FOLIA PARASITOLOGICA 42: 135–142, 1995

On the development and life cycle of *Spirocamallanus mysti* (Nematoda: Camallanidae)

N. C. De

Helminthology Laboratory, Zoology Department, Kalyani University, Kalyani, West Bengal, 741235, India

Key words: *Spirocamallanus*, copepods, life cycle, *Mystus*

Abstract. The development of *Spirocamallanus mysti* (Karve, 1952) was studied in the copepod hosts *Mesocyclops crassus* (Fischer) and *M. leuckarti* (Claus) and in the fish host *Mystus vittatus* (Bloch). When eaten by copepods the first-stage larvae burrow through the intestinal wall into the haemocoel and there they moulted twice to become the third, infective stage. The first moulting occurred on day 4 p.i. at 18–21°C (on day 6 p.i. at 16–20°C) and the second moultingoccurred on day 8 p.i. at 18–19.5°C (on day 11 p.i. at 16–20°C. Further development occurred only after reaching the stomach of the fish definitive host. In the fish stomach two more larval moultings occurred, the third on day 15 p.i. and the fourth (final) on day 37 p.i. in "male" larvae and day 67 p.i. in "female" larvae. The individual developmental stages and the morphological changes occurring during development are described in detail.

Spirocamallanus mysti (Karve, 1952) is a common nematode parasite inhabiting the stomach of different species of freshwater fishes that are widely distributed over the Indian subcontinent (see De et al. 1986a). Bashirullah and Ahmed (1976) studied the larval development of *S. intestinecolas* (Bashirullah, 1973) (= *S. mysti*) in the intermediate copepod host only. The present study was undertaken to work out the life cycle and development of the species in the intermediate copepod host as wall as in the definitive fish host.

MATERIALS AND METHODS

The nematodes *S. mysti* were recovered from the stomach of *Mystus vittatus* (Bloch) collected from the local fish market at Kalyani, West Bengal. The worms were washed thoroughly in 0.85% saline and examined under light microscope. The gravid females were sorted out for experimental use and others were fixed and conserved in 10% formalin. The females separated were kept in the small petri dishes (5 cm) (four females in each petri dish) containing filtered tap water and kept overnight at room temperature to allow them to release larvae. The evacuated females were then taken out and fixed, and stored in 10% formalin. The content of each petri dish was then poured into glass beaker (250 ml) each containing filtered pond water up to one third. Fifty previously starved, laboratory reared copepods, *Mesocyclops crassus* (Fischer) and *M. leuckarti* (Claus) were then added in each beaker. After three hours' exposure the copepods from each beaker were transferred to separate beakers (500 ml), three fourth of which had been previously filled up with filtered pond water. Few algal filaments were given to each such beaker. The beakers were then kept at the average laboratory temperature ranging between 16–20°C, 18–19.5°C, 19–20°C and 18–21°C. The copepods from each beaker were then teased by fine needles on the glass slide and examined under light microscope at regular intervals starting from 1 hour p.i. For experiments, a total of 36 *M. vittatus* reared in the laboratory for more than one year were used. To infect the fishes four infected copepods were forcibly pushed into the stomach of each fish by means of a long nozzled dropper. Then, following a regular schedule, the fishes were examined at autopsy on different days p.i. between 7 and 83. All larvae i.e. those obtained from the uteri of the female worms and those from the haemocoel of the copepods and the stomach of the fishes were studied following the methods adopted by De et al. (1986b). All measurements are in micrometres (μm) unless mentioned otherwise.

RESULTS

Experimental infection of copepods

Larvae released by the gravid females tend to stay at the bottom of the water column in the beaker and coil and uncoil their bodies at regular short intervals with their tails attached to the substratum. The copepods seem to be attracted by such body movements of the larvae and start to ingest them. The ingested larvae on reaching the intestine of the host bore through its wall and lodge themselves into the haemocoel by the 3rd hour and there they continue to develop further. The first-stage larvae initially grow but no morphological changes occur. Later, both cuticle and wall of the oesophagus become thickened and consequently, the spacious oesophageal cavity turns to a narrow canal. Oesophageal gland cells with distinct nuclei are seen at posterior end of oesophagus. At water temperatures of

Table 1. Measurements of different larval stages of *Spirocamallanus mysti* in *Mesocyclops crassus* and *M. leuckarti.*

	Free larvae in utero	From copepods				
		First stage 1–5 d.p.i.	First moulting 4, 6 d.p.i.	Second stage 5–9 d.p.i.	Second moulting 8–11 d.p.i.	Third stage 8–16 d.p.i.
Body length	312 – 352	329 – 486	463 – 484	490 – 862	650 – 692	616 – 860
Body width	15 – 18	15 – 30	26 – 28	28 – 40	25 – 30	23 – 42
Length of buccal capsule	–	–	–	–	19 – 21	19 – 24
Length of muscular oesophagus	–	–	69 – 74	79 – 149	129 – 138	108 – 146
Length of glandular oesophagus	–	–	32 – 36	57 – 108	98 – 102	85 – 117
Length of entire oesophagus	57 – 64	48 – 113	101 – 110	136 – 257	231 – 238	193 – 263
Nerve ring from anterior end	30	23 – 68	66 – 70	40 – 89	59 – 78	55 – 72
Excretory pore from anterior end	45 – 55	36 – 57	44 – 62	57 – 127	83 – 95	72 – 119
Tail length	85 – 113	66 – 117	62 – 68	42 – 82	35 – 41	32 – 42

16–20°C almost all larvae become sheathed (i.e. they lie within old cuticular exuviae) between day 4 and 6 p.i. The first moulting occurred on day 4 p.i. at 18–21°C (on day 6 p.i. at 16–20°C). The second moult after which the larvae attain the third, infective stage occurred on day 8 p.i. at 18–19.5°C (on day 11 p.i. at water temperatures 16–20°C). The 3rd-stage larvae were found located mainly in the cephalothorax of copepods.

In the present series of experiments the prevalence of infection of copepods was 39% and the intensity of infection ranged from 1–4.

Experimental infection of fishes

The 3rd-stage larvae grow and moult while they are in the stomach of the fish host. The 3rd moulting occurred on days 15 and 16 p.i. The fourth i.e. final moulting occurred at different times for the "male" and "female" larvae, on day 37 p.i. in "male" larva but on day 67 p.i. in "female" larva.

Descriptions of the developmental stages of *S. mysti*

a) First-stage larvae from uterus Fig. 1

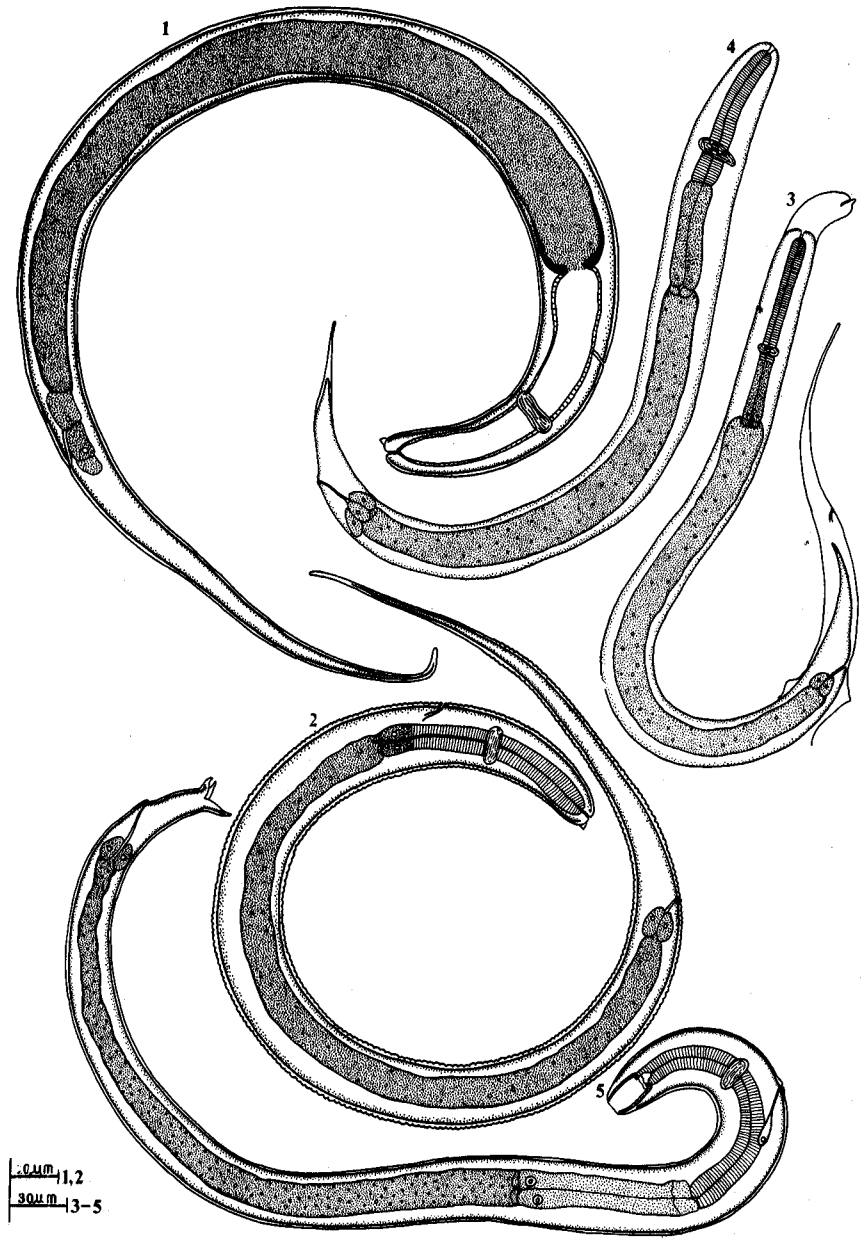
Body translucent, slender with attenuated posterior part. Cuticle thick, transversely striated. Length of body 312–352, width 15–18. Anterior end rounded, provided with dorsal dentate process. Short buccal tube (length 2) leads to thin-walled oesophagus (length57–64) with specious cavity inside. Nerve-ring surrounds oesophagus at distance of 30 from anterior end. Excretory pore lies posterior to nerve-ring (45–55 from cephalic end). Intestine thin-walled, wide with fine granulations inside. A short, narrow rectum follows intestine, large rectal glands present at their junction. Tail elongate 85–113 long, slender.

b) First-stage larvae from copepod host Fig. 2

Larvae studied between day 1 and 5 p.i. measure 329–486 in length, 15–30 in width (for other measurements see Table 1). Thick-walled oesophagus with narrow lumen inside; anterior muscular part continuous with posterior glandular part. Intestine with small granules. Tail elongate, slender.

c) Second-stage larvae Figs. 3–4,6a,b

At onset of first moulting old cuticle becomes loosened starting both from anterior and posterior ends. Larva within exuviae of old cuticle bears long, slender body (463–484 in length) with thin smooth cuticle. Anterior end of larvae rounded, without dorsal dentate process. Oesophagus indistinctly divided into anterior muscular (69–74 long), and posterior glandular (32–36 long) parts. Two small valves present at junction of oesophagus and intestine. Nerve-ring surrounds posterior half of muscular oesophagus (66–70 from cephalic end). Excretory pore lies a little ahead of nerve-ring, 44–62 from anterior extremity. Intestine long, wide, followed by short, narrow rectum; three large, oval rectal glands present at their junction. Tail elongated conical (62–68 long) ending in a sharp point. Second-stage larvae first obtained from copepods on day 5 p.i. at 16–20°C. At early stage cuticle remains thin, indistinctly striated. On days 5, 7, 8, and 9 p.i., larvae measure

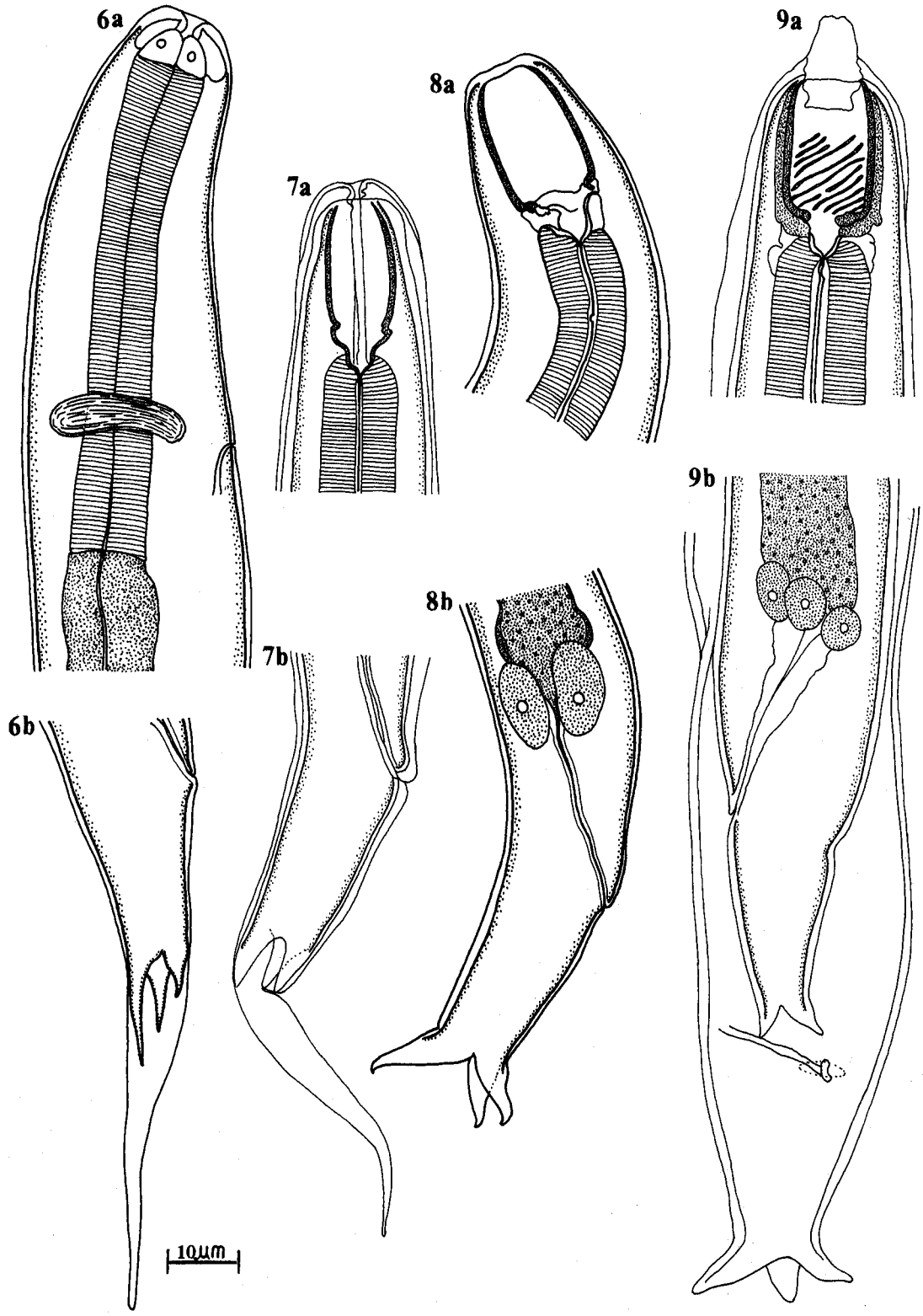


Figs. 1–5. *Spirocamallanus mysti.* **Fig. 1.** Free first-stage larva. **Figs. 2–5.** Development of larva in copepod. **Fig. 2.** First-stage larva on day 2 p.i. **Fig. 3.** Larva undergoing first moult on day 4 p.i. **Fig. 4.** Second-stage larva on day 7 p.i. **Fig. 5.** Third-stage larva on day 8 p.i.

490–862 in length, 28–40 in width. Anterior end rounded, without dorsal dentate process. Short buccal tube (4 long) opens into oesophagus. Small sclerotized "cap" appears at anterior end of muscular oesophagus. In older second-stage larvae "cap" enlarges, becomes bell-shaped, subsequently forms buccal capsule. Bipartite oesophagus with longer anterior muscular (79–149 long), and posterior glandular (57–108 long) parts. Distinct cell nuclei present at posterior wider part of glandular oesophagus. Two distinct valves present at oesophageo-intestinal junction. Nerve-ring encircles anterior muscular oesophagus at its second half (40–89 from head end). Excretory pore lies little posterior to nerve-ring (57–127 from anterior extremity). Intestine long, wide tube leading to a short, narrow rectum; three large rectal glands present at their junction. Elongated conical tail (42–82 long) ends in a pointed tip. Genital primordium could not be located.

d) Third-stage larvae (infective stage) from copepods
Figs. 5, 7a,b, 8a,b

Shortly prior to second moult, larvae within exuviae of old cuticle possess feebly sclerotized but well formed



Figs 6–9. *Spirocamallanus mysti* (a – anterior end of body, b – tail end). **Fig. 6.** Late second-stage larva on day 5 p.i. **Fig. 7.** Larva undergoing second moult on day 11 p.i. **Fig. 8.** Third-stage larva on day 8 p.i. **Fig. 9.** Larva undergoing third moult on day 15 p.i.

buccal capsule, short and wide tail ending in three large mucrones. At second moulting stage larvae measure 650–692 in length, 25–30 in width. Cuticle indistinctly striated. Buccal capsule 19–21 long. Oesophagus with 129–138 long muscular, 98–102 long glandular parts. Part of cuticular inner lining of oesophagus seen to pass out through buccal capsule at this stage. Nerve-ring and excretory pore lie at 59–78 and 83–95 respectively from cephalic end. Tail measures 35–41.

Fully formed third-stage larvae first appeared on day 8 p.i. at 18–19.5°C temperature. Larval body relatively large (616–860 long, 23–42 wide) with thick, distinctly striated cuticle. Buccal capsule well sclerotized, without any ridge on inner surface, measures 19–24 in length, 12–15 in width. Muscular oesophagus measures 108–146. Shorter, wider glandular oesophagus (85–117 long) with distinct cell nuclei at its end. Oesophageo-intestinal junction possesses a valvular apparatus. Nerve ring and excretory pore lie at 55–72 and 72–119 respectively, from head end. Thin-walled, wide intestine leads to short, narrow rectum with cuticular inner lining; three small unicellular rectal glands present at their junction. Tail (32–42 long) ends in one large dorsal and two smaller subventral mucrones.

e) Third-stage larvae from fish

Third-stage larvae of *S. mysti* were obtained from the stomach of experimentally infected fishes (*Mystus vittatus*) between day 13 and 16 p.i. Morphology of these larvae was similar to that of infective stage larvae from copepods except for the slightly larger body measurements (Table 2).

f) Fourth-stage larvae Figs. 9a,b, 10a,b, 12

During 3rd moulting larvae measure 988 × 36, bear indistinctly striated cuticle. Weakly sclerotized newly formed buccal capsule (23 long, 17 wide) bearing two sets of 11–12 spiral ridges on inner surface lies outside old one. Distinct oesophageal cup present at base of buccal capsule. Oesophagus with 132 long anterior muscular 123 long posterior glandular parts. Nerve-ring and excretory pore lie at distance of 85 and 146 respectively, from cephalic end. Tail (35 long) bears one large dorsal and two smaller subventral mucrones. Fourth-stage larvae first appeared on day 15 p.i. Fully formed fourth-stage larvae (1164–1714 long, 34–53 wide) possess thick, distinctly striated cuticle. Buccal capsule (28–38 long) well sclerotized, with two sets of spiral thickenings (12–14 in number on each set) on inner surface. Short oesophageal cup lies at base of buccal capsule. Muscular part of oesophagus 117–204. glandular part 144–208. Nerve-ring, measures deirids and excretory pore lie at 72–104, 90–122 and 149–178, respectively, from head end. Tail 28–43 long, bears three large mucrones distally. Sheathed "male" larvae were found on day 31 p.i. Larvae within exuviae of old cuticle measure 1472–1692 in length, 36–43 in width. Cuticle faintly striated. Feebly sclerotized new buccal capsule (23 long) appears outside old one. Caudal papillae as also spicules and gubernaculumbecome visible. Tail conical (30 long), with blunt tip baring very small mucrones.

g) Adults Figs. 11a–c, 13

Male. Fourth moulting first occurred on day 37 p.i. Morphology of moulting male is almost in complete keeping with that of fully mature male, differs mainly in degree of sclerotisation and number of spiral ridges of buccal capsule, presence of minute tail mucrones. During 4th moulting the male measures 1578 in length, 43 in width. Buccal capsule 28 long. Muscular part of oesophagus measures 147, glandular part 136. Nerve-ring 83 from anterior end. Excretory pore could not be located. Tail (32 long) still bears two very small mucrones.

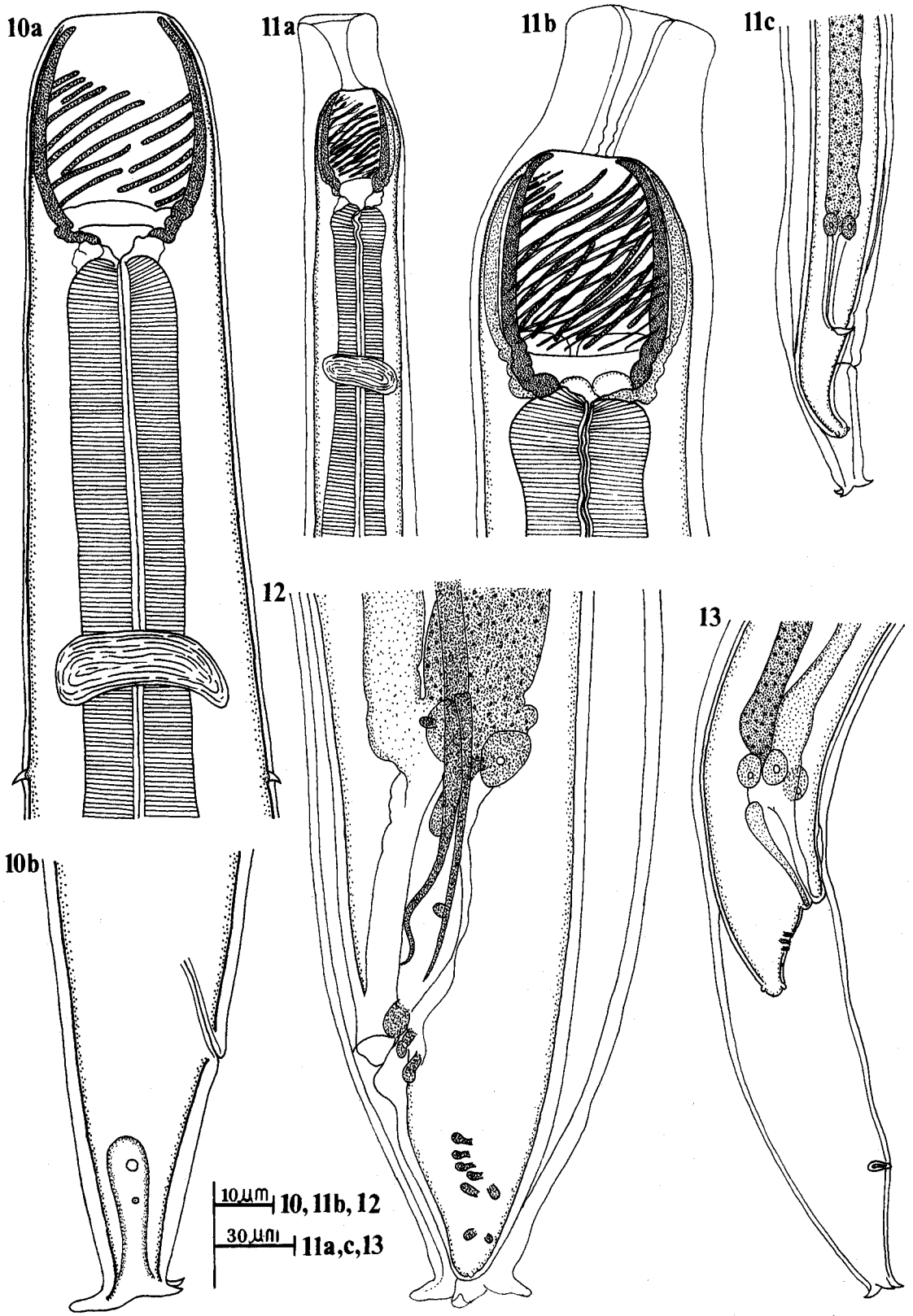
Female. Fourth moulting female first appeared on day 67 p.i. At this stage worm measures 1790 in length, 36 in width. Buccal capsule 37 long. Oesophagus with 163 long muscular, 172 long glandular parts. Nerve-ring lies at distance of 104 from cephalic end. Excretory pore not found. Both vulva and vagina developed. Tail (40 long) conical with blunt tip bearing three small mucrones. Young females start to grow. Maturation time of female could not be determined due to insufficient number of experimental fishes.

DISCUSSION

As far as the author is aware, the development of only four fish infecting species of the genus *Spirocamallanus* has so far been studied: *S. fluvidraconis* (Li, 1935) by Li (1935); *S. cearensis* (Pereira, Dias et Azevedo, 1936) (= *S. hilarii* Vaz et Pereira, 1954) by Pereira et al. (1936); *S. intestinecolas* Bashirullah, 1973 (= *S. mysti*) by Bashirullah and Ahmed (1976) and *S. cricotus* Fusco et Overstreet, 1978 by Fusco (1980). In general, *S. mysti* follows the course of development similar to that of other members of the family Camallanidae. The first-stage larvae moult twice to give third, infective stage. Li (1935) and Pereira et al. (1936) reported the occurrence of only one moult in *S. fluvidraconis* and *S. carensis*. Their descriptions of the first and second-stage, however, led Ivashkin et al. (1971) and Moravec (1975) to conclude that the first moult in both the cases were overlooked. The first stage has

Table 2. Measurements of different larval stage and adults of *Spirocamallanus mysti* in *Mystus vittatus* (in micrometres unless otherwise stated).

	Third- stage		Third moulting stage 15 d.p.i.	Fourth stage unsheathed 15, 16, 31, 36 d. p.i.	Fourth stage sheathed male 31 d.p.i.	Fourth moulting stage		Adult	
	unsheathed 13–16 d.p.i.	sheathed 13–16 d.p.i.				male 37 d.p.i.	female 67 d.p.i.	male 42–75 d.p.i.	female 72–83 d.p.i.
Body length (mm)	0.851 – 0.983	0.996 – 1.370	0.988	1.164 – 1.714	1.472 – 1.692	1.578	1.790	1.675 – 4.551	3.029 – 4.980
Body width	36 – 40	32 – 47	36	34 – 53	36 – 43	43	36	50 – 89	69 – 100
Length of buccal capsule	21 – 26	24 – 32	23	28 – 38	23	28	37	43 – 47	53 – 59
Length of muscular oesophagus	130 – 142	130 – 180	132	117 – 204	149 – 174	147	163	198 – 230	225 – 310
Length of glandular oesophagus	113 – 136	120 – 173	123	144 – 208	152 – 166	136	172	225 – 300	278 – 408
Length of entire oesophagus	244 – 276	251 – 353	255	289 – 410	301 – 340	283	335	423 – 529	503 – 716
Nerve ring from anterior end	70 – 81	76 – 98	85	72 – 104	78 – 96	83	104	98 – 108	115 – 132
Excretory pore from anterior end	113 – 146	146 – 163	146	149 – 178	166 – 178	–	–	197 – 297	261 – 285
Tail length	28 – 40	38 – 45	35	28 – 43	30	32	40	33 – 36	36 – 38



Figs. 10–13. Development of *Spirocamallanus mysti* larva in *Mystus vittatus*. **Fig. 10.** Fourth stage on day 15 p.i. (a – anterior end of body, b – tail end). **Fig. 11.** Female during fourth moulting, on day 67 p.i. (a – anterior end of body, b – anterior end of body, enlarged view, c – posterior end of body). **Fig. 12.** Tail end of sheathed fourth-stage larva ("male") on day 31 p.i. **Fig. 13.** Male during fourth moulting, on day 37 p.i.

characteristic dorsal dentate process at the cephalic end and an elongated tail. Later, the body cuticle and the oesophageal wall become thick and the dorsal dentate process becomes reduced and eventually casts off along with the cuticle during first moulting. Bashirullah and Ahmed (1976) recorded the presence of oesophagus with muscular wall throughout and two large anterior nuclei in the first-stage larvae of *S. intestinecolas*. In the present case, however, the first-stage larvae from the uterus of the female possess the oesophagus with membranous wall and spacious lumen inside. In the second-stage the larval body enlarges but tail becomes shorter. Early second-stage larvae still retain the buccal tube like the first-stage but later a small sclerotised "cap" appears and leads to the formation of buccal capsule prior to second moult. The oesophagus at the second-stage is clearly differentiated into anterior muscular and posterior glandular parts but Bashirullah and Ahmed (1976) reported that such differentiated oesophagus found only during the second moulting. In the late second-stage (prior to second moult) larvae a short and wide tail terminating in three distinct mucrones is seen within the old cuticle. Present third-stage larvae possess well formed buccal capsule but without any spiral ridge on inner surface. Spiral thickenings, however, were reported to be present on inner surface of the buccal capsule of the third-stage larvae of *S. cearensis* and *S. cricotus*. Tail mucrones are distinct in the present third-stage larvae.

Completion of the life cycle directly from the copepod to the final host was not attempted in any of the four above referred species of the genus *Spirocamallanus*. In the present case the copepods bearing infective stage larvae were forcibly pushed into the stomach of the laboratory reared *M. vittatus*. In the stomach of the fish host the larvae grow; their buccal capsule becomes large and well sclerotised, cuticle becomes thick and distinctly striated but tail end still bears three mucrones. Two larval moultings occur, the first moulting occurs on day 15 p.i. During moulting new buccal capsule with faint spiral ridges is seen outside the old smooth-walled buccal capsule. Relatively short conical tail baring three mucrones (similar as that of third-stage larva) is seen. Pereira et al. (1936) noted that larval development in small characid, *Curimatus elegans*, continued only up to fourth-stage. However, the present fourth-stage larvae could not be compared with the corresponding larval form of *S. cearensis*. In the stomach the "male" and "female" larvae undergo their final moult on day 37 p.i., and day 67 p.i. respectively. Morphology and measurements of the males and the females obtained in the present series of experiments conform with the data on the males and females of *S. mysti* as given by De et al. (1986a).

REFERENCES

BASHIRULLAH A. K. M., AHMED B. 1976: Larval development of *Spirocamallanus intestinecolas* (Bashirullah, 1973) Bashirullah, 1974 in copepods. Riv. Parasitol. 37: 303–311.

DE N. C., ROY R., MAJUMDAR G. 1986a: Redescription of *Spirocamallanus mysti* (Karve, 1952) (Nematoda: Camallanidae) with notes on related forms. Folia Parasitol. 33: 353–361.

DE N. C., SINHA R. K., MAJUMDAR G. 1986b: Larval development of *Procamallanus spiculogubernaculus* Agarwal, 1958 (Nematoda: Camallanidae) in copepods. Folia Parasitol. 33: 51–60.

FUSCO A. C. 1980: Larval development of *Spirocamallanus cricotus* (Nematoda: Camallanidae). Proc. Helminthol. Soc. Wash. 47: 63–71.

IVASHKIN V. M. A., SOBOLEV A., CHROMOVA L. A. 1971: Camallanids as Agents of Diseases of Man and Animals. Osnovy nematodol. 22, Moskva, 388 pp. (In Russian.)

LI H. C. 1935: The taxonomy and early development of *Procamallanus fluvidraconis* n sp. J. Parasitol. 21: 103–113.

MORAVEC F. 1975: The development of *Procamallanus laeviconchus* (Wedl, 1862) (Nematoda: Camallanidae). Věstn. Česk. Spol. Zool. 39: 23–38.

PEREIRA C., DIAS M. V., AZEVEDO P. 1936: Biologica do nematoide *Procamallanus cearensis* sp. n. Arch. Inst. biol. 5: 209–226.

Received 20 April 1994

Accepted 18 April 1995