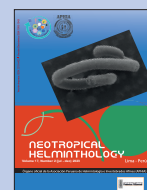




Neotropical Helminthology



ORIGINAL ARTICLE / ARTÍCULO ORIGINAL

NEW SPECIES OF *PROPARASITYLENCHUS* WACHEK (TYLENCHIDA, ALLANTONEMATIDAE) PARASITIZING *HARMONIA AXYRIDIS* PALLAS, 1773 (COLEOPTERA, COCCINELLIDAE) IN ARGENTINA

NUEVA ESPECIE DE *PROPARASITYLENCHUS* WACHEK (TYLENCHIDA, ALLANTONEMATIDAE) PARÁSITO DE *HARMONIA AXYRIDIS* PALLAS, 1773 (COLEOPTERA, COCCINELLIDAE) EN ARGENTINA

Nora B. Camino^{1,2,3*}, Sergio Rodríguez-Gil^{1,4}, Guillermo R. Reboredo^{1,4} & Sandra E. González^{1,3}

¹ Centro de Estudios Parasitológicos y de Vectores, CEPAVE, Argentina.

² Universidad Nacional de La Plata, UNLP, Facultad de Ciencias Naturales y Museo, Argentina.

³ Investigador CIC, Comisión de Investigaciones Científicas de la provincia de Buenos Aires, CIC, Argentina.

⁴ Consejo Nacional de Investigaciones Científicas y Técnicas, CONICET, Argentina.

* Corresponding author: nemainst@cepave.edu.ar

Nora B. Camino: <https://orcid.org/0000-0003-4910-3986>

Sergio Rodríguez-Gil: <https://orcid.org/0000-0003-4608-1961>

Guillermo R. Reboredo: <https://orcid.org/0000-0002-1214-316X>

Sandra E. González: <https://orcid.org/0000-0002-0609-0516>

ABSTRACT

A new species *Proparasylenchus inalienatus* sp. n. is described and illustrated, as a parasite of the coccinellid *Harmonia axyridis* Pallas, 1773 in a locality near Gran La Plata, Colonia Urquiza, Argentina. Of the 13 species described, this species is characterized by having a cephalic end projecting, faint stylet in its normal place, vulva posterior, tail appendage conoid, with a bifurcated tip in both sexes, male with two spicules and a tiny gubernaculum, and leptoderan bursa. We obtained the 18S rDNA partial sequence.

Keywords: Allantonematidae – bursa leptodera – Coccinellidae – Argentina

RESUMEN

Una nueva especie *Proparasylenchus inalienatus* sp. n., se describe parasitando al coccinélido *Harmonia axyridis* Pallas, 1773, de una localidad próxima al Gran La Plata, Colonia Urquiza. De las 13 especies descritas, esta se caracteriza por tener en el extremo cefálico una proyección, estilete tenue en su lugar normal, vulva posterior, apéndice caudal conoide,



con punta bifurcada en ambos sexos, macho con dos espículas y un diminuto gubernáculo y bursa leptodera. Obtuvimos la primera secuencia parcial 18S rDNA para el género.

Palabras clave: Allantonematidae – bursa leptodera – Coccinellidae – Argentina

INTRODUCTION

In search of parasitic nematodes of insects of horticultural interest in the surroundings of the city of La Plata, Argentina, the genus *Proparasitylenchus* was found parasitizing the coccinellid *Harmonia axyridis* Pallas, 1773. At present, the following genera: *Proparasitylenchus* Wachek, 1955; *Parasitylenchoides* Wachek, 1955, *Neoparasitylenchus* Nickle, 1967, and *Protylechus* Wachek, 1955 are poorly defined and they would need a better and expanded description. The species of the genus *Proparasitylenchus* are parasites of Coleoptera. Staphylinidae in Europe and North America. At this moment seven species have been described from the genus *Proparasitylenchus*, *P. athetae* (Wachek, 1955) Nickle, 1967; *P. platystethi* (Wachek, 1955) Nickle, 1967; *P. medonis* (Wachek, 1955) Nickle, 1967; *P. myrmedoniae* (Wachek, 1955) Nickle, 1967; *P. oxyteli* (Wachek, 1955) Nickle, 1967; *P. trogophoei* (Wachek, 1955) Nickle, 1967 from Europe; and *P. californicus* Poinar, G.O. Jr. *et al.*, 2015 from USA, all parasites of Coleoptera, Staphylinidae, except for *P. myrmedoniae* which parasitizing ants. In this paper we describe a new species *P. inalienatus* n. sp., a parasite of coccinellids from Argentina.

MATERIAL AND METHODS

Insects: adult coccinellid insects were collected by hand from September 2021 to April 2022 on plant species *Cucurbita maxima* var. *zapallito* (Carrière) Millán, 1947 (round green zucchini), at a locality near Gran La Plata, Colonia Urquiza (34°96'72"S, 58°04'96"W), Argentina. The beetles were distributed in individual plastic containers. In total, 260 adults of *Harmonia axyridis* (Pallas, 1773) (Coleoptera, Coccinellidae), were sampled and dissected. The coccinellids adults were dissected in Petri dishes filled with distilled water under a stereomicroscope.

Nematodes: living nematodes were removed from the hemocel of adult host, and then they were killed by placing them in distilled water at 60°C for 2 pure TAF (Poinar, 1975). For the molecular study, 20 specimens

were fixed in absolute alcohol. All the specimens were used for photographing in Olympus BX51 microscope with Olympus DP71 camera. Measurements of common nematode body features were performed on 16 fixed nematode specimens of each nematode stage of life cycle. Mean and in parenthesis minimum and maximum.

Total genomic DNA was extracted from four specimens using PURO-Genomic DNA (PB-L Productos Biológicos, Buenos Aires, Argentina) according to the manufacturer's protocol. The partial fragment of the 18S rDNA gene was amplified from individual worms. Amplification was conducted using polymerase chain reaction (PCR) on an Eppendorf Mastercycler thermal cycler (Hamburg, Germany) using forward primer Nem 18SF (5'-CGCGAATRGCTCATTACAACAGC-3') and Nem 18SR (5'-GGGCGGTATCTGATCGCC-3') according to Singh *et al.* (2013). The thermocycler conditions were: 94°C for 5 min; 94°C for 30 s, 35 cycles of 60°C for 40 s and 72°C for 60 s; and 72°C for 10 min. PCR products were analyzed by electrophoresis on 1% agarose gels and visualized by staining with ethidium bromide. The amplicons were sequenced in Unidad de Genómica del Instituto de Biotecnología del Instituto Nacional de Tecnología Agropecuaria, Argentina (INTA). The 18S rDNA partial sequence generated from this study were submitted to the National Center for Biotechnology Information (NCBI) GenBank database (<http://www.ncbi.nlm.nih.gov>) and can be accessed using the GenBank accession numbers: OP889000

Ethic aspect: The ethical biosafety standards established for laboratory animals and good animal experimentation practices were followed.

RESULTS

Proparasitylenchus Wachek, 1955 (Nickle, 1967)

Proparasitylenchus inalienatus sp.n.

Description (Figs. 1-5)

Entomoparasitic female: body cylindroid and arcuate, yellow. Body surface neither wavy nor with constrictions

and swellings. Cephalic end projecting (Fig. 1), faint stylet in its normal place. Excretory pore is behind the nerve ring. Anus present. Vulva posterior (Fig. 2). The

postvulval uterine sac is absent. Tail appendage conoid, with bifurcated tip (Fig. 3).



Figures 1-5. *Proparasitylenchus inalienatus* sp. n. 1. Head of the female; 2. Posterior end of female; 3. Posterior end of the female; 4. Posterior end of the male; 5. Posterior end of the larva. Bars = 50 μ m.

Female (n = 12): diameter of head at level of cephalic papillae: 40.90 μm (38.65-45.23); width of body at level of nerve ring: 65.60 μm (62.40-68.32); maximum body diameter: 84.35 μm (82.35-90.12); width of body at level of posterior end: 35.42 μm (32.50-40.10); distance anterior end to nerve ring: 135 μm (132-142); distance from anterior end to excretory pore: 170.80 μm (164.32-175.90); vagina length: 38.55 μm (32.42-40.22); V (distance anterior end to vulva/body length x 100): 86% (82-90); tail appendage length: 8.41 μm (7.98-9.25).

Male (n = 10): smaller than female. Weak, dorsal and paired subventral pharyngeal glands. Two spicules short and a tiny gubernaculum, narrow, subterminal and small leptoderan bursa (Fig. 4).

Male (n = 19): diameter of head at level of cephalic papillae: 6.25 μm (5.82-7.12); width of body at level of nerve ring: 10.41 μm (9.35-14.26); maximum body diameter: 20.50 μm (18.32-24.12); width of body at level of posterior end: 130 μm (125-142); distance anterior end to nerve ring: 20.85 μm (18.30-22.24); distance from anterior end to excretory pore: 43.75 μm (39.45-45.60); spicule length: 14.12 μm (12.34-18.50); gubernaculum length: 3.97 μm (3.25-4.43); tail appendage length: 4.41 μm (3.92-5.35)

Juveniles (n= 30): free living form very small and transparent, with stylet distinctly knobbed, excretory pore at less than 100 μm from anterior end. Tail appendage ends forked into two points (Fig. 5).

Type host: *Harmonia axyridis* Pallas, 1773 (Coleoptera, Coccinellidae)

Type locality: near Gran La Plata, Colonia Urquiza (34°56'21.199"S, 58°6'56.902"W), Buenos Aires, Argentina.

Etymology: *inalienatus* means unspoiled.

Type material: Holotype y Allotype deposited in Helminológica collection of Museo de La Plata, and Paratypes in CEPAVE TY 156.

ADN Characterization

Massey (1974) grouped four related genera: *Proparasitylenchus*, *Parasitylenchoides*, *Protilyenchus* and *Neoparasitylenchus*, proposing further study of them. There is no molecular study of these genera so we cannot make a comparison or discussion, we only contribute to this paper the 18S rDNA partial sequence. Future studies are needed to fill in this unknown.

REMARKS AND DISCUSSION

There are 13 species of the genus *Proparasitylenchus*, which are parasites of Coleoptera - Staphylinidae, and one species *P. myrmedoniae* (Wachek, 1955) Nickle, 1967, which parasites ants, that we will not be considered in this paper. All are from Germany, Europe and USA. Our new species shares with the described species the characters of the genus: body cylindroid and arcuate, yellow, excretory pore is behind the nerve ring, vulva posterior, two spicules short, gubernaculum small, and leptoderan bursa. *P. athetae* (Wachek, 1955) Nickle, 1967 the head of the parasitic female is sunk into the body, the tail appendage is stumpy, and the free-living stages have blunt tail tips and short stylets. *P. platystethi* (Wachek, 1955) Nickle, 1967 can be separated by the cuticle anullata, and the tail appendage in mucron shaped. *P. medonis* (Wachek, 1955) Nickle, 1967 has narrow head in the parasitic female. *P. oxyteli* (Wachek, 1955) Nickle, 1967 has the spicules thick with irregular edges and the stylet is very short. *P. trogophoei* (Wachek, 1955) Nickle, 1967 is distinguished by the structure of the spicule, pear-shaped spicule body and long, slender tip. *P. californicus* Poinar, G.O. Jr., et al., 2015 has the head of the parasitic female is sausage-shaped, not have knobbed but not cleft stylets.

Although the species is identified morphometrically, the phylogenetic tree could not be represented, this is because there are few genetic data deposited in the genus GenBank and these references have been geographically isolated.

Author contributions: CRediT (Contributor Roles Taxonomy)

NBC= Nora B. Camino

SRG = Sergio Rodríguez Gil

GR = Guillermo Reboredo

SG= Sandra González

Conceptualization: NBC, SRG, GR, SG

Data curation: NBC, SRG, GR, SG

Formal Analysis: NBC, SRG, GR, SG

Funding acquisition: NBC, SRG, GR, SG

Investigation: NBC, SRG, GR, SG

Methodology: NBC, SRG, GR, SG

Project administration: NBC, SRG, GR, SG

Resources: NBC, SRG, GR, SG

Software: NBC, SRG, GR, SG

Supervision: NBC, SRG, GR, SG

Validation: NBC, SRG, GR, SG

Visualization: NBC, SRG, GR, SG

Writing – original draft: NBC, SRG, GR, SG

Writing – review & editing: NBC, SRG, GR, SG

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