# The Japanese Journal of PHYCOLOGY

#### CONTENTS

Masahiro Notoya and Yusho Aruga: The life history of Dudresnaya japonica Okamura (Cryptonemiales, Rhodophyta) in cultuse  263 Tadao Yoshida: Solieria pacifica (Yamada) Yoshida, comb. nov. (Solieriaceae, Rhodophyta) from Japan  268 Tadao Yoshida: Notes on Spirocladia loochooensis (Yendo) Yoshida, comb. nov. (Rhodomelaceae, Rhodophyta)  271 Hiroo Satoh, Yukuya Yamaguchi, Kentaro Watanabe and Yusho Aruga: Light conditions and photosynthetic productivity of ice algal assemblages in Lake Saroma, Hokkaido  274 Takeo Okuda and Yukari Satoh: Conceptacular development in Sargassum filicinum and autumnal S. horneri (Phaeophyceae)  279 Sung-Min Boo, Jan Rueness and In Kyu Lee: Life history and taxonomy of Callithamnion callophyllidicola Yamada (Ceramiaceae, Rhodophyta)  284 Yoshihiko Iriki, Takeo Horiguchi and Emiko Masuo: Mucilage from Collinsiella cava (Yendo) Printz (Chlorophyta) with special reference to its sugar components	Yumiko Amemiya and Oki Nakayama: Separation and sugar composition of	0.59
(Cryptonemiales, Rhodophyta) in cultuse Tadao Yoshida: Solieria pacifica (YAMADA) YOSHIDA, comb. nov. (Solieriaceae, Rhodophyta) from Japan	gelatinous sheath carbohydrates produced by Microcystis	233
Tadao Yoshida: Solieria pacifica (YAMADA) YOSHIDA, comb. nov. (Solieriaceae, Rhodophyta) from Japan		263
Rhodophyta) from Japan		203
Tadao Yoshida: Notes on Spirocladia loochooensis (YENDO) YOSHIDA, comb. nov. (Rhodomelaceae, Rhodophyta)		260
(Rhodomelaceae, Rhodophyta)		200
Hiroo Satoh, Yukuya Yamaguchi, Kentaro Watanabe and Yusho Aruga: Light conditions and photosynthetic productivity of ice algal assemblages in Lake Saroma, Hokkaido	. , , , , , , , , , , , , , , , , , , ,	271
ditions and photosynthetic productivity of ice algal assemblages in Lake Saroma, Hokkaido		
Takeo Okuda and Yukari Satoh: Conceptacular development in Sargassum filicinum and autumnal S. horneri (Phaeophyceae)		
and autumnal S. horneri (Phaeophyceae)		274
Sung-Min Boo, Jan Rueness and In Kyu Lee: Life history and taxonomy of Callithamnion callophyllidicola Yamada (Ceramiaceae, Rhodophyta)	Takeo Okuda and Yukari Satoh: Conceptacular development in Sargassum filicinum	
Note  Masahiro Notoya and Yusho Aruga: Tissue culture from the explant of Ecklonia cava (Kellamiariales, Phaeophyta)  Note  Masahiro Notoya and Fumie Kasai: Genome differentiation in speciation of desmids (Chlorophyta)  Review  Terunobu Ichimura and Fumie Kasai: Genome differentiation in speciation of desmids (Chlorophyta)  Abstracts of the Symposium of the Japanese Society of Phycology		279
Yoshihiko Iriki, Takeo Horiguchi and Emiko Masuo: Mucilage from Collinsiella cava (Yendo) Printz (Chlorophyta) with special reference to its sugar components	Sung-Min Boo, Jan Rueness and In Kyu Lee: Life history and taxonomy of Callitham-	
Cava (YENDO) PRINTZ (Chlorophyta) with special reference to its sugar components	nion callophyllidicola Yamada (Ceramiaceae, Rhodophyta)	284
S. F. Friedlander, L. D. Melton and M. T. Brown: Ascorbic acid in the New Zealand seaweeds Porphyra columbina Mont. and Porphyra subtumens J. Ac. (Rhodophyceae)	Yoshihiko Iriki, Takeo Horiguchi and Emiko Masuo: Mucilage from Collinsiella	
Zealand seaweeds Porphyra columbina Mont. and Porphyra subtumens J. Ac. (Rhodophyceae)	cava (Yendo) Printz (Chlorophyta) with special reference to its sugar components(in Japanese)	291
(Rhodophyceae) 295  Article Masahiro Notoya and Yusho Aruga: Tissue culture from the explant of Ecklonia cava  KJELLMAN (Laminariales, Phaeophyta) 302  Article Masahiro Notoya and Yusho Aruga: Tissue culture from the explant of Ecklonia cava  KJELLMAN (Laminariales, Phaeophyta) 302  Article Masahiro Notoya and Yusho Aruga: Tissue culture from the explant of Ecklonia cava  KJELLMAN (Laminariales, Phaeophyta) 302  Article Masahiro Notoya and Yusho Aruga: Tissue culture from the explant of Ecklonia cava  (in Japanese) 302  Article Masahiro Notoya and Yusho Aruga: Tissue culture from the explant of Ecklonia cava  (in Japanese) 305  Abstracts of the Symposium of the Japanese Society of Phycology (in Japanese) 320  Book Review (in Japanese) 321	S. F. Friedlander, L. D. Melton and M. T. Brown: Ascorbic acid in the New	
Note  Masahiro Notoya and Yusho Aruga: Tissue culture from the explant of Ecklonia cava  KJELLMAN (Laminariales, Phaeophyta)	Zealand seaweeds Porphyra columbina Mont. and Porphyra subtumens J. Ag.	
Masahiro Notoya and Yusho Aruga: Tissue culture from the explant of Ecklonia cava  KJELLMAN (Laminariales, Phaeophyta)	(Rhodophyceae)	295
Masahiro Notoya and Yusho Aruga: Tissue culture from the explant of Ecklonia cava  KJELLMAN (Laminariales, Phaeophyta)		
Masahiro Notoya and Yusho Aruga: Tissue culture from the explant of Ecklonia cava  KJELLMAN (Laminariales, Phaeophyta)	•••	
Masahiro Notoya and Yusho Aruga: Tissue culture from the explant of Ecklonia cava  KJELLMAN (Laminariales, Phaeophyta)	Note	
KJELLMAN (Laminariales, Phaeophyta)		
Review  Terunobu Ichimura and Fumie Kasai: Genome differentiation in speciation of desmids (Chlorophyta)		300
Terunobu Ichimura and Fumie Kasai: Genome differentiation in speciation of desmids (Chlorophyta)	Кјепли (Lammariares, Friacophyta).	302
Terunobu Ichimura and Fumie Kasai: Genome differentiation in speciation of desmids (Chlorophyta)		
Terunobu Ichimura and Fumie Kasai: Genome differentiation in speciation of desmids (Chlorophyta)	***	
desmids (Chlorophyta)	Review	
Abstracts of the Symposium of the Japanese Society of Phycology		
Book Review(in Japanese) 321	desmids (Chlorophyta)(in Japanese)	305
Book Review(in Japanese) 321		
Book Review(in Japanese) 321	<b>*·*</b>	
Book Review(in Japanese) 321	Abstracts of the Symposium of the Japanese Society of Phycology	320
The state of the s		
( 5.1	The state of the s	
Contents of Volume 37		

#### 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。

入会,退会,会費の納入および住所変更等についての通信は 113 東京都文京区弥生2-4-16 学会センター ビル内 日本学会事務センター宛に、原稿の送付は 108 東京都港区港南4-5-7 東京水産大学 有賀祐勝気付 日本藻類学会編集委員会宛に、また、庶務一般およびバックナンバー等については 184 東京都小金井市貫井北町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. The annual dues (1989) for overseas members are 8,500 Yen (send the remittance to the Business Center for Academic Societies Japan, 4–16, Yayoi 2-chome, Bunkyo-ku, Tokyo, 113 Japan).

Manuscript for publication should be submitted directly to the Editor-in-Chief, Prof. Y. Aruga, Tokyo University of Fisheries, Konan-4, Minato-ku, Tokyo, 108 Japan. Inquiries and other information regarding the society should be addressed to the Japanese Society of Phycology, c/o Department of Biology, Tokyo Gakugei University, Nukuikitamachi, Koganei-shi, Tokyo, 184 Japan.

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As of January 1, 1990 (Vol. 38~), the annual membership fee and subscription fee will be as follows:

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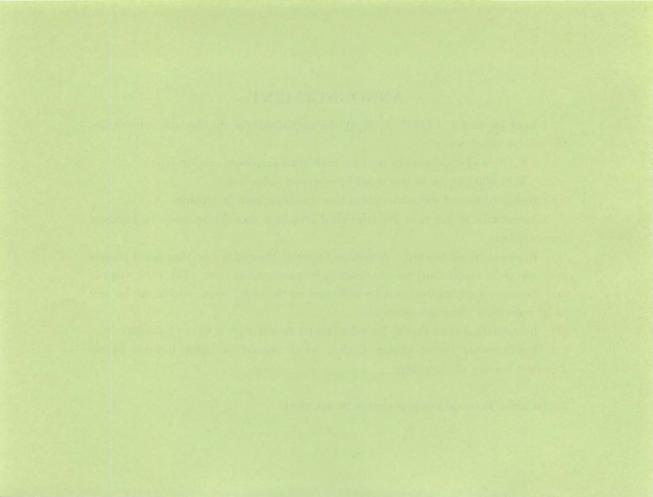
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- (3) International Postal Money Order, which should be made payable to the Japanese Society of Phycology

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#### 日本藻類学会第14回大会のお知らせ

日本藻類学会第14回大会を下記の要領で開催致します。奮って御参加下さい。

会 場:神戸大学教養部 〒657 神戸市灘区鶴甲1-2-1 〒078 (881) 1212 代表

会 期:1990年3月28日(水)編集委員会・評議員会

29日(木)口頭発表・総会・懇親会

30日(金)口頭発表·展示発表

4月2日(月) 」於 神戸大学理学部附属臨海実験所

#### 申 込 先:

大会・懇親会・発表の申込票,発表要旨の送付,連絡は下記宛にお願いします。 - 5657 神戸市灘区鶴甲1-2-1

神戸大学教養部生物学教室内

日本藻類学会第14回大会準備委員会

∞078 (881) 1212 内線 6261 (坪) 又は内線 4429 (能野)

#### 参加申込:

- 1) 大会参加者は、発表の有無にかかわらず、本誌に綴じ込みの大会申込票に必要事項を記入して、上記の 第14回大会準備委員会あて、お送りください。
- 2)大会費2,500円 (学生2,000円), および懇親会出席の方は会費3,000円を同封の振替用紙でお送り下さい。 送金先:振替 神戸 9-2222 日本藻類学会第14回大会準備委員会
- 3) 大会参加申込,送金,下記の発表要旨送付の締切りは1990年1月10日です。
- 発 表:発表を希望される方は、本誌に綴じ込みの発表申込票に必要事項を記入し、発表要旨の原稿を添え て、お申し込み下さい。
  - 1) 発表には、口頭発表と展示発表の2種類があります。希望する方を○で囲んで下さい。
  - 2) ロ頭発表:発表時間は、質疑応答の時間を含めて15分です。 使用スライドは 35 mm 版、スライド枠には、図1のように発表者氏名、発表番号スライド総枚数、映 写順序、上辺マークを御記入下さい。同じスライドを繰り返し映写する場合は、それに見合う枚数を御 用意下さい。
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4) 発表 (ロ頭発表・展示発表) の申込者は、本誌に綴じ込みの原稿用紙に要旨を記入して、1990年1月 10日必着で、上記の準備委員会宛にお送り下さい。

原稿はそのままオフセット印刷に回します。タイプライター, ワード・プロセッサー, パソコン等何を使っても結構ですが, 印字は明瞭な黒色でお願いします。

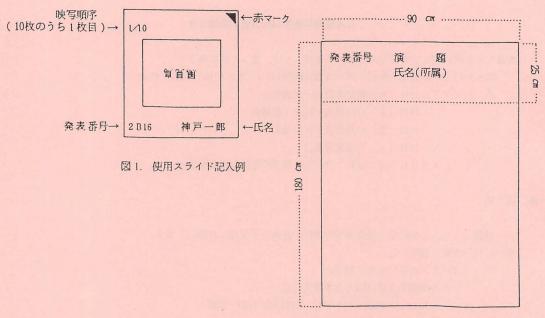


図2. 展示パネルの説明図

宿 泊:会場付近には、適当な宿泊施設がありません。JR三の宮、阪急三の宮付近から、JR六甲道駅、または阪急六甲駅を経由して、神戸市バスで、神戸大学教養部前に来られるのが便利と思います。三の宮付近から会場まで凡そ1時間かかります。JR三の宮、阪急三の宮付近の宿舎を紹介しますので、下記の表を参考に直接予約して下さい。

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共済関係				
六甲荘 (公)	6,458	241-2451	新神戸駅	徒歩15分
兵庫共済会館(市)	3,300	222-2600	JR 元町駅	徒歩10分
パレス神戸(警)	6,000	371-7800	JR 元町駅	徒歩5分
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ビジネスホテル				
神戸東急イン	7,700	291-0109	JR 三宮駅	徒歩5分
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神戸プラザホテル	5,200	332-1141	JR 元町駅	徒歩3分
神戸ワシントンホテル	7,750	331-6111	JR 三宮駅	徒歩10分
神戸ユニオンホテル	6,000	222-6500	JR 三宮駅	徒歩10分
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#### 日本藻類学会主催海藻採集会のお知らせ

下記の要領により瀬戸内海での海藻採集会を開催いたします。ご希望の方は下記の神戸大学理学部附属臨海実験所に直接お申し込み下さい。

1. 期 日:1990年3月30日(金)-4月2日(月)

参加者は当初定刻に集合していただきますが、以後、下記日程のご希望の時点でお帰りいただいても結構です。

2. 日程: (天候等により一部変更する場合もあります)

3月30日(金)17:00 大会受付前集合(実験所に移動)

或は 19:00 臨海実験所に直接到着のこと

日程説明, 夕食·懇親会, 実験所宿泊

3月31日(土)播磨灘北部 家島群島 上島,加島 採集,実験所宿泊

4月1日(日)備讃瀬戸 女木島 採集

高松港, 児島港 寄港 (両港とも下船可)

途中 瀬戸大橋を海上より見物,実験所宿泊

4月2日(月)淡路島南部 由良海岸 採集

3. 会 場:神戸大学理学部附属臨海実験所

〒656-24 兵庫県津名郡淡路町岩屋2746

電話 0799-72-2374

4. 採集船:実験所所属 実習船 おのころ 使用

5. 講 師:北海道大学理学部 吉田忠生,北海道大学理学部 増田道夫,京都大学農学部 鯵坂哲朗,

東京水産大学 大葉英雄

6. 参加費:宿泊費 1泊 500円

食 費 朝食 300円, 昼食 700円, 夕食 800円

懇親会 1000円

雜費 1日 200円

参加希望者に参加の可否、日程等の詳細をお送りし、参加希望日程、希望事項等を御返信いただき、 各参加者の参加日程から参加費を算出、納入期日など参加者各位に直接連絡いたします。

7. 定 員:10名 (実習船の乗船定員の都合から10名とします)

希望者多数の場合には先着順としますので予めご了承下さい。

参加資格は特に問いませんが、小型船に船酔する方はご遠慮下さい。

- 8. 申 込:**葉書にて1990年1月末日までに** 1) 氏名, 2) 連絡先, 3) 所属を明記の上, 上記の臨海実験所に直接お申込み下さい。
- 9. その他:潜水器具,採集具,標本作製・整理用品,薬品などはご希望により出来る限り用意いたします。

#### 要旨原稿の体裁見本

〇田中二郎\* ・伊藤真理\*\*: 褐葉アミジグサ科のフクリンアミジとサナダグサの形態

アミジグサ目アミジグサ科のニセアミジ属とサナダ グサ属には日本産の種類としてそれぞれフクリンアミ ジ(<u>Dilophus okamurae</u> Dawson)と・・・・・

・・・・・・が中央部付近

で多糖になることがある。精子のうは表皮上に盛り上 がって形成される。

(\*国立科博·植物研, \*\*日本女子大·家政)

○渡辺 信\* ・ L. GARY\*\*: クラミドモナス目とクロロコックム目 (緑葉綱) の 6 属における 2 本鞭毛遊走細胞の微細構造

<u>Dunaliella lateralis</u> (クラミドモナス目), <u>Spon-giochloris spongiosa</u>, <u>Protosiphon botryoides</u>,
<u>Tetracystis aeria</u>, ・・・・・・

る。

・・・・・・には ABBがみられず, BBのなす角度が大きく変化し, 細胞は裸であ

(\*富山大、\*\*オハイオ州立大)

## 日本藻類学会第14回大会申込用紙

### 大 会・懇 親 会 申 込 票

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原稿用紙

# Separation and sugar composition of gelatinous sheath carbohydrates produced by *Microcystis*

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Amemiya, Y. and Nakayama, O. 1989. Separation and sugar composition of gelatinous sheath carbohydrates produced by *Microcystis*. Jpn. J. Phycol. 37: 253–262.

Sugar compositions of polysaccharides of the gelatinous sheath produced by colonies and single cells of 3 species of Microcystis were compared. Single cells of M. aeruginosa without gelatinous sheath were obtained by changing the culture conditions. Single cells of M. wesenbergii and M. viridis were surrounded by a thin layer of gelatinous sheath, and were unable to form colonies. Carbohydrates of the gelatinous sheath and intracellular carbohydrates were separated by column chromatography on DEAE-Sephadex A-25. Polysaccharides of the gelatinous sheath produced by colonies showed marked heterogeneity in their sugar residues, while those of single cells were mainly composed of glucose. In the gelatinous sheath of colonies, xylose or glucose was abundant in two strains of M. aeruginosa, while in M. wesenbergii glucose, galactose and xylose were the main constituents and in M. viridis mannose was the characteristic major constituent. The relative amounts of the various sugar constituents of gelatinous sheath carbohydrates differed markedly among the three species of Microcystis cultured under the same conditions.

Key Index Words: colonies—gelatinous sheath—Microcystis aeruginosa—Microcystis viridis—Microcystis wesenbergii—single cells—sugar composition.

Microcystis, belonging to cyanobacteria, often causes waterblooms in eutrophic lakes. M. aeruginosa, M. wesenbergii and M. viridis are found together in the same lakes, but the dominant species varies depending on lakes, seasons, and years (WATANABE et al. 1986; TAKAMURA and WATANABE 1987). A conspicuous feature of *Microcystis* is the gelatinous sheath that surrounds cells. Drews and Weckesser (1982) called the structured external layer 'sheath', and the undefined, unstructured zones 'slime'. It is difficult to separate the sheath and the slime, although they can be differentiated microscopically. In this paper, slime and sheath are treated together as 'gelatinous sheath'. Saito (1985) showed that the properties of the gelatinous sheath of colonies of the three species of Microcystis and the rate of cellular dispersion after chlorination differed from each other.

The gelatinous sheath of *Microcystis* was found to be mainly composed of heteropolysaccharides containing uronic

acids (Amemiya and Nakayama 1986; Nakagawa et al. 1987), and its intracellular carbohydrates were composed mainly of glucose polymers (Hama and Handa 1982; Nakagawa et al. 1987). However, details of the differences in the gelatinous sheath of these species remain obscure because it is difficult to isolate the gelatinous sheath without injuring cells.

We, therefore, examined the methods for isolating the gelatinous sheath of the three species mentioned Microcystis (AMEMIYA and NAKAYAMA 1988). Solubilization of the gelatinous sheath was attempted at that time by physical treatments such as heat, shaking and sonication, and by chemical treatment with a chelator or in alkaline solution. Of the three Microcystis species, slime of M. aeruginosa was solubilized most easily, followed by that of M. wesenbergii, although it was partially solubilized, while that of M. viridis was only solubilized in an alkaline solution. The sheath of M. wesenbergii was almost totally insoluble by these procedures.

Based on the results of the solubilization experiment, we conducted further detailed investigation on the sugar constituents of polysaccharides from the gelatinous sheath produced by Microcystis colonies, and compared them with those of single cells which were unable to form colonies, thereby we expected to reveal possible differences in the characteristics of the gelatinous sheath between colonies and single cells of the same strain. In the present paper, we report the of carbohydrates separation from gelatinous sheath in addition to intracellular carbohydrates using ion-exchange chromatography, together with determination of their sugar constituents.

#### Materials and Methods

Cultures and growth conditions

Microorganisms: Unialgal colonies of two strains of M. aeruginosa and one strain of M. wesenbergii were isolated from a waterbloom in Lake Suwa in August 1980. M. viridis NIES-102 was obtained from the National Institute for Environmental Studies. This strain is axenic and composed of single cells which lack the ability to form colonies.

Culture conditions: Algal cells were cultured in B12 medium (Shibukawa et al. 1986) at 25°C and 1500 lux as standard conditions.

Isolation of single cells: Single cells were isolated from two strains of *M. aeruginosa* No. 1 and No. 2 and one strain of *M. wesenbergii*, all of which formed colonies. Details of the isolation method used are described in the Results.

Fractionation of carbohydrates from cell contents and gelatinous sheath

Single cells were precipitated by centrifugation at  $10,000 \times g$  for 10 min, followed by two washings with distilled water, and then lyophilized. Colonies were collected by filtration with a Nuclepore filter  $(1.0~\mu\text{m})$ , washed with water and lyophilized. Thirty milligrams of dried cells were suspended in 0.05~M Tris buffer (pH 8.0), sonicated at

90 W for 6 min and then centrifuged at  $1,500 \times g$ . The precipitate was repeatedly sonicated and centrifuged. The supernatant was applied to a column  $(2.2 \times 35 \text{ cm})$  packed with DEAE-Sephadex A-25, and carbohydrates were eluted with a gradient of sodium chloride in Tris buffer.

#### Analytical methods

Total organic carbon of algal cells was measured by an element analyzer (Perkin-Elmer, Type 240). Total carbohydrates were determined by the phenol-sulfuric acid method, and uronic acid residues were determined by Dishe's carbazole method modified by Galambos (1967). Monosaccharides were determined according to the method of NAKAGAWA et al. (1987). After hydrolysis with sulfuric acid, resulting individual monosaccharides were converted to their respective alditol acetate derivatives, and subjected to gas chromatography on a Hitachi chromatograph (Model 163) with a 2-m glass column. For determination of glucose and galactose, a column was packed with 3% ECNSS-M and heated at 190°C; for other neutral monosaccharides, a column packed with 3% EGSS-X was used and heated at 200°C.

#### Results

Preparation of single cells and colonies

Single cells were prepared from colonies of two strains of M. aeruginosa and a strain of M. wesenbergii. For M. aeruginosa, single cells are reported to grow faster than colonies (GANF and SCHACHE 1983). Therefore, temperature, light intensity and concentration of sodium nitrate were increased to obtain faster growth. Furthermore, the concentration of calcium and ferric ions was decreased and that of EDTA was increased, unless gelatinous sheath was produced, or extracellularly released material became insoluble gelatinous sheath. So, the colonies of M. aeruginosa No. 1 were cultured at 64 (26) sorts of combinations of culture conditions, i.e. temperature (25 and 30°C), light intensity

(1500 and 3000 lux), sodium nitrate (100 and 200 mg/l), calcium chloride (40 and 10 mg/l), ferric citrate (6 and 2 mg/l) and ED-TA (1 and 3 mg/l). Single cells and paired cells undergoing binary division grew with colonies under 29 conditions. The culture conditions and the number of cultures in which single cells appeared from colonies are shown in Table 1. Single cells showed a tendency to grow better at 30°C, 3000 lux, 200 mg/l sodium nitrate, and 10 mg/l calcium chloride, which are considered to be more favorable for single cells than the standard conditions. After repeated subculture on agar plates, single cells were obtained without gelatinous sheath. When cultured in the standard B12 medium under the standard conditions, they still grew singly.

Since single cells lacking the ability to form colonies became detached during culture of colonies of M. aeruginosa No. 2, they were isolated. There was no difference in the colonial form and size of a single cell between M. aeruginosa No. 1 and M. aeruginosa No. 2.

Single cells of *M. wesenbergii* which lack the ability to form colonies were also obtained during colony culture. However, each of the isolated single cells had a thin surrounding layer of gelatinous sheath. Hence, no single cells lacking gelatinous sheath could be prepared, but single cells with thin gelatinous sheath were treated as single cells of *M. wesenbergii* because they lacked the ability to form colonies.

The single cells obtained from *M. viridis* had gelatinous sheath similar to that of single cells of *M. wesenbergii*. These single cells were

Table 1. Number of cultures in which single cells appeared from colonies of *M. aeruginosa* No. 1 in each condition. Total number of cultures 29.

	Condition *	Number	Condition	Number
Temperature	25℃	11	30℃	18
Light intensity	1500 lux	6	3000 lux	23
Sodium nitrate	100 mg/l	11	200 mg/l	18
Cacium chlorid	le 40 mg/l	12	10 mg/l	17
Ferric citrate	6 mg/l	14	2 mg/l	15
EDTA	1 mg/l	14	3 mg/l	15

<sup>\*</sup> Standard culture conditions.

also classified as single cells because of their inability to form colonies. Preparation of colonies from single cells was made by employing conditions reverse to those under which single cells were prepared from colonies of M. aeruginosa No. 1. Accordingly, single cells of M. viridis were cultured under  $2^4 \times 3^2 = 144$ conditions involving all combinations of the six factors, i.e. temperature (25 and 15°C), light intensity (1500 and 500 lux), sodium nitrate (100 and 30 mg/l), EDTA (1 and 0 mg/l), calcium chloride (40, 120  $360 \,\mathrm{mg}/l$ ) and ferric citrate (3, 9 27 mg/l). Small granular colonies and slightly larger rod-shaped colonies were formed under 20 and 21 out of the 144 conditions, respectively. Table 2 summarizes the conditions under which the rod-shaped colonies grew: the temperature was lowered from 25°C to 15°C, the concentration of sodium nitrate was decreased from 100 mg/l to 30 mg/l, and the concentration of calcium chloride was increased from 40 mg/l to 360 mg/l for preparation of colonies from

Table 2. Number of cultures in which rod-shaped colonies of *M. viridis* appeared in each condition. Total number of cultures 21.

	Condition	Number	Condition	Number	Condition	Number
Temperature	25℃	4	15℃	17	••	
Light intensity	1500 lux	11	500 lux	10		
Sodium nitrate	100 mg/l	7	30 mg/l	14		
Calcium chloride	40 mg/l	3	120 mg/l	3	360 mg/l	15
Ferric citrate	3 mg/l	6	9 mg/l	11	27 mg/l	4
EDTA	1 mg/l	11	0  mg/l	10		

single cells. The single cells were, then, cultured under the above conditions using 1-l flasks. The results showed that, although some colonies were formed, single cells grew well. We then substituted the natural samples of M. viridis collected from the bloom in Lake Suwa for colonies of this species. These samples were used after partial removal of the gelatinous sheath of M. aeruginosa and M. wesenbergii by shaking and sonication procedures (Amemiya et al. 1988).

Organic material and carbohydrates produced in culture

Amounts of grown cells in cultures for 21 days, and their organic and carbohydrate carbon contents are summarized in Table 3. Single cells grew better than colonies during the culture for 21 days. This tendency was especially evident in *M. aeruginosa* No. 2. Similarly the production of carbohydrates was higher in single cells of *M. aeruginosa*, especially in those of *M. aeruginosa* No. 2. Carbohydrate carbon content of cultured cells ranged from 14% to 30% of organic carbon.

#### Separation of cellular carbohydrate

Separation of carbohydrates present within the cell from those in the gelatinous sheath

ion-exchange performed using was chromatography. After the cells had been sonicated and centrifuged, the supernatant was applied to columns of CM-Sephadex A-25 and DEAE-Sephadex A-25. Some of the carbohydates were adsorbed by DEAE-Sephadex A-25. Carbohydrates in single cells were intracellular, while those in colonies comprised intracellular carbohydrates and gelatinous sheath carbohydrates. carbohydrate fraction eluted in the same volume upon chromatography of extracts from both single cells and colonies was considered to be the intracellular carbohydrate fraction, whereas the carbohydrate fraction found only in colonies was regarded as that from the gelatinous sheath.

Figure 1 shows carbohydrates of *M. aeruginosa* No. 1 eluted with 0.05 M Tris buffer stepwise with increasing concentrations of sodium chloride. Most carbohydrates of single cells passed through without adsorption. Little was eluted with the buffer containing sodium chloride at a concentration of 0.1 M. In colonies, the fraction passing through accounted for 45.2% of total carbohydrates, and the fraction eluted with 0.1 M sodium chloride amounted to 3.2%; then, 19.5% of the carbohydrates were eluted

Table 3.	Comparison of carbohydrate carbon content in organic carbon between single cells and
colonies of thr	ree Microcystis species after culture for 21 days.

	Algal cells mg/l	Organic carbon mg/l	Carbohydrate carbon mg/l	Carbohydrate carbon content %
M. aeruginosa No. 1				
single cells	60.1	24.1	7.3	30.3
colonies	43.0	17.1	2.4	14.0
M. aeruginosa No. 2				
single cells	82.1	37.2	11.1	29.8
colonies	30.9	14.0	3.5	25.0
M. wesenbergii				
single cells	85.0	33.9	7.0	20.6
colonies	74.1	29.6	8.2	27.7
M. viridis				
single cells	55.3	22.2	4.9	22.1
colonies*	35.5	15.3	4.9	32.0

<sup>\*</sup> Natural colonies collected from a waterbloom in Lake Suwa in August 1986.

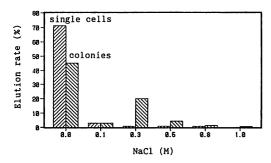


Fig. 1. Carbohydrates eluted stepwise in increasing concentrations of sodium chloride by anion exchange column chromatography (*M. aeruginosa* No. 1).

with 0.3 M sodium chloride, and only 4.7% of the carbohydrates were eluted at sodium chloride concentrations higher than 0.3 M. Accordingly, cellular carbohydrates were found to be divisible into two fractions; intracellular carbohydrates eluted without adsorption to the resin and carbohydrates from the gelatinous sheath eluted with a sodium chloride concentration of at 0.3 M or higher. Some carbohydrates of single cells and colonies, which were eluted with a sodium chloride concentration of 0.1 M, were also fractionated separately so that each of the two appeared as a discrete fraction.

From the results shown in Fig. 1, the method of carbohydrate separation was modified. Figure 2 shows carbohydrate fractions separated into three peaks by the modifi-

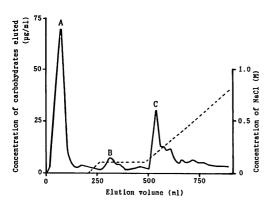


Fig. 2. Separation of carbohydrates in colonies of M. aeruginosa No. 1 by anion exchange column chromatography.

ed method. Fractions A, B and C were eluted with the buffer lacking sodium chloride, the buffer containing sodium chloride at a concentration of 0.1 M, and the buffer with a linear gradient from 0.1 to 0.8 M sodium chloride, respectively. Accordingly, intracellular carbohydrates were contained in fraction A, and carbohydrates of the gelatinous sheath were found in fraction C.

Fractionation of carbohydrates with DEAE-Sephadex A-25

Carbohydrates obtained in each fraction of the three species of Microcystis are shown in Table 4. In single cells of M. aeruginosa No. 1 and No. 2, about 70% of total carbohydrates were eluted in fraction A, 3-6% in fraction B, and 2-5% in fraction C. Nearly 40 to 45% of carbohydrates present in colonies of M. aeruginosa were eluted in fraction A, and about 25% in fraction C, indicating that intracellular carbohydrates and carbohydrates of the gelatinous sheath were present in an approximate ratio of 1.5-1.9:1. wesenbergii and M. viridis, as observed from the thin-layered gelatinous sheath, the percentage of carbohydrates of single cells eluted in fraction C was similar to that of colonies. Intracellular carbohydrates and carbohydrates

Table 4. Separation of carbohydrates by DEAE-Sephadex A-25 (%).

		Fraction		
	A	В	С	- Recovery
M. aeruginosa No. 1				
single cells	71.3	3.3	4.7	79.3
colonies	45.2	3.2	24.2	72.6
M. aeruginosa No. 2				
single cells	70.6	5.8	2.0	78.4
colonies	40.9	3.5	26.4	70.8
M. wesenbergii				
single cells	36.5	1.0	34.3	71.8
colonies	32.3	9.4	37.5	79.2
M. viridis				
single cells	37.5	16.4	20.3	74.2
colonies*	48.7	9.2	26.0	83.9

<sup>\*</sup> Natural colonies collected from a waterbloom in Lake Suwa in August 1986.

of the gelatinous sheath fraction of M. wesenbergii and M. viridis were present in ratios of 1:1 and 1.8:1, respectively.

Table 5 shows the percentages of uronic acid residues to carbohydrates. Single cells of M. aeruginosa did not contain uronic acids in fraction A, whereas single cells and colonies of other species contained uronic acids between 1.6% and 4.0% in this fraction. Uronic acids in fraction B increased from 3.5% to 7.4%, and those in fraction C showed further increases in amounts ranging from 9.1% to 22.7%. Gelatinous sheath fractions contained most of the uronic acids. In fraction C, the content of uronic acids in colonies was higher than that in single cells; the content in colonies of M. wesenbergii was highest, amounting to 22.7%. It was found that neutral sugars accounted for most of the carbohydrates in each fraction, exceeding 77.3%.

#### Sugar composition of the three species

The compositions of neutral sugars in each fraction of the three species of *Microcystis* are shown in Figs. 3–6. Glucose, galactose, mannose, xylose, fucose, rhamnose, ribose, and arabinose were present. However, only the first six monosaccharides are presented

Table 5. Uronic acid content (%) in carbohydrates of each fraction.

		Fraction	
	A	В	C
M. aeruginosa No. 1			
single cells	0.0	7.1	9.1
colonies	4.0	6.6	12.3
M. aeruginosa No. 2			
single cells	0.0	3.5	10.5
colonies	4.5	6.3	16.2
M. wesenbergii			
single cells	1.6	6.5	14.2
colonies	4.0	7.4	22.7
M. viridis			
single cells	3.6	4.3	9.6
colonies*	2.1	7.3	15.5

<sup>\*</sup> Natural colonies collected from a waterbloom in Lake Suwa in August 1986.

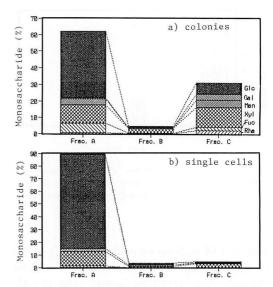


Fig. 3. Sugar composition in three fractions of M. aeruginosa No. 1.

graphically, and the last two monosaccharides were not included because they accounted for less than 2% of the total content. In M. aeruginosa No. 1 (Fig. 3), the main constituent of intracellular carbohydrate in fraction A was glucose, which amounted to 82% and 65% in single cells and colonies, respectively.

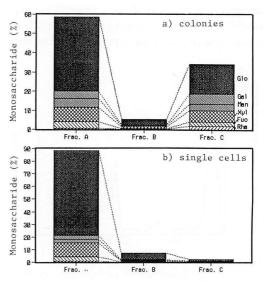


Fig. 4. Sugar composition in three fractions of M. aeruginosa No. 2.

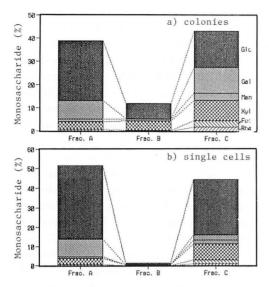


Fig. 5. Sugar composition in three fractions of M. wesenbergii.

Carbohydrates of the gelatinous sheath in fraction C consisted of 39% xylose, 22% glucose, 14% mannose, 11% galactose, 7% rhamnose and 6% fucose, suggesting the presence of heteropolysaccharides. Carbohydrates of *M. aeruginosa* No. 2 showed similar compositional patterns to those of *M.* 

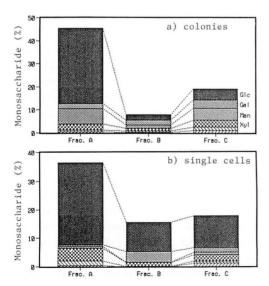


Fig. 6. Sugar composition in three fractions of M. viridis.

aeruginosa No. 1. However, carbohydrates of the gelatinous sheath were composed mainly of glucose often as much as 45% (Fig. 4). The sugar composition of M. wesenbergii is shown in Fig. 5. Intracellular carbohydrates were composed mainly of glucose, accounting for 73% and 64% in single cells and colonies. respectively. The gelatinous sheath fraction contained glucose (36%), galactose (26%) and xylose (20%). In colonies, much galactose was characteristically present not only in the gelatinous sheath fraction but also in the intracellular fraction. In single cells, glucose, the most significant constituent, also comprised more than half of the gelatinous sheath fraction. Figure 6 shows that the carbohydrates of single cells of M. viridis consisted of much larger proportions of glucose than those of colonies in fractions B and C. In single cells, glucose accounted for up to 79% of intracellular carbohydrates, and as much as 66% and 62% of the other two fractions, respectively, whereas the sugar contents except glucose were larger than those in colonies. The gelatinous sheath of colonies contained four kinds of residue, i.e. mannose, glucose, galactose and xylose in decreasing order. Mannose was also present in intracellular carbohydrates in large amounts, possibly being a characteristic feature of M. viridis colonies.

The sugar compositions of gelatinous sheath carbohydrates of the three species of *Microcystis* are shown comparatively in Fig. 7. Xylose or glucose was abundant in the gelatinous sheath carbohydrates from the two

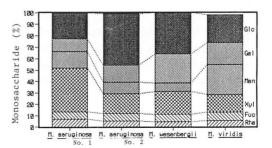


Fig. 7. Sugar composition of gelatinous sheath carbohydrates of three species of *Microcystis*.

strains of *M. aeruginosa*, while in *M. wesenbergii* glucose, galactose and xylose were abundant, and in *M. viridis* mannose was the major constituent except for glucose.

#### Discussion

Mutual transformation between colonies and single cells

Colonies may clearly lose their gelatinous sheath under certain culture conditions, and in fact light intensity is known to affect the appearance of the gelatinous sheath. Although colonies are present in nature, subculture during colony preservation often results in the occurrence of single cells (KRUGER and ELOFF 1979). Olsen and Ganf (1986) indicated that photosynthetic activity affected the synthesis of proteins and carbohydrates. Under conditions where synthesis of proteins is higher than that of carbohydrates, the increase in light intensity, water temperature and nitrogen concentration might induce an increase in cell growth. Therefore the formation of gelatinous sheath containing much carbohydrates is assumed to be difficult. PARKER (1982) suggested that Microcystis formed single cells in low concentrations of cations, and that culture with addition of a chelator could result in disappearance of the gelatinous sheath. A decrease in the concentration of calcium ion to one-quarter is considered to result in predominance of single cells. In contrast, gelatinous sheath might be produced under the reverse conditions. When the calcium concentration was increased nine times that of the normal B12 medium. colonies tended to grow. However, it seemed to be more difficult to produce a shift from single cells to colonies. Culture of colonies without single cells may be obtained by repeated isolation and subculture of several colonies under the conditions mentioned above.

Comparison of sugar composition of colonies and single cells

When carbohydrates from the cells were separated into three fractions using an anion-

exchanger, fractions A and C were found to be different not only in uronic acid content but also in sugar composition. In colonies, glucose accounted for most of the intracellular carbohydrates in fraction A, several monosaccharides except glucose were increased in fractions B and C, and the degree of heterogeneity in the amounts of constituent sugars was increased. With axenic M. aeruginosa K-3A, NAKAGAWA et al. (1987) reported that the constituent sugar unit of intracellular carbohydrates was mostly glucose, while the carbohydrates of slime were composed of heterogeneous sugar units. Our experimental findings after separation by anion-exchanger were in approximate agreement with their results. In single cells forming no colonies, glucose was found in the majority of intracellular carbohydrates, and more than 50% of carbohydrates were glucose even in fractions B and C of cells with layered thin gelatinous sheath. The thin layered gelatinous sheath of single cells contained less heterogeneous polysaccharides, and uronic acid residues were fewer in the gelatinous sheath of single cells than in the gelatinous sheath of colonies. Glucose is considered to play a central role in carbohydrate metabolism in single cells. The present results suggest that single cells and colonies may differ in their mode of carbohydrate synthesis.

Although colonies with gelatinous sheath produced less organic material and carbohydrates than did single cells, the existence of apparently highly heterogeneous polysaccharides might have significance in the physiology of *Microcystis*.

Sugar composition of the gelatinous sheath of three species

TAKAMURA et al. (1986) reported that rhamnose and xylose were abundant among the sugars present in the slime of axenic M. aeruginosa K-3A, and that glucose or xylose was abundant in the gelatinous sheath of other cyanobacteria. In the present study, xylose and glucose were shown to be the main sugar constituents of the gelatinous sheath of

M. aeruginosa No. 1 and No. 2, respectively. In addition, the amounts of galactose and mannose were higher in the gelatinous sheath of M. wesenbergii and M. viridis, respectively.

M. wesenbergii and M. viridis can be identified easily because their formation of colonies is a relatively consistent feature. However, M. aeruginosa shows various characteristics, and has to be reclassified on the basis of cell size, cellular arrangement, colony shape, staining of the gelatinous sheath by alcian blue, chemical properties and so on. WATANABE et al. (1986) showed that M. aeruginosa can be further divided into M. aeruginosa f. aeruginosa and M. aeruginosa f. flos-aquae. In the case of M. aeruginosa, the difference in carbohydrate composition of gelatinous sheath between the two strains might be due to the difference in the level of forma. In the present study, the sugar composition of gelatinous sheath differed among the three species of Microcystis cultured under the same conditions. general, the properties of polysaccharides are known to depend on the nature of their constituent sugars, particularly the mode of their arrangement, linkage configuration and position. It is suggested that these features of the main polysaccharides of the gelatinous sheath differ to a great extent among the three species of *Microcystis* examined in the present study.

#### Acknowledgement

We thank Professor Y. TAKAMURA, Ibaraki University, for his helpful advice.

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#### 雨宮由美子・中山大樹:Microcystis 属藍藻の生産する粘質鞘多糖の分離と糖組成

Microcystis 属藍藻について、M. aeruginosa 2 株、M. wesenbergii 1 株、M. viridis 1 株のコロニー株と群体を作っていない単細胞株を用い、コロニー株と単細胞株の生産する糖の組成を調べ、粘質鞘の糖組成の特徴を明らかにした。超音波処理により各株の糖を可溶化し、陰イオン交換クロマトグラフィー (DEAE-Sephadex A-25) を用いて、粘質鞘多糖と細胞内多糖の分離を行った。細胞内多糖は吸着せずに溶出する画分に、粘質鞘多糖は 0.1-0.8 M NaCl の濃度勾配で溶出する画分に分離された。細胞内構成糖はグルコースが大半を占めて種による差はなかったが、粘質鞘はヘテロ多糖であり、種により特徴がみられた。粘質鞘は、M. aeruginosa ではグルコースの多い株とキシロースの多い株があり、M. wesenbergii ではグルコース、ガラクトース、キシロースを多く含み、M. viridisではマンノースを多く含んでいた。 (400 山梨県甲府市武田4-3-11 山梨大学工学部環境整備工学科)

# The life history of *Dudresnaya japonica* Okamura (Cryptonemiales, Rhodophyta) in culture

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NOTOYA, M. and ARUGA, Y. 1989. The life history of *Dudresnaya japonica* OKAMURA (Cryptonemiales, Rhodophyta) in culture. Jpn. J. Phycol. 37: 263-267.

The life history of a red alga *Dudresnaya japonica* Okamura collected at Bishamon, Miura Peninsula, Kanagawa Prefecture, was studied in laboratory culture. The carpospores released from a natural mature erect female gametophyte gave rise to compact crustose tetrasporophytes. The tetraspores were produced from surface cell layer of the crustose frond by cruciate division. They germinated and grew up to crustose fronds, which issued erect male and female gametophytes. Both of the two species, *D. minima* and *D. japonica*, occurring in Japan have crustose tetrasporophytes in the life history.

Key Index Word: Cryptonemiales-culture-Dudresnaya japonica-life history-Rhodophyta.

Two species of *Dudresnaya* (Cryptonemiales, Rhodophyta) are known in Japan. *Dudresnaya japonica* Okamura is distributed along the Pacific coast of southern Honshu (Okamura 1936) and *D. minima* along the coasts from Kyushu to Okushiri Island, Hokkaido (Okamura 1936, Hasegawa 1949). Both of the two species were endemic.

Among thirteen species hitherto known in the genus *Dudresnaya* (Robins and Kraft 1985, Seales and Ballantine 1986), four species were reported to have isomorphic tetrasporophyte and gametophyte, and one species (*D. minima*) was recently repoted to have heteromorphic gametophyte and tetrasporophyte in the life history in laboratory culture; the crustose tetrasporophyte and the erect gametophyte (Notoya 1989). Umezaki (1968) carried out laboratory culture of *D. japonica* and showed only the early development of carpospores without success in completing its life history.

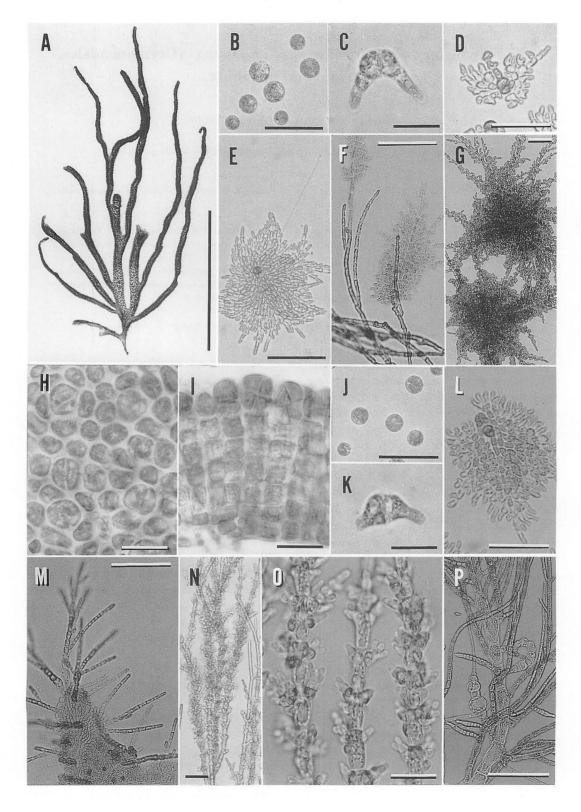
In this paper we report the life history of D. japonica having heteromorphic tetrasporophyte and gametophyte in a similar way to that of D. minima.

#### Material and Methods

Mature female gametophytes of *D. japonica* (Fig. 1, A) were collected on April 14, 1986, from a depth of 8 m, where *Ecklonia cava* and *Undaria pinnatifida* were mainly observed, at Bishamon, Miura Peninsula, Kanagawa Prefecture. They were put in a bottle of 3 *l* with natural seawater, and immediatly transported to the Aquaculture Center, Aomori Prefecture, for culture.

Well-mature cystocarpic branches 3-5 cm long were cut off, washed five or six times with brush in the sterilized seawater to take off contaminants attached to the surface of branches and finally set in Petri dishes with strilized seawater added with 5 ppm GeO<sub>2</sub> overnight. Next morning, released carpospores were collected by drawing with a glass capillary micropipet and were transferred into a new vessel containing sterilized seawater. Washing carpospores with sterilized seawater was repeated three or four times, and the spores were set on slide glasses for some hours in Petri dishes. Unialgal cultures were thus established.

The cultures were maintained at 25°C



under a photoperiod of 14L:10D. During the light period, light of 2000-4000 lux was supplied from white fluorescent lamps. Modified Grund medium (McLachlan 1973) was used for the cultures and renewed once a week.

#### Results

The size of the liberated carpospores ranged from 11 to 18  $\mu$ m (mean 14  $\mu$ m) in diameter (Fig. 1, B) about one half that of D. minima.

In the early developmental stage from carpospore the germling of diprotocellular type was observed one day after germination (Fig. 1, C). Three-day-old germlings were monostromatic and prostrate with cells spread around the original spore (Fig. 1, D). Ten-day-old germlings spread more and became compact disks with fine hair cells appeared, but having one cell layer yet (Fig. Sometimes, the filamentous cells were issued from surface or margin cells of the compact disk, and these filaments grew unattached. When attached to the substrate like a slide glass, the filaments often became reprostrate monostromatic disks and grew to compact disks (Fig. 1, F). However, the filaments were not observed to be upright fronds.

About fourty five days after carpospore germination, compact disks were formed. They were composed of seven or nine cell layers, but their margins were thin, composed of one layer, and more spread (Fig. 1, G). The discoidal fronds were 1-2 mm in diameter,

and they were not circular but like *Himenoclonium*.

In about two months of culture, the tetrasporangia were observed to lie scattered in the surface layer of the compact disks. In surface view, the tetrasporangia were 12–15  $\mu$ m in diameter and bigger than other surface cells (Fig. 1, H).

Vertical section of the compact disk showed that the tetrasporangia were situated at the top of cell rows each of which were composed of seven or eight sterile cells. The mature tetrasporangia were globular in shape, 15-19  $\mu$ m in height and 14-17  $\mu$ m in diameter, showing cruciate division (Fig. 1, I).

Liberated tetraspores from the crustose fronds were spherical, red in color, and 8–9  $\mu$ m in diameter (Fig. 1, J). The process of tetraspore germination was basically the same in their appearance as that of carpospore germination. One-day-old germlings of tetraspores were diprotocellular type (Fig. 1, K). Ten-day-old tetraspore germlings were quite similar to carpospore germlings in that they were monostromatic and prostrate in all directions around the original spore and became to form compact disks (Fig. 1, L).

After one and half a month in culture, branched upright fronds developed from the surface or marginal cells of the crustose compact disks (Fig. 1, M). The upright fronds grew up with alternate branches from the axis. After four weeks from the development of upright fronds, they reached about 1 cm in height and the spermatangia were observed at upper part of branches (Fig. 1, N & O).

In further three weeks, the fronds without

Fig. 1. Dudresnaya japonica Okamura in culture. (A) A female gametophyte collected at Bishamon, Miura Peninsula, Kanagawa Prefecture, Japan, on April 14, 1986. (B) Carpospores released from a natural gametophyte. (C) A carpospore germling of diprotocellular type after one day in culture. (D) A three-day-old germling, monostoromatic with prostrate cells around the original spore. (E) A ten-day-old germling, discoidal with fine hair cells. (F) Re-prostrate filamentous cells forming compact disk. (G) Fourty-five-day old germlings with several layers of cells in the centeral part. (H) Surface view of a mature tetrasporophyte, showing the tetrasporangia. (I) Vertical section of a mature tetrasporophyte, showing the tetrasporangia at the top of sterile cell row. (J) Tetraspores liberated from the crustose disk (tetrasporophyte). (K) A one-day-old tetraspore germling. (L) A ten-day-old tetraspore germling. (M) Initial upright growths from a tetraspore germling. (N) Branches of a mature male gametophyte with developed spermatangia. (O) Close up of parts of branches of a mature male gametophyte. (P) Parts of branches of a female gametophyte with young procarp-like cells. Scale bar; 10 cm in (A); 20 μm in (C), (H), (I), (K), (O); 50 μm in (B), (D), (J), (L); 100 μm in (E)–(G), (M), (N), (P).

spermatangia reached about 1.5 mm long, and young procarp-like cell rows were observed on axial filaments of the upright fronds (Fig. 1, P). Even after three months more in culture, upright fronds with fruiting organs were not obtained.

#### Discussion

The early development of sporelings has been reported in Dudresnaya sp. by Tobler (1903) and Killian (1914). Okamura (1908) reported the germlings of carpospore within cystocarp in D. japonica, and UMEZAKI (1968) reported the development of carpospore germlings from early to later stages for one year until the prostrate crustose disk phase was reached in D. japonica. NOTOYA (1989) reported the early stage of sporeling development in D. minima. In the present paper the early stages of tetraspore and carpospore germlings of D. japonica were described. From these reports, it may be said that in the genus Dudresnaya the early stage of sporelings is diprotocellular type and the sporelings became prostrate crustose disks in more developed stage. The tetrasporophyte of D. japonica is a branched Himenoclonium-like crustose disk and its shape is quite similar to that of D. minima (NOTOYA 1989), Schimmelmania (CHIHARA 1972), Acrosymphyton (CORTEL-BREEMAN 1975), Farlowia molis (DeCew and West 1981) and Gloeophycus (Nоточа 1983, 1984).

The branched filaments issued from a tetrasporophytic crustose disk were often observed in the present culture. They are similar to the frond reported by UMEZAKI (1986), but did not develop to upright gametophytic fronds.

The tetrasporangial division pattern of *Dudresnaya japonica* was completely cruciate type and differed from irregularly cruciate type of *D. minima* (NOTOYA 1989).

In the genus *Dudresnaya*, four species were hitherto known to have isomorphic gametophyte and tetrasporophyte; *D. australis*, *D. capricornica*, *D. crassa* and *D. verticillata*. Two species, *D. japonica* and *D. minima* 

which were endemic in Japan, possess the life history in which upright gametophytes alternate with crustose heteromorphic tetrasporophytes. The present results suggest the possibility that the remaining seven species, in which only the gametophyte is known up to the present, may have the heteromorphic tetrasporophyte.

#### Acknowledgement

The authors wish to express their sincere thanks to Mr. Shogo Arai for his kindness in collecting the samples for this study.

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#### 能登谷正浩・有賀祐勝:紅藻ヒビロウドの生活史

神奈川県三浦半島の毘沙門で採集した成熟したヒビロウド (Dudresnaya japonica Okamura) の雌性体(配偶体)から放出された果胞子を培養したところ、小さな殻状の薬体(胞子体)に発達し、それに四分胞子が形成された。放出された四分胞子は発芽して直立する配偶体に生長し、精子嚢をもつ体とプロカルブ様細胞をもつ体にまで発達した。従って、既に報告したヒメヒビロウド (D. minima Okamura) と同様に形態の異なる四分胞子体と配偶体からなる生活史を持つことが明らかになった。従って、本邦産のヒビロウド属2種は共に異形世代交代の生活史を持つことになる。(108東京都港区港南4-5-7東京水産大学藻類学研究室)

# Solieria pacifica (Yamada) Yoshida, comb. nov. (Solieriaceae, Rhodophyta) from Japan\*

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Yoshida, T. 1989. Solieria pacifica (Yamada) Yoshida, comb. nov. (Rhodophyta, Solieriaceae) from Japan. Jpn. J. Phycol. 37: 268–270.

An alga known as Solieria robusta from Japan was shown to be different from that of Australia, especially in basal morphology. A new combination, Solieria pacifica was proposed for the Japanese species, based on Chrysymenia pacifica Yamada.

Key Index Words: Chrysymenia pacifica—Rhodophyta—Solieria pacifica—Solieria robusta—Solieriaceae—taxonomy.

An alga of Solieriaceae from Japan was first identified as Rhabdonia robusta J. AGARDH by YENDO (1914), based on the specimens from southern part of Japan. This species was later called Solieria robusta (GREVILLE) KYLIN by Okamura (1936). The same species was also reported from Chinese coast (TSENG 1983) and Korea (LEE and KANG 1986). Comparison of Japanese specimens with those of Australia revealed certain differences in external morphology. Therefore, it is concluded that the alga in question is a different taxon at specific level from the southern hemisphere S. robusta (type locality: western Australia). Nomenclatural problem Japanese taxon is discussed in relation with Chrysymenia pacifica YAMADA (1933).

#### Materials

Many specimens from several localities of Chiba, Kanagawa, Wakayama, Hiroshima, Fukuoka, and Kumamoto Prefectures in southern parts of Japan, deposited in the herbarium (SAP) of Faculty of Science, Hokkaido University, were used for observation. A collection of *S. robusta* from Queenscliff, Vic-

toria, Australia (Aug. 20, 1988, leg. T. Yoshida, SAP 052979, 052980) was used for comparison. Herbarium specimens of Womersley collection in the University of Adelaide (now in AD) were also examined.

#### Observations

Morphology of Japanese specimens: One or several thalli arise from a discoid or irregular scutate basal disc (Fig. 1). thalli are up to 40 cm high, and are attenuating below into a short cylindrical stem, gradually dilating above, cylindrical when young, then becoming compressed above and nearly flat, up to 15 mm wide in the broadest part in older plants. The texture of the thallus is soft fleshy when young and almost cartilaginous in well grown plants. are 2-3 times branched alternately, with the bases of branchlets constricted. Apices of the branches are acutely pointed. Thallus structure is multiaxial in construction typical of this genus, with medulla composed by loosely arranged elongate filaments and cortex with inner large round cells and outer small cells. Carpogonial branches are usually composed of 3 cells, directing inwardly with trichogyne turned to the surface. Mature cystocarps are embedded in the medullary region and with a

<sup>\*</sup> Dedicated to the memory of the late Dr. Munenao Kurogi (1921–1988), Professor Emeritus of Hokkaido University.

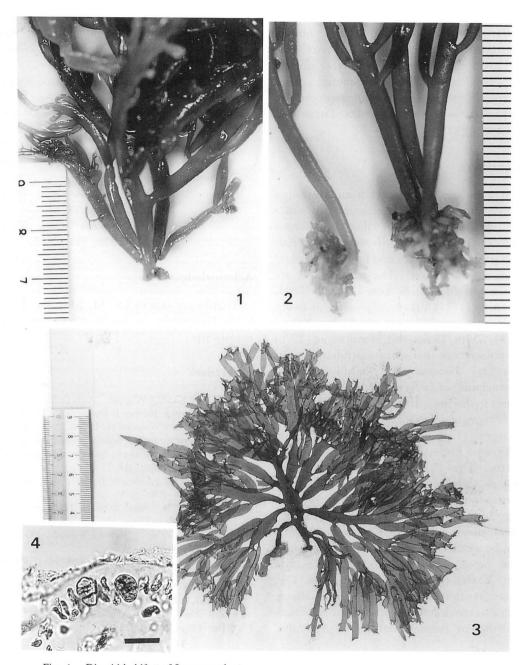


Fig. 1. Discoid holdfast of Japanese plant.

- Fig. 2. Basal fibrous holdfast of Solieria robusta from Queenscliff, Australia.
- ig. 3. Holotype of Chrysymenia pacifica Yamada, SAP 21250.

Fig. 4. Cross section of Chrysymenia pacifica, showing zonate tetrasporangia. Scale bar 30 μm.

distinct ostiole and are slightly raised on the surface of the branches. Each cystocarp has a large central fusion cell. Tetrasporangia are scattered over the thallus surface, dividing zonately. Okamura (1921) illustrated the morphological features of the Japanese plant.

Morphology of Australian Solieria robusta: The specimens at hand are young and im270 Yoshida, T.

mature individuals about 10 cm high. A holdfast consists of a group of fibrous outgrowths issued from the basal part (Fig. 2). Erect thalli are usually terete, slightly compressed, about 2 mm in diameter. A full description of vegetative and reproductive morphology was given by MIN THEIN and WOMERSLEY (1976).

#### Discussion

Japanese specimens differ from Australian material in morphology, especially in structure of basal parts in that no fibrous outgrowth is present in Japanese material. Erect part is much stout and larger in size and becomes compressed. These differences are sufficient to distinguish them at specific level. OKAMURA (1921) already expressed his doubt about the identification made by YENDO (1914). Min Thein and Womersley (1976) also pointed out that extra-australian records must be reexamined carefully. nomenclature of Japanese material becomes a subject to discuss. In 1933, YAMADA described Chrysymenia pacifica, based on a single specimen (Fig. 3) collected at Oshima, Wakayama Prefecture and sent to him by Dr. OKADA. This is a tetrasporic individual. structure that of Solieria. Tetrasporangia divide zonately (Fig. 4). Just after the publication of the name, OKAMURA (1934) mentioned that Chrysymenia pacifica must be treated as a synonym of Solieria robusta (auct. japon), according to the information from YAMADA himself. Identity of Chr. pacifica is thus clear. As shown above, Japanese specimens attributed to S. robusta are different from S. robusta of Australia.

New name is thus necessary for the Japanese taxon. In this circumstance, specific epithet "pacifica" is available for it, and the following combination is proposed:

Solieria pacifica (Yamada) Yoshida, comb. nov.

Basionym: Chrysymenia pacifica YAMADA,

1933: 279, pl. 10.

Holotype: SAP 21250 (Aug. 6, 1928.

Oshima, Wakayama Prefecture)

Synonym: Rhabdonia robusta sensu Yendo,

1914, non J. AGARDH. OKA-MURA, 1921: 102, pl. 174. Solieria robusta sensu OKA-MURA, 1936: 591, f. 277.

#### Acknowledgements

Thanks are due to Dr. M. Masuda, Hokkaido University, for his kind advice on the manuscript.

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#### 吉田忠生:ミリン(紅藻、スギノリ目)の学名について

日本中・南部に産する紅藻ミリンにはこれまでオーストラリア産の Solieria robusta の学名が当てられてきた。しかし、付着器の形態などに違いがあって、同一種とはいえない。日本産の種にはすでに Chrysymenia pacifica YAMADA の学名があるので、Solieria pacifica の組合せを提案する。(060 札幌市北区北10条西8丁目 北海道大学理学部植物学教室)

# Notes on Spirocladia loochooensis (Yendo) Yoshida, comb. nov. (Rhodomelaceae, Rhodophyta)

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YOSHIDA, T. 1989. Notes on *Spirocladia loochooensis* (YENDO) YOSHIDA comb. nov. (Rhodomelaceae, Rhodophyta). Jpn. J. Phycol. 37: 271–273.

Examination of newly collected materials of Wrightiella loochooensis Yendo showed that this species is properly accommodated in the genus Spirocladia Boergesen, because it has no spinous adventitious branchlet. Therefore, a new combination, Spirocladia loochooensis (Yendo) Yoshida, is proposed.

Key Index Words: Rhodomelaceae—Rhodophyta—Spirocladia loochooensis—taxonomy—Wrightiella loochooensis.

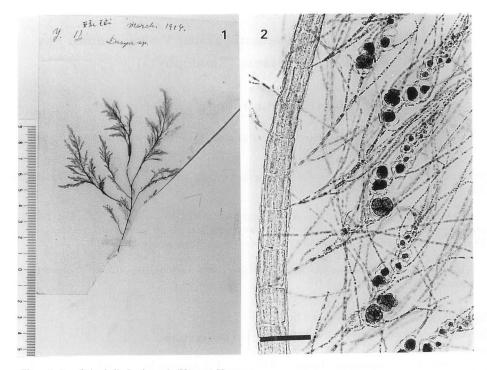
Wrightiella loochooensis was described by Yendo (1920) on a single specimen from an unknown locality of Okinawa Prefecture (Loochoo=Ryukyu), southern Japan. He gave a brief description without a figure. This species was rarely encountered since that time (Yamada and Tanaka 1938, Segawa and Yoshida 1961). A recent collection of an alga identifiable to this species afforded a chance to consider generic attribution of this taxon.

#### Materials

In addition to the holotype [Ryukyu (Okinawa March Prefecture), anonymous, ex herb. S. Narita, Yendo collection in TI, Fig. 1], following specimens deposited in the herbarium (SAP) of Faculty of Science, Hokkaido University were examined: Ryukyu, no data, anonymous (herb. Okamura). Kimu-wan, Okinawa Prefecture, February 3, 1989, anonymous (SAP 052366). Yonakuni Island, Okinawa Prefecture, April 1935, leg. T. Tanaka (SAP 21173). Amakusa, Kumamoto Prefecture, April 1933, leg. T. TANAKA (SAP 024213). Tomioka, Kumamoto Prefecture, July 1, 1957, leg. T. YOSHIDA (SAP 049889).

#### Observation

Tetrasporophytes only are available for study, including the holotype. The holotype specimen is 9.5 cm high without a basal part (Fig. 1). Thalli collected recently are up to about 10 cm high, terete throughout, irregulary branched. Basal parts are buried in Terete fronds branch several coral sand. times with wide angle, up to 1.5 mm in diameter. Erect axes are composed of a central cell and 4 pericentral cells, thickly corticated below. On the upper part of the thallus, many trichoblasts about 2 mm long issued spirally from the are Trichoblasts are uniseriate, sending off spirally uniseriate side branches from each cell of the axial filament, except the basal cell, and every cell of the trichoblast contains plastids. These deciduous trichoblasts fall off from the lower parts of the thallus, leaving basal cell. Tetrasporangial stichidia (Fig. 2) are formed by the transformation of an axial filament of the trichoblast, so that uniseriate lateral filaments are issued from the surface of stichidia. Tetrasporangia are produced spirally in the stichidia, one in each segment.



Figs. 1–2. Spirocladia loochooensis (Yendo) Yoshida. Fig. 1. The holotype specimen, TI. Fig. 2. A part of branch bearing stichidia. Scale 200  $\mu$ m.

#### Discussion

In describing this species, Yendo (1920) stated that "Affinis Wrightiella tumanowiczia ramulis spinaeformibus SCHMITZ, sed destitutis eadem facile diagnoscitur (similar to W. tumanowiczia, but easily distinguished by the absence of spinous branchlets)", as a distinction from the type species of the genus Later, Boergesen Wrightiella. established his new genus Spirocladia, with the type and only species, S. barodensis, from Okhamandal, in the State of Baroda, India, basing on the difference in that his genus had no spinous short branchlets of adventitious endogenous origin on the branches. In comparison with Yendo's species, it is clear that his species is properly accommodated to Spirocladia, not to Wrightiella. Boergesen (1933) observed only male individual. As the specimens at hand are all tetrasporic, identity with Indian species is not certain for the moment. Therefore, I propose the following

#### combination:

Spirocladia loochooensis (Yendo) Yoshida, comb. nov.

Basionym: Wrightiella loochooensis Yendo, Bot. Mag. Tokyo **34**: 8. 1920.

Holotype: Loochoo (Okinawa Prefecture), March 1914, ex herb. Narita, TI (Fig. 1).

Another species of the genus *Spirocladia*, *S. minuta* NASR (1939), from the Red Sea is very small (up to 3 mm high), and has decumbent lower parts and poorly corticated branches. These characters quite differ from our material.

I wish to acknowledge Miss N. Uesugi, Tokyo Kyuei Co., for suggesting the problem here considered.

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#### 吉田忠生: 新組合せ Spirocladia loochooensis (ヒゲヨレミグサ, 新称) について

ライチエラ Wrightiella loochooensis YENDO の記載後, BOERGESEN はインド産の材料について刺状の不定内生枝がないことを主な特徴として1933年に Spirocladia 属を設立した。日本産の種もそのような刺状小枝がないことから, Spirocladia 属に移すべきであるので, Spirocladia loochooensis (YENDO) comb. nov. の新組合せを提案し, あわせてヒゲョレミグサの新和名も提案する。(060 札幌市北区北10条西8丁目 北海道大学理学部植物学教室)

# Light conditions and photosynthetic productivity of ice algal assemblages in Lake Saroma, Hokkaido

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SATOH, H., YAMAGUCHI, Y., WATANABE, K. and ARUGA, Y. 1989. Light conditions and photosynthetic productivity of ice algal assemblages in Lake Saroma, Hokkaido. Jpn. J. Phycol. 37: 274–278.

Photosynthetic productivity of ice algal assemblages was investigated in Lake Saroma on the Okhotsk Sea coast of Hokkaido in early March of 1987 and 1988. Relative light intensity at the bottom of ice was about 1.0% of incident solar radiation in 1987, whereas it was about 4.4% in 1988. In photosynthesis-light curves, the photosynthetic rate was saturated at 27.5  $\mu$ E m<sup>-2</sup> s<sup>-1</sup> in 1987, the saturated rate being 0.42 mgC mgchl. $a^{-1}$  h<sup>-1</sup>, while it was not saturated even at 162  $\mu$ E m<sup>-2</sup> s<sup>-1</sup> in 1988, the maximum rate being 0.43 mgC mgchl. $a^{-1}$  h<sup>-1</sup>. The compensation light level of ice algae was 0.04–0.05% of the incident light intensity. The daily production of ice algae in 1987 (14.3 mgC m<sup>-2</sup> day<sup>-1</sup>) was 6.2 times greater than that in 1988 (2.3 mgC m<sup>-2</sup> day<sup>-1</sup>). The specific growth rate ( $\mu$ ) of ice algae was 0.12 and 0.027 div. day<sup>-1</sup> in 1987 and 1988, respectively. The present study demonstrates that the growth of ice algae is strongly controlled by light conditions at the bottom of ice in the ice-covered season.

Key Index Words: compensation point—growth rate—ice algae—Lake Saroma—photosynthesis.

Ice algae (ice algal assemblages) are present in all areas where sea ice is a regular feature of the environment (HORNER 1985). The role of ice algae is doubtlessly important in such ecosystems of ice-covered seas (McRoy et al. 1972, HORNER 1977, DUNBAR 1979, PLATT et al. 1982, PALMISANO and SULLIVAN 1983, WATANABE 1988, SATOH and WATANABE 1988). In the previous paper (Satoh et al. 1989), the present authors reported the photosynthetic production of ice algae under extremely low light conditions in Lake Saroma, which is known as the southernmost area of seasonal sea ice extension in the northern hemisphere, and suggested that their contribution to primary production in the lake was considerablely large. The obtained values of primary production of ice algae were comparable to those reported in the polar regions (Bunt 1964, Burkholder and Mandelli 1965, Alexander 1979, Satoh and WATANABE 1986).

In Lake Saroma, ice algae were found in the bottom layer of ice core and grew most extensively between mid-February and early March (Hoshiai and Fukuchi 1981). The light intensity at the bottom layer of ice was very low (Satoh et al. 1989) because of light extinction by ice and snow. Thus, it is important to know the photosynthetic rate accurately at light regimes near the compensation point for estimating primary production in the ice-covered season.

In this paper, the authors describe the under-ice light conditions with reference to the photosynthetic nature of ice algae in Lake Saroma. The results obtained in the ice-covered season of 1988 are compared with those in the preceding year (SATOH et al. 1989).

#### Material and methods

Field studies were carried out on 7-9

March in both 1987 and 1988 at the same site (44°10'N, 143°46'E) in Lake Saroma, Hokkaido, which is a lagoon of seawater flowing in through the two channels from the Sea of Okhotsk. For collection of ice algal samples, ice was cut in a mass of about 1 m<sup>2</sup> surface with a chain saw. The colored part within a few cm from the bottom of ice was scraped off into filtered seawater, and maintained at temperatures below 0°C for experiments. Three ice cores were collected with a SIPRE coring auger, and average concentrations of chlorophyll a within 4 cm from the bottom of ice cores were used as an index of biomass of ice algae. For this purpose, after the ice cores were melted at room temperatures, the samples were immediately filtered through glass fiber filters (Whatman GF/C). Pigments of ice algae retained on the filters were extracted with 90% acetone. Chlorophyll a and pheopigment concentrations were determined by the fluorometric method of STRICKLAND and Parsons (1972) modified by Aruga (1979) with a Hitachi 650-40 fluorometer or a Turner Designs 10-005R fluorometer.

Estimations of daily primary production of ice algae were done with two different methods; the *in situ* method and the chlorophyll method based on photosynthesislight curve. All measurements of photosynthetic activity of ice algae were made by the stable <sup>13</sup>C isotope method (SATOH *et al.* 1985).

To obtain the photosynthesis-light curves, Na<sup>13</sup>CO<sub>3</sub> (Prochem) was added to algal samples in 100 ml DO bottles with 9.9% of the final atom percent of <sup>13</sup>C. The samples were exposed to different light intensities from 0 to 162  $\mu$ E m<sup>-2</sup> s<sup>-1</sup> by using a projector lamp (Kondo 100V-150W) as the light source. Light intensities were regulated with neutral density filters (Toshiba TND-50, 20, 10, 5, 1, 0.5, 0.2). Measurements of light intensity (400-700 nm) were done with an LI-188B quantum meter equipped with an LI-190SB quantum sensor (LI-COR). After the incubation for 3 hours, the samples were filtered through glass fiber filters (Whatman GF/C) precombusted at 450°C for 4 hours. Isotope ratios of <sup>12</sup>C and <sup>13</sup>C in the samples were determined by infrared absorption spectrometry with a JASCO <sup>13</sup>C analyzer EX-130. Calculation of photosynthetic activity was made using the equation of HAMA *et al.* (1983). Daily production (mgC m<sup>-2</sup> day<sup>-1</sup>) was calculated on the basis of the daily photosynthetic rate (mgC mgchl.*a*<sup>-1</sup> day<sup>-1</sup>) multiplied by the chlorophyll *a* concentration (mg m<sup>-2</sup>) of ice core, because the ice algal samples were diluted with filtered lake water for measurements of photosynthetic activity.

In situ primary production of ice algae was also measured. The samples were incubated at the layer immediately beneath the ice during the period from noon to sunset on a clear day. The obtained value was multiplied by a factor of 2 to obtained the in situ daily production (mgC m<sup>-2</sup> day<sup>-1</sup>). Incident and underice photosynthetically active radiations (PAR, 400-700 nm) were measured with an LI-1000 integrating quantum meter equipped with an LI-190SB quantum sensor on snow cover and an LI-192SB underwater quantum sensor.

#### Results and Discussion

Environmental conditions of the study area

Lake Saroma is iced over usually from January to April. Comparing the environmental conditions in early March of 1987 with those in 1988, the thickness of ice in

Table 1. Environmental parameters, chlorophyll a, POC, chl.a/(chl.a+pheopigments), and POC/chl.a of ice algae in early March of 1987 and 1988. The light conditions were measured at local noon.

	1987	1988
Solar radiation (µE m <sup>-2</sup> s <sup>-1</sup> )	1325	1395
Thickness of ice (cm)	38	19
Overlying snow (cm)	8	8
PAR beneath ice ( $\mu$ E m <sup>-2</sup> s <sup>-1</sup> )	14	62
Water temperature (°C)	-1.4	-1.4
Salinity	32.4	31.6
Chl.a (mg m <sup>-2</sup> )	5.68	1.68
POC (mgC m <sup>-2</sup> )	160.2	120.0
Chl.a/(chl.a+pheopigments)	0.99	0.86
POC/chl.a	28.2	71.4

1988 was as half that in 1987 and snow covering the ice attained 8 cm in both years (Table 1). Differences in water temperature and salinity were very small between the two years. The relative light levels at the bottom of ice were 1.0% and 4.4% of the incident solar radiation at around local noon on a clear 1987 and 1988, respectively. dav in Although the level of solar radiation in the ice-covered period of 1988 was almost the same as that in 1987, the light intensity at the bottom of ice was more than 4 times greater in 1988 than in 1987. In considering these facts, it can be said that the under-ice light conditions were controlled by the thickness of ice and snow cover (WATANABE and SATOH 1987).

The standing stock of ice algae was concentrated within the bottom 4 cm layer of ice in Lake Saroma (Satoh et al. 1989), the situation being quite similar to that in the Antarctic ice algae (Watanabe and Satoh 1987). The standing stock of ice algae was 5.68 and 1.68 mg chl.a m<sup>-2</sup> in 1987 and 1988, respectively. The dominant species of ice algae were pennate diatoms Nitzschia spp. in both years.

#### Photosynthesis-light curves

Photosynthesis-light curves of ice algae obtained in 1987 and 1988 are shown in Fig. 1. In the curve in 1987 the photosynthetic rate was  $0.42 \text{ mgC mgchl.} a^{-1} h^{-1}$  at the saturated

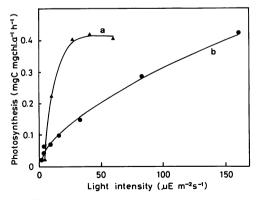


Fig. 1. Photosynthesis-light curves at 0°C of ice algae obtained in 1987 (a) and 1988 (b). The available values in oxygen were converted to those in carbon in the curve of 1987 (Satoh et al. 1988).

point of about 27.5  $\mu$ E m<sup>-2</sup> s<sup>-1</sup>. Contrarily, the photosyntheis-light curve in 1988 did not show the light saturation. The maximum photosynthetic rate was 0.43 mgC mgchl.a<sup>-1</sup>  $h^{-1}$  at 162  $\mu$ E m<sup>-2</sup> s<sup>-1</sup>. The steep initial slope and low saturating light intensity obtained in 1987 indicate the shade adaptation of ice algae (Platt et al. 1982, Palmisano and Sullivan 1985, Satoh and Watanabe 1986). On the other hand, the gentle initial slope with expected higher saturating light intensity obtained in 1988 suggests that the ice algae in that season had adapted to higher light intensities. The variations in such parameters as the saturated rate and the initial slope of photosynthesis-light curves of ice algae might be caused by the light conditions at the bottom of ice.

The compensation light intensity of ice algae both in 1987 and 1988 was about 0.5  $\mu$ E  $m^{-2}$  s<sup>-1</sup>, which corresponds to 0.04-0.05% of the down-welling irradiance in early March in the area. The compensation point was almost the same as those of ice algae obtained in the Antarctic region (PALMISANO and Sullivan 1983, Satoh and Watanabe 1986). The light intensity in the habitat of ice algae was remarkablely low as compared with that of phytoplankton obtained in the ice-free waters of the Antarctic (JACQUES 1983, Sakshaug and Holm-Hansen 1986). Thus, it is concluded that photosynthetic production of ice algae proceeds usually under extremely low light conditions.

Daily production and growth rate of ice algae in 1987 and 1988

The standing stock of ice algae in 1987 (5.68 mgchl.a m $^{-2}$ ) was 3.4 times greater than that in 1988 (1.68 mgchl.a m $^{-2}$ ). The *in situ* photosynthetic rate was 2.52 and 1.34 mgC mgchl. $a^{-1}$  day $^{-1}$  in 1987 and 1988, respectively. Based on these values, the daily production of ice algae was estimated to be 14.3 and 2.3 mgC m $^{-2}$  day $^{-1}$  in 1987 and 1988, respectively. It was 6.2 times greater in 1987 than in 1988.

The daily production was also estimated indirectly by the chlorophyll method (Існімика et al. 1962) and compared with that obtained by in situ measurements. On the basis of the diurnal change of incident light instensity, relative light intensity at the bottom of ice, photosynthesis-light curve and chlorophyll a concentration of ice algae, the daily photosynthetic rate is calculated as 1.82 mgC mgchl.a<sup>-1</sup> day<sup>-1</sup> in 1987 and 1.38 mgC mgchl.a<sup>-1</sup> day<sup>-1</sup> in 1988 (Table 2). The daily photosynthetic rates and production thus estimated agreed well with those obtained by in situ measurements (Table 2).

Based on the *in situ* daily production of ice algae, the growth rates ( $\mu$ ) were calculated by the following equation (Parsons and Takahashi 1973):

$$\mu = \frac{1}{t} \times \log_2 \frac{C_o + \Delta C}{C_o},$$

where  $C_0$  is the initial algal biomass in carbon calculated from the POC/Chl.a ratio, and  $\Delta C$  is the increase of algal biomass during time t estimated directly by the photosynthetic production. The calculated growth rates were 0.12 and 0.027 div. day<sup>-1</sup> in 1987 and 1988, respectively. The growth rate in 1987 was coincident well with those estimated for Antarctic ice algae in the spring seasons (Sullivan et al. 1985, Satoh and Watanabe 1986). The growth rate in 1988 was remarkablely low as compared with that in 1987. The high POC/chl.a ratio and low chl.a/(chl.a and pheopigments) ratio were the main causes for low growth rate of ice algae in

Table 2. Daily photosynthetic rate and production obtained by in situ measurements or estimated from photosynthesis-light curves, and grwoth rate calculated on the basis of in situ production of ice algae in early March of 1987 and 1988.

	1987	1988
In situ measurments:		
Photosynthetic rate (mgC mgchl.a <sup>-1</sup> day <sup>-1</sup> )	2.52	1.34
Production (mgC m <sup>-2</sup> day <sup>-1</sup> )	14.3	2.3
Estimation from P-L curves:		
Photosynthetic rate (mgC mgchl.a <sup>-1</sup> day <sup>-1</sup> )	1.82	1.38
Production (mgC m <sup>-2</sup> day <sup>-1</sup> )	10.3	2.3
Growth rate (div. day-1)	0.12	0.027

the season of 1988.

In conclusion, the photosynthetic nature and growth rate of ice algae were strongly influenced by the light conditions at the bottom of ice which were controlled by thickness of ice and snow cover, if any, as well as by solar radiation.

#### Acknowledgments

The authors express their sincere gratitude to Prof. T. Hoshiai (National Institute of Polar Research) for his encouragement during the present study, and also to Mr. A. Otomo and Mr. S. Miura (Yubetsu Fisheries Cooperative) for their cooperation during the field work. This study was supported by a research grant from the Ministry of Education, Science and Culture No. 6176070 and No. 62760162.

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## 佐藤博雄\*・山口征矢\*\*・渡辺研太郎\*\*\*・有賀祐勝\*:サロマ湖における ice algae の光合成生産力

1987年および1988年の 3 月上旬, サロマ湖の結氷期に ice algae (氷に付着する徴細藻類)の生産力を調査した。 氷直下の相対光強度は、1987年 (氷厚、38 cm)には氷上の日射量の1.0%、1988年 (氷厚、19 cm)には4.4%であった。1987年の光合成—光曲線で、光合成速度は 27.5  $\mu$ E m $^{-2}$  s $^{-1}$  で光飽和に達し 0.42 mgC mg.chla $^{-1}$  h $^{-1}$  であったが、1988年には 162  $\mu$ E m $^{-2}$  s $^{-1}$  のもとでも光飽和に達しなかった(最高値は 0.43 mgC mg.chla $^{-1}$  h $^{-1}$ )。 ice algae の推定光補償点は著しく低く、相対光強度として0.04 $^{-1}$ 0.05%であった。現場法によって得られた ice algae の生産量は、1987年に 14.3 mgC m $^{-2}$  day $^{-1}$ 、1988年には 2.3 mgC m $^{-2}$  day $^{-1}$  であった。生産量から求めた ice algae の増殖速度は、1987年は 0.12 div. day $^{-1}$ 、1988年は 0.027 div. day $^{-1}$ であり、ice algae の増殖は氷下の光条件によって大きく支配されていることが推察された。(\*108 東京都港区港南4 $^{-1}$ 5 $^{-7}$  東京水産大学、\*\*338 埼玉県浦和市下大久保255 埼玉大学教養部、\*\*\*173 東京都板橋区加賀1 $^{-9}$ 10 国立極地研究所)

## Conceptacular development in Sargassum filicinum and autumnal S. horneri (Phaeophyceae)

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OKUDA, T. and SATOH, Y. 1989. Conceptacular development in Sargassum filicinum and autumnal S. horneri (Phaeophyceae). Jpn. J. Phycol. 37: 279–283.

Conceptacular development was studied in Sargassum filicinum and autumnal S. horneri, and was compared with that of S. horneri. The initial cell is divided unequally to produce the tongue cell and the lower cell. Further longitudinal divisions of the lower cell follow, thus forming a lining for the depression. The tongue cell, with no divisions in it, leaves the floor when there are eleven cells surrounding and is transferred to the ostiole, where it forms a loose plug. The time of the tongue-cell's dislodgement and its conditions at the ostiole are almost the same as those reported for S. enerve (=S. fulvellum), though not for S. horneri.

Key Index Words: autumnal Sargassum horneri—conceptacular development—Fucales—Phaeophyceae—Sargassum filicinum—tongue cell.

In his three reports, Tahara (1940, 1941a, b) described some different modes of conceptacular development in the sixteen fucaleans found in Japanese waters. As Sargassum filicinum had been considered to be a rare species until 1950s, this alga was not dealt with by Tahara. Though Sawada (1955, 1956) reported some characteristics of S. filicinum from much more material, conceptacular development was not included in his papers. On the other hand, a strong resemblance in appearance between S. filicinum and S. horneri, in addition to opposite or complex characters in sexuality, led the present authors to investigate the conceptacular development.

The senior author (Okuda 1987) reported a finding in the western Seto Inland Sea of a population of *S. horneri* that became fertile late in the fall instead of the spring—the established time for this population of alga to be fertile. With cylindrical vesicles, these autumnal plants are presently identified with *S. horneri*. Other than the vesicles, however, there are still some features which are in need of examination before reaching a satisfactory identification. In this paper an attempt is made

to compare these algae with S. horneri from a viewpoint of conceptacular development.

#### Materials and Methods

Material of Sargassum filicinum was collected at the mouth of Omura Bay, near Sasebo, Nagasaki Pref., on May 8 and June 7, 1986. Plants were abundant on both these days and each sample turned out to be successful for this study. Plants with small receptacles were dominant on May 8, but only a few on June 7 were found. Samples of autumnal S. horneri were collected near the shores of Yanai, Yamaguchi Pref., on October 15, 1986. Sterile plants were then dominant, and 40-50% of the vegetation seemed to form small receptacles.

An apical section 2–3 mm long of each receptacle was cut from freshly collected thalli and immersed in the fixing fluid (1 g chromic acid, 1 ml propionic acid, 90 ml of seawater) for about 24 hours. Microtome sections were made from paraffin 6  $\mu$ m in thickness and stained with Heidenhain's iron haematoxylin.

#### Results

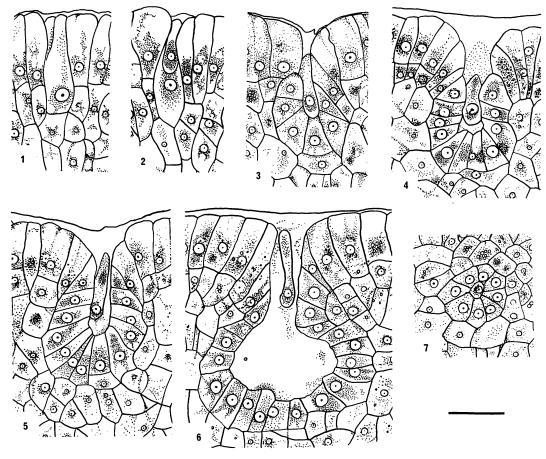
#### 1) S. filicinum

The conceptacle originates from an initial cell which is characteristically flask-shaped and is located near the apical cell of a receptacle (Fig. 1). The initial cell is divided by a downwardly curving transverse wall, thus producing two very dissimilar cells, namely, the tongue cell and the lower cell (Fig. 2). No further divisions occur in the tongue cell. In the lower cell, however, longitudinal divisions continue, and the cells derived from the lower cell partially surround the tongue cell (Fig. 3). Though the tongue cell is firmly attached to these surrounding cells in the early stages,

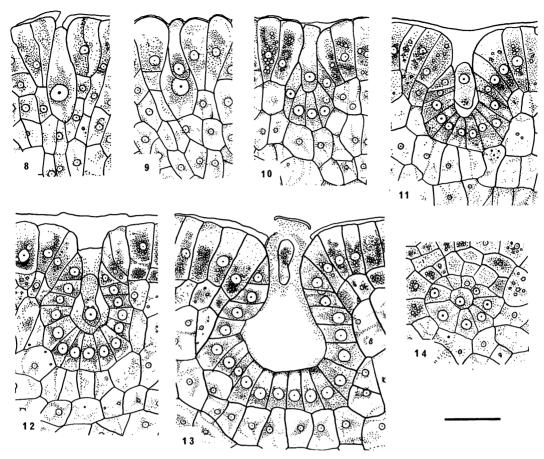
it becomes detached first at its base and is soon transferred to the ostiole (Fig. 4). A mass of mucilage is secreted around the tongue cell, thus leaving a gap between the ostiole and the tongue cell (Fig. 6). In a cross-section through the base of young conceptacles, the conceptacular lining-cells, or the cells derived from the longitudinal divisions of the lower cell, are arranged radially around the tongue cell (Fig. 7).

#### 2) Autumnal S. horneri

The development of the conceptacle follows the same process as *S. filicinum* described above. An initial cell is flask-shaped and is located near the apical cell of a receptacle (Fig. 8). The initial cell is divided by a



Figs. 1-7. Conceptacular development in Sargassum filicinum. 1. Initial cell. 2. Divided initial cell with tongue cell and lower cell. 3. Longitudinal division of the lower cell. 4. Dislodgement of the tongue cell. 5-6. Further development of the conceptacle. 6. Tongue cell as a loose plug in the ostiole. 7. Cross-section through the base of a young conceptacle with cells radially arranged. Scale bar is 20 μm.

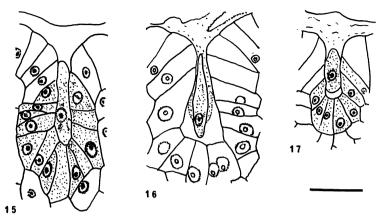


Figs. 8-14. Conceptacular development in autumnal Sargassum horneri. 8. Initial cell. 9. Divided initial cell with tongue cell and lower cell. 10. Longitudinal division of the lower cell. 11. Dislodgement of the tongue cell. 12-13. Further development of the conceptacle. 14. Cross-section through the base of a young conceptacle with cells radially arranged. Scale bar is  $20 \, \mu m$ .

downwardly curving transverse wall to produce two unequal cells, namely, the tongue cell and the lower cell (Fig. 9). No further divisions occur in the tongue cell. In the lower cell, on the other hand, longitudinal divisions continue, and there is no space between the tongue cell and the derived cells from the lower cell in the early stages (Fig. The tongue cell, however, becomes detached first from the inner wall of the conceptacle at its base and is shortly after transferred to the ostiole (Fig. 11). A mass of mucilage is secreted around the tongue cell, thus forming a gap between the ostiole and the tongue cell (Fig. 13). In a cross-section through the base of young receptacles, the conceptacular lining-cells, or the cells derived from the longitudinal divisions of the basal cell, are arranged radially around the tongue cell (Fig. 14).

#### Discussion

The present concept of the conceptacular development in the Fucales was first introduced by Simons (1906) on Sargassum filipendula. According to her designation, she showed that an initial cell is unequally divided to produce a tongue cell and a lower cell, and that the latter is responsible for the later development of the conceptacle. Nienburg (1913) studied seven species in seven genera and showed that the mode of the development varies somewhat in these members. Fen-



Figs. 15-17. Young conceptacles around the tongue-cell dislodgement (after Tahara 1940, 1941a). 15. Sargassum horneri. 16. S. enerve. 17. S. hemiphyllum. Scale bar is 20 µm.

SHOLT (1955) employed conceptacular development as one of five characteristics to discuss the Cystoseira-Cystophyllum complex. TAHARA (1940, 1941a, b) examined sixteen species in three genera around Japan, namely, Cystophyllum (Myagropsis), Sargassum, Coccophora. According to him, from a viewpoint of the tongue-cell behavior, there are some different paths even in Sargassum. Among thirteen species of Sargassum dealt with by him, S. horneri has attracted attention because of its growing tongue cell.

Based on some characteristics previously reported concerning S. filicinum (SAWADA 1955, 1956; OKUDA 1977) and autumnal S. horneri (Okuda 1987; Honda and Okuda 1989), the authors surmised that the conceptacular development in these algae would follow the same progress as S. horneri. However, as the results show, both algae follow the same pattern as that reported by Tahara (1940) on S. enerve (=S. fulvellum). The tongue cell of S. horneri enlarges, along with the divisions of the lower cell, in the early stages. According to Tahara's figure, redrawn in Fig. 15, there is no space between the enlarged tongue cell and the lower-cell derivatives when they number thirteen. The tongue cell becomes detached and transfers to the ostiole some time in the conceptacular development. In S. enerve (Fig. 16) and S. hemiphyllum (Fig. 17), the tongue cell leaves the base when the derivatives number about ten or earlier. In S. filicinum and autumnal S. horneri, the tongue cell has already been dislodged when the derivatives number eleven. As far as the number of the derivative cells at the time of the tongue-cell's dislodgement is concerned, thirteen is still early for S. horneri and eleven is late for S. filicinum and autumnal S. horneri. The authors examined S. horneri from Tsuyazaki, Fukuoka Pref., and found that the tongue cell is firmly attached to the floor of the conceptacle when the derivatives number eleven.

In more advanced stages, the tongue cell of both algae studied here secretes a mass of mucilage around it, thus leaving a space between the ostiole and the tongue cell. This is also true of *S. enerve*. In *S. horneri*, however, the tongue cell forms a tight plug according to Tahara (1940). This has been reconfirmed by the present authors as well.

Concerning the tongue-cell behavior when it leaves the floor and clogs the ostiole, the results are quite unexpected because autumnal *S. horneri* follows a different path than that of *S. horneri*. The authors will study this unexpected behavior further to find if there is a certain range in the cell number of the lower-cell derivatives at the time of the tongue-cell's dislodgement.

#### Acknowledgements

The authors stayed at the Tana Marine

Biological Station, Shimonoseki University of Fisheries, to collect and fix the autumnal S. horneri. They wish to thank Drs. Toshio Matsui, Kei Takizawa, and Mr. Kohichi Miki for providing them free use of the Station.

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#### 奥田武男・佐藤由香利:シダモク及び秋に成熟するアカモクの生殖器巣形成

春秋別べつに成熟するアカモクの2群のうち秋に成熟する1群,及びシダモクにおける生殖器巣の初期形成過程を調べ,すでに報告されている標準的な春のアカモクの場合と比較した。始原細胞が舌状細胞と下位細胞に不等割すること,下位細胞のみが分裂を続けて生殖器巣の内壁を形成して行くこと,舌状細胞はやがて基部から離れて巣口に移ること,下位細胞の分裂によって新成された細胞は,舌状細胞を中心に放射状に配列されること等は3者とも同じである。しかし今回取上げた2者では舌状細胞の離れる時期が早いこと,舌状細胞が巣口でアカモクのようには固い栓状とならず,粘液を分泌してゆるい栓状となること等は,ホンダワラでの結果と同じである。(812 福岡市東区箱崎6-10-1 九州大学農学部水産学教室)

## Life history and taxonomy of Callithannion callophyllidicola Yamada (Ceramiaceae, Rhodophyta)

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Boo, S.-M., Rueness, J. and Lee, I.K. 1989. Life history and taxonomy of Callithannion callophyllidicola Yamada (Ceramiaceae, Rhodophyta). Jpn. J. Phycol. 37: 284-290.

We report a life history of Callithamnion callophyllidicola Yamada from Korean water and discuss its taxonomic problems in species limit. The plants grow well in most coasts of Korea and all phases of Polysiphonia-type of life history are usually collected at one sampling. Though dioecism is a general form of the gametophytes, monoecious plants in culture produce dioecious gametophytes in next generation. Of factors tested in culture, growth rate depends much on light period than on light intensity, and much on light intensity than on temperature. All tetrasporelings are synchronously fertile under long-day condition with high light intensity, but male gametophytes are first fertile under short-day or low light condition. Induction of tetrasporangia also depends much on light period. In morphology, C. callophyllidicola resembles C. minutissima Yamada and Aglaothamnion oosumiense Itono, and their taxonomic relationship should be critically reassessed with thorough examination of their type specimens.

Key Index Words: Callithamnion callophyllidicola—Geramiaceae—growth rate—life history—reproductive organs' induction—taxonomy.

Modern concept of species needs the full interpretation of life history of organisms because they are quite variable, and some species may vary by altering their pattern of growth in response to environmental differences (Jones and Luchsinger 1986). Furthermore it is a life history which maximizes the potential for genetic recombination and genetic diversity from the union of one pair of gametes (Searles 1980). Therefore the developmental and environmental differences at all stages of the life history should be observed on a taxonomic view in field as well as in laboratory.

Recent taxonomic studies of the red algal genus Callithamnion have clearly demonstrated that much of confusion in species delimitation of the genus is due to failure in previous works to recognize the diverse form range of the taxa (Rueness and Rueness 1980). Furthermore Price et al. (1986) pointed out that Callithamnion and its allied genera,

Aglaothamnion, Pleonosporium and Seirospora, should critically be reexamined about their taxonomic status based on herbarium, laboratory and field studies in the various locations concerned.

In Korea just three species of Callithamnion have been described in flora (Boo 1985; LEE and KANG 1986) and until now there has not been any report on its experimental taxonomy. Callithamnion callophyllidicola YAMADA grows well in most coasts of Korea and is subject to be collected year round, even though its small size and delicateness. It was first described by YAMADA for the plants epiphytic on Callophyllis crispata Okamura and C. japonica Okamura at Enoshima, Sagami Province, Japan (YAMADA 1932). The long specific epithet was derived from the generic name of its host plants. Afterwards it has been known at most coasts of Japan and Korea, but is still restricted to the Japanese and Korean coasts (Yoshida et al. 1985; Lee and KANG 1986). In this paper we report its life history in the various laboratory conditions and discuss taxonomic problems in species delimitation.

#### Materials and Methods

Many field samplings were haphazardly carried out at the intertidal zones of coasts of Korea (Fig. 1). All plants collected were stored in 4% formalin-seawater and transported to the laboratory, where the morphological features were observed as thoroughly as possible.

Cultures for life history were established from excised apices from tetrasporophytes collected at the intertidal zone of Dokdo, East Sea of Korea, in September 1981. The other cultivation techniques followed Boo and Lee (1983). The experiments on growth rate and

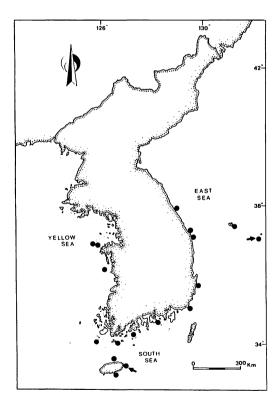


Fig. 1. Geographical distribution of Callithamnion callophyllidicola YAMADA along the coasts of Korea. Arrow represents sampling site for culturing plants.

induction of reproductive organs were carried out with the plants collected at a small tidepool in Sungsan, Chejudo, in August 1986. The isolates were maintained in IMR 1/2 medium (EPPLY et al. 1967) under cool white fluorescent lighting. The growth rate was measured at varied light intensities, photoperiods, and temperatures with the tetrasporelings. The tetraspores were allowed to settle on coverslips at the bottom of Petri dishes for one day under  $17\pm1^{\circ}$ C, 16:8LD and 50  $\mu$ mol m<sup>-2</sup>s<sup>-1</sup> and then transferred into each experimental conditions on a temperature-gradient table. The cell number of the tetrasporelings was counted at the seventh day after their settlement on coverglasses for comparison of growth rate. Eight tetraspores from two tetrasporangia were used for induction of sexual reproductive organs and five carpospores for induction of tetrasporangia. All reproductive organs were checked out every three days, when medium was exchanged to avoid nutrient limitation.

#### Results

PLANTS IN FIELD: Callithamnion callophyllidicola grows well in the intertidal and subtidal zones of most coasts of Korea (Fig. 1), where the plants are epizoic on sponges and Hydrozoa spp. or epiphytic on various seaweeds including Callophyllis spp. They are small, delicate and beautiful.

The plants were ca. 2 cm high and attached to substrata with their digitate rhizoids. The axial cells were formed from apical cells by oblique division and were, in the middle portion of plants,  $70-90~\mu m$  broad and  $200-220~\mu m$  long, thus the L/B being 2-3:1. The primary branches were derived alternately from every axial cells except lower ones. The third to fourth branches were formed in series similarly to the primary branches. All branches were usually distichous and could grow ultimately. The gland cells were not observed in Korean plants.

The gametophytes were dioecious. The spermatangia were formed on the adaxial por-

tion of branches of male plants. The carpogonial branches were observed on the 5th to 6th axial cells from apex and arranged in zig-zag form in female plants. After fertilization young gonimolobes were formed, which became lobed cystocarps and 300-500  $\mu$ m long when mature. The tetrasporophytes gametophytes. were isomorphic with Tetrasporangia were formed on the adaxial portion of branches. They were divided tetrahedrally, and  $40-55 \mu m \times 60-70 \mu m$  in Phenologically size. all phases Polysiphonia-type of life history were usually collected at one sampling. In January, April and August gametophytes and tetrasporophytes were observed at Sungsan, Chejudo. In February they were also collected at Imwon, Hupo and Gampo, eastern coast of Korea, and in April at Whanggumdo, western coast of Korea. In September and October, they occurred at Dokdo and Ulreungdo.

LIFE HISTORY IN CULTURE: Germination of tetraspores in culture resulted in the typical bipolar or irregular growth within two to three days after shedding on coverglasses. At sixth to seventh day the tetrasporelings produced some primary branches, which were first irregular but later regularly alternate. Filamentous rhizoids became digitate at this time. At fifteenth day spermatangia were observed on the adaxial portion of branches of male plants and at eighteenth day carpogonial branches began to occur on the 5th to 6th axial cells from the apex of female plants. At twenty-fourth day young cystocarps were

Table 1. Influence of photoperiod and light intensity on growth rate of Callithamnion callo-bhllidicola.\*

Photoperiod (hr)	Photon flu	x density (µmo	ol m <sup>-2</sup> s <sup>-1</sup> )
	199	122	73
16:8	77.1±6.72	50.1±6.27	42.4±5.36
8:16	$24.4 \pm 2.39$	$19.0 \pm 2.39$	$12.1 \pm 1.39$

<sup>\*</sup> Growth rate is expressed as mean value of cell yield with standard deviation. Fifteen to twenty tetrasporelings were investigated under each condition.

observed on the upper portion of female plants. At 30th day carpospores were shed from the mature cystocarps and began to germinate bipolarly as tetraspores. After two weeks the carposporelings became tetrasporophytes which were isomorphic with the gametophytes. Thus Korean plants of Callithamnion callophyllidicola in culture completed the typical Polysiphonia-type of life history during 44 days. Several successive life history cycles of two isolates were achieved. The cultured plants were basically same to plants in field.

On the other hand, some monoecious plants (below 1% of frequency) were observed. They were suspected to be derived from male plants because most branches were covered with spermatangia while just one to three carpogonial branches were observed. When isolated and cultured, they produced mature cystocarps of which carpospores were functional as those of dioecious plants. The carposporelings grew mature tetrasporophytes which produced tetraspores, and the

Table 2. Influence of temperature and light intensity on growth rate of  $Callithamnion\ callophyllidicola.*$ 

Temperature		Photon fl	ux density (µmol	m <sup>-2</sup> s <sup>-1</sup> )	
(°C)	269	132	83	43	23
27	dead	dead	dead	7.6±1.17	4.6±1.11
22	$35.2 \pm 6.69$	$80.2 \pm 16.58$	$39.9 \pm 6.61$	$17.8 \pm 3.06$	$10.6 \pm 0.80$
17	$23.8 \pm 5.01$	$57.5 \pm 12.19$	$61.8 \pm 7.68$	$20.1 \pm 2.02$	$10.7 \pm 1.42$
12	$6.0 \pm 0.80$	$23.1 \pm 5.99$	$25.9 \pm 4.11$	$13.2 \pm 2.60$	$8.7 \pm 0.75$

<sup>\*</sup> Growth rate is expressed as mean value of cell yield with standard deviation. Fifteen to twenty tetrasporelings were investigated under each condition.

Photoperiod (hr)	Light intensity		Numl	per of days	from tetra	spores	
	$(\mu \text{mol m}^{-2} \text{ s}^{-1})$	12	15	18	21	24	28
16:8	199	_	4M4F*				
	122	_	4M1F	2F	4F		
	73	_	1 <b>M</b>	4M2F	4F		
8:16	199		1 <b>M</b>	4M3F	4F		
	122	_	_	2M	3M	3F	
	73		_	1 <b>M</b>	3 <b>M</b>	1 F	3F

Table 3. Time course on induction of sexual reproductive organs from tetraspores of Callithannion callophyllidicola.

Table 4. Time course on induction of tetrasporangia from carpospores of Callithannion callophyllidicola.

Photoperiod Light intensity (hr) (\(\mu\mod \mod \mod \mod \mod \mod \mod \mod	Light intensity		Nu	mber of days from	ber of days from carpospores		
	(μmol m <sup>-2</sup> s <sup>-1</sup> )	11	14	17	20	23	27
16:8	199		5T*	shedding			
122	122		5T	shedding			
	73	_	5 <b>T</b>	shedding			
8:16	199	_	_			<b>2T</b>	5T
122	_	_	_		1 <b>T</b>	5T	
	73	_	_		_	1T	5T

<sup>\*</sup> T, plant with tetrasporangia.

tetrasporelings grew to mature dioecious gemetophytes. Thus the monoecious plants became normal dioecious gametophytes.

GROWTH RATE ON VARIED LABORATORY CONDITIONS: Tetrasporelings which received much light usually grew much fast (Table 1). However tetrasporelings under short-day condition with high light intensity grew much slowly than those under long-day condition with low light intensity, even though the former received 1692  $\mu$ mol m<sup>-2</sup>s<sup>-1</sup> per day and the latter 1168  $\mu$ mol m<sup>-2</sup>s<sup>-1</sup> per day. On temperature gradiant table tetrasporelings showed various growth rate. They were dead or grew slowly under high temperature (27°C). It is interesting that their growth rate at 22°C became maximum under 132  $\mu$ mol m<sup>-2</sup>s<sup>-1</sup>, while at 17 and 12°C it became maximum under 83  $\mu$ mol m<sup>-2</sup>s<sup>-1</sup>. In all conditions tested, the thallus form was most typical under 17°C and 83  $\mu$ mol m<sup>-2</sup>s<sup>-1</sup> (Table 2).

INDUCTION OF REPRODUCTIVE ORGANS: The time course on induction of sexual reproductive organs was very similar both under long-day condition with low light intensity and under short-day condition with high light intensity even though the growth rate was not. In long-day condition all gametophytes became synchronously fertile under 199  $\mu$ mol m<sup>-2</sup>s<sup>-1</sup>, but male plants became first fertile under 73 µmol m<sup>-2</sup>s<sup>-1</sup> Time course on induction of (Table 3). tetrasporangia was same under three different light intensities tested in long-day condition. Carposporelings produced tetrasporangia at the 14th day. In short-day condition, they also produced tetrasporangia at nearly the same time (Table 4).

#### Discussion

Callithamnion callophyllidicola was characterized by its corymbose to dichotomous ramifica-

<sup>\*</sup> M, male plant with spermatangia; F, female plant with carpogonial branches.

tion, distichous branches, and gland cells commonly in tetrasporophytes (YAMADA 1932). Its Japanese plants were investigated morphologically by SEGAWA (1942, 1949) and Kawashima (1960). general, In vegetative and reproductive features Korean plants were concordant with original description and figure of YAMADA (1932). The gland cells which were reported on Japanese tetrasporophytes (YAMADA 1932; KAWASHIMA 1960) were never observed in Judging from YAMADA's Korean plants. figure, we suppose that he would mistake small protuberances on branches for gland cells.

C. callophyllidicola has a relatively rapid life history in culture. Though dioecism is a general form of gametophytes in Callithamnion, monoecism often occurs in field (KNAGGS 1969) and laboratory (WHITTICK and West 1979; Rueness and Rueness 1980). Monoecious plants of C. bayleyi HARVEY and C. bipinnatum CROUAN isolated from field produced monoecious gametophytes in next generation in culture (WHITTICK and WEST 1979; RUENESS and RUENESS 1980). interesting that our result contrasts with monoecism of C. bayleyi and C. bipinnatum. Abnormalities in expression of sex or phases of life history have been reported to occur often in field and cultured plants, Rhodophyta (KNAGGS 1969; RUENESS and RUENESS 1980). Until now it is also very difficult to understand how abnormalities in sex and phases of life history might occur in field or cultured plants of marine red algae.

The growth rate can be directly expressed as the number of cells in a filament, since the plants of Callithannion basically consist of an uniseriate filament and intercalary transverse divisions are absent (DIXON 1973). Of factors tested in culture, growth rate of tetrasporelings in our plants depends much on light period than light intensity and much on light intensity than on temperature. Thus our data are in agreement with the findings of Kain (1987), while contrast with those of Edwards (1977, 1979) and Whittick (1981).

The induction time required for sporelings to become fertile can be correlated with

growth rate and photosynthesis (WHITTICK 1981). All tetrasporelings are synchronously fertile under long-day condition with high light intensity. The less tetrasporelings receive light, the more slowly the female gametophytes are fertile. This result suggests that induction of sexual reproductive organs may be directly correlated with photosynthesis and much energy is required for the formation of female reproductive Induction of tetrasporangia also depends much on light period because they are produced at nearly the same time under varied light intensities. In C. hookeri, daylength has no effct on fertility (EDWARDS 1979), while Newfoundland materials require long daylength (Whittick 1981). Thus it is also difficult for us to explain environmental factors controlling induction of reproductive organs. It is also interesting that induction time is similar in conditions tested while growth rate is not similar even though the former may be directly correlated with the latter.

(1944),According YAMADA callophyllidicola resembles C. minutissima YAMADA but can be easily distinguished by its more slender frond, longer cells and ultimate ramuli which do not taper conspicuously. As HARRIS (1962) pointed out, the quantitative characters noted by YAMADA (1944) are usually not stable taxonomically and are subject to change in varied environments. Therefore C. minutissima may be a same species with C. callophyllidicola, but rejection of the species should be possible after thorough examination of type specimens of two species. In addi-Dawson (1962) pointed that minutissima seems to be closely related to C. paschale Börgesen, but the latter may be distinguished in cortication of lower part of frond and multifarious branch (Table 5).

On the other hand, *C. callophyllidicola* also resembles *Aglaothamnion oosumiense* Itono in having uninucleus in axial cells and carpogonial branches being a zig-zag form (Segawa 1949), of which characters established the genus *Aglaothamnion* (Feldmann-Mazoyer 1940). Even so, Boddecke (1958) and Harris (1962) rejected identity of

Table 5. A comparison of some taxonomic characters among Callithamion callophyllidicola and its related species.

Characters	C. paschale	C. minutissima	Aglaothamnion oosumiense	C. callophyllidicola	C. callophyllidicola
Type locality	Easter Island, Chile	Hayama, Sagami Province, Japan	Tajiri, Oosumi Peninsula, Japan	Enoshima, Sagami Province, Japan	_
Thallus	erect (2.5cm)	erect (0.2-0.5cm)	erect (2.5cm)	erect (0.2-0.8cm)	erect (0.5-2cm)
Axis	naked	naked	naked	naked	naked
Lower axial cell	1.5-2.5:1 L/B	70–100µm in dia. 1–1.5:1 L/B	45μm in dia. 2.5:1 L/B	160μm in dia. 1.5-2:1 L/B	70–90μm × 200–220μm 2.4–2.8:1 L/B
Branching pattern	alternate distichous multifarious in part	dichotomous in general	alternate to pinnate distichous	alternate to dichot- omous-pinnate distichous	alternate to subdichotomous distichous
Lateral branch	4-5 order	_	3 order	3 order	3-4 order
Apex	_	obtuse	blunt	obtuse	blunt
Gland cell	absent	absent	absent	present*	absent
Spermatangia	seriate	seriate	seriate	seriate	seriate
Cystocarp	nearly spherical	lobed	lobed irregular	nearly spherical subterminal	lobed
Tetrasporangia	$45-55\mu m$ in dia. solitary sessile	$70 \mu \text{m} \times 45 \mu \text{m}$ tetrahedral sessile	70μm×27μm tetrahedral sessile	$70\mu m \times 60\mu m$ tetrahedral sessile	$40-55\mu\mathrm{m} \times 60-70\mu\mathrm{m}$ tetrahedral sessile
References	Börgesen (1924)	YAMADA (1944)	Itono (1971)	YAMADA (1932)	This paper

<sup>\*</sup> We suppose that YAMADA (1932) would mistake small protuberances for gland cells.

Aglaothamnion because the diagnostic characters of the genus overlapped those of Callithannion. In recent Price et al. (1986) also pointed out that Callithamnion and its allied genera including Aglaothamnion should critically be reexamined about their taxstatus based on herbarium, laboratory and field studies in the various locations concerned. However, if the genus Aglaothamnion is considered as a legitimate taxonomic category, C. callophyllidicola should be recombined into Aglaothamnion callo-We will discuss the taxonomic phyllidicola. relationship between CallithamnionAglaothamnion in a next chance.

#### Acknowledgements

This work was in part carried out at the

Section of Marine Botany, Department of Biology, Oslo University, and funded by Postdoctoral Fellowship of Korean Science and Engineering Foundation to SMB in the Department of Biology, Oslo University, Norway.

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### Sung-Min Boo\*・Jan Rueness\*\*・In Kyu Lee\*\*\*: キヌイトグサ(紅藻, イギス科)の生活史と分類

韓国沿岸産の紅藻キヌイトグサ (Callithamnion callophyllidicola Yamada) の生活史を報告し、その分類学的諸問題を論じた。本種は韓国のほとんどの沿岸でよく生育し、通常 Polysiphonia 型生活史のすべての段階のものが同時に採集される。配偶体は一般に雌雄異体であるが、培養では雌雄同体の薬体から次の世代には雌雄異体の配偶体がつくられる。成長速度は、培養で調べた要因の中では、光強度より光周期に、また温度より光強度に、より依存していた。四分胞子発芽体は、強光長日条件下ではすべて同調的に成熟したが、短日または弱光条件下では雄性配偶体がまず成熟した。四分胞子嚢の形成も、より光周期に依存していた。本種は、形態的にはヒナノキヌイトグサ (C. minutissima Yamada) および Aglaothamnion oosumiense Itono に似ており、これらの分類学的相互関係はタイプ標本の比較によって検討されなければならない。(\*Department of Biology, Chungnam National University, Daejeon 301-764, Korea; \*\*Department of Biology, Marine Botany, Oslo University, Oslo 3, Norway; \*\*\*Department of Botany, Seoul National University, Seoul 151-742, Korea)

## シワランソウモドキ Collinsiella cava (YENDO) PRINTZ (緑藻, ランソウモドキ科) の粘質様物質

#### 一構成する中性糖類を中心として―

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IRIKI, Y., HORIGUCHI, T. and MASUO, E. 1989. Mucilage from *Collinsiella cava* (YENDO) PRINTZ (Chlorophyta) with special reference to its sugar components. Jpn. J. Phycol. 37: 291–294.

Mucilage of a green alga, Collinsiella cava (Yendo) Printz (Collinsiellaceae, Chlorosphaerales) was investigated. Judging from the paper chromatogram, gas chromatogram and specific rotation, sugars in hydrolyzate of the mucilage were L-arabinose, rhamnose and glucose in the ratio 77:11:1. The specific rotation of the mucilage was  $[\alpha]_D^{20} = -11.75$ . The absorption maximum of the mucilage-iodine complex was 504 nm.

Key index words: L-arabinose—Chlorophyta—Collinsiella cava—glucose—mucilage—mucilage-iodine complex—rhamnose.

クロロスフェラ目 Chlorosphaerales ランソウモドキ科 Collinsiellaceae に属するランソウモドキ属 Collinsiella は、細胞が多量の粘質の中にあって群体をつくる緑藻類である。しかし、その粘質に関する生化学的知見は、現在まで見当らない。

今回,われわれは、シワランソウモドキの粘質を構成する中性糖類および粘質の物理化学的性質に関して若干の知見を得たので、ここに報告する。

#### 材料および方法

材料:シワランソウモドキ Collinsiella cava (YENDO) PRINTZ は静岡県下田市鍋田湾で岩に附着しているものを小刀ではぎとった。採集後,他の藻類などの夾雑物を除き,さらに水洗して塩分を除去したのち,風乾して実験材料に供した。

灰分:常法により定量した。

窒素: micro-Kjeldahl 法により定量した。

ペントザンおよびメチルペントザン: Krüger-Tollens-Kröber 法 (van der Haar 1920) および Ellet-Tollens-Mayer 法 (van der Haar 1920) により 定量した。

糖類:全糖は Dubois *et al.* (1956) の方法,還元糖は Somogyi 法 (1945)により定量した。

ペーパークロマトグラフィー:加水分解により生成した単糖類は,一次元上昇法(同一方向に2回)により,定性分析・単離を行った。展開液:n-ブタノールーメタノールー水 (8:2:3 v/v); 発色剤: アルカリ性硝酸銀試薬(Trevelyan et al. 1951), ベンチジン試薬(BACON and EDELMAN 1951), アニリン・蓚酸試薬(HORROCKS and MANNING 1949); 濾紙:東洋濾紙 No. 52(定性用),Whatman 3MM(単離用); 温度:室温。

「加水分解法 I 〕熱水可溶性多糖類(以後,粘質様物質 mucilage とよぶ)は,4%硫酸とともに,高圧釜中,120°C,60分加熱し,加水分解した後,硫酸を水酸化バリウム・炭酸バリウムで除き,減圧濃縮して,糖類は,N,N ジメチルホルムアミド(以後,DFA とよぶ)に溶解し,ペーパークロマトグラフィーにより定性分析した。

多糖類の加水分解:

「加水分解法Ⅱ〕上記の方法では加熱による加水分解中にペントース・メチルペントースの分解が生ずる。これを防ぐためにつぎのように行った。粘質様物質を10倍量の72%硫酸中に、0°C、24時間放置し、水を加えて16%硫酸とし、30日間室温中に放置し、完全に加水分解した後、硫酸を水酸化バリウム・炭酸バリウムで除き、減圧濃縮して、糖類は DFA に溶解させ、ペーパークロマトグラフィーにより定性分析した。

ガスクロマトグラフィー: 粘質様物質の構成糖の定性および量比測定のため、上記 DFA 溶液中の糖類をTFA 化したのち、ガスクロマトグラフィーを行った。機器 Shimadzu GC-3BF; カラム 1.2% XE-60 on Chromosorb W;  $N_2$  流速 40 ml/min; 温度 120°C。

吸収スペクトル:粘質様物質のヨード試薬による可 視部吸収スペクトルは、日立分光光度計 EPS 3型によ り測定した。

#### 結 果

組織化学的検索——風乾した薬体 10g を SOXHLET 法により,エーテル・クロロホルムの順に用いて,脂肪および葉緑素などを除去した。ついで,ヨード反応を検したところ粘質様物質の部位が赤色を呈したが,澱粉様顆粒は認められなかった。

全薬の一般分析――全薬中の灰分,窒素,ペントザン,メチルペントザンの定量結果は,Table 1 に示すとおりである。

粘質様物質の調製——風乾した藻体約 100 g を10倍量の80%エタノールとともに、還流冷却下、水浴中、1時間加熱することを2回行った。ついで、10倍量の水とともに、還流冷却下、直火、1時間加熱し、粘質様物質を抽出した。得られた上清を遠心分離(3000×g,15分)し、淡黄色の液を600 m/に濃縮し、99%エタノール 4500 m/に注入し、白色の沈澱 14.6 g を得た。

上記粉末をさらに 2000 ml の蒸溜水中に溶解し、遠心分離( $18,000\times g,20$ 分)を行い、得られた無色透明な上清を  $300\,ml$  に濃縮し、透析を 4 日間行った。この液を99% エタノールに注入し、白色沈澱(粘質様物質)  $13.5\,g$  を得た。

得られた粘質様物質の窒素含有量は0.083%, 灰分含有量は1.54%であった。

灰分含有量が比較的多量であるので、電気透析など の処理を行ったが、灰分量の減少は認められなかった ので、以後の純化は行わずに該糖質様物質の化学的性 質をつぎのように調べた。

粘質様物質の構成糖――粘質様物質 400 mg を〔加

Table 1. Results of analysis of the whole fronds (% on dry weight basis).

Ash	Total N	Pentosan	Methylpentosan
25.56	2.05	51.2	10.2

水分解法 1 ]により加水分解し,得られた単糖類をペーパークロマトグラフィーにより調べた結果は, Fig. 1 に示すとおりである。

なお,数種の発色剤を用いて,粘質様物質の加水分解液中の物質をペーパークロマトグラフィーにより定性分析した結果は,Fig.2 に示すとおりである。

Fig. 2 より明らかなように、 $R_G$ =1.55 のものは、 $R_G$  値およびベンチジン試薬で褐色 (dark brown) に呈色することからラムノース、 $R_G$ =1.17 のものは、ベンチジン試薬で赤褐色 (chocolate brown) に呈色すること、アニリン・蓚酸試薬で赤色に呈色すること、および  $R_G$  値からアラビノース、 $R_G$ =1.00 のものは  $R_G$  値 およびベンチジン試薬で褐色 (dark brown) に呈色することからグルコースと推定される。

なお、 $R_G=0.56$ , 0.48, 0.36 のものは微量のため上

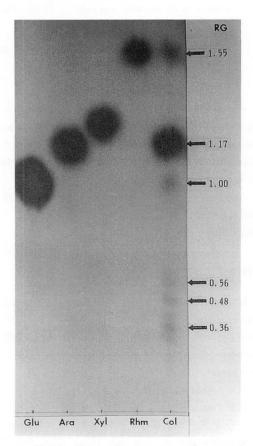


Fig. 1. Paper chromatogram of sugars in hydrolyzate of the mucilage from *Collinsiella cava*. Solvent: n-butanol-methanol-water (8:2:3 v/v). Color reagent: alkaline silver nitrate. Glu, glucose; Xyl, xylose; Ara, arabinose; Rhm, rhamnose; Col, hydrolyzate of *Collinsiella cava*.

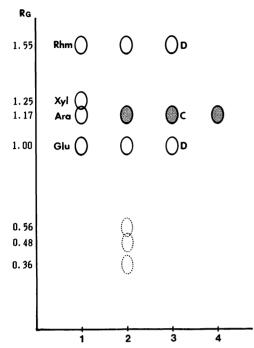


Fig. 2. Paper chromatographic identification of sugars in hydrolyzate of the mucilage of Collinsiella cava. 1: Control. 2: Alkaline silver nitrate reagent. 3: Benzidine-trichloroacetic acid reagent. 4: Aniline-oxalic acid reagent. Solvent: n-butanol-methanol-water (8:2:3 v/v). C, chocolate brown in color; D, dark brown in color; Rhm, rhamnose; Xyl, xylose; Ara, arabinose; Glu, glucose.

述の発色剤(アルカリ性硝酸銀試薬を除く)では明瞭 な呈色が認められず、また、後述のガスクロマトグラフィーでもその存在は証明できなかった。したがって、これらは中性糖類ではないものと推定される。

また、この加水分解液の比旋光度は、 $[\alpha]_0^0$  +87.04° (C=1, 水) である。一方、L-アラビノース、L-ラムノースの比旋光度が、それぞれ  $[\alpha]_D$ =+105°、+8.4° であることから、この粘質様物質は L-アラビノースを主体とする多糖類であると推定される。

L-アラビノースの単離——上記加水分解液中の糖類をペーパークロマトグラフィー(Whatman 3MM 濾紙を用いた)により分別し, $R_G=1.17$  の部位の糖類を単離した。得られた糖類の比旋光度は  $[\alpha]_0^{20}=+100.7^{\circ}$  (C=2.98, 水) で L-アラビノースの比旋光度  $[\alpha]_D=+105^{\circ}$  に近かった。

粘質様物質の構成糖の量比――粘質様物質 100 mg を [加水分解法 2]により加水分解し、生成した糖類の量比をガスクロマトグラフィーにより測定した。結

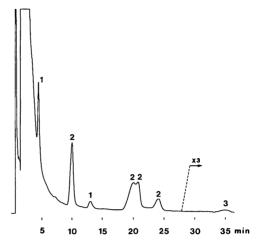


Fig. 3. Gas chromatogram of trifluoroacetylated sugars in hydrolyzate of the mucilage from Collinsiella cava. Condition for gas chromatography: column 1.2% XE60 on chromosorb W; temp.  $120^{\circ}\text{C}$ ;  $N_2$  flow speed 40 ml/min; column size 1.7 m (L)×3 mm (I.D.); Model Shimadzu GC-3BF. 1, rhamnose; 2, arabinose; 3, glucose.

果は Fig. 3 に示すとおりである。

ガスクロマトグラムの面積比から、この粘質様物質 を構成する糖類の量比は、アラビノース:ラムノー ス:グルコース=77:11:1 であることが明らかであ る。

粘質様物質の物理化学的性質——この粘質様物質の 物理化学的性質はつぎのようである。

- (i) 比旋光度:比旋光度は [α]%=-11.75° (C=1, 水)でやや左旋性を示した。
- (ii) ヨード試薬による呈色:この粘質様物質はヨード試薬により深紅色を呈する。この可視部における吸収をつぎのように調べた。すなわち、1%粘質様物質1mlに水2mlを加え、さらに0.02%ヨード試薬1mlを加え最大吸収波長を自記分光光度計により測定した。その結果、最大吸収波長は504nmであることが明らかになった。(澱粉―ヨウ素複合体の最大吸収波長は650nmである。)

#### 考 察

以上のようにシワランソウモドキの粘質様物質を構成する中性糖類は、L-アラビノース:ラムノース: グルコース=77:11:1 であることを明らかにした。また、粘質様物質に関しては、ヨード試薬により 504

nm の最大吸収波長を示す深紅色に呈色し、比旋光度は  $[\alpha]_0^\infty = -11.75^\circ$  (C=1, 水) であることが明らかになった。

植物界における L-アラビノースを主成分とするアラバンの分離例としては、高等植物では、リンゴ (Hirst and Jones 1939), ラッカセイ (Hirst and Jones 1947), テンサイ (Hirst and Jones 1948) などがあるが、藻類からの報告は見当らない。

また、現在まで、生体より単離された多糖類のうちョード反応により紅色を呈するものとしては、p-グルコースを構成成分とする紅藻澱粉のみが知られている。

以上の諸事実から、今回得られた粘質様物質は、生 化学的見地よりみて特殊な物質であるといえる。

今後さらに,この粘質様物質の構造について生理化 学的方法により解明したい。

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# Ascorbic acid in the New Zealand seaweeds *Porphyra columbina* Mont. and *Porphyra subtumens* J. Ag. (Rhodophyceae)

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FRIEDLANDER, S. F., MELTON, L. D. and BROWN, M. T. 1989. Ascorbic acid in the New Zealand seaweeds *Porphyra columbina* MONT. and *Porphyra subtumens* J. Ag. (Rhodophyceae). Jpn. J. Phycol. 37: 295-301.

Ascorbic acid in *Porphyra columbina* and *P. subtumens* (Rhodophyceae) was determined by two independent techniques: high performance liquid chromatography (HPLC) and titration with 2,6-dichlorophenolindophenol. Variations in the levels of ascorbic acid in the two species were investigated during the period of active growth. Values obtained on freshly harvested seaweed ranged from 402 to 186 mg 100 g<sup>-1</sup> dry weight for *P. columbina* and from 274 to 187 mg 100 g<sup>-1</sup> dry weight for *P. subtumens*. After seven months storage the ascorbic acid content of the dried seaweeds had decreased to less than 100 mg 100 g<sup>-1</sup> dry weight in *P. columbina* and to less than 30 mg 100 g<sup>-1</sup> dry weight in *P. subtumens*. Nori sheets were made from *P. columbina* and toasted without detectable loss of ascorbic acid.

Key Index Words: ascorbic acid—2,6-dichlorophenolindophenol—New Zealand—HPLC—nori—Porphyra columbina—Porphyra subtumens—Rhodophyceae.

Porphyra, a cosmopolitan genus, is eaten in many parts of the world but is particularly important in Japan and China where it is popularly known as nori and zicai, respectively. In these countries seven species are grown commercially with P. yezoensis UEDA, P. tenera KJELLMAN and P. haitanensis T.J. CHANG et ZHENG BAOFU being the most important (MIURA 1975, TSENG 1981, NISIZAWA et al. 1987, TSENG and FEI 1987).

In New Zealand the two most widely distributed species of *Porphyra* are *P. columbina* Mont., a winter annual, and *P. subtumens* J. Ag., reported to be a summer annual (Chapman 1969). However, our observations on *P. subtumens* indicate it is found throughout the year with a plentiful number of larger plants in September and October while there were only small plants present between February and April (Melton and Brown unpublished observations). *P. columbina* grows in the intertidal zone of rocky shores along

most of the New Zealand coastline whereas *P. subtumens* is an epiphyte growing on *Durvillaea antarctica* (Chamisso) Hariot and *D. willana* Lindauer in the upper sublittoral. *P. columbina*, known as karengo, is eaten by the Maori and is being considered for aquaculture. Recent investigations on *P. columbina* have been concerned with the structure of the porphyran (Brasch *et al.* 1981a, 1981b, 1984), cell culture (Liu and Gordon 1987) and resistance to desiccation (Brown 1987).

Analyses of several species of *Porphyra* indicate that they contain significant amounts of proteins, minerals and vitamins essential for human nutrition (Levring *et al.* 1969). Vague claims as to the vitamin C content of nori sheets such as that it contains more than the juice of tangerines and lemons (Noda and Iwata 1978) and one and a half times the content of oranges (Chapman and Chapman 1980) have no accompanying analytical evidence to support them. The analytical evidence which does exist shows a large varia-

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tion in values: 10-831 mg 100 g<sup>-1</sup> for dried *P. tenera* (Kanazawa 1963), 112.5 mg 100 g<sup>-1</sup> for nori sheets (hoshi-nori) (Nisizawa *et al.* 1987). Differences in ascorbic acid levels between genera of the Rhodophyceae have been reported (Lunde and Lie 1938; Creac'h 1960; Munda 1987). Seasonal variation has been observed within species (Lunde and Lie 1938; Munda 1987) including *Porphyra umbilicalis* (L.) J. Ag. (Lunde and Lie 1938). Furthermore vitamin C values decreased in dried *P. yezoensis* (Araki *et al.* 1982; Ogawa *et al.* 1983) and in nori sheets (Oohusa 1984) during storage.

The aim of the present study was to determine the ascorbic acid contents of two New Zealand *Porphyra* species. Levels in dried seaweeds immediately after collection and on storage were determined. The effect of making nori sheets on the ascorbic acid contents was also investigated. Two independent analytical procedures, HPLC and 2,6-dichlorophenolindophenol titration, were used.

#### Material and Methods

Samples of Porphyra columbina were collected at intervals from June to October 1986 (winter, spring) from Brighton (45°57'S, 170°20'E) and on three occasions from St Clair (45°55'S, 170°29'E) near Dunedin, New Zealand. The two locations are exposed, coastal sites 14 km apart. The epiphyte, Porphyra subtumens was collected only from the Brighton site over the same period of time. Plants were picked off Durvillaea antarctica and D. willana while uncovered at low tide. Whole plants were collected for both seaweeds and were washed with seawater at the point of collection to remove sand and other debris.

Freshly collected samples were dried at 30°C to a water content of approximately 8% in a circulating air oven. Drying commenced within 3 hr of collection. Dried samples were stored under air in sealed plastic bags in the dark at ca. 18 to 20°C. Samples (5 to 10 g) equivalent to 20 to 40 P. columbina plants or at least 120 P. subtumens plants were ground on the day of analysis in a Falling Number

mill (Type 3303, Huddinge, Sweden, zero setting) to a fine powder.

Ascorbic acid (AA) was extracted from a portion of the ground seaweed (0.3 to 2.0 g) by shaking for three minutes with the appropriate extracting solution (100 ml). Preliminary work showed that increasing the extraction time to 6 minutes or doing 2 successive extractions did not increase the yield of AA. The suspension was filtered through glass wool and then through filter paper (Whatman No. 4).

Ascorbic acid analyses were performed on 7 ml aliquots using the titrimetric method (AOAC 1984) with metaphosphoric-acetic acid as the extracting solution and the 2,6dichlorophenolindophenol reagent diluted 1 in 5. Analyses were also performed following the HPLC method of WIMALASIRI and WILLS (1983) with 3\% citric acid as the extracting solution. The HPLC system comprised a Waters Associates 6000A pump, a U6K injector, a Lambda-Max Model 480 spectrophotometer (Waters Associates) set at 254 nm, and a Shimadzu integrator recorder. The column was a Waters NH2 µ Bondapak. The flow rate was  $2 \text{ m} l \text{ min}^{-1}$ . The mobile phase was 70:30 acetonitrile to water with 10.0 mM potassium dihydrogen phosphate with the pH adjusted to 4.3 using concentrated phosphoric acid. A standard curve was constructed using five AA concentrations of 5 to 25  $\mu l$  m $l^{-1}$ . Samples were passed through a C<sub>18</sub> Sep-pak (Waters Associates), which had been previously washed first with 4 ml methanol and then with 10 ml distilled deionised water, before injection into the HPLC. Each sample (50 µl) was injected four times.

Repeatability was assessed by repeating the extraction and analysis of samples of *Porphyra* from the same collection eight times on the same day.

Recovery tests were performed by addition of AA (2.5  $\mu$ g m $l^{-1}$  and 5.0  $\mu$ g m $l^{-1}$ ) to the sample in the extracting solution and proceeding as before. The AA present was compared with an identical sample which did not have AA added to the extracting solution.

Ascorbic acid determinations were carried out on the sample immediately after drying at 30°C and at intervals varying from one to several weeks. Water determinations were also performed at the same time. These were graphed against number of days of storage, and the line of best fit drawn from the linear regression. Water values used for any particular day were read off this graph.

Nori sheets "hoshi-nori" were made from samples of P. columbina collected from Warrington beach, north of Dunedin (45°43'S, 170°36'E), on 22 February 1987. seaweed was kept in seawater at 4°C until it was processed. Nori sheets were prepared by blending wet seaweed with HPLC grade water at 5°C in a blender (Waring, New Hartford, USA) for 30 seconds until small pieces (5 mm across) were obtained. The suspension was immediately poured onto a wire mesh mat covered with muslin gauze (supported by a wooden frame, 24 cm × 24 cm) and spread out to form a sheet approximately 5 mm thick. Excess water was removed by pushing the mat down with the fingertips. The tray was placed in an oven with circulating air at 30°C for three to five hours until dry. The sheet of nori was toasted by moving it back and forth approximately 8 cm above a Bunsen flame. It took approximately 15 seconds for the colour to change to bright green which indicates the toasting is complete (Chapman and Chapman 1980). The HPLC and water analyses were done on the fresh wet P. columbina (weighing the sample immediately after spinning three revolutions in a plastic salad drier), on the fresh dried sample (30°C, four hours), on the blender water, on the final nori product and on the nori after toasting.

All AA values given in this paper are expressed as mg AA per 100 g dry weight. The dry weight was obtained by drying the samples for approximately 16 hours in vacuum oven at 115°C until constant weight was achieved. This is not to be confused with the initial drying process for 6 to 36 hours at 30°C, after which the seaweed had a moisture content of approximately 8%. No AA values

have been expressed in these latter terms.

#### Results and Discussion

During July and August, Porphyra columbina from Brighton had higher AA levels than P. columbina from St Clair (Table 1). The value obtained for St Clair remained relatively constant (~200 mg AA 100 g<sup>-1</sup> dry weight) whereas at Brighton a peak value was observed in August (402 mg AA 100 g<sup>-1</sup>) and declined thereafter. Differences between the two sites may be related to different environmental conditions although light, temperature and salinity were similar. The growth period did, however, appear to differ. The St Clair plants were evidently earlier, were at a more advanced stage of growth and were more plentiful at any particular time up to and including July. The maximum AA values in plants from Brighton corresponded with the time the seaweed was tender, relatively large, and prior to yellowing. This would appear to be the optimal time for collecting Porphyra from this site to produce nori sheets. The

Table 1. Changes in ascorbic acid in *Porphyra columbina* collected from the midlittoral of Brighton and St Clair and in *Porphyra subtumens* from the sublittoral fringe of Brighton. All values are in mg AA 100 g<sup>-1</sup> dry weight. Standard deviations are given in brackets.

	Ascorbic acid			
Sample date and site	HPLC	Titration		
P. columbina Brighton				
25 July 1986	322 (15)	342 (3)		
22 August 1986	376 (13)	402 (3)		
19 September 1986	304 (10)	293 (5)		
6 October 1986	253 (2)	249 (4)		
P. columbina St Clair				
9 June 1986	199 ( 6)	205 (1)		
22 July 1986	186 (18)	207 (2)		
7 August 1986	195 (10)	180 (2)		
P. subtumens Brighton				
14 July 1986	220 (13)	233 (2)		
22 August 1986	187 (13)	196 (4)		
19 September 1986	261 (14)	274 (5)		
6 October 1986	263 (11)	251 (2)		

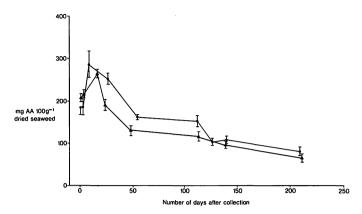


Fig. 1. Effect of storage on ascorbic acid (AA) content of *Porphyra columbina* collected from St Clair on 22 July 1986. HPLC data ●. Titration with 2,6-dichlorophenolindophenol data ▲. Error bars indicate ±1 standard deviation.

high AA values at this stage correlate with LISO and CALBRESE's (1974) finding that AA levels in red algae were significantly higher in actively growing parts of the plant.

Porphyra subtumens from Brighton had noticeably lower AA levels than P. columbina from the same site in the months of July and August (Table 1). In contrast in September and October values rose to be comparable to P. columbina values. While Chapman (1969) considered P. subtumens to be a summer annual, our own observations indicate rapid growth during September and October, the time of higher AA values.

Porphyra columbina collected from both St

Clair and Brighton sites, in July, showed a marked decrease in AA levels during storage of the dried samples (Figs. 1 and 2). Similarly, AA levels in *P. subtumens* decreased on storage (Fig. 3). Indeed, all dried samples of *P. columbina* and *P. subtumens*, no matter when collected, showed decreases in AA on storage (FRIEDLANDER 1987). The rate of destruction of AA is influenced *inter alia* by the temperature, the presence of oxygen and the water activity of food (TANNENBAUM *et al.* 1985). Considerable losses of AA in Japanese dried *P. yezoensis* and nori sheets stored in air have previously been reported (ARAKI *et al.* 1982; OGAWA *et al.* 1983;

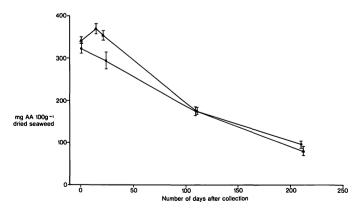


Fig. 2. Effect of storage on ascorbic acid (AA) content of *Porphyra columbina* collected from Brighton on 25 July 1986. HPLC data ●. Titration with 2,6-dichlorophenolindophenol data ▲. Error bars indicate ±1 standard deviation.

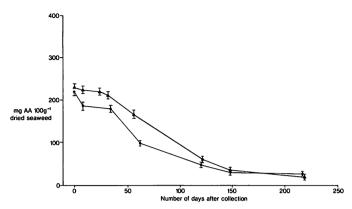


Fig. 3. Effect of storage on ascorbic acid (AA) content of *Porphyra subtumens* collected from Brighton on 14 July 1986. HPLC data ●. Titration with 2,6-dichlorophenolindophenol data ▲. Error bars indicate ±1 standard deviation.

Oohusa 1984). The 3 groups of investigators found loss of AA was directly related to the water activity. In our investigation, after the initial drying process at 30°C the seaweeds reached a water content of 6-10%. On storage the water content increased steadily to approximately 10-13% after 150 days. Ascorbic acid calculations were adjusted accordingly, however this increase in water content undoubtedly contributed to the decrease in AA values observed during the storage period.

The HPLC and titrimetric techniques produced comparable results (Table 1, Figs. 1, 2 and 3). The coefficient of variation for both methods was 3-5%, as determined by repeatability trials. Recovery tests gave a 107% recovery for HPLC and 81% for titration. JENSEN (1963) showed the titrimetric method for determining AA in brown seaweeds produced unrealistically high values unless phenolic compounds in the seaweed, which interfere with the titration, are removed prior to analysis. In the present study the effect of phenolics was not investigated. Although the recovery was only 81% by the titration method, it is possible that phenolic compounds increased the AA values, thus fortuitously giving values comparable to those obtained by HPLC.

The AA levels obtained in the present study fall within the range of  $10-831 \text{ mg } 100 \text{ g}^{-1}$ 

previously determined for *Porphyra tenera* (Kanazawa 1963) and are higher than the value of 112.5 mg 100 g<sup>-1</sup> reported for nori sheets (hoshi-nori) made probably from *Porphyra yezoensis* (Nisizawa et al. 1987). Comparisons with values for *P. umbilicalis* (Lunde and Lie 1938) which are quoted per wet weight without giving a water content, are not possible since the water content of fresh seaweed varies markedly depending on whether the seaweed is shaken to remove excess water or blotted dry.

No significant change in AA levels was noted on drying of the wet seaweed (30°C, 4 hours). Nor did nori sheet production or subsequent toasting significantly alter AA values (Table 2). Loss of AA might have been expected during nori sheet production since the Porphyra was blended with water, and AA is water soluble. However, analysis of the water initially drained from the nori sheets showed that leaching of AA was not detected. The volume of water used is important with respect to AA losses (EHEART and GOTT 1965). The present study used 100 to 150 ml of water per 10 g wet Porphyra. authors were unable to find a reference to the seaweed to water ratio used in nori making so a comparison could not be made.

Ascorbic acid is generally considered to be heat sensitive. The nori sheets were toasted at what was assumed to be high temperature

Table 2. Ascorbic acid levels during nori sheet making using the same *Porphyra columbina* sample on two different occasions. All values in mg AA  $100~\rm g^{-1}$  dry weight.

2	25 February 1987	2 March 1987
Wet seaweed	298a	251 <i>b</i>
Freshly dried seaweed	311a	_
Nori sheet	325a	248b
Water drained from nori she	et ND	ND
Nori sheet toasted	329a	233b

Values are the mean of four HPLC injections. ND=Not detected. Different letters (a, b) between treatments represent significant differences (P < 0.01).

for only 15 seconds. Hence, it is noteworthy that no loss of AA was observed on toasting the nori sheets (Table 2).

Therefore, nori sheets can contain as much AA as the seaweed itself providing the seaweed used is fresh, chopped in minimal water, dried and toasted for a minimal length of time and consumed relatively soon after making.

HPLC analysis on Japanese nori ("Westbrae Natural" brand sushi-nori) purchased in Dunedin could detect no AA. This was not surprising since the sample would have been over twelve months old before analysis, due to importing and storage. The absence of AA is not unexpected as some values reported by MIURA (1975) for Japanese nori sheets are very low (2 mg 100 g<sup>-1</sup>).

It must be borne in mind that a sheet of nori weighs ca. 3.0 g. Therefore the AA present in one sheet of nori, even if the nori is freshly prepared or stored in the absence of oxygen, will only make a small contribution to an individual's AA nutritional requirements. However, if P. columbina was eaten in larger quantities, either freshly harvested, or in a recently dried form, or as freshly made nori sheets, it would make an important contribution to human AA requirements.

#### Conclusions

Claims of high levels of AA in dried Porphyra have been confirmed for P. columbina

and *P. subtumens* but the actual values are dependent upon species, location, time of collection and length of storage. The maximum levels of AA in *P. columbina* occur during the phase of vigorous growth when the seaweed is tender and richly pigmented. This would appear to be the optimal time for harvesting. Differences between collection dates were also found in *P. subtumens*. During the period of sampling *P. columbina* from the same site generally had higher or similar AA values to *P. subtumens*.

The method of storage has an important influence on conserving the ascorbic acid content of dried *Porphyra*. When stored under air in plastic bags at ca. 18–20°C for 7 months all dried samples of *P. columbina* and *P. subtumens* showed marked decreases in AA levels.

Our method of processing *P. columbina* into nori sheets and its subsequent toasting did not affect the AA content. However, further investigations are required to find ways of maintaining the high levels of AA in the nori sheets during storage.

The HPLC and 2,6-dichlorophenolindophenol titrimetric methods have been commonly used to determine AA contents of foods. However, we believe this is the first time they have both been used to measure AA levels in the same seaweed samples. Comparable data were obtained by the two analytical procedures, but this may have been fortuitous.

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### S. F. Friedlander\*・L. D. Melton\*・M. T. Brown\*\*: ニュージーランド産紅藻 Porphyra columbina Mont. 及び Porphyra subtumens J. Ag. のアスコルビン酸

紅藻 Porphyra columbina と P. subtumens のアスコルビン酸を高速液体クロマトグラフィー及び2,6-ジクロロフェノールインドフェノールによる適定で定量し、両種の成育の活発な時期におけるアスコルビン酸レベルの変動を調べた。採集直後の試料のアスコルビン酸含量は、P. columbina では 402-186 mg 100 g<sup>-1</sup> (乾重)、P. subtumens では 247-187 mg 100 g<sup>-1</sup> (乾重) であた。 7 カ月貯蔵した試料では、アスコルビン酸含量の減少が認められ、P. columbina では 100 mg 100 g<sup>-1</sup> (乾重) 以下に、P. subtumens では 30 mg 100 g<sup>-1</sup> (乾重) 以下になっていた。P. columbina をすいて焼海苔を作ったが、検出できるほどのアスコルビン酸の減少はなかった。(\*Food Science Department, University of Otago, P.O. Box 56, Dunedin, New Zealand; \*\*Botany Department, University of Otago, P.O. Box 56, Dunedin, New Zealand)

## Masahiro Notoya and Yusho Aruga: Tissue culture from the explant of Ecklonia cava Kjellman (Laminariales, Phaeophyta)

Key Index Words: Ecklonia cava—Phaeophyta—tissue culture.

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There have been eight reports on tissue culture of Laminariales, of which three were concerned with development of callus (Saga and Sakai 1983, Polne-Fuller et al. 1986, Notoya 1988) and five were concerned with formation of sporophyte or aposporous gametophytes from the callus (Saga et al. 1978, Fries 1980, Fang et al. 1983, Yan 1984, Lee 1985).

FRIES (1980) reproted on Laminaria digitata and L. hyperborea, and LEE (1985) reported on L. saccharina. They observed explants of tissue to form callus which developed to aposporous male and female gametophytes. From these gametophytes were formed sporophytes by fertilization. SAGA et al. (1978) reported in L. angustata that single cells from callus-like structure formed new sporophytes. FANG et al. (1983) and YAN (1984) reported in L. japonica and Undaria pinnatifida that the tissue pieces developed to callus, and the callus cells differentiated and developed to new young sporophytes.

In this paper we describe the tissue culture of *Ecklonia cava* Kjellman in which explants from the blade formed callus-like filamentous cells and the filamentous cells developed to sporophyte-like plantlets.

A sporophyte of *Ecklonia cava* was collected in Nabeta Bay, Shimoda, Shizuoka Prefecture, on July 21, 1988. The tissues were excised from the blade meristematic zone, stipe and holdfast, and their surface was cleaned up with paper towels. The sterilization procedures of explants for tissue cultures were the same as described in a previous report (Notoya 1988).

The liquid and solid culture media were prepared using artificial seawater "Jamarin

S" (Jamarin Laboratory) enriched with PES. The culture media were solidified with 1.5% agar in 90 × 15 mm Petri dishes. The liquid medium was renewed at two-month intervals. All cultures were incubated at 20°C in 12:12 h light-dark cycle and illuminated by cool white fluorescent lamps at 500–1000 lux.

The explants of tissue from blade meristematic zone, stipe and holdfast were cultured on the solid media for about one month. Many callus-like filamentous cells were developed from all these tissues; mostly from medullary parts and little from the undersurface of inner cortex cells. These callus-like cells were unpigmented at the early stage of development. Mass of filamentous cells were gradually grown densely, and round cells were observed sparsely among the massive filaments.

The developmental process of callus-like filamentous cells on agar plates observed in the present experiment was basically the same as that previously reported with explants from the blade, stipe and holdfast of *Ecklonia stolonifera* (Notoya 1988). It was also similar to the results of other reports on *Laminaria digitata*, *L. hyperborea* (Fries 1980) and *L. saccharina* (Lee 1985).

The explants grown to the filamentous callus-like tissues were transferred into the liquid media.

Two months after transferred into the liquid media, the growth was not observed in the callus-like tissue from stipe and holdfast, and color of the whole tissues became reduced.

The growth of filamentous callus-like tissue was not so fast in the explant from the blade, and color of the central part of the tissue

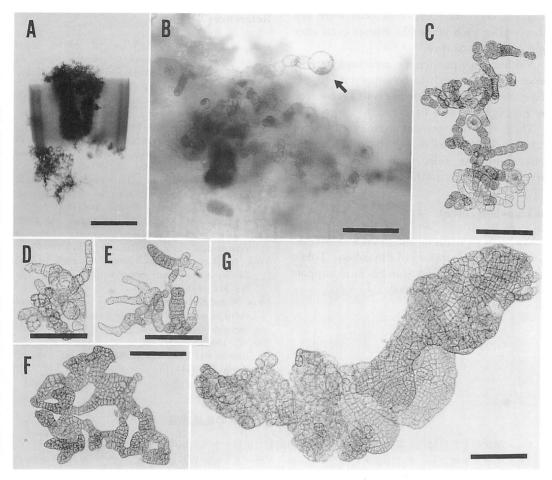


Fig. 1. Tissue culture from the blade explant of *Ecklonia cava* Kjellman. (A) Filamentous callus-like cells developed from the explant of blade in four months after transferred to the liquid culture medium. (B) Round cells (arrow) issued from the explant tissue. (C) Uniseriate cells developed from the detached round cells. (D) Clustered cells. (E) Early stage of blade-like plantlet developed from detached round cells. (F) Blade-like plantlet which was like net by irregular cell divisions. (G) More developed plantlet having partially one- or two-cell layers, spreading like sporophyte. Scale bar: (A) 2 mm, (B)–(G) 200  $\mu$ m.

became brownish, while color of the cells in the peripheral tissue became reduced. The uniseriate round cells which developed from the inner part of tissue were colored or pigmented. These uniseriate round cells were partly detached easily when the culture flask was shaken strongly.

After four months in culture, all the tissues from stipe and holdfast explants were completely bleached and died, while in the tissues from the blade explant the filamentous or round cells were growing slowly and especially the round uniseriate cells grew well and pigmented (Fig. 1, A-B).

After six months in culture, round filaments (Fig. 1, C), cluster (Fig. 1, D) or one- or two-layered blade-like tissues were observed (Fig. 1, E-G) in the culture of detached cells. In the early stage of blade-like development the filamentous tissue was narrow due to the repeating transverse cell divisions (Fig. 1, E). After longitudinal or irregular cell divisions occurred, the tissues became irregular in shape comparable to the early stage of sporophyte produced from female gametophyte (Fig. 1, F & G). This, however, developed to tissues of partially one- or two-cell layers, spreading like sporophyte

(Fig. 1, G). The rhizoidal cells were not observed in each blade-like tissues even after one month in culture.

Neither male nor female gametophyte cells have been observed in the uniseriate tissues developed from the callus-like cells as observed by Fries (1980) and Lee (1985).

Thus, in the present tissue culture of *Ecklonia cava*, it was shown that the filamentous callus-like cells from blade explants developed directly to the new sporophytes.

#### Acknowledgement

We wish to express our thanks to Mr. A. Kurashima, Laboratory of Phycology, Tokyo University of Fisheries, for his kind support in collection of the material.

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#### 能登谷正浩・有賀祐勝:カジメの組織培養

カジメ葉状部生長点付近,茎状部,仮根部から切り出した組織片を寒天培地で1か月間培養したところ,各組織片からカルス様細胞の形成が認められた。その後,このカルス様細胞を液体培地に移して6か月間培養した結果,葉状部生長点付近の組織からの培養では,カルス様細胞から発達した連続した球形細胞に色素体の形成が顕著に認められ,これらの細胞から葉状体への分化がみられた。(108 東京都港区港南4-5-7 東京水産大学藻類学研究室)

### 総 説

### 鼓藻類の種分化における染色体構成の変動1)

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Ichimura, T. and Kasai, F. 1989. Genome differentiation in speciation of desmids (Chlorophyta). Jpn. J. Phycol. 37: 305-319.

Genome differentiation in speciation of desmids is reviewed based on not only published but also unpublished information. The high chromosome numbers of desmids might be explained by the hypothesis held by some cytologists that chromosomes of desmids, like those of the Zygnemataceae, are polycentric. But recent studies on meiosis in some species of Pleurotaenium, Micrasterias, Closterium and Triploceras show clear evidence for the conventional type of meiosis and monocentric chromosomes. Genetic studies of mating type inheritance are consistent with the conventional type but not with the postreductional type of meiosis. Spontaneous, or artificially induced, giant cells have been studied to show their diploidy in some species of Cosmarium, Closterium, Staurastrum and Pleurotaenium. Mating systems (homothallism and heterothallism) and normal sexual expression are maintained undisturbed through ploidy changes in these desmids. Triploid zygospores in Cosmarium spp. are inviable because of irregular meiosis. However, those in the Pleurotaenium mamillatum and the Closterium ehrenbergii species complex are viable, although slightly less than the normal diploid zygospores. Studies on meiosis and F<sub>1</sub> viability in crosses within and between normal and giant-cell clones suggest that both the genomes of these two complexes are polyploid cytologically. Chromosome counts and cell size analyses show that speciation in the Cl. ehrenbergii complex appears to be accompanied by polyploidization and chromosome rearrangements. Genetic studies on mating type genes and some zygotic lethal factors indicate that homoeologous chromosome sets within the polyploid genome of each mating group (biological species) of the Cl. ehrenbergii complex are differentiated to such an extent that vegetative cells of these mating groups, including Group P of the highest level of polyploidy, appear to be haploid genetically.

Key Index Words: chromosomes—desmids—heterothallic—homothallic—mating type—polyploidy—speciation.

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#### はじめに

による。

 では Prescott et al. (1972, 1975, 1977, 1981, 1982) 及び Růžička (1977, 1981) 等の大著も刊行されている。その他,各国や地方の鼓藻類相の著書や論文も多数存在し,鼓藻類の形態分類は概ね完成していると考えても良い状態である。他の微細藻類とは比較にならないほど精細な分類系が知られている鼓藻類は,種分化の研究にとって非常に良い研究材料であると我々は考えている。

種分化の研究には様々なアプローチや方法が考えられるが、我々は"種"を客観的に認識することが最も 重要であり、種分化研究の出発点であると考え、主と

<sup>1)</sup> 黒木宗尚博士追悼論文 (Dedicated to the memory of the late Dr. Munenao Kurogi). 文部省科学研究費補助金一般研究 B No. 61480013

して、交配実験に基づく生殖的隔離機構の研究を行い その大要を既に発表した(市村 1982)。本総説では、 生殖的隔離機構によって認識された"種"の形態変異、 地理的及び生態的分布の研究と平行した細胞遺伝学的 研究より得られた最近の知見、即ち種分化に伴う染色 体構成の変動及びそのような変動を生み出す機構についての大要を紹介したい。

#### 鼓藻類の染色体数

鼓藻類の染色体数の研究は、一部減数分裂像によるものもあるが、大半は体細胞分裂像によるものである。 SARMA (1982, 1983) によると約14属65種数変種の染色体数が報告されている。一般に鼓藻類の染色体は小型で非常に数が多く、正確な染色体数を知ることは困難であることから、上記の研究には更に検討を要する研究例も含まれていると思われる。 鼓藻類の属の起源が非常に古いことに加えて、各属に含まれるごく一部の種類についてしか染色体数が知られていないことから、属の基本数については現在のところ全く解っていない。

比較的数の少ない例はチリモ Desmidium aptogonum の n=8 (ABHAYAVARDHARI and SARMA 1982), スポンディ ロシウム Spondylosium papillosum の n=9 (Brandham 1965a), ツヅミモ Cosmarium formulosum と C. subtumidum の n=10 (NIZAM 1974) である。最も染色体 数の多い例はネトリウム Netrium digitus のM系統の n=約592 (King 1960) であり, クンショウチリモ Micrasterias americana 及び M. rotata では n=200 以上の 系統が知られている。多くの鼓藻類は n=20~200 で, ホシミドロ科と比較しても染色体数が多い種類が多 い。微細藻類の中ではミドリムシ綱,渦鞭毛藻綱,ク リプト藻綱でも同程度に染色体数が多い種類が多数知 られているが、緑藻綱のオオヒゲマワリ目、クロロコ ックム目, ヒビミドロ目, カエトフォラ目, サヤミド ロ目では比較的染色体数の少ない種類が多い (SARMA 1982, 1983)。接合藻類で染色体が多いこと及び同一形 態種に染色体数の異なる系統が多いことについて Godward (1966) 及び Sarma (1982, 1983) は次のよう に考えている。即ち、接合藻類ではこれらの現象に、 通常知られている倍数性 (polyploidy) や異数性 (aneuploidy) の他に、接合藻類の染色体が特殊な構造 (後述) をしていることから染色体の分断のみによっ て染色体数を増加させる agmatoploidy が大きく関与 しているものと考えている。しかし,我々は,

agmatoploidy の可能性を全く否定するわけではないが、後述するように鼓薬類の種分化においても高等植物の進化におけるのと同様の過程によって倍数化と異数化が重要な役割を果たしてきたものと考えている。

#### 鼓藻類の動原体は局在型か分散型か?

通常、ほとんどの真核生物では1個の染色体にはた だ1個の動原体 (kinetochore 又は centromere)が存在 し, 局在型 (localized centromeric type) と呼ばれてい る。これに対して、昆虫の一部やイグサ (Luzula) 等の 一部の植物では1個の染色体に動原体が複数又は染色 体全域に広がって存在することが知られており、前者 は多動原体型 (polycentric type), 後者は分散型 (diffuse 或は holocentric type) と呼ばれている (White 1973)。 しかし両者は実際上区別するのが難しく、同義に解釈 されることが多い。分散型動原体生物では分裂中期か ら後期にかけて染色体が平行に分離することやX線等 によって分断された染色体片が極に移動すること等が 知られており、前述の agmatoploidy を生み出す要因 と考えられている。又,局在型動原体生物とは異なり, 分散型動原体生物の減数分裂では第一分裂が均等分裂 であり, 第二分裂において母方と父方の染色体が分離 すると言う後還元減数分裂 (postreductional meiosis) を 行うものが多いことが知られている (WHITE 1973)。 Godward は1954年にアオミドロ Spirogyra の染色体が 分散型動原体を持つと報告して以来 (Godward 1954), ホシミドロ Zygnema 及びヒザオリ Mougeotia (PRASAD and Godward 1962, 1966) 等のホシミドロ科だけでは なく鼓藻類 (King 1960, Brandham and Godward 1965b) を含めた接合藻類は分散型もしくは多動原体 染色体を持つ生物の顕著な細胞学的特徴を示すと主張 している (GODWARD 1966)。そして Spirogyra crassa (GOD-WARD 1961) 及び Cosmarium botrytis (Brandham and GODWARD 1965b) の減数分裂を後還元型と解釈してい る。その後、電子顕微鏡の発達に伴いミカヅキモ Closterium ehrenbergii (BRANDHAM and GODWARD 1965c) を 始めとしてかなりの数の鼓藻類を含めた接合藻類の核 分裂中の染色体が観察されたが (PICKETT-HEAPS 1975), 明確な動原体構造が観察されたのは Mougeotia sp. (Bech-Hansen and Fowke 1972) & Spirogyra majuscula (Mughal and Godward 1973) の2例のみである。そ してこの2例においても1個の染色体に複数の動原体 が認められたことから, 分散型ではないとしても多動 原体染色体が接合藻類一般の細胞学的特徴であるとす

る Godward の見解が、現在でも広く認められている (Sarma 1982, 1983, Coesel and Menken 1986, Hoshaw and Mccourt 1988)。しかし、最近コウガイチリモ Pleurotaenium mamillatum (Ling and Tyler 1976), Micrasterias mahabuleshwarensis 及び M. thomasiana var. notata (Blackburn and Tyler 1980, 1981), Cl. ehrenbergii (Kasai and Ichimura 1983),そしてオニノカナボウ Triploceras gracile (Ichimura and Kasai 1989)等の鼓藻類の減数分裂の研究において明確な局在型動原体の染色体像が観察されている。又,後述する交配型遺伝の研究結果は、少なくとも鼓藻類の減数分裂は後還元型ではなく、局在型動原体染色体を持つ多くの真核生物と同じ型であることを示している。

#### 鼓藻類の倍数体

鼓藻類の各属の染色体の基本数が知られていないこ とからも理解できるように、鼓藻類の種間又は系統間 の倍数関係を知ることは多くの場合非常に困難である。 Cosmarium turpinii (STARR 1958), ホシガタモ Staurastrum denticulatum 及び St. dilatatum (Brandham 1965b), Pleurotaenium mamillatum (LING and TYLER 1976), Closterium siliqua (Brandham 1965b) 及び Cl. ehrenbergii (Kasai and ICHIMURA 1987) の栄養増殖又は接合胞子の発芽におい て偶然に,或は Micrasterias spp. を遠心又は低温処理 で (Kallio 1951), C. turpinii (Berliner and Wenc 1976) や Cl. ehrenbergii (Kasai and Ichimura in press) を高張液 処理することによって,通常の栄養細胞より極端に大 きな巨大細胞を得ることがある。Cosmarium, Micrasterias 及び Staurastrum では細胞の巨大化ととも に放射相称 (radial symmetry) の程度も増加するが、細 胞形態の外観や細胞壁上の模様などに関しては巨大細 胞と正常細胞とはほぼ相似形である。C. turpinii (STARR 1958), C. botrytis (BRANDHAM 1965b), Pleurotaenium mamillatum (LING and TYLER 1976) 及び Closterium ehrenbergii (Kasai and Ichimura 1987) では染色体数か ら(後述),他の例では間接的証拠から,正常細胞を 1倍体 (haploid) とすると巨大細胞は2倍体 (diploid) であることが知られている。

#### 倍数体接合胞子の減数分裂

倍数体の鼓藻類が接合することによって生じた3倍 体又は4倍体の接合胞子は、正常な2倍体接合胞子と 同様によく発芽する。しかし、発芽個体が生存できる

かどうかは、発芽時に起こる減数分裂が正常であるか どうかによっているようである。一般的に、高等動植 物の奇数倍数体の減数分裂は異常を伴い、偶数倍数体 では正常な減数分裂が観察されることが多い (Steb-BINS 1971, WHITE 1978)。STARR (1958) の研究した Cosmarium turpinii の 3 倍体及び 4 倍体接合胞子, そし て Brandham (1965b) の C. botrytis の 3 倍体接合胞子か らは生存能のある発芽個体は得られておらず、両者と も減数分裂の異常が観察されている。Ling and Tyler (1974) はオーストラリアの Victoria 及び Tasmania 産 の主として細胞の大きさ等で区別されるが類似した Pleurotaenium の個体群間の交配実験を行い、P. ehrenbergii, P. mamillatum 及び P. coronatum と同定される 系統間でも自由に接合胞子が形成されることから,こ れら3種は同種であるとした。後に Ling and Tyler (1976) はこれらの接合胞子を発芽させ、減数分裂を観 察した結果を報告している。P. ehrenbergii と同定され た系統間の接合胞子では常に正常な2価染色体が認め られ、これらの系統は n=53 の染色体を持つことが 明かとなった。又これらの P. ehrenbergii の系統と P. mamillatum と同定された系統間の接合胞子でも53対 の2価染色体が数えられ、P. mamillatum の系統も n=53 であることが判明した。P. ehrenbegii の系統より 生じた2倍体を用いた3倍体及び4倍体接合胞子の減 数分裂もほぼ正常であり、正常な発芽個体が得られて いる。3倍体接合胞子では65~70の2価染色体, 8~12の1価染色体と少数の多価染色体と思われるも のが観察されるが、発芽個体の生存率は2倍体接合胞 子に比較して僅かに下がる程度で, 1倍体と2倍体の 中間の大きさの F<sub>1</sub> 個体が得られている。 4 倍体接合 胞子では106対の2価染色体が観察されており、発芽 個体の生存率は3倍体接合胞子と同程度であった。こ れに対して P. coronatum と同定された系統は,接合過 程の研究 (Ling and Tyler 1974) からも P. ehrenbergii 系 統の2倍体であろうと推察されていた。即ち1個の P. coronatum 細胞に最大16個の P. ehrenbergii の細胞が接 合しようと集合し、しばしば1個の P. coronatum と2 個の P. ehrenbergii が接合突起を形成し、1個又は2個 の接合胞子を形成する。又、P. coronatum は2倍体の P. ehrenbergii と細胞の大きさが同じであり、形態も先 端の突起の数が少し異なるだけでよく似ている。とこ ろが減数分裂による研究では P. coronatum の系統は n=145 又は147の染色体数を持ち, 2倍体の P. ehrengbergii の染色体数 n=106 より約40個染色体が 多いことから、単純に P. coronatum は P. ehrenbergii の 2

倍体であると断言するのには問題があることが判明し た。P. coronatum 系統間の接合胞子の減数分裂は P. ehrenbergii の 4 倍体接合胞子のそれと同様ほとんど が 2 価染色体であり、 F<sub>1</sub> 個体も正常であった。 P. coronatum 系統と P. ehrengbergii の 2 倍体間の接合胞子で は減数第一分裂後期の染色体分離に乱れが生じる等の かなりの異常も観察されているが、生存能のある Fi 個体が得られている。P. coronatum 系統の交配で得ら れた接合胞子では、まれではあるが融合していない核 内で2価染色体形成が観察されることからも、P. coronatum の栄養細胞には少なくとも2組の相同染色体 が存在することが推察されている。このような P. coronatum 系統からも巨大細胞が発見され、2倍体が得 られており、P. coronatum の1倍体とは勿論のこと、 P. ehrenbergii の 1 倍体及び 2 倍体とも接合し何段階か の倍数レベルの異なる接合胞子が得られ、発芽、減数 分裂も観察されている。Ling and Tyler (1976) はこれ らオーストラリア産の Pleurotaenium はすべて同一の生 物学的種 (P. mamillatum) と結論している。しかし我々 は、同じ結論に至るには P. coronatum 系統についてな お今後の研究,特に F1 及び F2 世代の子孫の生存率を 調べる必要があると考えている。(今後, 我々は Ling and Tyler (1976) の扱った Pleurotaenium を P. mamillatum 複合体と呼ぶ。)なお P. ehrenbergii と P. coronatum については,オーストラリア産とヨーロッパ 産のものが形態的にも同一種でないという分類同定上 の問題点が指摘されている。このように微細藻類の地 理的分布と種分化の問題を研究する上で,Pleurotaenium は非常に興味深い鼓藻類である。我々が種分 化の研究を行っている Closterium ehrenbergii 複合体でも Pleurotaenium と同様に3倍体接合胞子の減数分裂で 専ら2価染色体が観察され、かなり高い生存率で F, が得られており、F<sub>1</sub> 個体の大きさは1倍体と2倍体 の中間の値に分散することが知られている (Kasai and ICHIMURA 1987)

#### 倍数体と性決定機構

鼓藻類の交配様式はクローン内で接合が起こるホモタリズム (homothallism) と交配型 (mating type) の異なる クローン間で接合が起こるヘテロタリズム (heterothallism) の2通りに大別できる。 鼓藻類の倍数体でこれまでに有性生殖の知られている例では、倍数化に伴って上記の交配様式が変化したという例はない。ホモタリックの Closterium siliqua と Staurastrum den-

ticulata より得られた倍数体はそれぞれホモタリックであり、ヘテロタリックの Cosmarium turpinii, C. botrylis, Pleurolaenium mamillatum 及び Cl. ehrengbergii の倍数体はすべてヘテロタリックである(文献は前項に同じ)。又、Pleurolaenium と Closterium の 3 倍体接合胞子から生じる  $F_1$  個体には前述したような減数分裂像及び  $F_1$  個体の大きさの分布から、かなりの異数体が含まれているものと考えられる。しかし、生存した  $F_1$  個体には性表現を欠くものや明確なホモタリズムを示す個体は全く認められなかったことから、異数体もヘテロタリックであり、プラス又はマイナスの何れかの交配型を示すものと結論できる。

鼓藻類の交配型の遺伝はこれまでに C. turpinii (STARR 1954, 1959), C. botrytis (Brandham and Godward 1965a), Cl. moniliferum (LIPPERT 1967), Cl. ehrenbergii (LIP-PERT 1967, HAMADA et al. 1982, 市村 1982, ICHIMURA 1983, ICHIMURA and KASAI 1984a, KASAI and ICHIMURA 1987), Cl. peracerosum-strigosum-littorale 複合体 (WATA-NABE and ICHIMURA 1982) 及び Netrium digitus var. lamellosum (BIEBEL and REID 1965) 等のヘテロタリッ ク株を用いて研究されている。これらの鼓藻類では, 何れの種類でも1個の接合胞子より2個の発芽個体が 生じること、及び減数分裂の観察されている種類では 第二分裂後に娘核の何れか1個が退化し, 非姉妹核の 2核が生き残ることが知られている (KLEBAHN 1891, STARR 1954, LIPPERT 1967, BRANDHAM and GODWARD 1965b, Dubois-Tylski 1972, Kasai and Ichimura 1983)。上記の交配型遺伝の研究において、統計処理 ができるほどの数の F<sub>1</sub> 個体が調べられているほとん どの例において、F<sub>1</sub> 個体の交配型はプラスとマイナ スがほぼ1対1であり、又、大多数の接合子では交配 型の異なる2個の発芽個体が出現し、少数の接合胞子 でのみプラス又はマイナスの同じ交配型の2個体が発 芽個体として得られることが確認されている。このこ とは上記の細胞学的研究に基づいて、鼓藻類の性は交 配型遺伝子座 (mating type locus) に存在する1組の遺 伝子(交配型プラス  $mt^+$  と交配型マイナス  $mt^-$ )によ って決定され、通常減数第一分裂においてプラスとマ イナスの交配型遺伝子が分離するため、交配型の異な った2個の発芽個体が生じ,動原体と交配型遺伝子座 間で乗り換え (crossing over) が起こる場合にのみ同じ 交配型の2個の発芽個体が生じると解釈されている (Starr 1954, Biebel 1976, Hamada et al. 1982)。前述の 後還元減数分裂では上記の交配型遺伝解析の結果を解 釈するのは困難である。Brandham and Godward

(1965a) が後還元減数分裂と調和すると考えた Cosmarium botrytis の交配型遺伝の研究結果は、上に述 べた他の多くの研究例とは相反している。

我々は Closterium ehrenbergii の交配群 (生物学的種) 内の交配における F. 個体の生存率を調べる間に稀に 1個の接合胞子より2個の巨大細胞の発芽個体が出現 することを発見し、その場合に必ず発芽個体の両方と もが例外なくマイナスの交配型を示すことに興味を持 った。染色体数を調べるとこれらの巨大細胞は明らか に2倍体であり,その出現の細胞学的過程を考えると, これらの2倍体は mt+/mt- のヘテロ接合体であると考 えられることから, mt- は mt+ に対して優性であるは ずだと考えた (Kasai and Ichimura 1987)。又, mt+ 1 倍体のプラス株と mt+/mt- ヘテロ 2 倍体のマイナス株 の交配によって得た3倍体接合胞子の F. 個体の交配 型が1対1になることから、おそらく細胞学的には倍 数体であろうと考えられる Cl. ehrenbergii (後述) の性 は優性の1遺伝子 $mt^-$ によって決定される(即ち $mt^-$ を1個以上持つ個体はマイナスの交配型,mt- を持た ない個体はプラスの交配型を示す)のであろうと推論 した。そこで交配型マイナスの栄養細胞を高張液処理 し mt-/mt- のホモ接合体の 2 倍体のマイナス株を作出 し,プラス株と交配を行い,その3倍体接合胞子の F<sub>1</sub> 個体の交配型を調べた。その結果、予想通りにマイナ スの F<sub>1</sub> 個体が多く出現することから明らかに mt- は mt+ に対して優性であり、上記の推論が正しいと言 う結論に至った (Kasai and Ichimura in press)。

古くから多くの遺伝学者や細胞遺伝学者は両性生殖 をする倍数体 (bisexually reproducing polyploid) の性決 定機構に強い関心を抱いてきた (MULLER 1925, Westergaard 1958, Stebbins 1950, White 1978)。 性染 色体と常染色体の遺伝子のバランスで性が決定する Drosophila 型として知られている型の性決定機構を示 すほとんどの動物やスイバ (Rumex) 等の高等植物で は、倍数化によって子孫に不稔の間性個体を生ずると いう不利のために倍数化が抑制されている。しかし優 性Y型として知られるカイコ (Bombyx) や Melandrium (ナデシコ科マンテバの仲間) 等の植物ではY染色体 が強い優性を示すことによって性が決定する。この様 な生物では倍数化によっても上記のような性の異常な 個体が出現することがないので、倍数化による種分化 は全く抑制されることなく、進化の過程で多くの倍数 体の種を生み出してきた。種子植物、シダ植物、コケ 植物の倍数体種のほとんどが雌雄同株 (monoecious) であるか無性又は栄養繁殖するという事実は、雌雄の

個体が遺伝的に決められる性決定機構は倍数化によって攪乱され易いことを物語っているようである。

鼓藻類と同じ程度に染色体数が多いシダ植物は高次 の倍数体と考えられており、ほとんどの種類は1個の 前葉体に雌雄の両方の生殖器官を形成する雌雄同株で ある (Soltis and Soltis 1987 参照)。ほとんどの鼓藻 類もおそらく高次の倍数体と考えられるが (後述), 雌雄同株に対応するホモタリックのものだけでなく、 雌雄異株 (dioecious) に対応するヘテロタリックのも のもかなり知られている (STARR and ZEIKUS 1987)。又, 高等植物で倍数化が進んだ種類には無性生殖をするも のが多いことと関連して,一部の鼓藻類は有性生殖を 全く行わずに無性生殖のみを行っているのではないか と明言している研究者を想起することができる。 Fritsch (1930) はメソテニウム科やミカヅキモ及びペ ニウム Penium 等の比較的形態の単純な鼓藻類では頻 繁に接合胞子が観察されるのに対して、ユウアストル ム Euastrum やクンショウチリモ等の形態の複雑な属 の中には、これまでに多数の報告があるにも拘わらず 接合胞子が全く観察されていない種類が存在すること から、これらの鼓藻類は進化の過程で形態を分化させ る一方で有性生殖能を失ったのではないかと仮定して いる。Coesel (1974, 1988a), Coesel and Teixeira (1974), 及び Francke and Coesel (1985) 等は, 野外観 察だけでなく多数のクローン培養を行い有性生殖誘起 を試みた結果、ヨーロッパ産の鼓藻類の一部には有性 生殖能を失った個体群が存在すると主張している。上 記の研究者達は性を失う要因として倍数性を考えてい るわけではない。しかし、少し問題が異なるかも知れ ないが、Godward (1966) は agmatoploidy による染色 体数の異なる個体の出現と多くの鼓藻類で性が欠如す ることを直接関連づけて考えている。何れにしても、 これまでに数多くの人達が野外で鼓藻類を採集し、多 くの観察をしているにもかかわらず接合胞子が発見さ れていないことだけで、それらの鼓藻類で性が欠如し ていると断言するのは少し短絡的過ぎるように思われ る。

形態種 Closterium moniliferum と Cl. ehrenbergii の栄養細胞はほぼ相似形であり、細胞の大きさと色素体内でのピレノイドの配列状態が異なることでのみ区別されることから、前者より後者が進化したものと考えられている (Růžička 1977)。接合胞子形成過程の研究 (LIPPERT 1967, ICHIMURA and KASAI 1984b) 及びアイソザイムの研究 (FRANCKE and COESEL 1985, COESEL and MENKEN 1988, COESEL 1988a, b, 1989) も両形態種が近

縁であることを示している。ところが、形態種 Cl. ehrenbergii 内に少なくとも12以上の交配群(生物学 的種) が存在するだけでなく(市村 1982, 1985, ICHIMURA 1985 参照), 形態種 Cl. moniliferum 内にも複 数の交配群が存在することが市村 (1979) の予備調査 で判明している。SARMA (1982, 1983) によると Cl. moniliferum の染色体数は系統により異なり n= 70~180 である。Cl. ehrenbergii では交配群により異な り n=約100~250 である (後述)。このことは両形態 種内に倍数化(及び異数化)を伴って種分化した複数 の交配群が存在することを示しているようである。そ して, Cl. ehrenbergii の一部の交配群は同じ形態種の他 の交配群とよりも Cl. moniliferum の一部の交配群との 方がより近縁な場合も考えられる。事実、上記のアイ ソザイム研究では、オランダ産の Cl. ehrenbergii の個体 群は、英国産の同種の個体群とよりもオランダ産及び 英国産の Cl. moniliferum の個体群との方がより近縁で あるという結果が得られている。このように Cl. moniliferum から Cl. ehrenbergii への進化は生物学的種 のレベルまで分化した多数の個体群が関与しており、 その進化の過程は複雑であろうと思われるが、大きな 流れから考えると, Cl. moniliferum から Cl. ehrenbergii に 向かって倍数化が進んでいると考えてもさしつかえな いように思われる。染色体数の比較的少ない Cl. moniliferum にホモタリズムとヘテロタリズムが観察 され、染色体数の多い Cl. ehrenbergii ではヘテロタリズ ムの個体群がほとんどでホモタリズムの個体群は稀で ある。この事実は、鼓藻類では高等植物のように倍数 化によって交配様式が雌雄同株に限定されるのでな く、倍数化によってもヘテロタリズムが維持されてい ることを示している。

ヘテロタリックの Cl. ehrenbergii のプラスとマイナスの性が 1 個の優性遺伝子  $mt^-$  で決定される機構は、外見上或は現象的には高等植物で知られている Y 優性型の性決定機構とよく似ている。おそらく他の鼓薬類のヘテロタリズムでも同様な機構が働いているのであろう。 何故ならば、前述したように Pleurotaenium mamillatum 複合体では、数段階の倍数性の異なる系統間でもヘテロタリズムが正常に維持されており、交配型遺伝の調べられた他の鼓薬類でも  $F_1$  における交配型の分離が正常に 1 対 1 となることが知られているからである。

鼓藻類以外の藻類の性決定機構と倍数性との問題に関して、クラミドモナス Chlamydomonas reinhardtii では、ずっと以前から  $mt^+/mt^-$  のヘテロ接合体の 2 倍体の配

偶子が交配型マイナスの性を表現することが知られて いた (EBERSOLD 1967)。最近になって葉緑体遺伝の研 究と関連して, mt+ は葉緑体遺伝を, mt- は配偶子の 件表現を制御していることが、細胞融合による倍数体 作成だけでなく種々の遺伝学的解析からも明かとなっ てきた (GILLHAM et al. 1974, MATAGNE and MATHIEU 1983, Eves and Chiang 1984, Tsubo and Matsuda 1984, 松田 1982)。そして, Ch. reinhardtii では, 配偶子 の分化、受精、接合胞子形成に関する種々の突然変異 株を用いた遺伝、生化学、分子生物学的研究から、交 配型遺伝子は単一の遺伝子ではなく, mt+ 及び mt- は ともにいくつかの構造遺伝子と制御遺伝子を含む遺伝 子群から構成されていると考えられている (GOODENOUGH and FERRIS 1987)。 クラミドモナスで知 られている遺伝子制御系を念頭において考えると、ミ カヅキモにおける mi- による性決定機構はさらによ く理解できると思われる。(クラミドモナスとミカヅ キモの交配型の優劣が同じであるのは偶然の一致であ る。)

#### 近縁種の形態変異と分布域

互いに近縁と考えられるいくつかの種が存在する場合、各々の種の形態変異の範囲や地理的分布を研究することにより、種分化の機構や道程を解明する糸口が与えられる場合が多いと考えられる。又、比較的研究が進んでいる高等動植物等では、近縁の種の間でも分布域の広い種や狭い種が知られていたり、種分化に伴って形態が著しく変異する場合とそうでない場合や、種間で形態変異の程度が著しく異なる場合が知られている(Stebbins 1950、Mayr 1963、Grant 1981、館岡1983)。これに対して微生物では、一般に伝播能力が強く分布域は世界的な規模の広さと考えられていたり、その系統進化の位置が示すような進化的制約(evolutionary constraint)から種分化に伴う形態変異の程度は高等動植物と比較すると著しく小さいため、上記のような研究成果はまだほとんど得られていない。

微細藻類ではアストレフォメネ Astrephomene (BROOKS 1966), Chlamydomonas (WIESE 1974, WIESE and WIESE 1977, 1978), クリプセコディニウム Crypthecodinium (BEAM and HIMES 1977, 1982, HIMES and BEAM 1978), ユードリナ Eudorina (GOLDSTEIN 1964), ゴニウム Gonium (STEIN 1958, 1965, STEIN and MCCAULEY 1976, NOZAKI 1989), クワノミモ Pandorina (COLEMAN 1977), ボルブリナ Volvulina (CAREFOOT 1966) 等

の鞭毛藻類の形態種の中に、性的隔離機構によって区別される複数の生物学的種が存在する場合でも、それらの間の形態的差異は認識されていない。但しオオヒゲマワリの一種 Volvox carteri では、生殖的に隔離した個体群間である程度の形態的差異が認められ、しかも地理的分布もある程度限られているようである。このような V. carteri の変異個体群は品種 (formae) レベルの分類群として分類されているが (Nozaki 1988)、生物学的種概念からすると独立の種と考えるべきものであろう。

鼓藻類では上記の鞭毛藻類の研究結果とは異なり, 生殖的隔離機構の発達した個体群間で形態的差異が認 められる研究結果が多いように思われる。しかし, Cosmarium turpinii (STARR 1959), C. botrytis (BRANDHAM and GODWARD 1965a), Closterium ehrenbergii (LIPPERT 1967, ICHIMURA 1981, ICHIMURA and KASAI 1982) 及び Cl. moniliferum (LIPPERT 1967) 等の初期の研究では、生殖的隔離の現象を同一形態種内の個体群間で認めるだけで形態変異についてはほとんど研究されていない。 ICHIMURA and WATANABE (1974) は初めて形態種 Cl. calosporum 内に、細胞の大きさや形で区別できる複数の個体群が存在することを交配様式や接合胞子形成の条件の相違と関連づけて報告している。後にWATANABE (1978, 1979) は、Cl. calosporum の個体群について生理生態的研究と合わせた精細な形態変異の分析を行い、3種7変種に分類することを提唱してい

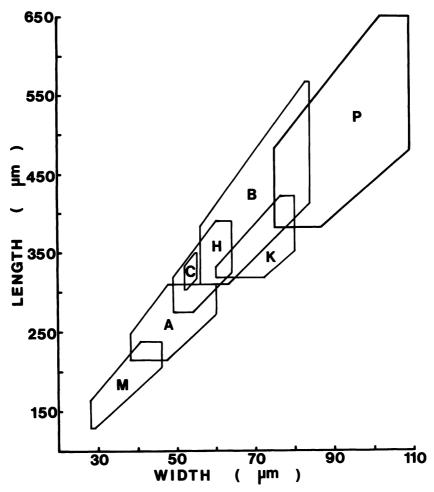


Fig. 1. Variation ranges in cell size for seven closely related mating groups of the *Closterium ehrenbergii* species complex. A, Group A from Japan and Australia; B, Group B from Japan and Taiwan; C, Group C from Japan; H, Group H from Nepal; K, Group K from Japan and Australia; M, Group M from Japan and Nepal; P, Group P from Japan, Denmark and U.S.A.

る。又一方, Watanabe and Ichimura (1978a, b, 1982) は Cl. peracerosum-strigosum-littorale 複合体のヘテロタリ ックの個体群について, 生殖的に隔離された個体群間 で明確な形態的差異が存在することを報告している。 最近 Kasai and Ichimura (1986) は Cl. ehrenbergii の交配 群(生物学的種)の中で、遺伝学的にも細胞学的にも 研究が進んでおり, かなり多くの試料が整っている A, B, H 群の形態変異の比較研究を行っている。その 大要は次の通りである。3群とも細胞の大きさ等の変 異の範囲は、これまで形態分類学者が種又は変種レベ ルで認めていた範囲と比較すると遙かに小さく, 3群 の平均値は明確に異なる。又、同所的分布をするA群 とB群は細胞の大きさと湾曲度によってはっきりと区 別されるのに対して、A及びB群とは異所的に分布す るH群は、細胞の大きさはA群とB群の中間の値を示 し、形は概ねB群と似ていることから、地理的分布の 情報がない場合には,交配実験以外に 3 群を明確に識別することはできない。

交配群 A, B, H はすべて平滑壁の接合胞子を形成し、交配実験によって群間でもかなりよく接合胞子が得られることから、これら 3 群は特に近縁の交配群と考えられている(市村 1982, 1985, Ichimura 1985)。交配群 C, K, M, P もすべて平滑壁の接合胞子を形成し、上記 3 群ほどではないが群間で接合胞子の得られる場合もあるし、少なくともかなりの性的反応を示す。このことからこれら 4 群も上記の 3 群と近縁であると思われる。まだ試料不足の交配群もあるが、未発表データに基づいてこれら 7 群の形態変異と地理的分布について考察を行いたい。群内又は群間の分類形質の変異を統計的に解析するために、各クローンを一定条件(温度に関してはP群のみ15°C、他は25°C、他の条件はKasai and Ichimura 1986 と同じ)で培養し、50又は

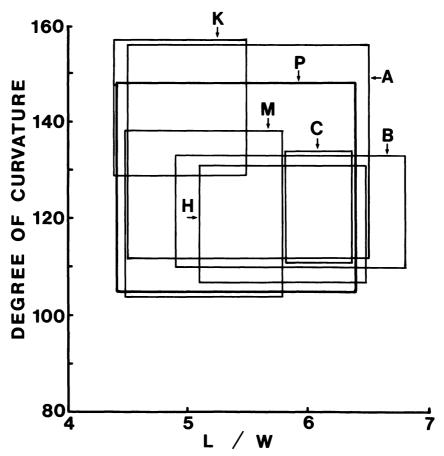


Fig. 2. Variation ranges in cell shape for seven closely related mating groups of the *Closterium ehrenbergii* species complex. A-P, see Fig. 1.

Mating groups	Width (µm)		Len	Length (μm)		L/W		Curvature (°)				
	Range	Mean	SD	Range	Mean	SD	Range	Mean	SD	Range	Mean	SD
A (47)*	38-60	50	4.2	216-310	250	21.6	4.5-6.5	5.0	0.4	112-156	137	8.4
B (44)	56-84	67	5.2	311-567	404	52.5	4.9-6.8	6.0	0.4	110-133	122	4.8
C (9)	52-55	54	1.0	303-350	323	13.6	5.8-6.4	6.0	0.2	111-137	121	8.0
H (38)	49-64	57	3.2	276-390	333	27.2	5.1-6.5	5.8	0.4	107-131	121	5.1
K (20)	60-80	73	5.4	317-420	363	26.6	4.4-5.5	5.0	0.3	129-157	145	7.6
M (17)	28-46	36	4.1	130-238	183	23.6	4.5-5.8	5.1	0.4	104-138	124	9.7
P (30)	75-109	93	7.7	381-650	513	62.4	4.4-6.4	5.5	0.5	104-148	126	10.1

Table 1. Morphological characters of seven closely related mating groups of the *Closterium ehrenbergii* species complex.

20細胞について、細胞の幅、長さ、幅と長さの比及び 湾曲度を計測し、それぞれの平均値を各クローンの値 として用いることにした。交配群 A, B, C, H, K, M 及びPの栄養細胞の大きさを比較するために細胞の幅 と長さの変異の範囲を Fig. 1 に、形を比較するために 細胞の幅と長さの比と湾曲度の範囲を Fig. 2 に示す。

地理的分布に関して、A群は日本とオーストラリア、B群は日本と台湾、C群は日本、H群はネパール、K群は日本とオーストラリア、M群は日本とネパール、P群は日本、デンマーク、米国に分布することを我々はこれまでに確認している。又、Coesel とその共同研究者が種生物学的研究 (Coesel 1988a, b, 1989、Coesel and Menken 1988) に用いた英国及び北フランスの Cl. ehrenbergii の多数の株の分与を昨年 Coesel 博士より受けた結果、P群が広く北ヨーロッパに分布することが明らかになってきた。

日本には分布していないH群を除くと、日本に同所 的分布する(細胞の小さい順に) M, A, C, K, B, P の 6群は細胞の大きさが相互にかなり明確に異なってい ることが Fig. 1 から理解できる。又,この図からも細 胞の幅と長さの比がほぼ一定で細胞の大きさが増大し ていることも読み取ることができる。しかし、各群の 細胞の形の比較は Fig. 2 を見るとさらによく理解でき る。A群とP群の細胞の形は変異にとみ、最も大きな 範囲に広がっている。その原因は、P群では世界の広 い範囲から試料が集められていること、又A群では日 本産の個体群ではこれほど大きな変異を示さないが オーストラリア産の個体群に細胞の形態の変異が非常 に大きなものが含まれていることによると思われる。 (後者については Kasai and Ichimura 1986 を参照され たい。前者については論文準備中である。)A群とP群 以外では形の変異の範囲が比較的小さいので、ある程 度個々の交配群の細胞の形の特徴を把握することができる。最も特徴的なのはK群であり、太くて(細胞の長さと幅の比 L/W が小さい)強く湾曲している。これに対してM群はK群と同程度に太いがそれほど湾曲していない。C群は細長く(L/W が大きい)、それほど湾曲していない。B群とH群は前述したように形が似ており、湾曲度はそれほどでもないが、やや太いものから細長いものまで少し変異が認められる。以上のようにこれら Cl. ehrenbergii の近縁交配群間では、細胞の形については細胞の大きさほど明確な差異は認められない。しかし、大きさと形を組み合わせると、同所的分布をする上記6群の交配群は形態的にある程度まで正確に同定することができる。参考のために各交配群の分類形質の平均値、標準偏差及び最大最小値と測定に供した各交配群のクローン数を Table 1 に示す。

#### 近縁種の染色体構成

一般に細胞の大きさと核の大きさ及び DNA 量とは正の相関があり、真核単細胞生物では0.92の相関係数が得られている (Cavalier-smith 1985)。このことに関してミカヅキモだけが例外であるとは考えられないことから、先に Fig. 1 で示した細胞の小さなM群から大きなP群の順に DNA 量は増加しているものと考えられる。染色体数についてはA群は  $n=100\sim106$ , B群は  $n=97\sim104$ , H群は  $n=105\sim127$ , P群は  $n=181\sim246$  であることが調べられているが (Kasai and Ichimura 1984, 未発表)、他の交配群についてはまだ研究されていない。細胞の大きさに従って DNA量が異なるであろう A, B, H の 3 群がほぼ同じレベルの染色体数であるのは染色体の大きさが異なるためであろうと思われる。しかし、実際にはこれら 3 群の染

<sup>\*</sup> Number of clones measured.

Cross	Individuals	Percent	Mating	No. of pairs			
Plus × minus	isolated	survival	type (+:-)	(+-)	(++)	()	
M-16-4a × M-16-4b	418	94.0	191 : 202	183	1	2	
×R-11-16	250	96.4	121:120	116	1	0	
× R-13-20	128	93.0	57:62	54	0	1	
$80-1-2 \times M-16-4b$	212	84.0	89:89	82	0	0	
GN-4-29×	44	84.1	17:20	16	0	0	
J5-48-19×	320	94.7	153:150	144	0	0	
× R-13-20	146	94.5	67:71	65	0	0	
$R-11-4 \times M-16-4b$	444	91.0	208 : 196	183	3	2	
R-13-131×	422	97.4	202:209	195	2	4	
× 80-1-1	102	94.1	49:47	45	0	0	
× R-13-20	76	88.2	35 : 32	30	0	0	
Subtotal	2562	93.2	1189 : 1198	1113	7	9	
M-16-4a×J6-73-4	102	70.6	34 : 38	23	1	0	
R-11-4×	72	75.0	25:29	20	0	1	
$J6-73-3 \times M-16-4b$	76	60.5	21:25	12	1	1	

54.2

64.6

66.4

67.7

68.8

50.9

56.0

61.3

85.4

24

164

438

238

32

108

100

478

3478

Table 2. Viability and mating type of F<sub>1</sub> progeny in crosses within Japanese populations of Group A.

色体は何れも非常に小型であるために、現在の我々の 技術では定量的には区別できないほどの差異である。 A, B, H の3群と比較するとP群は明らかに染色体数

×80-1-1

Subtotal

Subtotal

Total

 $M-16-4a \times J5-48-2$ 

80-1-2×

J5-48-19 ×

R-13-131 ×

× R-11-16

Table 3. Viability and mating type ratio of  $F_1$  progeny in crosses involving clones with or without a genetic factor causing lethality and mating type aberration.

Minus Plus	M-16-4b	R-13-20	J5-48-12
M-16-4a	94.0	93.0	67.7
	191:200	57:62	77:83 (12)*
R-13-131	97.4	88.2	56.0
	202:207	35:32	30:26 (4)*
J5-48-19	94.7	94.5	50.9
	153:150	67:71	19:36
			G=5.34, P<0.02

<sup>\*</sup> Number of selfing minus F<sub>1</sub> clones. G, value in the G-test (likelifood ratio test) for goodness of fit; P, probability.

が倍加している。P群の個体群は、これまでの我々の 調査では冷凉な流水にほとんど分布が限られており、 室内実験でもP群の最適増殖温度は5~15°Cの範囲に あり、A,B,H 群が20~30°Cであるのと明らかに異な っている。最近の Coesel (1989) の報告によると、北 フランスではP群の個体群は第二次大戦時の爆弾投下 によって生じた浅い水溜りにも生息することが確認さ れている。このことは, この地域に A, B, H 群等のよ うに好んで止水に生息する近縁の交配群が存在してい ないためであろうと思われる。P群が世界中の北方地 域に広く分布すること、他の多くの交配群が好んで生 息する温暖な止水域ではなく冷涼な流水域を好む性質 と合わせて、その染色体数の多さから、氷河期以後に 開けた生態的場に侵入し拡大分布した倍数体の高等植 物の種とP群を対比できるのではないかと我々は考え ている。そしてP群には染色体数がかなり異なる個体 が含まれていたり、減数分裂における染色体対合を低 下させる遺伝因子を持っている個体が含まれているこ

2

33

90

51

7

13

12

83

1285

5:8

53:53

138:153

77:83

14:8

19:36

30:26

140:153

1467:1504

0

0

1

1

0

0

2

0

1

0

0

0

1

13

となどから、P群は比較的歴史の新しい倍数体である ことが推察されている。

交配群 A, B, H の群間交配で得られた雑種接合胞子 の減数分裂では、染色体の対合はかなり異常で、1価 及び2価染色体とかなり多数の多価染色体が観察され る (Kasai and Ichimura 1984)。しかし,交配群A内の 3倍体接合胞子(前述)の減数分裂では少数の1価染 色体を除いてほとんどが 2 価染色体である (Kasai and ICHIMURA 1987)。このように群間交配で多価染色体が 形成され、群内の奇数倍数体の減数分裂が正常である ことから考えると、A,B,H の3群の栄養細胞は細胞 学的には倍数体であると結論することができる。そう するとP群はさらに高次の倍数体ということになる。 ところが交配型遺伝子や接合胞子形成に関与する遺伝 子の遺伝解析をすると, A, B, H, P, の 4 群とも遺伝 学的には1倍体と考えざるを得ない結果が最近得られ ている(市村・笠井未発表)。Closterium ehrenbergii の各 交配群は、Cl. moniliferum 等の小型の祖先種からおそ らく雑種形成をも伴った倍数化によって進化し、その 過程で前述した性決定機構が有効に働いてきたと思わ れる。しかし、上記のような遺伝学的結果から、倍数 化したゲノム間の分化はかなり進んでおり、P群をも 含めた交配群の栄養細胞には機能的に有効な遺伝子は 1組しか含まれていないと考えられる。

小型で多数の染色体を持つミカヅキモの近縁交配群 の個体群について、種分化の結果生じた染色体構造の 差異や、種分化の要因として働く可能性のある染色体 構造の変化を、直接細胞学的に調べることは現在ほと んど不可能と思われる。しかし、高等植物でも外見上 染色体構造が非常に似ており減数分裂においても正常 な染色体対合を示す近縁種間の染色体が、小さな染色 体部位の重複 (duplication), 欠失 (delation), 逆位 (inversion) 又は転座 (translocation) 等によって構造が必 ずしも全く同じでないことが、種間又は同種の生態型 間の雑種の花粉の稔性の低下から推論されている (Stebbins 1950, 1971)。高等植物の花粉の稔性とミカ ヅキモの接合胞子からの発芽個体の生存率は、両者と もその減数分裂の正常さの度合と1倍体世代の持つゲ ノムの適応度に依存している。Table 2 に我々がこれ までに得た交配群A内の種々のクローンの組合せの交 配で得た接合胞子からの F<sub>1</sub> 個体の生存率を示す。上 段11組の交配では約85%以上の生存率を示しており、 これらのクローンの相同染色体はほぼ同じ構造をして いると考えられる。中段の5組では約55~75%,下段 の4組では約50~70%とかなり生存率が低下してい る。中段ではブラス株 J6-73-3 及びマイナス株 J6-73-4 が生存率の低下要因となっていると思われるが,残念ながらまだ J6-73-3×J6-73-4 の交配結果が得られていない。これに対して下段の 4 組ではマイナス株 J5-48-2 が生存率低下の遺伝要因を持っていることが Table 3 から明かである。この J5-48-2 株の持つ遺伝因子は F<sub>1</sub> 個体の生存率の低下だけでなく交配型の分離や性 表現の異常にも影響を与えていることから,交配型遺伝子を含む染色体の構造に何等かの変化があるのではないかと推察される。

#### おわりに

**鼓藻類の形態分類学者の中には、鼓藻類、特に形態** 分化の著しい種類では有性生殖は鼓藻類の進化にとっ てそれほど大きな役割を果していないと考えている人 がかなりいる。一方で鼓藻類には染色体数が多い種類 が多いことは以前からよく知られていた。無性生殖で いくらでも個体群を増加させることができる鼓藻類に とって、倍数化又は異数化は高等植物と比較するとそ れほど大きな障害なく種分化を促進させてきたとも考 えられる。STEBBINS and HILL (1980) によると接合藻類 の起源となった生物は陸上の湿った環境で生まれ、そ の後進化してきたシダ植物やコケ植物との生存競争に よって、二次的に水の中に住むようになった生物群が 現生の接合藻類であろうと考えられる。もしそうだと すれば、現生の多くの鼓藻類が浅い水溜りを好むよう に、鼓藻類は年に少なくとも1度は完全に水分が無く なるような環境で進化してきたと思われる。鼓藻類が その様な環境に適応するためには、乾燥に強い耐久胞 子を形成する必要がある。多くの種類では有性生殖に よってのみその様な耐久型の接合胞子を形成する。有 性生殖によらない種類では単為胞子 (parthenospore) 又は偽似接合胞子 (azygospore) と呼ばれる耐久細胞を 形成する。これは、その名が示すように正常な接合過 程における2個の配偶子の融合過程が省略されたもの である。このように耐久細胞形成と有性生殖過程が細 胞分化を制御する機構でよく結合していることから考 えると、鼓藻類の進化において有性生殖が重要な働き をしてきたことは明白であろう。又、鼓藻類の接合胞 子は栄養増殖に不適な環境に耐えるだけではなく,環 境が好転したときや新しい環境に運ばれた場合に、減 数分裂を経て,その場,その時に適応した新しい遺伝 子の組合せの変異個体を生むという重要な役割を果し ている。染色体数が多い生物は、当然、少ない生物よ

りも遺伝的組換えの頻度が高く、生物の集団遺伝学的 特性や進化のパターンはその生物の染色体数によって 大きく影響されると考えられる。鼓藻類の種類の多さ、 形態変異の多様さはその染色体数の多さと無関係では ないように思われる。

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#### 日本藻類学会秋季シンポジウム講演要旨

## 1)漁場におけるコンブの生活研究と漁場造成技術への展開

川嶋 昭二

コンブの増殖手段の一つとして,古くから天然石やコンクリートブロックを漁場に投入する事業が行われているが,その形や大きさとコンブの着生,生産効果の関連についてはほとんど関心が払われていない。

演者は漁場に投入された円筒型、台型、三角錐型のコンクリートブロックを対象にし、これらの各基質のどの部分にコンブが着生し、良質な葉体として残るかを調査した。3種のブロックのうち、円筒型と台型は直立、横転あるいは倒立し、さらに海底地形に応じて傾斜するなど、相対的な形が全く変るため、コンブの着生部位もまた著しく異なったものになる。しかし、どのような場合であっても(1)最も高い部位の上向き水平面か、(2)ゆるい傾斜面の上縁、(3)特にその稜角(または稜線)部に集中的に着生し、(4)垂直面や傾斜面下部に着生した葉体はやがて消失するという共通性が認められた。また、各ブロックのそれぞれの姿勢ごとに全面型、縁辺型、対面型、偏在型および点在不定型の5つの着生様式が記録された。

これに対し三角錐型ブロックは海底地形が著しく複雑でない限り常に同じ姿勢で安定し、上向き平面を持たないためにコンブは傾斜した側面の一つに、上から下まで疎生し、円筒型や台型とは全く異なる着生様式を示した。

コンプの着生面積(根の占める面積)はブロックの型や姿勢にかかわらず前表面積のほぼ5-10% にすぎない。ブロックを大きくしても着生コンプ本数は必ずしも増えないし、多数のコンプが着生した場合、生長良好な葉体の下草となって生長が抑制されるものの割合が高くなる。このことから考えると生産効果を最大にするためのブロックの大きさがあることが推測できる。

コンブ増殖のための効率的基質を形や大きさの点から検討することは大規模な礁造りの基本となるもので、単にコンブ研究の課題としてだけではなく、工学的立場からのアプローチが重要であると考える。

#### 2) 褐藻卵の受精と発生

安部 守(山形大・理・生)

海産褐藻の Fucus 属および Pelvetia 属の卵は1)多量 に採取できること、2) in vitro で同調的に受精・発生 ができること、3)受精直後の zygote は球形で、無極性であること、などから受精、極性の発現の研究などの好材料として用いられている。

未受精卵から分泌される性誘引物質 fucoserraten を介して卵と精子が出会うと、瞬間的に細胞壁が形成され、精子が卵内に侵入したことが確められる。精核と精子ミトコンドリアが卵内に入り、精核は卵核に向って直進する。精核が卵核に接近すると、卵核膜が精核に向って突起を生じ、精核を包むようにして両核が融合し、受精が完了するり。

受精後は zygote 内に細胞小器官の不均等分布が現れる。たとえば、葉緑体は予定葉状体半球側に、ミトコンドリアは予定仮根半球側に多く分布するようになる<sup>2)</sup>。また、振動電極による細胞内外の電流測定の結果、受精後まもなく予定仮根半球から流入し、予定葉状体半球から流出する電流が認められた。この流れのパターンは仮根形成後も継続する<sup>3)</sup>。この電流によって zygote 内の顆粒の不均等分布が促され、また細胞膜内の荷電物質が電気泳動的に移動して不均等に分布し、これが極性軸(発生軸)の形成に寄与するものと考えられている。

Zygote には受精後 6~9 時間で極性軸が出現するが、この時期の極性軸は不安定で、外的条件の変化によって容易に変更される。しかし受精後 9~12時間で極性軸は安定し、外的条件によって左右されなくなるか。以上の経過は Fucus 属、Pelvetia 属に共通な現象である。

最近,極性軸の安定化に,F-actin(microfilament)が関与することが明らかにされている。microfilament の脱重合剤である cytochalasin を,極性軸の不安定な時期に投与すると極性軸の発現が阻害される。しかし極性軸が安定化した後の投与では阻害効果がみられない。 = ワトリの actin のモノクローナル抗体を結合する actin が zygote に存在することも確められた。また,actin と特異的に結合するファロイジンを用いて,zygote 内の actin の動態追跡も行われ,極性軸が安定化すると,F-actin が仮根形成予定域に局在するようになることも確められた。 F-actin は zygote の生長端(仮根の先端)に局在していることも知られている。

極性軸の安定化には、細胞壁の存在が不可欠であることも、zygote のプロトプラストの再生実験で明らかにされている<sup>7</sup>。

- 1) Quatrano, R. S. (1972), Exp. Cell Res, 70, 1.
- 2) Quatrano, R. S. (1977), J. Cell Sci., 24, 275.
- 3) Nuccitelli, R. (1978), Dev. Biol., 62, 13.
- 4) Quatrano, R. S. (1978), Ann. Rev. Plant Physiol., 29, 487.
- 5) Quatrano, R. S. (1973), Dev. Biol., **30**, 209. 安部 守 (1981), 遺伝, **35**, 10.
- 6) Kropf, D. L. et al. (1989), Plant Cell, 1, 191.
- 7) Kropf D. L. et al. (1988), Science, 239, 187.

#### 新刊紹介

藤田善彦・大城 香: ラン藻という生きもの 東京大学出版会. iv+134頁 (1989). 1442円.

光学顕微鏡による藍藻の形態やそれに基づく分類の 研究は古く1800年代の後半から行われてきたが、生理 化学や細胞構造の研究あるいは分子生物学の手法を取 り入れた研究が盛んになったので最近のことで、およ そ1960年以降といってよいだろう。バクテリアと共通 した多くの性質をもつ原核生物の藍藻、そして約30億 年も昔の古い時代からの酸素供給者であり有機物生産 者でもある藍藻についての研究者は、藍藻の培養法の 開発とカルチャーコレクション制度の確立と相俟っ て,近年飛躍的に増加した。1960年代に急速に蓄積さ れた藍藻についての知見は1973年刊行の次の2冊の本 にその大要を見ることが出来る。1) Carr, N.G. & B. A. Whitton ed. (1973) The Biology of Blue-Green Algae. Univ. California Press. 2) Fogg, G. E., W. D. P. Stewart, P. Fay & A. E. Walsby, (1973) The Blue-Green Algae. Academic Press.

しかし藍藻の研究の進歩は速く、上記の本に盛られた知識は既に1980年代には最新のものでなく、このため改訂版ともいうべき本が再度同じ編者等により1982年と1987年に出版された。3) Carr, N. G. & B. A. Whitton ed. (1982) The Biology of Cyanobacteria. Blackwell. 4) Fay, P. & C. Van Baalen ed. (1987) The Cyanobacteria. Elsevier.

著者等は今回の本の中で,さらに詳しく知りたい読 者のための参考書として上記の4冊を含む出版物の

名を挙げ、3), 4)が最近の情報を含む, 4)は特に 高度な専門書である, 1 ), 2 )を手にしたうえでこれ に挑戦されるほうがよいだろうと述べている。事実, 上記の英文の4冊の本は各章を専門の研究者が担当 し、最新の知見を総説しているので、専門分野を異に する人々や学生にとっては高度に過ぎ、理解は容易で ない。藍藻について最近の知見を平易に解説した本の 出現は藻学の分野に携わる人々の多くが望むところで あった。今回出版の「ラン藻という生きもの」は私達 のこうした要望に応えてくれる。2人の著者は共に国 立基礎生物学研究所に勤務し、主として藍藻の生理化 学の研究に携わる第一線の研究者である。本書は次の 6章から成り、他に参考書名と索引が添えられる。第 1章は「植物の世界の中のラン藻」で、藍藻の分類上 の位置、系統と進化の問題等が簡潔に記述される。第 2章は「ラン藻のかたち」で、細胞構造と主な細胞小 器官のはたらきが述べられる。第3章は「ラン藻の生 き方」で、著者等が最も得意とする分野であり、光合 成・呼吸・N。固定・藍藻運動・それに生き方の戦略 ―補色適応等の機構が解説される。第4章は「ラン藻 の生きる世界」で、生態学を扱い、第5章は「ラン藻 の歴史」で、進化・系統の問題が扱われる。第6章は 結章で,藍藻と細菌との関係,人間社会と藍藻等が取 り上げられ、最後に今後の藍藻研究の展望が述べられ る。藻学研究に携わる者は一読すべき本であろう。

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#### 新 刊 紹 介

片田 實:浅草海苔盛衰記—海苔の五百年— 成山堂書店. 202頁 (1989). 2884円.

日本の浅草海苔養殖が江戸時代以前から現在に至るまでどのような変遷の歴史を辿ってきたか。その移り変りは時代々々の社会情勢とどのような係わりをもって進んだかを東京湾の海苔養殖を中心に記述が進められる。水産植物学・海藻増殖学を専門とする著者(東京水産大学名誉教授)であるだけに、記述には海苔養殖のあり方やノリについての生物学的知見も随所に披歴される。本書は海苔養殖の社会史の秀れた読みものであると同時に、海苔養殖の生物学的側面も伝えてくれる。本書にはまた事実はそのようなことであったのかとか、成程そうした理由だったのかというような、これまでの定説を砕いて新たな根拠に立った新説が幾つも登場して興味をひく。

12章から成る。第1章 養殖以前一赤い海苔,黒い 海苔一。江戸の初期に果して浅草でノリが採れたのか, 「いろあかし」と出ている当時の浅草海苔、品川海苔 が本当に赤い海苔であったかの二つの問題を追究し、 特に後者について思いもよらない結論を出している。 第2章 大江戸名産浅草海苔。江戸期の海苔養殖は特 定の御用商人と村落による独占企業であり、生産技術 は外部に洩らされなかった。しかし、幕末近くにそれ は崩れて行く。第3章 混乱から競争へ。幕末から明 治にかけては海苔養殖にとって混乱期ともいうべき時 代であったが、明治に入ると、東京湾では海苔漁場拡 張競争が盛んとなり、浅草海苔は販路を地方都市に拡 げ、庶民の愛好食品となって行く。第4章 近代化へ の歩み。明治政府は水産とその教育に無関心であった。 移植法等僅かな海苔養殖技術の進歩は先覚者の犠牲的 努力により行われた。しかし昭和に入るや、浮きひび、 網ひびの発明があり、海苔養殖に近代化が訪れる。第 5章 寄せ来たる開発の怒涛。明治より昭和に至る漁 場の陸化と漁民の抵抗運動の様子等が語られる。第6 章 荒廃の里,蘇る海。第二次大戦後の海苔養殖の目 覚ましい復興の様子―ノリ種苗網の全国的交換の実 施、ドリュー女史によるノリ生活史の解明、人口採苗

技術の確立等--が述べられる。この章には生物学的知 見や養殖技術の記述が多い。第7章 天災か人災か。 昭和38~43年に経験した海苔生産の不安定性の問題の 解析、種苗網の低温保存法の確立等が述べられる。第 8章 本場浅草海苔の終焉。東京港や東京湾の工場地 帯の拡大整備による漁場の縮小、続く水質汚染による ノリ病害の蔓延、生産の低下と品質の低下の問題等が 論じられる。第9章 豊かなる荒廃。昭和30年代東京 水産大学木更津実習場に駐在した著者が見た木更津を 中心とする東京内湾漁業の衰微への経過が、コウイカ の性の生態や高級魚の水揚げの低下などとともに述べ られる。第10章 沖合へ、外海へ。内湾域の漁場を失 った海苔養殖が水深の大きい沖合や外海へ浮き流し式 養殖を発展させながら漁場を求めて行く様子が述べら れる。ここでは外海岸の富栄養化の問題がノリの品質 との関係で論じられ、またノリの栽培品種の出現が語 られる。第11章 衰運到来―再建に向けて―。ノリの 過当生産と海苔業者の廃業の様子が述べられ、次いで 著者は品質の向上と生産費の切下げこそが海苔養殖再 建の方策と力説する。第12章 海苔の常識,非常識。 韓国ノリが大量に輸入されていると信じられている が、事実は長らくゼロであること、次いでノリの人口 採苗法の確立をもたらしたコンコセリス相の発見者ド リュー女史の顕彰碑建立の経緯が語られ、最後の「拝 啓高橋 治殿 | では、ノリや海藻及び水産養殖につい ての世間の非常識や誤った認識と偏見を正すべく,著 者が敬愛する高橋 治氏への公開書簡の形で敢えて筆 を進めている。片田教授の面目躍如たるものがある。

本書はまさに著者が愛した東京湾の浅草海苔へのレクイエムであり、それはまた豊かな明日の海苔養殖への意見の書でもある。記述は平易であり、全編を通じて肩の凝らない一般向けの楽しい読みものとなっている。海産植物に関係をもつ人だけでなく広く一般の方にも一読を薦めたい好著である。

(筑波大学生物科学系 千原光雄)

#### 一学 会 録 事一

#### 1. 日本藻類学会臨時評議員会

1989年9月26日10:00~11:30に東北大学教養部において臨時評議員会を開催した。

#### I. 審議事項

#### 1. 山田基金による賞について

名称は「日本藻類学会賞」とする。前年度の学会誌「藻類」に掲載された論文の中から一つを選び、著者全員に賞を贈る。但し、受賞者は日本藻類学会の会員に限る。選考は会長、評議員及び編集委員会構成員の投票(3 名連記)による。授賞式は総会において行い、当分の間賞状だけを贈る。受賞講演は行わない。

#### 2. 寄付の受け入れについて

条件をつけずに受け入れる。使途は評議員会で決め、 総会に報告する。会費納入時にも受け入れの窓口(欄) を設けることを今後検討する。

#### Ⅱ. 報告事項

#### 1. 学会誌の発行状況について

編集委員長から「藻類」No. 3 は10月上旬に発送されるとの報告があった。

#### 2. 韓日合同シンポジウムについて

日本側の有賀祐勝実行委員から,「両国の藻類学会とは無関係に,(直接関わらない形で)運営する。今回の開催は,今後の継続開催を前提としない。両国それぞれ,招待講演5名,一般講演(研究発表)10名とする。研究発表の申し込みは10月16日まで。使用語は英語とし、Proceedings を出版の予定。案内を藻類No.3に出し、一般参加者を募集する。」との報告があった。

#### 3. その他

文部省から平成元年度研究成果刊行費助成金93万円 が交付された。

評議員会出席者:小林 弘会長, 鰺坂哲朗, 有賀祐勝 (編集委員長を兼任),大森長朗,小河久朗,奥田武男, 舘脇正和,原 慶明,三浦昭雄の各評議員,片山舒康, 真山茂樹各幹事。

#### 2. 日本藻類学会第5回秋季シンポジウム

1989年9月26日,日本植物学会第54回大会の関連集会として秋季シンポジウムが小河久朗氏(東北大学)を世話人として東北大学教養部において開催された。 演題は2つで,「褐藻卵の受精と発生」を安部 守氏 (山形大学)が,「漁場におけるコンプの生活研究と漁 場造成技術への展開」を川嶋昭二氏(前北海道水産試験場)が講演した。座長は谷口和也氏(東北区水産研究所)であった。全国から57名の参加者があり、2時間にわたって講演と論議が行われた。シンボジウム終了後、引き続いて同大学川内厚生会館軽食堂において小河久朗氏の司会で懇親会が開催された。小林 弘会長の開会の辞に始まり、安部 守氏(山形大)の乾杯の音頭で幕を開け、料理を楽しみながら2時間近くにわたってなごやかに行われた。

シンポジウム出席者 (○懇親会出席者) は次のとお り。

○秋山和夫, ○秋山 優, 阿久津健一, ○鯵坂哲朗, 阿部清美, 〇安部 守, 〇有賀祐勝, 〇石川依久子, ○石川 豊, ○出井雅彦, ○井上 勲, ○岩崎富生, ○大葉英雄、○大森長朗、○大森正之、○小河久朗、 ○奥田一雄, ○奥田武男, ○葛西広海, ○加崎英男, ○片山舒康、○亀井博一、○川合正允、○川嶋昭二、 ○ギタンジャリ・デシュムケ、○工藤利彦、○小林 弘、○小林芳江、○嵯峨直恒、○佐藤恵美、○高橋季 之, 〇舘脇正和, 〇田中次郎, 〇谷口和也, 〇徳田 広,○長島秀行,○西洞孝広,○野崎久義,○能登谷 正浩, 〇林田久美子, 〇原 慶明, フィリピーナ・ソ ット, ○福島 博, ○堀口健雄, ○松本正喜, ○真部 永地、○真山茂樹、○三浦昭雄、○宮田昌彦、○宮地 和幸, 〇本村泰三, 〇山岸高旺, 〇山中良一, 〇山本 虎夫,○吉崎 誠,○若菜 勇,○渡辺 信(50音順) シンポジウム運営に当たり、東北大学の教官諸氏お よび学生諸君には全てに行き届いた配慮を頂いた。記 してお礼申し上げる。

#### 3. 工場見学

1989年9月26日、秋季シンポジウムに先立ち、仙台市近郊の多賀城市にある大規模なワカメ加工工場の見学が行われた。工場では約1時間にわたり、ビデオによる説明と、実際に工場内でワカメが加工され製品化されていく過程を見学した。工場見学を企画して頂いた小河久朗氏(東北大学)および見学を快く引き受けて頂いた理研食品(株)の諸氏にお礼申し上げる。参加者は次のとおりである。

鯵坂哲朗,有賀祐勝,大森長朗,奥田武男,片山舒康,川合正允,川嶋昭二,小林 弘,徳田 広,原 慶明,フィリピーナ・ソット,松本正喜,真山茂樹, 三浦昭雄(50音順)

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## 一会 員 移 動一 新 人 会

住 所 変 更

#### 訃 報

本会会員 新崎盛敏氏は去る1989年10月26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

福原英司 (北海道), L.T. アンドレ (U.S.A.)

#### Acknowledgements to reviewers for Vol. 37

The Editorial Board is grateful to the following persons for their cooperation in reviewing the manuscripts submitted to the Japanese Journal of Phycology Volume 37.

M. CHIHARA, S. ENOMOTO, Y. FUJITA, Y. HARA, T. HORI, T. ICHIMURA, I. INO-UE, K. IWASA, T. KATO, H. KAWAI, H. KITO, H. KOBAYASI, K. KOHATA, M. MASUDA, H. MIKAMI, M. MIZUNO, H. NAKAHARA, K. NISIZAWA, H. NODA, T. OHMORI, T. OKUDA, I. SHIHIRA-ISHIKAWA, F. B. SOTTO, E. TAKAHASHI, M. TAKAHASHI, J. TANAKA, M. TATEWAKI, H. TOKUDA, K. UEDA, M. M. WATANABE, H. YABU, I. YAMADA, T. YAMAGISHI, Y. YAMAGUCHI, Y. YOKOHAMA, Y. YOSHIDA, M. YOSHIZAKI

#### Erratum (Addendum)

#### 訂 正(追加)

The following note has been erroneously missed from page 235 of this volume. Please insert the following below the Japanese abstract on page 235 in No. 3 of this volume.

第37巻第3号の235頁で下記のnote が誤って欠落してしまいました。お詫びして訂正いたします。同頁の和文要旨の下に挿入してください。

#### Note added in proof:

CORMACI and FURNARI (Jpn. J. Phycol. 37: 27, 1989) recently transferred Antithamnion simulans to Scagelia. Above, we synonymize it with S. pylaisaei.

#### 日本藻類学会事務局住所変更のお知らせ

1990年1月1日から学会事務局の住所は下記に変わります。編集関係以外の連絡はすべて下記宛にお願いします。

〒602 京都市上京区下立売小川東入 中西印刷株式会社内 (TEL. 075-441-3155) 日 本 藻 類 学 会

#### Change of Address of the Office

From January 1, 1990, all correspondence to the Society excepting the editorial business should be addressed to:

The Japanese Society of Phycology c/o Nakanishi Printing Co., Shimotachiuri-Ogawahigashi, Kamikyo-ku, Kyoto, 602 Japan

## 日本学術会議だより №15

# 「ヒト・ゲノム・プロジェクトの 推進について(勧告)」を採択

平成元年11月 日本学術会議広報委員会

日本学術会議は、去る10月18日から20日まで第108回総会(第14期4回目の総会)を開催しました。今回の日本学術会議だよりでは、その総会で採択された勧告を中心に、同総会の議事内容等について、お知らせいたします。

#### 日本学術会議第108回総会報告

第108回総会の主な議事概要は次のとおりであった。

第1日 (10月18日) の午前。まず、会長から、前回総会 以後の経過報告が行われ、続いて、各部・委員会の報告が 行われた。さらに、今回総会に提案されている3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。

第1日の午後。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等の審議が行われた。

第2日 (10月19日) の午前。前日提案された案件の審議・採決が順次行われた。

まず、第7部の専門別の会員定数の変更並びに同部世話 担当の研究連絡委員会の再編成(統合3件、分割2件、新 設1件、名称変更6件)を内容とする、会則の一部改正が 採択された。

続いて、第4部及び第7部の「会員の推薦に係る研究連絡委員会」の指定の変更を内容とする、関係規則の一部改正が採択された。

これらの改正は、具体的には第15期からの組織・活動に 係るものである。

さらに、生命科学と生命工学特別委員会の提案による「ヒト・ゲノム・プロジェクトの推進について(勧告)」が採択された。なお、この件の審議の際には、研究成果公開の原則とプライバシー保護等の問題、「プロジェクト・チェック機構(仮称)」の果たす具体的役割等について、討議が行われた。この勧告は、同日午後直ちに内閣総理大臣に提出され、関係省庁に送付された(この勧告の詳細は、別掲参照)。

また、本総会においては、会長から、日本学術会議の移転問題に関し、前回総会以降の主な動きとして、①去る8月「国の行政機関等移転推進連絡会議」において、本会議の移転先が横浜市「みなとみらい21地区」となったこと、②これに対し三役及び運営審議会のとった対応、などについて報告があった。種々質疑応答が行われた後、これまでの三役及び運営審議会の対応については、基本的な了承がなされた。また、今後の移転に関する諸問題については、当面、三役及び運営審議会にその処理を一任することで了承された。

第2日の午後。「地球環境問題」について、活発な自由討議が行われた(この自由討議の詳細は、別掲参照)。

第3日(10月20日)午前には各常置委員会が、午後には 各特別委員会が、それぞれ開催されさ。

#### ヒト・ゲノム・プロジェクト の推進について(勧告)

#### (勧告本文)

ヒト・ゲノムの全DNA塩基配列決定を主たる目標とする ヒト・ゲノム・プロジェクトは、関連諸分野の学術研究に 極めて大きなインパクトを与えると期待され、我が国とし て早急かつ重点的に推進すべきである。

そのためには、ヒト・ゲノム・プロジェクト推進組織(仮称)を設け、基本計画の立案、実施計画の策定、省庁間などの協議、国際協力、データ・ベースとレポジトリーの整備などを総合的に行うべきである。

また、この推進組織との緊密な連携のもとに、研究計画の実施に伴う社会的、法律的及び倫理的諸問題を客観的かつ公正に判断するとともに、これらの諸問題に適正に対処することを目的とするプロジェクト・チェック機構(仮称)を設立し全体として調和のとれた施策を進める必要がある。よって、日本学術会議は我が国における本プロジェクトの推進を図りそのために必要な措置を講ずるよう勧告する。(説明)[要旨]

- 1 ヒト・ゲノム・プロジェクトは、人類の遺伝情報の解読と遺伝子機能の解明を目指した研究計画であり、早急かつ重点的に推進すべきである。このプロジェクトによって生命科学等の領域の技術開発が進めば、人類福祉に貢献することは疑いない。
- 2 ヒト・ゲノム・プロジェクトを推進するために、ヒト・ゲノム・プロジェクト推進組織(仮称)を設置する。 その主たる目的は勧告本文のとおりである。

この組織は、研究の進展に弾力的に対処できるものと し、具体的問題の処理のため委員会等を設ける。運営に 当たっては個々の研究者とその集団の自主性を最大限に 尊重すべきである。

このプロジェクトの実施に伴って生じる社会的, 法律 的及び倫理的諸問題に適切に対処するために, プロジェ クト・チェック機構(仮称)を設立する。その主たる目 的は, 検体提供者の保護のための基準を作成すること, 情報の管理, 研究計画と成果の一般への公開の基準を作 成すること, 知的所有権問題についての方針を作成する こと, 研究成果の応用段階における倫理的問題について の指針を作成することであり, 目的達成のための必要な 権限が与えられるべきである。

#### 総会中の自由討議一地球環境問題一

本会議総会中の重要な行事である,自由討議が,総会2日目の10月19日の午後1時から3時間にわたって開催された。この総会中の自由討議は,会員のための一種の勉強会で,その時々の学術上の重要課題を取り上げて行われている。今回は「地球環境問題」という課題のもとに行われた。自由討議は,渡辺格副会長の司会のもとに,5人の会員による意見発表があり,さらにこれらの意見発表をもとに会員間で活発な討議が行われた。

今回の自由討議の概要は次のとおりである。

#### [5人の会員による意見発表]

まず、近藤次郎第5部会員から、9月の「地球環境保全に関する東京会議」の出席報告として、①環境保全と発展途上国の資源開発、産業振興等との関わりについての討議状況、②ソ連における環境問題、③大気中の炭酸ガスの増加に伴う温暖化による地球環境への影響に関する注目すべき研究結果の発表等について紹介があった。

次に、吉野正敏第4部会員から、「環境」というもののとらえ方、考え方について、人文・社会科学的な立場からの例をも引いて意見が述べられ、また、IGBPについて、同計画は、様々な環境問題を基礎科学分野としてとらえるものであり、その実施の結果、直ちに、環境問題が全て明らかになるものではない等の発言があった。

次いで、大谷茂盛第5部会員から、同会員が委員長を務めている化学工学研究連絡委員会が新たに提唱した「地球生態工学」に関し、提唱に至った経緯及びその学問内容等について説明がなされるとともに、この件に関連する、関係学会及び関係省庁における動きについて説明が行われた。

続いて、大島康行第4部会員から、生物界と地球環境と の関わりについて、①生物を媒介にした人間への影響の問題をどう捉えるかが、地球環境問題を考えていく上で非常 に重要な観点である。②環境汚染が生物資源に与える影響 は非常に大きく、様々な問題を生じ、結局、これが人間に はねかえってくる等の発言があった。

最後に稲田献一第3部会員から、経済活動特に生産活動 あるいは技術革新と環境問題との関わりについて意見が述 べられ、環境問題を考える際には、人間社会が様々な技術 をどのように使用するかという、人間社会の仕組みの問題 が最も重要な問題である等の発言があった。

#### 「討議

5人の会員による意見発表の後行われた討議では、第1 部から第7部まですべての部にわたる20人を超える会員より、それぞれ専門の立場から活発な発言がなされた。

この討議の中で述べられた意見は多岐多様にわたったが、その主なものを項目にして列挙すると、「コジェネレーションによるエネルギーの有効利用」、「IGBPへの人文・社会科学の関わり方」、「海洋と環境問題」、「畜産廃棄物の処理問題」、「徹生物利用による都市河川、下水の浄化システム」、「環境問題と人間性との関わり」、「環境問題に対する学術会議の体制及び取り組み方」、「地球環境に関する国際協力事業についての学術会議が果たすべき役割」等であった。

なお,この総会の自由討議の内容については,後日,日 学双書として出版される予定である。

#### 「委員会報告」3 件を発表

本会議では、その意思の表出の形態の一つとして、各部・各委員会がその審議結果をとりまとめたものを、総会又は運営審議会の承認を得て、外部に発表する「報告」(通称「対外報告」と言っている。)というものがある。 ノ

この日本学術会議だよりでも、今までに、この対外報告 を紹介してきたが、今回、最近発表されたもののうち、ま だ紹介していない3件を、次に紹介する。

#### 電子工学の体系化に向けて一電子・通信工 学研究連絡委員会報告(要旨)

電子工学はそれを基礎にした通信工学や情報工学、制御工学などの急激な進展に伴って、これらの技術分野・工学分野の相互関係が極めて複雑になって来ているので、電子工学の学問・技術体系の根幹、電子工学の基盤となっている方法論、通信工学、情報工学等の応用工学とのかかわりを明らかにするために方法論的体系化を試みた。

即ち電子工学を共通基礎理論,基礎電子工学,電子デバイス・回路工学,電子装置・システム工学,応用分野と階層化し、さらに「集積回路」と「光エレクトロニクス」が電子工学の中心分野を形成しつつある重要な技術領域であるとの認識に立ち電子工学の技術要素と通信工学、情報工学との相互関連を検討した。

また電子工学体系の範囲を大学学部での教育対象分野および大学院での教育を含む研究対象分野に分類した。

#### 「国際防災の十年」一災害科学研究者からの 提言一災害工学研究連絡委員会報告(要旨)

1987年、第42回国連総会で決議された「国際防災の十年」(IDNDR)のスタートがいよいよ迫ってきた。行政レベルでは、内閣総理大臣を本部長とする推進本部が設けられているが、この実施には科学者、研究者の各専門領域からの積極的な関与が不可欠である。国連決議に先立ち、災害工学研究連絡委員会ではこの活動の支持を表明し、またその後も米国アカデミーや国連諸機関の活動に協力してきたが、スタートに当たり、わが国の関係科学者、研究者のなすべき、あるいは行う得る事柄を提言という形で表明したものである。その内容は、①「国際防災の十年」で何をするのか、②国際共同研究の展開、③基礎研究の推進、④「国際防災の十年」の実現に向けて、より成っている。今後はその実現に向けて積極的な運動を進めていきたい。

#### 電圧,抵抗および温度の維持方式の 1990年1月1日からの変更について― 標準研究連絡委員会報告(要旨)

第77回国際度量衡委員会(1988年10月開催)は、電圧、抵抗の各標準の維持方式の変更と、温度目盛の変更の3つの勧告を採択し、1990年初頭からの実施を求めた。この変更を国内に事前に周知するため、関心の高い9学会に、勧告の要旨を掲載した。以下に勧告の骨子を記す。

電圧標準はジョセフソン効果で実現し、電圧周波数係数を $K_J$ と記号し、その値を483 597.9 GHz/Vと定義した。抵抗標準は量子ホール効果で実現し、量子化ホール抵抗を $R_K$ と記号し、その値を25 812.807  $\Omega$  と定義した。温度スケールは、IPTS-68に変わり、ITS-90(1990年国際温度目盛)を定義した。変更は多岐に亘るので、詳細は関連学会誌を参照されたい。

以上の変更により、電磁気計測の精度と国際的整合性が 著しく改善され、温度計測の熱力学温度との一致と低温域 への拡張が達成される。

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。

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日本学術会議広報委員会 電話03(403)6291

## The Japanese Journal of PHYCOLOGY

#### Volume 37, 1989

#### Contents

No. 1 (10 March)	
P. M. Sivalingam: Biochemical relationships of Valonia fastingiata and Valoniopsis	
pachynema from the tropics [P.M. Sivalingam: 熱帯産 Valonia fastigiata と	
Valoniopsis pachynema の生化学的関係]	1- 3
P. M. Sivalingam: Cytoplasmic electrolyte fluctuations in Valonia fastigiata and	
Valoniopsis pachynema with regard to the external environmental medium [P. M.	
Sivalingam: 外液に関連する Valonia fastigiata と Valonipsis pachynema の細胞質	
内電解質変動]	4− €
Kunshan Gao and Isamu Umezaki: Comparative studies of photosynthesis in dif-	
ferent parts of Sargassum thunbergii [高 坤 山・梅崎 勇:ウミトラノオの藻体部 位別の光合成活性に関する比較研究]	7-16
Tetsuro Ajisaka: Plurilocular sporangia and the development of plurispores in Ishige	
okamurai Yendo (Phaeophyceae) from the Kada coast, Wakayama Prefecture in	
Japan [ <b>鯵坂哲朗</b> :和歌山県加太産のインゲ(褐藻類)の複子 <b>嚢と遊走細胞の</b> 発生]	17-22
Mario Cormaci and Giovanni Furnari: World distribution of the genus Antithamnion	
NAEGELI (Rhodophyta, Ceramiaceae) [Mario Cormaci·Giovanni Furnari: 紅	
藻イギス科のフタツガサネ属の世界における分布	23-30
John P. Kociolek and Ronald K. Mahoney: Observations on valve morphology and	
the systematic position of Rouxia californica M. Peragallo (Bacillariophyceae) [John	
P. Kociolek · Ronald K. Mahoney: 珪藻 Rouxia californica M. PERAGALLO の殻形	
態の観察および系統的位置]	31-38
Michael J. Wynne: Observations on Pseudolaingia larsenii (Skottsb.) Levr.	
(Delesseriaceae, Rhodophyta) [Michael J. Wynne: 紅藻コノハノリ科の	
Pseudolaingia larsenii (Skottsb.) Levr. の観察]	39–45
本多正樹・奥田武男:秋に成熟するアカモクの卵放出,胚発生および光合成速度の	
季節変化〔Masaki Honda and Takeo Okuda: Egg liberation, germling develop-	46 50
ment and seasonal changes in photosynthetic rates of autumnal Sargassum horneri]  W. A. Nelson and R. E. De Wreede: Reproductive phenology of Analipus japonicus	46-52
(HARV.) WYNNE (Phaeophyta) in the eastern North Pacific [W. A. Nelson R. E. De	
Wreede: 東部北太平洋における褐藻マツモの生殖の季節性]	53-56
Wang Xiao Yang: Observations on pyrenoid ultrastructure of Cladophora conchopheria	33 30
(Chlorophyceae) 〔王 暁 陽:緑藻カイゴロモ (Cladophora conchopheria) のピレノイ	
ドについての電頭観察]	57-79
計 報 [Obituary]	60-62
新刊紹介〔Book Review〕	22, 52
ニュース [News]	63
学会録事〔Announcement〕	64
日本藻類学会第13回大会プログラム・講演要旨〔Program and abstracts of the XIIIth	
Annual Meeting of the Japanese Society of Phycology]	65–86
日本学術会議だより〔Japan Science Council News〕	87–88
No. 2 (20 June)	
Kunshan Gao and Isamu Umezaki: Studies on diurnal photosynthetic performance of	
Sargassum thunbergii I. Changes in photosynthesis under natural sunlight [高坤山	
・梅崎 勇:ウミトラノオの光合成に関する研究 I. 太陽光下での光合成の日	00.00
変化]	89–98
Kunshan Gao and Isamu Umezaki: Studies on diurnal photosynthetic performance of	
Sargassum thunbergii II.Explanation of diurnal photosynthesis patterns from examina- tions in the laboratory〔高 坤 山・梅崎 勇:ウミトラノオの光合成に関する研	

	99–104
Taizo Motomura: Ultrastructural study of sperm in Laminaria angustata (Lami-	
nariales, Phaeophyta), especially on the flagellar apparatus 〔木村泰三:ミツイシ	
コンブ(褐藻,コンブ目)の精子の微細構造,特に鞭毛基部構造について〕	105–116
Hiroyuki Ito: Seasonal fluctuation of Chrysochromulina parva (Prymnesiophyceae) in	
four ponds and lakes in the Kinki district, Japan〔伊藤裕之:近畿地方の 4 湖沼に	
おける Chrysochromulina parva LACKEY (プリムネシウム藻綱) の季節的消長]	117–122
Hiroshi Yabu: Notes on Urospora penicilliformis Areschouc from Hokkaido (數	
<b>駕:北海道函館産のシリオミドロについて]</b>	123–128
Naotsune Saga, Yoshihiko Sakanishi and Takashi Ogishima: Method for quick	
evaluation of cell viability in marine macroalgae [嵯峨直恒・坂西芳彦・荻島 隆:大型海産藻類の細胞生存率の簡易評価法]	129–136
Masahiko Idei and Hiromu Kobayasi: Examination of the type material of Diploneis	
boldtiana Cl. (Bacillariophyceae) 〔出井雅彦・小林 弘:Diploneis boldtiana Cl. の	
タイプ材料の調査]	137–143
松本正喜・吉田忠生:日本新産紅藻ヒメベニヤバネグサ (新称) Membranoptera	
spinulosa (Ruprecht) Kuntze(紅藻 コノハノリ科)について〔Masaki Ma-	
tsumoto and Tadao Yoshida: On Membranoptera spinulosa (RUPRECHT) KUNTZE	
(Delesseriaceae, Rhodophyta) new to Japan]	145-149
Masakazu Tatewaki, Wang Xiao-Yang and Isamu Wakana: A simple method of red	
seaweed axenic culture by spore-washing〔舘脇正和·王 暁 陽·若菜 勇:胞子	
洗浄を利用した簡易な紅藻の無菌培養法」	150-152
Mitsuo Kajimura: Lectotypification of Predaea tokidae, Predaea bisporifera (Nema-	
stomataceae, Rhodophyta) and Antithamnion okiense (Ceramiaceae, Rhodophyta)	
[梶村光男:Predaea tokidae, Predaea bisporifera (ヒカゲノイト科, 紅藻植物門) 及	
び Antithamnion okiense(イギス科,紅藻植物門)の選定基準標本の選定〕	153-155
新刊紹介〔Book Review〕	
学会録事〔Announcement〕	
学会会則〔Regulations of the Society〕	
投稿案内〔Information for authors〕	161 165
伎備条門 (Information for authors)	
伎偷条内(Information for authors)	
校備条约(Information for authors)  No. 3 (20 September)	
No. 3 (20 September)	164–165
No. 3 (20 September) Suzanne Fredericq, Max H. Hommersand and James N. Norris: Morphological	164–165
No. 3 (20 September)  Suzanne Fredericq, Max H. Hommersand and James N. Norris: Morphological observations on the adelphoparasite Gracilariophila oryzoides (Gracilariales,	
No. 3 (20 September)  Suzanne Fredericq, Max H. Hommersand and James N. Norris: Morphological observations on the adelphoparasite Gracilariophila oryzoides (Gracilariales, Rhodophyta) [Suzanne Fredericq·Max H. Hommersand·James N. Norris: 7	
No. 3 (20 September)  Suzanne Fredericq, Max H. Hommersand and James N. Norris: Morphological observations on the adelphoparasite Gracilariophila oryzoides (Gracilariales, Rhodophyta) [Suzanne Fredericq・Max H. Hommersand・James N. Norris: フデルフォ寄生薬 Gracilariophila oryzoides (紅薬、オゴノリ科) の形態観察]	
No. 3 (20 September)  Suzanne Fredericq, Max H. Hommersand and James N. Norris: Morphological observations on the adelphoparasite Gracilariophila oryzoides (Gracilariales, Rhodophyta) [Suzanne Fredericq・Max H. Hommersand・James N. Norris: アデルフォ寄生藻 Gracilariophila oryzoides (紅藻, オゴノリ科) の形態観察]	
No. 3 (20 September)  Suzanne Fredericq, Max H. Hommersand and James N. Norris: Morphological observations on the adelphoparasite Gracilariophila oryzoides (Gracilariales, Rhodophyta) [Suzanne Fredericq・Max H. Hommersand・James N. Norris: フデルフォ寄生藻 Gracilariophila oryzoides (紅藻, オゴノリ科) の形態観察]	167–179
No. 3 (20 September)  Suzanne Fredericq, Max H. Hommersand and James N. Norris: Morphological observations on the adelphoparasite Gracilariophila oryzoides (Gracilariales, Rhodophyta) [Suzanne Fredericq・Max H. Hommersand・James N. Norris: フデルフォ寄生藻 Gracilariophila oryzoides (紅藻, オゴノリ科) の形態観察]	167–179
No. 3 (20 September)  Suzanne Fredericq, Max H. Hommersand and James N. Norris: Morphological observations on the adelphoparasite Gracilariophila oryzoides (Gracilariales, Rhodophyta) [Suzanne Fredericq・Max H. Hommersand・James N. Norris: フデルフォ寄生薬 Gracilariophila oryzoides (紅藻, オゴノリ科) の形態観察]	167–179
No. 3 (20 September)  Suzanne Fredericq, Max H. Hommersand and James N. Norris: Morphological observations on the adelphoparasite Gracilariophila oryzoides (Gracilariales, Rhodophyta) [Suzanne Fredericq・Max H. Hommersand・James N. Norris: フデルフォ寄生薬 Gracilariophila oryzoides (紅藻, オゴノリ科) の形態観察]	167–179
No. 3 (20 September)  Suzanne Fredericq, Max H. Hommersand and James N. Norris: Morphological observations on the adelphoparasite Gracilariophila oryzoides (Gracilariales, Rhodophyta) [Suzanne Fredericq・Max H. Hommersand・James N. Norris: フデルフォ寄生薬 Gracilariophila oryzoides (紅藻, オゴノリ科) の形態観察]	167–179
No. 3 (20 September)  Suzanne Fredericq, Max H. Hommersand and James N. Norris: Morphological observations on the adelphoparasite Gracilariophila oryzoides (Gracilariales, Rhodophyta) [Suzanne Fredericq・Max H. Hommersand・James N. Norris: フデルフォ寄生藻 Gracilariophila oryzoides (紅藻, オゴノリ科) の形態観察]	167–179
No. 3 (20 September)  Suzanne Fredericq, Max H. Hommersand and James N. Norris: Morphological observations on the adelphoparasite Gracilariophila oryzoides (Gracilariales, Rhodophyta) [Suzanne Fredericq・Max H. Hommersand・James N. Norris: フデルフォ寄生薬 Gracilariophila oryzoides (紅藻, オゴノリ科) の形態観察]	167–179 180–186 187–193
No. 3 (20 September)  Suzanne Fredericq, Max H. Hommersand and James N. Norris: Morphological observations on the adelphoparasite Gracilariophila oryzoides (Gracilariales, Rhodophyta) [Suzanne Fredericq・Max H. Hommersand・James N. Norris: フデルフォ寄生薬 Gracilariophila oryzoides (紅藻, オゴノリ科) の形態観察]	167–179 180–186 187–193
No. 3 (20 September)  Suzanne Fredericq, Max H. Hommersand and James N. Norris: Morphological observations on the adelphoparasite Gracilariophila oryzoides (Gracilariales, Rhodophyta) [Suzanne Fredericq・Max H. Hommersand・James N. Norris: フデルフォ寄生藻 Gracilariophila oryzoides (紅藻, オゴノリ科) の形態観察]	167–179 180–186 187–193
No. 3 (20 September)  Suzanne Fredericq, Max H. Hommersand and James N. Norris: Morphological observations on the adelphoparasite Gracilariophila oryzoides (Gracilariales, Rhodophyta) [Suzanne Fredericq・Max H. Hommersand・James N. Norris: フデルフォ寄生藻 Gracilariophila oryzoides (紅藻, オゴノリ科) の形態観察]	167–179 180–186 187–193
No. 3 (20 September)  Suzanne Fredericq, Max H. Hommersand and James N. Norris: Morphological observations on the adelphoparasite Gracilariophila oryzoides (Gracilariales, Rhodophyta) [Suzanne Fredericq・Max H. Hommersand・James N. Norris: フデルフォ寄生藻 Gracilariophila oryzoides (紅藻, オゴノリ科) の形態観察]	167–179 180–186 187–193 194–200
No. 3 (20 September)  Suzanne Fredericq, Max H. Hommersand and James N. Norris: Morphological observations on the adelphoparasite Gracilariophila oryzoides (Gracilariales, Rhodophyta) [Suzanne Fredericq・Max H. Hommersand・James N. Norris: フデルフォ客生薬 Gracilariophila oryzoides (紅薬, オゴノリ科) の形態観察]	167–179 180–186 187–193 194–200
No. 3 (20 September)  Suzanne Fredericq, Max H. Hommersand and James N. Norris: Morphological observations on the adelphoparasite Gracilariophila oryzoides (Gracilariales, Rhodophyta) [Suzanne Fredericq・Max H. Hommersand・James N. Norris: フデルフォ寄生藻 Gracilariophila oryzoides (紅藻, オゴノリ科) の形態観察]	167–179 180–186 187–193 194–200
No. 3 (20 September)  Suzanne Fredericq, Max H. Hommersand and James N. Norris: Morphological observations on the adelphoparasite Gracilariophila oryzoides (Gracilariales, Rhodophyta) [Suzanne Fredericq・Max H. Hommersand・James N. Norris: フデルフォ客生薬 Gracilariophila oryzoides (紅薬, オゴノリ科) の形態観察]	167–179 180–186 187–193 194–200
No. 3 (20 September)  Suzanne Fredericq, Max H. Hommersand and James N. Norris: Morphological observations on the adelphoparasite Gracilariophila oryzoides (Gracilariales, Rhodophyta) [Suzanne Fredericq・Max H. Hommersand・James N. Norris: フデルフォ寄生薬 Gracilariophila oryzoides (紅薬, オゴノリ科) の形態観察]	167–179 180–186 187–193 194–200
No. 3 (20 September)  Suzanne Fredericq, Max H. Hommersand and James N. Norris: Morphological observations on the adelphoparasite Gracilariophila oryzoides (Gracilariales, Rhodophyta) [Suzanne Fredericq・Max H. Hommersand・James N. Norris: フデルフォ寄生藻 Gracilariophila oryzoides (紅藻, オゴノリ科) の形態観察]	167–179 180–186 187–193 194–200
No. 3 (20 September)  Suzanne Fredericq, Max H. Hommersand and James N. Norris: Morphological observations on the adelphoparasite Gracilariophila oryzoides (Gracilariales, Rhodophyta) [Suzanne Fredericq・Max H. Hommersand・James N. Norris: フデルフォ寄生薬 Gracilariophila oryzoides (紅薬, オゴノリ科) の形態観察]	167–179 180–186 187–193 194–200 201–207 208–212
No. 3 (20 September)  Suzanne Fredericq, Max H. Hommersand and James N. Norris: Morphological observations on the adelphoparasite Gracilariophila oryzoides (Gracilariales, Rhodophyta) [Suzanne Fredericq·Max H. Hommersand·James N. Norris: アデルフォ寄生藻 Gracilariophila oryzoides (紅薬, オゴノリ科) の形態観察]	167–179 180–186 187–193 194–200 201–207 208–212
No. 3 (20 September)  Suzanne Fredericq, Max H. Hommersand and James N. Norris: Morphological observations on the adelphoparasite Gracilariophila oryzoides (Gracilariales, Rhodophyta) [Suzanne Fredericq・Max H. Hommersand・James N. Norris: フデルフォ寄生薬 Gracilariophila oryzoides (紅薬, オゴノリ科) の形態観察]	167–179 180–186 187–193 194–200 201–207 208–212
No. 3 (20 September)  Suzanne Fredericq, Max H. Hommersand and James N. Norris: Morphological observations on the adelphoparasite Gracilariophila oryzoides (Gracilariales, Rhodophyta) [Suzanne Fredericq・Max H. Hommersand・James N. Norris: フデルフォ寄生藻 Gracilariophila oryzoides (紅藻, オゴノリ科) の形態観察]	167–179 180–186 187–193 194–200 201–207 208–212

の分類学と分布上の知見]	221–235
数] Hiroshi Kawai: First report of <i>Phaeosaccion collinsii</i> Farlow (Chrysophyceae, Sar-	236–238
cinochrysidales) from Japan [川井浩史:日本新産黄金色藻 Phaeosaccion collinsii FARLOW(フクロコガネモ:新称)	
新刊紹介〔Book Review〕 学会録事〔Announcement〕	
子云球事(Announcement) 日本学術会議だより〔Japan Science Council News〕	
No. 4 (10 December)	
Yumiko Amemiya and Oki Nakayama: Separation and sugar composition of	
gelatinous sheath carbohydrates produced by <i>Microcystis</i> 〔雨宮由美子・中山大樹: <i>Microcystis</i> 属藍藻の生産する粘質鞘多糖の分離と糖組成〕	253-262
Masahiro Notoy a and Yusho Aruga: The life history of Dudresnaya japonica Okamura	
(Cryptonemiales, Rhodophyta) in culture〔能 <b>登谷正浩・有賀祐勝</b> :紅藻ヒビロウ ドの生活史]	263-267
Tadao Yoshida: Solieria pacifica (YAMADA) YOSHIDA, comb. nov. (Solieriaceae,	203 207
Rhodophyta) from Japan [吉田忠生:ミリン(紅藻,スギノリ目)の学名について]	268-270
Tadao Yoshida: Notes on Spirocladia loochooensis (YENDO) YOSHIDA, comb. nov.	200 210
(Rhodomelaceae, Rhodophyta)〔吉田忠生:新組合せ Spirocladia loochooensis(ヒゲ ヨレミグサ,新称)について	271-273
Hiroo Satoh, Yukuya Yamaguchi, Kentaro Watanabe and Yusho Aruga: Light con-	
ditions and photosynthetic productivity of ice algal assemblages in Lake Saroma, Hokkaido 〔佐藤博雄・山口征矢・渡辺研太郎・有賀祐勝:サロマ湖における	
ice algae の光合成生産力〕	274-278
Takeo Okuda and Yukari Satoh: Conceptacular development in Sargassum filicinum	
and autumnal S. horneri (Phaeophyceae) 〔奥田武男・佐藤由香利: ンダモク及び 秋に成熟するアカモクの生殖器巣形成〕	279-283
Sung-Min Boo, Jan Rueness and In Kyu Lee: Life history and taxonomy of Callitham-	
nion callophyllidicola Yamada (Ceramiaceae, Rhodophyta) [Sung-Min Boo·Jan	
Rueness·In Kyu Lee: キヌイトグサ(紅藻, イギス科)の生活史と分類]	284-290
入来義彦・堀口健雄・増尾恵美子:シワランソウモドキ Collinsiella cava (YENDO)	
Printz(緑藻,ランソウモドキ科)の粘質様物質一構成する中性糖類を中心と	
して一 [Yoshihiko Iriki, Takeo Horiguchi and Emiko Masuo: Mucilage from Collinsiella cava (YENDO) PRINTZ (Chlorophyta) with special reference to its suger com-	
ponents]ponents	291-294
S. F. Friedlander, L. D. Melton and M. T. Brown: Ascorbic acid in the New	
Zealand seaweeds Porphyra columbina MONT. and Porphyra subtumens J. Ag.	
(Rhodophyceae) [S. F. Friedlander L. D. Melton M. T. Brown: = =	
ランド産紅藻 Porphyra columbina MONT. 及び Porphyra subtumens J. Ag. のアスコル	
ビン酸]	295-301
Masahiro Notoya and Yusho Aruga: Tissue culture from the explant of <i>Ecklonia cava</i> KJELLMAN (Laminariales, Phaeophyta) [能登谷正浩・有賀祐勝:カジメの組織培	
養〕	302-304
市村輝宜・笠井文絵:鼓藻類の種分化における染色体構成の変動〔Terunobu	
Ichimura and Fumie Kasai: Genome differentiation in speciation of desmids	
(Chlorophyta)]	305-319
日本藻類学会秋季シンポジウム講演要旨〔Abstracts of the Sumposium of the Japanese Society of Phycology〕	320_321
新刊紹介「Book Review」	
学会録事 [Announcement]	
日本学術会議だより〔Japan Science Council News〕	
第37巻総目次〔Contents of Volume 37〕	

## AUTHOR INDEX

Ajisaka, T. 鯵坂哲朗17	Mahoney, R. K31
Amemiya, Y. 雨宮由美子253	Masuda, M. 增田道夫180
Aruga, Y. 有賀祐勝263, 274, 302	Masuo, E. 增尾恵美子291
Bailey, J. C201	Matsumoto, M. 松本正喜145
Boo, SM284	Melton, L. D295
Brown, M. T295	Motomura, T. 本村泰三105
Chiang, YM. 江 永 棉187	Murase, N. 村瀬 昇213
Cormaci, M23	Nakayama, O. 中山大樹253
De Wreede, R. E53	Nelson, W. A53
Fredericq, S167	Norris, J. N167
Friedlander, S. F295	Notoya, M. 能登谷正浩263, 302
Furnari, G23	Ogishima, T. 荻島 隆129
Gabrielson, P. W221	Okuda, T. 奥田武男46, 279
Gao, K. 高坤山	Rueness, J284
Hommersand, M. H167	Saga, N. 嵯峨直恒129
Honda, M. 本多正樹46	Saito, Y. 斎藤 譲208
Horiguchi, T. 堀口健雄291	Sakanishi, Y. 坂西芳彦129
Ichimura, T. 市村輝宜305	Satoh, H. 佐藤博雄274
Idei, M. 出井雅彦137	Satoh, Y. 佐藤由香利279
Iriki, Y. 入来義彦291	Selivanova, O. N180
Ito, H. 伊藤裕之117	Sivalingam, P. M
Kajimura. M. 梶村光男153	Tatewaki, M. 舘脇正和150
Kapraun, D, F201	Umezaki, I. 梅崎 勇
Kasai, F. 笠井文絵305	Wakana, I. 若菜 勇150
Kawai, H. 川井浩史239	Wang, XY. 王 暁 陽57, 150
Kida, Y. 喜田和四郎194, 213	Watanabe, K. 渡辺研太郎274
Kobayasi, H. 小林 弘137	Wynne, M. J39
Kociolek, J. P31	Yabu, H. 籔
Lee, I. K284	Yamaguchi, Y. 山口征夫274
Lin, JL. 林 俊 亮187	Yamamoto, H. 山本弘敏236
Lindstrom, S. C221	Yoshida, T. 吉田忠生145, 268, 27
Maerowa M 前川行去 104 212	

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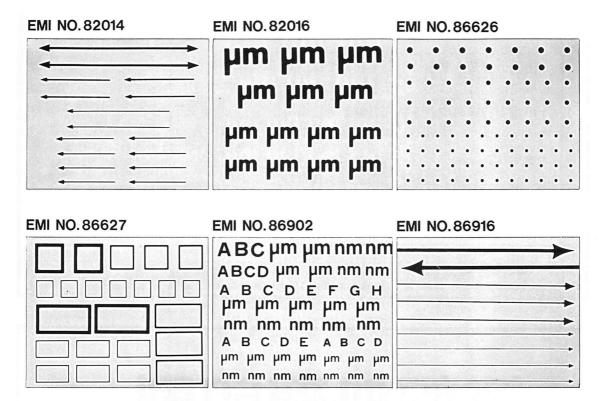
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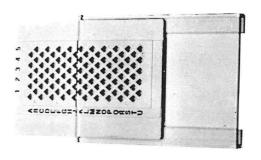
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本誌の出版費の一部は文部省科学研究費補助金「研究成果公開促進費」による。

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.

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# 类頁

#### 目 次

雨宮由美子・中山大樹:Microcystis 属藍藻の生産する粘質鞘多糖の分離と糖組成	(英文)	253
能登谷正浩・有賀祐勝:紅藻ヒビロウドの生活史	(英文)	263
吉田忠生: ミリン(紅葉, スギノリ目)の学名について	(英文)	268
吉田忠生:新組合せ Spirocladia loochooensis (ヒゲョレミグサ, 新称) について	(英文)	271
佐藤博雄・山口征矢・渡辺研太郎・有賀祐勝:サロマ湖における ice algae の光合成		
生産力	(英文)	274
奥田武男・佐藤由香利:シダモク及び秋に成熟するアカモクの生殖器巣形成	(英文)	279
Sung-Min Boo · Jan Rueness · In Kyu Lee:キヌイトグサ (紅藻, イギス科) の生		5
活史と分類	(英文)	284
入来義彦・堀口健雄・増尾恵美子:シワランソウモドキ Collinsiella cava (YENDO)		
Printz (緑藻,ランソウモドキ科) の粘質様物質-構成する中性糖類を中心と		
LT		291
S. F. Friedlander · L. D. Melton · M. T. Brown: ニュージーランド産紅藻 Porphyra		
columbina MONT. 及び Porphyra subtumens J. Ag. のアスコルビン酸	(英文)	295
<b>/</b> − <b>├</b>		
能登谷正浩・有賀祐勝:カジメの組織培養	(英文)	302
総 説		
市村輝宜・笠井文絵: 鼓藻類の種分化における染色体構成の変動		305
<b>***</b>		
日本藻類学会秋季シンポジウム講演要旨		320
新刊紹介		321
学会録事		323
第37巻絵目次		i-iv