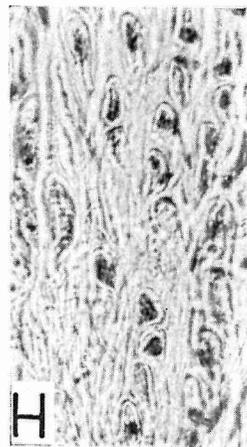
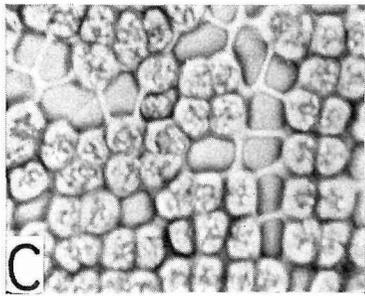
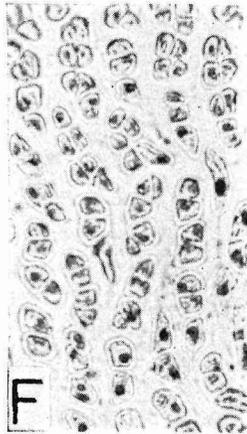
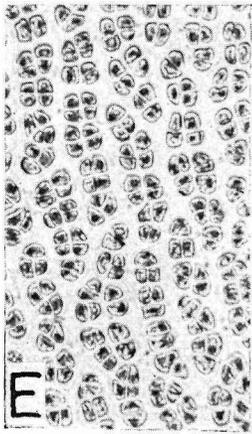
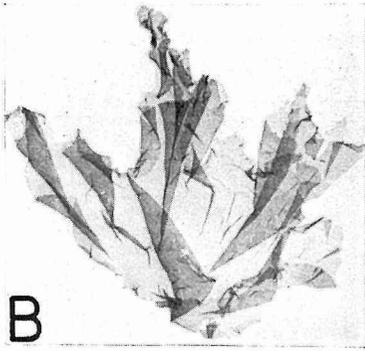
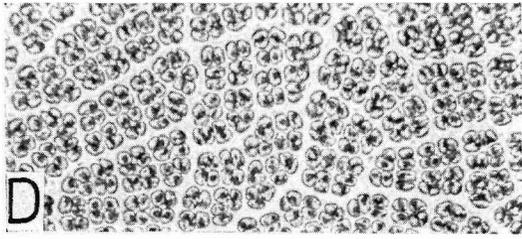
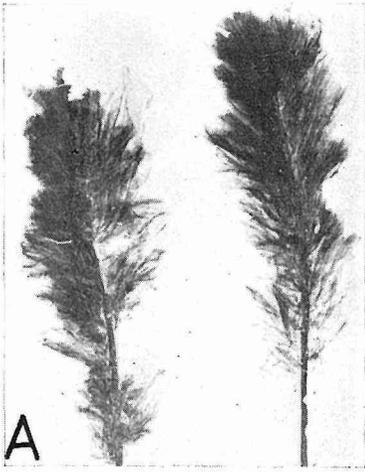


PLATE XIV

*Monostroma zostericola* TILDEN

- A. Surface view of emptied sporangia stained with safranin, showing liberation-pores.
- B. Side view of the same.
- C. Zoospores stained with a weak solution of iodine.
- D. Germination of zoospores.
- E. Germlings derived from zoospores, from 7-day old culture.
- F. Prostrate disc derived from a zoospore, from 20-day old culture.
- G. Surface view of the fertile part (gametangia) of a disc.
- H-I. Side view of the same.

A-B,  $\times 2000$ . C-E & G,  $\times 1000$ . F,  $\times 665$ . H-I,  $\times 500$ .

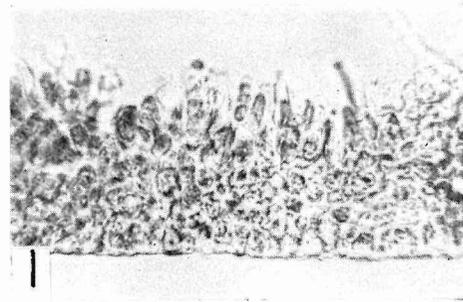
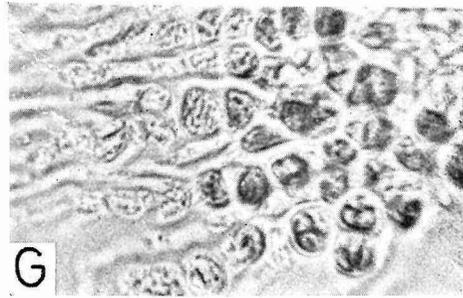
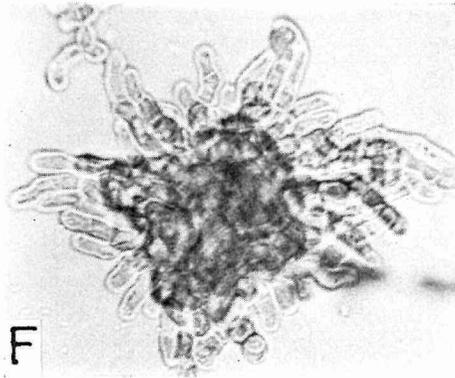
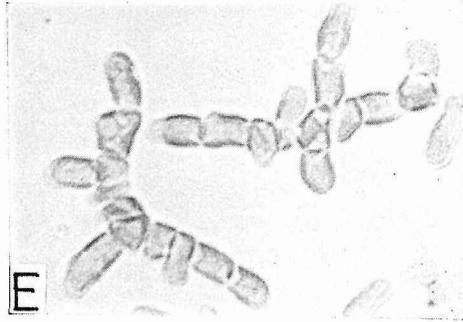
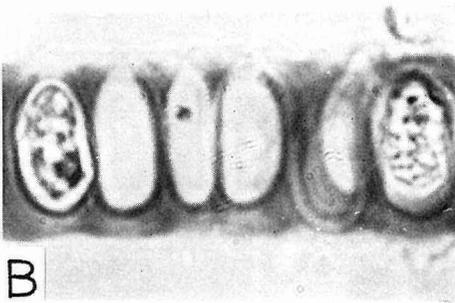
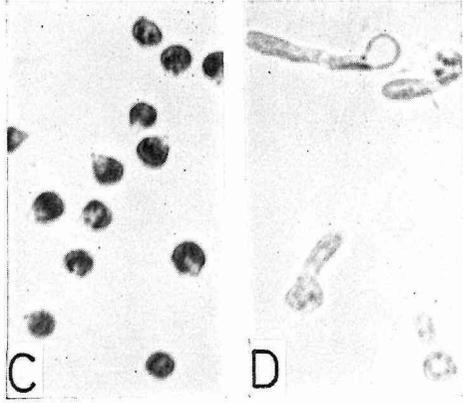
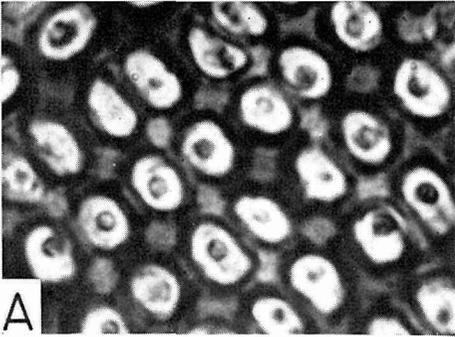


PLATE XV

*Monostroma zostericola* TILDEN

- A. Gametes and their conjugation.
- B. Germination of zygotes.
- C-D. Prostrate discs derived from zygotes, showing an upheaval.
- E. Saccate frond arising from the monostromatic disc.
- F. Young funnel-shaped frond.
- G-I. Monostromatic fronds developed directly from the disc without passing through a saccate stage.

(D-E & G-I. Photographed from the cultures grown at 5°C. in a 10-hr photoperiod and others from the cultures grown at room temperature).

A-C, × 1000. D, G & H, × 500. E, × 400. F, × 270.  
I, × 200.

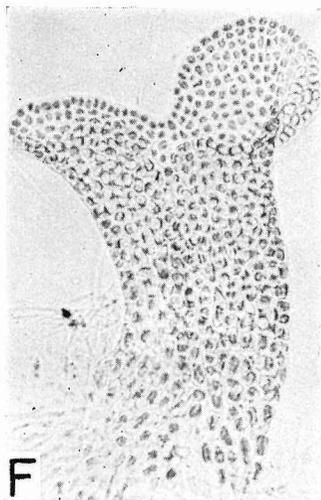
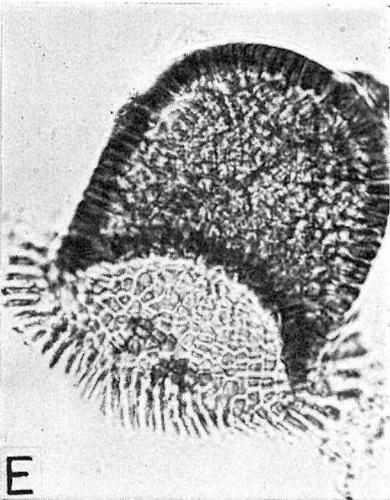
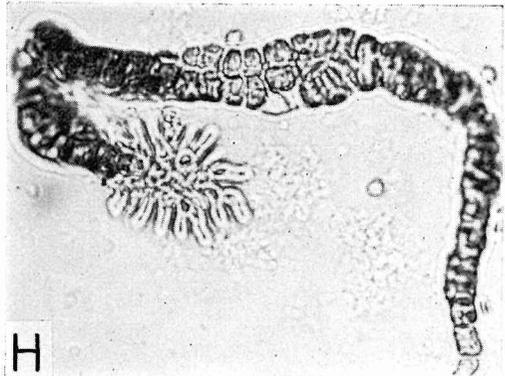
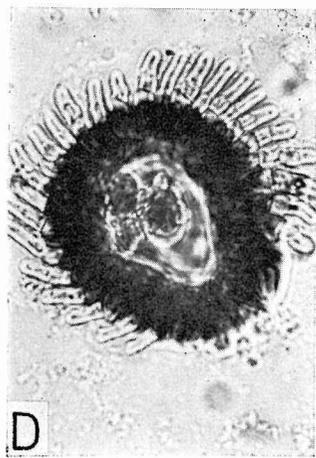
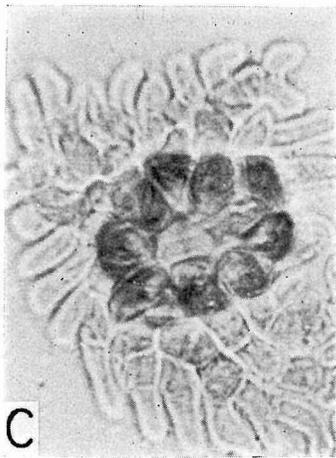
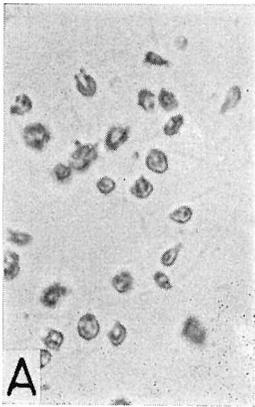


PLATE XVI

*Monostroma fuscum*

var. *splendens* (RUPR.) ROSENVINGE

A-B. Fertile plants.

C. Surface view of the upper part of a frond.

D. Surface view of the lower part of a frond.

E. Cross section of the upper part of a frond.

F. Cross section of the rhizoid-bearing part of a frond

G. Vertical section of the rhizoid-bearing part of a frond.

A-B,  $\times 1/5$ . C & E,  $\times 1000$ . D,  $\times 400$ . F-G,  $\times 200$ .

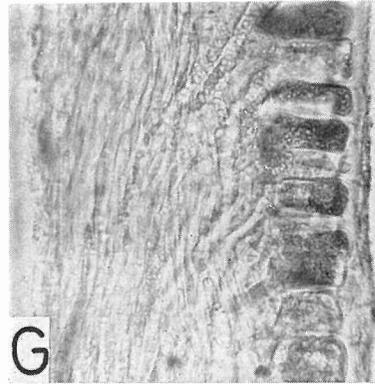
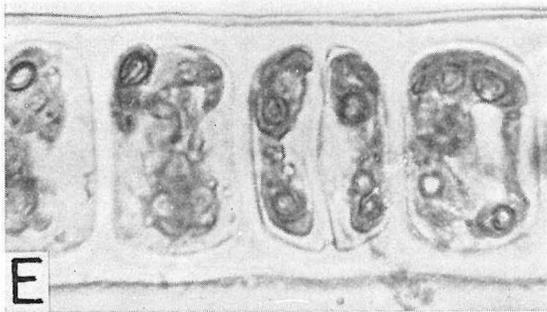
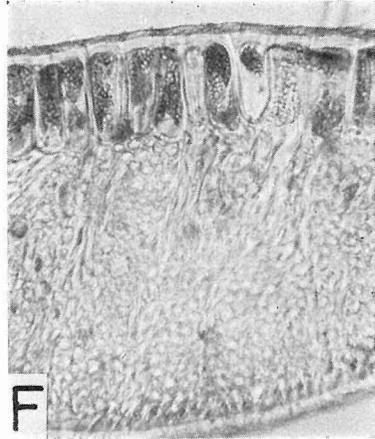
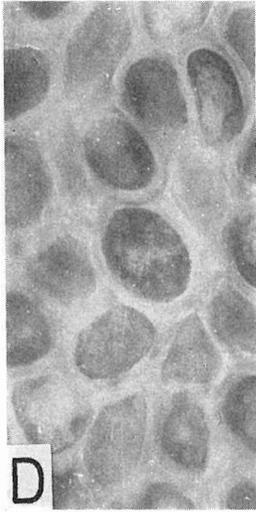
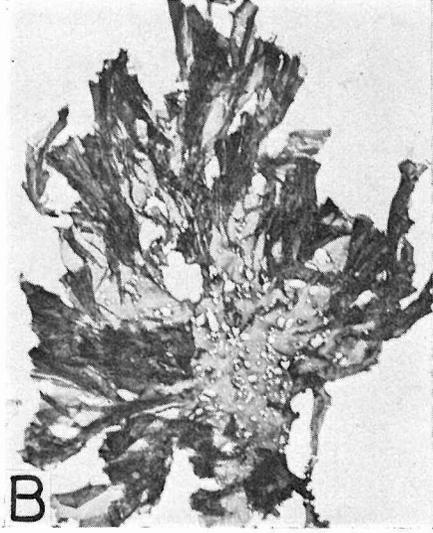
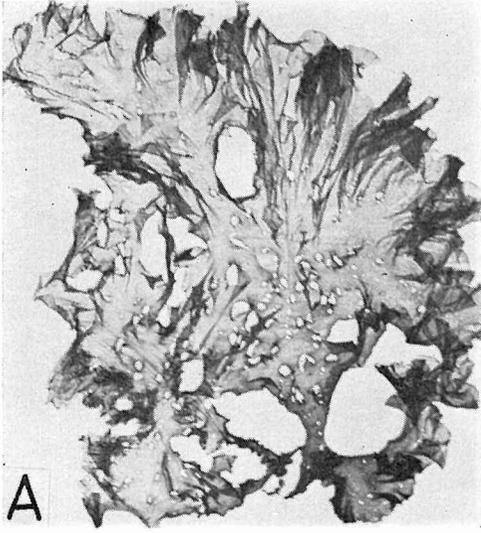


PLATE XVII

*Monostroma fuscum*

var. *splendens* (RUPR.) ROSENVINGE

- A. Surface view of the fertile part (sporangia) of a frond.
- B. Side view of the same.
- C. Surface view of emptied gametangia, showing liberation-pores.
- D. Side view of emptied sporangia stained with alum carmine.
- E. Surface view of emptied gametangia with sterile cells at the margin of a frond.
- F-G. Gametes.
- H. Conjugation of gametes.
- I. Zygotes from 3-day old culture.
- J. Germination of zygotes from 6-day old culture.
- K. Zoospores.
- L. Germination of zoospores from 5-day old culture.
- M. Germlings derived from zoospores, from 10-day old culture.

A-D & F-L,  $\times 1000$ .    E & M,  $\times 400$ .

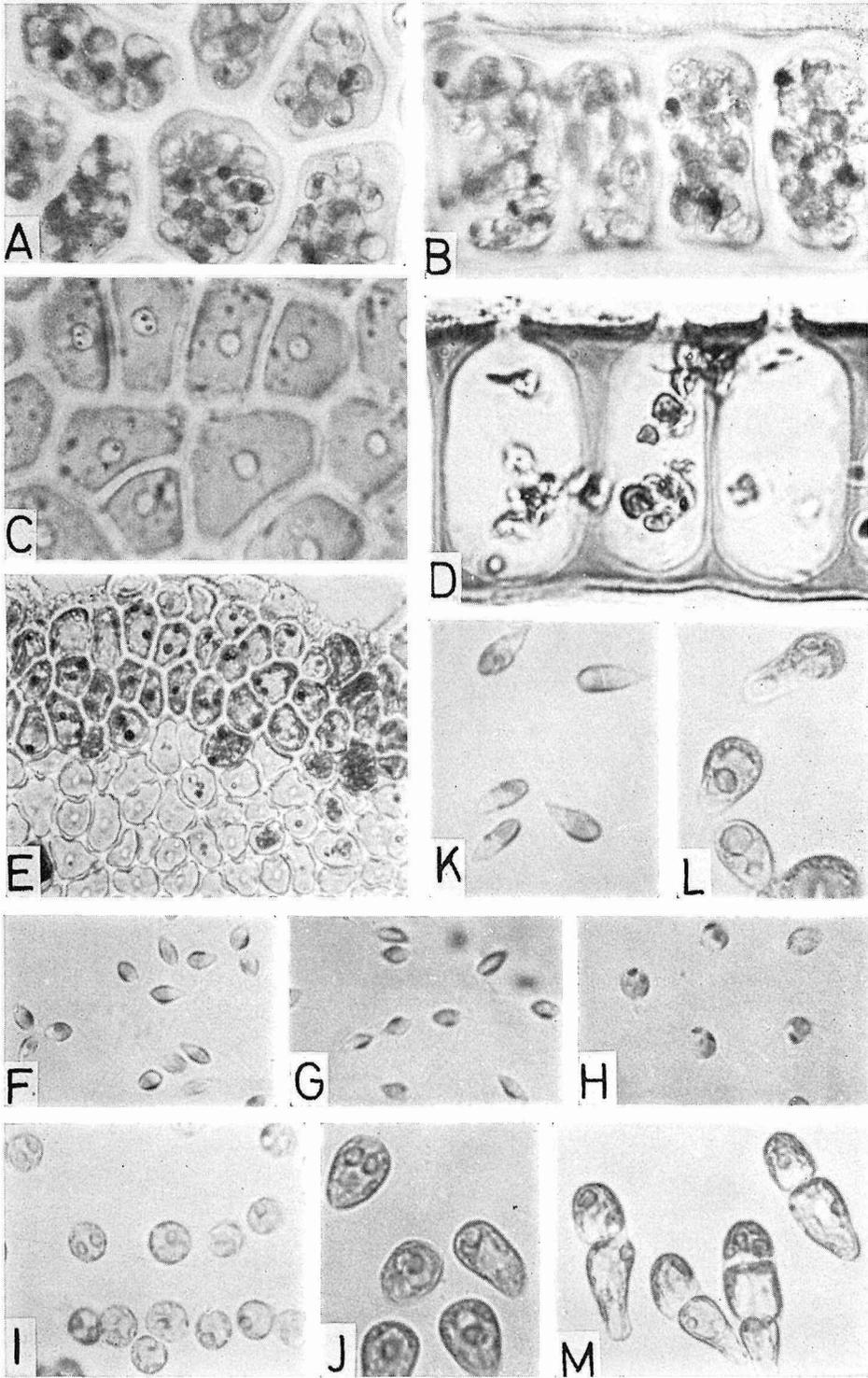


PLATE XVIII

*Monostroma fuscum*

var. *splendens* (RUPR.) ROSENVINGE

- A. Germlings derived from zoospores, from 12-day old culture.
- B. Germling derived from a zygote, from 13-day old culture.
- C. Germlings derived from zoospores, from 20-day old culture.
- D. Cylindrical frond derived from a zygote, from 30-day old culture.
- E. Saccate frond opened near the tip.
- F. Young monostromatic fronds with a tubular stipe.
- G. Surface view of the enlarged cells which break to occur an opening.

A, × 400. B, × 1000. C, × 100. D, × 87. E-F, × 50. G, × 665.

