New Findings on the Life History of *Monostroma zostericola* TILDEN

By

YUKIO YAMADA and MASAKAZU TATEWAKI

The life histories of several species of the genus *Monostroma* have been studied by many workers and several types of life-cycles have been found. YAMADA and KANDA (1941), working on *M. zostericola* TILDEN, reported that the leafy thallus is the sporophyte; it produces 4-flagellated zoospores which germinate developing again into the leafy thallus.

Since 1957 we have been studying the life histories of several species of *Monostroma* at the Marine Laboratory of the Institute of Algological Research, Hokkaido University in Muroran. In these studies we made some new findings in the life-cycle of *M. zostericola* and reported them preliminarily at the IX International Botanical Congress, Montreal, Canada in 1959. This paper gives these results in detail.

Material and Methods

The material was collected in the vicinity of the Marine Laboratory at Muroran. Usually the leafy thallus is found from January to June and it always grows epiphytically on the leaves of *Phyllospadix* in the middle-littoral or the lower-littoral belt of this area. The leafy thallus is the sporophyte and produces zoospores. Collected fertile fronds rinsed thrice in filtered seawater were placed singly on glass slides and then several drops of the seawater poured over them. Soon the zoospores were discharged and they attached themselves to the glass slides. They were cultured in a glass vessel containing about 200 ml. of medium. The cultures were grown in SCHREIBER's solution and kept near the north-east window at room temperature, as shown in figure 1. Since 1963 the cultures have been grown in ES medium (PROVASOLI's enriched seawater) and kept in refrigerators illuminated with 1500–2000 lux of fluorescent light (cool white) for 10–12 hours a day, at 5°C. or illuminated for 8 hours a day, at 0°C.. Some cultures were also kept at 13–14°C., and illuminated for 14 hours daily.

Zoospores and their development

The cells of the upper margin of the fertile fronds develop into sporangia

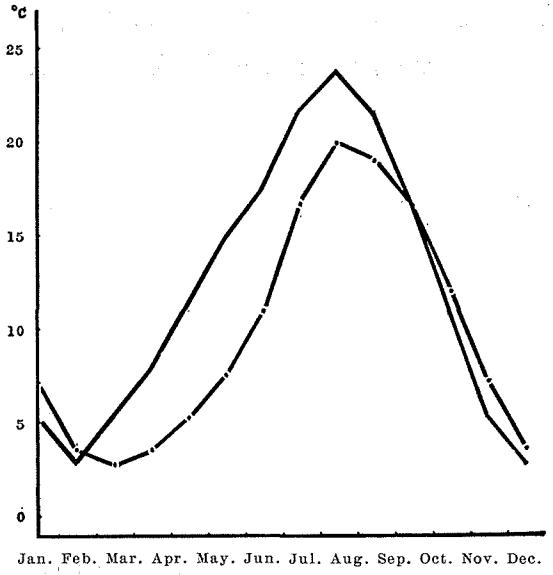

Fig. 1. Seawater temperature (——·——) at Charatsunai, Muroran and room temperature (————) in which cultures were grown. (Average of three years, 1958–1960).

containing each 4–(8) zoospores. The zoospores are liberated one by one through openings all placed on the same side of the frond. The zoospores are pear-shaped measuring 4.2–6.8 μ×3.3–5.2 μ, average, 5.35 μ×4.27 μ and they have four flagella of equal length (7.6–10.6 μ long) at the anterior end (Fig. 2, A). Their protoplast contains one chloroplast with a pyrenoid. They seem to have no eyespot and are negatively phototactic. After swarming for 10–20 minutes they lose their flagella, settle down on the substratum and soon form a wall (Fig. 2, B–C). After resting for 2–3 days they begin to germinate, taking an elongated shape, and divide transversally first into two cells and after form a 3–4 cells sporeling. Each cell of the sporeling then begins to branch off (Fig. 2, F–J). Other resting zoospores send out a germination tube in which all the cytoplasm migrates, leaving the original cell empty (Fig. 2, K–M). Within 2–4 days such sporelings divide into 2–4 cells with the transverse walls (Fig. 2, N–O).

Most cultures of zoospores were begun between March and May, 1957–1960.

In the cultures grown at room temperature, the sporelings in 20 days develop into a small disc by successive branchings and cell-divisions (Fig. 2, P–S). In one-month culture the discs give off prostrate branches radially. The central part of the discs then upheaves taking an irregular shape (Fig. 3, A–B). From July to September (during summer, the room temperature range is from 20°C. to 24°C.)

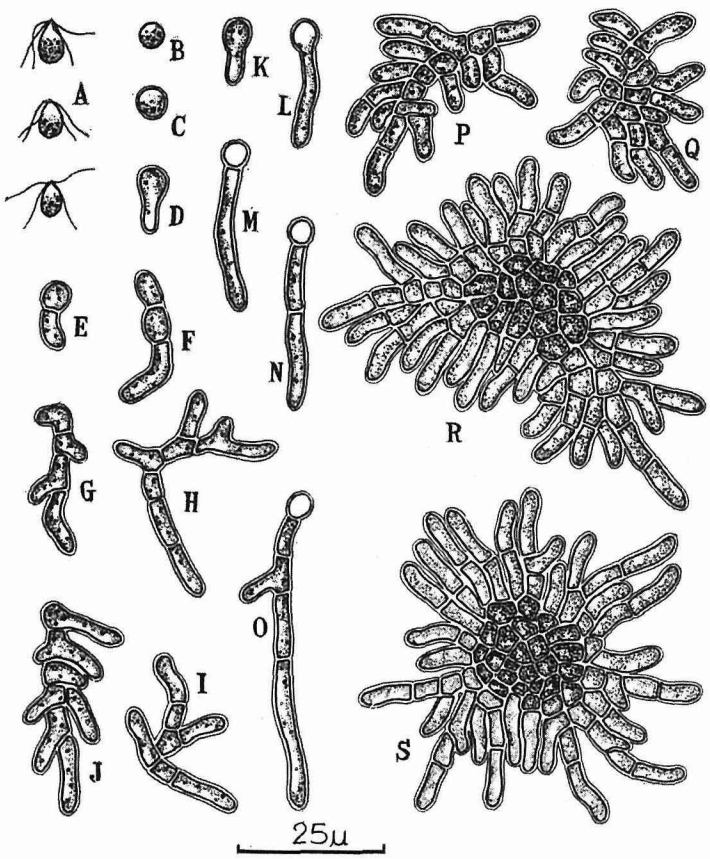

Fig. 2. *Monostroma zostericola* TILDEN

A. Zoospores. B–C. Resting zoospores. D. Germination of zoospore. E. 2-celled stage. F. 3-celled stage. G–J. Further development of sporelings. K–O. Another germination type of zoospores; L. migration of cytoplasm. P–Q. 10 days old sporelings (discs). R–S. 20 days old discs (grown in SCHREIBER's solution at room temperature).

the discs grow very slowly and they do not show any marked change. At the end of October (10–12°C.) the discs begin to grow actively and attain about 500 μ in diameter. Some discs produce not only the prostrate branches, but also produce many erect and tangled branches; such discs look like a green tuft. In November most surface cells of the disc gradually begin to enlarge and their cytoplasm becomes deep green. These enlarged cells change into gametangia and their cytoplasm divides into 2–8 or more portions, producing finally 8–32 gametes (Fig. 4, A–B). In a side view the gametangia are club-shaped and measure 15–35 μ in

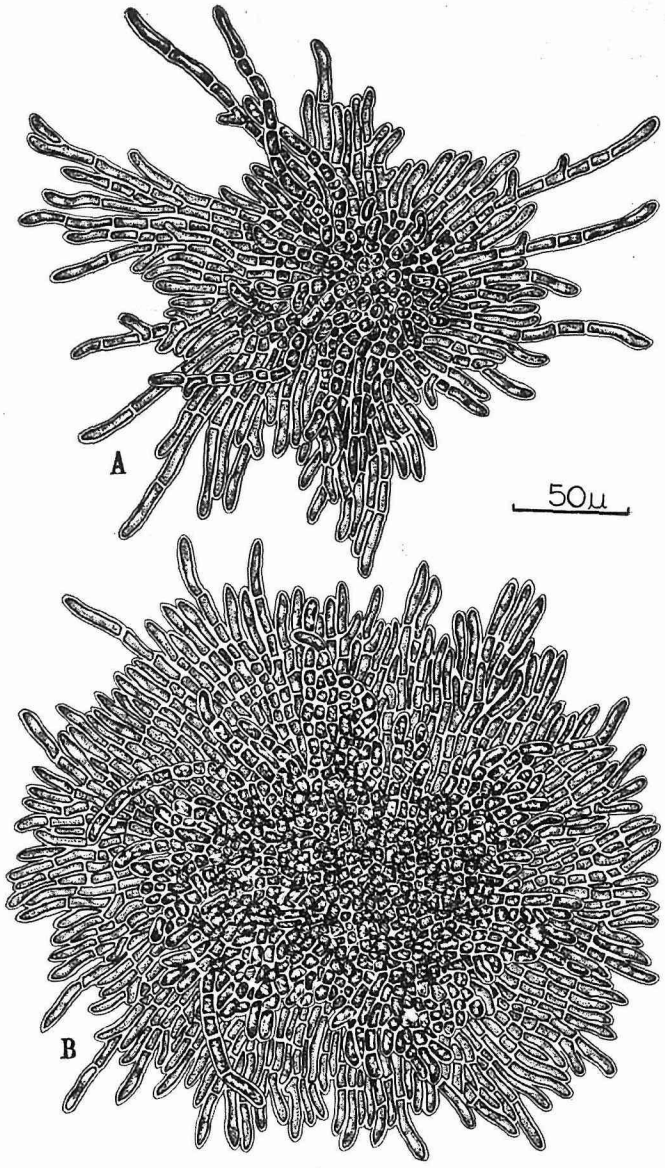

Fig. 3. *M. zostericola* TILDEN

A–B. Further development of discs; from 40–50 day culture (at room temperature).

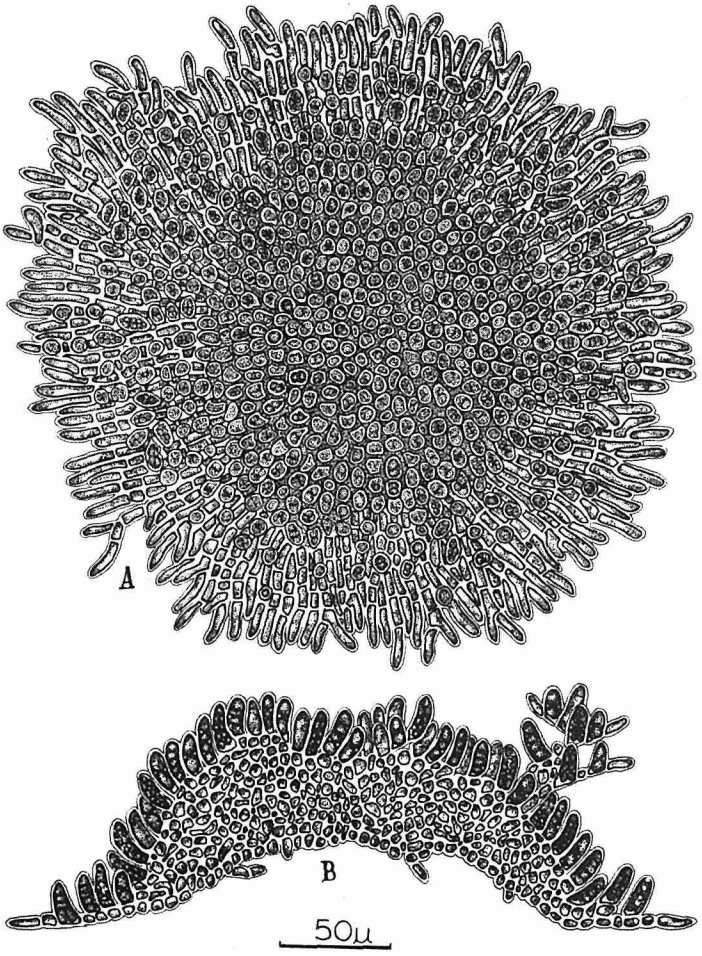

Fig. 4. *M. zostericola* TILDEN

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

height and 4.5–6.1 μ in breadth (Fig. 5, A–D). Generally at room temperature ranging 3–7°C. (winter) the gametangia reach maturity and the gametes are liberated one by one through an opening at the tip of each gametangium. In cultures started in March–May at room temperature, the discs derived from zoospores become fertile in 6–10 months.

In cultures started on January 8–11, 1958, the resting zoospores developed into a large flat disc (300–500 μ in diameter) within 50 days and the discs became fertile.

In this experiment (room temperature range was from 3°C. to 8°C.), the discs discharged the gametes in 60 days. Consequently, we can reduce the developmental period of this species.

In cultures grown in ES medium at 5°C. and a daily photoperiod of 10–12 hours (1500–2000 lux), the 30–35 days old discs develop into a fertile gametophyte discharging the gametes. (In cultures grown in SCHREIBER's solution under similar conditions, the discs grew slower than those in ES medium and became fertile in 45–50 days.) The zoospores cultured at 0°C. under illumination of 8 hours daily, grow very slowly and the one-month old sporelings are still a prostrate irregular filament consisting of 20–30 cells. These filaments develop into a small disc in 2–3 months. In cultures grown at 13–14°C. and illuminated 14 hours a day, the discs grow fastest and develop into a big disc or tuft (500–700 μ in diameter) in 30–40 days. Under this condition, however, these discs do not reach maturity.

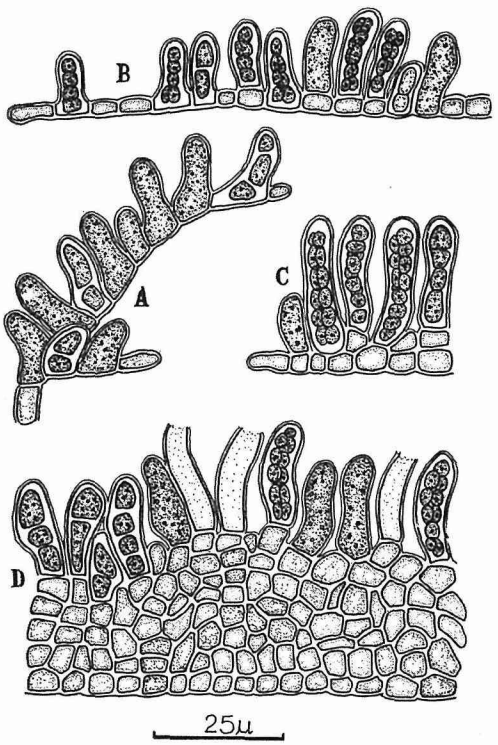

Fig. 5. *M. zostericola* TILDEN

A–D. Formation of gametes. A. branching gametangia, B–C. Gametangia of peripheral part of disc (cross section), D. Gametangia of central part of disc (cross section).

Gametes and their movement

The gametes are pear-shaped measuring 2.7–5.7 $\mu \times$ 1.8–4.5 μ, average, 4.12 $\mu \times$ 2.91 μ and have two flagella of equal length (7.6–10.6 μ long) at the anterior end (Fig. 6, A–B). Their protoplast contains one chloroplast with a pyrenoid. No eyespot is recognizable and they are negatively phototactic. After liberation from the gametangia, the gametes swim rapidly and soon conjugate even between gametes derived from the same gametophytic disc. Under a microscope, there is no marked difference in size between the conjugating gametes of a pair. The gametes conjugate side by side or end to end anteriorly and form a zygote (Fig. 6, C–E).

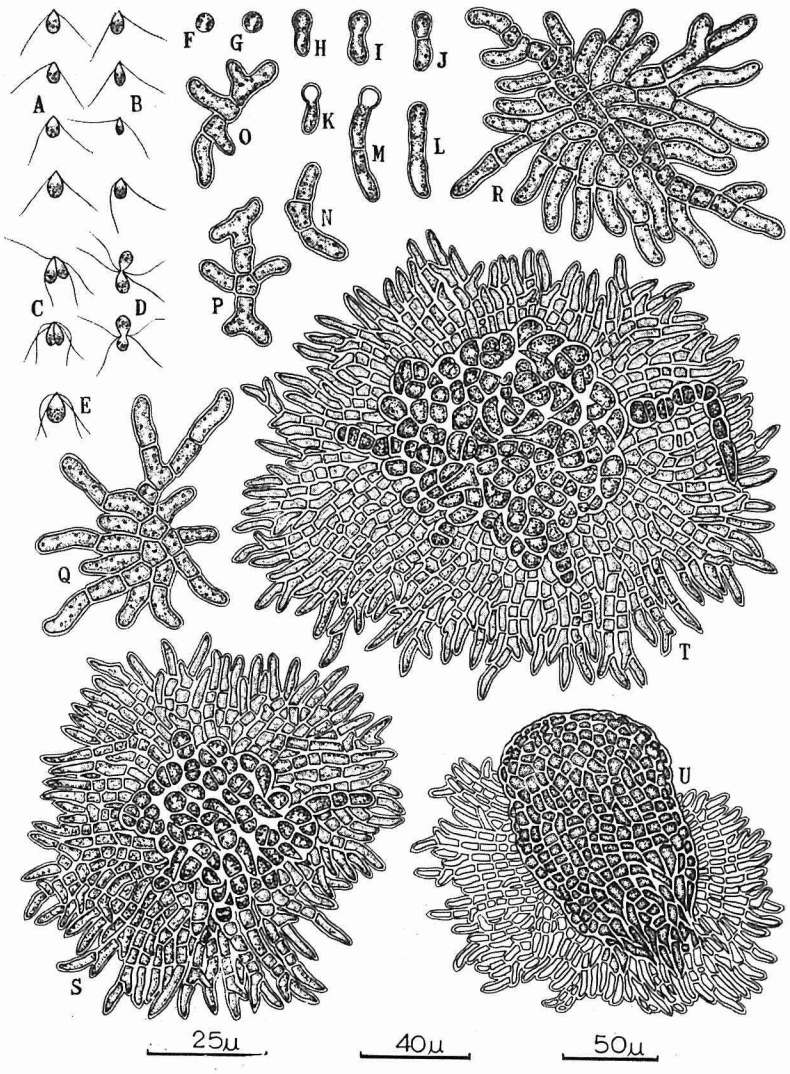

Fig. 6. *M. zostericola* TILDEN

A–B. Gametes. C–D. Conjugation of gametes. E. Planozygote. F–G. Resting zygotes. H–I. Germination of zygotes. J. 2-celled stage. K–M. Another germination type of zygotes, showing the cytoplasmic migration. N. 3-celled stage. O–U. Further development of discs (grown in SCHREIBER's solution at room temperature). O–P. 25 days old discs. Q–R. One month old discs. S–T. 45 days old discs, giving rise a saccate frond at the central part. U. Young saccate plant. A–R drawn to 25 μ scale. S–T drawn to 40 μ scale. U drawn to 50 μ scale.

Zygotes and their development

After swarming for a while the zygotes lose their flagella, settle down on the substratum and soon form a wall (Fig. 6, F–G). After resting for 3–4 days the zygotes begin to germinate, taking an elongated shape, and divide transversally into two cells (Fig. 6, H–J) and then each cell begins to branch off. Some zygotes send out a germination tube in which the cytoplasm migrates, leaving the original cell empty (Fig. 6, K–M). Then a small disc is formed in the same way as the one originated from the zoospores (Fig. 6, N–R).

Most cultures of zygotes (at room temperature) were begun between December and March, 1958–1960, temperature range was from 3°C. to 10°C..

In 35–40 days the discs begin to upheave at the central part and the swelling gradually becomes a standing saccate or tubular frond (Fig. 6, S–U). Such a structure opens at the tip and splits to the base and finally forms an expanded monostromatic frond (Fig. 7, A–D). The discs, however, often form a monostromatic frond directly without passing through the saccate stage. In this case one or two uniseriated filaments appear at the central part of the disc and develop directly into a monostromatic frond. Usually at 5–10°C. the monostromatic thalli reach maturity and dis-

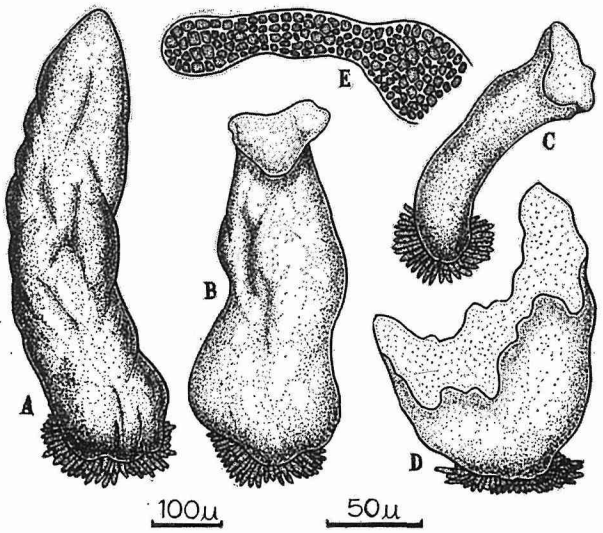

Fig. 7. *M. zostericola* TILDEN
A–C. Young saccate or tubular plants. D. Young plant found on a leaf of *Phyllospadix* in nature. E. A part of monostromatic frond formed directly without passing through the saccate stage.
A–D drawn to 100 μ scale. E drawn to 50 μ scale.

charge 4-flagellated zoospores in 50–60 days. The thallus derived from zygotes is identical to the plant of *M. zostericola* growing on the leaves of *Phyllospadix* in nature.

Of course, it is possible to accelerate the growth of the sporophytes and the formation of zoospores by regulating the conditions of cultivation as it was done with the gametophytes.

Plants derived from zygotes and grown in ES medium at 13–14°C., under illumination of 14 hours daily (1500–2000 lux), become mature and discharge zoospores in 20 days. However, in this case, the plant did not form a normal leafy thallus but became fertile at the disc stage (figure 6, T). In general, at temperature of 15°C. or more, the sporophytic disc (derived from zygotes) does not form a normal leafy thallus.

In cultures grown in ES medium at 5°C. and a daily photoperiod of 10–12 hours, the zygotes develop into a normal leafy thallus and become fertile in 30–40 days. (In cultures grown in SCHREIBER's solution under similar conditions, the plants grew slowly and were 20 days or more behind.)

Discussion

In their experiments on *M. zostericola*, YAMADA and KANDA (1941) observed that the *Monostroma*-plant is the sporophyte: it produces 4-flagellated zoospores which develop into a disc developing in a *Monostroma*-plant again. In the present experiment, however, we found that the discoid stage derived from the zoospores is the gametophyte which produces biflagellate gametes: the zygotes derived from these gametes develop into the *Monostroma*-plant (sporophyte). So in *M. zostericola*, there is an alternation of a large multicellular sporophyte with a small multicellular gametophyte. In this experiment, we did not observe that the zoospores developed directly into a leafy thallus. It is possible, however, that there are different ecological strains in *M. zostericola* as in *Enteromorpha linza*: one strain of *E. linza* has both the sexual and the asexual reproductions with an alternation of generations (MOEWUS, 1938 and ARASAKI, 1946), while another strain has only asexual plants repeating successive asexual reproduction (BLIDING, 1933 and YAMADA & SAITO, 1938).

Seasonal factors, as temperature, photoperiod and media influence the speed of growth of *M. zostericola* but do not change the pattern of the life-cycle. (Unfortunately, we have insufficient data concerning the optimum photoperiod and light intensities for growth of this plant. These problems will be studied in more detail later.)

In cultures started on January 8–11, 1958 at room temperature, we found that

the discs derived from zoospores formed gametangia at the beginning of March of the same year: in 50–60 days the discs became fertile gametophytes discharging gametes. The zygotes derived from these gametes were cultured on March 9–11 and these zygotes developed into a fertile sporophyte (leafy thallus) at the beginning of May. In this case one life-cycle was completed in 4 months.

On the other hand, the discs derived from zoospores cultured in March–May became fertile gametophytes and produced gametes and zygotes from the middle of November to the middle of next March (6–10 months). The zygotes developed into fertile sporophytes from the end of January to the beginning of May. In this case one life-cycle was completed in 8–12 months.

Evidently this plant does not grow well from summer to autumn at room temperatures from 18°C. to 24°C.. Also the gametophyte needs a temperature of 3–7°C. for maturity. The sporophyte (leafy thallus) grows well from winter to spring at room temperatures ranging from 3°C. to 10°C. or at least less than 14°C.. At temperature of 15°C. and more than 15°C. the sporophytic disc does not develop into a leafy thallus.

Since 1963 the cultures were grown in ES medium and kept under various temperatures and photoperiods. In the cultures grown at 5°C. and a daily photoperiod of 10–12 hours, the discs derived from zoospores developed in 30–35 days into a fertile gametophyte and produced gametes and zygotes. These zygotes developed into mature leafy thalli in 30–40 days. Under this condition, one life-cycle was completed in 60–75 days. (In the cultures grown in SCHREIBER's solution under similar conditions, both the gametophyte and the sporophyte grew slower than those grown in ES medium and one life-cycle was completed in 90–100 days.) The zygotes obtained at 5°C. but grown at 13–14°C. and with 14 hours light daily became fertile sporophytes (but dwarf) in 20 days. In this case one life-cycle was completed in 50 days. Under illumination of 14 hours daily at 13–14°C., the gametophytic discs derived from zoospores grew very fast and attained 500–700 μ in diameter within 30–40 days, but they did not mature. These discs became a normal fertile gametophyte only when they were removed to 5°C.. Under illumination of 8 hours daily at 0°C., both the gametophyte and the sporophyte grew poorly.

The predominant life-cycle of *Monostroma* so far described is an heteromorphic alternation of a macroscopic gametophyte (leafy thallus) with a microscopic unicellular sporophyte (cyst): *Monostroma* sp. (KUNIEDA, 1934), *M. wittrockii* (MOEWUS, 1938), *M. angicava* (YAMADA & SAITO, 1938), *M. grevillei* (SUNESON, 1947; KORNMANN & SAHLING, 1962), *M. latissimum* (ARASAKI, 1946 and 1951; SEGI & GOTO, 1956) and *M. nitidum* (ARASAKI, 1946 and 1951; SEGI & GOTO, 1956). In *M. pulchrum* (YAMADA & SAITO, 1938; YOSHIDA, 1964)=*M. undulatum* (KORNMANN

& SAHLING, 1962), there is an alternation of a macroscopic leafy thallus with a microscopic unicellular cyst, though they may both be considered as a sporophytic generation.

Isomorphic alternation has been reported in *M. fuscum* var. *splendens* (DUBE, 1962; TATEWAKI, 1963) and *M. obscurum* (GAYRAL, 1962). In addition, only isomorphic generation (no alternation) has been reported in *M. arcticum* (KORNMANN & SAHLING, 1962) and *M. leptodermum*—in cultures grown at 3–4°C. (KORNMANN & SAHLING, 1962).

In *M. zostericola*, though the alternation is heteromorphic, it is quite different from that of other described species; namely, an alternation of a macroscopic multicellular sporophyte (leafy thallus) with a microscopic multicellular gametophyte (disc). According to KORNMANN and SAHLING working on *M. leptodermum* from Helgoland, produced successive isomorphic generations as a sporophytic leafy thallus at a constant low temperature (3–4°C.) but at 15°C. the zoospores derived from leafy thallus developed into a prostrate filament which produced zoospores. These zoospores developed again into a leafy thallus only when grown at 3–4°C.. *M. leptodermum* resembles closely *M. zostericola* in cell size and cell arrangement, but it has a different life-cycle.

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Summary

In *Monostroma zostericola*, the leafy thallus is the sporophyte and produces 4-flagellated zoospores. No eyespot is recognizable in the zoospores and they are negatively phototactic. The zoospores germinate and develop into a disc. The disc derived from the zoospores is the gametophyte and produces biflagellate gametes (temperature range for maturity seems to be 3–7°C.). The gametes also seem to have no eyespot and are negatively phototactic. Sexual reproduction is isogamous. The gametes conjugate side by side or end to end anteriorly and form a zygote. The zygotes germinate and develop into a small disc. The disc derived from the zygotes begins to upheave at its central part and the swelling develops into a saccate frond which splits forming a monostromatic leafy thallus (a temperature ranging from 5°C. to 10°C., gives good growth).

The life-cycle of *M. zostericola* is an alternation of heteromorphic generations: a large multicellular sporophyte (leafy thallus) alternates with a small multicellular gametophyte (disc).

Under suitable temperature, photoperiod and media, the life-cycle can be completed in 50–60 days.

Literature Cited

ARASAKI, S.

1946. Studies on the swarmers and their development in *Ulvaceae* and *Monostromaceae*. Seibutsu (Life) I, 5-6 : 281–287.

1951. On the *Monostroma* found in Ise- and Mikawa-Bay. Suisangakuzasshi (J. Fisheries) 15, 3 : 137–143.

BLIDING, C.

1933. Ueber Sexualität und Entwicklung bei der Gattung *Enteromorpha*. Svensk Bot. Tidskr., 27 : 233–256.

1935. Sexualität und Entwicklung bei einigen marinen Chlorophyceen. Svensk Bot. Tidskr., 29 : 57–64.

DUBE, M.

1962. Life history of *Monostroma fuscum* var. *splendens* (RUPR.) ROSENV. Ameri. J. Bot. (Abstr. of Pap. at meeting of Bot. Soc. Ameri., 1962). 49 : 6, 2. 671.

GAYRAL, P.

1962. Reproduction et développement de *Monostroma obscurum* (KÜTZ.) J. AGARDH. Bull. Soc. bot. Fr., 109 ; nos 3-4. 53–59.

KORNMANN, P. & SAHLING, P. H.

1962. Zur Taxonomie und Entwicklung der *Monostroma*-Arten von Helgoland. Helgol. Wiss. Meeresunters., 8 (3) : 302–320.

KUNIEDA, H.

1934. On the life history of *Monostroma*. Proc. Imp. Acad. Tokyo, 10 : 103–106.

MOEWUS, F.

1938. Die Sexualität und der Generationswechsel der *Ulvaceen* und Untersuchungen über die Parthenogenese der Gameten. Arch. Protistenk., 91 : 375–441.

PAPENFUSS, G. F.

1962. On the genera of *Ulvales* and the status of the order. J. Linn. Soc. (Bot.) 56, 367 : 303–316.

SEGI, T. & GOTO, W.

1956. On *Monostroma* and its culture. Sorui (Bull. Jap. Soc. Phyc.) 4, 2 : 55–60.

SUNESON, S.

1947. Notes on the life history of *Monostroma*. Svensk. Bot. Tidskr., 41 : 235–246.

TATEWAKI, M.

1963. The life history of *Monostroma fuscum* var. *splendens*. Bot. Mag. Tokyo, 76 : 381–387.

YAMADA, Y. & KANDA, T.

1941. On the culture experiment of *Monostroma zostericola* and *Enteromorpha nana* var. *minima*. Sci. Pap. Inst. Algolog. Res., Fac. Sci., Hokkaido Imp. Univ., 2, 2 : 217–221.

YAMADA, Y. & SAITO, E.

1938. On some culture experiments with the swarmers of certain species belonging to the *Ulvaceae*. Sci. Pap. Inst. Algolog. Res., Fac. Sci., Hokkaido Imp. Univ. 2, 1 : 43–49.

YAMADA, Y. & TATEWAKI, M.

1959. Life history of *Monostroma*. Proc. IX Int. Bot. Congr., 2 : 483.

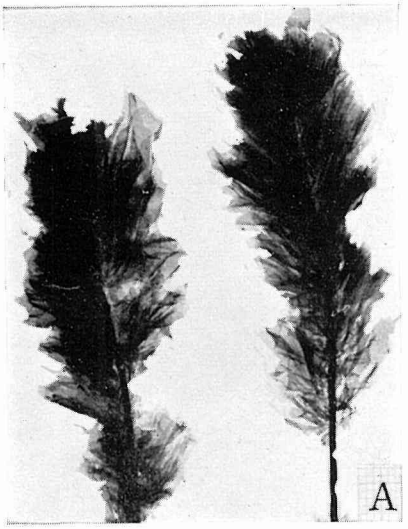
YOSHIDA, K.

1964. On the development of the sporelings of *Monostroma pulchrum* FARLOW. Sorui (Bull. Jap. Soc. Phyc.) 12, 1 : 8–14.

PLATE I

Monostroma zostericola

A. Habit of mature plants growing on the leaves of *Phyllospadix*. × 1/2.
B. A mature plant. × 1.
C. Surface view of emptied sporangia. × 1000.
D. Surface view of the upper part of the frond. × 400.
E. Surface view of the middle part of the frond. × 400.
F. Surface view of the lower part of the frond. × 400.
G. Cross section of the upper part of the frond. × 1000.
H. Cross section of the lower part of the frond. × 1000.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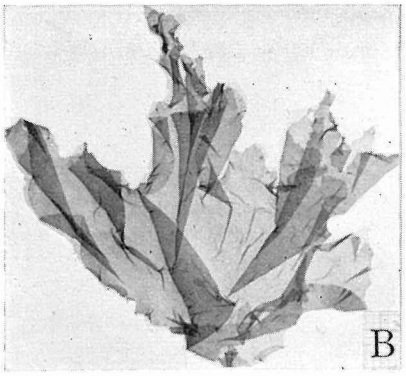
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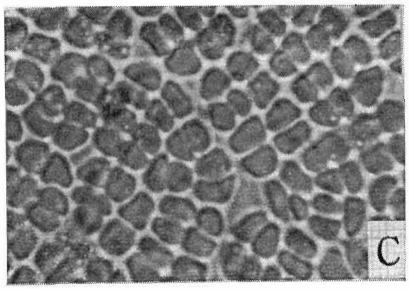
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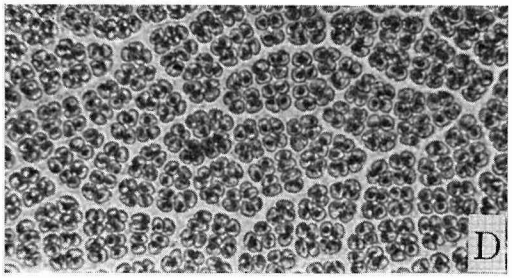
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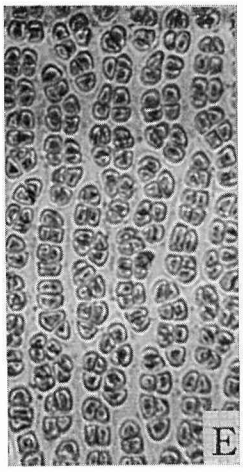
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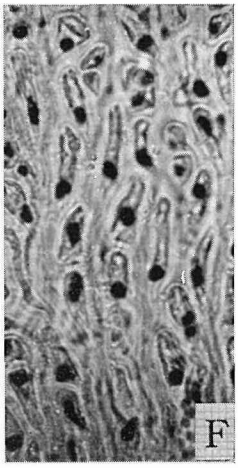
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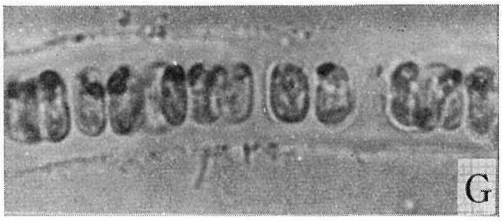
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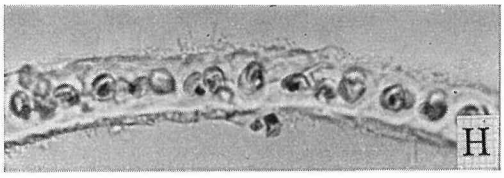
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PLATE II

Monostroma zostericola

A. Zoospores.

B. 20 days old disc derived from a zoospore.

C. Surface view of mature gametangia.

D–E. Side view of mature gametangia. A–E. × 1000.

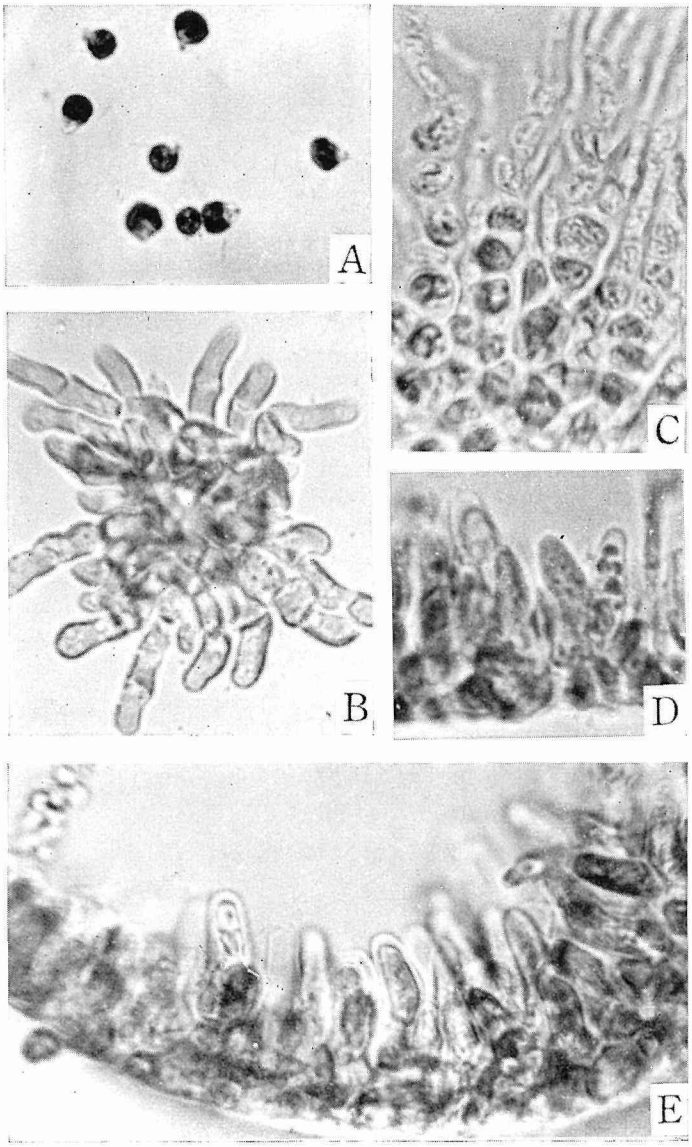

A
B
C
D
E

PLATE III

Monostroma zostericola

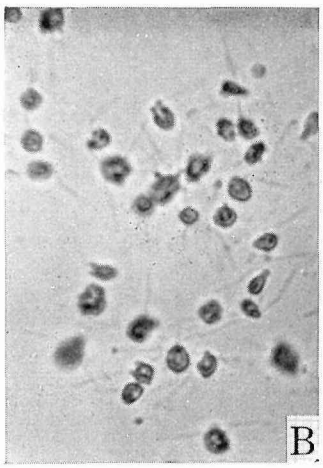
A. Gametes.

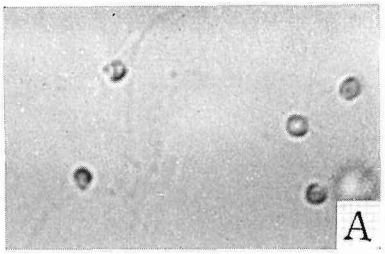
B. Conjugation of gametes.

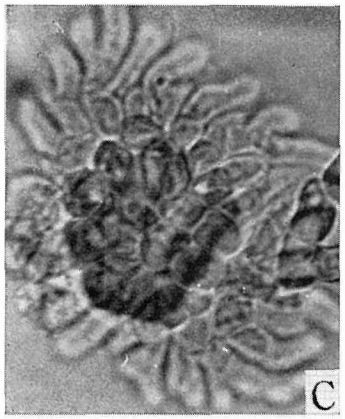
C. A disc which begins to upheave at the central part.

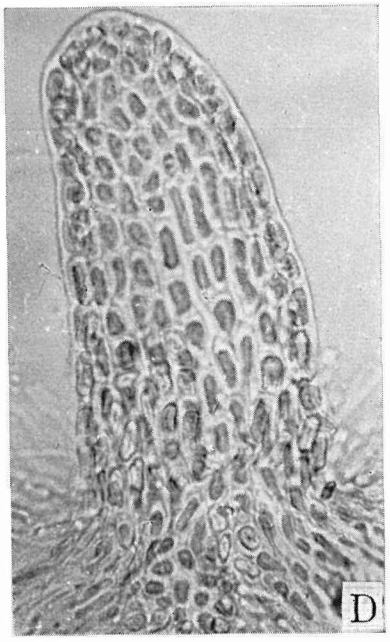
D. A young saccate plant.

A–C. × 1000. D. × 400.


B


A


C


D