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Spermatozoids of *Dictyopteris divaricata* and *Dictyota dichotoma* (Dictyotales, Phaeophyceae) from Japan

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Abstract

Male gametophytes produced from the cultured tetraspores of *Dictyopteris divaricata* (Okam.) Okamura and *Dictyota dichotoma* (Huds.) Lamouroux were shown to discharge spermatozoids with two flagella (anterior flagellum: 20-30 μm long; posterior flagellum: 7-10 μm long). The anterior flagellum bears numerous mastigonemes and the posterior flagellum is string-like. Spermatozoids with two flagella are characteristic of Japanese species, unlike related species from other regions which have a single flagellum. The number of flagella is one of the basic taxonomic criteria in the Dictyotales. These results suggest a taxonomic review is required for the Dictyotales.

Key Words: *Dictyopteris divaricata*, *Dictyota dichotoma*, Dictyotales, Flagella, Male gametophyte, Phaeophyceae, Spermatozoid

Introduction

Dictyotales (Phaeophyceae) plants are distributed widely throughout the world. The two species in this study, *Dictyopteris divaricata* (Okam.) Okamura and *Dictyota dichotoma* (Huds.) Lamouroux, are very common on rocks in the tidal zone from summer through autumn in Japan. Male gametophytes of these two species have been reported only once in Japan (Matsunaga, 1966). Usually, only the tetrasporophytes are collected. Recently, the female and male reproductive organs of these taxa were found to form on very minute gametophytes derived from tetraspores in culture, but the release of spermatozoids was never observed (Yabu et al., 1981, 1991). In this study, tetraspores from tetrasporophytes of these two species were cultured and developed into male gametophytes bearing antheridia which released spermatozoids. The characteristics of the spermatozoid flagella are discussed.

Materials and Methods

Tetrasporophytes of *Dictyopteris divaricata* and *Dictyota dichotoma* were collected on 7 July 1994 (Fig. 1(a)) and 3 October 1994 (Fig. 1(g)), respectively, at Usujiri near Hakodate (Hokkaido Pref.). Tetraspores released were cultured in the laboratory at 15°C, with a 14L : 10D light period and illumination of 2000 Lux. Modified Grund medium (McLachlan, 1973) was used throughout the culture.

Swimming spermatozoids were fixed in 2% formaldehyde seawater and stained with carbolfuchsin, and observed under a light microscope. For observation with a

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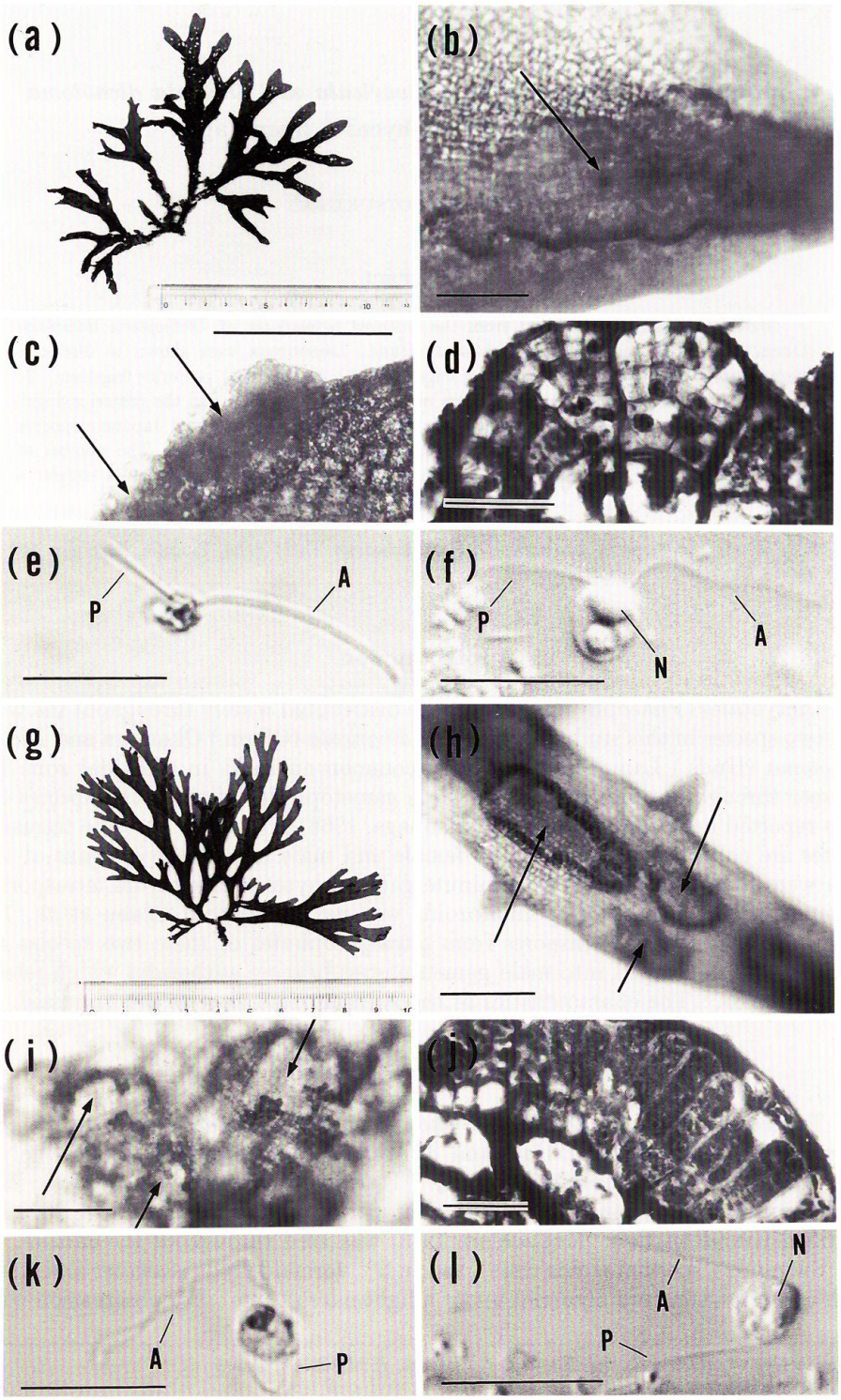
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scanning electron microscope (HITACHI S-2300), spermatozoids were placed on micro slide glasses (4×4 mm), fixed in a solution of 1% paraformaldehyde, and 2% glutaraldehyde seawater for 50 minutes, and washed with filtered seawater. The electron microscope was operated at 15KV.

Results and Discussion

In the culture of tetraspore-germlings, antheridia were formed after 40 days for *D. divaricata* (Fig. 1(b)-(d)) and after 50 days for *D. dichotoma* (Fig. 1(h)-(j)). Spermatozoids were released after 60 days for both species. Spermatozoids from both species have two flagella (Fig. 1(e), (k)). The anterior flagellum beats to advance the spermatozoids at speeds of 10-20 μm/s.

The spermatozoids of both species have a large nucleus close to the base of the flagella, and one or two plastids in the remnant portion (Fig. 1(f), (l)). A spermatozoid of *D. divaricata* is pear-shaped (3×4 μm) and extends two flagella at an angle of approximately 45° (Fig. 2(a), (c)). The anterior flagellum is 20-25 μm in length and bears numerous mastigonemes (2 μm long) from top to base (Fig. 2(b), (d)). The top of the flagellum is round and lacks a terminal acronema. No rows of spines are present. The posterior flagellum is string-like and measures 7-10 μm in length. A spermatozoid of *D. dichotoma* is spherical (3×3 μm) and has a long anterior flagellum (25-30 μm long) with mastigonemes (3 μm long), and a short posterior flagullum (7-10 μm long) (Fig. 2(e)-(h)). The terminal acronema and rows of spines are also absent in *D. divaricata*.

Spermatozoids of the Dictyotales have been reported to be biflagellate for 14 species in 9 genera: *Dictyota*, *Dilophus*, *Pachydictyon*, *Lobospira*, *Dictyopteris*, *Padina*, *Taonia*, *Homoeostrichus* and *Zonaria* (Williams, 1897; Manton et al., 1953; Manton, 1959; Phillips et al., 1990; Phillips & Clayton, 1991; 1993); 11 species of the first 8 genera are uniflagellate and 3 species of *Zonaria* are biflagellate. Observations in the present study indicate that spermatozoids from Japanese species of *Dictyopteris* and *Dictyota* are biflagellate, not uniflagellate as described from foreign species of the same taxa. Terminal acronema and rows of spines, which were reported in spermatozoids of European and Australian species, were not found.

Fig. 1(a)-(f). *Dictyopteris divaricata*. (a), Tetrasporophyte collected at Usujiri on July 7, 1994. (b), Part of a male gametophyte bearing initiatives of antheridia (indicated by arrow) (57-day culture). Bar: 50 μm. (c), Part of a male gametophyte bearing mature antheridia (indicated by arrow) (70-day culture). Bar: 10 μm. (d), Cross section of mature antheridia, stained with Heidenhain's haematoxylin. Bar: 20 μm. (e), Spermatozoid, stained with carbolfuchsin. A: anterior flagellum. P: posterior flagellum. Bar: 10 μm. (f), Micrograph of a spermatozoid by differential interference microscope. N: nucleus. A: anterior flagellum. P: posterior flagellum. Bar: 10 μm.

Fig. 1(g)-(l). *Dictyota dichotoma*. (g), Tetrasporophyte, collected at Usujiri on October 3, 1994. (h), Part of a male gametophyte bearing initiatives of antheridia (indicated by arrow) (60-day culture). Bar: 50 μm. (i), Part of a male gametophyte bearing mature antheridia (indicated by arrow) (71-day culture). Bar: 10 μm. (j), Cross section of mature antheridia, stained with Heidenhain's haematoxylin. Bar: 30 μm. (k), Spermatozoid, stained with carbolfuchsin. A: anterior flagellum. P: posterior flagellum. Bar: 10 μm. (l), Micrograph of a spermatozoid by differential interference microscope. N: nucleus. A: anterior flagellum. P: posterior flagellum. Bar: 10 μm.

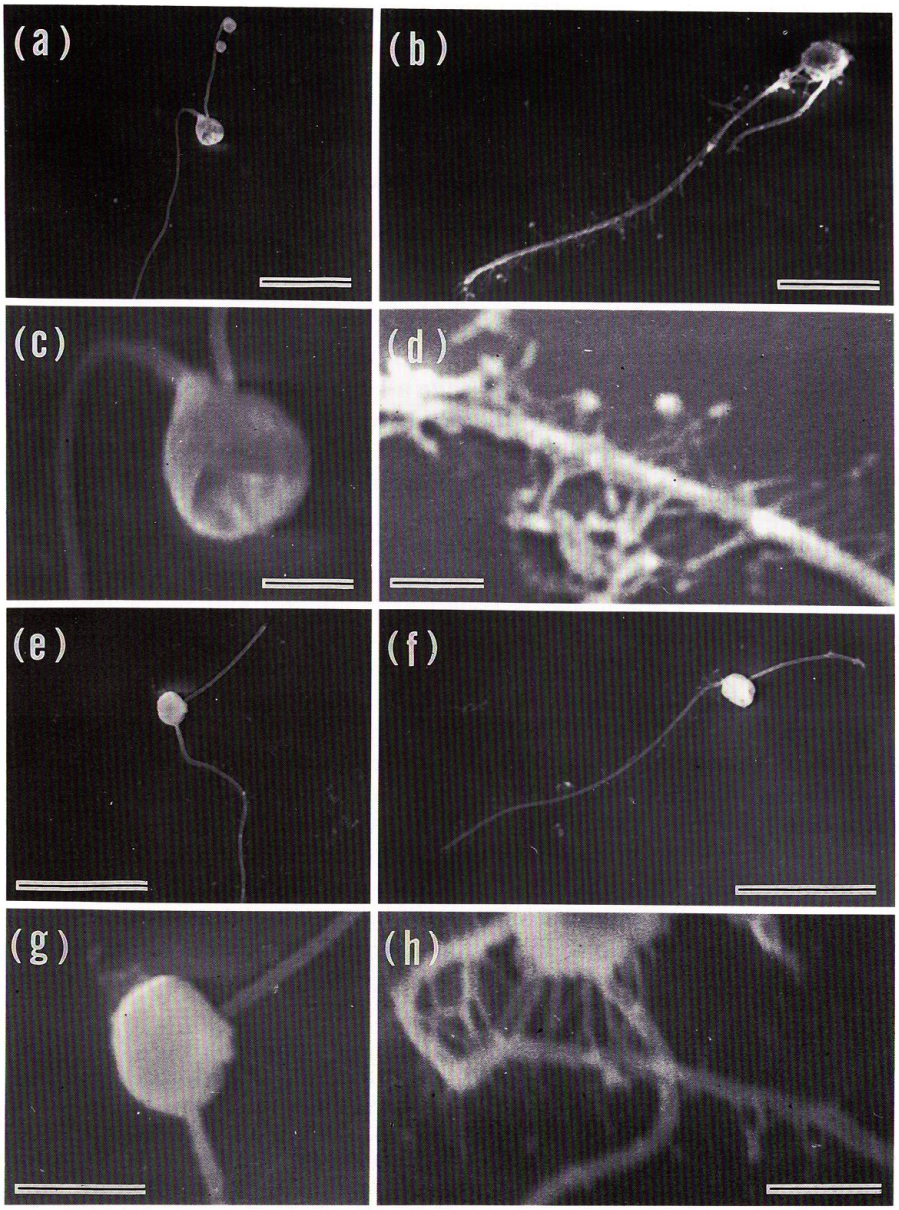

Fig. 2(a)-(d). Scanning electron micrograph of spermatozoids of *Dictyopteris divaricata*. (a) and (b), Biflagellate spermatozoid. Bar: 10 μm in Fig. (a), 5 μm in Fig. (b). (c), Magnification of Fig. (a). Bar: 1 μm. (d), Detail of an anterior flagellum, bearing mastigonemes from top to base. Bar: 1 μm.

Fig. 2(e)-(h). Scanning electron micrograph of spermatozoids of *Dictyota dichotoma*. (e) and (f), Biflagellate spermatozoid. Bar: 10 μm. (g), Magnification of Fig. (e). Bar: 2 μm. (h), Detail of an anterior flagellum, bearing mastigonemes from top to base. Bar: 1 μm.

Phillips and Clayton (1991, 1993) discussed that "the axoneme of the posterior flagellum was lost early in the evolution of the order", and concluded that *Zonaria* was a primitive genus of the Dictyotales. In this study, spermatozoids of Japanese *Dictyopteris divaricata* and *Dictyota dichotoma* were found to have posterior flagella longer than those of *Zonaria*. Thus, it is not reasonable to separate *Zonaria* from the other genera in the evolution of Dictyotales plants. Accordingly, observation of spermatozoids from the Dictyotales from Japan and other regions is required to elucidate the phylogenetic relationships within Dictyotales, and between Dictyotales and related orders.

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