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# The tetrasporophyte of *Gymnogongrus flabelliformis* HARVEY (Gigartinales, Phyllophoraceae)

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Tetraspores of the crustose red alga *Erythrodermis* sp. from Oshoro Bay, Hokkaido gave rise to upright plants, morphologically and anatomically similar to *Gymnogongrus flabelliformis* HARVEY, in unialgal culture. Sexual reproductive structure did not develop on these plants. Carpospores of *G. flabelliformis* from Muroran, Hokkaido germinated in culture to form crustose plants anatomically similar to *Erythrodermis*. Transfer of these crusts from full strength enriched seawater medium to unenriched seawater resulted in the formation of seriate intercalary tetrasporangia. Tetraspores germinated to form *Gymnogongrus* plants. The specific determination of the crustose tetrasporophyte (*Erythrodermis*) awaits further comparison with species from other geographic areas. On the basis of our studies it is evident that *Gymnogongrus flabelliformis* exhibits an alternation of independent, heteromorphic gametophytes and tetrasporophytes.

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Recent life-history studies of several species of the *Gymnogongrus* have demonstrated the existence of two types of life history within the genus. In the first type the *Gymnogongrus* plant alternates with a tetrasporangial crust. These include *G. leptophyllus* J. AGARDH (DECEW and WEST 1977 a), *G. furcellatus* (C. AG.) J. AGARDH (CANDIA and KIM 1977) and *G. martinensis* SETCHELL et GARDNER (?) (WEST and DECEW unpublished observation). In the second type, a tetrasporangial phase develops on the gametophyte, termed "tetrasporoblastic" by SCHOTTER (1968). These include *G. crenulatus* (TURN.) J. AGARDH (ARDRÉ 1978) and *G. platyphyllus* GARDNER (DECEW and WEST, unpublished observation). In addition, KASAHARA (1977) reported a dsitinct type of life history for *G. flabelliformis* HARVEY. Its alternate crustose phase produced "monospores" which gave rise to an upright thallus. Thus, the situation regarding the life history of *Gymnogongrus* seems to be complicated.

However, our culture studies of *Gymnogongrus flabelliformis* started from tetraspores and carpospores have shown this species to have a crustose tetrasporophyte. In this paper the naturally occurring and cultured tetrasporophyte of *G. flabelliformis* is reported.

## Materials and Methods

Fertile crustose tetrasporophytes were collected intertidally in Oshoro Bay, Hok-

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kaido in Japan, on November 4, 1977 and November 16, 1977 by M. MASUDA. Unialgal cultures were established according to methods reported for *Neodilsea crispata* (MASUDA 1973) and kept in freezer-incubators at the laboratory of Department of Botany, Faculty of Science, Hokkaido University, Sapporo. Fertile cystocarpic plants were collected intertidally at Muroran, Hokkaido in Japan, on November 29, 1975 and shipped on ice to Berkeley by J. A. WEST. Unialgal cultures were obtained using methods reported for *Hildenbrandia occidentalis* and *H. prototypus* (DECEW and WEST 1977b) and maintained in plant growth chambers at the laboratory of Department of Botany, University of California, Berkeley. At Sapporo laboratory, the cultures were kept at 5°C, 16 : 8 LD (light and dark cycle); 5°C, 8 : 16 LD; 10°C, 16 : 8 LD; 10°C, 8 : 16 LD; 15°C, 16 : 8 LD; 15°C, 8 : 16 LD; 20°C, 16 : 8 LD; and 20°C, 8 : 16 LD, each of which was illuminated with cool-white fluorescent lamps (2500–3000 lux). At the Berkeley laboratory, cultures were kept at 15°C, 16 : 8 LD, 210 lux; 15°C, 8 : 16 LD, 500 lux; and 20°C, 16 : 8 LD, 3100 lux, each of which was illuminated with cool-white fluorescent lamps.

## Results

*Observations of tetrasporangial crusts collected in the field*: The crusts grow on stones or rocks and are associated with *Gymnogongrus flabelliformis*, *Rhodoglossum japonicum* and *Dictyopteris divaricata*. They are circular to elliptical in shape (Fig. 1) and dark red in color. Fertile crusts reach 1.6–2.6 cm in diameter and are 300–580 μm thick in the central portion (including the nemathecia). They are composed of a monostromatic hypothallus, which consists of radiating filaments and attaches firmly to the substrate, and a polystromatic perithallus, which consists of tightly packed erect filaments (Fig. 2, A–C). The cells of the hypothallus are rectangular and 7.3–12.5 μm high and 9.4–30.5 μm broad in tangential section. The perithallus is

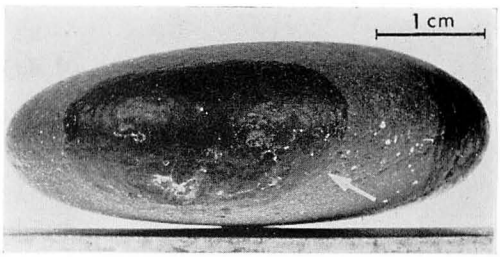



Fig. 1. Habit photograph of a field-collected crustose tetrasporophyte of *Gymnogongrus flabelliformis* (arrow) on a pebble.

thick and composed of 18–40 cell layers at the central portion of the thallus but becomes thinner toward the growing margin. The erect filaments of the perithallus are simple or dichotomously divided (Fig. 2, A–D). The cells of the erect filaments are 8.8–12.5 μm broad near the hypothallus, becoming slightly narrower distally, and are 5.3–7.5 μm broad at the distal end. They are 0.5 to 2 times as long as broad. No lateral fusion between cells of adjacent erect filaments was found.

The tetrasporangia are formed in large nemathecia, a characteristic of the Phyllophoraceae (Fig. 2, D–E), so that the nemathecia can be discriminated under a dissecting microscope. The nemathecia are circular to elliptical and 1250–2500 μm in diameter and 110–280 μm thick in the center. The tetrasporangium initials are transformed from intercalary cells of the erect filaments constituting the nemathecia (Fig. 2, D). The tetrasporangia are formed in 3–9 successive cells of a single filament in the center of the nemathecia and in 1 or 2 cells in the marginal portion (Fig. 2, D–E). All erect filaments of the nemathecium except the marginal ones produce tetrasporangia. Therefore, the nemathecium is packed with numerous sporangia. The terminal one or two cells of the fertile filaments remain sterile (Fig. 2, D–E). The mature tetrasporangia are ellipsoidal or globular in shape, measuring 20.0–30.0 μm × 17.5–22.5 μm, and are cruciately divided (Fig. 2, E).

*Culture experiments with tetraspores*:

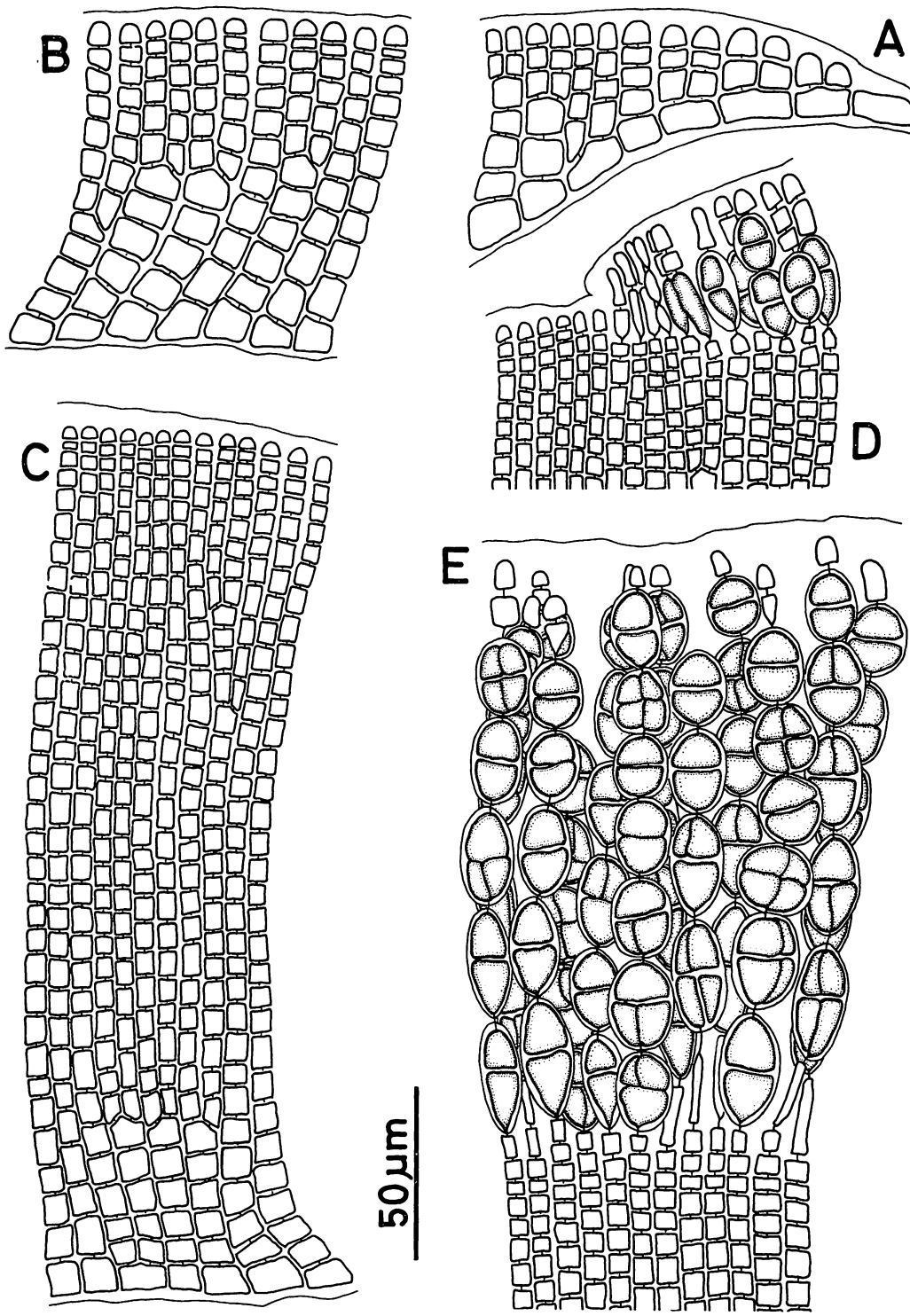
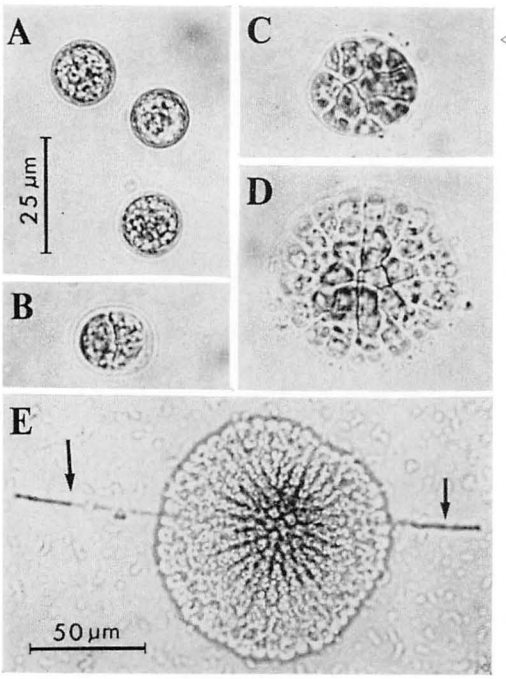



Fig. 2. Tangential sections of field-collected tetrasporophytes of *Gymnogongrus flabelliformis*. A. Marginal portion. B. Near marginal portion. C. Central portion. D. Marginal portion of the nemathecium. E. Central portion of the nemathecium.




⇦ Fig. 3. Tetraspores and the early development of *Gymnogongrus flabelliformis*. A. Three tetraspores. B–E. Tetraspore germlings grown at 10°C, 16 : 8 LD, B, two-day old; C, seven-day old; D, fourteen-day old; E, one-month old. (Arrows indicate two colorless hairs.) Scale in A applies also to B–D.

Fig. 4. Upright thalli derived from tetraspore germlings and field-collected *Gymnogongrus flabelliformis*. A. Two-month-old plant issuing a young upright thallus (arrow) from the crustose base grown at 10°C, 16 : 8 LD for 1 month and transferred to 15°C, 16 : 8 LD. B. Three-month-old plants grown at 10°C, 16 : 8 LD for 1 month and then transferred to 15°C, 16 : 8 LD. C–D. Seven-month-old plants grown at 10°C, 16 : 8 LD for 1 month and then transferred to 20°C, 16 : 8 LD. E. Young thallus of *G. flabelliformis* collected in Oshoro Bay on June 22, 1978. Scale in D applies also to E.

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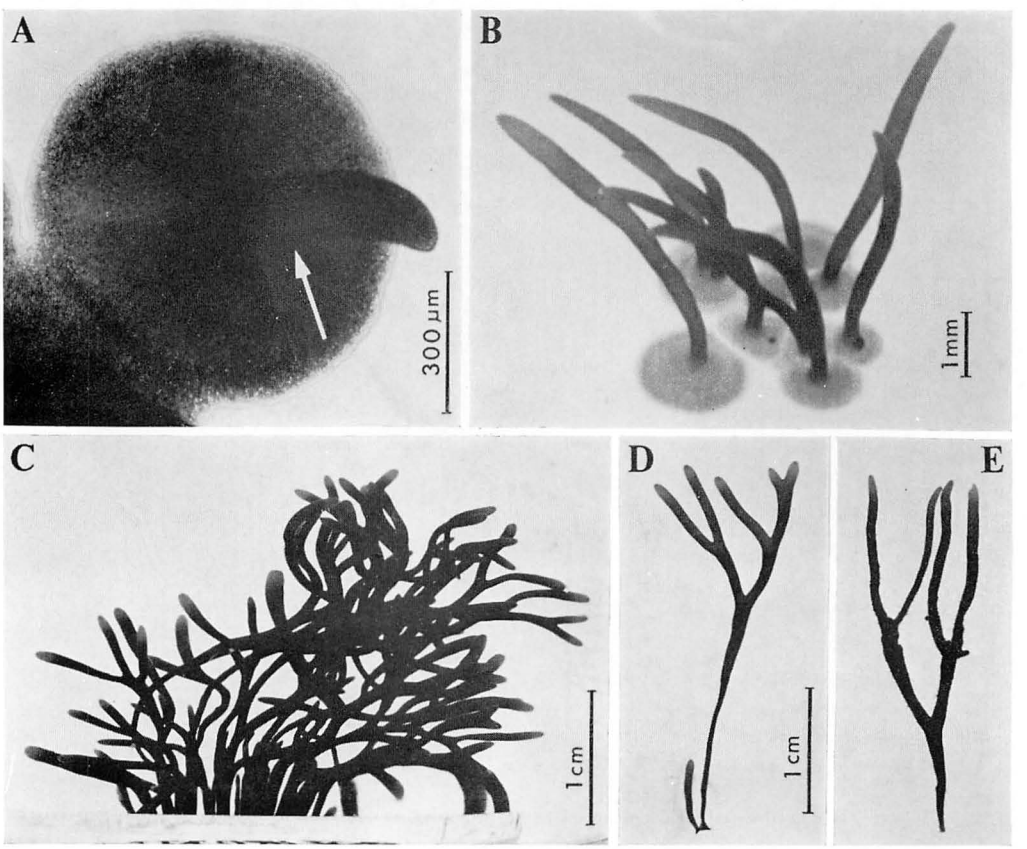

Liberated tetraspores are globular, pale rose in color, and 13.8–18.8 μm in diameter (Fig. 3, A). They were first cultured at 10°C, 16 : 8 LD. They attach to the substrate and divide into two cells within 1 or 2 days after inoculation (Fig. 3, B). The observed developmental stages are essentially similar to those described for carpospores of this species (KASAHARA 1977), and the germlings grow into polystromatic crusts (Fig. 3, C–E). However, the majority of the crusts formed colorless hairs (Fig. 3, E) which were not mentioned by KASAHARA for the carpospore germlings. After one month, the crusts reached 90–140 μm in diameter and formed 1–4 hairs which were 200–320 μm long and 2.5–3.0 μm broad.

One month-old cultures were divided into eight groups and grown under four different temperatures and two different light regimes (see Materials and Methods). Three weeks after transfer, the crusts grown at 15°C, 16 : 8 LD and 20°C, 16 : 8 LD, each formed an upright thallus near the center of the crust (Fig. 4, A). Seventy days after culture initiation, the crusts maintained at 10°C, 16 : 8 LD also produced upright thalli. By three months after culture initiation, the crusts grown at 10°C, 8 : 16 LD, 15°C, 8 : 16 LD and 20°C, 8 : 16 LD formed upright blades, and six and a half months after culture initiation those grown at 5°C, 16 : 8 LD and 5°C, 8 : 16 LD produced upright blades.

The crusts subsequently produced several upright blades. The upright blades grew well at 10–20°C and more rapidly under long-day conditions than under short-day conditions. By three months after culture initiation, the upright thalli grown at 15°C, 16 : 8 LD and 20°C, 16 : 8 LD had reached 4–7 mm in length (Fig. 4, B). The thalli were terete below but became flattened above. By seven months, the plants grown under these two conditions had developed into dichotomously divided upright thalli (Fig. 4, C–D), but the plants grown under the other culture conditions remained undivided. None of the cultured plants had become reproductive as of August, 1978, but they resemble field-collected *Gymnogongrus*

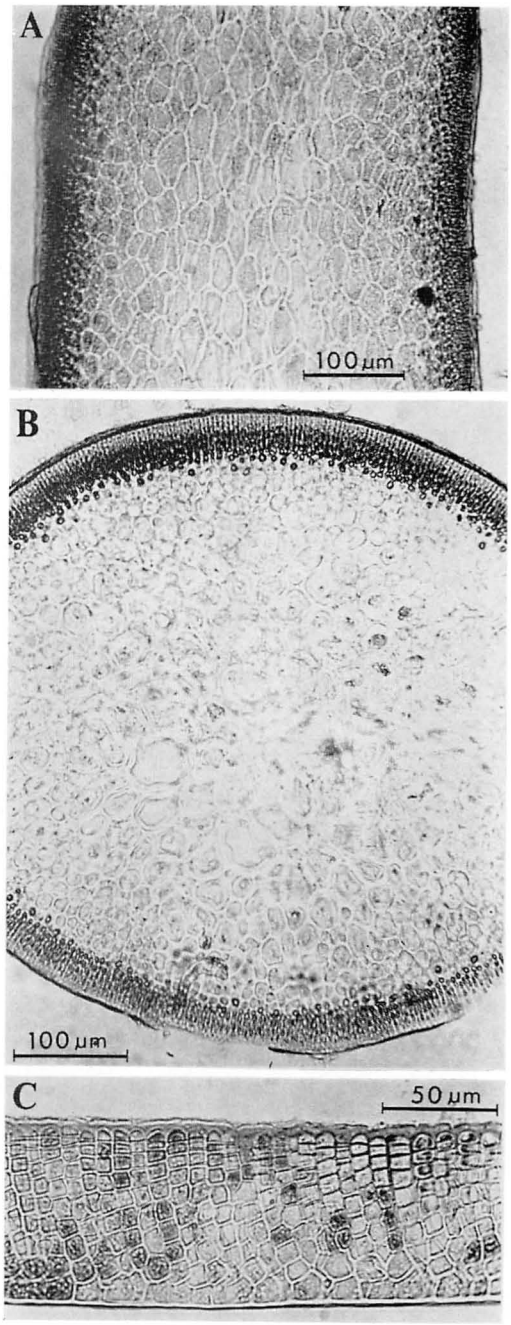


Fig. 5. Anatomical structure of cultured plants of *Gymnogongrus flabelliformis*. A. Longitudinal section of an upright thallus at 1 mm from the apex. B. Cross section of the middle portion of an upright thallus. C. Tangential section of a basal holdfast.

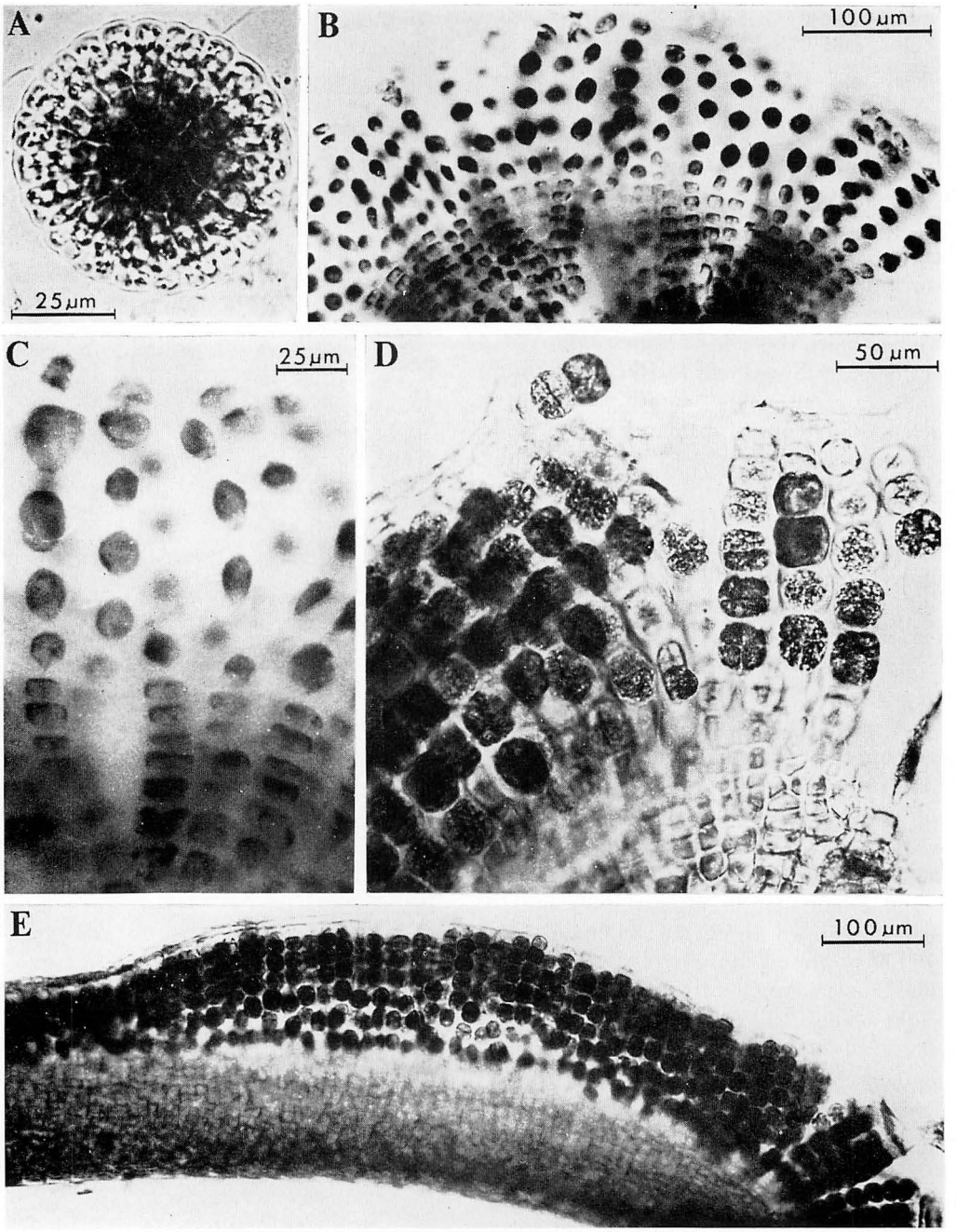


Fig. 6. Cultured tetrasporophytes of *Gymnogongrus flabelliformis*. A. Young tetrasporophyte grown at 15°C, 16 : 8 LD. B–D. Portions of tetrasporangial nemathecia, showing tetrasporangial mother cells in B–C and cleaved tetrasporangia in D. E. Tetrasporangial nemathecium on a crust.

*flabelliformis* in both external and internal structure (Fig. 4, C–E, 5, A–B). The structure of the basal crusts in both cultured and field *G. flabelliformis* is similar to that of the tetrasporangial crusts described above (Fig. 5, C).

*Culture experiments with carpospores*: Carpospores measuring 11–13 μm in diameter germinated either directly or by formation of a germ tube. Discs subsequently formed and continued to grow in thickness and diameter (Fig. 6, A). When the discs were about 1.2 cm in diameter (about 19 months after culture initiation), several treatments were tried to induce tetrasporogenesis: abrasion, changing daylength, dehydration, and reduction of nutrient levels. Two weeks after reducing nutrient levels from full strength PESW to only sterile seawater, tetrasporogenesis occurred.

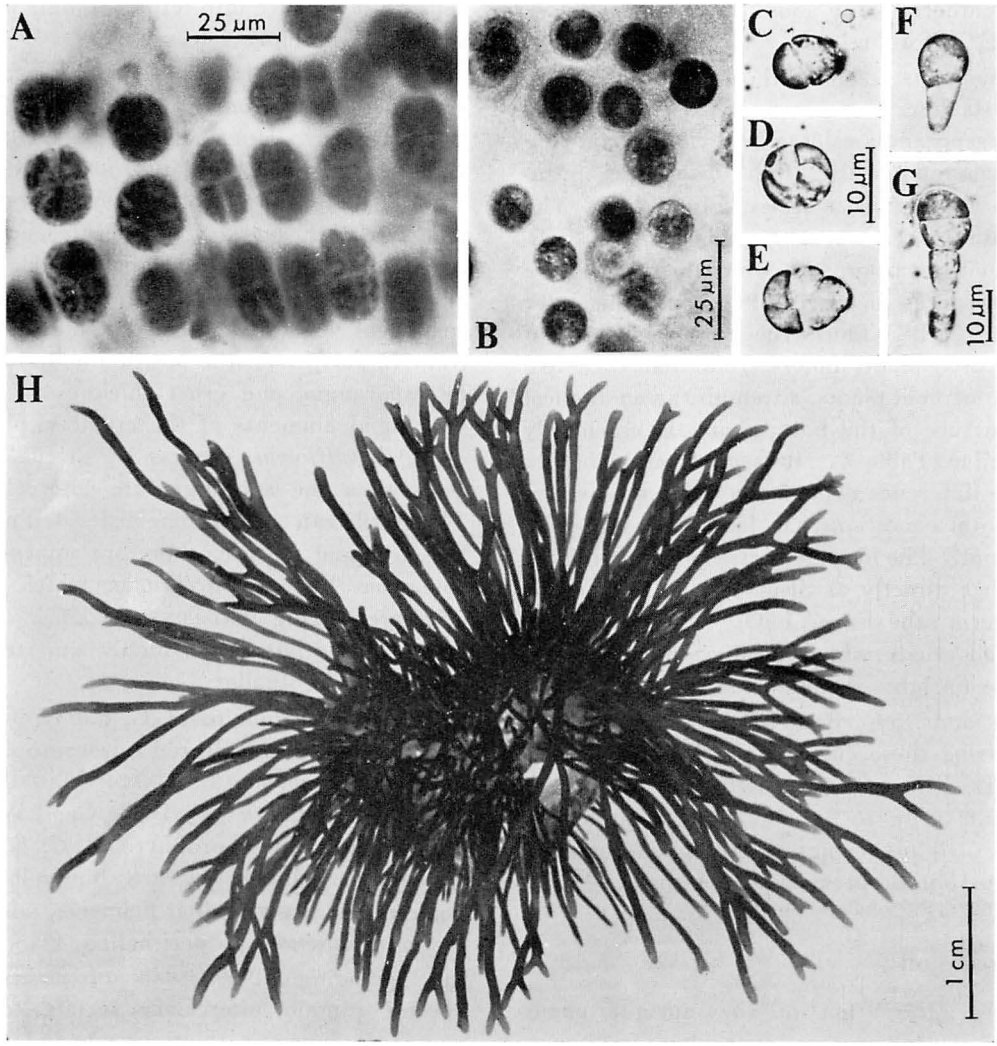


Fig. 7. Tetraspores, germinating tetraspores and upright thalli derived from cultured tetrasporophytes of *Gymnogongrus flabelliformis*. A. Series of tetrasporangia releasing. B. Tetraspores releasing from tetrasporangial wall. C–G. Germinating tetraspores. H. Eight-month-old plants grown at 15°C, 16 : 8 LD for 5 months and then shifted to 20°C, 16 : 8 LD. Scale in D applies also to E; scale in G applies also to C and F.

None of the other caused tetrasporogenesis. Further investigation into the developmental pattern indicated the following. The tetrasporangial mother cells become noticeable about five days after the nutrient reduction. They increase in size and cleave into tetrasporangia about five days later. Cleavage of the catenate series of 4–6 mother cells generally takes place basipetally, the first division taking place transversely (Fig. 6, B–C). The tetrasporangia remain in a nemathecium for about three days (Fig. 6, D–E) before release. The tetrasporangial nemathecia resemble those of field-collected crusts described above. The entire series of tetrasporangia release as a chain, following abscission below the lowermost tetrasporangium in a series (Fig. 7, A). The chain of tetrasporangia then breaks individual tetrasporangia which in turn release tetraspores measuring 9–12 $\mu$m in diameter (Fig. 7, B). Thus, the tetraspore size of cultured plants differs from that (13.8–18.8 $\mu$m) of field plants, although the anatomical structure of the both plants is essentially similar (Table 1). It is uncertain whether this difference depends on different environmental conditions, *i.e.*, laboratory and field, or not. The liberated tetraspores germinate either directly as discs (Fig. 7, C–E) or by a germ tube (Fig. 7, F–G). These grow into small crusts which produce upright axes. The uprights remain small in 15°C, 16 : 8 LD and 15°C, 8 : 16 LD. However, upon shifting these cultures to 20°C, 16 : 8 LD, a marked increase in growth rate took place. Plants grew to 3 cm in eight months (Fig. 7, F). Thus far none of the plants has produced either procarps or spermatia.

## Discussion

An alternation of the upright gametophyte and crustose sporophyte in *Gymnogongrus flabelliformis* was reported by KASAHARA (1977). According to him, the sporophyte formed "monosporangia". This is contrary to our results described above. A comparison of the development and morphology of the plants cultured by KASAHARA with our cultured plants indicates that while KASAHARA's observations were partially correct, they may be incomplete. What he interpreted to be "monosporangia" may have actually been tetrasporangial mother cells. Interpretation of the tetrasporangial mother cells as monosporangia is certainly conceivable, since the mother cells do not cleave into tetrasporangia until several days before release.

The tetrasporophyte of *G. flabelliformis* is similar to *Erythrodermis haematis* (HOLLENB.) DENIZOT (HOLLENBERG 1943; DENIZOT 1968) and *E. pacifica* HOLLENBERG (1969). The tetrasporophyte of *G. flabelliformis* differs from *E. haematis* in the presence of coalescent filaments. It can be more closely allied to *E. pacifica* in the possession of coalescent vegetative filaments, but differs from *E. pacifica* in the position and size of tetrasporangia, and crust thickness. The sporangial filaments of the crustose phase of *G. flabelliformis* possess 1–2 sterile cap cells, thus the sporangia are intercalary. The sterile cap cells become dislocated prior to sporangial release, therefore appearing somewhat like *E. pacifica* in which cap cells are lacking (HOLLENBERG 1969). The crusts of the latter are thinner and tetrasporangia are smaller.

The tetrasporophyte of *G. flabelliformis* also resembles the cultured tetrasporophyte of *Ahnfeltia concinna* J. AGARDH (MAGRUDER 1977) and *Ahnfeltia* sp.[1] (DECEW and WEST 1977 c). The tetrasporophyte of *G. flabelliformis* and *A. concinna* are similar in the coalescence of perithallial filaments, whereas in *Ahnfeltia* sp. perithallial filaments are spreading. The crusts of all three species contain intercalary seriate tetrasporangia; however, their position and number varies. The cultured tetrasporophyte of *A. concinna* possesses 2–4 sterile cap cells, 2–4 tetrasporangia in a series, and the sori

1) This isolate originally reported as *Ahnfeltia gigartinoides* is now considered to be an undescribed species.

Table 1. Comparative structure of tetrasporophytes from culture and field material of *Gymnogongrus* and *Ahnfeltia*. The data is derived from unpublished observations of the authors

| Species / Characters | *Erythrodermis* sp. & *Gymnogongrus furcellatus* | *Erythrodermis haematis* & *Gymnogongrus leptophyllus* | *Erythrodermis* sp.[1] & *Gymnogongrus flabelliformis* | *Petrocelis anastomosans* & *Gymnogongrus martinensis*? | *Petrocelis* sp. & *Ahnfeltia* sp.[2] | *Erythrodermis* sp. & *Ahnfeltia gigartinoides* |
|---|---|---|---|---|---|---|
| Number of tetrasporangia in series | 4-5 | 4 | 4-8<br>Muroran<br>3-9<br>Oshoro Bay | 3-4 | 1-3 | 1-2 |
| Tetrasporangial dimensions ($\mu$m) | 18-20 long<br>×<br>16-18 wide | 10-12 long<br>×<br>8-10 wide | 20-23×16-18<br>Muroran<br>20-30×17.5-22.5<br>Oshoro Bay | 8 long×8 wide | 12-15 long<br>×<br>10-11 wide | 18-39 long<br>×<br>8-13 wide |
| Tetrasporangial configuration | cruciate | cruciate | cruciate | "bisporangial"[3] | cruciate | cruciate to zonate |
| Tetrasporangial sori | nemathecial | nemathecial | nemathecial | non-nemathecial | non-nemathecial | nemathecial |
| Number of cap cells | 1 | 1 | 1-2 | 5-10 | 3-6 | 1-2 |
| Thickness of reproductive crust ($\mu$m) | 170-200 | 200-280 | 250-300<br>Muroran<br>300-580<br>Oshoro Bay | 180-200 | 180-230 | 130-210 |
| Number of cells in perithallial filaments | 8-10 | 8-10 | 11-15<br>Muroran<br>18-40<br>Oshoro Bay | 20-23 | 14-16 | 8-10 |
| Number of cells in subhypothallial row[4] | 1-4 | 1-4 | — | — | 1-2 | — |
| Perithallial cell dimensions ($\mu$m) | 8 long<br>6-8 wide | 5 long<br>5 wide | 8-13 wide (basal)<br>4-6 wide (distal)<br>0.5-1× long as broad<br>Muroran<br>8.8-12.5 wide (basal)<br>5.3-7.5 wide (distal)<br>0.5-2× long as broad<br>Oshoro Bay | 4 long<br>4 wide | 10 long<br>5 wide | 5 long<br>5 wide |
| Perithallial filament association | semi-spreading | spreading | coalescent | spreading | spreading | coalescent |

1) The data in the upper half of eachs quare is for lab cultured tetrasporophytes of the Muroran isolate and in the lower half for field-collected tetrasporophytes from Oshoro Bay.
2) This isolate originally reported as *Ahnfeltia gigartinoides* (DECEW and WEST 1977 c) is now considered to be an undescribed species.
3) The "bisporangia" observed in these specimens were probably in the first cleavage of tetrasporangial formation.
4) In culture crusts often become separated from the glass substrate and a series of coalescent cells develop downward from the hypothallus.

are non-nemathecial[1]. The crustose tetrasporophytes of *G. flabelliformis* possess 1–2 sterile cap cells, 3–9 tetrasporangia in a series, and are nemathecial. The crustose stage of *Ahnfeltia* sp. possesses 3–6 cap cells, 1–3 tetrasporangia in a series, and is non-nemathecial.

The presence of a nemathecium may be influenced by both the number of vegetative cells in a series which are differentiated into tetrasporangia, and the depth of this series within the crust (*i.e.*, the number of sterile cap cells above the tetrasporangial series). At present we do not know to what degree the above mentioned characters are subject to environmental variation.

In all species of *Gymnogongrus* and *Ahnfeltia* which have been discussed the single unifying character appears to be the presence of seriate tetrasporangia (Table 1). The presence of this character in the tetrasporophyte of genera with heteromorphic life histories is consistent with its existence in the tetrasporophyte of genera with isomorphic life histories in the Phyllophoraceae.

We wish to acknowledge Professor Munenao KUROGI of Hokkaido University for his criticism of the manuscript. Our thanks are also extended to the members of the Institute of Algological Research, Hokkaido University and of the Oshoro Marine Biological Station, Hokkaido University for permitting use of the laboratories.

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1) "Nemathecium" as defined here indicates an elevated reproductive structure.

**増田道夫*・T. C. ドゥキュウ**・J. A. ウエスト**：**

## オキツノリ (*Gymnogongrus flabelliformis*) の四分胞子体

北海道忍路湾に生育する *Erythrodermis* 属の一種と考えられる藻の四分胞子を培養して，有性生殖器官の形成は未だみられないが，外部及び内部形態がオキツノリに酷似する直立体が得られた。また，北海道室蘭産のオキツノリの果胞子の培養からは上述した *Erythrodermis* によく似た四分胞子体が得られ，この四分胞子の発芽体はオキツノリに生長した。四分胞子嚢の形成は栄養塩補強培養液 (PESW) から栄養塩を補強しない培養液に移した時にみられた。これらの事実から，オキツノリにはそれぞれ独立した世代として形態的に異なる配偶体と四分胞子体が存在することが明らかとなった。(*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・**Department of Botany, University of California, Berkeley, California 94720, U. S. A.)