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The life history of *Sphaerotrichia divaricata* (AG.) KYLIN (Phaeophyta, Chordariales) in culture

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The heteromorphic and haplo-diplontic life history of *Sphaerotrichia divaricata* from Wakasa Bay, Japan Sea, has been completed in culture. Zoospores from unilocular sporangium of macroscopic sporophyte developed into microscopic haploid gametophytes ($n=9-12$). Two different types of tufts occurred in the gametophytic stage according to conditions given in culture. The gametophytes grew more rapidly at higher temperatures and in longer photoperiods. Conjugation between gametes from uniseriate plurilocular sporangium (gametangium) of the gametophyte was either isogamous or anisogamous and the zygotes developed into macroscopic diploid sporophytes ($2n=18$-24). The sporophytes grew larger under cooler conditions and they matured earlier to bear unilocular sporangia under warmer conditions. Unfused gametes germinated asexually and developed into gametophytes, repeating the same gametophytic generation.

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Introduction

Sphaerotrichia divaricata (AG.) KYLIN taxonomically belongs to the Chordariaceae of Chordariales, Phaeophyta (INAGAKI, 1958). It is commonly distributed in Japan along both the coasts of Pacific and Japan Sea. However, the south region of the Pacific coast is an exception. This species is commercially important as it is used as tasteful food in Japan.

HYGEN (1934) reported that the Swedish species of *Sphaerotrichia divaricata* showed an alternation of heteromorphic generations : an alternation of a microscopic gametophyte with plurilocular sporangia and a macroscopic sporophyte with unilocular sporangia.

ARASAKI (1943), studying the life history of the species from Mikawa Bay, Pacific coast, found that the sparsely branched gametophytes had the dormant stage in hot summer months and matured in autumn when sea-water temperature dropped.

In this paper, the life history of *Sphaerotrichia divaricata* from Wakasa Bay, Japan Sea, its karyological observations, and the growth and maturity of both sporophytes and gametophytes under culture conditions are reported.

Materials and Methods

The sporophytes of *Sphaerotrichia divaricata* were collected at Takahama in Wakasa Bay facing the Japan Sea during the summer of 1974. The plants were found growing on *Sargassum confusum* C. AG. and *S. piluliferum* C. AG. or on rocks which were one or two meters below the low tide mark.

We used the same culture techniques and medium prescriptions as given by NAKAMURA and TATEWAKI (1975).

Cultures were incubated in 1500-3000 lux light 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-$\overline{10}$ (Set 5).

For the karyological observations, aceto-iron-haematoxylin-chloral hydrate method (WITTMANN, 1965) was used.

Results

Cultures from zoospores of the frond in nature were started on June 21, 1974. The fertile fronds in nature bear only unilocular sporangia from June to July. Mature unilocular sporangia are usually elongated obovoid, measuring 61-75×26-41 μm. Zoospores (4.7-6.6×2.8-3.8 μm in size) from the unilocular sporangium are pear-shaped with a single chromatophore and an eyespot, and are laterally biflagellated (**Fig. 1-A, B**). Settled zoospores become spherical and measure 2.8-4.7 (4.0 at an average) μm in diameter (**Fig. 1-C**).

Within 1-2 days, they germinated by pushing out a protuberance and then divided into two cells transversely (**Fig. 1-G, H**). By successive transverse divisions, the germlings developed into creeping uniseriate filaments consisting of 3-7 cells, and branched laterally (**Fig. 1-D, E, I, J**).

In Sets 1 and 2, as the result of an extensive formation of prostrate and upright branches (**Fig. 1-F**), most of the filamentous germlings developed into dense tufts composed of large basal layer and profusely branched erect portion (**Fig. 3-A**).

In Set 1, the upper part of some erect filaments transformed into uniseriate plurilocular sporangia (gametangia) within 13 days (**Fig. 3-C**). However, in Set 2, further growth made tufts larger (over 1 cm in diam.) and they never bore plurilocular sporangia even after 2 months.

In Set 5, 8-day-old filamentous germlings were usually provided with hairs (**Fig. 1-K**). By successive branchings and cell divisions, they developed into simple tufts composed

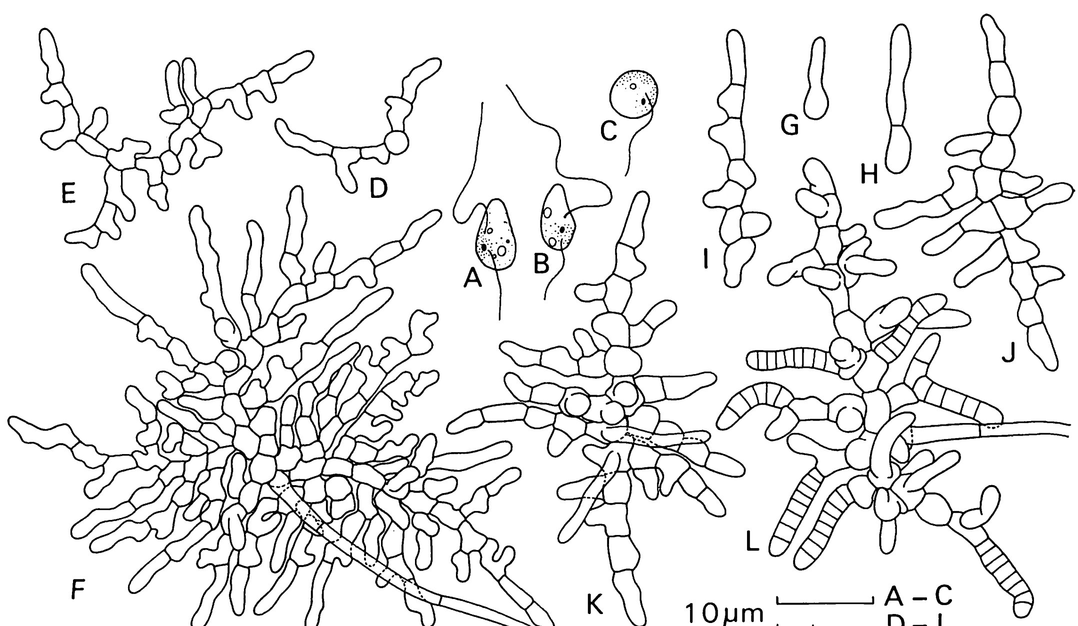


Fig. 1. *Sphaerotrichia divaricata.* Zoospores released from unilocular sporangia and development of their germlings. A, B: Zoospore. C: Settled zoospore with a flagellium. D-F: 5(D)-, 7(E)-, and 10(F)-day-old gametophytes in Set 1. G-L: 3(G, H)-, 4(I)-, 5(J)-, 8(K)-, and 13(L)-day-old gametophytes in Set 5.

of small basal layer and sparsely branched erect portion (**Fig. 1-L; 3-B**). They reached maturity within 20 days and most of the erect filaments transformed into uniseriate gametangia (**Fig. 3-B, C**).

In Sets 3 and 4, two types of germlings, dense tufts found in Sets 1 and 2, and simple ones in Set 5, appeared at a nearly same rate, and the gametangia released gametes in 18 days.

The morphology of gametes (4.7-6.2× 3.3-5.2 μm in size) was quite similar to that of zoospores (**Fig. 2-A, B**).

Sexual conjugation was observed between the gametes. The gametes were promptly released from gametangia when transferring from dark to right photoperiod regime (**Fig. 3-D**). Two swimming gametes accidentally fused on their heads. It seems that sometimes one flagellum of both the fusing gametes or of only one of them was lost during the process of conjugation (**Fig. 2-D, E**). The conjugation was either isogamous or anisogamous.

In Set 5, conjugation between the gametes occurred at 70-80%, and formed the zygotes (**Fig. 3-E**). However, in Sets 1 and 2, the conjugation scarcely occurred and most of the gametes germinated without fusion and each developed into a gametophyte, repeating the same gametophytic generation. In the Japanese species, gametophytes are sometimes monoecious. In such cases, the conjugation occurred between the gametes from one and the same thallus or even between the gametes from one gametangium.

Within 1-2 days, settled zygotes measured 5.7-12.4 (8.0 at an average) μm in diam. and soon germinated (**Fig. 2-F**). By successive branchings and transverse cell divisions (**Fig. 2-G, H, I**), the zygote developed into pseudoparenchymatous discs and at the center of which a hair was produced (**Fig. 2-J, K**). The disc was irregularly rounded in outline, consisting of a single layer of cells (**Fig. 2-L**). Within 15 days, the primary assimilating filaments arose from the central part of the disc (ca. 100 μm in diam.)

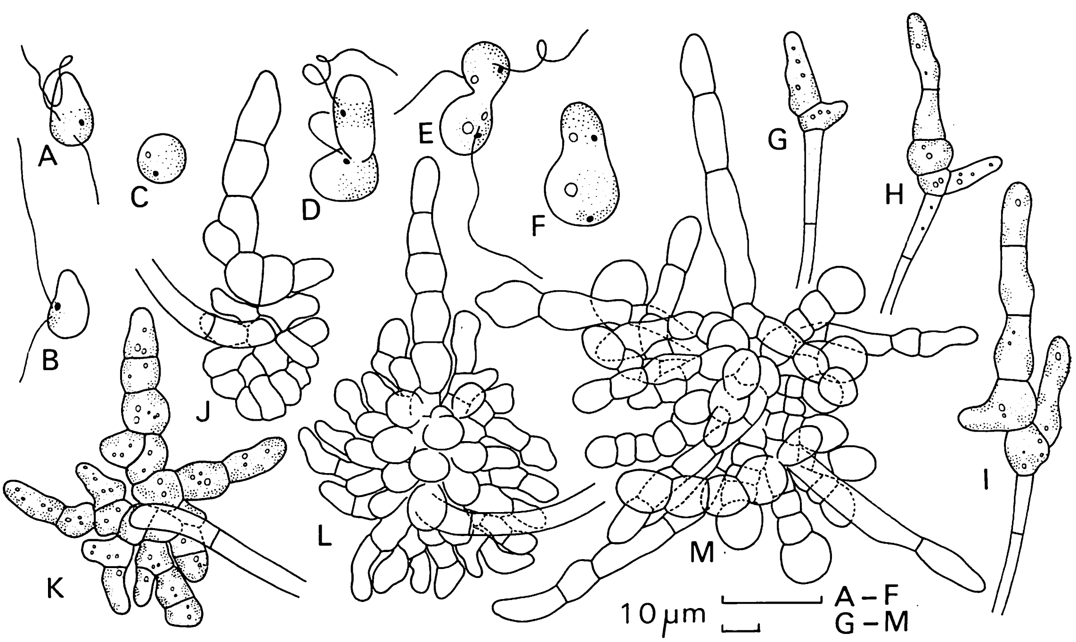


Fig. 2. *Sphaerotrichia divaricata.* Gametes released from gametangia, their zygotes, and their developmental stages. A, B: Gamete. C: Settled gamete. D, E: Conjugation of gametes. One flagellium of each gamete is lost during the process of conjugation. F: Settled zygote. G-L: 3(G)-, 4(H)-, 7(I)-, 10(J)-, 11(K)-, and 14(L)-day-old sporophytes in Set 5. M: 14-day-old sporophyte in Set 2.

and rhizoidal filaments were produced from the marginal portion (**Fig. 2-M**). The rhizoidal filaments and cylindrical cells arose from the lower cells of each primary assimilating filament and cohered together to build up a medullary layer. The growing point was terminated by some enlarged cells (**Fig. 3-F**). The secondary assimilating filaments arose profusely from the medullary cells to construct a cortical layer (**Fig. 3-G**), developing into a cylindrical plant (**Fig. 3-H**). In Set 5, within 3 months, the cylindrical plant (filiform, somewhat cartilaginous) laterally branched in all directions and grew about 4 cm in height (**Fig. 3-I**). Within one month in Sets 1 and 2, after 2 months in Sets 3 and 4, and after 3 months in Set 5, the cylindrical plants bore many unilocular sporangia (50×30 μm in size) and released many zoospores (**Fig. 3-J**).

Unfused gametes germinated and developed asexually into gametophtes. The settled gametes became spherical, measuring 2.9-6.7 (5.1 at an average) μm in diameter (**Fig. 2-C**). Under culture conditions, as seen in zoospore's germlings, two types of gametophytes were observed. Under warmer and longer-day conditions (e.g. Set 1), the growth and fertility of gametophytes were faster than those under cooler and shorter-day conditions (e.g. Set 5). Under cooler conditions, phaeophytic hairs were numerously produced on gametophytes. The loculus number of gametangia was more numerous in culture under cooler conditions than those under warmer conditions. The gametangia in Set 3 had loculi from 4-16, those in Set 4 from 6-20, and those in Set 5 from 10-28 (**Fig. 4**).

The number of chromosome was 23-30 in the original sporophytes obtained from nature (**Fig.5-A**), 7-17 (of which 80% was 9-12) in the gametophytes derived from zoospores or unfused gametes (**Fig. 5-B**), and 15-27 (of which 90% was 18-24) in the sporophytes in culture (**Fig. 5-C**).

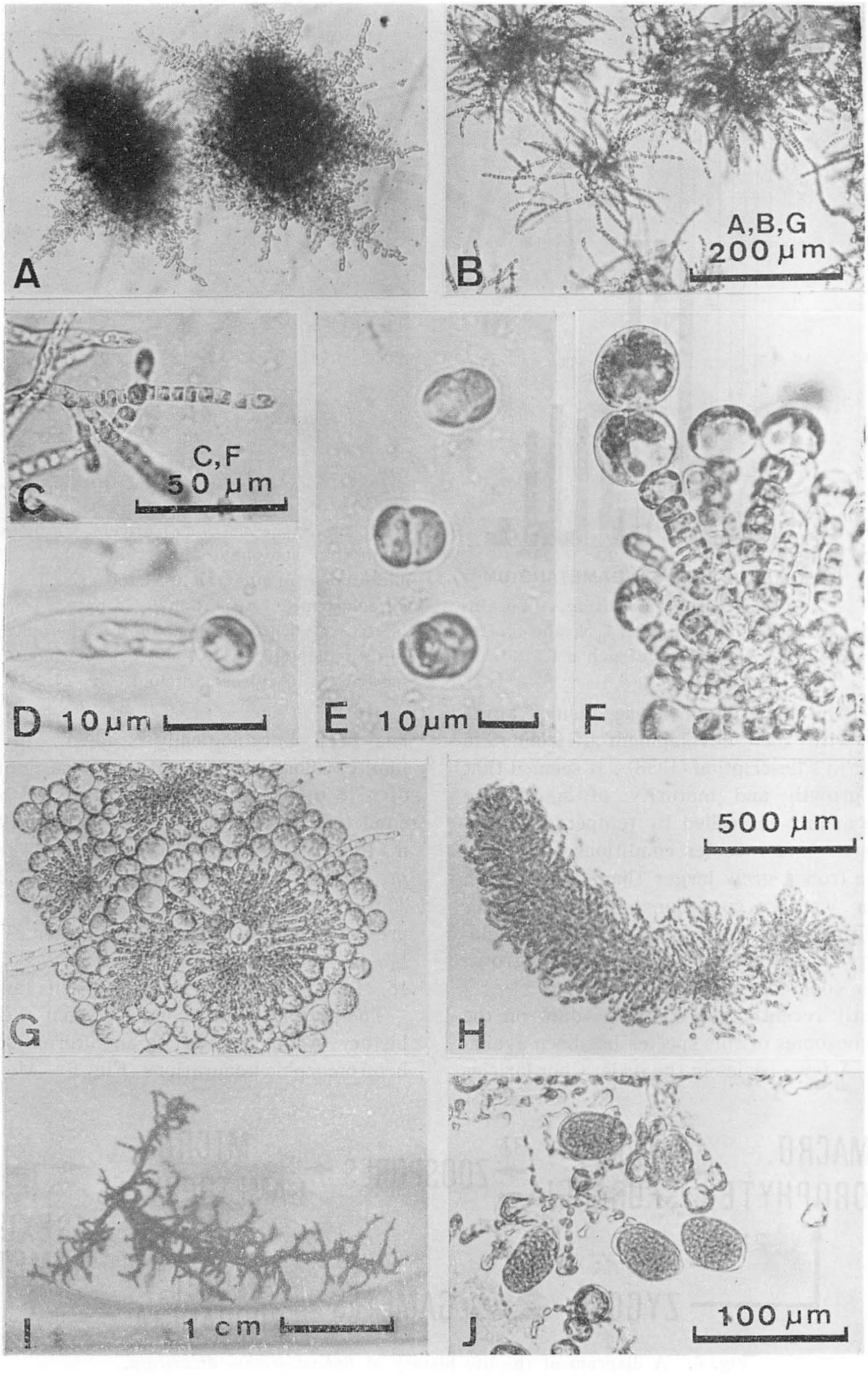
Discussion

HYGEN (1934), who studied the life history of the Swedish *Sphaerotrichia divaricata*, reported that zoospores from the unilocular sporangium developed into the 'pseudoparenchymatisch Basalscheibe' and that many zygotes occurred among the gametes from gametangia of the microscopic thallus. In his culture, anisogamous conjugation was observed. However, he said that only female gametes grew asexually to repeat more gametophytic generations. Most of the zygotes, this worker has observed, developed through 'Diplonema' stage into sporophytes. These sporophytes became fertile without construction of cylindrical fronds.

In our study, zoospores from the unilocular sporangiun and unfused gametes from the gametangium developed into the filamentous gametophytes. In this stage, dense tufts having profusely branched filaments were formed under warmer conditions. On the other hand, simple tufts having sparsely branched filaments were produced under cooler conditions. These simple tufts resemble the gametophytes of *Chordaria flagelliformis* which were cultured under cooler condition (culture temperature 6-10°C) by CARAM (1955) and (15°C) by KORNMANN (1962). The dormant stage, as seen in ARASAKI's culture (1943), was never observed under our culture conditions.

Zygotes grew to form the pseudoparenchymatous discs on which cylindrical sporophyte fronds arose. The 'Diplonema' stage thalli in HYGEN's culture have not been ob-

Fig. 3. *Sphaerotrichia divaricata*. Stages in development from gametophyte, through zygotic stage, to fertile sporophytes. A: Two dense tufts. 16-day-old gametophyte in Set 1. B: Simple tufts. 19-day-old gametophytes in Set 5. C: Uniseriate plurilocular sporangium (gametangium) in Set 5. D: Releasing gamete from gametangium in Set 3. E: Three settled zygotes in Set 3. F: Growing point of young sporophyte in Set 5. G: 27-day-old sporophyte in Set 3. H: Young cylindrical frond (sporophyte) in Set 5. I: 3-month-old sporophyte in Set 5. J: Unilocular sporangia of 2-month-old sporophyte in Set 4.


A
B
A,B,G
200 μm
C
C,F
50 μm
D 10 μm
E 10 μm
F
G
H
500 μm
I
1 cm
J
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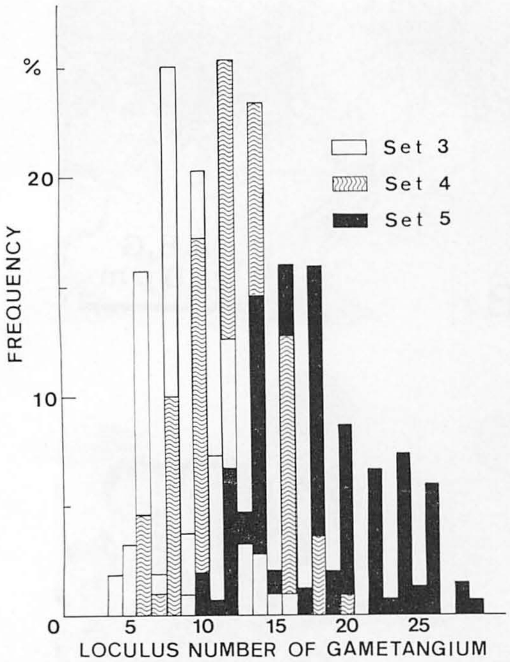

Fig. 4. *Sphaerotrichia divaricata.* Loculus number in gametangium in Set 3, 4, and 5. (200 gametangia were observed in each set.)

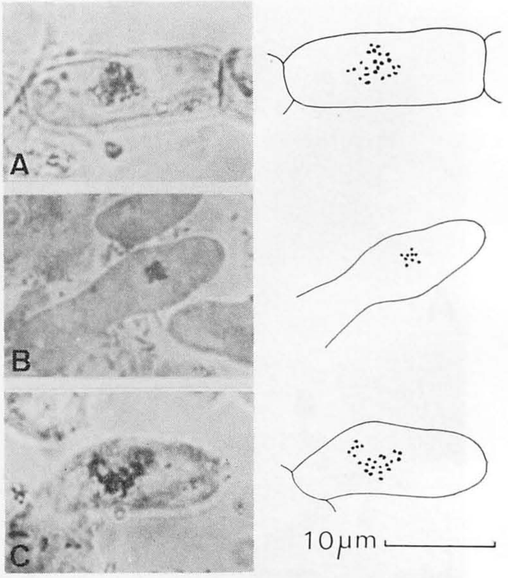

Fig. 5. *Sphaerotrichia divaricata.* Chromosome number in sporophyte and gametophyte. (left: microscopic photos; right: hand sketches.) A: Chromosomes of a sporophyte in nature (diploid) 2n=23. B: Chromosomes of a gametophyte in culture (haploid) n=9. B: Chromosomes of a sporophyte in culture (diploid) 2n=24.

served in this study. These results agree well with the 'development of fronds' in INAGAKI's description (1958). It seemed that the growth and maturity of sporophyte were mainly controlled by temperature conditions. Under cooler conditions, the sporophyte fronds grew larger than those grown under warmer conditions. Under warmer conditions, the sporophytes produced unilocular sporangia earlier than those grown under cooler conditions.

Until recently no complete data on the chromosomes of this species has been available. A few species in the order Chordariales has been karyologically studied. In the family Chordariaceae, chromosome number of n=8 or 9 and 2n=16 or 18 have been cited for *Myriogloea sciurus* (PARKE, 1933), n=10 and 2n=20 for *Sauvageaugloea griffithsiana* (CARAM, 1965), and n=10 and 2n=20 for *Eudesme virescens* (COLE, 1967). The present counts of the chromosomes of *Sphaerotrichia divaricata* (n=9-12 and 2n=18-24) show to fit the pattern for this family.

The present results confirmed that the life history of this species is an alternation of heteromorphic generations (**Fig. 6**). Meiosis

Fig. 6. A diagram of the life history of *Sphaerotrichia divaricata.*

may occur during the formation of zoospores in the unilocular sporangium on diploid thallus which was derived from the zygote.

The present result partially explains the appearance and disappearance of fronds in nature. In summer, the gametophytes grow well and mature faster, but most of the gametes develop asexually to repeat the same gametophytic generation. The rate of sexual conjugation increases from autumn to winter when sea-water temperature drops, and sporophytes derived from zygotes develop well into the branched cylindrical fronds during the seasons of winter and spring. The branched cylindrical fronds bear unilocular sporangia in early summer when sea-water temperature rises.

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鰺坂哲朗・梅崎 勇：培養によるイシモヅク（褐藻類ナガマツモ目）の生活史の研究

日本海若狭湾産の褐藻イシモヅクの生活史を室内培養によって完結した。自然に生育するいわゆるイシモヅクは，胞子体である。この藻体上の単子嚢から放出された遊走子は，そのまま発芽して顕微鏡的な単相 (n=9-12) の配偶体になる。この配偶体は，高温では密に分枝した叢状発芽体になり，低温では粗に分枝したものになる。そして高温長日ほど成長が速く，高温ほど早く成熟する。配偶体の複子嚢（配偶子嚢）から放出された配偶子の間で接合が行なわれる。接合子は，発芽して肉眼的な複相 (2n=12-24) の胞子体になる。この胞子体は低温で良く成長するし，高温ほど早く単子嚢をつけて成熟する。接合しなかった配偶子は，無性的に発芽して，再び配偶体世代を繰り返す。(606 京都市左京区北白川追分町 京都大学農学部水産学教室)

津村孝平：珪藻混種プレパラートの指示標識

Kôhei TSUMURA: The marking on diatom-slides.

珪藻の既製の混種プレパラート中の特定個体の封入位置を示す標識をそのプレパラートへ直接につけようとすると従来は刻印式の標本指示器を用いて，カバーグラスの表面に小円を刻印するよりほかに良い方法がなかったので，私は次善の策として下記の方法を案出して試用してみたところ，多少の不便はあるが，十分に実用になることがわかったので，ここに紹介する。

既製の混種プレパラートを鏡検して指示標識をつけたい個体が見付かったら，鏡検しながら，その個体の真上にカバーグラスへインキの点などを一時仮りに打って，そのプレパラートを裏返えして，裏面から鏡検して，その個体を確認し，プレパラートを載物台上へ