View metadata, citation and similar papers at core.ac.uk
brought to you by CORE
provided by CONICET Digital

ISSN 0373-5680 (impresa), ISSN 1851-7471 (en línea) Revista de la Sociedad Entomológica Argentina 75 (1-2): 101-104, 2016

New host and distribution for the mosquito parasite *Strelkovimermis spiculatus*

LOPEZ, Rocio P.*, Leonardo M. DÍAZ-NIETO* & Corina M. BERÓN

Instituto de Investigaciones en Biodiversidad y Biotecnología (INBIOTEC) – CONICET and Fundación para Investigaciones Biológicas Aplicadas (FIBA). Mar del Plata, Argentina.
E-mail: cberon@fiba.org.ar
* These authors contributed equally to this work.

Nuevo hospedador y distribución del parásito de mosquitos *Strelkovimermis spiculatus*

RESUMEN. *Strelkovimermis spiculatus*, Poinar & Camino 1986 (Nematoda: Mermithidae) ha sido encontrado parasitando algunos géneros de mosquitos tales como *Aedes* (*Ochlerotatus*), *Culex* y *Psorophora.* En un proyecto sobre distribución de mosquitos en la Provincia de Buenos Aires, Argentina, fueron encontradas especies de mosquitos parasitadas por nematodos en criaderos naturales, en los alrededores de la ciudad de Mar del Plata. El objetivo de este trabajo es identificar este parásito detectado en esta área de distribución y determinar las especies de mosquitos hospedadoras. Se describe la utilidad de secuencias correspondientes a los genes COI y 18S RNAr-ITS1-5.8S RNAr-ITS2-28S RNAr, en la identificación molecular de este nematodo, como complemento de la identificación de acuerdo con caracteres morfológicos, confirmando la identidad de *S. spiculatus.* En este trabajo se describe por primera vez a este nematodo infestando larvas de *Culex eduardoi* en un criadero natural de mosquitos, registrando la expansión de la distribución sudeste de este agente de control biológico de poblaciones de mosquitos de importancia sanitaria.

PALABRAS CLAVE. Nematodos. Nuevos registros. *Culex eduardoi.*

ABSTRACT. *Strelkovimermis spiculatus*, Poinar & Camino 1986 (Nematoda: Mermithidae) was found parasitizing some mosquito genera as *Aedes* (*Ochlerotatus*), *Culex* and *Psorophora*. In a mosquito distribution project in Buenos Aires, Argentina, we found nematodes infecting mosquito larvae in natural breeding sites in the outskirts of Mar del Plata city. The aim of this work was to identify this parasite in this distribution area and determine the mosquito species host. COI and 18S rRNA-ITS1-5.8S rRNA-ITS2-28S rRNA fragment genes were described and were used for molecular identification of this nematode, confirming the morphological diagnostic traits. In this report, a new host of *S. spiculatus*, the mosquito larvae of *Culex eduardoi* was detected, expanding its southeastern distribution.

KEY WORDS. Nematodes. New records. *Culex eduardoi*.

Some nematodes are considered potential agents for biological control programs because they are primarily obligate parasites of arthropods and other invertebrates. In particular, members of the Mermithidae family have proved to be effective in parasitizing natural populations of mosquito larvae (Stock & Goodrich-Blair, 2012). *Strelkovimermis spiculatus* was found for the first time in Argentina infecting larvae of the floodwater mosquito *Aedes* (*Ochlerotatus*) *albifasciatus* by Poinar & Camino (1986). Thus far, this parasite has been isolated in natural habi-

Recibido: 29-X-2015; aceptado: 14-IV-2016

tats from the immature stages of five species of *Culex* L., and a few *Aedes* (*Ochlerotatus*) sp. and *Psorophora* sp. collected in La Plata, Buenos Aires Province (34°S, 57-58° W) (García & Camino, 1990; Campos & Sy, 2003; Achinelly & Micieli, 2012; Di Battista *et al*., 2015). Moreover, it has been demonstrated that this nematode infected some *Anopheles* sp., *Aedes* sp. and *Culex* sp. in laboratory bioassays (García & Camino, 1990; Achinelly & Micieli, 2012).

As part of the mosquito distribution and identification project in General Pueyrredon district, Buenos Aires, Argentina, we found nematodes infecting mosquito larvae in natural breeding sites in the outskirts of Mar del Plata city (37°53`32``S, 57°36`02``W). The aim of this work was to identify this parasite in this new distribution area and determine the mosquito species host.

Mosquito larvae from temporary floodwaters were collected from two natural breeding sites on Provincial Route Nº 2, in summer 2015. Larvae were separated in individual containers for their identification and to determine the presence and the emergence of nematodes. Larvae and pupae of *Aedes albifasciatus*, *Aedes crinifer* (Theobald) and *Culex eduardoi* Casal & García were analyzed in order to determine parasitism in mosquito immature stages (Table I). The results obtained demonstrated that all the mosquito species were infected. The morphological identification of the nematodes was carried out by the analysis of morphological diagnostic traits through the observation by stereoscopic microscopy (at 100-630 amplification times), confirming the presence of *S. spiculatus* in all mosquito species (Poinar & Camino, 1986).

As a complement of the morphological identification, a molecular analysis of the nematodes was carried out. Genomic DNA from individual nematodes from each mosquito species and free parasites belonging to the breeding water were obtained by conventional techniques (Sambrook & Russel, 2001), considering free nematodes, those that were collected in the environment, outside of any host. In order to obtain gene sequences useful for the molecular identification of nematodes, the Polymerase Chain Reaction (PCR) technique was carried out using ribosomal and mitochondrial primers designed for eukaryotes, previously described (Folmer *et al.,* 1994; Díaz-Nieto *et al.,* 2013). Due to the fact that the ribosomal primer combination did not allow the amplification of the nematodes 18S rRNA or 28S rRNA complete genes, a set of specific primers were designed (Fig. 1). Therefore, the alignment of nucleotide sequences of ribosomal genes of different nematodes including mermithids, available in the database of the National Center for Biotechnology Information (NCBI, http://www.ncbi.nlm.nih.gov) was performed. Consensus regions useful as primer sequences were selected, in some cases with degenerate bases. Primer combinations and PCR conditions are detailed in Table II. The amplification products obtained from the cytochrome *c* oxidase I (COI) and 18S rRNA-

Table I. Parasitism rates describing the nematode infection of mosquito larvae species collected in natural breeding sites.

	Aedes albifasciatus	*Aedes crinifer*	*Culex eduardoi*
Number of examined hosts	77	193	11
Number of parasite hosts	40	11	1
Total number of nematodes	74	16	2
Prevalence[a]	51.95	5.70	9.00
Intensity[b]	1.85	1.45	2
Abundance[c]	0.96	0.083	0.18

[a]Number of parasitized hosts/number of examined hosts x 100
[b]Number of parasites/number of parasitized hosts
[c]Number of parasites/number of examined hosts
a, b and c according to Morales & Arelis Pino, 1987

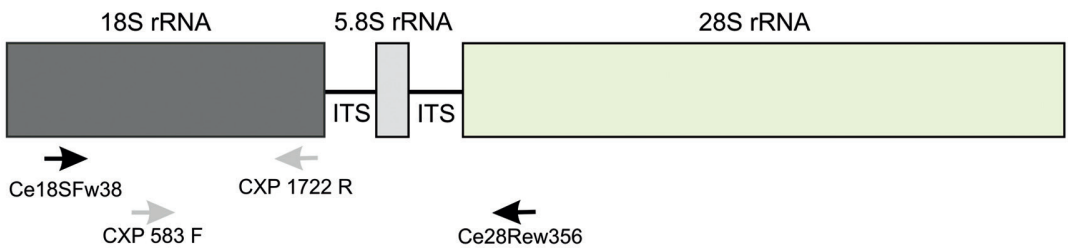

Fig. 1. Nuclear ribosomal genes operon showing the hybridization sites of primers used for the nematodes 18S rRNA - 28S rRNA amplification. The specific primer pairs used in this report for the amplification of the complete sequence are indicated in black arrows.

ITS1-5.8S rRNA-ITS2-28S rRNA fragment genes of each mosquito species and free nematodes were sequenced (Macrogen, Korea), manually assembled and deposited in the EMBL database under the accession numbers LN879495 and LN879496, respectively. The results were analyzed by BLASTn and multiple-sequence alignment.

The multiple sequences alignments analysis of the 636 bp COI fragment (accession N° LN879495) showed 100 % identity with *S. spiculatus* sequences availably in the GeneBank. On the other hand, the 1550 bp partial sequence obtained with ribosomal primers Ce18SFw38 and Ce28Rew356 (accession N° LN879496) (according to Fig. 1), showed 100 % identity only with 276 bp of *S. spiculatus* sequences (DQ665654, KP270701, KP270702, KP270700, KP270703 and KP270704) present in the Genebank (unpublished sequence and Belaich *et al.,* 2015). However, the complete fragment obtained shows a high identity with 18S rRNA-ITS1-5.8S rRNA-ITS2-28S rRNA partial fragments from other nematodes that were available in sequence databases (like *Mermis nigrescens, Pheromermis* sp. or *Caenorhabditis elegans*).

The results obtained from the COI and partial 18S rRNA confirm the molecular identification of *S. spiculatus* according to the morphological di-

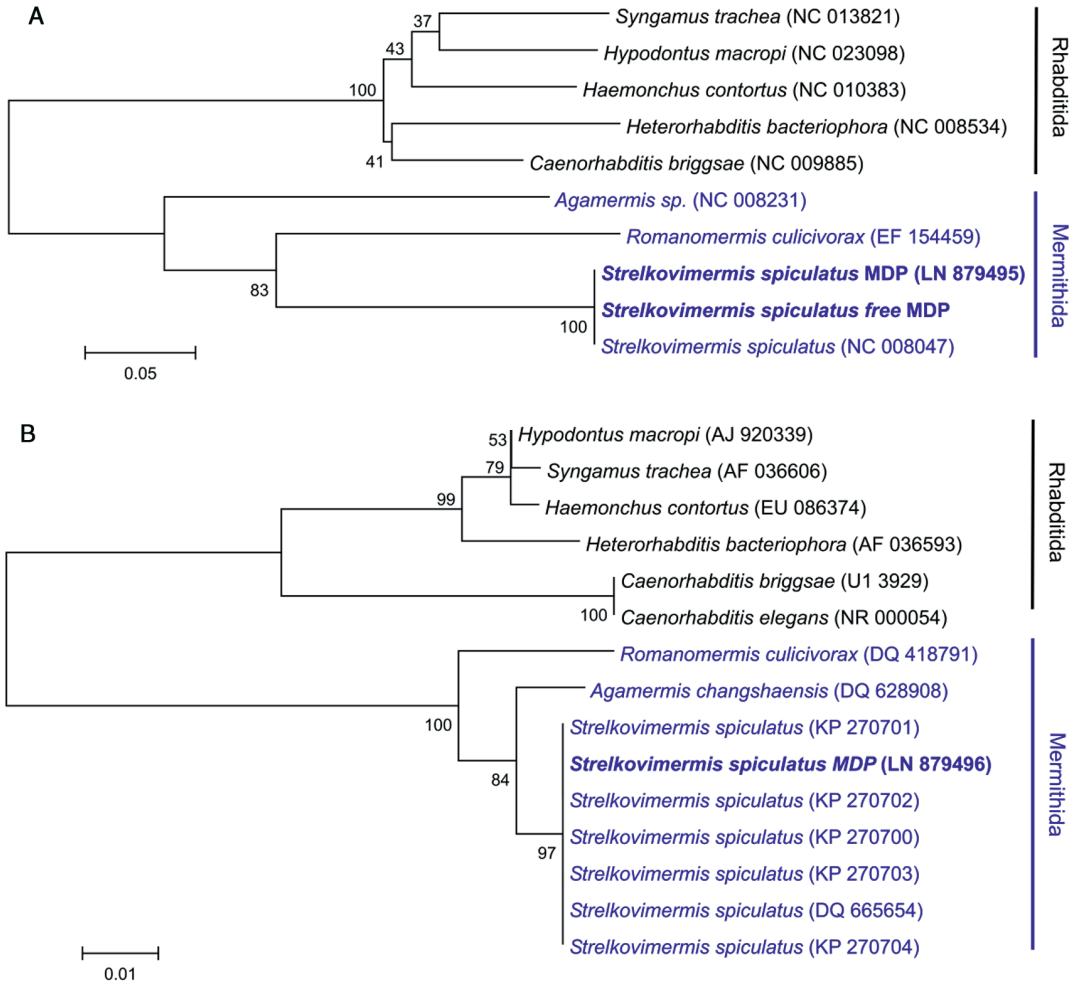

Fig. 2. Phylogenetic estimation by Neighbor Joining. A, Using COI and B, 18S rDNA coding-sequences. The phylogenetic trees were constructed with the distance matrix model (Neighbor Joining) of MEGA5 program. The node numbers represent the percentage corresponding to the values of 1000 cycles resampling (bootstrap). Species identified in this report are highlighted in bold text. The accession numbers of the sequences of the databases used for the analysis are listed below the name of each nematode species in brackets.

Table II. Primers used for amplification of fragments corresponding to 18S rRNA, 28S rRNA and COI nematode genes.

Primer pair	Gen amplified	Primer sequence	Annealing temperature in PCR reaction	Reference
Ce18SFw38 CXP 1722 R	18S rRNA	5´ AAAGAYTAAGCCATGCATG 3´ 5´ GTAGCGACGGGCGGTGTGTACAAAG 3´	48°C	This report Díaz-Nieto et al., 2013
CXP 583 F Ce28Rew356	18-28S rRNA	5´ CCAGCAGCCGCGGTAATTCCAGC 3´ 5´ CTTTGCAACTTTCCHTCACDGTACTT 3´	52°C	Díaz-Nieto et al., 2013 This report
HCO 2198 LCO 1490	COI	5´ TAAACTTCAGGGTGACCAAAAAATC 3´ 5´ GGTCAACAAATCATAAAGATATTGG 3´	52°C	Folmer et al., 1994 Folmer et al., 1994

agnostic traits. Dendograms were built using the Neighbor Joining model of the matrix distance program MEGA5, comparing the obtained sequences with others from public databases (NCBI) (Fig. 2).

In this study, we report the infection of *C. eduardoi* by *S. spiculatus* for the first time and provide tools to contribute with an accurate molecular identification of nematode mosquito parasites, obtaining the first partial sequence of 18S rRNA-ITS1-5.8S rRNA-ITS2-28S rRNA genes. This detection in the outskirts of Mar del Plata city expanded the southeastern distribution of *S. spiculatus.*

ACKNOWLEDGEMENTS

We especially thank Prof. Ana Tassi (former professor Universidad Nacional de Mar del Plata) for critical revision of the manuscript and Dr. María Fernanda Achinelly for her help in morphological nematode identification. This work was supported by grants of the Agencia Nacional de Promoción Científica y Tecnológica (ANPCyT PICT-2013-0431), Universidad Nacional de Mar del Plata (15/E692-EXA742) and CONICET (PIP 2012 Nº 112 20110100963).

LITERATURE CITED

ACHINELLY, M. F. & M. V. MICIELI. 2012. Host range of the parasite *Strelkovimermis spiculatus* (Nematoda: Mermithidae) in Argentina mosquitoes. *Journal of Vector Ecology* 38(1): 69-73.

BELAICH M. N., D. BULDAIN , P. D. GHIRINGHELLI, B. HYMAN, M. MICIELI & M. F. ACHINELLY. 2015. Nucleotide sequence differentiation of argentine isolates of the mosquito parasitic nematode *Strelkovimermis spiculatus* (Nematoda: Mermithidae). *Journal of Vector Ecology* 40(2): 415-418.

CAMPOS, R. E. & V. E. SY. 2003. Mortality in immatures of the floodwater mosquito *Ochlerotatus albifasciatus* (Diptera: Culicidae) and effects of parasitism by *Strelkovimermis spiculatus* (Nematoda: Mermithidae) in Buenos Aires Province, Argentina. *Memórias do Instituto Oswaldo Cruz* 98(2): 199-208.

DI BATTISTA, C. M., S. FISCHER & R. E. CAMPOS. 2015. Prevalence of parasitism and adult survival time of *Aedes albifasciatus* (Diptera: Culicidae) parasitized by *Strelkovimermis spiculatus* (Nematoda: Mermithidae). *Journal of Vector Ecology* 40(2): 393-397.

DÍAZ-NIETO, L. M., A. MACIÁ, G. PARISI, J. L. FARINA, M. E. VIDAL-DOMÍNGUEZ, M. A. PEROTTI & C. M. BERÓN. 2013. Distribution of mosquitoes in the South East of Argentina and first report on the analysis based on 18S rDNA and COI Sequences. *PloS One* 8: e75516.

FOLMER, O., M. BLACK, W. HOEH, R. LUTZ & R. VRIJENHOEK. 1994. DNA primers for amplification of mitochondrial cytochrome *c* oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology* 3(5): 294-299.

GARCÍA, J. J. & N. B. CAMINO. 1990. First record of natural infections of larvae of *Culex pipiens* (L.) from Argentina (Diptera: Culicidae). *Neotropica* 36(96): 83-86.

MORALES, G. & L. ARELIS PINO. 1987. *Parasitología Cuantitativa*, Fundación Fondo Editorial Act. Cient. Venezolana, Caracas, Venezuela, 132 pp.

POINAR Jr., G. O. & N. B. CAMINO. 1986. *Strelkovimermis spiculatus* n. sp. (Mermithidae: Nematoda) parasitizing *Aedes albifasciatus* Mac. (Culicidae: Diptera) in Argentina. *Journal of Nematology* 18(3): 317-319.

SAMBROOK, J. & D. W. RUSSELL 2001. *Molecular cloning. A laboratory manual.* 1rd ed. New York: Cold pring Harbor Laboratory Press.

STOCK, S. P. & H. GOODRICH-BLAIR. 2012. Nematode parasites, pathogens and associates of insects and invertebrates of economic importance. *Manual of techniques in invertebrate pathology,* 2nd ed. Academic, San Diego, 373-426.