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Electron Microscopy on the Myoneme of the Ciliate, *Zoothamnium* sp.

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It is well known that many of protozoans equipped with contractile systems by which they enable their bodies to move. A peritrich ciliate, such as *Vorticella*, *Carchesium* or *Zoothamnium*, has a special contractile system in a long stalk projected from the end of the zooid.

The stalk of *Carchesium* has been studied by both the physiological and morphological methods. Sugi (1961) measured changes of the volume and the length of a *Carchesium* stalk, and reported that the stalk shortened by 22–33% and the volume decreased by 24–38% of its resting state during contraction. Yagiu and Shigenaka (1960) carried out electron microscopic studies on fibrillar systems in ciliates. They classified *Vorticella* myonemes, each myoneme being composed of a bundle of fine myofilaments, into 2 types by their distribution in the zooid, body myoneme and circular myoneme. Recently, many papers have been published on peritrich ciliates with electron microscope. Amos (1972) revealed the structures of stalks in *Vorticella* and *Carchesium* in relation to a coiling mechanism on the stalk contraction. Allen (1973) suggested that the linking structures of myoneme, endoplasmic reticulum and surface reticulum in the stalk of *Vorticella* were the evidence of a message transfer from the cell surface to the sites of calcium release to trigger myonemal contraction. From the comparative studies on structures with 4 species of *Vorticella*, Kawamura (1973) reported that on the myoneme systems of these 4 species, there was a little difference in arrangements of the outer canaliculi of body myoneme each other.

A peritrich ciliate, *Zoothamnium*, has also a long stalk, but few papers have been published on this species. This paper is dealt with fine structure of the myoneme system in the stalk and zooid of *Zoothamnium*.

MATERIAL AND METHODS

Zoothamnium was collected from a brackish water lake, Shinji-ko, in Matsue City and kept in laboratory condition to eliminate clay particles in vacuoles within the organism for several days. Filtered brackish water collected from the habitat of the animals was used as a culture medium. After culturing for several days, organisms were employed for the electron microscopic observation.

The methods adapated for electron microscopic observations were mainly the conventional. The organisms were fixed at 0–3°C in a solution containing 1% glutaraldehyde and 75 mM phosphate buffer (pH=7.4). After pre-fixing in this medium for 90 min, specimens were washed for 30 min by exchanging 2 times with the buffer solution without glutaraldehyde. They were then post-fixed in 1% osmium tetroxide with 0.2 M sucrose and 75 mM phosphate buffer (pH=7.4). Dehydration was carried out in a series of ethanol and followed by propylene oxide. After dehydration the specimens were embedded in Epon 812 and hardened in a 50°C oven for several days. Silver-gold sections were cut with glass knives and picked up on formvar-coated grids. Thin sections were stained with saturated uranyl acetate for 60 min, followed by Reynoids' lead citrate for 3 min and examined in a Hitachi HU–11A electron microscope.

RESULTS AND DISCUSSION

A) *Zooid*

Zoothamnium was composed of bell-shaped zooid (30 μm × 55 μm) and an elongated stalk (100 μm in length) projecting from the posterior end of the zooid. The zooid contained a macronucleus in the form of a horseshoe, food vacuoles, mitochondria, endoplasmic reticula (ER), ribosomes and other cell organelles. The surface of the zooid was covered with many of pellicle ridges arranged in circumferential rows with perpendicular direction to the zooid axis (Fig. 1).

Contractile elements in the zooid were found as a number of longitudinal bundles of thin filaments lying under the pellicle and ones extending from the adoral zone to the scopular region (Fig. 2). Each zooid myoneme bundle originated from a less electron dense layer under the pellicle at the oral region. The bundles were lined in the layer with a regular arrangement at intervals of about 2.2 μm each other. A single bundle showed an elliptical form in cross section, measuring 0.8 μm × 0.3 μm in diameter and contained several numbers of tubular-ER (60 nm in diameter) in the central region (Fig. 3). The bundles were apart from the pellicle as they came down and closed to the scopular region. The longitudinal section at the middle of the zooid showed that the myonemal bundle containing tubular-ER in the central region was surrounded by ER measuring about 0.12 μm in width and 1.6 μm in length (Fig. 2). There was no membrane separating the myonemal bundle from the cytoplasm. The ER often enclosed the myonemal bundle completely. The ER associated with the bundle were not observed at the oral region and the scopular region of the zooid. The tubular-ER distributed in the central region throughout the entire length of the myoneme and filled with electron dense deposits in their cavities. A zooid myoneme consisted of a single population of longitudinally oriented microfilaments each with a diameter of 3–4 nm. Junctional structures with which microfilaments (myofila-

ments) were connected each other were not observed in the myonemal bundle.

B) *Scopula (junction between the stalk and the zooid)*

The scopula was highly organized region where many cilia were recognized and zooid myonemes merged into one large stalk myoneme (Fig. 4). In a longitudinal section, many cilia on the zooid were observed in penetrating about 1.5 μm into the stalk sheath, but not found in the stalk myoneme (Fig. 4). These cilia were seen to differ from the oral cilia only in their length, but the fine structure was somewhat similar to that of the oral ones. These cilia were counted about 150 in number in the cross section, distributing irregularly, and their basal bodies were embedded in the zooid (Fig. 5).

The annular tubes originating from the surface of each cilium ran longitudinally in a sheath from the scopula to the distal part of the stalk (left arrow in Fig. 6). The stalk contained annular tubes of about 150 in number, as their number corresponded to that of cilia. An annular tube measured 0.2 μm in diameter. On the surface of a cilium, a file of dots, which represented ring structure of an annular tube, was recognized in longitudinal section.

The sheath portion of a stalk was separated completely from the zooid by a thick plasmalemma which were composed of 4 membranes, except the part of pores that were frequently seen in the plasmalemma. A cilialy membrane was continued with the outer membrane. The plasmalemma elongated into the stalk and wrapped the stalk "spasmoneme" in it.

Contractile elements showed some changes in this scopular region. The bundles of the zooid myonemes converged into one large stalk myoneme which ran longitudinally throughout the entire length of the stalk in the middle part of the stalk. The ER surrounding the zooid myoneme disappeared at the upper site of the scopular region (arrow in Fig. 4 indicated as ER). Tubular-ER, on the other hand, were gathered and formed a regular arrangement in the stalk myoneme (arrows in Fig. 4 indicated as TER).

C) *Stalk*

The contractile stalk was consisted of three structures, a stalk sheath, annular tubes and a "spasmoneme". It may be in no doubt that these structures serve to bent the stalk.

The stalk sheath had a limiting membrane and was filled with the less electron dense matrix containing annular tubes (Fig. 8). Annular tubes at the peripheral of the sheath showed dense distribution than at the inner area close to the spasmoneme, although they were not so regularly distributed. The annular tubes (0.2 μm in diameter) in the outer area were seen larger than those in the inner area in a cross section (Fig. 8). In a longitudinal section, transverse stripes at interval of about 45 nm were found on the surface of annular tubes (Fig. 8). In a cross section in Fig. 8, the stalk myoneme was placed asymmetrically within the spasmoneme. This asymmetry ap-

peared to show a bent of the stalk into the left side. On the stalk contraction, of special interest was numerous fine filaments which appeared in the opposite site of a bent in the stalk sheath. These fine filaments connected adjacent annular tubes together and also annular tubes to the sheath membrane.

The spasmoneme, which was composed of a stalk myoneme and cytoplasm containing mitochondria in clusters, was enclosed with a thick plasmalemma. The stalk myoneme was consisted of several decades of bundles of myofilaments limited with tubular-ER. The bundles were found in a regular arrangement throughout the entire length of the stalk myoneme (Fig. 9). This arrangement seems to resemble to that of myofibriles in the smooth muscle cells.

A stimulus for the stalk contraction may be transmitted through the zooid myoneme, because the stalk myoneme is covered with the thick plasmalemma. The contraction of the stalk must be caused primarily by myofilaments (3–4 nm in diameter) which resemble musclar thinfilaments in their dimension. The contraction is further controlled by the interaction between myonemal bundles and annular tubes with fine filements. The conception can readily be explained by the idea that the fine filaments connect with annular tubes to make the state stable and the structure constricted thus prevent to bend to the side of the sheath. Annular tubes may have two main functions, one is to keep the stable state on the outer side of the bending portion of the stalk, and another is a role in the relaxation of the stalk. Annular tubes may have the elasticity owing to their tubular structure. Under a light microscope, *Zoothamnium* shows imperfect spiral contraction, while the other peritrich ciliates, *Vorticella* and *Carchesium* contract completely spirally. The reason why *Zoothamnium* contract imperfectly spirally may be explained by a difference in the structure that annular tubes in *Zoothamnium* distribute irregularly in the sheath.

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ABBREVIATIONS ON PLATES

AT	annular tube	BB	basal body
C	cilium	Cp	cytopharynx
Cy	cytoplasm	ER	endoplasmic reticulum
FV	food vacuole	N	nucleus
P	pore	Pl	plasmalemma
Sh	sheath	SM	stalk myoneme
TER	tubular-ER	ZM	zooid myoneme

PLATE I

Fig. 1. Longitudinal section of the zooid. The pellicle shows projected ridges. Two edges of the horseshoe-shape macronucleus are seen. ×1700

Fig. 2. Longitudinal section at the middle region of the zooid. The bundle of zooid myoneme (ZM) bears tubular-ER (TER) in the middle and ER (ER) at the periphery. ×15000

Fig. 3. Cross section at the oral portion. The bundle of a zooid myoneme (ZM) are shown just under the pellicle. They arrange at regular intervals. Upper bundles belong to oral myonemes which do not penetrate into the stalk myonene. ×15600

PLATE II

Fig. 4. Longitudinal section of the scopula. Many cilia (C) from the zooid insert into the sheath (Sh). ER found around the zooid myoneme disappeares at the upper site of this region (arrow indicated as ER), but tubular-ER (TER) are gathered in a regular arrangement with myonemal bundles. ×14000

Fig. 5. Cross section at the scopula. The basal bodies (BB) of the cilia in the zooid and the cilia in the sheath are seen, but the cilia not seen in the myonemal region (SM). ×7500

Fig. 6. Enlarged view of the scopula. The sheath is separated by the plasmalemma (Pl) which consist of 4 membranes (right arrow). Annular tubes (AT) originate from surface of cilia (left arrow). ×20000

PLATE III

Fig. 7. Englarged view of the stalk in cross section. The myonemal bundles are shown in a regular arrangement limited by the tubular-ER (TER). There are electron dense deposits in the tubular-ER (arrow). ×18000

Fig. 8. Cross section of the stalk. The spasmoneme is composed of the stalk myoneme (SM) and cytoplasm (Cy). Larger annular tubes are seen at the outer side and smaller ones at the inner side in the sheath (Sh). ×4600

Fig. 9. Longitudinal section of the stalk. Regular stripes are found on the surface of annular tubes in the stalk sheath. The bundles of the stalk myoneme run longitudinally in a regular arrangement in the spasmonem. ×11000

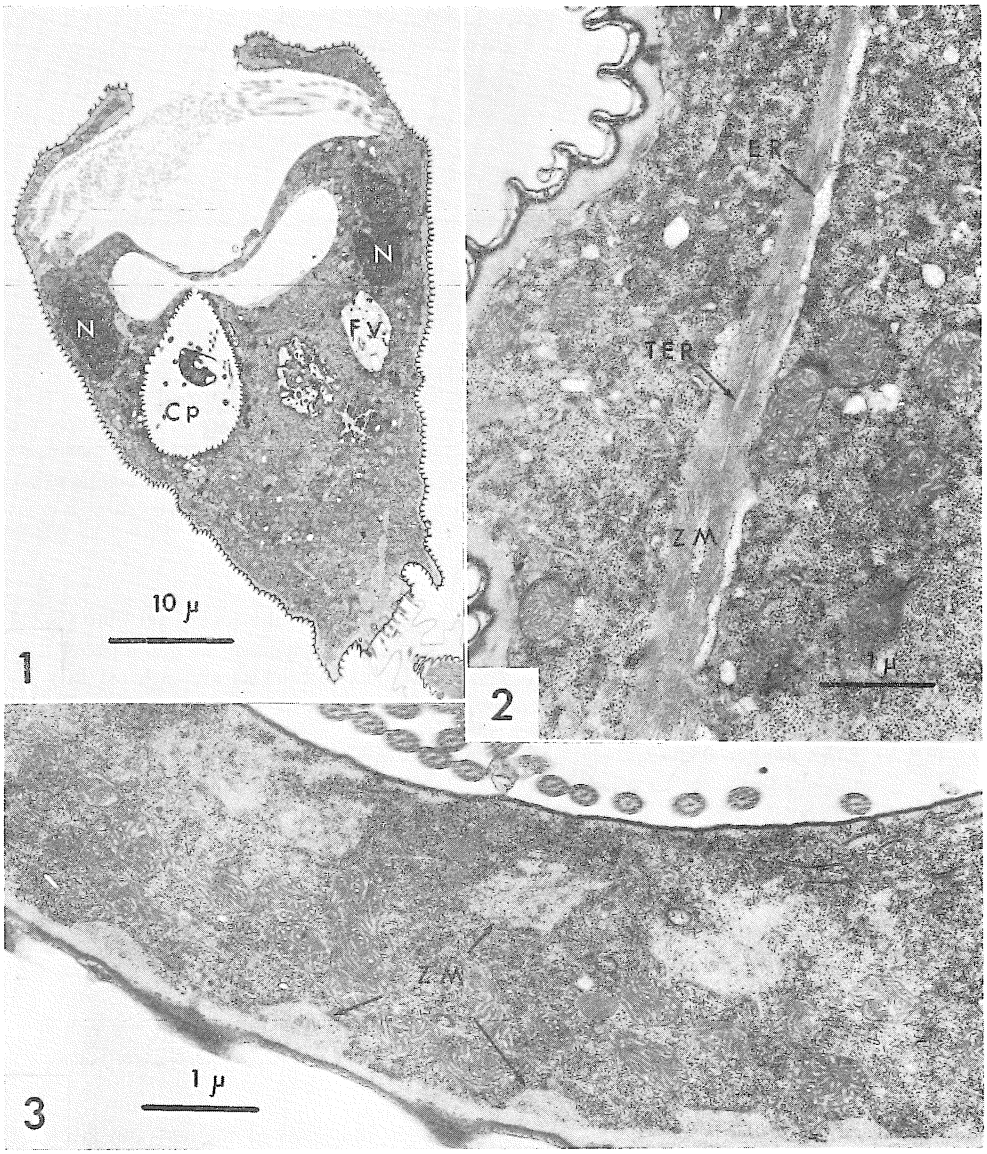
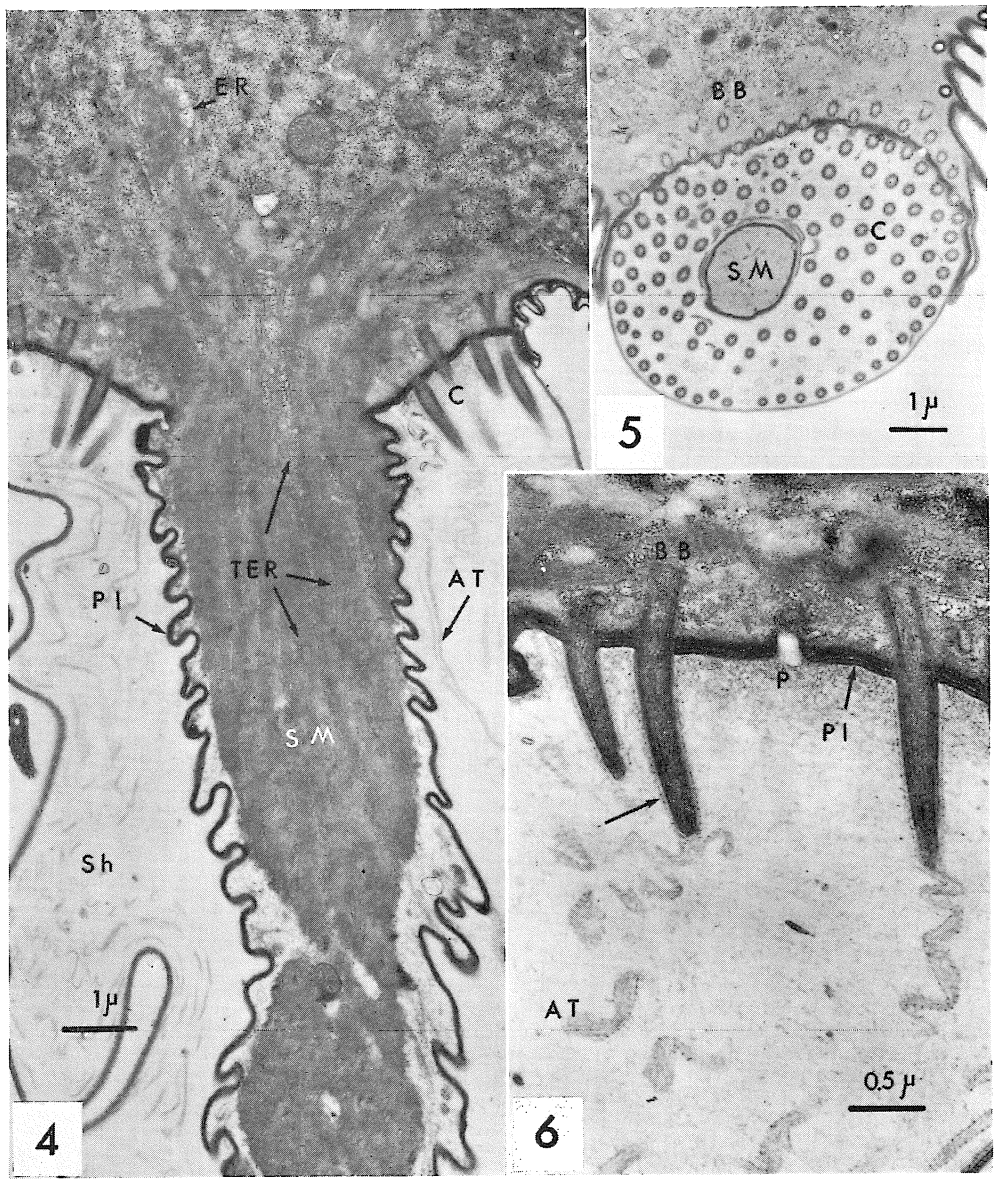


PLATE I



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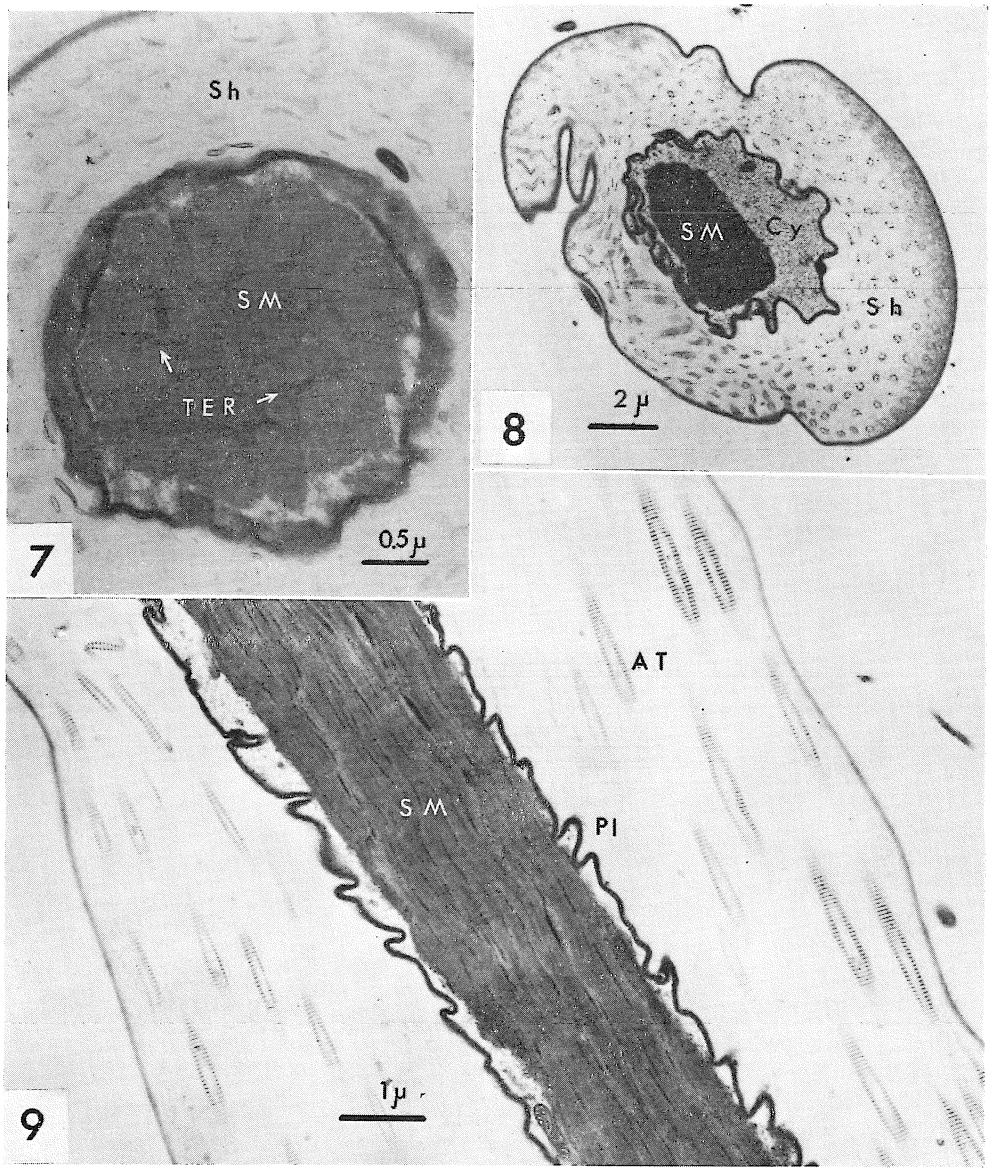

PLATE III