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## The Life History of Some Species of the Scytosiphonales

By

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## Introduction

The order Scytosiphonales established by FELDMANN (1949) has long been puzzling because life histories of members of this order have remained unsolved until quite recently. Since DANGEARD (1963) found unilocular sporangia of *Petalonia zosterifolia* in culture, NAKAMURA (1965), TATEWAKI (1966), LUND (1966), WYNNE (1969), HSIAO (1969) and EDELSTEIN *et al.* (1970) subsequently have observed the occurrence of unilocular sporangia-bearing plants (crusts and microthalli) in the genera *Scytosiphon* and *Petalonia* in both culture and nature, and the status of this order has become more clear.

Since 1963 we have been studying the life history of some species belonging to the Scytosiphonales at the Institute of Algological Research, Hokkaido University, Muroran. The present work was carried out to complete their entire life cycles by culturing them from generation to generation in the laboratory and to find more species characteristics. During this succeeding cultural experiment, we preliminarily reported new findings about the occurrence of ralfsioid thalli with unilocular sporangia in *Scytosiphon lomentaria*, *Petalonia fascia* and *Colpomenia bullosa*.

This paper deals with investigations on the life history of five species; Scytosiphon lomentaria, Petalonia zosterifolia, P. fascia, Colpomenia bullosa and Endarachne binghamiae belonging to the Scytosiphonaceae.

## Materials and Methods

Materials used for the present investigation were collected at Muroran (42°19' N, 140°59' E) during the winter and spring of 1963–1968, with the exception of *Endarachne binghamiae*, which was obtained at Amatsu-kominato (35°07' N, 140°10' E), Boso Peninsula, Chiba Prefecture on April 8, 1967.

For the culture study, fragments of the fertile frond were rinsed with autoclaved seawater and were placed one by one in separate petri dishes containing sterilized seawater. Newly liberated swarmers showing a negative phototaxis were washed 3-5 times in sterilized seawater by the micropipette method. Then they were pipetted on a glass slide unialgally. After their settlement this slide was transferred to a glass vessel ( $6.5 \text{ cm.} \times 8.0 \text{ cm.}$ ) containing 180 ml. of medium. Sexual reproduction was examined by mixing swarmers liberated from different individuals under a microscope. Zygotes could be easily detected by two eyespots which were retained for a few days. They were isolated by removing unfused gametes which attached themselves to the glass slide together with the zygotes, with a fine needle or a micropipette under a microscope. For a single plant culture, the 5~10-day-old germlings attached to the glass slide were isolated with a micropipette and transferred to the culture test tube ( $2 \text{ cm.} \times 13 \text{ cm.}$ ) containing 10 ml. of medium.

The culture medium used in this study was PESI, a slight modification of PROVASOLI'S ES medium and it was replenished at intervals of about 30 days. Cultures were grown in freezer-incubators illuminated with cool white fluorescent lamps (ca. 1500–3000 lux) at specified temperatures and photoperiods in the following combinations.

Number of incubator	Temperature (°C)	Photoperiod (Hr light-Hr dark)
No. 1	10	14-10
No. 2	12	10-14
No. 3	14	14-10
No. 4	14	10-14
No. 5	18	16- 8

These variables were selected to correspond approximately to intertidal conditions at the area of Muroran. During winter (December to February) water temperature was measured to range from 3 to 7°C. and photoperiod was a shortday condition, ranging from 9 to 11 hours of light. In this season, the fronds of *Scytosiphon, Petalonia* and *Colpomenia* grow well in nature. However, they grow poorly under conditions of 5°C and a 10-hr photoperiod in the laboratory culture. Conditions of No. 1 and No. 3 correspond to those of April and May at Muroran, when *Scytosiphon* and *Colpomenia* plants thrive most luxuriantly. Conditions of No. 2 and No. 4 correspond to those of October and November when ralfsioid plants with unilocular sporangia of *Scytosiphon* and *Petalonia* are found in nature. Conditions of No. 5 correspond to those of the end of June and July-August when the fronds of *Scytosiphon* and *Colpomenia* disappear in nature.

For the cytological observation, small pieces of the fronds or young germlings were fixed in the fixative of 1:3 acetic alcohol during night-time in nature and dark-period in culture. The staining of chromosomes was made by application of aceto-iron-haematoxylin-chloral hydrate method (YABU and TOKIDA, 1966).

#### Observations

#### Scytosiphon lomentaria (Lyngbye) Link

Cultures from swarmers of the cylindrical frond of *Scytosiphon lomentaria* in nature were begun on February 12, 1965, March 16 and April 26, 1967. The cultures were first kept in No. 1, No. 3 and No. 5 incubators. Some cultures were afterwards transferred to No. 2 incubator.

Scytosiphon lomentaria is very commonly found from December to June of the next year, growing on stones and rocks in the intertidal zone at the area of Muroran. This alga has an unbranched cylindrical frond, which is solid when young and later on becomes tubular with occational constrictions. When fertile the frond forms only plurilocular organs (gametangia) covering the entire surface, except at the base. The plurilocular organs possess 10–20 or more compartments with scattered unicellular paraphyses (Fig. 1, A–B).

In *Scytosiphon lomentaria* from Muroran, sexual plants are isomorphic and heterothallic. Gametes are slightly anisogamous and conjugate in pairs. They are pear-shaped or ovoid, containing a single chromatophore and one eyespot, and are laterally biflagellate (Fig. 1, C-D). Their flagella are commonly attached rather



Fig. 1. Scytosiphon lomentaria.

A-B. Sections of a part of plurilocular gametangia. A. female. B. male. C. Female gametes. D. Male gametes.

nearer to the posterior end of the pear-shaped body and are of unequal length; the longer (15–23  $\mu$ m long) usually directed forwards and the shorter (7.0–7.6  $\mu$ m long) backwards during movement. The eyespot is usually adjacent to the point of attachment of the flagella. The female gametes measure 6.4–8.4  $\mu$ m×3.0–3.8  $\mu$ m (average 7.6  $\mu$ m×3.6  $\mu$ m) and the male 6.1–7.6  $\mu$ m×2.7–3.6  $\mu$ m (average 7.0 $\mu$ m×3.1  $\mu$ m). Both kinds of the gametes show a negative phototaxis. The female gametes



Fig. 2. Scytosiphon lomentaria. Development of zygotes.

A. Conjugation of gametes. B-C. Settled zygotes. D. Germination of a zygote.
E-G. 4-day-old germlings. H-I. Uniseriated filamentous, 7-day-old germlings.
J-K. 7-10-day-old germlings. L. 12-day-old germling. M. 18-day-old discal germling. (All grown in No. 3 incubator.)

have a shorter period of motility than the male. The former becomes sluggish soon after liberation, settling down on the substratum and the latter swims vigorously for one to several hours. When the female and male gametes are mixed, conjugation occurs at ca. 50-60 per cent between them to form zygotes (Fig. 2, A) and unfused gametes usually develop parthenogenetically.

**Zygotes and their development**—After sexual fusion, the zygotes soon settle down on the substratum and become spherical, measuring 6.0–7.6  $\mu$ m (average 6.7  $\mu$ m) in



Fig. 3. Scytosiphon lomentaria. Mature sporophytes.

A. Section of a part of a crust. B-E. Side views of a part of mature crusts and unilocular sporangia. F. Unilocular sporangia borne on filaments of a tuft.

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diameter (Fig. 2, B-C). Within 1-2 days, settled zygotes begin to germinate by pushing out a protuberance (Fig. 2, D; Pl. I, A) and then divide into two cells transversally (Fig. 2, E-G). Zygotic germlings, by transverse cell divisions, at first become uniseriate filaments consisting of 4-6 cells, an apical one of which provides a hair (Fig. 2, H-I). Within 10 days, cells of the filament begin to form branches (Fig. 2, J-L). By successive branchings and transverse cell divisions, the germlings usually develop into monostromatic discs with several hairs at their central part (Fig. 2, M). Then they become gradually thickened by cell divisions parallel to the substratum. The 20-day-old discs measure about 150-250  $\mu$ m in diameter and the 60-day-old discs about 5-8 mm. or more. The one- to four-layered basal stratum bears assimilatory filaments, paraphyses, composed of 2-8 cells (Fig. 3, A), forming crusts. Sometimes, the germlings develop into microthalli consisting of branched uni- and bi-seriate filaments, forming tufts.

These crusts and tufts grew well under warm and long-day conditions (No. 3 and No. 5 incubators), but they did not become fertile. When they were transferred to No. 2 incubator, however, the paraphyses of the crusts elongated more and more, and bore unilocular organs laterally near their base (Fig. 3, B-E; Pl. I, D). In the tufted plantlets, the unilocular organs were borne laterally on the unior bi-seriate filaments directly (Fig. 3, F; Pl. I, F), or on branchlets of the filaments, which are identical to the paraphyses of the crusts. Within 40-50 days after transfer, the unilocular sporangia reached maturity and liberated zoospores. In the cultures started on March 16, 1967, zygotic germlings grown in No. 3 incubator for 30 days formed unilocular sporangia about 50 days after transfer to No. 2 incubator. Similarly, in the cultures started on April 26, 1967, zygotic germlings grown in No. 5 incubator for 30 days became fertile, bearing unilocular sporangia within 40 days after transfer to No. 2 incubator. Namely, sterile zygotic germlings grown under long-day conditions became fertile by transferring to short-day conditions.

Mature unilocular sporangia are usually elongated obovoid (or ovoid in the tufted plantlets), measuring 70-120  $\mu$ m×18-34  $\mu$ m and each of them contains about 60-260 or more zoospores. Meiosis occurs during the formation of zoospores in the unilocular sporangium and the number of reduced (haploid) chromosomes is counted *ca.* 22-24 (Pl. I, J).

**Zoospores and their development**—The structure of zoospores is quite similar to that of gametes. The zoospores are pear-shaped or ovoid, measuring 6.0-8.4  $\mu$ m× 3.0-4.5  $\mu$ m (average 7.8  $\mu$ m×3.7  $\mu$ m). They contain a single chromatophore and one eyespot, and are laterally biflagellate (Fig. 4, A). The anterior flagellum is longer (15-20  $\mu$ m) than the posterior one (6.0-10.6  $\mu$ m). After liberation the zoospores swim actively for a while showing a negative phototaxis and then settle

down on the substratum. Settled zoospores become spherical and meaure 4.5-5.3  $\mu$ m (average 4.9  $\mu$ m) in diameter (Fig. 4, B-C; Pl. I, H). Within 2-3 days, they begin to germinate by pushing out a protuberance and then divide into two cells transversally (Fig. 4, D-F). By successive transverse cell divisions, germlings develop into uniseriate filaments consisting of 3-7 cells, an apical one of which usually provides a hair (Fig. 4, G). In most cases, the filamentous germlings develop into minute discs by successive branchings and cell divisions (Fig. 4, H-J). Then several erect filaments arise from the discs and they develop into cylindrical *Scytosiphon* plants by successive transverse and longitudinal cell divisions (Fig. 5, A). Some germlings develop into creeping filaments with sparse branchings (Fig. 4, K-L)



Fig. 4. Scytosiphon lomentaria. Development of zoospores.

A. Zoospores liberated from unilocular sporangia. B-C. Settled zoospores. D. Germination of a zoospore. E-F. 2-celled germlings. G. 3-day-old germling. H-I. 6-7-day-old germlings. J. 10-day-old discal germling. K. 6day-old filamentous germling. L. 10-day-old filamentous germling. (Grown in No. 1 and No. 3 incubators.)



Fig. 5. Scytosiphon lomentaria. Further development of zoospores.

A-B. Young *Scytosiphon* plants arising from a minute disc and a filamentous plantlet, grown in No. 1 and No. 3 incubators.

giving off many cylindrical *Scytosiphon* plants as lateral outgrowths (Fig. 5, B). The *Scytosiphon* plants grew well under conditions of No. 1 (or No. 3) where are relatively cool and long-day conditions. In 30-day-old cultures the plants attained a height of about 5–10 cm. and within 60 days they reached maturity forming plurilocular gametangia. Male and female gametangia are produced on separate plants.

**Parthenogenesis**—Gametes of either sex develop parthenogenetically. After swiming the gametes settle down on the substratum and become spherical. Settled female gametes measure  $4.5-6.1 \ \mu m$  (average  $5.2 \ \mu m$ ) in diameter, and male ones  $4.2-5.7 \ \mu m$  (average  $4.7 \ \mu m$ ). After 1-2 days the settled gametes begin to germinate

by pushing out a protuberance (Pl. I, B) and in 4 days germlings divide into two cells transversally. They at first develop into uniseriate filaments consisting of 3-7 or more cells, an apical one of which usually provides a hair.

In early development, germlings from parthenogametes expressed various types of thalli, involving a progression from uniseriate to minute discal through branched filamentous structures, as those from zygotes and zoospores. However, their subsequent development exhibited significant variations as follows. In cultures grown in No. 1 incubator, the germlings mostly developed into minute discs and often into creeping branched filaments. They gave off cylindrical Scytosiphon plants as in the development of zoospores. In 50-60 days the Scytosiphon plants attained a height of 5-10 cm. and reached maturity. They bore plurilocular gametangia and liberated gametes of respective sex; plants from female parthenogametes were female and those from male ones were male. Contrary, in No. 5 incubator most of the germlings developed into sterile crustaceous discs or tufted plantlets without formaton of the Scytosiphon plants as in the development of zygotes. On the other hand, in No. 3 incubator some germlings developed into minute discs or creeping filaments, giving off the fertile Scytosiphon plants and others developed into sterile crustaceous discs or tufted plantlets without formation of the Scytosiphon plants. These sterile crustaceous discs and tufted plantlets grown in No. 3 and No. 5 incubators became fertile, bearing unilocular sporangia when they were transferred to No. 2 incubator (Pl. I, E and G). In this case, meiosis does not occur during the formation of swarmers in the unilocular sporangia. Swarmers from the unilocular sporangia of haploid crusts and tufts developed into the Scytosiphon plants in the same way as the zoospores. These Scytosiphon plants grew well under No. 1 (or No. 3) conditions. In 50-60 days they attained a height of 5-10 cm. and reached maturity, bearing plurilocular gametangia of respective sex. Sexuality did not change, even passing through the unilocular sporangia-bearing haploid ralfsioid phase.

The results of this culture experiment indicate that there is an alternation of heteromorphic generations in *Scytosiphon lomentaria*; an alternation of a large polystichous gametophyte with plurilocular gametangia (*Scytosiphon* phase) and a small haplostichous sporophyte with unilocular sporangia (ralfsioid or tufted phase). Further, parthenogametes develop into the *Scytosiphon* plants directly or passing through the ralfsioid phase, according to culture conditions of temperature and photoperiod.

#### Petalonia zosterifolia (REINKE) KUNTZE

Cultures of *Petalonia zosterifolia* were started on March 16 and 30, 1967. They were first kept in No. 1, No. 3 and No. 5 incubators. Some cultures were afterwards transferred to No. 2 and No. 4 incubators. *Petalonia zosterifolia* is found from January to April growing on stones and other algae in the intertidal zone at the area of Muroran. This alga has a narrow unbranched compressed frond and forms only plurilocular organs (gametangia) spreading over the surface of the whole frond. The plurilocular organs usually possess 4–10 rows of compartments with scattered unicellular paraphyses (Fig. 6, A–B).



Fig. 6. *Petalonia zosterifolia.* A-B. Sections of a part of plurilocular gametangia. C. Female gametes. D. Male gametes.

In *Petalonia zosterifolia* from Muroran, sexual plants are isomorphic and heterothallic. The gametes are pear-shaped or ovoid, containing a single chromatophore and one eyespot, and are laterally biflagellate (Fig. 6, C-D). Their flagella are of unequal length; the anterior ones  $(18-23 \,\mu\text{m})$  are longer than the posterior (7.6-9.1  $\mu$ m). Sexual reproduction is isogamous. There is no marked difference in size between conjugating gametes of a pair, measuring 5.3-7.6  $\mu$ m× 2.3-3.8  $\mu$ m (average 6.5  $\mu$ m×3.3  $\mu$ m). The two kinds of gametes are negatively phototactic. However, they are distinguished by the period of motility. One soon becomes sluggish and settles down on the substratum, while the other swims vigorously for a few hours before settlement. It is considered that the former is female and the latter is male. When both gametes are mixed, conjugation occurs at *ca*. 50-60 per cent to form zygotes and unfused gametes usually develop parthenogenetically.

**Zygotes and their development**—After sexual fusion, the zygotes soon settle down on the substratum and become spherical, measuring 6.0–6.4  $\mu$ m (average 6.2  $\mu$ m) in diameter (Fig. 7, A–B; Pl. II, A, a). Within 1–2 days settled zygotes germinate

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Fig. 7. Petalonia zosterifolia. Development of zygotes.

A-B. Settled zygotes. C-D. Germination of zygotes. E. 2-celled germling. F-G. Uniseriated filamentous germlings. H-J. 6-7-day-old germlings. K. 16-day-old discal germling. (All grown in No. 3 incubator.)

by pushing out a protuberance (Fig. 7, C-D) and then divide into two cells transversally (Fig. 7, E). Germlings usually develop into uniseriate filaments consisting of 3-5 cells, an apical one of which often provides a hair (Fig. 7, F-G) and in 5-7 days cells of the filaments begin to form branches (Fig. 7, H, J). Sometimes such branchings occur at the 2-celled stage (Fig. 7, I), especially grown under warm conditions. By successive branchings and transverse cell divisions, most of the germlings develop into minute discs with several hairs at the central part (Fig. 7, K). They gradually increase in diameter becoming crustaceous (Pl. II, C) and in 60 days attain a diameter of 5-8 mm. or more.

In No. 1, No. 3 and No. 5 incubators, the crusts from zygotes did not become fertile and finally fell off from the substratum, becoming rough. The  $75 \sim 90$ -day-old crusts grown under these culture conditions were transferred to No. 2 and No. 4 incubators in order to obtain fertile ones. Consequently, the fertile crusts



Fig. 8. *Petalonia zosterifolia*. Mature sporophytes. A. Section of a part of a crustaceous disc. B-E. Side views of a part of mature crusts and unilocular sporangia.

were observed in cultures grown in No. 4 incubator only. In No. 4 incubator the paraphyses of crustaceous discs elongated more and more, and 30-50 days after transfer unilocular sporangia were borne laterally near the basal cell of the paraphyses (Fig. 8, B-E; Pl. II, F), reaching maturity. In the cultures started on July 5, 1967, zygotic germlings (obtained by mixing of female and male gametes from the second generation plants in culture) were at first maintained under No. 3 conditions. In 45 days they developed into crustaceous discs, attaining about 2-3 mm. in diameter. These crustaceous discs formed unilocular sporangia (September 20-22) about 30 days after transfer to No. 4 incubator. Namely, the fertile crusts were obtained by changing culture conditions. Some zygotic germlings developed into tufted plantlets consisting of creeping branched filaments, often with minute discs, as described in *Scytosiphon lomentaria*. In this experiment, these tufts did not become fertile even under No. 4 culture conditions.

Unilocular sporangia are elongated obovoid, measuring  $64-95 \ \mu m \times 22-34 \ \mu m$ and each sporangium contains about 60-260 zoospores. Meiosis occurs during the formation of zoospores in the unilocular sporangium and the number of haploid chromosomes is counted *ca.* 21-22 (Pl. II, J).

**Zoospores and their development**—The zoospores are pear-shaped or ovoid, measuring 6.1–7.6  $\mu$ m × 3.0–4.5  $\mu$ m (average 6.5  $\mu$ m × 3.9  $\mu$ m). They contain a single chromatophore and one eyespot, and are laterally biflagellate (Fig. 9, A). The anterior flagellum is longer (18–20  $\mu$ m) than the posterior one (6.0–7.6  $\mu$ m). The zoospores swim actively for a while after liberation, showing a negative phototaxis and then settle down on the substratum. Settled zoospores become spherical, measuring 4.5–5.7  $\mu$ m (average 5.0  $\mu$ m) in diameter (Fig. 9, B–C; Pl. II, B). Within 1–2 days



Fig. 9. Petalonia zosterifolia. Development of zoospores.

A. Zoospores liberated from unilocular sporangia. B-C. Settled zoospores. D-E. Germination of zoospores. F. 2-celled germling. G-I. 4-day-old germlings. J-K. 6-day-old germlings. L. 10-day-old discal germling. (Grown in No. 1 and No. 3 incubators.) they begin to germinate by pushing out a protuberance (Fig. 9, D-E) and then divide into two cells transversally (Fig. 9, F). These germlings develop into uniseriate filaments consisting of 3-5 cells, an apical one of which usually provides a hair, and soon form minute discs by successive branchings and transverse cell divisions (Fig. 9, G-K). The minute discs usually provide several hairs at the central part (Fig. 9, L) and in 10-15 days several erect filamentous thalli arise from the discs (Fig. 10, A). These erect filaments develop into *Petalonia* plants by successive transverse and longitudinal cell divisions (Fig. 10, C; Pl. II, G). Some germlings develop into creeping and branched filaments, often with minute discs. These filaments also form many *Petalonia* plants as lateral outgrowths (Fig. 10, B).

The *Petalonia* plants grew well in No. 1 (or No. 3) incubator. In 30-day-old cultures the plants attained a height of about 1-5 cm. and reached maturity, bear-



Fig. 10. *Petalonia zosterifolia*. Further development of zoospores.

A-C. Young *Petalonia* plants arising from minute discs and a filamentous plantlet, grown in No. 1 and No. 3 incubators.

ing plurilocular gametangia. Female and male gametangia are produced on separate plants.

**Parthenogenesis**—The gametes of either sex develop parthenogenetically. The gametes, after swimming, settle down on the substratum and become spherical (Pl. II, A, b), measuring  $4.5-5.3 \,\mu\text{m}$  (average  $4.8 \,\mu\text{m}$ ) in diameter. Within 2 days settled gametes begin to germinate by pushing out a protuberance and in 5-6 days they divide into two cells transversally. The early and subsequent developments of parthenogametes of *Petalonia zosterifolia* are similar to those of *Scytosiphon lomentaria*.

In cultures grown in No. 1 incubator, germlings from parthenogametes mostly developed into minute discs and often filamentous plantlets. They gave off *Petalonia* plants in the same way as in the development of the zoospores (Pl. II, H). In 35-45 days the *Petalonia* plants attained a height of 1-5 cm. and reached maturity. They bore plurilocular gametangia and produced gametes of respective sex. Contrary, most of the germlings grown in No. 5 incubator developed into crustaceous discs or tufted plantlets without formation of the *Petalonia* plants as described in the development of zygotes. On the other hand, in No. 3 incubator some germlings developed into minute discs or creeping branched filaments, giving off the *Petalonia* plants and others developed into crustaceous discs or tufted plants and others developed into crustaceous discs or tufted plants and others developed into crustaceous discs or tufted plants and others developed into crustaceous discs or tufted plants and others developed into crustaceous discs or tufted plantlets without formation of the *Petalonia* plants. These crustaceous discs obtained in No. 3 and No. 5 incubator became fertile, bearing unilocular sporangia (Pl. II, E) when they were transferred to No. 4 incubator. In the case of tufted plantlets, fertile plants with unilocular sporangia were not obtained under similar experimental treatments.

In the unilocular sporangia derived from parthenogametes, meiosis does not occur during the formation of swarmers. Swarmers released from these unilocular sporangia developed into minute discs or branched filaments, giving off the *Petalonia* plants in the same way as the zoospores. The *Petalonia* plants grew well under conditions of No. 1 and No. 3. In about 30 days they attained a height of 1-5 cm. and reached maturity, bearing plurilocular gametangia. Sexuality was unchangeable in the respective plant.

Consequently, it is clear that in *Petalonia zosterifolia* there is an alternation of heteromorphic generations; an alternation of a large polystichous gametophyte with plurilocular gametangia (*Petalonia* phase) and a small haplostichous sporophyte with unilocular sporangia (ralfsioid phase). Parthenogametes develop into the *Petalonia* plants directly or passing through the ralfsioid phase, depending upon culture conditions.

#### Petalonia fascia (O. F. Müller) Kuntze

Cultures of *Petalonia fascia* were started on February 6 and 8, 1965, January 14, 1966, December 15, 1967 and March 4, 1968. The cultures were first kept in No. 1–No. 5 incubators. Some cultures were afterwards transferred from the long-day condition to the short-day.

*Petalonia fascia* is commonly found from December to early March of the next year, growing on stones, rocks and wooden work in the intertidal zone at the area of Muroran. The frond of this alga is flat and lanceolate, and broader than that of *P. zosterifolia*. When fertile, it forms only pluriloculor organs, spreading over the entire surface. The plurilocular organs usually possess 4-8 rows of compartments without scattered unicellular paraphyses (Fig. 11, A).



Fig. 11. Petalonia fascia.

A. Section of a part of plurilocular organs. B. Swarmers liberated from plurilocular organs. C-D. Settled swarmers. E. Germination of a swarmer.

**Swarmers from plurilocular organs and their development**—Since 1965, conjugation tests have been tried many a time on swarmers from plurilocular organs derived from a number of fronds collected in different seasons and at different loculities of the Muroran area, but no conjugation has occurred.

The swarmers from plurilocular organs are pear-shaped or ovoid, containing a single chromatophore and one eyespot, and are laterally biflagellate (Fig. 11, B). They measure  $6.1-7.6 \ \mu m \times 3.0-4.5 \ \mu m$  (average  $6.7 \ \mu m \times 3.4 \ \mu m$ ), and their longer flagella are  $18-22 \ \mu m$  long and their shorter ones  $6.1-7.6 \ \mu m$ . They swim actively for a while after liberation and show a negative phototaxis. Finally they become sluggish and settle down on the substratum. Settled swarmers become spherical, measuring  $4.2-5.3 \ \mu m$  (average  $4.5 \ \mu m$ ) in diameter (Fig. 11, C-D; Pl. III, A). Within 2 days the settled swarmers begin to germinate by pushing out a protuberance (Fig. 11, E) and then divide into two cells transversally (Fig. 12, A-B). By successive transverse cell divisions, germlings develop into uniseriate filaments consisting of 3-6 cells, an apical one of which usually provides a hair (Fig. 12, C-D). Within 7 days cells of the filaments begin to form branches (Fig. 12, E-F). By successive transverse cell divisions and branchings, the germlings develop into minute discs (Fig. 12, G) or branched filamentous plantlets.

Under various culture conditions, some of the minute discs and the branched filamentous plantlets gave off the *Petalonia* plants (Fig. 12, H), and others grew into crusts or tufts (Pl. III, C-D).

In the former case, the *Petalonia* plants grew well especially under the conditions of No. 1, No. 2 and No. 3. In 30 days the plants attained a height of



Fig. 12. Petalonia fascia. Development of swarmers from plurilocular organs.

A-B. 2-celled germlings. C-D. Uniseriated filamentous, 6-day-old germlings. E-F. 7-10-day-old germlings. G. 12-day-old discal germling. H. Young *Petalonia* plants arising from a minute disc. (From the materials grown in No. 1 and No. 3 incubators.)

about 3-5 cm. and in 40-50 days they reached maturity, forming plurilocular organs. Swarmers liberated from these plants developed into the *Petalonia* plants, or the crusts and the tufts.

In the latter case, the crusts or the tufts grew well especially in No. 3 and No. 5 incubators, and in 60 days they attained a diameter of 3-8 mm. When they were retained under No. 3 and No. 5 conditions, except a few case, they did not



Fig. 13. Petalonia fascia. Mature crusts.A. Section of a part of a crustaceous disc with paraphyses.B-D. Paraphyses with mature unilocular organs.

show any marked development and some of the crusts fell off from the substratum, becoming rough. In this experiment, they became fertile, bearing unilocular sporangia when they were transferred to No. 4 incubator.

In the cultures started with swarmers from the plurilocular organs on March 4, 1968 and grown in No. 5 incubator, germlings also developed into crusts or tufts in 80 days. Within 20 days after transfer to No. 4 incubator they became fertile, bearing unilocular sporangia.

Under warm and short-day conditions, the paraphyses of the crusts elongate and unilocular organs are borne laterally near the basal cells of the paraphyses (Fig. 13, B-D; Pl. III, F). The unilocular sporangia are elongated obovoid, measuring 55-90  $\mu$ m or more  $\times 25$ -30  $\mu$ m. Each unilocular sporangium produces about 60-260 swarmers. On the other hand, in the case of the tufts, some filaments give off branchlets with unilocular organs at their basal cell (Pl. III, E). These unilocular sporangia usually are globular or somewhat obovoid, measuring 20-40  $\mu$ m in diameter and each produces about 30 or more swarmers.

Meiosis does not occur in the unilocular sporangium of this alga (Pl. III, I-J). Swarmers from unilocular organs and their development-The swarmers from unilocular organs are pear-shaped or ovoid, measuring 6.0–8.4  $\mu$ mimes3.5–5.0  $\mu$ m (average 7.4  $\mu$ m × 4.4  $\mu$ m). They contain a single chromatophore and one eyespot, and are laterally biflagellate (Fig. 14, A). Their anterior flagellum is longer (15- $21 \,\mu\text{m}$ ) than their posterior one (6-9  $\mu\text{m}$ ). After liberation the swarmers swim actively for a while, showing a negative phototaxis and finally settle down on the substratum. Settled swarmers become spherical (Fig. 14, B-C; Pl. III, B) and measure 4.5-5.5  $\mu$ m (average 4.8  $\mu$ m) in diameter. Within 2-3 days they begin to germinate by pushing out a protuberance and then divide into two cells transversally (Fig. 14, D). In 5-7 days germlings divide into 3-4 cells uniseriately (Fig. 14, E) and each cell of the filament begins to branch off (Fig. 14, F-G). By successive cell divisions and branchings, the germlings develop into minute discs (Fig. 14, H) or branched filamentous plantlets. Within 15 days several Petalonia plants arise from the minute disc (Fig. 14, I-J; Pl. III, G) or from the filamentous plantlet (Pl. III, H). These Petalonia plants grew well especially in No. 1, No. 2 and No. 3 incubators. In 30-40 days they attained a height of 3-5 cm. or more and reached maturity, forming plurilocular organs. Swarmers from these plurilocular organs showed no sexuality and developed into the Petalonia plants or the unilocular sporangia-bearing crusts and tufts.

As mentioned above, *Petalonia fascia* from Muroran exhibited no sexuality. However, sometimes two generations alternate; one is a large polystichous plant with plurilocular organs (*Petalonia* phase) and the other is a small haplostichous plant with unilocular organs (ralfsioid or tufted phase), sometimes the *Petalonia* 



Fig. 14. *Petalonia fascia*. Development of swarmers from unilocular organs.

A. Swarmers liberated from unilocular organs. B-C. Settled swarmers. D. 2-celled germling. E-G. 7-10-day-old germling grown in No. 4 incubator. H. 7-day-old discal germling grown in No. 1 incubator. I-J. Young *Petalonia* plants arising from minute discs grown in No. 1 incubator; I, from a 13-day-old culture and J, from a 18-day-old culture.

phase is repeated. Such a life cycle seems to occur according to the environmental conditions of temperature, photoperiod and other factors. This type of life cycles is quite identical to that of parthenogenesis in *Scytosiphon lomentaria* and *Petalonia zosterifolia*.

## Colpomenia bullosa Yamada

Cultures of *Colpomenia bullosa* were begun on January 18, 1966 and March 13, 1967. The cultures were usually grown in No. 1 and No. 3 incubators consistently.

*Colpomenia bullosa* is very commonly found from late November to early June of the next year, growing on rocks in the intertidal zone at the area of Muroran. This alga has a saccate frond, which is solid in the juvenile stage and soon becomes hollow. When fertile, it forms only plurilocular organs, spreading over the surface of the whole frond. The plurilocular organs are divided uniseriately, often biseriately, into 10–30 compartments with scattered unicellular paraphyses (Fig. 15, A–B).



A-B. Sections of a part of plurilocular gametangia. C. Female gametes. D. Male gametes.

In Colpomenia bullosa from Muroran, sexual plants are isomorphic and heterothallic, and form only plurilocular gametangia. The gametes are pear-shaped or ovoid, containing a single chromatophore and one eyespot, and are laterally biflagellate (Fig. 15, C-D). Their flagella are of unequal length; the anterior flagellum (15-20  $\mu$ m) is longer than the posterior one (7.6-9.0  $\mu$ m). Sexual reproduction is isogamous; no marked difference in size is found between conjugating gametes of a pair. The gametes measure 5.3-7.6  $\mu$ m×3.0-3.8  $\mu$ m (average 6.5  $\mu$ m×3.3  $\mu$ m). They show a negative phototaxis and are distinguishable by the period of motility. One (female) soon becomes sluggish and settles down on the substratum, while the other (male) swims vigorously for a few hours before settlement.

When both kinds of the gametes are mixed, conjugation occurs at ca. 50-60 per cent to form zygotes (Fig. 16, A). Unfused gametes usually develop parthenogenetically.

**Zygotes and their development**—After sexual fusion, the zygotes soon settle down on the substratum and become spherical, measuring 5.7–7.9  $\mu$ m (average 6.9  $\mu$ m) in diameter (Fig. 16, B-C; Pl. IV, A). Within 1–2 days settled zygotes begin to germinate by pushing out a protuberance (Fig. 16, D) and then divide into two cells transversally (Fig. 16, E). By successive transverse cell divisions, germlings



Fig. 16. Colpomenia bullosa. Development of zygotes.

A. Conjugation of gametes. B-C. Settled zygotes. D. Germination of a zygote. E. 2-celled germling. F. Uniseriated filamentous, 5-day-old germling. G. 5-day-old germling. H. 10-day-old germling. I. 14-day-old germling. (All grown in No. 3 incubator.)

become uniseriate filaments, consisting of 4-7 or more cells, an apical one of which usually provides a hair (Fig. 16, F). In 5-7 days cells of the filaments begin to form branches (Fig. 16, G). The filamentous germlings often become creeping, loosely branched plantlets (Fig. 16, H-I; Pl. IV, D) and finally grow into crustaceous discs or tufted plantlets (Pl. IV, E-F).



Fig. 17. Colpomenia bullosa. Mature sporophytes.A-D. Side views of a part of mature crusts and unilocular sporangia.E. Unilocular sporangia borne on filaments of a tuft.

#### Y. Nakamura and M. Tatewaki

In 40~50-day-old cultures grown in No. 3 incubator, the crustaceous discs attained a diameter of about 1-3 mm. and produced the paraphyses on the whole surface. The paraphyses elongated more and more, and in 70-80 days bore unilocular sporangia laterally near their basal cells (Fig. 17, A-D; Pl. IV, G). In the case of the tufted plantlets, some filaments gave off branchlets with unilocular sporangia (Pl. IV, H) and others formed unilocular sporangia directly without formation of branchlets (Fig. 17, E). In the cultures grown in No. 1 incubator, the crustaceous discs and tufted plantlets did not become fertile.

Unilocular sporangia are elongated obovoid or ovoid, measuring  $80-150 \ \mu m \times 30-40 \ \mu m$  and each sporangium contains about 60-260 or more zoospores. Meiosis occurs during the formation of zoospores in the unilocular sporangium and the number of haploid chromosomes is counted *ca.* 18 (Pl. IV, L).

Zoospores and their development—The zoospores are pear-shaped or ovoid, measuring 6.0-7.6  $\mu$ m  $\times$  3.3-4.5  $\mu$ m (average 7.1  $\mu$ m  $\times$  4.0  $\mu$ m). They contain a single chromatophore and one eyespot, and laterally biflagellate (Fig. 18, A). Their flagella are of unequal length; the anterior ones (16-20  $\mu$ m) are longer than the posterior  $(6.0-7.6 \,\mu\text{m})$ . After liberation the zoospores swim actively for a while, showing a negative phototaxis and then settle down on the substratum. Settled zoospores become spherical (Fig. 18, B-C ; Pl. IV, C) and measure 4.5-5.4  $\mu$ m (average 4.7  $\mu$ m) in diameter. Within 1-2 days they begin to germinate by pushing out a protuberance (Fig. 18, D) and then divide into two cells transversally (Fig. 18, E). By successive transverse cell divisions, germlings develop into uniseriate filaments consisting of 3-7 cells (Fig. 18, F-G), an apical one of which usually provides a hair. In 5-7 days cells of the filaments give off branches. By successive cell divisions and branchings, the germlings develop into minute discoid plantlets (Fig. 18, H-I) and these gradually begin to upheave at their central part (Fig. 18, J). Such an upheaval rapidly increases in size and develops into a saccate Colpomenia plant.

The *Colpomenia* plants grew well in No. 1 and No. 3 incubators. In 40-50 days the saccate plants attained a height of about 1-2 cm. (Pl. IV, J) and reached maturity. They bore plurilocular gametangia, producing female and male gametangia on separate plants.

**Parthenogenesis**—Gametes of either sex develop parthenogenetically. After swimming, the gametes settle down on the substratum and become spherical, measuring 4.2-5.3  $\mu$ m (average 4.7  $\mu$ m) in diameter (Pl. IV, B). Within 2 days settled gametes begin to germinate by pushing out a protuberance and then divide into two cells transversally. Subsequent development of germlings involves a progression from uniseriate filaments to minute discs through intermediate structures by successive cell divisions and branchings.



Fig. 18. Colpomenia bullosa. Development of zoospores.

A. Zoospores liberated from unilocular sporangia. B-C. Settled zoospores. D. Germination of a zoospore. E. 2-celled germling. F-G. Uniseriated filamentous, 6-day-old germlings. H-I. 10-day-old germlings. J. Saccate *Colpomenia* plant arising from a prostrate system. (All grown in No. 3 incubator.)

In No. 1 and No. 3 incubators, the germlings mostly developed into crustaceous discs or tufted plantlets and some germlings produced saccate *Colpomenia* plants. In the latter case, the *Colpomenia* plants reached maturity in 50-80 days, bearing plurilocular gamentangia. In the former case, the crusts and the tufts (especially grown in No. 3 incubator) became fertile in 100-120 days, producing a number of swarmers from unilocular sporangia (Pl. IV, I). In this case, meiosis does not occur during the formation of swarmers in the unilocular sporangium. The swarmers from the unilocular sporangia derived from parthenogametes developed into the saccate *Colpomenia* plants in the same way as the zoospores. In 60–70 days these *Colpomenia* plants attained a height of about 0.5–1.0 cm. and reached maturity under No. 1 and No. 3 conditions. They bore plurilocular gametangia and produced gametes of respective sex.

Consequently, in *Colpomenia bullosa*, there is an alternation of heteromorphic generations; an alternation of a large polystichous gametophyte with plurilocular gametangia (*Colpomenia* phase) and a small haplostichous sporophyte with unilocular sporangia (ralfsioid or tufted phase). Parthenogametes develop into the *Colpomenia* plants either directly or passing through the ralfsioid phase with unilocular sporangia.

#### Endarachne binghamiae J. Agardh

Cultures of *Endarachne binghamiae* were started on April 10, 1967. The cultures were first kept in No. 3 and No. 5 incubators. Some cultures were afterwards transferred to No. 4 incubator.

Endarachne binghamiae is common on the coast of Honshu washed by warm currents. Materials used in this experiment were collected at Amatsu-kominato, Boso Peninsula, Chiba Prefecture on April 8, 1967, growing on rocks in the intertidal zone. This alga has a flat and lanceolate frond, superficially resembling Petalonia fascia. When fertile, the frond forms plurilocular organs, spreading



Fig. 19. Endarachne binghamiae. A-B. Sections of a part of plurilocular gametangia.

C. Female gametes. D. Male gametes.

over the entire surface. The plurilocular organs possess 4-12 rows of compartments (Fig. 19, A-B) and no unicellular paraphyses are found.

In Endarachne binghamiae from Amatsu-kominato, sexual plants are isomorphic and heterothallic, and bear only plurilocular gametangia. The gametes are pear-shaped or ovoid, containing a single chromatophore and one eyespot, and are laterally biflagellate (Fig. 19, C-D). Their flagella are of unequal length; the anterior ones (16-20  $\mu$ m) are longer than the posterior (6.0-7.6  $\mu$ m). Sexual reproduction is isogamous. The gametes measure 4.8-6.9  $\mu$ m×2.7-3.8  $\mu$ m (average 5.9  $\mu$ m×3.1  $\mu$ m). They show a negative phototaxis, swimming towards the darkest part of a vessel. They become sluggish after swimming for about one hour and finally settle down on the substratum. No significant difference is found in motility between female and male gametes.

When female and male gametes are mixed, conjugation occurs at 50-60 per cent to form zygotes and unfused gametes usually develop parthenogenetically.

**Zygotes and their development**—After sexual fusion, the zygotes soon settle down on the substratum and become spherical, measuring 5.0–6.5  $\mu$ m in diameter (Fig. 20, A–B; Pl. V, A, a and B). Within 1–3 days settled zygotes begin to germinate by pushing out a protuberance (Fig. 20, C–D) and divide into two cells transversally (Fig. 20, E). Then they develop into uniseriate filaments consisting of 3–5 or more cells (Fig. 20, F), an apical one of which often provides a hair.

In No. 3 incubator, cells of the filaments gave rise to branches (Fig. 20, G–H), developing into minute discs within 10 days and the growth of discs was very slow. On the other hand, in the cultures grown in No. 5 incubator, the growth of discs was very rapid (Fig. 20, I). The  $20 \sim 23$ -day-old discs attained a diameter of 2–3 mm. and the 30-day-old ones 5–10 mm., developing into crustraceous discs (Pl. V, D) or tufted plantlets. When retained in No. 3 and No. 5 incubators (long-day conditions) the crusts did not show any marked development. However, they became mature when they were transferred to No. 4 incubator. About one month after transfer some 4-month-old crusts (obtained from cultures in No. 5 incubator) reached maturity, bearing unilocular sporangia; the paraphyses did not grow well but they bore unilocular sporangia laterally near the basal cells (Fig. 20, K–L; Pl. V, E).

Unilocular sporangia are obovoid or more elongated one, measuring 65–75  $\mu$ m ×26–30  $\mu$ m. Meiosis occurs during the formation of zoospores in the unilocular sporangium, and number of haploid chromosomes is counted *ca.* 20–22 (Pl. V, J). **Zoospores and their development**—Unfortunately, zoospore-liberation from the unilocular sporangia and early development of zoospores of this alga could not be observed. However, many new germlings were found growing on the wall of culture vessels which contained mature crusts with unilocular sporangia. These



Fig. 20 Endarachne binghamiae. Development of zygotes.

A-B. Settled zygotes. C-D. Germination of zygotes. E. 2-celled germling. F. Uniseriated filamentous, 5-day-old germling. G-H. 11-day-old germlings. I. 14-day-old discal germling. J. Section of a part of a crustaceous disc. K-L. Side views of a part of mature crusts and unilocular sporangia. (C-H grown in No. 3 incubator, I-J in No. 5 and K-L in No. 4.)

new germlings composed of 5-10 cells were isolated with a micropipette and transferred to new culture vessels. They developed into minute discs and within 20 days after tranfer many *Endarachne* plants arose from these discs (Pl. V, G). The *Endarachne* plants grew well in No. 3 and No. 5 incubators. In 60-65 days they attained a height of about 2 cm. and reached maturity, bearing plurilocular gametangia. Female and male gametangia are produced on separate plants.

**Parthenogenesis**—The gametes of either sex develop parthenogenetically. After swimming they settle down on the substratum and become spherical, measuring  $3.7-4.8 \ \mu m$  (average  $4.5 \ \mu m$ ) in diameter (Pl. V, A, b). Within 2-3 days settled parthenogametes begin to germinate by pushing out a protuberance and divide into two cells transversally. By successive transverse cell divisions germlings at first develop into uniseriate filaments and then cells of the filaments give off branches, developing into minute discs (Pl. V, C).

In No. 3 and No. 5 incubators, most of the discs produced the *Endarachne* plants (Pl. V, H). In about 60–65 days the plants attained a height of 1–2 cm. and reached maturity, bearing plurilocular gametangia. On the other hand, some discs increased enormously in size and became crustaceous in the same way as the zygotes. These crustaceous discs became mature when they were transferred to No. 4 incubator, bearing unilocular sporangia on the paraphyses laterally near the basal cells (Pl. V, F).

The results of this experiment indicate that there is an alternation of heteromorphic generations in this species; an alternation of a large polystichous gametophyte with plurilocular gametangia (*Endarachne* phase) and a small haplostichous sporophyte with unilocular sporangia (ralfsioid phase). Parthenogametes develop into the *Endarachne* plants directly or passing through the ralfsioid phase.

## Discussion

The order Scytosiphonales was established by FELDMANN in 1949, on the basis of the cellular structure containing a single plastid with one large pyrenoid, and the presence of only one type of reproductive organs, the plurilocular sporangia. The Phaeosaccionaceae, which was included in this order, was recently removed to the Chrysophyceae by the morphology, life history and chemical composition of both the zoospore and the thallus (PARKE and DIXON, 1964; CRAIGIE *et al.*, 1971; MCLACHLAN *et al.*, 1971; CHEN *et al.*, 1974). Thus this order includes two families; Scytosiphonaceae and Chnoosporaceae, the latter with a single genus. The Scytosiphonaceae includes eight genera; Scytosiphon, Petalonia, Colpomenia, Endarachne, Hydroclathrus, Rosenvingea, Iyengaria and Utriculidium. FELDMANN considered that members of this order have an alternation of isomorphic generations and hypothesized that meiosis occurs during germination of zygotes.

Investigations on the life history of the species belonging to this order made by previous investigators in various loculities have been fully reviewed by WYNNE in 1969. As pointed out by him, in most of the previous culture studies the entire life cycle of these species has not been completed and thus the controversy has continued up to the present.

DANGEARD (1963) first found microthalli bearing both unilocular sporangia and erect blades in culture of *Petalonia zosterifolia*. NAKAMURA (1965) demonstrated that swarmers from the unilocular sporangia of *Ralfsia*-like crustaceous thalli obtained from nature, develop into *Scytosiphon lomentaria* and *Petalonia fascia* plants, and expressed the view that both species have an alternation of heteromorphic generations. TATEWAKI (1966) was able to complete the entire life cycle of *Scytosiphon lomentaria* in culture and demonstrated that this species exhibits an alternation of heteromorphic generations. LUND (1966) also reported the presence of microthalli bearing unilocular sporangia and young erect plants in *Scytosiphon lomentaria* in nature.

WYNNE (1969), investigating Scytosiphon, Petalonia and Endarachne, observed that the expression of crusts with unilocular sporangia or cylindrical thalli with plurilocular sporangia (Scytosiphon lomentaria) and crusts and blades (Petalonia fascia) is influenced in most experiments by culture conditions. He noted that swarmers from either unilocular or plurilocular organs tend to develop into cylindrical thalli or blades under relatively cool and short-day conditions, whereas the development of similar swarmers into crusts is favored by relatively warm and long-day conditions. Intermediate growth conditions bring about intermediate percentages of both morphological expressions. Further, HSIAO (1969), working on *Petalonia fascia*, concluded that iodine is essential for growth and reproduction of this species; the minimal concentrations required for development of the *Ralfsia*like thalli and blades are  $4 \times 10^{-5}$  M KI and  $4 \times 10^{-6}$  M KI, respectively, and that the other two morphological types of thalli, protonemata and plethysmothalli, are formed especially in iodide-free medium.

The major difference between the present results and those of the previous workers with the species of the Scytosiphonaceae is that the species examined, with the exception of *Petalonia fascia*, exhibit sexual reproduction. Sexual plants with plurilocular gametangia are isomorphic and heterothallic. Sexual reproduction ranges from isogamy (*Petalonia zosterifolia*, *Colpomenia bullosa* and *Endarachne binghamiae*) to slight anisogamy (*Scytosiphon lomentaria*). Conjugation between swarmers from the plurilocular organs has been observed in *Scytosiphon lomentaria* (BERTHOLD, 1881; KUCKUCK, 1898, 1912; FRYE, 1930; ABE, 1935 and TATEWAKI, 1966), *Petalonia zosterifolia* (KUCKUCK, 1912), *P. fascia* (KUNIEDA and ARASAKI, 1947), *Colpomenia sinuosa* (KUNIEDA and SUTO, 1938), *C. bullosa* (KUNIEDA and SUTO, 1948; NAKAMURA and TATEWAKI, 1966) and *Endarachne binghamiae* (KUNI EDA and SUTO, 1948). In these previous investigations except a few cases, however, it occurred in rare instances. In our experiments, the conjugation occurred commonly at 50-60 per cent and unfused gametes mostly developed parthenogenetically.

Zygotes, parthenogametes, zoospores, and swarmers expressed similarly various morphological types of thalli in early development. They develop into uniseriate filaments, prostrate branched filaments, erect branched filaments forming tufts, and uni- or multistratose discs, taking various intermediate structures. However, their subsequent development exhibited significant variations. In most experiments germlings from zygotes developed into discal structures, forming crusts (often tufts) without the formation of erect fronds. The growth of the crusts or tufts is promoted under relatively warm and long-day conditions, but they never became fertile, except Colpomenia bullosa. They bore unilocular sporangia when they were transferred to short-day and relatively cool conditions. Germlings from zoospores liberated from these unilocular sporangia gave rise to erect fronds identical with the parent plants. They bore plurilocular gametangia under relatively cool condition with long or short day-length (No. 1, No. 2 and No. 3 incubators). On the other hand, germlings from parthenogametes gave off the erect fronds with plurilocular gametangia under cool, long-day conditions (No. 1 incubator). Contrary, the similar germlings grown under warm, long-day conditions (No. 5 incubator) mostly developed into the crusts or the tufts without the formation of erect fronds. They formed unilocular organs when they were tranferred to short-day conditions (No. 2 or No. 4 incubator). Germlings from swarmers liberated from these unilocular organs of haploid thalli gave off the erect fronds in the same way as those from the zoospores. Further, under relatively cool, long-day conditions (No. 3 incubator) some germlings gave off the erect fronds with plurilocular gametangia and others developed into sterile crusts or tufts without the formation of erect fronds.

The results of our culture experiments on the development of parthenogametes are in accordance with WYNNE's observations on *Scytosiphon lomentaria* and *Petalonia fascia*, and also with those of DANGEARD (1963) on *Petalonia zosterifolia* from culture and of LUND (1966) on *Scytosiphon lomentaria* from nature, as cited above. It appears that LÜNING and DRING's observations (1973, 1975 a, b) regarding the influence of light quality, photoperiod, and temperature on the development of *Petalonia* and *Scytosiphon* were based on the development of parthenogametes liberated from plurilocular organs of the erect frond phase.

No morphological difference was found between crusts with unilocular sporangia derived from zygotes and those from parthenogametes. In our cytological observations, however, meiosis occurred only in unilocular sporangia of diploid crusts from the zygotes during the formation of zoospores, while it did not occur in those of haploid crusts from the parthenogametes.

From this, it was concluded that the pattern of life cycles with an alternation of erect fronds (cylindrical, leafy or saccate) and crustose discs was brought in response to a difference in ploidy level rather than to culture conditions, accompanying an alternation of nuclear phases.

In *Petalonia fascia*, we found no sexual phenomena as described by the previous investigators. Its life cycle is identical to that of parthenogenesis of the other species examined, exhibiting the dimorphic development, erect fronds and crusts in response to culture conditions, as reported by WYNNE in the same species from the North America. However, a variance in chromosome numbers was often found according to individuals, ranging from 24 to 50, though no evidence of conjugation of swarmers from plurilocular organs was found. In this respect it will require more precise investigations.

Further, DANGEARD (1963) has illustrated prostrate ectocarpoid plantlets with plurilocular organs in his succeeding cultures on the members of the Scytosiphonaceae, and also CARAM (1965) reported the similar ectocarpoid thalli with plurilocular organs in *Petalonia fascia*. HSIAO (1969), investigating *Petalonia fascia*, obtained the ectocarpoid thalli (protonemata) with plurilocular organs by changing iodine concentrations of culture medium. In the present cultures, we have commonly observed filamentous microthalli (tufts) with unilocular organs in *Scytosiphon lomentaria*, *Petalonia fascia* and *Colpomenia bullosa*, but we never obtained the ectocarpoid thalli with plurilocular organs in the species examined.

As to the taxonomic status of the crustose stage of *Scytosiphon*, LUND (1966) stated that it belongs to the genus *Microspongium*, and he was supported by MCLACHLAN *et al.* (1971). WYNNE (1969) expressed the view that the crust of *Petalonia fascia* presents the morphological characteristics of the subgenus *Stragularia* of *Ralfsia*, i. e., *R. californica*. EDELSTEIN *et al.* (1970), working on *Ralfsia clavata* and *R. bornetii*, reported that both species are the crustose stage of *Petalonia fascia*.

Naturally occurring crusts of *Scytosiphon* and *Petalonia* from Muroran grow on pebbles and stones together with *Ralfsia verrucosa*. Both crusts of *Scytosiphon* and *Petalonia* are able to be distinguished from *Ralfsia verrucosa* by an outer appearance and frond structure; the former two have a growing margin of discrete apical cells as pointed out by WYNNE (1969), and are easy to squash under a slight pressure. Furthermore, swarmers liberated from the former two crusts germinated primarily into creeping filaments which were simply or densely branched, but they did not germinate into minute parenchymatous discs as in *Ralfsia verrucosa* (NAKAMURA, 1963, 1972). This is one of the most distinguishing characters between the crustose stage of the Scytosiphonaceae and the genus *Ralfsia*. Regarding the development of swarmers of *Ralfsia clavata* (*=R. tenuis*), KYLIN and LOISEAUX described a discal type of development (KYLIN, 1934, Fig. 10; LOISEAUX, 1968, Fig. 5, D), while EDELSTEIN *et al.* (1970) reported that it had a creeping filamentous type of development. From this, it is supposed that the plants identified as *R. clavata* contain at least two different kinds of species. On the other hand, naturally occurring crusts of *Scytosiphon* and *Petalonia* at Muroran were unable to be distinguished from each other by the gross structure of their frond. They were distinguished by thickness of young germlings composed of several cells. Cells of the germlings from swarmers liberated from naturally occurring crusts of *Scytosiphon* were more slender (about 1  $\mu$ m in diameter) than those from the crusts of *Petalonia* (NAKAMURA, 1965). The clarification of relationship between crustose brown algae hitherto known and crustose stages in the life cylces of various Scytosiphonaceae should await further investigations by culturing them.

As mentioned above, among five species examined, one species, *Petalonia* fascia, is lacking in sexual reproduction and others, *Scytosiphon lomentaria*, *Petalonia zosterifolia*, *Colpomenia bullosa* and *Endarachne binghamiae* have sexual reproduction. The present results confirmed that the life cycle of members of the Scytosiphonaceae has an alternation of heteromorphic generations; a large polystichous plurilocular gametangia-bearing plant (cylindrical, leafy or saccate frond) and a small haplostichous unilocular sporangia-bearing plant (crusts or tufts). Further, meiosis occurs during the formation of zoospores in the unilocular sporangia of diploid thalli from zygotes. Parthenogenesis is a very common feature in all the species examined, exhibiting the dimorphic development, erect fronds and crustaceous discs (or tufted plantlets), according to culture conditions.

#### Summary

Five species of the Scytosiphonaceae from Pacific Coast of Japan, mainly from Muroran, Hokkaido, were investigated regarding the entire life cycle with cytological observations in laboratory cultures permitting temperatures and photoperiods.

Of five species examined, Scytosiphon lomentaria, Petalonia zosterifolia, Colpomenia bullosa, and Endarachne binghamiae have a sexual cycle with an alternation of heteromorphic generations.

Sexual plants are isomorphic and heterothallic, and are large polystichous cylindrical, saccate, or leafy fronds with plurilocular organs. Sexual reproduction is isogamous, with the exception of *Scytosiphon lomentaria* which is slightly anisogamous.

Zygotes develop into small haplostichous crustaceous discs or tufted plantlets with unilocular organs. Zoospores liberated from these unilocular organs develop into minute discs or filamentous plantlets, giving off cylindrical, saccate, or leafy erect fronds with plurilocular organs.

The pattern of the life cycle with an alternation of a sexual generation (the erect fronds) and an asexual generation (the crusts or tufts) occurs with an alter-

nation of nuclear phases. Meiosis occurs during the formation of zoospores in the unilocular ograns of the crusts or tufts from zygotes. The number of chromosomes in haploid phase is as follows; 22–24 in *Scytosiphon lomentaria*, 21– 22 in *Petalonia zosterifolia*, 20–24 in *P. fascia*, ca. 18 in *Colpomenia bullosa*, and 20–22 in *Endarachne binghamiae*.

Gametes of either sex develop parthenogenetically, undergoing two different developmental pathways; one develops into erect fronds with plurilocular organs and the other develops into crusts or tufts with unilocular organs. The expression of these two types of development, the erect fronds and the crusts (or tufts) is influenced by culture conditions; the former occurs under relatively cool conditions and the latter under relatively warm conditions.

The growth of crusts (or tufts) from zygotes and parthenogametes is promoted by relatively warm, long-day conditions and their maturation is governed by shortday conditions.

*Petalonia fascia* exhibited no sexuality. Its life cycle with dimorphic generations, erect fronds and crusts (or tufts) is identical to that of parthenogenesis of the other species examined.

The life cycles demonstrated in the present experiments may be summarized by the following two diagrams:

Scytosiphon lomentaria, Petalonia zosterifolia, Colpomenia bullosa and Endarachne binghamiae:



#### Petalonia fascia:



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## Plate I

## Scytosiphon lomentaria (LYNGBYE) LINK

- A. Settled zygotes and their germination.  $\times$  1000.
- B. Settled female parthenogametes and their germination.  $\times$  1000.
- C. One-month-old crustaceous disc derived from a female parthenogamete (No. 3).  $\times$  70.
- D. Section of a crust with unilocular sporangia, derived from a zygote (No. 2).  $\times$  300.
- E. Section of a crust with unilocular sporangia, derived from a male parthenogamete (No. 2).  $\times$  300.
- F. Unilocular sporangia borne on filaments of a tuft, derived from a zygote (No. 2).  $\times$  200.
- G. Branchlets with unilocular sporangia borne on a filament of a tuft, derived from a male parthenogamete (No. 2).  $\times$  200.
- H. Settled zoospores.  $\times$  1000.
- I. Chromosomes at metaphase in vegetative nucleus of a germling from a zygote, showing diploid phase.  $\times$  5000.
- J. Chromosomes at metaphase in vegetative nucleus of a germling from a zoospore, showing haploid phase.  $\times$  5000.
- K. Chromosomes at metaphase in vegetative nucleus of a germling from a female parthenogamete, showing haploid phase.  $\times$  5000.

Parenthesized No. is shown number of incubator in which the materials were cultured.

# [NAKAMURA-TATEWAKI] PLATE I



## Plate II

## Petalonia zosterifolia (REINKE) KUNTZE

- A. Settled zygotes (a) and parthenogametes (b).  $\times$  1000.
- B. Settled zoospores.  $\times$  1000.
- C. Twenty-day-old crustaceous disc derived from a zygote (No. 3).  $\times$  75.
- D. Section of a 40-day-old crust from a female parthenogamete (No. 3).  $\times$  200.
- E. Section of a crust with unilocular sporangia, derived from a male parthenogamete (No. 4).  $\times$  300.
- F. Section of a crust with unilocular sporangia, derived from a zygote (No. 4).  $\times$  300.
- G. Petalonia plants arising from a 15-day-old minute disc from a zoospore (No. 3).  $\times$  100.
- H. *Petalonia* plant borne on 1-month-old creeping filaments from a male parthenogamete (No. 1).  $\times$  50.
- I. Chromosomes at metaphase in vegetative nucleus of a germling from a zygote, showing diploid phase.  $\times$  5000.
- J. Chromosomes at metaphase in vegetative nucleus of a germling from a zoospore, showing haploid phase.  $\times$  5000.

# [NAKAMURA-TATEWAKI] PLATE II



## Plate III

Petalonia fascia (O. F. MÜLLER) KUNTZE

- A. Settled swarmers from plurilocular organs.  $\times$  1000.
- B. Settled swarmers from unilocular organs.  $\times$  1000.
- C. Twenty-day-old tufted germling from a swarmer of a plurilocular organ (No. 3). × 50.
- D. Two-month-old crustaceous disc from a swarmer of a pluri-locular organ (No. 3).  $\times$  65.
- E. Unilocular organs borne on loose filaments of a tuft (No. 4).  $\times$  200.
- F. Section of a crust with unilocular organs (No. 4).  $\times$  300.
- G. Petalonia plants arising from a 18-day-old minute disc derived from a swarmer of a unilocular organ (No. 1). × 75.
- H. *Petalonia* plants borne on a 20-day-old filamentous plantlet derived from a swarmer of a unilocular organ (No. 1).  $\times$  50.
- I. Chromosomes at metaphase in vegetative nucleus of a germling from a swarmer of a plurilocular organ.  $\times$  5000.
- J. Chromosomes at metaphase in vegetative nucleus of a germling from a swarmer of a unilocular organ.  $\times$  5000.

[NAKAMURA-TATEWAKI] PLATE III



## Plate IV

## Colpomenia bullosa YAMADA

- A. Settled zygotes.  $\times$  1000.
- B. Settled female parthenogametes.  $\times$  1000.
- C. Settled zoospores.  $\times$  1000.
- D. Short, filamentous 2-week-old tuft from a zygote (No. 3).  $\times$  100.
- E. Crustaceous, 1-month-old disc from a zygote (No. 3).  $\times$  52.
- F. Long filamentous 3-week-old tuft from a zygote (No. 3).  $\times$  30.
- G. Section of a mature crust derived from a zygote, bearing unilocular sporangia (No. 3). × 300.
- H. Unilocular sporangium borne on a filament of a tuft (No. 3).  $\times$  300.
- I. Mature unilocular sporangium borne on a paraphysis of a crust from a female parthenogamete (No. 3).  $\times$  500.
- J. Colpomenia plant from 50-day-old culture of a zoospore (No. 3).  $\times$  5.
- K. Chromosomes at metaphase in vegetative nucleus of a germling from a zygote, showing diploid phase.  $\times$  5000.
- L. Chromosomes at metaphase in vegetative nucleus of a germling from a zoospore, showing haploid phase.  $\times$  5000.

# [NAKAMURA-TATEWAKI] PLATE IV



## Plate V

## Endarachne binghamiae J. AGARDH

- A. Settled zygotes (a) just fused, and parthenogametes (b).  $\times$  1000.
- B. Settled zygotes.  $\times$  1000.
- C. Two-week-old germlings from female parthenogametes (No. 5).  $\times$  200.
- D. Crustaceous 4-month-old discs from zygotes (No. 5).  $\times$  3.
- E. Section of a crust from a zygote, bearing unilocular sporangia (No. 4). × 300.
- F. Section of a crust from a male parthenogamete, bearing unilocular sporangia (No. 4).  $\times$  300.
- G. Endarachne plants arising from an about 1-month-old minute disc from a zoospore (No. 3).  $\times$  70.
- H. Endarachne plants arising from a 20-day-old minute disc from a female parthenogamete (No. 3).  $\times$  100.
- I. Chromosomes at metaphase in vegetative nucleus of a germling from a zygote, showing diploid phase.  $\times$  5000.
- J. Chromosomes at metaphase in vegetative nucleus of a germling from a zoospore, showing haploid phase.  $\times$  5000.
- K. Chromosomes at metaphase in vegetative nucleus of a germling from a female parthenogamete, showing haploid phase.  $\times$  5000.

# [NAKAMURA-TATEWAKI] PLATE V

