Scanning electron microscopic observations of *Thelazia callipaeda* from human

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**Abstract:** Four females and a male nematode isolated from 2 patients who visited eye clinics in Seoul were identified as *Thelazia callipaeda* and their ultrastructures were observed by scanning electron microscopy (SEM).

General features of the worms were slender and attenuated at both ends. Vaginal opening was located at 0.27 mm from the anterior end, and in front of the esophageo-gastrointestinal junction. In the body cuticle transverse striations varied characteristicstically through the body. The number of cuticular transverse striations was 400 to 650/mm at head portion, 250/mm at middle portion and 300 to 350/mm at tail portion.

The SEM observation of the mouth part of the females showed 6 cord-like cuticular thickenings in hexagonal arrangement and an amphid was observed. A lateral line, a vaginal opening, a pair of phasmids, and an anus were identified in the body portion. A pair of papillae and 6 cord-like cuticular thickenings were on the mouth part of the male. It was difficult to observe structures at the tail of the male except wrinkle-like structures. Most of the larvae isolated from the uterus of a female worm were sheathed and thus cuticular striations were not seen. Others were unsheathed and revealed cuticular striations. The oval membrane which encysted sheathed larvae was also observed.

These are the 18th and 19th record of human thelaziasis in Korea as the literature are concerned.

**Key words:** *Thelazia callipaeda*, SEM, amphid, phasmid, papilla, transverse striation

**INTRODUCTION**

Genus *Thelazia* is small nematodes which parasitize in the conjunctival sac of mammals and birds. So far about 13 species have been recorded since Rhodes in 1676 first described an eye worm belonging to the genus *Thelazia* (Yamaguti, 1961). Among these species, *T. callipaeda* and *T. californiensis* were known to cause human thelaziasis. The former distri-
microscopic observation. Hong et al. (1988) described the internal structures of *T. callipaeda*, male and female. Surface ultrastructures of this worm were reported by several Japanese researchers (Arizono et al., 1976; Kagei et al., 1983).

This study was performed to observe the ultrastructures of *T. callipaeda* collected from two humans by scanning electron microscopy.

**MATERIALS AND METHODS**

One female worm from a 49-year-old man and 3 female and 1 male worms from a 50-year-old man were obtained. Larvae were drawn out of the uteri of a female worm. To prepare the specimens for SEM observation samples were washed, fixed and dehydrated by conventional methods. Specimens were coated with gold in 70A thickness using an ion sputtering coater, JFC-1100 and were observed by a scanning electron microscope, JSM-1100, at accelerating voltage of 15 KV.

**RESULTS**

A 50-year-old man who lived in Seoul visited Park Sang Yun Eye Clinic, complaining of foreign body sensation and itching of the eye. A female worm was picked out of his left eye. Also a 49-year-old man who lived in Seoul and suffered from the same symptoms visited Oh Young Whan Eye Clinic. He experienced a fly attack into his eye while climbing the Mt. Womyun in Socho-Dong. Four adults and a juvenile worm were isolated. These worms were white, filiform, and attenuated at both ends. Measurement data of the worms are described in Table 1.

The vaginal opening was located at 0.14 mm anterior to esophage-intestinal junction. The number of cuticular transverse striations was 400~650/mm at head portion, 250/mm at middle portion and 300~350/mm at tail portion. Caudal papillae could not be observed. In comparison of morphological characters, the location of vaginal opening and the number of cuticular transverse striations were compatible with *T. callipaeda*.

The SEM observation of the head portion of the females showed narrow transverse striations and smooth region under the mouth opening (Fig. 1). On the inner surface of the mouth

<table>
<thead>
<tr>
<th>Table 1. Measurement data of the worms (dimension: mm)</th>
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<tr>
<td>Structure</td>
</tr>
<tr>
<td>Body: length</td>
</tr>
<tr>
<td>width(max.)</td>
</tr>
<tr>
<td>Buccal cavity: length</td>
</tr>
<tr>
<td>Anterior end to vaginal opening</td>
</tr>
<tr>
<td>Spicule: length</td>
</tr>
<tr>
<td>Anterior end to esophage-intestinal junction</td>
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<tr>
<td>Posterior end to anus</td>
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<tr>
<td>No. of transverse striations/mm</td>
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Figs. 1-11. Scanning electron micrographs of female *T. callipaeda*. (bar; µm)

Fig. 1. Head portion of adult female. (×430)
Fig. 2. Cord-like cuticular thickenings (CD) at the margin of mouth. (×1500)
Fig. 3. Amphid (AM) on the outside of mouth. (×3000)
Fig. 4. Vaginal opening. (×4400)
Fig. 5. Lateral line in middle part. (×2000)
Fig. 6. Tail en face view. PH; phasmid. (×1000)
Fig. 7. Phasmid opening. (×2000)
Fig. 8. Anus. (×1500)
Figs. 9-11. Variation of cuticular transverse striations.
Fig. 9. Striations at anterior part. (×1800)
Fig. 10. Striations at middle part. (×1800)
Fig. 11. Striations at posterior part. (×2000)
opening 6 cord-like cuticular thickenings were arranged hexagonally (Fig. 2) which might be the rudimentary 6 lips of nematodes (Chitwood and Chitwood, 1977). The amphid opening which have been known as a chemical receptor was observed in the outside of the mouth (Fig. 3). It was pocket-shaped.

In the middle portion of the females, the vaginal opening (Fig. 4) and the lateral line (Fig. 5) tracking the body were observed. At the tails of the females a pair of papillae named as phasmids (Figs. 6-7) which are important in categorizing parasitic nematodes into class level and the anus (Fig. 8) were detected. These phasmids projected at the terminal. Their tips were slightly swollen.

The variation of cuticular transverse striations was characteristic not only in the number but also in the morphology (Figs. 9-11). The intervals between transverse striations were getting longer toward the posterior part. The transverse striations came off the cuticle in the posterior part and were frilling. There were also dense corrugations on the transverse striations. They were arranged perpendicularly to the transverse striations.

In the male a pair of head papillae (Fig. 12) as well as 6 cord-like cuticular thickenings (Fig. 13) were found on the inner surface of the mouth opening. The tail of the male was curled and thus caudal papillae could not be shown. They had two adjacent invaginated structures at the posterior part (Fig. 14). Their openings were slightly wrinkled. There were also transverse striations and dense corrugations on the cuticle (Fig. 15).

Most of the larvae which were isolated from the uterus were disc-shaped and sheathed (Fig. 16). As they were sheathed cuticular transverse striations were not found (Fig. 17). Some larvae which were partially unsheathed (Fig. 18) probably by larval movement appeared to be striated on their cuticle (Fig. 19). All the larvae we found were encysted with the oval membrane (Fig. 20) at that moment they were isolated.

DISCUSSION

Arizono et al. (1976) studied on the morphology of adult worms of *T. callipaeda* by scanning electron microscopy. They observed several pairs of the cephalic papillae which had nipple-like shape in the outside of the mouth part. The cord-like cuticular thickenings were seagull-shaped in lateral view. A pair of phasmids were projected at the terminal and had a pore at the center of each phasmid. Kagei et al. (1983) examined the caudal papillae of male worms by light and scanning electron microscopy.

We observed two large head papillae on the mouth opening of the male. They were distinct from the cephalic papillae in their morphology and orientation. They were absent in the females.

Min and Chun (1988) mentioned several wrinkle-like structures in the posterior part of male with light microscope. We also found two adjacent invaginated structures. Their openings were slightly wrinkled.

Compared with previous reports the vaginal

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Figs. 12-15. Scanning electron micrographs of male *T. callipaeda*. (bar; \(\mu m\))
Fig. 12. Mouth part showing a pair of head papilla (P). \(\times 1000\)
Fig. 13. Cord-like cuticular thickenings at the margin of mouth opening. \(\times 3000\)
Fig. 14. Wrinkle-like structures (W) at the tail. \(\times 1000\)
Fig. 15. Cuticular striations at middle part. \(\times 1000\)
Figs. 16-20. Scanning electron micrographs of larvae. (bar; \(\mu m\))
Fig. 16. Sheathed larva. \(\times 1200\)
Fig. 17. Higher magnification of Fig. 16. \(\times 4000\)
Figs. 18-19. Unsheathed larva showing cuticular striations. SH; sheath. (Fig. 18: \(\times 7200\); Fig. 19: \(\times 4000\))
Fig. 20. Oval membrane (OV). \(\times 220\)
opening of this worm was relatively nearer to the anterior end, 0.27 mm, than others, average 0.50 mm (Cho and Choi, 1978; Hong et al., 1981; Min and Chun, 1988). The number of transverse striations varied from 250 to 650 per millimeter according to the portion of the measurement. About 650 per millimeter striations could be discriminated at head portion.

Through the observations of the larvae we identified the sheath which covered intact larvae and the oval membrane which encysted sheathed larvae. All the larvae from the uterus were thought to be encysted with the oval membrane. After they were drawn out of the uterus they moved actively in saline solution, thus they came to be unsheathed. These results supported *T. callipaeda* to be ooviviparous rather than viviparous (Nagada, 1964; Hong et al., 1988).

Reports of human thelaziasis are increasing recently because of environmental and social factors (Lee et al., 1986). Their vectors have not been fully understood in Korea but *Amiota* spp. have been suggested to mediate the infection. In these cases one of the patients stated the existence of neighboring dogs and the other experienced a fly attack into his eye. These statements offered a way to deduce the life cycle of these worms. It would be meaningful to investigate flies as a vector and dogs as a reservoir host.

REFERENCES


인체 기생 Thelazia callipeda의 주사전자현미경적 관찰

기록

가을

향약

오영환


눈에 이물감, 갈락 자극 증상, 소양감 등을 호소하며 서울 지역 안과의원을 내원한 2명의 환자로부터 백색 설모양의 선충 자충 4마리, 응충 1마리 및 유충 여러 마리를 얻었다. 응체들은 동상적인 방법으로 처리하여 주사전자현미경으로 표피 미세구조를 관찰하였다.

응충의 경우, 체장은 11.00 mm, 체폭은 0.18 mm, 교미점의 길이는 0.70 mm, 식도의 길이는 0.35 mm이었다. 자충의 경우 체장은 13.50~18.00 mm, 체폭은 0.20~0.30 mm, 앞 끝에서 vaginal opening까지 0.27 mm, 식도의 길이는 0.41~0.80 mm, 뒷 끝에서 향문까지는 0.062 mm이었다.

특징적으로 체표에 가로로 주름이 있었는데 절단에서는 주름의 갭이 좁고 몸통 부위에서 그 수는 점차적으로 감소하며 표피가 일이나 나폴거리는 형태를 나타내는데 주름의 수는 달이 mm당 250~650개의 범위이었다. 위측정 결과를 통해 본 종은 동향인충(Thelazia callipeda)으로 동정하였다.

본 종의 전체 모양은 구부와 머루에서 관찰한 동충 중앙 부분의 점점 꼭대지는 형태였고 응충의 경우 머루가 가로로 주름이 있었다. 자충에서는 구부에 6개형 구조로 배열된 6개의 큰 모양의 각절 구조의 그 중에서 가장 자극 수용체인 amphid, 몸통 부위에 열출과 vaginal opening, 머루에서 또다른 자극 수용체인 1쌍의 phasmid와 anus의 미세구조를 관찰할 수 있었다. 응충에서는 무수의 큰 모양의 각절 구조 이외에 1쌍의 papillae와 머루에는 수바내어진 형태의 구조물을 확인하였으며 교미점과 caudal papillae는 응충이 1마리이고 그 발달이 되어 있어 확인이 어려웠다.

자충의 자궁에서 얇은 유충은 얇은 막에 서여 있었고 이 퇴출 방어사한 특징적인 가로 주름을 볼 수 있었다. 본 연구의 Thelazia callipeda는 우리 나라에서 보고된 것 중 18번 및 19번째 인체 감염의 예이다.