

## 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 esophago-intestinal junction. In the body cuticle transverse striations varied characteristically through the body. 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.

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-

butes mostly in Asia containing the USSR and the latter in California, Nevada and Oregon of the USA. They have morphological differences such as the location of vaginal opening, the number of caudal papillae and the number of cuticular transverse striations.

In Korea 17 cases of human thelaziasis have been reported and most of the worms were identified as *T. callipaeda* (Oh *et al.*, 1975; Choi *et al.*, 1982; Ryu *et al.*, 1986; Min and Chun, 1988; Hong *et al.*, 1988) through light

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 70Å 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.

**Table 1.** Measurement data of the worms (dimension: mm)

Structure	Worms	
	Male	Female
Body: length	11.00	13.50~18.00
width(max.)	0.18	0.20~0.30
Buccal cavity: length	0.015	0.015~0.020
Anterior end to vaginal opening	—	0.27
Spicule: length	0.70	—
Anterior end to esophago-intestinal junction	0.35	0.41~0.80
Posterior end to anus	—	0.062
No. of transverse striations/mm	250~350	250~650

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 esophago-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

(→)

**Figs. 1-11.** Scanning electron micrographs of female *T. callipaeda*. (bar;  $\mu$ m)

**Fig. 1.** Head portion of adult female. ( $\times 430$ )

**Fig. 2.** Cord-like cuticular thickenings (CD) at the margin of mouth. ( $\times 1500$ )

**Fig. 3.** Amphid (AM) on the outside of mouth. ( $\times 3000$ )

**Fig. 4.** Vaginal opening. ( $\times 4400$ )

**Fig. 5.** Lateral line in middle part. ( $\times 2000$ )

**Fig. 6.** Tail *en face* view. PH; phasmid. ( $\times 1000$ )

**Fig. 7.** Phasmid opening. ( $\times 2000$ )

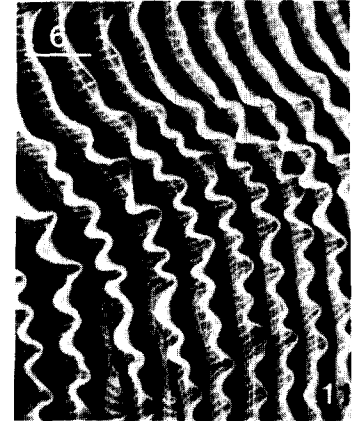
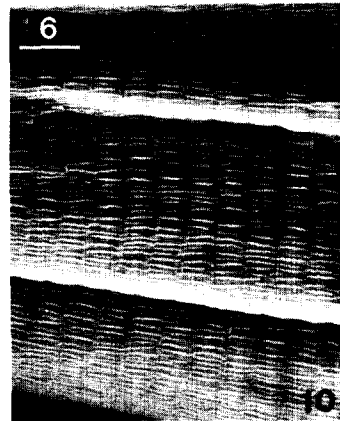
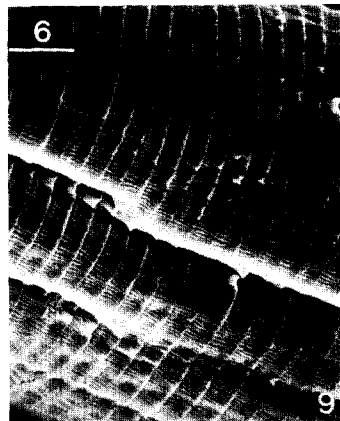
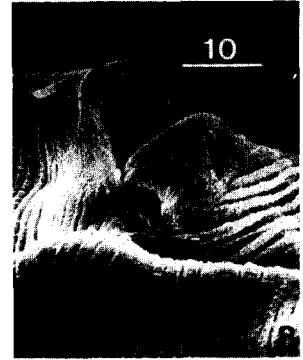
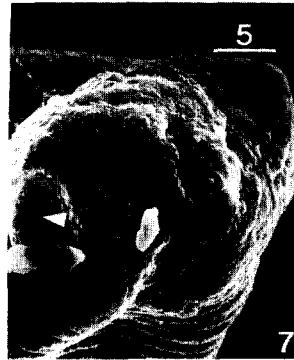
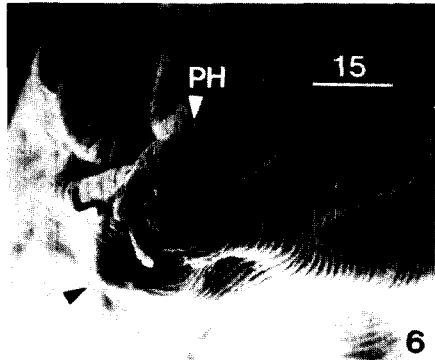
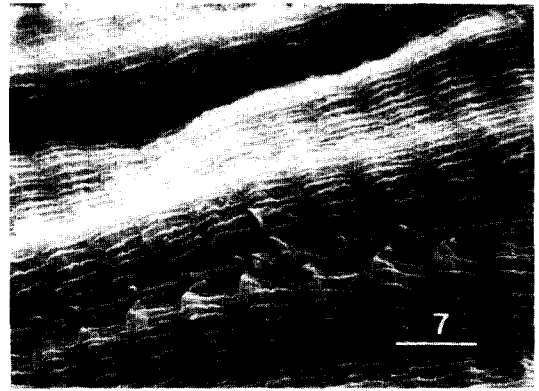
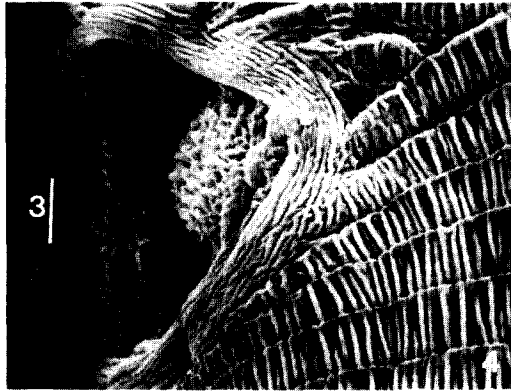
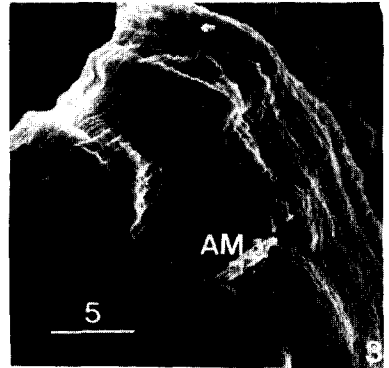
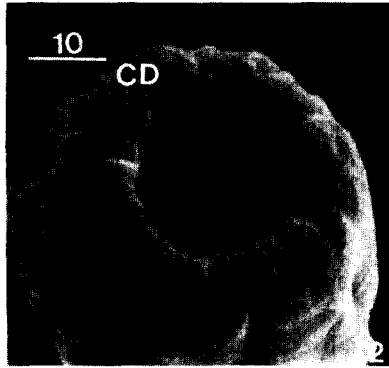
**Fig. 8.** Anus. ( $\times 1500$ )

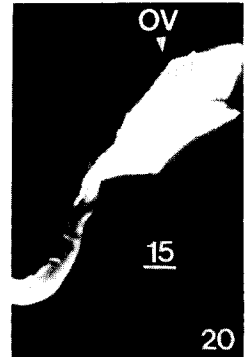
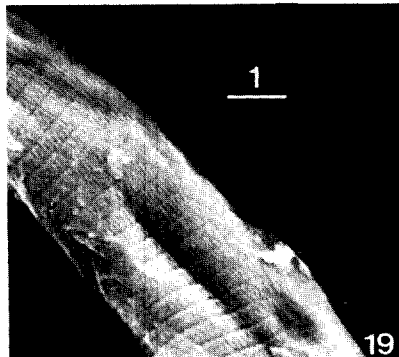
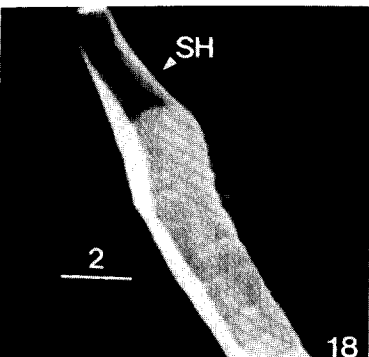
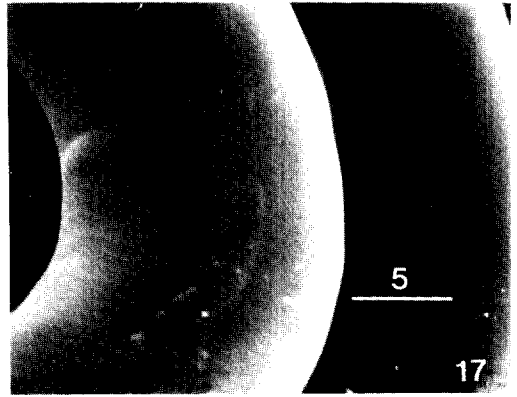
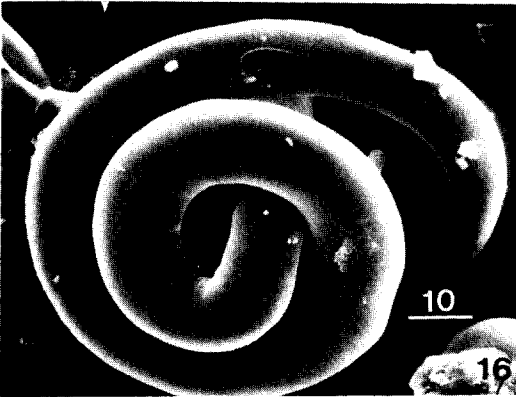
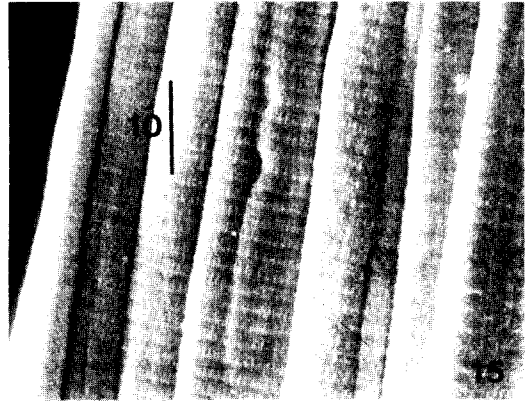
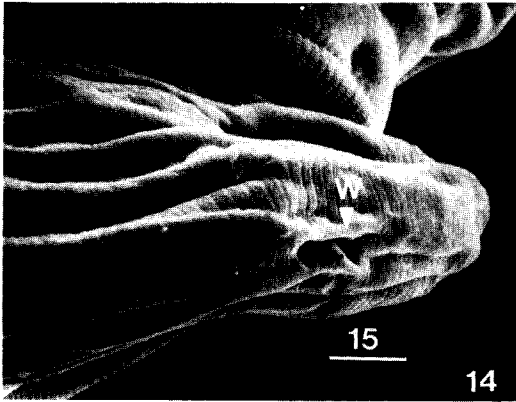
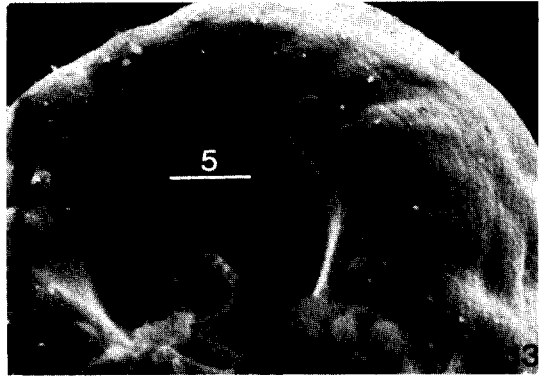
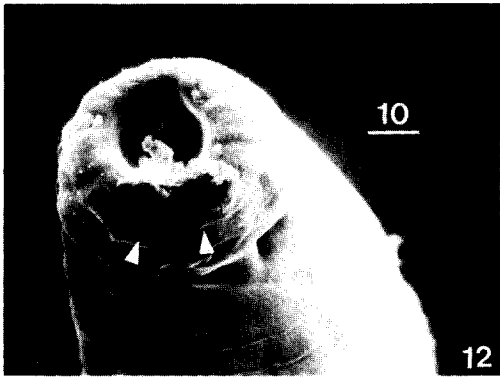
**Figs. 9-11.** Variation of cuticular transverse striations.

**Fig. 9.** Striations at anterior part. ( $\times 1800$ )

**Fig. 10.** Striations at middle part. ( $\times 1800$ )

**Fig. 11.** Striations at posterior part. ( $\times 2000$ )





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

(←)

**Figs. 12-15.** Scanning electron micrographs of male *T. callipaeda*. (bar;  $\mu\text{m}$ )

**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;  $\mu\text{m}$ )

**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 unshathed. These results supported *T. callipaeda* to be ovoviviparous 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.

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### 인체 기생 *Thelazia callipaeda*의 주사전자현미경적 관찰

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눈에 이물감, 결막 자극 증상, 소양감 등을 호소하며 서울 지역 안과의원을 내원한 2명의 환자로부터 백색 실 모양의 선충 자충 4마리, 웅충 1마리 및 유충 여러 마리를 얻었다. 충체들을 통상적인 방법으로 처리하여 주사 전자현미경으로 표피 미세구조를 관찰하였다.

웅충의 경우, 체장은 11.00 mm, 체폭은 0.18 mm, 교미침의 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, 뒤 끝에서 항문까지는 0.062 mm이었다.

특징적으로 체표에 가로 주름이 있었는데 전단에서는 주름의 간격이 좁고 몸통 부위에서 그 수는 점차적으로 감소하며 표피가 일어나 나풀거리는 형태를 나타내는데 주름의 수는 단위 mm당 250~650개의 범위이었다. 위 측정 결과를 통해 본 충을 동양안충(*Thelazia callipaeda*)으로 동정하였다.

본 충의 전체 모양은 구부와 미부에서 가늘고 몸통 중앙 부위는 점점 굵어지는 형태였고 웅충의 경우 미부가 꼬여 있었다. 자충에서는 구부에 6각형 구조로 배열된 6개의 끈 모양의 각질 구조와 그 측면으로 화학적 자극 수용체인 amphid, 몸통 부위에 옆줄과 vaginal opening, 미부에서 또다른 자극 수용체인 1쌍의 phasmid와 anus의 미세구조를 관찰할 수 있었다. 웅충에서는 두부에 끈 모양의 각질 구조 이외에 1쌍의 papillae와 미부에서는 우버내어진 형태의 구조물을 확인하였으며 교미침과 caudal papillae는 웅충이 1마리이고 그 말단이 꼬여 있어 확인이 어려웠다.

자충의 자궁에서 얻은 유충은 얇은 막에 싸여 있었고 이 막을 벗어야만 특징적인 가로 주름을 볼 수 있었다.

본 연구의 *Thelazia callipaeda*는 우리 나라에서 보고된 것 중 18번 및 19번째 인체 감염의 예이다.