대한의생명과학회지: 제5권 제1호 1999
Korean J. Biomed. Lab. Sci.
Vol. 5, No. 1, p.41~49, June, 1999

# Scanning Electron Microscopy of *Thelazia callipaeda* Railliet and Henry, 1910 in the Eye of a Dog

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**Abstract:** We isolated 7 oriental eye worms, *Thelazia callipaeda*, from right eye of a military dog that had been reared in the military base of Namyangju-Gun, Kyungki province in May, 1999. Some of them were observed with light microscope after fixation and mounting with lactophenol, the others with scanning electron microscope after fixation with glutaraldehyde. As the result, the morphological differences of head part of females and males, tail part of females and males, cuticular characteristics of the surface and location of vaginal opening, we identified of these worms with *T. callipaeda.*

**Key Words:** *Thelazia callipaeda*, Dog, Eye, Scanning electron microscope

## INTRODUCTION

Oriental eye worm, *Thelazia callipaeda*, as the first case of human thelaziasis in Korea was found at the conjunctiva of a girl lived in Hwanghae province in 1927 and has been reported in 1934[13]. According to Yamaguti (1961)[22], 13 species belong to genus *Thelazia* and *T. callipaeda*[19] is the main cause of human infection. Many cases of human thelaziasis have been reported in Korea[1,6,7,8,10,11,13,16,21]. Surface ultrastructure of oriental eye worm from the human eye that had entered a private ophthalmology clinic in Seoul area was shown and reported as the 18th and 19th human infection cases (Choi *et al.*, 1989)[6]. Hong *et al.* (1995)[8] reported the 23th and 24th human infection cases using scanning electron microscope after they had collected the oriental eye worms from 7-month-old infant lived in Uijungbu-City, Kyungki province and 42-year-old man who lived in Anyang-City, Kyungki province. Oriental eye worm belongs to nematodes and parasitize in the conjunctival sac and the lachrymal duct of dog, cat and mouse[13]. The intermediate host of this parasite is known to be small insects such as fruit-fly but it is not clear[7]. When this insects contact with the eye of animals such as a dog and suck the eye discharge and the tears, larvae enter the insects, grow into a infectious type and are transmitted to the 3rd host[2]. According to Choi *and* Cho (1978)[5] 31 adult worms were collected from a military dog after oriental eye worms were found in the eye of the soldier at the military base in the suburbs of Seoul, but the insect vector expected were not found. The reports of human thelaziasis are increasing recently,

* Received : May 4, 1999
Accepted after revision : June 15, 1999
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**Table 1.** Measurements of male and female of *Thelazia callipaeda* from dog

| Structure | Male (mm) (3 worms) | Female (mm) (4 worms) |
|---|---|---|
| Body: Length | 8.93~10.32 | 11.15~12.84 |
| Width | 0.29~ 0.34 | 0.28~ 0.37 |
| Buccal cavity: Length | 0.023~0.024 | 0.023~ 0.025 |
| Anterior end to nerve ring | 0.290~0.310 | 0.300~ 0.320 |
| Anterior end to vaginal opening | | 0.480~ 0.590 |
| Anterior end to esophagointestinal junction | 0.570~0.600 | 0.590~ 0.620 |
| Number of cuticular striations/mm: | | |
| Anterior portion | 274~280 | 235~249 |
| Middle portion | 196~201 | 150~160 |
| Precloacal papillae in male | 8 pairs | |
| Spicule: Left | 1.850~1.950 | |
| Right | 0.155~0.166 | |

especially in the field of ophthalmology. It seems that the human infection may be distributed nationwide in Korea[1,5,6,7,8,10,11,13,16,21].

We reported some of the morphological characteristics of oriental eye worms isolated from the eye of a military dog that had been reared at the militaries in Namyangju-Gun, Kyungki province by the light microscope and scanning electron microscope.

## ISOLATION AND FIXATION OF WORMS

We observed the eye-wax and found the worms like a white thread in the right eye of a 2-year-old military dog, the dog showed severe hyperemia and inflammation. Four females and three males worm that were milky and filiform were isolated from upper conjunctiva of the dog. Among them one female and one male worm were fixed with 2% glutaraldehyde solution for scanning electron microscopic observation. The others were fixed with 70% ethanol immediately after isolation. And then we observed them with light microscope and scanning electron microscope.

**Table 2.** Measurements of larvae of *Thelazia callipaeda* in uterus of worm

| Structure | Size (μm) |
|---|---|
| Encyst larvae, diameter | 89.6~102.4 |
| Mouth: Length | 5.12 |
| Width | 5.88 |
| Body: Length (without sheath) | 345.6~448.0 |
| Width (with sheath) | 20.5~ 25.6 (without sheath 12.8~ 15.4) |
| Sheath length | |
| from anterior part to head part | 12.8~ 23.0 |
| from posterior part to tail part | 140.8~153.6 |
| Immature egg in uterus: | |
| Length | 48.9~ 56.3 |
| Width | 28.2~ 33.28 |

*Ten larvae were measured

## RESULTS

### 1. Observation through light microscope

Three males and four females of *Thelazia callipaeda* collected from the dog's eye their

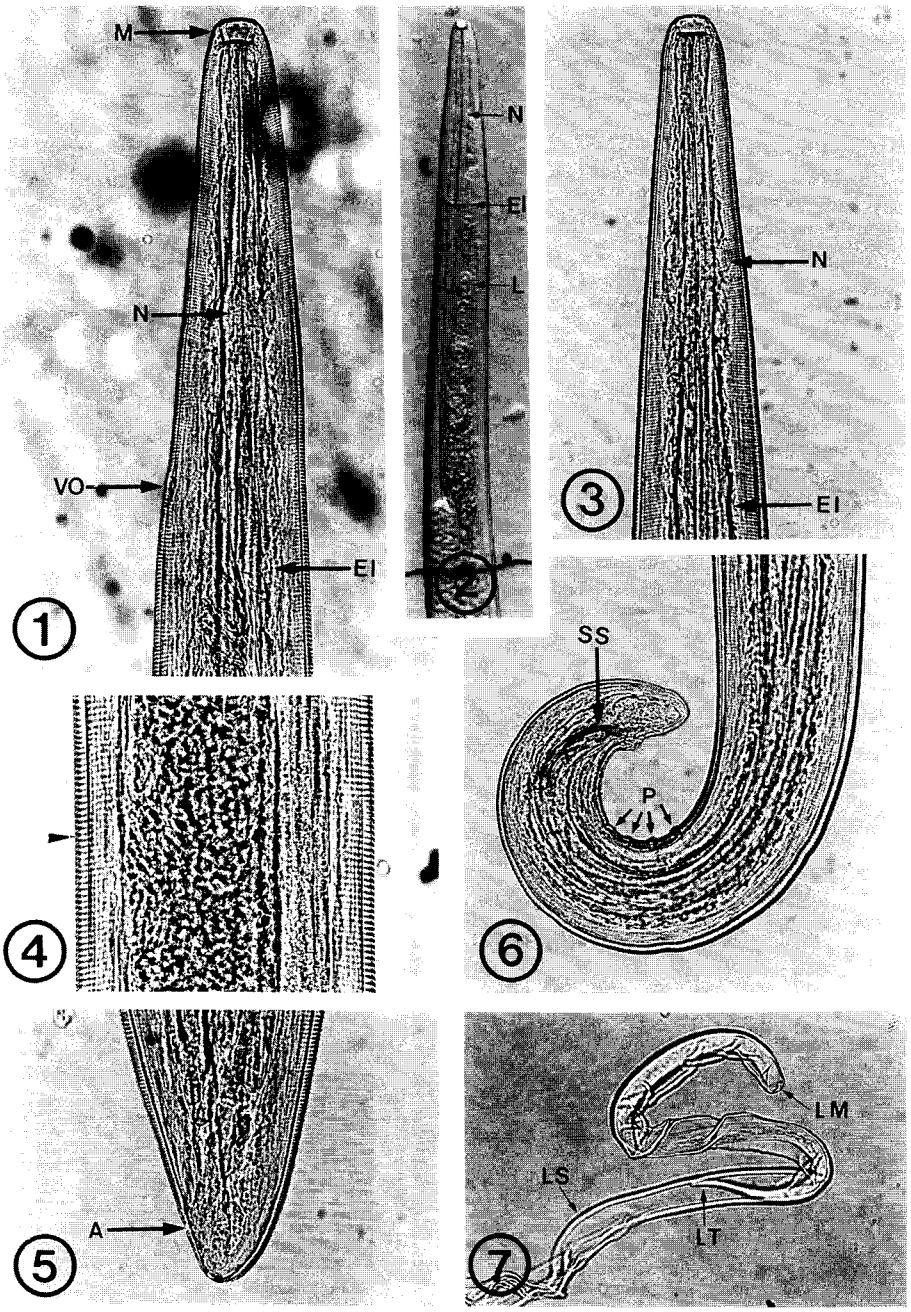


**Fig. 1-6.** Male and female worms of *Thelazia callipaeda* removed from the case. **Fig. 1.** Anterior part of the female worm showing mouth part, esophagus, nerve ring portion, vaginal opening and uterus. (X 100) **Fig. 2.** Anterior part of the female worm observed by steroscope. (X 40). **Fig. 3.** Anterior part of the male worm showing mouth part. (X 100) **Fig. 4.** Partial magnification of figure 1 showing (arrow) cuticular striations. (X 400). **Fig. 5.** Posterior end of the female worm. (X 100). **Fig. 6.** Posterior part of the male worm showing precloacal papillae and a part of spicule. (X 100). **Fig. 7.** Exuviated larvae in uterus of *Thelazia callipaeda*. (X 4500).

Abbreviation: A, anus; EI, esophago-intestinal junction; L, larva in uterus; LM, mouth part of larvae; LS, sheathed of larvae; LT, tail part of larvae; M, mouth part; N, nerve ring ; P, precloacal papillae; SS, cloaca with spicule; VO, vaginal opening.

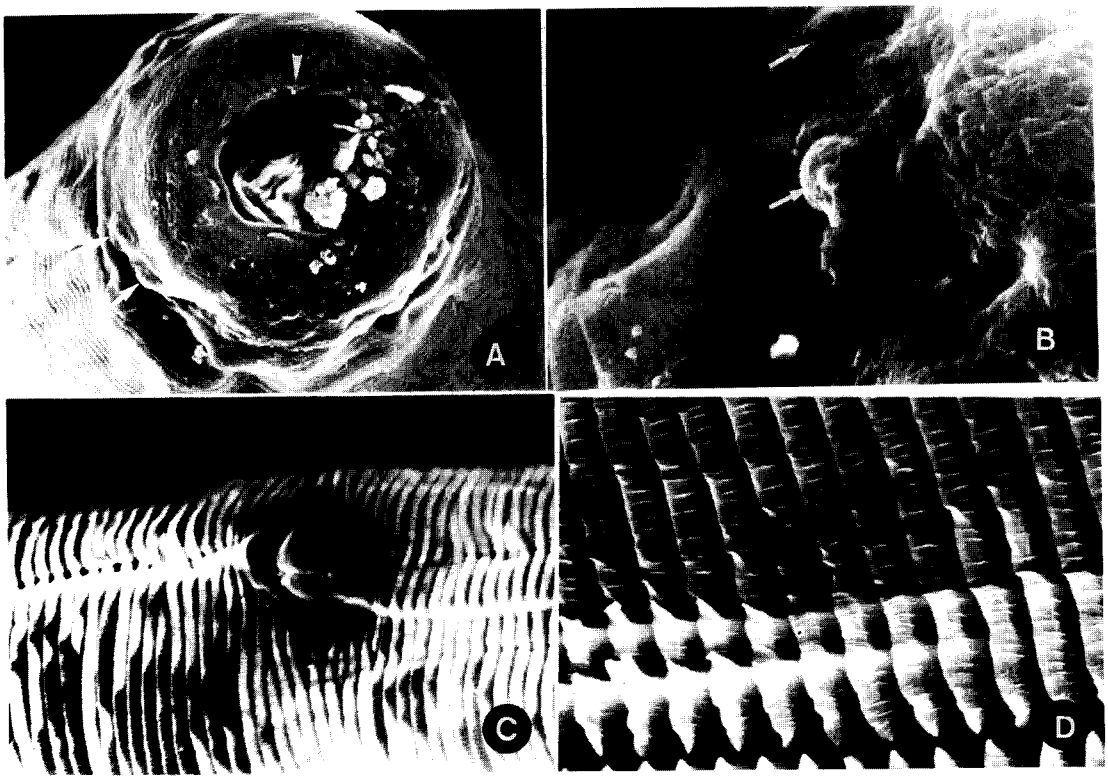


**Plate A-E.** Scanning electron micrographs of female *T. callipaeda*.
**Plate A.** Cord-like cuticular thickenings (▶) and cephalic papillae (→) at the margin of mouth (X 1,500).
**Plate B.** Cephalic papillae (arrow) at the margin mouth (X 5,000).
**Plate C.** Vaginal opening (X 1,000).
**Plate D.** Transverse cuticular striations at the middle portion of a female (X 2,000).

color were white and filiform. The size of male and female worms were 8.93~10.32×0.29~0.34 mm and 11.15~12.84×0.28~0.37 mm, respectively. In the buccal cavity, the size of male and female were 0.02 mm and 0.03 mm, respectively. The length from anterior end to an esophago-intestinal junction of male and female were 0.57 to 0.60 mm and 0.59 to 0.62 mm each (Table 1; Fig. 1, 2 & 3).

Male worm typically had 8 pairs of the irregular sized precloacal papillae (Fig. 6) and 3 to 4 pairs of postcloacal papillae in the cloacal opening. The cloacal papillae on one side were clearly showed by light microscope. The papillae were arranged on both sides of the worm symmetrically. The size of papillae showed a little differences but precloacal papillae were observed more clear than postcloacal papillae (Fig. 6). The spicule was concave or protruded from the body. The size of left longer spicule and right shorter spicule were 1.85 to 1.95 mm and 0.16 to 1.17 mm, respectively (Fig. 6). The vaginal opening of female worm was located anterior to esophago-intestinal junction, and the length from anterior end to vaginal opening was 0.48 to 0.59 mm (Fig. 1). Transverse cuticular striations was arranged in the mid-portion of the body surface regularly (Fig. 4). Female worms have 196 to 201 transverse cuticular striations and male worms have 150 to 160 cuticular striations, so female had striations more than male. Uterus was filled with the encysted larvae which was coil form and covered with thin membrane (Fig. 1). The encysted larvae were disc-shaped in uterus and the diameter was 89.6 to 102.4 μm (Table 2).

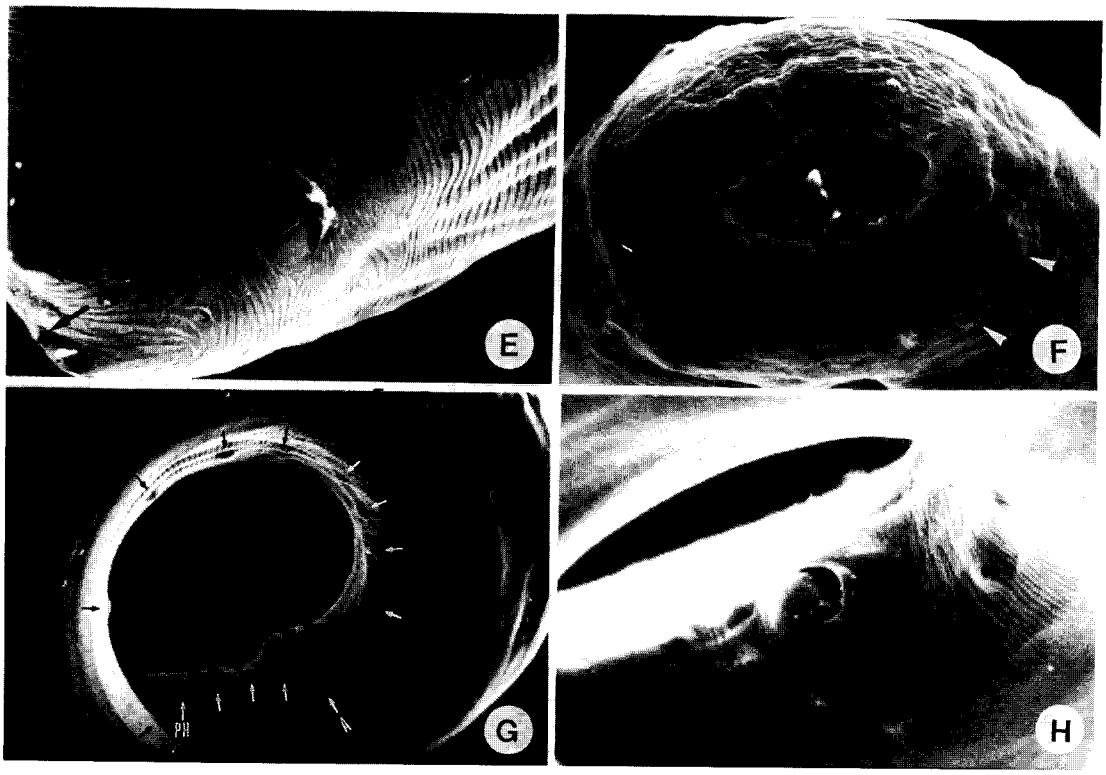


**Plate F-H.** Scanning electron micrographs of male *T. callipaeda*.
**Plate E.** Tail portion of a female *T. callipaeda*. P, papillae; PH, phasmid (X 1,000).
**Plate F.** Cord-like cuticular thickenings and cephalic papillae (arrow) at the margin of mouth (X 2,000).
**Plate G.** Tail portion of a male of *T. callipaeda*, showing 9 pair of precloacal papillae, 1 pair of adcloacal, 3 pair of postcloacal and phasmid. A, adcloacal; PH, phasmid (X 500).
**Plate H.** Higher magnification of the plate G (X 1,000).

The larvae had sheath covering with them and had not transverse cuticular striations in the surface after removing its oval membrane (Fig. 7). Particularly the sheath was longer than the tail portion and its length was 140.8 to 153.5 μm (Table 2; Fig. 7).

### 2. Observation by scanning electron microscopy

The inner surface of the mouth opening at the head portion of the female and male showed deep and six-angular shaped buccal cavity (Plates A & F). The V-shaped esophageal lumen was also observed at the lower part of the buccal cavity (Plate F). Six cord-shaped cuticular thickenings were observed on the inner surface of the mouth and a pair of papillae was observed on the outside of the mouth opening (Plates A & F). Vaginal opening looks like pocket locating in the lateral part of the body (Plate C). Cuticular transverse striations were distributed in the left and right sides of vaginal opening like armor and the transverse slit at the center of the vaginal opening was digged like a ravine (Plate C). The body surface was surrounded with cuticular transverse striations like armor. It looks like the tiles continuously arranged on the roof (Plate D).

In the female worms, the end of tail were obtuse and a anus was opened (Plate E). A pair of papillae named as phasmids were observed in both sides of the end of tail and a nipple-shaped form was projected to the upper ventral part of the anus (Plate E). In the male

worms, the tail was curled toward ventral and several papillae and a cloaca were observed at the tail portion (Plates G & H). The cloaca was opened toward ventral part and 9 pairs of precloacal papillae was arranged on the anterior part of cloaca (Plates G & H). A pair of adcloacal papillae were observed at the both sides of cloaca and 3 pairs of postcloacal papillae at the posterior part of cloaca (Plate G and H). A pair of phasmids were found at the end of the tail and its center of phasmidal gland was opened (Plate G and H).

## DISCUSSION

*Thelazia* belongs to nematodes was first found in the eye of a cow by Rhodes and named the parasite *Thelazia de Rhodes* by Bosc (1819)[4]. Among genus *Thelazia, T. callipaeda* and *T. californiensis* were known to cause human thelaziasis[18,20,22]. *Thelazia callipaeda* parasitize in the conjunctival sac or the lachrymal gland of a vertebrate such as dog, rabbit, cat, badger, rat and monkey[22]. Human case was first reported four worms from the eye of a 25-year-old chinese man by Stuckey in 1917[20]. This parasite was mostly found in dog, deer and sheep in California, Nevada and Oregon of the USA and had been frequently reported as a human infection cases[18]. Two species causing thelaziasis have morphological differences such as the location of vaginal opening, the number of caudal papillae of male, and the number of cuticular transverse striations[3,18,19].

The morphological characteristics of oriental eye worm had known the number of caudal papillae arranged in the anterior and posterior part of cloaca of the male[9]. Kagei *et al.* (1983)[9] reported that this worm had 8 pairs of precloacal papillae, a pair of adcloacal papillae in, 3 to 4 pairs of postcloacal papillae and a pair of phasmid in the caudal part. Bhaivulaya *et al.* (1970)[3] reported that vaginal opening of *T. callipaeda* was located in the anterior part of esophago-intestinal junction. Price (1930)[18] reported that vaginal opening at *T. californiensis* was located at the posterior part.

We observed the vaginal opening of female was located in the anterior portion of esophago-intestinal junction (Figs. 1 & 2) and a number of encysted larvae in uterus. By the scanning electron microscopical examination, the head of male and female, 6 cord-like cuticular thickening was observed on the margin of mouth opening (Plates A & F). This morphological characteristics was the same with result of Arizono *et al.* (1976)[2], Chai *et al.* (1989)[6], Hong *et al.* (1995)[8] and Naoki *et al.* (1975)[15].

A pair of nipple-shaped papillae were observed in the exterior and the lateral part of the head (Plates A & F), and that also coincides with the result of above authors. These nipple-shaped papillae are among 4 pairs of papillae arranged around the head of male and female and have been known as a chemical receptor together with a pair of slit-shaped amphids having a pore in both sides of the head.

The terminal of tail in female was blunt and a pair of phasmids with a opening were observed at the anal and the tail part (Plate E). Arizono *et al.* (1976)[2] have been reported the same structures and to be known a kind of phasmid.

In this study, the morphological characters of tail part in male were clearly observed.

In tail of male, anterior, lateral of cloaca and posterior of cloaca of papillae were observed nine pairs, one pair and three pairs (Plates G & H), respectively. A pair of phasmids with pore had observed at tail end (Plate H). These results were the same about the tail of oriental eye worm observed by Arizono *et al.* (1976)[2], Chai *et al.* (1989)[6] and Hong *et al.* (1995)[8].

The cuticular transverse striations of female and male investigated in this study were also important morphological characters of oriental eye worm[5,7].

The life cycle of genus *Thelazia* has not been manifested clearly. Nakada[5,14] reported that intermediate hosts, *Amiota variegata, A.*

*magna* and *Amiota*. sp. were known as the intermediate host in Japan and among them *A. variegata* was suitable host for experiment[5,14]. Fruit-fly is infected by encyst larvae when the fly takes in tears of dog that was infected with oriental eye worm. The encyst larvae taken are changed to larvae in the stomach of the fly, grow after migration from digestive organ to reproductive organ and arrive at the head through the abdominal cavity and thorax of fly. Human being and dogs are infected with the 3rd stage larvae (filariform larva) from the fly when the fly attacks into the eye of a human or a dog. It takes about ten to twenty days to grow into the infectious type in the intermediate host. The larva infected to the final host is known to grow into a adult worm molting twice in the conjunctival sac or the post-pouch of nictitating membrane for about 4 weeks[5,14].

A dog is considered as the most important reservoir host causing human thelaziasis epidemiologically. According to Okamura *et al.* (1965)[17] when they investigated 407 domestic dogs in Gumamoto area, the thelaziasis epidemic area, Japan, 17% among the dogs had been infected with oriental eye worm and the infection rate between the districts was so different. The infection rate was in the range of 0% to 64%. In Korea, the cases of human thelaziasis were reported several times but there was very few research for vectors to mediate the infection. Choi *and* Cho (1978)[5] reported that they found the case of human thelaziasis from soldiers in the suburbs of Seoul, and isolated a oriental eye *T. callipaeda* as a result of investigate ten dogs in the village including the militaries and one dog in the militaries. The military dog was presumed as a reservoir host because of being closely concerned with soldiers, so human infection mediated by the dog needs to be prevent. The infectious state of oriental eye worm which infect to human as well as animal will be investigated more widely. Especially, infectious state of insects as a intermediate host and the life cycle of these worms will be studied further more.

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=국문초록=

## 군견 안부위에 기생한 동양안충에 대한 주사전자현미경적 관찰 소견

연세대학교 보건과학대학 임상병리학과, 원주의과대학 기생충학교실* 및 내과학교실**

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저자들은 1999년 5월에 경기도 남양주군 군부대에서 사육하고 있는 한 마리의 군견 오른쪽 안부위에서 7마리 (암놈 4마리, 수놈 3마리)의 동양안충을 검출하였다. 이 중 일부는 고정과 lactophenol 액으로 봉입한 후 광학현미경으로 관찰하였으며 일부는 glutaraldehyde액에 고정하여 주사전자현미경으로 관찰하였다. 충체의 암놈 및 수놈의 두부와 꼬리부위의 형태 특징 그리고 충체 표피의 각질, 암놈 음문의 위치 등을 관찰한 결과 동양안충으로 동정하였다.

**[대한의생명과학회지 5(1): 41-49, 1999년 6월]**

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