

## Two new species of philometrid nematodes (Nematoda: Philometridae) from the southern flounder *Paralichthys lethostigma* in the estuaries of South Carolina, USA

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**Abstract.** Two new philometrid nematodes, *Philometra overstreeti* sp. n. and *Philometroides paralichthydis* sp. n., are described from female specimens collected from the southern flounder *Paralichthys lethostigma* Jordan et Gilbert (Paralichthyidae, Pleuronectiformes) in estuaries of South Carolina, USA. *P. overstreeti* is characterized by the presence of eight large, equal in size cephalic papillae, the absence of caudal projections, the body length of gravid females (14.10–27.06 mm) and the location in the host (among teeth). *P. paralichthydis* from the host's fins and the buccal cavity differs from its congeners mainly in possessing longitudinal cuticular ornamentations in addition to transverse ones or individual rounded bosses. It is the first species of *Philometroides* reported from flatfishes (Pleuronectiformes). A re-examination of type specimens of *Margolisionum bulbosum* Blaylock et Overstreet, 1999 showed that, in fact, nematodes belonging to two different species (now described to be new to science) and genera were confused in the original description of this taxon and in the diagnosis of *Margolisionum*. Since the male holotype of *M. bulbosum* cannot be identified to genus, *Margolisionum bulbosum* is a *species inquirenda* and a *species incertae sedis*. Consequently, *Margolisionum* becomes a *genus inquirendus*.

During recent investigations into the parasites of fishes in the estuarine systems of South Carolina carried out by the research team of the junior author (I. de Buron), the southern flounder *Paralichthys lethostigma* Jordan et Gilbert was found to harbour philometrid nematodes located in the host's musculature at the bases of fins and in the buccal cavity. These proved to represent gravid and subgravid females of two new species, which are described herein.

*Paralichthys lethostigma* is an important commercial fish and gamefish distributed in the West Atlantic from North Carolina to Texas in the USA (Froese and Pauly 2006).

### MATERIALS AND METHODS

Southern flounder were collected using trammel netting and electrofishing during daytime ebbing tides primarily over mud and oyster shell substrates adjacent to the marsh (<2 m depth). Fish were measured, placed on ice and transported to the laboratory for immediate dissection. Nematodes recovered were washed in physiological saline and then fixed in 70% ethanol or in 4% or 10% formaldehyde solution in physiological saline. Some specimens were fixed and stored in 95% ethanol for subsequent DNA examination. For light microscopy, specimens were cleared with glycerine. Drawings were made with the aid of a Zeiss drawing attachment. After examination, specimens were maintained in 70% ethanol in vials. Specimens used for scanning electron microscopy

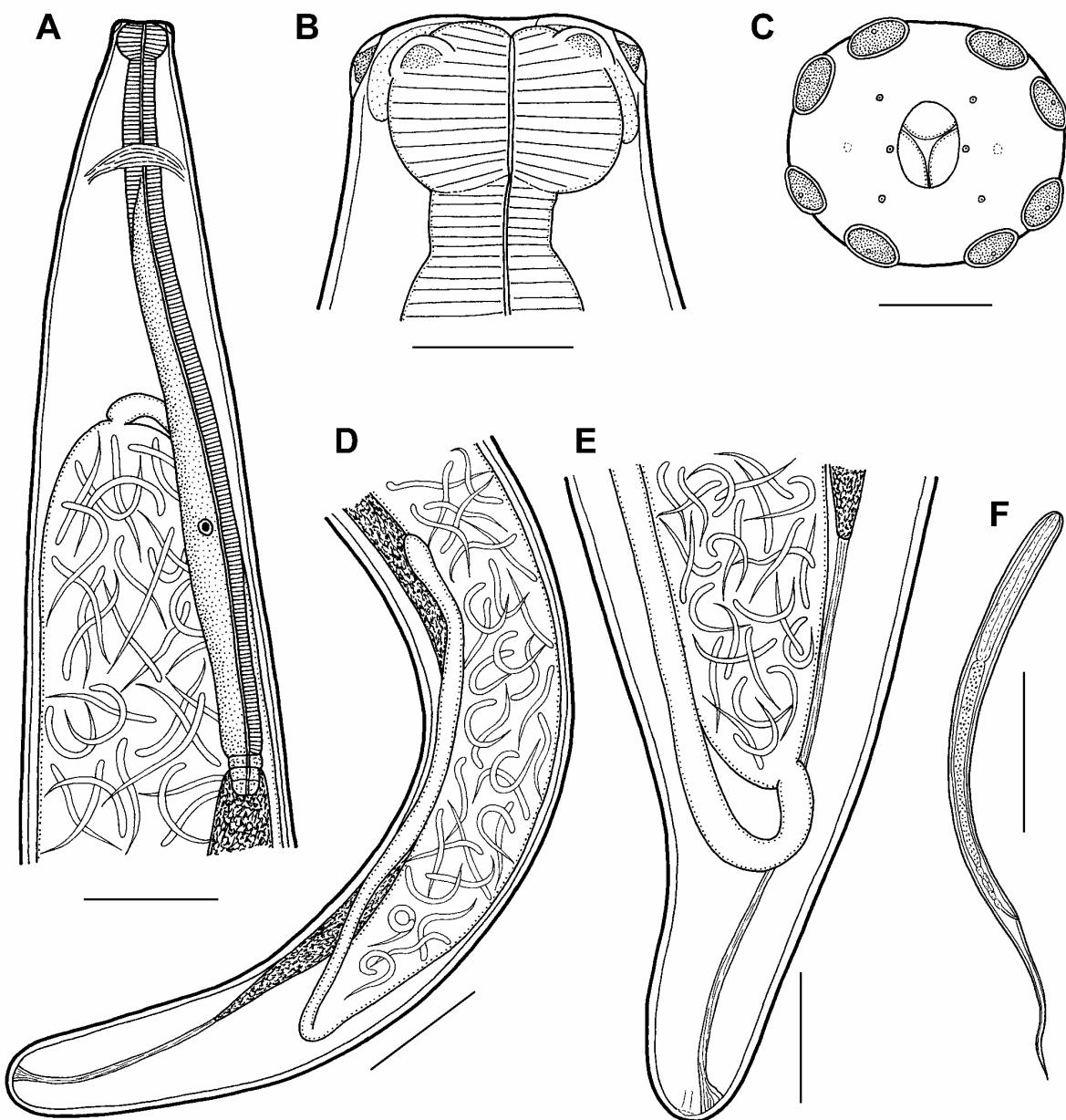
(SEM) were transferred to 4% formalin and then postfixed in 1% osmium tetroxide, dehydrated through a graded ethanol series, critical point dried and sputter-coated with gold; they were examined using a JEOL JSM-6300 scanning electron microscope at an accelerating voltage of 15 kV. For comparison, the female allotype and 1 female paratype of *Margolisionum bulbosum* were borrowed from the US National Parasite Collection, Beltsville, Maryland (catalogue numbers USNPC 087787.00 and USNPC 087788.00, respectively). All measurements are in micrometres unless otherwise stated. The names of fishes follow Nelson et al. (2004) and Froese and Pauly (2006).

### DESCRIPTIONS

#### *Philometra overstreeti* sp. n.

Figs. 1, 2

**Gravid female** (5 larvigerous specimens; measurements of holotype in parentheses): Body of fixed specimens brownish, cylindrical, somewhat tapering at both ends, 14.10–27.06 (27.06) mm long, maximum width 354–680 (680). Maximum width/length ratio of body 1:30–40 (1:40). Cephalic end and caudal end 78–95 (95) and 95–122 (95) wide, respectively. Cuticle smooth. Cephalic end blunt, with marked cephalic papillae. Oral aperture relative large, oval, surrounded by four pairs of large submedian papillae arranged in outer circle and six (2 lateral and 4 submedian) small papillae of inner circle; pair of small amphids present. Bottom of mouth



**Fig. 1.** *Philometra overstreeti* sp. n., gravid female from mouth of *Paralichthys lethostigma*. **A** – anterior part of body, lateral view; **B, C** – cephalic end, lateral and apical views; **D** – posterior part of body, lateral view; **E** – caudal end, lateral view; **F** – larva from uterus. Scale bars: A, D = 200  $\mu$ m; B = 50  $\mu$ m; C = 30  $\mu$ m; E, F = 100  $\mu$ m.

formed by flat surfaces of three oesophageal sectors. Anterior end of oesophagus swollen to form well-developed bulb 51–68 (60) long and 63–87 (87) wide. Long posterior portion of oesophagus almost cylindrical, provided with well-developed oesophageal gland extending anteriorly to level of nerve ring; oesophageal gland contains large cell nucleus located somewhat posterior to its middle, at 530–925 (925) from anterior extremity. Overall length of oesophagus including anterior bulb 1.02–1.33 (1.33) mm, representing 5–7 (7) %

of body length; maximum width of cylindrical part including oesophageal gland 68–95 (95). Small ventriculus, 12–15 (15) long and 27–33 (30) wide, opening into intestine through valve. Nerve ring 204–231 (218) from anterior end of body. Intestine relatively narrow, its posterior end atrophied, forming ligament attached ventrally to body wall near posterior extremity. Posterior end of body somewhat narrowed, rounded, without any caudal projections. Ovaries short, narrow, reflected. Uterus occupying major part of body, extending anteri-

orly to about mid-length of oesophagus, posteriorly to intestinal ligament. Uterus filled with numerous larvae and eggs. Larvae 378–393 (not measured in holotype) long, maximum width 12–15 (n = 5); length of their oesophagus and sharply pointed tail 111–114 (28–30% of body length) and 93–102 (24–27% of body length), respectively.

**Male.** Unknown.

**Type host:** Southern flounder *Paralichthys lethostigma* (Paralichthyidae, Pleuronectiformes) (total body length 17.5–55 cm).

**Site of infection:** In capsules among teeth.

**Type locality:** Estuarine system of South Carolina (Charleston), USA (32°30'–33°18'N, 79°12'–80°35'W) (holotype collected June 21, 2005 in the Charleston Harbor).

**Prevalence and intensity:** In June 2005: 33% (14 fish infected / 42 fish examined); 1–6 (mean 2) specimens per fish.

**Total number of specimens studied:** 12.

**Entomology:** This species has been named in honour of Prof. Robin M. Overstreet from the University of Southern Mississippi, Ocean Springs, Mississippi, who contributed greatly to the knowledge of fish parasites.

**Deposition of types:** Holotype USNPC 97632 and paratypes USNPC 97633 in the US National Parasite Collection, Beltsville, Maryland. Some paratypes in the helminthological collection of the Institute of Parasitology, ASCR, České Budějovice (Cat. No. N-857).

**Comments.** Blaylock and Overstreet (1999) erected a new genus *Margolisionum* to accommodate their species *M. bulbosum* Blaylock et Overstreet, 1999, newly described from the southern flounder *Paralichthys lethostigma* in the Mississippi Sound. The genus was mainly characterized by the presence of eight large cephalic papillae and the absence of an inner circle of papillae in gravid females, variable irregularly distributed cuticular bosses (except in the cephalic region), and by cuticularized beadlike teeth around the mouth. Moravec and Van As (2001) did not consider the size and the arrangement of cephalic papillae a suitable generic feature in *Philometridae* and synonymized *Margolisionum* with *Philometroides* Yamaguti, 1935. However, Golléty et al. (2005) argue that the absence of cuticular bosses in the female cephalic region as well as the size and arrangement of female cephalic papillae should be given generic consideration and, therefore, they re-established *Margolisionum*. According to them, *Margolisionum* differs from *Philometroides* in the absence of the inner circle of cephalic papillae and of cuticular bosses at the cephalic region in large females.

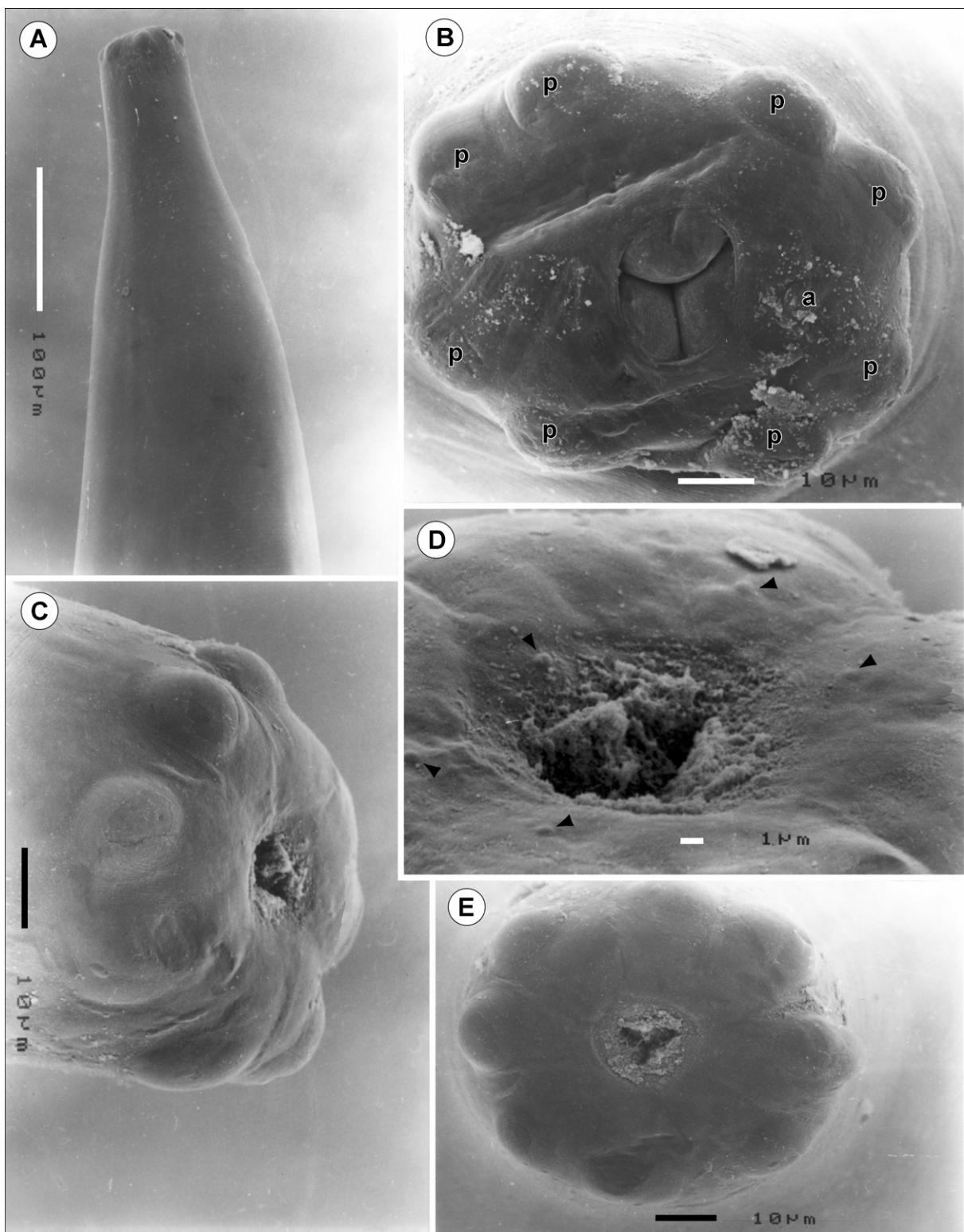
Recent examination of philometrid nematodes of the present material from *Paralichthys lethostigma* from the estuaries of South Carolina has shown that this fish host is parasitized by two philometrid species, one belonging to *Philometra* Costa, 1845 and one to *Philometroides* Yamaguti, 1935. Although they may appear to be simi-

lar and gravid females of both species possess eight markedly large cephalic papillae of the external circle, they distinctly differ in the presence/absence of cuticular ornamentations (bosses) and the presence/absence of an internal circle of cephalic papillae in the gravid female, in the shape of body (maximum body width/length ratio 1:30–40 vs. 1:11–22), the relative length of the oesophagus to the length of body (5–6% vs. 6–14%) and the size of the anterior oesophageal bulb (51–68 × 63–87 vs. 136–190 × 177–299 µm) in the gravid female and, partly, in the location of gravid females in the host's body (among teeth vs. bases of fins or buccal cavity).

While describing *M. bulbosum*, Blaylock and Overstreet (1999) mentioned that cuticular bosses were not always visible on fixed specimens using light microscopy and these were neither illustrated by them on the drawings of the female anterior and posterior ends (figs. 1 and 2). Consequently, it could not be excluded that the material of Blaylock and Overstreet (1999) contained nematodes belonging to two genera, as it is found in *P. lethostigma* in South Carolina (present study). This was confirmed by a recent re-examination of the allotype (subgravid female) and one paratype (damaged gravid female) of *M. bulbosum*, which both proved to be members of *Philometra*. Now it is apparent that two species of different genera were confused in the original description of *M. bulbosum*.

Unfortunately, the male of *M. bulbosum* was determined to be the holotype so that it is impossible to assign it to a genus; the cuticular bosses are generally absent from the body surface of conspecific males and mature (nongravid) females in *Philometroides* spp., as already observed by Rasheed (1963). Since only one type of the philometrid male has so far been discovered in *P. lethostigma* and its generic and species appurtenance is unknown (it can be determined only experimentally), it is necessary to consider *Margolisionum bulbosum* a *species inquirenda* and a *species incertae sedis*. Consequently, in accordance with the International Code of Zoological Nomenclature (Article 67.2.5), *Margolisionum* is a *genus inquirendus*. Therefore, both members of *Philometra* and *Philometroides* from *P. lethostigma* should be described as new species. The male of *M. bulbosum* was described from the host's musculature posterior to the head near the dorsal fin (Blaylock and Overstreet 1999).

Blaylock and Overstreet (1999) characterized *Margolisionum* [genus *inquirendus*] females as having a trilobed mouth, with a ring of cuticularized beadlike teeth. However, in contrast to their statement in the original description of *M. bulbosum*, the SEM micrograph (fig. 11) in their paper shows clearly that the mouth aperture is circular as in most philometrids and the structures described by them as 'beadlike teeth' do not surround the mouth aperture as in members of *Alinema* Rasheed, 1963 or *Neophilometroides* Moravec,



**Fig. 2.** *Philometra overstreeti* sp. n., scanning electron micrographs of gravid female from mouth of *Paralichthys lethostigma*. **A** – anterior end of body; **B, C** – cephalic end, apical and dorsoventral views; **D** – region of mouth with cephalic papillae of inner circle (arrows); **E** – apical view of cephalic end of another specimen. Abbreviations: a – amphid; p – cephalic papilla of outer circle.

Salgado-Maldonado et Aguilar-Aguilar, 2002 (see Moravec et al. 2002, 2006a) but are on the surface of the three sectors of the oesophagus; they are probably artefacts (impurity), as also suggested by the present study.

The morphology of gravid specimens of *P. overstreeti* is very similar to that of *Philometra cynoscionis* Moravec, de Buron et Roumillat, 2006, a species recently described from the mouth of the spotted seatrout *Cynoscion nebulosus* (Cuvier) (Sciaenidae) in the same locality (South Carolina estuarine system, USA) (Moravec et al. 2006b). Gravid females of both species are characterized by the presence of eight large cephalic papillae of the external circle and six small papillae of the internal circle, a rounded caudal end without any caudal projections, and by similar body measurements.

However, the external cephalic papillae of the gravid female of *P. overstreeti* are distinctly larger in relation to the size of the nematode cephalic end and all are of approximately the same size (Figs. 1C, 2B, C, D); in contrast, those in *P. cynoscionis* are relatively smaller and the dorso- and ventrolateral papillae are distinctly smaller than the dorsodorsal and ventroventral papillae. Moreover, the anterior oesophageal bulb in *P. overstreeti* is smaller than that in *P. cynoscionis* (51–68 × 63–87 µm vs. 90–135 × 114–165 µm) and the body is usually somewhat broader (maximum width/length ratio 1:30–40 vs. 1: 28–32), although the body length is approximately the same in both species. Distinction of *P. overstreeti* from species of *Philometra* parasitizing the host's subcutaneous tissues, fins or musculature is identical with the distinction of *P. cynoscionis* as already published by Moravec et al. (2006b).

*Philometra overstreeti* was found only in the mouth of the infected flounder, where it sometimes co-occurred with *Philometroides paralichthydis*. Of the 114 gravid and subgravid philometrid specimens collected from the fish mouth in August 2005, 7 proved to be *P. overstreeti* and 89 *P. paralichthydis*. However, the latter species (gravid and subgravid females) occurred also in the bases of fins.

***Philometroides paralichthydis* sp. n.** Figs. 3, 4

**Gravid female** (5 larvigerous specimens from fins; measurements of holotype in parentheses, those of 1 specimen from mouth in square brackets): Body of live specimens red-coloured, that of fixed specimens brownish, 10.54–29.31 (13.03) [15.67] mm long. Maximum width, 952–1,319 (952) [1,047], at region of oesophagus end; posterior part of body somewhat narrower; body tapering at both ends. Width of cephalic end 150–218 (190) [231], of posterior end 68–177 (82) [136]. Maximum width/length ratio of body 1:11–22 (1:14) [1:15]. Almost whole body covered with numerous, irregularly scattered cuticular ornamentations in form of small bosses and transverse mounds (Figs. 1A, D, E, H–K, 2 C–E), on posterior part of body also with

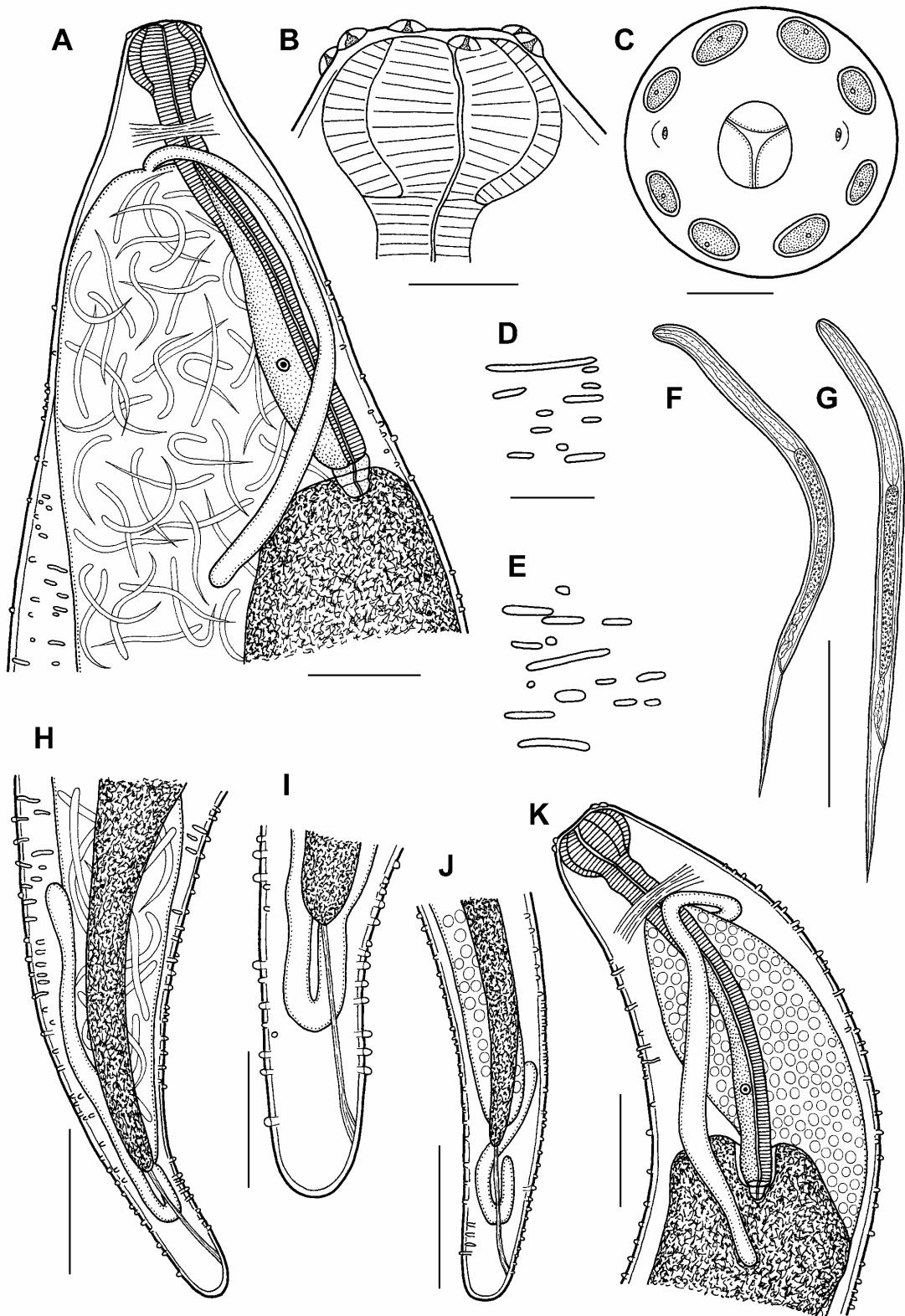
longitudinal mounds (Fig. 2E). Cuticular ornamentations present on all specimens, starting anteriorly at level of about middle of oesophagus and extending posteriorly to body end; ornamentations less distinct and more sparse in larger specimens. Cephalic end blunt, with marked cephalic papillae. Oral aperture large, oval, surrounded by eight large, outer cephalic papillae and small lateral amphids; papillae of inner circle not observed. Anterior end of oesophagus forming conspicuous bulb 136–190 (163) [150] long and 177–299 (204) [218] wide. Greater posterior portion of oesophagus almost cylindrical, with well-developed dorsal oesophageal gland provided with large cell nucleus at middle; gland opening anteriorly just posterior to level of nerve ring; maximum width of this part of oesophagus including oesophageal gland 109–190 (122) [109]. Entire oesophagus including anterior bulb 1.02–1.67 (1.17) [1.51] mm long, representing 6–10 (9) [10] % of body length. Small ventriculus present. Oesophagus opening into intestine through distinct valve. Nerve ring and cell nucleus of oesophageal gland 231–299 (272) [313] and 775–1,047 (816) [not measured] from anterior extremity. Intestine very broad at its anterior part; its posterior part narrow, attached by ligament to ventral body wall near posterior extremity. Posterior end of body rounded, without any projections. Ovaries reflected, situated near body ends. Uterus occupying almost entire space of body, reaching anteriorly to level of nerve ring and posteriorly to about end of intestine. Uterus contains large number of larvae and eggs. Larvae 345–390 (not measured) [not measured] long and 15–18 wide, with rounded anterior and sharply pointed posterior ends; length of oesophagus 105–117 (27–34% of body length), of tail 81–102 (21–27% of body length).

**Subgravid female** (1 specimen from fin; measurements of 1 specimen from mouth in parentheses): Length of body 6.72 mm (5.67 mm), maximum width 476 (354). Width of cephalic end 136 (136), of caudal end 82 (54). Maximum width/length ratio of body 1:14 (1:16). Cuticle with distinct ornamentations. Overall length of oesophagus 870 (816), representing 13% (14%) of body length; anterior bulb 109 (126) long, 163 (87) wide; maximum width of cylindrical part of oesophagus including oesophageal gland 68 (75). Small ventriculus 15 (12) long, 69 (27) wide. Oesophageal cell nucleus and nerve ring 652 (not found) and 218 (204), respectively, from anterior extremity. Posterior end rounded. Uterus filled with numerous eggs.

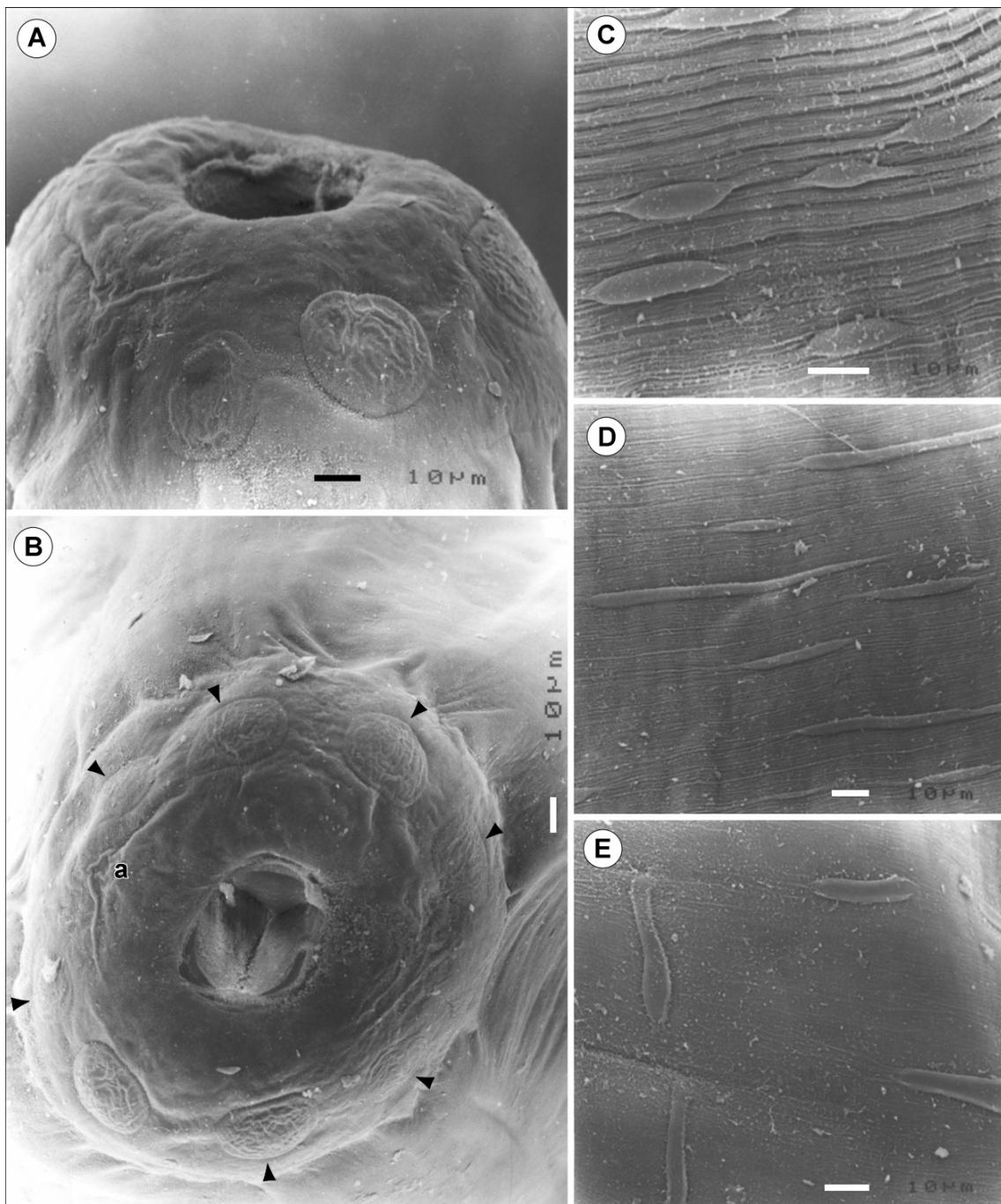
**Male.** Unknown.

**Type host:** Southern flounder *Paralichthys lethostigma* (Paralichthyidae, Pleuronectiformes) (total body length 8.5–55 cm).

**Site of infection:** Intramuscular tissue at base of fins and buccal cavity (tongue, between bones, on palatine archs and on rakers).



**Fig. 3.** *Philometrodes paralichthydis* sp. n., females from fins of *Paralichthys lethostigma*. **A** – anterior part of body of gravid specimen, lateral view; **B, C** – cephalic end, lateral and apical views; **D, E** – transverse cuticular ornamentations on anterior and posterior parts of body, respectively; **F, G** – larva from uterus; **H** – posterior part of body of gravid specimen, lateral view; **I** – caudal end of gravid specimen, lateral view; **J** – posterior end of subgravid specimen, lateral view; **K** – anterior end of subgravid specimen, lateral view. Scale bars: A, H, J, K = 200 µm; B, F, G, I = 100 µm; C = 40 µm; D, E = 50 µm.



**Fig. 4.** *Philometrodes paralichthys* sp. n., scanning electron micrographs of gravid female from fins of *Paralichthys lethostigma*. A, B – cephalic end, sublateral and apical views (arrows show cephalic papillae); C, D – transverse cuticular ornate on anterior and posterior parts of body, respectively; E – longitudinal and transverse cuticular ornate on posterior part of body. Abbreviation: a – amphid.

**Type locality:** Estuarine system of South Carolina (Charleston), USA (32°30'–33°18'N, 79°12'–80°35'W) (holotype collected June 14, 2005 in the Wando River).

**Prevalence and intensity:** In June 2005: 21% (9 fish infected / 42 fish examined); 1–28 (mean 7) specimens per fish.

**Total number of specimens studied:** 64.

**Entomology:** The specific name of this nematode is derived from the generic name of its fish host.

**Position of types:** Holotype USNPC 97634 and paratypes USNPC 9765 and USNPC 97636 in the US National Parasite Collection, Beltsville, Maryland. Some paratypes in the helminthological collection of the Institute of Parasitology, ASCR, České Budějovice (Cat. No. N-858).

**Comments.** The structure of the cephalic end and the embossment of the cuticle of gravid and subgravid females show that the specimens of the present material belong to *Philometrodes*. According to Moravec et al. (2005), at present this genus includes a total of 23 species parasitizing freshwater, brackish-water and marine fishes. Males are known only for 9 of them. Accordingly, most morphological differential features are found in gravid females.

While discussing the systematic status of *Margolisionum* [genus *inquirendus*], Golléty et al. (2005) distinguished it from *Philometrodes* by the absence of the internal circle of cephalic papillae and of cuticular bosses at the cephalic region in large females. However, if present, cuticular ornamentations (e.g. bosses) and their distribution on the female body are rather variable in philometrids and, in fact, they are lacking in the cephalic region in most *Philometrodes* spp. (Moravec 2006). Regarding the number, size and arrangement of female cephalic papillae, there is a high degree of interspecific variability in congeneric philometrids and these characters can hardly be considered to be of generic importance (Moravec 2004, 2006). The morphology of *P. seriola* (Ishii, 1931), a type species of *Philometrodes*, is insufficiently known, but there are several species of *Philometrodes* [e.g., *P. atropi* (Parukhin, 1966), *P. fulvidraconi* Wang, Yu et Wu, 1995, *P. moraveci* Vismanis et Yunchis, 1994, *P. pseudaspisii* Mo-

ravec et Ergens, 1970, *P. pseudorasbori* Wang, Yu et Wu, 1995)] possessing only eight cephalic papillae in gravid females.

A unique morphological feature of *P. paralichthydis* is the presence of longitudinal cuticular ornamentations, in addition to transverse ones or individual round bosses, by which it can be easily distinguished from its congeners. It also differs in some other features, as visible from the key to *Philometrodes* spp. provided by Moravec (2006). *P. paralichthydis* is the first species of *Philometrodes* reported from flatfishes (Pleuronectiformes).

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