JOURNAL OF INVERTEBRATE PATHOLOGY 37, 66–72 (1981)

Life Cycle of a New Species of *Amblyospora* (Microspora:Amblyosporidae) in the Mosquito *Aedes taeniorhynchus*[1,2]

JEFFREY C. LORD, DONALD W. HALL, AND E. ANN ELLIS

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

Received September 27, 1979

A new species of Microspora, *Amblyospora polykarya*, is described from the mosquito *Aedes taeniorhynchus*. The parasite is transovarially transmitted for one generation only. Spores in adult females extrude binucleate sporoplasms which infect developing eggs. Merogony occurs in larval oenocytes with diplokaryotic stages in early instars giving rise to plasmodia with many diplokarya. Plasmodia undergo cytokinesis to form diplokaryotic sporonts. In fat body cells, these sporonts secrete pansporoblastic membranes and undergo two nuclear divisions to form octonucleate sporonts. Cytokinesis and differentiation result in uninucleate spores in packets of eight. These spores are not transmissible per os and are of different morphotype from those in adult females. Infected larvae die in the fourth stadium.

KEY WORDS: *Amblyospora polykarya; Aedes taeniorhynchus*, transovarial transmission.

INTRODUCTION

A microsporidium recorded from *Aedes taeniorhynchus*, the black salt-marsh mosquito, by Chapman et al. (1966) has been assigned to the genus *Amblyospora* by Hazard and Oldacre (1975). As is the case for all members of the family Amblyosporidae, development is dimorphic with one spore type functioning in transovarial transmission and the other having unknown function (Hazard and Weiser, 1968; Andreadis and Hall, 1979a; Weiser, 1977). This species was assigned to the type III host–parasite relationship of Kellen et al. (1965) in which oenocytes and adipose tissue are invaded, sporogony (spore formation) occurs in both sexes, and mortality results in the fourth stadium. Lack of information about the vegetative and sporogonic stages and the spore type functioning in transovarial transmission has delayed assignment of species name. This study deals with these gaps and describes the species.

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% aqueous NaCl for oviposition. These females were then provided with raisins and maintained at 24°C under a 16:8-hr light–dark regimen. 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. A sample of each egg batch was hatched, and the larvae were reared to the fourth instar to screen for infection by examination against a black background to detect discoloration. Diseased larvae appeared lighter in color. The remainders of those batches found infected were subsequently hatched, and some of the larvae were smeared during each

[1] Florida Agricultural Experiment Station Journal Series No. 1936.

[2] This study was supported by Federal Hatch Project EY 01889 and by Environmental Grant 8210 from the Institute of Food and Agricultural Sciences, University of Florida.

0022-2011/81/010066-07$01.00/0

stadium. Smears were then air dried, fixed in 95% methanol, and stained with buffered Giemsa stain, *p*H 7.41. Other larvae were fixed in Carnoy's solution, embedded in paraffin, sectioned at 6 μm, and stained with Heidenhain's hematoxylin and eosin Y (Barbosa, 1974).

Tissue from females which had laid infected eggs was smeared in saline solution and examined first by phase-contrast and Nomarski interference-contrast microscopy and then stained with Giemsa.

For ultrastructural studies, infected specimens were dissected in 2.5% glutaraldehyde buffered with 0.1 M sodium cacodylate, *p*H 7.5, and fixed for 2 hr at room temperature in the dark in 2.5% glutaraldehyde, 0.1% peroxide in 0.1 M cacodylate buffer, *p*H 7.5 (Peracchia and Mittler, 1972). After several buffer washes, specimens were postfixed in 1% OsO_4, dehydrated in an ethanol series, en bloc stained with 0.5% uranyl acetate in 70% ethanol, and embedded in a Spurr–Epon mixture (Ellis and Avery, 1978). Ultrathin sections were poststained with 5% methanolic uranyl acetate, followed by lead citrate (Reynolds, 1963), and examined with a Hitachi HU125E electron microscope at an acceleration voltage of 75 kV.

Transmission studies. Surviving females from infected egg batches were either smeared and examined under phase contrast for the presence of spores or mated to colony males and placed in individual vials for oviposition. Because female *A. taeniorhynchus* are reluctant to mate when in captivity (O'Meara and Evans, 1974), it was necessary to force-mate the survivors using a modification of the technique of McDanial and Horsfall (1957). The females were fed blood prior to mating to ensure extension of the cerci, anesthetized with ether for 4 min, and placed on a grooved rubber surface in the supine position. Males were immobilized briefly by chilling and impaled on minuten pin probes. After removal of their legs, the males were brought into contact with females at a 60–90° angle, looping the claspers over the tip of the females' cerci until copulation was achieved.

Newly hatched larvae of *A. taeniorhynchus, Culex salinarius,* and *Anopheles albimanus* were exposed to spores from infected fourth-instar larvae at concentrations of 3.5×10^5, 3.5×10^6, and 7.0×10^6 spores/ml. All larvae exposed to spores were screened for infection in the fourth instar, reared through to maturity, and permitted to lay eggs. Their progeny were then reared and screened as fourth instars.

RESULTS AND DISCUSSION

Natural Infection Rates

Egg batches were obtained from 705 female *A. taeniorhynchus* collected from June to October 1978. Fourteen (2.0%) contained eggs infected with the microsporidium. From individual egg batches the percentage of larvae which developed patent infections ranged from 3.75 (3 of 80) to 100 (39 of 39). Only one infected female produced a second egg batch, which was uninfected suggesting a decreasing infection rate with successive gonotrophic cycles, as observed by Andreadis and Hall (1979b) for *Amblyospora* sp. in *Culex salinarius.*

Life Cycle

The presumptive developmental sequence of this microsporidium from the spores in adult females to the spores in mature larvae is shown in Figure 1. Spores are of two distinct morphotypes. The larger cylindrical binucleate spores (Figs. 2, 3) of adult females measure approximately 7.3 × 2.5 μm. The smaller uninucleate long subelliptical (Jamback, 1970) spores measuring approximately 4.4 × 2.4 μm (Figs. 13, 14, 19) which are packaged in groups of eight within a pansporoblastic membrane are found in mature larvae. Infection of the eggs is initiated within the female host when the spore polar filaments are everted extruding binucleate sporoplasms (Fig. 4).

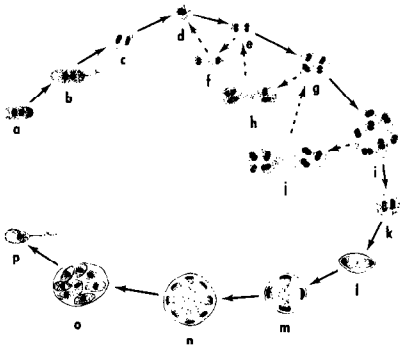


FIG. 1. Life cycle of *Amblyospora polykarya* in *Aedes taeniorhynchus*. (a) Binucleate spore in adult ♀; (b) extruded binucleate spore; (c) binucleate meront of eggs and young larvae; (d) diplokaryotic meront in young larvae; (e–j) plasmodia of second-, third-, and fourth-instar larvae showing hypothetical multiplication; (k) early binucleate sporont of fat body cells in third and fourth instar, (l) binucleate sporont with pansporoblast membrane; (m) tetranucleate sporont; (n) octonucleate sporont; (o) pansporoblast containing eight sporoblasts; (p) mature uninucleate spore.

The exact mechanism by which eggs are penetrated is unknown.

Within oenocytes of embryonated eggs and first-instar larvae of both sexes, small round binucleate stages (Fig. 5) undergo the initial stages of multiplication (merogony). In the first and second instars, after several divisions, the nuclei of the binucleate stages become closely appressed forming diplokarya (Figs. 6, 16) which undergo repeated karyokinesis, probably accompanied by some cytokinesis, resulting in a large number of darkly staining plasmodia (Fig. 7). These plasmodia are present in the second, third, and early fourth instars. Beginning in the third instar, these plasmodia become lobed (Figs. 8, 17) and undergo cytokinesis forming diplokaryotes which initiate sporogony. At some point in the above process the fat body is invaded. It is there that spores are formed.

Sporogony involves additional multiplication and morphogenesis resulting in uninucleate spores in groups of eight. At the beginning of this process, the nuclei of the diplokaryon separate and pansporoblastic membrane is secreted (Figs. 9, 18). The two nuclei of the sporont divide synchronously to form a tetranucleate stage (Fig. 10) in which karyokinesis again occurs resulting in an octonucleate sporont (Fig. 11). Cytokinesis is completed in the octonucleate sporont producing eight sporoblasts within the pansporoblastic membrane (Fig. 12). Each sporoblast develops into a pyriform uninucleate spore characterized by a thin exospore, and a polar filament which is abruptly constricted near its middle (Fig. 19).

The massive spore accumulation and fat body tissue destruction result in death in the fourth instar. The fourth stadium is ex-

FIGS. 2–15. Photomicrographs of Giemsa-stained and living *Amblyospora polykarya* (Figs. 2–13, ×1900; Figs. 14, 15 ×900).

FIG. 2. Free spore, phase contrast.

FIG. 3. Free spore, Nomarski interference contrast.

FIG. 4. Binucleate sporoplasms from adult female, Giemsa stain.

FIG. 5. Binucleate meronts, Giemsa stain.

FIG. 6. Diplokaryotic meronts, Giemsa stain.

FIG. 7. Plasmodium, Giemsa stain.

FIG. 8. Budding plasmodium, Giemsa stain.

FIG. 9. Binucleate sporont, Giemsa stain.

FIG. 10. Tetranucleate sporont, Giemsa stain.

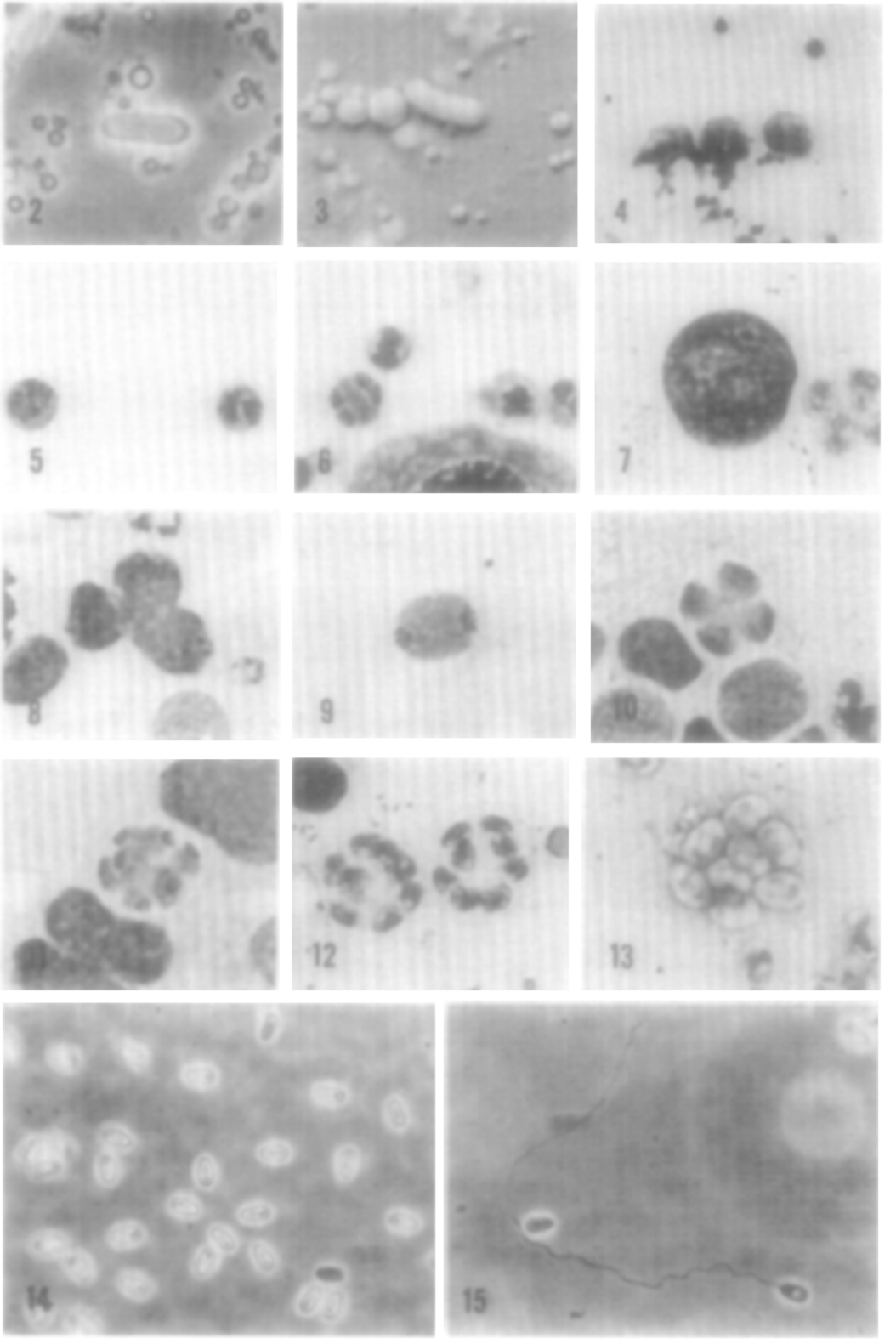
FIG. 11. Octonucleate sporont, Giemsa stain.

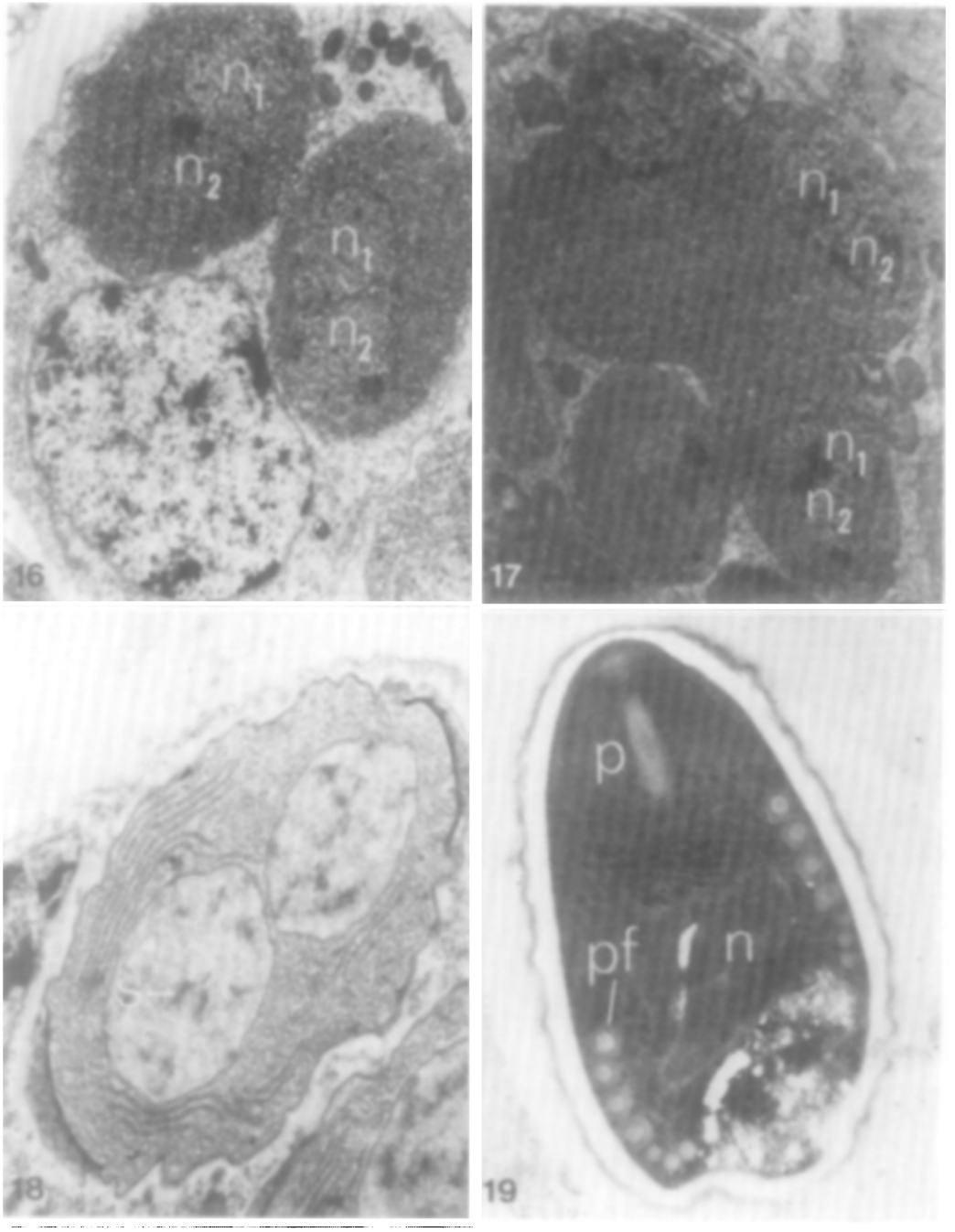
FIG. 12. Pansporoblast with eight sporoblasts, Giemsa stain.

FIG. 13. Mature uninucleate spores, Giemsa stain.

FIG. 14. Mature uninucleate spores, phase contrast.

FIG. 15. Extruded uninucleate spore, phase contrast.


2
3
4
5
6
7
8
9
10
12
13
14
15



FIGS. 16–19. Electron micrographs of developmental stages of *Amblyospora polykarya* in *Aedes taeniorhynchus* larvae.

FIG. 16. Host oenocyte containing two diplokaryotic meronts. N_1-N_2, diplokaryotic nuclei. ×10,000.

FIG. 17. Budding plasmodium with diplokaryotic nuclei. N_1-N_2, nuclei. ×8500.

FIG. 18. Binucleate sporont with nuclei in early stage of separation. (S) Synaptonemal complexes. ×8000.

FIG. 19. Mature uninucleate spore. (N) Nucleus; (P) polaroplast; (PF) polar filament. ×10,700.

tended by up to 10 days beyond that of healthy larvae.

Transmission

Of females surviving from infected egg batches, 14 were smeared and no evidence of infection was found. Twenty-two of these surviving females were force-mated with colony males and produced uninfected progeny indicating termination of transovarial transmission after one cycle. These results indicate that another means of transmission is required for maintenance of the parasite. Attempts to transmit the parasite by direct feeding of uninucleate spores to mosquito larvae were unsuccessful.

The origin of the binucleate spores in adult female *A. taeniorhynchus* and the fate of the uninucleate spores is still unknown. All attempts to transmit *Amblyospora* and related genera by direct feeding of uninucleate spores to healthy larvae have been unsuccessful both in our lab and by previous investigators (Kellen and Wills, 1962; Kellen et al, 1966). Also, binucleate sporonts of this microsporidium appear to have synaptonemal complexes (Fig. 18) indicating meiosis as reported by Hazard et al. (1979). These authors suggest the possible occurrence of a sexual process involving an alternate host.

Amblyospora, as defined by Hazard and Oldacre (1975), is characterized by dimorphic development. The sporogonic sequence in larvae produces eight spores by endogenous budding within a pansporoblastic membrane. These spores are oval with broadly rounded ends, tending to be truncate when preserved, with a thick exospore and usually with a mucus envelope. The polar filament is reduced in diameter in the distal portion. The sporulation sequence in oenocytes of female hosts produces isolated spores which have thin walls, a large posterior vacuole, and a polar filament of uniform diameter.

Although the uninucleate spores in larvae are long subelliptical in shape, have a thin exospore, and a plasmodium in their developmental sequence, and the development in adult females is unknown, we have chosen to temporarily leave this microsporidium in the genus *Amblyospora*. It is likely that this genus will require revision in the future as more species are thoroughly studied. The specific epithet is *polykarya* which means many nuclei, in reference to the plasmodium in its development.

Amblyospora polykarya sp. n.

Host. The black salt-marsh mosquito, *Aedes taeniorhynchus.*

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

Site of infection. Meronts are found in larval oenocytes. Uninucleate spores are found in larval fat body.

Vegetative stages. In larvae, diplokaryotic meronts give rise to plasmodia. These form sporonts by budding.

Sporulation stages. Sporonts in larvae contain two, four, or eight nuclei. Sporonts with eight nuclei produce eight sporoblasts within a pansporoblast membrane.

Spores. Two types of spores are produced; nearly pyriform, uninucleate spores with thin exospore in larvae and cylindrical, uninucleate spores in adult females.

ACKNOWLEDGMENTS

The authors express appreciation to Dr. Gary Hendrix, Research Director, Everglades National Park, and his staff for their assistance with collecting permits for this study. We also gratefully acknowledge the advice and encouragement of Mr. Edwin I. Hazard of the Insects Affecting Man Research Laboratory, U.S. Department of Agriculture.

REFERENCES

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

ANDREADIS, T. G., AND HALL, D. W. 1979b. Significance of transovarial infections of *Amblyospora* sp. (Microspora: Amblyosporidae) in relation to parasite maintenance in the mosquito *Culex salinarius*. *J. Invertebr. Pathol.*, **34**, 152–157.

BARBOSA, P. 1974. "Manual of Basic Techniques in

Insect Histology." Autumn Publishers, Amherst, Mass.

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., JOSLYN, 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 OLDACRE, S. W. 1975. Revision of Microsporida (Protozoa) close to *Thelohania*, with descriptions of one new family, eight new genera and thirteen new species. *U.S. Dep. Agr. Tech. Bull.*, **1530**, 1–104.

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.

JAMBACK, H. A. 1970. *Caudospora* and *Weiseria*, two genera of Microsporidia parasitic in blackflies. *J. Invertebr. Pathol.*, **16**, 3–13.

KELLEN, W. R., CHAPMAN, H. C., CLARK, T. B., AND LINDEGREN, J. E. 1965. Host–parasite relationships of some *Thelohania* from mosquitoes (Nosematidae:Microsporidia). *J. Invertebr. Pathol.*, **7**, 161–166.

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.

KELLEN, W. R., AND WILLS, W. 1962. The transovarian transmission of *Thelohania california* Kellen and Lipa in *Culex tarsalis* Coquillett. *J. Insect Pathol.*, **4**, 321–326.

MCDANIEL, I. N., AND HORSFALL, W. R. 1957. Induced copulation of aedine mosquitoes. *Science*, **124**, 754.

O'MEARA, G. F., AND EVANS, D. B. 1974. Female dependent stenogamy in the mosquito *Aedes taeniorhynchus*. *Anim. Behav.*, **22**, 376–381.

PERACCHIA, C., AND MITTLER, B. S. 1972. Fixation by means of gluteraldehyde-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.

WEISER, J. 1977. Contribution to the classification of microsporidia. *Vestn. Cesk. Spolecnosti Zool.*, **41**, 308–321.