

Spinitectus aguapeiensis n. sp. (Nematoda: Cystidicolidae) from Pimelodella avanhandavae Eigenmann (Siluriformes: Heptapteridae) in the River Aguapeí, Upper Paraná River Basin, Brazil

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Abstract Nematodes belonging to Spinitectus Fourment, 1883 (Nematoda: Cystidicolidae) were found in the intestine of Pimelodella avanhandavae Eigenmann (Siluriformes: Heptapteridae) from the Aguapeí River, Brazil. They represent a new species, Spinitectus aguapeiensis n. sp., which differs morphologically from its congeners in the body length, the number of spinose rings, the location of the excretory pore, the number of precloacal papillae and the length of the

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spicules. The new species is the first South American species within the genus with a remarkably spirally coiled posterior extremity in males and the largest spicules. It is also the second species with the highest number of precloacal papillae and has unique shape of the small spicule. *Spinitectus aguapeiensis* n. sp. is the first helminth species found in *P. avanhandavae*, the fourth species of this genus recorded in the River Paraná Basin and the sixth species of *Spinitectus* in South America.

Introduction

The nematode genus Spinitectus Fourment, 1883 includes parasitic species that are mainly found in freshwater and marine fishes (Moravec et al., 2002). In America, 15 species of this genus have been described from freshwater fishes, namely: S. acipenseri Choudhury & Dick, 1992, S. carolini Holl, 1928, S. gracilis Ward & Magath, 1917, S. humbertoi Caspeta-Mandujano & Moravec, 2000, S. mariaisabelae Caspeta-Mandujano, Cabañas-Carranza & Salgado-Maldonado, 2007, S. mexicanus Caspeta-Mandujano, Moravec & Salgado-Maldonado, 2000, S. micracanthus Christian, 1972, S. osorioi Choudhury & Pérez-Ponce de León, 2001 and S. tabascoensis Moravec, Salgado-Maldonado, Caspeta-Mandujano & González-Solís, 2009 (syn. S. macrospinosus Choudhury & Perryman, 2003) in North America; S. agonostomi Moravec & Baruš, 1971 in Central America; S. asperus Travassos,



Artigas & Pereira, 1928 (syn. *S. jamudensis* Thatcher & Padilha, 1977), *S. multipapillatus* Petter, 1987, *S. pachyuri* Petter, 1984, *S. rodolphiheringi* Vaz & Pereira, 1934 (syn. *S. sternopygi* Petter, 1984) and *S. yorkei* Travassos, Artigas & Pereira, 1928 in South America (Moravec, 1998; Salgado-Maldonado et al., 2005; Salgado-Maldonado, 2006; Caspeta-Mandujano et al., 2007; Moravec et al., 2009, 2010). Of these, *S. multipapillatus*, *S. rodolphiheringi* and *S. yorkei* were recorded in species of *Pimelodella* Eigenmann & Eigenmann in South America (Travassos et al., 1928; Vaz & Pereira, 1934; Petter, 1987; Luque et al., 2011 and references therein).

Pimelodella is the most species-rich genus within the family Heptapteridae, with 71 valid species largely distributed in South America (Bockmann & Guazzelli, 2003; Froese & Pauly, 2016). Its representatives are small to medium-sized catfishes (less than 15 to 20 cm in length), dwelling in several habitats, like streams, large rivers and surface or subterranean environments (Dazzani et al., 2012). Pimelodella avanhandavae Eigenmann is an endemic species to South America, distributed mostly in Brazil, along the Upper River Paraná, and is popularly known as "mandizinho" and/ or "mandi-chorão". This species is non-migratory, with external fertilization, no parental care and most likely with carnivorous feeding habits (Britto, 2008). During a parasitological survey of specimens of P. avanhandavae sampled in a Brazilian river, a new species of Spinitectus was found in the intestine, which is described herein.

Materials and methods

A total of 40 specimens of *P. avanhandavae* (standard length 9–17.5 cm; weight 7.8–58.5 g) were collected by fish hooks in August 2013 and November 2014 in the River Aguapeí (21°3′36.20″S, 51°45′38.58″W), Upper River Paraná Basin, located in a preserved area in Castilho municipality, São Paulo State, Brazil. Nematodes were recovered from the intestine, fixed in 70% ethanol and cleared with glycerine for light microscopical examination. Drawings were made with the aid of a drawing tube. Measurements are given in micrometres unless otherwise stated. For scanning electron microscopy, specimens were post-fixed in 1% osmium tetroxide (in phosphate buffer), dehydrated through a graded acetone series, critical point-dried

and sputter-coated with gold; they were examined using a JEOL JSM-7401F scanning electron microscope at an accelerating voltage of 4 kV (GB low mode). The host scientific name is in accordance with FishBase (Froese & Pauly, 2016).

Family Cystidicolidae Skrjabin, 1946

Spinitectus aguapeiensis n. sp.

Type-host: Pimelodella avanhandavae Eigenmann (Siluriformes: Heptapteridae).

Type-locality: River Aguapeí (21°3′36.20″S, 51°45′38.58″W), municipality of Castilho, São Paulo State, Brazil.

Site in host: Intestine.

Prevalence and mean intensity: 50% (20 infected fish/ 40 examined fish) and 5 (range 1–21) nematodes per infected host.

Type-material: Holotype (CHIOC 38513a), allotype (CHIOC 38513b) and paratypes (CHIOC 38514a-b, 38515, 38516 - 6 females and 4 males) in the Helminthological Collection of the Institute Oswaldo Cruz, Rio de Janeiro, Brazil, paratypes in the Helminthological Collection of the Instituto de Biociências, Universidade Estadual Paulista, Botucatu, Brazil (CHIBB 7964-7966 - 3 males) and the Helminthological Collection of the Institute of Parasitology, Biology Centre of the Czech Academy of Sciences, České Budějovice, Czech Republic (IPCAS N-1130 - 2 females and 3 males).

ZooBank registration: To comply with the regulations set out in article 8.5 of the amended 2012 version of the *International Code of Zoological Nomenclature* (ICZN, 2012), details of the new species have been submitted to ZooBank. The Life Science Identifier (LSID) for *Spinitectus aguapeiensis* n. sp. is urn:lsid:zoobank.org:act:6523E5C9-E7AF-48D1-8106-C3B4F4228D66.

Etymology: The specific name relates to the river where hosts were collected.

Description (Figs. 1–3)

General. Whitish small-sized nematodes. Cuticle with transverse striations and longitudinal wrinkles between spinose rings (Fig. 2B, D). Cephalic end rounded. Mouth oval to rectangular, surrounded by 2



large lateral pseudolabia, 2 subdorsal and 2 subventral sublabia, 4 single submedian papillae and 2 lateral amphids. Inner base of pseudolabia widening inside oral cavity and attach to inner wall of prostom (Figs. 1C, 2A). Surface of body with transverse rows of spines, being interrupted by 2 lateral, 1 dorsal and 1 ventral longitudinal empty lines, thus forming 4 distinct sectors (Fig. 2D-F). First spiny row situated between first and second thirds of vestibule (Fig. 1B, D). Rows 1-9 with 16 well-developed spines (4 per sector), rows 10-15 with 20 spines (5 per sector), row 15 and posteriormost with 24-32 spines (6-8 per sector). Some rings incomplete, asymmetrical and not forming a circle (Fig. 1B); some missing spines in rings from posteriormost part of body (Fig. 3B). Spination weakly visible in posterior extremity of body and finishing before anus/cloaca opening. Vestibule long, with proximal end forming small prostom, distal end reaching between second and third rows of spines. Oesophagus divided into muscular and glandular portions, latter much longer and broader. Nerve-ring encircles muscular oesophagus between 4th and 5th rows of spines (Fig. 1A, B). Excretory pore situated between 6th and 7th rows of spines (Figs. 1B, 2D). Deirids small, pit-like with central emerging structure, located between first and second rows of spines (Figs. 1B, D, 2B, C).

Male. [Based on 6 specimens, measurements of the holotype in parentheses.] Body length 7.12–9.21 (8.03) mm, maximum width 82-110 (98). First spinose ring 41–74 (57) from anterior extremity. Spines 12–17 (13–15) long and distance between rows 14-33 (15-21) in anterior body part; spines 7-13 (8-10) long and distance between rows 26-43 (31-35)in mid-body length. Length of vestibule including prostom 116-135 (126); muscular oesophagus 245–337 (287) long, 17–21 (20) wide; glandular oesophagus 1.16-2.07 (1.72) mm long, 38-52 (52) wide. Length ratio of both parts of oesophagus 1:4-6 (6). Deirids 67–110 (87), nerve-ring 152–190 (173), and excretory pore 209-274 (247) from anterior extremity of body. Posterior extremity of body remarkably spirally coiled, with narrow caudal alae 394–455 (–) long, supported by 21 caudal pedunculate papillae. Precloacal papillae 16 subventral pairs; postcloacal papillae 5 subventral pairs (Figs. 1J, 3D, E). Phasmids probably at same level as last postcloacal pair of papillae. Area rugosa absent. Large (left) spicule slender, 325–420 (347) long, 9–15 (15) wide, with slightly expanded proximal part and pointed distal end. Small (right) spicule shorter and broader than large spicule, 162–197 (162) long, 15–28 (20) wide, with hook-like distal end and two internal supports in mid-length (Fig. 1I, J). Length ratio of spicules 1:1–2 (2). Tail 178–199 (181) long, with small dorsal and ventral rounded protuberances on tip (Figs. 1J, 3D–F).

Female. [Based on 3 gravid specimens with mature eggs, measurements of the allotype in parentheses.] Body length 11.50-12.38 (12.38) mm, maximum width 132-169 (169). First row of spines 41-66 (52) from anterior extremity. Spines 8–18 (12–18) long and distance between rows 16-26 (16-21) in anterior part of body; spines 8-18 (8-14) long and distance between rows 33-40 (33-37) in mid-body length. Length of vestibule including prostom 122–125 (125); muscular oesophagus 316–489 (489) long, 19–20 (–) wide; glandular oesophagus 1.02-2.32 (1.07) mm long, 41–45 (-) wide. Length ratio of muscular and glandular oesophagus 1:2–7 (3). Deirids 62–99 (99), nerve-ring 175–181 (181) and excretory pore 231–259 (239) from anterior extremity of body. Vulva equatorial, with non-elevated lips, 6.23–6.53 (6.53) mm from anterior extremity, at 53-55% of body length. Vagina muscular, shortly directed anteriorly and immediately directed posteriorly (Fig. 1F). Eggs embryonated, $29-36 \times 19-21$ (29-32 × 20-21). Egg shell thickness 2-4 (3) (Fig. 1G). Tail elongate, 186-206 (206) long, with small mucron on tip and pair of lateral phasmids (Figs. 1E, 3A, C).

Female. [Gravid specimen with immature eggs, measurements of young female in parentheses.] Body length 9.07 (3.58) mm, maximum width 128 (72). First row of spines 77 (29) from anterior extremity. Spines 13–15 (10–12) long and distance between rows 19–25 (11–15) in anterior part of body; spines 8–10 (10–13) long and distance between rows 33–35 (21–24) in mid-body length. Length of vestibule including prostom 120 (90); muscular oesophagus 350 (173) long, 30 (18) wide; glandular oesophagus 1.70 (1.06) mm long, 61 (36) wide. Length ratio of muscular and glandular oesophagus 1:5 (6). Deirids 102 (61), nerve-ring 182 (132) and excretory pore 265 (162) from anterior extremity of body. Vulva postequatorial, with non-elevated lips, 5.255 (2.314) mm from anterior



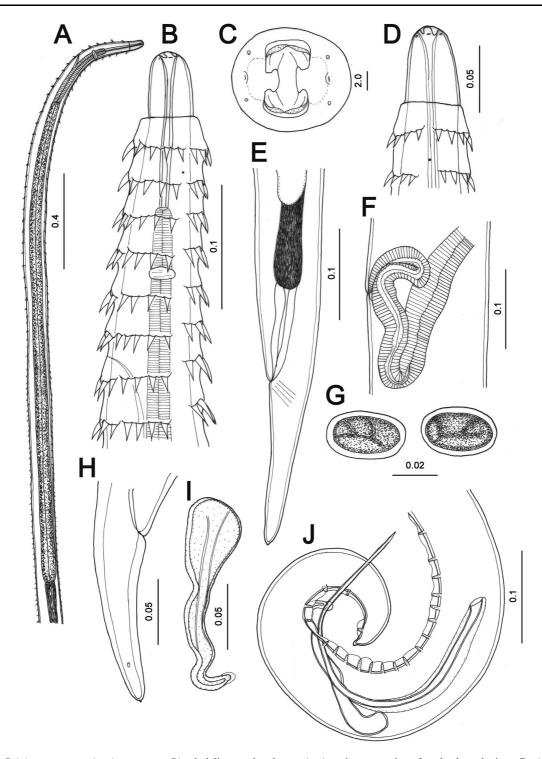


Fig. 1 *Spinitectus aguapeiensis* n. sp. ex *Pimelodella avanhandavae.* A, Anterior extremity of male, lateral view; B, Anterior extremity of female, sublateral view; C, D, Cephalic end, apical and lateral views, respectively; E, Posterior extremity of gravid female, lateral view; F, Region of vulva, lateral view; G, Eggs; H, Tail of non-gravid female, lateral view; I, Small spicule, subventral view; J, Posterior extremity of male, lateral view. *Scale-bars* are in millimetres



extremity, at 58 (65%) of body length. Vagina muscular, shortly directed anteriorly and immediately directed posteriorly. Eggs with not yet developed larvae, $17-18 \times 10-13$ (–). Egg shell thickness 2 (–). Tail 177 (125) long, with small mucron on tip (Fig. 1H).

Remarks

The specimens described herein belong to Spinitectus due to the presence of a cuticle provided with transverse rows of spines, two pseudolabia, a relatively long vestibule and caudal alae supported by pedunculate papillae (Moravec, 1998). Main morphological features used to differentiate *Spinitectus* spp. are the pattern of spinose rings arrangement, the structure of the mouth, the location of the vulva, the number and distribution of male caudal papillae, the length of spicules, the character of eggs and the position of the excretory pore and deirids (Moravec, 1979; Caspeta-Mandujano et al., 2000). To date, there are five valid species of Spinitectus recorded from South America (Moravec, 1998): S. asperus from Prochilodus scrofa (Valenciennes) (Prochilodontidae), S. multipapillatus from Pimelodella sp. (Heptapteridae), S. pachyuri from Pachyurus bonariensis Steindachner (Sciaenidae), S. rodolphiheringi and S. yorkei both from Pimelodella lateristriga (Lichtenstein) (Heptapteridae) (see Travassos et al., 1928; Vaz & Pereira, 1934; Petter, 1984, 1987). Of these, S. multipapillatus and S. yorkei were considered as possibly conspecific, even though the latter was inadequately described and a redescription is required (Moravec, 1998). Spinitectus asperus, S. multipapillatus and S. rodolphiheringi differ from the new species in having smaller body length (males: 2.42–4.60, 6.3–7.4 and 3.7–4.9 mm, respectively, vs 7.12–9.21 mm; females: 6-8.3, 6.5 and 5.9-7 mm, respectively, vs 11.50–12.38 mm), higher number of spines per row (60–90, 15–25 and 30–40, respectively, vs 16–20), different location of the excretory pore, number of precloacal papillae (4, 7–8 and 4, respectively, vs 16 pairs) and length of spicules (left: 220–230, 220–240 and 200–210 µm, respectively, vs 325–420 µm; right: 90, 50–55 and 120–125 μm, respectively, vs 162–197 μm). Spinitectus multipapillatus also possesses rows of cuticular spines divided into four sectors, but it differs from the new species in the above mentioned features. Even though S. pachyuri presents a similar body length for both males and females, it differs in having six pairs of precloacal papillae and shorter spicules (left: 100 μm, right: 50–55 μm). *Spinitectus yorkei* has a similar number of caudal papillae and although it was poorly described and many important features were not reported, it is clear that the species has smaller males (4.2 mm long), shorter spicules (left: 162 μm, right: 40 μm) and more cuticular spines per row (26–29 in males and 38–40 in females). *Spinitectus aguapeiensis* n. sp. has a remarkably spirally coiled posterior end in males, which is not present in any of the species described from South America.

Regarding species of Spinitectus reported in North and Central America, it is possible to find some resemblances with S. aguapeiensis n. sp. As for the body length, S. micracanthus (males: 7.6–8.2 mm), S. gracilis (males: 8–10; females: 10–15 mm) and S. mariaisabelae (males: 6.56-8.28; females: 11.82–14.58 mm) are within the same range, although they have a considerably lower number (2–4) of pairs of precloacal papillae. Spinitectus agostonomi, S. humbertoi, S. mariaisabelae, S. mexicanus, S. osorioi and S. tabascoensis also possess spines arranged in four sectors, but they all differ in the number (4) of pairs of precloacal papillae. Spinitectus aguapeiensis n. sp. is the second species with the largest number of precloacal papillae and that with the largest spicules amongst its congeners in South America. Moreover, this species has a unique shape of the small spicule that resembles a small shovel in its proximal extremity, with undulations and internal supports in mid-length and hook-like shape of the distal end.

Interestingly, four *Spinitectus* spp. (including the new species) have been reported in *Pimelodella* spp. from South America (Travassos et al., 1928; Vaz & Pereira, 1934; Petter, 1987; Luque et al., 2011; present study). Since *Pimelodella* spp. are carnivore or omnivore fish that consume mainly arthropods, fishes, free-living nematodes, algae and debris, they probably become infected after feeding on arthropods or small fish. Members of *Spinitectus* normally use arthropods as intermediate hosts and fish may act as paratenic hosts (Anderson, 2000; Bockmann & Guazzelli, 2003). Members of *Pimelodella* can be considered to serve as definitive hosts for *Spinitectus* spp. A certain degree of host specificity can be supposed for these nematodes.

Spinitectus aguapeiensis n. sp. represents the first record of a helminth species in *P. avanhandavae*, the



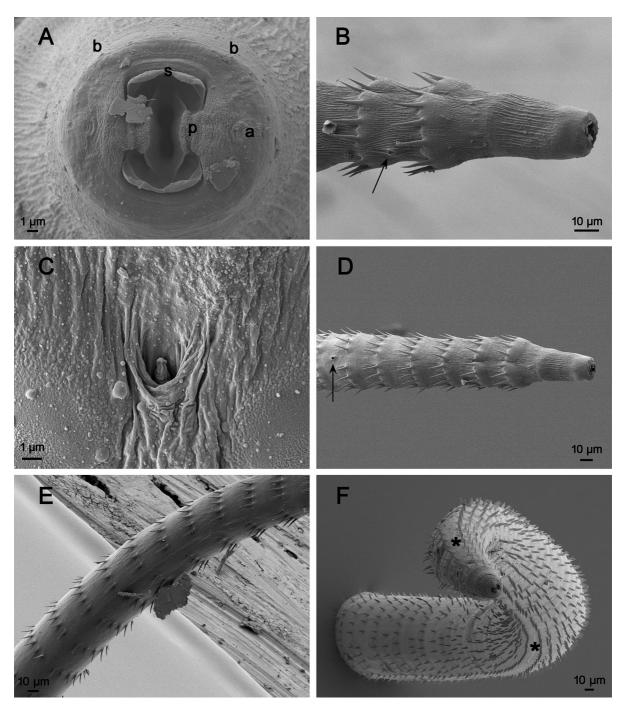


Fig. 2 *Spinitectus aguapeiensis* n. sp. ex *Pimelodella avanhandavae*. Scanning electron micrographs: A, Cephalic end, apical view; B, Anterior extremity of body showing first spinose rings (*arrow* indicates deirid), sublateral view; C, Detail of deirid; D, Anterior extremity of body (*arrow* indicates excretory pore), ventral view; E, Mid-part of body showing spinose rings, lateral view; F, Anterior extremity of male (*asterisks* indicate longitudinal empty sectors on spination). *Abbreviations*: a, amphid; b, cephalic submedian papilla; p, pseudolabium; s, sublabium



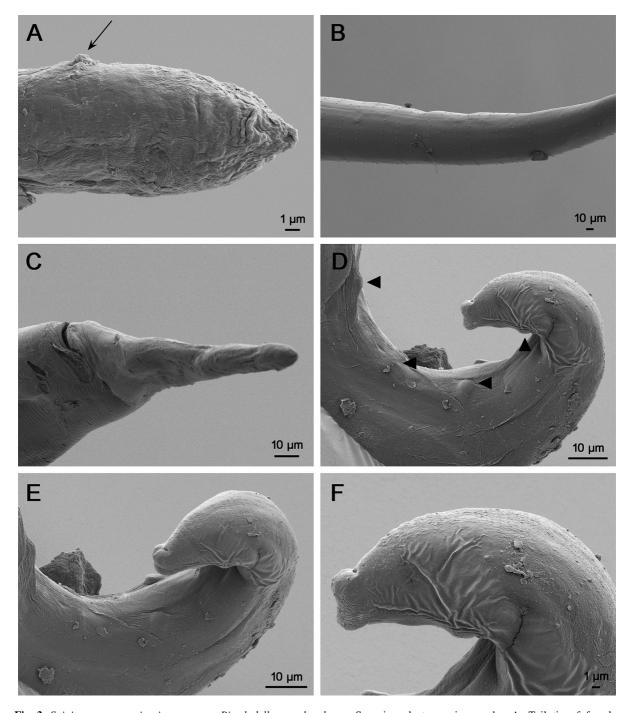


Fig. 3 Spinitectus aguapeiensis n. sp. ex Pimelodella avanhandavae. Scanning electron micrographs: A, Tail tip of female, dorsoventral view (arrow indicates phasmid); B, Posteriormost part of body, lateral view; C, Tail of female, sublateral view; D, E, Posterior extremity of male, subventral views (arrowheads indicate caudal papillae); F, Tail tip of male, sublateral view

fourth record of a nematode of this genus in the Paraná River Basin and the sixth species of *Spinitectus* in South America. **Acknowledgements** The authors thank the Companhia Elétrica de São Paulo (CESP) for the logistical support during sampling. Thanks are also due to Tomáš Scholz for hosting



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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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

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