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THE DEVELOPMENT OF THE NEMATODE PHILOMETRA ABDOMINALIS NYBELIN, 1928 IN THE INTERMEDIATE HOST

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Abstract. The development of *Philometra abdominalis* was studied experimentally in the copepod intermediate hosts, *Macrocyclops albidus*, *M. fuscus*, *Megacyclops viridis*, *Acanthocyclops vernalis* and *Diacyclops bisetosus*, kept at the temperatures of 20—24 °C. Free larvae of the first stage live in water for 2—25 days (dependently on the temperature). The nematode larvae molt twice (on the 5—6th and 7—9th day p.i.) in the haemocoel of the intermediate host before they attain the third, infective stage. A description is given of the first-, second- and third-stage larvae. The mode of the development of *Ph. abdominalis* larvae in the intermediate host is compared with that of larvae of other genera of the superfamily Dracunculoidea.

The nematode *Philometra abdominalis* is a widely distributed parasite of the abdominal cavity of some cyprinid fishes, particularly in Europe. According to Molnár (1967), obligate hosts of this species are minnow (*Phoxinus phoxinus*) and gudgeon (*Gobio gobio*), but it occurs also in fishes of the genera *Leuciscus* and *Barbus*, in which it reaches maturity only exceptionally.

The development of this parasite in the intermediate host has not been studied in detail. Only Molnár (1967) infected experimentally with *Ph. abdominalis* larvae the cyclops originating from the localities where this parasite occurs. Also the data on the development of other members of the genus *Philometra* are mostly inadequate or completely lacking. In the years 1967—1968 and 1974 the author carried out some experimental observations on the development of *Ph. abdominalis* and the reults are presented in this paper.

MATERIAL AND METHODS

Gravid females of *Ph. abdominalis* with moving larvae in the uterus were recovered from gudgeon (*G. gobio*) from the River Bystřice near Olomouc and from minnow (*Ph. phoxinus*) from the Rokytka Brook near Říčany caught in spring and summer. The nematodes were transferred to a small vessel with water, where their body ruptured due to osmotic pressure and a large number of larvae were released. The suspension of larvae was diluted and their concentration in water was determined. Feeding experiments were carried out in glass vessels (5 to 10 cm in diameter) filled with water up to the height of about 7 cm. Each vessel contained some 50 copepods to which about 1000 nematode larvae were added. These were kept at the laboratory temperatures of 20—24 °C. The copepods were examined every day. The nematode larvae were killed with 2% formaldehyde or by heating the slide with larvae in a drop of water above the flame; the larvae were examined in the same liquids.

RESULTS

EXPERIMENTAL INFECTION OF INTERMEDIATE HOSTS

Similarly as other representatives of the superfamily Dracunculoidea, the species *Ph. abdominalis* is ovoviviparous, i.e., first-stage larvae emerge from the egg shells already in the uterus of the female. The gravid female is located in the abdominal

cavity of the fish host, from where it actively penetrates into the rectum. The parasite pulls a part of its body out of the rectum into the water, where it ruptures under osmotic pressure and the larvae are released into the external environment. According to Molnár (1967), the larvae may be also expelled with the spawn or sperms of the host.

After the transfer of gravid females into water their body ruptures within 45 sec and a mass of larvae are released. In five specimens used in our experiments, the number of larvae in the uterus was estimated to be 42—45 thousands per female. The life span of these larvae depends on water temperature (Table 1) and ranges from 2 to 25 days.

Table 1. Dependence of surviving of *Ph. abdominalis* first-stage larvae in water on temperature

Water temperature °C	Length of larval surviving
7	25 days
15 to 18	16 days
20 to 24	7 days
30 to 34	2 days

The larvae of *Ph. abdominalis* move in the water and thus attract the attention of copepods and are swallowed by them. During 45 min the larvae penetrate through the wall of the intestine (in the region of cephalothorax) of copepods, serving as intermediate hosts, and get into their body cavity where further development occurs. The larvae move freely in the haemocoel of the intermediate host; most often they are located in the dorsal part of cephalothorax, in heavy infections also in other parts of body (abdomen, furca and antennule). During further development in the intermediate host the larvae undergo two molts (at the temperatures of 20—24 °C, 5—6 and 7—9 days after infection) before they reach the third, infective stage.

It was found that the following copepod species may serve as intermediate hosts of *Philometra abdominalis: Macrocyclops albidus* (Jurine), *M. fuscus* (Jurine), *Megacyclops viridis* (Jurine), *Acanthocyclops vernalis* (Fischer) and *Diacyclops bisetosus* (Rehberg). The first four species occur in the same places as this parasite. Adult forms of all these copepod species were easily infected with the larvae of the parasite and no significant differences in the incidence and intensity of infection were observed in individual species of intermediate hosts. At the dose of about 20 larvae per copepod the incidence was 80—100% and intensity of infection 1—23 larvae per copepod. The double dose of larvae caused all the copepods to die within 24 hours.

DEVELOPMENT OF LARVAE

a) First-stage larva

Fig. 1

First-stage larvae obtained from the uterus of female measure 0.480—0.543 mm in length and 0.021—0.024 mm in maximum width (other measurements are given in Table 2). The head end of the larva is provided with several minute mouth papillae, the exact number of which was not determined, and a very small dorsal cuticular tooth, which serves the larva for boring the intestine of the intermediate host when penetrating to its body cavity. The oesophagus is rather long, hyaline, in form of

a fine, thin-walled tube. The last third of the oesophagus length is formed by three oesophageal glands of light colour. Each of these glands has a large cellular nucleus. The nerve ring is conspicuous and surrounds the oesophagus approximately at the end of its first third; the excretory pore was not observed. The intestine is straight,

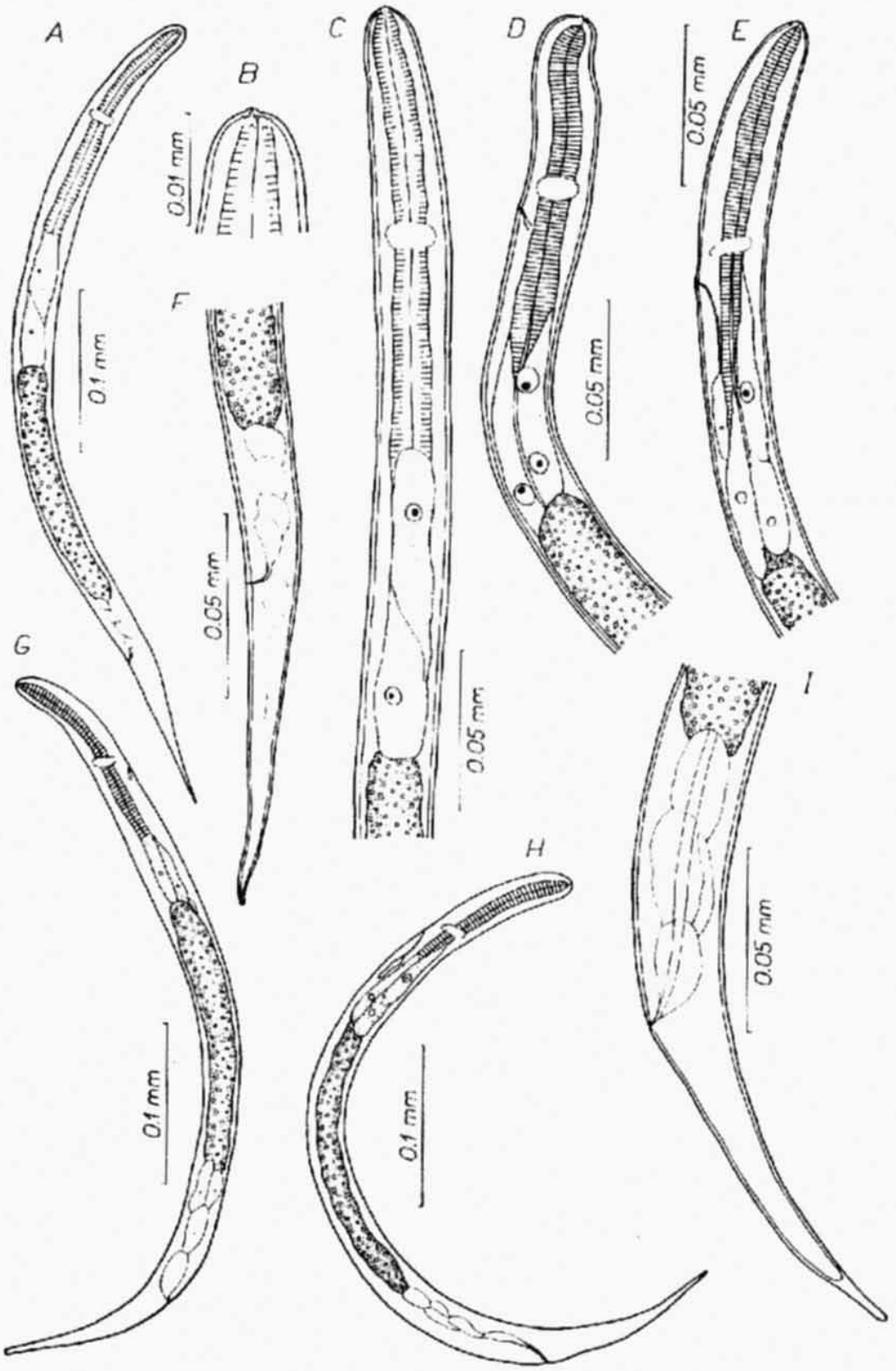

Fig. 1. *Philometra abdominalis* — first-stage larva. A, C, F — larva from the uterus of female (A — general view, C — anterior part of body, F — posterior part of body); G — larva from the copepod on day 1 p. i.; D — head end of larva on day 2 p. i.; I — tail of larva on day 3 p. i.; B, E — larva on day 4 p. i. (B — anterior end, E — anterior part of body, H — general view).

relatively wide and densely granulated. The rectum is a thin hyaline tube covered with several large unicellular rectal glands. The phasmids are large and situated immediately below the anus. The tail is slender and conical.

The development of the larvae continues after the penetration through the intestinal wall into the body cavity of the intermediate host. During the first 24 hours of development in the intermediate host the cuticle of larvae gets markedly thicker and the inner organs become more distinct than in free larvae. The excretory pore, lying slightly

Table 2. Measurements of first-stage larvae of *Ph. abdominalis* (in mm)

	Free larvae	Larvae from copepods			
		1 day p.i.	2 days p.i.	3 days p.i.	4 days p.i.
Length of body	0.480—0.543	0.543—0.549	0.465—0.540	0.525—0.579	0.555—0.570
Width of body	0.021—0.024	0.021—0.024	0.021—0.027	0.021—0.027	0.024—0.027
Length of oesophagus	0.210—0.246	0.168—0.177	0.150—0.165	0.165—0.189	0.174—0.180
Distance of nerve ring	0.069—0.081	0.072—0.078	0.056—0.081	0.072—0.081	0.075
Distance of excret. pore		0.084—0.090	0.060—0.084	0.084—0.090	0.090
Length of tail	0.087—0.096	0.093—0.108	0.081—0.105	0.099—0.111	0.111—0.114

below the level of nerve ring, is already visible. The intestine gets darker. During 3—4 days the larvae become a little thicker and a new cuticle begins to form under the old one. On day 5—6 p. i. the larvae undergo the first molt and develop to second-stage larvae.

q) Second-stage larva

Fig. 2

During the following 1—2 days, or possibly longer, the second-stage larvae remain inside the exuviae of the cuticle from the first molt and then leave it. Both larvae shedding the old cuticle and empty exuviae of the cuticle were observed in the haemocoel of the intermediate host 6 days p. i. The second-stage larvae (Table 3) only slightly

Table 3. Measurements of second-stage larvae of *Ph. abdominalis* from copepods (in mm)

	5 days p.i.	6 days p.i.	7 days p.i.
Length of body	0.495—0.561	0.480—0.498	0.603—0.645
Width of body	0.024—0.027	0.021—0.024	0.018
Length of oesophagus	0.216—0.255	0.162—0.270	0.258—0.300
Distance of nerve ring	0.078—0.081	0.057—0.078	0.102
Distance of excret. pore	0.088	0.069—0.096	
Length of tail	0.078—0.087	0.069—0.075	0.081

differ in their morphology from advanced first-stage larvae. The dorsal oesophageal gland is more marked and enlarged in length. The intestine, which is still densely granulated in these larvae, gets a yellowish to light orange colour. The tail is again conical. The existence of second-stage larvae is very short and they usually start further molt already in the exuviae of the first molt (Fig. 2 d). The old cuticle loosens most distinctly at the anterior and posterior ends of the body.

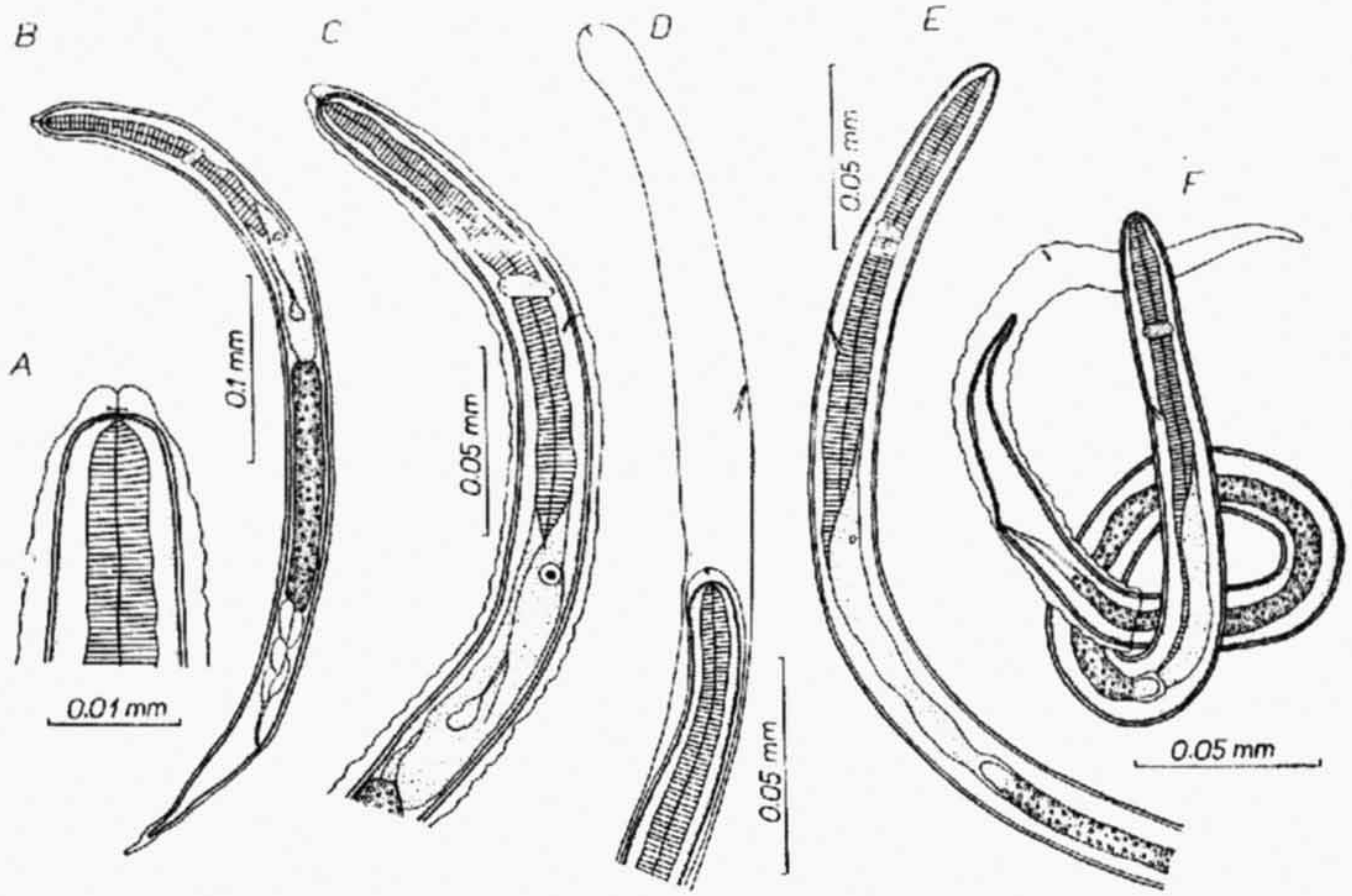

Fig. 2. *Philometra abdominalis* — second-stage larva from copepods. A-C — larva at first molt on day 5 p. i. (A — anterior end, B — general view, C — anterior part of body); D — head end of larva during the first molt at the onset of the second molt (on day 7 p. i.); E — anterior part of body of second-stage larva 6 days p. i.; F — larva leaving the excuviae of cuticle from the first molt (on day 6 p. i.).

c) Third-stage larva (infective larva) Figs. 3, 4

The second molt, during which the larvae change to third-stage larvae, follows immediately after the first one and was observed as early as on the 7th day p. i. The third-stage larvae are encased in the exuviae of the cuticle from the second molt and do not leave it even after 21 days of staying in the body of the intermediate host. During this second molt the larvae become more slender and their dorsal oesophageal gland is markedly elongated. The tail is somewhat shortened and its tip becomes blunt.

The body length of third-stage larvae is almost the same as that of first-stage larvae (Table 4), but the width is distinctly smaller. With the exception of the oesophageal

Table 4. Measurements of third-stage larvae of *Ph. abdominalis* from copepods (in mm)

	9 days p.i.	11 days p.i.	12 days p.i.	21 days p.i.
Length of body	0.516—0.531	0.570	0.495—0.525	0.459—0.531
Width of body	0.015—0.016	0.015	0.012—0.015	0.015
Length of oesophagus	0.258—0.300	0.282	0.240—0.246	0.249—0.273
Distance of nerv. ring	0.090—0.093	0.057	0.078	0.066—0.087
Distance of excret. pore	0.102	0.075	0.084	0.072—0.090
Length of tail	0.066—0.069	0.069	0.048	0.051—0.069

gland and intestine, the body of the larva is almost transparent. The head end is somewhat narrowed. The oesophagus is feebly muscular, rather long; the dorsal oesophageal gland, forming approximately one half of the oesophagus length, is compact and

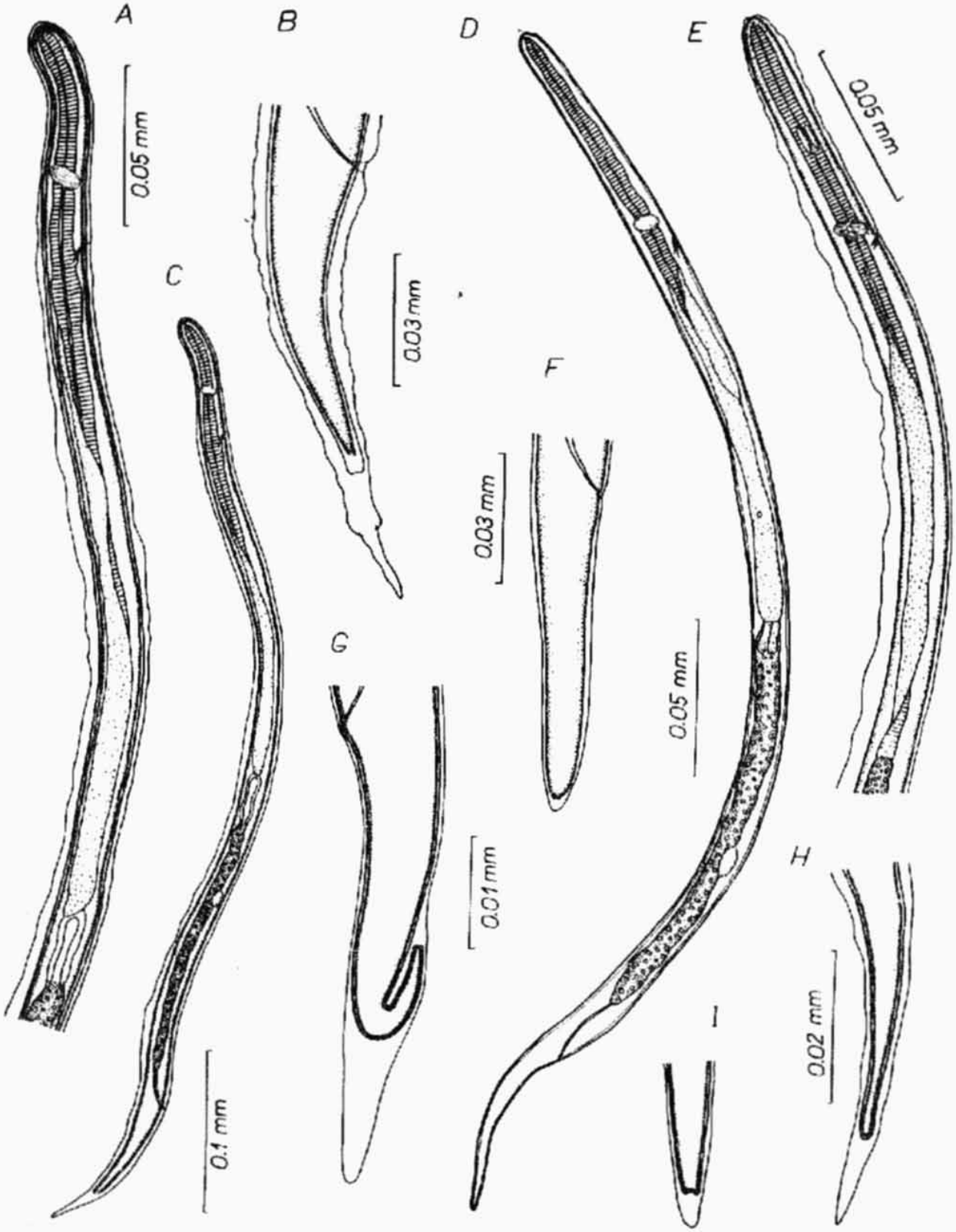

Fig. 3. *Philometra abdominalis* — third-stage larva from copepods. A,C,H — larva at the second molt on day 8 p. i. (A — anterior part of body, C — general view, H — tail tip); B — tail of larva during the second molt on day 9 p. i. (still inside the exuviae from the first molt); D — infective larva of the third stage on day 11 p. i.; E — anterior part of body of larva on day 12 p. i.; F,G — tail of infective larva on day 9 p. i.; I — tail tip of infective larva on day 9 p. i.

well visible. It opens a small distance below the nerve ring. The cellular nuclei of oesophageal glands are inconspicuous. Approximately half the distance between the anterior end of body and nerve ring there are two small, oval, refractile formations –probably penetration glands, which are usually present also in infective larvae of other nematodes. The excretory pore is situated just below the level of the nerve ring and is almost indistinct. The intestine is straight, densely granulated and of light yellow to orange colour. The rectum is formed as a thin hyaline tube. The oval genital primordium lies near the middle of length of intestine. The tail is conical and somewhat shorter than in larvae of the previous stage. Its end is blunt and provided with three minute papillar processes. These larvae can move and change their location in the body of the intermediate host.

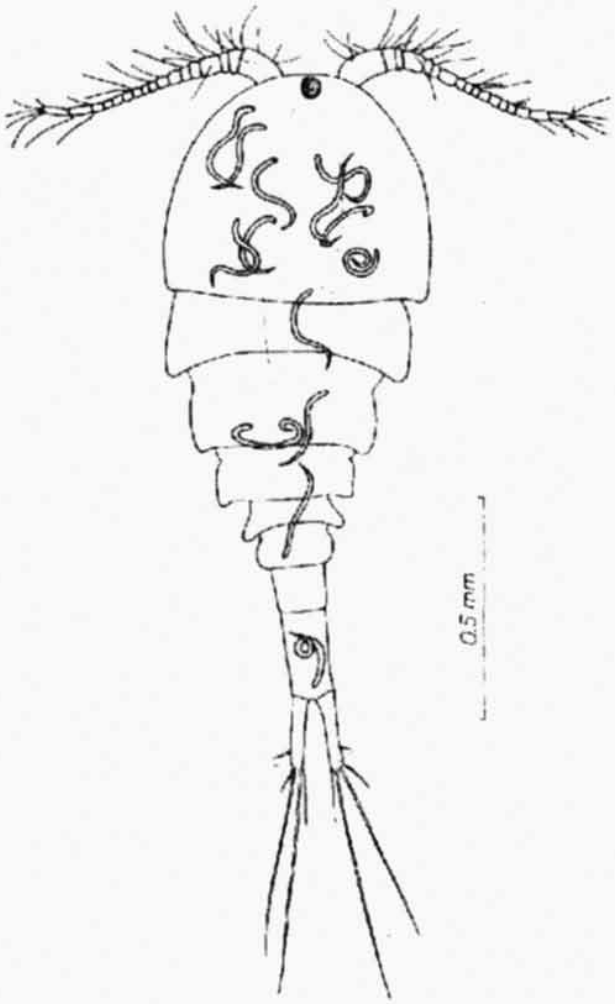

Fig. 4. *Acanthocyclops vernalis* with infective larvae of *Philometra abdominalis* in body cavity.

DISCUSSION

Life cycles of nematodes of the genus *Philometra* and some other related genera have already been studied by several authors, but the data on morphological changes which they undergo during their development are very incomplete. The life cycle of the genus *Philometra* was first dealt with by Strassen (1907), who assumed that the life cycle of the genus *Ichthyonema* (= *Philometra*, in part) would be similar as that of the species *Dracunculus medinensis*, due to similar morphological characters of adult forms. This hypothesis was supported by experimental infection of the copepods (*Cyclops* sp. and *Diaptomus* sp.) with the larvae of *Ichthyonema sanguinea* (= *Ph. abdominalis*?) carried out by the same author. The representatives of the class Copepoda were later found to serve as the only intermediate hosts of other species of the genus *Philometra* (Kuitunen-Ekbaum 1933, Furuyama 1934, Molnár 1966, 1967, 1969, Molnár and Fernando 1975), as well as of related genera *Philometroides* (Thomas 1929, Nybelin 1931, Wierzbicki 1960, Vismanis 1966, 1970, Vasilkov 1967, 1968, Ouk and Chun 1973, Yashchuk and Vasilkov 1970) and *Philonema* (Platzer and Adams 1967, Ko and Adams 1969).

Individual larval stages of *Ph. abdominalis* resemble in their general morphology the larvae of other members of the superfamily Dracunculoidea (presence of the dorsal tooth on anterior end of first-stage larva, character and development of oesophageal glands etc.). It is remarkable that the end of the conical tail of third-stage larva of *Ph. abdominalis* is provided with three minute processes; these are present and better developed in infective larvae of the genera *Micropleura*, *Avioserpens* and *Dracunculus*, whereas in the larvae of the genera *Philometra*, *Philometroides* and *Philonema* they

have not been mentioned. It is also of interest that in contrast to other members of this superfamily, the life-cycle of which has been studied, the infective larvae of *Ph. abdominalis* do not leave the exuviae of the cuticle of the last (second) molt, but remain inside it in the body of the intermediate host.

Most of the authors who studied the life-cycle of philometrid nematodes have not mentioned the number of larval molts in the intermediate host. According to Nybelin (1931), the larvae of *Philometroides sanguinea* molt only once in the intermediate host, 6 days after infection. But the author apparently did not distinguish the first and second molts which follow immediately one after another. Our finding that *Ph. abdominalis* larvae molt twice in the intermediate host confirms that the development of *Philometra* larvae in the intermediate host is similar to that in other genera of the superfamily Dracunculoidea. The larval development has been studied in detail in the genera *Dracunculus* (Moorthy 1938, Desportes 1938), *Micropleura* (Siddiqi and Jairajpuri 1963), *Avioserpens* (Supryaga 1965), *Philonema* (Platzer and Adams 1967, Ko and Adams 1969) and *Philometroides* (Vasilkov 1968, Vismanis 1970).

According to Wierzbicki (1960), the larvae of *Philometroides sanguinea* distinctly grow in the body of the intermediate host. The growth of larvae in the body of the intermediate host was observed also by Nybelin (1931) in *Philometroides sanguinea*, by Vismanis (1970) and Vasilkov (1968) in *Philometroides lusiana* and by Furuyama (1934) in *Philometra fujimotoi*. Some authors (Platzer and Adams 1967, Ko and Adams 1969) noted a considerable increase in body length during the development of *Ph. oncorhynchi* larvae in the copepods. During the development of *Ph. abdominalis* in the intermediate host, the larvae do not grow in length (third-stage larvae are approximately of the same length as first-stage larvae), but, on the contrary, they become more slender. This way of metrical development seems to be associated with the mode of infection in the definitive host, when the infective larvae have to penetrate through the gut wall into the abdominal cavity of the fish. Having compared the metrical development of larvae of representatives belonging to various genera of the superfamily Dracunculoidea, I found a certain sequence in the stage of modification of this development related to the phylogenetic age of hosts and, obviously, also of nematodes. In larvae of the genus *Philonema*, which parasitize phylogenetically old salmonid fishes, the metrical development in the intermediate host is similar to that of nematodes of the related family Camallanidae, i. e., they grow considerably in the intermediate host. On the other hand, larvae of the genera *Philometra* and *Philometroides*, which are parasites of phylogenetically younger fishes (mainly Cypriniformes), grow in size only slightly or do not grow at all. Larvae of the genera *Micropleura*, *Avioserpens* and *Dracunculus*, which are parasites of reptiles, birds and mammals, distinctly diminish during their development in the intermediate host. Accordingly, there is a tendency towards the gradual size decrease of the body of the infective larvae to facilitate their passage through the host tissues.

РАЗВИТИЕ НЕМАТОДЫ *PHILOMETRA ABDOMINALIS* NYBELIN, 1928 В ПРОМЕЖУТОЧНОМ ХОЗЯИНЕ

Ф. Моравец

Резюме. Изучали развитие нематоды *Philometra abdominalis* при экспериментальном заражении промежуточных хозяев, *Macrocyclops albidus*, *M. fuscus*, *Megacyclops viridis*, *Acanthocyclops vernalis* и *Diacyclops bisetosus*, содержасмых при температуре 20—24 °С. Свободные личинки 1-й стадии живут в воде в продолжение 2—25 дней (в зависимости от температуры). В гемоцеле промежуточного хозяина личинки нематоды линяют два раза (на 5—6-й

и 7– 9-й день после заражения), прежде чем достигнут 3-й, инвазионной стадии. Дано описание личинок 1-й, 2-й и 3-й стадий. Способ развития личинок *Ph. abdominalis* в промежуточном хозяине сравнивается с развитием личинок представителей других родов надсемейства Dracunculoidea.

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