Host range of the parasite *Strelkovimermis spiculatus* (Nematoda: Mermithidae) in Argentina mosquitoes

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ABSTRACT: *Strelkovimermis spiculatus* (Poinar and Camino 1986) is a nematode parasite of mosquitoes isolated from the Neotropical region. We investigated the host range of this parasite in mosquito populations for a better understanding of the dynamics of temporary breeding sites. Five grassy-pool habitats filled by rainwater were sampled from the summer 2007 through the fall 2008. Eight mosquito species were collected: *Anopheles albitarsis, Culex chidesteri, Culex dolosus, Culex maxi, Aedes albifasciatus, Psorophora ciliata, Psorophora cyanescens*, and *Psorophora albigenu.* Six of these species were parasitized: *Cx. chidesteri, Cx. dolosus, Cx. maxi, Ae. albifasciatus, Ps. ciliata,* and *Ps. cyanescens.* The occurrence of this mermithid in natural mosquito populations was increased from the end of winter to the end of the spring. Prevalence ranged from 11% to 100%. High levels of infections were registered only in *O. albifasciatus* larvae, the most abundant mosquito species (95%), followed by *Cx. dolosus* (2.7%). *Strelkovimermis spiculatus* completed its development in all infected mosquito larvae. The presence of *S. spiculatus* in six natural mosquito populations increases the number of susceptible species to 24. ***Journal of Vector Ecology* 38: (1): xxx-xxx. 2013.**

Keyword Index: Culicidae, Mermithidae, parasite, *Strelkovimermis spiculatus*; Argentina.

INTRODUCTION

The mermithids are a large group of nematode parasites, principally of insects, which are almost always lethal to their hosts (Poinar 1979). In the neotropical region, two mermithid species have been isolated from mosquitoes, *Hydromermis* sp. for *Psorophora ferox* larvae (Camino 1989) and *Strelkovimermis spiculatus* Poinar and Camino 1986 for the floodwater mosquito *Aedes albifasciatus* (Macquart), a serious pest in rural and urban areas of Argentina (García et al. 1994). This mosquito species becomes a pest during its population peaks of adult abundance that occur principally in the spring and fall (Maciá et al. 1995, Fontanarrosa et al. 2000, García and Micieli 2000) and was also identified in 1982 as a vector of western equine encephalitis in this country (Mitchell et al. 1987).

Strelkovimermis spiculatus has a life cycle similar to other aquatic mermithids. Infective pre-parasitic juveniles (J2) hatch from eggs and actively seek and penetrate larvae of the mosquito host. The third stage juvenile (J3) develops within the mosquito larva for six to eight days, at which time the post-parasitic juvenile (J4) emerges, killing the host. The J4 develop into adults, which mate and lay eggs in the aquatic substrate to complete the cycle (Camino and Reboredo 1994). This parasite produces epizootics in natural populations of *Ae. albifasciatus*, constituting one of the most interesting bioregulatory agents of this culicid (Micieli and García 1999, Campos and Sy 2006, Platzer 2007, Micieli et al. 2012).

Studies of this mermithid report a wide range of susceptible mosquito species under laboratory conditions (Becnel and Johnson 1998, Achinelly et al. 2004, Rodriguez Rodriguez et al. 2005). However, little is known about the parasitism in mosquito populations (García et al. 1994, Maciá et al. 1995, Micieli and García 1999, Campos and Sy 2003).

This work was conducted to study the parasitism by this mermithid in mosquito populations of temporary ponds within an area of the Argentine Pampean region in order to determine the natural host range of this parasite. We include a checklist of the mosquito species susceptible to *S. spiculatus.*

MATERIALS AND METHODS

Study area

Over a period of one year, five grassy-pool habitats were selected at random. These sites became flooded exclusively with local rain and were formed by depressions in the grass-covered ground. The five habitats were situated within suburbs (Manuel B. Gonnet, Melchor Romero, Punta Lara, Villa Elisa, and City Bell) of the city of La Plata, Buenos Aires province, Argentina. In this geographical region, the climate is temperate (annual average temperature, from 13° to 17° C) with rainfall occurring throughout the entire year (Cabrera and Willink 1980).

Site 1 (Manuel B. Gonnet; 34° 52′ 29″ S, 58° 00′ 31″ W) was a puddle (20x20 cm and 50 cm in depth) remaining flooded for no more than two weeks.

Site 2 (Melchor Romero; 34° 56′ 57″ S, 58° 02′ 50″ W) was a pond (50x50x50 cm) with a high variation in the water level throughout the year. One area of this pond was exposed to direct sunlight and thus would dry out periodically, whereas another sector was shaded and constituted a zone of permanent flooding.

Site 3 (City Bel; 34° 52′ 11.8″ S, 57° 57′ 28.9″ W) was a puddle formed by depressions in the ground of approximately 30x20x50 cm that were exposed to direct sunlight. This site generally remained flooded for a period of between two to six weeks, depending upon the rainfall.

Site 4 (Punta Lara; 34° 52′ 13″ S, 57° 57′ 30″ W), was a pond with dimensions about 15x25x30 cm, shaded and ephemeral, but containing a small area (3x2 cm) within the center of the depression where water remained for several days to a few weeks.

Site 5 (Villa Elisa; 34° 51′ 12″ S 58° 04′ 45″ W) was a grassy puddle of approximately 50x30x50 cm that was exposed to direct sunlight. This site remained flooded generally for a period between two and four weeks, depending upon the rainfall.

Collection and identification of mosquito and nematode populations

Weekly observations were conducted at the five selected habitats for a year extending from the summer of 2007 through the fall of 2008. In each pool, 50 samples, considered as a sampling unit, were collected after each flooding using a standard 300 ml dipper. Immature-stage mosquitos were transported to the laboratory, where the field-collected larvae were transferred to plastic pans containing dechlorinated water, usually within two h after the removal of the samples from the ponds. They were reared until the 4th instar and fed daily with finely ground rabbit chow (dehydrated alfalfa meal, wheat middlings, ground soybean hulls, dehulled soybean meal, ground corn, wheat flour, and cane molasses). The number of total larvae and the percentage parasitized by *S. spiculatus* were determined for each larval site. Mosquitoes were identified using appropriate taxonomic keys (Lane 1953, Darsie and Mitchell 1985). The prevalence for each mosquito species and the number of emerged nematodes per larva was determined by placing 50 4th instar larvae from each mosquito in the wells of multi-well plates and counting the number of emerged post-parasites. For identification, nematodes were killed in distilled water at 60° C for 2 min. They were removed to a 50% TAF solution (water-triethanolamine-formalin) (1:1) for 48 h, and then fixed into pure TAF (Poinar 1975). They were then transferred from the fixative to glycerol for slow evaporation and to clear parasites (Seinhorst 1959). The identification used the key of Poinar (1977).

RESULTS

The eight mosquito species collected in temporary ponds at the five locations in La Plata city throughout all seasons are shown in Table 1. Of these eight species, six were parasitized by the mermithid *Strelkovimermis spiculatus*: *Cx. chidesteri, Cx. dolosus, Cx. maxi, Ae. albifasciatus, Ps. ciliata*, and *Ps. cyanescens* (Table 2). Parasitism by this nematode in *Psorophora ciliata* and *Ps. cyanecens* larvae was recorded in our study for the first time. This is also the first report of infection of *Cx. chidesteri* and *Cx. maxi* larvae by this nematode in the field, although they were susceptible at laboratory exposure in previous assays (Table 3). *Strelkovimermis spiculatus* was not detected in larvae of *Ae. albitarsis* and *Ps. albigenu*.

The number of mosquito species in all environments varied between two and six (Table 1). Parasitism by *Strelkovimermis spiculatus* was observed in all sites from the end of the winter to the end of the spring. Infections were observed once per month in September, November, and December, 2007, while in summer the nematode was found exclusively in March, 2007 (at sites 1, 4, and 5) and January, 2008 (at sites 2, 3, and 4). Prevalence in the fall was recorded only at Site 5 in June, 2007.

The prevalence of *S. spiculatus* was variable among the eight mosquito species and in the five breeding sites and ranged from 11% to 100%. High prevalence was registered only in *Ae. albifasciatus* larvae and in all four ponds where this mosquito was collected (Table 2). Prevalence of *S. spiculatus* in other mosquito species, however, did not exceed 50%, the value reached in *Cx. dolosus* larvae at Site 2 (Table 2). The intensity of the number of nematodes per infected larva varied between one and eight, with the highest value (i.e., eight) being observed in *Ps. cyanencens* larvae at Site 4 (Table 2).

Ae. albifasciatus was the most abundant mosquito species in these temporary ponds at an occurrence of about 95% of the total mosquito larvae sampled, followed by *Cx. dolosus* at less than 3%. In contrast, the occurrence for other mosquito species did not exceed 1% (Table 1).

The presence of *S. spiculatus* in these six natural mosquito populations increases the number of susceptible species to 24 under natural and/or laboratory conditions (Table 3).

DISCUSSION

The genus *Strelkovimermis* includes ten mermithid nematode species that have been described only in dipterans of the family Chironomidae and Culicidae (Johnson and Kleve 1996). Only two of these species, *S. peterseni* (Poinar) and *S. spiculatus*, were recorded in hosts belonging to the family Culicidae. *Strelkovimermis peterseni* was found parasitizing larvae of the genus *Anopheles* in the Nearctic region, but infections were not recorded after exposure of immature larval stages of the genera *Aedes, Culex*, or *Psorophora* to that nematode species under natural or experimental conditions (Petersen and Chapman 1970). *Strelkovimermis spiculatus* was able to parasitize larvae of several

Table 1. Number of mosquito larvae sampled in temporary ponds within the suburbs of La Plata city, Buenos Aires province, Argentina. Occurrence: percentage of mosquito larvae recorded of each species on the total number of mosquito larvae sampled (n=15980) in all breeding sites.

	Ae. albifasciatus	*Cx. dolosus*	*Cx. maxi*	*Cx. chidesteri*	*Ps. ciliata*	*Ps. cyanecens*	*Ae. albitarsis*	*Ps. albigenu*
site 1	1,790	21	0	0	0	0	0	0
site 2	1,489	62	0	0	0	0	3	0
site 3	11,325	173	28	0	0	56	0	40
site 4	85	24	42	0	6	60	2	0
site 5	522	156	0	93	0	0	0	0
total	**15,211**	**436**	**70**	**93**	**6**	**119**	**5**	**40**
ocurrence	95.18	2.72	0.44	0.58	0.034	0.76	0.036	0.25

Table 2. Prevalence of infection (P) and number of emerged nematodes per larva (I) of *Strelkovimermis spiculatus* in mosquito populations at five larval habitats during a year of study.

	Ae. albifasciatus		*Cx. dolosus*		*Cx. maxi*		*Cx. chidesteri*		*Ps. cilliata*		*Ps. cyanecens*		*Ae. albitarsis*		*Ps. albigenu*	
	P (%) Mean ± SD	**I Mean ± SD**	**P (%) Mean ± SD**	**I Mean ± SD**	**P (%) Mean ± SD**	**I Mean ± SD**	**P (%) Mean ± SD**	**I Mean ± SD**	**P (%) Mean ± SD**	**I Mean ± SD**	**P (%) Mean ± SD**	**I Mean ± SD**	**P (%) Mean ± SD**	**I Mean ± SD**	**P (%) Mean ± SD**	**I Mean ± SD**
S1	0	0	29.0 ± 0	3 ± 0	0	0	0	0	0	0	0	0	0	0	0	0
									0	0	0	0	0	0	0	0
S2	63.3 ± 40.4 (20-100)*	2.0 ± 0.4 (1.6-2.5)*	27.5 ± 31.8 (5-50)*	1.8 ± 0.7 (1.3-2.3)*	0	0	0	0	0	0	0	0	0	0	0	0
S3	60.3 ± 45.3 (11-100)*	1.3 ± 0.3 (1.0-1.6)*	21 ± 0	1.0 ± 0	0	0	0	0	0	0	0	0	0	0	0	0
S4	54.5 ± 17.6 (33 -75)*	1.0 ± 0	37. 5 ± 17.7 (25-50)*	1.5 ± 0	5 ± 0	1.5 ± 0	0	0	5 ± 0	1.5 ± 0	28 ± 0	8 ± 0	0	0	0	0
S5	80.0 ± 26.4 (50-100)*	3.05 ± 0.9 (2.4-3.7)*	15.0 ± 14.1 (5-25)*	1.5 ± 0.7 (1.0-2.0)*	0	0	18 ± 0	1.0 ± 0	0	0	0	0	0	0	0	0

()* range of prevalence and intensity, S: site.

species of Neotropical culicids under natural and laboratory conditions (Achinelly et al. 2004, Becnel and Johnson 1998, Maciá et al. 1995, Achinelly and Micieli 2009). Although a broad range of mosquito species had been recorded as susceptible to infection by this mermithid under laboratory conditions, in the present study prevalence of this mermithid was found to be variable in the field. Epizootic levels were registered only in natural populations of *Ae. albifasciatus*, with prevalences that reached 100%, consistent with the observations of Campos and Sy (2003). Those authors considered this nematode to be a significant bioregulatory agent for the density of the immature stages of that mosquito pest within the Neotropical region. In this regard, enzootic levels of parasitism by this mermithid were registered in other mosquito species (Maciá et al. 1995, Campos et al. 1993, Micieli et al. 2012) as well as in our study. Infection by this mermithid was not recorded either in *Ae. albitarsis* nor in *Ps. albigenu*. *Strelkovimermis spiculatus* have been observed to complete development inside of mosquito larvae and emerge, in all the infected mosquito larvae, unlike the circumstance with *Agamermis culicids*, where that mermithid could not develop in the 4th instar of *Ae. sollicitans* larvae after infection (Petersen et al. 1967).

The number of nematodes emerging from each mosquito larvae in the present work was less than four parasites per infected mosquito larva with the single exception of *P. cyanecens*, where double this value was recorded. A lower value was obtained for the mermithids *Romanomermis culicivorax* and *Romanomermis iyengari* in natural populations of mosquitoes, which does not exceed three (Santamarina et al. 1992, Paily and Balaraman 2000, Perez-Pacheco et al. 2004, 2009). The reasons for the high number of nematodes that emerged from *P. cyanecens* larvae in our study are not known.

Thirteen mosquito species were susceptible to *S. spiculatus* infection with an overall mean of 79.6% ranging from 12.5% to 100% under laboratory conditions (Achinelly et al. 2004). In that study, *Ae. albifasciatus* larvae were among the most susceptible species to this nematode (97.5%). The differences in the susceptibility to *S. spiculatus* under field and laboratory conditions could be due to behavioral, physiological, genetic, and physical characteristics of each species evaluated. Kerwin et al. (1990), working with *R. culicivorax*, demonstrated that the components of the cuticle of Culicidae affected their susceptibility to infection by that nematode since variations in the stiffness of the Culicidae cuticle could either prevent or facilitate the mermithid's entry. In this regard, the composition and structure of the cuticle of *S. spiculatus* and its hosts have not yet been investigated. Also, the spatial distribution of the host and the parasite, and the characteristic of the habitat, could be determinants for creating opportunities for pathogen and parasite transmission and therefore on susceptibility, as was suggested by Lafferty and Holt (2003).

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Table 3. Susceptibility of inmature stages of mosquito species to the nematode *Strelkovimermis spiculatus* (N, natural infection; ER, experimental releases; L, laboratory infection).

Mosquito larvae	**N**	**E**	**L**	**References**
Aedes albopictus			x	4
Ae. aegypti		x	x	1, 2, 3, 4, 38
Ae. taeniorhynchus			x	4
Ae. triseriatus			x	4
Anopheles albitarsis			x	1
An. albimanus			x	4
An. quadrimaculatus			x	4
Culex apicinus		x	x	1, 3
Cx. castroi			x	1
Cx. chidesteri	x		x	*,1
Cx. dolosus	x		x	*, 1, 16
Cx. maxi	x		x	*, 1
Cx. mollis	x			16
Cx. pipiens	x	x	x	1, 2, 3, 4, 6, 8, 11, 15
Cx. quinquefasciatus			x	37
Cx. renatoi			x	1
Cx. restuans			x	4
Isostomya paranensis			x	1
Ae. albifasciatus	x		x	*, 1, 10, 12, 16, 23, 24, 25, 36
Ae. crinifer	x		x	1, 10, 16, 23
Psorophora ferox			x	1
Ps. ciliata	x			*
Ps.cyanescens	x			*
Toxorhynchites r. septentrionalis			x	4

* Species recorded in this study.

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