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Ultrastructure of the flagellar apparatus in the stephanokont zoospores of *Pseudobryopsis hainanensis* (Chlorophyceae)*

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The stephanokont flagellar apparatus of the zoospores of *Pseudobryopsis hainanensis* is examined and compared to that of stephanokont swarmers of other green algae, especially with that of *Derbesia* zoospores. The flagellar apparatus of this alga is composed of three connecting elements and flagellar roots; the most anteriorly located non-striated fibrous ring (band A), a socket-like structure that is composed of non-striated filaments similar to band A and that connects the proximal halves of the basal bodies with band A, and a supportive electron dense ring band without striation (band C). All these the major components lie at the junction of the body and dome.

The basal bodies of this alga are fixed in two ways to two of these three components. The posterior halves of each basal body are capped by the socket-structure which hang from the band A and are accomodated in the depressions of an electron dense band. The lack of striation in any component of the flagellar apparatus and the partial covering of the proximal end of the basal bodies by one of the band structure resemble those of the flagellar connection system described for *Derbesia*, though band B in *Derbesia* is morphologically modified in *Pseudobryospsis*. This may support the opinion that siphonous geen algae should be classified in the separate class rather than in the Chlorophyceae and Charophyceae sensu STEWART and MATTOX.

Key Index Words: *Chlorophyceae*; *flagellar apparatus*; Pseudobryopsis; *siphonous green alga*; *stephanokont zoospore*; *vltrastructure.*

The recent comparative studies of mitosis and flagellar apparatus in the cellular green algae have suggested the phylogenetic significance of these features (PICKETT-HEAPS and MARCHANT 1972, PICKETT-HEAPS 1975, STEWART and MATTOX 1975, 1978, MELKONIAN 1980, MOESTRUP 1978). However, these characteristics in the siphonous green algae have been less investigated than those of other green algae (see HORI 1977, HORI and ENOMOTO 1978a, c). Studies of the biflagellated cells of siphonous green algae have been published for some coenocytic species (CRAWLEY 1966, BURR and WEST 1970, WOODCOCK and MILLER 1973, WHEELER and PAGE 1974, MOESTRUP and HOFFMAN 1975, HORI 1977, 1981, HORI and ENOMOTO 1978b, MELKONIAN 1980) but the only ultrastructural study of stephanokont zoospores is concerned with *Derbesia tenuissima* (ROBERTS *et al.* 1980). As part of a series of comparative studies on reproductive cell formation and their ultrastructure in the coenocytic green algae, the stephanokont zoospores of

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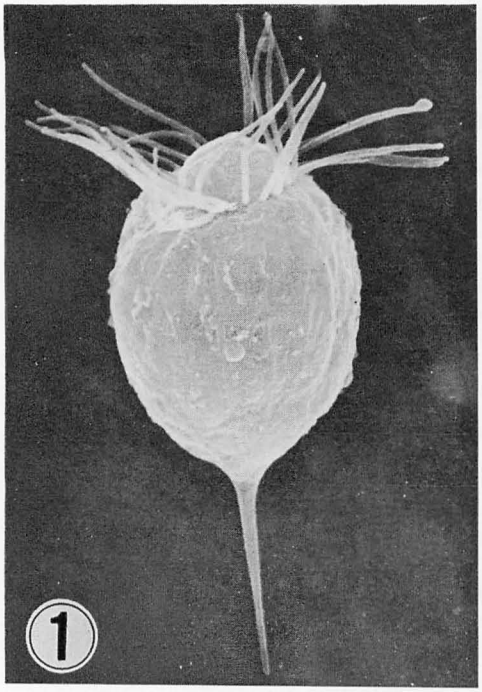

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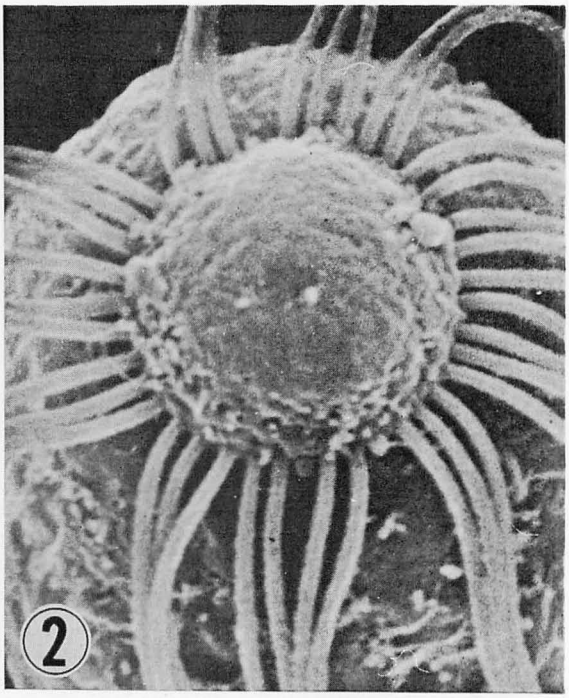

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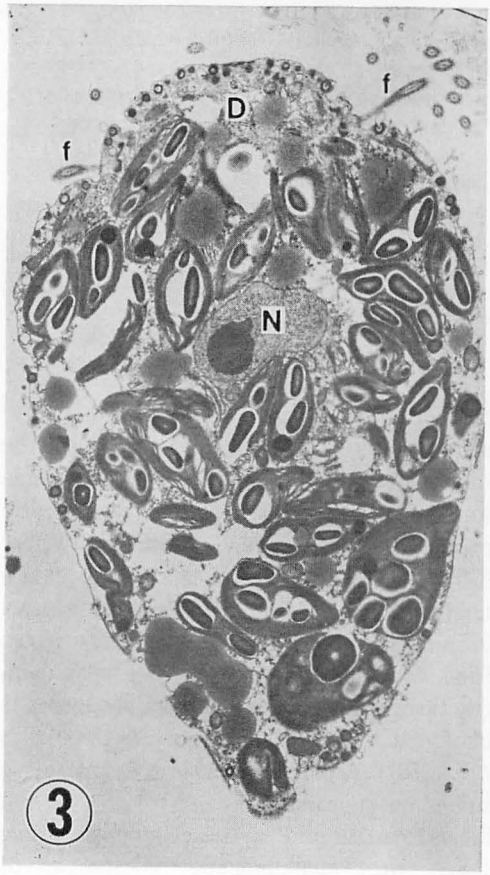

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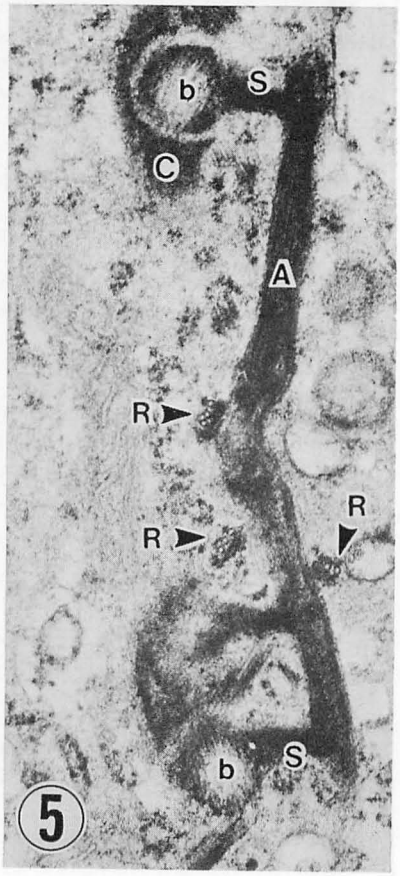

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Pseudobryospsis are examined in the present investigation.

The life history of the genus *Pseudobryopsis* was recently revealed through laboratory culture study by KOBARA and CHIHARA (1978 a, b) and OKUDA *et al.*, (1979), independently. They observed that the filamentous sporophytic germling derived from a zygote produces many stephanokont zoospores having a conspicuous tail-like appendage.

Materials and Methods

Liberation of zoospores was previously described (KOBARA and CHIHARA 1978a, b).

TEM fixation: Zoospores less than 1 hr old after liberation were processed for electron microscopy. 50% glutaraldehyde solution was added drop by drop to the zoospore suspension to a final concentration of 5 % and maintained for 3 hr in room temparature. The zoospores were then concentrated in the fixative by gentle centrifugation and rinsed twice in a seawater for 3 hr. Post fixation was carried out in unbuffered 2% OsO_4 for 2 hr (original 4% OsO_4 solutin dissolved in distilled water was diluted by adding of equal volume of seawater to final concentration of 2%).

Specimens were dehydrated in a graded ethanol series and embedded in Epon. The material was sectioned on a LKB 8800 ultrotome using a diamond knife and double stained by uranyl acetate and lead citrate and examined in a Hitachi H-12A electron microscope.

SEM fixation: 4% OsO_4 solution was added to the suspension of zoospores to a final concentration of 2% OsO_4 for 1 hr, washed in seawater, dehydrated in a graded ethanol series, critical-point dried, and placed onto aluminum support stubs with double-back adhesive tape. Samples were then coated with gold in a sputter and examined using a JEOL JSM-T20 scanning electron microscope at an accelerating voltage of 19 kV.

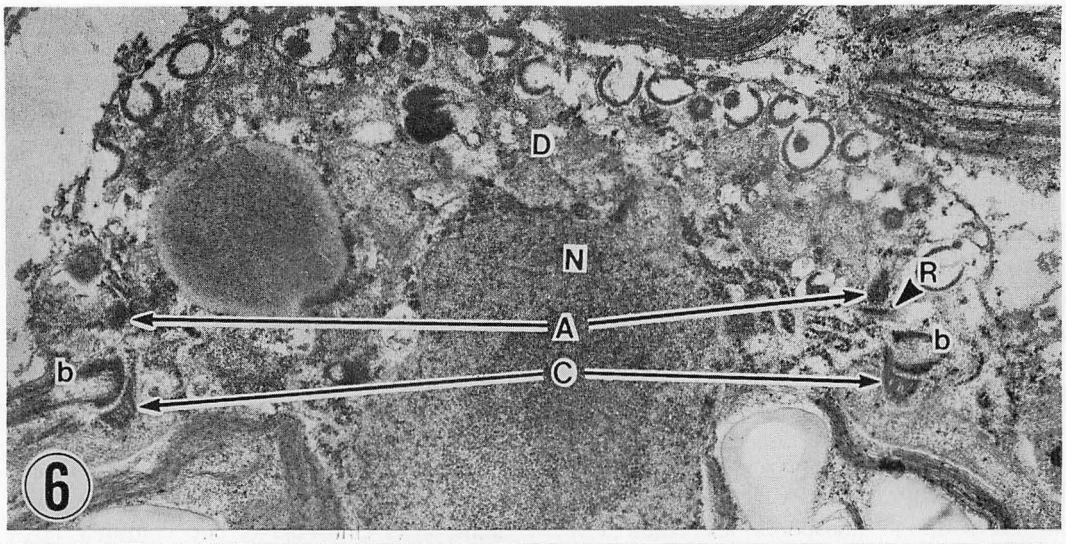
Results

General morphology: The cell body of *Psudobryopsis* zoospores is generally obovate to pyriform in shape, with a distinct anterior dome and a long posterior projection (Fig. 1). A crown of flagella encircles the most basal portion of the dome (Fig. 2). The dome lacks chloroplasts but contains large lipid granules, endoplasmic reticulum and a number of vesicles with a dense periphery and less dense content (Figs. 3, 6, 8). The cell body contains many discoidal chloroplasts without a pyrenoid, and a nucleus usually lies below the level of the flagellar ring (Fig. 3). The posterior tail (about 7-10 μm) contains endoplasmic reticulum and many microtubles (Fig. 4) which are extension of the microtubular roots and cytoskeletal microtubules.

Flagellar apparatus: The flagellar apparatus consists of a ring of about 34~39 basal bodies, two ring bands, the socket-like structures, and flagellar roots. All these major components of the flagellar apparatus are arranged in circular fashion at the proximal portion of the dome joining with the cell body (Figs. 6, 8).

Fig. 5 is a tangential section through a portion of the most anteriorly located, nonstriated fibrous band (band A in the terminogy of ROBERTS *et al.* 1980). In a median

Note: Legend abbreviations: A=band A; b=basal body; C=band C; D=dome; f=flagellum; N=nucleus; R=flagellar root; S=socket-like structure. Figs. 1-2. Scanning electron micrographs of a zoospore, emphasizing the shape of the cell body and the crown of 39 flagella. 1. Lateral view, ×1600; 2. Top view, ×7600. Figs. 3-5. Electron micrographs of the zoospore of *Pseudobryopsis hainanensis*. 3. A slightly oblique, median longitudinal section of a zoospore. ×5000; 4. Longitudinal section of part of a posterior tail which contains many microtubules and endoplasmic reticulum. ×19000; 5. Tangential section of band A and socket-like material surrounding the terminal ends of basal bodies. ×40000.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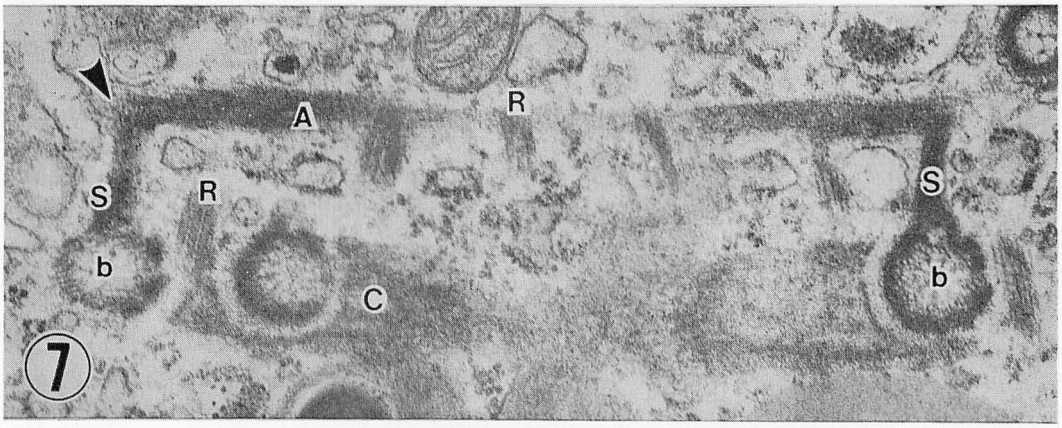
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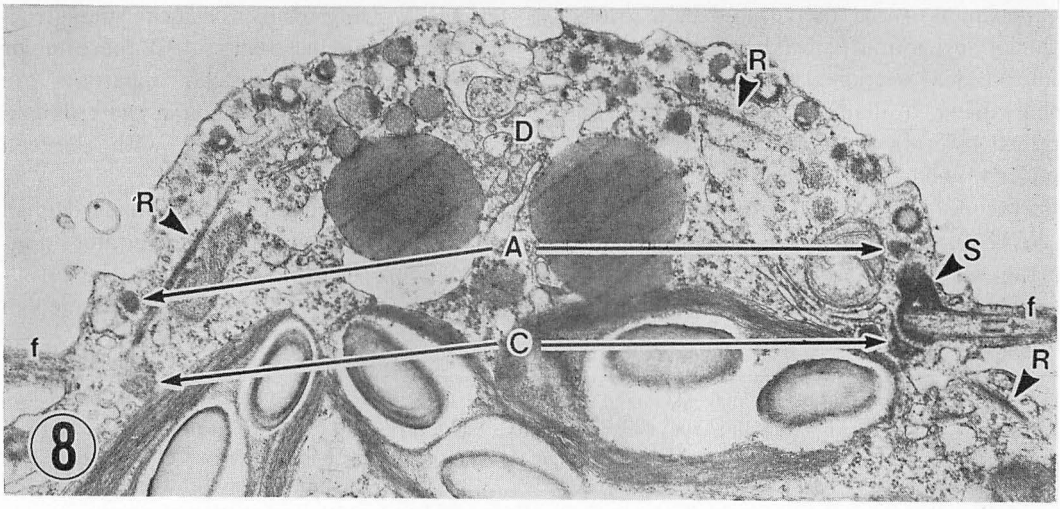
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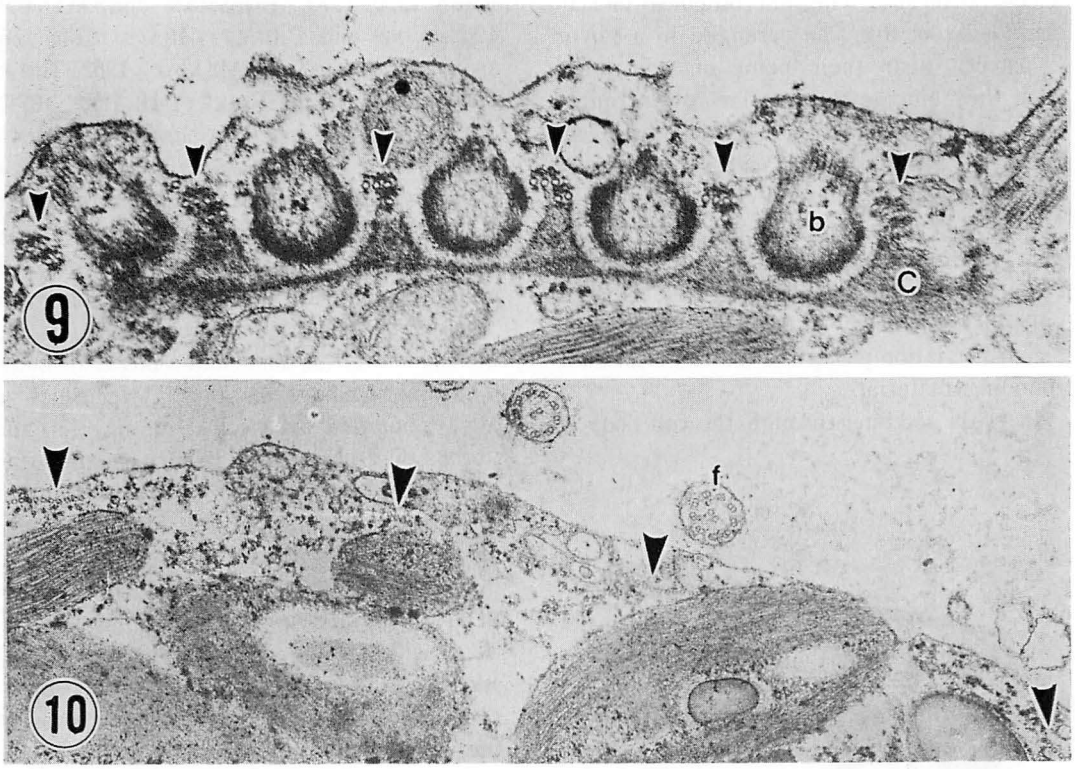
longitudinal section through the dome this is generally seen as the spherical aggregate of a finely granular matrix which presumably represents the cross section profiles of the constituent fibres (Figs. 6, 8).

The second type of electron dense material, corresponding in its positional relationship to the band B of ROBERTS *et al.* (1980), shows greatest modification in *Pseudobryopsis*. Each basal body is held by the socket-like material which hangs down from the bands A (Figs. 5, 7, 8). This appears to be composed of fibrous material similar to those of the anterior band A, because their constituent fibrils are seen to cross the fibrilar components of the band A (Fig. 7, arrow). A longitudinal section through the basal body shows that this material surrounds the proximal half of the basal bodies (Fig. 8).

In cross section the third type of electron dense material (referred to as band C after ROBERTS *et al.* 1980) extends to support the terminal end and underside surface of the basal bodies (Figs. 6, 8). When viewed in tangential sections, it is a plate-like structure composed of electron dense homogeneous material with repetative hollows opposite the basal bodies (Fig. 7). These hollows represent depressions to accomodate the



Figs. 6-8. Electron micrographs of the zoospore of *P. hainanensis*. 6. Longitudinal section through the dome, showing cross section of band A and C, and oblique or longitudinal section of basal bodies and their associated electron dense material. ×18000; 7. Part of the flagellar ring including all the components of the apparatus, band A and C, socket structure, microtubular roots and basal bodies. ×45000; 8. Longitudinal section through the dome, showing that the flagellar roots descend from the top of dome towards the cell posterior. ×20000.

Figs. 9-10. Electron micrographs of the zoospore of *P. hainanensis*. 9. Cross section of the flagellar bases, showing two types of microtubular roots (arrows) alternating with the basal bodies. ×47000; 10. Cross section of the cell body at the level of the nucleus, showing a number of grouped microtubules. ×30000.

terminal ends of the basal bodies. As their dimension is larger in diameter than that of the basal bodies, including their associated material of the second type, electron lucent spaces are found around each basal body.

Flagellar roots alternating with the basal bodies extend anteriorly inside in the dome and beneath the plasmalemma posteriorly in the cell body itself (Fig. 8). The flagellar roots in the dome extend towards the top of the dome (Fig. 8). Each root is composed of four to six microtubules arranged in two ways. In a 4-stranded root near its point of origin at the flagellar bases, the 4 microtubules are arranged in a 3 over 1 pattern which changes 4 microtubules in a row in a cell posterior. The microtubules in a 6-stranded root are also arranged in a 4 over 2 pattern near their point of origin, but soon they change to a similar row arrangement in the cell body. In Fig. 9 these two types of flagellar roots appear to alternate with one another, but they are not always arranged in such a regular manner in other part of band A. Both types of flagellar roots are embedded in electron dense material only in the vicinity of the basal bodies (Fig. 9). No striation pattern is found in the surrounding material.

In cross sections through the cell body at the level of the nucleus, some microtubular roots contain 10 microtubules arranged in a row (Fig. 10). It was not determined whether or not all of these groups extend into the long posterior tail of the cell body, but the full length of the tail contains microtubules (Fig. 4). The spatial arrangement of these components is shown diagramatically in Fig. 11.

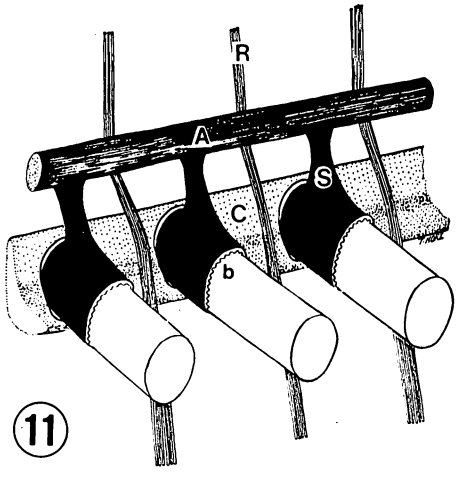


Fig. 11. Diagrammatic representation of the flagellar apparatus in the stephanokont zoospore of *P. hainanensis*.

Discussion

The ultrastructure of the flagellar apparatus in stephanokont reproductive cellls has been studied in the oedogonialean algae, *Oedogonium cardiacum* (*spermatozoid*: HOFFMAN 1970, 1973. HOFFMAN and MANTON 1963, COSS and PICKETT-HEAPS 1974; *zoospore*: HOFFMAN and MANTON 1962, HOFFMAN 1967, 1970, PICKETT-HEAPS 1971), *Bulbochaete hiloensis* (*zoospore*: RETALLACK and BUTLER 1972), *Oedocladium carolinianum* (*zoospore*: MALKOWITZ 1978), and the green siphon, *Derbesia tenuissima* (*zoospore*: ROBERTS *et al.* 1980). The stephanokont cells of these algae all bear a common feature. The cell body is divided into two distinct regions, an anterior dome and a posterior body. The junction between these two zones is always marked by a crown of flagella and fibrous ring-like bands. In all oedogonialean algae examined so far, the fibrous rings of the flagellar apparatus are striated, while all the major components are non-striated in the zoospores of the green siphon (ROBERTS *et al.* 1980). The present investigation facilitates the comparison of the substructural features of the flagellar apparatus among the stephanokont zoospores of the siphonous green algae.

The flagellar apparatus in the zoospores of *Pseudobryopsis* has several features shared with those of *Derbesia* (ROBERTS *et al.* 1980): the presence of an anteriorly positioned fibrous band (band A), and terminal band (band C) which supports the posterior ends of the basal bodies. Band A has no direct association with the basal bodies in both algae, but other components are closely

attached to the basal bodies.

However, there are also some variations in both algae. In *Pseudobryopsis*, a different type of non-striated electron dense connective structure associated with band A hangs down to attach the basal bodies. This is the socket-like structure that attaches at one end to the band A and at the other end cups the posterior half of the basal body. In the zoospores of *Derbesia*, instead, an usual type of fibrous band, morpologically identical to two other bands in both algae is present (ROBERTS *et al.* 1980). In some of their micrographs of bands B, however, slight disjunctive areas in electron density are found at regions between adjacent basal bodies (ex. Figs. 12-16 in ROBERTS *et al.* 1980); the areas in band B over each basal body is slightly more electron dense than those between the basal bodies. Furthermore, cross sectional micrographs including the three bands clearly indicate that band B is different from bands A and C, because the former is always less electron dense the than the others (Figs. 4-7 in ROBERTS *et al.* 1980). Preliminary observations on the zoospore of *Bryopsis maxima* (HORI, unpub. data) also indicate the presence of a connective structure similar to, but not identical with, that of *Pseudobryopsis* rather than the band B of *Derbesia*. Therefore, these features possibly imply various modifications of the band B structure in the stephanokont zoospores of green siphons.

Another difference is concerned with the cupping material lying around the terminal ends of the basal bodies. ROBERTS *et al.* (1980) states that the proximal part of the basal bodies in *Derbesia* is surrounded by an extension of band C and they interpreted that band C may correspond to the terminal caps found in the ulvalean swarmers (MEKLONIAN 1979, 1980). In *Pseudobryopsis*, however, the proximal part of the basal bodies is cupped by the socket structure.

The structure of the compound flagellar roots in *Pseudobryopsis* is different from that of *Derbesia*, in which they consist of two microtubules (ROBERTS *et al.* 1980). In *Pseudobryopsis* they are composed of four or six microtubules which are embedded in electron dense material only in the vicinity of the basal bodies. In *Derbesia* the flagellar roots extend from between the basal bodies only towards the cell posterior and not anteriorly in the dome, while in *Pseudobryopsis* they descend from close to the top of the dome to the most posterior end of the cell body passing through a crown of flagella.

ROBERTS *et al.* (1980) judged that all non-striated major components of the flagellar ring in the zoospores of *Derbesia* correspond to a feature of ulvalean swarmers in which a non-striated capping plate connects the basal bodies (MELKONIAN 1979, 1980, SLUIMAN *et al.* 1980) and suggested a phylogenetic link between the ulvalean algae and green siphons. Although the present observations on *Pseudobryopsis* appear possibly to support their concept, it is not concluded that all coenocytic and siphonous green algae should be included, because a striated connective is found in the siphonocladalean alga, *Dictyosphaeria cavernosa* (HORI and ENOMOTO 1978b). Before a phylogenetic affinity among this complex is solved, more detailed work on other genera is necessary.

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堀　輝三・高原隆明*：緑藻ハネモモドキ *Pseudobryopsis hainanensis* の多鞭毛遊走子の鞭毛装置構造

P. hainanensis の多鞭毛遊走子の鞭毛基部装置を電子顕微鏡を使って調べた。遊走子は，先端突起と後方の細胞本体部からなり，両者の接合部に沿って34～39本の鞭毛が環状に配位する。鞭毛基部を支える構造は，2種類の繊維性あるいは高電子密度物質からなる環帯構造と，各鞭毛基部を直接支え，かつ上記の繊維性環帯と直接連絡をもつ繊維性物質からなるソケット構造（同じく環状に配列している）である。いずれの要素にも紋様はみられない。これらの構造的特徴は，サヤミドロ目植物が生ずる多鞭毛遊走細胞のそれとは異なり，嚢状多核緑藻類のツユノイト属，ハネモ属の遊走子と類似する点が多い。以上のことから，多核緑藻類群の系統について簡単に論議した。(305 茨城県新治郡桜村天王台 1-1-1 筑波大学生物科学系　* 214　神奈川県川崎市多摩区東三田 2-1-1 専修大学商学部生物学教室)