FOLIA PARASITOLOGICA 43: 61–70, 1996

The development of *Procamallanus* (*Spirocamallanus*) *neocaballeroi* (Nematoda: Camallanidae), a parasite of *Astyanax fasciatus* (Pisces) in Mexico

František Moravec[1,2] and **Joaquín Vargas-Vázquez**[1]

[1]Laboratory of Parasitology, Centre for Research and Advanced Studies, National Polytechnic Institute, Carretera 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*

Key words: Nematoda, *Procamallanus*, *Spirocamallanus*, *Astyanax*, Copepoda, development, Mexico

Abstract. The development of the nematode *Procamallanus* (*Spirocamallanus*) *neocaballeroi* (Caballero-Deloya, 1977), an intestinal parasite of the characid fish, *Astyanax fasciatus* (Cuvier) in Mexico, was studied in the experimental copepod intermediate host, *Mesocyclops* sp. After the copepod's ingestion of free first-stage larvae of the nematode, these penetrate into the haemocoel of the intermediate host; they moult twice (on the 3rd and 4–5th day p.i. at 21–22°C) before they attain the third, infective stage. The third-stage larva already possesses the large buccal capsule subdivided into an anterior broad portion with eight spiral thickenings (as observed in lateral view) and a narrow posterior portion, and its tail tip bears three conical processes. The definitive host acquires infection by feeding on infected copepods; in the intestine of this fish, the nematode larvae undergo two more moults (on the 10th and 14–15th day p.i. at 25–32°C) before attaining their maturity. The prepatent period is approximately two months.

The nematode *Procamallanus* (*Spirocamallanus*) *neocaballeroi* (Caballero-Deloya, 1977) is a specific intestinal parasite of the freshwater characid, *Astyanax fasciatus* (Cuvier) in Mexico. It was originally described from Lake Catemaco in Veracruz (Caballero-Deloya 1977) and recently it has also been recorded from cenotes (sinkholes) in the coastal region of the State of Quintana Roo of the Yucatan Peninsula (Moravec et al. 1995a). The life cycle of this nematode species has been unknown. Although the development of a few American and Asian species of the subgenus *Spirocamallanus* from fishes has already been studied (Li 1935, Pereira et al. 1936, Bashirullah and Ahmed 1976, Fusco 1980, De 1995, Moravec et al. 1995b), information on the development of these nematodes and their larval morphogenesis remains still 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. 1986, Sinha 1988). In 1994, the present authors carried out some experimental observations on the development of *P.* (*S.*) *neocaballeroi* in the intermediate and definitive hosts and the results are presented in this paper.

*address for correspondence

MATERIALS AND METHODS

Gravid females of *Procamallanus* (*S.*) *neocaballeroi* with motile first-stage larvae in the uterus were recovered from the intestine of *Astyanax fasciatus*, originating from two near-by cenotes, Gran Cenote and Kawash, in a coastal region of the State of Quintana Roo, southeastern Mexico, caught in September 1994. The nematodes were individually placed in small glass vessels (diameter 13 cm) filled with water. Each vessel contained about 150–200 copepods. The nematodes' bodies were torn up by fine needles to release the larvae from the uteri. Only *Mesocyclops* sp. originating from the well in the village of Chemuan about 20 km north-west of Mérida was used as the experimental intermediate host. A small amount of detritus and plant remnants were then added into 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 24 hours.

The feeding experiment with fish definitive hosts was carried out in small aquaria in the laboratory where 10 small-sized *A. fasciatus* (body length 6–16 cm) were allowed to feed spontaneously on infected copepods harbouring the parasite's third-stage larvae. The experimental fishes originated from the cenote Noc-choncunchey in western Yucatan, where this nematode does not occur in the local population of *A. fasciatus*. After 24 hours, experimental fishes were placed outside in larger tanks with aerated water where the daily water temperature ranged between 25–32°C. Ten control specimens of *A.*

fasciatus were kept in another tank. All fishes were maintained on a diet of commercially produced fish food pellets.

The nematode larvae from infected copepods were immobilized by heating them in a drop of physiological saline on a 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 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 *Procamallanus* (*S.*) *neocaballeroi* infection in fishes in cenotes

The nematodes used in this study originated from the two near-by cenotes, Gran Cenote and Kawash (Moravec et al. 1995a). Both cenotes are populated only by two native species of fish, namely *Astyanax fasciatus* and the pimelodid catfish *Rhamdia guatemalensis* (Günther). *Procamallanus* (*S.*) *neocaballeroi* was found only in the first species in which it occurred rather frequently. Of 98 *A. fasciatus* examined from Gran Cenote, 47 (48 %) harboured this parasite, with the intensity of 1–8 (mean 2) nematodes per fish. Of 56 *A. fasciatus* from Kawash Cenote, 36 (64 %) were infected, with the intensity 1–3 (mean 2). A similar rate of *P.* (*S.*) *neocaballeroi* infection was found in 19 *A. fasciatus* from Box-Toro Cenote, where prevalence was 37 % and intensity 1–3 (mean 2) nematodes per fish (Moravec et al. 1995a); two *Cichlasoma* sp. specimens examined from the same locality were not infected by this nematode. Gravid females with larvae in the uterus represented only about 5 % of the nematodes recovered.

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

	First-stage larvae			
				First moult
	Free larvae	1 day p.i.	3 days p.i.	3 days p.i.
Length of body	0.593–0.613	0.443–0.630	0.570–0.588	0.618/0.660
Width of body	0.033–0.035	0.033–0.035	0.035	0.040
Length of oesophagus	0.133–0.135	0.130–0.140	0.120	0.175
Distance of nerve ring	0.075–0.083	0.090–0.093	0.063–0.068	0.080
Distance of excretory pore	0.100–0.110	0.128–0.133	0.088–0.098	0.125
Genital primordium from anterior extremity	0.248–0.253	0.245–0.313	0.275–0.298	0.393
Length of tail: old/new	0.220–0.225	0.225–0.253	0.188–0.228	0.078/0.120
% of oesophagus from body length	22	22–29	20–21	28
% of tail from body length	37–38	36–57	40	13
	Second-stage larva		Third-stage larvae	
	Second moult			
	5 days p.i.		5 days p.i.	6 days p.i.
Length of body	0.728		0.645–0.680	0.685
Width of body	0.040		0.035–0.043	0.040
Length of oesophagus	0.130		0.250–0.253	0.283
Muscular oesophagus			0.150–0.153	0.170
Glandular oesophagus			0.100	0.113
Buccal capsule – length			0.025–0.028	0.025
– width			0.020	0.020
– thickness			0.003	0.003
Distance of nerve ring	0.080		0.063–0.073	0.093
Distance of excretory pore	0.120		0.103–0.118	0.115
Genital primordium from anterior extremity	0.305		0.438–0.463	0.463
Length of tail: old/new	0.260/0.133		0.073–0.083	0.068
% of oesophagus from body length	18		41	45
% of tail from body length	36		11–12	10

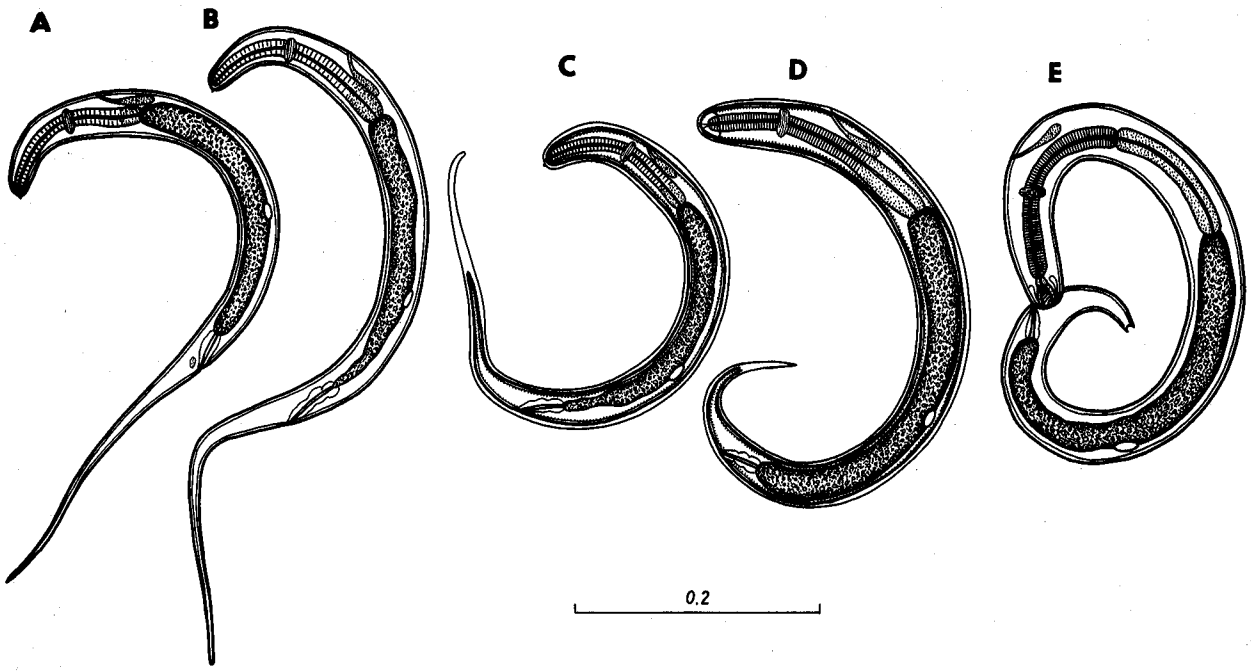

Fig. 1. *Procamallanus* (*S.*) *neocaballeroi*, development of larvae from the first to the third stage. **A** – first-stage larva from female's uterus; **B** – first-stage larva from copepods 1 day p.i.; **C** – larva undergoing first moult in copepods 3 days p.i; **D** – larva starting second moult in copepods 3 days p.i.; **E** – third-stage larva from copepods 5 days p.i.

Experimental infection of copepod intermediate hosts

Gravid females of *P.* (*S.*) *neocaballeroi*, like other representatives of the family Camallanidae, are ovoviviparous which means that their uteri already contain hatched first-stage larvae. The uteri of females used in experiments 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 motionless on the bottom of the vessel. Other larvae actively moved in the water, being usually attached by the tail to the bottom and waving with their bodies from one side to the other or they alternately rolled up and unrolled. The copepods feed on them actively. Within a few hours after ingestion, the larvae bore through the wall of the copepod's digestive tract with the help of their dorsal tooth into the haemocoel, where further development takes place. During the first phases of development in the body of copepods the larvae are still very active, but as they grow they become less motile. The third-stage larvae tend to rest in a coiled position. At the temperature of 21–22°C, the first larval moult occurs 3 days p.i.

The second moult after which the larvae attain the third, infective stage, occurs 4–5 days p.i. The fully formed third-stage larvae, already liberated from the cuticular sheath of the second moult, were found in copepods 5 and 6 days p.i. During the development in the copepod, the larvae undergo substantial morphological changes but their size remains much the same, the third-stage larvae being only slightly larger than the first-stage larvae (Table 1). The tail becomes relatively shorter (forming 10–12 % of the body length in third-stage larvae vs. 37–38 % in free first-stage larvae) and the infective third-stage larvae already possess big sclerotized buccal capsules supported by spiral thickenings. The oesophagus is distinctly divided into muscular and glandular portions, and the tail tip bears three conical processes. The infective larvae are located mostly in the cephalothorax of the intermediate host, less often in the abdomen or other parts of the body.

Only a *Mesocyclops* sp. was used in these experiments. During the first 1–2 days, about 50 % of the copepods became infected, but because of frequent deaths, only 20 % remained alive by the time infective larvae were present. The intensity was low, usually one, rarely 2, larvae being present in a copepod. All copepods remaining by 6 days p.i. were used to infect the fish.

Experimental infection of fish definitive hosts

In this experiment, 10 *A. fasciatus* were allowed to ingest spontaneously about 30 live copepods harbouring infective third-stage larvae (6 days p.i.).

Of the 10 fishes, only 5, examined on days 10, 12, 18 and 31 p.i., harboured larvae or adults of *P.* (*S.*)

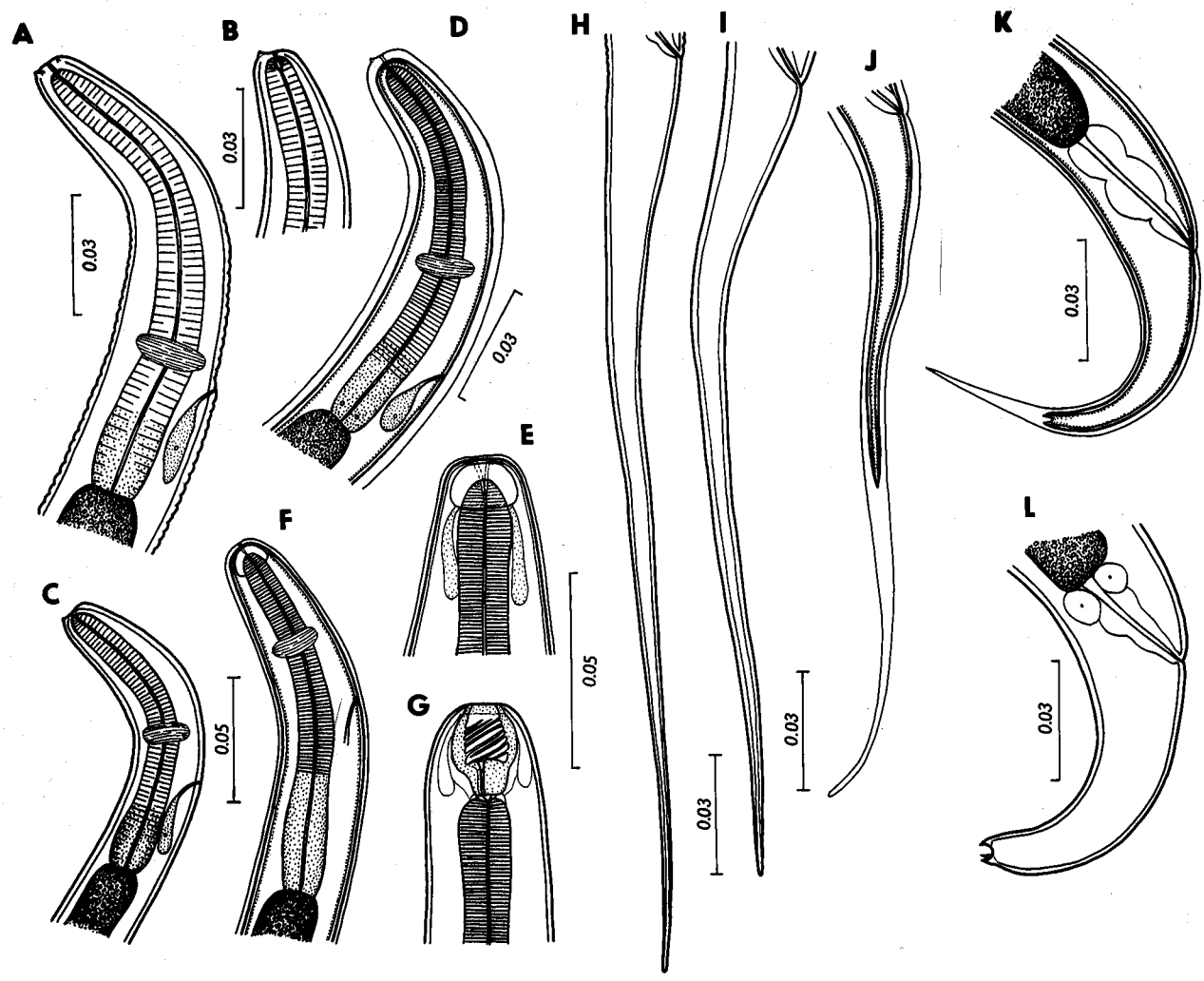

Fig. 2. *Procamallanus* (*S.*) *neocaballeroi*, cephalic and caudal ends of first-, second- and third-stage larvae. **A, H** – first-stage larva from female's uterus; **B, C, I** – first-stage larva from copepods 1 day p.i.; **D, J** – larva undergoing first moult in copepods 3 days p.i.; **E, F, K** – late second-stage larva starting second moult in copepods 3 days p.i.; **G, L** – third-stage larva from copepods 6 days p.i.

neocaballeroi. The remaining 5 fishes examined on days 15, 18, 31 and 47 p.i. were uninfected, as were all the control fishes. The nematodes were located in the middle and posterior parts of the host's intestine and in the pyloric caeca.

Morphometrical data of the nematodes shows that the third moult (first in the definitive host) occurs 10 days p.i., when the larva is approximately 1.4 mm long. The fourth-stage larvae obtained two days later (12 days p.i.) were already 1.6–2.0 mm long and the first signs of the next (fourth) moult (e.g. the presence of developing caudal papillae and spicules) were observed in the male larva; it can be estimated that this moult occurs 2–3 days later (14–15 days p.i.), i. e. after approximately 2 weeks after the infection. The finding of one female with eggs in uterus 31 days p.i. indicates that the prepatent period of this nematode may be about two months.

During the development of *P.* (*S.*) *neocaballeroi* in the definitive host, the following morphological changes were observed: shortening of the posterior portion of the buccal capsule and an increase in the number of spiral thickenings, the glandular portion of the oesophagus gradually becoming much longer than the muscular portion, and the disappearance of the conical processes on the tail tip of the larval nematodes.

Morphology and larval development

a) **First-stage larva** Figs. 1 A, B; 2 A–C, H, I; 3 A

The body of first-stage larvae from the uterus measures 0.593–0.613 in length and 0.033–0.035 in maximum width (see Table 1 for other measurements). The body is transparent, slender, with slightly transversely striated cuticle. The head end is rounded, with a small dorsal cuticular tooth. Oral papillae are hardly visible in lateral view. The mouth is formed by a short, thin, feebly sclerotized tube. The oesophagus is

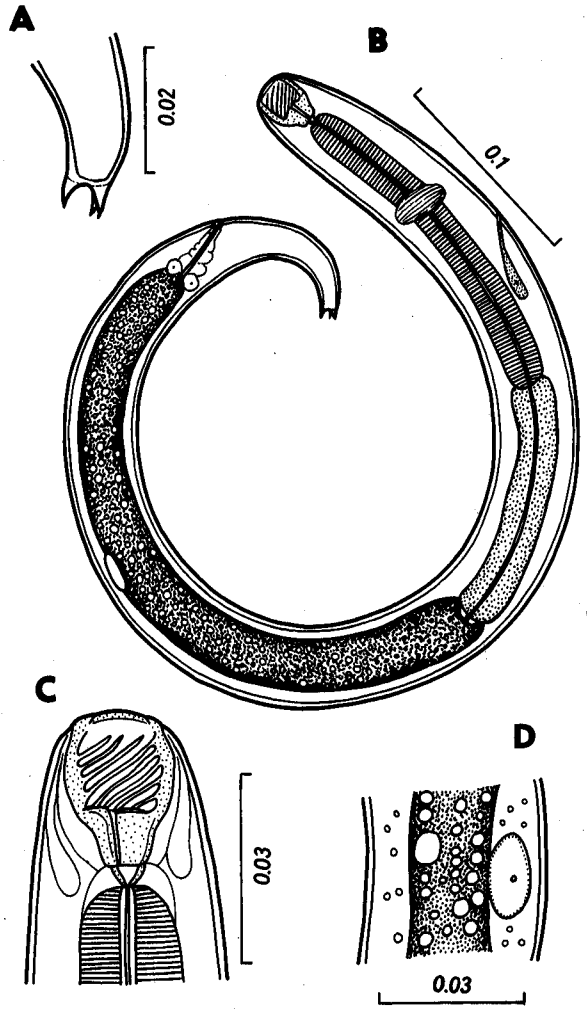

Fig. 3. *Procamallanus (S.) neocaballeroi*, third-stage larva from copepods. **A** – tip of tail; **B** – general view; **C** – buccal capsule in lateral view; **D** – region of genital primordium.

undivided, without a distinct lumen, representing 40–45 % of the whole body length. The posterior end of the oesophagus contains glandular tissue. The nerve ring encircles the oesophagus approximately at its middle. The excretory pore is located somewhat posterior to the nerve ring. The intestine is wide, light-coloured and finely granular. The rectum is a colourless tube; rectal glands are present, but not well visible. The small oval genital primordium is situated ventrally, slightly posterior to the middle of the intestine. The tail is conical, slender, with a sharply pointed tip; its length is 37–38 % of the total length of the body.

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

b) **Second-stage larva** Figs. 1 C, D; 2 D–F, J, K

The second-stage larvae undergoing the first larval moult were observed in the haemocoel of copepods 3 days p.i. (Figs. 1 C; 2 D, J). Their body is light-coloured, approximately of the same size as that of the first-stage larva (Table 1). The old cuticle is smooth and loosened along the whole length of body, and is best visible on both ends of the body, particularly on the tail. The newly formed tail resembles that of the first-stage larva in that it is rather long and sharply pointed. However, it is relatively shorter, representing 20–21 % of the body length. The inner organization of the body is almost the same as in the first-stage larvae, but the dorsal cuticular tooth is absent. The intestine is straight, wide, containing numerous fine granules. The rectum is colourless. The small oval genital primordium is shifted more posteriorly as compared with larvae of the first stage.

The late second-stage larvae (Figs. 1 D; 2 E, F, K) recovered 3 days p.i. were slightly larger (Table 1). Their body is still light-coloured and their cuticle smooth. The cephalic end is rounded, the mouth is formed by a short fine tube opening into the oesophagus. The anterior end of the oesophagus is covered with a thick, hyaline bell-shaped structure, surrounded by several drop-like glandular formations. This is apparently the anlage of the future buccal capsule. The posterior glandular portion of the oesophagus becomes longer, but a distinct division between the muscular and glandular parts of the oesophagus is not yet apparent. The intestine is wide and straight. Rectal glands become visible. The larva is preparing for its second moult which is best visible on the tail. Inside the old sheath of the caudal cuticle of the second-stage larva, a new tail typical of the third-stage larva is being already formed. The second moult of the larvae is completed day 5 p.i.

c) **Third-stage (infective) larva from copepods** Figs. 1 E; 2 G, L; 3; 5 A

The third-stage larvae obtained on days 5 and 6 p.i. are 0.645–0.685 in length and 0.035–0.043 in maximum width (Table 1), with a smooth cuticle. The mouth opening is circular. The buccal capsule is almost colourless or slightly golden, elongate, thick-walled and consists of the anterior wide portion supported by 8 spiral thickenings and the posterior narrow portion. The entire buccal capsule is 0.025–0.028 long and 0.020 wide; its anterior and posterior portions are 0.015–0.018 and 0.010 long, respectively. The oesophagus is distinctly divided into an anterior, almost cylindrical muscular portion with a strong cuticular lining and a somewhat shorter posterior glandular portion. The buccal capsule opens into the oesophagus through a distinct

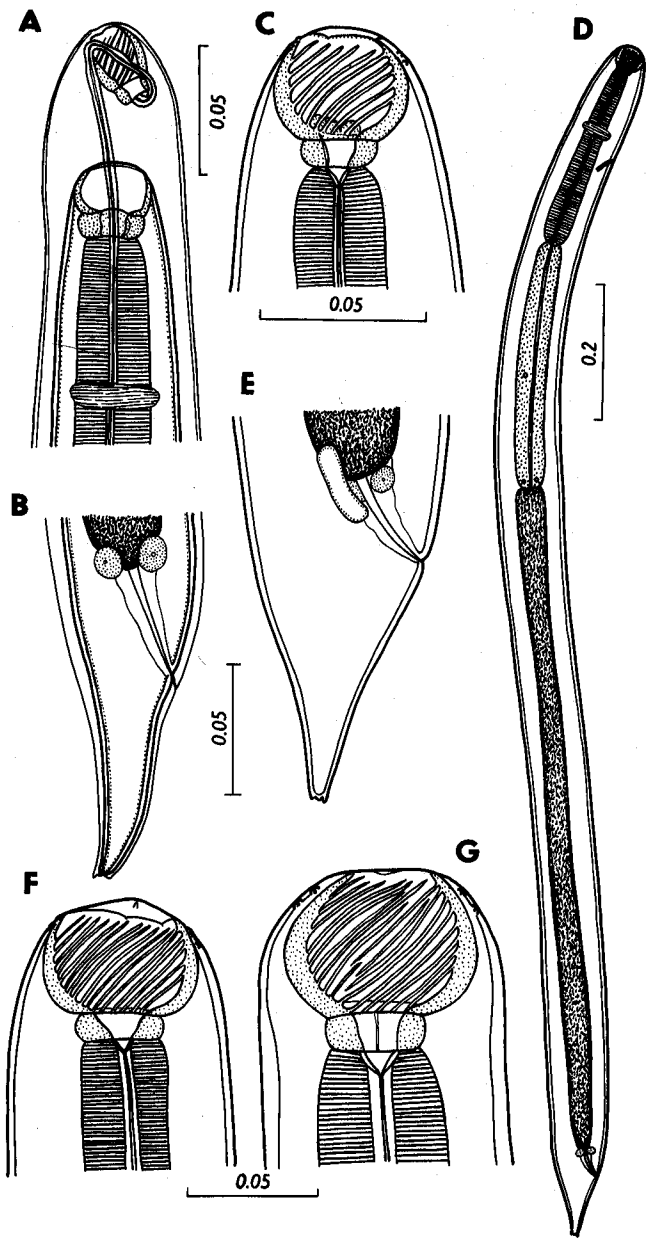

Fig. 4. *Procamallanus* (*S.*) *neocaballeroi*, development of nematodes from third-stage larvae to adults in the definitive host. **A, B** – cephalic and caudal ends of larva undergoing its third moult 10 days p.i.; **C–E** – fourth-stage larva 12 days p.i. (C – buccal capsule, D – total view, E – tail of male larva with developing spicules); **F** – buccal capsule of male 18 days p.i.; **G** – buccal capsule of female 31 days p.i.

oesophageal cup. The nerve ring encircles the muscular oesophagus approximately at its middle and the excretory pore is slightly posterior to the nerve ring. The oesophagus opens into the intestine through a small valve. The intestine is wide, orange–brown in colour, containing numerous granules and three large, uncoloured rectal glands surround the anterior end of the colourless rectum. The tail is conical, its tip bears one dorsal and two ventrolateral conical, sharply pointed caudal processes. The tail forms 10–12 % of the body length. The small oval genital primordium is located at the posterior half of the body.

d) **Fourth-stage larva from the definitive host**

Figs. 4 A–E; 5 B, D

One larva undergoing the third moult (first in the definitive host) was recovered from experimental fish on day 10 p.i. (Fig. 4 A, B). This was longer than third-stage larvae from copepods (Table 2) but its general morphology was rather similar. The main differences were the very wide, weakly sclerotized buccal capsule and smaller caudal processes. The larva was still inside the cuticle of the previous stage.

The fourth-stage larvae obtained two days later (12 days p.i.) are 1.618–2.054 long and 0.082–0.105 wide (Table 2), with a smooth cuticle. Their morphology is very similar to that of the third-stage larvae, the main difference being the markedly wide buccal capsule with the posterior portion being considerably reduced to form a typical basal ring. The anterior portion of the capsule is 0.036–0.039 long and 0.045 wide and its inner surface is supported by 11 spiral thickenings (in lateral view). The bottom of the capsule contains three distinct, anteriorly protruding sclerotized protuberances. The basal ring is thick-walled, 0.009 long and 0.024 wide. In contrast to the third-stage larvae from copepods, the glandular oesophagus of fourth-stage larvae is distinctly longer than the muscular one. The intestine is straight and brown-coloured. The tail is short and conical, with rudimentary caudal mucrones. The genital primordia in both the male and female larvae are tubular. In one male larva, it was already possible to observe small developing spicules and genital papillae still covered by cuticle.

e) **Adults** Figs. 4 F, G; 5 E, F

One fully mature male, 3.400 long and 0.136 wide, was recovered from experimental fish on day 18 p.i., whereas one female, 8.323 long and 0.177 wide, containing many eggs in the uterus was found on day 31 p.i. (Table 2). Their morphology fully corresponds to the redescription of this species provided by Moravec et al. (1995a). According to the state of development of the female it can be estimated that the prepatent period of this species is not less than two months.

DISCUSSION

The present data show that, in general, the development of *Procamallanus* (*S.*) *neocaballeroi* is similar to that in other members of the family Camallanidae.

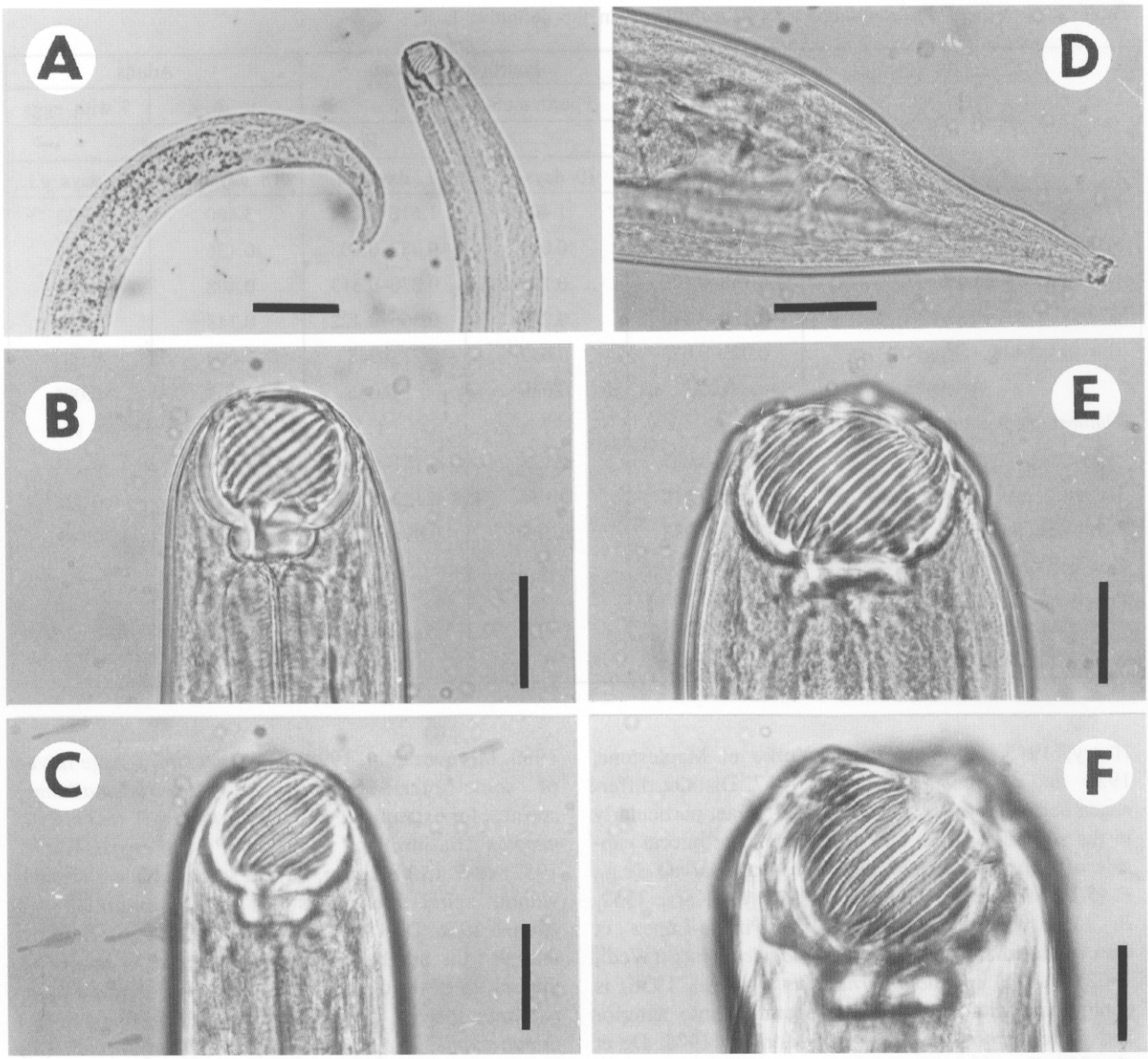

Fig. 5. Larvae and adults of *Procamallanus* (*S.*) *neocaballeroi* from experimental infections. **A** – cephalic and caudal ends of third-stage larva from *Mesocyclops* sp. 5 days p.i.; **B** – buccal capsule of male fourth-stage larva from *Astyanax fasciatus* 12 days p.i.; **C** – same, focused on spiral thickenings; **D** – tail of same larva; **E** – buccal capsule of male from *A. fasciatus* 18 days p.i.; **F** – buccal capsule of female from *A. fasciatus* 31 days p.i. Scale bars: A = 0.05 mm, B–E = 0.03 mm.

Although in our experiments we infected only one intermediate host species, a variety of copepods can probably be infected. Moravec (1994) stated 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. Pereira et al. (1936) reported *Diaptomus caerensis* and *D. azevedoi* as the intermediate hosts of the closely related species *P.* (*S.*) *caerensis* (Pereira, Vianna Dias et Azevedo, 1936) (= *P.* (*S.*) *hilarii* Vaz et Pereira, 1934), a parasite of *Astyanax bimaculatus vittatus* (Castelnau) in Brazil, but the rate of both natural and experimental infection was markedly low (0.2 % and 4 %, respectively). It is highly probable that also in this species the principal intermediate hosts are cyclopid copepods, like in other camallanids, whereas diaptomids become infected much less frequently. First-stage larvae of all camallanids keep to the bottom and, hence, are not readily available to floating copepods as diaptomids.

Regarding the morphology of individual larval stages of *P.* (*S.*) *neocaballeroi*, first- and second-stage larvae are similar to those described in other *Procamallanus* species as well as in the genera *Camallanus* Railliet et

Table 2. Growth of *Procamallanus (S.) neocaballeroi* in the definitive host.

	Third-stage larvae	Fourth-stage larvae		Adults	
		Fourth moult		♂	♀ with eggs
	From copepods	From *Astyanax fasciatus*			
	5–6 days p.i.	10 days p.i.	12 days p.i.	18 days p.i.	31 days p.i.
Length of body	0.645–0.685	1.401	1.618–2.054	3.400	8.323
Width of body	0.035–0.043	0.095	0.082–0.105	0.136	0.177
Muscular oesophagus	0.150–0.170	0.195	0.270–0.313	0.408	0.476
Glandular oesophagus	0.100–0.113	0.258	0.366–0.422	0.748	1.319
Buccal capsule – length	0.025–0.028	0.033	0.048	0.060	0.087
– width	0.020	0.036	0.045	0.063	0.081
Number of spiral thickenings	8	?	11	15	17
Nerve ring	0.063–0.093	0.123	0.141–0.147	0.195	0.231
Excretory pore	0.103–0.118	0.174	0.150–0.192	0.245	0.326
Lenth of tail	0.068–0.083	0.105	0.096–0.108	0.122	0.082
% of oesophagus from body length	41–45	34	36–39	34	22
Length ratio of muscular and glandular oesophagus	1 : 0.7	1 : 1.3	1 : 1.3–1.4	1 : 1.8	1 : 2.8
% of tail length	10–12	7	5–7	4	1

Henry, 1915, *Paracamallanus* Yorke et Maplestone, 1926 and *Neocamallanus* Ali, 1957. Distinct differences occur, however, in third-stage larvae, particularly in the structure of the buccal capsule. The buccal capsule of some *Procamallanus* third-stage larvae (e.g., *P. (S.) fulvidraconis* Li, 1935, *P. (S.) mysti* Karve 1952, *P. (S.) rebecae* (Andrade-Salas, Pineda-López et García-Magaña, 1994), *P. (P.) laeviconchus* (Wedl, 1862), *P. (P.) spiculogubernaculus* Agarwal, 1958) is continuous, without a distinct separation into anterior and posterior portions (Li 1935, Moravec 1975, De et al. 1986, De 1995, Moravec et al. 1995b), while those of *P. (S.) neocaballeroi*, *P. (S.) hilarii*, *P. (S.) pimelodus* Pinto, Fábio, Noronha et Rolas, 1974 and *P. (S.) cricotus* (Fusco et Overstreet, 1978) possess a capsule with a well separated, spacious posterior portion (Pereira et al. 1936, Fusco 1980, Moravec et al. 1993). A similar type of buccal capsule provided with a spacious posterior portion, usually separated from the anterior portion by several teeth, is also typical of the third-stage larvae of the camallanid genera *Camallanus*, *Neocamallanus*, *Serpinema* Yeh, 1960 and *Paracamallanus* (Campana-Rouget 1961, Moravec 1969, 1974, De et al. 1984, Bartlett and Anderson 1985). An interesting feature of the third-stage larva of *P. (S.) neocaballeroi*, *P. (S.) hilarii*, *P. (S.) pimelodus* and *P. (S.) cricotus* is that the anterior portion of the buccal capsule is provided with spiral thickenings, a feature typical of adults of the subgenus *Spirocamallanus* (Pereira et al. 1936, Fusco 1980, Moravec et al. 1993). However, third-stage larvae of some other *Procamallanus* (*Spirocamallanus*) species, for example *P. (S.) fulvidraconis*, *P. (S.) intestinecolas* (Bashirullah, 1973) (= *P. (S.) mysti* Karve, 1952) or *P. (S.) rebecae*, have a smooth buccal capsule without spiral thickenings (Li 1935, Bashirullah and Ahmed 1976, De 1995, Moravec et al. 1995b). It seems that also the number of conical processes or spines on the tail tip of third-stage larvae is typical of the *Procamallanus* species. Larvae of some species (e.g., *P. (S.) fulvidraconis*, *P. (S.) cricotus*, *P. (S.) mysti*, *P. (S.) rebecae*, *P. (P.) spiculogubernaculus*), including *P. (S.) neocaballeroi*, have three caudal processes, but there are four processes in the larvae of *P. (S.) hilarii* and *P. (P.) laeviconchus*.

It is also interesting that the larvae of *P. (S.) neocaballeroi* practically do not increase in size during their development from the first to the third stage in the copepod intermediate host. The third-stage larvae of other *Procamallanus* species from copepods are distinctly larger than conspecific first-stage larvae and, for example, the third-stage larvae of *P. (S.) rebecae* are even twice as long as first-stage larvae (Moravec et al. 1995b). According to Moravec (1978), a distinct increase in body sizes of larvae during their development in the copepod intermediate host can be regarded as a primitive character among dracunculoid nematodes, but it is not yet clear whether this is also valid for camallanids.

The fourth-stage larvae of *P. (S.) neocaballeroi* from the definitive host differ from conspecific third-stage larvae principally in the structure of the buccal capsule, presence of tubular genital primordia, reduced caudal processes and in the larger body (Table 2). In contrast to third-stage larvae, the buccal capsule of fourth-stage larvae is more similar to adult nematodes, i.e. its posterior portion is reduced to form the basal ring and spiral thickenings of the capsule are more numerous (11 in number). This structure of the buccal capsule is rather similar to that of the fourth-stage larvae of *P. (S.) pimelodus*, but differs markedly from that of the fourth-stage larvae of *P. (S.) inopinatus*, the latter having an unusually thick-walled buccal capsule (Petter and Thatcher 1988, Moravec et al. 1993). The third-stage larva of *P. (S.) hilarii* described from fish by Pereira et al. (1936) was in the fact the fourth-stage larva as can be seen from the illustration of its buccal capsule whose structure is almost identical with that of larvae of this stage in *P. (S.) neocaballeroi*. In addition, the tail tip of this larva was without any caudal processes, it possessed a tubular genital primordium and its body length was 16.2 mm.

The caudal processes of *P. (S.) neocaballeroi* are rudimentary in fourth-stage larvae. In contrast, the same larval stage of *P. (S.) pimelodus* has no caudal processes, although these are present in conspecific third-stage larvae (Moravec et al. 1993).

The rate of the larval development of fish nematodes in their invertebrate intermediate hosts is considerably affected by the water temperature (Moravec 1994). In our experiments with *P. (S.) neocaballeroi*, infective third-stage larvae developed in copepods in 5–6 days at 21–22°C. This is comparable with the same development of *P. (P.) laeviconchus* (6 days at 23–24°C), *P. (P.) spiculogubernacus* (4 and 6 days at 26°C and 38°C, respectively) and *P. (S.) rebecae* (6 days at 21–22°C) (Moravec 1975, Sinha 1988, Moravec et al. 1995b). 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).

Of all *Procamallanus* species, the rate of the development in the definitive host has so far been studied experimentally only in *P. (S.) mysti* (De 1995), where the last (fourth) moult in the definitive host was observed to occur in "male" and "female" larvae on days 37 and 67 p.i., respectively (water temperature not mentioned), and in *P. (S.) rebecae* (Moravec et al. 1995b), where adult nematodes developed in 42 days at 25–32°C. The development of *P. (S.) neocaballeroi* in *A. fasciatus* seems to be faster. At the same temperature (25–32°C), adults had already developed in about 16 days and the prepatent period can be estimated to be about two months. Moravec et al. (1995b) have estimated the prepatent period of *P. (S.) rebecae* to be about 2–3 months and Li (1935) has presupposed the prepatent period of *P. (S.) fulvidraconis* to be about 4 months.

Pereira et al. (1936) have considered copepods to be the first intermediate host of *P. (S.) hilarii* and small fishes (*Curimatus elegans* Steindachner) the second intermediate hosts. Now it is clear, however, that there is only one intermediate host (copepod) in all camallanids and that small fishes may act as paratenic hosts only and are not necessary for completing the nematode's development (Moravec 1994, Moravec et al. 1995b). Our experiments with *P. (S.) neocaballeroi* have confirmed that the fish definitive host may acquire infection directly by feeding on the copepods harbouring the parasite's third-stage larvae. However, considering data on the closely related South American species *P. (S.) hilarii* from the congeneric fish host (Pereira et al. 1936), it is highly probable that paratenic hosts (e.g. fish fry) may be another source of *P. (S.) neocaballeroi* infection of the definitive host.

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 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 illustrations. This study was supported by the grant no. PO99 from the Comisión Nacional para el Conocimiento y Uso de la Biodiversidad (CONABIO), Mexico.

REFERENCES

ANDERSON R. C. 1992: Nematode Parasites of Vertebrates. Their Development and Transmission. C.A.B. International, Wallingford, Oxon, 578 pp.

BARTLETT C. M., ANDERSON R. C. 1985: Larval nematodes (Ascaridida and Spirurida) in the aquatic snail, *Lymnaea stagnalis*. J. Invertebr. Pathol. 46: 153–159.

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

CABALLERO-DELOYA J. 1977: Estudio helmintológico de los animales silvestres de la estación de biología tropical "Los Tuxtlas", Veracruz. Nematoda II. Descripción de *Spirocamallanus neocaballeroi* sp. nov. (Nematoda: Camallanidae), del intestino de *Astyanax fasciatus* (Cuvier). Excerta Parasitológica en memoria del doctor Eduardo Caballero y Caballero, Instituto de Biología, Publicaciones Especiales 4, pp. 409–414.

CAMPANA-ROUGET Y. 1961: Remarques sur le cycle évolutif de *Camallanus lacustris* (Zoega, 1776) et la

logenie des Camallanidae. Ann. Parasitol. Hum. Comp. 36: 425–434.

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

DE N. C., SAMANTA P., MAJUMDAR G. 1984: Aspects of the developmental cycle of *Neocamallanus singhi* Ali, 1957 (Nematoda: Camallanidae). Folia Parasitol. 31: 303–310.

DE N. C., SINHA R. K., MAJUMDAR G. 1986: 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.

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

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

MORAVEC F. 1974: The development of *Paracamallanus cyathopharynx* (Baylis, 1923) (Nematoda: Camallanidae). Folia Parasitol. 21: 333–343.

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

MORAVEC F. 1978: The development of the nematode *Philometra obturans* (Prenant, 1886) in the intermediate host. Folia Parasitol. 25: 303–315.

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

MORAVEC F., KOHN A., FERNANDES B. M. M. 1993: Nematode parasites of fishes of the Paraná River, Brazil. Part 3. Camallanoidea and Dracunculoidea. Folia Parasitol. 40: 211–229.

MORAVEC F., MENDOZA-FRANCO E., VARGAS-VÁZQUEZ J., VIVAS-RODRÍGUEZ C. 1995b: Studies on the development of *Procamallanus* (*Spirocamallanus*) *rebecae* (Nematoda: Camallanidae), a parasite of cichlid fishes in Mexico. Folia Parasitol. 42: 281–292.

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

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.

PETTER A. J., THATCHER E. V. 1988: Observations sur le structure de la capsule buccale de *Spirocamallanus inopinatus* (Nematoda), parasite de poissons brésiliens. Bull. Mus. Natn. Hist. Nat., Paris, 4e sér., 10, sect. A, no. 4: 685–692.

SINHA A. K. 1988: On the life cycle of *Procamallanus spiculogubernaculus* (Camallanidae) (Agrawal, 1958) – a nematode parasite of fishes. Riv. Parassitol. 5 (49): 111–116.

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 1 August 1995

Accepted 20 December 1995