

# SCANNING ELECTRON MICROSCOPY OF HOOKWORMS

## 1. ADULTS AND INFECTIVE-STAGE LARVAE OF *NECATOR AMERICANUS* (STILES, 1902)

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### INTRODUCTION

Since 1968, the scanning electron microscope has been utilized in the field of parasitology. As this equipment has high resolving power and great depth of focus, it is expected to obtain further new information in the field mentioned above.

Studies of helminths with this equipment have been already reported on *Schistosoma* (Hockley, 1968; Silk *et al.*, 1970; Robson and Erasmus, 1970; Race *et al.*, 1971) *Acanthocephala* (Hammond, 1968), *Paragonimus* (Ishii and Miyazaki, 1968, 1970), *Ascaris* (Madden *et al.*, 1970; Weise, 1973; Maung, 1973), *Diplostomum* (Erasmus, 1970), helminth ova (Ishii and Miyazaki, 1971) and *Hymenolepis* (Berger *et al.*, 1971).

With regard to hookworms, Yoshida (1971a, b) demonstrated morphological differences of the transverse cuticular striations between *Ancylostoma braziliense* and *A. ceylanicum*, and Ishii (1971 a, b) graphically showed the mouth parts and some other parts of *A. duodenale* and *Necator americanus*.

The present authors carried out a series of morphological studies with the scanning electron microscope on eight species of hookworms, namely *N. americanus*, *A. duodenale*, *A. braziliense*, *A. ceylanicum*, *A. kusimaense*, *A. caninum*, *A. tubaeforme* and *A. malayanum*. As the first report of the series, the present paper describes the morphology of adult and infective-stage larvae of *N. americanus*.

### MATERIALS AND METHODS

The adult worms of *N. americanus* were obtained from a patient by anthelmintic

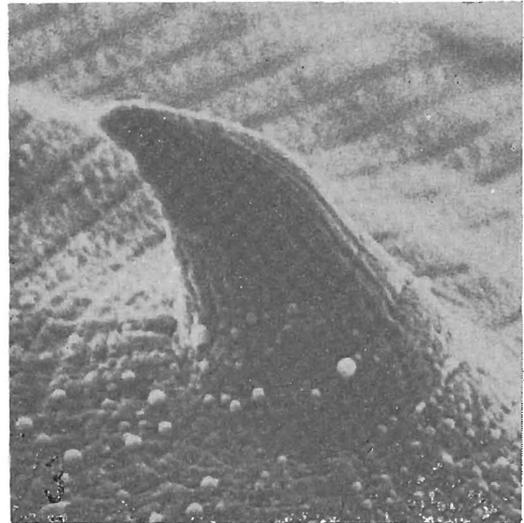
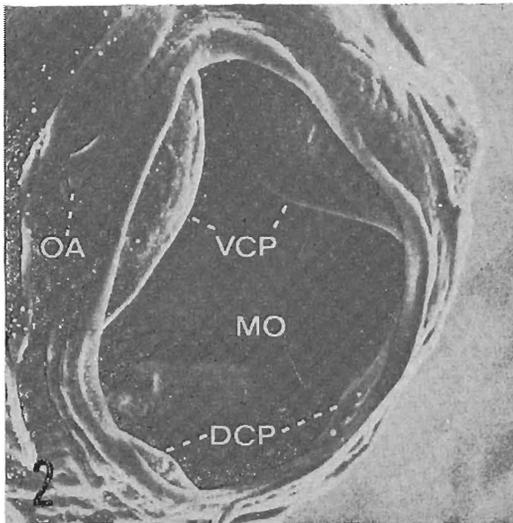
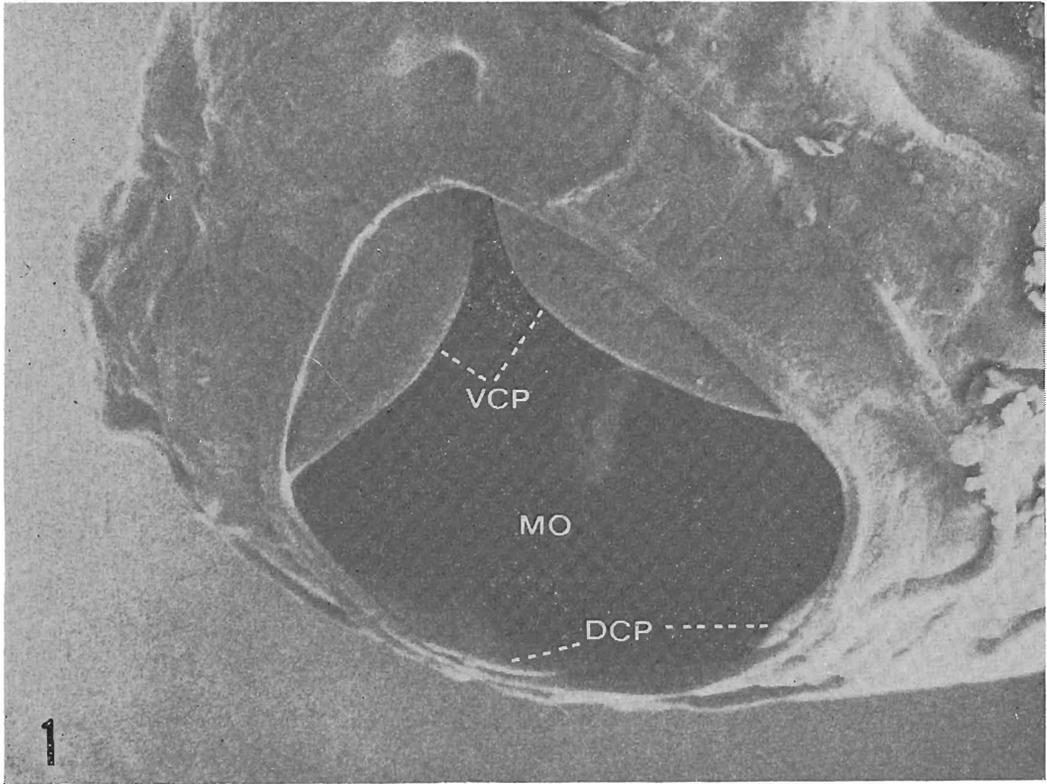
treatment with tetrachlorethylene followed by purgation. The worms were first washed gently with saline in order to remove dust from the surface, then fixed by immersion in 10% formalin. Worms were then dehydrated in an ascending series of acetone solutions (60, 70, 80, 90, 100, 100 and 100%). After drying in air, these specimens were coated first with carbon, then with gold in a vacuum evaporator.

The infective-stage larvae of this hookworm were obtained by cultivation (Harada-Mori method) of eggs in faeces from a patient having a pure infection of this hookworm. The method of fixation of the larvae was different from that used with the adult worms. The larvae were killed by heating (80°-90°C) under the wet condition and were fixed in 2.5% glutaraldehyde for one hour, then in 1% osmic acid for one hour. The procedure of dehydration and coating was the same as mentioned above.

For the present investigation a JSM-S1 scanning electron microscope (Japan Electron Optics Laboratory Co. Ltd., Tokyo) was used.

### RESULTS

The structures of mouth opening (MO), cutting plates and surface around the mouth opening are shown in Figs. 1 and 2. From these three-dimensional figures we can easily understand the constitution of mouth parts of this hookworm although the impression is somewhat different from that taken by the light microscope. Each of the ventral cutting



Figs. 1, 2—Mouth parts of adult *N. americanus* showing mouth opening (MO), ventral cutting plates (VCP), dorsal cutting plates (DCP), and orifice of amphid (OA) (Fig. 1, 1540 X; Fig. 2, 880 X).

Fig. 3—Cervical papilla (3000 X).

plates (VCP) is semilunar, stout, and sharp at the inner edge. The surface of the outer half of each ventral cutting plate is slightly uneven. The dorsal cutting plates (DCP) are visible at the dorsal margin of the mouth opening. The mouth opening is bordered with thickening of the cuticle. A small cleft on the subventral area which is well seen in Fig. 2 is considered to be the orifice of an amphid (OA).

Fig. 3 shows one of the cervical papillae which exist at the lateral side of the body at the level of the middle of the esophagus. It becomes evident by the scanning electron microscopic study that the shape of the cervical papillae varies somewhat with the species of hookworms. The cervical papilla of *N. americanus* is characterized by the following points: it is conical in shape, has a sharp point, and bends posteriorly like the horn of a rhinoceros. There are many longitudinal fine wrinkles on the surface of the papillae, and several lines encircle its base.

The nature of the transverse cuticular striations on the body surface of adult worm also varies with the species of hookworm. The striations of *N. americanus* are single, straight and deep, as shown in Fig. 4. The distance between the striations is 4.7 to 6.5  $\mu$ . Many fine wrinkles are seen on the surface between them.

The copulatory bursa is shown in Figs. 5 and 6. The bursa of *N. americanus* is longer in the direction of the lateral rays than of the dorsal ray. In Fig. 5, the pointed ends of the prebursal papillae (PBP) and probably the externo-lateral ray (ELR) are recognized. Fig. 6 shows the fine surface structure of the bursa. The striations on the surface of the bursa are not circular but are complicated by crossing and radiating.

The tail of an adult female is shown in Fig. 7. The anus (A), transverse cuticular striations and tip of the tail are well shown. The fact

that there is no mucron at the tip of the female tail is characteristic of *N. americanus*.

The surface structure of the sheath of an infective-stage larva is shown in Figs. 8 and 9. The distance between the transverse striations is about 1.1 $\mu$ . The lateral line (LL) which runs along the sheath is band-like and concave in the middle. The line is about 2.6 $\mu$  wide.

## DISCUSSION

The use of scanning electron microscopy on hookworms has been reported by two authors up to the present. Ishii (1971 a, b) graphically showed the mouth parts and transverse cuticular striations of *N. americanus*, and the mouth parts, cervical papillae and excretory pores of *A. duodenale*. Yoshida (1971 a, b) studied the transverse cuticular striations of adult worms and infective-stage larvae of *A. braziliense* and *A. ceylanicum*, and stated that the nature of the striations and the distance between the striations of the two species were quite different in the adult worms although those in the infective-stage larvae were not different.

The present paper offers new information on the morphology of adult worm and infective-stage larvae of *N. americanus*. The shape of the lateral line of the infective-stage larvae in this study coincides well with its cross section as obtained by transmission electron microscopy (Inatomi *et al.*, 1970).

## SUMMARY

The surface structures of adult worms and infective-stage larvae of *N. americanus* were studied with the aid of the scanning electron microscope. At the cephalic end of the adult worm, the mouth opening, ventral and dorsal cutting plates, and the orifice of an amphid were three-dimensionally demonstrated. The cervical papilla was conical and fine at the point, and bent posteriorly. The transverse

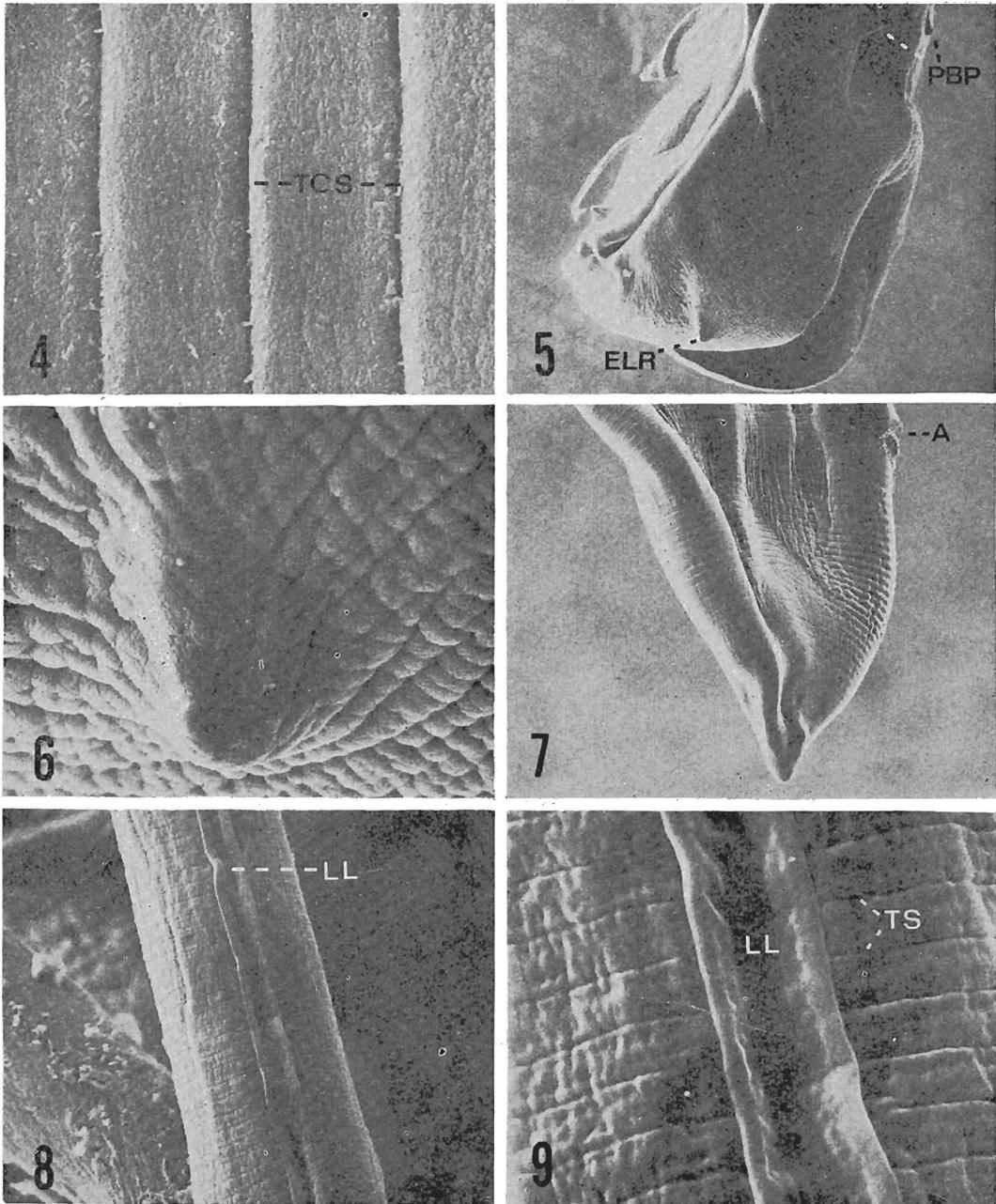


Fig. 4—Transverse cuticular striations (TCS) of adult worm (5190 X).

Figs. 5, 6—Copulatory bursa showing prebursal papillae (PBP), externo-lateral ray (ELR), and complicated surface striations on the bursa (Fig. 5, 220 X; Fig. 6, 2200 X).

Fig. 7—Tail part of female adult showing anus (A) and tail end which has no mucron (450 X).

Figs. 8, 9—Surface of sheath of the infective-stage larva of *N. americanus* showing lateral line (LL) and transverse striations (TS) (Fig. 8, 2100 X; Fig. 9, 7600 X).

cuticular striations of the adult worm were single, straight, deep and 4.7-6.5 $\mu$  apart. There were complicated wrinkles on the surface of the copulatory bursa. No mucron was seen at the tail end of the female adult worm. The lateral line on the sheath of an infective-stage larvae was band-like, concave in the middle, and about 2.6 $\mu$  wide.

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