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Cytoskeletal Construction and Alteration of Microtriches of *Diphyllobothrium hottai*, During Early Developmental Stages

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Abstract

The fine structure of the microthrix of *Diphyllobothrium hottai* Yazaki *et al.*, 1988 was examined using a scanning and transmission electron microscopy. Three types of microtriches, conoid type, digitiform type and filamentous type, were observed in the plerocercoid and the early developmental stages after infection in the experimental final host, *Mesocricetus auratus*. The worms cast off the filamentous microtriches during the first 2 ~ 3 hr PI. Each type of microthrix showed a peculiar fine structure in cross section and in longitudinal section. The core-microfilamentous structure of the conoid- and filamentous-type microthrix was somewhat scanty in contrast with the microvilli of human intestine. In addition, a tubular microfilamentous structure, about 6nm in diameter, was observed in all three types of microthrix. In each base, they were observed as a thick walled tube in longitudinal sections and as a dense inner ring, consisting of about 30 tubular subunits, in cross sections. In each shaft, the same tubular subunits were seen in layers in the central clear zone of filamentous microtriches and as a meshlike structure in the conoid-type microtriches.

Key words: *Diphyllobothrium*, microthrix, cytoskeleton, microfilament, tapeworm

Introduction

Cestode microtriches are changeable during development from the procercoid to the adult worm (Bräten, 1968 a, b; Grammeltvedt, 1973; Lumsden *et al.*, 1974). They are roughly classified into three types by shape; the blade-like or conoid type, the digitiform type and the filamentous type. The ultrastructure of microthrix has so far been reported by Jha and Smyth (1968), Lumsden (1975), Hess and Guggenheim (1977), Engelkirk and Williams (1983) and Holy and Oaks (1986). Hess and Guggenheim (1977) and Engelkirk and Williams (1988) have especially referred to the fine structure of the filamentous microthrix in *Mesocestoides* and in *Taenia*. The ultrastructure of the filamentous microthrix in Diphyllobothriid cestodes has not been reported. The present study aims to observe the cytoskeletal construction and the alteration of microtriches of *D. hottai* during the development from plerocercoids to adult stages (eight~twenty-four hr, post infection, PI) in the experimental final host, *Mesocricetus auratus*. It was revealed for the first time in Diphyllobothriid cestodes that the cytoskeletal construction of filamentous microthrix differed from those of two other types of microthrix and that filamentous microtriches were cast off during the first two or three hr PI.

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Materials and Methods

Plerocercoids of *D. hottai* were collected from the body cavities of Japanese surf smelts (*Hypomesus pretiosus japonicus*) from Hokkaido, Japan. Several plerocercoids were directly intubated into the stomachs of four golden hamsters, and recovered from the ileocaecal region at 2 and 3 hr PI and from the anterior region of the small intestine at 8 and 24 hr PI. The preparation for transmission electron microscope (TEM) examination was as follows: specimens dissected into small pieces were fixed for 2 hr at room temperature in 3% glutaralde-

hyde containing 2% tannic acid in 0.1M cacodylate buffer, pH 7.0. Following a buffer rinse, the specimens were postfixed for 2 hr at 4°C in 2% OsO_4, dehydrated through an ethanol series, placed in propylene oxide, and embedded in Epon 812. Ultrathin sections were double stained with uranyl acetate and lead, and examined in a Hitati H-500 TEM. The preparation for scanning electron microscope (SEM) examination was as follows: specimens were dehydrated through an ethanol series, soaked in amylacetate, transferred to critical point drying apparatus, coated with gold and examined in a Hitati S-450 SEM.

Results

The body surface of the plerocercoid covered with elongated filamentous microtriches (about 18 μm long) was observed using a SEM (Figs. 1a, b). Further observation of the microtriches of the plerocercoid using a TEM revealed two other types of microtriches such as the conoid and digitiform types (Fig. 3). The filamentous microtriches were cast off within 2~3 hr PI, when the worms attached to the ileocaecal region of the hosts (Figs. 2a, b), and the worms reserved the conoid-type microtriches, few in number and digitiform-type microtriches, predominant in number (Fig. 4). The digitiform type of plerocercoids and early developmental stages (2~3 hr PI), with a short base (proximal part) and long shaft (distal part), altered to that of adult stages (8~24 hr PI), with a long base and short shaft (Fig. 5).

— Ultrastructural characteristics of the filamentous microthrix —

Observation using a TEM: The base was about 0.4 μm in length, about 0.1 μm in diameter and was bound by a plasma membrane. A dense inner ring consisting of about 30 subunits was observed in the cross sections of the base (Fig. 6; arrow). In the longitudinal sections of the base, an electron dense layer was clearly observed just under the plasma membrane (Fig. 8; small arrows) and core-microfilamentous structures were shown in the central zone of the base. In comparison with the core-microfilamentous structures of an adult stage (Figs. 9, 10), they were somewhat obscure and few in number (Figs. 6, 8). The shaft was about 17 μm in length, and about 0.08 μm in diameter in the case of the round shaft; and 0.1 μm by 0.08 μm in the case of the ellipsoidal one. It was also bound by a plasma membrane. The central zone of the shaft was clear (Figs. 7, 8; large arrow). The cortex, about 15nm wide, was slightly electron dense, and bisected by a thin opaque lamina (Fig. 7; arrows). The inside of the cortex showed a thin electron dense layer (Fig. 7; large arrowheads), and tubular structures, about 6nm in diameter, were arranged in one or two layers in the thin electron dense layer (Fig. 7; small arrowheads). The tubular structures appeared to be the same as those of the base.

— Ultrastructural characteristics of the conoid-type microthrix —

The core-microfilamentous structure was as scanty as that of filamentous microthrix. In the cross section of the base, the electron dense layer, 14nm wide, was detected just under the plasma membrane (Fig. 6; large arrowheads) and the core-microfilamentous structure was indistinctly shown as the less electron dense material in the medulla (Fig. 6; cb). The shaft of microthrix was bound by a plasma membrane, and the medulla, surrounded with a less electron dense cortex layer (Fig. 6; small arrowheads), showed a meshlike structure (Fig. 6; cs). It appeared that the shaft of the conoid-type microthrix was occupied by tubular core-microfilamentous structures.

— Ultrastructural characteristics of the digitiform microthrix —

The digitiform microthrix of the plerocercoids and the early stages (2~3 hr PI) had a shorter base and more elongate shaft than that of the adult stages (8~24 hr PI) (Figs. 4, 5). The core-microfilamentous structure of the worms (8~24 hr PI) was more distinct than those of the filamentous-type and the conoid-type microthrix (Figs. 6, 8, 9). A clear central zone was not observed in the shaft of digitiform microthrix.

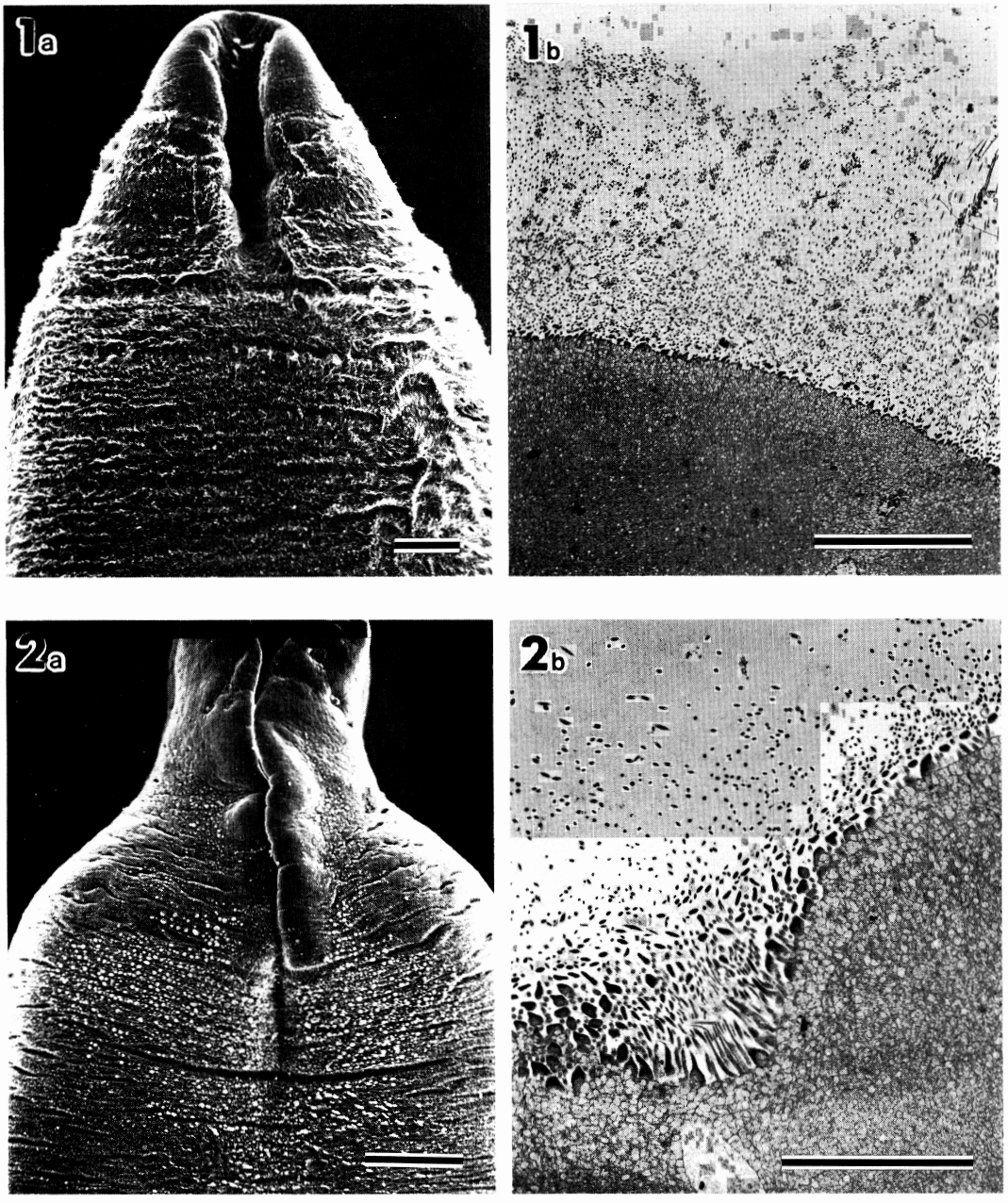
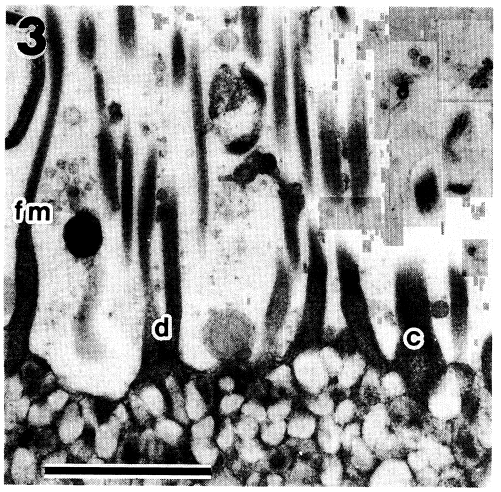


Fig. 1 Long filamentous microtriches of plerocercoid. a: scanning electron micrograph (Bar: 100 μm), b: transmission electron micrograph (Bar: 10 μm).

Fig. 2 Body surface of early stage worm in experimental final host (2hr PI). a: scanning electron micrograph (Bar: 100 μm)

b: transmission electron micrograph (Bar: 10 μm)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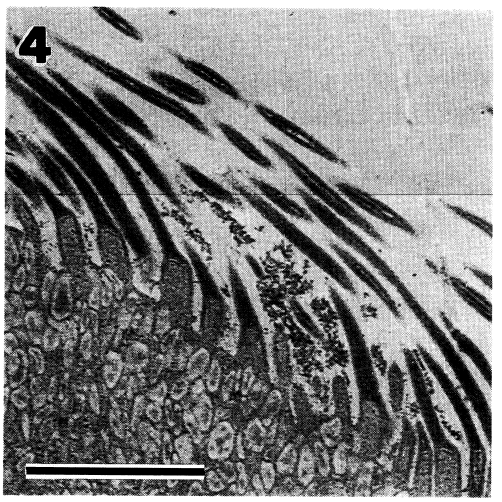
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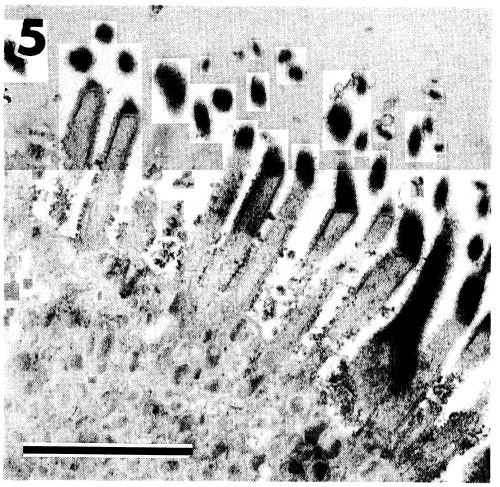
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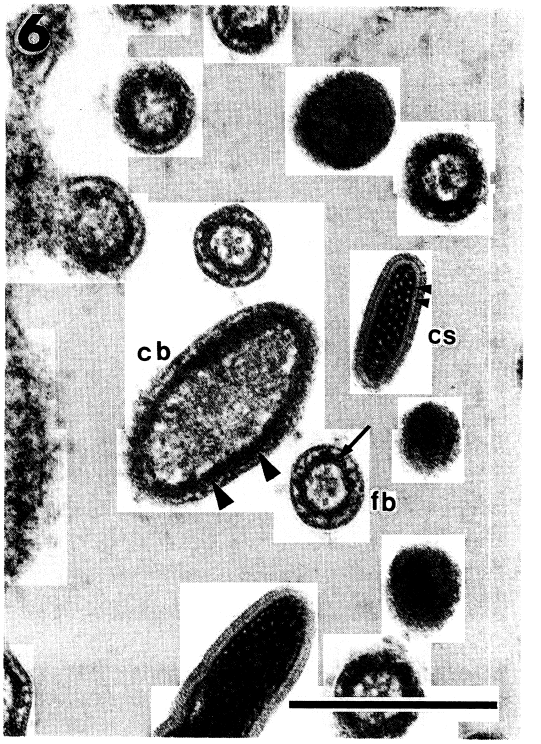
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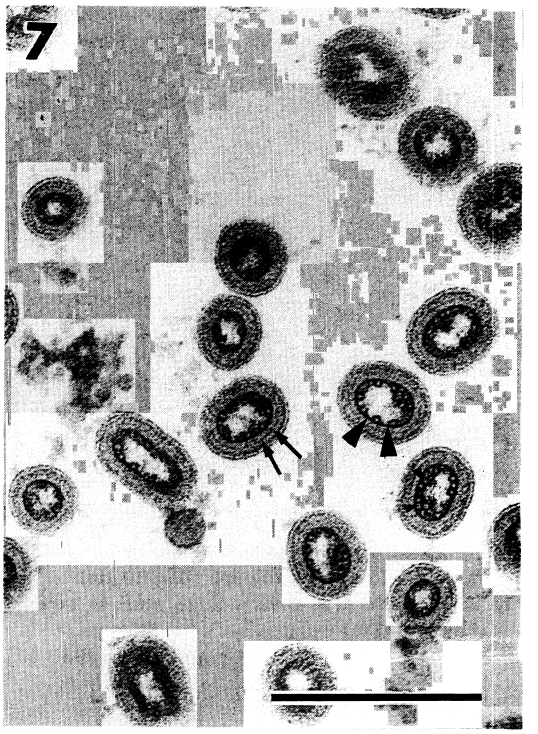
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The cross section of digitiform microthrix (2～3 hr PI) was not observed in this examination.

Discussion

Many authors have previously undertaken the ultrastructural observations of microthrix in various cestodes (Yamane, 1968; Jha and Smyth, 1969; Lumsden *et al.*, 1975; Thompson *et al.*, 1980; Holy and Oaks, 1986). The microthrix is clearly divided into two distinguishable parts, a shaft and a base by the multilaminated basal plate (Jha and Smyth, 1969). Different types of microtriches, in each part of the base and the shaft, have been observed according to their species, developmental stages, and locations on the body surface. Our observation suggests that the type of microthrix in cestodes is altered according to the host, adapting to their given environment. Microtriches have been analogized with microvilli, which constitute the brush border of transport epithelia in many invertebrates and vertebrates, although the microvilli have no densely fibrillar distal tip (shaft). Previous studies have shown that microtriches differ from microvilli in certain details of their fine inner structure (Belton, 1977; Yamane *et al.*, 1982; Conder *et al.*, 1983; Holy and Oaks, 1986). Observations of the longitudinal section of the microthrix base revealed an electron dense layer just under the plasma membrane, called a thick walled tube by Tompson *et al.* (1980). The dense inner ring consisting of about 30 units coincided with the thick walled tube. The fine structure of the base of the filamentous microthrix, which is cast off in early stages, coincides with the filamentous microthrix of other species (Tetrathyridium of *Mesocestoides corti* and *Taenia taeniaeformis*) reported by Hess and Guggenheim (1977) and Engelkirk and Williams (1983). Engelkirk and Williams (1983) observed the clear central zone of the shaft of filamentous microthrix of *Taenia taeniaeformis,* which was cast off by 18 days PI in rats, although they did not show tubular structures in the clear zone. Mukherjee and Williams (1967) described a tubular appearance of the microfilament (core microfilament of mouse intestine). The tubular microfilament detected in microtriches must be a definite cytoskeleton in the core microfilament of cestodes. The core-microfilamentous structure is observed in the medulla of the microthrix base, whereas they are scanty in early stages. The difference of core-microfilamentous structure between the early development stages and the adult stages must be further investigated in detail. Morphological differences between microtriches and microvilli suggest functional differences, such as the roles of protection and nutrient absorption. In the present study, the morphological alteration of microtriches in *D. hottai* plerocercoids was observed during the early developmental stages in the final host. The cytoskeleton of the microthrix has so far been reported by many authors (Jha and Smyth, 1969; Lumsden, 1975; Hess and Guggenheim, 1977; Tompson *et al.*, 1980; Engelkirk and Williams, 1983; Holy and Oaks, 1986). To observe the variety of microthrix cytoskeletons, differences in various cestode species, developmental stages, and location on the

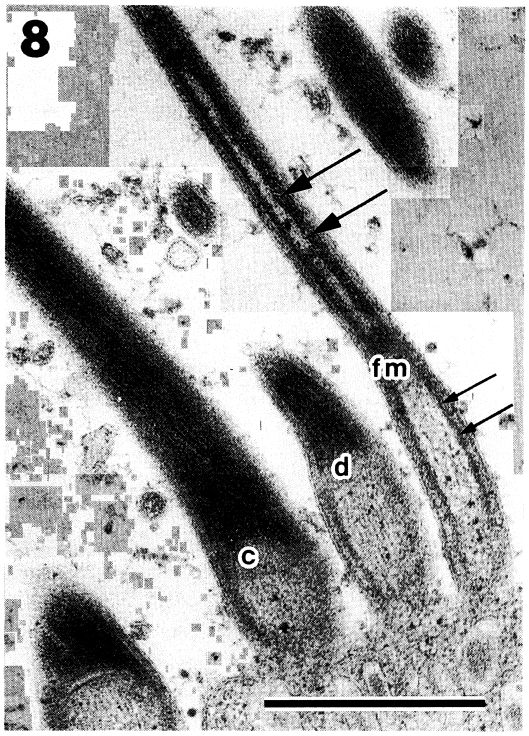
Fig. 3 Longitudinal section of plerocercoid microtriches, showing three types of microthrix (Bar: 1 μm).
c: conoid-type, d: digitiform-type, fm: filamentous-type

Fig. 4 Cross section through body surface of early stage worm (2hr PI). Digitiform-microthrix is dominant (Bar: 1 μm).

Fig. 5 Adult type of microtriches (24hr PI), longitudinal section (Bar: 0.5 μm).
It shows a long base and short shaft. A conoid type microthrix still remains.

Fig. 6 Cross section of base and shaft of the microtriches (2hr PI), (Bar: 0.2 μm).
An electron dense layer was detected just under the plasma membrane in the base (large arrowheads) and a less electron dense cortex layer in the shaft (small arrowheads). The dense inner ring (arrow) consists of about 30 tubular units.
cb: base of conoid type, cs: shaft of conoid type, fb: base of filamentous type

Fig. 7 Filamentous microtriches. Cross sections of shaft (Bar: 0.2 μm).
The cortex bisected by a opaque lamina (arrows). The outside of the clear zone shows a thin electron dense layer (large arrowheads). Tubular structure with amorphous material are arranged in one or two layers (small arrowheads).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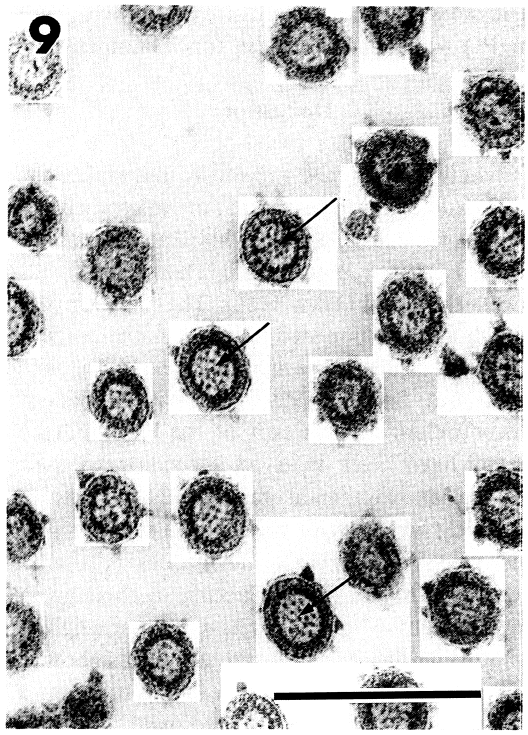
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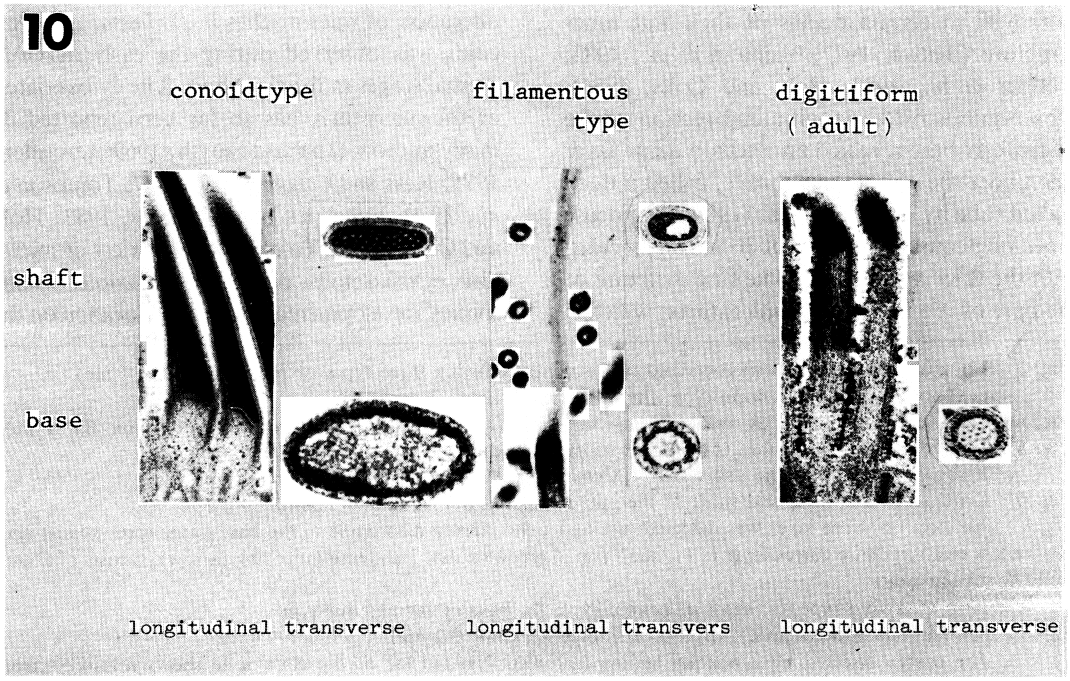
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conoidtype
filamentous type
digitiform (adult)
shaft
base
longitudinal transverse
longitudinal transvers
longitudinal transverse

body surfaces must be taken account for. In the present study, three types of microtriches were found from different developmental stages of one cestode species, *D. hottai,* and each type of microthrix differed in the structures of the base and shaft (Fig. 10). An newly-developed technique for the preparation of electron microscopic specimens will contribute to the detection of a more distinct image of the microthrix cytoskeleton, and it will be interesting to investigate the evolutional comparison between microthrix in cestodes and microvilli in the vertebrate intestine.

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Fig. 8 Three types of microthrix. Comparison of core-microfilamentous structures (Bar: 0.2 μm).
Fig. 9 Adult type of microtriches (8hr PI). Cross section of base (Bar: 0.2 μm). Core-microfilamentous structures are clear (arrows).
Fig. 10 The summarized fine structures of three types of microtriches.