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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ABSTRACT

Poinar, G., Jr., J. C. P. da Silva, and C. A. H. Flechtmann. 2014. *Protocylindrocorpus brasiliensis* n. sp. (Diplogastroidea: Cylindrocorporidae) associated with the ambrosia beetle, *Euplatypus parallelus* (F.) (Curculionidae: Platypodinae) in Para rubber trees (*Hevea brasiliensis*). Nematropica 44:51-56.

Protocylindrocorpus brasiliensis n. sp. (Diplogastroidea: Cylindrocorporidae) is described from reproductive stages removed from galleries of the ambrosia beetle, *Euplatypus parallelus* (F.) (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 72% of their total body length. The adults exhibit "conspecific agglutination" where they congregate in a slimy substance that serves to maintain them in a coherent group for mating. Some of the adults were infected by fungal and protozoan pathogens, implying 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, Cylindrocorpidae, elongate spicules, nematode diseases, *Protocylindrocorpus.*

RESUMEN

Poinar, G., Jr., J. C. P. da Silva, and C. A. H. Flechtmann. 2014. *Protocylindrocorpus brasiliensis* n. sp. (Diplogastroidea: Cylindrocorporidae) asociado con el escarabajo ambrosía, *Euplatypus parallelus* (F.) (Curculionidae: Platypodinae) en arboles del caucho Pará (*Hevea brasiliensis*). Nematropica 44:51-56.

Protocylindrocorpus brasiliensis n. sp. (Diplogastroidea: Cylindrocorporidae) se describe a partir de estadíos reproductivos extraídos de las galerías de escarabajos ambrosía, *Euplatypus parallelus* (F.) (Coleoptera: Curculionidae: Platypodinae), en árboles de caucho Pará (*Hevea brasiliensis*) en Brasil. Éste es el primer registro del género *Protocylindrocorpus* en los Neotrópicos. Los machos de *P. brasiliensis* son llamativos ya que sus largas espículas se extienden hasta el 72% de la longitud total del cuerpo. Los adultos presentan "aglutinación coespecífica", es decir, se congregan en una sustancia pegajosa que sirve para mantenerlos en un grupo cohesionado para el apareamiento. Algunos de los adultos fueron atacados por hongos y protozoos patógenicos, lo cual demuestra que las enfermedades juegan un papel en la regulación de las poblaciones naturales. El descubrimiento de *P. brasiliensis* proporciona nueva información sobre la estructura de los nemátodos, al igual que sobre su comportamiento y ecología.

Palabras clave: escarabajo ambrosia, aglutinación coespecífica, Cylindrocorpidae, espículas alargadas, enfermedades de nemátodos, *Protocylindrocorpus*.

INTRODUCTION

Several species of wood boring beetles are known to attack the Para rubber tree (*Hevea brasiliensis*) in Brazil (Silva *et al*., 2013). While investigating damage caused to Para rubber trees by the native ambrosia beetle, *Euplatypus parallelus* (F.) (Curculionidae: Platypodinae) in the northwestern region of the state of São Paulo, Brazil, nematodes were recovered from the beetle galleries and on the bodies of both male and female beetles. These nematodes were identified as belonging to the genus *Protocylindrocorpus* Rühm in the diplogasteridid family Cylindrocorpidae. Representatives of the Cylindrocorpidae are rare with less than 12 species known, including only two in the genus *Protocylindrocorpus* (Goodey, 1930; Rühm,

1956, 1959; Massey, 1960; Baker, 1962; Hunt, 1980; Kinn, 1984; Harman *et al*., 2000; Poinar *et al*., 2003; Kanzaki and Futai, 2004). The present study describes this nematode and discusses some biological features of this new species.

MATERIALS AND METHODS

The nematodes were discovered while examining the contents of galleries of the ambrosia beetle, *E. parallelus* (F.) (Curculionidae: Platypodinae) in Para rubber trees in the northwestern region of the state of São Paulo, Brazil. In addition to galleries, nematodes were also found on the surface of the adult beetles. A total of 2,367 adult beetles were examined in this study; 37 male and 47 female beetles were infested with these nematodes. After the nematodes were removed from living beetles, they were fixed in 70% ethanol and processed to glycerin. Observations, measurements, and photographs were made with a Nikon Optiphot light microscope (Nikon Instruments, Inc, Melville, NY). All measurements are in micrometres, unless otherwise indicated.

RESULTS

Systematics

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 genus level, and this action was followed by Andrassy (1976).

Protocylindrocorpus brasiliensis *n. sp. Description*

Nematode body 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 1.5 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 spicate. (Table 1).

Females. Monodelphic nematodes; 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 carried in uterus (Fig. 1; Fig. 2A,B).

Males. Nematodes 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 spicate, extending beyond border of bursal membrane (Fig. 3; Fig. 4; Fig. 5).

Type specimens. Female (Holotype), paratypes, and allotypes 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.) (Curculionidae: Platypodinae) in Para rubber trees (*Hevea brasiliensis*) in the northwestern region of the state of Sao Paulo, Brazil.

Etymology. The specific epithet reflects the geographic location where the specimens were found.

Diagnosis and Relationships

Protocylindrocorpus brasiliensis n. sp. is characterized by a smaller adult size {438-793 µm vs. 943-1,515 µm in *P. goodeyi* Rühm (1959) and 906-1,544 µm in *P. dendrophilus* Kinn, (1984); 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, which is not immersed in the anterior portion of intestine in *P. goodeyi* and *P. dendrophilus*; length of isthmus-basal bulb nearly equal to length of the corpus vs. 2× the length of the corpus in *P. goodeyi* and *P. dendrophilus*; presence of only 9 pairs of genital papillae while *P. goodeyi* and *P. dendrophilus* are described with 10 pairs of genital papillae. These characters separate *P. brasiliensis* from the other two species in the genus. In addition, the length of the spicules in *P. brasiliensis* [403 (280-458)] is smaller than those in *P. dendrophilus* [754 (586-925)] and *P. goodeyi* (72-600).

DISCUSSION

At the time of capture, the nematodes had congregated together (Fig. 8), exhibiting "conspecific agglutination". Such congregations were common, and 58% of the nematodes recovered in the galleries exhibited similar behavior. It is interesting that Rühm

Table 1. Mean, range, and standard deviation measurements (µm) of males (n = 12) and females (n = 12) of *Protocylindropcorpus brasiliensis.*

Character	Male	Female
Length	562 (438-682) 0.26	690 (615-793) 0.21
Greatest width	37 (30-43) 1.09	46 (39-53) 1.11
Length lips	3 (3-4) 1.09	3 (3-4) 1.11
Length stoma	23 (15-27) 0.77	24 (19-28) 0.76
Length stomal sleeve	5 (4-10) 0.81	8 (5-12) 0.82
Length corpus	37 (32-42) 1.02	43 (35-48) 1.08
Length isthmus-basal bulb	52 (48-59) 1.01	55 (48-64) 1.12
Head to excretory pore	54 (40-62) 1.45	77 (65-90) 1.53
Head to nerve ring	57 (48-70) 1.38	70 (65-78) 1.07
Length tail	30 (25-37) 1.06	45 (35-55) 1.41
Length tail spike	17 (14-25) 1.02	19 (13-22) 0.96
Length vagina	----	12 (10-17) 1.03
Percent vulva	----	64 (60-72) 1.01
Length post vulvar-sac	----	66 (43-137) 8.42
Length egg	----	74 (53-110) 4.20
Width egg	----	24 (21-32)1.02
Length spicule	403 (280-458) 5.06	----
Width spicule	3 (2-4) 0.16	----
Length gubernaculum	18 (14-23) 0.46	----
Width gubernaculum	4 (3-6) 0.28	----
Spicule L/body L	0.68 (0.64-0.72) 1.34	
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)

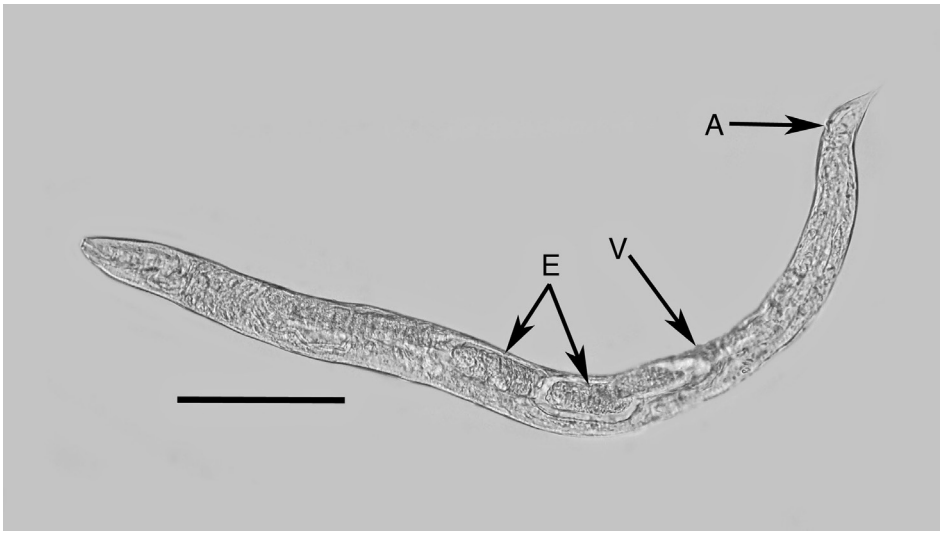

Fig 1. Female of *Protocylindrocorpus brasiliensis*. A = anus; E= eggs; V= vulva. Bar = 115 µm.

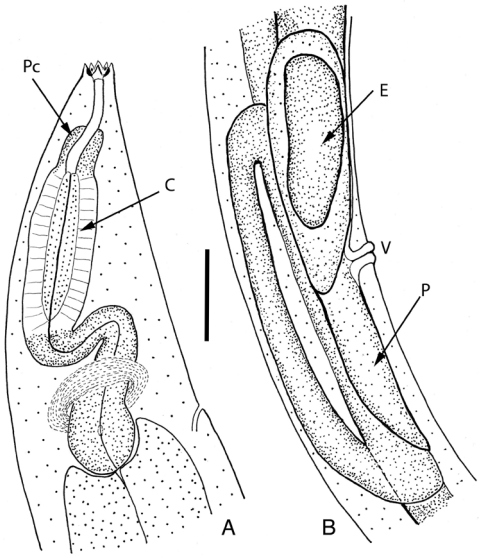

Fig. 2. Head and vulval region of *Protocylindrocorpus brasiliensis*. C= corpus; Pc = pharyngeal collar; E= egg; P= post vulvar sac; V= vulva. Bar for head region = 20 µm. Bar for vulval region = 30 µm.

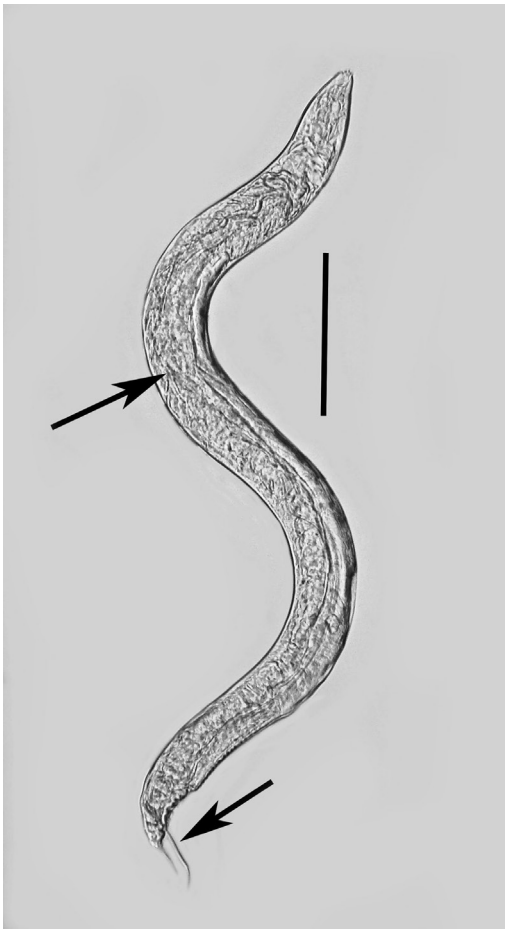

Fig. 3. Male of *Protocylindrocorpus brasiliensis*. Top arrow shows position of spicule head. Lower arrow shows spicules protruding from cloaca. Bar = 90 µm.

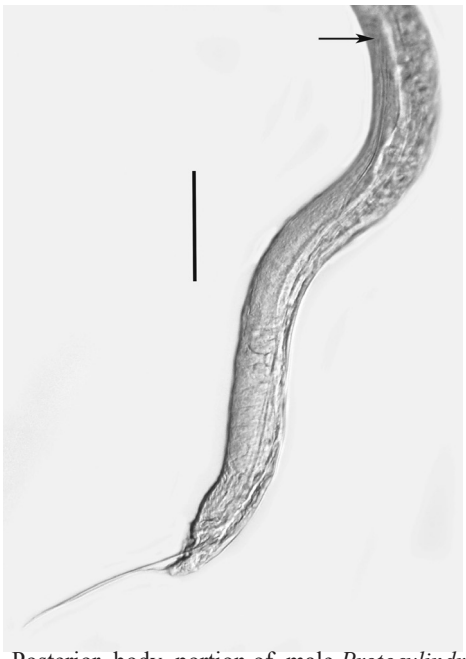

Fig. 4. Posterior body portion of male *Protocylindrocorpus brasiliensis* showing spicule length. Arrow shows position of spicule head. Note needlelike spicular shafts protruding from cloacal opening. Bar = 60 µm.

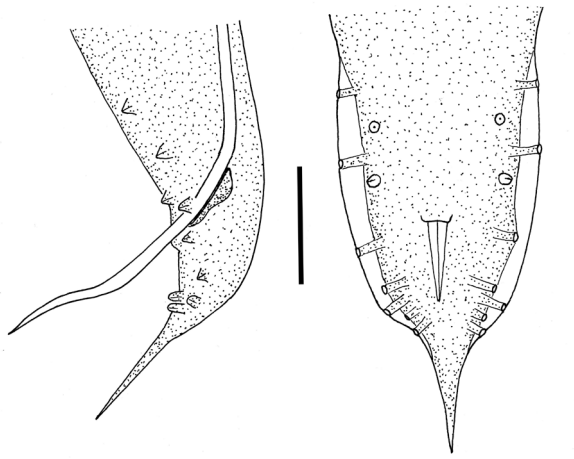

Fig. 5. Lateral and ventral views of male tail of *Protocylindrocorpus brasiliensis* showing position of genital papillae. Bar = 30 µm.

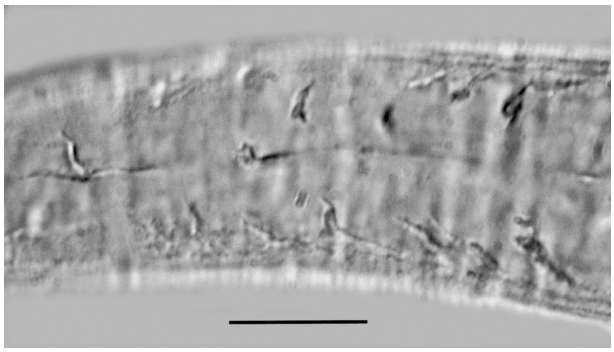

Fig. 6. Fungal mycelium growing on a *Protocylindrocorpus brasiliensis* female. Bar = 25 µm.

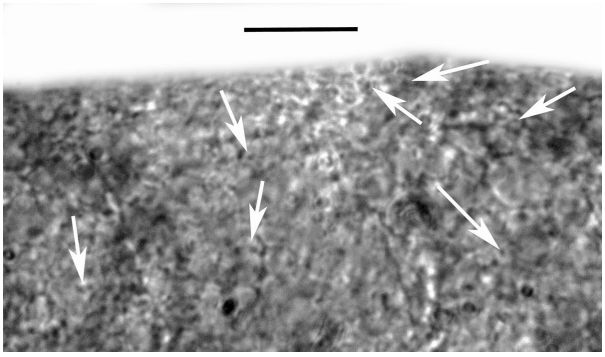

Fig. 7. Body cavity of a female *Protocylindrocorpus brasiliensis* filled with pansporoblasts (arrows) of a microsporidian parasite. Bar = 30 µm.

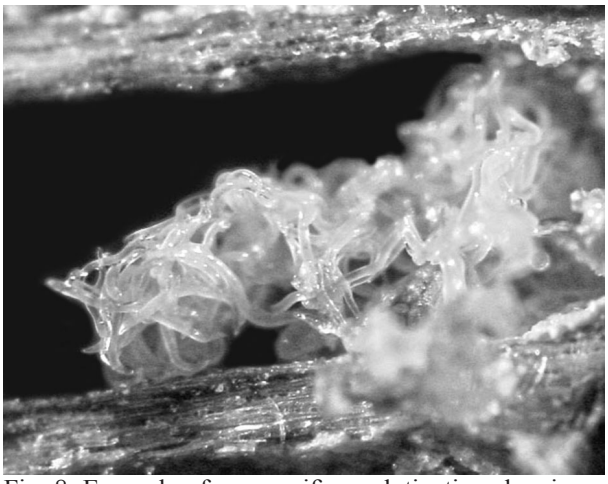

Fig. 8. Example of conspecific agglutination showing a mating assemblage of *Protocylindrocorpus brasiliensis* in a cavity of the ambrosia beetle, *Euplatypus parallelus*.

(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* n. sp. 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 long spicules of *P. brasiliensis* n. sp. and the other two species of the genus are noteworthy. Spicule size varies greatly in nematodes although most free-living forms have short spicules less than twice the tail diameter. Many nematodes have longer spicules than those of *P. brasiliensis* n. sp. 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, and 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, but the gut contents of *P. brasiliensis* contained mostly viscous deposits, and it was difficult to discern any definite structures. Kinn (1984) noted fungal spores in the pharyngeal lumen of some members of *P. dendrophilus*, and some females of *P. brasiliensis* n. sp. were parasitized by fungi (Fig. 6) and other females by a microsporidian (Fig. 7), showing that disease may play a role in regulating their numbers.

Amber fossils have shown that representatives of the Diplogastroidea have had associations with platypodine beetles for 20 to 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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