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# Geographical Variation of Development and Life History of *Blidingia minima* (Chlorophyceae) from Japan\*

by  
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## Introduction

*Blidingia minima* (NÄGELI ex KÜTZING) KYLIN is one of the widespread green algae in the upper littoral zone of seashore quite often dominating parts of the zone. This taxon was established by KYLIN in 1947 on the basis of culture experiments by BLIDING (1938) who showed that the following features distinguished it from genus *Enteromorpha* and proposed to establish a new genus: 1) Quadriflagellate zoospores lacking eyespots are the only reproductive cells produced; 2) During germination, all of the cytoplasm of the settled zoospore migrates into a germination tube and is walled off from the empty spore; 3) The germling produces prostrate branches which form an initially monostromatic disc that becomes distromatic in the center and gives rise to an erect, tubular frond with many initial cells. Nearly a hundred years before that, *Blidingia minima* had been described under the names *Ulva intestinalis* var. *nana* by SOMMERFELT (1826), *Enteromorpha minima* by KÜTZING (1849), *E. micrococca* by KÜTZING (1856) and *E. nana* by SJÖSTEDT (1939) on the basis of anatomical features. These species were distinguished from other *Enteromorpha* species by their small cell size (less than 10  $\mu$ m in surface view), but the criterion among these species was obscure, so there was a taxonomical confusion. KYLIN (1947) combined them as the synonym of *Blidingia minima*, since then the developmental features were added as a taxonomical criterion of *Blidingia* species with the anatomical features. After that several other species have been added to genus *Blidingia*.

Since BLIDING (1938) first reported the development of quadriflagellate zoospores in *B. minima* (as *Enteromorpha minima*) from Kristineberg, Göthenberg and Karlshamn, Sweden, many workers have investigated the life history of *Blidingia* species from various localities in Europe and Japan: YAMADA and KANDA (1941, 1944) and TATEWAKI (1972) on *B. minima* from Muroran, Japan; ARASAKI (1946) on *B. minima* from Mikawa,

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Japan; KYLIN (1947) on *B. minima* from Kristineberg, Sweden; AKIYAMA (1959) on *B. minima* from Matsushima, Japan; DANGEARD (1961) on *B. minima* and *B. marginata* from Brittany, France; BLIDING (1963, 1968) on *B. minima*, *B. chadefaudii* and *B. marginata* from different places in Europe; GAYRAL (1967) on *B. minima* from France and Morocco; YOSHIDA (1970) on *B. minima* from Suma and Shirahama, Japan; KORNMAN and SAHLING (1977, 1978) on *B. minima*, *B. chadefaudii*, *B. marginata* and *B. subsalsa* from Helgoland, West Germany. Among these many investigations, most workers of *Blidingia* species have reported one pattern of life history—an asexual, monophasic cycle in which erect, tubular fronds are repeated by quadriflagellate zoospores. Only TATEWAKI (1972) reported sexual reproduction by biflagellate gametes and isomorphic life history in his brief description.

On the developmental type in *Blidingia* species, BLIDING (1968) described differences in early ontogeny between *B. minima* and *B. chadefaudii* in Scandinavia and Mediterranean France; young discs in the former were tightly closed with short cells, whereas those in the latter were more open with longer cells. KORNMAN and SAHLING (1978) also pointed out the importance of early developmental differences between these two species in Helgoland, and concluded that plants with short-celled compact discs can be referred to as *B. minima* and those with longer-celled open discs can be referred to as *B. chadefaudii*. Recently, TATEWAKI and IMA (1984) have re-examined the life history of *B. minima* from Muroran and its vicinity. They reported three types of early development and four patterns of life history including two types of sexual generations in laboratory cultures. They have also found that in some populations the type of development is changeable from the *chadefaudii* type to the *minima* type with successive cultures; from that they suggested that differences in early ontogeny could not be used to separate the Muroran plants into two species.

On the other hand, however, it was also a recognized fact that most of Muroran plants repeated the same types of development which are either in *minima* or in *chadefaudii*, showing a genetic stability and they were considered to consist of two species, *B. minima* and *B. chadefaudii*. Therefore, the writer has collected *Blidingia* species from various localities in Japan, studying and comparing their development and life history.

The purpose of this study was to re-examine *Blidingia* species from Japan by culture experiments and to confirm the geographical variation of development and life history including the degree of scarcity in sexual reproduction.

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## Materials and Methods

### Collection and transportation

*Blidingia minima* (NÄG. ex KÜTZ.) KYLIN is widely distributed throughout Japan, growing on rocks and breakwaters in the upper and middle intertidal zones. The materials were collected partly by the writer own and partly by many cooperators at the various localities (26 areas 52 points) listed in Table 1 and shown in Figure 1. In each point, materials were collected from one to over ten populations. From several points, *B. marginata* (J. AGARDH) DANGEARD was also collected.

Collected plants were wrapped with paper towels to remove as much water as possible and placed in small polyethylene bags or glass bottles with caps in each habitat. They were maintained cool in an ice box during transport to the laboratory at Muroran. Under this semi-dried condition in the ice box, the plants showed usually no noticeable damage for 7-10 days. In plants collected from Miyagi, Chiba, Kochi, Maizuru, Yamaguchi, Amami-Oshima etc., semi-dried materials placed in glass bottles or polyethylene bags without keeping cool were sent by mail to the laboratory. Even by such methods, most of them were alive but some with little damage during the transit time.

In all populations collected, half of the plants were used for culture experiments and the others were preserved as herbarium specimens or liquid preserved materials in 10% formalin

in seawater in every population.

#### Phenological observation of *B. minima*

Phenological observation was carried out at Charatsunai, Muroran, Hokkaido. From several fixed populations of *B. minima* at Charatsunai-Muroran, in front of the laboratory, plants were collected each month from April 1982 to December 1983 except for the period of disappearance, and the size of plants was measured and released swarmers were cultured.

#### Isolation and culture

For culture experiments, fertile materials were rinsed several times in autoclaved seawater and each plant was introduced into a glass depression slide containing 1-2 ml sterile seawater. Most plants discharged swarmers after several minutes. Infertile materials from distant places were cleaned by brushing with a soft water-color brush and rinsed several times in autoclaved seawater. Each plant was cultured in a Petri dish (9 cm×2 cm) containing 50 ml enriched seawater medium until reproductive maturity. Swarmers were examined under a microscope (×800) after being fixed with formalin vapors for a few seconds. When biflagellate swarmers were found, conjugation tests were done by mixing on a glass slide a drop containing swarmers from a different individual and by observing aggregation and conjugation. Following a positive conjugation test, the gametes from each individual were washed two or three times in sterile seawater with a capillary pipette and drops containing gametes in approximately equal number were mixed together on a glass slide with a few drops of seawater or medium. Zoospores and parthenogametes were also transferred to glass slides after being washed two or three times with a capillary pipette. The glass slides with attached swarmers (zoospores, zygotes or parthenogametes) were placed in culture vessels (6.5 cm×8.0 cm) containing 180-200 ml medium, and unialgal cultures were established.

The culture medium employed was PES (PROVASOLI 1966), and was renewed every month. Culture experiments were conducted in 12 incubators equipped with Cool-White 40 W fluorescent lamps (3,000-4,000 lux) under the following temperature and photoperiod regimes: 5°C, 14:10 (5L) or 10:14 (5S), 10°C, 14:10 (10L) or 10:14 (10S), 14°C, 14:10 (14L) or 10:14 (14S), 18°C, 14:10 (18L) or 10:14 (18S), 22°C, 14:10 (22L) or 10:14 (22S), 26°C, 14:10 (26L), 28°C, 14:10 (28L). Among them, 14L incubator was mainly used, because this culture condition produced the best growth of *Blidingia* plants. But 18L and 22L conditions were also used for the plants collected from Shikoku, Kyushu and Amami-Oshima.

#### Morphological observation

Sections were made by hand using the straight-edge razor and pith stick or by a freeze microtome and stained with acetoorcein.

**Table 1** Collection data and culture results in *Blidingia minima* and its groups according to developmental types.

Abbreviations and symbols used in the table.

(SR)=Sexual reproduction; found in nature (++) ; found in culture (+) ; not found in both nature and culture (-) ; not found, but plants from nature or culture produced biflagellate swimmers (\*).

N=The northern group ; S=The southern group ;

M=The middle group.

# =The locality where collections of *B. marginata* with *B. minima* were made.

| Collection<br>Locality and Point | Date      | Type of<br>development | Life history<br>pattern (SR) | Group   |
|----------------------------------|-----------|------------------------|------------------------------|---------|
| 1 Kushiro                        |           |                        |                              |         |
| Shireto                          | 83/5/27   | F                      | III(-)                       | N       |
| Okotsu                           | 83/5/27   | F                      | III( *)                      | N       |
| Katsurakoi                       | 83/5/27   | F                      | III( *)                      | N       |
| 2 Abashiri                       |           |                        |                              |         |
| Abashiri                         | 83/5/28   | F                      | III( *)                      | N       |
| 3 Monbetsu                       |           |                        |                              |         |
| Monbetsu Port                    | 83/5/29   | F                      | III(-)                       | N       |
| Minamigaoka                      | 83/5/29   | F                      | III(-)                       | N       |
| Saruru                           | 83/5/29   | F                      | III(-)                       | N       |
| Saruru Port                      | 83/5/29   | F                      | III( *)                      | N       |
| 4 Mashike                        |           |                        |                              |         |
| Ofuyu                            | 84/6/15   | F                      | I, II(++)                    | N       |
| 5 Otaru                          |           |                        |                              |         |
| Zenibako                         | 83/6/17   | F, F→D                 | I(++)                        | N, M    |
| Asari                            | 83/6/17   | D                      | IV(-)                        | S       |
| Oshoro                           | 84/6/4    | F                      | I(++)                        | N       |
| 6 Hakodate                       |           |                        |                              |         |
| Hakodate                         | 84/4/10   | F-M-D                  | II, III, IV(+)               | M       |
| 7 Muroran                        |           |                        |                              |         |
| Charatsunai                      | 82/4-87/2 | F, D, F→D, FD mix      | I, II, III, IV(++)           | N, M    |
| Denshinama                       | 82/4-83/5 | F, D, F→D              | I, II, III, IV(++)           | N, S, M |
| Sakimori                         | 82/4-84/5 | F, D, F→D              | I, II, III, IV(++)           | N, N, M |
| Washibetsu                       | 84/5/29   | F                      | I(++)                        | N       |
| Horobetsu                        | 84/5/23   | F                      | III(-)                       | N       |
| Noboribetsu                      | 84/5/23   | F, D                   | II, III, IV(+)               | N, S    |
| 8 Aomori                         |           |                        |                              |         |
| Aomori Port                      | 84/4/10   | F                      | I(++)                        | N       |
| 9 Ohma#                          |           |                        |                              |         |
| Ohma Port                        | 84/6/23   | FD mix                 | III, IV( *)                  | M       |
|                                  | 86/1/13   | D                      | IV(-)                        |         |
| 10 Hachinohe                     |           |                        |                              |         |
| Tanesashi                        | 84/6/17   | D                      | IV(-)                        | S       |
| 11 Akita                         |           |                        |                              |         |
| Oga Port                         | 84/3/29   | F, FD, mix             | III, IV(-)                   | N, M    |

| Collection<br>Locality and Point | Date         | Type of<br>development | Life history<br>pattern (SR) | Group |
|----------------------------------|--------------|------------------------|------------------------------|-------|
| 12 Miyagi                        |              |                        |                              |       |
| Naruse                           | 84/4/2       | F                      | III(-)                       | N     |
| Ojika                            | 84/4/18      | F-M-D                  | I(++)                        | M     |
| Onagawa                          | 84/6/12      | D                      | IV(-)                        | S     |
| 13 Yamagata                      |              |                        |                              |       |
| Fukuura                          | 85/4/14      | F-M-D                  | III(-)                       | M     |
| 14 Ibaraki                       |              |                        |                              |       |
| Hiraiso                          | 84/4/30      | D                      | IV(-)                        | S     |
| 15 Chiba#                        |              |                        |                              |       |
| Choshi                           | 84/2/18      | D                      | IV(-)                        | S     |
| Magamegawa                       | 84/2/5       | D                      | IV(-)                        | S     |
| Katsuura                         | 84/7/5       | D                      | IV(-)                        | S     |
| Tateyama                         | 83/3/31      | D                      | IV(-)                        | S     |
| Narashino                        | 84/3         | D                      | IV(-)                        | S     |
| 16 Kanagawa                      |              |                        |                              |       |
| Enoshima                         | 83/3/29      | D                      | IV(-)                        | S     |
| Hayama                           | 83/3/29      | D                      | IV(*)                        | S     |
| Yuigahama                        | 85/5/5       | D                      | IV(-)                        |       |
| 17 Shizuoka                      |              |                        |                              |       |
| Heta                             | 83/1/1       | D                      | IV(-)                        | S     |
| Shimoda                          | 84/5/4, 86/4 | D                      | IV(*)                        |       |
| 18 Nagoya#                       |              |                        |                              |       |
| Nagoya Port                      | 84/3/14      | D                      | IV(-)                        | S     |
| 19 Maizuru                       |              |                        |                              |       |
| Nagahama                         | 84/4/28      | M                      | III, IV(-)                   | M     |
| 20 Hiroshima#                    |              |                        |                              |       |
| Ujina                            | 85/3/23      | D                      | IV(-)                        | S     |
| 21 Kochi                         |              |                        |                              |       |
| Usa-Bay                          | 83/2/9       | D                      | IV(-)                        | S     |
| Muroto Port                      | 83/3/6       | D                      | IV(-)                        | S     |
| 22 Yamaguchi                     |              |                        |                              |       |
| Senzaki-Bay                      | 85/12/25     | M                      | IV(-)                        | M     |
| 23 Shimonoseki                   |              |                        |                              |       |
| Yoshimo                          | 86/3/12      | M                      | I(+)                         | M     |
| Dannoura                         | 86/3/12      | D                      | IV(-)                        | S     |
| 24 Nagasaki                      |              |                        |                              |       |
| Nomozaiki                        | 85/4/24      | M                      | IV(*)                        | M     |
| 25 Kagoshima#                    |              |                        |                              |       |
| Shibushi                         | 85/3/26      | D                      | IV(-)                        | S     |
| Yamakawa                         | 85/3/25      | M                      | IV(*)                        | M     |
| Nejime                           | 85/3/25      | D                      | IV(-)                        | S     |
| 26 Amami-Ohshima                 |              |                        |                              |       |
| Kasari                           | 85/3/12      | D                      | IV(-)                        | S     |

## RESULTS

## 1. Population

*Blidingia* plants usually grow on rocks, breakwaters or concrete blocks in the upper intertidal zone, especially sheltered area as a green velvet mat during a flourish season. They are distributed throughout the Archipelago in the coasts of both the Japan Sea including a part of East China Sea and the Pacific Ocean in Japan. *B. minima* was collected from 26 areas and 52 points, from Kushiro, Hokkaido (surface seawater temperature: ca. 0°C in February—ca. 16°C in August) to Kagoshima, Kyushu (ca. 18–27°C) and Amami-Oh-

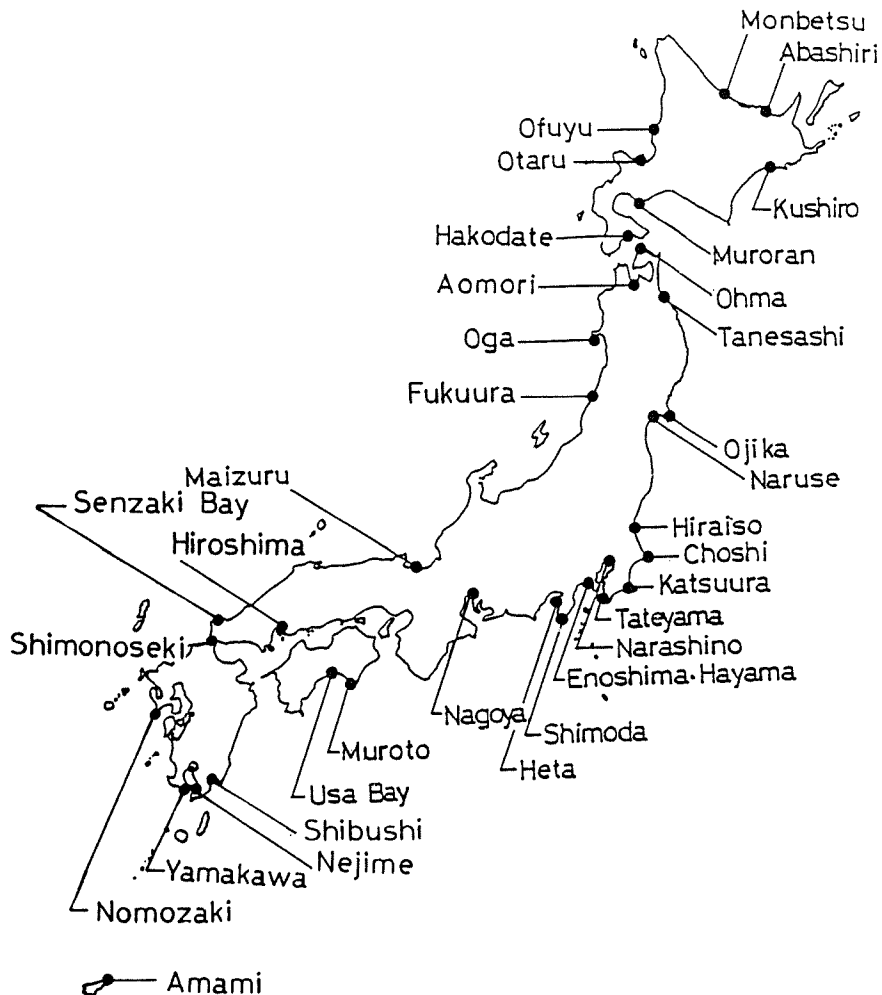


Fig. 1 Map of Japan showing where *Blidingia* plants for cultures were collected.

shima (ca. 20–28°C) as shown in Table 1 and Fig. 1. They are more abundant in the Pacific coast of Japan than the Japan Sea coast. Most populations grow at the highest intertidal level of any of the enteromorphoid algae.

At several points, especially in estuary regions in Honshu and Kyushu (Ohma, Chiba, Nagoya, Hiroshima and Shibushi-Kagoshima), *B. marginata* is more abundant than *B. minima*, but in Hokkaido, it has been not yet found to grow.

The result of phenological observation at Charatsunai, Muroran is as follows: it grows abundantly from March to June (sea water temperature; ca. 5–15°C) and disappears in August (ca. 20°C), but re-appears in October or November (ca. under 15°C).

Although the plants were collected from several different places at each locality, sexual populations in nature were found only in the following localities; Muroran (Charatsunai, Denshinama, Sakimori and Washibetsu), Ofuyu, Otaru (Oshoro and Zenibako), Aomori and Ojika (Table 1, Fig. 2; ++). Moreover, at Hakodate, Muroran (Charatsunai, Denshinama, Sakimori, and Noboribetsu) and Yoshimo-Shimonoseki, sexual reproduction occurred in culture, although only asexual plants were found in natural populations examined (Table 1, Fig. 2; +). In other populations from many other localities, only asexual plants which released quadriflagellate zoospores were found in culture or in nature. However, in some populations from 8 different localities; Kushiro, Abashiri, Monbetsu, Ohma, Hayama-Kanagawa, Shimoda-Shizuoka, Nomozaki and Yamakawa-Kagoshima (Table 1, Fig. 2;\*), a few individual plants released biflagellate swimmers in addition to quadriflagellate zoospores, but these swimmers did not show sexual behavior (Pl. IV-K,L).

All populations of *B. marginata* were found asexual reproduction and only quadriflagellate zoospores were produced both in natural and culture plants (Pl. X-F).

## 2. Frond morphology of *B. minima*

Erect, tubular fronds from the present study sites varied considerably in external morphology, especially in length, breadth and the existence of a few ramification (Pls. I and II). In Muroran, during flourish season (March–June) these fronds were usually 3–10 cm long and 2–5 mm broad and other seasons 0.5–3 cm and 1–2 mm broad. The longest frond collected from Charatsunai, Muroran in June was 15 cm in length and 7 mm in breadth (Pl. I-C), and the most slender fronds were 100–200  $\mu\text{m}$  in breadth and often ramified (Pls. I-E and II-H-L). Such a slender frond population was often found to grow on wet shady bluffs throughout the year at Charatsunai, Muroran. This slender form should be classified as *B. minima* var. *ramifera*, but there is no marked difference in the development between this slender form and other bigger or longer plants (cf. Pl. VI-E). In spite of variation of the external morphology, sterile cells are angular, constantly 4.5–7.0  $\mu\text{m}$  but not exceeding 10  $\mu\text{m}$  in diameter in surface view and arranged in no definite order from the upper part to the base of frond in living material (Pl. II-A-C and I-J). In cross section, sterile cells appear rectangular, 4.5–7.0  $\mu\text{m}$   $\times$  6–9  $\mu\text{m}$ , with cell walls that vary in thickness in the same individ-

ual depending on age and portion of the frond (Pl. II-E, F, K, L and Pl. III-A-H). However the thickness of inner cell wall seems to be still an important criterion in *Blidingia*, especially between *B. minima* and *B. chadefaudii*, so plants from most of populations collected from Japan were re-examined, especially among plants from 8 typical popula-

**Table 2-a** Variation of the thickness of inner cell wall in natural plants of several populations (mean  $\pm$  SD, n=30).

| Locality           | Type of development | Thickness of inner wall ( $\mu$ m) |                       |
|--------------------|---------------------|------------------------------------|-----------------------|
|                    |                     | basal part                         | upper part            |
| Shireto-Kushiro    | F                   | 8-20(9.8 $\pm$ 3.0)                | 16-28(17.2 $\pm$ 5.4) |
| Okotsu-Kushiro     | F                   | 10-16                              | 12-16                 |
| Katsurakoi-Kushiro | F                   | ca. 8                              | ca. 16                |
| Abashiri           | F                   | 6-12(7.3 $\pm$ 2.4)                | 6-10( 6.7 $\pm$ 2.0)  |
| Monbetsu Port      | F                   | 12-14                              | ca. 4                 |
| Ofuyu              | F                   | 6-12                               | ca. 8                 |
| Zenibako-Otaru     | F $\rightarrow$ D   | ca. 8                              | ca. 6                 |
| Asari-Otaru        | D                   | 6-10(7.2 $\pm$ 1.5)                | 4- 6( 5.1 $\pm$ 1.1)  |
| Hakodate           | F-M-D               | 6-10(8.3 $\pm$ 1.4)                | 6- 8( 7.1 $\pm$ 1.0)  |
| Charatsunai        | F                   | 8-20                               | 8-10                  |
| -Muroran           | D                   | ca. 6                              | 2- 6                  |
|                    | F $\rightarrow$ D   | 8-10                               | ca. 4                 |
| Denshinhamma       | F                   | 20-28                              | 20-24                 |
| -Muroran           | D                   | ca. 6                              | ca. 6                 |
| Sakimori           | F                   | 6- 8                               | ca. 8                 |
| -Muroran           | D                   | ca. 6                              | ca. 6                 |
|                    | F-D                 | 6-12(8.4 $\pm$ 2.2)                | 4-10( 6.2 $\pm$ 1.6)  |
| Noboribetsu        | F                   | 12-18                              | ca. 6                 |
| -Mnroran           | D                   | ca. 8                              | ca. 2                 |
| Oga                | FD mix              | ca. 12                             | 6- 8                  |
| Naruse-Miyagi      | F                   | 7- 8                               | 4- 5                  |
| Ojika-Miyagi       | F-M-D               | 6- 8                               | 4- 5                  |
| Onagawa-Miyagi     | D                   | 5- 8                               | ca. 6                 |
| Tateyama-Chiba     | D                   | 4- 8                               | ca. 2                 |
| Hayama-Kanagawa    | D                   | ca. 6                              | ca. 6                 |
| Shimoda-Shizuoka   | D                   | 6-10(7.0 $\pm$ 1.7)                | 3- 7( 4.9 $\pm$ 1.1)  |
| Maizuru            | M                   | ca. 8                              | ca. 2                 |
| Usa Bay-Kochi      | D                   | ca. 6                              | 4- 5                  |
| Nomozaki           | M                   | 5-12(7.4 $\pm$ 2.1)                | 2- 5( 3.8 $\pm$ 1.1)  |
| Shibushi           | D                   | 2- 4                               | 2- 4                  |
| Amami-Ohshima      | D                   | 4- 8(5.9 $\pm$ 1.1)                | 4- 6( 4.9 $\pm$ 1.1)  |

**Table 2-b** Results of *t* test in the comparison of the thickness of inner cell wall of basal and upper parts among natural plants collected from 8 populations ( $- = P < 0.01$ ,  $+ = 0.05 > P > 0.01$ ,  $++ = 0.1 > P > 0.05$ ,  $+++ = P > 0.1$ ). D, F, M in the parenthesis indicates the type of development of germlings of each population plant. Other capital letters show population codes.

| basal part            | K | AB  | M  | H   | N   | O   | S   | AM |
|-----------------------|---|-----|----|-----|-----|-----|-----|----|
| Shireto-Kushiro(F)    |   | -   | +  | -   | -   | -   | -   | -  |
| Abashiri(F)           | - |     | ++ | +   | +++ | +++ | +++ | -  |
| Sakimori-Muroran(F→D) | - | +++ |    | +++ | ++  | +   | -   | -  |
| Hakodate(F-M-D)       | - | +++ | +  |     | +   | -   | -   | -  |
| Nomozaki(M)           | - | -   | -  | -   |     | +++ | +++ | -  |
| Asari-Otaru(D)        | - | -   | -  | -   | -   |     | +++ | -  |
| Shimoda(D)            | - | -   | -  | -   | -   | +++ |     | -  |
| Amami-Ohshima(D)      | - | -   | -  | -   | -   | +++ | +++ |    |
| upper part            | K | AB  | M  | H   | N   | O   | S   | AM |

tions, *t* test was examined. The results are shown in Table 2-a and b. Each cell contains a parietal chloroplast with one pyrenoid. Fertile cells are swollen and oval. Formation of swarmers begins in the upper of the frond, extending gradually downwards. Fertile parts can be detected by their yellowish green color. Fertile cells produce 4-8 swarmers (4 zoospores or 4-8 gametes) (Pl. II-D). The swarmers are liberated one by one through a tear in the wall. In fronds collected at several sexual populations, dioecious gametophytes producing biflagellate gametes were easily found as well as sporophytes producing quadriflagellate zoospores.

Gametophytes were dioecious in all sexual populations examined and could not distinguished from sporophytes by their external appearance (Pl. I-D).

### 3. Reproduction and development of *B. minima*

Sexual reproduction is isogamous. The gametes are elongate pyriform and measure  $5-6\ \mu\text{m} \times 2-3\ \mu\text{m}$  (Pl. IV-A, B, E, F). They have two anterior flagella of equal length ( $10-12\ \mu\text{m}$ ) and a chloroplast with one pyrenoid, but no eye-spot in the posterior of the cell. They are not phototactic and after swimming for 10-20 minutes they attach to substratum and develop parthenogenetically. When gametes of both sexes are mixed, aggregation occurs and two gametes conjugate to form a zygote (Pl. IV-C, G, I, J).

Zoospores are pyriform of subspherical and measure  $6-7\ \mu\text{m} \times 4-5\ \mu\text{m}$  (Pl. II-D, H). They have four anterior flagella of equal length ( $10-12\ \mu\text{m}$ ) and a chloroplast with one pyrenoid, but no eye-spot in the posterior of the cell. They also show no phototaxis and after swimming for 5-10 minutes attach to the substratum (Pl. V-A).

The development of swimmers (zoospores, zygotes and parthenogametes) is identical, and developmental patterns are shown the following three types.

1) *Compact disc-type (D-type)*: Attached swimmers produce a short germination tube into which all cytoplasm migrates, leaving the original cell empty (Pl. V-B). The first cell of the germling, which is walled off from the empty base, elongates and divides into 2-3 cells which soon produce short side-branches (Pl. V-C, D). Then the germlings develop into a small prostrate monostromatic disc which is tightly closed in cell-arrangement (Pl. V-E). In 10- or 20-day-old cultures when grown at 14-22°C in long day conditions, the discs became di- or polystromatic in the center with several initial cells for each erect, tubular *Blidingia* frond (Pl. V-F-J).

2) *Filamentous microthallus type (F-type)*: Attached swimmers produce a long germination tube into which all the cytoplasm migrates, leaving the original cell empty (Pl. VI-A). The first cell of germlings, which is walled off from the empty cell, elongates and divides into 2-3 cells (Pl. VI-B). Such germlings produce long prostrate branches radially (Pl. VI-C-F) and finally develop into a prostrate disc which more open in cell-arrangement (Pl. VI-G). The discs increase in size and become polystromatic in the center (Pl. VI-H, I). In 30- or 40-day-old cultures, the discs attained a diameter about 1-2 mm (Pl. VI-J, K). In general, under high temperatures ranging from 14-22°C, these discs were irregularly raised in the center by diffuse growth and some surface cells produced swimmers—zoospores and gametes (Pl. VI-M). Under lower temperatures ranging from 5-10°C, however, the filamentous discs produce erect, tubular fronds in the center in 2-month-old culture without reproductive maturing in this prostrate microthallus stage. Some microthalli which produced erect, tubular fronds in the central area in 4-5-month-old cultures (Pl. VI-O), but usually did not produce erect fronds over one year culture.

3) *Middle type (M-type)*: This type shows the complete intermediate form between D-type and F-type, or continuous variation from D-type to F-type. Germination of attached swimmers is similar to that of the former two types (Pl. VII-A, B). Then the germlings develop into slight opened prostrate discs (Pl. VII-C-F) or those with various shapes from tightly closed to completely opened (Pl. VIII-A-E). However as different from F-type, these filamentous discs soon produced erect, tubular frond in the center of the disc in 20- or 25-day-old culture (Pl. VII-F-J). Filamentous discs rarely matured in 22L condition.

The variation of these three types of development in each populations is shown in Fig. 2 and Table 1. The D-type development was found in plants collected in the regions from south-western Hokkaido (including Muroran and Otaru) to the Pacific coast of Honshu, Shikoku, Kyushu, Amami-Oshima and Seto Inland Sea coast (Fig. 2, Table 1; D). The F-type development was found in plants collected in the regions of the whole of Hokkaido, north-eastern Honshu (northward from Naruse and Oga). In the regions occurring the F-type, plants of Hokkaido ordinary became fertile in the prostrate microthallus-stage (Fig.

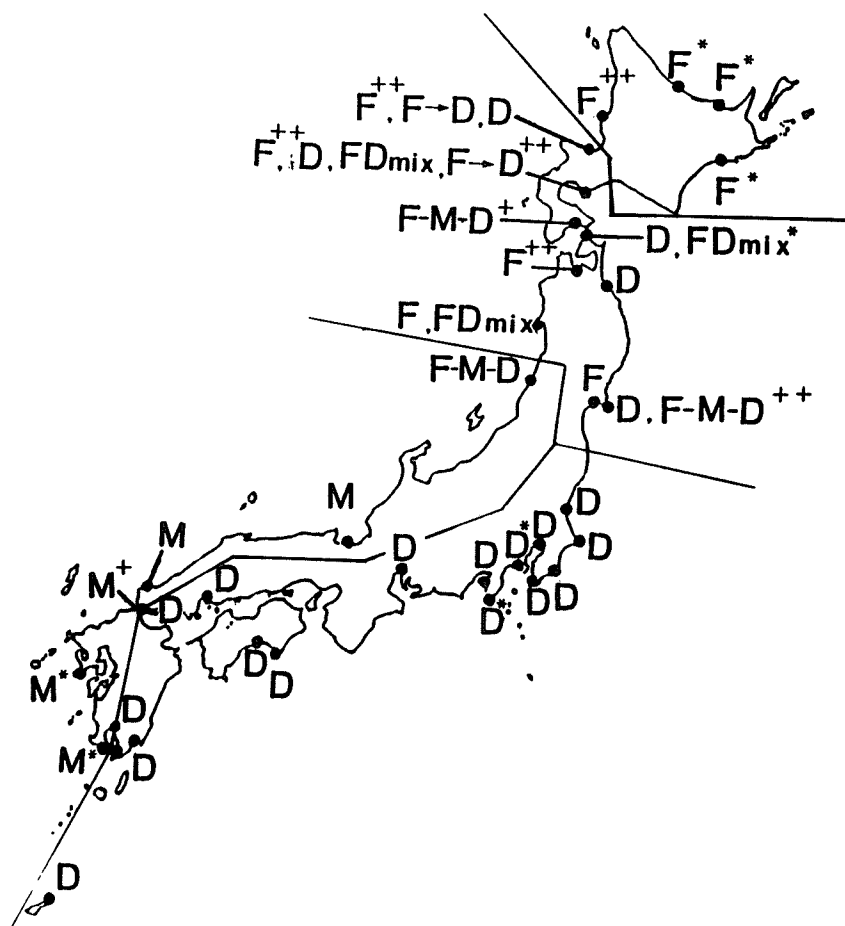


Fig. 2 Map of Japan showing distributions of *B. minima* divided into three groups according to the difference of developmental types:

D = Disc type; F = Filament type; M = Middle type.

FDmix = Mixed occurrence of both D- and F-type from the same individual.

F→D = Change of developmental type from F-type to D-type in successive cultures.

F-M-D = Continuous variation from D-type to F-type.

++ = Sexual population was found in nature.

+ = Sexual reproduction occurred in culture.

\* = Biflagellate swimmers were found in culture, but sexual reproduction could not be observed.

2, Table 1; F). The M-type development was found in plants collected in the regions of south-western Hokkaido, north-eastern Honshu, the Japan Sea coast of Honshu and the East China Sea coast of Kyushu. Plants from Maizuru, Senzaki, Yoshimo-Shimonoseki,

Nomozaki and Yamakawa showed a complete intermediate type (Fig. 2, Table 1; M), and plants from Hakodate, Fukuura and Ojika showed continuous variation from D-type to F-type (Fig. 2, Table 1; F-M-D).

In populations from Hiraiso, along the Pacific coast of Honshu, Shikoku, Kyushu to Amami-Ohshima and the coast of Seto Inland Sea (Hiroshima and Dannoura at Shimonoseki), only the D-type occurred, but in those from eastern Hokkaido (Kushiro, Abashiri and Monbetsu) only the F-type was observed.

In populations from south-western Hokkaido, north-eastern Honshu, where the M-type (continuous variation from D-type to F-type, Fig. 2, Table 1; F-M-D) occurred, the other two types were also found with one another. The mode of mixed appearance between D-type and F-type is divided into the following four cases.

a) The population whose plants occurred only the D-type and the population whose plants occurred only the F-type appeared separately; Denshinama, Sakimori and Noboribetsu around Muroran.

b) In the same population, the developmental type varied according to the season; Charatsunai at Muroran.

c) Swarmers from the same individual plant developed into clearly divided both D-type and F-type: Charatsunai and Sakimori at Muroran, Ohma and Oga (Fig. 2, Table 1; FDMix and Pl. VIII-F, G).

d) Swarmers derived from the same strain developed first into a polystromatic disc or pulvinus (F-type), but those from the second and third generations showed the D-type development repeatedly; Charatsunai, Sakimori, Denshinama at Muroran, and Zenibako at Otaru (Fig. 2, Table 1; F→D and Pl. VIII-H, I).

#### 4. Life history of *B. minima*

The life history of *Blidingia minima* from Japan was found to vary in populations collected at different localities and in different seasons, and divided into the following four patterns. These four patterns are the same as described previously on the Muroran's materials (TATEWAKI and IIMA 1984) (Fig. 3).

I. The first is a life history which is a fundamentally an alternation of erect, tubular, isomorphic gametophytes and sporophytes, but which always includes the occasional formation of prostrate microthalli producing gametes or zoospores.

II. The second is an irregular alternation of heteromorphic phases, with the erect, tubular frond producing only zoospores and the microthallus producing gametes or zoospores.

III. The third is an asexual alternation of phases with both the tubular frond and the microthallus producing only zoospores.

IV. The fourth is a monophasic cycle in which zoospores are the only reproductive cells produced and erect, tubular frond recycles itself. Germlings developed sometimes into filamentous prostrate discs with longer-celled branches, but never produced reproductive

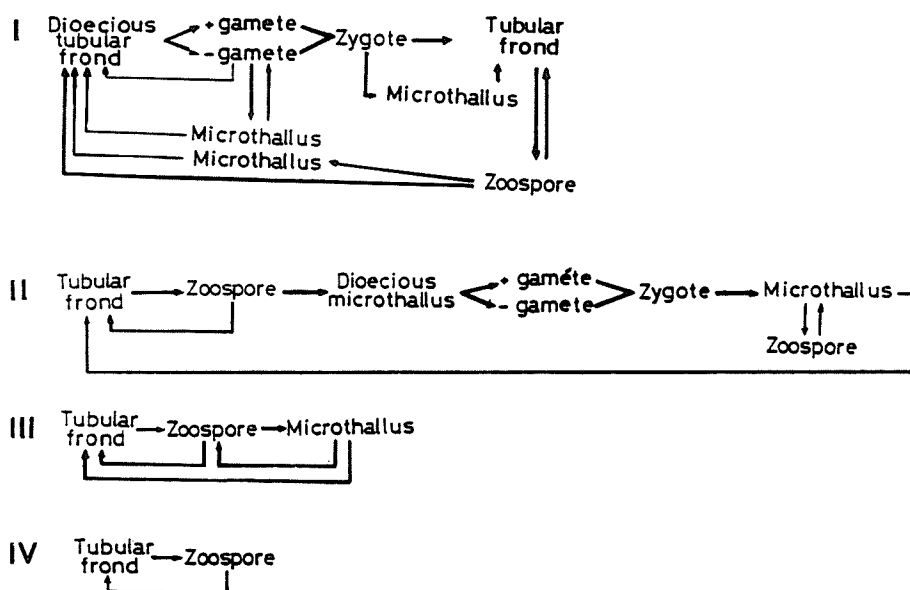


Fig. 3 Four patterns of life history of *B. minima* from Japan.

cells on the discs.

The first pattern was found in plants collected at the sites where sexual populations appeared. In these sexual populations, the plants from Muroran often showed an irregular alternation of generations and sometimes repeated asexual generations successively. However, the plants from Ofuyu, Otaru (Zenibako and Oshoro), Aomori and Ojika always showed an alternation of generations regularly. In plants collected from Yoshimo at Shimonoseki, more than 20 individual fronds were all sporophytes producing zoospores, but in culture these zoospores from 5 culture materials developed into gametophytes with the erect, tubular frond releasing only gametes. Plants from Yoshimo have sexual reproduction and show an isomorphic alternation of generation regularly in culture. So they seem to have the same pattern of life history.

The second pattern was found in plants collected at Hakodate and Muroran (Charatsunai, Denshinama, Sakimori and Noboribetsu). In these populations, sexual individuals (erect, tubular fronds) were not found in nature and the gametophyte occurred in culture hardly formed erect, tubular frond. At three points of Muroran; Charatsunai, Denshinama and Sakimori, there were found sexual individuals in other populations separately, but at Noboribetsu of Muroran and Hakodate, there were not found other sexual populations in nature. The third pattern occurred in populations from the whole of Hokkaido, north-eastern Honshu and Maizuru.

The fourth pattern occurred in populations from Muroran, Otaru of Hokkaido to

Honshu, Shikoku, Kyushu and Amami-Ohshima (Table 1).

### 5. Influence of temperature and photoperiods on the growth and maturation of *B. minima*

Generally, the formation of erect, tubular frond was much more influenced by temperatures than photoperiods in culture. Under the same temperature, germlings grew much better in long-day condition than in short-day condition. However the photoperiod did not influence reproductive maturity. Among the F-type strains from Hokkaido, the formation of erect, tubular frond was suppressed at 22°C in the strains from south-western regions (such as Muroran area), and at 18-22°C in those from eastern regions (Kushiro, Abashiri and Monbetsu). Under these high temperatures, most germlings developed into prostrate microthalli producing swarmers. In the F-type strains from north-eastern Honshu, however, prostrate microthalli became fertile only at 22 °C and the erect, tubular fronds were often produced in the center of filamentous open discs even at 22°C. In the both M-type and D-type strains from Honshu, Shikoku, Kyushu and Amami-Ohshima, the formation of erect, tubular fronds and their maturity were not suppressed at 14-22°C, but they were suppressed at 5-10°C and 28°C. Moreover, the D-type strains often showed the filamentous fringe of a basal disc at high temperatures (18-22°C) after occurring the normal D-type development and erect fronds, but reproductive maturity of filamentous sites like F-type strains could not be observed. Low temperatures influenced the development of the germlings of southern plants. In 5S and 5L conditions, germlings of the Senzaki plant showed an imperfect migration of cytoplasm and remained as it consisted of several cells in 1 month (Pl. IX-A). In 10S condition, germlings of the Nejime plant became complete prostrate discs producing a stolon-like vegetative reproduction (Pl. IX-B). Amami-Ohshima plants did not grow at 5°C at all, though other strains from Kyushu showed a slight growth at 5°C.

**Table 3** Temperature responses of 6 culture strains after successive cultures for over 1 year in the 14 L condition. Growth measurement was obtained from the 1-month-cultures after transfer to each condition.

|                     | 5 L | 14 L | 26 L |
|---------------------|-----|------|------|
| Moubetsu            | ++  | +++m | —    |
| Shireto-Kushiro     | ++  | +++m | —    |
| Charatsunai-Muroran | ++  | +++m | ++   |
| Nomozaki            | +   | +++  | ++   |
| Nejime              | +   | ++   | +++  |
| Amami-Ohshima       | —   | ++m  | +++  |

—=no growth, +=slight growth, ++=middle growth, +++=best growth, m=occurrence of maturity in 1-month-culture.

But the highest temperature (28°C) examined markedly suppressed the growth of germlings in all strains from Hokkaido to Amami-Oshima (Pl. IX-C, D, F).

Sexual reproduction was not inhibited by temperatures ranging from 10-22°C in the sexual strains of both F-type and M-type.

Temperature responses of culture plants from Hokkaido (Shireto-Kushiro, Monbetsu and Charatsunai-Muroran) and Kyushu (Nomozaki, Nejime and Amami-Oshima) were examined. About 1 month-old germlings cultured successively for over 1 year in the same 14L condition were transferred to 5L and 26L conditions and results are shown in Table 3. It is clear that the growth of germlings of Monbetsu and Kushiro plants were suppressed at 26°C, while those of Nejime and Amami-Oshima plants showed the best growth in that condition, even though they had been cultured in the same condition. On the other hand, the growth of 3 strains from Kyushu were suppressed at 5°C, especially the germlings of Amami-Oshima plant did not grow at all, while 3 strains from Hokkaido showed well growth. The culture plant from Charatsunai-Muroran showed well growth both at 5°C and 26°C, though it showed best growth at 14°C.

#### 6. Crossing experiments among sexual strains of *B. minima*

From 7 localities, 12 points and 20 field and cultural sexual populations, about 50 strains (both+ and -) were isolated. All sexual strains produce gametes repeatedly, which can develop parthenogenetically, and maintains sexuality from generation to generation.

As the results, when each gamete was heterosexual, all combinations examined could conjugate and zygotes developed into sporophytic plants producing zoospores. The crossing between the different populations were confirmed in the following combinations: Sakimori-Muroran vs. Zenibako-Otaru, Sakimori-Muroran vs. Denshinama-Muroran (Pl. IV-I), Charatsunai-Muroran vs. Aomori, Zenibako-Otaru vs. Aomori, Oshoro-Otaru vs.

**Table 4** Results of crossing experiments among sexual strains obtained from 8 populations.

|             |   |   |   |   |    |   |    |   |
|-------------|---|---|---|---|----|---|----|---|
| Sakimori    | + |   |   |   |    |   |    |   |
| Denshinama  | + | + |   |   |    |   |    |   |
| Charatsunai |   |   | + |   |    |   |    |   |
| Zenibako    | + |   |   | + |    |   |    |   |
| Oshoro      |   |   |   |   | +  |   |    |   |
| Aomori      |   |   | + | + | +  | + |    |   |
| Ojika       |   |   |   |   |    | + | +  |   |
| Yoshimo     |   |   |   |   |    |   | +  | + |
|             | S | D | C | Z | OS | A | OJ | Y |

+ = conjugation was confirmed and zygotes developed into sporophytic plants producing zoospores.

Aomori, Aomori vs. Ojika and Ojika vs. Yoshimo-Shimonoseki (Pl. IV-J) (Table 4).

All other sexual strains with the exception of Ojika vs. Yoshimo-Shimonoseki developing M-type development, showed the F-type development.

## 7. Morphology, development and life history of *B. marginata*

1) *Frond Morphology*: Tubular fronds of *B. marginata* are much more slender than those of *B. minima* and usually tangled each other (Pl. X-A). They are 1-3 cm long and up to 300  $\mu\text{m}$  broad. In surface view sterile cells are angular and a little smaller than those of *minima*, 4-6  $\mu\text{m}$  (Pl. X-B, C). As distinguished from *minima*, the cells arranged in definite order especially in lower parts. In cross section the thallus wall is very thin (2-4  $\mu\text{m}$ ) and sterile cells appear roundish measuring 4-6  $\mu\text{m}$  and each cell contains a parietal chloroplast with one pyrenoid (Pl. X-E). When fertile, cells are swollen and oval. Formation of swarmers begins in the upper part of the frond as in *minima* (Pl. X-D).

2) *Reproduction and Development*: Swarmers are only asexual, quadriflagellate zoospores. The zoospores are subspherical and measure 5-6  $\mu\text{m} \times 4-5 \mu\text{m}$ . They have four anterior flagella of equal length and a chloroplast with one pyrenoid, but no eye-spot in the posterior of the cell (Pl. X-F). They are not phototactic and after swimming for a while attach to the substratum. The development of zoospores is quite similar to that of European species described previously (DANGEARD 1961, BLIDING 1963, KORNMAN and SAHLING 1977, 1978). Attached zoospore does not produce a germination tube (Pl. X-G) and is divided into two cells without cytoplasm migration or empty cell. Then the germling develops into a small disc containing 4-8 cells by successive cell division (Pl. X-H). The prostrate disc becomes soon distromatic and polystromatic even less 1-week-old culture (Pl. X-I). A 10-day-old germling produced an erect, tubular frond in the center of a small disc (Pl. X-J). Under 14L condition, the erect, tubular frond only a few mm in high reached maturity in 3 weeks (Pl. X-M) and produced quadriflagellate zoospores again. The frond obtained in culture had the cells ordered in distinct rows as in the natural plant (Pl. X-K, L). The geographical variation of developmental type as like in *B. minima* was not observed.

3) *Life history*: *B. marginata* from Japan showed the monophasic life history by quadriflagellate zoospores without sexual reproduction, which corresponds to the results of European species previously reported.

## Discussion

*Blidingia minima* from Japan shows various life histories including sexual reproduction in the erect frond and prostrate microthallus stage. Moreover, by the difference of developmental types and life history patterns, *B. minima* from Japan can be divided to three groups: the northern, the southern and the middle group (Table 1, Fig. 2). Various frond morphology of *B. minima* have no relation with the developmental types and the life history patterns.

The plant of the southern group shows a clear D-type development and its life history is the pattern IV in which erect fronds repeat only asexual reproduction lacking the prostrate microthallic stage. Only this group exists from Amami-Oshima, which seems to be the southern growth limit of *Blidingia* in Japan, to Hiraiso, along the Pacific coast of Kyushu, Shikoku and Honshu and the coast of Seto Inland Sea (Hiroshima and Dannoura at Shimonoseki), is distributed. In north-eastern Honshu and south-western Hokkaido populations this group is found to grow in several places (Tanesashi, some populations of Muroran and Asari at Otaru).

Most of the plants of northern group show a clear F-type development and the life history pattern III, which is an asexual alternation of erect fronds and microthalli. Only this group exists in eastern Hokkaido and it is scattered existence in western Hokkaido and north-eastern Honshu. A few sexual populations are found in this group from western Hokkaido and north-eastern Honshu where plants of the other two groups are also found and their life history is of the pattern I or II.

In the middle group which is distributed from south-western Hokkaido to north-eastern Honshu and from north-western Honshu to southern Kyushu along the coast of the Japan and East China Seas, there are M-type or both D- and F-type development. These types differentiate from the same basic strains and these from the second and/or third generations can produce another type. The majority of sexual populations and strains is found in this group (Charatsunai, Sakimori and Denshinama at Muroran, Zenibako at Otaru, Ojika, Hakodate, Yoshimo at Shimonoseki), and all four patterns of life history are seen. So the middle group shows variety in both development and life histories.

Some cultured *Blidingia* plants collected at eastern Hokkaido (Kushiro, Abashiri and Monbetsu), Ohma, Hayama, Shimoda, Nomozaki and Yamakawa (Fig. 2, Table 1; \*) produced biflagellate swimmers which did not show sexual behavior (Pl. IV-K, L), although sexual reproduction was not observed in nature or culture. These biflagellate swimmers usually developed into sporophytes which produced quadriflagellate zoospores. However some strains from eastern Hokkaido (Abashiri and Katsurakoi-Kushiro), which belong to the northern group, repeated the gametophytic phase producing biflagellate swimmers through several generations. Unfortunately they could not be examined in a crossing experiment with other sexual strains. These strains are considered to have the potential of sexual reproduction. BLIDING (1968) and KORNMAN and SAHLING (1978) described the developmental differences between *Blidingia minima* and *B. chadefaudii* especially in early ontogeny. Germlings in *B. minima* produce short celled branches and develop into compact discs and germlings in *B. chadefaudii* produce longer-celled branches and develop into more open discs. Agreeably to their description, *Blidingia minima* from Japan may include *B. chadefaudii*; the southern plants with D-type development can clearly be referred to as *B. minima*, but the northern plants with F-type and the M-type plants of the middle group may be referred to as *B. chadefaudii*.

However, in the middle group, some plants showed both D- and F-types of development from the same individual (e. g. Muroran plants, Pl. VIII-F, G) and some others showed the M-type (e. g. Maizuru plants, Pl. VII-H), or continuous variation between D- and F-types (e. g. Hakodate plants, Pl. VIII-A-E). Moreover, swarmers derived in successive cultures from the same strain developed into F-type prostrate discs, but those from the second and third generations developed into D-type discs and soon initiate erect, tubular fronds (Murooran and Zenibako-Otaru plants, Pl. VIII-H, I). Therefore it is still believed that differences in early ontogeny cannot be used to separate the two species just as described in the previous paper (TATEWAKI and IIMA 1984).

In the other previous culture studies of *Blidingia minima* from Japan, only the D-type development has been reported. All of these studies were carried out with the plants from the Pacific coast or Seto Inland Sea where there were regions of the southern groups; plants from Muroran by YAMADA and KANDA (1941, 1944), from Mikawa by ARASAKI (1946), from Matsushima by AKIYAMA (1959) and from Suma and Shirahama by YOSHIDA (1970).

On the other hand, in the previous culture studies of *B. minima* from Europe, two distinct types and an intermediate type of development have been reported. In the first culture experiment of *B. minima* (as *Enteromorpha minima*) from Sweden, germlings appeared to show the intermediate type (BLIDING 1938, Figs. 3-4). Germlings of *B. minima* described by DANGEARD (1961) and GAYRAL (1967) from France showed both typical F-type development (DANGEARD 1961, Fig. 1 and Plate III-H; GAYRAL 1967, Plate I-3) and D-type development (DANGEARD 1961, Fig. 2 and Plate I; GAYRAL 1967, Plate I; GAYRAL 1967, Plate I-1, 2, 4). So there are surely two types of development in plants from France, but it is unknown whether these are derived from the same strain or not. KORN-MANN and SAHLING (1978) pointed out that some figures of *B. minima* reported by DANGEARD (1961) was *B. chadefaudii*, and that both species were defined as *B. minima* by GAYRAL (1967, Plate I) and in an earlier investigation of *B. minima* by themselves (KORN-MANN and SAHLING 1977, Fig. 38-I), two discs belonged to *B. chadefaudii* and only one was *B. minima*. In the present study, however, these differences can commonly occur from the same individual in Japanese plants. Therefore it is possible that European plants of *B. minima* show the mixed appearance of two types of development.

In the original description of *B. chadefaudii* (BLIDING 1963, 1968, VINOGRADOVA 1974, CHADEFAUD 1957 as *Feldmannodra* [= *Enteromorpha*] *chadefaudii*), this species has a typical anatomical feature as the thickness of the inner cell wall of tubular fronds (e. g. BLIDING 1963: ca. 30  $\mu\text{m}$ , VINOGRADOVA 1974: 15-80  $\mu\text{m}$ ). The plants from Sweden which are supposed to have this feature, because anatomical figures are Spanish materials and developmental figures are Swedish ones, show D-type development (BLIDING 1963, p. 33). Contrary, *B. chadefaudii* of KORN-MANN and SAHLING (1978) does not have this feature. The thickness of the inner cell wall of *B. minima* from Japan varies from thin (2-4  $\mu\text{m}$ ) to thick (20-28  $\mu\text{m}$ ), but very thick wall (over 30  $\mu\text{m}$ ) like that of BLIDING (1963) or VINO-

GRADOVA (1974, 1979) is not found (Table. 2, Pl. II-E, F, K, L and Pl. III-A-H).

In the present study, in fact, the plants of the northern group tend to have a little thicker wall and those of the southern group tend to have a thinner wall (cf. Table 2-a). The results of the *t* test on the thickness of the inner cell wall for plants collected from 8 populations are shown in Table 2-b. In the upper part, there is a significant difference between plants of the southern group (Asari-Otaru, Shimoda and Amami-Oshima) and two the other groups. But in the basal part there are no significant differences among 6 populations of three groups (Abashiri, Sakimori-Muroran, Hakodate, Nomozaki, Asari-Otaru and Shimoda) and plants from Shireto-Kushiro and Amami-Oshima show a significant difference from all other 6 populations. Moreover, between plants collected from Abashiri and Kushiro, which show the same F-type development (Pl. VI-C, G and I), there is a significant difference in both the basal and upper parts. Therefore the thickness of the inner cell wall seems to vary considerably even in the same group. The difference between thicker (20-28  $\mu\text{m}$ ) and thinner wall (2-6  $\mu\text{m}$ ) shown in *B. minima* from Japan probably corresponds to that of early species *minima* and *micrococca* or *minima* and *nana*. But these species were united into one species as *Blidingia minima* by KYLIN (1947). Although there is the tendency that thin-walled plants show D-type development and thick-walled plants show F-type development, thin-walled plants occasionally show the F-type development and a few thick-walled plants show the D-type development occasionally. Plate VIII-F, H and I show zoospores derived from the plant from Muroran with thick wall produce the D-type development partly or in the next generation. The thickness of inner cell wall varies depending on environment, age, length of frond and position of the same frond. In fact, the thickness of inner cell wall remarkably varies in culture condition or age of culture plants, and culture plants do not reflect the thickness of inner wall of the natural plants (Pl. III-I-N, all these culture plants showed D-type development).

On the other hand, both F-type development in northern plants and D-type development in southern plants did not change in different culture conditions. Also both types of development in the plants of the middle group could not be regulated by culture conditions.

These results suggest that genetic differentiation occurs in type of development both in the northern and the southern group, but the thickness of inner cell wall easily change by environmental conditions.

Although *B. chadefaudii* and *B. minima* showed a close similarity in the inner cell wall thickness at the early stages of development, but the former exhibited a characteristic thickness of inner cell wall in later stages. Due to this reason VINOGRADOVA (1974) treated these two as an independent species as there was no intermediate type.

*Blidingia minima* which is widely distributed in Japan however showed a wide variation in inner cell wall thickness and this variation seems to be an ecological and geographical phenomenon, therefore *Blidingia* which is widely distributed in Japan is one species *B. minima* previously reported by TATEWAKI and IIMA (1984) at Muroran.

As another species of *Blidingia* from Japan, *B. marginata* was found in 5 estuary-regions of Honshu and Kyushu. Its morphology, development and life history correspond with the previous descriptions obtained from European strains (DANGEARD 1961, BLIDING 1963, KORNMAN and SAHLING 1977, 1978). The erect, tubular frond has ordered cell arrangement in surface view and this typical feature always appears in the fronds of culture plants (Pl. X-B, C and J-L). The germinating zoospores do not produce a migration tube in early development and its erect, tubular frond is formed from a few celled basal disc. So *B. marginata* is considered to be clearly a different species from *B. minima*.

Accordingly, there are only two *Blidingia* species from Japan: *B. minima* and *B. marginata*. The former is on coasts around Japan, and the latter grows in some estuaries in Honshu and Kyushu.

CHAPMAN (1964) described that the *Enteromorpha* species germlings of some species develop into prostrate discs in early ontogeny and cells of some species are small, as in the *Blidingia* species. Furthermore, in *B. marginata* the germling lacks a migration tube which cuts off an empty cell. Therefore, Chapman pointed out that there is no reason for the definition of a separate genus *Blidingia* from the genus *Enteromorpha*. In the present study it is confirmed that sexual reproduction in *B. minima* from Japan sometimes occurs in populations of the northern and the middle group. Nevertheless the present writer does not agree with CHAPMAN's opinion because common features of *B. minima* and *B. marginata* can be found as follows: They have small cell size; They lack rhizoidal cells in the frond's basal part and eye-spots in swarmers; Attached swarmers develop into prostrate discs and then erect, tubular fronds upheave from the center of those discs; These two species have the same habitat. These features seem to be typical of the independent genus *Blidingia*. Although without exception, germlings with a germination tube or an empty cell could not be found in *B. marginata* from Japan, some germlings of *B. minima* from Amami-Oshima lacked germination tubes. According to BLIDING (1963), in germlings of *B. marginata* from Norway a few ones produce empty cells. So the writer considers the existence or nonexistence of the germination tube is the general feature of species *B. minima* and *B. marginata*, although there are some exceptional plants.

From the geographical infraspecific variation point of view, however, it is possible that *B. minima* from Japan is divided into three groups: *minima* (the southern), *chadefaudii* (the northern) and *minima-chadefaudii* complex groups (the middle). The former two groups always show definite developmental types, D-type and F-type respectively, which do not change in culture. So these developmental types are stable genetically and these groups seem to be differentiated in the species *Blidingia minima*. Thus the two groups show an allopatric distribution in eastern Hokkaido and the Pacific coast from Hiraio to Amami-Oshima.

From Fukuura to Yamakawa only the plants with M-type development grow. However, in the regions of south-western Hokkaido and north-eastern Honshu, all three develop-

mental types are found and the three groups show a sympatric distribution. Moreover it seems that, in these regions, the complexity of development types of the middle group occurs markedly and it may reflect the process of this geographical infraspecific variation.

In the present study, the fundamental difference between two types in *Blidingia* seems to reflect the environmental conditions especially temperature and light. Although in any culture conditions most of plants do not change their developmental types, old discs of plants of the southern group with D-type development commonly have filamentous fringes at higher temperature and short-day conditions (18S, 22S, 22L) and plants of the northern group with F-type development develop into small tightly closed discs in the lower temperature, short-day condition (10S). These are similar to the normal M-type development. These results and stability of temperature response in culture plants (cf. Table 3) suggest that all developmental types are reflected in all natural environmental conditions, especially temperature condition and that they are stable genetically.

Concerning the temperature resistance of *B. minima*, plants from north-eastern Hokkaido (Kushiro and Monbetsu) and plants from Amami-Oshima can not grow at 26°C or 5°C, respectively (Table 3). However, Muroran plants can grow within a 5-28°C range (Table 3, Plate IX-C, D) and southern Kyushu plants can grow within a 5-26°C range (Table 3, Plate IX-B), although at very high temperatures (26-28°C) for Muroran plants or very low temperatures (5-10°C) for Kyushu plants, normal growth or maturity does not occur. The only exception to this is the occurrence of the stolon-like propagation of the Nejime plants in 10S condition. These Hokkaido and Kyushu plants never experience such high or low temperatures in nature. Therefore the ability to grow in wide temperature ranges is considered to reflect the ecological feature of *Blidingia* species to grow in the most upper intertidal zone which is exposed to high or low temperature.

GOLDEN and COLE (1986) criticized the interpretation of heteromorphy in the life history of *Blidingia* from Muroran (TATEWAKI and IIMA 1984) as a culture artifact that was an essentially isomorphic life history. Certainly the writer has not yet found a microthallal stage bearing reproductive organ of *B. minima* in the field and he speculates that these stages are of ecological importance in the life history of the species. The erect, tubular fronds of *B. minima* grow abundantly as a green mat on rocks or concrete blocks in the upper intertidal zone from March or April to July, but they usually disappear in August at Muroran. These field observations agree with the results obtained in culture of plants of the northern and the middle groups: the formation of erect, tubular fronds is suppressed by high temperatures (Miyagi, Oga, Maizuru and Muroran plants at 22°C, Kushiro, Abashiri and Monbetsu plants at 18-22°C), while the growth of germlings is very slow at low temperatures (5-10°C). It may be that *Blidingia* of the northern and the middle groups grows as prostrate microthalli which can propagate themselves by zoospores and gametes in the lower intertidal zone from late summer to autumn. Such microthalli produce swimmers which attach and grow on rocks in the upper intertidal zone from winter to early summer. The

existence of heteromorphic life histories in the northern group of *Blidingia* supports the results of increase of the ratio of isomorphic algae to heteromorphic algae of green and brown algae (I/H ratio) in colder regions of Japan (NAKAHARA and MASUDA 1971).

As mentioned above, the complete intermediate type (Maizuru, Senzaki, Yoshimo-Shimonoseki, Nomozaki and Yamakawa), the continuous variety from D-type to F-type in the same clone (Hakodate, Fukuura, Ojika) and the appearance of both types in the same clone (Muroran, Ohma and Oga) were shown. Furthermore, it was confirmed that there is a change from F-type to D-type in the cultures from the second or third generations in the materials from Muroran and Zenibako. Accordingly, an instability of types of development may have something to do with the high frequency of sexual reproduction in the middle group.

CLAYTON (1982) described that asexual and vegetative reproduction stabilize pre-existing patterns of variation. Genotypic stability from one generation to the next ensures the perpetuation of well adapted types. She mainly described the apomictic direct life history in the Ectocarpales (Phaeophyta) and discussed the possibility of the evolution of apomixis.

In other marine algae there are many reports that life history or the existence of sexual reproduction is commonly influenced by geographical and environmental factors. DIXON (1965) described that tetrasporic plants occur further to the north than the sexual plants, and only sterile plants occur at the extreme northern limit of distribution in some European Rhodophyta. EDWARDS (1973) suggested that the absence of fertile gametophytic plants of two species of *Ceramium* from northern Scotland in the northern part of their geographical range might be attributable to the inhibition of gametangial production by the environment, since tetraspores always developed into gametophytic plants in culture. In Chlorophyta, FRIEDMANN (1969) reported that growth of *Prasiola stipitata* on the eastern shore of the Atlantic is perennial and meiotic plants are infrequent or absent from 55° latitude northwards, while south of this line growth is seasonal and meiotic plants are abundant. On the western shores of the Atlantic growth is perennial and gametophytes are absent from 44° latitude northward. CLAYTON (1982) also commented briefly that several species of the warm water green alga *Caulerpa* are flourish in Victoria in southern Australia, a cool temperate region, but fertile gametophytes are rarely found.

Moreover in Ulvales, it has been reported various life histories in *Ulva mutabilis* (cf. TANNER 1981). TANNER (1981) described that various life histories in *U. mutabilis* could explain the occurrence of asexual strains of algae at the border of the distribution of a given species. According to LØVLIE and BRYHNI (1978), in border populations of *U. mutabilis*, where population density is low and temperature is not optimal for gamete fusion, sexual reproduction would be disturbed and parthenogenetic reproduction would become predominant. This could also explain the existence of strains in *Ulva*, *Enteromorpha* and *Ulvaria* that reproduce strictly by biflagellate neutral swarmers (BLIDING 1963, 1968; KAPRAUN

1970).

The phenomenon of releasing both bi- and quadriflagellate swarmers from the same thallus has been reported in some species of Ulvales (*Enteromorpha linza*: YAMADA and SAITO 1938; *Ulva*: FÖYN 1958, BLIDING 1968, HOXMARK 1975; *Percursaria percursea*: KORNMAN 1956 and *Blidingia minima*: TATEWAKI and IIMA 1984, present study). This phenomenon is considered to show the potential of sexual reproduction in asexual strains.

*B. minima* from Japan shows a clear growth limit in the sexual populations or strains. Most of the sexual populations and strains found in nature and culture are from south-western Hokkaido and north-eastern Honshu. Some strains from Yoshimo-Shimonoseki are the only exception. This limitation seems to be closely related to temperature conditions in each place. Surface seawater temperatures of some places where sexual plants were found is as follows (minimum in February-maximum in August): Muroran (ca. 2°C-ca. 20°C), Oshoro (ca. 4°C-ca. 20°C), Shiogama near Ojika (ca. 4°C-ca. 25°C) and Yoshimo-Shimonoseki (ca. 7°C-ca. 24°C).

On the other hand, some of asexual regions are as follows: Kushiro (ca. 0°C-ca. 16°C), Choshi (ca. 9°C-ca. 24°C), Shimoda (ca. 12°C-ca. 26°C) and Amami-Oshima (ca. 19°C-ca. 28°C). A minimum seawater temperature limit for sexual regions is 2-7°C and that in asexual regions is more than that. Since plants releasing biflagellate swarmers were found in several asexual regions, many asexual plants may have the potential of sexual reproduction, but only a limited temperature range in winter may be suitable for sexual reproduction; lower or higher extreme temperature regions (northern and southern parts of Japan) may be unsuitable for sexual reproduction.

As most of the northern and all the southern group of *B. minima* from Japan lack sexual reproduction, each phenotype is genetically fixed, and developmental types are also fixed, but the some populations in the northern and the middle group are sexual, and genetic exchange can occur and furthermore mutation of genes or chromosomes seems to be apt to occur. Sexual isolation among sexual populations could not be found in tested combinations.

It had not been reported about the sexual population of *Blidingia* of the world, but *B. minima* is a species widely distributed throughout the world.

In spite of the wide distribution of *B. minima*, culture studies have only been carried out on European and Japanese materials. So the universal geographical variation of developmental type and existence of sexual population are not known at all. Therefore culture experiments are needed to clarify the relation between *B. minima* and *B. chadefaudii* or other species.

The geographical distribution of *B. minima* according to the difference of developmental type and life histories, that is, the existence of three groups, seems to be related to the level of genetic differentiation affected by environmental conditions. There are some methods to investigate the level of genetic differentiation, but these are unsuitable in the present case of *Blidingia* from Japan. Crossing tests could hardly be carried out in the

present study between the northern and southern group because there is not found that there are sexual populations in the southern group. However, it is confirmed that reproductive isolation does not occur between the northern and the middle groups, because Ojika and Muroran strains (the middle group) could be crossed by Aomori strains (the northern group).

Karyotype analysis is also important, but not carried out. INNES (1984) reviewed genetic differentiation among populations of marine algae, and INNES and YARISH (1984) reported that in *Enteromorpha linza* there are different isozyme patterns between sexual and asexual populations. So in *Blidingia*, the mode of reproduction and development should also be studied by such electrophoretically detectable genetic markers in the near future.

### Summary

*Blidingia* plants were collected from various localities along coasts all around Japan and investigated regarding their frond morphology, reproduction, development and life history. It is confirmed that there are two taxa in Japan: *Blidingia minima* and *B. marginata*.

*B. minima* has been shown to exhibit three types of development and four patterns of life history in laboratory cultures as follows.

*Developmental type*: 1) D-type—Germlings with short cells develop into compact discs which soon grow erect, tubular fronds; 2) F-type—Germlings with long cells develop into filamentous prostrate microthalli, which produce reproductive cells repeatedly and later grow erect, tubular fronds; 3) M-type—Germlings with moderately long cells develop into filamentous discs or prostrate microthalli, which produce few reproductive cells, but soon grow erect, tubular fronds. This is an intermediate type of development showing continuous variation between D- and F-type.

*Life history pattern*: I. Fundamentally an alternation of erect, tubular isomorphic gametophytes and sporophytes, but including an occasional formation of gamete- or zoospore producing microthalli; II. An irregular alternation of heteromorphic phases, with the erect, tubular frond producing only zoospores and microthallus producing gametes or zoospores; III. An asexual alternation of phases with both the tubular frond and the microthallus producing only zoospores; IV. A monophasic cycle in which zoospores are the only reproductive cells produced and the erect, tubular frond recycles itself.

*B. minima* from Japan has been divided into three groups according to the way they differ in types of development and life history patterns: 1) The southern group—There, there is D-type development and pattern IV life history; 2) The northern group—Here there is F-type development. The life history is almost pattern III but a few populations show sexual reproduction, *i. e.* pattern I and II; 3) The middle group—M-type or mixed occurrence of F-type and D-type development in the same strain occurs. The majority of sexual populations and all four patterns of life history are found. However, this group is an intermediate group between the former two and it is not well defined, both in development

and life history.

These three groups show clear geographical divisions. Only the southern group ranges from Amami-Oshima to Hiraiso, along the Pacific coast of Kyushu, Shikoku and Honshu, including the coast of Seto Inland Sea. Only the northern group is in eastern Hokkaido. Only the middle group is found from Fukuura to Yamakawa along the Japan and East China Seas. However, the three groups are mixed in south-western Hokkaido and north-eastern Honshu regions.

*Blidingia minima* from Japan seems to be divided into two taxa, such as *B. minima* and *B. chadefaudii*, according to differences in their early ontogeny and the thickness of inner cell wall of the frond; with *minima* the germling has short cells and the frond has a thin cell wall, and with *chadefaudii* the germling has long cells and the frond has a thick cell wall. However, because of the existence of the middle group and finding no correlation between the frond's anatomical features and type of development, it is concluded that these two taxa should be combined into the *Blidingia minima* species.

Another taxon, *Blidingia marginata*, found in several parts in Japan is considered to be a distinct independent species because it has a particular development; it has no germination tube which detaches as an empty cell, and its cells are ordered in definite longitudinal rows.

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PLATE I

Different forms in natural plants of *Blidingia minima*  
from Hokkaido to Kyushu and Amami-Ohshima  
(Field-collected specimens)

- A. Plants from Monbetsu Port, Hokkaido.
- B. Plants from Shireto-Kushiro, Hokkaido.
- C. Plants from Charatsunai-Muroran, Hokkaido.
- D. Plants from Sakimori-Muroran, Hokkaido (sexual plants; +, - = gametophytes, s = sporophytes).
- E. Charatsunai-Muroran, Hokkaido.
- F. Plants from Maizuru, Kyoto Prefecture.
- G. Plants from Usa Bay, Kochi Prefecture.
- C. Plants from Yoshimo-Shimonoseki, Yamaguchi Prefecture.
- I. Plants from Enoshima, Kanagawa Prefecture.
- J. Plants from Nejime, Kagoshima Prefecture.
- K. Plants from Amami-Ohshima.

Scale in K applies also to A-J.

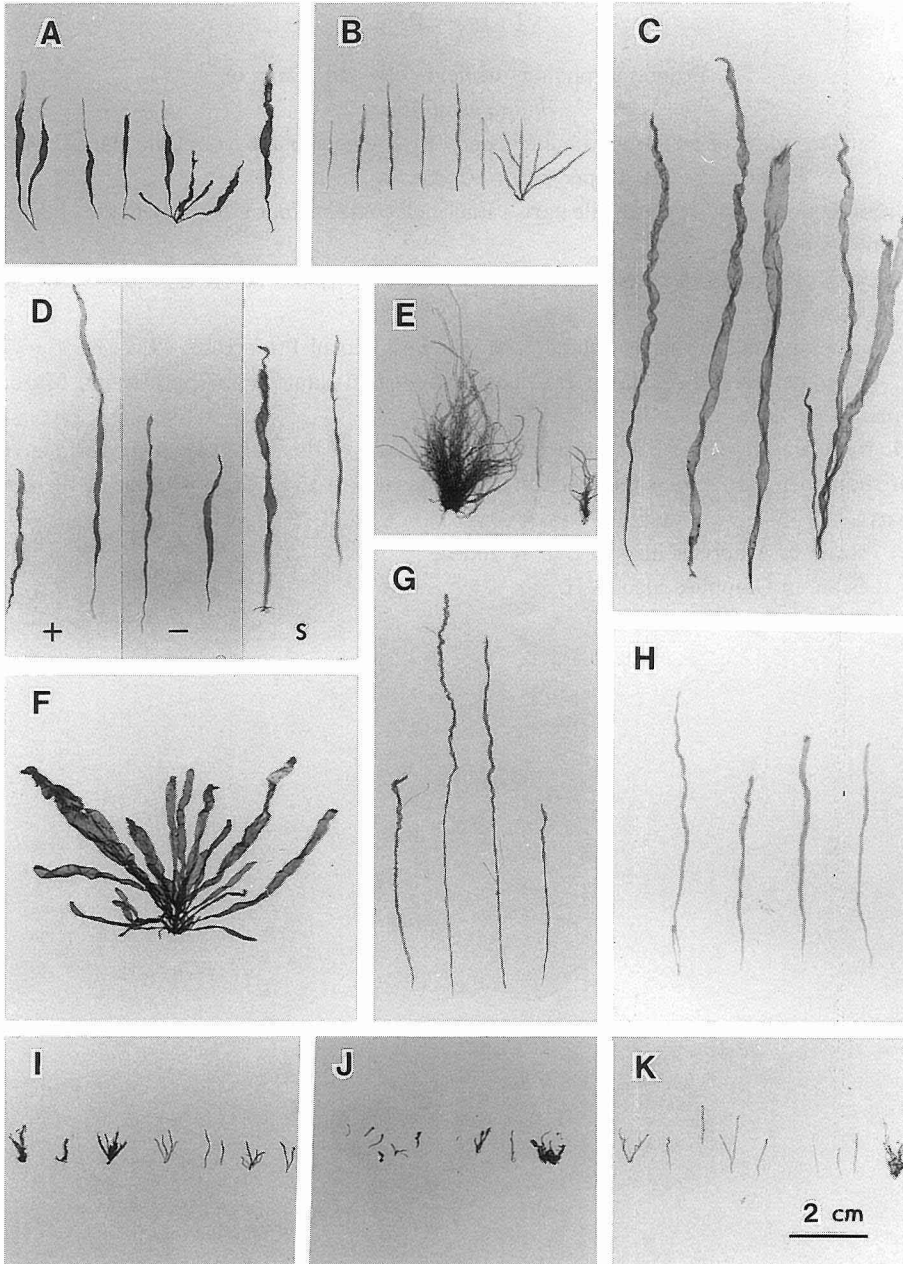


PLATE II

Frond morphology of field collected plants of

*Blidingia minima*

- A-C. Surface view of an erect, tubular frond of the longer plant from Charatsunai, Muro-ran; A. Basal part; B. Upper part; C. Fertile Part.
- D. Cross section of the upper fertile part. Each cell contains four swarmers in the Hakodate plant (sporophyte).
- E-F. Cross section of the erect tubular frond (the same plant as A-C): E. Basal part; F. Upper part.
- G. Frond with small branches (plant from Usa Bay, Kochi Prefecture, Pl. I-G).
- H-L. Frond morphology of the most slender and tangled plants from Charatsunai, Muroran (plants Pl. I-E):
- H. Ramification of the basal part; I. Surface view of the basal part; J. Surface view of the upper part; K. Cross section of the basal part; L. Cross section of the upper part.

Scale in A applies also to B-I, K and L.

Scale in G applies also to H.

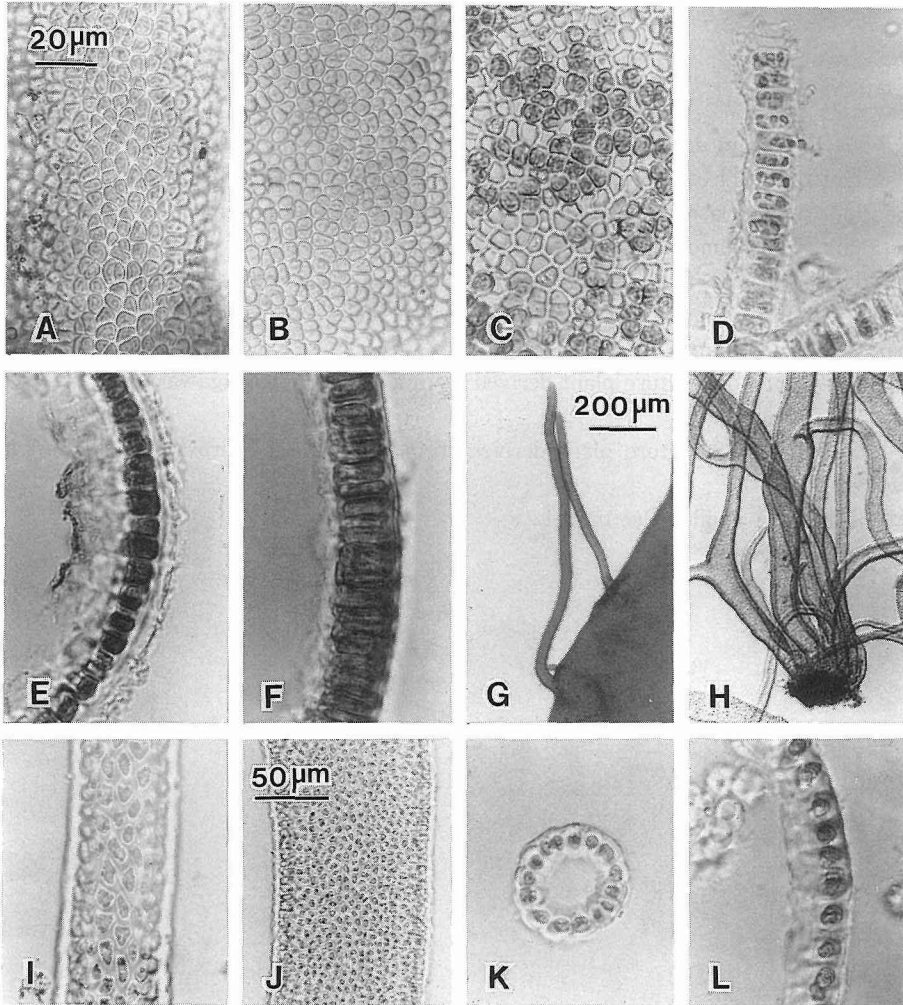


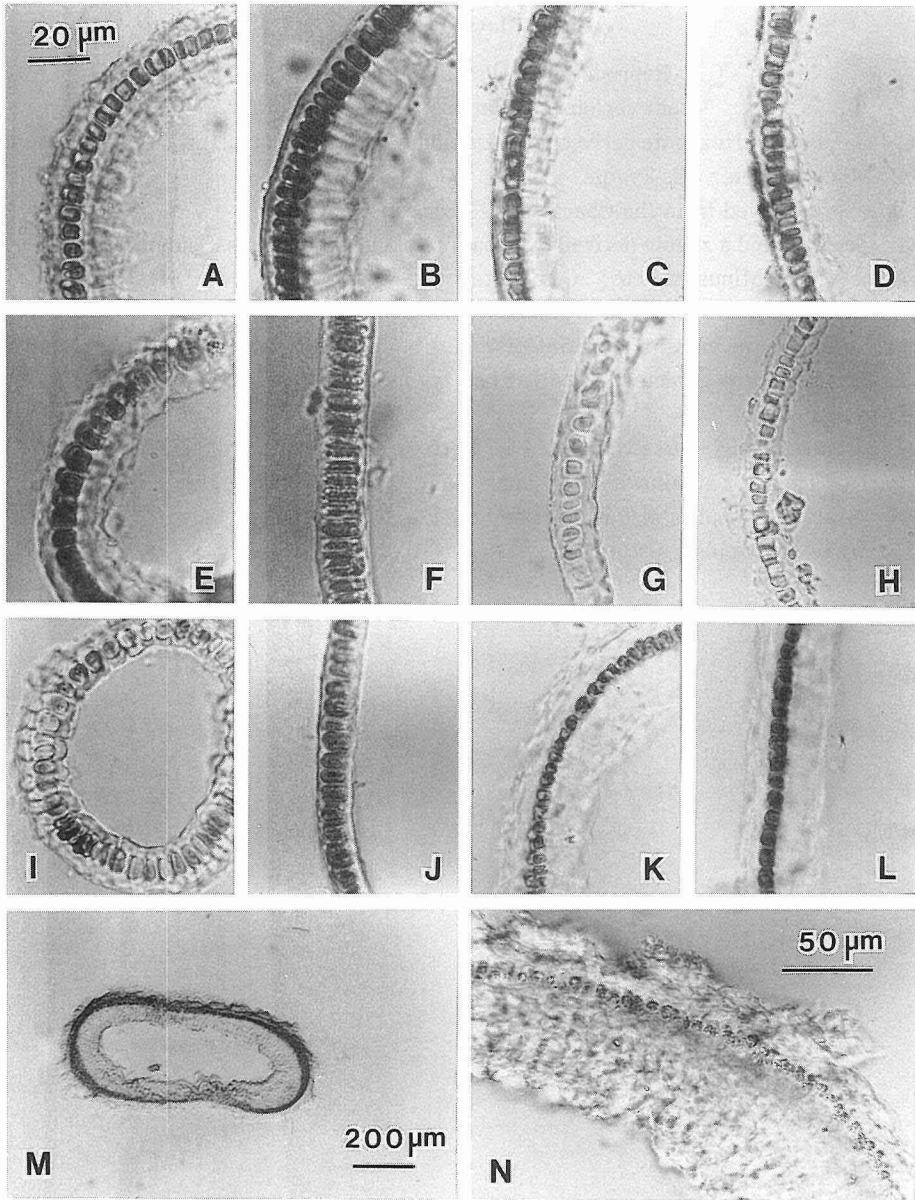
PLATE III

*Blidingia minima*

Cross section of the erect, tubular frond  
of natural plants (A-H) and culture plants (I-M)

- A-B. Plant from Shireto, Kushiro, Hokkaido: A. Basal part; B. Upper part.  
C-D. Plant from Abashiri, Hokkaido: C. Basal part; D. Upper part.  
E-F. Plant from Nomozaki, Nagasaki Prefecture: E. Basal part; F. Upper part.  
G-H. Plant from Hayama, Kanagawa Prefecture: G. Basal part; H. Upper part.  
I - J. One-month-old culture plant derived from a Charatsunai-Muroran plant: I. Basal part; J. Upper part.  
K-L. Five-month-old culture plant derived from a Hayama-Kanagawa plant: K. Basal part; L. Upper part.  
M-N. Ten-month-old culture plant derived from a Sakimori-Muroran plant (the same figures).

Scale in A applies also to B-L.



## PLATE IV

Reproductive cells of *Blidingia minima*  
from various populations and culture strains

- A-C. Gametes and a zygote derived from Denshinama-Muroran plants: A. Plus gamete; B. Minus gamete; C. Zygote.
- D. Zoospore derived from the Charatsunai-Muroran plant.
- E-G. Gametes and a zygote derived from culture plants of Yoshimo-Shimonoseki: E. Plus gamete; F. Minus gamete; G. Zygote.
- H. Zoospore derived from a Heta-Shizuoka plant.
- I-J. Results of the crossing experiments: I. Conjugation between Sakimori gamete (+) and Denshinama gamete (-); J. Planozygote from Yoshimo gamete (+) and Ojika gamete (-).
- K-L. Asexual biflagellate swimmers: K. Derived from a Katsurakoi-Kushiro plant; L. Derived from a Yamakawa-Kagoshima plant.
- Scale in C applies also to A-B and E-H.
- Scale in I applies also to J-L.

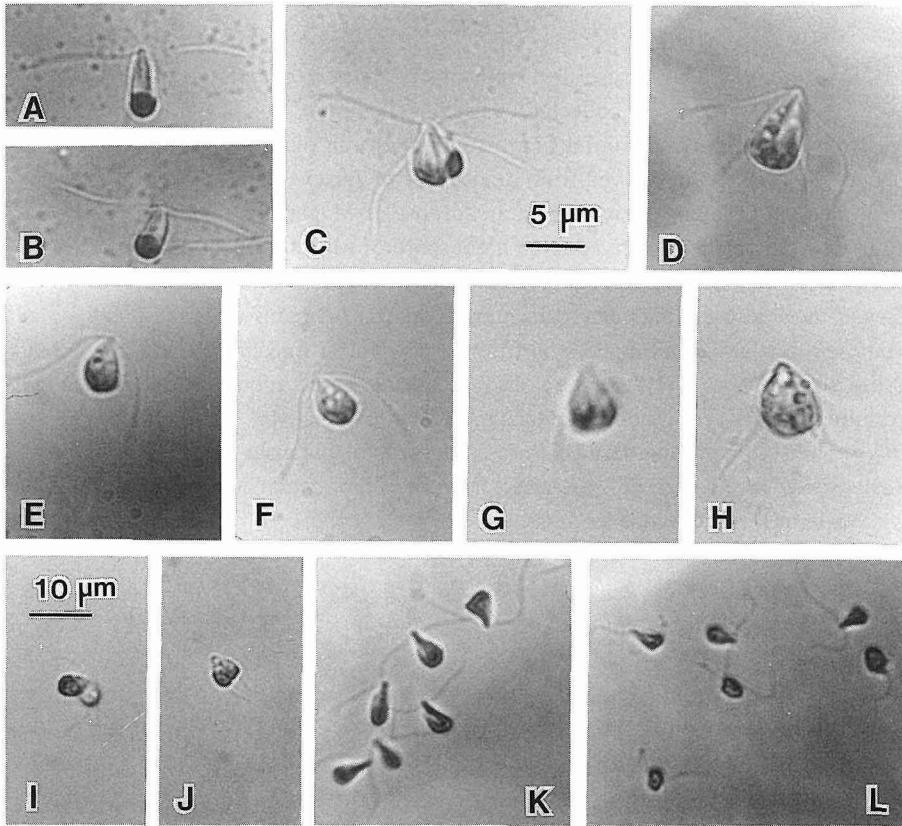
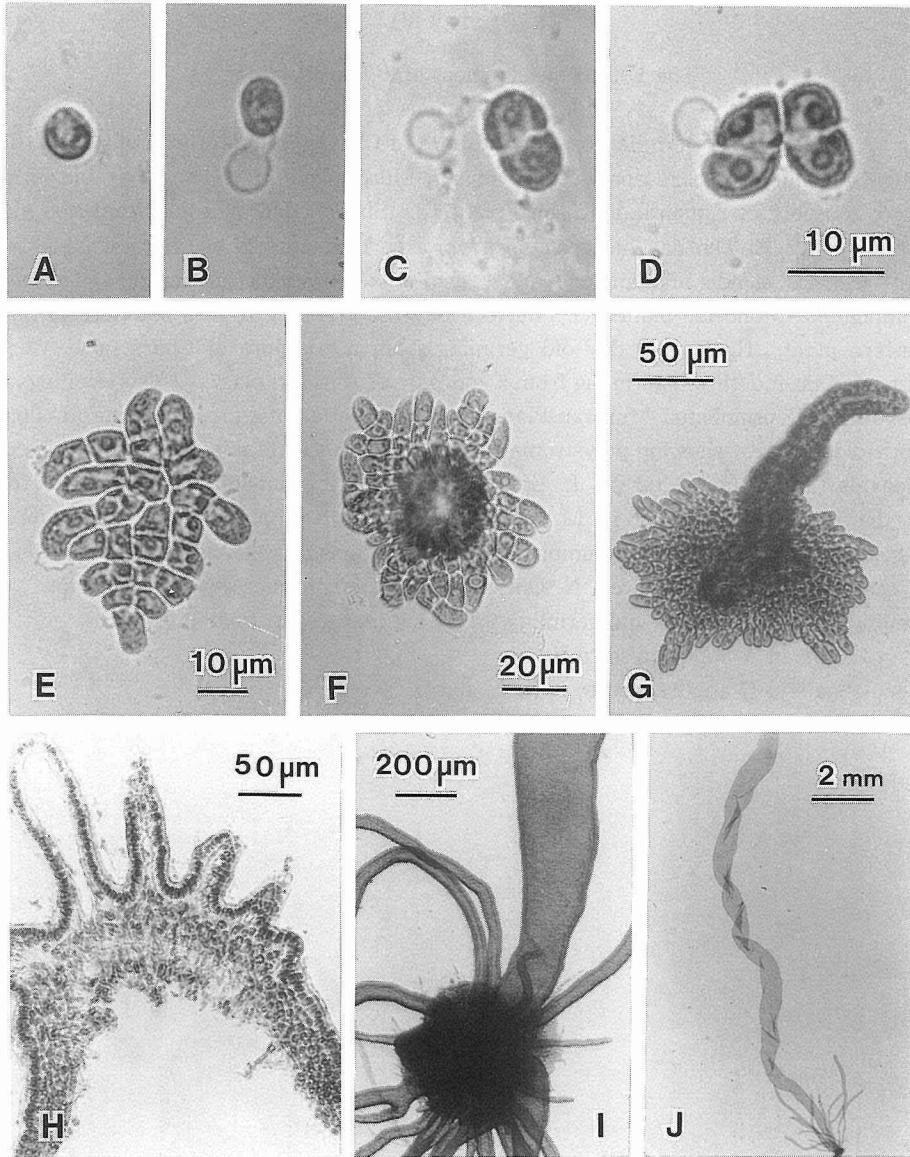


PLATE V

The D-type development of *Blidingia minima*  
collected at various localities

- A. Settled zoospore derived from a Charatsunai-Muroran plant.
- B. One-day-old germling derived from a zoospore of Asari-Otaru plant.
- C. Two-day-old germling derived from a zoospore of Magamegawa-Chiba plant.
- D. Four-day-old germling derived from a zoospore of Muroto-Kochi plant.
- E. Seven-day-old germling derived from a zoospore of Heta-Shizuoka plant.
- F. Eleven-day-old germling derived from a zoospore of Amami-Ohshima plant.
- G. Fourteen-day-old germling derived from a zoospore of Enoshima-Kanagawa plant.
- H. Cross section of a polystromatic disc producing erect, tubular fronds (1-month-old germling derived from the Charatsunai-Muroran plant).
- I. One-month-old germling derived from a zoospore of Shibushi-Kagoshima plant.
- J. Forty-seven-day-old germling derived from the Charatsunai-Muroran plant.

Scale in D applies also to A-C.



## PLATE VI

The F-type development of *Blidingia minima*  
collected at various localities

A. One-day-old germling from a zoospore of Charatsunai-Muroran plant. B. Two-day-old germling from a zoospore of Charatsunai-Muroran plant. C. Five-day-old germling from a zoospore of Katsurakoi-Kushiro plant. D. Six-day-old germling from a zygote of Aomori plant. E. Ten-day-old germling from a parthenogamete of Charatsunai-Muroran plant (the most slender and tangled plant). F. Twelve-day-old germling from a zoospore of Minamigaoka-Monbetsu plant. G. Fourteen-day-old germling from a zoospore of Shireto-Kushiro plant. H. Twenty-day-old germling from a zoospore of Charatsunai-Muroran plant. I. One-month-old germling from a zoospore of Abashiri plant. J-O. Germlings from zoospores of Denshinama-Muroran Plant. J. Cross section of a prostrate microthallus (1-month-old culture), showing polystromatic layer. K. Prostrate microthallus with irregular upheavals (40-day-old culture). L. Erect filaments bearing gametangia on fertile microthallus (the same plant as Fig. K). M. Release of gametes from plus and minus gametophytic prostrate microthalli, showing clumpings and conjugations between gametes of both sexes in the central area of the figure. N. One-month-old fertile microthallus and many young germlings. O. Five-month-old germling producing many erect tubular fronds.

Scale in A applies also to B.

Scale in H applies also to I, K, N and O.

Scale in G applies also to J and M.

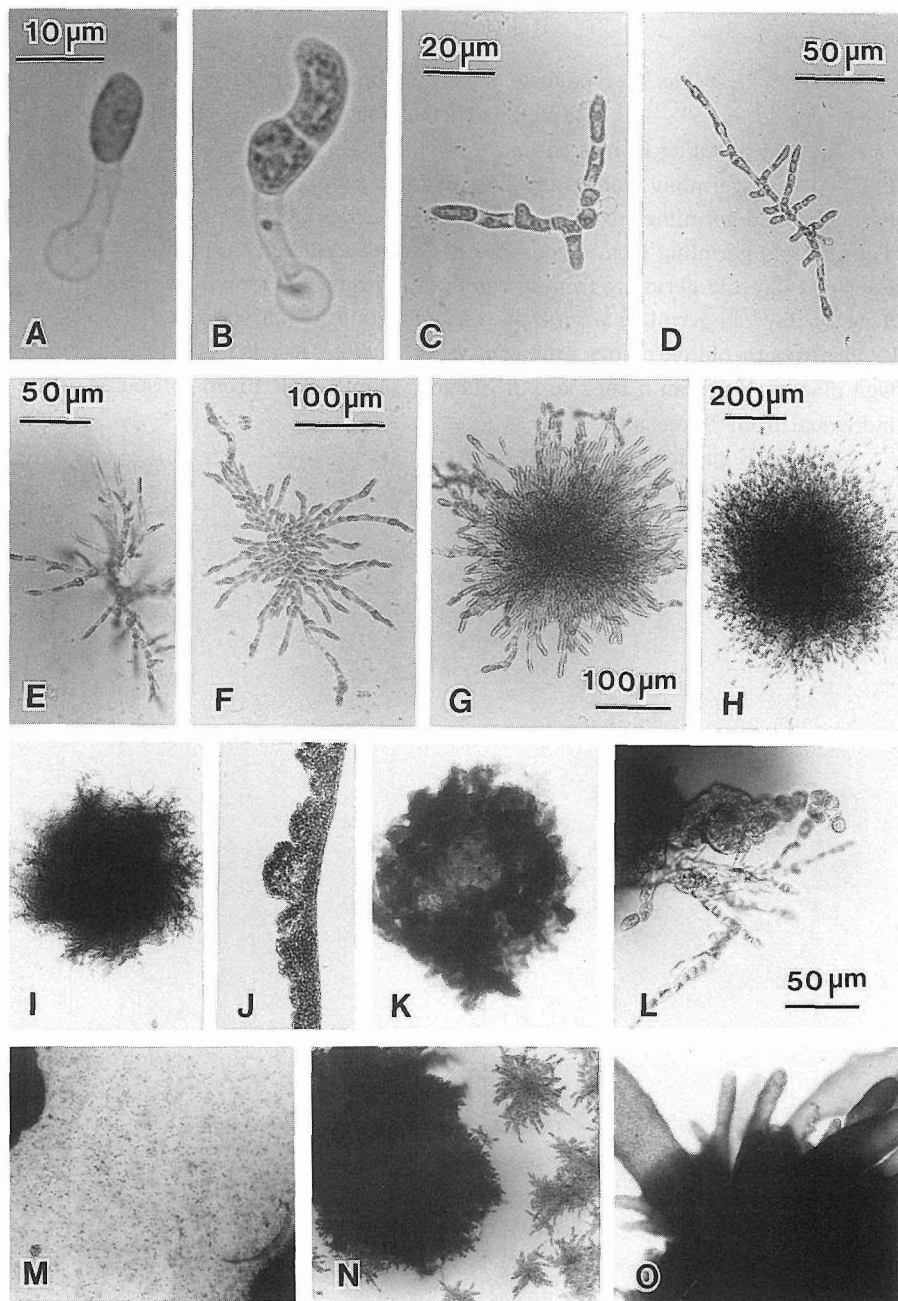


PLATE VII

The M-type development of *Blidingia minima*  
collected at various localities

- A. One-day-old germling from a zoospore of Nomozaki plant.
- B. Four-day-old germling from a zoospore of Maizuru plant.
- C. Eight-day-old germling from a zoospore of Hakodate plant.
- D. Ten-day-old germling from a zoospore of Senzaki-Yamaguchi plant.
- E. Fourteen-day-old germling from a zygote of Yoshimo-Shimonoseki strain.
- F. Twenty-day-old germling from a parthenogamete of Ojika plant.
- G-J. One-month-old germlings producing erect, tubular fronds: G. From a zoospore of Oga plant; H. From a zoospore of Maizuru plant; I-J. From zoospores of the same individual from Nomozaki.

Scale in E applies also to F.

Scale in G applies also to H-J.

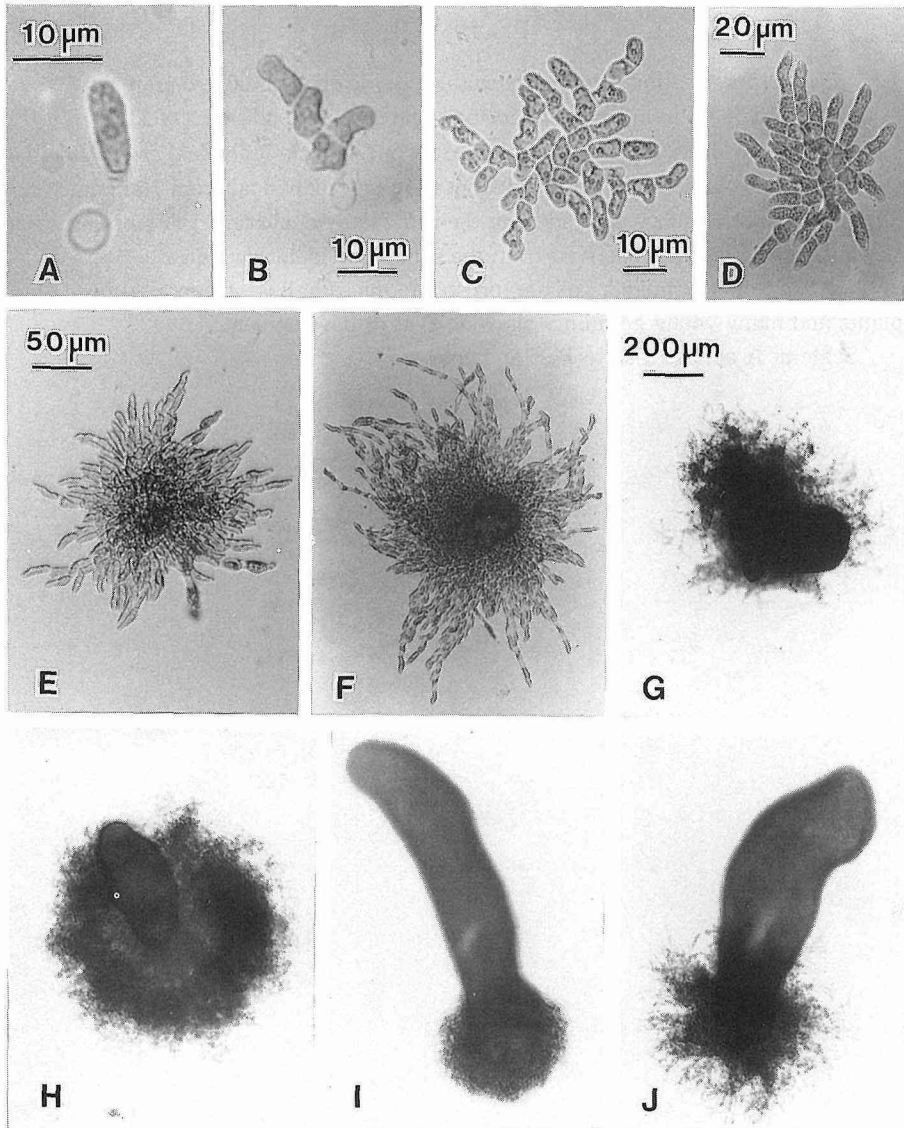


PLATE VIII

Differentiation of developmental types in the middle group of  
*Blidingia minima* grown in the same culture condition (14 L)

- A-B. Twenty-two-day-old germlings derived from the same individual from Hakodate.
- C-E. One-month-old germlings derived from the same individual from Hakodate.
- F. Seven-day-old germlings derived from the same individual from Sakimori-Muroran.
- G. One-month-old germling derived from the same individual from Oga.
- H-I. Fifty-day-old germlings (fertile prostrate microthallus) from Sakimori-Muroran plants and many young germlings showing D-type development.

Scale in A applies also to B-E and G-I.

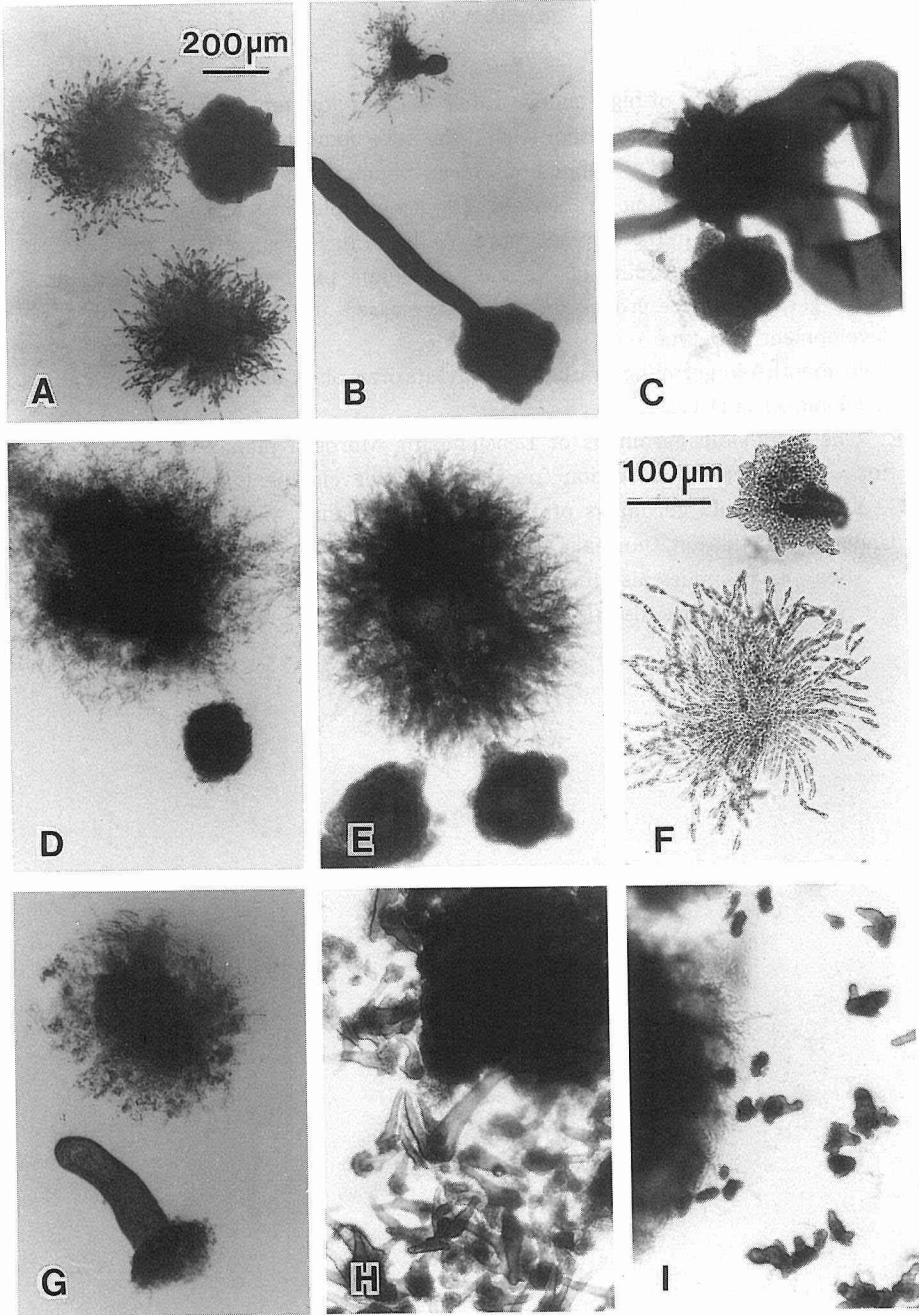


PLATE IX

*Blidingia minima*

Influence of high temperature (28 L) or low temperature and  
shortday (5-10 S) conditions in the development of zoospores from  
plants of various populations

- A. One-month-old germling of Senzaki-Yamaguchi plant under 5 S condition, showing leaving cytoplasm in the original spore. (Normal development is M-type.)
- B. Three-month-old germling of Nejime-Kagoshima plant under 10 S condition. Many young germlings were produced from a large parent disc by stolon-like tubes. (Normal development is D-type.)
- C. Two-month-old germling of Charatsunai-Muroran plant under 28 L condition. (Normal development is D-type.)
- D-E. One-month-old germlings of Denshinhamma-Muroran plant: D. Under 28 L condition; E. Under 22 L condition (normal F-type development).
- F-G. One-month-old germlings of Heta-Shizuoka plant: F. Under 28 L condition; G. Under 14 L condition (normal D-type development).

Scale in B applies also to D.

Scale in C applies also to E-G.

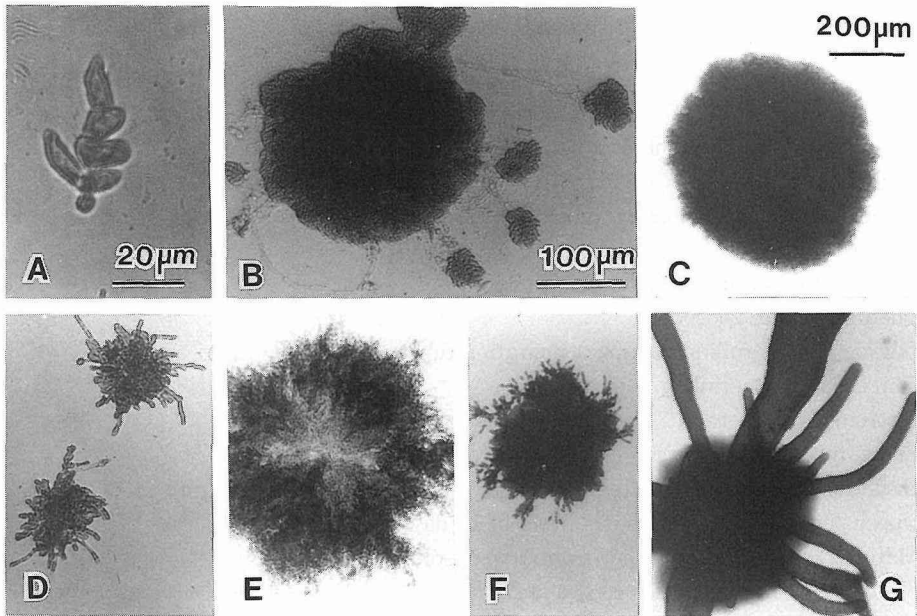


PLATE X

*Blidingia marginata*

Morphology and life history

of the materials from Kidogawa, Chiba Prefecture

- A. Natural plants.
- B-D. Surface view of an erect, tubular frond: B. Basal part; C. Upper part; D. Fertile part.
- E. Cross section of an erect, tubular frond.
- F. Quadriflagellate zoospore.
- G. One-day-old germling lacking a migration tube.
- H. Three-day-old germling.
- I. Distromatic disc, from 7-day-old culture.
- J. Erect, tubular frond arisen on the disc, from 10-day-old culture.
- K. Basal disc producing many erect, tubular fronds in 15-day-old culture.
- L. Upper part of an erect, tubular frond in 15-day-old culture.
- M. Matured erect, tubular frond, from 3-week-old culture.

Scale in B applies also to C and D.

Scale in G applies also to H and I.

