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Plurilocular sporangia and the development of plurispores in *Ishige okamurai* YENDO (Phaeophyceae) from the Kada coast, Wakayama Prefecture in Japan

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Plurilocular sporangia on the frond of *Ishige okamurai* were observed for the first time. They were uniseriate and transformed from the assimilatory filaments of the cortical layer. In culture, plurispores developed into new fronds through an initial pseudoparenchymatous disc stage.

Key Index Words: Ishige okamurai—*life history*—*Phaeophyceae*—*plurilocular sporangium*—*plurispore.*

The genus *Ishige* YENDO was originally placed in the Fucaceae (YENDO 1907). But with the observation of zoospores from unilocular sporangia in *I. sinicola* (SETCHELL and GARDNER) CHIHARA (ONDA and AZUMA in SEGAWA 1935), a new family Ishigeaceae (including 2 species) was established (OKAMURA in SEGAWA 1935). In *I. sinicola*, unilocular sporangia are transformed from the apical cells of the assimilatory filament, and zoospores have been observed (MIYAKE and KUNIEDA in OKAMURA 1936, fig. 131).

Since the discovery of a microthallus stage derived from the zoospores of *I. sinicola* (ARASAKI 1943), this family has been placed usually within the Chordariales (e.g., YOSHIDA *et al.* 1985).

The author has observed no unilocular sporangia on thalli of *I. okamurai* YENDO collected year-round on the Kada coast in Wakayama Prefecture. However, sori of plurilocular sporangia were observed on September 27, 1981. The plurilocular sporangia and the resultant developmental stages derived from these plurispores in culture are described for the first time.

Materials and Methods

The thalli of *Ishige okamurai* were collected on the Kada coast in Wakayama Prefecture facing the Pacific Ocean on 27 September 1981. They were found growing on rocks in the upper littoral zone.

The mature fronds were carried to the laboratory in a cool condition (ca. 5°C). For the culture study, the fertile fronds were rinsed thoroughly with several changes of autoclaved seawater and were dried for about one hour. They were then placed in Petri dishes containing sterilized seawater until plurispores were obtained. Plurispores, showing negative phototaxis, were washed 3–4 times in Petri dishes containing sterilized seawater using a micropipette under a microscope. A small suspension of plurispores was poured over glass slides and left for half an hour to settle. After settlement of the plurispores, these slides were washed with a jet of sterilized seawater and placed in glass vessels (6.5 cm × 8.0 cm) containing 180 m*l* of medium.

For single algal cultures, individual germlings adhering to the glass slide were isolated with a micropipette and transferred to a culture test tube (2 cm × 13 cm) containing

10 m*l* of medium.

The culture medium used in this study was PES medium (PROVASOLI 1968). The cultures were maintained in the incubators illuminated with the cool-white fluorescent lamps (ca. 3000 lux) under the following temperature-photoperiod regimes: 20°C, 16-$\overline{8}$ hr (Set 1); 20°C, 10-$\overline{14}$ hr (Set 2); 15°C, 14-$\overline{10}$ hr (Set 3); 15°C, 10-$\overline{14}$ hr (Set 4); 10°C, 14-$\overline{10}$ hr (Set 5) and 25°C, 16-$\overline{8}$ hr (Set 8).

Results

1. Plurilocular sporangia and plurispores

When a frond of *Ishige okamurai* was placed in a Petri dish, a mass of yellow-brownish swarmers was observed near the middle to upper branches (Fig. 2 A, arrows). The surface of these branches was covered with several raised sori of plurilocular sporangia (Fig. 2 B). At the cortical layer of these portions, the assimilatory filaments were divided dichotomously once or twice. The outermost 4-6 cells were transformed into uniseriate plurilocular sporangia, each cell of which had a red eyespot (Fig. 1 A, 2 C). Plurilocular sporangia were not observed on the basal portion of the frond. Plurispores were released from an apical pore of the plurilocular sporangium. They crowded around the parent frond for a while. Then, they showed negative phototaxis and swam to the wall of Petri dish.

The plurispores measured 7.4-9.8 μm × 4.9-6.1 μm in size. A plurispore was pear-shaped with a single chromatophore and an eyespot, and was laterally biflagellated (Fig. 1 B). Settled plurispores became spherical, measuring 6.1-7.5 μm in diameter (Fig. 1 C).

2. Development of plurispores

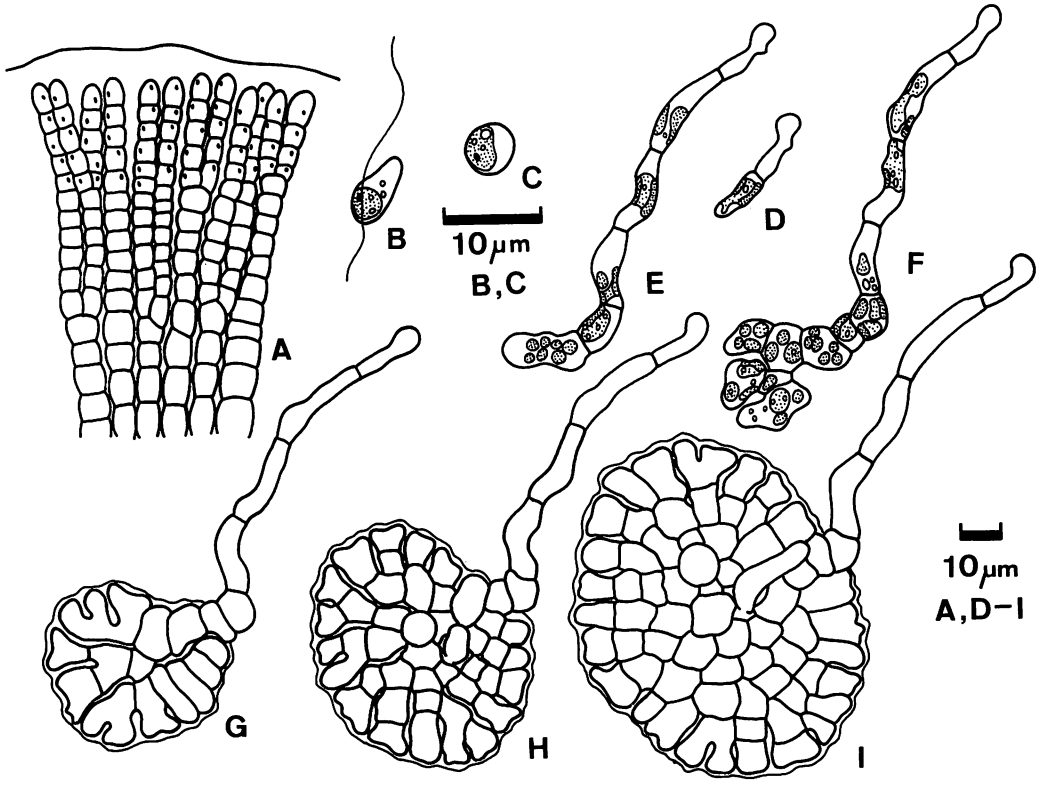


Fig. 1. *Ishige okamurai.* Plurilocular sporangia, plurispores and developmental stages of the plurispore: A. Plurilocular sporangia; B. Plurispore; C. Settled plurispore; D–I. 2-(D), 6-(E), 10-(F), 12-(G), 14-(H) and 16-(I)-day-old germlings in Set 2.

The following developmental stages of the plurispores were recorded mainly in Set 2.

Within 1-2 days, the settled plurispore germinated by pushing out a protuberance, and it divided transversely into two cells (Fig. 1 D). Usually, all contents of the plurispore moved into the protuberance.

The germling developed by the successive transverse divisions into a creeping uniseriate filaments consisting of 4–5 cells and 50–60 μm in length (Fig. 1 E). Primary lateral branches were produced near an apical cell of the protuberance (Fig. 1 F). Secondary lateral branches developed on the basal cells of the primary branches.

Within 2 weeks, as a result of the extensive branching (Fig. 1 G, H), 50–60% of the germlings soon developed into pseudoparenchymatous compact discs (Fig. 1 I). Since outermost cells of the disc sent forth prostrate branches dichotomously, the discs developed radiately into large ones (Fig. 2 D). They measured 200–300 μm in diameter within 1 month in Sets 1, 2 and 8, and within 2 months in Sets 3, 4 and 5.

On the other hand, other germlings developed into long filamentous prostrate thalli (200–600 μm in length), which produced long uniseriate upright branches, within 2 weeks. However, tips of branches made contact with the substrate here and there, and then new discs were formed (Fig. 2 E, F).

Initial developmental stages of the plurispore germlings were the same in the other Sets. In Sets 1-4, 50-60% of plurispore germlings soon developed into the compact discs, and others developed into the filamentous thalli. However, in warmer condition (Set 8) and cooler condition (Set 5), a ratio of the discs increased to 70-90%. The growth of the discs in warmer conditions was faster than that in cooler conditions. Within one month, both types of germlings developed into large discs.

Profusely branched upright filaments were produced from the center of the discs (Fig. 2 G). When these filaments were cut off and cultured in the test tube, new discs were produced from the cells which attached to the substratum. In Set 8, the upright filaments usually became dark brown. Then, a part of them fell off and made a new disc on the substratum. The discs enlarged (usually 5 mm in diam.), and eventually coalesced into a large mass (Fig. 2 H).

The disc has not produced any reproductive organs under any of the culturing conditions. The discs also have not made any erect fronds even after a year in Set 8. However, when a disc was transferred from Set 8 and cultured in Set 5 for 5 months, an erect frond was developed on the disc (Fig. 2 H). The erect frond produced some dichotomous branches and grew into a typical *Ishige okamurai* thallus, but it has produced no reproductive organs even after a year.

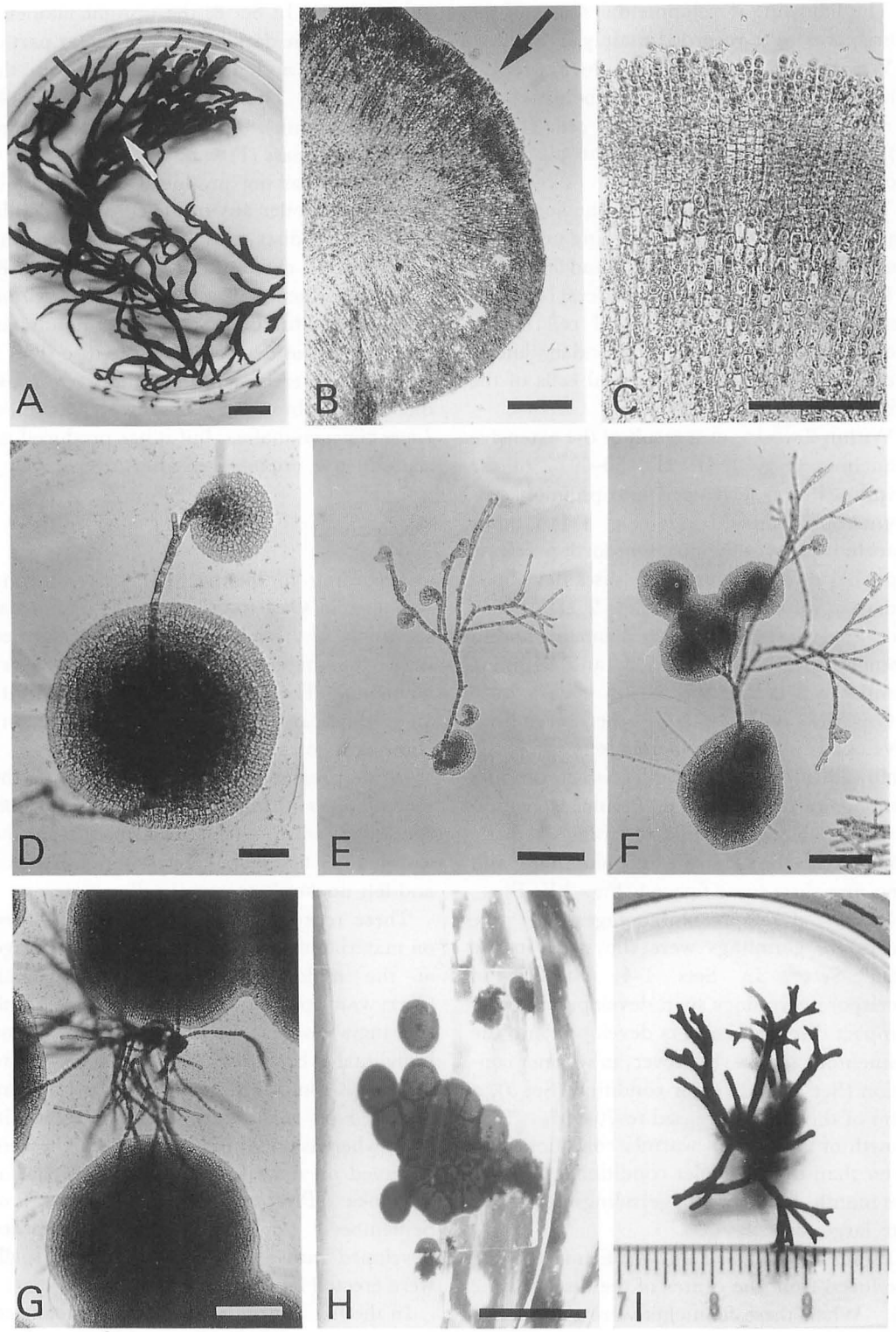
Discussion

According to the fig. 131 (Miyake and Kunieda in Okamura 1936), the unilocular sporangia of *Ishige sinicola* were transformed from the outermost cell of assimilatory filaments. The outermost cell enlarged into an elliptical cell, containing many chromatophores.

In the present study of *Ishige okamurai*, the macrothallus bore only plurilocular sporangia, which were transformed from the assimilatory filaments. They were uniseriate and left no sterile terminal cell.

These reproductive organs were observed on material of *Ishige okamurai* ca. 20 years ago at the middle part of Honshu, Japan (Tatewaki, personal correspondence). Both sporangia were observed on the same individual at Enoshima, Kanagawa Prefecture in June. Only plurilocular sporangia were observed on thalli from the same place in July, whereas only unilocular sporangia were observed on material collected from Atami, Shizuoka Prefecture at the end of September. Zoospores and plurispores developed into discs, on which *Ishige* thalli were erected later.

In the life history of *I. sinicola* as reported by Arasaki (1943) from Mikawa Bay, zoospores from unilocular sporangia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developed into filamentous microthalli, which formed uniseriate plurilocular sporangia. Gametes from the plurilocular sporangia showed sexual conjugation, and zygotes developed into filamentous microthalli. But they did not grow into *Ishige* thalli. ARASAKI (1943) concluded that the Ishigeaceae belongs to the Chordariales for the following reasons: 1) *I. sinicola* showed an alternation of heteromorphic generations, 2) microscopic gametophytes had motile gametes, and 3) plurilocular sporangia (gametangia) of *I. sinicola* in culture resembled those of certain species in the Chordariales.

In this study of *I. okamurai*, plurilocular sporangia were uniseriate and lacked sterile terminal cells. Plurispores from the frond developed directly into the typical fronds through a pseudoparenchymatous prostrate discoid stage. The disc was always sterile and functioned as a perennial holdfast system. The taxonomic position of Ishigeaceae is thus uncertain. Next, the author wishes to compare the characteristics of Ishigeaceae with those of members of the orders Chordariales, Scytosiphonales and the genera *Analipus, Myelophycus* and *Melanosiphon*. In the Chordariales, some species have both types of sporangia on the sporophyte. However, unilocular sporangia are always formed on the basal or middle part of the assimilatory filaments. Plurilocular sporangia of *I. okamurai* resembled those in the Scytosiphonales. However in the Scytosiphonales, the macroscopic frond with plurilocular sporangia is the gametophyte stage and alternates with a microscopic sporophyte stage with unilocular sporangia (NAKAMURA and TATEWAKI 1975). *Analipus* has both types of sporangia on the macroscopic fronds. But the developmental stage of zoospores and plurispores are parenchymatous, and plurilocular sporangia have typically two (1–3) sterile terminal cells (WYNNE 1971). *Myelophycus* and *Melanosiphon* have both types of sporangia but on separate individuals. These genera show polystichous growth and form parenchymatous tissue (WYNNE 1969; TANAKA and CHIHARA 1984).

According to TATEWAKI's personal correspondence, there may be several *I. okamurai* populations, in which *Ishige* thalli produced different types of reproductive organ. However, the question remains whether the frond with unilocular sporangia is really absent or not at Kada coast, Wakayama Prefecture and whether the species of the Ishigeaceae have an alternation of isomorphic or heteromorphic generation.

To clarify the taxonomic position of the Ishigeaceae in Phaeophyceae, it is necessary to survey critically the presence of unilocular sporangia and to complete the life history on materials collected from various localities in Japan.

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Fig. 2. *Ishige okamurai*. Plurilocular sporangia and developmental stages of the plurispore: A. The mature frond releasing plurispores from the middle and upper portions (arrows); B, C. A sorus of plurilocular sporangia (arrow) in a transverse section of the frond; D. 44-day-old germling in Set 4; E, F. 30-(E), 44-(F)-day-old germlings in Set 3; G. 44-day-old germlings in Set 8; H. 5-month-old germlings in Set 8; I. 5-month-old thallus in Set 5, after having been transferred from Set 8. Scale: A, H, I: 1 cm; B, E–G: 200 μm; C, D: 100 μm.

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鯵坂哲朗：和歌山県加太産のイシゲ（褐藻類）の複子嚢と遊走細胞の発生

イシゲの藻体で，今回初めて複子嚢が観察された。それらは単列で皮層の同化糸が変成してできる。この複子嚢からの遊走細胞は培養すると，偽柔組織の盤状体ステージを経て，直立するイシゲ藻体に生長した。（606 京都市左京区北白川追分町　京都大学農学部熱帯農学専攻）

新 刊 紹 介

CROASDALE, H. & E. A. FLINT: Flora of New Zealand Desmids. Volume II. Botany Division, D.S.I.R., Christchurch, New Zealand. NZ $57.50（邦価約 7,400 円）

先に藻類35巻2号90頁で紹介した第1巻と同じ2名の著者らによる第2巻で，ニュージーランド産の *Actinotaenium* 属14種，*Cosmarium* 属129種，*Cosmocladium* 属2種，*Xanthidium* 属13種，*Spinoclosterium* 属未同定種，変種，品種合わせて267分類群を含む。第1巻とは印刷及び出版元が異なるため，紙質と装幀がほんの少し変化したが，第1巻と同質の美しい書物である。カバーの水彩画は各巻で取扱っている鼓藻類の種類を示しており，書棚から必要とする巻を無意識に取り出させてくれる。ニュージーランドの鼓藻類の生息地のカラー写真は新たに全頁版1葉と半頁版16葉が含まれており，第1巻と合わせて彼地に対する臨場感を強めている。採集地の地図2葉と植生や水質等の環境条件を附記した採集地リスト（表1～7）及びニュージーランド産鼓藻類の属の検索表は第1巻と同じものが本巻にも掲載されており，便利である。各種の記載は33～125頁に，続いて126～128頁に用語説明（本巻で必要とされる用語が附加されている）があり，129～140頁に約400の引用文献を掲載し，142～147頁は索引である。記載種の線画スケッチは1巻と同じく図と種名が見開きになっており，1巻に続いてプレート28からプレート61の34頁にまとめられている。本巻では，J. RALFS (1807–1890), W. M. MASKELL (1840–1898), C. F. O. NORDSTEDT (1838–1924) と H. L. SKUJA (1892–1972) の4人の著名な鼓藻類学者の略伝がそれぞれ1頁に写真1葉とともに記述されている。また著者らの写真1葉がカバーの綴じ込み部分に掲載されている。本シリーズでは新分類群の記載がほとんどないのは残念であるが，彼地の鼓藻類の研究はまだ十分に行われたと言える状態であるとは思えないことから，今後ニュージーランドの鼓藻類の研究を始める人にとっては，古い資料を網羅整理した重要な文献の1つであることは確かである。

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