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Research Note

STUDIES ON *POLIDISPYRENIA SIMULII* (MICROSPORA; PLEISTOPHORIDAE) IN *SIMULIUM PERTINAX* (DIPTERA; SIMULIIDAE) IN BRAZIL

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Despite of the existence of extensive information about host pathogen relationships, ecology and epizootiology for a few species of microsporidia, little is known about most of the described species of this group of worldwide insect pathogens. One of the most common microsporidia species occuring in blackfly larvae is *Polidispyrenia simulii.* Even though there are several studies of *P. simulii* life cycle, little is known about its transmission. The first report of this microsporidiosis in blackfly larvae from Brazil was done by A. Lutz & A. Splendore (1904, *Z. Bact. Parasitenkd. Infektionskr. Hyg. Abt. I Orig., 36:* 645-650). The present work intends to investigate the life cycle and transmission aspects of this pathogen in a *Simulium pertinax* population from São Paulo State, Brazil.

Larvae of *S. pertinax* infected with microsporidia were collected from their natural breeding sites and kept in a laboratory rearing system adapted from the J. N. Raybould & J. Grunewald (1975, *Tropenmed. Parasitol., 27:* 155-168) system, where adults were obtained from infected larvae. Smears of infected larvae were air dried, fixed in absolute ethanol and stained with Giemsa, buffered at pH 7.0 to reveal the presence of microsporidian spines. Acid hydrolisis followed the method of J. Weiser (1976, *J. Invertebr. Pathol., 28:* 147-149).

For electron microscopy studies, small portions of infected hosts (larvae and adults) were fixed in 3.0% glutaraldehyde buffered in 0.1 M phosphate for 3 hr at room temperature. Calcium chloretum and sacarose 0.1 M were added to the buffer. The material was post-fixed in 4.0% osmium tetroxide for 1 hr and then in 2.0% tanic acid for more 1 hr. The dehydration process was through a graded ethanol series into acetone and then the material was embedded in Epon-Araldite (H. H. Mollenhauer, 1964, *Stain technol., 39:* 111-114). Sections were post-stained in methanolic uranyl acetate followed by lead citrate (E. S. Reynolds, 1963, *J. Cell Biol., 17:* 208-212). The sections were examined with a Zeiss MT-2 electron microscope.

The fat body of the larvae was the site of infection for this microsporidia. Spores of *P. simulii* from infected larvae of *S. pertinax* measured 6.3 x 8.27 μm and are consistent with those reported by G. Marino et al. (1979, *Neotropica, 25:* 127-132) and J. J. Garcia et al. (1989, *J. Amer. Mosq. Control Assoc., 5:* 64-69) for *P. simulii* infecting several blackfly species from Argentina. The polar tube was extruded and measured ca. 70.0 μm. Sinaptonemal complexes were present in the nuclei of spores, showing that meiotic divisions are involved in the production of sporoblasts.

Fourteen adult females of *S. pertinax* originated from heavily infected larvae, were examined and there was no sign of microsporidium spores, even in the oenocytes. These results suggest either that vertical transmission does not occur, or that it occurs at very low levels. Nevertheless, the synaptonemal complexes observed suggest that a sexual fusion must occurs at some stage of the microsporidia life cycle, forming diploid spores. E. U. Canning & E. I. Hazard (1982, *J. Protozool., 29:* 39-49) pointed out that these diploid spores could be involved in vertical transmission. Further studies are necessary to understand the life cycle of this widely distributed microsporidium and its maintenance in blackfly host populations.

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