# *Orientatractis moraveci* n. sp. and *Rondonia rondoni* Travassos, 1920 (Nematoda: Atractidae), parasites of *Pimelodus blochii* (Osteichthyes, Pimelodidae) from the Acre and Xapuri Rivers, Western Amazon, Brazil

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SUMMARY

The fish fauna in the State of Acre represents 10·7% of all fish species recorded from Brazil, but, despite this, there are few fish parasite studies in this area. The recent expansion of fish farming in Acre prompted a need for helminthological studies of the most commonly consumed fish species in the area, *Pimelodus blochii* (Pimelodidae). The aim of this study was to analyse the helminth fauna of *P. blochii* from the Acre and Xapuri Rivers in Northwestern Brazil. Numerous nematodes were collected from the intestine and two species of the family Atractidae were identified: *Rondonia rondoni* Travassos, 1920 and *Orientatractis moraveci* n. sp. The new species is distinguished from its congeners mainly by having: 10 pairs of caudal papillae (3 pairs pre-cloacal, 2 pairs ad-cloacal and 5 pairs post-cloacal); unequal spicules of 161–198 and 69–100 μM long; and a gubernaculum 38–58 μM long with an antero-lateral process. Morphological and ultrastructural data on *O. moraveci* n. sp. and *R. rondoni* are presented, in addition to new genetic data based on partial 18S rDNA and 28S rDNA. The taxonomic status of *Labeonema synodontisi* (Vassiliadès, 1973) is discussed, suggesting that it should be returned to the genus *Raillietnema*.

Key words: Nematoda, Cosmocercoidea, Atractidae, Brazil, scanning electron microscopy, 18S rDNA, 28S rDNA.

## INTRODUCTION

The Amazon basin contains the richest ichthyofauna in the world, with estimated 2500 of the 6000 species of Neotropical species (Goulding, 1980; Reis *et al*. 2003). In the State of Acre in Western Amazonia, Brazil, *c*. 310 species of fish were catalogued, representing nearly 11% of all fish species recorded in Brazil (Acre, 2010).

According to the structural census report of fishing in the continental waters of North Brazil, the fishing practiced in the State of Acre is basically artisanal and for subsistence, representing the main protein source for local people (Vieira *et al*. 2006). In recent years, as a measure to reduce deforestation and improve the local economy, fish farming, mostly in already deforested and abandoned areas near or within small-dams, has been encouraged as a sustainable alternative to cattle-farming (Reis *et al*. 2015). Thus, ichthyoparasitological studies in the Amazon should encompass, in addition to cultivated species, native species generally consumed by the local population.

In the State of Acre, *Pimelodus blochii* Valenciennes, 1840 is the fish eaten most often by the locals. The Pimelodidae are exclusively freshwater fishes, occurring in South America and Panama (Lundberg and Littmann, 2003; Ribeiro and Lucena, 2006). The genus *Pimelodus* Lacépède, 1803, is composed of *c*. 24 valid species, with 8, including *P. blochii*, from the Amazon Basin (Ribeiro and Lucena, 2006).

This, the first helminthological survey on *P. blochii* from the Acre and Xapuri Rivers, Northwest Brazil, revealed 2 species of atractid nematodes in the intestine of this fish. The present paper includes the results of this survey, with the description of *Orientatractis moraveci* n. sp. and new morphological and ultrastructural data on *Rondonia rondoni* Travassos, 1920. In addition, new 18S rDNA and 28S rDNA sequences are presented with a phylogenetic analysis based on new and existing sequences of partial 18S rDNA.

## MATERIALS AND METHODS

Specimens of the catfish *P. blochii*, collected between November, 2013 and April, 2014 by local fisherman

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operating in the Acre River ($n = 120$) (10°39′40″S, 68°30′19″W) and the Xapuri River ($n = 120$) (10°38′20″S, 68°32′08″W), were transferred fresh to the laboratory. *Pimelodus blochii* from Acre River were 8·8–25·7 (18·58 ± 2·12) cm long and from Xapuri River were 13·7–23·2 (14·27 ± 1·12) cm long. The organs were separated in Petri dishes and examined under a stereomicroscope. Prevalence and intensity data follow Bush *et al*. (1997). The nematodes recovered were washed in physiological saline and then fixed in 70% alcohol or hot 4% formaldehyde solution. For light microscopy, the nematodes were cleared with glycerine. Drawings were made with the aid of a Leica drawing attachment. After examination, the specimens were stored in vials of 70% ethanol. Measurements are presented in micrometres as the range followed by the mean in parentheses, unless otherwise stated. Specimens were deposited in the Helminthological Collection of the Instituto Oswaldo Cruz (CHIOC), Rio de Janeiro, Brazil.

For scanning electron microscopy (SEM), specimens were postfixed for 24 h at room temperature in a solution of 1% osmium tetroxide and 0·8% potassium ferrocyanide, dehydrated through a graded alcohol series, critical-point dried and sputter-coated with gold. The samples were examined using a JEOL JSM-6390 LV SEM, from the Plataforma de Microscopia Eletrônica do Instituto Oswaldo Cruz, at an accelerating voltage of 15 kV.

### *Genetic analysis*

Genomic DNA was extracted using a QIAamp DNA Mini Kit (QIAGEN) according to the manufacturer's instructions. The partial region spanning 18S rDNA was amplified by polymerase chain reaction (PCR) using a newly designed set of primers, Atractid_18SF (5′-CGT ATC GTT GCG TGA GAG GTG-3′) and Atractid_18SR (5′-AAA GTG TCG AAA CAG CAT TCC-3′), and PCR condition was performed under the following conditions: 94 °C for 3 min, followed by 40 cycles of 95 °C, 30 s, 53·7 °C, 30 s, 72 °C, 60 s and 72 °C for 5 min. PCR assays were carried out in a total volume of 15 μL containing 7·5 μL of 2 × GoTaq® Colorless Master Mix (Promega), 0·5 μL $Mg^{2+}$ (50 mM concentration), 1·5 μL of each primers with final concentration at 0·5 μM, 2·0 μL of cDNA sample and ultrapure water to complete. The partial region spanning 28S rRNA was amplified by PCR using the primers C1 (5′-ACCCGCTGAATTTAAGCAT-3′) and D2 (5′-TGGTCCGTGTTTCAAGAC-3′) (Hassouna *et al*. 1984, after Chisholm *et al*. 2001) under the following conditions: 95 °C for 5 min, 40 cycles at 95 °C, 60 s, 56·2 °C, 45 s, 72 °C, 60 s and 72 °C for 5 min.

PCR products were visualized after electrophoresis on a 1·5% agarose gel stained with SYBR green (Invitrogen). Amplified PCR products were purified with ExoSAP-IT (Affymetrix) following the manufacturer instructions. DNA cycle sequencing reactions were performed using BigDye v.3.1 chemistry (Applied Biosystems). The sequencing was performed in an ABI Prism 3100 sequence analyser using the same primer set. Sequences of both strands were generated, edited and aligned using the CLUSTAL W algorithm of the MEGA 6.0 package (Thompson *et al*. 1994; Tamura *et al*. 2011). The comparison for similarities with sequences from GenBank was performed using BLAST 2.0 (Altschul *et al*. 1990). The best-fit substitution model of partial 18S dataset was GTR + I + G model of nucleotide substitution selected under the Akaike information criterion (AIC) using MrModelTest 2 with aid of PAUP4.0a147 (Nylander, 2004). Tree reconstructions were carried out using Bayesian Inference (BI) via the software MrBayes 3.2.6, where the Markov chain Monte Carlo (MCMC) was set to $2 \times 10^6$ generations, every 100th tree was sampled and the first $10^5$ generations were omitted from phylogeny reconstruction; gamma shape parameter and proportion of invariants (PINVAR) were estimated from the dataset (Ronquist and Huelsenbeck, 2003). The remaining trees were used to generate a consensus tree and to calculate the Bayesian posterior probabilities (Bpp) of all branches using a majority-rule consensus approach. Maximum-likelihood (ML) analysis was constructed with PhyML 3·1 and nodal support was estimated by performing 1000 bootstrap replicates (Swofford, 2002). Tree topologies were visualized in FigTree 1.4.2 (2014). Taxa, for which sequences from GenBank were used for phylogenetic analysis, were KR139827 *Spectatus spectatus*, U94371 *Cruzia americana*, DQ503461 *Raillietnema* sp., EF375487 *Labeonema* sp., DQ118537 *Nemhelix bakeri*, LC018444 *Cosmocercoides pulcher*, DQ442679 *R. rondoni*, EF375487 *Labeonema synodontisi* and AF036607 *Teratocephalus lirellus* (as Outgroup), in addition to the new sequences of KX524511 *R. rondoni* and KX524513 *O. moraveci* n. sp.

## RESULTS

A total of 240 *P. blochii* were examined, 120 from the Xapuri River and 120 from the Acre River. The two atractid nematode species found are described below.

*Orientatractis moraveci* n. sp. (Figs 1–3)

Cosmocercoidea Railliet, 1916

Atractidae Railliet, 1917

*Orientatractis* Petter, 1966

*Type host: P. blochii* Valenciennes 1840.

*Site*: Intestine.

*Prevalence*: Acre River: 13% (16 infected fishes of 120 examined). Xapuri River: 23% (28 infected fishes from 120 examined).

*Intensity of infection*: Acre River: 1–138 (26); Xapuri River: 1–273 (29).

*Mean abundance:* Acre River: 4; Xapuri River: 7.

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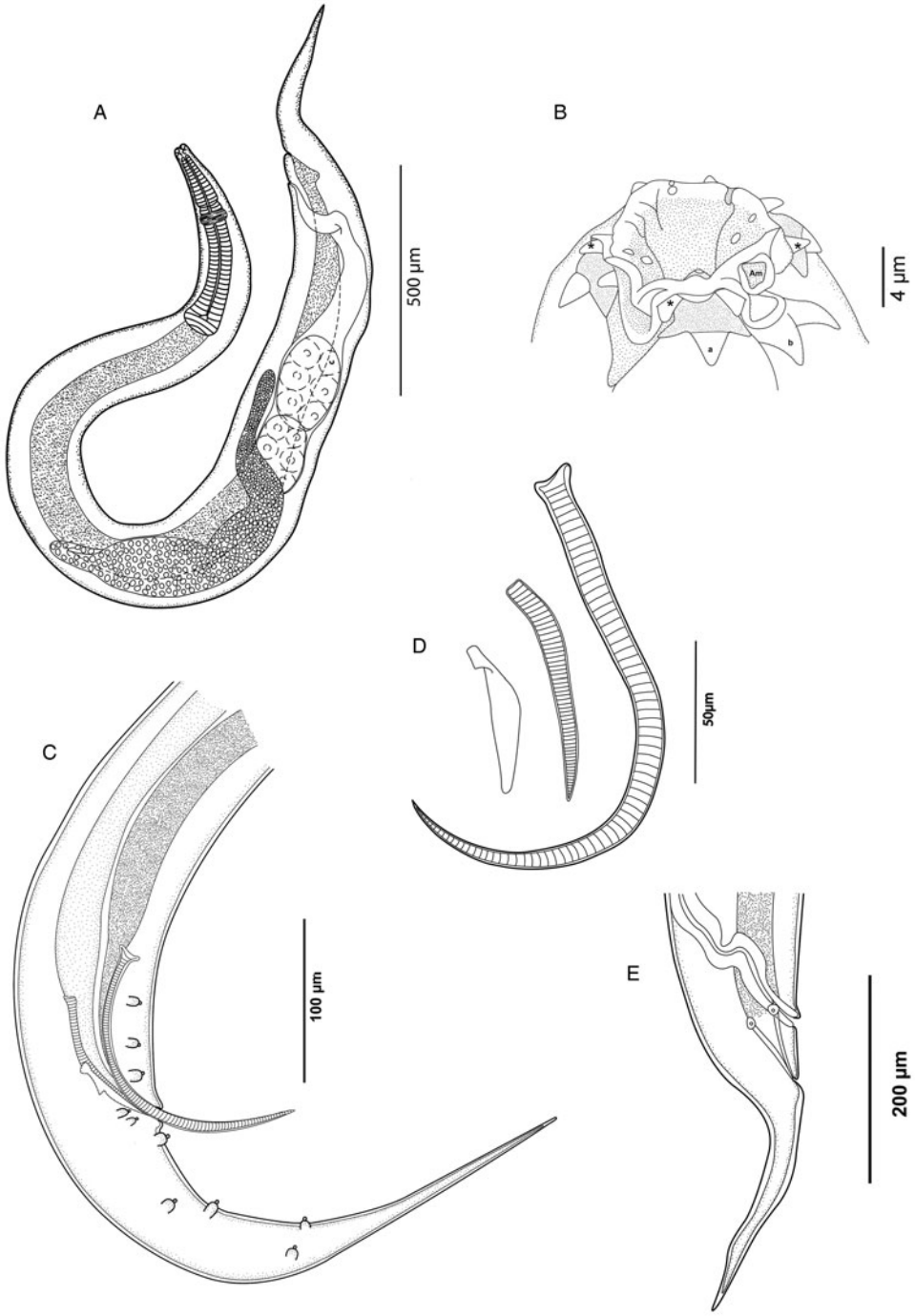


Fig. 1. *Orientatractis moraveci* n. sp. (A) Whole body showing oesophagus and sharply pointed tail; (B) cephalic end with four submedian lips with bicornuate (asterisks) and simple horns (a); two lateral lips with inverted U-shaped structure (b); amphids (Am) and small papillae surrounding the oral opening; (C) tail of male with spicules, gubernaculum and papillae; (D) detail of spicules and gubernaculum; (E) female tail with vulva and anal aperture.

*Type locality*: Xapuri River, Acre State, Brazil.

*Other locality:* Acre River, Acre State, Brazil.

*Material deposited:* CHIOC numbers 38326a (holotype) and 38326b (alotype) and 38326c (paratypes).

### *Morphological and ultrastructural data*

Small sized whitish nematodes. Cuticle with transverse striations. Cephalic end of body blunt; posterior end of body slender, with very long, sharply pointed tail (Figs 1A, 2A and E). Oral opening surrounded by 2 lateral amphids and 8 papillae arranged in 2 circles (1B, 2B–D). Cephalic end with 6 lips: 4 submedian lips with bicornuate structures (2 subdorsal and 2 subventral) and single horned structure immediately posterior each, and 2 lateral lips with sclerotised, inverted U-shaped structure with free tips (Figs 1B, 2B–D). Large lateral amphids are located anterior to inverted U-shaped structure (Figs 2B and C). Dorsal and ventral grooves separate

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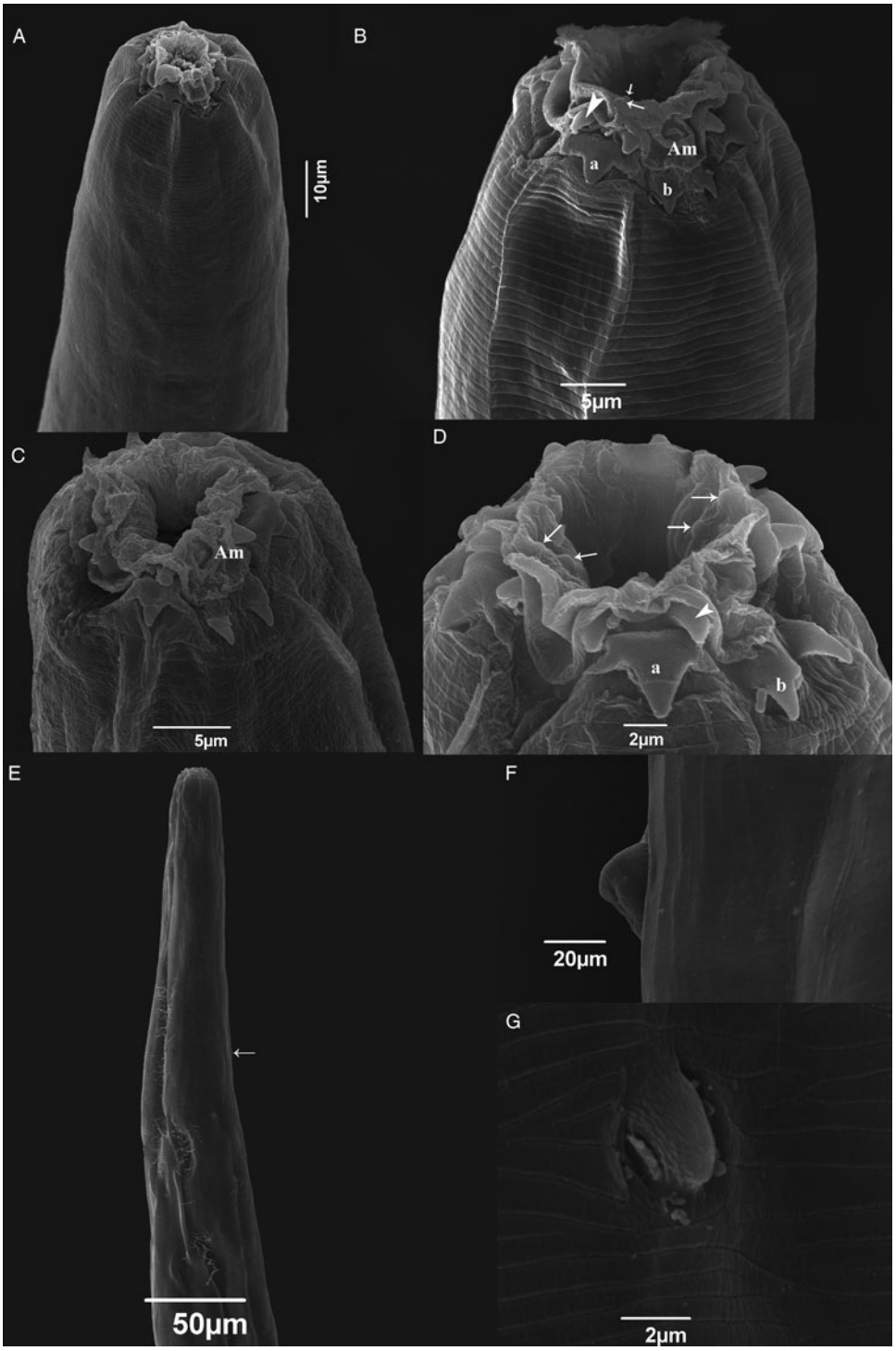


Fig. 2. Scanning electron microscopy of *Orientatractis moraveci* n. sp. (A) Anterior region of the body with oral aperture; (B) Cephalic end showing submedian lips with bicornuate (arrowhead) and single horns (a); lateral lips showing inverted U-shaped structure with free tips (b), amphids (Am) and two rows of papillae (arrows); (C) lateral lip (l) with inverted U-shaped tips between two submedian lips (s) with single horn structure and amphid (Am); (D) detail of submedian lip with bicornuate tips (arrowhead) and single horn (a) and tips of lateral structure (b); (E) knob-like deirid (arrow) and excretory pore (*) in anterior third of the body; (F)–(G) detail of knob-like deirid.

the right and left sides of cephalic extremity (Fig. 2D).

*Male (measurements based on 10 adult specimens)*. Body 2·09–3·13 (2·55) mm long; maximum width 95–135 (118). Length of entire oesophagus 271–386 (362); anterior part of oesophagus (corpus) 120–138 (133) long and 20–30 (26) wide; posterior part, including bulb, 138–253 (230) long and 50–75 (62) wide. Nerve ring and excretory pore 103–200 (155) and 126–208 (162), respectively, from anterior extremity (Figs 2E, G). Deirids located 238–333 (279) from

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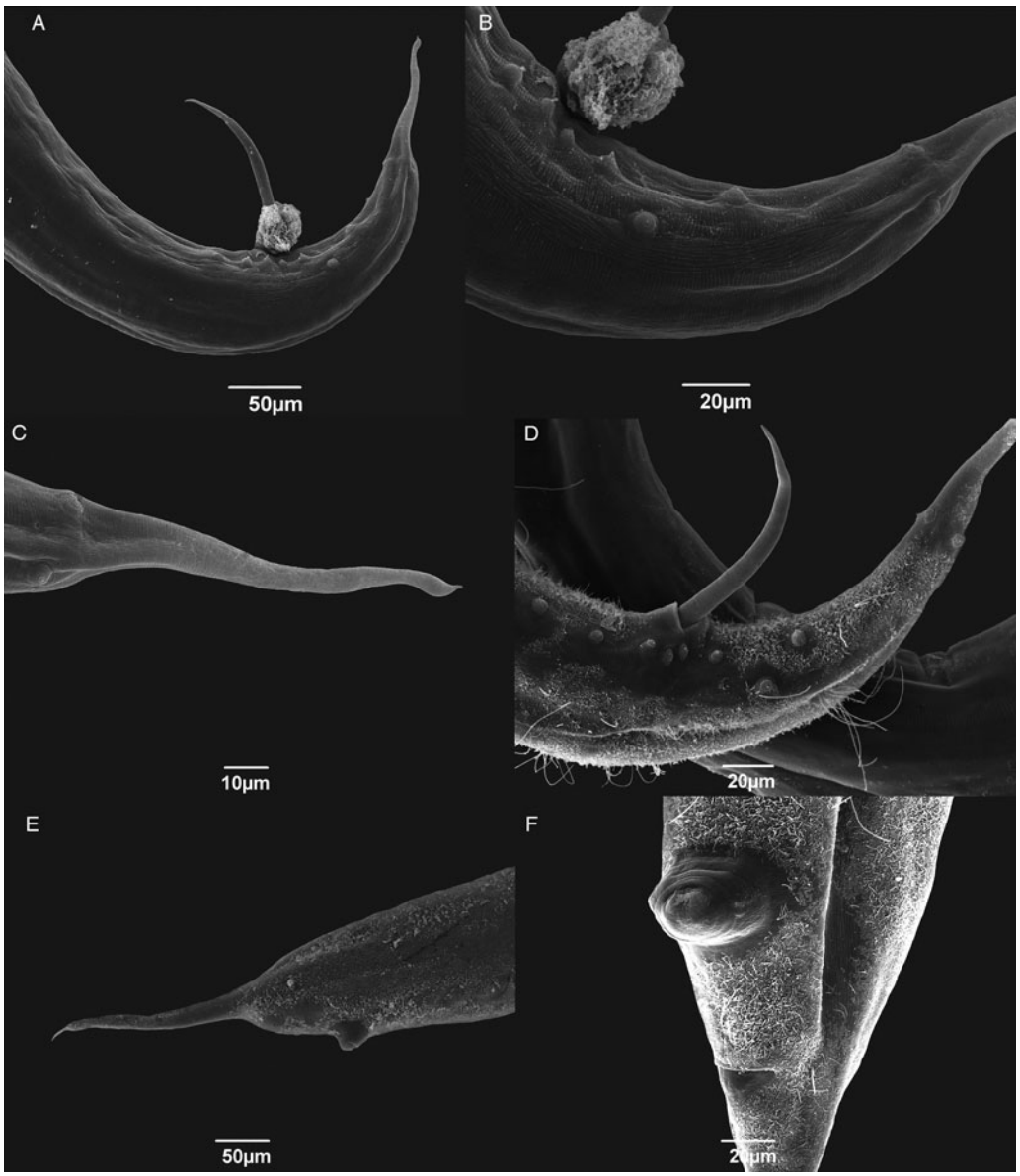


Fig. 3. Scanning electron microscopy of *Orientatractis moraveci* n. sp. (A) general view of posterior region of male showing spicule and papillae; (B) detail of ad-cloacal and post-cloacal papillae and lateral longitudinal cuticular striation; (C) detail of tip tail of male; (D) male caudal papillae in lateral view; (E) lateral view of posterior region of female showing protuberant vulva and pointed tail; (F) detail of protuberant vulva and anus.

anterior extremity (Figs 2E and F). Tail slender, 225–270 (252) long, sharply pointed (Figs 1C, 3A and C). Longitudinal cuticular striations observed laterally on posterior extremity (Figs 3A and B). There are 10 pairs of caudal papillae: 3 subventral pairs precloacal, 2 pairs ad-cloacal (sometimes only 1 pair is visible) and 5 pairs post-cloacal. The post-cloacal are disposed as: 1 pair subventral followed by 1 pair lateral, and 1 pair subventral; 2 last pairs (1 subventral and 1 lateral) are close to each other, at the end of the second third of length of tail (Figs 1C, D, 3A–D). Spicules unequal, well sclerotized; larger (left) spicule 161–198 (181) long; smaller (right) 69–100 (87) long (Figs 3A, B and D). Gubernaculum 38–58 (50) long, with anterior lateral process (Figs 1C and D).

*Female (measurements based on 10 specimens).* Body 1·98–2·71 (2·39) mm long; maximum width 142–200 (172) (Fig. 1A). Length of entire oesophagus 322–400 (360); anterior part of oesophagus (corpus) 101–147 (132); posterior part of oesophagus, including bulb, 202–253 (231) (Fig. 1A). Nerve ring and excretory pore 115–184 (135) and 138–232 (181), respectively, from anterior extremity. Deirids 230–333 (275) from anterior end. Vulva with extended lips, situated 57–76 (67) anterior to anus (Figs 1E, 3E and F). Vagina short, oriented anteriorly. Monodelphic uterus contains developing eggs, 175–266 (218) long and 92–142 (117) wide, and free larvae (Fig. 1A). Tail 237–294 (272) long.

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*Genetic data*. Two new sequences of *O. moraveci* n. sp. were obtained in this study and were deposited in the GenBank under the accession numbers KX524513 (18S rDNA region with 731 base pairs) and KX524514 (28S rDNA with 734 base pairs). The sequence most similar to *O. moraveci* n. sp. with respect to the partial 18S rDNA region was *Dirofilaria repens* (AB973229) with a 97% similarity, 100% query cover and a maximum score of 1229. For the new sequence of the partial 28S rDNA region, the most similar sequence was *C. pulcher* (LCO18444) with an 84% similarity, 79% query cover and a maximum score of 571.

*Remarks*. *Orientatractis moraveci* n. sp. is the first species of the genus parasitizing fish in South America. A comparison of the data between *O. moraveci* n. sp. and the other species of the genus shows that the new species is mainly distinguished from its congeners by having: 10 pairs of caudal papillae (3 pairs pre-cloacal, 2 pairs ad-cloacal and 5 pairs post-cloacal); unequal spicules 161–198 and 69–100 long; and a gubernaculum 38–58 long with an anterior lateral process.

*Etymology*. The new species is named for Prof. Frantisek Moravec, from the Czech Academy of Sciences, Czech Republic, in recognition of his valuable contributions to our knowledge of the nematode parasites of fishes and his willingness to work with and teach researchers from all over the world.

*Rondonia rondoni* Travassos, 1920 (Figs 4–6)

*Site*: Intestine.

*Host: P. blochii* Valenciennes 1840.

*Prevalence*: Acre River: 8% (9 infected fishes from 120 examined); Xapuri River: 19% (23 infected fishes from 120 examined).

*Intensity of infection*: Acre River: 1–20 (6); Xapuri River: 1–226 (29).

*Mean abundance:* Acre River: 0·5; Xapuri River: 6.

*Locality*: Acre and Xapuri Rivers, Acre State, Brazil.

*Material deposited:* CHIC n. 38324 (vouchers)

*Morphological and ultrastructural data*. Medium sized nematodes. Cuticle smooth, transversally striated. Head end rounded. Mouth with 3 rudimentary bi-lobed lips (1 dorsal and 2 ventral) surrounded by inner and outer circles each of 4 papillae and 2 amphids (Figs 4A, 5A and B).

*Male (measurements based on 10 specimens)*. Body 3·9–4·9 (4·4) mm long and 165–230 (204) wide. Oesophagus 555–610 (589) in total length; anterior end forms pharynx 13–20 (17) long; oesophageal corpus 310–340 (326) long and 43–68 (54) wide; posterior part of oesophagus, including bulb, 240–275 (263) long and 50–105 (63) wide at anterior narrow part; bulb 88–130 (104) long and 88–103 (93) wide. Nerve ring and excretory pore 358–457 (406) and 715–1040 (902), respectively, from anterior extremity. Tail conical, slender, 230–325 (278) long (Figs 4B, 5C and D). Caudal papillae: 4 pairs pre-cloacal (3 simple and 1 double pair), 2 pairs ad-cloacal and 6 pairs post-cloacal (4 subventral and 2 lateral) (Figs 4B, 5D–F). Spicules unequal and dissimilar; large spicule 150–183 (165) long; small spicule 78–100 (87) long; gubernaculum simple, 33–40 (36) long (Figs 4B and C). SEM observations demonstrated 2 parallel ventral rows of minute spines on posterior third of body, ending close to cloaca (Fig. 5C).

*Female (measurements based on 10 specimens)*. Body of gravid females 4·65–7·02 (5·57) mm long and 330–460 (407) wide (Fig. 4D). Oesophagus 615–695 (658) in total length; anterior pharynx 13–18 (16) long; oesophageal corpus 345–380 (367) long and 53–75 (66) wide; posterior part, including bulb, 270–315 (291) long and 60–95 (74) wide at narrowest region; bulb 108–123 (115) long and 108–120 (116) wide (Figs 4D and E). Nerve ring and excretory pore 430–470 (450) and 830–1240 (991), respectively, from anterior extremity. Ovary in mid-body; uterus contains developing eggs, 305–435 (364) long and 185–220 (203) wide, and free larvae; vagina short; vulva opens into rectum (Figs 4D–F, 6A and B). Tail conical, slender, 230–450 (322) long, with different patterns of cuticular striations close to tip (Figs 4F and 6C).

*Genetic data*. A new sequence of 28S rDNA of *R. rondoni* obtained in this study was deposited in the GenBank under the accession number KX524512 (722 base pairs). An additional sequence of the 18S rDNA region with 745 base pairs was also deposited under the accession number KX524511. The BLAST results for the new 28S rDNA sequence showed that the closest similarity was with *C. americana* (U94757) with a 86% similarity, a 55% query cover and a maximum score of 422. The result for the partial 18S rDNA sequence of *R. rondoni* indicated a 99% similarity, 100% query cover and a maximum score of 1360 with *R. rondoni* (DQ442679).

*Phylogenetic tree of the Cosmocercoidea*

Our sequences of the partial 18S rDNA gene of *R. rondoni* and *O. moraveci* n. sp. were used in a phylogenetic analysis with other sequences from the Cosmocercoidea previously deposited in the GenBank, with the free-living nematode *T. lirellus* used as the outgroup (Fig. 7). The topology of the tree included representatives of three families: Atractidae, Cosmocercidae and Kathlaniidae. Atractids, represented by *R. rondoni* and *O. moraveci* n. sp. sequences, compose a clade with a statistical

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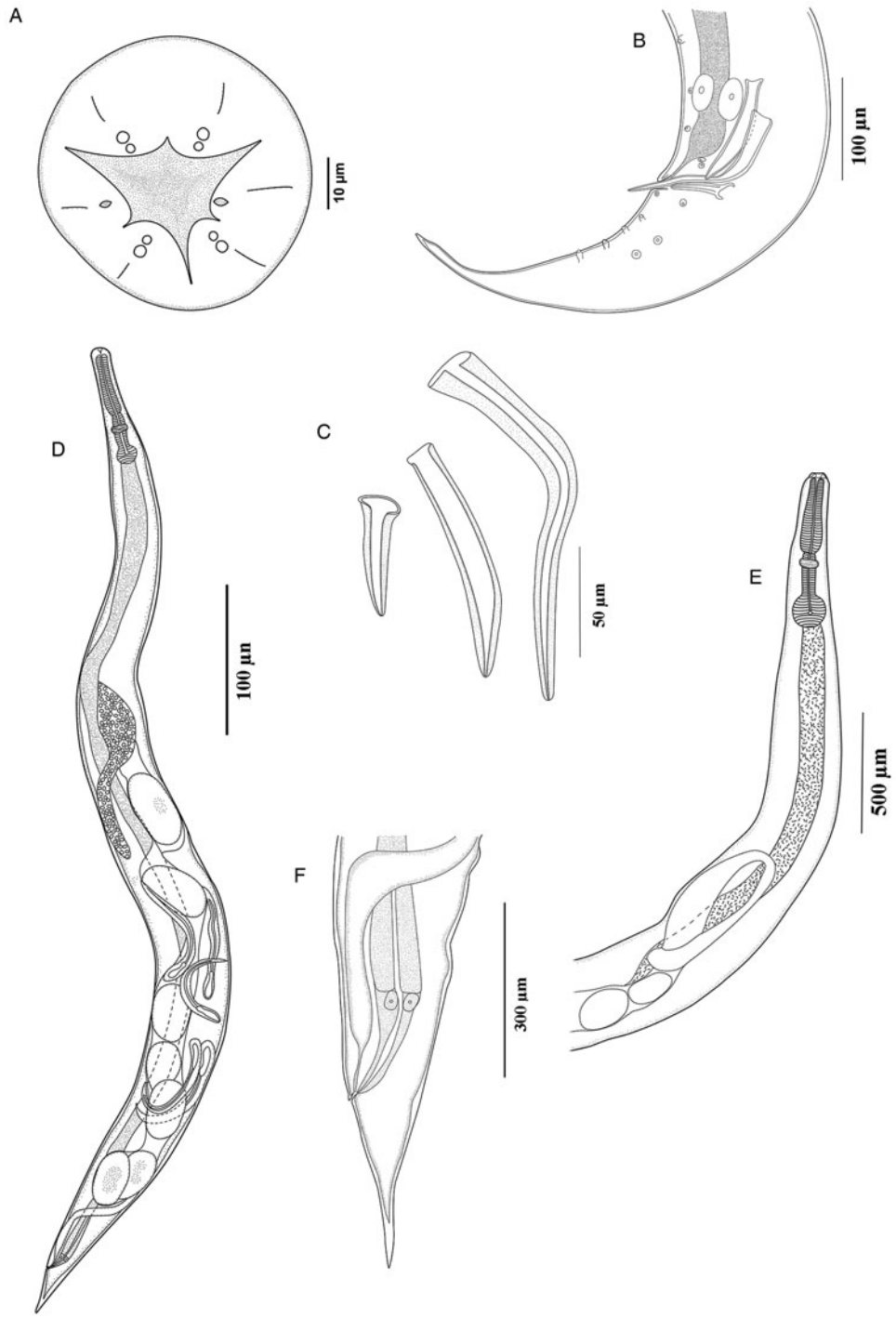


Fig. 4. *Rondonia rondoni*. (A) Three rudimentary bi-lobed lips surrounded by inner and outer circles of four papillae and two amphids; (B) tail of male with spicules, gubernaculum and papillae; (C) detail of spicules and gubernaculum; (D) entire body of female showing oesophagus, eggs and larvae; (E) detail of the oesophagus; (F) tail of female with common aperture.

support of 0·88 (Bpp) and 82% (ML). The cosmocercid clade with *N. bakeri, C. pulcher* and *Railletnema* sp., had a statistical support of 0.0.93 (Bpp) and 68% (ML). *Cruzia americana*, a kathlaniid, was in the same clade with the cosmocercids (Bpp = 1 and ML = 97%), whereas another kathlaniid, *S. spectatus*, appeared in a well separated clade (Bpp = 1; value not showed in ML). *Labeonema synodontisi*, an atractid, appears close to *Raillietnema* sp., nesting with the Cosmocercidae (Bpp = 1 and ML = 100%). Consequently, the Atractidae appears closer to the Cosmocercidae than to the Kathlaniidae (Bpp = 1 and ML = 100%).

## DISCUSSION

The *Orientatractis* Petter, 1966 and *Klossinemella* Costa, 1961 are close related genera differing in the

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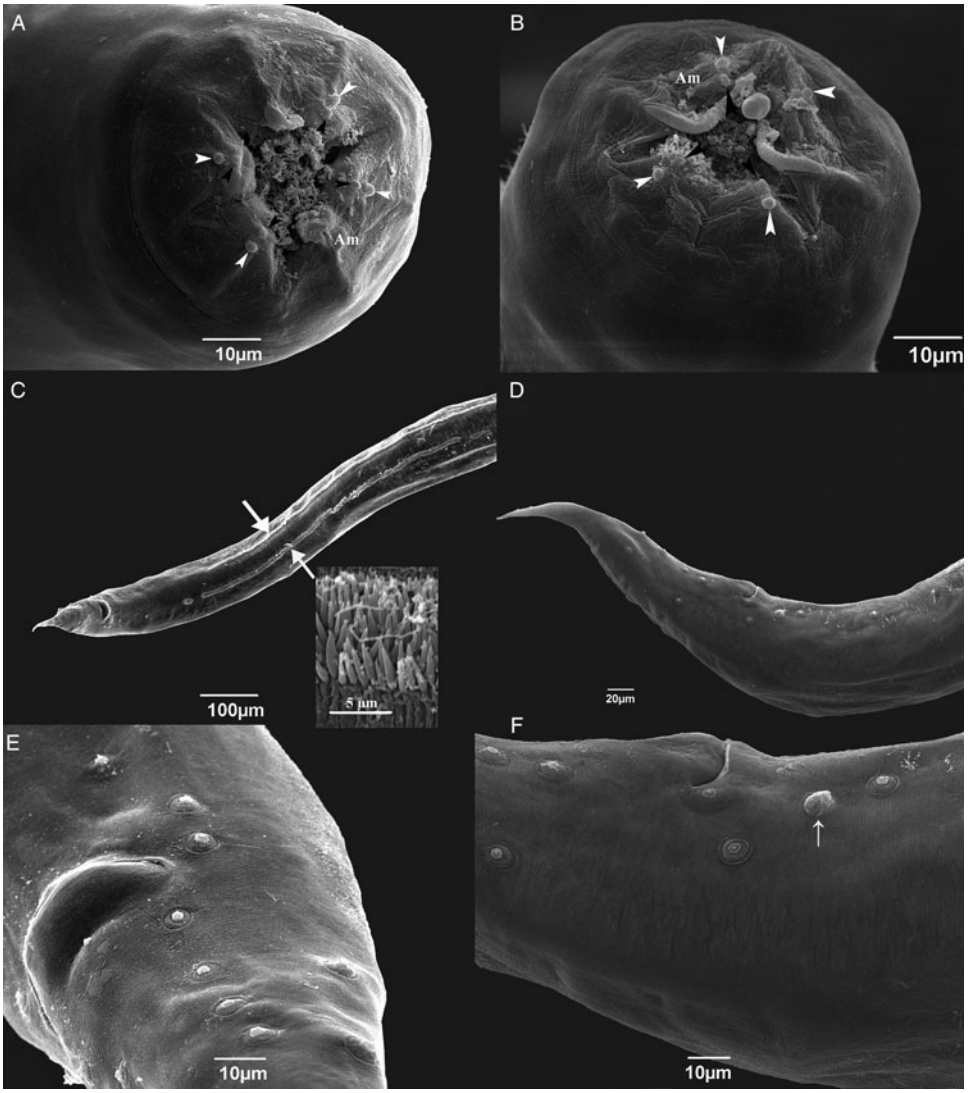


Fig. 5. Scanning electron and light microscopy of *Rondonia rondoni*. (A and B) Anterior region showing three bi-lobed lips, oral aperture, inner (black arrowhead) and outer (white arrowhead) circles of four papillae and amphids (Am); (C) general view of posterior region of male with sharply pointed tail and two parallel sub-lateral bands of small spines (arrows); inset: detail of small spines; (D) male tail with papillae; (E) ventro-lateral view of papillae; (F) lateral view of posterior region showing double papilla (arrow).

number and shape of sclerotized structures surrounding the mouth. After González-Solís and Moravec (2004), members of *Klossinemella* have 8 Y-shaped sclerotized structures and 4 single horns whereas in *Orientatractis* there are 4 bicornuate pieces and 4 single horns. The *O. moraveci* n. sp. is included in this genus by the presence of 4 bicornuate pieces and 4 single horns.

Species of the genus *Orientatractis* have been reported parasitizing fish, reptiles and anurans from Mexico, Thailand, Vietnam, Costa Rica and New Guinea (Petter, 1966; Buckley, 1969; González-Solís and Moravec, 2004; Gibbons and Platt, 2006; Bursey *et al*. 2014; Moravec *et al*. 2015). Currently, this genus comprises the species *Orientatractis levanhoai* Petter, 1966, *Orientatractis leiperi* Buckley, 1969, *Orientatractis campechensis* González-Solís and Moravec, 2004, *Orientatractis chiapasensis* González-Solís and Moravec, 2004, *Orientatractis asymmetrica* Gibbons and Platt, 2006, *Orientatractis hamabatrachos* Bursey *et al*. 2014 and *Orientatractis mekongensis* Moravec *et al*. 2015. Only three of these species parasitize fishes: *O. chiapasensis* from *Vieja intermedia* and *Tomocichla tuba*, and *O. campechensis* from *Vieja bifasciata*, all cichlid fishes from México, and *O. mekongensis* from *Helicophagus leptorhynchus*, a pangasiid fish from Thailand. *Orientatractis moraveci* n. sp. represents the first report of a species of the genus parasitizing a pimelodid fish in South America.

*Orientatractis moraveci* n.sp. differs from the *O. chiapasensis* mainly by the size of the large spicule (161–198 *vs* 204–238 µM), distance vulva–anus (57–76 *vs* 92–129 µM) and the size of tail of both males (225–270 *vs* 460–505 µM) and females (237–294 *vs*

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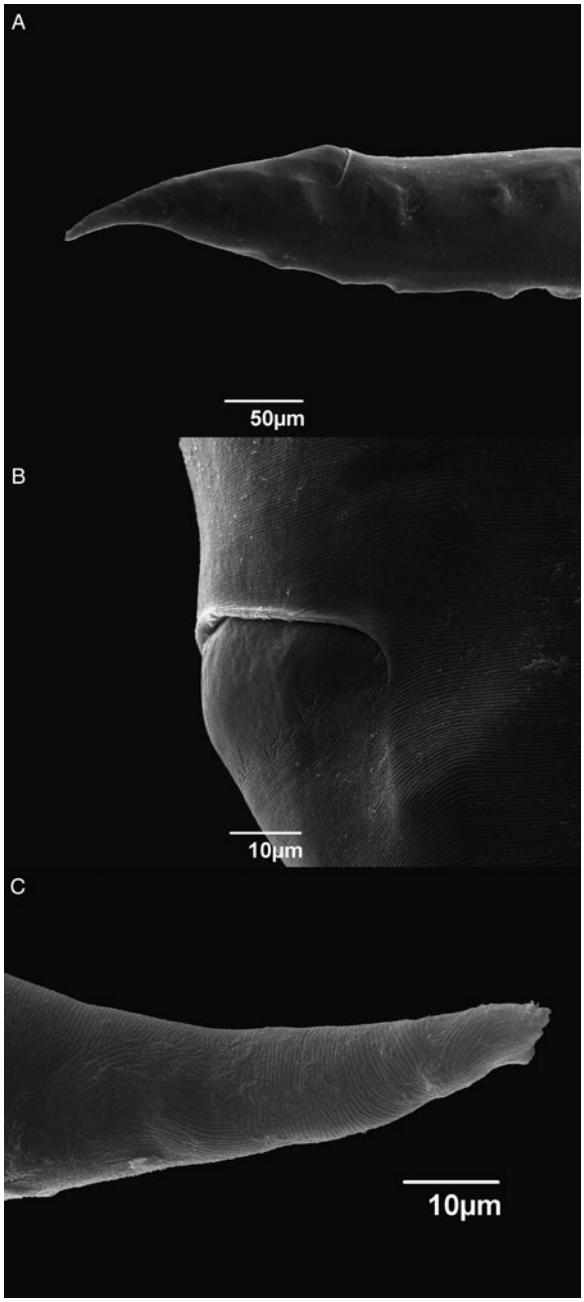


Fig. 6. Scanning electron microscopy of females of *Rondonia rondoni*. (A) posterior region of female showing cloacal aperture; (B) detail of cloacal aperture; (C) female tail tip detail showing different patterns of cuticular striations.

380–722 µM). It is also differentiated from *O. campechensis* in the size of large spicule (161–198 *vs* 430–506) µM, the vulva-anus distance (57–76 *vs* 76–106 µM) and the size of tail of the males (225–270 *vs* 608–722 µM) and females (237–294 *vs* 866–1246 µM). Compared with these species, the number of post-cloacal papillae is also different (5 pairs *vs* 4 pairs). In the case of *O. mekongensis*, in addition to the host and considerable geographical separation, differences included the body length of the male (2099–3135 *vs* 5350–6660 µM) and female (1985–2710 *vs* 7750–8950 µM), the size of the large spicule (161–198 *vs* 306–384 µM), the vulva-anus distance (57–76 *vs* 108–150 µM) and the size of tail of the males (225–270 *vs* 435–680 µM) and females (237–294 *vs* 952–1074 µM).

*Orientatractis moraveci* n. sp. differs from *O. hamabatrachos*, an amphibian parasite from New Guinea, mostly by the vulva-anus distance (57–76 *vs* 102–153 µM) and the size of the tail of the males (225–270 *vs* 306–408 µM) and females (237–294 *vs* 510–612 µM). The species parasitizing reptiles include *O. asymmetrica* from Costa Rica, which differs in the body length of the males (2099–3135 *vs* 4050–4290 µM) and females (1985–2710 *vs* 3740–5500 µM), the vulva-anus distance (57–76 *vs* 100–112 µM) and the size of the tail of the males (225–270 *vs* 836–952 µM) and females (237–294 *vs* 820–1080 µM). The other reptile parasite, *O. levanhoai*, can be differentiated mainly by the body length of the males (2099–3135 *vs* 3300 µM) and females (1985–2710 *vs* 3400 µM) and the size of the tail of the males (225–270 *vs* 800 µM) and females (237–294 *vs* 1000 µM). The number of male caudal papillae reported in the species from amphibians and reptiles was also different.

*Rondonia rondonia* was briefly described by Travassos (1920), with more detailed data being given a few years later (Travassos *et al*. 1928). Subsequently, only two additional nominal species have been assigned to the genus, namely *Rondonia lophii* Gállego-Berenger, 1947, a fish parasite from Spain based only on female specimens, and *Rondonia batrachogena* Bursey *et al*. 2014, from amphibians in New Guinea. *Rondonia rondoni* has been reported from varies fishes, such as members of the Characidae, Mulidae, Douradidae and Pimelodidae, in different river basins, such as the Paraguay, Paraná, Amazonas and São Francisco (Gállego-Berenger, 1947; Costa, 1963; Moravec *et al*. 1992). Despite a number of previous studies, our SEM analyses enabled us to detect a pair of double papillae in the pre-cloacal region. Travassos *et al*. (1928), when described *R. rondoni*, reported the presence of two parallel, chitinous projections in the pre-cloacal region of the male homologous to those observed in species of the genus *Cosmocerca* Diesing, 1861. These structures, seen for the first time using the SEM, are composed of rows of minute spines directed posteriorly.

The two nematodes from *P. blochii* studied here from the Acre and Xapuri Rivers presented differences in terms of their ecological indices (prevalence, intensity and abundance). *Orientatractis moraveci* n. sp. from the Xapuri River showed a higher prevalence (13·33 *vs* 23·33%), mean intensity (26·31 *vs* 29·18) and mean abundance (3·51 *vs* 6·08). The same result was obtained for *R. rondoni*, with especially large differences in prevalence (7·5 *vs* 19·17%), mean intensity (6 *vs* 29·22) and mean abundance (0·45 *vs* 5·6). These data showed that

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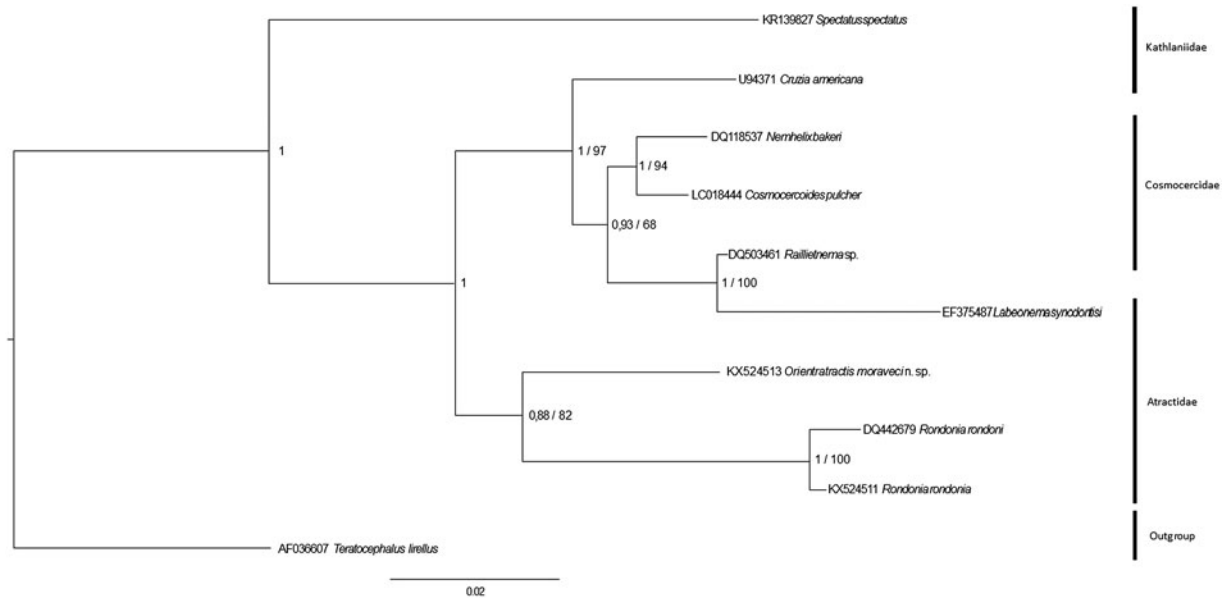


Fig. 7. Bayesian and ML trees from phylogenetic analyses of the 18S rDNA sequences from cosmocercoid nematodes (families Atractidae, Cosmocercidae and Kathlaniidae). First number represents branch support of Bayesian posterior probability (for $2 \times 10^6$ generations; burn-in 105); the second number shows the ML bootstrap value (from 1000 replications). ML, maximum-likelihood.

*O. moraveci* n. sp. and *R. rondoni* share the same host, site and geographical distribution, with increased ecological indexes in the Xapuri River, which is a smaller tributary of the Acre River.

In general, our phylogenetic reconstruction of the 18S rDNA region exhibited consistent data, with a high statistical support, for the studied representative members of the Cosmocercoidea. Species of this superfamily are able to parasitize a variety of hosts groups, such as freshwater fish (*S. spectatus, O. moraveci* n. sp. and *R. rondoni*), mammals (*C. americana*), amphibian (*Raillietnema* sp. and *C. pulcher*) and land snails (*N. bakeri*). Our sequences of *R. rondoni* and *O. moraveci* n. sp., together with a sequence of *R. rondoni* previously deposited in GenBank, grouped with the Atractidae clade with moderate statistical support. Additional analysis using a greater number of species of atractids might offer stronger phylogenetic support.

The new sequence of *O. moraveci* n. sp. confirms the position of this species within the Atractidae, with the Cosmocercidae as a sister group and the Kathlaniidae as a basal group. However, Pereira *et al.* (2015) found that the kathlaniid *C. americana* nested within the Cosmocercidae, and the inconsistent position of this species was also pointed out by Nadler *et al.* (2007) when analyzing the same 18S rDNA region. *Raillietnema* sp., *C. pulcher* and *N. bakeri* grouped within the Cosmocercidae with high Bpp support (0.0.93), but much less so for the bootstrap analysis (68%).

The position of *Labeonema synodotisi* (Vassiliadès, 1973) (=EF375487 *Labeonema* sp. in GenBank) collected from the African fish *Synodontis ocellifer* is also inconsistent. Koubková *et al.* (2008) proposed this new combination for this species, previously described as *Raillietnema synodontisi* Vassiliadès, 1973. However, Moravec and Van As (2004) pointed out that *R. synodontisi* differs from *Labeonema* spp. in possessing two ovaries instead of one, and its larvae do not hatch *in utero*. Pereira *et al.* (2015) also used this *Labeonema* sequence in a phylogenetic analysis of the Cosmocercoidea, considering it very similar to the cosmocercoid genus *Raillietnema* Travassos, 1927. *Labeonema synodontisi* also nested with *Raillietnema* sp. in our analysis, with strong support, using new and existing atractid sequences. Therefore, both molecules (present study and Pereira *et al.* 2015) and morphology (Moravec and Van As, 2004) suggest that *L. synodontisi* should be returned to its original genus as *R. synodontisi.*

The description of *O. moraveci* n. sp., a parasite of *P. blochii*, represents the first occurrence of a member of the genus in fishes of South America, and *R. rondoni* is reported for the first time parasitizing this same host from the Acre and Xapuri Rivers, Western Amazon, Brazil. New ultrastructural and molecular data derived from these species will assist the future phylogenetic analyses of groups within the Cosmocercoidea. The molecular data and previous morphological studies of *L. synodontisi* indicate that it should be returned to the genus *Raillietnema.*

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ETHICAL STANDARDS

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