JOURNAL OF INVERTEBRATE PATHOLOGY 41, 301–304 (1983)

A New Microsporidian Parasite of the Mosquito *Aedes taeniorhynchus*[1,2]

JEFFREY C. LORD AND DONALD W. HALL

Department of Entomology and Nematology, University of Florida, Gainesville, Florida 32611

Received March 24, 1982; accepted May 24, 1982

A new species of microsporidium is described from the mosquito *Aedes taeniorhynchus*. This microsporidium is vertically transmitted and exhibits dimorphic development with one sequence leading to formation of short pyriform, uninucleate spores in male fourth instar larvae, pupae, and adults, and the other sequence leading to formation of diplocaryotic stages and cylindrical spores in adult females. Vertical transmission is apparently limited to a single generation, and the uninucleate spores are not transmissible per os to larvae.

KEY WORDS: *Aedes taeniorhynchus; Microsporidium fimbriatum* sp.n.; mosquito; verticle transmission.

INTRODUCTION

The black saltmarsh mosquito, *Aedes taeniorhynchus*, is sometimes infected by the dimorphic microsporidium *Amblyospora polykarya* (Lord et al., 1981). *A. polykarya* is vertically transmitted for a single generation, and produces fulminating infections and mortality in some fourth instar male and female progeny of infected females. In the course of screening the progeny of field-collected *A. taeniorhynchus* for *A. polykarya*, we have found a few egg batches infected with a new vertically transmitted microsporidium. The new microsporidium is described in this report.

MATERIALS AND METHODS

Experimental animals. Biting female *A. taeniorhynchus* were collected by power aspirator on the Coastal Prairie Trail, Flamingo, Everglades National Park, Florida. Each female was given a blood meal either from human or rabbit and placed in a glass vial with cotton gauze moistened with 0.15% NaCl for oviposition. These females were then provided with raisins and maintained at 24°C under a 16:8 hr light:dark regimen until oviposition. Larvae were reared under the same photoperiod and temperature conditions in 0.15% NaCl in enamel pans and fed an infusion of 3:2 powdered liver and brewer's yeast daily.

Life cycle studies. Fourth instar larvae were screened for infection by examination against a black background to detect discoloration. Adults were squashed for examination by phase-contrast microscopy. Some individuals from one of the infected egg batches were smeared at intervals throughout their development and stained with Giemsa, while others were prepared for electron microscopy. Smears were air dried, fixed in 95% methanol, and stained with buffered Giemsa stain, *p*H 7.41.

Specimens were prepared for electron microscopy as described by Lord et al. (1981).

Transmission studies. Uninucleate spores from a patently infected larva were fed to 24-hr-old *A. taeniorhynchus* larvae. These larvae were then reared to the fourth instar and examined for infection.

RESULTS AND DISCUSSION

The smear of the parent female contained diplocaryotic stages of the parasite and empty cylindrical spore walls (Fig. 1) which

[1] Florida Agricultural Experiment Station Journal Series No. 1937.

[2] This study was supported by Office of Naval Research Contract N00014-80-C-0172 and by Federal Hatch Project EY01889.

0022-2011/83 $1.50

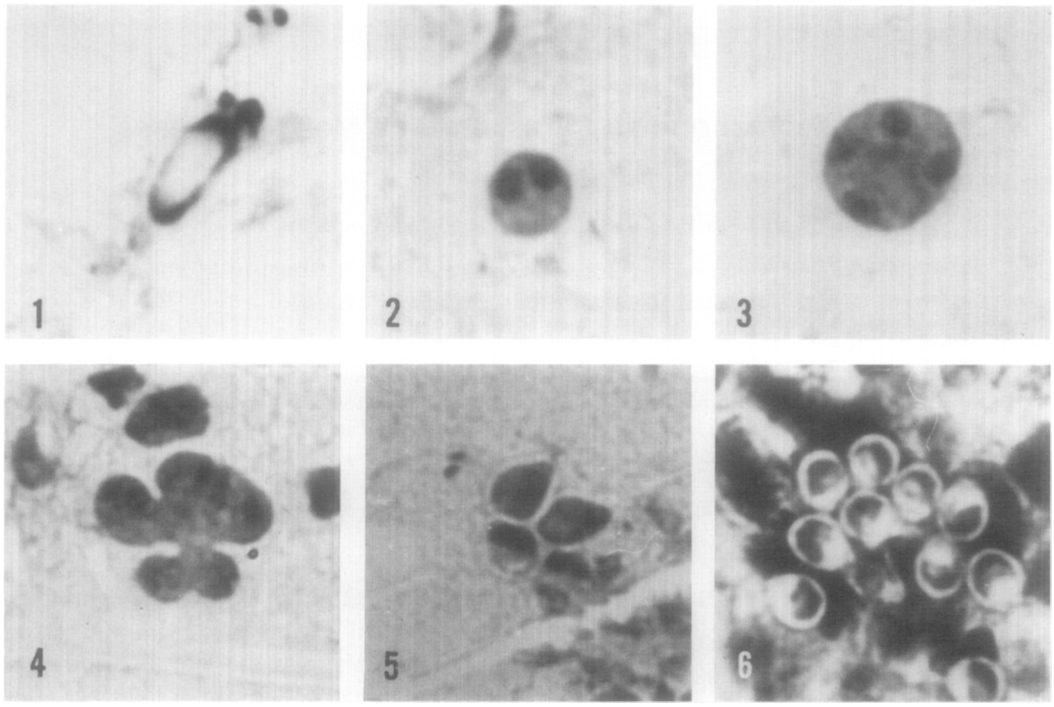

FIG. 1. Empty spore case from adult female mosquito. Giemsa stain. ×2400.
FIG. 2. Binucleate meront from male larva. Giemsa stain. ×2400.
FIG. 3. Quadrinucleate sporont from male larva. Giemsa stain. ×2400.
FIG. 4. Octonucleate sporont from male larva. Giemsa stain. ×2400.
FIG. 5. Sporoblasts from male larva. Giemsa stain. ×2800.
FIG. 6. Mature spores from adult male. Giemsa stain. ×2300.

appeared similar to the walls of the spores which function in vertical transmission in members of the family Thelohaniidae (Hazard and Weiser, 1968; Andreadis and Hall, 1979).

Sixteen adult progeny were obtained from one infected field-collected adult female *A. taeniorhynchus*. Of these adults, 12 were females and showed no signs of infection, and all four males contained numerous uninucleate spores in the fat body. This result suggests that uninucleate spores are probably formed only in males, a phenomenon common to certain other dimorphic microsporidia (Chapman et al., 1966; Kellen et al., 1966). The fact that none of the female progeny were infected also suggests that vertical transmission is limited to a single cycle as has also been described for *A. polykarya* (Lord et al., 1981).

Three of the smeared individuals, a third instar larva, a fourth instar larva, and a pupa, were found to harbor the parasite.

The third instar larva contained many presumptive meronts (Fig. 2) which were shown by electron microscopy to be diplocaryotic (Fig. 9). They also contained a smaller number of binucleate and quadrinucleate sporonts (Fig. 3), as well as a few scattered sporonts with six or eight nuclei (Fig. 4), sporoblasts (Fig. 5), and spores (Figs. 6, 8). Sporogony continues through the fourth instar and pupal stage. Multinucleate sporonts appear to produce uninucleate sporoblasts by budding. No pansporoblastic membrane was observed.

Spores from larvae and adult males appear short, pyriform, and with a sharp point at the anterior end when viewed in fresh smears. However, the point is difficult to demonstrate in Giemsa stained smears and electron micrographs (Figs. 5–8). The exo-

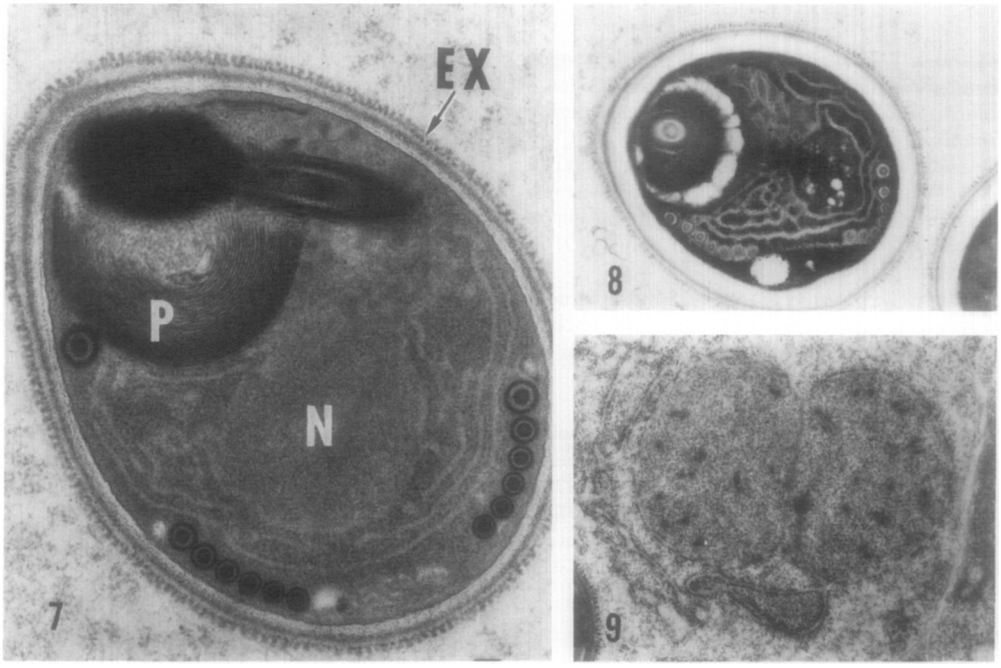

FIG. 7. Immature spore from adult male. EX, exospore; N, nucleus; P, polaroplast. × 26,000.
FIG. 8. Mature spore from adult male. × 14,000.
FIG. 9. Diplocaryotic stage in fourth instar larva. × 15,000.

spore is characterized by the presence of a fine, dense fringe.

This microsporidium is apparently fairly rare. We found infections in only three of over 2000 egg batches from wild-caught females. However, it is possible that some infections were missed early in the study due to the fact that sporulation does not occur until the adult stage in some individuals and only larvae were screened in these early studies.

Because of the uncertain taxonomic position of this parasite, we have assigned it to the collective genus *Microsporidium* suggested by Sprague (1977). Its development is most similar to dimorphic members of the family Thelohaniidae (Sprague, 1977), but the form of the spores found in males and the absence of the pansporoblastic membrane preclude placement in this family at this time. We also do not know whether there is meiosis in the developmental sequence of the uninucleate spores as has been reported for certain other microsporidia with dimorphic development (Hazard et al., 1979). The specific epithet, *fimbriatum*, means "fringed" in reference to the exospore of spores in male larvae.

Microsporidium fimbriatum sp.n.

Host. The black saltmarsh mosquito *Aedes taeniorhynchus* (Wiedemann).

Type locality. Coastal Prairie Trail, Everglades National Park, Florida.

Vegetative stages. Meronts have one or two diplocarya.

Sporulation stages. Sporonts found in third and fourth instar larvae and pupae contain two, four, six, or eight nuclei. No pansporoblastic membrane is evident.

Spores. Two types of spores are produced, cylindrical spores (approximately 8 × 3 μm) in the adult female and uninucleate, short pyriform spores (approximately 4 × 3 μm), having fringed exospores and seven coils in the polar filament, in the last instar larvae, pupae, and adult males.

Type material. Holotype slides will be sent to the United States National Museum.

REFERENCES

ANDREADIS, T. G., AND HALL, D. W. 1979. Development, ultrastructure, and mode of transmission of *Amblyospora* sp. (Microspora) in the mosquito. *J. Protozool.*, **26**, 444–452.

CHAPMAN, H. C., WOODWARD, D. B., KELLEN, W. R., AND CLARK, T. B. 1966. Host–parasite relationships of *Thelohania* associated with mosquitoes in Louisiana (Nosematidae: Microsporidia). *J. Invertebr. Pathol.*, **8**, 452–456.

ELLIS, E. A., AND AVERY, S. W. 1978. Resin formulations incorporating Epon 812 into a low viscosity embedding medium. *Proc. Southeast Electron Microsc. Soc.*, **1**, 20.

HAZARD, E. I., JOSYLN, D. J., ELLIS, E. A., AND ANDREADIS, T. C. 1979. Meiosis and its implications in the life cycles of *Amblyospora* and *Parathelohania* (Microspora). *J. Parasitol.*, **65**, 117–122.

HAZARD, E. I., AND WEISER, J. 1968. Spores of *Thelohania* in adult female *Anopheles:* Development and transovarial transmission, and redescriptions of *T. legeri* Hesse and *T. obesa* Kudo. *J. Protozool.*, **15**, 817–823.

KELLEN, W. R., CHAPMAN, H. C., CLARK, T. B., AND LINDEGREN, J. E. 1966. Transovarian transmission of some *Thelohania* (Nosematidae: Microsporidia) in mosquitoes of California and Louisiana. *J. Invertebr. Pathol.*, **8**, 355–359.

LORD, J. C., HALL, D. W., AND ELLIS, E. A. 1981. Life cycle of a new species of *Amblyospora* (Microspora: Amblyosporidae) in the mosquito *Aedes taeniorhynchus*. *J. Invertebr. Pathol.*, **37**, 66–72.

PERACCHIA, C., AND MITTLER, B. S. 1972. Fixation by means of glutaraldehyde–hydrogen peroxide reaction products. *J. Cell Biol.*, **53**, 234–238.

REYNOLDS, E. S. 1963. The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. *J. Cell Biol.*, **17**, 208–211.

SPRAGUE, V. 1977. Classification and phylogeny of the microsporidia. *In* "*Comparative Pathobiology*" (L. A. Bulla and T. C. Cheng, eds.), Vol. 2, "Systematics of the Microsporidia," pp. 1–30, Plenum, New York.