

Parasitism by *Tylenchid Nematodes* in Natural Populations of *Pintomyia fischeri* (Diptera: Psychodidae: Phlebotominae) in ArgentinaFernández MS^{1,2}, Santini MS^{2,3}, Diaz JI^{2,4}, Villarquide L⁵, Lestani E¹, Salomón OD^{1,2} and Achinelly M^{2,4*}¹Instituto Nacional de Medicina Tropical (INMeT), Puerto Iguazú, Misiones, Argentina²Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Argentina³Centro Nacional de Diagnóstico e Investigaciones Endemo-epidemicas (CeNDIE), Administración Nacional de Laboratorios e Institutos de Salud (ANLIS), Argentina⁴Centro de Estudios Parasitológicos y de Vectores, CCT La Plata (CONICET-UNLP), Argentina⁵Laboratorio de Control de Vectores Entomológicos de Importancia Sanitaria (LaCVEIS) Fundación H. A. Barceló, Argentina

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CC-BY 4.0Keywords Sand Flies; Nematoda;
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Abstract

Pintomyia fischeri adults collected in different eco-epidemiological studies in the northeastern of Argentina were found parasitized by juvenile nematodes (Tylenchida) isolated from the body cavity. The percentage of infected females and males was 3.8% and 2.9% respectively. Part of the life cycle of sand flies and tylenchid nematodes take place in humid and dark sites, where infection of immature stage of Phlebotominae insects is possible. Biology of this parasite could help to determine the breeding sites of sand flies. This study constituted the first report of tylenchid nematodes infecting sand flies at field conditions in South-America.

Phlebotominae are insects of public health importance because their role in the transmission of the etiological agents of several diseases, being the different leishmaniasis the best known around the world.

There are 28 species of Phlebotominae recorded in Argentina [1], some of them involved in the transmission of the etiological agent of Visceral Leishmaniasis (VL) and Cutaneous Leishmaniasis (CL) [2]. *Pintomyia fischeri* (Pinto, 1926) constitutes the third more abundant species in a deforestation area in northeast Argentina [3] where CL due to *Leishmania braziliensis* Vianna, 1911, is endemic [4,5]. There are not records of *Pi. fischeri* as *Leishmania* (Viannia) spp. vector in Argentina [1], although adults infected by *Leishmania* spp. have been observed in an endemic area of ACL in Brazil, suggesting this sand fly species as a probable secondary vector [6]. Taking into account the public health importance of sand flies, any contribution regarding a possible agent for its biological control is relevant.

Tylenchid nematodes were reported from the body cavity of sand flies [7]. The infective stage (gravid females) penetrates through the body wall of first stage larvae of phlebotomine insects. The eggs develop to third and fourth juvenile stage, and they are accumulated in the infected sand fly adults. Nematodes leave the insect host via the anus or reproductive pore and mature to adult in the environment where mated females reinvade the insect to restart the cycle. Heavy infections greatly reduced egg production and in several cases, completely sterilized female flies. So, these nematodes could be a natural bioregulator agent of sand fly populations [8].

In this work parasitism by tylenchid nematodes is reported in natural populations of the sand fly *Pi. fischeri*, in the northeastern Argentina.

The study was carry out in a rural zone of Puerto Iguazú (25° 39' S, 54° 33' W), Misiones province, at the northeastern border of Argentina. The area known as "Dos mil hectáreas", which covers an area of 2000 hectares, was originally a native forest, but after its partial deforestation, many farms have been established since 2003, with a peak of human intervention and settlement during 2004 [4]. A mosaic composed by primary and secondary forest, pigsties, chicken houses, crops and houses (farms) arrange the landscape of the area which is surrounded by natural protected forest reservations and the Paraná River.

Phlebotominae were collected in the framework of different eco-epidemiological studies from winter 2006 to summer 2010 using CDC mini light traps (408 traps-night) operated from 5:00 PM to 09:00 AM and placed at 1.5 m above the ground [9]. Sand flies were preserved in 1% lactophenol and identified according to Galati (2003).

Nematodes recovered from the body cavity of sand flies were identified using a light microscope, following specific bibliography [11,12]. Photographs were taken using a Q-Imaging Go-3 camera. The length and width were determined; measurements were given in micrometers, with the mean followed by the range in brackets.

A total of 898 *Pi. fischeri* adults were captured in 97 trap-night (557 females and 341 males). Thirty-one *Pi. fischeri* specimens were found parasitized by filiform juvenile nematodes in the haemocele representing the 3.5% of the total *Pi. fischeri* capture (14 light traps). The percentage of infected females and males was 3.8% and 2.9% respectively.

Nematodes presented buccal stylet and pharyngeal glands characteristic of Tylenchida larvae (Figures 1 A-D). The mean length (n=98) was 233 μm (160-263), and the width 17.6 μm (10-23). Unfortunately due to the nature of the sand flies diaphanization methodology -lactophenol-, which prevented the maturation of juvenile nematodes to female adult stage, taxonomic identification was not possible.

Parasitized individuals were collected in peridomestic capture sites (henhouse and pigsties), except one from the edge of forest. They were captured mainly in summer (n=23), but with some records in spring (n=5) and fall (n=3).

Poinar et al. (1993) [8] observed that the elimination of tylenchid nematode juveniles and parasite infection could be take place in humid and dark sites, similar than those suggested for the oviposition and larval growing of Phlebotominae as tree buttresses, and rodent burrows with organic debris like leaf litter, dung and crevices [7].

Pintomyia fischeri was found in Argentina in a secondary forest and peridomestic environments [3,4]. In the same way, Rangel and Lainson (2009) [13], suggested that this species in Brazil is adapting to the domiciliary environment, with anthropophilic behavior, but also feeding on dogs and birds. Although the breeding sites of the immature stages of *Pi. fischeri* in Argentina has not been found yet, it could take place under humidity and darkness soil conditions at the sampling sites considered in our study. Knowledge of the biology and dynamics of this nematode at field may be useful for determining

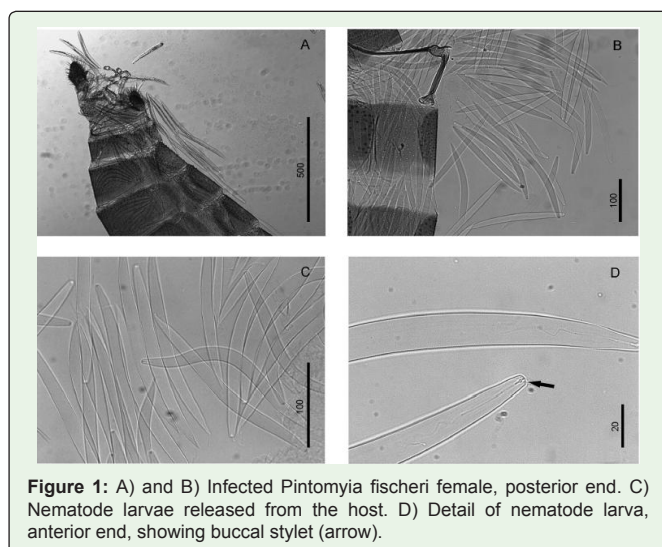


Figure 1: A) and B) Infected *Pintomyia fischeri* female, posterior end. C) Nematode larvae released from the host. D) Detail of nematode larva, anterior end, showing buccal stylet (arrow).

the breeding sites of the immature stages of these insects, which is necessary to develop control strategies. Pathogenicity and deleterious effects on the reproductive success of *Pi. fischeri* (and potentially other sand flies species), could contribute to look for new ways for vector bioregulation.

Tylenchid nematodes were reported from different species of Phlebotominae sand flies from Africa, the Middle East and Central America [7,14-16]. Individuals of *Lutzomyia longipalpis* (Lutz & Neiva 1912) were observed infected in a laboratory colony where progenitors were originally collected in Cundinamarca, Colombia [8].

This report constituted as our knowledge the first report of tylenchid nematodes parasitizing natural populations of Phlebotominae in South-America.

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