FOLIA PARASITOLOGICA 58[4]: 318–321, 2011
ISSN 0015-5683 (print), ISSN 1803-6465 (online)

http://www.paru.cas.cz/folia/

RESEARCH NOTE

NEW MORPHOLOGICAL DATA ON THE FIRST-STAGE LARVAE OF TWO *PROCAMALLANUS* SPECIES (NEMATODA: CAMALLANIDAE) BASED ON SEM STUDIES

Šárka Mašová[1], **Vlastimil Baruš**[2] and **František Moravec**[3]

[1] Department of Botany and Zoology, Faculty of Science, Masaryk University, Kotlářská 2, 611 37 Brno, Czech Republic;

[2] Institute of Vertebrate Biology, Academy of Sciences of the Czech Republic, Květná 8, 603 65 Brno, Czech Republic;

[3] Institute of Parasitology, Biology Centre of the Academy of Sciences of the Czech Republic, Branišovská 31, 370 05 České Budějovice, Czech Republic

Abstract: First-stage larvae of camallanid nematodes *Procamallanus* (*Procamallanus*) *laeviconchus* (Wedl, 1862) and *Procamallanus* (*Procamallanus*) sp. from naturally infected *Distichodus niloticus* (Hasselquist) and *Clarias gariepinus* (Burchell), respectively, from Lake Turkana, Kenya (new geographical records) are described, being for the first time studied by scanning electron microscopy. Larvae of both species are characterised by the presence of a dorsal cephalic tooth, four submedian cephalic papillae and a pair of amphids, and by the elongate tail with several terminal digit-like processes. The latter formations probably serve for the attachment of larvae to the substrate in water when the larvae attract copepod intermediate hosts by their movements; these structures, especially their numbers, may be of taxonomic importance in camallanid nematodes.

The nematode genus *Procamallanus* Baylis, 1923 contains many species parasitizing freshwater, brackish-water and marine fishes and, less often, amphibians. The following five subgenera of *Procamallanus* were recognised by Moravec and Thatcher (1997) (see also Gibbons 2010): *Procamallanus* Baylis, 1923, *Spirocamallanus* Olsen, 1952, *Spirocamallanoides* Moravec et Sey, 1988, *Punctocamallanus* Moravec et Scholz, 1991 and *Denticamallanus* Moravec et Thatcher, 1997. Of them, only representatives of *Procamallanus* and *Spirocamallanus* are known from the African continent (totally 7 species), whereby *P.* (*P.*) *laeviconchus* (Wedl, 1862) (the type species of the genus) has so far been the only species of the former subgenus reported from freshwater fishes in Africa. This is also the only African *Procamallanus* species in which the life cycle has hitherto been studied and the first-stage larvae described (Moravec 1975).

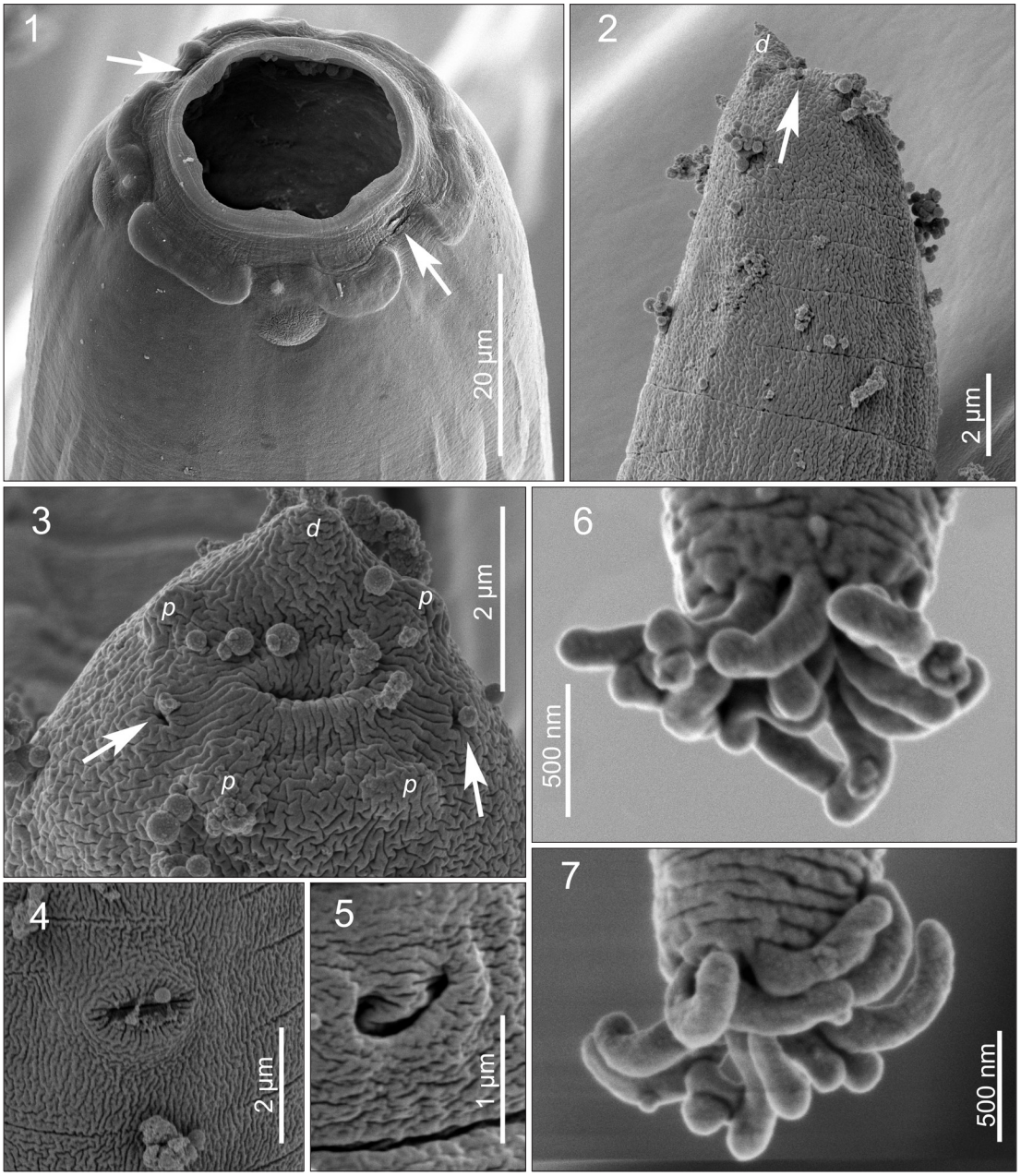
While redescribing *P. laeviconchus* from *Synodontis* spp. (congeneric with its type host) in Botswana (including SEM study), Moravec and Van As (2004) drew attention to the fact that this species was reported from about 30 fish species belonging to seven families and the specimens often exhibited morphological differences; they considered the presence of an unusually lobed circumoral flange a characteristic feature of *P. laeviconchus*, already illustrated by Wedl (1862) in the original description.

The first-stage larvae used in this study were obtained from the uteri of broken nematode females dissected out from the stomachs of *Distichodus niloticus* (Hasselquist) (Citharinidae, Characiformes) and *Clarias gariepinus* (Burchell) (Clariidae, Siluriformes), collected in Lake Turkana, north-western Kenya, in 2008 and 2009. Since the adult specimens from the former host were morphometrically in accordance with the redescription of *P. laeviconchus* given by Moravec and Van As (2004), including the presence of a characteristic circumoral flange (Fig. 1), they were assigned to this species. In contrast, the morphology of adult specimens from the latter host was somewhat different and the characteristic circumoral flange was missing (Fig. 8); for the time being, this form is designated *Procamallanus* (*Procamallanus*) sp., even though further studies may show it represents a new species.

All nematode specimens were fixed in hot 4% formaldehyde solution in saline. They were examined using an Olympus BX51 light microscope equipped with differential interference contrast (Nomarski DIC) optics, and the digital image analysis system (Stream Motion) was used for measurements. All measurements are given in micrometres. For scanning electron microscopy (SEM), the specimens (both adults and larvae) were dehydrated through a graded ethanol series, critical-point dried (in a Bal-Tec CPD 030 Critical Point Dryer) using liquid CO_2, mounted on aluminium stubs with double-sided adhesive disc and sputter-coated with gold (in a Balzers SCD 040). The samples of larvae were examined using a Quanta™ 3D FEG scanning electron microscope at an accelerating voltage of 3−5 kV, adults at 10 kV.

Description of first-stage larvae of *P. laeviconchus* (n = 5) and *Procamallanus* sp. (n = 5) (measurements of the latter in parentheses) (Figs. 2–7, 9–11): Body very small, colourless, 481–538 (414–459) long, maximum width 18–19 (18–20). Width of anterior end 9–12 (8–11), at level of anal pore 14–16 (13–15). Cuticle thin, slightly transversely striated (Figs. 2, 9). Cephalic end with distinct dorsal tooth (Figs. 2, 3, 9). Mouth aperture slit-shaped, laterally elongated, surrounded by four large submedian cephalic papillae and pair of small lateral amphids (Figs. 3, 9). Short, narrow buccal tube present. Oesophagus undivided, 77–84 (69–82) long. Nerve ring and excretory pore usually not

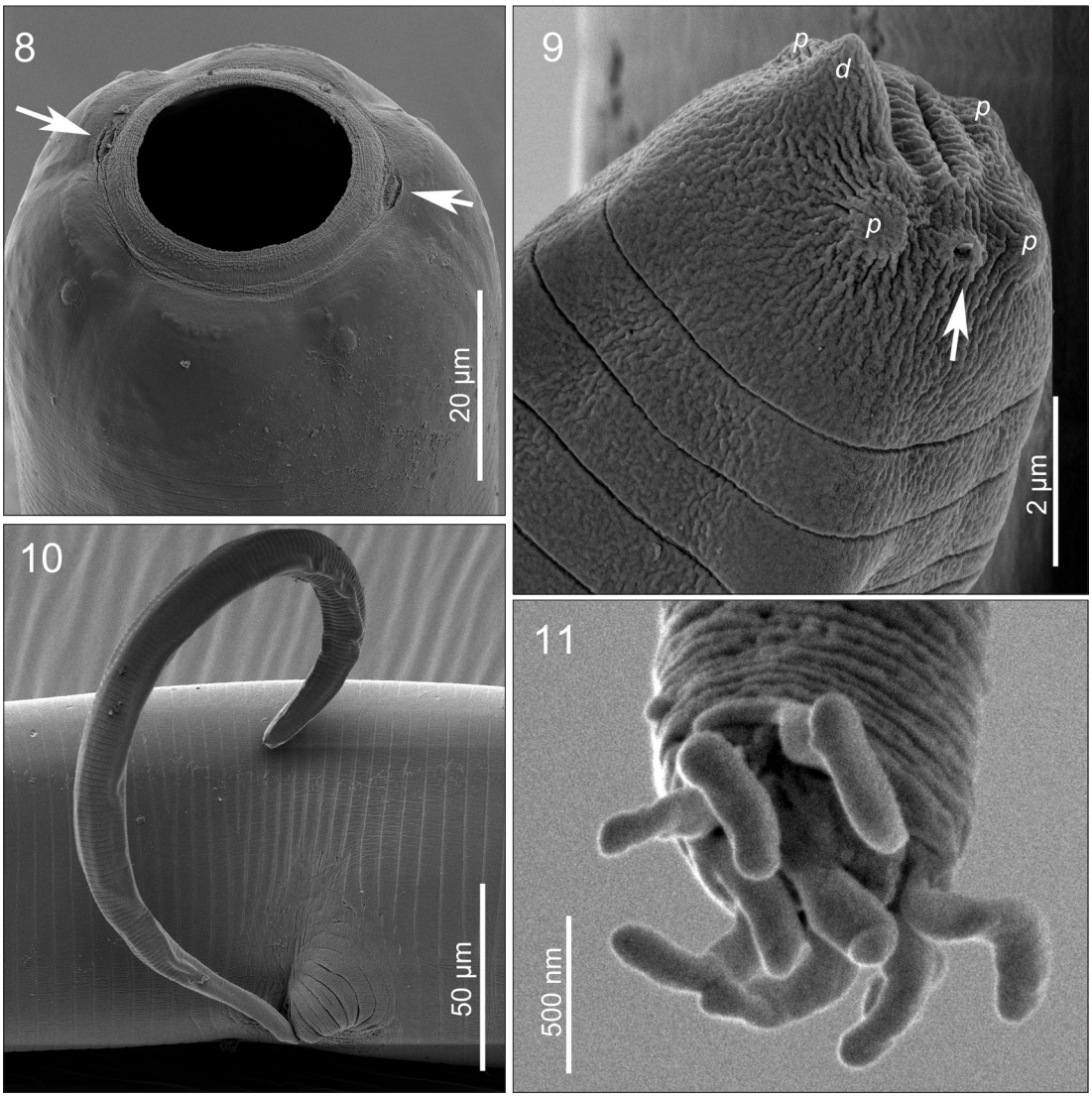
Address for correspondence: Š. Mašová, Department of Botany and Zoology, Faculty of Science, Masaryk University, Kotlářská 2, 611 37 Brno, Czech Republic. Phone: +420 549 494 447; Fax: +420 541 211 214; E-mail: masova@sci.muni.cz



Figs. 1–7. *Procamallanus* (*Procamallanus*) *laeviconchus* (Wedl, 1862) from *Distichodus niloticus*, scanning electron micrographs. **Fig. 1.** Anterior end of adult with circumoral flange, dorsoventral view (arrows indicate amphids). **Fig. 2.** Cephalic end of first-stage larva, lateral view (arrow indicates amphid). **Fig. 3.** Cephalic end of first-stage larva, apical view (arrows indicate amphids). **Fig. 4.** Larval anal pore. **Fig. 5.** Larval excretory pore. **Figs. 6, 7.** Tail tips of first-stage larvae (different specimens) with digit-like processes, lateral views. *Abbreviations*: d – dorsal tooth; p – cephalic papilla.

well visible. Tail elongate, slender, 192–221 (172–186) long, representing 39–44 (40–41)% of larval body length. Tail tip bearing approximately 14–15 (9–11) minute digit-like processes 0.60–0.75 (0.56–0.60) long and 0.15–0.19 (0.13–0.20) wide (Figs. 6, 7, 11).

All camallanid nematodes, including species of *Procamallanus*, are ovoviviparous, producing first-stage larvae, which are usually released from the female's vulva (Fig. 10) (Ivashkin et al. 1971, Anderson 2000). These larvae get into the water where they are eaten by copepod intermediate hosts in the body of which they continue to grow and attain the infective third larval stage. Although the life cycles of many camallanid species of *Camallanus* Railliet et Henry, 1915, *Neocamallanus* Ali, 1957, *Paracamallanus* Yorke et Maplestone, 1926 and *Procamallanus* Baylis, 1923 were studied and the first-stage larvae of these species were described (see Anderson 2000), all these studies were based on light microscopical observations. These larvae were reported to possess a dorsal tooth, enabling their better penetration through intermediate host tissues, and their tail was generally described as conical, slender, with a sharply pointed tip; the same concerned the larvae of the African species *Procamallanus laeviconchus* (see Moravec 1975).



Figs. 8–11. *Procamallanus* (*Procamallanus*) sp. from *Clarias gariepinus*, scanning electron micrographs. **Fig. 8.** Anterior end of adult without circumoral flange, dorsoventral view (arrows indicate amphids). **Fig. 9.** Cephalic end of first-stage larva, dorsolateral view (arrow indicates amphid). **Fig. 10.** Larva releasing from vulva. **Fig. 11.** Tail tip of first-stage larva with digit-like processes, apical view. *Abbreviations*: d – dorsal tooth; p – cephalic papilla.

The only camallanid species whose first-stage larvae were examined by SEM was *Camallanus oxycephalus* Ward et Magath, 1917 (see Kelly et al. 1989); the authors observed the tail tip of these larvae to form "an oval-spatulate area containing rows of muscle", assumed to be utilised as a muscular grasping organ or sucker enabling the larvae to attach to the substrate. On the contrary, later Moravec and Justine (2006) found the tail tips of first-stage larvae of two *Camallanus* species, *C. cotti* Fujita, 1927 and *C. lacustris* (Zoega, 1776), to possess a crown of several minute digit-like processes (called also mucrons or terminal spikes), distinctly visible by SEM, but almost invisible under the light microscope.

The purpose of the present paper is to put data about presence and number of digit-like processes at the tail tip of first-stage larvae of a representative of *Procamallanus* and *P. laeviconchus* on published record. Moravec and Justine (2011) and Yooyen et al. (2011) reported such digit-like processes in larvae of four *Procamallanus* spp. parasitizing marine fishes off New Caledonia [*P.* (*P.*) *annulatus* Yamaguti, 1955; *P.* (*S.*) *monotaxis* (Olsen, 1952)] and Thailand [*P.* (*S.*) *rigbyi* Yooyen, Moravec et Wongsawad, 2011; *P.* (*S.*) *similis* Yooyen, Moravec et Wongsawad, 2011], but since these larvae were not studied by SEM, the processes at the tail tips are poorly visible in drawings. Apparently, caudal processes serve the larva for a better attachment of its tail to the bottom or aquatic vegetation. The larvae attach to the substrate using these processes and wave with their bodies from side to the side or they alternately roll up and unroll, attracting copepod intermediate hosts. Further studies may prove the character of caudal processes or other modifications in first-stage larvae to be an important taxonomic feature differentiating camallanid species (Moravec and Justine 2006).

The structure of the cephalic end of first-stage larvae of *Procamallanus* spp., as revealed by SEM in this study, i.e. the presence of a dorsal tooth and a slit-shaped oral aperture sur-

rounded by four submedian papillae and a pair of lateral amphids, is probably also typical of other camallanid genera at this stage. The same structure of the larval cephalic end was found in *Camallanus* spp. studied by SEM (Kelly et al. 1989, Moravec, unpublished) and even in the first-stage larvae of *Dracunculus medinensis* (Linnaeus, 1758) of the related superfamily Dracunculoidea (Harada et al. 1989).

This study was financially supported by the Ichthyoparasitology Research Centre project No. LC522, the long-term research plan MSM 0021622416 funded by the Ministry of Education, Youth and Sports of the Czech Republic, the research project of the Institute of Parasitology, BC ASCR No. Z60220518, the Czech Science Foundation grant No. 526/09/H025 and the FEI Czech Republic. The authors are grateful to Mikuláš Oros, Dagmar Jirsová and Miloslav Jirků, Institute of Parasitology, BC ASCR, Czech Republic, for their great help in the field and for contributing the specimens.

References

Anderson R.C. 2000: Nematode Parasites of Vertebrates. Their Development and Transmission. 2nd Ed. CABI Publishing, Wallingford, 650 pp.

Gibbons L.M. 2010: Keys to the Nematode Parasites of Vertebrates. Supplementary Volume. CABI, Wallingford, 416 pp.

Harada M., Suguri S., Inatomi S., Katakura K., Kobayashi A. 1989: Scanning electron microscopy of the first-stage larvae of *Dracunculus medinensis*. Jpn. J. Parasitol. 38: 17–21.

Ivashkin V.M., Sobolev A.A., Khromova L.A. 1971: [Camallanata of Animals and Man and the Diseases Caused by Them.] Osnovy Nematodologii 22. Nauka, Moscow, 388 pp. (In Russian.)

Kelly R., Crites J.L., Mergo J.C. 1989: Comparative morphology of the first-stage larvae of two species of *Philometra* (Nematoda: Philometridae) and one species of *Camallanus* (Nematoda: Camallanidae) from Lake Erie fishes. Ohio J. Sci. 89: 70–73.

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

Moravec F., Justine J.-L. 2006: *Camallanus cotti* (Nematoda: Camallanidae), an introduced parasite of fishes in New Caledonia. Folia Parasitol. 53: 287–296.

Moravec F., Justine J.-L. 2011: New data on the morphology of *Procamallanus* (*Procamallanus*) *annulatus* and *Procamallanus* (*Spirocamallanus*) *monotaxis* (Nematoda: Camallanidae) from marine fishes off New Caledonia. Helminthologia 48: 41–50.

Moravec F., Thatcher V.E. 1997: *Procamallanus* (*Denticamallanus* subgen. n.) *dentatus* sp. n. (Nematoda: Camallanidae) from the characid fish, *Bryconops alburnoides*, in the Brazilian Amazon. Parasite 4: 239–243.

Moravec F., Van As J. 2004: Nematodes from the squeaker fishes *Synodontis nigromaculatus* and *S. vanderwaali* from the Okavango River, Botswana, including three new species. Syst. Parasitol. 59: 169–187.

Wedl K. 1862: Zur Helminthenfauna Äegyptens. Sitzungsber. Kaiser. Akad. Wiss., Math.-Naturwiss. Cl., 44: 463–482.

Yooyen T., Moravec F., Wongsawad C. 2011: Two new sibling species of *Procamallanus* (*Spirocamallanus*) (Nematoda: Camallanidae) from marine fishes in the Gulf of Thailand. J. Parasitol. 97: 931–938.

Received 11 April 2011
Accepted 23 May 2011