ISSN 0038-1578

Vol. 41 No. 3 20 September 1993

The Japanese Journal of PHYCOLOGY

CONTENTS

THE JAPANESE SOCIETY OF PHYCOLOGY

日 本 藻 類 学 会

日本藻類学会は1952年に設立され，藻学に関心をもち，本会の趣旨に賛同する個人及び団体の会員からなる。本会は定期刊行物「藻類」を年4回刊行し，会員に無料で頒布する。普通会員は本年度の年会費7,000円（学生は5,000円）を前納するものとする。団体会員の会費は12,000円，賛助会員の会費は1口20,000円とする。

庶務および会計に関する通信は，**602 京都市上京区下立売通小川東入　日本藻類学会**宛に，また「藻類」への原稿の送付は　**184 小金井市貫井北町4-1-1　東京学芸大学生物学教室内　日本藻類学会編集委員会**宛にされたい。

The Japanese Society of Phycology

The Japanese Society of Phycology, founded in 1952, is open to all who are interested in any aspect of phycology. Either individuals or organizations may become members of the Society. The Japanese Journal of Phycology (SÔRUI) is published quarterly and distributed to members free of charge.

Inquiries and other information regarding the society should be addressed to **The Japanese Society of Phycology, Shimotachiuri Ogawa Higashi, Kamikyoku, Kyoto, 602 Japan.** The annual dues (1993) for overseas members are 7,000 Yen (Send the remittance to The Japanese Society of Phycology at the above address).

Manuscript for publication should be submitted directly to the Editor-in-Chief, **Prof. I. Shihira-Ishikawa, Department of Biology, Tokyo Gakugei University, Nukuikita-machi, Koganei-shi, Tokyo,184 Japan.**

1993-1994年役員

会　　長：	有賀　祐勝（東京水産大学）
庶務幹事：	佐藤　博雄（東京水産大学）
会計幹事：	能登谷正浩（東京水産大学）
評 議 員：	
	鰺坂　哲朗（京都大学）
	千原　光雄（日本赤十字看護大学）
	榎本　幸人（神戸大学）
	原　　慶明（筑波大学）
	井上　　勲（筑波大学）
	喜田和四郎（三重大学）
	右田　清治
	大野　正夫（高知大学）
	岡崎　恵視（東京学芸大学）
	奥田　武男（九州大学）
	田中　次郎（国立科学博物館）
	谷口　和也（東北区水産研究所）
	秋山　　優
	山本　弘敏（北海道大学）
	横浜　康継（筑波大学）
	吉田　忠生（北海道大学）
編集委員会：	
委 員 長：	石川依久子（東京学芸大学）
幹　　事：	真山　茂樹（東京学芸大学）
実行委員：	片山　舒康（東京学芸大学）
	川井　浩史（神戸大学）
	前川　行幸（三重大学）
	岡崎　恵視（東京学芸大学）
	渡辺　　信（国立環境研究所）
委　　員：	千原　光雄（日本赤十字看護大学）
	藤田　雄二（長崎大学）
	堀　　輝三（筑波大学）
	井上　　勲（筑波大学）
	加藤　哲也（京都大学）
	喜田和四郎（三重大学）
	小林　　弘（東京珪藻研究所）
	大野　正夫（高知大学）
	舘脇　正和（北海道大学）
	都築　幹夫（東京大学）
	横浜　康継（筑波大学）
	吉田　忠生（北海道大学）

Officers for 1993-1994

President:	Yusho ARUGA (Tokyo University of Fisheries)
Secretary:	Hiroo SATOH (Tokyo University of Fisheries)
Treasurer:	Masahiro NOTOYA (Tokyo University of Fisheries)
Members of Executive Council:	
	Tetsuro AJISAKA (Kyoto University)
	Mitsuo CHIHARA (The Japanese Red Cross College of Nursing)
	Sachito ENOMOTO (Kobe University)
	Yoshiaki HARA (University of Tsukuba)
	Isao INOUYE (University of Tsukuba)
	Washiro KIDA (Mie University)
	Seiji MIGITA
	Masao OHNO (Kochi University)
	Megumi OKAZAKI (Tokyo Gakugei University)
	Takeo OKUDA (Kyushu University)
	Jiro TANAKA (National Science Museum)
	Kazuya TANIGUCHI (Tohoku National Fisheries Research Institute)
	Masaru AKIYAMA
	Hirotoshi YAMAMOTO (Hokkaido University)
	Yasutsugu YOKOHAMA (University of Tsukuba)
	Tadao YOSHIDA (Hokkaido University)
Editorial Board:	
	Ikuko SHIHIRA-ISHIKAWA (Tokyo Gakugei University), Editor-in-Chief
	Shigeki MAYAMA (Tokyo Gakugei University), Secretary
	Nobuyasu KATAYAMA (Tokyo Gakugei University), Associate Editor
	Hiroshi KAWAI (Kobe University), Associate Editor
	Miyuki MAEGAWA (Mie University), Associate Editor
	Megumi OKAZAKI (Tokyo Gakugei University), Associate Editor
	Makoto M. WATANABE (National Institute for Environmental Studies), Associate Editor
	Mitsuo CHIHARA (The Japanese Red Cross College of Nursing)
	Yuji FUJITA (Nagasaki University)
	Terumitsu HORI (University of Tsukuba)
	Isao INOUYE (University of Tsukuba)
	Tetzuya KATO (Kyoto University)
	Washiro KIDA (Mie University)
	Hiromu KOBAYASI (Tokyo Diatom Institute)
	Masao OHNO (Kochi University)
	Masakazu TATEWAKI (Hokkaido University)
	Mikio TSUZUKI (University of Tokyo)
	Yasutsugu YOKOHAMA (University of Tsukuba)
	Tadao YOSHIDA (Hokkaido University)

Jpn. J. Phycol. (Sôrui) 41: 191–198, September 20, 1993

Taxonomic studies on *Ulva pertusa* (Ulvophyceae). I. Morphological study

Mitsunobu Kamiya*, Koji Doi*, Yoshiaki Hara* and Mitsuo Chihara**

*Institute of Biological Sciences, University of Tsukuba, Tsukuba, Ibaraki, 305 Japan
**Japanese Red Cross College of Nursing, Hiro-o, Shibuya, Tokyo, 150 Japan

Kamiya M., Doi K., Hara Y. and Chihara M. 1993. Taxonomic studies on *Ulva pertusa* (Ulvophyceae). I. Morphological study. Jpn. J. Phycol. **41**: 191–198.

Ulva pertusa Kjellman (Ulvales, Ulvophyceae) is abundant at Ebisujima (=Ebisu Island), Shimoda, Shizuoka Prefecture, and at this locality plants with a distinct stalked appearance, but otherwise similar to *U. pertusa*, occur with a limited distribution on the southern side of the island. The gross morphology and the distribution of rhizoidal cells between cell layers differ at extremes, but intermediate forms exist and neither of these characters proved to be taxonomically useful. In addition, test crosses showed that there was no reproductive isolation among any combinations of *U. pertusa* and "stalked-*Ulva*". It is concluded that *U. pertusa* is morphologically variable species and forms with a petiolate morphology are included within this taxon.

Key Index Words: *crossing experiment, morphological variation, stalked*-Ulva, *taxonomy*, Ulva pertusa.

Ulva species are common components of the marine intertidal flora of Japan and at least 10 species are recognized in Japan (Yoshida *et al.* 1990). Among them, *U. pertusa* is abundant throughout the year and widely distributed. The gross morphology of the thallus can be highly variable, making specific assignment of individual specimens difficult.

Blades of *Ulva pertusa* found at Ebisujima, Shimoda, Izu Peninsula, are light to dark green, irregularly orbicular or broad, and often perforated. The blades are flat and sheet-like with a short stipe and small discoid holdfast.

They are seasonally abundant from early spring through summer. However, some of the plants have narrow, extended stipes, which branch one to several times and are sometimes twisted; the terminal blades are fan-shaped. Such plants are referred to here as "stalked-*Ulva*".

Plants with this morphology have been placed in the genus *Letterstedtia* (Areschoug 1851). He distinguished *Letterstedtia* from *Ulva* on the basis of the gross morphology and the existence of the so-called hyphae forming a medulla-like layer in the stipe, similar to the rhizoidal cells at the base of the thallus in *Ulva*. Papenfuss (1960) considered that the gross morphology was too variable for such a distinction to be valid and further noted that the "hyphae" could also be seen in some *Ulva* species. He therefore transferred the 3 species formerly ascribed to *Letterstedtia* to *Ulva*.

The aim of the present study was to compare the morphology of "stalked-*Ulva*" with typical *U. pertusa* and to clarify their relationships. Test crosses were also performed to investigate potential reproductive isolation between the two morphological entities.

Materials and Methods

Several plants were intermittently collected from each of 8 sites at Ebisujima (Fig. 1) from 1989 to 1992 in order to observe their morphology. The size of vegetative and reproductive cells of *Ulva pertusa* and "stalked-*Ulva*"

Contribution from Shimoda Marine Biological Research Center, University of Tsukuba, no. 552

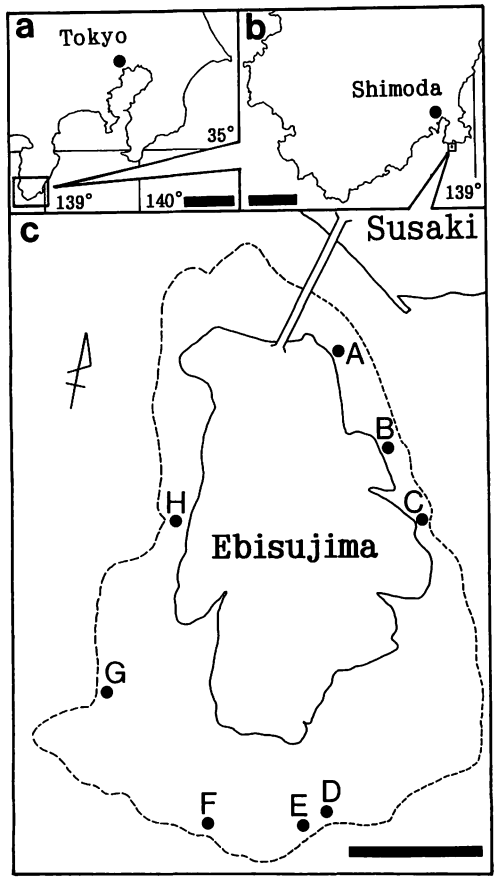


Fig. 1. The location of Ebisujima (a; scale bar=50 km, b; scale bar=5 km) and the sampling sites (c; scale bar=50 m). A broken line indicates 0 meter sea level.

were measured in the specimens collected in Ebisujima on August 7, 1992 and October 21, 1992. Ten plants were randomly collected from each site for measurement of thallus thickness during April, May, July, November and December, 1989. The thickness of thallus was measured in the center of the expanded thallus, using freezing microtome sections and light microscopy.

To test for reproductive isolation several fertile thalli of *Ulva pertusa* from site B and of "stalked-*Ulva*" from site D were collected (see Fig. 1c), and placed in filtered sea water in the laboratory. A pair of male and female gametes of "stalked-*Ulva*" was selected by preliminary crosses between biflagellate gametes released from each fertile thallus, because it was difficult to distinguish definitely male and female gametes from their size and color. Five individuals of *U. pertusa* and "stalked-*Ulva*" were examined to cross with each mating type of "stalked-*Ulva*" on sterilized glass slides. When positive crossing was judged by observing aggregation and conjugation, the zygotes were isolated by using their negative photoaccumulation and cultured in small plastic vessels containing PES medium (Starr and Zeikus 1993).

Results

Morphology

The following morphological characters of *Ulva pertusa* and "stalked-*Ulva*" were considered to have taxonomic significance and recorded: (1) gross morphology, (2) shape and size of cells in surface and sectional view and the number of pyrenoid in a vegetative cell, (3) thallus thickness, (4) the internal structure of the stipe and the basal region, (5) shape and size of reproductive cells.

Gross morphology. "Stalked-*Ulva*" specimens were typically medium green, perforated, 3-15 cm in height and with long and narrow stipes (5-25 mm long and 0.5-3.5 mm wide) branching one to several times (Fig. 2f). Plants with a morphology intermediate between *U. pertusa* and "stalked-*Ulva*" were collected at the south of the island where both forms existed together (Fig. 1c, sites D, E and F). Figure 2 shows a range of plants of the intermediate type. The thalli of these plants were similar in height to those of "stalked-*Ulva*". The stipes of the plants were relatively wide and scarcely differentiated from upper blades. The expanded fronds of the plants were partially interconnected.

Shape and size of cells in surface and sectional view and the number of pyrenoid in a vegetative cell. Cells in middle and apical regions in surface view were irregularly polygonal, with 3-5 corners that were a little rounded. These cells were arranged in indistinct groups, or formed short straight to curved rows with diverse orientations. Ordinary cells in the basal regions were relatively round, arranged in short curved rows. The abundant dark

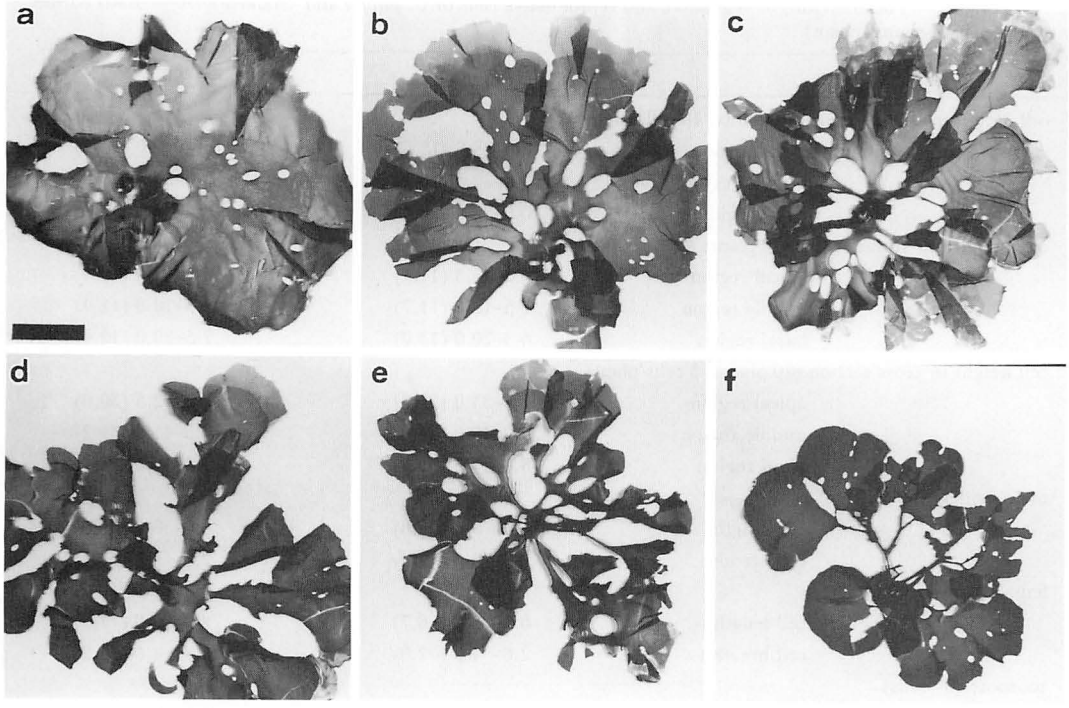


Fig. 2. Typical and intermediate morphologies of *U. pertusa* and "stalked-*Ulva*" with various forms from sites D-F in Fig. 1. Scale bar (2 cm) in Fig. 2a applies to all the figures. a. Typical *U. pertusa*. b–e. Intermediate form between *U. pertusa* and "stalked-*Ulva*". f. Typical "stalked-*Ulva*".

colored or colorless rhizoidal cells were slightly larger than the ordinary cells. In cross-section, cells were subrectangular to cylindrical in shape with rounded corners. The number of pyrenoids was mostly 1 or 2 in a vegetative cell and rarely more than 3. These characteristics of "stalked-*Ulva*" were quite similar to those of *U. pertusa*, and the cell size in the middle region showed no significant difference ($P>0.5$) between them (Table 1).

Thallus thickness. The thallus thickness in the middle region of blades was compared throughout the year (Table 2), but there was no significant difference between specimens assigned to *Ulva pertusa* and "stalked-*Ulva*". The only significant difference ($P<0.01$) among specimens from different sites was that the plants at site H were thinner than elsewhere.

The internal structure of the stipe and the basal region. Algae in the genus *Ulva* are composed of two layers of the cells, with numerous cells having rhizoidal extensions between the cell layers in the basal region that are directed towards the base of the plant. Figure 3 shows the distribution of rhizoidal cells of *U. pertusa* (a) and "stalked-*Ulva*" (b). In typical *U. pertusa* several chlorophyllous rhizoidal cells (Fig. 4a-c) appeared at the position 1 in Fig. 3a, and the abundance of rhizoidal cells increased toward the holdfast (position 2 in Fig. 3a, Fig. 4d-f). Numerous colorless rhizoidal cells (Fig. 4g-i) were seen only near the holdfast (position 3 in Fig. 3a). In "stalked-*Ulva*" several chlorophyllous rhizoidal cells (Fig. 5a-c) appeared in the thallus near the stipe (position 1 in Fig. 3b). Many rhizoidal cells (Fig. 5d-i) were seen throughout the stipes (position 2 and 3 in Fig. 3b). Rhizoidal cells often occurred in narrowed parts of the upper thallus of the "stalked-*Ulva*" as indicated in Fig. 3b. The distribution of the rhizoidal cells of typical "stalked-*Ulva*" differed from typical *U. pertusa*. However, this character could not be used to separate clearly "stalked-*Ulva*" from

Table 1. The size (μm) of vegetative and reproductive cells of *U. pertusa* and "stalked-*Ulva*". Each datum indicates range and (mean).

		U. pertusa	stalked-*Ulva*
cell length in surface view (20 plants, 10 cells/plant)			
	apical region	8.8–23.8 (15.6)	10.0–20.0 (14.2)
	middle region	10.0–22.5 (15.9)	10.0–22.5 (15.7)
	basal region	10.0–27.5 (18.3)	12.5–27.5 (19.4)
cell breadth in surface view (20 plants, 10 cells/plant)			
	apical region	6.3–16.3 (11.1)	6.3–15.0 (10.4)
	middle region	7.5–18.8 (11.7)	7.5–18.8 (11.4)
	basal region	6.3–20.0 (13.0)	7.5–20.0 (12.8)
cell height in cross section (20 plants, 5 cells/plant)			
	apical region	22.5–35.0 (27.3)	22.5–42.5 (30.0)
	middle region	25.0–45.0 (33.9)	27.5–45.0 (34.2)
	basal region	18.8–47.5 (31.2)	17.5–47.5 (27.7)
male gamete (50 cells)			
	cell length	6.0– 8.0 (6.6)	6.0– 8.0 (7.0)
	cell breadth	2.0– 3.0 (2.4)	2.0– 3.7 (3.1)
female gamete (50 cells)			
	cell length	6.0– 8.2 (6.7)	7.0– 9.0 (7.5)
	cell breadth	2.0– 4.0 (2.9)	2.3– 4.0 (3.2)
zoospore (50 cells)			
	cell length	6.8–10.8 (9.4)	7.5–12.0 (9.6)
	cell breadth	3.3– 6.8 (4.8)	4.0– 7.0 (5.5)

U. pertusa because specimens were often observed with intermediate morphologies.

Shape and size of reproductive cells. In all seasons, most plants in the collected materials of both *Ulva pertusa* and "stalked-*Ulva*" were gametophytes, which produced slightly narrower swarmers of male gamete or slightly broader swarmers of female. These differences could be found by careful observations after crossing tests. Sporophytes produced larger quadriflagellated zoospores (Table 1).

Reproductive isolation

Different mating types of "stalked-*Ulva*" (D1 and D2 in Table 3) confirmed by preliminary crosses were selected. This pair was used for test crosses with gametes from each of 5 individuals of *U. pertusa* and "stalked-*Ulva*", including D1 and D2. Almost all gametic swarmers crossed between different mating types showed aggregation and conjuga-

Table 2. Mean thallus thickness (μm) in the middle region of blade and standard deviation of 10 plants from each site. Bars indicate that plants were missed the chance to be collected from the sites.

Sites	April 5	May 5	July 1	November 11	December 26
A	87.08± 9.38	90.30±11.5	102.9 ±11.4	81.15± 7.57	68.95± 5.80
B	70.01± 6.28	75.10± 9.20	—	85.15±11.6	94.06±11.4
C	93.20±23.0	61.45±11.3	100.5 ±10.1	81.10±17.1	104.5 ±12.2
D	61.88± 4.35	72.35± 3.93	70.30±10.5	83.30± 9.35	84.45±14.0
E	64.40± 5.40	81.28± 5.04	67.15±12.7	80.90±11.6	88.95± 1.20
F	79.45± 9.50	61.38±10.4	57.25± 4.40	77.25± 7.70	91.50± 7.47
G	78.23±12.9	71.30± 6.18	—	78.65± 4.18	84.05±20.2
H	62.68±16.7	—	47.90± 7.80	63.30± 8.48	62.20±15.7

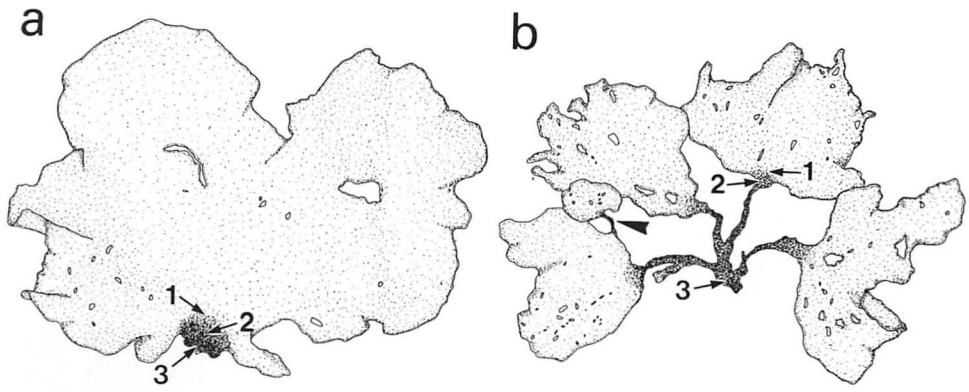


Fig. 3. The distribution of rhizoidal cells of *U. pertusa* (a) and "stalked-*Ulva* (b) is indicated by higher dense dots. Rhizoidal cells are also seen in a narrowed part of the upper thallus (arrowhead).

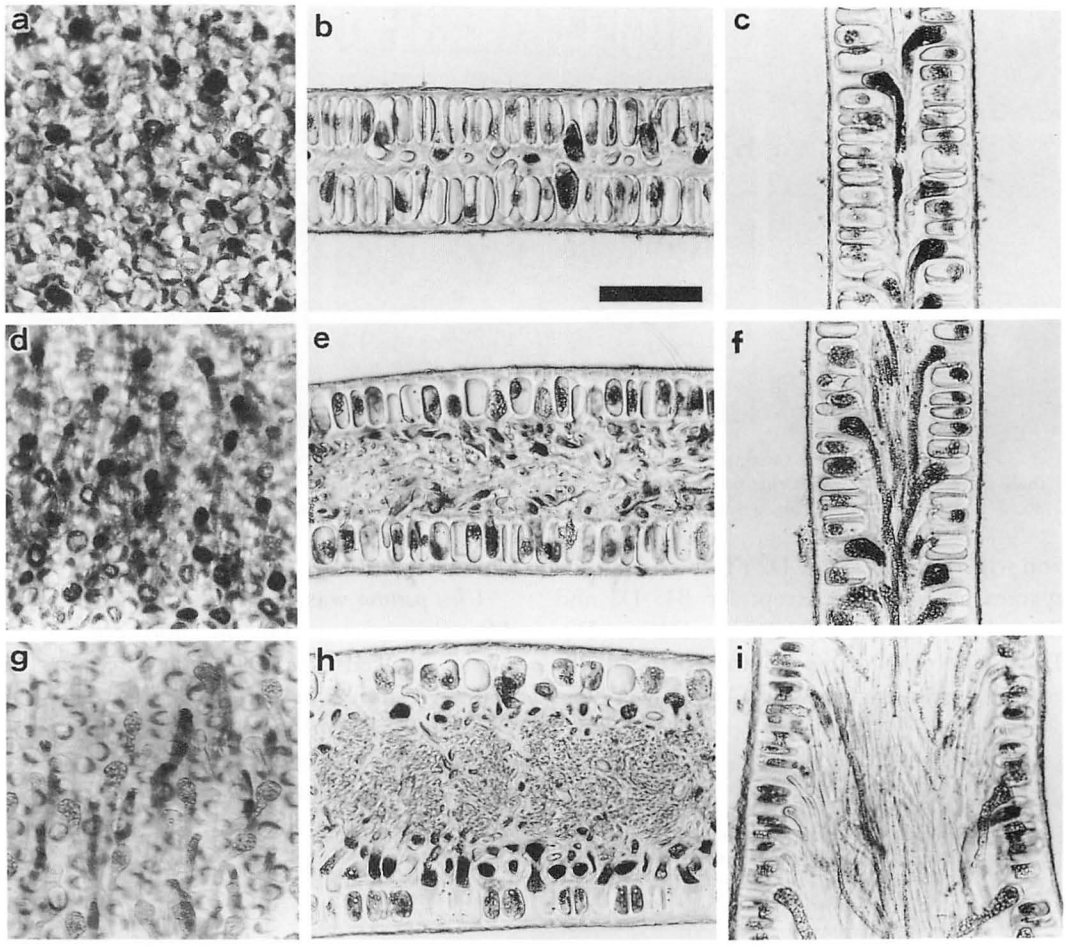


Fig. 4. Surface view (a, d, g), transversal section (b, e, h) and longitudinal section (c, f, i) of vegetative thalli of *U. pertusa* with rhizoidal cells that are directed towards the base of the plant. a–c, position 1 in Fig. 3a; d–f, position 2 in Fig. 3a; g–i, position 3 in Fig. 3a. Scale bar (50 μm) in Fig. 4b applies to all the figures.

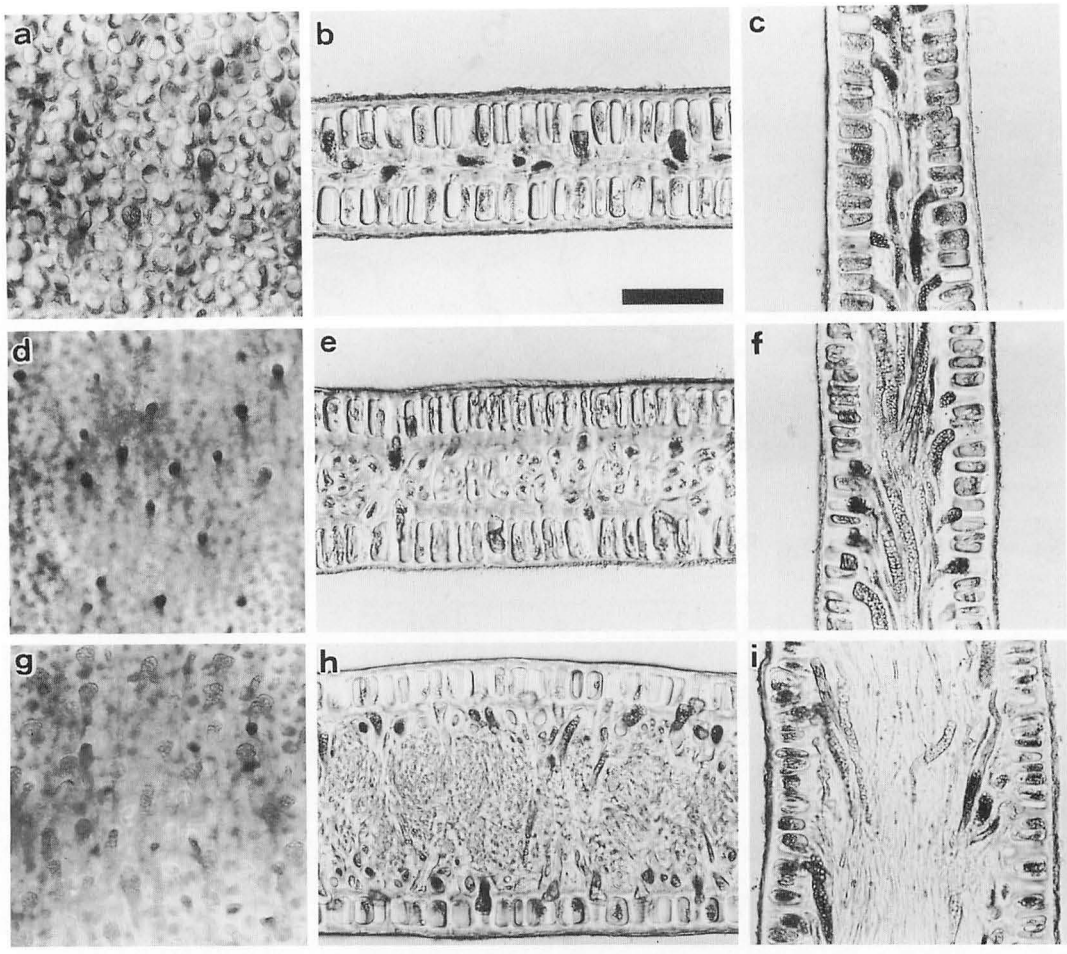


Fig. 5. Surface view (a, d, g), transversal section (b, e, h) and longitudinal section (c, f, i) of vegetative thalli of "stalked-*Ulva*" with rhizoidal cells that are directed towards tha base of the plant. a–c, position 1 in Fig. 3b; d–f, position 2 in Fig. 3b; g–i, position 3 in Fig. 3b. Scale bar (50 μm) in Fig. 5b applies to all the figures.

tion with those of D1 or D2 (Table 3) and the zygotes germinated, except for B4, D3 and D4, which crossed with neither D1 nor D2. The germlings are developing to the thalli, however, releasing zoospores have never been observed.

Table 3. The result of test crosses between *U. pertusa* and "stalked-*Ulva*". Each number designates one plant, and B and D indicate *U. pertusa* from site B and "stalked-*Ulva*" from site D, respectively. + shows that gametes crossed and the zygotes germinated, and − shows not crossed.

	B1	B2	B3	B4	B5	D1	D2	D3	D4	D5
D1	+	+	+	−	−	−	+	−	−	−
D2	−	−	−	−	+	+	−	−	−	+

Habitat

Ulva pertusa was widely distributed around Ebisujima except on the northern shore, while "stalked-*Ulva*" was restricted to the southern shore which consists of a broad flat rock platform with strong wave action. *U. pertusa* grew inshore of "stalked-*Ulva*" at the southern shore, but there was no clear boundary between the populations.

Discussion

Ulva pertusa was described by Kjellman (1897) on the basis of specimens from Japan. According to the description, thalli are 15–20 cm high, perforated by many roundish or

irregularly shaped foramina, at least 125 μm thick in the lower part and about 40 μm thick in the marginal region. Kjellman's description was augmented with habit photos of five specimens and illustrations of thallus structure. He cited Hakodate, Enoshima and Yokohama as their localities, with a comment that the species seemed to be widely distributed. In the present study, the *Ulva* species growing abundantly at Ebisujima had thalli up to 17 cm high, and a thickness of middle and basal regions of 40-130 μm and 80-400 μm, respectively. These plants agree well with the description and illustrations of *U. pertusa* given by Kjellman. Incidentally, Shimoda is near the TYPE localities of Enoshima and Yokohama.

When typical *Ulva pertusa* and "stalked-*Ulva*" are compared, their gross morphology is quite distinct. However, the existence of plants with morphology intermediate between *U. pertusa* and "stalked-*Ulva*" makes it difficult to distinguish them. Similar difficulties are reported in distinguishing Australian plants of *U. rigida* C. Agardh, which is a most common species in southern Australia (Phillips 1988). *U. spatulata* Papenfuss (previously referred to *Letterstedtia*) has long stipes like those of "stalked-*Ulva*"; and *U. australis* Areschoug shows transitional stages of the gross morphology between *U. spatulata* and *U. rigida*. Phillips (1988) regarded *U. australis* and *U. spatulata* as synonyms of *U. rigida* because the two taxa were identical to *U. rigida* in diagnostic characters and developmental patterns.

Rhizoidal cells in "stalked-*Ulva*" were more highly developed than those in *U. pertusa*, but the distinction of rhizoidal cell development was not clear. The rhizoidal cells were found near the base of the plants as well as in narrow parts of the upper thallus (Fig. 3b). So the development of rhizoidal cells may simply relate to the width of the thallus.

The process of the formation of the stipes has not been observed, but it is possible to infer the process by comparing plants with intermediate morphologies between *Ulva pertusa* and "stalked-*Ulva*". The holes of the thalli become larger and join with one another near the holdfast (Fig. 2b, c). The thalli divide into several main portions by the further enlargement of the perforations (Fig. 2d, e) and the upper expanded thalli are then attached by narrow stipes (Fig. 2f). This process may occur throughout the thallus so that "stipes" can be observed in upper thallus parts (Fig. 3b).

Some workers (Bliding 1968; Koeman and van den Hoek 1981; Hoeksema and van den Hoek 1983) considered pyrenoid number, thallus thickness, and size and arrangement of vegetative cells to be useful taxonomic characters, whereas others (Titlyanov *et al.* 1975; Steffensen 1976; Phillips 1988) reported that, in some *Ulva* species, thallus thickness, cell size and pyrenoid number were too variable for taxonomic use. In this study the cell size of middle and apical region, measured in surface view and cross-section, exhibited considerable variations and overlapped between *U. pertusa* and "stalked-*Ulva*" (Table 1). It was obvious that there was no critical difference between them in the shape and arrangement of their vegetative cells and the pyrenoid number in a cell. The thallus thickness was also highly variable in the individual plants and there was no significant difference between those of *U. pertusa* and "stalked-*Ulva*". It may be attributed to thallus size, season and/or habitat in *U. pertusa*.

The crossing of gametes between *Ulva pertusa* and "stalked-*Ulva*" and germination of the zygotes were observed, but the progeny development has not been observed yet. Now zygotes, gametes and zoospores of "stalked-*Ulva*" and zygotes between *U. pertusa* and "stalked-*Ulva*" are continued to culture, so it will become clarified in the near future whether the gross morphology of "stalked-*Ulva*" is genetically stable or not.

Gametes of several plants (B4, D3 and D4 in Table 3) crossed with neither D1 nor D2, but it is not clear whether the results were caused by the absence of their crossing ability or some difference of the experimental conditions.

Plants of the stalked form were also seen with *Ulva pertusa* on relatively flat rock plat-

forms with strong wave action in Arashima, Miyagi Prefecture, Tanesashi, Aomori Pref. and Ooarai, Ibaraki Pref. The formative process of the morphology of "stalked-*Ulva*" has not been observed, but it may be related to environmental factors. However, the possibility remains that genetic differences exist between the ecological forms. Consequently, a molecular taxonomic analysis using isozymes was initiated. Results of this investigation will be reported separately.

Acknowledgements

Special thanks are due to Dr. Robert J. King, the University of New South Wales, for his critical reading of the draft. We also thank Dr. Yasutsugu Yokohama, Dr. Isao Inouye and other members of the Laboratory of Plant Phylogeny and Taxonomy, University of Tsukuba, for their encouragement and advice, and the members of the Shimoda Marine Biological Research Center, University of Tsukuba, for their help in collecting materials.

References

Areschoug, J. E. 1851. *Letterstedtia*, ny alg-form från Port Natal. Öfvers. [Sv.] Vetensk. Akad. Förh. Stockh. **1**: 1–4.

Bliding, C. 1968. A critical survey of European taxa in Ulvales. II. *Ulva , Ulvaria, Monostroma, Kornmannia*. Bot. Not. **121**: 535–629.

Hoeksema, B. W. and van den Hoek, C. 1983. The taxonomy of *Ulva* (Chlorophyceae) from the coastal region of Roscoff (Brittany, France). Bot. Mar. **26**: 65–86.

Kjellman, F. R. 1897. Marina Chlorophyceen från Japan. Bihang Till K. Svenska Vet. -Akad. Handlingar. **23**: III. 1–44, pl. 3.

Koeman, R. P. T. and van den Hoek, C. 1981. The taxonomy of *Ulva* (Chlorophyceae) in the Netherlands. Br. phycol. J. **16**: 9–35.

Papenfuss, G. F. 1960. On the genera of the Ulvales and the status of the order. J. Linn, Soc. (Bot.) **56**: 303–318.

Phillips, J. A. 1988. Field, anatomical and developmental studies on southern Australian species of *Ulva* (Ulvaceae, Chlorophyta). Aust. Syst. Bot. **1**: 411–456.

Starr, R. C. and Zeikus, J. 1993. UTEX - the culture of algae at the University of Texas at Austin. J. Phycol. **29**(suppl.): 1–106.

Steffensen, D. A. 1976. Morphological variation in *Ulva* in the Avon-Heathcote Estuary, Christchurch. N. Z. J. Mar. Freshwater Res. **10**: 329–341.

Titlyanov, E. A., Glebova, N. T. and Kotlyarova, L. S. 1975. Seasonal changes in structure of the thalli of *Ulva fenestrata* P. *et* R. Ekologiya **6**: 320–324.

Yoshida, T., Nakajima, Y. and Nakata, Y. 1990. Check-list of marine algae of Japan (in Japanese). Jpn. J. Phycol. **38**: 269–320.

神谷充伸*・土井考爾*・原　慶明*・千原光雄**：アナアオサ（アオサ藻綱）の分類学的研究．I．形態学的研究

恵比須島（静岡県下田市）の周囲の潮間帯にはアナアオサが繁茂するが，この島の南側のアナアオサの集団に，柄を有するアオサ（エツキアオサと仮称）がみられる。本研究では，形態学的研究および交配実験を行い，両藻の分類学的な関係を検討した。エツキアオサは外部形態や基部付近の仮根細胞の分布においてアナアオサと明らかに異なるが，南側の集団の個体を詳細に調査すると，両者の中間の形態を示す個体も存在し，形態的特徴からは両者を区別できないことが判明した。交配実験ではアナアオサとエツキアオサの配偶子は交雑し，その接合子は正常に発芽することが確認された。これらの結果から，アナアオサは形態的に変異に富んだ種であり，エツキアオサはこの分類群に含まれると考えられる。（*305 つくば市天王台1-1-1　筑波大学生物科学系，**150 渋谷区広尾4-1-3　日本赤十字看護大学）

(*Received March 18, 1993, Accepted June 22, 1993*)

Jpn. J. Phycol. (Sôrui) 41: 199–206, September 20, 1993

Taxonomic studies on *Ulva pertusa* (Ulvophyceae). II. Preliminary isozyme analysis

Koji Doi*, Mitsunobu Kamiya*, Yoshiaki Hara* and Mitsuo Chihara**

*Institute of Biological Sciences, University of Tsukuba, Tsukuba, Ibaraki, 305 Japan
**Japanese Red Cross College of Nursing, Hiro-o, Shibuya, Tokyo, 150 Japan

Koji Doi, Mitsunobu Kamiya, Yoshiaki Hara and Mitsuo Chihara. 1993. Taxonomic studies on *Ulva pertusa* (Ulvophyceae). II. Preliminary isozyme analysis. Jpn. J. Phycol. 41: 199–206.

Preliminary comparisons of isozyme banding patterns were carried out between *Ulva pertusa* Kjellman and *U. arasakii* Chihara, and between *U. pertusa* and "stalked-*Ulva*". Several electrophoretic differences were detected for enzyme-species including GDH, IDH and GOT between specimens of *U. pertusa* and *U. arasakii*. Otherwise, no differences for any of assayed enzymes were detected between specimens of *U. pertusa* and "stalked-*Ulva*" at the same population in Ebisujima (Shimoda, Shizuoka Pref.).

Overall electrophoretic patterns among specimens of *U. pertusa* from fourteen localities from Hokkaido to Yamaguchi were almost identical in spite of their wide morphological variability. However, a variation in GDH was found for the typical form of *U. pertusa* in populations on the eastern side of Ebisujima. Other variations in GDH and IDH were recognized in the populations of Kabushima (Hachinohe, Aomori Pref.).

Cross experiments of the gametes from two electroforms of GDH from Ebisujima revealed that they are interfertile.

*Key Index Words: crossing test—genetic variability—isozyme analysis—"stalked-*Ulva*"—taxonomy—* Ulva arasakii—Ulva pertusa.

The plants of the genus *Ulva* (Ulvales, Ulvophyceae) are among the most common coastal seaweeds in Japan. Classification of the numerous species assigned to this genus is based on gross morphology of the thallus. However, shape, size and thickness of *Ulva* thalli generally vary with age and habitat. Specimens of *U. pertusa* show significant morphological variation, ranging from an *U. conglobata*-type to an *U. arasakii*-type. The previously reported "stalked-*Ulva*" represents an additional morphological form assigned to this species (Kamiya *et al.* 1993).

Genetic data of isozyme analysis have been applied to taxonomic studies of red algae (Lindstrom and South 1989), dinoflagellates (Hayhome *et al.* 1987), charophytes (Grant and Procter 1980), and desmids (Francke and Coesel 1985; Coesel and Menken 1986, 1988; Jurgenson and Biebel 1989). In the Ulvales, Innes and Yarish (1984) and Innes (1987, 1988) serveyed intraspecific genetic variability in *Enteromorpha linza* (L.) J. Agardh. Relatively fewer isozyme studies have been conducted on algae compared to higher plants, and the techniques suitable for algae tissue have not been satisfactorily established. Consequently, the number of enzymes examined in most studies of algae has been insufficient, making precise genetic interpretation difficult. In addition, the intra- and interspecific taxonomy of many algal species investigated has not been established by means of crossing tests or morphology and genetic analysis.

In this paper, the preliminary interspecific comparison of isozyme patterns between *U. pertusa* and *U. arasakii* growing sympatrically was carried out. It was followed by intraspecific comparison of *U. pertusa* among allopatric populations, and between typical

Contribution from Shimoda Marine Research Center, University of Tsukuba, No. 553.

U. pertusa and "stalked-*Ulva*". The effectiveness of isozyme analysis for the taxonomy of these algae is discussed.

Materials and Methods

Collection and preservation of algae. Specimens showing the typical appearances of both *Ulva pertusa* and "stalked-*Ulva*" were collected from populations in Ebisujima, Shimoda, Izu Peninsula, (Kamiya *et al.* 1993) from March, 1989 to May, 1991. At the same time, specimens of the typical *U. pertusa* were collected from various localities in Hokkaido and Honshu (Fig. 1) for comparison. Specimens of *U. arasakii* were collected from Ooarai (Ibaraki Pref.) and Kimigahama (Chiba Pref.) as well.

Living specimens from which surface sea water was removed were placed into individ-

Morphologically typical *Ulva pertusa*

1. Monbetsu (Monbetsu-shi, Hokkaido)
2. Ougonmisaki (Rumoi-shi, Hokkaido)
3. Zenibako (Otaru-shi, Hokkaido)
4. Haruka (Otaru-shi, Hokkaido)
5. Aomori (Aomori-shi, Aomori)
6. Kabushima (Hachinohe-shi, Aomori)
7. Areshima (Shizugawa-cho, Miyagi)
8. Ooarai (Ooarai-machi, Ibaraki)
9. Kimigahama (Choushi-shi, Chiba)
10. Kominato (Amatsukominato-machi, Chiba)
11. Ebisujima (Shimoda-shi, Shizuoka)
12. Nabeta (Shimoda-shi, Shizuoka)
13. Tokiwa (Ube-shi, Yamaguchi)

Ulva arasakii

8. Ooarai (Ooarai-machi, Ibaraki)
9. Kimigahama (Choushi-shi, Chiba)

"stalked-*Ulva*"

11. Ebisujima (Shimoda-shi, Shizuoka)

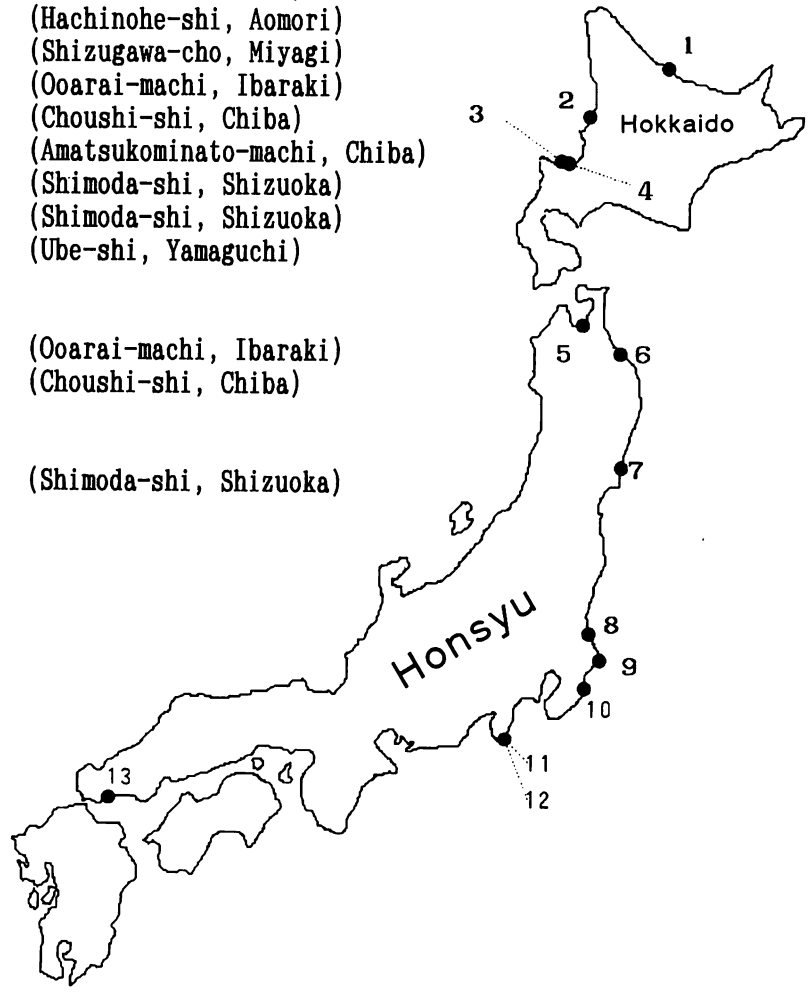


Fig. 1. Localities of *Ulva pertusa* and related taxa collected in Japan.

ual vinyl bags, maintained at approximately 10°C and transported rapidly to University of Tsukuba.

Motile cells released from matured specimens were observed to identify gametophyte and sporophyte thalli. Gametes determined to have compatible mating types were used for the crossing tests. For isozyme analysis, fresh specimens were ground in liquid nitrogen, and stored at −80°C until being assayed.

Isozyme analysis. Crude extracts were obtained by grinding material (at least 500 mg wet weight) in 1.0 m*l* of cold extraction buffer; 0.5 M Tris-HCl buffer, pH 8.0, containing 70 mM 2-mercaptoethanol, 26 mM sodium metabisulfite, 0.5 mM EDTA, 5 mM sodium ascorbate, 0.1% Tween 80 and 4% soluble polyvinylpyrrolidone with an average molecular weight of 40,000. Extracts were centrifuged at 6,200 G for 10 min. The supernatants were immediately filtered through a simplified gel filtration method (Kato 1987) for purifying isozyme samples.

For electrophoresis, starch gel (12.8% w/v) made up with the system 5 and 10 buffers of Soltis *et al.* (1983) was used. Samples were absorbed onto rectangular wickes of Advantec 51B chromatography paper, inserted into a slice made across the gel ca. 5 cm from the cathode. Electrophoresis was done at 4°C and 150 volts (constant voltage) for 3–5 hours, until the bromphenol blue marker, which was inserted in the gel with the samples, had migrated 10 cm from the origin.

The following fifteen enzymes were preliminarily tested: esterase (EST), glutamate dehydrogenase (GDH), isocitrate dehydrogenase (IDH), malate dehydrogenase (MDH), malic enzyme (ME), glucose-6-phosphate dehydrogenase (G6PDH), hexokinase (HK), phosphoglucoisomerase (PGI), phosphoglucomutase (PGM), triphosphate isomerase (TPI), 6-phosphogluconate dehydrogenase (6-PGD), acid phosphatase (ACPH), glutamate oxaloacetate transaminase (GOT), superoxide dismutase (SOD), and shikimate dehydrogenase (SKDH). The protocol of staining was followed by Soltis *et al.* (1983) with some modifications (Table 1).

Table 1. The staining protocol of enzymes employed in this study.

Enzyme	Reactant/Stain
GDH	100 m*l* 0.1 M Tris-HCl, pH 8.0
	2.94 g L-glutamic acid
	20 mg NAD
	10 mg MTT
	2 mg PMS
IDH	100 m*l* 0.1 M Tris-HCl, pH 8.0
	100 mg isocitric acid, trisodium salt
	5 m*l* 1.0 M $MgCl_2$
	20 mg NADP
	10 mg MTT
	2 mg PMS
GOT	100 m*l* 0.1 M Tris-HCl, pH 8.0
	100 mg L-aspartic acid
	100 mg α-ketoglutaric acid
	5 mg pyridoxal-5'-phosphate
	90 mg Fast blue BB salt
G6PDH	100 m*l* 0.1 M Tris-HCl, pH 8.0
	200 mg glucose-6-phosphate
	20 mg NADP
	10 mg MTT
	2 mg PMS

Abbreviations: ATP, adenosine-5'-triphosphate; EDTA, disodium ethylenediamine tetra-acetic acid; MTT, (3-[4, 5-dimethylthiazol-2-1]-2, 5-diphenyltetrazolium bromide; NAD, β-nicotinamide adenine dinucleotide; NADP, β-nicotinamide adenine dinucleotide phosphate; PMS, phenazine methosulphate.

Crossing tests. Crossing tests were carried out between gametes from different electroforms of morphologically typical *U. pertusa* at Ebisujima. The technique was outlined in the previous paper (Kamiya *et al.* 1993).

Results

Preliminary observations using typical *Ulva pertusa* (Fig. 2) for the fifteen enzymes listed above showed that stable bands could be obtained from four enzymes; IDH, GDH, G6PDH, and GOT.

U. arasakii (Fig. 3) was provided to analyze its isozymes in comparison with those of *U. pertusa*. Clear differences were recognized for three of four enzyme species examined (Fig. 5), whereas no difference was found for G6PDH between two entities (Table 2).

When the banding patterns of morphologically typical *U. pertusa* and "stalked-*Ulva*"

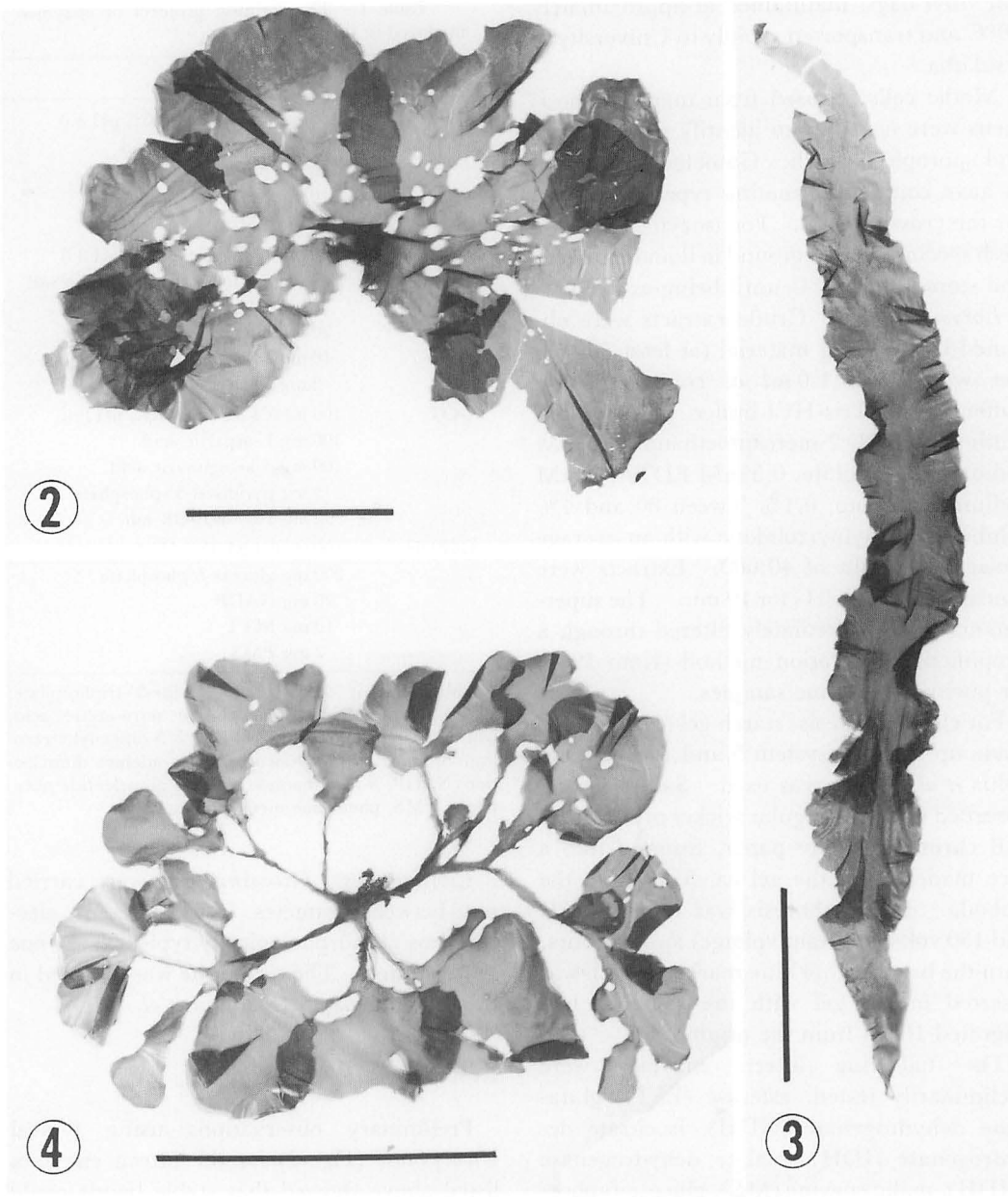


Fig. 2. Specimen of typical *Ulva pertusa* collected at Ebisujima in December, 1989. Scale bar=5.0 cm.
Fig. 3. Specimen of typical *Ulva arasakii* collected at Kimigahama (Chiba Pref.) in May, 1990. Scale bar=5.0 cm.
Fig. 4. Specimen of "stalked-*Ulva*" collected at southern population in Ebisujima in December, 1989. Scale bar=5.0 cm.

(Fig. 4) in Ebisujima were compared, no specific banding patterns were found (Fig. 6). Otherwise, two banding patterns for GDH were found in typical *U. pertusa* from different populations in Ebisujima (Fig. 7). Specimens of the populations on the eastern side of this island (sites A, B and C in Fig. 1 of Kamiya *et al.*, 1993) exhibited one banding pattern, labelled "GDH-B", whereas specimens of the other sites had a banding pattern,

Table 2. The summarized results of electrophoretic patterns of *U. pertusa*, *U. arasakii* and "stalked-*Ulva*". Letters designate the electrophoretic forms in each enzyme recognized within each population.

Populations	Enzyme species			
	IDH	GDH	GOT	G6PDH
U. pertusa				
Ebisujima	A	A, B	A	A
Kabushima	A, B	A, C	A	A
Other populations	A	A	A	A
U. arasakii	B'*	C'*	B	A
"Stalked-*Ulva*"	A	A	A	A

* Migration distance of these electromorphs not compared directly with specimens from Kabushima.

"GDH-A", common to populations of all other Japanese localities investigated (Fig. 1). The specimens showing "GDH-B" were similar to other populations for other enzymes examined (Table 2), and could not be distinguished morphologically.

Other variations of GDH and IDH were also found in the population of typical *U. pertusa* from Kabushima (Fig. 7). Some specimens showed unusual banding patterns for GDH and IDH (labelled "GDH-C" and "IDH-B") while the others in the same populations showed the pattern of "GDH-A" and "IDH-A" which were widely spread. No characteristics of gross morphology were associated with this genetic variation.

No difference in the electrophoretic behavior specific to sporophytic and gametophytic

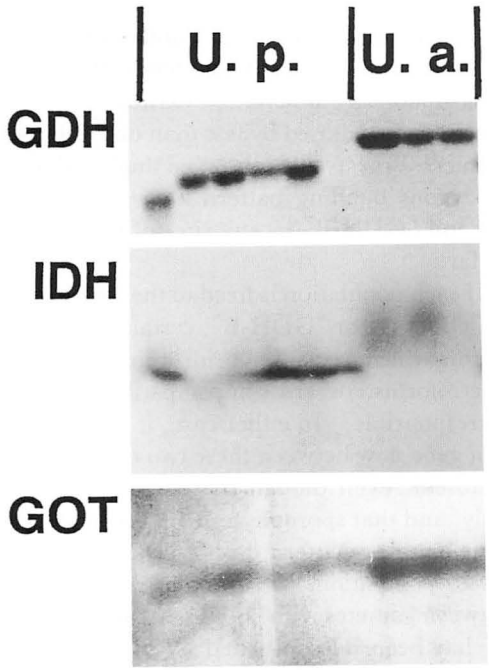


Fig. 5. Representative zymogram patterns for *Ulva pertusa* and *U. arasakii*. U.p., *U. pertusa*. U.a., *U. arasakii*.

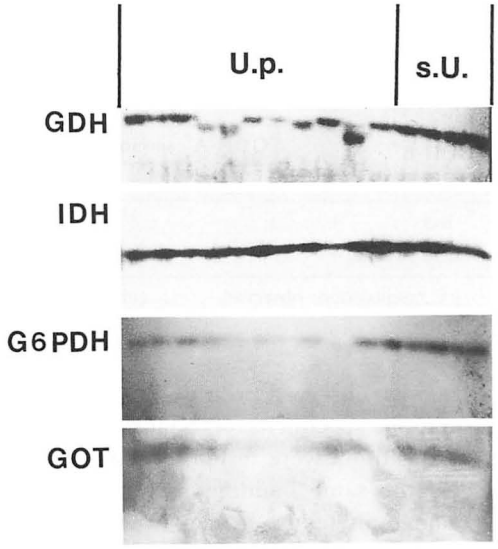


Fig. 6. Representative zymogram patterns for *Ulva pertusa* and "stalked-*Ulva*" in Ebisujima. U.p., *U. pertusa*. s.U., "stalked-*Ulva*".

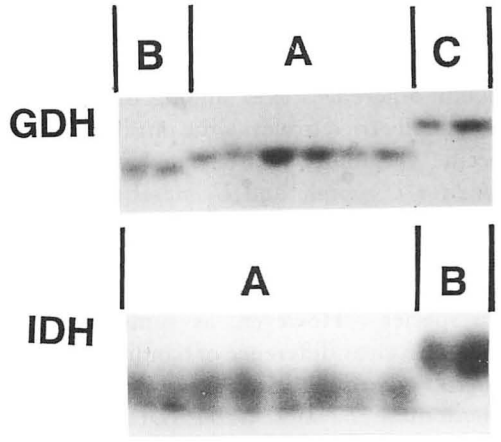


Fig. 7. Representative zymogram patterns for *Ulva pertusa* in Kabushima. Letters designate electrophoretic forms (see Table 2).

Table 3. The results of test crosses between typical specimens of *Ulva pertusa* showing "GDH-A" and "GDH-B" banding patterns. Alphabets designate populations (see Kamiya *et al.* 1993). Numbers represent specimens examined.

"GDH-B" specimens	"GDH-A" specimens					
	B-1	B-2	B-5	B-6	C-1	C-2
B-3	+	+	−	−	+	+
B-4	+	−	−	−	+	+

+: conjugation observed. −: conjugation not observed.

plants was observed in this study. Eight sporophytic specimens of typical *U. pertusa* from Ebisujima were tested, and all of them showed the same banding pattern found in the gametophytes, i. e. they were homozygous for "GDH-A" or "GDH-B" genotypes.

On the basis of isozyme data obtained in this study, crossing tests were performed between specimens representing different electroforms of *U. pertusa* from Ebisujima showing "GDH-A" and "GDH-B" respectively. Conjugations were observed among gametes of compatible mating types, except for the cases that experimental errors happened (Table 3).

Discussion

Previously, we reported that *Ulva pertusa* is morphologically variable, and that petiolate forms are included within this taxon (Kamiya *et al.* 1993). Molecular taxonomic analysis with isozymes was initiated to determine if genetic differences exist among these forms. We initially tried to clarify the intra- and interspecific variability of isozyme banding patterns for *U. pertusa* and *U. arasakii*. Results indicate the presence of main bands for four enzyme-species mentioned above. The banding pattern of G6PDH was common for the two species. However, as summarized in Table 2, a clear difference of banding patterns was recognized between these two species for at least three enzymes: GDH, IDH and GOT. In contrast, intraspecific variability was found in only one enzyme, GDH, from two populations of *U. pertusa*.

For all of the examined enzyme-species, no differences in isozyme banding patterns were detected between specimens of "stalked-*Ulva*" and typical *U. pertusa* growing at the same locality. These results suggest that the two forms are not genetically isolated.

Kamiya *et al.* (1993) showed clear morphological differences between typical *U. pertusa* and "stalked-*Ulva*". However, the presence of numerous intermediate forms made assignment of individual specimens to either species problematic. Also, sexual reproduction was shown between gametes from typical *U. pertusa* and "stalked-*Ulva*" under laboratory culture condition. Consequently, it was concluded that "stalked-*Ulva*" could not be treated as an autonomous entity of *U. pertusa*. Isozyme data from the present study supports this previous thesis (Kamiya *et al.* 1993). In addition, it indicates that isozyme analysis is effective for observing genetic structure of algae such as "stalked-*Ulva*", and possible to use for evaluating these intraspecific relationships.

It is noteworthy that two electrophoretic forms of typical *U. pertusa*, "GDH-A" and "GDH-B", were found in Ebisujima. Through interpopulational comparison of isozymes in this island, it was found that clear electrophoretic difference existed between specimens separated by less than one hundred meters. We have not observed thalli with heterozygous banding pattern between "GDH-A" and "GDH-B" in any sites of this island, so far.

If each population is fixed to the single form of "GDH-A" or "GDH-B", certain ecological factors which prevent hybridization of the two electroforms, or cross incompatibility could be responsible. In either case, it is supposed that gene flow between these two electroforms is absent, even though they grow sympatrically, and that sporophytes with the heterozygous banding pattern do not occur in any of these populations. Although conjugation between gametes with "GDH-A" and "GDH-B" has been demonstrated by crossing tests under laboratory culture condition, the viability of these hybrids has not been evaluated. Efforts continue to identify heterozygous

sporophytes from the natural populations from Ebisujima. However, probability of success is slight because populations of *U. pertusa* are considered to have a high gametophyte/sporophyte ratio since gametophytes can be reproduced by their own parthenogenesis and through zoospore formation of the sporophytes, whereas the sporophytes can be reproduced only by conjugation of the gametophytes.

Innes (1987) described genetic differentiation for *Enteromorpha linza* among localities separated by less than a few hundred meters. He suggested that the genetic differentiation was maintained by some environmental factors such as difference of salinity. It is possible in our case that the difference of environmental factors such as the amount of solar radiation relates to the genetic differentiation between these populations. The eastern population with "GDH-B" is considerably shaded by the steep cliff and canopies of trees in contrast to the southern population inhabiting broad flat rock platform with no shadings. Further examination is necessary to study their physiological differences induced by the environmental factors.

Acknowledgements

We would like to express our gratitude to Dr. Tatsumi Kato, Tsukuba Botanical Garden, National Science Museum, for aid in experiments of isozyme analysis and for helpful advice, and Prof. Yasutsugu Yokohama, Shimoda Marine Research Center, University of Tsukuba, for his advice in morphological and ecological aspects. We would like to express our thanks to Dr. Robert J. King, The University of New South Wales, for reading and criticizing this manuscript. Special thanks must be accorded to all the members of the laboratory of plant taxonomy and phylogeny in University of Tsukuba, for their generous advice and encouragement throughout the work.

References

Coesel, P. F. M. and Menken, S. B. J. 1986. Allozymic evidence for aneuploidy in *Closterium ehrenbergii* Mengehini (Desmidiaceae, Chlorophyta) (Research note). Phycologia **25**: 579–582.

Coesel, P. F. M. and Menken, S. B. J. 1988. Biosystematic studies on the *Closterium moniliferum/ehrenbergii* complex (Chlorophyta, Conjugatophyceae) in Western Europe. I. Isozyme patterns. Br. phycol. J. **23**: 193–198.

Francke, J. A. and Coesel, P. F. M. 1985. Isozyme variation within and between Dutch populations of *Closterium ehrenbergii* and *C. moniliferum* (Chlorophyta, Conjugatophyceae). Br. phycol. J. **20**: 201–209.

Grant, M. C. and Proctor, V. W. 1980. Electrophoretic analysis of genetic variation in the Charophyta. I. Gene duplication via polyploidy. J. Phycol. **16**: 109–115.

Hayhome, B. A., Whitten, D. J., Harkins, K. R. and Pfiester, L. A. 1987. Intraspecific variation in the dinoflagellate *Peridinium volzii*. J. Phycol. **23**: 573–580.

Innes, D. J. 1987. Genetic structure of asexually reproducing *Enteromorpha linza* (Ulvales: Chlorophyta) in Long Island Sound. Marine Biology **94**: 459–467.

Innes, D. J. 1988. Genetic differentiation in the intertidal zone in populations of the alga *Enteromorpha linza* (Ulvales: Chlorophyta). Marine Biology **97**: 9–16.

Innes, D. J. and Yarish, C. 1984. Genetic evidence for the occurrence of asexual reproduction in populations of *Enteromorpha linza* (L.) J. Ag. (Chlorophyta, Ulvales) from Long Island Sound. Phycologia **23**: 311–320.

Jurgenson, J. E. and Biebel, P. 1989. Taxonomy of a mixed *Cylindrocystis* assemblage from Vermont soil. II. Isozyme variation. Arch. Protistenkd. **137**: 299–307.

Kamiya, M., Doi, K., Hara, Y. and Chihara, M. 1993. Taxonomic studies of *Ulva pertusa* (Ulvophyceae). I. Morphological analysis. Jpn. J. Phycol. **41**: 191–198.

Kato, T. 1987. Hybridization between *Dianthus superbus* var. *longicalycinus* and *D. shinanensis* evidenced by resolvable esterase isozymes from herbarium specimens. Ann. Tsukuba Bot. Gard. **6**: 9–18.

Lindstrom, S. C. and South, G. R. 1989. Evidence of species relationships in the Palmariaceae (Palmariales, Rhodophyta) based on starch gel electrophoresis. Crypt. Bot. **1**: 32–41.

Soltis, D. E., Haufler, C. H., Darrow, D. C. and Gastony, G. J. 1983. Starch gel electrophoresis of ferns: A complication of grinding buffers, gel and electrode buffers, and staining schedules. Amer. Fern J. **73**: 9–27.

土井考爾*・神谷充伸*・原　慶明*・千原光雄**：アナアオサ（アオサ藻綱）の分類学的研究．II．アイソザイムの分析

アナアオサ，ナガアオサ，及び通称エツキアオサのアイソザイムを解析し比較した。アナアオサとナガアオサの間では，4種類のアイソザイムのうち3種類にバンドパターンの明瞭な差異が認められたが，エツキアオサはアナアオサと同じパターンを示した。一方，全国14地点のアナアオサ集団間では，静岡県下田市恵比須島および青森県八戸市蕪島を除き，バンドパターンは共通であった。恵比須島のアナアオサの集団を調査したところ，典型的なバンドパターンを示す個体と共に，他のどの集団にも見られないバンドパターンを示す個体がみられた。両パターンを示すタイプの個体間では，配偶子が接合し，正常に交雑が起こることが確認された。(*305 茨城県つくば市天王台1-1-1　筑波大学生物科学系，**150 東京都渋谷区広尾4-1-3　日本赤十字看護大学)

(*Received March 18, 1993, Accepted June 22, 1993*)

Jpn. J. Phycol. (Sôrui) 41: 207–214, September 20, 1993

The influence of ultraviolet radiation on the photosynthetic activity of several red algae from different depths

Miyuki Maegawa*, Masayo Kunieda** and Washiro Kida*

*Laboratory of Phycology, Faculty of Bioresources, Mie University, kamihama 1515, Tsu, Mie, 514 Japan
**Toukai Technology Center, Inokoishi 2–710, Meitou-ku, Nagoya, 465 Japan.

Maegawa, M., Kunieda, M. and Kida, W. 1993. The influence of ultraviolet radiation on the photosynthetic activity of several red algae from different depths. Jpn. J. Phycol. 41: 207–214.

Influence of UV (ultraviolet) radiation on the photosynthetic activity of several red algae collected from the shallow and deep waters was studied. Additionally, light environment in the coastal waters was determined. Direct sun light including PAR (photosynthetically active radiation) and UV radiation had a little effect on the photosynthetic activities of shallow-water species. They seem to be adapted to strong solar radiation and resist UV radiation. Direct sun light for only 30 minutes at noon on a fine day was a serious obstacle to the photosynthesis of deep-water species from the depth of about 25 m. UV radiation amounting to only 1–5% of solar radiation may seriously inhibit the photosynthetic activity of deep-water species used in this study. UV radiation from the sun can be regarded as one of the most important factors limiting the vertical distribution of red algae.

Key Index Words: PAR—photosynthesis—Rhodophyta—solar radiation—UV radiation—vertical distribution.

It is generally believed that red algae are adapted to the deep waters. However, there are many species of red algae growing in the shallow waters. The previous studies (Yokohama 1973, Murase *et al.* 1989) indicated a kind of chromatic adaptation among red algae. The shallow-water species seem to be adapted to white light and the deep-water species to green and blue light. The difference of photosynthetic characteristics between shallow- and deep-water species is dependent on phycoerythrin content, which was closely related to the light conditions in growing site. However, several problems have been remained. One of the most important problems is "Why the deep-water species can not grow in the shallow waters?" The deep-water species adapting to dim and blue or green light might be able to grow in high and white light condition.

In this study, our attention was focused on ultraviolet (UV) radiation affecting the algal photosynthetic activity. Interest in the impact of UV radiation on marine organisms has recently increased because of the possible elevation of UV radiation to the earth's surface, which is caused by thinning of the ozone layer in the upper atmosphere (Hofmann 1989). UV radiation is shorter than 400 nm in wavelength, and it is divided arbitrarily into three groups; UV-A (400–320 nm), UV-B (320–280 nm) and UV-C (280–200 nm). In the outer space UV radiation occupies 9 to 10 %, and on the water surface it occupies only 1 to 5% depending on the weather (Fleishmann, 1989). UV-C is absorbed by ozone layer and oxygen in the atmosphere, so UV-C does not reach to the sea surface. UV-A and UV-B are injurious to organisms on the earth (Calkins 1980). We have a hypothesis that UV radiation from the sun acts as one of the factors limiting the vertical distribution of red algae in the coastal ecosystem, i.e. shallow-water species have an ability to resist strong sun light and UV radiation, in contrast deep-water species do not have such an ability.

There are several studies which have examined the sensitivity for phytoplanktons to

UV radiation (Lorenzen 1979, Smith and Baker 1980, Hobson and Hartley 1983, Bühlmann *et al.* 1987). There was no doubt that increasing UV radiation could depress the photosynthetic activity by bleaching or altering the composition of photosynthetic pigments (Häder and Häder 1988, El-Sayed *et al.* 1990). However, there have been no information on the effects of UV radiation on macroalgae, which play an important role in the coastal ecosystem. In the present paper we have conducted a measurement of UV flux in the coastal water, and estimation of the influence of various intensities of direct sun light, photosynthetically active radiation (PAR) and UV radiation from the sun on photosynthetic activity of red algae from different depths.

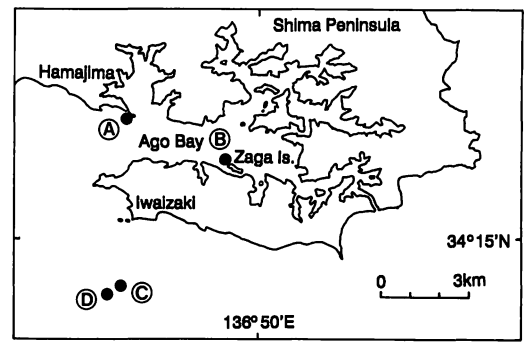


Fig. 1. A map showing the stations where the materials were collected. *Chondrus verrucosa* and *Phyllymenia sparsa* were collected from near the low water level at the point A, and *Gracilaria textorii* from a floating buoy from the point B. *Meristotheca papulosa* and *Peyssonnelia caulifera* were collected at the depth of about 25 m at the point C. At the point D the vertical profile of solar energy spectra were determined.

Materials and Methods

UV radiation above the water surface and in water was measured by Maycum underwater UV sensor (UV103AB). The detection band of this sensor is from 250 nm to 410 nm, and have high sensitivity more than 80% at the range of UV-A and UV-B. PAR above the water surface and in water was measured by LI-COR LI-192SB. These light data were stored in LI-COR LI-1000 data logger. The energy spectrum above the water surface and in water from 300 nm to 1000 nm was measured by LI-COR LI-1800UWC. The light measurements were carried out near the mouth of Ago Bay as shown in Fig. 1.

Five species of the Rhodophyceae were used in this study. Three of them were collected from the shallow waters and the other species were from the deep waters. The former three are regarded as shallow-water species since they can not be found in the deep waters, while the latter two as deep-water species since they can not be found in the shallow waters. The shallow-water species were *Chondrus verrucosa* Mikami and *Phyllymenia sparsa* (Okamura) Kylin collected from near the low water level at the coast of Hamajima and *Gracilaria textorii* Sringer from floating buoy used for pearl oyster cultivation near Zaga Island in Ago bay. The deep-water species were *Meristotheca papulosa* (Montague) J. Agardh and *Peyssonnelia caulifera* Okamura collected from the depth of about 25 m off Iwaizaki.

Collected samples were transported to the Fisheries Research Laboratory of Mie University in Zaga Island and were rinsed with filtered sea water to make them free of obvious epiphytes with careful handling not to wound the fronds and were protected from direct sun light. Sample pieces of 15 cm^2 (3 cm × 5 cm) were cut off fronds, and were kept in running sea water overnight to avoid abnormal results caused by cutting (Sakanishi *et al.* 1988).

Fig. 2 shows schematic diagrams of exposure examination under direct sun light and PAR for shallow- and deep-water species. A piece of sample was set in the water bath in which fresh sea water was poured continuously. Samples were separated into the two groups, which were exposed to direct sun light and PAR respectively. The PAR exposure was made by shielding the sample with an acrylic plate of 3 mm thick, cutting the UV band less then 350 nm in wavelength as shown in Fig. 3. Regulations of the light intensity were served by the sheets of black mesh nets covered in piles. One sheet of the

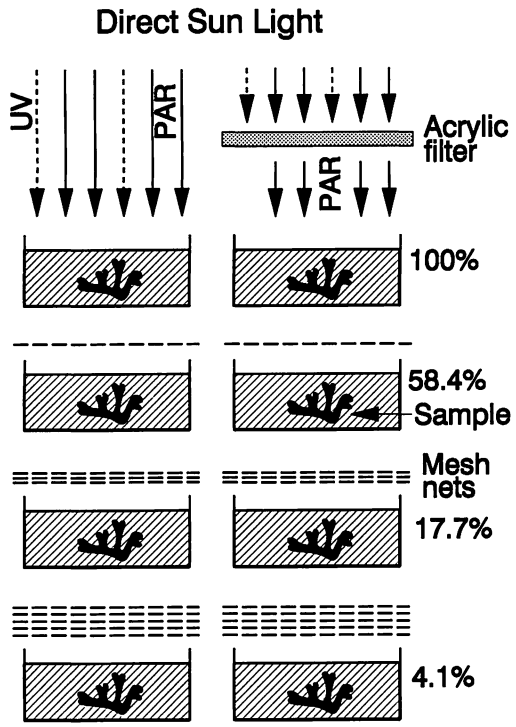


Fig. 2. Schematic diagram of exposure examination under the various intensity of direct sun light and PAR for shallow and deep-water species. Acrylic filter cuts UV radiation and transparent only PAR. Dosage of direct sun light and PAR were regulated by the number of black mesh nets covered in piles and by the exposure period. Relative values of the light intensity are also shown.

net transmitted the light of 58.4%. Samples of *Meristotheca papulosa* and *Phyllymenia sparsa* shielded with 0-5 sheets of black mesh nets were exposed to direct sun light and PAR for 1 and 2 hours, respectively. The samples of *Chondrus verrucosa* shielded with the black mesh nets were exposed for 2 hours, and only the samples under direct sun light and PAR with no black mesh net exposed for 6 hours. This examination was carried out at noon on a fine and sometimes cloudy day in June 19, 1989. During this examination photon flux density ($\mu Em^{-2}s^{-1}$) was monitored and conversed to the unit of integrated photon flux density (Em^{-2}). Therefore, the dosage of photon flux density was regulated by changing the exposure period and the number of black mesh nets. After exposure examination, photosynthetic activities were measured

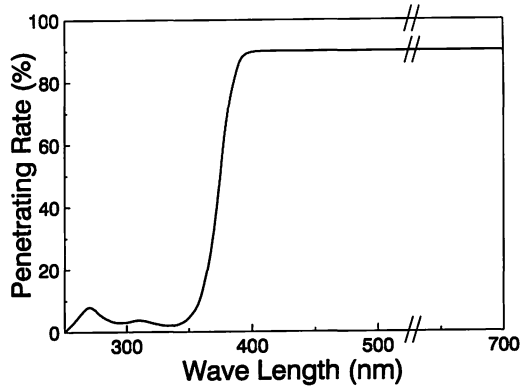


Fig. 3. Transmission spectrum of acrylic plate of 3 mm thick from 250 to 700 nm in wavelength used in the exposure examinations.

at 400 $\mu Em^{-2}s^{-1}$ with the Productmeter, an improved type of differential gas-volumeter (Yokohama *et al.* 1986, Yokohama and Maegawa 1988).

Further exposure examination was designed

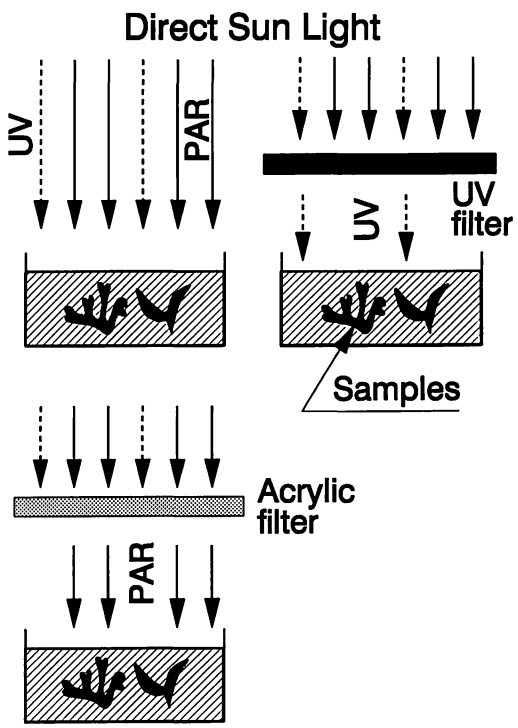


Fig. 4. Schematic diagram of exposure examination under direct sun light, PAR and UV radiation. Acrylic filter cuts UV radiation and UV filter cuts PAR and transparent only UV radiation. Both of shallow- and deep-water species are put in each water bath.

as shown in Fig. 4. Solar radiation was divided into the two bands of UV radiation and PAR by using a UV transmittance filter (Corning 7-54) and the acrylic plate. The Corning 7-54 filter almost cuts PAR longer than 400 nm, and transmitted only UV radiation of 240-400 nm. Three sets of the exposure experiments, direct sun light, PAR and UV radiation, were served by the same way as mentioned in Fig. 2. Each set in the examination included two species of red algae, *Gracilaria textorii* as a shallow-water species and *Peyssonnelia caulifera* as a deep-water species. Each of samples was exposed for 30 minutes at noon on a fine day. During this examination photon flux density and UV radiation were monitored. The amounts of dosage were 200 Whm^{-2} for direct sunlight, 1.5 Em^{-2} or 100 Whm^{-2} for PAR and 9 Whm^{-2} for UV radiation. The radiometric units (Whm^{-2}) of direct sun light and PAR were conversed by the manual of LI-COR (1979). This exposure examination was carried out in June 21, 1990.

After exposure examination, photosynthesis and respiration were measured by the Productmeter at 8 different intensities from 0 to 400 $\mu Em^{-2}s^{-1}$ of artificial light and at 24.5°C, which was near the *in situ* sea water temperature of sampling area. The light source was a projector lamp (Kondo 100 V-300 W) and the light intensity was adjusted using neutral density filters (Toshiba TND-50, -25, -12.5). Photon flux density was measured with a quantum meter system (LI-COR LI-192SB, LI-1000). UV radiation from the projector lamp is assumed to be cut by the bottom of the water bath made of acrylic plate. Photosynthetic and respiratory rates were measured in the same manner as we used in our previous experiment (Murase *et al.* 1989).

Results

Fig. 5 shows a vertical profile of solar energy spectra from 300 nm to 1000 nm in wavelength at a point near the sampling station of deep-water species off Iwaizaki at noon on a fine day in July 22, 1992. When the sun light penetrates the ocean it is altered in both quality and quantity. Water itself absorbs strongly the component above 600 nm including red light, far red and infrared radiation. UV radiation below 400 nm and blue light in shorter part were attenuated strongly by the water and suspended matters. So, in the deep waters blue to green light from 450 nm to 600 nm occupied a large part of light energy. The maximum transmittance can be seen at around 500 nm in this area.

Fig. 6 shows a typical vertical profile of UV radiation below 400 nm and PAR from 400 nm to 700 nm calculated from the data of Fig. 5. Attenuation rate of UV radiation was higher

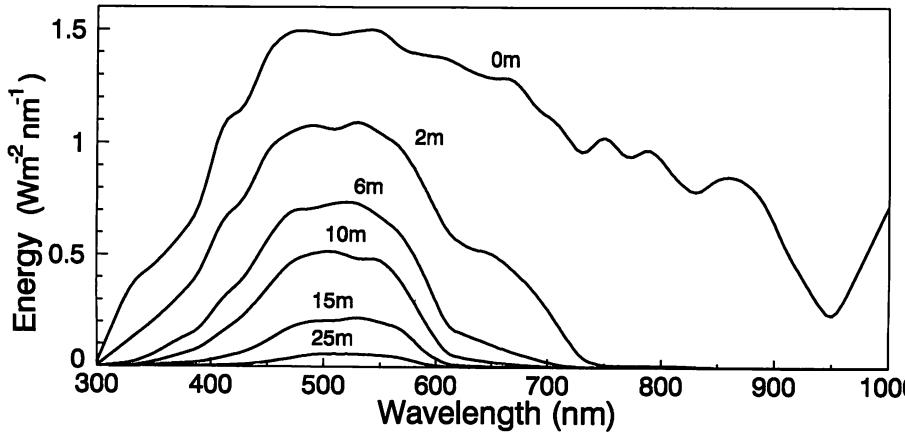


Fig. 5. Vertical profile of solar energy spectra from 300 nm to 1000 nm in wavelength at a point near the sampling station for deep-water species.

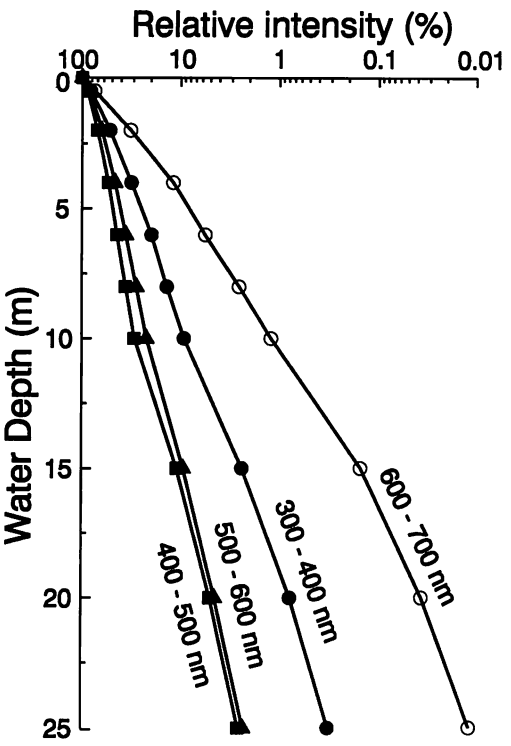


Fig. 6. Vertical profile of UV radiation and PAR calculated from the data in Fig. 5. Each line shows the average value of 300–400 nm (-●-), 400–500 nm (-■-), 500–600 nm (-▲-) and 600–700 nm (-○-).

than PAR from 400 nm to 600 nm, and was lower than red light from 600 nm to 700 nm. UV radiation decreased to about 11% at 10 m and to below 1% at 20 m in depth.

Effects of the pre-exposure to different amounts of direct sun light and PAR on the photosynthetic activity of shallow- and deep-water species were shown in Fig. 7. *Chondrus verrucosa* from the lower level of intertidal zone decreased in photosynthetic activity gradually with increasing in direct sun light and PAR, and maintained about 50-65% after exposure to 9 Em^{-2} of PAR or direct sun light, which corresponded to exposure to direct sun light for 6 hours about noon on a fine day in summer. The photosynthetic activity of *Phyllymenia sparsa* from the upper level of sublittoral zone decreased to 30% and 22% after exposure to 6 Em^{-2} of PAR and direct sun light, respectively. Shallow-water species were a little more sensitive to direct sun light containing UV radiation than PAR. As compared with the shallow-water species, enhanced solar radiation greatly reduced the photosynthetic activity of a deep-water species, *Meristotheca papulosa*, collected from 25 m in depth. After exposure to 1 Em^{-2} of PAR, the photosynthetic activity of *M. papulosa* maintained 78% under PAR, and decreased to only 16% under direct sun light. After exposure to about 2 Em^{-2} of PAR, the photosynthetic activity of this species was lost.

Figs. 8 and 9 show the photosynthesis-light curves of a shallow-water species, *Gracilaria textorii*, and a deep-water species, *Peyssonnelia caulifera*, respectively, after exposure to direct sun light, PAR and UV radiation for 30 minutes as shown in Fig. 4. The photosynthetic activity of each sample showed relative value to the control samples, which was exposed neither to direct sun light nor to UV radiation. Saturated photosynthetic activity of *G. textorii* after exposure to sun light decreased to 75-80% of control samples as shown in Fig. 8. There is also no marked difference in the photosynthetic-light curves among samples exposed to direct sun light, PAR and UV radiation, besides a slightly gentle initial slope of the sample exposed UV radiation. As for the deep-water species *Peyssonnelia caulifera* in Fig. 9, decreases in the saturated photosynthetic activity and the angle of intial slope in the photosynthesis-light curve of the sample exposed to direct sun light, PAR or UV radiation were more pronounced than those observed in the shallow-water species. Direct sun light was the most injurious to deep-water species, and depressed the saturated photosynthetic activity to only 5-10% of that in the control after exposure to 1.5 Em^{-2} of PAR. Enhanced UV radiation and PAR alone also affected the photosynthetic activity of *P. caulifera*, and depressed the saturated photosynthetic activity of this species to 50-60% and 20-30%, respectively.

Discussion

Numerous studies have shown that increasing levels of UV exposure result in reducing

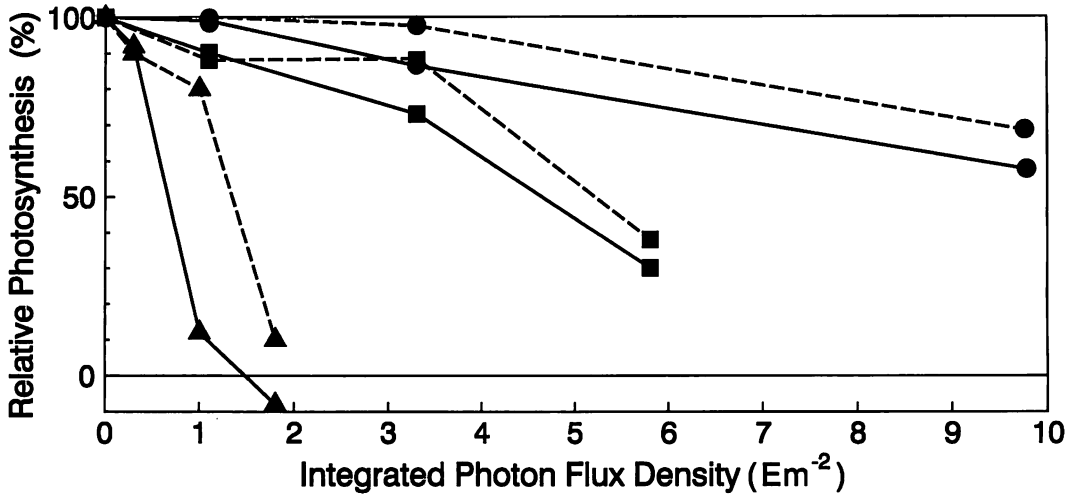


Fig. 7. Changes in photosynthetic activity after exposing to direct sun light and PAR. Dosage of direct sun light and PAR was adjusted by changing the exposure period and the number of black mesh nets (cf. Fig. 2). After the exposure examination, photosynthetic rate was mesured at 400 μEm^{-2}s^{-1}. Samples were *Condrus verrucosa* (●) collected from intertidal zone, *Phyllymenia sparsa* (■) collected from upper subtidal zone and *Meristotheca papulosa* (▲) collected from depth of 25 m. Straight lines and broken lines show the data under direct sun light and PAR, respectively.

primary production of phytoplanktons (Steeman-Nielsen 1954, Calkins 1980, Worrest *et al.* 1980, Hobson and Hartley 1983, Bühlman *et al.* 1987). Bülmann *et al.* (1987) pointed out that there was no photoinhibition without UV or under weak UV radiation, and under more than 70 μEm^{-2}s^{-1} of UV radiation photoinhibition is always observed to a certain degree depending on the combination of PAR and UV ray on phytoplanktonic C-assimila-

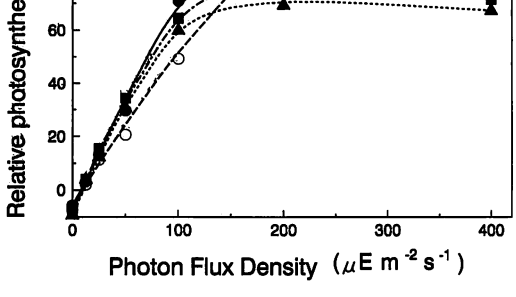


Fig. 8. Photosynthesis-light curves of *Gracilaria textorii*, a shallow-water species determined, after exposing to direct sun light (-▲-), PAR (-■-) and UV radiation (-○-) for 30 minutes at noon on a fine day in summer (cf. Fig. 4). Photosynthetic activity was shown in relative values against data obtained with the control sample's data kept in a running seawater bath without exposing to direct sun light (-●-). Dosage of direct sun light for 30 minutes at noon on a fine day corresponded to 1.5 Em^{-2} or nearly 100 Whm^{-2} of PAR, and 9 Whm^{-2} of UV radiation.

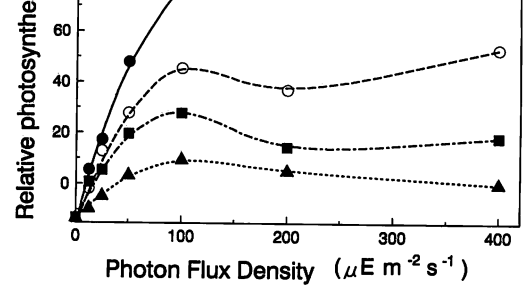


Fig. 9. Photosynthesis-light curves of *Peyssonnelia caulifera*, a deep-water species determined after exposing to direct sun light (-▲-), PAR (-■-) and UV radiation (-○-) for 30 minutes at noon on a fine day in summer (cf. Fig. 4). Photosynthetic activity was shown in the relative values against data obtained with the control sample kept in a running seawater bath without exposing to direct sun light (-●-). Dosage of direct sun light for 30 minutes at noon on a fine day corresponded to 1.5 Em^{-2} or about 100 Whm^{-2} of PAR, and 9 Whm^{-2} of UV radiation.

tion. Also, Jokiel and York (1984) observed that photosynthetic activities of phytoplanktons in the shallow waters had an ability to resist strong UV radiation, and deep-water species did not have such an ability. The results obtained in the present study on macroalgae were essentially similar to those observed in phytoplanktons.

The photosynthesis of the deep-water species was seriously inhibited by the exposure to direct sun light for only 30 minutes at noon on a fine day in summer, while that of the shallow-water species was little affected. A considerable part of the inhibition on the photosynthesis of the deep-water species seems to attribute to UV radiation, which amounts to only 4–5% of direct sun light at noon on a day in summer. However, the effect of PAR on the photosynthesis of the deep-water species was larger than that of UV radiation as shown in Fig. 9. This fact suggests that the mechanism of the photoinhibition on deep-water red algae is different from that of the photoinhibition on phytoplankton since Bühlmann *et al.* (1987) pointed out that there was no photoinhibition without UV radiation in phytoplankton.

Although the total effect of PAR in the inhibition on the photosynthesis of the deep-water species was larger than that of the UV radiation, the specific effect of UV radiation was much larger than that of PAR. In the case of the shallow-water species, the specific effect of UV radiation was not so larger than that of PAR. It can be assumed that shallow water red algae possessed strategies to cope with UV radiation. Several authors pointed that UV absorbing substance contained in some algae played an important role as a biofilter against the solar UV radiation (Shibata 1969, Sivalingam and Nisizawa 1990). In fact, this substance is contained in almost of all groups in algae, particularly much in red and blue-green algae. Whereas, the deep-water red algae used in this study had little ability to resist the surplus direct sun light and UV radiation. This fact suggests that the deep-water species have a little or no UV absorbing substance. Further investigation on the UV absorbing substance will be carried out in a next research.

The previous papers (Yokohama 1973, Murase *et al.* 1989) indicated that one of the most important factors limiting vertical distributions of red algae was the vertical variation in light intensity and spectral properties. From the results of the present study, however, it is assumed that the intensity of UV radiation is another most important factor limiting vertical distributions of red algae. About 11% of UV radiation at the water surface penetrated to the depth of 10 m at a point near the station where the deep-water species were collected (Figs. 5 and 6). This fact indicates that the photosynthesis of the deep-water species is seriously inhibited by the exposure to under-water light at the depth of 10 m for 4.5 hours at noon on a fine day in summer. The excessive UV radiation in the shallow waters may function as the most important factor determining the upper limit in the vertical distribution of a red alga.

References

Bühlmann, B., Bossard, P. and Uehlinger, U. 1987. The influence of long wave ultraviolet radiation (u.v.-A) on the photosynthetic activity (^{14}C-assimilation) of phytoplankton. J. Plankton Res. **9**: 935–943.

Calkins, J. 1980. The ecological significance of solar UV radiation on aquatic organisms. Nature **283**: 563–566.

El-Sayed, S. Z., Stephens, F. C., Bidigare, R. R. and Ondrused, M. E. 1990. Effect of ultraviolet radiation on Antarctic marine phytoplankton. p279–385. *In* K. R. Kerry and G. Hempel [ed.] Antarctic Ecosystems. Ecological Change and Conservation. Springer-Verlag, Berlin.

Fleischmann, E. M. 1989. The measurement and penetration of ultraviolet radiation into tropical marine water. Limnol. Oceanogr. **34**: 1623–1629.

Häder, D. P. and Häder, M. A. 1988. Ultraviolet-B inhibition of mortality in green and dark bleached *Euglena gracilis*. Curr. Microbiol. **17**: 215–220.

Hobson, L. A. and Hartley, F. A. 1983. Ultraviolet irradiance and primary production in a Vancouver Island fjord, Columbia, Canada. J. Plankton Res. **5**: 325–331.

Hofman, D. J. 1989. Direct ozone depletion in springtime Antarctic lower stratospheric clouds. Nature **337**: 444–449.

Jokiel. P. L. and York. R. H. 1984. Importance of ultraviolet radiation in photoinhibition of microalgal growth. Limnol. Oceanogr. **29:** 192–199.

LI-COR 1979. Radiation measurement. LI-COR, inc. Lincoln.

Lorenzen, C. J. 1979. Ultraviolet radiation and photoplankton phytosynthesis. Limnol. Oceanogr. **24:** 1117–1120.

Murase, N., Maegawa, M. and Kida, W. 1989. Photosynthetic characteristics of several species of Rhodophyceae from different depths in the coastal area of Shima Peninsula, central Japan. Jpn. J. Phycol. **37:** 213–220.

Sakanishi, Y., Yokohama, Y. and Aruga, Y. 1988. Photosynthesis measurements of blade segments of brown algae *Ecklonia cava* Kjellman and *Eisenia bicyclis* Setchell. Jpn. J. Phycol. **36:** 24–28.

Shibata, K. 1969. Pigments and a UV-absorbing substance in corals and blue-green alga in the great barrier reef. Plant Cell Phsiol. **10:** 325–333.

Sivalingam, P. M. and Nisizawa, K. 1990. Ozone hole and its correlation with the characteristic UV-absorbing substance in marine algae. Jpn. J. Phycol. **38:** 365–370.

Smith, R. C. and Baker, K. S. 1980. Stratosperic ozone, middle ultraviolet radiation, and carbon-14 measurements of marine productivity. Science **208:** 592–593.

Steeman-Nielsen. E. 1954. On organic production in oceans. J. Conseil **19:** 309–328.

Worrest, R. C., Brooker, D. L. and Van Dyke, H. 1980. Results of a primary productivity study as affected by the type of glass in the culture bottle. Limnol. Oceanogr. **25:** 360–364.

Yokohama, Y. 1973. Photosynthetic properties of marine benthic red algae from different depths in the coastal area. Bull. Jap. Soc. Phycol. **21:** 119–124 (in Japanese).

Yokohama, Y., Katayama, N. and Furuya, K. 1986. An improved type 'Productmeter', a differential gas-volumeter, and its application to measuring photosynthesis of seaweeds. Jpn. J. Phycol. **34:** 37–42 (in Japanese).

Yokohama, Y. and Maegawa, M. 1988. Measurements of photosynthesis and respiration of large samples by 'Productmeter', a differential gas-volumeter. Jpn. J. Phycol. **36:** 29–36 (in Japanese).

前川行幸*・国枝昌代・喜田和四郎*：紫外線が生育深度の異なる紅藻の光合成におよぼす影響**

浅所から採集された3種の紅藻および深所（水深約25 m）から採集された2種の紅藻について，UV（紫外線）が光合成活性におよぼす影響を調べた。また，沿岸域での光環境も合わせて測定した。PAR（光合成有効波長域）や紫外線を含む太陽からの直射光による前照射は，浅所産紅藻の光合成活性をほとんど低下させることがなかったのに対し，深所産紅藻は夏の晴天の正午前後30分間の直射光，そのPAR成分およびUV成分のいずれの照射によっても著しい光合成活性の低下が見られた。海面上で太陽放射の1–5%を占めるすぎない紫外線が深所産紅藻の阻害作用に占める割合はかなり大きいものといえるが，PARとUVの相乗効果は深所紅藻の光合成活性をさらに大きく低下させるものと考えられる。水中の光環境の測定から，5–10 mの水深に到達するUVの量は深所産紅藻の光合成に障害をおよぼすに十分であるとみなされ，したがって，本研究に用いられた深所産紅藻はこのような浅所では生育できないものと考えられる。紫外線は紅藻の垂直分布を規制する大きな要因の一つであると言えよう。（*514 三重県津市上浜町1515　三重大学生物資源学部藻類増殖学研究室，**465 名古屋市東区猪子石2-710　㈶東海技術センター）

(Received November 19, 1992, Accepted July 7, 1993)

Jpn. J. Phycol. (Sôrui) 41: 215–220, September 20, 1993

Occurrence of heterotrophic bacteria causing lysis of cyanobacteria in a eutrophic lake

Yoko Yamamoto*, Seiichi Niizuma*,***, Nobuo Kuroda**,**** and Mitsuru Sakamoto**

*Faculty of Agriculture, Meiji University, Higashimita, Kawasaki, 214 Japan
**Water Research Institute, Nagoya University, Chikusaku, Nagoya, 464–01 Japan

Yamamoto, Y., Niizuma, S., Kuroda, N. and Sakamoto, M. 1993. Occurrence of heterotrophic bacteria causing lysis of cyanobacteria in a eutrophic lake. Jpn. J. Phycol. 41: 215–220.

192 strains of heterotrophic bacteria were isolated from the eutrophic Lake Suwa and examined for their ability to lyse cyanobacteria. Approximately 40% of the strains were able to lyse at least one strain of cyanobacteria. The ratio of cyanobacteria-lytic to total isolates in each month was highest in September, when *Microcystis* blooms began to disintegrate. The genera *Alcaligenes, Flavobacterium/Cytophaga* group and *Pseudomonas* accounted for the great majority of strains capable of lysing cyanobacteria.

Key Index Words: alga-lysing bacteria—cyanobacteria—lake water

The distribution and production of heterotrophic bacteria in natural aquatic ecosystems depend on the supply of organic matter from primary producers, particularly phytoplankton. For instance, Coveney et al. (1977) and Straskrabova and Komarikova (1976), reported that an increased abundance of heterotrophic bacteria follows the development and decline of phytoplankton in reservoirs. Kuroda and Sakamoto (1986) studied seasonal changes of the heterotrophic bacterial community in the eutrophic Lake Suwa and found changes in the composition of the bacterial and phytoplankton community. *Pseudomonas* spp. accounted for a larger percentage of the isolates during June and July when cyanobacterial blooms were present, whereas, *Alcaligenes* spp. dominated in September when the cyanobacteria bloom disintegrated. They suggested that the change of organic matter supply caused the observed changes in the bacterial flora.

Much information has accumulated on the potential role of cyanophages and other lytic agents for regulating cyanobacterial blooms (Burnham et al.1976, Cannon et al. 1976, Daft and Stewart 1971, Daft et al. 1975, Granhall and Berg 1972, Mitsutani et al. 1988, Rein et al. 1974, Shilo 1969, 1970, Yamamoto 1981, Yamamoto and Suzuki 1977, 1990, Yamamoto et al. 1991).

The present study examines the ability of bacteria from Lake Suwa to lyse cyanobacteria.

Materials and Methods

1. *Study area*

Lake Suwa, located in Nagano Prefecture, central Japan (36°3N, 138°E), is a eutrophic lake in which cyanobacteria, *Microcystis* spp., are dominant every year from May to September. The lake is about 13.3 km^2 in area with a maximum depth of 6.8 m and a mean depth of 4.6 m.

2. *Heterotrophic bacteria*

The heterotrophic bacterial strains used in this study were 192 isolates obtained from the surface water of Lake Suwa in 1981 and 1984 (Table 1). The isolates were maintained on

*** Present adress: ZEN-NOH Agricultural Technical Center, Higashi-Yahata, Hiratsuka 254, Japan
**** Present address: Aichi Fishery Research Institute, Miyacho, Gamagori 443, Japan
Correspondence: Yoko Yamamoto

Table 1. Number of heterotrophic bacterial strains isolated from Lake Suwa during the study.

Generic group	1981					1984			Total numbers
	May	Jun	Jul	Aug	Sep	Jun	oct	Nov	
Alcaligenes	2		3	10	18	3	1	4	41
Flavobacterium-Cytophaga group	6	5	21	13	4	6	1	3	59
Pseudomonas	4	14	18			1	6	2	45
Enterobacter	3	1		3	1	1	1		10
Xanthomonas	4	4					1		9
Moraxella-Acinetobacter group	3	1					1		5
Acinetobacter		1				1	1		3
Agromobacterium-Alcaligenes		1							1
Micrococcus	2								2
Vibrio							2		2
Unidentified	1	7	2	2	1	1	1		15
Total numbers	25	34	44	28	24	13	15	9	192

basal medium containing 200 mg tryptocase (BBL), 100 mg yeast extract (Difco), 50 mg glucose (Wako), 12 g agar in 1 l of distilled water at 20°C.

3. *Cyanobacteria*

The cyanobacteria employed as test organisms for lytic activity were grown in media (Table 2) under continuous cool-white fluorescent lamps (50–75 $\mu E \cdot m^{-2} \cdot s^{-1}$) at 28°C.

Table 2. Media for algal culture.

Species and strains	Medium***
Microcystis aeruginosa Kützing (NIES 99*)	CT
M. aeruginosa f. aeruginosa Kützing (NIES 44)	CT
M. aeruginosa (M-11)	M-11
M. aeruginosa (F-F)	CT
M. flos-aquae (IAM-M-178**)	CT
M. flos-aquae	CT
M. viridis Lemmermann	MA
M. wesenbergii Komárek (NIES 108)	CB
M. wesenbergii Komárek (NIES 112)	CT
Anabaena affinis Lemmermann (NIES 40)	CT
A. circinalis Rabenhorst (NIES 41)	CT
A. solitaria f. solitaria Klebahn	CT
A. cylindrica Lemmermann (IAM-M-1)	MDM
Anacystis nidulans (IAM-M-6)	MDM

* NIES=Microbial Culture Collection at National Institute for Environmental Studies.

** IAM=Institute of Applied Microbiology, University of Tokyo.

*** CT, MA and CB medium: Ichimura (1979)
M-11 medium: Hagiwara et al. (1984)
MDM medium: Watanabe (1960)

4. *Assay method of lytic activity*

Each strain of bacteria was streaked onto

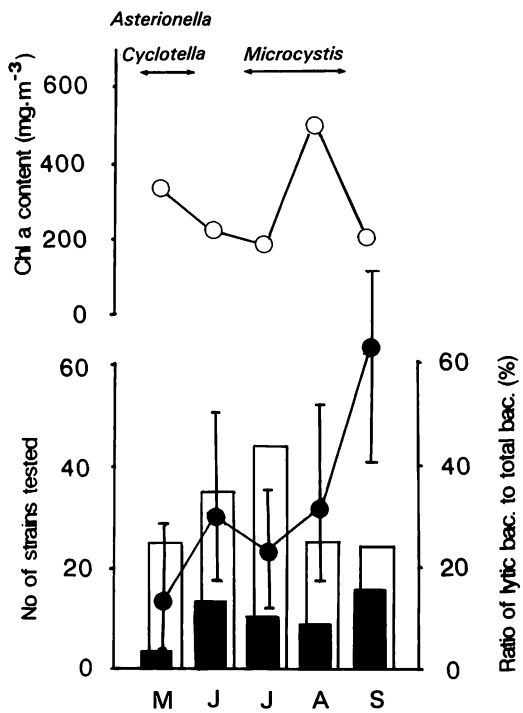


Fig. 1. Seasonal changes in cyanobacteria-lytic heterotrophs, dominant species of phytoplankton and Chl *a* content in 1981. Symbols; □, number of bacterial strains tested; ■, number of lytic strains; ●, percentages of lytic strains; ○, chlorophyll *a* content in the surface water. Bar indicates 95% confidence interval.

1% agar plates of the basal medium and incubated at 25°C. When there was detectable growth, streaks were cut into small blocks and placed on mats of cyanobacterial cells spread onto the surface of Whatman GF/C filters. The results were determined by the appearance of a clear zone formed on the cyanobacterial mats after several days (Yamamoto and Suzuki, 1990). When a tested strain showed lytic ability for at least one strain of cyanobacteria, it was judged to be lytic.

Results

Seasonal changes in the ratios of cyanobacteria-lytic bacteria, the dominant species of phytoplankton, the Chl *a* content (Fig. 1) and environmental parameters were measured in surface water of the lake from May to September in 1981. The pH of the water ranged from 7.1 to 9.6 and the temperature range between 15.5 to 25.6°C. *Cyclotella meneghiniana* Kützing and *Asterionella gracillima* (Hantzsch) Heiberg appeared in April and dominated throughout May. In July *Microcystis* spp. (Aoyama, 1985) were dominant. The chlorophyll *a* content was maximum on August in 1981. The phytoplankton responsible for an increase in the chlorophyll during this period were *M. wesenbergii* and *M. viridis*. These populations declined in September. The contribution of lytic bacteria was low (16%), in May, when the water temperature was low. Subsequently, it increased to 20-25%, but did not show significant variation in the period from June to August. In September when *Microcystis* spp. began to decline, more than 50% of the hetrotrophs were lytic.

The cyanobacteria-lytic spectrum of heterotrophic bacteria and the ratio (as percentage) of the cyanobacteria-lytic heterotrophs to the total number of isolates tested are shown in

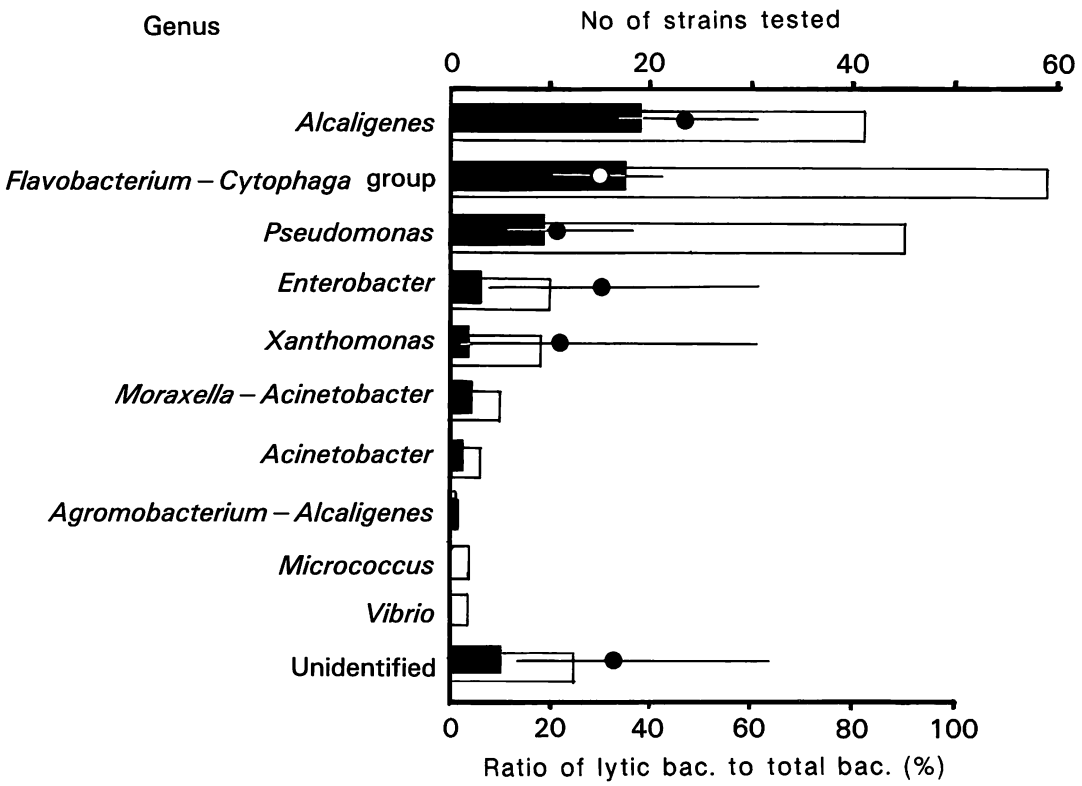


Fig. 2. Occurrence of heterotrophic bacteria lysing cyanobacteria. Symbols; □, number of bacterial strains tested; ■, number of lytic strains; ●, percentages of strains lysing cyanobacteria. Bar indicates 95% confidence interval.

Table 3. Lytic potential of heterotrophic bacteria.

Heterotrophic bacteria	Cyanobacteria strains													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Alcaligenes	[a]41/6[b]	41/2	40/0	37/3	41/3	41/3	41/7	41/1	41/0	41/2	41/6	41/0	40/0	36/0
Flavobacterium-Cytophaga group	50/3	53/0	42/0	48/1	51/1	48/4	50/3	53/0	47/2	50/2	52/1	52/0	52/0	54/0
Pseudomonas	44/0	42/0	42/0	44/0	44/0	41/3	41/3	44/0	42/2	44/0	42/2	42/0	44/0	43/0
Enterobacter	9/0	9/0	9/0	6/2	8/1	8/1	9/0	8/1	9/0	9/0	8/1	9/0	8/0	8/0
Xanthomonas	9/0	9/0	9/0	7/2	9/0	8/1	9/0	9/0	9/0	9/0	9/0	9/0	9/0	9/0
Moraxella-Acinetobacter group	4/1	5/0	5/0	5/0	5/0	5/0	4/1	5/0	5/0	4/1	5/0	5/0	5/0	5/0
Acinetobacter	3/0	3/0	3/0	2/1	2/1	3/0	3/0	3/0	3/0	2/1	3/0	3/0	3/0	3/0
Agromobacterium-Alcaligenes	1/0	1/0	1/0	1/0	1/0	1/0	1/0	1/0	1/0	1/0	1/0	1/0	1/0	1/0
Micrococcus	2/0	2/0	2/0	2/0	2/0	2/0	2/0	2/0	2/0	2/0	2/0	2/0	2/0	2/0
Vibrio	2/0	2/0	2/0	2/0	2/0	2/0	2/0	2/0	2/0	2/0	2/0	2/0	2/0	2/0
Unidentified	12/1	14/0	14/0	10/1	12/0	13/1	11/1	12/0	12/0	8/4	12/0	12/2	11/0	11/0

1) *Microcystis aeruginosa* Kützing (NIES 99); 2) *M. aeruginosa f. aeruginosa* Kützing (NIES 44)=176; 3) *M. aeruginosa* (M-11); 4) *M. aeruginosa* (F-F); 5) *M. elabens* (NIES 42); 6) *M. flos-aquae* (IAM-M-178); 7) *M. flos-aquae*; 8) *M. viridis* (A. Brown) Lemmermann (NIES 102); 9) *M. wesenbergii* Komárek (NIES 108); 10) *M. wesenbergii* Komárek (NIES 112); 11) *Anabaena circinalis* Rabenhorst (NIES 41); 12) *A. solitaria f. solitaria* Klebahn; 13) *A. cylindrica* Lemmermann (IAM-M-1); 14) *Anacystis nidulans* (IAM-M-6).

[a] The total number of heterotrophic bacteria strains tested.

[b] The number of heterotrophic bacteria strains that were active against the test substrate organisms (cyanobacteria).

Figure 2 and Table 3. *Alcaligenes, Flavobacterium/Cytophaga* group and *Pseudomonas* spp. accounted for the great part of strains showing lytic activity against cyanobacteria (Fig. 2). Of 41 strains of *Alcaligenes*, 19 showed lytic ability (46%); 17 strains constituting the 59 *Flavobacterium/Cytophaga* group (29%), and 9 of the 44 *Pseudomonas* spp. (20%) were capable of lysing cyanobacteria. The others did not cause lysis. Although the number of cyanobacteria-lytic heterotrophic bacteria differs from strains to strains, there were almost always at least a few heterotrophic bacteria lytic for *Microcystis* spp. (except strain M-11), *Anabaena circinalis* and *A. solitaria* which occur abundantly in hypereutrophic lakes such as Lake Suwa. No bacterium lytic for soil (*Anabaena cylindrica*) or mesotrophic (*Anacystis nidulans*) isolates was detected.

Discussion

In the present study, a considerable number of bacteria showed lytic activity for cyanobacteria, which occur abundantly in eutrophic lakes, but no bacteria showed it for cyanobacteria originating from the other habitats. Cyanobacteria-lytic heterotrophic bacteria in a eutrophic lake, may perhaps be active only against cyanobacteria occuring in such lakes.

According to Fallon and Brock (1979), the abundance of cyanobacteria-lytic heterotrophic bacteria was correlated positively with cyanobacterial biomass in Lake Mendota, Wisconsin. However, in Lake Suwa surface water and other eutrophic lakes in Japan, cyanobacteria-lytic organisms appear to coincide with the disintegration of cyanobacterial blooms in the fall (Yamamoto 1981, 1988, Yamamoto and Suzuki 1990, Yamamoto et al. 1988). This was also confirmed by the present study; abundant heterotrophs lysing cyanobacteria were also observed when the bloom of *Microcystis* declined (Fig. 1). The cause of the sudden decline of cyanobacterial blooms is not well understood. Increases of cyanobacteria lytic-heterotrophs may play an important role in this process.

As shown in Figure 2, most of the cyanobacteria-lytic bacterial strains consisted of *Alcaligenes*, the *Flavobacterium/Cytophaga* group and *Pseudomonas* spp. These genera are believed to be widely distributed in marine and freshwater environments (Konda 1982, 1985, Starr et al. 1981). *Alcaligenes* spp.

(Day and Withers 1985, Martin et al. 1978) and *Pseudomonas* spp. (Ramasamy and Verachtert 1980) produce exoenzymes such as β-glucosidase, and many *Cytophaga* can produce protease (Christison and Martin 1971) and β-lactam antibiotics (Redhead and Wright 1980) capable of lysing cyanobacteria. β-Glucosidase is one of the broad-specificity enzymes that catalyze the hydrolysis of β-linked glucose carbohydrates and is widely distributed in aquatic environments. Chróst (1989) showed that β-glucosidase activity was low during spring when phytoplankton grew rapidly, began to increase gradually during the bloom break-down and reached the highest values during the late stage of phytoplankton collapse; a similar study has been done in brackish water (Somville, 1984). These enzymes and antibiotics may play a role in the breakdown of organic matter including cyanobacteria.

References

Aoyama, R. 1985. Report of Suwa Hydrobiological Station, Shinsiu university. In T. Okino (ed.)

Burnham, J. C., Steak, T. and Locher, G. 1976. Extracellular lysis of the blue-green alga *Phormidium luridum* by *Bdellovibrio bacterioborus*. J. Phycol. **12:** 306–313.

Cannon, R. E., Shane, M. S. and Whitaker, J. M. 1976. Interaction of *Plectonema boryanum* (Cyanophyceae) and LPP-cyanophages in continuous culture. J. Phycol. **12:** 418–421.

Christison, J. and Martin, S. M. 1971. Isolation and preliminary characteristics of an extracellular protease of *Cytophaga* sp. Can. J. Microbiol., **17:** 1207–1216.

Chróst, R. J. 1989. Characterization and significance of β-glucosidase activity in lake water. Limnol. Oceanogr. **34:** 660–670.

Coveney, M. F., Cronberg, G., Enell, M., Larsson, M. and Olofsen, L. 1977. Phytoplankton, zooplankton and bacteria-standing crops and production relationships in eutrophic lakes. Oikos **29:** 5–21.

Daft, M. J. and Stewart, W. D. P. 1971. Bacterial pathogens of freshwater blue-green algae. New Phytol. **70:** 819–829.

Daft, M. J., McCord, S. B. and Stewart, W. D. P. 1975. Ecological studies on algal-lysing bacteria in fresh waters. Freshwater Biol. **5:** 577–596.

Day, A. G. and Withers, S. G. 1985. The purification and characterization of a α-glucosidase from *Alcaligenes faecalis*. Biochem. Cell Biol. **64:** 914–922.

Fallon, R. D. and Brock. T. D. 1979. Lytic organisms and photooxidative effects: Infuluences on blue-green algae (Cyanobacteria) in lake Mendota, Wisconsin. Appl. Environ. Microbiol. **38:** 499–505.

Granhall, U. and Berg, B. 1972. Antimicrobial effects of *Cellvibrio* on blue-green algae, Arch. Microbiol. **84:** 234–242.

Hagiwara, T., Yagi, O., Takamura, Y. and Sudo, R. 1984. Isolation of bacteria-free *Microcystis aeruginosa* from Lake Kasumigaura. Water Poll. **7:** 437–422.

Ichimura, T. 1979. Media for the cultivation of algae. p. 295–296. In K. Nishizawa and M. Chihara (eds.). Methods in Phycological Studies. Kyouritu Press, Tokyo, In Japanese.

Konda, T. 1982. Distribution of aerobic heterotrophic bacteria in lake waters. Water Temp. Res. **25:** 19–28.

Konda, T. 1985. Difference in bacterial floras among different size fractions of suspended particles in a hypertrophic pond. Jpn. J. Limnol., **46:** 247–255.

Kuroda, N. and Sakamoto, M. 1986. A study on the structure of the heterotrophic bacterial community in Lake Suwa by colony grouping method. Jpn. J. Limnol. **47:** 229–267.

Martin, E. L., Leach, J. E. and Kuo, K. J. 1987. Biological regulation of bloom-causing blue green algae. p. 62–67. In M. W. Loutit and J. A. R. Miles (eds.). Microbial ecology. Springer-Verlag.

Mitsutani, A., Uchida, A. and Ishida, Y. 1988. Occurrence of blue-green algae and algal lytic bacteria in Lake Biwa. Bull. Jap. Soc. Microbiol. Ecol. **2:** 21–28.

Ramasamy, K. and Verachtert, H. 1980. Locallization of cellulase components in *Pseudomonas* sp. isolated from activated sludge. J. Gen. Microbiol., **117:** 181–191.

Redhead, K. and Wright, S. J. L. 1980. Lysis of the cyanobacterium *Anabaena flos-aquae* by antibiotic-producing fungi. J. Gen. Microbiol., **119:** 95–101.

Rein, R. L., Shane, M. S. and Cannon, R. E. 1974. The characterization of *Bacillus* capable of blue-green bactericidal activity. Can. J. Microbiol. **20:** 981–986.

Shilo, M. 1969. Morphological and physiological aspects of the interaction of *Bdellovibrio* with host bacteria. Curr. Top. Microbiol. Immunol. **50:** 174–204.

Shilo, M. 1970. Lysis of blue-green algae by myxobacter. J. Bacteriol. **104:** 453–460.

Somville, M. 1984. Measurment and study of substrate of exoglucosidase activity in eutrophic water. Appl. Environ. Microbiol., **48:** 1181–1185.

Starr, M. P., Stolp, H., Truper, H. G., Balows, A. and Schlegel, H. G. 1981. The procaryotes. p. 2284. A handbook of habitats, isolation and identification of bacteria. Springer, Berlin, Heiderberg, New York.

Straskrabova, V. and Komarikova, J. 1976. Seasonal

changes of bacterioplankton in a reservoir related to algae. 1. Nutrients and biomass. Int. Rev. Ges. Hydrobiol. **64**: 285–302.

Watanabe, A. 1960. List of algal strains in the collection at the Institute of Applied Microbiology, University of Tokyo. J. Gen Appl. Microbiol. **6**: 283–292.

Yamamoto, Y. 1981. Observation on the occurrence of microbial agents which cause lysis of blue-green algae in Lake Kasumigaura. Jpn. J. Limnol. **42**: 20–27.

Yamamoto, Y. 1988. Cyanobacteria-lysing agents and their distribution patterns of lakes and rivers in Japan. Bull. Jap. Soc. Microbial Ecol. **2**: 77–88.

Yamamoto, Y. and Suzuki, K. 1977. Ultrastructural studies on lysis of blue-green algae by a bacterium. J. Gen. Appl. Microbiol. **23**: 285–295.

Yamamoto, Y. and Suzuki, K. 1990. Distribution and algal-lysing activity of fruiting *Myxobacteria* in Lake Suwa. J. Phycol. **26**: 457–462.

Yamamoto, Y., Hayashi, H. and Kanno, N. 1988. Seasonal changes of algal-lysing agents in polluted water. Bull. Jap. Soc. Microbial Ecol. **2**: 45–51.

Yamamoto, Y., Kanno, N. and Hayashi, H. 1991. The distribution of alga-lysing microbes of sediments in Lake Kizaki and Lake Suwa. Jpn. J. Phycol. **30**: 271–279.

山本鎔子*・新妻成一**・黒田伸郎***・坂本　充****：富栄養湖におけるラン藻溶解性細菌について

諏訪湖の表層水から無作為に分離した192株の従属栄養細菌を用いて，ラン藻 *Microcystis* 溶解能の有無を調べた。溶藻能をもつ主な従属栄養細菌は，*Alcaligenes, Flavobacterium/Cytophaga* および *Pseudomonas* 属であった。湖沼中の *Microcystis* ブルームが減少しはじめる9月に分離された株は，その60%が溶藻能を示した。（*214 神奈川県川崎市多摩区東三田　明治大学，**254 神奈川県平塚市東八幡　全農・農業技術センター，***443 愛知県蒲郡市三谷町　愛知県水産試験場，****464 名古屋市千種区　名古屋大学）

(*Received March 2, 1993, Accepted July 7, 1993*)

Jpn. J. Phycol. (Sôrui) 41: 221–230, September 20, 1993

Chrysochromulina quadrikonta sp. nov., a quadriflagellate member of the genus *Chrysochromulina* (Prymnesiophyceae=Haptophyceae)

Masanobu Kawachi and Isao Inouye

Institute of Biological Sciences, University of Tsukuba, Tsukuba, Ibaraki, 305 Japan

Kawachi, M. and Inouye, I. 1993. *Chrysochromulina quadrikonta* sp. nov., a quadriflagellate member of the genus *Chrysochromulina* (Prymnesiophyceae=Haptophyceae). Jpn. J. Phycol. 41: 221–230.

An unusual quadriflagellate species of *Chrysochromulina, Chrysochromulina quadrikonta* sp. nov. (Prymnesiophyceae=Haptophyceae) is described based on observations of cultured material isolated from sea-water samples collected from Tokyo Bay. The four flagella are equal and homodynamic and only one flagellum shows a green autofluorescence. The cells were covered with two types of scales, a plate-like scale and a spiny scale, which are morphologically similar to those of a previously described species, *Chrysochromulina ericina*. This indicates the close affinity of these two species. However, *C. quadrikonta* is distinguished from *C. ericina* in various respects, including scale sizes, cell shape and the peculiar distribution of the spiny scales.

Key index words: Chrysochromulina—*flagellum—haptonema—Haptophyceae—Prymnesiophyceae—quadriflagellate.*

The genus *Chrysochromulina* is a member of the Prymnesiophyceae, and is characterized by both a well developed haptonema that emerges between the two flagella and unmineralized organic scales that cover the cell surface (Green *et al.* 1989). Species of *Chrysochromulina* are widely distributed in the oceans, and the genus contains ca. 50 species (Estep *et al.* 1984; Estep and MacIntyre 1989).

Recently, we discovered a quadriflagellate species that exhibits a prymnesiophycean nature, including a long haptonema and many spiny scales that cover the cell surface. The organism was observed in Tokyo Bay, Japan, August to October 1988, September 1989 and September and October 1990. This alga was also observed in oyster farms in Kesen-numa, Miyagi Prefecture (the northern part of Honshu Island), Japan, in October and November 1991, where it formed a bloom and caused a brown coloration of oyster gills, although no toxin was detected (M. Fujita, pers. comm.). The same species was recently observed in Melbourne, Australia (D. Hill pers. comm.) and in Nelson, New Zealand (L. Rhodes, pers. comm.). Obviously, this unusual prymnesiophyte species is widely distributed in the Western Pacific.

In this paper, light microscopy and external cell morphology are presented, and based on these results we discuss the taxonomy of this organism and we propose a new name, *Chrysochromulina quadrikonta* sp. nov. for it.

Materials and Methods

Chrysochromulina quadrikonta occurred in enriched seawater cultures of water samples collected at the surface in the port of Yokohama (34°28′N; 139°09′E), Tokyo Bay, Kanagawa Prefecture, Japan, on 11 September 1988. Some other species of *Chrysochromulina*, *C. hirta* Manton (Manton 1978), *C. spinifera* (Fournier) Pienaar et Norris (Pienaar and Norris 1979), *C. pringsheimii* Parke et Manton (Parke and Manton 1962), *C. alifera* Parke et Manton. (Parke *et al.* 1956) and several undescribed species, were also present in the same samples. The unialgal culture was established by single cell isolations using micropipettes and dilution techniques. For

both enrichment and unialgal cultures, an ESM medium (Okaichi *et al.* 1982) was used and cultures were grown at 20°C, and about 46 μmol m^{-2} s^{-1} were provided from cool-white fluorescent tubes for 14 : $\overline{10}$h. 1 mg l^{-1} GeO_2 was added to the enrichment cultures to inhibit diatom growth.

To observe the living and fixed specimens, we used a Nikon Optiphoto microscope with differential interference contrast (DIC) optics as well as an Olympus BH-2 epifluorescence microscope. A high-speed (200 frames s^{-1}) video (NAC MHS-200, NAC Inc., Tokyo 106, Japan) mounted on a Nikon Optiphoto bright-field microscope was used to observe the cell behaviour, especially the flagellar movement. The video system was carried out in the negative mode.

Whole mount specimens (Moestrup and Thomsen 1980) were prepared for observations of the cell coverings. A drop of the cell suspension was placed on formvar-coated grids and exposed for 30 s to OsO_4 vapour provided from drops of a 4% solution. The grids were then dried, rinsed in distilled water, and dried again prior to shadowcasting with platinum/palladium at an angle of about 30° or staining with 2% uranyl acetate for 15 min (McFadden *et al.* 1986). The uranyl acetate-stained specimens were rinsed in distilled water so that the positively stained specimens could be obtained. The specimens were examined with a JEOL 100 CX II transmission electron microscope.

The following two prymnesiophytes were examined to compare scale morphology with the quadriflagellate alga. A biflagellate organism that resembles *C. quadrikonta* in cell shape and scale morphology produced a bloom on 20 July 1986 at the same location in Tokyo Bay, where *C. quadrikonta* had been collected in 1988. A unialgal culture of this organism was established in the same way as indicated above. *Chrysochromulina ericina* Parke et Manton (Parke *et al.* 1956) was present in enriched cultures of the water samples collected at the surface in the port of Nagoya (35°02′N; 136°48′E), Aichi Prefecture, Japan, on 3 April 1989. These were also used to observe the scale morphology.

Results

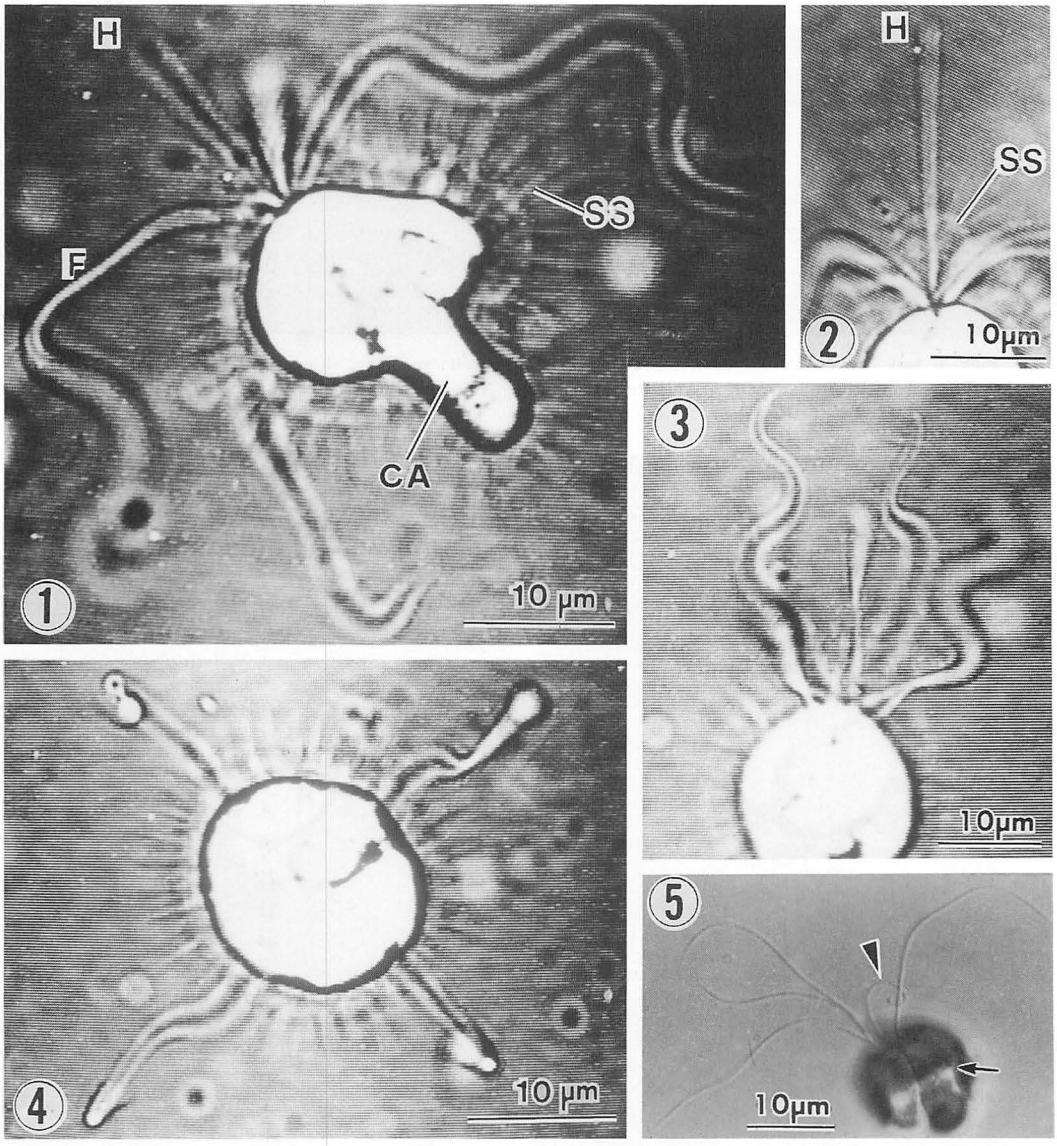
Chrysochromulina quadrikonta sp. nov. (Figs. 1-17)

DIAGNOSIS: Cellula subsphaerica, 10-25 μm longa, 10-18 μm lata, appendicem caudata, 2-5 μm longa; flagella 4, aequalia, 30-40 μm longa. Haptonema, 25-30 μm longa. Chloroplasti, 2 vel 4, parietales, lobis pofundes, quisque pyrenoides unicum immersum fovens. Periplastus et basis haptonemae squamais dimorphis obtectus. Squama strati exteriori cylindrica, 4-6 μm longa, 0.35 μm lata, basis conico, 0.8-1.2 μm lata. Squama strati interioris laminaris, suborbicularis, 1.2-1.6 μm × 1.4-2.0 μm, margine paulum incrassato, ora inconspicua 0.1 μm lata, cristis radiantibus et fibris inconspicuis. Affinis *Chrysochromulinae ericinae*, sed numero flagelli, forma cellulae, magnitudine squamae et distributione squamae diversa.

HOLOTYPUS: Fig. 1

Cell subspherical, 10-25 μm long and 10-18 μm wide, with a caudate appendage, 2-5 μm long; flagella 4, equal, 30-40 μm long. Haptonema, 25-30 μm long. Chloroplasts, 2 or 4, parietal, deeply lobed, each with an immersed pyrenoid. Periplast and haptonematal base coated with two different types of scales. Outer scales, cylindrical, 4-6 μm long and 0.35 μm wide, with a conical base, 0.8-1.2 μm wide. Inner scales plate-like and subcircular, 1.2-1.6 μm × 1.4-2.0 μm, with a slightly thickened margin and an inconspicuous rim, 0.1 μm wide, with a pattern of radiating ridges and inconspicuous fibrils. *Chrysochromulina quadrikonta* is a close relative of *Chrysochromulina ericina*, however, there are differences in the number of flagella, cell shape, scale sizes and distribution pattern of scales.

HOLOTYPE: Fig. 1, from the culture established from a water sample collected on 11 September 1988 at the port of Yokohama, Tokyo Bay, Kanagawa Prefecture, Japan (34°28′N; 139°09′E).

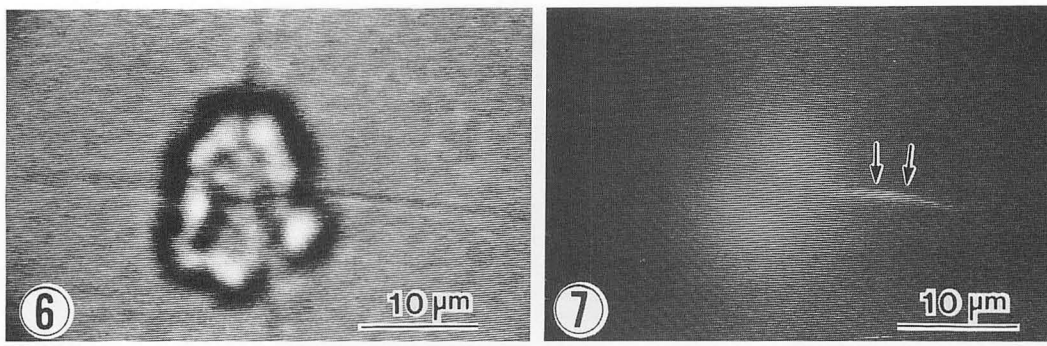


Figs. 1–5. *Chrysochromulina quadrikonta* sp. nov. Figs. 1–4. High-speed video images of the cells. Fig. 1. A typical cell during forward swimming, showing four flagella (F), a haptonema (H), spiny scales (SS) and a caudate appendage (CA). Fig. 2. Anterior part of the cell. Note the distribution of the spiny scales (SS) around the base of the haptonema. Fig. 3. Backward swimming of the cell. Fig. 4. Proximal view of the cell, showing flagella radiating in a cruciform pattern. Fig. 5. Light micrograph of a fixed cell, showing the four flagella, a coiled haptonema (arrowhead) and a deeply lobed chloroplast (arrow).

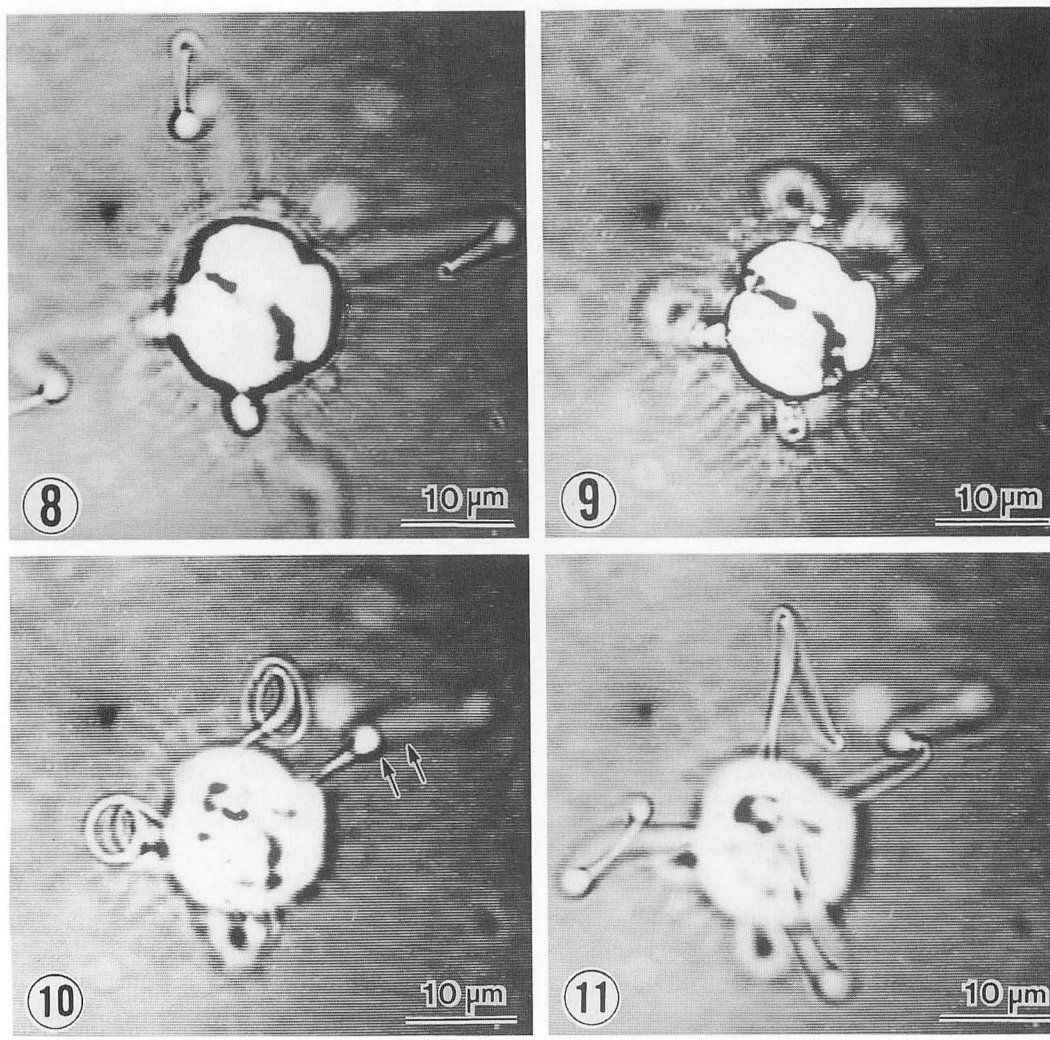
DISTRIBUTION: Yokohama, Kanagawa Prefecture, Kesen-numa, Miyagi Prefecture, Japan; Melbourne, Australia; Nelson, New Zealand.

ETYMOLOGY: quadrikonta (Latin), meaning four-flagella.

The cells are subspherical and usually possess a posteriorly projecting caudate appendage (Figs. 1, 17). Each cell has four flagella and a haptonema that arise from the anterior side of the cell, opposite the caudate appendage, and the entire surface of the cell is covered by spiny scales (Figs. 1, 17). The caudate appendage is distinctive in actively grow-



Figs. 6 and 7. Video images of *Chrysochromulina quadrikonta* sp. nov., showing flagellar autofluorescence. Fig. 6. Phase contrast image. Fig. 7. Fluorescent image. Note autofluorescence in one flagellum (arrows).



Figs. 8–11. High-speed video images of *Chrysochromulina quadrikonta* sp. nov., showing the successive processes of flagellar coiling (Fig. 8, 9) and uncoiling (Fig. 10, 11). Arrows in Fig. 10 show the first uncoiling flagellum. Time elapsed (milli-second): Fig. 8 (0), Fig. 9 (20), Fig. 10 (160), Fig. 11 (260).

ing cells, however, it is lost or disappears in both the fixed cells (Fig. 5) and the living cells in the old cultures.

Internally, two or four lateral chloroplasts are visible under the light microscope. Each chloroplast is deeply lobed into two sections and possesses an embedded pyrenoid (Figs. 5, 17).

Prior to cell division, the flagella and haptonema duplicate and then segregate to opposite sides.

The four flagella are equal in length, and they radiate forming a cruciform pattern when viewed from above the cell (Fig. 4). All the flagella are homodynamic. There is a green autofluorescence in only one of the four flagella (Figs. 6, 7).

The haptonema usually extends straight (Figs. 1, 2, 17). It is capable of coiling (Fig. 5), although coiling occurs only occasionally. Cells usually swim extending the haptonema ahead, with the flagella beating alongside the cell body, extending their distal ends backward (Fig. 1). Sometimes flagellar coiling was also observed. It coils into a helix with two or three gyres (Figs. 8–11). Coiling occurs simultaneously in all four flagella when the cells cease swimming and stop flagellar beating (Fig. 7). In a few seconds after coiling, one flagellum uncoils (Fig. 10), and then other three flagella follow (Fig. 11). Then the cells start to swim again. The haptonema is extended and never coils during the time period that flagellar coiling and uncoiling occur. Backward swimming was also observed. The cells extend all the flagella forward and generate enough propulsive force to swim backward (Fig. 3). The haptonema was usually kept stretched during backward swimming.

Haptonematal activities such as prey capturing, transportation and aggregation of prey particles that occurs in the mixotrophic species, *Chrysochromulina hirta* Manton (Kawachi et. al. 1991), have never been observed in *C. quadrikonta*. *C. quadrikonta* does not take up any food particles, and food vacuole have not been detected.

Individual spiny scales can be easily observed with the light microscope. They are distributed not only on the cell body but also around the proximal part of the haptonema, having a dome-like appearance (Fig. 2). Shadowcast whole mounts revealed that *C. quadrikonta* has two types of unmineralized scales, spiny scales and plate-like scales (Figs. 12–14). The spiny scale consists of a cylindrical upper part and a conical base (Fig. 14). The conical base bears a pattern of concentric and radiating ridges (Fig. 14). The proximal surface of the conical base has obvious radiating ridges at the edge (Fig. 14). The inner plate-like scale is subcircular and the margin is slightly thickened (Fig. 13). Each surface of the plate-like scale shows different patterns (Fig. 13). The distal surface (Fig. 13, A) bears a pattern of fine fibrils, and there is a rim at the edge (Fig. 13, arrows). The proximal surface (Fig. 13, B) is characterized by a pattern of radiating ridges.

Discussion

Regardless of possessing four flagella, it is obvious based on the presence of the haptonema and the characteristics of the scales that *C. quadrikonta* belongs to the genus *Chrysochromulina* (Prymnesiophyceae), i.e. the haptonema is well developed and the scales are different types. Ultrastructural observations of the scales indicate that this organism is closely related to the previously described species, *Chrysochromulina ericina* Parke et Manton (Parke *et al.* 1956). *C. ericina* has two different types of scales, spiny scales and plate-like scales (Fig. 16) (Parke *et al.* 1956; Manton and Leedale 1961) that resemble those of *C. quadrikonta*, i.e. the shape and surface patterns are almost identical. However, there are obvious size differences (Table 1). The plate-like scale of *C. quadrikonta* is considerably larger than that of *C. ericina*. The cylindrical part of the spiny scale of *C. quadrikonta* is wider than that of *C. ericina*, while both the length of the spiny scale and the width of the conical base of *C. ericina* are much longer than those of *C. quadrikonta*. These differences are so clear that *C. quadrikonta* is distinguishable

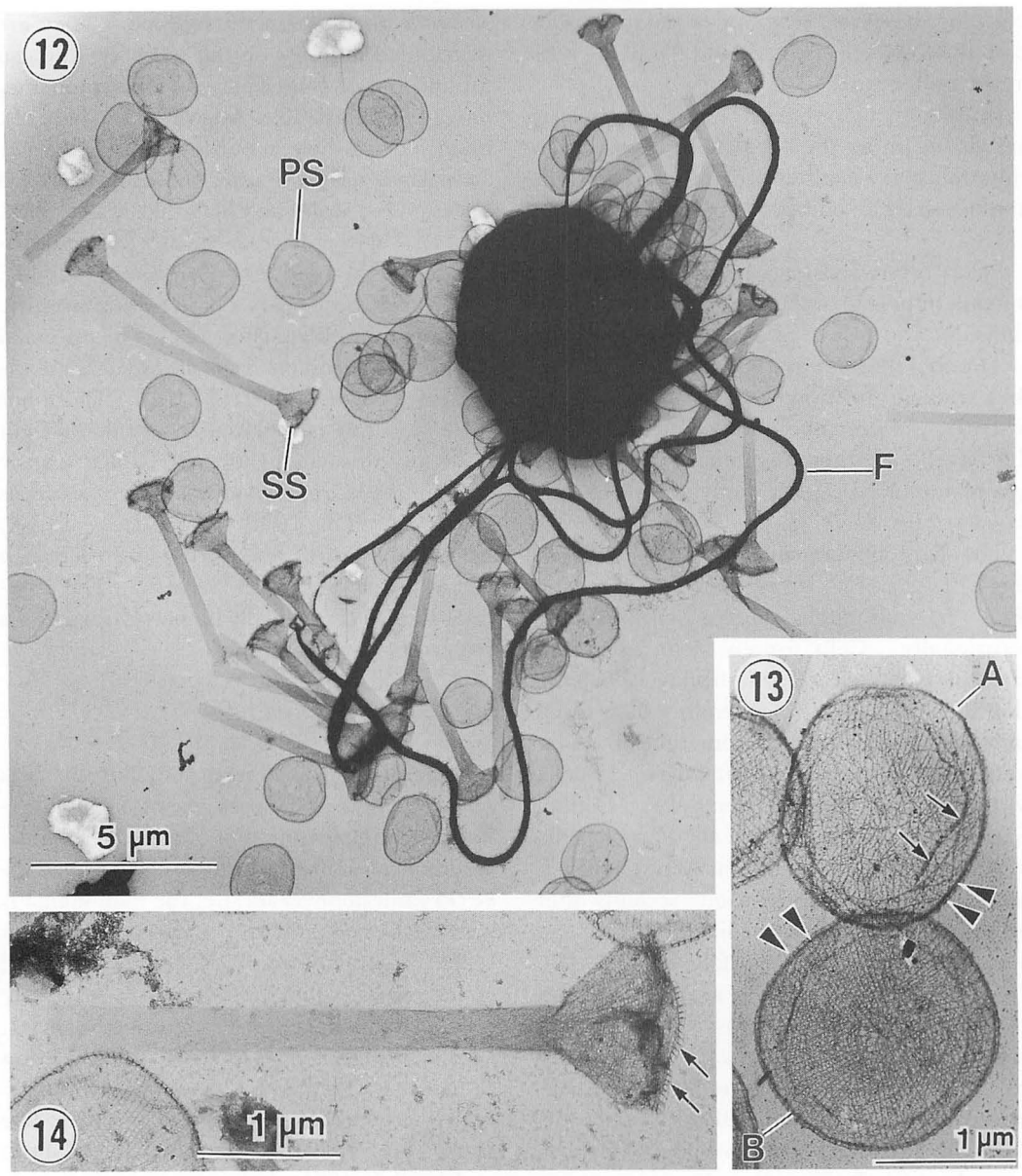


Fig. 12–14. Uranyl acetate-stained whole mount specimens of *Chrysochromulina quadrikonta* sp. nov. Fig. 12. A cell showing flagellum (F), spiny scales (SS) and plate-like scales (PS). Fig. 13. Two plate-like scales bearing different patterns. The upper plate-like scale (A) shows the pattern of the distal surface. Fine fibrils and a rim (arrows) are visible. The lower plate-like scale (B) shows the pattern of the proximal surface. The margin of plate-like scales is slightly thickened (arrowheads). Fig. 14. A spiny scale. The pattern of the conical base is visible. Note the proximal surface of the conical base bearing radiating ridges (arrows).

from *C. ericina* based only on the morphology of the scales. The dome-like distribution of spiny scales is also a characteristic feature of *C. quadrikonta* which has never been observed in other *Chrysochromulina*. Moreover, there are many distinctive differences between *C. quadrikonta* and *C. ericina* such as the shape and size of the cell, and the length of both the flagella and haptonema (Table 2). The coiling ability of the haptonema and prey capture also appears to be a difference between these two species. Parke *et al.* (1956) reported a

Table 1. Comparisons of scale size between *Chrysochromulina quadrikonta* sp. nov. and *C. ericina*.

	C. quadrikonta	*C. ericina**
plate-like scale		
width	1.2–1.6 μm	0.5–0.8 μm
length	1.4–2.0 μm	0.6–1.0 μm
spiny scale		
length	4–6 μm	8–15 μm
width of cylinder	0.35 μm	0.2–0.3 μm
width of conical base	0.8–1.2 μm	1.0–1.4 μm

* Parke et. al. (1956) and present study.

phagotrophic ability in *C. ericina*; whereas, no food capture and uptake was even observed in *C. quadrikonta*. However, *C. quadrikonta* is a close relative of *C. ericina*; therefore, based on these differences, we can conclude that the quadriflagellate prymnesiophyte is a natural taxon and should be recognized as an independent taxonomic entity.

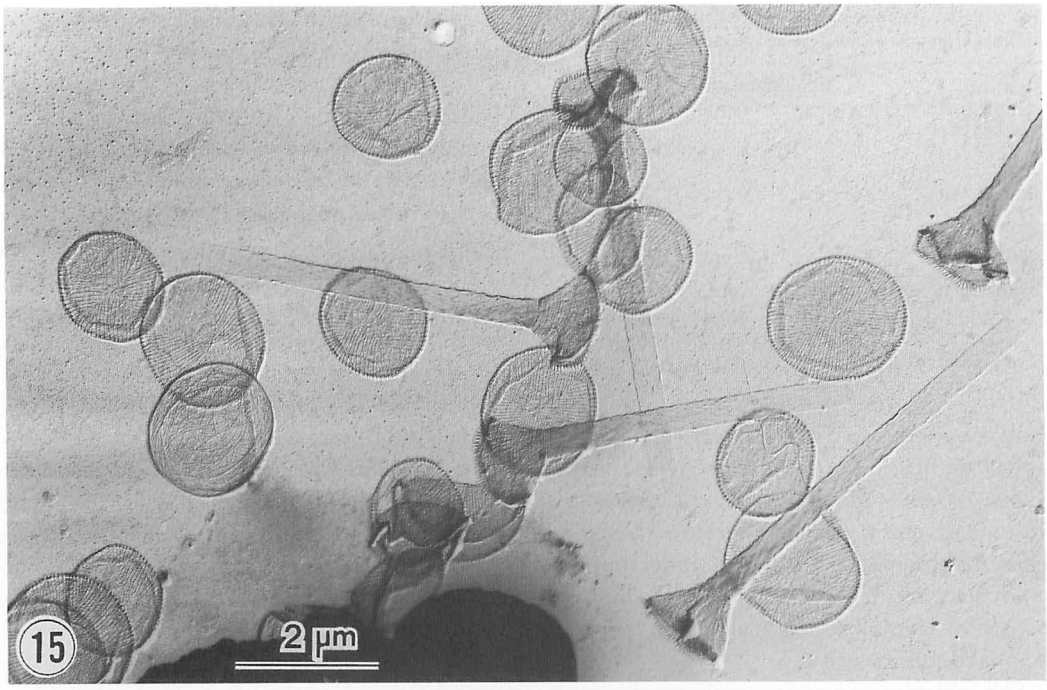
The majority of prymnesiophytes possesses two flagella, and only *Chrysochromulina birgeri* Hallfors et Niemi (Hallfors and Niemi 1974) has been described as a species possessing four flagella. However, it should be noted that the population of *C. birgeri* in the natural sea water sample consisted of not only quadriflagellate but also biflagellate forms, i.e. the number of flagella of this species are not so strict. In contrast, in the culture of *C. quadrikonta*, biflagellate cells have never been observed. Therefore, the presence of four flagella is one of the most characteristic and stable features of *C. quadrikonta*, hence the species epithet.

In green algae, the number of flagella is often regarded as a diagnostic feature of generic rank (e.g. *Chlamydomonas* vs. *Carteria* in the Chlorophyceae). However, there are examples of algae that possess different numbers of flagella that are taxonomically treated as members of the same genus. For example, in the genus *Pyramimonas* (Prasinophyceae), the majority of the species have four flagella; however, species possessing eight (e.g. *Pyramimonas octopus* Moestrup, Hori et Christeusen, Moestrup *et al.* 1987) or 16 flagella (e.g. *Pyramimonas cyrtoptera* Daugbjerg et Moestrup, Daugbjerg and Moestrup 1992) were also found in *Pyramimonas*. It is postulated that these species originated from the quadriflagellate species in the subgenus *Pyramimonas*, because of the similarities in the morphological characteristics such as scales and intracellular ultrastructures, (Moestrup *et al.* 1987; Daugbjerg and Moestrup 1992). On the other hand, in the life cycle of various species of the Ulvophyceae, quadriflagellates often occur as zoospores, while gametes are biflagellates. As mentioned above, the phenomenon of duplication of the number of flagella is not very unusual in green algal lineages. However, it is most unusual and has never been recorded in chlorophyll *c*-containing algae. Of these, only prymnesiophytes have nearly equal and homodynamic flagella. Other chlorophyll *c*-containing algae have heterokont and heterodynamic flagella so that functional differentiation of flagella may be much larger than that of prymnesiophytes. Therefore, the situation of prym-

Table 2. Comparisons of cellular characters between *Chrysochromulina quadrikonta* sp. nov. and *C. ericina*.

	C. quadrikonta	*C. ericina**
cell size	10–25 μm	5–12 μm
flagellar length	30–40 μm	20–30 μm
haptonemal length	25–30 μm	40–50 μm
cell shape	subspherical and caudate	spherical to oblong
distribution of spiny scales	periplast, haptonemal base	periplast only
coiling ability of haptonema	yes (but rare)	yes
phagotrophy	probably no	yes
cell form	motile	motile, amoeboid, walled

* Parke et. al. (1956).



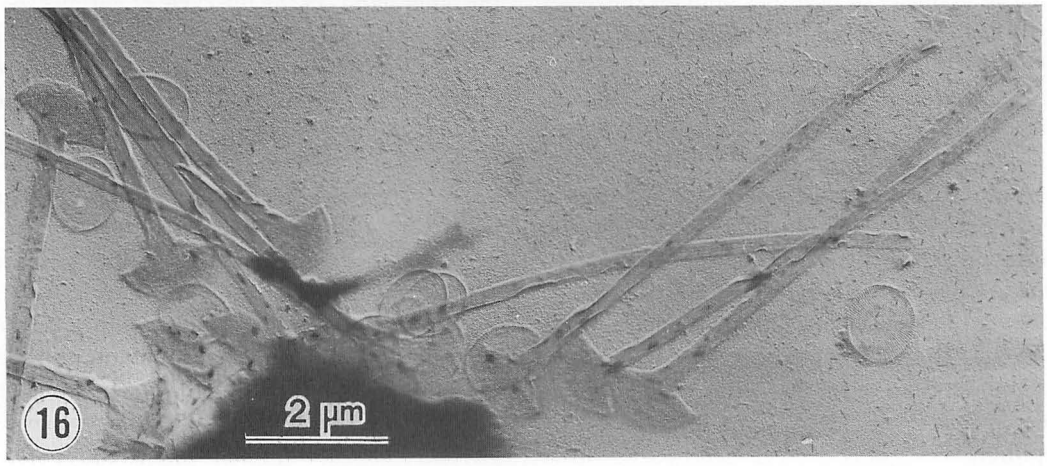


Fig. 15 and 16. Shadowed whole mount specimens allied algae. Fig. 15. Scales of a biflagellte organism collected in 1986 from Tokyo Bay. Fig. 16. Scales of *Chrysochromulina ericina*.

nesiophytes is almost the same as in green algae. Hence, it tempts us to speculate that the doubling of flagella is apt to happen in the evolution of organisms that possess equal and homodynamic flagella and whose functions are more or less the same.

Despite its distinct morphology and its ability of forming a bloom, it is strange that *C. quadrikonta* has not been described before. It may have been overlooked in previous floristic studies; however, its rather sudden emergence in various parts of the Western Pacific during the last several years may require other explanations. One possible postulation is that *C. quadrikonta* may be a species recently established from a biflagellate species of *Chrysochromulina* by the "doubling" of the flagella. It should be noted in relationship to this interpretation that a biflagellate organism exists which has a cell form and scales (Fig.

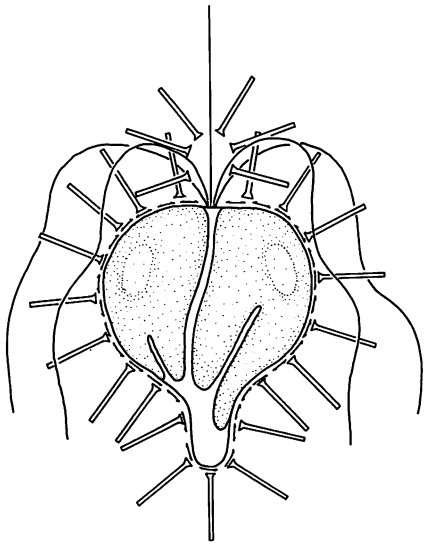


Fig. 17. Diagram of *Chrysochromulina quadrikonta* sp. nov.

15) almost the same as *C. quadrikonta*. We collected this alga from Tokyo Bay in July 1986, two years before the first record of *C. quadrikonta*. In the natural sea water samples and a culture of the biflagellate, we did not notice a quadriflagellate form. At that time we identified the biflagellate alga as *Chrysochromulina ericina* and provided it as a material for a previous work on flagellar autofluorescence of chlorophyll *c*-containing algae (Kawai and Inouye 1989, Figs. 16, 17). Since 1988, however, only the quadriflagellate form has been collected from this location. It is interesting to know how the quadriflagellate species was established, and what type of cytological changes made such a drastic transfiguration possible. Careful investigations on the flagellar-haptonematal apparatus architecture and morphological changes during cytokinesis may provide clues to answer these questions.

Acknowledgments

We are grateful to Mr. K. Ishida, University of Tsukuba, for providing the holotype specimens. This study was supported by a Grant-in-Aid for Scientific Research (No. 03454014) from the Ministry of Education, Science and Culture, Japan.

References

Daugbjerg, N. and Moestrup, ϕ. 1992. Ultrastructure of *Pyramimonas cyrtoptera* sp. nov. (Prasinophyceae), a species with 16 flagella from northern Foxe Basin, Arctic Canada, including observations on growth rates. Can. J. Bot. **70:** 1259–1273.

Estep, K. W., Davis, P. G., Hargraves, P. E. and Sieburth, J. McN. 1984. Chloroplast containing microflagellates in natural populations of North Atlantic nanoplankton, their identification and distribution; including a description of five new species of *Chrysochromulina* (Prymnesiophyceae). Protistologica **20:** 613–634.

Estep, K. W. and MacIntyre, F. 1989. Taxonomy, life cycle, distribution and dasmotrophy of *Chrysochromulina*: a theory accounting for scales, haptonema, muciferous bodies and toxicity. Mar. Ecol. Prog. Ser. **57:** 11–21.

Green, J. C., Perch-Nielsen, K., and Westbroek, P. 1989. Phylum Prymnesiophyta. p. 293–317. *In* L. Margulis, J. O. Corliss, M. Melkonian and D. J. Chapman [ed.] Handbook of the Protoctista. Jones & Bartlett, Boston.

Hallfors, G. and Niemi, A. 1974. A *Chrysochromulina* (Haptophyceae) bloom, under the ice in the Tvarminne Archipelago, southern coast of Finland. Mem. Soc. Fauna Flora Fenn. **50:** 89–104.

Kawachi, M., Inouye, I., Maeda, O. and Chihara, M. 1991. The haptonema as a food-capturing device: observations on *Chrysochromulina hirta* (Prymnesiophyceae). Phycologia **30:** 563–573.

Kawai, H. and Inouye, I. 1989. Flagellar autofluorescence in forty-four chlorophyll *c*-containing algae. Phycologia **28:** 222–227.

Manton, I. and Leedale, G. F. 1961. Further observations on the fine structure of *Chrysochromulina ericina* Parke and Manton. J. mar. biol. Ass. U. K. **41:** 145–155.

Manton I. 1978. *Chrysochromulina hirta* sp. nov., a widely distributed species with unusual spines. Br. phycol. J. **13:** 3–14.

McFadden, G. I., Hill, D. R. A. and Wetherbee, R. 1986. A study of the genus *Pyramimonas* (Prasinophyceae) from southeastern Australia. Nord. J. Bot. **6:** 209–234.

Moestrup, ϕ. and Thomsen, H. A. 1980. Preparation of shadowcast whole mounts. p. 386–390. *In* E. Gantt [ed.] Handbook of phycological methods, developmental and cytological methods. Cambridge University Press, Cambridge.

Moestrup, ϕ., Hori, T. and Kristiansen, A. 1987. Fine structure of *Pyramimonas octopus* sp. nov., an octoflagellated benthic species of *Pyramimonas* (Prasinophyceae), with some observations on its ecology. Nord. J. Bot. **7:** 339–352.

Okaichi, T., Nishino, S. and Imatomi, Y. 1982. Collection and mass culture. p. 23–34. *In* Soc. Sci. Fish.

[ed.] Toxic phytoplankton-occurrence, mode of action, and toxins (in Japanese). Koseisha-Koseikaku, Tokyo.

Parke, M. and Manton, I. 1962. Strudies on marine flagellates. VI. *Chrysochromulina pringsheimii* sp. nov., J. mar. biol. Ass. U. K. **42:** 391–404.

Parke, M., Manton, I. and Clarke, B. 1956. Studies on marine flagellates. III. Three further species of *Chrysochromulina*. J. mar. biol. Ass. U. K. **35:** 387–414.

Pienaar, R. and Norris, R. 1979. The ultrastructure of the flagellate *Chrysochromulina spinifera* (Fournier) comb. nov. (Prymnesiophyceae) with special reference to scale production. Phycologia **18:** 99–108.

河地正伸・井上　勲：4本鞭毛をもつハプト藻，*Chrysochromulina quadrikonta* の新種記載

東京湾から分離，培養した，4本鞭毛をもつハプト藻を記載した。本種の鞭毛は等長で，同調した運動を示し，1本のみが自家蛍光をもつ。2種類の鱗片形態は *Chrysochromulina ericina* のそれに類似するが，サイズは異なり，容易に区別される。更に，本種と *C. ericina* は細胞形態や刺状鱗片の分布様式も異なる。これらの差異に基づいて，本種を *Chrysochromulina quadrikonta* と命名した。(305 茨城県つくば市天王台1-1-1　筑波大学生物科学系)

(*Received March 25, 1993, Accepted July 12, 1993*)

Jpn. J. Phycol. (Sôrui) 41: 231–236, September 20, 1993

Use of fluorescent staining to monitor the temporal pattern of cell wall resynthesis in *Ulva fasciata* (Ulvales, Chlorophyta) protoplasts

Yean-Chang Chen* and Chung-Sing Chen

Institute of Aquaculture, National Taiwan Ocean University, Keelung Taiwan, Republic of China

Chen, Y. C. and Chen, C. S. 1993. Use of fluorescent staining to monitor the temporal pattern of cell wall resynthesis in *Ulva fasciata* (Chlorophyta: Ulvales, Ulvaceae) protoplasts. Jpn. J. Phycol. 41: 231–236.

Fluorescent brightener agent (FBA) was used to continuously check the development of new cell walls of an *Ulva fasciata* protoplast population. Cell wall resynthesis began within 6 hrs after the isolation of protoplasts. Maximum cell wall formation was reached at the 8th day of incubation, when about 81% of the protoplasts had formed new cell walls. Resynthesis of cell walls was delayed when they were stained earlier than 4 hrs after isolation. However, after 24 hrs, the influence was small.

Key Index Words: Chlorophyta—fluorescent brightener agent—new cell wall—protoplasts—stain—Ulva fasciata.

Algal protoplasts have considerable potential for use in physiological investigations. Practical applications are varied. Protoplasts can be subjected to genetic modification, used for mass production of protoplast-fusion hybrids (Saga *et al.* 1986) or for establishment of cell suspensions (Chen, L. C-M. 1989). However, since they lack a cell wall, protoplasts are fragile, and in culture they are particularly susceptible to changes in the osmotic concentration of the medium. Thus, protoplasts regenerate only when incubated in a medium with suitable and well-regulated osmotic oncentration (Ahuia 1982; Evans and Bravo 1983; Chen, L. C-M. 1989). Also, when protoplasts form new cell walls, they must be transferred from the hyperosmotic medium and incubated in enriched seawater (i.e. Provasoli 1968). This counteracts the increase in turgor pressure resulting from the regeneration of the cell walls (Kirst and Bission 1979, Berliner 1981). In a previous study of the green alga *Ulva fasciata* (Chen and Chen 1991). It was found that protoplasts grow best at hyperosmotic concentrations, but that the osmotic concentration shoud be gradually decreased as cell-wall resynthesis occurs to obtain a high number of regenerated protoplasts (Chen, Y. C. 1989). However, neither the optimal time to begin decreasing the osmolarity of the medium nor the rate at which the reduction should occur are known and no doubt differ with taxon and culture conditions. If there was a way to identify those protoplasts that had already formed cell walls, and these protoplasts were directly transferred to enriched seawater, then it might be easier to obtain a high yield of regenerated protoplasts. This paper investigates one possible method.

FBA was used to follow the course of resynthesis of cell wall in protoplast cultures. This agent specifically binds with cell wall materials (Maeda and Ishida 1967). Fluorescent staining, to distinguish whether or not the protoplasts have regenerated new cell walls, can be used to address these problems in batch culture to which FBA has been added. Fluorescent staining has often been used to observe the biosynthesis of cell walls with an electron microscope (Berliner *et al.* 1978, Haigler and

* Present address: Institute of Oceanography, National Taiwan University, Taipei, Taiwan, Republic of China.

Brown 1980, Herth 1980, Galbraith 1981, Itoh *et al.* 1984), and to distinguish whether or not protoplasts have retained remnants of the mother-cell walls. However, they have rarely been used to follow, *in vivo*, the development of the new walls of protoplasts during incubation.

The purpose of this paper is to describe a method of fluorescent staining which can be used with protoplasts of *Ulva fasciata* and similar Chlorophyta to determine the temporal pattern of cell wall resynthesis in a process of culture procedure, with less negative side effects.

Materials and Methods

Fronds of the marine macroalgae *Ulva fasciata* Delile were collected at Keelung, Taiwan on May 5, 1989. Immediately after the collection, plants were washed with autoclaved seawater several times, and transported to the laboratory.

Protoplasts were obtained by digestion of the cell walls with enzymes as decribed by Chen and Chen (1991). Selected pieces of healthy fronds (approx. 2 cm^2) were thoroughly cleaned in filtered seawater. Then they were incubated for 24 hrs in 100 ml of autoclaved Provasoli's enriched seawater (PES) medium (Provasoli 1968) containing 10 ml of antiboitic mixture (Polne-Fuller and Gibor 1987). The culture room was 24°C and had a 12 : $\overline{12}$ L : D regime with irradiation of 20 $\mu Em^{-2}s^{-1}$. Fronds of *U. fasciata* were cut to 0.5–1 mm square pieces on a clean bench with a sterile knife blade. To obtain protoplasts 0.1 g of pieces was incubated on a rotary shaker (50 rpm) for 12 hrs in darkness at 24 °C, in 10 ml of sorbitol-enzyme solution (1.2 M sorbitol, 4% cellulase, Onozuka R-10, 2% marcerozyme, Onozuka R-10). Then the protoplast-enzyme suspension was layered onto the top of a 35% (w/v) density buffer (Ficoll-400, Sigma) solution, and centrifuged at 200 × g (or 1200 rpm, HERMLEZ 320) for 30 min, to remove detritus. Protoplasts were separated (purified) from the interface between the density buffer and the enzyme solution with a sterile Pasteur pipette. Purified protoplasts of *U. fasciata* were cultured in mannitol-PES medium. Osmotic concentration was adjusted to 0.84 M mannitol. Fluorescent brightening agent (FBA) (Calcofluor White ST, Sigma) was used to stain the new cell wall of protoplasts.

One-tenth of a ml of stock 1% FBA per 10 ml of culture medium was added to six cultures of purified protoplasts at 0, 2, 4, 8, 12 and 24 hrs after isolation. After staining, protoplasts with resynthesized cell walls appear yellow or green when viewed with a fluorescent microscope. The protoplasts without cell walls appear red. Changes in the number of fluorescent protoplasts in each culture were monitored continuously until the 8th day of incubation. At each monitoring period, five fields (ca. 100–500 cells in a field) were randomly sampled from each of the six cultures. In addition, another seven cultures of purified protoplasts were incubated in FBA-free media until stained with 0.01% FBA at days 2, 3, 4, 5, 6, 7, and 8 after isolation, and the average percentages with new cell walls were determined immediately. These groups provide comparative information to determine whether or not FBA influences the process of cell wall formation. They are considered to be control groups. An inverted fluorescent microscope (Nikon, Diaphot-TMD with TMD-EF) was used to examine protoplasts for cell wall synthesis. Except for protoplast isolation all laboratory procedures were carried out under 12 : $\overline{12}$ L : D regime and irradiation of 166 $\mu Em^{-2}s^{-1}$ at 24°C in a culture room.

Results and Discussion

Newly synthesized cell walls of algal protoplast are difficult to observe under a normal optical microscope. Fluorescent complexes, resulting from the binding of FBA with amorphous cellulose, facilitate the observation of the cell wall resynthesis. With FBA as the staining agent, protoplasts with new cell walls exhibit either green or yellow fluorescence, depending on the stage of formation of the cell

Table 1. Percentage of appearance of new cell wall in FBA-stained *U. fasciata* protoplasts at hrs 0, 2, 4, 8, 12 and 24 after isolation.

Age of protoplasts (hrs)	Percentage* of protoplasts with new cell walls					
	Hours after isolation for protoplasts staining					
	0 hr	2 hrs	4 hrs	8 hrs	12 hrs	24 hrs
6		0.46	0.48			
8	0.29	0.62	0.66	0.50		
10	0.38	0.94	1.16	1.56		
12	0.48	1	1.48	1.65	0.62	
14	0.57	1.06	1.53	1.85	0.82	
16	0.67	0.12	1.57	5.08	1.97	
18	0.76	1.18	1.62	8.13	9.06	
20	0.86	1.23	1.66	11.18	16.46	
24	1.05	1.35	1.75	17.28	20.19	
48	16.89	16	19.34	46.63	49.26	37.91
72	21.97	33.78	39.36	52.92	53.96	47.21
96	28.16	38.21	45.63	59.01	58.83	64.83
120	32.6	42.17	59.09	63.62	63.72	68.34
144	43.01	49.05	62.84	66.82	68.72	73.28
168	49.67	51.27	64.05	68.65	73.49	78.52
192	49.67	51.42	64.24	72.46	74.83	80.85

Culture conditions: 166 $\mu Em^{-2}s^{-1}$, 12 : $\overline{12}$ L : D at 24°C.

*The percentage is expressed as the average value of five randomly selected fields under an inverted microscope with fluorescent equipment.

wall. In protoplast populations of *U. fasciata*, cell wall resynthesis in FBA-enhanced medium does not proceed synchronously, due to the variant physiological conditions of cells.

The resynthesis of cell walls of *U. fasciata* protoplasts that were stained at 2 hrs after isolation, was initiated at hour 6 after isolation (Talbe 1). At this time about 0.5% of the protoplasts had cell walls. This could be due to the remaining old cell walls. However, the percentage of regenerated cell walls increased at hr 8, verifying that the protoplasts had formed new walls. The highest percentage (81%) was reached after 8 days of incubation in the group of protoplasts that was stained after 24 hrs of isolation.

Through eight days of monitoring, the number of green fluorescent protoplasts (those with a cell wall) increased in every experimental group irrespective of the time of staining (Fig. 1). Protoplasts stained at 0, 2 and 4 hrs after isolation took the longest time after staining (ca. 48-44 hrs) for evident appearance (11-16%) of green fluorescent protoplasts. Protoplasts stained at 8, 12 and 24 hrs after isolation took 8, 6 and 3 hrs, respectively. This indicates that there is considerable inhibition of cell wall resynthesis in protoplasts stained earlier than 4 hrs after isolation.

Initiation of cell wall resynthesis in *Boergesenia forbesii* protoplasts occurs within 2 to 3 hrs of incubation (Itoh *et al.* 1986). The temporal differences in resynthesis of cell walls between the studies of *B. forbesii* and this study of *U. fasciata* could reflect the considerable taxonomic differences between the species, or differences in the techniques used to obtain the protoplasts, the osmotic concentration, pH, the physiological status and growth stage of the plant material. Itoh *et al.* (1984, 1986) physically cut the coenocytic plant of *B. forbesii* to obtain protoplasts. However, the protoplasts from *Ulva* which is not coenocytic, were obtained through the digestion of the original cell wall and subsequent centrifugation to separate the protoplasts from the debris. The protoplasts of *Ulva* were, there-

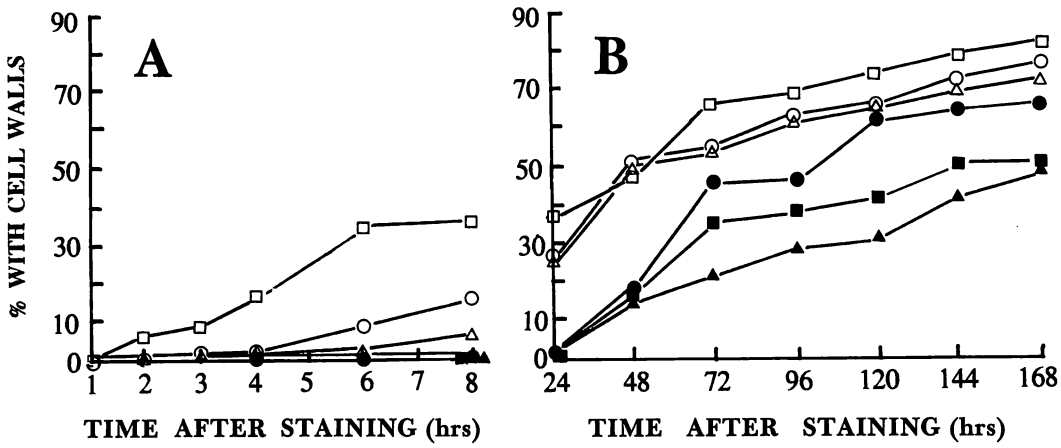


Fig. 1. Appearance of new cell walls of *Ulva fasciata* protoplasts stained with FBA at hrs 0, 2, 4, 8, 12 and 24 after isolation under 166 $\mu Em^{-2}s^{-1}$, 12 : $\overline{12}$ L : D at 24°C conditions (A, early stage; B, late stage). The percentage is expressed as the average value of five randomly sampled fields under a microscope. ▲, protoplasts stained at 0 hr after isolation; ■, stained at 2 hrs; ●, stained at 4 hrs; △, stained at 8 hrs; ○, stained at 12 hrs; □, stained at 24 hrs.

fore, subjected to considerably more stress than were those of *B. forbesii*. Such stresses are known to affect the physiology of the protoplasts (Galun 1981).

Itoh *et al.* (1984) also reported that cell wall synthesis can be negatively affected by FBA in studies of the green alga *B. forbesii*. They found that 95 μM FBA (ca. 0.01%) was the highest concentration which was not toxic to terminal complexes. However, even at these concentrations, FBA disfigured the microfibrils. This presumably also influenced cell wall resynthesis.

Cell wall formation of the protoplasts that were stained at 24 hrs after isolation showed no apparent negative influence. In fact cell wall resynthesis was almost the same between this group and the control groups (Fig. 2). The rates of increase in presence of cell walls are similar, indicating that the delay in cell wall synthesis occurs only in the early phases of resynthesis. Although early staining with FBA delays the resynthesis of cell wall, the method described here has proved faster and less harmful to the protoplasts than other methods, such as those which distinguish the

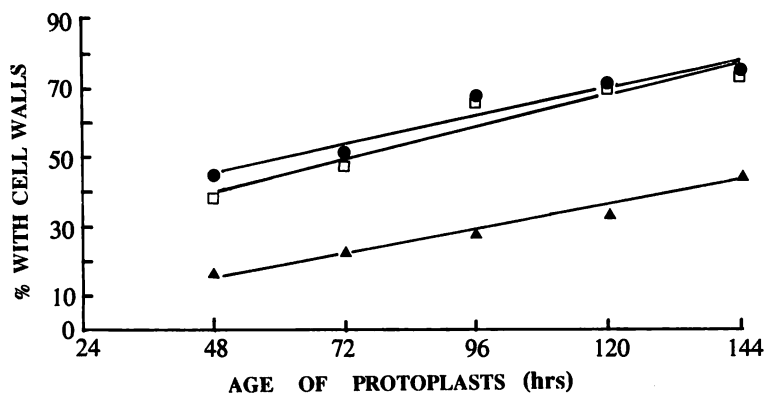


Fig. 2. The relationship between time (hr) and increase rate in protoplast with new cell walls from group 0, 24 and control groups. Group 0 and group 24 were stained at the start of incubation (0 hr) and after 24 hrs of isolation respectively. Control groups were incubated in FBA-free madia until they were stained at days 2, 3, 4, 5 and 6 after isolation. For group 0, $Y=2.29+0.276X$ with $r^2=0.98$, for group 24, $Y=25.07+0.339X$ with $r^2=0.929$, and for control groups, $Y=32.86+0.285X$ with $r^2=0.925$. (▲, Group 0; □, Group 24; ●, Control groups.

new cell walls through the use of electron microscopes (Burgess *et al.* 1978, Itoh *et al.* 1984) and protein-synthesis inhibitors (Itoh *et al.* 1986). The information provided here should encourage use of the FBA staining technique in continuously recording the number (percentage) of protoplasts with new cell walls of *Ulva fasciata* and of other marine macroalgae.

Information on the time of cell wall resynthesis will allow us to transfer protoplasts from a hyperosmotic concentration to normal enriched seawater with optimal timing. This should lead to more efficient propagation of marine algae.

Acknowledgments

Sincere gratitude is extended to Dr. Jane, E. Lewis, Institute of Marine Biology, National Taiwan Ocean University, and Dr. Stephen G. Nelson, Marine Laboratory, University of Guam, for helpful suggestions on the manuscript.

References

Ahuia, M. R. 1982. Isolation, culture and fusion of protoplast: Problems and prospects. Silvae Genetica **31:** 2–3.

Berliner, M. D. 1981. Protoplasts of eukaryotic algae. Inter. Rev. Cytology **73:** 1–19.

Berliner, M. D., Wood, N. L. and Damico, J. 1978. Vital and calcofluor staining of *Cosmarium* and its protoplasts. Protoplasma **96:** 39–46.

Burgess, J., Linstead, P. J. and Bonsall, V. E. 1978. Observation on the time course of wall development at the surface of isolated protoplast. Planta **139:** 85–91.

Chen, C. S. and Chen, Y. C. 1991. Isolation and regeneration of protoplasts from the green alga *Ulva fasciata* Delile. Proceedings of N.S.C., ROC. Vol. 15, No. 4, pp. 244–250.

Chen, L, C-M. 1989. Cell suspension culture form *Porphyra lineares* (Rhodophyta) a multicellular marine red alga. J. Appl. Phycol. **1:** 153–159.

Chen, Y. C. 1989. Studies on the preparation and regeneration of protoplasts from green algae *Ulva fasciata* Delile: The effect of osmotic pressure, temperature, shaking speed and duration on the yield and regenration rate of protoplast. Master thesis of National Taiwan Ocean University. p. 56, Keelung Taiwan R.O.C.

Evans, D. A. and Bravo, J. E. 1983. Protoplast isolation and culture. *In* Handbook of plant cell culture. Vol. 1. D. A. Evans, W. R. Sharp, P. V. Ammirato and Y. Yamamoto (Eds). pp. 124–176.

Galbraith, D. W. 1981. Microfluorimetri quantitation of cellulose biosynthesis by plant protoplasts using calcofluor white. Physiol. Plant. **53:** 111–116.

Galun, E. 1981. Plant protoplasts as physiological tools. Ann. Rev. Plant Physiol. **32:** 237–266.

Haigler, C. H. and Brown, B. M., Jr. 1980. Calcofluor white ST alters the *in vivo* assembly of cellulose microfibrils. Science **210:** 903–905.

Herth, W. 1980. Calcofluor white and congo red inhibit chitin microfibril assmbly of *Poterioochromonas*: Evidence for a gap between polymerization and microfibril formation. J. Cell Biol. **87:** 442–450.

Itoh, T., O'Neil, R. M. and Brown, R. M., Jr. 1984. Interference of cell wall regeneration of *Boergesenia forbesii* protoplasts by Tinopal LPW, a fluorescent brightening agent. Protoplasma **123:** 174–183.

Itoh, T., Legge, R. L. and Brown, R. M., Jr. 1986. The effects of selected inhibitors on cellulose microfibril assmbly in *Boergesenia forbesii* (Chlorophyta) protoplasts. J. Phycol. **22:** 224–233.

Kirst, G. O. and Bisson, M. A. 1979. Regulation of turgor pressure in marine algae: lons and low-molecular-weight organic compounds. Aust. J. Plant Physiol. **6:** 539–556.

Maeda, H. and Ishida, N. 1967. Specificity of binding of hexopyranosyl polysaccharides with fluorescent brightener. J. Biochem. **12:** 276–278.

Polne-Fuller, and Gibor, A. 1987. Tissue culture of seaweeds. p. 219–239. *In* K. T. Bird and P. H. Benson [Eds.], Seaweed cultivation for renewable resources. Elsevier, Amsterdam, Oxford, New York, Tokyo.

Provasoli, L. 1968. Media and products for the cultivation of marine algae. p. 63–75. *In* A. Watanabe and A. Hattori [Eds.] Cultures and collections of algae. Jap. Soc. Plant Physiol., Tokyo.

Saga, N., Polne-Fuller, M. and Gibor, A. 1986. Protoplasts from seaweeds: production and fusion. Beihefte Zur Nur Nova Hedwigea **83:** 37–43.

Zhang, D. 1983. Study on the protoplast preparation, culture and fusion of somatic cells from two species of green algae *Ulva linza* and *Monostroma angicava* Kjellm, J. Shandong College Oceanog. **13:** 57–65 (Chinese with English abstract).

陳　衍昌・陳　忠信：リボンアオサ（緑色植物門，アオサ目，アオサ科）のプロトプラストの細胞壁再成を経時的に調べるための蛍光染色

リボンアオサのプロトプラストを蛍光染色 (FBA) を含むメディウム中で培養し，細胞壁の再生を経時的に追跡した。細胞壁再生はプロトプラスト単離後 6 時間以内に始まった。細胞壁の形成率は 8 日後に最大となり，約 81％のプロトプラストが細胞壁を再生した。細胞壁の再生は，プロトプラスト単離後 4 時間以内に染色した場合には遅れたが，24時間後に染色したものでは，染色による阻害は小さかった。この方法は，細胞壁再生状態を知るための簡便法として利用できる。(Institute of Aquaculture, National Taiwan Ocean University, Keelung Taiwan, Republic of Chaina)

(*Received November 19, 1992, Accepted June 14, 1993*)

Jpn. J. Phycol. (Sôrui) 41: 237–241, September 20, 1993

Morgan L. Vis and Robert G. Sheath: Distribution and systematics of *Chroodactylon* and *Kyliniella* (Porphyridiales, Rhodophyta) from North American streams

Key Index Words: Chroodactylon—*Cladophoraceae*—*epiphyte*—*freshwater streams*—Kyliniella—*North America.*

Morgan L. Vis and Robert G. Sheath, Department of Biology, Memorial University of Newfoundland, St. John's, Newfoundland, Canada A1B 3X9.

The freshwater pseudofilamentous members of the rhodophyte order Porphyridiales, *Chroodactylon* and *Kyliniella*, have been reported from North American streams but there has been no detailed study of their geographic distribution or taxonomic status. Hence, this study was undertaken as part of a survey of 1,000 stream segments in North America (Sheath and Cole 1992).

a) *Chroodactylon*

Populations of Cladophoraceae (151) were collected from streams throughout North America from the Northwest Territories (68°N) to Costa Rica (10°N) (Sheath and Cole 1992). The samples were fixed in 2.5% gluteraldehyde and maximum depth and width, pH, specific conductance, temperature and mean current velocity were measured from each stream segment as described by Sheath *et al.* (1989).

From each cladophoracean population, branches were randomly sampled and at least 0.2 g fresh weight of material was thoroughly searched for *Chroodactylon*. The length and diameter of the middle cell from the main filament of *Chroodactylon* was measured using an ocular micrometer. The number of false branches was enumerated, total filament length was measured and number of *Chroodactylon* plants g^{-1} fresh weight of Cladophoraceae was calculated.

Only one type specimen of *Chroodactylon* species reported from freshwaters was available for examination.

Chroodactylon ornatum (C. Agardh) Basson 1979. *Bot. Mar.* **22:** 67. (Basionym: *Conferva ornata* C. Agardh 1824. *Systema Algarum*: 104) (holotype) PC herbarium G. Thuret No. 69. in Lacu Mälaren.

The type specimens of *Chroodactylon ramosum* (Thwaites) Hansg. and *Asterocytis smaragdina* (Reinsch) Forti could not be located and probably no longer exist (BM, IB, M, TCD). A portion of the type specimen was moistened and removed for examination. Each population and the type specimen was measured in replicates of ten with the exception of ON 65, from which only six plants were available. Sample size was determined and statistical tests were done according to Vis and Sheath (1992).

Seven populations of *Chroodactylon* were found, one from northern Manitoba, three from southern Ontario, two from western New York and one from southern Arizona (Table 1). The host was *Cladophora glomerata* (L.) Kütz. in each case, with the exception of *Rhizoclonium hookeri* Kütz. in Manitoba. The densities of *Chroodactylon* plants ranged from 60 to 570 filaments g^{-1} fresh weight host. The streams containing *Chroodactylon* tended to be large (maximum width 8–20 m, maximum depth 60–>100 cm), moderately flowing (0–62 cm s^{-1}), warm (12–23°C) and alkaline (pH 6.8–8.5, specific conductance 220–540 μS cm^{-1}). These trends are similar to those previously reported for the occurrence of *Chroodactylon* in North American freshwaters (Sheath and Hymes 1980; Sheath and Morison 1982).

Cell dimensions varied considerably within the populations and type specimen, such that there was overlap in the ranges of diameter

Table 1. Morphometric features of *Chroodactylon* types and populations in North America. Mean and standard deviation in parentheses.

Population[a] or type	Cell diameter (μm)	Cell length (μm)	Filament length (μm)	False branch number
a) *Populations measured*				
MAN11	5.8- 8.7 (6.4±1.0)	8.7-14.5 (11.3±2.1)	47- 372 (159±117)	0-1
ON23	5.8- 9.3 (7.7±1.1)	7.3-11.6 (9.5±1.6)	71- 560 (326±178)	0-1
ON50	6.1- 7.2 (7.1±0.9)	7.2-11.9 (10.2±1.5)	118-1,180 (294±320)	0-6
ON65	8.8-11.6 (10.2±0.9)	8.8-11.9 (10.7±1.2)	53- 442 (204±178)	0-4
NY113	6.5-11.6 (8.6±1.6)	7.1-10.2 (9.9±2.3)	24- 974 (188±324)	0-2
NY114	8.7-11.6 (10.8±1.0)	10.2-14.5 (11.9±1.9)	59- 295 (142± 77)	0-3
AZ5	7.4-10.2 (8.4±0.9)	10.3-16.0 (12.2±2.4)	88- 643 (362±199)	0-4
b) *Types*				
ornatum	4.9- 7.4 (6.4±0.9)	8.6-14.8 (10.9±1.7)	345-1,240 (817±332)	1-4
ramosum[b]	c. 17	c. 28	—	—
smaragdinum[c]	4.1-8.4	9.7-11.2	700-1,100	max. 5

[a] MAN=Manitoba, ON=Ontario, NY=New York, AZ=Arizona
[b] from Plate 213 in Harvey (1848) as *Hormospora ramosa*
[c] from protologue of Reinsch (1875) as *Callonema smaragdinum*

and length (Table 1). In addition, the type of *C. ornatum* did not statistically differ from three populations in cell diameter and all seven populations in cell length ($p<0.05$). The cell sizes from this study (5.8-11.6×7.1-16.6 μm, Table 1) are also similar to other freshwater studies (4.0-16.0×6.4-17.8 μm) (Daily 1943; Prescott 1962; Taft and Taft 1971; Sheath and Hymes 1980; Sheath and Morison 1982) and some marine accounts (3-8×8-20 μm) (Taylor 1957; Schneider and Searles 1991). However, cell sizes of marine populations from Jamaica and California are larger (13-28×9-19 μm) (Chapman 1961; Abbott and Hollenberg 1976).

Filament lengths of *Chroodactylon* also vary considerably and the number of false branches was significantly correlated to this feature ($p<0.05$, Table 2). The maximum filament length measured was an order of magnitude smaller than that reported for some marine populations (Taylor 1957; Schneider and Searles 1991).

Based on the similarity in morphometry among the North American populations of *Chroodactylon* and the type specimen of *C. ornatum* and the protologue of *C. smaragdinum* (Table 1, Reinsch 1875), we consider them to be synonymous. The oldest specific epithet is *C. ornatum* and the North American populations are referred to this taxon. The fact that the cell dimensions of *C. ramosum* determined

Table 2. Morphometric characteristics of *Kyliniella latvica* populations. Mean and standard in parentheses. Measurements from Skuja (1926) and Flint (1953) below. All measurements in μm.

Population[a] or type	Cell diameter	Cell length	Rhizoid diameter	Rhizoid length	Filament diameter
K. latvica type	9.9-14.8	9.9-17.3	7.4- 9.9	17.3-24.7	17.3-29.6
	(12.7±1.8)	(13.4±2.6)	(8.4±1.3)	(19.8±3.1)	(20.8±4.7)
	(10-19)	—	(10)	(150)	(16)
NH	7.4-14.8	4.9-11.1	7.4- 9.9	17.3-24.7	12.4-32.1
	(11.3±2.1)	(7.7±2.1)	(8.5±1.2)	(20.3±2.5)	(25.7±6.9)
	(c. 15)	—	—	(≤50)	—
RIA3	8 -11	5 -9	7 -10	18 -25	24
	(9.7±1.0)	(6.1±1.4)	(8.3±1.5)	(21.3±2.9)	

[a] NH=New Hampshire, RI=Rhode Island

from the original plate differ considerably from those of *C. ornatum* (Table 1, Harvey 1848) is in disagreement with previous studies which have synonymized these two taxa (John *et al.* 1979; Entwisle and Kraft 1984).

Description

Pseudofilaments with variable number of false branches (0-6) composed of rectangular to ellipsoidal cells loosely arranged in a linear fashion within a broad gelatinous matrix (Figs. 1-2). Cells with axial blue-colored chloroplast containing a prominent central pyrenoid (Fig. 3). Cell diameter 5.8-11.6 μm, cell length 7.1-16.6 μm and filament length 24-1,240 μm (Table 1). Occasional component of the epiphyton of *Cladophora* and *Rhizoclonium* in warm, alkaline streams of North America.

b) *Kyliniella*

Kyliniella was collected from the only two sites in North America known to contain this alga, Rhode Island (Sheath and Burkholder 1985) and New Hampshire (Flint 1953) (Table 2). The type specimen of *K. latvica* was obtained as follows:

> *Kyliniella latvica* Skuja 1926. *Acta Horti. Bot. Univ. Latv.* **1:** 4 (holotype) RIG. in Latvia in Lacu Usma (Sinus Brūždanga) epiphytic on *Phragmites*, Aug. 20, 1925.

The populations and type were measured for cell diameter and length, rhizoid diameter

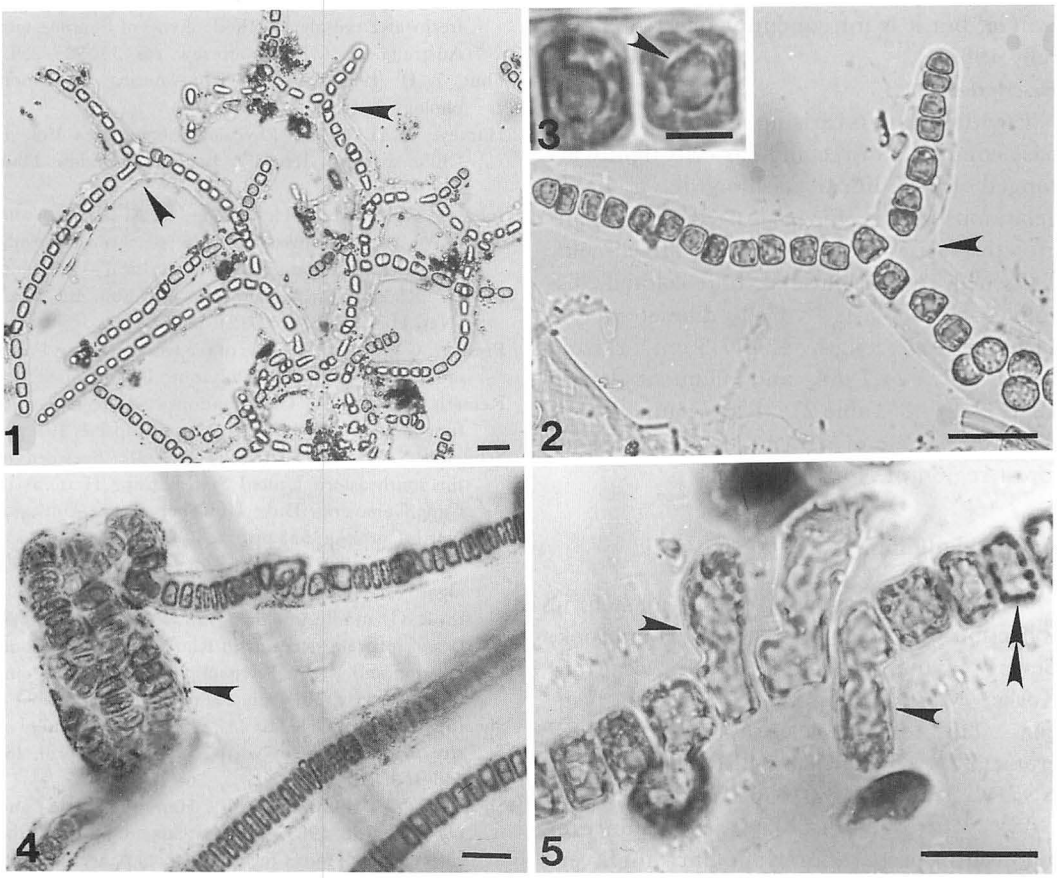


Fig. 1-3. *Chroodactylon ornatum* (NY114). Scale bar=20 μm except in Fig. 3 where it is 5 μm. 1, complex of pseudofilaments with false branches (arrowheads). 2, linear arrangement of cells in a broad gelatinous matrix with a false branch (arrowhead). 3, Two cells showing the axial, stellate chloroplast with prominent, central pyrenoid (arrowhead). Figs. 4-5. *Kyliniella latvica* (from Sheath 1984 with permission). Scale bar=20 μm. 4, Pseudofilament arising from discoidal base (arrowhead). 5, Densely packed cells with parietal, discoidal chloroplasts (double arrowhead); some cells produce rhizoidal outgrowths (arrowheads).

and length, and filament diameter. Each population was examined in replicates of ten with the exception of RI A3 from which only 4–8 measurements could be made.

The two populations and the type specimen had similar ranges of morphometric features except for a significantly larger cell length in the type ($p < 0.05$). However, this feature is quite variable and cannot be used alone to distinguish taxa. Therefore, we conclude that the North American populations are synonymous with *K. latvica*.

Kyliniella latvica appears to be quite rare in that it has been found in only two streams out of 1,000 surveyed from North America (Sheath and Cole 1992). Worldwide, it has also been reported from Austria, France and Latvia, but it is infrequently collected (Bourrelly 1985).

Description

Pseudofilaments arising from a discoidal base composed of rectangular cells tightly arranged in a linear fashion in a broad gelatinous matrix (Figs. 4–5). Rhizoidal outgrowths arise from cells for attachment. Cells with several parietal, blue-colored, discoidal chloroplasts. Cell diameter 7.4–14.8 μm, cell length 4.9–17.3 μm, rhizoid length 17.3–24.7 μm and filament length 12.4–32.1 μm (Table 2). Rare component of the littoral zone of streams in the deciduous forest region of North America.

Acknowledgments

We thank the following people for help in collection of specimens: Julie Hambrook, Beverly Hymes, Donald Kaczmarczyk, Mary Koske, Mary Morison and Roxanna Viquez. This research was supported by NSF grants BSR 8607092 and BSR 8906986 and NSERC grant OGP 0105629 to R.G.S. and OGP 2016 to Charles Davis. Assistance in manuscript preparation from Christine Everson is appreciated. Type specimens were kindly loaned by PC and RIG and an additional specimen from the Lewis H. Flint collection from Louisiana State University was made available by Russell Chapman.

References

Abbott, I. A. and Hollenberg, G. J. 1976. Marine algae of California. Stanford University Press, Stanford, California, 827 pp.

Agardh, C. A. 1824. Systema Algarum. Literis Berlingianis, Lund, 312 pp.

Basson, P. W. 1979. Marine algae of the Arabian Gulf coast of Saudi Arabia. Bot. Mar. **22:** 65–82.

Bourrelly, P. 1985. Les algues d'eau douce, initiations à la systematique, Vol. 3. Les Algues bleues et rouges, Les Eugléeniens, Peridiniens et Cryptomonadines 2nd ed. N. Boubée, Paris, 606 pp.

Chapman, V. J. 1961. The marine algae of Jamaica. Part I. Myxophyceae and Chlorophyceae. Bull. Inst. Jamaica Sci. Ser. **12:** 201 pp.

Daily, W. A. 1943. First reports for the algae *Borzia, Aulosira* and *Asterocytis* in Indiana. Butler Univ. Bot. Stud. **6:** 84–86.

Entwisle, T. J. and Kraft, G. T. 1984. Survey of the freshwater red algae (Rhodophyta) of Southeastern Australia. Aust. J. mar. freshw. res. **35:** 213–259.

Flint, L. H. 1953. *Kyliniella* in America. Phytomorphology **3:** 76–80.

Harvey, W. H. 1848. Phycologia Britannica Vol. 4, Chlorospermae. Reeve & Benham, London, Plate 213.

John, D. M., Price, J. H., Maggs, C. A. and Lawson, G. W. 1979. Seaweeds of the western coast of tropical Africa and adjacent islands: a critical assessment. III. Rhodophyta (Bangiophyceae). Bull. Br. Mus. (Nat. Hist.). bot. ser. **7(2):** 69–82.

Prescott, G. W. 1962. Algae of the western Great Lakes area. Wm. C. Brown, Dubuque, Iowa, 977 pp.

Reinsch, P. F. 1875. Contributiones ad algologiam et fungologiam Vol. 1. T. O. Weigel, Lipsiae, 103 pp.

Schneider, C. W. and Searles, R. B. 1991. Seaweeds of the southeastern United States: Cape Hatteras to Cape Canaveral. Duke University Press, Durham, North Carolina, 553 pp.

Sheath, R. G. 1984. The biology of freshwater red algae. *Prog. Phycol. Res.* **3:** 89–157.

Sheath, R. G. and Burkholder, J. M. 1985. Characteristics of softwater streams in Rhode Island. II. Composition and seasonal dynamics of macroalgal communities. Hydrobiologia **128:** 109–118.

Sheath, R. G. and Cole, K. M. 1992. Biogeography of stream macroalgae in North America. J. Phycol. **28:** 448–460.

Sheath, R. G., Hamilton, P. B., Hambrook, J. A. and Cole, K. M. 1989. Stream macroalgae of the eastern boreal forest region of North America. Can. J. Bot. **67:** 3553–3562.

Sheath, R. G. and Hymes, B. J. 1980. A preliminary investigation of the fresh water algae in streams of southern Ontario, Canada. Can. J. Bot. **58:** 1295–1318.

Sheath, R. G. and Morison, M. O. 1982. Epiphytes on

Cladophora glomerata in the Great Lakes and St. Lawrence Seaway with particular reference to the red alga *Chroodactylon ramosum* (=*Asterocytis smargdina*). J. Phycol. **18:** 385–391.

Skuja, 1926. Eine neue Süsswasserbangiacee *Kyliniella latvica* n.g., n. sp. Acta Horti Bot. Univ. Latv. **1:** 1–5.

Taft, C. E. and Taft, C. W. 1971. The algae of western Lake Erie. Bull. Ohio biol. survey New Ser. **4:** 1–189.

Taylor, W. R. 1957. Marine algae of the northeastern coast of North America. University of Michigan Press, Ann Arbor,Michigan, 509 pp.

Vis, M. L. and Sheath, R. G. 1992. Systematics of the freshwater red algal family Lemaneaceae in North America. Phycologia **31:** 164–179.

Morgan L. Vis・Robert G. Sheath：*Chroodactylon* と *Kyliniella*（紅藻；ヒナノリ目）の北アメリカの河川における分布と系統分類

北米の1000地点にわたる淡水藻の分布調査の結果に基づき，紅藻 *Chroodactylon* と *Kyliniella* の分布と形態学的観察の結果につき報告する。*Chroodactylon ornatum* はマニトバ北部，オンタリオ南部，ニューヨーク西部，アリゾナ南部から7つの個体群が採集された。北米の材料とタイプ標本の観察から *Chroodactylon ornatum* と *C. smaragdinum* は同種であると結論した。*Kyliniella* はロードアイランドとニューハンプシャーの2地点でのみ採集された。

(Department of Biology, Memorial University of Newfoundland, St. John's, Newfoundland, Canada A1B 3X9)

(*Received January 28, 1993, Accepted June 4, 1993*)

Jpn. J. Phycol. (Sôrui) 41: 243–246, September 20, 1993

Taku Misonou, Mamiko Seto and Takeshi Nitta: Phylogenetic relationship of three *Bryopsis* species (Derbesiales, Chlorophyta) based on 16S ribosomal RNA sequences

Key Index Words: Bryopsis *(Chlorophyta)—chloroplast 16S rRNA—molecular evolution—phylogeny—sequence divergence.*
Taku Misonou and Mamiko Seto, Department of Biology, Faculty of Education, Yamanashi University, Kofu, Yamanashi, 400 Japan
Takeshi Nitta, Laboratory of Biology, Tokyo University of Agriculture and Technology, Fuchu, Tokyo, 183 Japan

We attempted a molecular phylogenetic approach based on small-subunit rRNA (ribosomal RNA) sequences in three members of genus *Bryopsis*. In this study, we targeted the chloroplast 16S rRNA molecule for the partial sequencing. Their phylogenetic relationship previewed by organelle DNA RFLP (Restriction Fragment Length Polymorphism) analysis (Misonou *et al.* 1989) and some characteristics of *Bryopsis* 16S rRNA were discussed. The algae used were *Bryopsis maxima* Okamura, *B. plumosa* (Hudson) C. Agardh, and *B.* sp. (Misonou *et al.* 1989). The first was collected at Choshi in Chiba, on the Pacific coast, the second at Yokosuka in Kanagawa, and the third at Futtsu in Chiba, from November 1986 to May 1990. The second and third sites are on Tokyo Bay.

Chloroplasts were isolated from algal thalli in an alkaline buffer containing EDTA (Misonou *et al.* 1989, Ishihara *et al.* 1992). Total nucleic acids were extracted by the chloroform/phenol method from this fraction and RNAs were purified with the DNase I treatment. 16S rRNA partial sequences of each species were determined directly with dideoxynucleotide chain termination method using reverse transcriptase accrding to Lane *et al.* (1985). The three primers used are complimentary to the *Escherichia coli* 16S rRNA highly conserved region. Their positions and sequences are I: 357–342 (5′CTGCTGCCTCCCGTAGOH3′), II: 1242–1227 (5′CCATTGTAGCACGTGTOH3′) and III: 1510–1495 (5′GGCTACCTTGTTACGAOH3′) according to *E. coli* nomenclature (Sawada *et al.* in press).

The sequences read with each primer were 466 bases and aligned with the homologous sequence of *Chlorella vulgaris* strain 211-11b 16S rRNA (Neefs *et al.* 1990) as an outgroup in Fig. 1. *Bryopsis* 16S rRNAs show intrageneric sequence divergence between each species (Fig. 1). It might be considered that the speciation of these organisms is an ancient incident or the rate of 16S rRNA molecule evolution in this genus is faster than that of higher plant.

Of the total 466 bases, 443 bases were comparable to each other in sequence. The remaining of 23 bases could not be identified by anomalous bands across the lanes on the gel owing to the nucleotide modification. While the sequences with primer II were homologous, the primer I region of *B. plumosa* has 1 deletion (No. 103 in Fig. 1 I), and sequences with primer III were heterogeneous (Fig. 1). In this region, the chain termination reaction continued only up to 100 bases with 19 anomalous bands. It seems that primer III complimentary to *E. coli* sequence anneals to *Bryopsis* RNAs in low extent due to the sequence heterogeneity, and/or this region is abundant in modified nucleotides.

The genetic distances of these three regions were calculated according to Kimura's 2 parameter method (Kimura 1980) and presented in Table 1. The primer III region of 3 *Bryopsis* is not only heterogeneous each other but also shows low homology with *Chlorella* sequence. Moreover, no homologous sequence with this region was found in GEN-

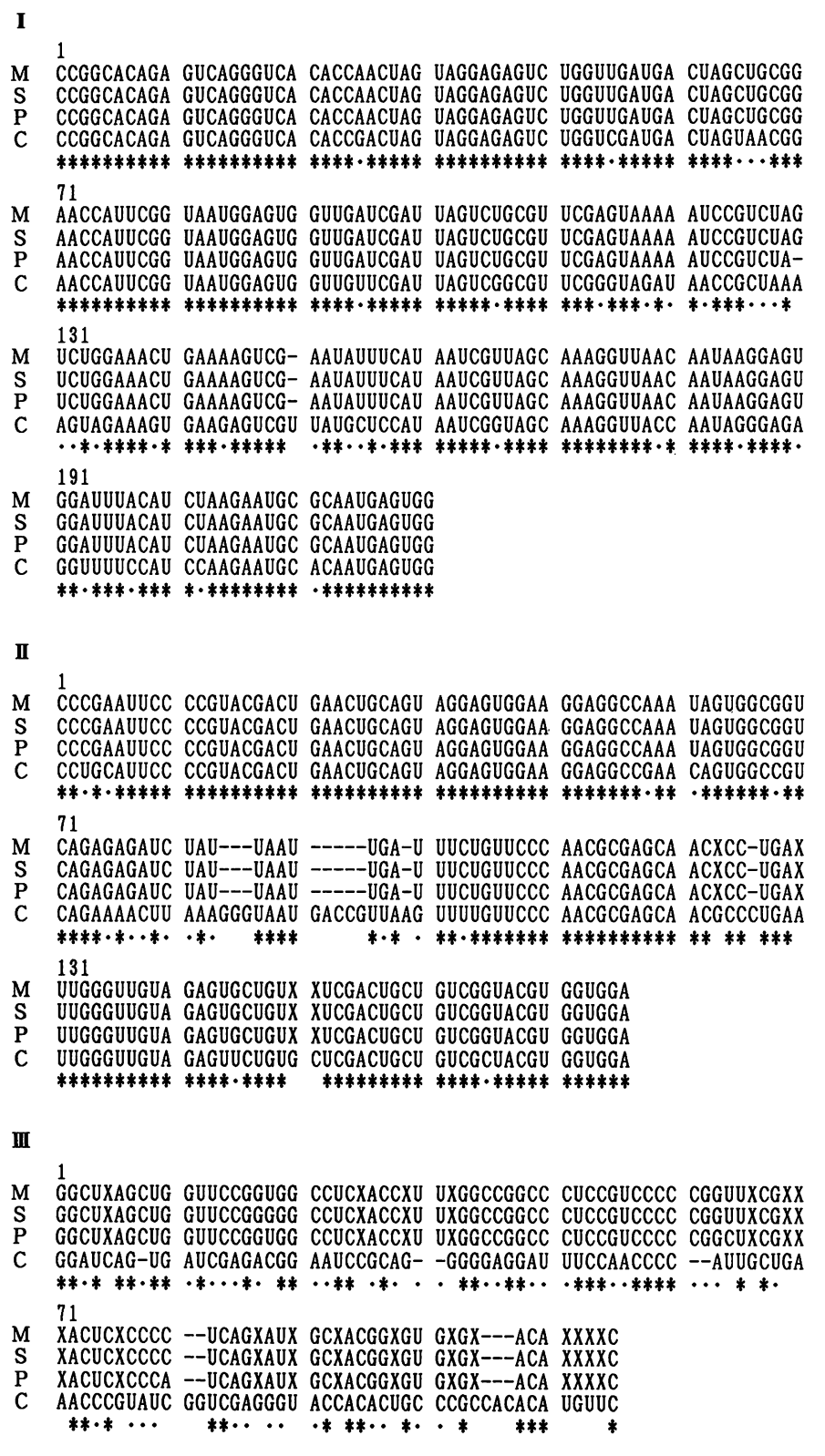

I
1
M CCGGCACAGA GUCAGGGUCA CACCAACUAG UAGGAGAGUC UGGUUGAUGA CUAGCUGCGG
S CCGGCACAGA GUCAGGGUCA CACCAACUAG UAGGAGAGUC UGGUUGAUGA CUAGCUGCGG
P CCGGCACAGA GUCAGGGUCA CACCAACUAG UAGGAGAGUC UGGUUGAUGA CUAGCUGCGG
C CCGGCACAGA GUCAGGGUCA CACCGACUAG UAGGAGAGUC UGGUCGAUGA CUAGUAACGG
71
M AACCAUUCGG UAAUGGAGUG GUUGAUCGAU UAGUCUGCGU UCGAGUAAAA AUCCGUCUAG
S AACCAUUCGG UAAUGGAGUG GUUGAUCGAU UAGUCUGCGU UCGAGUAAAA AUCCGUCUAG
P AACCAUUCGG UAAUGGAGUG GUUGAUCGAU UAGUCUGCGU UCGAGUAAAA AUCCGUCUA-
C AACCAUUCGG UAAUGGAGUG GUUGUUCGAU UAGUCGGCGU UCGGGUAGAU AACCGCUAAA
131
M UCUGGAAACU GAAAAGUCG- AAUAUUUCAU AAUCGUUAGC AAAGGUUAAC AAUAAGGAGU
S UCUGGAAACU GAAAAGUCG- AAUAUUUCAU AAUCGUUAGC AAAGGUUAAC AAUAAGGAGU
P UCUGGAAACU GAAAAGUCG- AAUAUUUCAU AAUCGUUAGC AAAGGUUAAC AAUAAGGAGU
C AGUAGAAAGU GAAGAGUCGU UAUGCUCCAU AAUCGGUAGC AAAGGUUACC AAUAGGGAGA
191
M GGAUUUACAU CUAAGAAUGC GCAAUGAGUGG
S GGAUUUACAU CUAAGAAUGC GCAAUGAGUGG
P GGAUUUACAU CUAAGAAUGC GCAAUGAGUGG
C GGUUUUCCAU CCAAGAAUGC ACAAUGAGUGG
II
1
M CCCGAAUUCC CCGUACGACU GAACUGCAGU AGGAGUGGAA GGAGGCCAAA UAGUGGCGGU
S CCCGAAUUCC CCGUACGACU GAACUGCAGU AGGAGUGGAA GGAGGCCAAA UAGUGGCGGU
P CCCGAAUUCC CCGUACGACU GAACUGCAGU AGGAGUGGAA GGAGGCCAAA UAGUGGCGGU
C CCUGCAUUCC CCGUACGACU GAACUGCAGU AGGAGUGGAA GGAGGCCGAA CAGUGGCCGU
71
M CAGAGAGAUC UAU---UAAU -----UGA-U UUCUGUUCCC AACGCGAGCA ACXCC-UGAX
S CAGAGAGAUC UAU---UAAU -----UGA-U UUCUGUUCCC AACGCGAGCA ACXCC-UGAX
P CAGAGAGAUC UAU---UAAU -----UGA-U UUCUGUUCCC AACGCGAGCA ACXCC-UGAX
C CAGAAAACUU AAAGGGUAAU GACCGUUAAG UUUUGUUCCC AACGCGAGCA ACGCCCUGAA
131
M UUGGGUUGUA GAGUGCUGUX XUCGACUGCU GUCGGUACGU GGUGGA
S UUGGGUUGUA GAGUGCUGUX XUCGACUGCU GUCGGUACGU GGUGGA
P UUGGGUUGUA GAGUGCUGUX XUCGACUGCU GUCGGUACGU GGUGGA
C UUGGGUUGUA GAGUUCUGUG CUCGACUGCU GUCGCUACGU GGUGGA
III
1
M GGCUXAGCUG GUUCCGGUGG CCUCXACCXU UXGGCCGGCC CUCCGUCCCC CGGUUXCGXX
S GGCUXAGCUG GUUCCGGGGG CCUCXACCXU UXGGCCGGCC CUCCGUCCCC CGGUUXCGXX
P GGCUXAGCUG GUUCCGGUGG CCUCXACCXU UXGGCCGGCC CUCCGUCCCC CGGCUXCGXX
C GGAUCAG-UG AUCGAGACGG AAUCCGCAG- -GGGGAGGAU UUCCAACCCC --AUUGCUGA
71
M XACUCXCCCC --UCAGXAUX GCXACGGXGU GXGX---ACA XXXXC
S XACUCXCCCC --UCAGXAUX GCXACGGXGU GXGX---ACA XXXXC
P XACUCXCCCA --UCAGXAUX GCXACGGXGU GXGX---ACA XXXXC
C AACCCGUAUC GGUCGAGGGU ACCACACUGC CCGCCACACA UGUUC

Table 1. Genetic distances ($\times 10^{-3}$ Knuc) of three regions between each of three *Bryopsis* and *Chlorella* 16S rRNA.

region	M-S	M-P	S-P	M-C	S-C	P-C
I	0.0	0.0	0.0	171.4	171.4	166.1
II	0.0	0.0	0.0	113.5	113.5	113.5
III	12.5	25.1	12.3	681.5	673.0	756.5
total	2.3	4.5	6.8	213.9	213.7	217.4

M: *Bryopsis maxima*; S: *B.* sp.; P: *B. plumosa*; C: *Chlorella vulgaris*.

BANK database. These results show that *Bryopsis* 16S RNA sequences around the position 1400-1500 are not conservative even in the primer III region considered homologous from bacteria to higher plant chloroplast. This may suggest that these algae occupy a unique position in plant kingdom.

From these genetic distances, a phylogenetic tree was inferred in Fig. 2 by UPGMA (Sokal and Michener 1958), and NJ method (Saitou and Nei 1987) with bootstrap confidence limits using the Clustal V computer program (Higgins 1991). In both trees, *B.* sp. is distinguished from other 2 species. It is shown that this alga, having regarded as an intraspecific variation of *B. plumosa*, is an independent species and has rather far relationship with *B. plumosa*. In the UPGMA tree, *B.* sp. is clustered with *B. maxima* as previous RFLP analysis (Misonou *et al.* 1989). On the other hand, although the bootstrap confidence interval is not sufficiently high, *B.*. sp. is separated from *B. maxima*—*B. plumosa* cluster in the NJ tree. In the case of this study, UPGMA tree seems to be more reliable for the close relationship of these algae. More sequence informations may be required for further phylogenetic analyses of *Bryopsis*.

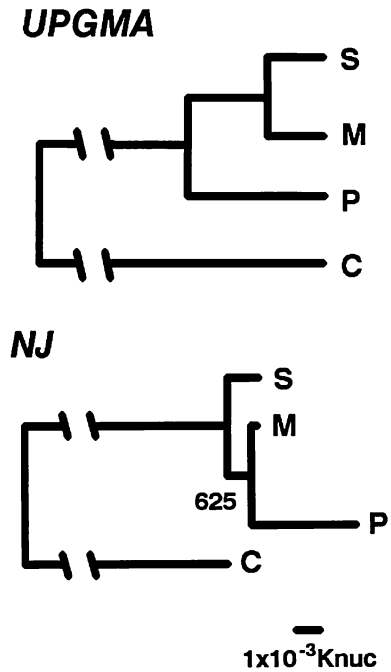


Fig.2. Phylogenetic trees drawn with genetic distances of three *Bryopsis* and *Chlorella* 16S rRNA partial sequences using 2 tree construction methods. M, *Bryopsis maxima*; S, *B.* sp.; P, *B. plumosa*; C, *Chlorella vulgaris* 211-11b. The number under the branch in NJ tree is the confidence interval for nodes based upon 1,000 bootstrap samples.

Acknowledgments

We are much indebted to Dr. H. Oyaizu, The University of Tokyo for the primers and to Dr. Kohbara, Senshu University for a useful suggestion pertaining to *Bryopsis* classification. We also thank Prof. K. Wakabayashi, Yamanashi Medical College for his kind help to this study.

This research was supported by a Grant-in-Aid from the Ministry of Education, Science and Culture of Japan.

References

Higgins, D. G. 1991. Clustal V Documentation. EMBL.

Ishihara, J., Pak, J. Y., Fukuhara, T. and Nitta, T. 1992. Association of particles that contain double-stranded RNAs with algal chloroplasts and mitochondria. Planta **187**: 475-482.

Kimura, M. 1980. A simple method for estimating evolutionary rates of base substitutions through com-

Fig. 1. Partial sequences of three *Bryopsis* 16S rRNA. The sequences determined with three primers are aligned. M, *Bryopsis maxima*; S, *B.* sp.; P, *B. plumosa*; C, *Chlorella vulgaris* 211-11b. Hyphens and Xs show deletions and uncleotides that could not be identified, respectively. Asterisks are the homologous nucleotides with three *Bryopsis* and *Chlorella*. Dots are the nucleotides homologous within three *Bryopsis* while not with *Chlorella*.

parative studies of nucleotide sequences. J. Mol. Evol. **16:** 111–120.

Lane, J., Pace, B., Olsen, G. J., Stahl, D. A., Sogin, M. L. and Pace, N. R. 1985. Rapid determination of 16S ribosomal RNA sequences for phylogenetic analyses. Proc. Natl. Acad. Sci.USA **82:** 6955–6959.

Misonou, T., Ishihara, J., Pak, J. Y. and Nitta, T. 1989. Restriction endonuclease analysis of chloroplast and mitochondrial DNAs from *Bryopsis* (Derbesiales, Chlorophyta). Phycologia **28:** 422–428.

Neefs, J-M., Van de Peer, Y., Hendriks, L. and De Wachter, R. 1990. Compilation of small ribosomal subunit RNA sequences. Nuc. Acid Res. **18:** 2237–2317.

Saitou, N. and Nei, M. 1987. The neighbor-joining method: A new method for reconstructing phylogenetic trees. Mol. Biol. Evol. **4:** 406–425.

Sawada, H., Ieki, H., Oyaizu, H. and Matsumoto, S. 1993. Proposal for rejection of *Agrobacterium tumefaciens* and revised descriptions for the genus *Agrobacterium* and *Agrobacterium radiobacter* and *Agrobacterium rhizogenes*. Int. J. Syst. Bacteriol. (in press)

Sokal, R. R. and Michener, C. D. 1958. A statistical method for evaluating systematic relationships. Univ. Kansas Sci. Bull. **28:** 1409–1438.

御園生　拓*・瀬戸麻美子*・新田　毅**：16S リボソーム RNA 塩基配列による 3 種のハネモ属藻（ツユノイト目，緑藻植物門）の系統関係

大型緑藻ハネモ属の系統を明らかにするために，葉緑体 16S rRNA（リボソーム RNA）の塩基配列を比較し解析することを試みた。逆転写酵素を利用したサンガー反応のプライマーには 16S rRNA に特異的な保存配列を 3 種類用い，計466塩基を読んだ。ミナトハネモ (*Bryopsis* sp.) は独立した種であり，ハネモ (*B. plumosa*) やオオハモネ (*B. maxima*) とは遺伝的に離れているという結果を得た。また，これらのハネモ 16S rRNA の塩基 No. 1400～1500に相当する部分は既知の生物の配列との相同性が低く，これらが他の植物と系統的に離れている可能性が示唆された。（*400 甲府市武田 4　山梨大学教育学部生物学教室，**183 府中市幸町 3　東京農工大学一般教育部生物）

(*Received April 7, 1993, Accepted July 7, 1993*)

Jpn. J. Phycol. (Sôrui) 41: 247–252, September 20, 1993

Inderdeep Kaur and M. R. Vijayaraghavan: Histogenesis and conceptacle organization in *Sargassum vulgare* C. Agardh (Fucales, Phaeophyta).

Key Index Words: conceptacle organization—Fucales—Phaeophyta—Sargassum vulgare—Sulphated polysaccharides.
Inderdeep Kaur and M. R. Vijayaraghavan, Department of Botany, University of Delhi, Delhi 110007, India

Sargassum vulgare C. Agardh has a three sided apical cell lodged in the apical groove. The derivatives cut by this cell lead to the formation of meristoderm, cortex and medulla. During the reproductive period, unisexual conceptacles are borne in the receptacles. The growth of the receptacle takes place by means of pyramidal truncate initial that lies at the base of a groove. The conceptacles develop near the receptacle initial from the meristoderm layer of the receptacle and later are embedded in the cortex of the receptacle.

The conceptacle development in Fucales is known (Moss 1965, 1967, 1970; McCully 1966, 1968; Critchley et al. 1991) but a correlative study on histogenesis and histochemistry is lacking. The present communication in *Sargassum vulgare* deals with histogenesis and histochemistry of the apical cell, the receptacle and the conceptacle initials and the hairs in cryptostomata.

The plants of *Sargassum vulgare* were collected during the low tide periods from Port Okha, Gujarat, through the months of January, February and November 1987-89. The desired plant parts were fixed in 10% v/v acrolein at 4°C for 24 h, washed thrice in distilled water and postfixed in 1% $HgCl_2$ for 12 h to stabilize phenolic compounds. The material was again washed twice with distilled water to remove the traces of fixatives; dehydrated through methoxy-ethanol (3 changes, 24 h); ethanol (1 change, 24 h), propanol (1 change, 24 h) and n-butanol (1 change, 24 h). The material was infiltrated and embedded in glycol methacrylate (Feder and O'Brien 1968) and sectioned on a Spencer rotary microtome fitted with a locally made adaptor to hold glass knives. Two micron thick sections were cut and serially transferred to small drops of distilled water kept on precleaned and dried slides and later stained with PAS reagent (Feder and O'Brien 1968) and TBO (McCully 1966).

For scanning electron microscopy, the selected plant parts were fixed in 4% formaldehyde, dehydrated in a graded acetone series, critical point dried and scanned for topographical detailes.

Apical cell—Organization of the vegetative apex: The plants grow by means of a three sided apical cell lodged in a groove. This cell in transverse section, appears triangular (Fig. 1) and in longitudinal section more or less biconvex (Fig. 2). The promeristem of this taxon is identical in structure and behaviour as reported for other taxa of this order. The mucilage that fills the apical groove stains reddish-violet with TBO and magenta with PAS reagent. This indicates a mixture of sulphated and carboxylated polysaccharides (Fig. 2). The apical cell walls are thin and stain identical to the mucilage.

The apical cell cuts off derivatives parallel to all its sides; these have wavy cell walls and undergo divisions and enclose a few intercellular spaces (Fig. 3). The apical cell cytoplasm reveals small vacuoles and few sulphated polysaccharides; large, polarized nucleus with prominent nucleoli (Fig. 3). The meristoderm cells near the cavity are gorged with physodes (Fig. 3).

Receptacle and conceptacle development: During the reproductive phase, the plants bear abundant receptacles. The growth of the receptacle also takes place by means of a pyramidal initial that lies at the base of the groove (Fig. 4). One of the cells in the

meristoderm layer of the receptacle becomes large and functions as the conceptacle initial (Fig. 5). The cell wall of conceptacle initial contains a mixture of carboxylated and sulphated polysaccharides but the cytoplasm reveals negligible polysaccharides. The initial undergoes an unequal transverse division (Fig. 6). The upper small cell elongates and points towards the exterior and later becomes the tongue-cell which has a distinct cap that is rich in sulphated polysaccharides. The tongue-cell eventually degenerates and the lysate contributes to the ostiolar plug material (Fig. 8). The lower large cell is the mother cell of the conceptacle wall (Fig. 7) and undergoes divisions.

The young conceptacle is oval to pear-shaped and has a narrow neck (Fig. 9). As the plants reach sexual maturity, oogonia (Fig. 9) and antheridia as well as the associated paraphyses differentiate from the conceptacle wall and mature inside the cavity. The ostiole further narrows but concomitantly the base widens. The receptacles become spherical and accommodate both developing and developed sex organs.

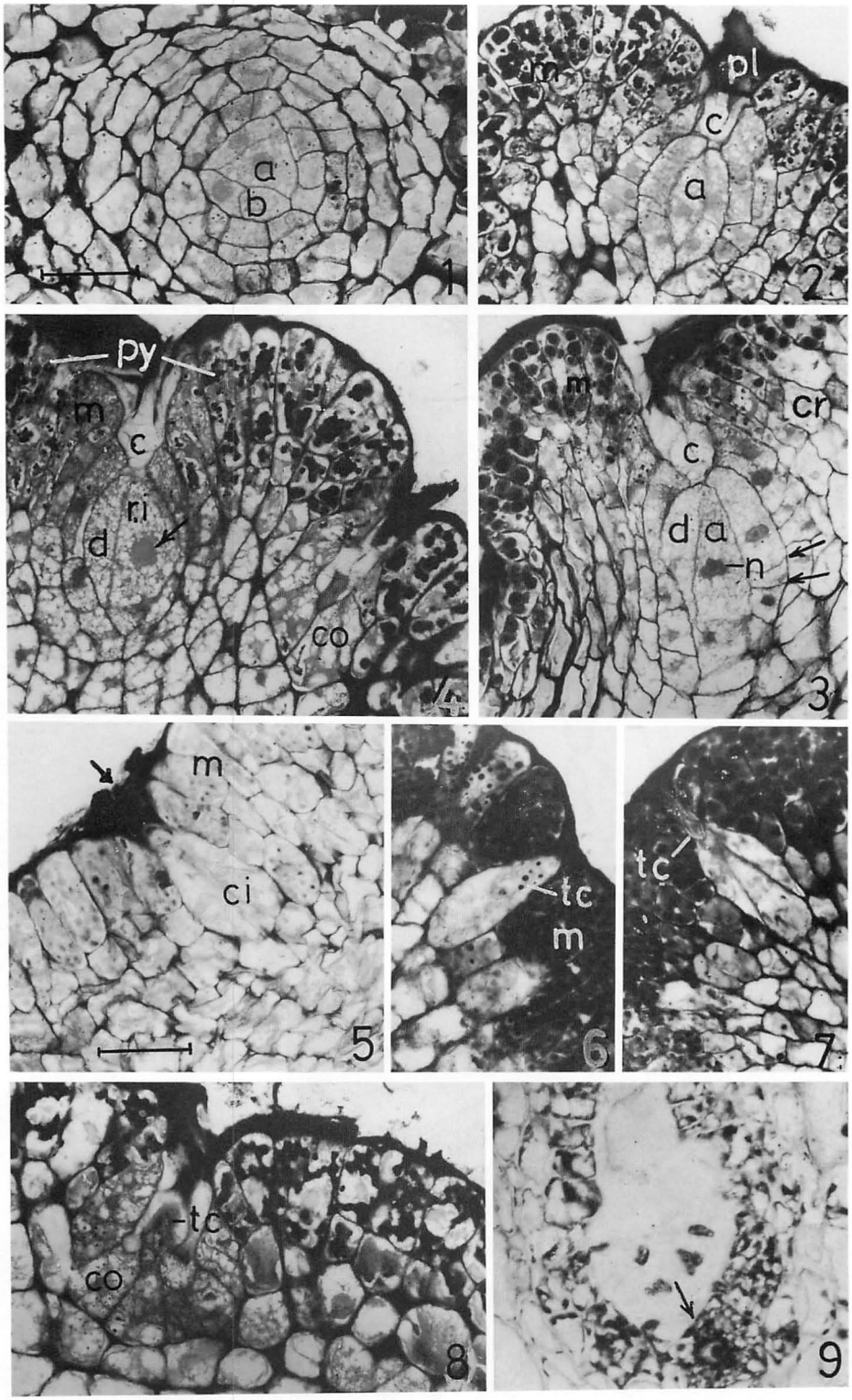
The mature conceptacle wall cell's cytoplasm reveals abundant vacuoles and physodes. At the time of gamete release, the wall cells are vacuolate. The paraphyses rich in physodes are intermingled with reproductive organs.

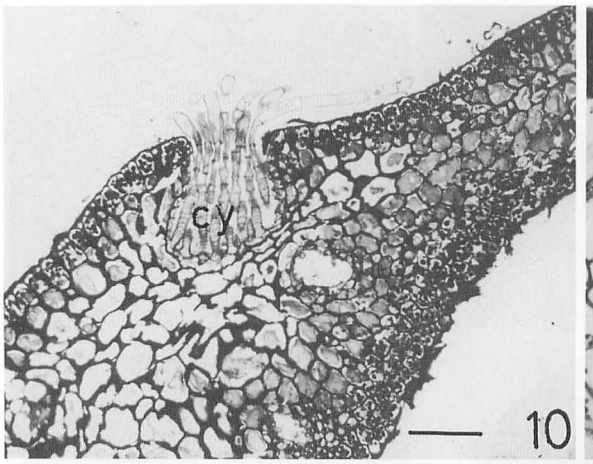
Cryptostomata: Besides the fertile conceptacles, sterile conceptacles (cryptostomata) occur both on the vegetative (Fig. 13) and the reproductive (Fig. 10) thalli. Cryptostomata appear as dots, and are freely scattered on the vegetative regions. In the reproductive regions, they coexist with the conceptacles. The cryptostomata show identical developmental pattern as the fertile conceptacles. The cryptostomata have abundant hairs which show only trichothallic growth (Figs. 12, 14). During the initial stages, the hairs reveal moderate polysaccharides and few physodes (Fig. 11). The nuclei are large. A mature hair has three regions. The lowermost region has meristematically active basal cell; the middle region has four to six recently formed short cells which have not yet elongated and the upper region has hairs which are partly within and partly outside the cavity (Fig. 14). The portions of hairs that emerge out, are replete with physodes, numerous vacuoles and moderate polysaccharides (Fig.15).

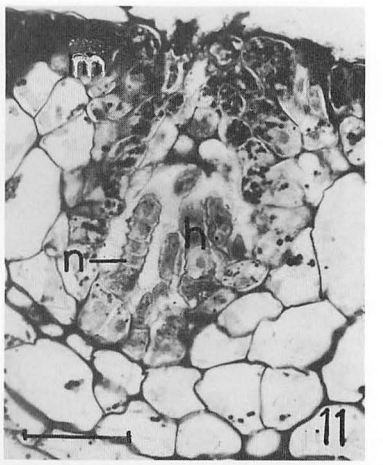
In *Sargassum vulgare*, the apical cell and the conceptacle initials are deep seated occupying the base of the cavity that is filled with a mixture of sulphated and carboxylated polysaccharides which protect the thalli against desiccation when the plants are exposed

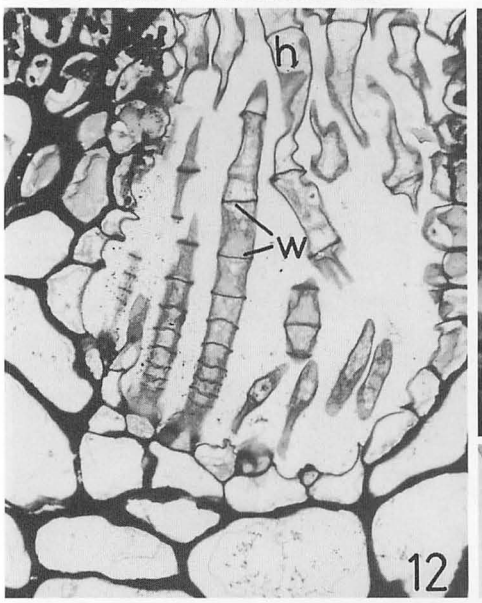
Figs. 1–9. *Sargassum vulgare*, (Figs. 1–9 TBO stained, Scale bars=9.4 μm). Fig. 1. Transverse section of a vegetative thallus apex showing a triangular apical cell (a) with three cutting faces. The derivatives (d) result in formation of a promeristem. Figs. 2, 3. Longitudinal sections through the apical regions showing biconvex apical cells (a) lodged in cavity (c) filled with polysaccharide plug materials (pl). The apical cell cytoplasm bears abundant vacuoles, a few polysaccharides and a prominent nucleus (n). The derivatives (d) undergo divisions to form meristoderm (m), cortex (cr) and medulla (not seen in figure). Fig. 4. Longitudinal section through apical region of the receptacle showing a cavity (c) at the base of which is the receptacle initial (ri) with distinct nucleus (arrow). The derivatives (d) possess wavy walls. The meristoderm cells (m) are rich in physodes (py). A developing conceptacle (co) is also seen. Fig. 5. Conceptacle initial (ci) differentiates from amongst the meristoderm (m) cells. The initial is lodged in a cavity filled with polysaccharides (arrow). Fig. 6. The initial undergoes unequal transverse division resulting in tongue-cell (tc) and a basal cell. Figs. 7, 8. The basal cell divides resulting in conceptacle (co) wall formation. In 8, a polysaccharide cap covers the tongue-cell (tc). Fig. 9. The oogonial (arrow)/antheridial initials differentiate from the conceptacle wall cells.

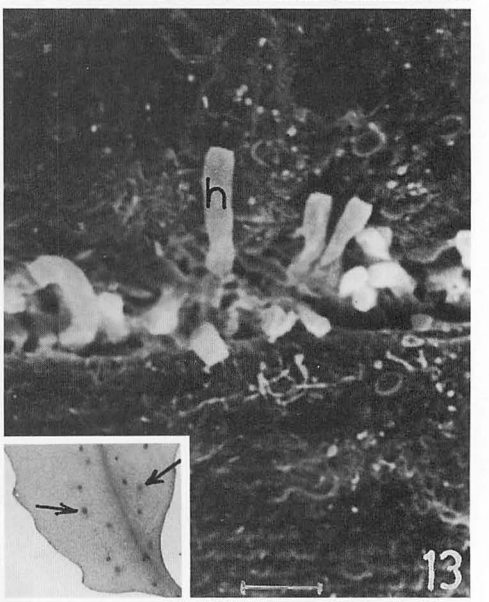
Figs. 10–15. *Sargassum vulgare*. Figs. 10–11, 14, 15. TBO stained. Fig. 10. Longitudinal section of a receptacle passing through a cryptostoma (cy) showing a profuse growth of hairs. Scale bar=25 μm. Fig. 11. Cryptostoma cut in a longitudinal section. The young hairs (h) reveal cells that are small, rectangular with prominent nuclei (n) occupying the major portion of the cytoplasm. Scale bar=9.4 μm. Fig. 12. In the hairs (h) a few lower cells remain small whereas those towards the ostiole elongate. The cell walls (w) stain intense megenta with PAS reagent. Scale bar=9.4 μm. Fig. 13. Scanning electron micrograph of the leaf to show top view of cryptostoma with emergent hairs (h). The inset shows freely distributed cryptostomata (arrows) on a leaf portion. Scale bar=6.8 μm. Figs. 14, 15. Mature cryptostomata showing hairs (h) that have elongated. These reveal cytoplasm with a few vacuoles (v) and nucleus (n). The portion of hairs that emerges out is highly vacuolate (v). Scale bar=9.4 μm.

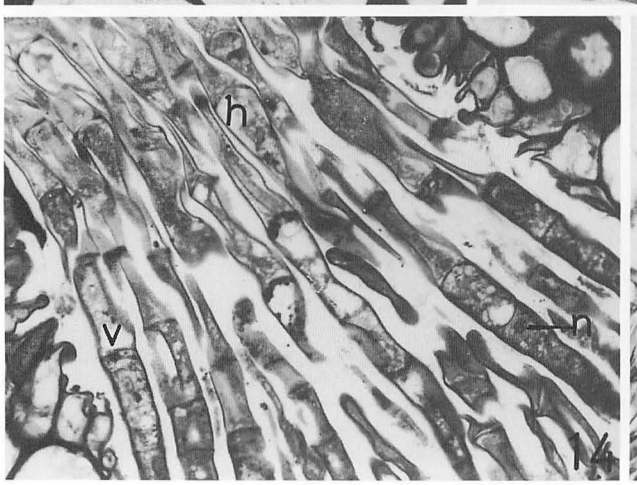

a
b
1
pl
c
a
2
py
m
c
ri
d
co
4
m
cr
c
d a
n
3
m
ci
5
tc
m
6
tc
7
tc
co
8
9

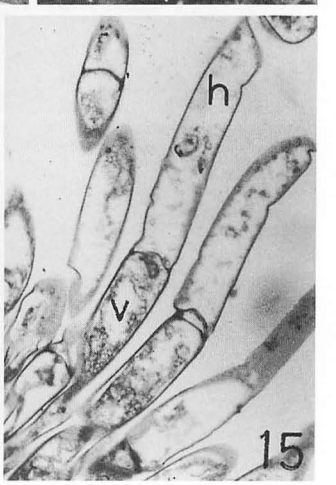

cy
10


m
n
h
11


h
w
12


h
13


h
v
n
14


h
v
15

(Vijayaraghavan and Kaur, 1991).

Tahara (1940) in *Sargassum horneri* (Turner) C. Agardh and Fensholt (1955) in *Cystophyllum* sp. referred to the formation of a "conceptacle-stopper" through the action of a tongue-cell. Critchley et al. (1991). in *S. heterophyllum* observed sulphated polysaccharides in the ostiole plug, and these are identical to the paraphysial secretions. In *S. vulgare* (present work) the ostiole plug of the female conceptacle accrues materials from: 1) tongue-cell lysis, 2) paraphyses secretions, 3) conceptacle wall cell secretions (see also Vijayaraghavan and Kaur 1991). In contrast the male conceptacle lacks ostiole plug and the conceptacle closure occurs by adpressing meristoderm cells (Vijayaraghavan and Kaur 1992).

Further, in *Sargassum vulgare* the ostiole closure is a presaged and coordinated phenomenon persisting until the oogonia/antheridia mature and lyse to pave way for the eventual release of respective reproductive bodies.

The distribution pattern morphology of the cryptostomata and the conceptacles on the thallus surface is noteworthy. The conceptacles are more closely placed than the cryptostomata. The young conceptacles occupy very little space in the apex of a receptacle but further down the developing, flask-shaped, conceptacles require more space. The cryptostomata are scattered on both the vegetative and reproductive structures and are bereft of plug materials. The hairs in cryptostomata show trichothallic growth. This type of basal meristem is found in many phaeophycean taxa belonging to Ectocarpales, Desmarestiales, Tilopteridales, Cutlariales, and Laminariales. (Fritsch 1945). Thus, the hairs in Fucales, as in other phaeophycean taxa represent an evolutionary primitive mode of growth. The thallus shows more advanced apical growth.

The occurrence of conceptacles in receptacles and the appearance of cryptostomata on both vegetative and reproductive branches and differential amount of plug materials suggest a line of evolution from plants which discriminately bear scattered conceptacles over the leaf and branch surfaces to those taxa with conceptacles localised on special branches. Certain branches were set apart to bear conceptacles as these conceptacles in other parts of the plant body were rendered sterile and thus changed into cryptostomata (Simons 1906). The present work supports these observations.

The authors thank the two learned referees for their valuable suggestions.

References

Critchley, A. T., Peddemors, V. M. and Pienaar, R. N. 1991. Reproduction and establishment of *Sargassum heterophyllum* (Turner) C. Ag. (Phaeophyceae, Fucales). Br. Phycol. J. **26:** 303–314.

Feder, N. and O'Brien, T. P. 1968. Plant microtechnique: Some principles and new methods. Am. J. Bot. **55:** 123–142.

Fensholt, D. E. 1955. An emendation of the genus *Cystophyllum* (Fucales). Am. J. Bot. **42:** 305–322.

Fritsch, F. E. 1945. The Structure and Reproduction of the Algae. Vol. II. Cambridge University Press, London

McCully, M. E. 1966. Histological studies on the genus *Fucus*. 1. Light microscopy of the mature vegetative plant. Protoplasma **62:** 287–305.

McCully, M. E. 1968. Histological studies on the genus *Fucus*. II. Histology of the reproductive tissues. Protoplasma **66:** 205–230.

Moss, B. L. 1965. Apical dominance in *Fucus vesiculosus*. New Phytol. **64:** 387–392.

Moss, B. L. 1967. The apical meristem of *Fucus*. New Phytol. **66:** 67.

Moss, B. L. 1970. Meristems and growth control in *Ascophyllm nodosum* (L.) Le Jol. New Phytol. **69:** 253–260.

Simons, E. B. 1906. A morphological study of *Sargassum filipendula*. Bot. Gaz. **41:** 161–182.

Tahara, M. 1940. On the development of the conceptacle of *Sargassum, Coccophora* and *Cystophyllum*. Sci. Rep. Tohoku Imp. Univ., **15:** 321–330.

Vijayaraghavan, M. R. and Kaur, I. 1991. Histochemistry and ultrastructure of paraphyses in *Sargassum vulgare* C. Agardh and *S. johnstonii* Setchell & Gardner. Jpn. J. Phycol. **39:** 347–353.

Vijayaraghavan, M. R. and Kaur, I. 1992. Antheridium development and spermatozoid release in *Sargassum vulgare* C. Agardh and *S. johnstonii* Setchell & Gardner. Jpn. J. Phycol. **40:** 325–332.

Inderdeep Kaur・M. R. Vijayaraghavan：*Sargassum vulgare* C. Agardh（褐藻；ヒバマタ目）の組織発生と生殖器巣形成

Sargassum vulgare C. Agardh は三稜形の頂端細胞を頂端部のくぼみに有する。この頂端細胞から切り出された細胞が形成表皮、皮層および髄層を形成する。成熟期には生殖器床に単性の生殖器巣が生じる。生殖器床の発達は頂端のくぼみの基部に位置する先端を切った三角錐形のイニシャルによって起こる。生殖器巣は生殖器床のイニシャルの近くに生殖器床の形成表皮から発達し、後に生殖器床の皮層の中に埋まるようになる。(Department of Botany, University of Delhi, Delhi 110007, India)

(*Received January 6, 1993: Accepted July 11, 1993*)

Jpn. J. Phycol. (Sôrui) 41: 253–274, September 20, 1993

総　説

Taxonomy of the family Batrachospermaceae (Batrachospermales, Rhodophyta)

Shigeru Kumano

Department of Biology, Faculty of Science, Kobe Univesity, Rokko-dai, Nada-ku, Kobe, 657 Japan

Kumano, S. 1993. Taxonomy of the family Batrachospermaceae (Batrachospermales, Rhodophyta). Jpn. J. Phycol. 41: 253–274.

In this review some reappraisals of the classification system of the family Batrachospermaceae are introduced. An aspect of morphological relationships among the sections and the genera and a check list of the hitherto-described 104 taxa of the family Batrachospermaceae are given with taxonomic notes. This review deals with four genera, namely, genus *Batrachospermum* (91 taxa), genus *Sirodotia* (9 species), genus *Tuomeya* (1 species) and genus *Nothocladus* (3 species). The genus *Batrachospermum* (91 taxa) consists of two subgenera, namely, subgenus *Acarposporophytum* (1 species) and subgenus *Batrachospermum* (90 taxa) including 8 section, namely, section *Helminthoidea* (4 taxa), section *Batrachospermum* (18 taxa), section *Setacea* (3 taxa), section *Turfosa* (5 taxa), section *Virescentia* (14 taxa), section (*Hybrida* (3 taxa), section *Aristatae* (8 taxa) including 2 subsections, namely, subsection *Aristatae* and subsection *Macrosporum* and section *Contorta* (35 taxa) including 5 subsections, namely, subsection *Intortum*, subsection *Torridum*, subsection *Procarpum*, subsection *Kushiroense* and subsection *Ambiguum*.

Key Index Words: Batrachospermum—*Batrachospermaceae*—*Rhodophyta*—Nothocladus—Sirodotia—Tuomeya—*taxonomy*

The family Batrachospermaceae (as familia Batrachospermae) was established by C. Agardh (1824) including four genera, namely, the genus *Mesogloia*, the genus *Batrachospermum*, the genus *Thorea* and the genus *Draparnaldia*.

Kuetzing (1857) described *Baileya americana* based on the specimen collected by Bailey. Harvey (1858) established the genus *Tuomeya* and described the same plant under the binomial *Tuomeya fluviatilis* based on the specimen collected by Tuomey and Bailey.

Harvey (1858) treated the family Batrachospermaceae as the order Batrachospermeae, which grouped into two suborders: suborder Batrachospermeae (*Batrachospermum*) and suboder Lemanieae (*Lemanea* and *Tuomeya*), placed in the class Chlorospermeae, "green algae". Rabenhorst (1868) first removed the family Batrachospermaceae together with some other freshwater taxa from "green alage" to the Rhodophyceae.

Kylin (1912) established the genus *Sirodotia* based on the type species, *S. suecica*, then Skuja (1934) established the genus *Nothcladus* based on the type species, *N. nodosus*.

The above-mentioned four genera are delineated primarily on the basis of the development of gonimblast filaments, the size of carpogonia and the vegetative structures of thallus (Kylin 1956).

Recently, some reappraisals were made on the taxonomic frame work of the genus *Batrachospermum* (Necchi 1990a, Compère 1991) and of the family Batrachospermaceae (Necchi & Entwisle 1990).

The aim of this paper is to review such reappraisals, to consider an aspect of morphological relationships mainly among the sections of the family Batrachospermaceae, and to show a check list of the hitherto-described 104 taxa of the family with taxonomic notes.

Type elements of the genus *Batrachospermum*

The genus *Batrachospermum* was established by Roth (1797) for an unnamed species, for which he cited the synonyms, *Conferva nodosa* L., *Chara batrachosperma* Weiss and *Chara gelatinosa* (L.) Roth. The first of these names is a typographical error by Roth, the second name would result in a tautonym, not allowed under the art. 55.1 (a) of ICBN (Greuter et al. 1988), and the third name was transferred to the genus *Batrachospermum* by De Candolle (1802). Roth (1800) described *Batrachospermum moniliforme*, the first species formally assigned to the genus, which includes *Chara gelatinosa* and *Conferva gelatinosa* as synonyms.

As corroborated by Skuja in Farr et al. (1979), Roth did not propose a new species but only a new name for *Conferva gelatinosa*. *Batrachospermum moniliforme* is illegitimate and a superfluous name for *Batrachospermum gelatinosum* under the art. 63.1 of ICBN, because Roth cited as synonym the earlier, validly published *Conferva gelatinosa* L. Therefore, the correct name of this species is *Batrachospermum gelatinosum* (L.) DC. as stated by Necchi (1990a) and Compère (1991).

The type element of *Conferva gelatinosa* L. and of the genus *Batrachospermum* Roth could be the specimen from Sweden described by the phrase "*Conferva filis ramosis moniliformibus, articulis globosis gelationosis*" (Linnaeus 1753), because the same phrase was used by Linnaeus (1755) in Flora Suecica. Unifortunately, the specimen labeled *Conferva gelatinosa* in the Linnean Herbarium would have been included since 1753 and therefore can neither be the holotype nor the lectotype (Compère 1991).

Among the other elements cited in the protologue, an available lectotype could be associated with the illustration from Dillenius' Historia Muscorum for *Conferva fontana nodosa spermatis ranarum instar lubrica, major* et *fucosa* (Dillenius 1741, pl. 7, fig. 42), which has always been associated with *Conferva gelatinosa* and *Batrachospermum moniliforme*.

Compère (1991) examined the specimen on which Dillennius' drawing was based, deposited in the Dillenius' Herbarium of Oxford University (OXF), and found the characteristic carposporophytes and trichogynes of *Batrachospermum moniliforme*. Therefore, Compère chose the Dillenius' specimen of *Conferva fontana nodosa spermatis ranarum instar lubrica, major* et *fucosa* (Dillenius 1741, pl. 7, fig. 42) as the lectotype specimen for *Conferva gelatinosa* [=*B. gelatinosum* (L.) DC].

Another illustration from Dillenius' Historia Muscorum (1741, pl. 7, fig. 43) cited by Linnaeus is referred by Bory (1808) to his *Batrachospermum ludibonda stagnilis*. Còmpere (1991) examined the specimen illustrated by Dillenius and found the gonimoblasts inserted in the whorls, not exerted as *Batrachospermum stagnale* [=*B. ectocarpum*], so that this specimen agreed rather well with the current concept of *Batrachospermum gelatinosum* [=*B. moniliforme*].

Sirodot (1884) proposed the subdivision of the genus *Batrachospermum* into six sections in his monograph, "Les Batrachospermes", in which all the section names were not written in Latin but French, for example, *Helmintoides, Moniliformes, Setaces, Turficoles, Vertes* and *Hybride*. Most authors such as Hamel (1925), Israelson (1942), Bourrelly (1970), Reis (1974) and Starmach (1977) have agreed with a somewhat enlarged version of the subdivision of the genus into the sections proposed by Sirodot (1884), generally translating the French section names into the Latin names.

Compère (1991) pointed out that, before the publication of "Les Batrachospermes" (1884), Sirodot (1873, 1875) had proposed a first sketch of his subdivisions of *Batrachospermum* into sections and subsections and the Latin names published there have priority on the French names of "Les Batrachospermes". Compère (1991) did a reappraisal of section names of the genus *Batrachospermum* and showed the correct names.

Since then, four sections, namely, section *Contorta* Skuja (1931a), section *Aristatae* Skuja (1933), section *Claviformia* Reis (1973) and section *Carpocontorta* Sheath et al. (1986) were successively proposed.

Moreover, the subgenus *Acarposporophytum* Necchi (1987) was proposed to accommodate *B. brasiliense* Necchi, a species without gonimoblasts and carposporangia but with direct formation of the filaments of *Chantransia*-phase from the fertilized carpogonium.

An aspect of phylogenetic relationships among the sections of the family Batrachospermaceae.

The carpogonia of the taxa of the section *Helminthoidea* are small and with ovoid or ellipsoidal trichogynes. Carpogonium-bearing branches are not differentiated from primary branchlets. Carposporophytes are small and numerous often more than ten in each whorl. Judging from these characteristics, the section *Helminthoidea* is considered as the most primitive section among the family Batrachospermaceae.

Placing the section *Helminthoidea* on the main trunk of the phylogenetic tree, other genera and sections of the family Batrachospermaceae fall into the following six evolutional lines: 1) *Batrachospermum* line, 2) *Hybrida-Setacea* line, 3) *Aristatae-Acarposporophytum* line, 4) *Virescentia* line, 5) *Turfosa-Sirodotia* line and 6) *Contorta* line.

1. *Batrachospermum* line.

One of the most primitive taxa among the section *Batrachospermum* is considered to be *B. stagnale*, for which carpogonia are with ovoid or club-shaped trichogynes, carpogonium-bearing branches are differentiated after fertilization, carposporophytes are numerous and scattered on periphery of a whorl.

The degree of the differentiation of carpogonium-bearing branches from primary branchlets is considered to reflect the degree of the phylogenetic changes. The more highly differentiated taxa are regarded as the more highly advanced ones. With the comparison of the differentiation of carpogonia and carpogonium-bearing branches from laterals of the whorl, it may be considered that *B. stagnale* [=*B. arcuatum*] is rather primitive, *B. gelatinosum* is more advanced (Kumano et al. 1970).

Among the section *Batrachospermum, B. nova-guineense* is regarded as such an advanced taxon, for which carpogonium-bearing branches are short, more differentiated, slightly curved. Some taxa of the section *Contorta* such as *B. tortuosum* have curved carpogonium-bearing branches. Therefore, *B. nova-guineense* is regarded as one of the connecting links between the both sections (Kumano & Johnstone 1983).

There is another trend toward the reduction of laterals of long carpogonium-bearing branches. In the early stage of development, long carpogonium-bearing branches of *B. cylindro-cellulare* closely resemble those of *B. cayennense* of the section *Aristatae*. Therefore, the former is regarded as an intermediate taxon between the section *Batrachospermum* and *Aristatae* (Kumano 1984a).

Sheath & Cole (1990) assigned *B. heterocorticum* to the section *Batrachospermum*, because of straight carpogonium-bearing branches, large lateral whirls and the presence of several carposporophytes in middle to outer portion of the whorl. However, the shape of trichogyne of this species is similar to that of *B. sirodotii* (section *Virescentia*). So that *B. heterocorticum* is regarded as an intermediate taxon between the section *Batrachospermum* and the section *Virescentia*.

2. *Hybrida-Setacea* line

Carpogonium-bearing branches of *B. virgato-decaisneanum* of the section *Hybrida* are short and well-differentiated, one or two carposporophytes are semiglobular and inserted central within a whorl. Carpogonium-bearing branches of *B. atrum* of the section *Setacea* are very short and composed of a few cells; a carposporophyte develops a wart-like protuberance on central axis; cortical filaments are well-developed like a pseudoparenchyma. So that, *B. atrum* is regarded as one of the most advanced taxa on the *Hybrida-Setacea* line.

3. *Aristatae-Acarposporophytum* line

As mentioned above, some members of the

section *Aristatae* are thought to be derived from some taxa of the section *Batrachospermum*. In *B. hypogynum* (Kumano & Ratnasabapathy 1982) and *B. macrosporum* (Kumano & Necchi 1990) with large carpospores germinating within carposporagia, the protoplasmic connections between carpogonia and rosette-like hypogynous cells are observed. These characteristics of the above-mentioned two taxa indicate that both taxa are regarded as more advanced taxa.

B. brasiliense of the subgenus *Acarposporophytum* (Necchi 1990a), in which a carposporophyte is reduced only one-celled zygote (Necchi 1987), is considered to be derived from some taxa of the section *Aristatae* and regarded as one of the most advanced taxon on the *Aristatae-Acarposporophytum* line.

4. *Virescentia* line

Concerning to *B. sirodotii* of the section *Virescentia*, a carpogonium with a cylindrical large trichogyne, a carpogonium-bearing branch is short and differentiated, one or two carposporophytes are inserted centrally within a whorl and bigger than those found in the section *Batrachospermum*.

B. bakarense, in which a short carpogonium-bearing branch is composed of two to five cells and slightly curved, resembles that of some taxa of the section *Contorta*. This fact suggests that there is a close relationship between the section *Virescentia* and the section *Contorta* (Kumano & Ratnasabapathy 1984).

5. *Turfosa-Sirodotia* line

Concerning to *B. turfosum* var. *undulatopedicellatum* (Kumano & M. Watanabe 1983), *B. periplocum* (Skuja 1969) and *B. orthostichum* (Skuja 1931a) of the section *Turfosa*, gonimoblast filaments spread along the central axis and carposporangia are developed in a similar mode as in the taxa of the genus *Sirodotia* (Kumano 1982c). Concerning to *B. tapirense*, two types of gonimoblast filaments are observed; one is the erect (*Batrachospermum*) type, another is the diffused (*Sirodotia*) type (Kumano & Phang 1987). Because of these characteristics, the above-mentioned taxa are considered as apparently intermediate taxa linking the genus *Batrachospermum* and the genus *Sirodotia*.

6. *Contorta* line

As mentioned previously, *B. tortuosum* having a slightly curved carpogonium-bearing branch (Kumano 1978) is thought to be derived from taxa such as *B. nova-guineense* (Kumano & Johnstone 1983) of the section *Batrachospermum* and *B. bakarense* (Kumano & Ratnasabapathy 1984) of the section *Virescentia*. Among this evolutional line, a carpogonium-bearing branch of *B. gibberosum* of the section *Contorta* is composed of thick-walled cells and differentiated very much, cortical filaments and secondary branchlets stick to axial cells and develop into pseudoparenchymatous structures (Kumano 1978). Therefore, *B. gibberosum* is regarded as one of the most advanced taxa on the *Contorta* line and seems to be an intermediate taxon linking the genus *Batrachospermum* and the genus *Tuomeya* (Kumano 1978, 1986).

A check list of the hitherto-described 104 taxa in the family Batrachospermaceae.

A check list is compiled of the hitherto-described 104 taxa with taxonomic notes.

Family Batrachospermaceae Agardh 1824

Genus *Batrachospermum* Roth 1800

I. Subgenus *Acarposporophytum* Necchi 1990a
Type: *B. brasiliense* Necchi 1987.

Gonimoblast filaments and carposporangia absent. A carposporophyte reduced to only one cell, a zygote. Filaments of the *Chantransia*-phase developed directly from fertilized carpogonium.

1) *B. brasiliense* Necchi 1987

II. Subgenus Batrachospermum Necchi 1990a
Type: *B. gelatinosa* (L.) DC. 1802

Syn.: genus *Batrachospermum* Roth 1800

Carposporophyte, gonimoblast filaments and carposporangia, present. Filaments of the *Chantransia*-phase developed from the germination of carpospores.

1. Section *Helminthoidea* De Toni 1897
   Type: *B. confusum* (Bory) Hassal 1845 [=*B. helminthosum* Sirodot 1884, non Bory 1808]
   Syn.: section *Helminthosa* Sirodot 1873, section "*Helminthoides*" Sirodot 1884.

Fronds not saturate green. Carpogonium-bearing branches not so differentiated, arising from the cells of the fascicles or from pericentral cells of primary branchlets. Laterals of carpogonium-bearing branches short. Carpogonia small in size, with ellipsoidal or ovoid trichogynes. Carposporophytes pedunculate, small, globular, numerous, scattered in the outer half to the whorl.

Compère (1991) pointed out that the name *Helminthosa* Sirodot (1873) could not be applied to this section, though it includes the same taxa, because Sirodot (1873) cited as type *B. helmintosum* Bory (1808) which was included in section "*Verts*" in 1884, under the new illegitimate name *B. coerulescens* Sirodot (1884). The section name "*Helminthoides*" first published in a French name by Sirodot (1884) could not be taken into account under the art. 18.4 of ICBN. De Toni (1897) was the first to treat this section name as the Latin name and has to be accepted as author of the name of section *Helminthoidea*.

The section *Helminthoidea* was included in the section *Batrachospermum* by Necchi and Entwisle (1990), however, I prefer to keep separated the above both sections.

1) *B. confusum* (Bory) Hassal 1845
   [=*B. ludibonda* Bory var. *confusa* Bory 1808, *B. helminthosum* Sirodot 1884, non *B. helmentosum* Bory 1808, *B. crouanianum* Sirodot 1884]

According to Compère (1991), the species name proposed by Sirodot (1884) was illegitimate for two reasons, 1) as a superfluous later renaming of the previous *B. confusum* Bory (1808), 2) as a later homonym (orthographic variant) of the earlier *B. helmentosum* Bory (1808).

Compère (1991) found that the Bory's holotype specimen had carpogonia with ovoid trichogynes and spermatangia occurred on the same specimen (monoecious species).

2) *B. boryanum* Sirodot 1884
   [=*B. anatinum* Sirodot 1884]

3) *B. boryanum* var. distensum (Kylin) Israelson 1942
   [=*B. distensum* Kylin 1912]

4) *B. szschwanense* Jao 1941

Jao (1941) stated that this species has resemblances of *B. boryanum* Sirodot, but differs from the latter chiefly in having male and female plants being similar in general appearance, an internodal filament entirely wanting and a curved trichogyne. It should be compared with the monoecious *B. distensum* Kylin (1912) and the polygamous *B. anatinum* Sirodot (1884).

2. Section *Batrachospermum*
   Type: *B. gelatinosa* (L.) DC. 1802 [=*B. moniliforme* Roth 1800]
   Syn.: section *Moniliformia* Sirodot 1873; section *Moniliformes* Sirodot 1884.

Fronds not saturate green. Carpogonium-bearing branches somewhat differentiated, usually arising from the cells of the fascicles, sometimes from the pericentral cells of the primary branchlets. Carpogonia small to large, with club- or finally often urn-shaped trichogynes. Carposporophytes pedunculate, globular, small, numerous, scattered within the whorl at various distances from the center. Laterals of carpogonium-bearing branches elongate, usually embracing the carpogonia and carposporophytes.

1) *B. gelatinosum* (L.) DC. 1802
[=*Conferva gelatinosa* L. 1753, *B. moniliforme* Roth 1800, *B. corbula* Sirodot 1884, *B. decaisneanum* Sirodot 1884, *B. gelatinosum* var. decaisneanum (Sirodot) Reis 1969, *B. radians* Sirodot 1884, *B. Moniliforme* f. *lipsiensis* Roth 1800, *B. moniliforme* var. *scopula* Sirodot 1884]

2) *B. gelatinosum* f. *pyramidale* (Sirodot) Compère 1991
[=*B. pyramidale* Sirodot 1884, *B. moniliforme* f. *pyramidale* (Sirodot) Israelson 1942, *B. pygmaeum* Sirodot 1884]

3) *B. gelatinosum* var. *obtrullatum* Kumano et M. Watanabe 1983

4) *B. durum* C. A. Agardh 1824
[=*B. densum* Sirodot 1884, *B. moniliforme* f. *densum* (Sirodot) Israelson 1942, *B. gelatinosum* f. *densum* (Sirodot) Compère 1991]

Starmach (1982) stated that the species name *B. durum* must be used, because the reason given by Sirodot (1884) for rejecting C. A. Agardh's specific name and for proposing a new name for this species are not accepted by ICBN.

5) *B. helminthoideum* (Sirodot) Mori 1975
[=*B. moniliform* var. *helminthoideum* Sirodot 1884]

6) *B. sporulans* Sirodot 1884

7) *B. godronianum* Sirodot 1884

8) *B. reginense* Sirodot 1884

9) *B. stagnale* (Bory) Hassal 1845
[=*B. ludibonda* Bory var. *stagnalis* Bory 1808, *B. ectocarpum* Sirodot 1875, 1884, *B. arcuatum* Kylin 1912]

According to Compère's observation (1991), the Bory's specimens (PC), clearly show the exerted gonimoblasts characteristic of *B. ectocarpum*. Sirodot himself (1884) considered both names as synonyms but did not accept Bory's epithet. Reis (1973), working on Portuguese material, distinguished *B. stagnale* as dioecious from the monoecious *B. ectocarpum*.

Sheath and Burkholder (1983) synonymized *B. ectocarpum* Sirodot with the dioecious *B. boryanum* Sirodot because of the variability of trichogynes in populations from Rhode Island, and included the section *Helminthoidea* into the section *Batrachospermum*.

However, Compère (1991) observed a few spermatia on the specimens bearing carpogonia in Bory's holotype (PC) and preferred to keep *B. boryanum* of the section *Helminthoidea* and *B. ectocarpum* of the section *Batrachospermum* separated on the basis of the exerted gonimoblasts in the monoecious *B. stagnale* (Bory) Hassal 1845 [=*B. ectocarpum* Sirodot 1884].

10) *B. sinense* Jao 1941

Jao (1941) assigned this species to the section *Turfosa*. A young trichogyne of this species is cuneate, but the mature one becomes round or obovate, sometimes inflad like a balloon, and a carpogonium-bearing branch is composed of barrel-shaped cells and provides many elongated laterals. These characteristics are also observed in the taxa of the section *Batrachospermum*, so that, this species resembles more closely those of the section *Batrachospermum* rather than those of the section *Turfosa* (Kumano 1984b).

11) *B. arcuatoideum* Reis 1973

12) *B. sporiferum* Mori 1975

13) *B. japonicum* Mori 1975

14) *B. polycarpum* Mori 1975

15) *B. cylindro-cellulare* Kumano 1978

The early stage of development of a carpogonium-bearing branch of this species is similar to that of *B. caynnenense* Montagne of the section *Aristatae*.

16) *B. nova-guineense* Kumano et Johnstone 1983

Johnstone et al. (1980) assigned this species to the section *Hybrida*, however, I prefer to assign this species to the section *Batrachospermum*.

17) *B. heterocorticum* Sheath et Cole 1990

Sheath & Cole (1990) mentioned that this species was assigned to the section *Batrachospermum*, because of straight carpogonium-bearing branches, large lateral whorls and presence of several carposporophytes in middle to outer portion of the whorl, on the other hand, the shape of trichogyne of this species was similar to that of *B. sirodotii* Skuja ex P. Reis (1974) of the section *Virescentia*.

[Section *Carpocontorta* Sheath et al. 1986]
Type: *B. carpocontortum* Sheath et al. 1986

Sheath et al. (1986) established a monotypic section *Carpocontorta* based primarily on the presence of protrusions and bends in a trichogyne, and on the size and localization of carposporophytes. However, other species have a similar shaped trichogyne (Sirodot 1884, Reis 1969, Mori 1975), and in these cases, the shape of the trichogyne has been treated as a characteristics at species rank. So that, *B. carpocontortum* Sheath et al. can be readily included in the section *Batrachospermum* as pointed out by Necchi and Entwisle (1990).

18) *B. carpocontortum* Sheath et al. 1986

3. Section *Setacea* De Toni 1897
Type: *B. dillenii* Sirodot 1884 [=*B. atrum* (Huds.) Harvey 1841]
Syn.: section *Moniliformia* subsection *Capillacea* Sirodot 1873, section *Moniliformia* subsection *Setacea* Sirodot 1875, section *Setaces* Sirodot 1884

Fascicles reduced and very short. Carpogonia with club- or urn-shaped trichogynes. A carpogonium-bearing branch well- differentiated and reduced to a few cells. Carposporophytes appearing as wart-like protuberances on the central axis.

Compère (1991) stated that the French name of this section given by Sirodot (1884) was first treated as the Latin name by De Toni (1897). The earlier epithet *Capillacea* Sirodot (1873) has priority only at the subsection rank; at the section rank, *Setacea* Sirodot (1875) is illegitimate as a later superfluous synonym of *Capillacea* Sirodot (1873) according to the art. 63.1 of ICBN and cannot be considered as a basionym for the section *Setacea*. At the section rank, however, *Setacea* is the correct epithet.

The section *Setacea* is included in the section *Viridia* [=*Virescentia*] by Necchi and Entwisle (1990), however, I prefer to keep the above both sections separated.

1) *B. atrum* (Huds.) Harvey 1841
[=*Conferva atra* Hudson 1798, *B. gallaei* Sirodot 1884, *B. dillenii* Sirodot 1884, *B. tenuissimum* Bory, *B. angolense* W. West & G. S. West 1897, *Sirodotia angolensis* (W. West & G. S. West) Skuja in Reis 1960]

2) *B. puiggarianum* Grunow in Wittrock et Nordstedt 1883
[=*B. atraum* var. *puiggarianum* (Grunow) Necchi 1989, *B. schwackeanum* Moebius 1892, *B. nigrescens* W. West et G. S. West 1897, *Sirodotia nigrescens* (W. West & G. S. West) Skuja in Reis 1960]

3) *B. diatyches* Entwisle 1992
[=*B. nothogeae* Skuja, nom. nud.]

4. Section *Turfosa* Sirodot 1873
Type: *B. vagum* (Roth) Ag. 1824 [=*B. turfosum* Bory 1808]

Syn.: section *Turficola* De Toni 1897; section *Turficoles* Sirodot 1884.

Fronds pseudo-dichotomously branched. Carpogonium-bearing branches straight, short and arising from pericentral cells of fascicles. Carpogonia sessile or indistinctly stalked elongate conical trichogyne with the largest diameter distal. Carposporophytes big, globular or semiglobular, single (sometimes couple, central within a whorl. Gonimoblast filaments of two types of erect and diffused.

In the section *Turfosa* emended by Necchi (1990a), the following taxa was recognized as possessing two types of gonimoblast filaments (erect and diffused), namely *B. orthostichum* Skuja, *B. periplocum* (Skuja) Necchi, *B. turfosum* Bory, *B. tapirense* Kumano et Phang. Originally classified taxa in the section *Turfosa* such as *B. vogesiacum* T. G. Schults ex Skuja and *B. gombakense* Kumano et Ratnasabapathy must be transferred to the section *Virescentia*, as redefined in Kumano and Phang (1987) and Necchi (1990a), because these taxa have only an erect type of gonimoblast filament.

1) *B. turfosum* Bory 1808
[=*Chara batrachosperma* var. *vaga* Roth 1797, *Batrachospermum moniliforme* var. *vagum* (Roth) Roth 1800, *B. vagum* (Roth) Ag. 1824, *B. keratophytum* Bory 1808, *B. vagum* var. *keratophytum* (Bory) Sirodot 1884]

Compère (1991) mentioned that at the species rank, the name *B. turfosum* Bory (1808) antedated *B. vagum* (Roth) Ag. (1824) and has to be used, even though the epithet *vagum* is older at the variety rank.

According to Compère's observation (1991), the Bory's type specimen has a few carpogonia with elongated, club-shaped trichogynes and spherical spermatia; it belongs to a monoecious species, but the reproduction is ensured by ovoid or elliptical monospores.

2) *B. turfosum* var. *undulato-pedicellatum* Kumano et M. Watanabe 1983

3) *B. orthostichum* Skuja 1931a

Although Skuja (1931a) originally assigned this species with *atrum*-like fascicles to the section *Setacea*, it seems better to assign it to the section *Turfosa* based on the shape of trichogyne and a little long carpogonium-bearing branch. Skuja (1931a) observed some diffused gonimoblast filaments extending out from a globular carposporophyte of this species.

4) *B. periplocum* (Skuja) Necchi 1990a
[=*B. vagum* var. *periclocum* Skuja 1969]

Skuja (1969) observed some diffused gonimblast filaments extending out from a globular carposporophyte of this species.

5) *B. tapirense* Kumano et Phang 1987

This species resembles *B. bakarense* Kumano et Ratnasabapathy of the section *Virescentia* in having a short carpogonium-bearing branch and a carpogonium with club-shaped trichogyne. However, the former differs from the latter in having the carpogonium-bearing branch growing toward the same direction that cortical filaments are formed, moreover, in having both the radially branched and the diffused gonimoblast filaments.

5. Section *Virescentia* Sirodot 1873, 1875
Type: not designated in 1875, but only one species cited by Sirodot 1875, *B. helmentosum* Bory 1808 [=*B. coerulescens* Sirodot 1884]
Syn.: section *Viridia* De Toni 1897; section *Verts* Sirodot 1884.

Fronds saturate green. Carpogonium-bearing branches differentiated, short, arising from the pericentral cells. Carpopogonia with distinctly pedunculate, cylindrical trichogynes. A carposporophyte big, globular, single (rarely in couple), central within a whorl.

1) *B. helmentosum* Bory 1808
[=*B. coerulescens* Sirodot 1884, non *B. helminthosum* Sirodot 1884]

Compère (1991) stated that the reason given by Sirodot (1884) for rejecting Bory's specific name and for proposing a new name for this species are not accepted under ICBN and the former name must be used. The Bory's holotype specimen (PC) is a female plant, with a big carposporophyte in the center of a whorls and carpogonium with a stalked trichogyne.

2) *B. sirodotii* Skuja ex P. Reis 1974
[=*B. virgatum* Sirodot 1884 nom. illeg., non *B. moniliforme* var. *virgatum* Kuetzing]

According to Compère (1991), when published the species name *B. virgatum* Sirodot (1884) cited the earlier *B. julianum* (Memegh.) Arc. as a synonym, therefore this species name is illegitimate as superfluous according to the art. 63 of ICBN. It cannot be considered as a new combination based on *B. moniliforme* var. *virgatum* Sirodot (1884), as suggested by Necchi (1989, 1990a) because Sirodot indicate by a question mark that he was not sure that his name could apply to Kuetzing's taxon. Skuja indicated that *B. virgatum* Sirodot was different from *B. moniliforme* var. *virgatum* Kuetzing. Consequently, Skuja proposed, but did not publish, the new name *B. sirodotii* for the illegitimate *B. virgatum* Sirodot. This name was accepted and validly published by Reis (1974) with a full and direct reference to the replaced name.

The lectotype of this species was chosen by Compère (1991) among the specimens cited by Sirodot (1884), which was determined by Sirodot and examined by Skuja.

3) *B. graibussoniense* Sirodot 1884

4) *B. bruziense* Sirodot 1884

5) *B. testale* Sirodot 1884

6) *B. lochmodes* Skuja 1938

7) *B. vogesiacum* T. G. Schultz ex Skuja 1938
[=*B. vagum* var. *flagelliforme* Sirodot 1884, *B. flagelliforme* (Sirodot) Necchi 1989]

Compère (1991) mentioned that this species name was proposed by Skuja (1938a) as a new name, at the species rank, for *B. vagum* var. *flagelliforme*, which was not a nomen nudum as stated by Necchi (1989, 1990a), but a nomen novum and validated before 1953, by the mere reference to the validly published *B. vagum* var. *flagelliforme* according to the art. 32.3, 33.2 of ICBN.

According to Compère (1991) the type specimen of this species is not the Schultz's specimen, not cited in the protologue, but one of the specimens cited by Sirodot (1884) for this variety. Among these specimens, Compère (1991) designated the specimen in Herbier Thuret (PC) as the lectotype of this species; this specimen bears the determination "*B. vagum* variete *flagelliforme* Sirodot fructifie" and has been determined by Skuja as "*Batrachospermum vogesianum* F. G. Schultz".

8) *B. gulbenkianum* Reis 1965b

9) *B. transtaganum* Reis 1970

10) *B. crispatum* Kumano et Ratnasabapathy
(Ratnasabapathy and Kumano 1982a)

11) *B. bakarense* Kumano et Ratnasabapathy 1984

Although this species resembles some taxa such as *B. tortuosum* of the section *Comtorta* in having a slightly curved carpogonium-bearing branch, it resembles more closely species of the section *Virescentia* in having a short carpogonium-bearing branch and a carpogoium with a club-shaped trichogyne.

12) *B. gombakense* Kumano et Ratnasa-

bapathy
(Ratnasabapathy and Kumano 1982b)

Ratnasabapathy and Kumano (1982b) assigned this species to the section *Turfosa*. However, this species had better to be assigned to the section *Virescentia* based on the characteristics; a carpogonium with an indistictly stalked and inverted conical trichogyne and a single large carposporophyte inserted centrally in a whorl.

[Section *Claviformia* Reis 1973]
Type: *B. azeredoi* Reis 1967

The section *Claviformia* was established by Reis (1973) by having basically a carpogonium with a sessile and club-shaped trichogyne and central carposporophytes.

According to these circumscription, I agree with Necchi's opinion (1990a) that the section *Claviformia* can be merged in the section *Virescentia*, whose members have a stalked or sessile, cylindrical or club-shaped trichogyne and sessile or central carposporophytes.

13) *B. azeredoi* Reis 1967

14) *B. ferreri* Reis 1967

6. Section *Hybrida* De Tni 1897
Type: *B. virgato-decaisneanum* Sirodot 1884
Syn.: section *Hybride* Sirodot 1884

Fronds saturate green. Carpogonium-bearing branches short and arising from the basal cells of the primary branchlets. Carpogonia with trichogynes somewhat asymmetric, sessile or indistinctly stalked, ellipsoidal. Carposporophytes globular, big, single (or couple), central within the whorl.

This section name was first treated as the Latin name by De Toni (1897).

1) *B. virgato-decaisneanum* Sirodot 1884

2) *B. abilii* Reis 1965a

3) *B. mikrogyne* Flint et Skuja in Flint 1953

7. Section *Aristatae* Skuja 1933
Lectotype: *B. cayennense* Montagne in Kuetzing 1849 designated by Necchi (1990a).

Fronds irregularly branched. Carpogonium-bearing branches straight, long and differentiated from the fascicles. Carpogonia symmetrical. Carposporophytes pedunculate, spherical.

Skuja (1933) proposed the section *Aristatae* to include *B. macrosporum* Montagne, *B. cayennense* Montagne, *B. aristatae* Skuja sp. nov. and probably *B. breutelii* Rabenhorst, however, he did not designate the type species of this section, which presumably would be *B. aristatum*, which was not formally described. The earliest effectively and valid published species in the section *Aristatae* is *B. cayennense* Montagne is Kuetzing (1849). Thus, this species was disignated as the lectotype of the section *Aristatae* by Necchi (1990a).

It is proposed that this section is divided into the following two subsections based on the occurrence of the hypogynous cells forming the rosette-like laterals.

7–1. *Aristatae* subsect. nov.
Typus: *B. cayennense* Montagne in Kuetzing 1849

Frons plus minusve irregulariterque ramosa. Ramuli carpogoniferi stricti, longi, e cellulis pericentralis orientes, sine cellulis hypogynis rosulatis. Trichogyne indistincte pedicellata. Carposporophytum in peripherias verticilli insertum, aux ex verticillis exertum.

Subsection *Aristatae*

Fronds more or less irregularly branched. Carpogonium-bearing branches straight, long, arising from pericentral cells, without rosette-like hypogynous cells. Trichogyne indistinctly stalked. Carposporophytes spherical, inserted within or exerted from the periphery of a whorl.

1) *B. cayennense* Montagne in Kuetzing 1849

2) *B. turgidum* Kumano 1982b

3) *B. longiarticulatum* Necchi 1990a

4) *B. beraense* Kumano 1978

The taxa of the family Batrachospermaceae exhibited the primitive characteristics in the absence of any fusion of a fertilized carpogonium with a hypogynous cell and in the formation of the gonimoblast filaments produced directly from the undivided fertilized carpogonium. However, the gonimoblast filaments of *B. beraense* Kumano were developed from the divided carpogonium, the lower cell of which did not fuse with the hypogynous cell, as in certain members of the family Helmintho-cladiaceae (Kumano and Ratnasabapathy 1982).

5) *B. breutelii* Rabenhorst 1855
[=*B. dimorphum* Kuetzing 1857]

B. breutelii firstly described without illustrations by Rabenhorst (1855) based on the specimen collected by Breutel from Gnadenthal near Cape of Good Hope in South Africa. Kuetzing (1857) described *B. dimorphum* with illustrations based on the specimen collected by Pfarrwe Wenck from the same locality, Gnadenthal in South Africa. Skuja (1933) examined the both type specimens of *S. breutelii* Rabenhorst (1855) and *B. dimorphum* Kuetzing (1857) and pointed out that the latter was regarded as conspecific with the former, which was validly published.

Skuja (1933) examined this species and found the unusual gonimoblast filaments developed into the large gemmae, "Brutkoerper".

7-2. *Macrosporum* subsect. nov.
Typus: *B. macrosporum* Montagne 1850

Frons plus minusve irregulariterque ramosa. Ramuli carpogoniferi stricti, longi, e cellulis pericentralis orientes. Cellulae hypogynae rosulatae, lateraliter prolatae, magnae. Carpogonia symmetrica, trichogyne indistincte pedicellata, urniformis. Carposporophytum in peripherias verticilli insertum, aux ex verticillis exertum.

Subsection *Macrosporum*

Fronds more or less irregularly branched. Carpogonium-bearing branches straight, long, arising from pericantral cells. Hypogynous cell in rosette, laterally elongate, large. Carpogonia symmetrical, trichogyne indistinctly stalked, urn-shaped. Carposporophytes spherical, inserted within or exterted from the periphery of a whorl.

6) *B. equisetifolium* Montagne 1850

Based on the examination of type specimen, Kumano (1990) stated that this species resembled *B. macrosporum* Montagne in having hypogynous cells forming rosette-like laterals, but differed from the latter in the size of the trichogyne.

7) *B. hypogynum* Kumano et Ratnasabapathy
(Ratnasabapathy and Kumano 1982b)

The rosette-like nutritive hypogynous cell, connected with a carpogonium and the underlying cells of a carpogonium-bearing branch, took part an important role in the formation of a large carposporangia in this species (Kumano and Ratnasabapathy 1982).

8) *B. macrosporum* Montagne 1850
[=*B. oxycladum* Montagne 1850, *B. macrosporum* var. *oxycladum* (Montagne) Sirodot 1884, *B. excelsum* Montagne 1850, *B. macrosporum* var. *excelsum* (Montagne) Sirodot 1884]

Kumano (1990) examined the type specimen of *S. oxycladum* Montagne and pointed out that this species was regarded as a synonym of *B. macrosporum* Montagne. This species had nutritive hypogynous cells which

formed rosette-like laterals and the largest carposporangia among the taxa of the genus *Batrachospermum* Roth.

8. Section *Contorta* Skuja 1931a
Type: *B. procarpum* Skuja 1931a

Fronds irregularly or pseudo-dichotomously branched. Carpogonium-bearing branches curved or twinsted and coiled, differentiated from the fascicles. Carpogonia symmetrical. Carposporophytes sessile, semiglobular. Gonimoblast filaments of an erect type.

Skuja (1931a) proposed the division of the genus *Batrachospermum* into two subgenera, subgenus *Eu-Batrachospermum* and subgenus *Condea*, the latter with the single section *Contorta* to accommodate *B. procarpum* Skuja (1931a). The section *Contorta* as part of the genus *Batrachospermum* has been widely accepted and numerous species have been assigned to this section.

It is proposed that this section is divided into the following five subsections based on the occurrences of monosporangia, curved or spirally coiled carpogonium-bearing branches, loosely or compactly agglomerated carposporophytes.

8-1. *Intortum* sect. nov.
Typus: *B. intortum* Jao 1941

Frons plus minusve irregulariterque ramosa. Ramuli carpogoniferi tortuosi, e cellulis pericentralis orientes. Trichogyne claviformis, indistincte pedicellata, ad basim saepe flexa. Carposporophytum globosus vel semiglobosus, in centro verticilli insertum. Monosporangia in ramulis carpogoniferiis, secundariis vel primaiis terminalia.

Subsection *Intortum*

Frons irregularly branched. Carpogonium-bearing branches twisted, arising from pericentral cells. Trichogyne club-shaped, indistinctly stalked, often bent at the base. Carposporophytes globular or semiglobular, inserted centrally. Monosporangia terminating laterals of carpogonium-bearing branches, primary and secondary branchlets.

1) *B. intortum* Jao 1941

This taxon hitherto known only from China (Jao 1941) was found on several localities in the mountainous eastern part of Cuba, Prov. Oriente, by Rieth (1979).

2) *B. pseudocarpum* Reis 1973

3) *B. woitapense* Kumano 1983c

4) *B. lusitanicum* Reis 1965a

8-2. *Torridum* sect. nov.
Typus: *B. torridum* Montagne 1850 [=*B. vagum* var. *torridum* (Montagne) Sirodot 1884]

Frons plus minusve irregulariterque ramosa. Ramuli carpogoniferi plus minisve curvi, e cellulis pericentralis orientes. Trichogyne indistincte pedicellata, claviformis, ad basim saepe flexa. Carposporophytum semiglobosi, breves, centro verticillii inserti.

Subsection *Torridum*

Fronds more or less irregularly branched. Carpogonium-bearing branches more or less curved, arising from pericentral cells. Trichogyne club-shaped, indistinctly stalked, often bent at the base. Carpogonia asymmetrical. Carposporophytes semiglobular, big, inserted centrally.

5) *B. tortousum* Kumano 1978

6) *B. tortousum* var. *majus* Kumano 1982a

7) *B. torridum* Montagne 1850
[=*B. vagum* var. *torridum* (Montagne) Sirodot 1884]

Based on the examination of the type specimen, Kumano (1990) did not assign this species to the section *Turfosa* but to the section

Contorta and proposed to use Montagne's species name.

8) *B. doboense* Kumano et Borden-Kerby 1986

9) *B. faroense* Kumano et Borden-Kerby 1986

8–3. *Procarpum* sect. nov.
Typus: *B. procarpum* Skuja 1931a

Frons plus minusve irregulariterque ramosa. Ramuli primarii audouinelloidei, alterne vel unilaterliter raminificantes. Ramuli carpogoniferi tortuosi, e cellulis pericentralis orientes. Trichogyne indistincte pedicellata, ellipsoidea vel claviformis. Carposporophytum semiglobosi, breves, centro verticillii inserti. Fila gonimoblastorum longa, plus minisve laxe agglomerata.

Subsection *Procarpum*

Frons more or less irregularly branched. Primary branchlets *Audouinella*-like, alternately or unilaterally branched. Carpogonium-bearing branches twisted or spirally coiled, arising from pericentral cells. Trichogyne indistinctly stalked, ellipsoidal or club-shaped. Carposporophytes semiglobular, big, centrally inserted. Gonimoblast filaments long, more or less loosely agglomerated.

10) *B. procarpum* Skuja 1931a

11) *B. procarpum* var. *americanum* Sheath et al. 1992

12) *B. equisetoideum* Kumano et Necchi 1985

13) *B. cipoense* Kumano et Necchi 1985

Sheath et al. (1992) stated that this species was considered to be a synonym of *B. globosporum* Israelson (1942) because ellipsoidal cells were found in the outer portion of facicles of the type material and the graduation of cell shapes was found from the basal portion to the outer portion of fascicles in the original figures by Israelson (1942). However, I do not agree with the opinions of Sheath *et al.* because the characteristics of the audouinelloid fascicles of this species were different from those of *B. globosporum* Israelson (1942).

14) *B. jolyi* Necchi 1986

Sheath et al. (1992) considered that this species was a synonym of *B. globosporum*, because ellipsoidal and ovoid cells of fascicles were found in the type materials and also in the original figures by Necchi (1986). However, I agree with Necchi's opinion (1986, 1990a) that this species is classified with *B. procarpum* having audouinelloid fascicles.

15) *B. densiverticillatum* Necchi 1990a

8–4. *Kushiroense* subsect. nov.
Typus: *B. kushiroense* Kumano et Ohsaki 1983

Frons plus minusve irregulariterque ramosa. Ramuli carpogoniferi valde tortuosi, e cellulis percentralis orientes. Trichogyne indistincte pedicellata, urnformis, ad basim saepe flexa. Carposporophytum globosi vel semiglobosi, centro verticillii inserti. Fila gonimoblastorum longa, laxe agglomerata.

Subsection *Kushiroense*

Fronds irregularly branched. Carpogonium-bearing branches strongly twisted, arising from pericentral cells. Trichogyne indistinctly stalked, often bent at the base. Carposporphytes globular or semiglobular, centrally inserted. Gonimoblast filaments long, loosely agglomerated.

16) *B. kushiroense* Kumano et Ohsaki 1983

17) *B. guyanense* (Montagne) Kumano 1990 [=*B. vagum* var. *guyanense* Montagne 1850]

Based on the examination of the type specimen, Kumano (1990) did not assign this species to the section *Turfosa* but to the section *Contorta* and proposed a new combination.

18) *B. tabagatense* Kumano et Borden-Kerby 1986

19) *B. nechochoense* Kumano et Borden-Kerby 1986

20) *B. nonocense* Kumano et Liao 1987

21) *B. iriomotense* Kumano 1982a

22) *B. globosporum* Israelson 1942

This species seemed better to be assigned to the section *Contorta* rather than to the section *Turfosa* (Israelson 1942), because the carpogonium-bearing branch of this species was strongly curved and the trichogyne was formed asymmetrically (Kumano 1984a).

23) *B. breviarticulatus* (Necchi et Kumano) Necchi 1990a
[=*B. capense* Starmach ex Necchi et Kumano var. *breviarticulatum* Necchi et Kumano 1984]

24) *B. capense* Starmach ex Necchi et Kumano 1984
[=*B. capense* Starmach 1975, nom. illeg.]

25) *B. skujanum* Necchi 1986

8–5. *Ambiguum* subsect. nov.

Typus: *B. ambiguum* Montagne 1850 [=*B. bicudoi* Necchi 1986, *B. exsertum* Necchi 1986, *B. basilare* Flint et Skuja in Flint 1953].

Fronds plus minusve irregulariterque ramosa. Ramuli carpogoniferi valde tortuosi, e cellulis pericentralis orientes. Trichogyne indistincte pedicellata, urnformis, ad basim saepe flexa. Carposporophytum globosi vel semiglobosi, centro verticillii inserti. Fila gonimoblastorum langa, confertim agglomerata.

Subsection *Ambiguum*

Fronds irregularly branched. Carpogonium-bearing branches strongly twisted. Trichogyne indistinctly stalked, often bent at the base. Carposporophytes globular or semiglobular, centrally inserted. Gonimoblast filaments compactly agglomerated.

26) *B. tiomanense* Kumano et Ratnasabapathy
(Ratnasabapathy and Kumano 1982a)

27) *B. omodoense* Kumano et Borden-Kerby 1986

28) *B. hirosei* Ratnasabapathy et Kumano 1982b

29) *B. nodiflorum* Montagne 1850
[=*B. vagum* var. *nodiflorum* (Montagne) Sirodot 1884]

Based on the examination of the type specimen, Kumano (1990) stated that this species was not assigned to the section *Turfosa* but to the section *Contorta*, and proposed to use Montagne's species name.

30) *B. ambiguum* Montagne 1850
[=*B. bicudoi* Necchi 1986, *B. exsertum* Necchi 1986, *B. basilare* Flint et Skuja in Flint 1953].

Based on the examination of the type specimen Kumano (1990) stated that this species was assigned to the section *Contorta*, because of the occurrence of the spirally twisted carpogonium-bearing branch of this species.

31) *B. louisianae* Skuja in Flint 1949

Flint (1949) pointed out this species was assigned to the section *Contorta*. Based on the examination of the lectotype and other specimens, Sheath et al. (1992) confirmed a curved carpogonium-bearing branch.

32) *B. henriquesianum* Reis 1972

33) *B. mahlacense* Kumano et Borden-Kerby 1986

34) *B. gracillimum* W. West et G. S. West emend. Necchi 1989

35) *B. gibberosum* (Kumano) Kumano 1986 [=*Tuomeya gibberosa* Kumano 1978]

Kumano (1986) stated that this species differed from *Tuomeya americana* (Kuetzing) Papenfuss, the type species of the genus *Tuomeya*, in the presence of the secondary branchlets and the absence of the gonimoblast placenta, the latter of which was characteristic of the genus *Tuomeya*. Because this species is similar to the taxa of the genus *Batrachospermum* in the structure of thallus and the process of the fertilization, it was transferred from the genus *Tuomeya* to the genus *Batrachospermum*.

Genus *Sirodotia* Kylin 1912
Type: *Sirodotia suecica* Kylin 1912
Syn.: section *Sirodotia* (Kylin) Necchi et Entwisle 1990

Fronds irregularly branched. Carpogonium-bearing branches short, arising from pericentral cells and or cells of fascicles. Carpogonia asymmetrical with elongate conical or club-shaped trichogynes. Carposporophytes indefinite in shape, extend over along the cortical filaments. Gonimoblast filaments of a diffused type.

Necchi and Entwisle (1990) treated the genus *Sirodotia* Kylin 1912 as a section *Sirodotia* of the genus *Batrachospermum* Roth, however, I prefer to keep the above both genera separated, because the taxa of the genus *Sirodotia* is similar to those of the genus *Batrachospermum* in the vegetative structures but differs in the reproductive structures, in having a diffuse gonimoblast rather than a dense globular gonimoblast and a lobed rather than an isodiametric carpogonium base.

1) *S. suecica* Kylin 1912
[=*Batrachospermum suecicum* (Kylin) Necchi et Entwisle 1990, *S. fennica* Skuja 1931b, *Batrachospermum fennicum* (Skuja) Necchi et Entwisle 1990, *S. acuminata* Skuja in Flint 1950]

2) *S. huilensis* (Welw., W. et G. S. West) Skuja 1931b
[=*Batrachospermum huillense* Welwitsch ex W. et G. S. West 1897, *S. ateleia* Skuja 1938]

3) *S. delicatula* Skuja 1938
[=*Batrachospermum delicatulum* (Skuja) Necchi et Entwisle 1990]

4) *S. sinica* Jao 1941

5) *S. segawae* Kumano 1982c

This species was reported from Japan without the Latin name and description by Segawa (1939), so that, a new species name was validly published by Kumano (1982c).

6) *S. yutakae* Kumano 1982c

This species was reported from Japan without the Latin name and description by Segawa (1939), so that, a new species name was validly published by Kumano (1982c).

7) *S. tenuissima* (Collins) Skuja in Flint 1948
[*Batrachospermum vagum* (Roth) C. Agardh var. *flagelliforme* Sirodot f. *tennuissima* Collins 1895]

8) *S. gardneri* Skuja in Flint 1950

Based on the examination of the type specimen, Necchi et al. (1993) mentioned that *Sirodotia gardneri* could not be compared in all characteristics with the other species of *Sirodotia* because the type specimen was a male plants.

9) *S. polygama* Skuja in Flint 1948

According to Necchi et al. (1993), this species can be assigned to the section *Turfosa* sensu Necchi et Entwisle (1990).

Genus *Tuomeya* Harvey 1858
Type: *Tuomeya americana* (Harv.) Papenfuss 1958 [=*Baileya americana* Kuetzing 1857, *Tuomeya fluviatilis* Harvey 1858]
Syn.: section *Tuomeya* (Harvey) Necchi et Entwisle 1990.

Necchi and Entwisle (1990) treated the genus *Tuomeya* Harvey as a section *Tuomeya* of the genus *Batrachospermum* Roth, however, I prefer to keep the genus *Batrachospermum* and the genus *Tuomeya* separated.

1) *Tuomeya americana* (Harv.) Papenfuss 1958
[=*Baileya americana* Kuetzing 1857, *Tuomeya fluviatilis* Harvey 1858, *Batrachospermum americanum* (Kuetzing) Necchi et Entwisle 1990]

Genus *Nothocladus* Skuja 1934
Type: *N. nodosus* Skuja 1934 [=*N. tasmanicus* Skuja 1934]
Syn.: section *Nothocladus* (Skuja) Necchi et Entwisle 1990

Necchi and Entwisle (1990) treated the genus *Nothocladus* Skuja as a section *Nothocladus* of the genus *Batrachospermum* Roth, however, I prefer to keep the above both genera separated, because *Nothocladus* differs from *Batrachospermum* in diffuse gonimblast filaments and from *Sirodotia* in symmetrical carpogonia.

1) *N. nodosus* Skuja 1934
[=*N. tasmanicus* Skuja 1934, *Batrachospermum nodosum* (Skuja) Necchi et Entwisle 1990]

2) *N. lindaueri* Skuja 1944
[=*Batrachospermum lindaueri* (Skuja) Necchi et Entwisle 1990]

3) *N. afroaustralis* Skuja 1964

The taxa of the family Batrachospermaceae treated in this paper.

Family Batrachospermaceae Agardh 1824
Genus *Batrachospermum* Roth 1800
I. Subgenus *Acarposporophytum* Necchi 1990a
1) *B. brasiliense* Necchi 1987
II. Subgenus *Batrachospermum* Necchi 1990a
[=genus *Batrachospermum* Roth 1800]
1. Section *Helminthoidea* De Toni 1897
[=section *Helminthosa* Sirodot 1873, section "*Helminthoides*" Sirodot 1884]
1) *B. confusum* (Bory) Hassal 1845
[*B. ludibonda* Bory var. *confusa* Bory 1808, *B. helminthosum* Sirodot 1884, non *B. helmentosum* Bory 1808, *B. crouanianum* Sirodot 1884]
2) *B. boryanum* Sirodot 1884
[=*B anatinum* Sirodot 1884]
3) *B. boryanum* var. distensum (Kylin) Israelson 1942
[=*B. distensum* Kylin 1912]
4) *B. szschwanense* Jao 1941
2. Section *Batrachospermum*
[=section *Moniliformia* Sirodot 1873; section *Moniliformes* Sirodot 1884, section *Carpocontorta* Sheath et al. 1986]
1) *B. gelatinosum* (L.) Dc. 1802
[=*Conferva gelatinosa* L. 1753, *B. moniliforme* Roth 1800, *B. corbula* Sirodot 1884, *B. decaisneanum* Sirodot 1884, *B. gelatinosum* var. *decaisneanum* (Sirodot) Reis 1969, *B. radians* Sirodot 1884, *B. moniliforme* f. *lipsiensis* Roth 1800, *B. moniliforme* var. *scopula* Sirodot 1884]
2) *B. gelatinosum* f. *pyramidale* (Sirodot) Compère 1991
[=*B. pyramidale* Sirodot 1884, *B. moniliforme* f. *pyramidale* (Sirodot) Israelson 1942, *B. pygmaeum* Sirodot 1884]
3) *B. gelatinosum* var. *obtrullatum* Kumano et Watanabe 1983
4) *B. durum* C. A. Agardh 1824
[=*B. densum* Sirodot 1884, *B. moniliforme* f. *densum* (Sirodot) Israelson 1942, *B. gelatinosum* f. *densum* (Sirodot) Compère 1991]

5) *B. helminthoideum* (Sirodot) Mori 1975 [=*B. moniliform* var. *helminthoideum* Sirodot 1884]
6) *B. sporulans* Sirodot 1884
7) *B. godronianum* Sirodot 1884
8) *B. reginense* Sirodot 1884
9) *B. stagnale* (Bory) Hassal 1845 [=*B. ludibonda* Bory var. *stagnalis* Bory 1808, *B. ectocarpum* Sirodot 1875, 1884, *B. arcuatum* Kylin 1912]
10) *B. sinense* Jao 1941
11) *B. arcuatoideum* Reis 1973
12) *B. sporiferum* Mori 1975
13) *B. japonicum* Mori 1975
14) *B. polycarpum* Mori 1975
15) *B. cylindro-cellulare* Kumano 1978
16) *B. nova-guineense* Kumano et Johnstone 1983
17) *B. heterocorticum* Sheath et Cole 1990
18) *B. carpocontortum* Sheath et al. 1986.

3. Section *Setacea* De Toni 1897 [=section *Moniliformia* subsection *Capillacea* Sirodot 1873, section *Moriliformia* subsection *Setacea* Sirodot 1875, section *Setaces* Sirodot 1884]
1) *B. atrum* (Huds.) Harvey 1841 [=*Conferva atra* Hudson 1798, *B. gallaei* Sirodot 1884, *B. dillenii* Sirodot 1884, *B. tenuissimun* Bory, *B. angolense* W. West & G. S. West 1897, *Sirodotia angolensis* (W. West & G. S. West) Skuja in Reis 1960]
2) *B. puiggarianum* Grunow in Wittrock et Nordstedt 1883 [=*B. atrum* var. *puiggarianum* (Grunow) Necchi 1989, *B. schwackeanum* Moebius 1892, *B. nigrescens* W. West et G. S. West 1897, *Sirodotia nigrescens* (W. West & G. S. West) Skuja in Reis 1960]
3) *B. diatyches* Entwisle 1991 [=*B. nothogeae* Skuja, nom. nud.]

4. Section *Turfosa* Sirodot 1873 [=section *Turficola* De Toni 1897; section *Turficoles* Sirodot 1884]
1) *B. turfosum* Bory 1808 [=*Chara batrachosperma* var. *vega* Roth 1797, *Batrachospermum moniliforme* var. *vagum* (Roth) Roth, *B. vagum* (Roth) Ag. 1824, *B. keratophytum* Bory, *B. vegum* var. *keratophytum* (Bory) Sirodot]
2) *B. turfosum* var. *undulato-pdeicellatum* Kumano et M. Watanabe 1983
3) *B. orthostichum* Skuja 1931a
4) *B. periplocum* (Skuja) Necchi 1990a [=*B. vagum* var. *periclocum* Skuja 1969]
5) *B. tapirense* Kumano et Phang 1987

5. Section *Virescentia* Sirodot 1873, 1875 [=section *Viridia* De Toni 1897; section *Verts* Sirodot 1884, section *Claviformia* Reis 1973]
1) *B. helmentosum* Bory 1808 [=*B. coerulescens* Sirodot 1884, non *B. helminthosum* Sirodot 1884]
2) *B. sirodotii* Skuja ex P. Reis 1974 [=*B. virgatum* Sirodot 1884 nom. illeg., non *B. moniliforme* var. *virgatum* Kuetzing]
3) *B. graibussoniense* Sirodot 1884
4) *B. bruziense* Sirodot 1884
5) *B. testale* Sirodot 1884
6) *B. lochmodes* Skuja 1938
7) *B. vogesiacum* T. G. Schultz ex Skuja 1938 [=*B. vagum* var. *flagelliforme* Sirodot 1884, *B. flagelliforme* (Sirodot) Necchi 1989]
8) *B. gulbenkianum* Reis 1965b
9) *B. transtaganum* Reis 1970
10) *B. crispatum* Kumano et Ratnasabapathy (Ratnasabapathy and Kumano 1982a)
11) *B. bakarense* Kumano et Ratnasabapathy 1984
12) *B. gombakense* Kumano et Ratnasabapathy (Ratnasabapathy and Kumano 1982b)
13) *B. azeredoi* Reis 1967
14) *B. ferreri* Reis 1967

6. Section *Hybrida* De Toni 1897 [=section *Hybride* Sirodot 1884]
1) *B. virgato-decaisneanum* Sirodot 1884
2) *B. abilii* Reis 1965a
3) *B. mikrogyne* Flint et Skuja in Flint 1953

7. Section *Aristatae* Skuja 1933

7-1. Subsection *Aristatae* Kumano 1993
1) *B. cayennense* Montagne in Kuetzing 1849

2) *B. turgidum* Kumano 1982b
3) *B. longiarticulatum* Necchi 1990a
4) *B. beraense* Kumano 1978
5) *B. breutelii* Rabenhorst 1855
[=*B. dimorphum* Kuetzing 1857]

7-2. Subsection *Macrosporum* Kumano 1993
6) *B. equisetifolium* Montagne 1850
7) *B. hypogynum* Kumano et Ratnasabapathy
(Ratnasabapathy and Kumano 1982b)
8) *B. macrosporum* Montagne 1850
[=*B. oxycladum* Montagne 1850, *B. macrosporum* var. *oxycladum* (Montagne) Sirodot 1884, *B. excelsum* Montagne 1850, *B. macrosporum* var. *excelsum* (Montagne) Sirodot 1884]

8. Section *Contorta* Skuja 1931a
8-1. Subsection *Intortum* Kumano 1993
1) *B. intortum* Jao 1941
2) *B. pseudocarpum* Reis 1973
3) *B. woitapense* Kumano 1983c
4) *B. lusitanicum* Reis 1965a

8-2. Subsection *Torridum* Kumano 1993
5) *B. tortuosum* Kumano 1978
6) *B. tortuosum* var. *majus* Kumano 1982a
7) *B. torridum* Montagne 1850
[=*B. vagum* var. *torridum* (Montagne) Sirodot 1884]
8) *B. doboense* Kumano et Borden-Kerby 1986
9) *B. faroense* Kumano et Borden-Kerby 1986

8-3. Subsection *Procarpum* Kumano 1993
10) *B. procarpum* Skuja 1931a
11) *B. procarpum* var. *americanum* Sheath et al. 1992
12) *B. equisetoideum* Kumano et Necchi 1985
13) *B. cipoense* Kumano et Necchi 1985
14) *B. jolyi* Necchi 1986
15) *B. densiverticillatum* Necchi 1990a

8-4. Subsection *Kushiroense* Kumano 1993
16) *B. kushiroense* Kumano et Ohsaki 1983
17) *B. guyanense* (Montagne) Kumano 1990
[=*B. vagum* var. *guyanense* Montagne 1850]
18) *B. tabagatense* Kumano et Borden-Kerby 1986
19) *B. nechochoense* Kumano et Borden-Kerby 1986
20) *B. nonocense* Kumano et Liao 1987
21) *B. iriomotense* Kumano 1982a
22) *B. globosporum* Israelson 1942
23) *B. breviarticulatus* (Necchi et Kumano) Necchi 1990a
[=*B. capense* Starmach ex Necchi et Kumano var. *breviarticulatum* Necchi et Kumano 1984]
24) *B. capense* Starmach ex Necchi et Kumano 1984
[=*B. capense* Starmach 1975, nom. illeg.]
25) *B. skujanum* Necchi 1986

8-5. Subsection *Ambiguum* Kumano 1993
26) *B. tiomanense* Kumano et Ratnasabapathy
(Ratnasabapathy and Kumano 1982a)
27) *B. omodoense* Kumano et Borden-Kerby 1986
29) *B. nodiflorum* Montagne 1850
[=*B. vagum* var. *nodiflorum* (Montagne) Sirodot 1884]
30) *B. ambiguum* Montagne 1850
[=*B. bicudoi* Necchi 1986, *B. exsertum* Necchi 1986, *B. basilare* Flint et Skuja in Flint 1953].
31) *B. louisianae* Skuja in Flint 1949
32) *B. henriquesianum* Reis 1972
33) *B. mahlacense* Kumano et Borden-Kerby 1986
34) *B. gracillimum* W. West et G. S. West emend. Necchi 1989
35) *B. gibberosum* (Kumano) Kumano 1986
[=*Tuomeya gibberosa* Kumano 1978]

Genus *Sirodotia* Kylin 1912
[=section *Sirodotia* (Kylin) Necchi et Entwisle 1990]
1) *S. suecica* Kylin 1912
[=*Batrachospermum suecicum* (Kylin) Necchi et Entwisle 1990, *S. fennica* Skuja 1931b, *Batrachospermum fennicum* (Skuja) Necchi et Entwisle 1990, *S. acuminata* Skuja ex Flint 1950]
2) *S. huillensis* (Welw., W et G. S. West) Skuja 1931b
[=*Batrachospermum huillense* Welwitsch ex W. et G. S. West 1897, *S. ateleia* Skuja 1938]

3) *S. delicatula* Skuja 1938
[=*Batrachospermum delicatulum* (Skuja) Necchi et Entwisle 1990]
4) *S. sinica* Jao 1941
5) *S. segawae* Kumano 1982c
6) *S. yutakae* Kumano 1982c
7) *S. tenuissima* (Collins) Skuja ex Flint 1948
[=*Batrachospermum vagum* (Roth) C. Agardh var. *flagelliforme* Sirodot f. *tenuissima* Collins 1895]
8) *S. gardneri* Skuja in Flint 1950
9) *S. polygama* Skuja in Flint 1948

Genus *Tuomeya* Harvey 1858
[=*section Tuomeya* (Harvey) Necchi et Entwisle 1990]
1) *Tuomeya americana* (Harv.) Papenfuss 1958
[=*Baileya americana* Kuetzing 1857, *Tuomeya fluviatilis* Harvey 1858, *Batrachospermum americanum* (Kuetzing) Necchi et Entwisle 1990]

Genus *Nothocladus* Skuja 1934
[=section *Nothocladus* (Skuja) Necchi et Entwisle 1990]
1) *N. nodosus* Skuja 1934
[=*N. tasmanicus* Skuja 1934, *Batrachospermum nodosum* (Skuja) Necchi et Entwisle 1990]
2) *N. lindaueri* Skuja 1944
[=*Batrachospermum lindaueri* (Skuja) Necchi et Entwisle 1990]
3) *N. afroaustralis* Skuja 1964

Acknowledgments

The author wishes to express his sincere thanks to Prof. T. Yoshida of Hokkaido University for his valuable suggestions and criticism.

References

Agardh, C. A. 1824. Systema Algarum. Literis Berlifgianis, Lundae.

Bory, de Sait-Vincent 1808. Memoire sur le genre *Batrachosperma* de la famille des Conferves. Ann .Mus. Hist. Nat. Paris **12:** 310–332, pl. 29–31.

Bourrelly, P. 1970. Les algues d'eau douce. 3 Les algues bleues et rouges. N. Boubee & Cie, Paris.

Compère, P. 1991. Taxonomic and nomenclatural notes on some taxa of the genus *Batrachospermum* (Rhodophyceae) Belg. Journ. Bot. **124:** 21–26.

De Candolle, A. P. 1802. Rapport sur les Conferves. J. Phys. Chim Hist. Nat. **54:** 421–441.

De Toni, J. B. 1897. Sylloge algarum. 4: Florideae, Sectio I. Typis Seminarii, Patavii

Dillenius, J. J. 1741. Historia Muscorum. Oxonii: e Theatro Sheldoniano.

Entwisle, T. J. 1992. The setaceous species of *Batrachospermum* (Rhodophyta): A re-evaluation of *B. atrum* (Hudson) Harvey and *B. puggarianum* Grunow including the description of *B. diatyches* sp. nov. from Tasmania, Australia. Muelleria **7:** 425–445.

Farr, E. R., Leussink, J. A. & Staflue F. A. 1979. Index nominum genericorum (plantarum). I. Regn. Veget. 100.

Flint, L. N. 1948. Studies on freshwater red algae. Am. J. Bot. **35:** 428–433.

Flint, L. N. 1949. Studies on freshwater red algae. Am. J. Bot. **36:** 549–552.

Flint, L. N. 1950. Studies on freshwater red algae. Am. J. Bot. **37:** 754–757.

Flint, L. N. 1953. Two new species of *Batrachospermum*. Proc. La. Acad. Sci. **16:** 10–15.

Greuter, W. et al. 1988. International Code of Botanical Nomenclature. Regn. Veget. 118. Koeltz Scientific Books, Koenigstein.

Hamel, G. 1925. Floridees de France. IV. Rev. Algol. **2:** 280–309.

Harvey, W. H. 1841. A manual of the British Algae. van Voorst, London.

Harvey, W. H. 1858. Nereis Borealis-Americana: or a contribution to a history of the marine algae of North America. part 3. Published by the Smithonian Institution, Washington.

Hassall, A. H. 1845. A history of the British freshwater algae. Taylor, Walton & Maberly, London.

Hudson, G. 1798. Flora Anglica ed. III. London.

Israelson, G. 1942. The freshwater Florideae of Sweden. Symb. Bot. Upsal. **6:** 1–135.

Jao, C. C. 1941. Studies on the freshwater red algae of China. VIII. A preliminary account of the Chinese freshwater Rhodophyceae. Sinensia **12:** 245–290.

Johnstone, I. M., Mukiu, J., Nagari, T. Pokihian, M. & Rau, M. 1980. *Batrachospermum*; first freshwater red algal record for New Guinea. Science in New Guinea **7:** 1–5.

Kumano, S. 1978. Notes on freshwater red algae from West Malaysia. Bot Mag. Tokyo **91:** 97–107.

Kumano, S. 1982a. Four taxa of the section *Contorta* of the genus *Batrachospermum* (Rhodophyta, Nemalionales) from Iriomote Jima and Ishigaki Jima, subtropical Japan. Jpn. J. Phycol. **30:** 181–187.

Kumano, S. 1982b. Four taxa of the section *Moniliformia, Hybrida* and *Setacea* of the genus *Batracho-*

spermum (Rhodophyta, Nemalionales) from temperate Japan. Jpn. J. Phycol. **30:** 289–296.

Kumano, S. 1982c. Development of carpogonium and taxonomy of six species of the genus *Sirodotia*, Rhodophyta, from Japan and west Malaysia, Bot. Mag. Tokyo **95:** 125–137.

Kumano, S. 1983. Studies on freshwater Rhodophyta of Papua New Guinea II. *Batrachospermum woitapense*, sp. nov. from the Papuan Highlands. Jpn. J. Phycol. **31:** 76–80.

Kumano, S. 1984a. Studies on freshwater red algae of Malaysia V. Early development of carposporophytes of *Batrachospermum cylindrocellulare* Kumano and *B. turtuosum* Kumano. Jpn. J. Phycol. **32:** 24–28.

Kumano, S. 1984b. Some observations on *Batrachospermum intortum* Jao and *B. sinense* Jao (Rhodophyta, Nemalionales) from Szechwan in China. Jpn. J. Phycol. **32:** 221–226.

Kumano, S. 1986. Studies on freshwater red algae of Malaysia VI. Morphylogy of *Batrachospermum giberosum* (Kumano), comb. nov. Jpn. J. Phycol. **34:** 19–24.

Kumano, S. 1990. Carpogonium and carposporophytes of Montagne's taxa of *Batrachospermum* (Rhodophyta) from French Guiana. Cryptogam. Algol. **11:** 281–192.

Kumano, S. & Bowden-Kerby, W. A. 1986. Studies on the freshwater Rhodophyta of Micronesia I. Six new species of *Batrachospermum* Roth. Jpn. J. Phycol. **34:** 107–128.

Kumano, S. & Johnstone, I. M. 1983. Studies on the freshwater Rhodophyta of Papua New Guinea I. *Batrachospermum nova-guineense* sp. nov. from the papuan Lowlands, Jpn. J. Phycol. **31:** 65–70.

Kumano, S. & Liao, L. M. 1987. A new species of the section *Contorta* of the genus *Batrachospermum* (Rhodophyta, Nemalionales) from Nonoc Island, the Philippines. Jpn J. Phycol. **35:** 99–105.

Kumano, S. & Necchi, O. Jr. 1985. Studies on the freshwater Rhodophyta of Brazil II. Two new species of *Batrachospermum* from states of Amazonas and Minas Gerais. Jpn. J. Phycol. **32:** 221–226.

Kumano, S. & Necchi, O. Jr. 1990. *Batrachospermum macrosporum* Montagne from South America. Jpn. J. Phycol. **38:** 119–123.

Kumano, S. & Ohsaki, M. 1983. *Batrachospermum kushiroense*, sp. nov. (Rhodophyta, Nemalionales) from Kushiro Moor in cool temperate Japan. Jpn. J. Phycol. **31:** 156–160.

Kumano, S. & Phang, S. M. 1987. Studies on freshwater red algae of Malaysia VII. *Batrachospermum tapirense*, sp. nov. from Sungai Tapir, Johor, Peninsula Malaysia Jpn. J. Phycol. **35:** 259–264.

Kumano, S. & Ratnasabapathy, M. 1982. Studies on freshwater red algae of Malaysia III. Development of carposporotphytes of *Batrachospermum cayennense* Montagne *B. beraense* Kumano and *B. hypogynum* Kumano et Ratnasabapathy. Bot. Mag. Tokyo **95:** 219–228.

Kumano, S. & Ratnasabapathy, M. 1984. Studies on freshwater red algae of Malaysia IV. *Batrachospermum bakarense*, sp. nov. from Sungai Bakar, Kelanta, West Malaysia. Jpn. J. Phycol. **32:** 19–23.

Kumano, S., Seto, R. & Hirose, H. 1970. On the development of the carposporophytes in several species of the Batrachospermaceae with special refernce to their phylogenetic relations. Bull. Jpn. Soc. Phycol. **18:** 116–120.

Kumano, S. & Watanabe, M. 1983. Two new varieties of *Batrachospermum* (Rhodophyta) from Mt. Albert Edward, Papua New Guinea. Bull. Natn. Sci. Mus. Tokyo Ser. B. **9:** 85–94.

Kuetzing, F. T. 1849. Species Algarum. p. 537, Lipsiae.

Kuetzing, F. T. 1857. Tabulae Phycologicae. Nordhausen.

Kylin, H. 1912. Studien ueber die schwedischen Arten der Gattung *Batrachospermum* Roth und *Sirodotia* nov. gen. Nova Acta Regiae Societatis Scientarum Upsalinsis ser. IV. **3:** 1–40.

Kylin, H. 1956. Die Gattungen der Rhodophyceen. CWK Gleerups, Lund.

Linnaeus, C. 1753. Species plantarum. Imp. Laurentii Salvii, Holmiae.

Linnaeus, C. 1755. Flora Suecica. Laurentii Salvii, Stopckholmiae.

Moebius, M. 1892. Ueber einige brasilianische Algen. Ber. dt. Bot. Ges. **10:** 18–26.

Montagne, C. 1850. Cryptogamia Guyanensis, seu plantarum cellarrium in Guyana gallica annis 1835–1849 a cl. Leprieur collectarum enumeratio universalis. Ann. Sci. Nat. Bot. ser. 3, **14:** 83–309.

Mori, M. 1975. Studies on the genus *Batrachospermum* in Japan. Jpn. J. Bot **20:** 461–484.

Necchi, O. Jr. 1986. Studies on the freshwater Rhodophyta of Brazil 4. Four new species of *Batrachospermum* (Section *Contorta*) from the southern states of São Paulo. Rev. Bras. Biol. **46:** 17–25.

Necchi, O. Jr. 1987. Studies on the freshwater Rhodophyta of Brazil 3. *Batrachospermum brasiliense* sp. nov. from the state of São Paulo, southern Brazil. Revista Brasileira de Biologia **47:** 441–446.

Necchi, O. Jr. 1989. Rhodophyta dea água doce do estado de São Paulo: Levantamento taxonômico. Bol. Bot. Univ. S. Paulo **2:** 11–69.

Necchi, O. Jr. 1990a. Revision of the genus *Batrachospermum* Roth (Rhodophyta, Batrachospermales) in Brazil. Bibl. Phycol. **84:** 1–201.

Necchi, O. Jr. 1990b. Evaluation of numeric taxonomic characters in Brazilian species of *Batrachospermum* (Rhodophyta, Batrachospermales) in Brazil. Rev. Bras. Biol. **50:** 627–641.

Necchi, O. Jr. & Entwisle, T. J. 1990. A reappraisal of generic and subgeneric classification in the Batrachospermaceae (Rhodophyta). Phycologia **29:**

478–488.

Necchi, O. Jr. & Kumano, S. 1984. Studies on the freshwater Rhodophyta of Brazil 1. Three taxa of *Batrachospermum* Roth from the Northeastern State of Sergipe. Jpn. J. Phycol. **32:** 348–353.

Necchi, O. Jr., Sheath, R. G. & Cole, K. M. 1993. Distribution and systematics of the freshwater genus *Sirodotia* (Batrachospermales, Rhodophyta) in North America. J. Phycol. **29:** 236–243.

Papenfuss, G. F. 1958. Notes on algal nomenclature IV. Taxon **7:** 104–109.

Rabenhorst, L. 1855. Beitrag zur Kryptogamenflora Sudafrica. Pilze und Algen. Allgem. deutsche Naturalist Zeitung **1:** 282.

Rabenhorst, L. 1868. Flora Europaea algarum aquae dulcis et submarinae. Eduardum Kummerum, Lipsiae.

Ratnasabapathy M. & Kumano, S. 1982a. Studies on freshwater red algae of Malaysia I. Some taxa of the genera *Batrachospermum, Ballia* and *Caloglossa* from Pulau Tioman, West Malaysia. Jpn. J. Phycol. **30:** 15–22.

Ratnasabapathy M. & Kumano, S. 1982b. Studies on freshwater red algae of Malaysia II. Three species of *Batrachospermum* from Sungai Gombak and Sungai Pusu, Selangor, West Malaysia. Jpn. J. Phycol. **30:** 119–124.

Reis, M. P. dos 1960. Revião dos espécime de *Batrachospermum* Roth e *Sirodotia* Kylin dos herbarios des Institutos Botânicos de Coimbra e Lisboa. Bol. Soc. Brot. ser. 2, **34:** 37–55.

Reis, M. P. dos 1965a. Subsídios para o conhecimento das Rodofíceas de água doce de Portugal V. Bol. Soc. Brot. ser. 2, **39:** 137–145.

Reis, M. P. dos 1965b. *Batrachospermum gulbenkianum*, sp nov. Anuario da Sociedade Brotteriana **31:** 31–37.

Reis, M. P. dos 1967. Duas espécies novas de *Batrachospermum* Roth: *B. azerdoi* e *B. ferreri*. Bol. Soc. Brot. ser. 2, **41:** 167–179.

Reis, M. P. dos 1969. Subsídos para o conhecimento das Rodofíceas de água doce de Portugal VII. Bol. Soc. Brot. ser. 2, **43:** 183–192.

Reis, M. P. dos 1970. Rhodophyceae novae. Memórais da Sociedade Broteriana **21:** 23–26.

Reis, M. P. dos 1972. *Batrachospermum henriquesianum*, sp nov. Bol. Soc. Brot. ser. 2, **46:** 181–185.

Reis, M. P. dos 1973. Subsídios para o conhecimento das Rodofíceas de água doce de Portugal VIII. Bol. Soc. Brot. ser. 2, **47:** 139–157, 5pl.

Reis, M. P. dos 1974. Chaves para a identificação das espécies portuguesas *Batrachospermum* Roth. Anúario da Sociedade Brotteriana **40:** 37–129.

Reith, A. 1979. Ein *Batrachospermum* der Sektion *Contorta* Skuja aus Kuba. Kulturpflanze **27:** 265–281.

Roth, A. W. 1797. Bermerkungen über das Studium der cryptogamische Wassergewachse. Gebudern Hahn., Hannover.

Roth, A. W. 1800. Tentamen Florae Germanicae 3. Bibl. Gleditschiano, Lipsiae.

Sagawa, S. 1939. Two species of *Sirodotia* found in Japan. Shokubutu to Doubutu **7:** 2033–2036.

Sheath, R. G. & Burkholder, J. M. 1983. Morphometry of *Batrachospermum* populations intermediate between *B. boryanum* and *B. ectocarpum* (Rhodophyta). J. Phycol. **19:** 324–331.

Sheath, R. G. & cole, K. M. 1990. *Batrachospermum heterocorticum* sp. nov. and *Polysiphonia subtilissima* (Rhodophyta) from Florida spring-fed streams. J. Phycol. **26:** 563–568.

Sheath, R. G. & Morison, M. O., Cole, K. M. & Vanalstyne, K. L. 1986. A new species of freshwater Rhodophyta, *Batrachospermum carpocontorum*. Phycologia. **23:** 321–330.

Sheath, R. G., Vis, M. L. & Cole, K. M. 1992. Distribution and systematics of *Batrachospermum* (Batrachospermales, Rhodophyta) in North America. J. Phycol. **28:** 234–346.

Sirodot, S. 1873. Nouvelle classification des algues d'eau douce du genre *Batrachospermum*; développement; générations alternantes. C. R. Hebd. Seances Acad. Sci. Paris **76:** 1216–1220.

Sirodot, S. 1875. Observations sur le développement des algues d'eau douce composant le genre *Batrachospermum*. Bul. Soc. Bot. France **22:** 128–145.

Sirodot, S. 1884. Les Batrachospermes, organisation, fonction, développement, classification. G. Masson, Paris.

Skuja, H. 1931a. Einiges zur Kenntnis der brasilianschen Batrachospermen. Hedwigia **71:** 78–87.

Skuja, H. 1931b. Untersuchungen uber die Rhodophyceen des Süsswassers. I–II. Archiv Prot. **74:** 297–309.

Skuja, H. 1933. Untersuchungen über die Rhodophyceen des Süsswassers. III. Arch. Prot. **80:** 357–366.

Skuja, H. 1934. Untersuchungen über die Rhodophyceen des Süsswassers. V. Beihefte Botanischen Zentralblatt **52B:** 179–188.

Skuja, H. 1938. Die Süsswasserrhodophyceen der deutschen limnologischen Sunda-Expedition. Arch. Hydrobiol. suppl. **15:** 603–637, pl. 29–35.

Skuja, H. 1944. Untersuchungen über die Rhodophyceen des Süsswassers. VII-XII. Acta Horti Botanici Universitatis, Latvia **14:** 1–64.

Skuja, H. 1964. Weiteres zur Kenntnis der Süsswasserrhodophyceen der Gattung *Nothocladus*. Rev. Algol. N. S. **7:** 304–314.

Skuja, H. 1969. Eigentumliche morphologische Anpassung eines *Batrachospermum* gegen mechanische Schadigung in fliessendum Wasser. Öester. bot. Zeit. **116:** 55–64.

Starmach, K. 1975. Algae from montane streams on the Island Mahe, in the Seychelles. Acta Hydrobiol. **17:** 210–209.

Starmach, K. 1982. Red algae in the Kryniczanka

stream. Fragmenta Floristica et Geobotanica ann. **28**: 257–293.
West, W. & West, G. S. 1897. Welwitsch's African freshwater algae (part 6). J. Bot. Lond. **35**: 235–243.
Wittrock & Nordstedt 1883. Algae Exsiccatae **11**: 1, no. 501.

熊野　茂：カワモズク科（カワモズク目，紅藻植物）の分類

この総説では，カワモズク科の分類に関する最近の改変，形態学的観点から見た属・節間の類縁関係，及び，現在までに記載された104分類群のリストを，分類学的ノートを付して紹介した。この総説で取り扱った分類群はカワモズク科の4属である，即ち，カワモズク属（91分類群），ユタカカワモズク属（9種），ツオメヤ属（1種），及びノトクラヅス属（3種）。カワモズク属(91分類群)は2つの亜属からなる，即ち，無果胞子体亜属（1種）とカワモズク亜属（90分類群)。カワモズク亜属は次の8つの節から構成される，即ち，ヘルミントイデア節（4分類群）：カワモズク節（18分類群）：セタケア節（3分類群）：ツルフォサ節（5分類群）：ヴィレンセンチア節（14分類群）：ヒブリダ節（3分類群）：2つの亜節，即ち，マクロスポルム亜節とアリスタタエ節亜節からなるアリスタタエ節（8分類群）：5つの亜節，即ち，イントルタ亜節，トリヅム亜節，ブロカルブム亜節，クシロエンシス亜節及びアムビグウム亜節からなるコントルタ節（35分類群)。(657 神戸市灘区六甲台1–1　神戸大学理学部生物学教室)

(*Received April 19, 1993, Accpeted July 9, 1993*)

藻類 Jpn. J. Phycol. 41: 275–276, September 20, 1993

斎藤　譲：*Janczewskia tokidae* は *J. morimotoi* の若い大形個体

Yuzuru Saito: *Janczewskia tokidae* is a young and large frond of *J. morimotoi*

Yuzuru Saito, Faculty of Fisheries, Hokkaido University, Hakodate, Hokkaido, 041 Japan

Janczewskia tokidae Saito was established from material collected on July 1, 1965 at Kinaoshi, Pacific coast of southern Hokkaido. The type specimen was remarkably large, with further branched free branches on both male and female gametophytes, which occurred only on the tetrasporangial fronds of *Janczewskia morimotoi* Tokida. The materials collected in August 1975 from the same locality were similar in habit to *J. morimotoi*, in that the free branches possessed branchlets only on the tetrasporangial fronds. The seasonal observations of the Kinaoshi materials strongly indicate that *J. tokidae* is synonymous with the earlier described *J. morimotoi*, and that the branchlets represent early seasonal forms of the same species. Thus the author now proposes to relegate *J. tokidae* as a synonym of *J. morimotoi*.

はじめに：筆者（Saito 1971）が *Janczewskia tokidae* として報告した材料は，1965年7月1日，北海道渡島半島の太平洋沿岸に位置する木直（きなおし，南茅部町の一部）で採集したものであった。かつて，筆者の恩師時田　郁先生が樺太海馬島で森本忠夫氏の1943年9月採集の材料にもとづいて設立した *Janczewskia morimotoi* Tokida (1947) は，その発出する枝も含めるならば直径 4–5 mm の球状で，四分胞子体だけが更に分岐する枝を有する，といった植物だったのに比較すると，体は発出する枝まで含めれば直径が約 15 mm と大きく，雄性体や雌性体でも更に分岐する小枝を有する，等の相違が見られた。

筆者は上の記載を試みた後も，上記した新種の極端な枝状部の発達，枝や生殖器官の宿主との酷似，等から，宿主のウラソゾとの関係にさえ疑問を抱き，1975年採集の材料で宿主と寄生物の性別の関係を調べたことがあった（斎藤ら 1977）。しかしながら，両者の性別に特記すべき関係は見出されず，更にその年8月に同じ木直で採集した宿主，寄生物両者の果胞子と四分胞子の大きさにもかなりの差異が認められ，結局両者は別個の植物体，との結論を得た。

この1975年8月に採集した材料は体の寸法を調べた他，胞子発生についても観察した。そこで8月の材料はかなり小さく，時田　郁先生の報告した樺太の *J. morimotoi* との差別を認めるのは無理と知って，その秋に日本の *Janczewskia* 研究のため北大を訪問した Dr. A. M. Nonomura に，当地の材料は *J. morimotoi* として仕事を進めるべき，を助言したので，彼が北大で実施した研究の報告はすべてその学名を使用している。

ここでは，主として1989年の4月から9月までの期間，同じ木直で採集した材料を計測した値から，*J. tokidae* は *J. morimotoi* の synonym と見なされるべきもの，とした結論に就いて記述したい。

1989年の観察：4月29日に木直で5個体の四分胞子体を採集したが，発出する枝状部も含めた体の直径は平均で 6.5 mm であった。7月6日には2個体が得られ，直径の平均は 6.9 mm，7月20日にも同じく2個体を採集して平均 7.3 mm を得たので，この期間に多少の生長を示した。なお，7月20日には雄性体，雌性体もそれぞれ2個体ずつ採集されたが，前者は 8.5 mm，後者は 8.2 mm で，同日採集の四分胞子体よりいくぶん大きい。それ以後の採集は8月30日であったが，最大形だった雌性体でも19個体の平均が 2.5 mm，さらに9月19日ともなれば最大の雄性体で 1.6 mm であった。したがって，目立って体が小型化したのは7月20日以降8月30日の間だったと言えよう。

個々の生殖器官を有する枝や嚢果等の寸法では，雄性体の末端枝の頂端に形成される精子器托の季節を追っての小型化が最も目立ち，7月12日に直径 (1011±162) μm（20–50個に就いて計測して平均した。以下同様）を示していたものが，8月30日に到って (705±94) μm，9月19日には (507±156) μm と，ほぼ直線的に小型化が進行した。続いて雌性体上の嚢果の直径も，7月12日には (683±114) μm 有ったものが，8月30日には (457±93) μm，9月19日に (380±152) μm と直線的ながらも，雄性体の精子器托より幾分ゆるやかな小型化を示した。それに比して四分胞子体の成実枝の直径は，7月6日に (330±28) μm，9月19日になって

も (287±40) μm と，小型化はさして顕著であったとは言いがたい。

塊状実質部の周辺に発出する枝の更なる分岐を，まず雌性体に就いて見ると，7月20日以前の材料では二回分岐も稀でなく，約20%の枝で観察されたし，一回だけの分岐を示したものは半数以上に上っていた。8月30日，9月19日採集材料の枝では，二回の分岐を示したものは殆ど無く，8月30日採集材料の枝で一回の分岐を示したものは約40%であり，9月19日採集材料の枝では10%以下になった。7月12日採集の雄性体では，一回だけの分岐を示したものが90%以上であったのに，8月30日，9月19日採集材料の枝では，殆ど分岐は見られなかった。しかしながら四分胞子体では，7月20日採集材料の枝で，ほぼ半数ずつ，が一回分岐と二回分岐を示した。さらに8月30日，9月19日採集の材料になると約10%の枝が一回の分岐を示し，他は分岐の見られない枝であった。

考　察：以上のように，筆者が最初 *Janczewskia tokidae* として報告した材料は，季節を追って小型化することが明らかで，その小型化の程度も著しいとは言え，変化はおおむね連続的であり，秋に小型化した材料は時田先生が樺太から報告した *Janczewskia morimotoi* モリモトソゾマクラで報告された寸法の範囲に納まる事が明らかになった。この性質は，筆者らが以前から報告して来たハネソゾ（工藤・斎藤 1985）やミツデソゾ（斎藤 1989）の秋の小型化と質的に同様なものと見なすべきものと言えよう。

さらに，木直で9月19日に採集した個体の枝は，ほぼ10%が更なる分岐を示しただけであった。とは言え，統計処理を目的とした採集以外は一般に大形個体を目指して採集するのが普通で，森本忠夫氏の9月の採集品が比較的大型で，四分胞子体が分岐する枝を有していた，と言うのは普通のことで，特記するには当らないのでは無かろうか。

引　用　文　献

工藤清見・斎藤　譲　1985. *Laurencia pinnata* Yamada ハネソゾの秋型. 藻類 **33**: 77–78.

Saito, Y. 1971. Two species of *Janczewskia* from Japan and their systematic relationships. Proc. 7th Internat. Seaweed Symposium. 146–149.

斎藤　譲　1989. 日本産オモテソゾはミツデソゾと同一物. 藻類 **37**: 208–212.

斎藤　譲・米田哲朗・吉川元秀　1977. 寄生性紅藻 *Janczewskia tokidae* と宿主 *Laurencia nipponica* ウラソゾの関係. 藻類 **25**(山田先生追悼号): 311–317.

Tokida, J. 1947. Notes on some new or little known marine algae, 1. J. Jap. Bot. **21**: 127–130.

ニュース

13th International Diatom Symposium

Hotel Villa del Mare, Aquafredda di Maratea (Potenza), Italy
September 1-7, 1994

(第13回国際珪藻シンポジウムのご案内)

標記の国際シンポジウムが1994年9月1日—7日にわたって,南イタリーのAquafredda di Maratea (Potenza) の Hotel Villa del Mare で(コンビーナーはナポリ臨海実験所植物研究室主任 Donata Marino 教授)でおこなわれます。

このシンポジウムには珪藻に関係のある全ての分野,即ち,形態学,細胞学,遺伝学,生殖生物学,生理学,生化学,分子生物学,古生物学,系統学,分類学などを含み,また,海産珪藻,淡水産珪藻,現生の珪藻,化石の珪藻,全てにわたってのロ頭発表と展示発表が求められています。

会場にはナポリ臨海実験所から約 200 Km 南のホテル (Hotel Villa del Mare) が予定されていますが,このあたりは空も海も青く素晴らしいところと聞いています。興味のある方のご参加を希望します。講演申込,宿泊,経費などについての詳細は後日連絡のあり次第ご案内申し上げますが,詳細についての問い合わせは下記の現地事務局か,または,東京珪藻研究所宛でお願いします。

現地事務局:Jean Gilder Congressi snc, 13th Int. Diatom Symposium, via G. Quagliariello 35/E, 1-80131 Napoli, Italy.
Tel: +39 (0) 81-546-3779 or 545-4617. Fax: +39 (0) 81-546-4617

日本珪藻学会:〒184 東京都小金井市本町3-8-9-813
東京珪藻研究所 小林 弘
Tel. & Fax.: 0423-84-7795.

—学 会 録 事—

1. 学会誌改革実務委員会報告

1993年3月の総会で承認された学会誌改革のための実務委員会が有賀祐勝，石川依久子，渡辺信，岡崎恵視，片山舒康，川井浩史，小林弘，田中次郎，原慶明，出井雅彦，中原紘之，堀口健雄，藤田大介，大野正夫，真山茂樹，野崎久義，横浜康継のメンバーで発足し，第1回の委員会を5月29日，第2回を6月26日に東京水産大学資源育成学科会議室で開催した。

2. 持回り評議委員会報告

6月11日付けの郵送で次の2件について審議し，いずれも承認された。

(1) 学会誌改革実務委員会要項ならびに委員（上記1参照）を承認した。

(2) 海藻の明日を考えるシンポジウム実行委員会（伊藤啓二会長）から依頼のあったシンポジウム「海藻の明日を考える」(1993年9月3日）の後援を承認した。

—会 員 移 動—

新 人 会

住 所 変 更

訃　　報

本会会員　安藤一男氏は去る1993年8月9日逝去されました。謹んで哀悼の意を表します。　日本藻類学会

退　　会

日本学術会議だより　No.29

「学術分野における国際貢献についての基本的提言」を採択

平成５年５月　日本学術会議広報委員会

日本学術会議は，去る4月21日から23日まで第116回総会を開催しました。今回の日本学術会議だよりでは，同総会の議事内容及び同総会で採択された「学術分野における国際貢献についての基本的提言」等についてお知らせいたします。

日本学術会議第116回総会報告

日本学術会議第116回総会（第15期・第５回）が，４月21日～23日の３日間にわたって開催された。

総会の初日の午前は，会長からの前回総会以降の経過報告に続いて，各部，各委員会等の報告が行われた。次いで，今回総会に提案されている２案件について，それぞれ提案説明がなされた後，質疑応答が行われた。

午後からも提案案件に対する質疑応答が行われた後，引き続き各部会が開催され，午前中に提案説明された総会提案案件の審議が行われた。

総会２日目の午前は，前日提案された２案件及び緊急に提案された１案件の審議・採決が順次行われた。

まず，「国際対応委員会の改組について(申合せ)」が採択された。これは，学術の国際化の急速な進展に伴い，国際学術団体及び国際学術協力事業への対応の重要性がますます増大してきており，日本学術会議としてもその職務を遂行する上で，学術の国際化に関する状況の迅速かつ的確な把握が不可欠であるという観点から，より広範囲にわたる国際学術情報の収集と，それに基づく適切な対応ができるよう，国際対応組織の充実強化を図るために，必要な措置を講じたものである。

次いで，「学術分野における国際貢献についての基本的提言」が採択された。本件については，日本学術会議第15期活動計画の中の重点目標として掲げられており，また，一昨年秋の第113回総会において内閣官房長官から，「学術研究の分野で我が国がどのような国際的貢献をなすべきかについて全学問領域から総合的に検討し，意見を出すよう」求められ，以来，日本学術会議における重要案件として鋭意審議してきたものである。

提言は，１．学術分野における国際貢献の意義，２．学術分野における国際貢献の在り方，３．学術分野における国際貢献を進めるための提案という構成内容になっており，日本学術会議は，今後とも，本提言に基づき，具体的な諸課題について検討していくこととしている。

最後に，上記の提言に基づき，日本学術会議は，国際貢献のための新しいシステムを構築するための具体的方策を直ちに検討し，その速やかな推進を図るという内容の「学術分野における国際貢献についての基本的提言に関する附帯決議」が採択された。

また，「学術分野における国際貢献についての基本的提言」に関する会長談話を22日付けで発表した。

午後からは，現在，常置委員会，特別委員会で審議されている懸案事項について，自由討議が行われた。

総会３日目は，午前は各特別委員会，午後は各常置委員会・国際対応委員会がそれぞれ開催された。

なお，近藤会長が，４月22日に河野内閣官房長官と，また，同27日に宮澤内閣総理大臣とそれぞれ会見し，「学術分野における国際貢献についての基本的提言」を手渡すとともに，同提言について報告した。

学術分野における国際貢献についての基本的提言（抜粋）

（前文略）

１．学術分野における国際貢献の意義

（本文略）

２．学術分野における国際貢献の在り方

（本文略。項目のみ）

(1) 対等・互恵の原則に基づいた国際学術協力の強化
(2) 国際学術協力の積極的発議等
(3) 人材育成への協力による国際貢献の推進
(4) 我が国の学術情報の提供・紹介の促進
(5) 学術に関する国際団体への対応強化

３．学術分野における国際貢献を進めるための提案

前節で述べた我が国の学術分野における国際貢献の在り方を踏まえ，これを推進していくために，以下の事項を提案する。

(1) 我が国からの情報提供機能等の充実・強化

① 学会の支援・育成

我が国の学会は，高等教育研究機関や産業界の研究成果の発表の場として重要な役割を果たしてきた。また，研究者相互の活発な国際交流等を通じて，情報の提供に努めているところである。しかしながら，ほとんどの学会は，資金の不足から，必要な活動も十分にできない状況にある。

学術分野における国際貢献という観点において，非政府機関（ＮＧＯ）としての学会の果たす役割は極めて大きく，それらが有する情報提供機能を最大限に発揮できるよう，学会の支援・育成を図る必要がある。

② アジア地域における学術研究に関する連携の強化

我が国と地理的・歴史的・文化的な関係の深いアジア地域の学術の発展に資するため，アジア地域の科学者や学術研究機関の間の学術研究ネットワークを拡充・強化することが必要である。また，将来的には，アジアの学術振興のための国際的な組織の在り方について，関係各国の科学者と協議していく必

要がある。

(2) 国際学術交流のための支援の充実

① 学術研究機関の整備等

新しい知識の創造と発展は，優れた研究者が集い，切磋琢磨するところから生まれるものであり，研究者の未知への挑戦に対して最も適切な施設・資金・支援システムなどの研究環境を提供することが必要である。したがって，全世界の研究者が日本で研究することに魅力を感じ，充実した研究生活が送れるように，学術研究機関の整備及び適切な運営を図るべきである。

② 来日研究者・留学生への支援の充実

学術分野における国際貢献の第一歩として，各国の人材育成への協力，とりわけ来日研究者・留学生の支援に十分な配慮がなされなければならない。したがって，内外における日本語教育の充実や，来日研究者・留学生の住居，日本人研究者・学生や地域の人々との交流を可能とする交流施設など生活・文化施設の整備・充実を早急に図るべきである。

③ 海外派遣研究者への支援の拡充

国際学術交流は，相手国の国情に応じた総合的配慮の下に行われる必要がある。したがって，その国の研究者との恒常的な連携・協力を維持するとともに我が国からの海外派遣研究者が必要とする各種情報の提供や連絡・調整などもできる人材の当該国への配置など，海外派遣研究者の支援体制の拡充を検討する必要がある。

(3) 学術分野における国際貢献のための新しいシステムの構築

国際的な学術協力については，我が国においても，既に多くの機関がその努力を重ねているところである。しかしながら，投入されている資金等そのための支援は，質・量ともに，未だ国際的な要求に応える水準にまで達しているとは言えない。しかも，現在個別に推進されている学術協力の相互の連絡・調整は，必ずしも十分ではなく，我が国の総力を挙げてこれを推進しているとは言えない状態にある。

また，今後ますます増えていくと思われる各種の国際的な学術協力プロジェクトの立案や協力，参加，推進については，これまで以上に，科学者の総意を反映しつつ，総合的かつ適切な判断を機動的になし得る場を確保しなければならない。

さらに，我が国が国際的な学術協力のための諸施策を強力に推進するためには，科学者の力のみならず，政府・産業界の協力，更には国民の理解等総合的な支援が必要である。

これらの問題点を改善し，学術分野において国際社会の期待に応える貢献をなし得るように，国民の理解の下に，諸課題の整理，必要な資金の確保・配分等を行う新しいシステム（例えば「学術協力機構」）を構築するなど，今後真剣に検討を進める必要がある。

終わりに

日本学術会議は，人類共通の資産としての学術の発展こそが人類の繁栄と世界の平和の礎となるとの見地から，本提言を取りまとめたものである。

なお，日本学術会議は，今後とも，本提言に基づき，内外の科学者を始め，広く関係各方面の意見を聴きながら，具体的な諸課題について引き続き検討していくことを付言したい。

平成5年(1993年)度共同主催国際会議

日本学術会議では，我が国において開催される学術関係国際会議のうち毎年おおむね6件について，学・協会と共同主催している。

本年もまた，6件の国際会議を共同主催することとしており，その概要は，次のとおりである。

◆第7回太平洋学術中間会議（6月27日～7月3日）

太平洋地域の住民の繁栄と福祉に直接関わる学術上の問題に関する研究を発展させるため，討論を行い，最新の研究情報を交換することを目的として宜野湾市（沖縄コンベンションセンター，沖縄都ホテル，メルパルク沖縄）において開催される。

参加予定人数500人（国外300人，国内200人）参加予定国数29か国。

◆第6回国際気象学大気物理学協会科学会議及び第4回国際水文科学協会科学会議合同国際会議（7月11日～23日）

気象学，大気物理学及び陸水・水文科学に関する研究を発展させるため，討論を行い，最新の研究情報を交換することを目的として横浜市（横浜国際平和会議場）において開催される。

参加予定人数1,500人（国外700人，国内800人），参加予定国数68か国。

◆第15回国際植物科学会議（8月23日～9月3日）

植物科学に関する研究を発展させるため，討論を行い，最新の研究情報を交換することを目的として横浜市（横浜国際平和会議場）において開催される。

参加予定人数4,000人（国外1,500人，国内2,500人），参加予定国数81か国。

◆第24回国際電波科学連合総会（8月23日～9月3日）

電波科学に関する研究を発展させるため，討論を行い，最新の研究情報を交換することを目的として京都市（国立京都国際会館）において開催される。

参加予定人数1,200人（国外800人，国内400人），参加予定国数49か国。

◆アジア社会科学研究協議会連盟第10回総会（9月5日～11日）

アジア・太平洋地域における社会科学の教育，研究，訓練及び普及を促進するため，討論を行い，最新の研究情報を交換することを目的として川崎市（かながわサイエンスパーク）において開催される。

参加予定人数120人（国外60人，国内60人），参加予定国数17か国。

◆第21回国際純粋・応用物理学連合総会（9月20日～25日）

物理学を発展させるため，討論を行い，最新の研究情報を交換することを目的として奈良市（奈良県新公会堂）において開催される。

参加予定人数300人（国外150人，国内150人），参加予定国数41か国。

御意見・お問い合わせ等がありましたら，下記までお寄せください。

〒106 東京都港区六本木7-22-34

日本学術会議広報委員会　電話03(3403)6291(代)

賛助会員

北海道栽培漁業振興公社　060 札幌市中央区北3条西7丁目
北海道第二水産ビル4階
阿寒観光汽船株式会社　085-04 北海道阿寒郡阿寒町字阿寒湖畔
株式会社　シロク商会　260 千葉市春日1-12-9-103
全国海苔貝類漁業協同組合連合会　108 東京都港区高輪2-16-5
有限会社　浜野顕微鏡　113 東京都文京区本郷5-25-18
株式会社ヤクルト本社研究所　189 東京都国立市谷保1769
田崎真珠株式会社田崎海洋生物研究所　779-23 徳島県海部郡日和佐町外ノ牟井
神協産業株式会社　742-15 山口県熊毛郡田布施町波野962-1
理研食品株式会社　985 宮城県多賀城市宮内2丁目5番60号
株式会社白寿保健科学研究所　原　昭邦　351 朝霞市栄町3-3-7

藻類のライフヒストリーをオリジナルの線図に解説をつけ見開きで示す！

藻類の生活史集成 全3巻 堀 輝三編 (送料各巻450円)

第1巻 緑色藻類	(185種)	B5判・450頁・定価8240円	94年2月刊行
第2巻 褐藻・紅藻類	(171種)	B5判・424頁・定価8240円	93年9月刊行
第3巻 単細胞・鞭毛藻類	(146種)	B5判・372頁・定価7210円	93年11月刊行

藻類の研究者115名が自らの研究成果と資料をもとに執筆に当たり，現時点で明らかになっている藻（502種）の生活史・生活環を線図で集大成した初めての本。

本書の構成は，図を左頁に対面する頁に和英の解説文をつけて，2ページを1単位として組み立ててある。執筆者によるオリジナルの線図は，藻類のライフサイクルを一見して理解させそれに簡明な解説を付す。さらに教育的配慮から多くの種について分布図を，そして各巻ごとに同義語を，各巻の巻末に学名総索引・和名索引を収録して読者が使いやすいよう工夫した。藻類を専門とする研究者や中学・高校の生物の先生，水に関連する研究所や企業の方々を初め，藻類に興味をもつ人々にとって，長い間出版が望まれていた本である。

刊行は9月に第2巻を：11月に第3巻を：94年2月に第1巻を刊行し完結。[呈内容案内]

第2期がスタート——第11巻刊行のお知らせ——

写真で見る種の同定と分類！

淡水藻類写真集 第11巻

山岸高旺・秋山 優編

B5判・100シート 定価7210円

製本様式を下記のようにいたしました。

2穴・並製箱入り 〒各380円

第1期10巻に続き今秋から年2～3冊を刊行して10巻（1000種）を目標とする。これにより2000種となり利用価値も高まる。(12巻進行中)

既刊 第1期10冊1・2巻 定価4120円／3～10巻 定価5150円（〒各380円）

藻類の生態

秋山 優・有賀祐勝 共編
坂本 充・横浜康継

A5判(上製函入) 640頁
定価13,184円(〒450円)

日本淡水藻図鑑

廣瀬弘幸・山岸高旺編 日本ではじめて創られた本格的な図鑑。淡水藻類の研究者や水に関係する方々にとっては貴重な文献である。

定価39140円

植物組織学

猪野俊平著 植物組織学の定義・内容・発達史から研究方法を幅広く詳述した唯一の書。

定価18540円

藻類学総説

廣瀬弘幸著 藻類の分類と形態を重点に置いて，克明な図により丁寧に解説する。 定価10300円

ナマコとウニ

——民謡と酒のさかなの話——

大島廣著 定価1009円

水の環境科学

鈴木静夫著 公害防止から環境保全へと時代が変わり本書は水の環境の現実を解説する。

定価2472円

生物学史展望

井上清恒著 五千年にわたる生物学の流れを時代の経緯と共に語る。 定価5974円

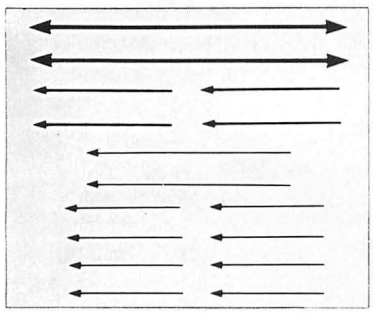
内田老鶴圃 〒112 東京都文京区大塚3－34－3
電話(03)3945-6781 FAX(03)3945-6782

(価格は税込)

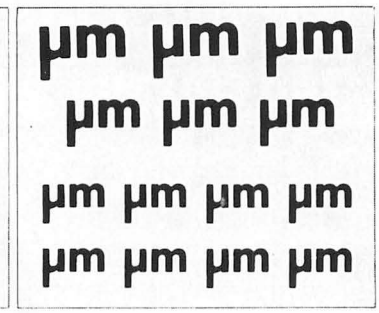
新製品ご案内!!

レタリングシート（ブラック アンド ホワイト）

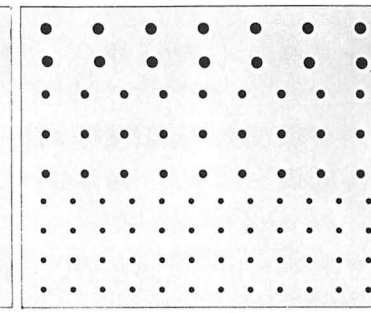
EMI NO.82014



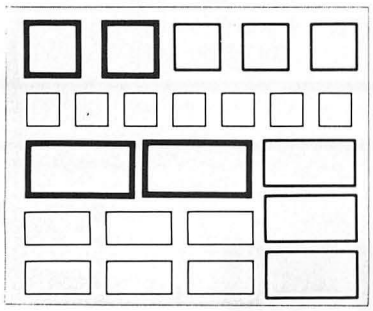
EMI NO.82016



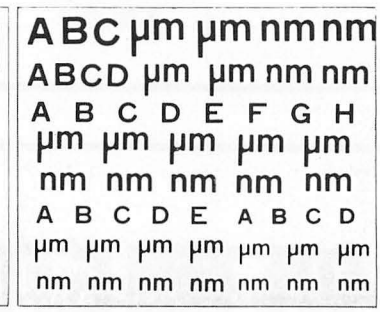
EMI NO.86626



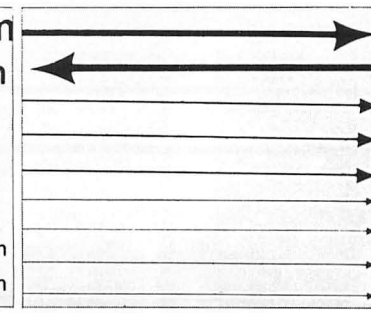
EMI NO.86627



EMI NO.86902

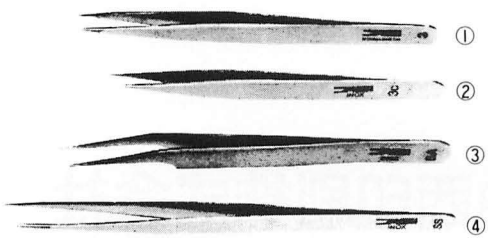


EMI NO.86916



※レタリングシートの総合カタログが出来ました。下記の住所へカタログをご請求下さい。

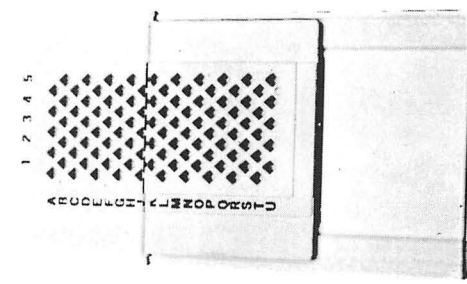
西独製 精密ピンセット



①時計ピンセット　③5型変形ピンセット
②3Cピンセット　④SS型ピンセット

各1本：￥2,200

EMグリッドボックス



1個：￥1,800　10個：￥15,000

EM資材直販センター
〒274 千葉県船橋市三山5-6-1 TEL.0474(75)5783
東京営業所：TEL.03(988)9906

学 会 出 版 物

下記の出版物をご希望の方に頒布致しますので，学会事務局までお申し込み下さい。（価格は送料を含む）

1. **「藻類」バックナンバー** 価格，会員各号1,750円，非会員各号3,000円，30巻4号（創立30周年記念増大号，1—30巻索引付）のみ会員5,000円，非会員7,000円，欠号：1—2号，4巻1，3号，5巻1—2号，6—9巻全号。

2. **「藻類」索引** 1—10巻，価格，会員1,500円，非会員2,000円，11—20巻，会員2,000円，非会員3,000円，創立30周年記念「藻類」索引，1—30巻，会員3,000円，非会員4,000円。

3. **山田幸男先生追悼号** 藻類25巻増補．1977．A5版，xxviii＋418頁．山田先生の遺影・経歴・業績一覧・追悼文及び内外の藻類学者より寄稿された論文50編（英文26，和文24）を掲載，価格7,000円。

4. **日米科学セミナー記録** Contributions to the systematics of the benthic marine algae of the North Pacific. I. A. Abbott・黒木宗尚共編．1972．B5版，xiv＋280頁，6図版．昭和46年8月に札幌で開催された北太平洋産海藻に関する日米科学セミナーの記録で，20編の研究報告（英文）を掲載。価格4,000円。

5. **北海道周辺のコンブ類と最近の増養殖学的研究．** 1977．B5版，65頁。昭和49年9月に札幌で行なわれた日本藻類学会主催「コンブに関する講演会」の記録。4論文と討論の要旨。価格1,000円。

Publications of the Society

Inquiries concerning copies of the following publications should be sent to the **Japanese Society of Phycology, Shimotachiuri Ogawa Higashi, Kamikyoku, Kyoto, 602 Japan.**

1. **Back numbers of the Japanese Journal of Phycology** (Vols. 1–28, Bulletin of Japanese Society of Phycology). Price, 2,000 Yen per issue for member, or 3, 500 Yen per issue for nonmember; price of Vol. 30, No. 4 (30th Anniversary Issue), with cumulative index (Vols. 1–30), 6,000 Yen for member, or 7,500 Yen for nonmember (incl. postage, surface mail). Lack: Vol. 1, Nos. 1–2; Vol. 4, Nos. 1, 3; Vol. 5, Nos. 1–2; Vol. 6–Vol. 9, Nos. 1–3.

2. **Index of the Bulletin of Japanese Society of Phycology.** Vol. 1 (1953)–Vol. 10 (1962), Price 2,000 Yen for member, or 2,500 Yen for nonmember; Vol. 11 (1963)–Vol. 20 (1972), Price 3,000 Yen for member, or 4,000 Yen for nonmember. Vol. 1 (1953)–Vol. 30 (1982), Price 4,000 Yen for member, or 5,000 Yen for nonmember (incl. postage, surface mail).

3. **A Memorial Issue Honouring the late Professor Yukio Yamada** (Supplement to Volume 25, the Bulletin of Japanese Society of Phycology). 1977. xxviii＋418 pages. This issue includes 50 articles (26 in English, 24 in Japanese with English summary) on phycology, with photographs and list of publications of the late Professor Yukio Yamada. 8,500 Yen (incl. postage, surface mail).

4. **Contribution to the Systematics of the Benthic Marine Algae of the North Pacific.** Edited by I. A. Abbott and M. Kurogi, 1972. xiv＋280 pages, 6 plates. Twenty papers followed by discussions are included, which were presented in the U.S.-Japan Seminar on the North Pacific Benthic Marine Algae, held in Sapporo, Japan, August 13–16, 1971. 5,000 Yen (incl. postage, surface mail).

5. **Recent Studies on the Cultivation of *Laminaria* in Hokkaido** (in Japanese). 1977. 65 pages. Four papers followed by discussion are included, which were presented in a symposium on *Laminaria,* sponsored by the Society, held in Sapporo, September 1977. 1,200 Yen (incl. postage, surface mail).

1993年9月15日 印刷
1993年9月20日 発行

Printed by Nakanishi Printing Co., Ltd.

編集兼発行者 石 川 依 久 子
〒184 小金井市貫井北町 4-1-1
東京学芸大学生物学教室内
Tel. 0423-25-2111 内線 2665

印 刷 所 中 西 印 刷 株 式 会 社
〒602 京都市上京区下立売通小川東入
Tel. 075-441-3155

発 行 所 日 本 藻 類 学 会
〒602 京都市上京区下立売通小川東入
Tel. 075-441-3155
振替口座：京都 1-50488

本誌の出版費の一部は文部省科学研究費補助金「研究成果公開促進費」による。
Publication of The Japanese Journal of Phycology has been supported in part by a Grant-in-Aid for Publication of Scientific Research Result from the Ministry of Education, Science and Culture, Japan.

第41巻　第3号　1993年9月20日

藻　類

目　　次

◆・◆

ノート

◆・◆

総　説

◆・◆

雑　録

日　本　藻　類　学　会