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Two new species of philometrid nematodes (Philometridae), Barracudia notabilis n. sp. and Philometra consimilis n. sp., from the ovary of the hound needlefish Tylosurus crocodilus (Belonidae) off Florida, USA

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Article info	Summary
Received February 2, 2021 Accepted March 3, 2021	Two new species of philometrid nematodes (Philometridae), <i>Barracudia notabilis</i> n. sp. and <i>Philometra consimilis</i> n. sp., are described from males found in the ovary of the hound needlefish <i>Tylosurus crocodilus</i> (Péron et Lesueur) (Belonidae) from off the Atlantic coast of Florida, USA. Both species are described and illustrated based on light and scanning electron microscopical examinations. <i>Barracudia notabilis</i> n. sp., the first representative of this genus from fishes in the Atlantic Ocean, can be differentiated from other congeners by the body length of the male (2.69 mm), the length of the gubernaculum (57 μ m) and spicules (81 μ m), and by the shape of the gubernaculum. <i>Philometra consimilis</i> n. sp. differs from all congeners mainly in the unique structure of the distal tip of the gubernaculum (bearing two smooth dorsal barbs) and the species is also characterized by the length of spicules (111 μ m) and the length of the gubernaculum (84 μ m). Further studies are needed to discover and describe so far unknown gravid females of <i>Barracudia</i> spp.
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Introduction

Nematodes of the dracunculoid family Philometridae Baylis et Daubney, 1926 represent a large group of important fish parasites many species of which occur in marine fishes. The taxonomy of these histozoic parasites is complicated by their marked sexual dimorphism in which females are highly modified and considerably larger than the males. In contrast to conspicuous, large-sized and often red-coloured philometrid females, conspecific minute males pass usually unnoticed during routine examinations of fish and that is why the males of many philometrids remain unknown (Moravec, 2006).

Needlefishes (Beloniformes: Belonidae) have frequently been reported as hosts of philometrid nematodes. They are small to medium sized, torpedo-shaped, predominantly piscivorous, surface-oriented species prized in some areas as food fish or bait for large offshore predators like marlin (Istiophoridae) (Collete, 2016). The common name for the family is derived from members' needle-like toothy beaks. Moravec *et al.* (2021) recently provided a review of belonid-infecting philometrids globally, and described as new two philometrid species infecting the Atlantic needlefish *Strongylura marina* (Walbaum) and the redfin needlefish *Strongylura notata* (Poey) from estuarine waters along the Gulf of Mexico coast of Florida, USA. The field collections of Moravec *et al.* (2021) also yielded specimens of hound needlefish *Tylosurus crocodilus* (Péron et Lesueur) that were hosts to the philometrid species described herein. *Tylosurus crocodilus*, known locally as houndfish (Page *et al.*, 2013), is primarily considered a coastal species but is also found offshore (Collete, 2016) through much of the tropical and temperate oceans of the world (Froese & Pauly, 2021). It is

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Fig 1. Map of Tampa Bay, Florida, USA, showing three collection localities for hosts, *Tylosurus crocodilus*, found to be infected by *Philometra consimilis* n. sp. or both that species and *Barracudia notabilis* n. sp.

large among belonids, reaching lengths in excess of 1 m, and it is notable for being traumatogenic. It has caused serious, even fatal, puncture wounds in humans owing to its shape and habit of leaping horizontally over the water surface at high speeds (Manh *et al.*, 2020).

Regarding previous records of philometrids from *T. crocodilus*, Nikolaeva and Parukhin (1968) recorded gravid females of *Philometra* sp. from the swimbladder of this host species (reported as *Strongylura raphidoma*) in the Gulf of Mexico, Moravec and Ali (2005) the females of *Philometra tylosuri* Moravec et Ali, 2005 from the subcutaneous tissue and musculature of *T. crocodilus* in the Persian Gulf (off Iraq), Moravec and Justine (2009) the males of *Philometra dentigubernaculata* Moravec et Justine, 2009 from the oculo-orbits of *T. crocodilus* from off New Caledonia, and Moravec *et al.* (2012) the gravid female of *Philometroides indonensiensis* Moravec, Walter et Yuniar, 2012 from the musculature of *T. crocodilus* from off Indonesia. Apparently, also the females reported as *Philometra* sp. from the musculature of this host species in the Philippines and Indonesia by Petersen *et al.* (1993) and Jacob and

Palm (2006), respectively, belonged to this species (see Moravec *et al.*, 2021); *Philometra* sp. was also listed as a parasite of *T. crocodilus* in the Persian Gulf off Al Qatif, Saudi Arabia by Mohamed *et al.* (2010).

Records of philometrids from other congeneric hosts include that of Linton (1907) reporting female specimens of *Philometra* sp. (misidentified as *Ichthyonema* [= *Philometra*] *globiceps* - see Moravec & Tedesco, 2015) from the musculature of *Tylosurus acutus* (Lacepède) from Bermuda and those of Moravec and Rohde (1992) reporting females of *Philometra kohnae* Moravec et Rohde, 1992 and *P. Iomi* Moravec et Rohde, 1992 from the subcutaneous tissues of *Tylosurus gavialoides* (Castelnau) from off Australia.

Materials and Methods

Fish were collected in lower Tampa Bay (Fig. 1) during routine fish population monitoring conducted by the Florida Fish and Wildlife Conservation Commision' Fish and Wildlife Research Institute (FWRI). Haul seines, 183 m in length, were deployed along the



Fig. 2. Barracudia notabilis n. sp. from T. crocodilus, male. A – anterior end of body, lateral view; B – cephalic end, apical view; C, D – caudal end, lateral and ventral views, respectively; E – gubernaculum, lateral view; F – caudal end, apical view

shoreline from net wells of mullet skiffs (see Wessel & Winner, 2003). Captured fish were bagged in plastic and immediately packed in ice until crews returned at the end of field days to FWRI Headquarters in St. Petersburg Florida for further processing (time on ice < 12 hrs).

In the laboratory, fish were measured for standard length, and examined for parasites. Gonad-infecting philometrids were surveyed and fixed for morphological study in 5 % formalin by methods of Moravec *et al.* (2021). So fixed, specimens were shipped by commercial courier to the Institute of Parasitology, Biology Centre of the Czech Academy of Sciences, České Budějovice, Czech Republic for microscopic evaluation and description.

For light microscopical examination, the nematodes were cleared by use of glycerine. Drawings were made with the aid of a Zeiss drawing attachment. Specimens used for scanning electron microscopy (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 with 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. The fish nomenclature adopted follows FishBase (Froese & Pauly, 2021).

Ethical Approval and/or Informed Consent

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

Results

Family Philometridae Baylis et Daubney, 1926

Barracudia notabilis n. sp.

(Figs. 2 – 4)

Description: Male (1 specimen, holotype): Body filiform, whitish, 2.69 mm long, maximum width 36; anterior part of body somewhat narrowed towards cephalic extremity (Fig. 2A). Maximum width/ body length ratio 1:18. Cuticle smooth. Cephalic end rounded, 27 wide. Oral aperture small, circular. Fourteen minute cephalic papillae arranged in 2 circles present: external circle formed by 4 submedian pairs of papillae; internal circle by 4 submedian and 2 lateral papillae. Small lateral amphids just posterior to lateral papillae of internal circle (Figs. 2B, 3A,B). Oesophagus 432 long, comprising 16 % of body length, with inflation at anterior end measuring 27 × 12; posterior part of muscular oesophagus overlapped by well-developed oesophageal gland with large cell nucleus; maximum width of gland 18 (Fig. 2A). Small ventriculus present, its length 6, width 9. Nerve ring and oesophageal nucleus 96 and 267 from anterior extremity, respectively (Fig. 2A). Excretory pore 162 from anterior end. Testis extending anteriorly approximately to level of oesophago-intestinal junction, overlapping posterior portion of oesophagus (Fig. 2A). Posterior end of body blunt, 39 wide, provided with broad U-shaped mound situated laterally and dorsally to cloacal opening (Figs. 2F, 3C,D). At least 2 adanal pairs of very flat, hardly visible caudal papillae present between ventral ends of cloacal aperture and caudal mound (Figs. 2F, 3C). Pair of small phasmids present dorsoventrally on caudal mound (Figs. 2F, 3C,D). Caudal mound with pair of minute circular depression (Figs. 2F, 3C,D). Cloacal aperture oval, markedly dorsoventrally elongated (Figs. 2F, 3C). Spicules sickle-shaped in lateral view, equally long, with somewhat expanded proximal and sharply pointed, ventrally oriented distal tips (Fig. 2C,D, 4); in ventral view, middle portion of spicules markedly broad, 9 wide (Fig. 2D); length of spicules 101, representing 3 % of body length. Gubernaculum 57 long, with narrow, weakly-sclerotized tubular proximal portion; distal portion of gubernaculum broad, heavily sclerotized, wedge-shaped in lateral view, its distal tip rounded, dorsally curved (Fig. 2C,E, 4). Length ratio of gubernaculum and spicules 1:1.42. Spicules and gubernaculum well sclerotized, brownish (Fig. 4). Female: Not known.

Taxonomic summary

Type host: Hound needlefish *Tylosurus crocodilus* (Péron et Lesueur) (Belonidae, Beloniformes); 503 mm (SL).

Site of infection: Ovary.

Type locality: Tampa Bay, Florida, Rattlesnake Key at mouth of Terra Ceia Bay (27.5474, -82.633250), USA (collected 7 November 2017).

Prevalence and intensity: 1 fish infected/5 fish examined); 1 nematode.

Type specimen: Male holotype (mounted on SEM stub) in the Helminthological Collection of the Institute of Parasitology, BC CAS, České Budějovice, Czech Republic (Cat. No. N–1229).

Etymology: The specific name *notabilis* (= remarkable, notable) is a Latin adjective that refers to the unusual shape of the gubernaculum.

Remarks

In having ventrally curved, sickle-shaped spicules, the distal tip of the gubernaculum oriented dorsally and the cloacal aperture markedly dorsoventrally elongate, this nematode belongs to the philometrid genus *Barracudia* Moravec et Shamsi, 2017. To date, only two species of *Barracudia* are known, both parasitizing barracudas (Sphyraenidae): *B. australiensis* Moravec et Shamsi, 2017 from the gall-bladder of *Sphyraena obtusata* Cuvier off Australia and *B. philippinensis* (Quiazon et Yoshinaga, 2013) from the ovary of *Sphyraena forsteri* Cuvier off the Philippines (Quiazon & Yoshinaga, 2013; Moravec & Shamsi, 2017).

Barracudia notabilis n. sp. differs from two other congeners mainly in the shape and structure of the gubernaculum (proximal portion of the gubernaculum narrow, almost straight, posterior portion conspicuously broad, wedge-shaped in lateral view, with a narrowed,



Fig. 3. Barracudia notabilis n. sp. from T. crocodilus, scanning electron micrographs of male. A, B – cephalic end, lateral and apical views, respectively (arrows indicate amphids); C, D – caudal end, sublateral and dorsal views, respectively (arrow indicates phasmid).

Abbreviations: a – pair of cephalic papillae of outer circle; b – submedian cephalic papilla of inner circle; c – lateral cephalic papilla of inner circle; d – caudal mound; e – two adanal papillae; f – cuticular depression; o – oral aperture

smooth, dorsally curved distal tip vs. proximal end of the gubernaculum narrowed, strongly curved dorsally, distal tip smooth, laterally expanded, with a minute terminal digital projection in *B. australiensis* or proximal end of the gubernaculum rather broad, slightly dorsally bent, distal tip with a reflected dorsal barb and lamellate structure in *B. philippinensis*). It also differs in the male body length (2.69 mm vs. 5.02 mm or 3.05 - 3.78 mm), the length of the gubernaculum and spicules (gubernaculum 57 µm vs. 63 µm or 90 – 103 µm; spicules 81 µm vs. 108 µm or 82 – 88 µm) and in the gubernaculum/spicules length ratio (1:1.42 vs. 1:1.71 or 1:1.06 – 1.24). The new species also differs in the family and order of fish hosts (Belontidae, Belontiformes vs Sphyraenidae, Perciformes) and in the geographical region (Atlantic Ocean vs South Pacific Ocean or South China Sea).

Worth noting, however, is that three sphyraenids occur sympatrically with *T. crocodilus* in the Gulf of Mexico, *Sphyraena barracuda* (Edwards), *S. borealis* DeKay and *S. guachancho* Cuvier (Russell, 2002); these likely prey on the same potential intermediate host species, leaving open the intriguing possibility that members of *Barracudia*, including the new species, are typically parasites of sphyraenids and that *T. crocodilus* is a paradefinitive host. Reproducing females of *Barracudia notabilis* must be found to test this hypothesis, and special care would be warrented in identification or first description of any philometrids that might be collected in the future from Western Atlantic *Sphyraena* spp.



Fig. 4. Caudal end of Barracudia notabilis n. sp., lateral view

Philometra consimilis n. sp. (Figs. 5, 6)

Description: Male (6 specimens; measurements of holotype in parentheses): Body filiform, whitish, 2.19 – 3.81 (3.44) mm long, maximum width at middle of body 33 - 48 (48); anterior part of body tapering to anterior extremity, with slightly outlined constriction just posterior to cephalic end (Fig. 5A). Maximum width/body length ratio 1:66 - 94 (1:72). Cuticle smooth. Cephalic end rounded, 21 - 33 (33) wide, width at cephalic constriction 18 - 30 (30). Small oral aperture surrounded by 14 cephalic papillae arranged in 2 circles and pair of amphids, as in males of all other philometrid species (cephalic ends used for SEM proved to be very damaged and unsuitable for illustrations). Oesophagus 282 - 441 (441) long, comprising 7 - 13 (13) % of body length, with slight inflation at anterior end measuring $21 - 36 \times 12 - 21$ (36×21); posterior part of muscular oesophagus overlapped by well-developed oesophageal gland with large cell nucleus; maximum width of gland 15 – 21 (21) (Fig. 4A). Ventriculus small, 6 – 15 (15) long,

9 - 18 (18) wide. Nerve ring and oesophageal nucleus 126 - 198 (198) and 201 - 327 (327), respectively, from anterior extremity. Excretory pore 171 - 216 (216) from anterior end. Posterior end of body blunt, 30 - 45 (45) wide, with broad caudal mound formed by 2 lateral reniform portions well separated dorsally (Figs. 5D, 6A,B). Four pairs of small adanal papillae present in space between caudal mound and cloacal aperture: 2 pairs of small, markedly elevated papillae situated closely to cloacal aperture and 2 pairs of very flat, hardly visible papillae located externally to them; pair of small phasmids present dorsoventrally on caudal mound (Figs. 5D, 6B - F). Each portion of caudal mound with 1 subdorsal, minute circular depression (Figs. 5D, 6B). Spicules needle-like, equally or nearly equally long, with somewhat expanded proximal and sharply pointed distal tips (Fig. 5B,C); length of spicules 105-117 (117), maximum width 6 (6); length ratio of spicules 1:1.00-1.03 (1:1.00); spicules representing 2.9 - 4.8 (3.4) % of body length. Gubernaculum narrow, 78 - 90 (90) long, with anterior portion somewhat dorsally bent; length of anterior bent part 45 - 57 (48), comprising 53 - 63 (53) % of entire gubernaculum length (Fig. 5B,C,E - G).



Fig. 5. *Philometra consimilis* n. sp. from *T. crocodilus*, male. A – anterior end of male, lateral view; B, C – posterior end of male, lateral and ventral views, respectively; D – caudal end, apical view; E – gubemaculum, lateral view; F, G – distal tip of gubemaculum, lateral and subdorsal views, respectively

In lateral view, distal tip of gubernaculum with 2 smooth, reflexed dorsal barbs (1 large and 1, more distal barb small) and with several transverse, dorsally uninterrupted lamellae in region anterior to barbs; proper extremity of gubernaculum posterior to barbs rounded (Figs. 5F,G, 6C – F). Length ratio of gubernaculum and spicules 1:1.23 – 1.35 (1:1.30). Spicules and gubernaculum well sclerotized, yellowish, anterior part of gubernaculum and proximal ends of spicules colourless.

Female: Not known.

Taxonomic summary

Type host: Hound needlefish *Tylosurus crocodilus* (Péron et Lesueur) (Belonidae, Beloniformes); 503 mm (SL).

Site of infection: Ovary.

Type locality: Tampa Bay (27.632516, -82.574166), Gulf of Mexico, USA (collected 9 December 2019).

Other locality: Tampa Bay (27.588783, -82.602266; 27.5474, -82.633250), Gulf of Mexico, USA (collected 15 September and 7 November 2017).

Prevalence and intensity: 4 fish infected/5 fish examined; 1 - 4 nematodes per fish.

Type specimens: Holotype and 2 paratypes in the Smithsonian National Museum of Natural History, Washington, USA (Cat. Nos. USNM 1640967 and USNM 1640968, respectively); 2 paratypes in the Helminthological Collection of the Institute of Parasitology, BC CAS, České Budějovice, Czech Republic (Cat. No. N–1230). Etymology: The specific name *consimilis* (= much like) is the Latin adjective relating to the fact that this species is very similar to *Philometra aequispiculata* Moravec, Bakenhaster, Seyoum et Tringali, 2021, a recently described gonad-infecting species parasitizing *Strongylura* spp. in the Gulf of Mexico (Moravec *et al.*, 2021).

Remarks

The males of this new species are morphologically and biometrically very similar to those of *P. aequispiculata*, but differ in the longer gubernaculum (78 – 90 μ m vs. 60 – 69 μ m) and a differnt length ratio of the gubernaculum and spicules (1:1.23 – 1.35 vs. 1:1.50 – 1.67). However, the main difference is in the structure of the distal gubernaculum tip: whereas that of *P. consimilis* n. sp. possesses two smooth dorsal barbs, the larger barb of *P. aequispiculata* bears three distinct lateral transverse grooves and there are two uninterrupted lamellar structures posterior to it. Moreover, the fish hosts of these species belong to different genera (*Tylosurus* Cocco vs Strongylura van Hasselt).

One male of *P. consimilis* n. sp. was collected from the ovary of the same specimen of *T. crocodilus*, in which also a single specimen of *Barracudia notablilis* n. sp. was found.

Discussion

While the morphology of P. consimilis n. sp. is typical of gonad-in-

fecting species of *Philometra* Costa, 1845, the finding of a representative of another philometrid genus, *B. notabilis* n. sp., in the ovary of *T. crocodilus* is remarkable, especially in a co-infection with *P. consimilis*. It should be noted that all hitherto described species of *Barracudia* are known only by males and juvenile females. Whereas the specimens of *B. philippinensis* and *B. notabilis* n. sp. were found in the hosts' ovary, those of *B. australiensis* were recovered from the host's gall bladder.

This indicates a possibility that the location of large, subgravid and gravid females of *Barracudia* spp. in the host is other than fish gonads. In fact, *T. crocodilus* is reported as the host of *P. tylosuri*, a species described from a single nongravid female from the fish musculature in the Persian Gulf, or of *Philometra* sp. based on females found in the musculature or the swimbladder from off the Philippines and Indonesia and in the Gulf of Mexico (see above). Since the males of these nematodes remain unknown and the genus *Barracudia* was based solely on male morphology, it cannot be excluded that at least some of these philometrid females might belong to *Barracudia*. Therefore, new studies in this respect are needed to elucidate this problem.

The present description of *P. consimilis* n. sp. demonstrates again the importance of detailed studies of male morphology for the distinction between similar species of philometrids. It is believed that subsequent studies on the fauna of philometrids parasitizing fishes in the Gulf of Mexico will enable the discovery and description of the hitherto unknown gravid females of *P. consimilis* n. sp.

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Fig. 6. *Philometra consimilis* sp. n. from *T. crocodilus*, scanning electron micrographs of male. A, B – caudal end, lateral and apical views, respectively; C, D – enlarged region of cloaca, apical and sublateral views, respectively; E, F – distal tip of gubernaculum, lateral and subdorsal views, respectively. *Abbreviations*: a – two flat adanal papillae; b – two elevated adanal papillae; d – caudal mound; e – cuticular depression; g – gubernaculum; s – spicule

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Conflict of Interest

The authors declare that they have no conflict of interest.

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