

Observations on nematodes from the Indonesian shortfin eel *Anguilla bicolor bicolor* McClelland in India, including a revalidation of *Heliconema ahiri* Karve, 1941 (Physalopteridae)

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Abstract

Two species of parasitic nematodes, *Heliconema ahiri* Karve, 1941 (Physalopteridae) and *Procamallanus* (*Spirocamallanus*) *anguillae* Moravec *et al.*, 2006 (Camallanidae), were recorded from the Indonesian shortfin eel *Anguilla bicolor bicolor* McClelland in the Chalakkudi River and Veli Lake, respectively, both Kerala, India. The former species (*H. ahiri*), rediscovered from eels in India after 82 years from its original description, has been redescribed and revalidated based on its specific feature (presence of groups of minute denticles in the mouth) distinguishing it from other congeners; its finding in *A. b. bicolor* represents a new host record. The latter species (*P. anguillae*) has been recorded in India for the first time. SEM examination of its first-stage larvae from uterus has shown that *P. anguillae* is another species of *Procamallanus* whose larvae possess a crown of digit-like processes at the tail tip.

Keywords

Parasitic nematode, *Heliconema*, *Procamallanus*, *Spirocamallanus*, freshwater eel, *Anguilla bicolor bicolor*, Kerala, India

Introduction

The present knowledge of nematode parasites of freshwater eels (*Anguilla* spp.) in India is scarce (Soota 1983; Sood 1989). Four species were reported from *Anguilla bengalensis bengalensis* (Gray), viz. *Heliconema ahiri* Karve, 1941, *Paraquimperia anguillae* Karve, 1941, *Procamallanus ahiri* Karve, 1952 and *Raphidascaris* sp. larva (Karve 1941, 1952; Naidu 1983), and one, *Itheringascaris neocornutum* (Rajyalakshmi, Rao et Shyamasundari, 1992), considered a *species inquirenda* by Moravec *et al.* (2012a), from *Anguilla bicolor bicolor* McClelland (see Rajyalakshmi *et al.* 1992). During recent studies on parasites of freshwater fishes carried out in the State of Kerala, southern India, nematodes were recovered from both the above-mentioned species of eels, *A. b. bengalensis* and *A. b. bicolor*. Results of the systematic evaluation of these parasites from *A. bengalensis* have been published by Moravec *et al.* (2012b), whereas those from *A. bicolor* are presented herein.

Anguilla bicolor bicolor (maximum body length 120 cm) is a tropical commercial migratory fish breeding in the ocean, widespread in the Indian Ocean and the western Pacific. It lives in freshwater areas as an adult and in estuaries and sea as young (Froese and Pauly 2013).

Materials and Methods

Fish were collected using hook and line and gill netting. Nematodes recovered from the digestive tract were fixed in hot 70% ethanol and preserved in 70% ethanol. For light microscopical (LM) examination, they were cleared with glycerine. Drawings were made with the aid of a Zeiss microscope drawing attachment. Specimens used for scanning electron microscopy (SEM) were postfixed in 1% osmium tetroxide, dehydrated through a graded acetone series, critical point dried and sputter-coated with gold; they were examined using a JEOL JSM-7401F scanning electron microscope at an accele-

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rating voltage of 4 kV (GB low mode). All measurements are in micrometres unless otherwise indicated. Fish names follow FishBase (Froese and Pauly 2013).

Results

The following two nematode species were found to parasitize *Anguilla bicolor bicolor* in the localities of Kerala, India:

Family Physalopteridae Railliet, 1893

Heliconema ahiri Karve, 1941

Description: Medium sized, whitish nematodes with thick, finely transversely striated cuticle (Fig. 2A). Cephalic end rounded. Cuticle in cephalic region inflated to form cephalic vesicle extending posteriorly to level of deirids and anteriorly forming cephalic collar which may extend beyond interlabia anteriorly

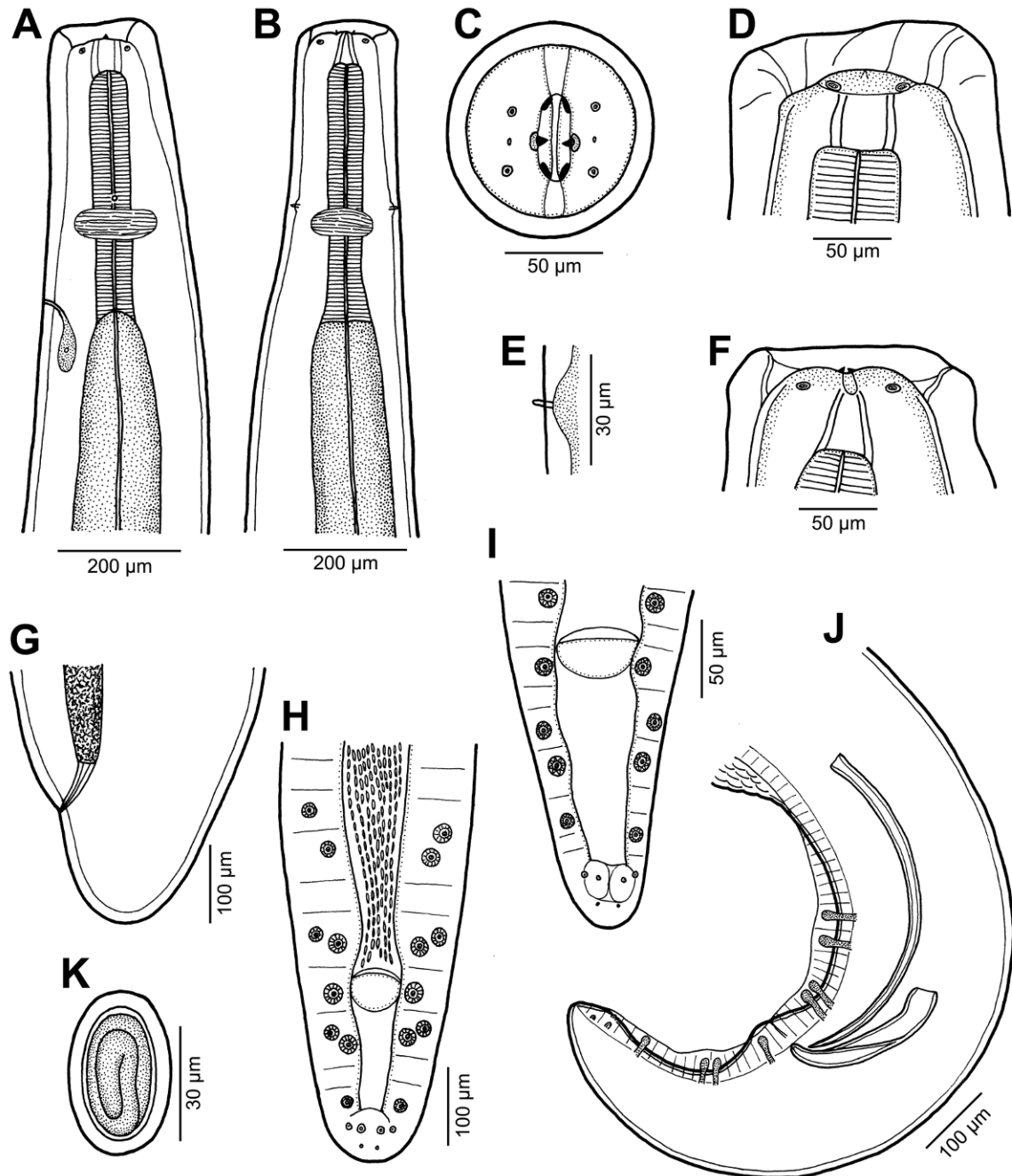


Fig. 1. *Heliconema ahiri* Karve, 1941: **A** and **B** – anterior end of male, lateral and dorsoventral views. **C** – cephalic end of female, apical view. **D** – cephalic end of male, lateral view. **E** – deirid. **F** – cephalic end of male, dorsoventral view. **G** – tail of gravid female, lateral view; **H** – posterior end of male, ventral view. **I** – tail of male, ventral view. **J** – posterior end of male, lateral view. **K** – egg

(Figs 1A, B, D, F; 2A, B, D). Oral aperture dorsoventrally elongate, oval, rather large, surrounded by two massive, rounded lateral pseudolabia (Fig 1C; 2A–E). Each pseudolabium bears two large submedian (dorsolateral and ventrolateral) cephalic papillae and terminal, kidney-shaped lateral depression containing lobular mass (Figs 1C; 2A–E); small lateral amphid situated between cephalic papillae (Figs 1C; 2A, C). Inner surface of each pseudolabium with elongate lateral mound bearing marked triangular terminal lateral tooth (internolateral tooth) and simple flat tooth at each dorsoventral extremity (Figs 1C; 2A–E); in addition, row of about 5 minute denticles present near

each flat tooth (Fig. 2C, E). In apical view, interlabia separated from each other by narrow, dorsoventrally elongate dorsal and ventral fields distinctly demarcated by grooves (Figs 1C; 2A–E). Buccal cavity short (Fig. 1A, B, D, F). Oesophagus divided into short, narrow anterior muscular portion and much longer, wide glandular portion (Fig. 1A, B). Nerve ring encircles muscular oesophagus at its posterior half (Fig. 1A, B). Small simple deirids situated approximately at level of nerve ring (Fig. 1A, B, E). Excretory pore slightly posterior to anterior end of glandular oesophagus (Fig. 1A). Tail of both sexes with rounded tip (Figs 1 G, H–J; 3A–C, E–G).

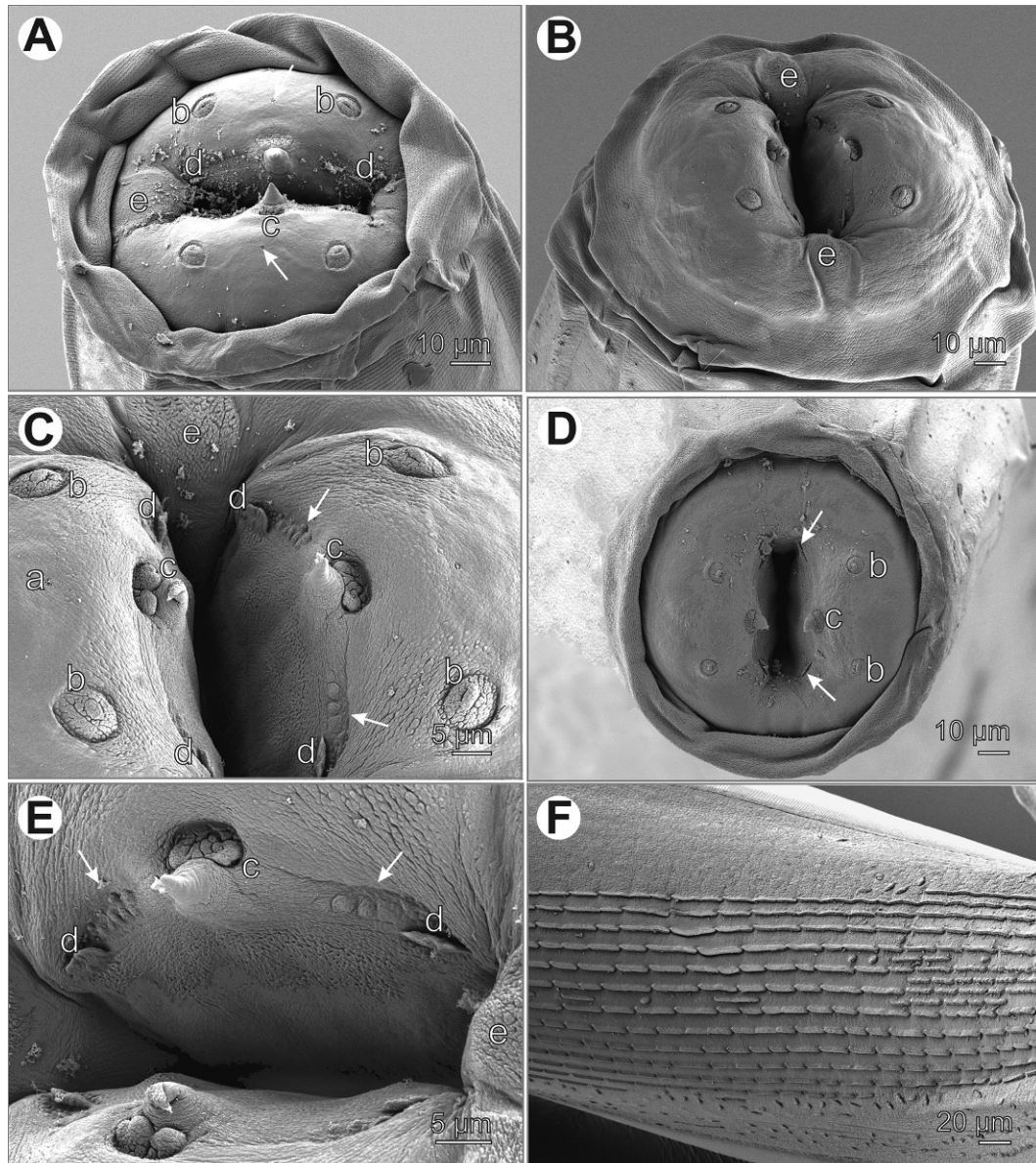


Fig. 2. *Heliconema ahiri* Karve, 1941, scanning electron micrographs: **A** – cephalic end, apical view (arrow indicates amphid). **B** – same of another specimen, subapical view. **C** – region of mouth, apical view (arrow indicates groups of minute denticles). **D** – cephalic end, apical view (arrow indicates submedian tooth). **E** – region of mouth, apical view (arrow indicates groups of minute denticles). **F** – ventral precloacal ridges of male. **Abbreviations:** a – amphid; b – cephalic papilla; c – lateral tooth with lateroapical depression; d – submedian tooth; e – dorsal/ventral field between pseudolabia

Male (6 specimens). Length of body 22.89–31.80 mm, maximum width 408–558. Pseudolabia 12–18 long. Cephalic vesicle 218–517 long and 163–299 wide. Entire oesophagus 3.25–4.27 mm long, representing 12–15% of body length; muscular oesophagus 313–503 long, 42–82 wide; glandular oesophagus 2.94–3.7 mm long, 150–204 wide; length ratio of parts of oesophagus 1:6.43–9.39. Nerve ring, deirids and excretory pore 326–367, 258–326 and 237–544, respectively, from anterior extremity. Caudal end spirally coiled, provided with broad lateral alae supported by 4 pairs of subventral pedunculate preanal papillae arranged in couples and 5 pairs of subventral postanal papillae, of which papillae of first four pairs are rather large, pedunculate and those of last pair are very small, sessile; an additional pair of small postanal sessile papillae situated ventrally at level of last subventral postanal pair (Figs 1H–J; 3A–F). Pair of minute phasmids present just posterior to last pair of postanal papillae (Figs 1H–J; 3B,C). Cloacal lips somewhat elevated. Ventral precloacal surface with about 16 longitudinal tessellated ridges (area rugosa) (Figs 1H; 3F). Spicules unequal and dissimilar (Fig. 1J); left spicule 408–501 long, with sharply pointed distal tip; right spicule broader, boat-shaped, 168–237 long, tapered towards distal tip. Length ratio of spicules 1:1.72–2.86. Length of tail 272–381.

Female (3 gravid specimens). Length of body 34.54–37.60 mm, maximum width 666–748. Pseudolabia 15–18 long. Cephalic vesicle 381–721 long, 204–326 wide. Entire oesophagus 3.99–4.35 mm long, 11–13% of body length; muscular oesophagus 503–544 long, 68–95 wide; glandular oesophagus 3.48–3.84 mm long, 163–204 wide; length ratio of parts of oesophagus 1: 6.92–7.44. Nerve ring, deirids and excretory pore 258–354, 272–381 and 571–707, respectively, from anterior extremity. Rectum a hyaline tube. Vulva situated 16.23–20.54 mm from anterior end of body, at 47–55% of body length. Vagina narrow, muscular, directed first anteriorly from vulva and then oriented posteriorly. Uteri containing numerous oval, thick-shelled, embryonated (larvated) eggs (Fig. 1K); eggs 39–48 × 27–30, with wall 5–6 thick. Tail short, 163–231 long, with rounded tip (Figs 1G; 3G).

Host: Indonesian shortfin eel, *Anguilla bicolor bicolor* (Anguillidae, Anguilliformes).

Site of infection: Stomach.

Locality: Chalakkudi River, Kerala, India (collected 12 February 2010).

Prevalence and intensity: 5 fish infected/9 fish examined; totally 26 nematodes.

Deposition of voucher specimens: Institute of Parasitology, ASCR, in České Budějovice (Cat. No. N – 1009).

Comments: The general morphology of these nematodes shows that they belong to the subfamily Proleptinae of the family Physalopteridae following the conception of Chabaud (1975) (see also Chabaud 2009, Gibbons 2010). However, the taxonomy of this group, especially regarding the delimitation of the genera, still remains somewhat confused. Moravec *et al.* (2007) have indicated that genera in this subfamily should be differentiated mainly based on cephalic structures,

particularly on the cephalic dentation, which corresponds to a great extent with a distribution in one or another group of hosts. According to those authors, the characters of three of the four recognized proleptine genera, *Heliconema* Travassos, 1919, *Paraleptus* Wu, 1924 and *Proleptus* Dujardin, 1845, are rather similar and, in fact, there were frequent confusions in assigning different species to these genera. For the time until a relevant revision is made on the basis of a detailed study of the cephalic structures of their type species, Moravec *et al.* (2007) suggested to distinguish these three genera according to their cephalic dentation.

Considering the conception proposed by Moravec *et al.* (2007), the morphology (cephalic dentation) and the type of host (an anguilliform fish) of specimens of the present material correspond to *Heliconema*, except for the presence of minute denticles on each pseudolabium in addition to an internolateral tooth and a simple tooth at each dorsoventral extremity. In contrast, the internal border of each pseudolabium in representatives of *Paraleptus* bears an internolateral tooth and a series of larger denticles, whereas that of *Proleptus* spp. bears a single internolateral tooth and a circumoral row of serrations; hosts of species of both these genera are mainly elasmobranchs (Moravec *et al.* 2007).

Unfortunately, the cephalic structures of the type species of the three above-mentioned genera have not yet been studied by SEM. Moreover, it is evident that most proleptine species are inadequately described, because some morphological structures of these nematodes are difficult or practically impossible to observe under the light microscope (Moravec 2007). Of many described *Heliconema* spp., cephalic structures have so far been studied using the SEM in only four species, *H. africanum* (Linstow, 1899), *H. brooksi* Crites et Overstreet, 1991, *H. hainanensis* Li, Liu, Liu et Zhang, 2013 and *H. longissimum* (Ortlepp, 1922) (see Crites and Overstreet 1991, Moravec *et al.* 2007, 2013, Li *et al.* 2013). However, minute denticles on each pseudolabium (in addition to an internolateral tooth and a simple tooth at each dorsoventral extremity), which were found in specimens of the present material, were observed in none of them. Nevertheless, since the type species of *Heliconema* (*H. heliconema* Travassos, 1919) has not yet been studied by SEM and because the above-mentioned minute denticles may be also present in other species of this genus, the specimens of the present material are provisionally assigned to *Heliconema*.

The presence of the minute denticles on pseudolabia, as found in our specimens (Fig. 2C, E), is a unique feature among all *Heliconema* spp. by which the Indian specimens from *A. b. bicolor* also differ from the morphometrically very similar species *H. africanum* and *H. longissimum* (see Moravec *et al.* 2007, 2013). The only *Heliconema* species described from freshwater eels in India is *H. ahiri* from *Anguilla b. bengalensis* in Pune, Maharashtra (Karve 1941), which was considered a junior synonym of *H. longissimum* by Moravec *et al.* (2007). However, results of the present study indicate that our specimens represent a distinct species, different from *H. lon-*

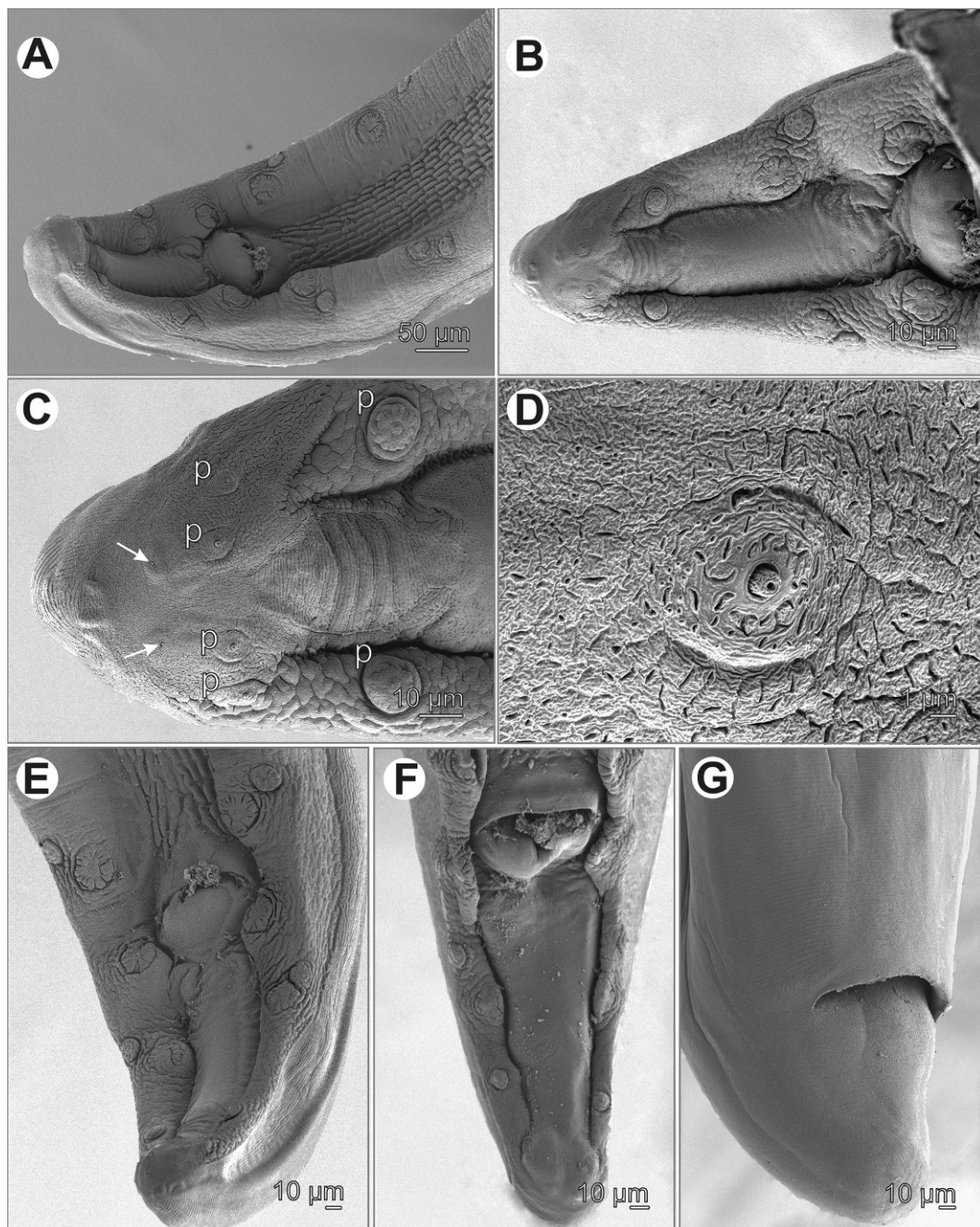


Fig. 3. *Heliconema ahiri* Karve, 1941, scanning electron micrographs: **A** – posterior end of male, subventral view. **B** – tail of male, ventral view. **C** – tip of male tail, ventral view (arrow indicates phasmid). **D** – detail of ventral postanal papilla. **E** – tail of male, subventral view. **F** – same of another specimen, ventral view. **G** – caudal end of female, sublateral view. **Abbreviation:** p – postanal papilla

gissimum. Considering that specimens of the present material were collected from congeneric hosts in the same geographical region as *H. ahiri*, it is apparent that both these forms are identical. The measurements of both forms are similar except for somewhat longer spicules in *H. ahiri* (630–770 and 250–270 vs. 408–501 and 168–237), which is probably within the intraspecific variability of this species. According to the original description (Karve 1941), *H. ahiri* was characterized by the presence of seven pairs of postanal papillae; however, the

distribution of caudal papillae was studied only in lateral view and it is clear that in fact phasmids were considered to be the last pair of postanals. So, the number and distribution of postanal papillae in *H. ahiri* are the same as in the present specimens (Figs. 1H–J; 3A–C, E, F). Therefore, *H. ahiri* should be revalidated and its occurrence in *A. b. bicolor* represents a new host record. *Heliconema africanum* is also known to parasitize two species of eels (*A. mossambica* and *A. marmorata*) in South Africa (Moravec *et al.* 2013).

Family Camallanidae Railliet et Henry, 1915

Procamallanus (*Spirocamallanus*) *anguillae* Moravec, Taraschewski, Anantaphruti, Maipanich et Laoprasert, 2006

Description: Medium-sized nematodes with finely transversely striated cuticle (Fig. 4D, E). Mouth aperture oval, surrounded by 12 submedian cephalic papillae arranged in three circles, each formed by four papillae; papillae of external circle distinctly larger (Fig. 4A). Each papilla of second circlet with distinct pore (Fig. 4B). Pair of small lateral amphids present. Buccal capsule orange-brown, thick-walled, slightly

longer than wide, with simple, well developed basal ring. Inner surface of capsule provided with 11–15 spiral ridges in lateral view, 2–3 of them being incomplete. Muscular oesophagus claviform, distinctly shorter than glandular oesophagus. Intestine brown, narrow. Deirids small, simple, with rounded end situated at about mid-way between posterior end of buccal capsule and nerve ring (Fig. 4E). Excretory pore at about level of end of muscular oesophagus. Tail of both sexes with two (dorsal and ventral) terminal cuticular spikes.

Male (10 specimens). Length of body 17.57–25.00 mm, maximum width 258–381. Buccal capsule including basal ring 90–105 long, its width 78–87; basal ring 9–15 long and 51–63

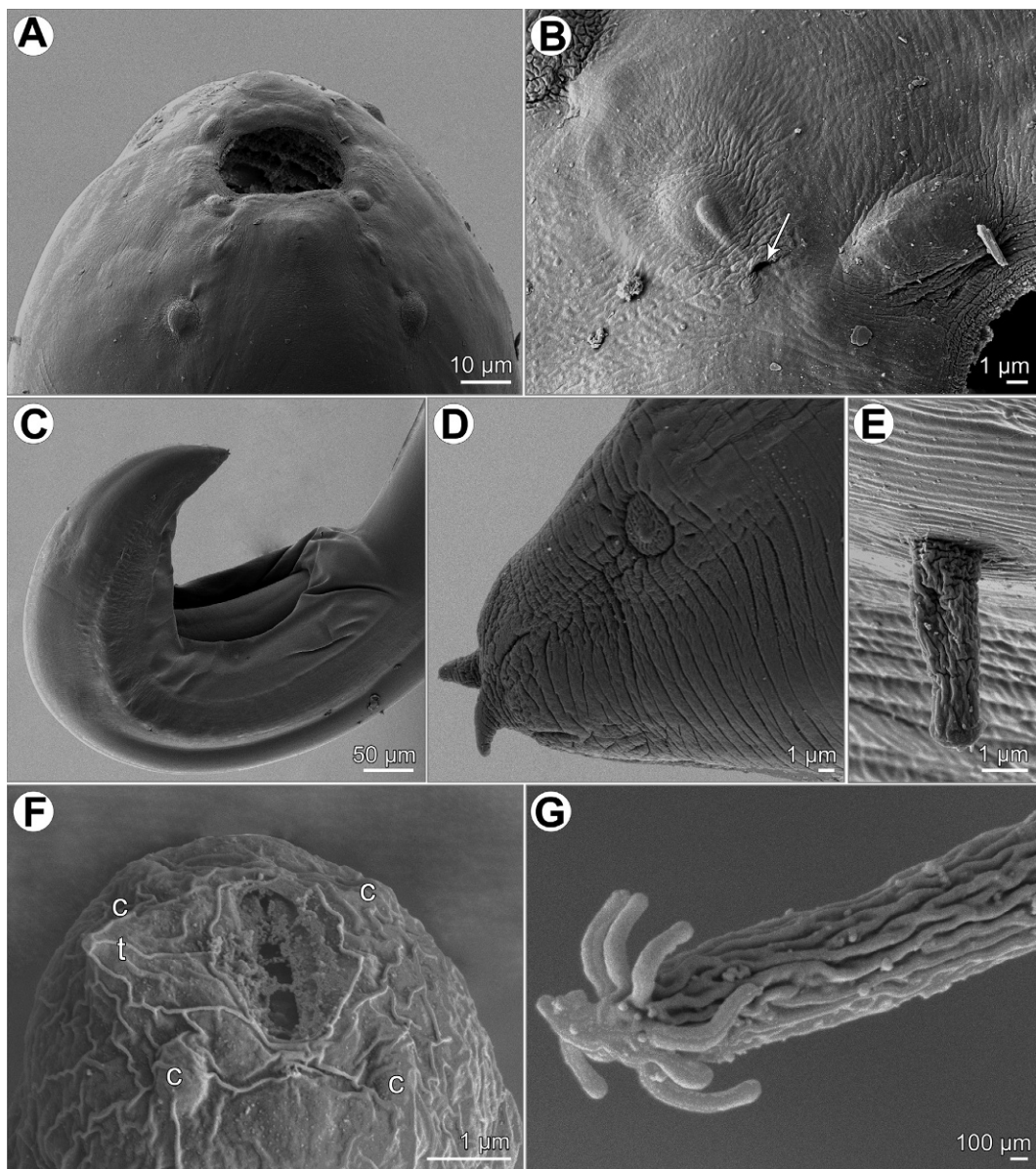


Fig. 4. *Procamallanus* (*Spirocamallanus*) *anguillae* Moravec *et al.*, 2006, scanning electron micrographs: **A** – cephalic end of male, lateral view. **B** – detail of submedian cephalic papilla of middle circle (arrow indicates distinct porus). **C** – posterior end of male, sublateral view. **D** – tip of male tail, lateral view. **E** – deirid. **F** – cephalic end of first-stage larva, subapical view. **G** – tail tip of first-stage larva. **Abbreviations:** c – cephalic papilla; t – dorsal cephalic tooth

wide. Maximum width/length ratio of buccal capsule 1:1.7–1.25. Spiral ridges 12–15, of which 2–3 incomplete. Length of muscular oesophagus 435–490, maximum width 75–123; length of glandular oesophagus 789–1020, maximum width 60–138; length ratio of muscular and glandular oesophagus 1:1.7–2.3. Length of entire oesophagus and buccal capsule representing 6–8% of body length. Nerve ring, deirids and excretory pore 258–340, 156–201 and 503–830, respectively, from anterior extremity. Posterior end of body ventrally bent, provided with wide, vesiculated caudal alae supported by pedunculate papillae; anteriorly alae interconnected by mound, forming thus a kind of pseudosucker, and posteriorly reaching to base of caudal terminal spikes (Fig. 4C). Preanal papillae: 3 pairs of subventral pedunculate papillae; postanal papillae: 4 pairs of subventral and 2 pairs of lateral papillae (last pair representing phasmids – see Fig. 4D); additional 2 pairs of small transversely elongate sessile ventral papillae surrounding cloacal opening. Region of cloaca somewhat elevated. Spicules similar in shape, unequal, with sharply pointed distal ends; large (right) spicule 289–384 long; small (left) spicule less sclerotized, 189–249 long. Length ratio of spicules 1:1.40–1.78. A small gubernaculum about 30 long present. Tail conical, 216–270 long (Fig. 4D); length of terminal spikes 5–6.

Female (5 larvigerous specimens). Length of body 35.17–43.17 mm, maximum width 625–816. Buccal capsule including basal ring 102–114 long and 96–99 wide; basal ring 15 long and 63–69 wide. Maximum width/length ratio of buccal capsule 1:1.06–1.19. Number of spiral ridges 11–12, of which 2–3 incomplete. Length of muscular oesophagus 503–585, maximum width 114–141; length of glandular oesophagus 938–1183, maximum width 135–150; length ratio of muscular and glandular oesophagus 1:1.9–2.7. Length of entire oesophagus and buccal capsule representing 4–5% of body length. Nerve ring, deirids and excretory pore 270–340, 138–201 and 666–775, respectively, from anterior extremity. Vulva covered by small, elevated flap, pre-equatorial, 15.64–19.19 mm from anterior extremity, at 43–47% of body length. Vagina muscular, directed posteriorly from vulva. Uterus filled with numerous larvae about 400 long; cephalic end of larva with distinct dorsal tooth and four submedian papillae (Fig. 4F); tail tip of larva bearing several digit-like projections (Fig. 4G). Female tail broadly rounded, with digit-like projection bearing two (dorsal and ventral) minute cuticular spikes. Length of entire tail 219–285; digit-like projection including spikes 39–69 long and 21–27 wide; spikes 5–6 long.

Host: Indonesian shortfin eel, *Anguilla bicolor bicolor* (Anguillidae, Anguilliformes).

Site of infection: Intestine.

Locality: Veli Lake, Kerala, India (collected 20 October 2009 and 20 January 2010).

Prevalence and intensity: 83% (19 fish infected/23 fish examined); totally 190 nematodes.

Deposition of specimens: Institute of Parasitology, BC ASCR, České Budějovice (N – 793).

Comments: The morphology and measurements of specimens of the present material are more or less in agreement with the original description of *Procamallanus* (*Spirocamallanus*) *anguillae* Moravec *et al.*, 2006 and, therefore, they are assigned to this species. Small biometrical differences are apparently within the intraspecific variability of *P. anguillae*. This species was described by Moravec *et al.* (2006) from the same host species (*A. bicolor bicolor*) from a few freshwater and brackish-water localities in southern Thailand and has not been recorded since. The present finding of *P. anguillae* in India represents a new geographical record.

Within the Camallanidae, Moravec and Justine (2006) were the first to report a crown of several minute digit-like processes on the tail tips of first-stage larvae of two species of *Camallanus* Railliet et Henry, 1915, *C. cotti* Fujita, 1927 and *C. lacustris* (Zoega, 1776); they considered these structures, distinctly visible by SEM but almost invisible under the light microscope, to be possibly an important taxonomic feature for different camallanid species. Later digit-like processes on the tail tip of first-stage larvae were also found in two species of *Procamallanus* (*Procamallanus*), *P. laevisconchus* (Wedl, 1862) and *Procamallanus* sp., parasitizing freshwater fishes in Africa (Mašová *et al.* 2011) and in two species of *Procamallanus* (*Spirocamallanus*), *P. rigbyi* Yooyen, Moravec et Wongsawad, 2011 and *P. similis* Yooyen, Moravec et Wongsawad, 2011, parasites of marine fishes in the Gulf of Thailand (Yooyen *et al.* 2011). These structures are reported in *P. anguillae* for the first time.

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