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Anatomical Study of *Chordodes koreensis* in the Parasitic Phase Using Electron Microscopy

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Abstract : Horsehair worms (*Chordodes koreensis)* develop as parasites in the bodies of grasshoppers, crickets, cockroaches, and some beetles. *Chordodes koreensis* is an accidental parasite of humans, livestock, or pets and poses no public health threat. The male of *Chordodes koreensis* in the later larval stage from canine vomitus was investigated by the scanning and transmission electron microscopy. In cross sections, the body wall is composed of four components namely epicuticle, cuticle, epidermis, and muscle layers. The parenchymal tissue fills the rest of the body and surrounds the visceral organs such as intestine, and ventral nerve cord but testes were not found. The epicuticle is a thin superficial layer whose surface shows rows of polygonal elevations called areoles. The cuticle has 17 layers of collagenous fibers spirally wound about the long axis of the worm. The section through the cuticle reveals the layers of large fibers cut obliquely lengthwise, alternating with layers of fibers sectioned obliquely crosswise. The layers of large fiber formed a double helix about longitudinal axis of the worm. The epidermis is a single layer. The muscles were interrupted by the nervous lamella in the only midventral portion. The medulla of muscle plate is composed of lightly stained cytoplasm, mitochondria, weakly developed endoplasmic reticulum, and glycogen granules. Between the medulla of a cell and the plasmalemma lies a broad cortical zone of myofilaments. The circular muscles are absent. The characteristic feature of the cytoplasm is that there was no content in peripheral mesenchyme, but was an abundance of large clear vacuoles which give the cytosome a foamy appearance. The nucleus of mesenchyme is not easily identified in our specimens.

Key words **:** horsehair worm, *Chordodes koreensis,* ultrastructure, cuticle.

Introduction

Nematomorphs, commonly called horsehair worms or gordian worms, have long been exciting to mythologists and biologists. They are distributed world widely and represent a primitive group, with invertebrates, especially insects, serving as a developmental hosts (10,12). Nematomorpha are a defined monophyletic group represented by two sister taxa, freshwater species (Gordiida) and marine species (Nectonema) (7).

The life cycle of horsehair worms includes two different developmental stages, a microscopically small larva in aquatic phase and macroscopic adults in parasitic phase. In Gordiida, the main layer of the cuticle is composed of several layers of fibers, followed by a thin epidermis and longitudinal musculature (1,2,13). The cuticle of worms in Gordiida is very thick and includes an outer epicuticle, which has a various structure depending on the genus and species. The external layer can be either smooth or sculptured by elevations of different shapes and sizes known as areoles. The surface of them often bears apical spines, bristles or pores. They are main tool for nematomorph determination (6,8,11). Schmidt-Rhaesa (9) reported that two main layers (distal and proximal layer) can be distinguished when the cuticle is cross sectioned. The proximal layer is continuous in all regions of the cuticle, while the distal layer differs according to the areolar type.

However, the internal anatomy of gordiid species is a little known. Especially the cuticular structure has major importance for the taxonomy of Nematomorpha. Therefore, the aim of this study is to describe the cuticular ultrastructural characteristics of a *Chordodes koreensis* isolated from canine vomitus.

Materials and Methods

We had been reported the later stage male of *C. koreensis* from canine vomitus both under light and scanning electron microscopy (SEM) (11). The mid part of the worm was cut and fixed with NBF. For SEM, the worm was previously washed with 0.1M phosphate buffer pH 7.4 (PB) and fixed with 2.5% glutaraldehyde in PB at 4°C for 4 hrs. After washing

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with PB, the specimens were post-fixed with 1% osmium tetroxide at 4°C for 4 hrs. The specimens were dehydrated in a graded ethyl alcohol series, dried by CO_2 critical point, coated osmium and examined by SEM (S-4800, Hitachi) at 15 kV. For transmission electron microscopy (TEM), the dehydrated specimens were twice passed in propylene oxide, and embedded in epon mixture. The ultrathin sections were stained with uranyl acetate and lead citrate and examined in a Hitachi H-600 electron microscope at 75 kV.

Results

In the SEM view of cross section, the body wall is composed of four components namely epicuticle, cuticle, epidermis, and muscle layers. The parenchyme tissue fills the rest of the body and surrounds the visceral organs such as intestine, and ventral nerve cord but testes were not found (Fig 1-A,C). The epicuticle is very thin. The cuticle appears thick and is composed of radial fibrillar layers (Fig 1-B). The fibers of proximal layer are comparatively large, and the number of fiber sheets are counted as many as 17 layers (Fig 1-D). Fibers are arranged in one layer parallely aligned with all fibers (Fig 2-A). In the TEM view of cross section, the section through the cuticle reveals the layers of large fibers cut obliquely lengthwise (LF), alternating with layers of fibers sectioned obliquely crosswise (XF) (Figs 1-D, 2-A). The LF layers formed a double helix about longitudinal axis of the worm (Fig 2-A). The fibers were organized into layers in the same planes and with the same helical pitch were the strata of large fibers.

The epidermis is separated from the muscle layer, the dominant cell type is dense and roughly cuboidal. The cytological features are many clear vacuoles near the outer surface of the cell. Between the tonofilaments lie the clear vacuoles which appear to be opened beneath the cuticle. The tonofilaments terminate at the cell boundaries in hemidesmosomes, which are so numerous, especially at the upper surface of the cell, that they are almost a continuous belt (Fig 2-B). Ventral nerve cord is connected to the epidermis by a thin lamella in only midventral portion (Fig 1-C).

The muscle layer consists of longitudinal, thin and long muscle cells, which is connected to the epidermis by a thin lamella in the only midventral portion. Scattered muscle cells can be seen in the mesenchyme adjacent to the ventral neural lamella (Fig 1-C). The musculature of *C. koreensis* was the inner most circular band wall. The unit of layer is flat plate-like cells radially arranged. It has a uniform thickness (about 45 μm) except mid-ventrally (Fig 1-B,C).

The fine structure of the muscle plates is seen in Figs 2-C, D,E. The medulla of each plate is composed of lightly stained cytoplasm, mitochondria, weakly developed endoplasmic reticulum, and glycogen granules (Fig 2-C,D,E). Between the medulla of a cell and the plasmalemma lies a broad cortical zone of myofilaments. An electron lucent matrix, composed of fibrils of collagen, completely isolates each plate (Fig 2-D). Distal to the plates the matrix merges with the basal lam-

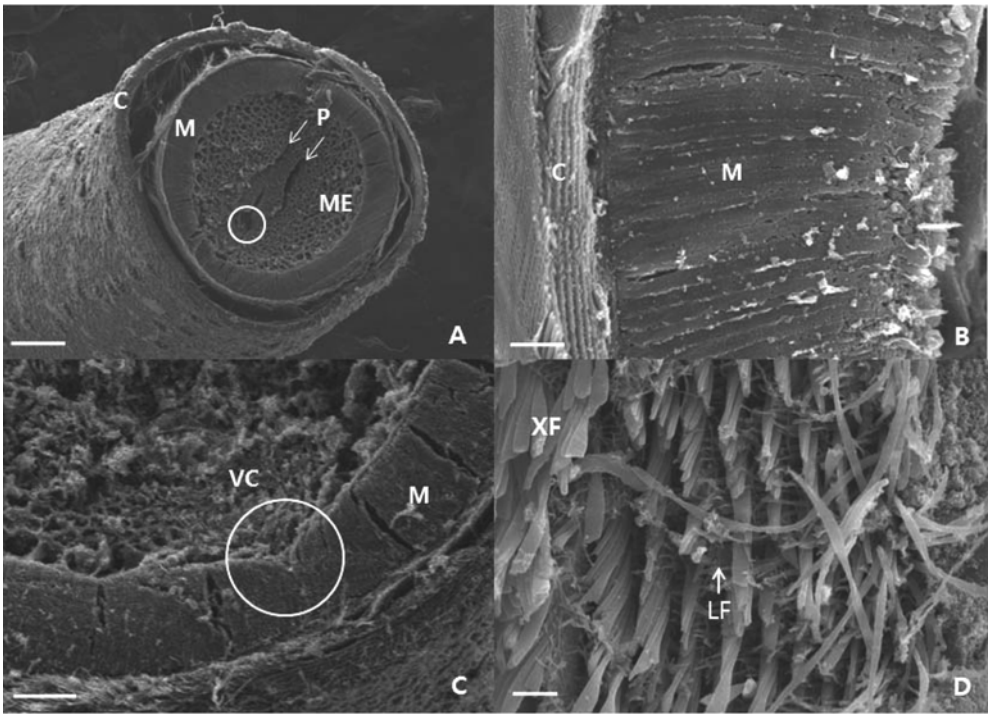

Fig 1. Scanning electron microscopy of the transverse sectioned soma. (A) Whole section of worm. Intestine (circle). Bar = 5 μm. (B) Longitudinal muscle fibers. Bar = 10 μm. (C) The layer of longitudinal muscle fibers interrupted by the nervous lamella (circle). Bar = 100 μm. (D) Cuticular fibers. Bar = 1 μm. *C* cuticle, *LF* large fibers, *M* muscle, *ME* mesenchyme, *P* pseudocoel. *VC* ventral cord, *XF* cuticular fiber cut obliquely crosswise.

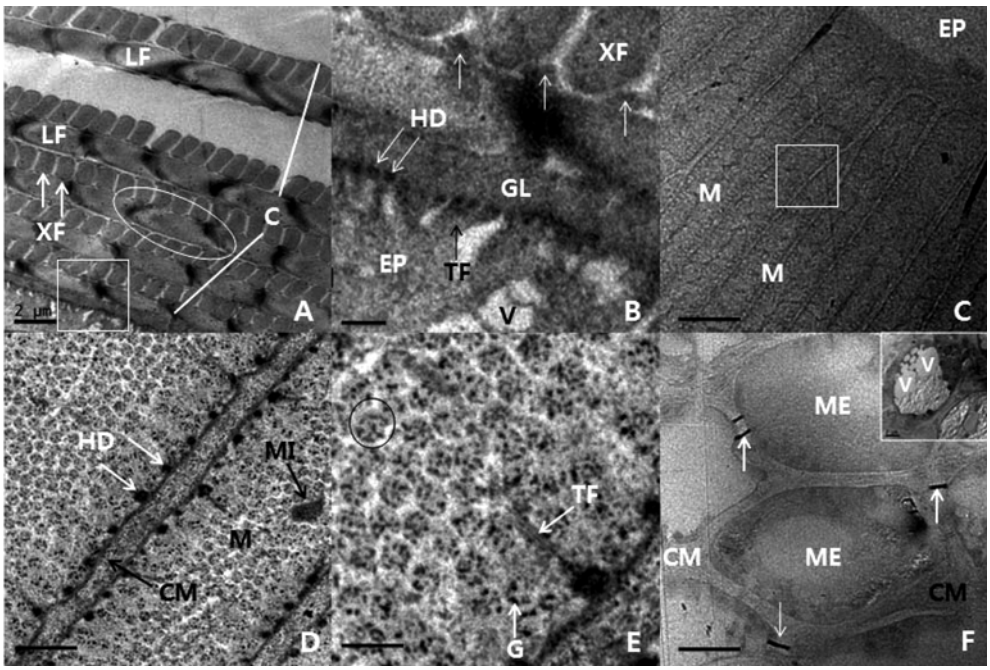

Fig 2. TEM of cross section of *C. koreensis*. (A) Cuticle and part of epidermis near middle part of worm. Single strata of cuticular fibers cut longitudinally. The LF layers formed a double helix (circle). (B) Higher magnification of square in A. Hemidermosomes bind epidermal cell to germinal layer of cuticle. Fibers in a stage of differentation (arrow). Bar = 0.2 μm. (C) Cross section of several muscle plates. Bar = 5 μm. (D) Higher magnification of square in C. The muscle layer situated under the basal lamina of epidermis. Bar = 1 μm. (E) Single myosin filament (cycle), Bar = 1 μm. (F) Peripheral mesenchyme. Arrows indicate fusion of basal lamina of mesenchymal cells. Bar = 5 μm. Square: central mesenchyme. *C* cuticle, *CM* collagenous matrix, *EP* epidermis, *G* glycogen granules, *GL* germinal layer of cuticle, *HD* hemidesmosomes, *LF* cuticular fiber sectioned obliquely longitudinally, *M* muscle, *MI* mitochondria, *ME* mesenchyme, *TF* tonofilaments, *V* vacuoles, *XF* cuticular fiber cut obliquely crosswise.

ina of the epidermis. The matrix about the muscle units is continuous proximally with that enclosing the mesenchyme (Fig 2-C). The muscle plates are anchored on all sides to the matrix by numerous hemidesmosomes which appear in transverse view as spherical or ovoidal densities beneath the plasma membranes of the muscle plates (Fig 2-D). The tonofilaments extend mesiad from the hemidesmosomes into the muscle plates as far as their medullae (Fig 2-E). The glycogen is scattered in the near of collagenous matrix (Fig 2-E).

Internal to the musculature of *C. koreensis* is a mesh of mesenchyme. The characteristic feature of the cytoplasm is that there was no content in peripheral mesenchyme (Fig 2-F), but was an abundance of large clear vacuoles which give the cytosome a foamy appearance. The nucleus is not easily identified in our specimens. Enclosing the mesenchymal cells is a basal lamina, and to that is the collagenous matrix that isolates the mesenchymal cells from one another, except at the points (arrows, Fig 2-F) where two or more cells are separated by only a bridge of basal lamina. The collagenous fibers are arranged in collagenous matrix.

Discussion

We previously identified our Nematomorpha specimen as *C. koreensis* according to the cuticular structures, general shape of posterior end of the male. The cuticle of *C. koreensis* contains the simple, tubercle, bulging, crowned and circumcluster areoles (11). This study shows the structural and morphology characteristics of the *C. koreensis*.

The cuticle of horsehair worms has two structurally different layers, a proximal layer containing fibers and a distal one. The fibers of the proximal layer were arranged in one layer parallely aligned. The number of fiber sheets varies widely according to species and researchers. Also, the cuticle of horsehair worms has two fundamentally different functions. One is for protection during the free-living phase and the other is for absorbing nutrients through the cuticle (9,10). The cuticle of larval stage is replaced towards the end of the parasitic phase by the adult cuticle. In the parasitic phase, it is thin, homogeneous and flexible (9). In the present study, the cuticle of *C. koreensis* appears thick and is composed of radial fibrillar layers. The fibers of proximal layer are comparatively large, and the number of fiber sheets is counted as 17 layers.

Schmidt-Rhaesa (9) reported that the epidermis is very thin, formed by single low cuboidal cells and has a connection with the ventral nerve cord through a thin lamella. The epidermal cells contain numerous filaments connecting the abundant hemidesmosomes on the apical and the basal layer. The epidermis of *C. koreensis* has many clear vacuoles, tonofilaments, and numerous hemidesmosome. The tonofilaments ter-

minate at the cell boundaries in hemidesmosomes, which are so numerous, especially at the upper surface of the cell, that they are almost a continuous belt (Fig 2-B). But we did not find rough ER, mitochondria, and glycogen in epidermis.

In an experimental infection study of *Paragordius varius*, muscle cells of *P. varius* vary in size and shape, and cytoplasm of muscle cells includes the round nucleus, few mitochondria and abundant glycogen at day 10 after infection. At first, muscle cells are small and do not form a continuous sheet. They quickly increase in size and number. At day 30 after infection, the thickness of the sheet of muscle cells is 43 um. The shape of the muscle cells change during development from a platymyarian condition to a coelomyarian condition (9). In the present study, the muscle layer consists of longitudinal, thin and long muscle cells which connected to the epidermis by a thin lamella in the only midventral portion. The unit of layer is flat platelike cells radially arranged. It has a uniform thickness (about 45 μm) except mid-ventrally (Fig 1-B,C).

It is well known that the nematomorphs and nematods share the characteristic musculature, which have only longitudinal muscles differ from Nemathelminthes. Nematomorphs, like nematodes, possess only longitudinal and no circular musculature (1). The longitudinal and circular muscles of worm are detected in a plesiomorphic condition of Nemathelminthes and in all nemathelminth taxa (5). However, Nematoda and Nematomorpha differ in the organization of muscle fibers (3). Although the simple type (platymyarian type) or the more complex type (coelomyarian type) is separated in the structure of muscle fibers, nematodes show uniformally muscle fiber arrangement (3). The musculature of Gordiida is unusual, with ribbon like muscle fibers containing the thick and long filaments of paramyosin and thin filaments (4). In our experiment, the worm possesses only longitudinal musculature and no circular musculature.

In gordian worm, the mesenchymal cells are irregular, stellate, or spindle-shaped cells (2). The most characteristic feature of the cytoplasm, like our experiment is an abundance of large vacuoles which give the cytosome a foamy appearance. Cytoplasmic organelles are not easily identified in this specimen.

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개의 구토물에서 분리한 오디흑연가시(철선충)의 전자현미경을 이용한 해부학적 소견

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요 약 : 철선충은 주로 사마귀와 같은 곤충에 기생하는 선충류로 사람이나 동물은 우연히 감염될 수 있는 것으로 알려져 왔다. 철선충의 생활사는 크게 곤충에 기생하는 기생생활기, 그리고 발육이 완료된 후에 숙주를 죽이고 외계로 나와 자유생활을 하는 자유생활기로 구분할 수 있다. 본 연구는 개의 구토물에서 오디흑연가시 수컷을 발견하여 이의 횡단면을 주사 및 투과 전자현미경으로 관찰한 것이다. 전자현미경하에서 표피는 4층(epicuticle, cuticle, epidermis, muscle)으로 이루어졌고, 내강에는 장, 배신경절이 발견되었다. 그러나 고환은 발견되지 않았다. 표피의 최상층에서 여러 형태의 areoles이 관찰되었으며, cuticle은 17열의 섬유로 이루어져 있었다. Epidermis는 1열로 구성되어 있었으며, muscle층은 여러 개의 근세포가 종으로 일정하게 배열되어 있었다. 그리고 내강에 배신경절이 충체의 정중앙 배측에서만 존재하는 것으로 확인되었다. 이러한 결과로 본 철선충은 기생생활기를 끝내지 못한 미성숙충으로 판단된다.

주요어 : 철선충, 오디흑연가시, 개, 표피.