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Notes on the Life Cycle of *Spiroxys japonica* Morishita, 1926 (Nematoda : Gnathostomatidae)

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During the epidemiological surveys on the larva migrans due to nematodes, a species of larval spiruroid belonging to the genus *Spiroxys* was recovered from the loach, *Misgurnus anguillicaudatus*. Since larval forms of several *Spiroxys* species parasitizing turtles have been known from freshwater fishes (Hedrick, 1935; Moravec and Barus, 1971; Wiles, 1975), the present species was at first considered to be a reptilian parasite (Hasegawa and Otsuru, 1977). However, experiments carried out subsequently revealed that this species was an amphibian nematode, *Spiroxys japonica* Morishita, 1926.

This paper deals with the results of these experiments and discussion on the life cycle of this parasite.

Materials and Methods

1. Collection and maintenance of hosts.

The loaches, *Misgurnus anguillicaudatus*, infected with *Spiroxys* larvae, were captured at Hachiro-Gata, Akita Prefecture. The loaches as experimental hosts were bought from a dealer in Niigata City (locality unknown). These fish were reared in aquaria (23×15×18 cm) at room temperature and allowed to eat the laboratory mouse foods (MF of Oriental Kobo Kogyo Ltd., Tokyo).

The frogs, *Rana tagoi*, *R. brevipoda porosa* and *R. nigromaculata*, were collected at Mt. Kakuda of Maki Town, suberbs of Niigata City and Kanazuka Area of Kajigawa Village, Niigata Prefecture, respectively. They were maintained at room temperature in aquaria of the same size used for the loaches. Up to six frogs were reared in each aquarium. They were forced to eat porcine kidney or liver at every second or third day.

The tadpoles of *Rana rugosa* were captured in Akita City.

The copepods, *Mesocyclops leuckarti*, were collected from a culture pond of the Laboratory of Animal Physiolgy, Faculty of Science, Niigata University. They were maintained in the pond water at 26±3 C.

2. Examination of natural infection.

The loaches of Hachiro-Gata, the tadpoles of *Rana rugosa* and the adults of *R. nigromaculata* were examined for *Spiroxys* larvae and adults. They were cut into chips and digested with the artificial gastric juice at 37 C. Worms were picked out from the residues. The digestive tracts of the adult frogs and some loaches were dissected out, incised, put between two glass plates and observed under dissecting microscope.

3. Experiment I (May-June, 1977).

Each of 4 frogs (*Rana tagoi*) was forced to eat the viscera of naturally infected loaches containing 50 larvae and was examined at various intervals. At autopsy, the digestive tracts were examined with dissecting microscope. When frogs died before a given period, necropsies were per-

formed subsequently. Six frogs served as controls. The body length of these frogs was 3.8 to 4.2 cm.

4. Experiment II (June-August 1977).

The eggs of *Spiroxys japonica* were obtained from the adults collected from *Rana nigromaculata*. They were cultured in tap water containing streptomycin at 26±3 C. When they hatched, the copepods, *Mesocyclops leuckarti*, were introduced into the culture dishes and allowed to eat the larvae for 3 hours. Subsequently, these copepods were put back to glass beaker containing the pond water without larvae and maintained at 26±3 C. They were autopsied at various intervals.

5, Experiment III (July-August, 1977).

The copepods, experimentally infected with *Spiroxys japonica* 15 to 25 days before, were put into aquaria containing the loaches (Body length 7.7 to 10.6 cm). Three days later, water in aquaria was exchanged to remove remaining copepods. Five or 10 days after the exchange of water, the loaches were killed and the viscera of them were examined with dissecting microscope.

6. Experiment IV (August, 1977).

The frogs, *Rana brevipoda porosa*, divided into three groups, namely A, B and C, composing of 3, 3 and 4 individuals, respectively. The body length of these frogs was 3.7 to 5.0 cm. Each frog of groups A and B was given orally the copepods which were on the 10 th and 15 th days after the experimental infection with the hatched larvae, respectively. Number of the larvae in one copepod was one to 3. Group C served as controls. Nine days later, the frogs were killed and the digestive tracts were examined.

7. Fixation and preparation.

Worms recovered from the frogs, tadpoles and loaches were preserved in physiological saline before fixation with 70 C 70% ethanol. For microscopical survey, they were cleared with glycerin alcohol solution and mounted on slides with 50% glycerin jelly. The larvae obtained from the copepods were moderately heated to halt their movement and observed. But after the 6 th day of infection, the worms from the copepods were treated as the larvae recovered from the other hosts.

Results

1. Natural infection

a) The larvae collected from loaches (*Misgurnus anguillicaudatus*) of Hachiro-Gata.

In the loaches the larvae were found encysted in the stomach and intestine wall and the mesentery (Figs. 1, 2, 13 and 16).

Description of larvae: Body small and slightly tapered in both extremities. Cuticle with fine transverse striations. Somatic musculature platymyarian. Head with two lateral pseudolabia which primitively tri-lobed. Cephalic papillae indistinct. Buccal cavity poorly developed. Esophagus club-shaped and composed of anterior muscular and posterior glandular portions but junction between them always inconspicuous. Near posterior end of esophagus three large nuclei seen. Tail conical. Measurements are shown in Table 1.

b) The larvae collected from tadpoles of *Rana rugosa*.

Morphological characteristics of the larvae collected from the tadpoles are identical with those of the larvae from the loaches. Measurements are stated in Table 1.

c) The adult worms and the larvae collected from *Rana nigromaculata*.

The incidence and intensity of infection of *Spiroxys* in the frogs are shown in Table 2. Adult worms parasitized the duodenum embedding their neck in the mucosa, while larvae were coiled or threaded within the muscle of stomach wall (Fig. 9).

Description of adult worms (Figs. 3 to 7 and 10): Body cylindrical and tapered in anterior extremity. Body around level of anterior end of intestine strongly constricted. Head with two large tri-lobed lips which seen clover-leaf-like in lateral view. Two spines, one in dorsal midline and the other

Table 1 Measurements of *Spiroxys* larvae recovered from naturally or experimentally infected hosts

Host	*Misgurnus anguillicaudatus*	*Rana rugosa* (tadpole)	*Rana nigromaculata*	*Rana tagoi*	*Mesocyclops leuckarti*	*Misgurnus anguillicaudatus*	*Rana brevipoda porosa*
Condition of infection	Natural	Natural	Natural	Experimental (34 days later)	Experimental (7 days later)	Experimental (10 days later)	Experimental (9 days later)
Locality	Hachiro-Gata	Akita City	Kanazuka				
No. of worms measured	14	13	15	11	5	5	6
Body length, mm	1.13−2.81	1.18−1.87	2.07−3.83	1.99−3.21	1.03−1.16	1.00−1.48	1.67−2.48
〃 (mean±S.D.)	1.79±0.54	1.59±0.18	3.30±0.52	2.78±0.34	1.09±0.04	1.24±0.15	2.04±0.29
Maximum body width	44−122	44− 70	93−181	78−144	39− 40	41− 44	59− 93
Length of esophagus	300−656	342−492	493−711	493−696	244−315	259−281	392−515
Distance from cephalic apex to nerve ring	118−148	124−178	174−259	176−226	104−126	100−118	133−170
Distance from cephalic apex to excretory pore	148−285	157−242	218−344	234−315	−	163−204	181−278
Length of tail	41− 74	48− 70	56− 78	48− 67	48− 56	43− 47	44− 63
Experiment No.				I	II	III	IV

Measurements in microns unless stated otherwise.

Table 2 Natural infection of *Spiroxys japonica* in *Rana nigromaculata* of Kanazuka area, Kajigawa Village, Niigata Prefecture

Date	Body length of frogs (cm)	No. of frogs	Incidence of adult worms (%)	Intensity of adult worms (mean)	Incidence of larval worms (%)	Intensity of larval worms (mean)
June 5, 1977						
	3.0−3.9	5	40.0	13.0	0.0	−
	4.0−4.9	3	0.0	−	0.0	−
	5.0−5.9	2	100.0	12.5	0.0	−
	6.0−6.9	4	75.0	12.5	0.0	−
	7.0−7.9	3	100.0	29.0	0.0	−
	Total	17	58.8	17.4	0.0	−
Sept. 5, 1977						
	3.0−3.9	9	0.0	−	44.4	8.3
	4.0−4.9	2	50.0	1.0	50.0	4.0
	5.0−5.9	2	0.0	−	100.0	4.5
	6.0−6.9	6	16.7	1.0	66.7	>50.0
	7.0−7.9	2	50.0	3.0	100.0	>50.0
	Total	21	14.3	1.7	61.9	>27.0

in ventral midline present between lips and body. Esophagus club-shaped and divided into two portions, anterior muscular and posterior glandular. Junction between them at the same level of nerve ring but inconspicuous.

Male (based on 20 specimens) : Body length 8.9 to 13.8 mm. Maximum body width 334 to 450 μ. Length of esophagus 1.33 to 1.85 mm. Distance from cephalic apex to the middle of nerve ring 0.52 to 0.64 mm and to excretory pore 0.73 to 0.99 mm. Tail conical, curved ventrad and 160 to 247 μ in length. Caudal alae moderately developed. Eleven pairs of caudal papillae present ; 4 pairs preanal and 7 pairs postanal. Last pair of preanal papillae and second pair of postanal papillae are very small and situated ventrally. Two spicules, almost equal and 0.12 to 0.19 mm in length.

Female (based on 20 specimens) : Body length 7.28 to 19.78 mm. Maximum body width 247 to 798 μ. Length of esophagus 1.16 to 1.85 mm. Distance from cephalic apex to the middle of nerve ring 0.45 to 0.75 mm, and to excretory pore 0.73 to 1.15 mm. Tail conical and 137 to 247 μ in length. Ditance from caudal apex to vulva 2.00 to 7.14 mm. Eggs short-elliptical, 59 to 67×44 to 52 μ in size. Egg shell thin and its surface stippled finely. At deposition, eggs were one- to two-cell stage.

Description of larvae : Morphology of the larvae were almost similar to those of the larvae recovered from naturally infected loaches or tadpoles, although the body size was markedly larger. Primordial reproductive organs in the larvae over 3 mm in body length also developed. In one type (probably male), they are packed in one rod ventrad to intestine, and the anterior part of this rod turns posteriorly. In the other type (probably female), they form three branches, one anterior and two posterior. One of the posterior branches is shorter and more ventral. Measurements of larvae are stated in Table 1.

2. Experiment I.

As shown in Table 3, the adult *Spiroxys japonica* were recovered from 3 of 4 frogs which were administrated infected loaches. Many larvae, coiled or threaded, were also found in the alimentary canal, especially in the stomach muscles of all frogs. These larvae were morphologically identical to those in the naturally infected loaches although the body size was markedly increased. Measurements of these larvae are stated in Table 1. Measuremets of adults are as follows. Male : Body length 6.87 to 9.54 mm. Maximum body width 218 to 276 μ. Esophagus length 0.87 to 1.12 mm. Distance from cephalic apex to the middle of nerve ring 363 to 486 μ, and to excretory pore 493 to 711 μ. Tail length 122 to 192 μ. Spicule length 0.82 to 1.03 mm. Female : Body length 8.22 and 10.71 mm. Maximum body width 290 and 305 μ. Esophagus length 0.99 and 1.26 mm. Distance from cephalic apex to the middle of nerve ring 392 to 522 μ, and to excretory pore 595 μ (only one specimen measurable). Tail length 141 and 167 μ. Distance from caudal apex to vulva 2.63 and 3.54 mm. Specimens were immature and eggs were not found.

Neither adult nor larva was found in the

Table 3 Infection experiment of *Spiroxys* larvae recovered from loaches to *Rana tagoi*

Frog No.	Duration of infection (days)	No. of recovered adults	Habitat and No. of recovered larvae				No. of total larvae recovered
			Stomach wall	Duodenum wall	Intestine wall	Rectum wall	
1	25	2	22	1	1	1	25
2	29	2	17	3	5	0	25
3	34	1	30	0	0	0	30
4	44	0	++	0	0	0	++

six control frogs.

3. Experiment II.

The eggs of *Spiroxys japonica* from naturally infected *Rana nigromaculata* developed in tap water, and sheathed larvae were seen within eggs on the 6 th day of culture. They hatched on the 7 th day and the larvae lashed vigorously in water (Fig. 11). The copepods, *Mesocyclops leuckarti*, readily ingested these larvae. The ingested larvae soon penetrated the wall of alimentary canal and entered the haemocoelom of copepods. The sheathes of larvae were shed during this process. Number of larvae in one copepod was 1 to 5. Movement of the infected copepods became slowly and easily to be caught.

Description of exsheathed larva : Body relatively thick, tapered in posterior extremity. Cuticle with fine and irregular striations. Body length 148 to 207 μ. Maximum body width 15 to 19 μ. Head without lips. Cephalic hook present. Esophagus club-shaped. Intestine somewhat inconspicuous but with granules. Tail long-conical and with dull tip.

On the third day after infection, the nerve ring appeared and outline of the intestine became obvious although the body sizes remained almost same as those at onset of infection. Many larvae grew quickly thereafter and molted on the 6 th to 8 th day after infection (Fig. 14), but some larvae, especially those in abdomen of copepods, remained in smaller sizes. The larvae of premolt stage ruptured at their head part unless dissection was performed in physiological saline.

Description of the larvae at premolt stage (Figs. 8 and 12) : Body slender 511 to 856 μ in length and 34 to 52 μ in maximum width. Cuticle with fine striations. Head rounded and with cephalic hook. Mouth opens at apex of head and funnel-shaped. Esophagus 178 to 278 μ in length. Nerve ring at about two-fifths of esophagus. Excretory pore at same level as nerve ring. Intestine with brown granules of various sizes. Primordial germ cells seen at level of the middle of intestine. Anus at 52 to 70 μ from caudal tip. A pair of lateral rounded projection present at base of tail. Body tapered suddenly behind anus.

Description of molted larvae recovered on the 7 th day after infection : The morphology of the molted larvae are identical with those collected from the naturally infected loaches or tadpoles. Measurements of larvae are shown in Table 1.

The molted larvae grew slowly thereafter. The body length of three larvae recovered on the 25 th day after infection was 1.10 to 1.19 mm.

More than 50 control copepods were examined and found free from any nematode.

4. Experiment III.

Spiroxys larvae were found from 4 of 5 loaches autopsied on the 5 th day after the termination of infection. One to 7 (mean 3.8) larvae were found coiled or threaded in the stomach serosa. Six of 10 loaches examined on the 10 th day also harboured 1 to 5 (mean 3.3) *Spiroxys* larvae. Eighty % of the recovered larvae were collected from stomach serosae, 15 % from intestine serosae and 5 % from mesenteries. Eight controls were free from *Spiroxys*.

Morphology of these larvae were identical with those of worms from naturally infected loaches. Measurements of larvae are shown in Table 1.

5. Experiment IV.

At autopsy, all frogs of groups A and B were parasitized by *Spiroxys* larvae. These larvae were found coiled or threaded within the stomach musculature (Fig. 15). One larva was found encysted on the stomach serosa. Number of larvae per one frog was 2 to 4 (mean 2.8). The body length of the larvae recovered from groups A and B were 2.04 ± 0.29 mm (n=6) and 1.99 ± 0.33 mm (n=6) (mean$\pm$SD), respectively. Morphological features were identical to those of the larvae obtained from naturally infected loaches although the body sizes were generally larger. Measurements are stated in Table 1.

Discussion

The results of the present experiments may suggest the life cycle of *Spiroxys japonica* to be as follows: The eggs are discharged into water with faeces, where they develop and sheathed larvae hatch out. They are ingested by the fresh-water copepods and they enter the haemocoelom of the copepods, shedding their sheathes. In the haemocoelom, they molt to infective larvae. These infective larvae may reach to the final host using one of the following routes; (1) the copepods are swallowed by the frogs, (2) the copepods are eaten by the fresh-water fishes which play as paratenic hosts, (3) the copepods are ingested by the tadpoles and the larvae become adults after the metamorphosis of the hosts, (4) the tadpoles eat the copepods and are eaten by large frogs after or before the metamorphosis. In the final hosts, the larvae firstly migrate into the stomach wall and grow slowly. Then, they move to the duodenum and became adult after one (probably) molt.

In this cycle, the copepod is intermediate host and the freshwater fishes and the tadpoles are thought to be paratenic hosts, because the larvae did not show any essential development in these vertebrates.

The authors suppose that the routes (1) and (2) among previously mentioned ones may not be involved in the usual life cycle because the frogs are terrestrial feeders generally and they must have limited chances to feed on the aquatic animals. The route (3) seems to be possible although no evidence regarding this route has ever been observed. If possible, this route is not the main one since the number of larvae in the stomach wall is generally larger in bigger (2 to 3 years old) frogs than in smaller (1 year old) frogs (Table 2). From the fact that adult frogs prey upon smaller ones time by time, the authors speculate that small frogs, metamorphosed from tadpoles harbouring the larvae, may play role as reservoir hosts and therefore the route (4) may be the most probable one.

The presence of the annual rythm in the development of *S. japonica* is strongly suggested by the survey of natural infection in the frogs. The authors suppose that the larvae in the frog stomach wall start to develop when the hibernation of the hosts are over. The larvae which are ingested during late summer and autumn may remain as larval stage until next spring although some developments, especially in the body size and the reproductive organs, occur in the stomach wall of the frogs.

The life cycle of *S. japonica* closely resemble that of *S. contortus* Rudolphi, the stomach parasite of the fresh-water turtle (Hedrick, 1935) and also that of *Gnathostoma* spp. Hedrick described the infective-stage larvae in the copepods as third-stage larvae. He might consider the sheath of the hatched larva as the cuticle of the first-stage larva and the larva with cephalic hook as the second stage. However, this interpretation does not agree with the evidence that in the spiruroid nematodes the cephalic hook is highly characteristic to the first-stage larvae. Miyazaki (1966) also pointed out the presence of same discrepancies on the larval stages of *Gnathostoma* spp. These confusions should be solved by the careful comparative study on the whole life cycle of various gnathostomatid nematodes.

Besides fishes and tadpoles, reptiles and molluscs were known to play role as paratenic hosts of *S. contortus* and *S.* sp., respectively (Khromova, 1969; Sharpilo and Sharpilo, 1969), but the participation of these animals in the life cycle of *S. japonica* remains to be elucidated in future.

Summary

The life cycle of *Spiroxys japonica* Morishita, 1926, was studied. Copepod, *Mesocyclops leuckarti*, was found to be the intermediate host and the loach, *Misgurnus anguillicaudatus*, and the tadpole of *Rana rugosa* were known to play as paratenic hosts. The mode of infection, annual rythm in the life cycle and stages of the larvae

were discussed.

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Spiroxys japonica Morishita, 1926 (Nematoda: Gnathostomatidae) の生活史

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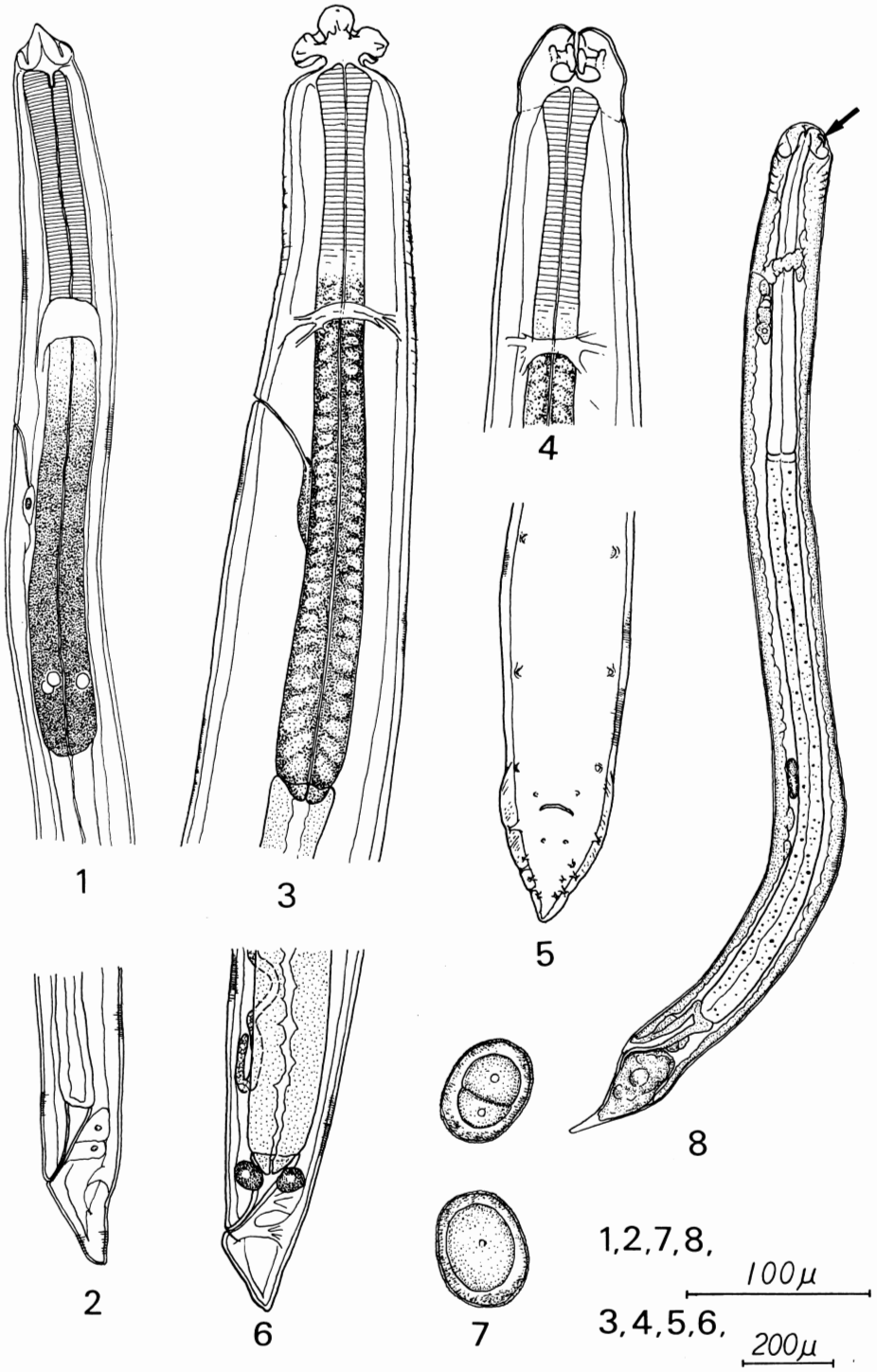
Spiroxys japonica の虫卵は，水中で孵化し，被鞘を有する幼虫となつて遊泳する．この幼虫は中間宿主のケンミジンコ *Mesocyclops leuckarti* に摂食されると，被鞘を脱して血体腔に入り，1回脱皮して感染幼虫となる．ドジョウ *Misgurnus anguillicaudatus* およびツチガエル *Rana rugosa* の蝌蚪が paratenic host となりうることが示された．

生活史における食物連鎖と季節の関与，および幼虫の期について考察した．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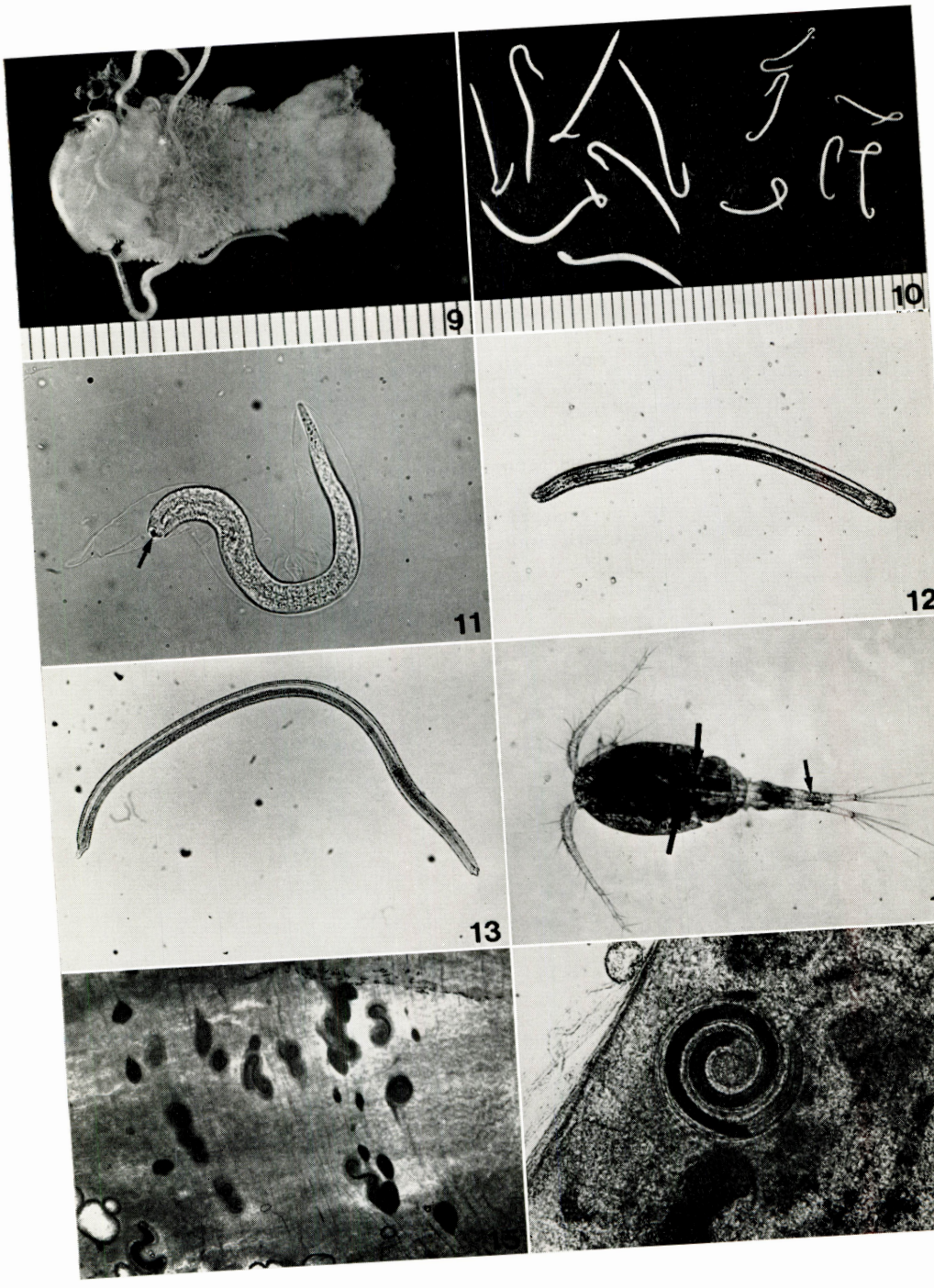
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Explanation of figures

Figs. 1 and 2 *Spiroxys* larva recovered from naturally infected loach. 1, head and 2, tail in lateral view.

Figs. 3 to 7 *Spiroxys japonica* adults from *Rana nigromaculata*. 3, head in lateral view. 4, head in dorsal view. 5, male tail in ventral view. 6, female tail in lateral view. 7, eggs.

Fig. 8 *S. japonica* larva recovered from *Mesocyclops leuckarti* on the 7 th day after infection in lateral view. Arrow indicates cephalic hook.

Fig. 9 Duodenum of *Rana nigromaculata* parasitized by *S. japonica*, natural infection. (× 1.9)

Fig. 10 Adults of *S. japonica*. Females (left) and males (right). (× 1.4)

Fig. 11 Sheathed larva of *S. japonica*. Arrow indicates cephalic hook. (× 303)

Fig. 12 Premolt larva recovered from *Mesocyclops leuckarti* on the 6 th day after infection. (× 87)

Fig. 13 *Spiroxys* larva recovered from naturally infected loach. (× 46)

Fig. 14 *Mesocyclops leuckarti* infected with *S. japonica*. Large arrows indicate molted larvae and small arrow shows undeveloped larva. (× 24)

Fig. 15 *Spiroxys* larvae in stomach wall of experimentally infected *Rana brevipoda porosa*. (× 8)

Fig. 16 *Spiroxys* larva in intestine serosa of naturally infected loach. (× 49)