Scientific Note

Nucleotide sequence differentiation of argentine isolates of the mosquito parasitic nematode Strelkovimermis spiculatus (Nematoda: Mermithidae)

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The mermithids are a large, important group of nematode parasites, principally of insects; infection is almost always lethal to their host. Their potential as biological control agents against mosquitoes has been well documented (Platzer 2007). Strelkovimermis spiculatus Poinar and Camino 1986 (Nematoda: Mermithidae) is a parasite isolated initially from Aedes (Ochlerotatus) albifasciatus (Macquart) larvae in rain-flooded ponds (Poinar and Camino 1986) and subsequently from other mosquito species (Micieli et al. 2012) and from Culex pipiens in house drainage ditches (Muttis et al. 2013) within the Buenos Aires, Argentina region. This nematode was morphologically characterized by a ventrally-shifted mouth opening and spicules fused at the tips (Poinar and Camino 1986). Epizootics produced by this nematode are commonly observed in natural populations of Ae. albifasciatus, since S. spiculatus is a natural enemy of the immature insect stages (Campos and Sy 2003). Infective parasitic (second stage, J2) juveniles hatch from eggs when mosquito breeding sites become flooded and then actively seek out and penetrate host mosquito larvae. Third stage juvenile (J3) develop within the mosquito in six to eight days, at which time postparasitic juveniles (J4) emerge, killing the host. Adults mate and lay eggs in an aquatic environment where the cycle is completed (Platzer 2007). Previous studies of Strelkovimermis spiculatus include reports of their mass rearing (Camino and Reboreda 1996), the effects of biotic and abiotic factors on their infectivity (Micieli et al. 2012), and mosquito host range (Achinelly et al. 2004). However, little is known about the population dynamics of this nematode that could be easily addressed if robust molecular markers were available to identify populations and characterize genetic variation within and among populations.

In this study, Strelkovimermis spiculatus isolates from two different environments (permanent and temporary flood ponds) and three hosts (Aedes albifasciatus, Culex pipiens, and Culex dolosus) were discriminated using simple cloning and PCR techniques.

Nematodes were collected from two different types of mosquito breeding habitats located in the suburbs of La Plata and Ensenada, Buenos Aires province, Argentina. Permanent flood ponds were characterized by household drainage systems, a common breeding site for Cx. pipiens. Two sites were selected: Site 1 (34° 54' 29" S, 58° 2' 25" W) located in San Carlos, La Plata City and Site 2 (34° 52' 49" S, 58° 0' 38" W) at Gónnet, La Plata. Temporary flood ponds correspond to soil depressions where water accumulates exclusively from rainfall, the larval site of the mosquito host Ae. albifasciatus; depressions ranged in size from 2 to 5 m wide and 0.5 cm deep, depending on ambient precipitation amounts. Site 3 was located in a coastal suburb of Punta Lara, Partido de la Ensenada (34° 49 19.9 "S, 57° 58 7.5" W). Site 4, located in Los Hornos, 7 km from the La Plata city center (34° 59 16.1 "S, 57° 59 43" W); this breeding ground was a shaded and ephemeral pond, filled by rainwater, persisting from several days to a few weeks. More specifically, this site was a drainage ditch where water pooled as runoff from an adjacent field after its flooding by rain. For collection of S. spiculatus, water samples containing mosquito larvae were collected using a 300 ml dipper and filtered through a fine mesh net. The mosquito larvae retained in the net were placed in plastic buckets and transported to the laboratory for further analysis. Approximately 10% of the mosquito larvae in each collection were randomly sampled and both stereo and optical microscopes were used to determine the presence of the nematode. Mosquito larvae were placed in plastic trays of 38 x 28 cm with 1,000 ml of tap water and allowed to mature to stage IV. At that time, individual larvae were placed in 10 ml distilled water and post-parasitic nematodes allowed to emerge. Adults were killed in 60° C and transferred to a fixative of 50% aqueous triethanolamine formalin for 48 h and then placed in 100% triethanolamine formalin before transfer to glycerol for slow evaporation in order to clear the parasites (Seinhorst 1959). The fixed specimens were used for taxonomic identification following the key of Poinar (1977) and description by Poinar and Camino (1986). The nematodes were frozen in distilled water at -20° C and were stored for subsequent molecular analysis. Strelkovimermis spiculatus DNA was purified as in Kobylinski et al. (2012). DNA preparations were used as templates in PCR amplification assays using Taq DNA polymerase (Promega). For all sites, DNA samples were obtained from 50 pooled postparasitic nematodes. Nuclear 18S rDNA gene forward