

***Heliconema monopecteri* n. sp. (Nematoda: Physalopteridae)
from *Monopterus cuchia* (Hamilton) (Osteichthyes: Synbranchidae) in India,
with notes on the taxonomy of *Heliconema* spp.**

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Summary

A new nematode species, *Heliconema monopecteri* n. sp. (Physalopteridae), is described from the stomach and intestine of the freshwater fish *Monopterus cuchia* (Hamilton) (Synbranchidae) in Bijnor district, Uttar Pradesh, India. It is mainly characterized by the lengths of spicules (468 – 510 µm and 186 – 225 µm), the postequatorial vulva without elevated lips, the presence of pseudolabial lateroterminal depressions and by the number and arrangement of caudal papillae. This is the first representative of the genus reported from a synbranchiform fish. Another new congeneric species, *Heliconema pisodonophidis* n. sp. is established based on a re-examination of nematodes previously reported as *H. longissimum* (Ortlepp, 1922) from *Pisodonophis boro* (Hamilton) (Ophichthidae) in Thailand; ovoviviparity in this species is a unique feature among all physalopterids. *Heliconema hamiltonii* Bilqees et Khanum, 1970 is designated as a *species dubia* and the nematodes previously reported as *H. longissimum* from *Mastacembelus armatus* (Lacépède) in India are considered to belong to *H. kherai* Gupta et Duggal, 1989. A key to species of *Heliconema* Travassos, 1919 is provided.

Keywords: parasitic nematode; Physalopteroidea; new species; freshwater fish; Synbranchiformes; South Asia

Introduction

Recent parasitological examinations of some freshwater fishes in Bijnor district, Uttar Pradesh, India carried out in August 2018, revealed the presence of physalopterid nematodes in the digestive tract of the *cuchia* *Monopterus cuchia* (Hamilton) (Synbranchidae, Synbranchiformes). A detailed study of their morphology by both light (LM) and scanning electron microscopy (SEM) shows that they represent a new species of the genus *Heliconema* Travassos, 1919, which is described below.

The *cuchia* (maximum length 70 cm) is a tropical commercial fish that occurs in freshwater and brackish-water habitats of South Asia (Pakistan, India, Nepal, Bangladesh and Myanmar). Adults

are known to hibernate in mud during cold season (Froese and Pauly, 2018).

Material and Methods

Fish were obtained from the fish market in Bijnor (fish allegedly caught in the River Ganga), Bijnor district (29.3724°N, 78.1358°E), Uttar Pradesh, India in August 2018 and these were kept alive in aquaria at 24 °C until dissection. A total of two specimens of *Monopterus cuchia* (total body length 37 and 45 cm) were examined for the presence of parasites. The nematodes obtained were washed in physiological saline and then fixed in hot 70% ethanol. For LM, the nematodes were cleared using glycerine. Drawings

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were made with the aid of a Zeiss drawing attachment. Specimens used for SEM were postfixed in 1 % osmium tetroxide (in phosphate buffer), 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 accelerating voltage of 4 kV (GB low mode). All measurements are in micrometres unless otherwise indicated. For comparison, specimens of *Heliconema* from *Pisodonophis boro* (Hamilton) in Thailand, identified by Moravec *et al.* (2007) as *H. longissimum* (Ortlepp, 1923) and deposited in the Helminthological Collection of the Institute of Parasitology, CAS, České Budějovice (Cat. No. N – 862), were re-examined. The fish nomenclature adopted follows FishBase (Froese and Pauly, 2018).

Ethical Approval and/or Informed Consent

All applicable institutional, national and international guidelines for the care and use of animals were followed.

Results

Family Physalopteridae Railliet, 1893

Heliconema monopteri n. sp.

(Figs. 1, 2)

Description: Medium sized, whitish nematodes with thick, transversely striated cuticle. Cephalic end rounded. Cuticle in cephalic region inflated to form cephalic vesicle extending posteriorly to about level of deirids and anteriorly forming somewhat extended collar (Figs. 1A–C, 2C). Oral aperture dorsoventrally elongate, oval, rather large, surrounded by 2 massive, rounded lateral pseudolabia. Each pseudolabium bears 2 large submedian (dorsolateral and ventrolateral) cephalic papillae and oval lateroterminal depression filled with irregularly lobular mass; small lateral amphids situated between both cephalic papillae (Figs. 1C, 2A–C). Inner surface of each pseudolabium with elongate lateral mound bearing marked triangular terminal lateral tooth (internolateral tooth) situated immediately near inner border of cephalic depression and simple flat tooth at each dorsoventral extremity; no denticles present near terminal lateral teeth (Figs. 1C, 2A–C). Buccal cavity short. Oesophagus divided into short, narrow anterior muscular portion and much longer, wide glandular portion. Nerve ring encircles muscular oesophagus approximately at its middle or somewhat posterior to it. Small simple deirids situated at about level of nerve ring (Fig. 1B). Excretory pore slightly anterior to anterior end of glandular oesophagus (Fig. 1A). Tail of both sexes with rounded tip.

Male (3 specimens; measurements of holotype in parentheses): Length of body 23.39 – 27.35 (23.39) mm, maximum width 340 – 394 (340). Pseudolabia 24 – 27 (24) long. Cephalic vesicle 286 – 299 (286) long and 258 – 272 (272) wide. Buccal cavity 24 – 27 (27) long. Entire oesophagus 3.48 – 3.55 (3.48) mm long, repre-

sented 11 – 15 (15)% of body length; muscular oesophagus 449 – 490 (490) long and 82 – 95 (82) wide; glandular oesophagus 2.99 – 3.10 (3.10) mm long and 163 – 177 (163) wide; length ratio of two parts of oesophagus 1:6.1 – 6.9 (1:6.1). Nerve ring, deirids and excretory pore 313 – 354 (326), 299 – 313 (313) and 420 – 530 (420) from anterior extremity, respectively. Caudal end spirally coiled, provided with lateral alae supported by 4 pairs of subventral pedunculate preanal papillae arranged in couples, and 5 single pairs of subventral postanal papillae, which are rather large and pedunculate; an additional pair of small postanal sessile papillae situated ventrally slightly posterior to level of last subventral postanal pair (Figs. 1E,G, 2E). Pair of minute phasmids present posterior to ventral pair of posteriormost postanal papillae (Fig. 1E,G). Ventral surface between posteriormost ventral postanal papillae and phasmids elevated to form distinct small protuberance. Cloacal lips somewhat elevated (Fig. 2E). Ventral precloacal surface with about 12 longitudinal tessellated ridges (area rugosa) (Figs. 1E, 2D,E). Spicules unequal and dissimilar; left spicule 465 – 510 (468) long, with sharply pointed tip; right spicule broader, boat-shaped, 186 – 225 (186) long, tapered towards distal tip. Length ratio of spicules 1:2.07 – 2.62 (1:2.52). Length of tail 340 – 367 (340).

Female (7 ovigerous specimens; measurements of allotype in parentheses): Length of body 29.29 – 34.07 (34.07) mm, maximum width 517 – 571 (571). Pseudolabia 27 – 41 (41) long. Cephalic vesicle 272 – 340 (272) long and 258 – 326 (272) wide. Buccal cavity 27 – 36 (36) long, 27 – 36 (36) wide. Entire oesophagus 3.80 – 4.62 (4.58) mm long, representing 11 – 17 (13)% of body length; muscular oesophagus 490 – 585 (571) long and 95 (95) wide; glandular oesophagus 3.31 – 4.04 (4.01) mm long and 150 – 190 (190) wide; length ratio of two parts of oesophagus 1:1.6 – 7.0 (1:7.0). Nerve ring, deirids and excretory pore 313 – 381 (340), 299 – 367 (354) and 510 – 544 (535) from anterior extremity, respectively. Vulva postequatorial, situated 16.32 – 20.03 (20.03) mm from anterior end of body, at 54 – 66 (59)% of body length. Vulval lips not elevated. Vagina narrow, muscular, directed posteriorly from vulva. Uteri containing numerous oval, thick-shelled, embryonated (larvated) eggs (Fig. 1F); eggs 39 – 45 × 27 – 30 (42 – 45 × 27 – 30), with wall 4 – 5 (4 – 5) thick. Tail short, 177 – 190 (190), with rounded tip; pair of small lateral phasmids situated near tail tip (Fig. 1D).

Taxonomic summary

Type host: *Cuchia*, *Monopterusuchia* (Hamilton) (Synbranchidae, Synbranchiformes).

Site of infection: Stomach and intestine.

Type locality: Fish market in Bijnor (fish allegedly caught in the River Ganga), Bijnor district (29.3724°N, 78.1358°E), Uttar Pradesh, India (collected in August 2018).

Prevalence and intensity: 2 fish infected/2 fish examined; 30 and 40 nematode specimens.

Type specimens: Helminthological Collection of the Institute of

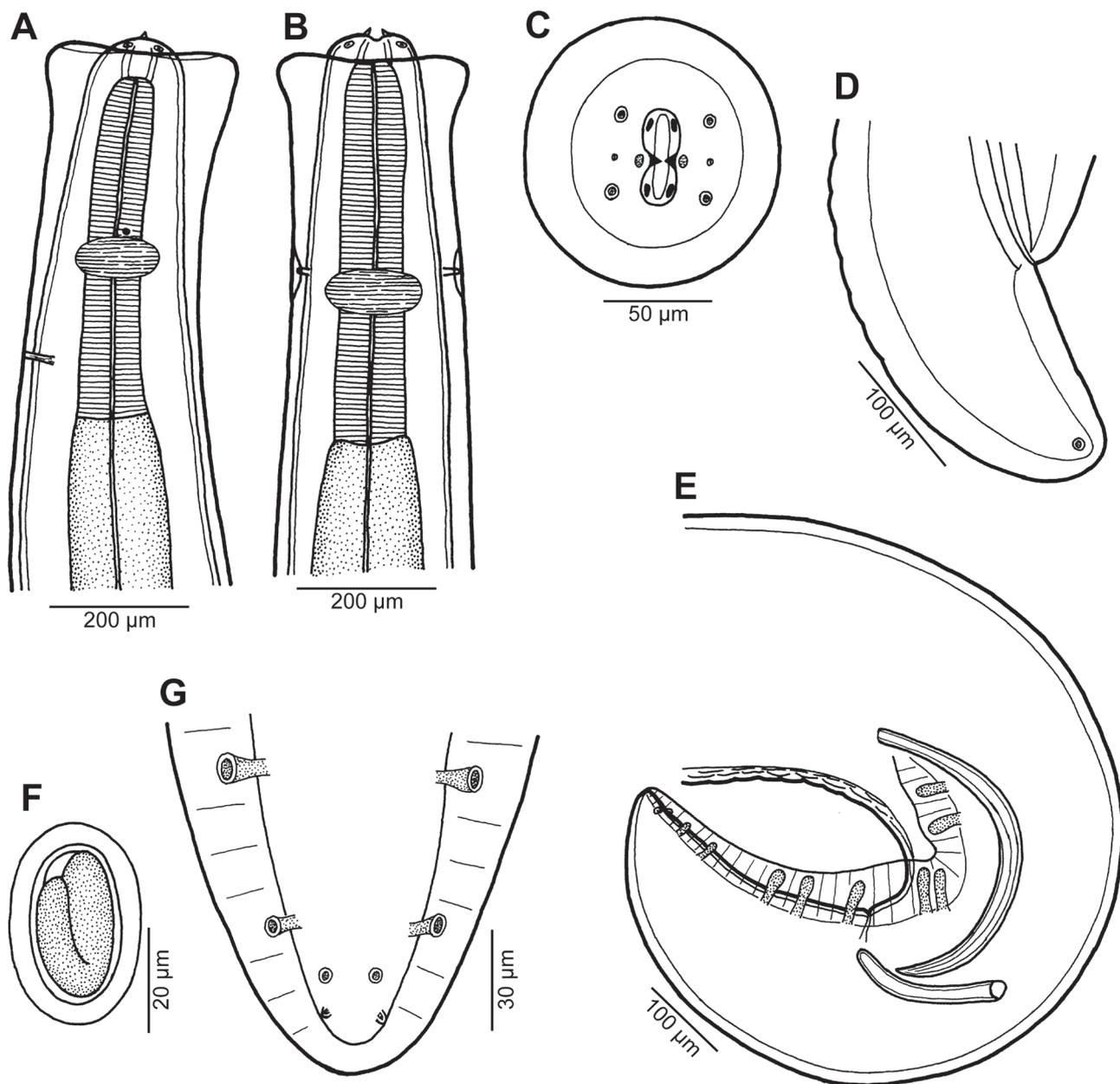


Fig. 1. *Heliconema monopteri* n. sp. A,B – anterior end, lateral and dorsoventral views, respectively; C – cephalic end, apical view; D – tail of female, lateral view; E – posterior end of male, lateral view; F – egg; G – tail tip of male, ventral view

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Etymology: The specific name of this nematode relates to the genitive form of the generic name of the host.

Discussion

The following ten recognisable species of *Heliconema* were reported by Li *et al.* (2013): *H. baylisi* Ogden, 1969, *H. brevispiculum* Baylis, 1934, *H. brooksi* Crites et Overstreet, 1991, *H. hainanense*

Li, Liu, Liu et Zhang, 2013, *H. heliconema* Travassos, 1919, *H. kherai* Gupta et Duggal, 1989, *H. longissimum*, *H. psammobatidus* Threlfall et Carvajal, 1984, *H. savala* Akram, 1996 and *H. serpens* Fusco et Palmieri, 1980. However, as mentioned by Moravec and Nagasawa (2018), later this list was extended for an additional two congeneric species, *H. africanum* (Linstow, 1899) and *H. ahiri* Karve, 1941, parasites of freshwater eels (*Anguilla* spp.) in South Africa and India, respectively (Linstow, 1899; Karve, 1941; Moravec *et al.*, 2013a,b).

The present taxonomy of *Heliconema* spp. is rather problemat-

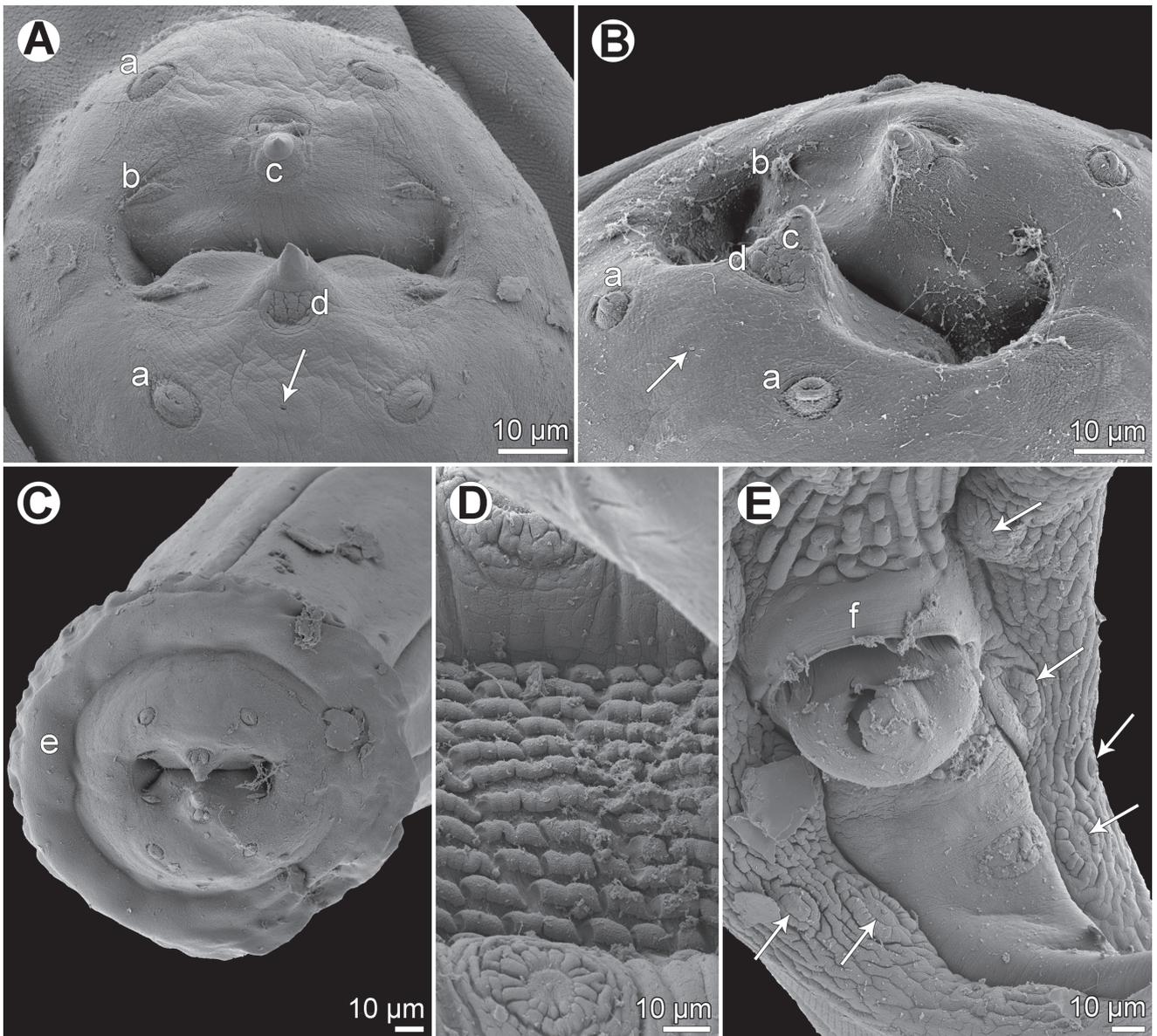


Fig. 2. *Heliconema monopteri* n. sp., scanning electron micrographs. A,B – cephalic end, two different subapical views (arrows indicate amphids); C – cephalic end, apical view; D – detail of ventral precloacal cuticular ridges (area rugosa), ventral view; E – region of cloacal aperture, ventral view (arrows indicate caudal papillae).
 Abbreviations: a – cephalic papilla; b – submedian tooth; c – lateral tooth; d – lateroapical pseudolabial depression; e – cephalic hood; f – cloacal aperture

ic, because the descriptions of older species are solely based on LM observations, whereas some important morphological features, such as the cephalic structures or the exact number and distribution of caudal papillae, require the use of SEM. The situation in *Heliconema* is further complicated by the fact that many congeneric species have similar measurements of the body and of spicules, but they can be discriminated by features difficult or impossible to observe by LM. Apparently, this often led to wrong species identifications.

Such an example is the species *H. longissimum*, originally inadequately described as *Physaloptera longissima* by Ortlepp (1922)

from snakes in Australia. Later it was redescribed from poorly preserved paratype specimens by Ogden (1969), who had also identified the nematodes at his disposal from *Mastacembelus armatus* (Lacépède) in India and those from *Anguilla mossambica* (Peters) in South Africa as *H. longissimum*; he also considered *H. anguillae* to be conspecific with *H. longissimum*. Subsequently, De *et al.* (1978) and De (1988) designated *Paraleptus komiyai* Sood, 1970 and *Notopterooides alatae* Majumdar, 1965, both parasites of *M. armatus* in India, as junior synonyms of *H. longissimum* and Moravec *et al.* (2007) synonymized *H. ahiri*, described from *Anguilla bengalensis* (Gray) in India, with this species.

However, the subsequent study of Moravec *et al.* (2013b) showed that the records of *H. longissimum* from *Anguilla mossambica* reported by Ogden (1969) and Taraschewski *et al.* (2005) in South Africa concerned in fact *H. africanum* and that the insufficiently described *H. longissimum* might be its junior synonym. Until *H. longissimum* is redescribed in detail (including the use of SEM) based on a newly collected topotypic material and the validity of this species is confirmed, this name should only be used for the type specimens originally studied by Ortlepp (1922). Chabaud and Campana-Rouget (1956) suggested that the host (unidentified Australian snakes) of Ortlepp's type material of the species is doubtful, but, according to Moravec *et al.* (2013b), apparently the snakes served only as postcyclic hosts for this parasite, which acquired the infection by feeding on its true definitive hosts (fish); some species of sea snakes are known to be specialised to feed on eels. Based on newly collected materials, *H. ahiri* and *H. anguillae* were resurrected by Moravec *et al.* (2013a) and Katahira and Nagasawa (2015), respectively. Accordingly, the nematodes reported as *H. longissimum* from *Anguilla japonica* Temminck et Schlegel in China by Li (1934) and others (see Li *et al.*, 2013) belonged to *H. anguillae*.

Ogden (1969), De *et al.* (1978) and De (1988) reported *H. longissimum* from *Mastacembelus armatus* in India; De *et al.* (1978) and De (1988) considered *Paraleptus komiyai* and *Notopteroides alatae*, respectively, parasites of the same host species in the same region, to be synonyms of *H. longissimum* (see above). Later, Gupta and Duggal (1989) described *H. kherai* Gupta et Duggal, 1989 from *M. armatus* in India. Because of the morphological similarity of all these Indian forms, occurring in the same host species (*M. armatus*) that, in contrast to hosts of other *Heliconema* spp., belongs to the fish family Mastacembelidae, we consider them to represent one and the same species, *H. kherai*. Unfortunately, the original description of *H. kherai* is inadequate, but conspecific nematodes (as *H. longissimum*) were relatively well described by other above-mentioned authors. The species has not yet been examined by SEM.

Moravec *et al.* (2007) described *Heliconema* specimens from the rice-paddy eel *Pisodonophis boro* (Ophichthidae) in Thailand and identified them as *H. longissimum*. However, due to an insufficiently known morphology of *H. longissimum*, the validity of this species is uncertain (see above). Moravec *et al.* (2007) reported markedly large eggs (as compared with other *Heliconema* spp.) in the Thai specimens from *P. boro*, which was questioned by Katahira and Nagasawa (2015). However, the recent re-examination of these specimens deposited in the Helminthological Collection of the Institute of Parasitology, Czech Academy of Sciences surprisingly revealed that their uteri were largely filled with very numerous free first-stage larvae and small, non-shelled developing eggs, whereas typical embryonated, shelled eggs were quite rare. The presence of free larvae instead of shelled eggs is a unique feature among all hitherto known representatives of *Heliconema* as well as all physalopterid nematodes, in which only shelled eggs have

been described. Considering also the type of the host (a representative of the Ophichthidae), we propose to establish a new species for these Thai specimens from *P. boro*, based on the description provided by Moravec *et al.* (2007); we propose the name ***H. pisodonophidis* n. sp.** for this species (syntypes deposited in the Helminthological Collection, Institute of Parasitology CAS, České Budějovice, Czech Republic; Cat. No. N – 862). The distinction of *H. pisodonophidis* from other congeners is apparent from the key at the end of Discussion.

By the general morphology, the new species *H. monopteri* n. sp. is most similar to *H. africanum*, *H. anguillae* and *H. pisodonophidis* n. sp., differing from them mainly in having a shorter left spicule. Differences from all species of *Heliconema* are apparent from the key at the end of Discussion.

To date, six nominal species of *Heliconema* have been reported from South Asia (Bangladesh, India and Pakistan) (see also Sood, 2017): *H. ahiri* from *Anguilla bicolor* McClelland and *A. bengalensis* in India (Karve, 1941; Moravec *et al.*, 2013a), *H. brevispiculum* from *Channa marulius* (Hamilton) in Bangladesh (Khan and Yaseen, 1969), *H. hamiltonii* Bilqees et Khanum, 1970 from *Sicamugil hamiltonii* (Day) in Pakistan (Bilqees and Khanum, 1970), *H. heliconema* from *Muraenesox cinereus* (Forsskål) in India and Pakistan (Khan and Begum, 1971; Gupta and Garg, 1976), *H. kherai* from *Mastacembelus armatus* in India (De *et al.*, 1978; De, 1988; Gupta and Duggal, 1989) and *H. savala* from *Lepturacanthus savala* (Cuvier) in Pakistan (Akram, 1996); nematodes of the seventh species, *H. longissimum*, reported from India, are now considered to belong to *H. kherai* (see above).

However, except for *H. ahiri* and *H. kherai*, the congeneric nematodes from South Asia were poorly described and, judging from their unusual hosts, their species or generic identification seems to be doubtful. In particular this concerns females allegedly of *H. brevispiculum* reported from a freshwater fish (*C. marulius*) or the female nematodes described as a new species *H. hamiltonii*; the latter species was evidently based on anisakid larvae (!), as visible from illustrations, and it should be designated a *species dubia*. Consequently, *H. monopteri* n. sp. represents an additional species of *Heliconema* in South Asia with well-known morphology and the second species in this region studied by SEM.

Conflict of Interest

The authors declare that they have no conflict of interest.

Acknowledgements

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Key to species of *Heliconema*:

- 1 Left spicule more than 2 mm long 2
 - Left spicule less than 1 mm long 4
- 2 Adanal papillae absent; left spicule 2.09 – 3.50 mm long. Parasitic in Ophichthidae (*Ophichthus*); Gulf of Mexico *H. brooksi*
 - Adanal papillae 1 – 2 pairs; left spicule equal or longer than 3.5 mm 3
- 3 Three pairs preanal papillae, 2 pairs adanal papillae; left spicule 4 mm long, right spicule 270 µm; vulva at approximately first third of body. Parasitic in Trichiuridae (*Lepturacanthus*); off Pakistan *H. savala*
 - Four pairs preanal papillae, 1 pair adanal papillae; left spicule 3.5 – 7.3 mm, right spicule 300 – 350 µm; vulva at approximately second quarter of body. Parasitic in Muraenidae (*Echidna*) and Muraenesocidae (*Muraenesox*); Atlantic and Indian Oceans (off Trinidad, Senegal and Pakistan) *H. heliconema*
- 4 Pair of ventral postanal papillae just posterior to cloacal aperture present; pseudolabial lateroterminal depression present 5
 - Pair of ventral postanal papillae just posterior to cloacal aperture absent; pseudolabial lateroterminal depression present or absent 6
- 5 Postanal papillae of fifth subventral pair pedunculate; vulva preequatorial; size of eggs 39 – 51 × 20 – 30 µm; length of left and right spicule 538 – 820 µm and 200 – 306 µm, respectively. Parasitic in Anguillidae (*Anguilla*); East Asia (Japan, China) *H. anguillae*
 - Postanal papillae of fifth subventral pair sessile; vulva preequatorial to somewhat postequatorial; eggs 75 – 81 × 42 – 48 µm, but uterus mostly filled with free larvae; length of left and right spicule 520–734 µm and 232 – 286 µm, respectively. Parasitic in Ophichthidae (*Pisodonophis*); South-East Asia (Thailand)
 *H. pisodonophidis* n. sp.
- 6 Vulva distinctly postequatorial; pseudolabial lateroterminal depression present; dorsoventral flat teeth on each pseudolabium without nearby denticles; length of left and right spicule 468 – 510 µm and 186 – 225 µm, respectively. Parasitic in Synbranchidae (*Monopterus*); South Asia (India) *H. monopteri* n. sp.
 - Vulva equatorial, exceptionally may be slightly postequatorial; pseudolabial lateroterminal depression present, absent or not reported; dorsoventral flat teeth on each pseudolabium with or without nearby denticles 7
- 7 Dorsoventral flat teeth on each pseudolabium with nearby denticles; pseudolabial lateroterminal depressions present; length of left and right spicule 408 – 770 µm and 168 – 270 µm, respectively. Parasitic in Anguillidae (*Anguilla*); South Asia (India) *H. ahiri*
 - Dorsoventral flat teeth on each pseudolabium without nearby denticles 8
- 8 Pseudolabial lateroterminal depressions present; vulva preequatorial; length of left and right spicule 650 – 857 µm and 290 – 400 µm, respectively. Parasitic in Anguillidae (*Anguilla*); South Africa
 *H. africanum*
 - Pseudolabial lateroterminal depressions absent or not reported; vulva equatorial or postequatorial. Parasitic in other host families 9
- 9 Parasites of teleosts or elasmobranchs 10
 - Parasites reported from snakes 14
- 10 Pseudolabial lateroterminal depressions absent; vulva preequatorial; length of left and right spicule 420 – 630 µm and 190 – 300 µm, respectively. Parasitic in Congridae (*Uroconger*) and Muraenidae (*Congresox*, *Muraenesox*); South China Sea *H. hainanense*
 - Pseudolabial lateroterminal depressions not reported 11

- 11 Parasites of Anguilliformes or Synbranchiformes 12
 - Parasites of Rajiformes: Vulva equatorial, with elevated anterior lip; length of left and right spicule 558 – 621 µm and 131 – 151 µm, respectively. Parasitic in Arhynchobatidae (*Sympterygia*); off Chile
 *H. psammobatidus*
- 12 Parasites of Synbranchiformes: Vulva equatorial to slightly postequatorial, with non- elevated anterior lip; length of left and right spicule 420 – 630 µm and 190 – 300 µm, respectively. Parasitic in Mastacembelidae (*Mastacembelus*); South Asia (India) *H. kherai*
 - Parasites of Anguilliformes 13
- 13 Length of left and right spicule 380 – 440 µm and 280 – 380 µm, respectively. Parasitic in Muraenidae (*Echidna*); Indian Ocean *H. baylisi*
 - Length of left and right spicule 500 – 520 µm and 230 – 250 µm, respectively; vulva equatorial with elevated anterior lip. Parasitic in Muraenesocidae (*Muraenesox*); off Australia *H. brevispiculum*
- 14 Vulva preequatorial; length of left and right spicule 516 µm and 228 – 300 µm, respectively; size of eggs 59 × 32 µm. Reported from unidentified snakes; Australia *H. longissimum*
 - Vulva situated in about mid-length of body, anterior vulval lip non-elevated; length of left and right spicule 404 – 525 µm and 247 – 314 µm, respectively; size of eggs 49 – 54 × 27 – 31 µm. Parasitic in Homalopsidae (*Cerberus*); Malaysia *H. serpens*

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