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Spirurids from *Gracilinanus agilis* (Marsupialia: Didelphidae) in Brazilian Pantanal wetlands with a new species of *Physaloptera* (Nematoda: Spirurida)

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ABSTRACT

Gastrointestinal nematodes were recovered from thirty four *Gracilinanus agilis* from forty four collected in Pantanal, Mato Grosso do Sul State, Brazil. Two hundred seventy four spirurids were recovered from the esophagus, stomach and intestines, comprising three species from three different genera. These were identified as *Pterygodermatites* (*Paucipectines*) *jägerskiöldi*, *Spirura guianensis* and *Physaloptera herthameyerae* n. sp. is first described. This is the first record of nematodes of the genera *Physaloptera* and *Spirura* in hosts of the genus *Gracilinanus*. The high prevalence of spirurids in 72.3% of the *G. agilis* collected probably is influenced by the arboreal and diet behaviors.

1. Introduction

The geographic distribution of the marsupial *Gracilinanus agilis* Burmeister, 1854 is the northeast, central west and southeast of Brazil, Paraguay, Uruguay, Argentina, Colombia; also, in eastern Peru and eastern Bolivia (Brito et al., 2008).

The biodiversity of faunal and floral species of Pantanal is still not well known, and has justified studies to establish the critical knowledge required to support a sustainable use of natural resources and preservation of the biome.

The knowledge of the parasitism in Neotropical small marsupials is scarce. Pujol-Luz et al. (2004) registered ectoparasites as maggots of the botfly *Metacuterebra apicalis* in *G. agilis* and Herrera et al. (2005) considered this marsupial an accidental host for *Trypanosoma cruzi*. Recently, our group registered the spirurid *Pterygodermatites* (*Paucipectines*) *jägerskiöldi* parasitizing the small intestine of *G. agilis* and *G. microtarsus* Wagner, 1842 (Lopes Torres et al., 2007) and *Gracilioxyuris agilisis* Feijó, Torres, Maldonado and Lanfredi, 2008, infecting the large intestine of *G. agilis* (Feijó et al., 2008) and these were first reports of helminths parasites from these genera of marsupials.

The spirurid nematodes, in the adult stage, are obligatory parasites of vertebrates and require one or more intermediate hosts, which one is an arthropod (Stunkard, 1953). The infection of *G. agilis* by spirurids may occur due to their insectivorous–omnivorous alimentary habit, mainly based on insects (Pardini et al., 2005).

Spirurids have been documented as cause of morbidity and mortality in the wild marsupial *Didelphis virginiana* Kerr, 1792 and the predictive factors to their occurrence were mainly related to biological and ecological parameters, such as age and weather season (Nichelason et al., 2008). Morbidity includes the development of immediate

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type hypersensity as a result of chronic infection with gastric spirurids (Harari et al., 1991).

The genus *Physaloptera* Rudolphi, 1819 have insects as intermediate host (Gray and Anderson, 1982) and reptiles as paratenic hosts (Widmer, 1970). *Physaloptera* species were recorded as parasites of amphibians, reptiles, birds and mammals, mainly from the stomach of these animals but sometimes also in the small intestine (Ortlepp, 1922, 1937).

In the present study a new species of *Physaloptera* is described and the co-parasitism of spirurids in *G. agilis* is discussed.

2. Materials and methods

2.1. Study area

Forty four *G. agilis* were trapped with Sherman live traps, necropsied at Pantanal, Mato Grosso do Sul State, Brazil between July 2002 and August 2005. Animals were euthanatized following Cardoso (2002). The study was carried out in the Rio Negro Farm (19°34′54″S, 56°14′62″W), sub-region of Aquidauana (19°15′01″S, 57°01′29″W), Nhumirim Farm (19°15′01″S, 57°01′29″W) and Alegria Farm (19°15′01″S, 57°01′29″W), both in sub-region of Nhecolândia. The landscape in this region is composed by patches of semideciduous forest interspersed with open grassy areas. *G. agilis* were captured in collaboration between the Laboratório de Biologia de Helmintos Otto Wucherer and Laboratório de Biologia, Parasitologia de Mamíferos Silvestres and ONGs Conservation International/EarthWatch in the project health status and diagnosis, population ecology and use of space of small mammals species in the corridor Cerrado-Pantanal, and were authorized by the Instituto Brasileiro de Recursos Naturais Renováveis (IBAMA). License numbers: CGFAU 009/2002, 032/2002 and 091/2004.

2.2. Helminthes examination

The abdominal and thoracic cavities were opened and the organs were placed separately in Petri dishes, kept in physiological saline and dissected under stereomicroscope to remove the small helminths. Worms were washed twice in saline to remove tissue debris and fixed in AFA at 60 °C (2% glacial acetic acid, 3% formaldehyde, and 95% of 70° ethanol). For morphological studies the nematodes were clarified in phenol 80% (70% ethanol and phenolic acid), mounted as temporary slides and examined using a Zeiss Standart 20 light microscope. The drawings for the morphometric analyses were made with the aid of a camera lucida. For scanning electron microscopy (SEM) specimens were post-fixed in 1% OsO_4 and 0.8% $K_3Fe(CN)_6$, dehydrated in graded ethanol (50°–100° GL), critical point dried in CO_2, mounted on stubs, coated with gold and examined under a scanning electron microscope Jeol JSM-5310 (Mafra and Lanfredi, 1998). Measurements were taken from all mature nematodes of both sexes and were expressed in μm, except measurements indicated in millimeters (mm), the means and standard deviation are followed by the range enclosed in parentheses. For parasitological analysis, the prevalence, intensity and abundance of infection for each species were calculated according to Bush et al. (1997).

The type-species of *Physaloptera herthameyerae* n. sp., and paratypes of *Spirura guianensis* and *Pterygodermatites* (*Paucipectines*) *jägerskiöldi* were deposited in the Coleção Helmintológica do Instituto Oswaldo Cruz – Fundação Oswaldo Cruz, numbers 35651a, 35651b, 35652a and 35652b.

3. Results and discussion

The aim of this study was to record the gastrointestinal spirurids, parasites of the small marsupial *G. agilis*, a species of physalopterid is described by light microscopy and scanning electron microscopy and proposed as a new species. These results are helpful to add knowledge to the helminthological diversity found in small marsupials of Pantanal wetlands, Brazil.

Physaloptera herthameyerae n. sp.

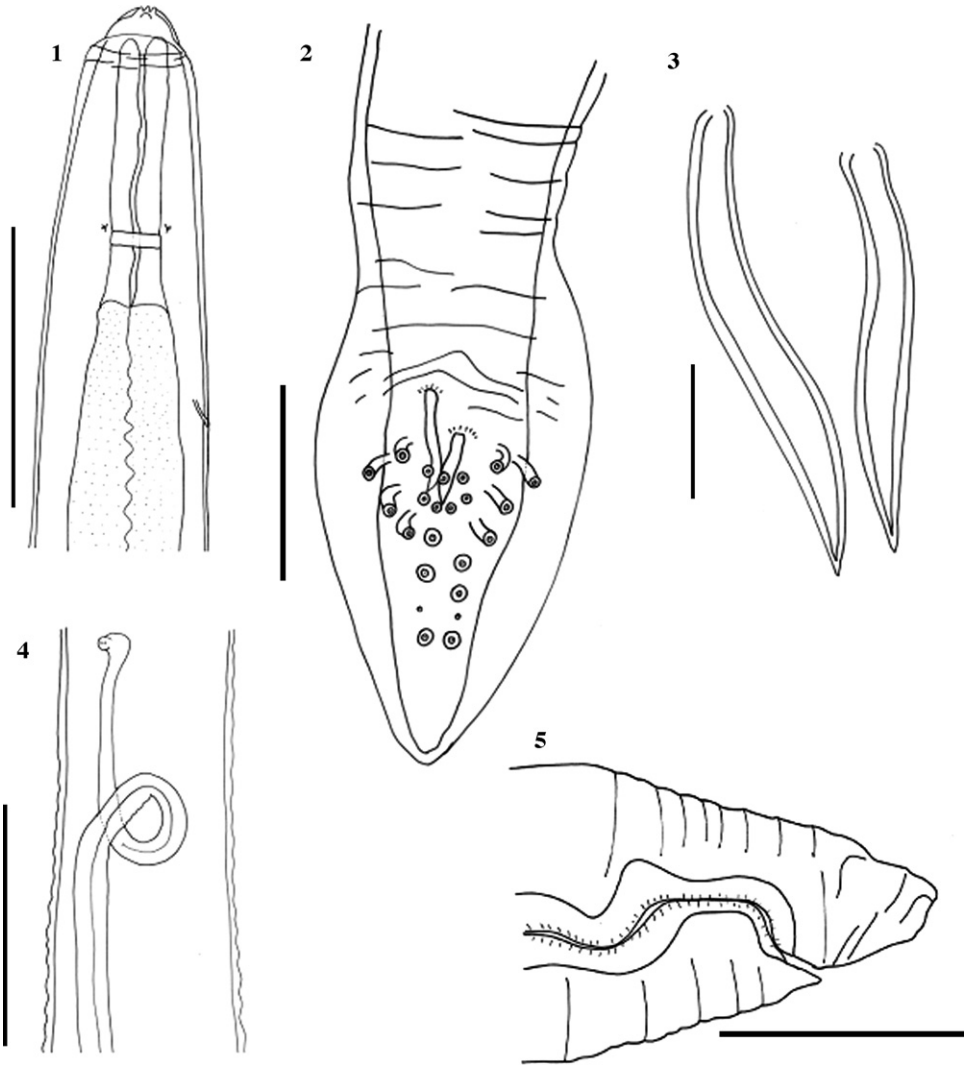
General. Five males, four females and one immature form, totalizing ten nematodes of genus *Physaloptera* were found attached by the anterior end on stomach wall of three *G. agilis*.

These nematodes present thick cuticle, slightly dilated at the cervical region, extending from the posterior margins of the lips and forming a cephalic collar around them (Figs. 1, 6 and 7). Two similar deirids are laterally located in a short distance of the nervous ring level (Figs. 1 and 6).

The cephalic end is dome-shaped, composed of two semicircular and convex pseudolips that laterally surround the oral opening (Figs. 6 and 7). Each pseudolip bears a pair of papillae, three porous-like circumscribed regions, with a cuticle pattern different from the surface of the anterior end (Figs. 6–12). The amphids are located just lateral to the central circumscribed region (Figs. 7 and 8). The internal margins of the lips have a pair of cuticular folds and between them a tripartite structure, called teeth, is projected, each one shows a pore on the internal face and a fourth tooth is just externally to them (Figs. 7, 10–12).

Male. Body length 18.6–35.2 mm (27.6 ± 7.98) and body width 714–977 (834 ± 94.07). Length of muscular esophagus 547–811 (694 ± 133.24); glandular 3.19–7.05 mm (6.05 ± 1.89). Nerve ring, deirids and excretory pore 407–575 (484 ± 69.75), 393–657 (575 ± 147.07) and 761–953 (850 ± 65.67) from the anterior end respectively.

Male posterior end ventrally bent and the cuticle in this region appears loose forming lateral caudal wings. The ventral surface presents 3 different cuticular patterns: the transversal striations of the body are interrupted on the wings becoming smooth and the ventral region is decorated by rows of cuticular bead-like structures, becoming small ridges flanking the cloaca and the 3 precloacal papillae (Figs. 13–15) again forming beaded rows posterior the cloaca. The central region posterior the second pair of postcloacal papillae is smooth toward the tail tip. The 21 papillae are button-like. Three papillae are situated just anterior the cloacal aperture and five pairs of posteroventral papillae are located as follows: 2 pairs on a



Figs. 1–5. Adult specimens of *Physaloptera herthameyerae* n. sp. (1) anterior end of male showing the nervous ring, deirids, muscular–glandular esophagus junction and excretor pore; (2) ventral view of posterior end of male showing cloacal papilae and one pair of phasmids. Scale bars: 0.5 mm; (3) spicules dissected. Scale bar: 100 μm; (4) anterior region of female showing vulva opening and the uterus opistodelphic; (5) posterior end showing anus. Scale bars: 1 mm.

protuberance just posterior the cloaca plus 3 pairs equidistant from each other spanning the midline between the cloaca and near the tip of the tail (Fig. 2). Four externolateral pedunculated papillae are on each side of the cloaca, a pair of phasmids is located between the fourth and fifth pairs of posteroventral papillae. (Figs. 2 and 15). The cloacal aperture is 739–758 (752 ± 157.24) from the posterior end. The spicules are unequal, the left 375–426 (414 ± 10.59) and the right 315–379 (346 ± 23.29) (Fig. 3).

Female. Body length 26.5–41.5 mm (31.8 ± 8.34) and width 928–1.32 mm (1.0 ± 233.24), muscular and glandular esophagus length 750–996 (887 ± 125.48) 4.0–8.2 mm (5.5 ± 2.32) respectively. Nerve ring, deirids and excretory pore situated 476–680 (568 ± 103.46), 861–1.1 mm (998 ± 125.48) and 903–1.2 mm (1.0 ± 148.84) from the anterior end, respectively. Uterus is didelphys of the 2-B type of Morgan (1943), vulva opening 5.71–10.1 mm (7.34 ± 2.40) from the anterior end (Fig. 4). Uterine eggs 46–48 (47 ± 1.00) long and 26–31 (29 ± 2.64) wide. Anus opening 370–660 (480 ± 147.58) from the posterior end (Fig. 5).

Type host. *G. agilis* Burmeister, 1854 (Marsupialia: Didelphidae).

Type locality. *G. agilis* – Alegria Farm, Pantanal, Mato Grosso do Sul State, Brazil (19°15′01″S, 57°01′29″W).

Habitat. Stomach.

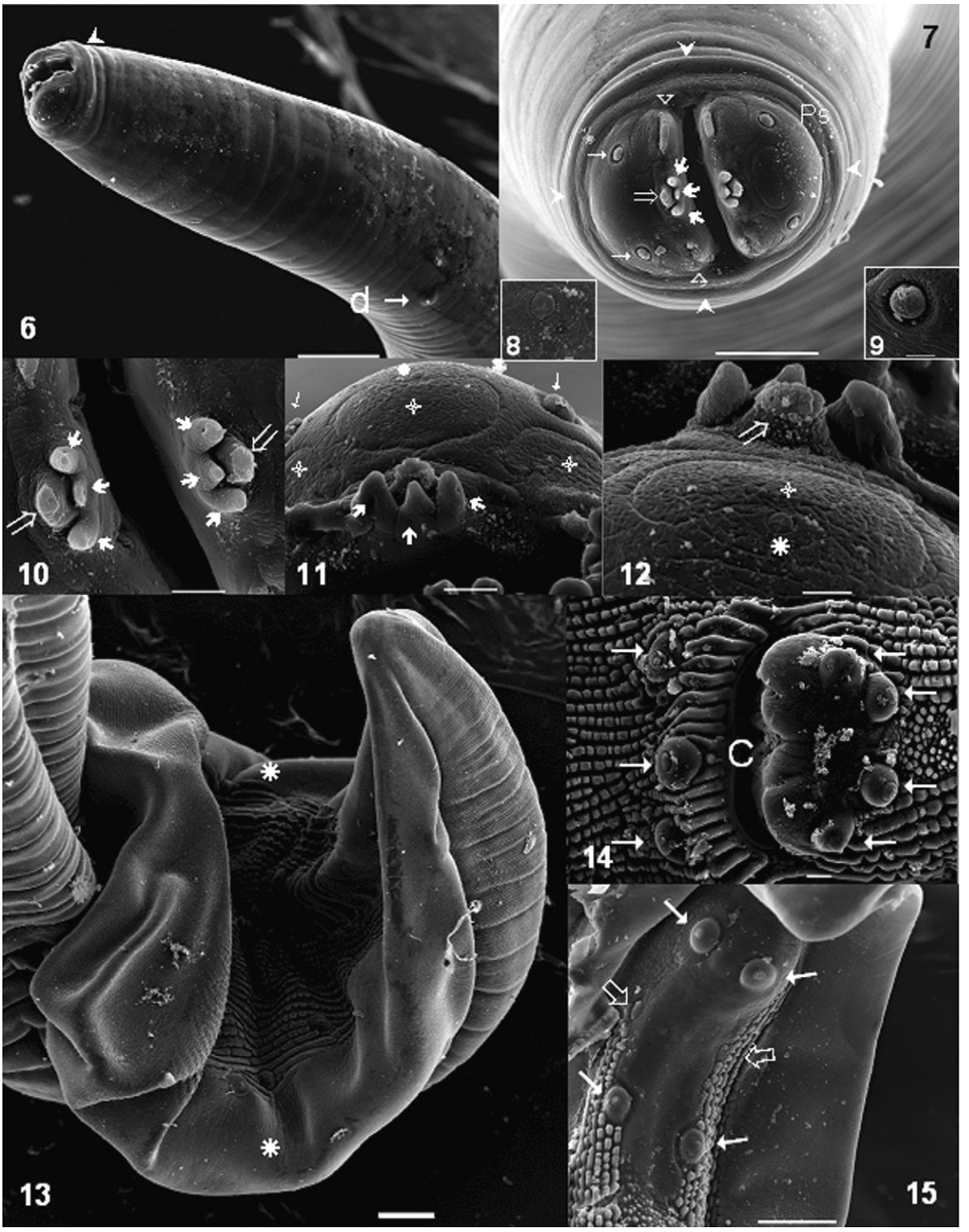
Prevalence. *G. agilis*: 6.81% (3 positive of 44 collected).

Intensity of infection. *G. agilis*: 3.0 ± 3.46.

Mean abundance. *G. agilis*: 0.20 ± 1.07.

Type specimens. Holotype: 1 male (CHIOC 35651a); Allotype: 1 female (CHIOC 35652a); Paratypes: 1 male (CHIOC 35651b), 1 female (CHIOC 35652b).

Etymology. The species name is in honor to Hertha Meyer pioneer in cell culture and electron microscopy in Brazil.



Figs. 6–15. Scanning electron microscopy of *Physaloptera herthameyerae* n. sp. male: (6) anterior region, longitudinal view of the lips (pseudolabia) and cervical region showing cephalic collarette (➤) and deirids (d). Scale bar: 100 μm; (7) apical view of the anterior end, showing each pseudolip (Ps) with 2 cephalic papillae (→), 2 folds (◗), tripartite (➔) and external teeth (⇒) and cephalic collarette (➤). Scale bar: 50 μm; (8) detail of amphid. Scale bar: 1 μm; (9) detail of cephalic papillae. Scale bar: 5 μm; (10) apical view of the pseudolip showing the tripartite tooth (➔) and external tooth (⇒); (11) internal view of a tripartite tooth showing the pore-like structures (➔), porous areas (✣), cephalic papillae (→) and amphid (✱). Scale bars: 10 μm; (12) externolateral view of the pseudolip showing external teeth (⇒), central porous area (✣) and amphid (✱). Scale bar: 5 μm; (13) lateroventral view of posterior end

4. Remarks

The morphology of the anterior end in both sexes shows two large lateral simple pseudolips, delimiting the cephalic end from the body and are provided with papillae; the cuticle forming a cephalic collaret; the deirids located anterior to the nerve ring and the pattern of the male caudal region, and cloacal papillae allowed to identify the studied specimens in the genus *Physaloptera* Rudolphi, 1819.

The genus *Physaloptera* is well known in mammals but few data are found in the literature of nematodes of this genus parasitizing marsupials. *Physaloptera herthameyerae* n. sp. was compared with other species of the same genus, using mainly the following features: number and pattern of male caudal papillae; morphology and length of spicules and uterus pattern.

Physaloptera herthameyerae n. sp. closely related species are *Physaloptera clausa* Rudolphi, 1819; *P. maxillaris* Molin, 1860; *P. bispiculata* Vaz and Pereira, 1935; *P. ackerti* Hill, 1939; *P. hispida* Schell, 1950; and *P. longispicula* Quentin, 1968, since all present 21 male caudal papillae, however all have longer spicules.

Physaloptera herthameyerae n. sp. also can be differed from *P. clausa*, *P. maxillaries* and *P. hispida* by the absence of phasmids, from *P. bispiculata* by the morphology of the caudal wings, especially by scanning electron microscopy (Mafra and Lanfredi, 1998) and *P. ackerti* females that presents polydelphic uterus while in *P. herthameyerae* n. sp. is didelphic.

Physaloptera hispida is the most closely related species to *Physaloptera herthameyerae* n. sp. by the similarities in the morphology of spicules, pattern of caudal papillae and in the morphology of the uterus, but it differ in the body length of males and females, both spicules are longer, besides the relative length of right and left spicules to be the opposite in *P. herthameyerae* n. sp. and the position of the vulvar opening of *P. hispida* to be posterior.

Physaloptera felidis Ackert, 1936 (Marchiondo and Vicente, 1978); *P. praeputialis* Von Linstow, 1988; *P. rara* Hall and Wigdor, 1918 (Tiekotter, 1981); and *P. bispiculata* Vaz and Pereira, 1935 (Mafra and Lanfredi, 1998) were analyzed also by SEM. *Physaloptera herthameyerae* n. sp. presents the same general surface features of physalopterid, as the cephalic structures and males caudal structures but can be differentiated by small details of some structures: as the porous areas, the tripartite teeth and margins of the pseudolips in males and females and cuticular decoration of the caudal region of males. Further studies of other species by SEM are needed to discuss the real taxonomic value of these structures.

Based on morphological and morphometric analyzes by light and scanning electron microscopy of the gastrointestinal spirurids parasite from *G. agilis* we are proposing a new specie *Physaloptera herthameyerae* n. sp.

Besides the description of a new species, our study evidenced that 72.3% of the forty four *G. agilis* collected were parasitized by spirurids, totaling 272 nematodes. Spirurids were present in all segments of the gastrointestinal tract, being: 24 specimens of *Spirura guianensis* attached to the esophagus wall, 7 in the stomach and 17 in the small intestine; 10 specimens of *Physaloptera herthameyerae* n. sp. attached to the stomach wall and 214 *Pterygodermatites* (*Paucipectines*) *jägerskiöldi* in the small intestine.

The comprehension of the structure of the parasites community in natural environments, in special the spirurids, can provide information that can indicate perspective to deal with different outbreaks promoted by anthropogenic disturbances on mammals' habitat and whether environmental stress (Wells et al., 2007). Vicente et al. (1997) listed helminths parasites of 16 marsupials species in Brazil, although neither was parasite of the genus *Gracilinanus*.

Pterygodermatites (*Paucipectines*) *jägerskiöldi* has already been recovered from other host species, as *Caluromys* (*Caluromys*) *philander* (Lent and Freitas, 1935). *G. microtarsus* and *G. agilis* (Lopes Torres et al., 2007), while *Spirura guianensis* was cited infecting the marsupials *Caluromys* (*Caluromys*) *philander* (Quentin, 1973), *Marmosa murina*, *Micoreus demerarae*, *Philander opossum* (Pinto and Gomes, 1980; Pinto et al., 1982) and in primates "Monki-monki" (Ortlepp, 1924), *Saguinus nigricollis* (Cosgrove et al., 1963) and *Saguinus geoffroyi* (Thatcher and Porter, 1968). In addition *Physaloptera herthameyerae* n. sp. is the first species of the genus described from marsupial of the genus *Gracilinanus*.

The spirurid *Turgida turgida* Rudolphi, 1819 was found attached to the stomach of *Didelphis virginiana*, causing granulomatous inflammation, ulcers and fibrosis at the point of attachment. These lesions could potentially lead to gastric perforation, bacteremia or sepsis (Nettles et al., 1975; Alden, 1995). Nichelason et al. (2008) pointed out *T. turgida* as one of the species that has potential to cause significant morbidity and mortality in opossum.

In the present study *Physaloptera herthameyerae* n. sp., *Spirura guianensis* and *Pterygodermatites* (*Paucipectines*) *jägerskiöldi* were in high prevalence in *G. agilis*. *Physaloptera herthameyerae* n. sp., probably causes similar lesions as the described for *T. turgida* (Nettles et al., 1975; Alden, 1995) since it was also found attached to the stomach wall, causing the same lesions (data not shown). On the other hand *Spirura guianensis* and *Pterygodermatites* (*Paucipectines*) *jägerskiöldi* were also firmly fixed to the epithelium of the esophagus and small intestine and in high parasite loads.

Similarities in spirurids communities need to be seen in the context of habitat overlap of host species, which might increase interspecific transmission of helminths via intimate hosts' contact, use of contaminated substrate, or feeding the same intermediate hosts (Ezenwa, 2003; van der Wal et al., 2000). In addition, Püttker et al. (2008) emphasizes that some nematode species are not host specific, because were recorded in different rodents and

showing caudal wings (✱). Scale bar: 100 μm; (14) detail of the cloacal opening (C) showing 3 precloacal papillae (→), 2 pairs of papillae postcloacal (→) and the longitudinal cuticular ridges. Scale bar: 10 μm; (15) ventral view showing 2 last pairs of postcloacal papillae (→) and between papillae the one pair of phasmid (⇨). Scale bar: 50 μm.

marsupials' species. Our results corroborate with Wells et al. (2007) and Püttker et al. (2008) once these authors proposed that the arboreal activity reduces exposure of the host to the soil, minimizing a contact with infective larval and eggs of the hosts' feces. *G. agilis* is an arboreal marsupial and insectivorous–omnivorous which could have influenced the prevalence of parasitism. *G. agilis* did not present infection with strongylids and the prevalence of nematodes as the trichonstrongylids is lower compared with arthropod-transmitted spirurids (unpublished data). Nematodes spirurids could have two main ways to infect *G. agilis*, i.e. when invertebrates are eaten on the soil or in the trees dossel. Further researches are needed to investigate parasite communities in small marsupials and its relationship within their habit as the use of alimentary resources or forage behavior.

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Tribute

On March 15th, 2009 the Parasitology World lost Dra. Reinalda Marisa Lanfredi an important Brazilian helmintologist researcher. She dedicated about 28 years to morphological and ultrastructural helminthes analyzes. All your students and collaborates have a little of you on their hearts, mind and research. Your contribution to science is immortal. Thanks. Students, Teachers and Friends of Laboratorio de Biologia de Helmintos Otto Wucherer, IBCCF- UFRJ, Brazil.

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