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SNAIL HOSTS OF *ANGIOSTRONGYLUS CANTONENSIS* IN TAIPEI, TAIWAN

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ABSTRACT

The snail host of *Angiostrongylus cantonensis* in Taipei, Taiwan was determined. Two species of land snails, *Bradybaena similaris* and *Achatina fulica,* were incriminated as intermediate hosts of the parasite. The adults were obtained from the lungs and heart of laboratory rats which were fed with infective larvae of *A. cantonensis* found in these snails. The rats passed first stage larvae in feces 40-42 days after infection. The larval development in snail hosts were studied, and the life cycle of this nematode was established in the laboratory.

The possible role of these two species of snails in transmission of human angiostrongyliasis in Taiwan is discussed.

Angiostrongylus cantonensis was first recorded in Taiwan in 1935 when Matsumoto found the nematodes from wild rats collected in Hualien County(1). These parasites were sent to Yokogawa for identification and were described as a new species, *Haemostrongylus ratti,* in 1937 (2). The nematode was re-assigned to the genus *Pulmonema* by Yokogawa and Morishita in 1949 (3). However, these names are synonymous with *Angiostrongylus cantonensis* (Chen) Dougherty, 1946 (4) originally described as *Pulmonema cantonensis* in 1935 (5).

A. cantonensis in the adult stage lives in the lungs and heart of the rat. Man is an accidental host for this nematode. It is a causative agent of the disease "eosinophilic meningoencephalitis" in man. In the past few years this syndrome has been reported in the Pacific islands and Southeast Asia where the parasites were found in rats. The first human case of *A. cantonensis* was reported in Taiwan by Nomura and Lin in 1945(6). They recovered more than ten immature *A. cantonensis* from the cerebrospinal fluid of a 15-year-old Japanese male admitted to the hospital on the suspicion of meningitis. Two other human cases were also diagnosed by the recovery of the worms. Rosen *et al.*(1962) found young adults of *A. cantonensis* in the brain of a 50-year-old male Filipino who died in Hawaii (7), and suspected that another patient might have been infected with this parasite by the appearance of pathological sections of the brain. Prommindaroj *et al.* in 1962 (8) found an adult male worm of a species of *Angiostrongylus,* probably *A. cantonensis,* in the anterior chamber of the eye of a 34-year-old male in Thailand.

The life cycle of *A. cantonensis* was elucidated in detail by Mackerras and Sandars in Australia in 1955 (9). Two species of slugs, *Agriolimax laevis* and *Limax arborum,* were found as the intermediate hosts of the parasite. From then on, a wide range of mollusks, including snails and slugs, has been found to serve

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as intermediate hosts for this parasite naturally and experimentally (10–14).

In spite of the presence of *A. cantonensis* in local rats and the occurrence of human angiostrongyliasis on this island, no further reports have been made since 1945 except for the recent study by Kuntz and Myers, dealing with the survey of rodent parasites(15). About five years ago, the author examined two rats in Taipei infected with *A. cantonensis*. This finding promoted an investigation to determine the snail host of the nematode in this city. Two species of land snails were found to be parasitized with infective larvae (3rd stage larvae) of *A. cantonensis*. The larval development of the parasite in these snail hosts was studied and the possible role of these snails in transmission of the disease is discussed in the present paper.

While this work was in progress, Huang *et al.* (16) found the second case of human angiostrongyliasis in Taiwan. They detected a young male worm of *A. cantonensis* from the eye of a 11-month-old female.

MATERIALS AND METHODS

Two common species of land snails were collected around the places where two wild rats infected with *A. cantonensis* were captured in Taipei city. These included 218 *Bradybaena similaris* (Férussac) and 83 *Achatina fulica* (Férussac). The head-foot of the snail was examined after digestion by artificial pepsin-hydrochloric acid (1% HCl solution containing 0.5 gm of pepsin). Not more than 15 *B. similaris* or 5 *A. fulica* were digested at a time. The digested material was then searched for infective larvae of *A. cantonensis* under a dissecting microscope. To ascertain the identification of larvae, they were fed to the rats of Long Evans strain together with a piece of crab liver. Fecal examinations were started on the 40th day after feeding. The rats were sacrificed to recover the adult worms from the lungs and hearts for identification.

Experimental infections of *A. cantonensis* were conducted to study the larval development in *B. similaris* and *A. fulica*. Snails were exposed to fresh feces containing first stage larvae of *A. cantonensis* for 2 to 5 hours in petri dish. They were then reared in a terrarium, covered with wet soil and kept in the laboratory. The development of the larvae was observed by examining snails at different times after infection.

The morphology of the parasite was briefly described since it had been dealt with by Chen (5), Yokogawa(2), and Mackerras and Sandars (9) in detail. All measurements of the parasties were made on adult worms and each stage larva fixed in 5% formalin solution, under slight cover glass pressure.

RESULTS

(1) Description of the larvae and adult of *A. cantonensis* and experimental infection of the rats:

Forty-eight and 3 infective larvae of *A. cantonensis* were found from a total of 218 *B. similaris* (*Fig. 1*) and 83 *A. fulica* (*Fig. 2*) examined, respectively. The morphological characteristics of the larvae detected in both species of snails were identically the same. The infective larvae measure 466.1 to 497.7 microns long by 23.7 to 29.6 microns wide. Esophagus 160.0 to 189.6 microns long, nerve ring about 79 microns and genital primordium 276.5 to 320.0 microns from the tip of head; and anus 39.5 microns from the tip of tail. There are two well developed chitinous rods in the buccal cavity. Refractile granules, seen in the intestinal wall of second stage larvae, disappear in this stage so that the whole larva appears transparent. Larvae remain quiet in muscles of the snail and become very active once they are released (*Figs. 5 and 6*).

The infective larvae were fed to three laboratory rats. As shown in TABLE I, the rats passed first stage larvae in feces 40–42 days after infection. Larvae were continuously discharged until autopsy.

TABLE I
The results of experimental infections of rats with infective larvae of ***A. cantonensis***

Rat no.	No. of larvae fed	Date of infection	Finding of larvae in feces		Autopsy		
			Date	Days after infection	Date	Days after infection	No. & locations of worms
1	12	Dec. 7, '63	Jan. 17, '64	41	Feb. 24, '64	79	3♂ +5♀ (Lungs)
2	25	Jan. 3, '64	Feb. 12, '64	40	July 18, '64	197	1♂ +1♀ (Heart) 6♂ +7♀ (Lungs)
3	11	Jan. 15, '64	Feb. 26, '64	42	June 23, '64	160	1♂ (Heart) 2♂ +2♀ (Lungs)

First stage larvae measure 263.1 to 299.7 microns long by 11.3 to 14.0 microns wide. Rhabditoid esophagus long, nearly half the length of body, 126.5 to 139.9 microns; nerve ring 66.7 to 83.3 microns from anterior end; genital primordium posterior to midpoint of body, 183.2 to 199.8 microns from anterior end. They remain quiet in rat feces till the droppings were smeared on slide. The larvae move around actively (*Fig. 3*).

The adults were recovered in the pulmonary arteries and hearts of infected rats at autopsy. Twenty-eight (58.3%) of 48 larvae fed developed to mature worms. There were slightly more female (15) than male (13) worms recovered from the rats. Rats showed no symptoms of the infection of *A. cantonensis* through the course of the experiment.

Adult worms: the anterior end is rounded; esophagus is short as compared with that of each stage larva; intestine is wider than esophagus at junction (*Fig. 8*); nerve ring is about 203 microns from anterior end. Males measure 22.0 to 25.0 mm long by 0.335 to 0.385 mm wide (average 23.2×0.364). Esophagus 290.0 to 311.8 microns long by 50.8 to 65.3 microns wide (average 301.6×58.0); and intestine 87 microns wide on an average at junction with esophagus. The posterior end is provided with an inconspicuous copulatory bursa which is supported by rays having common pattern for each half of bursa (*Fig.10*). The dorsal ray short and not distinct, ending in three small digitations; externo-dorsal ray isolated; three laterals extend outwardly from a common trunk, postero- and medio-lateral rays close together; two ventrals fused to form a single trunk at root. Spicules are subequal in length, yellow-brown in color, measuring 1.19 to 1.32 mm long. Females measure 29.0 to 31.0 mm long by 0.462 to 0.505 mm wide (average 30.0×0.485). Esophagus 275.5 to 319.0 microns long by 65.3 to 79.8 microns wide (average 298.7×72.5); and intestines average 116 microns wide at junction with esophagus. The posterior end is obliquely truncated; anus 50.8 microns and vulva 166.8 microns ventrally from posterior end (*Fig. 9*). The most conspicuous feature of female worms is the "barber's pole" pattern which is made of spirally coiled, colorless uterus and blood-filled straight intestine (*Fig. 7*).

In contrast, the adults collected from a naturally infected wild rat, *Rattus norvegicus norvegicus*, at Alilao village of Taipei County in 1960 gave smaller measurements than the worms from experimentally infected rats because of heavey infection. Eighty-eight males and 122 females were collected in the lungs and heart of that rat. Males measure 12.5 to 23.0 mm long by 0.232 to 0.368 mm wide (average 16.7×0.268), and females 18.5 to 30.0 mm long by 0.255 to 0.480 mm wide (average 21.6×0.327). The two rats which were found infected with *A. cantonensis* in Taipei city were also parasitized with more than one hundred parasites in the lungs and heart.

The eggs of different developing stages, from one cell eggs to embryonated eggs, as well as first stage larvae, were seen in

the lungs of rats. The eggs are thin-shelled, transparent, elliptical, and measure 75.1 to 83.0 microns long by 41.5 to 47.4 microns wide (average 77.4×44.2). The eggs develop and hatch out in the lungs of the rat in 5–6 days(13). First stage larvae seen in the lungs are morphologically the same as those in feces. These larvae later break through into the respiratory tract, go up the trachea and then are swallowed (9).

(2) Experimental infection of the snail hosts with *A. cantonensis:*

The larval development of *A. cantonensis* in the snail host was studied in the laboratory. *B. similaris* and *A. fulica* were exposed to rat feces containing first stage larvae. Number 2 rat was used as the source of first stage larvae for all experiments. Not many differences of the larval development were observed between the two species of snails. In general, both the infection and the larval development occurred easier in *B. similaris* than in *A. fulica.* More time was needed to expose *A. fulica* to first stage larvae to establish the infection. In the snail phase, tissue reaction around the larvae was observed in *A. fulica.* The temperature is the most important factor in limiting the development. The larvae developed to second stage larvae in 14–16 days, and third stage larvae in 23–26 days at room temperature of 25–30 C. It was noticed, regardless of the species of snails, that the development of larvae was not uniform if the snail was heavily infected.

The larval development slowed down or even stopped under lower temperatures. It took 90 days for the infective stage larvae to develop in *A. fulica* during the period of January to April, 1964 (room temperature: 10–27 C). The larvae actually started growing in the latter parts of the period when the range of room temperature were risen to 18–27 C.

Second stage larvae measure 445.0 to 478.0 microns long by 23.7 to 27.7 microns wide. Esophagus 158.0 to 185.7 microns long; nerve ring 67.2 to 86.9 microns and genital primordium 240.4 to 284.4 microns from the tip of head. Two chitinous rods in buccal cavity appear in this stage. (*Fig. 4*). The larvae are easy to find in muscles of the snail because the intestinal wall is composed of large cells filled with refractile granules. They are contained in a sheath and are sluggish.

The infective larvae collected from experimentally infected snails were not morphologically different from those of naturally infected snails. Survival of larvae in the snail host up to 10 months after infection was observed in *B. similaris* and 11 months in *A. fulica.* The infective larvae were fed to two laboratory rats. First stage larvae began to appear in the rat feces 40 and 41 days after infection, and adults were recovered in the lungs and heart at autopsy. The life cycle of *A. cantonensis* was therefore completed, and the attempt to establish the strain in the laboratory was successful.

DISCUSSION

Three species of snails were found to be naturally parasitized with infective larvae of *A. cantonensis, i. e., Achatina fulica*(Férussac)in Hawaii(11); *Bradybaena similaris* (Férussac), and *Subulina octona* (Bruguière) in New Caledonia (12). The present study revealed *B. similaris* and *A. fulica* served as snail hosts of *A. cantonensis* in Taiwan for the first time.

In regard to the avenues of human infection with *A. cantonensis,* it is obviously the result of eating raw or improperly cooked snails or food contaminated with mollusks harboring the infective larvae. It is also within the range of possibility that infection can be attained from paratenic or carrier hosts. Fresh water prawns in Tahiti (17) and land crabs on Saipan (18) harbored infective larvae of *A. cantonensis.* Alicata (19) also reported that pigs and calves could be carrier hosts for infective larvae of the parasite. Live and unchanged larvae were recovered from the stomach, spleen, liver, and lungs of pig and calf two weeks after feeding 5,000

and 50,000 larvae, respectively. Therefore, the eating habits and personal hygiene in different areas are the most important factors in the occurrence of human angiostrongyliasis.

B. similaris is a native snail, inhabiting the garden and ground closely associated with residence sections. Although this species is not consumed as food yet it can not be excluded from being the source of contaminating vegetables, soil, and water following the death of snails. There is also the possibility that the snail is eaten by an appropriate paratenic or carrier hosts which are consumed by man.

On the contrary, *A. fulica* is eaten by some inhabitants, especially during the time of the World War II when meat ran short. This species of snail was first introduced to Taiwan by Shimojo from Singapore in 1932 (20). He brought 20 snails in January, but all of them died from the cold weather. In July of the same year, he brought back again 20 snails, and was able to establish the strain in Taiwan. The snails then spread out quickly all over the island through the medium of Tazawa and Miyazima. Nowadays, this snail has become well established, and causes damage to vegetables, ornamentals, and other plants everywhere on the island.

Tazawa is the first man to commercialize snail raising in Taiwan. He pointed out that this snail was good food and told people how to cook it. It may be of interest to describe one of the methods cited by Sonan (20) as follows:

Snails are heated in a pot with a little water. The animal will come out the shell and soon be killed as the water gradually gets hot. It is suggested not to boil snails for a long time for the animal becomes too hard and loses its taste. The animal is then pulled out of the shell, washed in a salt solution to remove the mucous, and finally cut into small pieces to serve with seasoning such as vinegar, soy, salt, sugar, or essence.

It is not eaten raw, however, contamination with infective larvae of *A. cantonensis* on the fingers during the process of cooking, and eating insufficiently cooked snails are direct avenues of infection with this parasite. On the whole, it appears that *A. fulica* is a more important host than *B. similaris* as a potential source of human angiostrongyliasis in Taiwan, although the infection was lower in the former than in the latter snail. Little attention has been given to *A. cantonensis* in Taiwan thus far. To what extent human infection with this parasite takes places remains to be solved.

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Fig. 1. Land snail, *Bradybaena similaris.*

Fig. 2. Giant African land snail, *Achatina fulica.*

Fig. 3. First stage *A. cantonensis* larva in rat feces.

Fig. 4. Second stage *A. cantonensis* larva removed from snail muscles, showing chitinous rods in buccal cavity.

Fig. 5. Coiled infective third stage *A. cantonensis* larva in the snail muscles.

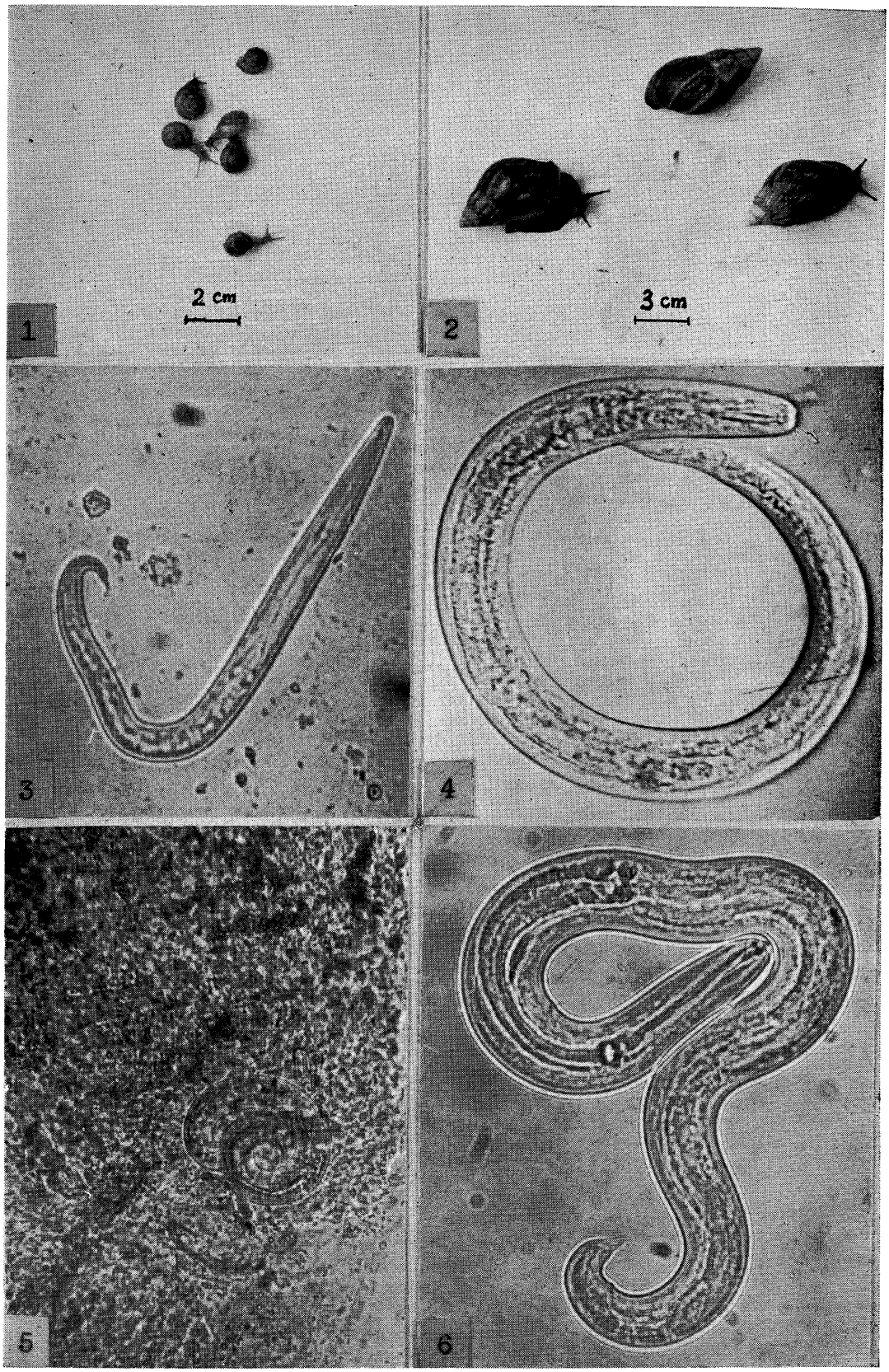
Fig. 6. Third stage *A. cantonensis* larva.

Fig. 7. Living adult worms of *A. cantonensis, left,* females; *right,* males.

Fig. 8. Anterior part of adult *A. cantonensis,* showing intestine wider than esophagus at junction.

Fig. 9. Obliquely truncated posterior end of adult female, showing anus and vulva (Lateral view).

Fig. 10. Posterior end of adult male, showing bursa and spicules (Dorsal view).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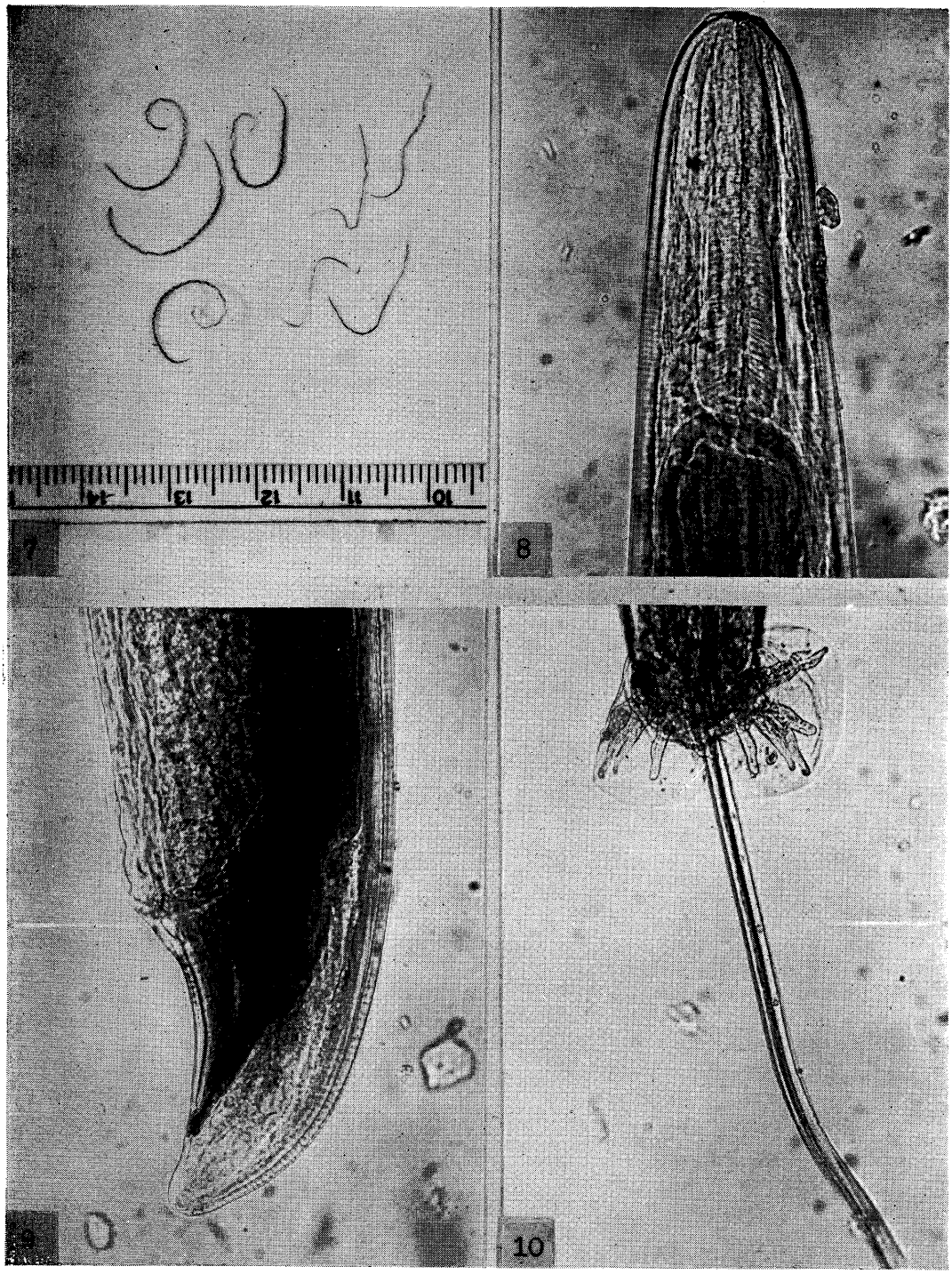
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