# Anandranema phlebotophaga n. gen., n. sp. (Allantonematidae : Tylenchida), a new nematode parasite of phlebotomine sand flies (Psychodidae : Diptera) with notes on experimental infections of these insects with parasitic rhabditoids

George O. POINAR Jr., \* Cristina FERRO \*\*, Alberto MORALES \*\* and Robert B. TESH \*\*\*

\* Department of Entomological Sciences, University of California, Berkeley, CA 94720. \*\* Entomology Group. National Institute of Health, Bogota, Colombia, and \*\*\* Yale Arbovirus Research Unit, School of Medicine, Yale University, New Haven, CT 06510, USA. Accepted for publication 16 March 1992.

**Summary** – Anandranema phlebotophaga n. gen., n. sp. (Allantonematidae : Tylenchida) is described from the phlebotomine sand fly, Lutzomyia longipalpis (Psychodidae : Diptera) from South America. This parasite is characterized by the absence of males, the small size and inconspicuous vulvar opening of the infective hermaphrodites and the pleomorphic shape of the mature parasitic hermaphrodites. The infective stage hermaphrodites of *A. phlebotophaga* n. sp. penetrate through the body wall of first stage. L. longipalpis larvae and are carried into the pupa and adult stage of the host. Infected female hosts deposit juvenile nematodes while ovipositing. Heavy infections reduced egg production and sometimes completely sterilized female flies. Laboratory trials using *Heterorhabditis* sp. (Heterorhabditidae : Rhabditida) and *Steinernema carpocapsae* (Steinernematidae : Rhabditida) against larvae of the sand fly, *Phlebotomus papatasi* (Psychodidae : Diptera) showed that infection with both nematodes could occur. Possibilities of the above nematodes for the biological control of sand flies are discussed.

Résumé – Anandranema phlebotophaga n. gen., n. sp. (Allantonematidae : Tylenchida), un nouveau nématode Tylenchide parasite de phlébotomes (Psychodidae : Diptera) et note sur les infestations expérimentales de ces insectes par des Rhabditoïdes parasites – Description est donné d'Anandranema phlebotophaga n. gen., n. sp. (Allantonematidae : Tylenchida) provenant de Lutzomyia longipalpis, phlébotome (Psychodidae : Diptera) d'Amérique du Sud. Ce parasite est caractérisé par l'absence de mâles, la petite taille, l'ouverture vulvaire très discrète des hermaphrodites infestants et le type pléomorphique des hermaphrodites matures parasites. Les femelles infestantes pénètrent à travers la paroi des larves de premier stade de L. longipalpis et se propagent dans les pupes et les stades adultes. Les femelles infestées relâchent les nématodes juvéniles lors de l'oviposition. Les infestations importantes réduisent la production des œufs et peuvent totalement stériliser les phlébotomes femelles. Des essais au laboratoire ont montré que Heterorhabditis sp. (Heterorhabditidae : Rhabditida) et Steinernema carpocapsae Steinernematidae : Rhabditida) peuvent infester les larves de Phlebotomus papatasi (Psychodidae : Diptera). Un lâcher de ces nématodes dans la perspective d'un contrôle biologique des phlébotomes est discuté.

Key-words : Nematodes, Anandranema, rhabditoids, phlebotomids

Phlebotomine sand flies serve as vectors of bartonellosis, leishmaniasis and several arboviral diseases and consequently are of considerable public health importance. Spirurid, filariid and tetradonematid nematodes have been recovered from the body cavity of phlebotomine sand flies (Poinar, 1975; Killick-Kendrick *et al.*, 1989) and although tylenchid parasites have been noted (Young & Lewis, 1977) none have been described. Thus, when a tylenchid nematode infection was discovered in a laboratory colony of *Lutzomyia longipalpis*, it provided adequate material for detailed study of this association.

The present paper describes the parasite and discusses its life history and effect on sand fly hosts. The biological control potential of this nematode is compared with that of *Steinernema* and *Heterorhabditis* species which were used to infect sand fly larvae in the laboratory.

## Materials and methods

The nematode-infected individuals of *Lutzomyia* longipalpis (Lutz & Neiva) were discovered in a laboratory colony of the insects maintained at the National Institue of Health in Bogota, Colombia, a portion of which was transferred to the Yale Arbovirus Research Unit. The progenitors of this laboratory colony were originally collected in El Callejon, Department of Cundinamarca, Colombia in 1990.

Juvenile nematodes were allowed to emerge from infected adult flies maintained on water agar cultures and were also collected directly from the body cavity of similar flies. The nematodes were reared to the adult stage in water in small Petri dishes and hanging drop slides.

Infected L. longipalpis larvae, pupae and imagoes were

dissected to examine the adult and developmental stages of the parasites. For taxonomic studies, the nematodes were killed in hot water (55 °C), fixed in TAF and processed to glycerin. All examinations were conducted with a Nikon Optiphot microscope equipped with differential interference contrast.

Since the small number of L. longipalpis larvae available were infected with A. phlebotophaga, experimental infections with heterorhabditids and steinernemadids were conducted with larvae from a laboratory colony of Phlebotomus papatasi (Scopoli) in plaster of Paris sand fly rearing containers (Modi & Tesh, 1983). Approximately 10 000 infective stage juveniles of the 42 strain of Steinernema carpocapsae, the SC strain of Heterorhabditis bacteriophora and the H-127 strain of H. bacteriophora were added to the bottom of three rearing containers, respectively, each containing between 300 and 400 second, third and fourth instar larvae of P. papatasi. All three treatments were replicated three times. The controls received no nematodes. Insect mortality was evaluated 18 days after adding the nematodes by dissecting each dead larva and looking for signs of nematode activity.

## Anandranema \* n. gen.

*Diagnosis*: Allantonematidae (Pereira); Tylenchida (Filipjev). Hermaphroditic; small infective stages with well-developed stylet and stylet knobs; vulvar opening inconspicuous. Parasitic hermaphrodites elongate – sausage shaped at first, eventually becoming pleomorphic; oviparous; males absent.

Anandranema n. gen. is the only allantonematid species lacking males and possessing hermaphroditic females. The small size and inconspicuous vulvar opening in the infective hermaphrodites and the pleomorphic shape of the mature parasitic hermaphrodites separate this genus from previously described members of the family.

## Anandranema phlebotophaga n. sp. (Figs 1, 2 A-E)

# Measurements

Infective hermaphrodites (n = 10). L = 277-365 (324  $\pm$  112)  $\mu$ m; max. body diam. = 16-23 (19  $\pm$  3)  $\mu$ m; ant. end – excr. pore = 63-73 (71  $\pm$  4)  $\mu$ m; ant. end – nerve ring = 49-59 (53  $\pm$  3)  $\mu$ m; tail length = 19-37 (30  $\pm$  6)  $\mu$ m; body diam. at anus = 11-13 (12  $\pm$  2)  $\mu$ m; tail tip – vulva = 33-53 (43  $\pm$  3)  $\mu$ m; stylet = 8-11 (10  $\pm$  1)  $\mu$ m; gonad primordium = 29-95 (48  $\pm$  10)  $\mu$ m.

Mature parasitic hermaphrodites (n = 15). L = 460-1090 (676 ± 14) µm; max. body diam. = 48-128

 $(73 \pm 9) \ \mu\text{m}$ ; tail length = 37-96 (63 ± 4)  $\mu\text{m}$ ; tail tipvulva = 45-120 (66 ± 8)  $\mu\text{m}$ . L × width fertilized egg (in utero) = 38-54 (47 ± 3) × 19-24 (22 ± 2)  $\mu\text{m}$ .

# Description

Characters as defined in the generic description.

Infective stage hermaphrodite (Figs 1 A, 2 E): Tip of stylet set off and thicker than remainder; guiding ring behind thickened portion; dorsal gland opening immediately behind stylet knobs; subventral gland openings approximately two stylet lengths behind stylet knobs; basal portion of glands with their respective nuclei overlapping the pharynx and the dorsal portion of the intestine; excretory pore faint and located posterior to the nerve ring; hemizonid located just posterior to the excretory pore; gonad anlag composed of four to eight cells depending on the state of development; anus faint; tail tip pointed; the adult infective stage is enclosed within the fourth stage cuticle which has pronounced annular rings and distinct paired lateral lines extending from the region of the pharyngeal glands to the anal region.

Parasitic hermaphrodite (Figs 1 B, C; 2 D) : Normally elongate at first (in host larva) but often becoming short and wide and exhibiting pleomorphism later (in the pupal and adult hosts), body wall containing a " bush border " composed of fine, short lines and a mucoid layer on the surface. A distinct spermatheca is present (most conspicuous in the younger hermaphrodites) and filled with spherical microsperms (1-2  $\mu$ m in diameter); ovary elongated, reaching the anterior region in older hermaphrodites, may be straight, bent once or twice; vulva opening terminal, subterminal or ventral in position; body may be straight, curved ventrally, dorsally or in various coiled positions; tail rounded; stylet, excretory pore, nerve ring, pharynx and anus not visible.

## Type host and locality

*Lutzomyia longipalpis* (Phlebotominae : Psychodidae : Diptera). Laboratory colony originating from El Callejon, Cundinamarca Department, Colombia.

#### Type specimens

Holotype (infective hermaphrodite) and allotype (mature parasitic hermaphrodite) deposited in the Nematology collection, Department of Nematology, Davis, CA, USA; paratypes deposited in the collection of the Laboratoire de Biologie Parasitaire, Protistologie, Helminthologie, Muséum National d'Histoire Naturelle, Paris.

## Life cycle

The infective stage hermaphrodites of A. phlebotophaga (Fig. 1 A) were observed to penetrate directly through the body wall of first stage L. longipalpis larvae. The nematodes first developed into elongate cylindrical forms (Fig. 1 C) that consisted essentially of a reproductive unit protected and nourished by the nematodes

<sup>\*</sup> Derived from the Greek noun "anandros" which means without a mate or husbandless.



Fig. 1. Anandranema phlebotophaga n. gen., n. sp. A : Infective stage hermaphrodite; B : Mature parasitic hermaphrodite; C : Immature parasite hermaphrodite.

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**Fig. 2.** A : Tail of an infected female *Lutzomyia longipalpis* showing parasitic juveniles (arrows) of *Anandranema phlebotophaga* n. gen., n. sp. in the body cavity; B : Young parasitic juveniles of *Anandranema phlebotophaga* removed from an infected adult *Lutzomyia longipalpis*. (Arrow shows loose cuticle in a molting juvenile); C : A juvenile of *Anandranema phlebotophaga* attempting to exit from the egg. Note stylet (arrow); D : A mature parasitic hermaphrodite of *Anandranema phlebotophaga* removed from the body cavity of *Lutzomyia longipalpis*; E : Head of an infective hermaphrodite of *Anandranema phlebotophaga*. Note stylet and subventral gland openings (arrow); F : A larva of *Phlebotomus papatasi* infected with *Heterorhabditis bacteriophora* (NC strain). (Arrow shows nematode in host's body cavity).

original body wall. A mucoid deposit and bush border on the outside of the body wall suggested that the hermaphrodite absorbed nutrients directly through the cuticle. Egg development was initiated in the late larval host stages and continued in the pupal and adult sand flies. At this time, the parasitic hermaphrodites had become wider and assumed a variety of shapes. Very little embryonic development occured in the hermaphrodite, and the eggs were deposited soon after fertilization into the host's hemocoel. Juvenile development continued to the late third stage in the host's body cavity and these juveniles accumulated in the infected adult fly, sometimes packing the body cavity (Fig. 2 A). The nematodes exited via the hosts alimentary (anus) and reproductive systems. Infected female flies could actively deposit juvenile nematodes during oviposition procedure.

Upon reaching the environment, the nematodes molted twice to reach the adult stage. These molts took approximately 2 weeks at 15 °C and sometimes both cuticles were shed simultaneously; however in general cuticle C-4 was maintained and the infective hermaphrodites were thus ensheathed in the last juvenile cuticle. The infective stages could be maintained in water at 15 °C for 12 weeks.

## Effect on the host

The nematodes occured in both male and female hosts. Up to eight hermaphrodites and their progeny occured in a single fly. Heavy infections greatly reduced egg production and in some cases, completely sterilized female flies.

## EXPERIMENTAL INFECTIONS

The results of challenging larvae of *P. papatasi* with the infectives of *Heterorhabditis* and *Steinernema* are presented in Table 1. Although some infection occured

**Table 1.** Mortality of *Phlebotomus papatasi* larvae when challenged with infective juveniles of *Heterorhabditis* and *Steinerne-ma*.

Nematode	Larval mortality	
	Second-third instar	Fourth instar
H. bacteriophora (NC strain)	30 % (144)*	60 % (142)
H. sp. (H-127 strain)	13 % (256)	23 % (168)
S. carpocapsae (42 strain)	7 % (237)	7 % (162)
Control	1 % (273)	0 % (157)

\* Percent mortality followed by total number of that particular instar in the container.

with all three nematode strains, the highest mortality rates were obtained with *Heterorhabditis*, especially *H. bacteriophora* against fourth instar larvae (60 % mortality).

## Discussion

This is the first allantonematid that multiplies exclusively by autotoky (production of progeny by a single parent), in this case hermaphroditism (Poinar & Hansen, 1983). The microsperm found in the hermaphroditic females of *A. phlebotophaga* (1-2  $\mu$ m in diameter) shares the distinction with those of *Deladenus wilsoni* Bedding (1-2  $\mu$ m in diameter) (Bedding, 1968) and *Ty-lenchulus semipenetrans* Cobb (2  $\mu$ m in diameter) (Baccetti *et al.*, 1983) in representing the smallest known sperm in the phylum Nematoda.

Two different types of nematode infections of sand flies are presented in this study. The first, dealing with *A. phlebotophaga*, represents a natural infection by an obligate parasite. The second deals with experimental infections caused by the commercially available rhabditoid parasites *Heterorhabditis* and *Steinernema*.

The former nematode is a naturally occuring parasite of sand fly larvae in infested areas. From notes presented in the literature, it would appear that tylenchid nematodes are fairly widespread in phelbotomine sand flies. They have been reported from species of Lutzomyia, Phlebotomus and Sergentomyia in Africa, the Middle East and Central America (Young & Lewis, 1977). It is likely that these nematodes constitute a means of natural control of phlebotomine sand flies in many parts of the world. Could this infection be augmented by releasing infective stage hermaphrodites in the environment? Probably so. The effect of A. phlebotophaga on its sand fly host (partial to complete sterilization) is similar to that of the related nematode Tripius sciarae (Bovien) which parasitizes sciarid flies (Sciaridae: Diptera). When soil containing the infective stage females of T. sciarae was distributed over several flats in a section of a greenhouse, the sciarid population rapidly declined and by the end of four weeks, the flies were almost completely eliminated from that portion of the greenhouse (Poinar, 1965). The nematode stages of A. phlebotophaga could be distributed in two ways - by releasing infected female sand flies that would search out and deposit nematodes in breeding sites or by applying the infective stages directly to known breeding sites.

Although the exact larval breeding sites of *L. longipalpis* and of most other sand fly species have been difficult to locate, the available evidence suggests that the immature forms of this group of insects develop in moist soil with high organic content in tree buttresses, caves, termite mounds, animal burrows, barns, basements and other similar habitats which are protected from direct sunlight, temperature extremes and flooding (Rutledge & Mosses, 1972; Bettini & Melis, 1988; Basimike & Mutinga, 1990). If high density breeding sites can be located, then these would be ideal locations to liberate the infective stages of heterorhabditids. The advantage of these rhabditoids is that they normally kill the host within 24 hours after entering the hemocoel, however, there would be essentially no transporting of the nematode from site to site by adult flies as would be the case with *A. phlebotophaga*. Future studies are needed to elucidate the biological control potential of these nematodes against phlebotomine sand flies.

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