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The morphology of free-living stages and immature parasites of *Rhabdias paraensis* (Nematoda: Rhabdiasidae), a parasite of *Rhinella marina* (Anura: Bufonidae) in Brazil

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Abstract

Rhabdias paraensis Santos, Melo, Nascimento, Nascimento, Giese et Furtado, 2011 was described based on fully gravid worms. Further investigations on the free-living stages, immature worms and young individuals were facilitated by cultivation in the laboratory, which allowed us to add new information about the morphology and development of the species. Observations on the free-living development of *R. paraensis* showed that the life cycle is typical of *Rhabdias*, with alternation of gonochoristic and hermaphroditic generations and without homogony. Males of the free-living generation were different from those in several species of the genus studied previously. In the original description, the excretory glands and duct were absent in gravid specimens of *R. paraensis*, while in this study, distinct excretory glands and a duct were observed in immature and young individuals. Additionally, we recognised the separation of the buccal capsule walls into anterior and posterior portions and described the specific shapes of these portions in lateral and apical view. Studies on the morphology and development of free-living stages of *Rhabdias* spp. from Neotropical regions may provide additional information for species determination.

Keywords

Amazon, *Rhinella marina*, Rhabdiasidae, free-living generation, immature hermaphrodites

Introduction

Nematodes of the genus *Rhabdias* Stiles et Hassall, 1905 are lung-dwelling parasites of amphibians and some reptiles. The alternation of hermaphroditic and gonochoristic generations is a characteristic feature of their biology (Anderson 2000). Information on their free-living development and/or the morphology of free-living stages is available for less than a third of more than 70 species. The free-living gonochoristic generation was studied and described in 3 of the approximately 13 species of *Rhabdias* occurring in Neotropical amphibians (Kloss 1971, 1974). According to Kuzmin (2013), the free-living stages of these species are rather similar; however, differences in the number of hermaphroditic-generation larvae developing in the free-living females of Brazilian *Rhabdias* spp. were used as additional characteristics for species differentiation (Kloss 1971, 1974). Similarly, fine details of the morphology of infective larvae of some *Rhabdias* spp. also added information for species determination (Lhermitte-Vallarino *et al.* 2009, 2010a; Junker *et al.* 2010; Kuzmin *et al*. 2014).

The immature (subgravid) parasitic stage of *Rhabdias* spp. draws attention due to its morphology (Baker 1979; Ballantyne 1991; Kuzmin 2013). In contrast to the parasitic larval stages, the immature stage may be prolonged due to the host immune response to high infection intensity or because of environmental conditions (Kuzmin 2013). Soon after the fourth moult, the individuals from the host body cavity or lungs develop specific morphological characteristics and usually differ from the gravid worms in features other than body size or underdeveloped genital systems. The study of such specimens may add to our knowledge of the morphology of various *Rhabdias* species and complete the formal description of the species.

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Rhabdias paraensis Santos, Melo, Nascimento, Nascimento, Giese et Furtado, 2011 was described based on morphological studies of fully gravid worms from the lungs of the cane toad, *Rhinella marina* (L.) in Belém (Pará, Brazil). In further investigations, we obtained and examined its free-living stages (early-stage larvae, males and females of the gonochoristic generation, infective larvae) through cultivation in the laboratory, along with immature and young individuals from experimentally infected toads. The results of these studies are presented here.

Materials and Methods

Cultivation of free-living stages

One *Rhi. marina* from the area around the Universidade Federal do Pará (Belém, Brazil) was euthanised by injection of lidocaine hydrochloride 2% and was examined for parasites on February 4, 2014. Approximately 100 gravid hermaphrodite specimens of *R. paraensis* were found in its lungs. Two cultures were established using the method of Langford and Janovy (2009). Faeces of the toad containing numerous eggs of *R. paraensis* were used as a culture medium, and 10 gravid parasitic specimens of *R. paraensis* from the host's lungs were placed in each culture. Samples were collected from the cultures 18, 24, 40, 48 and 114 h (5 days) after the beginning of cultivation.

Another toad from the same population was examined on February 17, 2014. Six gravid hermaphrodite specimens of *R. paraensis* were found in the lungs. Cultivation of free-living stages was performed using the same method, and three parasitic specimens of *R. paraensis* were placed into the culture. Samples were collected from the culture 30, 52, 72, 80 and 96 h after the beginning of cultivation. As the culture appeared to contain free-living stages of *Strongyloides* sp., the nematodes collected were not used for morphological studies, except for the free-living females with developing larvae inside. The latter were identified as belonging to Rhabdiasidae based on the morphology of the infective larvae.

All cultures were kept at room temperature, 21–26°C.

Experimental infection

Since *Rhabdias* spp. infect their hosts percutaneously (Anderson 2000), an adult *Rhi. marina* (snout-vent length 13 cm) collected from the same locality was placed in a box with a surface area of 92 cm^2 (bottom size 11.5 × 8 cm) on a suspension of *ca*. 1,000 infective larvae for 1.5 h. The infection procedure was repeated four days after, with *ca*. 700 infective larvae and the same exposure time. The toad was examined seven days after the first infection. One immature *R. paraensis* was found in the body cavity of the toad, and 18 immature and young parasite specimens were collected from the lungs and used for further morphological studies.

Collection and examination of the nematodes

All nematodes collected in the experimental studies and those found in naturally infected hosts were examined alive in 0.98% saline, washed in clean saline and fixed with hot 70% alcohol. For morphological studies, nematodes were cleared in lactophenol and examined on temporary slides. Apical sections were made manually. Morphological examination and drawings were made using an Olympus BX41 microscope equipped with a drawing tube. Measurements were made from the drawings.

All measurements presented in the text are given as mean values followed by ranges in parentheses.

Results

The rate of free-living development

During the first day of cultivation, development of male and female rhabditoid larvae was observed. By the end of the first day (24 h of cultivation), third- and fourth-stage larvae predominated in the cultures, and a small number of adult males were also found. Adult males and females were observed mainly 30–36 h from the beginning of cultivation. The male:female ratio calculated in samples collected at the beginning of day two of cultivation was 1:1.2. By the end of the second day, most individuals in the cultures were females with one to four (usually two or three) developing eggs in utero. Some males were still present 40–48 h after the beginning of cultivation. During the third day of cultivation, development and hatching of eggs in females was observed. By the end of day three, the first infective larvae were noticed inside females, and some were free in the culture medium. During the fourth day of cultivation, most larvae reached the third stage inside the females. The larvae (usually two, less frequently three) actively moved inside the female cuticle, and some managed to break the cuticle.

No homogonic stages of *Rhabdias* were observed in the three cultures examined.

The morphology of free-living stages

Rhabditiform larvae of the gonochoristic generation. The first-stage larvae (18 specimens measured) have spindle-shaped bodies 385 (365–405) µm long, with a maximum width of 21 (21–26) µm at mid-length, tapering towards the anterior and posterior extremities (Fig. 1A). The anterior end is rounded, and the posterior end is pointed. The stoma and oesophagus are typically rhabditoid, though the stomal parts are not distinct. The stoma is 5 (4–5) µm long, and the oesophagus is 101 (90–108) µm long, or 25.6 (23.8–29.0)% of the body length. The oesophageal bulb is 14 (13–16) µm wide, and a valve is present. The nerve ring surrounds the isthmus of the oesophagus 68 (58–75) µm from the anterior end. The

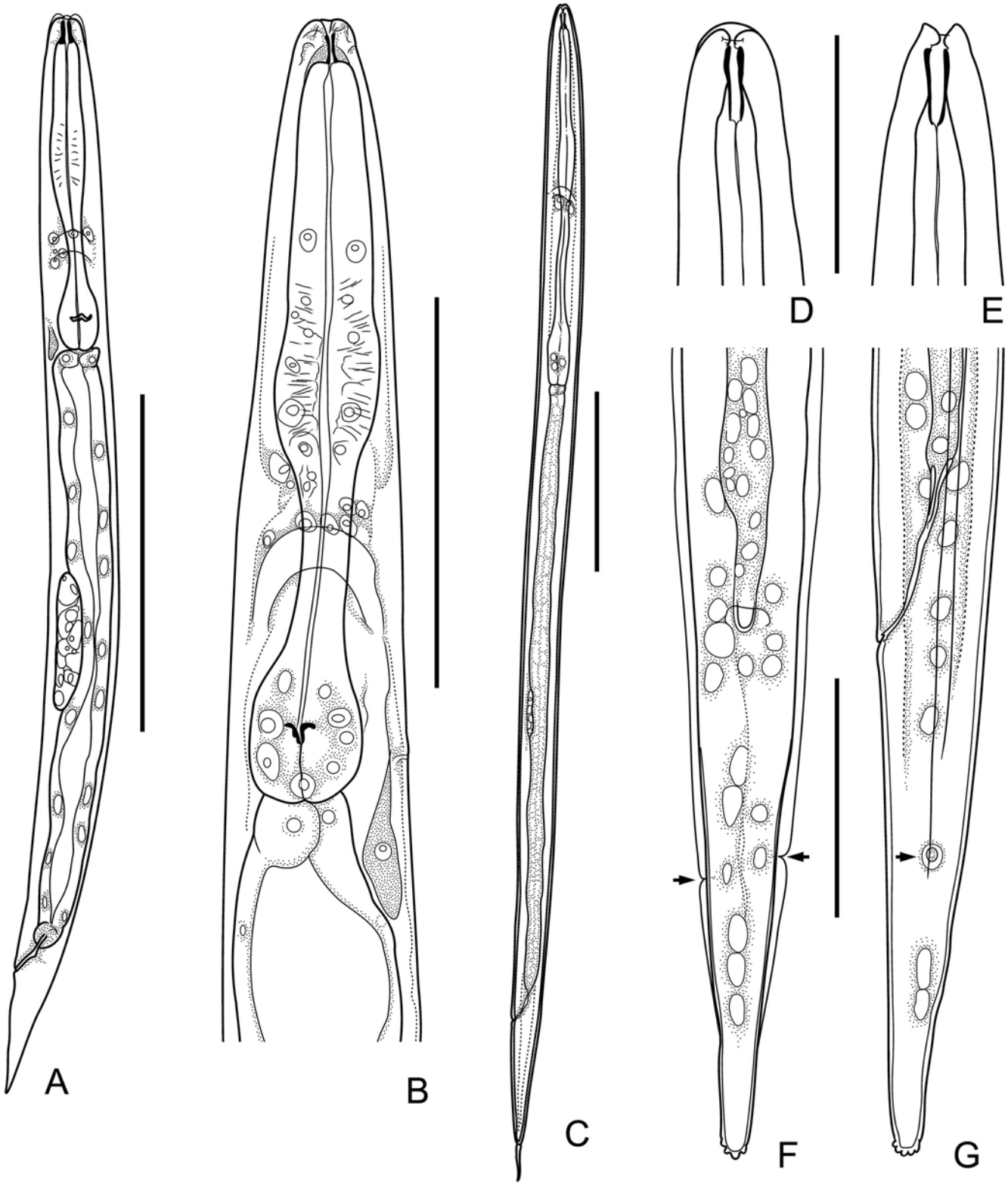

Fig. 1. Free-living larval stages of *Rhabdias paraensis*. A – Newly hatched L1, general view (lateral); B – L2, anterior portion of body, lateral view; C – Ensheathed infective larva, general view (lateral); D – Anterior end of infective larva, dorsoventral view; E – Anterior end of infective larva, lateral view; F – Posterior portion of infective larva, ventral view; G – Posterior portion of infective larva, left lateral view. Sheath was removed in D–G; arrowheads indicate phasmids. Scale bars: A, C – 100 µm; B – 50 µm; D–G – 30 µm

excretory cells are small and drop-like, with an excretory pore located on the ventral side, approximately at the middle of the oesophageal bulb. The intestine is thick-walled, with a distinct, wide lumen. Sixteen large nuclei were observed in the intestine walls in lateral view (Fig. 1A), excluding the smaller nuclei of the oesophageal-intestinal sphincter. The rectum is tubular and thin-walled. The conical tail is 43 (39–49) µm long, constituting 11.2 (10.5–13.2)% of the body length. The genital primordium is elongated, oval or lens-shaped in lateral view and 56 (43–82) µm long; it is wide, almost rectangular, with rounded corners in ventral view. The distance from the anterior end to the middle of the genital primordium is 197 (171–222) µm, or 51.7 (44.5–60.4)% of the body length.

In the later-stage larvae, the oesophagus is more distinctly rhabditoid, with well-recognised parts (Fig. 1B). The genital primordium grows mostly posteriorly in male larvae, reaching the level of the anus in late L3 (Fig. 2A); it grows in both anterior and posterior directions in female larvae. In female L3, the anterior and posterior portions of the primordium reflect upon themselves (Fig. 3A). Males and females may be distinguished based on the body shape by the third stage, because females retain the body shape of L1, whereas in males, the posterior half of the body is wider and the tail is shorter and tapers more abruptly on the dorsal side (Fig. 2A).

Free-living males (12 specimens). In adult males, the body is elongated: 508 (453–587) µm long, 21 (19–24) µm wide at the oesophageal-intestinal junction and 25 (23–28) µm wide at mid-length; the posterior portion is curved ventrally (Fig. 2B). The anterior part of the body (Fig. 2C) is morphologically similar to that of rhabditoid larvae. The stoma is 6

Fig. 2. Free-living males of *Rhabdias paraensis*. A – Moulting L3 (M3), lateral view; B – Adult male, lateral view; C – Anterior portion of body, ventro-lateral view; D – Posterior portion of body, lateral view; E – Posterior portion of body, ventral view. Scale bars: A, B – 200 µm; C-E – 50 µm

(6–8) µm long; the oesophagus is 104 (84–128) µm long, or 20.6 (18.0–24.4)% of the body length; the oesophageal bulb is 13 (12–14) µm wide. The nerve ring is situated 80 (61–107) µm from the anterior end. The intestine is wide and comparatively thin-walled.

The short anterior portion of the testis bends posteriorly 201 (159–253) µm from the anterior end. The length of the genital tube, except for its bent portion, is 262 (185–305) µm, or 52.8 (40.9–59.2)% of the body length. The testis is narrow in the anterior portion and widens posteriorly (Fig. 2B). The *vas deferens* is wide and thick-walled, narrowing posteriorly, with two lateral diverticuli directed anteriorly in the anterior portion (Fig. 2E). The ejaculatory duct is short and thin-walled. The spicules are short, equal, similar, joined in the posterior portion, slightly curved ventrally (Fig. 2 D, E), and 24 (22–26) µm long in lateral view. The gubernaculum is shield-shaped in ventral view and 11 (10–13) µm long in lateral view. There are seven pairs of ventrolateral genital papillae: three pairs are pre-cloacal, and four pairs are post-cloacal (Fig. 2 D, E). The posterior two (subterminal) pairs are situated close to the tail tip. Additionally, one unpaired ventral pre-cloacal papilla is situated close to the cloacal aperture, and a pair of lateral papillae is anterior to the subterminal pairs of ventro-lateral papillae (Fig. 2D). Thin sub-lateral caudal alae are present, beginning from the level of the second pair of ventrolateral papillae and running posteriorly to the end of the tail. All papillae are free from the alae, except for the last two ventro-lateral pairs (Fig. 2E). The tail is short and conical, with a short spike on the tip. The tail is 36 (29–45) µm long, or 7.3 (6.5–8.6)% of the body length.

Free-living females (26 specimens). The body is 733 (565–802) µm long, tapering gradually from mid-region towards the anterior and posterior extremities (Fig. 3B–D). The body is 33 (24–47) µm wide at the oesophageal-intestinal junction, 43 (29–56) µm at the level of the vulva. The stoma is 8 (6–8) µm long; the oesophagus is 125 (113–145) µm long, or 17.6 (15.1–20.5)% of the body length; the oesophageal bulb is 18 (14–20) µm wide. The nerve ring is situated 97 (89–108) µm from the anterior end. The intestine is comparatively thick-walled, with dilatation of the lumen in the anterior and posterior portions (Fig. 3B, C). The tail is 59 (48–71) µm long, or 8.0 (6.5–11.3)% of the body length, conical and elongated in shape. The genital system is amphidelphic, with similar and equal anterior and posterior limbs. The vulva is located 401 (288–465) µm from the anterior end, this distance corresponding to 55.2 (44.2–61.9)% of the body length. In gravid females, the vulva and vagina are reduced, and the vulvar aperture is indistinct. Larvated eggs in uteri are 56–78 µm long and 28–41 µm wide.

Usually, two to three eggs were observed in gravid females (Fig. 3B); four eggs were found in one female only. The eggs developed and hatched only inside females (Fig. 3C, D); i. e., no eggs were oviposited. After hatching, the larvae first fed on and destroyed the female's genital system (Fig. 3D) and then consumed all other inner organs (Fig. 3 E, F). The infective third-stage larvae disrupted the mother's cuticle and entered the outer medium (Fig. 3G).

Free-living larvae of the hermaphroditic generation

Rhabditiform larvae are present inside free-living females (Fig. 3E, F; 6 specimens measured). Their total length is 284 (278–290) µm, and their maximum width is 10 (9–10) µm. The oesophagus is rhabditoid and 59 (57–62) µm long, or 20.9 (19.9–21.8)% of the body length. The genital primordium is small and ovoid.

Infective third-stage larvae are found outside females (Fig. 1C–G; 20 specimens measured), enclosed in sheaths 619 (549–669) µm long. The sheath has a typical chequered appearance, with a combination of external longitudinal and internal transverse striations. After fixation, some larvae become shorter than their sheaths, measuring 594 (504–645) µm long and 22 (19–27) µm wide at mid-length. The dorsal and ventral pseudolabia are present beside the oral opening. The pseudolabia are broad and rounded in dorsoventral view and triangular in lateral view (Fig. 1D, E). The stoma is 8 (8–9) µm long, wider at the anterior portion and cylindrical in the posterior half. The posterior portion of the stoma is surrounded with oesophageal tissue (Fig. 1D, E). The oesophagus is 179 (160–197) µm long, occupying 30.2 (25.2–33.3)% of the body length and not strictly rhabditoid in shape. It is connected through dilation in the anterior portion (metacorpus) to the posterior bulb by a conspicuous and narrow isthmus (Fig. 1C). The oesophageal bulb is elongated and 10 (8–12) µm wide. The nerve ring surrounds the oesophagus at the anterior part of the isthmus, 98 (83–120) µm from the anterior end. The lumen of the intestine is inconspicuous. The intestinal cells and pseudocoelom are filled with small spherical inclusions. The genital primordium is small, oval-shaped, and 18 (16–19) µm long, situated 357 (323–413) µm from the anterior end of the larva [59.7 (54.5–69.2)% of the body length]. Two inconspicuous, low lateral alae ending in the posterior third of the tail are present on each side in the posterior portion of the larva (Fig. 1 F, G). The rectum is distinct, thin, and slightly curved ventrally in its posterior portion (Fig. 1G). The tail is elongated, 50 (43–53) µm long [8.5 (7.5–9.6)% of the body length], and gradually narrows posteriorly, with a rounded extremity (Fig. 1 F, G). Several (6–7) minute buds are present on the tail tip. Phasmids are situated near the middle of the tail (Fig. 1 F, G).

Morphological peculiarities of immature and young individuals of the hermaphroditic generation – an immature specimen from the body cavity of the host

This specimen is comparatively small, whitish in colour, and lacks the inflation of the cuticle characteristic of specimens from the host's lungs. The body is 1.274 mm long, 48 µm wide at the oesophageal-intestinal junction and 51 µm at mid-length. The anterior end is rounded (Fig. 4B, C). Lips are absent, but prominent circumoral papillae are visible in lateral view. The buccal capsule is clearly divided into two portions: the anterior portion, 3 µm deep, and the posterior portion, 4 µm deep. The posterior portion has optically denser walls. The

capsule is generally barrel-shaped, with a maximum outer diameter of 13 µm and a cylindrical lumen 9 µm wide. The oesophagus is club-shaped, 296 µm long (23.2% of body length) and 29 µm wide at the anterior end; the bulb is 32 µm wide. Prominent excretory glands stretch from the excretory duct posterior to the nerve ring up to the anterior portion of the intestine. Similar to immature specimens from the lungs, the genital system is poorly developed, with narrow syngonia, filled with large oogonia and oocytes arranged in a single line (Fig. 4 H, I) interrupted by the testis zone at approximately the middle of each syngonium (Fig. 4I). The uteri are still empty, tubular and narrow. The vulva is situated 726 µm from the anterior end (57% of body length). The tail is conical and 88 µm long (6.9% of body length).

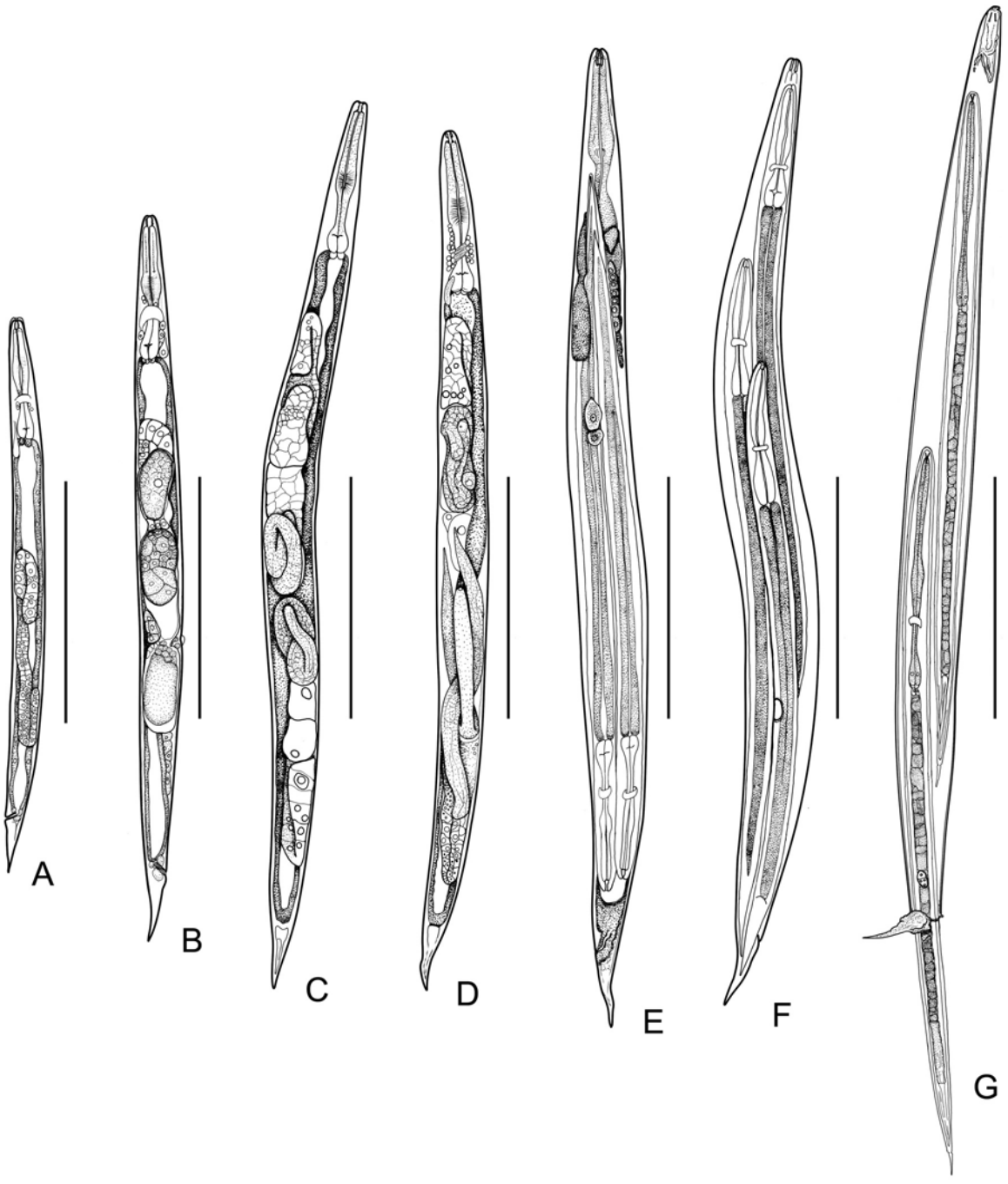

Fig. 3. Free-living females and developing larvae of the hermaphroditic generation of *Rhabdias paraensis*. A – Female L3, lateral view; B – Adult female with fertilised eggs, lateral view; C – Female with developing larvae in eggs; D – Female with hatching larvae; E – Female with developing larvae and partly ruined inner organs; F – Female cuticle with L2 of the hermaphroditic generation inside; G – Infective L3 escaping from the maternal cuticle. Scale bars: 200 µm

Immature and young (possessing few eggs in uteri) specimens in the host's lungs (5 specimens measured)

Immature specimens are generally similar to fully gravid adult specimens of *R. paraensis*. Body cuticle inflation becomes prominent, especially in the anterior portion of the body (Fig. 4A, K), though it is less prominent than in gravid worms. The intestine is distinctly brown, with black contents in the posterior portion (Fig. 4K). The body is 1.853–3.906 mm long, 59–109 μm wide at the oesophageal-intestinal junction, 74–126 μm wide at the vulva. The anterior end is similar to that in mature individuals (Fig. 4A), though more truncated. Four short, rounded longitudinal elevations are present on the dorsal, ventral and both lateral sides of the anterior extremity (Fig. 4D, E). Lips are absent; 6 lip-shaped projections on the outer surface of the apical extremity are present, separated from each

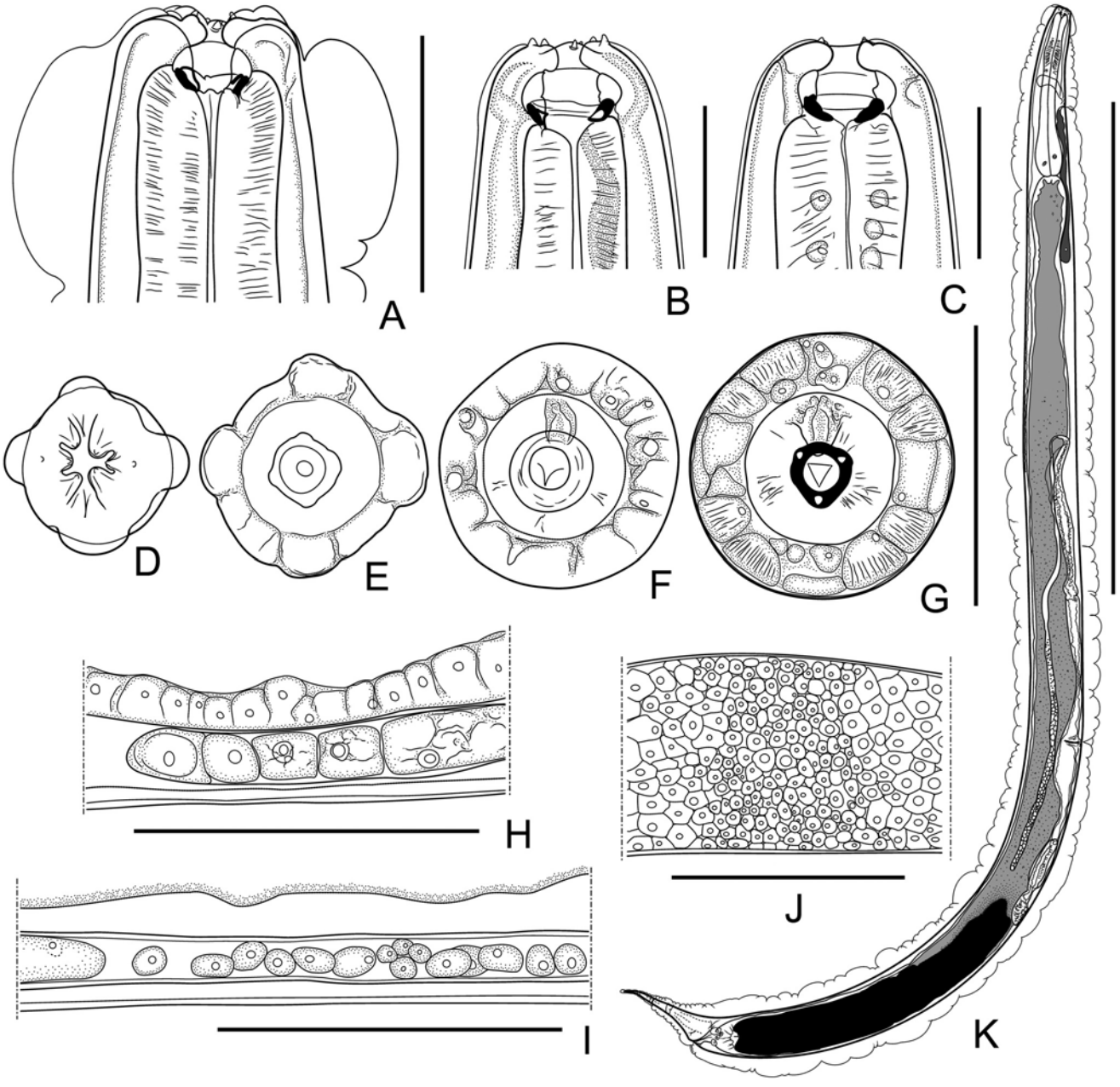

Fig. 4. Immature and young parasitic specimens of *Rhabdias paraensis*. A – Immature specimen from host lungs, anterior end, lateral view; B, C – Immature specimen from body cavity of host, anterior end: B – Lateral view, C – Dorsal view; D, E, F, G – Optical transverse sections through anterior end of young specimen: D – Apical view of anterior extremity; E – Section at level of anterior edge of buccal capsule; F – Section at level of buccal capsule, middle; G – Section through posterior portion of buccal capsule; H – Apex of posterior syngonium in an immature specimen from the lungs; I – Oogonia and testis zone in an immature specimen from the lungs; J – Testis zone in a young specimen; K – An immature specimen from the lungs, general view (lateral). Scale bars: A, B – 20 μm; C–I – 50 μm; J – 100 μm; K – 1 mm

other by concave folds (Fig. 4D). Submedian projections are situated closer to the oral opening than the lateral ones. Each projection bears a prominent internal labial papilla on the inner edge. External labial papillae are indistinct. Amphidial openings are situated posterior to the lateral projections (Fig. 4D). The vestibulum is narrow, shorter than the buccal capsule, and is outlined by the rounded inner portions of the submedian apical projections (Fig. 4A). The buccal capsule is the same shape as that in the immature specimen from the body cavity: 14–15 µm wide and 8–9 µm deep. The posterior portion of the buccal capsule is surrounded by the apex of the oesophagus (Fig. 4A). The entrance to the buccal capsule is circular in apical view, with four thickenings on the lateral, dorsal and ventral sides (Fig. 4E). The anterior portion of the buccal capsule is thick-walled, translucent and circular in apical view (Fig. 4F). The posterior portion has optically denser walls and 3 rounded and bright thickenings coinciding with the borders between the sectors of the oesophagus: two dorso-lateral and one ventral (Fig. 4G). The duct of the dorsal oesophageal gland enters the posterior part of the buccal capsule (Fig. 4G). The oesophagus is 336–373 µm long, (9.3–20.1% of the body length), 22-30 µm wide at the anterior portion, and 45-59 µm wide at the bulb. The nerve ring encircling the oesophagus is located 141–171 µm from the anterior end. The excretory glands are prominent, stretching from the excretory duct located posterior to the nerve ring up to the anterior portion of the intestine (Fig. 4K). The genital system is amphidelphic, composed of two opposing sets of uteri, oviducts and syngonia. The testis zone has small ovoid sperm cells grouped among larger polygonal oogonia (Fig. 4J). The position of the vulva is variable. The distance from the anterior end to the vulva is 0.994–1.705 µm, or 39.5–53.6% of the body length.

Discussion

The present observations on the free-living development of *R. paraensis* have demonstrated that the life cycle of the species is typical of *Rhabdias*, with alternation of gonochoristic and hermaphroditic generations and without homogony ("rhabdiasoid type" after Kuzmin 2013). Hermaphroditism of the *R. paraensis* parasitic generation was ascertained by the observed presence of zones of spermatogenesis in the syngonia of immature and young parasitic individuals. The male:female ratio (which is close to 1:1) and the matricidal hatching strategy (*endotokia matricida*) in the development of hermaphroditic-generation larvae are also similar to traits of other studied species of the genus.

Free-living-generation females had an average of one to four eggs and two to three developing larvae. This characteristic does not differentiate the species from other *Rhabdias* spp. parasitising amphibians in the Neotropical region; one to two larvae were reported for *R. fuelleborni* Travassos, 1926 females (Kloss 1971), and up to three to four larvae were reported for *R. hermaphrodita* Kloss, 1971, *R. elegans* Gutierrez, 1945, and *R. androgyna* Kloss, 1971 (Kloss 1971, 1974). Similarly, two to three larvae inside each female were observed in *R. americanus* Baker, 1978, *R. bakeri* Tkach, Kuzmin et Pulis, 2006, *R. brachylaimus* (Linstow, 1903), *R. joaquinensis* Ingles, 1935, and *R. ranae* Walton, 1929, all of which are parasitic in amphibians from other geographical regions (Yuen 1965; Baker 1979; Langford and Janovy 2009).

Slightly different numbers of eggs and/or larvae in females have been reported in other species of the genus. Not more than two eggs were observed in *R. bermani* Rausch, Rausch et Atrashkevich, 1984, and *R. rubrovenosa* (Schneider, 1866), which are parasitic in amphibians (Kuzmin 2013), and in *R. chamaeleonis* (Skrjabin, 1916), *R. gemellipara* Chabaud, Brygoo et Petter, 1961, *R. mariauxi* Lhermitte-Vallarino et Bain, 2009, and *R. brygooi* Lhermitte-Vallarino, Barbuto et Bain, 2010, which are parasitic in reptiles (chameleons) (Chabaud *et al.* 1961, Lhermitte-Vallarino and Bain 2004; Lhermitte-Vallarino *et al*. 2009, 2010a). Up to four eggs were observed in females of *R. sphaerocephala* Goodey, 1924 from amphibians (Kuzmin 2013) and in *R. okuensis* Lhermitte-Vallarino et Bain, 2008 and *R. jarki* Lhermitte-Vallarino et Bain, 2004 from chameleons (Lhermitte-Vallarino and Bain 2004; Lhermitte-Vallarino *et al.* 2008). A larger number of eggs was reported in only one species, *R. multiproles* Yuen, 1965 from amphibians; its females possess three to eight eggs (Yuen 1965).

As the fecundity of free-living females may depend on cultivation conditions (Baker 1979), the differences in the number of larvae in free-living females within the range of one to four cannot be used for differentiation of Neotropical *Rhabdias* spp.

Males of the free-living generation of *R. paraensis* were different from those in several species of the genus studied previously. Baker (1979) described three pairs of precloacal and five pairs of postcloacal ventro-lateral genital papillae, all connected to thin caudal alae, in males of *R. americanus* and *R. bakeri*. Yuen (1965) observed four precloacal and four postcloacal pairs of papillae in males of *R. brachylaimus* and *R. multiproles*. Lhermitte-Vallarino *et al*. (2009) found three precloacal and three postcloacal ventro-lateral pairs in *R. mariauxi*, of which only the two sub-terminal pairs were connected to the sub-lateral alae. In this study, males of *R. paraensis* possessed sub-lateral alae connected only to the two sub-terminal pairs of papillae (similarly to *R. mariauxi*), and ventro-lateral papillae were arranged into three precloacal pairs (similar to *R. americanus*, *R. bakeri* and *R. mariauxi*) and four postcloacal pairs (similar to *R. multiproles* and *R. brachylaimus*). Presumably, the number and position of the genital papillae and their connection to caudal alae in free-living males may be a diagnostic characteristic of *Rhabdias* species.

From the shape of the pseudolabia in lateral view and by the presence of small "buds" on the rounded tail tip, the infective larvae of *R. paraensis* appeared to be morphologically similar to those of *R. sphaerocephala*; however, the pseudolabia in *R. paraensis* infective larvae are broad and rounded in

dorsoventral view, whereas those of *R. sphaerocephala* are triangular, with rounded tips (Kuzmin *et al.* 2014). The shape of the stoma and the oesophagus in the examined infective larvae of *R. paraensis* and the combined longitudinal and transverse striations of the sheath were similar to those in the infective larvae of other *Rhabdias* species (Lhermitte-Vallarino *et al*. 2010b; Junker *et al.* 2010; Kuzmin *et al*. 2014).

In the description of *R. paraensis* based on gravid specimens only, the absence of excretory glands and ducts was revealed by both morphological observations of entire worms and histological sections (Santos *et al.* 2011). In this study, we observed distinct excretory glands and ducts in immature and young individuals of the species. Presumably, the glands disappear during maturation. It would be interesting to examine immature specimens of other species of *Rhabdias* in which the excretory glands were not described, e.g., *R. pseudosphaerocephala*, *R. fuelleborni*, *R. elegans*, *R. androgyna* and *R. hermaphrodita* occurring in South America (Travassos 1926; Kloss 1971; Kuzmin *et al*. 2007; González and Hamann 2008).

In the original description of *R. paraensis* (Santos *et al*. 2011), the detailed structure and shape of the buccal capsule are not specified, because the thick body wall in the apical region of gravid specimens made it impossible to observe details of the capsule morphology. In this study, we recognised the separation of the buccal capsule wall into an anterior and posterior portion and revealed the specific shape of these parts in lateral and apical view. In the presence of separated anterior and posterior portions in the buccal capsule, *R. paraensis* is similar to some other species of *Rhabdias*, mostly parasites of reptiles (see Lhermitte-Vallarino *et al*. 2010a), and also to several species from amphibian hosts in the Afrotropical region: *R. ohlerae* Junker, Lhermitte-Vallarino et Bain, 2010, *R. picardiae* Junker, Lhermitte-Vallarino et Bain, 2010, and *R. tanyai* Junker, Lhermitte-Vallarino et Bain, 2010 (Junker *et al.* 2010). The specific shape of the posterior portion of the buccal capsule differentiates *R. paraensis* from all mentioned species. In our opinion, detailed studies on the buccal capsule morphology of other *Rhabdias* spp. from the Neotropical region may provide additional information for species differentiation.

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