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Life Cycle of a Frog Nematode, *Spinitectus ranae* Morishita, 1926 (Cystidicolidae)

HIDEO HASEGAWA and MASAMITSU OTSURU

(Department of Medical Zoology, Niigata University School of Medicine, Niigata, Japan)

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Introduction

During the epidemiological surveys on the causative agent of two human cases of zoonotic infection due to some larval spiruroid nematode, Otsuru *et al.* (1974) recovered three larval types of spiruroid nematodes from the freshwater shrimp, *Paratya compressa improvisa*. One of them was considered as *Spinitectus* sp. by the same authors, but species name has been unsettled. This study was carried out in order to infect the appropriate host with this type larva and obtain the adult stage to be identified precisely.

Materials and Methods

1. Recovery of larvae.

The freshwater shrimps, *Paratya compressa improvisa*, were collected from a small pond in Hiatari area of Kaneyama Town, Yamagata Prefecture. They were digested with artificial gastric juice at room temperature. The larvae were picked out using a small needle and preserved in physiological saline at 4 C before administration.

2. Infection experiments.

Frogs and newts were used as experimental final hosts.

Rana tagoi, *R. brevipoda porosa* and *R. rugosa* were captured at Kakuda Mountain of Maki Town, suburbs of Niigata City, and Kanazuka area of Kajigawa Village, Niigata Prefecture, respectively. Newts, *Cynops pyrrhogaster*, were netted at Echigo-Hayakawa of Murakami City, Niigata Prefecture. These hosts were reared in aquaria (27×15×20 cm) at room temperature in spring to autumn and at 24±2 C in winter. Up to six frogs or newts were maintained in each aquarium. Frogs were forced to eat porcine kidney or liver once per two or three days. Newts were also allowed to eat the same foods at the same intervals.

Spinitectus larvae, suspended in physiological saline, were given orally using a small glass pipette. After administration, these frogs and newts were autopsied at various intervals. When they died before a given period, necropsies were performed subsequently.

3. Survey on natural infection.

In order to investigate the naturally infected hosts, two species of frogs, *Rana rugosa* and *R. ornativentris*, and newts, *Cynops pyrrhogaster*, were captured in the small pond where *Paratya compressa improvisa* was collected.

4. Autopsy and preparation.

At autopsy, the frogs and newts were killed with ether inhalation, digestive tracts were incised and examined under dissecting microscope. Worms were picked up with a fine needle and preserved in physiological saline before fixation. For microscopical survey, worms were fixed with 70% ethanol at 70 C, cleared in glycerin alcohol solution and mounted on glass slides with 50% glycerin jerry. Figures of nematodes were drawn with the aid of a camera lucida.

Results

1. Infection experiments.

At autopsy of the frogs and newts, almost all of the recovered worms were found in

their stomachs (Fig. 10). They attached their heads to the epithelium but none of them penetrated the stomach wall. Although some worms were found in the intestine or the rectum in a few instances, they were usually damaged or died. Pathological changes of the stomach epithelium were not noticeable.

(a) *Rana brevipoda porosa*.

In spring and summer, most larvae molted on the 6th or 7th day after infection. They molted again between the 25th and 45th day after infection and became adults. However, the larvae could not develop when infections were made in late autumn or winter though the frogs were maintained at relatively warmer condition. Control frogs (4 in autumn and 15 in summer) were found free from *Spinitectus*.

(b) *Rana tagoi*

On the 25th day after infection, the larvae were found molting to adult stage. Six control frogs were not parasitized by *Spinitectus*.

(c) *Rana rugosa*.

Spinitectus larvae grew quickly in this species and mature adults were recovered on the 15th day after infection. No *Spinitectus* were found in three controls.

(d) *Cynops pyrrhogaster*.

The larvae could survive in the newt stomach for relatively long period without development. Eight controls were not parasitized by *Spinitectus*.

Results of the infection experiments were summarized in Table 1.

2. Description of worms.

(a) Third-stage larva (Based on 10 specimens collected from the shrimps; Figs. 1, 2, 9 and 12).

The larvae of this stage were found encysted in the muscles of various parts of the shrimps.

Body filiform, slightly tapered anteriorly. Cuticle with rings of posteriorly directed spines and transverse striations. Body length 1.19 to 1.79 mm and maximum body width 56 to 89 μ. Head with a pair of lateral pseudolabia each of which has submedian papillae and an amphid. Buccal cavity funnel-shaped and 44 to 63 μ in length. Esophagus consisting of two portions, anterior muscular and posterior glandular. Muscular esophagus 111 to 148 μ long and glandular 226 to 337 μ long. Tail conical, 41 to 55 μ long and its tip has a small process covered with minuts spines. Nerve ring large, and distance from cephalic apex 85 to 100 μ. Excretory pore at base of fifth spinous ring, 161 to 167 μ from cephalic apex. Primitive reproductive organs are seen and sexes distinguishable: in male, germ cells form a longitudinal rod ventrad to alimentary canal and posterior end of this rod is apart from anus, while in female, the reproductive organs are filiform and arise from preanal area, wind and extend anteriorly. First spinous ring at 61 to 74 μ from cephalic apex and composed of 26 spines. First, second and third rings are set closely. Spinous rings continue to posterior end, decreasing in number and size of spines. Number of spinous rings 66 to 79.

(b) Fourth-stage larva (Based on 12 specimens recovered on the 7th and 10th days after infection from *Rana brevipoda porosa*; Figs. 3, 4, 5 and 13).

Body filiform. Sexes are easily distinguishable by reproductive organs, especially vagina. First and second spinous rings are set closely. This is the result of the preceding molt during which first ring of third-stage larva was eliminated (Fig. 11). Number of spines on first ring 32. These spinous rings disappear in posterior part of body, but they reappear again in perianal area in female. Head and alimentary canal closely resemble those of third-stage larva. Excretory pore at base of fourth spinous ring. Tail tip without spine-covered process.

Male: Body length 1.80 to 2.20 mm and maximum body width 74 to 90 μ. Length of buccal cavity 44 to 52 μ, muscular esophagus 159 to 200 μ, and glandular esophagus 389 to 463 μ. Distance from cephalic apex to middle of nerve ring 100 to 107 μ, to excretory pore 181 to 189 μ, and to first spinous ring 85 to 87 μ. Number of spinous

rings 49 to 56. Tail conical and 63 to 68 μ in length. Reproductive organs fairly developed and testis and vas deferens observable. Anlage of spicule also seen.

Female : Body length 2.16 to 2.59 mm and maximum body width 74 to 104 μ. Length of buccal cavity 43 to 56 μ, muscular esophagus 159 to 222 μ, and glandular esophagus 381 to 514 μ. Distance from cephalic apex to middle of nerve ring 104 to 122 μ, to excretory pore 185 to 213 μ, and to first spinous ring 81 to 102 μ. Number of spinous rings 54 to 96. Tail conical and somewhat inclines ventrad. Length of tail 43 to 57 μ. Anlage of vulva still closed, at 174 to 233 μ from caudal tip.

(c) Adult (Based on 49 specimens recovered from *Rana tagoi*, *R. brevipoda porosa* and

Table 1 Infection trials of *Spinitectus* larvae collected from *Paratya compressa improvisa* to various amphibians

Period of infection (days)	No. of amphibian hosts	No. of larvae given (per one host)	No. of worms recovered	Recovery rate (%)	Stages of recovered worms
(A) *Rana brevipoda porosa*; Oct., 1976-Jan., 1977.					
2	2	20	27	67.5	3 rd.
6	1	〃	16	80.0	3 rd.
12	1	40	3	7.5	3 rd.
21	2	〃	6	7.5	3 rd.
25	2	80	1	0.6	3 rd.
51	1	40	1	2.5	3 rd. (?)
100	2	20	0	0.0	
(B) *Rana brevipoda porosa*; May-Aug., 1977.					
6	2	100	18	9.0	3 rd, 3 rd (molt), 4 th.
10	4	〃	6	1.5	3 rd (molt), 4 th.
15	1	〃	2	2.0	3 rd. 4 th.
25	2	〃	6	3.0	4 th.
45	4	50	6	3.0	5 th (mature).
51	4	〃	1	0.5	5 th (mature).
(C) *Rana tagoi*; May-July, 1977.					
25	1	100	5	5.0	4 th, 4 th (molt), 5 th (immature).
35	1	〃	24	24.0	5 th (mature).
50	2	〃	0	0.0	
(D) *Rana rugosa*: July-Aug., 1977.					
15	2	50	7	7.0	5 th (mature).
21	2	〃	3	3.0	5 th (mature).
(E) *Cynops pyrrhogaster*; Oct.-Nov., 1976.					
2	2	20	5	12.5	3 rd.
6	2	〃	2	5.0	3 rd.
12	1	40	0	0.0	
21	1	〃	0	0.0	
23	4	20	0	0.0	
(F) *Cynops pyrrhogaster*; May-June, 1977.					
25	1	50	0	0.0	
45	2	〃	0	0.0	
〃	1	36	0	0.0	

R. rugosa on the 35th, 45th, and 15th and 21st days after infection, respectively: Figs. 6, 7, 8, 10, 14 and 15).

Body filiform, slightly tapered in anterior extremity. Caudal part of male bends ventrad. Cuticle with spinous rings and inconspicuous annulations. Fine striations are seen at posterior part of male. First and second spinous rings are closer together. First ring composed of 32 to 34 spines. In succeeding rings, spines decrease their sizes gradually and disappear in posterior part of male while in female they persist to tail. Mouth with two small lateral pseudolabia. Submedian papillae and amphids are seen. Buccal cavity funnel-shaped and very narrow in dorsoventral view. Esophagus divided into anterior muscular and posterior glandular portions. Glandular esophagus broader and longer than muscular portion. Excretory pore at base of fourth spinous ring. In male testis is a narrow tube, runs anteriorly and turns posteriorly behind the esophagointestinal junction, and is followed by vas deferens. Tail with caudal alae and 10 pairs of pedunculate papillae on them; four pairs preanal, one pair adanal and five pairs postanal. Last pair of postanal papillae very inconspicuous. Spicules dissimilar; one is very long, thin and with lateral membranes while the other is short and robust. In female, ovaries filiform, begin near the level of posterior end of esophagus, run posteriorly and are followed by two large seminal receptacles; one is anterior to vulva and the other at the same level of it. Vagina strong and very long, and runs posteriorly. Vulva at a short distance in front of anus. Eggs, thick-shelled, elliptical and embryonated at deposition.

Morphometric data are presented in Tables 2 and 3.

3. Natural infection.

Of 7 *Rana rugosa*, 2 *R. ornativentris* and 3 *Cynops pyrrhogaster*, only one *R. rugosa* was parasitized by 5 female *Spinitectus*. Morphology of these worms is same as those of experimentally infected ones. Morphometric

Table 2 Comparison of morphometric data among male adults of *Spinitectus* recovered from experimentally and naturally infected frogs; measurements in mm

Host	*Rana tagoi*	*Rana rugosa*	*Rana nigromaculata*	*Rana nigromaculata*
Experimental/Natural	experimental	experimental	natural	natural
Period of infection (days)	35	15	–	–
No. worms measured	10	3	–	–
Reporter	present authors	present authors	Morishita (1926)	Yamaguti (1941)
Body length	2.34 -2.68	3.40 -3.66	4 -5.2	3.6 -5.3
Body width	0.081-0.107	0.111-0.141	0.15 -0.16	0.1 -0.12
Length of buccal cavity	0.048-0.056	0.048-0.056	0.043	0.042-0.048
Length of muscular esophagus	0.167-0.207	0.207-0.215	0.33 -0.35	0.21 -0.3
Length of glandular esophagus	0.421-0.522	0.479-0.558	0.92 -1.03	0.71 -0.855
Length of tail	0.059-0.068	0.073-0.089	0.11	0.09 -0.12
Distance from cephalic apex to nerve ring	0.104-0.126	0.115-0.126	0.086*	
Distance from cephalic apex to excretory pore	0.155-0.207	0.211-0.215		
Distance from cephalic apex to first spinous ring	0.085-0.104	0.096-0.104	0.052-0.082	0.04 -0.16
No. of spinous rings	45-76	43-58	65-75	
Length of longer spicule	0.379-0.507	0.443-0.540	0.9 -0.93	0.57 -0.63
Length of shorter spicule	0.067-0.074	0.079-0.085	0.11 -0.12	0.1 -0.12

* Distance from anterior end of muscular esophagus.

Table 3 Comparison of morphometric data among females adults of *Spinitectus* recovered from experimentally and naturally infected frogs; measurments in mm unless stated otherwise

Host	*Rana brevipoda porosa*	*Rana rugosa*	*Rana rugosa*	*Rana nigromaculata*	*Rana nigromaculata*
Experimental/Natural	experimental	experimental	natural	natural	natural
Period of infection (days)	45	15, 21	–	–	–
No. worms measured	5	6	5	–	–
Reporter	present authors	present authors	present authors	Morishita (1926)	Yamaguti (1941)
Body length	3.60 -4.05	4.11 -5.04	7.46 -7.94	4. -6.7	4.0 -5.2
Body width	0.093-0.133	0.157-0.194	0.263-0.289	0.2 -0.23	0.12 -0.15
Length of buccal cavity	0.056-0.063	0.056-0.063	0.059-0.074	0.043	
Length of muscular esophagus	0.229-0.263	0.235-0.259	0.303-0.344	0.32 -0.37	0.25 -0.3
Length of glandular esophagus	0.522-0.680	0.595-0.674	0.972-1.102	1-1.32	0.78 -0.9
Length of tail	0.056-0.067	0.054-0.074	0.067-0.083	0.078-0.095	0.078-0.084
Distance from cephalic apex to nerve ring	0.107-0.133	0.133-0.141	0.130-0.185	0.082*	
Distance from cephalic apex to excretory pore	0.222-0.252	0.222-0.289	0.289-0.315		
Distance from cephalic apex to first spinous ring	0.115-0.126	0.115-0.130	0.141-0.159	0.06 -0.073	0.080-0.100
No. of spinous rings	103-131	106-127	122-218	150-175	
Distance from caudal end to vulva	0.280-0.377	0.289-0.337	0.428-0.595	0.35 -0.38	0.32 -0.38
Dimensions of eggs, μ	37×22	37-41×22		34×22	30-33×18-21

* Distance from anterior end of muscular esophagus.

data are given in Table 3.

Discussion

The morphological characteristics of the present adults clearly indicate their name as *Spinitectus ranae* Morishita, 1926. The morphometric data in Tables 2 and 3 show also close correlation with those presented by Morishita (1926) and Yamaguti (1941) although the present specimens are generally smaller. In spite of their sizes, the length of buccal cavity and the distance from cephalic apex to first spinous ring of the present specimens are longer than those observed by these investigators. These disaccordances are, however, thought to be resulted from the difference in conditions of fixation because cold fixatives cause shrinkage of head while hot ethanol makes head straight. Although Morishita described 9 pairs of caudal papillae, number and position of them in the present specimens agree with the report by Yamaguti (1941).

To the authors' knowledge, the life history of *S. ranae* has not been studied, and in this study the larval stages and development in the final host are believed to be firstly elucidated. Although the development in the intermediate host is not observed yet, the life cycle of *S. ranae* is supposed as follows. The embryonated eggs deposited in the feces do not hatch until they are eaten by the intermediate host, the freshwater shrimp. They hatch in the digestive tract of the host and penetrate into the muscles where they encyst and develop to the infective third-stage. The encysted larvae in muscles wait to be eaten by the final host, the frog. In the final host, they inhabit in the stomach and become adults.

Many factors such as temperature and nutrition of the host might influence on the growth of *S. ranae* in the final hosts. The season might be also involved in these

factors because the development of the larvae was greatly retarded in late autumn and winter even though the frogs were maintained in relatively warm condition (Table 1). In the present experiments, differences in the growth rate of worms were noticed among host species. Captive and reared condition of frog is also considered to influence on the growth. Of three species of frogs used, *R. rugosa* seems to be the most favorable host for *S. ranae* under the experimental condition.

Up to date, four contributions have been made on the life histories of *Spinitectus*. In 1939, Gustafson reported that he had obtained adult worms of *S. gracilis* from experimentally infected fish on the third day after infection. His finding seems to be incorrect because such a short period is not enough to allow the parasites to grow into adults in the cold-blooded vertebrates. Yamaguti and Nishimura (1944) and Johnson (1966) recorded *Spinitectus* larvae from the freshwater shrimps in Japan and India, respectively, and regarded these larvae as the parasites of fish. The authors, however, speculate that the larvae from *Neocaridina denticulata* (Yamaguti and Nishimura, 1944) might be *S. ranae*.

Recently, Keppner (1975) studied precisely the life cycle of *S. micracanthus*, the parasite of the freshwater fish. He observed only one molt in the final host, and discussed his results in two manners, namely, (1) one more molt, not observed, might occur in the intermediate host or in the final host before the molt observed by him in the fish, (2) the third-stage larvae might molt directly to juvenile adults without an intervening fourth-stage larva. However, the authors suppose that another interpretation of his data is also possible. The worm in Fig. 22 (Juvenile adult after him) of his report shows a "dart-like process" at tail tip while the adult in Fig. 23 lacks it. Since such cuticular structures may not be eliminated without passing through the molt, it is strongly suggested that a molt might occur in the final host of his experiments after the observed molt. In this view the worm in Fig. 22 is considered to be the fourth-stage larva.

Summary

1. *Spinitectus* larvae from the freshwater shrimps, *Paratya compressa improvisa*, were administrated orally to the frogs, *Rana tagoi*, *R. brevipoda porosa* and *R. rugosa*.

2. The larvae developed to the adult stage in these three species of frogs, and were identified as *S. ranae* Morishita, 1926 (Cystidicolidae).

3. This is considered to be the first report on the life history of *S. ranae*.

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Spinitectus ranae Morishita, 1926 (Cystidicolidae : Nematoda) の生活史

長谷川英男　大鶴　正満
(新潟大学医学部医動物学教室)

ヌカエビ *Paratya compressa improvisa* より得た *Spinitectus* 属線虫幼虫をタゴガエル *Rana tagoi*, ツチガエル *R. rugosa* およびトウキョウダルマガエル *R. brevipoda porosa* に経口投与した．その結果は

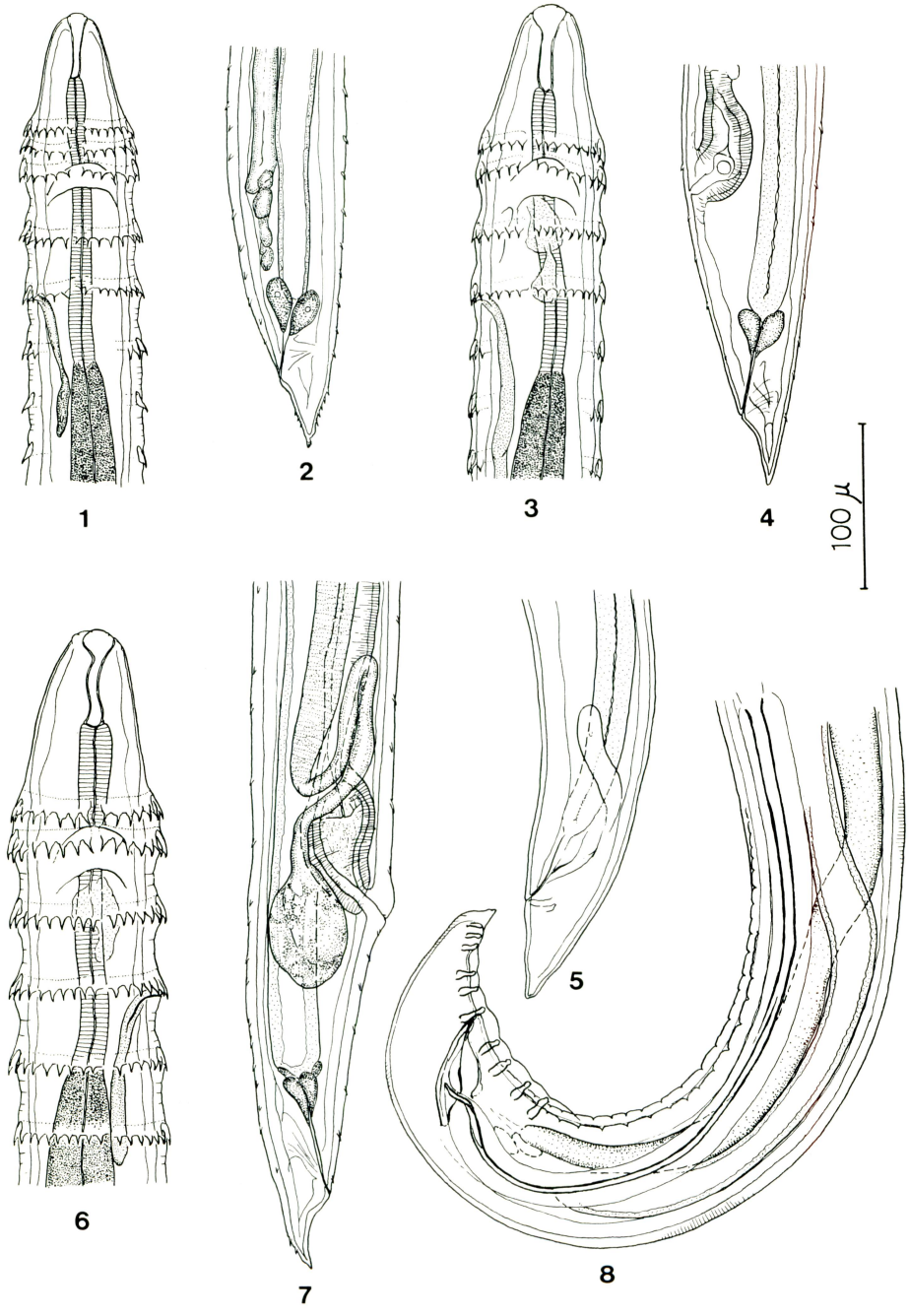
1) この幼虫は，これら3種の実験的終宿主内で発育して成虫となり，その形態から *S. ranae* Morishita, 1926 と同定された．

2) 本報は *S. ranae* の生活史についての最初のものと考えられる．

Explanation of Figures

Figs. 1 and 2, Third-stage larva recovered from *Paratya compressa improvisa*. 1, head (female), 2, tail (female). Figs. 3-5. Fourth-stage larvae recovered from *Rana brevipoda porosa*. 3, head (female), 4, tail (female), 5, tail (male). Figs. 6-8. Adults recovered from *Rana tagoi* on the 35th day after infection. 6, head (female), 7, tail (female), 8, tail (male).

Fig. 9 Third-stage larva in abdominal leg of *Paratya compressa improvisa*. (× 43)
Fig. 10 Adult in stomach of *Rana rugosa*. (× 30)
Fig. 11 Molting third-stage larva. Orders of spinous rings of third and fourth stages are presented in Roman and Arabic numerals, respectively. (× 430)
Fig. 12 Third-stage larva (× 30)
Fig. 13 Fourth-stage female. (× 30)
Fig. 14 Mature female. (× 30)
Fig. 15 Mature male. (× 30)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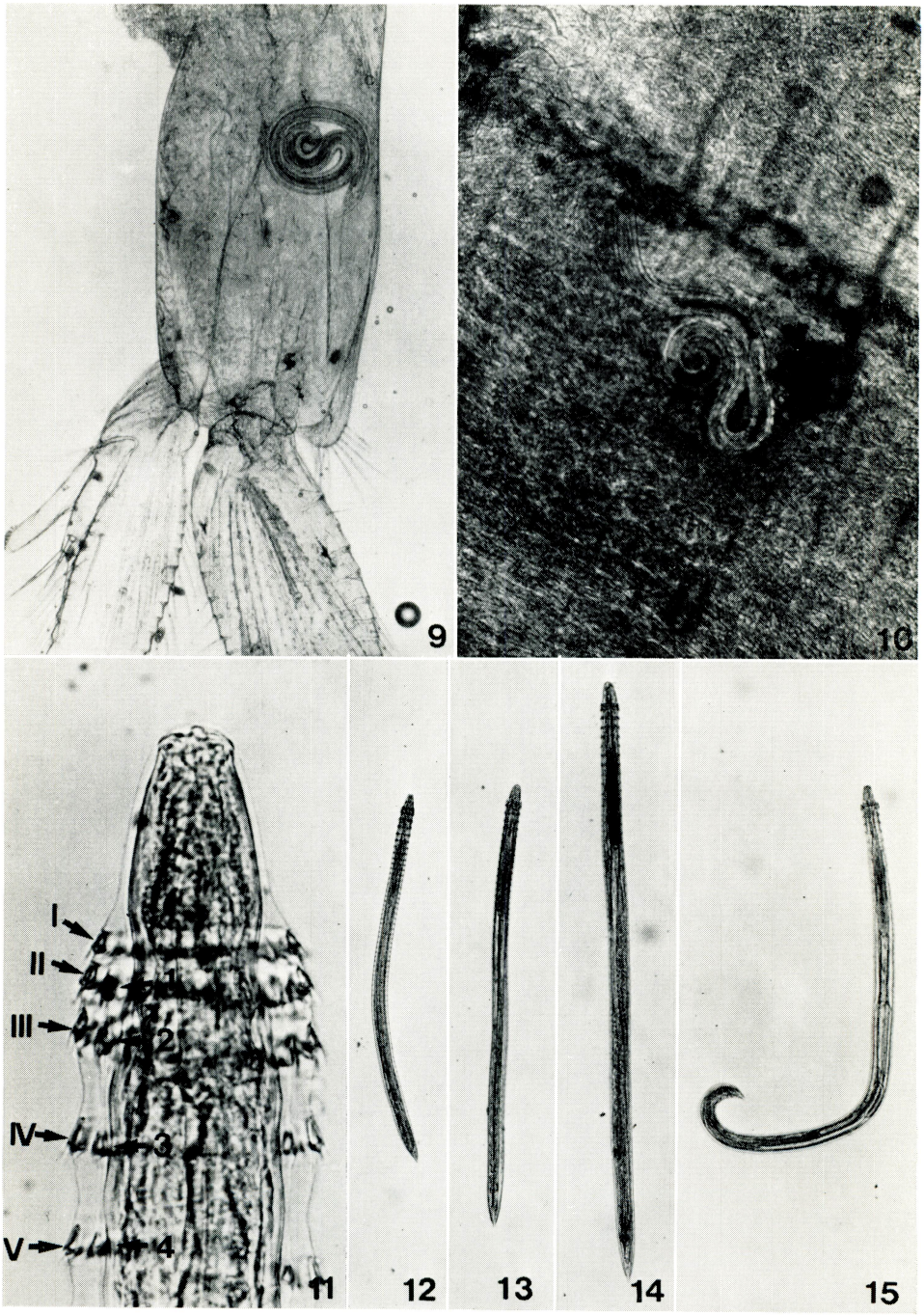
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