

Ultrastructural study of sperm in *Laminaria angustata* (Laminariales, Phaeophyta), especially on the flagellar apparatus*

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The ultrastructure of sperm in *Laminaria angustata* KJELLMAN, especially its flagellar apparatus, was observed by SEM and TEM, and compared with *L. angustata* zoospore and zoospores of the Laminariales previously examined. In addition to a long posterior flagellum, the sperm has the characteristic feature in that the both flagella protrude through an anterior gullet and a posterior one respectively. Two or three chloroplasts are located in the cell periphery. They are not associated with the flagellar basal bodies. Four flagellar rootlets exist; major anterior rootlet (MAR), minor anterior rootlet (mar), minor posterior rootlet (mpr) and bypassing rootlet (BR). The number of microtubules consisting the MAR and the BR is five and four respectively. The mar and the mpr respectively consist of a single microtubule. The BR extends posteriorly along the nuclear membrane. Both flagellar basal bodies are connected to each other by an electron-dense, connecting band. These features are unique among the brown algal motile cells and different from zoospores of this species.

Key Index Words: Flagellar apparatus—*Laminaria angustata*—Phaeophyta—sperm—ultrastructure.

Almost all swimmers in the Phaeophyta have common features; 1) a longer, mastigonemes-bearing anterior flagellum and a shorter, undecorated posterior one with a lateral, ventral insertion of the flagella and 2) one bilobed chloroplast containing an eyespot. However, sperms of several groups in the Phaeophyta in which oogamy is prevalent (Dictyotales, Sporochnales, Desmarestiales, Laminariales and Fucales) are different from these typical characteristics. HENRY and COLE (1982a, b) observed zoospores of 17 species and sperms of 13 species belonging to the Laminariaceae, Alariaceae and Lessoniaceae of the Laminariales. They showed that the characteristic aspects of laminariacean sperms

(except for *Chorda* sperm) are as follows: 1) a very long and tapering posterior flagellum (the anterior flagellum of laminariacean sperm appears identical to that of the zoospore), 2) two or three chloroplasts per sperm, and 3) the lack of an eyespot (zoospores of most laminariacean plants also lack eyespots). Recently, CLAYTON (1984b) reviewed phylogenetically close relationships between the Sporochnales, Desmarestiales and Laminariales based on the physiological characteristics of fertilization (their sexual pheromones secreted from eggs have a sperm-releasing function as well as a sperm-attracting one), the manner of gametogenesis which is influenced by blue and red light (LÜNING and DRING 1972, MÜLLER and LÜTHE 1981) or by the iron concentration in the medium (MOTOMURA and SAKAI 1984a), and especially, characteristic features of their sperms as mentioned above.

Recently, O'KELLY and FLOYD (1984) determined the absolute configuration of the

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flagellar apparatus of zoospores of *Laminaria digitata* and *Nereocystis luetkeana*. They suggested that the flagellar apparatus in brown algal motile cells might possess basically an absolute configuration with minor variations in component array which could be contributed to phylogenetic studies on the Phaeophyta.

In this study, the ultrastructure of *Laminaria angustata* sperm, especially its flagellar apparatus is investigated, and the differences in flagellar apparatus features between zoospores and sperms are discussed.

Materials and methods

Culture

Sporophytes of *Laminaria angustata* KJELLMAN were collected at Charatsunai, Muroran, Hokkaido, Japan in November and December of 1984 and 1985. Cultured specimens of female and male gametophytes were obtained from germlings of zoospores and maintained in Fe-free ASP₁₂NTA medium (MOTOMURA and SAKAI 1984a, b). To induce gametogenesis, small tufts (ca. 0.5–1.0 mm diam.) of female and male vegetative gametophytes were inoculated into PESI medium (TATEWAKI 1966) separately. Cultures were incubated at 10°C, 14:10 LD cycle and 55 $\mu\text{mol m}^{-2}\text{s}^{-1}$ from cool-white fluorescent lamps.

Electron microscopy

Preparation of liberated sperms for electron microscopy was conducted as follows: About one week after the separate inoculation of the male and female gametophytes into PESI medium, eggs and oogonia of female gametophytes and antheridia of male gametophytes were matured in respective cultures. At this time, many sperms were

liberated within seconds by the addition of one or two drops of conditioned medium from the female gametophyte culture to the mature male gametophytes (the effect of this phenomenon is presumed to be by "lamoxirene", LÜNING and MÜLLER 1978, MÜLLER *et al.* 1979, MARNER *et al.* 1984). Liberated sperms around the mature male gametophytes were immediately fixed for about 15 min. in 1% glutaraldehyde and 0.5% OsO₄ in PESI medium. After block staining in 0.5% uranyl acetate solution for 15 min. at 4°C, specimens were dehydrated in an acetone series and embedded in Spurr's epoxy resin. Serial sectionings were done with a diamond knife on a Porter-Blum MT-1 ultramicrotome, and sections were mounted on formvar-coated slot grids with careful monitoring of the orientation and sequence of the sections. Sections were stained with uranyl acetate and lead citrate, or only lead citrate, and observed with a Hitachi H-300 electron microscope. Following O'KELLY and FLOYD (1983), analysis of basal body triplet implication in micrograph of serial sections confirmed the absolute orientations derived from section mapping.

For whole-mount preparations, a drop of suspension containing liberated sperms was placed on formvar-coated grids, and fixed in a vapor of OsO₄ for 30–60 sec. After rinsing and drying, the grids were shadowed with platinum.

For SEM specimens, liberated zoospores and sperms fixed as above were placed on TOYO membrane filters (regenerated cellulose type, with 1.0 μm and 0.2 μm pore sizes), and dehydrated in an acetone series. They were critical-point-dried using dry ice, coated with gold, and observed with a Hitachi S-510 electron microscope.

Fig. 1. TEM micrograph of a liberated sperm of *Laminaria angustata* by whole-mount preparation. Note an anterior flagellum (AF) decorated with mastigonemes and a long posterior flagellum (PF).

Fig. 2. SEM micrograph of a zoospore of *L. angustata* bearing AF and PF. Arrow shows the cytoplasmic hollow along the AF.

Fig. 3. SEM micrograph of a sperm of *L. angustata* bearing AF and PF. The bases of both flagella are surrounded by the cytoplasm (arrows). Mastigonemes on an anterior flagellum are wavy or detached.

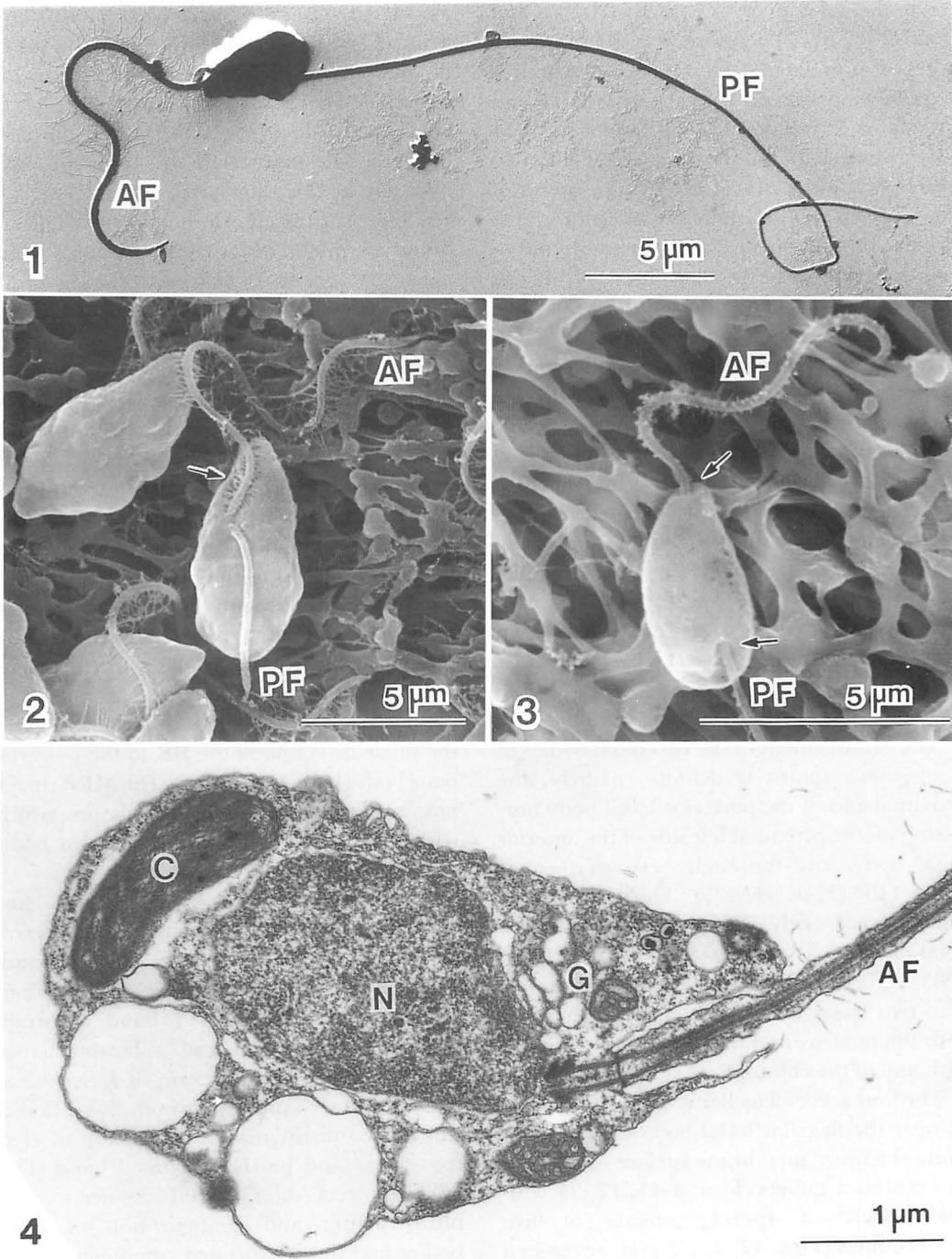
Fig. 4. TEM micrograph of a sperm of *L. angustata*. The nucleus (N) and Golgi body (G) exist near the basal body of anterior flagellum (AF) but the chloroplast (C) does not. Note that the AF protrudes through the anterior gullet.

Flagellar apparatus absolute configuration conventions and terminology are those of O'KELLY and FLOYD (1984), and BARR and ALLAN (1985).

Results

General ultrastructure of liberated sperm

General ultrastructure (Fig. 4) of liberated



sperm (ca. $5\ \mu\text{m} \times 3\ \mu\text{m}$) of *Laminaria angustata*, including a heterochromatin-rich nucleus, several small chloroplasts, mitochondria and the Golgi body, is fundamentally consistent with those of previous investigations about the related organisms by HENRY and COLE (1982 b). Therefore, it is not necessary to report it in detail. However, differences of external appearance between zoospores and sperms of *L. angustata* newly found by SEM observation will be shown. As mentioned by HENRY and COLE (1982 b), the sperm of this species has an anterior flagellum (ca. $15\ \mu\text{m}$ long) bearing mastigonemes and a long tapering posterior flagellum (ca. $40\ \mu\text{m}$ long) (Fig. 1). The zoospore does not have such a long posterior flagellum (Fig. 2). Both flagella are inserted asymmetrically and a shallow groove extends anteriorly with the anterior flagellum. On the contrary, this groove can not be observed on the sperm surface. The appearance of sperm is not so uniform as that of zoospore. However, it is confirmed that both flagella of almost all sperms protrude through an anterior gullet and a posterior gullet respectively (Fig. 3). This arrangement can also be observed in thin sections (Figs. 4, 5, 8–11, 24, 25).

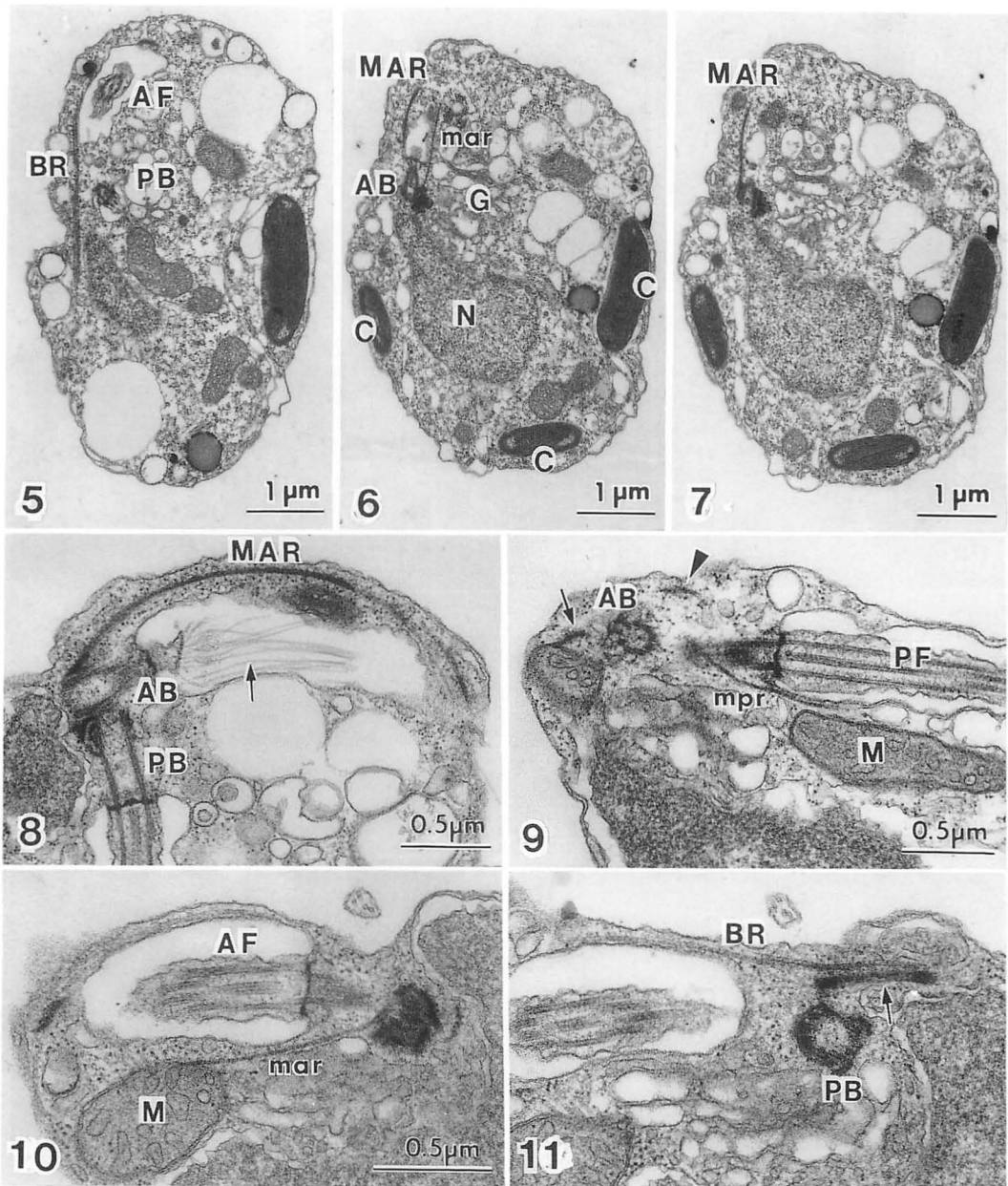
The flagellar apparatus

The arrangement of the two basal bodies in *L. angustata* sperm is definite, namely, the proximal end of the posterior basal body bordering on the proximal left side of the anterior basal body, and the angle between them is about 110° (Figs. 41, 42). This is one of the typical basal body configurations in brown algal motile cells (O'KELLY and FLOYD 1984, 1985, O'KELLY, personal communication). The two basal bodies are closely associated with the nucleus and the Golgi body, but not with any of the chloroplasts (Figs. 4, 6, 9–11).

The four sets of flagellar rootlets are observed near the flagellar basal bodies. They run along the inner membrane surface of anterior and posterior gullets (Figs. 8–11, 12, 24, 25). The **MAR** of sperm consists of five microtubules (Figs. 12, 13, 23) (*vs.* seven in a zoospore of *L. angustata* (MOTOMURA, un-

published data.) and other Laminariales (O'KELLY and FLOYD 1984) and runs anteriorly through the right side of sperm from the proximal part of the anterior basal body (Figs. 5–8, 21–23, 41, 42). The **MAR** is closely associated with the surface of a mitochondrion (Figs. 19–21) and forms an arc at the tip of the sperm before terminating in this region. The **mar** and **mpr** rootlets run respectively along the anterior and posterior gullets. The **mar** originates from the proximal part of the anterior basal body (Figs. 6, 10, 39). It runs anteriorly along the left side of the sperm and terminates posteriorly to the apex. It consists of one microtubule and is associated with a mitochondrion located at the anterior cytoplasm (Fig. 24). The **mpr** extends posteriorly from the proximal part of the posterior basal body (Fig. 9). It consists of a single microtubule, and is also associated with a mitochondrion (Fig. 25). The **BR** which is composed of four microtubules (Figs. 12–14) originates near the anterior end of the **MAR** and extends posteriorly, passing immediately ventral to the basal bodies and apparently terminating in the cytoplasm ventral to the nucleus (Fig. 5). No associations of the rootlets with any chloroplasts are seen. A posterior fibrous band, which extends from the posterior basal body to the anterior end of the nucleus, connects the **BR** to the posterior basal body (Figs. 11, 15–17). The **MPR** (major posterior rootlet), which extends posteriorly with the **BR** from the posterior basal body, does not exist (Figs. 12–18).

In zoospores of *Laminaria digitata* and *Nereocystis luetkeana*, O'KELLY and FLOYD (1984) reported a set of three striated bands that connect the basal bodies to each other, namely a deltoid striated band, a strap-shaped striated band and a button-shaped striated band. In the sperm of *L. angustata*, these structure connecting both basal bodies can not be distinguished. Instead, an electron-dense and partially striated band (Fig. 22) connects both basal bodies. The ultrastructure and configuration of basal bodies and their associated components were observed by serial section in the views from

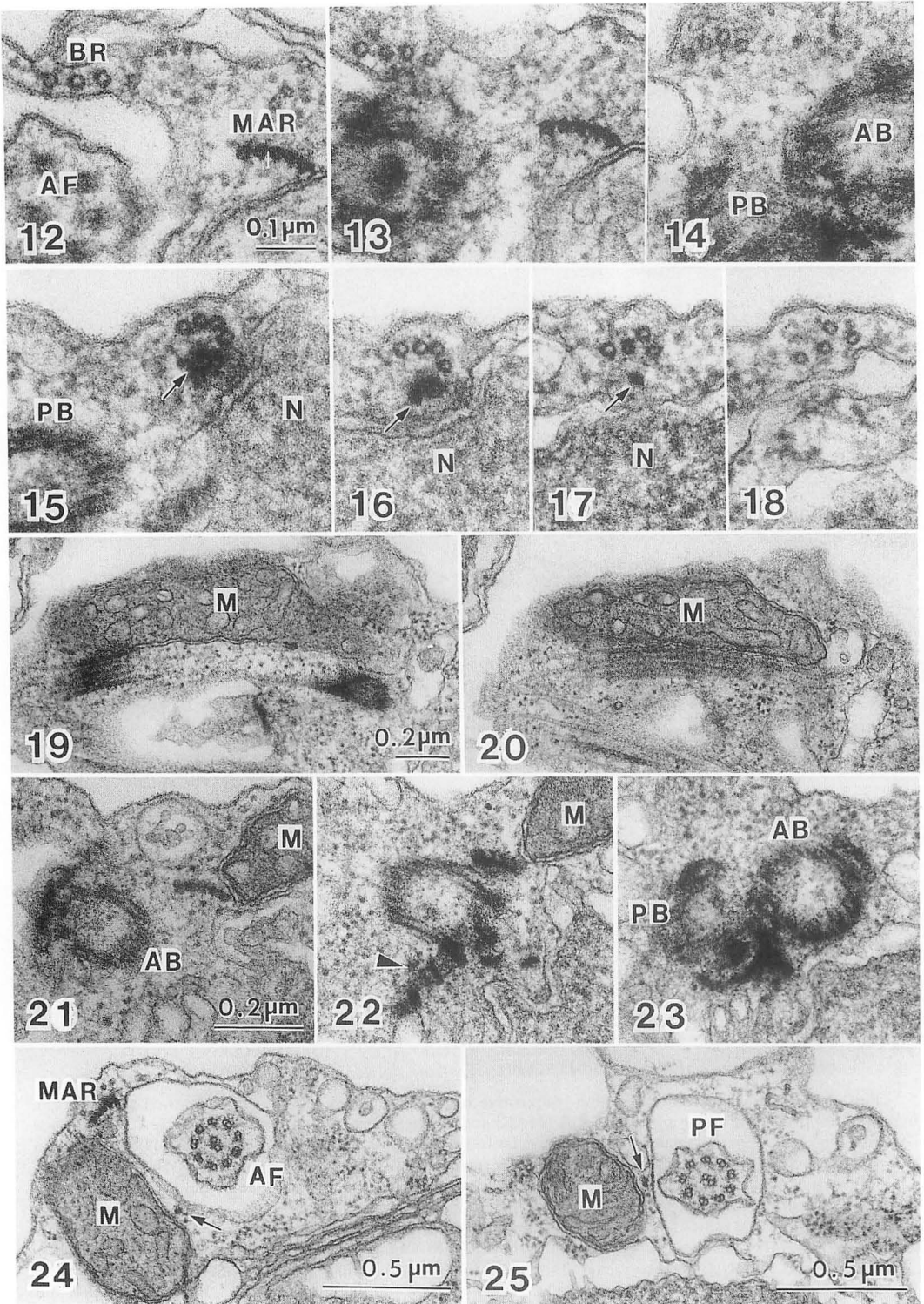


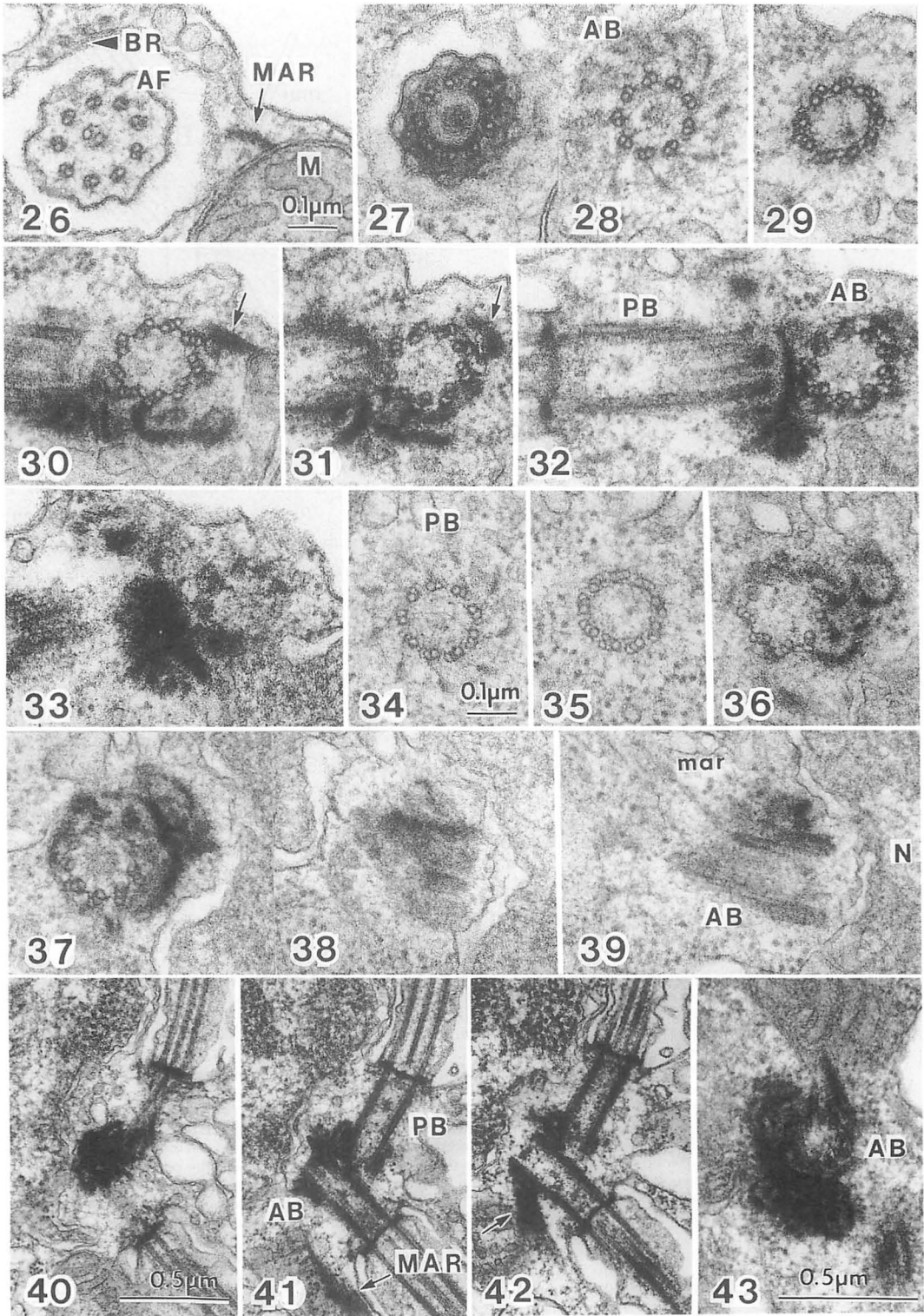
Figs. 5-7. TEM micrograph of non-consecutive serial longitudinal sections of a liberated sperm. The bypassing rootlet (BR), major anterior rootlet (MAR) and minor anterior rootlet (mar) are visible. The nucleus (N) and Golgi body (G) exist near the anterior flagellar basal body (AB) and posterior flagellar basal body (PB). In this case, three chloroplasts (C) are observed in the cytoplasm, but they do not exist near the basal bodies.

Fig. 8. MAR runs anteriorly from the proximal side of the anterior basal body. Arrow shows mastigonemes on the anterior flagellum.

Fig. 9. Note that minor posterior rootlet (mpr) runs from the proximal part of the posterior basal body and it is associated with the mitochondria (M). Arrow shows the MAR and arrowhead shows the BR.

Figs. 10 and 11. Non-consecutive serial sections. In Fig. 10, the minor anterior rootlet (mar) runs anteriorly from the proximal part of the anterior basal body and is associated with the mitochondria. In Fig. 11, the BR and the posterior fibrous band (arrow) are visible. Note that the posterior fibrous band is associated with the nuclear membrane.





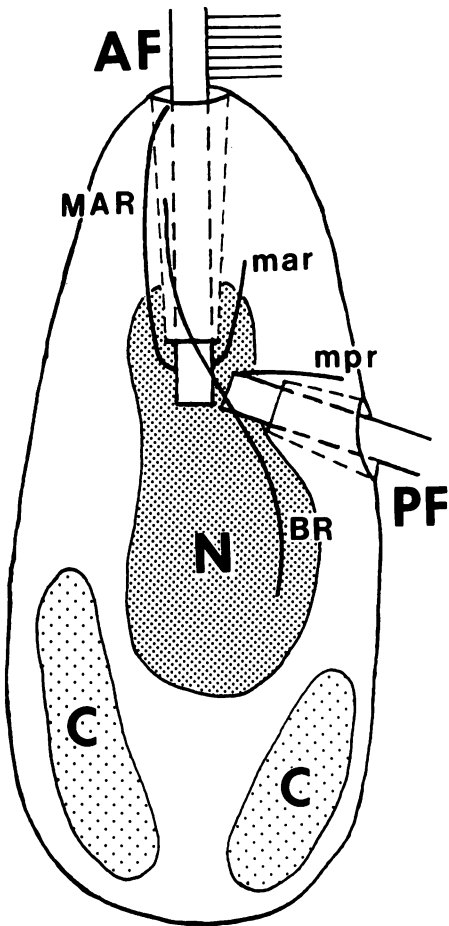


Fig. 44. Diagrammatic representation of a sperm in ventral view.

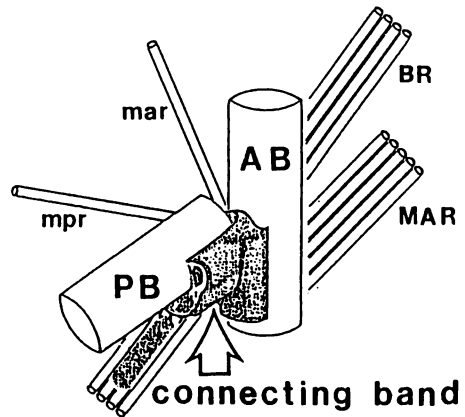


Fig. 45. Diagrammatic representation of the basal bodies and associated flagellar apparatus components.

the top of the anterior basal body to the bottom (Figs. 26–33), from the top of the posterior basal body to the bottom (Figs. 34–39) and from one side of both basal bodies (Figs. 40–42). Figure 27 shows the transverse section of the basal plate at the top of the anterior flagellum. The curved fibrils typically found at the distal end of basal bodies are shown in Figures 28 and 34. The connecting band covers the half round of the proximal side of the posterior basal body (Figs. 31–33, 36, 37, 41) and is three-lobed (Figs. 36, 37, 43). Electron dense material

Figs. 12–18. Non-consecutive serial sections of the **BR**. In Figs. 12 and 13, the **BR** consisted of four microtubules and the **MAR** consisted of five microtubules are visible. Arrow indicates the posterior fibrous band which is associated with the nuclear membrane. It is also noted that **MPR** does not occur.

Figs. 19 and 20. Consecutive serial sections grazing the **MAR**. It is noted that the **MAR** is associated with the mitochondria.

Figs. 21–23. Non-consecutive serial sections of the **MAR**. The **MAR** composed of five microtubules is deviated from the proximal side of the anterior basal body (Fig. 23) and associated with the mitochondria. In Fig. 22, striation of the connecting structure is visible (arrowhead).

Fig. 24. Transverse section of the anterior flagellum. The **MAR** and **mpr** (arrow) are visible and they are associated with the mitochondria.

Fig. 25. Transverse section of the posterior flagellum. It is noted that the **mpr** (arrow) is associated with the mitochondria.

Figs. 26–33. Non-consecutive (Figs. 26–28, 30–33 are consecutive) cross sections through the anterior flagellum (**AF**) and basal body (**AB**). Arrow shows the **MAR** and arrowhead shows the **BR**. Fig. 27 shows the curved fibrils. Profiles of the connecting band are observed in Figs. 30–33.

Figs. 34–39. Non-consecutive (Figs. 36–39 are consecutive) cross sections through the posterior basal body (**PB**), as seen from the distal end to the proximal end of the posterior basal body, indicated by counterclockwise implications of the nine triplets. Fig. 34 shows the curved fibrils at the distal end of the posterior basal body. In Figs. 36 and 37, the profiles of the connecting band are observed. In Fig. 39, the anterior basal body (**AB**) and **mar** appear.

Figs. 40–42. Consecutive serial sections through the flagellar apparatus. Arrow shows the **MAR**. The proximal end of the posterior basal body (**PB**) arranges as bordering on the proximal side of the anterior flagellar basal body (**AB**), and the angle between **PB** and **AB** is about 110° . Note that the connecting band attaches to the nuclear membrane (Fig. 40).

Fig. 43. Connecting band between the anterior basal body (**AB**) and the posterior basal body is visible as three-lobed. Electron-dense material is seen in each lobe of connecting structure.

can be observed in each lobe between the connecting band and the posterior basal body (Figs. 36, 37, 41, 43). This band also covers the proximal end of the posterior basal body (Fig. 32) and the side of the anterior basal body adjacent to the posterior basal body (Figs. 30–32). The nuclear protrusion extends to the basal bodies and attaches to the connecting band (Figs. 6, 40, 41). Based on the results, schematic diagrams showing the configuration of flagellar apparatus of *Laminaria angustata* sperms and its position in the cell were presented in Figures 44 and 45, respectively.

Discussion

In zoospores of *Laminaria angustata*, a shallow groove extends with the anterior flagellum from the flagellar base to the anterior top of the cell. Similar grooves have been found in TEM photographs of zoospores of *Laminaria digitata* (O'KELLY and FLOYD 1984), gametes of *Scytosiphon* sp. (CLAYTON 1984a), zoospores of *Desmarestia viridis*, *D. ligulata* and several species of the Laminariales (MOTOMURA, unpublished). It seems that these structures exist in many phaeophycean swimmers which have a typical flagellation. On the contrary, sperms of this species have no such groove, but both flagella protrude through an anterior gullet and a posterior one respectively. These structures have not been reported in the brown algal swimmers. It is probably an important characteristic of *Laminaria* that the structural differences of flagellar insertion between zoospore and sperm are possessed.

It has been demonstrated that a chloroplast with or without an eyespot always exists near the basal bodies in many swimmers of phaeophycean algae: zoospores and gametes of *Ectocarpus* and *Pylaiella* (BAKER and EVANS 1973a, b, LOFTHOUSE and CAPON 1975, MARKEY and WILCE 1976a, b), gametes of *Scytosiphon* (MANTON 1964, CLAYTON 1984a), male gametes of *Cutleria* (LA CLAIRE and WEST 1979), and zoospores of the Laminariales (TOTH 1976, HENRY and COLE 1982a, O'KELLY

and FLOYD 1984). Female gametes of *Cutleria* have many chloroplasts, but one of them is close to the basal bodies (LA CLAIRE and WEST 1978). Even in the Fucales, the sperm has one small chloroplast near the basal bodies (MANTON and CLARKE 1956, MANTON 1964, BOUCK 1969, BERKALOFF and ROUSSEAU 1979). A sperm of *L. angustata* has two or three small chloroplasts which are located in the cell periphery. No connection between the nuclear envelope and the chloroplast endoplasmic reticulum could be detected. Despite the existence of a nucleus and a Golgi body near the flagellar basal bodies, intimate relationship between chloroplasts and the flagellar basal bodies is not present. Therefore, it is a very unique character that no chloroplast is associated with the basal bodies in *Laminaria* sperm.

There have been few reports of detailed observations on the flagellar apparatus in the brown algal swimmers. But it is now clear that five flagellar rootlets (**MAR**, **mar**, **MPR**, **mpr** and **BR**) compose the flagellar apparatus and their configurations are nearly identical (O'KELLY and FLOYD 1984, 1985). Among them, the **MPR** is observed in many phaeophycean swimmers having an eyespot (O'KELLY, personal communication) and sperms of several species of the Fucales, *Halidrys*, *Cystoseira* and *Bifurcaria* (MANTON 1964) and *Phyllospora* (MOESTRUP 1982). However, O'KELLY and FLOYD (1985) reported that there are several variations in the number of microtubules of flagellar rootlets. They suggested that the number of microtubules in both the **MAR** and **BR** of almost all brown algal motile cells is basically seven and variations from "seven and seven" in sperms of the Fucales and zoospores of the Laminariales probably represent a derived status. Therefore, such variations may be useful in determining the phylogenetic relationships within and among the more advanced brown algal orders. Additionally, it must be noticed that especially in the oogamous groups, for example in the Laminariales in this experiment, gamete ultrastructure differs considerably from

almost typical zoospore ultrastructure. Therefore, it is also necessary to observe reproductive cells derived from both the gametophytic and the sporophytic generations throughout the life history of the species.

Compared with ultrastructures of phaeophycean reproductive cells examined so far, it has become clear that those of *Laminaria* have several unique characters as follows: 1) **MPR** does not exist in zoospore and sperm of *Laminaria angustata*; 2) the rootlet formula of **MAR** and **BR** of *Laminaria* sperm is "five and four", differing from "seven and five" of laminarialean zoospores and "seven and seven" of most other phaeophycean swimmers; 3) sperm has two or three small chloroplasts, which do not exist near the flagellar basal bodies; 4) the **BR** of *Laminaria* sperm extends posteriorly along the nuclear envelope, even though in almost all brown algal swimmers, including laminarialean zoospores, it extends posteriorly along the ventral surface of the chloroplast; 5) an electron-dense band with partial striation connects the basal bodies to each other in *Laminaria angustata* sperms like other laminarialean sperms (HENRY 1982b), while in zoospore, a set of three striated bands connects the basal bodies (O'KELLY and FLOYD 1984); 6) sperm has a long tapering posterior flagellum; 7) both flagella of sperm protrude through the gullets respectively; 8) **mar** and **mpr** of sperms are associated with mitochondria; and 9) egg has the vestigial flagella and unique flagellar apparatus (MOTOMURA and SAKAI 1988). These characters of reproductive cells of *Laminaria* would be important to consider the phylogenetic position of the members of the Laminariales within the brown algae.

CLAYTON (1984b) hypothesized a close phylogenetic relationships among the Sporochnales, Desmarestiales and Laminariales based on possessing their common features in that their sperms have several chloroplasts and no eyespot. Actually, it is also obvious that sperms of *Perithalia caudata* (Sporochnales) (MÜLLER *et al.* 1985) and *Desmarestia* spp. (RAMIREZ *et al.* 1986) and *Arthrocladia villosa* (Desmarestiales) (MÜLLER

and MEEL 1982) have a long, tapering posterior flagellum like sperms of the Laminariales. Then, it might be possible to be more precise about the phylogenetic relationships among these three orders by examining the detailed ultrastructures of sperms and eggs on the above-mentioned features.

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本村泰三：ミツイシコンブ（褐藻，コンブ目）の精子の微細構造，特に鞭毛基部構造について

ミツイシコンブの精子の微細構造，特にその鞭毛基部構造を透過型・走査型電子顕微鏡を用いて明らかにし，その遊走子および今までに報告されている褐藻類の遊走細胞との比較を行った。精子は長い後鞭毛を有しており，両鞭毛の付け根付近を細胞質が襟状となって取り囲んでいる。細胞質中には2-3個の葉緑体が存在しているが，それらと鞭毛基部構造との密接な関係は見られない。4組の鞭毛根 (MAR, mar, mpr, BR) が観察でき，それぞれ5, 1, 1, 4本の微小管からなる。BRは精子前方より核膜に沿って後方に伸び，MAR, mar, mprはそれぞれミトコンドリアとの連結が認められた。2本の鞭毛基部は特徴的な形態をした電子密度の高い1枚の帯状構造物でつながれている。上記の微細構造のいくつかは他の褐藻類の遊走細胞では報告されていない特徴であり，今後，褐藻植物内でのコンブ類植物の系統関係を考える上で重要であると考えられる。(051 北海道室蘭市母恋南町1-13 北海道大学理学部附属海藻研究施設)