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THE JAPANESE SOCIETY OF PHYCOLOGY

日 本 藻 類 学 会

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日本藻類学会第16回大会のお知らせ

日本藻類学会第15回大会を下記の要領で開催します。奮ってご参加下さい。

会　　場：東京水産大学　〒108 東京都港区港南4–5–7
　　電話　03 (3471) 1251（代）

会　　期：1992年 3 月27日（金）
　　28日（土）　海苔栽培業見学会
　　29日（日）編集委員会・評議員会
　　30日（月）口頭発表・総会・懇親会
　　31日（火）口頭発表・展示発表

申 込 先：

大会・懇親会・発表の申込票および発表要旨の送付，見学会の申込，その他連絡は下記宛にお願いします。

〒108 東京都港区港南4–5–7
東京水産大学　資源育成学科　大葉英雄気付
日本藻類学会第16回大会準備委員会
電話　03 (3471) 1251　内線 437（大葉）
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1）大会参加者は，発表の有無にかかわらず，本誌に綴込みの大会申込票に必要事項を記入して，上記の第16回大会準備委員会宛にお送り下さい。
2）大会参加費4,000円（学生3,000円），および懇親会費3,000円を同封の振替用紙でお送り下さい。
　送金先：郵便振替口座　東京2–604139
　　日本藻類学会第16回大会準備委員会
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発　　表：発表を希望される方は，本誌に綴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込み下さい（**1992年 1 月10日必着**）。

1）発表は，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。
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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（大会プログラムに記されているもの），スライド総枚数，映写順序，上辺マークをご記入下さい。同じスライドを繰返し映写する場合は，それに見合う枚数をご用意下さい。
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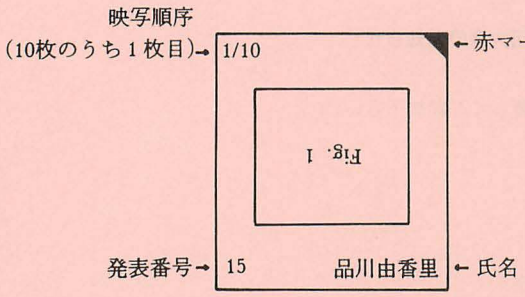

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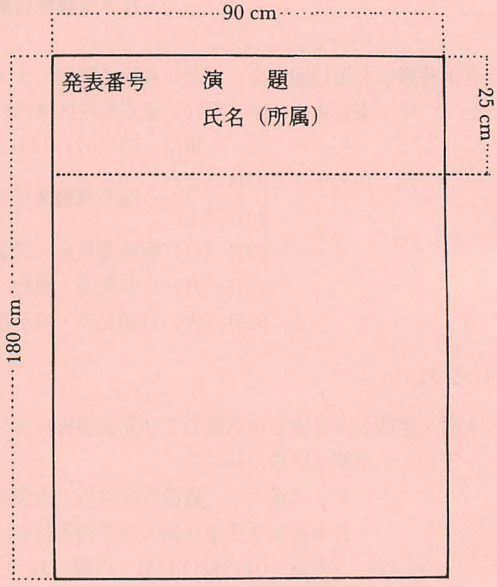

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4) 要旨原稿は，以下の要領・見本にしたがって，タイプライター，ワープロまたはパソコン（24ドット以上の印字）で作成して下さい。

①原稿は，B5 用紙（オニオンペーパーなどの薄手の用紙は避けてください）に明瞭な黒色（カーボンリボン等を使用）で印字して下さい。

②原稿は演題等も含めて，縦 150 mm × 横 100 mm の範囲内に24字× 22行の印字を標準とします（枠で囲まないで下さい）。

③著者名，演題，要旨本文，所属の順に書いて下さい。

④著者が複数の場合は，演者名の前に○をつけて下さい。また，所属が異なるときは著者名の後に＊印を付し，区別して下さい。

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⑥所属は（ ）内に入れて，最下段に表示して下さい。

⑦和文原稿の場合，句読点は「,」（コンマ）と「。」（マル）を使用して下さい。

⑧学名等，イタリックで表示する場合，イタリック文字を使用するか，その部分に下線をつけて下さい。

⑨原稿は約 2/3 に縮小し，そのままオフセット印刷されます。折り曲げずに郵送して下さい。なお，著者校正はありませんので，ご注意下さい。

日本藻類学会主催‘海苔栽培業見学会’のお知らせ

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1. 期　日：1992年３月27日（金）～28日（土）
2. 日　程：３月27日（金）JR 内房線上総湊駅前に集合
　　海苔栽培業見学
　　富津市「鈴孝荘」宿泊
　　３月28日（土）海苔栽培業見学
　　JR 内房線大貫駅前にて解散
3. 見学先：天羽漁業協同組合湊のり生産組合
　　千葉県富津市湊1155
　　新富津漁業協同組合
　　千葉県富津市富津2430-1
　　（社）千葉県のり種苗センター
　　千葉県富津市小久保3090
4. 参加費：交 通 費　実費
　　昼 食 費　実費
　　宿 泊 費　約6,000円
　　懇親会費　2,000円
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6. 申　込：参加希望者は，往復葉書に１）氏名，２）連絡先，３）所属を明記の上，**1992年１月10日までに**，上記の第16回大会準備委員会宛にお申込下さい。
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　　なお，日程等の詳細につきましては，追って参加決定者にご通知致します。

要旨原稿の見本

○工藤利彦*・増田道夫**：紅藻ショウジョウケノリの形態学的研究

日本沿岸各地に生育する紅藻ショウジョウケノリには，Polysiphonia urceolata (Dillwyn) Greville の学名が与えられてきた。……

……したがって，本邦産ショウジョウケノリの学名は P. senticulosa に変更されるべきであると結論された。

(*札幌大・生物，**北大・理・植物)

○Boo, S. M.,* J. Rueness,** I. K. Lee*** and T. Yoshida****: A New Combination in Aglaothamnion (Ceramiaceae: Rhodophyta)

Examination of the type specimens of Callithamnion callophyllidicola and living materials collected from Tyoshi and……

……between *A. callophyllidicola* and *C. minutissima* is discussed.

(*Chungnam Nat'l Univ., **Oslo Univ.,*** Seoul Nat'l Univ.,****Hokkaido Univ.)

(原稿には枠をつけないで下さい)

日本藻類学会第16回大会申込用紙

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（フリガナ）
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発　　表：　す　る（単独・連名），しない。

懇親会：　参　加，不参加。

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大会参加費　4,000円（学生　3,000円）

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発表の種類（希望する方を◯で囲んで下さい）：

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演　題：＿＿＿＿＿＿＿＿＿＿

連絡先：（連名の場合は演者）＿＿＿＿＿＿＿＿＿＿ ☎＿＿＿＿＿＿

Jpn. J. Phycol. (Sôrui) 39: 301–306, December 10, 1991

Aglaothamnion callophyllidicola (Yamada) comb. nov. (Ceramiaceae, Rhodophyta)

Sung Min Boo*, In Kyu Lee**, Jan Rueness*** and Tadao Yoshida****

* *Department of Biology, Chungnam National University, Daejon 305–764, Korea*
** *Department of Biology, Seoul National University, Seoul 151–742, Korea*
*** *Department of Biology, Marine Botany, Oslo University, Oslo 3, Norway*
**** *Department of Botany, Faculty of Science, Hokkaido University, Sapporo 060, Japan*

Boo, S. M., Lee, I. K., Rueness, J. and Yoshida, T. 1991. *Aglaothamnion callophyllidicola* (Yamada) comb. nov. (Ceramiaceae, Rhodophyta). Jpn. J. Phycol. **39**: 301–306.

A ceramiaceous red alga *Callithamnion callophyllidicola* Yamada was critically examined and compared with a related taxon, *C. minutissima* Yamada. The type and newly collected materials were found to have uninucleate vegetative cells and lobed cystocarps that link it closely with the related genus *Aglaothamnion* Feldmann-Mazoyer. The new binomial *Aglaothamnion callophyllidicola* (Yamada) comb. nov. is proposed and *C. minutissima* Yamada is here treated as a later synonym.

Key Index Words: Aglaothamnion callophyllidicola—Callithamnion callophyllidicola—Callithamnion minutissima—*Ceramiaceae*—*Rhodophyta*—*Taxonomy*.

Aglaothamnion was segregated from *Callithamnion* on the basis of *C. furcellariae* J. Agardh by Feldmann-Mazoyer (1940), but the genus had not been well defined (Dixon and Price 1981). The taxonomic relationship between two genera has recently been reappraised by L'Hardy-Halos and Rueness (1990), who proposed reinstatement of *Aglaothamnion* for the species of *Callithamnion* complex with only one nucleus in each vegetative cell. Thus several species previously assigned to *Callithamnion* in Europe are newly attributed to *Aglaothamnion*. In Korea and Japan three *Aglaothamnion* and nine *Callithamnion* species have been reported (Lee and Kang 1986, Yoshida *et al.* 1990), but their taxonomic positions have not been fully investigated.

Callithamnion callophyllidicola Yamada is a small ceramiaceous alga that occurs near the low water mark and in the subtidal habitats. It was first described from Enoshima in central Japan by Yamada (1932) as epiphytes on *Callophyllis crispata* Okamura and *C. japonica* Okamura. Although the diagnosis for *Callithamnion callophyllidicola* included information on its vegetative and reproductive morphology, some described features are inconsistent with those now known to be characteristic of the genus (Segawa 1942, Boo *et al.* 1989). A reexamination of the type and newly collected materials of *C. callophyllidicola* and the related taxon, *C. minutissima* Yamada, was undertaken in order to reassess its taxonomic position.

Material and Methods

One envelope with three herbarium sheets as *Callithamnion callophyllidicola*, which were designated as types by Yamada (1932), has been housed in the herbarium of Faculty of Science, Hokkaido University (SAP). The first sheet is annotated as "Type! Enoshima, Apr. 6, 1932". Many plants are epiphytic on *Callophyllis crispata* (Fig. 1A). They are tetrasporic plants, males or females with cystocarps. The second sheet is annotated as "cotype! Enoshima, IV-1932" and has many plants epiphytic on the same host plant. The third is also annotated as "cotype! Enoshima, 6/4, 1932" and has seven plants (Fig. 1B).

The annotations were made by Y. Yamada himself.

Small fragments of dry specimens from the herbarium sheets were softened for a while in distilled water and then prepared for microscopic observation. When possible, the preparations were stained with aniline blue/acetic acid and washed for observation.

The live tetrasporic plants were collected for staining nucleus at the low water mark in Choshi (Jan. 28, 1990), Chiba Prefecture and Kikonai (Feb. 15, 1990) of southern Hok-

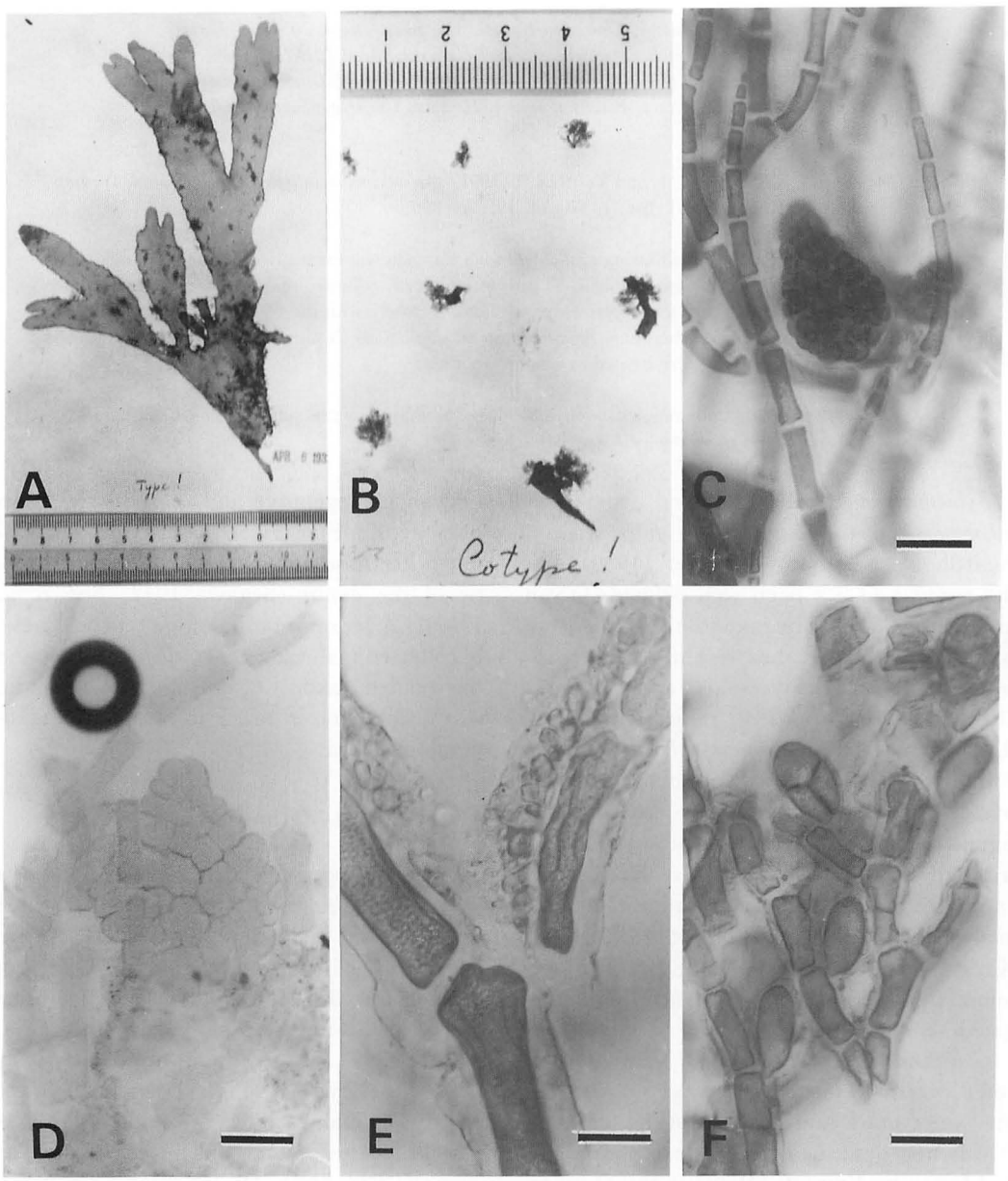

Fig. 1. *Aglaothamnion callophyllidicola*, habit and morphology. A. Holotype of *A. callophyllidicola* epiphytic on *Callophyllis crispata* Okamura (SAP 13082). B. Isotype sheet. C. Cystocarp of a female plant from Choshi. D. Cystocarp of a female plant on type sheet. E. Spermatangial branches of a male plant on type sheet. F. Tetrasporangia of a tetrasporic plant on type sheet. Scale: 50 μm for C, D, and 40 μm for E, F.

kaido. They were fixed in 2% glutaraldehyde and stained with the fluorochrome 4'-6 diamidino-2-phenylindole (DAPI, Goff and Coleman 1984) for observation under photomicroscope equipped with epifluorescence filters.

The original materials of *Callithamnion minutissima* Yamada are kept in SAP. They are mounted on slide glass and are given collection numbers of 368 and 1837.

Results

Callithamnion callophyllidicola Yamada: The plants grow with a maximal height of about 8 mm and are attached to the host by thin rhizoidal filaments which come out of the basal cells of the frond. The rhizoids are simply branched to digitate (Fig. 3B). The axial cells are formed from apical cells by oblique division and 70-90 μm broad and 200-220 μm long in the middle portion of plants, thus the L/B ratio being 2-3: 1. The branching pattern is alternate to subdichotomo-pinnate (Fig. 3A). The primary branches are derived alternately from every axial cell except lower ones (Fig. 2A). The third or fourth are usually formed similarly to the primary branches. All branches are distichous and can grow ultimately. Only one nucleus is observed in every vegetative cell of the axis and branches (Fig. 2A-B).

The gametophytes are dioecious. Spermatangial mother cells are cut off from the adaxial portion of the branches and give rise to spermatangia. Thus small spermatangial patches are seen on the adaxial portion of branches of male plants (Figs. 1E, 3D) and the branches with spermatangial patches are often curved. Carpogonial branches are formed on the axial vegetative cells in the upper portion of female plants. They are composed of four cells and are arranged in zig-zag (Fig. 3E-F). Two sterile cells are accompanied with the carpogonial branches. After fertilization young gonimolobes are formed (Fig. 3G-H), which become spherical to irregular carposporophytes and 300-500 μm long when mature (Fig. 1C-D).

Tetrasporophytes are isomorphic to gametophytes. Tetrasporangia are formed on the adaxial portion of branches (Fig. 2A). They are divided tetrahedrally and 40-55 μm $\times$ 60-70 μm in size (Figs. 1F, 3C). The gland cells, which were described on the tetrasporophytes by Yamada (1932) and Kawashima

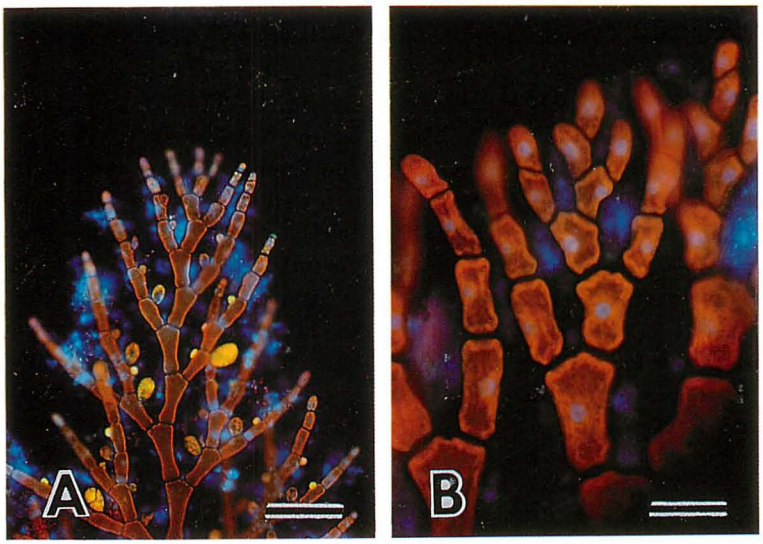

Fig. 2. *Aglaothamnion callophyllidicola* stained with DAPI. A. A tetrasporic plant from Choshi under photomicroscope eqipped with epifluorescence filters. B. One nucleus in each vegetative cell. Scale: 100 μm for A and 40 μm for B.

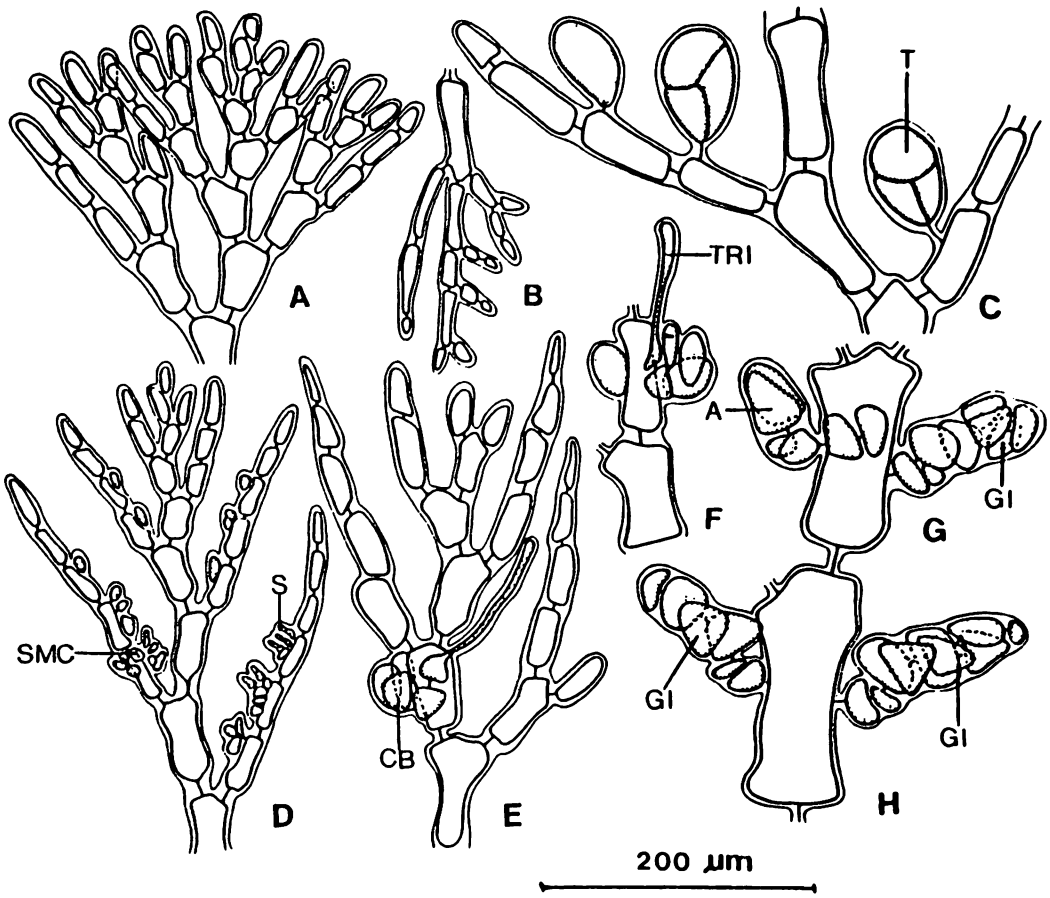

Fig. 3. *Aglaothamnion callophyllidicola*, morphology. A. Actively dividing apex. B. Digitate rhizoid. C. Development of tetrasporangia. D. Development of spermatangia. E–F. Carpogonial branches and postfertilization process. G–H. Development of gonimolobes. A; axiliary cell, CB; carpogonial branch, GI; gonimolobe initial, S; spermatangia, SMC; spermatangial mother cells T; tetrasporangia, TRI; trichogyne.

(1960), are never observed in this study.

Callithamnion minutissima Yamada: The original materials are kept only on slides (Nos. 368 and 1837). They are crushed to piece and bleached, from which we couldn't observe any characteristic features. As there is no accompanying illustration in the original paper (Yamada 1944, p. 14) and the species has scarcely been reported in flora, checklist and monograph, we have no choice but to refer the original description.

Discussion

The genus *Aglaothamnion* is typified by *A. furcellariae* (J. Agardh) Feldmann-Mazoyer, which was lectotypified with Agardh Herbarium no. 19087 (LD) collected from Bohuslan on the Swedish west coast (L'Hardy-Halos and Rueness 1990). Although it has been circumscribed in the uninucleate vegetative cells, a zig-zag alignment of cells of the carpogonial branches and the lobed, not rounded cystocarps (Feldmann-Mazoyer 1940), the zig-zag type of the carpogonial branches occurs in multinucleate taxa like *Callithamnion corymbosum* (Smith) Lyngbye and the cystocarps of *A. byssoides* (Arnott *ex* Harvey in Hooker) L'Hardy-Halos *et* Rueness are spherical to irregular in shape. Thus, validity of *Aglaothamnion* has recently been insisted for the species with only one nucleus in each vegetative cell because of its consistency (L'Hardy-Halos and Rueness 1990).

Although there have been reports that *Callithamnion callophyllidicola* Yamada has some diagnostic characters of the genus *Aglaothamnion*, a taxonomic combination has not been made because the generic concept of *Aglaothamnion* was obscure (Segawa 1942) and type specimens had not been critically examined (Boo *et al.* 1989). Our observation on type materials of *C. callophyllidicola* agrees well to the protologue (Yamada 1932) and the previous observations (Segawa 1942, 1949, Kawashima 1960), but some features are inconsistent with them. One nucleus is found in each vegetative cell of the live plants collected in Choshi, although we could not observe nucleus from dry type materials. In addition, as our observation confirms no gland cells on type plants, Yamada must have mistaken small protuberances on branches for gland cells (Boo *et al.* 1989).

The shape of the cystocarps in the protologue is shown to be spherical (Yamada 1932, Pl. VIb), but they have been reported to be lobed (Segawa 1942, p. 208, Kawashima 1960, p. 107) and irregularly spherical (Kawashima 1960, p. 107). In this study the cystocarps of type materials present lobed forms (Fig. 1D), that is also included in the genus range of *Aglaothamnion*. Features on male and tetrasporic plants agree well to the above reports.

The foregoing sections mention that the sheet (SAP 13082) designated as type by Yamada (1932) has many individuals epiphytic on *Callophyllis crispata*. We confirmed that all specimens on the type sheet are not heterogeneous, so Yamada's type of *C. callophyllidicola* is regarded as correct according to the Article 9.1 of the Berlin code (Greuter *et al.* 1988).

It was reported that *Callithamnion minutissima* resembled *C. callophyllidicola* but was easily distinguished by its more slender frond, longer axial cells and not tapered ultimate ramuli (Yamada 1944). As is pointed out in European *Callithamnion* species (Harris 1962), Yamada's diagnostic characters are quantitative or unstable and subject to be changed in different environment (Boo *et al.* 1989). However, since the same name was preoccupied for species of Adriatic Sea described by Zanardini (1842) and Kützing (1843 p. 371), Yamada's *C. minutissima* is an illegitimate name and should be replaced or rejected. The original materials in the herbarium of Hokkaido University (SAP) are kept so crushed in piece and bleached that we could not observe any features from them and the protologue has no illustration (Yamada 1944). The type locality, Hayama of Kanagawa Prefecture, is also situated near Enoshima, the type locality of *C. callophyllidicola*. Furthermore, as *C. minutissima* overlaps *C. callophyllidicola* in protologue (Boo *et al.* 1989, Table 1), we conclude *C. minutissima* Yamada as a later synonym.

According to Dawson (1962), *Callithamnion paschale* Børgesen is closely related to *C. minutissima*, but he did not state whether the alga had one or more nuclei in vegetative cells. Since both Dawson (1962) and Abbott and Hollenberg (1976) distinguished *Aglaothamnion* from *Callithamnion*, it seems implicit that at least *C. paschale* is a multinucleate species. *Aglaothamnion oosumiense* Itono (1971) is another related species (Boo *et al.* 1989), that needs a further study.

There are still some questions to be answered about the genus *Aglaothamnion* and its species. However, from the view discussed above, it is obvious that *Callithamnion callophyllidicola* is a distinct and an endemic species to Japan and the surrounding coasts, and includes the related taxon, *C. minutissima*. The following new binomial combination is therefore proposed:

Aglaothamnion callophyllidicola (Yamada), comb. nov.

Basionym: *Callithamnion callophyllidicola* Yamada 1932, p. 270. fig. 3a-b. pl. V, VIb., Holotype SAP 13082 "Enoshima, Sagami Province, Japan, Y. Yamada, April 6, 1932" Herbarium of Faculty of Science, Hokkaido University.

Synonym: *Callithamnion minutissima* Yamada 1944, p. 14., Types SAP, collection number 368 and 1837 "Hayama, Kanagawa Prefecture, Japan" Herbarium of Faculty of

Science, Hokkaido University.

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Boo, Sung Min* · Lee, In Kyu · Jan Rueness*** · 吉田忠生**** :**
キヌイトグサ（紅藻，イギス科）について

キヌイトグサのタイプ標本および新たに採集した生材料の詳しい観察から，この種の栄養細胞は単核であり，嚢果の外形が浅裂することを確認した。これらの特徴は近縁属であるアグラオタムニオン *Aglaothamnion* 属と一致するので，キヌイトグサに対して *Aglaothamnion callophyllidicola* の組合せを提案する。またヒナノキヌイトグサ *Callithamnion minutissima* Yamada は種のレベルで区別することができないので，キヌイトグサの異名であると結論される。（*Department of Biology, Chungnam National University, Daejon 305-764, Korea. **Department of Biology, Seoul National University, Seoul 151-742, Korea. ***Department of Biology, Marine Botany, Oslo University, Oslo 3 Norway, ****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）

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Seasonal protein variation in the New Zealand seaweeds *Porphyra columbina* Mont. and *Porphyra subtumens* J. Ag. (Rhodophyceae)

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Seasonal variation in tissue protein was measured in the red algae *Porphyra columbina* Mont. and *Porphyra subtumens* J. Ag. by three methods (total nitrogen multiplied by 6.25, biuret protein, and sum of anhydroamino acids). The protein levels in both species showed a seasonal trend, with maximum levels occurring in the winter. The measured protein content varied between the methods used, with the nitrogen value multiplied by 6.25 giving the highest value, followed by the sum of anhydroamino acids and then the biuret method. The sum of amino acids appeared to be the most accurate method of determination. Based on the sum of anhydroamino acids, a new multiplication factor of 5.0 for the conversion of nitrogen to protein has been proposed. The predominant protein-bound amino acids were Ala, Glu, Asp and Leu, followed by Val, Lys and Arg. Ala was the main amino acid in *P. columbina*, but Glu was the main amino acid in *P. subtumens*. Similarly, Ala and Glu were the main free amino acids in *P. columbina* and *P. subtumens*, respectively. The protein-bound amino acids and the major free amino acids showed specific seasonality.

Key Index Words: amino acids—biuret—nitrogen—nori—Porphyra columbina—Porphyra subtumens—*protein—Rhodophyceae—seasonal.*

Mariculture of the red algae *Porphyra* and its processing into thin, purple-black sheets, called "hoshi nori", is a prominent food industry in Japan. *P. yezoensis* Ueda is now the main species used although *P. tenera* Kjellman was important in the past (Miura 1975; Nisizawa *et al.* 1987).

Porphyra is used as food in other parts of the world also. It is farmed in China, where it is known as "zicai" (Tseng 1981), and in Korea, where it is known as "kim" (Mumford and Miura 1984). It is consumed in smaller quantities in Wales and New Zealand, where it is known as "laver" and "karengo", respectively (Chapman and Chapman 1980). The most common species of *Porphyra* found in New Zealand are *P. columbina* Mont. and *P. subtumens* J. Ag. (Chapman 1969). *P. columbina*, a traditional food of the Maori, grows on rocky substrate in the intertidal zone on most of the coastline around New Zealand (Nelson 1984). Mariculture of *P. columbina* is being investigated (Brown *et al.* 1990). *P. subtumens* is an endemic epiphyte on *Durvillaea antarctica* (Chamisso) Hariot and *D. willana* Lindauer (Chapman 1969). Recent studies on New Zealand *Porphyra columbina* and *P. subtumens* include the determination of their ascorbic acid contents (Friedlander *et al.* 1989). The cell culture (Xue-Wu and Gordon 1987) and resistance to desiccation (Brown 1987) of *P. columbina* have also been investigated.

The protein content determined by different investigators for the same *Porphyra* species show considerable variation. Protein in *P. tenera* is reported to range from 14.9%, dry weight, (Mukai *et al.* 1981) to 56.1% (Noda and Horiguchi 1975). The only available

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literature on the protein content of *P. columbina* Mont. was 5.5%, dry weight, as reported by Quilhot (1970). Ji *et al.* (1981) studied the seasonal variation of amino acids in *P. yezoensis* in three locations differing in dissolved nitrogen content (30 μg to 300 μg NH_4-N/*l* seawater). The total amino acids ranged from approximately 10 to 30%, air dry weight. Lower protein contents occurred in regions with lower seawater nitrogen.

Several studies have been carried out on seasonal tissue nitrogen variation. Brown *et al.* (1990) found higher values in *P. columbina* Mont. (New Zealand) during the peak growth period (winter). The same trend was observed by Ji *et al.* (1981) in *P. yezoensis*, and by Takagi (1951) in several *Porphyra* spp.

Various methods have been used to study protein in *Porphyra*. Mukai *et al.* (1981) compared protein in the cell walls of *P. tenera* obtained by multiplying the nitrogen content by 6.25, summation of amino acids and a biuret-Folin method. They found the protein content calculated from nitrogen gave the same value as the sum of anhydroamino acids. This suggests that all nitrogen was present as amino acids, and the protein molecules contained 16% nitrogen. However, protein determined by the Lowry-biuret method gave considerably higher results than the other two methods. The nitrogen conversion factor 6.25 indicates 16% protein nitrogen, and assumes no non-protein nitrogen is present. This factor is applicable to egg, meat and legumes. The factor for refined flour is 5.70 and for milk and milk products is 6.38. The 6.25 conversion factor is often employed when the protein nitrogen content is not known. Protein calculated by this method should be referred to as "crude protein" (FAO/WHO 1973; FAO/WHO/UNU 1985). The nitrogen content of proteins can vary from 12 to 30% (Lillevik 1970). Arasaki and Mino (1973) found the nitrogen content of alkali soluble seaweed proteins, which they reported to be the major type of protein in *Porphyra*, ranged from approximately 12 to 14%. Determination of the protein nitrogen content usually involves extraction of the protein, and assumes the extraction is complete. However, Coulson (1955) and Smith and Young (1953) found polysaccharides can interfere with the extraction of protein from Phaeophyta. Three or four types of soluble proteins have been found in seaweeds (Takagi 1950; Arasaki and Mino 1973; Amano and Noda 1990). Seaweeds often contain non-protein nitrogenous compounds. These may include ammonia compounds, free amino acids, peptides, nitrates and pigments (Rosell and Srivastava 1985), and seaweeds produce non-protein amino acids (Impellizzeri *et al.* 1975; Fattorusso and Piattelli 1980).

Amino acids in algae may occur in combined (protein-bound) or free form (Young and Smith 1958). Ala, Glu and Asp have been found to be the predominant amino acids in *Porphyra* (Munda and Gubensek 1976; Ji *et al.* 1981; Amano and Noda 1990), while Harada *et al.* (1990) found Ala, Glu and Tau were the predominant free amino acids, with Asp also present. Tau has been found to be extensively distributed in the seaweeds of Rhodophyta (Impellizzeri *et al.* 1975; Fattorusso and Piattelli 1980). The biuret method, a commonly used colorimetric method, does not measure free amino acids or dipeptides. It is based on the solubility of protein in alkali, which varies for different proteins. Arasaki and Mino (1973) reported that *P. tenera* contained 65% alkali soluble protein.

The present study investigates the seasonal protein variation in *P. columbina* and *P. subtumens* measured by three methods, and looks at the possibility of using a conversion factor other than 6.25 for the nitrogen value. The study looks briefly at the quality of the protein present, and at the free amino acids, some of which have been reported to contribute to the flavour of nori.

Materials and Methods

Porphyra columbina was collected from rocks in the littoral zone of the rocky shore at St. Clair, Dunedin. *Porphyra subtumens*, an epiphyte on *Durvillaea antarctica* and *D. willana*,

was collected from the sublittoral zone at Brighton, 14 km south west of Dunedin. Collections of *P. subtumens* were restricted to periods of low tide (0.1 to 0.2 m below mean tide level). Whole plants were washed with seawater at the time of collection, to remove sand and epiphytes. Samples were pooled and drying at 30°C in an air circulating oven commenced within three hours of collection. Dried samples were ground in a Wiley Mill, to pass through mesh size 0.5 mm. The samples were then stored at room temperature (approximately 20°C) in air tight containers until analyzed. Each sample analyzed contained portions from at least 50 plants.

Total nitrogen was determined by the Dumas method, using a Coleman Model 29 Nitrogen Analyzer, and had an error of ±0.3%, dry weight. The measured nitrogen content was multiplied by 6.25 to obtain the "crude protein content". The biuret method (Goa 1953) as modified by Bergersen (1980) was used to determine the total protein content of the samples. Dialyzed bovine serum albumin (2.5 mg/ml distilled water) was used as a standard solution. Preliminary studies showed a green pigment, formed or unmasked by the addition of NaOH to the samples, caused interference. Consequently, determinations were carried out by simultaneously subtracting the pigment absorbance of samples that did not have Benedicts reagent added. Analyses were done in triplicate.

Amino acid analysis was used to determine the amino acids: aspartic acid, threonine, serine, glutamic acid, proline, glycine, alanine, valine, isoleucine, leucine, tyrosine, phenylalanine, histidine, lysine, arginine,

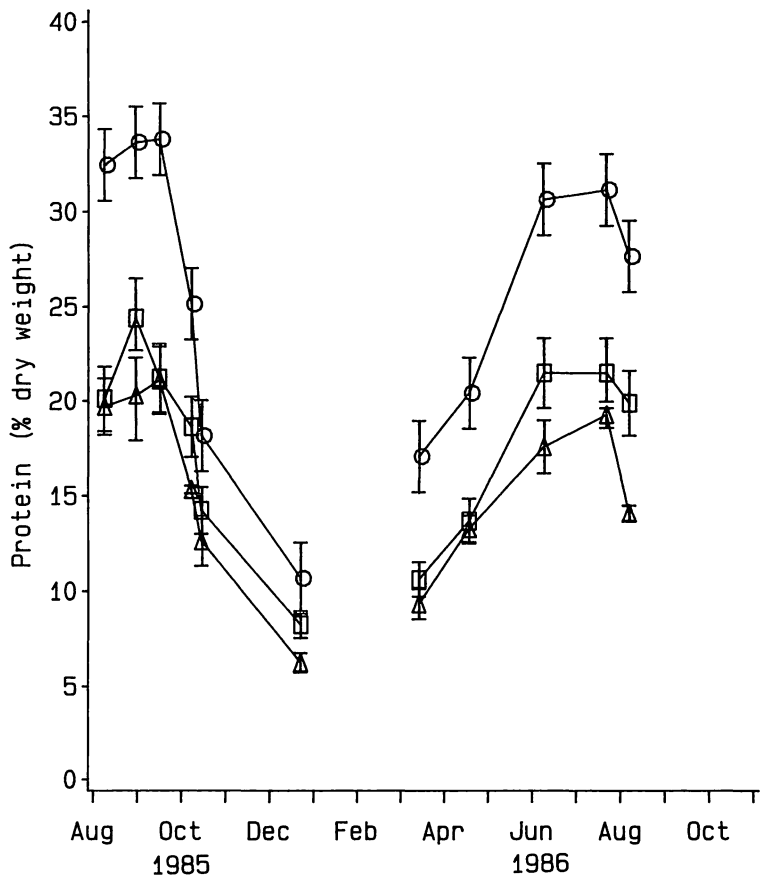

Fig. 1a. Seasonal variation in the protein content of *Porphyra columbina* (○, crude protein using conversion factor of 6.25; □, sum of anhydroamino acids; △, biuret). Error bars indicate ±1 standard deviation.

and cysteine as cysteic acid. Three samples were analyzed for taurine and methionine.

Samples of air dried seaweed were oxidized with performic acid to stabilize cysteine as cysteic acid (Hirs 1967). The samples were then hydrolyzed in 6M HCl *in vacuo* for 24 hours at 110°C (Moore *et al.* 1958). The resulting amino acids were measured using a Waters Millipore HPLC Amino Acid Analyzer, with a sulphonated polystyrene column, using a halide buffer system. Post column fluorescence with the addition of hypochlorite was used for the detection of proline. The three samples analyzed for taurine and methionine were not oxidized with performic acid. In this study "total amino acids" refers to the sum of amino acids obtained from acid hydrolysis, and, therefore, consists of protein-bound and free amino acids. The "total amino acid" summations were based on anhydroamino acid molecular weights. Single analyses were carried out. The coefficient of variation (cv^2) was measured for a duplicate sample (*P. columbina*, 9 August 1985). The cv^2 of the sum of the individual amino acids was 8.6%, and this was used as an approximation of the error.

Free amino acids were extracted from the ground, air dried samples (10 mg) in Eppendorf tubes, using 0.5 ml 0.2 M Na citrate/HCl (pH 3.0). Samples of *P. columbina* were sonicated for 30 minutes, whereas *P. subtumens* required 60 minutes. The samples were left standing at ambient temperature for two hours before centrifuging. The amino acids were measured using the Amino Acid Analyzer, as for the acid hydrolysis. Summations were based on the molecular weight of the "free" amino acid form. Single analyses were carried out. The coefficient of variation (cv^2) was measured for a duplicate sample (*P. columbina*, 9 August 1985). The cv^2 of the

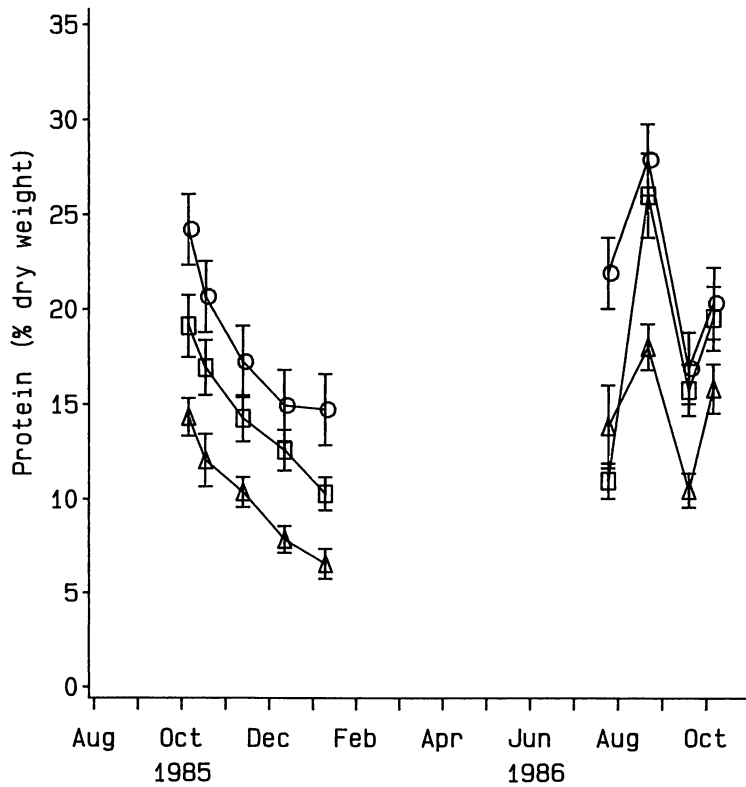

Fig. 1b. Seasonal variation in the protein content of *Porphyra subtumens* (○, crude protein using conversion factor of 6.25; □, sum of anhydroamino acids; △, biuret). Error bars indicate ±1 standard deviation.

sum of the invididual amino acids was 5.0%, and this was used as an approximation of the error.

The water content of the air dried (at 30°C) samples, was determined by drying duplicate portions under vacuum at 110°C until constant weight was achieved ("oven dried"). "Dry weight" in this study refers to "oven dry weight".

Results

Seasonal variation in protein content was observed in *P. columbina* and *P. subtumens* by three methods (Fig. 1a, b). The highest levels occurred in the winter months in both species. Using the same method, the protein contents of the two species were comparable for samples collected at similar times. The protein levels in *P. columbina* samples analyzed ranged from 10.6 to 33.8%, dry weight, when measured by nitrogen multiplied by 6.25; 8.2 to 24.5% by sum of anhydroamino acids (not including Met, Trp, Tau); 6.2 to 21.1% by the biuret method. For *P. subtumens* the ranges were 14.8 to 28.2%, dry weight; 10.2 to 26.0% and 6.5 to 18.0%, respectively. The results of the three methods were correlated (Pearson's correlation, $P<0.05$). However, the average protein content varied significantly between the three methods (Duncan's multiple range test, $P<0.01$). For both seaweeds multiplying nitrogen by 6.25 gave the highest average value (*P. columbina*: 25.5%±7.9%, dry weight and *P. subtumens*: 20.0%±4.5%), followed by sum of anhydroamino acids (17.6%±5.2% and 16.1%±5.0%, respectively (not including Met, Trp and Tau)) and the biuret method (15.3%±4.8% and 12.1%±3.7%, respectively).

Amino acid analysis showed Ala followed by Glu, Asp and Leu were the major amino acids in *P. columbina* (Table 1). In *P. subtu-*

Table 1. Seasonal variation in the amino acid composition of *P. columbina*, St Clair (g/100 g dry weight).

Amino acids	Collection date										
	9 Aug[1]	31 Aug	16 Sep	8 Oct	15 Oct	23 Dec	14 Mar	18 Apr	9 Jun	22 Jul	7 Aug
Ala	3.49	4.82	3.30	2.87	1.65	1.40	1.81	2.08	4.18	4.58	3.50
Arg	1.64	1.47	1.56	1.57	1.16	0.22	0.67	0.98	1.26	1.36	1.27
Asp	2.01	2.54	2.73	1.92	1.68	0.89	1.22	1.38	2.37	2.20	2.38
$CysO_3H$	0.82	0.56	0.77	0.73	0.56	0.29	0.32	0.44	0.50	0.57	0.77
Glu	2.63	2.90	2.77	2.28	1.69	0.76	1.16	1.64	2.73	2.52	2.07
Gly	1.30	1.45	1.31	1.25	1.08	0.70	0.93	0.92	1.40	0.74	0.84
His	0.26	0.36	0.90	0.90	0.77	0.12	0.12	0.13	0.54	0.16	0.73
Ile	0.66	0.70	0.62	0.60	0.49	0.27	0.34	0.51	0.73	0.66	0.65
Leu	1.29	2.63	1.27	0.95	0.51	0.62	0.80	1.03	2.31	2.41	1.32
Lys	1.26	1.22	1.17	1.15	0.97	0.56	0.81	0.88	1.24	1.16	1.25
Met	nd[2]	0.33[3]	nd	nd	nd	nd	nd	nd	nd	0.27[3]	nd
Phe	0.50	0.60	0.48	0.47	0.35	0.19	0.21	0.30	0.50	0.46	0.36
Pro	0.83	0.78	0.88	0.72	0.62	0.28	0.38	0.82	0.78	0.84	0.81
Ser	1.23	1.28	1.23	1.12	0.91	0.49	0.55	0.83	0.97	1.05	1.28
Tau	nd	0.31[3]	nd	nd	nd	nd	nd	nd	nd	0.25[3]	nd
Thr	1.13	1.33	1.15	1.04	0.89	0.53	0.57	0.90	1.04	1.11	1.23
Tyr	0	0.24[3]	0	0	0	0	0	0	0	0.10[3]	0
Val	1.10	1.76	1.10	1.02	0.85	0.85	0.71	0.91	1.05	1.67	1.43
Total	20.13	25.27	21.21	18.59	14.19	8.17	10.59	13.74	21.57	22.11	19.87

[1] Mean of duplicate determinations.
[2] Not determined.
[3] No performic acid treatment.

Table 2. Seasonal variation in the amino acid composition of *P. subtumens*, Brighton (g/100 g dry weight).

Amino acids	Collection date								
	6 Oct	18 Oct	13 Nov	12 Dec	10 Jan	25 Jul	22 Aug	19 Sep	6 Oct
Ala	1.55	1.20	1.08	0.90	0.76	1.00	2.59	1.14	1.35
Arg	1.33	1.51	1.33	1.10	0.76	0.78	1.75	0.74	1.48
Asp	2.62	2.00	1.80	1.97	0.86	1.46	2.81	1.84	2.68
$CysO_3H$	0.64	0.53	0.48	0.51	0.57	0.40	0.71	0.48	0.58
Glu	2.93	2.53	2.01	1.69	1.28	1.57	3.41	2.26	2.75
Gly	1.17	0.89	0.76	0.70	0.67	0.71	1.24	0.82	0.99
His	0.06	0.39	0.23	0.06	0.10	0.02	0.88	0.14	1.06
Ile	0.85	0.73	0.64	0.56	0.54	0.41	1.05	0.68	0.83
Leu	1.52	1.30	1.19	0.99	0.93	0.70	3.00	2.57	1.52
Lys	1.25	1.07	0.98	0.76	0.64	0.93	1.65	0.98	1.23
Met	nd[1]	0.29[2]	nd	nd	nd	nd	nd	nd	nd
Phe	0.54	0.65	0.44	0.22	0.16	0.06	1.61	0.30	0.65
Pro	0.95	0.85	0.64	0.61	0.93	0.64	1.01	0.71	0.86
Ser	1.36	1.06	0.97	0.87	0.63	0.80	1.55	1.00	1.21
Tau	nd	0.32[2]	nd	nd	nd	nd	nd	nd	nd
Thr	1.19	0.91	0.76	0.74	0.62	0.70	1.23	0.82	1.08
Tyr	tr[3]	0.14	tr	tr	tr	tr	0.26	0.22	0.10
Val	1.22	1.12	0.93	0.82	0.80	0.72	1.25	1.00	1.09
Total	19.15	17.48	14.23	12.49	10.24	10.92	26.00	15.69	19.46

[1] Not determined.
[2] No performic acid treatment.
[3] Trace.

mens the same four major amino acids were found (Table 2), but Glu, and not Ala, was the predominant amino acid. Other prominent amino acids found in both seaweeds included Val, Lys and Arg. The levels of amino acids were similar in both species, except for Ala, which was notably higher in *P. columbina*. Tyr was detected only in *P. columbina* when the samples had not been oxidized with performic acid. Therefore, it would appear that Tyr was destroyed in *P. columbina* that had been treated with performic acid prior to the acid hydrolysis. The seasonal fluctuation of most of the individual amino acids was correlated to protein (biuret method), nitrogen and the total amino acids (Pearson's correlation, $P<0.05$).

The total level of free amino acids ranged from 0.5 to 4.1% in *P. columbina* and from 1.0 to 2.3% in *P. subtumens* (not including Met, Trp and Tau) (Tables 3 and 4). For *P. columbina*, the total free amino acids were correlated seasonally to the total amino acids, obtained by acid hydrolysis, and to the nitrogen content (Pearson's correlation, $P<0.05$), but correlation was weak for *P. subtumens*.

Discussion

Seasonal changes in the protein content of *P. columbina* and *P. subtumens* were found (Fig. 1) with the highest values observed in the winter months. Our results are in agreement with the seasonal fluctuation in nitrogen previously reported (Brown *et al.* 1990) for *P. columbina*, in *P. yezoensis* sampled on four occasions (Ji *et al.* 1981) and other *Porphyra* spp. (Takagi 1951). The higher values in winter correspond to the period of maximum growth.

Eighteen amino acids were found in *P. columbina* and *P. subtumens* (Tables 1 and 2) with Ala, Glu, Asp and Leu, the major amino acids. Some loss of Thr and Ser may have occurred because these amino acids can be par-

Table 3. Seasonal variation in the free amino acid composition of *P. columbina*, St Clair (g/100 g dry weight).

Amino acids	Collection date								
	9 Aug[1]	16 Sep	8 Oct	15 Oct*	23 Dec	18 Apr	9 Jun	22 Jul	7 Aug
Ala	1.80	1.51	1.26	0.57	0.17	1.26	2.23	2.22	2.28
Arg	tr[2]	tr	tr	—	tr	tr	tr	tr	tr
Asp	0.30	0.15	0.12	—	0.07	0.19	0.21	0.16	0.16
$CysO_3H$	0.07	0.06	0.05	—	0.02	0.04	0.04	0.05	0.03
Glu	0.68	0.54	0.55	0.30	0.15	0.29	0.68	0.92	0.77
Gly	0.04	0.03	0.04	0.03	0.01	0.06	0.02	0.03	0.03
His	0.02	0.01	0.02	—	0.02	0.02	0.02	0.02	0.02
Ile	0.02	0.02	0.01	—	tr	tr	0.01	0.01	0.01
Leu	0.03	0.03	0.03	—	0.01	tr	0.02	0.03	0.02
Lys	0.04	0.04	0.03	—	0.02	0.03	0.03	0.05	0.03
Met	nd[3]	nd	nd	nd	nd	nd	nd	nd	nd
Phe	0	0	0	—	0	0	0	0	0
Pro	0.31	0.44	0.27	0.21	tr	0.21	0.46	0.42	0.47
Ser	0.17	0.20	0.14	—	0.02	0.10	0.20	0.17	0.11
Tau	nd	nd	nd	nd	nd	nd	nd	0.27	nd
Thr	0.03	0.04	0.03	—	0.02	0.05	0.03	0.03	0.03
Tyr	0	0	0	—	0	0	0	0	0
Val	0.02	0.03	0.03	—	0.01	0.02	0.02	0.02	0.03
Total	3.52	3.08	2.58	—	0.53	2.26	3.97	4.38	3.99

[1] Mean of duplicate determinations.
[2] Trace.
[3] Not determined.
* Insufficient sample.
— denotes inaccurate peak integration.

tially destroyed by acid hydrolysis (Heimann 1980). The amino acid analyses generally agree with what has been reported for other *Porphyra* spp. (Munda and Gubensek 1976; Ji *et al.* 1981; Amano and Noda 1990). The seasonal changes in the individual amino acids present in highest concentrations are similar to those we found for protein by three different methods and are comparable to those observed (Ji *et al.* 1981) for *P. yezoensis*.

The free amino acids Ala, Glu and Gly (Nisizawa *et al.* 1987; Noda *et al.* 1975) and Tau (Noda and Iwata 1978) are thought to contribute to the desirable flavour of nori. *P. columbina* attained higher levels of Glu, Ala and total free amino acids than *P. subtumens* (Tables 3 and 4). Tau was present in reasonable quantities in both species (0.21 g/100 g, dry weight, in *P. subtumens* and 0.27 g/100 g in *P. columbina*). Harada (1988) recorded 0.48 g/100 g, dry weight, for Tau in *P. columbina*. Noda *et al.* (1975) reported levels of 1.21 to 1.62 g/100 g, dry weight, in *P. tenera*. Harada *et al.* (1990) found Tau varied seasonally (December to April, Japan) in *Porphyra* spp. from approximately 0.80 to 1.50 g/100 g, dry weight. Gly was not a prominent free amino acid in either species in the present study.

It is not surprising that the three protein methods we used gave different results, as they each measure a somewhat different group of compounds. The higher crude protein values could be due to the fact that non-protein nitrogen is present and the conversion factor of 6.25 is not appropriate. The biuret method generally gave the lowest values. In theory the biuret method would under-estimate the protein content, because it does not include free amino acids or dipeptides and relies on protein solubility. The most accurate estimation of protein would be obtained with

Table 4. Seasonal variation in the free amino acid composition of *P. subtumens*, Brighton (g/100 g dry weight).

Amino acids	Collection date							
	18 Oct*	13 Nov	12 Dec	10 Jan*	25 Jul	22 Aug	19 Sep	6 Oct
Ala	—	0.14	0.11	0.04	0.59	0.18	0.13	0.23
Arg	—	tr[2]	tr	tr	tr	tr	tr	tr
Asp	—	0.06	0.09	0.03	0.29	0.07	0.16	0.18
$CysO_3H$	—	0.13	0.06	—	0.09	0.12	0.02	0.03
Glu	0.51	0.41	0.40	0.09	0.78	0.60	0.56	0.55
Gly	0.02	0.01	0.01	0.01	0.04	0.02	0.01	0.01
His	—	0.04	0.02	—	0.01	0.01	0.02	0.02
Ile	—	0.02	0.02	0.01	0.03	0.04	0.02	0.02
Leu	—	0.03	0.04	0.02	0.03	0.07	0.04	0.04
Lys	—	0.04	0.04	0.03	0.05	0.07	0.06	0.06
Met	nd[1]	nd	nd	nd	nd	nd	nd	nd
Phe	—	tr	tr	tr	tr	tr	tr	tr
Pro	—	tr	tr	tr	tr	0.10	tr	tr
Ser	0.04	0.06	0.06	0.02	0.14	0.14	0.08	0.12
Tau	0.21	nd	nd	nd	nd	nd	nd	nd
Thr	—	0.03	0.02	tr	0.07	0.03	0.02	0.02
Tyr	0.08	0.08	0.05	tr	0.14	0.21	0.26	0.11
Val	0.03	0.03	0.09	0.01	0.03	0.06	0.09	0.08
Total	—	1.09	1.01	—	2.30	1.71	1.47	1.48

[1] Not determined.
[2] Trace.
* Insufficient sample.
— denotes inaccurate peak integration.

the knowledge of the molecular weights of the "in-chain" sequence of the amino acids. However, this is impracticable and the sum of anhydroamino acids was chosen for this study. Thus the total level of amino acids will be under-estimated. Free amino acids, that were inclusive in the hydrolysis, were summed as anhydroamino acids. This represents another source of under-estimation of total amino acids. Other investigators generally did not state whether amino acids were summed in anhydroamino or free form. Exceptions are Mukai *et al.* (1981) who used anhydroamino acids and Rosell and Srivastava (1985) who used the free form. In the present study it appeared that the sum of amino acids gave the most accurate protein measurement. However, the method did not include Trp. Literature values for Trp range from 0.11 g/100 g, dry weight, in *P. columbina* (Quilhot 1970) to 0.78 g/100 g in *P. tenera* (Arasaki and Mino 1973). Protein quality based on the amino acid composition, "amino acid score" or "chemical score", (using FAO/WHO/UNU (1985) suggested requirements for adults) was adequate at maximal winter levels in *P. columbina*. When egg was used as a reference protein, as recommended by Passmore and Eastwood (1986), Phe and Tyr were limiting throughout the year in both *Porphyra* species. The egg composition of Paul and Southgate (1978) was used. The amino acid score ranged from 12 to 19, air dried weight, in *P. columbina* and from 3 to 65 in *P. subtumens*. Air dried weight was used in order to approximate nori, which has a similar water content (9 to 12%). The consumption of nori in Japan has been reported to average 72 sheets per capita, per year (Freeman 1985). Consumption would be higher for some individuals than others, but because each sheet weighs only about 3 grams (Miura

1975), nori would not appear to be a major source of protein.

The conversion factors for nitrogen to protein obtained by summation of amino acids, ranged from 3.08 to 5.96 for *P. columbina* and *P. subtumens* (not including Tau, Met and Trp). When the measurements for Tau and Met were included (Tables 1 and 2), as well as an average literature data of 0.43 ± 0.34 g/100 g, dry weight, for Trp (data from Quilhot 1970; Rao and Polacchi 1972; Arasaki and Mino 1973), a conversion factor of 5.0 was obtained. This estimated conversion factor was lower than the factor 6.25, frequently used by researchers studying seaweed, but compares favourably with conversion factors that we have calculated from literature data, using nitrogen in *Porphyra* spp. and corresponding sum of amino acids. These ranged from 3.47 for *P. tenera* (Arasaki and Mino 1973) to 6.17 (Mukai *et al.* 1981) for *P. tenera*, with an average of 4.98. These conversion factors do not contain Tau, and often do not contain Met or Trp.

Rosell and Srivastava (1985) found that multiplying the nitrogen content of the seaweed residue by 6.25, after alcohol extraction (80% ethanol), gave results comparable to those obtained by summation of amino acids. Ito and Hori (1989) noted that 70% ethanol extraction removes soluble nitrogen compounds, including free amino acids, amines and pigments. These usually account for 10 to 20% of the total nitrogen compounds. Leung *et al.* (1972) reported that several seaweeds contained approximately 20% non-protein nitrogen. This indicates 80% of the nitrogen in seaweed is protein nitrogen. Therefore, once again, a factor of 5.0 multiplied by total nitrogen would appear to give a satisfactory estimate of protein content. We conclude, the best method for determining protein levels would be the summation of amino acids, followed by using the conversion factor of 5.0 for the nitrogen value.

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K. A. Aitken・L. D. Melton・M. T. Brown*：ニュージーランド海藻 *Porphyra columbina* Mont. と *Porphyra subtumens* J. Ag.（紅藻植物）におけるタンパク質量の季節変化

紅藻の *Porphyra columbina* Mont. と *Porphyra subtumens* J. Ag. の組織中のタンパク質量の季節変化を測定した。分析方法としては，全窒素量の6.25倍法，Biuret 法，アンヒドロアミノ酸量の合計法の 3 種の方法を用いた。両種 *Porphyra* のタンパク質量は似たような季節変化を示し，冬期に最大となった。タンパク質量は測定法により異り，常法に従って全窒素量を6.25倍して得た値が最も高い値となり，次いで総アンヒドロアミノ酸法による値がつずき，Biuret 法では最小の値となった。このうち，二番目の方法が *Porphyra* タンパク質の定量法としては最も正確なものと思われた。この方法によって得られた結果に基づくと，全窒素量を5.0倍することにより，より正確なタンパク質量が求められることが提唱できる。2 種の *Porphyra* の主要アミノ酸は Ala, Glu, Asp, Leu で，次いで Val, Lys, Arg であった。そのうち Ala は特に *P. columbina* の主要なものであるのに対して，*P. subtumens* では Glu が主であった。これと同じことが両種 *Porphyra* の遊離アミノ酸においてもみられた。またタンパク質構成アミノ酸と主な遊離アミノ酸の季節変化は，それぞれ特異的な様相を示した。(Food Science Department, University of Otago, P.O. Box 56, Ounedin, New Zealand *Botany Department, University of Otago, P.O. Box 56, Dunedin, New Zealand)

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Critical review of the taxonomy and life history of *Kjellmania arasakii* (Dictyosiphonales, Phaeophyceae)

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Kawai, H. 1991. Critical review of the taxonomy and life history of *Kjellmania arasakii* (Dictyosiphonales, Phaeophyceae). Jpn. J. Phycol. 39: 319–328.

A taxonomic review of *Kjellmania arasakii* based on the materials newly collected at Yashiro Island, Seto Inland Sea, Japan revealed that the species is identical with *Stictyosiphon soriferus*, which is distributed widely in North Atlantic Ocean. At Yashiro Island, *Stictyosiphon soriferus* grows on subtidal artificial substrata at a depth of 3–4 m. The thallus is 3–4 times irregularly branched, delicate, attaining about 20 cm in height and forms abundant plurilocular sporangia. In culture the swarmers from plurilocular sporangia developed into protonemata on which erect thalli directly arose. The polystichous, branched erect thalli grew well and formed plurilocular sporangia between 5–20°C, although best between 10 and 15°C. Unilocular sporangia were formed only in 5°C short day conditions.

Key Index Words: *Dictyosiphonales*—Kjellmania arasakii—*Life history*—*Phaeophyceae*—Stictyosiphon soriferus—*Taxonomy*.

Yamada (1953) described *Kjellmania arasakii* based on some drift materials collected at Fukagawa in Tokyo Bay by S. Arasaki during 1951 and 1952. However, this report was preliminary, lacking Latin descriptions and detailed descriptions of the species. Yamada mentioned that he intended to publish a second report including a detailed description, but it has not been published. Arasaki and Nozawa (1953) cultured the same material as Yamada studied and reported the occurrence of erect filamentous gametophytes from the swarmers of the field plant; however, they could not complete the life history. They reported a *Laminaria*-type of germination of the swarmers in which most cellular contents migrated into the germination tube forming a emptied embryospore. However, such a germination pattern is rather uncommon in the Dictyosiphonales in which the genus is placed, and does not agree with previous reports on the life history of *Kjellmania* and its closely related genus *Stictyosiphon* (Sauvageau 1929, Rosenvinge 1935, South and Hooper 1976).

The genus *Kjellmania* was established by Reinke (1889) based on *Kjellmania sorifera* Reinke collected at Kiel, Baltic Sea. However, the distinction between *Kjellmania* and *Stictyosiphon* has been disputed, and *Kjellmania* is generally now recognized as a taxonomic synonym of *Stictyosiphon* (Rosenvinge 1935, Rosenvinge and Lund 1947, Naylor 1958, South and Hooper 1976). On the other hand, the characters used to distinguish *Kjellmania arasakii* from *Kjellmania sorifera* (=*Stictyosiphon soriferus*), such as the manner of branching, and presence or absence of intercalary plurilocular sporangia, seem to be rather variable and need revision. In addition, the life history of *K. arasakii* is not yet fully clear. Accordingly, this paper aims to reexamine the morphology and life history of *K. arasakii* using the type specimen and newly collected materials referable to the species, and to compare it with specimens of Atlantic *Stictyosiphon soriferus*, including authentic materials, to clarify its systematic status.

Materials and Methods

Collections of specimens were made at

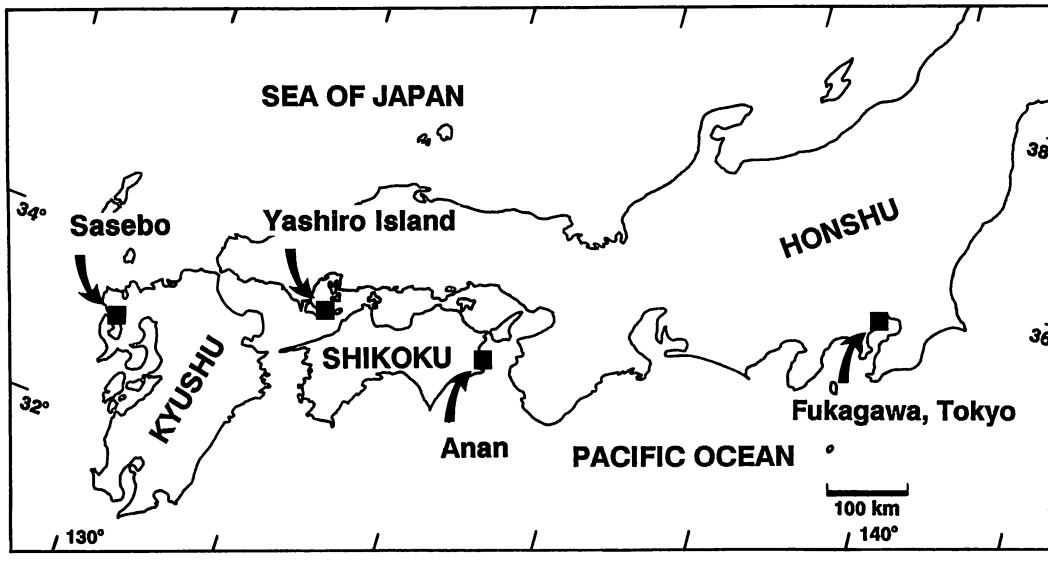


Fig. 1. Distribution of *Stictyosiphon soriferus* (Reinke) Rosenvinge in Japan. Squares show the localities of collections.

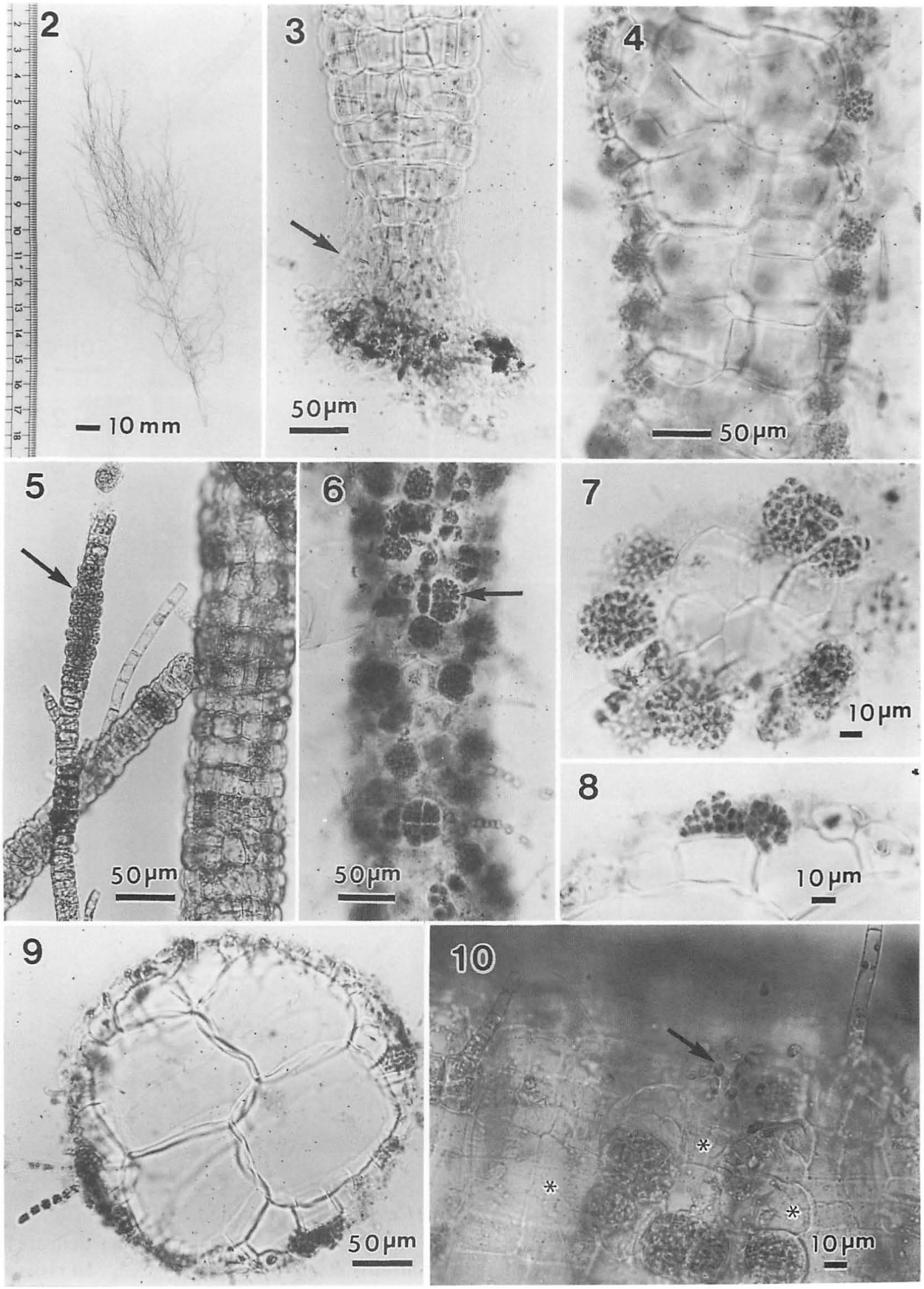
Yashiro Island, Yamaguchi Prefecture, Seto Inland Sea (Fig. 1, 33°55′N, 132°20′E) on 15 May 1986 using SCUBA. The type specimen of *Kjellmania arasakii* (SAP 027286, Fukagawa, Tokyo Bay, Fig. 1, leg. S. Arasaki, 4 February 1951) and permanent preparations made from the type material by Y. Yamada, as well as some additional herbarium specimens collected by Arasaki (2 January 1951, 26 January, 2, 23, 25, 26 February 1952) were examined. Additional materials as follow were also examined: Japanese herbarium specimens referable to *Kjellmania arasakii* collected at Sasebo, Nagasaki Pref. (Fig. 1) (leg. S. Migita, April 1955) and Anan, Tokushima Pref. (Fig. 1) (−5 m deep, leg. S. Arai, 14 February 1984); herbarium sheet of *Stictyosiphon soriferus* (as *Kjellmania sorifera* Reinke, January and June 1888, Kiel, collected and identified by Reinke placed in B. M.); permanent preparation of *Stictyosiphon soriferus* (leg. P. Kornmann and P.-H. Sahling, 26 August 1960, Helgoland); fresh and liquid preserved material of *Stictyosiphon soriferus* (15 April 1989, Portsmouth, England).

Morphological observations by light microscopy were made on living materials, specimens preserved in 3-5% formaldehyde-seawater and permanent preparations made from dried herbarium specimens.

Cultures were started from swarmers released from plurilocular sporangia on the erect thalli collected on 15 May 1986 at Yashiro Island, Seto Inland Sea. For comparisons in the morphology, life history and response to day length-temperature conditions, culture strains of *Stictyosiphon soriferus* from Portsmouth, England (Kawai SSO-1), Denmark (Pedersen strain no. 42), and Bergen, Norway (Kawai SSO-3) were also cultured. Swarmers were pipetted onto glass slides and cultured in glass vessels containing 200 ml of PESI medium (Tatewaki 1966). The sets of culture conditions used were 5°C SD (short day; 8: 16hLD), 5°C LD (long day; 16: 8hLD), 10°C SD, 10°C LD, 15°C SD, 15°C LD, 20°C SD and 20°C LD, under white fluorescent lighting of approximately 30 μmolm^{-2}s^{-1} (5°C) or 50 μmolm^{-2}s^{-1} (10°C, 15°C, 20°C). A temperature gradient plate was also used to investigate high lethal temperatures under long-day conditions (16: 8hLD).

Results

Habitat and morphology of the plant at Yashiro Is-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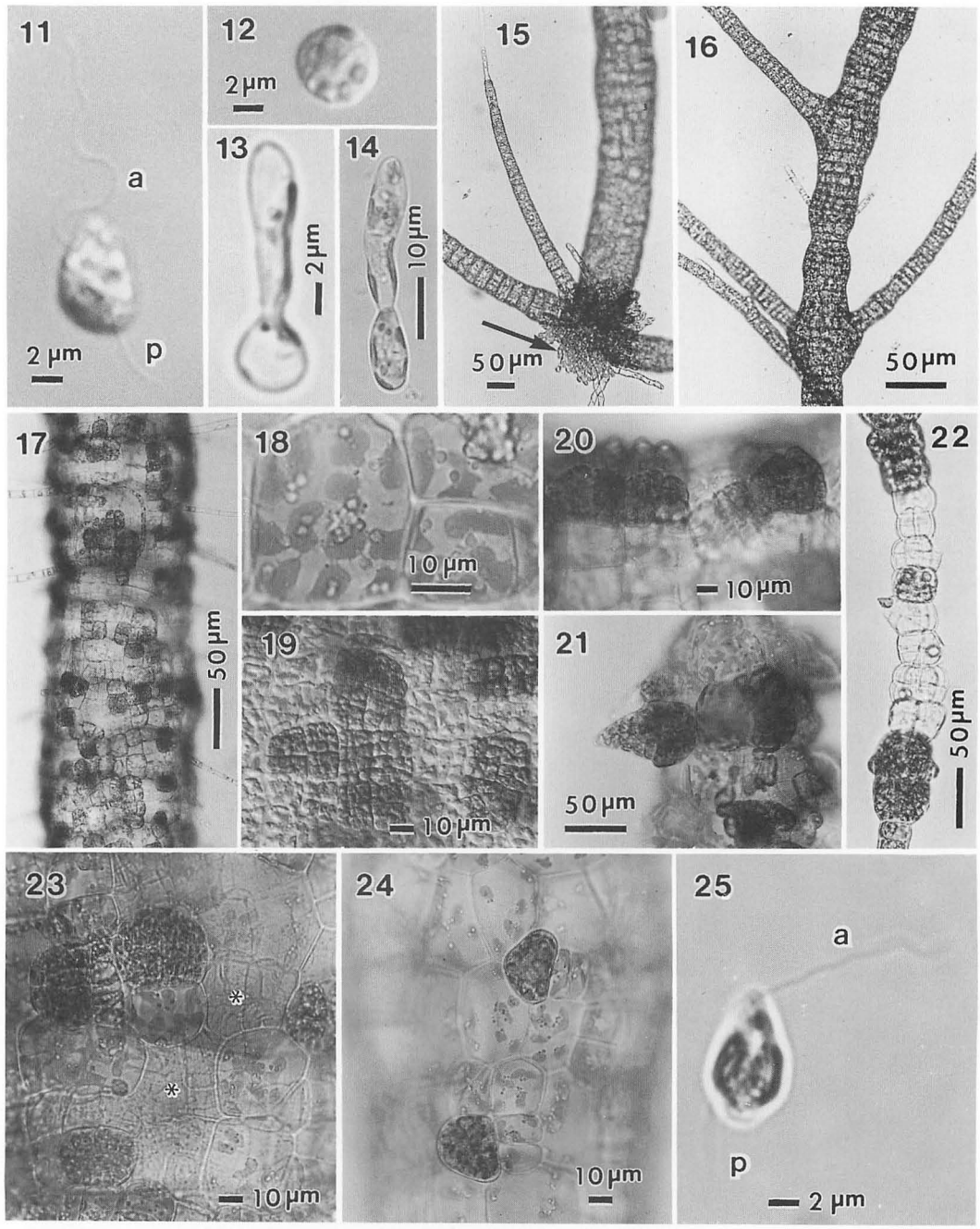


land and morphology of other Japanese materials

The plants grow densely on concrete experimental substrata for algae at a depth of 3-4 m below Mean Low Water Level, together with *Punctaria* sp. The erect thallus is 3-4 times irregularly branched, without an obvious main axis, pale yellow in color, delicate and attains 20 cm in height (Fig. 2). In cross section the thallus consists of four large colorless central cells surrounded by 1-2 layers of

small pigmented peripheral cells (Fig. 9). In longitudinal section the central cells are rounded, 80–110 × 70–100 μm in size (Fig. 4). Terminal and lateral (often opposite) phaeophycean hairs are present (Figs. 5, 6, 9, 10). Plurilocular sporangia form by repeated divisions of peripheral cells (Figs. 6–10). They are usually formed in groups of 2–4 sporangia each containing 4–8 loculi in the surface view, square to rectangular in the shape, 12–16 × 10–14 μm in length and width in surface view (Figs. 5, 10). In the terminal thinner branches of the thallus where inner cells are not differentiated, entire peripheral cells transform into plurilocular sporangia which appear as intercalary sporangia (Fig. 5). The basal part of the plant attaches to the substratum by rhizoidal filaments (Fig. 3). Unilocular sporangia were not detected.

The materials from Sasebo and Anan (Fig. 32) agreed well with the specimens from Yashiro Island in general appearance and internal structure. Both materials had plurilocular sporangia which agreed with those of Yashiro Island in morphology (Figs. 33, 34). Unilocular sporangia were not detected.

Culture experiments on the plant from Yashiro Island

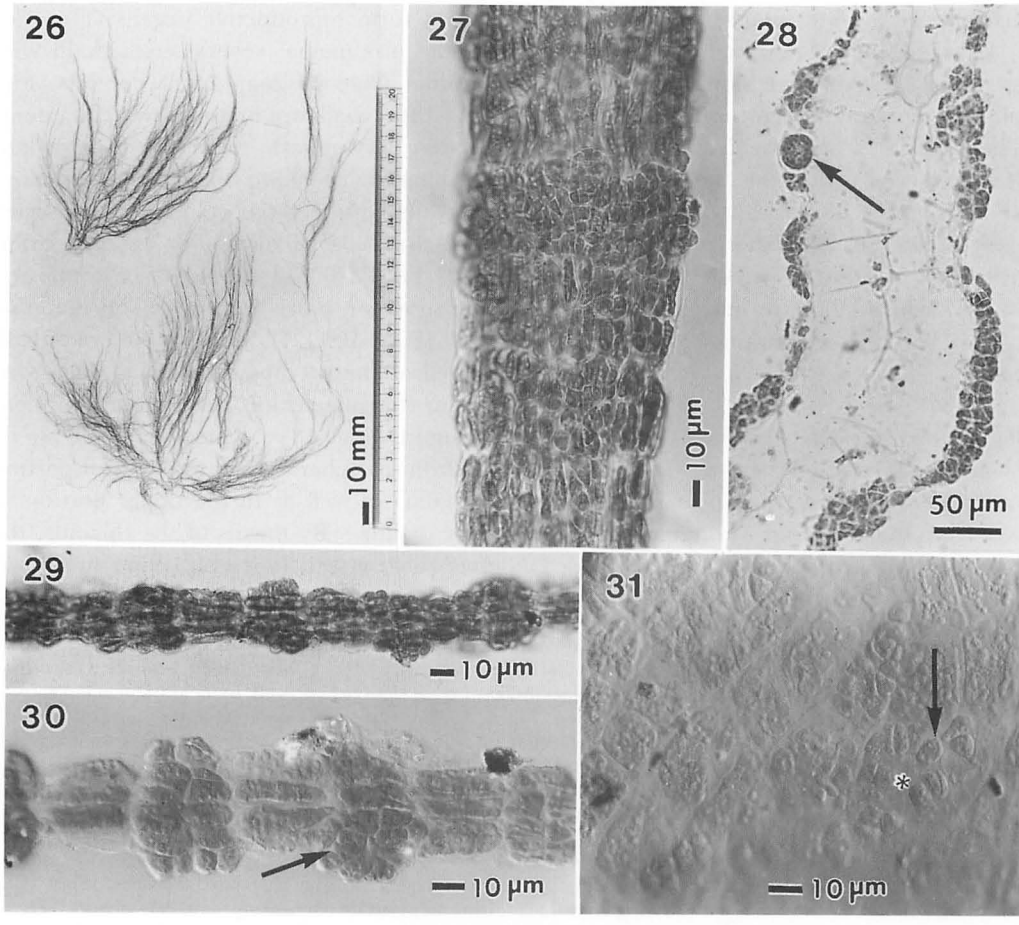
Swarmers released from plurilocular sporangia (plurispores) are pear-shaped, 6–11 × 5–7 μm in length and width, containing a chloroplast with an eyespot, and provided with two laterally inserted flagella, a longer anterior (14–16 μm in length) and a shorter posterior one (ca. 6 μm in length) (Fig. 11). Released swarmers swim for some minutes and then settle on the glass (Fig. 12). Sexual fusions among the swarmers were not observed. Abnormal swarmers in which two or more individual swarmers coalesced with each other, when released through the release pores of the sporangia, were often observed. Such abnormal swarmers settled and developed in the same way as normal swarmers. In 1–2 days they germinate forming a germ tube (Figs. 13, 14), and develop into branched prostrate filaments (protonemata) which do not form reproductive organs (Fig. 15). On the protonema, several erect thalli with terminal phaeophycean hairs develop (Fig. 15). Erect thalli are first uniseriate filaments of intercalary growth, then they undergo longitudinal divisions to become polystichous and differentiate large colorless inner cells. Erect thalli form irregular or opposite branches (Fig. 16). Lateral, often opposite, phaeophycean hairs are present on the erect thalli (Figs. 16, 17). Cells of the erect thalli as well as the protonema contain many discoid or elongated chloroplasts with prominent pyrenoids (Fig. 18). Rhizoidal filaments issue from peripheral cells in the basal portion of thallus as well as in the upper portion in older thallus. By means of the rhizoids, the erect thalli attach to the substrata firmly, or

Figs. 2–10. *Stictyosiphon soriferus* (Reinke) Rosenvinge in the field. (Figs. 3–9. Preserved materials stained with toluidine blue). Fig. 2. Habit of the field plant collected at Yashiro Island, Seto Inland Sea on 15 May 1986. Fig. 3. Basal part of the erect thallus showing rhizoidal filaments (arrow). Fig. 4. Micrograph focused on the central part of the thallus showing large rounded inner cells. Fig. 5. Parenchymatous branches and intercalary plurilocular sporangia on a terminal branch (arrow). Fig. 6. Surface view of mature erect thallus forming abundant plurilocular sporangia (arrow). Fig. 7. Cross section of upper thin part of mature erect thallus forming protruded conical plurilocular sporangia. Fig. 8. Cross section of thicker part of mature thallus forming immersed plurilocular sporangia. Fig. 9. Cross section of thicker part of thallus showing rounded inner cells. Fig. 10. Surface view of erect thallus showing plurilocular sporangia, released swarmers (arrow) and emptied sporangia (asterisks).

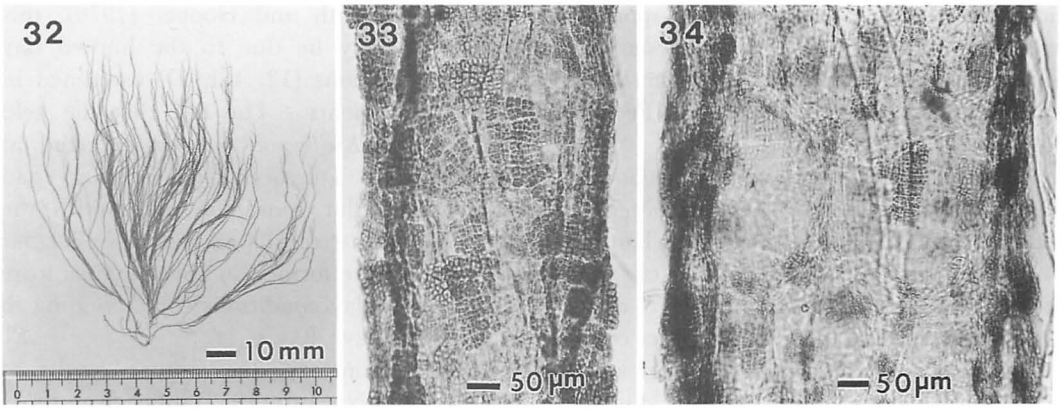
Figs. 11–25. *Stictyosiphon soriferus* (Reinke) Rosenvinge in culture. Fig. 11. Swarmer (pluri-spore) released from plurilocular sporangium. a, anterior flagellum; p, posterior flagellum. Fig. 12. Settled swarmer. Figs. 13, 14. Germination of swarmer. Fig. 15. Young erect thalli arising on prostrate filaments (protonema, arrow). Fig. 16. Opposite and unilateral branches on erect thallus. Fig. 17. Surface view of mature erect thallus. Fig. 18. Peripheral cells containing many discoid chloroplasts with prominent pyrenoids. Fig. 19. Surface view of thicker part of mature thallus showing grouped plurilocular sporangia among vegetative peripheral cells. Figs. 20, 21. Lateral views of immersed and protruded type of plurilocular sporangia. Fig. 22. Intercalary plurilocular sporangia formed on terminal thin part of the thallus. Fig. 23. Emptied plurilocular sporangia (asterisks). Fig. 24. Unilocular sporangia. Fig. 25. Zoospore (uni-spore) released from unilocular sporangium. a, anterior flagellum; p, posterior flagellum.

entangle with each other.

The erect thalli developed and formed plurilocular sporangia in all culture conditions examined, but they grew faster, and attained their maximum length of 15-20 cm in 10°C and 15°C. In 5°C short day conditions they formed unilocular sporangia, sometimes mixed with plurilocular ones. Plurilocular sporangia are formed by repeated divisions of the peripheral cells. The shapes of them are rather variable depending on their position on the thallus, age and culture conditions (Figs. 17, 19-23). Almost flat plurilocular sporangia as well as protruded ectocarpoid ones are observed. In the terminal part of the thallus where inner cells do not develop, the whole surface of the thallus transforms into plurilocular sporangia, which appear as intercalary sporangia (Fig. 22). Unilocular sporangia are conical or irregularly spherical, sessile, formed from the peripheral cells, 28-30 × 20-28 μm in length and width (Fig. 24). Zoospores (uni-spores) released from unilocular sporangia are about 7 × 5 μm in length and width provided with two, longer anterior and shorter posterior, flagella (Fig. 25). Swarmers from plurilocular sporangia as well as zoospores from unilocular sporangia germi-



Figs. 26–31. Type specimen and permanent preparations of *Kjellmania arasakii* Yamada. Fig. 26. Habit of the type material (SAP 027286). Fig. 27. Surface view of the type material forming immersed plurilocular sporangia. Fig. 28. Longitudinal section of mature erect thallus forming both unilocular (arrow) and plurilocular sporangia in the preparation of Y. Yamada. Figs. 29, 30. Terminal thinner part of the thallus forming plurilocular sporangia (arrow) in Yamada's preparation. Fig. 31. Surface view of mature erect thallus in Yamada's preparation showing individual swarmers (arrow) and emptied loculi (asterisk) of plurilocular sporangia.



Figs. 32–34. Herbarium specimens of Japanese *Stictyosiphon soriferus* (Reinke) Rosenvinge. Figs. 32, 33. Habit and surface view of the specimen forming plurilocular sporangia collected at Anan, Tokusima Pref. on 14 February 1984 by S. Arai. Fig. 34. Surface view of the specimen forming plurilocular sporangia collected at Sasebo, Nagasaki Pref. on April 1955 by S. Migita.

nated and developed in the same manner as the original swarmers. *In situ* germinations of the swarmers or zoospores in the sporangia were often observed, especially in old cultures. The high lethal temperature was 25–26°C in the Yashiro Island strain.

Morphological observations on the type specimen of Kjellmania arasakii

The erect thallus is 3–4 times irregularly branched, without an obvious main axis, and attains 15 cm in height (Fig. 26). In longitudinal section the thallus consists of large rounded colorless central cells of about 100×90 μm in longitudinal section, surrounded by 1–2 layers of small pigmented cells (Fig. 28). Plurilocular sporangia are abundant, formed by divisions of peripheral cells, $35\text{–}40 \times 25\text{–}30$ μm in surface view, somewhat protruded from the surface (Fig. 27, 31). In the terminal thinner part of the thallus each loculus of the sporangia is strongly protruded (Figs. 29, 30), however, not completely independent as shown in fig. 15 in Arasaki & Nozawa (1953). Rounded sessile unilocular sporangia immersed in the peripheral cells are also present, about 30×25 μm in length and width (Fig. 28).

Culture experiments on Atlantic Stictyosiphon soriferus

All of the three European strains of *Stictyosiphon soriferus* (SSO-1, SSO-3, Pedersen no. 42) showed direct type of life histories and formed plurilocular sporangia. In the Denmark strain a few unilocular sporangia were formed in 3°C short day conditions. The high lethal temperature in the Portsmouth strain was 24–25°C.

Discussion

The specimens from Yashiro Island agreed well with the type material of *Kjellmania arasakii*, in appearance and general anatomical features, such as size and branching pattern of erect thalli, and shape, arrangement and size of inner and peripheral cells. The plurilocular sporangia of the Yashiro Island plant also agreed with figs. 1–4 of Yamada (1953) and those of the type specimen and his permanent preparations. Plurilocular sporangia are formed by repeated divisions of peripheral cells, as in *Kjellmania* and *Stictyosiphon*. Although the nature of the sporangia is a little obscure in figs. 1–3 of Yamada (1953), the examination of type permanent preparations (Figs. 29, 30) revealed that they are the same as those of Yashiro Island plant (Figs. 2, 22). However, the plurilocular sporangia shown in fig. 15 of Arasaki and Nozawa (1953) seem to be rather different from them, because each loculus of the sporangium is independent at the base in cross sec-

tion. Such kinds of plurilocular sporangia are reminiscent of those of *Coelocladia arctica* Rosenvinge and *Litosiphon subcontinuus* (Rosenvinge) Lund (Rosenvinge 1898, Pedersen 1976).

Naylor (1958) reviewed the genus *Stictyosiphon* and its four European species (e.g. *S. tortilis* (Ruprecht) Reinke, *S. adriaticus* Kützing, *S. soriferus*, and *S. griffithsianus* (Le Jolis) Holmes et Batters). According to her, *S. adriaticus* and *S. soriferus* have rounded inner cells in longitudinal sections, while *S. tortilis* and *S. griffithsianus* have longer ones. Furthermore, *S. soriferus* is distinguished from *S. adriaticus* in having a solid thallus and more uniformly sized and arranged inner cells. In Europe the former has a more northern distribution than the latter. Compared with these species, *Kjellmania arasakii* agrees well with *Stictyosiphon soriferus* in its anatomical features. Yamada (1953) distinguished *Kjellmania sorifera* and *K. arasakii* in the manner of branching and absence of intercalary sporangia in the latter. However, the differences in the branching manner are apparently within the range of variations caused by age and growing conditions. As mentioned above, in this species the intercalary sporangia are essentially the same as those formed on the surface of the thicker thallus. Furthermore, since the specimens Yamada (1953) examined were all drift materials, terminal thinner mature portions could have been already lost. Detailed comparisons with the herbarium specimens and live or liquid preserved materials of European specimens of *Stictyosiphon soriferus* (cited in Materials and Methods) with those of *Kjellmania arasakii* in the present study also showed no distinctive differences between the two taxa.

In culture, the plants from Yashiro Island showed a direct type of life history and formed unilocular and plurilocular sporangia. This life history pattern agreed well with the culture results of Rosenvinge (1935) and South and Hooper (1976) on *Stictyosiphon soriferus*, as well as the results on the Atlantic culture strains in the present study. Although unilocular sporangia were not reported in the culture of South and Hooper (1976), this difference may be due to the limited day length conditions (12: 12hLD) examined in their experiments. The fact that the field material of *Kjellmania arasakii* collected at Fukagawa in Tokyo Bay on February had some unilocular sporangia agree with the present culture results in which unilocular sporangia were formed in lower temperature (5°C) short day conditions corresponding to winter at Tokyo Bay.

On the contrary, the culture results of Arasaki and Nozawa (1953) differ from those in the previous reports and present work on *S. soriferus* in the following points; 1) *Laminaria*-type of germination in which most cellular contents migrate into germ tube and the embryospores become almost empty; 2) the thalli derived from swarmers developed into erect (gametophytic) filaments and formed clustered plurilocular sporangia resembling those of *Botrytella* (=*Sorocarpus*); 3) swarmers from the plurilocular sporangia were supposed to be gametes, (actual copulations were not observed but presumptive zygotes with two stigmata were found) and the presumptive zygotes developed into parenchymatous sporophytes. With regard to 1), *Laminaria*-type (mediate filamentous type) germination is rather rare in the order Dictyosiphonales and not reported in *Stictyosiphon*. The rare known exception in the order is the case in *Coelocladia arctica* (Pedersen 1976). About 2), as Arasaki and Nozawa (1953) also mentioned, such plurilocular sporangia differ from those known in *Stictyosiphon*, and are rather similar to those of *Coelocladia arctica*. Concerning 3), since they did not observe actual copulations nor complete the life cycle, their explanation of the erect filaments derived from the original swarmer as gametophytes is questionable. In fact, the presumptive gametophytic filament illustrated in fig. 7 of Arasaki and Nozawa (1953) has some longitudinal walls, resembling those in the sporophytic thallus in their fig. 13. Settled swarmers provided with two (or more) stigmata can also be caused by abnormal coalescences of the swarmers as observed in the present culture. This

phenomenon may occur by too low or high temperature treatment during transportation or storage. Accordingly, it is presumed that the material Arasaki and Nozawa (1953) used for culture (at least the swarmers from which cultures were started) was somehow different from the specimens sent to Yamada. Judging from the germination pattern and the morphology of the erect thalli and sporangia, the entity of the material Arasaki and Nozawa cultured is most likely a *Coelocladia*. This hypothesis is also supported by the recent finding of *Coelocladia arctica* from Japanese waters (at Oshoro, Japan Sea coast of Hokkaido; Kawai and Sato 1991). However, specimens referable to *Coelocladia* were not found in the herbarium specimens sent by Arasaki to Yamada and deposited in SAP.

In conclusion, through the detailed morphological comparisons and culture experiments in the present work, there are no essential differences distinguishing *Kjellmania arasakii* from *Stictyosiphon soriferus*. Accordingly, *Kjellmania arasakii* is considered to be taxonomically identical with *Stictyosiphon soriferus* at the species level. The species is distributed widely in the central part of Japan as shown in Fig. 1. Although the geographical distribution of the species in Japan seems to be a little more to the south than in Europe, there were no remarkable differences in the high lethal temperatures between Japanese and European strins. Judging from the culture results, the species could be expected to have a much wider distribution in Northern Japan and other Pacific Ocean areas.

Acknowledgments

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川井浩史：褐藻サメズグサ（*Kjellmania arasakii*, ウイキョウモ目）の分類と生活史の再検討

褐藻サメズグサの分類について瀬戸内海・屋代島において新たに採集した材料などにもとづき再検討した結果，本種は北大西洋に広く生育する *Stictyosiphon soriferus* と同一種であると結論した。本種は屋代島においては水深 3–4 m の亜潮間帯の人工的な基物の上に生育しており，藻体は高さ 20 cm に達し，3–4回不規則に枝分かれし，柔らかく，表面に複子嚢を生ずる。培養下では複子嚢に由来する遊走細胞はプロトネマに発達し，その上に直接直立藻体を形成した。直立藻体は多列形成的となり，分枝しながらよく成長し 5–20°C で複子嚢を生じたが，10°C と 15°C で特によく成長した。単子嚢は 5°C 短日条件でのみ形成された。（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）

Jpn. J. Phycol. (Sôrui) 39: 329–336, December 10, 1991

Taxonomic notes on the Halymeniaceae (Rhodophyta) from Japan. I. *Halymenia acuminata* (Holmes) J. Agardh.

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Kawaguchi, S. 1991. Taxonomic notes on the Halymeniaceae (Rhodophyta) from Japan. I. *Halymenia acuminata* (Holmes) J. Agardh. Jpn. J. Phycol. 39: 329–336.

The vegetative and reproductive morphology of *Halymenia acuminata* (Holmes) J. Agardh is described and illustrated from materials collected near the type locality. This species lacks distinctive anticlinal medullary filaments a critical feature of *Halymenia*. The structure of its auxiliary cell ampullae and the developmental pattern of its pericarps are most like *Grateloupia* within the family Halymeniaceae. A mediate discal type of spore germination pattern also differs from that of *Halymenia* and is typical of *Grateloupia*. The resurrection of ***Grateloupia acuminata*** Holmes is therefore proposed.

Key Index Words: Grateloupia—Grateloupia acuminata—Halymenia—Halymenia acuminata—*Halymeniaceae—Rhodophyta—taxonomy.*

In 1896, Holmes described *Grateloupia acuminata* based on a specimen collected at Enoshima, on the central Pacific coast of Japan. Subsequently, J. Agardh (1901) transferred the species to *Halymenia*. Since then, the binomial *H. acuminata* has been accepted by Okamura (1908) and other workers (Segawa 1956, Lee and Kang 1986).

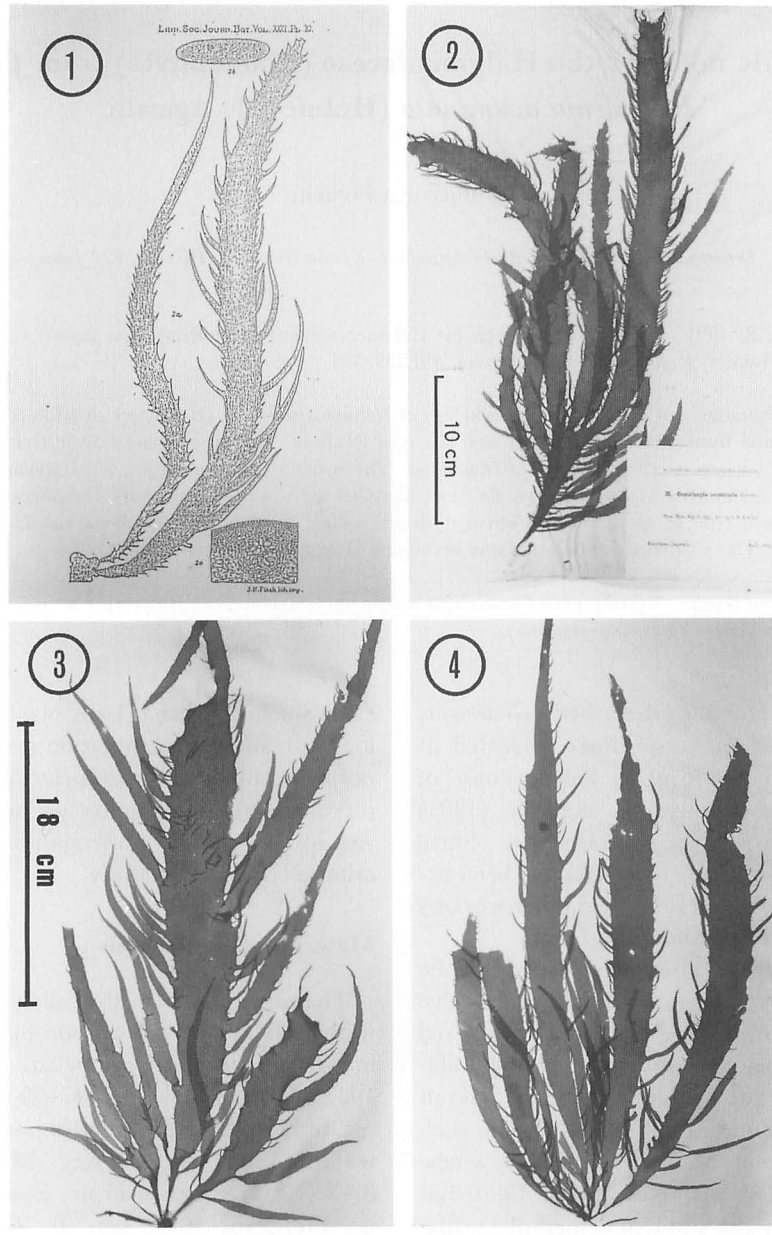
Recent authors consider genera within the family Halymeniaceae defined on vegetative features, and *Halymenia* is often characterized by the presence of conspicuous anticlinal filaments in the medulla (Abbott 1967, Kraft 1977, Maggs and Guiry 1982, Gargiulo *et al.* 1986). However, my recent study of a new species of *Grateloupia* (Kawaguchi 1990) has suggested that the anticlinal medullary filaments may not be as strong a generic characteristic as previously thought. The distinction between the two genera could rather alternatively made on a combination of reproductive features and a spore germination pattern. The ultimate characterization of these genera thus remains to be resolved.

In the present study, vegetative and reproductive details of *Halymenia acuminata* are described principally from specimens collected at Shichirigahama (1 km east from the type locality), and the germination mode of its carpospores and tetraspores is clarified in laboratory culture. The results are presented as a step toward a better understanding of generic criteria within this family.

Materials and Methods

The materials from the following localities were used for study: tetrasporangial, Shirahama, Wakayama Pref., 30. iii. 1957, 5. iv. 1957, leg. Y. Tsuji, SAP 047499-50; cystocarpic, tetrasporangial, Inamuragasaki, Kanagawa Pref., 26. ii. 1967, leg. M. Yoshizaki, SAP 031317-8; cystocarpic, Zushi, Kanagawa Pref., iii. 1940, leg. T. Tanaka, SAP 021553; cystocarpic, Shichirigahama, Kanagawa Pref., 14. v. 1955, leg. Y. Tsuji, SAP 047504; cystocarpic, tetrasporangial, Shichirigahama, Kanagawa Pref., 2. iv. 1984, leg. S. Kawaguchi, *Kawaguchi* 1037-8 (cast up ashore); cystocarpic, tetrasporangial, Shichirigahama, Kanagawa Pref., 28. iii. 1990, leg. S. Kawaguchi, *Kawaguchi* 1017-9 (cast up ashore).

Although the type specimen (probably in



Figs. 1–4. *Halymenia acuminata* (Holmes) J. Agardh. Fig. 1. Holmes' original illustration. Fig. 2. Okamura's specimen used in Algae Japonicae Exsiccatae (no. 31, SAP Okamura herb. as *Grateloupia acuminata* Holmes). Figs. 3, 4. Cystocarpic plants (*Kawaguchi* 1038, 1037). Scale in Fig. 3 applies also to Fig. 4.

BM) has not been examined, the above specimens agree well with Holmes' illustrations (Fig. 1) and Okamura's specimen collected at the type locality in April 1897 and used in his Algae Japonicae Exsiccatae (as *Grateloupia acuminata* in SAP Okamura herb., Fig. 2).

Sections were made by hand using a razor blade, stained with 0.5% (w/v) cotton blue in a lactic acid/phenol/glycerol/water (1 : 1 : 1 : 1) solution and mounted in 50% glycerol-seawater mixture on microscope slides.

Carpospores and tetraspores were obtained

from the drift specimens collected by the author at Shichirigahama on April 1 1989 and March 28 1990. Liberated spores were inoculated into small petri dishes (6 cm in diameter) containing full strength Provasoli's Enriched Seawater (PES). Plants were grown at 20°C, 12 : 12 light and dark cycle under white fluorescent light 1500-2000 lux.

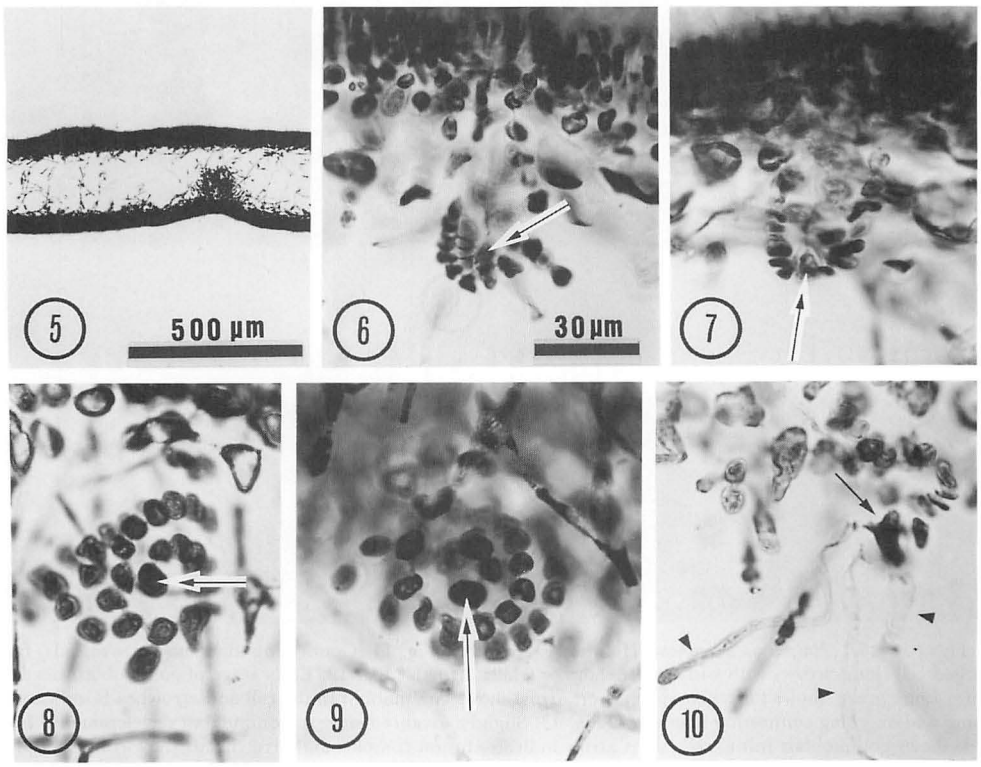
Abbreviations of herbaria follow Holmgren *et al.* (1981).

Observations

Vegetative morphology

The upright thalli arise caespitosely from a discoid holdfast. The stipe, up to 3 cm long and 2 mm in diameter, gradually expands into a simple or a few times branched, flat, linear-lanceolate blade. Branching may occur also in the stipe. The margin of the blade is beset with pinnate proliferations that are beset again with pinnate ramuli. Minute proliferations may also arise from the surfaces. Some of the marginal proliferations grow into bladelets with similar appearance to the main blade. The main blade reaches 50 cm high and 4 cm wide (Figs. 2-4). The texture is very gelatinous when young, but becomes somewhat firmer with age. The color is rose red to blood red.

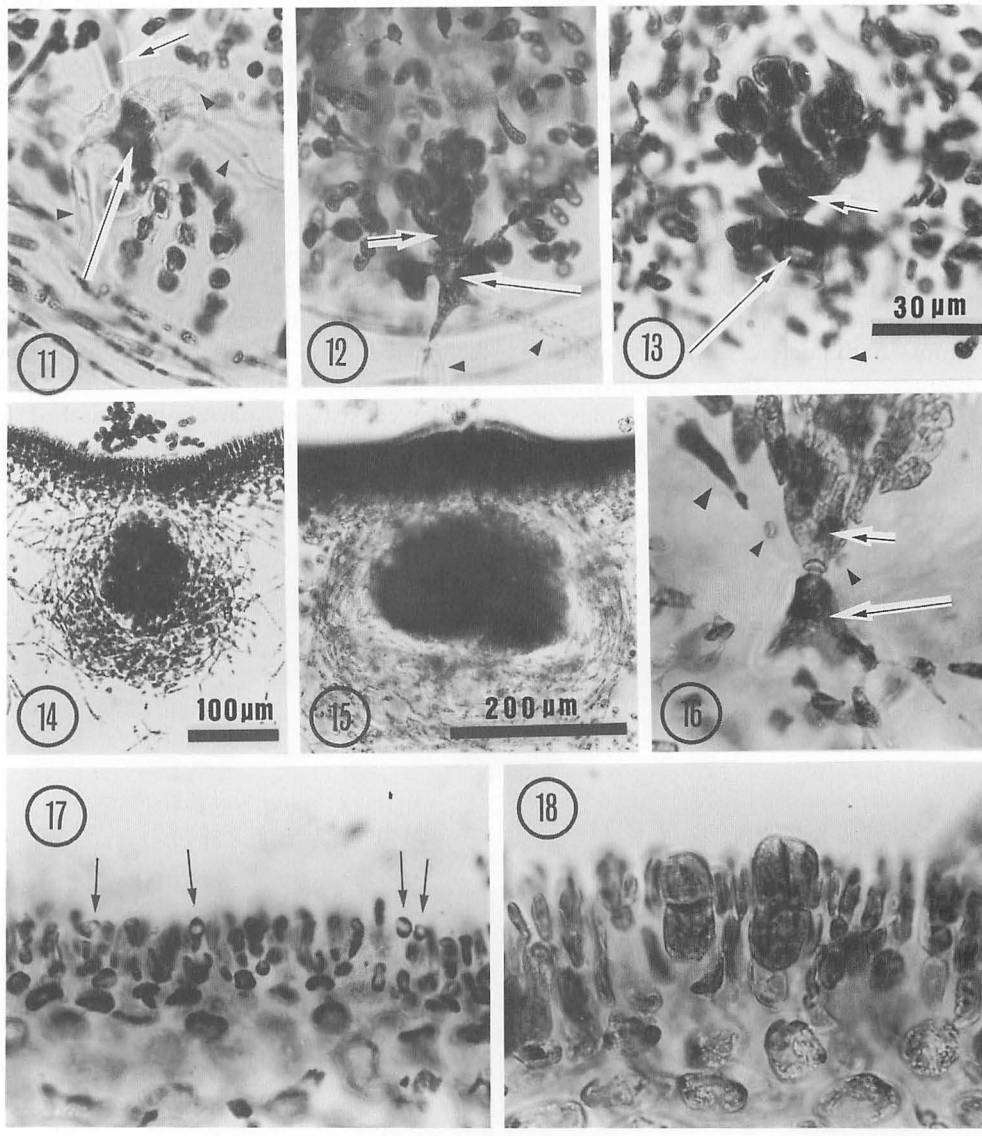
The blade is up to 600 μm thick, composed of a rather thin cortex and lax medulla (Fig. 5). The cortex consists of about 6 cells, with an outer of 2-3 elliptical to rounded cells arranged in anticlinal rows and an inner of 3-4 larger, irregularly-shaped cells often laterally connected by secondary pit-connections. The medulla, about the three-fourths of the blade, consists of sparsely-intermeshed filaments, running in various directions (Fig. 5).



Figs. 5–10. *Halymenia acuminata* (Holmes) J. Agardh. Fig. 5. Transverse section of young branch showing sparse medulla. Figs. 6, 7. Carpogonial ampullae. Arrow shows carpogonium with trichogyne. Figs. 8, 9. Auxiliary cell ampullae branched to the second order. Arrow shows auxiliary cell. Fig. 10. Connecting filaments (arrowheads) produced from slightly enlarged cell (arrow). Scale in Fig. 6 applies also to Figs. 7–10.

Reproduction

Carpogonial branches and auxiliary cells are formed in separate flask-shaped ampullae of filaments branched to the second order. The carpogonial branch is two-celled and positions in the center of the ampulla (Figs. 6, 7). The auxiliary cell ampulla is somewhat larger in size and usually the fifth cell of the



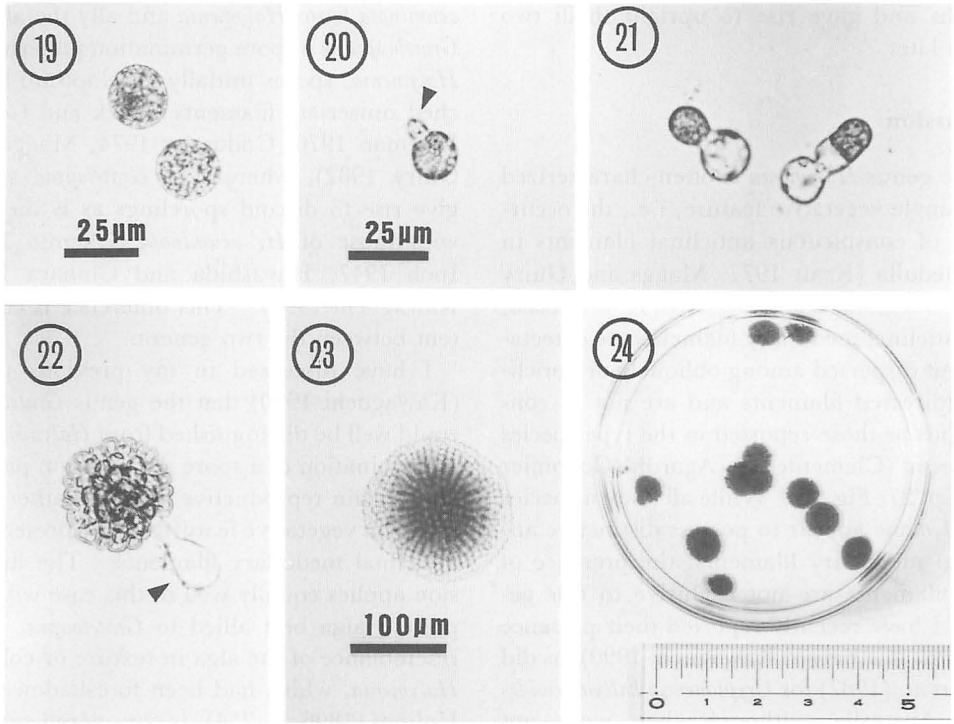
Figs. 11–18. *Halymenia acuminata* (Holmes) J. Agardh. Fig. 11. Connecting filaments (arrowheads) from enlarged cell (long arrow) with withered trichogyne (short arrow). Fig. 12. Early stage of gonimoblast development. Long arrow shows fusion complex, short arrow shows gonimoblast initial cell and arrowheads indicate incoming and outgoing connecting filaments. Fig. 13. Slightly advanced stage of gonimoblast development. Long arrow shows gonimoblast initial cell, short arrow indicates fusion complex and arrowheads show connecting filament. Fig. 14. More advanced stage. Note that pericarpial filaments are well developed. Fig. 15. Mature cystocarp with pericarp. Fig. 16. Basal portion of mature cystocarp. Long arrow shows fusion complex, short arrow indicates elongated gonimoblast initial. Note that ring-like structures (small arrowhead) and foliar radiating processes (large arrowhead) are seen along the initial. Fig. 17. Spermatangia formation. Fig. 18. Tetrasporangia formation. Scale in Fig. 13 applies also to Figs. 11, 12 and 16–18.

primary filament functions as an auxiliary cell (Figs. 8, 9).

The early stages of fertilization were not traced with certainty. However, several connecting filaments were observed to be cut off from a slightly enlarged, irregularly-shaped cell with a withered trichogyne (Figs. 10, 11). The connecting filament fuses with an auxiliary cell. The filament may terminate here, but often an outgoing filament is produced from the other side of the auxiliary cell (Fig. 12). After fusion a gonimoblast initial cell is cut off from the auxiliary cell toward the surface, and the ampullary cells then begin to produce lateral branchlets. The initial cell successively cuts off several gonimoblast cells and these in turn divide to form carposporangia (Figs. 12, 13). As the gonimoblast develops further, the ampullary filaments and the derivative branchlets become elongated to form a thick involucre (=pericarp). Neighboring vegetative cells and their derivatives also contribute to the pericarp formation (Fig. 14). The pericarp remains distinct around the fully developed carposporophyte (Fig. 15). The mature cystocarp is spherical in outline, 300-400 μm in diameter, deeply immersed in the thallus and opens by a pore (an ostiole) in the cortex (Fig. 15). In old specimens, foliar radiating processes and small ring-like structures were observed along the side of the elongated gonimoblast initial (Fig. 16).

Male reproductive structures were found scattered on the plants bearing cystocarps. Spermatangia are cut off singly or in pairs from the outermost cortical cells (Fig. 17).

Tetrasporangia are dispersed over the blade as are the sexual organs. Tetrasporangial initials are cut off laterally from the cortical cells in the third layer from the surface. Mature sporangia are broadly ellipsoidal, 15-



Figs. 19–24. *Halymenia acuminata* (Holmes) J. Agardh. Fig. 19. Liberated carpospores. Fig. 20. Germtube formation (arrowhead) two days after inoculation. Fig. 21. 6-day-old germling. Note that spore content evacuated into germtube. Fig. 22. 10-day-old sporeling developing into crust with marginal meristem and empty spore wall (arrowhead). Fig. 23. 14-day-old crust. Fig. 24. Two-month-old crusts beginning to develop erect thalli. Scale in Fig. 20 applies also to Figs. 21, 22.

18 μm wide by 33–40 μm long, submerged in the cortex, and cruciately or decussately divided (Fig. 18).

Development of spores

Liberated carpospores are 18–22 μm in diameter (Fig. 19). One or two days after settling, the spore developed a germ tube (Fig. 20) into which the spore content migrated (Fig. 21). A septum was then formed, leaving only the original spore wall behind. Irregular divisions of the germ tube cell gave rise to a multicellular, discoid sporeling (Fig. 22). The disc grew by divisions of the marginal meristem and after one week reached 50 μm in diameter. In two weeks, the disc reached 100 μm in diameter (Fig. 23), and in five months, 3–5 mm (Fig. 24). Upright thalli arose from near the center of the disc after two months. Tetraspores developed in a similar manner to carpospores. The developed discs reached 2 mm in diameter in two months and gave rise to upright thalli two weeks later.

Discussion

The genus *Halymenia* is often characterized by a single vegetative feature, i.e., the occurrence of conspicuous anticlinal filaments in the medulla (Kraft 1977, Maggs and Guiry 1982, Gargiulo, *et al.* 1986). In *H. acuminata*, the anticlinal medullary filaments are detectable, but dispersed among obliquely or periclinally directed filaments and are not as conspicuous as those reported in the type species *H. floresia* (Clemente) C. Agardh (Codomier 1974, p. 27, Fig. 4). While all known species of *Halymenia* appear to possess distinctive anticlinal medullary filaments, the presence of such filaments are not exclusive to this genus. I have recently reported their presence in *Grateloupia kurogii* (Kawaguchi 1990), as did Scott *et al.* (1982) for *Cryptonemia kallymenioides* from Australia, although they were not present as regularly nor predominantly as is usual in the genus *Halymenia*.

Chiang (1970) proposed that the morphology of auxiliary cell ampullae is generically diagnostic within this family. The ampulla of *Halymenia acuminata* is sparingly branched up to the second order and conical in outline. This type of ampulla corresponds well with the *Grateloupia*-type in Chiang's scheme, and in contrast to the *Halymenia*-type which is profusely branched to the fourth order and tends to be wide across the top (Chiang 1970). Although Kraft (1977) and Guiry and Maggs (1982) have shown that the ampullar types are not invariably a consistent generic characteristic, the present alga is allied more to the genus *Grateloupia* than to *Halymenia* in this regard.

The similarity of this alga to *Grateloupia* is also found in its comparatively thick pericarp constructed not only of ampullary filaments but also of neighboring vegetative cells. This differs to *Halymenia* where the pericarp is reported to be derived only from ampullary filaments (Balakrishnan 1961, Chiang 1970, Maggs and Guiry 1982, Gargiulo *et al.* 1986).

The most decisive feature to separate *H. acuminata* from *Halymenia* and ally the alga to *Grateloupia* is a spore germination pattern. In *Halymenia*, spores initially develop into branched uniseriate filaments (Hoek and Cortel-Breeman 1970, Codomier 1974, Maggs and Guiry 1982), whereas in *Grateloupia* spores give rise to discoid sporelings as is the case with those of *H. acuminata* (Chemin 1937, Inoh 1947, Hayashida and Chihara 1967, Kawaguchi 1990). This difference is consistent between the two genera.

I have discussed in my previous paper (Kawaguchi 1990) that the genus *Grateloupia* could well be distinguished from *Halymenia* on a combination of a spore germination pattern and certain reproductive features rather than solely on vegetative features as the presence of anticlinal medullary filaments. The discussion applies equally well in this case with the present alga best allied to *Grateloupia*. The resemblance of the alga in texture or color to *Halymenia*, which had been foreshadowed by Holmes (1896, p. 254), is considered superficial. *Grateloupia turuturu* Yamada is a good example that supports this consideration. *G. turuturu* has lubricous texture and bright red color, but its reproductive features and spore

germination pattern are typical of *Grateloupia* (Kawabata 1962, Hayashida and Chihara 1967).

On these grounds, I conclude that *H. acuminata* does not belong in the genus *Halymenia* but in *Grateloupia*. I therefore propose the resurrection of the following binomial:

Grateloupia acuminata Holmes, Linn. Soc. J. Bot. **31:** 254, fig. 2a-c. 1896.

Synonym: *Halymenia acuminata* (Holmes) J. Agardh, Sp. Alg. III(4): 130-131. 1901.

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川口栄男：日本産紅藻ムカデノリ科に関する分類ノート

I. *Halymenia acuminata* (Holmes) J. Agardh.

Holmes (1896) が江ノ島から *Grateloupia acuminata* の名で記載し，J. Agardh (1901) によってイソノハナ属 *Halymenia* に移されたオオムカデノリ *H. acuminata* の栄養体，生殖器官を精査した。本藻にはイソノハナ属に特徴的とされる垂直な髄糸が顕著には認められず，その助細胞 ampulla, 嚢果を包む pericarp の構造はむしろムカデノリ属 *Grateloupia* に最も近い特徴を示した。さらに，間接盤状型の胞子発芽様式はこれまでイソノハナ属で報告された様式とは明らかに異なり，ムカデノリ属に典型的である。従って，本藻はイソノハナ属ではなくムカデノリ属の種として扱うのが妥当であると結論した。元の名である *Grateloupia acuminata* Holmes を復活することを提案した。(812 福岡市東区箱崎6-10-1　九州大学農学部水産学第二教室)

Jpn. J. Phycol. (Sôrui) 39: 337–345, December 10, 1991

Ferredoxin-nitrite reductase from a cyanobacterium *Spirulina platensis*

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Miyaji, T., Masada, M. and Tamura, G. 1991. Ferredoxin-nitrite reductase from a cyanobacterium *Spirulina platensis*. Jpn. J. Phycol. **39**: 337–345.

Ferredoxin-dependent nitrite reductase (Fd-NiR) (EC 1. 7. 7. 1) was purified about 4,100-fold, with a yield of 14%, from a cyanobacterium, *Spirulina platensis* by a procedure involving acetone fractionation, DEAE-cellulose chromatography, Butyl-Toyopearl chromatography, Sephadex G-75 gel filtration and ferredoxin-Sepharose affinity chromatography. The purified enzyme was apparently homogeneous, as judged on polyacrylamide disc gel electrophoresis, with a specific activity of 222 units/mg of protein. The molecular weight of the enzyme was estimated to be 57 kilodaltons by gel filtration. On subunit analysis by SDS-PAGE, a single band corresponding to molecular weight of 58 kilodaltons appeared. The purified enzyme (Fd-NiR) showed 1.4 times higher methyl viologen-linked activity than ferredoxin-dependent activity. In the oxidized form, the enzyme exhibited absorption maxima at 277, 390 (Soret band), 573 (α band) and 695 (CT band) nm, indicating that siroheme is involved in the catalysis of nitrite reduction. The absorbance ratios, A_{390}: A_{277} and A_{573}: A_{390} were 0.58 and 0.26, respectively. The Km values calculated from Lineweaver-Burk plot of the data were 4.8×10^{-4}M (nitrite) and 2.0×10^{-6}M (reduced ferredoxin).

Key Index Words: cyanobacterium—ferredoxin linked enzyme—nitrite reductase—Spirulina platensis.

The nitrite reductases (NiRs) from plants catalyze the reduction of nitrite to ammonia in the presence of reduced ferredoxin, which is the physiological donor of electrons, or in the presence of reduced methyl viologen, which is an artificial electron donor. This enzyme has been purified from several plants and characterized (Ho and Tamura 1973, Shimizu and Tamura 1974, Hirasawa and Tamura 1980, Hirasawa-Soga and Tamura 1981, Hirasawa-Soga *et al.* 1982, Hirasawa-Soga *et al.* 1983, Nagaoka *et al.* 1984, Hirasawa *et al.* 1984, Ishiyama and Tamura 1985, Ishiyama *et al.* 1985, Ide and Tamura 1987). However, nitrite reductases from cyanobacteria have not received the same attention as those from higher plants. NiRs from *Anabaena cylindrica* (Hattori and Uesugi 1968), *Anacystis nidulans* (Manzano 1977), *Anabaena* sp. 7119 (Mendez and Vega 1981, Mendez *et al.* 1981), *Spirulina platensis* (Yabuki *et al.* 1985) and *Phormidium laminosum* (Arizmendi and Serra 1990) have been partially characterized, but only the enzymes from *S. platensis* and *P. laminosum* have been purified to a high degree.

In the present report, we highly purified NiR from *S. platensis* and described several properties of the NiR.

Materials and Methods

Materials

The following chemicals were purchased from commercial sources: DEAE-cellulose DE-52 (Whatman, Maidstone, U. K.); Butyl Toyopearl 650S (Toso, Tokyo, Japan); Sephadex G-75, CNBr-activated Sepharose 4B, Electrophoresis Calibration Kit, Blue Dextran 2000 (Pharmacia, Uppsala, Sweden); Coomassie brilliant blue G-250, R-250 (Fluka, Buchs, Switzerland); sodium dithionite (Koso Chemical, Tokyo, Japan); methyl viologen (Tokyo Kasei, Tokyo, Japan); albumin fraction V, Calibration Proteins II (Boehringer Mannheim, Mannheim, F. R. G.); finely granulated sugar (local market). Other chemicals were reagents of analytical grade from Wako Pure Chemical (Tokyo, Japan).

Cell culture

Spirulina platensis strain OU-1, kindly supplied by professor K. Wada of Kanazawa University, was grown photoautotrophically at 35°C in inorganic artificial medium (SOT medium; Ogawa and Terui 1970) in a 100 liter plastic container (AL-LL Reactor, supplied by Mitsubishi kakoki, Tokyo, Japan). The culture medium was agitated by a stream of air from a pump and continuous illumination was provided by four white fluorescent lamps (FLR40SW/M40W; Mitsubishi) at 10,000 lux. About 300 g (wet wt.) of cells were harvested by suction filtration at the stationary phase, which was reached after one week. The harvested cells were immediately placed in a freezer at −30°C and stored until use.

Assay of enzymatic activity

The method for the assay of nitrite reductase (NiR) activity was essentially the same as that described in our previous paper (Yabuki and Tamura 1985). The reaction mixture contained, in a total volume of 1 ml, 20 μmol of Tris-HCl buffer, pH 7.5, 2 μmol of $NaNO_2$, 3 μmol of methyl viologen or 2 mg of ferredoxin from *S. platensis*, 3.75 mg of sodium dithionite (freshly dissolved in 0.3 M $NaHCO_3$) and an aliquot of enzyme preparation. In the enzymatic reaction, reduced methyl viologen was used as electron donor unless otherwise stated.

The reaction was started by the addition of sodium dithionite after a 2 min of preincubation at 35°C and was incubated for 4 min at the same temperature. The reaction was terminated by vigorous shaking in a cyclomixer. The decrease in the level of nitrite was measured by the method of Snell and Snell with sulfanilamide and N-1-naphthylethylenediamine dihydrochloride.

In the inhibitory experiments, the effect of inhibitors on the reduced form of NiR was measured by addition of sodium nitrite after the preincubation of the enzyme in a solution containing Tris-HCl, methyl viologen, sodium dithionite and each inhibitor for 7 min at 35°C. The inhibitory effects on the oxidized form was measured by addition of sodium dithionite.

One unit of NiR activity was defined as the amount of enzyme that reduced 1 μmol of nitrite per min under the conditions of the assay.

Purification of ferredoxin

Ferredoxin from *S. platensis* were purified by acetone fractionation, chromatography twice on DEAE-cellulose and once on Sephadex G-75. In this study, an absorbance ratio for ferredoxin (A_{423}/A_{277}) of more than 0.45 was used. Amounts of ferredoxin were estimated using a molar absorption coefficient of 9.2 $mM^{-1}cm^{-1}$ at 423 nm (Hall *et al.* 1972).

Preparation of ferredoxin-Sepharose

Ferredoxin-Sepharose 4B was prepared by the method of Shin and Oshino (1978).

Purification of NiR

All the purification procedures were performed in a cold room at 4°C. Dialysis was performed overnight against the indicated buffer, and centrifugation was carried out at 10,000 × g for 15 min.

(1) *Extraction of the Enzyme*

Frozen cells of *S. platensis* (300 g) were thawed and mixed in the four volumes (1,200 ml) of extraction buffer (20 mM Tris-HCl buffer, pH 7.5, containing 200 mM NaCl). The suspension was sonicated (Sonicator; Otake seisakusho, Tokyo, Japan) at 15 kHz for 4 min, and a crude extract was obtained after removal of cell debris by centrifugation.

(2) *Acetone fractionation*

The precipitate formed from the crude extract (1340 ml) after the addition of cold acetone (−30°C) to a final concentration of 35% (v/v) was removed by centrifugation and discarded. Cold acetone (−30°C) was further added to the supernatant to give a final concentration of 75%, then the resultant precipitate was collected by centrifugation, resuspended in 200 ml of 20 mM Tris-HCl

buffer, pH 7.5, and dialyzed overnight against the same buffer. The dialyzed sample was centrifuged to remove the precipitate.

(3) *Column chromatography on DEAE-cellulose*

The dialyzed solution (300 ml) was loaded on an anion-exchange column of a DEAE-cellulose DE-52 (5 cm × 22 cm) equilibrated with 20 mM Tris-HCl buffer, pH 7.5. After the column had been washed with same buffer, the elution was performed as follows. A linear concentration gradient of NaCl was established with 600 ml of 20 mM Tris-HCl buffer, pH 7.5, in the mixing vessel and 600 ml of 20 mM Tris-HCl buffer, pH 7.5, containing 300 mM NaCl in the reservoir. The flow rate of elution was 30 ml/hr, and fractions of about 10 ml each were collected. The fractions containing NiR activity were pooled.

(4) *Column chromatography on Butyl-Toyopearl*

The pooled fractions (70 ml) were supplemented with ammonium sulfate to bring them to 40% saturation. The precipitate formed was removed by centrifugation. The resultant supernatant was loaded on a hydrophobic column of Butyl-Toyopearl 650S (2.2 cm × 21 cm) equilibrated with 20 mM Tris-HCl buffer, pH 7.5, that was 40% saturated with ammonium sulfate. After the column was washed with the same buffer, a linear concentration gradient of ammonium sulfate was established with 250 ml of equilibration buffer in the mixing vessel and the same volume of 20 mM Tris-HCl buffer, pH 7.5, in the reservoir. The flow rate of elution was 20 ml/hr and fractions of about 4 ml were collected. The fractions containing NiR activity were combined.

(5) *Column chromatography on Sephadex G-75*

The pooled fractions (35 ml) were concentrated in a dialysis bag immersed in finely granulated sugar overnight and then dialyzed against 20 mM Tris-HCl buffer, pH 7.5, containing 200 mM NaCl. The resultant dialyzed solution of the enzyme was filtered through a gel filtration column of Sephadex G-75 (3 cm × 90 cm) equilibrated with the dialysis buffer. The flow rate of elution was 10 ml/hr and fractions of about 3 ml were collected. The fractions active for NiR were pooled.

(6) *Column chromatography on ferredoxin-Sepharose 4B*

After the pooled (20 ml) fractions has been dialyzed against 20 mM Tris-HCl buffer, pH 7.5, they were loaded on an affinity column of ferredoxin-Sepharose 4B (1 cm × 2 cm) equilibrated with the same buffer. After washing with same buffer, elution of the enzyme was performed with 20 mM Tris-HCl buffer, pH 7.5, containing 400 mM NaCl. The flow rate of elution was 15 ml/hr and fractions of about 1 ml were collected. The fractions containing NiR activity were pooled.

Storage of the enzyme

After the enzyme solution purified by the procedure described above was dialyzed against 20 mM Tris-HCl buffer, pH 7.5, containing 200 mM NaCl and 10% glycerol (v/v, final concentration), the preparation was stored in a freezer (−80°C) and aliquots were used for subsequent analysis.

Absorption spectra

The absorption spectra of oxidized form of the enzyme were recorded at room temperature against a buffer blank in a spectrophotometer Hitachi U-3200 in cuvettes of 1 cm path length.

Other analytical methods

Protein was determined by the methods of Bradford (1976) with albumin fraction V as a standard. Analytical gel electrophoresis was carried out by the method of Davis (1964) for PAGE and by that of Laemmli (1970) for SDS-PAGE. Protein bands on the gel slab were stained with Coomassie brilliant blue R-250. Activity staining of methyl viologen-dependent NiR activity was performed by the modified version of the method of Hucklesby and Hageman (Vega and Kamin 1977).

Table 1. Summary of purification of nitrite reductase from *Spirulina platensis*

Step	Activity (units)	Protein (mg)	Specific Activity (units/mg protein)	Purification	Yield (%)
Crude extract	411	7560	0.0544	1.0	100
Acetone 35–75%	528	1130	0.467	8.6	129
DEAE-Cellulose	230	224	1.03	19	56
Butyl Toyopearl	163	14.7	11.1	200	40
Sephadex G-75	83.5	0.980	85.2	1600	20
Ferredoxin Sepharose	55.6	0.250	222.4	4100	14

These data were obtained during the processing of 300 g (wet wt.) of the cells.

Results

Purification of enzyme

Table 1 shows the summary of a typical purification that started with 300 g (wet wt.) of *Spirulina platensis* cell. Nitrite reductase (NiR) was purified more than 4,100-fold, with a yield of 14%, to a specific activity of 222 units/mg protein at 35°C. The ratio of the activity with ferredoxin to that with methyl viologen was 0.72.

After the last step (Fig. 1.) of the purification, PAGE in 7% gels and SDS-PAGE in 10% gels gave a single band of protein upon staining with Coomassie brilliant blue R-250 (Fig. 2.). Furthermore, the band visualized by activity staining corresponded to the band of purified enzyme after non-denaturing PAGE (Fig. 2a).

Absorption spectrum

The solution of oxidized form of NiR gave the absorption spectrum shown in Fig. 3. In addition to the protein peak at 277 nm, the purified NiR exhibited absorption maximum at 390 nm (Soret band), 573 nm (α band) and 695 nm (CT band), respectively. The ratio of A_{390}/A_{277} was 0.58, and the ratio of α band to Soret peak was 0.26.

Molecular weight

The molecular weight of the purified NiR

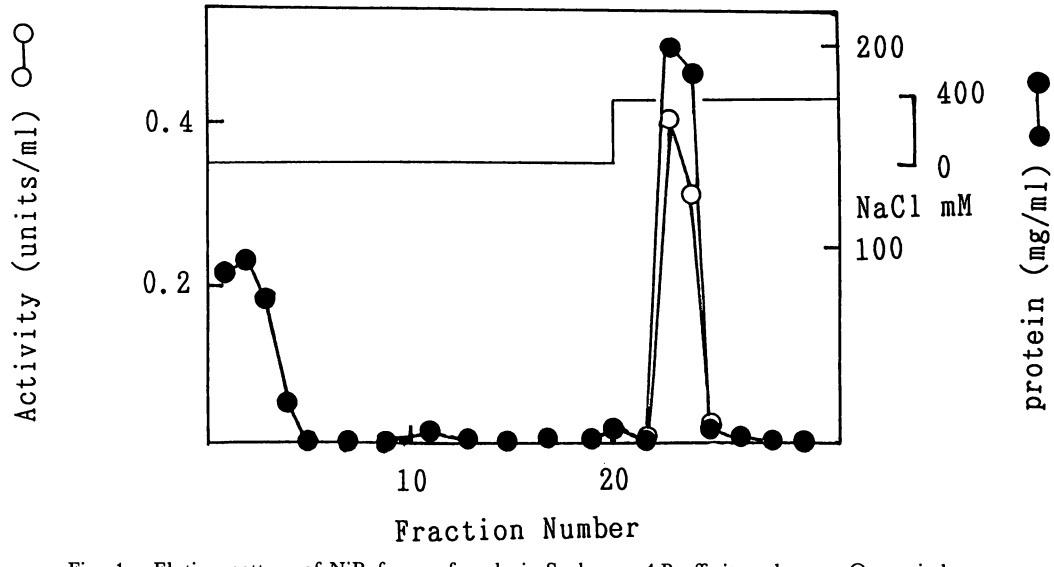

Fig. 1. Elution pattern of NiR from a ferredoxin-Sepharose 4 B affinity column. Open circle, enzyme activity in units per ml; closed circle, amount of protein in mg per ml. Experimental conditions are described in the text. Nos. 23–24 of the fractions were pooled.

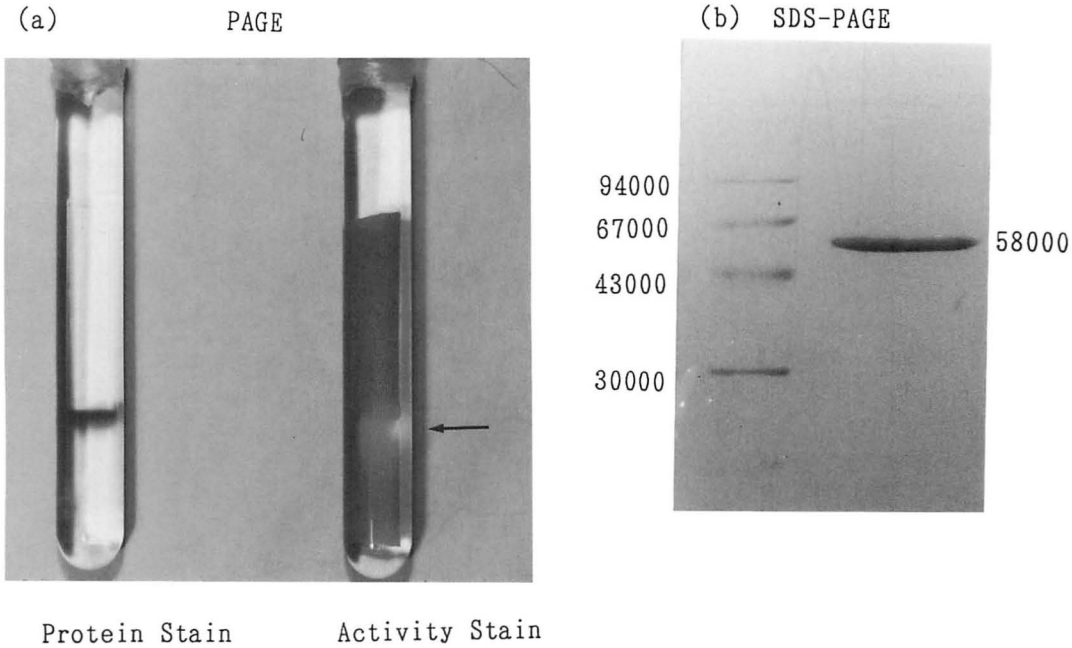

Fig. 2. Electrophoretograms of purified NiR on PAGE and SDS-PAG. (a): about 50 μg of the enzyme was subjected to electrophoresis on a 7% polyacrylamide gel disc. A constant electric current (5 mA per tubes) was applied for 5 hr. The NiR was detected by protein staining and activity staining. Activity is shown by an arrow. (b): about 50 μg of the enzyme was incubated at 100°C in a water bath for 5 min with 0.1% SDS and 1% β-mercaptoethanol. The treated enzyme was subjected to electrophoresis on a 10% SDS-polyacrylamide gel. A constant electric current (5 mA) was applied for 6 hr. The NiR was detected by protein staining.

was estimated by gel filtration on Sephadex G-75 (Fig. 4a) and SDS-PAGE (Fig. 2b). In Figure 4, the elution volumes for the marker proteins are plotted against their molecular weights. The molecular weight of NiR was estimated to be 57 kDa by gel filtration and 58 kDa by SDS-PAGE.

Effect of substrate concentration on enzyme activity

The effects of sodium nitrite and ferredoxin on NiR were determined by varing their concentration. From the double-reciprocal (Lineweaver-Burk) plot (Fig. 5), Km values for sodium nitrite and ferredoxin were determined to be 4.8×10^{-4} M and 2.0×10^{-6} M, respectively.

Optimum pH of NiR

The effects of pH on the NiR activity were studied under the same conditions as those described in Materials and Methods, except that the pH of the reaction mixture was varied. The pH-activity curve obtained were rather flat with an optimum pH around pH 7.5 (data not shown).

Effects of inhibitors

The effects of various compounds on the reaction catalyzed by NiR were examined (Table 2). Both the oxidized and the reduced form of the enzyme were strongly inhibited by cyanide. p-Chloromercuribenzoic acid (p-CMB) had an inhibitory effect only at high concentration as 2 mM, and this reagent inhibited more than two times oxidized form of NiR than reduced one. An inhibitor of hemoproteins, NaN_3 and a metal-chelating reagent, EDTA, had slightly inhibitory effect at 2 mM.

Heat stability

Methyl viologen-supported and ferredoxin-supported activities were both stable to heating at 40°C for 5 min, but more than 90% of

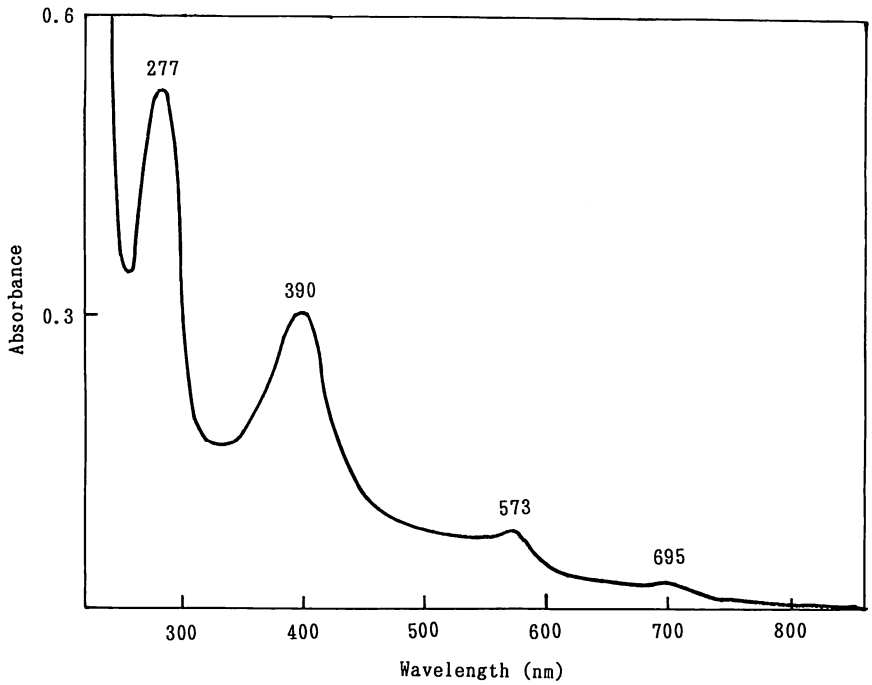

Fig. 3. Absorption spectrum of NiR. The purified enzyme preparation (400 μg/ml; oxidized form) was used. The protein was dissolved in 20 mM Tris-HCl buffer, pH 7.5 containing 200 mM NaCl and 10% glycerol (v/v). The absorption spectra were measured at room temperature.

both activities was lost after heating at 60°C for 5 min. Heating at 70°C for 5 min completely eliminated both activities. Ferredoxin-supported activity was somewhat less stable than methyl viologen-supported activity (Fig. 6).

Discussion

The present paper described a new methods for purifying ferredoxin dependent-nitrite reductase (Fd-NiR) from cells of a cyanobacterium *Spirulina platensis*. This enzyme was purified 4,100-fold (Table 1), and behaved as an almost homogeneous protein on polyacrylamide gel electrophoresis (Fig. 2). This purified enzyme showed a specific activity of 222 units/mg protein at 35°C. Compared with the specific activity reported in other works on NiRs from cyanobacteria, this value is very high, and it was almost same value as that reported previously from our laboratory (194 units/mg protein, Yabuki *et al.* 1985) and lower than the value for NiR from *Phormidium laminosum* (625 units/mg protein at 50°C, Arizmendi and Serra 1990). The ratio of the activities with ferredoxin and methyl viologen was 0.72. This ratio is close as that for the enzyme from *Anabaena cylindrica* (0.88, Hattori and Uesugi 1968) and *Anabaena* sp. 7119 (0.93, Mendez *et al.* 1981). However, NiRs from spinach leaves (Hirasawa and Tamura 1980) and green shoots of bean (Ishiyama *et al.* 1985) have the ratio of 1.74 and 1.43, respectivery. This difference may be due to the nature of the individual enzymes.

The absorption spectrum of this enzyme was similar to that of NiRs from other cyanobacteria and eukaryotic organisms, suggesting the presence of a siroheme as the prosthetic group. Yabuki *et al.* (1985) purified the NiR from *S. platensis*, a same strain in our study, 4,200-fold. However their purified enzyme showed many minor peaks such as at 350, 360, 462, 534, 588 and 658 nm in addition to the three peaks characteristic of siroheme. These small peaks might be due to

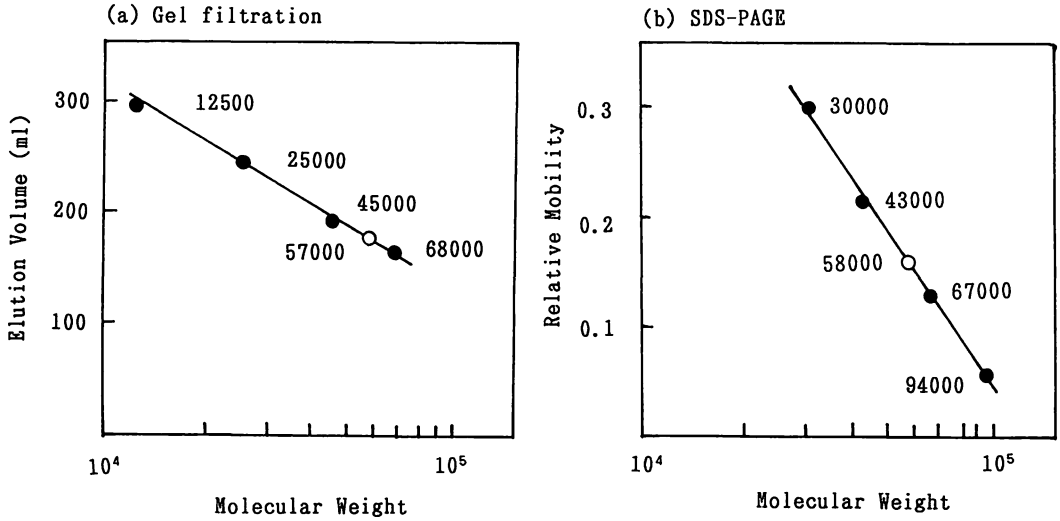

Fig. 4. Determination of the molecular weight of NiR. (a): NiR (500 μg/ml) and each marker protein (10 mg/ml) were separately filtered through a column of Sephadex G-75 (2 × 90 cm) equilibrated with 20 mM Tris-HCl buffer, pH 7.5, containing 200 mM NaCl and 10% glycerol (v/v). The marker proteins used were cytochrome c (12500), chymotrypsinogen A (25000), ovalbumin (45000), bovine serum albumin (68000). ○, NiR. (b): Electrophoretic mobilities of marker proteins and NiR are shown in the figure. The condition of SDS-PAGE was shown in the legend of Fig. 2(b). The marker proteins have following molecular weights: carbonic anhydrase (30000), ovalbumin (43000), bovine serum albumin (67000), phosphorylase b (94000).

some contaminants in their preparation.

The molecular weight of this enzyme (57-58 kDa) is slightly larger than that of other cyanobacterial NiRs (52-54 kDa, Manzano 1977, Mendez and Vega 1981, Arizmendi and Serra 1990) with the exception of NiR from *A. cylindrica* (68 kDa, Hattori and Uesugi 1968).

The Km values for sodium nitrite reported from other cyanobacterial NiR are 5×10^{-5} M (*A. cylindrica*, Hattori and Uesugi 1968) and 4×10^{-5} M (*P. laminosum*, Arizmendi and Serra 1990). The Km value for sodium nitrite of NiR from *S. platensis* was calculated to be 4.8×10^{-4} M. However, this value is a little larger than those of other cyanobacterial

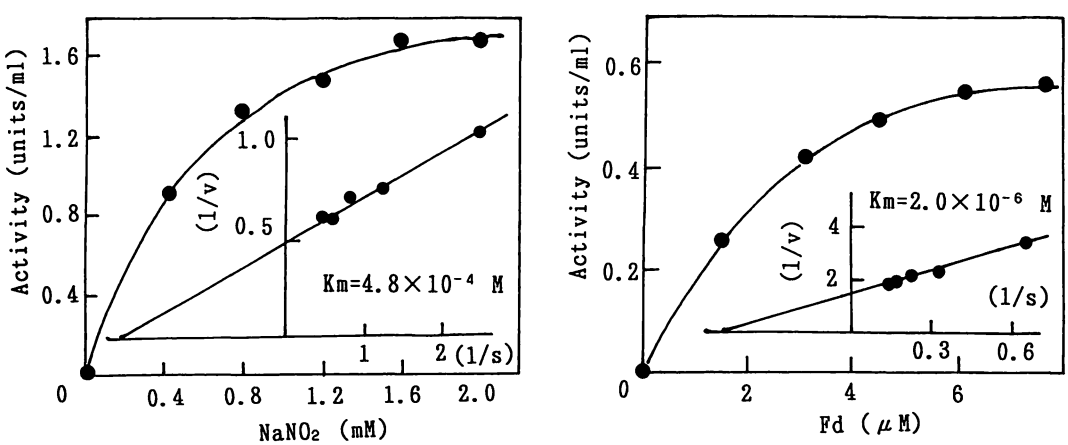

Fig. 5. Effects of nitrite and ferredoxin concentrations on NiR. The reaction mixture was varied in nitrite concentration (a) and ferredoxin concentration (b). For detailed see Materials and Methods. The amount of protein used for assay was 10 μg. Km values were calculated from Lineweaver-Burk plots.

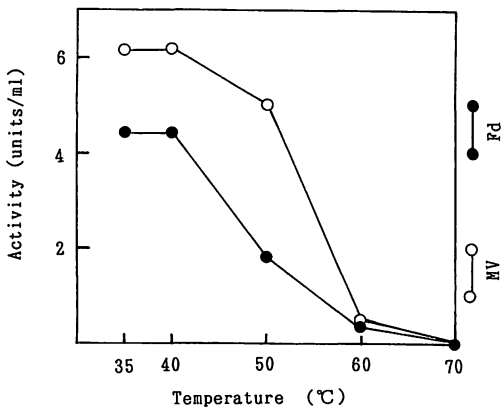

Fig. 6. Heat stability of NiR. The remaining activities were measured by the methods as described in Materials and Methods after a heat treatment of the enzyme for 5 min. The amount of protein used in each assay was 10 μg. Fd, ferredoxin-supported activity; MV, methyl viologen-supported activity.

NiRs. The Km value for ferredoxin of NiR from *Spirulina platensis* was 2.0×10^{-6} M. This value was almost same as that of NiR from *A. cylindrica* (5×10^{-6} M, Hattori and Uesugi 1968).

The optimum pH (7.5) for activity of this enzyme is almost same as that of NiR from *A. cylindrica* (pH 7.6, Hattori and Uesugi 1968).

The effect of inhibitors on the NiR from *S. platensis* were similar to those on NiRs from other cyanobacteria (Mendez *et al.* 1981, Arizmendi and Serra 1990).

The NiR from *S. platensis* is a little more stable to heating than the NiR from spinach leaves (Ho and Tamura 1973).

From our present study on NiR from *S. platensis*, we conclude that this enzyme is similar to NiRs from other plants, with the exception of its molecular weight.

Table 2. Effects of inhibitors on NiR.

Inhibitor	Concentration (mM)	Inhibition (%)	
		Oxidized form	Reduced form
KCN	0.02	55.9	57.8
	0.2	100	100
NaN_3	0.2	0	0
	2	6.4	13.7
p-CMB	0.2	6.1	6.1
	2	87.7	39.0
EDTA	0.2	0	0
	2	7.9	6.1

Purified enzyme (10 μg of protein) was employed in each reaction. In the control experiment, inhibitors were omitted. Other experimental conditions were as described in Materials and Methods.

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宮地竜郎・政田正弘・田村五郎：藍藻 *Spirulina platensis* のフェレドキシン依存性亜硝酸還元酵素

窒素固定を行わない藍藻である *Spirulina platensis* の同化型フェレドキシン依存性亜硝酸還元酵素を簡便な方法で4100倍に精製した。精製酵素は比活性が 222 units/mg protein であり，PAGE 及び SDS-PAGE 的に均一な標品であった。精製酵素の吸収スペクトルは 277, 390 (Soret band), 573 (α band), 695 (CT band) nm に吸収極大が見られた。分子量はゲル濾過法により 57 kDa, SDS-PAGE により 58 kDa と推定された。亜硝酸とフェレドキシンに対する Km 値はそれぞれ 4.8×10^{-4} M, 2.0×10^{-6} M と算出された。(271 千葉県松戸市松戸648　千葉大学園芸学部生物化学研究室)

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Histochemistry and ultrastructure of paraphyses in *Sargassum vulgare* C. Agardh and *S. johnstonii* Setchell & Gardner

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Sargassum vulgare and *S. johnstonii* bear androgynous receptacles with unisexual conceptacles. The fully developed conceptacles are filled with secretions and communicate with the external environment through the ostiole plugged with polysaccharidic material. The conceptacles are lined by flat cells–the conceptacle-wall cells, which are the progenitors of either antheridia or oogonia or paraphyses. In a young conceptacle, the paraphysial and the conceptacle-cell walls contain alginic acid and sulphated polysaccharides. During the early stages of conceptacle development, the cytoplasm is organelle-rich but in the old conceptacles, the organelles lyse and the paraphysial and conceptacle-wall cell cytoplasm becomes vacuolate.

Key Index Words: alginic acid—histochemistry—paraphysis—Phaeophyceae—Sargassum—*secretions—sulphated polysaccharides.*

In Sargassaceae (Fucales), the histochemical and ultrastructural information on the paraphyses and the conceptacle-wall cells is meagre. The mucilaginous secretions have been reported from the conceptacles of *Fucus edentatus* (McCully 1968) whereas elaborate wall projections have been observed in the conceptacle wall cells of *Durvillaea potatorum* (Clayton et al. 1987). The present study on *Sargassum vulgare* C. Agardh and *S. johnstonii* Setchell & Gardner is undertaken to correlate the histochemical, developmental and ultrastructural aspects of the paraphyses and the conceptacle-wall cells.

Materials and Methods

The plants of *Sargassum vulgare* and *S. johnstonii* were collected during the low tide period from Port Okha, Gujarat, through the months of January, February and November of 1987–89. The selected parts of the plants thus collected were processed for light, transmission and scanning electron microscopy.

For light microscopy, the plant parts were fixed in 10% acrolein, washed in distilled water and postfixed in 1% $HgGl_2$. This was followed by dehydration, and infiltration. The infiltrated materials were embedded in glycol methacrylate (Vijayaraghavan & Shukla 1990), and sectioned on a Spencer (AO) rotary microtome fitted with a locally made adaptor to hold glass knives. Two micron thick sections were serially transferred to small drops of distilled water and later stained with PAS reagent for insoluble polysaccharides (Vijayaraghavan and Shukla 1990); with Alcian Blue for sulphated polysaccharides (Parker and Diboll 1966); with TBO for sulphated and carboxylated polysaccharides (McCully 1966); with Coomassie Brilliant Blue for proteins (Weber and Osborn 1975) and with Feulgen reagent for DNA (Vijayaraghavan and Shukla 1990). For SEM, the plant parts were fixed in 4% formalin, dehydrated in graded acetone series, critical point dried and scanned for topographical details. For transmission electron microscopy, the desired stages were cut into small pieces, fixed in 6% glutaraldehyde prepared in phosphate buffer, dehydrated, infiltrated and embedded in Epon-Araldite mixture (Mollenhauer 1964). Ultrathin sections were stained and then observed through

Philips EM 300.

Results

Light microscopic Studies

The reproductive phases are seasonal in *Sargassum vulgare* and *S. johnstonii*. The axillary receptacles occur in clusters (Figs. 1, 2). Unisexual conceptacles are embedded in the receptacular tissues. The antheridial and oogonial conceptacles are borne in the same receptacle but in the conceptacles, either the antheridia or oogonia cooccur with paraphyses. Thus, the plants are neither monoecious nor dioecious in the generally accepted sense as both types of reproductive organs are present in the same receptacle but always in separate conceptacles. Androgynous condition thus prevails in these two species of *Sargassum*.

Early in the ontogeny, the conceptacle-wall cells enlarge and develop into papillae which through repeated transverse divisions develop into multicellular paraphyses whose terminal cells are globose (Fig. 3). Along with these changes either the oogonia or the antheridia also codevelop inside the conceptacle.

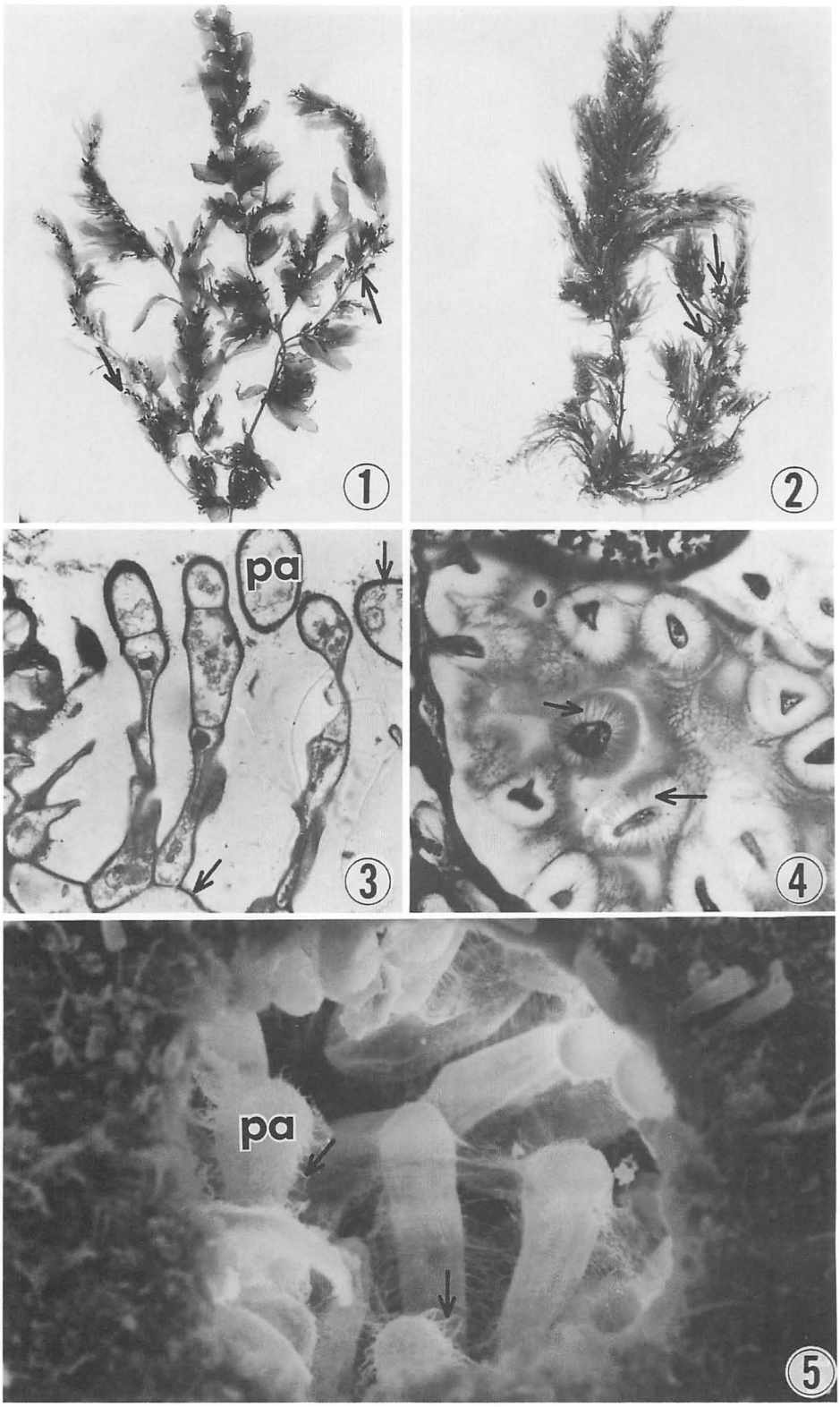
The young paraphysial cells show walls which stain reddish-violet with TBO and moderate magenta with PAS reagent and are hence rich in alginic acid. The cytoplasm stains well for proteins and contains moderate amount of polysaccharides. Also enclosed in the cytoplasm are a few small vacuoles and phenolic compounds which occur in large amounts. In the developing paraphyses, the cell walls reveal identical staining reactions. The cell walls, in addition, reveal small thread-like structures that stain intense violet with TBO (Figs. 3, 4). The paraphysial cells, in transverse sections, reveal core filled with phenolic materials (Fig. 4). The cytoplasm gradually becomes vacuolate and reveals a low amount of proteins and moderate polysaccharides. The cells are uninucleate and the nucleus stains well for DNA. Hence, the paraphyses which codevelop with sex organs, remain rich in cytoplasmic contents during the early stages of oogonial/antheridial development. Later as the sex organs are ready for gamete release, the paraphysial cytoplasm shows vacuolation.

The conceptacle-wall cells when young contain abundant proteins, moderate polysaccharides and phenolic materials. At maturity, the wall cells become more vacuolate and the proteins are meagre. The conceptacular cavity reveals polysaccharidic secretions which stain turquoise with Alcian Blue, magenta with PAS reagent and violet with TBO–thus indicating a mixture of sulphated and carboxylated polysaccharides (Fig. 4). This polysaccharidic material also acts as a plug in the female conceptacle where it keeps the ostiole closed while the oogonia are developing.

Electron Microscopic Studies

The paraphysial cell when viewed under scanning electron microscope reveals many strands that radiate from the wall into the conceptacle cavity (Fig. 5). At ultrastructural level, this wall is well differentiated, thick, lacks an orderly arrangement of microfibrils and is overlaid by material that is differentiated into an inner light and an outer dark zones. The latter radiates a few electron dense strands into the conceptacle cavity (Fig. 7). Adjacent to the wall lie many vacuoles that contain electron dense materials. The material is released from the vacuoles and

Figs. 1–5. Fig. 1. *Sargassum vulgare*, a mature plant showing forked branches, leaves, bladders and receptacles (arrows). ×0.47. Fig. 2. *Sargassum johnstonii*, whole mount of a plant bearing dense leaves and receptacles (arrows). ×0.47. Fig. 3. *S. vulgare*, Mature female conceptacle showing paraphyses (pa) near the ostiolar region. The terminal paraphysial cells are globular and veneered by polysaccharidic materials. The paraphysis cytoplasm reveals phenolic materials and carboxylated polysaccharides. The paraphysial and the conceptacle-cell walls reveal small polysaccharidic projections (arrows). ×1474 (TBO stained). Fig. 4. *S. vulgare*, Transverse section of a conceptacle, showing paraphyses surrounded by metachromatic material. The core of the paraphysis encloses phenolic materials and polysaccharides. From the core radiate thin strands into the conceptacle cavity. ×1474 (TBO stained). Fig. 5. *S. johnstonii*. Scanning electron micrograph. The paraphyses that occlude the ostiole show blunt tips from which emerge many, small, thread-like structures (arrows). ×1180.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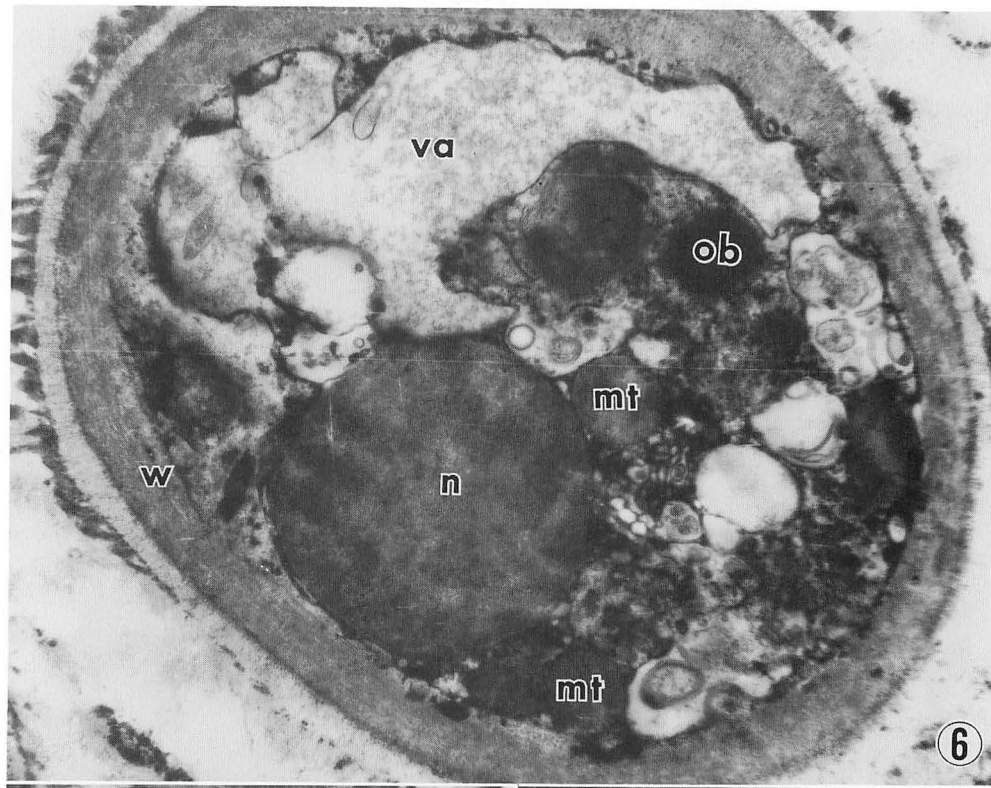
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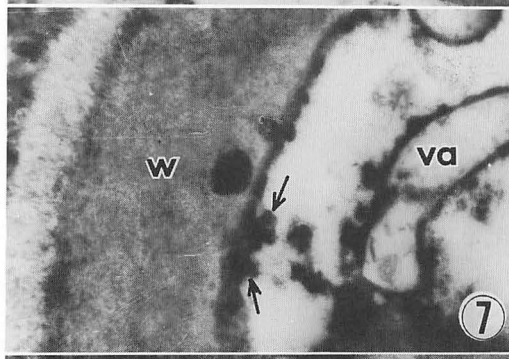
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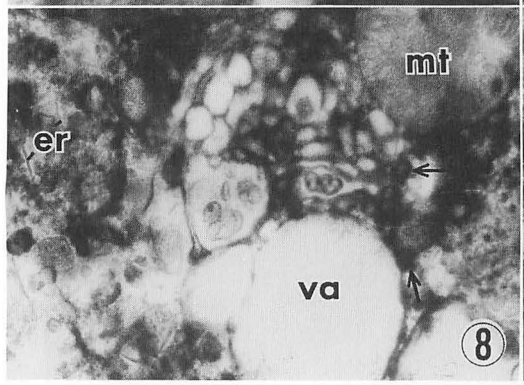
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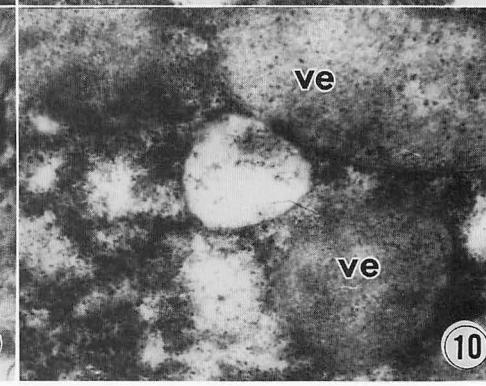
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deposited on the inner side of the wall (Fig. 7). The vacuoles containing electron dense materials are also abundant elsewhere in the cytoplasm.

The paraphyses possess vesicles that contain particulate electron-dense materials. These vesicles are identical to those found in the conceptacle-wall cell. The paraphyses have organelle-rich cytoplasm (Fig. 6). The nucleus is large, peripherally placed and has a well-defined nuclear membrane. Many vacuoles of different shapes and sizes; pleomorphic mitochondria, golgi bodies (Fig. 8), abundant osmiophilic droplets and endoplasmic reticula (Fig. 8) are present. In the mature paraphyses, organelles undergo lyses and the lysate is added to the wall material. Thus, the cell has an elaborate wall but vacuolate cytoplasm.

The conceptacle-wall cell cytoplasm reveals plastids, mitochondria, and golgi bodies. The golgi bodies are well developed and occur in both the formative and maturation faces. At the formative face many vesicles with fibrous material are produced (Fig. 9). The vesicles are pinched-off and later fuse between themselves. The golgi derived vesicles that are produced in the vicinity of the conceptacle-cell wall fuse with the wall and thus add to the wall material (Fig. 10). Also present inside the cell cytoplasm are vesicles which are filled with particulate, and electron-dense materials (Fig. 10).

Discussion

The walls of the paraphyses in *Sargassum johnstonii* and *S. vulgare* show short outgrowths that extend into the conceptacle cavities as seen in *Durvillaea potatorum* (Clayton *et al.* 1987). In addition, in *Sargassum* spp. the paraphysis cytoplasm has three types of vacuoles; 1) vacuoles with electron-dense particulate material, 2) vacuoles with electron-dense material and 3) with fibrous material. Such vacuoles are also present in the conceptacle-wall cells of *Sargassum* spp. In *Fucus edentatus*, the paraphyses cytoplasm contain vacuoles with different inclusions (McCully 1968).

In *Sargassum johnstonii* and *S. vulgare* the presence of numerous mitochondria and a few chloroplasts is another noteworthy feature of paraphysial cytoplasm which indicates a high rate of metabolic activity. Living *Fucus edentatus* paraphyses when viewed with fluoresence microscope suggest that the paraphyses cells possess abundant mitochondria (McCully 1968). Further, the conceptacle cavity in *Sargassum vulgare* and *S. johnstonii* is filled with sulphated and carboxylated polysaccharides that are perhaps secreted both by the paraphyses and the conceptacle-wall cells. Mucilage in the reproductive conceptacles in *Pelvetia canaliculata* is produced in cells lining the walls (Evans *et al.* 1973). A similar situation is found in *Fucus edentatus* (McCully 1968).

The role of alginic acid and sulphated polysaccharides is well known. According to Percival and McDowell (1967) they are mainly involved in prevention of desiccation. The polysaccharides in *Sargassum vulgare* and *S. johnstonii*, are presumed to form a soft, slippery cushion in which the sex organs develop. Once these organs are ready for release, the polysaccharides are at a low level in the conceptacle cavity. In *Cystophyllum sisymbrioides* paraphyses push their way through before the oogonia appear. The paraphyses are at first stiff but become soft after extrusion and form a slimy matrix in which the oogonia are en-

Figs. 6–10. *Sargassum johnstonii*. Transmission electron micrograph. Fig. 6. A paraphysis in transverse section to show a thick wall (w), peripherally placed nucleus (n), mitochondria (mt), vacuoles (va) of various sizes and osmiophilic bodies (ob). ×7,830. Fig. 7. Paraphysis wall (w) is the site of active discharge of phenolic materials (arrows). Many vacuoles (va) containing electron dense particulate material lie near the wall. ×21,140. Fig. 8. Portion of paraphyses to show abundant, electron dense osmiophilic bodies (arrows), and endoplasmic reticula (er) in the cytoplasm and different types of vacuoles (va) and pleomorphic mitochondria (mt). ×21,140. Fig. 9. Well formed golgi apparatus (gb) cuts off vesicles (ve) that aggregate below the conceptacle-cell wall (w). A vesicle is seen in the process of releasing its contents toward the wall (arrow). ×32,790. Fig. 10. Portion of conceptacle-wall cell cytoplasm to show coexistence of large and small vesicles filled with electron dense particulate materials. ×32,790.

closed (Tahara 1913). In *Bifurcaria brassiciformis*, the oogonia could be seen carried up on stalks which were themselves embedded in a tenacious common jelly, presumably derived from the walls of the paraphyses (Delf 1935).

The presence of phenolic materials in the conceptacular tissues is of great ecological significance. The ostioles in *Sargassum* spp. of the old receptacle are devoid of polysaccharidic plug-materials. The disappearance of plug-material upon release of oogonia/spermatozoids makes conceptacles susceptible to the attack by epiphytic and endophytic microbes. The abundance of the phenolic deposits in the persistent tissues of old conceptacles help to deter the attack of the microbes through invasion route. Phenolic materials also help to prevent attack by the herbivores (Clayton and Shankly, 1987). The paraphysial cytoplasm is packed with phenolic materials in *Sargassum* spp. throughout the conceptacle development. In *Scytosiphon* sp. the paraphyses have a protective role during gamete discharge, perhaps they help in preventing the excessive spread of lytic enzymes to the walls of immature gametangia (Clayton, 1984).

The conceptacles, during the last phase of reproductive season (January end to February) are totally devoid of contents. In *Sargassum johnstonii* and *S. vulgare* the conceptacle-cell wall, during this stage, is elaborate and possesses prominent, thread-like, projections as seen in *Durvillaea potatorum* (Clayton *et al.* 1987), where the conceptacle cells immediately adjacent to the conceptacle contents produce short filamentous outgrowths which extend into the central cavity.

The conceptacle-wall cells also reveal perinuclear golgi bodies which produce many vacuoles. The fibrous materials are frequently incorporated into the vacuoles at the formative region, undergo condensation and are released at the cell surface (present work). Such vacuoles have been termed as secretory vacuoles and have been shown to be released at the cell surface (Bouck 1962; Mollenhauer and Whaley 1963). The production of sulphated material by the meristoderm cells of *Pelvetia canaliculata* and secretory cells of *Laminaria saccharina* occurs in the golgi-rich, perinuclear areas (Evans et al., 1973). In pancreatic acinar cells, sulphation of material occurs in golgi bodies (Berg and Young 1971). Discharge of fibrous materials into the wall and cavity in *Sargassum johnstonii* and *S. vulgare* presumably occurs by the activity of golgi bodies present in the conceptacle-wall cells and the paraphyses and the energy required for the secretory process is provided by numerous mitochondria in the cell cytoplasm. These two cell populations, therefore, may be designated at least functionally as secretory cells.

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M. R. Vijayaraghavan · Inderdeep Kaur: *Sargassum vulgare* C. Agardh と *Sargassum johnstonii* Setchell & Gardner の側糸の組織化学と微細構造

Sargassum vulgare C. Agordh と *S. johnstonii Setchell & Gardner* は雌雄異巣の生殖器托をもつ。十分発達した生殖器巣は分泌液で満たされており，巣口にある多糖物質を介して外界と連絡している。生殖器巣には，平板状の生殖器巣壁細胞が並んでおり，それらは造精器か造卵器のいづれか，または側糸となる。若い生殖器巣では，側糸および生殖器巣壁細胞は，アルギン酸と硫酸多糖を含んでいる。生殖器巣の発達初期過程では，細胞内小器官が多くみられるが，老成した細胞では，それらは溶解し，側糸や生殖器巣壁細胞は液胞化する。(Department of Botany, University of Delhi, Delhi 110007, India)

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pH-dependent regulation of carbonic anhydrase induction and change in photosynthesis during adaptation of *Chlorella* cells to low CO_2

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Induction of carbonic anhydrase (CA) activity was pH-dependent when *Chlorella* grown under 3% CO_2 in air was transferred to low CO_2 conditions (ordinary air) at various pHs. Optimum pH for CA induction was 8.0 in *C. ellipsoidea* C-27, which has both intracellular and extracellular CAs, and 7.0 to 8.0 in *C. regularis* which has CA mostly on the cell surface. Below pH 5.5, CA induction was suppressed in both species even under low CO_2 conditions. As photosynthetic O_2 evolution in *C. ellipsoidea* C-27 adapted to low CO_2 for 3 h was independent of external pH when measured under the condition used for adaptation, the pH-dependency of CA induction cannot be attributed to that of the photosynthetic activity.

The rate of photosynthesis was kept constant at both pH 8.0 and 5.5 during adaptation to low CO_2. However, the rate measured with ethoxyzolamide (EZA), an inhibitor of CA, decreased gradually at both pHs. The suppression by EZA was not observed in the presence of 10 mM $NaHCO_3$. Rate of photosynthesis under CO_2-limiting conditions in cells adapted to low CO_2 with cycloheximide decreased gradually at both pH 8.0 and 5.5.

These results suggest that high rate of photosynthesis under CO_2-limiting conditions in cells adapted to low CO_2 is due to the function of carbonic anhydrase at high pH and due to an EZA-sensitive protein factor, which may enhance CO_2 transport, at low pH.

Key Index Words: carbonic anhydrase—Chlorella ellipsoidea—Chlorella regularis—*CO_2 acquisition—enzyme induction—low-CO_2 adaptation—pH effect—photosynthesis.*

Algal cells grown in ordinary air (low-CO_2 cells) exhibit higher affinity for CO_2 in photosynthesis and higher activity of carbonic anhydrase (CA) than those grown in CO_2-enriched air (high-CO_2 cells) (see review by Raven 1984, Aizawa and Miyachi 1986, Badger 1987). Those changes induced during adaptation to low CO_2 have been reported to be regulated by several environmental factors. For example, light plays an important role in CA induction and its effect is different depending on algal species. Namely, CA induction in *Chlamydomonas reinhardtii*, which has mainly extracellular CA, showed a requirement of both high energy of light for photosynthesis and low energy of blue light as a photosignal (Kimplel *et al.* 1983, Dionisio *et al.* 1989a, b, 1990). However, only photosignal is essential for CA induction in *Chlorella vulgaris* 11 h, which has only intracellular CA (Shiraiwa *et al.* 1981, Shiraiwa and Miyachi 1983) and in *Chlorella regularis*, which has mostly extracellular CA (Umino *et al.* 1991). Effect of other factors such as CO_2 concentration (Shiraiwa and Miyachi 1985), temperature (Shiraiwa and Miyachi 1985) and O_2 concentration (Shiraiwa *et al.* 1988) on CA induction were examined mainly in *Chlorella vulgaris* 11 h, but few works in other algae.

Recently, pH was shown to be an important factor regulating CA induction in *Chlamydomonas*. Patel and Merrett (1986)

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Abbreviations: CA, carbonic anhydrase; DIC, dissolved inorganic carbon; EZA, ethoxyzolamide; CHI, cycloheximide; high-CO_2 cells, algal cells grown in air enriched with 3% CO_2; low-CO_2 cells, algal cells grown in air; pcv, packed cell volume.

showed that CA induction during adaptation of high-CO_2 cells to air was enhanced concomitantly with pH, although how the induction of CA is controlled by pH is not elucidated yet. As dissociation of DIC is strongly dependent on pH of the medium, phenomena induced by a change in DIC concentration, such as CA induction and change in an affinity of photosynthesis, may be probable to be affected directly and/or indirectly by change in pH. Response of the induction of CAs with different locations in a cell to external pH is also interesting to be compared.

In the present study, we therefore investigated the effect of pH on CA induction within a wide range of pH, from 3 to 9, in two species of *Chlorella* which have different CA localization. Effect of pH on CO_2 acquisition in photosynthesis during adaptation of high-CO_2 cells to low CO_2 conditions was also tested.

Materials and Methods

Algal materials and culture—Chlorella ellipsoidea Gerneck (IAM C-27) was obtained by courtesy of Prof. T. Hirokawa of Niigata University. *Chlorella regularis* (Endo *et al.* 1974) was a kind gift of Prof. S. Miyachi of University of Tokyo. These algae were grown autotrophically in a flat oblong glass vessel containing *ca.* 1.3 liter of the inorganic MC medium (Watanabe 1960). The suspensions were continuously aerated with ordinary air enriched with 3% CO_2 to obtain cells adapted to high CO_2 (high-CO_2 cells). To obtain air-adapted cells (low-CO_2 cells), the cells harvested by centrifugation were suepended in an appropriate buffer at a density of 3 m*l* pcv·liter^{-1}, and then transferred to air. The algal suspension was continuously illuminated by 200 W-incandescent reflector lamp (Toshiba, Tokyo) at 1.2 kW·m^{-2} (16 klux). The temperature during the growth and the adaptation to air was kept under the optimum conditions for photosynthesis and growth in each alga, namely at 25°C in *Chlorella ellipsoidea* and at 30°C in *Chlorella regularis*.

Determination of photosynthetic O_2 evolution—Algal suspension (5 ml) of *Chlorella ellipsoidea* harvested from the culture was immediately transferred into a water-jacketed transparent glass-cylinder equipped with a Clark-type oxygen probe (Rank Brothers, London). After 1-min incubation in the dark, photosynthesis was initiated by illumination by a tungsten projector lamp at 1.4 kW·m^{-2} (18 klux), and change in O_2 concentration in the medium was continuously measured by the O_2-electrode. The temperature was kept at 25°C.

Carbonic anhydrase assay—Enzyme assay was carried out according to the method of Wilbur and Anderson (1948). To measure CA activity localized on the cell surface of intact cells (E), 2 m*l* of CO_2-saturated water was added to 3 m*l* of 20 mM (final concentration, 12 mM) sodium veronal buffer (pH 8.3) containing 100 μl of the suspension of intact cells suspended in 100 mM Tris-H_2SO_4 buffer (pH 8.3). The time required for pH change from 8.3 to 7.3 was measured at 2°C. The reaction mixture was continuously stirred by a magnetic stirrer. To determine CA activity in cell homogenates (H), 100 μl of homogenates obtained by disruption of algal cells suspended in 100 mM Tris-H_2SO_4 buffer (pH 8.3) with a French Pressure Cell (Ohtake Seisakusho, Tokyo) at 147 MPa (1,500 kg·cm^{-2}) was used for the CA assay. Enzyme units were calculated using the following equation:

$$\text{units} = t_b \cdot t_e^{-1} - 1$$

where t_b and t_e represent the time (seconds) needed for the pH change with or without sample, respectively. Internal CA activity (I) was calculated by H−E.

Results

Effect of pH on the increase in CA activity during adaptation of high-CO_2 cells to low CO_2 for 3 h was tested in *Chlorella ellipsoidea* C-27 and *Chlorella regularis* (Fig. 1). CA activity detected both inside the cells and on the cell surface was almost equal in *Chlorella ellipsoidea* C-27, whereas more than 97% of CA was located on the cell surface in *Chlorella regularis*. Both intra- and extracellular CA ac-

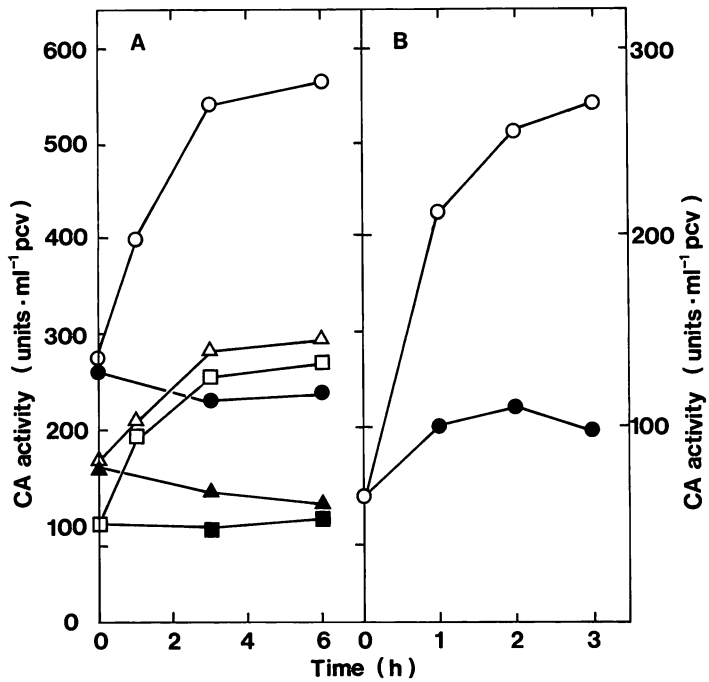

Fig. 1. Time course of CA induction in *Chlorella* after the transfer of high-CO_2 cells to air-level CO_2. A: *Chlorella ellipsoidea* C-27. Open and closed marks, in cells adapted to low CO_2 at pH 8.0 and pH 3.0, respectively. Circles, CA activity in cell homogenate; triangles, extracelular CA activity; squares, intracellular CA activity. B: *Chlorella regularis*. Open and closed circles, extracellular CA activity in cells adapted to low CO_2 at pH 8.0 and pH 5.5, respectively.

tivities of *Chlorella ellipsoidea* took a paralel time course and increased 2-3 times during 3-h adaptation to air at pH 8.0, but no increase in any CA activities was observed at pH 3.0 (Fig. 1A). Extracellular CA activity of *Chlorella regularis* also increased 3 times during 3-h adaptation to air at pH 8.0, but only slight increase was observed at pH 5.5 (Fig. 1B). Optimum pH for the increase in intra- and extracellular CA activities was 8.0 in *Chlorella ellipsoidea* C-27 (Fig. 2A). Intracellular and extracellular CA activities were similarly changed depending on pH. The optimum pH for the increase in external CA in *Chlorella regularis* was 7-8 (Fig. 2B).

In *Chlorella ellipsoidea* cells adapted to low CO_2 at various pHs from 5.5 to 9.0 for 3 h, photosynthetic activity was measured immediately after the transfer of algal suspension from the culture to the reaction vessel. The activity measured without any additives showed no marked variation between pH 5.5 and 9.0. However, the activity measured with 10 mM $NaHCO_3$ clearly exhibited pH-dependence with a peak at pH 8.0 (Fig. 3). The rate of photosynthetic O_2 evolution was suppressed at 10 mM $NaHCO_3$ below pH 6.5, but enhanced above the pH.

When high-CO_2 cells of *Chlorella ellipsoidea* C-27 were transferred to air at pH 8.0 and 5.5, the rates of photosynthesis were almost constant during the adaptation to air at pH 8.0 and 5.5 (Fig. 4). At pH 8.0, the rate was gradually decreased by the addition of 0.1 mM EZA, a membrane-permeable inhibitor of CA, to reach about one-half value of the control. The suppression was recovered by the addition of 10 mM $NaHCO_3$. At pH 5.5, the rate of photosynthesis was strongly decreased at 0.01 mM EZA, but the rate was recovered at 1 mM $NaHCO_3$. The recovery rated almost 100% at the beginning of air-adaptation, but almost linearly decreased by 50% of the value in control for 200 min

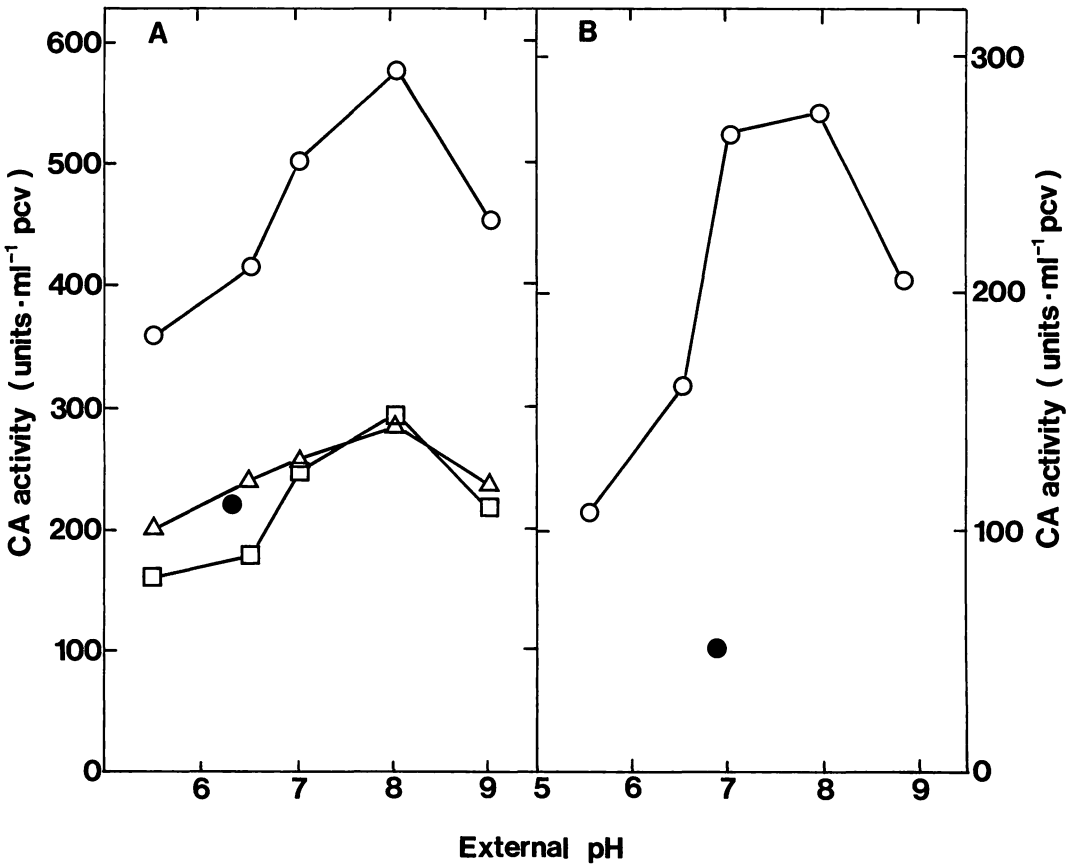

Fig. 2. pH-dependent curves of CA induction in *Chlorella* when high-CO_2 cells were adapted to air-level CO_2 for 3 h. A: *Chlorella ellipsoidea* C-27. Circles, CA activity in cell homogenate; triangles, extracellular CA activity; squares, intracellular CA activity; closed circles, CA activity in the cell homogenate of high-CO_2 cells. pH was kept nearly constant at respective pH by 20 mM MES-Tris buffer containing 1/20 concentration of the culture medium during the adaptation to air. B: *Chlorella regularis*. Open circles, extracellular CA activity in low-CO_2 cells; closed circles, extracellular CA activity in high-CO_2 cells. pH was constantly maintained at respective pH by 50 mM MES-NaOH buffer below pH 6.5 and by 50 mM Tris-H_2SO_4 buffer above pH 7. Both buffer contained 1/20 concentration of the culture medium.

(Fig. 4C). The rate of photosynthesis in control decreased with time when high-CO_2 cells were transferred into air with cycloheximide (CHI), an inhibitor of translation of protein synthesis on 80 S ribosomes (Fig. 4B, D). The rate was hardly affected by EZA at pH 8.0 (Fig. 4B), but strongly limited at pH 5.5 for about 2 h after the start of the adaptation (Fig. 4D). Photosynthesis under CO_2-saturating conditions in CHI-treated cells did not change during the adaptation to low CO_2 at both pH 8.0 and 5.5 (Fig. 4B, D). 0.1 mM EZA was inhibitory to the maximum photosynthesis at pH 5.5, but not at pH 8.0 (unpublished data and Fig. 4A).

Discussion

CA induction during the adaptation of high-CO_2 cells to low CO_2 preferred alkaline pH rather than acidic pH where it was strongly suppressed (Fig. 1). The result is similar to that observed in *Chlamydomonas* (Patel and Merrett 1986). The optimum pH for CA induction was around 8 in both *Chlorella ellipsoidea* and *Chlorella regularis* which have different localization of CA (Fig. 2). Optimum pH for photosynthesis under CO_2-saturating

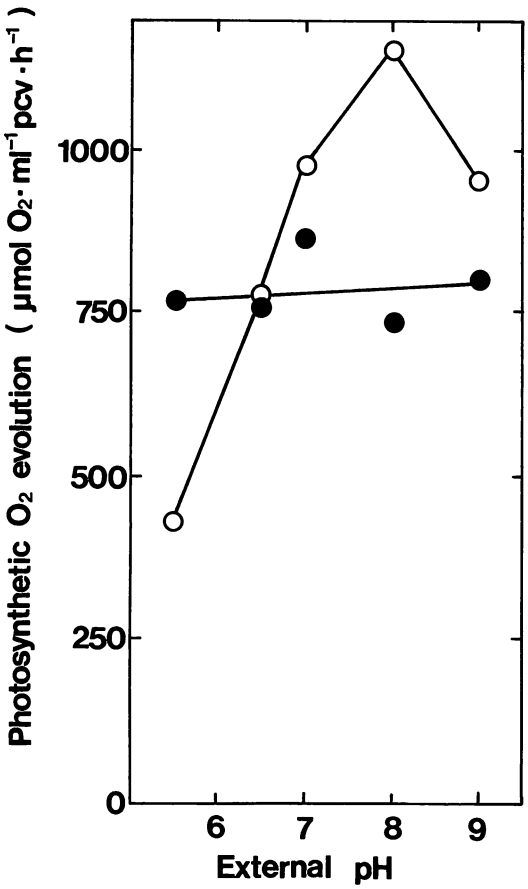

Fig. 3. Effect of pH during adaptation on photosynthesis in *Chlorella ellipsoidea* C-27 adapted to air-level CO_2 for 3 h. Algal cells were adapted to air at various pHs from 5.5 to 9.0 for 3 h, and thereafter the suspension was transferred to the vessel equipped with O_2-electrode. After 1-min incubation in the dark, the rate of photosynthetic O_2 evolution was determined with or without addition of 10 mM $NaHCO_3$. Open and closed circles, rates of photosynthetic O_2 evolution measured with or without the addition of 10 mM $NaHCO_3$, respectively.

conditions was also 8 in both *Chlorella ellipsoidea* (Fig. 3) and *Chlorella regularis* (data not shown). As photosynthesis measured under CO_2-limiting conditions in cells adapted to air for 3 h was similar among various pHs (Fig. 3), difference in CA activity induced at various pHs would be independent of that in photosynthetic activity.

For it has been reported that internal pH was not so strictly affected by changes in external pH in *Chlorella* (Tsuzuki *et al.* 1985), change in CA induction at various pHs could be due to effect of changes in external pH rather than internal one. Active DIC absorbed by cells for photosynthesis in both high- and low-CO_2 cells of *Chlorella ellipsoidea* (Nara *et al.* 1990) and *Chlorella regularis* (Satoh and Shiraiwa, unpublished) is free CO_2, not HCO_3^-. The concentration of free CO_2 dissolved in the culture medium may be equilibrated with that of atmospheric CO_2 because of strong aeration. Therefore, the concentrations are thought to be same among various pHs because the solubility of CO_2 is not affected by pH. These things also suggest that DIC acquisition and CA induction are controlled mainly by changes in pH of the medium.

When high-CO_2 cells were transferred to low CO_2, photosynthesis can be limited by CO_2 transfer from the medium to the site of CO_2 fixation by ribulose-1,5-bisphosphate carboxylase/oxygenase. Under the conditions, physiological basis of adaptation to low CO_2 conditions seems to vary depending on pH. The photosynthetic activity of the cells adapted at pH 8.0 was sensitive to EZA, indicating that CA induction is the major strategy of adaptation to low CO_2 at this pH. The data from the experiments with cycloheximide (Fig. 4A, B) are in fair agreement with this. On the other hand, the adaptation to low CO_2 at pH 5.5 is assumed not to be achieved by the induction of CA, because CA is not functional at acidic pH where DIC mostly exists in the form of free CO_2, an active species of DIC absorbed by *Chlorella* (Nara *et al.* 1990). A reason for inhibition of photosynthesis by EZA at pH 5.5 (Fig. 4C, D) is not elucidated yet. A possible speculation may be the inhibitory effect of EZA on the membrane transport of DIC, since EZA affected the membrane permeability to glycolate in *Chlorella vulgaris* (Shiraiwa and Schmid 1986). Notable in this respect is the fact that the adaptation to low CO_2 at pH 5.5 is sensitive to cycloheximide, as is at alkaline pHs (Fig. 4). This indicates that protein(s) other than CA has to be synthesized for the adaptation, in order to enhance DIC utilization at low pH.

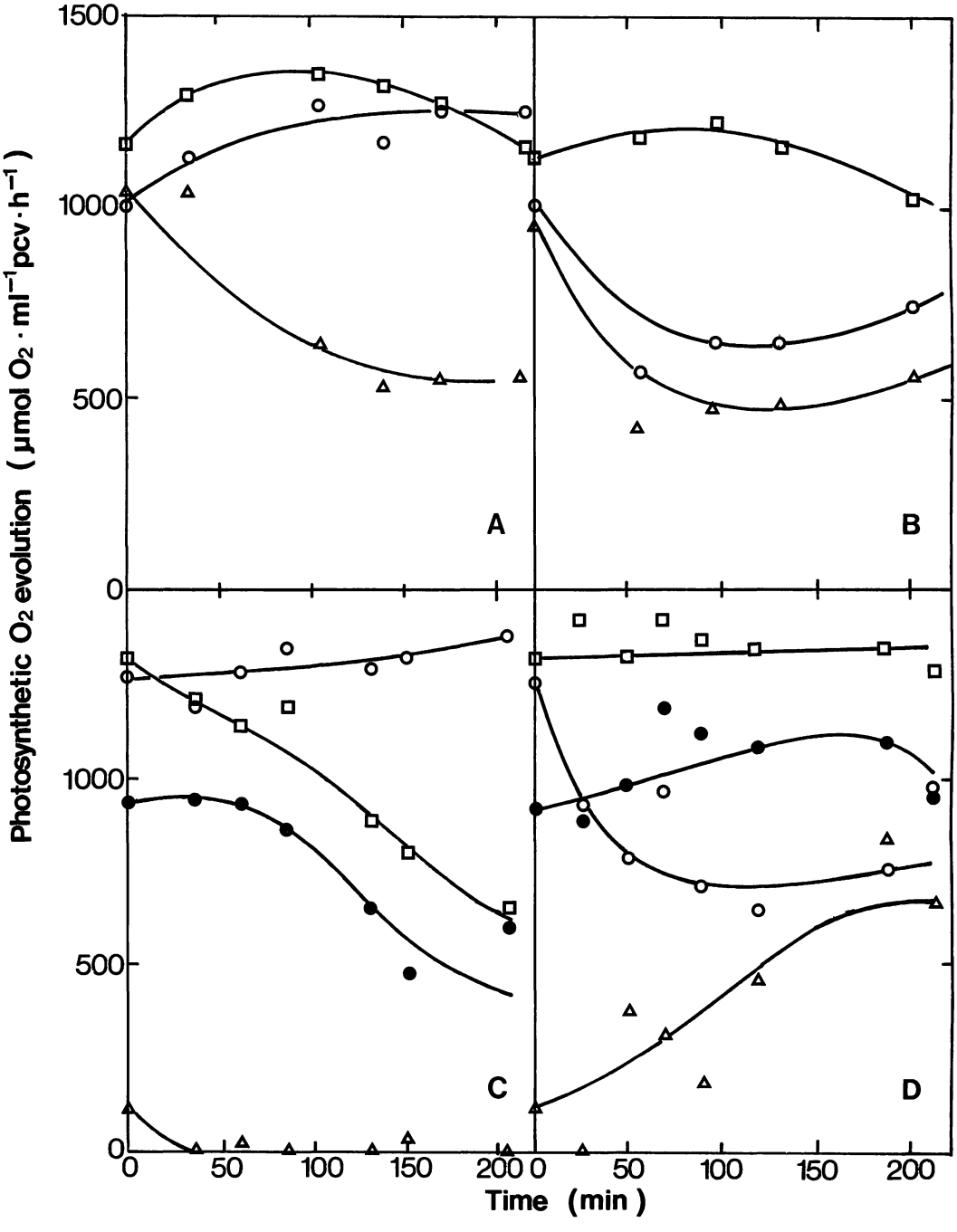

Fig. 4. Change in the rate of photosynthetic O_2 evolution in *Chlorella ellipsoidea* C-27 after the transfer of high-CO_2 cells to air-level CO_2. A and B: Cells adapted to air at pH 8.0 in the absence and presence of 17.8 μM cycloheximide, respectively. Circles, control; triangles, +0.1 mM EZA; squares, +0.1 mM EZA+10 mM $NaHCO_3$. C and D: Cells adapted to air at pH 5.5 in the absence and presence of 17.8 μM cycloheximide, respectively. circles, control; triangles, +0.01 mM EZA; squares, +0.01 mM EZA+1 mM $NaHCO_3$; closed circles, +0.01 mM EZA+10 mM $NaHCO_3$. Buffers used at respective pHs were the same ones as in Fig. 2.

As shown in Fig. 3, photosynthesis in low-CO_2 cells of *Chlorella ellipsoidea* under CO_2-saturated conditions was several times higher at pH 8.0 than pH 5.5, although that under CO_2-limiting conditions was almost same at various pHs. Photosynthesis at pH 5.5 was diminished by the addition of high concentration of DIC. The reason for the suppression is still unclear. One possibility, as assumed by Hogetsu and Miyachi (1979), is a strong drop of internal pH caused by absorbing huge amount of CO_2 and the subsequent conversion to HCO_3^- and H^+. These results in *Chlorella* are inconsistent with those, reported in *Chlamydomonas* by Patel and Merrett (1986), showing that photosynthesis under CO_2-saturated conditions at pH 5.5 was three times higher than that at pH 7.5 in both high- and low-CO_2 cells. As the concentration of DIC accumulated in the cells was two times higher at pH 7.5 than pH 5.5 in low-CO_2 cells, but was independent of pH in high-CO_2 cells, it is considered that photosynthetic activity is dependent on external pH proper, but independent of internal DIC accumulated. The mechanism how external pH affects the CA induction and the change in photosynthetic CO_2 fixation during adaptation to low CO_2, and the reason why pH-dependency of photosynthesis is different between *Chlorella* and *Chlamydomonas* remains to be elucidated.

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白岩善博・横山真也・佐藤　朗：クロレラの低 CO_2 条件への適応に伴うカルボニックアンヒドラーゼ誘導と光合成活性変動の pH による調節

カルボニックアンヒドラーゼ(CA)の局在性が異なる二種の単細胞緑藻 *Chlorella ellipsoidea* C-27 および *Chlorella regularis* を用いて低 CO_2 条件への適応に伴う CA 誘導および光合成の CO_2 に対する親和性の変動に及ぼす pH の影響を調べた。CA 誘導の至適 pH は藻種および CA の局在性に関わらず pH 8 付近であり，pH 5.5 以下では CA の誘導は認められなかった。高（3％）CO_2 条件に適応した *C. ellipsoidea* を低（0.03％）CO_2 条件に移した場合，CO_2 律速条件における光合成活性は pH 5.5 および8.0のいずれの pH でも変動しなかった。しかし，CA 阻害剤であるエトキシゾルアミド添加条件下では CO_2 律速条件下での光合成は時間と共に減少した。また，いづれの pH でも，低 CO_2 への適応時にシクロヘキシミドを添加すると，光合成の CO_2 に対する親和性の増大が阻害された。以上の結果より，これらの藻種が，アルカリ域では CA を誘導することにより，また，酸性域では CO_2 利用を促進する CA 以外のタンパク性因子を誘導することにより CO_2 律速条件下での光合成活性を維持していることが示唆された。（950-21 新潟市五十嵐二の町8050　新潟大学理学部生物学科）

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Diatom assemblages in a high moor: an observed correlation between species composition and pool size

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Katoh, K. 1991. Diatom assemblages in a high moor: an observed correlation between species composition and pool size. Jpn. J. Phycol. **39**: 363–368.

The species composition of periphytic diatom assemblages collected from Kinunuma Moor, an high moor at an altitude of about 2000 m, was analyzed. A correlation between the species composition and pool size was observed. *Eunotia* species, such as *E. curvata* (Kütz.) Lagerst., *E. curvata* v. *subarcuata* (Kütz.) Woodhead et Tweed, *E. pectinalis* v. *minor* (Kütz.) Rabh., *E. tenelloides* H. Kob. *et al.*, were dominant in large pools (more than 10 m across), whereas *Frustulia rhomboides* v. *saxonica* (Rabh.) De Toni was dominant in smaller pools (less than 1 m across). In medium sized pools the relative abundance of *Eunotia* species and *Frustulia* species was almost equal, but *Asterionella ralfsii* W. Sm. was sometimes dominant. The water temperature was likely to be higher in smaller pools than in larger pools. Therefore, water temperature, its stability, or the stability of total environment is assumed to have some relation to the observed variation in species composition.

Key Index Words: Asterionella ralfsii—*diatoms—ecological stress*—Eunotia curvata—Frustulia rhomboides *v.* saxonica—*high moor—water temperature.*

Diatom assemblages in moors have been surveyed by some diatomists (e.g. Hirano 1976, 1977). But there are few reports about the relation between environmental conditions except for pH and species composition of diatom assemblages in moors: the pH preference of diatom species was analyzed by many authors (e.g. Van Dam *et al.* 1981, Watanabe and Yasuda 1982).

The species composition of diatom assemblages is influenced by conditions other than pH. Van Dam *et al.* (1981) showed that the abundance of *Eunotia exigua* (Bréb.) Rabh. increases with the concentration of sulfate. Scherer (1988) discussed the importance of local variation in trophic status and some restrictive conditions, such as occasional desiccation, to species composition. Van Dam (1988) also suggested the importance of desiccation to species composition.

In high moors smaller pools often dry up whereas larger pools always contain water. Considering the results of the works mentioned above, it appears that species composition of diatom assemblage has some relation to pool size. The aim of this study is mainly to analyze the relation between the species composition of diatom assemblages and pool size.

Materials and Methods

Kinunuma Moor was selected as the study field. The moor is located on the mountain ridge on the border of Tochigi Prefecture and Gunma Prefecture, at an altitude of about 2000 m (Fig. 1), and the latitude and longitude of the moor are 36°52.5′N and 139°22.5′E, respectively. The area of this moor is almost 800 m (north to south) × 400 m (east to west). There are about 50 pools in it. The largest one is "Kin-Numa" (Kin pool) with dimension of about 80 m × 40 m, and small pools are less than 1 m across.

Field surveys were made on 29 June, 21 July and 29 September in 1986. Eleven sampling stations were set in ten pools and one

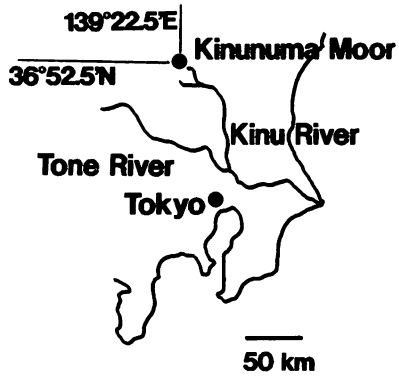

Fig. 1. The location of Kinunuma Moor.

brooklet which issued from a large pool. As the flow of the brooklet stopped before the third survey (29 September) because of a drop in the water level of the source, a sample was collected from a puddle which was a remnant of the brooklet.

At the second and the third surveys, water temperature (WT) and pH were measured by alcohol thermometer and test papers (Toyo Roshi Co. Ltd.), respectively. Electric conductivity (EC) was measured at the second survey by portable conductivity meter (TOA model CM-1K). Pool size was recorded in three ranks, large (more than 10 m across), medium (from 1 m to 10 m across) and small (less than 1 m across). Usually, pool area and pool depth were positively correlated although the depth of some small or medium sized pools was almost equal to that of the large pools. Station 9 was located at such a medium sized and deep pool.

Diatoms were collected from the surface of dead grasses in water. Samples were cleaned with H_2SO_4 and mounted in Pleurax. The relative abundance of diatom taxa occurring in each sample was obtained by counting the number of valves; more than 400 valves were counted for each sample.

Data were analyzed using multivariate analysis. Hierarchical cluster analysis and principal component analysis (PCA) were carried out for the classification and ordination of the samples and species. Bray-Curtis similarity index (Bray and Curtis 1957) was used in cluster analysis. It is known by a variety of names such as Czekanowski's index, Least Common Percentage Index, is said to be robust, and reflects accurately true similarity (Bloom 1981, Faith *et al.* 1987). In PCA each sample and taxon become "variable" and "sample" of the data matrix, respectively. Based on the result, the effect of pool size and other environmental factors were analyzed.

Results

The measured parameters of the pool water are listed in Table 1. Water temperature had

Table 1. Characters of water at stations 1–11.

Station No.	Area	pH (July)	EC (μS/cm) (July)	WT (°C) (July)	WT (°C) (Sept.)
1	(brooklet)	5.4	8.4	15.1	10.4
2	small	—[1]	4.2	—	*[2]
3	("Kin-numa") large	5.4	7.5	15.1	11.0
4	small	5.2	6.7	18.1	11.0
5	medium	5.2	11.2	16.5	13.5
6	small	—	—	—	14.0
7	medium	5.2	8.6	16.5	—
8	medium	5.6	7.9	17.2	12.0
9	medium[3]	—	—	*	11.0
10	small	—	—	*	14.0
11	large	—	—	*	*

[1] "—" indicates that the parameter was not measured whereas diatom sampling was carried out.
[2] "*" indicates that no work was done at the station.
[3] Deeper than the other medium sized pools (see text).

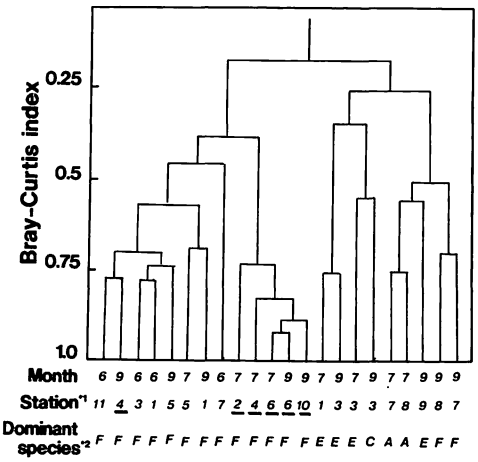

Fig. 2. The result of the cluster analysis (furthest neighbor method). *1: The underlined stations are for small pools. *2: "F", "E", "C" and "A" are for *Frustulia rhomboides* v. *saxonica, Eunotia curvata* or its varieties, *Fragilaria construens* v. *bidens*, and *Asterionella ralfsii*, respectively.

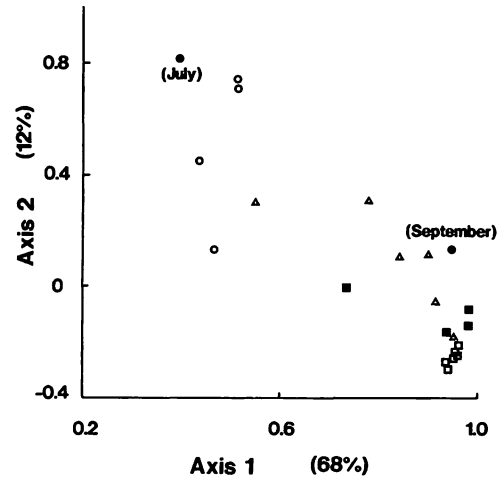

Fig. 3. Factor loadings of the principal component analysis. The parenthesized values beside the axis number are proportions of the eigenvalue of each axis (i.e. principal component). Small, medium and large pools are indicated by squares, triangles and circles, respectively. The samples collected in June are indicated by solid squares. The samples collected from Station 1 (brooklet) are indicated by solid circles with the sampling month parenthesized.

a tendency to increase in small pools, though the number of samples was small. In the brooklet and larger pools WT was relatively low.

Figure 2 gives the result of the cluster analysis. The samples were classified into three categories and the classification was correlated with pool size. This correlation suggested that pool size was an index of the most important environmental factor affecting the species composition of diatom assemblages.

The first category is the group of samples in which *Eunotia curvata* (Kütz.) Lagerst. and its varieties were dominant. The samples from larger pools tend to belong to this category. Station 9 was a medium sized pool but deeper than any other medium sized pools. Samples of the second category contained *Asterionella ralfsii* W. Sm. as the foremost or secondmost abundant taxon. All samples belonging to this category were collected from medium sized pools. Samples in which *Frustulia rhomboides* v. *saxonica* (Rabh.) De Toni was the most dominant belong to the third category. All the samples from small pools belonged to this category. In the brooklet (Station 1) diatom assemblage was similar to that in large pools as long as the flow did not stop. In the samples collected on 29 June, *Frustulia rhomboides* v. *saxonica* was the most dominant and the samples belonged to the third category.

Figure 3 shows the factor loadings of the variables (=diatom samples) of the PCA. This figure shows that, according to the samples collected, a predictable difference is always found in the species composition of

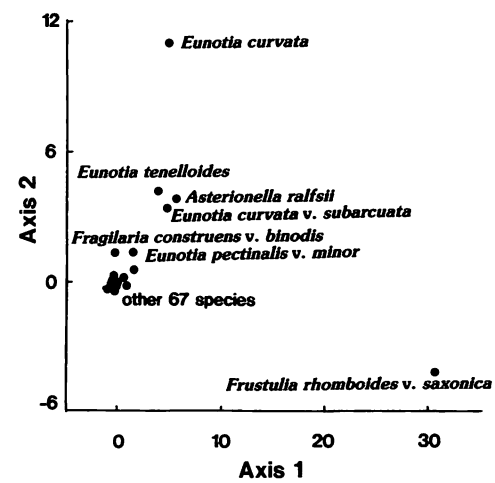

Fig. 4. Principal component scores of the principal component analysis.

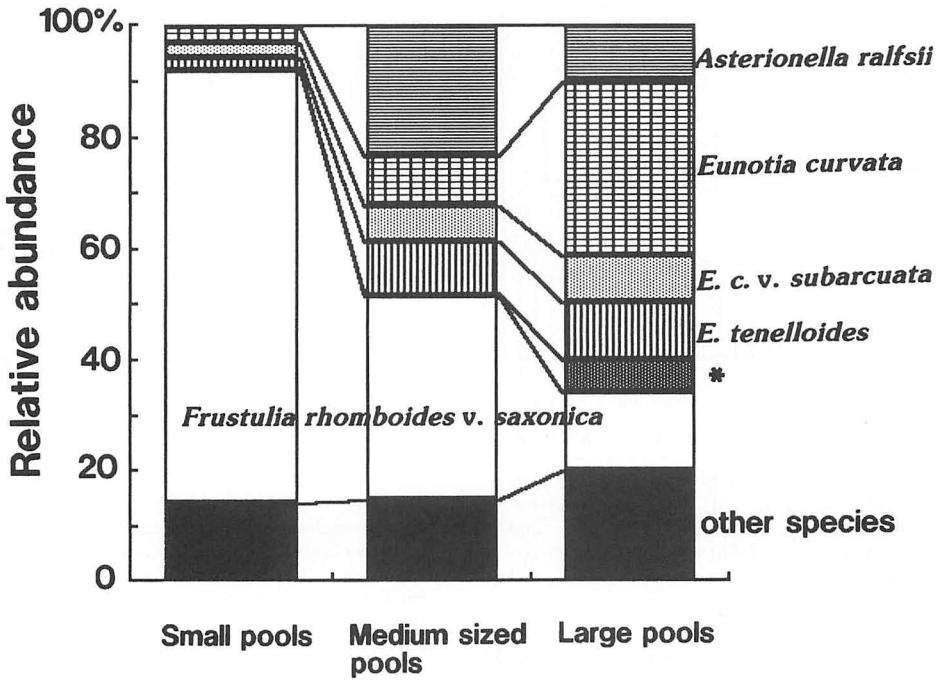

Fig. 5. Average species composition of the diatom assemblages in the small, medium and large pools. *: *Fragilaria construens* v. *binodis*.

small and large pools. This difference seems to be proportional to the size of the pools as the medium pool samples range between the small and large samples. This figure also shows that the samples collected in June were similar to the samples collected from small pools or the samples collected from some of the medium sized pools.

Figure 4 shows the principal component score of each taxon. The preference of each taxon for a particular pool size is understood by Figures 3 and 4. *Eunotia curvata, E. curvata* v. *subarcuata* (Kütz.) Woodhead *et* Tweed, *E. pectinalis* v. *minor* (Kütz.) Rabh., *E. tenelloides* H. Kob. *et al., Fragilaria construens* v. *binodis* (Ehr.) Grun. and *Asterionella ralfsii* each had large second principal component score. It appears that these taxa prefer larger pools to smaller ones. This preference was confirmed by the average relative abundance of diatoms in small, medium and large pools (Fig. 5).

Discussion

It is evident that the genera *Eunotia* and *Frustulia* were dominant in the pools of the Kinunuma Moor. This result coincides with those of other studies such as Hirano (1976, 1977). The genus *Pinnularia*, which is known as one of the popular genera in high pools (Hirano 1977, Hirano and Iwaki 1982), did not appear frequently, but more than a few taxa (10 species and 1 variety) of this genus were found. *Asterionella ralfsii* was dominant in some of the samples. This taxon prefers acidic and humic water (Patrick and Reimer 1966). It is concluded that the diatom assemblages in the pools of Kinunuma Moor were typical of high moors.

The water temperature was likely to be higher in small pools than in large pools (Table 1). A similar correlation between water temperature and pool size was observed at high moors in the Minamiaizu district, Fukushima Prefecture, Japan (Katoh, in printing). It is assumed that the heat capacity of water bodies affects the water temperature, and that water temperature is more stable in large pools than in small pools.

The correlation between species composi-

tion and pool size observed in the present study was also observed at 23 high moors (119 samples) in the Minamiaizu district (Katoh, in printing). Scherer (1988) states that the dominant species of diatom assemblage in marshes corresponds to the level of "ecological stress": *Eunotia exigua, Frustulia rhomboides* v. *saxonica* and *Asterionella ralfsii* correspond to quite severe, moderately severe and less severe "ecological stress", respectively. In the present study *E. exigua* was not dominant because no station was in an extremely severe environment: this species was often dominant in the samples collected from the wet ground of high moors but not dominant in pools (Katoh, in printing). As for *Frustulia rhomboides* v. *saxonica* and *Asterionella ralfsii* the result of the present study is similar to that of Scherer (1988), if "ecological stress" includes the instability of water temperature, or if "ecological stress" becomes more severe as the pool size decreases. Van Dam (1988) suggested the importance of desiccation to species composition, and it is reasonable that desiccation is more likely to occur in smaller pools than in larger pools. Therefore, it appears that the species composition of diatom assemblages in high moors has some association with pool size through environmental instability such as the instability of water temperature and occasional desiccation, or "ecological stress".

It is possible that an unstable environment itself limits the species composition. It is also possible that the development of diatom assemblages is disturbed and species which normally occur at later stages of this development can not dominate in an unstable environment. It was reported that *Eunotia curvata* and *E. pectinalis* are abundant at later stages of this development under acidic conditions (Planas *et al.*, 1989). Further studies are needed on the causes of different species composition in small and large pools.

Based on the results, one possible explanation is as below for the species composition of the samples collected from Station 1 or collected at the first survey. In the stable brooklet, environmental conditions were similar to those in large pools (in July). When the flow stopped (in September), its conditions became unstable like those in smaller pools. The environmental conditions of pools in the season of thaw (June) also seem to be unstable because of the irregular inflows from melting snow.

It is concluded that environmental instability (or "ecological stress") influences species composition: pool size is regarded as an index of environmental instability. Assemblages dominated by *E. exigua*, which were not dominant in the present study, are established in the most unstable habitat. Assemblages dominated by *F. rhomboides* v. *saxonica* develop under a moderately unstable environment. Assemblages dominated by *Asterionella ralfsii* occur when the environment becomes more stable. Assemblages in which *Eunotia curvata* and its varieties are dominant are found in quite stable habitats.

Acknowledgments

I would like to express my sincere thanks to Dr. H. Kobayasi, President of the Japanese Society of Diatomology, and Dr. S. Mayama, Tokyo Gakugei University, for their advice on the identification of diatoms.

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加藤和弘：高層湿原の珪藻群集—種組成と池塘の大きさとの間に見られた関係

鬼怒沼湿原は標高約2000 mに位置する高層湿原である。同湿原で付着珪藻群集の調査を行った結果，群集の種組成と池塘の大きさとの間に関連がみられた。小型の池塘では *Frustulia rhomboides* v. *saxonica* (Rabh.) DeToni が優占し，大型の池塘では *Eunotia curvata* (Kütz.) Lagerst., *E. curvata* v. *subarcuata* (Kütz.) Woodhead et Tweed, *E. pectinalis* v. *minor* (Kütz.) Rabh., *E. tenelloides* H. Kob. *et al.* が優占した。中程度の大きさの池塘では，*Frusturia* 各種と *Eunotia* 各種はほぼ同じくらい出現したが，時に *Asterionella ralfsii* W. Sm. が優占した。大型の池塘でより低い水温が記録されたことから，水温またはその変わりやすさが種組成に関わるとも考えられるが，環境全般の安定性が関与した可能性もある。（153 東京都目黒区駒場3-8-1　東京大学教養学部生物学教室）

Jpn. J. Phycol. (Sôrui) 39: 369-373, December 10, 1991

Ryozo Seto, R. N. Yadava and Shigeru Kumano: Development of short spinous branchlets of *Compsopogon aeruginosus* var. *catenatum* (Compsopogonaceae, Rhodophyta)

Key Index Words: central cells—Compsopogon aeruginosus *var.* catenatum—*freshwater-Rhodophyta—spinous branchlets.*
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Krishnamurthy (1962) mentioned that *Compsopogon aeruginosa* is very similar to *C. coeruleus*, but can be distinguished by two sharp features: 1) the monospores are comparatively small, and 2) the older thallus and the main axis bear short spinous branchlets. The latter feature, short spinous branchlets, is observed on the Indian specimens collected from various parts of Gujrat by Patel and Francis (1969) and the Japanese specimens from Lake Shinji by Nakamura and Chihara (1983).

Compsopogon aeruginosus var. *catenatum*, which has spinous branchlets in the nodulated axis, was described as a new variety by Yadava and Pandey (1980) based on the Indian specimens collected from Dorania River at Bareilly. A new form of *Compsopogon aeruginosus* with spinous branchlets was also reported by Singh and Pandey (1986) from Nakatia River. This paper deals with the development of spinous branchlets based on the specimen of *Compsopogon aeruginosus* var. *catenatum* collected from Nakatia River in India.

Observations

1. **Specimens examined in the present study:** The specimens of *Compsopogon aeruginosus* var. *catenatum* collected by R. N. Yadava from Nakatia River near Bareilly in India in August 1988 were deposited in the herbaria of Department of Botany, University of Allahabad in India, and Department of Biology, Faculty of Science, Kobe University in Japan.

2. **Development of filamentous branches prior to cortication:** The younger portion of the thallus remains uniseriate. Prior to cortication, an erect thallus may give rise to several angular branches by oblique divisions of axial central cells. The cell destined to form a filamentous branch pushes out laterally and distally and the protuberance thus formed is delimited by a cross-wall as the initial cell of a filamentous branch. The initial cell then undergoes successive transverse divisions to give rise to a uniseriate filamentous branch (Figs. 1-3). This filamentous branch may eventually develop cortical cells in the same manner as the main axis. Filamentous branches generally originate to form an angle of 30 to 60 degrees with reference to the main axis. Leteral filamentous branches of main thalli are also richly branched, arise alternatively, and are uniseriate.

Then, a uniseriate filamentous branch is formed by a series of discoid cells developed by repeated transverse divisions of the dome-shaped apical cell. A few cells below the apical cell of a branch do not divide while other axial rows of cells divide periclinally and form a number of peripheral segments. These cells subsequently transform into a single layer of cortical cells. Further anticlinal and transverse divisions result in the formation of one or two layers of cortical cells.

3. **Development of short spinous branchlets:** The old thallus bears many short spinous branchlets. They usually originate at right angles to the main axis from central cells of the old thalli, which have well-deve-

loped cortical cells, usually more than one layer (Figs. 4-7, 10-14, 15-16 and 18).

In the old thallus, a spinous short branchlet is formed by a division of a central cell. On the apical half of a central cell, a small protuberance is formed, which is delimited by a cross-wall as the initial cell of a short spinous branchlet (Fig. 4). This initial cell undergoes successive transverse divisions to give rise to a short spinous branchlet (Figs. 5-7). In the young stage short spinous branchlets do not have cortical cells (Figs. 5-7).

These short spinous branchlets eventually develop central cells and a large number of surrounding cortical cells in the same manner as filamentous branches (Figs. 8, 10, 17-18). Sometimes, short spinous branchlets may newly develop on the old spinous branchlets in the same manner (Figs. 8-9 and 17). In other words, short spinous branchlets may grow out into uniseriate filamentous branches of limited length (Figs. 9, 13-16).

Discussion

Krishnamurthy (1962) observed the Indian specimens of *Compsopogon aeruginosus* and mentioned that the old thallus bears short spinous branchlets. These branchlets are laterals of thalli, which developed from an uncorticated segment of the axis the development of which was arrested, while the main axis formed a cortex. Patel and Francis (1969) observed short spinous branchlets on the main axis as well as on the older parts of lateral branches of *C. aeruginosus*. Based on the Japanese specimens of *C. aeruginosus*, Nakamura and Chihara (1983) observed many short spinous branchlets, which originated from the outermost cortical cells in the old part of main axis, and they did not differentiate into central and cortical cells.

In *C. aeruginosus* var. *catenatum*, Yadava and Pandey (1980) mentioned that peripheral cells of the thallus serve as initials, which divide transversely resulting into short spinous branchlets and these spine-like structures are not morphologically different from filamentous branches. Thus, short spinous branchlets are in young or arrested states of the filamentous ones.

Singh and Pandey (1986) observed short spinous branchlets of a new form of *C. aeruginosus* and mentioned that short spinous branchlets originate directly from the central cells or from large cortical cells of the innermost layer of thallus. The short spinous branchlets may grow out into branched uniseriate filamentous short laterals of limited growth.

In the present study, it is observed that a short spinous branchlet of *C. aeruginosus* var. *catenatum* originates from a central cell of main axis. In the old thallus, an initial cell of a short spinous branchlet is formed by the divi-

Figs. 1-3. Development of a filamentous lateral branch of *Compsopogon aeruginosus* (J. Ag.) Kuetzing var. *catenatum*. 1. A protuberance formed on a central cell. 2. An initial cell formed by the oblique division of a central cell. 3. Three-celled stage of a filamentous lateral branch.

Figs. 4-7. Development of a short spinous branchlet. 4. An initial cell of a short spinous branchlet formed by the division of a central cell. 5. Side view of two-celled stage of short spinous branchlet originated at right angle to the main axis. 6. Surface view of two-celled stage of a short spinous branchlet. 7. Five-celled stage of a short spinous branchlet.

Figs. 8-9. Development of short spinous branchlets and an uniseriate filamentous branch on the old spinous branchlet. 8. Three short spinous branchlets newly formed by the division of central cells. 9. Forming the uniseriate filamemtous branch of limited length.

Fig. 10. Forming several central cells and cortical cells in a short spinous branchlet.

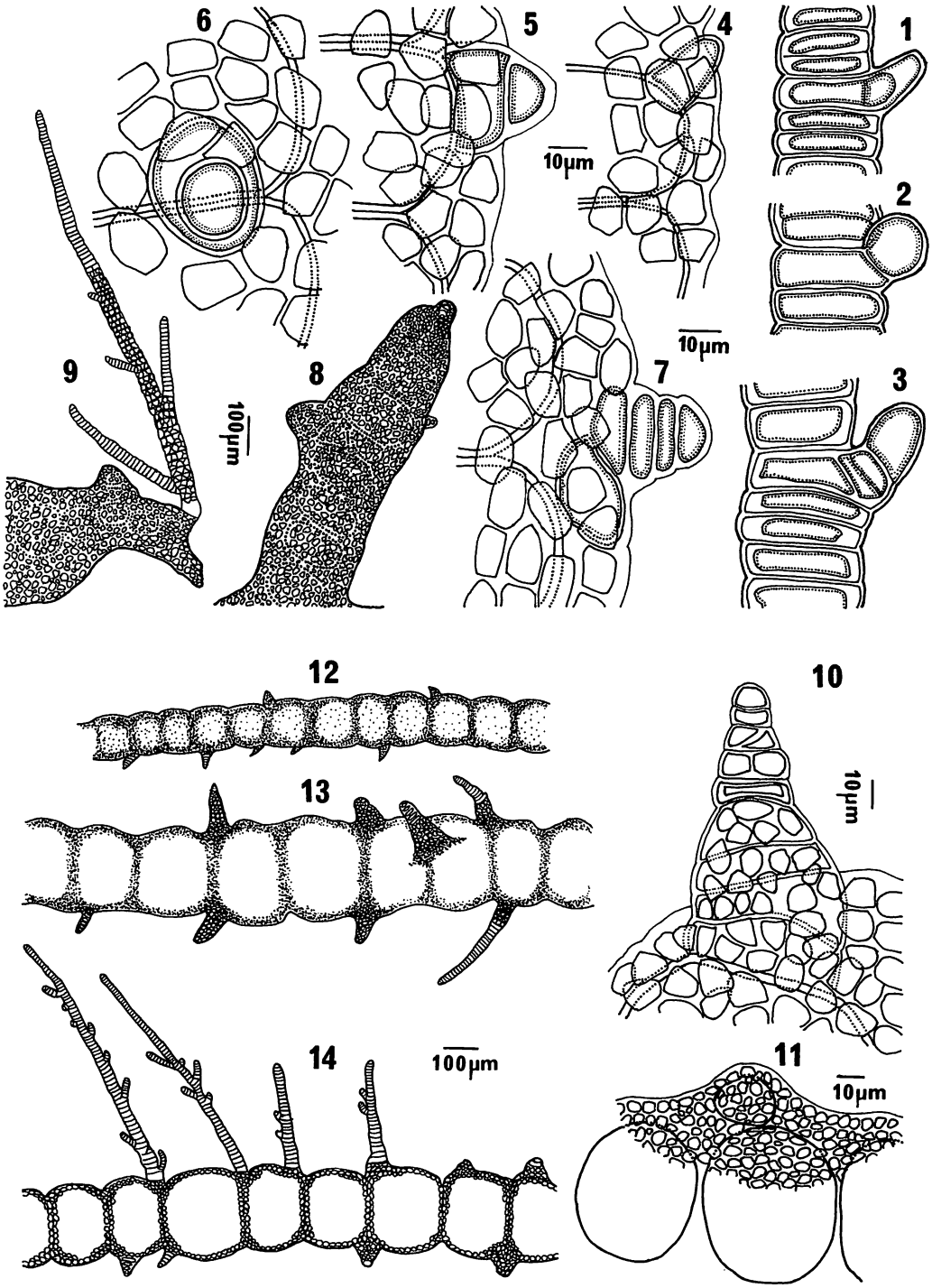
Fig. 11. A short spinous branchlet showing arrested stage of development.

Figs. 12-14. Various developmental stages of short spinous branchlets. 12. A corticated thallus with many short spinous branchlets. 13. An nodulated thallus with many well-developed short spinous branchlets. 14. A thallus with four uniseriate filamentous branches and several short spinous branchlets in arrested stages.

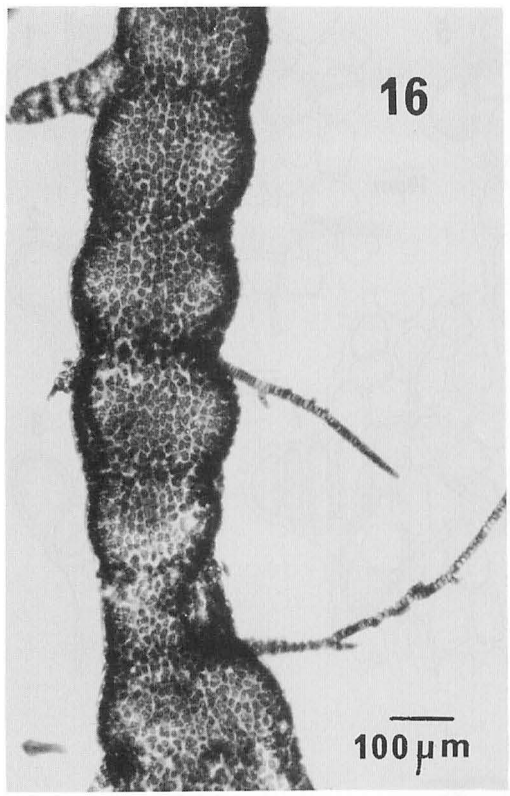
Figs. 15-16. Various developmental stages of short spinous branchlets. 15a. A corticated thallus with many short spinous branchlets. 15b. A nodulated thallus with many well-developed short spinous branchlets. 16. A thallus with two uniseriate filamentous branchlets and three short spinous branchlets.

Fig. 17. Two spinous branchlets (arrows) newly formed by the division of each central cell on the old spinous branchlet.

Fig. 18. Development of a short spinous branchlet formed by the division of a central cell (arrow).



sion of a central cell. On the apical half of a central cell, a small protuberance is formed at the right angle to the main axis, which is delimited by a cross-wall as an initial of a short spinous branchlet. This initial cell develops into short spinous branchlets consist-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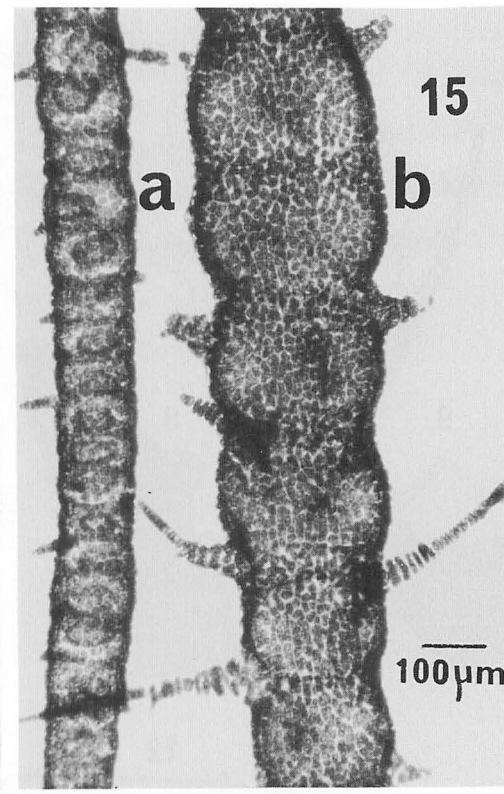
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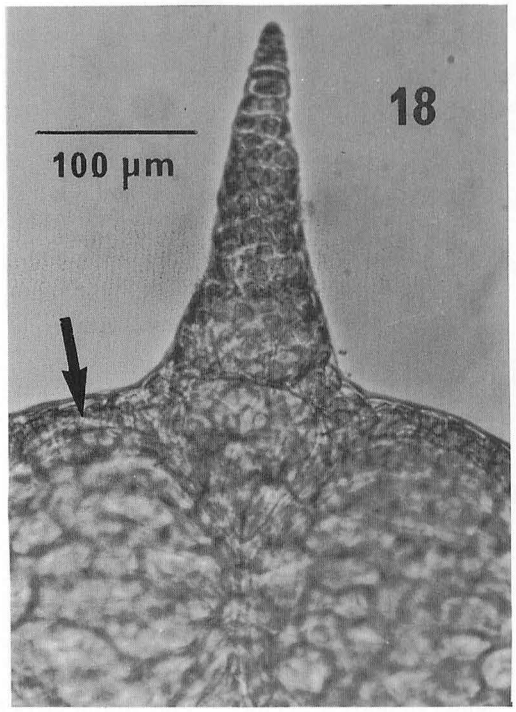
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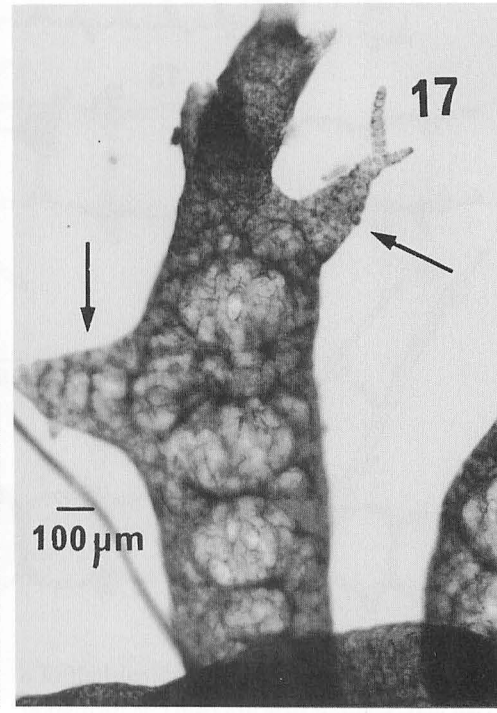
17
100µm

ing of central cells and cortical cells, then it undergoes successive transverse divisions to give rise to a short uniseriate filamentous branch.

The short spinous branchlet does not originate from the central cell in the uncorticated uniseriate portion of thallus, but from those in the corticated portion of thallus, while the uncorticated uniseriate thallus produces only filamentous lateral branches. A series of developmental events from an arrested stage of spinous branchlet to a well-developed filamentous branch are observed. It appears that each central cell has the ability to produce lateral branchlets at various stages of development.

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瀬戸良三*・R. N. Yadava・熊野　茂***：インド産淡水紅藻 *Compsopogon aeruginosus* var. *catenatum* の刺状小枝の形成**

インドで採集された *Compsopogon aeruginosus* var. *catenatum* の標本によって，本変種の顕著な特徴である刺状小枝の形成を明らかにした。本変種の通常の側枝は，本属の他の種と同様，単列糸状体の中軸細胞から新生される。一方，皮層細胞のよく発達した枝の中軸細胞から刺状小枝の始原細胞が必ず生じ，この細胞の分裂によって刺状小枝が形成される。このことから，刺状小枝形成過程は通常の側枝のそれと本質的に異ならないと考えられる。

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Michio Masuda, Masao Ohno and Gavino C. Trono, Jr.: A taxonomic assessment of *Porphyra suborbiculata* Kjellman, a food species from the Philippines*

Key Index Words: *Nori sheets—Philippines*—Porphyra—Porphyra crispata—Porphyra suborbiculata—*taxonomy.*

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Five species of *Porphyra* have been reported form the Philippine Islands (Silva *et al.* 1987). These are mainly limited to the northern coasts of Luzon Island. *Porphyra* thalli are locally known as "gamet" and are collected along the coast of Ilocos Norte. They are sold in the form of dried sheets called "pedazo" at the local market (Trono and Ganzon-Fortes 1988). The materials for preparation of the dried "gamet" are washed with freshwater and are made into sheets, 100–150 × 100 cm in size and about 5 mm thick and are dried under the sun. The dried sheets are expensive in comparison with other seaweeds such as *Enteromorpha, Codium, Caulerpa, Halymenia, Gracilaria* and *Laurencia.*

"Gamet" is prepared in the form of salad or used in soups. The utilization of *Porphyra* as food is limited to the populations along coastal communities in northern Luzon. This report presents observations on the ecology, morphology and taxonomy of *Porphyra* in Ilocos Norte.

Materials and Methods: Plants were collected at Ablan, Burgos, Ilocos Norte, Luzon Island on February 1 and 3, 1990, fixed and preserved in 10% formalin in seawater. A portion of the collections was dried for herbarium sheets, or was transported to Japan. Voucher specimens are deposited in the herbarium, Faculty of Science, Hokkaido University at Sapporo (SAP). Dried sheets were purchased from a local gamet gatherer.

Ecology: *Porphyra* plants were found growing on rocky, wave-exposed upper intertidal zone in Ilocos Norte from November to February. At the time the survey was done, the water temperature was 25.9°C and salinity was 34.8‰.

Two *Porphyra* entities have been noted previously (Trono unpublished observations): one with roundish to reniform thalli and the other with lanceolate thalli. The entity with lanceolate thalli appears to be dominant during the early period of the growing season in November, but was not seen or collected during this survey. The rocky habitat is exposed to very rough water, and the gatherers were only able to collect the "gamet" for short periods during low tides for one or two calm days a week during the winter months from November to February.

Morphology: Specimens collected and materials of dried sheets purchased have the following features in common. Foliose thalli are purplish red in color, round, reniform or funnel-shaped, 1-3 cm high, rolled toward one side (Fig. 1). Several thalli are deeply lobate near the base (Fig. 2) and show a habit similar to the green alga *Ulva conglobata.* Each thallus possesses a discoid holdfast, 0.8–1.2 mm in diameter, and an obscure stipe. The thalli are monostromatic, 35–45 μm thick at the center (1 cm above the base), 22–25 μm thick at the vegetative margin. Vegetative cells in surface view are polygonal in shape, 20–30 μm long × 12–22 μm wide. The cells are irregularly arranged, contain a single stellate

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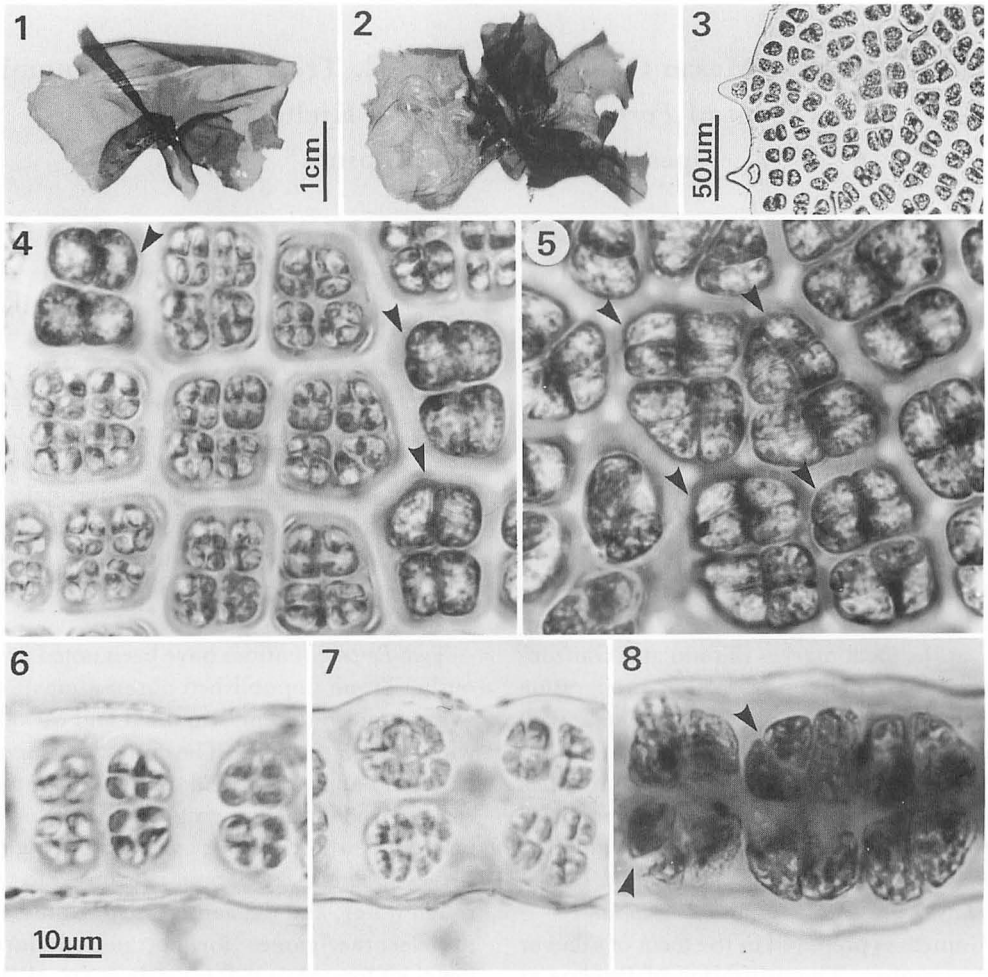

Figs. 1–8. *Porphyra suborbiculata* Kjellman. Figs. 1, 2. Herbarium specimens collected at Ablan, Burgos, Ilocos Norte, northern Luzon Island on February 3, 1990 (SAP 054606). Fig. 3. Marginal portion of a thallus, showing microsopic serrations. Fig. 4. Surface view of a spermatangial area intermixed with zygotosporangia (arrowheads); note each spermatangium composed of 16 cells (a/4, b/4), each of three zygotosporangia of 4 cells (a/2, b/2). Fig. 5. Surface view of a zygotosporangial area; note each of four zygotosporangia (arrowheads) composed of 8 cells (a/2, b/4). Figs. 6, 7. Transverse section of a spermatangial area; note spermatangia composed of 4 layers (c/4 stage) in Fig. 6 and those of 8 layers (c/8) in Fig. 7. Fig. 8. Transverse section of a zygotosporangial area; note each of two zygotosporangia composed of 4 layers (left and center, c/4; arrowheads indicating one of the last divisions, others out of focus) and a zygotosporangium of 2 layers (right, c/2 stage). Scale in Fig. 1 applies also to Fig. 2; scale in Fig. 6 applies also to Figs. 4, 5, 7 and 8.

chloroplast, are elliptical to rounded rectangular in transverse section, 20-30 μm high × 15-30 μm wide at the center of the thallus. The lowermost cells of thalli bearing rhizoidal filaments are variable in shape and size in surface view, angulate-capitate to oblong-capitate, 20-60 μm long × 20-35 μm wide. Microscopic serrations are present along the margins of the blades (Fig. 3).

Plants are monoecious. Spermatangia occur in a continuous marginal band without intervening vegetative cells. Zygotosporangia[1)] occur adjacent and just inside the spermatangial areas and also form a continuous broad

[1)] This new term was new term was proposed on the basis of a peculiar ontogeny of the zygote in *Bangia* and *Porphyra* by Guiry (1990).

band without intervening vegetative cells. These two areas are sometimes intermixed at certain portions (Fig. 4). Fertile cells are rounded rectangular to elliptical in surface view and fertile portions of the thalli are 35–50 μm thick. Spermatangial parent cells are divided into 16 cells in surface view (the division formula=a/4, b/4, Fig. 4) and 8 layers in sectional view (the division formula=c/8, Fig. 7). After presumed fertilization, zygotes are dividied into 8 cells in surface view (the division formula=a/2, b/4, Fig. 5) and 4 layers in sectional view (the division formula=c/4, Fig. 8). The number of divisions represents maxima. In many spermatangia the division formula is a/4, b/4, c/4 (Fig. 6) and in many zygotosporangia it is a/2, b/4, c/2 (Fig. 8, right). Release of spermatia and zygotospores was not confirmed.

Taxonomic remarks: Five species of *Porphyra* have been reported from Ilocos Norte, northern Luzon Island (Silva *et al.* 1987): *P. atropurpurea* (Olivi) De Toni, *P. crispata* Kjellman, *P. denticulata* Levring, *P. marcosii* Cordero and *P. suborbiculata* Kjellman. The last four species have microscopic serrations along the margins of the thallus. This peculiar feature characterizes several species in the genus. *Porphyra denticulata* differs from the alga in question in its lanceolate form of thalli, 32 spermatia formed within a spermatangial pocket, and 8 zygotospores formed per zygotosporangium (Levring 1953). *Porphyra marcosii* is distinguished from the present alga by its linear-lanceolate thalli and 8 zygotospores per zygotosporangium (Cordero 1977). In addition to these four species, the following seven species of *Porphyra*, all of which grow in Asiatic waters, are known to possess microscopic serrations along margins of their blades: *P. dentata* Kjellman (1897), *P. dentimarginata* C. Y. Chu et S. C. Wang (1960), *P. guangdongensis* Tseng et T. J. Chang (1978), *P. haitanensis* T. J. Chang et B. F. Zheng (1960), *P. okamurae* Ueda (1932), *P. tanegashimensis* Shinmura (1974), and *P. vietnamensis* Tanaka et P.-H. Ho (1962). These species, except for *P. dentimarginata*, can be distinguished from our alga by their lanceolate thalli. *P. dentimarginata* has round to elliptic thalli, but it differs from the Ilocos Norte plants in its exceedingly thicker (76–120 μm) thalli (Chu and Wang 1960).

The alga under study is similar in many respects, except for the division formula of spermatangia, to *Porphyra suborbiculata* (Table 1). According to Kurogi (1972), division numbers of spermatangia and zygotosporangia in the species of *Porphyra* he studied vary with age. The division numbers of young plants are smaller than those of old plants and these young plants can release spermatia and zygotospores. Furthermore, Miura (1968) reported geographical variation in the division formula of zygotosporangia for *Porphyra katadae* Miura. Our alga can therefore be identified with *Porphyra suborbiculata* Kjellman despite the variation in spermatangial division.

One other alga that requires discussion is *Porphyra crispata* Kjellman. This species has been reported from various localities in Asiatic waters (Ueda 1932, Tanaka 1952, Dawson 1954, Miura 1967, Tseng *et al.* 1983). According to Kurogi and Yamada (1986), however, Kjellman's type materials of *P. crispata* were not *Porphyra* but a green alga *Monostroma nitidum*. *Porphyra crispata* Kjellman (1897) should be reduced to be a synonym of *Monostroma nitidum* Wittrock (1866). Kjellman (1897) described neither marginal serrations nor reproductive structures for *P. crispata*. Ueda (1932), Tanaka (1952) and Miura (1967) gave detailed descriptions of a species of *Porphyra* which they referred to *P. crispata* Kjellmen. It is likely that further study will result in a new name for this entity.

The taxonomic relationship between *Porphyra suborbiculata* and *P. crispata* sensu Ueda requires further comment. Taxonomic features of both algae are summarized in Table 1. As these algae were first reported from Japanese waters (the type locality of *P. suborbiculata* is Goto Islands), references are limited to papers of the three major Japanese authors, Ueda (1932), Tanaka (1952) and Miura (1967). Ueda (1932) characterized his *P.*

Table 1. A comparison of *Porphyra suborbiculata* Kjellman and *P. crispata* sensu Ueda

Characters	*P. suborbiculata*			*P. crispata*		
	Ueda (1932)	Tanaka (1952)	Present authors	Ueda (1932)	Tanaka (1952)	Miura (1967)
Thallus shape	round, reniform or funnel-shaped	ovate or reniform	round, reniform or funnel-shaped	elliptical or reniform	ovate or reniform	reniform to linear
Thallus size	3–7 cm high	3–10 cm high, 3–7 cm wide	1–3 cm high	2–4(–8) cm high	2–5 cm high, 2–4 cm wide	up to 10 cm high
Thallus division	present only in old plants	not described	present	present	present	present
Thallus margin	rolled in old plants	slightly undulate	rolled toward one side	undulate	laciniate with slightly crenate	not described
Serration	present	present	present	present	present	present
Color	purplish red	light pink or purplish red	purplish red	light red or russet	light red or russet or light russet	glossy blackish purple
Thickness of vegetative part	25–35 μm	30–48 μm	35–45 μm	45–50 μm	45–68 μm	60 μm
Vegetative cell in surface view	nearly round	angular with rounded angles	polygonal; 20–30 μm long, 12–22 μm wide	long elliptical	oblong-elliptical; 15–20 μm in diam.	not described
Vegetative cell in cross section	quadrate with rounded angles; slightly higher than wide	quadrate with rounded angles; slightly higher than wide	quadrate with rounded angles; 20–30 μm high, 15–30 μm wide	elliptical; 32 × 20 μm, one half times as high as wide	elliptical; 55–60 × 20–27 μm, one half times as high as wide	not described
Rhizoidal cells	angulate capitate	angulate capitate	angular to oblong capitate; 20–60 μm long, 20–35 μm wide	oblong capitate; 55–60 μm long, 20–24 μm wide	oblong capitate	oblong or capitate
Thickness of fertile part	45–48 μm	40–50 μm	35–50 μm	55–65 μm	not described	not described
Female and male areas	not described	splashed	splashed	separated	separated	not described
Division formula of zygotosporangia	a/2, b/4, c/4 or a/2, b/2, c/4	a/2, b/4, c/4	a/2, b/4, c/4	a/2, b/2, c/6 or a/2, b/2, c/6+ 2(a/1, b/1, c/1)	a/2, b/4, c/4	a/2, b/2, c/4 or a/2, b/2, c/8
Division formula of spermatangia	a/4, b/4, c/4	a/4, b/4, c/4	a/4, b/4, c/8	a/4, b/4, c/8	a/4, b/4, c/8	a/4, b/4, c/8

crispata as follows: 1) a peculiar division of zygotosporangia, 2) a smaller thallus, 3) exceedingly elongated capitate cells bearing rhizoidal filaments, and 4) a caespitose thallus similar to *Ulva conglobata*. Tanaka (1952) and Miura (1967) seemed to agree with the opinion of Ueda. There is, however, a discrepancy in the description of the division formula of zygotosporangia by these authors. According to Ueda, the division formula of zygosporangia for his *P. crispata* is a/2, b/2, c/6 or a/2, b/2, c/6+2 (a/1, b/1, c/1), but according to Tanaka, it is a/2, b/4, c/4. The latter is the same as that of *P. suborbiculata* given by Ueda and Tanaka (Table 1). According to Miura (1967), the division formula of zygotosporangia of *P. crispata* sensu Ueda is usually a/2, b/2, c/4 and rarely a/2, b/2, c/8. Miura (1967) states that the division mode of zygotosporangia described by these three authors is basically similar. The size of thalli overlaps each other and is not clearly separated. The shape of lowermost cells bearing rhizoidal filaments is very variable within an individual, and so it is not a good diagnostic character. "Caespitose" thalli (sensu Ueda and Tanaka) may be formed by divisions of funnel-shaped thalli. Our observations on specimens in the field showed that the *Porphyra* population was composed of variable shapes of foliose thalli. Divided "caespitose" thalli resembling *Ulva conglobata* in outer appearance were found among round, reniform or funnel-shaped thalli.

Ueda (1932) and Tanaka (1952) were in agreement with regard to the division formulae of spermatangial parent cells for *Porphyra suborbiculata* and *P. crispata* sensu Ueda: the former species is a/4, b/4, c/4 and the latter is a/4, b/4, c/8. However, Kurogi (1972) described the division formula of *P. suborbiculata* as identical to that of *P. crispata*.

Tanaka (1952) emphasized the occurrence of splashed patches of spermatangial and zygotosporangial areas for *Porphyra suborbiculata*. We also found such intermixed areas in the Philippine materials. However, each area is widely distinct and intermixed areas are very narrowly restricted. It is questionable whether this character has taxonomic significance.

Finally, thallus thickness in both algae should be compared. *Porphyra crispata* has thicker thalli than *P. suborbiculata* (Table 1); however, it is hardly possible to distinguish *P. crispata* as described by Ueda (1932) from *P. suborbiculata* as described by Tanaka (1952) on the basis of thallus thickness. Thus, there is no clear distinction between *P. suborbiculata* and *P. crispata* sensu Ueda and it is suggested that the latter is "caespitose" and may be older thalli of the former. In order to clarify further the status of *Porphyra crispata* sensu Ueda it is necessary to analyze its seasonal and geographical variations.

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増田道夫[1]・大野正夫[2]・Gavino C. Trono, Jr.[3]：フィリピンで食用にされているマルバアマノリ

フィリピンのルソン島北部の Ilocos Norte 州では，11月から2月にかけて生育するアマノリ属から乾海苔を作成して食用にしている。生育期の後期の乾海苔製品並びに現地で2月に採集した標本は，藻体の形，大きさ，厚さ，鋸歯，及び生殖器官の分裂様式の特徴からマルバアマノリ (*Porphyra suborbiculata* Kjellman) であることが判明した。ツクシアマノリ (*P. crispata* sensu Ueda) と本種との関係についても論じた。([1]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；[2]781-11 土佐市宇佐町井尻194 高知大学海洋生物教育研究センター；[3]Marine Science Institute, College of Science, University of the Philippines, Diliman, Quezon, Philippines)

Jpn. J. Phycol. (Sôrui) 39: 381-384, December 10, 1991

Hirotoshi Yamamoto: Observations on the adelphoparasite *Congracilaria babae* Yamamoto (Gracilariaceae, Rhodophyta) of the Philippines

Key Index Words: adelphoparasite—bisporangium—Congracilaria—*Gracilariaceae—Rhodophyta.*
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Yamamoto (1986) established the monotypic genus *Congracilaria* on the basis of the materials from Okinoerabu Island in the subtropical region of Japan, characterizing it by its adelphoparasitic and bisporic nature, and deep pot-shaped spermatangial conceptacle. Its distribution has also been expected in southeast Asian regions where the host alga, *Gracilaria salicornia*, is much more luxuriant than in the type locality.

In 1988-1990, the author collected a lot of specimens growing on *G. salicornia* from various parts of the Philippines, and made detailed comparisons with the Japanese materials.

The materials in the Philippines were collected at the following sites and dates:

Luzon Island: Laoag, Jun. 1990; Batangas, Nov. 1989.

Mactan Island: Jan. 1988.

Palawan Island: Cowry Island, Jan. 1988; Barangay Tacduan, Jan. 1988; Barangay Bangkaw-bangkaw, Jan. 1988.

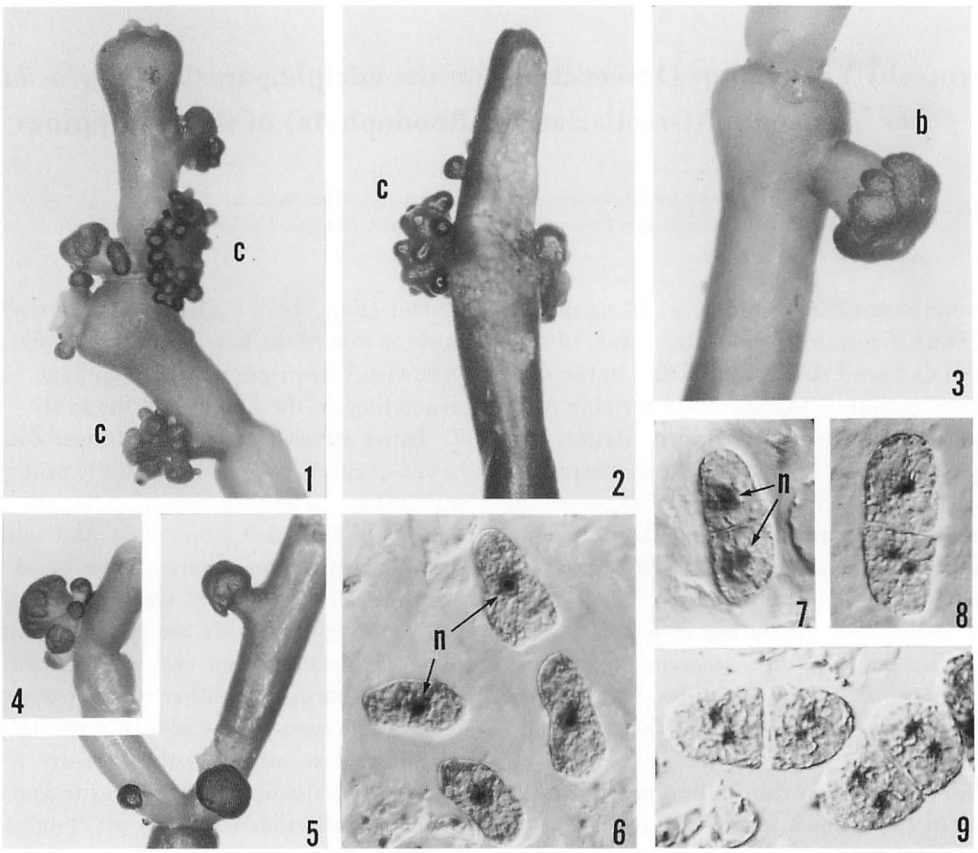
All specimens collected were monoecious and bisporic.

The materials were preserved in about 5% formol seawater and in ethanol acetic acid (3 : 1) fixative, and were sectioned using freezing microtome or squashed for microscopic observations. Wittmann's solution (Wittmann 1965) was used for observations on nuclei.

1. External morphology: The alga is parasitic on various parts of the host, *G. salicornia*. It has usually a mushroom-shaped appearance with a short stipe. The surface of the part corresponding to the cap of a mushroom is smooth, but sometimes slightly undulate or rarely lobed. The parasites are up to 3.5 mm high and 5 mm in diameter at the cap. The stipes are up to 1.2 mm high and 1.2 mm in diameter (Figs. 1-5). The color is almost the same as that of the host or slightly lighter, the yellowish brown-purplish brown tone varies depending on the habitats of the host.

2. Inner structure: Cortical layer consists of one or rarely two rows of densely protoplasmic cells. The outermost cells are 8.0-9.5 μm high, 5.5-9.5 μm wide and without secondary pit connections. Hair basal cells are present, with several nuclei. Medullary cells up to 450 μm wide are poorly protoplasmic. Transition from cortex to medulla in cell size is abrupt. Neither rhizoids penetrating into the tissue of the host, nor special cells which serve to attach to the host are recognized. The tissue between parasite and host appears continuous with only pit connections (cf. Figs. 5-7 in Yamamoto 1986).

3. Reproductive organs: Carpogonial branches consist of two cells and are formed all over the cap. After presumed fertilization, the cells around each carpogonial branch develop into cystocarps in the process similar to that of *Gracilaria* species. Cystocarps are dome-shaped, up to 600 μm high and 750 μm in diameter with an ostiole at the top. A number of cystocarps appear on a frond. Accordingly, the fronds with cystocarps show remarkably uneven or lobed appearance (Figs. 1-2). The vegetative tissue of gonimoblast consists of large cells which produce gonimoblasts and a few tubular cells. Cells close to the tips of gonimoblasts develop into carposporangia. Some of tubular cells reach the pericarp and connect with pericarp cells through several pits. Cystocarps almost always coexist with spermatangia. Spermatangia are formed in deep conceptacles up to 80 μm deep and 60 μm wide, and crowded in a sorus but usually separated



Figs. 1–9. *Congracilaria babae* of the Philippines.
Figs. 1–5. Various habits of *Congracilaria babae* growing on the host alga, *Gracilaria salicornia*. c: Carposporophytes, showing a lot of projections of cystocarps on a single frond. b: Bisporophytes, showing smooth surface of their caps and distinguishable stalks. Figs. 6–9. Process of bisporangium formation. Fig. 6. Sporangia before cell division. Fig. 7. A sporangium transversally divided, showing a single nucleus in each cell. Fig. 8. A dividing sporangium, showing a single nucleus in upper cell and two nuclei in lower cell. Fig. 9. Binucleate bispores. n: Nucleus. ×3.3 for Figs. 1–5; ×520 for Figs. 6–9.

from each other by vegetative cells. The conceptacles appear to be the *verrucosa* type of *Gracilaria* species (Yamamoto 1975). Bisporangia are produced superficially all over the cap (Figs. 3-5) instead of tetrasporangia (cf. Figs. 6, 10-14 in Yamamoto 1986). Initial sporangia divide transversally into two cells, each of which has a single nucleus (Fig. 6). Subsequently, the nucleus of each cell divides once again without cytokinesis, resulting in binucleate cells. Sporangia are 38.4–44.5 μm high and 18.2-22.2 μm wide and surrounded by rows of elongated vegetative cells. Surface of the cap with sporangia is usually smooth (Figs. 3-5).

The Philippine specimens are a little larger in frond size than Japanese ones, but there are no critical differences between the two in their external appearance, internal structures and reproductive organs (Table 1).

Bisporangium has been recognized as the most distinctive feature to characterize the genus *Congracilaria* from its related taxa. The existence of bisporangia in the materials from various parts of the Philippines proved that it is of value as a taxonomic criterion, because bisporangia are consistent structures common in populations of *Congracilaria* in Japan and the Philippines.

In addition to the similarity found in the structures and the reproductive organs between the two taxa, monoecism is prevalent.

Table 1. Comparative data on Japanese and Philippine materials of *Congracilaria babae*

	Japanese taxon	Philippine taxon
Host	*G. salicornia*	*G. salicornia*
Dimension	up to 3 mm high, up to 4.5(–5) mm diam.	up to 3.5 mm high, up to 5 mm diam.
Stipe	up to 1 mm high, up to 1.2 mm diam.	up to 1.2 mm high, up to 1.2 mm diam.
Surface	smooth, slightly or scarcely undulated	smooth, slightly undulated, rarely remarkably lobed
Color	same as host	same as host
Hair	present, but rare	present
Rhizoid	absent	absent
Outermost cell	7.9–9.6(–11.2) μm high, 5.6–9.6(–10.4) μm wide	8.0–9.5 μm high, 5.5–9.5 μm wide
Medullary cell	up to 560 μm wide	up to 450 μm wide
Transition in cell size	abrupt	abrupt
Carpogonial branch	two-celled	two-celled
Cystocarp	dome-shaped, single ostiole, a number of cystocarps in a single frond, up to 540 μm high, up to 700 μm diam.	dome-shaped, single ostiole, a number of cystocarps in a single frond, up to 600 μm high, up to 750 μm diam.
Tubular cell in cystocarp	present	present
Spermatangial conceptacle	*verrucosa* type of *Gracilaria* up to 50(–60) μm deep, up to 40 μm wide, in sorus	*verrucosa* type of *Gracilaria* up to 80 μm deep, up to 60 μm wide, in sorus
Sporangium	bisporangium, each cell with two nuclei, 38–50 μm high, 18–20 μm wide	bisporangium, each cell with two nuclei, 38.4–44.5 μm high, 18.2–22.2 μm wide
Coexistence of different reproductive phases	monoecious, rarely three phases coexisting	monoecious

As Yamamoto (1986) previously pointed out the result of Japanese alga which was examined by Goff's (1981) experiment, the monoecism of this alga may be a reflection of nature of binucleate bispores. During the two successive nuclear divisions in a sporangium to produce binucleate bispores, the reduction of chromosome number presumably occurs in the second division, resulting in binucleate spores which have heterogenotypic nuclei in each and may develop into a bisexual frond.

The author expects *Congracilaria babae* to be present in the regions including southern China, Indonesia, Micronesia, and possibly more widely distributed, corresponding to the broad geographical distribution of its host, *G. salicornia* from Mauritius to Hawaii in the tropical zone.

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山本弘敏：フィリピン産の寄生種フシクレタケの観察

現在まで鹿児島県沖永良部島からのみ知られていた寄生種フシクレタケ (*Congracilaria babae*) が，フィリピンにも広く分布することを確認した。フィリピン産の外部形態，内部組織，生殖器官はともに日本産のものとほぼ同じであり，この種の特徴とされている二分胞子のうと雌雄同体性は全ての採集地域からの標本で認められた。この結果，この特性は一地域（日本）の標本にのみ見られた例外的な形質ではなく，本種の特徴的な形質であることを確認した。(041-16 北海道南茅部町字臼尻152　北海道大学水産学部臼尻水産実験所)

Jpn. J. Phycol. (Sôrui) 39: 385–387, December 10, 1991

Yasushi Fujimori, Katsuhito Nakamura, Yuichi Maruoka, Tsuneo Matsubayashi and Goro Tamura: Immunological comparison of algal cysteine synthases

Key Index Words: Chlorella—*cysteine synthase*—*immunological comparison*—Nannochloropsis—Porphyra—Scenedesmus—Spirulina.

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Cysteine synthase (CSase, EC 4.2.99.8) catalyzes the synthesis of L-cysteine from *O*-acetyl-L-serine and sulfide. CSases from a variety of higher plants have been extensively purified and characterized (Masada *et al.* 1975, Tamura *et al.* 1976, Bertagnolli and Wedding 1977, Murakoshi *et al.* 1985, Ikegami *et al.* 1988, Nakamura and Tamura 1989), however relatively little is known about the properties of algal CSases (Schmidt 1977, Leon *et al.* 1987, Diessner and Schmidt 1981, Fujimori *et al.* 1991). In the present experiment, the antiserum raised against *Chlorella* and *Porphyra* CSases have been prepared and, using these antiserum, the immunological comparison of CSases from five species of algae [*Chlorella* and *Scenedesmus* (Chlorophyceae); *Nannochloropsis* (Eustigmatophyceae); *Porphyra* (Rodophyceae); *Spirulina* (Cyanophyceae)] was examined.

For the development of anti-*Chlorella* CSase serum, the purification of the enzyme was performed by the procedures described in the previous paper (Nakamura and Tamura 1989). The purified *Chlorella* CSase had a specific activity of 270 μmol L-cysteine formed per min per mg of protein and also showed a single band in PAGE. The purified enzyme was emulsified with Freund's complete adjuvant and injected subcutaneously into a previously unimmunized rabbit. Each rabbit was injected 4 times in the same manner. After 8 weeks, whole blood was collected by cardiac puncture and the serum was obtained after centrifugation. As for *Porphyra* CSase, the methods for purification and preparation of the antiserum were described in the previous paper (Fujimori *et al.* 1991). The *Porphyra* CSase had a specific activity of 408 μmol cysteine formed per min per mg protein and showed a single band in PAGE.

Table 1 shows the presence of CSase in several algae of different families. Each alga in the table was macerated in 30 mM Tris-HCl buffer (pH 8.0) containing 10 mM 2-mercaptoethanol and 0.5 mM EDTA and homogenized by sonication except *Porphyra* that was extracted by autolysis, and the homogenate was subjected to enzyme assay.

Specific activity is expressed as cysteine formation in 1 μmol per min per mg protein of crude extract. On a wet weight basis, 16 to 30-fold amount of activities compared with that from *Porphyra* and *Spirulina* were detected in *Chlorella* and *Nannochloropsis*, respectively. When expressed on a protein basis, however, algal preparations exhibited approximately the same value except for *Spirulina*.

As shown in Fig. 1, the anti-*Chlorella* CSase thoroughly inactivated the CSase from *Chlorella, Scenedesmus* and *Nannochloropsis*. However, this anti CSase serum was almost ineffective in inactivating the *Porphyra* and *Spirulina* enzymes (Fig. 1A). The amount of anti-*Chlorella* serum, which caused 50% inhibition of *Chlorella, Scenedesmus* and *Nannochloropsis*

Table 1. Cysteine synthase activity in several algae.

Species	CSase Activity (units/g wet weight)	Specific Activity (units/mg protein)
Chlorella[a]	24.0	1.09
Scenedesmus[a]	7.1	0.76
Nannochloropsis[b]	39.0	0.84
Porphyra[c]	1.3	0.34
Spirulina[d]	1.5	0.08

[a] *Chlorella* (*Chlorella* sp.) and *Scenedesmus* (*Scenedesmus* sp.) were grown in the medium which contained, per liter: KNO_3, 2.5 g; KH_2PO_4, 0.6 g; $MgSO_4 \cdot 7H_2O$, 0.6 g; Fe-EDTA, 15 mg; H_3BO_3, 2.86 mg; $MnCl_2 \cdot 4H_2O$, 1.81 mg; $ZnSO_4 \cdot 7H_2O$, 0.22 mg; $CuSO_4 \cdot 5H_2O$, 0.08 mg and Na_2MoO_4, 0.021 mg (pH 7.0).

[b] *Nannochloropsis* (*Nannochloropsis oculata*) were grown in the medium which contained, per liter: $(NH_4)_2SO_4 \cdot 5H_2O$, 100–300 mg; calcium superphosphate, 15–20 mg; urea, 5 mg; CLEWAT-32 (purchased from Teikoku Kagaku Sangyou Co.) (pH 8.0). After the cultivation in this medium for few days, following supplemental elements were added, per liter of medium: KNO_3, 100 mg; Na_2HPO_4, 20 mg; Fe-EDTA, 3 mg.

[c] *Porphyra* (*Porphyra yezoensis*) was grown in Tokyo bay in winter.

[d] *Spirulina* (*Spirulina platensis*) was cultured by the method of Ogawa and Terui (1970). It was done in the SOT medium (pH 9.0–9.2) at 33°C, under continuous light (7–10 klux).

[a,b] Light (5–10 klux) and air containing CO_2 (1–2%) were supplied.

CSase activities were 10, 7 and 16 μl, respectively.

On the other hand, anti-*Porphyra* CSase reacted most with *Porphyra* CSase only slightly with the *Chlorella, Nannochloropsis* and *Spirulina* CSase, and to an intermediate degree with the *Scenedesmus* CSase (Fig. 1B). Then, the 50% inhibition values for the anti-*Porphyra* CSase to *Porphyra, Scenedesmus, Nannochloropsis, Chlorella* and *Spirulina* CSase were calculated to be 9, 35, >140, >250 and >250 μl, respectively.

Based on the immuno-inactivation results it appears that *Chlorella* and *Nannochloropsis* CSases are immunologically closely related, although not identical, while both CSases differ substantially from the *Scenedesmus* CSase. Moreover, the total lack in cross-reactivity between these serum and the CSase from *Spirulina* (Fig. 1A, B) may indicate that the surface protein structure of Cyanophyceae CSase is widely different from that of Chlorophyceae, Eustigmatophyceae and Rodophyceae.

The magnitude of the reactivities of antiserum were as follows: anti-*Chlorella* CSase (*Scenedesmus* > *Chlorella* and *Nannochloropsis* > > *Porphyra* and *Spirulina*); anti-*Porphyra* CSase (*Porphyra* > *Scenedesmus* > > *Spirulina, Chlorella* and *Nannochlorosis*). Then we can speculate that the order of the antigenic

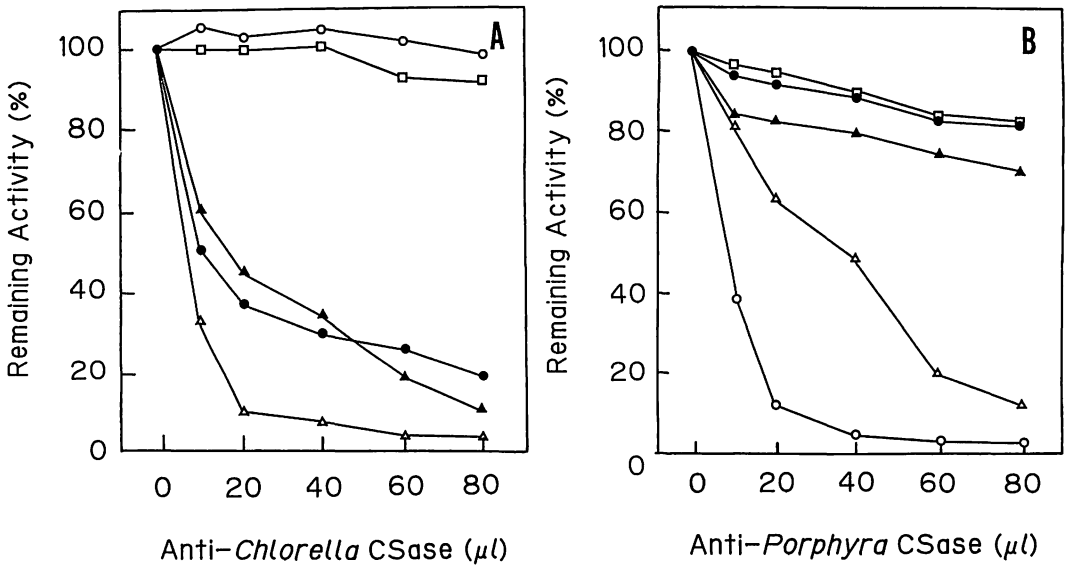

Fig. 1. Immuno-inactivation of the CSase from *Chlorella* (●), *Scenedesmus* (△), *Nannochloropsis* (▲), *Porphyra* (○) and *Spirulina* (□). Titrations were performed with anti-*Chlorella* CSase (A) and anti-*Porphyra* CSase (B). Extraction of CSases from the thalli was done by sonication except *Porphyra* enzyme which was extracted by autolysis.

reactivity between *Chlorella* and other four species are; *Chlorella-Nannochloropsis-Scenedesmus---Porphyra---Spirulina.*

Nannochloropsis had been belonging to Chlorophyceae group (Droop 1955). However, it was reclassified to Eustigmatophyceae (a kind of Xanthophyceae) for the particular difference of the chloroplast pigment composition and cell structures (Antia *et al.* 1975, Hibberd 1981). It is so curious that the *Chlorella* CSase is immunologically more closely related to the enzyme from *Nannochloropsis* than that from *Scenedesmus*. On the other hand, *Scenedesmus* CSase had some common antigenic determinants of the enzyme not only in *Chlorella* but also in *Porphyra.*

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藤森　泰・中村勝人・丸岡裕一・松林恒夫*・田村五郎：藻類システイン合成酵素の免疫学的比較

緑藻 *Chlorella* 及び紅藻 *Porphyra* からそれぞれ精製したシステイン合成酵素 (CSase) を抗原として用いて，それぞれの酵素に対する抗 CSase 血清を作製し，5 種類の藻類 (*Chlorella, Scenedesmus, Nannochloropsis, Porphyra, Spirulina*) の CSase について，免疫滴定を行ない比較を試みた。その結果，*Chlorella* と真正眼点藻 *Nannochloropsis* の CSase は共に 2 種の抗 CSase 血清に対し類似した反応を示し，免疫学的に近い関係が見られた。緑藻 *Scenedesmus* の CSase は，上記のものとは異なる滴定曲線を示し，*Chlorella* 及び *Nannochloropsis* の CSase と免疫学的にかなり異なることが示唆された。また，藍藻 *Spirulina* の CSase は，両抗 CSase 血清にほとんど反応を示さなかった。(271 千葉県松戸市松戸648　千葉大学園芸学部生物化学研究室，*833福岡県筑後市久富1343　クロレラ工業株式会社)

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月舘真理雄*・新井章吾**・成原淳一***：宮崎県門川地先のカジメ群落の観察

Mario Tsukidate, Shogo Arai and Junichi Narihara: Morphological features of *Ecklonia cava* Kjellman from Kadogawa, northern part of Miyazaki Prefecture, Kyushu.

Key Index Words: Ecklonia cava—*Laminariales*—*Morphology*—*Distribution*.
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岡村(1927) はカジメ *Ecklonia cava* Kjellm. の産地について「本州中部，太平洋沿岸ニ生ジ，専ラ東海道沿岸（三重茨城間）ニ産ス」，「分布ハ極メテ狭ク，三重県度合郡宿，田曽辺ヨリ専ラ東海道中部ニ普ク茨城県多賀郡大津辺ニ及ビ金華山迄ハ達セズ」とした。

その後，カジメの分布については，岡田（1933）は「三重県より茨城県に至る太平洋沿岸に分布する」，瀬川（1956）は「表日本中部」，新崎（1964）は「中南部・三重～茨城の間」，千原（1970）は「太平洋沿岸中部，九州，朝鮮」，川嶋（1989）は「太平洋沿岸ー茨城県大洗以南の関東，東海地方，志摩半島沿岸，四国南岸，瀬戸内海沿岸；九州ー天草地方，唐津湾；日本海沿岸ー隠岐島」としており，千原，川嶋は九州沿岸にもカジメの分布することを記述した。

九州におけるカジメの生育地に関する具体的な指摘は，山内ら（1979）が大分県沿岸について，百合野ら（1979）が宮崎県沿岸について，中村ら（1981）が熊本県沿岸について，また，松井ら（1984）は山口県の日本海沿岸にカジメの分布することを，報告した。

今回，筆者らは1989年6月22日および1990年7月12日の2回にわたって，宮崎県門川町地先の門川湾内の，カジメと同定される植物の群落とその藻体の形態を観察する機会を得たので報告する。

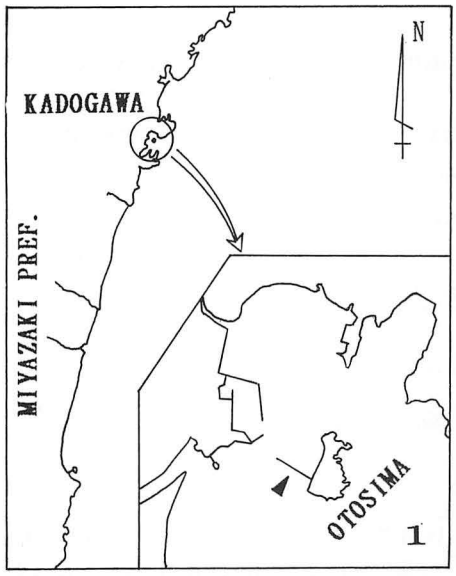
観察した場所は，宮崎県北部の東臼杵郡門川町乙島南側周辺の海域で(Fig. 1)，水深3～7 mの範囲の天然岩盤および防波堤基部斜面（コンクリート）にカジメ群落が形成されていた。当該海域は地形的にはやや閉鎖的で内湾性と考えられ，海域の透視度は数m以下で水中懸濁物が多く，葉部には浮泥がかなり付着していた(Fig. 2)。着生密度は32～44個体/m^2，年齢組成は幼体（初年体）から数令の成体にいたる複数の年齢群によって構成されていた(Figs. 3, 4)。平均的な成体(Fig. 4) の形態的特徴は以下の通りであった。

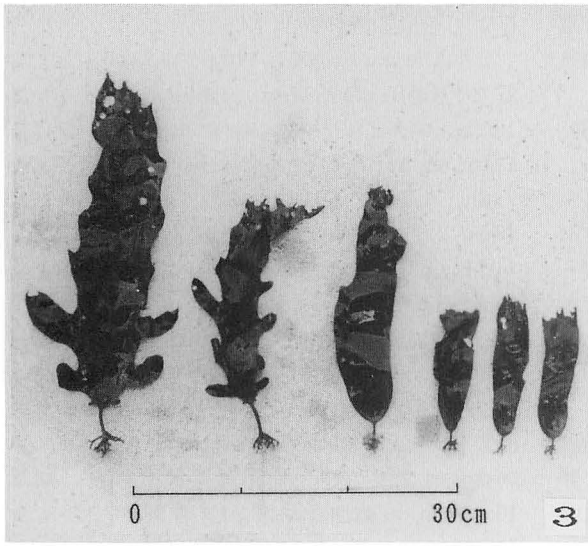
全長は74 cm，湿重量は840 gで，茎は円柱状，実質でやや偏圧し茎長17 cm，茎径（茎部最下部）は1.5 cm，中央部はやや太くなっている。中央葉は幅11 cm，厚さは下部（生長点付近）で4 mm，頂部で2 mm，側葉数は左30枚，右31枚，最大側葉長は45 cmであった。中央葉および側葉は平滑な革質でしわは無く，側葉は明瞭な鋸歯を有し単条で2次側葉は認められなかった。

25個体の成体について茎部断面の年輪を観察した結果では，2輪の個体が16個体と多く，このことから乙島南側周辺の海域に生育するカジメの年齢は比較的若く，およそ3年以下ではないかと推測される。成体には茎長の様々な個体が観察され，ほぼ15～30 cm の範囲にあるが，群落中では茎長15～20 cmの比較的茎の短い個体が優占していた。いずれの個体も中央葉の大きさに対し側葉数が多く，かつ，かなり伸長しており，2次側葉は認められなかった。

門川地先のカジメ成体の茎長は，岩橋（1968）が示した伊豆半島下田町須崎地先のカジメ成体の平均的な茎長110 cm，および林田（1984）の示した南伊豆町下流地先のカジメ成体の茎長100～200 cm，という値に比較し，明らかに短い茎長値となっている。

百合野ら（1979）によれば，宮崎県沿岸のカジメの分布は北部の門川地先および隣接する日向市平岩，幸脇地先とされ，これらの海域以外では認められていない。宮崎県沿岸にはカジメ以外の多年生コンブ科藻





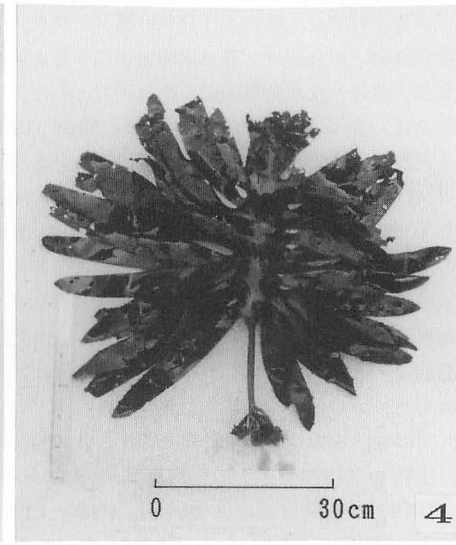


Fig. 1. The study site at Kadogawa Bay, Miyazaki Prefecture.
Fig. 2. An adult plant community of *Ecklonia cava*.
Fig. 3. Jevenile *Ecklonia cava* plants below one year of age.
Fig. 4. An adult plant of *Ecklonia cava*.

類としてクロメが認められるが，その分布は北部の門川地先および中央部の都農・川南地先海域に限られており，岡村（1927）が記載した県南部の油津には現在生育は認められていない。

今後は門川，川南地先海域のカジメ，クロメ両種について群落の生態的特徴の差違および形態的特徴の変異について，更に研究を進める予定である。

最後に，本稿のご校閲を賜った北海道大学理学部教授吉田忠生博士に厚くお礼申し上げる。

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新刊紹介

Akatsuka, I. (ed.)**: Introduction to applied phycology.** vi＋683 pp. 1990. SPB Academic Publishing bv, The Hague. Price: Dfl. 240（邦貨約2万円）

我が国でも藻類学のこれまでの研究をまとめた綜説論文集の英文著作が見られるが，その内容は主として国内に限られたものである。本書は日本人で初めて国際的視野に立って編集した応用藻類学の本で昨年オランダから出版された。掲載する論文数は30編と多く，それぞれの論文の長さは5頁から55頁と異なるが，全体として683頁からなる大著である。執筆者は共著も含めて51名，11名の日本人の方々のお名前も見られ，いずれもその方面で国際的に活躍している研究者ばかりである。本書の特徴の一つは，現在話題になっているものから将来その可能性が十分あるものを含めてこれまで余り紹介されていない色々な分野の総説を収めていることで，藻類が我々の周辺で広い範囲に関連していることを改めて認識させられる。それぞれの論文は藻類の応用の分野について極めて興味深い最先端の知識を提供し，各トピックの概略を知ることが出来て題名通り入門書としての役割も果たすが，いずれも豊富に列挙している関係文献により最近の詳細な背景を知ることが出来，むしろ専門書として研究者・学生のいずれにも勧めることが出来る。本書は次ぎの様に3つの部分に大別され，トピックスごとにこれまでの研究の経過，問題点更に将来展望などを述べている。

大略を示すと，

パート1は224頁，11編の論文からなり，細菌学，菌類学，薬学，理化学などほかの基礎的な学問分野に関連するものを含み，海藻の抗菌性；薬理作用；毒成分とその取り込み機構；解毒作用；特殊含有成分（赤血球凝集素）；金属イオン除去能力；海藻粘質物の物理化学的性質；植物プランクトンの餌料としての価値及びノリの病害と食品的価値（味と香り）について述べている。

パート2では157頁にわたって海藻と生息環境との関わりについての5編の論文からなる。即ち植物プランクトンと富栄養（湖沼では現在量の変化，海洋では赤潮）；動物の食圧；着生生物との相互関係及び海での汚染源（船底及び構築物の汚れ）について総説している。

パート3では幅広い利用や応用面，技術関係，バイオテクノロジーなどについての論文14編，277頁からなる。即ち海藻粘質物の食品，医科・歯科への利用；製紙・製塩技術；微細藻類の大量栽培技法；排水処理やビタミン定量測定法での藻類の役割；ノリ・オゴノリ・ツノマタの養殖についての海外事情；メタンガス生産の将来性；農耕・園芸への微量成分の効用；組織培養（細胞，原形質体，組織）などによる遺伝子工学と予想出来る今後の成果など広く紹介している。

以上掲載論文の数々は藻類の各方面への利用・応用についての知識を増し，理解を深め，より一層の興味を覚えさせるものであり是非一読をお勧めする。尚，本書は国際的にも評価が高く，逸早くJournal of Applied Phycology 3巻（1991年）が好意ある書評を掲載していることを付け加えておきます。

（226 横浜市緑区東本郷6-27-2-306　正置富太郎）

藻類 Jpn. J. Phycol. 39: 394, December 10, 1991

吉田忠生：第4回国際藻類学会議報告

T. Yoshida: Report on the Fourth International Phycological Congress in North Carolina

第4回国際藻類学会議は1991年8月4日から10日までの7日間アメリカ North Carolina 州 Durham の Duke University において行なわれた。松林に囲まれたキャンパスのなかで Bryan University Center を主会場とし，Biological Science Building, Chemistry Laboratory の建物も使用した。この期間中，Durham は東京の真夏と同じくらいの蒸し暑い気候だった。今回はアメリカ藻類学会との共催で開催された。このため講演要旨は Journal of Phycology 27巻3号のサプリメントとして印刷され，雑誌購読者には前以て配布されていたので，すでにご存じの方も多いはずである。会議の参加者は600名以上あり，アメリカ合衆国は地元ということもあって，約250名で1/3以上を占めていた。日本からは同伴者を含めて30名が出席した。

8月4日（日）には参加登録とともにいくつかの Workshop が開催された。夕方には野外で Barbeque Dinner を楽しんだ。5日朝から簡単な開会式とそれにつづく Plenary Session で会議が開始された。Plenary Session では，5日 J. Ramus: Photon capture: Beyond the notion of color, 6日 G. M. Hallegraeff: On the global increase of toxic algal bloom, 8日 G. O. Kirst: Salinity tolerance in algae: Organic osmolyties-necessary luxures for extreme conditions, 9日 R. S. Quatrano: Establishment of cell polarity in *Fucus* の4題の講演が行なわれた。

Congress の会期中に15の Symposia と，多くの Contributed papers のセッションが行なわれた。Symposia の主題はつぎのとうりであった。

Algal systematics: Modern concepts of algal phylogeny
Applied phycology: Recent advances in algal biotechnology
Ecology: Macroalgae in flowing water
Physiology: Algae and light
Cell and molecular biology: The cytoskeleton
Algal systematics: Morphological and systematic data
Applied phycology: Developments in large-scale culture
Ecology: Terrestrial algae
Physiology: Nutrition
Cell and molecular biology: Molecular organization of the chloroplast
Algal systematics: Algal typification
Applied phycology: Algae pollution assessment and remediation
Ecology: Algal propagules and recruitment
Physiology: Algae-atmosphere interactions
Cell and molecular biology: Developmental molecular biology

Systematics や Evolution の Session では DAN, RNA を用いた研究が多く発表され，これも一つの傾向であることが感じられた。

6日（火）の夕方はアメリカ藻類学会主催の Banquet があり，7日（水）はエクスカーションと Workshop が実施された。8日（木）の夜にはアメリカ藻類学会の auction が行なわれた。これは参加者が持ち寄った本や別刷り，その他の品物を競売して売り上げを学会の運営にあてるもののようで，この国の習慣のようであった。

9日（金）の夕方からは市内のホテルで Congress Banquet と閉会式が行なわれた。このとき Poster Session の表彰もあり，石田・中山・原グループと畠山・佐々・渡辺・高市グループが1，2位となった。次回は中国の青島市で開催されることが発表され，開催国を代表して曽呈奎氏のスピーチがあった。

（060 札幌市北区北10条西8丁目　北海道大学理学部）

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原　慶明・有賀祐勝：第2回日韓藻類学シンポジウム（1991年9月8–11日）

Y. Hara and Y. Aruga: The 2nd Japan-Korea Symposium on Phycology, 8-11 September 1991, in Tsukuba, Japan

日本藻類学会と韓国藻類学会の協力で，日韓藻類学シンポジウム組織委員会の主催により標記シンポジウムが筑波大学国際会議場を主会場として開催された．これは日本藻類学会が毎年開催している秋季シンポジウムを兼ねたものである．参加者は，韓国からの31名，タイからの1名，ペルーからの1名を含め，合計約110名であった．

9月8日夕方から登録をすませた人々が集まり，18時から筑波大学内のキャフェテリアで歓迎レセプションが開かれ，日韓両国の藻類研究者の交流がなごやかに始まった．

9月9日には9時半から開会式が行われ，日本藻類学会の有賀祐勝会長および韓国藻類学会の Koh Nam Pyo 会長の挨拶があった．引続き学術研究発表に移り，まず Lee In Kyu 教授の特別講演“Life history of Ceramiales specially referring to the mixed-phase reproduction”が行われた．その後，Session 1 Taxonomy: Red Algae I, Session 2 Cultivation and Utilization of Algae I, Session 3 Taxonomy: Diatoms, Session 4 Ecology: Macroalgae が休憩と昼食をはさんで行われた．また，夜には Young phycologists と Senior phycologists とに分かれて学外でナイトセッション（〝つくばの夜〟：懇親会）が開かれ，にぎやかな意見交換の機会がもたれた．

9月10日は川井浩史博士の特別講演“A perspective on the phylogeny of the Phaeophyceae”に始まり，Session 5 Taxonomy: Brown and Green Algae, Session 6 Molecular Taxonomy, Session 7 Physiology: Microalgae, Session 8 Physiology and Genetics: Macroalgae, Session 9 Taxonomy: Red Algae II, Session 10 Dinoflagellates and Environmental Sciences が休憩と昼食をはさんで行われた．日本藻類学会有賀会長の閉会の辞があり，学術研究発表の部は終了した．結局，この2日間に2題の特別講演，18題の招待講演，14題の応募講演が行われた．（講演要旨については本誌 39: 399–414を参照されたい．）

同日18時半から筑波大学管理棟の食堂で晩餐会が開催された．日本側からは有賀会長，韓国側からは Lee In Kyu 教授の挨拶があり，三浦昭雄教授の乾杯の音頭で開宴し，英語，韓国語，日本語を交えたにぎやかな交歓会となった．また，Lee 教授からは，本シンポジウムのプロシーディングスを Korean Journal of Phycology の特別号として出版することについて検討してみたい，次回のシンポジウムは西太平洋地域の他の国々にも呼びかけて1993年にソウルで開催できるよう努力したい旨の発言があった．

9月11日は，ワークショップ“Introduction to the Phycoflagellates”が筑波大学第二学群棟の実験室で行われ35名が参加した．午前は講義，午後は実習が行われた．その内容は次のとおりである．

講　義

I. Inouye: General Introduction: Microanatomy and Taxonomy of Microalgae. Prasinophytes.

H. Nozaki: Chlorophytes.

M. Idei: Diatoms.

I. Inouye: Chromophyte Algae Including Haptophytes.

T. Horiguchi: Taxonomy and Identification of Freshwater and Marine Dinoflagellates.

M. Erata: Cryptomonads and Other Miscelaneous Microalgae.

実　習

Culture Techniques and Light Microscopy

1) Inoculation, isolation and culture of microalgae.
2) Identification of microalgae.

Electron Microscopy

1) Scanning electron microscopy (SEM): Techniques for preparing algal cells.
2) Transmission electron microscopy (TEM): Coating grids with Formvar, whole mount preparations and for cutting sections and staining.
3) View with TEM and SEM.

また，講義・実習修了後も18時から21時まで，スライドやビデオを見ながら熱心な討論が行われた．

なお，本シンポジウムの開催に対して次の諸団体等から協賛または協力を戴いた．ここに記して感謝いたします．

筑波大学国際研究集会基金，角谷商店，㈱三洋，㈱高岡屋，日本海苔生産機械協会，全国海苔貝類漁業協同組合連合会，フルタ電気㈱，㈱山本海苔店，理研食品㈱，渡邊機開工業㈱，㈱ユニカフェ，㈱白子，ニチモウ㈱，つくば市観光課，国立科学博物館筑波実験植物園

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有賀祐勝，千原光雄，土井孝爾，恵良田眞由美，藤伊正，藤田大介，福代康夫，後藤敏一，原　慶明，畠山典子，本多大輔，堀志保美，堀　輝三，堀口健雄，市村輝宜，出井雅彦，猪川倫好，井上　勲，石川依久子，石田健一郎，海部英一郎，神谷充伸，片桐正幸，片山舒康，加藤辰巳，河地正伸，川井浩史，北山太樹，菊池則雄，小林　弘，工藤利彦，前川行幸，真山茂樹，峰　一郎，三浦昭雄，宮村新一，中村良子，中山　剛，長嶋美香子，能登谷正浩，新山優子，野崎久義，岡崎恵視，大房　剛，佐藤征矢，佐藤　忍，斉藤　譲，関本弘之，須田彰一郎，田中次郎，網川亜紀子，都筑幹夫，和田雅人，渡辺　信，山中良一，山田明子，吉崎誠，横浜康継

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第2回日韓藻類学シンポジウムに参加して

去る9月8～11日，茨城県つくば市の筑波大学において上記シンポジウムが開催され，日本と韓国の藻類研究者が一堂に会しました。台風17号の通過で天候はあまり良くなかったにもかかわらず，とても多くの参加者を得て会場は大変賑やかな様子でした。私は2日目より参加することができましたので，その経過と印象について報告します。

2日目，研究発表が筑波大学大学会館国際会議場において日本藻類学会会長の有賀祐勝先生と韓国藻類学会会長の Koh Nam Pyo 先生による開会の挨拶で始められました。紅藻イギス目に属する種の生活史を精力的に調べ上げた Lee In Kyu 先生の特別講演の後，4セッションで17題の講演が続き，夕方まで熱心な討論が行われました。夜になると，筑波大学周辺で有数の Japanese Sake Bar でいわゆる若手の会が開かれました。日本藻類学会若手の会幹事の本多大輔君の挨拶で乾杯した後，日韓両国の若き研究者の間で親密な交流が行われました。

3日目は褐藻の系統に関する諸研究を展望した川井浩史先生の特別講演の後，6セッションで15題の講演が行われました。同日夜には，大学会館で本シンポジウムの締め括りであるバンケットが盛大に開かれました。閉会の挨拶として，組織委員会のメンバーである有賀祐勝・Koh Nam Pyo・小林弘・Lee In Kyu・三浦昭雄先生による熱っぽいスピーチの後，宴は盛り上がり，時間が幾らあっても足りないようでした。

シンポジウムの最終日は実験室で Introduction to the Phytoflagellates と題したワークショップが開かれました。参加者の大部分は韓国の方々でしたので，その中に混じって受講していた私は，自分が北海道ではなく韓国から来日してきたかのような気分になってしまいました。午前中は井上勲先生による総論に続き，各分類群について造詣の深い研究家による各論が，午後は微細藻研究のための培養技術と電顕技術について実演がありました。ここでの大学院生による情熱的な解説は特に印象的でした。ホストとなった筑波大学生物科学系は微細藻の研究が盛んな研究室として知られているので，私だけでなく韓国からの参加者の期待も大きかったようですが，それを裏切らないどころか，350ページのテキスト，豊富な講師陣，最新の内容と，韓国の方々には非常に好評で，私は唯々圧倒されるばかりでした。

本シンポジウムの準備と運営には筑波大学を中心に多くの方々の労力と情熱が注がれました。本当にご苦労様でした。特に，有賀先生のお言葉を借りれば，原慶明先生の super human efforts は賞賛に値することでしょう。

「シンポジウム」は元々は「酒宴」を意味した言葉であるそうですが，本シンポジウムもその点において例外ではなく，酒杯を片手の議論や情報交換が重要な役割を果たしていたようです。今回，このシンポジウムに参加して特に印象深かったは韓国の（自分と同年代の）若い研究者の多さとその研究内容の多様さで，この事からも韓国の藻類学研究が年々盛んになっていることがうかがえます。また個人的には，一昨年北大理学部植物学教室を訪問された Boo Sung Min 先生や数カ月前に瀬戸内海の採集で御一緒した Oh Yoon Sik 先生と再会し，両先生のご研究の進展ぶりに驚かされました。加えて，若い研究者の中に独身女性が多かったことは，私にとって無視できないことで，韓国語会話の学習と韓国への留学も検討の価値ありと感じています。

講　演　の　様　子


ワークショップの様子

本シンポジウムは一昨年秋に韓国ソウルで行われた韓日藻類学シンポジウムを第1回とし，会場を日韓で交代しながら隔年で続けられる予定とのことですが，隣り合う両国の交流の場としていつまでもいつまでも続けられることを期待してやみません。

研究発表者および題目は次の通りであった（*は招待発表）。

*I. K. Lee and G. H. Kim: Life history of Ceramiales specially referring to the mixed-phase reproduction

*T. Kudo: Taxonomic Features of *Polysiphonia morrowii* Harvey (Ceramiales, Rhodophyta)

*S. M. Boo: Life history, phenology and taxonomy of *Campylaephora crassa* (Ceramiaceae, Rhodophyta)

G. H. Kim and I. K. Lee: *Aglaothamnion chejuense* sp. nov. (Rhodophyta, Ceramiaceae) from Korea

K. W. Nam and Y. Saito: A re-examination of some European and Californian *Laurencia* species (Ceramiales, Rhodophyta)

*C. H. Sohn and N. P. Koh: Cultivation and utilization of seaweeds in Korea

*M. Tsuzuki and N. Shimoyama: Utilization of microalgae and IAM culture collection

D. Fujita: Culture of *Laminaria japonica* using deep-ocean-water pumped up in Toyama Bay

J. A. Lee: Gametogenesis and early sporophyte development of *Laminaria religiosa* Miyabe in the east coast of Korea

*S. Mayama: Morphology and life cycle of *Eunotia multiplastidica* sp. nov. (Bacillariophyceae) with special reference to systematics of Raphidioid diatoms

*J. K. Choi and J. H. Noh: Morphologic and taxonomic investigations on the diatom genus *Diploneis* Ehr.

J. H. Lee, T. Gotoh and J. Chung: A study of diatom species *Gomphonema vibrio* Ehr. var. *subcapitatum* (Mayer) Lee, comb. nov.

J. H. Lee and Y. H. Jung: A study on the taxonomy of the marine diatom genus *Coscinodiscus* and their geographical variations in the Korean coastal waters

*M. Maegawa: Effect of UV radiation on the vertical distribution of red algae and contents of UV absorbing substance

*Y. H. Kim, J. S. Yoo and J. H. Kim: Ecological studies on succession of marine algae

N. Katayama, K. Takakura and Y. Yokohama: The effect of seawater dilution on the photosynthetic activity of seaweeds growing in tide pools

M. Notoya, M. Nagashima and Y. Aruga: Influence of light intensity and temperature an callus development in young sporophytes of some species of Laminariales (Phaeophyta)

*C. H. Koh and S. H. Oh: Distribution pattern of macroalgae in the west sea (Eastern Yellow Sea), Korea

*H. Kawai: A perspective on the phylogeny of the Phaeophyceae

Y. S. Oh and I. K. Lee: Taxonomy on the genus *Cladophora* (Cladophoraceae, Chlorophyta) from Korea

*J. Tanaka: Reproductive structure and taxonomy of *Spatoglossum* (Dictyotales, Phaeophyceae)

*S. A. Yoo and K. S. Lee: A chemotaxonomic study on geographical variations of Korean Fucales plants 4. the Isoenzymes

*T. Kato and M. Watanabe: Molecular taxonomy of *Microcystis* (Cyanophyceae) based on allozyme divergence

*H. Sekimoto, S. Satoh and T. Fujii: Biochemical and physiological properties of a gametic protoplast-release-inducing protein in *closterium*

*M. Wada: Characterization of a Na^+-activated ATPase of a marine Raphidophyte, *Heterosigma akashiwo*

Y. Nakamura and T. Ikawa: Purification and characterization of nitrate reductase from *Porphyra yezoensis* (Rhodophyta)

J. A. Shin and A. Miura: Genetic improvement of eating quality of dried sheets of *Porphyra* by using wild-type recombinant in *P. yezoensis*

I. K. Chung and M. K. Kim: Effects of heavy metals on *Ulva pertusa* Kjellman

*M. Yoshizaki: Taxonomy and phylogenetic analysis of the Nemaliales (Rhodophyta) on the basis of the thallus structure, initiation of carpogonial branch and carposporophyte formation

*H. B. Lee: Taxonomy of the genus *Grateloupia* (Halymeniaceae, Rhodophyta) in Korea

M. S. Kim, I. K. Lee and S. M. Boo: Phenology and morphological variability in a Korean population of *Gracilaria verrucosa* (Hudson) Papenfuss, Rhodophyta

P. Pholpunthin, Y. Fukuyo, H. Inoue and Y. Nimura: Sexual reproduction in the marine dinoflagellate *Pyrophacus stenii*

*M. M. Watanabe, S. Suda, I. Inouye and T. Sasa: Taxonomy and phylogeny of a green dinoflagellate, *Lepidodinium viride* (Dinophyta)

*M. Okazaki: Algal calcification. Its contribution to the "CO_2 problem"

（発表順）

（北海道大学理学部・北山太樹）

Abstracts of the 2nd Japan-Korea Symposium on Phycology

第2回日韓藻類学シンポジウム（日本藻類学会秋季シンポジウム）

講 演 要 旨

PLENARY LECTURES

LIFE HISTORY OF CERAMIALES SPECIALLY REFERRING TO THE MIXED-PHASE REPRODUCTION. In Kyu Lee and Gwang Hoon Kim. Department of Biology Seoul National University, Seoul, Korea

Most studies on life history of Ceramiales carried out in culture have not incorporated either cytological observations or genetic investigations of key features, and the unusual phenomena seen in Ceramiales, such as bisexuality and mixed-phase reproduction have been largely unexplained. This study, based on laboratory culture of about sixty Korean species of Ceramiales under the controlled environment, was aimed to elucidate the essential life history of ceramialecean species as well as the cause and meaning of the mixed phases by chromosome analysis and crossing experiment among isolates of each species.

The members of four families of the Ceramiales investigated characteristically showed a *Polysiphonia*-type life history with isomorphic gametophytic and tetrasporophytic generations. Most of the variations in the life history that have been encountered both in nature and under laboratory conditions involve only secondary modifications of the *Polysiphonia*-type life history. Among them, *Antithamnion nipponicum* Yamada et Inagaki, *Antithamnion sparsum* Tokida, *Antithamnion secundum* Itono, *Platythamnion yezoense* Inagaki, *Aglaothamnion oosumiense* Itono (Ceramia-ceae), *Dasysiphonia chejuensis* Lee et West (Dasyaceae), *Polysiphoina morrowii* Harvey and *Symphyocladia pennata* Okamura (Rhodomelaceae) showed mixed phases and/or bisexuality in laboratory culture. Mixed-phase plants of *Antithamnion sparsum*, *A. nipponicum*, *A. secundum* and *Platythamnion yezoense* were collected also from the field. In *Antithamnion sparsum*, *Platythamnion yezoense*, *Dasysiphonia chejuensis* and *Polysiphonia morrowii*, mixed phases and bisexuality often occurred on the same plants. Most species were regularly dioecious. However, dioecious species sometimes producing both sexes on the same plant as an aberrant behaviour. Most common is the occurrence of mixed phases with both sexual stages and tetrasporangia occurring on the same plant. The occurring percentage of mixed-phase plants varied greatly according to the species and populations, and it also varied according to environmental conditions of each isolates in *Antithamnion nipponicum*, *A. sparsum*, *A. secundum*, *Platythamnion yezoense*, *Aglaothamnion oosumiense* and *Dasysiphonia chejuensis*. All of the examined populations of above species showed a same pattern of life history. Therefore, mixed-phase reproduction was found to be one of the natural property and could be used as good character of each species.

The cause of mixed phases and/or bisexuality must be closely related to the sex determining mechanism of each species. The male and female sex ratio examined most of the species was always 1:1 regardless of environmental condition, so that the sex of these species was thought to be determined by sex chromosome. Some of the species which have only asexual life history were also observed: *Monosporus indicus* Boergesen and *Monosporus keomundoensis* Kim et Lee (*nom. inval.*) have monosporangium and *Callithamnion chejuense* Kim et Lee (*nom. inval.*) have only parasporagium for the reproductive structure, respectively. Sexual reproduction seems to be degenerated in these species.

A PERSPECTIVE ON THE PHYLOGENY OF THE PHEOPHYCEAE

Hiroshi Kawai, Department of Botany, Faculty of Science, Hokkaido University, Sapporo, 060 Japan

The class Phaeophyceae is a member of the so-called Chromophyta containing chlorophyll a and c as photosynthetic pigments, distinguished from other classes in having multicellular thalli with plasmodesmata, differentiation between vegetative and reproductive cells and abundant alginates and florotannins. The Phaeophyceae is generally believed to be derived from the Chrysophyceae, as supported by some fine structural and molecular data (Lim et al. 1985, O'Kelly & Floyd 1985, Bhattacharya et Druehl 1988).

Concerning the phylogeny in the Phaeophyceae, Kylin (1933) proposed a phylogenetic tree, in which he regarded the life history pattern and basic construction of the thallus as the most important characteristics in discussing the phylogeny. Since then his basic concept has been generally accepted, although some modified phylogenetic trees have been proposed (Scagel 1966, Wynne & Loiseaux 1976). However, several basic contradictions have become apparent as further knowledge accumulated especially from recent culture studies. For example, the orders Laminariales and Desmarestiales which were considered to belong to different phylogenetic lines, now appear to have much closer systematic relations. Therefore, a revision of our concept of the fundamental characteristics and phylogeny of the Phaeophyceae is needed. In the present paper, I will review some taxonomic characteristics and recent biological findings, paying special attention to their systematic implications.

The number and shape of the chloroplasts and presence and absence of the pyrenoids are the most prominent and important cytological features. They are generally constant among various species in an order irrespective of the life-stage or part of the thallus, and are regarded as good systematic characteristics. A single chloroplast with a pyrenoid in a cell is considered to be the most primitive condition in the Phaeophyceae, and many chloroplasts without pyrenoids in a cell to be the most derived.

Life history patterns are still one of the most important systematic characteristics. In Syringoderma, obvious variations in the morphology of the gametophytes (from branched filaments to only 2 gametophytic cells) are found, despite the rather uniform morphology of the sporophyte (Henry 1984). Monoecism and dioecism seem to occur widely in some orders such as Laminariales, Desmarestiales and Fucales.

Sexual pheromones are shown to have essential roles in the sexual reproduction of the Phaeophyceae (Maier & Müller 1986). About 10 sexual pheromones (often occurring as mixtures) have been identified to date and their distribution among phaeophycean taxa apparently has systematic implications. For example, lamoxirene is confirmed to be the functional pheromone in more than 30 species of the derived Laminariales, however, it is not found in the primitive laminarialean families nor in any other orders. Desmarestene, viridiene, fucoserratene and cystophorene are also known only in one order as actual pheromones, although others (e.g. ectocarpene and hormosirene) are found in several orders. In Analipus, a quantitatively minor substance (hormosirene) is suggested to act as the actual pheromone (Müller et al. 1990).

The structure of swarmers (i.e. the condition of the chloroplasts and flagella) is considered to have important systematic implications. Most phaeophycean zoospores have a chloroplast with a stigma and flagellar swelling on the basal part of posterior flagellum. Flagellar basal bodies are connected with the nucleus and are spatially associated with the chloroplasts. However, those of derived Laminariales lack a stigma and flagellar swelling. Green flagellar autofluorescence associated with the stigma and flagellar swelling, which is suggested to be involved in the photoreception of phototaxis, is noted to occur in phototactic swarmers (Müller et al. 1987, Kawai 1988). The stigma is shown to have a reflective function and focuses the reflected light on the flagellar swelling (Kawai et al. 1990, Kreimer et al. 1991). Some sperm (e.g. Laminariales) have longer posterior flagella and several fragmented chloroplasts which are not associated with the flagellar base, while others (e.g. Fucales) share the longer posterior flagellum but have a chloroplast with or without a stigma. Remnant flagella were found in some immotile cells such as laminarialean egg and dictyotalean spores (Motomura 1989, Phillips 1991), which suggest that the eggs and tetraspores in these orders originate from flagellate cells. The flagellar rootlet system is in principle similar among most phaeophycean swarmers, composed of bypassing rootlets which pass through the flagellar bases longitudinally, and four kinds of rootlets (MAR, mar, MPR, mpr) which issue from the flagellar bases (O'Kelly 1989). Laminarialean sperm lack MPR. In fucalean sperm a bundle of the MAR forms a proboscis. There are variations in the number of tubules in those rootlets, however, the basic number in phaeophycean swarmers is reported to be MAR/mar/MPR/mpr/BR = 7/1/3/1/7.

INVITED PAPERS

TAXONOMIC FEATURES OF *POLYSIPHONIA MORROWII* HARVEY (CERAMIALES, RHODOPHYTA). Toshihiko Kudo[1], Michio Masuda[2]. [1]Biol. Lab., Sapporo Univ., Toyohira-ku, Sapporo, Japan. [2]Dept. of Botany, Fac. of Science, Hokkaido Univ., Kita-ku, Sapporo, Japan

Taxonomic features of *Polysiphonia morrowii* were investigated through its life history stages by laboratory culture experiments and periodic field observations. The taxonomic criteria which had been used for this species were evaluated by comparing ontogenetic data of field-collected and cultured plants. Some of taxonomic features adopted previously show phenotypic variation according to environmental conditions or growth stages: the length/diameter ratio of segments, the external appearance of branches, number of axillary tetrasporangial branches. The significant specific features of *P. morrowii* were confirmed in this investigation.

This species is characterized by the following features: (1) ecorticated thalli with 4 pericentral cells, (2) thick and setaceous thalli in fully matured stage, (3) adventitious rhizoids without septations, (4) tightly intricated prostrating branches, (5) sharply pointed ultimate branchlets, (6) endogenous axillary branchlets formed from the central axial cells, (7) a few trichoblasts in matured stage, (8) tetrasporangia formed both on the ultimate and axillary branchlets, (9) axillary tetrasporangial branchlets tuftly (7-8 in number) formed on the axils of fully matured plants, (10) spermatangial branches replacing the whole trichoblasts, (11) urceolate cystocarps.

The occurrence of endogenous axillary branches is a peculiar feature characterizing some species in the genus *Polysiphonia*. The taxonomic relationship between *P. morrowii* and closely related species sharing the above-mentioned feature is discussed.

LIFE HISTORY, PHENOLOGY AND TAXONOMY OF *CAMPYLAEPHORA CRASSA* (CERAMIACAE, RHODOPHYTA). Boo, Sung-Min. Dept. of Biology. Chungnam National Univ., Daejon, 305-764, Korea.

The life history of an epiphytic species, *Campylaephora crassa* (Okamura) Nakamura, was investigated in the wild on the central eastern coast of Korea and under various laboratory conditions. Korean *C. crassa* plants showed no obvious seasonal differences in the occurrence of reproductive organs, whereas their overall habit was variable. In culture the species completed a *Polysiphonia* type of life history without the basiphyte, but parasporangia occurred at a frequency of about 1 % on young tetrasporophytes. Paraspores grew into tetrasporophytes, which later cycled a *Polysiphonia* type of life history. Crossability tests also documented that paraspores had the same nuclear phase as normal tetrasphorophytes. This fact indicates that paraspores of *C. crassa* may be mitotic diploids which show a temporary modification in the life history strategy. Maximal growth in the field occurred in May when the seawater temperature ranged from 14.9 to 18.2 ℃, while in culture the optimum temperature for growth was between 16 and 20 ℃ under each photon flux density. Plants in the field had tetrasporangia and cystocarps throughout year and the tetrasporophytes in culture formed tetrasporangia at most combinations of photon flux densities and temperatures.

CULTIVATION AND UTILIZATION OF SEAWEEDS IN KOREA. Chul Hyun Sohn[1] and Nam Pyo Koh[2]. [1]Dept. of Aquaculture, Nat'l. Fish. Univ. of Pusan, Pusan, Korea. [2]Dept. of Aquaculture, Yeosu Fish. College, Yeosu, Korea

Seaweed flora of the Korean coast is quite diverse, and more than 600 species has been reported. In Korea utilization of the seaweeds dates back to the early Korean history, and they have been cultivated since 17 C. The total annual production of the seaweed from the natural algal beds and by cultivation was recently estimated to be 483,000 M/T. The production by cultivation occupies more than 90% of the total algal production. In 1988, 34,619 M/T of seaweeds were exported, while 3,090 M/T were imported, and most of them were dried and salted. The major algae of economic importance include *Porphyra, Gelidium, Gracilaria, Pachymeniopsis, Undaria, Laminaria, Sargassum, Hizikia, Enteromorpha* and *Codium* etc. Of these *Porphyra, Undaria, Laminaria, Hizikia, Enteromorpha* have been cultivated. *Porphyra* is very popular, and its annual production is amounting to US $ 300 million which is more than half of the total aquaculture fisheries production in Korea. Traditionally the seaweeds have been consumed by various ways such as fresh salads, dried snacks, roasting lavers and seaweed soup. However industrial processing has been undeveloped. In Korea the agar production is made mainly by agar extraction from *Gelidium amansii*, while production of carrageenan/alginic acid is very few.

UTILIZATION OF MICROALGAE AND IAM CULTURE COLLECTION. Mikio Tsuzuki and Naomi Shimoyama. Institute of Applied Microbiology, Univ. of Tokyo, Yayoi, Bunkyo-ku, Tokyo 113 Japan

Microalgae has often been used as materials for research of plant science. It is because they can grow very fast under a certain condition and be taken out quantitatively with pipettes. Axenic culture and mutants have also been useful. However, the species usually used have been rather restricted so far, and the strain number of the species are sometimes important for physiological and biochemical researches, for each strain shows different characteristics at times. The same is the case in biotechnology. Though some chemicals such as β-carotene and polysaccharides are extracted from some strains, microalgae are still relatively untapped resources.

It is important for a researcher to obtain and then to keep strains of algae when he wants to use them. Since most of the algal species must be maintained by serial transfer at regular intervals, there is a great risk of disappearance. There needs the special center where algal strains are maintained and supplied by demand.

Algal Culture Collection in the Institute of Applied Microbiology (IAM), University of Tokyo was commenced in 1957. Many strains of microalgae were collected and identified there and were maintained to be distributed for scientific and technological studies and education. These works, however, were carried out in a research laboratory. In 1989 Microbial and Microalgal Research Center was established in the same institute and it took over the collection for the purpose of substantial culture center of microorganims including microalgae. As the first step of the center, we have been checking the strains we have and collecting information about them for a catalogue. Other activities of our collection will be also reported in this symposium.

MORPHOLOGY AND LIFE CYCLE OF *EUNOTIA MULTIPLASTIDICA* SP. NOV. (BACILLARIOPHYCEAE) WITH SPECIAL REFERENCE TO SYSTEMATICS OF RAPHIDIOID DIATOMS. Shigeki Mayama. Department of Biology, Tokyo Gakugei Univ., Koganei-shi, Tokyo, Japan.

Valve shapes of *Eunotia* species are usually simple and the taxonomic characteristics are scarce under light microscopy. In addition, the valve shape varies during the life cycle. However, there has been no observation throughout the life cycle using SEM in any species.

E. multiplastidica was originally found on wet moss in Sainokawa, Ehime Pref., Japan. Unialgar cultures were established from the original material. One to two months later, sexual reproduction was observed in these cultures. In this process, one auxospore was formed from two gametangia. An initial cell developed within each auxospore was followed by a formation of post-initial cells. The initial valves were 45-47 μm long and about three times longer than those of gametangial cells.

Observations of the valves and plastids were carried out throughout the life cycle using these cultured cells. Though the shape and length of the valves varied extremely, certain characteristics were stable throughout the life cycle, i.e., the numerousness of chloroplasts, the location of pattern center and raphe, areola morphology, striation density, the number of labiate process and epitheca depth. These characteristics were used as taxonomic criteria of this species.

In the pennate diatoms, there have been few reports on the fine structure of the perizonium. In this study, the structure of the perizonium was examined in detail using SEM. The traditional systematics of the raphidioid diatoms was constructed based on only the presence of the labiate process and the raphe. However, the particular structure in the perizonium, namely, the areolated primary transverse band with both a circular and linear pattern center, suggests a probable phylogenetic affinity with the centric diatoms and the araphid diatoms.

MORPHOLOGIC AND TAXONOMIC INVESTIGATIONS ON THE DIATOM GENUS *DIPLONEIS* EHR. Choi, Joong Ki and Jae Hoon Noh. Department of Oceanography, Inha University, Inchon 402-751, KOREA

A large number of *Diploneis* species collected from the Korean coastal waters have been investigated by light and scanning electron microscope. 27 species, 8 varieties and 1 forma are identified, however, 7 species are unidentified and photographed in this study. Among these species, 16 species, 5 varieties and 1 forma are newly recorded in Korea. These species are *D. bomboides*, *D. adonis* var. *oamaruensis*, *D. diplosticta*, *D. novaesleendae*, *D. bombus* var. *bombiformis*, *D. hospes*, *D. dalmatica*, *D. campylodiscus*, *D. nitescense*, *D. gemmata* var. *pristophora*, *D. vespa*, *D. notabilis*, *D. suborbicularis*, *D. crabro* var. *dirhombus*, *D. schmidtii*, *D. contigua*, *D. graefii*, *D. vacillans*, *D. parca*, *D. vacillans* var. *renitens*, *D. smithii* forma *rhombica* and *D. lacrimans*.

EFFECT OF UV RADIATION ON THE VERTICAL DISTRIBUTION OF RED ALGAE AND CONTENTS OF UV ABSORBING SUBSTANCE. Miyuki Maegawa, Masayo Kunieda and Washirou Kida. Faculty of Bioresources, Mie Univ., Tsu, Mie, Japan.

UV band occupies 9 % of solar radiation in the outer space and 1-4 % on the sea surface depending on weather conditions. UV radiation, dose not penetrate so much as PAR in the sea, decreased to 10 % at a depth of 5 m, and below 1 % at a depth of 10 m. We tested the influence of UV radiation, full solar radiation and PAR for several red algae collected from shallow water, intertidal zone to upper subtidal zone, and from deep water, 25-30 m depth. UV radiation, which occupied only a few ratios of solar radiation, depressed photosynthetic activity of deep water species significantly as well as full solar radiation and PAR did. Shallow water species were not affected so much by UV radiation, full solar radiation and PAR.

We have a hypothesis that UV absorbing substance containing much in red and blu-green algae protect the thallus from strong UV radiation. Shallow water species may have an ability to resist injurious UV radiation, and deep water species may have no such an ability. However, there have been a little information for ecological role of UV absorbing substance. Then, we measured the contents of UV absorbing substance for 33 species collected from shallow and deep waters. Shallow water species contained much UV absorbing substance, and deep water species contained little or no UV absorbing substance. Particularly, intertidal species had more UV absorbing substance than other species.

In our previous paper, deep water species had more phycoerythrin, which absorbed green to blue light, than shallow water species. So, deep water species adapted to deep water light condition. In this study, shallow water species have more UV absorbing substance than deep water species. So, shallow water species are protected from injurious UV radiation by high contents of UV absorbing substance. UV radiation in the sea is one of the most important factors to control the vertical distribution of red algae, with reference to the content levels of UV absorbing substance between shallow and deep water species.

ECOLOGICAL STUDIES ON SUCCESSION OF MARINE ALGAE. Young Hwan Kim, Jong Su Yoo and Jee Hwan Kim. Dept. of Biology, Chungbuk Nat. Univ., Chongju 360-763, Korea.

As the process of ecological succession occurs more rapidly in benthic marine habitats than in terrestrial communities, benthic marine communities provide a particularly convenient testing-ground for theories about ecosystem development and ecological succession. Since 1985 we have examined the variations of colonization, growth pattern and succession of benthic marine algae on various artificial substrata and also on cleared natural surfaces.

Firstly, seven kinds of artificial substrata, i.e., concrete, slate, glass, wood, rubber, aluminium and PVC plates, were placed at upper, middle and lower intertidal zones of Poryong and Sochon, western coast of Korea, during the period of January - November 1985. As a whole, coccoid blue-green algae and diatoms were observed as pioneer algae settled over newly placed substrata, and then filamentous green and crustose coralline algae were gradually luxuriant, whereas diatoms decreased in abundance. Colonization and growth of marine algae were significantly influenced by differences between tidal levels or the kind of artificial substrata.

Studies have also been made of the recolonization of cleared natural surfaces over a 21-mo period (July 1986 to April 1988) at intertidal zones of Muchangpo and Maryangri, western coast of Korea. Surfaces were sterilized by burning after clearing. In general, the successive stages found in the permanent quadrats were blue-greens or filamentous algae - membranous algae - perennial algae. However, the nature and position of the surface and length of time of exposure have been shown to influence the population which develops. It was concluded that a climax community can be attained after 18 months since the substrata were cleared.

DISTRIBUTION PATTERN OF MACROALGAE IN THE WEST SEA (EASTERN YELLOW SEA), KOREA. Chul-Hwan Koh, Sang-Hee Oh, Dept. of Oceanography, Seoul Nat. Univ. Seoul, Korea

Koh and Lee (1982) tried to differentiate the floral composition of benthic algae between coastal and open waters in the Kyunggi Bay, West Sea of Korea. The total number of species tends to increase from the coastal waters to the open sea. Brown algae are more sensitive in terms of species number. Song (1984), Sohn (1987) and Park & Kim (1990) reported also the same tendency in the distribution pattern of macroalgae in the whole area of West Sea.

Koh and Lee (1982) insisted that the floristic differences that are observed between the coastal waters and open sea area appears to be related to water turbidity rather than to temperature as reported by Kang (1966). Several authers have agreed with the importance of turbidity since 1982. The relationship between the distribution and environments are reviewed in this presentation.

REPRODUCTIVE STRUCTURE AND TAXONOMY OF *SPATOGLOSSUM* (DICTYOTALES, PHAEOPHYCEAE). Jiro Tanaka. Dept. of Botany, National Science Museum, Shinjuku-ku, Tokyo, Japan

Three species of *Spatoglossum*, *S. pacificum* YENDO, *S. crassum* sp. nov. and *S. latum* sp. nov. occur along the Pacific coasts of the central Japan. They form three kinds of reproductive organs, *i.e.* sporangia, oogonia and antheridia on separate plants. Sporangia can be distinguished from oogonia by bigger size and sometimes by possession of four spores. As the result of the morphological comparison among the Japanese species and the other established species of the world, the genus *Spatoglossum* can be separated into two groups based on the position of reproductive organs.

In the first group including *S. pacificum*: sporangia and oogonia aggregated in sori and project above the thallus surface; antheridial sori stand up above the general thallus surface. These characters are well known in the other genera of the Dictyotales, *i.e. Dictyota, Dilophus, Pachydictyon* and *Dictyopteris*. This group comprises *S. areschougii* J. AG. from Barbados in the Caribbean Sea and *S. chapmanii* LINDAUER from New Zealand.

In the second group including *S. crassum* and *S. latum*: sporangia scatter throughout the thallus and not in a sorus; sporangia and antheridial sori are lying completely within the cortex; oogonia scatter throughout the thallus and project both above and below the cortex. These features, particularly the buried sporangia into the cortex, have never been known in the other genera of the Dictyotales. This group comprises *S. crispata* HOWE from Peru and *S. macrodontum* J. AG. from Australia.

It can be concluded that these two groups are separated into two genera based on the above-mentioned remarkable differences on reproductive structures.

A CHEMOTAXONOMIC STUDY ON GEOGRAPHICAL VARIATIONS OF KOREAN FUCALES PLANTS. 4. THE ISOENZYMES. *Soon-Ae Yoo and Ki-Sung Lee. Department of Biology, Pai-Chai University, Daejon 302-735, Korea.*

To obtain chemotaxonomic characteristics of Korean Fucales plants, we had already compared the composition of pigments, phospholipids, neutral fats, inorganic polyphosphate, and haemagglutinin[1,2,3]. In this paper, we compare the patterns of 13 kinds of isoenzymes extracted from 10 species of Korean Fucales plants.

The electrophoretic zymograms showed that most of the carbohydrate-metabolizing enzymes(G-6-P DH, MDH, ADH, IDH, PGM, AMY) and catalase had mono- and/or di-morphic patterns. It seems that the genetic variations are small in those enzyme systems. Those enzyme systems seems to be genetically stable. They have the main enzyme activities in isoenzymatic zymograms mobilized to the cathode.

On the other hand, in the case of haydolase(AKP, ACP, EST), isomerase(GPI), oxidoreductase, they all showed polymorphic isoenzymatic zymograms. We believe that these enzymatic systems are genetically rather unstable. Most of these enzymes are involved in phosphate metabolism, and the activity of these enzymes varies according to the phosphate concentration of the environment. So these enzymes seem to be important in studying genetic variations in Fucales plants growing in different geographical habitat.

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2) Yoo, S. A., K. S. Lee, and K. S. Park. 1988. A chemotaxonomic study on geographical variations of Korean Fucales plants. 2. Lectin. *Korean J. Phycol.* 3(1):9-12.

3). Yoo, S. A. and K. S. Lee. 1988. A chemotaxonomic study on geographical variations of Korean Fucales plants. 3. The 3-dimensional analysis of total lipids. *Kor. Jour. Bot.* 31(2):83-89.

MOLECULAR TAXONOMY OF MICROCYSTIS (CYANOPHYCEAE) BASED ON ALLOZYME DIVERGENCE. KATO Tatsumi and WATANABE Masayuki. National Science Museum, Tsukuba 305, JAPAN.

Microcystis is a limnoplanktic, bloom-forming blue-green alga. As a part of molecular taxonomic revision, allozyme divergence of Japanese Microcystis was studied electrophoretically.

We investigated allozyme genotypes at four enzyme loci (IDH, 6PGD, PGI and PGM) on a total of 168 strains collected at fourty-three waters. The obtained data were statistically analyzed according to KATO & DOI's Minimum Replacement Method. As a result, the strains studied were classified into four genetic types: Microcystis aeruginosa S-type, M. aeruginosa L-type, M. viridis and M. wesenbergii. Each of the four types was found to exhibit characteristic morphology. M. aeruginosa S-type was characterized by its small size cells (3.0-4.2 μm). The other three types that have larger cells (4.5-6.5 μm) were discriminated by the colony formation: M. aeruginosa L-type, M. viridis and M. wesenbergii formed clathrate, cubic and pouched colonies, respectively. Accordingly, the four genetic types could be viewed as separate taxonomic entities.

The above achievements will provide a new and reliable taxonomic system of Microcystis. As clearly illustrated here, the allozyme study (i.e. molecular taxonomic approach based on allozyme divergence) is considered as one of the most effective approaches that would lead to the solution of many taxonomic problems on cyanophytes, especially at the species and/or genus level.

BIOCHEMICAL AND PHYSIOLOGICAL PROPERTIES OF A GAMETIC PROTOPLAST-RELEASE-INDUCING PROTEIN IN *CLOSTERIUM*.

Hiroyuki Sekimoto, Shinobu Satoh, and Tadashi Fujii. Institute of Biol. Sci., Univ. of Tsukuba, Tsukuba-shi, Ibaraki 305, Japan.

Heterothallic strains of *Closterium* proliferate asexually in nitrogen-sufficient medium while conjugation is induced in nitrogen-deficient mating medium (MI medium). The conjugation process consists of cell division, pairing, formation of conjugation papillas, release and fusion of gametic protoplasts, and formation of zygotes. Some cells formed a papilla and released their protoplasts without pairing. This indicated that the process of protoplast-releasing was independent upon pairing and there might be a substance, which was responsible for protoplast release, in the medium.

1). Detection of a substance that induces the gametic protoplasts. When mt^+ and mt^- cells were separately incubated in the MI medium obtained from 55h-old-mixed cultures of mt^+ and mt^- cells, release of protoplasts was observed only in mt^- cells. These data indicated that a substance responsible for protoplast release was released into the medium.

2). Purification of a protoplast-release-inducing protein (PR-IP). The substance with the ability to induce the release of protoplasts was purified from the medium by sequential column-chromatographic steps, and named PR-IP. The PR-IP had an apparent molecular mass of 95k on gel filtration and could be separated into several isoforms by anion-exchange chromatography. Each isoform consisted of two glycopolypeptides of M_rs of 42k and 19k, while the deglycosylated polypeptides had M_rs of 34k and 18k, respectively.

3). Physiological properties of PR-IP. Light was indispensable for the protoplast-release. From an analysis of dose-response curves, the concentration required for 50% of the maximum response (ED_{50}) was calculated as $4.1 \cdot 10^{-9}$ M. Moreover, the reduced protoplast-releasing reaction was observed by the treatment of high concentration of PR-IP (5.2 μg/2ml). These data indicated that PR-IP dose not act as cell wall lytic enzyme, which is well-known in conjugation of *Chlamydomonas*. We propose the PR-IP is a biologically active glycopolypeptide that induces the release of protoplasts by binding to receptors on the cell surface, as do animal peptide hormones.

CHARACTERIZATION OF A NA^+-ACTIVATED ATPASE OF A MARINE RAPHIDOPHYTE, *HETEROSIGMA AKASHIWO*.

Masato Wada. Institute of Biol. Sci., Univ. of Tsukuba, Tsukuba-shi, Ibaraki, Japan.

A marine raphidophycean unicellular biflagellate, *H. akashiwo*, is a naturally occurring, wall-less organism which is useful for both the preparation of plasma membrane and the investigations of its physiological functions. One of he major functions of plasma membranes is the regulation of ion transport at the cell surface. High purified plasma membranes were isolated from *H. akashiwo* with silica microbead method and the novel membrane associated Na^+-activated ATPase activity were characterized. The ionic requirements and spectra of effective inhibitors on the ATPase activity showed a close similarity to the animal Na^+,K^+-ATPase. This kind of ATPases which are sensitive to vanadate forms phosphorylated intermediate in their enzyme cycle. The phosphorylated intermediate of this ATPase were detected as 140 kDa polypeptide with acid SDS-polyacrylamide gel electrophoresis; this molecular weight was considerably bigger than the α subunit of animal Na^+,K^+-ATPase. However, the antiserum to animal Na^+,K^+-ATPase reacted to *H. akashiwo* 140 kDa ATPase. It was suggested that both Na^+-activated ATPases have a common epitope. The cDNA sequences of animal Ca^{2+}-ATPase and fungal H^+-ATPase has been already analyzed with biotechnological methods. The obtained informations on their functions have facillitated to understand the ion transport, ion selectivity and ATP hydrolysis mechanisms of these ATPases. The primary structure of the ATPase of *H. akashiwo* contribute the understanding of Na ion transport or Na ion selectivity mechanism. A cDNA cloning of the ATPase from *H. akashiwo* was achieved with PCR method and successed, the homology between those ATPases was discussed.

TAXONOMY AND PHYLOGENETIC ANALYSIS OF THE NEMALIALES (RHODOPHYTA) ON THE BASIS OF THE THALLUS STRUCTURE, ITIATION OF CARPOGONIAL BRANCH AND CARPOSPOROPHYTE FORMATION. Makoto Yoshizaki, Dept. of Biology, Toho University, Funabashi-shi, Chiba, Japan

The order Nemaliales was established by Schmitz (1892), on the basis of the lack of auxiliary cell in the formation of carposporopyte, and the following 3 families as taxa composing the order Nemaliales are proposed by Pueschal and Cole (1982): The Acrochaetiaceae, Helmithocladiaceae and Galaxauraceae.

The data available at present, the following keys can be made in connection with the taxonomy and phylogenetic relationships of the Nemaliales on the basis of the thallus structure, carpogonial branch and carposporophyte formation:

1. Carpogonial branch is directly formed on the filamentous thallus ---- 2
1. Carpogonial branch is formed on the assimilatory filament ---- 3
2. Compact carposporophyte is formed ---- Acrochaetiaceae
2. Diffused carposporophyte is formed ---- Woelkerlingiaceae fam. nov.
3. Carpogonial branch is formed terminally on a cell of assimilatory filament ---- 4
3. Carpogonial branch is formed laterally on a cell of assimilatory filament ---- 6
4. Hypogynous cell gives rise lateral cells with dense contents ---- Galaxauraceae
4. Hypogynous cell not produce lateral cells ---- 5
5. Compact carposporophyte is formed ---- Nemaliaceae
5. Diffused carposporophyte is formed ---- Dotyophycaceae fam. nov.
6. Compact carposporophyte is formed -- Helminthocladiaceae
6. Diffused carposporophyte is formed ---- Dermonamataceae

TAXONOMY OF THE GENUS *GRATELOUPIA* (HALYMENIACEAE, RHODOPHYTA) IN KOREA

Hae Bok Lee, Department of Biology, Chongju University, Chongju City 360-764, KOREA

The external morphology, internal structure and reproductive organ of nine species of *Grateloupia* (Halymeniaceae, Rhodophyta) analyzed and re-evaluated taxonomically. The species of *Grateloupia* reported until now in Korea is *Grateloupia divaricata, G. filicina, G. filicina* var. *porracea, G. imbricata, G. livida, G. okamurae, G. prolongata, G. sparsa* and *G. turuturu*. As a result of comparison of the taxonomic characters between *G. filicina* and *G. filicina* var. *porracea*, the latter is regarded as a independent species from the former. And the morphological characters of *G. okamurae* is compared to other species and re-evaluated taxonomically. The taxonomic characters of the plants belong to the genus *Grateloupia* are (1) lubricous texture, (2) 5-10 cell layers of cortex, (3) lobed inner cortical cell, (4) longitudinal arrangement of medullary filaments, (5) reproductive organs on frond surfaces, and (6) conical auxiliary cell ampulla with one primary and on to four secondary ampullar filaments.

TAXONOMY AND PHYLOGENY OF A GREEN DINOFLAGELLATE, *LEPIDODINIUM VIRIDE*.(DINOPHYTA).

Makoto M. Watanabe[1] Shoichiro Suda[1], Isao Inouye[2] and Tsutomu Sasa[1]. [1]National Institute for Environmental Studies, Tsukuba, Ibaraki, Japan. [2]Institute of Biol.Sci., Univ. Tsukuba, Tsukuba, Ibaraki, Japan.

A green-colored marine dinoflagellate, *Lepidodiunium viride* with a chlorophyll *a*- and *b*-containing vestigial endosymbiont is given with special emphasis on the morphology and the pigment composition.

The host dinoflagellate cell is unarmored and has a gymnodinoid overall appearance. The theca or amphiesma basically consists of the outer membrane and flattened thecal vesicles in which no thecal plates are developed. Unusual hand basket-shaped scales cover the entire cell surface together with a layer of mucilaginous material. These findings led us to conclude that the organism was a new member of the Gymnodiniaceae and to propose the above new genus and species name.The ultrastructure of the host cells is typical of the dinoflagellates; however, the organism has 1) an unusual cytoplasmic projection that may be a homologue of the peduncle, 2) a single membrane-bounded body containing membranous sheets, closely situated next to the endosymbiont, and 3) an electron opaque network-forming appendage surrounding the transverse flagellum. None of these features have been found in other dinoflagellates.

The vestigial endosymbiont is unlike anything that has been found in the dinoflagellates before. The cytoplasm of the endosymbiont is separated from the host cytoplasm by a double membrane and neither a nucleus or mitochondria occur within it. The endosymbiont contains chlorophylls a and b and the usual chlorophyte carotenoids, that are neoxanthin, violaxanthin, antheraxanthin, zeaxanthin and beta-carotene. In addition to these carotenoids, some unknown peaks were detected. One peak is situated at the identical retention time to that of lutein, but the absorption spectrum is slightly different from that of lutein. The other peaks are undecided, although there is a small peak which seems to show the identical retention time and absorption spectrum to those of siphonaxanthin. Based on these results, it is speculated that a prasinophyte would seem to be the likeliest candidate for a progenitor of the endosymbiont.

ALGAL CALCIFICATION. ITS CONTRIBUTION TO THE "CO_2 PROBLEM".

Megumi Okazaki. Department of Biology, Tokyo Gakugei University, Nukuikita-machi, Koganei-shi, Tokyo 184, Japan.

The recent increase in atmospheric CO_2 (350ppm at present) is a matter of anxiety because of rapid climatic changes from its greenhouse effect. However, the primitive atmosphere contained much more CO_2 (about 97%) and it has been suggested that photosynthesis and biological $CaCO_3$ deposition in ocean played an important role to obtain the present low level of CO_2 on the earth. In fact, nearly 10,000 times as much carbon of atmosphere is in undecayed organic matter in sediments and indeed 100,000 times as much in limestone ($CaCO_3$).

The certain corallinaean algae form algal limestone and Halimeda is important as a $CaCO_3$-sand former and as a sediment-consolidator in coral reefs.

Coccolithophorid such as Emiliania huxleyi is regarded as the chief producer of $CaCO_3$ in ocean.

$CaCO_3$ deposition in ocean takes place by a following reaction: $2\ HCO_3^- + Ca^{2+} \rightarrow CO_2 + CaCO_3 + H_2O$. Therefore, it should be noticed that $CaCO_3$ formation accompanies CO_2 evolution from dissolved HCO_3^- in seawater. Algal calcification, however, is coupled with their photosynthesis and the rate of photosynthesis is several times higher than calcification rate. Thus, CO_2 release from seawater never takes place during their calcification process. However, their photosynthetic products in ocean might be completely oxidized sooner or later. Therefore, contribution of calcification to fixing atmospheric CO_2 is dependent on what proportion of organic matter produced in ocean is not oxidized back to CO_2.

CONTRIBUTED PAPERS

AGLAOTHAMNION CHEJUENSE SP. NOV. RHODOPHYTA, CERAMIACEAE) FROM KOREA. Gwang Hoon Kim and In Kyu Lee. Department of Biology, Seoul National University, Seoul 151-742, Korea

Aglaothamnion chejuense, a new red alga bearing parasporangia, is described from Cheju island of Korea. Although, the plants showed alternate branching pattern and had only one nucleus in each vegetative cell, it was clearly distinguished from other members of the genus *Aglaothamnion* as well as *A. hookeri*, the only other species bearing parasporangia, by the characters of branching pattern, apical parasporangia and lack of sexual stages. Asexual life history which comprised of parasporophyte and tetrasporophyte was repeated six times without any sexual reproductive structure. The parasporangia developed from an apical cell of branch, whereas the tetrasporangia developed seriately on the adaxial side of lateral branches.

CULTURE OF LAMINARIA JAPONICA USING DEEP-OCEAN-WATER PUMPED UP IN TOYAMA BAY. Daisuke Fujita. Toyama Pref. Fish. Exp. Stn., Nameri-kawa-shi, Toyama, Japan.

Using flowing deep-ocean-water(DOW, 6℃) pumped up from the depth <200m of Toyama Bay, one year old plants of Laminaria japonica were cultured in a tank set on the artificial upwelling experimental facility for about one month in summer. They grew well at the rate of 9.6mm/day, while those cultured in the sea (25℃, 5m depth) deteriorated. Iron level was high at the old part of DOW-cultured kelp. In the laboratory culture(10℃, 3,000 lux) for one month, sporelings of the kelp grew the best in PEDOWI, and in PESI, in DOW and in SW in turn. Undaria pinnatifida sporelings also grew well in the order above.

A RE-EXAMINATION OF SOME EUROPEAN AND CALIFORNIAN *LAURENCIA* SPECIES(CERAMIALES,RHODOPHYTA). KiWan Nam and Yuzuru Saito*. Dept. of Mar. Biol., National Fish. Univ. of Pusan. *Lab. Mar. Bot., Fac. Fish., Hokkaido Univ.

Morphology of some European and Californian species, *L. pinnatifida, L. spectabilis, L. crispa, L. platycephala* and *L. hybrida*, was re-examined. Spermatangia and tetrasporangia essentially differ from those of *L. obtusa*, the type of the genus, as produced at alternately branched filaments derived from epidermal cells rather than at dichotomously branched trichoblasts done from axial cells and at epidermal cells rather than at pericentral cells, respectively. Those species probably occupy a different position in evolutionary line from the genus.Of those species, *L. hybrida* is distinctive in with six pericentral cells instead of five at fertile segment of female trichoblast.

GAMETOGENESIS AND EARLY SPOROPHYTE DEVELOPMENT OF *LAMINARIA RELIGIOSA* MIYABE IN THE EAST COAST OF KOREA. Jin Ae Lee, Dept. Environ. Sci., Inje Univ., Kimhae 621-749, Korea.

Ecotypic populations of *Laminaria religiosa* in the east coast of Korea were monitored from October 1989 to October 1990 to investigate growth and reproductive phenology at near southern limit of its distribution in East Sea of Korea. Plants exhibited an annual growth pattern with maximum in June. Most of the morphological parameters measured showed the similar pattern and reached maximum values in June. Blade disintegration occurred during the summer months. Reproductive sporophytes occurred from October to December 1989. Young sporophyte population was observed in March, which was assumed to be the result of the late fall sporogenesis activity. Reproduction and growth in gametophytes and growth in juvenile sporophytes were studied in relation to temperature and irradiance. Although no seasonal variation was found, higher irradiance (80 $\mu E \cdot m^{-2} \cdot sec^{-1}$), and temperature of 4 - 12 ℃ resulted in greater activities.

A STUDY OF DIATOM SPECIES GOMPHONEMA VIBRIO EHR. VAR. SUBCAPITATUM (MAYER) LEE, COMB. NOV. Jung Ho Lee[1], Toshikazu Gotoh[2] and Jun Chung[1]. [1]Dept. Biol., Kyungpook Univ., Taegu, Korea. Dept. Biol., [2]Kinki Univ., Osaka, Japan.

This taxon was described as Gomphonema intricatum var. vibrio f. subcapitata by Mayer (1928). Micromorphology of this taxon and G. vibrio were studied with ornamentation of girdle can be devided this taxon from G. angustum (Syn. G. intricatum). There is no basical difference between G. vibrio and V. intricatum var. vibrio f. subcapitata except for size and outline of valve and striae density. It therefore is proposed that the new combination, G. vibrio var. subcapitatum. And also, it is elucidate that G. nipponica Skv. is a synonym of this taxon.

A STUDY ON THE TAXONOMY OF THE MARINE DIATOM GENUS *COSCINODISCUS* AND THEIR GEOGRAPHICAL VARIATIONS IN THE KOREAN COASTAL WATERS. Jin Hwan Lee and Yoon Hee Jung. Sangmyung Women's University, Seoul 110-743, Korea

In order to identify the RADIATI group of the diatom genus *Coscinodiscus* from the eastern, western, and southern Korean coastal waters, it has been studied both light microscope (LM) and scanning electron microscope (SEM) observations of a fine structure and morphological patterns.

As a result, the genus *Coscinodiscus* was recorded six species and one unidentified species : *C. gigas*, *C. granii*, *C. asteromphalus* and *C.* sp.1.

Most of *Coscinodiscus* taxa have showed the insignificant morphological (the valve diameter, number of areolae, the number of marginal processes, etc.) variations in three coastal waters of Korea. As compared between Korean specimens and other waters, it showed geographical differences ; valve diameters, areolae number, feature of labiate processes, perforations of the cribra, etc.

THE EFFECT OF SEAWATER DILUTION ON THE PHOTOSYNTHETIC ACTIVITY OF SEAWEEDS GROWING IN TIDE POOLS. Nobuyasu Katayama[1], Kumi Takakura[1] and Yasutsugu Yokohama[2]. [1]Dept. of Biology, Tokyo Gakugei Univ., Koganei, Tokyo, Japan. [2]Shimoda Marine Research Center, Univ. of Tsukuba, Shimoda, Shizuoka, Japan.

The effect of a stepwise seawater concentration fall, diluted with distilled water or bufferred isotonic NaCl solution(pH 8.2), on the photosynthetic rate of *Ulva pertusa*, *Enteromorpha crinita* and *Grateloupia filicina*, growing in tide pools, was examined. *E. crinita* was most tolerant of the seawater dilution among them. The photosynthetic activity of *G. filicina* was affected mainly by the change in CO_2 concentration, while of *U. pertusa* the activity was affected by the changes in both pH and salinity. These seaweeds retained their photosynthetic activities even in freshwater in several hours. The result obtained indicates that the tolerance for the seawater dilution by a rainfall must be one of the important properties of seaweeds growing in tide pools.

INFLUENCE OF LIGHT INTENSITY AND TEMPERATURE ON CALLUS DEVELOPMENT IN YOUNG SPOROPHYTES OF SOME SPECIES OF LAMINARIALES (PHAEOPHYTA). Masahiro Notoya, Mikako Nagashima and Yusho Aruga. Lab. of Phycol., Tokyo Univ. of Fish., Konan-4, Minato-ku, Tokyo, 108 Japan

Excised rectangular blade pieces (0.5-1 mm) in young sporophytes (3-5 mm blade length) of four Laminariales species (Costaria costata, Eisenia bicyclis Laminaria japonica, Undaria pinnatifida) were cultured under various light intensities (1-8 klux) and temperatures (10-25℃), and callus development and differentiation were observed. Callus development from pieces of sporophyte explant was almost the same in each species. After 2-4 days in culture, callus cells were produced from the cut edge. In about 3-4 weeks, blade-like plantlets were differentiated from growing callus cells. Favorable conditions for callus development from explants were different with species: 15℃ and 2-4 klux in C. costata, 20 ℃ and 1 klux in E. bicyclis, 10-15℃ and 1 klux in L. japonica and 15-20℃ and 2 klux in U. pinnatifida.

TAXONOMY ON THE GENUS *CLADOPHORA* (CLADOPHORACEAE, CHLOROPHYTA) FROM KOREA
Yoon Sik Oh and In Kyu Lee. Department of Biology, Seoul National University, Seoul 151-742, Korea

A taxonomic study on the members of *Cladophora* commonly collected from the coasts of Korea was carried out to re-appraise the morphological characters and elucidate the interspecific delimitations among species. For the distinction of *Cladophora* species, the following taxonomic criteria along with traditional ones were used: architecture, color, texture, rhizoidal morphology of plants, density, basal fusion, and ramification with phyllotaxis of branches, and cell shape and dimension. On the basis of these criteria, seventeen species including a new one were described in this study. They also showed distributional pattern closely related to environmental factors and were divided into several groups with ecological characteristics in relation to the morphological variations.

GENETIC IMPROVEMENT OF EATING QUALITY OF DRIED SHEETS OF PORPHYRA BY USING WILD-TYPE RECOMBINANT IN P. YEZOENSIS. Jong-Ahm Shin and Akio Miura. Lab. Algae Cultivation, Tokyo Univ. Fish., Minato-ku, Tokyo, Japan.

Growing test of the gametophytes of ZGRW was performed for determining of characteristics pertaining to ZGRW. Crispness and free amino acids contents of dried sheets of Porphyra are related in eating quality. They were determined to compare ZGRW with growers' one. ZGRW showed better quality in crispness than growers' one. Concerning free amino acids contents, alanine, glutamic acid, aspartic acid and taurine in ZGRW were more abundant than those of growers' one. Alanine and glutamic acid are concerned in taste substances in dried sheets of Porphyra. Alanine was 522.86-2625.57mg/100g and glutamic acid was 592.87-1574.02mg/100g in ZGRW to that alanine 485.69-2525.89mg/100g and glutamic acid 476.70-1472.59mg/100g in growers' one. ZGRW presented more sweet flavor than growers' one.

PURIFICATION AND CHARACTERIZATION OF NITRATE REDUCTASE FROM *PORPHYRA YEZOENSIS* (RHODOPHYTA). Yoshiko Nakamura and Tomoyoshi Ikawa. Institute of Biol. Sci., Univ. of Tsukuba, Tsukuba-shi, Ibaraki, Japan.

Assimilatory nitrate reductase (NR) catalyzes the first step in the reduction of nitrate to ammonia. We developped an effective method to isolate the homogenious NR from *Porphyra yezoensis* using PEG treatment, ammonium sulfate fractionation, chromatography on butyl Toyopearl 650-M, blue Sepharose CL-6B, DEAE Cellulose, hydroxyapatite colums and Sephacryl S-400 gel filteration. The best enzyme preparation was purified to 5,700-fold and had a specific activity of 12.5 μmol $NO_2^- \cdot min^{-1} \cdot mg^{-1}$ protein. A molecular weight for the native enzyme was estimated to be 380,000 and for the subunit to be 100,000. Deduced from the native and subunit molecular weights, NR from *P. yezoensis* is a tetramer. The UV/visible absorption spectra of the oxidized and reduced NR indicated typical features of b-type cytochromes. The enzyme was NADH specific and had an optimal pH at pH 8.5.

EFFECTS OF HEAVY METALS ON *ULVA PERTUSA* KJELLMAN. Ik Kyo Chung & Mi Kyung Kim, Dept. of Marine Science, Pusan National Univ., Pusan 609-735, KOREA.

The effects of several metals (Cd, Cu, Zn, Hg) on *Ulva pertusa* were examined. Acclimated disc samples were continuously exposed for a maximum period of 2 weeks to a series of concentration of metals supplemented to enriched seawater.

Toxicity of metals assessed in terms of growth rate, photosynthetic pigment contents and chlorophyll fluorescence, were highest in Hg treated samples, severe in Cu, moderate in Zn and low in Cd in *U. pertusa*. Values of F_{max}/F_o ratio in photochemical quenching were remarkably decreased and growth rates were significantly reduced at higher concentrations of metals. These indicate that those are effective and reliable means of assessing toxicity of contaminants in relatively short term studies with this green alga.

PHENOLOGY AND MORPHOLOGICAL VARIABILITY IN A KOREAN POPULATION OF *GRACILARIA VERRUCOSA* (HUDSON) PAPENFUSS, RHODOPHYTA.
Kim, Myung-Sook, In Kyu Lee and Sung-Min Boo*. Dept. of Biology, Seoul Natl. Univ., Seoul 151-742 and *Dept. of Biology, Chungnam Natl. Univ., Daejon 305-764, Korea

The phenology and morphological variability of *Gracilaria verrucosa* (Huds) Papenfuss were analysed at the littoral habitat of Daechon, west coast of Korea from July 1988 to June 1989. Fifty or more plants were sampled haphazardly in field for phenology. Twenty-five plants were randomly selected and measured for the length, axis diameter, medullary cell diameter and constriction in basal portion of branches. Cystocarpic plants occurred at maximum from June to July, while tetrasporic ones dominated from August to September. This implied that ecological conditions related with summer were important for reproduction of our plants. Plant length, axis diameter, medullary cell diameter and constricton in basal portion of branches varied significantly throughout the year. The correlation coefficient between axis diameter and medullary cell diameter was positively correlated, whereas between plant length and constriction of basal portion of branches it was negatively correlated. In standardization of four investigated characters, the monthly variability of basal constriction of branches contrasted with those of other three features.

SEXUAL REPRODUCTION IN THE MARINE DINOFLAGELLATE PYROPHACUS STEINII.
Pornsilp Pholpunthin, Yasuwo Fukuyo, Hiroaki Inoue and Yoshihachiro Nimura. Department of Fisheries, Faculty of Agriculture, University of Tokyo, 1-1-1 Yayoi, Bunkyo-ku, Tokyo, Japan

The sexual reproduction in Pyrophacus steinii is anisogamous and heterothallic. Male gametes differ from female gametes and vegetative cells. The female gametes can not be differenciated from the vegetative cells. Cell fusion between the male and female gametes occurs in a few hours to several days after inoculation of the male gametes into a culture of non-male clone. Zygotes are similar to the vegetative cells in shape except possessing two longitudinal flagella. The transformation from the planozygote to the hypnozygote (resting cyst) requires five to eight days for completion.

―会 員 移 動―

新　入　会

住 所 変 更

退　　会

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日本学術会議だより　No.22

第15期最初の総会開催される

平成３年８月　日本学術会議広報委員会

日本学術会議の第15期が７月22日から発足し，７月22日～24日の３日間，第15期最初の総会が開催されましたので，その総会等についてお知らせします。

日本学術会議第112回総会報告

７月22日の第15期の発足に伴い，内閣総理大臣による日本学術会議会員の辞令交付が行われた。第15期の会員は，選出制度が学術研究団体を基礎とする推薦方式になって，３回目の会員である。この第15期会員による最初の総会である，第112回総会が７月22日から24日までの３日間，本会議講堂で開催された。

第１日目（22日）は，午前は新会員への辞令交付式があり，午後総会が開会され，直ちに，会長及び両副会長の選挙が行われた。会員による互選の結果，会長には近藤次郎第５部会員が13期，14期に引き続き三選された。人文科学部門の副会長には，川田侃第２部会員，自然科学部門の副会長には，渡邊格第４部会員が選出された（渡邊副会長は再選）。選挙終了後，近藤会長から「新人の方が半数以上おられ，大きな抱負をもっておられると思う。挫折感を持つことのないようできるだけの努力をしたい。皆様にも御協力をお願いしたい。」との就任のあいさつがあり，又，川田，渡邊両副会長からもそれぞれ就任のあいさつがあった。

会長，副会長選出後は，直ちに各部会が開催され，各部の部長，副部長，幹事の選出が行われた。（第15期の役員については，別掲を参照）

第２日目は10時に総会が開催され，近藤会長が14期の会長という資格で第14期の総括的な活動報告を行った。その報告の折々には，国際交流とか，将来計画委員会，学術会議の予算等，会長の感慨，または感想をも交えてその所感を述べた。続いて，会員推薦管理会報告として，久保亮五委員長の代理として事務総長が，第15期会員の推薦を決定するまでの経過報告を行った。

引き続き，会長から３日目の総会で提案・審議する予定の「第15期活動計画委員会の設置について（申合せ案）」に関する各部での事前討議について，並びに各常置委員会の各部での委員の選出について，それぞれ各部へ依頼した。

総会終了後，各部会が開催され，前述の申合せ案の討議及び各常置委員会委員の選出等が行われた。

第３日目（24日）。10時に総会が開会され，会長から「第15期活動計画委員会の設置について」の提案が行われた。これは，第15期の活動の基本計画の立案を目的とする臨時の委員会を次の定例総会までの間，設置するということを内容としている。そしてこの提案は原案どおり可決された。

総会終了後，直ちに各部会が開会され，設置が決定された第15期活動計画委員会委員の選出等が行われた。

なお，この第15期活動計画委員会は，総会期間中に第１回の会議を開き，全会員を対象にした第15期の学術会議の活動に関するアンケートの実施を決めるなど，早速その活動を開始した。

また，運営審議会附置委員会，常置委員会，国際対応委員会等も活動を開始した。

第15期日本学術会議の辞令交付式等について

第112回総会に先立ち，第15期日本学術会議会員の辞令交付式が７月22日（月）11時から、総理大臣官邸ホールで行われた。辞令交付式は，海部内閣総理大臣，坂本内閣官房長官，大島，石原両官房副長官，稲橋総理府次長等の出席を得て執り行われた。

第１部から第７部までの会員１人ずつの名前が読み上げられた後全会員の最年長である渡邊格第４部会員が代表して海部総理から辞令を手渡された。この後，海部総理大臣から「会員の皆様には，創造性豊かな科学技術の発展，総合的観点に立った学術研究に係る諸活動に御尽力いただきたい。」とのあいさつがあり，これに応えて第15期会員を代表して渡邊格会員が「微力ながら全力を尽くし，重要な責務を全うし，国民の期待に応えたい。」とあいさつがあり，式は終了した。式には192名の会員が出席した。

また，総会２日目の夕方には，学術会議ホールで，坂本官房長官主催の第15期会員就任パーティーが開催された。パーティーは坂本官房長官のあいさつで開会し，日本学士院院長代理の藤田良雄幹事の祝辞があり，これに対する近藤会長の答礼のあいさつ，沢田敏男日本学術振興会会長の発声による乾杯の後，懇談に入った。ホールには溢れんばかりの人々で歓談が続き盛会であった。

第15期日本学術会議役員

会　長　　近藤　次郎（第５部・経営工学）
副会長　　川田　侃（第２部・政治学）
副会長　　渡邊　格（第４部・生物科学）
＜各部役員＞

第１部	部　長	肥田野　直	（心理学）
	副部長	弓削　達	（歴史学）
	幹　事	一番ケ瀬康子	（社会学）
	〃	山本　信	（哲学）
第２部	部　長	西原　道雄	（民事法学）
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	幹　事	正田　彬	（社会法学）
	〃	山下　健次	（公法学）
第３部	部　長	大石　泰彦	（経済政策）
	副部長	島袋　嘉昌	（経営学）
	幹　事	岡本　康雄	（経営学）
	〃	藤井　隆	（経済政策）
第４部	部　長	中嶋　貞雄	（物理科学）
	副部長	田中　元治	（化学）
	幹　事	竹内　郁夫	（生物科学）
	〃	樋口　敬二	（地球物理学）
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	幹　事	内田　盛也	（応用化学）
	〃	増子　曻	（金属工学）
第６部	部　長	中川昭一郎	（農業総合科学）
	副部長	水間　豊	（畜産学）
	幹　事	志村　博康	（農業工学）
	〃	平田　熙	（農芸化学）
第７部	部　長	岡田　晃	（社会医学）
	副部長	伊藤　正男	（生理科学）
	幹　事	渥美　和彦	（内科系科学）
	〃	金岡　祐一	（薬科学）

（注）カッコ内は，所属部・専門

平成４年(1992年)度共同主催国際会議

本会議は，昭和28年以降，学術関係国際会議を関係学術研究団体と共同主催してきたが，平成４年（1992年）度には，次の６国際会議を開催することが，６月７日の閣議で了解された。(カッコ内は，各国際会議の開催期間と開催地)

・第９回国際光合成会議
（平成４年８月30日～９月５日，名古屋市）
共催団体：日本植物生理学会
・国際地質科学連合評議会及び第29回万国地質学会議
（平成４年８月24日～９月３日，京都市）
共催団体：(社)東京地学協会外５学会
・第５回世界臨床薬理学会議
（平成４年７月26日～31日，横浜市）
共催団体：日本臨床薬理学会
・第11回国際光生物学会議
（平成４年９月７日～12日，京都市）
共催団体：日本光生物学協会
・第14回国際平和研究学会総会
（平成４年７月27日～31日，京都市）
共催団体：日本平和学会
・第８回国際バイオレオロジー会議
（平成４年８月３日～８日，横浜市）
共催団体：日本バイオレオロジー学会

第15期日本学術会議会員の概要について

この度任命された210人の第15期日本学術会議会員の概要を以下に紹介する。（カッコ内は前期）

1	性別	男子207人(207人)	女子３人(３人)
2	年齢別	50～54歳　３人	55～59歳　29人
		60～64歳　105人	65～69歳　58人
		70～74歳　15人	
		最年長　74歳（76歳）	
		最年少　54歳（51歳）	
		平均年齢　63.5歳（63.1歳）	
3	勤務機関及び職名別		
(1)	大学関係	国立大学	71人(78人)
		公立大学	２人(４人)
		私立大学	93人(88人)
		その他	３人(２人)
		計	169人(172人)
(2)	国公私立試験研究機関・病院等		11人(９人)
(3)	その他	法人・団体関係	９人(10人)
		民間会社	９人(６人)
		無職	10人(13人)
		その他	２人(０人)
		計	30人(29人)
4	前・元・新別	前会員	88人(109人)
		元会員	３人(４人)
		新会員	119人(97人)
5	地方別（居住地）	北海道	４人(３人)
		東北	８人(６人)
		関東	133人(130人)
		中部	20人(17人)
		近畿	34人(42人)
		中国・四国	５人(４人)
		九州・沖縄	６人(８人)

（注）詳細については，日本学術会議月報７月号を参照

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(3403)6291

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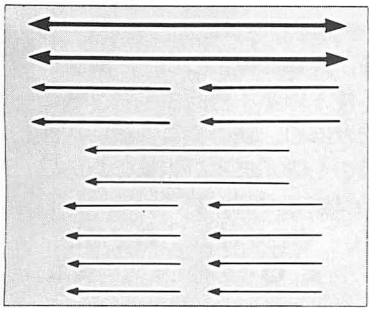

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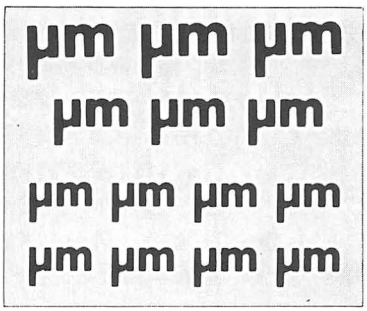

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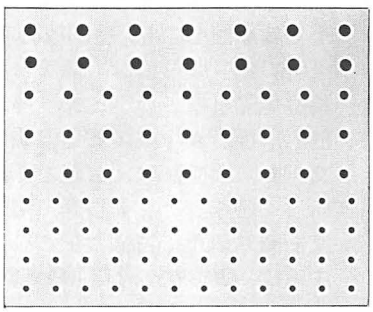

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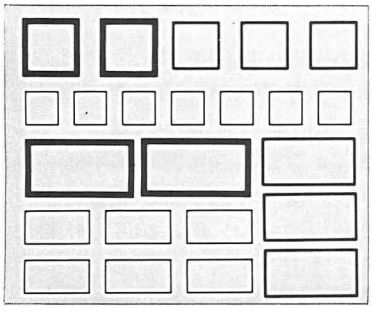

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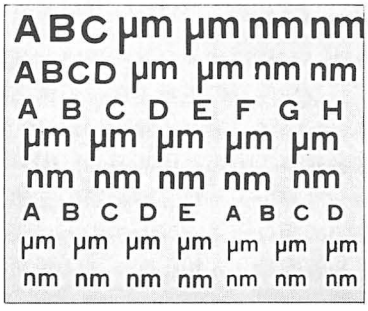

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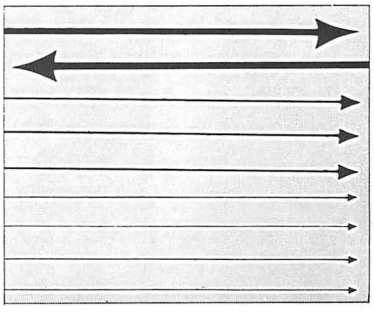

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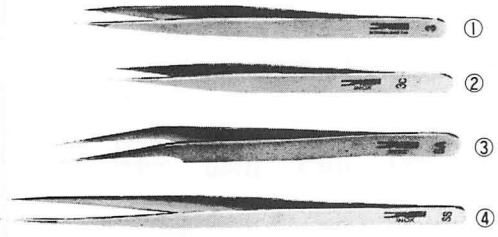

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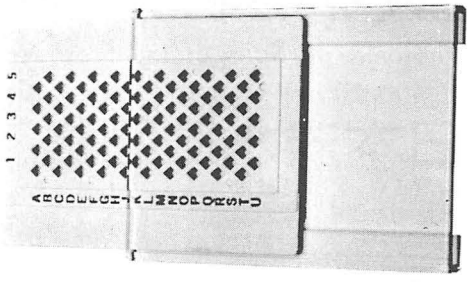

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3. **山田幸男先生追悼号**　藻類25巻増補．1977．A 5 版，xxviii + 418頁．山田先生の遺影・経歴・業績一覧・追悼文及び内外の藻類学者より寄稿された論文50編（英文26，和文24）を掲載，価格 7,000円。

4. **日米科学セミナー記録**　Contributions to the systematics of the benthic marine algae of the North Pacific. I. A. Abbott • 黒木宗尚共編．1972．B 5 版，xiv + 280頁，6 図版．昭和46年 8 月に札幌で開催された北太平洋産海藻に関する日米科学セミナーの記録で，20編の研究報告（英文）を掲載。価格 4,000円。

5. **北海道周辺のコンブ類と最近の増養殖学的研究**．1977．B 5 版，65頁。昭和49年 9 月に札幌で行なわれた日本藻類学会主催「コンブに関する講演会」の記録。4 論文と討論の要旨。価格 1,000円。

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1. **Back numbers of the Japanese Journal of Phycology** (Vols. 1–28, Bulletin of Japanese Society of Phycology). Price, 2,000 Yen per issue for member, or 3, 500 Yen per issue for nonmember; price of Vol. 30, No. 4 (30th Anniversary Issue), with cumulative index (Vols. 1–30), 6,000 Yen for member, or 7,500 Yen for nonmember (incl. postage, surface mail). Lack: Vol. 1, Nos. 1–2; Vol. 4, Nos. 1, 3; Vol. 5, Nos. 1–2; Vol. 6–Vol. 9, Nos. 1–3.

2. **Index of the Bulletin of Japanese Society of Phycology.** Vol. 1 (1953)–Vol. 10 (1962), Price 2,000 Yen for member, or 2,500 Yen for nonmember; Vol. 11 (1963)–Vol. 20 (1972), Price 3,000 Yen for member, or 4,000 Yen for nonmember. Vol. 1 (1953)–Vol. 30 (1982), Price 4,000 Yen for member, or 5,000 Yen for nonmember (incl. postage, surface mail).

3. **A Memorial Issue Honouring the late Professor Yukio Yamada** (Supplement to Volume 25, the Bulletin of Japanese Society of Phycology). 1977. xxviii + 418 pages. This issue includes 50 articles (26 in English, 24 in Japanese with English summary) on phycology, with photographs and list of publications of the late Professor Yukio YAMADA. 8,500 Yen (incl. postage, surface mail).

4. **Contribution to the Systematics of the Benthic Marine Algae of the North Pacific.** Edited by I. A. ABBOTT and M. KUROGI, 1972. xiv + 280 pages, 6 plates. Twenty papers followed by discussions are included, which were presented in the U.S.-Japan Seminar on the North Pacific Benthic Marine Algae, held in Sapporo, Japan, August 13–16, 1971. 5,000 Yen (incl. postage, surface mail).

5. **Recent Studies on the Cultivation of *Laminaria* in Hokkaido** (in Japanese). 1977. 65 pages. Four papers followed by discussion are included, which were presented in a symposium on *Laminaria,* sponsored by the Society, held in Sapporo, September 1977. 1,200 Yen (incl. postage, surface mail).

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