FOLIA PARASITOLOGICA 42: 281–292, 1995

Studies on the development of *Procamallanus* (*Spirocamallanus*) *rebecae* (Nematoda: Camallanidae), a parasite of cichlid fishes in Mexico

F. Moravec[*1,2], **E. Mendoza-Franco**[1], **J. Vargas-Vázquez**[1] and **C. Vivas-Rodríguez**[1]

[1]Laboratory of Parasitology, Centre for Research and Advanced Studies, National Polytechnic Institute (CINVESTAV-IPN), Carr. Antigua a Progreso Km 6, A.P. 73 "Cordemex", C.P. 973 10 Mérida, Yucatán, Mexico;

[2]Institute of Parasitology, Academy of Sciences of the Czech Republic, Branišovská 31, 370 05 České Budějovice, Czech Republic (address for correspondence)

Key words: Nematoda, *Procamallanus*, *Spirocamallanus*, *Cichlasoma*, Copepoda, development, paratenic hosts, Mexico

Abstract. A description is given of the life cycle of the nematode *Procamallanus* (*Spirocamallanus*) *rebecae* (Andrade-Salas, Pineda-López et García-Magaña, 1994), an intestinal parasite of cichlids in Mexico. The copepod *Mesocyclops* sp. was found to be a suitable experimental intermediate host. After the copepod's ingestion of free first-stage larvae of the nematode, these enter the haemocoel of the intermediate host; they moult twice (on the 3rd and 5–6th day p.i. at 21–22°C) before they attain the third, infective stage. The third-stage larva already possesses the large buccal capsule without spiral thickenings and its tail tip bears three cuticular spines. The larvae undergo two additional moults (13–14 days and 42 days p.i.) in the definitive host (*Cichlasoma urophthalmus*) before changing to adults; the prepatent period is about 2–3 months. Experimental infection of guppies, *Poecilia reticulata*, have shown that these fishes may become paratenic (metaparatenic) hosts of this parasite. The morphology of individual larval stages of this nematode is described.

The nematode *Procamallanus* (*Spirocamallanus*) *rebecae* (Andrade-Salas, Pineda-López et García-Magaña, 1994), an intestinal blood-sucking parasite of cichlids (*Cichlasoma* spp. and *Petenia splendida*), has only recently been described from fishes in the Mexican states of Campeche and Tabasco (Andrade-Salas et al. 1994), occurring as well in some sinkholes ("cenotes") of the Peninsula of Yucatan, southeastern Mexico (Moravec et al. 1995). The life cycle of this species has been unknown. Although the development of a few American and Asian species of the subgenus *Spirocamallanus* from fishes has already been studied experimentally (Li 1935, Pereira et al. 1936, Bashirullah and Ahmed 1976, Fusco 1980, De 1995), the present knowledge of the life cycle patterns and the morphogenesis of larvae of these remarkable nematodes remains insufficient (Anderson 1992). The same concerns congeneric species of the subgenus *Procamallanus* where the development of only three African and Asian species has so far been studied (Moravec 1975, Wang and Ling 1975, De et al. 1986a, Sinha 1988). In 1994, the present authors carried out some experimental observations on the development of *P.* (*S.*) *rebecae* in the intermediate, paratenic and definitive hosts and the results are presented in this paper.

*to whom correspondence should be sent

MATERIALS AND METHODS

Gravid females of *Procamallanus* (*S.*) *rebecae* with motile first-stage larvae in uterus were recovered from the intestine of the cichlid *Cichlasoma urophthalmus* (Günther), originating from the sinkhole Chen-há Cenote (Zona Chochola) in the State of Yucatan, southeastern Mexico, caught in June and September 1994. The nematodes were individually placed in small glass vessels (diameter 13 cm) filled with water, their bodies were torn by fine needles and the larvae were released from the uteri. Each vessel contained 100–120 copepods. A *Mesocyclops* sp. originating from the well in the village of Chemuan about 20 km north-west of Mérida was used as the only experimental intermediate host. A small amount of detritus and plant remnants were then added to each vessel which were kept at the laboratory temperatures of 21–22°C. The copepods were examined for the presence of nematode larvae at intervals of 1–3 days.

Feeding experiments with small fishes serving as paratenic hosts were carried out in small aquaria in the laboratory where fishes were allowed to feed spontaneously on the infected copepods harbouring the parasite's third-stage larvae. Afterwards, the fishes were kept in the laboratory at 21-22°C and fed with commercial dry food for aquarium fishes. Four guppies, *Poecilia reticulata* (Peters) (body length 3.2–3.4 cm) and 1 molly, *Poecilia latipinna* (LeSueur) (body length 3.4 cm),

all from a pet shop, were used. The feeding experiment with cichlids as definitive hosts was carried out in a larger aquarium where experimental fishes (5 specimens of *Cichlasoma urophthalmus* [Günther], body length 10–31 cm), originating from the breeding in the CINVESTAV, Mérida, were allowed to feed spontaneously on the infected copepods. The aquarium with aerated water and containing these experimental fishes was situated outside and, accordingly, the daily water temperature ranged between 25–32°C. Five control specimens of *C. urophthalmus* were kept in another aquarium. All cichlids were maintained on a diet of commercially produced fish food pellets.

The nematode larvae collected from infected copepods were killed by heating them in a drop of physiological saline on the glass slide over a flame for several seconds and then fixed by adding 4 % formaldehyde. Adults and larvae from fishes, as well as third-stage larvae from copepods were fixed in petri dishes by adding hot 4 % formaldehyde. The killed nematodes were examined with a light microscope and drawings were made with the aid of an Olympus microscope drawing attachment. All measurements are given in millimetres.

RESULTS

Natural infection of *Procamallanus* (*S.*) *rebecae* in fishes of the Chen-há Cenote

The nematodes used for this experimental work originated from the sinkhole Chen-há Cenote. Of the three native species of fish recorded from this cenote, *P.* (*S.*) *rebecae* was found only in *C. urophthalmus* although two other fish species, *Poecilia velifera* and *Rhamdia guatemalensis,* were also present. Of 25 small-sized *C. urophthalmus* examined on 14th September 1994 (body length 6–8 cm), 18 proved to harbour this parasite (prevalence 72 %) with the intensity 1–13 (mean 3) nematodes per fish. The nematodes were found only in the host's intestine, both at its anterior and posterior parts, where they sucked blood from the intestinal mucosa. In this sample, altogether 61 specimens of *P.* (*S.*) *rebecae* were recovered amongst which only 7 (11 %) gravid females with larvae in the uterus were present. Occasional collecting of plankton from this locality showed a frequent occurrence of the copepod *Macrocyclops albidus* (Jurine) which probably serves here as a natural intermediate host for this nematode species.

Experimental infections of copepod intermediate hosts

As other camallanids, the nematode *P. (S.) rebecae* is ovoviviparous which means that its first-stage larvae hatch *in utero* and are either passed into water with faeces of the host or gravid females protrude from the anus and rupture on contact with water (Anderson 1992).

The uteri of all nematode gravid females contained, in addition to first-stage larvae, a large proportion of eggs; after liberating the content of uterus into the water, many larvae, probably those not yet completely developed, remained on the bottom of the vessel without moving. Other larvae actively moved in the water, being attached by their tail tip to the bottom of the vessel, thus attracting the copepods which swallowed them.

After ingestion by a copepode intermediate host, the larvae penetrated the wall of the digestive tract into the haemocoel within a few hours to develop further. The penetration was accomplished with the aid of the larval cephalic tooth. During their entire development in the intermediate host, the larvae moved in the haemocoel, being located around the gut in the cephalothorax.

The larvae increase in size and undergo two moults, the first one 3 days p.i. and the second one 5–6 days p.i. at 21–22°C, before reaching the third stage, which is infective for the definitive or paratenic hosts (Table 1).

The first fully developed third-stage larva liberated from the cuticle of the second moult was recovered from a copepod 8 days p.i. The development of some larvae may probably be delayed, because a larva apparently just after the moult (with only a slightly sclerotized, thin-walled buccal capsule) was found in the copepod as late as 15 days p.i. The third-stage larvae already possess a big sclerotized buccal capsule and their tail is provided with three small terminal spikes; these larvae remain spirally coiled in the haemocoel of the intermediate host, but no capsule develops around them. After attaining the third stage, the larval development ceases in the body of the intermediate host. During the development from the first to the third stage the length of larvae increases approximately twice, whereas their tail becomes relatively shorter, representing 8 % (31–33 % in first-stage larvae) of the whole body length.

During the first 1–2 days, about 50 % of copepods were infected, but because of deaths, this percentage decreased to 15–30 % by the time when third-stage larvae were present. The intensity of infection was low, mostly one, rarely two nematode larvae occurring in one copepod. Some of the infected copepods kept mostly to the bottom of the vessel, being less motile than uninfected ones. Occasionally live copepods laying on the bottom and incapable of any motion were observed.

In addition to the copepods, one experimental vessel contained also a female specimen of the shrimp *Typhlatya* cf. *pearsei* Creaser (Decapoda, Natatia), collected from the same locality (the well in Chemuan) as copepods, but it did not become infected.

The development of larvae of *P.* (*S.*) *rebecae* was observed in copepods for the period of 34 days

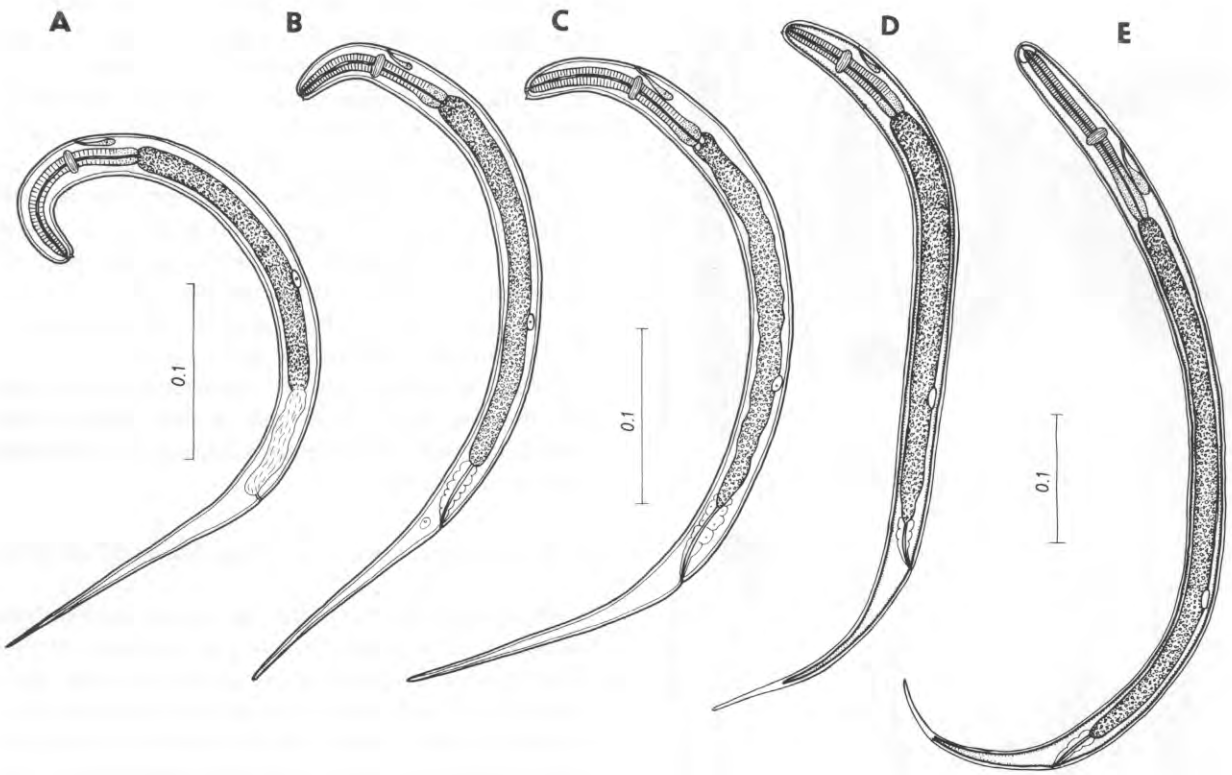

Fig. 1. *Procamallanus* (*S.*) *rebecae*, first- and second-stage larvae. **A** – first-stage larva from female's uterus; **B** – first-stage larva from copepods 1 day p.i.; **C** – same, 2 days p.i.; **D** – larva undergoing first moult in copepods 3 days p.i.; **E** – second-stage larva preparing for second moult in copepods 5 days p.i.

whereafter the remaining copepods were used for experimental infection of fishes.

Experimental infections of fish paratenic hosts

Thirty live copepods containing third-stage larvae of *P.* (*S.*) *rebecae* 34 days p.i. (the percentage of infected specimens about 30 %, intensity 1–2 nematode larvae per crustacean) were added to a 5-litre aquarium with 4 guppies and 1 molly, which were allowed to ingest them spontaneously. The molly and one guppy, both examined 13 days p.i., proved to be free of parasites. One guppy died 4 days p.i. and it contained 3 third-stage nematode larvae morphologically almost identical with those from copepods, but they were evidently larger (Table 2). The fishes killed and examined on days 13th and 36th contained two larvae and one larva, respectively. While the larvae in the first case were still in the third stage, the larva in the latter case was already a fourth stage (Table 2). All larvae of *P.* (*S.*) *rebecae* found in experimental poeciliids were free (non-encapsulated) in the lumen of the anterior part of intestine, being attached by their buccal capsule to the intestinal mucosa.

Experimental infections of fish definitive hosts

In this experiment, 5 specimens of *C. urophthalmus* were allowed to ingest spontaneously about 30 live, experimentally infected copepods harbouring third-stage nematode larvae (12 days p.i.); the percentage of infected copepods was about 20 %, intensity 1–2 larvae per crustacean.

The first two fishes examined on days 2 and 4 were uninfected, whereas the remaining fishes, examined on days 11, 18 and 39, harboured 3, 2 and 3 nematodes, respectively; all these were located in the posterior and middle parts of the host's intestine.

The morphometrical examination of the recovered larvae shows that the third-stage larvae were already prepared for the third moult (first in the definitive host) as early as day 11 p.i., being approximately 4.5 mm long. It can be estimated that this moult would occur about 2–3 days later, i.e. after approximately 13–14 days p.i.

After another week (18 days p.i.), only fourth-stage larvae were found. However, the larvae just before the fourth moult were found as late as 39 days p.i. It can be estimated that the last moult takes place some 3 days later, i.e. approximately on day 42 p.i. at this water temperature. The prepatent period could not be established.

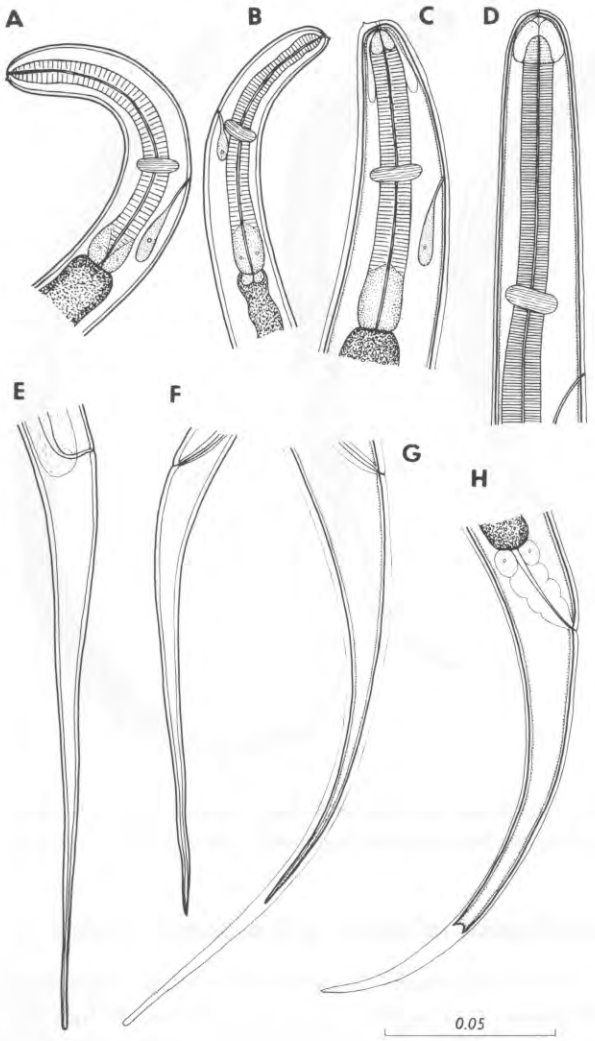

Fig. 2. *Procamallanus* (*S.*) *rebecae*, cephalic and caudal ends of first- and second-stage larvae. **A, E** – first-stage larva from female's uterus; **B, F** – first-stage larva from copepods 2 days p.i.; **C, G** – larva undergoing first moult in copepods 3 days p.i.; **D, H** – second-stage larva preparing for second moult in copepods 5 days p.i.

Morphology and larval development

a) First-stage larva Figs. 1A–C; 2A–B, E–F

The body of first-stage larvae from the female uterus is 0.488–0.508 mm long and 0.023–0.025 mm wide (Table 1). The body is transparent, slender, with an almost smooth cuticle. The head is blunt, armed with a small dorsal cuticular tooth. Oral papillae are indistinct. The mouth is formed by a short, thin, feebly sclerotized tube. The oesophagus is undivided, without a distinct lumen, representing 20–21 % of the whole body length. The posterior end of the oesophagus contains oesophageal glands with distinct cell nuclei. The oesophagus opens into the intestine through a small valve. The nerve ring encircles the oesophagus below its middle. The excretory pore is located immediately below the level of the nerve ring. The intestine is wide, light-coloured, with a fine granulation. The rectum is a colourless tube; rectal glands are present, but not well visible. The small oval genital primordium is situated ventrally, approximately at mid-length between the oesophagus end and the anal opening. The tail is conical, slender, with a sharply pointed tip. Its length is 31–33 % of the total length of the larval body.

Having penetrated into the haemocoel of the copepod, the first-stage larvae change only little in their morphology and measurements during the following two days (Table 1).

b) Second-stage larva Figs. 1D–E; 2C–D, G–H

The bodies of larvae in the first moult, present in the haemocoel of copepods 3 days p.i., are light-coloured and somewhat larger than those of the first-stage larvae (Table 1). The old cuticle is smooth and loosened along the whole length of body. The exuviae of this cuticle are best visible on both ends of the body, particularly on the tail. The newly formed tail resembles that of the first-stage larva, i.e., it is rather long and sharply pointed, but it is relatively shorter, representing only 25 % of the body length. The inner organisation of the body is almost the same as in the first-stage larvae, distinct changes being visible only at the cephalic end. The anterior end of the oesophagus is provided with a short hollow and it is surrounded by several elongate, drop-like glandular formations. The intestine is straight, relatively wide and contains numerous fine granules. The rectum is colourless. The oval genital primordium is more posterior when compared with first-stage larvae.

The second-stage larvae (Fig. 1E) recovered 5 days p.i. were distinctly longer and the relative length of their tail was markedly smaller (Table 1). Their bodies are still light-coloured, and the cuticles smooth. The cephalic end is rounded, the mouth is formed by a short thin tube opening into the oesophagus. The anterior end of the oesophagus is covered with a thick, hyaline bell-shaped formation; apparently, this "cap" is the anlage of the future buccal capsule. The posterior glandular part of the oesophagus becomes longer, but a distinct division between the muscular and the glandular parts of the oesophagus is not yet apparent. The intestine is wide and straight. Rectal glands become visible. The larva starts its second moult which is best visible on the tail. Inside the old cuticle of the second-stage larva, a new tail, typical of the third-stage larva, is already being formed. The second moult of the larvae is completed on the 6th day p.i.

Table 1. Growth of *Procamallanus (S.) rebecae* larvae in the intermediate host.

	First- stage larvae			First moult
	Free larvae	1 day p.i	2 days p.i.	3 days p.i.
Length of body	0.488–0.508	0.423	0.470	0.563
Width of body	0.023–0.025	0.020	0.023	0.033
Length of oesophagus	0.098–0.105	0.080	0.094	0.118
Distance of nerve ring	0.063–0.070	0.045	0.040	0.080
Distance of excretory pore	0.075–0.078	0.055	0.074	0.083
Genital primordium from anterior extremity	0.213–0250	0.188	0.235	0.325
Length of tail	0.150–0.165	0.123	0.148	0.183/0.138
% of oesophagus of body length	20–21	19	20	21
% of tail of body length	31–33	29	31	33/25
	Second-stage larva	Third-stage larvae		
	5 days p.i.	8 days p.i.	15 days p.i.	34 days p.i.
Length of body	0.793	1.103	1.360	0.870–1.105
Width of body	0.030	0.038	0.045	0.030–0.038
Length of oesophagus	0.168	0.248	–	0.201–0268
Muscular oesophagus	0.115	0.135	–	0.113–0.138
Glandular oesophagus	0.053	0.113	–	0.088–0.125
Buccal capsule – length	–	0.030	0.030	0.028–0.033
– width	–	0.018	0.023	0.016–0.018
– thickness	–	0.004	0.002	0.004–0.006
Distance of nerve ring	0.095	0.115	–	0.090–0.098
Distance of excretory pore	0.103	0.125	–	0.100–0.128
Genital primordium from anterior extremity	0.495	0.663	0.480	0.338–0.450
Length of tail	0.138/0.095	0.090	0.105	0.068–0.093
% of oesophagus of body length	21	25	27	26–30
% of tail of body length	17/12	8	8	8

c) Third-stage (infective) larva from copepods
Figs. 3–4

The third-stage larva, immediately after the second moult, was observed on 8th day p.i. (Table 1). Its body is light-coloured, with an almost smooth cuticle. The cephalic end is rounded, with distinct cephalic papillae arranged in two circlets surrounding the mouth. A thin-walled, oval, colourless buccal capsule with a spacious cavity is present. This is followed by a short, narrow, cuticularized ring-like formation connecting the capsule and the oesophagus. The oesophagus is relatively longer that that in the second-stage larvae, representing 25 % of the body length, but the division between the muscular and the glandular parts is still obscure.

During the following days, the general morphology and measurements of third-stage larvae do not change substantially (Table 1), only the walls of the buccal capsule become more sclerotized and markedly thicker. The larvae obtained 34 days p.i. are 0.870–1.105 mm long and 0.030–0.038 mm wide (Table 1), with a smooth cuticle. The cephalic end bears eight small papillae arranged in two circlets surrounding the circular mouth opening. The buccal capsule is yellowish, elongate, continuous, relatively thick-walled, with a slightly outlined transverse constriction at its basal portion; its inner surface is smooth, without spiral thickenings. The oesophagus is distinctly divided into an anterior, almost cylindrical muscular portion with a strong cuticular lining and a posterior glandular portion of approximately

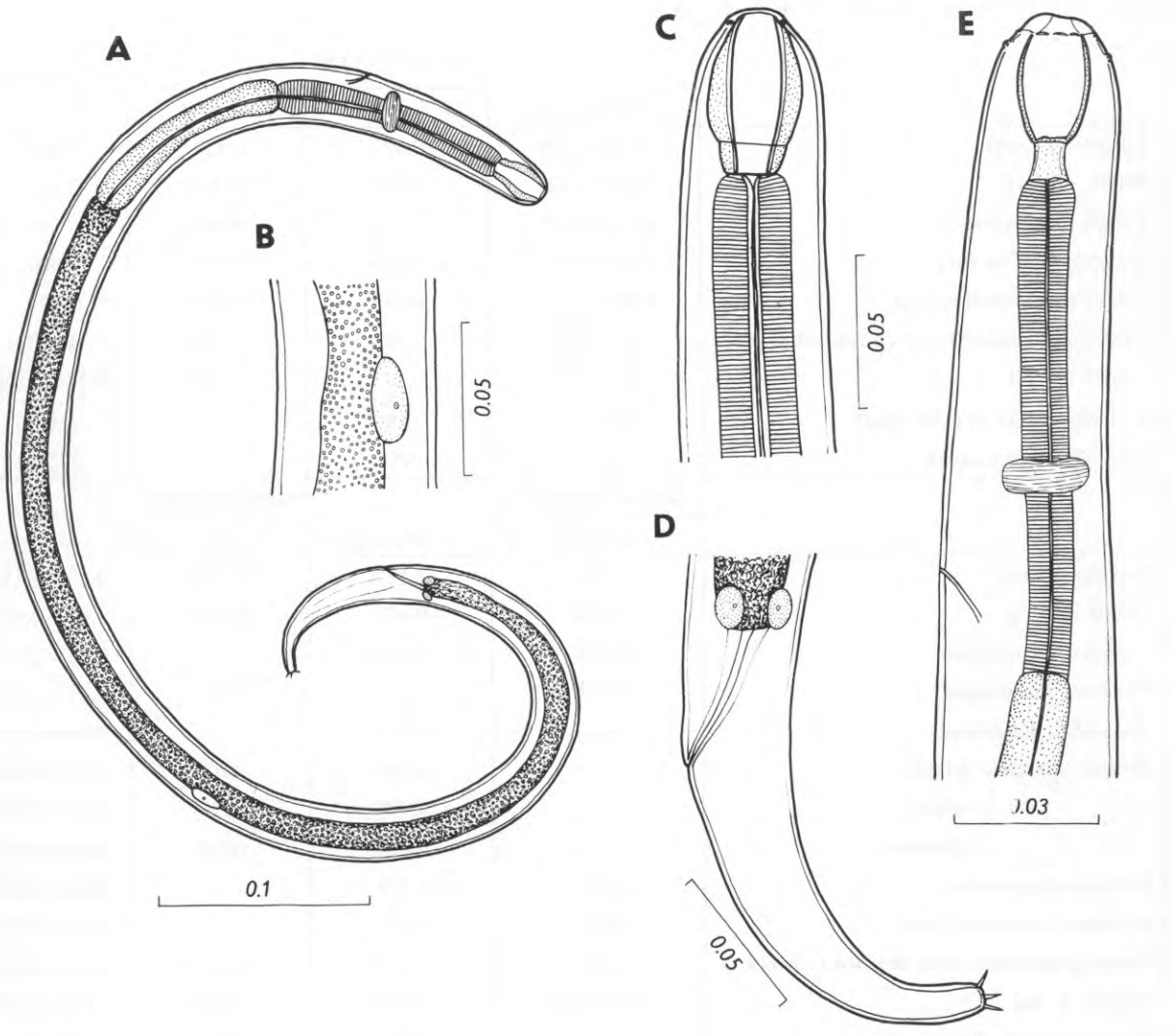

Fig. 3. *Procamallanus* (*S.*) *rebecae*, third-stage larva from copepods. **A** – general view; **B** – region of genital primordium; **C** – cephalic end; **D** – tail; **E** – cephalic end of larva just after second moult; (A–D – 34 days p.i; E – 15 days p.i.)

the same length. The nerve ring encircles the muscular oesophagus approximately at its mid-length, the excretory pore is slightly posterior to the nerve ring. The oesophagus opens into the intestine through a small valve. The intestine is straight, orange-brown in colour and contains numerous granules. Three large, uncoloured unicellular glands surround the rectum, which is a thin, straight hyaline tube. The tail is conical, elongate, with a rounded tip bearing one dorsal and two ventrolateral small thin terminal cuticular spines; the tail represents 8 % of the body length. The small oval genital primordium is located in the posterior half of the body. These larvae are approximately twice as long as the first-stage larvae from the uterus.

d) Third-stage larva from experimental fishes
Figs. 5D–I; 6A

Third-stage larvae were obtained from guppies on day 4 and 13 p.i. Their morphology was similar to that of infective larvae obtained from copepods except for the larger size of the body and some internal organs (Table 2). The size of the buccal capsule was almost identical to that in larvae from copepods. The genital primordium was oval, and the tail tip had three small, thin cuticular spines.

Larvae in the third moult obtained from *C. urophthalmus* on day 11 p.i. were larger (Table 2), their genital primordium was more elongate and, in addition to

Table 2. Growth of *Procamallanus (S.) rebecae* larvae in fish hosts.

	From copepode (intermediate host)	From *Poecilia reticulata* (paratenic host)		
		Third-stage larvae		Fourth-stage larvae
	34 days p.i.	4 days p.i.	13 days p.i.	36 days p.i.
Length of body	0.870–1.105	1.690–1.750	2.065	4.020
Width of body	0.030–0.038	0.035–0.045	0.050	0.090
Buccal capsule – length	0.018–0.033	0.030–0.033	0.038	0.045
– width	0.016–0.018	0.020	0.023	0.038
– thickness	0.004–0.006	0.005	0.005	0.010
Muscular oesophagus	0.113–0.138	0.135–0.155	0.180	0.230
Glandular oesophagus	0.088–0.125	0.073	0.185	0.260
Distance of nerve ring	0.090–0.098	0.103–0.108	0.125	0.150
Distance of excretory pore	0.100–0.128	0.118–0.125	0.138	0.225
Genital primordium from posterior extremity	0.388–0.450	0.750–0.760	1.065	1.910
Length of tail	0.068–0.093	0.125–0.130	0.125–0.150	0.180
% of oesophagus of body length	26–30	14–15	20	13
% of tail of body length	8	7–8	7	4
	From *Cichlasoma urophthalmus* (definitive host)			
	Third-stage larvae	Fourth-stage larvae	Juvenile ♂♂	Juvenile ♀
	11 days p.i.	18 days p.i.	39 days p.i.	39 days p.i.
Length of body	4.410–4.570	6.433–7.031	8.350–8.568	9.874
Width of body	0.068–0.109	0.122–0150	0.204–0.218	0.204
Buccal capsule – length	0.045–0.051	0.069–0.072	0.078–0.081	0.078
– width	0.027–0.030	0.045–0.051	0.045–0.048	0.057
– thickness	0.009	0.009–0.012	0.012	0.012
Muscular oesophagus	0.147–0.272	0.340–0.354	0.326–0.340	0.381
Glandular oesophagus	0.198–0.286	0.367–0.381	0.449	0.503
Distance of nerve ring	0.153–0.174	0.228–0.267	0.245	0.258
Distance of excretory pore	0.186–0.285	0.348–0.394	0.394–0.449	0.408
Genital primordium from posterior extremity	1.725–2.271	3.400–3.522	–	4.964
Length of tail	0.190–0.218	0.272	0.231–0.245	0.326
% of oesophagus of body length	13	11–12	10	10
% of tail of body length	4–5	4	3	3
Developing spicules – right	–	–	0.240–0.465	–
– left	–	–	0.201–0.210	–

the old buccal capsule typical of the third larval stage (size 0.045–0.051 × 0.027 mm), a newly formed, slightly sclerotized capsule of the fourth stage with spiral thickenings (size 0.051 × 0.039 mm) was already present (Fig. 5D). The relative length of the tail became smaller, it representing only 4–5 % of the body length. A newly formed cuticle was already visible under the old, thick cuticle. All these signs indicated that the larvae were prepared for their third moult.

e) Fourth-stage larva Figs. 5E–I; 6B–C

A fourth-stage larva 4.02 mm long and 0.090 mm wide was obtained from a guppy on day 36 p.i. (Table

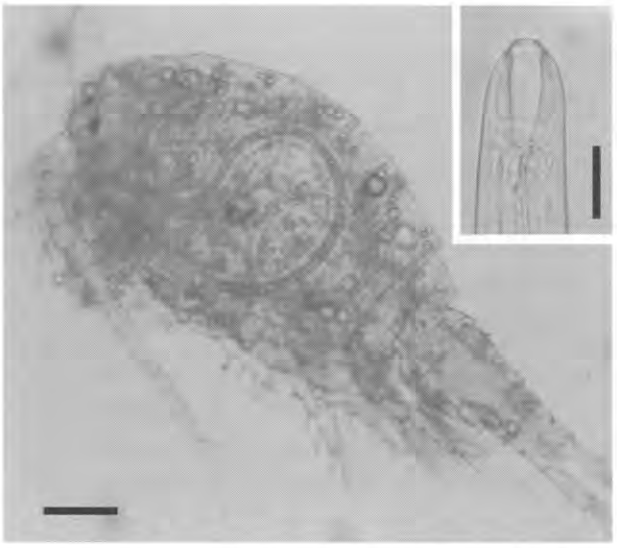

Fig. 4. Two third-stage larvae of *Procamallanus* (*S.*) *rebecae* in the haemocoel of the copepod; scale bar = 100 µm. Insert: cephalic end of the larva; scale bar = 20 µm.

2). Its body was whitish, with a thin, smooth cuticle. The buccal capsule was colourless, thick-walled, with 10 spiral thickenings. Otherwise the general morphology was similar to that of third-stage larvae, except for the genital primordium which was elongate, 0.458 mm long, with the anlage of the developing vagina; the vulva was still covered by the cuticle. The intestine was narrow and brownish. The tail was similar to that of the third-stage larva, bearing three small cuticular spines at the tip (Fig. 5H), and represented only 4 % of the body length.

Two fourth-stage larvae obtained from *C. urophthalmus* on day 18 p.i. were considerably larger, measuring 6.43–7.03 mm in length and 0.122–0.150 mm in width (Table 2). Their general morphology was practically identical with that of the fourth-stage larva from *P. reticulata*. Their buccal capsule had 12–13 spiral thickenings, 2–3 of them being incomplete.

On day 39 p.i., three larvae already in the fourth moult were recovered from *C. urophthalmus*. It was possible to distinguish their sex: the males were 8.350–8.568 mm long, and, in addition to the testis, caudal papillae, still covered by the cuticle, and developing, sclerotized spicules were present (Table 2). The female larva was larger (body length 9.874 mm), with a well-developed vulva (still covered by a cuticle), vagina, uterus and ovaries; the uterus already contained a few immature eggs. The cuticle of all these juveniles was markedly thick and it was possible to observe the onset of the separation of the old cuticle from the newly formed one. Apart from the buccal capsule, typical of the fourth-stage larvae (with 13 spiral thickenings), the new buccal capsule was visible in all of these larvae in 4th moults; it was wider than the former, already provided with spiral thickenings but still light-coloured due to its insufficient sclerotization. The oesophageal portion of these larvae represented 10 % of the body length, whereas the tail formed only 3 %.

DISCUSSION

Although at present the subgenus *Spirocamallanus* Olsen, 1952 includes more than a hundred nominal species parasitic in fish (see Andrade-Salas et al. 1994), the life cycles have so far been experimentally studied in only a few of them. Li (1935) was the first to describe the development of *Procamallanus* (*S.*) *fulvidraconis*, a parasite of freshwater catfishes in China, in the copepod intermediate host (*Cyclops magnus*, *C. serratus* and *C. vicinus*) and Pereira et al. (1936) studied the development of the South American species *P.* (*S.*) *caerensis* (= *P.* (*S.*) *hilarii* according to Kohn and Fernandes 1988), a parasite of the characid fish *Astyanax bimaculatus* in Brazil. In addition to intermediate hosts *Diaptomus caerensis* and *D. azevedoi*, the latter authors demonstrated experimentally that paratenic hosts, fry of the fish *Curimatus elegans*, may participate in the life cycle of this nematode. Later Bashirullah and Ahmed (1976) followed the larval development of *P.* (*S.*) *intestinecolas* (= *P.* (*S.*) *mysti* according to De et al. 1986b), a parasite of the freshwater catfish *Mystus vittatus* in Bangladesh, in the intermediate host (*Mesocyclops leuckarti* and *Thermocyclops crassus*); the whole development of *P.* (*S.*) *mysti* was experimentally studied by De (1995) in India, using *Mesocyclops leuckarti* and *M. crassus* as intermediate hosts and *Mystus vittatus* as the definitive host. Fusco (1980) reported on the development of *P.* (*S.*) *cricotus*, a parasite of *Micropogonias undulatus* and at least 12 other species of marine and estuarine fishes in the northern Gulf of Mexico, in the harpacticoid copepod *Tigriopus californicus*. In addition to copepods, Fusco (1980) successfully infected the shrimp *Penaeus setiferus*. Third-stage larvae of *P.* (*S.*) *cricotus* were previously obtained by Overstreet (1973) from experimentally and naturally infected shrimps *P. setiferus* in the Gulf of Mexico and by Feigenbaum (1975) from *P. vannamei* from the Pacific coast of Mexico (Fusco 1980).

In addition to species parasitic in fishes, Thurston (1970) reported that *Procamallanus* (*Spirocamallanus*) *xenopodis* of the stomach of African clawed toads (*Xenopus* spp.) developed to the third stage in *Thermocyclops infrequens* and *Mesocyclops leuckarti* in about 15 days at 22–25°C.

Within the subgenus *Procamallanus* Baylis, 1923, the development has so far been studied in only three species. Moravec (1975) followed the larval development in the species *P.* (*P.*) *laeviconchus*, a frequent parasite of African freshwater fishes of several families. In addition to the experimental intermediate

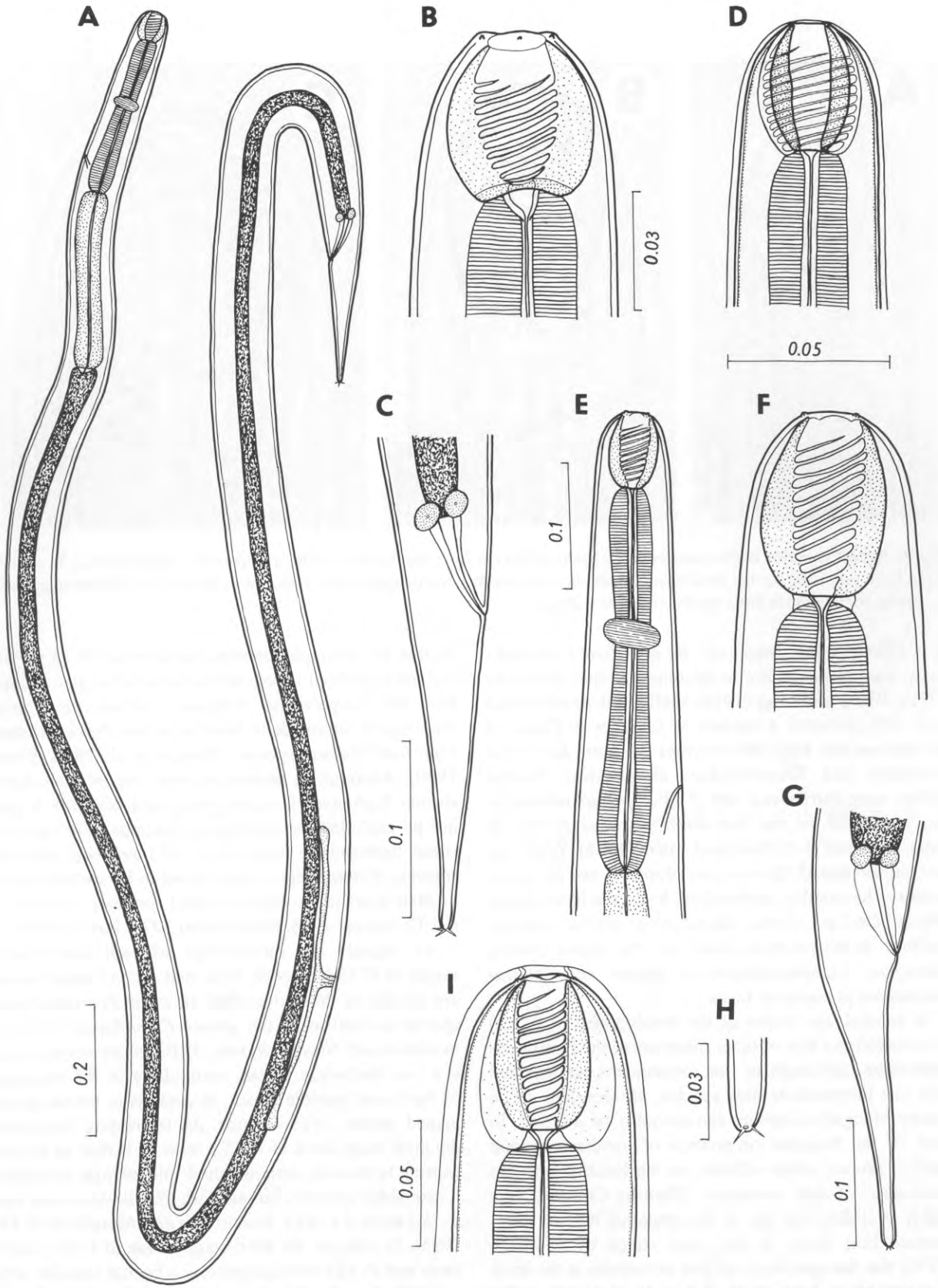

Fig. 5. *Procamallanus* (*S.*) *rebecae*, larvae from paratenic and definitive hosts. **A–C** – fourth-stage larva from *Poecilia reticulata* 36 days p.i. (**A** – general view, **B** – buccal capsule, **C** – tail); **D–I** – larvae from *Cichlasoma urophthalmus* (**D** – larva undergoing third moult 11 days p.i., **E** – cephalic end of fourth-stage larva 18 days p.i., **F** – buccal capsule of fourth-stage larva 18 days p.i., **G** – tail of fourth-stage larva 18 days p.i., **H** – tail tip of fourth-stage larva 18 days p.i., **I** – cephalic end of fourth-stage larva preparing for fourth (last) moult).

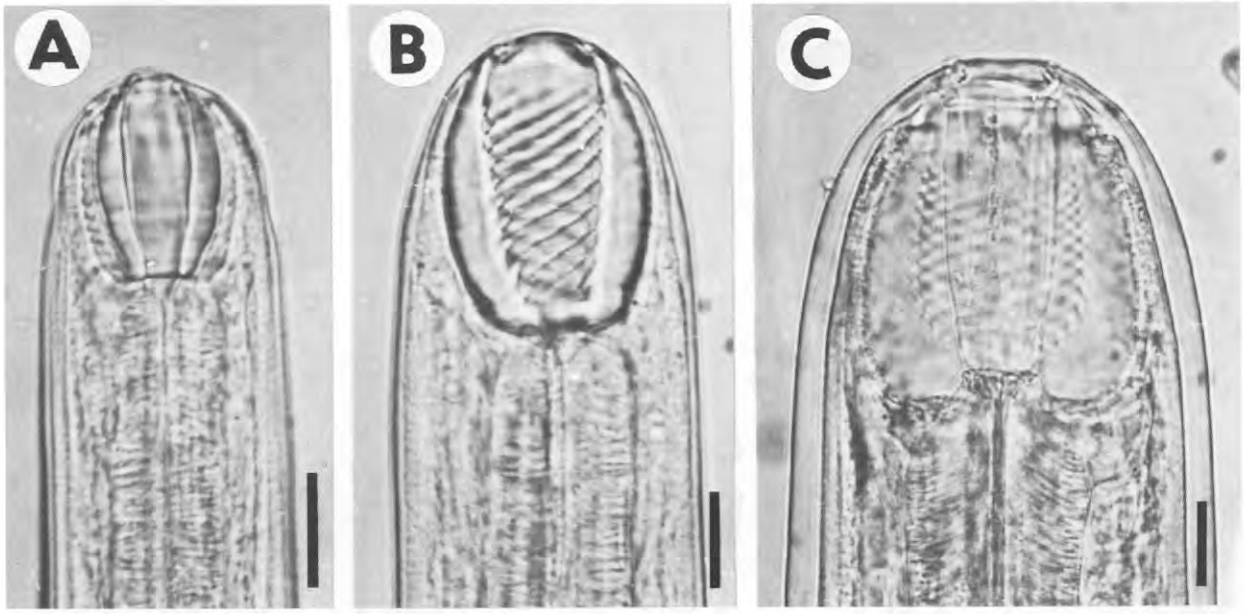

Fig. 6. Buccal capsules in *Procamallanus* (*S.*) *rebecae* larvae from the definitive host, *Cichlasoma urophthalmus*. **A** – third-stage larva preparing for the third moult; scale bar = 30 μm; **B** – fourth-stage larva; scale bar = 20 μm; **C** – fourth-stage larva preparing for the fourth (last) moult; scale bar = 20 μm.

host (*Mesocyclops leuckarti*), he also found paratenic hosts, *Gambusia affinis* to become infected (Moravec 1975). Wang and Ling (1975) studied the development of *P.* (*P.*) *fukiensis*, a parasite of catfishes in China, in the intermediate host (*Mesocyclops leuckarti*, *Eucyclops serrulatus* and *Thermocyclops oithonoides*). Similar studies were carried out with *P.* (*P.*) *spiculogubernaculus*, a parasite of the fish *Heteropneustes fossilis* in India, by De et al. (1986a) and Sinha (1988). While the first authors found *Mesocyclops obsolatus* and *M. oithonoides* to be suitable intermediate hosts, the latter author infected *Cyclops vicinus*, *Mesocyclops leuckarti* and *M. hyalinus* as intermediate hosts and the fishes *Clarias batrachus*, *Lepidocephalichthes guntea* and *Puntius conchonius* as paratenic hosts.

In general, the course of the development of *P.* (*S.*) *rebecae* follows that of other members of the family Camallanidae. Although in our experiments we infected only one intermediate host species, *Mesocyclops* sp., a variety of copepod species can probably be infected. In view of the frequent occurrence of another copepod species, *Macrocyclops albidus*, in the locality with the occurrence of this nematode (Chen-há Cenote), it is highly probable that this is the principal natural intermediate host there. It has been stated by Moravec (1994) that the specificity of fish nematodes at the level of intermediate hosts is much broader than that at the level of definitive hosts, which seems to be valid particularly for camallanids. Similarly to the situation with the species *Camallanus lacustris* in Europe, it may well be that the principle intermediate hosts of *P.* (*S.*) *rebecae* are cyclopoid copepods but, less often, also diaptomid and harpacticoid copepods, which have been reported as intermediate hosts in other *Procamallanus* (*Spirocamallanus*) species (Pereira et al. 1936, Fusco 1980). Although an attempt to infect the only available shrimp *Typhlatya* cf. *pearsei* was not successful, it cannot be excluded that freshwater shrimps may also become intermediate hosts of *P.* (*S.*) *rebecae*, because shrimps (*Penaeus* spp.) were found to be suitable intermediate hosts in the closely related species *P.* (*S.*) *cricotus* (Overstreet 1973, Feigenbaum 1975, Fusco 1980).

As regards the morphology of individual larval stages of *P.* (*S.*) *rebecae*, first- and second-stage larvae are similar to those described in other *Procamallanus* species as well as in the genera *Camallanus*, *Paracamallanus* and *Neocamallanus*. Differences occur, however, in third-stage larvae, particularly in the structure of the buccal capsule which, in contrast to the three last named genera, is continuous. An interesting feature of the third-stage larva of *P.* (*S.*) *rebecae* is that its buccal capsule is smooth, without spiral thickenings, by which it resembles infective larvae of *P.* (*S.*) *fulvidraconis* and *P.* (*S.*) *mysti* (Li 1935, Bashirullah and Ahmed 1976, De 1995). In contrast, the third-stage larvae of *P.* (*S.*) *caerensis* and *P.* (*S.*) *cricotus* possess a buccal capsule with spiral thickenings (Pereira et al. 1936, Fusco 1980). Li (1935) and Pereira et al. (1936), however, considered infective larvae from copepods to be the second stage, which is evidently an error (see Moravec 1975). It

seems that also the number of conical processes or spines on the tail tip of third-stage larvae is typical of the species. Larvae of some species (e.g., *P.* (*S.*) *fulvidraconis*, *P.* (*S.*) *cricotus*, *P.* (*S.*) *mysti*, *P.* (*P.*) *spiculogubernaculus*) including *P.* (*S.*) *rebecae* have three caudal processes, but there are four processes in the larvae of *P.* (*S.*) *caerensis* and *P.* (*P.*) *laeviconchus*.

Since *P.* (*S.*) *rebecae* belongs to the same morphological group of species as *P.* (*S.*) *cricotus* (see Moravec et al. 1995), it is interesting to compare their infective larvae from copepods. While the body of *P.* (*S.*) *cricotus* third-stage larvae is reddish, 0.55–1.00 mm long, with a golden buccal capsule provided with 16–23 spiral thickenings and the tail is 0.040–0.062 mm long (Fusco 1980), that of *P.* (*S.*) *rebecae* larvae is 0.87–1.36 mm long, with a colourless to yellowish buccal capsule without any spiral thickenings and the tail is 0.068–0.105 mm long. Accordingly, both species can be reliably distinguished already at the stage of infective larvae. Fusco (1980) has reported that the tail tip of the second-stage larvae of *P.* (*S.*) *cricotus* is blunt to three-spined but, judging from the presence of the buccal capsule and the body size (up to 0.911 mm), it is evident that the author included young third-stage larvae.

The rate of the larval development of fish nematodes in their invertebrate intermediate hosts depends considerably on the water temperature (Moravec 1994). In our experiments with *P.* (*S.*) *rebecae*, this development was relatively quick, when infective third-stage larvae developed in copepods in 6 days at 21–22°C. This is comparable with the same development of *P.* (*P.*) *laeviconchus* (6 days at 23–24°C) and *P.* (*P.*) *spiculogubernaculus* (4 and 6 days at 26°C and 38°C, respectively) (Moravec 1975, Sinha 1988). On the other hand, a slower development was observed by Li (1935) in *P.* (*S.*) *fulvidraconis* (8–9 days at laboratory temperature), by Fusco (1980) in *P.* (*S.*) *cricotus* (10–11 days at 23–26°C) and by Thurston (1970) in *P.* (*S.*) *xenopodis* (more than 12 days at 22–25°C).

Until recently, the rate of the development in the definitive host was not studied experimentally for any *Procamallanus* species, only Li (1935) presumed that it lasted up to four months in *P.* (*S.*) *fulvidraconis*. De (1995) observed the last (fourth) moult of "male" and "female" larvae of *P.* (*S.*) *mysti* in the definitive host to occur on day 37 p.i. and day 67 p.i., respectively. Our experimental observations on *P.* (*S.*) *rebecae* confirm a relatively slow development in the definitive host, *Cichlasoma urophthalmus*, when adults (both males and females) developed in about 42 days p.i. at 25–32°C. This is similar to the development in other camallanids. Stromberg and Crites (1974) reported that the adults of *Camallanus oxycephalus* first appeared in its fish definitive host after 18–24 days, whereas Moravec (1969) observed males and females of *Camallanus lacustris* as late as 35 and 67–69 days p.i., respectively, in its definitive host, the perch. However, the prepatent period of *C. lacustris* was three months according to the latter author. Consequently, it can be estimated that the prepatent period of *P.* (*S.*) *rebecae* lasts about 2–3 months.

The present study has shown that small planktonophagous fishes, for example the guppy, may serve as experimental paratenic hosts for *P.* (*S.*) *rebecae*. The infective nematode larvae from copepods can survive more than one month in the body of its paratenic host and they may not only increase in size in this host but they can even attain the next, fourth larval stage in it (metaparatenic parasitism); but this development is much slower than that in the definitive host. While the larvae already finished their third moult and changed to fourth-stage larvae in *C. urophthalmus* on day 13 p.i., the larva recorded on the same day (13 days p.i.) from *Poecilia reticulata* was in the third stage and its body length was only half of that of the larvae from *C. urophthalmus* on day 11 p.i. (Table 2). Paratenic (metaparatenic) hosts (small fish *Curimatus elegans*) were found as well in the South American species *P.* (*S.*) *caerensis* (Pereira et al. 1936). Also Moravec (1975) reported experimental paratenic hosts, the mosquito fish *Gambusia affinis*, in the African species *P.* (*S.*) *laeviconchus*, and Sinha (1988) found experimentally three species of fish paratenic hosts in the Indian species *P.* (*P.*) *spiculogubernaculus*. Paratenic parasitism seems to be widely distributed in nematodes of the family Camallanidae and it has also been recorded for members of the genera *Camallanus* (Kupryanova 1954, Moravec 1971, Stromberg and Crites 1974, Crites 1976) and *Paracamallanus* (Moorthy 1938).

Our experiments have demonstrated that the fish definitive host (*Cichlasoma* spp.) may acquire *P.* (*S.*) *rebecae* infection directly by feeding on the copepods harbouring the parasite's third-stage larvae. However, paratenic hosts (small fish) may apparently be another source of infection. In the locality from where *P.* (*S.*) *rebecae* was collected from *Cichlasoma urophthalmus* (Chen-há Cenote), the common poeciliid fish, *Poecilia velifera*, might play a role of a paratenic host of this nematode.

Acknowledgements. The authors' thanks are due to Dr. Frank Fiers of the Royal Belgium Institute of Natural Sciences in Brussels, Belgium, for the identification of copepods and the shrimp used in this study, and to Mrs. I. Husáková, a technician of the Institute of Parasitology, Academy of Sciences of the Czech Republic, in České Budějovice for her help with preparation of drawings. This study was supported by the grant no. PO99 from the Comisión Nacional para el Conocimiento y Uso de la Biodiversidad (CONABIO), Mexico. We also thank Dr. R. Simá-Alvarez, CINVESTAV-IPN Mérida, for making microphotographs of Fig. 4 .

REFERENCES

ANDERSON R. C. 1994: Nematode Parasites of Vertebrates. Their Development and Transmission. C.A.B. International, Wallingford, Oxon (UK), 578 pp.

ANDRADE-SALAS O., PINEDA-LÓPEZ R. F., GARCÍA-MAGAÑA L. 1994: *Spirocamallanus rebecae* sp. n. (Nematoda: Camallanidae) from freshwater fishes in south-eastern Mexico. Folia Parasitol. 41: 259–270.

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

CRITES J. L. 1976: An alternative pathway in the life cycle of *Camallanus oxycephalus* Ward et Magath 1916 (Nematoda: Camallanidae). J. Parasitol. 62: 166.

DE N. C. 1995: On the development and life cycle of *Spirocamallanus mysti* (Nematoda: Camallanidae). Folia Parasitol. 42: 135–142.

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

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

FEIGENBAUM D. L. 1975: Parasites of the commercial shrimp *Penaeus vannamei* Boone and *Penaeus brasiliensis* Latreille. Bull. Mar. Sci. 25: 491–514.

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

KOHN A., FERNANDES B. M. M. 1988: Helminth parasites of fishes from the hydroelectric power station of Eletrosul (Brazil). I. *Procamallanus annipetterae* n. sp. and *Spirocamallanus pintoi*, new species (Nematoda, Camallanidae) from the reservoir of "Salto Osorio". Mem. Inst. Oswaldo Cruz 83: 293–298.

KUPRYANOVA R. A. 1954: On the biology of fish nematodes *Camallanus lacustris* (Zoega, 1776) and *Camallanus truncatus* (Rudolphi, 1814) (Nematodes: Spirurida). Dokl. AN SSSR 97: 373–376. (In Russian.)

LI (1935): The taxonomy and early development of *Procamallanus fulvidraconis* n. sp. J. Parasitol. 21: 102–113.

MOORTHY V. N. 1938: Observations on the life history of *Camallanus sweeti*. J. Parasitol. 24: 323–342.

MORAVEC F. 1969: Observations on the development of *Camallanus lacustris* (Zoega, 1776) (Nematoda: Camallanidae). Acta Soc. Zool. Bohemoslov. 33: 15–33.

MORAVEC F. 1971: On the problem of host specificity, reservoir parasitism and secondary invasions of *Camallanus lacustris* (Nematoda; Camallanidae). Helminthologia 10 (Year 1969): 107–114.

MORAVEC F. 1975: The development of *Procamallanus laeviconchus* (Wedl, 1862) (Nematoda: Camallanidae). Acta Soc. Zool. Bohemoslov. 39: 23–38.

MORAVEC F. 1994: Parasitic Nematodes of Freshwater Fishes of Europe. Academia and Kluwer Acad. Publishers, Prague and Dordrecht, Boston, London, 473 pp.

MORAVEC F., VIVAS-RODRÍGUEZ C., SCHOLZ T., VARGAS-VÁZQUEZ J., MENDOZA-FRANCO E., GONZÁLEZ-SOLÍS D. 1995: Nematodes parasitic in fishes of cenotes (= sinkholes) of the Peninsula of Yucatan, Mexico. Part 1. Adults. Folia Parasitol. 42: 115–129.

OVERSTREET R. M. 1973: Parasites of some penaeid shrimps with emphasis on reared hosts. Aquaculture 2: 105–140.

PEREIRA C., DIAS M. V., AZEVEDO P. 1936: Biologia do nematóide "*Procamallanus caerensis*" n. sp. Arch. Inst. Biol. S. Paulo 7: 209–226 + 2 Plts.

SINHA A. K. 1988: On the life cycle of *Procamallanus spiculogubernaculus* (Camallanidae) (Agrawal, 1958) – a nematode parasite of fishes. Riv. Parassitol. 49: 111–116.

STROMBERG P. C., CRITES J. L. 1974: The life cycle and development of *Camallanus oxycephalus* Ward et Magath, 1916 (Nematoda: Camallanidae). J. Parasitol. 60: 117–124.

THURSTON J. P. 1970: Studies on some Protozoa and helminth parasites of *Xenopus*, the African clawed toad. Rev. Zool. Bot. Afr. 87: 349–369.

WANG P., LING X. 1975: Some nematodes of the suborder Camallanata from Fujian Province, with notes on their life histories. Acta Zool. Sinica 21: 350–358. (In Chinese, Engl. summary.)

Received 4 March 1995

Accepted 22 August 1995