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Source: Journal of Parasitology, 98(1):170-174. 2012.
Published By: American Society of Parasitologists
DOI: http://dx.doi.org/10.1645/GE-2902.1
URL: http://www.bioone.org/doi/full/10.1645/GE-2902.1

J. Parasitol., 98(1), 2012, pp. 170–174

MARMOSA PARAGUAYANA (MARSUPIALIA: DIDELPHIDAE) AS A NEW HOST FOR *GRACILIOXYURIS AGILISIS* (NEMATODA: OXYURIDAE) IN BRAZIL

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ABSTRACT: Didelphids (Didelphimorphia: Didelphidae) are a large and well-studied group of Neotropical marsupials. Although knowledge of the parasitic fauna of didelphids is still scarce, recent work has suggested that Neotropical marsupials are often hosts of pinworms. Here, we isolated oxyurid nematodes from fecal samples of *Marmosa paraguayana* (Marsupialia: Didelphidae) and provide a general description and measurements for male and female specimens. We concluded these specimens can be assigned to *Gracilioxyuris agilisis* (Ascaridida: Oxyuridae), an oxyurid recently described as a parasite of the didelphid *Gracilinanus agilis* (Didelphimorphia: Didelphidae). The finding of *G. agilisis* in a different, albeit closely related, host species strengthens the previous notion of a close association between pinworms and didelphids and contributes to the knowledge of the helminthic fauna of didelphid marsupials.

Didelphids (Didelphimorphia: Didelphidae) form a large group of small- to medium-sized marsupial mammals occurring in the Neotropics; in this group, there are more than 90 species that inhabit various habitats from the ground floor to the vegetation canopy (Gardner, 2008). Recently, there has been an increased effort to elucidate various aspects of their ecology (e.g., Pires and Fernandez, 1999; Martins et al., 2006; Leiner and Silva, 2007), taxonomy (e.g., Voss and Jansa, 2003, 2009), and behavior (Moraes Jr. and Chiarello, 2005; Delciellos and Vieira, 2009). However, although Neotropical marsupials are widespread and form a relatively well-known group, knowledge of their parasitic fauna is still scarce.

For example, studies on the habits of Tate's woolly mouse opossum, *Marmosa paraguayana* (*Micoureus paraguayanus* until recently; see Voss and Jansa, 2009), a medium-sized (58–132-g) didelphid that inhabits eastern Paraguay, northeastern Argentina, and southeastern and southern Brazil (Gardner and Creighton, 2008), have brought valuable information on its feeding ecology (Leite et al., 1996; Pinheiro et al., 2002), reproductive biology (Quental et al., 2001; Barros et al., 2008), and use of space (Pires and Fernandez, 1999). Nonetheless, to date, only a few recent studies have documented parasites in this genus. The protozoans *Eimeria micouri* and *Leishmania* (*Viannia*) *braziliensis*, for example, were observed in *M. constantinae* and *M. demerarae* in Bolivia and Colombia, respectively (Alexander et al., 1998; Heckscher et al., 1999). For *M. paraguayana*, there are records of both *Leishmania* (*Leishmania*) *amazonensis* and *Leishmania* (*Viannia*) *braziliensis* isolated from specimens from Brazil (Quintal et al., 2010). With respect to the helminth fauna, knowledge is even poorer. Thus, there is only 1 record of the cestode *Mathevotaenia bivittata* (Anoplocephalidae) found in *M. paraguayana* in Argentina (Campbell et al., 2003).

The description of parasites occurring in a given species is an essential step toward a more detailed description of the ecology of both host and parasites. Moreover, such information is valuable for future work aiming to establish phylogenetic relationships within both groups and to reconstruct the evolutionary history they share (Page, 1993; Hugot, 2003). Here, we report the finding of the oxyurid nematode *Gracilioxyuris agilisis*, previously described as a parasite of the didelphid *Gracilinanus agilis* (Feijó et al., 2008), in a different didelphid species, *Marmosa paraguayana*.

MATERIALS AND METHODS

Twenty *M. paraguayana* were trapped from September 2005 to August 2006 at the Reserva Biológica de Mogi Guaçu (22°15′/22°18′S, 47°08′/47°13′W), a Cerrado (Neotropical savannah) remnant in Mogi Guaçu, São Paulo, Brazil. Individuals were captured in an 11 × 11 trapping grid, with 121 trapping stations located 15 m from each other. A Sherman live trap (7.5 × 9.0 × 23.5 cm) was installed in trees at each trapping station about 1.75 m above ground and baited with banana and peanut butter.

Feces on the trap floor, and those defecated by individuals during manipulation, were collected and preserved in 70% ethanol. All hosts were released after data collection. Feces were transported to the laboratory and analyzed using a stereomicroscope to determine diet composition. Consequently, nematodes were found and isolated. Nematodes were cleared in lactophenol and observed by ordinary light microscopy. Several individuals were air-dried, mounted on metal stubs, coated with gold (Sputter Coater Balzers SCD050), and examined using a JSM 5800LV scanning electron microscope (JEOL, Tokyo, Japan). Nematodes were then identified by morphological comparison with published descriptions of those found in other didelphid species (Navone et al., 1990; Gardner and Hugot, 1995; Guerrero and Hugot, 2003; Feijó et al., 2008).

RESULTS

Nematodes were found in the feces of 14 of the 20 trapped individuals (prevalence of 70%). Morphological characteristics unambiguously indicate these specimens can be assigned to a single species of oxyurid. Below, we provide general morphological descriptions of the specimens found and describe sex-specific morphological features and body measurements (μm).

AMENDED DESCRIPTION

Gracilioxyuris agilisis

(Figs. 1, 2)

Diagnosis: Individuals with transverse grooves on cuticle (Fig. 1A); oral opening surrounded by 3 lips, 4 labial papillae, and 2 amphids (Fig. 1B, C). Lateral alae begin at cephalic region and continue as longitudinal crests (Fig. 1D). Males with area rugosa at midbody (Fig. 2A) and 4 pairs of genital papillae; first and second pairs adanal. Third pair postcloacal and last pair caudal (Fig. 1E). Phasmids also present at dorsum (Fig. 1F), with

Received 24 June 2011; revised 22 September 2011; accepted 28 September 2011.
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DOI: 10.1645/GE-2902.1

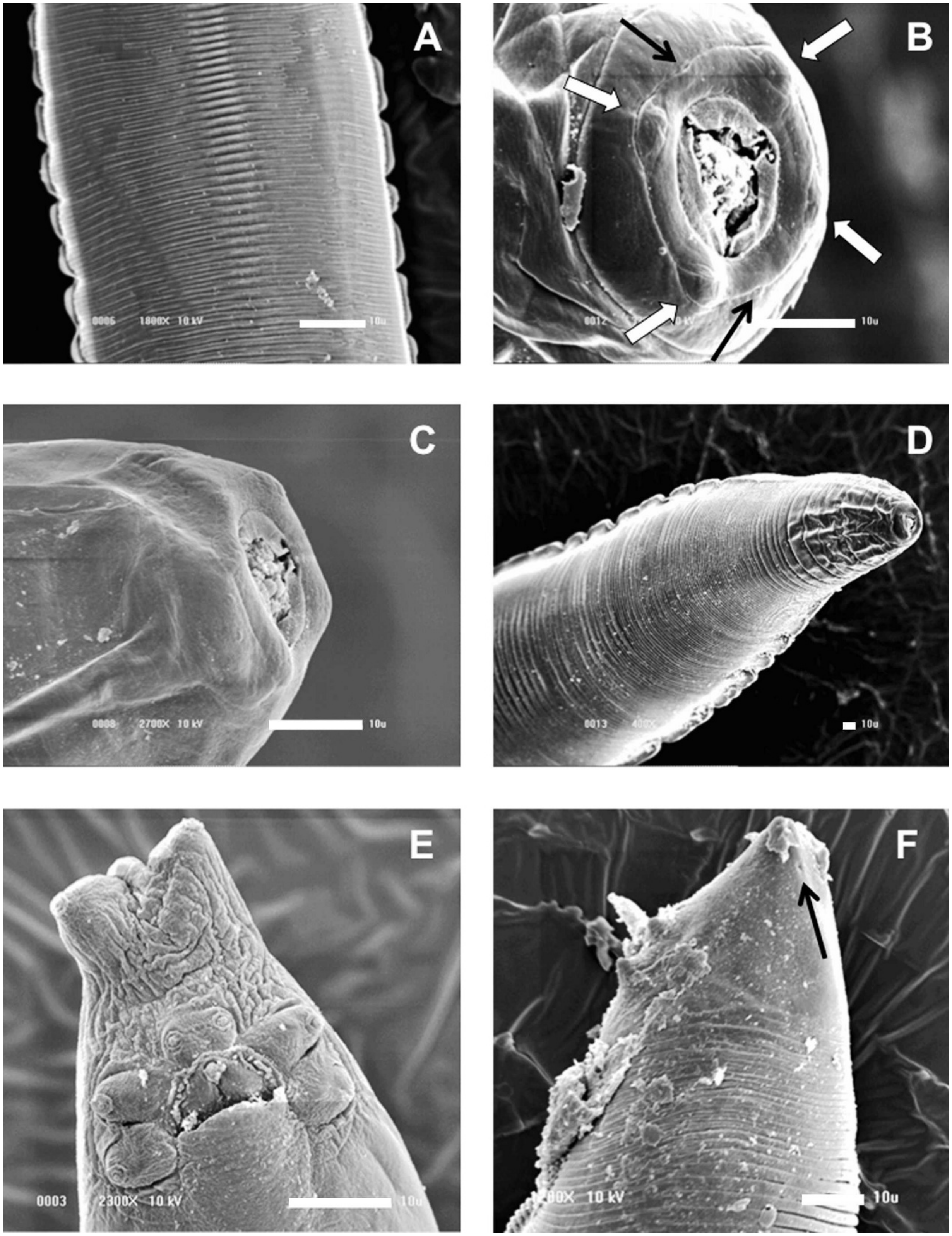

FIGURE 1. Scanning electron microscope images of oxyurid specimens collected from the feces of *Marmosa paraguayana*. (**A**) Cuticle. (**B, C**) oral opening surrounded by 3 lips, 4 labial papillae (white arrows) and 2 amphids (black arrows). (**D**) Lateral alae. (**E**) Genital papillae; the first and second pairs are adanal, the third pair is postcloacal, and the last pair are at the caudal end. (**F**) Phasmids (black arrow) present at the dorsum. Bars = 10 μm.

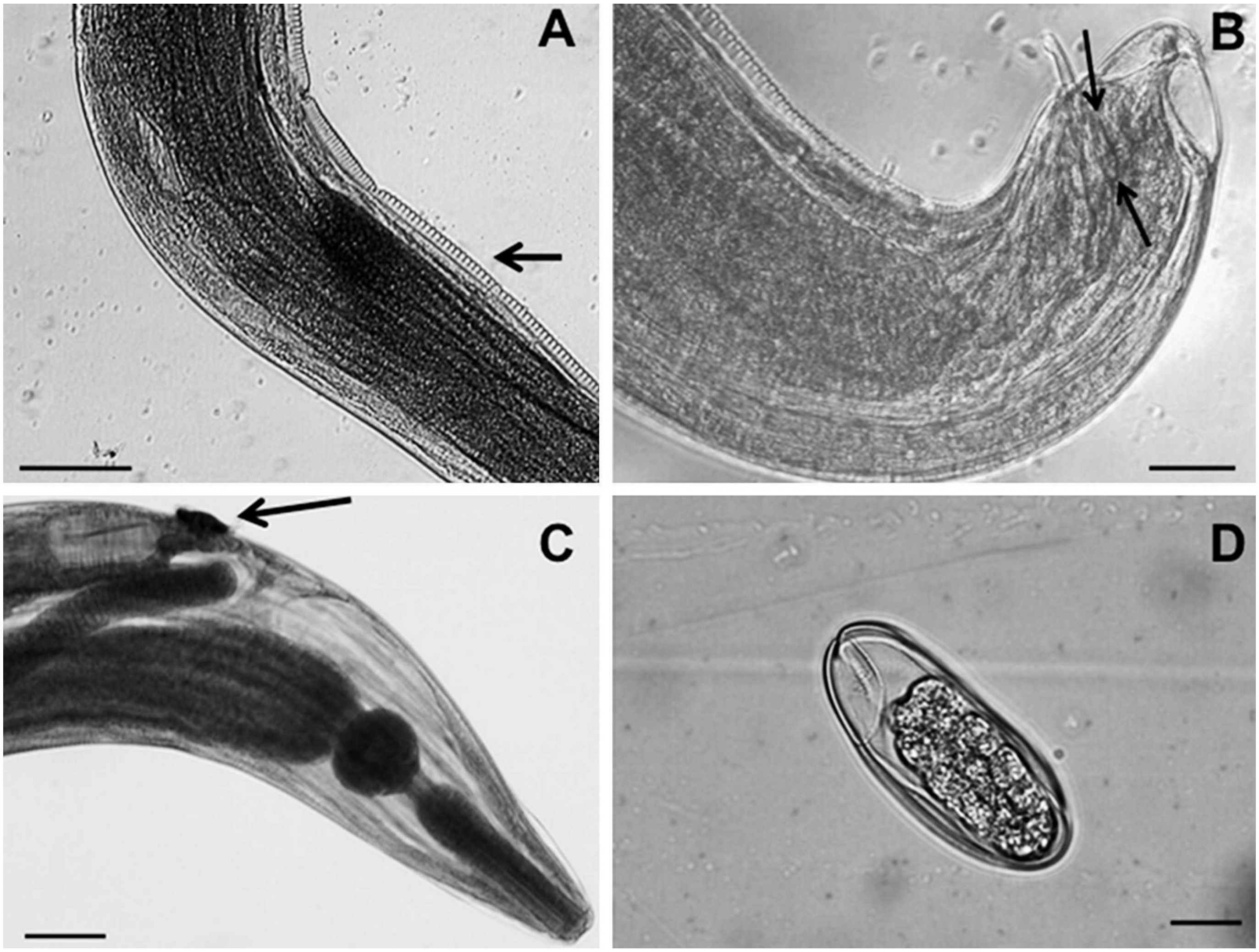

FIGURE 2. Optical microscope images of oxyurid specimens collected from the feces of *Marmosa paraguayana*. (**A**) Area rugosa (black arrow) at midbody in a male specimen. (**B**) Male posterior end showing the gubernaculum (length delimited by tips of black arrows) and reproductive spicule. (**C**) Female anterior end showing the vulva with cement. (**D**) Operculated egg. Bars = 100 µm (**A**), 50 µm (**B, C**); and 20 µm (**D**).

straight copulatory spicule (Figs. 1F and 2B), accompanied by rounded gubernaculum (Fig. 2B) located ventrally at caudal end. Male body measurements (mean ± SD and range within parentheses) based on 4 specimens as follows: total length = 1,194.0 ± 280.6 (951.0–1,585.0), width at level of mid-body = 138.0 ± 8.4 (131.0–150.0), esophagus length = 213.0 ± 10.1 (204.0–225.0), bulb length = 60.0 ± 3.9 (57.0–65.0), and bulb width = 56.0 ± 2.6 (54.0–59.0). Measurements from anterior end to various features as follows: to nerve ring = 74.0 ± 5.9 (70.0–81.0); to excretory pore = 278.0 ± 37.8 (235.0–306.0); and to area rugosa = 487.0 ± 212.7 (308.0–796.0). Spicule length = 91.0 ± 5.2 (86.0–97.0), and gubernaculum = 36.0 ± 5.1 (30.0–41.0).

Females with thick muscular vulva (Fig. 2C), frequently found closed by cement; genital tract didelphic. Vulva and excretory pore located in first third of body. Female body measurements based on 14 specimens as follows: total length = 2,406.0 ± 336.5 (1,912.0–3,173.0), width at level of midbody = 237.0 ± 91.4 (112.0–403.0), esophagus length = 280.0 ± 19.5 (249.0–312.0), bulb length = 80.0 ± 7.1 (68.0–87.0), bulb width = 83.0 ± 8.5 (68.0–97.0). Measurements from anterior end to various features as follows: to nerve ring = 92.0 ± 5.6 (81.0–101.0); to excretory pore = 236.0 ± 75.9 (156.0–306.0); to vulva = 390.0 ± 90.1 (254.0–533.0); and to tail = 479.0 ± 58.7 (405.0–556.0). Eggs (Fig. 2D) oval and operculated, 92.31 ± 1.90 (88.55–93.93; n =11) long × 38.76 ± 1.50 (35.38–40.35) wide.

Taxonomic summary

Type host: Marmosa paraguayana (Tate, 1931) (Didelphimorphia: Didelphidae).

Type locality: Reserva Biológica de Mogi Guaçu (22°18′S, 47°11′W), Mogi Guaçu, Brazil.

Type material: Museu de Zoologia do IB/UNICAMP (ZUEC), Campinas, Brazil (ZUEC NMA 01; ZUEC NMA 02)

Site of infection: Unknown.

Prevalence: Fourteen of 20 examined.

Remarks

Specimens of the pinworm found in *M. paraguayana* described here differ in morphology and morphometry from other oxyurids found in Neotropical marsupials such as *Neohilgertia venusti* in *Thylamys venustus cinderellus* (Navone et al., 1990), *Didelphoxyuris thylamisis* in *Thylamys elegans* (Gardner and Hugot, 1995), and *Monodelphoxyuris dollmeiri* in *Monodelphis emiliae* (Guerrero and Hugot, 2003). However, the general morphology of males, females, and eggs, as well as body measurements of the specimens reported here, are very similar to those of *Gracilioxyuris agilisis*, a new genus and species recently collected from the cecum of *Gracilinanus agilis* (Feijó et al., 2008). Nonetheless, there are minor differences between the specimens described here and previously described specimens of *G. agilisis*; female bulbs in the specimens measured here are proportionally larger than those previously recorded for *G. agilisis*, and the males' gubernaculum is short and rounded, whereas male *G. agilisis* gubernaculum was before observed to be longer and stretched (Feijó et al., 2008). Despite such differences, we opted for a conservative approach and classified the specimens found as members of *G. agilisis*. Further studies using molecular techniques are needed to assess whether such observed differences are the result of intraspecific variation.

Marmosa paraguayana co-occurs at the study site with *Gracilinanus microtarsus* (Fernandes et al., 2010), but we do not know at this point whether the latter also hosts *G. agilisis*. Such information might clarify the ecology and evolution of this nematode. The finding of a common parasitic species for *Marmosa* and *Gracilinanus*, coupled with other records of pinworms as didelphid parasites (Navone et al., 1990; Gardner and Hugot, 1995; Guerrero and Hugot, 2003), suggests that pinworms and didelphids share a close evolutionary history that is yet to be better explored. Furthermore, this finding expands the range of the pinworm–didelphid association to southeastern Brazil; formerly, the range was restricted to the southeastern slopes of the Andes (Guerrero and Hugot, 2003) and to central Brazil (Feijó et al., 2008).

DISCUSSION

Although helminthological records on Neotropical marsupials are scarce (Jiménez et al., 2008), the present study reveals valuable information regarding the parasitic fauna of such secretive animals. If, and how, pinworms affect didelphids' individual fitness are unanswered questions. Additional studies describing the parasites of other species, details on the consequences of parasitism on the host, and phylogenetic relationships among the pinworms of didelphids will illuminate the diversity of Neotropical marsupial endoparasites and the ecological and evolutionary implications of these interactions.

ACKNOWLEDGMENTS

We are very grateful to J. G. Neto and M. M. Pinto (both of the Mogi Guaçu Biological Reserve) for logistical support and Dr. D. C. G. M. S. Freire for helping with identifications of helminths. We thank the anonymous reviewers for comments on the manuscript. M.M.P. was supported by an FAPESP scholarship. M.V.S.S.-R. was supported by a CAPES scholarship. S.F.d.R. was partially supported by a research fellowship from CNPq. Research supported by grants from FAPESP.

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