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Author: Ivanova, Elena S.

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***Araguanema mutabile* sp. nov., a new species of a rare genus (Drilonematoidea: Cephalobomorpha) parasitic in earthworms of Ecuador**

Elena S. Ivanova

Centre of Parasitology of A.N. Severtsov Institute of Ecology and Evolution RAS, Leninskii prospect 33, 119071 Moscow, Russia. E-mail: elena_s_ivanova@rambler.ru

Abstract: A new species, *Araguanema mutabile* sp. nov., from the coelomic cavity of a glossoscolecoid earthworm *Aptodrilus fuhrmanni* Michaelsen, 1918 deposited in the collection of Natural History Museum of Geneva, is described and illustrated. The new species is characterised by the presence of two types of somatic sensory organs, i.e. vesicular and fimbriate, four cephalic papillae, a small cuticularised stoma, a muscular, clavate pharynx, a nerve ring encircling isthmus, no distinct spermatheca, and a posterior position of vulva and thick-shelled eggs. From the only known species of the genus, *A. venezuelae* Ivanova & Hope, 2004, it differs by the differently shaped pharynx, indistinct vs distinct amphids, a stoma with cuticularised vs non-cuticularised walls, the different arrangement of the sensory organs, and more numerous eggs with ornamented vs smooth egg-shells. The significance of the number of somatic organs for the species identification is discussed.

Keywords: Fimbriate organs - morphology - nematodes - somatic sensory organs - taxonomy - vesicular organs.

INTRODUCTION

During the visit to the Natural History Museum of Geneva in 2002, the author examined earthworms of the museum collection for the presence of parasitic nematodes. In total, 192 specimens of 142 earthworm species (from 1 to 6 specimens of each species) (Ivanova & Vaucher, 2005) were dissected and 19 specimens were found infected by Drilonematoidea nematodes. The intensity of infection ranged from 1 to 16 nematodes. The study had shown that many Neotropic earthworms from the collection were parasitized by nematodes. It also revealed that many nematodes were represented by new taxa. Two new genera and several new species had been described based on these findings, i.e. *Yagansiella longicollis*, *Patagoniella capitoporus*, *Ungella chileana*, *Ungella micronychium* (Ivanova & Vaucher, 2005) and *Creagrocercus braziliensis* (Ivanova & Spiridonov, 2011). Except for the latter, all nematodes belonged to the family Ungellidae (Drilonematoidea: Cephalobomorpha: Rhabditida) and were found in three species of *Yagansia* Michaelsen, 1899 (Acanthodrilidae). *Creagrocercus braziliensis* was recovered from a host from the phylogenetically distant family Glossoscolecidae (*Rhinodrilus contortus* Cernosvitov, 1938) and found to belong to the different, higher nematode taxon, Plectida (Ivanova & Spiridonov, 2011). Using the molecular phylogenetic analysis based on the molecular data obtained for

the species of the same genus, it was shown that creagrocercid nematodes represent the only known case of intra-coelomic parasitism within Plectida. Herein we present a description of the new nematode species from the collection of earthworms from Natural History Museum of Geneva. This new species was found in a host from the family Glossoscolecidae native of Central and South America and was characterised by the presence of numerous somatic sensory organs. This rare trait for secernentean nematodes (apart of deirids and postdeirids) is regarded as diagnostic for the subfamily Diceloidinae of Drilonematidae (Spiridonov & Ivanova, 2005) where the new species has been placed. Three genera constituting Diceloidinae (*Diceloides* Timm, 1967, *Mbanema* Spiridonov, 1992 and *Araguanema* Ivanova & Hope, 2004) are monotypic and known by single findings. *Araguanema venezuelae* Ivanova & Hope, 2004 was recovered from *Rhinodrilus* sp. (Glossoscolecidae) in Venezuela. The description of the type species of the genus *Araguanema* Ivanova & Hope, 2004 was based on a single female. The new species is also described on a limited material because only two females were found. Moreover, the present study has shown that two specimens differ in the number of somatic organs. Because both nematodes were recovered from the same host specimen it may be presumed that both represent the same species as the probability that these specimens belong to different species seems low.

It is the author's opinion that such a form with this very unusual morphology deserves nevertheless to be described despite a certain controversy concerning the diagnostic characters and the limited material allowing only light-microscopic examination.

MATERIAL AND METHODS

Two specimens of *A. mutabile* sp. nov. were recovered from the formaldehyde-preserved *Aptodrilus fuhrmanni* (Michaelsen, 1918), Glossoscolecidae collected in Ecuador, Prov. Bolivar, Cashka Totoraz, 3200 m Paramo, in the forest border in April 3, 1987 and deposited in the collection of the Natural History Museum in Geneva (MHNG). Nematodes were then processed to glycerin following the method of Seinhorst (1959) and mounted on permanent slides using the wax ring method. Measurements and drawings were obtained with Zeiss Jenaval and Nikon Eclipse E200 microscopes with drawing attachments. Illustrations were finalised with a WACOM Intuos A4 USB drawing tablet and Adobe Illustrator CS5 following Coleman (2003). De Man indices and absolute measurements are given, where Ph is pharynx length, Ex is a distance from apex to excretory pore. The name 'fimbriate organs' was used when describing structures of sensory nature with a radially striated, basal rim (Ivanova & Hope, 2004).

TAXONOMIC PART

Araguanema mutabile sp. nov.

Figs 1 & 2

Type specimens: Holotype female (catalogue number MHNG-INVE-92679) and a paratype female (MHNG-INVE-92681) deposited in the Muséum d'histoire naturelle, Geneva, Switzerland.

Diagnosis: *Araguanema mutabile* sp. nov. is characterised by the presence of somatic sensory organs of two types, the different number of fimbriate organs in different specimens, four cephalic papillae, a short, widened, cuticularised stoma, inconspicuous amphids, a muscular, clavate pharynx with the long corpus expanded at anterior, an isthmus and a basal bulb, a nerve ring encircling isthmus, no distinct spermatheca, a posterior position of vulva, eggs with thick, faintly-coloured egg-shells, and a wide anus with long protruding fibres. Males unknown. It was assigned to the genus *Araguanema* Ivanova & Hope, 2004 on the basis of the presence of somatic sensory organs of two types, four cephalic sensilla, a muscular pharynx, a nerve ring position anterior to the pharynx bulb, the similarly structured reproductive system without a differentiated spermatheca, the similar vulva position and similar, characteristic structure of anus. It closely resembles *A. venezuelae* Ivanova & Hope, 2004,

the only other known species of the genus, in general appearance and body proportions and the structure of sensory organs. The new species is distinguished from *A. venezuelae* by the differently shaped pharynx (vs cylindrical with the slight basal swelling), inconspicuous vs prominent amphids, a different stoma structure (vs shallow, infundibular, non-cuticularised), more numerous eggs with ornamented vs smooth egg-shells and the different arrangement of the sensory organs (more numerous, larger fimbriate organs and vesicular organs arranged in one file vs two files).

Other genera of Diceloidinae include *Diceloides* Timm, 1967 and *Mbanema* Spiridonov, 1992 (Timm, 1967; Spiridonov, 1992). The former genus was described from the glossoscolecoid host *Thamnodrilus yunkerii* Gates, 1968 from Panama (Timm, 1967). Male morphology is unknown for *Diceloides* as well as *Araguanema*. The original description of *Diceloides* was also based mostly on females because the only male specimen obtained was incomplete, lacking the tail end. The general morphology of *Diceloides* and *Araguanema* is similar in the structure of a head end, the female reproductive system and the presence of two types of sensory organs of a lateral field, fimbriate and vesicular. The main diagnostic feature for these genera is the position of a nerve ring which is located on an intestine in *Diceloides*, but on pharynx in *Araguanema*. Timm (1967) also reported the presence of an offset spermatheca in *Diceloides* which is absent in *Araguanema*. *Mbanema nigeriense* Spiridonov, 1992 was recovered from the earthworm *Eudrilus eugeniae* (Kinberg, 1867) (Eudrilidae) from Nigeria (Spiridonov, 1992). It is differentiated from the other genera of Diceloidinae in having the sensory organs of the only type - vesicular ones (Spiridonov, 1992). The salient spermatheca was also reported.

The different number of fimbriate organs in both specimens described below remains unexplained and calls in question the credibility of this trait for the species identification within *Araguanema*. For both species of this genus, the different disposition of fimbriate organs on left and right body sides was noted while their number remains unknown (Ivanova & Hope, 2004).

Etymology: The species name refers to the variability in the number of fimbriate organs.

Description

Female: Short, stout nematode. Body tapered to both ends. Head end bluntly rounded. Tail broadly conical. Short conical mucron 3-4 µm long present. Cuticle 2-3 µm thick, distinctly annulated (annuli ca. 2 µm wide). Lateral chords stretched from short distance from apex to nearly to tail tip, ca. 20 (19-23) µm wide. Left lateral chord of holotype bearing irregular row of vesicular sensory organs (VO) broken by large, prominent fimbriate organs (FO) (Fig. 1A, E, F). VO 6-9 µm in diam., with shallow cavity and seta inside observed only in portion of organs. VO arranged in one disorderly file with interval between

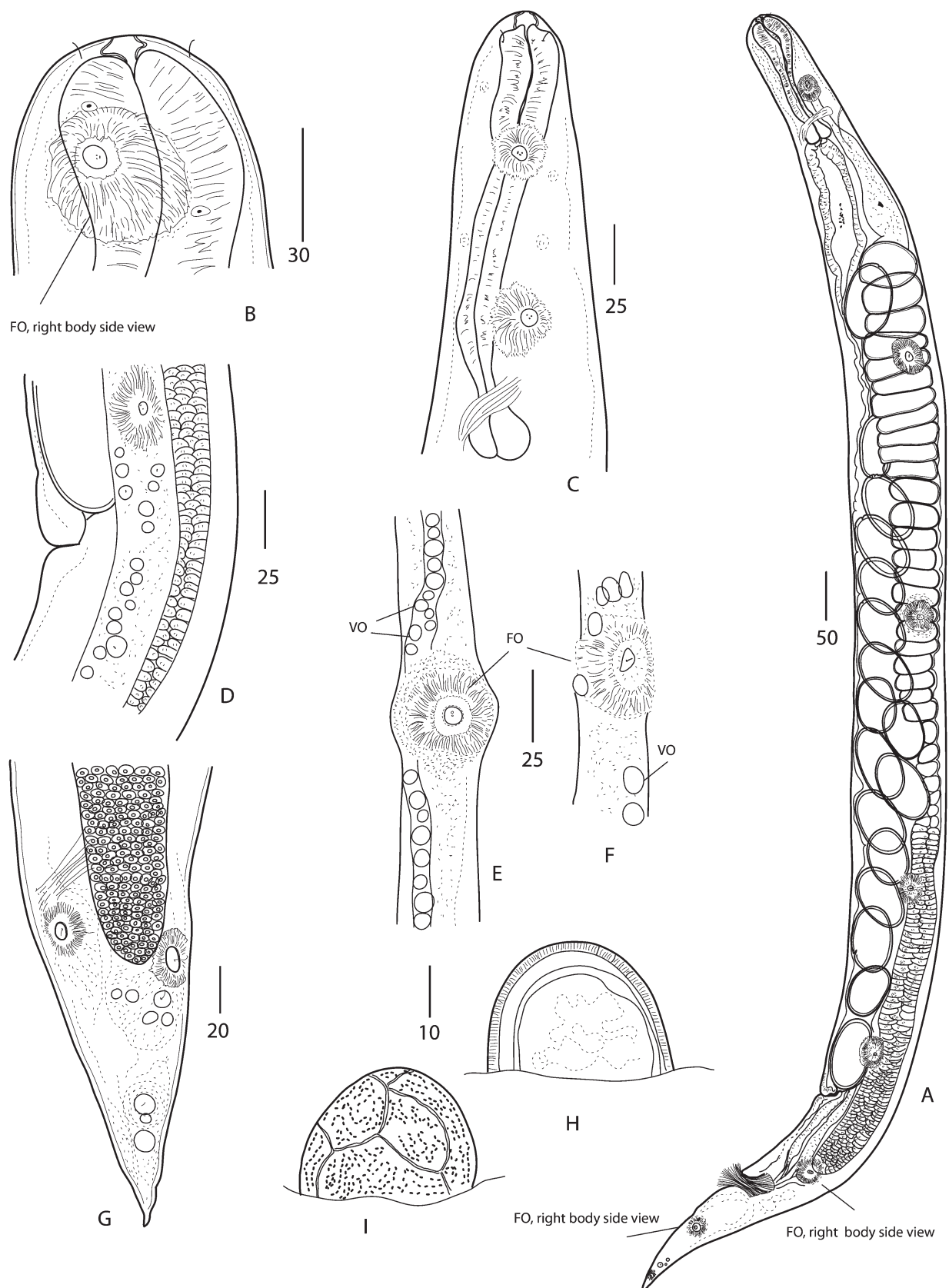


Fig. 1. *Araguanema mutabile* sp. nov. Female. A, B, D-F, H-I: holotype; C, G: paratype. Except G, all in lateral position. Scale bars in μm . (A) Entire worm. (B) Head. (C) Anterior part. (D) Vulval region. (E-F) Lateral chord at mid-body. (G) Tail, subventral view. (H-I) Egg-shell. Abbreviations: FO – fimbriate organ, VO – vesicular organ.

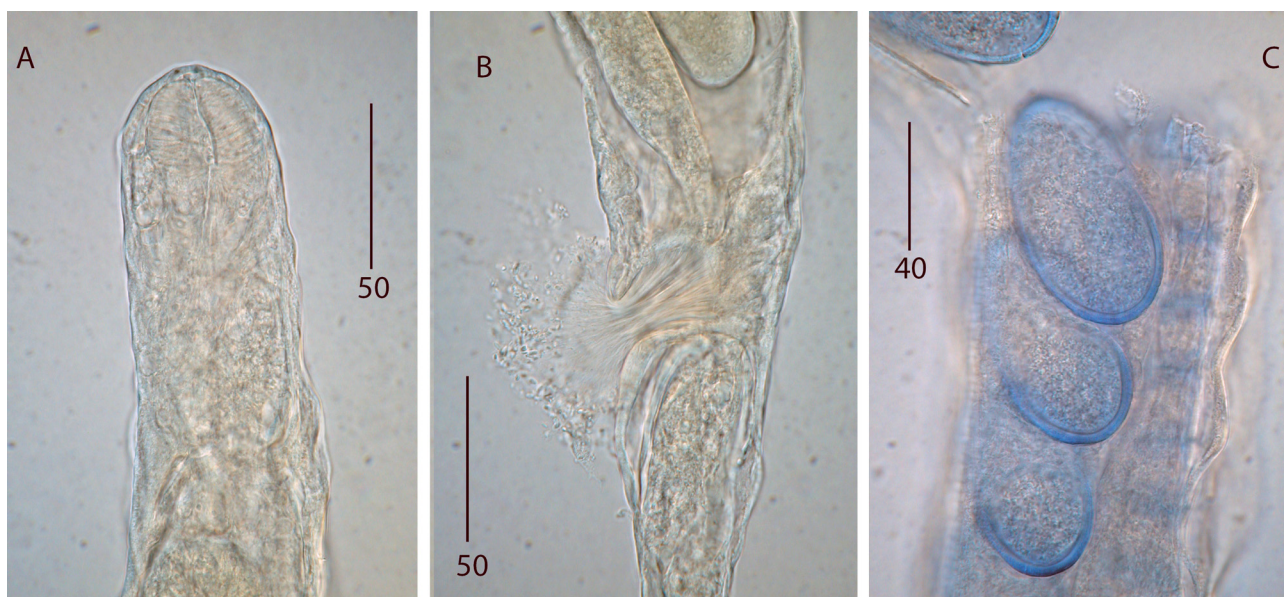


Fig. 2. *Araguanema mutabile* sp. nov. Female holotype, all from lateral, scale bars in μm . (A) Anterior part. (B) Region of anus. (C) Egg in uterus.

them 2–6 μm and located in the middle of lateral chord at body ends but slightly displaced dorso-laterally at mid-body. Total number of VO exceeding one hundred. Six FO present: anteriormost in 80 μm from apex; second in 300 μm from it or at the level of ovary reflexion; third in 370 μm posteriad; fourth in 190 μm further away; fifth just anterior to vulva level; sixth at mid-tail. Each FO crater-like, convex, radially striated, 32 ± 4 (27–36) μm in basal diam., with an aperture 7 ± 2 (5–9) μm in diam. and a single protruding sensillum; flanked by smaller vesicular organs 2–5 μm in diam. On the right lateral chord, the anteriormost and posteriormost FO located closely to body ends.

In the paratype, 24 FO positioned along lateral chord anterior to anus between VO in a pattern similar to the holotype, distanced from each other in 78 ± 10 (65–90) μm . Additionally, 2 FO located very slightly asymmetrically in caudal region in 120 μm from tail tip (Fig. 1G); all FO of paratype slightly smaller than those in holotype: 24 ± 3 (21–28) μm in basal diam. and aperture 7 ± 3 (3–10) μm in diam.

Mouth aperture small. Lips absent. Four bristle-like sensilla slightly distanced from mouth aperture. Amphids not detected. Stoma small, bowl-shaped, cuticularised, with walls ca. 1 μm thick (Fig. 1B); in paratype straighter than in holotype (Fig. 1C). Pharynx (Figs 1C; 2A) extending to anterior end, muscular, clavate, with long corpus expanding towards anterior, short, not demarcated morphologically isthmus and small pyriform basal bulb. Corpus 34 μm wide at anterior in holotype, 24 μm in paratype; isthmus 20 μm wide, bulb 20 μm wide and 27 μm long. Nerve ring encircling isthmus. Excretory

pore in holotype located opposite nerve ring in dorso-lateral position (Fig. 1A); not detected in paratype. Cardia small. Intestine with thickened walls and darkish content. Reproductive system monodelphic, prodelphic. Ovary tip situated between vulva and anus in holotype, posterior to anus in paratype. Ovary 34 μm wide distally, oocytes initially numerous, then placed in two, than one row. Mature oocytes large, with thick walls. Ovary running to anterior by dorsal body side and reversing at short distance from pharynx base. Spermatheca not demarcated. Oviduct indistinct. Up to 17 eggs in thin-walled uterus. Eggs ovoid, with egg-shells ca. 2 μm thick covered by minute tubercles divided by shallow grooves (Fig. 1H, I). Egg-shells and proximal part of ovary with ink-bluish colouration (Fig. 2B). Vagina short (18 μm), slightly inclined, directed posteriad (Fig. 1D). Vulval lips very slightly enlarged. Vulva posterior. Anus at a short distance to vulva, rectum a large chamber with numerous long, hair-like fibres protruding from anus (Fig. 2C). Anus position in paratype obscured and was estimated approximately. Rectal glands not detected.

Dimensions

Holotype female (broken): L = 1518 μm ; max width = 100 μm ; anal width = 51 μm ; Ph = 147 μm ; Ex = 128 μm ; NR = 125 μm ; anterior to ovary flexure = 268 μm ; V% = 81.4; egg 74 μm x 40 μm ; tail length = 154 μm ; a = 15.2; b = 10.3; c = 9.9.

Paratype female: L = 1953 μm ; max width = 105 μm ; anal width = 69 μm ; Ph = 153 μm ; anterior to ovary flexure = 370 μm ; V% = 81.3%; egg = 67 μm x 41 μm ; tail length = 135 μm ; a = 18.6; b = 12.8; c = 14.5.

Type habitat: Anterior region of coelomic cavity.

Type host and locality: *Aptodrilus fuhrmanni* (Michaelsen, 1918), (Annelida, Clitellata, Lumbricina, Glossoscolecidae), MHNG-INVE-92020, Ecuador, Prov. Bolivar, Cashka Totoraz, 3200 m Paramo, 03.04.1987. See Zicsi (1988) for description of these specimens.

Remark: The description is based mainly on the holotype female, which is in lateral position with the left side on top. The details of morphology of the right side of body (particularly, the number of fimbriate organs) were traced only on body ends due to the significant body thickness. The anterior part of the paratype female is positioned similarly while the posterior is in the subventral position. The condition of the paratype did not allow locating an excretory pore.

TAXONOMIC DISCUSSION

Of all Drilonematoidea, somatic sensory organs were found in Dicoloidinae and two members of the family Ungellidae, the monotypic genera *Acanthungella* Ivanova & Hope, 2004 and *Chabaudigella* Ivanova & Bain, 2012, both from the Ungellinae subfam. (Timm, 1967; Spiridonov, 1992; Ivanova & Hope, 2004; Ivanova & Bain, 2012). Wherever reported, sensory organs are confined to lateral chords. The organization of somatic organs in both ungellid genera and *M. nigeriense* is similar: vesicular organs are distributed along a lateral chord while fimbriate organs restricted to the tail region where they represent a pair of caudal organs (phasmids). The number of vesicular organs can vary in different individuals (Ivanova & Bain, 2012). The data on the number of fimbriate organs in species displaying such features are limited because of scant nematode material available, except for the observed difference in disposition of fimbriate organs on left and right body sides. No molecular data for any species displaying sensory organs are yet available. Still, phylogenetic relationships within Drilonematoidea were shown for the limited number of taxa (Ivanova & Spiridonov, 2015) parasitizing Old World hosts. Current knowledge of Drilonematoidea from Neotropic earthworms is limited by light microscopic studies.

Another puzzling character found is the position of the excretory pore in the holotype, which is located opposite the nerve ring and also the position of an excretory gland and pore which is clearly seen as dorso-lateral (Fig. 1A). This does not agree with the general body plan of the Nematoda. No explanations yet can be offered for this, moreover it was not detected in the paratype.

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