



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 ± 112) µm; max. body diam. = 16-23 (19 ± 3) µm; ant. end – excr. pore = 63-73 (71 ± 4) µm; ant. end – nerve ring = 49-59 (53 ± 3) µm; tail length = 19-37 (30 ± 6) µm; body diam. at anus = 11-13 (12 ± 2) µm; tail tip – vulva = 33-53 (43 ± 3) µm; stylet = 8-11 (10 ± 1) µm; gonad primordium = 29-95 (48 ± 10) µm.

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

(73 ± 9) µm; tail length = 37-96 (63 ± 4) µm; tail tip-vulva = 45-120 (66 ± 8) µm. L × width fertilized egg (in utero) = 38-54 (47 ± 3) × 19-24 (22 ± 2) µm.

#### 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 mucoïd layer on the surface. A distinct spermatheca is present (most conspicuous in the younger hermaphrodites) and filled with spherical microsperms (1-2 µm 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.

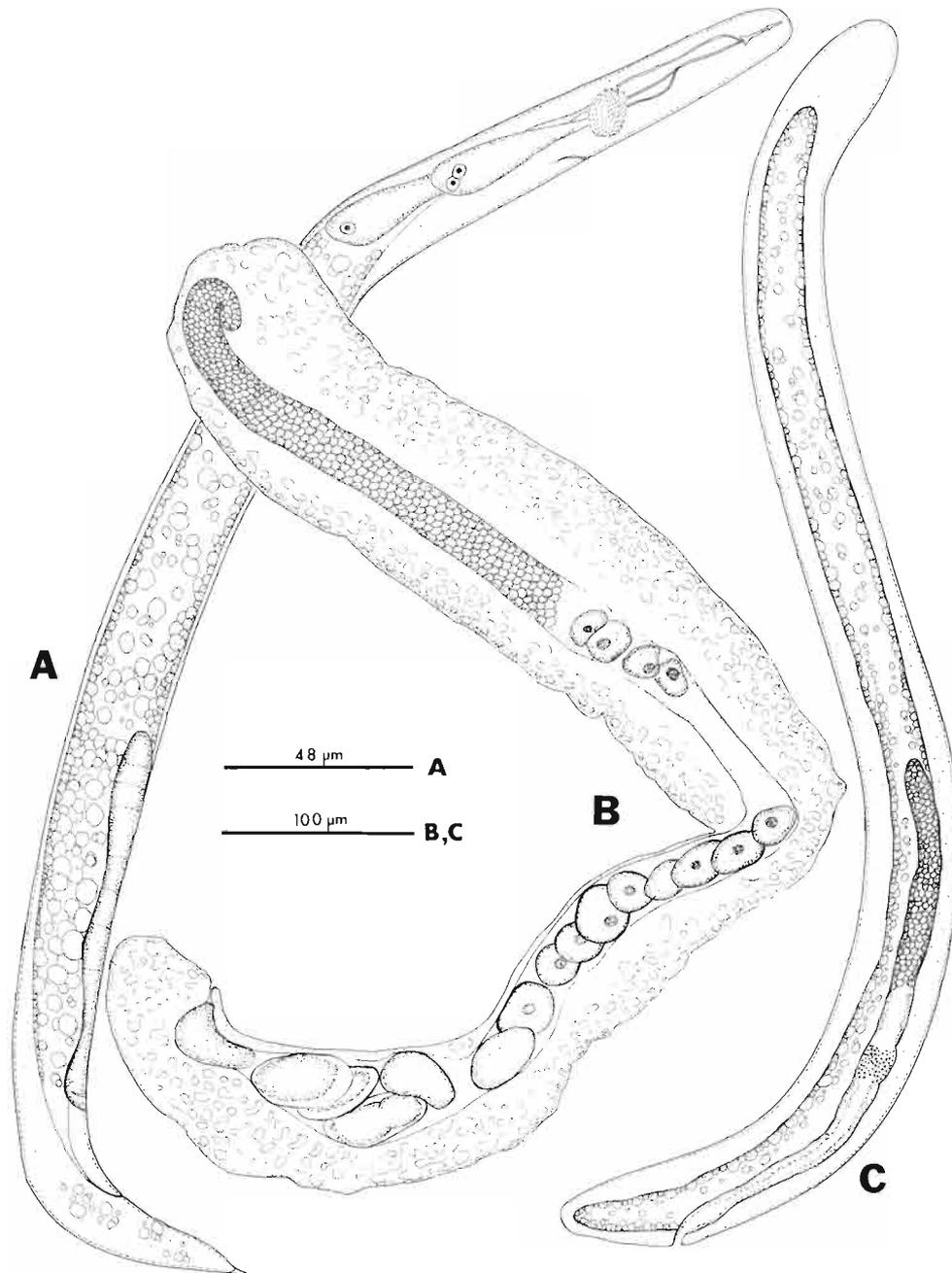
#### TYPE SPECIMENS

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.

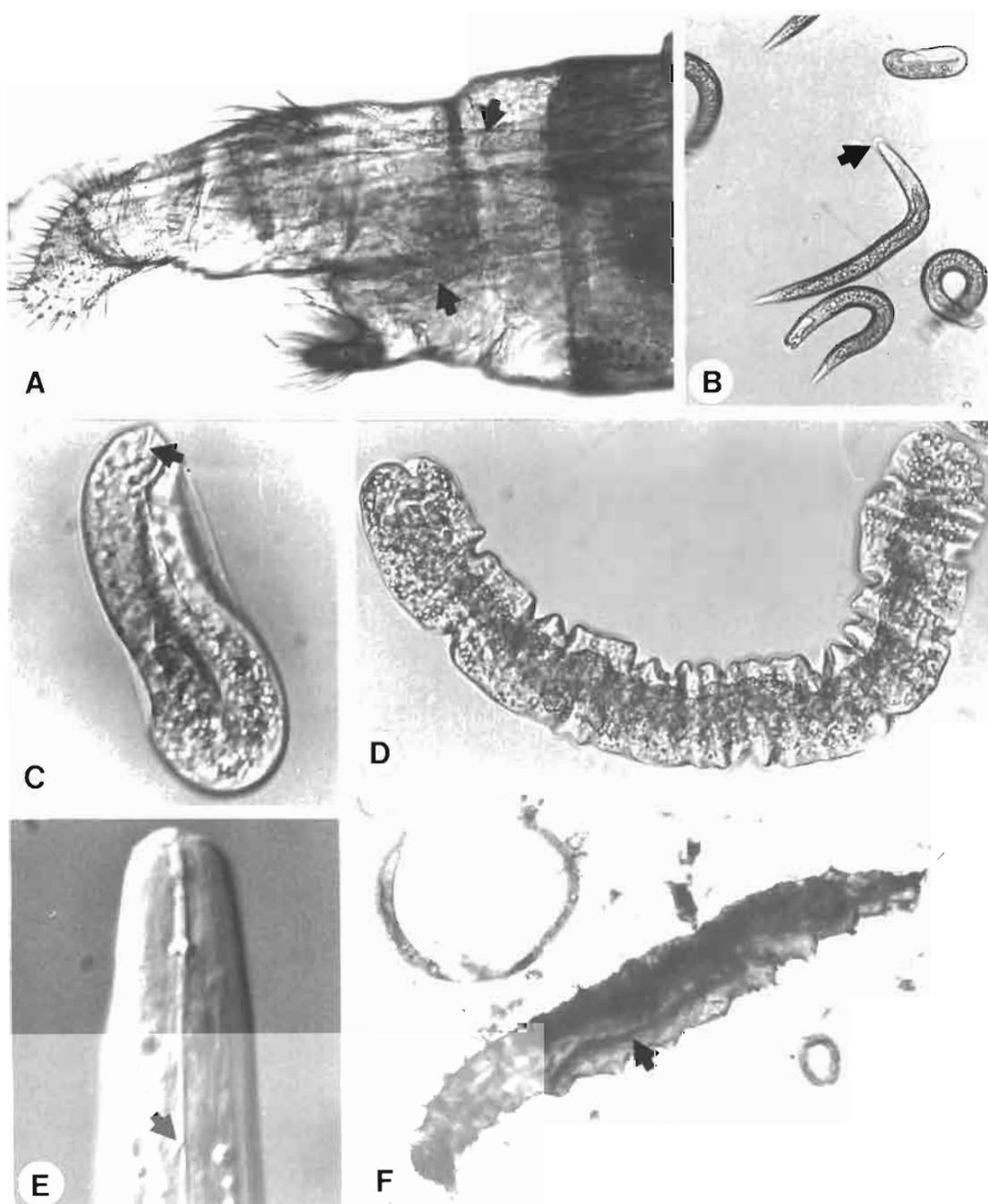
#### LIFE CYCLE

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

\* 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.



**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 occurred 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 occurred in both male and female hosts. Up to eight hermaphrodites and their progeny occurred 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 occurred

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

Nematode	Larval mortality	
	Second-third instar	Fourth instar
<i>H. bacteriophora</i> (NC strain)	30 % (144)*	60 % (142)
<i>H. sp.</i> (H-127 strain)	13 % (256)	23 % (168)
<i>S. carpocapsae</i> (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 µm in diameter) shares the distinction with those of *Deladenus wilsoni* Bedding (1-2 µm in diameter) (Bedding, 1968) and *Tylenchulus semipenetrans* Cobb (2 µm 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 occurring parasite of sand fly larvae in infested areas. From notes presented in the literature, it would appear that tylenchid nematodes are fairly widespread in phlebotomine 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 sciararum* (Bovien) which parasitizes sciarid flies (Sciaridae: Diptera). When soil containing the infective stage females of *T. sciararum* 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.

#### Acknowledgments

This work was supported in part by grants from the National Institutes of Health (AI 28528) and from the MacArthur Foundation (9008073 A).

#### References

- BACCETTI, B., DALLAI, R., GRIMALDI DE ZIO, S. & MARINARI, A. (1983). The evolution of the nematode spermatozoon. *Gamete Res.*, 8 : 309-323.
- BASIMIKE, M. & MUTINGA, M. J. (1990). Temperature and moisture content of soils of termite mounds and animal burrows in relation to relative abundance of adult phlebotomine sand flies (Diptera : Psychodidae) in Marigat Semiarid Area, Baringo District, Kenya. *Envir. Entomol.*, 19 : 486-489.
- BEDDING, R. A. (1968). *Deladenus wilsoni* n. sp. and *D. siri-cidicola* n. sp. (Neotylenchidae) entomophagous-mycetophagous nematodes parasitic in siricid woodwasps. *Nematologica*, 14 : 515-525.
- BETTINI, S. & MELIS, P. (1988). Leishmaniasis in Sardinia. III. Soil analysis of a breeding site of three species of sand flies. *Med. vet. Entomol.*, 2 : 67-71.
- KILLICK-KENDRICK, R., KILLICK-KENDRICK, M., NAWI, N. A. Q., ASHFORD, R. W. & TANG, Y. (1989). Preliminary observations on a tetradonematid nematode of phlebotomine sand flies of Afghanistan. *Annles Parasitol. hum. comp.*, 64 : 332-339.
- MODI, G. B. & TESH, R. B. (1983). A simple technique for mass rearing *Lutzomyia longipalpis* and *Phlebotomus papatasi* (Diptera : Psychodidae) in the laboratory. *J. med. Entomol.*, 20 : 568-569.
- POINAR, JR. G. O. (1965). The bionomics and parasitic development of *Tripus sciarae* (Bovien) (Sphaerulariidae : Aphelelenchoidea), a nematode parasite of sciarid flies (Sciaridae : Diptera). *Parasitology*, 55 : 559-569.
- POINAR, JR. G. O. (1975). *Entomogenous nematodes*. Leiden, E. J. Brill, 317 p.
- POINAR, JR. G. O. & HANSEN, E. (1983). Sex and reproductive modifications in nematodes. *Helminthol. Abstr. Ser. B*, 52 : 145-163.
- RUTLEDGE, L. C. & MOSSES, H. L. (1972). Biology of immature sandflies at the bases of trees in Panama. *Envir. Ent.* 1 : 300-309.
- YOUNG, D. G. & LEWIS, D. J. (1977). Pathogens of Psychodidae (phlebotomine sand flies). In: Roberts, D. W., & Strand, M. A. (Eds). *Pathogens of medically important arthropods*. Bull. World Health Organ. (Suppl. No. 1), 55 : 9-24.