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Gonad-infecting philometrid *Philometra philippinensis* sp. nov. (Nematoda, Philometridae) from the bigeye barracuda *Sphyraena forsteri* Cuvier (Sphyraenidae) off Mariveles, Bataan Province, Philippine archipelago

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Abstract

We conducted a parasitological study to identify nematodes infecting the gonads of bigeye barracuda *Sphyraena forsteri* Cuvier (Sphyraenidae) caught in the West Philippine Sea, off the Mariveles coast, Bataan Province, the Philippine archipelago. Males and very tiny nongravid female philometrid nematodes were collected from the ovaries of the fish. Based on light and scanning electron microscope observations, and supported by molecular analyses of the partial nucleotide sequences of the 18S small subunit ribosomal DNA, the philometrid nematode was determined to be a new species belonging to the genus *Philometra* Costa, 1845 in the family Philometridae (Nematoda: Dracunculoidea), and was named *P. philippinensis* sp. nov. This new species was clearly distinguishable morphologically from other congeners that parasitize the gonads of marine fishes. Males have unique sickle-shaped spicules and gubernaculum, and a vulva-like cloacal opening. Following *Dentirumai philippinensis* Quiazon et Moravec, 2013 and *Philometra robusta* Moravec, Möller et Heeger, 1992, this is the third philometrid species and the first gonad-infecting philometrid from the Philippine archipelago.

Keywords

Philometridae, 18S small subunit rDNA, gonad-infecting philometrid nematodes, *Philometra philippinensis*, *Sphyraena forsteri*, Philippine archipelago

Introduction

Nematodes of the spirurid superfamily Dracunculoidea Stiles, 1907 represent a large and diverse group of parasites that are distributed throughout the world (Moravec 2006). Among these are the philometrid nematodes belonging to the genus *Philometra* Costa, 1845. This genus includes a large number of species that parasitize the organs and cavities of a range of host fishes. To date, a total of 147 philometrid species parasitizing freshwater, brackish-water, and marine fishes have been identified (Moravec and de Buron 2013). Among these are 35 species that infect the body musculature and subcutaneous tissues and 32 species that primarily infect gonadal tissue (Moravec 2006; González-Solís *et al.* 2007; Moravec and Salgado-Maldonado 2007; Moravec *et al.* 2008a, b; Moravec and de Buron 2009; Moravec *et al*. 2010a, b, 2011a, b, 2012; Quiazon *et al*. 2008a, b, 2010; Moravec and Bakenhaster 2010; Quiazon and Moravec 2013).

Gonad-infecting philometrid nematodes are nonzoonotic parasites. Despite this, they seem to have a moderate to severe effect on fish reproduction (Sakaguchi *et al.* 1987; Kåll *et al.* 2004; Clarke *et al.* 2006; Moravec 2006; Deepthi *et al.* 2007; Moravec and Salgado-Maldonado 2007; Quiazon *et al*. 2008b; Perez *et al*. 2009; Radhakrishnan *et al.* 2010; Séguin *et al.* 2011). The ability to accurately identify these parasites is important for developing a full understanding of their biological and infection dynamics, and can also aid development of easy diagnosis, treatment, and control measures. To date, researchers have attempted to use some of the species in this group as temporary and/or permanent biological markers to study population and stock structure in marine fishes (Zischke *et al.* 2009; Hutson *et al.* 2011) and as

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potential sentinel parasites for heavy metal accumulation (Baruš *et al.* 2007).

The Philippines, an archipelagic country surrounded with vast marine fishery resources, is considered to be the center of marine fish biodiversity worldwide (Carpenter and Springer 2005). Despite this, there is very little information on the parasites, particularly the philometrid nematodes, that infect marine fishes in this region. To date, there have only been three reports on philometrid species in the Philippines, wherein only two species are currently recognized as valid, *Philometra robusta* Moravec, Möller et Heeger, 1992 and *Dentirumai philippinensis* Quiazon et Moravec, 2013 (Moravec *et al.* 1992; Quiazon and Moravec 2013). Although the impacts of philometrid nematodes on the fisheries industry cannot be ignored (Moravec 2004, 2006; Quiazon *et al*. 2008a, b, 2010; Portes Santos and Moravec 2009), there is still limited information on their species distribution, diversity, and infectivity, particularly in regions such as the Philippine archipelago (Quiazon and Moravec 2013). To address this gap in knowledge, we conducted a parasitological investigation for gonad-infecting philometrid nematodes in the ovaries of bigeye barracuda *Sphyraena forsteri* Cuvier (Sphyraenidae). As a result, we collected male and tiny nongravid female philometrid nematodes. Subsequent detailed morphological and molecular studies on the samples revealed that these gonad-infecting nematodes represent a new species.

Materials and Methods

Morphological studies. Male and nongravid female nematodes were collected from the ovaries of bigeye barracuda (*Sphyraena forsteri* Cuvier). The fish were captured in the West Philippine Sea, off the Mariveles coast, Bataan Province, Philippines (14° 21.51′N; 120° 26.27′E) in August 2011 (Fig. 1). The parasites were collected by opening and sectioning the ovary, shaking the pieces of tissue, then washing in physiological saline solution (PSS) in Petri dishes. The males were collected from the dishes using fine needles, washed again with fresh PSS, and fixed in 70% or absolute (99.5%) ethanol for morphological or molecular examination, respectively. The morphology was examined under both a light microscope (LM) and a scanning electron microscope (SEM). During LM examination, the whole body of the 70% ethanol-fixed specimens was cleared in glycerin, and individually mounted on glass slides. Some morphological features that were difficult to observe by LM were examined using SEM. Briefly, the 70% ethanol-fixed specimens were post-fixed in 1.2–1.5% glutaraldehyde, post-fixed in 1% osmium tetroxide, and dehydrated through a series of ascending ethanol concentrations. Samples were subjected to three changes of absolute butyl alcohol and freeze-dried. The freeze-dried samples were subsequently sputter-coated with gold and observed under a scanning electron microscope (SEM S-4000, Hitachi, Japan). Drawings of the nematodes were made with the aid of an Olympus photomicroscope and computer software (CorelDRAW® Graphics Suite – Version 12.0). The male holotype, paratypes, and allotype of the new nematode species were deposited at the Meguro Parasitological Museum, Tokyo (M.P.M. Coll. Nos. 20898–20900). All measurements are in micrometres unless otherwise stated. The fish name follows FishBase (Froese and Pauly 2013).

Molecular studies. Genomic DNA was extracted from three 99.5% ethanol-fixed male individuals using a DNeasy™ Tissue Kit (Qiagen, Hilden, Germany) (protocol for animal tissues). To amplify the 18S small subunit (SSU) ribosomal DNA gene, we used the forward primer D-1F (GCCTATAATGGTGAAACCGCGAAC) (Wijová *et al.* 2006) and a designed reverse primer SSU1300R (TCACTCCACCAACTAAGAACGG). The PCR assay was performed with 1 µL sample DNA (template) in a total volume of 20 µL which contained 0.6 µL forward and reverse primer, 14.1 µL DDW, and 3.7 µL Taq mix (containing 0.1 µL Takara Ex Taq™ HS; 2 µL [10×] Ex Taq Buffer; and 48 µL dNTP mixture) (Takara Bio Inc., Otsu, Japan). PCR was performed using an iCycler™ (Bio-Rad Laboratories, Tokyo, Japan) following the method in previous report (6 cycles of 95°C for 1 min, 44°C for 1 min, and 72°C for 2 min, followed by 24 cycles in which the annealing temperature was increased to 48°C) (Wijová *et al.* 2006). All sequencing reactions were carried out using the BigDye terminator kit version 3.1 and resolved with ABI 3730XL genetic analyzer (Applied Biosystems, Japan). Molecular analyses of the partial nucleotide sequences were carried out using BioEdit version 7.0.4.1 (Hall 1999) and MEGA 4 (Tamura *et al.* 2007). The phylogenetic tree (Neighbor-Joining, Kimura 2-parameter model, bootstrap, complete deletion) was generated using the latter software. The partial sequences were deposited and made available in GenBank under accession numbers KC342903– KC342905.

Results

Morphological Studies

***Philometra philippinensis* sp. nov.** (Figs 2, 3)

Description: Male (10 specimens; holotype; measurements of paratypes in parentheses). Body filiform, whitish, tapering at rounded posterior end; body tapers from mid-portion of body to anterior end near nerve ring area and slightly bulges at anteriormost end. Body 3.55 (3.05–3.78) mm long; maximum width 60 (45–62); representing 1.69% (1.2–2.0%) of the body length; minimum width at nerve ring area 32 (30–38); width of cephalic end 48 (30–45), of caudal end 49 (38–50). Cuticle smooth. Cephalic end rounded, cephalic papillae very small, indistinct when viewed laterally. Oral aperture small, (2.5–3.0) in diameter, surrounded by 14 small cephalic papillae arranged in two circles and large lateral amphids; internal circle consisting of four submedian and two lateral single

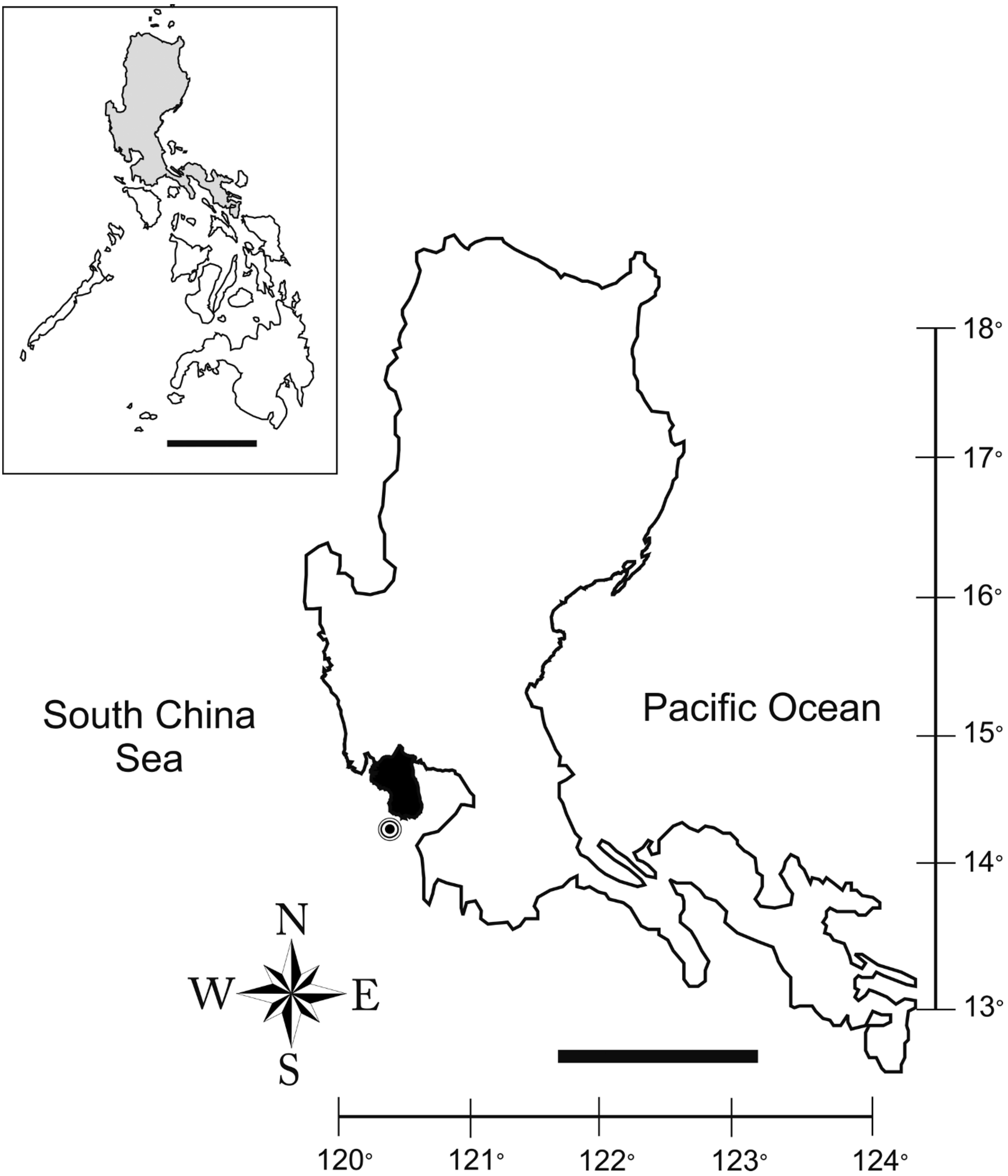

Fig. 1. Luzon Island in the Philippine archipelago (inset), illustrating the location of the fishing ground off Bataan Province where specimens of *P. philippinensis* sp. nov. were collected from bigeye barracuda *Sphyraena forsteri*. Gray shade-main Luzon Island; black shade-Bataan Province. (Scale bar: Inset = 200 miles; Luzon Island = 100 miles)

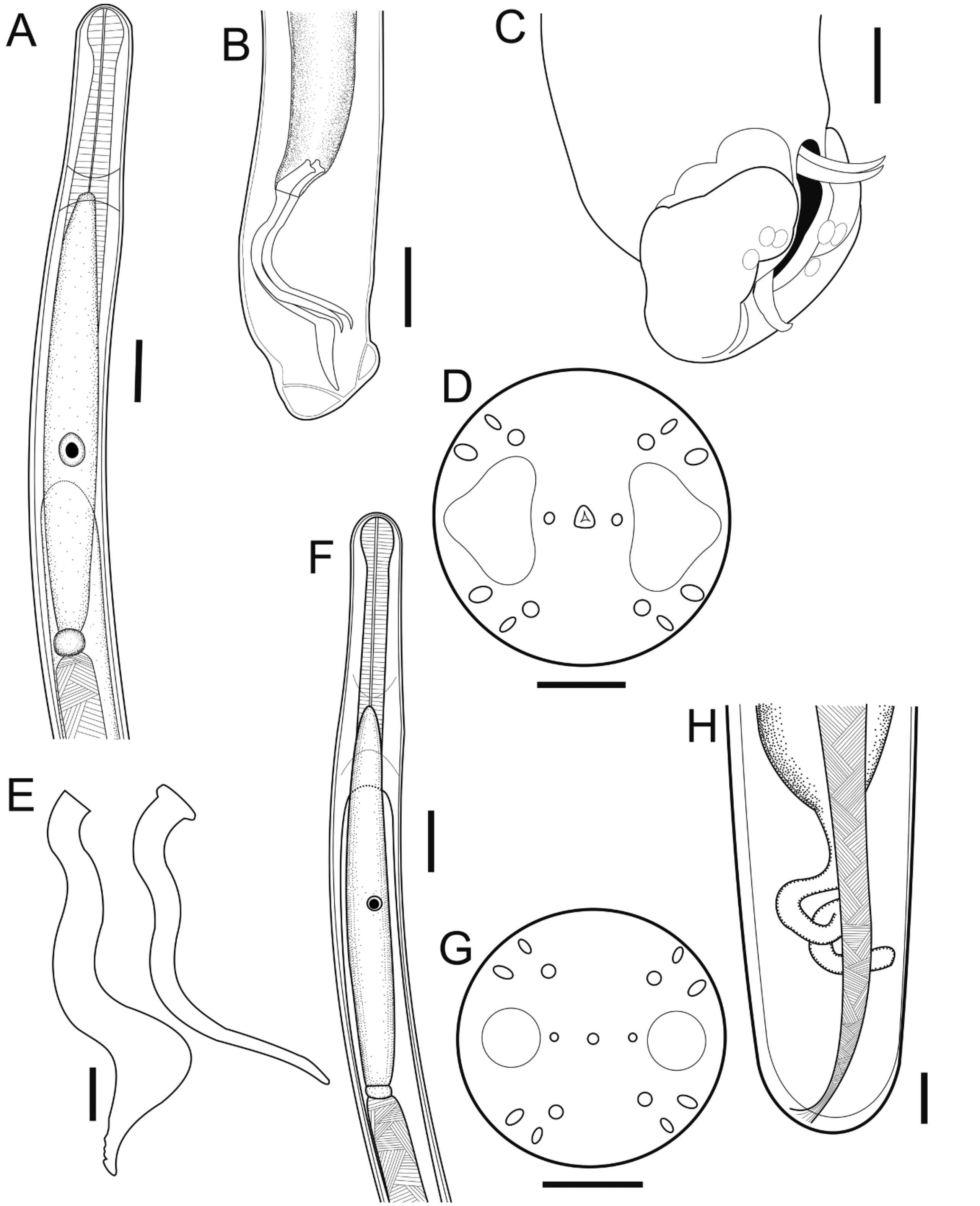

Fig. 2. *Philometra philippinensis* sp. nov. **A** – anterior end of male, lateral view; **B** – posterior end of male, lateral view; **C** – closer view of the posterior end of male showing vulva-like cloacal opening for the protrusion of the sickle-shaped spicules and gubernaculum; **D** – cephalic end of male, apical view; **E** – sickle-shaped gubernaculum (left) and spicule (right); **F** – anterior end of nongravid female, lateral view; **G** – cephalic end of nongravid female, apical view; **H** – posterior end of nongravid female, lateral view. Scale bars = 50 µm (A, F), 30 µm (B), 10 µm (C, D, E, G, H)

papilla; external circle consisting of four pairs of submedian papillae. Oesophagus narrow, 355 (325–400) long, representing 10% (9.4–13%) of the body length, with inflation at the anterior end (length 33 (28–33); width 28 (18–28)); length of anterior section of oesophagus 145 (130–175), that of posterior section overlapped by oesophageal gland 210 (200–270); minimum width of oesophagus at nerve ring 22 (10–20); maximum width in middle of oesophageal gland 28 (22–32); posterior portion of oesophagus with well-developed, long oesophageal gland with large cell nucleus at middle 10 (10–12.5) in diameter. Oesophageal nucleus and nerve ring 280 (250–280) and 125 (125–170), respectively, from anterior extremity. Excretory pore 125 (175–225) from anterior end of body. Small ventriculus present, 11–15 long and 13–15 wide. Intestine narrow, connected with genital tube to form the vulva-like cloacal opening (Figs. 2B, C, 3D–F). Posterior end of body blunt, with broad, U-shaped, lobular mound and hardly visible caudal papillae. Spicules yellowish-brownish, slender, bent, sickle-shaped (Figs 2B, E, 3G), equally long, with somewhat expanded proximal end and sharply pointed distal tip; length of spicules 82 (82–88), representing 2.3% (2.2–2.8%) of the body length; spicule width at proximal end 5.9 (5.6–6.5); spicule width at middle 4.1 (4.1–5.0). Gubernaculum narrow, 102 (90–103) long, sickle-shaped with approximately its proximal half dorsally bent and relatively widened, 12 (10–12), followed by tapering to distal end (Figs 2B, E, 3G); length of anterior bent part 26 (25–29), representing 26 (26–28)% of the entire gubernaculum length; width of gubernaculum at proximal end 9 (8–9); distal tip with distinct reflected dorsal barb and lamellate-like structure. Length ratio of gubernaculum and spicule 1:1.24 (1:1.06–1.23). Spicule and gubernaculum well sclerotized.

Nongravid female (10 specimens): body of live and fixed specimens whitish, somewhat tapering at rounded posterior end; body tapers from midportion of body to anterior end near nerve ring area and body slightly bulges towards anterior end as in males. Body 2.78–4.00 mm long, its maximum width at middle 55–85; minimum body width at nerve ring area 40–60. Cuticle smooth. Cephalic end rounded, 38–50 wide; cephalic papillae very small, indistinct when viewed laterally. Oral aperture small, 1.0–1.2 in diameter, surrounded by 14 small cephalic papillae arranged in two circles and slightly outlined lateral amphids; internal circle consisting of four submedian and two lateral single papilla; external circle consisting of four pairs of submedian papillae. Oesophagus including anterior bulbous inflation 460–600 long, representing 12–22% of the body length; bulb 32–42 long and 25–30 wide; muscular oesophagus posterior to bulb and not covered by oesophageal gland 100–155 long; length of posterior part overlapped by oesophageal gland 305–500; minimum width of oesophagus at nerve ring area 18–22; maximum width at midportion of the oesophageal gland, 20–40. Oesophageal gland marked, extending anteriorly from posterior end of oesophagus to near nerve ring area; large oesophageal gland nucleus 12–18 from anterior proximity. Nerve ring 132–175 from anterior end of body. Oesophagus opening into intestine through distinct valve. Small ventriculus present, 20 long and 12–22 wide. Intestine narrow, ending blindly; its posterior end attached to body wall near caudal end by narrow ligament. Vulva and anus absent. Ovaries long, narrow, reflected, situated near both body ends. Uterus empty. Posterior end of the body rounded.

Type host: Bigeye barracuda, *Sphyraena forsteri* Cuvier, 1829 (Sphyraenidae, Perciformes) (SL 55–70 mm).

Site of infection: Ovary.

Type locality: West Philippine Sea, off Mariveles coast, Bataan Province, Philippines (14°21.51´N; 120°26.27´E) (collected August 2011).

Prevalence and intensity: 40% (10 fish infected/25 fish examined); 1–10 worms per fish. Deposition of type specimens: Male holotype, paratypes, and allotype deposited at the Meguro Parasitological Museum, Tokyo (M.P.M. Coll. Nos. 20898–20900).

Etymology: The specific name *philippinensis* relates to the country near which the type locality of this philometrid is located.

Remarks: The male philometrids isolated from the ovaries of bigeye barracuda have two very unique morphological features that have never been described in any males to date; i.e., males have: (a) sickle-shaped spicules and gubernaculum (Figs 2B, E, 3G); and (b) a vulva-like cloacal opening capable of accommodating the protrusion of both sickle-shaped structures during copulation (Figs 2C, 3D-G). Further detailed morphological and biometric comparisons of the current male specimens with the reported gonad-infecting *P. sphyraenae* Moravec et Manoharan, 2013 from Pickhandle barracuda, *Sphyraena jello* Cuvier, a host fish of the same family (Sphyraenidae) (Moravec and Manoharan 2013), have revealed that they represent a new species. Major differences in males include the following: longer body length (2.07–2.76 vs 3.05–3.78 in the new species), broader caudal end (33–36 vs 38–50), a lower ratio of the oesophagus length in relation to the total body length (17–22 vs 9.4–13.0%), a shorter spicule (120–132 vs 82–88), a lower ratio of spicule length in relation to the total body length (4.7–6.0 vs 2.2–2.8%), a longer gubernaculum (66–75 vs 90–103), a lower ratio of the anterior bent part of the gubernaculum in relation to the total gubernaculum length (40–44 vs 26–28%) and lower length ratio of gubernaculum and spicules (1 : 1.68–1.87 vs 1 : 1.06–1.24).

Comparisons with other congeneric gonad-infecting philometrids reported to date (Moravec *et al.* 2011a) confirm their identity as a new species. Based on the total body length of males, the new species is most similar to *P. cephalus* Ramachandran, 1975, *P. genypteri* Moravec, Chávez et Oliva, 2011, *P. globiceps* (Rudolphi, 1819), *P. jordanoi* (López-Neyra, 1951), *P. lateolabracis* (Yamaguti, 1935); and *P. mira* Moravec et Justine, 2011 (Moravec 2006; Quiazon *et al.* 2008a; Moravec *et al.* 2011b; Moravec and Justine 2011). However, despite the similarity in the body size, a closer look at other biometric data reveals separate species. Although *P.*

cephalus has a similar range of spicule lengths, it differs in the following: a narrower body at the middle portion (30–40 vs 45–62 in the new species), a relatively shorter oesophagus (48–50 vs 325–400) and gubernaculum (76–81 vs 90–103). *Philometra genypteri* has a similar oesophagus length, ratio of the oesophagus length in relation to the parasite's total body

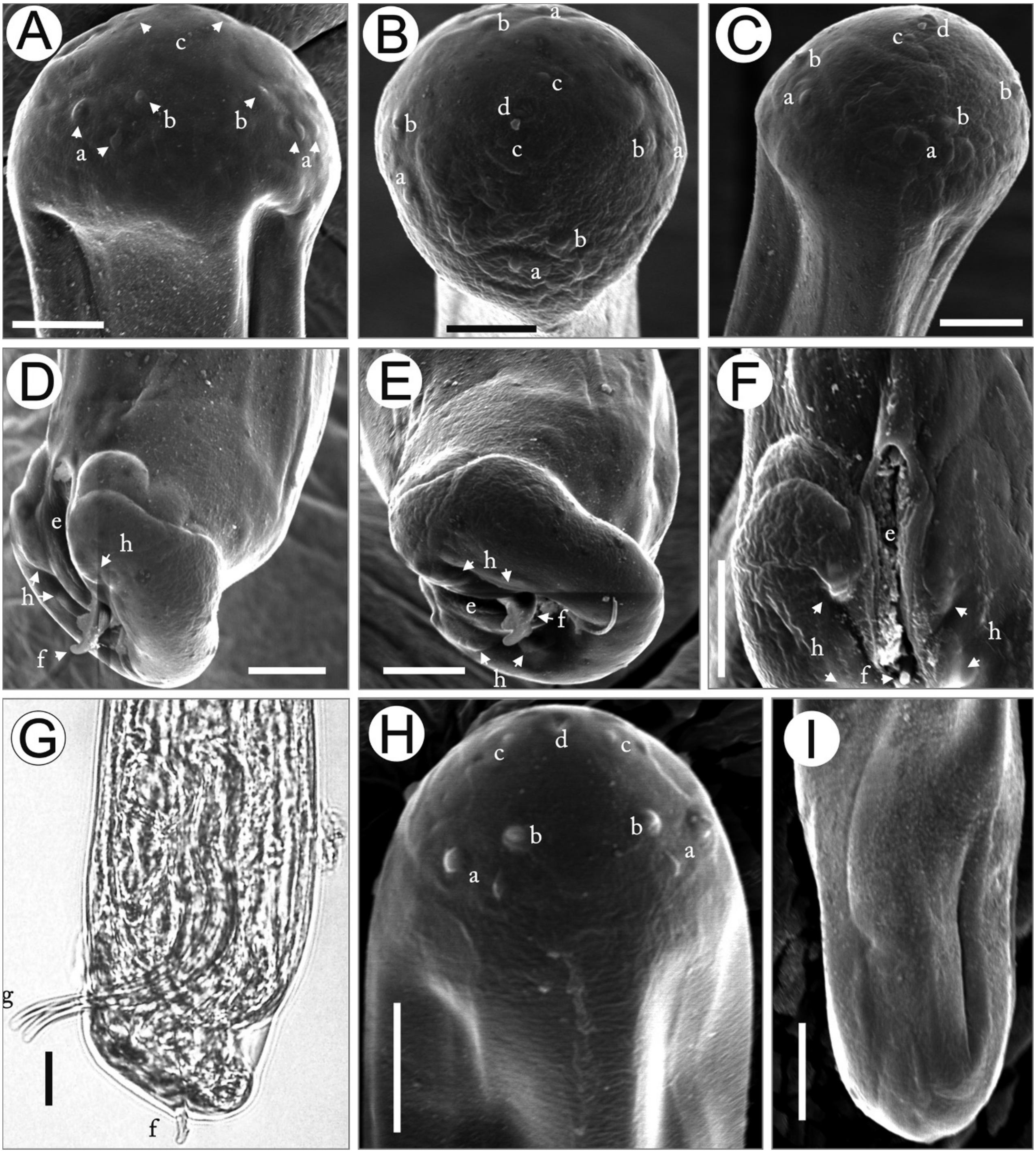

Fig. 3. *Philometra philippinensis* sp. nov., scanning electron micrographs (A–F, H, I) and light microscopic view (G): **A–C** – closer views of the anterior end of males showing the arrangement of the cephalic papillae; **D–F** – posterior end of males showing the vulva-like cloacal opening for the protrusion of sickle-shaped spicules and gubernaculum; **G** – posterior end of male showing the protruding sickle-shaped spicules and gubernaculum; **H** – anterior end of nongravid female showing the arrangement of the cephalic papillae; **I** – posterior end of nongravid female. **Abbreviations:** a – submedian pair of external cephalic papillae, b – submedian cephalic papilla of internal circle, c – lateral cephalic papilla of internal circle, d – mouth, e – vulva-like cloacal opening, f – gubernaculums, g – spicules, h – caudal papillae. Scale bars = 10 µm

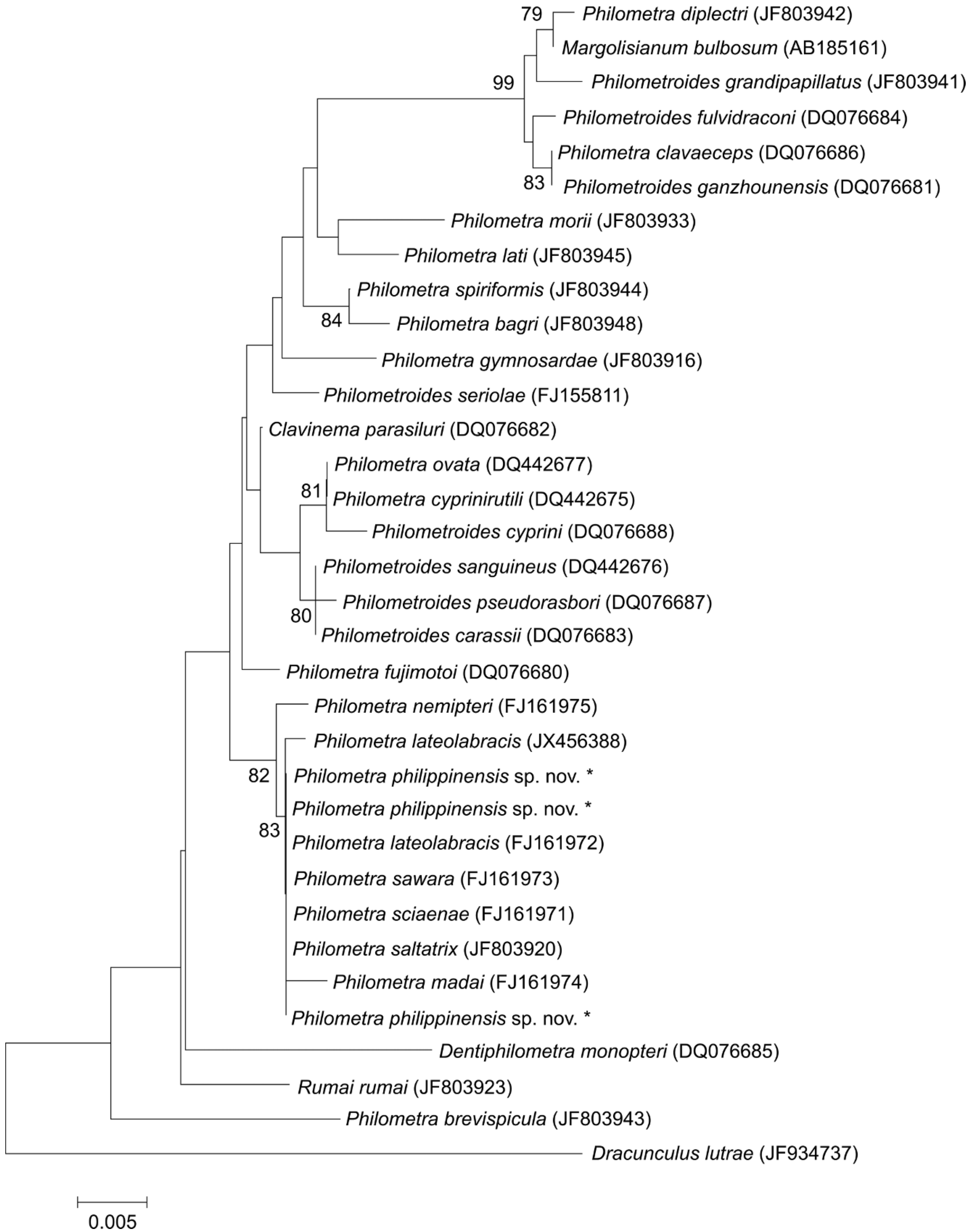

Fig. 4. Neighbor-Joining tree showing the phylogenetic position of *Philometra philippinensis* sp. nov. (with asterisks) among reported dracunculoid nematodes (Kimura 2-parameter model, bootstrap, complete deletion, MEGA4)

length, and distance of the oesophageal gland nucleus from the anterior extremity. However, there are differences between both species which include: a narrower body width (39–42 vs 45–62), longer spicules (126–135 vs 82–88), a higher ratio of the spicule length in relation to the total body length (4.0–4.3 vs 2.2–2.8%), a shorter gubernaculum (87 vs 90–103), a higher length ratio of the gubernaculum and spicules (1 : 1.45–1.55 vs 1 : 1.06–1.24), longer anterior bent part of the gubernaculum (33–39 vs 25–29), representing a higher ratio in relation to the total length of gubernaculum (38–45 vs 26–28%). *Philometra globiceps* has a similar size range for the oesophagus length, but differs substantially in the following: a broader body (80–88 vs 45–62), a shorter distance from the nerve ring to the anterior extremity (104 vs 125–170), longer spicules (137–156 vs 82–88), a shorter gubernaculum (44–78 vs 90–103), and a broader maximum width of the oesophagus overlapped by the oesophageal gland (88 vs 22–32). *Philometra jordanoi* has a similar range of body widths and lengths of the anterior part of the oesophagus, but differs in the following: having relatively longer spicules (265 vs 82–88), a shorter gubernaculum (84 vs 90–103), and a higher length ratio of the gubernaculum and spicules (1 : 3.15 vs 1 : 1.06–1.24). *Philometra lateolabracis*, as reported by Quiazon *et al.* (2008a), is similar in the range of body widths and lengths of the anterior part of oesophagus, but differs in the following: having a shorter distance of the nerve ring from the anterior extremity (30–116 vs 125–170), spicules slightly unequal, and a higher length ratio of the gubernaculum and spicules (1 : 1.30–2.02 vs 1 : 1.06–1.24). Last, *P. mira* differs from the new species in the following: having longer spicules (96–105 vs 82–88), a shorter gubernaculum (78–84 vs 90–103), and a larger inflation (bulb length) on the anterior end of the oesophagus (42–48 vs 28–33). Above all, the males of these six *Philometra* species have needle-shaped spicules and a small rounded cloacal opening (i.e., in contrast to the sickle-shaped spicules and vulva-like cloacal opening of the new species).

Currently, there are only two recognized philometrid species in Philippine waters. Moravec *et al.* (1992) reported *Philometra robusta* from the abdominal cavity of the map puffer *Arothron mappa* (Lesson) (Tetraodontidae), whereas Quiazon and Moravec (2013) reported *Dentirumai philippinensis* from the subcutaneous tissues (in the body musculature) of the loach goby *Rhyacichthys aspro* (Valenciennes) (Rhyacichthidae). Although Schmidt and Kuntz (1969) reported finding of *P. lateolabracis* in the tomato hind *Cephalopholis sonnerati* (Valenciennes) (Serranidae) and the Javan flounder *Pseudorhombus javanicus* (Bleeker) (Paralichthyidae) from Palawan, Philippines, the taxonomical identity was evidently wrong and is currently considered *Philometra* sp. (Quiazon *et al.* 2008a, Quiazon and Moravec 2013). Furthermore, Petersen *et al.* (1993) reported gravid *Philometra* sp. in the musculature of the hound needlefish *Tylosurus crocodilus crocodilus* (Péron et Lesueur) (Belonidae) in Philippine waters, which was later identified as belonging to the genus *Philometroides* (see Moravec *et al.* 2012). Also, based on the difference in habitat of the host fish (i.e., freshwater vs marine), the *Philometra* sp. isolated from an unspecified tissue of the Philippine catfish *Clarias batrachus*

Table I. Estimates of evolutionary divergence (Kimura 2-parameter model) and number of base differences (in parentheses) among congeneric gonad-infecting *Philometra* species (complete deletion, bootstrap, 1000 replicates, MEGA4)

***Philometra* species**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**GenBank Acc. Nos.**
1 *Philometra philippinensis*	–									KC342903*
2 *Philometra philippinensis*	0.003 (3)	–								KC342904*
3 *Philometra philippinensis*	0.003 (3)	0.000	–							KC342905*
4 *Philometra nemipteri*	0.002 (2)	0.005 (5)	0.005 (5)	–						FJ161975
5 *Philometra lateolabracis*	0.001 (1)	0.004 (4)	0.004 (4)	0.003 (3)	–					FJ161972
6 *Philometra lateolabracis*	0.002 (2)	0.005 (5)	0.005 (5)	0.004 (4)	0.001 (1)	–				JX456388
7 *Philometra sawara*	0.000	0.003 (3)	0.003 (3)	0.002 (2)	0.001 (1)	002 (2)	–			FJ161973
8 *Philometra sciaenae*	0.000	0.003 (3)	0.003 (3)	0.002 (2)	0.001 (1)	0.002 (2)	0.000	–		FJ161971
9 *Philometra saltatrix*	0.000	0.003 (3)	0.003 (3)	0.002 (2)	0.001 (1)	0.002 (2)	0.000	0.000	–	JF803920
10 *Philometra madai*	0.002 (2)	0.005 (5)	0.005 (5)	0.004 (4)	0.003 (3)	0.004 (4)	0.002 (2)	0.002 (2)	0.002 (2)	FJ161974

*Present study

Table II. Estimates of evolutionary divergence (Kimura 2-parameter model) and number of base differences (in parentheses) of *Philometra philippinensis* sp. nov. with other philometrid nematodes (complete deletion, bootstrap, 1000 replicates, MEGA4)

Philometrid nematodes	1	2	3	4	5	6	7	8	Sites of infection	GenBank Acc. Nos.
1 *Philometra philippinensis*	–								Gonad	KC342903*
2 *Philometra clavaeceps*	0.035 (28)	–							Gonad, abdominal cavity, swim bladder	DQ076686
3 *Philometroides fulvidraconi*	0.036 (29)	0.004 (3)	–						Orbit, swim bladder, abdominal cavity, gill cover	DQ076684
4 *Philometra morii*	0.031 (25)	0.031 (25)	0.035 (28)	–					Buccal cavity, sinuses	JF803933
5 *Philometra gymnosardae*	0.022 (18)	0.026 (21)	0.027 (22)	0.029 (23)	–				Body cavity	JF803916
6 *Philometroides seriolae*	0.020 (16)	0.027 (22)	0.029 (23)	0.025 (20)	0.012 (10)	–			Body muscles	FJ155811
7 *Clavinema parasiluri*	0.017 (14)	0.024 (19)	0.025 (20)	0.030 (24)	0.015 (12)	0.016 (13)	–		Operculum, gill arch, swim bladder, pleural cavity	DQ076682
8 *Rumai rumai*	0.024 (19)	0.033 (26)	0.034 (27)	0.035 (28)	0.030 (24)	0.027 (22)	0.022 (18)	–	Mouth, tongue, operculum, head	JF803923
9 *Dentiphilometra monopteri*	0.043 (34)	0.055 (43)	0.053 (42)	0.052 (41)	0.048 (38)	0.046 (36)	0.047 (37)	0.046 (36)	Abdominal cavity, mesentery	DQ076685

*Present study

(Linnaeus) (Clariidae) (Arthur and Lumanlan-Mayo 1997) is not identical with that found in the bigeye barracuda. Furthermore, among fishes of the family Sphyraenidae, Moravec (2006) reported *Philometra* sp. infecting an unspecified region of the body, based on data from Parukhin (1971), of the Japanese barracuda (*S. japonica* Bloch et Schneider) and the blackfin barracuda (*S. genie* Klunzinger). Since *P. philippinensis* is described from a congeneric host, it cannot be discounted that the above-mentioned *Philometra* sp. belonged to the same species.

Molecular Studies

Partial nucleotide sequences (1,145–1,153 bp) of the 18S SSU rDNA were obtained and compared with other related nematodes deposited in GenBank. The generated Neighbor-Joining tree revealed a grouping of *P. philippinensis* with congeneric gonad-infecting philometrids (i.e., *P. nemipteri*, *P. saltatrix*, *P. sciaenae*, *P. lateolabracis*, *P. sawara* and *P. madai*). The phylogenetic tree clearly separates all congeneric species infecting only gonads of marine fishes into one clade. Exception to the congeneric gonad-infecting species is the *P. clavaeceps* Dogiel et Akhmerov, 1959 that also infects abdominal cavity and serosa of swim bladder of freshwater fish. The molecular data support previous reports that individual philometrid species exhibits a high degree of host specificity, and are identified based on their sites of infection (Moravec 2004, 2006; Moravec *et al.* 2008c). Despite of the low estimated evolutionary divergence among the congeneric gonad-infecting *Philometra* species of marine fishes, morphological differences have clearly differentiated *P. philippinensis* with other species (Fig. 4, Tables I and II).

Discussion

Because only gravid females are used in philometrid taxonomy, the available females collected in the present study were not used for morphological comparisons. Thus, the classification of *P. philippinensis* is based entirely on morphological, molecular, host, and geographical differences between males from other congeneric species. Morphologically, *P. philippinensis* possess two unique morphological features (i.e., sickle-shaped spicules and gubernaculum and a vulva-like cloacal opening) that have never been reported among male philometrids. Molecular data also support the morphological data in confirming the species differences of *P. philippinensis* from other related nematodes deposited in GenBank, particularly among other genera and those within the genus *Philometra* that parasitize different body parts other than the gonads. Despite the low evolutionary divergence within congeneric gonad-infecting *Philometra* species in marine fishes, the 18S SSU rDNA region can be useful in the re-evaluation of the taxonomical position of this group, thus making it a very important tool for rapid identification of newly discovered species or confirmation of species in question. In addition, other gene regions have to be explored in support to the morphological data, particularly on closely related congeneric species.

There are a total of 32 recognized *Philometra* species that infect gonadal tissue. Among these, males have been described in only a few species. In contrast, the majority of species were classified based on observation of semigravid and/or gravid females (Merella *et al.* 2005; Moravec 2006; Moravec and Salgado-Maldonado 2007; Moravec *et al*. 2008a, b; 2010a, b; 2011a, b; Moravec and Justine 2008, 2009, 2011; Quiazon *et al.* 2008a, b; Gaglio *et al.* 2009; Moravec and de Buron 2009, 2013). In all likelihood, a number of philometrid nematodes have yet to be discovered and identified in Philippine waters. *Philometra philippinensis* is the third nominal philometrid species in the Philippines and the first gonad-infecting species in this region.

The impacts of gonad-infecting philometrids cannot be underestimated. Although these parasites have less direct impact, with respect to human consumption, compared with those infecting the edible part of the fish muscle tissue, they may have direct effects on fish populations by affecting the host's reproduction (Hesp *et al.* 2002; Clarke *et al*. 2006; Moravec 2006; Perez *et al*. 2009). Interestingly, these philometrid nematodes have been used as temporal biological markers to study the population and stock structure of marine fishes. For example, Hutson *et al.* (2011) concluded that unidentified *Philometra* species could be used as permanent biological markers. In contrast, Zischke *et al.* (2009) noted that *Philometra rajani* (Mukherjee, 1966) (considered a *species inquirenda* by Moravec and Manoharan 2013) was not suited for use, even as a temporary biological marker. Researchers have also evaluated the utility of the genus as indicators of environmental contamination. Baruš *et al.* (2007) tested the utility of *P. ovata* as an indicator of heavy metal pollution in freshwater ecosystems. Studies such as these could benefit from the ability to accurately identify the philometrid nematodes to species level.

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