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LARVAL DEVELOPMENT OF PROCAMALLANUS SPICULOGUBERNACULUS AGARWAL, 1958 (NEMATODA: CAMALLANIDAE) IN COPEPODS

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Abstract. Larval development of *Procamallanus spiculogubernaculus* was studied under laboratory conditions. Two copepods, *Mesocyclops obsolatus* and *M. oithonoides*, maintained at temperatures 20—28 °C, 29—35 °C and 35—37 °C were infected with larvae from gravid *P. spiculogubernaculus* occurring in gastro-intestinal tract of *Heteropneustes fossilis*. Developmental phases including moulting and attainment of infective stage took place in the haemocoelic cavity of the copepods. Larvae moulted twice and a phase of growth, development and morpho-metric changes occurred between the two moults. At the infective stage, third one, 3—7 caudal spines, were present, partly in consonance with the diagnostic features of Camallanata. An enhanced development and associated changes leading to the attainment of infective stage occurred at an increased temperature.

Of the piscine nematodes, the members of different genera of the family Camallanidae form the most prevalent group, particularly in catfishes. The life cycle studies on camallanids include *Camallanus* (Mecznikow 1866, Leuckart 1876, Linstow 1909, Leiper 1910, Kupryanova 1954, Campana-Rouget 1961, Moravec 1969, 1971a, b, Monchenko 1972, Stromberg 1973, Stromberg and Crites 1974, Bashirullah and Ahmed 1976a, Crites 1976), Zeylanema (Moorty 1938), *Procamallanus* (Moravec 1975), *Spirocamallanus* (Li 1935, Pereira et al. 1936, Bashirullah and Ahmed 1976b, Fusco 1980), *Neocamallanus* (De et al. 1984) and *Paracamallanus* (Moravec 1974). The present study was undertaken to work out the life cycle of *P. spiculogubernaculus*, a common parasite of *Heteropneustes fossilis* (Bloch). *Mesocyclops obsolatus* (Koch) and *M. oithonoides* Sars were successfully used as intermediate hosts under laboratory conditions.

MATERIAL AND METHODS

Gravid *Procamallanus spiculogubernaculus* containing motile larvae were recovered from the stomach of naturally infected fish, *Heteropneustes fossilis* collected from a fishpond at Burdwan, West Bengal. A total of 56 specimens (average length 14 cm) were sacrificed, out of which 30 were found infected. The average number of nematodes recovered was 6. To obtain the larvae, gravid females containing motile larvae in their uteri were selected and transferred to glass vessels (ø 7 cm) with a small amount of filtered pond water. Generally 3 females were placed in each vessel. Their bodies were then teased to release the larvae. The mutilated bodies of the worms were removed. Further, filtered pond water was added to increase the height of water column up to 8 cm. Copepods, *Mesocyclops obsolatus* and *M. oithonoides* were collected from nature, maintained at room temperature in glass jars, using algal filaments and detritus as food. Fifty copepods of each species were examined before experimentally used and none was found to be infected with any nematode. 40 laboratory-reared copepods, *M. obsolatus* and *M. oithonoides*, were released in vessels containing nematode larvae which were kept at room temperature.

The percentage of infection in the copepods was determined by examining 25 copepods under low magnification. After 24 hours' exposure the infected copepods were transferred to fresh culture vessels and maintained at room temperature at different time of the year (ranging from

20 to 28 °C, 29 to 35 °C and 35 to 37 °C). Few algal filaments and detritus were added to each vessel. During each experiment four such cultures were set to procure sufficient number of copepods.

To study the course of development, the larvae were dissected out from the haemocoel of the infected copepods initially at 3-hour intervals after the 24-hours infection period. Later the period of interval gradually increased.

All larvae, i.e. those obtained from cyclops and free larvae from the uteri,were studied in the following manner. The larvae were placed in a drop of water on the slide and covered with a cover slip. The cover slip was then fixed with sealing wax. In order to kill the larvae in stretched condition the slide was gently heated over the flame of a spirit lamp and a drop of 4 % formalin was added to the margin of the cover slip. Clearing was done by 2 % glycerine.

RESULTS

The larvae in the culture vessels displayed considerable motility. Mostly they remained fixed to a substrate by their tails and lashed their bodies vigorously and thus seemed to attract the cyclops for swallowing. The larvae were ingested by copepods after one hour. Soon thereafter the larvae pierced through the intestinal wall by boring with their dorsal dentate process and penetrated into the haemocoel where further development occurred. The susceptibility to infection was higher in the male than in female copepods. No early copepodites were found to get infection. Heavily infected copepods became sluggish and rested at the bottom of the vessel. In all the experiments the percentage of infection was 30 %. On an average, 2 larvae (ranging from 1 to 6) per copepod were recorded. In the first experiment, the larvae were found to attain the second stage between days 3—8 p.i. (first moulting recorded on day 7 p.i.) and the second moult, after which the larvae attain the third, infective stage, occurred between days 4—12 p.i. The third-stage larvae in cyclops remained mainly in the cephalothorax but, contrary to those of other genera of Camallanidae (*Camallanus*, *Paracamallanus* and *Neocamallanus*), they did not coil in a circle. They were easily discernible in the body of infested cyclops due to the orange coloured intestine. No further development occurred then inside the cyclop body.

DESCRIPTION OF THE DEVELOPMENTAL STAGES OF *P. spiculogubernaculus*

a) First-stage larvae (from uterus of female)

Figs. 1A, 2A, D

Larval body slender, translucent, 0.458—0.573* long and 0.017—0.022 wide. Thick cuticle with fine transverse striations. A small dentate process present on dorsal head end. Oral papillae though present their exact number cannot be determined for minuteness. Mouth opening leading to a thin, feebly sclerotized oral tube (0.002—0.004 long). Thin-walled oesophagus 0.081—0.097 long and 0.010—0.012 wide, bearing a spacious cavity inside. Large, unicellular oesophageal glands present on posterior part of oesophagus. Distinct nerve ring lying at a distance of 0.069—0.076 from anterior end; excretory pore situated slightly anterior to nerve ring (0.059—0.062 from cephalic extremity). Intestine straight, wide and with fine granulations inside. Short, straight rectum with thin sclerotized wall. Three distinct oval unicellular rectal glands present at junction of intestine and rectum. Tail elongated and slender, 0.192—0.216 in length.

* All measurements are in mm.

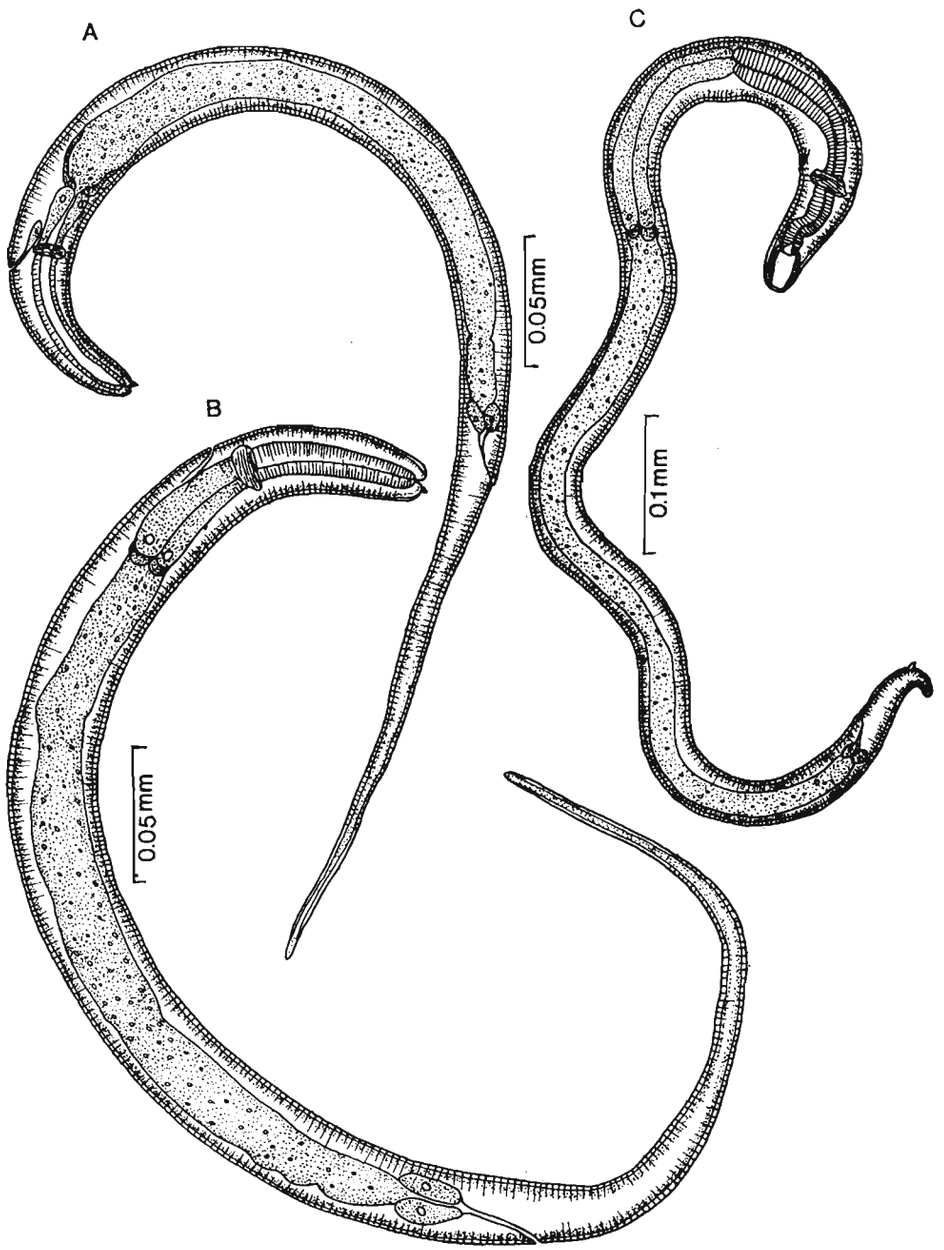


Fig. 1. *Procamallanus spiculogubernaculus* Agarwal, 1958. A—C — larva, overall view (A — free first stage, B — first stage from cyclops, 7 days p.i., C — third stage from cyclops, 13 days p.i.).

b) First-stage larvae from copepods Figs. 1B, 2B, E

First-stage larvae on entering the haemocoel of the cyclops initially do not change their morphology except for a slight change in body measurements (Table 1).

Larval body slender, translucent and measuring 0.405—0.616 in length and 0.017—0.034 in width. Body cuticle thickened and with dense transverse striations. Dorsal dentate process at cephalic end present. Oral tube almost unchanged in length (0.002—0.005). Oesophagus (0.064—0.114 long and 0.008—0.015 wide), muscular in its greater part except for a short posterior glandular region. No distinct divisional mark between two oesophageal regions observed. Oesophageal lumen narrow due to thickened oesophageal walls. Distinct valvular apparatus appearing at junction of oesophagus and intestine. Nerve ring and excretory pore at 0.030—0.076 and 0.032 to 0.081, respectively, from anterior end. Tail long and slender (0.096—0.216).

c) Sheathed first-stage larvae Figs. 2C, F

During the first moult the old cuticle becomes loosened at both anterior and posterior ends of larvae. Body inside the old cuticle 0.504—0.586 long and 0.027—0.032 wide. Anterior end of larva broad, rounded in shape and lacking dorsal dentate process. Oral tube measuring 0.003—0.005 in length. Oesophagus 0.130—0.140 long and 0.010—0.012 wide, indistinctly divided into anterior muscular and posterior glandular part. Nerve ring and excretory pore 0.064—0.069 and 0.079—0.089, respectively, from anterior extremity. Tail 0.049—0.140 long, shorter than that of first-stage larva in unsheathed condition.

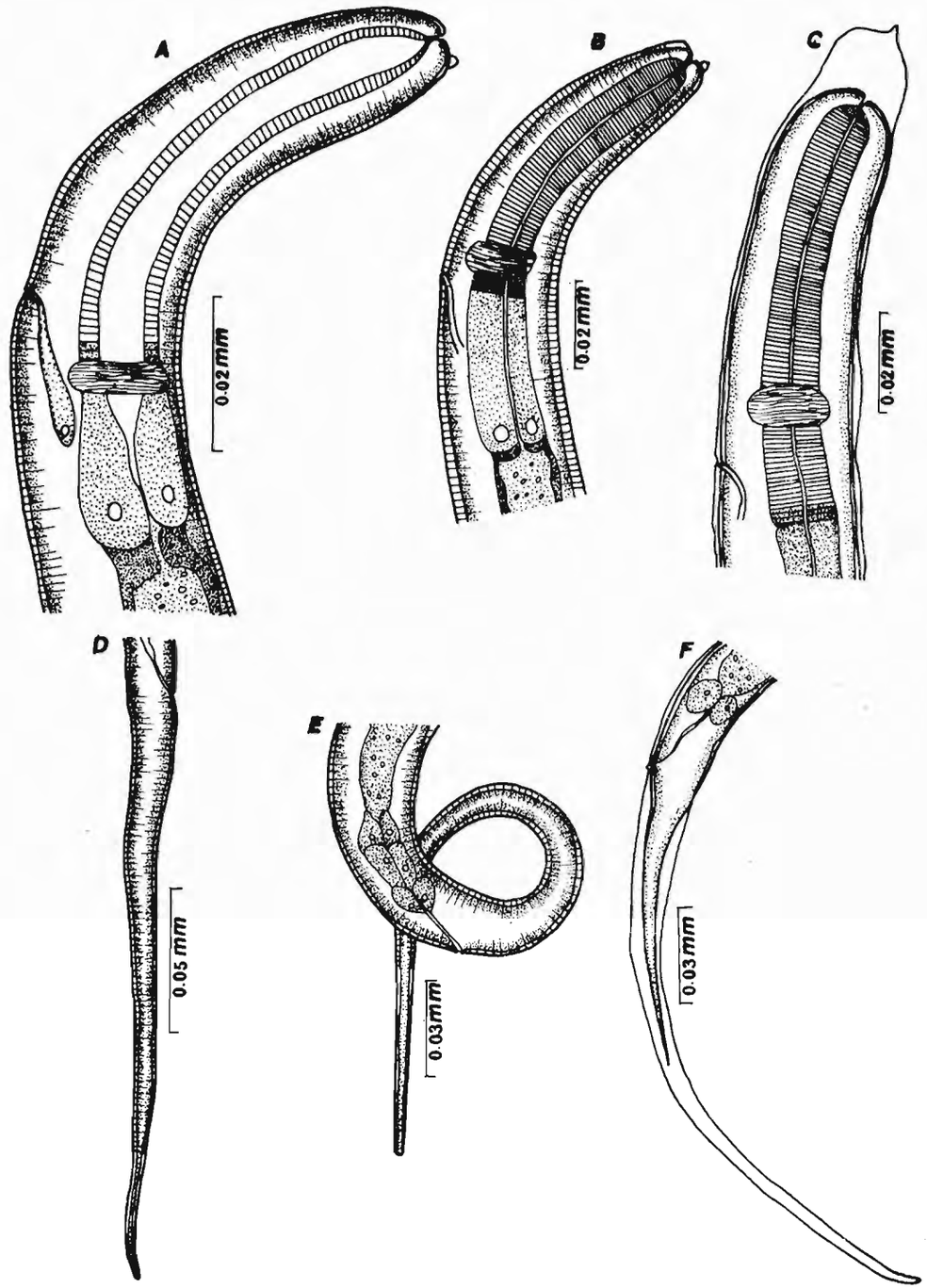
d) Second-stage larvae Fig. 3A

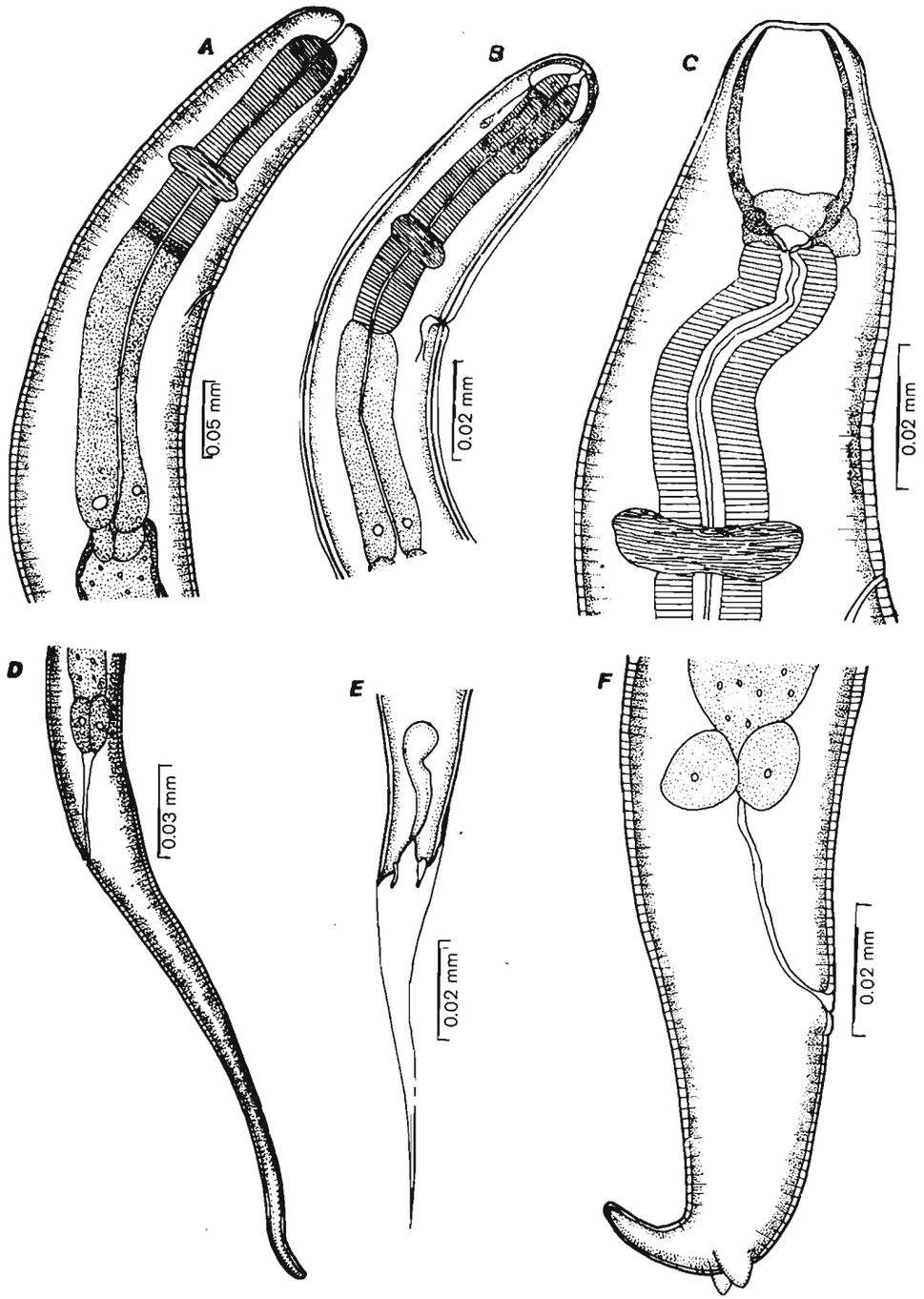
Young second-stage larvae are almost similar to unsheathed first-stage larvae and differ only in lacking dorsal dentate process on head end. Body 0.517—0.669 long and 0.030—0.034 wide, covered with thin and smooth cuticle. Oral tube measuring 0.004—0.005. Oesophagus 0.106—0.165 long, 0.012—0.015 wide and still indistinctly divided into anterior muscular and posterior glandular part. Nerve ring and excretory pore 0.049—0.084 and 0.059—0.106, respectively, from cephalic extremity. Orange-brown coloured straight and wide intestine bearing dense granules. Tail slender and 0.093—0.180 long.

e) Sheathed second-stage larvae Fig. 3B

Second larval moult occurs between days 4 and 12 p.i. at water temperature of 26—28 °C. At this stage, the larvae measure 0.600—0.856 in length and 0.030—0.039 in width. Buccal capsule in form of hyaline, poorly sclerotized bell, 0.009—0.013 long and 0.015—0.020 wide at anterior end of oesophagus. Oesophagus distinctly divided into anterior muscular (0.085—0.118 long) and posterior glandular (0.068 to 0.096 long) part. Nerve ring and excretory pore 0.071—0.084 and 0.084—0.121, respectively, from head end. Tail shorter (0.037—0.059 long) than that of unsheathed second-stage larva and bearing 3—7 mucrones.

Fig. 2. *Procamallanus spiculogubernaculus* Agarwal, 1958. A—C — anterior end of larva (lateral view) A — free first stage, B — first stage from cyclops, 7 days p.i., C — sheathed first stage from cyclops, 7 days p.i., D—F — posterior end of larva (lateral view) (D — first stage, E — first stage from cyclops, 7 days p.i., F — sheathed first stage from cyclops, 7 days p.i.).





f) Third-stage larvae (infective stage) Figs. 1C, 3C

Third-stage larvae first appear on day 4 p. i. at water temperature ranging between 26—28 °C. Larval body fairly large and measuring 0.807—1.159 in length and 0.032 to 0.049 in width. Buccal capsule large (0.032—0.039 long and 0.015—0.022 wide), well sclerotized and of golden brown colour. Anterior muscular oesophagus (0.130 to 0.209 long) larger (smaller in few cases) than posterior glandular part (0.123—0.207). Nerve ring surrounding muscular oesophagus at 0.062—0.162 from anterior end. Excretory pore situated behind nerve ring, 0.069—0.182 from head end. Valvular apparatus trilobate, present at oesophageo-intestinal junction. Intestine straight and wide, filled with dense granules and opening into short tubular rectum. Rectal glands (three) large, unicellular. Tail short (0.037—0.096), attenuated posteriorly and bearing a variable number of mucrones (3—7) at its tip.

DISCUSSION

The present study reveals that the larval development of *Procamallanus spiculogubernaculus* in copepod intermediate hosts proceeds as follows: the first-stage larvae are released by adult females and expelled out along with host's fecal matters. Under optimal conditions these larvae survive for several hours in water and attract cyclops by their persistent wriggling movements. They are then swallowed by cyclops. Soon thereafter, the first-stage larvae pierce through the intestinal wall with their dorsal cephalic dentate process and reach the haemocoel of the host where further development takes place. At the temperatures of 20—28 °C the larvae attain second stage on days 3—8 p.i. The second-stage larvae are devoid of dorsal cephalic dentate process and grow considerably in the host's body (Table 1). The second moult starts on day 4 p.i. at 20—28 °C (on day 3 p.i. at 35—37 °C) and the larvae attain the third, infective stage. This is accompanied by considerable morphological changes, like the development of large golden-brown buccal capsule and appearance of mucrones on the tail tip. The larvae after attaining the third infective stage show no further morphometrical changes (Table 1).

In general, the course of development of *P. spiculogubernaculus* follows that of other members of the family Camallanidae. In the present experiment, *Mesocyclops obsolatus* and *M. oithonoides* get the infection, but the incidence, as also the intensity, are comparatively low. This may be related with their size and capability of ingesting the first-stage larvae. The development may also occur in a number of other members of Cyclopoidea.

The first and second-stage larvae of *P. spiculogubernaculus* represent morphological structures which are characteristic of larval stages of the genera *Spirocamallanus*, *Camallanus* and *Zeylanema*. The third-stage larvae are, however, different in the structure of the buccal capsule which, by contrast to the latter two genera, is continuous. Li (1935) recorded three processes only on the tail tip of infective third-stage larvae of *S. fulvidraconis* and Pereira et al. (1936) and Moravec (1975) observed 4 conical processes on the tail tip of *S. caerensis* and *P. laeviconchus*, and 3—7 in the young third-stage larvae of *P. spiculogubernaculus*.

Fig. 3. *Procamallanus spiculogubernaculus* Agarwal, 1958. A—C — anterior end of larva (lateral view) (A — second stage from cyclops, 3 days p.i., B — sheathed second stage from cyclops, 8 days p.i., C — third stage from cyclops, 13 days p.i.), D—F — posterior end of larva (lateral view) (D — second stage from cyclops, 3 days p.i., E — sheathed second stage from cyclops, 8 days p.i., F — third stage from cyclops, 13 days p.i.).

Table 1. Growth of first-, second- and third-stage larvae of *Procamallanus spiculogubernaculus* in cyclops

	First-stage larvae				
	free larvae from uterus	unsheathed		sheathed	
		1—3 days p.i.	4—6 days p.i.	3—4 days p.i.	7 days p.i.
Length of body	0.458—0.573	0.405—0.563	0.502—0.605	0.586	0.504
Width of body	0.017—0.022	0.017—0.027	0.021—0.032	0.032	0.027
Length of oral tube	0.002—0.004	0.002—0.005	0.002—0.005	0.003	0.005
Lenght of buccal capsule	—	—	—	—	—
Width of buccal capsule	—	—	—	—	—
Length of muscular oesophagus	—	—	—	—	—
Length of glandular oesophagus	—	—	—	—	—
Total length of oesophagus	0.081—0.097	0.064—0.111	0.074—0.101	0.130—0.140	0.133
Distance of nerve ring from anterior end	0.069—0.076	0.038—0.071	0.030—0.064	0.064—0.069	0.064
Distance of excretory pore from ant. end	0.059—0.062	0.049—0.081	0.044—0.064	0.081—0.089	0.079
Length of tail	0.192—0.216	0.170—0.212	0.148—0.177	0.094—0.140	0.049

	Second-stage larvae			Third-stage larvae	
	unsheathed 3 days p.i.	sheathed		12 days p.i.	25 days p.i.
		4 days p.i.	8 days p.i.		
Length of body	0.517—0.598	0.770—0.856	0.854	0.807—1.378	1.112
Width of body	0.030—0.034	0.032—0.039	0.032	0.032—0.049	0.034
Length of oral tube	0.004—0.005	0.002—0.004	0.005	—	—
Length of buccal capsule	—	—	—	0.032—0.039	0.034
Width of buccal capsule	—	—	—	0.017—0.022	0.017
Length of muscular oesophagus	—	0.113—0.118	0.108	0.130—0.162	0.162
Length of glandular oesophagus	—	0.086—0.096	0.079	0.123—0.160	0.143
Total length of oesophagus	0.106—0.121	0.199—0.214	0.187	0.253—0.322	0.305
Distance of nerve ring from anterior end	0.049—0.064	0.071—0.084	0.074	0.062—0.130	0.089
Distance of excretory pore from ant. end	0.059—0.084	0.098—0.121	0.084	0.069—0.182	0.162
Length of tail	0.103—0.180	0.037—0.057	0.049	0.037—0.062	0.055

The present account is in conformity with the findings of Ko and Adams (1969), Stromberg and Crites (1974). Bashirullah and Ahmed (1976a, b) and De et al. (1984) relating to the accelerated rate of larval development at higher temperature.

In *P. spiculogubernaculus*, the second moult was found to occur 1 day earlier due to an increased temperature of 9 °C.

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РАЗВИТИЕ ЛИЧИНОК *PROCAMALLANUS SPICULOGUBERNACULUS* AGARWAL, 1958 (NEMATODA: CAMALLANIDAE) В РАКООБРАЗНЫХ

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Резюме. Изучали развитие личинок *Procamallanus spiculogubernaculus* в лабораторных условиях. Двух ракообразных, *Mesocyclops obsolatus* и *M. oithonoides*, поддерживаемых при температурах 20—28 °С, 29—35 °С и 35—37 °С, заражали личинками из гравидных *P. spiculogubernaculus*, выделенных из желудочно-кишечного тракта *Heteropneustes fossilis*. Фазы развития, включая линьку и достижение инфекционной стадии, происходили в полости гемоцела ракообразных. Личинки линяли два раза и фазы роста, развития и морфогенетических изменений происходили между линьками. В третьей, инфекционной стадии, встречались 3—7 хвостовых шипов, что отвечает диагностическим признакам Camallanata. Развитие и соединенные с ним изменения осуществлялись при повышенной температуре.

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C. W. Schwabe: Veterinary medicine and human health.

3rd ed. Williams and Wilkins, Baltimore — London 1984, 680pp., 193 Figs. Price $ 48.

At the beginning of the book is a motto by Rudolf Virchow: "Between animal and human medicine there is no dividing line — nor should there be. The object is different but the experience obtained constitutes the basis of all medicine." This idea is the leitmotiv of the entire volume. The text is divided into 26 chapters arranged in 7 sections: I — The challenge of "One medicine", II — Food and malnutrition, III — Zoonoses and medical research, IV — Epidemiology and population medicine, V — Environmental quality, VI — Mental health and human values, VII — Fulfillment. Already this enumeration shows the wide spectrum of problems discussed by the author. Each chapter opens with one or more mottoes and terminates with a list of references, suggestions for further reading, and other keys to the literature. The chapters are divided into many subchapters which are further divided. There is a large number of figures, photographs and tables which are numbered for each chapter separately. They show schemes of interrelations, various systems and classifications, maps, and graphs. The tables present surveys and characterizations and contain an immense number of data. Some of them cover even several pages. It is impossible to deal with in detail the contents of the monograph in this brief report. It is written in an unusual manner. In addition to the scientific facts it contains also excursions into the history, passages quoted from different books including poetry, and many case studies. A parasitologist will certainly read with a great interest the Chapter 15 devoted to medical ecology. It discusses multiple determinants of diseases, host-agent interactions, host-agent and environment, ecological changes and disease patterns, ecological balance and disease, qualitative analyses of direct zoonoses, cyclo-, meta- and saprozoonoses. Remarkable is also Chapter 20 dealing with medical zoology and concerning the veterinary connection between medicine and zoology, animals as agents of disease, animals as vectors of infection and nonarthropod vectors. Of course, other specialists will appreciate the topics of other chapters.

In general it may be said that the book is filled with facts raising the problems and showing their solution which get the reader to think them through. It was written and edited with utmost care. The author's wide knowledge of the problems, his knowledge of the history of science, as well as of scientific publications deserves admiration. Undoubtedly the book will become an invaluable source of rich information for the specialists of many fields.

Dr. V. Černý, C.Sc.