

## SCIENTIFIC NOTE

### AN IRIDESCENT VIRUS AND A MICROSPORIDIUM IN THE BITING MIDGE *CULICOIDES BARBOSAI* FROM FLORIDA

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**ABSTRACT.** An iridescent virus and a microsporidium (*Nosema* sp.) were found infecting larvae of the biting midge *Culicoides barbosai* collected in Boynton Beach, Palm Beach County, Florida. Larvae were extracted from mud collected weekly during a 22-month period from March 1998 to December 1999. Virus-infected larvae were present in 4 collections made in March, April, and May 1998 and the average infection level was 4.7% (range 2.3–7.1%). The virus infected the fat body and produced an iridescent blue color in larvae because of the crystalline arrays of the particles. These virus particles were hexagonal, measured 99 nm from side to side, and had an electron-dense inner core. The site of infection of the *Nosema* sp. also was the fat body of the larva, which became opaque white. These opaque areas were the result of masses of oval binucleate spores measuring  $2.7 \pm 0.1 \times 4.4 \pm 0.3 \mu\text{m}$  ( $n = 25$ ). The *Nosema* sp. was present in larvae of 14 collections made during the 22-month period, with an average infection level of 6.1% (range 0.3–21.4%). Although individual larvae infected with the iridescent virus and *Nosema* sp. occurred in 4 collections, no dual infections were observed. Larvae patently infected with the iridescent virus and *Nosema* sp. died before pupation.

**KEY WORDS** *Culicoides barbosai*, *Nosema* sp., biological control, biting midge, iridescent virus, microsporidium, pathogen

*Culicoides barbosai* Wirth and Blanton, a biting midge, can pose a tremendous nuisance problem to residents and adversely affect tourism in communities along the southern coast of Florida (Linley and Davies 1971). The lack of effective control of biting midges with chemical insecticides has stimulated investigations into the possible use of biological control, which requires continual discovery of new agents (Burgess 1981). Herein, we report an iridescent virus and a microsporidium found infecting *C. barbosai* larvae collected from the Atlantic coast of Florida and the prevalence of the 2 parasites during a 22-month period in 1998 and 1999.

Weekly mud collections from a tidal marsh in Boynton Beach, Palm Beach County, Florida, were made from March 1998 through December 1999. These collections originally were made for the purpose of monitoring larval populations during a removal trapping experiment. They also provided an opportunity to examine *C. barbosai* larvae for parasites and pathogens. Mud collected from the Boynton Beach sites was processed weekly at the Center for Medical and Veterinary Entomology, Gainesville, FL, to extract larvae. The collected larvae were counted, recorded, and then examined with a dissecting microscope at 12 $\times$  magnification with top lighting and a black background for gross

pathological symptoms. Not all collections produced larvae.

Infected tissue was further examined with light and electron microscopy. Fresh smears of tissues infected with the microsporidium were examined and photographed with a Zeiss Photomicroscope III (Carl Zeiss, Inc., Norcross, GA). Fresh spores were measured with a Vickers Image Splitting Micrometer (Vickers Instruments, Inc., Malden, MA) by using phase contrast at 1,250 $\times$ . Tissues were prepared for ultrastructural examination according to previously described protocols (Becnel 1992).

The gross appearance of *C. barbosai* larvae infected with the virus was an iridescent blue color. Early infections started out pale blue, but as the infection progressed, the blue color intensified. Ultrastructural studies revealed that the fat body was overtaken by crystalline arrays (Fig. 1a) of hexagonal particles with an electron-dense central core and a less dense outer layer, measuring  $98.9 \pm 0.6$  nm ( $n = 10$ ) in cross-section (Fig. 1b). Infections were observed in early and late instars, and all infected larvae died before pupation. No pupae were observed with the blue iridescence. The virus was present in 4 of 28 collections during the 22-month period (1 in March 1998, 2 in April 1998, and 1 in May 1998), with an average infection rate of 4.7% (range 2.3–7.1%); 23 of 589 larvae examined were infected.

The microsporidium was expressed as a white opaque mass, consisting of an accumulation of refractile oval spores in the larval fat body tissue, when examined by light microscopy (Fig. 1c).

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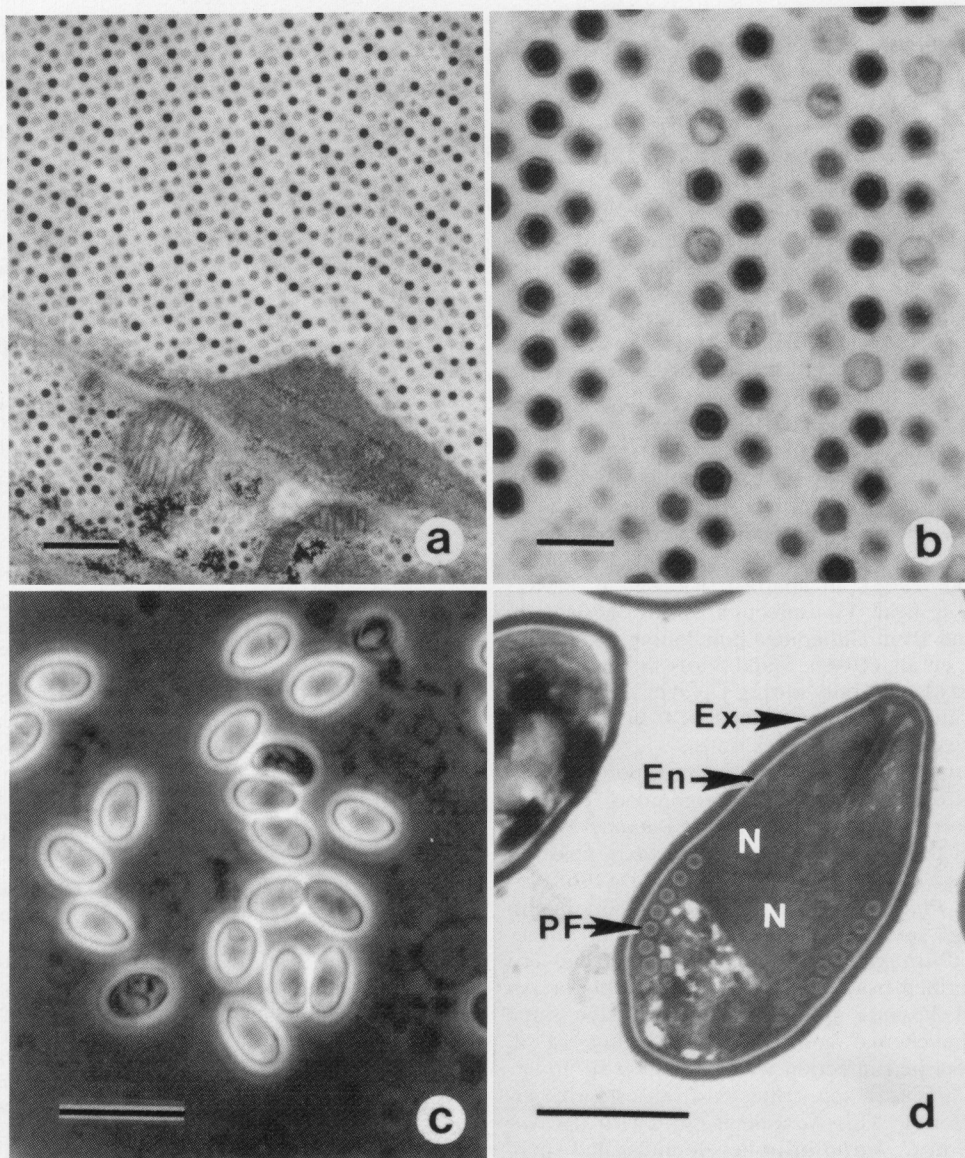


Fig. 1. Iridescent virus and *Nosema* sp. observed in larvae of *Culicoides barbosai*. (a) Electron micrograph of iridescent virus array in fat body of a *C. barbosai* larva. Bar, 0.1  $\mu\text{m}$ . (b) Electron micrograph of iridescent virus particles with electron-dense inner core and lighter staining outer layer. Bar, 100 nm. (c) Fresh spores of *Nosema* sp. from fat body of infected *C. barbosai* larva. Bar, 10  $\mu\text{m}$ . (d) Electron micrograph of *Nosema* sp. spore. En, endospore; Ex, exospore; N, nuclei; PF, polar filament. Bar, 1  $\mu\text{m}$ .

Fresh spores measured  $2.7 \pm 0.1 \times 4.4 \pm 0.3 \mu\text{m}$  ( $n = 25$ ). In ultrastructural observations, the spores were binucleate, had a thick exospore and a thin endospore wall, and contained an anisofilar polar filament with 9 coils (6 thick and 3 thin; Fig. 1d). Although this anisofilar polar filament is unusual, binucleate stages during development and the absence of a sporophorous vesicle indicated that this microsporidium was of the genus *Nosema* (Sprague et al. 1992). This *Nosema* sp. was observed in 14

of the 28 collections, with 12 of the positive collections occurring from February through May in both 1998 and 1999; the other 2 positive collections occurred in July and December 1999. The average infection rate in positive collections was 6.1% (range 0.3–21.4%); 63 of 1,440 larvae examined were infected. All patently infected larvae died before pupating.

Four collections contained both larvae infected with iridescent virus and others infected with *No-*

*sema* sp. These collections were made in March, April (2 collections), and May 1998. No dual infections were observed.

The 1st report of an iridescent virus in *Culicoides* was by Chapman et al. (1968), who described a virus 125–130 nm in diameter from a treehole *Culicoides* sp. in Louisiana. An iridescent virus with a side-to-side measurement of 130 nm was reported by Rieb et al. (1982) from 3 *Culicoides* in France. Recently, Mullens et al. (1999) reported an iridescent virus with a face-to-face measurement of 129 nm in *Culicoides variipennis sonorensis* Wirth and Jones from Riverside County, California. All of the above reports described infected larvae with a blue or blue-green iridescence similar to the virus reported here from infected *C. barbosai*. However, the size of the virus reported here is smaller, measuring 99 nm. The prevalence of the *C. barbosai* virus (February–May) coincides with that of the iridescent virus of the *Culicoides* sp. in Louisiana, which was reported to be February to April (Chapman et al. 1968). In contrast, Rieb et al. (1982) reported a near 1% infection level consistently year-round from *Culicoides* populations in France. Mullens et al. (1999) found virus-infected *C. v. sonorensis* larvae only during October and November in California, but it is unclear if negative collections were made at other times.

Few microsporidia have been reported from members of the family Ceratopogonidae. The earliest report was of *Nosema sphaeromiadis* Weiser from larval *Sphaeromyias* sp. collected in Czechoslovakia (Weiser 1957). Chapman et al. (1968) reported 2 *Plistophora* spp. producing mortality in *Culicoides* species collected in Louisiana. Subsequently, *Plistophora culicoidi* Levchenko and Issi was described from a *Culicoides* sp. in Russia. An unnamed *Nosema* sp. was reported in the same study (Levchenko and Issi 1973). Kline et al. (1985) reported infection with a *Nosema* sp. in larvae of *Culicoides* spp. collected from estuarine areas of Florida. This *Nosema* is similar to the *Nosema* sp. in *C. barbosai* in development and spore ultrastructure, because both have in common the same unique configuration of the anisofilar polar filament. A *Vavraia* sp. was found in adult *Culicoides edeni* Wirth and Blanton, also in Florida (Atkinson 1990). Because of the scarcity of these observations, little is known about the effect of microsporidia on *Culicoides* populations. Examination of our data indicates that although infection levels generally remain low, this *Nosema* sp. can persist in *C. barbosai* larval populations for several months (February–May). Kline et al. (1985) re-

ported a similar pattern of *Nosema*-type infections in *Culicoides* spp., with infection rates ranging from 1.1 to 4.5% during the coldest months (January–May).

Although infection levels of iridescent virus and microsporidia in natural *Culicoides* populations are generally low ( $\leq 10\%$ ), mortality of patently infected individuals was complete (Chapman et al. 1968, Reib et al. 1982, Mullens et al. 1999). Presently, limitations on the infectivity of these parasites preclude their consideration as effective control agents. Methods to increase infectivity, such as the introduction of a mermithid nematode parasite that increases viral virulence (Mullens et al. 1999), may lead to their more efficient use as biological control agents.

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#### REFERENCES CITED

- Atkinson CT. 1990. Fine structure and sporogonic development of a *Vavraia* sp. (Microsporidia: Pleistophoridae) in the biting midge *Culicoides edeni* (Diptera: Ceratopogonidae). *J Invertebr Pathol* 55:105–111.
- Becnel JJ. 1992. Horizontal transmission and subsequent development of *Amblyospora californica* (Microsporidia, Amblyosporidae) in the intermediate and definitive hosts. *Dis Aquat Org* 13:17–28.
- Burges HD. 1981. Strategy for the microbial control of pests in 1980 and beyond In: Burges HD, ed. *Microbial control of pests and plant diseases 1970–1980* London, United Kingdom: Academic Press. p 797–836.
- Chapman HC, Petersen JJ, Woodard DB, Clark TB. 1968. New records of parasites of ceratopogonidae. *Mosq News* 28:122–123.
- Kline DL, Kelly JF, Ellis EA. 1985. A *Nosema*-type microsporidian infection in larvae of *Culicoides* spp. from salt marshes in Florida. *J Invertebr Pathol* 45:60–65.
- Levchenko NG, Issi IV. 1973. Microsporidia of blood-sucking Diptera. In: Dubitskii AN, ed. *Regulators of the numbers of blood-sucking flies in southeastern Kazakhstan* Alma-Ata, USSR: Kazakhstan Academy of Science. p 42–64.
- Linley JR, Davies JB. 1971. Sandflies and tourism in Florida and the Bahamas and the Caribbean area. *J Econ Entomol* 64:264–278.
- Mullens BA, Velten RK, Federici BA. 1999. Iridescent virus infection in *Culicoides variipennis sonorensis* and interactions with the mermithid parasite *Heleidomermis magnapapula*. *J Invertebr Pathol* 73:231–233.
- Rieb J-P, Mialhe E, Quiot JM. 1982. Ceratopogonidae larvae infected by an iridovirus. *Mosq News* 42:529.
- Sprague V, Becnel JJ, Hazard EI. 1992. Taxonomy of the phylum Microspora. *Crit Rev Microbiol* 18:285–395.
- Weiser J. 1957. Cizopasnici nekterych druhu hmyzu ssa-jicoho krev. *Cesk Parasitol* 4:355–359.