Redescription of the females of *Hystrignathus rigidus* Leidy, 1850 (Nematoda: Hystrignathidae), parasites of *Odontotaenius disjunctus* (Coleoptera: Passalidae) from eastern USA

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Abstract

The female of *Hystrignathus rigidus* Leidy, 1850 (Nematoda: Hystrignathidae) is redescribed on the basis of new material from *Odontotaenius disjunctus* (Coleoptera: Passalidae) from Athens, Georgia, USA; which also constitutes a new locality record. SEM images are provided for the first time for the species. It is also first shown that *H. rigidus* presents ridged-shelled eggs. A differential diagnosis is provided. *H. rigidus* can be differentiated from the rest of the species of this genus by having a short, non inflated first cephalic annule; spines that surpass the level of the oesophagus, an absence of lateral alae, ridged-shelled eggs and its length of the body and tail. The material from the present study differs from a previous redescription by Christie (1934) by its shorter body (2.125–2.950 vs. 2.130–4.200), first cephalic annule (0.003–0.005 vs. 0.012) and oesophagus (0.350–0.430 vs. 0.650–0.670).

Key words: Nematoda, Hystrignathidae, *Hystrignathus*, Georgia, USA, taxonomy, redescription, SEM

Introduction

The family Hystrignathidae Travassos, 1920 (Nematoda: Thelastomatoidea) comprises a large group of parasites from the posterior intestine of passalid beetles (Adamson & van Waerebeke 1992). The first studies on this family correspond to Leidy (1850) with the description of *Hystrignathus rigidus* Leidy, 1850, parasite of *Odontotaenius disjunctus* from the eastern USA. Since that time, *H. rigidus* was redescribed and recorded for the same host species from several United States localities by Christie (1934). In terms of prevalence, field surveys of *O. disjunctus* collections in North and South Carolina, USA, indicated this parasite can be found in most beetles (70–80%), and throughout all months of the year (Pearse et al. 1936; Reinert 1973).

Outside of the United States, *H. rigidus* was recorded by Baker (1967) as parasitizing several species of *Pentalobus* (Passalidae) from Ghana. Gupta & Kaur (1978) also recorded the species from *Melolonthus melolonthus* from northern India.

*Hystrignathus* Leidy, 1850, the type genus of Hystrignathidae, is characterized by females having the cervical cuticle armed with opposite rows of spines, the first row with 16 elements; procorpus clavate and genital tract didelphic-amphidelphic (Adamson & van Waerebeke 1992).

In the current paper the female of *H. rigidus* is redescribed on the basis of material from a new locality within the United States. New data on their morphometrics and SEM studies are also given.

Material and methods

Two specimens of *O. disjunctus* were collected by hand in rotting logs from Athens, Georgia, in the eastern USA.
The beetles were maintained alive in 10-gal glass aquarium with rotting hardwood logs until processing. They were killed with an overdose of chloroform and immediately dissected. The intestines were withdrawn and excised in normal saline. The parasites were removed and killed with hot normal saline (60–70°C), then fixed in 70% ethanol.

Nematodes were transferred and cleared in glycerine via slow evaporation method and mounted in the same medium. The edges of the coverslips were sealed using nail polish. Measurements were made with a calibrated eyepiece micrometre attached to a compound microscope. De Man’s ratios a, b, c and V% were calculated. Each variable is shown as the range followed by the mean plus standard deviation in parentheses; the number of measurements is also given. All measurements, unless specified, are in millimetres.

Line drawings were made with the softwares CorelDRAW X3 and Adobe Photoshop CS2 using micrographs (taken with an AxioCam digital camera attached to a Carl Zeiss AxioScop 2 Plus compound microscope) as templates. Scale bars of all plates are given in millimetres.

Some specimens were prepared for SEM as follows: they were dehydrated in a graded ascendant ethanol series, critical point-dried in a Balzers CPD 030 critical point drier. Then, mounted in aluminum stubs and coated in gold in a Bal-Tec SCD 050 sputter coater. SEM micrographs were taken at an acceleration voltage of 20 kV.

The studied material is deposited in the Colección Helmintológica de las Colecciones Zoológicas (CZACC), Instituto de Ecología y Sistemática, Havana, Cuba; United States National Parasite Collection (USNPC), Washington, USA.; the Royal Belgian Institute of Natural Sciences (RIT), Brussels, Belgium and the Coleção Helmintológica do Instituto Oswaldo Cruz (CHIOC), Rio de Janeiro, Brazil.

Results

Family Hystrignathidae Travassos, 1920

Genus Hystrignathus Leidy, 1850

*Hystrignathus rigidus* Leidy, 1850

**Material studied.** Vouchers. 10♀♀, USA, Georgia, Athens; in *Odontotaenius disjunctus*; 26/IX/2013; A. Davis coll.; CZACC 11.4881-11.4890; 9♀♀, same data as the latter, USNPC; 2♀♀, same data as the latter, CHIOC; 2♀♀, same data as the latter, RIT 833-834.

**Measurements.** Females (n = 23) a = 11.41–15.15 (13.01 ± 1.05 n = 22), b = 5.79–7.02 (6.44 ± 0.42 n = 12), c = 4.40–6.40 (5.23 ± 0.53 n = 18), V% = 41.96–48.24 (45.88 ± 1.71 n = 15), total length = 2.125–2.950 (2.521 ± 0.221 n = 22), maximum body width = 0.150–0.230 (0.195 ± 0.018 n = 23), first cephalic annule (length×width) = 0.003–0.005×0.033–0.043 (0.004 ± 0.001×0.038 ± 0.003 n = 11) stoma length = 0.043–0.063 (0.057 ± 0.005 n = 22), procorpus length = 0.230–0.330 (0.291 ± 0.023 n = 22), isthmus length = 0.020–0.053 (0.037 ± 0.007 n = 13), diameter of basal bulb = 0.073–0.093 (0.084 ± 0.003 n = 23), total length of oesophagus = 0.350–0.430 (0.399 ± 0.023 n = 13), nerve ring to anterior end = 0.170–0.290 (0.201 ± 0.027 n = 21), excretory pore to anterior end = 0.470–0.750 (0.606 ± 0.055 n = 16), vulva to posterior end = 1.100–1.625 (1.366 ± 0.148 n = 15), tail length = 0.430–0.530 (0.479 ± 0.023 n = 18), eggs = 0.095–0.108×0.038–0.048 (0.102 ± 0.004×0.044 ± 0.003 n = 17).

**Redescription.** Female body robust, widening from the base of the first cephalic annule, maximum body diameter at level between the base of the oesophagus and the excretory pore, then tapering towards anus. Cuticle markedly annulated in the spiny region, annuli less marked in the rest of the body. Cervical cuticle armed by *ca* 92 opposite rows of spines, from the end of the first cephalic annule to *ca* a body width posterior the basal bulb. First row with 16 short spines, *ca* 4 µm in length, their tips rounded. Next to that row spines become sharp-pointed and increase their length to a maximum of *ca* 10 µm at rows from 4th to 26th. Spines of the posterior rows diminishing their length to *ca* 5 µm at level of the final rows. At level of the first third of the spiny region arise additional lines of spines, intercalated between the initial 16, increasing the number of elements of the rows to *ca* 18. Sub-cuticular longitudinal striae present. Lateral alae absent, but instead a very low ridge is visible extending laterally from the end of the spiny region and surpassing the level of the vulva, then becoming less conspicuous until disappear at the last third of the body. Head cone-like, truncated, set-off from the rest of body by a wide, deep groove. Eight paired, barely prominent papillae around the oral aperture, their shape rounded, with the center depressed, giving a ring-like appearance to the margins. Amphids lateral, pore-like. First cephalic annule very short, *ca* the half of the head.
length, its diameter similar to head. Stoma about four head-lengths long, surrounded by an oesophageal collar. Oesophagus consisting of a muscular procorpus, its base slightly inflated, well set-off from the isthmus. Basal bulb rounded, valve plate well developed. Intestine simple, sub-rectilinear. Rectum short, anus not prominent. Nerve ring encircling procorpus at about 40% of its length. Excretory pore situated at about a body width posterior to basal bulb. Vulva a median transverse slit near midbody, lips slightly prominent. Vagina muscular, forwardly directed. Genital tract didelphic-amphidelphic. Oocytes in single rows. Both ovaries reflexed. Anterior ovary reflexed at the level of the excretory pore, distal flexure $ca$ 2.3 body-widths length. Posterior ovary reflexed at $ca$ 2.5 body-widths anterior to the level of the anus, distal flexure $ca$ 2.2 body-widths long. Eggs ovoid, shell with eight rough, longitudinal, hardly prominent ridges. A number of 6 to 19 eggs at a time in the uteri. Tail conical, attenuate, ending in a fine tip.

**Host.** *Odontotaenius disjunctus* (Coleoptera: Passalidae).

**Site of infestation.** Hind gut.

**Locality.** Athens, Georgia, USA.

**Differential diagnosis.** *H. rigidus* can be differentiated from *H. coci* García, Ventosa & Morffe, 2009; *H. inflatus* Travassos & Kloss, 1957; *H. splendidus* Morffe & García, 2010 and *H. tarda* (Artigas, 1928) Travassos & Kloss, 1958 by having a short and not inflated first cephalic annule vs. the longer and notably inflated one of the latter species (García *et al.* 2009; Morffe & García 2010b; Travassos & Kloss 1957a; 1958). *H. rigidus* can also be readily segregated from several species of the genus by the extension of its spines, which surpasses the level of the oesophagus. Most of the *Hystrignathus* present spines that do not extend further down the oesophagus level: *H. cobbi* Travassos & Kloss, 1957; *H. dearmasi* Morffe & García, 2010; *H. egalis* van Waerebeke & Remillet, 1982; *H. heliae* Travassos & Kloss, 1957; *H. inegalis* van Waerebeke & Remillet, 1982; *H. insularis* van Waerebeke, 1973; *H. meridensis* Guerrero, 1980; *H. metropolitanus* Cordeiro, 1981; *H. papillosus* Cordeiro, 1981 and *H. popillosus* Guerrero, 1980 (Cordeiro 1981; Guerrero 1980; Morffe & García 2010a; Travassos & Kloss 1957b; van Waerebeke 1973; van Waerebeke & Remillet 1982).

The species with spines surpassing the level of the oesophagus are *H. ferox* Hunt, 1982; *H. paulistanus* Cordeiro, 1981; *H. pearsoni* Travassos & Kloss, 1958; *H. rosario* García, Ventosa & Morffe, 2009; *H. rugosus* Travassos & Kloss, 1958 and *H. spinosus* Travassos & Kloss, 1957 (Cordeiro 1981; García *et al.* 2009; Hunt 1982; Travassos & Kloss 1957a; 1958). From the latter taxa (excluding *H. paulistanus*) that present lateral alae well developed, *H. rigidus* differs by lacking of such structures, which are substituted by low cuticular ridges. Besides, *H. paulistanus*, *H. pearsoni* and *H. spinosus* present smooth-shelled eggs in opposition to the ridged-shelled eggs of *H. rigidus*.

*H. rigidus* can be differentiated from *H. ferox* by having a shorter body (present material 2.125–2.950, Christie’s material 2.130–4.200 vs. 4.500–5.070) (Christie 1934; Hunt 1982). *H. rosario* present the tail comparatively longer than *H. rigidus* ($c = 3.38–3.98$ vs. present material 4.40–6.40, Christie’s material 4.43–7.69) (Christie 1934; García *et al.* 2009).

In the original description of *H. paulistanus* Cordeiro did not mention the existence or absence of lateral alae. However, *H. paulistanus* differs (in addition to the smooth eggs) by having a longer first cephalic annule, not surpassed by the stoma (Cordeiro 1981).

**Comments.** The specimens from the present study mostly agree with the morphology and morphometrics given in the redescription by Christie (1934). The more evident differences are the longer body (2.130–4.200 vs. 2.125–2.950) and the oesophagus (0.650–0.670 vs. 0.350–0.430) in Christie’s specimens. Moreover, the first cephalic annule is shorter in our material (0.003–0.005 vs. 0.012). Also of note is that both Leidy (1850) and Christie (1934) did not mention the presence of ornamentations on the egg surfaces. In our material the eggs present eight evident ridges in the shell, as occurs in several species of the genus and hystrignathids as well.

*H. rigidus* has been recorded for multiple states in the eastern USA, including Illinois, Louisiana, Maryland, Pennsylvania and Virginia (Christie 1934), as well as North Carolina (Pearse *et al.* 1936) and South Carolina (Reinert 1973), consistent with the distribution of its host *O. disjunctus* (Schuster 1983). The current study constitutes a new locality record for the species.

Christie (1934) stated that in all the examined hosts, *H. rigidus* was associated to *Xyo pseudohystrix* Travassos & Kloss, 1958. Frequently, the author also found male specimens belonging to a single morphotype. Being unable to assign such males to one of the two species, the author assigned them, arbitrarily and provisionally, to *H. rigidus*. Before such problems, further studies on new material are needed in order to describe and assign properly the
males of the both, *H. rigidus* and *X. pseudohystrix*. In that sense, molecular markers such as the 28S rDNA have proven to be useful for confirming congenericity of males and females among Thelastomatoidea (Guzeeva et al. 2010; Spiridonov & Cribb 2012).

FIGURE 2. *Hystrignathus rigidus* Leidy, 1850. Female. SEM images. A. Habitus. B. Cervical region. D. Cephalic end, *en face* view. E. Cervical spines at midpoint of the spiny region. F. Cervical spines at the end of the spiny region. Scale bars: A. 0.5 mm. B. 0.2 mm. C. 0.1 mm. D. 0.02 mm. E. 0.03 mm. F. 0.05 mm.
Acknowledgements

The authors are indebted to Julien Cillis (RBINS) for his technical assistance with SEM. To Dr. Wilfrida Decraemer, Dr. Marie-Lucie Susini and Laetitia Despontin for their help during the stay of the senior author in the RBINS. We thank MSc. Eduardo Furrazola (Instituto de Ecología y Sistemática) for his help with the micrographs. SEM studies were financed by the Belgian Development Cooperation through the Belgian Focal Point of the Global Taxonomy Initiative (GTI), 2013 call, granted to the senior author.

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