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***Orientatractis brycini* sp. nov. (Nematoda: Atractidae) from characiform freshwater fishes in Gabon, Africa**

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Abstract: The nematode *Orientatractis brycini* sp. nov. (Atractidae) is described from the intestine of *Brycinus macrolepidotus* Valenciennes (Alestidae) and *Xenocharax spilurus* Günther (Distichodontidae) collected in two localities from Gabon, Africa. The new species is characterized by the presence of four submedian lips with well-sclerotized pieces armed with two recurved pointed spines and one median large spine on their distal part, along with two smaller spines posterior to amphidial pores. It differs from its congeners mainly in the length of both spicules, gubernaculum, presence of two lateral spines posterior to amphids, distribution and number of caudal papillae. An emended generic diagnosis is provided. This is the eighth species in the genus *Orientatractis*, the fourth from fish hosts and the first from Africa, which expands its geographical distribution.

Keywords: New species - nematode - *Brycinus* - *Xenocharax*.

INTRODUCTION

The family Atractidae Railliet, 1917 includes 26 genera separable into two groups according to the type of ovaries: *Atractis* Dujardin, 1845, *Buckleyatractis* Khalil & Gibbons, 1988, *Cobboldina* Leiper, 1911, *Crossocephalus* Railliet, 1909, *Cyrtosomum* Geddoelst, 1919, *Diceronema* Gibbons, Knapp & Krecek, 1995, *Grassenema* Petter, 1959, *Labeonema* Puylaert, 1970, *Labiduris* Schneider, 1866, *Leiperenia* Khalil, 1922, *Klossinemella* Costa, 1961, *Monhysterides* Baylis & Daubney, 1922, *Orientatractis* Petter, 1966, *Paraorientatractis* Gibbons, Khalil & Marinkelle, 1997, *Paratractis* Sarmiento, 1959, *Pneumotractis* Bursey, Reavill & Greiner, 2009, *Podocnematractis* Gibbons, Khalil & Marinkelle, 1995, *Proatractis* Caballero, 1971, *Pseudatractis* Yamaguti, 1961, *Pseudocyrtosomum* Gupta & Johri, 1987, *Rhinoceronema* Mondal & Manna, 2013, *Rhinoclemmysnema* Gibbons & Platt, 2006, and *Rondonia* Travassos, 1920 are in the monodelphic group; whereas *Fitzsimmons-nema* Petter, 1966, *Nouvelnema* Petter, 1959, *Probstmayria* Ransom, 1907, form the didelphic group (Adamson & Baccam, 1988; Bursey & Flanagan,

2002; Gibbons, 2010; Mondal & Manna, 2013). Of these, *Proatractis* was synonymized with *Klossinemella*, while *Cyrtosomum* and *Pseudatractis* were synonymized with *Atractis* (see Baker, 1987; Moravec & Thatcher, 1997), although Bursey & Flanagan (2002) retained both genera as valid.

During a short visit to the Muséum d'histoire naturelle de Genève in 2012, the examination of parasitic nematodes collected from freshwater fishes in Gabon, Africa, revealed the presence of very peculiar attractid specimens. These parasites were found in two characiform fishes, *Brycinus macrolepidotus* Valenciennes (Alestidae) and *Xenocharax spilurus* Günther (Distichodontidae) and belong to an unknown species of *Orientatractis*, which is described herein.

Brycinus macrolepidotus and *X. spilurus* are freshwater fishes that inhabits rivers and lakes in Lower Guinea, from Cameroon to the Chiloango River Basin, the Nile system and the Democratic Republic of Congo. They feed on insects, crustaceans and vegetation (Froese & Pauly, 2016).

MATERIAL AND METHODS

Fishes were collected by angling in 2 localities from the Franceville area, Southeast Gabon, in November 2010. All nematode specimens recovered were washed in physiological saline, fixed in hot 4% formaldehyde solution and cleared in different ratios of glycerine-water mixture for light microscopy. For scanning electron microscopy (SEM), specimens were postfixed 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). Drawings were done with the aid of a camera lucida. All measurements are in micrometers, unless otherwise indicated. Scientific names of fishes follow Froese & Pauly (2016). All collections were made in the frame of a Scientific Research Convention between the Centre International de Recherches Médicales de Franceville (CIRMF) and the Muséum d'histoire naturelle de Genève (MHNG) with the research permit AR0017/09/MESRSDT/CENAREST/CG/CST/CSAR.

TAXONOMIC PART

Family Atractidae Railliet, 1917
Genus *Orientattractis* Petter, 1966

Type species: *O. levanhoai* Petter, 1966

Emended generic diagnosis: Small nematodes with a complex structure of the apical extremity. Oral opening surrounded by 6 (2 lateral and 4 submedian) poorly-developed lips. One or two circlets of oral papillae. Each submedian lip bearing a chitinous piece formed by two well-sclerotized, recurved, pointed spines and a single large median spine. Lateral lips small, supporting large amphids; two small spines posterior to each amphidial pore present or not. Grooves or narrow lateral alae present, extending from first third of esophagus to posterior end of body, but not reaching tail tip. Esophagus divided in a cylindrical corpus, elongated isthmus, and posterior, well-developed or not, valved bulb. Nerve ring surrounding isthmus at its anterior end. Deirids small, knob-like, somewhat anterior or at level of nerve ring. Excretory pore at mid-length of corpus or slightly anterior to esophageal bulb. Tail long and thread-like. Males with two unequal, similar spicules and small gubernaculum. Females monodelphic, viviparous, with vulva near anus. Parasites of freshwater turtles, frogs and fish.

***Orientattractis brycini* sp. nov.**

Figs 1-3

Deposition of specimens: Holotype (MHNG-INVE-91071), allotype (MHNG-INVE-91072) and pa-

ratypes (MHNG-INVE-91073) in the Muséum d'histoire naturelle, Geneva. – Paratypes in the Helminthological Collection of the Institute of Parasitology, Biology Centre, Czech Academy of Sciences, České Budějovice (Cat. No. N-1072).

Type host: *Brycinus macrolepidotus* Valenciennes (Alestidae, Characiformes) (Body length 21.4 cm).

Other host: *Xenocharax spilurus* Günther (Distichodontidae, Characiformes) (Body length 15.5-20.2 cm).

Site of infection: Intestine.

Type locality: Bridge on Ogooué River, Haut-Ogooué, Gabon (01°38'24"S; 13°31'48"E; elev. 300 m), collected on 28/11/2010.

Other localities: Mpassa River, near Hotel Poubara, Franceville, Haut-Ogooué, Gabon (01°37'12"S; 13°36'00"E; elev. 300 m), 30/11/2010.

Prevalence and intensity: *Brycinus macrolepidotus*: prevalence 25% (1 fish infected/4 examined), mean intensity 24 nematodes (range 24). *Xenocharax spilurus*: 43% (3/7), 4.3 (2-8).

Etymology: The specific name relates to the generic name of the fish host (i.e., *Brycinus*).

Description

General: Whitish, small-sized nematodes, with cuticle finely transversely striated. Anterior end rounded, posterior end with very slender, long, pointed tail (Fig. 1A, E). Oral opening rhomboid or quadrangular, with 2 lateral and 4 submedian poorly-developed lips (Figs 1D, 2A, B). Each submedian lip bearing one large spherical papilla and external pair of well-sclerotized, recurved, pointed spines joined at the base and a single large median spine. Lateral lips supporting large amphids; two small spines posterior to each amphidial pore present (Figs 1D, 2A-D). Lateral grooves extending from first third of esophagus to posterior end of body, but not reaching tail tip (Figs 1E, G, 2F). Esophagus divided in a cylindrical corpus, elongated isthmus, and posterior, well-developed, valved bulb (Fig. 1A, B). Nerve ring surrounding isthmus at its anterior end. Deirids small, knob-like, somewhat anterior or at level of nerve ring (Figs 1A, B, 2E). Excretory pore anterior to esophageal bulb (Fig. 1A, B). Intestine straight. Rectum a hyaline tube.

Male (22 specimens, measurements of holotype in parentheses): Length of body 2.58-3.07 (3.04) mm, maximum width 52-93 (72). Length of corpus 130-163 (150), of isthmus 289-346 (305); entire esophagus 436-507 (455). Width of esophageal bulb 33-47 (43). Nerve ring, excretory pore, and deirids 162-226 (178), 294-350 (310), and 175-199 (189), respectively, from anterior end of body. Eight pairs of caudal papillae: 1 subventral precloacal pair, 3 subventral adcloacal pairs, close to each other (one pair anterior to cloacal opening,

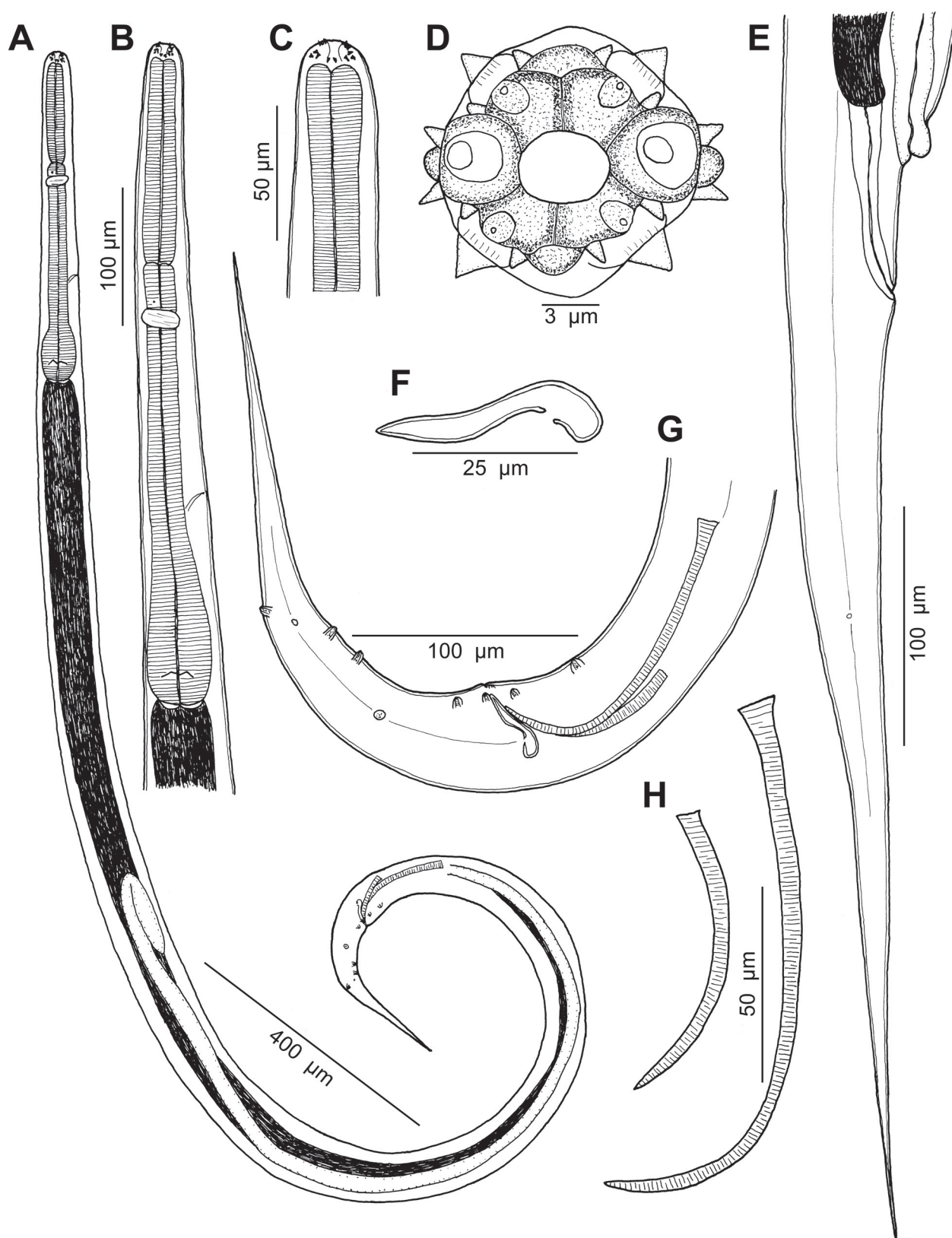


Fig. 1. *Orientatractis brycini* sp. nov. (A) Whole body of male, lateral view. (B, C) Anterior end of body, lateral views. (D) Cephalic end, apical view. (E) Posterior end of female, lateral view. (F) Gubernaculum, lateral view. (G) Posterior end of male, lateral view. (H) Spicules, lateral view.

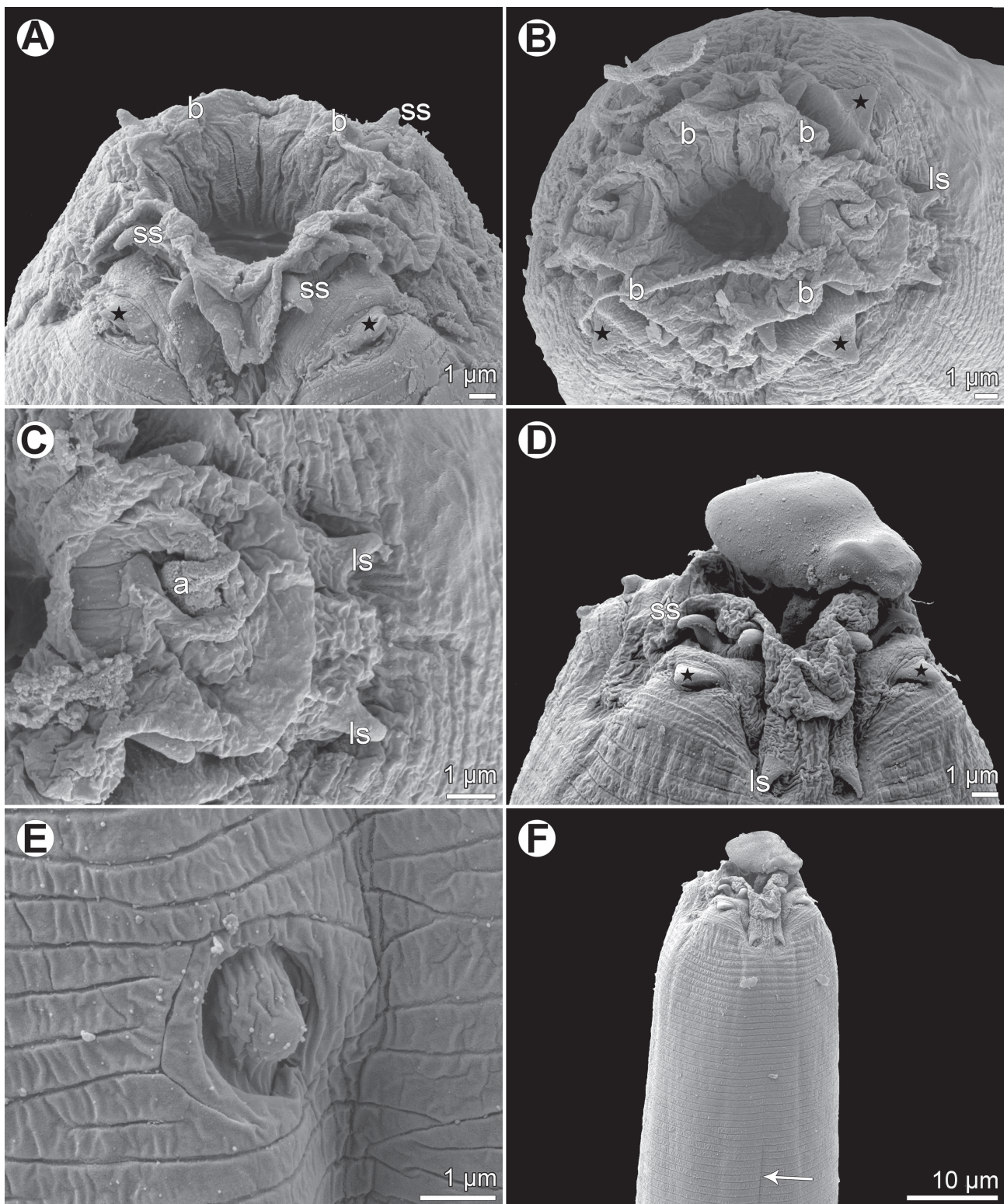


Fig. 2. *Orientatractis brycini* sp. nov., SEM micrographs. (A, B) Cephalic end, subapical and apical views, respectively (asterisks indicate single median spine). (C) Detail of lateral lip, amphid and lateral spines, apical view. (D) Cephalic end, sublateral view (asterisks indicate single median spine). (E) Deirid. (F) Anterior end of body (arrow indicates groove-like lateral ala). Abbreviations: a – amphid, b – cephalic submedian papilla, ls – lateral paired spine, ss – submedian paired spine.

one at same level and one posterior to it), 4 postcloacal pairs (first pair of postcloacals lateral, second and third pairs subventral and close to each other, fourth pair subdorsal) (Figs 1G, 3A). Pair of small, lateral outlets (probably representing phasmids) between pairs 3 and 4 of postcloacals (Fig. 3A, E). Single left-shifted papilla on anterior cloacal lip weakly-developed (Fig. 3C, D). Spicules unequal, similar, well-sclerotized. Left and right spicules 130-158 (148) and 75-90 (83) long, respectively. Both spicules with transverse striations along their lengths; proximal ends slightly expanded, distal ends sharply pointed (Fig. 1G, H). Gubernaculum 29-39 (37) long, well-sclerotized, proximal end rounded, with deep depression; distal end pointed and slightly ventrally curved (Fig. 1F). Tail 207-257 (229) long, with dorsal groove-like structure (Fig. 3B).

Female (13 gravid specimens; measurements of allotype in parentheses): Length of body 2.50-3.61 (3.30) mm, maximum width 60-129 (109). Length of corpus 126-170 (161), of isthmus 294-355 (318); entire esophagus 443-509 (479). Width of esophageal bulb 34-50 (43). Nerve ring, excretory pore, and deirids 158-221 (217), 292-347 (331), and 196-205 (-), respectively, from anterior end of body. Vulva with anterior lip slightly elevated, near the posterior end of body, 2.19-3.18 (2.91) mm from anterior end of body, somewhat anterior to anal opening (Figs 1E, 3F). Distance anus-vulva 39-72 (44). Vagina muscular, anteriorly directed. Uterus containing fully developed larvae 673-1176 (927) long; some females with developing eggs 236-305 × 93-110 (236-242 × 93-94). Tail 270-388 (348) long, with pore-like phasmids between first and second thirds of tail length (Fig. 1E).

Remarks

Petter (1966) erected the genus *Orientatractis* to allocate nematodes with a particular structure of the oral opening, specifically the presence of symmetrical groups of 3 sclerotized posteriorly directed spines surrounding mouth. Currently, this genus includes 7 valid species, namely: *O. asymmetrica* Gibbons & Platt, 2006 in *Rhinoclemmys pulcherrima* Gray (Testudines) from Costa Rica, *O. campechensis* González-Solís & Moravec, 2004 in *Paraneotroplus bifasciatus* (Steindachner) (reported as *Vieja bifasciata*) and *Cichlasoma pearsei* (Hubbs) (both Perciformes) from Southern Mexico, *O. chiapasensis* González-Solís & Moravec, 2004 in *Theraps intermedius* (Günther) (reported as *Vieja intermedia*) and *Tomocichla tuba* (Meek) (both Perciformes) from Southern Mexico, *O. hamabatrachos* Bursey, Goldberg & Kraus, 2014 in *Austrochaperina basipalmata* (van Kampen) (Anura) from New Guinea, *O. levanhoai* (type species) in *Indotestudo elongata* (Blyth) (reported as *Testudo elongata*) (Testudines) from Vietnam, *O. leiperi* Buckley, 1969 in *Podocnemis vogli* Müller (Testudines) from Colombia, and *O. mekongensis* Moravec, Kamchoo & Pachanawan, 2015 in *Pangasius bocourti* Sauvage (Siluriformes) from Thailand (Petter, 1966; Buckley,

1969; González-Solís & Moravec, 2004; Gibbons & Platt, 2006; Bursey *et al.*, 2014; Moravec *et al.*, 2015).

Even though the type species of the genus was not reviewed, we decided to emend the generic diagnosis, based on the already described species and present data, since several important features were not included in the original description (see Petter, 1966). Morphological features as the structure of the oral opening, presence of deirids, among others, were incorporated to the diagnosis for making it easier to distinguish *Orientatractis* from closely related genera (e.g., *Klossinemella* and *Paraorientatractis*) within the Atractidae. Thus, *Orientatractis* and *Paraorientatractis* have four bicornate submedian structures surrounding mouth, whereas *Klossinemella* shows eight pairs; the two first genera differ in the number of lips (6 vs. 4) and presence of ornamentations on the dorsal surface of body in *Paraorientatractis*. These changes do not modify the systematic position of the genus.

The four bicornate structures along with a pair of spines posterior to amphidial pore are only present in *O. brycini* sp. nov., *O. hamabatrachos*, and *O. leiperi*; while in *O. asymmetrica*, *O. campechensis*, *O. chiapasensis*, *O. levanhoai*, *O. mekongensis* are lacking.

Orientatractis brycini sp. nov. shows similar body length to that of *O. chiapasensis*, and is near the lower size range of *O. campechensis*, *O. leiperi* and *O. levanhoai*, whereas the remaining three species (*O. asymmetrica*, *O. hamabatrachos*, *O. mekongensis*) have larger bodies. However, *O. brycini* sp. nov. differs from all species within the genus in the size of both spicules (except in *O. hamabatrachos*), gubernaculum and number and distribution of caudal papillae (see Table 1).

The new species shares some similarities with *Paraorientatractis semiannulata* Gibbons, Khalil & Marinkelle, 1997, a nematode of *Podocnemis unifilis* Troschel (Testudines) in Brazil (Gibbons *et al.*, 1997). Both species harbour identical shape and structures surrounding mouth, such as each submedian lip with a pair of recurved pointed spines and single median spine near their distal margin, along with a pair of smaller spines posterior to amphidial pores. Moreover, both have two unequal, striated spicules, similar gubernaculum and number of caudal papillae. However, they differ in the ornamentations on the dorsal surface of body and striated, broad, well-developed lateral alae in *P. semiannulata*.

Caballero-Rodríguez (1971) described *Proatractis parvicapiticonata* from the tortoise *Staurotypus triporcatus* in Veracruz, Mexico. Later, this species was transferred to *Klossinemella* as *K. parvicapiticonata* by Moravec & Thatcher (1997). González-Solís & Moravec (2004) stated that it probably belongs to *Orientatractis* according to the shape of spicules, number and distribution of caudal papillae and structure of the anterior end, but until the type material of *K. parviticonata* is re-examined, it should be retained within the genus *Klossinemella*.

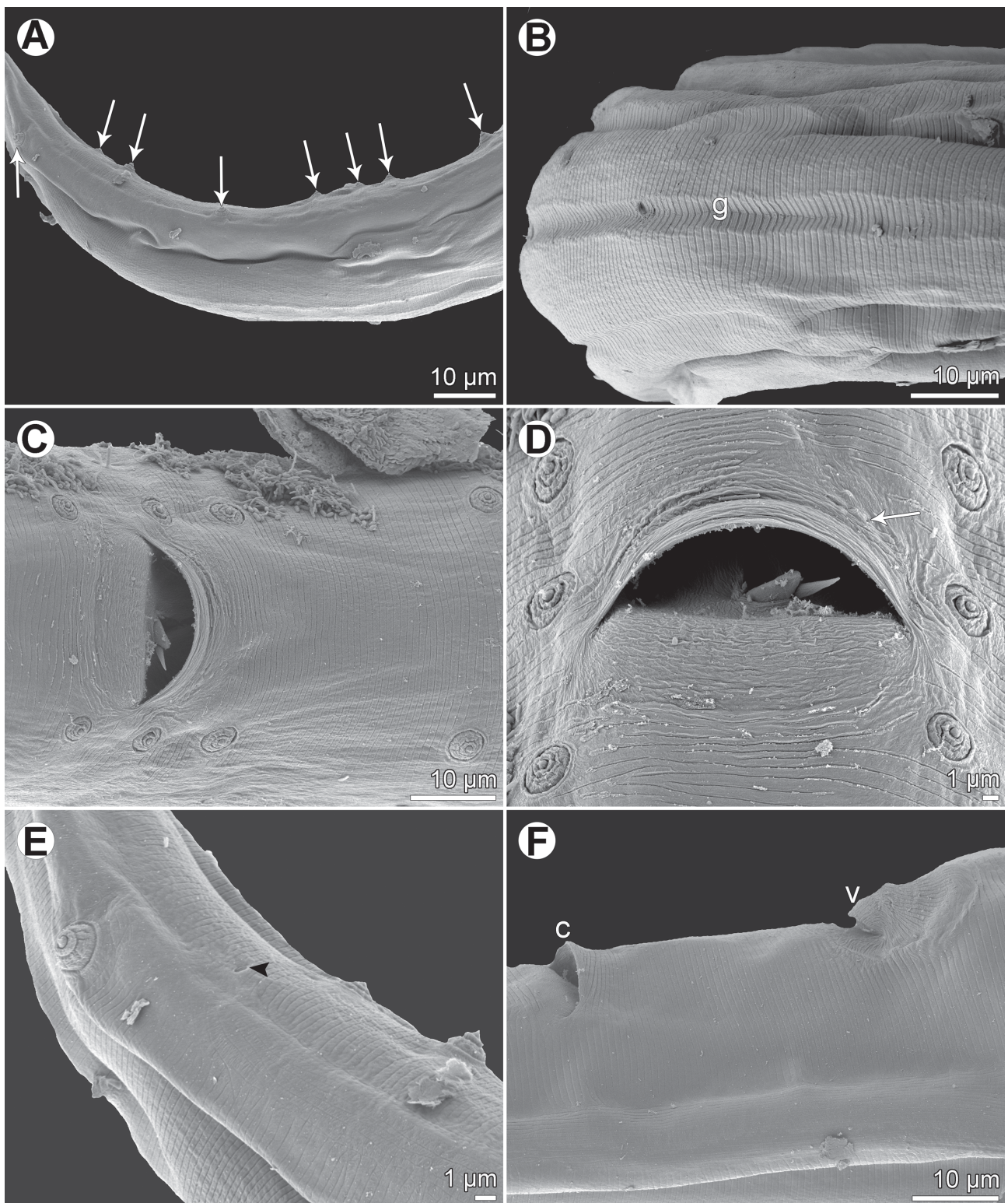


Fig. 3. *Orientatractis brycini* sp. nov., SEM micrographs. (A) Posterior end of male, sublateral view (arrows indicate caudal papillae). (B) Dorsal surface of male tail. (C, D) Region of cloaca, ventral views (arrow indicates left-shifted unpaired papilla). (E) Posterior end of male, lateral view (arrowhead indicates phasmids). (F) Region of anus and vulva, lateral view. Abbreviations: c – anus, g – groove-like dorsal structure, v – vulva.

Table 1. Comparison of some selected measurements of the valid species of *Orientattractis* around the world; measurements are in micrometers, unless otherwise stated.

	Body length (mm)		Spicule length		Gubernaculum length	Caudal papillae*
	male	female	right	left		
<i>O. asymmetrica</i>	4.1-4.3	3.7-5.5	86-104	220-239	56-66	4: 2: 3 + 1
<i>O. brycini</i> sp. nov.	2.6-3.1	2.5-3.6	75-90	130-158	29-39	1: 3: 4
<i>O. campechensis</i>	3.0-3.5	3.3-4.3	84-106	430-506	62-74	0: 5: 4 + 1
<i>O. chiapasensis</i>	2.7-3.0	2.0-3.8	60-68	204-238	44-49	0: 5: 4 + 1
<i>O. hamabatrachos</i>	3.2-4.3	3.4-4.9	79-98	146-165	55-67	2: 1: 5 + 1
<i>O. leiperi</i>	3.0-3.9	2.7-4.3	160	470	76	3: 1: 5 + 1
<i>O. levanhoai</i>	3.3	3.4	90	170	40	3: 0: 5
<i>O. mekongensis</i>	5.3-6.6	7.7-8.9	90-105	306-384	33-51	2: 1: 5

* pairs of caudal papillae (without considering phasmids): precloacal: adcloacal: postcloacal + single median papilla (left-shifted in *O. brycini* sp. nov.)

Interestingly, *O. brycini* sp. nov. was found in two fish species of the order Characiformes, but from different families (Alestidae and Distichodontidae) and sampling localities (Ogooue and Mpassa). Despite this, there were no differences in the morphology and biometrical values among the nematodes from both hosts, although certain morphometric variability always occurs intraspecifically. Such morphological and biometrical variability which might be associated with local ecological conditions and physiological traits of host species is not uncommon (see González-Solís & Moravec, 2004).

Nothing is known about the life cycle of these nematodes, but as in other members of Atractidae, larvae develop to the third stage in uterus, thus auto-infection is possible (Anderson, 2000). Viviparity has greatly helped attractid nematodes to parasitize several unrelated vertebrates (i.e., turtles, fish, amphibians, grazing mammals) by venereal and oral transmission (Baker, 1982), and to be distributed in different zoogeographical regions (America and Indonesia). The present finding represents the eighth species in the genus *Orientattractis* and the fourth being reported from fish hosts, since other members were reported in tortoises (*O. levanhoai*, *O. leiperi*), frog (*O. hamabatrachos*), and turtle (*O. asymmetrica*). This is also the first record of a species of *Orientattractis* in Africa, which expands the geographical distribution of the genus, since it was previously reported from Costa Rica, Colombia, Mexico (American continent), Thailand, Vietnam (Southeastern Asia) and New Guinea (Melanesia).

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