Margolisianum bulbosum n. gen., n. sp. (Nematoda: Philometridae) from the Southern Flounder, Paralichthys lethostigma (Pisces: Bothidae), in Mississippi Sound, USA

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**MARGOLISIANUM BULBOSUM** N. GEN., N. SP. (NEMATODA: PHILOMETRIDAE) FROM THE SOUTHERN FLOUNDER, **PARALICHTHYS LETHOSTIGMA** (PISCES: BOTHIDAE), IN MISSISSIPPI SOUND, USA

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**ABSTRACT:** A new species of a philometrid nematode, *Margarolisianum bulbosum*, is described from the subcutaneous tissue in the mouth (larvigerous females), head (males, ovigerous, and larvigerous females), and eye (previgorous females) of the southern flounder, *Paralichthys lethostigma*, from Mississippi Sound. It is placed in a new genus diagnosed by the combination of 8 large, paired but separate cephalic papillae; no inner cephalic papilla; an esophagus with a separate, muscular anterior bulb; a prominent mononuclear esophageal gland; and variable, irregularly distributed cuticular bosses in the females, as well as a vestigial rectum, particularly in larvigerous females. Some female specimens exhibit rows of lateral grooves and longitudinal ridges near the posterior end. Males have 2 small slightly subequal spicules, a barbed gubernaculum, 4 pairs of small cephalic papillae, and a biparite hypodermal extension within a membranous cuticle on the posterior end. Males, ovigerous females, and larvigerous females appear to be present year round in this sporadic infection in Mississippi.

Over the last 30 yr, a conspicuous red philometrid nematode has irregularly occurred in the mouth of the southern flounder, *Paralichthys lethostigma* in Mississippi (see Overstreet and Edwards, 1976). The fish supports a small but important (mostly recreational) fishery in the Mississippi Sound and Gulf of Mexico. As part of normal monitoring of the flounder population in Mississippi and as part of a tagging experiment, several previgorous flounders were found to have these large red worms in the mouth and under the skin around the head. Histological sections from the tagging experiment revealed more extensive infections in the subcutaneous tissue surrounding the head, including evidence of males. Careful dissection of fish known to harbor females revealed the enigmatic males. The present paper describes the new species in a new genus.

**MATERIALS AND METHODS**

Nematodes were killed and fixed in glacial acetic acid and then transferred to either 70% ethanol or 70% ethanol with 5 parts glycerin. For light microscopy, representative worms were cleared in glycerin by evaporation of the ethanol from the glycerin solution and mounted in glycerin jelly. En face views were prepared according to Anderson's (1958) method. Drawings were made with the aid of a drawing tube. Live specimens were examined in temporary wet mounts with both a saline and a saline-neutral red solution. Histological sections were prepared by injection into and overnight fixation of the whole fish followed by 3-day postfixation of excised tissue with Bouin's solution. Fixed tissue was washed overnight in tap water, dehydrated through an ethanol series, embedded in paraffin, sectioned at 4 μm, and stained with hematoxylin and eosin. Worms for scanning electron microscopy (SEM) were washed and shaken in several changes of 70% ethanol, brushed to remove debris, postfixed in OsO4, rinsed in 0.1 M NaCac, dehydrated through an ethanol series, critical-point dried, sputter-coated with gold, and viewed using a JEOL JSM-T330 scanning electron microscope. Measurements are given in micrometers as ranges, with those of the holotype (or allotype) given in parentheses.

**DESCRIPTION**

*Margarolisianum* n. gen.

*Diagnosis:* Philometridae, Philometrinae. Body stout (male body small), reddish as live specimens (male translucent). Cuticle of females with variable irregularly distributed bosses (except in cephalic region) most visible in living material and in SEM. Cephalic end with 8 large paired but separate circumoral papillae; inner papillae lacking. Amphids lateral; mouth trifoliated, with ring of cuticularized beadlike teeth. Esophagus muscular, with separate bulbous anterior region (in females), lacking vesicles. Esophageal gland extensive, prominent, dorsal, uninnucleate. Intestine wide, forming a vestigial rectum, particularly in larvigerous females. Reproductive system didelphic, amphidetic with ovaries reflected in vicinity of junction with larger oviduct. Vulva in posterior third of body, degenerate in larvigerous females. Tail biparite (particularly in males).

Type and only species: *Margarolisianum bulbosum* n. gen., n. sp.

*Etymology:* The generic name is a Latinization of the name Margoli in recognition of the late Dr. Leo Margoli of the Pacific Biological Station, Nanaimo, British Columbia, Canada, and his contributions to knowledge of fish nematodes. The Latinized name is considered nomimative neuter.

*Margarolisianum bulbosum* n. sp.
(Figs. 1-25)

* Larvigerous female (based on 5 specimens): Body generally widest at midlength, 5.67–10.27 long by 0.07–0.51 wide at anterior end, 0.44–0.59 wide in middle, and 0.11–0.59 wide at posterior end, usually coiled in situ. Living material reddish brown. Cephalic papillae equal in size, 0.02–0.04 in diameter (Figs. 2, 11). Amphids roughly separating papillae into 2 groups. Oral aperture circular, surrounded by a circle of cuticularized beadlike “teeth”; bottom of mouth formed by 3 sectors of esophagus (Fig. 11). Ova visible, motile, in two sinuous, overlapping loops of micrometer size. In vivo, females 15–20 μm long total length, with 2 separate bulbous anterior sector; anterior bulb 0.06–1.0 long by 0.06–0.18 wide. Esophageal gland approximately 0.56 by 0.13 (at widest point), prominent, extensive, mononuclear, joining esophagus at level of nerve ring. Nerve ring approximately 0.16 from anterior tip of body, connected to body wall by muscles (Figs. 1, 12). Intestine without cecum, with valvular esophageal intestinal junction, expanded to fill most of body width, emptying into ductlike vestigial rectum near posterior end. Tail blunt but slightly tapering into 2 equivocal lips (Fig. 14). Anus virtually terminal, atrophied, without apparent opening (Figs. 3, 13). Phasmids indistinct. Excretory pore not visible. Uterus filling entire body, packed with larvae. Body distorted in the most larvigerous worms, particularly after fixation. Ovaries generally obscured, reflexed when visible, connecting to oviducts in esophageal and preanal areas, sometimes looping around intestinal tract (Figs. 1, 3). Vulva in posterior 1/5 of body, degenerate (Fig. 25). Larvae (10 specimens) 0.3–0.35 long by 0.01 wide, with distinct larval tooth.

*Ovigerous female (based on 7 specimens):* Body generally widest at midlength, 5.67–8.90 (8.34) long by 0.07–0.31 (0.20) wide at anterior ...
end, 0.17–0.56 (0.31) wide in middle, and 0.14–0.41 (0.20) wide at posterior end, usually coiled in situ. Living material translucent to reddish. Cephalic papillae equal in size, 0.01–0.03 in diameter, with appearance similar to that in larvigerous females. Bosses more uniform in structure than in larvigerous females, visible on most live specimens and SEM preparations, but not always visible on fixed specimens using light microscopy (except in cross section). Longitudinal ridges in 2 rows on posterior end apparent in some specimens with SEM but usually indistinct with light microscopy (Figs. 15, 16, 20). Esophagus as in larvigerous females, 0.61–0.89 (0.61) in total length, with separate bulbous anterior section; anterior bulb 0.05–0.09 (0.08) long by 0.05–0.11 (0.10) wide. Esophageal gland 0.45–0.58 (0.58) by 0.13 (at widest point), structure as in larvigerous females. Nerve ring 0.12–0.20 (0.17) from anterior tip of body, connected to body wall by muscles. Intestinal structure as in larvigerous females but more prominent. Tail as in larvigerous females. Anus more prominent than in larvigerous females, virtually terminal (Figs. 3, 6, 13). Uterus filling most of body, at least partially filled with eggs. Ovaries reflexed, connecting to oviducts in
esophageal and preanal areas, sometimes looping around intestinal tract (Figs. 1, 3); loop of anterior ovary with anterior portion 0.24–0.38 (0.38) from anterior end of body; loop of posterior ovary with posterior portion 0.16–0.27 (0.27) from posterior end. Vulva in posterior ½ of body, in process of degenerating (Fig. 24). Excretory pore not visible.

**Preovigerous female** (based on 1 specimen): Body biliform, 2.17 long by 0.03–0.04 wide. Living material translucent. Prominent glands surrounding anterior end of esophagus (Fig. 4). Bosses not evident. Esophageal gland 0.28 long, prominent, mononuclear. Nerve ring indistinct, anterior edge approximately 0.08 from anterior tip of body (Fig. 4). Tail blunt, with equivocal bilobed lip (Fig. 6). Intestine without cecum, with valvar esophageal intestinal junction, expanded to fill most of body width, forming swollen “rectal” area at posterior end. Anus virtually terminal (Fig. 6). Uterus filling most of body, filled with granular material and developing eggs. Ovaries as in ovigerous females; loop of anterior ovary with anterior portion 0.12 from anterior end of body; loop of posterior ovary with posterior portion 0.043 from posterior end. Vulva in posterior ½ of body (Fig. 5). Phasmids indistinct. Excretory pore not visible.

**Male** (based on 6 specimens): Body 1.46–2.69 (2.69) by 0.03–0.08 (0.08), filiform, translucent, with curved posterior end; posterior end with a lobelike bipartite extension (apparently of the hypodermis) enclosed in membranous cuticle (Figs. 9, 18, 19). Caudal papillae possibly present, but sense siae on 3 pairs of potential papillae unconfirmed in SEM (Fig. 19). Cuticle smooth. Cephalic end claviform, surrounded by 4 pairs of minute individual papillae; papillae slightly offset from anterior tip (Figs. 7, 10, 17). Esophagus 0.21–0.30 (0.30) long, cylindrical, without distinctive anterior bulb, largely obscured by esophageal gland; esophageal gland 0.22–0.25 (0.25) long by 0.01–0.02 (0.02) wide; nucleus of esophageal gland 0.01 by 0.01. Excretory pore, 0.13–0.16 (0.14) from anterior tip of the body (Fig. 7). Anterior border of nerve ring 0.04–0.06 (0.05) from anterior tip of worm. Testis monorchic, packed with spermatozoa. Spicules slightly subequal, narrow, sclerotized, capitate, protruding through cuticular fold (Figs. 8, 9, 18, 19); right spicule 0.074–0.087 (0.077) long by 0.005 wide at maximum width; left spicule 0.069–0.084 (0.07) long by 0.005 wide at maximum width. Gubernaculum 0.045–0.060 (0.051) long, 0.003 wide, with barb 0.027–0.040 (0.031) from proximal end, not entirely sclerotized (Figs. 8, 9, 18). Length ratio of spicules to gubernaculum 1: 0.60–0.83 (1:0.66), averaging 1:0.68.

**Taxonomic summary**

**Type host:** *Paralichthys lethostigma* Jordan and Gilbert, 1884, southern flounder (Pisces: Bothidae).

**Sites:** Larvigerous and ovigerous females in subcutaneous tissue of mouth and head. Preovigerous female in eye. Males in muscle tissue just posterior to head near dorsal fin.

**Type locality:** Davis Bayou, Ocean Springs, Mississippi; other localities: Mississippi Sound, off Mississippi barrier islands, and Galveston Bay, Texas.

**Prevalence and mean intensity:** Females in 56% (10 of 18) of hosts examined from Mississippi in 1997 with a mean intensity (Bush et al., 1997) of 2.0 ± 0.5 (range 1–6); males confirmed in every examined host infected with females.

**Specimens deposited:** Holotype (male), United States National Parasite Collection, Beltsville, Maryland (USNPC), no. 87786; allotype (female) USNPC no. 87787; paratypes (1 male, 1 female) USNPC no. 87788. Paratypes (2 males, 2 females), H. W. Manter Laboratory, University of Nebraska State Museum (HWML), nos. 39712, 39715 and 39713, 39714, respectively.

**Etymology:** The Latin specific epithet bulbosum refers to the bulbous nature of the anterior portion of the esophagus.

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**Figures 7–10.** *Margolisianum bulbosum* n. gen., n. sp. from *Paralichthys lethostigma*. 7, Anterior end, male, dorsal view. Scale bar = 0.1 mm. 8, Posterior end, male, lateral view. Scale bar = 0.1 mm. 9, Posterior end, male, ventral view. Scale bar = 0.1 mm. 10, En face view, male. Scale bar = 0.025 mm.
Remarks

*Margolisianum* differs from all other philometrid genera in that the single species possesses 8 large, paired, but separate, cephalic papillae in an outer ring and lacks inner papillae. This arrangement, in addition to a ring of beadlike structures around the mouth, is most similar to that in species of *Philometra* Costa, 1845 in the subgenus *Alinema* Rasheed, 1963; however, the papillae in *Margolisianum* are much larger than those reported for members of *Philometra* (*Alinema*). *Margolisianum* is also similar to *Philometroides* Yamaguti, 1935, *Paraphilometroides* Moravec and Shaharom-Harrison, 1989, and *Buckleyella* Rasheed, 1963 in that species have cuticular bosses. The cuticular bosses of *M. bulbosum*, however, are most visible in living material and in electron microscopy and appear to be related directly to worm maturity. The new species of *Margolisianum* lacks the cuticular cephalic alae supported by muscles found in the members of *Paraphilometroides*, lacks the cuticular bosses in the cephalic region as well as the small cephalic papillae reported in members of *Philometroides*, and lacks the protruding teeth, posterior papillae, and flat cephalic papillae characteristic of the monotypic *Buckleyella*.

*Margolisianum bulbosum* differs from other species in related genera most noticeably in the size and arrangement of the single row of cephalic papillae. Only 19 of the approximately 60 nominal species currently assigned to *Philometra* are described as having 8 cephalic papillae in pairs in the outer row. Of those, only 5 have 8 in the outer row without any in an inner row (*Philometra macronesi* (Shendge and Deshmukh, 1977); *Philometra parasilius* Yamaguti, 1935; *Philometra kohnae* Moravec and Rohde, 1992; *Philometra iomi* Moravec and Rohde, 1992; and *Philometra balistii* (Rasheed, 1963)). Only *P. kohnae* resembles *M. bulbosum* in size and arrangement. *Margolisianum bulbosum*, however, is a smaller worm than *P. kohnae* with a shorter esophagus.
is found in a totally different group of hosts, and has bosses, particularly on the posterior end.

Only 3 (Philometroides anguillae (Ishii, 1916); Philometroides atropi (Parukhin, 1966); and Philometroides pseudaspii Moravec and Ergens, 1970) of the 15 or so nominal species in Philometroides (=Pseudophilometroides Parukhin, 1966) are even remotely similar to M. bulbosum. Philometroides anguillae is most similar to M. bulbosum, but M. bulbosum has a separate anterior esophageal bulb, occurs in flatfish rather than eels, is located in the subcutaneous tissue of the host, has smaller cephalic papillae, and is about 1/3, the size of P. anguillae. Philometroides atropi is also similar, but M. bulbosum has fewer, less visible bosses under light microscopy, is 1/2 the size of P. atropi, and has an esophagus with a separate anterior bulb. Margoliusianum bulbosum is much smaller than P. pseudaspii, lacks prominent bosses (excrecences) on the anterior end, has a separate anterior bulb on the esophagus, and is found in flatfish rather than cyprinids.

Males are known for only approximately 20 of the 60 or so species of Philometra and 4 of the approximately 15 species of Philometroides. The basis for separating males is largely by association with corresponding females, but the size and equality of the spicules as well as the size and shape of the gubernaculum have been considered important taxonomic features (Moravec, 1978; Moravec, Vidal-Martinez et al., 1995). Of the 5 species of Philometra and 3 species of Philometroides with females most similar to M. bulbosum, the males are unknown. Once more males are recognized and described, the natural classification of the group can be better evaluated.

Male philometrids are often much smaller than female counterparts and located far from the larvigerous females. Males of M. bulbosum are about the same size as the preovigerous females collected from flounders in 1997. Previous attempts between 1971 and 1997 to collect males by placing torn pieces of juvenile flounder in saline in a screened funnel were unsuccessful. Our technique of carefully teasing apart tissue in saline under the dissecting microscope revealed males in virtually every examined fish that was infected with females. Males within fibromas in the jaw were seen in 1971 (Overstreet and Edwards, 1976). Fibromas were not observed in 1997, and males were found in the jaws on only 1 occasion. Males were found almost exclusively in the dorsal musculature immediately posterior to the head. However, we cannot rule out the possibility that males were elsewhere as well because after establishing that males could consistently and easily be found in this site, we did not always examine the remainder of the muscle tissue. We also saw little pathological alteration associated with the worms in 1997. There was some inflammation around the females (Fig. 21) but none associated with the males.

Discussi

Philometriidae Baylis and Daubney, 1926 consists of 3 sub-families, the largest of which is Philometrinae Baylis and Daubney, 1926. Rasheed (1963, 1965) was one of the first to attempt a clarification of the taxonomy of the group. She recognized 7 genera, the largest of which (Philometra) she divided into 3 subgenera. Ishii (1971) and Chabaud (1975) essentially followed Rasheed’s classification but accepted an additional genus (Spirofilometra Parukhin, 1971). Moravec and Shaharom-Harrison (1989) added Paraphilometroides but recognized Clavinema Yamaguti, 1935, which Rasheed (1963) had synonymized with Philometra. They also synonymized Thwaitia Rasheed, 1963 with Philometra. We considered all current and previously recognized genera and subgenera as potentially acceptable genera for our differential diagnosis.

Genera within Philometrinae have been classified primarily on the basis of cuticular ornamentation and arrangement of cephalic papillae on the female (Rasheed, 1963; Chabaud, 1975; Moravec and Shaharom-Harrison, 1989). Both Rasheed (1963) and Moravec and Shaharom-Harrison (1989) used the presence or absence of cuticular ornamentation in keys to broadly divide the group into 2. Both keys also used types of ornamentation to further divide the ornamented group. Philometra, Clavinema, and Thwaitia are included among the nonornamented forms. Philometroides and Paraphilometroides are included among the ornamented forms because they have small, irregular bosses. Buckleyella is considered an ornamented form with large, regularly distributed, rod-shaped bosses. Our discovery that M. bulbosum has bosses readily visible only in living material or by SEM and irregularly occurring rows of lateral grooves and longitudinal ridges on the posterior ends of only some females suggests that this character is unreliable. Because bosses were variable and because the bosses on larvigerous females were elongated and less robust than on less mature specimens, we suspect that cuticular ornamentation is related to migration.
through host tissue and, therefore, to the habitat and maturity of the worm. As worms complete their migration and become larvigerous, the bosses take on a different, potentially less functional form. Whereas the presence of this feature is certainly diagnostic, its presence can be overlooked on some specimens or species, and, consequently, its use can be misleading.

Rasheed (1963) subdivided the largest genus (Philometra) into 3 subgenera based on the size and arrangement of the cephalic papillae. Moravec and Shaharom-Harrison (1989) apparently did not consider cephalic papillae to be as diagnostic as Rasheed did, because they did not recognize subgenera and considered as synonyms 2 genera with different types of papillae (Thwaitia a junior synonym of Philometra). We suspect that cephalic papillae are important and useful generic characters, particularly in light of the consistency of the character between males and females.

Rasheed (1963) and Margolis and Moravec (1987) also considered the shape of the esophagus to be of generic importance. Rasheed erected Thwaitia based in part on a swollen anterior esophagus. Margolis and Moravec resurrected Clavinema based in part on the distinctive shape of the anterior bulb of the esophagus. Margolisianum clearly has a distinctive esophageal bulb in that the bulb is separate and exhibits a different pattern in muscle arrangement. This bulb is apparently more similar to that shown in Clavinema than in Thwaitia because Rasheed (1963) shows no separation of the anterior bulb. However, we were unable to visualize the bulb (or the esophagus) in comparative material of Clavinema mariae (Layman, 1930) Margolis and Moravec, 1987 (coll. no. B1–52) from the Pacific Biological Station, Nanaimo, British Columbia, Canada. Thus, we note that caution should be exercised in evaluating this character because some descriptions use the term “bulb” but actually refer to a swelling without separation or a distinctive muscle arrangement pattern. The apparent lack of the bulb in males, however, makes this structure a potentially confusing phylogenetic and taxonomic character.
Margolis and Moravec (1987) considered the esophageal gland as a generic character. The absence of a prominent, well-developed esophageal gland in Clavinema was part of their argument in differentiating its species from those of Philometra. Recent studies (Moravec et al., 1998) have shown that the oral aperture of Clavinema is triangular in contrast to that in Philometra, Margolisianus, and other philometrids, where the opening is circular. Margolisianus bulbosum has a well-developed esophageal gland in both males and females. The esophageal gland, however, may be more characteristic of the habitat of the worm than of phylogenetic importance. Because of the esophageal gland's digestive function (see Lee and Atkinson, 1977), worms that undergo extensive migrations through tissue may have a need for a more prominent esophageal gland. The developmental stage of the worm may also be important in that once migration is complete, the esophageal gland may decrease in size. We thus agree with Moravec and Rohde (1992) and Vidal-Martínez et al. (1995) that the location of mature worms in the host tissue may be a more important taxonomic/phylogenetic character than the form of the esophageal gland.

We further recognize that other characters (or combination of characters) may serve as generic diagnostic features of Margolisianus. For example, the presence of a terminal “rectal” area in the intestine is unusual among mature philometrids. However, we are hesitant to make broad generalizations at present because, as Moravec, Scholz et al. (1995) indicated, there is great variability in some characters among species assigned to particular genera.

A fluctuating annual prevalence of M. bulbosum in Mississippi as well as highly variable year classes of its host P. lethostigma is recognized from 3 decades of usually unsystematic observations. The reasons for this variability have not been evaluated, but, if the species has a detrimental effect on the late juvenile and adult flounders, that effect is not evident. Further, based on our collection of males and ovigerous and larvigerous females throughout the year (January, April, May, August, November, December), it appears that worms are maintained in the flounders, but it does not rule out the possibility that mating or development is seasonal. In fact, the only time we found males and females (ovigerous) together in the same capsule was in spring. However, because of the location of the worms in the host, any seasonality is probably linked to environmental conditions such as temperature rather than the reproductive cycle of the host.

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LITERATURE CITED


