HOKKAIDO UNIVERSITY

Title	A taxonomic study of the family Scytosiphonaceae (Scytosiphonales, Phaeophyceae) in Japan
Author(s)	Kogame, Kazuhiro
Degree Grantor	北海道大学
Degree Name	博士(理学)
Dissertation Number	乙第4596号
Issue Date	1994-09-30
DOI	https://doi.org/10.11501/3079374
Doc URL	https://hdl.handle.net/2115/51224
Type	doctoral thesis
File Information	000000278135.pdf

Hokkaido University Collection of Scholarly and Academic Papers : HUSCAP

A taxonomic study of the family Scytosiphonaceae (Scytosiphonales, Phaeophyceae) in Japan

by

Kazuhiro Kogame

1994

A taxonomic study of the family Scytosiphonaceae (Scytosiphonales, Phaeophyceae) in Japan

by

Kazuhiro Kogame

1994

CONTENTS

Contents 1

Acknowledgements 2

Summary 3

Introduction 5

Materials and methods 9

Results and discussion on each species 11

Colpomenia (Endlicher) Derbès et Solier in Castagne 11

Colpomenia sinuosa (Mertens ex Roth) Derbès et Solier ... 12

Colpomenia peregrina (Sauvageau) Hamel 21

Colpomenia phaeodactyla Wynne et Norris 34

Colpomenia bullosa (Saunders) Yamada 39

Hydroclathrus Bory 48

Hydroclathrus clathratus (C.Agardh) Howe 49

Scytosiphon C.Agardh 56

Scytosiphon lomentaria (Lyngbye) Link 57

Scytosiphon nakamurae sp. nov. 69

Scytosiphon gracilis sp. nov. 82

Scytosiphon tenellus sp. nov. 94

Petalonia Derbès et Solier 104

Petalonia zosterifolia (Reinke) Kuntze 105

Petalonia fascia (O.F.Müller) Kuntze 114

Petalonia binghamiae (J.Agardh) Vinogradova 127

General discussion 134

Key to species examined in this study 147

References 149

ACKNOWLEDGEMENTS

I wish to thank cordially my supervisor Professor T. Yoshida, Hokkaido University, for his helpful advice and encouragement. Also I wish to thank Professor M. Masuda, Hokkaido University and Dr H. Kawai, Kobe University, for their helpful suggestions and providing materials. I appreciate the kindness of Dr T. Horiguchi, Hokkaido University, for his critical reading of the manuscript and helpful comments. I am grateful to Professor M. Tatewaki, Hokkaido University, for his critical comments and the loan of specimens of some species. I acknowledge cooperation of following persons for their help of collecting materials, providing materials and encouragement; Professor S. Enomoto, Kobe University, Professor S. Kamura, Ryukyu University, Dr S. Kawaguchi, Kyusyu University, Dr T. Kudo, Sapporo University, Dr T. Motomura, Hokkaido University, Dr M. Baba, Marine Biological Research Inst. of Japan, Mr. S. Arai, Marine Algae Research Co., Ltd., Dr T. Kitayama, National Science Museum, Tokyo, Mr M. Matsumoto, Japan NUS Co., Ltd., Dr I. Mine, Mr T. Abe and Mr Y. Yamagishi, Hokkaido University, and Mr K. Shida, Oshoro Marine Biological Station, Hokkaido University.

SUMMARY

The order Scytosiphonales is characterized by a heteromorphic life history with a macroscopic, polystichous gametophyte (macrothallus) and a microscopic, haplostichous sporophyte (microthallus), and by the possession of a single plastid with a large pyrenoid per cell. Two families are included: the Chnoosporaceae and the Scytosiphonaceae. The latter includes six or seven genera whose species are common in tropical to cold-temperate waters throughout the world. The following characters have been used in the taxonomy of the Scytosiphonaceae: external morphology, structure of thallus, plurilocular sporangial sori, ascocyst, phaeophycean hair, amongst others, although the validity of these characters is controversial. Microthalli are mainly known from laboratory cultures, and field microthalli have been reported for only a few species.

In the present study, an examination of taxonomic characters and life histories was carried out on the following twelve species from Japan: *Colpomenia sinuosa* (Mertens ex Roth) Derbès et Solier, *C. peregrina* (Sauvageau) Hamel, *C. phaeodactyla* Wynne et J. N. Norris, *C. bullosa* (Saunders) Yamada, *Hydroclathrus clathratus* (C. Agardh) Howe, *Scytosiphon lomentaria* (Lyngbye) Link, *S. nakamurae* sp. nov., *S. gracilis* sp. nov., *S. tenellus* sp. nov., *Petalonia zosterifolia* (Reinke) Kuntze, *P. fascia* (O. F. Müller) Kuntze, *P. binghamiae* (J. Agardh) Vinogradova. Morphological observations, using light microscopy and culture studies to observe each stage of life history under various conditions, were carried out on macrothalli and microthalli collected from various localities. Phenological observations were made for several species at Oshoro on the western coast of Hokkaido.

Specimens deposited in the herbarium of the Faculty of Science, Hokkaido University (SAP) were also examined.

A reassessment of taxonomic characters suggests that initial development of macrothalli, form of plurilocular sporangia, ascocyst and morphology of microthallus are important for generic and specific features. Uniseriate and spherical types are recognized in initial development of macrothallus. The former type characterizes the species of *Scytosiphon* and *Petalonia*, and the latter the species of *Colpomenia* and *Hydroclathrus*. Two types of plurilocular sporangia, loose type and packed type, are considered useful for species-level taxonomy. Microthalli can be classified into four groups morphologically, and these can be used as a taxonomic feature at species level. Thallus thickness, gamete size and the presence or absence of ascocysts are also important as species characters.

Culture studies carried out in the course of the present investigation elucidate heteromorphic life histories involving macrothalli and microthalli in eleven species (excluding *C. phaeodactyla*). Although microthalli bearing only unilocular sporangia have been known previously in the order Scytosiphonales, it is shown that microthalli also produce plurilocular sporangia in addition to unilocular sporangia in *C. sinuosa*, *C. peregrina* and *H. clathratus*. Gamete fusion is described for *C. peregrina*, *C. bullosa*, *S. lomentaria*, *S. nakamurae*, *S. gracilis*, *S. tenellus*, *P. fascia* and *P. binghamiae*. Life histories observed in culture during the present study can be classified into three patterns by reproductive styles of microthalli. These three life history patterns are in close relation to the morphology of microthalli, and conform with the phenologies of the species examined.

INTRODUCTION

Thuret (in Le Jolis 1864) established the tribe Scytosiphoneae to include the genera *Scytosiphon* C. Agardh and *Phyllitis* Kützing (*Petalonia* Derbès et Solier), recognizing affinities of plurilocular sporangia between the two genera. Thuret & Bornet (1878) added *Hydroclathrus* Bory along with *Colpomenia* Derbès et Solier to the tribe. Farlow (1881) was the first to treat the group as the rank of family.

Feldmann (1949) established the order Scytosiphonales which was characterized by two features: the cell has one plastid with a large pyrenoid; the thalli produce only plurilocular sporangia as a reproductive organ. He included the three families in the Scytosiphonales: Phaeosaccionaceae Feldmann, Scytosiphonaceae, and Chnoosporaceae Setchell & Gardner. *Phaeosaccion* Farlow, the only genus in the Phaeosaccionaceae, was later transferred to Chrysophyceae (Parke and Dixon 1964). Feldmann (1949) transferred the following four genera to the Scytosiphonaceae: *Rosenvingea* Børgesen, *Iyengaria* Børgesen, *Utriculidium* Skottsberg and *Endarachne* J. Agardh. Chnoosporaceae included only the genus *Chnoospora* J. Agardh. Although Feldmann (1949) did not ascertain that each cell of *Chnoospora* has one plastid with a large pyrenoid, Fotos (1981) made clear the feature in *Chnoospora minima* (Hering) Papenfuss. Feldmann (1949) believed that species of this order have isomorphic life histories. Unilocular sporangia of species of this order had been unknown, and these life histories had not been proved.

The occurrence of unilocular sporangia in the Scytosiphonales was first reported by Dangeard (1963). He observed that prostrate filaments formed unilocular sporangia in culture of

Petalonia zosterifolia (Reinke) O. Kuntze. Nakamura (1965) and Tatewaki (1966) clearly demonstrated that *Scytosiphon lomentaria* (Lyngbye) Link has a heteromorphic life history in which gametophytic macrothalli and sporophytic crustose thalli are alternated. Since then, other members of this order were also proved to exhibit alternations of heteromorphic generations by Wynne (1969), Edelstein *et al.* (1970), Nakamura and Tatewaki (1975) and Clayton (1980, 1981).

Therefore, it is now generally recognized that the order Scytosiphonales can be distinguished from other orders by the characteristic of plastids as proposed by Feldmann (1949) and a heteromorphic life history pattern mentioned above (Cole 1970, Bold and Wynne 1985) on the contrary to Feldmann's belief.

The following nine genera are currently included in the order Scytosiphonales; *Chnoospora* in Chnoosporaceae, *Colpomenia*, *Hydroclathrus*, *Iyengaria*, *Rosenvingea*, *Scytosiphon*, *Petalonia*, *Endarachne* and *Jolyna* in Scytosiphonaceae. Although *Utriculidium* was included in this order by Feldmann (1949), the character of its plastids is not yet to be determined. Seven genera, excluding *Iyengaria* and *Jolyna*, are reported from Japan (Yoshida *et al.* 1990).

Since the species of the family Scytosiphonaceae are usually common occurrence in the world, they have been used as materials for cytological (Clayton and Beakes 1983, Clayton 1984,), physiological (Hsiao 1969), photomorphogenetical (Lüning and Dring 1973, Dring and Lüning 1975), and ecological (Lubchenco and Cubit 1980, Dethier 1981) studies.

However, classification in the Scytosiphonaceae has been state of flux. Although there are taxonomic studies on the genus *Colpomenia* in the Gulf of California and New Zealand by Wynne and

Norris (1976) and Parsons (1982), respectively, and studies on the classification of the genera *Colpomenia* and *Scytosiphon* in Australia by Clayton (1975, 1978, 1981), most other reports merely described species of this family as parts of floral studies. Also in Japan, no monographical studies have been undertaken.

It also should be pointed out that because of poorly illustrated, original descriptions and subsequent taxonomic studies and because of negligence of external morphological variations, the definitions of species and genera often became ambiguous, and nomenclature of some taxa has been confused. Esteeming different characters as a key taxonomic criterion by different authors has resulted establishment of several different classification systems.

Although many culture studies have revealed that this family has a heteromorphic life history, minute sporophytes in nature are poorly known except for those of *Scytosiphon lomentaria* and *Petalonia fascia*. Because of it, only characters of gametophytes have been used for the classification in this family.

Bearing the above mentioned problems in mind, this taxonomic study has been carried out to achieve the following aims: 1) to describe as many species of this family in Japan as possible in detail; 2) to conduct laboratory culture to examine every stage in life history; 3) to search sporophytes in nature to use their morphological characters for classification; 4) to show stable morphological characters which are not effected by environmental conditions; 5) to characterize species and genera by critical key characters.

Terminology

Macrothallus: Parenchymatous thallus of polystichous development. This thallus forms plurilocular sporangia as a reproductive organ.

Microthallus: Filamentous or pseudoparenchymatous thallus of haplostichous development. This thallus forms unilocular sporangia and/or plurilocular sporangia.

Parthenogenetic microthallus: Microthallus derived from unfused gamete.

Zygotic microthallus: Microthallus derived from zygote.

Plurispore: The motile cell produced by a plurilocular sporangium.

Unispore: The motile cell produced by a unilocular sporangium.

Ascocyst: Enlarged or elongated cell containing rich fucosan vesicles. Although an elongate, sterile cell among plurilocular sporangia on macrothalli in *Colpomenia* and *Scytosiphon* is generally called a paraphysis, it contains a large amount of fucosan. Therefore, the paraphysis containing fucosan is called an "ascocyst" in this study.

MATERIALS AND METHODS

Localities and collection dates of the materials for morphological and culture studies are shown in Table 1 and Fig 1. For morphological observations living materials and preserved materials in 5% formaldehyde-seawater were used.

Anatomical observations with light microscopy were carried out on living materials and liquid-preserved materials. Sections were made by hand, using stainless razor blade and pith stick. Preparations were preserved in glycerin-sea water (1:1) containing phenol after staining with methylene blue.

Cultures were started from plurispores released from plurilocular sporangia on the macrothalli. The plurispores were pipetted onto cover glasses and cultured in glass vessels containing 100 ml of PESI medium (Tatewaki 1966).

Isolation of zygotes was carried out as follows. Female and male gametes were mixed to make zygotes in a drop of medium on a cover glass. After unfused gametes and zygotes around an objective zygote were removed under an inverted microscope, it was marked with a felt-tip pen on the under surface of a dish. The thallus developed from the zygote was taken off from the substratum with tweezers and isolated with a pipet or tweezers.

Unilocular sporangia excised from microthalli collected in fields were pipetted into drops of medium on cover glasses. After releases of unispores from the unilocular sporangia, the cover glasses on which the unispores settled were transferred to culture glass vessels containing medium. Several cultures were started from fragments of microthalli by pipetting them.

GeO_2 (0.005 mg/ml) and penicillin G potassium (48 U/ml) were

used to suppress respective growth of diatom and blue green algae, when they appeared in culture. The cultures of progeny were started by isolation of swarmers or sporangium onto cover glasses.

The sets of culture conditions used were 5°C SD (short day; 8:16h light and dark cycle), 5°C LD (long day; 16:8h light and dark cycle), 10°C SD, 10°C ND (neutral day; 12:12h light and dark cycle), 10°C LD, 15°C SD, 15°C ND, 15°C LD, 20°C SD and 20°C LD, under fluorescent lighting of approximately 30-50 μmol $m^{-2}s^{-1}$.

Phenological observations were mainly carried out at Oshoro, Hokkaido. Water temperatures and daylength at Oshoro are shown in Fig. 2. Additional phenological data were obtained from other collections for this study and herbarium specimens.

Abbreviation: Culture conditions shown in Tables are indicated by abbreviated form omitted "°C" and "D". For example, 15L means 15°C LD condition. In Tables, "P" and "U" mean "bearing plurilocular sporangia" and "bearing unilocular sporangia", respectively.

Table 1. Locality numbers (No.) and localities where materials used in this study were sampled. Locality numbers are plotted on Fig. 1.

No.	Locality	No.	Locality	No.	Locality
1	Oshoro	41	Fukaura	81	Tarumi
2	Iwanai	42	Iwadate	82	karimo
3	Setana	43	Niigata	83	Maruyama
4	Okushiri	44	Shiiya	84	Ieshima
5	Esashi	45	Kashiwazaki	85	Ogishima
6	Matsumae	46	Aikawa, Sado	86	Megishima
7	Kojima	47	Ogi, Sado	87	Kitagishima
8	Shirakamimisaki	48	Notojima	88	Manabeshima
9	Yoshioka	49	Abushima	89	Awashima
10	Kikonai	50	Yabuta	90	Uoshima
11	Esan	51	Takahama	91	Ohtobishima
12	Shikabe	52	Kasumi	92	Mukaijima
13	Rebunge	53	Tomari	93	Yugeshima
14	Muroran	54	Awano	94	Ohshima
15	Harutachi	55	Taneichi	95	Ohmishima
16	Urakawa	56	Ohtsuchi	96	Nakashima
17	Samani	57	Kesennuma	97	Katayamajima
18	Utabetsu	58	Naruse	98	Himeshima
19	Erimomisaki	59	Shiogama	99	Muroto
20	Syoya	60	Syobuta	100	Susaki
21	Hiroo	61	Nagasaki	101	Ikata
22	Akkeshi	62	Inubouzaki	102	Mimaizai
23	Hanasaki	63	Jougashima	103	Tsuyazaki
24	Tomoshiri	64	Kannonzaki	104	Shingu
25	Nosappu	65	Aburatsubo	105	Fukuoka
26	Nokkamappu	66	Hayama	106	Hadonomisaki
27	Seseki	67	Zushi	107	Hirado
28	Utoro	68	Enoshima	108	Nomozaki
29	Abashiri	69	Shimoda	109	Tomioka
30	Notorimisaki	70	Omaezaki	110	Ushibuka
31	Saruru	71	Iragomisaki	111	Ohyano
32	Oumu	72	Shinmaiko	112	Aoshima
33	Menashidomari	73	Uchiumi	113	Futo
34	Souyamisaki	74	Shizaki	114	Akasaki
35	Rishiri	75	Toushitou	115	Bise
36	Teuritou	76	Sugashima	116	Minnashima
37	Ofuyu	77	Nakiri	117	Sesoko
38	Ohma	78	Shingu	118	Henoko
39	Sai	79	Koza		
40	Tappizaki	80	Shirahama		

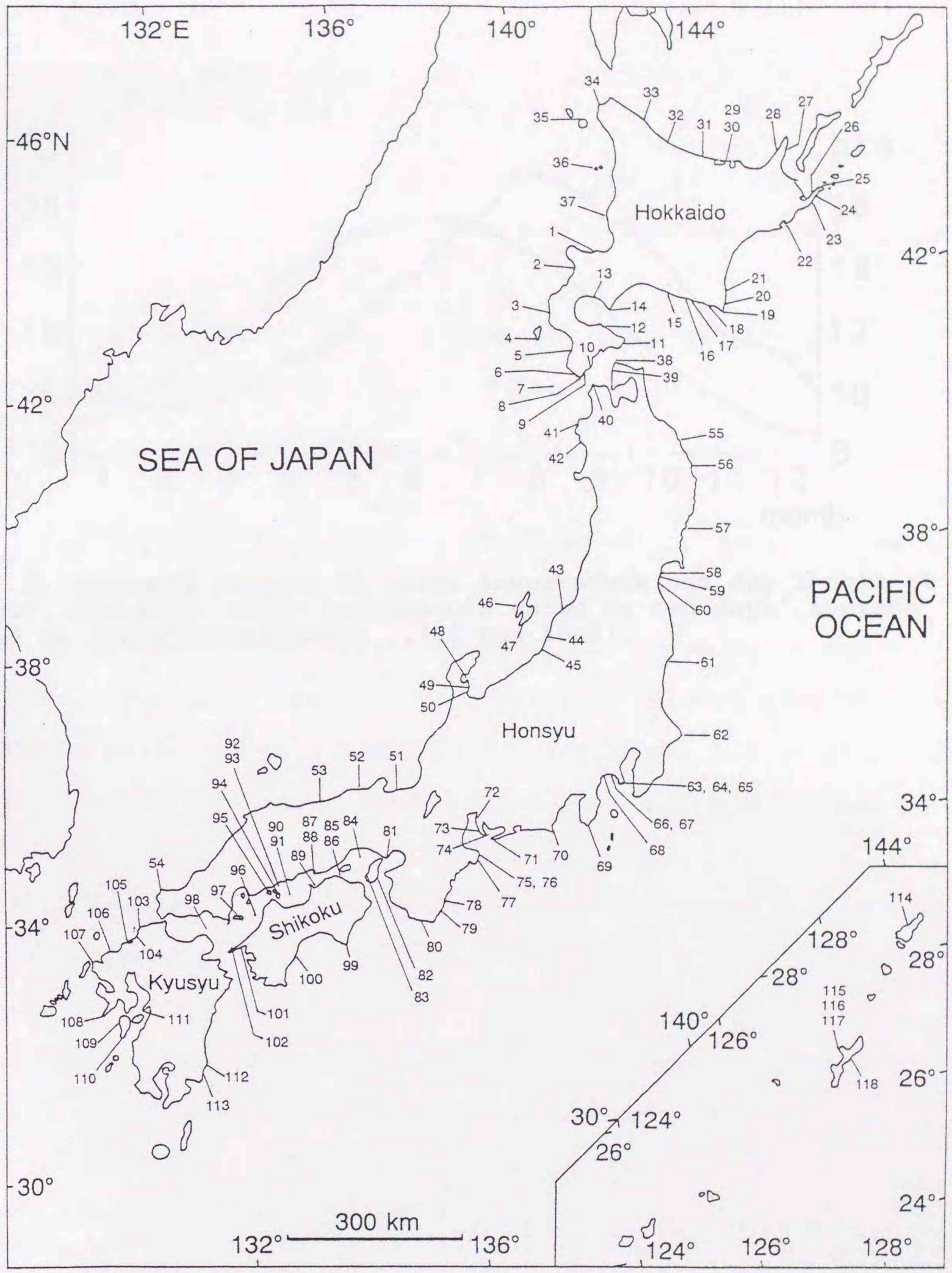


Fig. 1. Outline map of Japan and adjacent region showing localities where materials used in this study were collected. Numbers plotted correspond to those in Table 1.

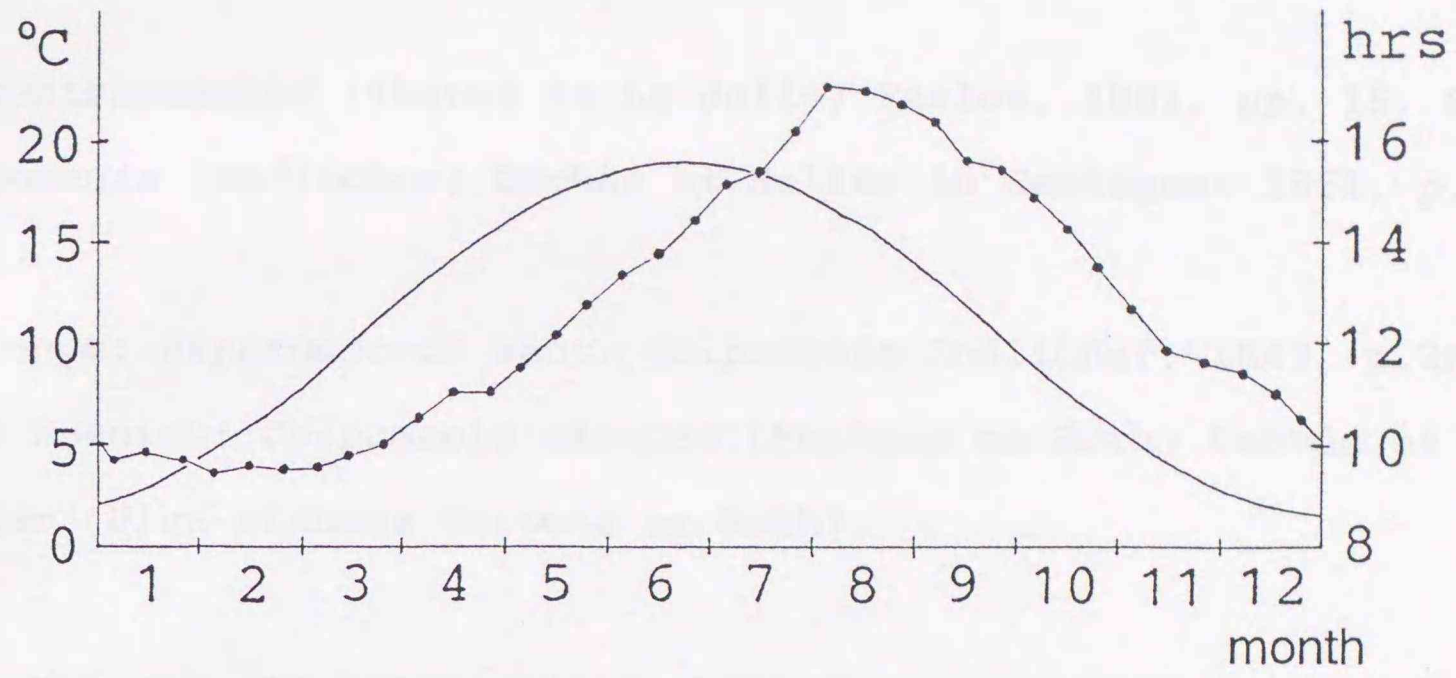


Fig. 2. Seasonal changes in water temperature and day length at Oshoro, Hokkaido. Water temperature shown is ten days' average based on daily measurements (1988 and 1989).

RESULTS AND DISCUSSION ON EACH SPECIES

SCYTOSIPHONACEAE (Thuret in Le Jolis) Farlow, 1881, pp. 15, 62.

***Colpomenia* (Endlicher) Derbès et Solier in Castagne, 1851, p.95.**

Basionym: *Asperococcus* sect. *Colpomenia* Endlicher, 1843, p.26.

Type species: *Colpomenia sinuosa* (Mertens ex Roth) Derbès et Solier (*Ulva sinuosa* Mertens ex Roth).

Macrothalli are globular, bullate or cylindrical, simple or branched, membranaceous, hollow. They arise from a small saccate base. Initials of macrothalli are spherical in shape. These thalli consist of a pigmented cortical layer and a colorless medullary layer. Hairs are scattered or grouped. Macrothalli attach to substrata with rhizoids arising from under surface cells. Plurilocular sporangia are formed acropetally on the surface of thalli, in sori or not in sori. Ascocysts are present among plurilocular sporangia.

Seven species of *Colpomenia; C. bullosa* (Saunders) Yamada (1948), *C. ecuticulata* Parsons (1982), *C. peregrina* (Sauvageau) Hamel (1931), *C. phaeodactyla* Wynne et Norris (1976), *C. ramosa* Taylor (1945), *C. sinuosa* (Roth) Derbès et Solier (1856) and *C. tuberculata* Saunders (1898), are distributed mainly in the Pacific area. Other species, *C. mollis* Taylor (1945) from Galapagos Islands and *C. nainativensis* Durairatnam (1962) from Ceylon, were described. Wynne and Norris (1976) suggested that *Iyengaria* should be merged with *Colpomenia*, and that *C. stellata* (Børg.) Børgesen (1930) is reinstated.

Colpomenia sinuosa (Mertens ex Roth) Derbès et Solier in Castagne

Derbès et Solier in Castagne, 1851, p. 95; Derbès et Solier, 1856, p.11, pl.22, figs 18-20; Clayton 1975, p.187-195, figs 5, 6, 7, 12, 13; Parsons 1982, p.289-301, figs 2, 3; Wynne and Norris 1976, p.1-18, figs 1, 2, 3, 11-a; Womersley 1987, p.297, figs 107A, 108E,F.

Basionym: *Ulva sinuosa* Mertens ex Roth, 1806, p.327, pl.12a-c.

Synonym: *Asperococcus sinuosus* (Mertens ex Roth) Bory 1832, p.326.

Type Locality: Cadiz, Spain.

Type : Location uncertain (see Clayton 1975, p.189; Parsons 1982, p.293).

Japanese name: Fukuronori

Materials

Materials used for morphological and culture studies are as follows. Localities are shown in Fig. 3. The numbers in square brackets after localities below correspond to those in Fig. 3.

Niigata Pref. : Aikawa, Sado [46] (7-vii-1990 leg. T. Kitayama), Toyama Pref. : Abugashima [49] (12-vii-1991, leg. T. Yoshida), Shizuoka Pref. : Susaki, Shimoda [69] (14-vi-1990, leg. T. Kitayama), Hyogo Pref. : Kasumi [52] (viii-1990 leg. S. Enomoto and T. Kitayama, 3-vi-1991 leg. K. Kogame), Karimo [82] (31-iii-1990 leg. S. Enomoto & K. Kogame), Matsushima, Ieshima Isls [84] (27-x-1989 leg. S. Enomoto & K. Kogame), Ehime Pref. : Uoshima [90] (8-v-1990 leg. S. Enomoto & K. Kogame), Yugeshima [93] (11-v-1990 leg. S. Enomoto & K. Kogame), Fukuoka Pref. : Tsuyazaki [103] (3-v-1989, leg. S. Kawaguchi), Okinawa Pref. : Sesoko [117] (8-iii-1990 leg. K. Kogame), Henoko [118] (9-iii-1990 leg. T. Yoshida).

Herbarium specimens examined are as follows. Kanagawa Pref. : Hayama [66] (6-v-1985, leg. J. Tanaka, SAP 054646), Aburatsubo

[65] (viii-1923, leg. T. Yoshida, SAP 021296), Mie Pref. : Sugashima [76] (21-iv-1955, leg. Y. Tsuji), Kochi Pref. : Konoura, Susaki [100] (19-iii-1957, leg. N. Tazawa), Miyazaki Pref. : Futo [113] (25-v-1982, leg. T. Yoshida, SAP 043852).

Morphology

Macrothalli are spherical or saccate, hollow, frequently folded or irregular in shape, up to 10-20 cm in diameter, somewhat firm in texture, yellowish brown to brown in color (Figs 4A-D). In section, thalli consist of a cortical layer and a medullary layer, being 400-1100 µm in thickness (Fig. 5A). Cortical layers consist of 1-3 layers of small pigmented cells (Figs 5B, 6A). Medullary layers consist of 3-4 layers of large colorless cells. The medullary cells are spherical to ellipsoid, and 10-350 × 30-350 µm in size (Fig. 5A). Hairs are tuft, arising from the cortical layer when young or from a deep pit when mature (Figs 5D, 6C). Thalli attach to the substratum with rhizoidal filaments produced on surface cells (Fig. 5I). They become mature acropetally forming plurilocular sporangia. Plurilocular sporangia form in punctate sori around tufts of hairs, up to 600 µm in diameter (Figs 5C,D, 6C). They are uniseriate or partly biseriate, loosely coherent with each other and 35-50 µm in length (Figs 5G, 6C). Unicellular ascocysts are present among plurilocular sporangia (Figs 5E-H, 6B,C). A cuticle layer on plurilocular sporangia is obvious (Figs 5E,F, 6C). The cuticle layer ruptures when mature (Figs 5F, 6C). Ascocysts remain after release of plurispores. Secondary plurilocular sporangia are common (Figs 5H, 6B). Unilocular sporangia are unknown on erect, saccate thalli.

Habitat and Phenology

Macrothalli usually grow on rocks or sometimes on other algae in the lower intertidal to subtidal zone. Fertile thalli are usually found during spring and summer.

Geographical distribution

This species is distributed in warm temperate and tropical coasts in all around the world (California, Wynne et Norris 1976; Australia, Clayton 1975; India, Børgesen 1935; France, Hamel 1931-39; Japan, present study).

Culture study

Collection data of cultured strains of this species are shown in Table 2.

In culture of certain strains, plurispores from macrothalli developed into microthalli which formed plurilocular or unilocular sporangia, although they rarely developed into macrothalli. Culture conditions of these strains are shown in Table 2 (28 Sesoko, 310 Yuge, 335 Kasumi, 358 Kasumi). No sexual reproduction could be observed.

Observations on strain 335 Kasumi are mentioned below.

Development of plurispores from macrothalli

Plurispores are of the normal phaeophycean type, pear drop-shaped, with a longer anterior and a shorter posterior flagellum, containing a plastid and a stigma, 6.0-7.2 × 3.8-4.8 μm in size (Fig. 7A). Plurispores from macrothalli were cultured at 5-20°C under both LD and SD conditions. Settled plurispores were round, 4.0-4.6 μm in diameter. They germinated without leaving an empty cell. The germlings were prostrate, branched filaments at first,

and grew into pseudodiscoid or tufted microthalli (Figs 7B-D) except for 5°C conditions. Plurispores germinated but did not grow more than a few cell-stage under 5°C conditions. The pseudodiscoid thalli consisted of prostrate filaments and uniseriate upright filaments (Figs 7E-H). Prostrate filaments of these thalli did not grow in a regularly radial manner. The microthalli grew into 1-3 mm in diameter after 3 months, and became fertile at 10-20°C both under LD and SD conditions. They produced plurilocular sporangia under long day conditions (10-20°C) (Fig. 7F), unilocular sporangia under 10°C SD and 15°C SD (Figs 7G,H), and both type of sporangia under 20°C SD. The plurilocular sporangia were ectocarpoid, pedicellate or sessile, on prostrate filaments or terminally on erect filaments (Figs 7G,H). Unilocular sporangia were ovoid or elongate ovoid, terminal on erect filaments. Tufted microthalli also formed plurilocular or unilocular sporangia on the branched filaments under the same conditions as pseudodiscoid thalli produced them. Microthalli bore unicellular ascocysts terminally on upright filaments (Figs 7E-H).

Development of plurispores from cultured microthalli

Plurispores from plurilocular sporangia borne on microthalli under a 20°C LD condition were cultured at 5-20°C under both SD and LD conditions (Table 3). These plurispores were morphologically the same as those from macrothalli, 6.4-8.0 × 3.6-4.4 µm in size (Fig. 8A). Settled plurispores were rounded, 3.8-5.0 µm in diameter. They germinated and developed into pseudodiscoid or tufted microthalli as the same manner with the germlings of plurispores from the macrothalli (Figs 8B-E). These microthalli

formed plurilocular sporangia under long day conditions (10-20°C) (Figs 8F,G), while they produced unilocular sporangia under 10°C SD and 15°C SD (Fig. 8H), and both sporangia under 20°C SD similar to those derived from plurispores from macrothalli. They also formed ascocysts (Fig. 8H). These fertile microthalli were also morphologically identical with those derived from plurispores from macrothalli. Germlings from plurispores from microthalli scarcely grew under 5°C conditions. When the three-month-old germlings kept under a 5°C LD condition were transferred to a 15°C LD condition, they grew into microthalli which formed plurilocular sporangia.

Development of unispores from cultured microthalli

Unispores from unilocular sporangia formed on microthalli under 15°C SD were cultured at 5-20°C under both LD and SD (Table 4). Unispores were of normal phaeophycean type, containing a plastid and a stigma, 5.8-6.6 x 3.6-4.2 µm in size. Settled unispores were rounded, 4.2-5.0 µm in diameter. They germinated without leaving a empty cell and grew into small disks (Figs 8J-L). These disks became swollen, and developed into spherical thalli under a 15°C LD condition (Fig. 8M). The prostrate systems did not grow further after forming spherical thalli. At 10-20°C under both LD and SD except 15°C LD, although spherical thalli grew better than prostrate systems, the prostrate systems became 3-5 mm in diameter after 2 months, and formed further several spherical thalli. After 2 months under a 20°C LD condition, the spherical thalli grew up to 1-1.5 cm in diameter, and became fertile with plurilocular sporangia. Plurilocular sporangia formed punctate sori around hair pit. Unicellular ascocysts were

observed among plurilocular sporangia. No reproductive organs were observed on the prostrate systems. Germlings from unispores did not grow under 5°C conditions.

Direct type life history

In some strains, plurispores from macrothalli developed into the identical macrothalli. Culture conditions of these strains are shown in Table 2 (235 Tsuyazaki, 250 Matsushima, 324 Susaki, 357 Kasumi).

In the strain 235 Tsuyazaki, plurispores from macrothalli were cultured at 5-20°C under both LD and SD conditions. Plurispores (Fig. 9A) germinated into prostrate branched filaments, and grew into small, pseudodiscoid, prostrate systems less than 0.5 mm in diameter (Fig. 9B). They became swollen, and developed into spherical macrothalli directly (Figs 9C-F). Under 15°C LD and 20°C LD, prostrate systems did not spread further after macrothalli developed. Under 15°C SD and 20°C SD, prostrate systems grew to more than 2-3 mm in diameter, bearing several macrothalli. Macrothalli grew over 10 mm in diameter, and became fertile with plurilocular sporangia under 15°C and 20°C conditions. The plurilocular sporangia were more abundantly produced at 20°C than 15°C. No reproductive organs were observed on the prostrate systems.

Discussion

Colpomenia sinuosa resembles to *C. peregrina* (Sauvageau) Hamel in external morphology. According to Sauvageau (1927) and Blackler (1964, 1967), *C. sinuosa* differed from *C. peregrina* by

thicker and folded thalli, yellowish brown color when dried, the deep origin of the hair, the position of the sori of plurilocular sporangia, presence of a cuticle layer over the sori. While, Clayton (1975) suggested that *C. sinuosa* and *C. peregrina* might be distinguished only on the basis of the shape of the sori and the presence of a cuticle layer on the plurilocular sporangia. *C. sinuosa* bears rounded, plurilocular sporangial soli with a cuticle layer over them, while *C. peregrina* forms irregular, plurilocular sporangial sori without a cuticle. She also showed the evidence that these species could be distinguished with the number of layers of colorless medullary cells in the thallus, using the ANOVA. Also in this study it was concluded that features of plurilocular sporangia were important to distinguish these species. The results of present morphological observations on *C. sinuosa* were in accordance with those of Sauvageau (1927), Blackler (1964, 1967), Parsons (1982), Wynne and Norris (1967) and Womersley (1987).

There are a few culture reports of *C. sinuosa*. Kunieda and Suto (1938) observed a sexual reproduction of *C. sinuosa* and reported that it represents anisogamy. However, their observations on the shape of plurilocular sporangia and size of gametes are correspond with those *C. peregrina* as revealed by this study. Materials used by Kunieda and Suto (1938) were highly probable to be *C. peregrina*. Blackler (1981) reported that plurispores from plurilocular sporangia on spherical thalli of *C. sinuosa* directly developed into spherical thalli in culture, and microthalli did not form any sporangia. Clayton (1982) also reported the same result in her culture study of *C. sinuosa* from Australia.

In this study, a life history which involved microthalli forming plurilocular and unilocular sporangia was observed although sexual reproduction was not found. A diagram of life history of this species in my culture is shown in Fig. 10. Microthalli formed plurilocular sporangia under long day conditions (10-20°C), unilocular sporangia under 10°C SD and 15°C SD, and both sporangia in 20°C SD. These results reveal that sporangium production on the microthalli is influenced by photoperiod and temperature. Plurispores from the microthallus play a role of propagating microthalli, since they develop into microthalli in all conditions tested whenever they grew. On the other hand, unispores from microthalli give rise to spherical macrothalli. Prostrate systems developing from unispores grew further under conditions except of 15°C LD, even after forming spherical macrothalli. However, these prostrate systems are different from those derived from plurispores in that they did not form any sporangia but spherical thalli. It is considered that prostrate systems from unispores have a property to develop into spherical thalli. These results are similar to those of *C. peregrina* which will be mentioned later.

Although phenological observations were not carried out in this study, based on the culture studies, it is inferred that in fields, plurispores develop into microthalli which form plurilocular sporangia in summer to propagate, while during autumn and winter they produce unilocular sporangia to form spherical thalli which are expected to appear in winter and spring.

In the culture of the strain Tsuyazaki, plurispores from plurilocular sporangia on spherical thalli developed into

spherical thalli directly, forming no sporangia on prostrate systems. This result agrees with those of Blackler (1981) and Clayton (1982) mentioned above. In the field populations which possess such a direct type life history, it seems that spherical thalli repeat the direct type of reproduction under their favorite conditions, arising from prostrate systems.

Morphology of microthalli of *C. sinuosa* resembles to those of *C. peregrina* in culture. However, microthalli of *C. sinuosa* represented pseudodiscoid, and the distal tips of prostrate filaments did not coherently grow and the edge of the thalli was not smooth. It is possible that the latter irregularity was due to unfavorable culture conditions for morphogenesis of microthalli of *C. sinuosa*. Therefore, it is necessary to observe the microthalli in nature. The microthalli are expected to resemble to those of *C. peregrina* also in nature.

It is apparent that *C. sinuosa* is found in warmer waters (Blackler 1964). Also in Japan, *C. sinuosa* is distributed along the coasts of central Honsyu and southern area. The result that plurispores and unispores did not grow in 5°C conditions in culture may support the reason why this species is not distributed in cooler temperate waters.

Table 2. Collection data of cultured strains of *Colpomenia sinuosa*. Culture conditions attempted and Progeny of plurispores from macrothalli are also shown.

strain no.	locality	date	culture conditions	progeny
235	Tsuyazaki	3 v 1989	5S, 5L	not grow
			10SL	macrothalli
			15S, 20S	pseudodisks bearing macrothalli (P)
			15L, 20L	macrothalli (P)
250	Matsushima	27 x 1989	15L	macrothalli (P)
281	Sesoko	8 iii 1990	20S	pseudodisks (U, P)
			20L	pseudodisks (P), macrothalli (P)
310	Yuge	11 v 1990	15L	pseudodisks (P), a few macrothalli (P)
			20L	pseudodisks (P)
			20S	pseudodisks (U)
324	Susaki	14 vi 1990	15L	macrothalli (P)
			20L	macrothalli (P)
335	Kasumi	viii 1990	5SL	not grow
			10S, 15S	pseudodisks (U)
			10-20L, 20S	pseudodisks (P)
357	Kasumi	3 vi 1991	15L	macrothalli
358	Kasumi	3 vi 1991	15L	pseudodisks (P)

Table 3. Culture of *Colpomenia sinuosa*. Progeny of plurispores from microthalli.

strain no.	locality	date	culture conditions	progeny
335	Kasumi	viii 1990	5SL	not grow
			10S, 15S	pseudodisks (U)
			10-20L	pseudodisks (P)
			20S	pseudodisks (U, P)

Table 4. Culture of *Colpomenia sinuosa*. Progeny of unispores from microthalli.

strain no.	locality	date	culture conditions	progeny
335	Kasumi	viii 1990	5SL	not grow
			10L, 15L	macrothalli
			10-20S	pseudodisks bearing macrothalli
			20L	pseudodisks bearing macrothalli (P)

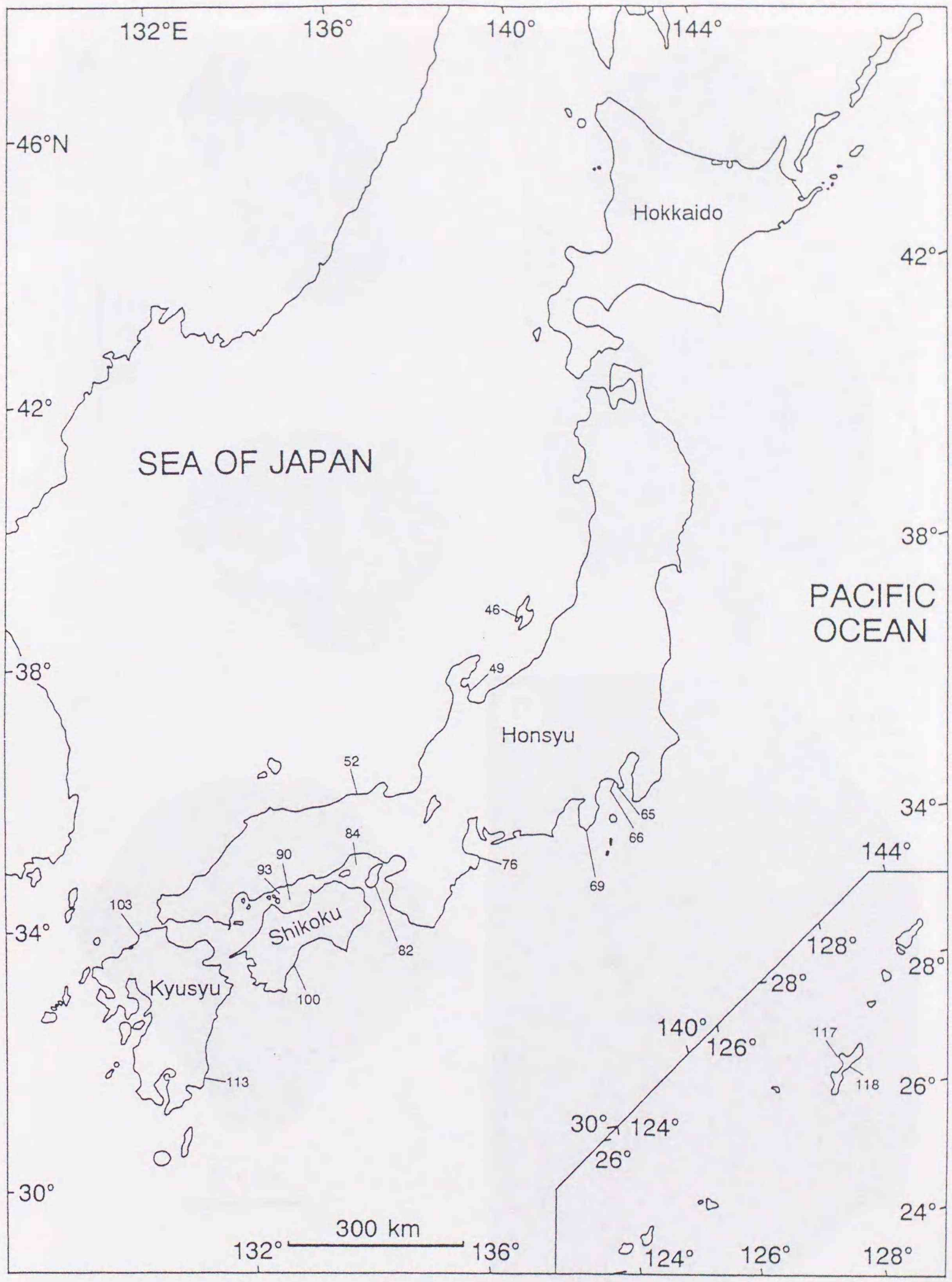


Fig. 3. Localities where materials of *Colpomenia sinuosa* were collected. Numbers plotted correspond to those in Table 1.

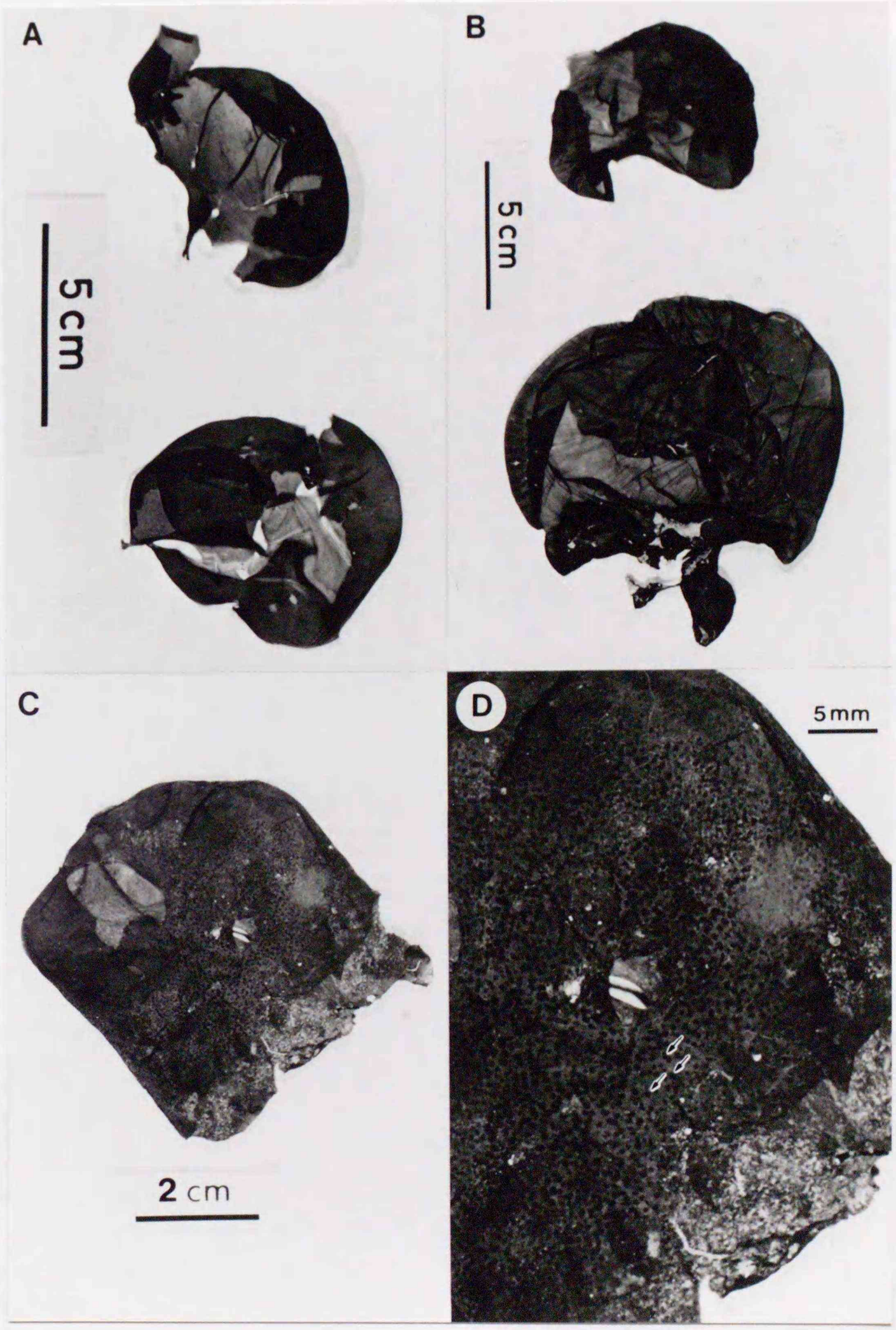


Fig. 4. Habit of dried herbarium specimens of *Colpomenia sinuosa*.

A. Collected at Kasumi, Hyogo Pref. on 3 June 1991. **B.** Collected at Henoko, Okinawa Pref. on 9 March 1990. **C.** Collected at Yugeshima, Ehime Pref. on 11 May 1990. **D.** Sori of plurilocular sporangia on a thallus surface (arrows).

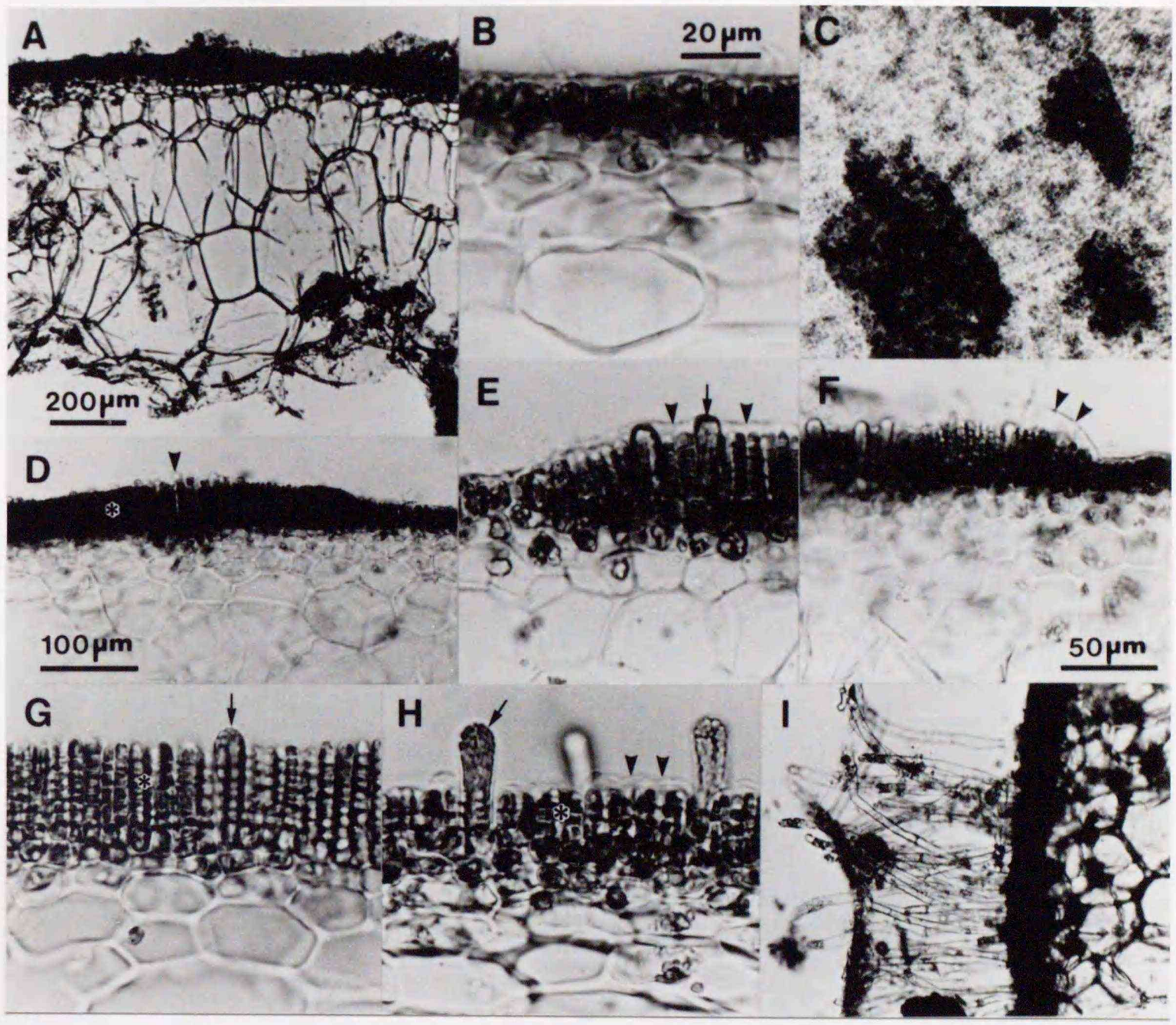


Fig. 5. Anatomy of *Colpomenia sinuosa*.

A. Section of thallus. **B.** Cortical cells of vegetative thallus in section. **C.** Plurilocular sporangial sori (dark portion stained with methylene blue) in surface view. **D.** Hair tufts (arrowhead) in plurilocular sporangial sori (asterisk) in section. **E, F.** Plurilocular sporangia and paraphyses (arrow) with a cuticular covering (arrowheads). **G.** Plurilocular sporangia (asterisk) and paraphyses (arrow) without a cuticular covering. **H.** Secondary plurilocular sporangia (asterisk) with a cuticular covering (arrowheads), and paraphyses (arrow). **I.** Section of thallus through a part with rhizoids for attachment to substratum.

Scale in **A** applies to **C**; Scale in **B** applies to **E**, **G** and **H**; Scale in **D** applies to **I**.

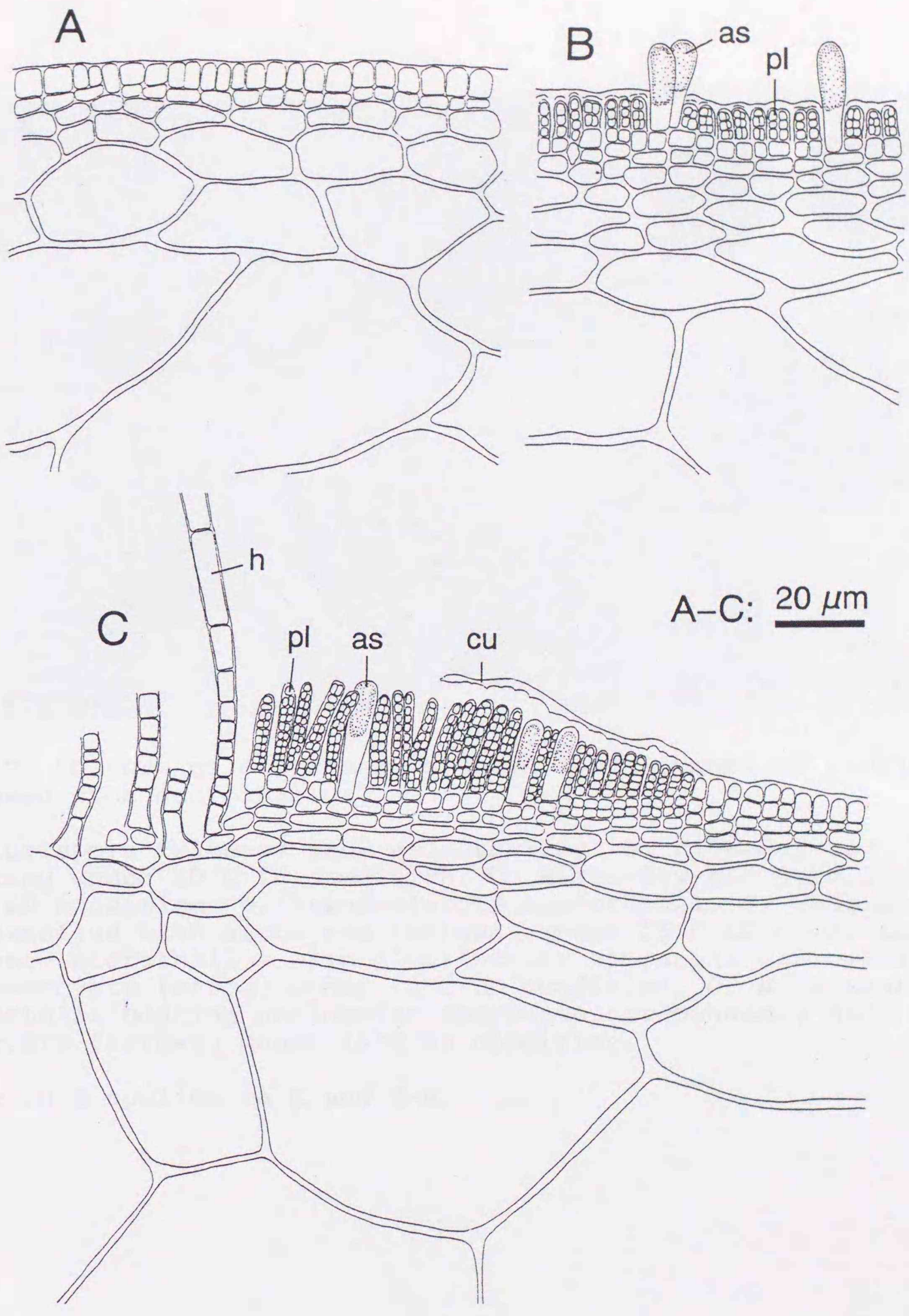


Fig. 6. Anatomy of *Colpomenia sinuosa*.

A. Section of vegetative thallus. **B.** Secondary plurilocular sporangia in section of thallus. **C.** Plurilocular sporangia and hair tuft in section of thallus.

as: ascocyst, cu: cuticle, h: phaeophycean hair, pl: plurilocular sporangia.

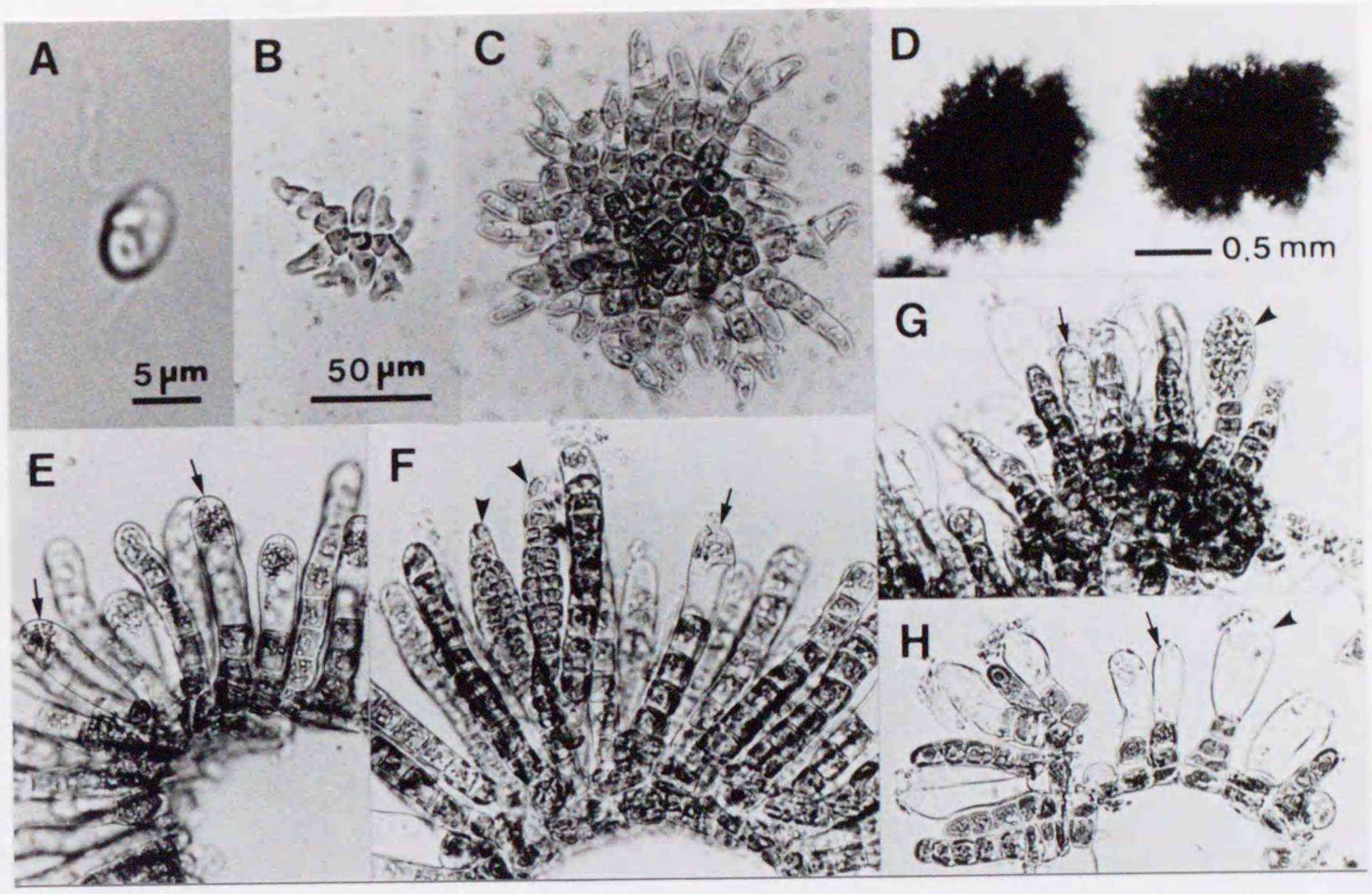


Fig. 7. Culture of *Colpomenia sinuosa*. Development of plurispores released from macrothallus.

A. Plurispore released from macrothallus. **B.** Five-day-old germling under 20°C LD condition. **C.** Eight-day-old germling under 20°C LD condition. **D.** Pseudodiscoid microthalli. **E.** Squashed microthallus with ascocysts (arrows) under 15°C LD condition. **F.** Squashed microthallus with plurilocular sporangia (arrowheads) and ascocysts (arrow) under 15°C LD condition. **G, H.** Squashed microthalli bearing unilocular sporangia (arrowheads) and ascocysts (arrows) under 15°C SD condition.

Scale in **B** applies to **C** and **E-H**.

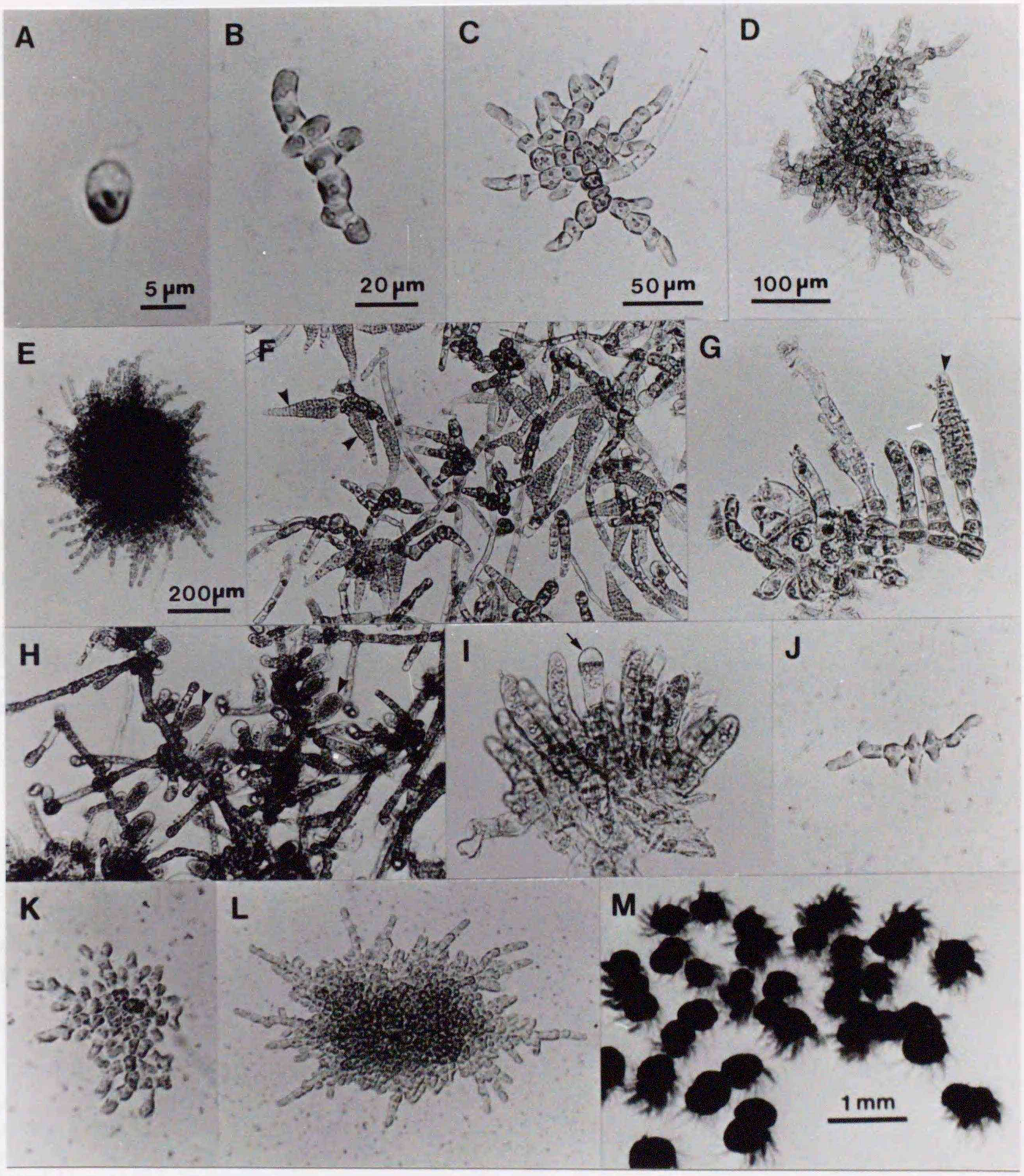


Fig. 8. Culture of *Colpomenia sinuosa*.

⇒

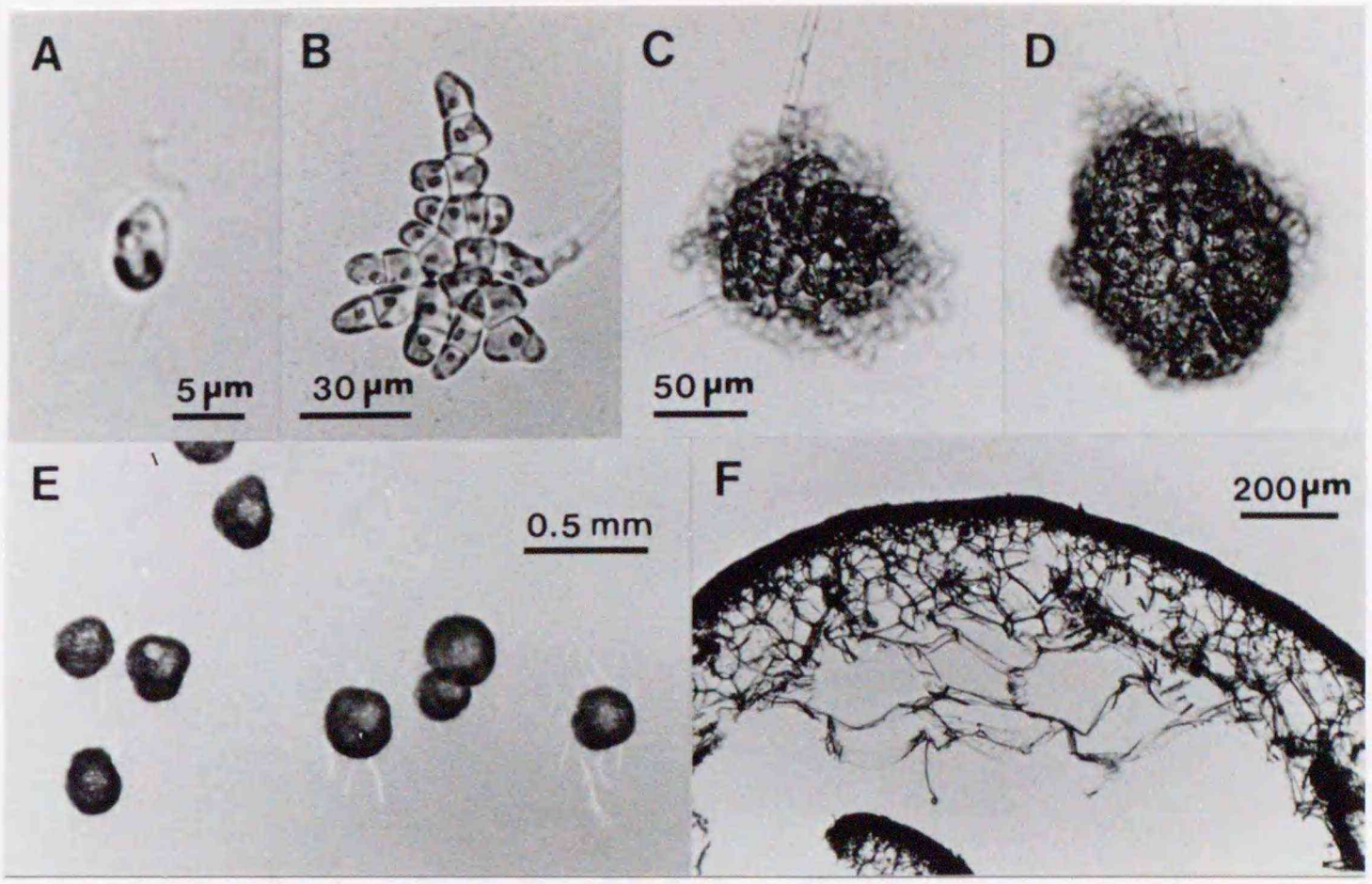


Fig. 9. Culture of *Colpomenia sinuosa* (strain 235 Tsuyazaki) which represents a direct-type life cycle. Development of plurispores released from plurilocular sporangia on macrothalli.

A. Plurispore from macrothallus. **B.** Eleven-day-old germling under 15°C LD condition. **C, D.** Sixteen-day-old germling under 15°C LD condition, rising in central part. **E.** Small, spherical macrothalli under 15°C LD condition. **F.** Section of macrothallus.

Scale in **C** applies to **D.**

⇐

Fig. 8. Culture of *Colpomenia sinuosa*. Development of plurispores released from plurilocular sporangia on microthallus (**A-I**) and development of unispores released from unilocular sporangia on microthallus (**J-M**).

A. Plurispore from microthallus. **B.** Four-day-old germling under 20°C LD condition. **C.** Seven-day-old germling under 20°C LD condition. **D.** Twelve-day-old germling under 20°C LD condition. **E.** Pseudodiscoid microthallus. **F, G.** Squashed microthalli forming plurilocular sporangia (arrowheads) under 20°C LD condition. **H.** Squashed microthallus forming unilocular sporangia (arrowheads) under 15°C LD condition. **I.** Squashed microthallus with an ascocyst (arrow). **J.** Eight-day-old germling under 15°C SD condition. **K.** Twelve-day-old germling under 15°C SD condition. **L.** Eighteen-day-old germling under 15°C SD condition. **M.** Small spherical macrothalli arising on pseudodisks.

Scale in **C** applies to **G, I, J** and **K**; Scale in **D** applies to **F, H** and **L.**

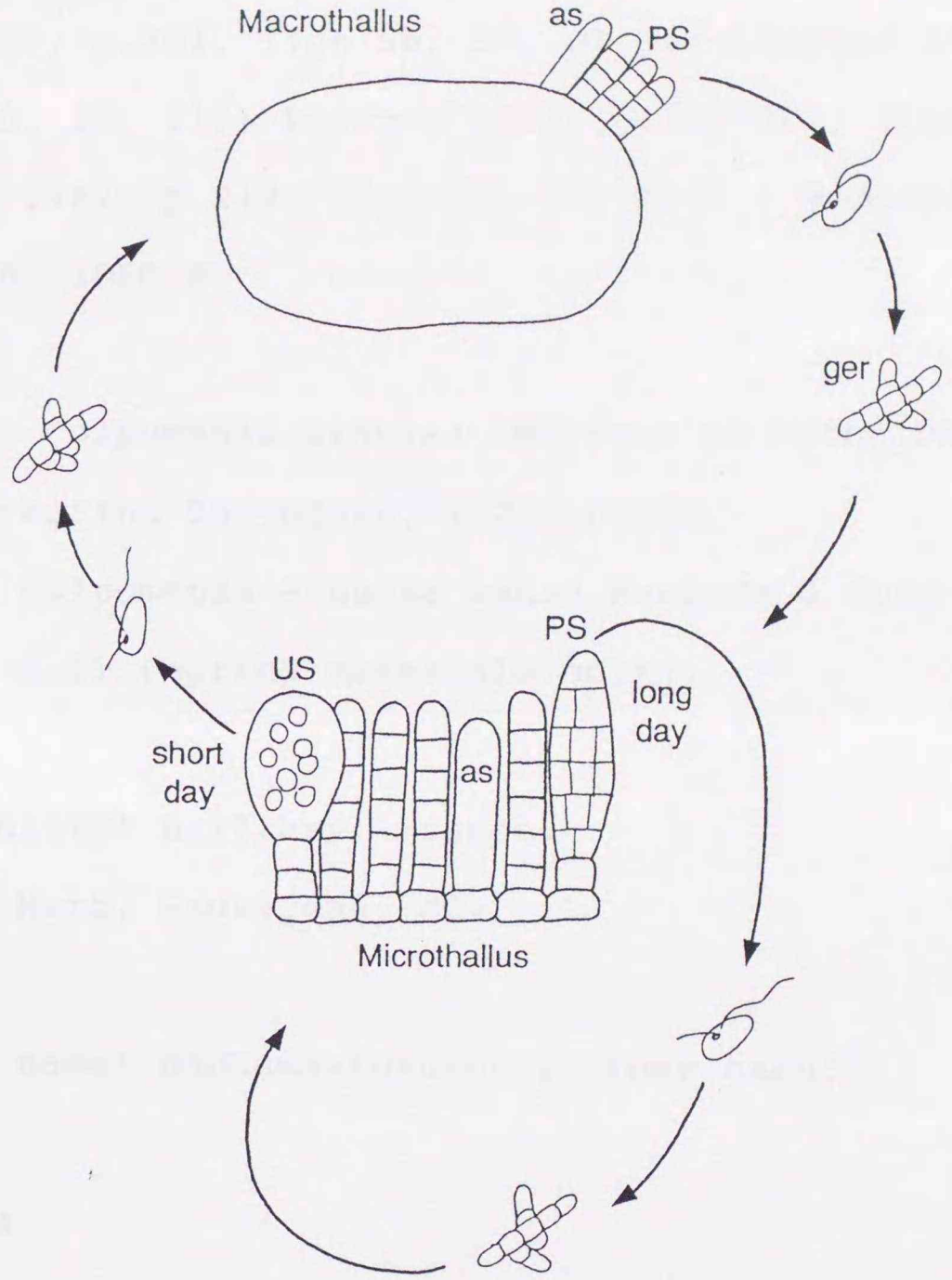


Fig. 10. Diagram of life history of *Colpomenia sinuosa* in culture.

as; ascocyst, ger; germling, PS; plurilocular sporangium, US; unilocular sporangium.

Colpomenia peregrina (Sauvageau) Hamel

Hamel 1937, p.201, figs 56, 57, pl.7.; Clayton 1975, p.187-195, figs 8, 9, 10, 11.; Parsons 1982, p.289-301, figs 6, 7, 13.; Fletcher 1987, p.219, figs 56, 57, Pl.7.; Womersley 1987, p.298, figs 107B, 108G,H.

Basionym: *Colpomenia sinuosa* (Mertens ex Roth) Derbès et Solier var. *peregrina* Sauvageau, 1927, p.321.

Synonym: *Colpomenia sinuosa* sensu Kunieda & Suto 1938, p.539-546, Fig. II, 1-11 (spring materials only).

Type locality: Brittary, France.

Type: in Herb. Sauvageau, PC.

Japanese name: Usukawa-fukuronori (new name)

Materials

Materials used for morphological and culture studies are as follows. Localities are shown in Fig. 11. The numbers in square brackets after each locality correspond to those in Fig. 11.

Macrothalli: Hokkaido: Oshoro [1] (11-iii-1987 leg. K. Kogame, 8-v-1987 leg. K. Kogame, 9-vi-1987 leg. K. Kogame, 21-vi-1987 leg. K. Kogame, 9-xii-1987 leg. K. Kogame, 21-i-1988 leg. K. Kogame, 17-ii-1988 leg. K. Kogame, 18-iii-1988 leg. K. Kogame, 14-v-1988 leg. M. Matsumoto, 24-v-1988 leg. K. Kogame, 8-vi-1988 leg. K. Kogame, 10-iii-1989 leg. K. Kogame, 22-vi-1989 leg. K. Kogame, 2-xii-1989 leg. K. Kogame, 28-ii-1990 leg. K. Kogame, 23-iii-1990 leg. K. Kogame, 17-iv-1990 leg. K. Kogame, 4-vi-1990 leg. K. Kogame, 24-xi-1990 leg. K. Kogame, 15-ii-1991 leg. K. Kogame, 17-iii-1992 leg. K. Kogame, 26-iv-1992 leg. K. Kogame, 15-v-1992 leg. K. Kogame), Setana [3] (18-iii-1987 leg. K. Kogame), Matsumae [6] (10-i-1989 leg. I. Mine), Shirakamimisaki [8] (19-iii-1987 leg. K. Kogame, 16-v-1988 leg. K. Kogame), Yoshioka [9] (16-v-1988 leg. K. Kogame), Kikonai [10] (18-iii-1987 leg. K. Kogame), Muroran [14] (18-iv-1987 leg. K. Kogame, 4-ii-1988 leg. K. Kogame, 12-xii-1988 leg. K. Kogame, 26-v-1990

leg. H. Kawai, 25-v-1993 leg. K. Kogame), Akkeshi [22] (14-vii-1988 leg. H. Kawai, 26-vi-1991 leg. K. Kogame, 29-vi-1991 leg. K. Kogame), Hanasaki [23] (7-ix-1987 leg. K. Kogame, 22-xii-1987 leg. K. Kogame), Aomori Pref. : Ohma [38] (3-iii-1987 leg. K. Kogame, 21-ii-1988 leg. H. Kawai, 2-ii-1990 leg. K. Kogame, 29-iv-1990 leg. K. Kogame), Niigata Pref. : Kashiwazaki [45] (27-iii-1989 leg. K. Kogame), Kanagawa Pref. : Aburatsubo [65] (2-xii-1987 (drifted) leg. T. Yoshida), Shizuoka Pref. : Susaki, Shimoda [69] (14-vi-1990 leg. T. Kitayama), Hyogo Pref. : Karimo, Awaji Isl. [82] (31-iii-1990 leg. S. Enomoto & K. Kogame), Matsushima, Ieshima Isls [84] (27-x-1989 leg. S. Enomoto & K. Kogame), Okayama Pref. : Kitagishima [87] (4-iv-1989 leg. S. Enomoto & K. Kogame), Hiroshima Pref. : Mukaijima [92] (28-x-1987 leg. S. Enomoto & T. Yoshida), Kagawa Pref. : Ogishima [85] (25-x-1989 leg. S. Enomoto & K. Kogame), Awashima [89] (5-iv-1989 leg. S. Enomoto & K. Kogame), Ehime Pref. : Nakashima [96] (16-iv-1988 leg. S. Enomoto & K. Kogame), Oomishima [95] (12-v-1990 leg. S. Enomoto & K. Kogame), Fukuoka Pref. : Tsuyazaki [103] (21-iv-1989 leg. S. Kawaguchi).

Microthalli: Hokkaido: Oshoro [1] (25-x-1988 leg. K. Kogame, 22-vi-1989 leg. K. Kogame, 12-viii-1989 leg. K. Kogame, 23-ix-1989 leg. K. Kogame, 4-xi-1989 leg. K. Kogame, 17-iv-1990 leg. K. Kogame, 21-v-1990 leg. K. Kogame, 4-vi-1990 leg. K. Kogame, 28-vii-1990 leg. K. Kogame, 13-ix-1990 leg. K. Kogame, 24-x-1990 leg. K. Kogame).

Herbarium specimens examined are follows. Miyagi Pref. : Shiogama [59] (23-vi-1952, leg. M. Kurogi), Fukushima Pref. : Nagasaki [61] (SAP 035852, 14-vii-1981, leg. T. Yoshida), Ishikawa Pref. : Notojima [48] (SAP 047928, 12-ii-1986, leg. K. Ikehara), Aichi Pref. : Shinmaiko [72] (SAP 024889, 11-iv-1944, leg. N. Segi), Mie Pref. : Sugashima [76] (21-iv-1955, leg. Y. Tsuji), Fukui Pref. : Takahama [51] (SAP 051029, 1-iv-1987, leg. K. Ikehara),

Morphology

Macrothalli

Macrothalli are spherical, saccate, membranous, hollow, frequently irregular in shape, 5-20 cm in diameter (Figs 12A,B). They are yellowish brown to brown in color, sometimes greenish in drying, usually delicate in texture. Young thalli less than 1 mm in diameter are still solid (Fig. 13H). In section, thalli consist of a cortical layer and a medullary layer, 160-480 µm in thickness (Figs 13A,B, 14A). The cortical layer consists of 1-3 layers of pigmented cells (Figs 13C, 14A). The medullary layer consists of 3-4(5) layers of cells which are colorless, large,

spherical to ellipsoid, 20-160 × 30-200 µm in size (Figs 13A,B). Hairs are tufted, arising from cortical layer or pits (Fig. 13B). They are attached to the substratum with rhizoidal filaments produced from under surface cells (Fig. 13G). Thalli become fertile acropetally, forming plurilocular sporangia on the surface. Plurilocular sporangia originate from surface cells (Figs 13D, 14B). Plurilocular sporangial sori are extended over the thallus surface without forming a punctate patch. Mature plurilocular sporangia are laterally coherent loosely each other, without a cuticle layer over them (Figs 13E,F, 14C,D). Larger plurilocular sporangia (female gametangia) are uniseriate or biseriate, 26-40 µm in length (Figs 13E, 14C). Smaller plurilocular sporangia (male gametangia) are 2-4 seriate, colorless, 30-36 µm in length (Figs 13F, 14D). Unicellular ascocysts are present among plurilocular sporangia (Figs 13D-F, 14B-D). Ascocysts remain intact after release of plurispores. Secondary plurilocular sporangia are commonly observed. No unilocular sporangia are known on macrothalli.

Microthalli

Microthalli are discoid, 1-3 mm in diameter (Fig. 15A). They are pseudoparenchymatous, consisting of prostrate filaments and erect filaments (Figs 15B,D,E, 16A-I). Erect filaments are uniseriate, usually unbranched, loosely adherent with each other, easily separable under pressure, up to 110-160 µm in length. Cells of the erect filaments are 8-14 µm in length and 10-13 µm in width, and they possess a plastid with a pyrenoid per cell (Fig. 15C). Plurilocular sporangia are ectocarpoid, pedicellate on prostrate filaments or apically on erect filaments, 50-60 µm

in length and 18-20 μm in width (Figs 15E, 16A-C,E). Unilocular sporangia are spherical to obovoid, sessile or pedicellate on prostrate filaments, rarely laterally on erect filaments, 30-70 × 17-30 μm in size (Figs 15D, 16F-I). Ascocysts are common, sessile or pedicellate on prostrate filaments, 40-70 μm in length and 10-14 μm in width (Figs 15B,E, 16A,B,D,G).

Habitat and Phenology

Saccate thalli grow in the lower intertidal and subtidal zones, usually on various other algae such as *Sargassum* spp. *Neorhodomela aculeata*, *Dictyopteris divaricata*, *Ahnfeltiopsis flabelliformis* and on rocks. They appear in late autumn persisting until early summer, and disappear from mid-summer onward at Oshoro, Hokkaido (Table 5). However, in some localities they are found also during summer and autumn.

At Oshoro, Hokkaido, microthalli grow on boulders and other algae such as *Sargassum confusum* and *Dictyopteris divaricata*, in the lower intertidal and subtidal zones. They can be found from spring to autumn at Oshoro and they have plurilocular sporangia during spring and summer, unilocular sporangia in autumn (Table 5).

Geographical distribution

This species is reported from California (Abbott & Hollenberg 1976), Australia (Clayton 1975), New Zealand (Parsons 1982), and Japan (present study). Although this species is found in Europe (Hamel 1931-39, Fletcher 1987), it may originate from the Pacific area, representing an introduced species to Europe (Jones 1974, Farnham 1980). In Japan, this species is distributed

in Hokkaido, Honsyu, Shikoku and Kyusyu (Fig. 11).

Culture study

Culture strains from macrothalli are shown in Table 6. Culture conditions and progeny of plurispores from macrothalli are also indicated in Table 6.

Sexual reproduction

Gamete fusions were observed in materials from Oshoro, Hokkaido.

Female gametes were pear drop-shaped, of normal phaeophycean type with one plastid and a stigma, 8.2-10.0 × 4.0-6.2 μm in size (Fig. 17A). They showed negative phototaxis, and then settled on the substratum. Settled female gametes were round, 5.4-7.0 μm in diameter. Male gametes were the same shape as female gametes, but, slender and smaller than the female, 4.2-6.4 × 2.0-2.8 μm in size (Fig. 17B). They contained a pale plastid with a stigma. They settled on the substratum after swimming showing negative phototaxis. Male gametes settled were rounded, 2.6-3.8 μm in diameter. When female and male gametes were mixed, a settled female gamete was surrounded by several male gametes which were attached to the settled female gamete by anterior flagellum, and then, one of the male gametes fused with the female gamete (Figs 17C-F). Zygotes were distinguished from unfused female gametes by possessing two stigmata.

Further development of zygotes could not be followed in this study.

Parthenogenetic development from female gametes

Settled female gametes germinated into prostrate branched filaments and developed into disks, pseudodisks, tufts or intermediate forms between them (Figs 18A-C). Tufted thalli consisted of branched filaments which freely grew with knot parts. Discoid and pseudodiscoid thalli consisted of prostrate branched filaments and erect, uniseriate, simple filaments arising from them (Figs 18D-F). Erect filaments loosely adjoined with each other. Microthalli became fertile in 1-3 months, reaching up to 1-3 mm in diameter. They formed plurilocular sporangia under long day conditions at 5-20°C (Fig. 18D), unilocular sporangia under 15°C SD (Fig. 18F), plurilocular or unilocular sporangia under 5°C SD, 10°C SD, 10°C ND, 15°C ND, and 20°C SD (Fig. 18E, Table 6). Plurilocular sporangia were ectocarpoid, pedicellate on prostrate filaments or apical on erect filaments. Unilocular sporangia were obovoid, pedicellate on prostrate filaments or terminal on erect filaments. Pedicellate or sessile unicellular ascocysts were sometimes observed on prostrate filaments (Fig. 18D).

Parthenogenetic development from male gametes

Most of male gametes did not germinate. However, a few germlings developed into disks, pseudodisks, tufts or intermediate forms between them, as did germlings from female gametes. These microthalli formed plurilocular sporangia at 5-20°C under long day conditions and 5°C SD, unilocular sporangia under 15°C SD and 20°C SD, and plurilocular or unilocular sporangia under a 10°C SD condition (Table 6). Morphology of these microthalli was the same as that of microthalli from female

gametes.

Development of plurispores from microthalli

Plurispores from microthalli derived parthenogenetically from female gametes which grew under 15°C LD were cultured under various conditions (Table 7; strain 240 Oshoro). Plurilocular sporangia on microthalli under 15°C ND where the thalli bore both plurilocular and unilocular sporangia were also isolated and cultured (Table 7; strain 319 Oshoro). The plurispores were of the normal phaeophycean type with a plastid and a stigma, 6.8-10.0 × 4.8-6.8 µm in size (Fig. 19A). Settled plurispores were round, 5.2-6.6 µm in diameter. They germinated and developed into disks, pseudodisks or tufts as did female gametes (Figs 19B-E). These microthalli formed plurilocular sporangia under long day conditions (Fig. 19F), unilocular sporangia under 10-20°C SD (Fig. 19G) in the same manner as microthalli derived from female gametes (Table 7).

Plurispores from microthalli derived parthenogenetically from male gametes which grew under a 20°C LD condition were cultured at 10-20°C under both LD and SD conditions (Table 7). These plurispores were of the same size of plurispores from microthalli derived from female gametes. These plurispores developed into microthalli similar in morphology to their parent microthalli and formed plurilocular sporangia under long day conditions at 10-20°C, unilocular sporangia under 15°C SD and 20°C SD, plurilocular or unilocular sporangia under 10°C SD.

Development of unispores from microthalli

Unispores from microthalli derived parthenogenetically from

female gametes under a 15°C SD condition were cultured at 5-20°C under both LD and SD conditions, and under 10°C ND and 15°C ND conditions (Table 8; strain 317 Oshoro). Unilocular sporangia were also isolated from microthalli forming both unilocular and plurilocular sporangia under 15°C ND, and they were cultured at 10°C and 20°C under both LD and SD. (Table 8; strain 319 Oshoro). Unispores were of the normal phaeophycean type, with a plastid and a stigma, 7.0-10.0 × 3.6-4.6 µm in size (Fig. 19H). Settled unispores were round, 4.4-6.0 µm in diameter. They germinated into small pseudodisks through prostrate branched filaments (Figs 19I,J). These small pseudodisks became swollen, and then developed into spherical thalli. Spherical thalli became fertile acropetally with female gametangia. Ascocysts were present among female gametangia.

Unispores from unilocular sporangia on microthalli derived from male gametes under 15°C SD, were cultured under 15°C SD and 15°C LD. These unispores developed into spherical thalli in the same manner as mentioned above. The spherical thalli formed male gametangia.

Direct type life history

In some strains (Table 6; 218 Megishima, 306 Kojima, 307 Ohshima, 309 Yuge), plurispores from plurilocular sporangia on spherical thalli developed into similar spherical thalli directly in the same manner as unispores from microthalli mentioned above.

Culture from field microthalli with unilocular sporangia

Results in strain 175 Oshoro are mentioned below.

Unilocular sporangia isolated were cultured under a 10°C LD condition. Germlings from unispores released from the unilocular sporangia developed into spherical thalli identical with *Colpomenia* (Figs 20A-F). After 3 months, these spherical thalli reached 5 mm in diameter, forming plurilocular sporangia with ascocysts.

Germlings from plurispores from the spherical thalli were cultured at 10°C and 15°C under both LD and SD conditions. They developed into microthalli which formed ectocarpoid plurilocular sporangia under 10°C LD and 15°C LD, unilocular sporangia under 10°C SD and 15°C SD. (Table 9).

Germlings from plurispores from these microthalli grown under a 15°C LD condition were cultured at 10°C under SD, ND and LD and at 15°C under SD and LD. (Table 10). They grew into microthalli reaching 1-2 mm in diameter, and formed plurilocular sporangia under long day conditions, unilocular sporangia under 15°C SD, and both type of sporangia under 10°C ND two months after.

Unispores from unilocular sporangia on the microthalli under 15°C SD were cultured at 5-20°C under LD and SD, and under 10°C ND (Table 11). These unispore germlings developed into spherical thalli under all conditions examined except for 20°C LD where 30% of germlings grew into tufts forming ectocarpoid plurilocular sporangia. These spherical thalli became fertile with plurilocular sporangia at 10-20°C under LD and SD conditions.

Culture from field microthalli with plurilocular sporangia

Results in strain 238 Oshoro are mentioned below.

Isolated fragments of microthalli bearing plurilocular

sporangia were cultured under 15°C LD condition. They grew into pseudodiscoid thalli which formed ectocarpoid plurilocular sporangia. Germlings from plurispores (Fig. 20G) obtained under 15°C LD were cultured at 5-20°C under LD and SD, and under 10°C ND and 15°C ND conditions. They developed into microthalli (Figs 20H-J) which formed ectocarpoid plurilocular sporangia (Fig. 20K) under long day conditions at 5-20°C and 5°C SD, unilocular sporangia (Fig. 20L) under 10-20°C SD, and both sporangia under 10°C ND and 15°C ND (Table 12). Ascocysts were sometimes observed on the microthalli. These microthalli were morphologically identical with those of *C. peregrina* in culture.

Unispores from unilocular sporangia on microthalli under 15°C SD were transferred under ten culture conditions mentioned above (Table 13). The majority of germlings from the unispores developed into spherical thalli identical with *Colpomenia* although tufts were sometimes observed. Spherical thalli were obtained under all conditions examined and formed plurilocular sporangia under 10°C LD and 15°C LD.

Discussion

Colpomenia peregrina (Sauvageau) Hamel was originally described as *C. sinuosa* (Mertens ex Roth) Derbès et Solier var. *peregrina* by Sauvageau (1927). Later Hamel (1937) recognized it as an independent species. Sauvageau (1927), Blackler (1964, 1967) and Clayton (1975) discussed morphological characters separating *C. peregrina* from *C. sinuosa* (see discussion on *C. sinuosa* in this study). This study also shows that features of plurilocular sporangia, such as sorus shape and presence/absence of a cuticular covering, are important to distinguish these

species. The results of the present morphological observations are in agreement with those of Sauvageau (1927), Blackler (1964, 1967), Parsons (1982) and Fletcher (1987). Although *C. peregrina* is found in temperate waters in Pacific and Atlantic Oceans, it has not been reported from Japan. It seems that Japanese phycologists have confused *C. peregrina* with *C. sinuosa*.

The present culture study on Japanese *C. peregrina* revealed that a heteromorphic life history which involved spherical macrothalli and discoid microthalli. Although sexual fusion was observed in materials from Oshoro, culture from zygotes was not carried out because zygotes could not be isolated. Diagram of life history of this species in my culture is shown in Fig. 21. In results on materials from Oshoro, plurispores developed into microthalli under all culture conditions examined. The microthalli formed plurilocular or unilocular sporangia, depending on temperature and photoperiod. Plurispores from microthalli developed into similar microthalli. Unispores from microthalli developed into spherical macrothalli. At Oshoro microthalli were found from spring to autumn. They were fertile with plurilocular sporangia during spring and summer, with unilocular sporangia in autumn. The observed reproductive phenology agrees well with results of culture studies: microthalli forming plurilocular sporangia under long day conditions and unilocular sporangia under short day conditions at 10-20°C. It is considered that plurispores from microthalli give rise to microthalli during spring and summer, since plurispores from microthalli developed into similar microthalli in culture. On the other hand, spherical macrothalli were present during winter and spring at Oshoro. The spherical thalli are expected to

develop from unispores from unilocular sporangia produced on microthalli in autumn, since unispores developed into spherical thalli in culture. Formation of reproductive organs on microthalli, which depends on temperature and photoperiod, accounts for the phenology of this species.

Clayton (1979) reported a sexual life history of Australian *C. peregrina* in culture: gametophytic spherical macrothalli forming gametangia alternate with sporophytic microthalli bearing unilocular sporangia. The microthalli reported by her are morphologically similar to those in the present study. However, Clayton (1979) did not mention the presence of plurilocular sporangia on the microthalli. She might not be able to observe them since most cultures were set under short day conditions where the plurilocular sporangia may not be formed on the microthalli. Sauvageau (1927) and Blackler (1964, 1981) reported plurilocular sporangia on microthalli in European materials as in the present study. It is necessary to reexamine Australian materials.

This is the first report of microthalli of *C. peregrina* in fields. The microthalli in fields are small, pseudoparenchymatous disks forming ascocysts and plurilocular and unilocular sporangia. Although the microthallus of *C. peregrina* resembles some discoid brown algae, it could not be identified with them. Discoid brown algae with ascocysts were previously grouped in the genus *Ascocyclus* Magnus until this was rejected by Loiseaux (1967) (see also Russell 1964). At present, these algae belong to *Myrionema*, *Chilonema* and *Hecatonema*. Species of *Myrionema* usually have uniseriate plurilocular sporangia which are different from those of microthalli of *C. peregrina*. *Chilonema foecundum*

(Streofelt) Fletcher (Fletcher 1987) and *C. hispanicum* (Sauvageau) Fletcher (Fletcher 1987) morphologically resemble the microthalli of *C. peregrina*. Plurispores of these two species, however, show a stellate-type germination according to Loiseaux (1967) (as *Hecatonema foecundum* and *H. hispanicum*, respectively), which is entirely different from the germination type of *C. peregrina*. *Symphyocarpus* has also discoid thalli with ascocysts and is similar to the microthalli of *C. peregrina*. Furthermore, it possesses a plastid with a pyrenoid per cell, which agrees with a critical feature of Scytosiphonales. However, only thalli bearing plurilocular sporangia are known for *Symphyocarpus*, and the plurilocular sporangia are different from those of microthalli of *C. peregrina* in shape.

Table 5. Collection data of *Colpomenia peregrina* at Oshoro, Hokkaido. (Ve, vegetative thalli; P, thalli with plurilocular sporangia; U, thalli with unilocular sporangia; (Ve), (P), (U), microthalli identified morphologically, not cultured; --, thallus was not found)

Macrothalli

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
1987			P		P	P		--	--		Ve	Ve
1988	P	P	P		P	P				--	Ve	
1989			P			P		--	--		Ve	Ve
1990		P	P	P	P	P	--		--	Ve	Ve	
1991		P										
1992		P	P	P	P							

Microthalli

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
1988										U	--	
1989		--				P		P	U		U	--
1990		--	--	(Ve)	P	(P)	(P)		(U)	(U)	--	

Table 6. Collection data of cultured strains of *Colpomenia peregrina*. Culture conditions attempted and progeny of plurispores from macrothalli are also shown. Sex is indicated only in materials from Oshoro, Hokkaido. It was identified by the plurispore size, but not by gamete fusion.

strain no.	locality	date	sex	culture conditions	progeny
40	Oshoro	21 vi 1987	female	10L	pseudodisks (P)
47	Nokkamappu	7 ix 1987		10L	pseudodisks (P), macrothalli (P)
110	Misaki	20 iv 1988		10L	pseudodisks (P)
121	Kumoishi	15 v 1988		5SL	disks (P), few macrothalli
				10-20S	pseudodisks (U)
				10-20L	pseudodisks (P)
128	Oshoro	21 v 1988	female	10L	pseudodisks (P)
129	Oshoro	21 v 1988	female	10L	pseudodisks (P)
130	Oshoro	24 v 1988	female	10L	pseudodisks (P)
131	Oshoro	24 v 1988	female	10L	pseudodisks (P)
143	Kikonai	21 vi 1988		10L	pseudodisks (P)
147	Akkeshi	29 vi 1988		10L	pseudodisks (P)
157	Sai	16 vii 1988		10L	pseudodisks (P)
218	Megisima	6 iv 1989		10L	macrothalli
				15L	macrothalli (P)
240	Oshoro	22 vi 1989	female	5S,5-20L,10N	pseudodisks (P)
				15S	pseudodisks (U)
				15N	pseudodisks (P, U)
276	Oshoro	28 ii 1990	female	10-20L, 10S, 15SN, 20S	pseudodisks (P) pseudodisks (U)
				5S, 10N,	pseudodisks (P, U)

Table 6. Continued

strain no.	locality	date	sex	culture conditions	progeny
288	Oshoro	23 iii 1990	female	5S, 10-20L	pseudodisks (P)
				15S, 20S	pseudodisks (U)
				10SN	pseudodisks (P,U)
292	Oshoro	17 iv 1990	female	5-20L	pseudodisks (P)
				10S, 15SN, 20S	pseudodisks (U)
				5S, 10N	pseudodisks (P, U)
306	Kojima	9 v 1990		10L, 15L	macrothalli (P)
307	Ohshima	10 v 1990		10L, 15L	macrothalli (P)
309	Yuge	11 v 1990		15L	macrothalli (P)
311	Munakata	12 v 1990		5S	pseudodisks (P), few macrothalli
				10-20L	pseudodisks (P)
				15S	pseudodisks (U)
				10S, 20S	pseudodisks (P, U)
317	Oshoro	21 v 1990	female	10-20L	pseudodisks (P)
				15S, 20S	pseudodisks (U)
				10SN, 15N	pseudodisks (P, U)
319	Oshoro	4 vi 1990	female	5-20L	pseudodisks (P)
				10-20S	pseudodisks (U)
				5S,10N, 15N	pseudodisks (P, U)
349	Oshoro	15 iv 1991	male	5SL, 10-20L	pseudodisks (P)
				15S, 20S	pseudodisks (U)
				10S	pseudodisks (P, U)

Table 7. Culture of *Colpomenia peregrina*. Progeny of plurispores from microthalli.

strain no.	locality	date	sex	culture conditions	progeny
240	Oshoro	22 vi 1989	female	5S, 10-20L 10-20S, 10N, 15N	pseudodisks (P) Pseudodisks (P,U)
319	Oshoro	4 vi 1990	female	10L, 20L 10S, 20S	pseudodisks (P) pseudodisks (U)
349	Oshoro	15 iv 1991	male	10-20L 15S, 20S 10S	pseudodisks (P) pseudodisks (U) pseudodisks (P,U)

Table 8. Culture of *Colpomenia peregrina*. Progeny of unispores from microthalli.

strain no.	locality	date	sex	culture conditions	progeny
317	Oshoro	21 v 1990	female	5-20SL, 10N, 15N	macrothalli (P; 10N, 15L, 20L)
319	Oshoro	4 vi 1990	female	10SL, 20SL	mostly macrothalli (P; 20L)
349	Oshoro	15 iv 1991	male	15SL	macrothalli (P; 15L)

Table 9-11. Culture of *Colpomenia peregrina*. Progeny of swarmers in strains isolated from field microthalli with unilocular sporangia.

Table 9. Progeny of plurispores from macrothalli.

Strain no.	locality	date	sex	culture conditions	progeny
175	Oshoro	25 x 1988	female	10S, 15S	pseudodisks (U)
				10L, 15L	pseudodisks (P)

Table 10. Progeny of plurispores from microthalli.

strain no.	locality	date	sex	culture conditions	progeny
175	Oshoro	25 x 1988	female	10S, 15S	pseudodisks (U)
				10N	pseudodisks (U, P)
				10L, 15L	pseudodisks (P)

Table 11. Progeny of unispores from microthalli.

strain no.	locality	date	sex	culture conditions	progeny
175	Oshoro	25 x 1988	female	5-15SL, 20S, 10N,15N	macrothalli (P, except 5SL)
				20L	macrothalli (P)
					tufts (P)

Table 12, 13. Culture of *Colpomenia peregrina*. Progeny of swarmers in strains isolated from field microthalli with plurilocular sporangia.

Table 12. Progeny of plurispores from microthalli.

strain no.	locality	date	sex	culture conditions	progeny
238	Oshoro	22 v 1989	female	5SL, 10-20L	pseudodisks (P)
				10-20S	pseudodisks (U)
				10N, 15N	pseudodisks (U, P)

Table 13. Progeny of unispores from microthalli.

strain no.	locality	date	sex	culture conditions	progeny
238	Oshoro	22 v 1989	female	5-20SL, 10N, 15N	mostly macrothalli (P, except 5SL and 10S) sometimes tufts (rarely P)

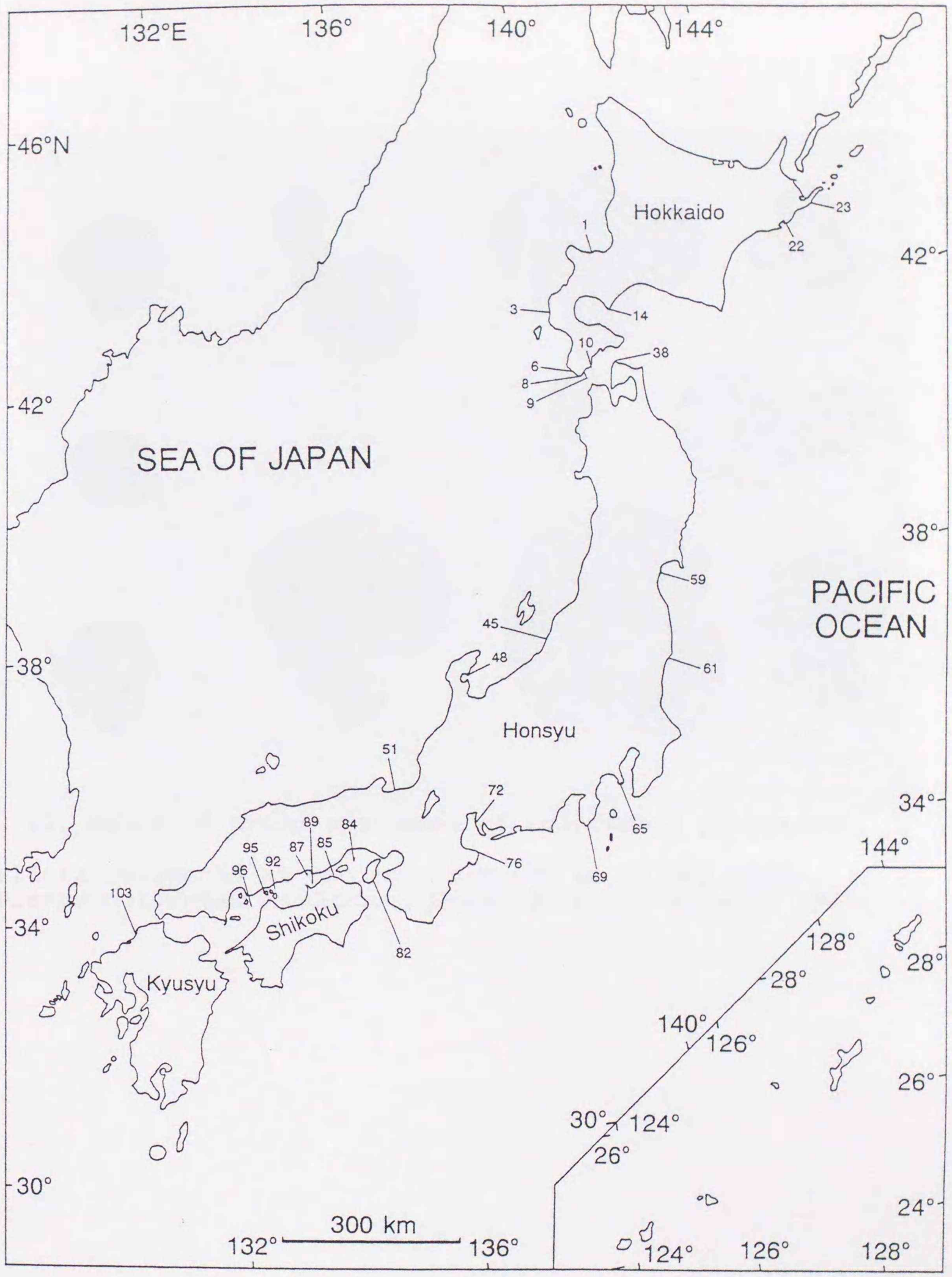


Fig. 11. Localities where materials of *Colpomenia peregrina* were collected. Numbers plotted correspond to those in Table 1.

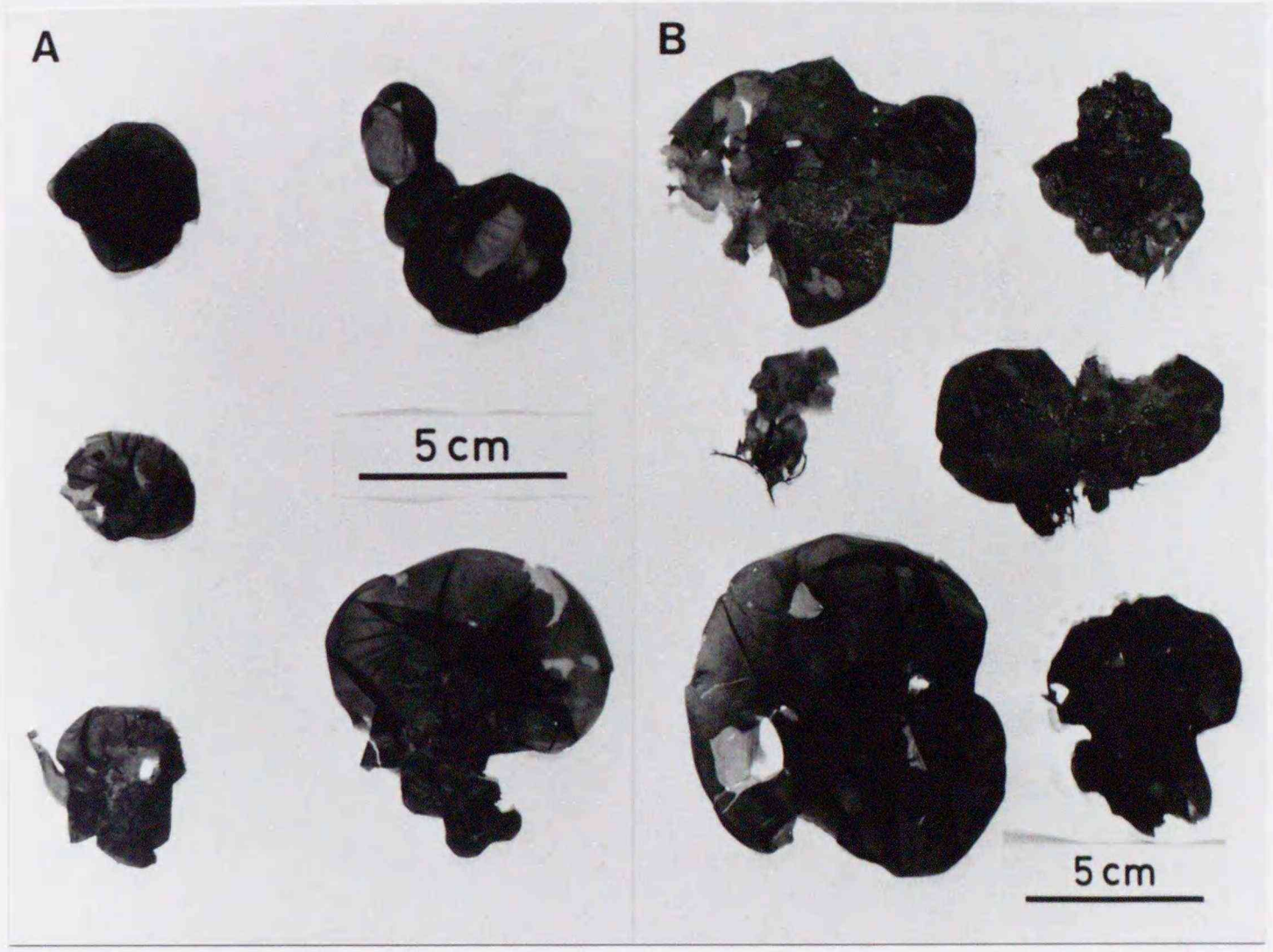


Fig. 12. Habit of dried specimens of *Colpomenia peregrina*.

A. Plants collected at Oshoro, Hokkaido on 29 May 1992.
B. Plants collected at Oshoro, Hokkaido on 21 January 1988.

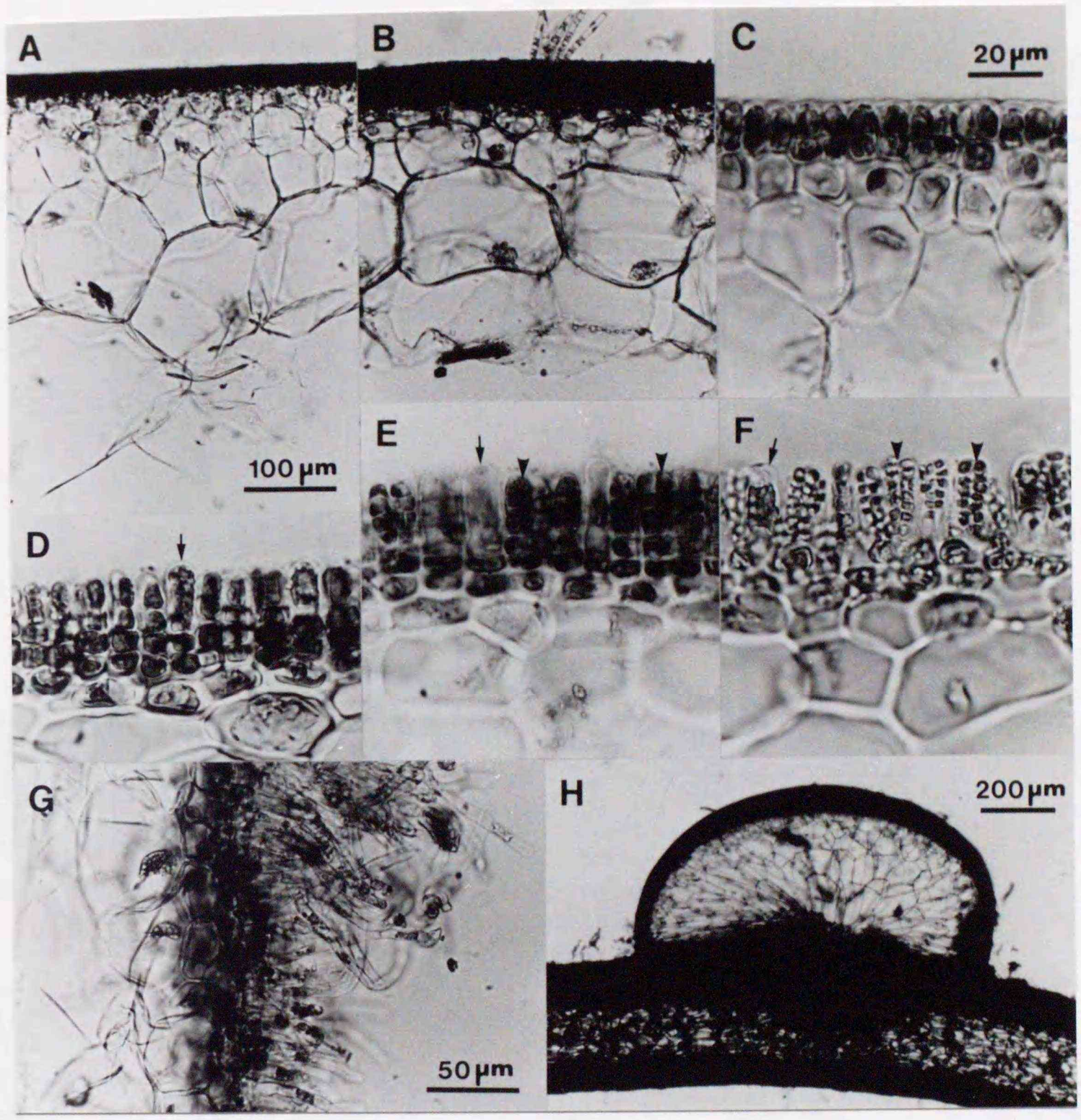


Fig. 13. Anatomy of *Colpomenia peregrina*.

A. Section of thallus. **B.** Section of thallus through phaeophycean hair tuft. **C.** Cortical part of vegetative thallus in section. **D.** Cortical part in section, showing young plurilocular sporangia and ascocysts (arrow). **E.** Female plurilocular sporangia (arrowheads) and ascocysts (arrow) in section of thallus. **F.** Male plurilocular sporangia (arrowheads) and ascocysts (arrow) in section of thallus. **G.** Section of thallus, showing rhizoids arising from thallus surface. **H.** Section of young macrothalli on leaf of *Sargassum confusum*.

Scale in **A** applies to **B**; Scale in **C** applies to **D-F**.

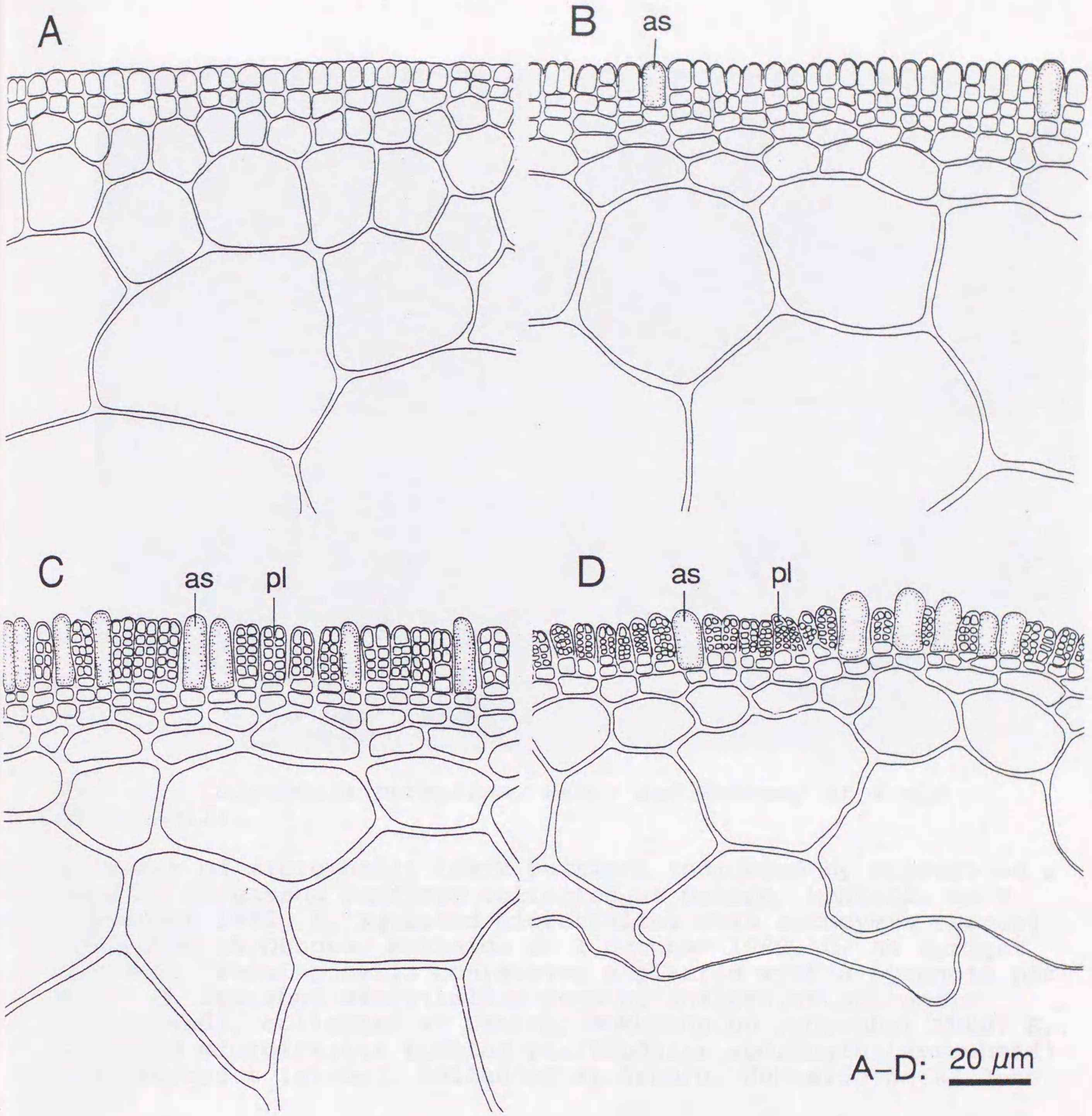


Fig. 14. Anatomy of *Colpomenia peregrina*.

A. Section of vegetative thallus. **B.** Young ascocysts (as) in section of thallus. **C.** Section of female thallus forming female plurilocular sporangia (pl) and ascocysts (as). **D.** Section of male thallus forming male plurilocular sporangia (pl) and ascocysts (as).

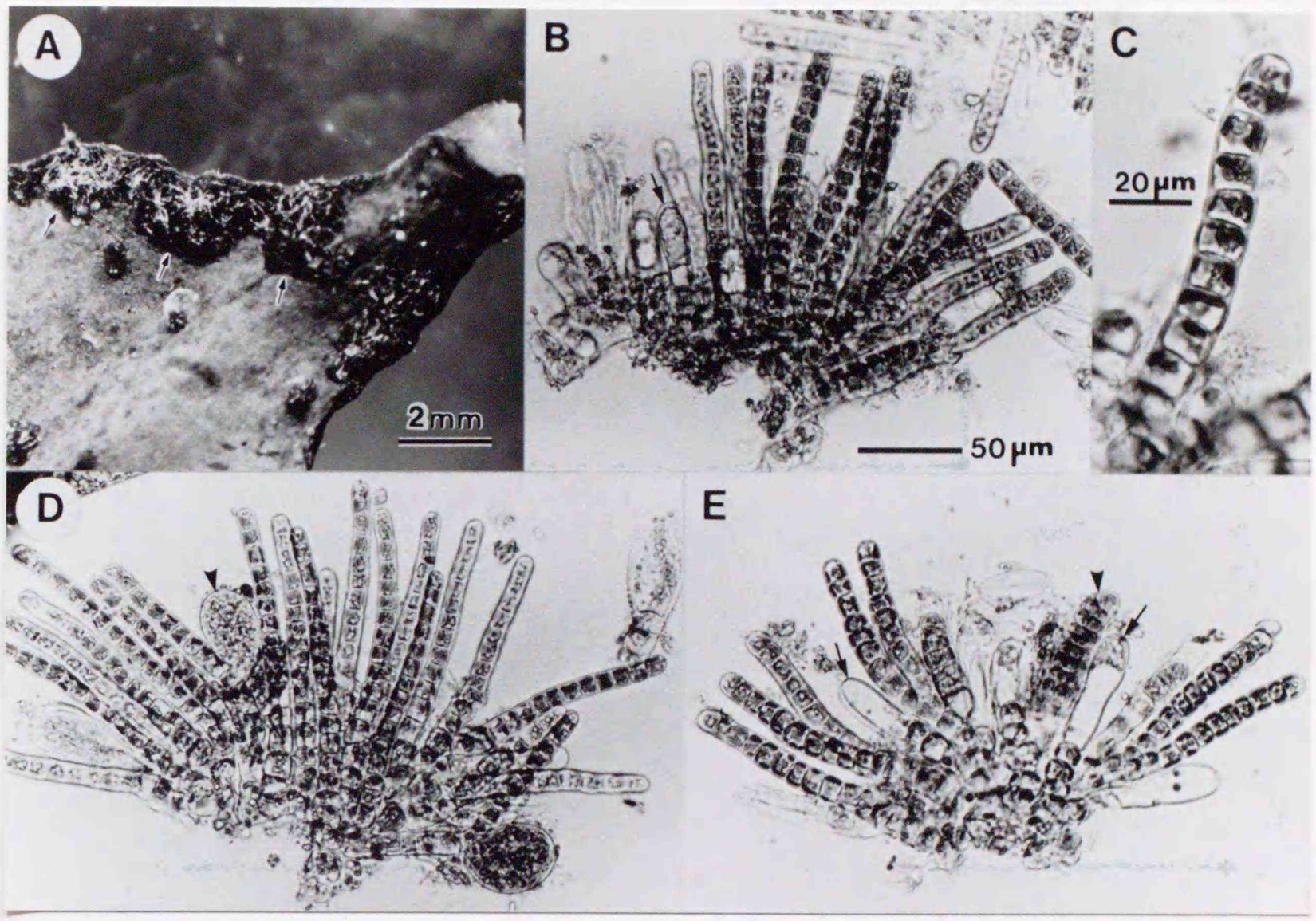


Fig. 15. *Colpomenia peregrina*. Habit and anatomy of field microthalli.

A. Habit of microthalli (dark portions indicated by arrows) on a leaf of *Sargassum confusum* collected at Oshoro, Hokkaido on 9 September 1992. **B.** Squashed microthallus with ascocysts (arrow), collected at Oshoro, Hokkaido on 1 October 1988. **C.** An upright filament, showing cells containing a plastid with a pyrenoid par cell. **D.** Squashed microthallus forming unilocular sporangia (arrowhead), collected at Oshoro, Hokkaido on 1 October 1988. **E.** Squashed microthallus forming plurilocular sporangia (arrowhead) and ascocysts (arrow), collected at Oshoro, Hokkaido on 22 June 1989.

Scale in **B** applies to **D** and **E**.

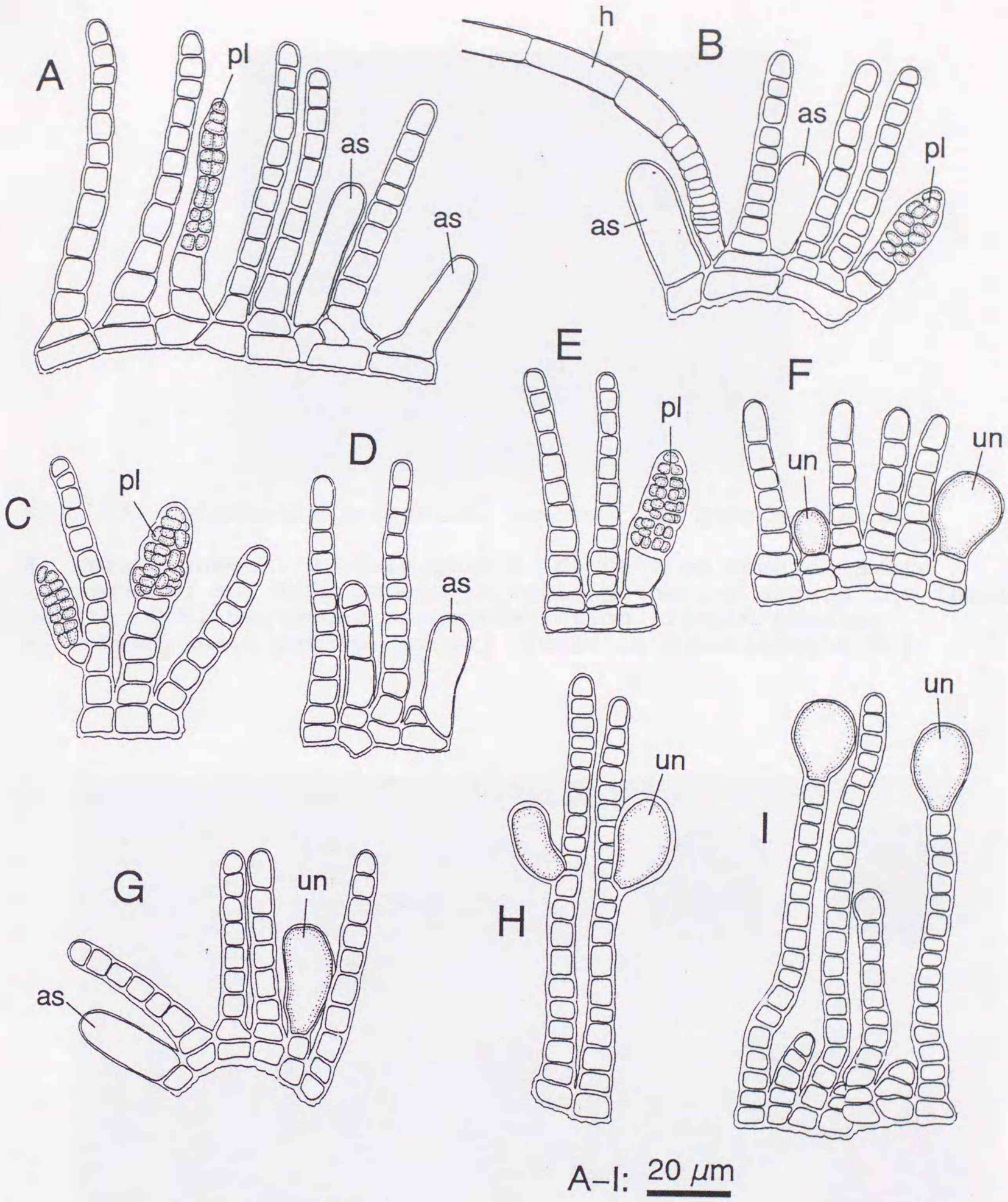


Fig. 16. *Colpomenia peregrina*. Anatomy of field microthalli.

A-E. Squashed thalli forming plurilocular sporangia (pl) and ascocysts (as). Thalli collected at Oshoro, Hokkaido on 28 July 1990 (**A, D, E**), on 12 August 1989 (**B**) and on 22 June 1989 (**C**). **F-I.** Squashed thalli forming unilocular sporangia (un) and ascocysts (as). Thalli collected at Oshoro, Hokkaido on 23 September 1989 (**F**) and on 25 October 1988 (**G-I**).

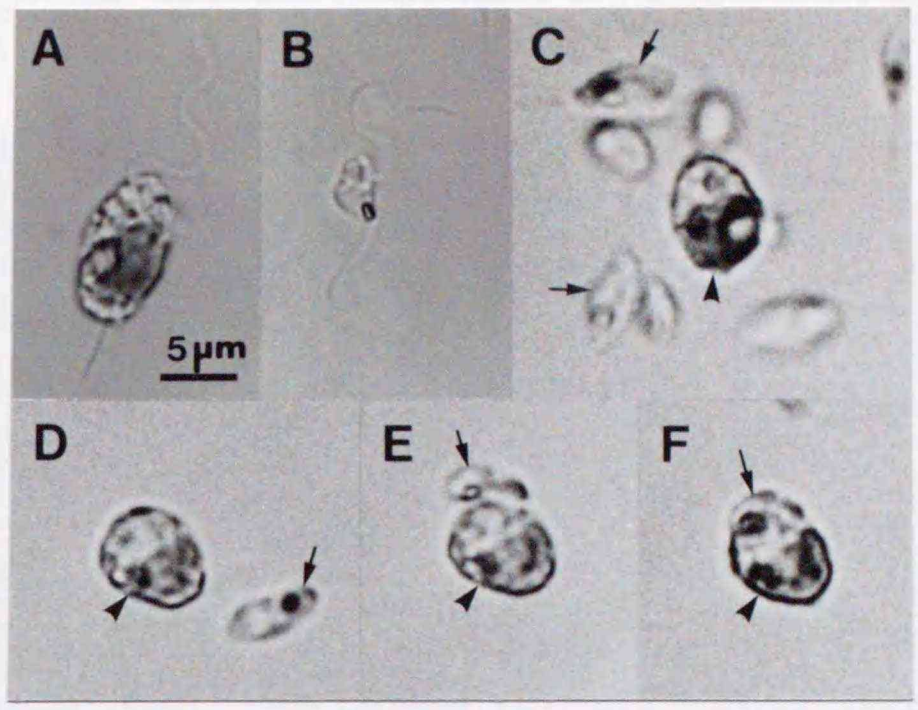


Fig. 17. *Colpomenia peregrina*. Gametes and gamete fusion.

A. Female gamete. **B.** Male gamete. **C.** Settled female gamete (arrowhead) and male gamete (arrows) gathering around the female gamete. **D-F.** Sequences of gamete fusion (female gamete, arrowhead; male gamete, arrow). Scale in **A** applies to **B-F.**

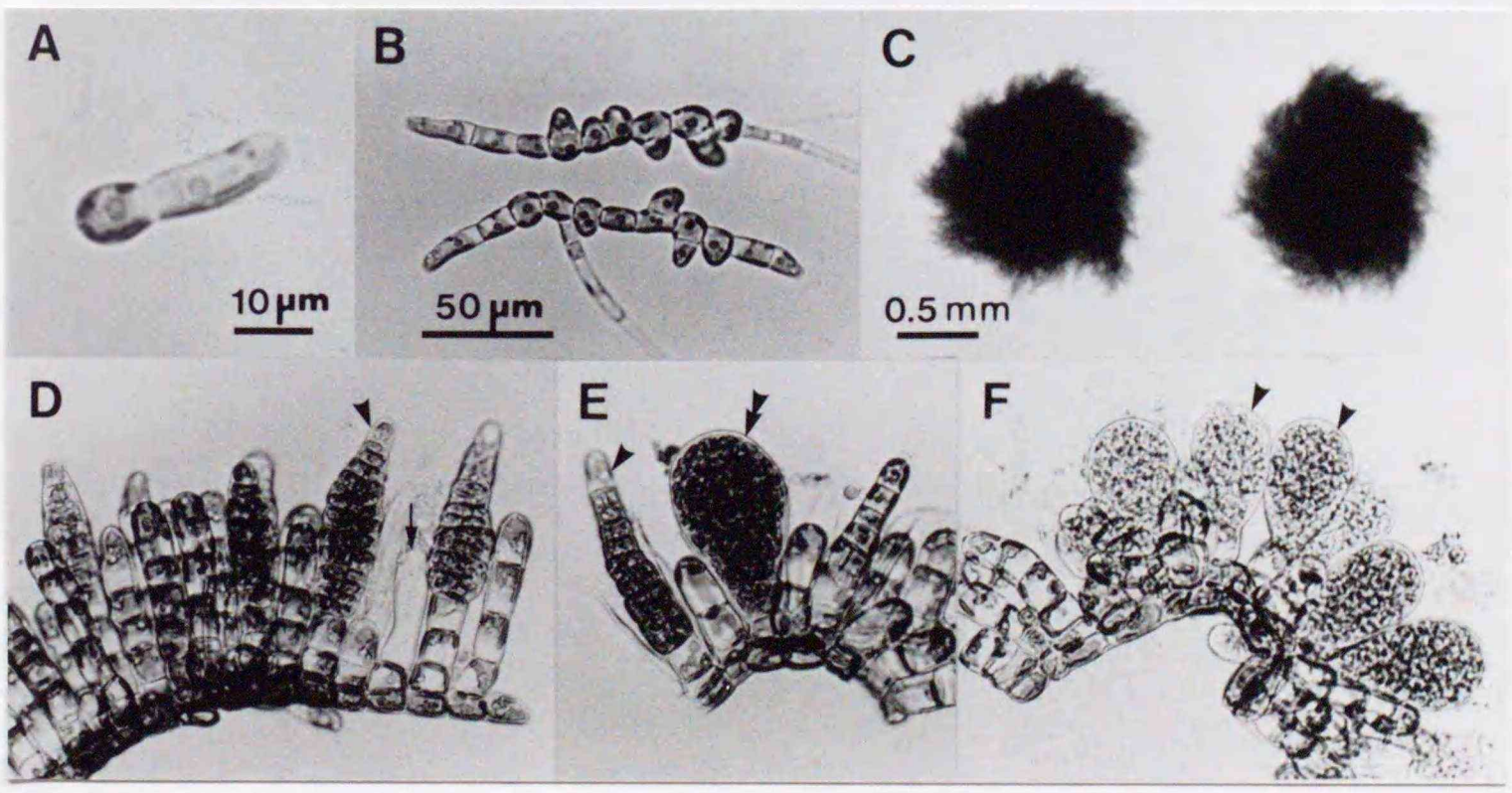


Fig. 18. Culture of *Colpomenia peregrina*. Parthenogenetic development of female gamete.

A. Three-day-old germling under 15°C LD condition. **B.** Eight-day-old under 15°C LD condition. **C.** Pseudodiscoid microthalli under 15°C ND condition. **D.** Squashed microthallus forming plurilocular sporangia (arrowhead) and ascocysts (arrow) under 15°C LD condition. **E.** Squashed microthallus forming plurilocular sporangia (arrowhead) and unilocular sporangia (double arrowhead) under 15°C ND condition. **F.** Squashed microthallus forming unilocular sporangia (arrowheads) under 15°C SD condition. Scale in **B** applies to **D-F.**

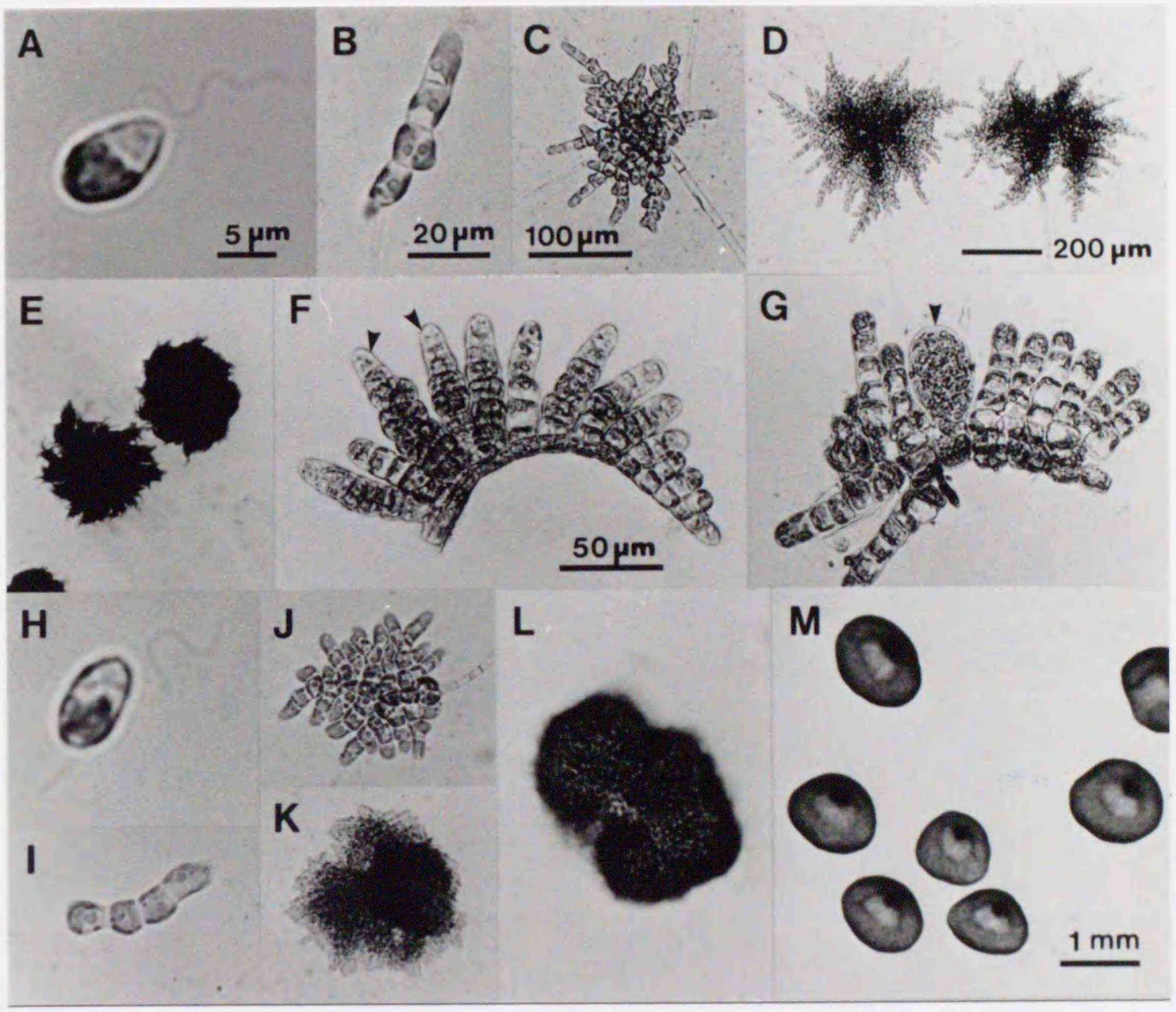


Fig. 19. Culture of *Colpomenia peregrina*. Development of plurispores (**A-G**) and unispores (**H-M**) from microthallus which developed from female gamete.

A. Plurispore released from plurilocular sporangium on microthallus. **B.** Five-day-old germling under 15°C LD condition. **C.** Nine-day-old germling under 15°C LD condition. **D.** Eleven-day-old germlings under 15°C LD condition. **E.** Pseudodiscoid microthalli. **F.** Squashed microthallus forming plurilocular sporangia (arrowheads) under 15°C LD condition. **G.** Squashed microthallus forming (arrowhead) unilocular sporangia under 15°C SD condition. **H.** Unispore released from unilocular sporangium on microthallus. **I.** Six-day-old germling under 15°C SD condition. **J.** Twelve-day-old germling under 15°C SD condition. **K, L.** Three-week-old germlings under 15°C SD condition, showing a swollen part on the thallus. **M.** Young, spherical macrothalli.

Scale in **A** applies to **H**; Scale in **B** applies to **I**; Scale in **C** applies to **K** and **L**; Scale in **F** applies to **G** and **J**.

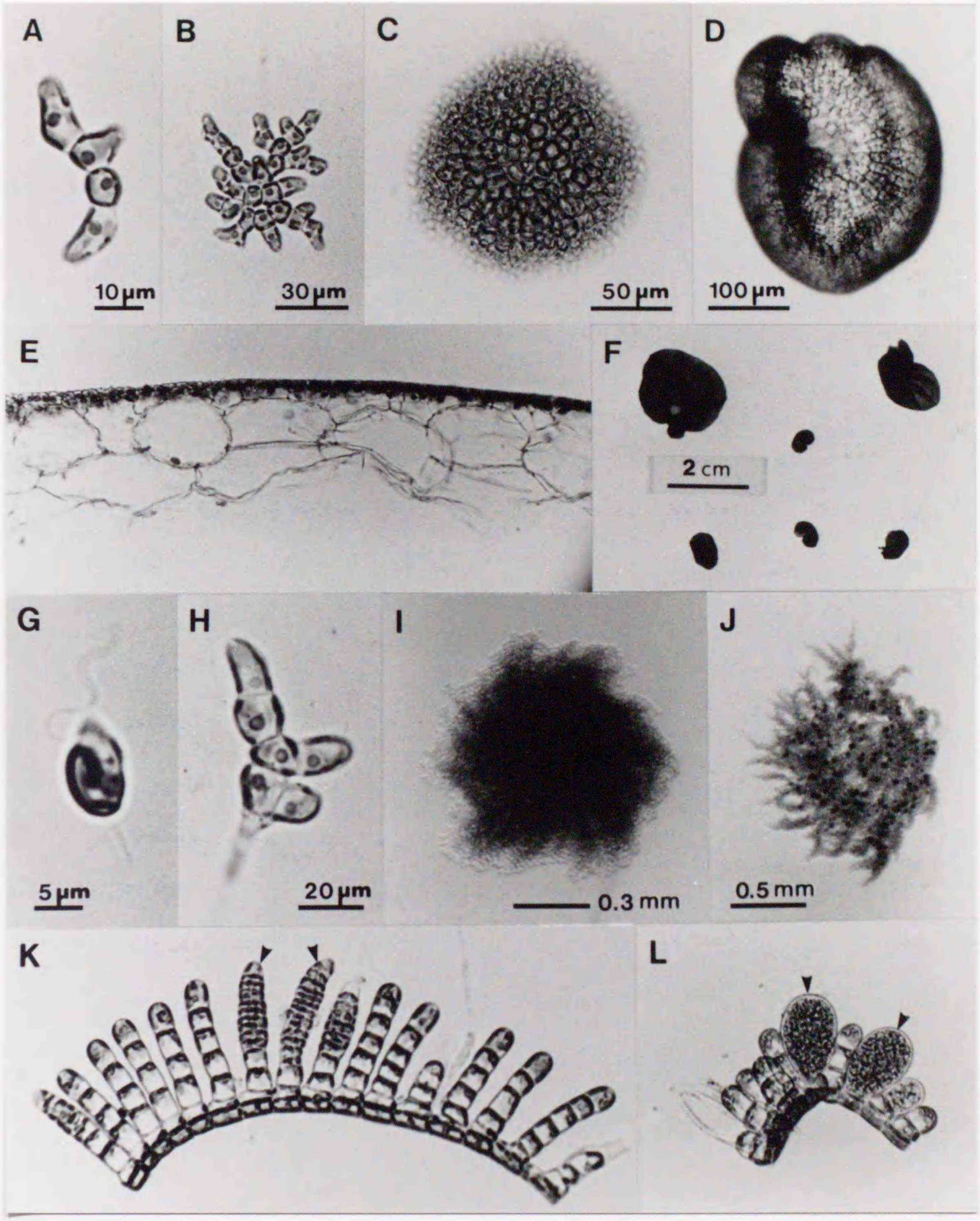


Fig. 20. *Colpomenia peregrina*. Cultures of field microthalli.

A-F. Progeny of unispores from microthallus. **A.** Seven-day-old germling under 15°C SD condition. **B.** Sixteen-day-old germling under 15°C SD condition. **C.** Four-week-old germling under 15°C SD condition, showing a swollen part which is developing into a spherical thallus. **D.** Young, spherical macrothallus. **E.** Section of a macrothallus. **F.** Dried specimen of macrothalli obtained in culture. **G-L.** Progeny of plurispores from microthallus. **G.** Plurispore from microthallus. **H.** Six-day-old germling under 15°C LD condition. **I.** Microthallus. **J.** Microthallus forming unilocular sporangia under 15°C SD condition. **K.** Squashed microthallus forming plurilocular sporangia (arrowheads) under 15°C LD condition. **L.** Squashed microthallus forming unilocular sporangia (arrowheads) under 15°C SD condition. Scale in **C** applies to **K** and **L**; Scale in **D** applies to **E**.

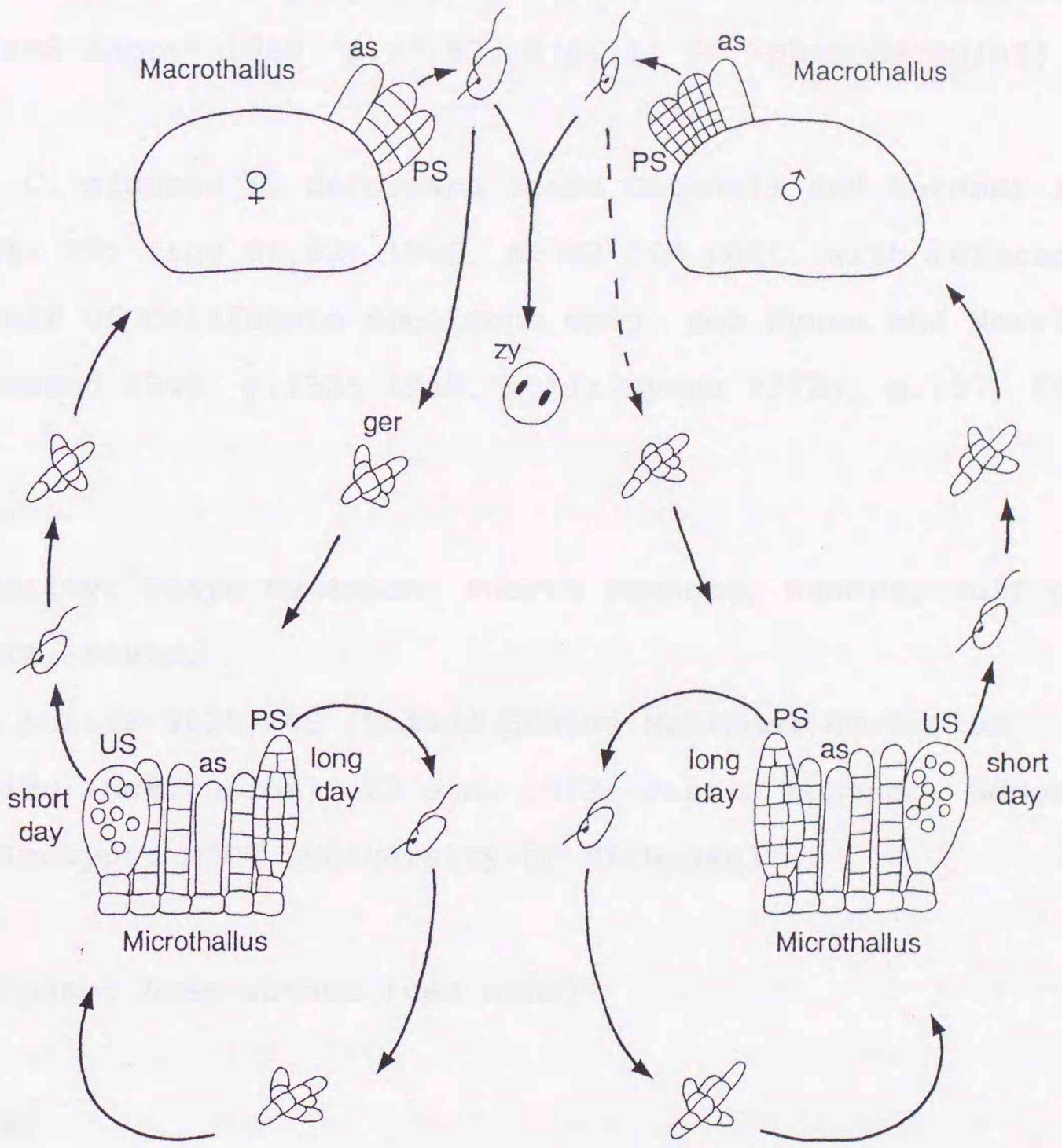


Fig. 21. Diagram of life history of *Colpomenia peregrina* in culture.

as, ascocyst; ger, germling; PS, plurilocular sporangium; US, unilocular sporangium; zy, zygote.

Colpomenia phaeodactyla **Wynne et Norris**

Wynne et Norris 1976, p.5, fig.4, 5a, 5b and 11c.; Santelices, Ramirez and Abbott 1989, p.73-82, Fig. 1. (*C. phaeodactyla*?).

Synonym: *C. sinuosa* f. *deformans* sensu Setchell and Gardner 1924, p.726, pl. 19: figs 61,62; 1925, p.542 (in part, with reference to the Gulf of California specimens only, see Wynne and Norris 1976); Dawson 1944, p.233; 1966, p.11; Wynne 1972a, p.137, fig. 1.

Type locality: Playa Estacion, Puerto Penasco, Sonora, Gulf of California, Mexico.
Type: J. Norris 3629, US (United States National Herbarium, Smithsonian Institution). 22 Jan. 1973, Puerto Penasco, Sonora, Mexico. Isotype: MICH (University of Michigan).

Japanese name: Hoso-watamo (new name)

Materials:

Localities of materials are shown in Fig. 22. The numbers in square brackets after each locality correspond to those in Fig. 22.

Materials used for morphological and culture observations are follows. Ehime Pref. : Yugeshima [93] (11-v-1990, leg. S. Enomoto & K. Kogame), Fukuoka Pref. : Tsuyazaki [103] (21-iv-1989 leg. S. Kawaguchi, 3-v-1989 leg. S. Kawaguchi, 23-ii-1990 leg. S. Kawaguchi).

Herbarium specimens examined are follows. Aichi Pref. : Shizaki [74] (SAP 054387, 10-iii-1941 leg. S. Arasaki), Uchiumi [73] (SAP 025028, iv-1938 leg. N. Segi), Mie Pref. : Nakiri [77] (ii-1923 leg. Y. Yamada), Wakayama Pref. : Shirahama [80] (SAP 035217, 12-ii-1954 leg. K. Mitsuhashi), Ehime Pref. : Ikata [101] (SAP 034945, 26-iii-1955 leg. Y. Nomura; SAP 035323, 27-iii-1959 leg. Y. Nomura), Kochi Pref. : Muroto [99] (18-iv-1957 leg. N.

Tazawa), Konoura, Susaki [100] (19-iii-1957 leg. N. Tazawa), Yamaguchi Pref. : Awano [54] (SAP 049271, 24-ii-1944 leg. T. Tanaka), Fukuoka Pref. : Shingu [104] (SAP 048296, 11-iii-1956 leg. M. Ichiki), Tsuyazaki [103] (SAP 031116, 3-ii-1958 leg. T. Sawada), Nagasaki Pref. : Nomozaki [108] (SAP 028380, 20-i-1961 leg. K. Matsubayashi; SAP 035155, 6-iv-1977 leg. T. Yoshida), Kumamoto Pref. : Ushibuka [110] (25-iii-1934 leg. Takamatsu), Ohyano [111] (SAP 048298 27-xii-1959 leg. M. Ichiki), Tomioka [109] (SAP 049828, 5-iv-1955 leg. T. Yoshida).

Morphology

Macrothalli

Macrothalli consist of a saccate base and tubes which arise from the saccate base in cluster (Figs 23A-D). They are light yellowish brown to brown in color, and delicate in tubes, somewhat firm in saccate bases in texture. The tubes are simple, hollow throughout the length, tapered towards upper and lower ends, up to 25 cm in length and 1 cm in width. They sometimes become flattened with the ruffled margin. The saccate bases are adherent, usually 0.5-1.5 cm in diameter. In cross section, tubes consist of cortical and medullary layers, 90-130 µm in thickness (Figs 24A,B, 25A,B). The cortical layer consists of 1-3 layers of small, pigmented cells (Fig. 24C). The medullary layer consists of 3-4 layers of large, colorless cells. In cross section, medullary cells are round to ellipsoid, larger in the inner portions, 20-60 × 20-90 µm in size. In longitudinal sections, the medullary cells are longer in the inner portion, up to 240 µm in length (Fig. 24E). In sections, the saccate bases are up to 450-600 µm, and the medullary layers consist of 5-7 cells (Fig. 24F). Hairs are usually grouped, arising from the cortical layer or somewhat deep pits. Thalli attach to the substrata with rhizoidal filaments arising from the under surface of the saccate base (Fig. 24G). They become fertile with plurilocular sporangia

acropetally from the bases of the tubes. Plurilocular sporangia are formed by divisions of surface cells. Mature plurilocular sporangia are loosely coherent to each other, without a cuticle layer (Figs 24D, 25B). These plurilocular sporangia are uni- or biseriate, up to 40 µm in length. Unicellular ascocysts are present among plurilocular sporangia, ovoid or conical in shape, 16-18 × 6-10 µm in size (Figs 24D, 25B). They are shorter than plurilocular sporangia. Ascocysts remain after release of plurispores. Secondary plurilocular sporangia are in common occurrence. No unilocular sporangia are found on macrothalli.

Habitat and Phenology

Macrothalli grow on rocks and boulders in the lower intertidal zone although I have collected them only a few times. The thalli are collected during winter and spring.

Geographical distribution

According to Wynne and Norris (1976), this species is distributed in northern Gulf of California, Pacific Baja California, Pacific coast of Costa Rica, and southern and central Japan.

Culture study

Strains used in this study are shown in Table 14 in which culture conditions and progeny of plurispores are also shown.

No sexual reproduction could not be observed.

Plurispores (Fig. 26A) from macrothalli were grown at 5-20°C under both SD and LD. Settled plurispores germinated into prostrate branched filaments and developed into disks (Figs 26B-

E); however, most of germlings developed to tufts (Fig. 26F). Knotted and massive portions were sometimes observed on filaments of the tufts. These thalli grew under all conditions examined, even at 5°C. When these thalli were cultured on a rotary shaker under 20°C LD, crustose thalli developed (Fig. 25G). The crustose thalli consist of prostrate filaments and assurgent filaments arising from them. When the thalli detached from substratum, descending filaments were issued from the basal surface (Fig. 26H). These microthalli did not form any sporangia nor macrothalli.

Discussion

Colpomenia phaeodactyla had been confused with *C. bullosa* until it was recognized by Wynne and Norris (1976) who originally described it as a new species from the Pacific coast of Mexico. According to them, this species is characteristic with the delicate nature of the sacs and their clustered aspect from a common basal system, by which it is distinct from *C. bullosa*. Wynne and Norris (1976) examined Japanese materials and mentioned that this species occurs also in Japan. Morphology of Japanese materials examined in this study agrees with the description of the Mexican entity given by Wynne and Norris (1976). However, there are minor differences in the thickness of thalli and the shape of ascocysts. In my Japanese materials the thickness of vegetative tubes is 90-130 µm, while it is 70-88(110) µm according to Wynne and Norris (1976). This difference is considered to be insignificant since those ranges are overlap each other. Shape of ascocysts is ovoid in Japanese materials, but cylindrical in Mexican materials (Wynne and Norris 1976, Fig.

5). Although there are these differences, the Japanese materials was identified with *C. phaeodactyla* in the present study.

There is only one culture study of *C. phaeodactyla* (Wynne 1972a). In the culture of Mexican materials plurispores from macrothalli developed into prostrate filaments forming conical plurilocular sporangia. Plurispores from the plurilocular sporangia on the microthalli developed to spherical thalli which formed plurilocular sporangia. Unilocular sporangia were unknown (Wynne 1972a). A diagram of life history in the present culture is shown in Fig. 27. Tufted, discoid and massive thalli did not form any sporangia nor macrothalli in this study. These results are different from those of Wynne and Norris (1976). However, dominance of tufted thalli in culture suggests that culture conditions are not suitable for this species to grow, because tufted thalli are considered as abnormal growths. Therefore, the discoid shape is considered to be essential in the microthallus morphology of this species. Massive portions may be expressions of discoid portions without attaching to substrata.

Microthalli of this species have not been found in fields. More examinations are necessary to compare Japanese entity with Mexican *C. phaeodactyla*.

Table 14. Collection data of culture strains of *Colpomenia phaeodactyla*. Culture conditions and progeny of plurispores from macrothalli are also shown.

strain no.	locality	date	culture conditions	progeny
233	Tsuyszaki	3 v 1989	5-20SL	mostly tufts, rarely small massive thalli
234	Tsuyazaki	3 v 1989	15L	mostly tufts, rarely small massive thalli

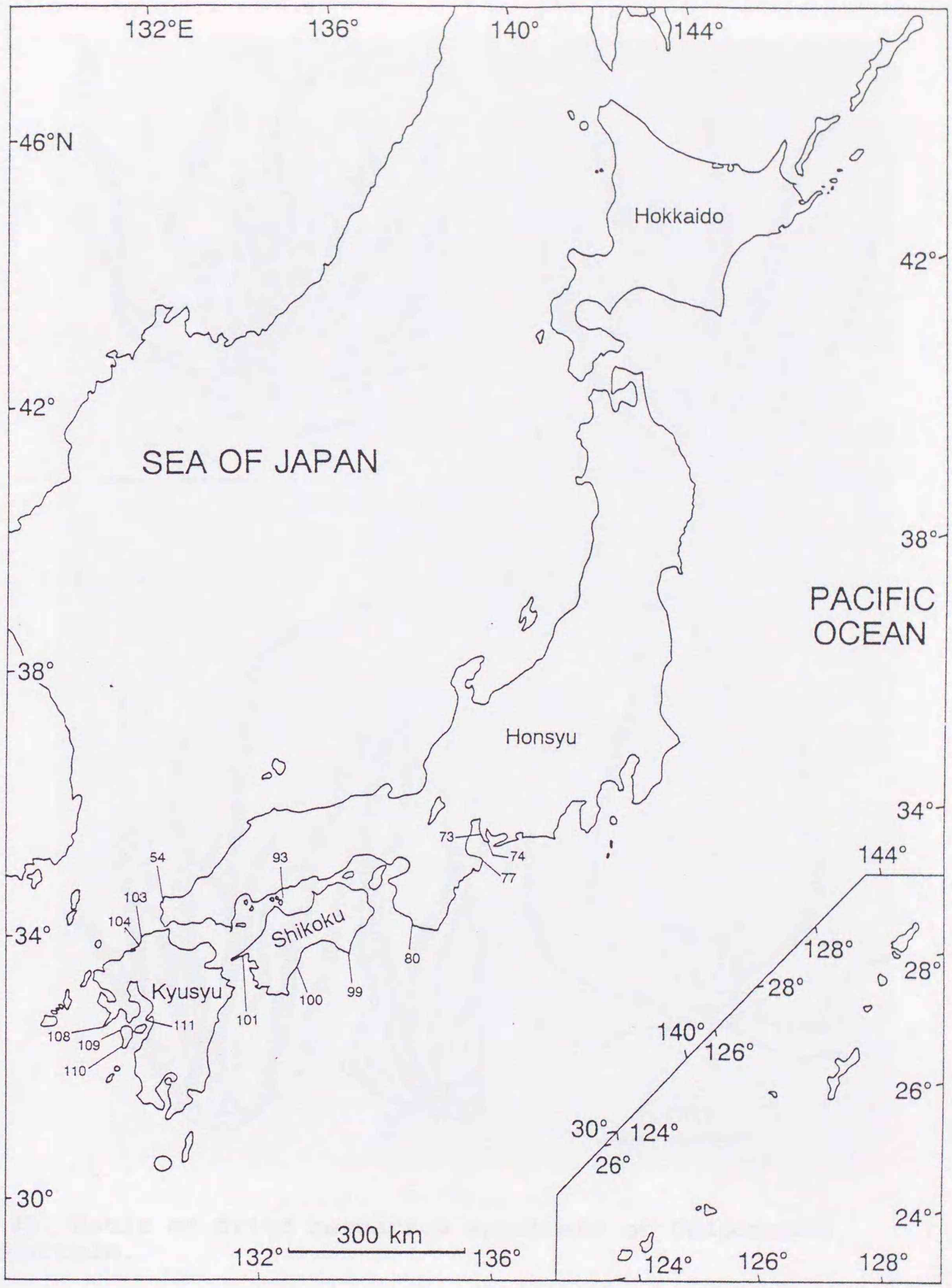


Fig. 22. Localities where materials of *Colpomenia phaeodactyla* were collected. Numbers plotted correspond to those in Table 1.

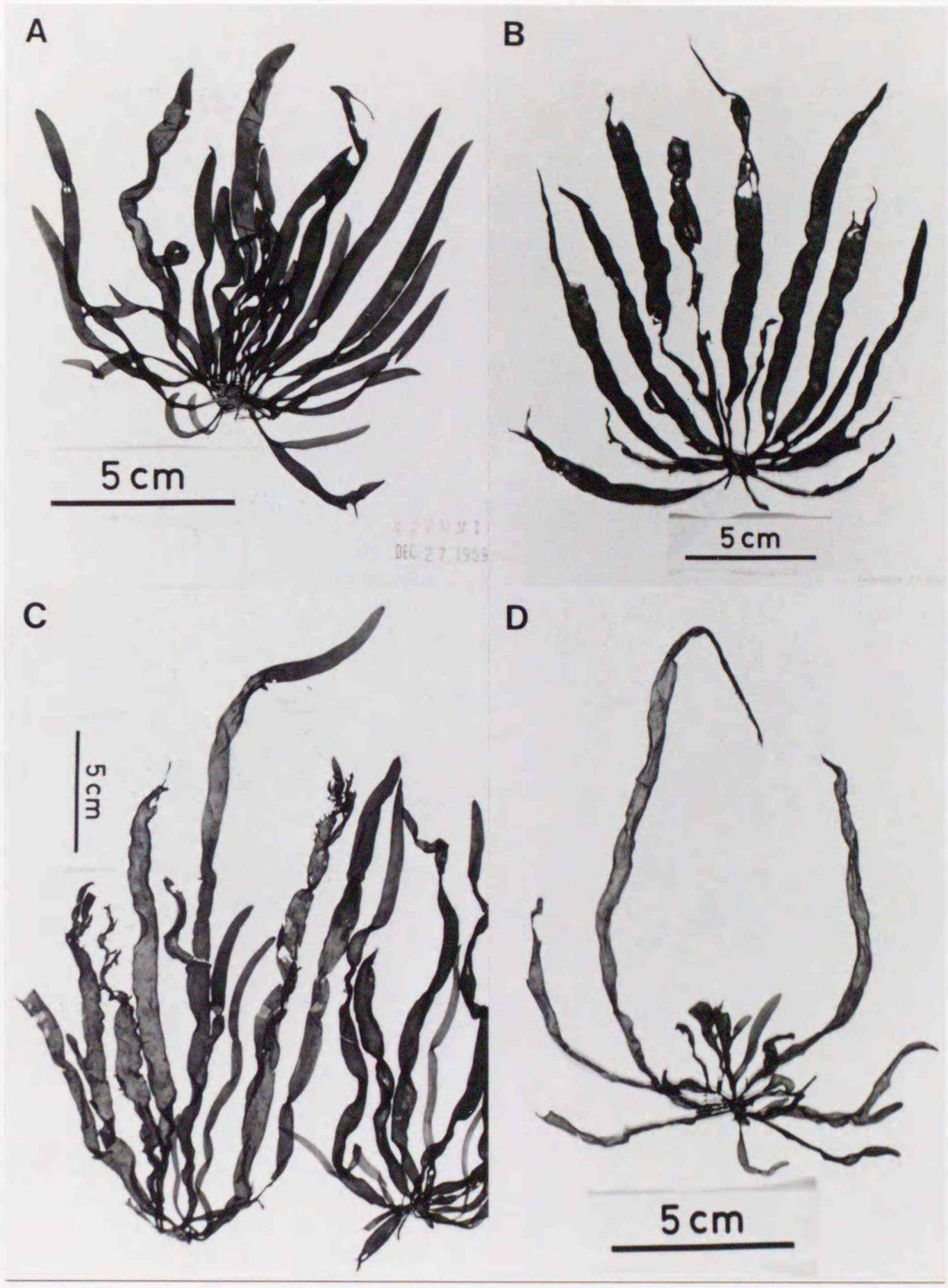


Fig. 23. Habit of dried herbarium specimens of *Colpomenia phaeodactyla*.

A. Herbarium specimen (SAP 048298, Misumi, Kumamoto Pref., 27 December 1959, leg. M. Ichiki). **B.** Plant collected at Tsuyazaki, Fukuoka Pref. on 21 April 1989 by S. Kawaguchi. **C.** Herbarium specimen (SAP 035333, Ikata, Ehime Pref., 27 March 1959, leg. Y. Yamada). **D.** Plant collected at Tsuyazaki, Fukuoka Pref. on 23 Feb. 1990 by S. Kawaguchi.

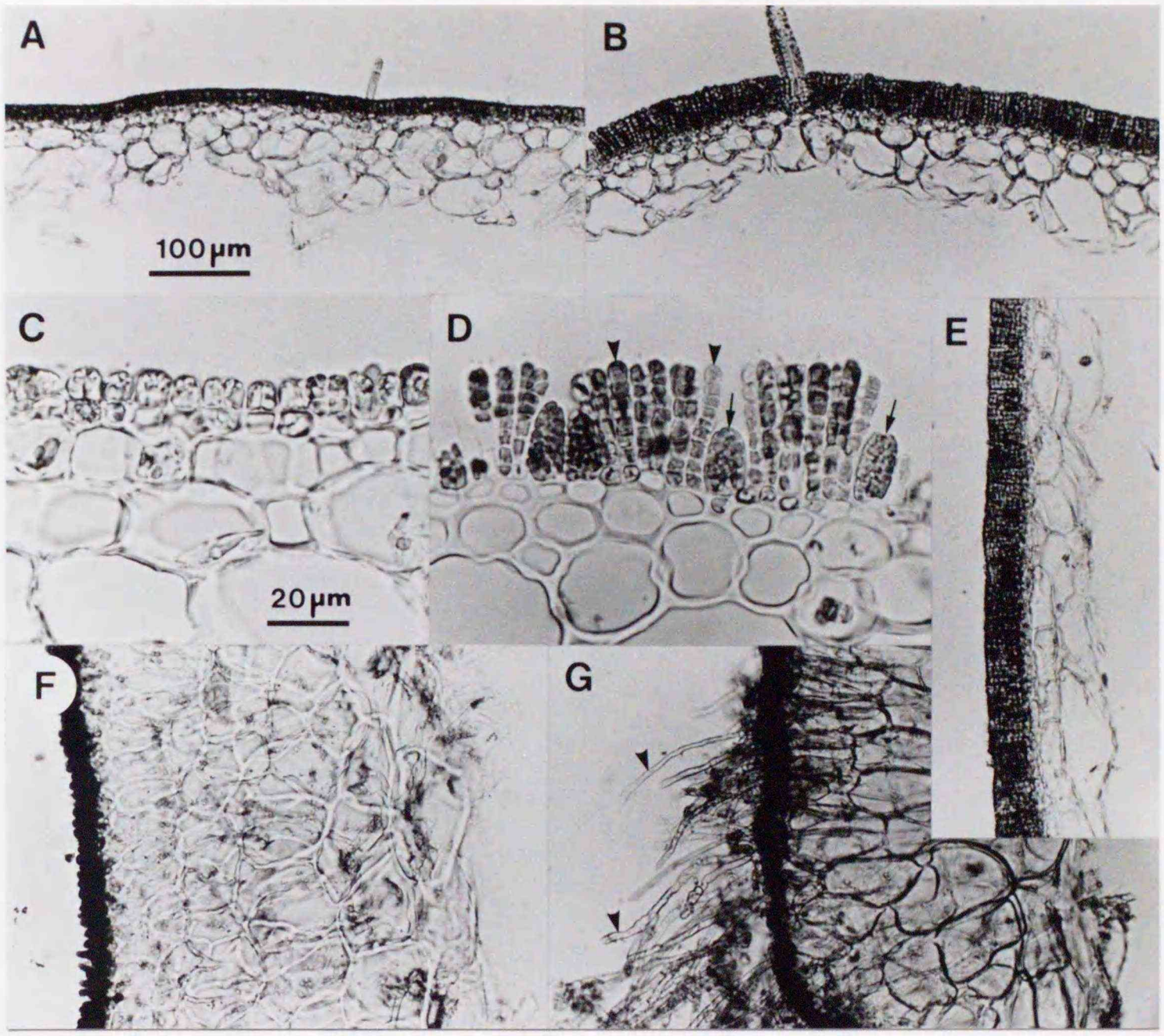


Fig. 24. Anatomy of *Colpomenia phaeodactyla*.

A, **B**. Cross sections of thalli. **C**. Cortical layer in cross section of thallus. **D**. Plurilocular sporangia (arrowheads) and ascocysts (arrows) in cross section of thallus. **E**. Longitudinal section of fertile thallus. **F**. Longitudinal section of basal saccate portion. **G**. Rhizoids (arrowheads) in longitudinal section of basal portion.

Scale in **A** applies to **B** and **E-G**; Scale in **C** applies to **D**.

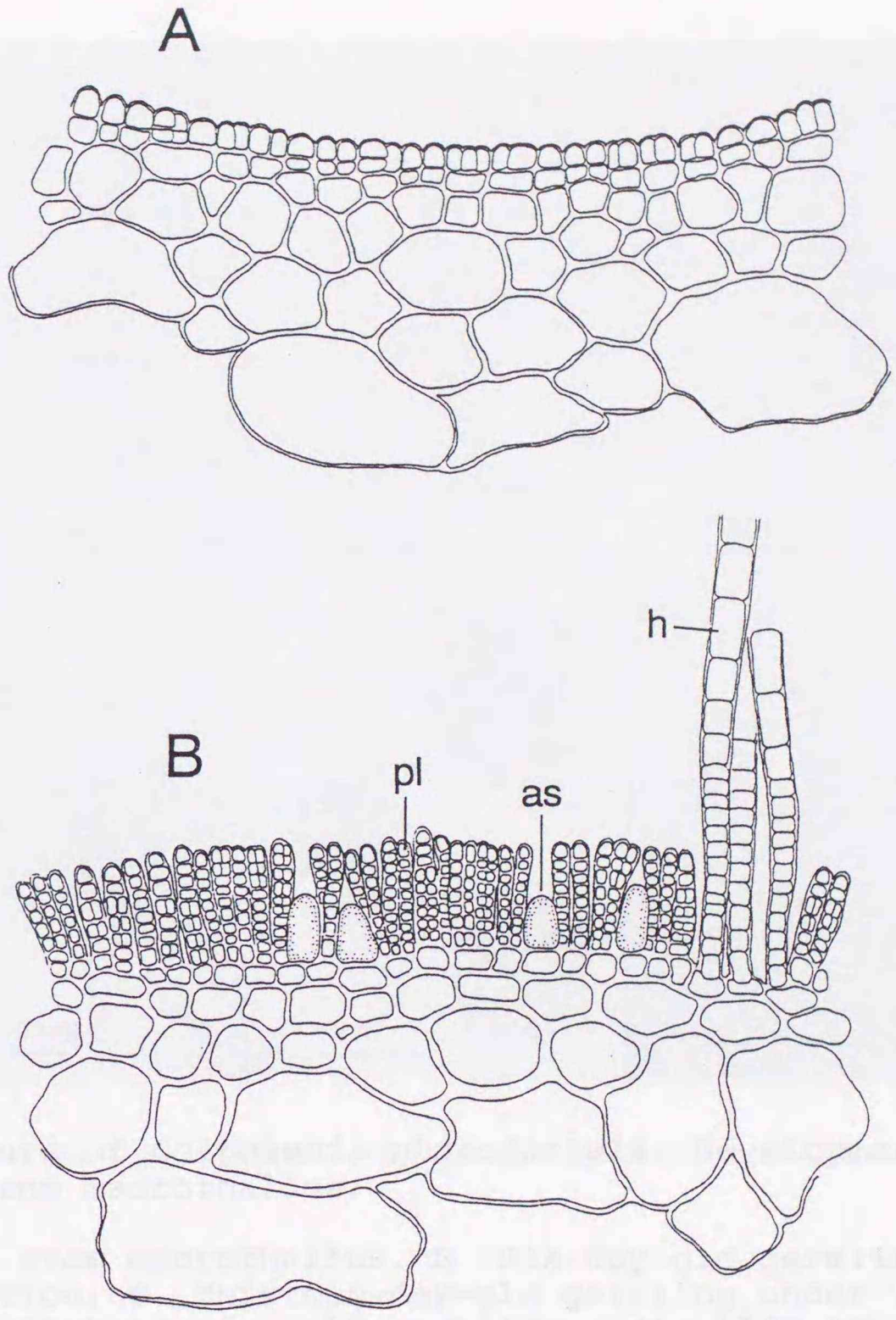


Fig. 25. Anatomy of *Colpomenia phaeodactyla*.

A. Cross section of vegetative thallus.
B. Cross section of fertile thallus, showing plurilocular sporangia (pl), ascocysts (as) and phaeophycean hair (h).

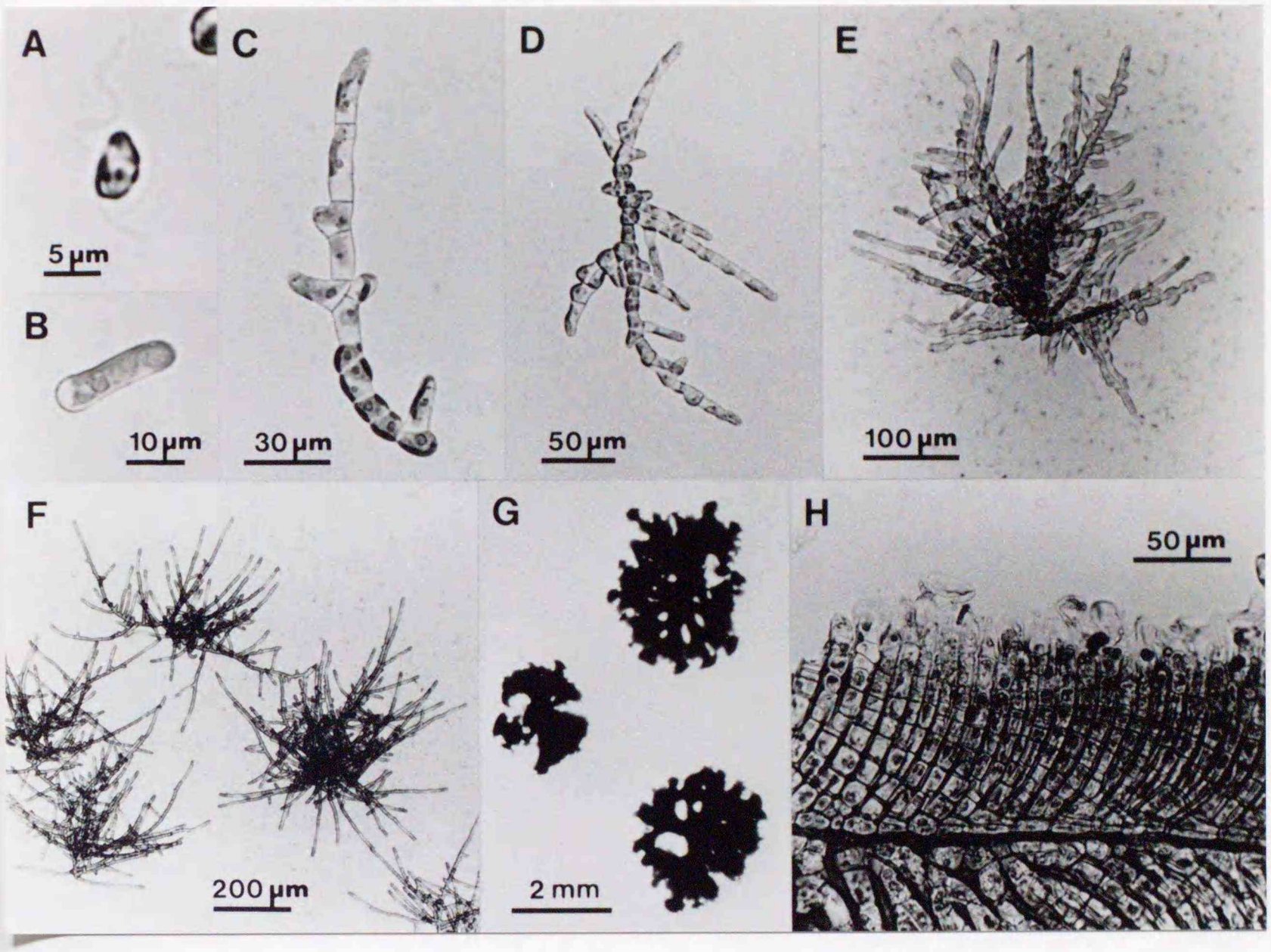


Fig. 26. Culture of *Colpomenia phaeodactyla*. Development of plurispores from macrothallus.

A. Plurispore from macrothallus. **B.** Six-day-old germling under 15°C LD condition. **C.** Thirteen-day-old germling under 15°C LD condition. **D.** Eighteen-day-old germling under 15°C LD condition. **E, F.** Twenty four-day-old germlings under 15°C LD condition, which are filamentous. **G.** Crustose microthalli cultured on rotary shaker under 20°C LD condition. **H.** Longitudinal section of crustose microthallus.

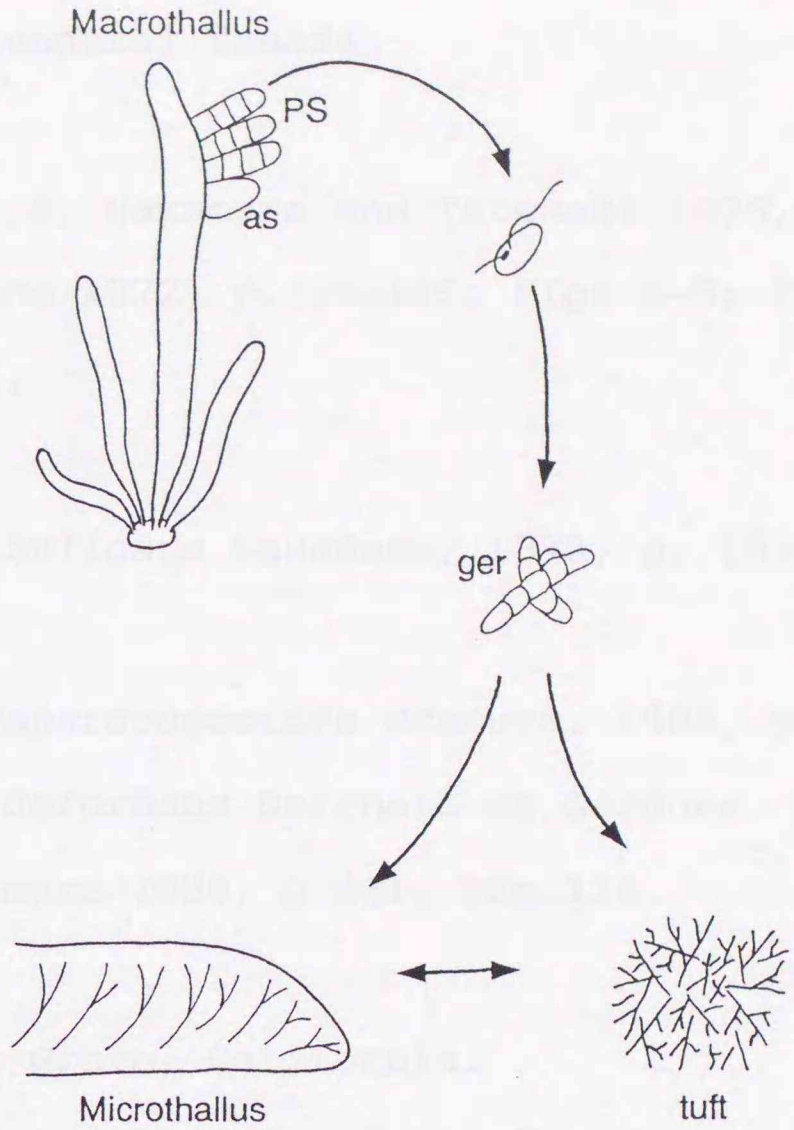


Fig. 27. Diagram of life history of *Colpomenia phaeodactyla* in culture.

as, ascocyst; ger, germling; PS, plurilocular sporangium.

Colpomenia bullosa (Saunders) Yamada

Yamada 1948, p.6-7, Pl.4; Nakamura and Tatewaki 1975, p.57-93, Figs 15-18, Pl.IV; Wynne 1972, p.133-145, Figs 3-5; Parsons 1982, p.289-301, Figs 4,5,14.

Basionym: *Scytosiphon bullosus* Saunders, 1898, p. 163, pl. 31, figs 1-7.

Synonym: *Macrosiphon asperococcoides* Okamura, 1902, p.118; *Colpomenia sinuosa* f. *deformans* Setchell et Gardner, 1903, p.242, pl.18, figs 13-15; Okamura 1936, p.231, Fig.124.

Type locality: Pacific Grove, California.

Lectotype: in herb. of De Alton Saunders, FH (Farlow Herbarium of Harvard University), *Scytosiphon bullosus*, Pacific Grove, Cal., Aug. 1896, near Bath House rocks off it (?) De A.S. The lectotype was designated by Parsons (1982).

Japanese name: Watamo

Materials

Localities of materials are shown in Fig. 28. The numbers in square brackets after each locality correspond to those in Fig. 28. Materials used for morphological and culture studies are as follows.

Macrothalli: Hokkaido: Oshoro [1] (11-iii-1987 leg. K. Kogame, 8-v-1987 leg. K. Kogame, 9-vi-1987 leg. K. Kogame, 18-iii-1988 leg. K. Kogame, 17-v-1992 leg. K. Kogame), Esashi [5] (15-v-1988 leg. K. Kogame), Matsumae [6] (10-i-1989 leg. I. Mine), Shirakamimisaki [8] (19-iii-1987 leg. K. Kogame, 16-v-1988 leg. K. Kogame), Kikonai [10] (18-iii-1987 leg. K. Kogame, 21-iv-1987 leg. H. Kawai), Esan [11] (17-v-1988 leg. K. Kogame), Muroran [14] (18-iv-1987 leg. K. Kogame, 28-i-1988 leg. K.

Kogame, 4-ii-1988 leg. K. Kogame, 21-ii-1988 leg. K. Kogame, 23-iii-1988 leg. K. Kogame, 12-xii-1988 leg. K. Kogame, 22-i-1989 leg. K. Kogame, 26-ii-1989 leg. K. Kogame, 18-iii-1990 leg. H. Kawai), Urakawa [16] (4-v-1989 leg. T. Kitayama), Harutachi [15] (15-v-1987 leg. K. Kogame, 5-iv-1988 leg. K. Kogame), Samani [17] (15-v-1987 leg. K. Kogame, 5-iv-1988 leg. K. Kogame, 11-ii-1989 leg. K. Kogame, 19-v-1991 leg. K. Kogame), Utabetsu [18] (6-iv-1988 leg. K. Kogame), Erimo [19] (16-v-1987 leg. K. Kogame, 19-v-1991 leg. K. Kogame), Syoya [20] (12-ii-1989 leg. K. Kogame), Hiroo [21] (19-v-1991 leg. K. Kogame), Akkeshi [22] (8-ix-1987 leg. K. Kogame, 29-vi-1988 leg. K. Kogame, 26-vi-1991 leg. K. Kogame, 29-vi-1991 leg. K. Kogame, 17-vi-1992 leg. K. Kogame), Nosappu [25] (21-xii-1987 leg. K. Kogame), Nokkamappu [26] (15-iv-1987 leg. K. Kogame, 22-xii-1987 leg. K. Kogame), Seseki [27] (1-vii-1988 leg. K. Kogame), Aomori Pref. : Ohma [38] (3-iii-1987 leg. K. Kogame, 21-ii-1988 leg. H. Kawai, 1-ii-1990 leg. K. Kogame, 29-iv-1990 leg. K. Kogame), Akita Pref. : Iwadate [42] (7-iv-1991 leg. T. Kitayama), Fukui Pref. : Takahama [51] (1-iv-1987 leg. H. Kawai), Hyogo Pref. : Maruyama, Awajishima [83] (3-iv-1992 leg. S. Enomoto & K. Kogame), Kamishima, Ieshima Isls [84] (31-iii-1990 leg. S. Arai), Kagawa Pref. : Awashima [89] (5-iv-1989 leg. S. Enomoto & K. Kogame), Megishima [86] (6-iv-1989 leg. S. Enomoto & K. Kogame), Okayama Pref. : Manabeshima [88] (4-iv-1989 leg. S. Enomoto & K. Kogame), Kitagishima [87] (3-iv-1989 leg. S. Enomoto & K. Kogame).

Microthalli: Hokkaido : Oshoro [1] (22-x-1988 leg. K. Kogame, 22-xi-1988 leg. K. Kogame, 23-ix-1989 leg. K. Kogame, 4-xi-1989 leg. K. Kogame, 2-xii-1989 leg. K. Kogame, 24-x-1990 leg. K. Kogame, 24-xi-1990 leg. K. Kogame), Muroran [14] (28-x-1988 leg. K. Kogame, 12-xi-1988 leg. K. Kogame, 12-xii-1988 leg. K. Kogame, 22-i-1989 leg. K. Kogame, 1-iii-1989 leg. K. Kogame, 12-xii-1989 leg. K. Kogame), Utabetsu [18] (11-ii-1989 leg. K. Kogame), Notorimisaki [30] (26-viii-1989 leg. K. Kogame), Aomori Pref. : Ohma [38] (1-ii-1990 leg. K. Kogame, 24-ix-1991 leg. K. Kogame).

Herbarium specimens examined are follows. Hokkaido: Teuri Isl. [36] (SAP 044223, 13-ii-1982 leg. M. Marui), Iwate Pref. : Ohtsuchi [56] (SAP 053179, 26-v-1979 leg. S. Kawaguchi; SAP 054641, 21-iii-1985 leg. J. Tanaka), Miyagi Pref. : Kesennuma [57] (x-1955 leg. M. Kurogi), Murohama, Naruse [58] (SAP 035282, 8-ii-1965 leg. T. Yoshida), Chiba Pref. : Inubosaki [62] (11-ii-1924, leg. M. Noda), Fukui Pref. : Takahama [51] (1-iv-1987 leg. K. Ikehara), Mie Pref. : Sugashima [76] (21-iv-1955 leg. Y. Tsuji), Kumamoto Pref. : Tomioka [109] (SAP 021533, iii-1939 leg. Y. Yamada), Ohyano [111] (SAP 048297, 15-iii-1960 leg. M. Ichiki).

Morphology

Macrothalli

Macrothalli consist of clusters of long, hollow tubes arising from a saccate base (Figs 29A-C). The tubes taper to the apex and base, up to 30 cm in length, 2-4 cm in width, sometimes

flattened, raffled in the margin, yellowish brown in color. Young thalli less than 5 mm in diameter are spherical with a small projection which grow into a tube (Fig. 29D). In transverse section, thalli consist of a cortical and a medullary layer, 120-230 µm in thickness (Figs 30A,B, 31A). The cortical layer consists of small, pigmented cells, 2-3 cells in thickness (Figs 30C, 31A). The medullary layer consists of round or ellipsoid, colorless cells, 3-5 cells in thickness. In longitudinal section, inner medullary cells are longer, up to 180-250 µm in length (Fig. 30G). The saccate base is folded, irregular in shape, 1-3 cm in diameter. In section the saccate bases are 350-700 µm in thickness, consisting of 4-6 medullary cell layers (Fig. 30H). Hairs are grouped, arising from the cortical layer or rather deep pits. Thalli attach to substrata with rhizoidal filaments from the under surface of the saccate base (Figs 30I,J). Thalli become fertile acropetally from the base of the tubes, forming plurilocular sporangia. Mature plurilocular sporangia are loosely coherent to each other, without a cuticle layer covering on them (Figs 30E, 31C). Plurilocular sporangia are uni- or biseriate, 30-36 µm in length. Unicellular ascocysts are cylindrical or clavate, among plurilocular sporangia (Figs 30D,E, 31B,C). The ascocysts remain after release of plurispores (Fig. 30F). Secondary plurilocular sporangia are common. No unilocular sporangia are observed on macrothalli.

Microthalli

Microthalli are discoid, small, 1-5 mm in diameter, yellowish brown to brown in color. Thalli consist of prostrate filaments and assurgent filaments developing from them and some

thalli bear also downwardly curved filaments (Figs 32A,C,D). Cells of assurgent filaments are smaller in the upper portions, forming the cortical layer (Fig. 31B). These filaments are firmly coherent to each other. Thickness of thalli is up to 200-700 µm. Cells of the medullary layer are sometimes reddish brown in color, 25-40 µm in length and 11-20 µm in width. Fertile thalli form unilocular sporangia laterally on the bases of paraphyses which arise from the cortical layer (Figs 32C-F, 33A-C). The paraphyses are 100-200 µm in length. Their cells are shorter in the upper portion, 8-14 µm in length and 9-12 µm in width, and they are long in the lower portion, 18-50 µm in length and 8.5-10 µm in width. Sessile unilocular sporangia are clavate in shape, 50-120 × 20-26 µm in size (Figs 32D-F, 33A-C). Unicellular ascocysts are present among the paraphyses (Figs 32F,G, 33A). They are cylindrical, clavate, 50-120 × 9-14 µm in size, usually pedicellate on the surface cells of the thalli. Cells of thalli contain one plastid with a large pyrenoid, which is typical of scytosiphonean algae (Fig. 32B).

Habitat and Phenology

Macrothalli grow on rocks or boulders, rarely on other algae, in the lower intertidal to subtidal zones of sheltered places from wave action. They are generally found during winter and spring. At Oshoro, Hokkaido macrothalli appear in winter, persisting until spring, and disappear from summer onward (Table 15). However, at Akkeshi and Nemuro, Hokkaido they are found also in summer.

Microthalli are epilithic on rocks or boulders or epizoic on limpet shells, in the lower intertidal to subtidal zones. At

Oshoro, Hokkaido microthalli with unilocular sporangia are found during autumn and winter (Table 15). Since identification of vegetative thalli are difficult, their collection data are not shown in this study.

Geographical distribution

Colpomenia bullosa is distributed on the coast of the north Pacific (Alaska to central California, Abbott and Hollenberg 1976; Japan, Yamada 1948). Although this species was found also in New Zealand by Parsons (1982), she mentioned that it might be imported by ships from Japan or North America. This species has not been found in Australia (Wormersly 1987) nor Europe (Hamel 1931-1939, Fletcher 1987). In Japan, this species is widely found along the coasts of Hokkaido, Honshu, Shikoku and Kyusyu (Fig. 28).

Culture study

Strains isolated from macrothalli are listed in Table 16. Culture conditions and progeny of plurispores are also shown in Table 16.

Sexual reproduction

Gamete fusions were observed in materials from Oshoro, Muroran and Harutachi, Hokkaido.

Gametophytic macrothalli are dioecious, and gametes are isogamous. Gametes are pear drop-shaped, of normal phaeophycean type with one plastid with a stigma (Fig. 34A). They showed negative phototaxis. When female and male gametes were mixed, a settled female gamete was surrounded by several male gametes

which touched the settled female gamete by anterior flagella, and then, one of male gametes fused with the female gamete (Figs 34B-E). Zygotes were distinguishable from unfused gametes by possessing two stigmata (Fig. 35F).

Further development of zygotes could not be followed.

Development of plurispores from macrothalli

Plurispores from macrothalli were grown at 5-20°C under both LD and SD conditions. Most germlings from plurispores grew into tufts (Fig. 35A), and some developed into firm, pseudo-parenchymatous masses, through small discoid thalli (Fig. 35B). The massive thalli were composed of closely packed branched filaments whose cells were large and long in the medulla and small, pigmented in the cortex (Fig. 35C). The massive thalli became free from the substrata, and grew up to 2-3 mm in diameter. They formed unilocular sporangia under 15°C SD, 20°C SD and 20°C LD. Unilocular sporangia formed laterally on the bases of paraphyses arising from the surface of the massive thalli (Figs 35D,E). Clavate ascocysts also formed among the paraphyses (Fig. 35F). Tufted thalli did not form any kind of sporangia.

Development of unispores from microthalli

Unispores released from unilocular sporangia on microthalli were cultured under 15°C SD and 20°C SD conditions. Unispores developed mostly into tufts, sometimes into massive thalli, as did plurispores from macrothalli (Table 17).

Culture from field microthalli

Strains isolated from field microthalli are shown in Table

18 in which culture conditions and progeny of unispores are also shown.

Cultures were started from unilocular sporangia isolated from field microthalli. The isolated unilocular sporangia were cultured mainly under a 5°C SD condition. Unispores released from the unilocular sporangia grew into tufted thalli which consisted of branched filaments with knots (Figs 36I,J). Some of thalli developed into disks, but they became tufted, giving rise to branched filaments from the surface of them. Firm massive thalli were sometimes observed (Figs 36K-L). The massive thalli became fertile with unilocular sporangia under a 15°C LD condition (only strain 243 Notorimisaki). Unilocular sporangia were formed laterally on the bases of paraphyses arising from the surface of the thalli. Clavate ascocysts were present among the paraphyses.

In strain 173 Muroran, unispores (Fig. 35A) from unilocular sporangia isolated were grown under 10°C LD. They developed into small disks (Figs 36B-F). The disks became swollen, and developed into spherical thalli (Figs 36G,H). After three months, spherical thalli grew to 1 mm in diameter and formed plurilocular sporangia associated with ascocysts (Fig. 36H). Plurispores from the plurilocular sporangia were gametes which fused well to form zygotes.

Discussion

Colpomenia bullosa (Saunders) Yamada was originally described as *Scytosiphon bullosus* from California by Saunders (1898). Later, Setchell and Gardner (1903) described *C. sinuosa* f. *deformans*, including *S. bullosus* as a synonym. In Japan, Okamura (1936) gave Japanese materials the name *C. sinuosa* f.

deformans S. et G., according to them. However, Yamada (1948) considered this species as a separate species from *C. sinuosa*, and named *Colpomenia bullosa* (Saunders) Yamada for it, which is generally accepted now.

Based on the present study, this species can be characterized by possession of a few of tubes arising from a saccate base, plurilocular sporangia loosely adhering with each other without a cuticle layer, isogamy, thickness of the sacs being 120-230 μm, and firm crustose microthalli with ascending and descending, branched filaments.

Culture study of *C. bullosa* had been carried out with materials from Muroran, Hokkaido by Nakamura and Tatewaki (1975). They clearly showed that this species has a sexual heteromorphic life history.

The diagram of life history of this species based on the present study is shown in Fig. 37. In this study, an isogamous sexual fusion was observed in the materials from Muroran, Harutachi and Oshoro, Hokkaido. Such sexual fusion was also reported by Nakamura and Tatewaki (1975). In culture of plurispores from macrothalli, tufted thalli were dominant under all conditions tested. It was difficult to obtain crustose microthalli and macrothalli, owing to the dominance of tufts in this culture. The tufted thalli were also observed by Nakamura and Tatewaki (1975). The tufts are considered to be filaments which did not undergo normal morphogenesis. Therefore, present culture methods may be not fit for this species.

In this study, microthalli identical with those of *C. bullosa* were collected in fields, and they were cultured in the laboratory. The microthalli are characteristic with discoid

thalli consisting of upwardly and downwardly curved branched filaments, forming unilocular sporangia laterally on the bases of the paraphyses and forming ascocysts among the paraphyses. These thalli have not been described nor named previously. In culture from field microthalli, tufts were dominant, and tubular macrothalli were not obtained. However, the field microthalli were recognized as those of *C. bullosa* from morphological comparison between field microthalli and cultured microthalli from macrothalli. Spherical thalli bearing plurilocular sporangia in culture from field microthalli are considered to have become fertile without producing tubular thalli. It is considered that culture conditions were not suitable as in the culture of plurispores from macrothalli, since tubular thalli were not obtained in the culture.

Table 15. Collection data of *Colpomenia bullosa* at Oshoro, Hokkaido. (P, thalli with plurilocular sporangia; U, thalli with unilocular sporangia; (U), microthalli bearing unilocular sporangia identified morphologically, not cultured; --, thallus was not found)

Macrothalli

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
1987					P	P		--	--		--	--
1988	--	--	P							--	--	
1989			P			--		--	--		--	--
1990		P	P	--			--		--	--	--	
1991		--										
1992			P	P								

Microthalli

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
1988										U	U	
1989								(U)			U	U
1990		--	--							(U)	(U)	
1991		--										

Table 16. Collection data of cultured strains of *Colpomenia bullosa*. Culture conditions and progeny of plurispores from macrothalli are also shown.

strain no.	locality	date	sex	culture conditions	progeny
4	Oshoro	11 iii 1987		10L, 20S	tufts, massive thalli (U; 20S)
18	Takahama	1 iv 1987		10SL, 15SL	tufts, massive thalli (U; 15S)
21	Nokkamappu	15 iv 1987		10-20L	tufts, massive thalli
87	Muroran	28 i 1988		10SL	tufts, massive thalli
338	Muroran	15 ii 1991	female	10-20SL	tufts, massive thalli (U; 15L→15S)
339	Muroran	15 ii 1991	male	5-20SL	tufts, massive thalli

Table 17. Culture of *Colpomenia bullosa*. Progeny of unispores from microthallus.

strain no.	locality	date	sex	culture conditions	progeny
4	Oshoro	11 iii 1987		20S	tufts, massive thalli
18	Takahama	1 iv 1987		15S	tufts, massive thalli
338	Muroran	15 ii 1991	female	15S	tufts, massive thalli

Table 18. Culture of *Colpomenia bullosa*. Progeny of unispores from field microthalli.

strain no.	locality	date	culture conditions	progeny
172	Oshoro	25 x 1988	5S	tufts, massive thalli
173	Muroran	28 x 1988	10L	tufts, macrothalli (P)
184	Muroran	12 xi 1988	5S, 10L	tufts, massive thalli
186	Oshoro	22 xi 1988	5SL	tufts, massive thalli
192	Muroran	12 xii 1988	5SL	tufts, massive thalli
243	Notorimisaki	26 viii 1989	5-20SL	tufts, massive thalli (U; 15L)
251	Oshoro	4 xi 1989	5S	tufts, massive thalli
258	Oshoro	2 xii 1989	5S	tufts, massive thalli
259	Muroran	12 xii 1989	5S	tufts, massive thalli

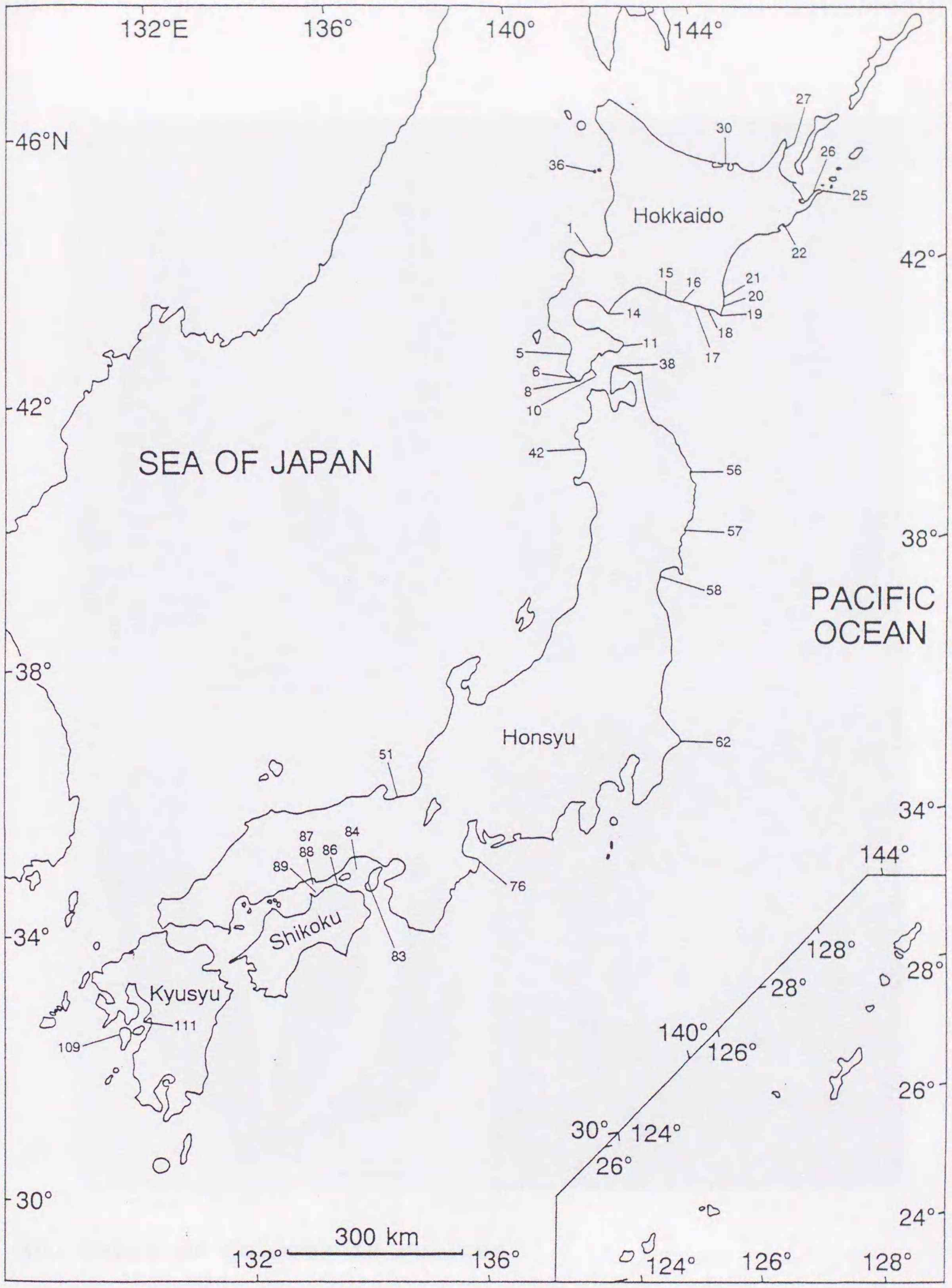


Fig. 28. Localities where materials of *Colpomenia bullosa* were collected. Numbers plotted correspond to those in Table 1.

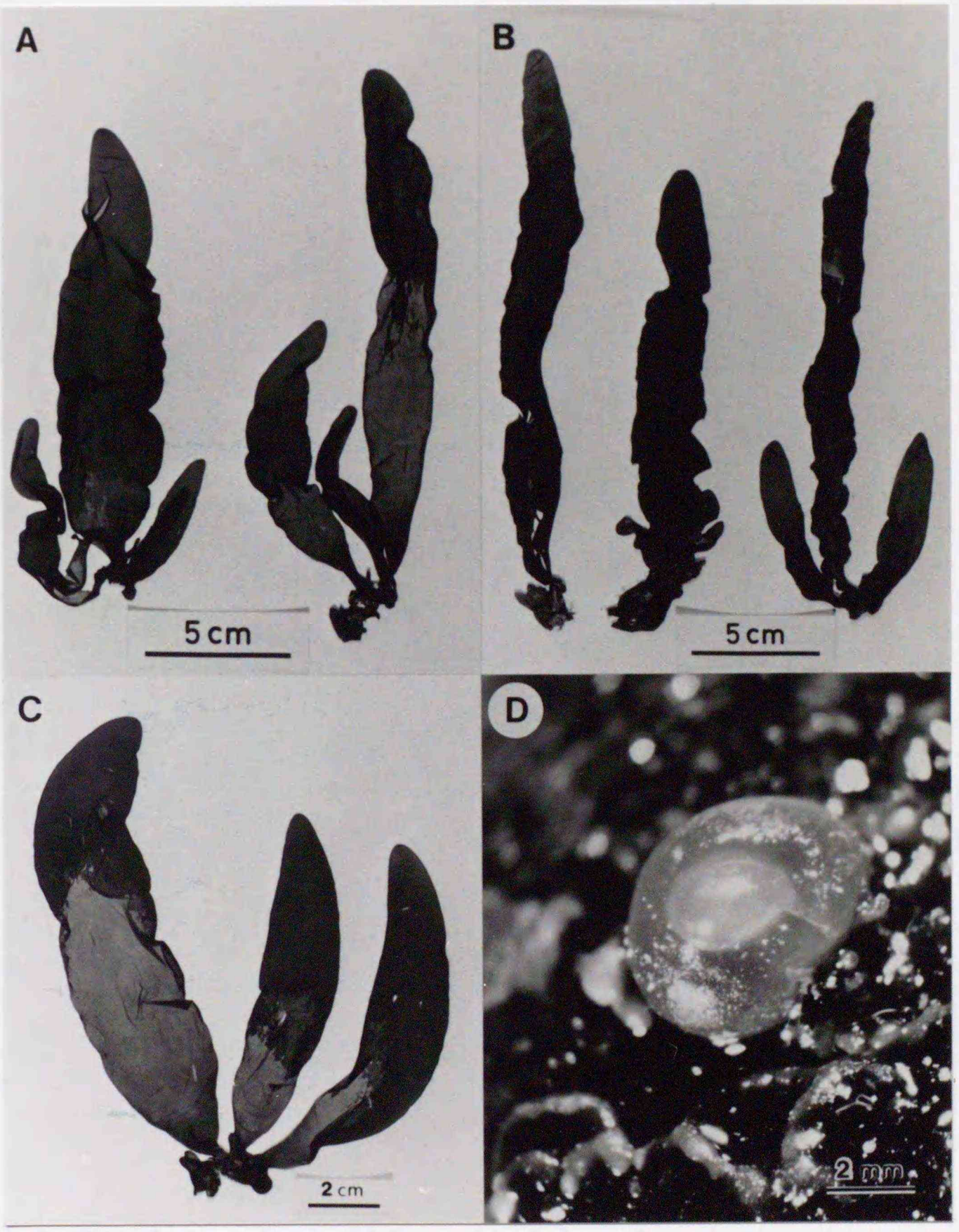


Fig. 29. Habit of *Colpomenia bullosa*.

A, C. Plants collected at Muroran, Hokkaido on 21 February 1988.
B. Plants collected at Harutachi, Hokkaido on 5 April 1988.
D. Juvenile macrothallus collected at Muroran, Hokkaido on 12 December 1989. Note an initial of a tube in the upper end of the thallus.

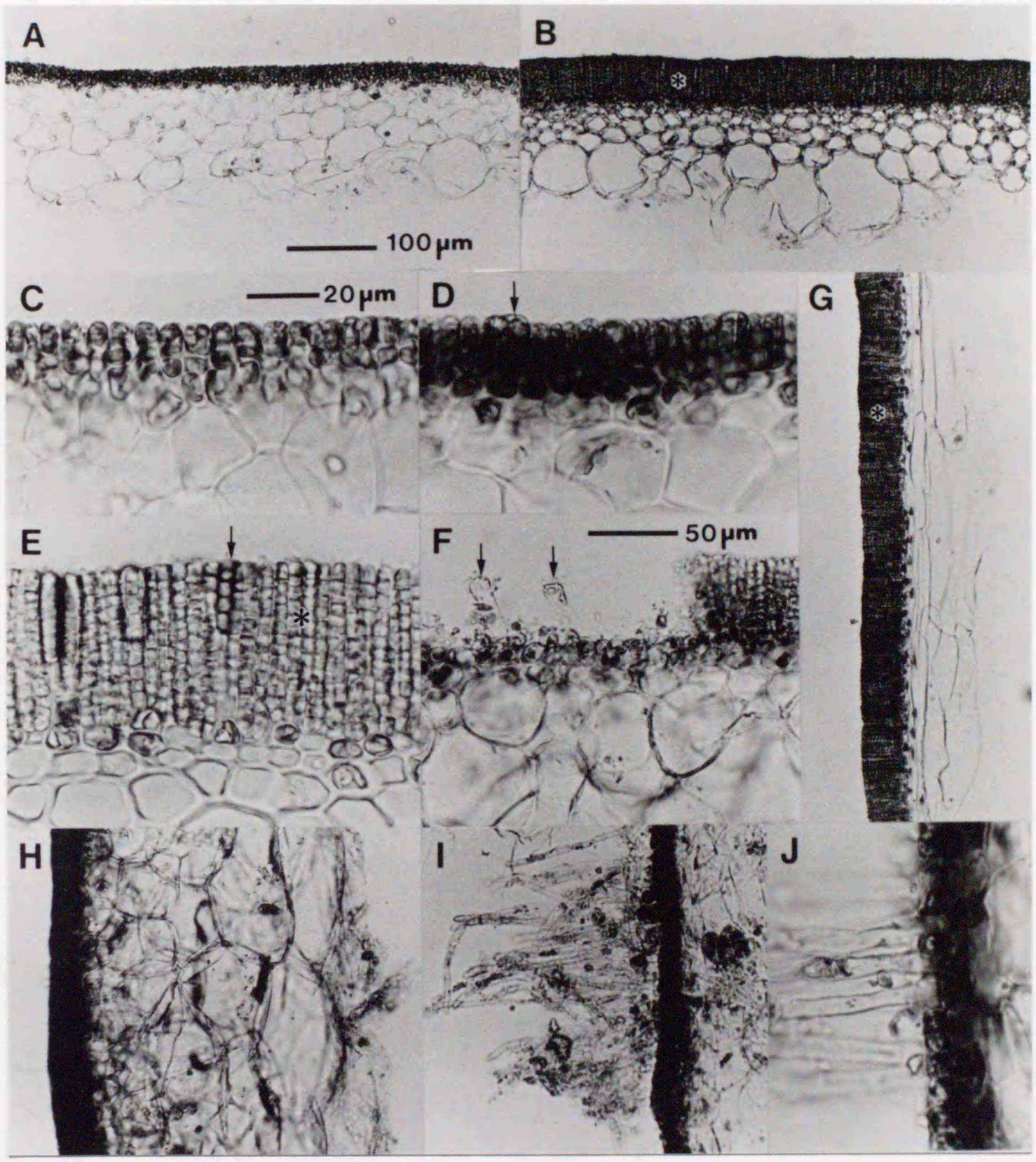


Fig. 30. Anatomy of *Colpomenia bullosa*.

A, B. Cross sections of thalli (asterisk, plurilocular sporangia). **C.** Cortical layer in cross section of thallus. **D.** Young plurilocular sporangia and ascocysts (arrow) in cross section of thallus. **E.** Plurilocular sporangia (asterisk) and ascocysts (arrow) in cross section of thallus. **F.** Ascocysts (arrows) after release of plurispores in cross section of thallus. **G.** Longitudinal section of thallus. **H.** Longitudinal section of basal spherical portion. **I, J.** Rhizoids arising from surface of basal spherical portion in longitudinal section.

Scale in **A** applies to **B** and **G-I**; Scale in **C** applies to **D**, **E** and **J**.

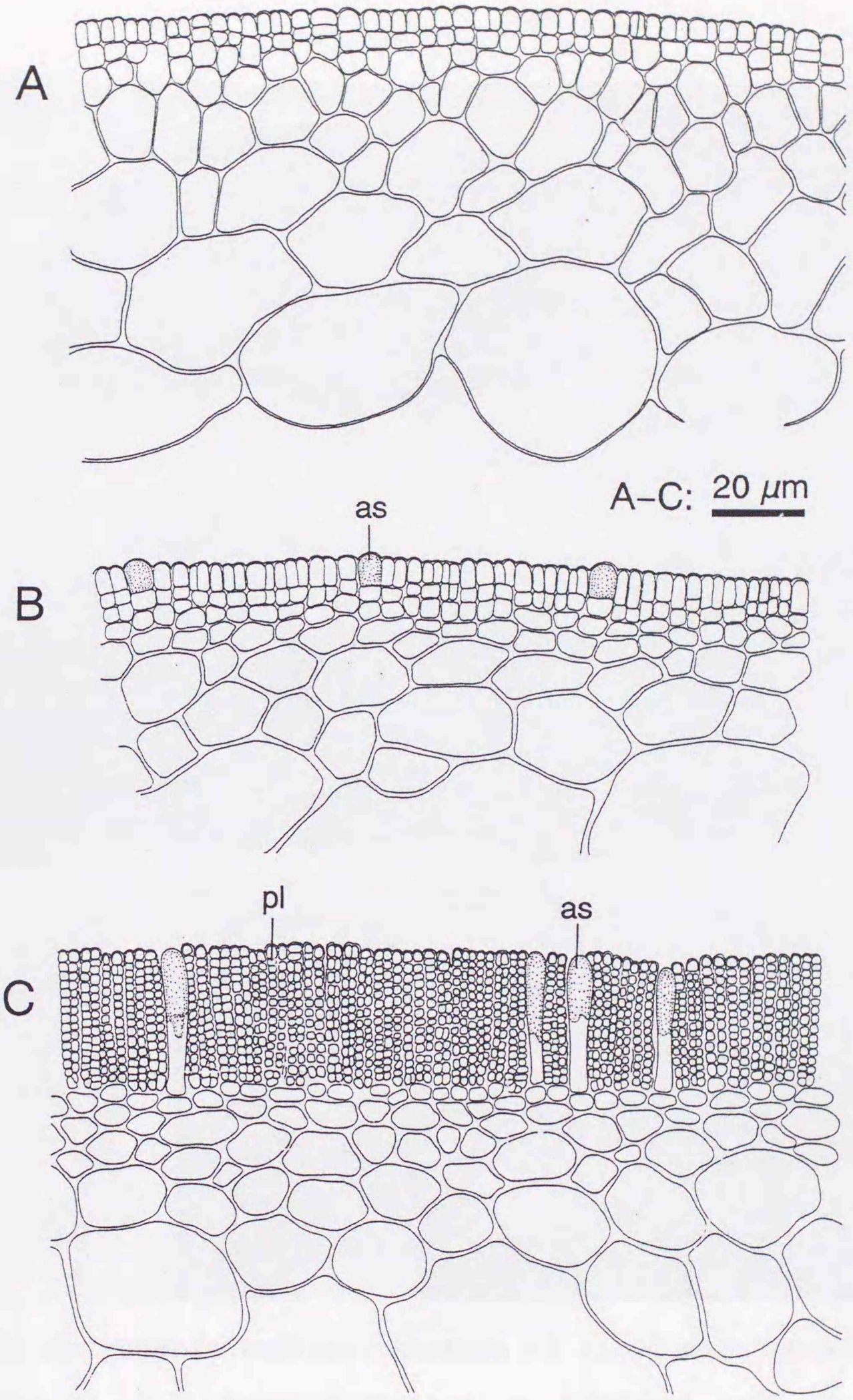


Fig. 31. Anatomy of *Colpomenia bullosa*.

A. Cross section of vegetative thallus. **B.** Cross section of thallus with young ascocysts (as) in the early stage of formation of plurilocular sporangia. **C.** Cross section of fertile thallus, showing plurilocular sporangia (pl) and ascocysts (as).

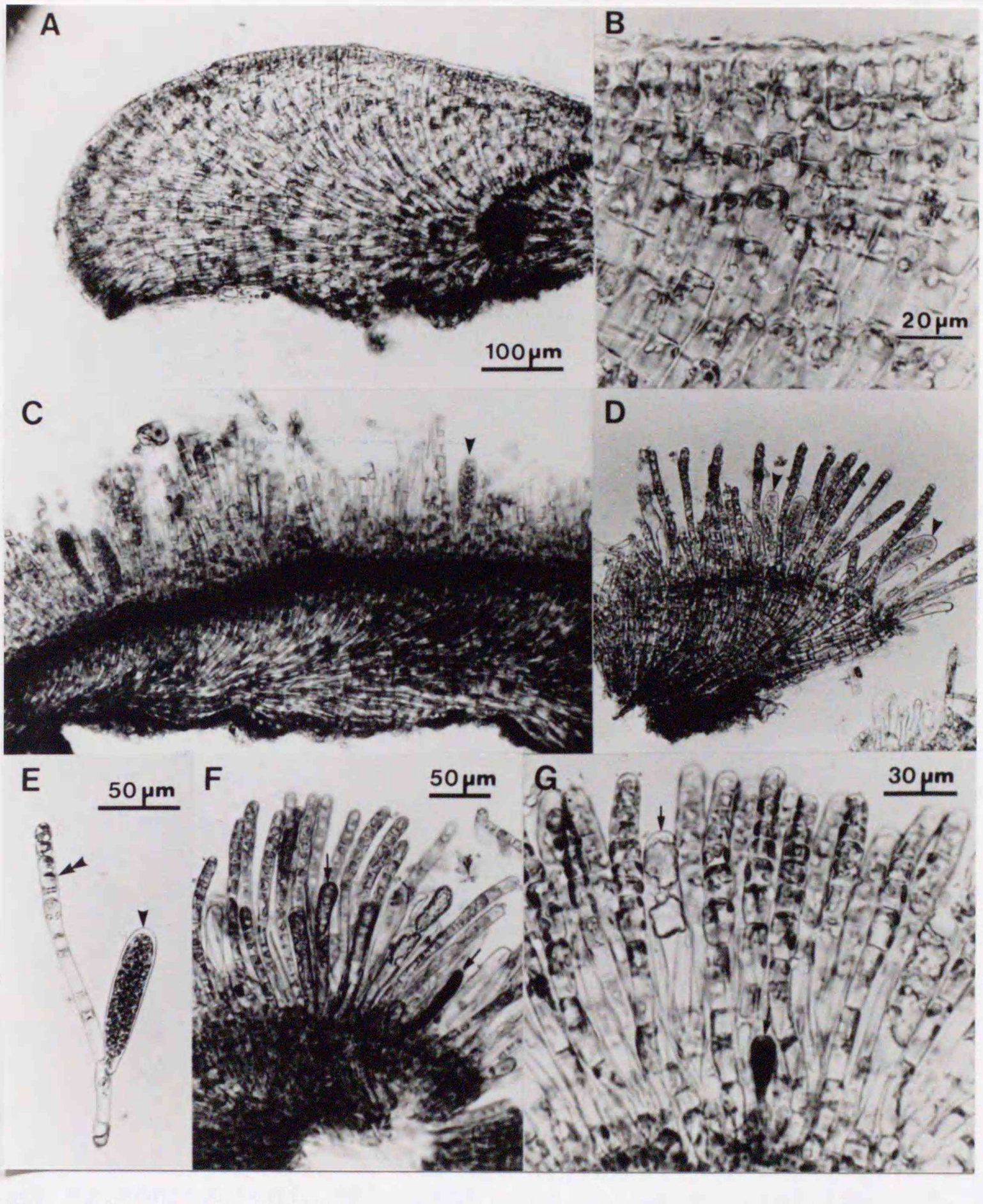


Fig. 32. *Colpomenia bullosa*. Anatomy of field microthalli.

A. Longitudinal section of thallus. **B.** Marginal part of longitudinal section of thallus. Note cells with a plastid and pyrenoid per cell. **C.** Longitudinal section of thallus forming unilocular sporangia (arrowhead), paraphyses and ascocysts. **D.** Squashed fertile thallus with unilocular sporangia (arrowheads). **E.** Unilocular sporangium (arrowhead) on a basal cell of a paraphysis (double arrowhead). **F, G.** Ascocysts (arrows) among paraphyses.

Scale in **A** applies to **C** and **D**.

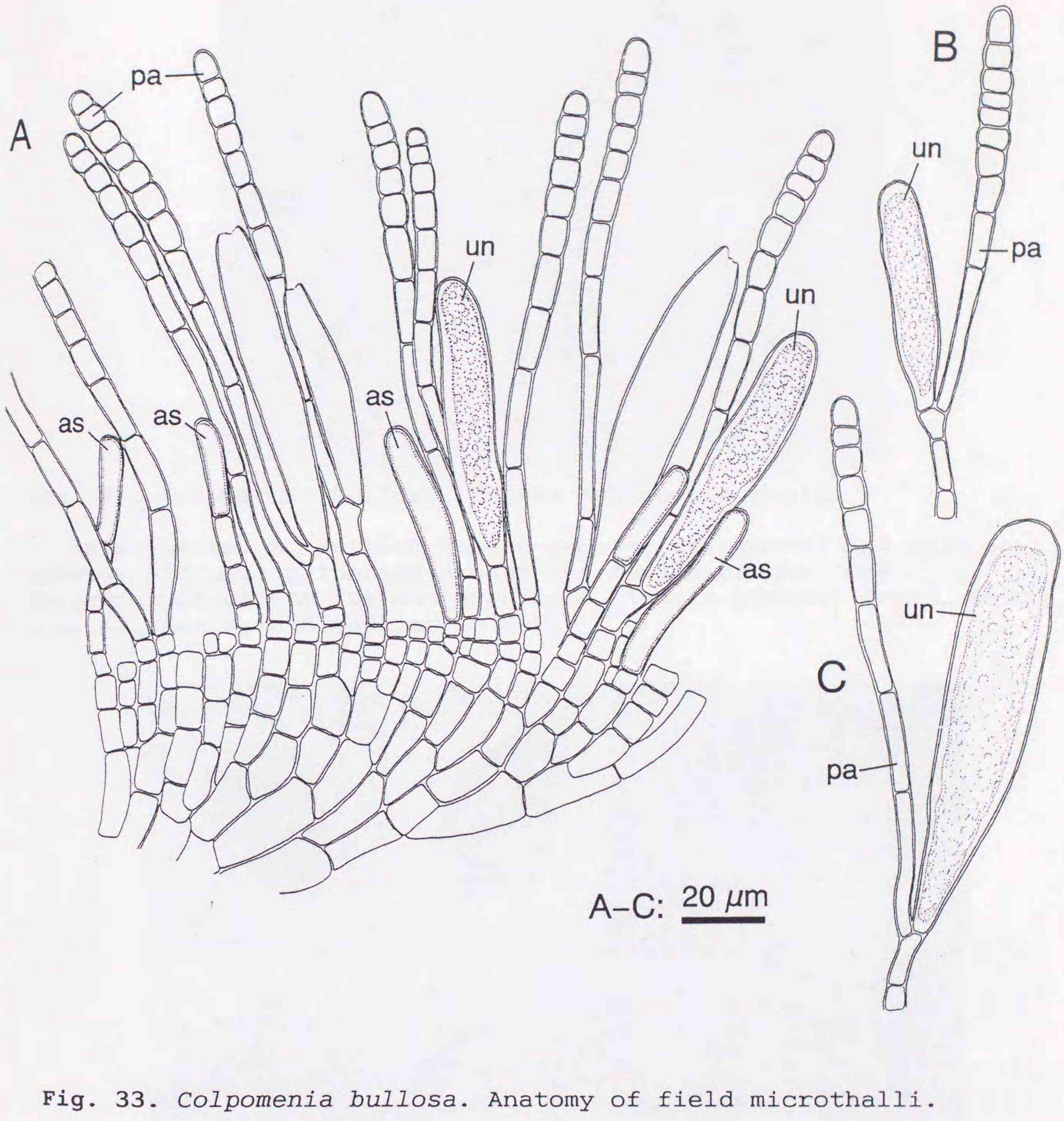


Fig. 33. *Colpomenia bullosa*. Anatomy of field microthalli.

A. Longitudinal section of fertile microthallus, showing unilocular sporangia (un), ascocysts (as) and paraphyses (pa). **B, C.** Unilocular sporangia (un) and paraphyses (pa).

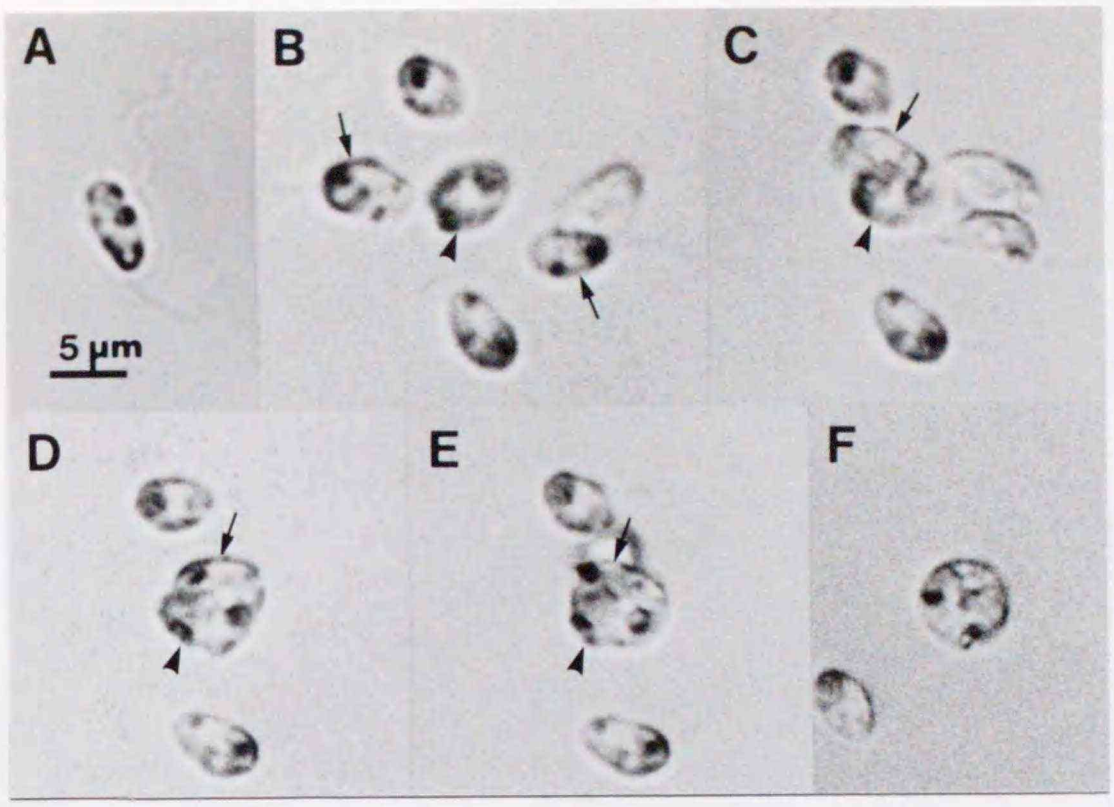


Fig. 34. *Colpomenia bullosa*. Gamete and gamete fusion.

A. Male gamete. **B.** Settled female gamete (arrowhead) and male gametes (arrows) gathering around the female gamete. **C-F.** Sequences of gamete fusion (arrowhead, female gamete; arrow, male gamete). Scale in **A** applies to **B-F.**

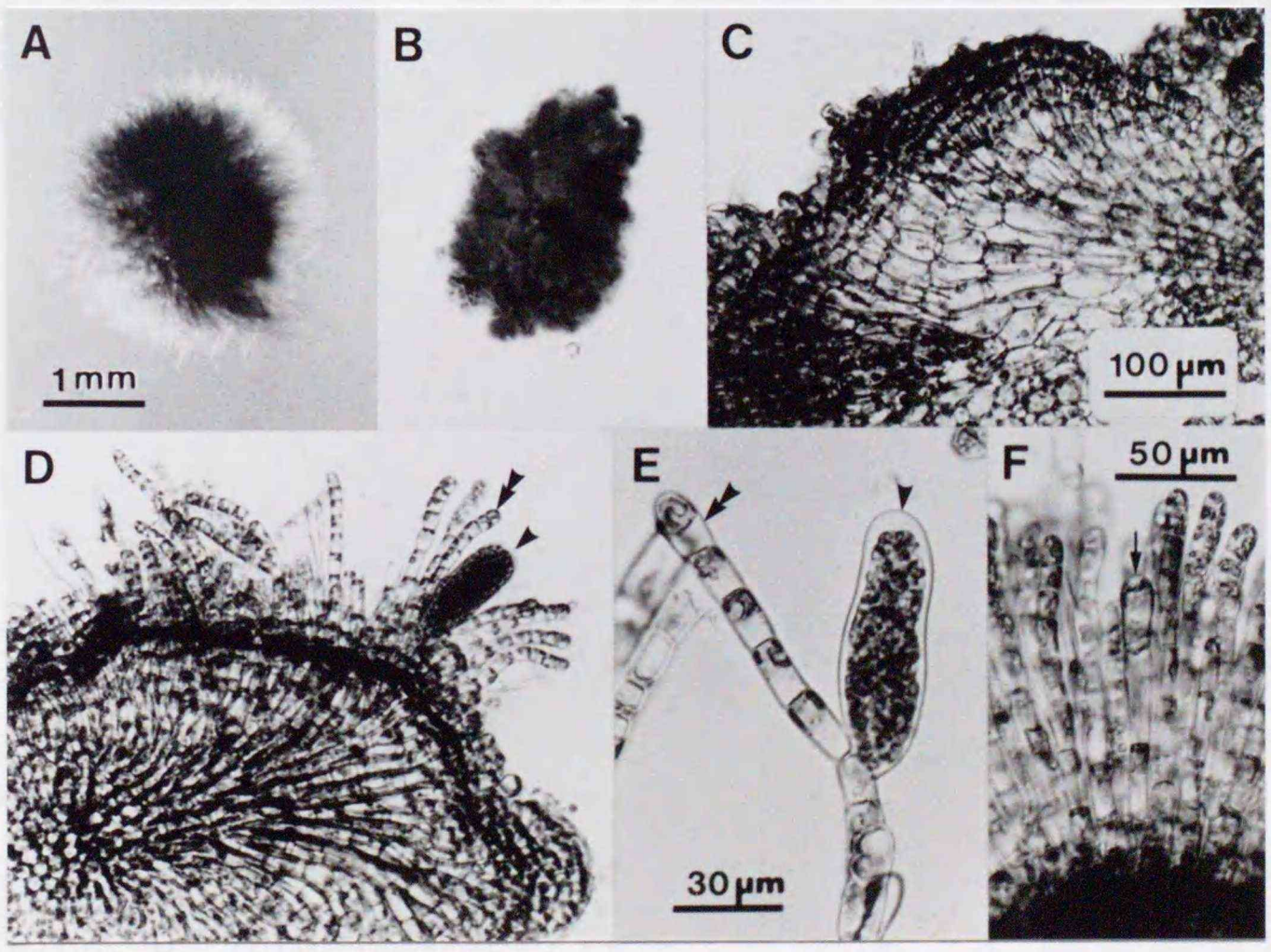


Fig. 35. Culture of *Colpomenia bullosa*. Parthenogenetic development of gametes.

A. Tufted microthalli under 20°C LD condition. **B.** Crustose microthalli under 20°C LD condition. **C.** Longitudinal section of microthallus under 20°C LD condition. **D.** Longitudinal section of microthallus forming unilocular sporangium (arrowhead) with paraphyses (double arrowhead) under 15°C SD condition. **E.** Unilocular sporangium (arrowhead) laterally on a basal cell of a paraphysis (double arrowhead). **F.** Ascocyst (arrow) among paraphyses on microthallus. Scale in **A** applies to **B**; Scale in **C** applies to **D.**

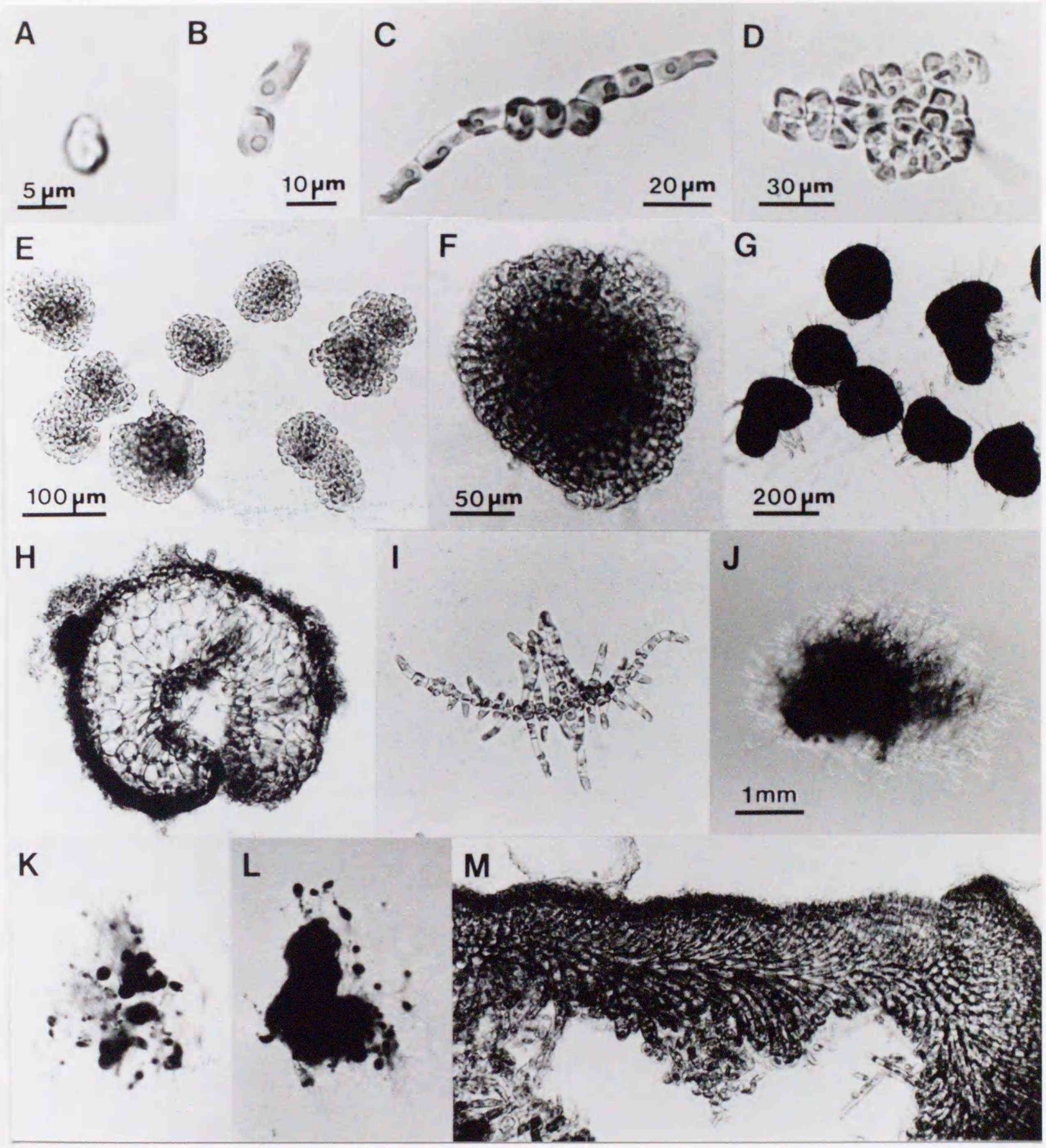


Fig. 36. Culture of *Colpomenia bullosa*. Development of unispores from field microthalli.

A. Unispore from field microthallus. **B.** Nine-day-old germling under 10°C LD condition. **C.** Seventeen-day-old germling under 10°C LD condition. **D.** Three-week-old germling under 5°C SD condition. **E.** Small disks under 5°C SD condition. **F.** Small disk forming a swollen part under 5°C SD condition. **G.** Young, spherical macrothalli under 5°C SD condition. **H.** Longitudinal section of spherical macrothallus. **I.** Filamentous germling. **J.** Tufted microthallus. **K.** Tufted thallus with massive parts. **L.** Crustose thallus with filaments. **M.** Longitudinal section of crustose thallus.

Scale in **E** applies to **H**, **I** and **M**; Scale in **J** applies to **K** and **L**.

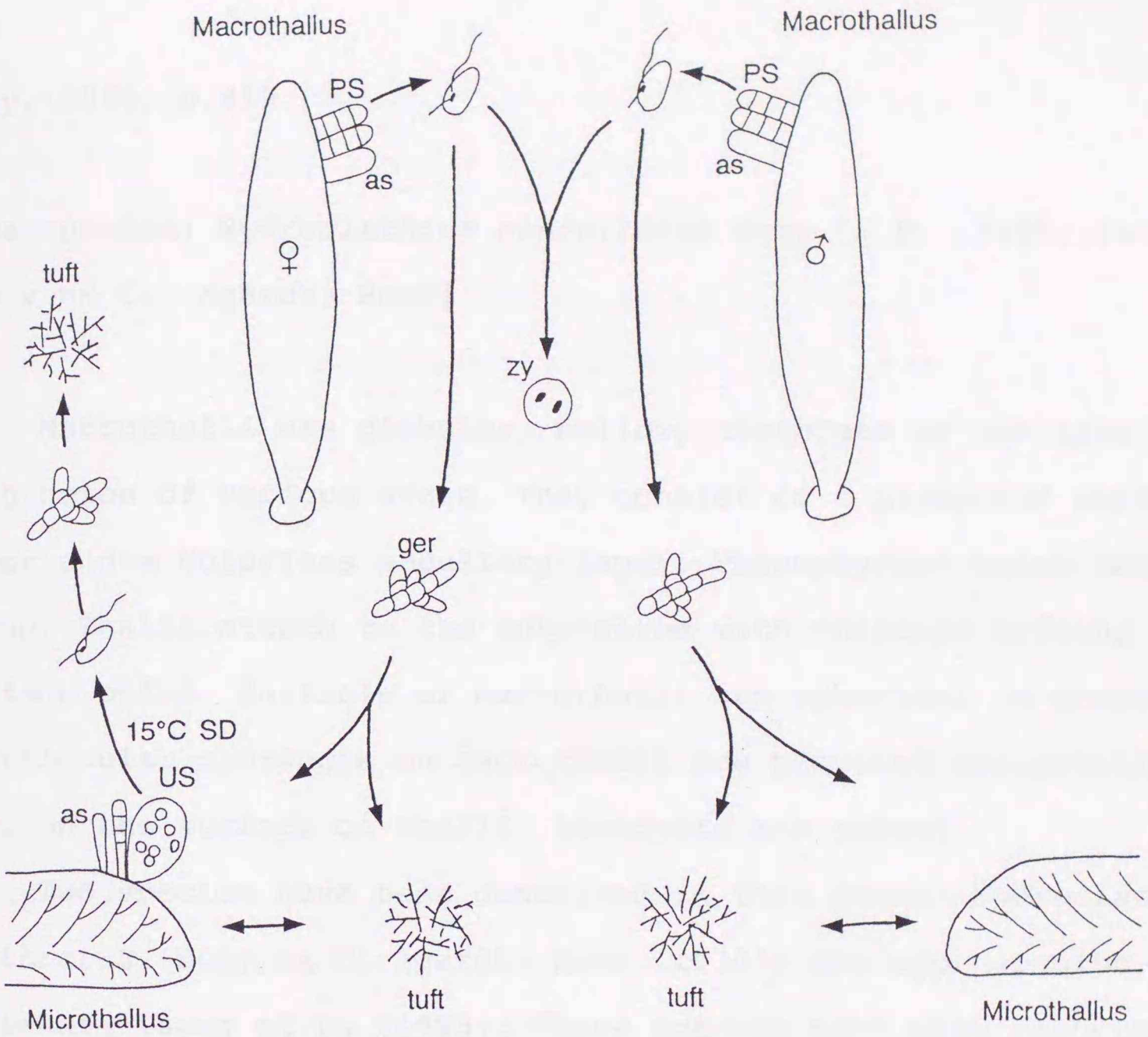


Fig. 37. Diagram of life history of *Colpomenia bullosa* in culture.

as, ascocyst; ger, germling; PS, plurilocular sporangium; US, unilocular sporangium; zy, zygote; SD, short day condition.

Hydroclathrus Bory

Bory, 1825, p.419.

Type species: *Hydroclathrus cancellatus* Bory [= *H. clathratus* (Bory ex C. Agardh) Howe]

Macrothalli are globular, hollow, clathrate or net-like, with holes of various sizes. They consist of a pigmented cortical layer and a colorless medullary layer. Phaeophycean hairs are in group. Thalli attach to the substratum with rhizoids arising from surface cells. Initials of macrothalli are spherical in shape. Plurilocular sporangia on macrothalli are produced acropetally in sori on the surface of thalli. Ascocysts are absent.

Two species have been described in this genus: *Hydroclathrus clathratus* (Bory ex C. Agardh) Howe (1920); the type species, and *H. tenuis* Tseng et Lu (1983). These species have been reported from Japanese waters (Okamura 1902, Tanaka 1990).

Hydroclathrus clathratus (C.Agardh) Howe

Howe 1920, p.590; Okamura 1902, I, p.18-19, Pl.4, Fig.10, Pl.5, Fig.7-13; Womersley 1987, p.300, Figs 109A, 110A,B.

Basionym: *Encoelium clathratus* C. Agardh, 1822, p.412

Synonym: *Hydroclathrus cancellatus* Bory, 1825, p.419.

Type locality: Belle Isle, France. (but see Hamel 1931-39, p.202).

Type: in Herb. Bornet, PC ? in Herb. Agardh, LD ? (see Womersley 1987).

Japanese name: Kagomenori

Materials

Localities of materials are shown in Fig. 38. The numbers in square brackets after each locality correspond to those in Fig. 38.

Hokkaido : Kojima, Matsumae [7] (23-viii-1988 leg. I. Mine), Aomori Pref. : Ohma [38] (21-ii-1988 leg. H. Kawai, 1-viii-1988 leg. T. Kitayama, 2-ii-1990 leg. K. Kogame, 29-iv-1990 leg. K. Kogame), Sai [39] (16-vii-1988 leg. K. Kogame, 31-vii-1988 leg. T. Kitayama), Tappizaki [40] (11-viii-1988 leg. M. Masuda), Fukaura [41] (10-viii-1988 leg. M. Masuda), Toyama Pref. : Abugashima [49] (12-vii-1991 leg. T. Yoshida), Shizuoka Pref. : Susaki, Shimoda [69] (14-vi-1990 leg. T. Kitayama), Wakayama Pref. : Miwasaki, Shingu [87] (4-v-1987 leg. M. Masuda), Ehime Pref. : Uoshima [90] (8-v-1990 leg. S. Enomoto & K. Kogame), Mimaizaki [102] (20-iv-1988 leg. S. Enomoto & K. Kogame), Fukuoka Pref. : Tsuyazaki [103] (21-iv-1989 leg. S. Kawaguchi), Okinawa Pref. : Henoko [118] (9-iii-1990 leg. T. Yoshida), Minnashima [116] (10-iii-1990 leg. T. Yoshida), Bise [115] (11-iii-1990 leg. K. Kogame).

Herbarium specimens examined are follows. Hokkaido: Okushiri [4] (******* leg. T. Abe), Niigata Pref. : Aikawa, Sado [46] (SAP 051037, 12-vi-1987 leg. K. Ikehara det. T. Yoshida), Toyama Pref. : Yabuta [50] (SAP 049270, 7-iv-1932 leg. K. Ohshima), Kanagawa Pref. : Misaki, Aburatsubo [65] (SAP 9491, 1-v-1927 leg. S. Akiyama), Mie Pref. : Wagu, Toshitou [75] (SAP 023084, vi-1942 leg. S. Inoh), Wakayama Pref. : Setosaki, Shirahama [80] (SAP

027250, vi-1940 leg. S. Inoh), Miwasaki Shinguh [78] (SAP 053324, 7-v-1989 leg. T. Yamamoto), Saga Pref. : Hadonomisaki [106] (SAP 043785, 28-v-1983 leg. T. Yoshida), Nagasaki Pref. : Hirado [107] (SAP 043620, 25-v-1983 leg. T. Yoshida), Miyazaki Pref. : Futo [113] (SAP 043840, 25-v-1982 leg. T. Yoshida), Kumamoto Pref. : Ohyano [111] (SAP 048299, 23-iii-1957 leg. M. Ichiki).

Morphology

Macrothalli are globular with holes, clathrate, irregular in shape, 5-20 cm in diameter (Figs 39A-C). They are yellowish brown to brown in color, somewhat firm in texture. Holes are round or ellipsoid, variable in size. Thalli consist of a cortical layer of 1-3 small, pigmented cells and a medullary layer of 3-6 large, colorless cells (Figs 40 A,B, 41A,B). In margins of the holes, surfaces of thalli are curved to the inside of the thalli. In section right angle to the margin of the hole, medullary cells are round or ellipsoid, larger in the inner portion, 15-140 × 20-220 µm in size. In section parallel to the margin of the hole, medullary cells are longer in the inner portion. Hairs arise in group from cortical layers when young or somewhat deep pits which form owing to growth except the pits (Figs 40C, 41A,B). Thalli attach broadly to substrata with rhizoidal filaments (Figs 40E,F). Plurilocular sporangia are produced in sori which usually include hair groups (Figs 39D, 40C, 41B). The sori are round to ellipsoid, 100-800 µm in diameter. Mature plurilocular sporangia are loosely coherent to each other without a cuticle layer over them (Figs 40D, 41B). Plurilocular sporangia are bi- or tetraseriate, to 15-30 µm in length. Ascocysts are absent. Secondary plurilocular sporangia are common. No unilocular sporangia are observed on macrothalli.

Habitat and phenology

Clathrate thalli grow on rocks, boulders or sometimes on other algae in the intertidal to subtidal zone. The thalli are annual and usually present from winter to early summer.

Geographical distribution

This species is widely distributed, being reported from warm temperate to tropical coasts in all areas of the world (California, Setchell and Gardner 1925; South Australia, Womersley 1987; France, Hamel 1931-39). In Japan, this species is distributed in coasts from Okushiri Island, Hokkaido (Abe 1993) to Okinawa except northern Pacific coasts of Honsyu.

Culture study

Strains used in this culture are listed in Table 19, and progeny of plurispores from macrothalli are also shown.

No sexual reproduction could be observed.

Development of plurispores from macrothalli

In some strains, plurispores from macrothalli grew into tufted or pseudodiscoid microthalli forming plurilocular or unilocular sporangia. In such strains, reproductive organs on the microthalli and culture conditions where the plurispores were cultured are shown in Table 19 (284 Bise, 334 Sado). Culture studies on strain 334 Sado are mentioned below.

Settled plurispores germinated into prostrate, branched filaments (Figs 42A-C) and developed into tufted or pseudodiscoid (Figs 42D,E,G) or massive thalli (Fig. 42F) which reached up to 1.5 mm in diameter after 3 months. These thalli consisted of branched filaments. Thalli grown on rotary shaker became discoid,

but they partly detached from the substrata (cover glasses) (Fig. 42H). These discoid thalli consisted of prostrate filaments and upright filaments arising from them (Figs 42I,J). These microthalli formed plurilocular sporangia (Fig. 42K) under 10°C LD and 15°C LD conditions, unilocular sporangia (Fig. 42L,M) under 10-20°C SD and 15°C ND, mostly plurilocular sporangia or sometimes both type of sporangia 20°C LD. The plurilocular sporangia were ectocarpoid, pedicellate, and sometimes branched (Fig. 42K). The unilocular sporangia were ellipsoid, oval, and sessile or pedicellate (Figs 42L,M).

Development of plurispores from microthalli

Plurispores (Fig. 43A) from plurilocular sporangia on microthalli kept under 20°C LD, were cultured at 5-20°C under SD and LD (Table 20).

Settled plurispores germinated into prostrate, branched filaments (Fig. 43B), and developed into tufted or pseudodiscoid (Figs 43C,D) or massive thalli as the same developmental manner of plurispores from macrothalli. No growth was observed under 5°C conditions. These microthalli formed plurilocular sporangia under 10°C LD and 15°C LD, unilocular sporangia under 10°C SD, 15°C SD and 20°C SD, and mostly plurilocular sporangia and rarely unilocular sporangia under a 20°C LD condition.

Development of unispores from microthalli

Unispores from unilocular sporangia on microthalli under 20°C SD, were cultured at 5-20°C under SD and LD. (Table 21).

Settled unispores germinated into prostrate, branched filaments, later into small disks. These small disks grew into

spherical thalli (macrothalli), becoming swollen (Figs 43F,G). No prostrate systems were developed under any conditions attempted. Germlings from unispores did not grow at 5°C. At 20°C, the spherical thalli reached to 2 mm in diameter and became fertile with plurilocular sporangia (Fig. 43I). Spherical thalli which produced holes on the surface were observed at 15°C after 3 months (Fig. 43H).

Direct type life history

In some strains, plurispores from macrothalli germinated and developed into spherical thalli. Such strains and culture conditions where the plurispores were grown are shown in Table 19 (strain no. ; 158 Sai, 160 Ohma, 161 Fukaura, 163 Tappizaki, 165 Kojima, 258 Bise).

In strain no. 158 Sai, plurispores from spherical thalli under 20°C LD were cultured at 5-20°C under both SD and LD conditions. Germlings grew into small disks through prostrate, branched filaments (Figs 44A,B); however, those cultured at 5°C did not grow. The small disks became swollen, and developed to spherical thalli (Fig. 44C). Prostrate systems were not developed. Under 10°C SD and 15°C SD conditions, spherical thalli reached to 1 cm and 3-4 cm in diameter, respectively, but did not become fertile. Under 20°C SD and 20°C LD conditions, they became fertile after 4 months and 2 months, respectively, producing plurilocular sporangia abundantly. Under 10°C LD and 15°C LD conditions, spherical thalli produced plurilocular sporangia less than under 20°C conditions.

Discussion

Recently, *Hydroclathrus tenuis* Tseng et Lu (1983) was described from the Xisha Islands, South China Sea. According to Tseng et Lu (1983), *H. tenuis* is distinct from *H. clathratus* in respects of its thinner thalli (250-300 μm), its smaller medullary cells (70-80 μm in diameter) and its longer plurilocular sporangia (22-25 μm). Characters of thallus thickness and size of medullary cells are considered to be useful to distinguish *H. tenuis* from *H. clathratus* based on the present study. However, length of plurilocular sporangia is unuseful as a distinct character between them, since ranges of the length of the plurilocular sporangia in *H. clathratus* in this study is 15-30 μm which overlap the length in *H. tenuis* described by Tseng and Lu (1983).

Clayton (1982) reported a direct type life history for Australian *H. clathratus*: plurispores from macrothalli developing into the same macrothalli. Japanese six strains isolated from different six localities also represent such a direct type life history in culture. While, microthalli bearing unilocular sporangia and plurilocular sporangia were observed in some strains. The present culture study first revealed that a heteromorphic life history involving macrothalli and microthalli, although sexual reproduction was not observed. A diagram of the life history of this species is shown in Fig. 45 based on the present culture study. The formation of plurilocular sporangia and unilocular sporangia depended on temperature and photoperiod. These results resemble those of *C. sinuosa* and *C. peregrina* in this study. Since both plurispores from macrothalli and microthalli developed into microthalli, their role is to reproduce the microthalli. Whereas, unispores play a role to

reproduce macrothalli. These results are also similar to those in *C. sinuosa* and *C. peregrina*.

Morphology of microthalli in culture was considered to be abnormal, since it is easy to become tufted or massive, and rare to be discoid. On a rotary shaker under a 15°C LD condition, they gave rise to discoid thalli, but construction of filaments became irregular when fertile. It is necessary to find microthalli in the field to examine their morphology.

In culture under 5°C conditions, swarmers did not grow. This result supports the fact that this species does not occur in coasts along cold temperate waters.

Table 19. Collection data of cultured strains of *Hydroclathrus clathratus*. Culture conditions attempted and progeny of plurispores from macrothalli are also shown.

strain no.	locality	date	culture conditions	progeny
158	Sai	16 vii 1988	5SL	not growing
			10SL	macrothalli
			15SL, 20SL	macrothalli (P)
160	Ohma	1 viii 1988	20L	macrothalli (P)
161	Fukaura	10 viii 1988	20L	macrothalli (P)
163	Tappizaki	11 viii 1988	15L	macrothalli
165	Kojima, Esashi	23 viii 1988	20L	macrothalli (P)
284	Bise	11 iii 1990	20L	pseudodisks (P)
			20S	pseudodisks (U)
285	Bise	11 iii 1990	20L	macrothalli (P)
334	Sado	8 vii 1990	10-20L	pseudodisks (P)
			15SN, 20S	pseudodisks (U)

Table 20. Culture of *Hydroclathrus clathratus*. Progeny of plurispores from microthalli

strain no.	locality	date	culture conditions	progeny
284	Bise	11 iii 1990	20L	pseudodisks (P)
334	Sado	8 vii 1990	5SL	not grow
			10L, 15L	pseudodisks (P)
			10-20S	pseudodisks (U)
			20L	pseudodisks (P,U)

Table 21. Culture of *Hydroclathrus clathratus*. Progeny of unispores from microthalli

strain no.	locality	date	culture conditions	progeny
284	Bise	11 iii 1990	20S	macrothalli
334	Sado	8 vii 1990	10-20SL	macrothalli (P; 20L)

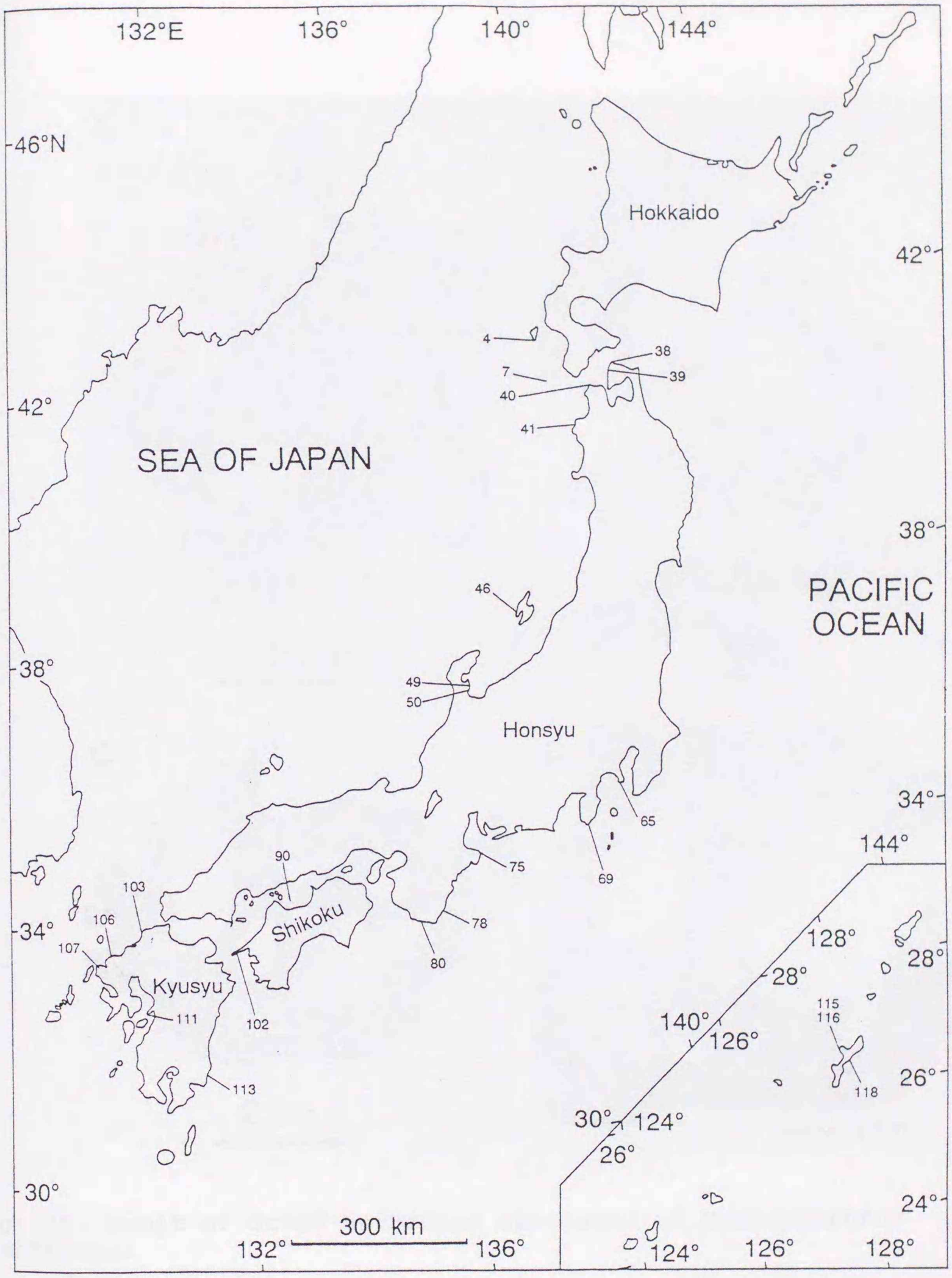


Fig. 38. Localities where materials of *Hydroclathrus clathratus* were collected. Numbers plotted correspond to those in Table 1.

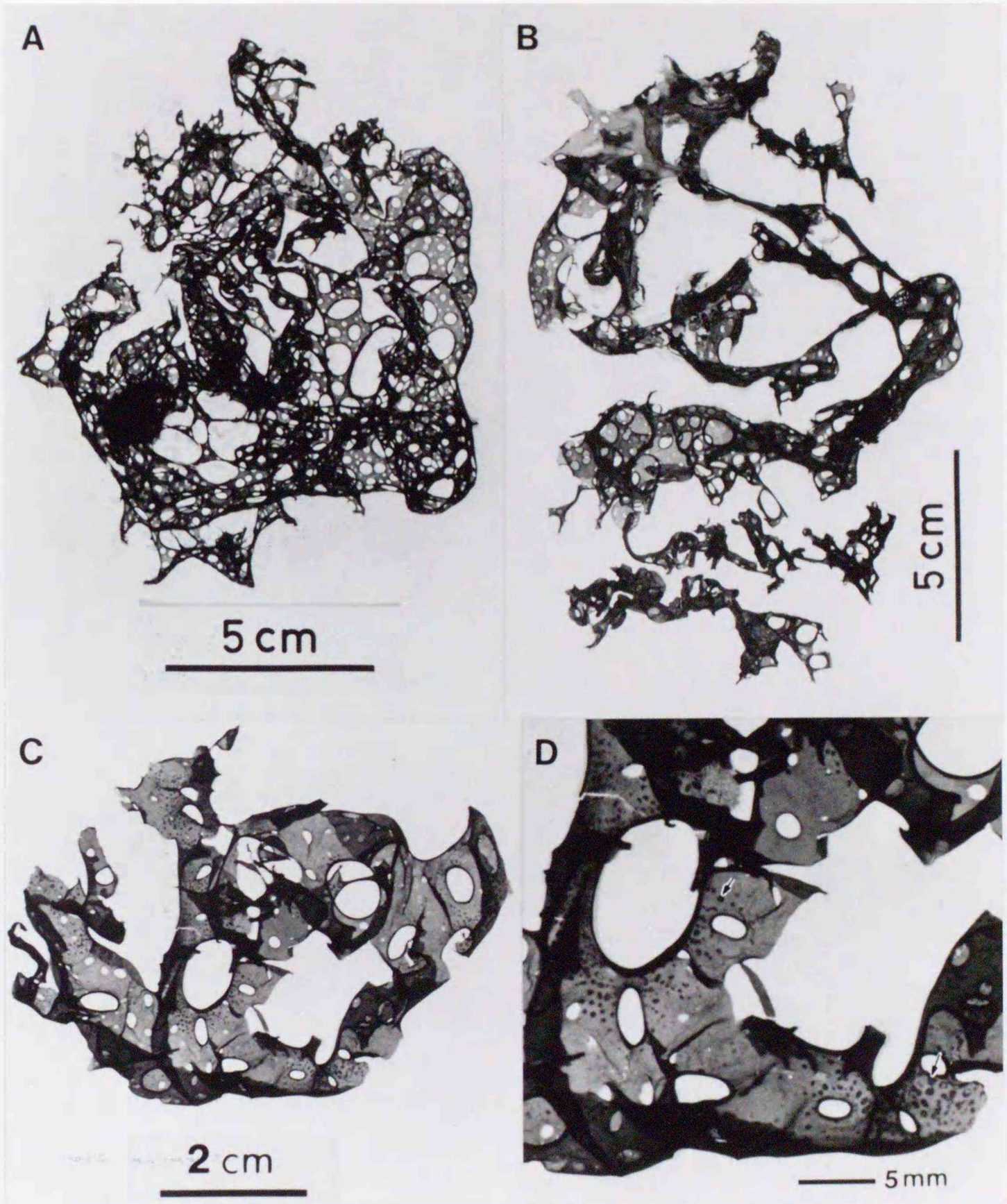


Fig. 39. Habit of dried herbarium specimens of *Hydroclathrus clathratus*.

A. Plant collected at Sai, Aomori Pref. on 16 July 1988. **B.** Plant collected at Mimaizaki, Ehime Pref. on 20 April 1988 by S. Enomoto. **C.** Plant collected at Aburatsubo, Kanagawa Pref. on 8 June 1989 by M. Matsumoto. **D.** Sori of plurilocular sporangia (arrows).

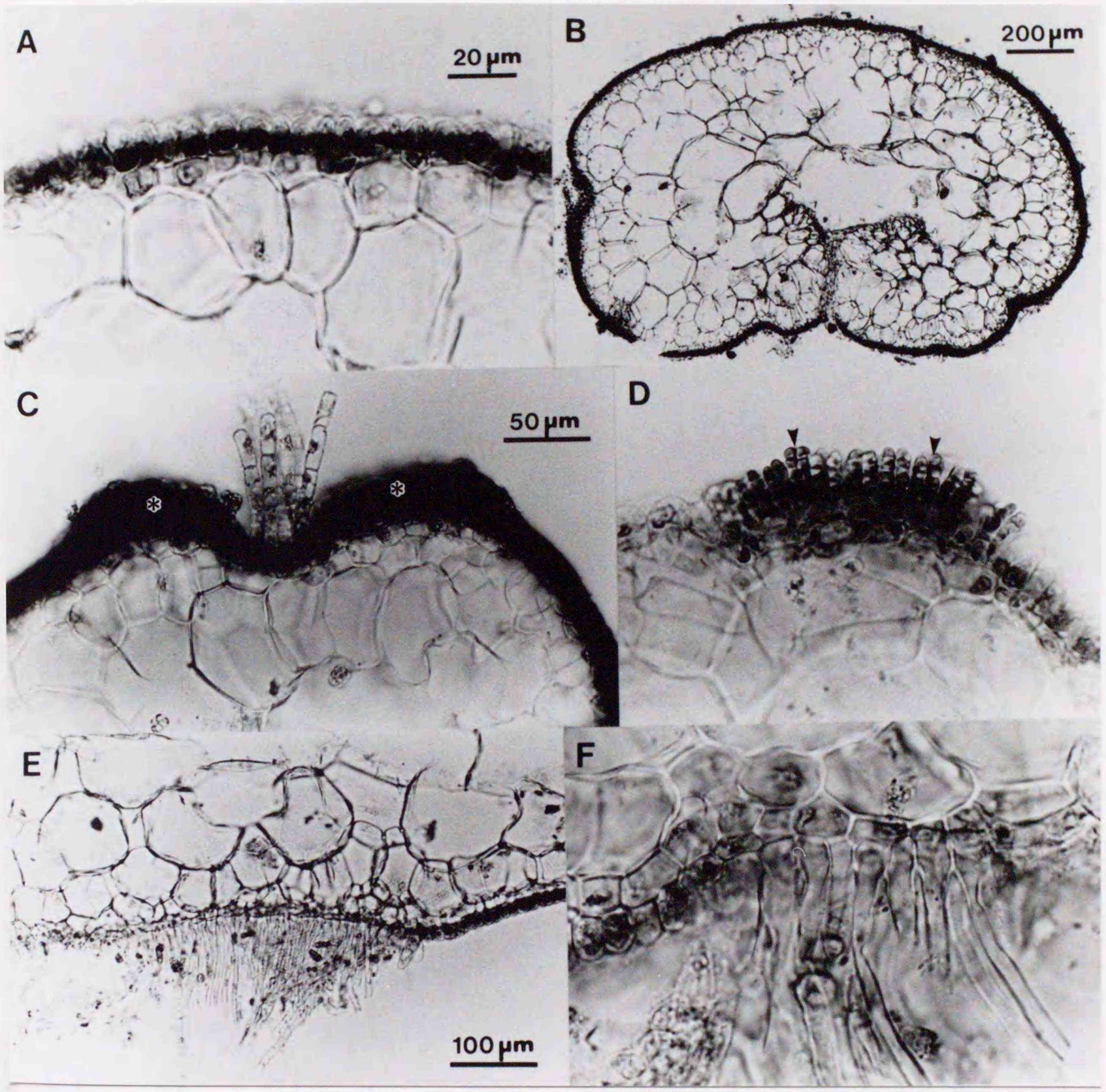


Fig. 40. Anatomy of *Hydroclathrus clathratus*.

A. Cortical part in cross section of thallus. **B.** Section of thallus. **C.** Phaeophycean hair tuft in section through plurilocular sporangial sori (asterisks). **D.** Plurilocular sporangia (arrowheads). **E, F.** Rhizoids arising from thallus surface where thallus attaches to substratum.

Scale in **A** applies to **D** and **F**.

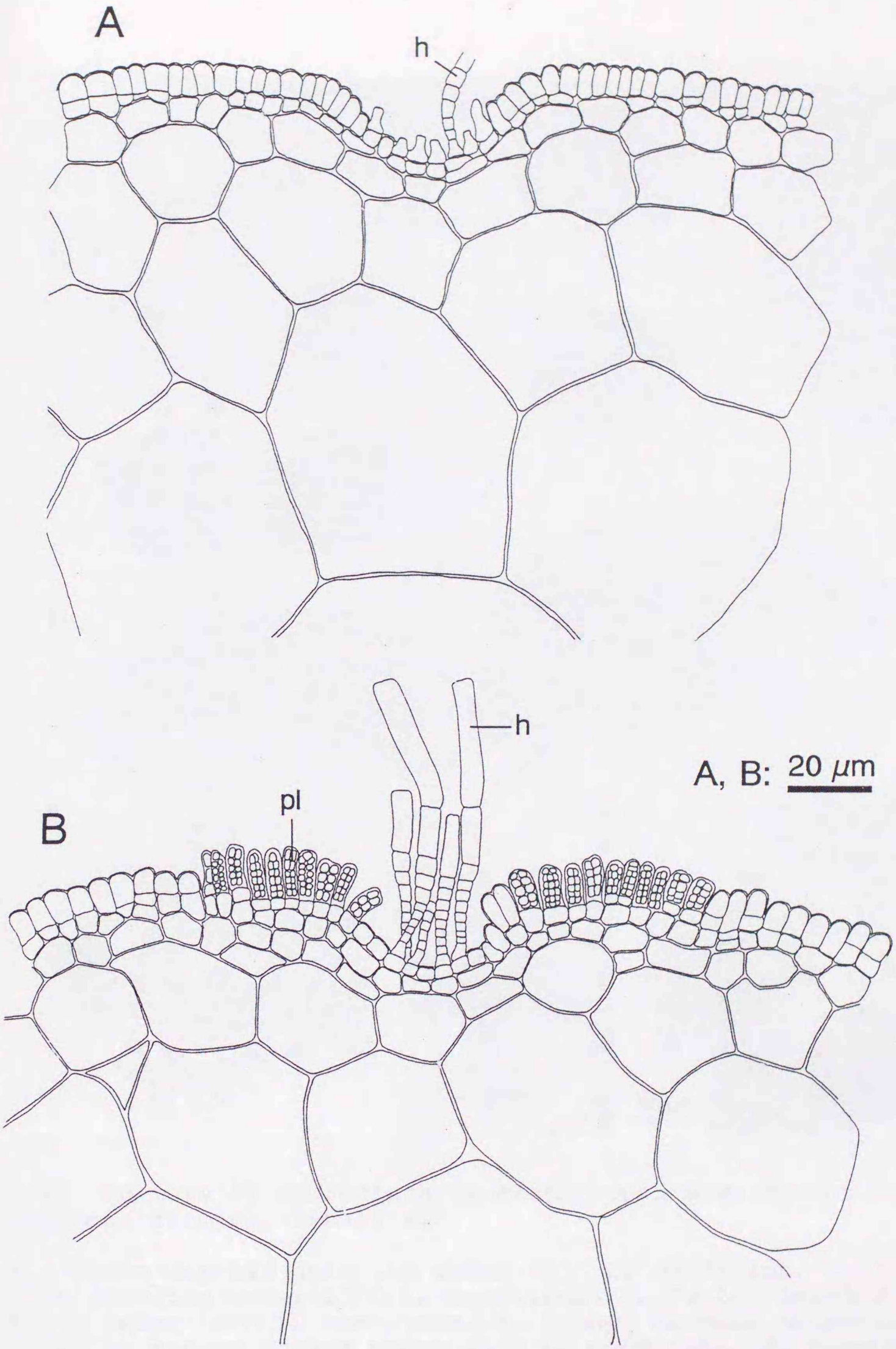


Fig. 41. Anatomy of *Hydroclathrus clathratus*

A. Cross section of vegetative thallus through a part with phaeophycean hair (h) tuft. **B.** Cross section of fertile thallus, showing plurilocular sporangia (pl) and phaeophycean hairs (h).

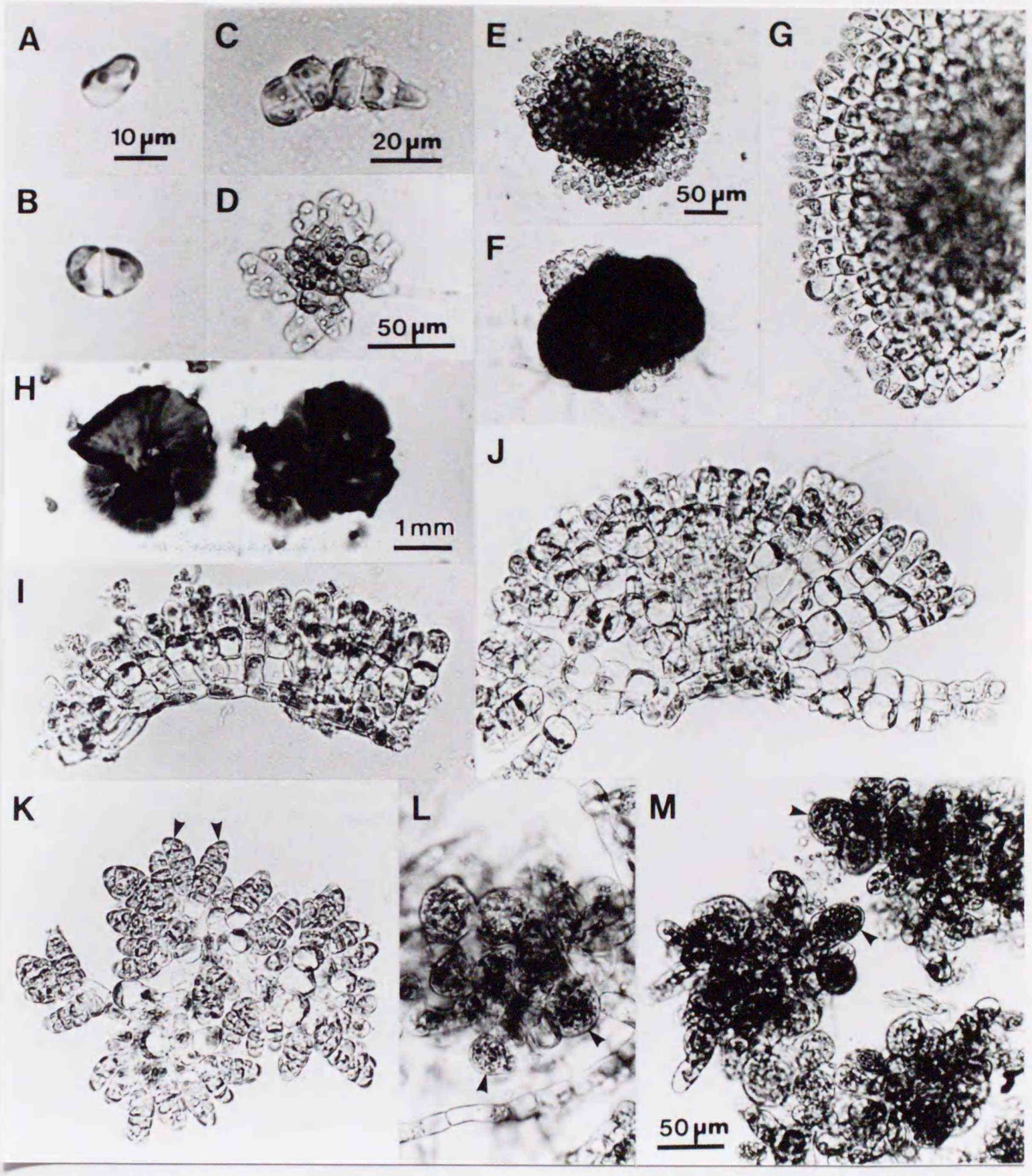


Fig. 42. Culture of *Hydroclathrus clathratus*. Development of plurispores from macrothallus.

A, B. Three-day-old germling under 20°C LD condition. **C.** Four-day-old germling under 15°C LD condition. **D.** Twelve-day-old germling under 15°C LD condition. **E.** Young, discoid microthallus cultured on rotary shaker under 15°C LD condition. **F.** Microthallus with massive part under 15°C LD condition. **G.** Marginal portion of microthallus. **H.** Discoid microthalli on rotary shaker under 15°C LD condition. **I, J.** Squashed discoid microthalli. **K.** Squashed microthallus forming plurilocular sporangia (arrowheads) under 15°C LD condition. **L, M.** Squashed microthalli forming unilocular sporangia (arrowheads) under 20°C SD condition.

Scale in **A** applies to **B**; Scale in **E** applies to **F**; Scale in **D** applies to **G** and **I-L**.

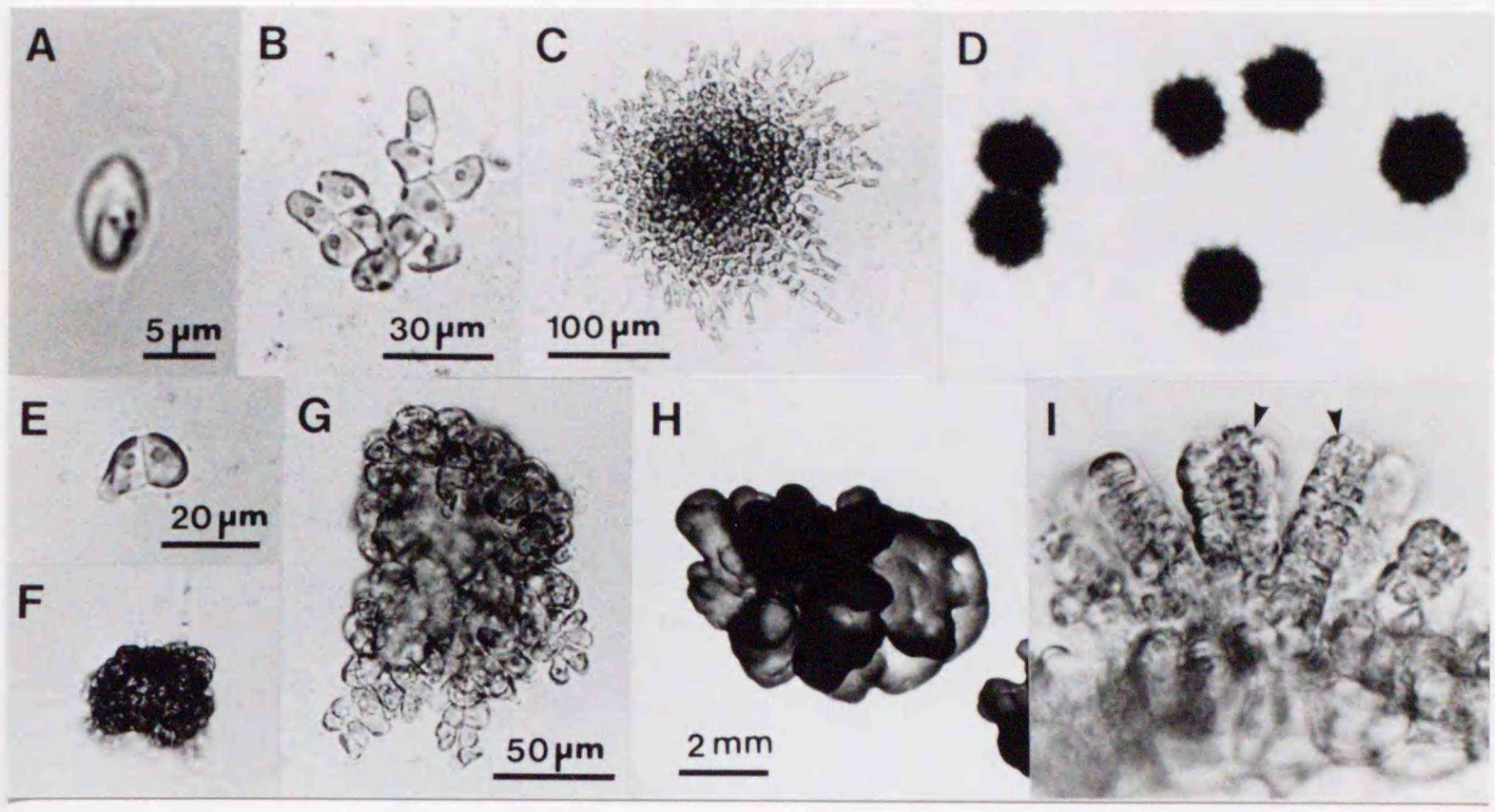


Fig. 43. Culture of *Hydroclathrus clathratus*. Development of plurispores (**A-D**) and unispores (**E-I**) from microthalli.

A. Plurispore from microthallus. **B.** Five-day-old germling under 20°C LD condition. **C.** Ten-day-old germling under 20°C LD condition. **D.** Pseudodiscoid microthalli. **E.** Three-day-old germling under 15°C SD condition. **F.** Sixteen-day-old germling under 15°C SD, showing a rise of a thallus. **G.** Swollen thallus in under side view through substratum of cover glass. **H.** Macrothallus with perforations under 15°C SD condition. **I.** Plurilocular sporangia (arrowheads) on macrothallus under 20°C LD condition. Scale in **C** applies to **F**; Scale in **E** applies to **I**.

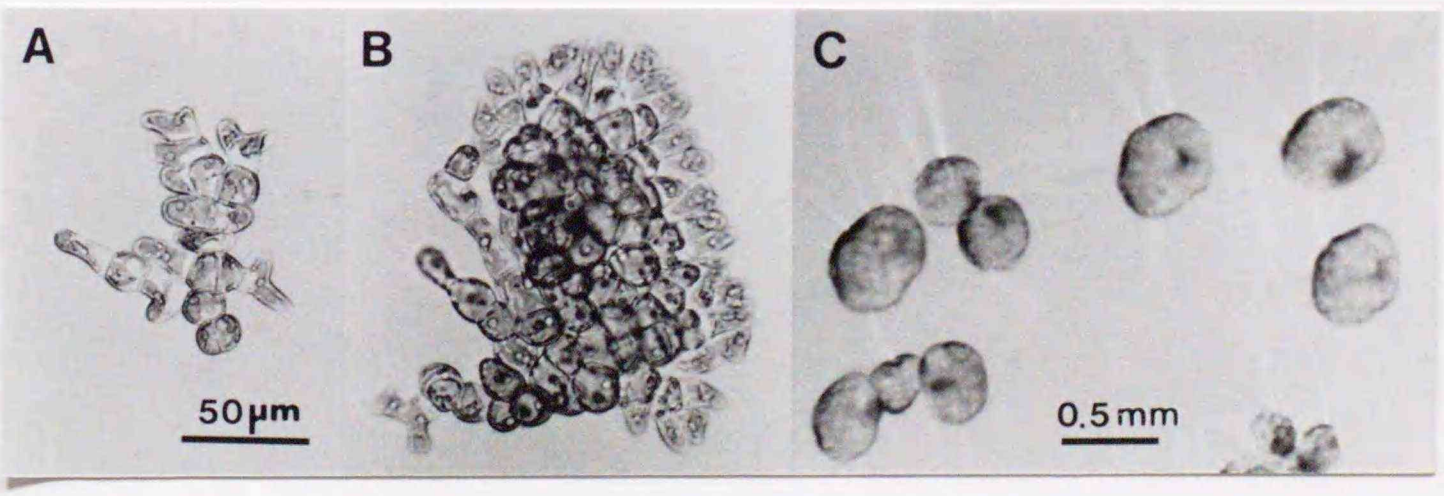


Fig. 44. Culture of *Hydroclathrus clathratus*. Development of plurispore from macrothallus in strain with direct type life history.

A. Eight-day-old germling under 20°C LD condition. **B.** Twelve-day-old germling under 20°C LD condition, showing small disk. **C.** Spherical macrothalli. Scale in **A** applies to **B**.

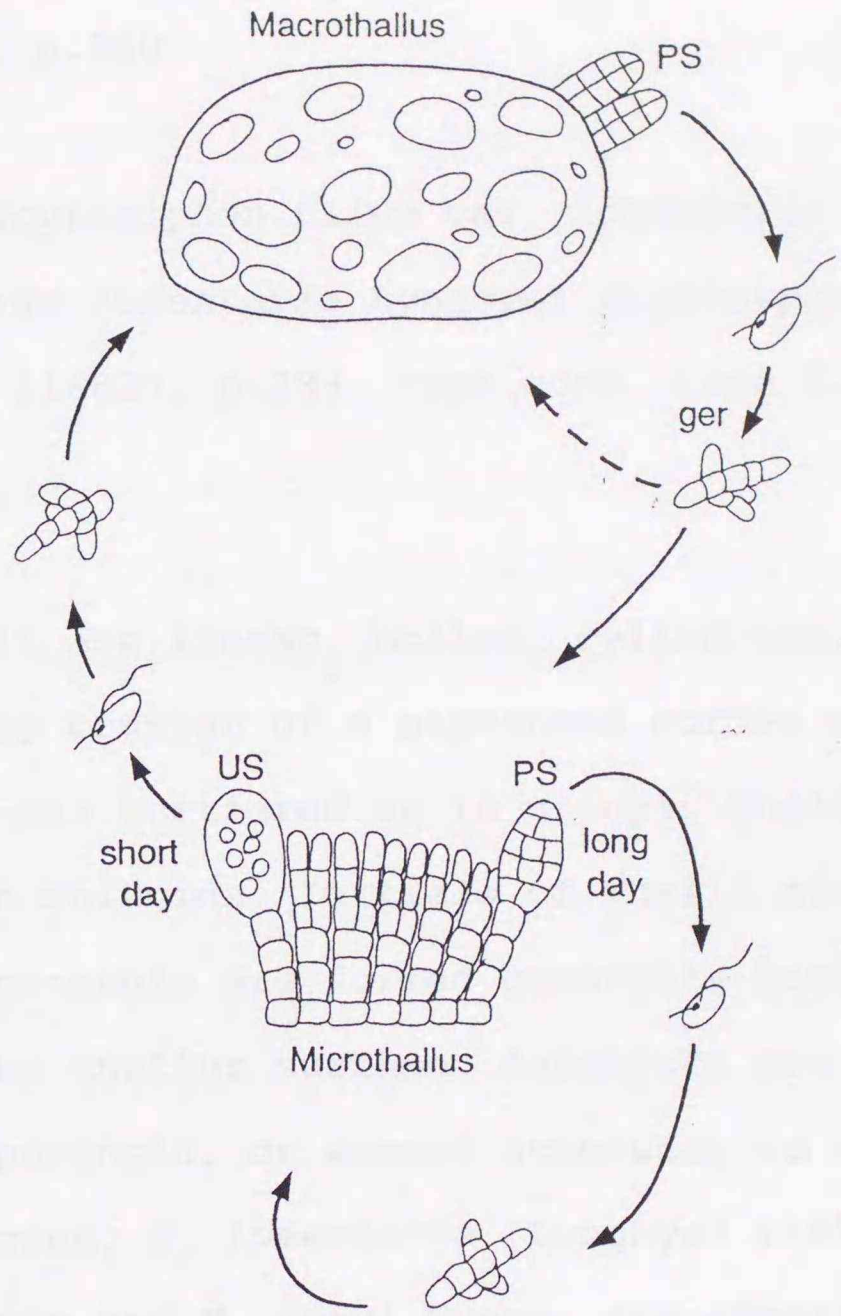


Fig. 45. Diagram of life history of *Hydroclathrus clathratus* in culture.

ger, germling; PS, plurilocular sporangium; US, unilocular sporangium; broken line, course in direct type life cycle.

Scytosiphon **C. Agardh** (nom. cons.)

C. Agardh 1820, p.160.

Type species: *Scytosiphon filum* var. *lomentaria* (Lyngbye) C. Agardh. [= *Chorda lomentaria* Lyngbye; *Scytosiphon lomentaria* (Lyngbye) Link (1883), p.233. type cons. (see I.C.B.N., 1961, p.213.)

Macrothalli are linear, hollow, cylindrical or flattened, gregarious. They consist of a pigmented cortex and a colorless medulla. Hairs are scattered or in groups. Thalli attach to the substratum with rhizoids. Initials of thalli are linear in shape. Plurilocular sporangia are formed generally basipetally, expanding on the thallus surface. Ascocysts are present among plurilocular sporangia, or absent according to species.

Three species, *S. lomentaria* (Lyngbye) Link, *S. complanatus* (Rosenvinge) Doty and *S. dotyi* Wynne, are generally recognized. Although several formae and varieties have been described as infraspecific taxa *S. lomentaria*, they are generally recognized as synonyms of above three species because their variable appearances depend on environmental factors (Wynne 1969, Clayton 1978). In this study four species, including *S. lomentaria* and three new species, are reported from Japan.

Although a combination of *Scytosiphon simplicissimus* (Clemente) Cremades (1990) was proposed, placing *S. lomentaria* (Lyngbye) Link as a synonym of it, in this study the latter name is used since the former name is still not used generally.

Scytosiphon lomentaria (Lyngbye) Link

Link 1883, p.233; Saunders 1889, p.41, Fig.8 (constricted thalli only); Okamura, 1907-1935, I, p.144, 146, Pl. 30; Smith 1944, p.129, Pl.19, Fig.1; Taylor 1957, p.168, Pl.15, Fig.2, Pl.16, Fig.3; Tatewaki 1966, p.62-66, Figs 1-10; Nakamura and Tatewaki 1975, p.57-93, Figs 1-5, Pl.I; Kristiansen and Pedersen 1979, p.31-56, Figs 7-8, 13-18; Clayton 1981, p.67-75, Figs 1, 4 (section of type); Fletcher 1987, P.248, Figs 67B, 69, Pl.11a,b; Womersley 1987, p.294, Figs 106A, 108C.

Basionym: *Chorda lomentaria* Lyngbye, 1819, p.74.

Synonym: *Scytosiphon lomentaria* var. *typica* Rosenvinge, 1893, p.863; *Scytosiphon lomentaria* f. *typicus* Setchell et Gardner, 1925, p.533; Tokida 1954, p.104.

Type: in herb. Lyngbye, C.

Type locality: Quivig, Farø Island, Denmark.

Japanese name: Kayamonori

Materials

Localities of materials are shown in Fig. 46. The numbers in square brackets after each locality correspond to those in Fig. 46.

Macrothalli: Hokkaido: Oshoro [1] (11-iii-1987 leg. K. Kogame, 8-v-1987 leg. K. Kogame, 14-v-1988 leg. K. Kogame, 24-v-1988 leg. K. Kogame, 22-vi-1989 leg. K. Kogame, 23-iii-1990 leg. K. Kogame, 17-iv-1990 leg. K. Kogame, 21-v-1990 leg. K. Kogame, 15-ii-1991 leg. K. Kogame, 17-iii-1992 leg. K. Kogame, 26-iv-1992 leg. K. Kogame), Iwanai [2] (15-v-1988 leg. K. Kogame), Setana [3] (18-iii-1987 leg. K. Kogame), Esashi [5] (15-v-1988 leg. K. Kogame), Matsumae [6] (11-i-1989 leg. K. Kogame), Shirakamimisaki

[8] (19-iii-1987 leg. K. Kogame, 16-v-1988 leg. K. Kogame), Kikonai [10] (19-iii-1987 leg. K. Kogame, 21-iv-1987 leg. H. Kawai, 16-v-1988 leg. K. Kogame, 21-vi-1988 leg. N. Sato & Y. Hattori), Esan [11] (17-v-1988 leg. K. Kogame), Souya [34] (3-v-1988 leg. K. Kogame), Rishiri [35] (10-vi-1989 leg. H. Kawai), Abashiri [29] (6-ix-1987 leg. K. Kogame, 2-vii-1988 leg. K. Kogame), Shikabe [12] (4-iii-1987 leg. K. Kogame), Rebunge [13] (8-v-1985 leg. K. Kogame), Muroran [14] (18-iv-1987 leg. K. Kogame, 18-v-1987 leg. H. Kawai, 5-ii-1988 leg. K. Kogame, 21-ii-1988 leg. K. Kogame, 12-xii-1988 leg. K. Kogame, 22-i-1989 leg. K. Kogame, 24-ii-1989 leg. K. Kogame, 26-ii-1989 leg. K. Kogame, 30-vii-1989 leg. H. Kawai, 18-iii-1990 leg. H. Kawai, 27-v-1990 leg. H. Kawai), Harutachi [15] (15-v-1988 leg. K. Kogame), Urakawa [16] (4-v-1989 leg. T. Kitayama), Samani [17] (19-v-1991 leg. K. Kogame), Erimo [19] (16-v-1987 leg. K. Kogame, 19-v-1991 leg. K. Kogame), Syoya [20] (16-v-1987 leg. K. Kogame), Hiroo [21] (19-v-1991 leg. K. Kogame), Akkeshi [22] (26-vi-1990 leg. K. Kogame, 17-v-1991 leg. K. Kogame, 26-vi-1991 leg. K. Kogame, 29-vi-1991 leg. K. Kogame, 17-vi-1992 leg. Kogame, 18-vi-1992 leg. Kogame), Nokkamappu [26] (15-iv-1987 leg. Kogame), Hanasaki [23] (18-v-1991 leg. Kogame), Seseki [27] (1-vii-1988 leg. Kogame), Utoro [28] (1-vii-1988 leg. Kogame), Aomori Pref. : Ohma [38] (3-iii-1987 leg. Kogame, 21-ii-1988 leg. H. Kawai, 1-ii-1990 leg. K. Kogame), Akita Pref. : Iwadate [42] (7-v-1991 leg. T. Kitayama), Niigata Pref. : Shiiya [44] (27-iii-1989 leg. K. Kogame), Kashiwazaki [45] (27-iii-1989 leg. K. Kogame), Hyogo Pref. : Kasumi [52] (3-vi-1991 leg. K. Kogame), Hyogo Pref. : Maruyama, Awajishima [83] (3-iv-1992 leg. S. Enomoto & K. Kogame), Kagawa Pref. : Awashima [89] (5-iv-1989 leg. S. Enomoto & K. Kogame), Megishima [86] (6-vi-1989 leg. S. Enomoto & K. Kogame), Okayama Pref. : Kitagishima [87] (4-iv-1989 leg. S. Enomoto & K. Kogame, 11-v-1990 leg. S. Enomoto & K. Kogame), Manabeshima [88] (4-iv-1989 leg. S. Enomoto & K. Kogame), Ehime Pref. : Nakashima [96] (16-iv-1988 leg. S. Enomoto & K. Kogame), Uoshima [90] (8-v-1990 leg. S. Enomoto & K. Kogame), Ohshima [94] (10-v-1990 leg. S. Enomoto & K. Kogame), Ohmishima [95] (12-v-1990 leg. S. Enomoto & K. Kogame).

Microthalli: Hokkaido: Oshoro [1] (30-ix-1987 leg. K. Kogame, 7-xi-1987 leg. K. Kogame, 1-x-1988 leg. K. Kogame, 25-x-1988 leg. K. Kogame, 22-xi-1988 leg. K. Kogame, 23-ix-1989 leg. K. Kogame, 4-xi-1989 leg. K. Kogame, 2-xii-1989 leg. K. Kogame, 28-vii-1990 leg. K. Kogame, 24-x-1990 leg. K. Kogame, 24-xi-1990 leg. K. Kogame, 15-ii-1991 leg. K. Kogame), Notorimisaki [30] (26-viii-1989 leg. K. Kogame), Seseki [27] (27-viii-1989 leg. K. Kogame), Aomori Pref. : Ohma [38] (24-ix-1991 leg. K. Kogame), Ehime Pref. : Uoshima [90] (28-x-1987 leg. S. Enomoto & K. Kogame), Ohtobishima [91] (30-x-1987 leg. S. Enomoto & K. Kogame).

Herbarium specimens examined are follows. Iwate Pref. : Taneichi [55] (SAP 026934, 14-iv-1952 leg. S. Kawashima), Ohtsuchi [56] (SAP 053188, 26-v-1979 leg. S. Kawaguchi), Miyagi Pref. : Syobuta [60] (SAP 035277, 2-v-1965 leg. T. Yoshida), Kanagawa Pref. : Zushi [67] (SAP 055416, i-1923 leg. Y. Yamada), Hayama [66] (SAP 054873, 14-i-1979 leg. Tsukidate), Shizuoka Pref. : Shimoda [69] (SAP 054662, 21-xii-1985 leg. J. Tanaka), Aichi Pref. : Shinmaiko [72] (SAP 024891, 11-iv-1944 leg. T. Segi), Nagasaki Pref. : Nomozaki [108] (SAP 028382, 20-i-1961 leg. K. Matsubayashi), Miyazaki Pref. : Aoshima [112] (SAP

025480, 28-iii-1946 leg. M. Kurogi).

Morphology

Macrothalli

Macrothalli are cylindrical or flattened, hollow, 10-50 cm in length, 3-8 mm in width, commonly gregarious, arising from a small holdfast (Figs 47A-D). They are simple, unconstricted when young, later usually constricted at regular intervals, but sometimes not constricted, yellowish brown when young, darker when mature. Thalli consist of cortical and medullary layers (Figs 48A,B, 49A). The cortex is composed of 2-3 layers of small, pigmented cells (Fig. 48C). The medulla is composed of 3-5 layers of large, colorless cells. In cross section medullary cells are round to ellipsoid, larger in the inner portion, 15-80 × 15-80 µm in size. In longitudinal section medullary cells are longer in inner portions, up to 240-320 µm in length (Fig. 48D). Hairs are single or grouped, arising from the cortical layer. Holdfasts are composed of rhizoidal filaments arising from the under surface of thalli (Figs 48E,F). Thalli form plurilocular sporangia usually basipetally. Plurilocular sporangia are produced by divisions of cortical cells. Mature plurilocular sporangia are uni- or biseriate, loosely coherent to each other without a cuticle layer covering them, 30-50 µm in length (Figs 48G,H, 49B). Unicellular ascocysts are associated with plurilocular sporangia (Figs 48G,H, 49B). They are scattered, and are variable in number. Ascocysts remain after releases of plurispores. Secondary plurilocular sporangia are usually produced (Fig. 48H).

Microthalli

Microthalli are discoid, 1-3 mm in diameter, dark brown in color and gelatinous in mature thalli. Thalli consist of prostrate, branched filaments and uniseriate, simple, erect filaments arising from the prostrate filaments (Figs 50A-C, 51A-E). Erect filaments are loosely adjoined with each other. It is easy to squash the thalli between a slide glass and a cover slip. Mature thalli are 160-300 µm in thickness. In mature thalli, cells of erect filaments are 9-20 µm in length and 7-12 µm in width. When mature, upper portions of erect filaments elongate and change to paraphyses. Upper cells of the paraphyses are 8-13 × 8.5-10 µm in size, 1.0-1.5 times as long as width, and their lower cells are 30-50 × 6.5-8 µm in size, 4.5-7.5 times as long as width. Cells contain a plastid with a pyrenoid per cell (Fig. 50D). Unilocular sporangia are formed laterally on basal cells of the paraphyses (Figs 50E, 51B-E). They are sessile, elongate oval or clavate, 60-150 × 18-28 µm in size.

Habitat and Phenology

Macrothalli grow on rocks, boulders or other algae in the lower intertidal and subtidal zones exposed to or sheltered from wave action. In Japan, macrothalli are generally found in winter and spring (Table 22). At Oshoro, Western Hokkaido, macrothalli appear in winter persisting until spring, but vanish during summer and autumn. However, at colder localities such as Akkeshi and Nemuro, northeastern Hokkaido, macrothalli are found also during summer and autumn.

Microthalli grow on rocks or boulders or shells in the low intertidal and subtidal zones. Microthalli are found more abundantly in sheltered area than in exposed area. At Oshoro,

Hokkaido, microthalli bearing unilocular sporangia are found during autumn and winter (Table 22). Collection data of vegetative microthalli are not shown since identification of the vegetative thalli could not been done owing to few morphological characters.

Distribution

This species is widely distributed along coasts of cold and warm waters throughout the world (Smith 1944, Taylor 1957, Clayton 1981).

Culture study

Culture strains isolated from plurispores from macrothalli are shown in Table 23 in which culture conditions and progeny of plurispores are also given.

Sexual reproduction

Gamete fusions were observed in materials from Muroran and Oshoro. Macrothalli are dioecious gametophytes and gametes are mostly isogamous although female gametes are somewhat larger than male ones. Gametes are of normal phaeophycean type, with a plastid and a stigma. They usually showed negative phototaxis. Female and male gametes measured 6.0-7.0 × 3.0-4.4 μm and 5.6-6.8 × 2.6-4.0 μm in size, respectively (Figs 52A,B). When female and male gametes were mixed, a settled female gamete was surrounded by several male gametes which touched the settled female gamete by anterior flagella, and then, one of male gametes fused with the female gamete (Figs 52C-E). Zygotes were distinguished from unfused female gametes by possessing two stigmata (Fig. 52F). The

fragrant odor of sexual pheromone was observed in culture where female gametes were released.

Parthenogenetic development of gametes

Female gametes were cultured at 5-20°C under both SD and LD conditions. Germlings first developed into prostrate, branched filaments, later discoid thalli (Figs 53A-C). The discoid thalli usually had free branched filaments. The filaments produced disks when they attached to substrata. These discoid microthalli consist of prostrate branched filaments and simple, uniseriate upright filaments which arise from the prostrate filaments (Fig. 53D). After 3 months, discoid thalli formed unilocular sporangia under 15°C SD. The discoid thalli formed unilocular sporangia also under 10°C SD and 20°C SD. They formed unilocular sporangia more abundantly under 15°C ND 2 months after transfer from 20°C LD (Fig. 53E). Unilocular sporangia were elongate oval, sessile, laterally on the base of paraphyses.

Macrothalli were formed on branched filaments and disks. Proportions of microthalli issuing macrothalli were less than 10 %. Frequency of formation of macrothalli was somewhat higher in short day conditions than in long day conditions. Macrothalli grew to 10 cm or more in length, and became fertile with plurilocular sporangia at 5-15°C under both SD and LD. They were the same sex as that of the parent thallus when it was ascertained. Under 20°C conditions, macrothalli grew to less than 1 cm in length, and formation of plurilocular sporangia and its releases were rarely observed. Constrictions on macrothalli were not observed under any culture conditions tested.

Processes of parthenogenetic development of male gametes

were mostly the same as those in female gametes.

Development of unispores from parthenogenetic microthalli

Unilocular sporangia isolated from discoid thalli under 15°C ND were cultured under 10°C SD and 15°C ND (Table 24). Unispores (Fig. 53F) released from these unilocular sporangia germinated into prostrate branched filaments, later developed to small disks from which macrothalli arose well (Figs 53G,H). The small disks scarcely grow further (Fig. 53J). After 3-4 months, macrothalli grew to 10 cm in length and 1 mm in width, and formed plurilocular sporangia. Plurispores represented the same sex as their original parents.

Direct-type life history

In some strains, frequency of macrothalli was high in culture of plurispores from macrothalli (Table 23; strain no. 37 Erimomisaki, 44 Akkeshi, 46 Abashiri, 152 Shiretoko, 156 Akkeshi, 385 Menashidomari, 386 Oumu, 387 Saru, 241 Muroran). In culture of 156 Akkeshi, no unilocular sporangia were formed under all conditions examined.

Culture from unilocular sporangia on field microthalli

Unilocular sporangia were isolated and were cultured from field microthalli. Culture strains, culture conditions and progeny from the unispores are shown in Table 25.

Results of culture in strain 225 Oshoro were mentioned in detail below.

Unilocular sporangia isolated were cultured at 5-20°C under both SD and LD conditions. Unispores released from these

sporangia germinated into prostrate branched filaments (Figs 54A,B), later developed to small disks which give rise to many macrothalli under 5-15°C conditions (Figs 54C,D). A macrothallus initial was first a uniseriate upright filament which elongates and forms longitudinal walls. Macrothalli grew to 5-15 cm in length and 1 mm in width (Figs 54E,F), and became fertile forming plurilocular sporangia after 2-4 months. These macrothalli were cylindrical, hollow, and their plurilocular sporangia were identical with those of *Scytosiphon lomentaria*, loosely coherent to each other without a cuticle (Fig. 54G). Unicellular, clavate ascocysts were present in fertile portions (Fig. 54G). Constrictions on the macrothalli were not observed. Discoid systems grew to less than 1 mm in diameter. Under 20°C conditions formation of macrothalli, growth of them and formation of plurilocular sporangia were not well. Under the conditions discoid systems grew to 2-5 mm in diameter.

Female and male thalli were obtained in culture from one unilocular sporangium. Their gametes were fused with those in culture from field macrothalli.

Discussion

Scytosiphon lomentaria (Lyngbye) Link is distinguished from other species of *Scytosiphon* by the following features: macrothalli (gametophytes) constricted, loosely coherent plurilocular sporangia loosely attaching each other, absence of cuticle layers covering plurilocular sporangia, presence of ascocysts among plurilocular sporangia, and *Microspongium*-phase microthalli (sporophytes).

Constrictions of macrothalli are variable in its number and

the degree. Mature thalli without constrictions, however, are frequently observed. Thallus size is also variable. Furthermore, thalli are usually cylindrical but sometimes flattened or twisted. However, such thalli representing large variations in gross morphology have the same features in respect of form of plurilocular sporangia and ascocysts.

Microthalli of *S. lomentaria* in fields have been found by several authors. Nakamura (1965) reported *Ralfsia*-like crust collected from nature which is a sporophyte of *S. lomentaria*. Lund (1966) suggested that *Microspongium gelatinosum* Reinke is a microthallus of *S. lomentaria*. McLachlan *et al.* (1971) and Kristiansen and Pedersen (1979) demonstrated that microthalli in culture of *S. lomentaria* from Denmark resemble *M. gelatinosum*. Fletcher (1987) reported that macrothalli identical with *S. lomentaria* were obtained in culture of *M. gelatinosum* from the south coast of England. He also mentioned that most of so called *Ralfsia*-like crusts obtained in culture of *S. lomentaria* (Nakamura 1965, Tatewaki 1966, Nakamura and Tatewaki 1975, Rhodes and Connell 1973) were *Microspongium*-like. *Microspongium gelatinosum* was originally described by Reinke (1888) based on materials with plurilocular sporangia. Thalli with unilocular sporangia were also included in this species by Reinke (1889). Therefore, both plurilocular sporangia bearing plants and unilocular sporangia bearing plants are included in this Reinke's species. However, plurilocular sporangia bearing plants have not been known to be related with *S. lomentaria*. Kristiansen and Pedersen (1979) suggested that *M. gelatinosum* must be recognized as plurilocular sporangia bearing plants by strict sense of Reinke, and that its typification is needed.

In the present culture of unilocular sporangia on discoid thalli collected at Oshoro, macrothalli identical with *S. lomentaria* were obtained. These macrothalli produced plurilocular sporangia which are also identical with those of *S. lomentaria*. Their plurispores represent female and male gametes which fused with those of *S. lomentaria* in culture. These results clearly prove that field microthalli cultured represent sporophytes of *S. lomentaria*. Unispore cultures from microthalli collected at localities other than Oshoro gave rise to *S. lomentaria*-phase macrothalli.

The microthalli of *S. lomentaria* under study resembled *Microspongium gelatinosum* (Reinke 1889, only plants with unilocular sporangia) described by Fletcher (1987). However, pedicellate unilocular sporangia were not observed in my materials, whereas they were sometimes found in British Isles (Fletcher 1987). *Microspongium gelatinosum* in Denmark (Lund 1966, Kristiansen and Pedersen 1979) is polystichous at the upper portion of erect filaments, which is different from that in present observations. Microthalli of *S. lomentaria* may show such minor local differences in morphology. In culture of *Microspongium* sp. from Nova Scotia, McLachlan *et al.* (1971) observed the occurrence of *Scytosiphon*-like macrothalli. However, plurilocular sporangia formed on their macrothalli were firmly packed, which is completely different from loosely coherent ones of *S. lomentaria*. Therefore, their materials are not considered as *S. lomentaria*.

Ralfsia bornetii sensu Tanaka and Chihara (1980) is very similar to microthalli of *S. lomentaria* recognized in this study. This result supports Fletcher's opinion (Fletcher 1987, p.228)

that *Ralfsia bornetii* sensu Tanaka and Chihara (1980) is a synonym of *M. gelatinosum*.

Tatewaki (1966) and Nakamura and Tatewaki (1975) concluded that *S. lomentaria* represents a dimorphic and biphasic life history including gametophytic macrothalli bearing plurilocular sporangia and sporophytic crustose thalli bearing unilocular sporangia. A diagram of life history of this species in my present culture is shown in Fig. 55. In this study, other pathways agree with those of Nakamura and Tatewaki (1975) although my culture from zygotes was not carried out. However, in their culture of parthenogenetic germlings formation of macrothalli was obviously effected by temperature and photoperiod, whereas in this study it is not obvious with most of them developing to microthalli. Responses of macrothallus formation to temperature and photoperiod were reported by Wynne (1969) (in materials from California), Rhodes and Connell (1973) (American east coast), Dring and Lüning (1975) (Helgoland): macrothalli arise abundantly in lower temperature and short day conditions. An example without such responses was reported by Clayton (1976, 1978) (Australia) and Kristiansen and Pedersen(1979) (Denmark). Responses of the macrothallus production to only temperature were also known (Correa *et al.* 1986, in *S. lomentaria* var. *complanatus* sensu Correa *et al.*). Furthermore, Roborts and Ring (1972) and Kristiansen and Pedersen (1979) (Denmark) reported the macrothallus production was effected by light intensity.

At Oshoro, Hokkaido, microthalli were fertile with unilocular sporangia during autumn and winter. Such phenological observations agree with the culture result that microthalli

become fertile under 10-20°C SD and 15°C ND conditions. Macrothalli begin to appear in winter at Oshoro, and in culture unispores from microthalli in fields developed to macrothalli under 5-15°C conditions. These results strongly suggest that macrothalli in fields develop from unispores released from microthalli during autumn and winter. Therefore, the unilocular sporangial production controlled by temperature and photoperiods may account for the phenology of macrothalli.

In development of unispores, Nakamura and Tatewaki (1975) also showed that they developed to macrothalli. However, Wynne (1969) reported that formation of macrothalli depended on temperature and photoperiods in development of unispores as in development of plurispores.

In culture of strain no. 156 Akkeshi, responses of formation of macrothalli to culture conditions were different from those in culture from Oshoro. In culture from Akkeshi, the vast majority of plurispores developed into macrothalli at 5-15°C. This result may suggest that this strain has a direct type life history.

Table 22. Collection data of *Scytosiphon lomentaria* at Oshoro, Hokkaido. (P, thalli with plurilocular sporangia; U, thalli with unilocular sporangia; (U), microthalli bearing unilocular sporangia identified morphologically, not cultured; --, thallus was not found)

Macrothalli

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
1987					P	P		--	--		--	P
1988	P	P	P							--	--	
1989			P		P	P		--	--		--	--
1990		P	P	P			--		--	--	--	
1991		P										
1992			P	P								

Microthalli

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
1987									U		U	
1988										U	U	
1989									(U)		U	U
1990							(U)			(U)	(U)	
1991		(U)										

Table 23. *Collection data of cultured strains of* *Scytosiphon lomentaria*. Culture conditions tested and progeny of plurispores from macrothalli are also shown. Tufts always appear with disks.

strain no.	locality	date	sex	culture conditions	progeny
15	Setana	18 iii 1987		10L, 15L	disks, a few macrothalli (P)
20	Takahama	1 iv 1987		10L	disks, a few macrothalli (P)
				20L→15S	disks (U)
22	Hanasaki	16 iv 1987		10L,15L	disks, a few macrothalli (P)
				20L	disks (U)
25	Muroran	18 iv 1987		10L	disks, a few macrothalli
31	Oshoro	8 v 1987		10L	disks, a few macrothalli
				15S	disks (U), a few macrothalli (P)
37	Erimomisaki	16 v 1987		5S, 10SL	disks, many macrothalli (P)
38	Syono	17 v 1987		10L	disks, a few macrothalli (P)
44	Akkeshi	1 vii 1987		10L	macrothalli (P)
46	Abashiri	6 ix 1987		10L	disks sprouting macrothalli (P)
84	Oshoro	21 i 1988		10L, 15L	disks, a few macrothalli (P)
106	Himeshima	17 iv 1988		10L	disks, a few macrothalli (P)
109	Kurotsuzaki	10 iv 1988		10L, 20L	disks
112	Nakajima	21 iv 1988		10L	disks, a few macrothalli (P)
116	Ofuyu	2 v 1988		10L	disks, a few macrothalli (P)
117	Bakkai	2 v 1988		10L	disks, a few macrothalli (P)
119	Kitamiesashi	3 v 1988		10L	disks, a few macrothalli
125	Shirakamimisaki	16 v 1988		10L	disks, a few macrothalli
132	Oshoro	24 v 1988	female	10L	disks, a few macrothalli (P)
148	Hanasaki	29 vi 1988		10L	disks, a few macrothalli
152	Shiretoko	1 vii 1988		10L, 15L	disks sprouting macrothalli
156	Akkeshi	14 vii 1988		5SL,10S,15S	macrothalli
				10L,15L,20S	disks sprouting macrothalli (P)
				20L	disks
381	Muroran	26 ii 1989	female	5S	disks, macrothalli (P)

Table 23. Continued.

strain no.	locality	date	sex	culture conditions	progeny
382	Muroran	26 ii 1989	male	5S	disks, macrothalli (P)
231	Oshoro	1 v 1989	female	5S, 5-20L	disks, a few macrothalli (P; 5-15L)
				10-20S	disks (U), a few macrothalli
				20L→15N	disks (U)
232	Oshoro	1 v 1989	male	5S, 5-20L	disks, a few macrothalli (P; 5-15L)
				10-20S	disks (U), a few macrothalli (P; 5S,10S)
				20L→15N	disks (U)
385	Menashidomari	11 vi 1989		10L	disks sprouting macrothalli (P)
386	Oumu	11 vi 1989		10L	disks sprouting macrothalli (P)
387	Saru	12 vi 1989		10L	disks sprouting macrothalli (P)
241	Muroran	30 vii 1989		5-15L	disks, a few macrothalli (P)
				20SL	disks
				10S, 15S	disks, many macrothalli

Table 24. Culture of *Scytosiphon lomentaria*. Progeny of unispores from microthalli.

strain no.	locality	date	sex	culture conditions	progeny
231	Oshoro	1 v 1989	female	10S, 15N	macrothalli (P; female gametes)
232	Oshoro	1 v 1989	male	10S, 15N	macrothalli (P; male gametes)

Table 25. Culture of *Scytosiphon lomentaria*. Progeny of unispores from field microthalli.

strain no.	locality	date	culture conditions	progeny
52	Oshoro	30 ix 1987	10L	macrothalli (P)
59	Ohtobisima	30 x 1987	15L	macrothalli (P)
68	Oshoro	7 xi 1987	15L	macrothalli (P; female and male gametes)
169	Oshoro	1 x 1988	10L	macrothalli (P)
180	Oshoro	25 x 1988	15L	macrothalli (P; female and male gametes)
188	Oshoro	22 xi 1988	10L	macrothalli (P)
244	Notorimisaki	26 viii 1989	10L	macrothalli (P)
246	Seseki	27 viii 1989	10L	macrothalli (P)
254	Oshoro	4 xi 1989	5-15SL	macrothalli (P)
			20SL	disks, disks sprouting macrothalli
255	Oshoro	2 xii 1989	5-15SL	macrothalli (P)
			20SL	disks, a few macrothalli
365	Ohma	24 ix 1991	10S	macrothalli (P)

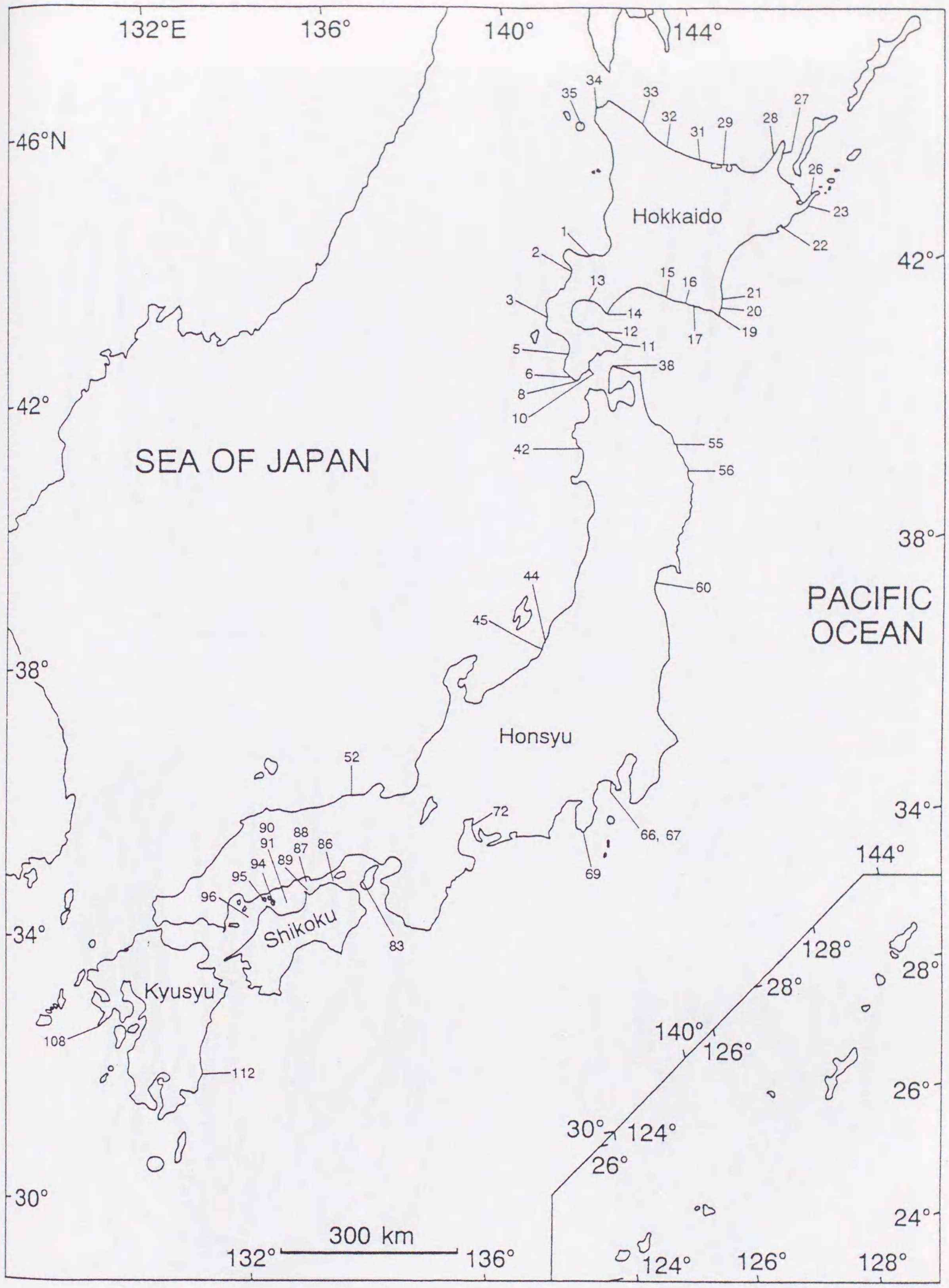


Fig. 46. Localities where materials of *Scytosiphon lomentaria* were collected. Numbers plotted correspond to those in Table 1.

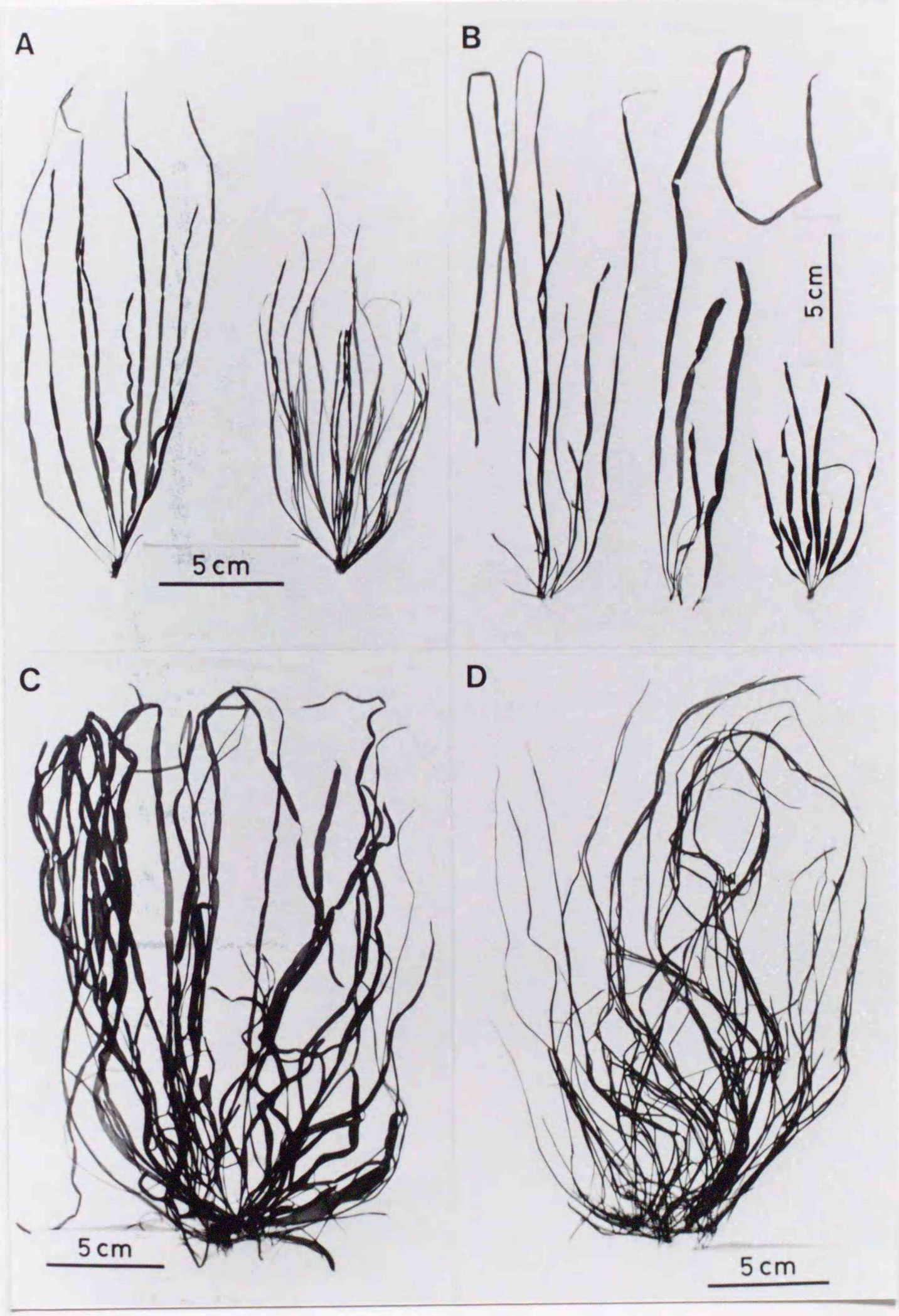


Fig. 47. Habit of dried herbarium specimens of *Scytosiphon lomentaria*.

A. Plants collected at Oshoro, Hokkaido on 21 January 1988. B. Plants collected at Oshoro, Hokkaido on 28 February 1990. C. Plants collected at Oshoro, Hokkaido on 17 April 1990. D. Plants collected at Oshoro, Hokkaido on 21 May 1990.

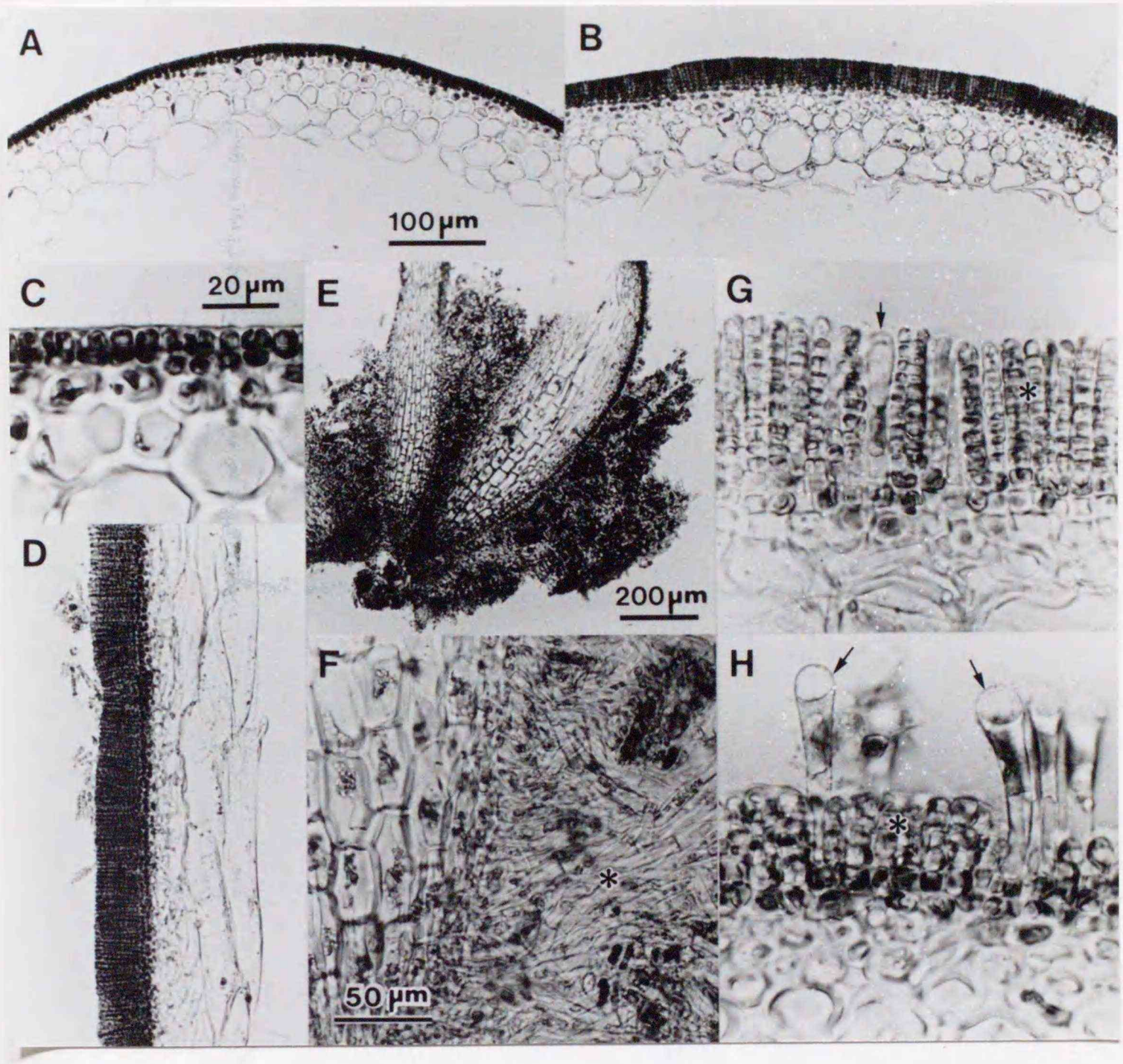


Fig. 48. Anatomy of *Scytosiphon lomentaria*.

A, B. Cross sections of thalli. C. Cortical layer in cross section of thallus. D. Longitudinal section of thallus. E. Longitudinal section of basal portion. F. Rhizoids (asterisk) constructing holdfast. G. Plurilocular sporangia (asterisk) and ascocyst (arrow). H. Ascocysts (arrows) and secondary plurilocular sporangia (asterisk).

Scale in **A** applies to **B** and **D**; Scale in **C** applies to **G** and **H**.

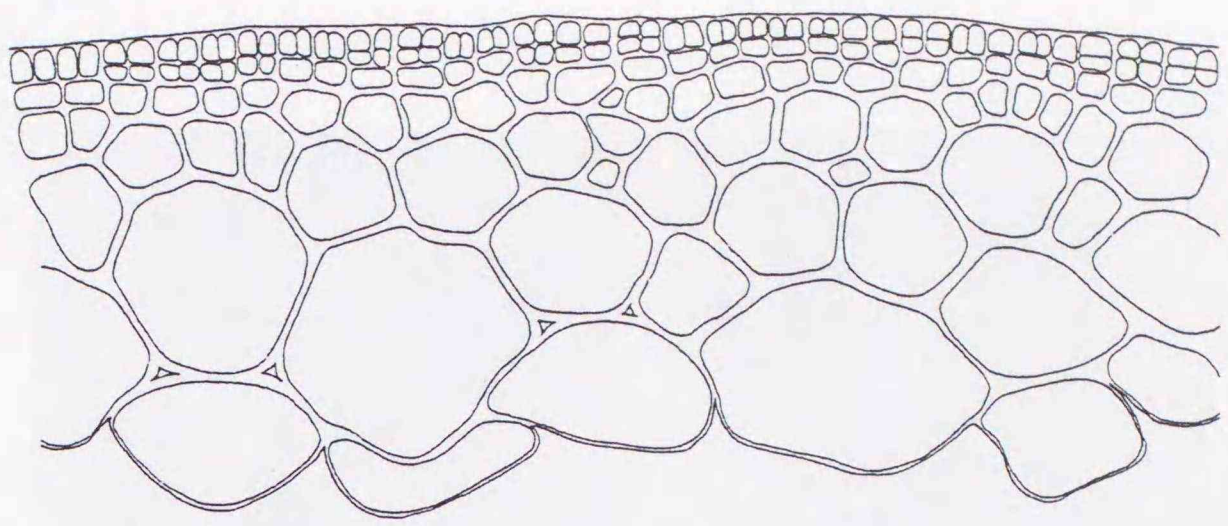


Fig. 49. Anatomy of *Scytosiphon lomentaria*.

A. Cross section of vegetative thallus. B. Cross section of fertile thallus, showing plurilocular sporangia (pl) and ascocysts (as).

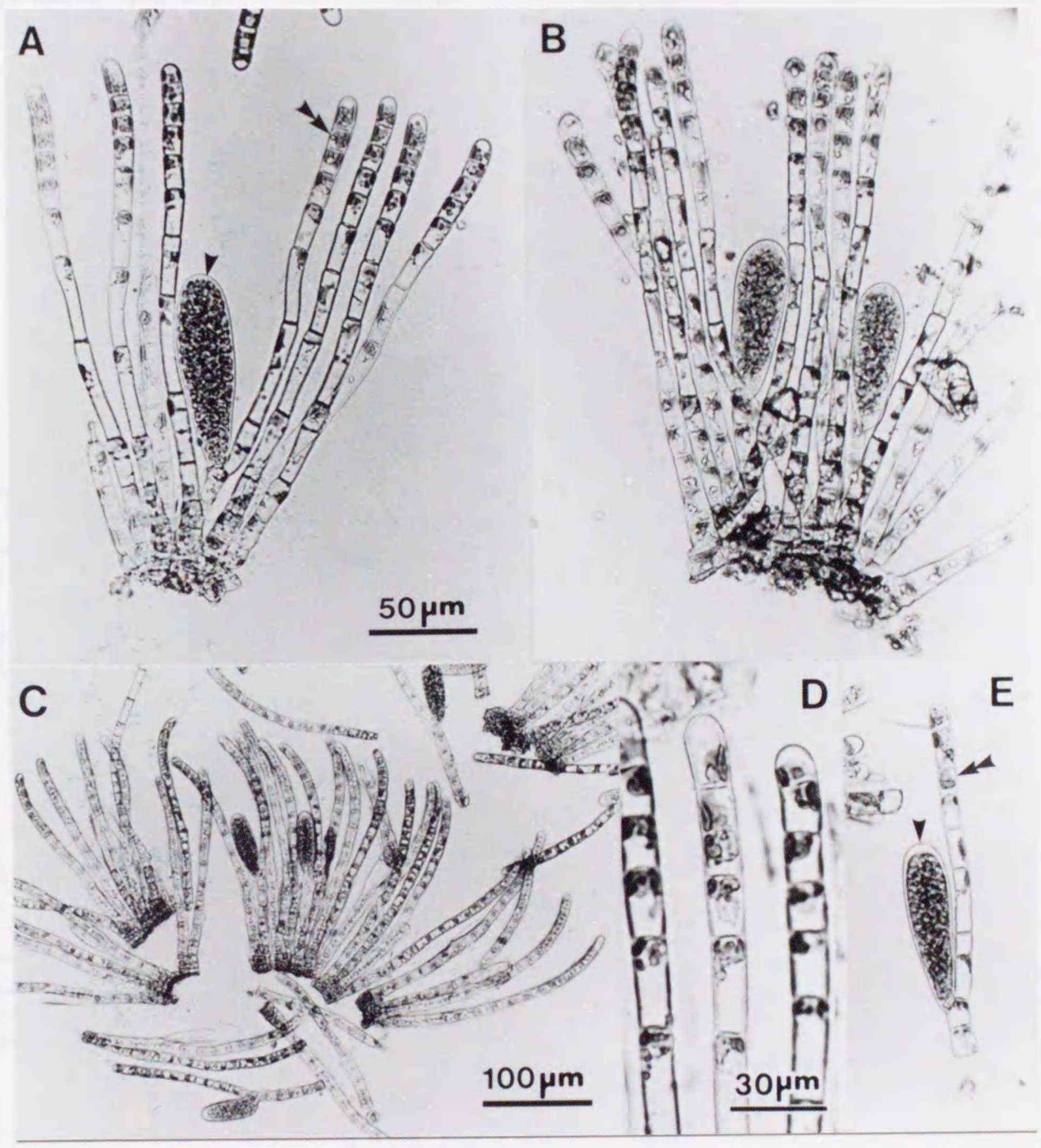


Fig. 50. *Scytosiphon lomentaria*. Anatomy of field microthalli.

A-C. Squashed fertile microthalli with unilocular sporangia (arrowhead) and paraphyses (double arrowhead) (**A:** Oshoro, Hokkaido, 25 October 1988, **B:** Oshoro, Hokkaido, 22 November 1988, **C:** Ohtobishima, Ehime Pref., 30 October 1987). **D.** Paraphyses, showing a plastid with a pyrenoid per cell. **E.** Unilocular sporangium (arrowhead) laterally on a basal cell of a paraphysis (double arrowhead).

Scale in **A** applies to **B** and **E**.

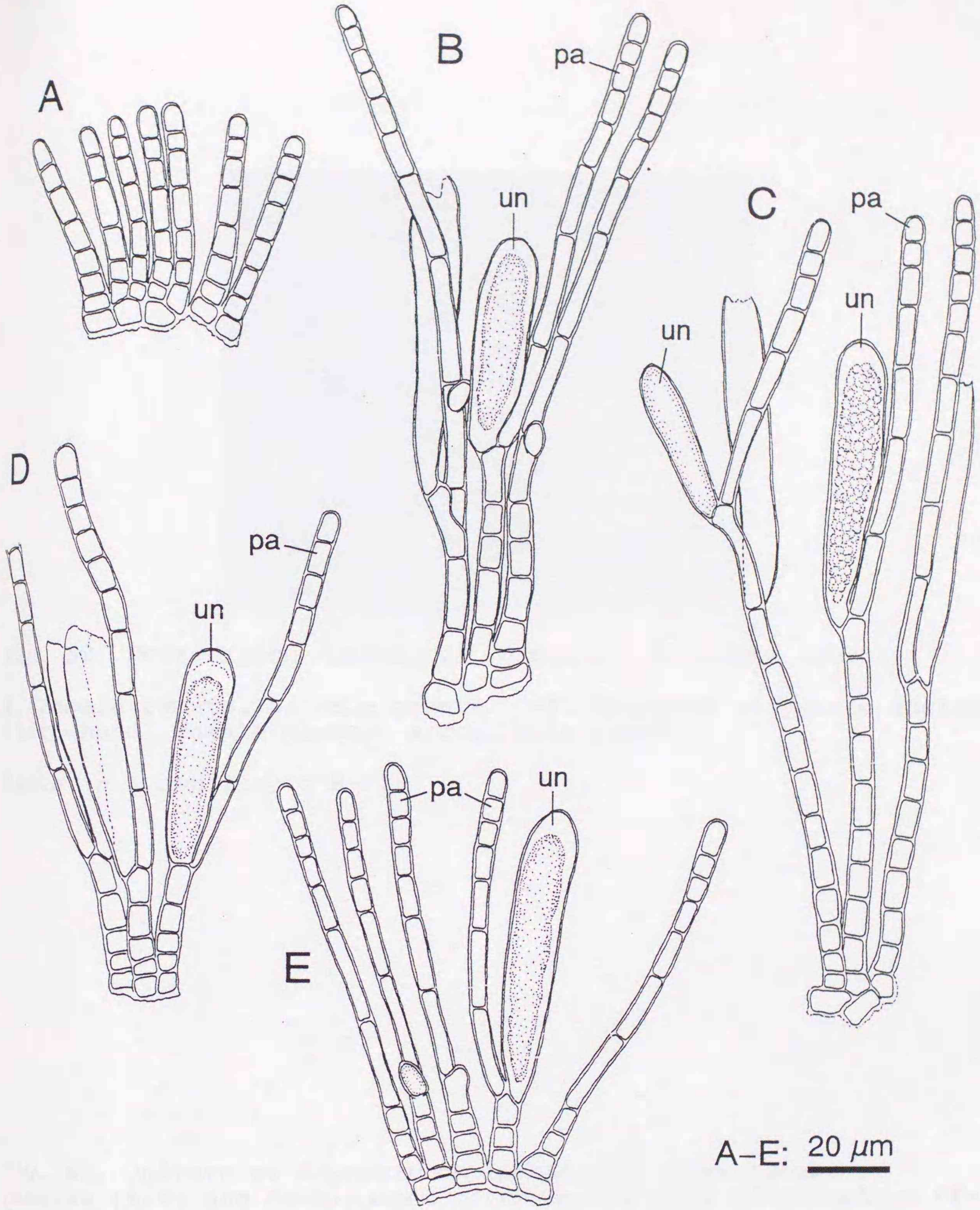


Fig. 51. *Scytosiphon lomentaria*. Anatomy of field microthalli.

A. Infertile thallus squashed. **B-E.** Fertile thalli squashed, showing unilocular sporangia (un) and paraphyses (pa).

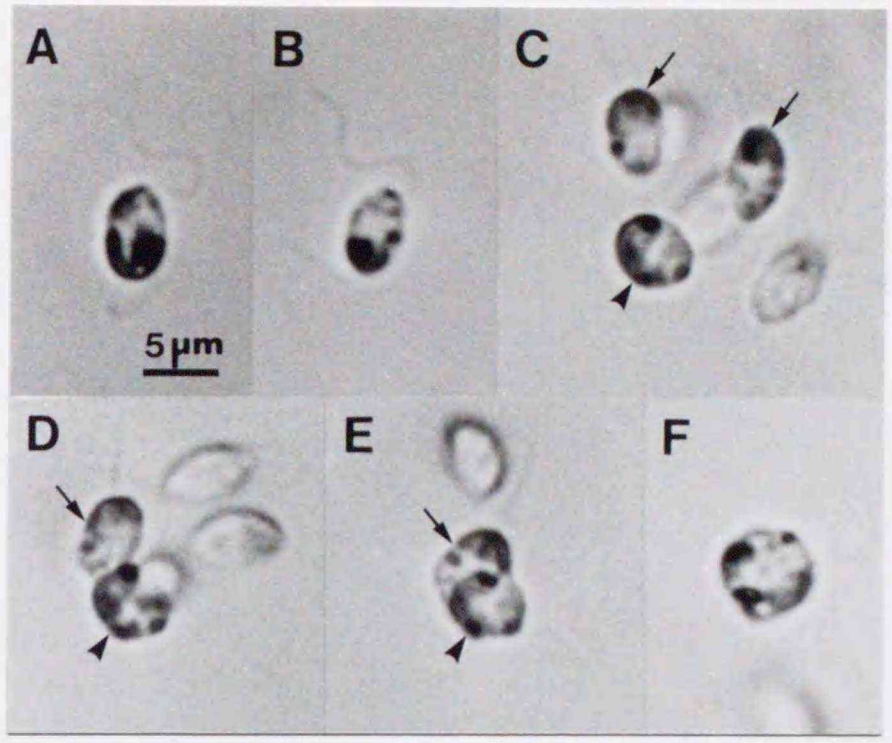


Fig. 52. *Scytosiphon lomentaria*. Gametes and gamete fusion.

A. Female gamete. **B.** Male gamete. **C-F.** Sequence of gamete fusion (arrowhead, female gamete; arrow, male gamete).

Scale in **A** applies to **B-F**.

⇒

Fig. 53. Culture of *Scytosiphon lomentaria*. Development of gametes (**A-E**) and development of unispores from microthallus (**F-J**).

A. Fifteen-day-old germling under 5°C SD condition. **B.** Germling with small discoid part under 10°C LD condition. **C.** Discoid microthallus under 10°C LD condition. **D.** Squashed discoid microthallus. **E.** Squashed microthallus forming unilocular sporangia (arrow) and paraphyses (arrowheads) under 10°C SD condition. **F.** Unispore from microthallus. **G.** Sixteen-day-old germling under 15°C ND condition. **H, I.** Squashed discoid thalli forming initials of macrothalli (arrowheads). **J.** Macrothalli under 15°C ND condition.

Scale in **A** applies to **H**.

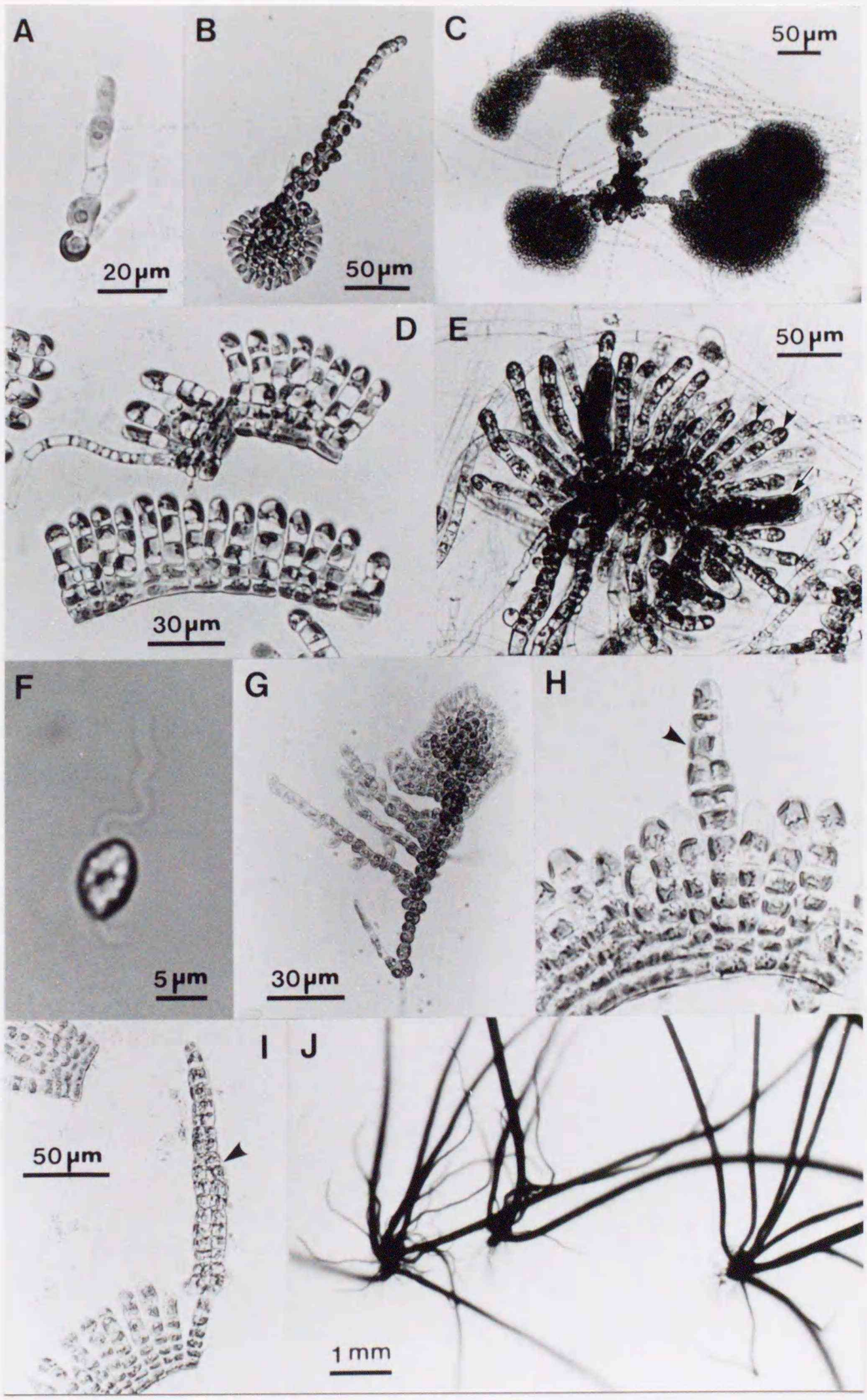


Fig. 53. Culture of *Scytosiphon lomentaria*.

⇐

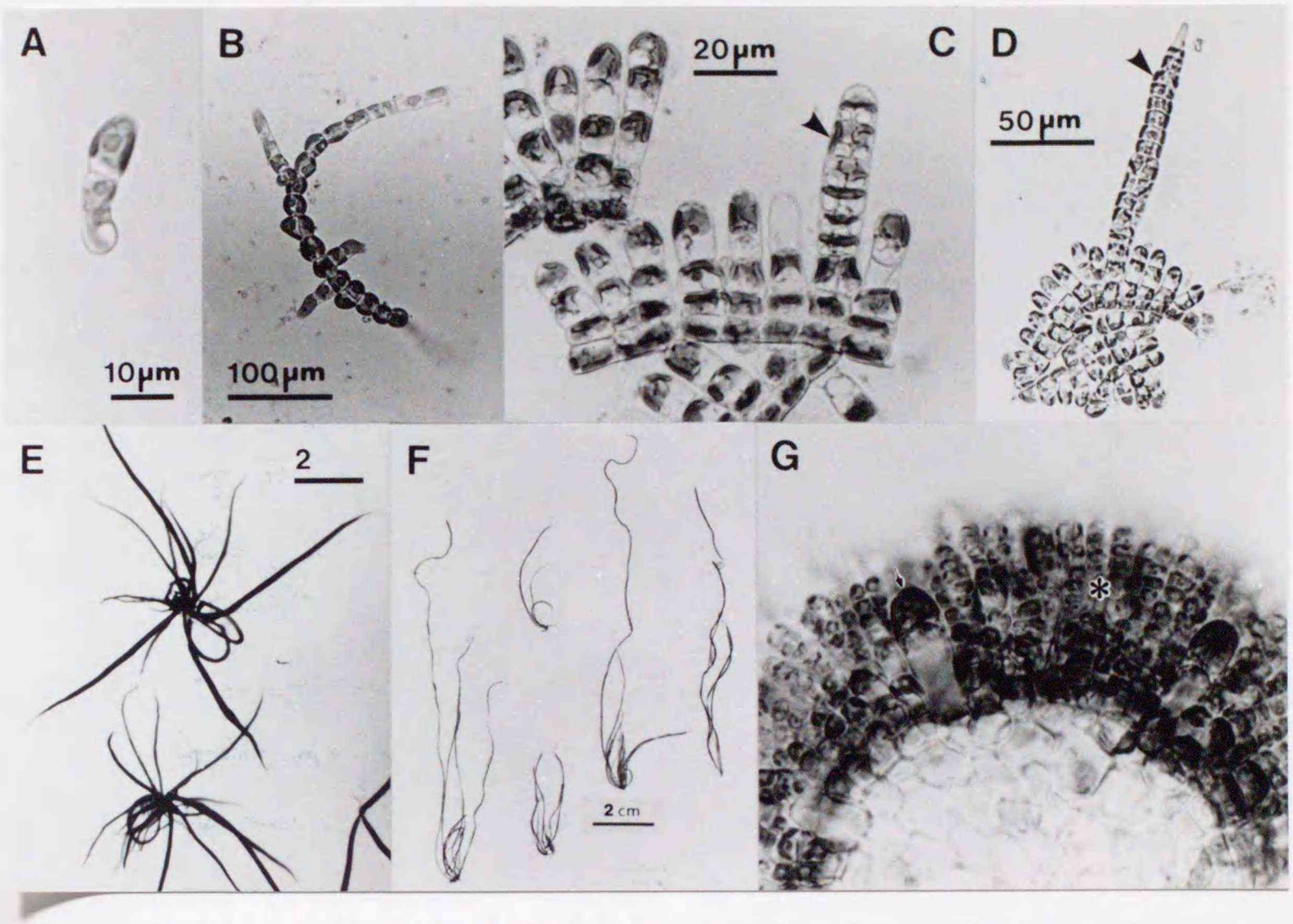


Fig. 54. Culture of *Scytosiphon lomentaria*. Development of unispores from field microthallus.

A. Six-day-old germling under 10°C LD condition. **B.** Twenty five-day-old germling under 10°C LD condition. **C, D.** Squashed small discoid thalli forming initials of macrothalli (arrowheads) (**C:** 10°C LD condition, **D:** 10°C SD condition). **E.** Macrothalli under 15°C LD condition. **F.** Dried specimens of macrothalli obtained under 10°C LD condition. **G.** Plurilocular sporangia (asterisk) and ascocysts (arrow) in cross section of macrothallus under 10°C LD condition.

Scale in **C** applies to **G.**

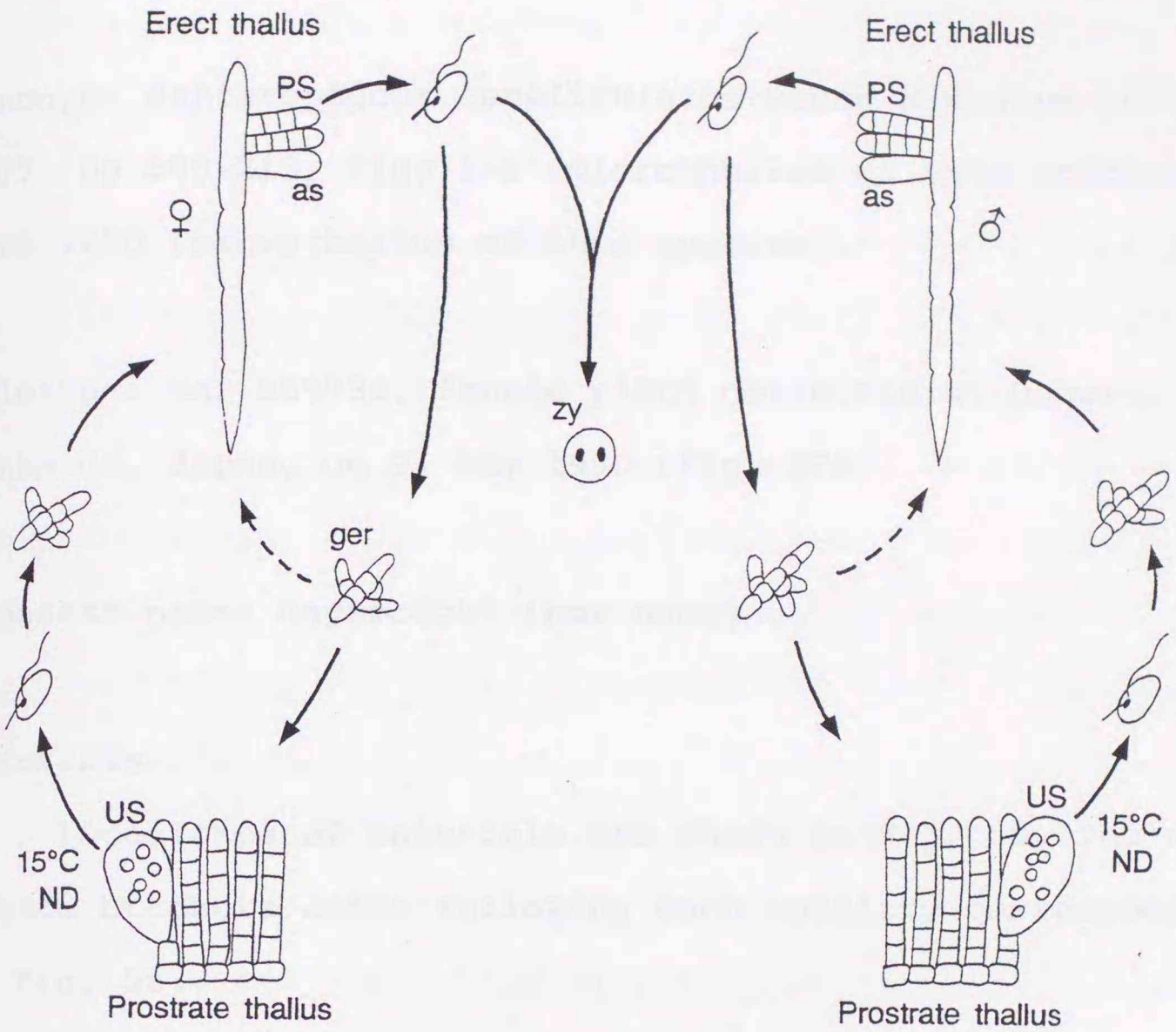


Fig. 55. Diagram of life history of *Scytosiphon lomentaria* in culture.

as, ascocyst; ger, germling; PS, plurilocular sporangium; US, unilocular sporangium; zy, zygote; ND, neutral day condition; broken line, rare course.

Scytosiphon nakamurae **sp. nov.**

Synonym: *Hapterophycus canaliculatus* sensu Nakamura and Nakahara 1977, pp.203-213, Figs 1-5 (microthallus of this species); sensu Mine 1990 (microthallus of this species).

Holotype: SAP 059732, female plant collected at Oshoro, Otaru, Hokkaido, Japan, on 21 May 1990 (Fig. 57A).

Japanese name: Kayamodoki (new name)

Materials

Localities of materials are shown in Fig. 56. The numbers in square brackets after following each locality correspond to those in Fig. 56.

Macrothalli: Hokkaido: Oshoro [1] (17-iv-1990 leg. K. Kogame, 16-v-1990 leg. M. Nagano, 21-v-1990 leg. K. Kogame, 4-vi-1990 leg. K. Kogame, 26-iv-1992 leg. K. Kogame), Notorimisaki [30] (2-vii-1988 leg. K. Kogame), Rishiri [35] (19-vi-1991 leg. H. Kawai), Aomori Pref. : Ohma [38] (29-iv-1990 leg. K. Kogame).
Microthalli: Hokkaido: Oshoro [1] (9-xii-1987 leg. K. Kogame, 21-i-1988 leg. K. Kogame, 17-ii-1988 leg. K. Kogame, 22-xi-1988 leg. K. Kogame, 2-xii-1989 leg. K. Kogame), Aomori Pref. : Ohma [38] (2-ii-1990 leg. K. Kogame).

Descriptio

Macrothalli simplices, tubulares, cylindrici, ad 7 mm in latitudine, ad 40 cm in longitudine, rare constricti, gregarii, sub basi parva exorientes, flavidi vel atrobrunnei; e cellulis corticalibus uni- ad tristratis pigmentifera et cellulis medullosis tri- ad quinquestratis incoloribus compositi; cellulae medullosae in sectione transversali rotundae vel ovales, 12-50 × 12-50(60) µm in amplitudine; longitudo cellularum medullosarum extus 15-35 µm intus 300-550 µm; pili achromi plerumque

aggregati, e cellulis corticalibus orientes; hapteron basale ex filamentis rhizoideis constans; plantae dioeciae; gametis anisogamicis; sporangia plurilocularia feminea uniserialia vel partim biserialia, 28-54 µm in longitudine; gametae femineae 7.8-9.8 × 4.2-5.8 µm in amplitudine; sporangia plurilocularia masculina pleurumque quadriserialia, 28-54 µm in longitudine; gametae masculae 4.4-5.8 × 1.8-2.6 µm in amplitudine; sporangia plurilocularia ambo generatim basipetaliter in pagina integera thallorum facta; sporangia plurilocularia matura laxe cohaerentia inter se, sine strato tegens cuticulare atque a presso facile discreta; ascocystae unicellulares inter sporangia plurilocularia adsunt; sporangia plurilocularia secundaria vulgaria.

Microthalli crustacei, irregulares in forma ad 3 cm in diametro flavidi ad atrobrunnei in colore, asperati, aliquantum ad margine divisi, pseudoparencymati, ex filamentis ramosis sursum et deorsum curvis constantes; cellulae medullosae 60-90 µm in longitudine, 16-30 µm in latitudine; mago inter strata medullosa et corticalia inliquida; cellulae thallorum plastidium unum cum pyrenoide una in quoque cellula continentes; sporangia unilocularia lateralia super basi paraphysium, sessilia aut pedicello uni- vel bicellulari, clavata vel elongate obovoidea, 80-130 × 24-34 µm in amplitudine; paraphyses uniseriales, 120-310 µm in longitudine, quinque- ad octocellulares; cellulae paraphysium superne 6-20 µm in longitudine et 9-13 µm in latitudine, inferne 90-110 µm in longitudine et 7-10 µm in latitudine.

Etymology: the epithet commemorates Dr Yositeru Nakamura, Professor Emeritus of Hokkaido University, for his many

contributions to phycology including the taxonomy of the family Scytosiphonaceae.

Description

Macrothalli simple, tubular, cylindrical, up to 7 mm in width, up to 40 cm in length, rarely constricted, gregarious, arising from a small base, yellowish to dark brown in color, composed of pigmented, 1-3-layered cortical cells and colorless, 3-5-layered medullary cells; medullary cells rounded or oval, 12-50 × 12-50(-60) µm in size in transverse section; length of medullary cells, 15-35 µm in outside and 300-550 µm in inside; phaeophycean hairs usually grouped, arising from cortical cells; basal holdfast consisting of rhizoidal filaments. Plants dioecious with anisogamous gametes; female plurilocular sporangia uniseriate or partly biseriate, 28-54 µm in length; female gametes 7.8-9.8 × 4.2-5.8 µm in size; male plurilocular sporangia usually quadriseriate, 28-54 µm in length; male gametes 4.4-5.8 × 1.8-2.6 µm in size; both plurilocular sporangia produced on the entire surface of the thalli, generally basipetally; mature plurilocular sporangia loosely coherent to each other without a covering cuticle layer, and easily separated by pressure; unicellular ascocysts present among plurilocular sporangia; secondary plurilocular sporangia common.

Microthalli crustose, irregular in shape, up to 3 cm in diameter, yellowish to blackish brown in color, confluent, somewhat divided at the margin, pseudoparenchymatous, consisting of upwardly curved branched filaments and downwardly curved branched filaments; medullary cells 60-90 µm in length and 16-30 µm in width; a border between medullary and cortical layers not

clear; cells of thalli containing a plastid with a pyrenoid per cell; unilocular sporangia lateral on the base of paraphyses, sessile, or with one- or two-celled pedicel, clavate or elongate obovoid, 80-130 × 24-34 µm in size; paraphyses uniseriate, 120-310 µm in length, 5- to 8- celled; cells of paraphyses 6-20 µm in length, 9-13 µm in width in the upper part, 90-110 µm in length, 7-10 µm in width in the lower part.

Morphology

Macrothallus

Macrothalli are simple, tubular, up to 7 mm in width, 40 cm in length, rarely constricted, gregarious, arising from a small base, yellowish to dark brown in color (Figs 57A-D). The thalli are composed of pigmented cortical cells (1-3 cell layers thick) and colorless medullary cells (3-5 cell layers thick) (Figs 58A,B, 59A). Medullary cells are rounded or oval, 12-50 × 12-50(60) µm in size in transverse section. In longitudinal section, medullary cells are longer in inside, and 15-35 µm in length in outside and 300-550 µm in length in inside (Fig. 58C). Phaeophycean hairs are usually grouped, arising from cortical cells, and are sunken in fertile thalli. A basal holdfast is composed of rhizoidal filaments (Fig. 58D).

Plurilocular sporangia are formed on the entire surface of the thalli, generally basipetally. The plants represent dioecism and produce anisogametes. Female plurilocular sporangia are uniseriate or partly biseriate, and 28-54 µm long with accompanying ascocysts (Figs 58E, 59C). Male plurilocular sporangia are usually tetraseriate and 28-54 µm in length with unicellular ascocysts which are the same as female ones (Figs

58F, 59D). Plurilocular sporangia are formed by divisions of cortical cells. They are covered with a cuticle layer in immature state (Fig. 59B). But, mature plurilocular sporangia are loosely adhered with each other without the cuticle, and easily separated by pressure. After release of plurispores ascocysts remain on the surface of the thalli. Secondary plurilocular sporangia are produced among the remaining ascocysts (Figs 58G, 59E).

Microthallus

Microthalli are crustose, irregular in shape, up to 3 cm in diameter, yellowish to blackish brown in color (Fig. 60A). The thalli are confluent and somewhat divided at the margin. Thalli are pseudoparenchymatous, consisting of upwardly and downwardly curved branched filaments whose cells are 60-90 µm in length and 16-30 µm in width in a medullary layer (Figs 60B,C). Terminal cells of upwardly curved filaments form a cortical layer. A border between medullary and cortical layer is not clear. Cells of thalli contain a plastid with a pyrenoid per cell (Fig. 60F).

Reproductive organs on the microthalli are only unilocular sporangia. They are formed laterally on the base of paraphyses which arise from the surface of the thallus (Figs 60C, 61A). Unilocular sporangia are usually pedicellate (with one- or two-celled pedicel) or sessile, clavate or elongate obovoid in shape, 80-130 × 24-34 µm in size (Figs 60D,E, 61A,B). Paraphyses are uniseriate, 120-310 µm in length, and they are composed of 5-8 cells. Cells of the paraphyses are 6-20 µm in length, 9-13 µm in width in the upper cells and 90-110 µm in length, 7-10 µm in width in the lower cells.

Habitat and Phenology

Macrothalli grow on rocks, boulders and shells in the lower intertidal zone in exposed or sheltered area. At Oshoro, Hokkaido macrothalli are present in spring but absent in other seasons (Table 26).

Microthalli grow on rocks, boulders and shells in the lower intertidal and subtidal zones. Microthalli with unilocular sporangia appear in late autumn and persist during winter months (Table 26). Since identification of vegetative thalli was difficult, their phenological information can not be given in this study.

Geographical distribution

This species has been known from only five localities: Oshoro, Shiriuchi (Mine 1990), Muroran (Nakamura and Nakahara 1977), Notorimisaki, Hokkaido and Ohma, Aomori Prefecture.

Culture study

Culture strains isolated from macrothalli are shown in Table 27. Culture conditions and progeny of plurispores are also indicated in Table 27.

Sexual reproduction

Female gametes released from plurilocular sporangia were pear drop-shaped, 7.8-9.8 × 4.2-5.8 μm in size, laterally biflagellate with a longer anterior and a shorter posterior flagella (Fig. 62A). They contained a plastid with a pyrenoid and a stigma, and physodes. They showed negative phototaxis, and then settled on the substratum. Settled female gametes are rounded,

5.4-6.4 µm in diameter. Male gametes were the same shape as female gametes, but, slender and smaller than the female, 4.4-5.8 x 1.8-2.6 µm in size (Fig. 62B). They contained a pale chloroplast with a stigma. They settled on the substratum after swimming showing negative phototaxis. Settled male gametes were rounded, 2.6-3.6 µm in diameter. When female and male gametes were mixed, a settled female gamete was surrounded by several male gametes which were attached to the settled female gamete by anterior flagellum, and then, one of male gametes fused with the female gamete (Figs 62C-E). Zygotes were distinguished from unfused female gametes by possessing two stigmata (Fig. 62F).

The fragrant odor of sexual pheromone was observed in culture where female gametes were released.

Development of zygote

Strains used, culture conditions and progeny of zygotes are shown in Table 28.

Zygotes isolated were cultured under a 20°C LD condition. Zygotes germinated and formed prostrate branched filaments, and then become small disks (Figs 63A-D). The small disks raised its surfaces and developed to spherical masses which became free from substrata. These masses grew into hapteron-like microthalli (Fig. 63E). They were pseudoparenchymatous, consisting of downwardly and upwardly curved branched filaments in vertical sections (Fig. 63F). Microthalli produced unilocular sporangia two months after transfer from 20°C LD to 15°C SD (Fig. 63G). Unilocular sporangia were formed laterally on the basal portions of uniseriate paraphyses which arose from the thallus surface (Figs 63G,H).

Development of unispore from zygotic crustose thallus

Unispores measured 6.4-7.8 × 4.0-4.8 µm in size, and settled unispores measured 4.4-5.4 µm in diameter. Culture of unispores were started from excised unilocular sporangia which were pipetted into dishes one by one. Progeny of unispores released from unilocular sporangia and culture conditions are shown in Table 29.

Unispores (Fig. 64A) germinated and formed small disks (Figs 64B,C) all of which subsequently produced numerous macrothalli. These macrothalli grew well under 5-15°C conditions and reached reproductive maturity. Fertile thalli measured up to 8 cm or more in length and 1-1.5 mm in diameter at 5-15°C (Figs 64D,E); however, those under 20°C conditions were less than 1-2 mm in length. Macrothalli became fertile, bearing plurilocular sporangia (Figs 64F,G). Female and male thalli were obtained from one unilocular sporangia, and their gametes fused well. Under 20°C conditions, plurispore release rarely occurred although macrothalli produced plurilocular sporangia.

Parthenogenetical development of female gamete

Settled female gametes germinated into small disks through prostrate branched filaments (Figs 65A-C). The small disks became swollen, and developed to spherical masses which became free from the substrata (Fig. 65D). These spherical thalli grew well under higher temperature and long day conditions, and became irregular and hapteron-like in shape (Fig. 65E). These thalli formed unilocular sporangia under 15°C SD after four months. Unilocular sporangia were also abundantly formed on thalli after transfer from 20°C LD to 15°C SD (Figs 65F-H). These thalli represented

the same morphology as zygotic hapteron-shaped thalli.

Macrothalli rarely occurred in the parthenogenetical development of female gametes. Macrothalli became fertile with female plurilocular sporangia.

Parthenogenetical development of male gamete

Settled male gametes mostly did not germinate, when they germinated, the majority did not grow well (Fig. 65I); however, a few germlings gave rise to hapteron-shaped thalli (Fig. 65J). These thalli were transferred from 20°C LD to 15°C SD. They became fertile 2 months after transferring and formed unilocular sporangia. Male hapteron-shaped thalli represented the same morphology as female ones.

Development of unispore from parthenogenetic crustose thallus

Unilocular sporangia on microthallus grown under a 15°C SD condition were isolated and maintained in various culture conditions. Culture strains, conditions and progeny of unispores are shown in Table 30.

Unispores (Fig. 65K) released from unilocular sporangia germinated and developed into macrothalli in the same manner as unispores from zygotic crustose thalli (Figs 65L-N). Macrothalli of female and male strains formed respective female and male plurilocular sporangia (Fig. 650). Gametes released from these plurilocular sporangia fused when female and male gametes were mixed.

Culture from field microthallus

Unilocular sporangia formed on field microthalli were

isolated and cultured. Culture strains, culture conditions and progeny of unispores are shown in Table 31.

Unispores (Fig. 66A) germinated and formed small disks (Figs 66B,D). These disks mostly developed into hapteron-shaped thalli under all conditions examined (Fig. 66E), sometimes giving rise to macrothalli (Figs 66C,H). Hapteron-shaped thalli formed unilocular sporangia under a 15°C SD condition after four months (Figs 66F,G). Thalli transferred from 20°C LD to 15°C SD condition also became fertile with unilocular sporangia. Unispores from these unilocular sporangia also mostly developed into crustose thalli; however, they sometimes developed into macrothalli except for 20°C conditions.

Macrothalli grew up to 6 cm or more in length, becoming tubular, under 5-15°C conditions. They formed female or male plurilocular sporangia on different thalli (Figs 66I,J). Female and male thalli were obtained in culture started from one unilocular sporangium. Female and male gametes fused well and formed zygotes. These gametes also fused with gametes obtained in culture from field macrothalli.

Female gametes which germinated parthenogenetically mostly developed into macrothalli under 5-15°C conditions.

Discussion

Scytosiphon nakamurae is similar to *Scytosiphon lomentaria* (Lyngbye) Link in the morphology of macrothallus. However, the former macrothallus does not have regular constrictions, whereas that of *S. lomentaria* usually have constrictions with regular intervals. Gametes of *S. nakamurae* are clearly anisogamous: female gametes are 7.5-9.5 × 4.5-5.5 µm in size and male gametes

4.5-5.5 × 2-2.5 µm in size. Those of *S. lomentaria*, however, are nearly isogamous: females are 6.4-8.4 × 3.0-3.8 µm in size and males 6.1-7.6 × 2.7-3.6 µm in size (Nakamura and Tatewaki 1975). In Australian complanate *Scytosiphon*, sizes of female and male gametes are described as 5-6 µm and 4-5 µm in length, respectively (Clayton 1980). These sizes of gametes are entirely different from those of *S. nakamurae*.

Scytosiphon nakamurae is peculiar in the morphology of microthallus which is composed of bilaterally curved branched filaments. However, the microthallus of *S. lomentaria* is discoid and it is composed of prostrate filaments and simple erect filaments arising from the prostrate filaments (Tatewaki 1966, Fletcher 1987). The microthallus of *S. nakamurae* is very similar to *Hapterophycus canaliculatus* Setchell and Gardner (Setchell 1912, Hollenberg 1941, Wynne 1969) and *Hapterophycus echigoensis* Noda (Noda 1973). In Japanese *H. canaliculatus*, *Scytosiphon-like* tubular macrothalli bearing plurilocular sporangia were reported in culture studies (Nakamura and Nakahara 1977, Mine 1990). The alga identified as *H. canaliculatus* by those investigations apparently corresponds to the microthallus of *S. nakamurae*. However, culture studies of *H. canaliculatus* from its type locality, California, show that any kinds of macrothalli are not involved in its life history (Hollenberg 1941, Wynne 1969). However, Californian *H. canaliculatus* is considered to belong to the order Scytosiphonales in possessing one chloroplast with a pyrenoid per cell (Wynne 1969). The culture study of *H. echigoensis* from its type locality is required to clarify its taxonomic status. If *H. echigoensis* has a *Scytosiphon*-phase macrothallus in the life cycle, it would be the same as *S.*

nakamurae which would be named *Scytosiphon echigoensis*. However, if did so, it would be very problematical that the type was a prostrate hapteron-shaped microthalli. It is better that the present species is treated as a new species.

The life history of *Scytosiphon nakamurae* in culture is summarized in Fig. 67. This life history pattern is the same as those of previous reports in Scytosiphonaceae. Female and male macrothalli were derived from culture of a single unilocular sporangium borne on zygotic microthallus. This result suggests that reduction divisions might occur in unilocular sporangia on microthalli which developed from zygotes. In development of plurispores it is reported that the final morphological expression (erect macrothallus or crustose microthallus) depends on photoperiods and/or temperature in *Scytosiphon lomentaria* (Tatewaki 1966, Wynne 1969, Rhodes and Connell 1973, Dring and Lüning 1975). However, in *S. nakamurae* progeny of female gametes represented microthalli under any culture conditions examined although a few macrothalli appeared. Unispores developed into macrothalli under 5-15°C conditions in culture.

In field observations at Oshoro, Hokkaido, macrothalli with plurilocular sporangia were present in only spring, microthalli with unilocular sporangia were found during only late autumn and winter. In culture, unilocular sporangia were formed on microthalli under a 15°C SD condition. They were formed more abundantly after microthalli were transferred from 20°C LD to 15°C SD. These results agree with field observations in which microthalli became fertile with unilocular sporangia in autumn and early winter. In culture, unispores developed into macrothalli under 5-15°C conditions. Therefore, seasonally

restricted occurrence of macrothalli in field is due to unilocular sporangial production which is controlled by temperature and photoperiod.

In culture of field microthalli, most unispores developed into microthalli and some gave rise to female or male macrothalli. In these culture, female plurispores developed into macrothalli. These results are contrary to unispore and plurispore development in culture from field macrothalli. In culture of *Hapterophycus canaliculatus* by Nakamura and Nakahara (1977), unispores developed into macrothalli in culture isolated in November, but into microthalli isolated in December. My result resembles their latter culture. However, reasons of these inconsistent results are unknown.

At Notoromisaki, only female plants were found. Strain from Notoromisaki represented that their plurispores developed into macrothalli under all culture conditions attempted except for 20°C conditions. Fertilization between female gametes of Notoromisaki and male gametes of Oshoro did not occur. It is possible that an asexual population of female-like plants exists at Notoromisaki. A similar case was reported in *Cutleria cylindrica*, in which populations with only female plants show a direct type life history in laboratory culture (Kitayama *et al.* 1992).

Table 26. Collection data of *Scytosiphon nakamurae* at Oshoro, Hokkaido. (P, thalli with plurilocular sporangia; U, thalli with unilocular sporangia; (U), microthalli bearing unilocular sporangia identified morphologically, not cultured; --, thallus was not found)

Macrothalli

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
1990				P	P	P	--		--	--	--	
1992				P								

Microthalli

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
1987												U
1988	U	U									U	
1989												U
1990		(U)	(U)								(U)	
1991		(U)										

Table 27. *Collection data of cultured strains of Scytosiphon nakamurae.* Culture conditions tested and progeny of plurispores from macrothalli are also shown.

strain no.	locality	date	sex	culture conditions	progeny
153	Notorimisaki	2 vii 1988		5-15SL	small massive thalli sprouting macrothalli
				20SL	massive thalli, small macrothalli
295	Oshoro	17 iv 1990	female	15L	massive thalli, a few macrothalli (P)
297	Ohma	29 iv 1990	female	5L, 10L	massive thalli, a few macrothalli (P)
				15L, 20L, 5-20S	massive thalli
298	Ohma	29 iv 1990	female	10L	massive thalli
313	Oshoro	16 v 1990	female	5SL, 10L, 15SL, 20SL	massive thalli
				10S	massive thalli, a few macrothalli
				20L→15S	massive thalli (U)
314	Oshoro	16 v 1990	male	5L, 20S	massive thalli
				5L, 10SL, 15SL, 20L	no progeny
				20L→15S	massive thalli (U)
320	Oshoro	4 vi 1990	female	10L	massive thalli
321	Oshoro	4 vi 1990	male	10L	massive thalli
362	Rishiri	19 vi 1991	male	10L	massive thalli, a few macrothalli

Table 28. Culture of *Scytosiphon nakamurae*. Progeny of zygotes.

strain no.	culture conditions	progeny
313 female x 314 male	20L	massive thalli
	20L→15S	massive thalli (U)

Table 29. Culture of *Scytosiphon nakamurae*. Progeny of unispores from zygotic microthalli.

strain no.	culture conditions	progeny
313 female x 314 male (Oshoro 16 v 1990)	5L, 10-15SL 20SL	macrothalli (male and female; 15L) massive thalli, small macrothalli

Table 30. Culture of *Scytosiphon nakamurae*. Progeny of unispores from microthalli.

strain no.	locality	date	sex	culture conditions	progeny
313	Oshoro	16 v 1990	female	5-15SL	macrothalli (P)
				20SL	massive thalli, small macrothalli
314	Oshoro	16 v 1990	male	5-15SL	macrothalli (P)
				20SL	massive thalli, small macrothalli

Table 31. Culture of *Scytosiphon nakamurae*. Progeny of unispores from field microthalli.

strain no.	locality	date	culture conditions	progeny
76	Oshoro	9 xii 1987	10L	massive thalli, a few macrothalli
85	Oshoro	21 i 1988	10L	massive thalli, a few macrothalli
95	Oshoro	17 ii 1988	10L	massive thalli, a few macrothalli (P: female, male)
257	Oshoro	2 xii 1989	5L,10S, 15L,20SL	massive thalli
			5S,10L,15S	massive thalli, a few macrothalli
274	Ohma	2 ii 1990	5S	massive thalli, a few macrothalli (P: female, male)

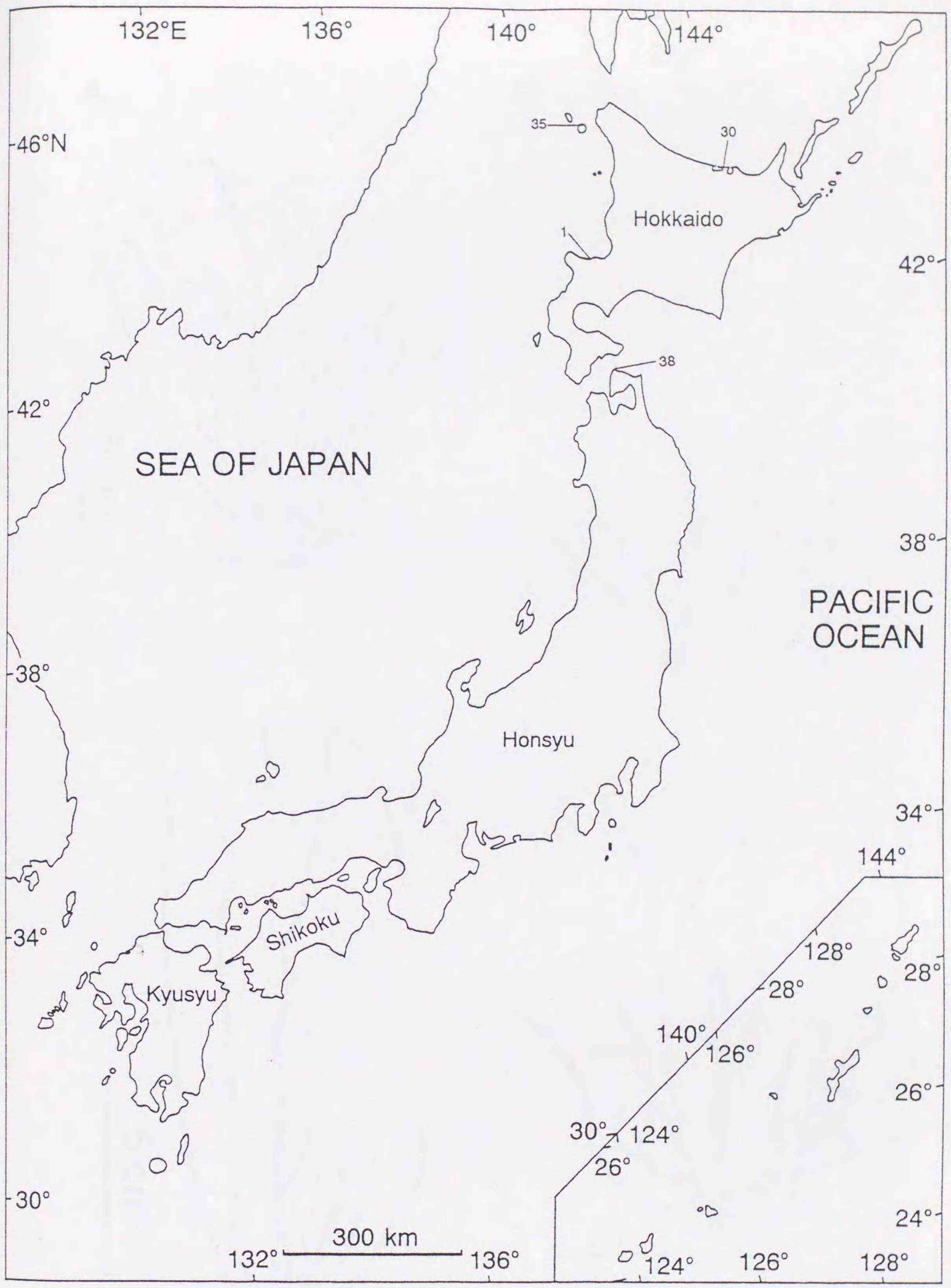


Fig. 56. Localities where materials of *Scytosiphon nakamurae* were collected. Numbers plotted correspond to those in Table 1.

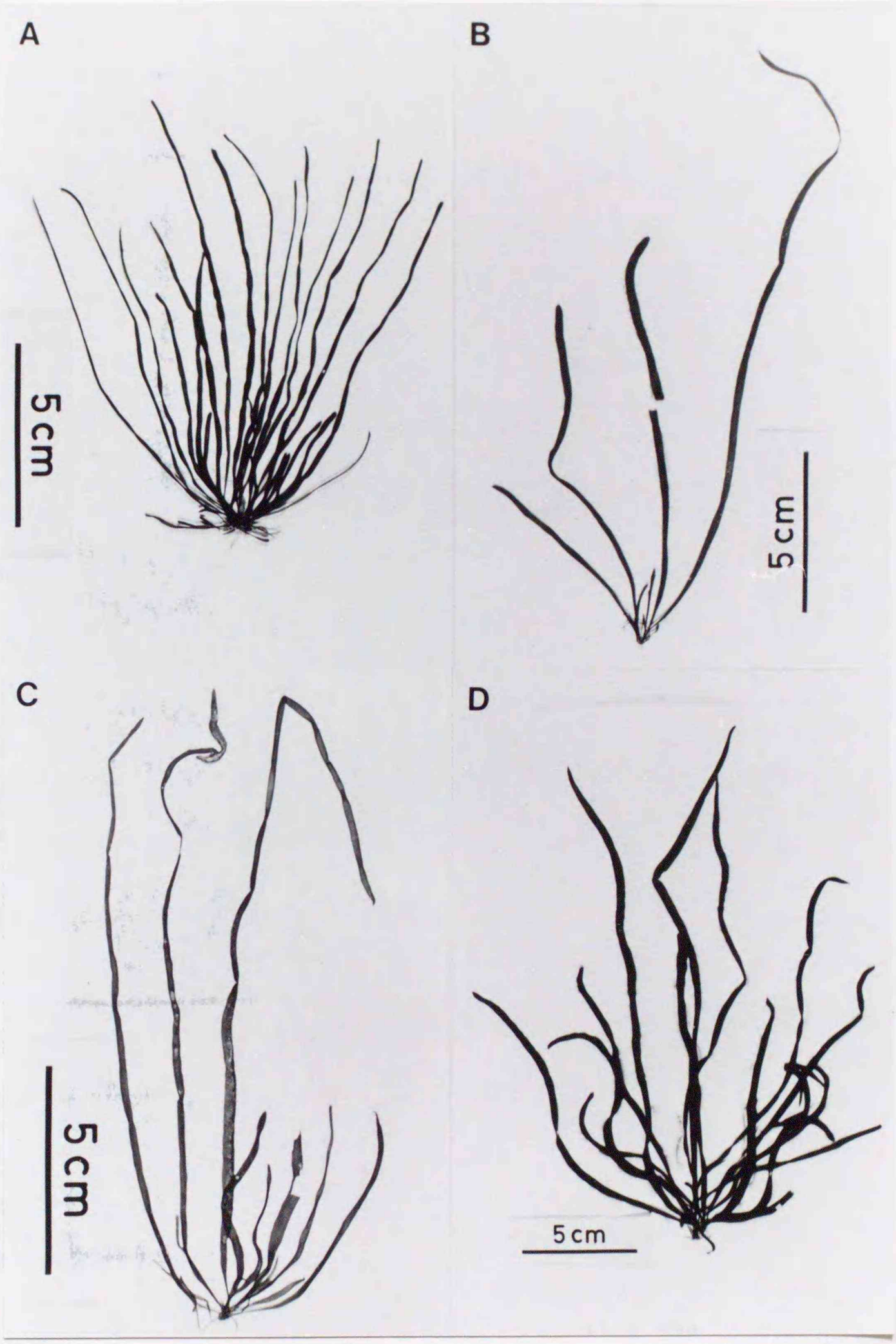


Fig. 57. Habit of specimens of *Scytosiphon nakamurae*.

A. Holotype specimen (SAP 059732). Female plant collected at Oshoro, Hokkaido on 21 May 1990. **B.** Female plant collected at Ohma, Aomori Pref. on 29 April 1990. **C.** Isotype specimen (SAP 059731). Male plant. **D.** Plant collected at Notorimisaki, Hokkaido on 2 July 1988.

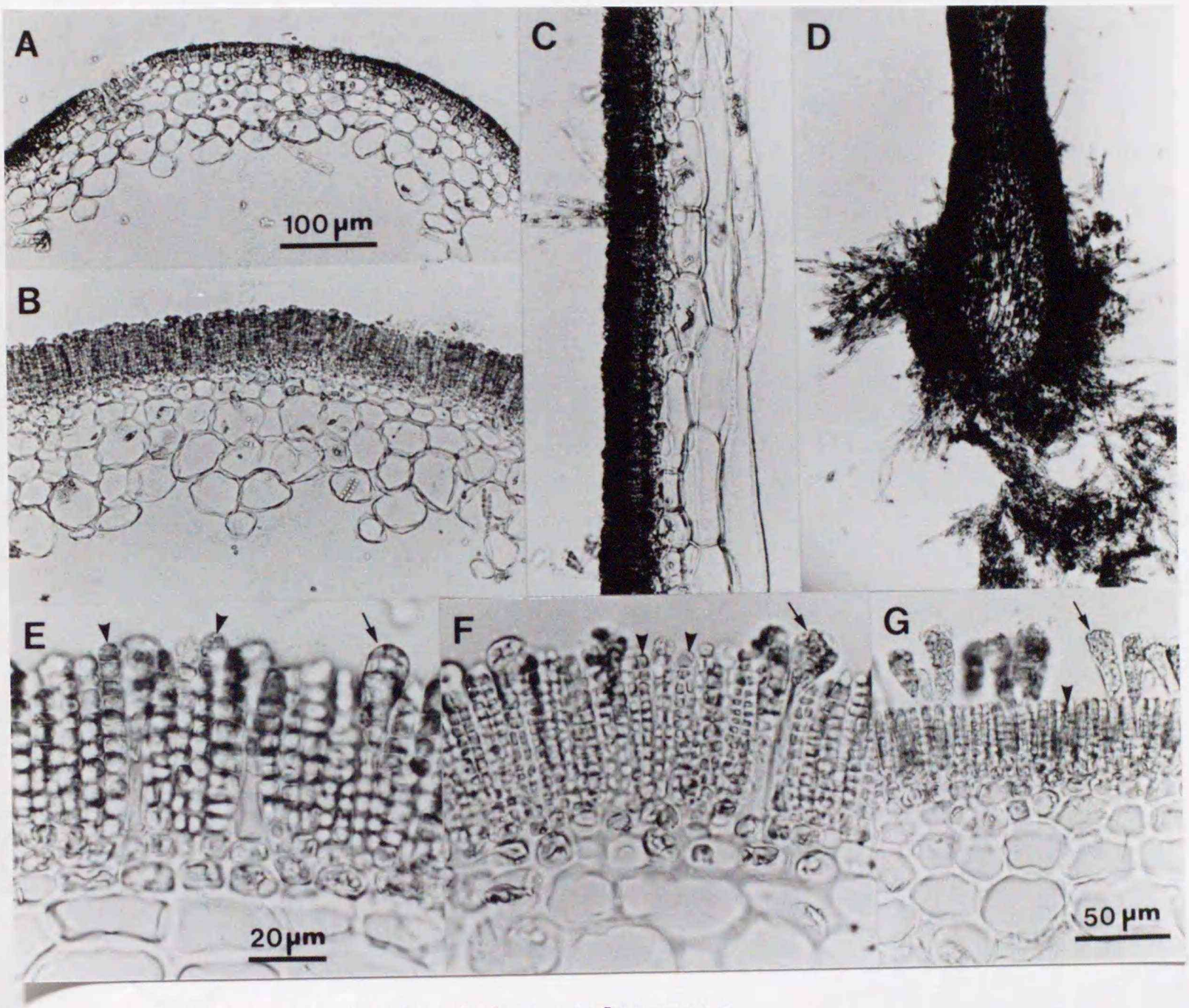


Fig. 58. Anatomy of *Scytosiphon nakamurae*.

A. Cross section of vegetative thallus. **B**. Cross section of fertile thallus. **C**. Longitudinal section of fertile thallus. **D**. Longitudinal section of basal portion of thallus, showing rhizoids. **E**. Female plurilocular sporangia (arrowheads) and ascocysts (arrow). **F**. Male plurilocular sporangia (arrowheads) and ascocysts (arrow). **G**. Secondary plurilocular sporangia (arrowhead) and ascocysts (arrow).

Scale in **A** applies to **B-D**; Scale in **E** applies to **F**.

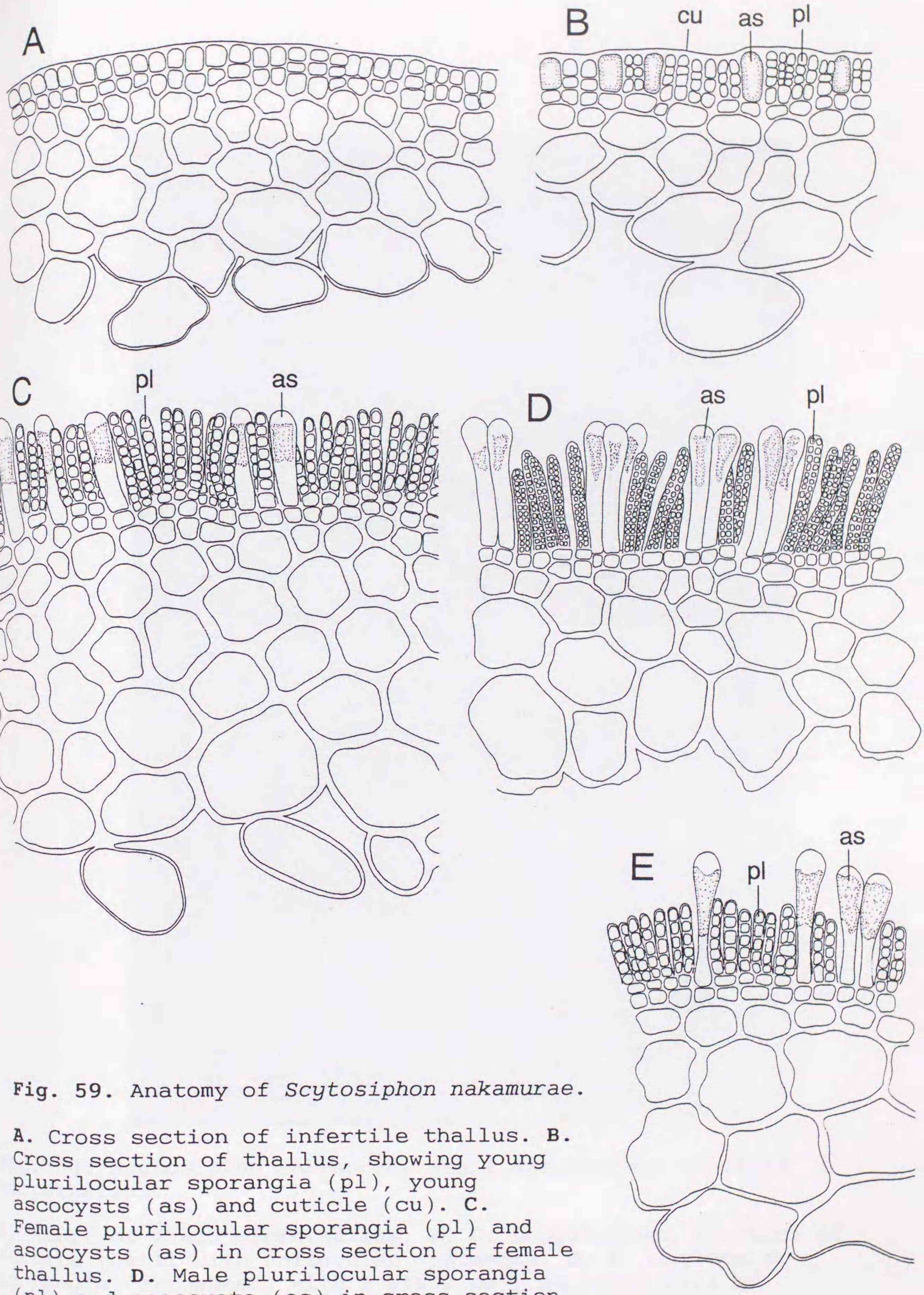


Fig. 59. Anatomy of *Scytosiphon nakamurae*.

A. Cross section of infertile thallus. **B.** Cross section of thallus, showing young plurilocular sporangia (pl), young ascocysts (as) and cuticle (cu). **C.** Female plurilocular sporangia (pl) and ascocysts (as) in cross section of female thallus. **D.** Male plurilocular sporangia (pl) and ascocysts (as) in cross section of male thallus. **E.** Secondary plurilocular sporangia (pl) and ascocysts (as) in cross section of female thallus.

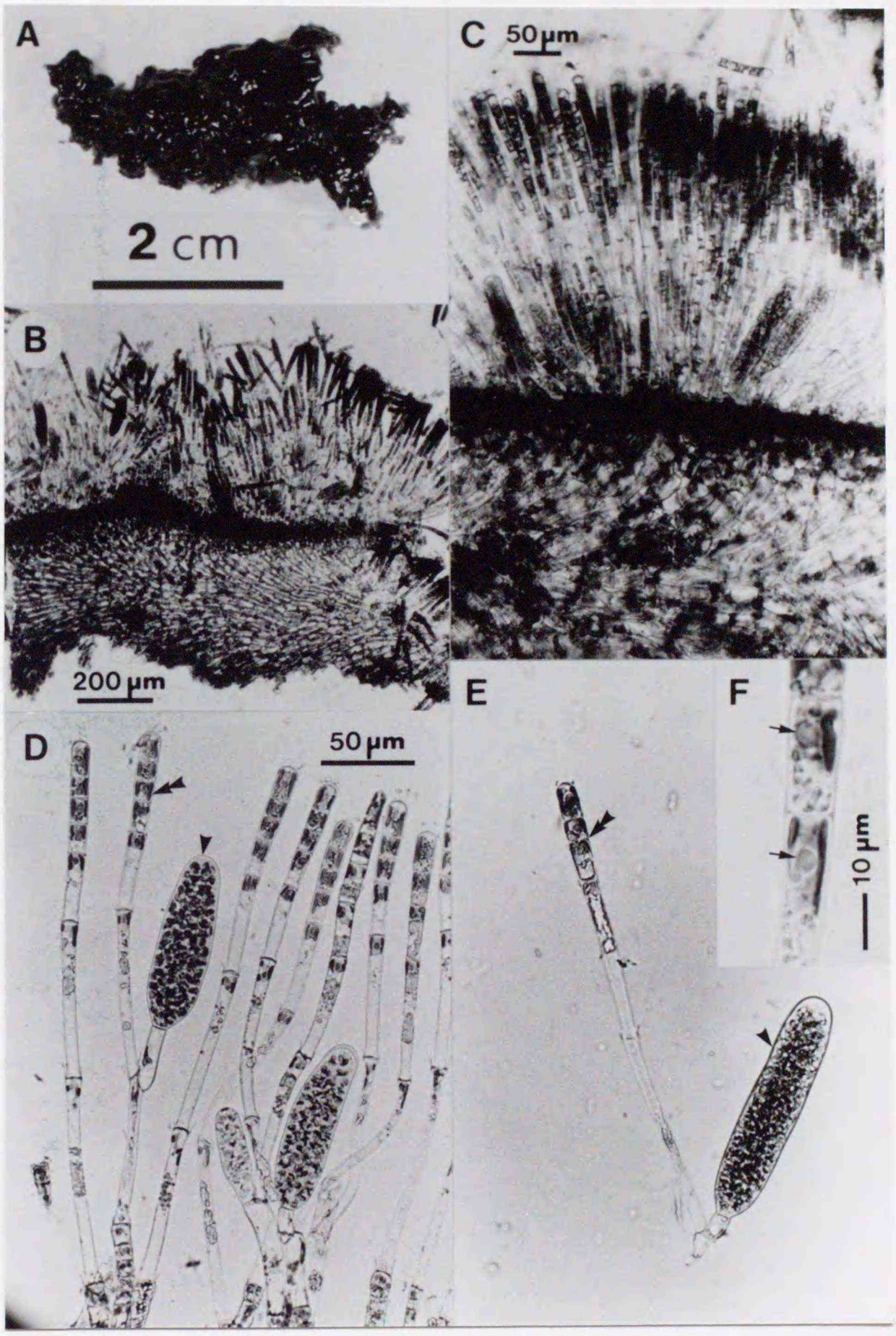


Fig. 60. *Scytosiphon nakamurae*. Habit and anatomy of field microthallus.

A. Habit of field microthallus. B, C. Longitudinal sections of fertile thalli with unilocular sporangia. D, E. Unilocular sporangia (arrowhead) with a stalk cell on basal cell of paraphyses (double arrowheads). F. Cells of paraphyses, showing one plastid with a pyrenoid (arrows) per cell.

Scale in D applies to E.

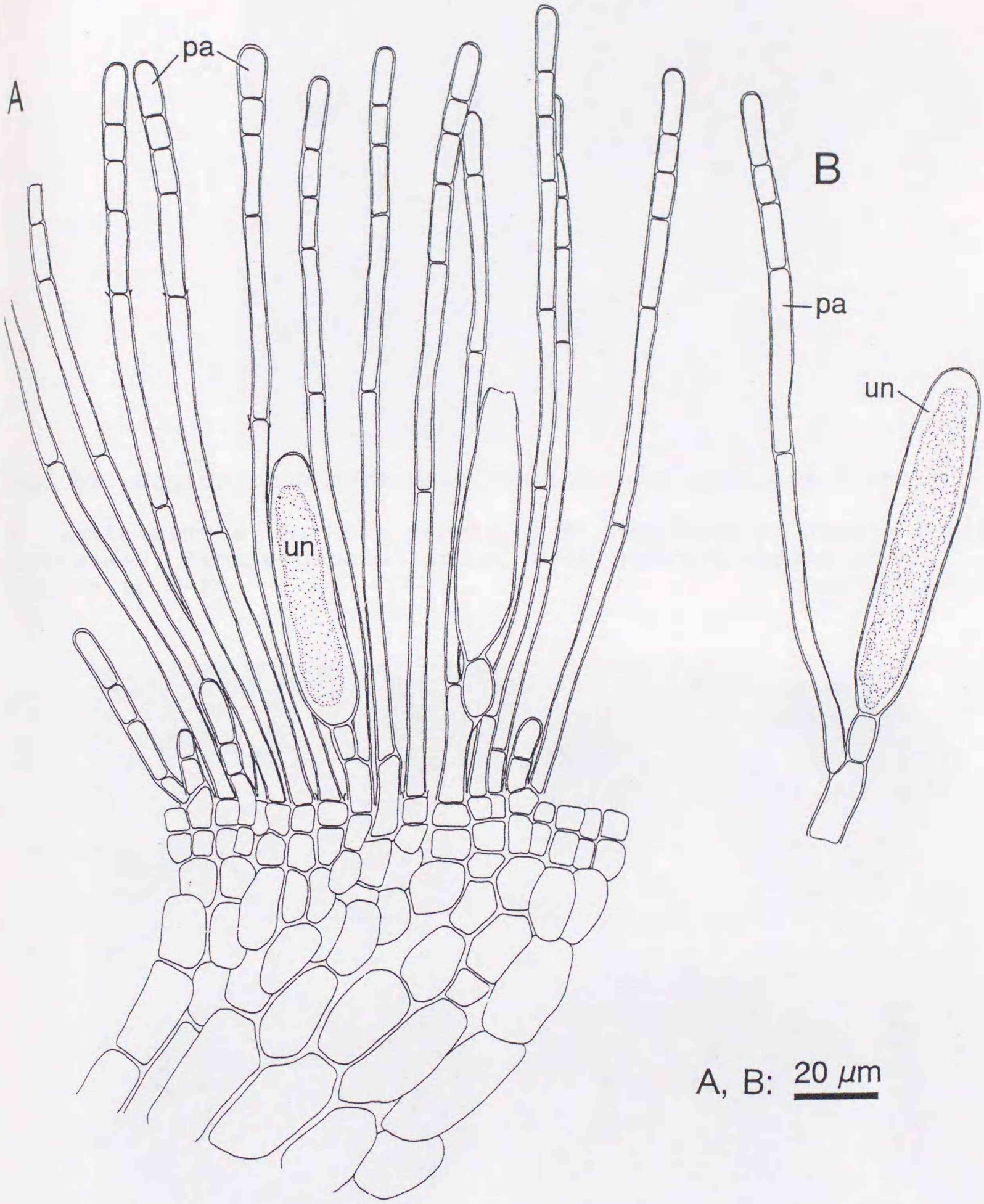


Fig. 61. *Scytosiphon nakamurae*. Anatomy of field microthallus.

A. Longitudinal section of thallus, showing unilocular sporangia (un) and paraphyses (pa). **B.** Unilocular sporangium (un) with a pedicel on a basal cell of paraphysis (pa).

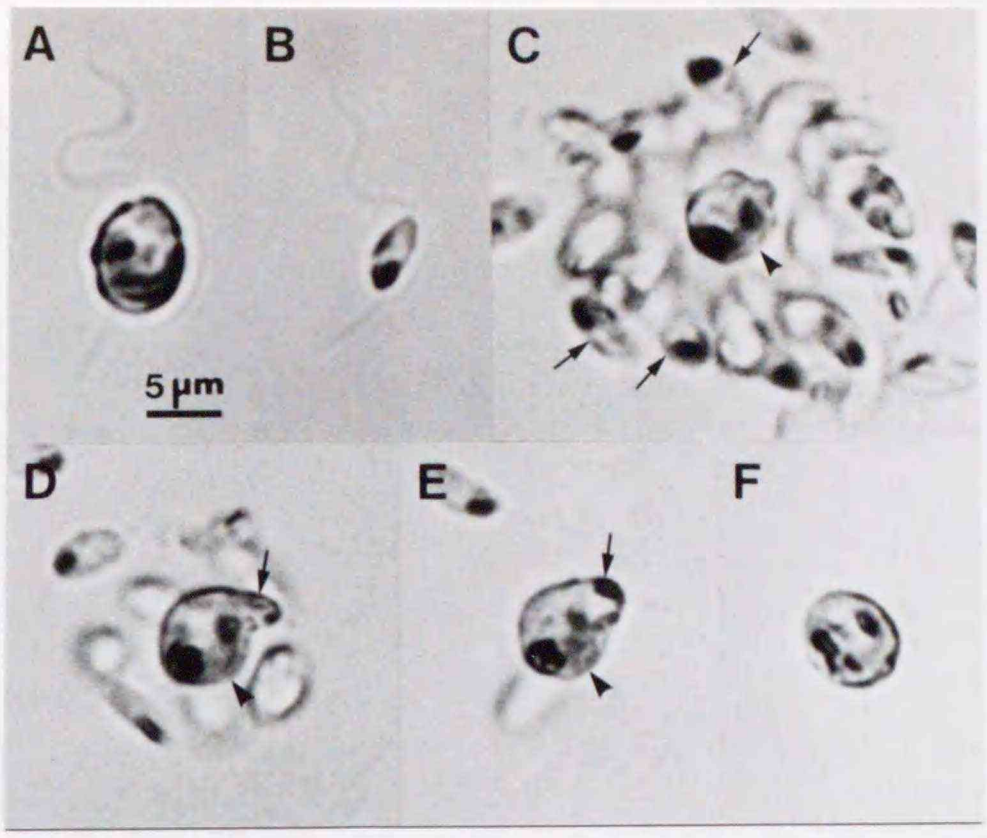


Fig. 62. *Scytosiphon nakamurae*. Gametes and gamete fusion.

A. Female gamete. B. Male gamete. C-F. Sequence of gamete fusion (arrowhead, female gamete; arrow, male gamete). Scale in **A** applies to **B-F**.

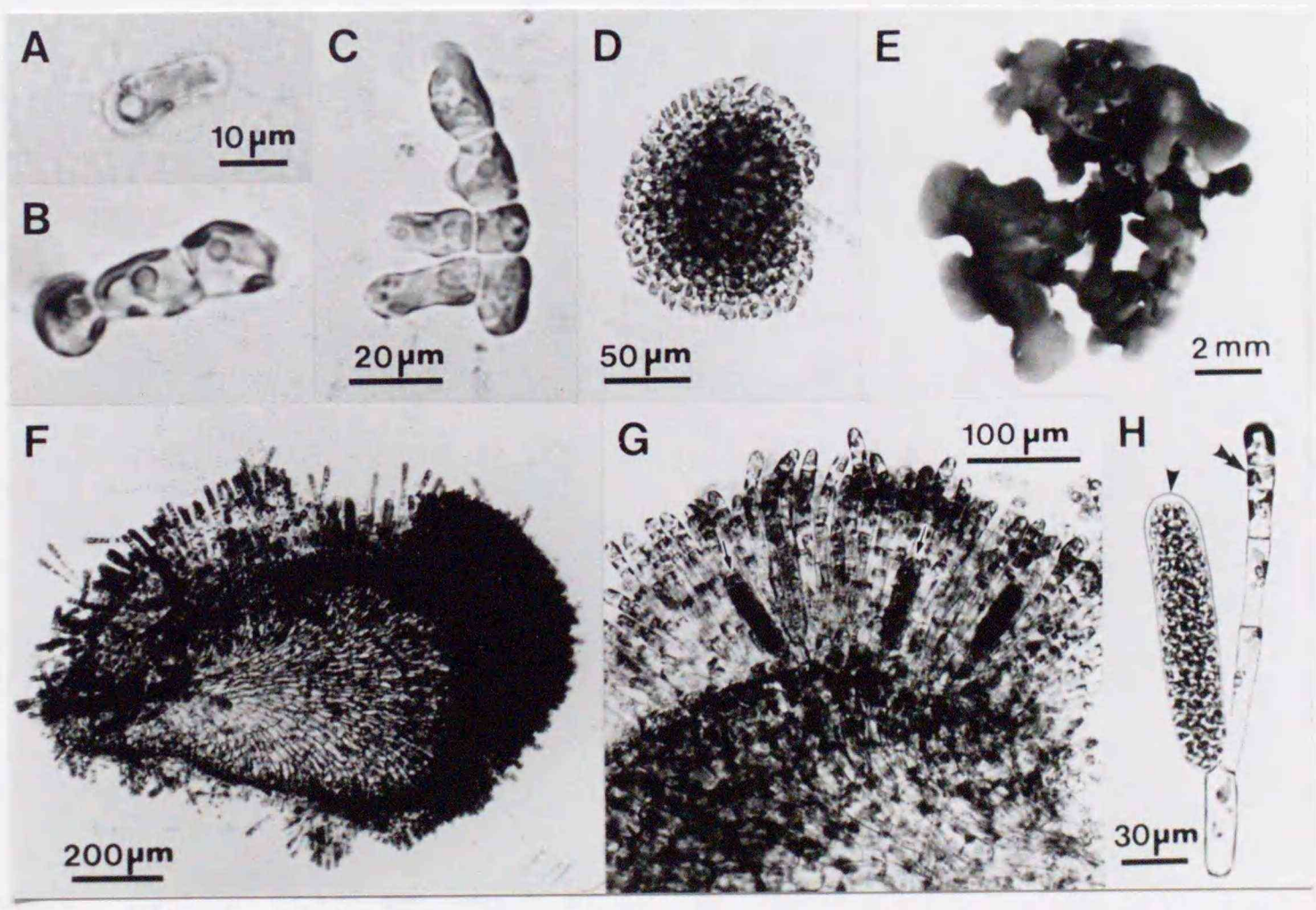


Fig. 63. Culture of *Scytosiphon nakamurae*. Development of zygote.

A. Two-day-old germling under 15°C LD condition. B. Six-day-old germling under 15°C LD condition. C. Eight-day-old germling in 15°C LD condition. D. Twenty-day-old germling under 15°C LD condition. E. Hapteron-like microthallus under 20°C LD condition. F. Longitudinal section of fertile microthallus with unilocular sporangia under 15°C SD condition. G. Unilocular sporangia (arrows) and paraphyses in section of microthallus under 15°C SD condition. H. Unilocular sporangium (arrowhead) lateral on a basal cell of a paraphysis (double arrowhead). Scale in **A** applies to **B**.

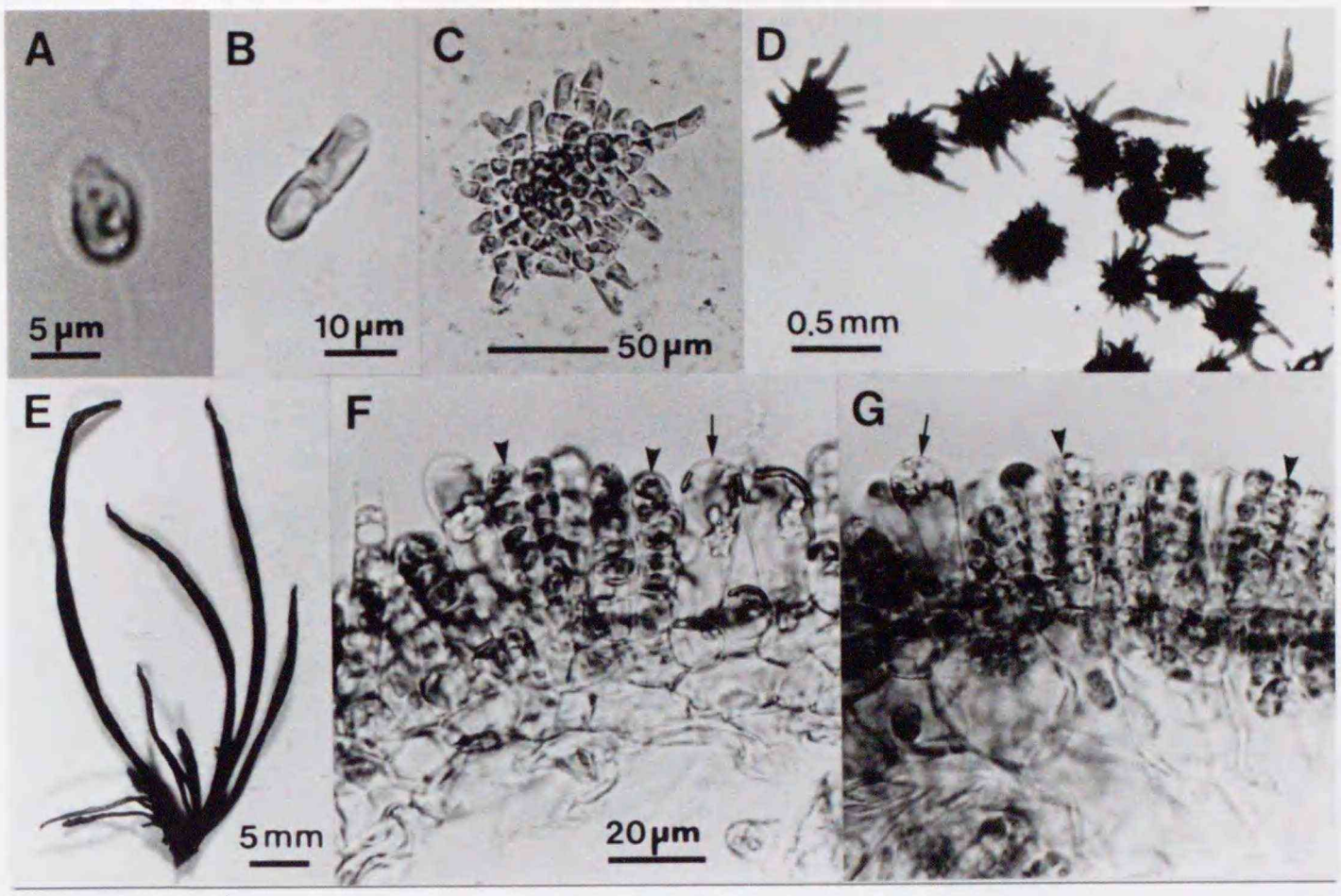


Fig. 64. Culture of *Scytosiphon nakamurae*. Development of unispore from zygotic microthallus.

A. Unispore. **B.** Four-day-old germling under 15°C SD condition. **C.** Twelve-day-old germling under 15°C SD condition. **D.** Small disks producing macrothalli under 15°C SD condition. **E.** Macrothalli. **F.** Female plurilocular sporangia (arrowhead) and ascocysts (arrow) in cross section of macrothallus. **G.** Male plurilocular sporangia (arrowheads) and ascocysts (arrow) in cross section of macrothallus. Scale in **F** applies to **G**.

⇒

Fig. 65. Culture of *Scytosiphon nakamurae*. Development of gametes (**A-J**) and development of unispores from parthenogenetic microthallus (**K-O**).

A-H. Parthenogenetic development of female gamete. **A.** Six-day-old germling under 10°C LD condition. **B.** Sixteen-day-old germling under 10°C LD condition. **C.** Three-week-old germling under 10°C LD condition. **D.** Massive microthalli under 10°C LD condition. **E.** Hapteron-like microthallus under 20°C LD condition. **F.** Longitudinal section of microthallus. **G.** Longitudinal section of microthallus forming unilocular sporangia (arrowhead) and paraphyses under 15°C SD condition. **H.** Unilocular sporangium (arrowhead) and paraphyses (double arrowhead). **I.** Five-day-old germling of male gamete under 10°C LD condition. **J.** Hapteron-like microthalli developed from male gametes. **K.** Unispore from parthenogenetic microthallus. **L.** Seven-day-old germling under 15°C SD condition. **M.** Sixteen-day-old germling under 15°C SD condition. **N.** Macrothalli arising from small disks under 15°C SD condition. **O.** Cross section of fertile macrothalli under 15°C LD condition. Scale in **A** applies to **I**; Scale in **E** applies to **J**; Scale in **C** applies to **H**; Scale in **G** applies to **M**.

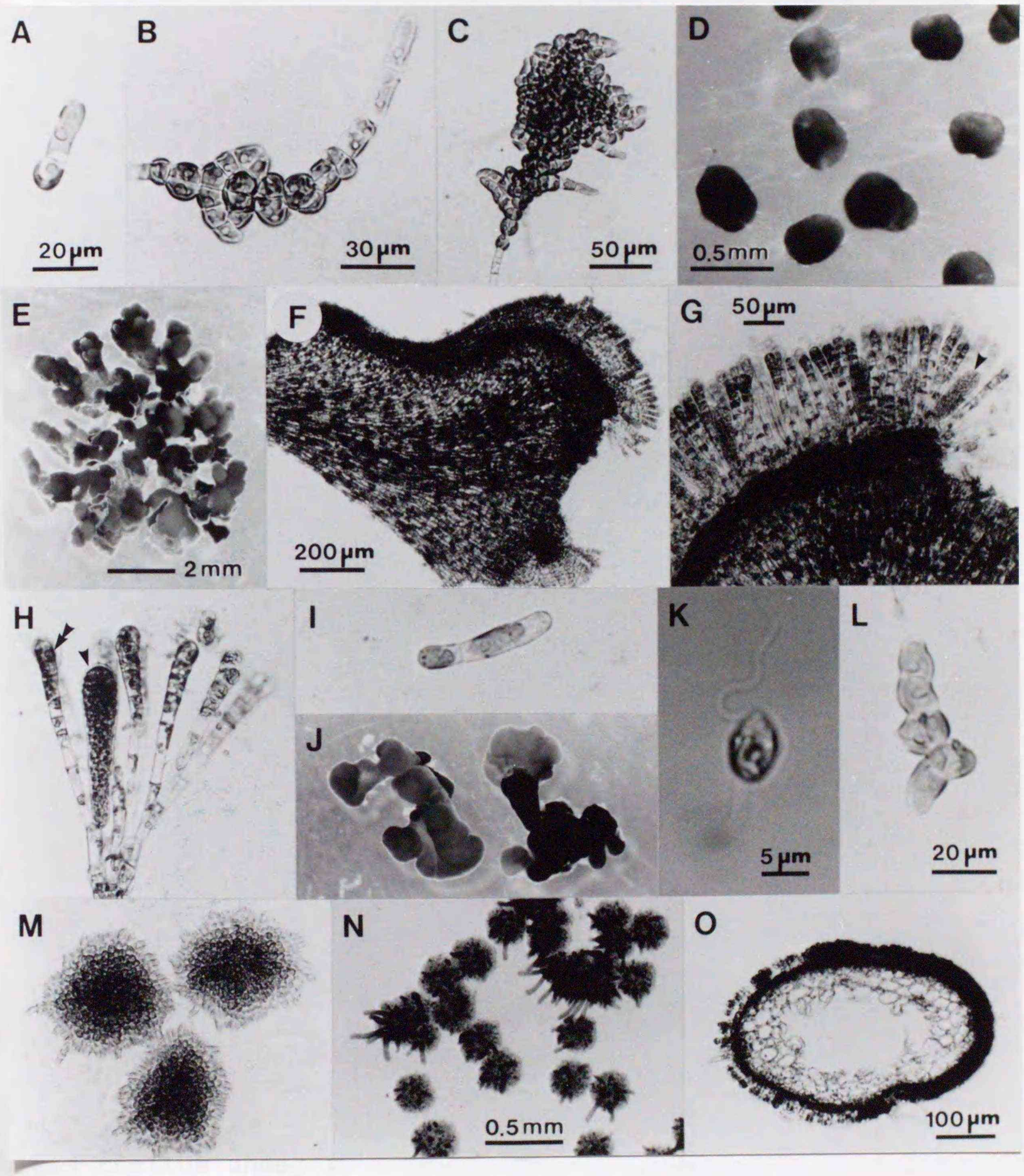


Fig. 65. Culture of *Scytosiphon nakamurae*.

⇐

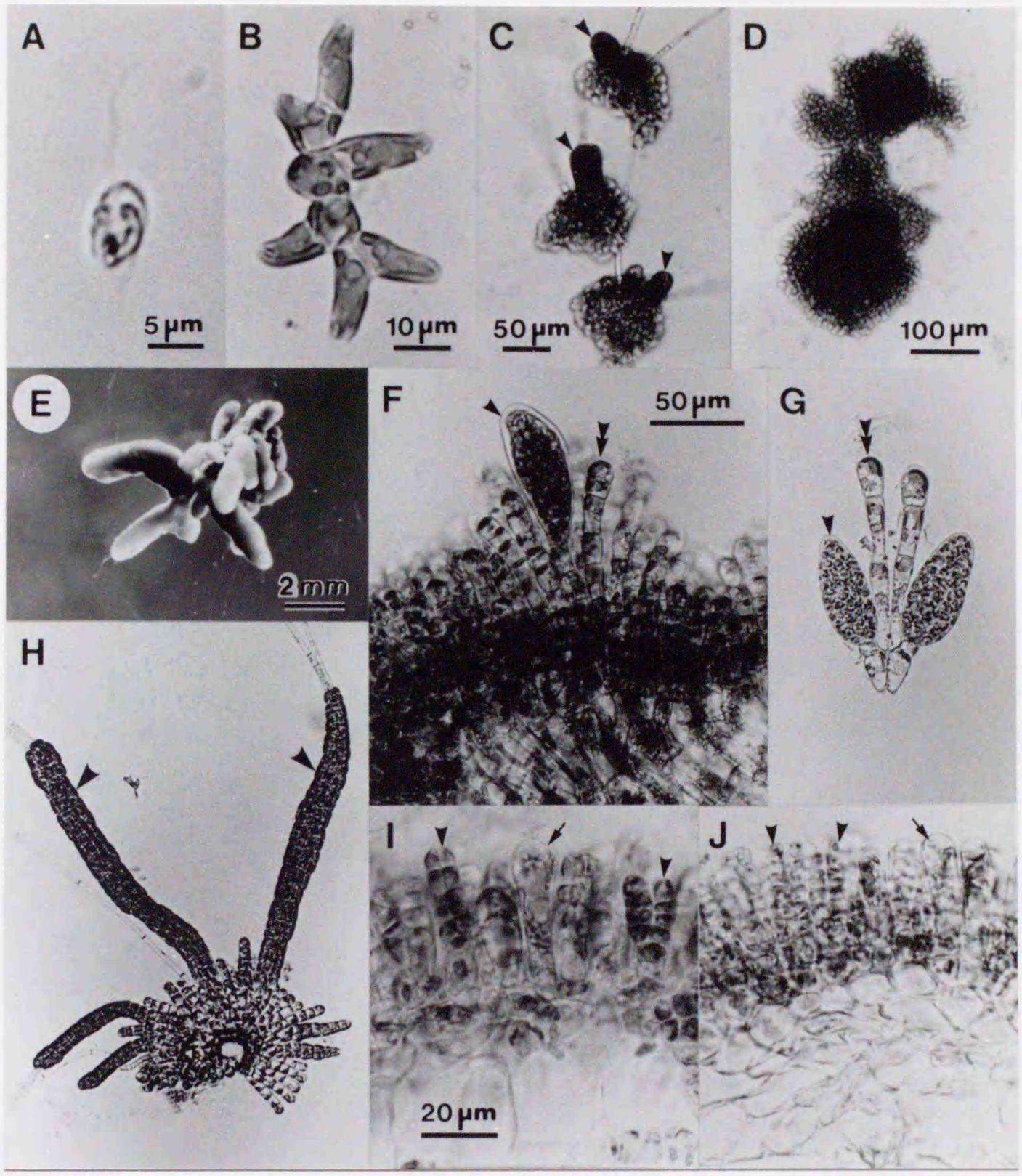


Fig. 66. Culture of *Scytosiphon nakamurae*. Development of unispores from field microthallus.

A. Unispore. **B.** Thirteen-day-old germling under 10°C LD condition. **C.** One-month-old thalli under 5°C SD condition, showing macrothalli (arrowheads) arising from small disks. **D.** Fifteen-day-old thallus under 10°C LD condition. **E.** Hapteron-like microthallus. **F.** Longitudinal section of microthallus forming unilocular sporangium (arrowhead) and paraphyses (double arrowhead) under 15°C SD condition. **G.** Unilocular sporangia (arrowhead) with a stalk cell on a basal cell of paraphyses (double arrowhead). **H.** Squashed small disk issuing macrothalli (arrowheads). **I.** Female plurilocular sporangia (arrowheads) and ascocysts (arrow) in section of macrothallus. **J.** Male plurilocular sporangia (arrowheads) and ascocysts (arrow) in section of macrothallus.

Scale in **F** applies to **G**; Scale in **C** applies to **H**.

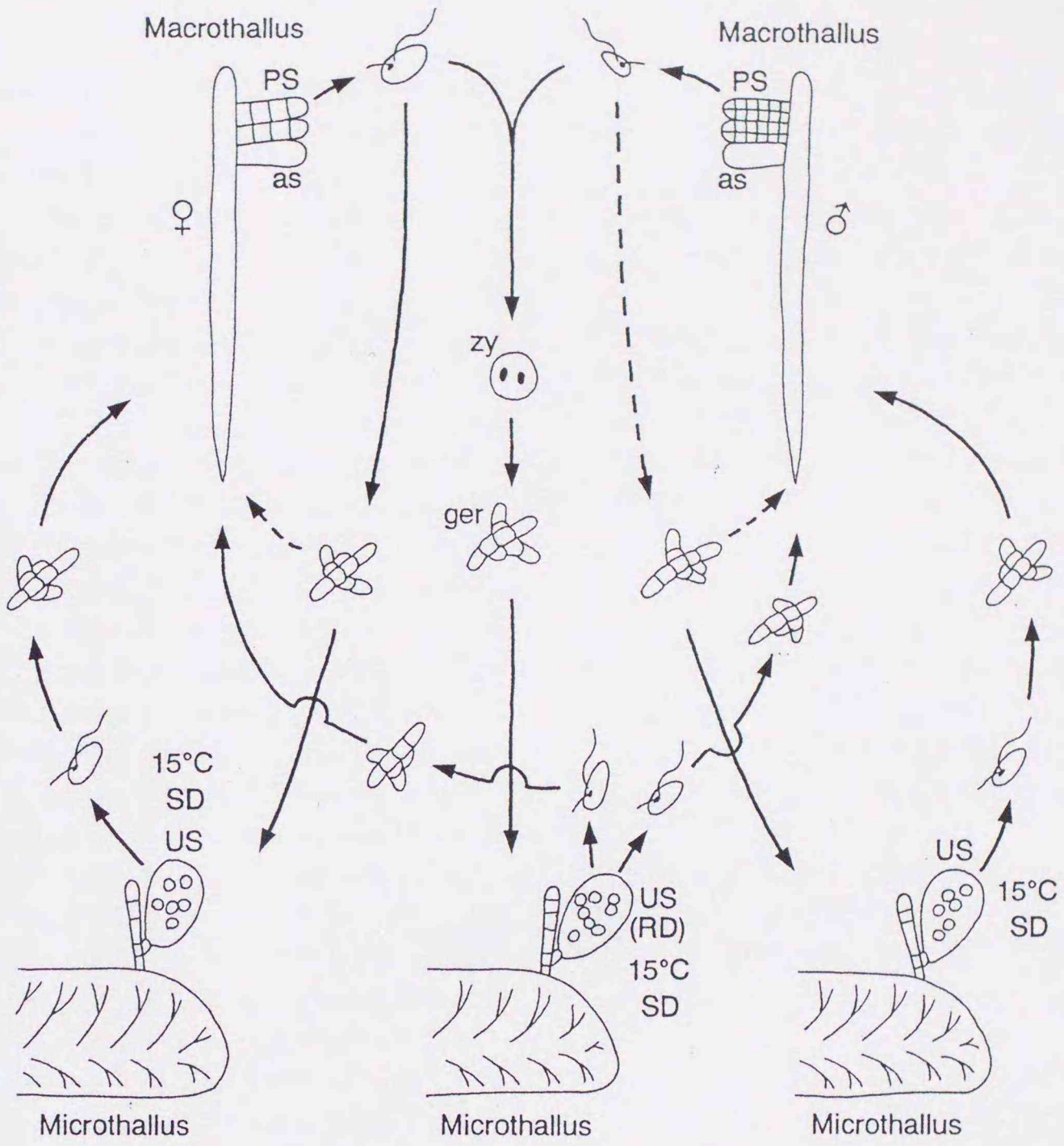


Fig. 67. Diagram of life history of *Scytosiphon nakamurae* in culture.

as, ascocyst; ger, germling; PS, plurilocular sporangium; RD, reduction division; SD, short day condition; US, unilocular sporangium; zy, zygote; broken line, rare course.

Scytosiphon gracilis **sp. nov.**

Synonym: *Scytosiphon dotyi* sensu Fletcher 1987, pp.245-248, Figs 67A,68, Pl.11c.

Holotype: SAP 059720, plant collected at Ohma, Aomori Pref., Japan, on 1 February 1990 (Fig. 69A).

Japanese name: Usukayamo (new name)

Materials

Localities are shown in Fig. 68. The numbers in square brackets after following each locality correspond to those in Fig. 68.

Macrothalli: Hokkaido: Oshoro [1] (10-iii-1989 leg. K. Kogame, 2-xii-1989 leg. K. Kogame, 11-ii-1993 leg. K. Kogame), Samani [17] (15-v-1987 leg. K. Kogame, 5-iv-1988 leg. K. Kogame, 11-ii-1989 leg. K. Kogame, 19-v-1991 leg. K. Kogame), Utabetsu [18] (6-iv-1988 leg. K. Kogame), Aomori Pref. : Ohma [38] (3-iii-1987 leg. K. Kogame, 21-ii-1988 leg. H. Kawai, 1-ii-1990 leg. K. Kogame), Kanagawa Pref. : Jogashima [63] (29-iii-1989 leg. T. Kitayama), Kannonzaki [64] (21-ii-1988 leg. S. Arai), Aichi Pref. : Iragomisaki [71] (2-iv-1988 leg. M. Masuda), Hyogo Pref. : Karimo, Awajishima [82] (31-iii-1990 leg. S. Enomoto & K. Kogame), Maruyama, Awajishima [83] (3-iv-1992 leg. S. Enomoto & K. Kogame), Kamishima, Ieshima Isls (1-iv-1988 leg. M. Masuda), Kagawa Pref. : Awashima [89] (5-iv-1989 leg. S. Enomoto & K. Kogame), Megishima [86] (6-iv-1989 leg. S. Enomoto & K. Kogame), Okayama Pref. : Kitagishima [87] (4-iv-1989 leg. S. Enomoto & K. Kogame), Ehime Pref. : Nakashima [96] (16-iv-1988 leg. S. Enomoto & K. Kogame), Ohita Pref. : Himeshima [98] (17-iv-1988 leg. S. Enomoto & K. Kogame), Fukuoka Pref. : Tsuyazaki [103] (21-iv-1989 leg. S. Kawaguchi).

Microthalli: Aomori Pref. : Ohma [38] (24-ix-1991 leg. K. Kogame).

Herbarium specimens examined: Aomori Pref. : Ohma [38] (SAP 052808, 21-i-1988 leg. T. Kitayama), Miyagi Pref. : Ishihamazaki, Shiogama [59] (18-xii-1951 leg. M. Kurogi), Hyogo Pref. : Tarumi [81] (SAP 028236, 14-xi-1959 leg. T. Yamamoto), Ehime Pref. : Ikata [101] (SAP 035203, 7-iv-1954 leg. Y. Nomura).

Descriptio

Macrothalli simplices, complanati vel cylindrici, cavi, 10-25(-40) cm longi, 0.5-4(8) mm lati, gregarii, fasciculati, flavidi -brunnei in colore quum juveniles atrobrunnescentes quum maturi, molles et subtiles, interdum rugosi in thallis grandibus, ex stratis corticalibus et medullosis constantes; cortex ex 1-3 stratis cellularum parvarum pigmentiferarum constans; medulla ex 2-3 stratis cellularum grandium incolorium constans; cellulae medullosae in sectione transversali rotundae vel ellipsoideae, 24-36 × 24-80 µm in amplitudine, in sectione longitudinali intus longiores, usque ad 400 µm longae; pili achromi singulares vel agregati, variabiles in quantitate; haptera parva, indistincta; thalli ad substrata per filamenta rhizoidea affixi; plantae gametis isogamicis; gametae 5.8-7.6 × 3.2-4.4 µm in amplitudine; sporangia plurilocularia basipetaliter in pagina integera thallorum facta; sporangia plurilocularia firme cohaerentia inter se, atrato tegens cuticulare; ascocystae absentes; partes thallos post liberationem plurisporarum incolorescentes.

Microthalli minuti, caespitosi, ad 1 mm in diametro, ex filamentis ramosis prostratis et ex filamentis erectis uniserialibus simplicibus constantes; cellulae filamentorum prostratorum interdum longitudinaliter dividentes, 7-17 × 6-9 µm in amplitudine; filamenta erecta e 15-60 cellulis composita, 200-480 µm longa; cellulae filamentorum erectorum 5-15 µm longae, 8-12 µm latae, plastidium unum cum pyrenoide una in quoque cellula continentes; filamenta erecta libera inter se; sporangia unilocularia obovoidea 45-110 × 24-38 µm in amplitudine, sessilia vel pedicellata in filamentis prostratis, interdum terminalia vel lateralia in filamentis erectis.

Description

Macrothalli simple, flattened or cylindrical, hollow, up to 10-25 (-40) cm long and 0.5-4 (8) mm wide, gregarious, yellowish brown in color in young, becoming dark brown when mature, soft and delicate; thalli sometimes wrinkled in large ones, consisting of cortical and medullary layers; cortex consisting of 1-3 layers of small pigmented cells; medulla consisting of 2-3 layers of large colorless cells; in transverse section medullary cells round or ellipsoid, 24-36 × 24-80 µm in size, in longitudinal section longer in inner portions, up to 400 µm long; phaeophycean hairs single or grouped, variable in its amount; holdfasts small, not distinct; thalli attaching to the substrata with rhizoidal filaments; plants dioecious with isogamous gametes; gametes 5.8-7.6 × 3.2-4.4 µm in size; plurilocular sporangia formed basipetally on the entire surface of thalli; plurilocular sporangia firmly coherent to each other, with a covering cuticular layer; ascocysts absent; portions of thalli becoming colorless after release of plurispores.

Microthalli, minute, tufted, to 1 mm in diameter, consisting of prostrate branched filaments and simple, uniseriate, upright filaments arising from them; cells of prostrate filaments sometimes dividing longitudinally, 7-17 × 6-9 µm in size; upright filaments composed of 15-60 cells, 200-480 µm in length; cells of the upright filaments 5-15 µm long and 8-12 µm wide, having a plastid with a pyrenoid per cell; upright filaments free from each other; unilocular sporangia obovoid, 45-110 × 24-38 µm in size, sessile or pedicellate on prostrate filaments, sometimes terminal or lateral on upright filaments.

Morphology

Macrothalli

Erect plants are gregarious, with many clustered macrothalli (Figs 69A-D). Macrothalli are flat or cylindrical, hollow, up to 10-25 (-40) cm in length and 0.5-4 (-8) mm in width, sometimes wrinkled in large ones. These macrothalli are yellowish brown in young, becoming dark brown in color when fertile, and are soft and delicate in texture. The thalli consist of cortical and medullary layers (Figs 70A,B, 71A). The cortical layer is composed of 1-3 layers of small pigmented cells (Fig. 70E). The medullary layer is composed of 2-3 layers of large colorless cells. In cross section medullary cells are round or ellipsoid, 24-36 × 24-80 µm in size. In longitudinal section they are longer in inner portions, to 400 µm in length (Fig. 70C). Phaeophycean hairs are solitary or grouped, variable in amount. Thalli attach to the substrata with rhizoidal filaments (Fig. 70D) and lack distinct holdfasts. They became fertile basipetally, forming plurilocular sporangia by divisions of surface cells (Figs 70F, 71B). Plurilocular sporangia are firmly adjoining with each other (Figs 70G, 71C,D). A cuticle layer covering plurilocular sporangia is present (Fig. 70G). Ascocysts are absent. Since pigmented cells in the cortical layer also usually become plurilocular sporangia, portions which released plurispores become colorless (Fig. 69A).

Microthalli

Plants are tufted, consisting of prostrate branched filaments and simple, uniseriate, upright filaments which arise from the prostrate filaments (Figs 72A,B, 73A,B, 74A,B). Plants

are minute, up to 1 mm in diameter. Cells of prostrate filaments sometimes divide longitudinally, 7-17 × 6-9 μm in size. Upright filaments are composed of 15-60 cells, 200-480 μm in length. Cells of the upright filaments are 5-15 μm in length and 8-12 μm in width, having a plastid with a pyrenoid per cell. Upright filaments are free from each other. Unilocular sporangia are obovoid, 45-110 × 24-38 μm in size, sessile or pedicellate on prostrate filaments (Figs 73A,B, 74B). They sometimes form terminally or laterally on upright filaments (Figs 72C,D, 74A).

Habitat and Phenology

Macrothalli grow on rocks, boulders and concrete blocks in the middle to lower intertidal zone in moderately exposed or sheltered areas, frequently in groups. The thalli are generally found during winter and spring, and are sometimes associated with *Scytosiphon lomentaria*.

Microthalli are found on boulders in the lower intertidal zone in sheltered area where macrothalli grow. Microthalli with unilocular sporangia were once found in September.

Geographical distribution

This species is widely distributed in Japan, found in Hokkaido, Honsyu, Shikoku and Kyusyu.

Culture study

Strains isolated from macrothalli are listed in Table 32. Culture conditions and progeny of plurispores are also shown in Table 32.

Sexual reproduction

Gamete fusions were observed in macrothalli collected at Ohma, Aomori Prefecture. This species is dioecious and gametes are nearly isogamous. Gametes released from plurilocular sporangia are of normal phaeophycean type, with a plastid and a stigma. Female and male gametes measured 6.2-7.6 × 3.8-4.4 μm and 5.8-7.0 × 3.2-4.0 μm in size, respectively (Figs 75A,B). When female and male gametes were mixed, a settled female gamete was surrounded by several male gametes which touched to the settled female gamete by anterior flagellum, and then, one of male gametes fused with the female gamete (Figs 75C-E). Zygotes were distinguished from unfused gametes by possessing two stigmata (Fig. 75F).

The fragrant odor of sexual pheromone was observed in culture where female gametes were released.

Development of zygotes

Isolated zygotes, which were formed by fusion of gametes from macrothalli grown under 5°C SD, were cultured under 10°C SD and 20°C LD conditions. Zygotes germinated into prostrate, branched filaments and developed into tufted microthalli (Figs 76A-D). These tufted microthalli consisted of branched filaments with longitudinal walls and with uniseriate upright filaments (Figs 76E,F). No discoid thalli were observed. After 2 months, the microthalli reached to 6 mm in diameter and became fertile, forming spherical to obovoid unilocular sporangia (Figs 76E,F). No macrothallus was formed on the microthalli (Table 33).

Parthenogenetic development of gametes

Female gametes were cultured at 5-20°C under both LD and SD. Germlings developed into tufted microthalli (Figs 77A-C). Cells of the branched filaments of the tufts sometimes divided longitudinally, and produced knot portions. Some prostrate filaments of the tufted thalli became coherent to each other but did not produce disks (Figs 77C,G). Prostrate filaments sometimes produced uniseriate upright filaments (Fig. 77I). The microthalli formed unilocular sporangia under all culture conditions tested except 5°C SD. Unilocular sporangia were produced more abundantly under higher temperatures. They were spherical to obovoid, sessile or pedicellate on prostrate filaments or on free, branched filaments.

The microthalli produced macrothalli under all conditions examined (Fig. 77D). Macrothalli were first uniseriate, later parenchymatous by longitudinal divisions, usually with a phaeophycean hair on the top. These macrothalli became hollow and reached to more than 10 cm in length (Fig. 77E). In lower temperatures and short day conditions, macrothalli arose more abundantly, and grew into longer thalli, whereas they grew no more than 5 mm in length under 20°C LD. Macrothalli formed plurilocular sporangia under all conditions attempted. The plurilocular sporangia were firmly packed, and sometimes massive (Fig. 77F). Plurispores were female gametes, when their sexuality was ascertained. No ascocysts among plurilocular sporangia were observed. Under 20°C SD and 20°C LD, formation of plurilocular sporangia and releases of plurispores were less frequent than other culture conditions. Macrothalli produced also unilocular sporangia under 20°C SD and 20°C LD. The unilocular sporangia were spherical to obovoid on the surface of the macrothalli.

Male gametes were cultured at 5-20°C under both SD and LD as were female gametes. Their development was almost the same as that of female gametes. Tufted microthalli formed unilocular sporangia under 15°C SD and 20°C SD, and gave rise to macrothalli abundantly.

Development of unispores from parthenogenetic microthalli

Culture strains used, culture conditions and progeny of unispores are shown in Table 34.

Unispores from microthalli developed from female gametes were cultured at 5-20°C under SD and LD. The unispores were pear drop-shaped, 6.4-7.8 × 4.0-4.6 µm in size (Fig. 78A). Settled spores were round, 4.2-5.6 µm in diameter. Germlings developed into prostrate filamentous thalli (Figs 78B-D) which formed unilocular sporangia and macrothalli (Fig. 78E) as did germlings derived from gametes.

Unispores from male parthenogenetic microthalli also developed in the same manner as gametes.

Culture from field microthalli

Unilocular sporangia were isolated from field microthalli, and cultured. Culture strains, culture conditions and progeny of unispores are shown in Table 35. Results of culture study on strain 367 Ohma are mentioned below.

Unilocular sporangia isolated from field microthalli were cultured under 10°C SD condition. Their Unispores (Fig. 79A) germinated to prostrate branched filaments (Fig. 79B) which formed macrothalli abundantly (Fig. 79C). The macrothalli were somewhat twisted, cylindrical, hollow (Figs 79D-F). They grew up

to 5-10 cm in length and 1 mm in diameter, and became fertile, forming firmly packed plurilocular sporangia with a cuticle cover (Fig. 79G). No ascocyst was observed. Plurispores from the macrothalli represented gametes, and they fused to form zygotes. Female and male gametes measured 5.6-6.2 × 3.4-4.0 µm and 5.2-6.2 × 3.0-4.0 µm in size, respectively. It was also observed that these gametes fused with those in culture from field macrothalli. Microthalli did not form unilocular sporangia under 10°C SD.

The female and male gametes parthenogenetically developed into prostrate filamentous thalli and produced macrothalli at 5-20°C under both SD and LD, and unilocular sporangia under 20°C SD.

In culture of three strains 374-376 Ohma unispores from unilocular sporangia isolated developed to prostrate, filamentous thalli, forming unilocular sporangia. Formation of macrothalli was less frequent and their growth was less conspicuous in these strains.

Discussion

Scytosiphon gracilis is distinguished from other species of *Scytosiphon* by its thin thallus, firmly packed plurilocular sporangia with cuticular covering, lack of ascocysts and microthalli similar to *Compsonema saxicolum*.

Scytosiphon lomentaria is clearly distinguished from *S. gracilis* by its thicker thallus, loosely adjoined plurilocular sporangia without cuticle, presence of ascocysts and *Microspongium*-like microthalli.

Several species and forms lacking ascocysts have been described. *Scytosiphon complanatus* (Rosenvinge) Doty (1947) was

originally described as *Scytosiphon lomentaria* var. *complanatus* Rosenvinge (1893) with characteristics of the complanate, unconstricted thallus and absence of ascocysts, based on materials from Greenland. Discoid microthalli were observed in culture of *S. complanatus* from the type locality, Greenland (Pedersen 1980). Therefore, *S. complanatus* differs from *S. gracilis* in morphology of the microthalli. *Scytosiphon dotyi* without ascocysts is also distinct from *S. gracilis* in having discoid microthalli (Wynne 1969).

Results reported by Clayton (1981) and Wynne (1969) do not agree with each other in respect to ascocysts of Californian complanate *Scytosiphon*. Clayton (1981) reported that *S. lomentaria* f. *complanatus major* lacks ascocysts and has a cuticle covered plurilocular sporangial sori on the basis of examinations of the type specimen, although ascocysts are not commented in the original description by Setchell and Gardner (1925). However, Wynne (1969) mentioned that California plants identical with *S. lomentaria* var. *complanatus* Rosenvinge agree with Rosenvinge's description (1893) except for the presence of ascocysts in local materials. Although Wynne included *S. lomentaria* f. *complanatus minor* in *S. dotyi*, he excluded *S. lomentaria* f. *complanatus major* because he believed that the latter possesses ascocysts. However, *S. lomentaria* f. *complanatus major* must be included in *S. dotyi* since it have no ascocysts according to Clayton's observation of the type specimen (Clayton 1981). *Scytosiphon lomentaria* var. *complanatus* sensu Correa *et al.* (1986) also has ascocysts. Species of complanate *Scytosiphon* having ascocysts must be excluded from *S. lomentaria* var. *complanatus*, according to Pedersen (1980).

Scytosiphon pygmaeus was described by Reinke (1892) on the basis of material dredged from a depth of 20 meters at northwest of Fehmarn in Kieler Bucht, Germany. This species was distinguished from *S. lomentaria* in its smaller size (length 5-10 mm and thickness less than 0.1 mm) and the lack of ascocysts. *Scytosiphon crispus* Skottsberg (1907), described from the Falkland Islands, likewise lacks ascocysts, and its length is less than 1 cm. Although *S. pygmaeus* and *S. crispus* may be related with *S. gracilis* in the absence of ascocysts, they are too minute to be compared with the latter species.

Recognition of ascocysts as a taxonomic character is not in general accordance. Pedersen (1980) recognized *S. complanatus* (Rosenvinge) Doty as a distinct species because it lacks ascocysts. Whereas Clayton (1976a, 1981) neglected importance of ascocysts as a taxonomic character, referring the report of Loiseaux (1967). However, Clayton's opinion is not convincing since the report of Loiseaux (1967) is on the genus *Ascocyclus* in Myrionemataceae. In the present study, presence or absence of ascocysts was demonstrated to be definite in each species examined. Therefore, ascocysts are treated as an important taxonomic character for distinction of species.

My culture study on *Scytosiphon dotyi* sensu Fletcher (1987) (strain Margarete, England) revealed that microthalli were not discoid thalli, but filamentous thalli (data not shown) which were different from those of *S. dotyi* Wynne (Wynne 1969). Plurispores in culture of this strain fused with male gametes of *Scytosiphon gracilis* (data not shown). Therefore, this strain is concluded to be the female plant of *S. gracilis*. *S. dotyi* sensu Fletcher is conspecific with *S. gracilis*, and this extends the

distribution of this species to Europe.

A diagram of life history of this species in culture is shown in Fig. 80. Life history of *S. gracilis* in culture is considered as dimorphic and biphasic type which agrees with those of other species of Scytosiphonaceae (Nakamura and Tatewaki 1975, Clayton 1980). In culture of strains from Ohma, macrothalli arose from microthalli under all conditions examined (5-20°C under SD and LD). These results suggest that formation of macrothalli do not depend on temperature and photoperiods. However, macrothalli grew larger in lower temperatures and short day conditions. In culture of strains of Samani and Utabetsu, macrothalli rarely arose under 10°C LD, but abundantly appeared under 10°C SD. Formation of macrothalli may be controlled by photoperiods in these strains.

Although unilocular sporangia were formed usually under 10-20°C conditions, they were not produced in some strains. These strains may represent a direct type life history. However, the border between a normal and a direct type life history is unclear since there are strains in which unilocular sporangia are rarely formed.

In culture of *S. gracilis*, microthalli were not in the form of disks, but rather filamentous systems. Their unilocular sporangia were formed without paraphyses. In this study, field microthalli of *S. gracilis* were examined. In culture of unilocular sporangia on *Compsonema saxicolum*-like thalli collected from Ohma, macrothalli identical with *S. gracilis* were obtained. Plurispores from the macrothalli fused with gametes of *S. gracilis*. These results strongly suggest that *Compsonema saxicolum*-like thalli collected in fields are microthalli of *S.*

gracilis. Female and male thalli appeared in culture of a single unilocular sporangium on a field microthallus. This result suggests that the microthallus is diploid and sporophytic, and that reduction division occurs in the unilocular sporangium.

Table 32. *Collection data of cultured strains of Scytosiphon gracilis.* Culture conditions tested and progeny of plurispores from macrothalli are also given. (fil. microthalli = filamentous microthalli)

strain no.	locality	date	sex	culture conditions	progeny
1	Ohma	3 iii 1987		5-20SL	fil. microthalli sprouting macrothalli (P)
36	Samani	15 v 1987		10L, 20L	fil. microthalli
				10S, 15S	fil. microthalli sprouting macrothalli (P)
98	Ohma	21 ii 1988	female	10L	fil. microthalli (U) sprouting macrothalli (P)
104	Samani	5 iv 1988		10L	fil. microthalli (U)
				20L	fil. microthalli
105	Utabetsu	6 iv 1988		10L	fil. microthalli
				10S	fil. microthalli sprouting macrothalli
107	Himeshima	17 iv 1988		10L, 20L	fil. microthalli sprouting macrothalli
113	Nakajima	21 iv 1988		10L	fil. microthalli sprouting macrothalli (P)
210	Oshoro	10 iii 1989		5S	fil. microthalli (U) sprouting macrothalli (P)
216	Kitagijima	4 iv 1989		10L	fil. microthalli sprouting macrothalli
217	Awashima	5 iv 1989		10L	fil. microthalli sprouting a few macrothalli (P)
				20L	fil. microthalli (a few U) sprouting macrothalli
220	Megishima	6 iv 1989		10L	fil. microthalli, a few macrothalli
265	Oshoro	2 xii 1989		5S	fil. microthalli sprouting macrothalli (P)
				20L	fil. microthalli (U) sprouting macrothalli (P)
271	Ohma	2 ii 1990	male	5-15SL	fil. microthalli sprouting macrothalli (P)
				20L	fil. microthalli sprouting a few macrothalli
				20S	fil. microthalli (a few U) sprouting macrothalli (P)
273	Ohma	2 ii 1990	female	5L, 20L	fil. microthalli (U) sprouting a few macrothalli
				5L,10SL,15SL	fil. microthalli (U) sprouting macrothalli (P)
				20S	fil. microthalli (U) sprouting macrothalli (P, U)
289	Karimo	31 iii 1990		5-20SL	fil. microthalli sprouting macrothalli (P)
352	Hanasaki	18 v 1991		10L	fil. microthalli
				10S	fil. microthalli sprouting macrothalli
355	Samani	19 v 1991		10L	fil. microthalli sprouting a few macrothalli
				10S	fil. microthalli sprouting macrothalli

Table 33. *Culture of Scytosiphon gracilis.* Progeny of zygotes.

strain no.	culture conditions	progeny
273 Ohma female × 272 Ohma male	10S, 20L	filamentous microthalli (U)

Table 34. Culture of *Scytosiphon gracilis*. Progeny of unispores from parthenogenetic microthalli. (fil. microthalli = filamentous microthalli)

strain no.	locality	date	sex	culture conditions	progeny
98	Ohma	21 ii 1988	female	5L	fil. microthalli sprouting a few macrothalli
				5S	fil. microthalli sprouting macrothalli (P)
				10SL, 15SL	fil. microthalli (U) sprouting macrothalli (P)
				20L	fil. microthalli (U) sprouting macrothalli (P, U)
				20S	fil. microthalli sprouting macrothalli
273	Ohma	2 ii 1990	female	10S	fil. microthalli sprouting macrothalli
				15S, 20S	fil. microthalli (U) sprouting macrothalli
				20L	fil. microthalli (U) sprouting a few macrothalli (P, U)
271	Ohma	2 ii 1990	male	20S	fil. microthalli sprouting macrothalli (P)

Table 35. Culture of *Scytosiphon gracilis*. Progeny of unispores from field microthalli. (fil. microthalli = filamentous microthalli)

strain no.	locality	date	culture conditions	progeny
367	Ohma	24 ix 1991	10S	fil. microthalli sprouting macrothalli (P: female, male)
374	Ohma	24 ix 1991	10S	fil. microthalli (U) sprouting a few macrothalli
375	Ohma	24 ix 1991	10S	fil. microthalli (U) sprouting a few macrothalli
376	Ohma	24 ix 1991	10S	fil. microthalli (U) sprouting a few macrothalli

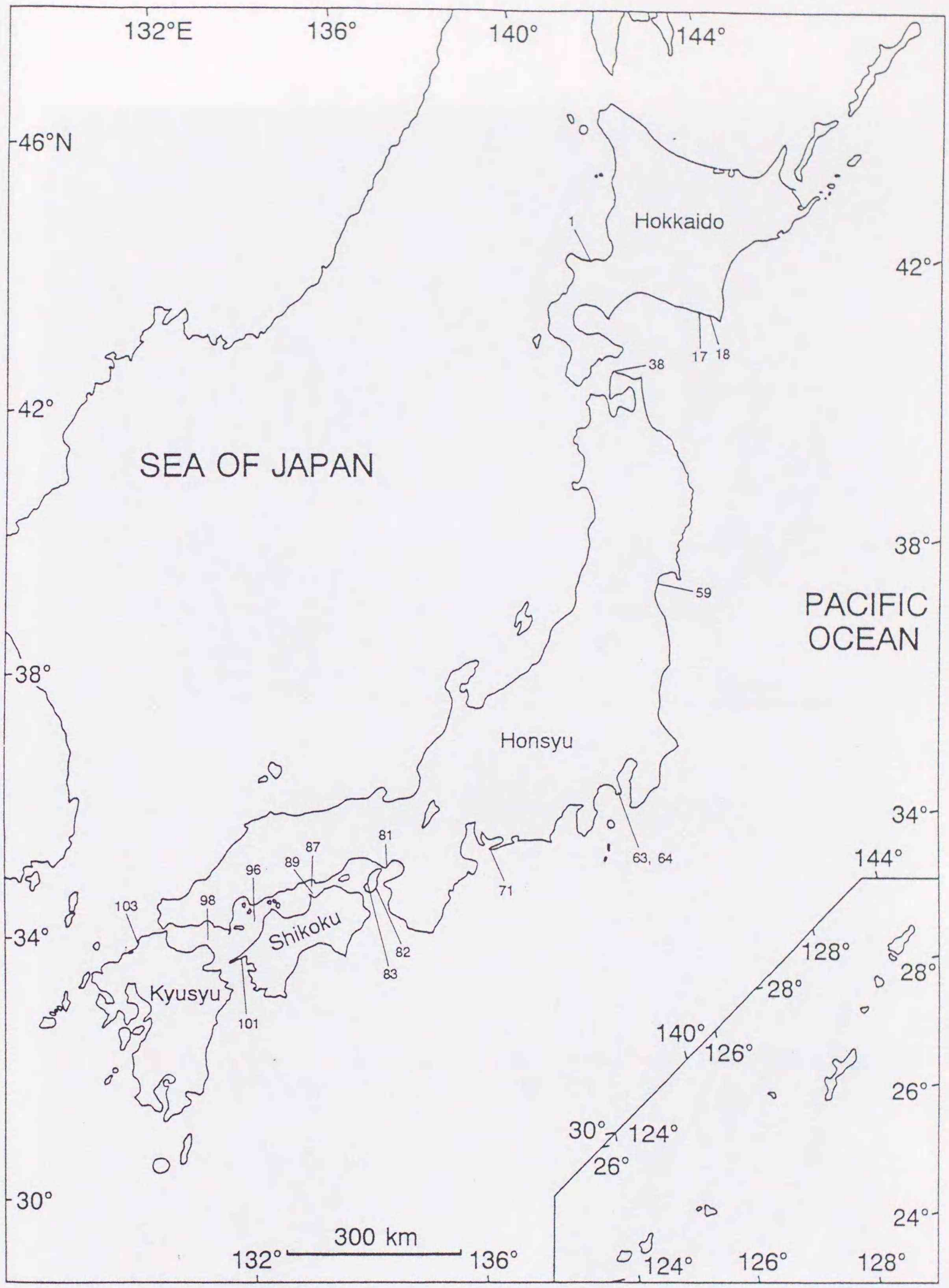


Fig. 68. Localities where materials of *Scytosiphon gracilis* were collected. Numbers plotted correspond to those in Table 1.

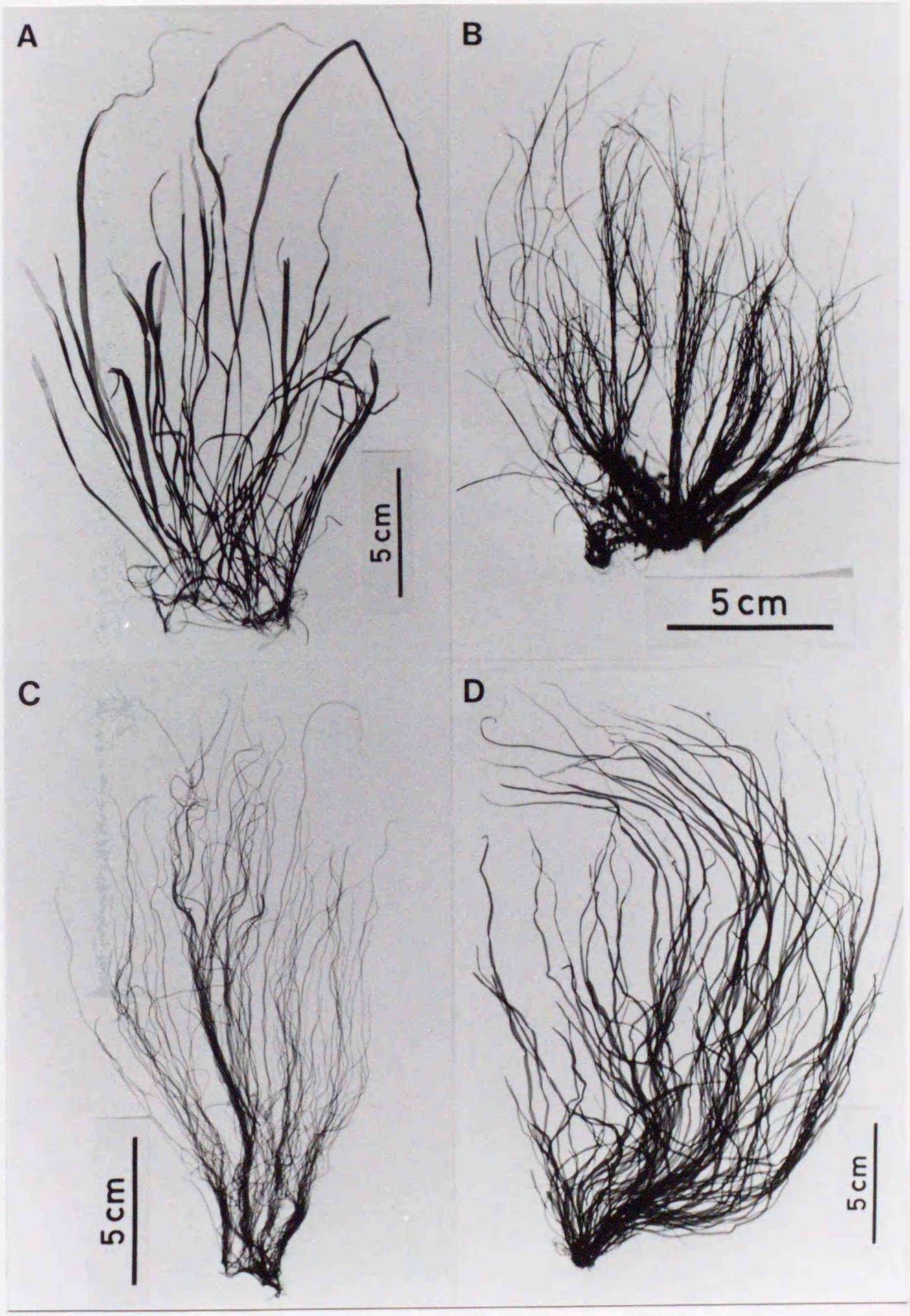


Fig. 69. Habit of specimens of *Scytosiphon gracilis*.

A. Holotype specimen (SAP 059720). Plant collected at Ohma on 1 February 1990. B. Plant collected at Ohma on 21 February 1988 (leg. H. Kawai). C, D. Plants collected at Kitagishima, Okayama Pref. on 4 April 1989 (leg. S. Enomoto & K. Kogame).

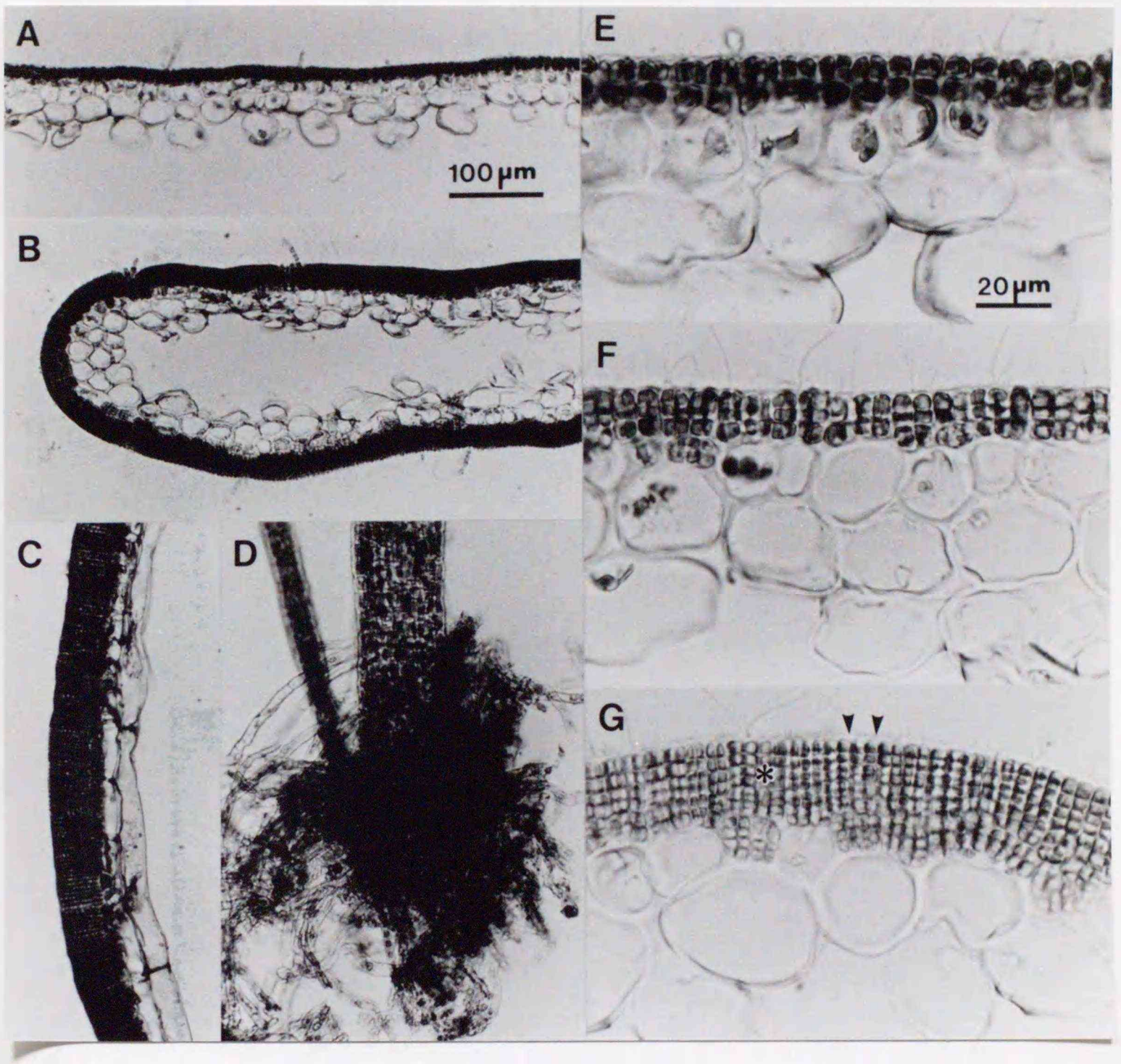


Fig. 70. Anatomy of *Scytosiphon gracilis*.

A, **B**. Cross sections of thalli. **C**. Longitudinal section of thallus. **D**. Basal portion of thalli, showing rhizoids. **E**. Cortical and medullary cells in cross section of thallus. **F**. Young plurilocular sporangia. **G**. Plurilocular sporangia (asterisk) with a cuticular covering (arrowheads).

Scale in **A** applies to **B-D**; Scale in **E** applies to **F** and **G**.

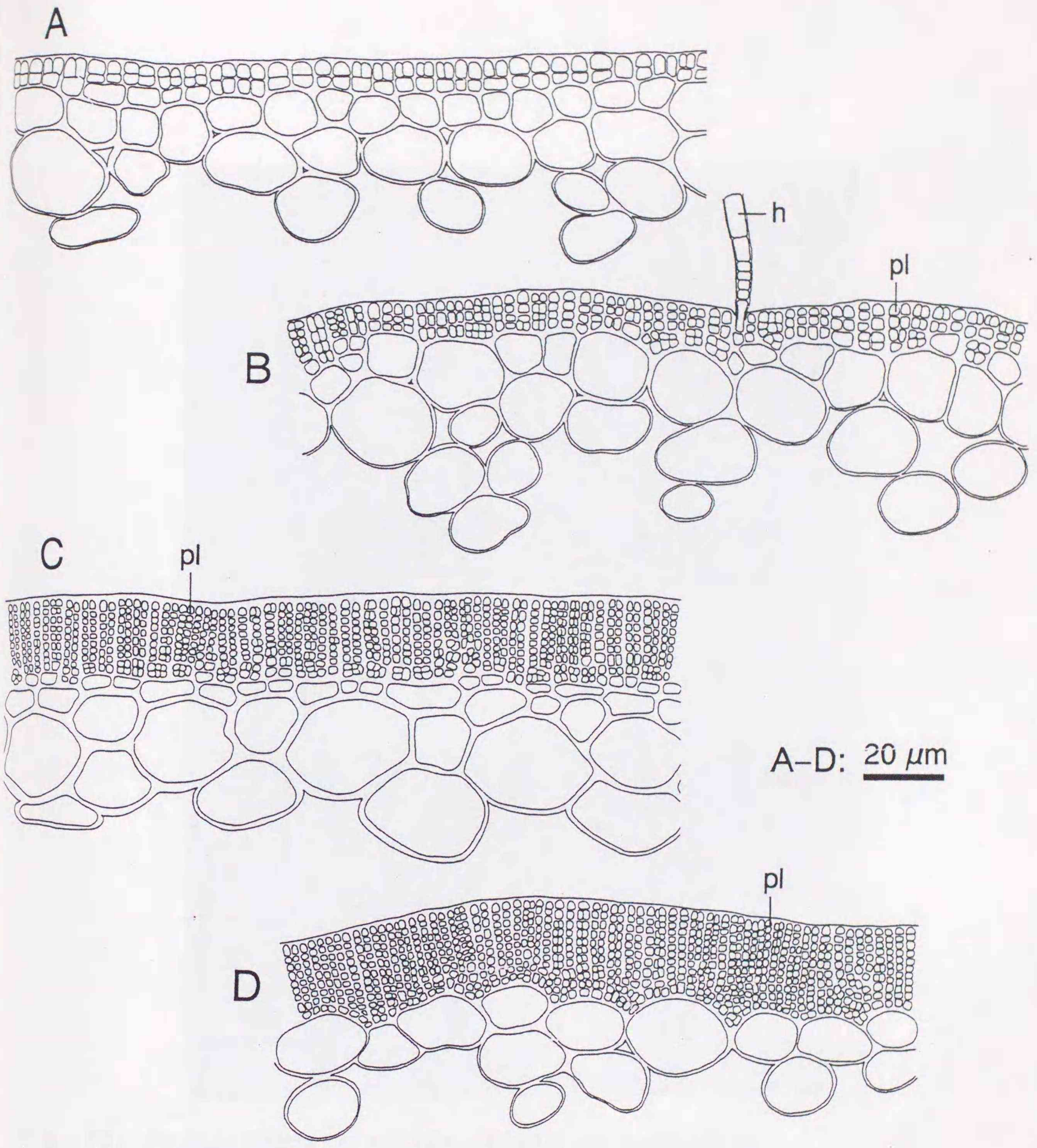


Fig. 71. Anatomy of *Scytosiphon gracilis*.

A. Cross section of vegetative thallus. **B.** Cross section of thallus in early stage of formation of plurilocular sporangia (pl). h: phaeophycean hair. **C, D.** Cross section of fertile thalli with plurilocular sporangia (pl).

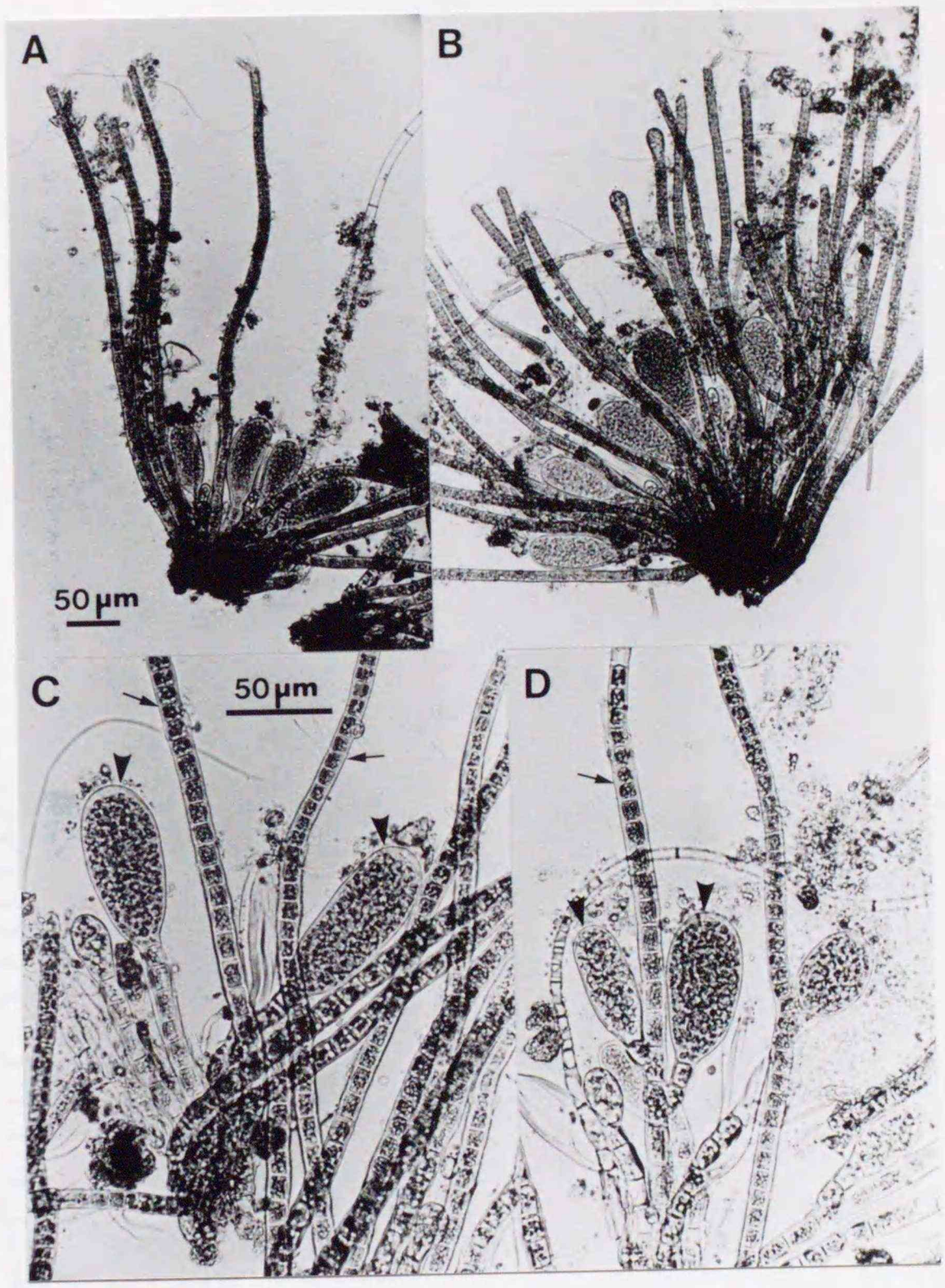


Fig. 72. *Scytosiphon gracilis*. Field microthalli.

A, **B**. Plants collected at Ohma on 24 September 1991. **C**. Unilocular sporangia (arrowheads) on stalk filament, and uniseriate upright filaments (arrows). **D**. Pedicellate unilocular sporangia (arrowheads) on upright filaments (arrow).

Scale in **A** applies to **B**; Scale in **C** applies to **D**.

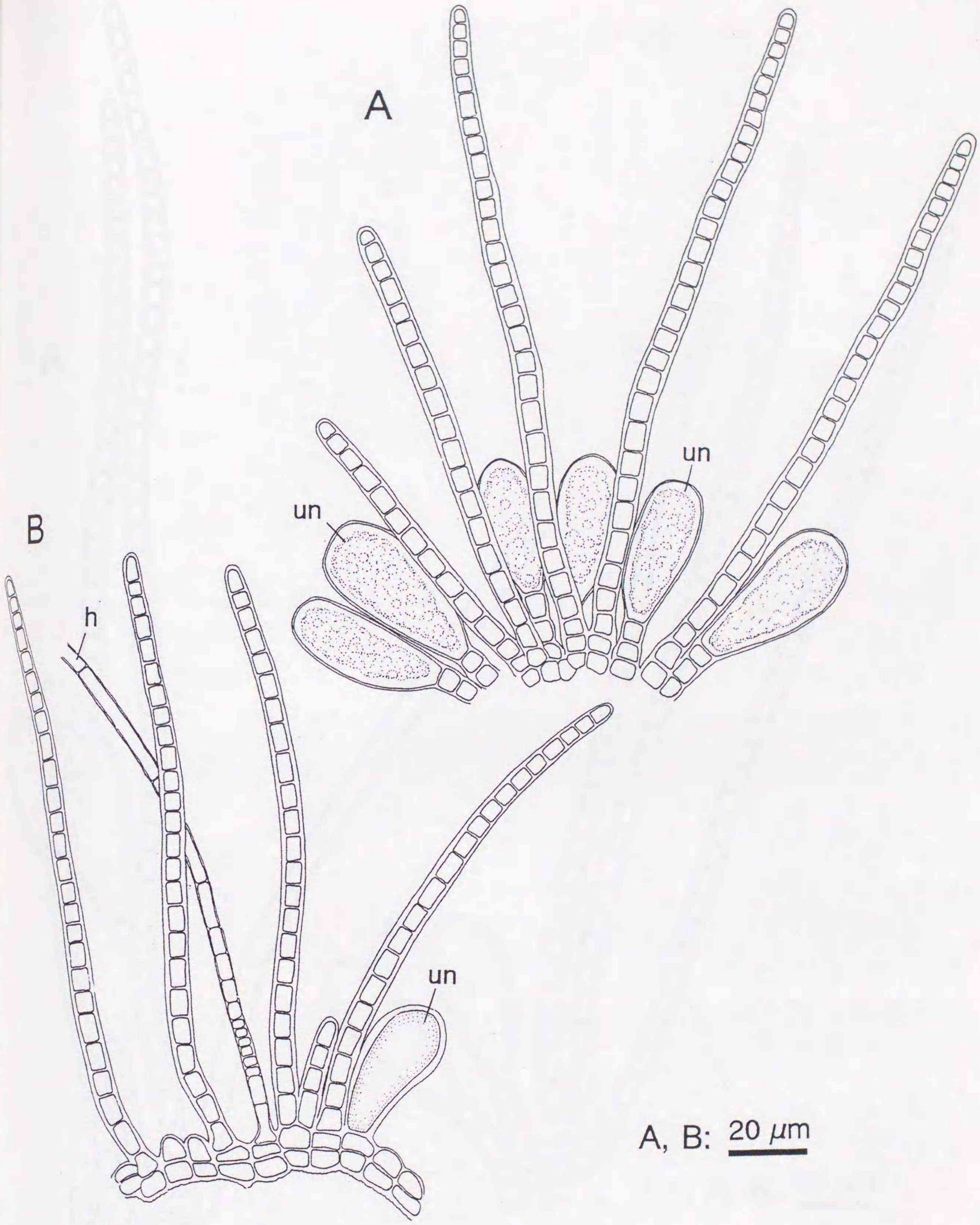


Fig. 73. *Scytosiphon gracilis*. Field microthalli.

A, B. Squashed thalli baring with unilocular sporangia (un). h: phaeophycean hair.

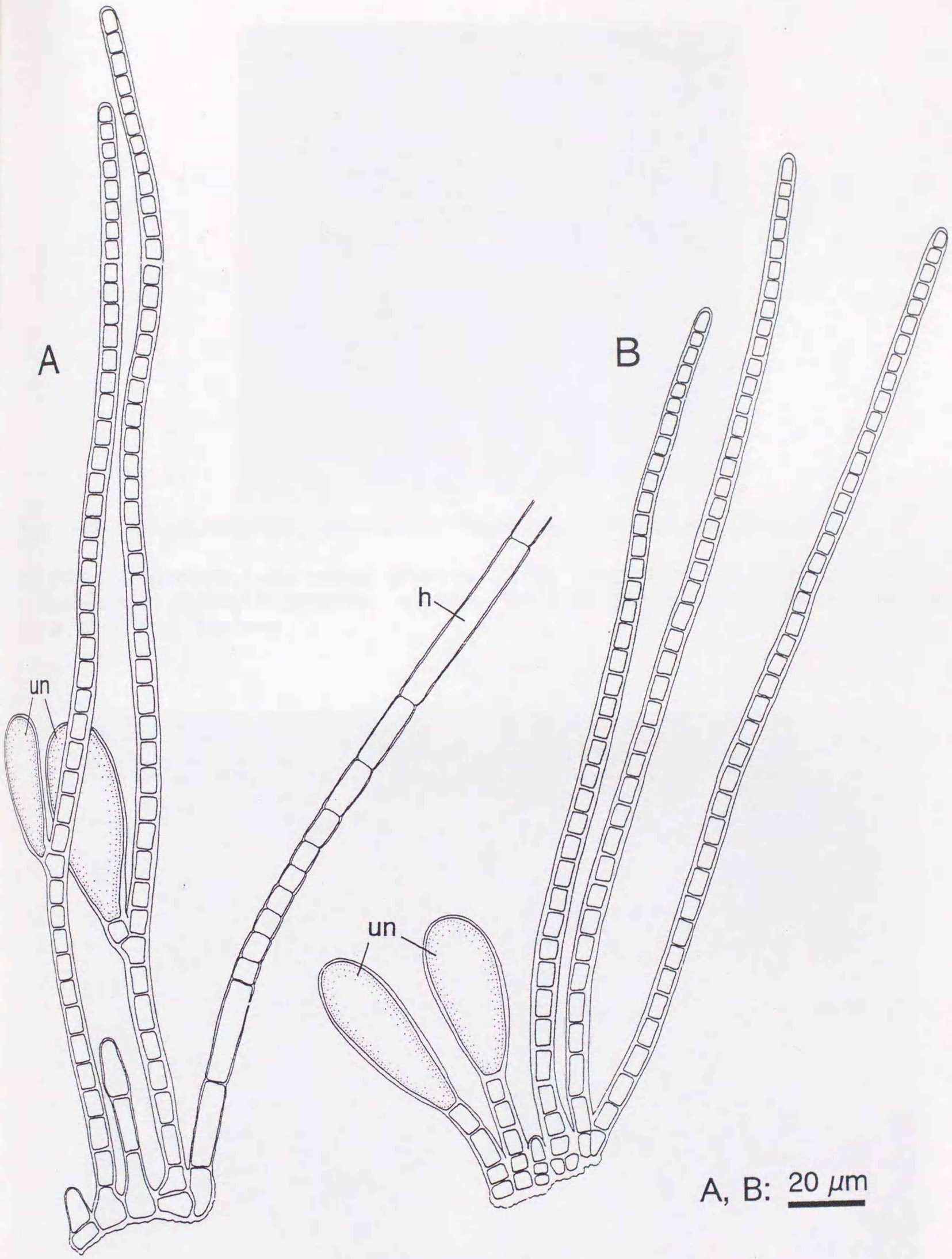


Fig. 74. *Scytosiphon gracilis*. Field microthalli.

A. Squashed microthallus, showing unilocular sporangia (un) laterally on uniseriate upright filaments. h: phaeophycean hair.
B. Squashed microthallus bearing unilocular sporangia (un).

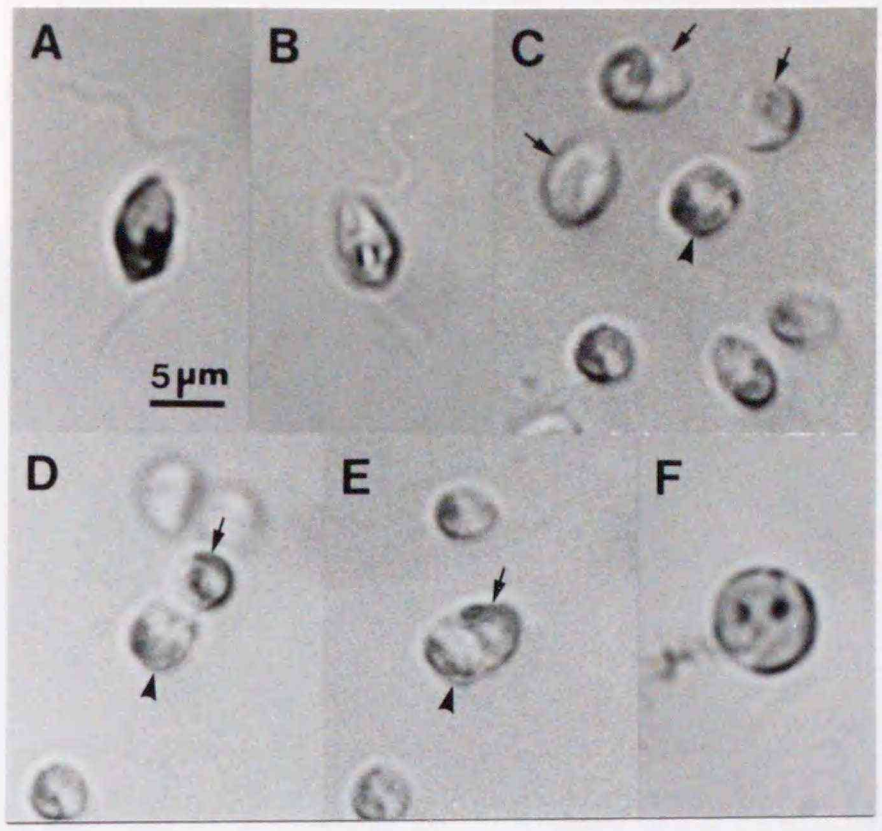


Fig. 75. *Scytosiphon gracilis*. Gametes and gamete fusion.

A. Female gamete. **B.** Male gamete. **C-E.** Sequence of gamete fusion (arrowhead, female gamete; arrow, male gamete). **F.** Zygote. Scale in **A** applies to **B-F**.

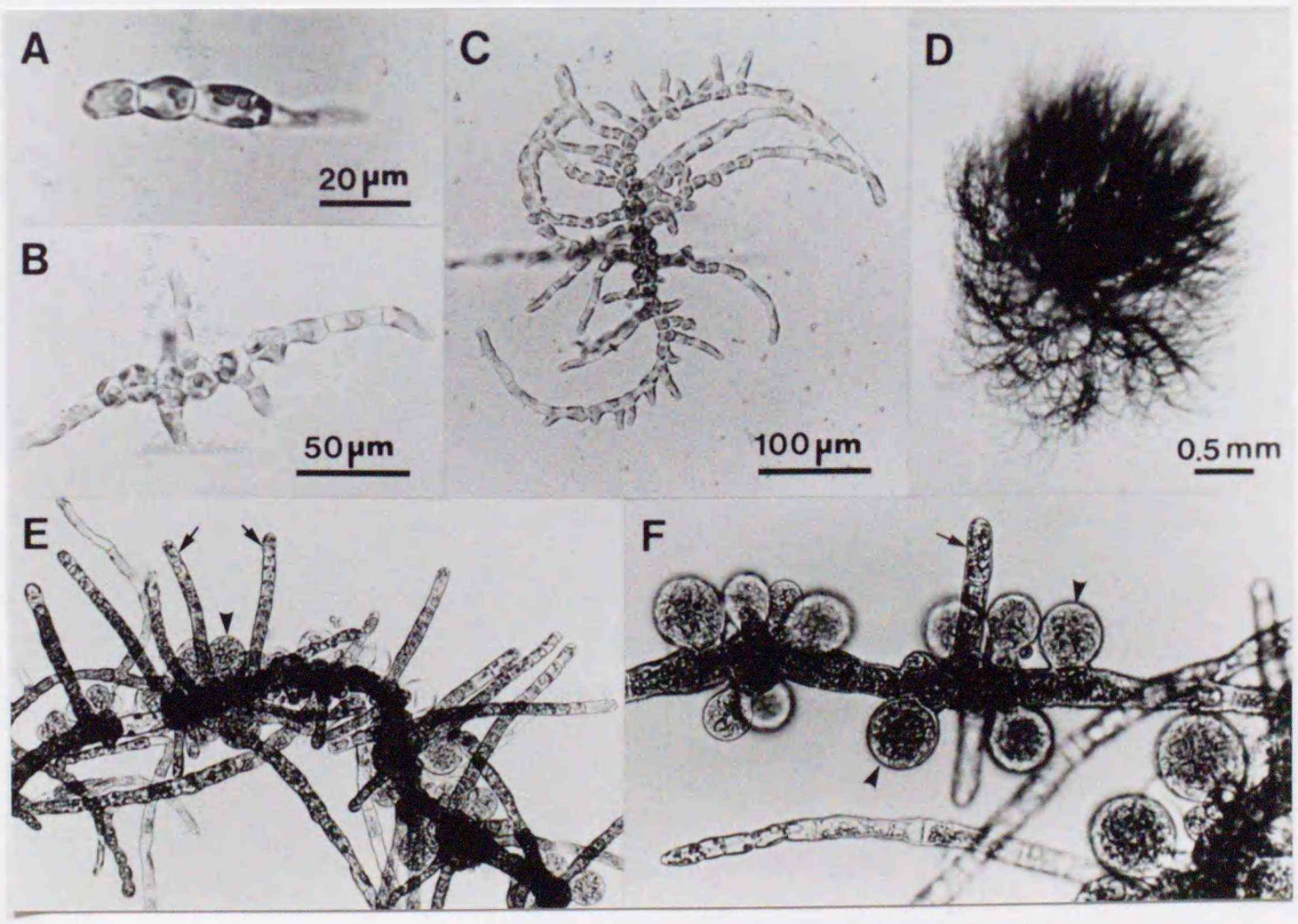


Fig. 76. Culture of *Scytosiphon gracilis*. Development of zygote.

A. Six-day-old germling under 10°C SD condition. **B.** Eleven-day-old germling under 10°C SD condition. **C.** Seventeen-day-old germling under 10°C SD condition. **D.** Seven-week-old microthallus under 10°C SD condition. **E, F.** Unilocular sporangia (arrowheads) on filaments of microthallus under 20°C LD condition (arrows: uniseriate upright filaments). Scale in **B** applies to **F**; Scale in **C** applies to **E**.

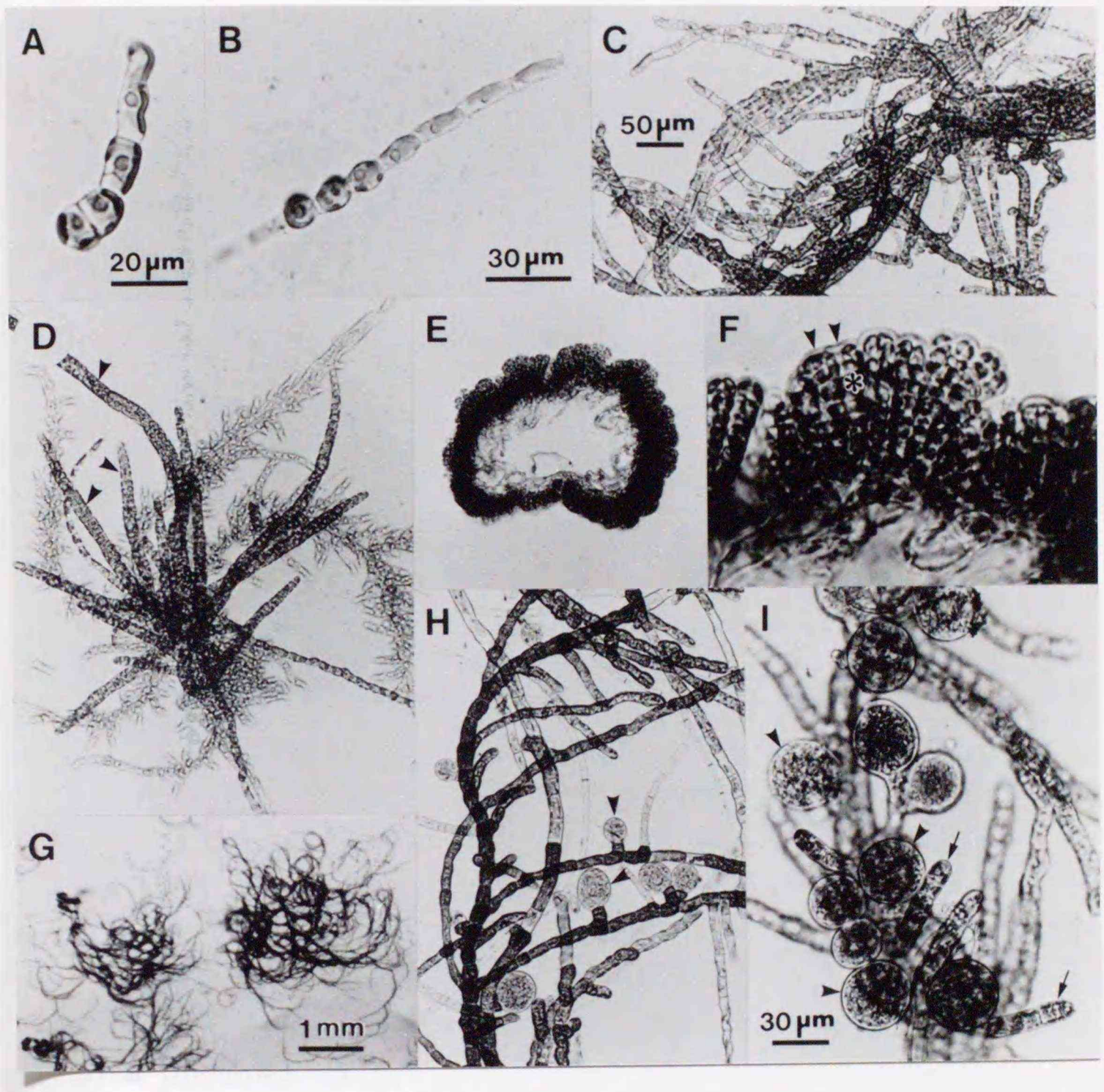


Fig. 77. Culture of *Scytosiphon gracilis*. Parthenogenetic development of gametes.

A. Four-day-old germling under 10°C SD condition. **B.** Eleven-day-old germling under 10°C LD condition. **C.** Microthallus under 10°C LD condition. **D.** Macrothalli (arrowheads) arising from microthallus under 10°C SD condition. **E.** Cross section of macrothallus with plurilocular sporangia under 10°C LD condition. **F.** Plurilocular sporangia (asterisk) in cross section of macrothallus (arrowheads: cuticle). **G.** Habit of microthalli under 10°C LD condition. **H, I.** Unilocular sporangia (arrowheads) on filaments of microthalli (arrows: uniseriate upright filaments).

Scale in **B** applies to **F**; Scale in **C** applies to **D**, **E** and **H**.

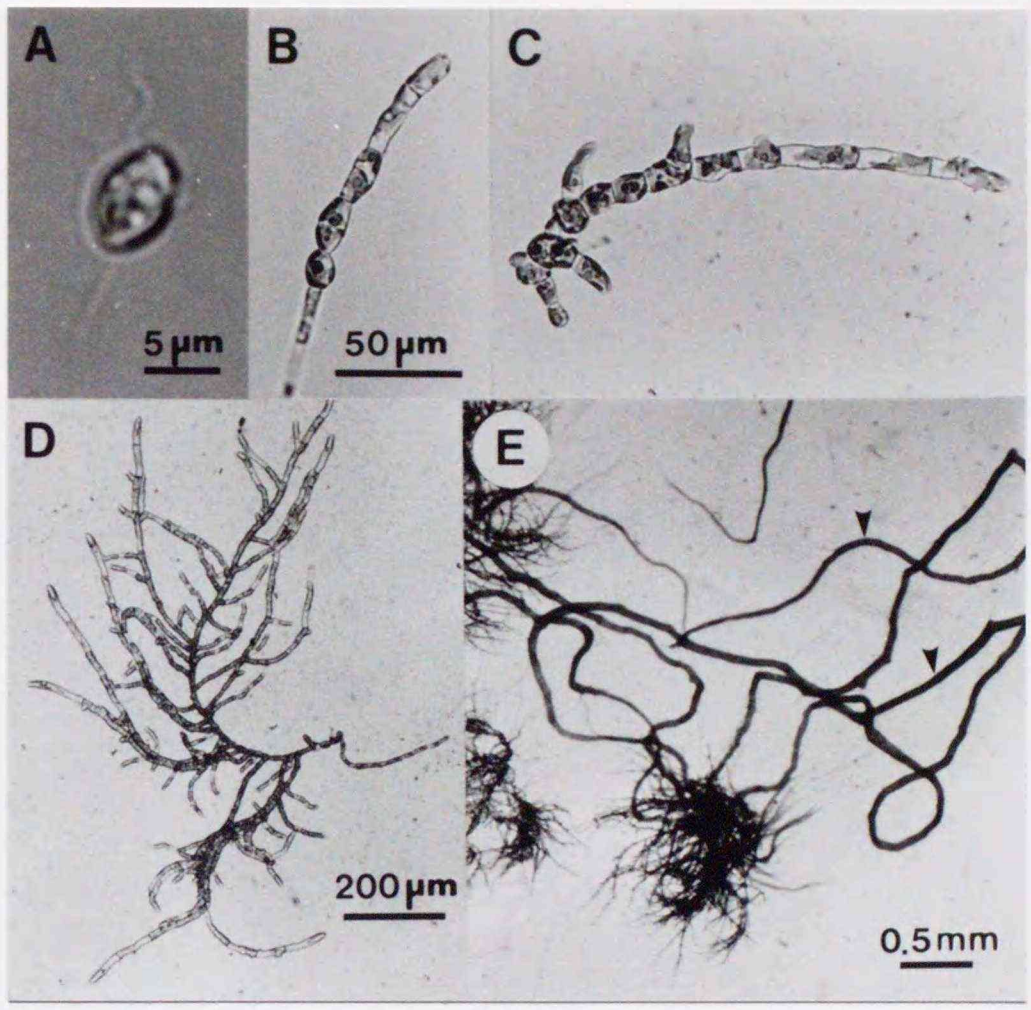


Fig. 78. Culture of *Scytosiphon gracilis*. Development of unispores from parthenogenetic microthallus.

A. Unispore. **B.** Eighteen-day-old germling under 10°C LD condition. **C.** Eight-day-old germling under 20°C LD condition. **D.** Twelve-day-old germling under 20°C LD condition. **E.** Four-week-old thalli under 20°C LD condition, showing macrothalli (arrowheads) arising from microthallus.

Scale in **B** applies to **C**.

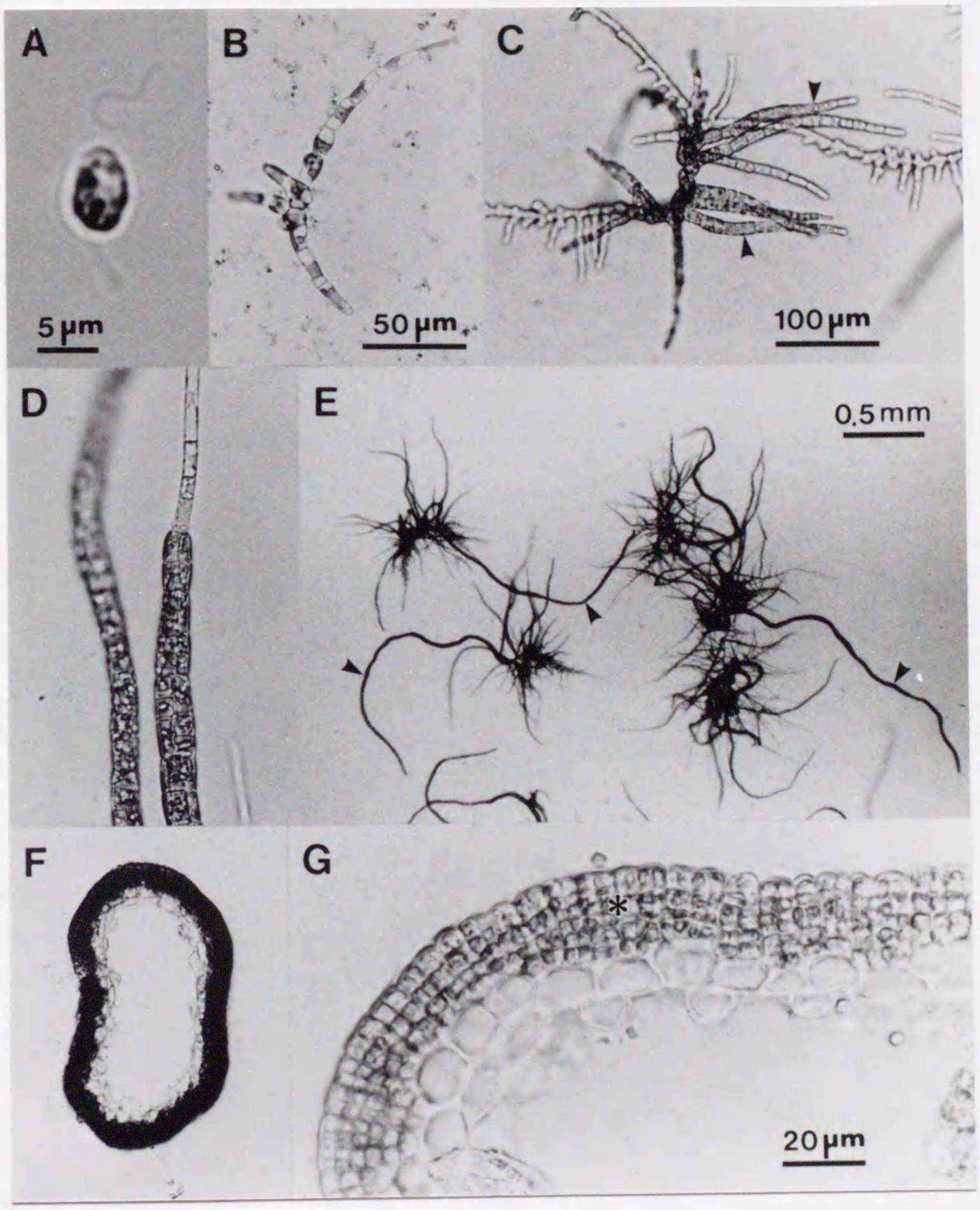


Fig. 79. Culture of *Scytosiphon gracilis*. Development of unispores from field microthallus.

A. Unispore. **B.** Seventeen-day-old germling under 10°C SD condition. **C.** Three-week-old thallus under 10°C SD condition, showing macrothalli (arrowheads) arising from prostrate filaments. **D.** Terminal portion of macrothalli with a phaeophycean hair. **E.** One-month-old thalli under 10°C SD condition, showing macrothalli (arrowheads) arising from microthalli. **F.** Cross section of fertile macrothallus with plurilocular sporangia. **G.** Plurilocular sporangia (asterisk) in cross section of macrothallus.

Scale in **B** applies to **D**; Scale in **C** applies to **F**.

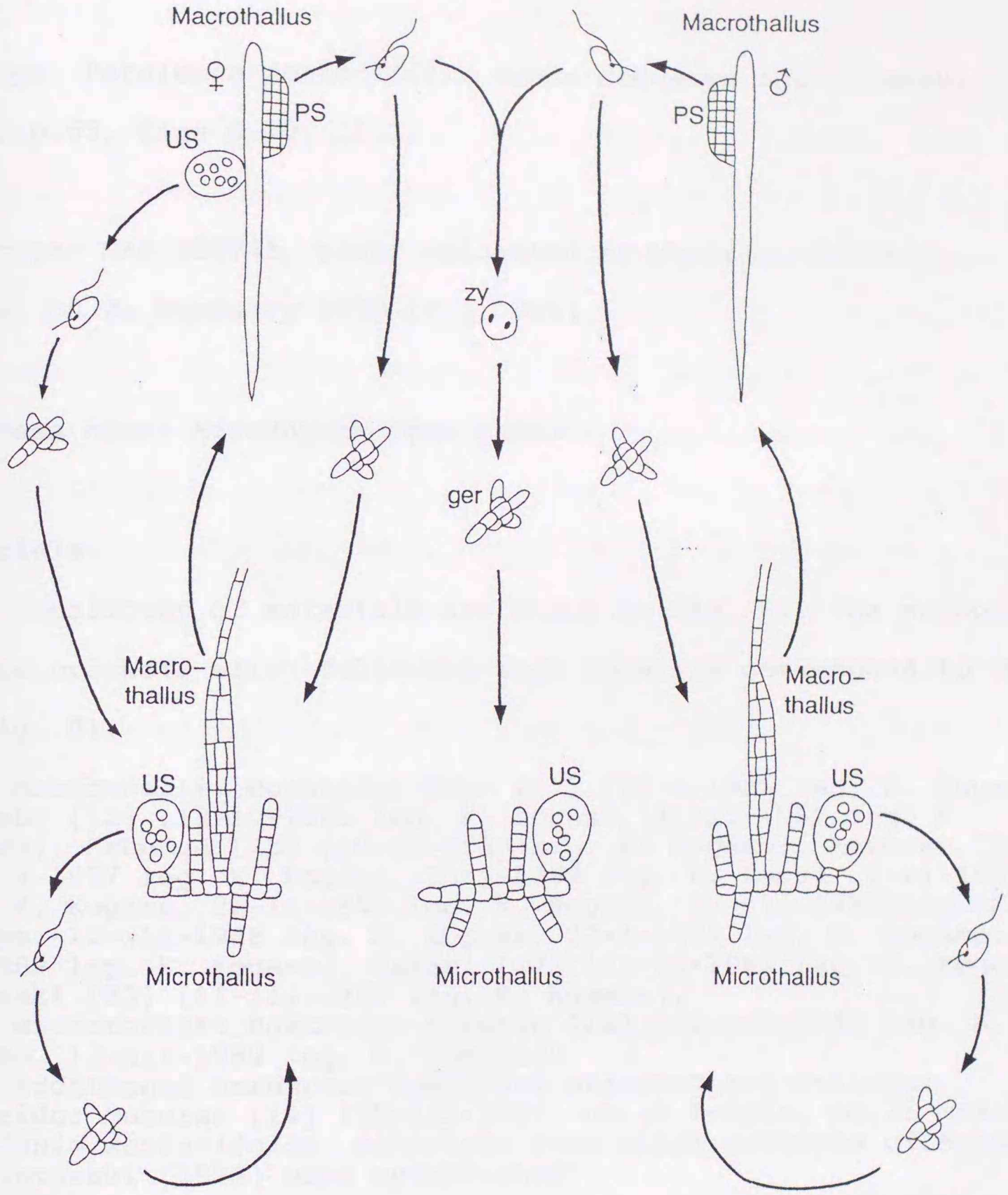


Fig. 80. Diagram of life history of *Scytosiphon gracilis* in culture.

ger, germling; PS, plurilocular sporangium; US, unilocular sporangium; zy, zygote.

Scytosiphon tenellus **sp. nov.**

Synonym: *Petalonia zosterifolia* sensu Nakamura and Tatewaki, 1975, p.65, figs 6-10, pl.2.

Holotype: SAP 059746, plant collected at Muroran, Hokkaido, Japan, on 21 February 1988 (Fig. 82A)

Japanese name: Hirakayamo (new name)

Materials

Localities of materials are shown in Fig. 81. The numbers in square brackets after following each locality correspond to those in Fig. 81.

Macrothalli: Hokkaido: Esan [11] (18-v-1988 leg. K. Kogame), Shikabe [12] (12-iv-1986 leg. K. Kogame, 4-iii-1987 leg. K. Kogame), Rebunge [13] (10-iv-1985 leg. K. Kogame), Muroran [14] (18-iv-1987 leg. K. Kogame, 28-i-1988 leg. H. Kawai, 5-ii-1988 leg. K. Kogame, 21-ii-1988 leg. K. Kogame, 23-iii-1988 leg. K. Kogame, 12-xii-1988 leg. K. Kogame, 22-i-1989 leg. K. Kogame, 24-ii-1989 leg. K. Kogame), Samani [17] (11-ii-1989 leg. K. Kogame), Hanasaki [23] (11-iii-1989 leg. K. Kogame).

Microthalli: Hokkaido: Muroran [14] (12-xii-1988 leg. K. Kogame, 12-xii-1989 leg. K. Kogame).

Additional herbarium specimens examined are follows: Hokkaido: Muroran [14] (30-iii-1967, no.18 female, no.25 male, as *Petalonia zosterifolia*, materials from which cultures of Nakamura and Tatewaki (1975) were established).

Descriptio

Macrothalli lineares, single, plerumque complanati, cavi, sine constrictionibus, 3-15 (-25) cm longi, 1-5 mm lati, sub haptero parvo exorientes, flavidi-brunnei in colore quum juveniles atrobrunnescentes quum maturi, subtiles; ex atratis corticalibus et medullosis constantes; cortex ex 1-3 stratis cellularum parvarum pigmentiferarum constans; medulla ex 1-3

stratis cellularum grandium incolorium constans; cellulae medullosae in sectione transversali rotundae vel ellipsoideae, 10-30 × 10-80 µm in amplitudine, in sectione longitudinali intus longiores, ad 300-460 µm longae; pili achromi aggregati vel singulares, variabiles in quantitate; hapteron ex filamentis rhizoides sub pagina thallorum exorientibus constans; plantae gametis isogamicis; gametae 5.8-6.6 × 3.0-4.0 µm in amplitudine; sporangia plurilocularia in pagina integera thallorum facta, firme contigua, 30-40 µm longa, strato tegens cuticulare; ascocystae inter sporanga plurilocularia, variabiles in quantitate; partes thallos post liberationem plurisporarum incolorescentes.

Microthalli discoidei, firmi, ad 5 mm in diametro, brunnei vel atrobrunnei in colore, ex filamentis prostratis ramosis et filamentis erectis uniserialibus simplicibus constantes; filamenta erecta nonnihil firme affixa inter se, 70-220 µm longa; cellulae filamentorum erectorum 7-20 µm longae, 7-12 µm latae, nonnihil cupiformes; sporangia unilocularia lateralia super cellulis sustinentibus paraphysium, sessilia clavata, 70-140 µm longa, 20-28 µm lata; paraphyses 100-260 µm longae, e 5-8 cellulis compositae; cellulae paraphysium in partibus superis breviores, 8-30 × 7-9 µm in amplitudine, partibus inferis longiores, 60-140 × 6.5-8 µm in amplitudine; cellulae thallorum plastidium unum cum pyrenoide una in quoque cellula continentes.

Description

Macrothalli linear, usually flat, hollow, without constrictions, 3-15 (-25) cm long, 1-5 mm wide, arising from a small holdfast, yellowish brown when young, becoming dark brown when

mature, delicate, consisting of cortical and medullary layers; cortex consisting of 1-3 small pigmented cells; medulla consisting of 1-3 large colorless cells; in transverse section medullary cells round to ellipsoid, 10-30 × 10-80 µm in size, in longitudinal section longer in inner portions, to 300-460 µm long; phaeophycean hairs grouped or single, variable in amount; holdfast consisting of rhizoidal filaments arising from the surface of the thalli; plants dioecious; gametes isogamous, 5.8-6.6 × 3.0-4.0 µm in size; plurilocular sporangia formed on the entire surface of thalli, firmly packed, 30-40 µm long, with a covering cuticular layer; ascocysts present among plurilocular sporangia, variable in amount; portions of thalli becoming colorless after release of plurispores.

Microthalli discoid, firm, to 5 mm in diameter, brown to dark brown in color, composed of branched, prostrate filaments and simple, uniseriate upright filaments arising from them; upright filaments somewhat firmly adjoined to each other, 70-220 µm long; cells of the upright filaments 7-20 µm long, 7-12 µm wide, somewhat barreled in shape; unilocular sporangia lateral on supporting cells of paraphyses, sessile, clavate, 70-140 µm long and 20-28 µm wide; paraphyses 100-260 µm long, composed of 5-8 cells; cells of the paraphyses shorter in upper portions, 8-30 × 7-9 µm in size, and longer in lower portions, 60-140 × 6.5-8 µm in size; cells of thalli containing one plastid with a large pyrenoid for each.

Morphology

Macrothalli

Thalli are linear, usually flat, hollow, without constric-

tions, 3-15 (-25) cm in length, 1-5 mm in width, arising from a small holdfast (Figs 82A-D). Thalli are yellowish brown when young, becoming darker in color in fertile condition, and delicate in texture. Thalli consist of a cortical and a medullary layers (Figs 83B, 84A-C). The cortex is composed of 1-3 layers of small pigmented cells. The medulla is composed of 1-3 layers of large colorless cells which are round to ellipsoid, 10-30 × 10-80 µm in size. In longitudinal section, medullary cells are larger in inner portions, to 300-460 µm in length (Fig. 83C). Phaeophycean hairs are variable in amount, grouped or solitary. Holdfasts are composed of rhizoidal filaments arising from the under surface of the thalli (Fig. 83D). Thalli became fertile basipetally, forming plurilocular sporangia by divisions of the surface cells. Usually cortical cells also change to plurilocular sporangia. Plurilocular sporangia are firmly packed, 30-40 µm in length, with a cuticle even when mature (Figs 83F,G, 84B,C, 85A,B). Ascocysts are present among plurilocular sporangia, variable in amount (Figs 83E-G, 84B,C, 85A,B). Portions which released plurispores are colorless.

Microthalli

Thalli are discoid, firm, up to 5 mm in diameter, brown to dark brown in color. Thalli are composed of branched prostrate filaments and simple, uniseriate upright filaments arising from them (Figs 86A,B, 87A,B). Upright filaments are somewhat firmly adjoined with each other, 70-220 µm in length. Cells of the upright filaments are 7-20 µm in length, 7-12 µm in width, somewhat swollen. In fertile thalli, upper portions of upright filaments elongate to become paraphyses, later unilocular spor-

angia are formed laterally on supporting cells of the paraphyses (Figs 86C-E, 87A,B). Paraphyses are 100-260 µm in length, composed of 5-8 cells. Cells of the paraphyses are shorter in upper portions, 8-30 × 7-9 µm in size, and longer in lower portions, 60-140 × 6.5-8 µm in size. Unilocular sporangia are sessile, clavate, 70-140 µm in length and 20-28 µm in width. Each cell of thalli contains one plastid with a large pyrenoid (Fig. 86D).

Habitat and Phenology

Macrothalli grow on rock, boulders, concrete blocks, or sea glasses (*Phyllospadix*) in the lower intertidal to subtidal zones in moderately exposed area. The thalli are found during winter and spring.

Microthalli are found on rocks or boulders in the lower intertidal to subtidal zones. Microthalli bearing unilocular sporangia were found in early winter.

Geographical distribution

This species has been collected from six localities on the Pacific coasts of Hokkaido to date (Fig. 81).

Culture study

Culture strains isolated from macrothalli are listed in Table 36 in which culture conditions and progeny of plurispores are also shown.

Sexual reproduction

Gamete fusions were observed only in materials from Muroran,

Hokkaido. This species is dioecious, and gametes are isogamous. Gametes released from plurilocular sporangia are of normal phaeophycean type, with a plastid and a stigma (Figs 88A,B). They usually showed negative phototaxis. Female and male gametes were measured 5.8-6.6 × 3.0-4.0 μm and 5.8-6.6 × 3.2-4.0 μm in size, respectively. When female and male gametes were mixed, a settled female gamete was surrounded by several male gametes which touched the settled female gamete by an anterior flagellum, and then, one of male gametes fused with the female gamete (Figs 88C-F).

The fragrant odor of sexual pheromones was observed in culture where female gametes were released.

Further development of zygotes could not be followed.

Parthenogenetic development of gametes

Female gametes germinated into prostrate branched filaments, later they became disks which consisted of prostrate branched filaments and simple, upright filaments arising from them (Figs 89A-E). Margin of disks were smooth and round (Fig. 89C). Free branched filaments with knots were usually observed. When these filaments attached to substrata, they produced disks. Discoid microthalli formed unilocular sporangia two months after transfer from 15°C LD to 10°C SD. Unilocular sporangia are ellipsoid, arising laterally on supporting cells of paraphyses (Fig. 89F).

Macrothalli arose on disks and branched filaments at 5-20°C under SD and LD. They arose more abundantly and grew larger in lower temperatures and short day conditions. Macrothalli reached to 5-10 cm or more in length, and became fertile with plurilocular sporangia. Plurispores represented female gametes.

Development from male gametes was similar to those from female gametes.

Development of unispores from parthenogenetic microthallus

A culture strain, culture conditions and progeny of unispores are shown in Table 37. Unispores germinated to prostrate systems which issued macrothalli under conditions except 20°C SD and 20°C LD (Figs 89G,H). The macrothalli produced plurilocular sporangia which were frequently massive (Figs 89I,J). Ascocysts were associated with the plurilocular sporangia (Figs 89K,L).

Culture from microthalli in fields

Unilocular sporangia were isolated from microthalli collected from Muroran. Culture strains, culture conditions and progeny of unilocular sporangia are shown in Table 38. Culture studies on strain 262 Muroran are mentioned below.

Unispores from isolated unilocular sporangia were cultured under a 5°C SD condition. They germinated into branched filaments, and became small disks which issued macrothalli abundantly (Figs 90A-D). Macrothalli were flat, hollow, sometimes twisted (Fig. 90E). After 3 months, they reached up to 10 cm in length, 1 mm in width, and formed firmly packed plurilocular sporangia (Fig. 90F). Plurispores were gametes, and they fused to form zygotes. Female and male thalli were obtained in culture of a single unilocular sporangium. Gametes released from these thalli also fused with gametes in culture established from field macrothalli of *S. tenellus*, between reciprocal female and male gametes.

Discussion

Scytosiphon tenellus is distinct from other members of *Scytosiphon* by the following characteristics: the flat and thin thallus; the firmly packed plurilocular sporangia covered with a cuticle layer; the presence of ascocysts; the *Stragularia*-like microthallus.

Scytosiphon lomentaria (Lyngbye) Link represents with thicker thalli, its plurilocular sporangia are loosely coherent to each other without a cuticular covering on sori. Further, microthalli of *S. lomentaria* represent *Microspongium*-like thalli. Therefore, *S. lomentaria* is clearly distinguished from *S. tenellus*. Although *S. complanatus* (Rosenvinge) Doty, *S. dotyi* Wynne and *S. lomentaria* f. *complanatus major* Setchell et Gardner have complanate thalli, they lack ascocysts among plurilocular sporangia. Therefore, these taxa are entirely different from *S. tenellus*.

Nakamura and Tatewaki (1975) reported a life history of *Petalonia zosterifolia* in culture from Muroran. However, their materials has ascocysts among plurilocular sporangia, which is not a feature of *P. zosterifolia*. In their culture, microthalli were discoid, while *P. zosterifolia* represents filamentous microthalli. In observations of their voucher specimens used for cultures, their materials are hollow. These characteristics differ from those of *P. zosterifolia*, and they agree with those of *S. tenellus*. Therefore, it is concluded that *P. zosterifolia* sensu Nakamura and Tatewaki (1975) is conspecific with *S. tenellus*.

In the culture of field microthalli, macrothalli identical with *S. tenellus* were obtained. Their plurispores are gametes,

showing fusion between female and male gametes. These gametes reciprocally fused with those of *S. tenellus*. These results prove that the field microthalli cultured are those of *S. tenellus*. The result, that female and male thalli were obtained in culture from one unilocular sporangium, suggests that the field microthalli are sporophytes, and that reduction division may occur in the unilocular sporangium.

Field microthalli of *S. tenellus* are very similar to *Stragularia clavata* (Harv. in Hook) Hamel (Fletcher 1987, including *Ralfsia bornetii* and *R. tenuis*). It is reported that *S. clavata* represents a phase in life history of *Petalonia fascia* (Edelstein *et al.* 1970 as *Ralfsia*, Rhodes and Connell 1973 as *Ralfsia*). Also in this study I found that the field microthalli of *S. tenellus* and *P. fascia* quite resemble each other and it is difficult to distinguish them.

Australian complanate *Scytosiphon* examined by Clayton (1980) has packed plurilocular sporangia covered with a cuticle, ascocysts among them and discoid microthalli. These characteristics agree with those of *S. tenellus*. However, the complanate *Scytosiphon* represents anisogamy in its sexual reproduction with female gamete of 5-6 µm and male gametes of 4-5 µm in length (Clayton 1980). Therefore, Australian complanate *Scytosiphon* is distinguished from isogamous *S. tenellus*.

In the present culture, germlings of gametes developed to disks or tufts consisting of branched filaments. When the free branched filaments attached to the substrata (coverslips or bottoms of glass vessels), they began to form disks there. From this result, the free branched filaments are considered to be abnormal growths which failed to form disks.

Table 36. Collection data and cultured strains of *Scytosiphon tenellus*. Culture conditions tested and progeny of plurispores from macrothalli are also shown. Tufts always appeared with disks.

strain no.	locality	date	sex	culture conditions	progeny
3	Shikabe	4 iii 1987		5SL,10S,15S	disks, macrothalli (P)
				10L	disks, a few macrothalli (P)
27	Muroran	18 iv 1987	female	10L, 15L	disks, a few macrothalli
				15L→10S	disks (U), macrothalli
89	Muroran	28 i 1988		10L, 20L	disks
91	Muroran	5 ii 1988	female	10L	disks, a few macrothalli (P)
92	Muroran	5 ii 1988	male	10L	disks, a few macrothalli (P)
99	Muroran	21 ii 1988	female	10L	disks, a few macrothalli (P)
100	Muroran	21 ii 1988	male	10L	disks, a few macrothalli (P)
127	Esan	17 v 1988		10L, 20L	disks
				10S	disks, macrothalli
394	Muroran	22 i 1989	female	5-15SL	disks, a few macrothalli (P)
				20SL	disks
395	Muroran	22 i 1989	male	5-10SL, 15S	disks, a few macrothalli (P)
				15L, 20SL	disks
207	Samani	11 ii 1989		5S	disks
209	Hiroo	12 ii 1989		5S	disks, macrothalli (P)
214	Nosappu	11 iii 1989		5S	disks, a few macrothalli (P)

Table 37. *Culture of Scytosiphon tenellus.* Progeny of unispores from parthenogenetic microthalli. Tufts always appear with disks.

strain no.	locality	date	sex	culture conditions	progeny
27	Muroran	18 iv 1987	female	5SL,10SL,15L 15S 20SL	small disks sprouting macrothalli (P) disks, a few macrothalli disks

Table 38. Culture of *Scytosiphon tenellus.* Progeny of unispores from field microthalli.

strain no.	locality	date	culture conditions	progeny
194	Muroran	12 xii 1988	5SL	macrothalli (P)
195	Muroran	12 xii 1988	5S	small disks sprouting macrothalli (P)
262	Muroran	12 xii 1989	5S	macrothalli (P: female, male)

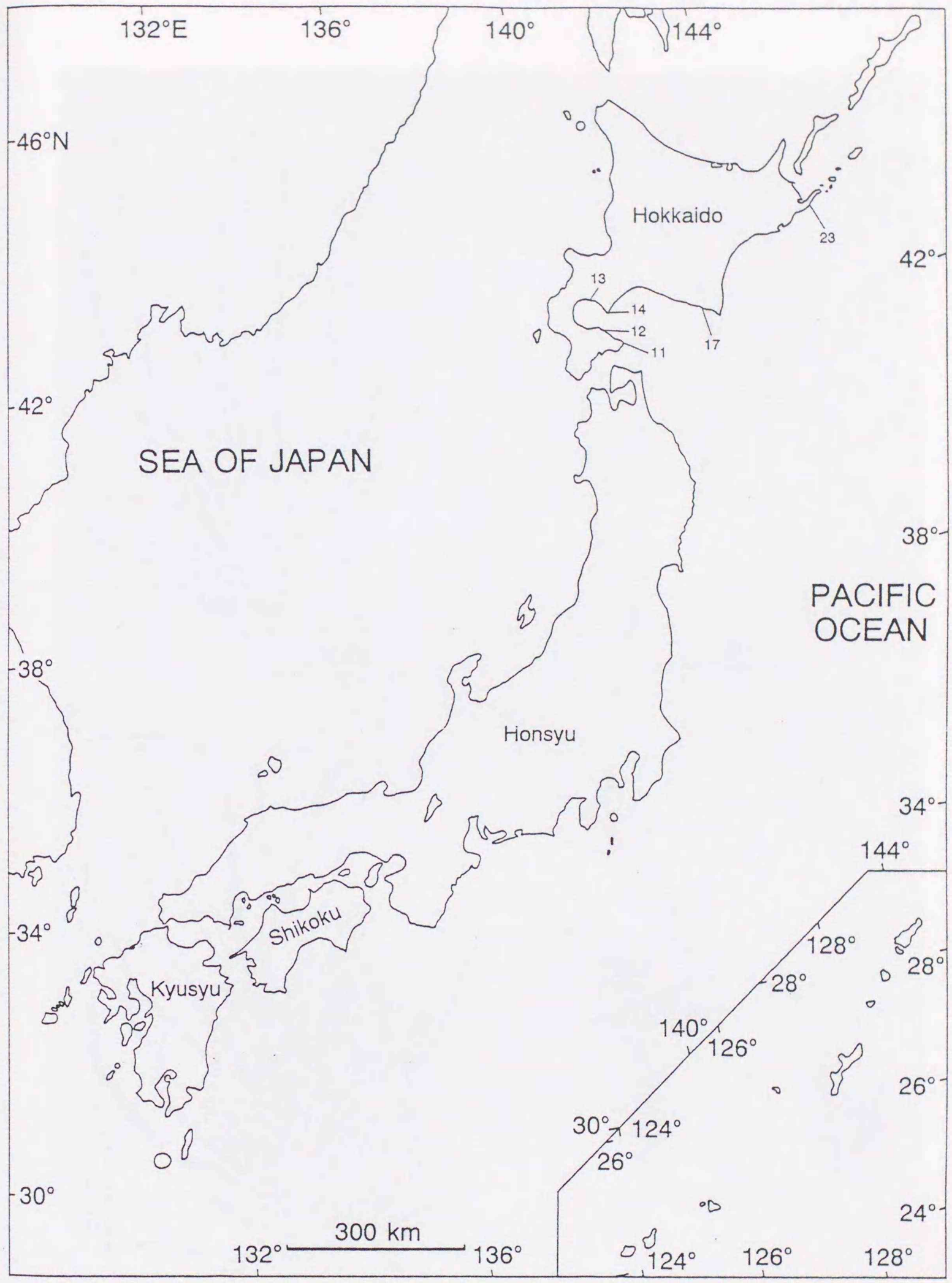


Fig. 81. Localities where materials of *Scytosiphon tenellus* were collected. Numbers plotted correspond to those in Table 1.

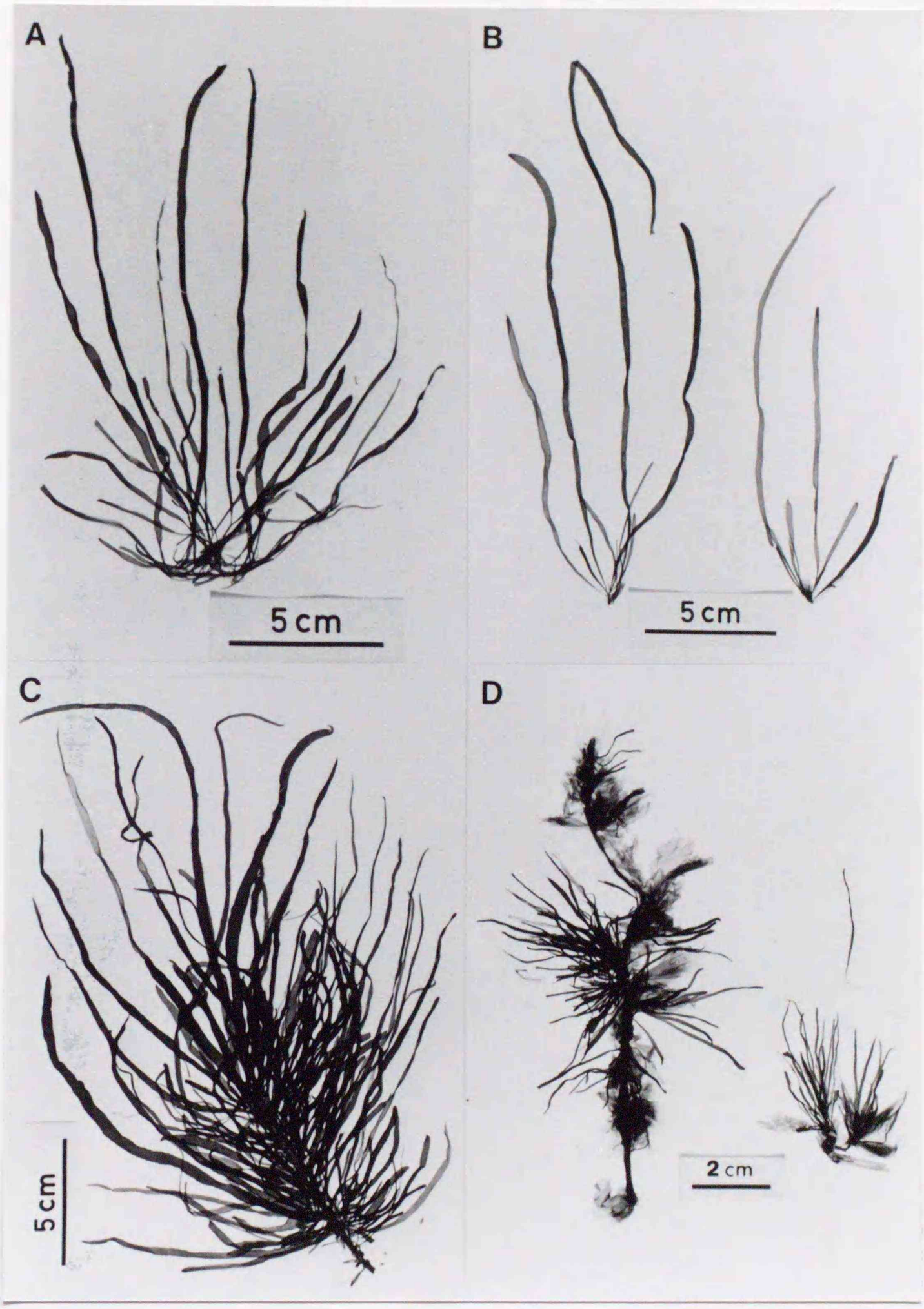


Fig. 82. Habit of specimens of *Scytosiphon tenellus*.

A. Holotype specimen (SAP 059746). Plant collected at Muroran, Hokkaido on 21 February 1988. B, C. Isotype specimens (SAP 059745, SAP 059744, respectively). D. Plants collected at Shikabe, Hokkaido on 12 April 1986.

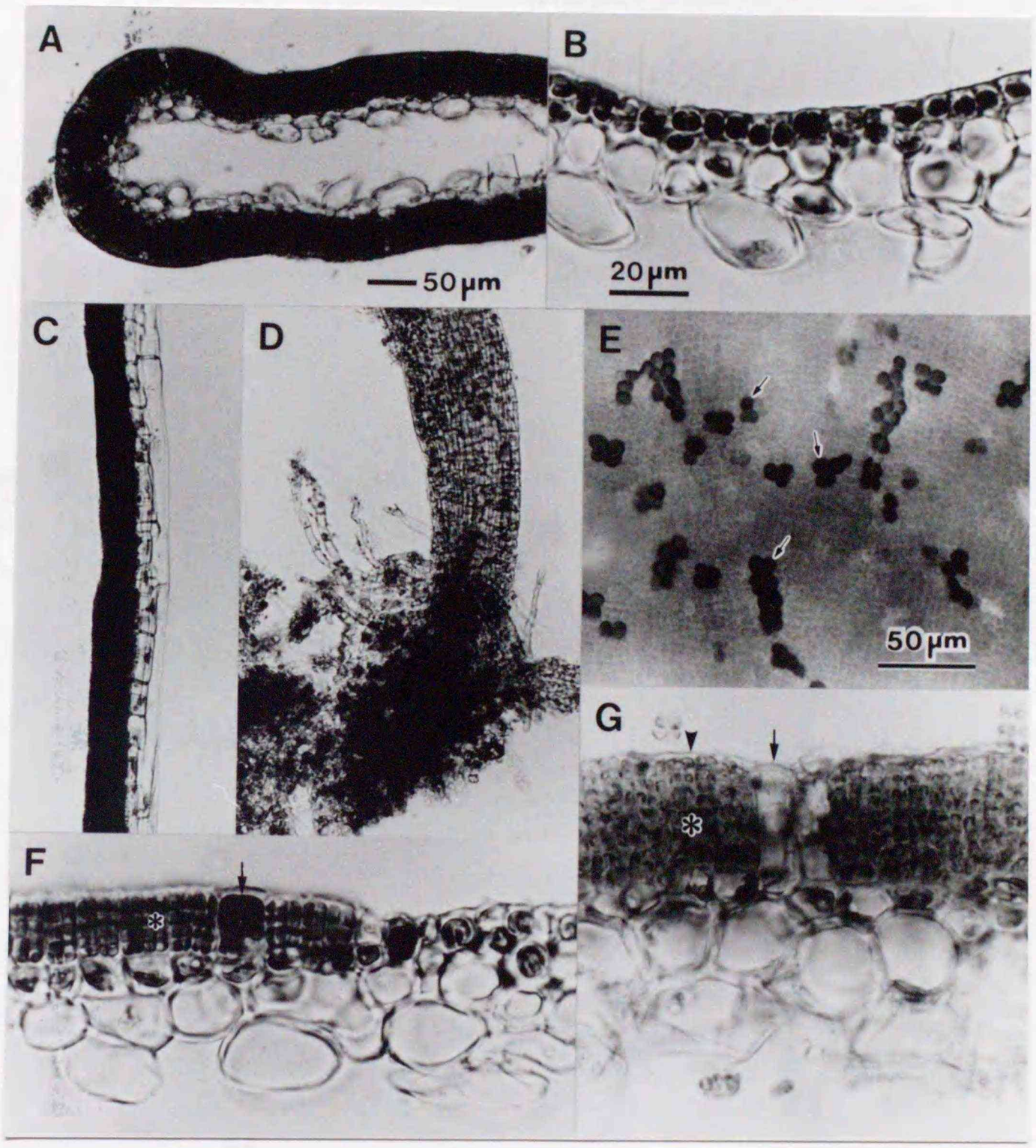


Fig. 83. Anatomy of *Scytosiphon tenellus*.

A. Cross section of fertile thallus. **B.** Cross section of vegetative thallus. **C.** Longitudinal section of fertile thallus **D.** Basal portion of thallus, showing rhizoids. **E.** Surface view of fertile thallus (arrows: ascocysts). **F, G.** Plurilocular sporangia (asterisk) and ascocysts (arrow) in longitudinal sections of thalli.

Scale in **A** applies to **C** and **D**; Scale in **B** applies to **F** and **G**.

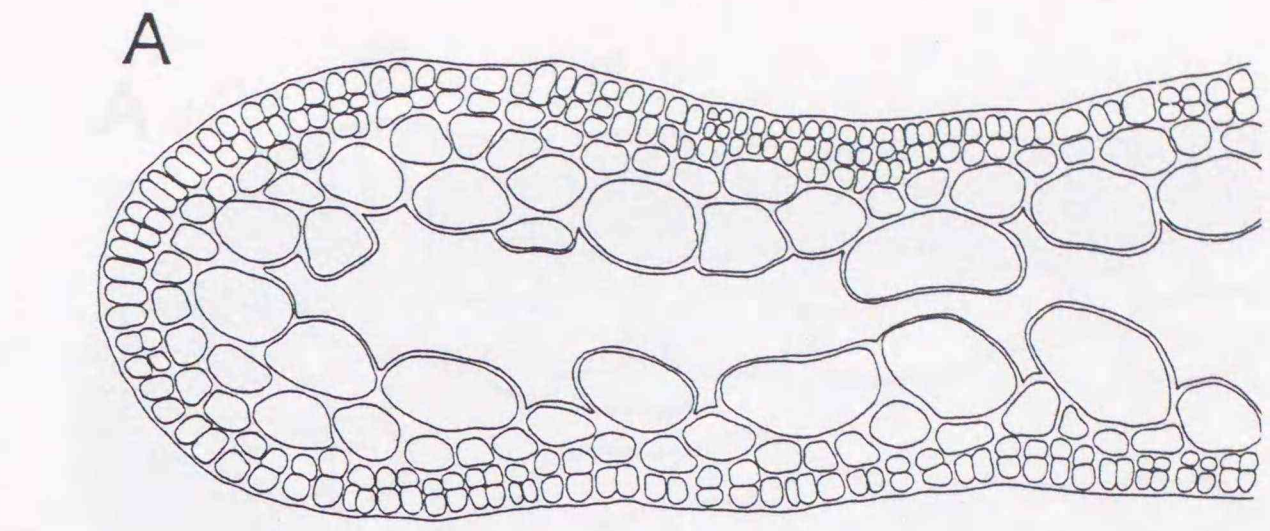


Fig. 84. Anatomy of *Scytosiphon tenellus*.

A. Cross section of vegetative thallus. B, C. Cross sections of thalli, showing young plurilocular sporangia (pl) and young paraphyses (pa).

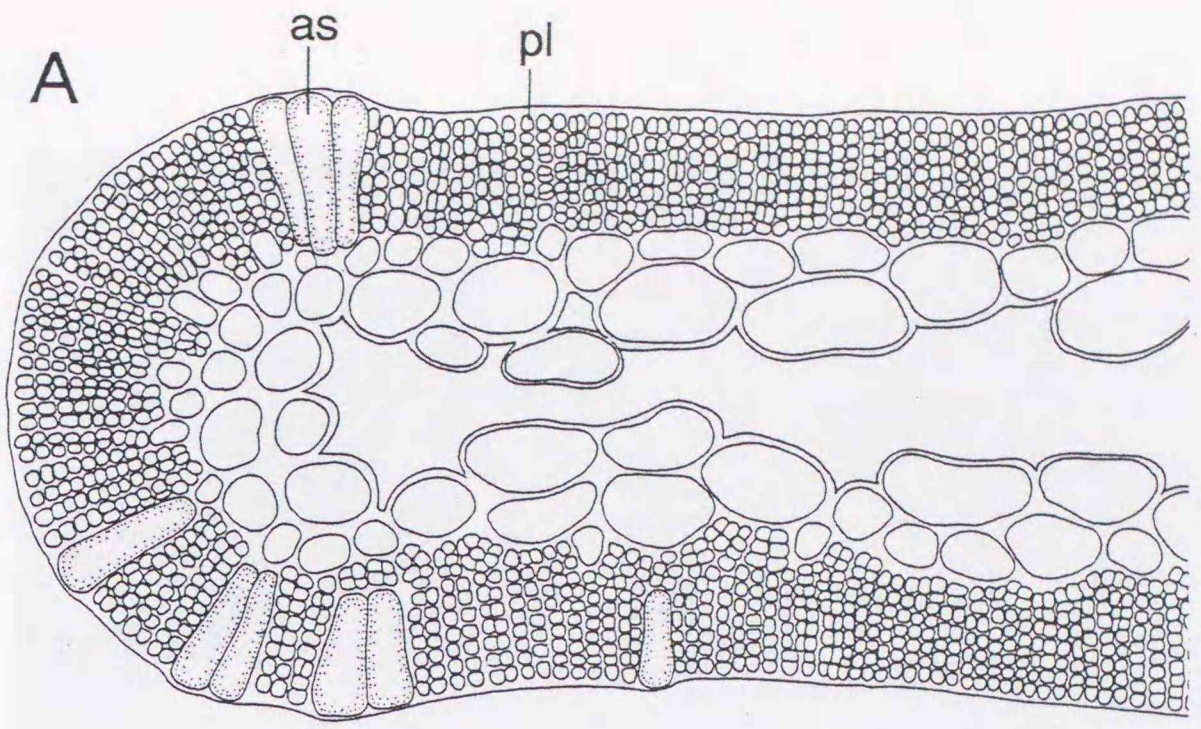


Fig. 85. Anatomy of *Scytosiphon tenellus*.

A, B. Cross section of fertile thalli with mature plurilocular sporangia (pl) and ascocysts (as).

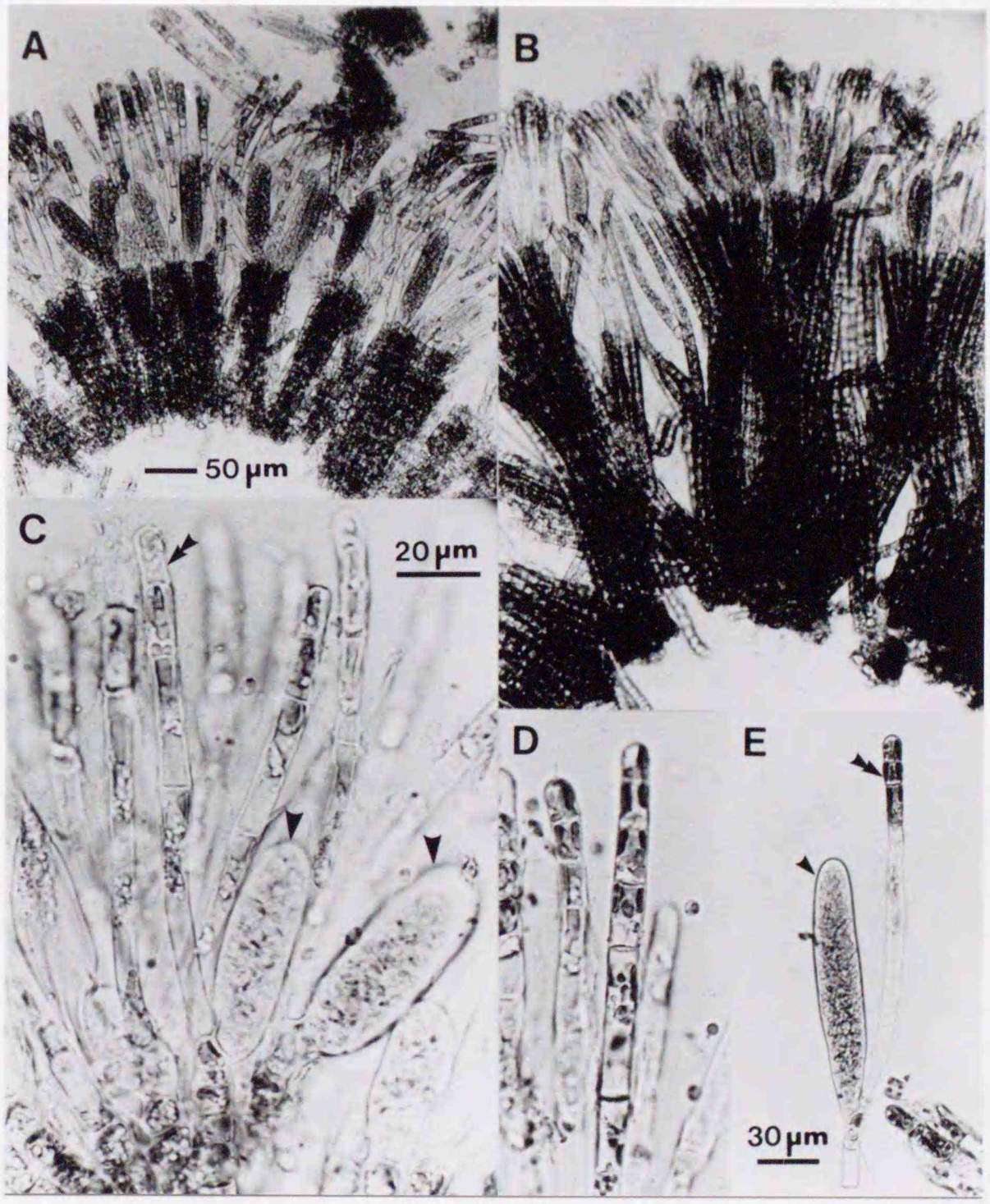


Fig. 86. *Scytosiphon tenellus*. Anatomy of field microthalli.

A. Squashed thallus collected at Muroran, Hokkaido on 12 December 1988. **B.** Squashed thallus collected at Muroran, Hokkaido on 12 December 1989. **C.** Unilocular sporangia (arrowheads) and paraphyses (double arrowhead). **D.** Paraphyses, showing a plastid with a pyrenoid per cell. **E.** Sessile unilocular sporangium (arrowhead) on a supporting cell of a paraphysis (double arrowhead).

Scale in **A** applies to **B**; Scale in **C** applies to **D**.

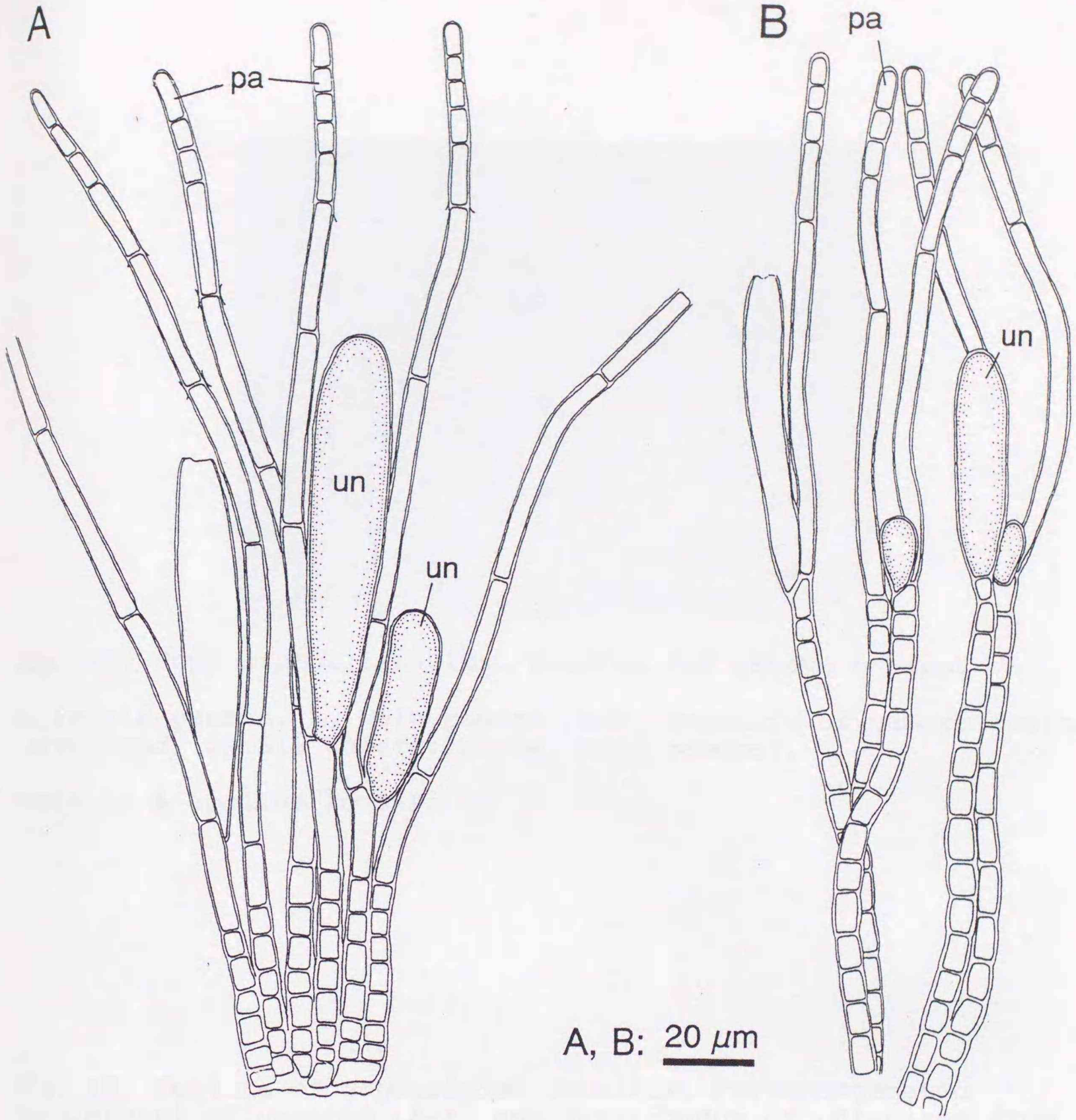


Fig. 87. *Scytosiphon tenellus*. Anatomy of field microthalli.

A, B. Squashed fertile thalli with unilocular sporangia (un) and paraphyses (pa).

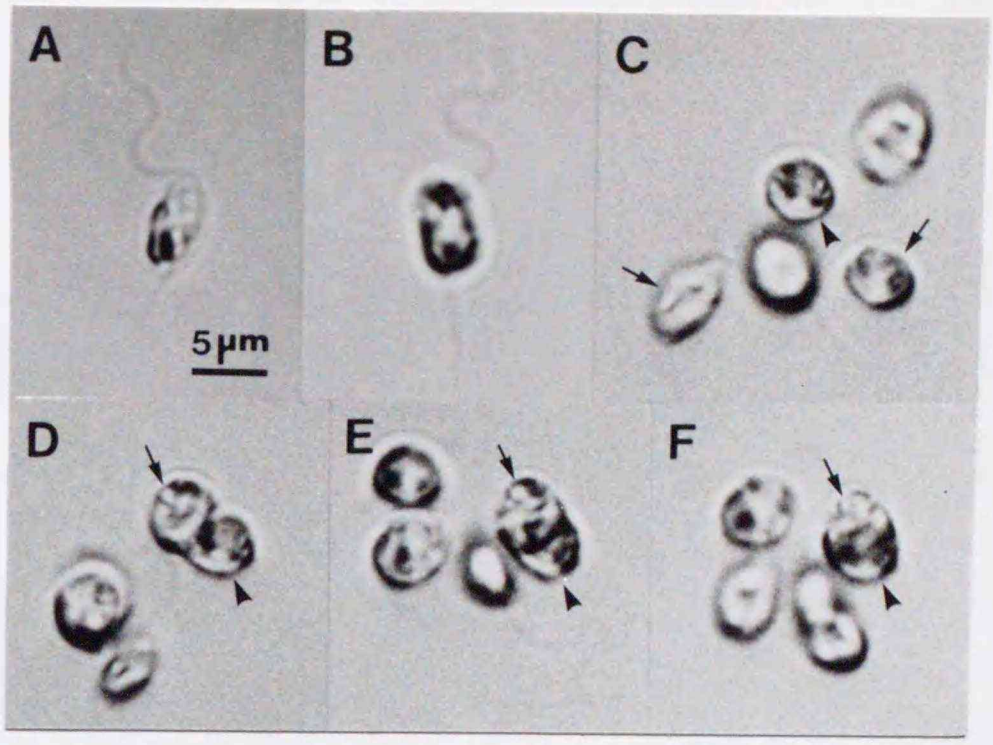


Fig. 88. *Scytosiphon tenellus*. Gametes and gamete fusion.

A. Female gamete. **B**. Male gamete. **C-F**. Sequence of gamete fusion (arrowhead, female gamete; arrow, male gamete).

Scale in **A** applies to **B-F**.

⇒

Fig. 89. Culture of *Scytosiphon tenellus*. Parthenogenetic development of gametes (**A-F**) and development of unispores from microthallus (**G-L**).

A, B. Sixteen-day-old germlings under 10°C LD condition. **C, D**. Five-week-old discoid microthalli under 10°C LD condition. **E**. Squashed microthallus under 10°C LD condition. **F**. Squashed microthallus forming unilocular sporangia (arrowheads) and paraphyses (arrows) under 10°C SD condition. **G**. Twenty four-day-old germling under 10°C SD condition. **H**. Macrothallus (arrowhead) arising from prostrate germling under 10°C SD condition. **I**. Macrothallus forming massive plurilocular sporangia (arrowhead). **J**. Cross section of macrothallus with plurilocular sporangia. **K**. Plurilocular sporangia and ascocysts (arrow) in cross section of macrothallus. **L**. Ascocysts (arrow) after release of plurispores in cross section of macrothallus.

Scale in **E** applies to **F**, **H** and **L**; Scale in **G** applies to **I**.

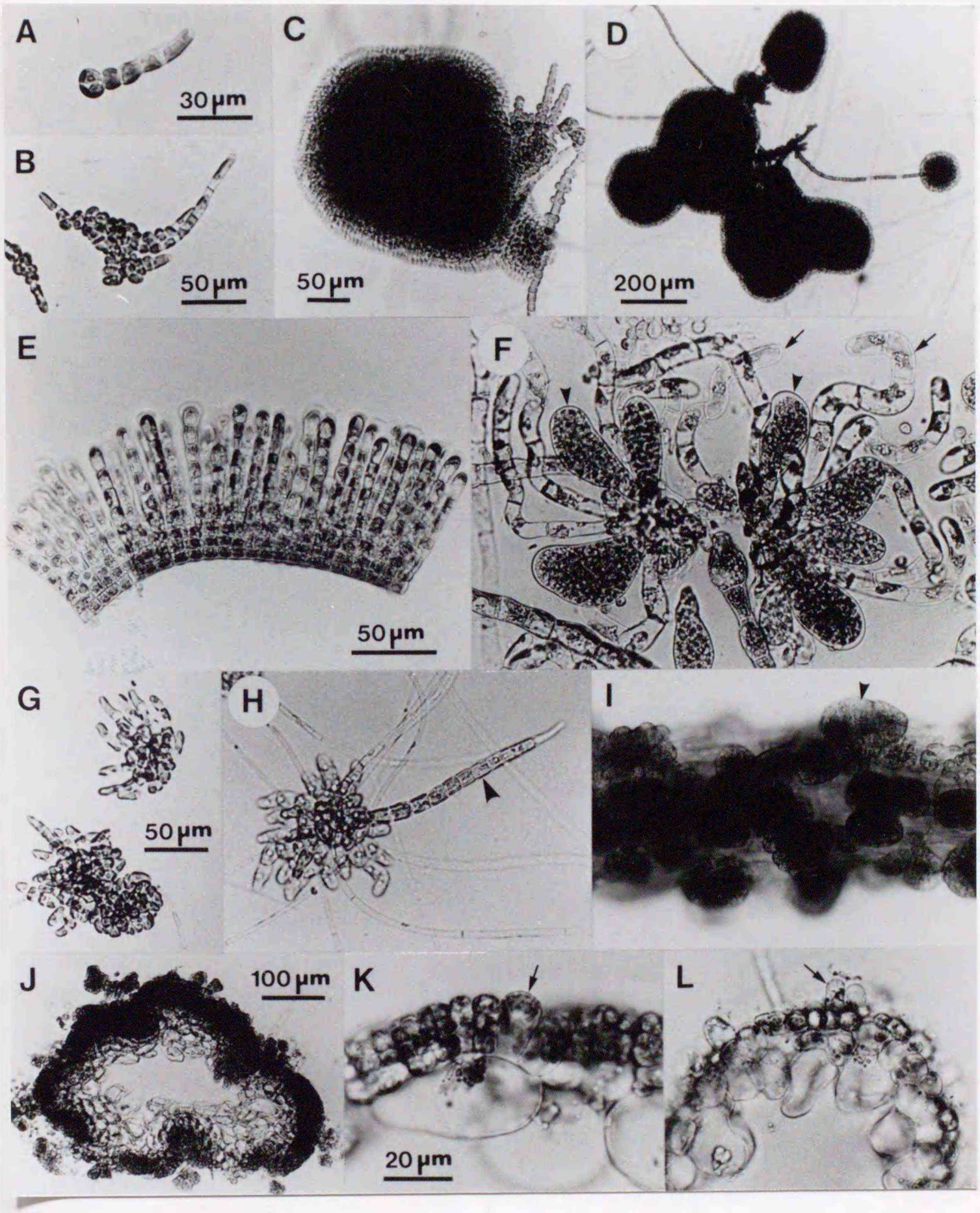


Fig. 89. Culture of *Scytosiphon tenellus*.

⇐

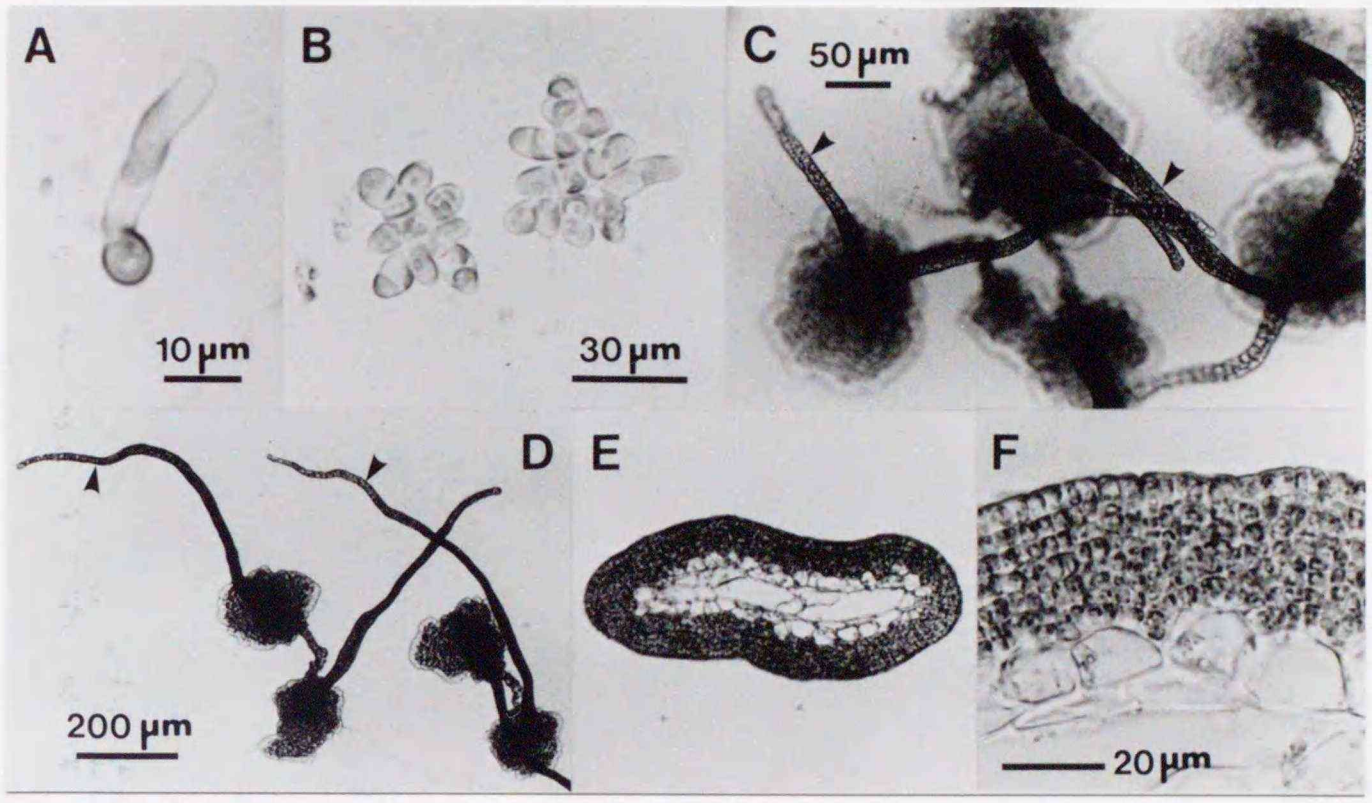


Fig. 90. *Scytosiphon tenellus*. Development of unispores from field microthallus.

A. Twelve-day-old germling under 5°C SD condition. **B.** One-month-old germling under 5°C SD condition. **C, D.** One-month-old germling under 10°C LD condition, showing macrothalli (arrowheads) arising from small disks. **E.** Cross section of macrothallus with plurilocular sporangia. **F.** Plurilocular sporangia in cross section of macrothallus.

Scale in **C** applies to **E.**

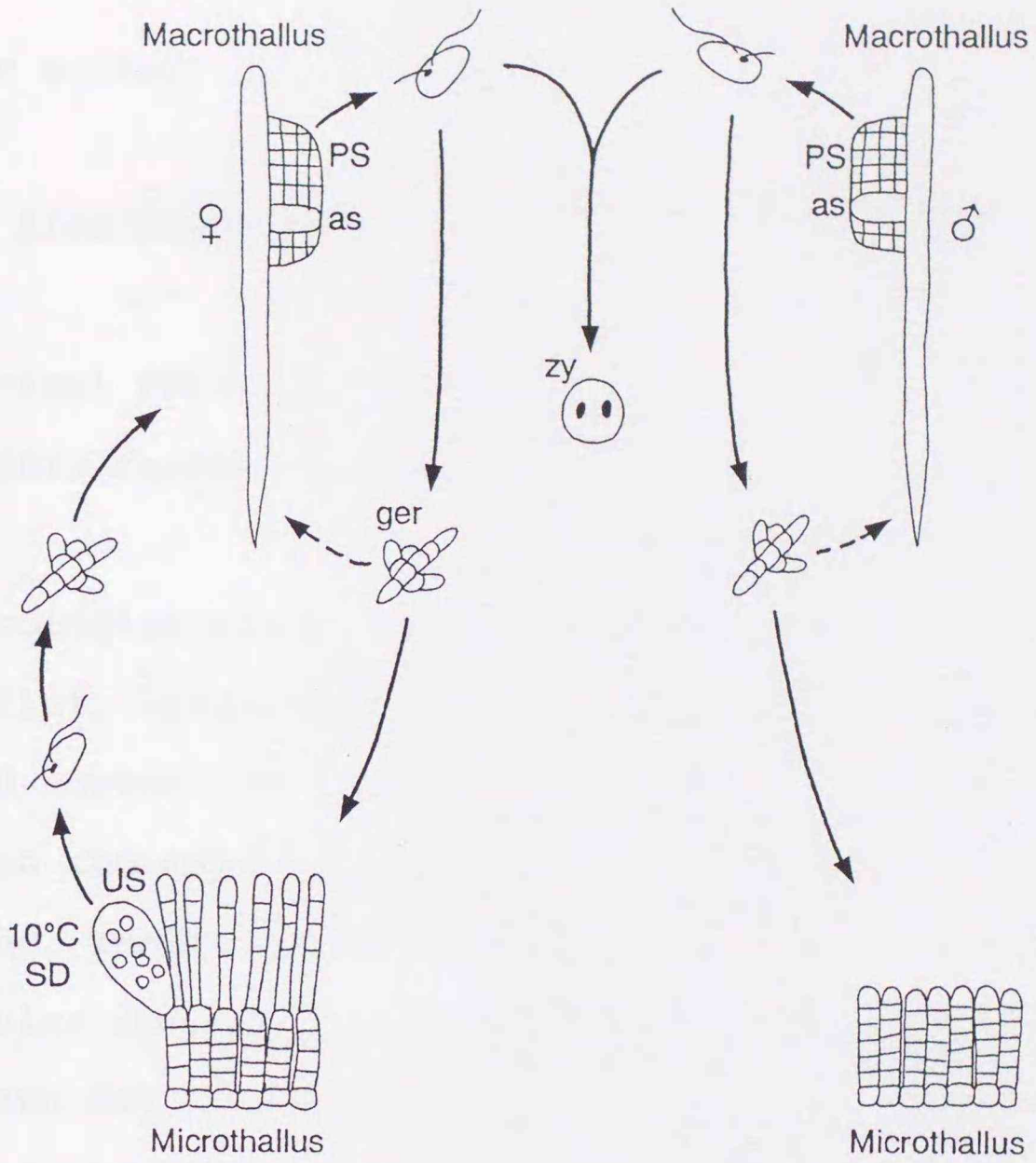


Fig. 91. Diagram of life history of *Scytosiphon tenellus* in culture.

as, ascocyst; ger, germling; PS, plurilocular sporangium; US, unilocular sporangium; zy, zygote; SD, short day condition; broken line, rare course.

Petalonia **Derbès et Solier, nom. cons.**

Derbès et Solier, 1850, p.265.

Synonym: *Ilea* Fries, 1835, p.321; *Phyllitis* Kützing, 1843, p.342.

Type species: *Petalonia debilis* (C. Agardh) Derbès et Solier
[= *Petalonia fascia* (O.F. Müller) O. Kuntze]

Macrothalli are linear or lanceolate, tapering to the base, and are flat, solid, simple, gregarious. Thalli consist of a pigmented cortex and a colorless medulla. Rhizoidal filaments are present in the medulla according to species. Hairs are scattered or grouped. Thalli attach to substratum with rhizoids. Plurilocular sporangia are formed basipetally on the surface of thalli, and are firmly packed with a cuticle covering them. Ascocyst is usually absent.

Microthalli are in the type of *Compsonema saxicolum* or *Stragularia* according to species.

Silva (1952) reviewed the nomenclatural history of this genus. Three species, which are *P. fascia* (O. F. Müller) O. Kuntze, *P. zosterifolia* (Reinke) O. Kuntze and *P. filiformis* (Batters) O. Kuntze, are generally recognized. In this study, a close relationship between *P. zosterifolia* and *P. filiformis* is pointed out. The placement of *Endarachne* under a synonymy of *Petalonia* is supported in accordance with an opinion of Vinogradova (1973).

Petalonia zosterifolia (Reinke) O. Kuntze

O. Kuntze, 1891-98, p.419; Newton 1931, p.176; Rosenvinge and Lund 1947, pp.34-37, Fig.11; Clayton 1981, pp.67-75, Fig.7 (section of type); Fletcher 1987, pp.236-239, Figs 64,65, Pl.10c; Kogame and Kawai 1993, pp. 29-37, figs 2-29.

Basionym: *Phyllitis zosterifolia* Reinke, 1889, p.61.

Synonym: *Ilea fascia* f. *zosterifolia* (Reinke) Setchell et Gardner, 1924, p.13; *Petalonia fascia* var. *zosterifolia* (Reinke) Taylor, 1957, p.167-168.

Type: K

Type locality: Moltenort, Kiel, Germany.

Japanese name: Hosoba-seiyohabanori

Materials

Localities of materials are shown in Fig. 92. The numbers in square brackets after following each locality correspond to those in Fig. 92.

Macrothalli: Hokkaido: Oshoro [1] (11-iii-1987 leg. K. Kogame, 8-v-1987 leg. K. Kogame, 7-xi-1987 leg. K. Kogame, 9-xii-1987 leg. K. Kogame, 21-i-1988 leg. K. Kogame, 17-ii-1988 leg. K. Kogame, 18-iii-1988 leg. K. Kogame, 14-v-1988 leg. K. Kogame, 19-xi-1988 leg. S. Ueda, 22-xi-1988 leg. K. Kogame, 26-xi-1989 leg. T. Kitayama, 2-xii-1989 leg. K. Kogame, 28-ii-1990 leg. K. Kogame, 15-ii-1991 leg. K. Kogame, 26-iv-1992 leg. K. Kogame, 11-ii-1993 leg. K. Kogame), Iwanai [2] (15-v-1988 leg. K. Kogame, 1-v-1989 leg. K. Kogame), Esashi [5] (15-v-1988 leg. K. Kogame), Setana [3] (18-iii-1987 leg. K. Kogame), Shirakamimisaki [8] (19-iii-1987 leg. K. Kogame), Muroran [14] (5-iv-1988 leg. K. Kogame, 22-i-1989 leg. K. Kogame), Samani [17] (11-ii-1989 leg. K. Kogame), Hiroo [21] (12-ii-1989 leg. K. Kogame), Akkeshi [22] (30-vi-1988 leg. K. Kogame, 14-vii-1988 leg. H. Kawai, 18-vi-1992 leg. K. Kogame), Hanasaki [23] (7-ix-1987 leg. K. Kogame), Seseki [27] (1-vii-1988 leg. K. Kogame), Ofuyu [37] (2-iii-1988 leg. K.

Kogame), Niigata Pref. : Niigata city [43] (15-iv-1987 leg. K. Ikehara), Shiiya [44] (27-iii-1989 leg. K. Kogame), Kashiwazaki [45] (27-iii-1989 leg. K. Kogame).

Microthalli: Hokkaido: Oshoro (23-ix-1989 leg. K. Kogame).

Herbarium specimens examined are as follows: Hokkaido : Setana [3] (SAP 025362, 28-iii-1945 leg. Y. Yamada), Esashi [5] (SAP 042481, 3-iv-1943 leg. M. Kurogi; SAP 025419, 3-iv-1944 leg. Y. Hasegawa), Shirakamimisaki [8] (SAP 042483, 7-iv-1947 leg. M. Kurogi).

Morphology

Macrothalli

Macrothalli are gregarious, linear, flattened, simple, twisted in narrower thalli, 10-20 (up to 30) cm long, (0.5-) 2-5 (-8) mm wide and taper towards the base (Figs 93A-D). The thalli are 60-120 µm in thickness, consisting of cortical cells (1-3 cell layers thick) and colorless medullary cells (Figs 94A, 95A). Each cell of the thallus contains a plastid with a prominent pyrenoid. Medullary cells are rounded or oval and 22-54 × 8-24 µm in size in transverse section and 20-270 µm (or more) in length in longitudinal section (Fig. 94C). The thalli are solid or sometimes partly hollow (Figs 94A,B, 95A,B). Rhizoidal filaments in the medullary layer and phaeophycean hairs are rare. The basal holdfast is composed of rhizoidal filaments (Fig. 94E), and never forms an expanded mat.

Plurilocular sporangia are produced on the surface of the thalli, sometimes in sori which are often longitudinally elongated. Maturation of the thallus occurs basipetally. Plurilocular sporangia are closely packed, covered with a cuticle and 14-40 µm (6-12 loculi) in height (Figs 94D, 95B). Ascocysts were not observed among plurilocular sporangia.

Microthalli

Microthalli consist of branched prostrate filaments and simple, uniseriate erect filaments, forming small tufts of less than 1 mm in diameter and up to 240 µm in height (figs 96A,C,D, 97A,B). Cells of erect filaments measure 6-14 µm in length and 7-12 µm in width. Cells of prostrate filaments measure 8-18 µm in length and 6-17 µm in width; they often form secondary longitudinal walls. Each cell contains a plastid with a prominent pyrenoid (Fig. 96B). Unilocular sporangia are formed on prostrate filaments with pedicel or sometimes terminally on erect filaments (figs 96A,C, 97A,B). They are obovoid, 30-50 µm in length and 15-25 µm in width. No plurilocular sporangia were observed.

Habitat and Phenology

Macrothalli grow in tufts on rocks, boulders, concrete blocks and on shells usually in the upper intertidal zone of exposed areas. Macrothalli appear in winter and disappear in spring at Oshoro (Table 39). Macrothalli were collected during winter and spring also at other localities. They were observed in summer at Akkeshi and Hanasaki. Fertile plants were found in all collections.

Microthalli are found on boulders in lower intertidal zone. In respect to phenology on microthalli, observations are insufficient to mention.

Geographical distribution

This species is reported from Europe (Fletcher 1987), east coast of north America (Taylor 1957), west coast of north America (Setchell and Gardner 1925), Pacific coast of Russia (Vinogradova 1973), and Japan. This species is not known in Indian Ocean and

southern Hemisphere. In Japan, this species is distributed in northern coasts of Japan Sea and in the Pacific coasts of Hokkaido.

Culture study

Culture strains isolated from macrothalli are listed in Table 40 in which culture conditions and progeny of plurispores are also shown.

Development of plurispores from macrothalli

Plurispores released from plurilocular sporangia were pear drop-shaped, 7-8 µm × 4-5 µm in size, laterally biflagellated with a longer anterior and a shorter posterior flagellum (Fig. 98A). They contained a plastid with a pyrenoid and a stigma. Sexual reproduction and fragrant odors of sexual pheromones were not observed. The plurispores showed negative phototaxis, and then settled on the substratum. Settled plurispores germinated in 1-5 days without leaving an emptied "embryospore" wall. The germlings developed into microthalli of branched filaments from which uniseriate upright filaments arose (Figs 98B-D,J). The cells of prostrate filaments also formed secondary longitudinal walls and then sometimes became knotted. The microthalli grew larger in higher temperature conditions. They remained filamentous, and never formed compact disks.

Within a month, erect filaments with a hair grew from the microthalli, developing into polystichous macrothalli (Figs 97E,F). The macrothalli were flattened and solid and often became twisted (Fig. 97G). The macrothalli developed in all conditions examined, but they were more abundant under short day conditions

and grew larger under lower temperature conditions. They attained 2 mm in width and 10 cm or more in length under a 5°C SD condition, but were only 0.2 mm in width and 1 cm in length under a 20°C LD condition. The macrothalli formed closely packed plurilocular sporangia covered with a cuticle (Fig. 98H). The sporangial walls were visible after plurispore release (Fig. 98I).

Microthalli formed unilocular sporangia only in strains no. 9, 64, 73, 74, 82 (collected from Oshoro) (Fig. 98J). They were formed under all culture conditions tested except 5°C in strain no. 73, under long day conditions at 10°C, 15°C, 20°C in strain no. 9, and under a 20°C LD condition in strain no. 64, 74 and 82. Unilocular sporangia were ovoid or ellipsoid, 60-70 µm × 30-40 µm in size, sessile or pedicellate on prostrate filaments.

Strain no. 9 often formed ovoid sessile unilocular sporangia on the macrothalli together with plurilocular sporangia under a 20°C LD condition (Fig. 98K).

Development of unispores from microthalli

Culture strain, culture conditions and progeny of unispores are shown in Table 41. Unispores released from unilocular sporangia measured 7-8 µm × 4-5 µm in size (Fig. 98L). They developed to filamentous microthalli that formed macrothalli in the same manner as plurispores from plurilocular sporangia.

Culture of field microthalli

Culture strain, culture conditions and progeny are shown in Table 42. In culture, microthalli are filamentous or tufted, consisting of branched filaments with knots and uniseriate

upright filaments (Figs 99A,B). Microthalli formed sessile or pedicellate unilocular sporangia on the prostrate filaments under 10°C LD, 15°C and 20°C conditions. Unispores (Fig. 99C) released from them developed into microthalli. The microthalli also formed macrothalli under all conditions examined. The macrothalli were flat or often twisted, similar to *Petalonia zosterifolia* in culture (Figs 99D,E). They formed densely packed plurilocular sporangia covered with a cuticle (Figs 99E,F). Plurispores (fig. 99G) released from them developed into microthalli forming unilocular sporangia and macrothalli.

Discussion

The morphology of plants collected from various localities in Japan agreed well with the original description of *Petalonia zosterifolia* (Reinke 1889) and other previous reports of the Atlantic entity (Rosenvinge & Lund 1947, Clayton 1981, Fletcher 1987). The occurrence of filamentous microthalli in Japanese plants also agrees with the results of the previous culture studies on the species (Fletcher 1974). *Petalonia zosterifolia* is taxonomically very close to *Petalonia filiformis* (Batters 1888, Kuckuck 1897). Newton (1931) and Fletcher (1987) distinguished *P. zosterifolia* from *P. filiformis* by the absence of fibrous rhizoidal mats at the bases, and in having broader thalli and different distributions, although these characteristics are not very clear. Therefore, considering that both of the species have filamentous microthalli (Fletcher 1974, 1981), there remains a possibility that *P. zosterifolia* is conspecific with *P. filiformis*, or differs only at a lower taxonomic rank (e.g. variety or form). However, until their taxonomic relations become

clearer in Atlantic materials, it is better for Japanese plants to be tentatively classified as *P. zosterifolia*.

In the present culture of *P. zosterifolia*, microthalli remained filamentous, and never formed *Ralfsia*-like crusts resembling that of *Scytosiphon lomentaria* and *Petalonia fascia* (Wynne 1969, Nakamura and Tatewaki 1975). Fletcher (1974) and Boone and Kapraun (1989) also mentioned that *Ralfsia*-like crusts were not observed in culture of *P. zosterifolia*. On the other hand, Dangeard (1963) reported glomerulus, myrionematoid and ralfsioid microthalli. However, his results can not be generalized because a part of his culture bearing ectocarpoid plurilocular sporangia on the microthalli contained more than one plastid in each vegetative cell (Wynne 1969).

The morphology of microthalli (sporophytes) are known to be more or less variable in the Scytosiphonaceae. For example, *Scytosiphon lomentaria* and *Petalonia fascia* occasionally form filamentous sporophytes in culture, although they are normally crustose. The quality of light or concentration of iodine in the culture media are shown to affect on their morphology (Lüning and Dring 1973, Hsiao 1969). However, in *P. zosterifolia*, the morphology of microthalli was uniformly filamentous in various conditions, and it was concluded that the microthalli of this species is basically filamentous.

It was confirmed that the filamentous microthalli of *P. zosterifolia* are identical with *Compsonema saxicolum* encountered in the field, because the field plants of *C. saxicolum* formed *P. zosterifolia*-like macrothalli in culture.

Compsonema saxicolum has been studied by several authors in culture. Fletcher (1987) reported the occurrence of macrothalli

resembling *Petalonia* or *Scytosiphon* in the life history of *C. saxicolum*. He (1981) also mentioned that microthalli of *Petalonia filiformis* in culture appear to show a marked similarity to *C. saxicolum*.

Although there has been a report on the life history of Japanese *P. zosterifolia* (Nakamura and Tatewaki 1975), detailed observations on their voucher material revealed that it was not *P. zosterifolia* but *Scytosiphon tenellus*, having hollow thalli and ascocysts among plurilocular sporangia (see this study on *S. tenellus*). The occurrence of *Ralfsia*-like microthalli in their culture, which is different from filamentous microthalli in our results, also supports this conclusion.

Petalonia zosterifolia is also similar to *Scytosiphon complanatus* (Rosenvinge) Doty in morphology. Pedersen *et al.* (1987) doubted their distinctiveness. However, the macrothalli of *P. zosterifolia* are solid when young, while those of *S. complanatus* are essentially hollow (Rosenvinge and Lund 1947). Furthermore, *S. complanatus* forms crustose microthalli (Pedersen 1980) which strongly contrasts with the filamentous ones in *P. zosterifolia*. Therefore, these two are distinct species.

Fig. 100 summarizes life history of Japanese *Petalonia zosterifolia* shown in the present culture study. This life history pattern agrees in principle with the reports of Fletcher (1974) and Boone & Kapraun (1989) on Atlantic materials. However, in our culture, in addition to the usual plurilocular sporangia, the macrothalli bore unilocular sporangia under 20°C LD conditions. However, they were not found in the field materials and can be artifact of culture experiment.

Culture results of Japanese *P. zosterifolia*, in which

macrothalli appeared under all culture conditions examined, do not explain why macrothalli could not be found during summer and autumn at Oshoro. However, 20°C culture conditions were less suitable for the growth of macrothalli and plurispore release than other conditions. Since *P. zosterifolia* grows in the upper intertidal zone, higher air temperature and strong solar irradiation during summer may cause serious damage to the growth of *P. zosterifolia*. Therefore, the occurrence of *P. zosterifolia* may be suppressed by these factors. This notion is supported by the fact that the species showed longer growth periods at Akkeshi and Hanasaki where the air and water temperatures are much lower than at Oshoro.

Table 39. Collection data of *Petalonia zosterifolia* at Oshoro, Hokkaido. (P, thalli with plurilocular sporangia; --, thallus was not found)

Macrothalli

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
1987				P		P	--		--	--		P	P
1988	P		P	P		P					--	P	
1989				P			--		--	--		P	P
1990			P	--	--			--		--	--	--	
1991			P										
1992				P	P								
1993			P										

Table 40. Collection data and cultured strain of *Petalonia zosterifolia*. Culture conditions tested and progeny of plurispores from macrothalli are also shown. (fil. microthalli = filamentous microthalli)

strain no.	locality	date	culture conditions	progeny
9	Oshoro	11 iii 1987	5-15S, 5L, 10-20L	fil. microthalli sprouting macrothalli (P) fil. microthalli (U) sprouting macrothalli (P) (U; 20L)
13	Shiraitomisaki	18 iii 1987	15S, 20L	fil. microthalli sprouting macrothalli (P)
14	Setana	18 iii 1987	10L, 15L	fil. microthalli sprouting macrothalli (P)
16	Shirakamimisaki	19 iii 1987	10L, 20L	fil. microthalli sprouting macrothalli (P)
49	Hanasaki	7 ix 1987	5L, 10L, 20L	fil. microthalli sprouting macrothalli (P)
64	Oshoro	7 xi 1987	10SL	fil. microthalli sprouting macrothalli (P)
			20L	fil. microthalli (U) sprouting macrothalli (P)
65	Oshoro	7 xi 1987	10SL, 20L	fil. microthalli sprouting macrothalli (P)
73	Oshoro	9 xii 1987	5-15SL, 20S	fil. microthalli sprouting macrothalli (P)
			20L	fil. microthalli (U) sprouting macrothalli (P)
74	Oshoro	9 xii 1987	10L	fil. microthalli sprouting macrothalli (P)
			20L	fil. microthalli (U) sprouting macrothalli (P)
82	Oshoro	21 i 1988	5L, 10L	fil. microthalli sprouting macrothalli (P)
83	Oshoro	21 i 1988	5-15L, 20S	fil. microthalli sprouting macrothalli (P)
			20L	fil. microthalli (U) sprouting macrothalli (P)
115	Ofuyu	2 v 1988	10L, 20L	fil. microthalli sprouting macrothalli (P)
120	Oshoro	14 v 1988	10L, 20L	fil. microthalli sprouting macrothalli (P)
149	Akkeshi	30 vi 1988	10L, 20L	fil. microthalli sprouting macrothalli (P)
151	Shiretoko	1 vii 1988	10L, 20L	fil. microthalli sprouting macrothalli (P)
155	Akkeshi	14 vii 1988	10L, 20L	fil. microthalli sprouting macrothalli (P)
202	Muroran	22 i 1989	5S, 20L	fil. microthalli sprouting macrothalli (P)
205	Samani	11 ii 1989	5S, 20L	fil. microthalli sprouting macrothalli (P)
208	Hiroo	12 ii 1989	5S, 15L	fil. microthalli sprouting macrothalli (P)
215	Shiiya	27 iii 1989	10L, 20L	fil. microthalli sprouting macrothalli (P)

Table 41. Culture of *Petalonia zosterifolia*. Progeny of unispores from microthalli. (fil. microthalli = filamentous microthalli)

strain no.	locality	date	culture conditions	progeny
74	Oshoro	9 xii 1987	5SL	fil. microthalli sprouting macrothalli (P)
			10-20SL	fil. microthalli (U) sprouting macrothalli (P)

Table 42. Culture of *Petalonia zosterifolia*. Progeny of unispores from field microthalli. (fil. microthalli = filamentous microthalli)

strain no.	locality	date	culture conditions	progeny
380	Oshoro	23 ix 1989	5S, 10S	fil. microthalli sprouting macrothalli (P)
			10-20L	fil. microthalli (U) sprouting macrothalli (P)

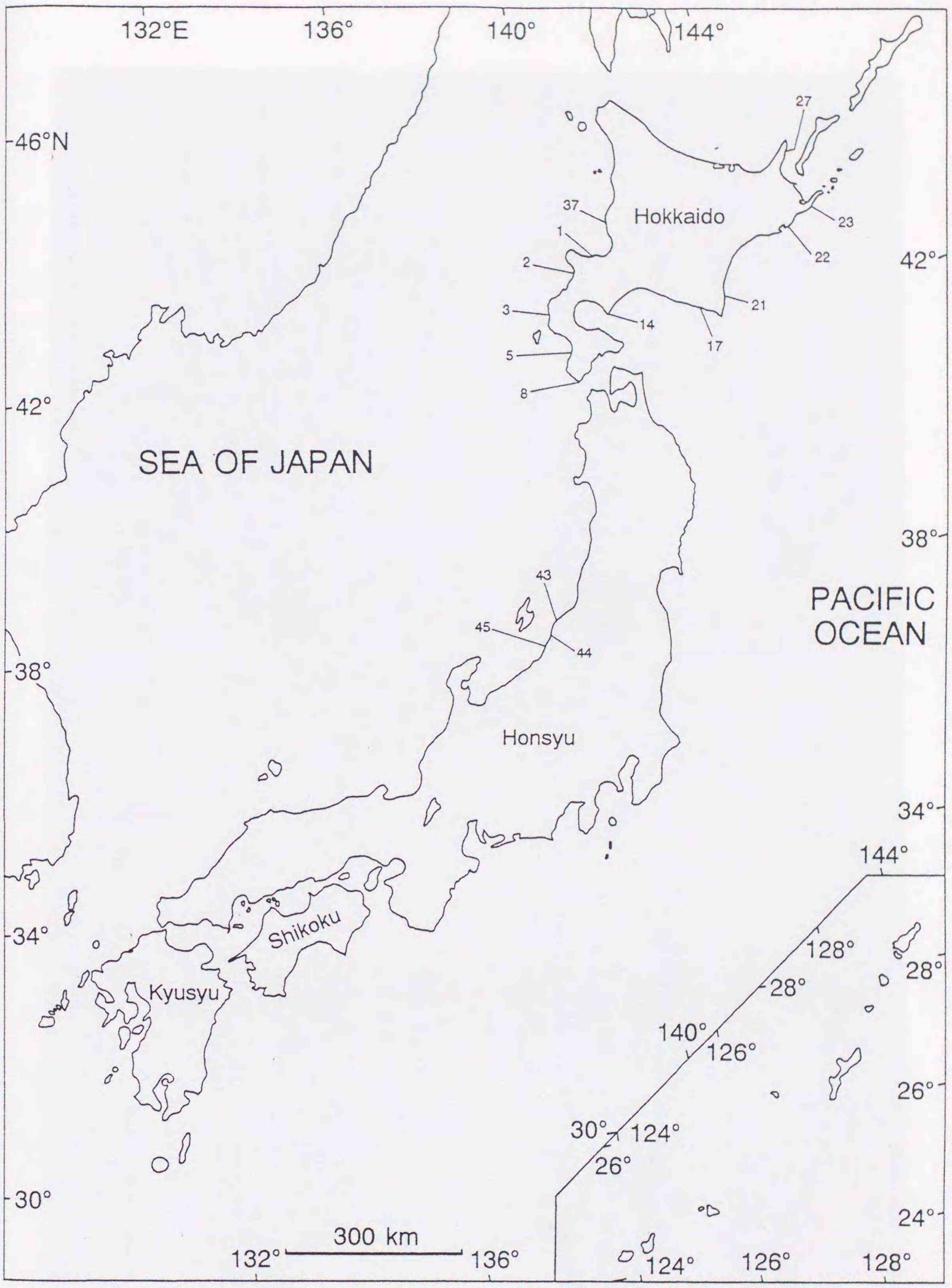


Fig. 92. Localities where materials of *Petalonia zosterifolia* were collected. Numbers plotted correspond to those in Table 1.

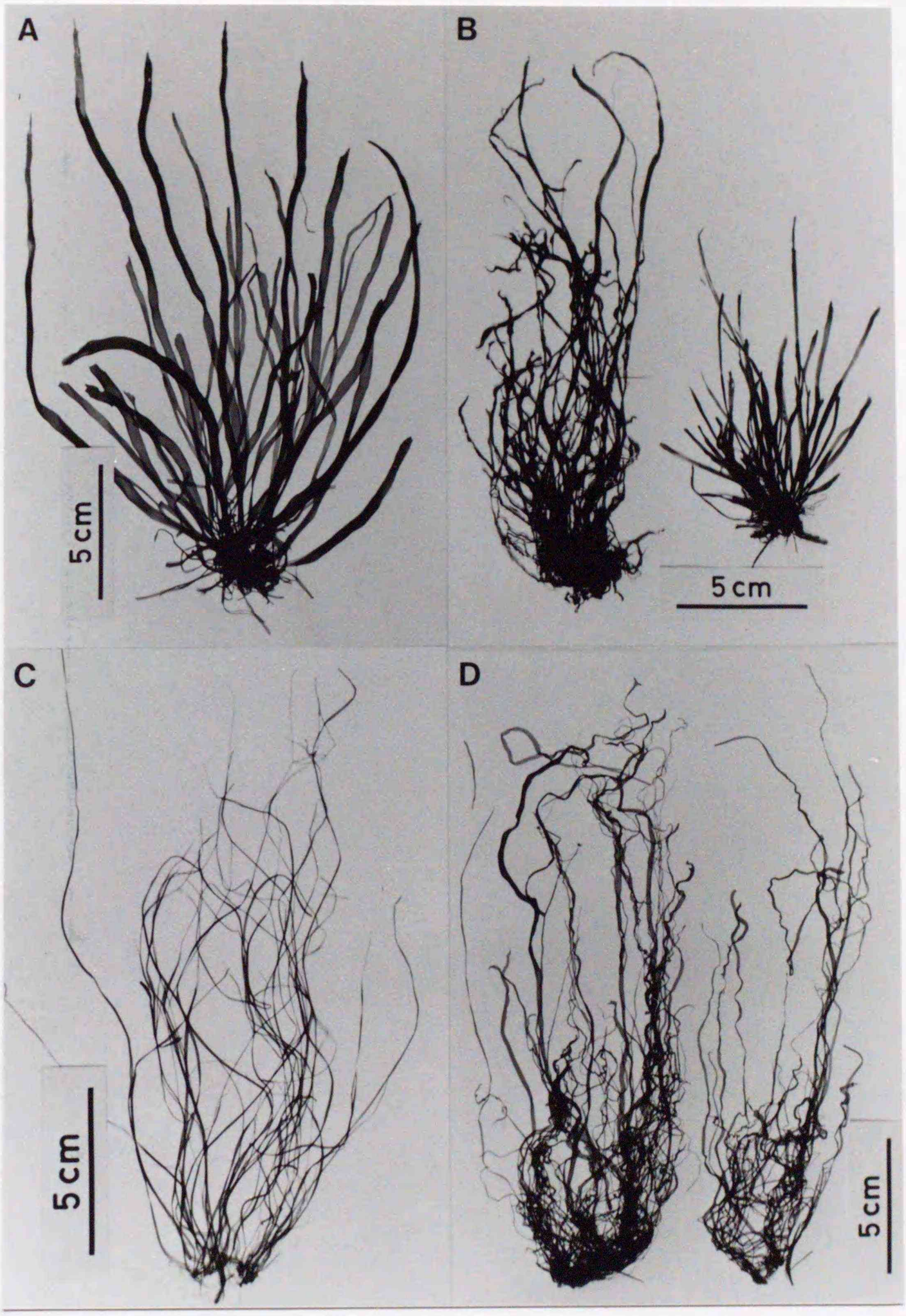


Fig. 93. Habit of specimens of *Petalonia zosterifolia*.

A. Plant collected at Oshoro, Hokkaido on 21 January 1988.
B. Plants collected at Oshoro, Hokkaido on 17 March 1992.
C. Plant collected at Oshoro, Hokkaido on 9 December 1987.
D. Plants collected at Oshoro, Hokkaido on 15 February 1991.

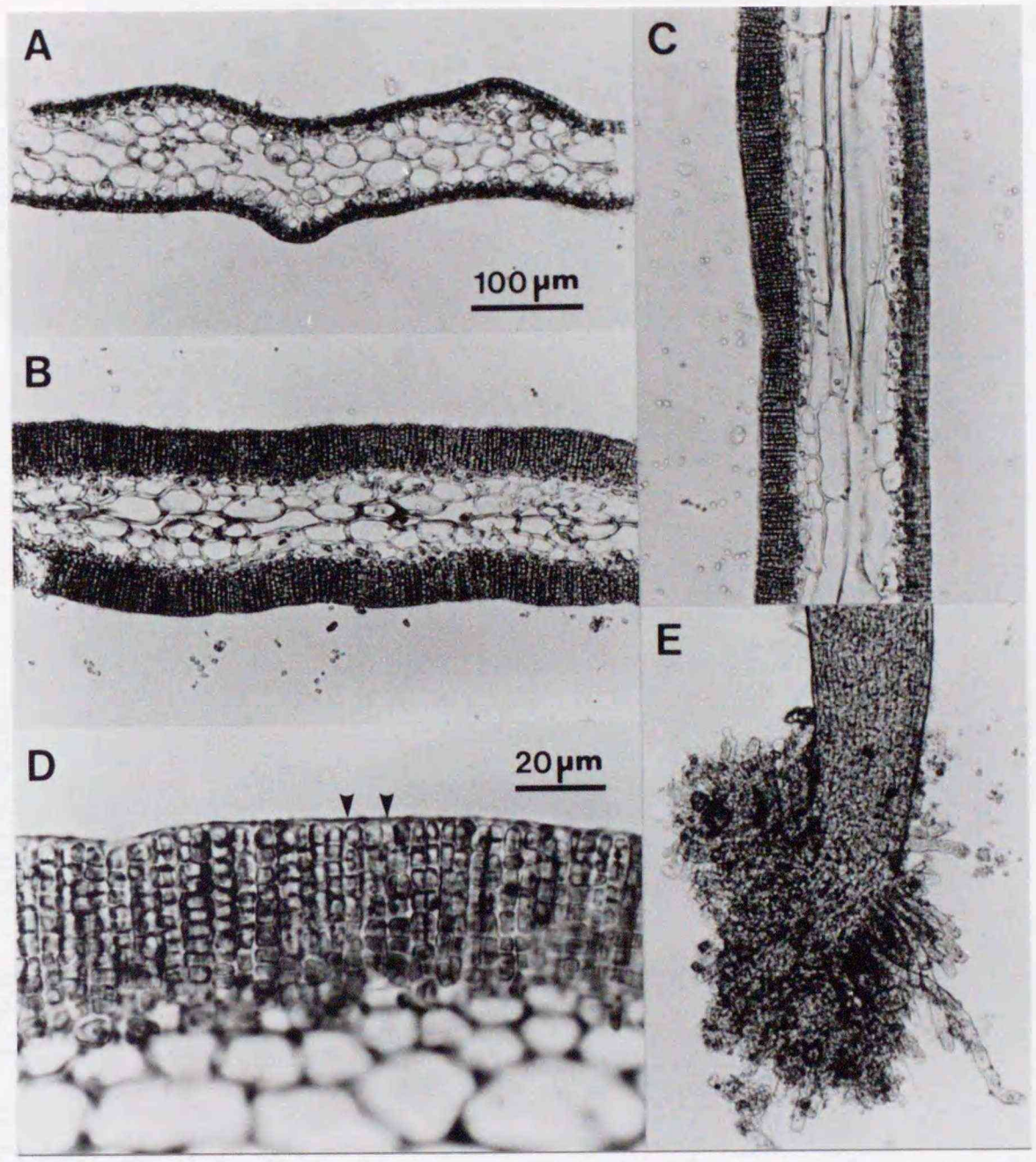


Fig. 94. Anatomy of *Petalonia zosterifolia*.

A. Cross section of vegetative thallus. **B.** Cross section of fertile thallus. **C.** Longitudinal section of fertile thallus. **D.** Plurilocular sporangia in cross section of thallus (arrowheads: cuticle). **E.** Basal portion of thallus, showing rhizoids.

Scale in **A** applies to **B**, **C** and **E**.

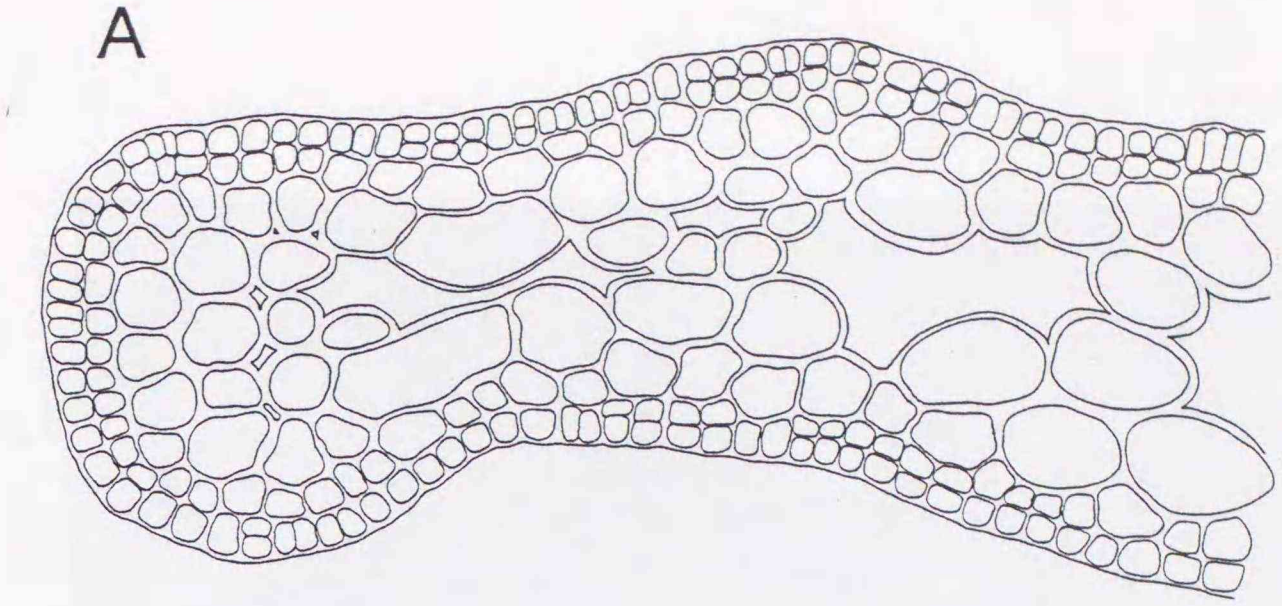


Fig. 95. Anatomy of *Petalonia zosterifolia*.

A. Cross section of vegetative thallus.
B. Cross section of fertile thallus with plurilocular sporangia (pl).

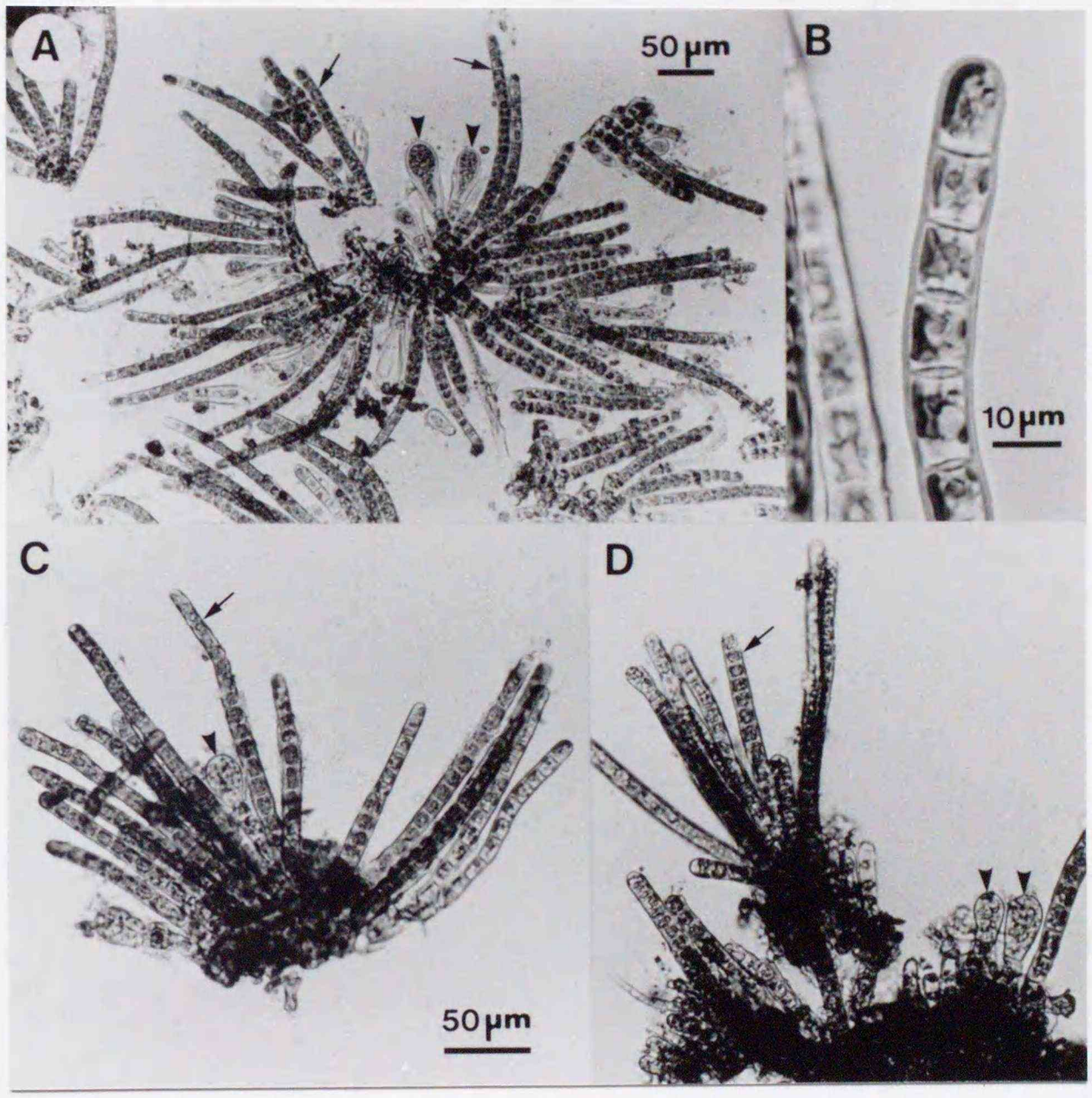


Fig. 96. *Petalonia zosterifolia*. Anatomy of field microthalli.

A, C, D. Squashed thalli with unilocular sporangia collected at Oshoro, Hokkaido on 23 September 1989 (arrow, uniseriate upright filament; arrowhead, unilocular sporangium). **B.** Uniseriate upright filament, showing a plastid with a pyrenoid per cell.

Scale in **C** applies to **D**.

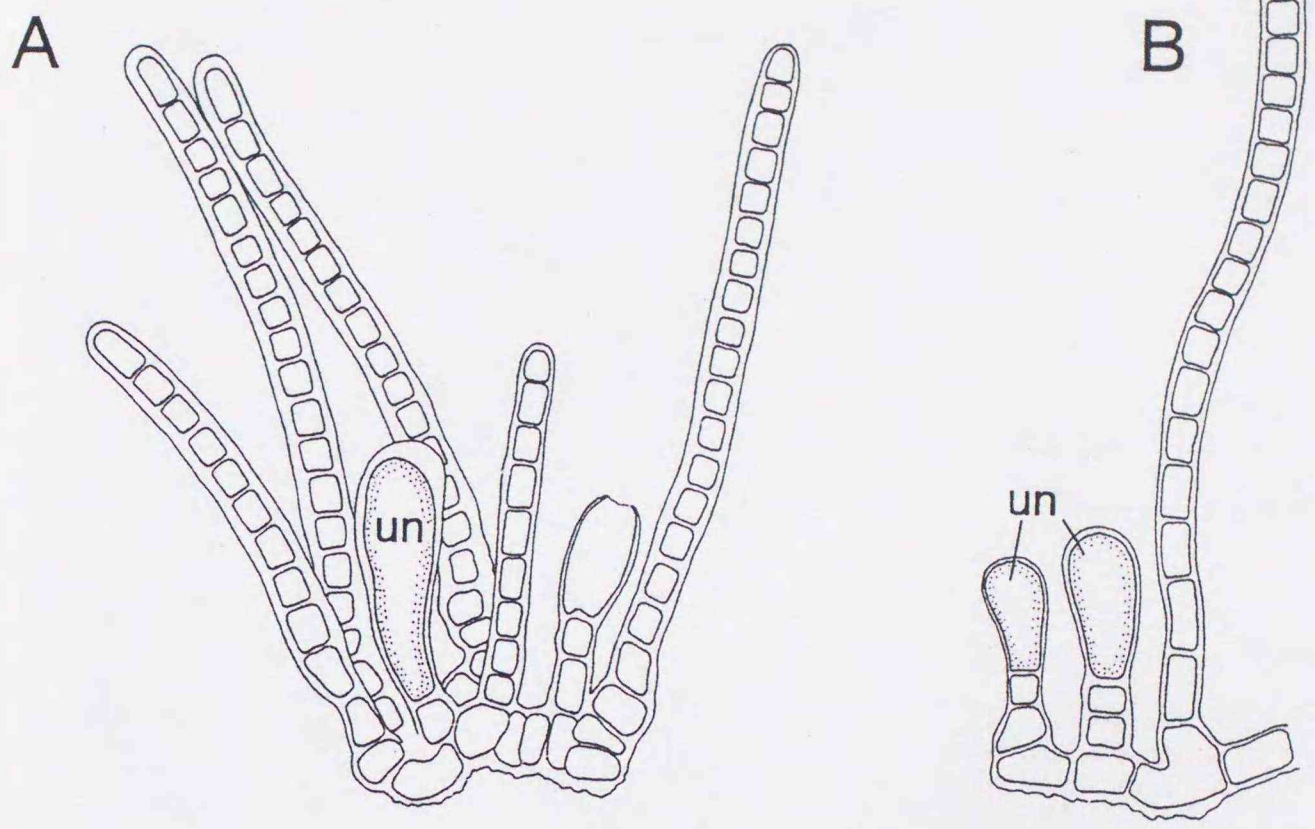


Fig. 97. *Petalonia zosterifolia*. Anatomy of field microthalli.

A, B. Squashed fertile microthalli with unilocular sporangia (un).

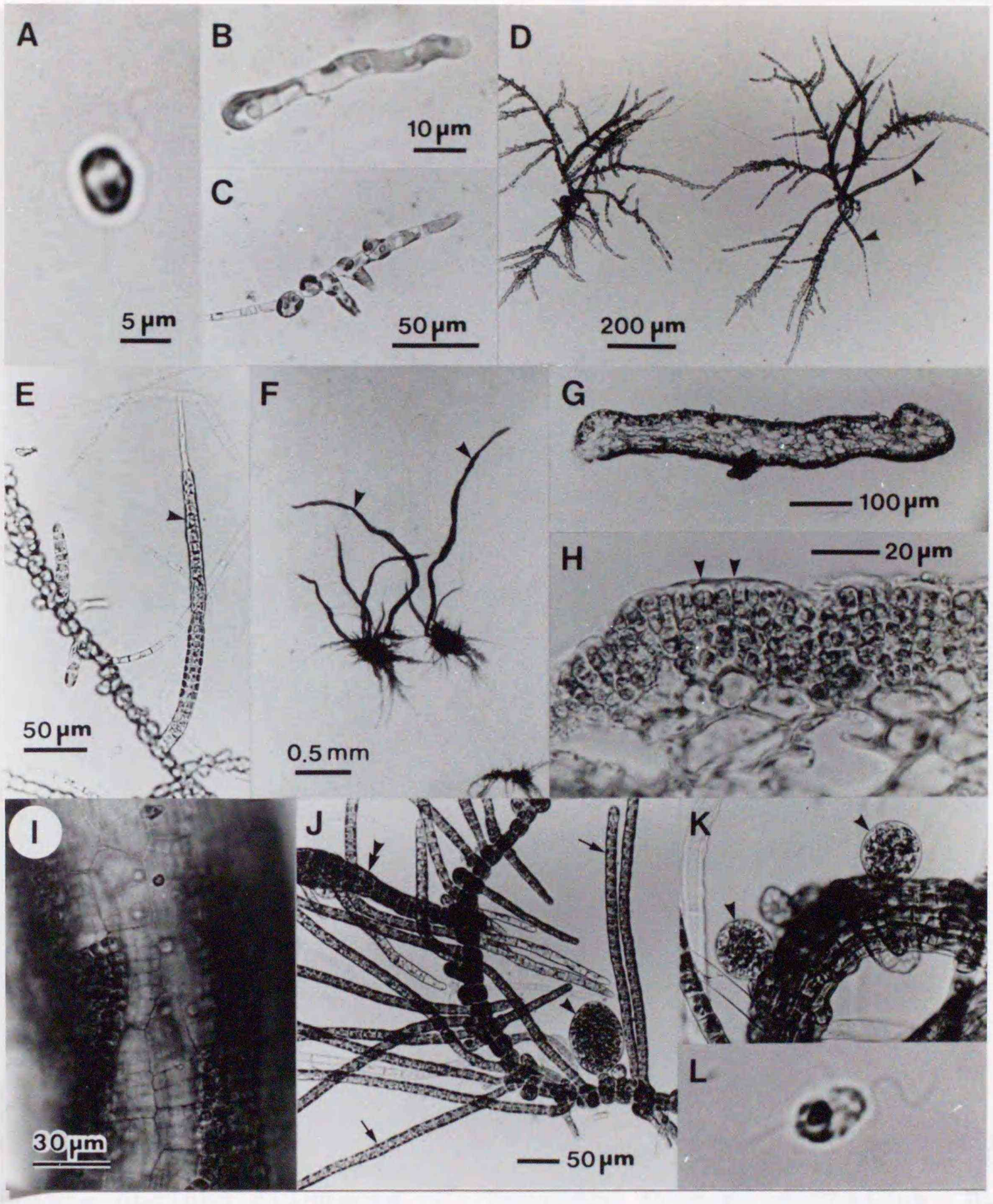


Fig. 98. *Petalonia zosterifolia*. Development of plurispores from macrothallus.

A. Plurispore. **B.** Four-day-old germling under 10°C LD condition. **C.** Eleven-day-old germling under 10°C LD condition. **D, E.** One-month-old microthalli under 10°C LD condition, bearing macrothalli (arrowheads). **F.** Macrothalli (arrowheads) arising from microthalli under 15°C LD condition. **G.** Cross section of macrothallus. **H.** Plurilocular sporangia in cross section of macrothallus (arrowheads: cuticle). **I.** Surface view of macrothallus, showing emptied plurilocular sporangia. **J.** Filaments of microthallus with unilocular sporangia (arrowhead), macrothallus (double arrowhead) and uniseriate upright filaments (arrows). **K.** Unilocular sporangia (arrowheads) on macrothallus. **L.** Unispore.

Scale in **A** applies to **L**; Scale in **C** applies to **K**.

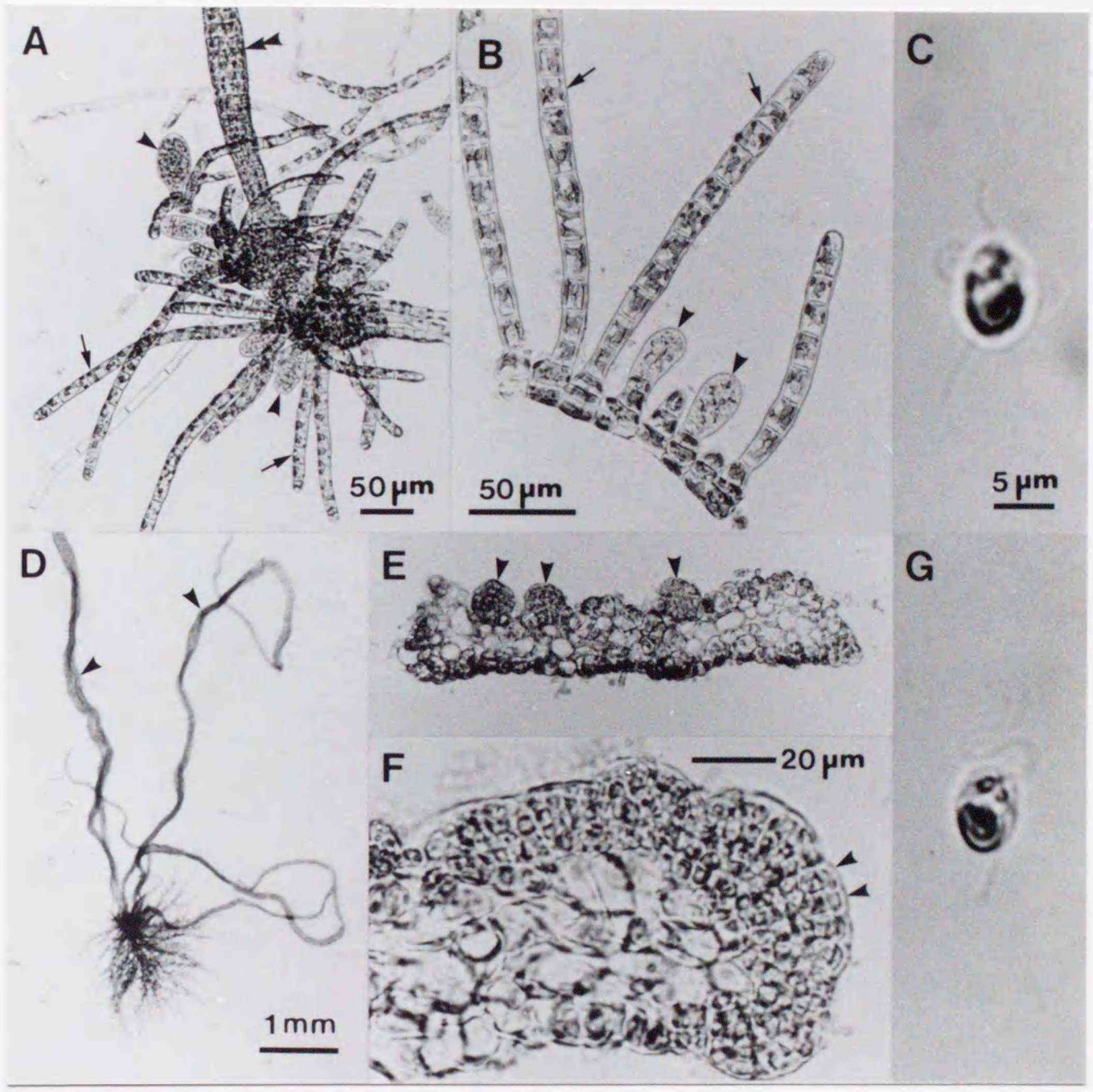


Fig. 99. *Petalonia zosterifolia*. Culture of field microthallus.

A. Squashed microthallus with macrothallus (double arrowhead), unilocular sporangia (arrowheads) and uniseriate upright filaments (arrows) under 20°C LD condition. **B.** Squashed microthallus with young unilocular sporangia (arrowheads). Arrows show uniseriate upright filaments. **C.** Unispore from microthallus. **D.** Macrothalli (arrowhead) on microthallus under 15°C LD condition. **E.** Cross section of macrothallus with plurilocular sporangia (arrowheads). **F.** Plurilocular sporangia in cross section of macrothallus (arrowheads: cuticle). **G.** Plurispore from macrothallus.

Scale in **A** applies to **E**; Scale in **C** applies to **G**.

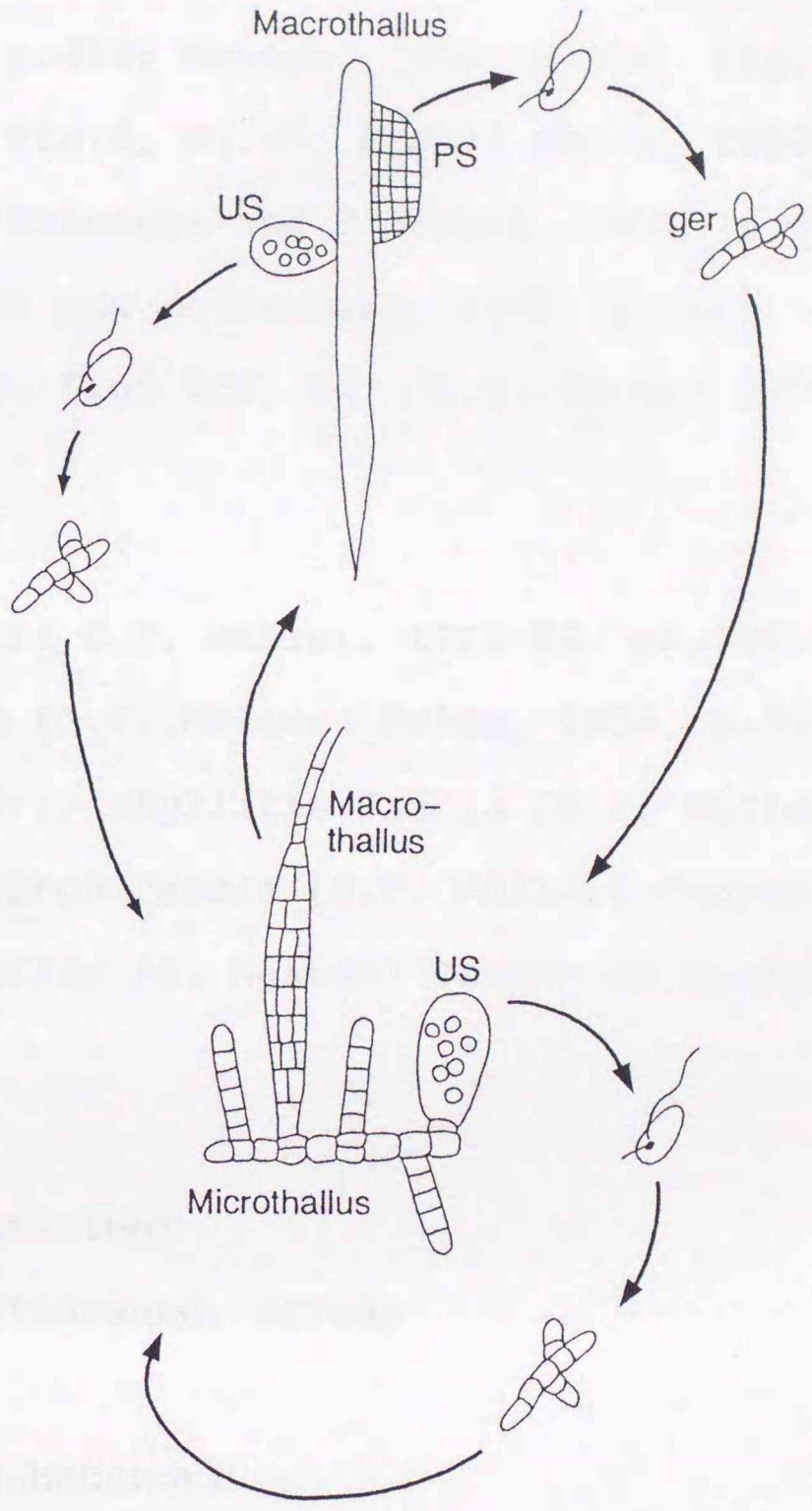


Fig. 100. Diagram of life history of *Petalonia zosterifolia* in culture.

ger, germling; PS, plurilocular sporangium; US, unilocular sporangium.

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

O. Kuntze, 1891-98, p.419; Newton, 1931, p.176, Fig.110; Taylor, 1957, p.167, Pl.14, Fig.5, Pl.15, Fig.3; Wynne, 1969, pp.17-31, Figs 6-8, Pls 6-13; Nakamura and Tatewaki, 1975, p.57-93, Figs 11-14, Pl.III; Abbott and Hollenberg, 1976, p.200, Fig.163; Fletcher 1987, p.230, Figs 61A, 62, Pl.9; Tanaka 1990, p.28, Fig.15.

Basionym: *Fucus fascia* O.F. Müller, 1771-82, pl.768.

Synonym: *Ilea fascia* (O.F. Müller) Fries, 1835, p.321; Okamura, 1936, p.234, Fig.127-1; *Phyllitis fascia* (O.F. Müller) Kützing, 1843, p.342; *Scytosiphon fascia* (O.F. Müller) Crouan frat., 1867, p.169; *Petalonia debilis* (C. Agardh) Derbès et Solier, 1850, p.266.

Type: probably non-existent

Type locality: Christiansund, Norway

Japanese name: Seiyo-habanori

Materials

Localities are shown in Fig. 101. The numbers in square brackets after following each locality correspond to those in Fig. 101.

Macrothalli: Hokkaido: Oshoro [1] (11-iii-1987 leg. K. Kogame, 1987-12-9 leg. K. Kogame, 21-i-1988 leg. K. Kogame, 17-ii-1988 leg. K. Kogame, 18-iii-1988 leg. K. Kogame, 2-xii-1989 leg. K. Kogame, 24-xi-1990 leg. K. Kogame, 15-ii-1991 leg. K. Kogame, 17-iii-1992 leg. K. Kogame), Setana [3] (18-iii-1987 leg. K. Kogame), Matsumae [6] (11-i-1989 leg. I. Mine), Yoshioka [9] (16-v-1988 leg. K. Kogame), Kikonai [10] (18-iii-1987 leg. K. Kogame), Shikabe [12] (4-iii-1987 leg. K. Kogame), Muroran [14]

(18-iv-1987 leg. K. Kogame, 28-i-1988 leg. K. Kogame, 5-ii-1988 leg. K. Kogame, 21-ii-1988 leg. K. Kogame, 23-iii-1988 leg. K. Kogame, 12-xii-1988 leg. K. Kogame, 22-i-1989 leg. K. Kogame, 18-iii-1990 leg. K. Kogame), Samani [17] (11-ii-1989 leg. K. Kogame), Hiroo [21] (19-v-1991 leg. K. Kogame), Akkeshi [22] (1-vii-1987 leg. H. Kawai, 14-vii-1988 leg. H. Kawai, 26-vi-1990 leg. K. Kogame, 17-v-1991 leg. K. Kogame, 26-vi-1991 leg. K. Kogame, 27-vi-1991 leg. K. Kogame, 29-vi-1991 leg. K. Kogame, 18-vi-1992 leg. K. Kogame), Hanasaki [23] (xii-1985 leg. H. Kawai, 7-ix-1987 leg. K. Kogame, 20-xii-1987 leg. K. Kogame, 18-v-1991 leg. K. Kogame), Tomoshiri [24] (30-vi-1987 leg. H. Kawai), Nosappu [25] (8-viii-1987 leg. M. Matsumoto, 21-xii-1987 leg. K. Kogame), Nokkamappu [26] (22-xii-1987 leg. K. Kogame), Aomori Pref. : Ohma [38] (3-iii-1987 leg. K. Kogame, 21-ii-1988 leg. H. Kawai, 2-ii-1990 leg. K. Kogame), Niigata Pref. : Kashiwazaki [45] (27-iii-1989 leg. K. Kogame), Hyogo Pref. : Awajishima Karikozaki (31-iii-1990 leg. S. Enomoto & K. Kogame), Karimo [82] (31-iii-1990 leg. S. Enomoto & K. Kogame), Maruyama [83] (3-iv-1992 leg. S. Enomoto & K. Kogame), Kamishima, Ieshima Isls [84] (1-iv-1988 leg. S. Enomoto & M. Masuda), Ehime Pref. : Nakashima [96] (16-iv-1988 leg. S. Enomoto & K. Kogame), Munakata, Ohmishima [95] (12-v-1990 leg. S. Enomoto & K. Kogame), Ohita Pref. : Himeshima [98] (18-iv-1988 leg. S. Enomoto & K. Kogame).

Microthalli: Hokkaido: Oshoro [1] (30-ix-1987 leg. K. Kogame, 7-xi-1987 leg. K. Kogame, 22-xi-1988 leg. K. Kogame, 24-x-1990 leg. K. Kogame), Seseki [27] (1-vii-1988 leg. K. Kogame, 27-viii-1989 leg. K. Kogame), Notorimisaki [30] (26-viii-1989 leg. K. Kogame), Hanasaki [23] (18-v-1991 leg. K. Kogame), Aomori Pref. : Ohma [38] (24-ix-1991 leg. K. Kogame), Kagawa Pref. : Ogishima [85] (25-x-1989 leg. S. Enomoto & K. Kogame).

Herbarium specimens examined are follows. Iwate Pref. : Taneichi [55] (SAP 026940, 13-i-1952 leg. S. Kawashima), Miyagi Pref. : Matsushima, Shiogama [59] (SAP 035275, 5-xi-1965 leg. T. Yoshida), Fukuoka Pref. : Nishi Park, Fukuoka [105] (SAP 048300, 2-ii-1957 leg. M. Ichiki), Kumamoto Pref. : Tomioka [109] (SAP 049822, 11-iv-1955 leg. T. Yoshida), Kagoshima Pref. : Akasaki, Amamiohshima [114] (SAP 028711, 30-iii-1963 leg. K. Matsunaga).

Morphology

Macrothalli

Macrothalli are lanceolate or elongate lanceolate, 10-20(-30) cm in length and 0.5-4 cm in width, tapering towards the base, gregariously arising from the base (Figs 102A-D). Thalli are yellowish brown when young, being darker in color when fertile. In cross section thalli are solid, consisting of cortical and medullary layers (Figs 103A-D, 104A-D). The cortex is composed of 1-3 layers of small, pigmented cells. The medulla

is composed of large, colorless cells which are round to ellipsoid, 15-35 × 20-50 µm in size in cross section. Medullary cells are sometimes collapsed, irregular in shape, issuing rhizoidal filaments which are variable in amount (Figs 103G-I, 104B-D). The rhizoidal filaments are 8-14 µm in width. Phaeophycean hairs are variable in amount, solitary or grouped, arising from the cortical cells. Holdfasts are more or less conical, consisting of rhizoidal filaments which arise from surface cells of the basal part of the thalli (Fig. 103F). Thalli become fertile basipetally with plurilocular sporangia. Plurilocular sporangia produced by divisions of cortical cells are firmly packed, being covered with a cuticle layer (Figs 103E, 104A,C). Ascocysts are absent among plurilocular sporangia. Plurilocular sporangia reached to 20-40 µm in length. Secondary plurilocular sporangia are common.

Microthalli

Microthalli are discoid, small, 1-5 mm in diameter, brown to dark brown in color (Fig. 105A). Thalli consist of prostrate branched filaments and erect filaments arising from them (Figs 105B,C, 106A-C). Erect filaments are usually simple, uniseriate, to 40-60 µm in length. They are firmly coherent to each other, not easily separable under pressure. Cells of erect filaments are 7-11 µm in length, 7-13 µm in width. In fertile thalli upper portions of erect filaments elongate, and change to paraphyses, unilocular sporangia are formed laterally on supporting cells of the paraphyses (Figs 105B-D, 106A-C). Paraphyses are to 100-180 µm in length. Cells of paraphyses are shorter in upper portions, 10-20 × 10-11 µm in size, longer in lower portions, 50-120 × 6-10

μm in size. They have a plastid with a conspicuous pyrenoid (Fig. 105E). Unilocular sporangia are elongate obovoid to clavate, 35-110 μm in length, 18-26 μm in width.

Habitat and Phenology

Macrothalli grow on rocks, boulders, shells, sea grasses (*Phyllospadix*, *Zostera*), and sometimes other algae (*Laminaria*, *Sargassum* etc.) in the intertidal and subtidal zones. In Japan, macrothalli are generally found during winter and spring. Also at Oshoro, Hokkaido, macrothalli appear in winter and disappear in summer (Table 43). However, at Akkeshi and Nemuro, Hokkaido, macrothalli are found during summer and autumn.

Microthalli grow on rocks, boulders and shells in the lower intertidal and subtidal zones, and are sometimes associated with microthalli of *Scytosiphon lomentaria*, *Colpomenia bullosa* and *Ralfsia* spp. At Oshoro, Hokkaido, microthalli bearing unilocular sporangia are found during autumn and winter (Table 43). However at Shiretoko and Nemuro, Hokkaido, they are found in summer. Since identification of vegetative thalli is difficult, no phenological data of microthalli can be given in this paper.

Geographical distribution

This species is distributed along the coasts of cold and warm temperate waters throughout the world (Abbott and Hollenberg 1976, Fletcher 1987). In Japan, this species is widely found on the coasts from Hokkaido to Amami Islands.

Culture study

Culture strains isolated from macrothalli are listed in

Table 44 in which culture conditions and progeny of plurispores are also shown.

Sexual reproduction

Fusions of gametes were observed in materials of Oshoro, Hokkaido and Ohma, Aomori Prefecture.

Sexual reproduction of this species was isogamy. Female gametes released from plurilocular sporangia on macrothalli were pear drop-shaped, 5.6-6.8 × 3.2-4.2 µm in length and width, laterally biflagellated with a longer anterior and a shorter posterior flagella (Fig. 107A). They contained a plastid with a pyrenoid and a stigma. Male gametes were the same shape as female gametes, 5.6-6.8 × 3.0-4.0 µm in size (Fig. 107B). Female and male gametes showed negative phototaxis. Female and male gametes settled on substrata were round. When female and male gametes were mixed, a settled female gamete was surrounded by several male gametes which touched the settled female gamete by anterior flagella, and then, one of male gametes fused with the female gamete (Figs 107C-E). Zygotes were distinguished from unfused gametes by possessing two stigmata (Fig. 107F). The fragrant odor of sexual pheromones was observed in culture where female gametes were released.

Development of zygotes

Zygotes, which were formed by gametes produced under 10°C SD, were isolated, and were cultured at 5-20°C under SD and LD (Table 45). Their germlings resulted prostrate branched filaments at first, later developed into disks (Figs 108A-C). The disks consisted of basal prostrate filaments and simple upright

filaments arising from them (Fig. 108D). Tufts composed of branched filaments with knots were usually observed on the disks. Macrothalli rarely arose on tufts under 15°C SD and 15°C LD conditions (Fig. 109A). They formed plurilocular sporangia. Microthalli formed unilocular sporangia laterally on supporting cells of paraphyses under 20°C SD after 4 months (Figs 108E,F).

Development of unispores from zygotic microthalli

Unilocular sporangium which were formed under 20°C SD were isolated and were cultured under 10°C SD and 10°C LD (Table 46). Unispores (Fig. 108G) released from the unilocular sporangium germinated to prostrate branched filaments, later became small prostrate systems which issued macrothalli (Figs 108H,I). The small prostrate systems did not grow further (Fig. 108J). Under 10°C SD, macrothalli reached to 10 cm in length and 8 mm in width after 3 months, and became fertile with plurilocular sporangia. Plurispores were gametes which fused to form zygotes. Female and male thalli were obtained in culture derived from one unilocular sporangium.

Development of plurispores from macrothalli on zygotic microthalli

Plurispores (Fig. 109B) produced on macrothalli in culture of zygotes were cultured at 5-20°C under SD and LD conditions (Table 47). Plurispores were pear drop-shaped, of normal phaeophycean-type, 5.8-7.2 × 3.6-4.4 µm in size, exhibiting a negative phototaxis. Settled plurispores were round, 4.0-5.0 µm in diameter. These plurispores did not fuse with female nor male gametes. They developed in the same manner as zygotes, forming

disks with tufts (Fig. 109C). These microthalli rarely issued macrothalli under 10°C LD, 15°C SD, 15°C LD and 20°C LD conditions. Under 20°C SD they formed unilocular sporangia (Fig. 109D).

Development of unispores from microthalli which were developed from plurispores produced by zygotic macrothalli

Unilocular sporangia on microthalli under 20°C SD were cultured under 5°C SD, 10°C SD, 15°C LD and 20°C LD (Table 48). Unispores developed to macrothalli in the same manner as unispores from zygotic microthalli (Fig. 109E). Under 10°C SD macrothalli formed plurilocular sporangia after 2 months. Female and male thalli were obtained in culture of one unilocular sporangium.

Parthenogenetic development of gametes

Results of culture studies on strain 269 Ohma (female) and 270 Ohma (male) are mentioned below.

Settled female gametes were cultured at 5-20°C under SD and LD conditions. Their germlings were at first prostrate branched filaments, and they developed to disks (Figs 108K-M) which consisted of basal prostrate branched filaments and simple upright filaments arising from them. Tufts which consisted of branched filaments with knot portions were usually observed, as well as disks. They formed disks when they attached to substrata. Disks grew to 1 cm in diameter (after 3 months under 20°C LD), but they were usually detached from substrata.

Macrothalli sometimes were formed on tufts under all conditions examined, rarely on disks (Fig. 108N). They were

formed more or less abundantly and grew larger in short day conditions than those in long day conditions. Macrothalli became fertile with plurilocular sporangia under all conditions attempted. However, plurispore release scarcely occurred at 20°C. Plurispores were female gametes when the sexuality was ascertained.

Microthalli became fertile, forming unilocular sporangia under a 20°C SD condition. Unilocular sporangia were formed laterally on supporting cells of paraphyses.

Parthenogenetic development of male gametes was almost the same as female gametes.

Development of unispores from parthenogenetic microthalli

Unispores from unilocular sporangia on parthenogenetic microthalli were cultured under 10°C SD and 20°C SD conditions (Table 49). Settled unispores germinated at first to prostrate branched filaments, later became small disks which issued macrothalli in abundance. Prostrate branched filaments also issued macrothalli before they became small disks. The small disks did not grow further. Sexuality of plurispores from macrothalli were ascertained under 10°C SD, and they were the same as those of original gametes.

Culture of strain 154 Akkeshi

Plurispores from macrothalli under 5°C SD were cultured at 5-20°C under SD and LD conditions. Most of their germlings gave rise to macrothalli under all conditions examined. Disks which sprouted macrothalli did not expanded further. Although they reached to 2 mm in diameter under 20°C SD after 2 months, they

also sprouted macrothalli abundantly. These disks did not form any sporangia under any conditions examined. Macrothalli formed plurilocular sporangia. Plurispore release scarcely occurred at 20°C.

Culture of field microthalli

Unilocular sporangia on field microthalli were isolated and cultured. Culture strains, culture conditions and its progeny are shown in Table 50. Results in strain 336 Oshoro were mentioned below.

Unispores from isolated unilocular sporangia were cultured under a 10°C SD condition. They germinated to prostrate branched filaments which sprouted macrothalli (Figs 110A-D). Although the prostrate filaments grew to disks, they remained small, not expanding. Macrothalli were morphologically identical with *Petalonia fascia*, being flat, lanceolate, solid (Figs 110E,F). They formed firmly packed plurilocular sporangia (Fig. 110G). Plurispores represented gametes which fused to form zygotes. Female and male thalli were obtained in culture of one unilocular sporangium. These gametes reciprocally fused with those of *Petalonia fascia* in culture (strain 269 Ohma, female; 270 Ohma, male).

In culture of strain 351 Hanasaki, disks and macrothalli which were identified with *P. fascia* were obtained. In this strain, macrothalli formed unilocular sporangia under a 20°C SD condition (Fig. 110H).

Discussion

Several formae have been established under *Petalonia fascia*:

for example, they are f. *fascia* (Hauck 1885), f. *debilis* (C. Agardh) Sechell et Gardner (1924), f. *caespitosa* (J. Agardh) Kjellman (1883), f. *zosterifolia* (Reinke) Setchell et Gardner (1924), f. *filiformis* (Batters) Setchell et Gardner (1924). However, these formae are rarely used in recent papers.

Ilea zosterifolia Noda (1964) [*Ilea* Fries (1835) is a synonym of *Petalonia* Derbès et Solier (1850) (O. Kuntze, 1891-98)] was described based on materials epiphytic on *Phyllospadix iwatensis* Makino from Shioyazaki, Fukushima Prefecture. According to Noda (1964), this species is distinguished from *P. fascia* in respects of small thalli (0.8-1 cm in length, 2-3 mm in width) and the presence of hairs. However, since the type material were collected in summer (16 July 1958), it might be small. Hairs also exist in *P. fascia*. Therefore, these characteristics do not distinguish *Ilea zostericola* from *P. fascia*. Reexamination on *Ilea zostericola* is necessary.

In this study on *P. fascia*, rhizoidal filaments in medulla of the thalli, which are similar to those of *Petalonia binghamiae* (*Endarachne binghamiae*), were sometimes found. The filaments were variable in amount, particularly abundant in materials of Akkeshi and Nemuro. Most previous descriptions of this species do not mention the presence of such rhizoidal filaments. For example, Abbott and Hollenberg (1976) (California), Womersley (1987) (Australia) and Fletcher (1987) (England) did not mention them. However, Vinogradova (1973) reported the presence of rhizoidal filaments in the medulla of *P. fascia*. Based on my observations, *P. fascia* and *P. binghamiae* can not be distinguished each other by presence or absence of such rhizoidal filaments. In this study, it is revealed that the width of the rhizoidal filaments

in medulla is useful to distinguish the two species: 8-14 µm in *P. fascia*, 4-9 µm in *P. binghamiae*.

Ascocysts among plurilocular sporangia are generally absent in *P. fascia*. Wynne (1969) pointed out an illustration of Setchell and Gardner (1925) which shows ascocysts, and mentioned that they were mistaken on this point. Wynne's examination of many populations of *P. fascia* on the west coast of North America could not find any thalli of this species with ascocysts. Also in Japanese *P. fascia*, ascocysts are absent. However, Womersley (1987) reported Australian *P. fascia* which has ascocysts-like cells in fertile or infertile portions of the thalli. The Australian *P. fascia* is of interest, since it may represent a separate species from *P. fascia*.

In this study, sexual reproduction has been confirmed in materials from two localities. It represents isogamy, and the manner of gamete fusion is the same as those reported previously in other species of Scytosiphonaceae (Nakamura and Tatewaki 1975, Clayton 1980), in which a settled female gamete is surrounded by male gametes, then it is fused with one of male gametes. The evidence of sexual reproduction in *P. fascia* has not been reported (Clayton 1988). Although there are some reports of sexual reproduction, they are doubtful. Kunieda and Arasaki (1947) reported sexual reproduction in *P. fascia*, but they did not observe the fusion of gametes. Shannon *et al.* (1988) reported sexual reproduction of *P. fascia* from New Hampshire. However, it should be pointed out that their results are not substantiated by photographs and observations of the fusions, and that frequency of the fusions is very low. Therefore, this is the first confirmed report on sexual reproduction of this species.

A diagram of life history of *P. fascia* in this culture is shown in Fig. 111. In this study, a heteromorphic life history involving sexual reproduction was shown in *P. fascia*, which is the same as those of *Petalonia binghamiae* (*Endarachne binghamiae*) and *Scytosiphon lomentaria* (Nakamura and Tatewaki 1975). Macrothalli represent gametophytes, and microthalli represent sporophytes. The results that female and male thalli were obtained in culture from one unilocular sporangium which was formed on zygotic microthalli (sporophyte), suggest that reduction division may occur in the unilocular sporangium.

In this culture, zygotic microthalli sprouted macrothalli although the frequency was low. No macrothallus on zygotic microthalli is known in Scytosiphonales except this study. Plurispores from these macrothalli on zygotic microthalli developed into microthalli which formed unilocular sporangia. In culture of the unilocular sporangium, female and male thalli were observed. These results suggest that reduction division occurred in the unilocular sporangium, and the macrothalli derived from zygotes were diploid.

There are several reports suggesting that morphogenesis of macrothalli was affected by temperature and photoperiods in development of plurispores (Edwards 1969, Wynne 1969, Roeleveld *et al.* 1974, Nakamura and Tatewaki 1975). However, in the present culture of strains from Ohma and Oshoro, such effect was not obviously observed, although macrothalli more or less abundantly developed in lower temperatures and short day conditions.

In Ohma and Oshoro, macrothalli appear in winter and spring, and disappear in summer and autumn. Microthalli forming unilocular sporangia were collected in autumn and winter. The

culture results that microthalli formed unilocular sporangia under 15°C SD and 20°C SD, agree with observation of field microthalli. Since unispores in culture mostly developed to macrothalli under 5-15°C conditions, they are considered to play a role of production of macrothalli. Therefore, macrothalli, which appear in winter, are considered to develop from unispores. Formation of unilocular sporangia which is controlled by temperature and photoperiods accounts for the appearance of macrothalli at Ohma and Oshoro.

In culture of strain no. 42 Akkeshi, plurispores from macrothalli developed to macrothalli at 5-20°C under SD and LD conditions. These results agree with the occurrence of macrothalli even in summer. Unilocular sporangia were not observed in culture of this strain. Therefore, this strain represents a direct type life history.

Wynne (1969, 1972b) suggested that blade forms which classified as f. *fascia*, f. *debilis* and f. *caespitosa* are related with the potentiality of the life history. In this study, it was difficult to classify Japanese materials as those forms, and such correlation was not recognized.

In culture of strain 351 Hanasaki, macrothalli formed unilocular sporangia under a 20°C SD condition, in addition to plurilocular sporangia. Unilocular sporangia on macrothalli were not found in field thalli, and they are unknown in this species. The unilocular sporangia on macrothalli are considered to be artifacts that might be induced under the higher temperature and short day condition under which the parent plant of this strain has not been experienced.

Table 43. Collection data of *Petalonia fascia* at Oshoro, Hokkaido. (P, thalli with plurilocular sporangia; U, thalli with unilocular sporangia; (U), microthalli bearing unilocular sporangia identified morphologically, not cultured; --, thallus was not found)

Macrothalli

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
1987			P	--	--		--	--		--	P	
1988	P	P	P							--	P	
1989			P		--		--	--		--	P	
1990		P	--	--			--		--	--	P	
1991		P	P									
1992			P	--								

Microthalli

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
1987									U		U	(U)
1988										(U)	U	(U)
1989									(U)		(U)	(U)
1990		--	--							U	(U)	
1991		(U)										

Table 44. Collection data of cultured strains of *Petalonia fascia*. Culture conditions tested and progeny of plurispores from macrothalli are also shown. Tufts always appeared with disks.

strain no.	locality	date	sex	culture conditions	progeny
2	Shikabe	4 iii 1987		5-20SL	disks, a few macrothalli
12	Setana	18 iii 1987		10L	disks, a few macrothalli (P)
24	Muroran	18 iv 1987		10L	disks
41	Tomoshiri	30 vi 1987		10L	macrothalli (P)
42	Akkeshi	1 vii 1987		5-15SL	macrothalli (P)
				20SL	macrothalli
45	Akkeshi	26 vii 1987		10L	disks, a few macrothalli
48	Hanasaki	7 ix 1987		10L	macrothalli (P)
70	Oshoro	9 xii 1987		10L	disks
				10S	disks, macrothalli (P)
77	Hanasaki	20 xii 1987		10L	disks (U)
78	Nosappu	21 xii 1987		10L	disks (U)
79	Nokkamappu	22 xii 1987		10L	disks (U)
80	Oshoro	21 i 1988		10L, 20L	disks
114	Ofuyu	2 v 1988		10L	disks, a few macrothalli
154	Akkeshi	14 vii 1988		10L	macrothalli (P)
200	Matsumae	11 i 1989		5S	disks, a few macrothalli
204	Samani	11 ii 1989		5S	disks
211	Ohma	11 iii 1989	female	5S	disks, a few macrothalli (P)
212	Ohma	11 iii 1989	male	5S	disks, a few macrothalli (P)
219	Awashima	6 iv 1989		10L	disks
269	Ohma	2 ii 1990	female	5-15SL, 20L	disks, a few macrothalli (P)
				20S	disks (U)
270	Ohma	2 ii 1990	male	5-15SL, 20L	disks, a few macrothalli (P)
				20S	disks (U)

Table 45. Culture of *Petalonia fascia*. Progeny of zygotes. Tufts always appeared with disks.

strain no.	culture conditions	progeny
269 Ohma female x 270 Ohma male	5SL, 10SL, 20L	disks
	15SL	disks, rarely macrothalli (P)
	20S	disks (U)

Table 46. Culture of *Petalonia fascia*. Progeny of unispores from zygotic prostrate thalli.

strain no.	culture conditions	progeny
269 Ohma female x 270 Ohma male	10S	macrothalli (P, female, male)
	10L	macrothalli (P)

Table 47. Culture of *Petalonia fascia*. Progeny of plurispores from macrothalli which arise from zygotic microthalli. Tufts always appeared with disks.

strain no.	culture conditions	progeny
269 Ohma female x 270 Ohma male	5SL, 10SL	disks
	15SL, 20L	disks, rare macrothalli (P)
	20S	disks (U)

Table 48. Culture of *Petalonia fascia*. Progeny of unispores from microthalli which were developed from plurispores from zygotic macrothalli.

strain no.	culture conditions	progeny
269 Ohma female x 270 Ohma male	5S, 15L,	macrothalli (P)
	20L	macrothalli
	10S	macrothalli (P; female, male)

Table 49. Culture of *Petalonia fascia*. Progeny of unispores from parthenogenetic microthalli.

strain no.	locality	date	sex	culture conditions	progeny
269	Ohma	2 ii 1990	female	10S, 20S	macrothalli (P)
270	Ohma	2 ii 1990	male	10S, 20S	macrothalli (P)

Table 50. Culture of *Petalonia fascia*. Progeny of unispores from field prostrate thalli.

strain no.	locality	date	culture conditions	progeny
63	Oshoro	7 xi 1987	10L	macrothalli (P)
150	Seseki	1 vii 1988	10L	macrothalli (P)
166	Notorimisaki	26 viii 1989	10S	macrothalli (P)
245	Seseki	27 viii 1989	5-20S, 5L, 10L	macrothalli (P)
			15L, 20L	macrothalli (P), a few disks (U)
248	Ogijima	25 x 1989	10S	macrothalli (P)
336	Oshoro	24 x 1990	10S	macrothalli (P; male, female)
351	Hanasaki	18 v 1991	5S, 10S	macrothalli (P)
			10L, 15S	disks (U), macrothalli (P)
			15L	disks (U), macrothalli (P, U)
			20S	disks (U), macrothalli (U)
363	Ohma	24 ix 1991	5S, 10SL, 15SL	macrothalli (P)
			20SL	macrothalli, a few disks

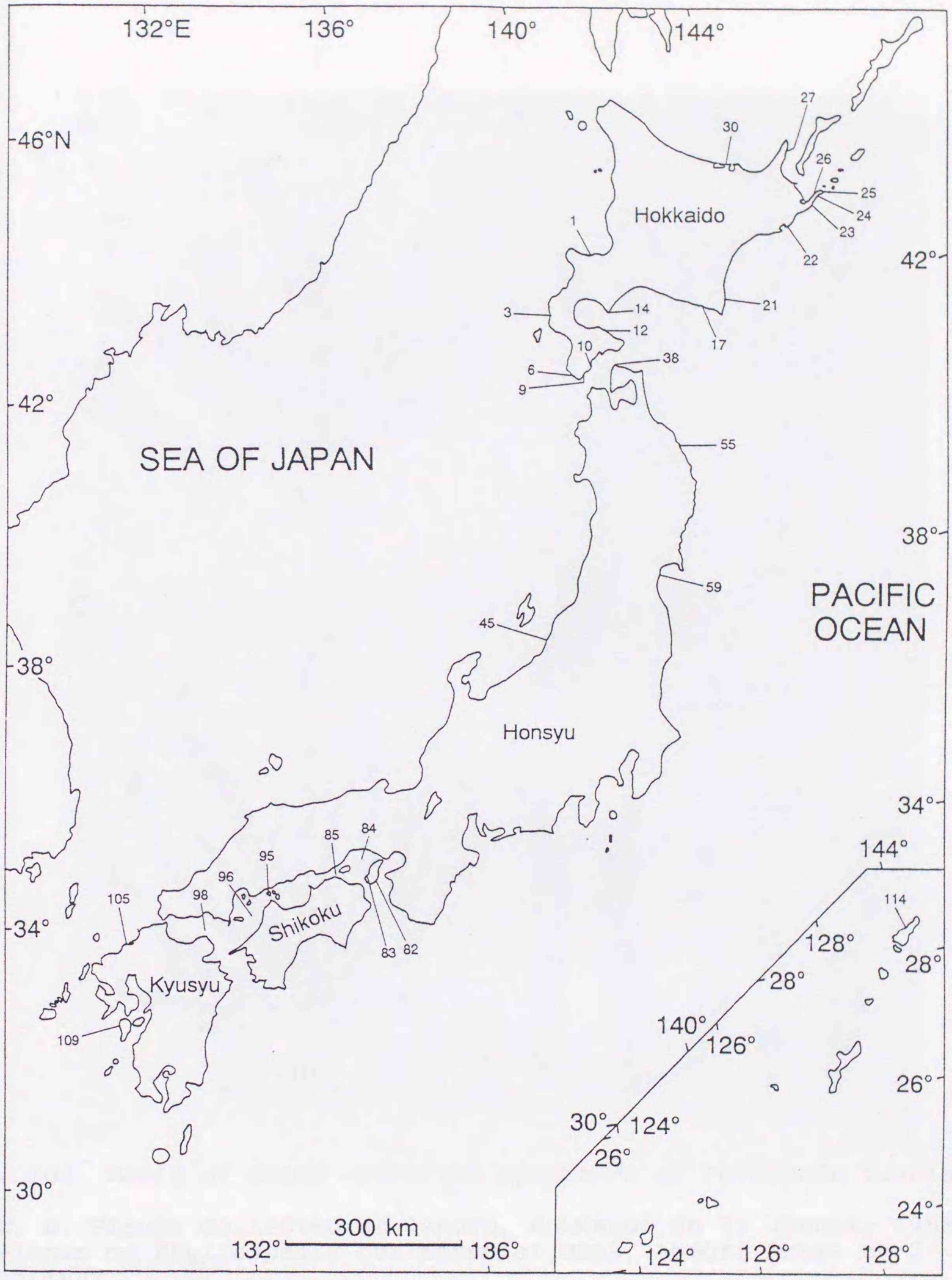


Fig. 101. Localities where materials of *Petalonia fascia* were collected. Numbers plotted correspond to those in Table 1.

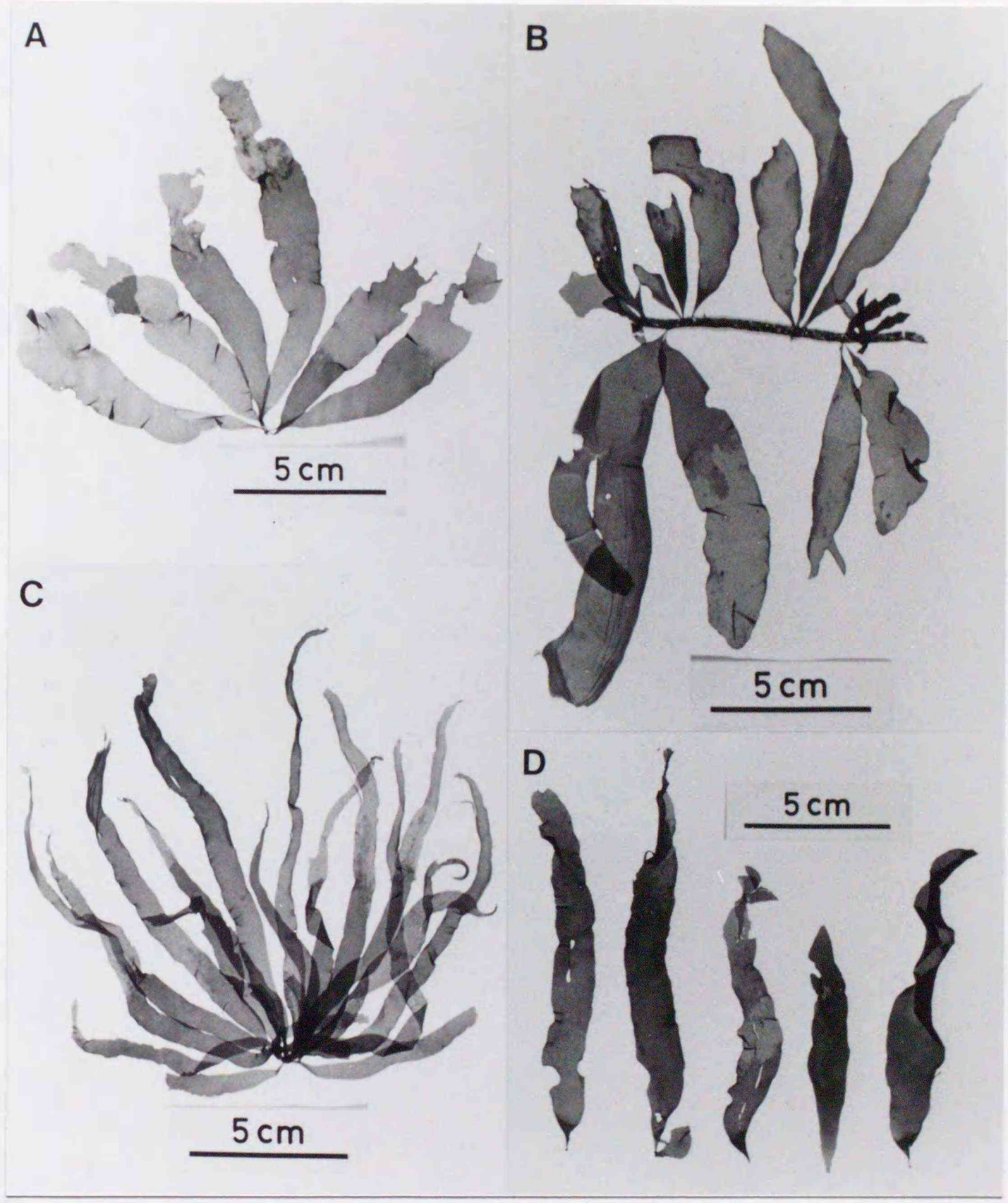


Fig. 102. Habit of dried herbarium specimens of *Petalonia fascia*.

A, C, D. Plants collected at Oshoro, Hokkaido on 21 January 1988.
B. Plants on *Phyllospadix* collected at Ohma, Aomori Pref. on 3 March 1987.

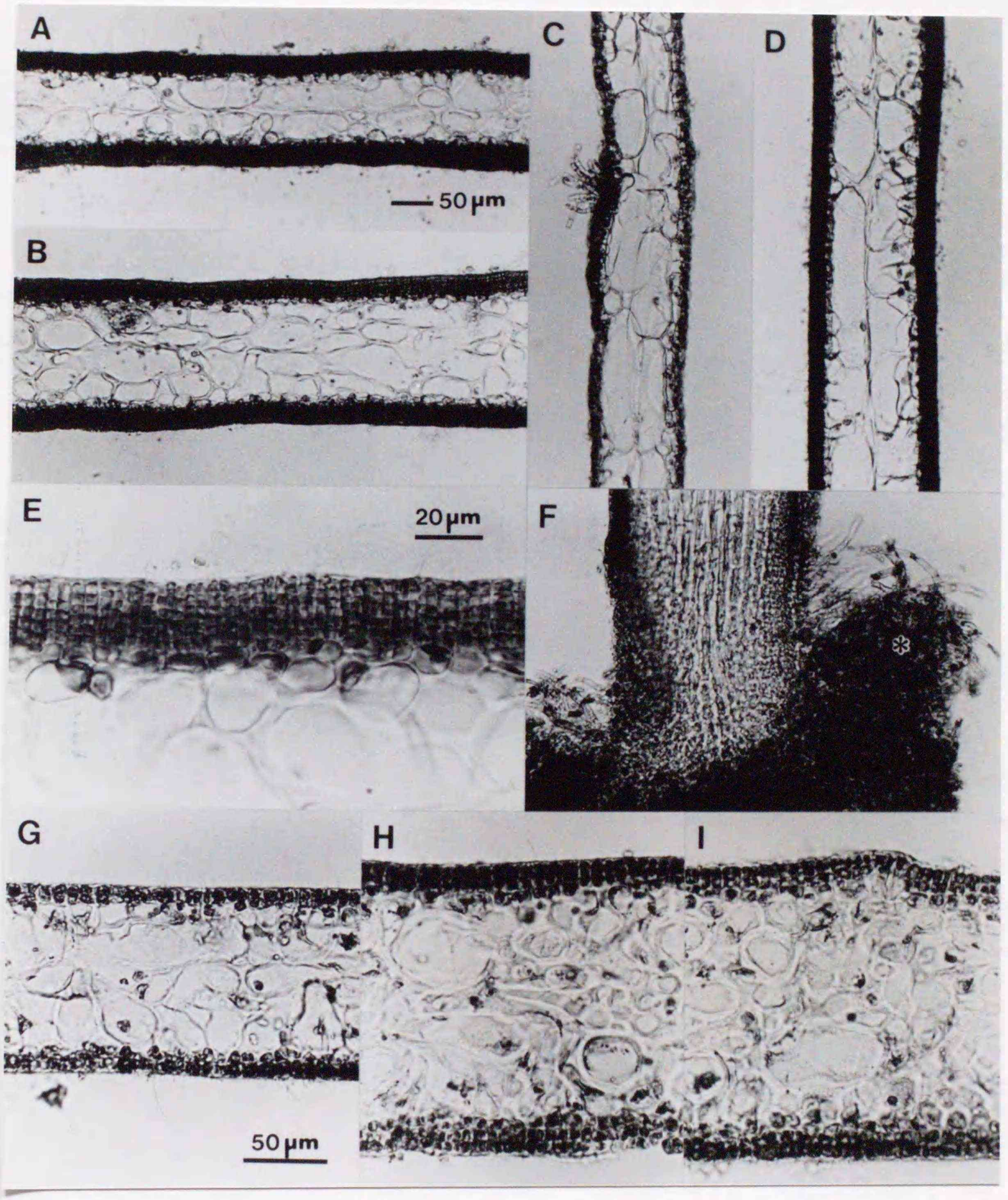


Fig. 103. Anatomy of *Petalonia fascia*.

A, **B**. Cross sections of fertile thalli. **C**. Longitudinal section of vegetative thallus. **D**. Longitudinal section of fertile thallus. **E**. Plurilocular sporangia in cross section of thallus. **F**. Longitudinal section of basal part of thallus, showing rhizoids which construct holdfast (asterisk). **G**. Medullary cells elongating towards inside in cross section of thallus. **H**, **I**. Rhizoidal filaments and ruptured cells in medulla in cross section of thalli.

Scale in **A** applies to **B-D** and **F**; Scale in **G** applies to **H** and **I**.

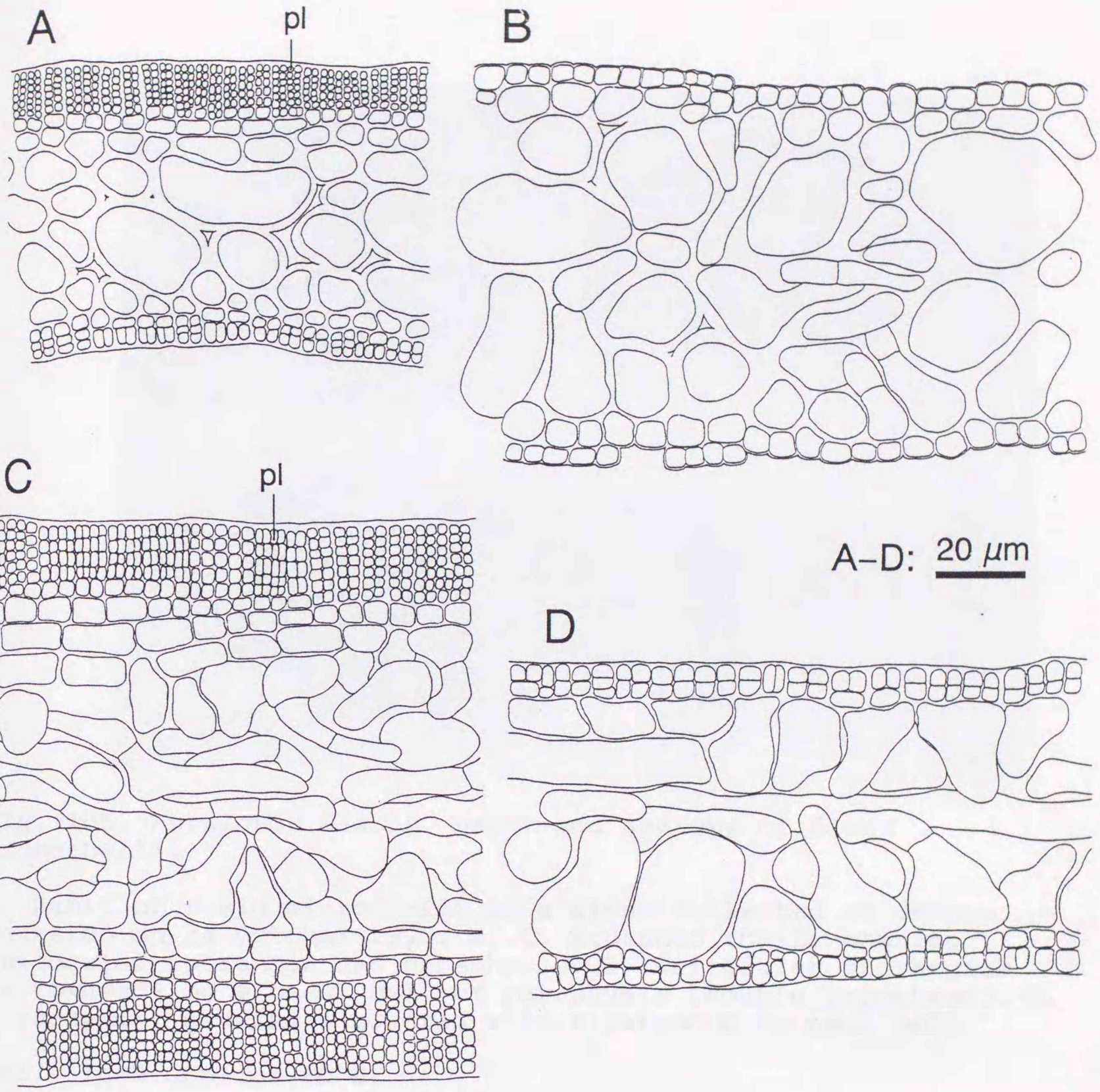


Fig. 104. Anatomy of *Petalonia fascia*.

A. Cross section of fertile thallus with plurilocular sporangia (pl). **B.** Cross section of thallus after release of plurispores. **C.** Longitudinal section of thallus with plurilocular sporangia (pl), showing rhizoidal filaments in medulla. **D.** Longitudinal section of vegetative thallus, showing inward elongation of medullary cells.

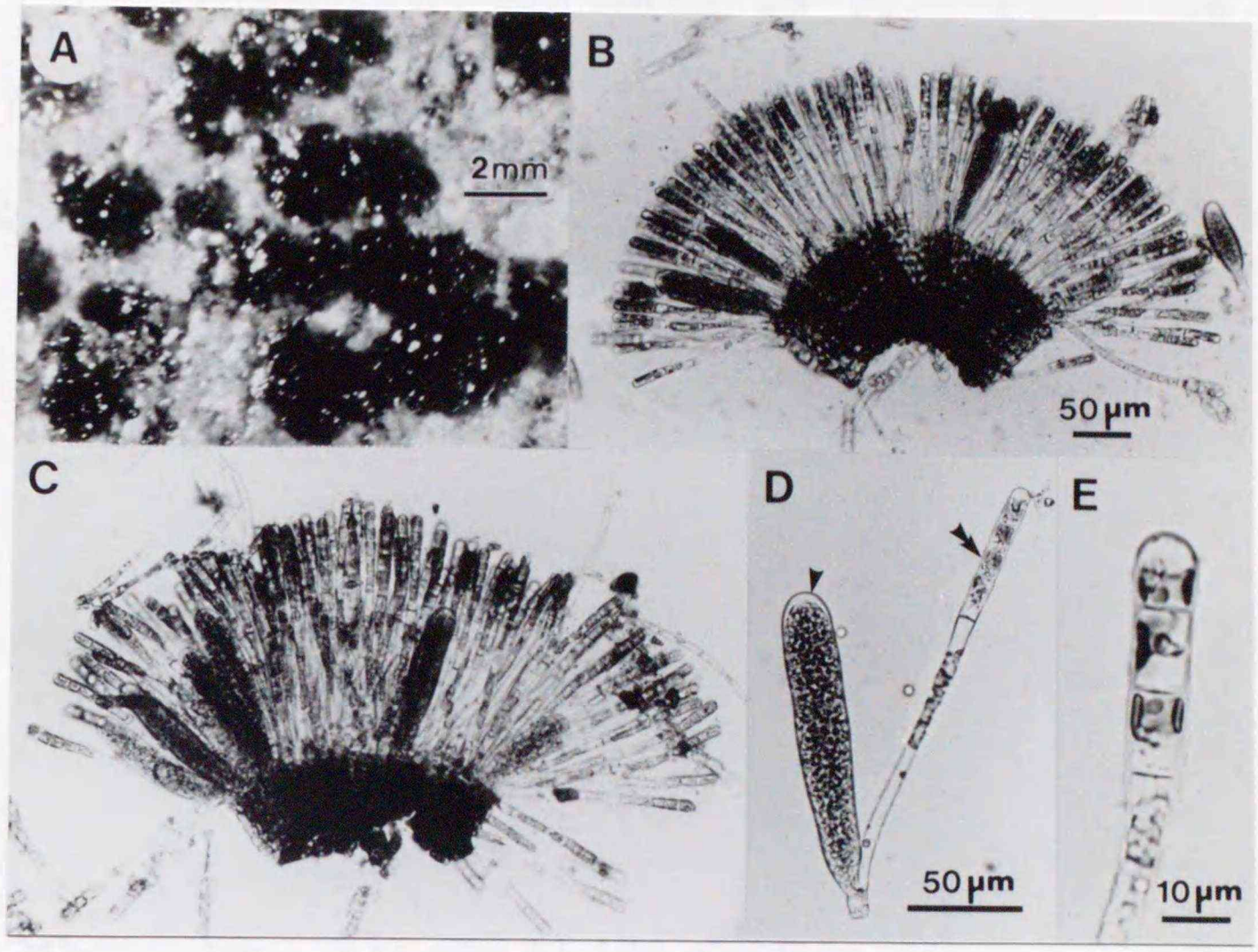


Fig. 105. *Petalonia fascia*. Habit and anatomy of field microthalli.

A. Habit of field microthalli on a stone collected at Oshoro, Hokkaido on 24 October 1990. **B, C.** Squashed thalli bearing unilocular sporangia and paraphyses. **D.** Unilocular sporangium (arrowhead) on a basal cell of paraphysis (double arrowhead). **E.** Paraphysis, showing a plastid with a pyrenoid in each cell.

Scale in **B** applies to **C**.

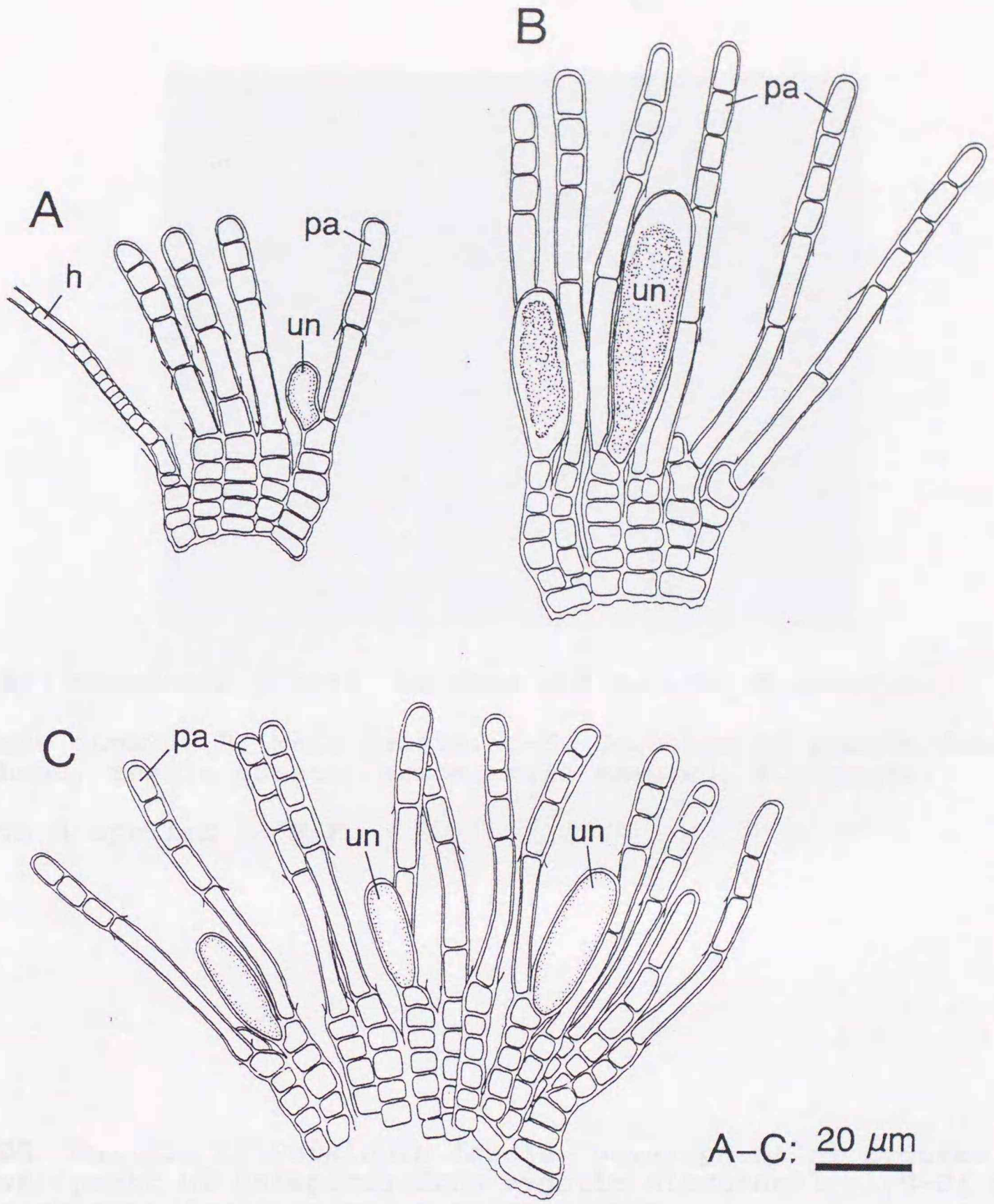


Fig. 106. *Petalonia fascia*. Anatomy of field microthalli.

A. Squashed thallus, showing young unilocular sporangium (un), young paraphyses (pa) and phaeophycean hair (h). **B, C.** Squashed thalli baring unilocular sporangia (un) and paraphyses (pa).

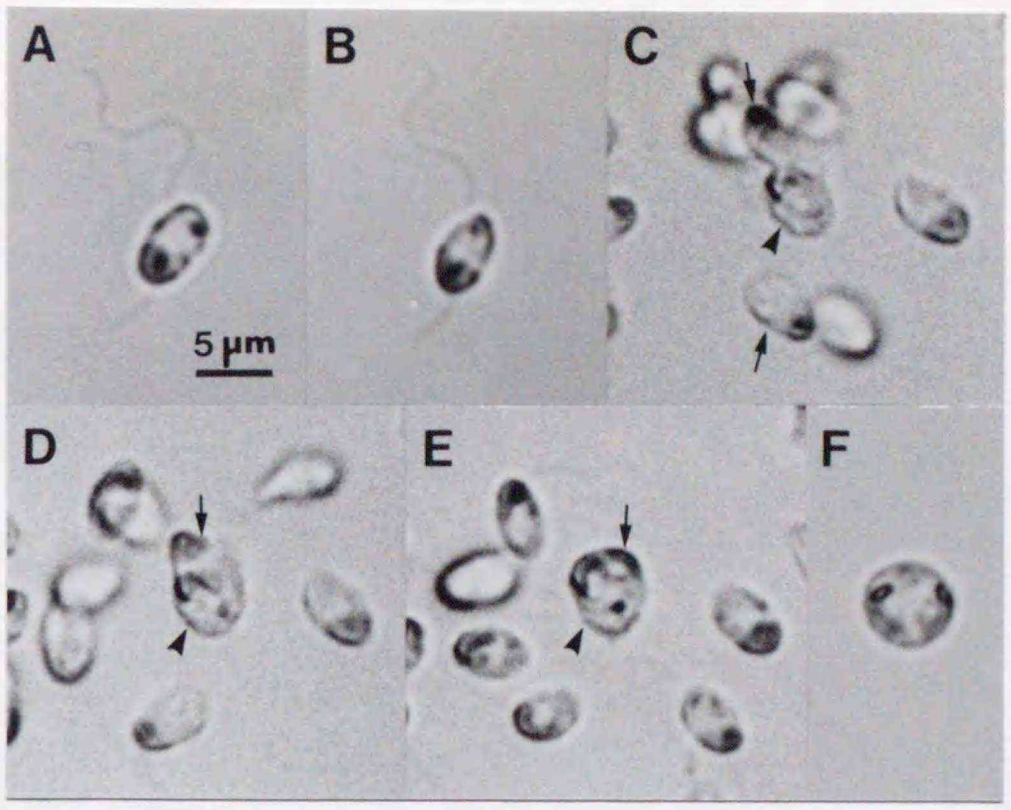


Fig. 107. *Petalonia fascia*. Gametes and fusion of gametes.

A. Female gamete. **B.** Male gamete. **C-E.** Sequence of gamete fusion (arrowhead, female gamete; arrow, male gamete). **F.** Zygote.

Scale in **A** applies to **B-F**.

⇒

Fig. 108. Culture of *Petalonia fascia*. Development of zygotes (**A-F**), development of unispores from zygotic microthallus (**G-J**) and parthenogenetic development of gametes (**K-N**).

A. Two-week-old germling under 10°C LD condition. **B.** One-month-old germling under 10°C LD condition. **C.** Zygotic microthallus under 20°C LD condition. **D.** Squashed discoid microthallus under 10°C SD condition. **E.** Squashed microthallus forming unilocular sporangia (arrowhead) and paraphyses (double arrowhead) under 20°C SD condition. **F.** Unilocular sporangium (arrowhead) on a basal cell of a paraphysis (double arrowhead). **G.** Unispore from zygotic microthallus. **H.** One-week-old germling under 10°C SD condition. **I.** Macrothalli (arrowheads) arising from prostrate part under 10°C SD condition. **J.** Macrothalli under 10°C LD condition. **K.** Nineteen-day-old germling under 10°C LD condition. **L.** One-month-old germling under 10°C LD condition. **M.** Discoid microthallus under 20°C SD condition. **N.** Discoid microthallus issuing macrothalli under 20°C SD condition.

Scale in **A** applies to **E**, **F**, **H** and **K**; Scale in **B** applies to **I** and **L**; Scale in **M** applies to **N**.

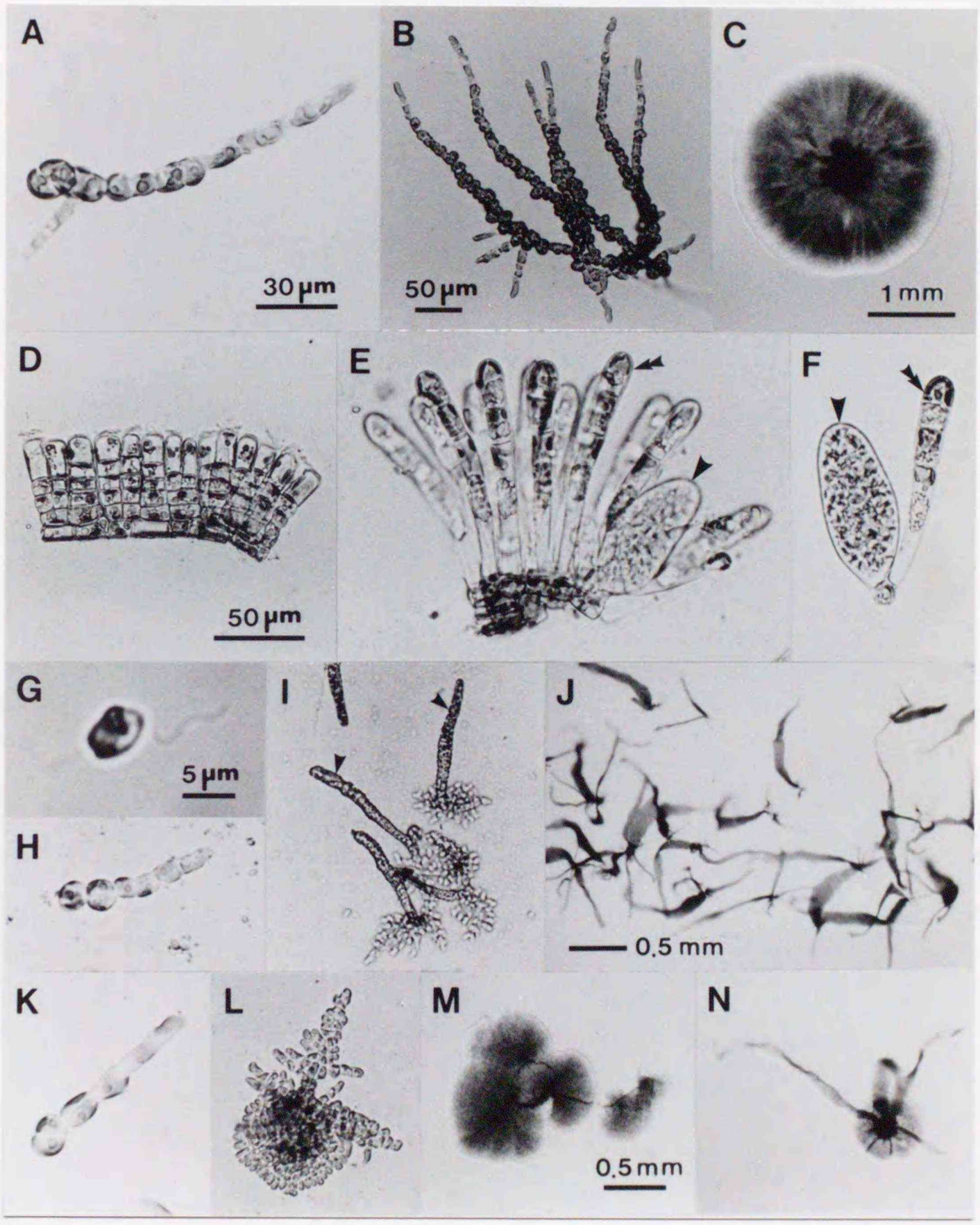


Fig. 108. Culture of *Petalonia fascia*.

⇐

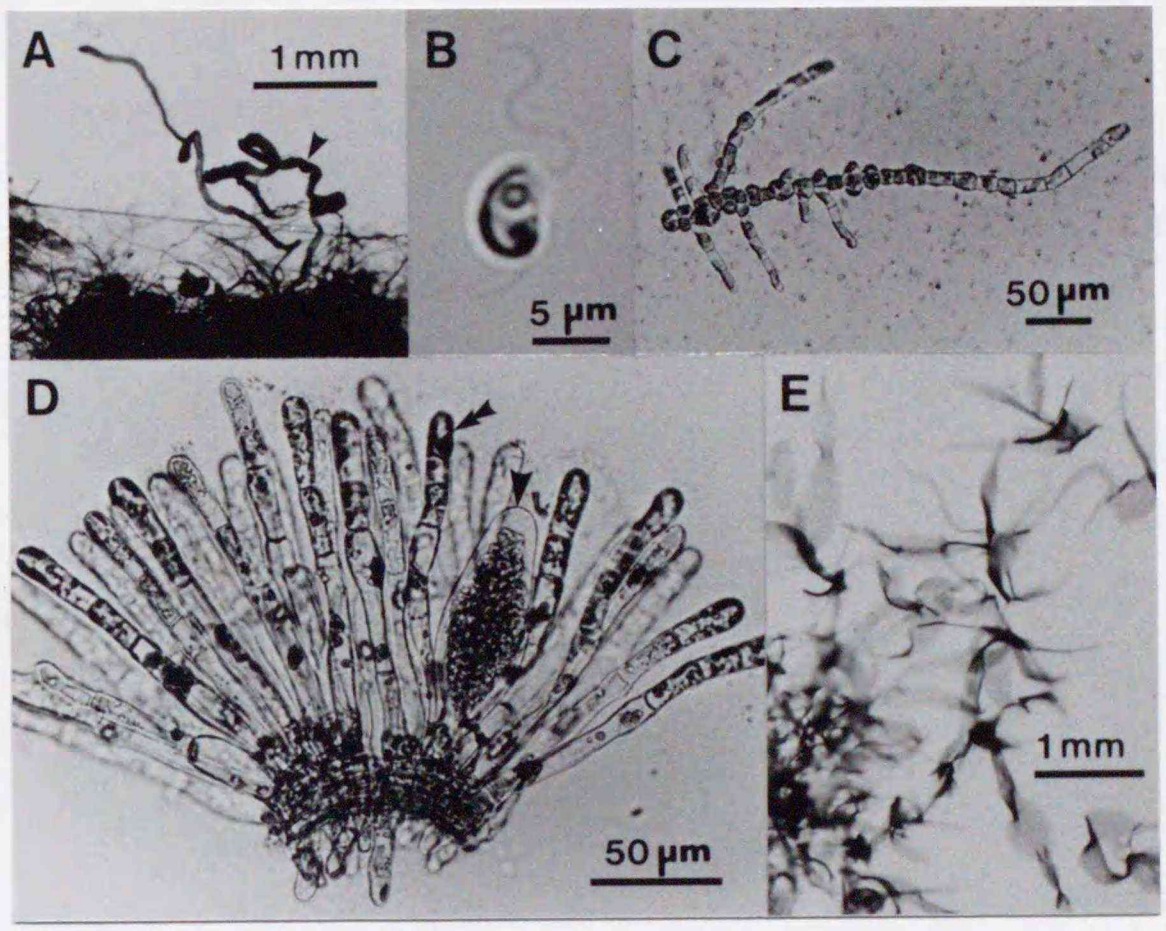


Fig. 109. Culture of *Petalonia fascia*. Progeny from macrothallus on zygotic microthallus.

A. Macrothalli (arrowhead) on filamentous part of zygotic microthallus under 20°C LD condition. **B.** Plurispore from macrothallus on zygotic microthallus. **C.** Two-week-old germling of plurispore from macrothallus on zygotic microthallus under 10°C SD condition. **D.** Squashed microthallus developed from plurispore from macrothallus on zygotic microthallus, showing unilocular sporangium (arrowhead) and paraphyses (double arrowhead). **E.** Macrothalli developed from unispores released from unilocular sporangium on microthallus shown in **Fig. D.**

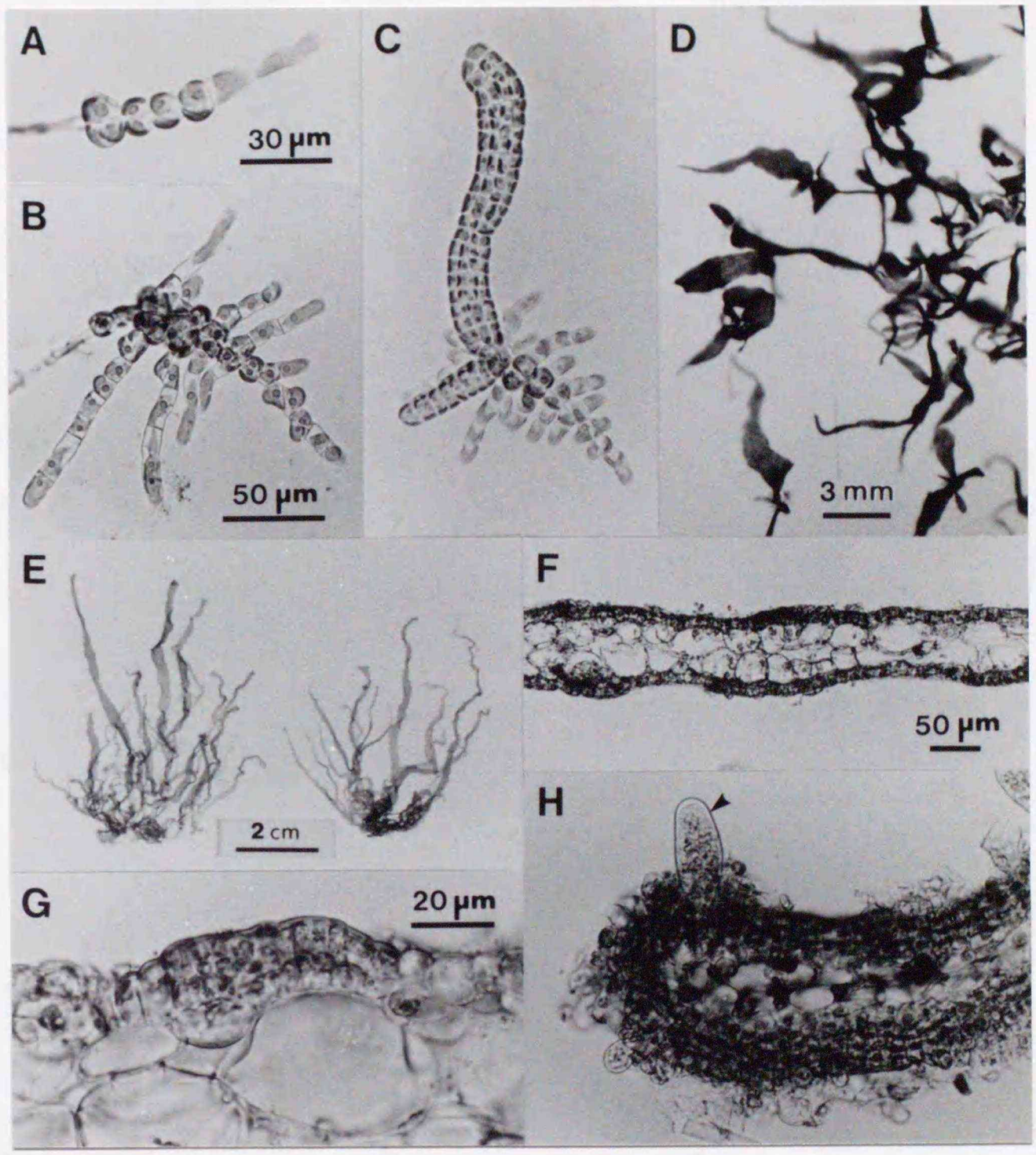


Fig. 110. Culture of *Petalonia fascia*. Development of unispores from field microthalli.

A. Two-week-old germling under 5°C SD condition. **B.** Nineteen-day-old germling under 5°C SD condition. **C.** Nineteen-day-old germling under 5°C SD condition, showing macrothalli arising from prostrate part. **D.** Macrothalli under 10°C SD condition. **E.** Dried specimens of macrothalli cultured under 5°C SD condition. **F.** Cross section of macrothallus. **G.** Plurilocular sporangia in cross section of macrothallus. **H.** Unilocular sporangia (arrowhead) in cross section of macrothallus in culture of strain 351 Hanasaki under 20°C SD condition.

Scale in **B** applies to **C** and **H**.

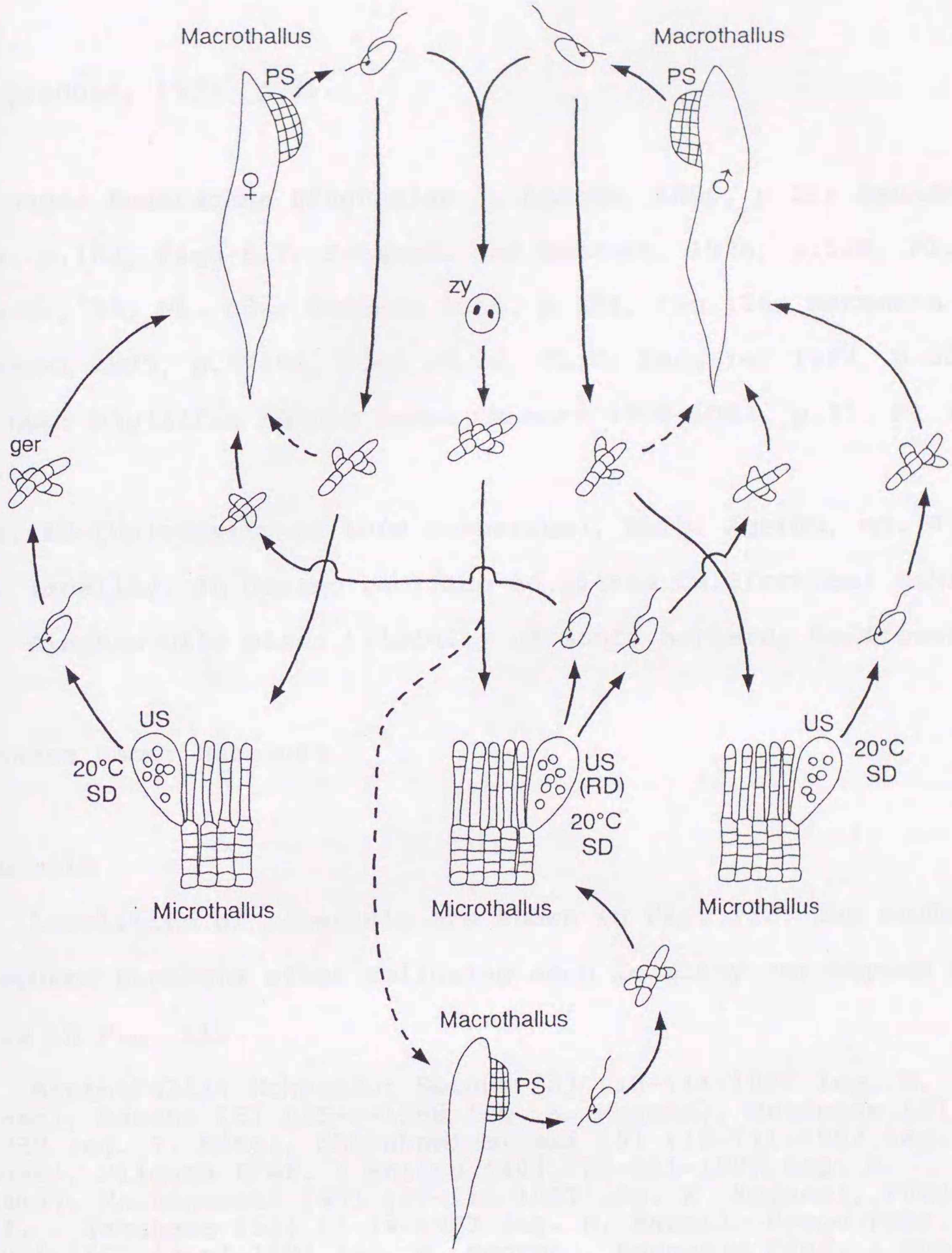


Fig. 111. Diagram of life history of *Petalonia fascia* in culture.

ger, germling; PS, plurilocular sporangium; US, unilocular sporangium; zy, zygote; RD, reduction division; SD, short day condition; broken line, rare course.

Petalonia binghamiae (J.Agardh) Vinogradova

Vinogradova, 1973, p.31.

Basionym: *Endarachne binghamiae* J. Agardh, 1896, p.26; Saunders 1898, p.162, Figs 6,7; Setchell and Gardner, 1925, p.538, Pl. 38, figs 37, 38, Pl. 83a; Okamura 1936, p.233, Fig.126; Nakamura and Tatewaki 1975, p.57-93, Figs 19,20, Pl.V; Seagrief 1967, p.50,51.
Synonym: *Phyllitis fascia* sense Okamura 1900-1902, p.35, Pl.10.

Type: LD (University of Lund Herbarium), Herb. Agardh, no. 47849
Type locality: In Oceano pacifico ad littus Californiae; mihi a Dina Bingham Olim missa (vicinity of Santa barbara, California).

Japanese name: Habanori

Materials

Localities of materials are shown in Fig. 112. The numbers in square brackets after following each locality correspond to those in Fig. 112.

Macrothalli: Hokkaido: Setana [3] (18-iii-1987 leg. K. Kogame), Esashi [5] (15-v-1988 leg. K. Kogame), Matsumae [6] (10-i-1989 leg. I. Mine), Shirakami-misaki [8] (19-iii-1987 leg. K. Kogame), Niigata Pref. : Shiiya [44] (27-iii-1987 leg. K. Kogame), Kashiwazaki [45] (27-iii-1987 leg. K. Kogame), Fukui Pref. : Takahama [51] (1-iv-1987 leg. H. Kawai), Hyogo Pref. : Kasumi [52] (3-vi-1991 leg. K. Kogame), Kanagawa Pref. : Enoshima [68] (26-i-1990 leg. S. Boo), Yamaguchi Pref. : Katayamajima [97] (13-vi-1987 leg. H. Kawai), Ehime Pref. : Mimaizaki [102] (20-iv-1988 leg. S. Enomoto & K. Kogame), Fukuoka Pref. : Tsuyasaki [103] (21-iv-1989 leg. S. Kawaguchi), Saga Pref. : Hadonomisaki [106] (9-iv-1990 leg. M. Masuda).

Herbarium specimens examined are as follows. All specimens were labeled as *Endarachne binghamiae*. Aomori Pref. : Ohma [38] (SAP 028752, 27-iii-1956 leg. S. Kawashima), Niigata Pref. : Ogi, Sado [47] (SAP 053216, 18-v-1963 leg. K. Akiyama), Kanagawa Pref. : Hayama [66] (SAP 054874, 14-i-1979 leg. Tsukidate), Enoshima [68] (SAP 13086, 6-iv-1932 leg. Y. Yamada), Shizuoka Pref. :

Omaezaki [70] (9-i-1969 leg. M. Masuda), Wakayama Pref. : Koza [79] (SAP 051530, 28-iii-1987 leg. T. Yamamoto), Tottori Pref. : Tomari [53] (SAP 054916, 4-vi-1991 leg. R. Kamiyama), Nagasaki Pref. : Nomozaki [108] (SAP 028381, 4-ii-1961 leg. K. Matsubayashi), Kumamoto Pref. : Tomioka [109] (SAP 021764, 11-v-1940 leg. S. Inoh).

Morphology

Macrothalli

Macrothalli are narrowly lanceolate to lanceolate, flat, solid, 5-25 cm in length, 0.5-3.5 cm in width, arising abundantly from a holdfast (Figs 113A-E). Thalli are yellowish brown to brown in color, somewhat firm in texture. The thalli consist of a cortical layer, an outer medullary layer and an inner medullary layer (Figs 114A-E, 115A,B). The cortex is composed of 1-3 layers of small, pigmented cells (Fig. 114G). In cross section the outer medulla is composed of somewhat large cells which are round to ellipsoid, 12-16 × 12-24 µm in size, in longitudinal section their cells are longer in inner portions, up to 70 µm in length (Fig. 114E). The inner medullary layer is composed of rhizoidal filaments which are entangled, sometimes branched, pale in color, 4-9 µm in diameter (Figs 114A-C,E,G, 115A,B). Phaeophycean hairs are grouped, arising from pits (Fig. 114C). Holdfasts are conical, being composed of rhizoidal filaments which issued from the surface of the basal part and are firmly coherent to each other (Fig. 114F). Thalli became fertile basipetally, forming plurilocular sporangia by divisions of surface cells. Plurilocular sporangia are firmly packed, and covered with a cuticle (Figs 114H, 115B). Ascocysts are absent among them. Secondary plurilocular sporangia are common.

Habitat and phenology

Macrothalli grow on rocks or concrete blocks generally in the middle to lower intertidal zone in exposed area. They are found in season from winter to early summer.

Geographical distribution

This species is distributed in warm temperate coasts of the north Pacific Ocean (California; Setchell and Gardner 1925, Japan; Okamura 1936) and in South Africa (Seagrief 1967, 1984). In Japan, this species is found on the coasts of southern Hokkaido, Honsyu and Kyusyu in the Sea of Japan, and on the Pacific coasts of central and southern Honsyu, Shikoku and Kyusyu, and on the coasts of Seto Inland Sea.

Culture study

Culture strains are listed in Table 51 in which culture conditions and progeny of plurispores are also shown.

Sexual reproduction

Fusions of gametes were observed in materials collected at Kasumi on 3 June 1991.

Sexual reproduction of this species was isogamy. Female gametes released from plurilocular sporangia on macrothalli were pear drop-shaped, 5.8-7.0 × 3.8-4.2 μm in length and width, laterally biflagellated with a longer anterior and a shorter posterior flagella (Fig. 116A). They contained a plastid with a pyrenoid and a stigma. Male gametes were the same shape as female gametes, 5.8-6.4 × 3.6-4.2 μm in size (Fig. 116B). Female and male gametes showed negative phototaxis. Settled female and male gametes were round, 4.0-5.0 μm in diameter. When female and male

gametes were mixed, a settled female gamete was surrounded by several male gametes which touched the settled female gamete by anterior flagella, and then, one of male gametes fused with the female gamete (Figs 116C-F). Zygotes were distinguished from unfused gametes by possessing two stigmata. The fragrant odor of sexual pheromone was observed in culture where female gametes were released.

Development of Zygotes

Zygotes, formed by fusions of gametes under a 15°C SD condition, were cultured under 20°C LD (Table 52). Their germlings were at first prostrate branched filaments and later developed into disks (Fig. 117A). The disks consisted of basal branched filaments and simple, upright filaments arising from them (Fig. 117B). Margins of disks were smooth. After 2 months the disks began to be free from the substrata. After 4 months they formed unilocular sporangia which were lateral on supporting cells of paraphyses (Figs 117C,D).

Development of unispores from zygotic disks

Unilocular sporangia, which formed on zygotic disks under a 20°C LD condition, were isolated from zygotic microthallus, and cultured under 10°C LD, 15°C SD, 15°C LD, 20°C SD and 20°C LD conditions (Table 53). Unispores (Fig. 118A) from isolated unilocular sporangia germinated to small disks (Figs 118B,C) which gave rise to macrothalli (Figs 118D,E). Disks did not spread further after formation of macrothalli. Under 15°C SD after 4 months macrothalli grew to 10 cm in length and 4 mm in width, and produced plurilocular sporangia (Figs 118F-H).

Macrothalli under 10°C LD and 20°C LD became fertile after 5 months and 3 months, respectively. Female and male thalli were observed under 15°C SD in culture which were started from one unilocular sporangium. Determinations of sexuality were carried out by observations of gamete fusions.

Parthenogenetic development

Settled female gametes were cultured at 15°C and 20°C under SD and LD conditions (Table 51; 360 Kasumi). Their germlings were at first prostrate branched filaments and later became small disks. All small disks gave rise to macrothalli under all conditions examined. The small disks ceased to grow further. Macrothalli grew to 2-3 cm in length and 3 mm in width, and produced plurilocular sporangia which were firmly packed without ascocysts. Plurispores represented gametes whose sexuality was the same as the original gametes, when it was ascertained.

Parthenogenetic development of male gametes (strain 359 Kasumi) was almost the same as that of female gametes.

Discussion

Petalonia binghamiae (J. Agardh) Vinogradova (1973) were first described as *Endarachne binghamiae* J. Agardh from California, establishing the monotypic genus *Endarachne* (J. Agardh 1896). Although *Endarachne* was distinguished from *Petalonia* by having entangled medullary filaments, Vinogradova (1973) observed that *P. fascia* and *P. zosterifolia* also have such rhizoidal filaments in the medulla. Therefore, Vinogradova (1973) proposed a combination of *Petalonia binghamiae* (J. Agardh) Vinogradova, placing *Endarachne* as a synonym of *Petalonia*. The

name of *E. binghamiae* is generally used for this species, owing to that Vinogradova's report in Russian publication has not been widely known.

Also in this observations of *P. fascia*, rhizoidal filaments in the medulla were sometimes found, especially abundant in materials from Akkeshi. It was difficult to distinguish such materials from *P. binghamiae*. However, I found that the width of the rhizoidal filaments is different between the two species: 4-9 μm in *P. binghamiae*, 8-14 μm in *P. fascia*.

Sexual reproduction of this species has been reported only by Kunieda and Suto (1948) and Nakamura and Tatewaki (1975). This species is isogamy in their results. Life history studies in culture have been carried out by Wynne (1969), Nakamura and Tatewaki (1975), and Clayton (1982). Wynne (1969) and Clayton (1982) reported a life cycle in which macrothalli directly arose from disks. Nakamura and Tatewaki (1975) reported a complete life history which is dimorphic and biphasic.

A diagram of life history of this species in the present culture is shown in Fig. 119. The results of the present culture study nearly agree with those of Nakamura and Tatewaki (1975). However, zygotic microthalli did not form unilocular sporangia under long day conditions in culture of Nakamura and Tatewaki (1975), whereas in this study they formed unilocular sporangia under a 20°C LD condition. The result, that female and male thalli were obtained in culture of one unilocular sporangium formed on zygotic microthalli, suggests that reduction division may occur in the unilocular sporangium.

In the present culture, microthalli were shown to be *Stragularia*-like, and they resembled those of *Petalonia fascia*.

These results agree with those of Nakamura and Tatewaki (1975). Although field microthalli of this species have not been known, they are also presumed to be *Stragularia*-like.

Table 51. Collection data of culture strains of *Petalonia binghamiae*. Culture conditions tested and progeny of plurispores from macrothalli are also shown.

strain no.	locality	date	sex	culture conditions	progeny
19	Takahama	1 iv 1987		5-15SL 20SL	macrothalli(P) disks
39	Katayamajima	13 vi 1987		10L	macrothalli
108	Kurotsuzaki	19 iv 1988		10L	disks
111	Mimaizaki	20 iv 1988		10L	macrothalli(P)
122	Esashi	15 v 1988		10L	macrothalli
198	Matsumae	10 i 1989		5S	macrothalli
203	Enoshima	26 i 1990		10L	disks
223	Hadonomisaki	9 iv 1989		10L	disks
226	Hadonomisaki	9 iv 1989		10L	macrothalli(P)
236	Tsuyazaki	3 v 1989		15L	macrothalli
359	Kasumi	3 vi 1991	male	15SL, 20SL	macrothalli(P)
360	Kasumi	3 vi 1991	female	15SL, 20SL	macrothalli(P)

Table 52. Progeny of zygotes in culture of *Petalonia binghamiae*.

strain no.	locality	culture conditions	progeny
360 Kasumi female x 359 Kasumi male		20L	disks (U)

Table 53. Culture of *Petalonia binghamiae*. Progeny of unispores from zygotic microthalli.

strain no.	locality	culture conditions	progeny
360 Kasumi female X 359 Kasumi male		10L, 15SL, 20L	macrothalli (P)

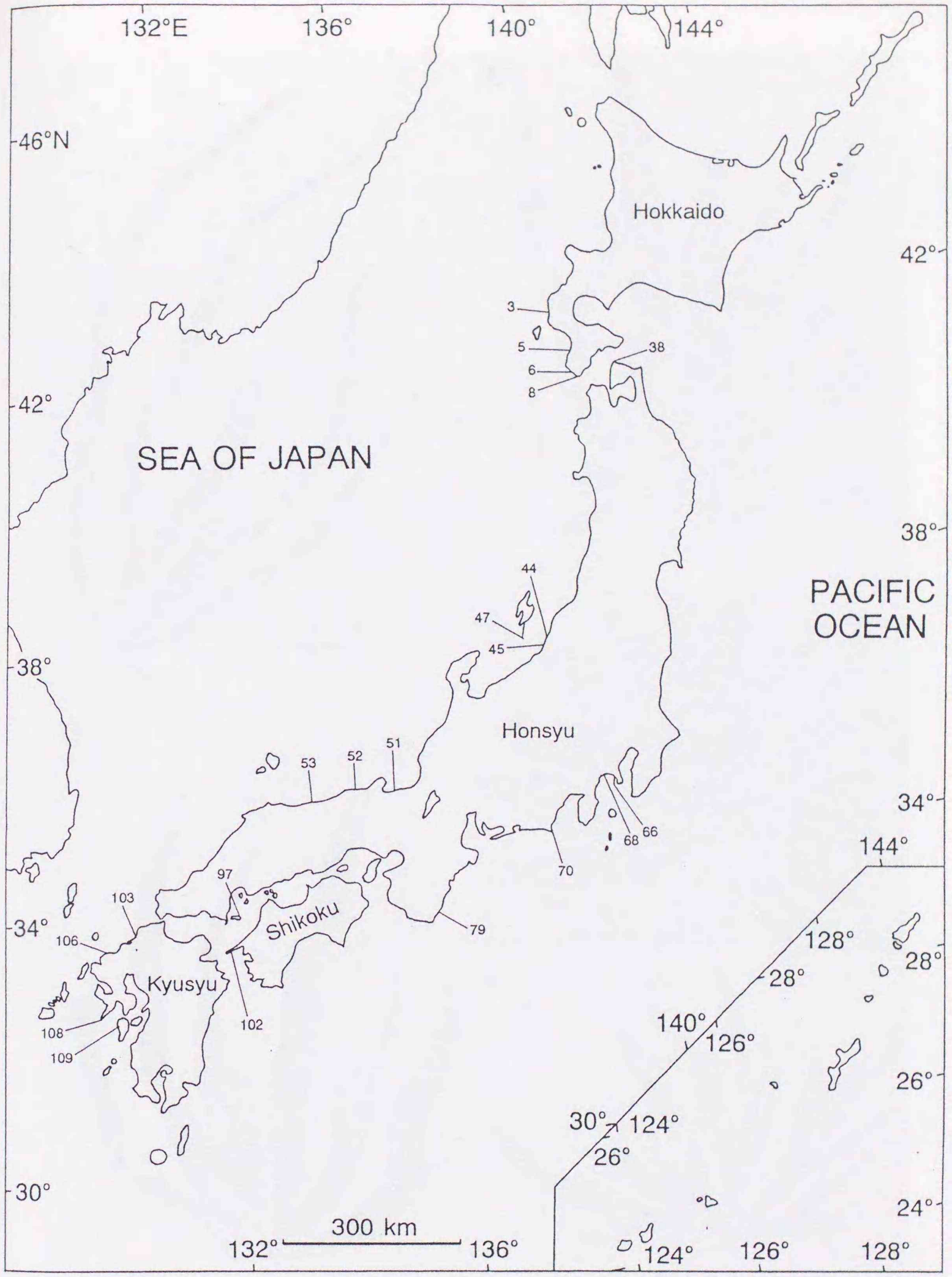


Fig. 112. Localities where materials of *Petalonia binghamiae* were collected. Numbers plotted correspond to those in Table 1.

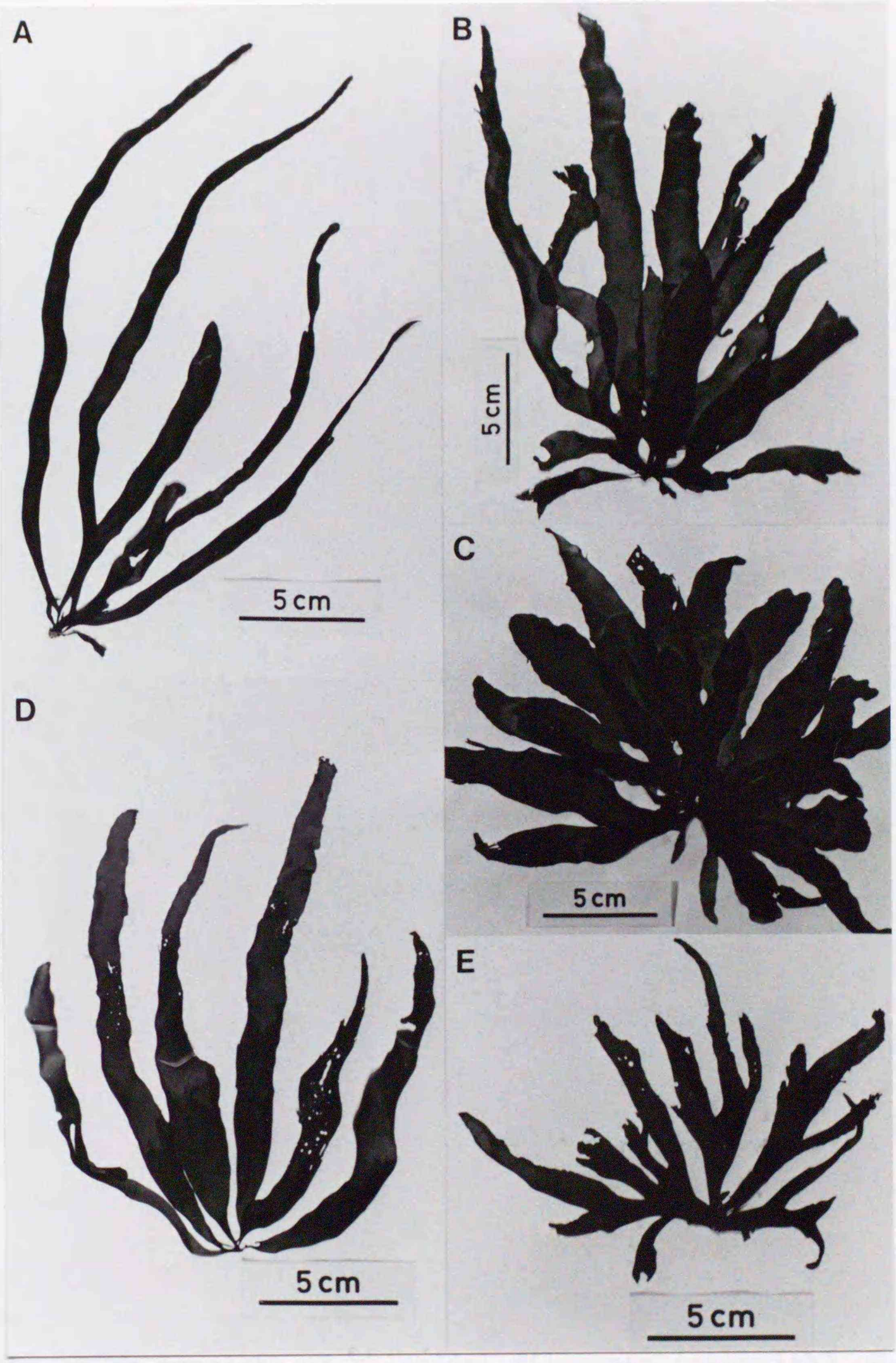


Fig. 113. Habit of dried specimens of *Petalonia binghamiae*.

A. Plant collected at Kasumi, Hyogo Pref. on 3 June 1991. B, C. Plants collected at Hadonomisaki, Saga Pref. on 9 April 1989 (leg. M. Masuda). C. Plant collected at Shirakamimisaki, Hokkaido on 19 March 1987. D. Plant collected at Esashi, Hokkaido on 15 May 1988.

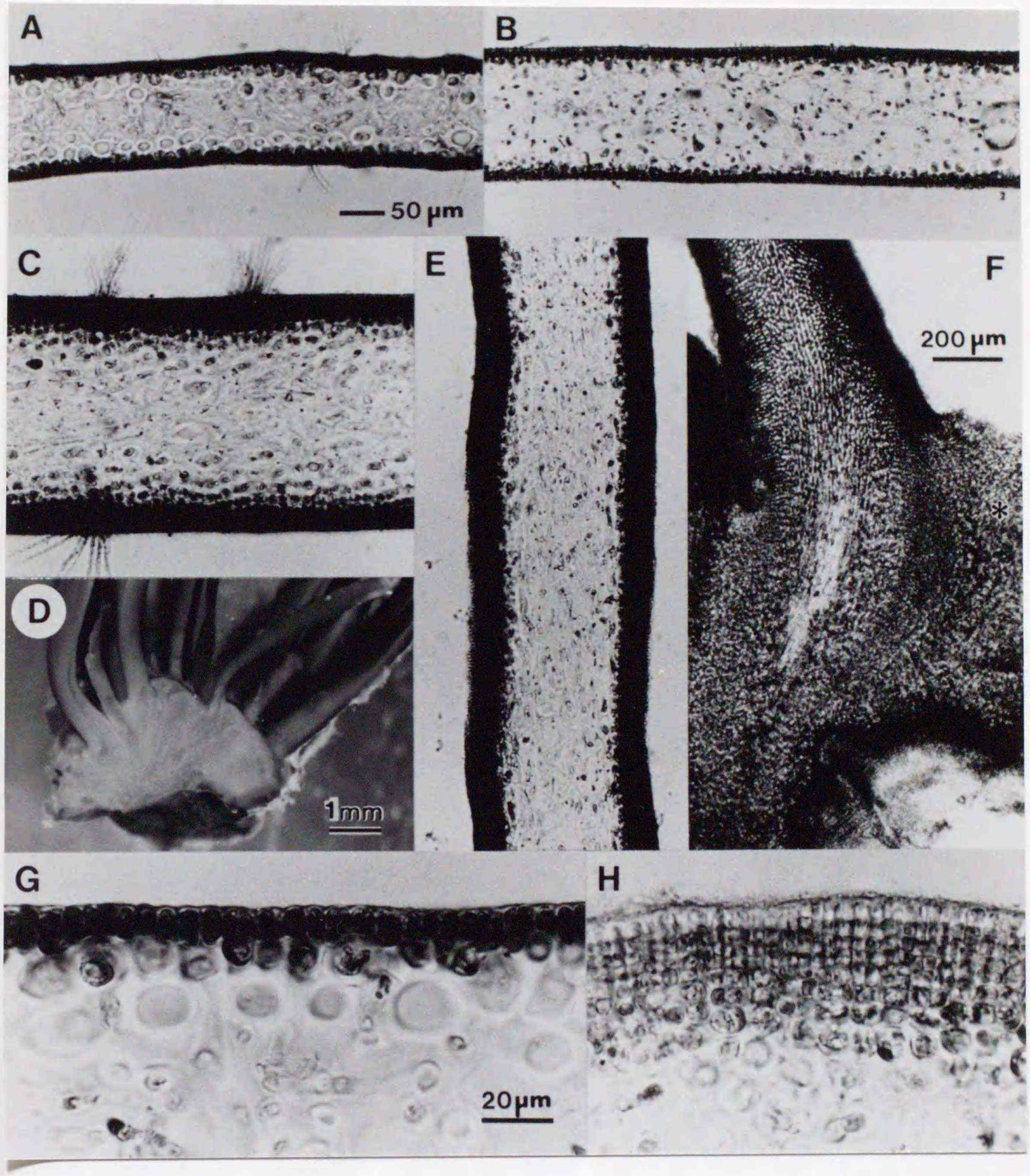


Fig. 114. Anatomy of *Petalonia binghamiae*.

A, **B**. Cross sections of vegetative thalli. **C**. Cross section of fertile thallus, showing phaeophycean hair tufts. **D**. Longitudinal section of holdfast. **E**. Longitudinal section of fertile macrothallus. **F**. Longitudinal section of holdfast, showing holdfast which consists of rhizoidal filaments (asterisk). **G**. Cross section of vegetative thallus, showing cortical cells. **H**. Plurilocular sporangia in cross section of thallus.

Scale in **A** applies to **B**, **C** and **E**; Scale in **G** applies to **H**.

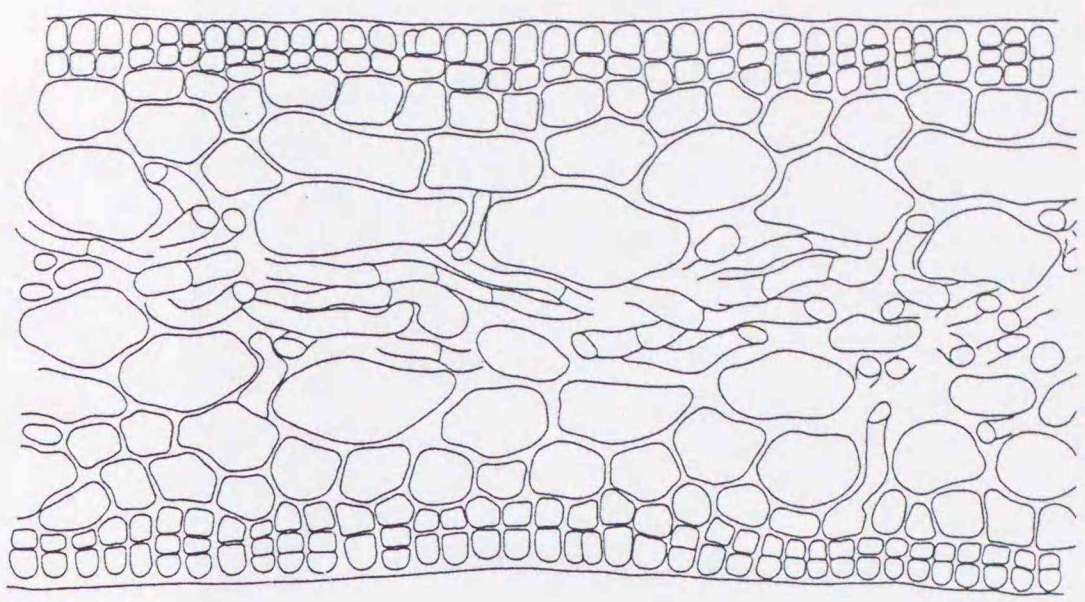


Fig. 115. Anatomy of *Petalonia binghamiae*.

A. Cross section of vegetative thallus.
B. Cross section of fertile thallus with plurilocular sporangia (pl).

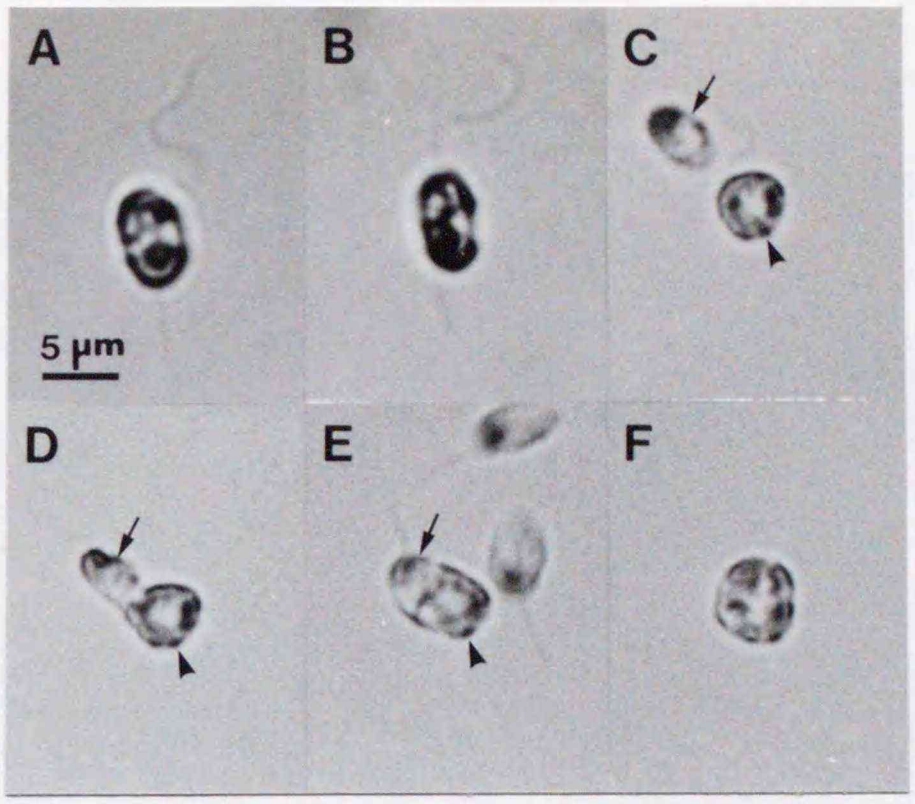


Fig. 116. *Petalonia binghamiae*. Gametes and sequence of gamete fusion.

A. Female gamete. **B**. Male gamete. **C-F**. Sequence of gamete fusion (arrowhead, female gamete; arrow, male gamete).

Scale in **A** applies to **B-F**.

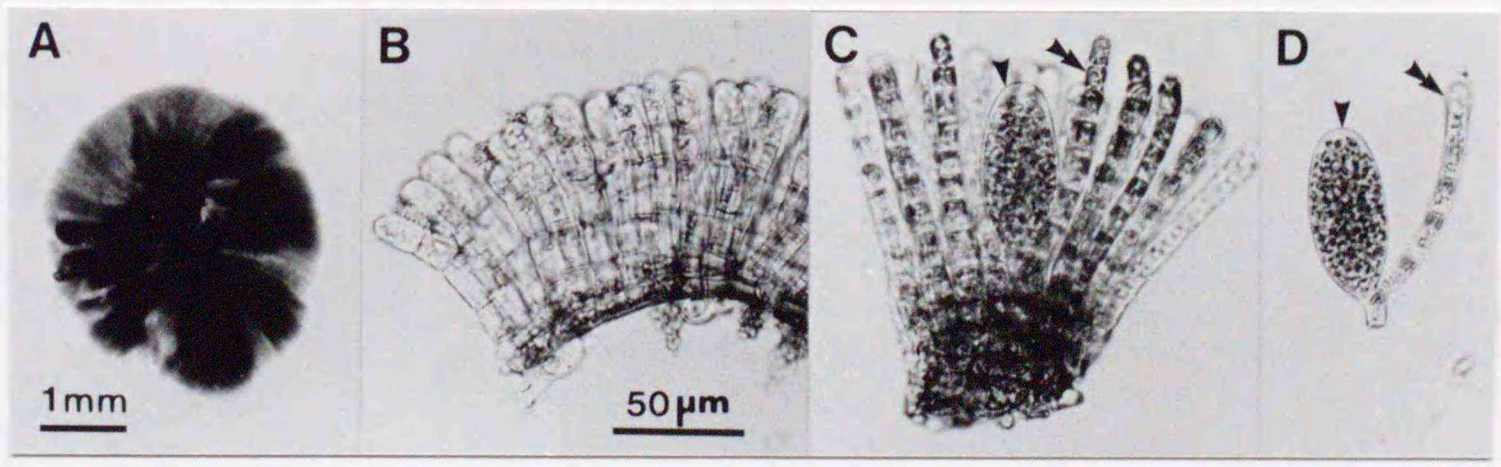


Fig. 117. Culture of *Petalonia binghamiae*. Zygotic microthalli.

A. Discoid thallus. **B**. Squashed discoid thallus. **C**. Squashed thallus forming unilocular sporangium (arrowhead) and paraphyses (double arrowhead) under 20°C LD condition. **D**. Unilocular sporangium (arrowhead) on a basal cell of a paraphysis (double arrowhead).

Scale in **B** applies to **C** and **D**.

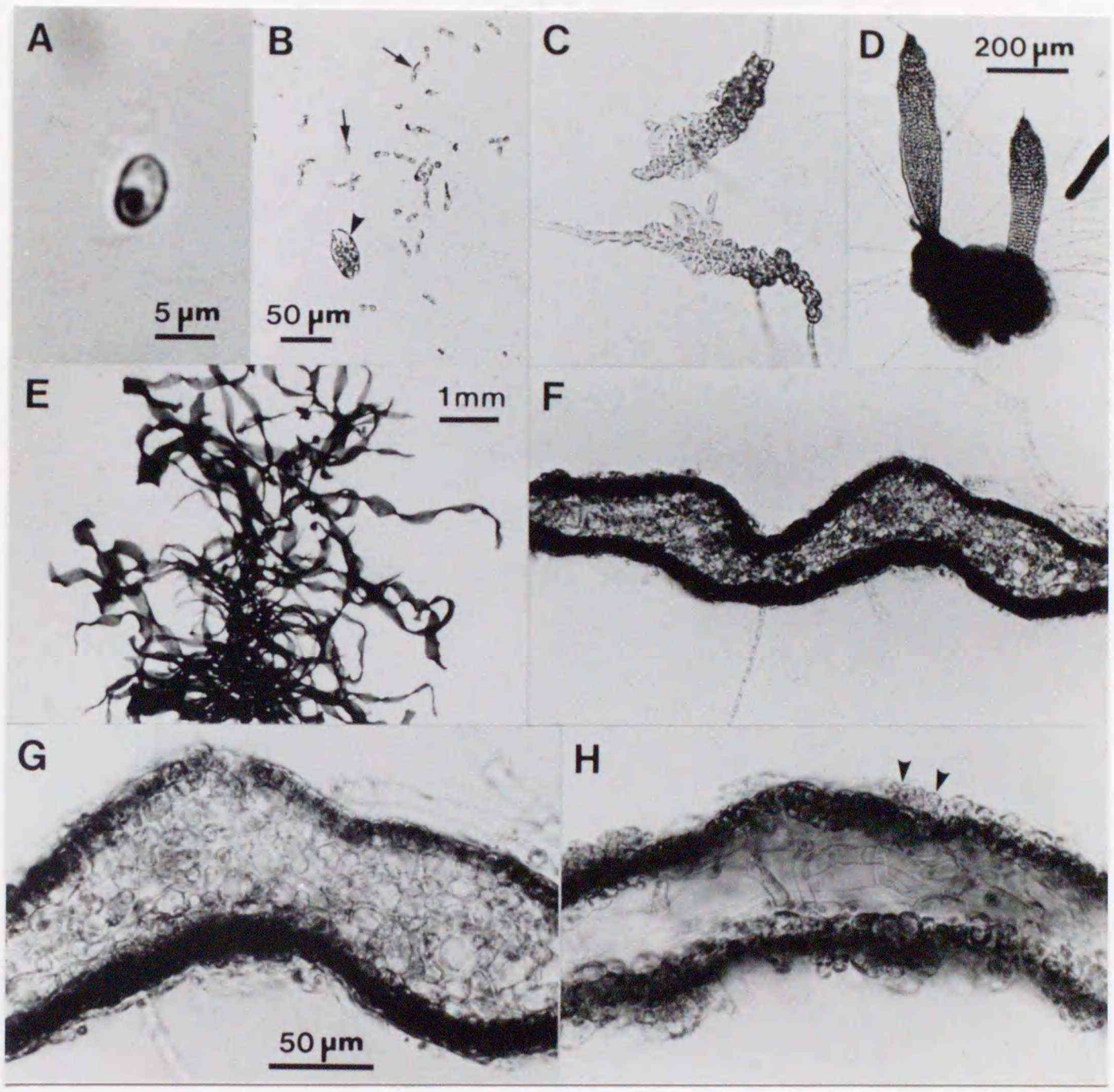


Fig. 118. Culture of *Petalonia binghamiae*. Development of unispores from zygotic microthallus.

A. Unispore. **B.** Three-day-old germlings of unispores under 20°C LD condition and unilocular sporangium from which the unispores were released. **C.** Two-week-old germlings under 15°C LD condition. **D.** Three-week-old small discoid thallus issuing macrothalli under 15°C LD condition. **E.** One-month-old thalli under 15°C LD condition, showing macrothalli. **F, G.** Cross section of macrothalli, showing rhizoidal filaments in medulla. **H.** Cross section of fertile macrothallus with plurilocular sporangia (arrowheads).

Scale in **B** applies to **C** and **F**; Scale in **G** applies to **H**.

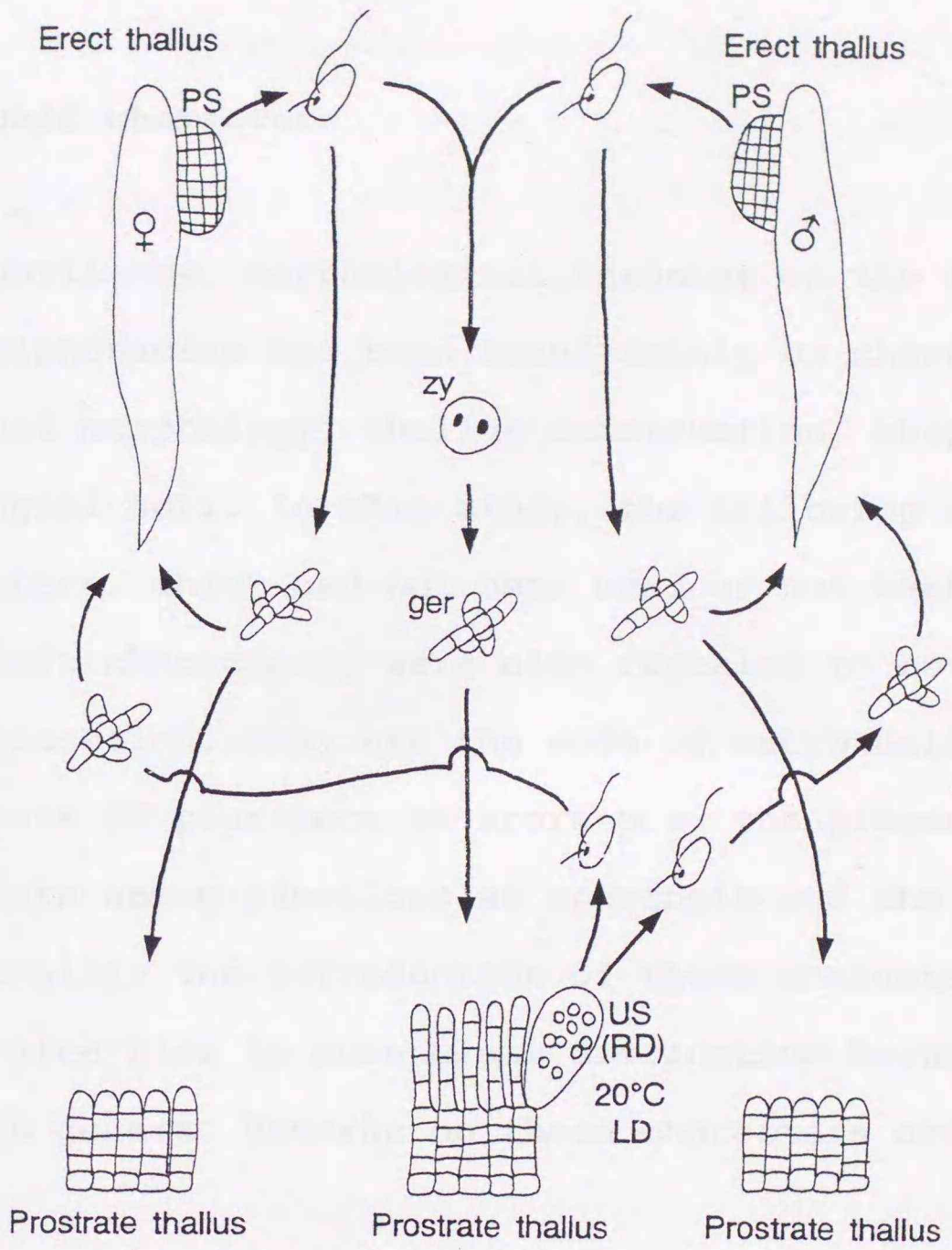


Fig. 119. Diagram of life history of *Petalonia binghamiae* in culture.

ger, germling; PS, plurilocular sporangium; US, unilocular sporangium; zy, zygote; RD, reduction division; LD, long day condition.

GENERAL DISCUSSION

Taxonomic characters

Until now, morphological taxonomy on the family Scytosiphonaceae has been based mainly on characters such as external morphology, thallus construction, shape of plurilocular sporangial sori. In this study, the following morphological characters, which had not been used or not been evaluated as taxonomic characters, were also revealed to be useful in the classification. They are the mode of macrothallus initiation, the structure of plurilocular sporangia, the presence or absence of ascocysts among plurilocular sporangia and the morphology of microthalli. The introduction of these characters by this study could give rise to more clear distinction between species or between genera. Remarks on these characters are mentioned below.

Initiation of macrothalli

Two types of macrothallus initiation are recognized in the species of the family Scytosiphonaceae examined in this study. The first type is named the uniseriate type (Figs 120A-D). In this type, initials of macrothalli are uniseriate filaments whose cells longitudinally divide to form polystichous macrothalli. In the second type, named the spherical type (Figs 120E-I), small microthalli become swollen to form spherical thalli.

The uniseriate type is represented in the species of *Scytosiphon* and *Petalonia* (Wynne 1969, Nakamura and Tatewaki 1975, present study). Macrothalli in these species are cylindrical or flat, linear to lanceolate, and generally several

to many macrothalli arise gregariously from the base. On the other hand, the spherical type is represented in species of *Colpomenia* and *Hydroclathrus* (Wynne 1972, Nakamura and Tatewaki 1975, Clayton 1979, present study). Although *Colpomenia bullosa* has tubular thalli, the tubular thalli are formed secondary by elongation from the saccate base which is first spherical in shape. In *Hydroclathrus clathratus*, spherical thalli are formed at first, later they became perforated. In the spherical type, one plant is generally consist of one spherical thallus as observed in the present culture.

Wynne and Norris (1976) distinguished *Colpomenia* from *Scytosiphon* in respects of initial morphology of macrothalli. However, this characteristic of macrothalli is considered to be more important as a taxonomic feature since it might be useful to divide the Scytosiphonaceae into two groups at family level.

Plurilocular sporangia on macrothalli and cuticular covering

Two types are recognized in the structure of plurilocular sporangia on macrothalli. One type is called the loose type where columns of plurilocular sporangia are loosely adhered to each other (Figs 120A-C). The columns of plurilocular sporangia are easily separable under pressure. The species of *Colpomenia*, *Hydroclathrus clathratus*, *Scytosiphon lomentaria* and *S. nakamurae* have this type of plurilocular sporangia. Another type is called the packed type where columns of plurilocular sporangia are firmly coherent to each other, being covered with a cuticle layer (Figs 120D-F). *Scytosiphon gracilis*, *S. tenellus* and the species of *Petalonia* have this type of plurilocular sporangia.

The structure of plurilocular sporangia (loose type or

packed type) are useful at least for the taxonomy of species level. In the early stage of development plurilocular sporangial adhesion of cells which change to them is firm, and a cuticle layer on the surface of thalli still remains in all the species examined (Fig. 121B,E). Later, in the loose type, plurilocular sporangia become loosely adhering to each other, and the cuticle layer peels off from these plurilocular sporangia (Fig. 121C). Although *C. sinuosa* has plurilocular sporangia of this loose type, a cuticle layer remains until plurilocular sporangia become mature: it is described that the cuticular covering on plurilocular sporangia is present in *C. sinuosa*. In other species having the loose type the cuticle peels off before maturation of plurilocular sporangia: it can be described that a cuticle layer on plurilocular sporangia is absent. On the other hand, in the packed type a cuticle layer do not peel off from plurilocular sporangia even when they are mature (Fig. 121F). In this type, plurispores are released from a pore in the cuticle or from parts where they had been released and the cuticle had peeled off already.

Ascocysts

Ascocysts are usually enlarged or elongated cells, initially pigmented, and packed with fucosan vesicles, and later often become empty or reddish in color (Fritsch 1945, Fletcher 1987). Ascocysts are distributed in the genera *Myrionema*, *Chilonema*, *Pseudolithoderma*, *Petroderma*, *Symphyocarpus*, *Laminariocolax*, etc. In Scytosiphonales, they are encountered in the genera *Colpomenia* and *Scytosiphon*, and have been generally called paraphyses owing to being located among plurilocular sporangia on macrothalli.

However, the paraphyses contain fucosan vesicles in rich in the same manner as ascocysts in other brown algae. Fletcher (1987) calls ascocyst-like cells for the paraphyses of *Colpomenia* and *Scytosiphon*.

Such cells containing many fucosan vesicles, including the paraphyses among plurilocular sporangia, are called ascocysts in this study. Ascocysts are also observed on microthalli in species of *Colpomenia* (*C. sinuosa*, *C. peregrina* and *C. bullosa*). The amount of ascocysts are often variable with individual thalli. However, the presence or absence of ascocysts is consistent in each species examined in the present study. This character is useful at least for the taxonomy at species level.

Morphology of microthalli

Microthalli have been revealed by previous observations in culture and in fields in some species of Scytosiphonales. Microthalli bearing terminal unilocular sporangia are known in culture of Australian *Colpomenia peregrina* (Clayton 1979). Fletcher (1981) mentioned that microthalli in culture of *Petalonia filiformis* resemble *Compsonema saxicolum* (Kuckuck) Kuckuck. Microthalli of *Scytosiphon lomentaria* are identified as *Microspongium gelatinosum* Reinke in fields (Lund 1966, McLachlan *et al.* 1971, Fletcher 1978). Microthalli of *Petalonia fascia* in fields represent plants identified as *Stragularia clavata* (Harvey) Hamel (Edelstein *et al.* 1979, Rhodes and Connel 1973, Roeleveld *et al.* 1974, Fletcher 1978) in Europe and as *Ralfsia clavata* Setchell and Gardner (Wynne 1969) in California. Microthalli are morphologically different from species to species as mentioned above. Morphology of microthalli in other species

has not been known.

Field microthalli of the following eight species were observed in this study: *Colpomenia peregrina*, *C. bullosa*, *Scytosiphon lomentaria*, *S. nakamurae*, *S. gracilis*, *S. tenellus*, *Petalonia zosterifolia* and *P. fascia*.

Microthalli of *Colpomenia peregrina* are minute discoid in shape, consisting of branched prostrate filaments and simple, upright filaments arising from them, and they produce plurilocular or unilocular sporangia on the prostrate filaments or terminally on the upright filaments. They also produced ascocysts. Although microthalli of *C. sinuosa* were not found in field observations, they were morphologically similar to those of *C. peregrina* in culture. Therefore, also in fields they may be similar to those of *C. peregrina*.

Microthalli of *Scytosiphon gracilis* and *Petalonia zosterifolia* can be identified as *Compsonema saxicolum* whose thalli represent minute tufts of simple, upright filaments arising from prostrate filaments. The microthalli produce unilocular sporangia usually on prostrate filaments with a pedicel.

Microthalli of *Scytosiphon lomentaria*, *S. tenellus* and *Petalonia fascia* are discoid in shape, consisting of prostrate, branched filaments and simple, upright filaments which are loosely or firmly adhered by side. When fertile, upper parts of upright filaments elongate into paraphyses, and unilocular sporangia are formed laterally on basal cells of the paraphyses. Although microthalli of *P. binghamiae* were not observed in field materials, they morphologically resemble those of *P. fascia* in the present culture. They are expected to represent similar

morphology to those of *P. fascia* also in field.

Microthalli are firm, crustose or hapteron-like in *Colpomenia bullosa* and *Scytosiphon nakamurae*, consisting of upwardly and downwardly curved, branched filaments. Unilocular sporangia are formed laterally on basal cells of paraphyses which are elongated and differentiated from surface cells of thalli.

Four types of microthalli are recognized as mentioned above (Figs 121A-E). They are *Colpomenia peregrina* type (*C. sinuosa*, *C. peregrina*), *Compsonema* type (*S. gracilis*, *P. zosterifolia*), *Microspongium-Stragularia* type (*S. lomentaria*, *S. tenellus*, *P. fascia*, *P. binghamiae*) and *Hapterophycus* type (*C. bullosa*, *S. nakamurae*).

Forms of microthalli are not always uniform in culture. For example, filamentous microthalli have been observed in addition to discoid thalli in culture of *Scytosiphon lomentaria* and *Petalonia fascia* (Wynne 1969, Nakamura and Tatewaki 1975, etc.). Also in this study filamentous thalli in addition to discoid thalli appeared in culture of *S. lomentaria* and *P. fascia*. There are some evidences that environmental factors, such as concentrations of iodine (Hsiao 1969), phosphate, nitrate (Roberts and Ring 1972), quality and intensity of light (Roberts and Ring 1972, Lüning and Dring 1973), and degree of attachment to the substratum and crowding (Wynne 1972b), influence the morphology of microthalli. Therefore, these filamentous microthalli in culture of *S. lomentaria* and *P. fascia* are considered to represent expressions of the algae under stressful or unfavorable growth conditions (Wynne 1972b). Although microthalli of *Compsonema* type in culture are described to be filamentous, they are essentially different from filamentous

expressions of microthalli of *S. lomentaria* and *P. fascia* mentioned above. They are not expressions which failed to become disks owing to unfavorable culture conditions, since in field they are also morphologically identified with *Compsonema saxicolum* which represents tufted thalli.

Although microthallus morphology has not been used as a taxonomic character, it is useful as the character at least at species level in the Scytosiphonaceae.

Life history

Life histories of the species of Scytosiphonaceae have been considered to be basically heteromorphic, alternating between large gametophyte (macrothallus) bearing plurilocular sporangia and small sporophyte (microthallus) bearing unilocular sporangia (Tatewaki 1966, Nakamura and Tatewaki 1975, Clayton 1979, 1980). However, most reports of culture studies on the species of this family did not show sexual reproduction. A direct type life history in which plants reproduce only with macrothalli have been reported by several authors (Clayton 1982 for review).

In life histories of scytosiphonaceous species, several reports reveal that formation of macrothalli are induced by photoperiods under a certain range of temperature (Tatewaki 1966, Wynne 1969, Nakamura and Tatewaki 1975, Dring and Lüning 1975 etc.). Although these responses to photoperiods may account for seasonal occurrence of macrothalli, some reports represent the case that photoperiods do not influence formation of macrothalli (Clayton 1976a, b, Kristiansen and Pedersen 1979, Pedersen 1980). From these features, one would know that photoperiodic and non-

photoperiodic races adapting respective local environment are present even in single species.

From the present results of culture studies, three patterns are recognized among heteromorphic life histories (Fig. 123). Although life history patterns must be classified in respect to sexual life cycles, parthenogenetic cycles are used in order to compare between more species. Direct type life histories are excluded here since such types have been observed in most species examined.

Pattern I of the three is represented with *Colpomenia sinuosa*, *C. peregrina* and *Hydroclathrus clathratus*. In this pattern plurispores from macrothalli develop into microthalli which form plurilocular sporangia under long day conditions and unilocular sporangia under short day conditions. These plurispores from microthalli give rise to similar microthalli, and the unispores from microthalli develop to macrothalli.

Pattern II is represented with *S. gracilis* and *P. zosterifolia*. In this pattern, plurispores from macrothalli develop to microthalli which sprout macrothalli under 5-20°C conditions. Furthermore, these microthalli form unilocular sporangia under higher temperature and both long and short day conditions. Unispores develop to the same microthalli as do plurispores.

Pattern III is represented with *C. bullosa*, *S. lomentaria*, *S. tenellus*, *S. nakamurae*, *P. fascia* and *P. binghamiae*. Plurispores from macrothalli develop into microthalli which rarely give rise to macrothalli. The microthalli produce unilocular sporangia mainly under 15°C and 20°C short day conditions. Unispores from microthalli develop into macrothalli.

These life history patterns are closely related with morphology of microthalli.

Species representing the life history pattern I have microthalli of *Colpomenia peregrina* type. These microthalli become fertile under any culture conditions tested and form plurilocular or unilocular sporangia terminally on upright filaments without paraphyses, and so they can easily form reproductive organs without elongating paraphyses. These microthalli are considered to have properties that reproduce themselves in short-term cycles. These microthalli are expected to form plurilocular sporangia and to reproduce themselves in summer. In fact, in *C. peregrina* they form plurilocular sporangia in summer at Oshoro. These species take a strategy that microthalli propagate themselves by plurispores in season when they live as microthalli.

Species representing the life history pattern II have microthalli of *Compsonema saxicolum* type. Although field microthalli were observed only in autumn, they may form unilocular sporangia also in summer, judging from my culture study. These species also take a strategy that microthalli reproduce themselves by unispores in the season when they live as microthalli.

Species representing the life history pattern III have microthalli of *Microspongium-Stragularia* type or bilateral type. These microthalli basically do not become fertile in summer, but form unilocular sporangia during autumn and winter. Unispores from unilocular sporangia on the microthalli develop into macrothalli, then, they do not repeat microthalli. The microthalli are generally firm or tight disks which may be

resistant to a seasonal change of environment and be able to survive until the next reproductive season. These microthalli form unilocular sporangia after elongation of paraphyses. These microthalli take a strategy that they spend as tight thalli without reproduction until autumn when they begin to produce unilocular sporangia.

As mentioned above, these life history patterns are related with microthallus morphology which is appropriate for short- and long-term life in fields. The species of the Scytosiphonaceae may take characteristic strategies according to the microthallus morphologies.

Remarks on circumscription of genera in Scytosiphonales

External morphology and structure of macrothalli have been used as generic characters in the Scytosiphonales. Growth form is also used in a certain case. However, definitions of genera are unclear, then they are sometimes in confusion.

Table 54 shows comparison of species of *Colpomenia* and *Hydroclathrus*. Table 57 shows comparison of genera examined in this study. The genus *Colpomenia* contains species whose shape of macrothalli is variable in this genus. For example, *C. phaeodactyla* has tubular macrothalli resembling species of *Scytosiphon*, and *C. ramosa* has tubular, branched macrothalli similar to species of *Rosenvingea*. Wynne and Norris (1976) mentioned that *C. phaeodactyla* has a small saccate base in contrast to plants of *Scytosiphon*, and that *C. ramosa* are basically prostrate expansions which are different from erect forms of *Rosenvingea*. Although the species of *Colpomenia* are

variable in the macrothallus shape and there is a considerable difference between spherical macrothalli (*C. sinuosa* and *C. peregrina*) and tubular ones (*C. bullosa* and *C. phaeodactyla*), an intermediate form is known *C. tuberculata* Saunders (1898) of which macrothalli are blunt tubercles. Their macrothallus initiation is consistently the spherical types and their plurilocular sporangia are of the loose type with ascocysts. The occurrence of ascocysts on microthalli ascertained in the present study may be recognized as a further generic feature.

The genus *Iyengaria*, monotypic genus based on *I. stellata* Børgesen was established by Børgesen (1939), distinguishing from *Colpomenia* in respects to the localization of growth to solid projections. However, Wynne and Norris (1976) mentioned that the difference is seemed to be in degree rather than in kind, and suggested that *Iyengaria* is merged with *Colpomenia*. Although *Iyengaria* lacks ascocysts, Wynne and Norris (1976) mentioned that the character is questionable, referring to its variation in the genus *Scytosiphon*. Culture study has not been carried out and plurilocular sporangial type (loose or packed) is unknown for *Iyengaria*. Further critical studies are needed to elucidate the taxonomic status of this genus.

Hydroclathrus has clathrate macrothalli which are so peculiar in shape. Therefore, distinction between this genus and other genera is clear-cut in external morphology of macrothalli. However, *Hydroclathrus* has been considered to be closely related to *Colpomenia* because any differences between the two genera except for the external morphology have not been shown. The present study, however, points out that the absence of ascocysts may distinguish *Hydroclathrus* from *Colpomenia*.

Tables 55 and 56 show comparison of species of *Scytosiphon* and *Petalonia* examined in this study, respectively. There are some opinions that distinctiveness between the genera *Scytosiphon* and *Petalonia* is unclear. Rosenvinge and Lund (1947) mentioned that distinctiveness between these genera is only originally hollow or originally solid, showing resemblance among *S. complanatus* and *P. zosterifolia*. It is not sufficient to examine the character of being hollow or solid for taxonomic character. In this study, hollow thallus and solid thallus are considered not to be confused, as in Wynne's opinion (Wynne 1969). Two characters, hollow/solid thalli and loose/packed plurilocular sporangia are apparently correlated in genera of the Scytosiphonaceae except for *Scytosiphon*. The species of *Petalonia* have solid thalli and packed plurilocular sporangia. However, in the species of *Scytosiphon* which have hollow thalli, both loose and packed plurilocular sporangia can be found. Thus, it is difficult to evaluate these combined characters as distinction of these two genera. Furthermore, the structure of plurilocular sporangia is still unknown for *Scytosiphon complanatus* and *S. dotyi*. At present, on the basis of the thallus form I prefer separating these genera to amalgamating them in accordance with the previous authors (Wynne 1969, Fletcher 1987), although packed plurilocular sporangia in *S. gracilis* and *S. tenellus* indicate the similarity to species of *Petalonia*.

The genus *Endarachne* is treated as a synonym of *Petalonia* in this study in accordance with Vinogradova (1973). However, recently, a new genus *Jolyna* Guimaraes in Scytosiphonaceae was established with *Jolyna laminarioides* Guimaraes as the type species (Guimaraes *et al*. 1986). This genus was characterized in

respects of the medulla consisting of long cylindrical fiber-like cells and networked ground tissue. However, This species resembles *Petalonia binghamiae* since the ground tissue cells seem to be kinds of rhizoidal filaments in medulla. The genus *Jolyna* is needed to be reexamined, because of its close relationship between *Endarachne* and *Petalonia*.

Remarks on circumscription of families in Scytosiphonales

Two families, Chnoosporaceae (Setchell and Gardner 1925) and Scytosiphonaceae (Farlow 1881), are recognized in the Scytosiphonales. However, definition of the families has not been reexamined since the time when the two families were assigned to the Scytosiphonales (Feldmann 1949). The Chnoosporaceae including only one genus *Chnoospora* was established by Setchell and Gardner (1925) based on the terminal growth. Although growth form of the Scytosiphonaceae is known to show diffuse growth, detailed examinations have not been carried out. Although *Iyengaria* is belonged to Scytosiphonaceae, it has a growth region at a terminal portion of projections of thalli (Børgesen 1939).

The present study reveals that the genera in Scytosiphonaceae are possible to separate into two groups in respect to initial forms of macrothalli. The two groups represent the uniseriate-type and spherical-type in initiation of macrothalli, respectively (Table 57). This character may be able to use as a taxonomic character at family level. For that purpose, it is necessary to examine initiation of macrothalli for *Rosenvingea* and *Chnoospora*.

Key to species examined in this study in Scytosiphonaceae

1 Macrothalli initially spherical 2

1 Macrothalli initially uniseriate 6

2 Macrothalli saccate or tubular .....(*Colpomenia*)....3

2 Macrothalli clathrate ..... *Hydroclathrus clathratus*

3 Macrothalli saccate 4

3 Macrothalli tubular 5

4 Plurilocular sporangia formed in circular sori with cuticular covering ..... *Colpomenia sinuosa*

4 Plurilocular sporangia formed in irregular sori lacking cuticular covering ..... *Colpomenia peregrina*

5 Tubular thalli abundantly arising from saccate base, 90-130 µm thick ..... *Colpomenia phaeodactyla*

5 One to several tubular thalli arising from saccate base, 120-230 µm thick ..... *Colpomenia bullosa*

6 Macrothalli hollow, cylindrical or flat linear, simple .....(*Scytosiphon*).....7

6 Macrothalli solid and flattened .....(*Petalonia*).....10

7 Plurilocular sporangia of loose-type without cuticular covering; medullary layers 3-5 cell layers thick 8

7 Plurilocular sporangia of packed-type with cuticular covering, medullary layers 1-3 cell layers thick 9

8 Macrothalli with regular constrictions; gametes isogamous; Microthalli of *Microspongium*-type ... *Scytosiphon lomentaria*

8 Macrothalli without regular constrictions; gametes anisogamous; Microthalli of *Hapterophycus*-type ..... *Scytosiphon nakamurae*

9 Ascocysts absent among plurilocular sporangia; microthalli of *Compsonema*-type ..... *Scytosiphon gracilis*

9 Ascocysts present among plurilocular sporangia; microthalli of *Stragularia*-type ..... *Scytosiphon tenellus*

10 Macrothalli narrow, linear, 0.5-8 mm in width; microthalli of *Compsonema*-type ..... *Petalonia zosterifolia*

10 Macrothalli lanceolate, 5-40 mm in width 11

11 Rhizoidal filaments absent or sometimes present in medulla of macrothalli, 8-14 µm in diameter ..... *Petalonia fascia*

11 Rhizoidal filaments abundant in medulla of macrothalli, 4-9 µm in diameter, forming thick layer ..... *Petalonia binghamiae*

Table. 54. Comparison of the species of *Colpomenia* and *Hydroclathrus* examined in this study.

	C. sinuosa	*C. peregrina*	*C. phaeodactyla*	*C. bullosa*	*H. clathratus*
shape	spherical	spherical	tubular with spherical base	tubular with spherical base	clathrate
medullary cell layers	4-7	3-4	3-4	3-5	3-6
plurilocular sporangium	loose	loose	loose	loose	loose
cuticle	present	absent	absent	absent	absent
ascocyst	present	present	present	present	absent
sexual repr.	?	anisogamy	?	isogamy	?
microthallus	*C.peregrina*-type	*C.peregrina*-type	?	bilateral type	?
pluril. spor. on microthallus	present	present	?	absent	present
ascocysts on microthallus	present	present	?	present	absent

Table. 57. Comparison of the genera examined in this study.

	Colpomenia	*Hydroclathrus*	*Scytosiphon*	*Petalonia*
shape	spherical cylindrical unbranched branched	clathrate	cylindrical flat unbranched	flat linear lanceolate unbranched
hollow or solid	hollow	hollow	hollow	solid
filaments in medulla	absent	absent	absent	absent or present
plurilocular sporangium	loose	loose	loose packed	packed
ascocyst	present	absent	present absent	absent
direction of maturation	acropetal	acropetal	basipetal	basipetal
initiation of macrothallus	spherical	spherical	uniseriate	uniseriate
microthallus	*C.peregrina*-type bilateral type	?	*Microspongium* *Hapterophycus* *Compsonema* *Stragularia*	*Compsonema* *Stragularia*

Table. 56. Comparison of three species of *Petalonia* from Japan.

	P.zosterifolia	*P.fascia*	*P.binghamiae*
shape	flat linear	flat lanceolate	flat lanceolate
filaments in medulla	rare	sometimes	abundant
plurilocular sporangium	packed	packed	packed
ascocyst	absent	absent	absent
sexual reproduction	?	isogamy	isogamy
microthallus	*Compsonema*	*Stragularia*	*Stragularia**

*: Microthalli were observed only in culture.

Table. 57. Comparison of the genera examined in this study.

	Colpomenia	*Hydroclathrus*	*Scytosiphon*	*Petalonia*
shape	spherical cylindrical unbranched branched	clathrate	cylindrical flat unbranched	flat linear lanceolate unbranched
hollow or solid	hollow	hollow	hollow	solid
filaments in medulla	absent	absent	absent	absent or present
plurilocular sporangium	loose	loose	loose packed	packed
ascocyst	present	absent	present absent	absent
direction of maturation	acropetal	acropetal	basipetal	basipetal
initiation of macrothallus	spherical	spherical	uniseriate	uniseriate
microthallus	*C.peregrina*-type bilateral type	?	*Microspongium* *Hapterophycus* *Compsonema* *Stragularia*	*Compsonema* *Stragularia*

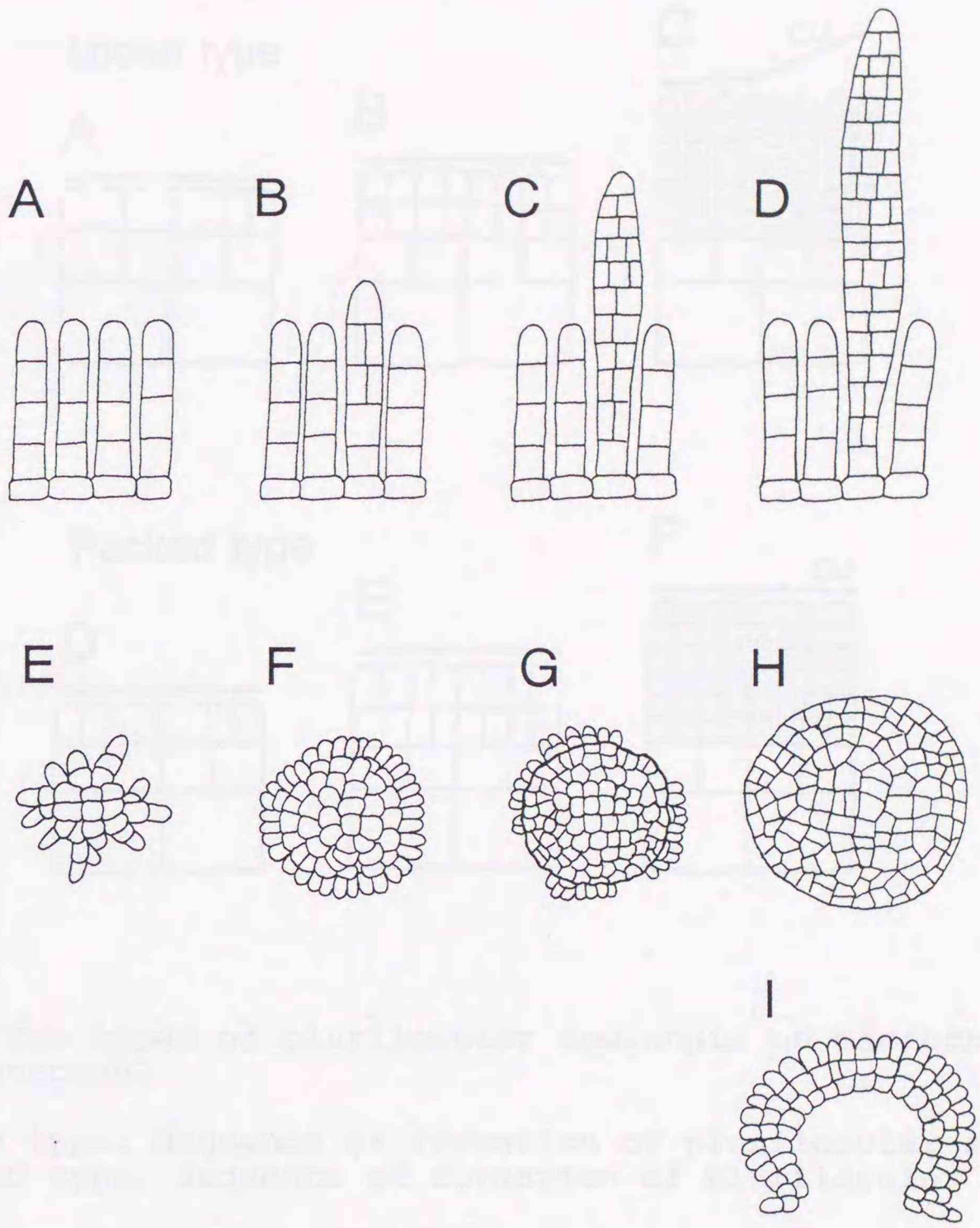


Fig. 120. Diagram of initiation of macrothalli in culture of Scytosiphonaceae.

A-D. Uniseriate type. Sequence of formation of macrothallus in vertical view of crust. **E-I.** Spherical type. Sequence of formation of macrothallus in surface view (**E-H**) and vertical view (**I**).

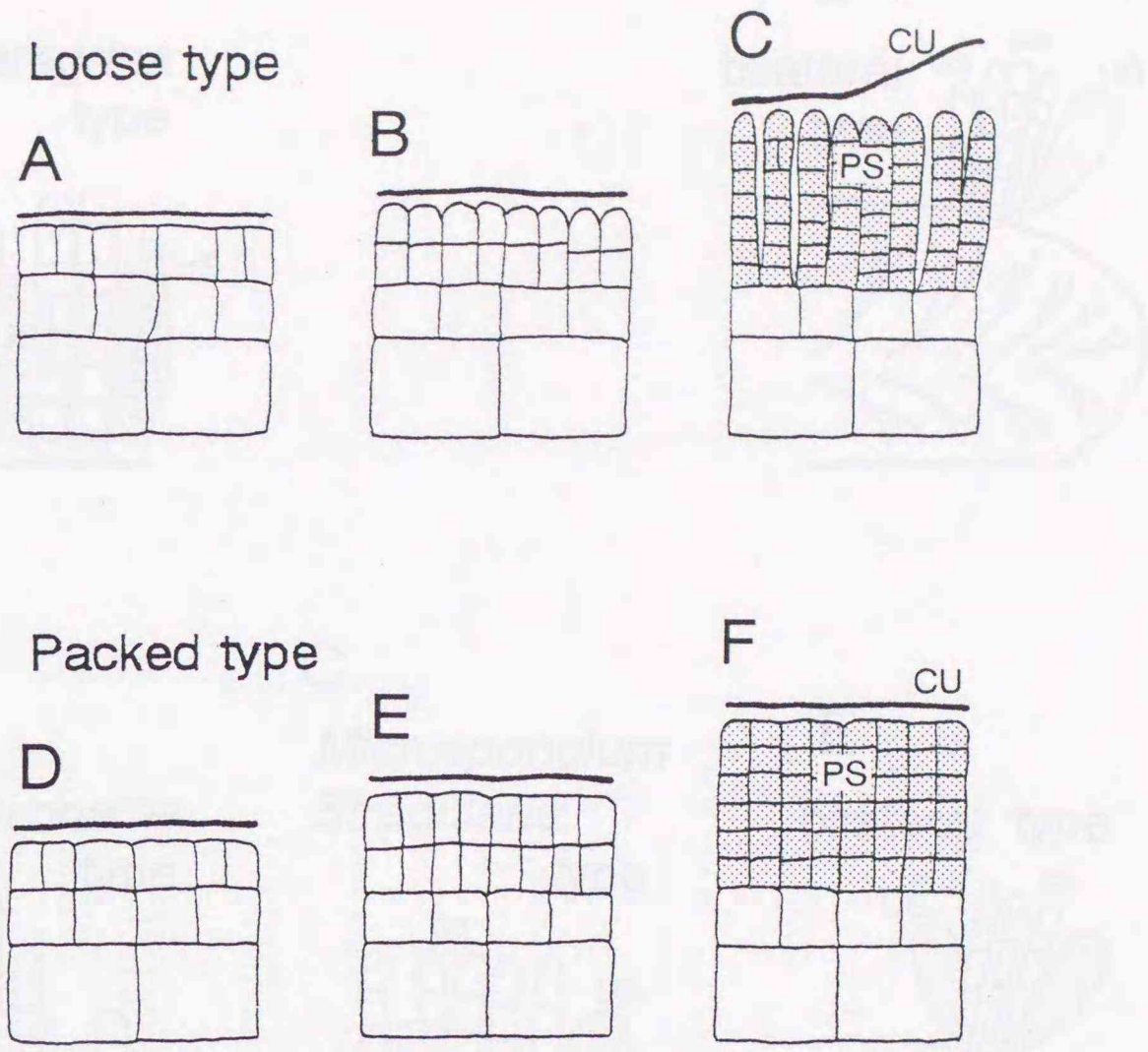


Fig. 121. Two types of plurilocular sporangia on macrothalli in Scytosiphonaceae.

A-C. Loose type. Sequence of formation of plurilocular sporangia.
D-F. Packed type. Sequence of formation of plurilocular sporangia.

CU, cuticle; PS, plurilocular sporangia.

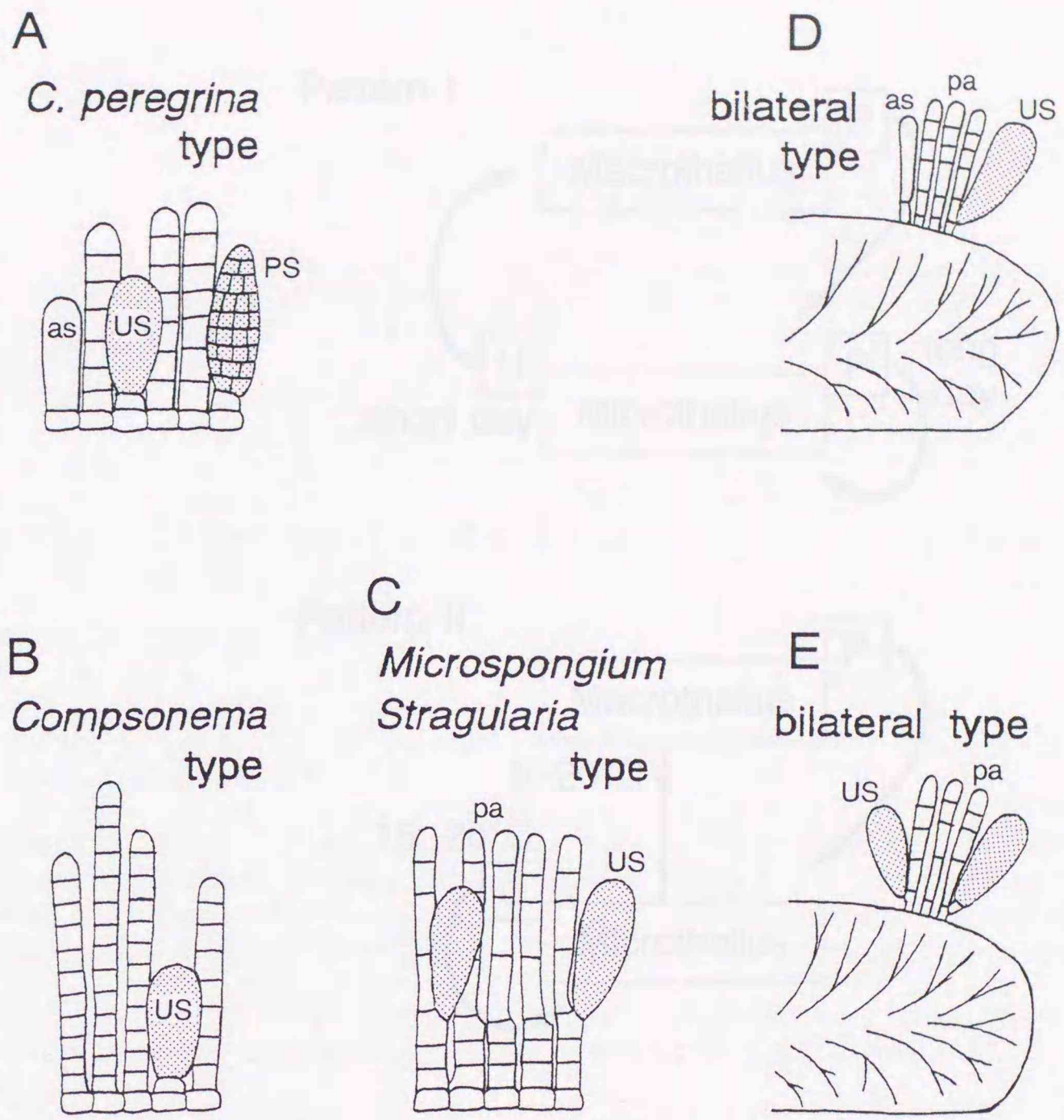


Fig. 122. Types of microthalli in Scytosiphonaceae.

A. *Colpomenia peregrina* type.
B. *Compsonema saxicolum* type.
C. *Microspongium-Stragularia* type.
D, E. Bilateral type.

as, ascocyst; pa, paraphyses; PS, plurilocular sporangium; US, unilocular sporangium.

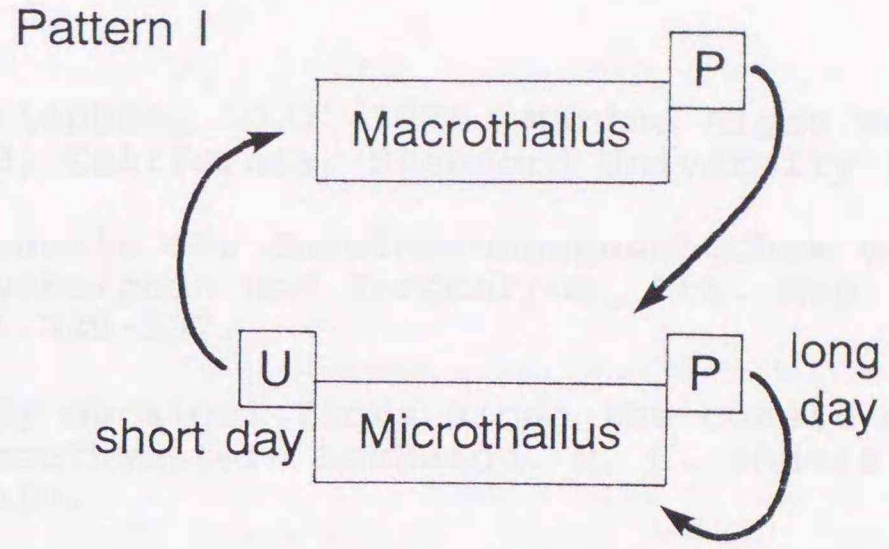


Fig. 123. Life history patterns of Scytosiphonaceae in culture.

P, plurilocular sporangia; U, unilocular sporangia; broken line, frequent course.

REFERENCES

Abbott, I.A. and Hollenberg, G.J. 1976. Marine Algae of California. Stanford, California, Stanford University Press.

Abe, K. 1935. Zur Kenntis der Entwicklungsgeschichte von *Heterochordaria*, *Scytosiphon* und *Sorocarpus*. Sci. Rep. Tohoku Imp. Univ., Biol. **9**: 329-337.

Abe, T. 1993. A study on algal flora along the coasts of Okushiri Island and Setana, southwestern Hokkaido. M. C. thesis of Fac. of Science, Hokkaido Univ.

Agardh, C.A. 1820. Species Algarum. Vol. 1, Part 1, Fucoideae, pp. 1-168. (Lund).

Agardh, J.G. 1896. Analecta algologica. Cont. III. Lunds Univ. Arsskr. **30**: 1-140, 1 pl.

Batters, E.A.L. 1888. A description of three new marine algae. J. Linn. Soc., Botany **24**: 450-453.

Blackler, H. 1964. Some observations of the genus *Colpomenia* (Endlicher) Derbès et Solier 1851. In: Davy de Virville, Ad.; Feldmann, J. eds. Proceedings of the fourth International Seaweed Symposium Biarritz Sept. 1961. London, Pergamon Press. pp. 50-54.

Blackler, H. 1967. The occurrence of *Colpomenia peregrina* (Sauv.) Hamel in the Mediterranean (Phaeophyta: Scytosiphonales). Blumea **15**: 5-8.

Blackler, H. 1981. Some algal problems with special reference to *Colpomenia peregrina* and other members of the Scytosiphonaceae. Br. phycol. J. **16**: 133.

Bold, H.C. and Wynne, M.J. 1985. Introduction to the Algae: Structure and reproduction. 2nd Edn. Prentice-Hall, New Jersey.

Boone, P.W. and Kapraun, D.F. 1989. Developmental studies of three Scytosiphonaceae (Phaeophyta) from North Carolina. Crypt. Bot. **1**: 15-25.

Børgesen, F. 1914. The marine algae of the Danish West Indies, Part II: Phaeophyceae. Dansk Botanisk Arkiv, **2**: 157-224.

Børgesen, F. 1928. On *Rosenvingea stellata*, a new Indian algae, and on an interesting littoral algal vegetation in which this species is a characteristic constituent. Dansk Botanisk Arkiv, **5**: 1-11.

Børgesen, F. 1930. Some Indian green and brown algae especially from the shores of the presidency of Bombay. J. Indian Botanical Society, **9**: 151-174.

Børgesen, F. 1935. A list of marine Algae from Bombay. Det Kgl.

Danske Videnskabernes Selskab. Biologiske Meddelelser XII, 2: 1-64 + Pl. I-X.

Børgesen, F. 1939. Marine algae from the Iranian Gulf. pp. 47-141. in Part 1 in K. Jessen and R. Sparck, editors, Danish Scientific Investigations in Iran. Copenhagen: E. Munksgaard.

Bory de St-Vincent, J.B. 1825. Hydroclathre. Dict. Class. d'Hist. Nat. **8**: 419.

Bory de St-Vincent, J.B. 1832. Expedition scientifique de Morée. Section des sciences physiques, T. III, 2 part. botanique. Paris.

Brophy, T.C. and Murray, S.N. 1989. Field and culture studies of a population of *Endarachne binghamiae* (Phaeophyta) from Southern California. J. Phycol. **25**: 6-15.

Chihara, M. 1970. Common seaweeds of Japan in color. xviii + 173 pp. Osaka: Hoikusha Publishing Company.

Clayton, M.N. 1975. A study of variation in Australian species of *Colpomenia* (Phaeophyta; Scytosiphonales). Phycologia **14**: 187-195.

Clayton, M.N. 1976a. The morphology, anatomy and life history of a complanate form of *Scytosiphon lomentaria* (Scytosiphonales, Phaeophyta) from southern Australia. Marine Biology **38**: 201-208.

Clayton, M.N. 1976b. Complanate *Scytosiphon lomentaria* (Lyngbye) J.Agardh (Scytosiphonales: Phaeophyta) from southern Australia: the effects of season, temperature, and daylength on the life history. J. exp. mar. Biol. Ecol. **25**: 187-198.

Clayton, M. N. 1978. Morphological variation and life history in cylindrical forms of *Scytosiphon lomentaria* (Scytosiphonaceae: Phaeophyta) from southern Australia. Marine Biology **47**: 349-357.

Clayton, M.N. 1979. The life history and sexual reproduction of *Colpomenia peregrina* (Scytosiphonaceae, Phaeophyta) in Australia. Br. phycol. J. **14**: 1-10.

Clayton, M.N. 1980. Sexual reproduction - a rare occurrence in the life history of the complanate form of *Scytosiphon* (Scytosiphonaceae, Phaeophyta) from southern Australia. Br. phycol. J. **15**: 105-118.

Clayton, M.N. 1981. Studies of variability in Australian species of *Scytosiphon*. p. 67-75. In Fogg, G.E. and Jones, W. Eifion (ed.), Proceedings of the Eighth International Seaweed Symposium, xxxv + 769 pp.

Clayton, M.N. 1982. Life history studies in the Ectocarpales (Phaeophyta): Contributions toward the understanding of evolutionary processes. Bot. Mar. **25**: 111-116.

Clayton, M.N. 1988. Evolution and life histories of brown algae. Bot. Mar. **31**: 379-387.

Cole, K. 1970. Ultrastructural characteristics in some species in the order Scytosiphonales. Phycologia **9**: 275-283.

Correa, J., Novaczek, I. and McLachlan, J. 1986. Effect of temperature and daylength on morphogenesis of *Scytosiphon lomentaria* (Scytosiphonales, Phaeophyta) from eastern Canada. Phycologia **25**: 469-475.

Cremades, J. and Pérez-Cirera, J.L. 1990. Nuevas combinaciones de algas bentónicas marinas, como resultado del estudio del herbario de Simón de Rojas Clemente y Rubio (1777-1827). Anales Jard. Bot. Madrid **47**: 489-492.

Crouan, P.L. and H.M. 1867. Florule du Finistère. Paris and Brest.

Dangeard, P. 1962. Sur la réproduction et le développement de *Petalonia zosterifolia* (Reinke) Kuntze. C. r. hebd. Séanc. Acad. Sci. **254**: 1895-1896.

Dangeard, P. 1963. Recherches sur le cycle évolutif de quelques Scytosiphonacées. Botaniste **46**: 5-129.

Dawson, E.Y. 1944. The marine algae of the Gulf of California. Allan Hancock Pacific Expeditions, **3**: 182-453.

Dawson, E.Y. 1966. New records of marine algae from the Gulf of California. J. Arizona Acad. Sci. **4**: 55-66.

Derbès, F. and Solier, A.J.J. 1850. Sur les organes reproducteurs des Algues. Ann. Sci. nat. (Bot.), Ser. 3, **14**: 261-282, Pls 32-37.

Derbès, F. and Solier, A.J.J. 1851. Algues. In Castagne, J.L.M., Supplément au Catalogue des plantes qui croissent naturellement aux environs de Marseille. pp. 93-121. (Nicot and Pardigon: Aix.).

Derbès, F. and Solier, A.J.J. 1856. Memoire sur quelques points de la physiologie des Algues. C.r. hebd. Séanc. Acad. Sci., Paris **I**: 1-20.

Doty, M.S. 1947. The marine algae of Oregon. Part I. Chlorophyta and Phaeophyta. Farlowia **3**: 1-65.

Durairatnum, M. 1962. Some marine algae from Ceylon, 1. Bulletin of the Fisheries Research Station, Department of Fisheries, Ceylon **15**: 16 pages.

Earle, S.A. 1969. Phaeophyta of the Eastern Gulf of Mexico. Phycologia **7**: 71-254.

Edelstein, T., Chen, L.C-M. and McLachlan, J. 1970. The life cycle of *Ralfsia clavata* and *R. bornetii*. Canadian Journal of Botany **48**: 527-531.

Edwards, P. 1969. Field and cultural studies on the seasonal

periodicity of growth and reproduction of selected Texas benthic marine algae. Contr. mar. Sci. Univ. Tex. **14**: 59-114.

Endlicher, S.L. 1843. Genera plantarum. suppl. 3.

Farlow, W.G. 1881. Marine algae of New England and adjacent coasts. Washington.

Farnham, W.F. 1980. Studies on aliens in the marine flora of southern England. In Price, J.H., Irvine, D.E.G. & Farnham, W.F. (Eds) The shore environment 2: ecosystems pp. 875-914. Systematics Association Special Volume 17(b). Academic Press, London and New York.

Feldmann, J. 1949. L'ordre des Scytosiphonales. Mém. Hist. Nat. Afr. Nord, Hors-Sér. **2**: 103-115.

Fletcher, R.L. 1974. Studies on the brown algal families Ralfsiaceae and Scytosiphonaceae. Br. phycol. J. **9**: 218.

Fletcher, R.L. 1978. Studies on the family Ralfsiaceae (Phaeophyta) around the British Isles. In Irvine, D.S.G. & Price, J.H. (Eds) Modern approaches to the taxonomy of red and brown algae pp. 371-398. Systematics Association Special Volume 10. Academic Press, London and New York.

Fletcher, R.L. 1981. Studies on the ecology, structure and life history of the brown alga *Petalonia filiformis* (Batt.) Kuntze (Scytosiphonaceae) around the British Isles. Phycologia **20**: 103-104.

Fletcher, R.L. 1987. Seaweeds of the British Isles. Vol.3. Fucophyceae (Phaeophyceae) Part 1. British Museum (Natural History), London, x + 359pp.

Fotos, S.F. 1981. Observations on *Chnoospora minima* (Hering) Papenfuss (Phaeophyta, Scytosiphonales) in the field and in culture. Jpn. J. Phycol. **29**: 101-108.

Fries, E.M. 1835. Corpus Florarum Provincialium Sueciae, 1 Floram Scanicam. Upsaliae.

Fritsch, F.E. 1945. The structure and reproduction of the algae Vol. 2. Cambridge University Press, Cambridge.

Guimarães, S.M.P.B., Braga, M.R.A., Cordeiro-Marino, M. and Pedrini, A.G. 1986. Morphology and taxonomy of *Jolyna laminarioides*, a new member of the Scytosiphonales (Phaeophyceae) from Brazil. Phycologia **25**: 99-108.

Hamel, G. 1931-1939. Phéophycées de France. Paris. XLVII + 432 pp.

Hollenberg, G. J. 1941. Culture studies of marine algae. II. *Hapterophycus canaliculatus* S. & G. Amer. J. Botany **28**: 676-683.

Howe, M.A. 1920. Algae. In Britton, N.L. and Millspaugh, C.F.,

The Bahama Flora. New York.

Hsiao, S.I.C. 1969. Life history and iodine nutrition of the marine brown alga, *Petalonia fascia* (O.F. Müll.) Kuntze. Can. J. Bot. **47**: 1611-1616.

Hsiao, S.I.C. 1970. Light and temperature effects on the growth, morphology, and reproduction of *Petalonia fascia*. Can. J. Bot. **48**: 1359-1361.

Jaasund, E. 1964. Marine algae from northern Norway. III. Bot. Mar. **6**: 129-133.

Jones, W.E. 1974. Changes in the seaweed flora of the British Iles. In Hawksworth, D.L. (Ed) The changing flora and fauna of Britain PP. 97-113. Systematics Association Special Volume 6. Academic Press, London and New York.

Kitayama, T., Kawai, H. and Yoshida, T. 1992. Dominance of female gametophytes in field populations of *Cutleria cylindrica* (Cutleriales, Phaeophyceae) in the Tsugaru Strait, Japan. Phycologia **31**: 449-461.

Kristiansen, Aa. and Pedersen, P.M. 1979. Studies on life history and seasonal variation of *Scytosiphon lomentaria* (Fucophyceae, Scytosiphonales) in Denmark. Bot. Tidsskr. **74**: 31-56.

Kuckuck, P. 1894. Bemerkungen zur marinen Algenvegetation von Helgoland. I. Wiss. Meeresunters. (Helgol.) N.F. **1**: 223-263.

Kuckuck, P. 1897. Bemerkungen zur marinen Algenvegetation von Helgoland. II. Wiss. Meeresunters. (Helgol.) N.F. **2**: 271-400.

Kunieda, H. and Suto, S. 1938. The life-history of *Colpomenia sinuosa* (Scytosiphonaceae), with special reference to the conjugation of anisogametes. Bot. Mag., Tokyo **52**: 539-546.

Kunieda, H. and Suto, S. 1948. On the isogamy and the life-history of *Endarachne binghamiae* and *Colpomenia bullosa*. Suisangakkai Ho **10**: 8-9.

Kuntze, O. 1891-98. Revisio generum plantarum, Part I-III. Leipzig.

Kützing, F.T. 1843. Phycologia generalis. Leipzig.

Kylin, H. 1947. Die Phaeophyceen der Schwedischen Westküste. Acta Univ. lund. **43**: 1-99.

Le Jolis, A. 1863. Liste des algues marines de Cherbourg. Mém. Imp. Soc. Sci. Nat. Cherbourg **10**: 1-168, Pls 1-6.

Lindauer, V.W., Chapmann, V.J. and Aiken, M. 1961. The marine Algae of New Zealand. Part II. Phaeophyceae. Nova Hedwigia 3: 129-350, 41 pls.

Link, J.H.F. 1833. Handbuch zur Erkennung der Nutzbarsten und am

häufigsten vorkommenden Gewächse. Vol. 3. Berlin.

Littler, M.M. and Littler, D.S. 1983. Heteromorphic life-history strategies in the brown alga *Scytosiphon lomentaria* (Lyngb.) Link. J. Phycol. **19**: 425-431.

Loiseaux, S. 1967. Morphologie et cytologie des Myrionémacées. Rev. Gén. Bot. **74**: 329-347, Pls 1-3.

Loiseaux, S. 1970. *Streblonema anomalum* S.et G. and *Compsonema porangiiferum* S. et G. stages in the life history of a minute *Scytosiphon*. Phycologia **9**: 185-191.

Lund, S. 1966. On a sporangia-bearing microthallus of *Scytosiphon lomentaria* from nature. Phycologia **6**: 67-78.

Lüning, K. and Dring, M.J. 1973. The influence of light quality on the development of the brown algae *Petalonia* and *Scytosiphon*. Br. phycol. J. **8**: 333-338.

Lüning, K. and Dring, M.J. 1975. A photoperiodic response mediated by blue light in the brown alga *Scytosiphon lomentaria*. Planta **125**: 25-32.

Lyngbye, H.C. 1819. Tentamen Hydrophytologiae Danicae. Hafniae.

Maier, I. and Müller, D.G. 21986. Sexual pheromones in algae. Biol. Bull. **170**: 145-175.

McLachlan, J., Chen, L. C-M. & Edelstein, T. 1971. The life history of *Microspongium* sp. Phycologia **10**: 83-87.

Müller, O.F. 1771-82. Flora Danica **4**, **5**. Havniae.

Mine, I 1990. Marine benthic algal flora from Kikonai to Matsumae, Hokkaido. M. Sci. Thesis. Hokkaido University.

Nakamura, Y. 1965. Development of zoospores in *Ralfsia*-like thallus, with special reference to the life cycle of the Scytosiphonales. Bot. Mag. Tokyo **78**: 109-110, 5fig.

Nakamura, Y. 1972. A proposal on the classification of the Phaeophyta. In contributions to the systematics of marine algae of the North Pacific, Abbott, I.A. and Kurogi, M. eds, pp. 147-156, Jpn. Soc. Phycol., Kobe.

Nakamura, Y. and Nakahara, H. 1977. The life cycle of *Hapterophycus canaliculatus* (Phaeophyta). Bull. Jap. Soc. Phycol. **25**, suppl. (Mem. Iss. Yamada): 203-213.

Nakamura, Y. and Tatewaki, M. 1975. The life history of some species of Scytosiphonales. Sci. Pap. Inst. Algol. Res., Fac. Sci., Hokkaido Univ. **6**: 57-93.

Newton, L. 1931. A Handbook of the British Seaweeds. British Museum (Natural History), London, viii + 478 pp.

Nizamuddin, M. and Farooqi, P.B. 1968. The morphology and structure of *Endarachne binghamiae* J. Agardh. Bot. Mar. **11**: 40-53.

Noda, M. 1973. Some marine algae collected on the coast of Kashiwazaki Prov. facing the Japan Sea (2). Sci. Rep. Niigata Univ. ser. D no. **10**: 1-10.

Noda, M. 1964. Marine algae in the vicinity of the Shioyazaki Cape, Fukushima Prefecture. J. Fac. Sci. Niigata Univ., Ser. II, **4**: 33-75.

Okamura, K. 1900-1902. Illustrations of the marine algae of Japan. Keigyosha, Tokyo.

Okamura, K. 1902. Nihon sourui mei. first ed. Keigyosha, Tokyo. (in Japanese).

Okamura, K. 1902-35. Icons of the Japanese algae. I-VII. Published by the author, Tokyo.

Okamura, K. 1936. Nihon kaiso-shi. Uchida Rokakuho, Tokyo. 9+6+964+11 pp. (in Japanese).

Parke, M. and Dixon, P.S. 1964. A revised check list of British Marine Algae. J. mar. biol Ass. U. K. **44**: 499-542.

Parsons M.J. 1982. *Colpomenia* (Endlicher) Derbès et Solier (Phaeophyta) in New Zealand. New Zealand J. Botany **20**: 289-301.

Pedersen, P.M. 1980. Culture studies on complanate and cylindrical *Scytosiphon* (Fucophyceae, Scytosiphonales) from Greenland. Br. phycol. J. **15**: 391-398.

Pedersen, P.M. 1981. Phaeophyta: Life histories. p. 194-217. In Lobban, C.S. & Wynne, M.J. (Eds), The Biology of Seaweeds. Blackwell Sci. Publs, Oxford, vi + 786 pp.

Pedersen, P.M. 1984. Studies on primitive brown algae (Fucophyceae). Opera Bot. **74**: 1-76.

Pedersen, P.M., Kristiansen, A. and Moseholm, L. 1987. The effect of temperature on growth and reproduction of *Scytosiphon complanatus* (Fucophyceae) from Greenland. Nord. J. Bot. **7**: 729-733.

Pedersen, P.M. and Sokhi, G. 1990. Studies on the type species of *Compsonema*, *C. minutum* (Fucophyceae, Scytosiphonales); aspects of life history, taxonomic position, shedding of wall elements and plasmodesmata. Nord. J. Bot. **10**: 547-555.

Reinke, J. 1889. Atlas deutsher Meeresalgen. Heft I. Paul Parey, Berlin.

Rhodes, R.G. & Connell, M.V. 1973. The biology of brown algae on the Atlantic coast of Virgina. II *Petalonia fascia* and *Scytosiphon lomentaria*. Chesapeake Sci. **14**: 211-215.

Ribera, M.A., Gomez Garreta, A., Gallardo, T., Cormaci, M., Furnari, G. and Giaccone, G. 1992. Check-list of Mediterranean Seaweeds. I. Fucophyceae (Warming, 1884). Botanica Marina **35**: 109-130.

Roberts, R. and Ring, F.M. 1972. Preliminary investigations into conditions affection the growth of the microscopic phase of *Scytosiphon lomentarius* (Lyngbye) Link. Soc. Bot. Fr., Memoires 1972: 117-128.

Roeleveld, J.G., Duisterhof, M. & Broman, M. 1974. On the year cycle of *Petalonia fascia* in the Netherlands. Neth. J. Sea Res. **8**: 410-426.

Rosenvinge, L.K. and Lund, S. 1947. The marine algae of Denmark: Contributions to their natural history. Vol. II. Phaeophyceae. III: Encoeliaceae, Myriotrichiaceae, Giraudiaceae, Striariaceae, Dictyosiphonaceae, Chordaceae and Laminariaceae. Kongel. Dansk. Vidensk. Selsk., Biol. Skr. **4**: 1-99.

Rosenvinge, L.K. 1893. Grønland havalger. Meddr Gronland **3**: 765-981.

Roth, A.W. 1806. Catalecta Botanica **3**. Leipzig.

Russell, G. 1964. Systematic position of *Pilayella littoralis* and status of the order Dictyosiphonales. Br. phycol. Bull. **2**: 332-336.

Santelices, B., Ramirez, M.E. and Abbott, I.A. 1989. A new species and new records of marine algae from Chile. Br. phycol. J. **24**: 73-82.

Saunders, De A. 1898. Phycological Memoirs. Proceedings of the California Academy of Sciences, third series, Botany, **1**: 147-168.

Sauvageau, C. 1927. Sur le *Colpomenia sinuosa* Derb. et Sol. Bull. Stn Biol. Arcachon **24**: 309-353.

Seagrief, S. C. 1967. The seaweeds of the Tsitsikama Coastal National Park. 147 pp. A publication of the National Parks Board of the Republic of South Africa.

Seagrief, S. C. 1984. A catalogue of South African green, brown and red marine algae. Memoirs Bot. Survey South Africa no. 47, vi + 72pp.

Setchell, W. A. 1912. Algae novae et minus cognitae. I. University of California Publications in Botany **4**: 229-268, pls. 25-31.

Setchell, W.A. and Gardner, N.L. 1903. Algae of Northwestern America. University of California Publications in Botany, **1**: 164-418.

Setchell, W.A. and Gardner, N.L. 1924. Phycological contributions. BII. Univ. Calif. Publ. Bot. **13**: 1-13.

Setchell, W.A. and Gardner, N.L. 1925. The marine algae of the Pacific coast of North America. III Melanophyceae. Univ. Calif. Publs Bot. **8**: 383-898, pls 34-107.

Shannon, R.K., Crow, G.E. and Mathieson, A.C. 1988a. Seasonal abundance and recruitment patterns of *Petalonia fascia* (O.F. Müller) Kuntze and *Scytosiphon lomentaria* (Lyngbye) Link var. lomentaria in New Hampshire, U.S.A. Bot. Mar. **31**: 207-214.

Shannon, R.K., Crow, G.E. and Mathieson, A.C. 1988b. Evidence for sexual reproduction in *Petalonia fascia* (O.F. Mull) Kuntze. Bot. Mar. **31**: 511-513.

Silva, P.C., 1952. A review of nomenclatural conservation in the algae from the point of view of the type method. Univ. Calif. Publs Bot. **25**: 241-323.

Skottsberg, C. 1907. Zur Kenntnis der subantarktischen und antarktischen Meeresalgen. I Phaeophyceen. Wiss. Ergebn. Schwed. Südpolar-Exped. 1901-3,4. No. 6.

Smith, G.M. 1944. Marine algae of the Montery Peninsula, Califolnia. Stanford University Press, Stanford.

Tanaka, J. 1990. Brown algae from the Amami Islands. Mem. Natn. Sci. Mus., Tokyo, **23**: 23-32.

Tanaka, J. and Chihara, M. 1980. Taxonomic study of the Japanese crustose brown algae (3) *Ralfsia* (Ralfsiaceae, Ralfsiales)(Part 2). J. Jap. Bot. **55**: 337-342.

Tatewaki, M. 1966. Formation of a crustaceous sporophyte with unilocular sporangia in *Scytosiphon lomentaria*. Phycologia **6**: 62-66.

Taylor, W.R. 1945. Pacific marine algae of the Allan Hancock Pacific Expeditions to the Galapagos Islands. Allan Hancock Pacific Expeditions, **12**: iv + 528 pp.

Taylor, W.R. 1957. Marine algae of the northeastern coast of North America. Revised edition. University of Michigan Press. Ann Arvor.

Thuret, G.A. and Bornet, J-B.É. 1878. Études phycologiques. Paris.

Tokida, J. 1954. The marine algae of southern Saghalien. Mem. Fac. Fish. Hokkaido Univ. **2**: 1-264.

Tseng, C.K. and Lu, B. 1983. Two new brown algae from the Xisha Islands, South China Sea. Chin. J. Ocean. Limn. **1**: 185-188. + 1 pl.

Vinogradova, K.L. 1973. K anatomii roda *Petalonia* Derb. et Sol. (Scytosiphonales). Nov. Sist. Nizsh. Rast., Bot. Inst. Akad. Nauk SSSR **10**: 28-32.

Womersley, H.B.S. 1987. The marine benthic flora of Southern Australia. Part II. 484 pp. South Australian Government Printing Division, Adelaide.

Wynne, M.J. 1969. Life history and systematic studies of some Pacific North American Phaeophyceae (brown algae). Univ. Calif. Publs Bot. **50**: 1-88.

Wynne, M.J. 1972a. Studies of the life forms in nature and in culture of selected brown algae. pp. 133-145. In I.A. Abbott and M. Kurogi (eds), Contributions to the Systematics of benthic marine algae of the north Pacific. Kobe, Japan: Japanese Society of Phycology.

Wynne, M.J. 1972b. Culture studies of Pacific coast Phaeophyceae. Soc. bot . Fr., Mémoires 1972: 129-144.

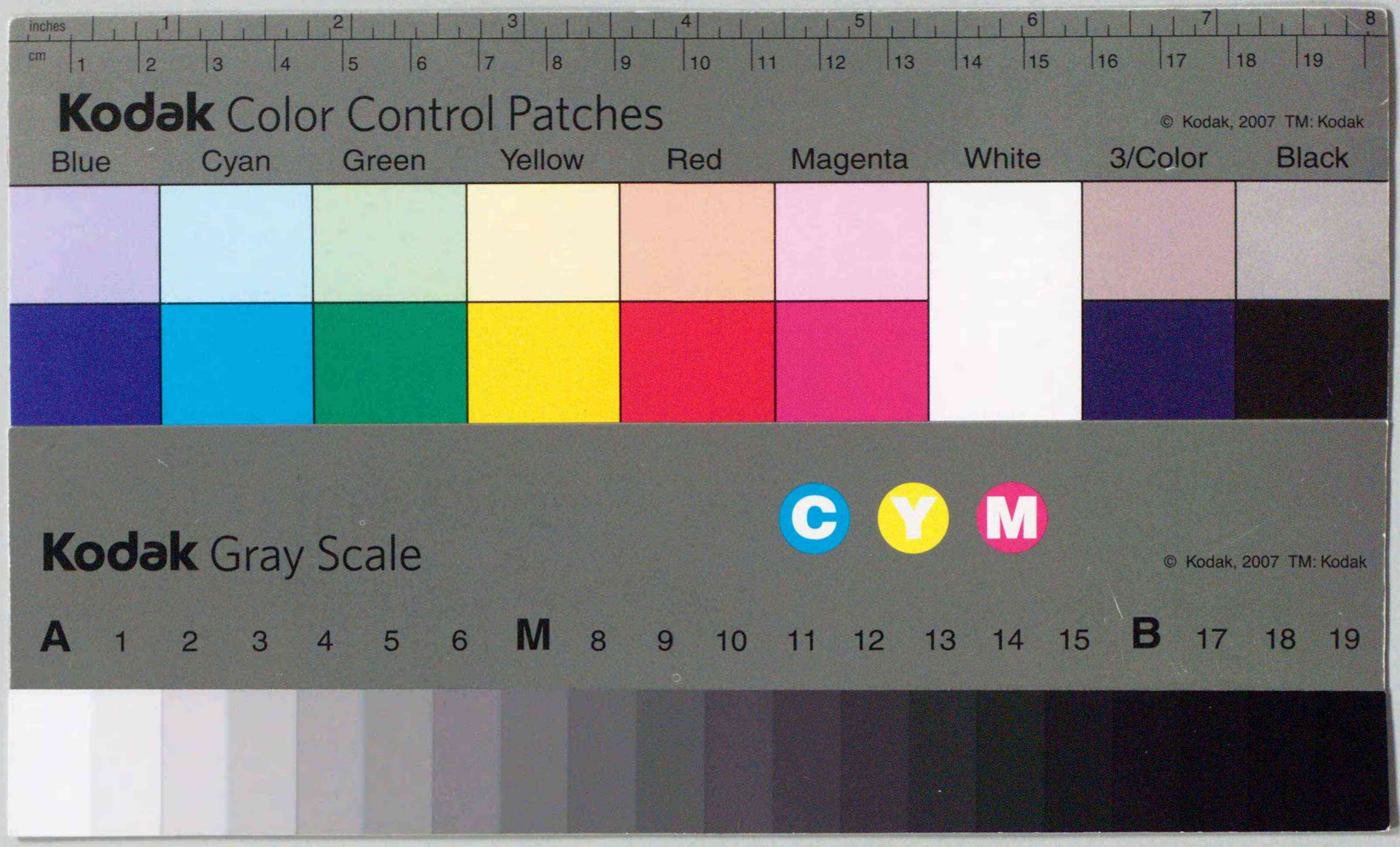
Wynne, M.J. and Banaimoon, S.A. 1990. The occurrence of *Jolyna laminarioides* (Phaeophyta) in the Arabian Sea and the Indian Ocean and a new report of *Melanothamnus somalensis* (Rhodophyta). Bot. Mar. **33**: 213-218.

Wynne, M.J. and Loiseaux, S. 1976. Recent advances in life history studies of the Phaeophyta. Phycologia **15**: 435-452.

Wynne, M.J. and Norris, J.N. 1976. The genus *Colpomenia* Derbès et Solier (Phaeophyta) in the Gulf of California. Smithsonian contributions to botany **35**: 1-18.

Yamada, Y. 1948. Marine algae. Number 1 in Y. Yamada and T. Kinoshita, Icones of the marine animals and plants of Hokkaido. 18 pp., pl.1-22. Yoichi, Hokkaido: Hokkaido Fisheries Scientific Institution.

Yoshida, T., Nakamura, Y. and Nakata, Y. 1990. Check-list of marine algae of Japan. Jpn. J. Phycol. **38**: 269-320.


inches
cm
Kodak Color Control Patches
© Kodak, 2007 TM: Kodak
Blue
Cyan
Green
Yellow
Red
Magenta
White
3/Color
Black
Kodak Gray Scale
C
Y
M
© Kodak, 2007 TM: Kodak
A 1 2 3 4 5 6 M 8 9 10 11 12 13 14 15 B 17 18 19