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Surface Topography of Larval and Adult *Trichinella spiralis* by Scanning Electron Microscopy*

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Introduction

The light microscopical morphology of *Trichinella spiralis* has been published by many investigators[3,4,7,8,11,12]. The ultrastructural findings of the parasite as determined by means of transmission electron microscope have been described extensively by several workers[1,2,6,10,12]. Recently, the surface morphology of larval and adult *Trichinella spiralis* studied by scanning electron microscopy has been reported by Kim and Ledbetter[5].

The author has studied the topographical relationships between the light microscopical findings and the ultrastructural ones concerning larval and adult *T. spiralis* using phase contrast microscope, scanning electron microscope and transmission electron microscope. The present paper reports firstly some observations on the fine structure of larval and adult of *Trichinella speralis* made by means of the scanning electron microscope.

Materials and Methods

The strain of *Trichinella spiralis* (Owen, 1835) Railliet, 1891 used was obtained from a polar bear, *Thalarctos maritimus* in 1968, and has been maintained in Hartley guinea pigs in the same manner as described in the previous report[9]. Hartley guinea pigs were inoculated with 1000 of muscle larvae at least 3 months prior to their being used for obtaining sufficient number of muscle larvae. The muscle was collected from the infected animals sacrified. The well-minced muscle was digested for 2 hours at 37°C in an artificial gastric juice, agitated on a magnetic stirrer. The digestant was passed through 100- and 250-mesh sieve and was allowed to settle for 15 minutes. The fluid was centrifuged at 1000 rpm for 5 minutes. The larvae were washed by alternating repetitions of centrifugation and dispersion in Hanks' solution. The procedures were repeated five times for the clearing of admixture. The cleaned larvae were used for scanning electron microscopy.

Wistar rats were inoculated orally with approximately 1000 muscle larvae. The rats were killed after starvation for 24 hours 7 to 12 days after infection. The entire small intestine was removed, slit longitudinary, and washed in warm physiological saline solution to remove the contents. The contents were washed out of small intestine with Hanks' solution using pipettes, and were treated in a solution of 0.2% trypsin in Rinaldini solution at pH 7.4 and 37°C for 30 minutes. The worms which were made to pass through a modified Baermann apparatus were collected. The

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worms were washed three times by alternating repetitions of sedimentation and dispersion at intervals of 10 minutes in centrifugation tube (40 ml in capacity) with Hanks' solution. The cleaned larval and adult parasites were immediately fixed in 1% cold glutaraldehyde solution with phosphate buffer at pH 7.4 for 2 hours. After rinse in buffer solution, the worms were followed by post-fixing in 1% osmium tetroxide solution with phosphate buffer at pH 7.4 for 2 hours, and were washed three times in the phosphate buffered solution.

The fixed specimens were dehydrated in a graded series of ethanol solution by routine method and were followed by three changes of pure isoamyl acetate. The specimens in pure isoamyl acetate were dried by the critical point method using liquid CO_2. The dried worms were placed on the block covered with double-sided adhesive tape or silver-paste, sputter-coated with gold, and observed at accelerating voltages of 5 to 30 kV. A part of the fixed worms were cracked in liquid N_2. The dried specimens were prepared by the treatment as described above. The cracked surface of the worms was observed for an understanding of the superficial findings of the worms.

Results

1. Muscle larva

The transverse striations at intervals of 0.7 to 1.7 μm were recognized on the surface of the body of muscle larva excepting the anterior and posterior ends. Shallow, longitudinal grooves run in parallel with the body axis on each of ventral and dorsal sides of the body. The lateral cords were distinguished on the lateral surface. Any of opening and depression was not found on the lateral surface. The oral opening is slit-like, about 1.5 μm in width. The anterior end is domical and free of transverse striation. The tip of the cephalic dome is slightly depressed below the surface. Mouth opens in the center of the depression, and is slit-like and 1.5 μm in width. A tiny stylet is sometimes seen protruding from the oral opening. The posterior end of female muscle larva is round, smooth and free of transverse striation. Anus is slit-like opening fringed in diameter of about 2.2 μm. A small appendage and tiny projection were found on each lateral side of the posterior of male muscle larva. The cloacal opening of the male larva appears to be similar to the anal opening of the female larva.

2. Adult intestinal worm

The transverse striations at intervals of 0.6 to 1.8 μm are recognized on the cuticular surface of the entire body excepting the anterior and posterior ends. A shallow, longitudinal groove runs in parallel to the body axis. Besides, longitudinal ridges are ranged perpendicular to the transversal striations at short and regular intervals on the cuticular surface. A number of crateriform depressions or pores in pairs are lined on the lateral surface. In the anterior part, those depressions are shallow and appear to be round, opaque patches. The pores in the center of each of the patches are tiny and indistinct. In the observation of cracked surface of the worms, the depressions are recognized forming two lines on the lateral cord containing a tubular structure. Mouth opens at the tip of the cephalic dome. The oral opening is flanked by two liberate elevations having two small depressions on each of them. The bilateral elevations are situated on a gentle bulge having a pair of amphids and ten small depressions which consist of a pair of lateral mostouter labial ones, two pairs of labial ones and two pairs of cephalic ones. The amphid is situated posterior to the lateralmost outer labial pore. A taper stylet protrudes from the oral opening.

In adult female, a vulva opens on the ventral side at the distance of about one-fifth of the

body-length from the anterior end. The vulval opening is transversal slit about 10 μm in width, and is surrounded by smooth elevation of cuticle.

At the posterior end of adult female, a pair of well-developed copulatory appendages protrude from lateral side of the posterior end. Four papillae consisting of two dorsal conical papillae and two ventral tubercles are situated in an area between copulatory appendage and cloacal opening. In some of the male worms, the copulatory bell (cloacal sheath) is noted everted through cloacal opening.

Discussion

There are many reports dealing with the light microscopical findings on the structures of adult and larval *Trichinella spiralis*. Several investigators observed the ultrastructures of muscle larva and intestinal adults by transmission electron microscopy for extending the light microscopical findings. The scanning electron microscopy, however, has been published only by Kim and Ledbetter[5]. They found a stylet elevating through the oral opening of the adult worms, although it has been assumed by some investigators[3,12] that the stylet seen in the larvae have been disappeared in the intestinal adults. In the present observation, also the stylets protruding through the oral openings of both larval and adult worms were recognized. The author (1979) described previously that a number of crateriform depressions were recognized arranged in two rows on the lateral surface of intestinal adults by the scanning electron microscopy[10]. In the present experiment also, those depressions which were lined in two rows on the lateral surface of intestinal adults were confirmed. Richels[8] reported that the opague structures stained by silver-staining method were noted lined on the lateral side of 2 days-old intestinal worms. The silver-stained structures have been recognized lined on the lateral side of the intestinal adults using light microscope by Cobb[4], Chitwood[3] and others, too. Kim and Ledbetter[5] reported that the presence of hypodermal gland cell openings, or pores, in the lateral cords was a constant feature of the adult of both sexes, although they were absent in the muscle larva. They assumed that the impregnated precursors of the gland cells developing below the larval cuticle were made to be visible by Richels' silver staining. The author is going to make a report on the transmission electron microscopical findings of the crateriform depressions considered to be the openings of hypodermal gland cells in the next paper.

Summary

The superficial structure and cracked surface of adult and larval *Trichinella spiralis* (Owen, 1835) Raillet, 1891 were observed by use of scanning electron microscope.

The transverse striations are seen at intervals of 0.7 to 1.7 μm on the cuticle of muscle larva. A tiny stylet protrudes from oral slit situated at the tip of cephalic dome. The posterior end of female muscle larva is round and smooth, and has an anus at its center. A small appendage and tiny projection are on each of the lateral sides of posterior end of male muscle larva. A cloaca opens on a tip of the posterior.

The transverse striations are seen at intervals of 0.6 to 1.8 μm on the cuticle of the adult worms. Besides, longitudinal ridges are closely ranged perpendicular to the transverse striation at short and regular intervals on the cuticular surface. A number of crater-shaped depressions, which are considered to be the openings of hypodermal gland cells, are ranged in pairs on the cuticle situated upon the lateral cord. The mouth of adult worms is slit-like, flanked with the bilateral elevations

surrounded by a gentle bulge. There are a pair of amphids and ten depressions on the cephalic dome. The vulva is transversal slit about 10 μm in width, surrounded with cuticular elevation, and situated on the ventral side about one-fifth of body length from the anterior end. There are a pair of copulatory appendages, four papillae or tubercles, two pairs of protuberances on the posterior end of adult male. Sometimes, the copulatory bell is recognized everted from the cloaca of the male.

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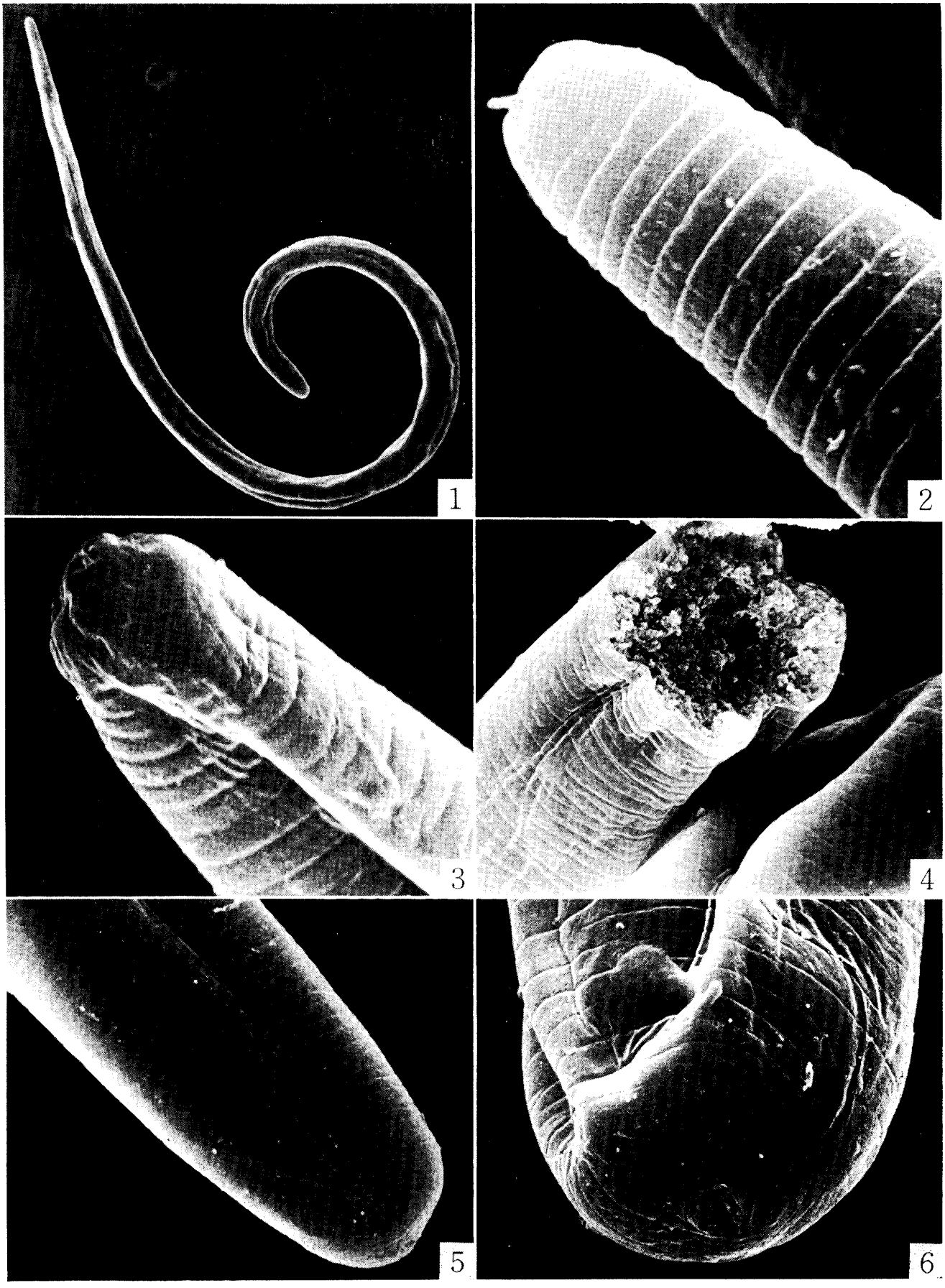
Explanation of figures

All figures are scanning electron photomicrographs of *Trichinella spiralis*.

Fig. 1. Whole body of muscle larva. ×240
Figs. 2 and 3. Anterior end of muscle larva. A stylet protrudes through oral opening. ×6,000
Fig. 4. Cracked surface of muscle larva in mid-gut level. ×2,400
Fig. 5. Posterior end of female muscle larva. ×2,400
Fig. 6. Posterior end of male muscle larva. Arrows show a projection and papilla on the lateral side of posterior end. ×6,000
Fig. 7. Anterior part of adult male. ×850
Fig. 8. Anterior end of adult male. A stylet elevates through oral opening. ×3,600
Fig. 9. Anterior end of adult male. A stylet elevates from mouth. Crateriformed depressions are located on the cephalic dome. ×12,000
Fig. 10. Cracked surface of adult male. Crateriformed pores arrange on the lateral cord. An arrow points lateral tube located in the lateral cord. ×2,400
Fig. 11. Lateral view of posterior end of adult male. ×3,600
Fig. 12. Dorsal view of posterior end of adult male. ×2,400
Fig. 13. Whole body of adult male. Crateriformed pores in pairs arrange on the lateral side. ×1,000
Fig. 14. Ventral view of posterior end of adult male. ×3,600
Fig. 15. Dorsal view of posterior end of adult male. ×2,400
Fig. 16. Anterior part of adult female. Pores in pairs are located on the lateral surface of adult female. ×1,200
Fig. 17. Vulva and pores are situated on the ventral and lateral sides of adult female, respectively. ×2,400
Fig. 18. Pores in pairs are arranged on the lateral surface of adult female. ×1,400
Fig. 19. Pores, and transverse and longitudinal striations of cuticle of adult female. ×7,500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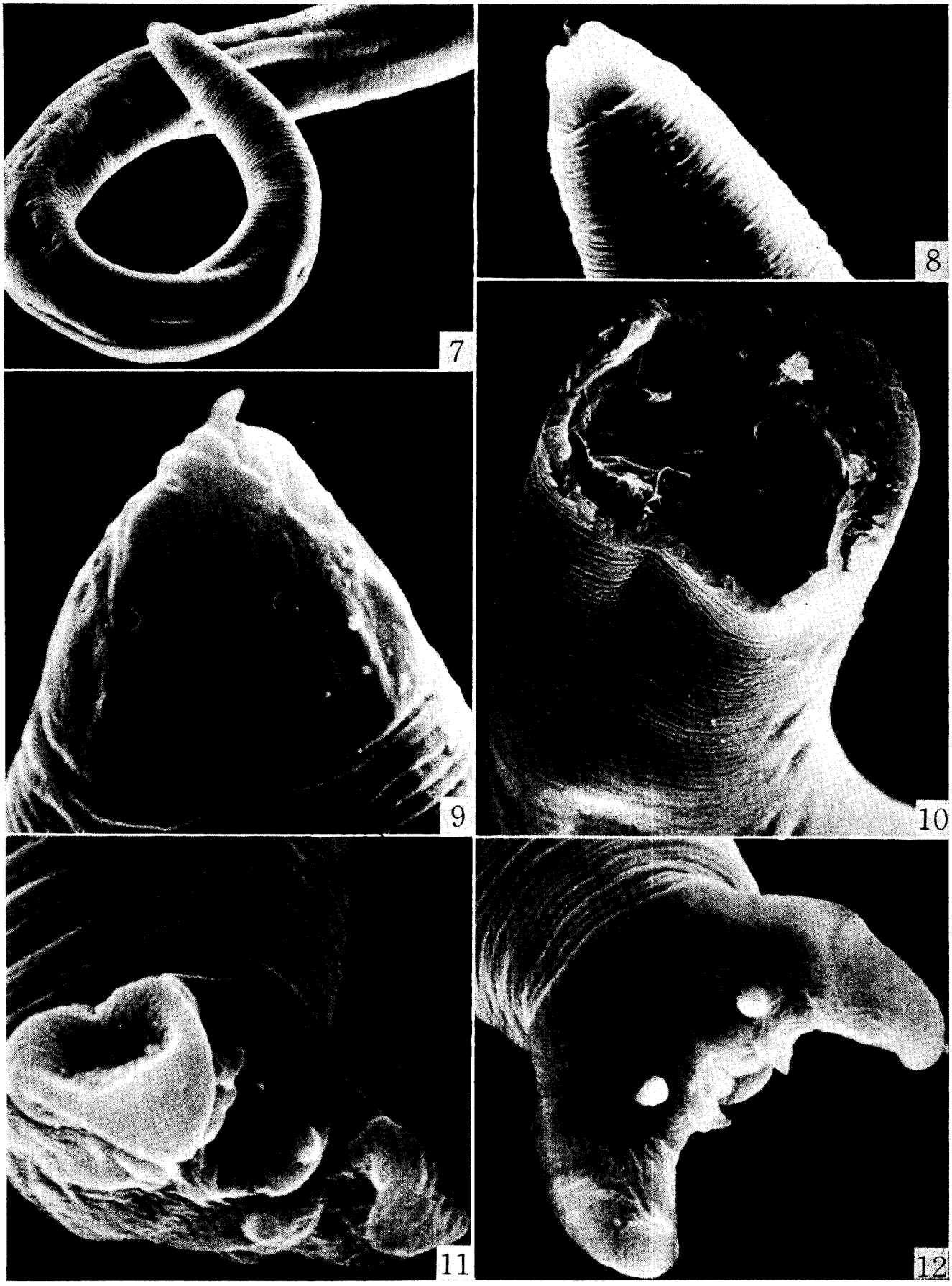
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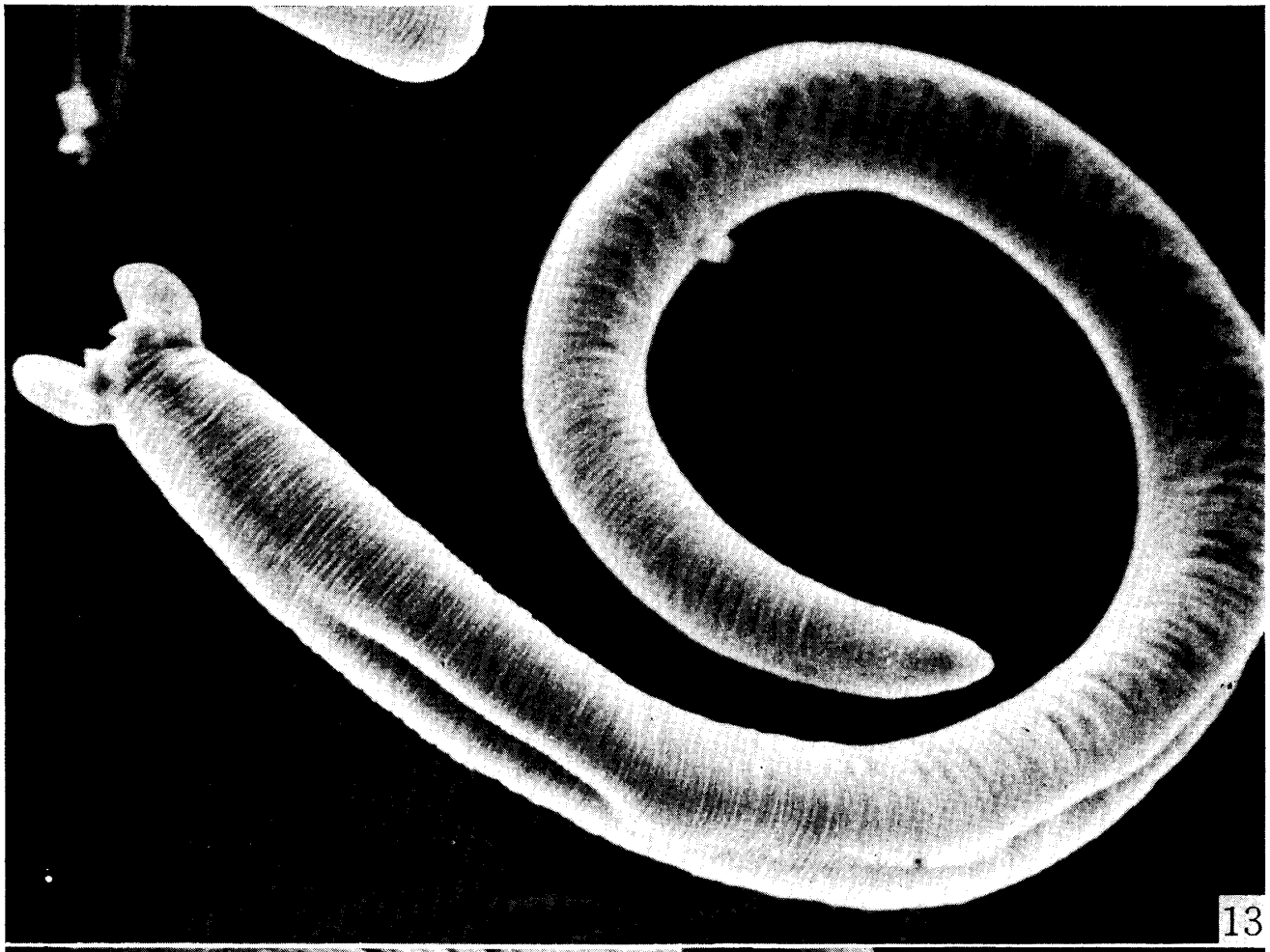

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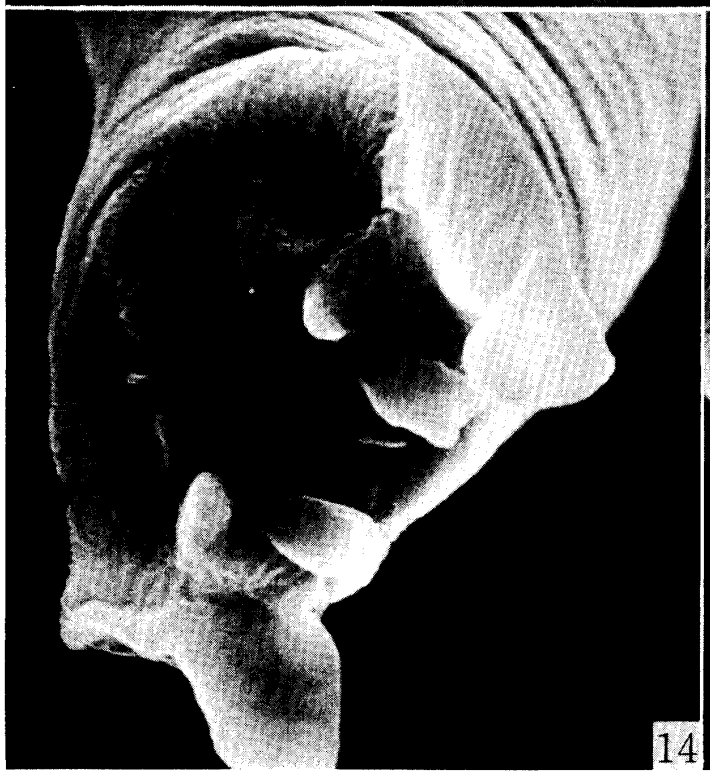

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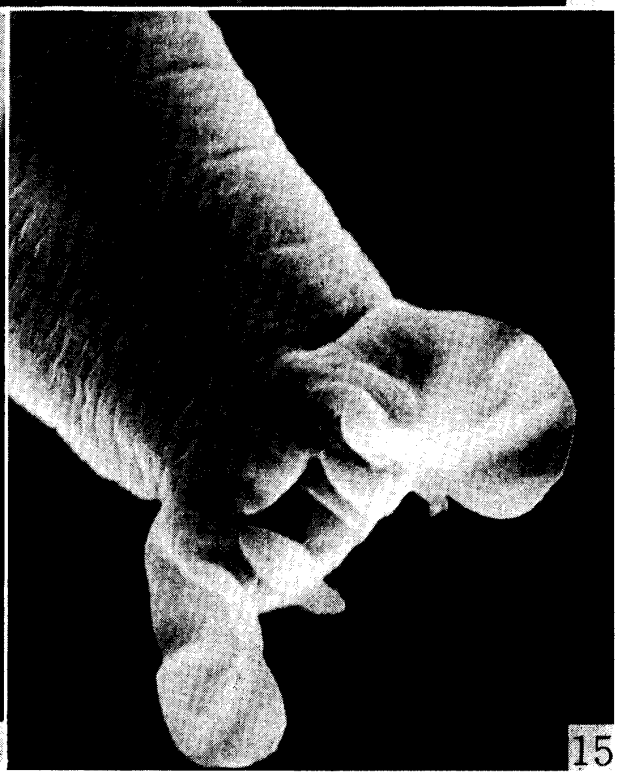

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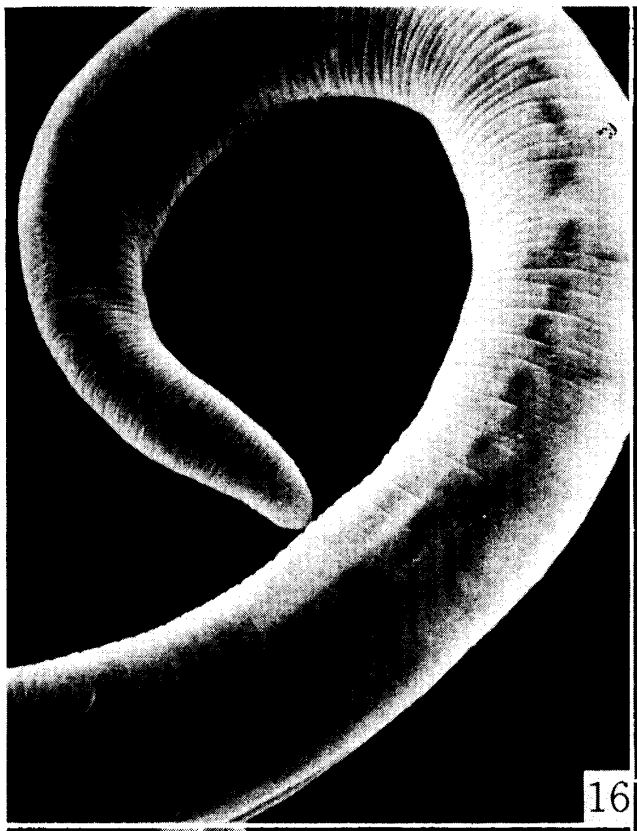

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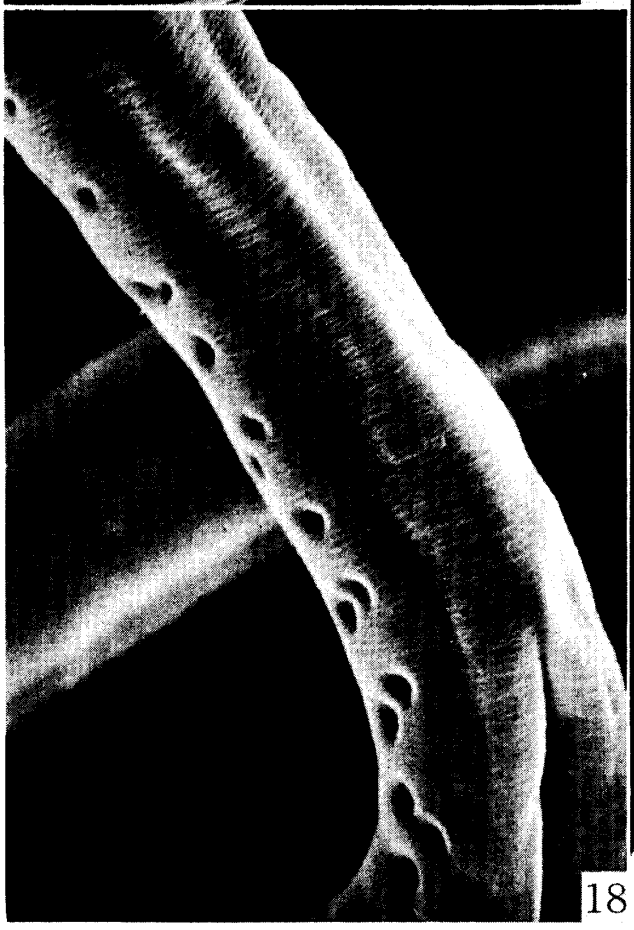

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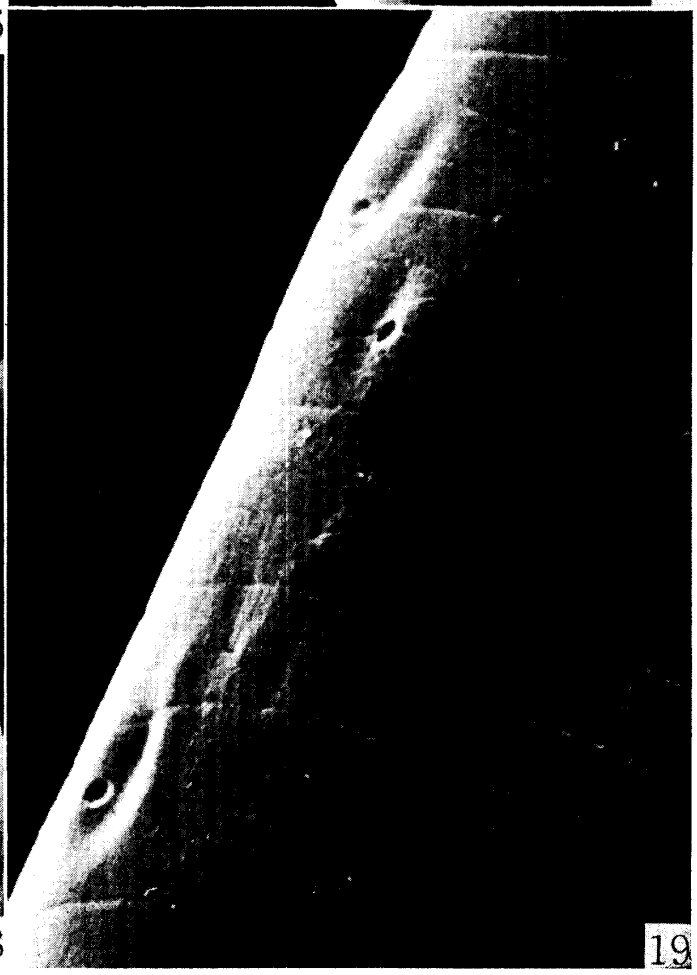

19