SCANNING ELECTRON MICROSCOPY OF HOOKWORMS 1. MORPHOLOGICAL DIFFERENCES BETWEEN INFECTIVE STAGES OF *ANCYLOSTOMA CANINUM* (ERCOLANI, 1859) AND *ANCYLOSTOMA TUBAEFORME* (ZEDER, 1800)

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INTRODUCTION

Svensson (1925), Heydon (1927) and Sasa *et al.*, (1958) found that *Ancylostoma duodenale* larvae could be separated morphologically from *Necator americanus* but not from other larvae of the genus *Ancylostoma.* Inatomi *et al.*, (1968) and Sakumoto *et al.*, (1971) found that the larvae of *A. duodenale*, *A. caninum*, *A. braziliense* and *A. ceylanicum* could be distinguished from each other by the patterns of the lateral alae. Yoshida (1971), using scanning electron microscopy to separate the infective stages of *A. braziliense* and *A. ceylanicum*, found that the distance between the striations of these two species were not different.

In the present paper, the scanning electron microscope was used to study morphological differences between infective stages of *A. caninum* and *A. tubaeforme.*

MATERIALS AND METHODS

Larvae of *A. tubaeforme* and *A. caninum* were obtained from cats and dogs, respectively, and were cultured by the Harada-Mori technique (Harada and Mori, 1951). Seven-day-old larvae of both species were exsheathed by the modified floating raft method of Goodney (1925). Larvae were fixed with 70% hot ethyl alcohol and dehydrated by grading through 70%, 80%, 90% and 100% alcohol. They were left for 3 days in absolute alcohol and a further 3 days in xylene. Larvae were then attached to aluminium mounting stubs using a minimum amount of adhesive which had been extracted by chloroform from cellotape. The stubs were transferred to a vacuum evaporation unit and coated with pure gold or a gold-silver alloy by evaporating the metal from a tungsten filament at a pressure of about 10^{-5} torr. The stubs were rotated during the evaporation to ensure that an even coating was obtained. The specimens were then examined in a Cambridge 'Stereoscan' Mark 11 A Electron Microscope, using the emissive mode (secondary electrons) and a beam potential of 5 kv. Photographs were recorded on Kodak Tri-X 35 mm film.

RESULTS

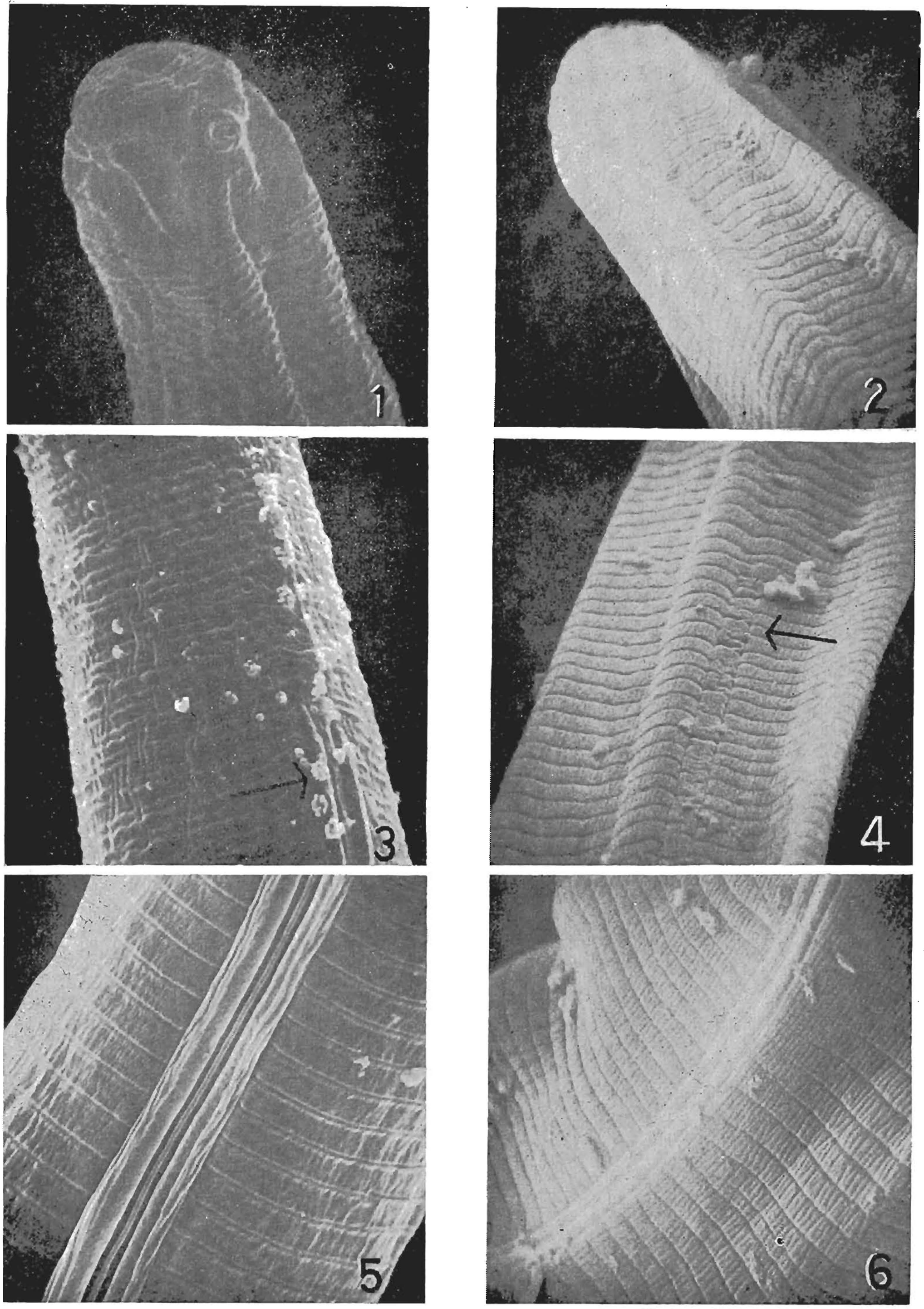
Anterior end

The shape of the anterior end of *A. caninum* (Fig. 1) was relatively wider and rounder than that of *A. tubaeforme* (Fig. 2). Otherwise this section of the larvae of both species appeared unremarkable.

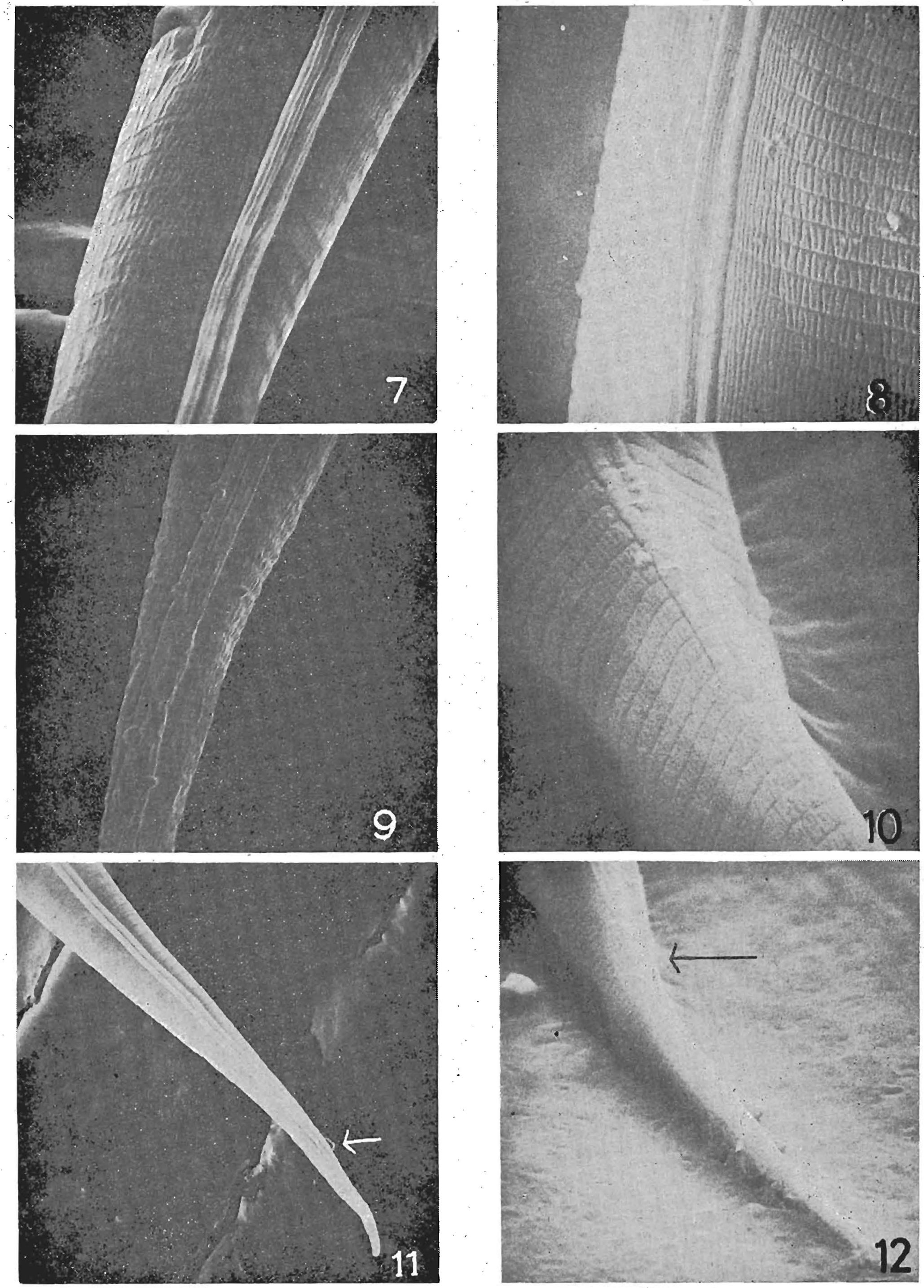
Lateral alae

In both species, the lateral alae could be distinguished beginning at approximately the anterior one-tenth of the body length. In *A. caninum* they emerged abruptly from the body surface (see arrow, Fig. 3). In *A. tubaeforme*, the emergence was much more gradual (see arrow, Fig. 4).

In both species, the lateral alae showed three branches running posteriorly (Figs. 5, 6).



Figs. 1, 2—Showing head end of *A. caninum* (1) X 6800 and *A. tubaeforme* (2) X 6800.
Figs. 3, 4—Showing the beginning of the lateral alae of *A. caninum* (3) X 6000 and *A. tubaeforme* (4) X 6000.
Figs. 5, 6—Showing the big three projections of the lateral alae of *A. caninum* (5) X 6000 and *A. tubaeforme* (6) X 6000.



Figs. 7, 8 —Showing two projections of the lateral alae of *A. caninum* (7) X 6000 and *A. tubaeforme* (8) X 6000.

Figs. 9, 10 —Showing the end of the lateral alae of *A. tubaeforme* (10) X 6000 and the presence of the lateral alae of *A. caninum* (9) X 6000 at the same level of 10.

Figs. 11, 12—Showing the space between the tip of tail and the end of the lateral alae of *A. caninum* (11) X 1800 and *A. tubaeforme*. (12) X 1800.

At the posterior third of the body in each species, the three branches of the alae became two (Figs. 7, 8) and these two branches continued toward the tip of the tail. In *A. tubaeforme* (Fig. 10), the alae terminated sooner than in *A. caninum* (Fig. 9), the distance between the tip of the tail and the termination of the alae in *A. tubaeforme* (Fig. 12) being about three times longer than in *A. caninum* (Fig. 11).

The alae of *A. caninum* (Figs. 3, 5, 7, 9) were more prominent than those of *A. tubaeforme* (Figs. 4, 6, 8, 10). The space between branches of the alae in *A. tubaeforme* was wide and shallow (Figs. 6, 8) whereas in *A. caninum* (Figs. 5, 7) it was deep and narrow.

Tail

In *A. caninum* the larval tail (see arrow, Fig. 11) tapered more gradually than in *A. tubaeforme* (see arrow, Fig. 12).

Transverse striations

No remarkable differences were detected in the transverse striations between the two species.

DISCUSSION

Yoshida (1971) used scanning electron microscopy to study the infective-stage larvae of *A. braziliense* and *A. ceylanicum*, but did not find any differences between the transverse striations between these two species. In the present work, transverse striations in *A. caninum* and *A. tubaeforme* also exhibited no outstanding differences. Yoshida (1971) did not mention lateral alae. The present author found that the lateral alae could be used to distinguish between the larvae of *A. caninum* and *A. tubaeforme*. In *A. caninum*, the lateral alae were prominent whereas in *A. tubaeforme* they were not. The lateral alae in *A. caninum* terminated closer to the tip of the tail than in *A. tubaeforme*.

SUMMARY

Infective larvae of *A. tubaeforme* and *A. caninum* could be distinguished by the aid of scanning electron microscopy. The anterior end in *A. caninum* was relatively rounder and bigger than in *A. tubaeforme*. The lateral alae of *A. caninum* were comparatively more prominent, rose up from the body abruptly and terminated closer to the tip of the tail. The tail in *A. caninum* tapered more gradually than in *A. tubaeforme*.

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