A NEW SPECIES OF NEMATODE, *SCLERORHABDITIS NEOTROPICALIS* SP.N. (RHABDITIDA), ASSOCIATED WITH *AZTECA* ANTS IN *CECROPIA OBTUSIFOLIA*

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ABSTRACT

Esquivel, A., J. Abolafia, P. Hanson, and A. Pinto. 2012. A new species of nematode, *Sclerorhabditis neotropicalis* sp.n. (Rhabditida), associated with *Azteca* ants in *Cecropia obtusifolia*. Nematropica 42:163-169.

Sclerorhabditis neotropicalis sp. n. is described and illustrated. It can be distinguished by its body length (515-560 µm in females and 363-455 µm in males), lip region with crown-shaped lips, having acute three acute thorns, oral aperture one third of lip region width, stoma 15-22 µm long or 1.0-1.6 times as long as diameter of lip region, stomatal tube about 1.5 times longer than wide, bearing rhabdia with slightly rugous walls at lumen side, stegostom lacking glottoid apparatus and denticles, pharynx 125-135 µm long in females and 107-131 µm long in males, excretory pore and hemizonid located at 21-22% of the distance from the anterior end. The female reproductive system is didelphic-amphidelphic, with ovaries straight or dorsally reflexed, and the vulva is a transverse slit, 55-61% from the anterior end, female tail conoid elongated, 62-76 µm long, having a posterior hyaline portion very slender and with acute tip. The male reproductive system is monorchic, with testis anterio-ventrally reflexed, spicules free, 36-47 µm long or 1.6-1.8 times longer than anal body width, with rounded manubrium, gubernaculum 15-18 µm long or 38-42% of spicule length, and bursa anteriorly open, peloderan, with scalloped margin and bearing eight pairs of genital papillae (1+1/1+1+1+1+2), tail conoid spicate, 23-30 µm long. The new species was found in close association with an ant colony (*Azteca constructor* Emery, 1876) inside a *Cecropia obtusifolia* Bertoloni, 1840 tree in a tropical rain forest in Costa Rica. Certain species of *Azteca* ants live in mutualistic association with Cecropia trees and preliminary evidence suggests that the new nematode species is invariably present in the colonies of at least some of these *Azteca* species. It is, therefore, possible that the new nematode species represents a third partner in the mutualism, although its role is still unknown.

Key words: ants, *Cecropia* tree, description, morphology, nematodes, taxonomy, tropical rain forest

RESUMEN

Esquivel, A., J. Abolafia, P. Hanson, and A. Pinto. 2012. Una nueva especie de nematodo *Sclerorhabditis neotropicalis* sp. n. (Rhabditida), asociado con hormigas *Azteca* en árboles de *Cecropia obtusifolia*. Nematropica 42:163-169.

Sclerorhabditis neotropicalis sp. n. se describe e ilustra. *S. neotropicalis* sp. n. se puede distinguir por la longitud de su cuerpo (515-560 µm en hembras y 363-455 en machos), región labial con labios en forma de corona, con aspecto de tres espinas puntiagudas, abertura oral un tercio de la anchura de la región labial, longitud del estoma 15-22 µm ó 1.0-1.6 veces la longitud del diámetro de la región labial, tubo estomatal 1.5 veces más largo que ancho, evidenciando rhabdia con paredes ligeramente rugosas en la pared del lumen, el estegostoma carece de aparato glotoide y dentículos, longitud de la faringe entre 107-135 µm de longitud, poro excretorio y hemizonidio localizado 21-22% de la región anterior a nivel del istmo del esófago. El sistema reproductivo de la hembra es didelfico-anfidelfico, con ovaries rectos o ligeramente flexionados dorsalmente, la vulva es una hendidura transversal, localizada 55-61% de la región anterior, la cola de la hembra es conoidea alargada, 62-76 µm de longitud, con una región posterior hialina muy fina con la punta afilada. El sistema reproductivo del macho es monórquico, con el testículo ventral y anteriormente flexionado, espículas de 36-47 µm de longitud ó 1.6-1.8 veces más largas que la anchura del cuerpo a nivel del ano, el manubrium es redondeado, gubernáculo de 15-18 µm de longitud ó 38-42% de la longitud de la espícula y la bursa anteriormente abierta, tipo pelodera con el borde redondeado con ocho pares de papilas genitales (1+1/1+1+1+1+2), con la cola conoidea de 23-30 µm de longitud. La nueva especie fue encontrada en estrecha relación con una colonia de hormigas (*Azteca constructor* Emery, 1876) dentro de un árbol de *Cecropia obtusifolia* Bertoloni, 1840 en el bosque lluvioso tropical de Costa Rica. Ciertas especies de hormigas *Azteca* viven en asociación mutualista con árboles de

Cecropia y la evidencia preliminar sugiere que la nueva especie de nematodo se encuentra invariablemente presente en las colonias de al menos algunas especies de *Azteca*. Por lo tanto es posible que la nueva especie de nematodo represente un tercer participante en la relación mutualista, aunque todavía se desconoce su papel

Palabras clave: árboles de *Cecropia*, bosque tropical lluvioso, descripción, hormigas, morfología, nematodos, taxonomía.

INTRODUCTION

Recently, Ahmad et al. (2007) proposed the new genus *Sclerorhabditis* to accommodate an interesting nematode species having a particular head shape and labial sclerotization. The new nematode was isolated from the decaying matter in a hollow cavity in the trunk of palm tree, *Oreodoxa regia* L. According to the authors, insects probably act as phoretic carriers enabling the nematode to colonize these habitats. This paper describes the second species of the genus, *S. neotropicalis* sp. n., which is associated with *Azteca constructor* Emery, 1876 and *Cecropia obtusifolia* Bertoloni, 1840 in lowland tropical rain forests of Costa Rica. This paper is devoted only to the taxonomic description of the new nematode species; the biology and ecology of this symbiotic relationship will be the subject of a future publication.

MATERIALS AND METHODS

A small sample of moist, paste-like material was collected from the inner walls of the internode of a *Cecropia obtusifolia* tree containing an active ant nest. The material was transferred to a small quantity of tap water to release the nematodes. The extracted nematodes were fixed with hot 4% formaldehyde and brought to pure glycerol by a modification of Seinhorst (1959). Adult nematodes were permanently mounted on Cobb slides using the paraffin wax ring method (de Maeseneer and d´Herde, 1963). Standard measurements and ratios were taken for each specimen (Table 1). Nematodes were photographed with a Nikon DS-Fi1 digital camera on a Nikon Eclipse 80i microscope. Drawings were made with a camera lucida on Olympus BX50 microscope.

RESULTS

Sclerorhabditis neotropicalis sp. n. (Figs. 1, 2)

Material examined. Sixteen females and seven males from the humid lowland tropical rain forest.

Measurements. See Table 1.

Adult.

Body small, 363-560 µm long, having a straight or slightly curved habitus when relaxed with hot formaldehyde. Body fusiform, with anterior end narrower than adjacent pharyngeal region; maximum body diameter is at anterior vulva lip. Cuticle with fine transverse annuli well demarcated only at the anterior and posterior regions. Lateral fields about one-fifth as wide as body-width, composed of two lines, beginning at the base of the stoma and extending to the posterior end, at level of anus aperture. Lip region offset from adjoining body by constriction. Lips strongly sclerotized, crown-shaped. Both dorsal and subventral pairs of lips with three curved thorn shape projections directed towards the stoma, the central one longer than both lateral and with a pointed process at the base (in lateral view); lateral lips hyaline and membranous, somewhat square in shape. Amphidial apertures very small, rounded. Oral aperture one third of lip region width. Stoma tubular, 2.0-2.8 as long as diameter of stoma or 1.0-1.4 as long as diameter of lip region; stomatal tube (gymno-stegostom) about 1.5 times longer than wide, lumen slightly wider anteriorly, bearing rhabdia with slightly rugous walls at lumen side; cheilostom with sclerotized rhabdia located among the crown-shape lips, gymnostom very long, the most part of stomatal tube; stegostom very short, lacking glottoid apparatus and denticles. Pharynx rhabditoid, one-fourth of total length of nematode; pharyngeal corpus muscular with procorpus cylindrical and metacorpus swollen, isthmus relatively robust, and terminal bulb ovoid with butterfly valve. Nerve ring at 69 % of neck length, at isthmus level. Excretory pore and hemizonid evident in the ventral side, located at level of isthmus or at 21-22% of body length from anterior end. Cardia conoid about 2.0 µm long. Intestine very wide with well defined lumen, having the cardia with thinner walls.

Female. Reproductive system didelphic-amphidelphic, both genital branches well developed, anterior branch on right and posterior branch on left side of intestine. Ovaries straight or dorsally reflexed, flexure usually small, with oocytes arranged in two or more rows. Oviduct very short, continued in a globular spermatheca with numerous and very small spermatozoa. Uterus more or less tubular, one third of the corresponding body diameter long with thick walls, usually with one uterine developed egg (40-44 x 17-26 µm) at the posterior branch. Vagina thin-walled, about 30% anal body diameter long. Vulva a transverse slit, slightly protruding, with clearly dilator muscles. Rectum tubular, with wider lumen at proximal portion, 1.1 - 1.5 times as long as anal body diameter. Tail

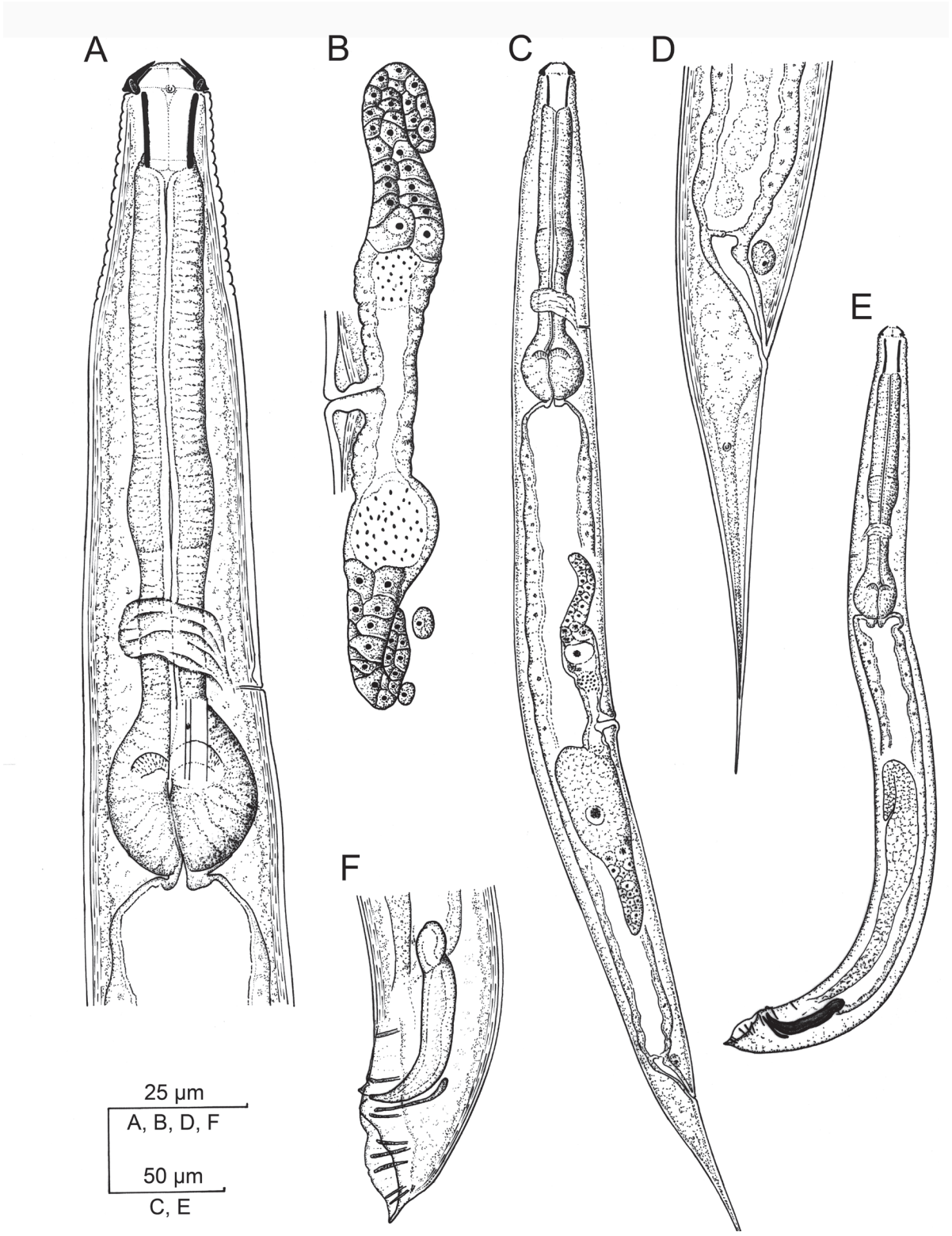

Fig. 1. *Sclerorhabditis neotropicalis* sp.n. A) Female pharyngeal region; B) Female reproductive system; C) Entire female; D) Female tail region; E) Entire male; F) Male tail region.

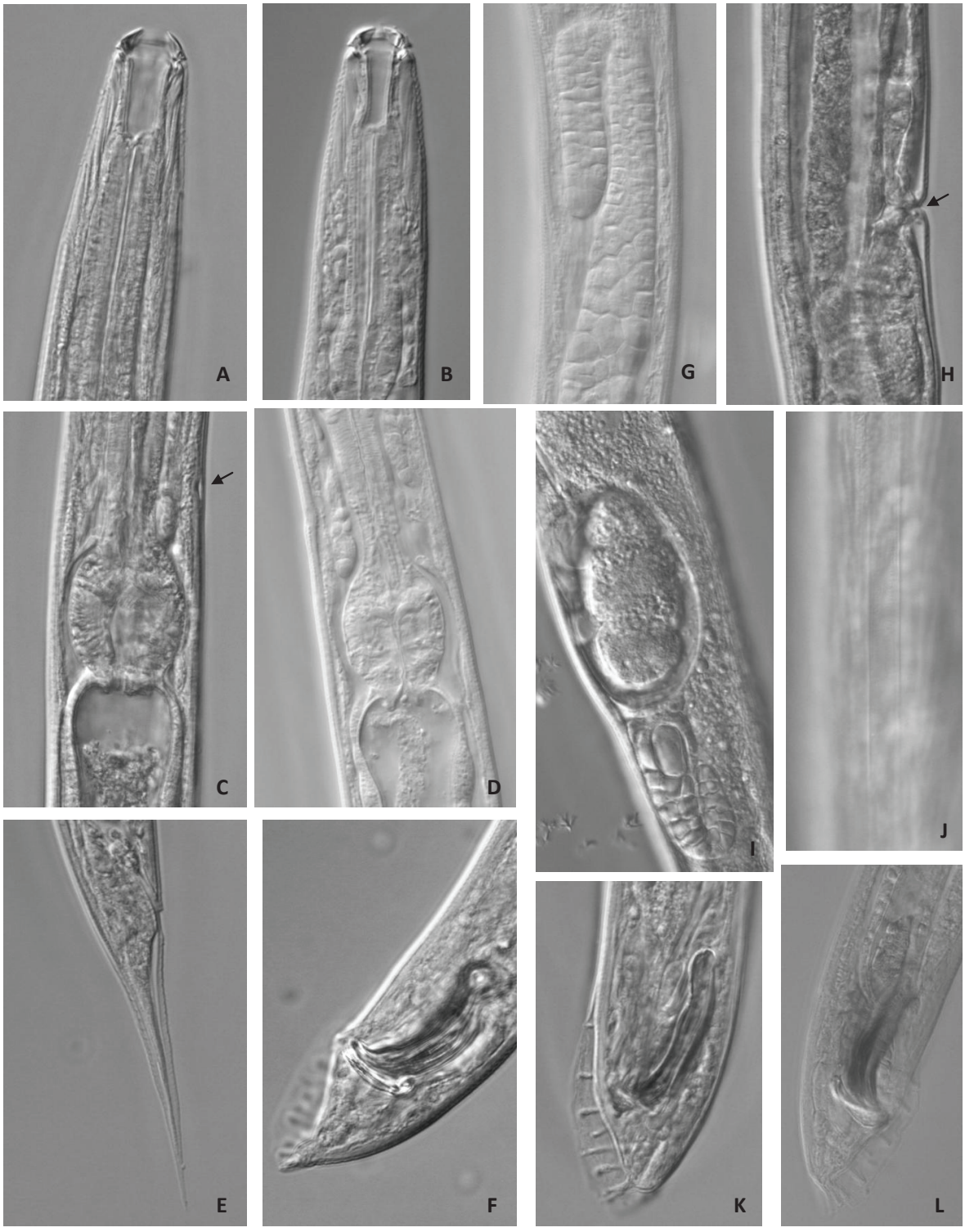

Fig.2. *Sclerorhabditis neotropicalis* sp.n. A) Female head region; B) Male head region; C) Hemizonid (arrow) and pharyngo-intestinal junction (female); D) Pharyngo-intestinal junction (male); E) Female tail region; F) Male tail; F) Spicules and Gubernaculum; G) Male testis; H) Vulva; I) Egg & posterior ovary; J) Lateral field (female); K-L) Bursa and genital papillae.

Table 1. Measurements and ratios of *Sclerorhabditis neotropicalis* sp. n. All measurements are in µm. Mean ± SD and range in parenthesis.

Character	Holotype female	Paratype females n= 15	Paratype males n= 7
L	550	542.1 ± 11 (515-560)	408 ± 28.9 (363-455)
a	16.2	14.6 ± 1 (13.1-16.2)	13.3 ± 0.4 (12.8-13.9)
b	4.1	4.1 ± 0.1 (4.0-4.4)	3.6 ± 0.1 (3.5-3.7)
c	8.6	7.9 ± 0.4 (7.2-8.6)	15.9 ± 0.9 (14.0-16.8)
c´	4.3	4.3 ± 0.4 (3.5-5.0)	1.0 ± 0.1 (1.0-1.2)
V (%)	56	56.1 ± 1.5 (55-61)	-
Lip region width	13	13.2 ± (12-14)	11.8 ± 1.3 (10-14)
Stoma length	17	16.5 ± 1 (15-18)	16.6 ± 2.5 (15-22)
Stoma width	6.5	6.9 ± 0.6 (6-8)	5.5 ± 0.5 (5-6)
Pharynx length*	134	131.8 ± 2.9 (125-135)	114 ± 8.2 (107-131)
Hemizonid from anterior end	117	117.3 ± 2.8 (114-123)	-
Body width at pharynx base	33	35.1 ± 2.0 (32-40)	30.4 ± 2.5 (26-34)
Length of anterior ovary/testis	73	78.9 ± 12.3 (60-101)	163 ± 18 (140-193)
Length of posterior ovary	65	68.2 ± 16.6 (48-111)	-
Distance of vulva to anterior end	310	302.8 ± 5.8 (290-313)	-
Maximum body diameter	34	37.2 ± 2.5 (34-42)	30.6 ± 2.2 (26-33)
Rectum length	20	21 ± 1.8 (18-24)	-
Anal body diameter	15	16 ± 1.2 (15-19)	24.1 ± 2.3 (21-28)
Tail length	64	68.6 ± 4.4 (62-76)	25.7 ± 2.3 (23-30)
Spicule length	-	-	40.7 ± 3.5 (36-47)
Gubernaculum length	-	-	16.5 ± 1.0 (15-18)

* Pharynx length (measured from the stoma base to basal bulb = pro + meta and postcorpus length).

elongate conoid, posterior hyaline portion very slender and tip acute. Phasmids located at 20% of tail length from anus.

Male. Smaller than female but similar in general morphology. Posterior end ventrally curved. Stoma slightly narrower than that in female. Reproductive system monorchic, with testis anterio-ventrally reflexed, on right side of intestine. Germinal zone with two or three rows of hexagonal cells with nuclei. *Vesicula seminalis* filled with small round sperm cells, *vas deferens* with granulated appearance. Spicules free, strong and thick, 1.6-1.8 times longer than anal body width, straight in the anterior portion and curved distally as a smooth J-shape, manubrium swollen and more or less rounded, calamus funnel-shaped and lamina ventrally curved with acute tip. Gubernaculum 38-42% as long as spicule length, proximal third bent anteriorly. Bursa open anteriorly, peloderan with scalloped margin and bearing eight pairs of genital papillae, two precloacal and six postcloacal, the two posterior papillae very close (1+1/1+1+1+1+2). Tail conoid spicate, with phasmid not well observed, but apparently located very posterior.

Diagnosis. Sclerorhabditis neotropicalis sp. n. can be distinguished by its body length (515-560 µm in females and 363-455 µm in males), lip region with crown-shaped lips having acute three acute thorns, oral aperture one third of lip region width, stoma 15-22 µm long or 1.0-1.6 times as long as lip width, stomatal tube about 1.5 times longer than wide, bearing rhabdia with slightly rugous walls at lumen side, stegostoma lacking glottoid apparatus and denticles, pharynx 107-135 µm long, excretory pore and hemizonid located at 21-22% of the distance from the anterior end at isthmus level. The female reproductive system is didelphic-amphidelphic, with ovaries straight or dorsally reflexed, and the vulva is a transverse slit, 55-61% from the anterior end, female tail conoid elongated, 62-76 µm long, having a posterior hyaline portion very slender and with acute tip. The male reproductive system is monorquic, with testis anterio-ventrally reflexed, spicules free, 36-47 µm long with rounded manubrium, gubernaculum 15-18 µm long or 38-42% of spicule length, and bursa anteriorly open, peloderan with scalloped margin and bearing

eight pairs of genital papillae (1+1/1+1+1+1+2), and male tail conoid spicate, 23-30 µm long.

Relationships. The material examined fits very well the description of *Sclerorhabditis* Ahmad et al., 2007. According to these authors, *Sclerorhabditis* is closely related to *Diploscapter* Cobb, 1913 and Carinoscapter Siddiqi, 1999, but is clearly distinguishable by the head shape and labial sclerotization. *Sclerorhabditis neotropicalis* sp. n. is distinguished from *S. tridentata* nom. emend. in having a larger body (515-560 vs 356-461 µm), broader diameter at midbody (34-42 vs 20-32 µm), and at anus (15-19 vs 9-12 µm), lip region with truncate appearance (vs with hemispherical appearance), having crown-shaped lips with more acute thorn (vs more rounded), and not prominent pointed process (vs very prominent), neck region with anterior portion narrower than adjacent body region and clearly annulated (vs anterior portion of neck not differentiated from adjacent body region and all annuli with similar size), oral aperture wider (one third of lip region width vs one fourth to one fifth), stoma with different proportion (1.0-1.6 vs 1.7-2.4 as long as diameter of lip region), with stomatal tube wider (about 1.5 times longer than wide vs 3.5 times), having anteriorly divergent walls (vs straight walls), and with thicker rhabdia and slightly rugous at lumen side (vs very thin and smooth), longer pharynx (125-135 vs 83-116 µm), large hemizonid (21-22 % from anterior end vs not indicated in S. tridentate), excretory pore at isthmus level (vs basal bulb level), and slightly longer tail (62-76 µm, c´= 3.5-5.0 vs 45-63 µm, c´= 3.8-6.5), having a hyaline and very slender portion region with acute tip (vs thicker not hyaline posterior region with finely rounded tip)

Type locality and habitat. Cordillera Volcánica Central Conservation Area. *Cecropia obtusifolia* plants, about two meters high, growing in the ditch along the side of the road (Highway 4), about two kilometers south of the entrance to the La Selva Biological Station (OTS), Costa Rica. The nematodes were extracted from mounds of moist (paste-like), dark brown material, apparently consisting of parenchyma tissue (pith) that the ants scrape from the inner walls of the internode.

Type material. Holotype female on slide L8m, paratypes on slides L7, L8, L9 and L10, males on slides L2, L3, L4 and L5 in the nematode reference collection at the Laboratorio de Nematología, Escuela de Ciencias Agrarias, Universidad Nacional, Costa Rica. Slides L1 & L6, L14 and L15 deposited in the nematode reference collection at the Nematology Laboratory, Jaén University, Spain.

Etymology. Named for its association with a neotropical tree (*Cecropia obtusifolia*).

DISCUSSION

The mutualism between *Cecropia* and species of *Azteca* ants has been studied by several authors (e.g. Davidson and Fisher, 1991; Longino, 1991; Davidson, 2005); however, practically nothing is known about the role of nematodes in the ant nest. Longino (1991) briefly mentioned the presence and abundance of nematodes in mounds of material inside the ant nest. These mounds are generally located very near the *Azteca* brood. Blatrix et al. (2009) demonstrated the multipartite symbiotic relation between plant, ant and fungus. It is possible that this new finding represents a similar symbiotic community, involving *Azteca, Cecropia, Sclerorhabditis,* bacteria and possibly fungi.

The material included in this paper was collected in a lowland tropical rain forest (60 m above sea level) in a *Cecropia obtusifolia* tree with an ant nest (*Azteca constructor*). Recently we observed the same nematode in *C. obtusifolia* colonized by *Azteca xanthochroa* in San Ramón (1100 m above sea level). Previous observations of at least 20 samples of *C. insignis* and *C. obtusifolia* (from various parts of the country) containing one or the other of these *Azteca* species, showed that nematodes are always present, although it remains to be determined if it is always the same nematode species. The nematodes are not associated with ant garbage dumps, but rather with mounds of plant tissue that the ants scrape from the inner walls of the stem, and these mounds are generally located near the ant brood. These observations strongly suggest that the association between the nematodes and the *Azteca* ants inhabiting *Cecropia* stems is more than a casual association. The role of the nematodes in this system is currently under investigation.

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LITERATURE CITED

Ahmad, I., A. A. Shah and M. Mahamood. 2007. Nematodes of the order Rhabditida from India. Description of *Sclerorhabditis tridentatus* gen. n., sp. n. (Nematoda: Rhabditidae). Nematology 9:43-47.

Blatrix, R., S. Bouamer, S. Morand and M. A. Selosse. 2009. Ant-plant mutualisms should be viewed as symbiotic communities. Plant Signaling & Behaviour 4:554-556.

Davidson, D. W. 2005. *Cecropia* and its biotic defenses. Pp. 214-226 *in*: C.C. Berg & P. Franco Rosselli, *Cecropia*. Flora Neotropica, New York Botanical Garden, New York.

Davidson, D. E. and B. L. Fisher. 1991. Symbiosis of ants with *Cecropia* as a function of light regime. Pp. 289-309 *in*: C. R. Huxley and D.F. Cutler (Eds.). Ant-Plant Interactions. Oxford University Press, Oxford.

de Maeseneer, J. and J. d´Herde. 1963. Méthodes utilisées pour l´etude des anguillules libres du sol. Revue de l´ Agriculture Bruxelles 16:441-447.

Longino, J. T. 1991. *Azteca* ants in *Cecropia* trees: taxonomy, colony structure, and behaviour. Pp. 271-288 *in:* Huxley, C.R. & Cutler D.F. (Eds). Ant-plant interactions. Oxford University Press, Oxford.

Seinhorst, J. W. 1959. A rapid method for the transfer of nematodes from fixative to anhydrous glycerine. Nematologica 4:67-69.

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