

Protocylindrocorpus brasiliensis n. sp. (Diplogastroidea: Cylindrocorporidae) associated with the ambrosia beetle, Euplatypus parallelus (F.) (Curculionidae: Platypodinae) in Para rubber trees (Hevea brasiliensis)

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PROTOCYLINDROCORPUS BRASILIENSIS N. SP. (DIPLOGASTROIDEA:
CYLINDROCORPORIDAE) ASSOCIATED WITH THE AMBROSIA BEETLE,
EUPLATYPUS PARALLELUS (F.)(COLEOPTERA: CURCULIONIDAE:
PLATYPODINAE) IN PARA RUBBER TREES (*HEVEA BRASILIENSIS*)

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Running head: *Protocylindrocorpus brasiliensis* in para rubber trees

ABSTRACT

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Protocylindrocorpus brasiliensis n. sp. (Diplogastroidea: Cylindrocorporidae) associated with the ambrosia beetle, *Euplatypus parallelus* (F.)(Coleoptera: Curculionidae: Platypodinae) in Para rubber trees (*Hevea brasiliensis*). Nematropica 00-00.

Protocylindrocorpus brasiliensis n. sp. (Diplogastroidea: Cylindrocorporidae) is described from reproductive stages removed from galleries of the ambrosia beetle, *Euplatypus parallelus* (F.)(Coleoptera: Curculionidae: Platypodinae) in Para rubber trees (*Hevea brasiliensis*) in Brazil. This is the first record of the genus *Protocylindrocorpus* from the Neotropics. Males of *P. brasiliensis* are quite striking because their long spicules extend up to 70% of their total body length. This spicule/body ratio is one of the largest if not the largest of any free-living nematode. The adults exhibit “conspecific agglutination” where they congregate in a slimy substance that serves to maintain them in a coherent group. Some of the adults were attacked by fungi and protozoan pathogens, showing that disease plays a role in regulating natural populations. The discovery of *P. brasiliensis* provides new information on nematode structure, behavior and ecology.

Key Words: ambrosia beetle, conspecific agglutination, Cylindrocorporidae, elongate spicules, nematode diseases, *Protocylindrocorpus*

RESUMEN

INTRODUCTION

Several species of wood boring beetles are known to attack the Para rubber tree in Brazil (da Silva et al., 2013). While investigating damage caused to Para rubber trees (*Hevea brasiliensis*) by the native ambrosia beetle, *Euplatypus parallelus* (F.)(Coleoptera: Curculionidae: Platypodinae) in the northwestern region of the state of Sao Paulo, Brazil, nematodes were recovered from some of the beetle galleries. These nematodes were identified as belonging to the genus *Protocylindrocorpis* Rühm in the diplogasteridid family Cylindrocorpidae. Representatives of the Cylindrocorpidae are rare nematodes with less than 12 species including only two in the genus *Protocylindrocorpis* (Goodey, 1930; Rühm, 1956, 1959; Massey, 1960; Baker, 1962; Hunt, 1980; Kinn, 1984; Harman et al., 2000; Poinar et al., 2003; Kanzali and Futai, 2004). Dauer stages of the nematodes were obtained from under the elytra of the beetles. The present study describes this nematode and discusses some of its interesting attributes, such as the presence of elongate needle-like spicules and the phenomenon of conspecific agglutination of the adults.

MATERIALS AND METHODS

The nematodes were discovered while examining the contents of galleries of the ambrosia beetle, *Euplatypus parallelus* (F.)(Coleoptera: Curculionidae: Platypodinae) in Para rubber trees (*Hevea brasiliensis*) in the northwestern region of the state of Sao Paulo, Brazil.

RESULTS

Diplogastroidea

Cylindrocorporidae

Protocylindrocorpus Rühm, 1959

Protocylindrocorpus brasiliensis n. sp.

The monodelphic females, leptoderan bursa, outstretched testis and extremely long spicules place the new species in the genus *Protocylindrocorpus* Rühm (1959). While Rühm (1959) originally described *Protocylindrocorpus* as a subgenus of the genus *Cylindrocorpus* with *C. goodeyi* Rühm as the type species, Paramonov (1964) raised *Protocylindrocorpus* to the generic level and this action was followed by Andrassy (1976).

Description

Cuticle with faint cross and longitudinal striae; lateral fields obscure; lips 6, acutely pointed, each lined on inner side with thick refractive cheilorhabdions; metastom slightly anisomorphic; Stoma elongate, narrow, teeth absent; pharyngeal collar present, length usually between one fourth and one half length of stoma; corpus muscular, approximately 1 ½ times length of stoma; isthmus and basal bulb continuous, glandular appearing, approximately equal to length of corpus, reflexed near middle; basal bulb with minute valve plates, base of bulb sunk in ventricular portion of intestine. Excretory pore at level of basal bulb; nerve ring encircling isthmus just anterior to basal bulb; hemizonid and phasmids obscure; tail spicata.

Female (Figs 1, 2AB): Monodelphic; ovary reflexed three times with double bend in region of vulva, tip extending to tail region; vulva a transverse slit located in posterior third of body, with slightly protuberant lips in ovipositing females; vagina short, straight, post-vulval sac variable in length, usually between one and two body widths; tail short, tapering to a narrow spine; eggs large, from one to seven in uterus at a time.

Male (Figs 3,4,5A,B): With single, outstretched testis; spicules paired, separate, needlelike, often sinuous in body, extremely long, reaching up to 70% of total body length. Gubernaculum simple, boat-shaped with tip bent upward against spicules; nine pairs of genital papillae, three pairs pre-anal papillae, fourth pair either pre-anal or ad-anal, five pairs post-anal; of post-anal pairs, first pair sub-anal, remaining pairs near tail base before tail constriction. Bursa, narrow, leptoderan with proximal end open; tail spicata, extending beyond border of bursal membrane.

Dauer juveniles not recovered.

Measurements

See table 1.

Type material

Female (Holotype) and paratypes deposited at the Department of Plant Protection FEIS/UNESP, Av. Brasil, 56 15385-000 Ilha Solteira, SP, Brazil.

Type host and locality: The nematodes were living in the galleries of the ambrosia beetle, *Euplatypus parallelus* (F.)(Coleoptera: Curculionidae: Platypodinae) in Para rubber trees (*Hevea brasiliensis*) in the northwestern region of the state of Sao Paulo, Brazil.

Diagnosis and relationships

The smaller adult size (438-793 μm in *P. brasiliensis* vs 943-1515 μm in *P. goodeyi* and 906- 1544 μm in *P. dendrophilus* Kinn, 1983), sharply pointed, rather than rounded lips as occurs in *P. goodeyi* and *P. dendrophilus*), presence of a pharyngeal sleeve (= collar) (lacking in both *P. goodeyi* and *P. dendrophilus*), basal bulb surrounded by the ventricular portion of the intestine (not immersed in anterior portion of intestine in *P. goodeyi* and *P. dendrophilus*), , length of isthmus-basal bulb subequal to length of the corpus (vs 2.0 or more times the length of the corpus in *P. goodeyi* and *P. dendrophilus*) and the presence of only 9 pairs of genital papillae (both *P. goodeyi* and *P. dendrophilus* are described with 10 pairs of genital papillae) separate *P. brasiliensis* from the other species in the genus, *P. goodeyi* Rühm (1959) and *P. dendrophilus* Kinn (1984).

DISCUSSION

At the time of capture, the nematodes had congregated together (Fig. 8), a condition we refer to here as “conspecific agglutination”. It is interesting that Rühm (1959) also commented that the adults of *P. goodeyi* were clustered together in a sticky mass under the bark of a tree. It is possible that the females of *P. brasiliensis* produce some type of

attractant similar to that produced by females of *Cylindrocorpus longistoma* and *C. curzii* (Chin and Taylor, 1969). These authors showed that such secretions were species-specific and attracted males to the mating site.

The extremely long spicules of *P. brasiliensis* and the other two species of the genus are fascinating. Spicule size varies greatly in nematodes however most free-living forms have short spicules less than twice the tail diameter. While many nematodes have longer spicules than those of *P. brasiliensis*, when the ratio of spicule length to body length expressed as a percentage is used rather than actual length, then the 70% ratio shown in the male depicted in figure 4 is one of the highest values for any free-living nematode, although Kinn (1984) reported up to 80% of the total body length for *P. dendrophilus*. Some vertebrate parasites, especially members of the Spirurida, can match and even surpass this figure (Skrjabin et al., 1967). Males of *Gongylonema falconis* Oschmarin from the esophagus of falcons has a spicule/body length value of 77% while the parasite of galliform birds, *G. ingluvicola* Ransom has spicules equal in length to its body (100%). Among invertebrate parasites, some mermithids are long-spiculed although the spicule/body length ratio is rarely over 20% (Poinar, 1981). In these animal parasites, usually only one of the two spicules is elongated. The other spicule is much reduced and rarely reaches more than twice the cloacal diameter. In *Protocylindrocorpus* spp., both spicules are equal or subequal. The perplexing question is why are the spicules of *Protocylindrocorpus* spp. so long? Chitwood and Chitwood (1950) suggested that there may be a correlation between long spicules and long, tubular vaginas. However the vagina of *Protocylindrocorpus* spp. is very short and other species in the family survive quite well with short spiculed males. The spicules are maintained

in a spicular pouch lodged in the cloacal chamber (Chitwood and Chitwood, 1950). During spicular growth, the surrounding sheath enlarges as the spicules increase in length. This growth of the spicular sheath is essential to keep the spicule ends from entering the body cavity, which would provide an opening for infections.

This is the first record of *Protocylindrocorpus* in the Neotropics and suggests that the genus may have a global distribution. The first described species, *P. goodeyi*, was collected in Basel, Switzerland and the second, *P. dendrophilus* was described from Louisiana, USA. Members of the Cylindrocorpidae are considered to be microbotrophs however the gut contents of *P. brasiliensis* contained mostly viscous deposits and it was difficult to discern any definite structures. However, Kinn (1984) noted fungal spores in the pharyngeal lumen of some members of *P. dendrophilus*. Some females of *P. brasiliensis* were parasitized by fungi (Fig. 6) and other females by a microsporidian (Fig.7), showing that disease played a role in regulating their numbers.

Amber fossils have shown that representatives of the Diplogastroidea have had associations with platypodine beetles for 20-30 million years. The dauer stages of *Scolytonema dominicana* Poinar and *S. hispaniolae* Poinar were associated with platypodine beetles in Dominican amber and 14 dauer juveniles of *S. mexicana* Poinar occurred adjacent to a platypodine beetle in Mexican amber (Poinar, 2011).

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Table 1. Measurements (μm) of males (n=12) and females (n=12) of *P. brasiliensis*.

Character	Male	Female
Length	562 (438-682)	690 (615-793)
Greatest width	37 (30-43)	46 (39-53)
Length lips	3 (3-4)	3 (3-4)
Length stoma	23 (15-27)	24 (19-28)
Length stomal sleeve	5 (4-10)	8 (5-12)
Length corpus	37 (32-42)	43 (35-48)
Length isthmus-basal bulb	52 (48-59)	55 (48-64)
Head to Excretory pore	54 (40-62)	77 (65-90)
Head to nerve ring	57 (48-70)	70 (65-78)
Length tail	30 (25-37)	45 (35-55)
Length tail spike	17 (14-25)	19 (13-22)
Length vagina	-----	12 (10-17)
Percent vulva	-----	64 (60-72)
Length post vulvar-sac	-----	66 (43-137)
Length egg	-----	74 (53-110)
Width egg	-----	24 (21-32)
Length spicule	385 (74-458)	-----
Width spicule	3 (2-4)	-----
Length gubernaculum	18 (14-23)	-----
Width gubernaculum	4 (3-6)	-----

Ratio a	15(13-17)	16(14-17)
Ratio b	5.6(4.9-6.1)	7.0(6.2-7.5)
Ratio c	17.5(16.3-18.7)	15.3(13.7-17.5)

FIGURES

1. Female of *Protocylindrocorpus brasiliensis*. A = anus; E= eggs; V= vulva. Bar = 115 μm .
2. Female of *Protocylindrocorpus brasiliensis*. A. head and pharynx. Bar = 20 μm . B. vulval area. Bar = 30 μm .
3. Male of *Protocylindrocorpus brasiliensis*. Top arrow shows position of spicule head. Lower arrow shows spicules protruding from cloaca. Bar = 90 μm .
4. Posterior body portion of male *Protocylindrocorpus brasiliensis* showing spicule length. Arrow shows position of spicule head. Note needlelike spicular shafts protruding from cloacal opening. Bar = 60 μm .
5. Tail region of *Protocylindrocorpus brasiliensis* showing position of genital papillae. A. Lateral view of tail. B. Ventral view of tail with leptoderan bursa. Bar = 30 μm .
6. Fungal mycelium growing on the body of a *Protocylindrocorpus brasiliensis* female. Bar = 25 μm .
7. Pansporoblasts of a microsporidian parasite developing inside the body of a *Protocylindrocorpus brasiliensis* female. Bar = 30 μm .
8. Example of conspecific agglutination showing a mating assemblage of *Protocylindrocorpus brasiliensis* in a bark beetle cavity in a rubber tree.