JOURNAL OF INVERTEBRATE PATHOLOGY 61, 267–274 (1993)

Ultrastructure and Life Cycle of *Merocinta davidii* gen et sp. nov, a Dimorphic Microsporidian Parasite of *Mansonia africana* (Diptera: Culicidae) from Tanzania

JUDITH K. PELL[1] AND ELIZABETH U. CANNING

Department of Biology, Imperial College of Science, Technology and Medicine, Prince Consort Rd., London SW7 2AZ, United Kingdom

Received December 16, 1991; accepted June 23, 1992

Mansonia africana **larvae were sampled at three sites in NE Tanzania, between June and September 1987. At each site between 2.5 and 5.7% were infected with a multisporous microsporidian parasite. The ovoid spores measured 2.5 × 1.5 μm. Meronts were diplokaryotic with electron-dense, thickened unit membranes. Repeated division of the diplokarya gave rise to multinucleate plasmodia, the plasma membrane of which was entirely surrounded by two other membranes interpreted as host endoplasmic reticulum. Both membranes were studded with ribosomes. Meronts became ribbon-like before division, the endoplasmic reticulum dividing with the parasite. At the onset of sporogony the two nuclei of the diplokaryon separated and the electron-dense coat was sloughed off. Nuclear division and cytoplasmic division gave rise to uninucleate sporoblasts and spores. Adult *M. africana* collected at one of the three sites were infected with two diplokaryotic microsporidia, designated Type A and Type B, which differed only in the size of the spores. Spores of Type A measured 3.4 × 1.8 μm and those of Type B measured 6.6 × 2.9 μm; both types were binucleate. Type A infected 10.8 and Type B 5.8% of the adults collected. Mixed infections were not observed. In transovarial transmission trials, larvae were not successfully reared from adults infected with Type B. However, 11.3% of progeny from females infected with Type A exhibited multisporous microsporidian infections indistinguishable from those found in larvae collected at that site, showing that the multisporous parasite in larvae and the Type A infection of adults represent different stages in the life cycle of a single species. The morphology and life cycle of this microsporidian exclude it from all other related genera and it is attributed here to a new genus and given the name *Merocinta davidii*.**

KEY WORDS: Microsporidium, dimorphism, culicine mosquito, taxonomy.

INTRODUCTION

In a previous publication, a new octosporous species of microsporidia, *Tricornia muhezae,* was described from larvae of *Mansonia africana* collected in three sites in NE Tanzania in 1985 and 1987 (Pell and Canning, 1992). In samples taken in 1987, larvae from each of the three sites were also infected with a multisporous species, although individuals infected with both species were never found. Also, adults of the same mosquito collected at one of the sites harbored two different diplokaryotic infections. Since many microsporidia are dimorphic, with both forms developing in different stages of the life cycle of the same host, transovarial transmission experiments were done to determine whether either of the two adult infections were linked to the larval infections. This article describes the results of these experiments and the ultrastructure of the multisporous larval infection. The taxonomic position of this parasite is discussed in the light of these findings.

MATERIALS AND METHODS

Mansonia spp. larvae respire by attaching their siphons to the roots of floating vegetation, particularly *Pistia stratiotes* (Laurence, 1960), from which they can be dislodged for collection by shaking (Service, 1976). Larvae were collected from three sites within 2 miles of the town of Muheza, Tanzania (5° 11′S, 38° 5′ E) from June to September 1987. These sites were Kisiwani Pond, Mamakingi Pond, and Muheza Sisal Estate Swamp (Pell and Canning, 1992). All dislodged larvae were collected and examined for the incidence of microsporidian infection at each of the three sites.

Larvae were dissected after identification and one part of each smeared, air dried, fixed in methanol, and stained for 30 min in 10% Giemsa's stain (Gurr's Improved R66), buffered at pH 7.2. The remaining pieces of larvae were fixed for electron microscopy. These

[1] Present address: AFRC Institute of Arable Crops Research, Rothamsted Experimental Station, Harpenden, Herts. AL5 2JQ, UK.

0022-2011/93 $4.00

pieces were cut into 1-mm lengths which were transferred to Karnovsky's fixative for 10 min at ambient temperature and then into fresh fixative for 1 hr at 4°C. After the material was washed twice in 0.12 M sodium cacodylate buffer, it was kept in buffer at 4°C and not processed further until returned to the United Kingdom laboratory 1–3 months later. It was then postfixed in 1.5% osmium tetroxide, block-stained in phosphotungstic acid and uranyl acetate, dehydrated in acetone, and embedded in Spurr's resin. Gold sections were cut on a Reichert ultramicrotome, further stained in alcoholic uranyl acetate and lead citrate, and viewed on a Phillips 300 transmission electron microscope (Canning *et al.*, 1982).

Mansonia spp. adults were caught using human bait catches at Muheza Sisal Estate Swamp on five evenings between June and September 1987. The following morning the mosquitoes were blood-fed using an anesthetised guinea pig.

Two days after blood-feeding, gravid females were transferred to ovipots for oviposition. These consisted of a paper cup containing a glass slide on which the mosquito could rest, and small pieces of *Pistia* leaves cut from the edges of larger leaves, floating on water, under which the egg batches were laid.

Each day, individuals that had laid eggs were recorded. The thorax and part of the abdomen of all such females were immediately smeared, stained in Giemsa's stain, and examined for microsporidian infection. When egg batches laid by infected females hatched, they were introduced into larval bowls (plastic washing-up bowls) and reared to third/fourth instar. In general, the majority of eggs in a batch hatched successfully. Reared larvae were smeared and stained for transovarially transmitted infections. Egg batches from healthy females were treated similarly and served as controls.

Larval rearing bowls contained 2 liters of water with approximately 10 g of guinea pig diet and 2 g of yeast. A 7-cm^2 tuft of grass with roots was also added, resting on an upturned cup. *P. stratiotes* plants were added as attachment sites for the larvae. These plants were collected from Kwamihayo Pond (1 mile from Muheza) where, although repeated collections were made, no *Mansonia* spp. were ever found. Plants were thoroughly washed in running water before use, to remove mud and other insect larvae, and regularly replaced as they died. Yeast was added every 3 days to maintain a large population of microorganisms and the bowls were covered in a fine meshed netting to prevent egg laying by other mosquitoes. The culture method was modified from that of Laurence *et al.* (1962).

RESULTS

Larval Infection

The mean prevalence of the multisporous infection from *M. africana* at the three sites was 2.5% (n = 159) at Kisiwani, 5.7% (n = 174) at Muheza Sisal Estate Swamp, and 2.5% (n = 121) at Mamakingi Pond. Larvae infected with multispores showed no overt symptoms even when heavily infected. *Coquilletidia* spp. and *M. uniformis* larvae from the same sites were not infected.

Light Microscopical Observations

The earliest stages seen were diplokaryotic meronts with variable numbers of paired nuclei from one (Fig. 1) to six or more (Fig. 2), forming multinucleate plasmodia. Long ribbon-like strings of diplokaryotic stages were noted consistently (Fig. 3).

As sporogony proceeded the two nuclei of the diplokaryon separated and each underwent further divisions to produce plasmodia with variable numbers of nuclei (Fig. 4). Cytoplasmic division gave uninucleate sporoblasts and spores (Fig. 5). Spores were oval and slightly flattened terminally. They measured 2.5 ± 0.3 μm × 1.5 ± 0.3 μm (n = 11). The number of spores in the sporophorous vesicle ranged from 40 to 60. The vesicle diameter varied in size from 27.5 to 12.5 μm diameter, according to the number of spores within.

Ultrastructural Observations

Infections were found in the tissues of the gut, often close to the microvilli. The earliest stages seen were

FIGS. 1–11. Photomicrographs and electron micrographs of *Merocinta davidii* from *Mansonia africana* larvae. Figs. 1–5: Giemsa-stained smears. Scale bar on Fig. 1 = 10 μm. Figs. 6–11: transmission electron micrographs; scale bars = 1 μm.

FIG. 1. Two meronts, one with a single pair of diplokaryotic nuclei and the other with three pairs.

FIG. 2. Multinucleate merogonic plasmodium with six pairs of diplokaryotic nuclei.

FIG. 3. Merogonic stages formed into long ribbons with diplokaryotic nuclei.

FIG. 4. Sporogonic plasmodium with numerous nuclei, no longer in diplokaryotic configuration.

FIG. 5. Uninucleate spores.

FIG. 6. Diplokaryotic meront (M) limited by a single, slightly thickened unit membrane.

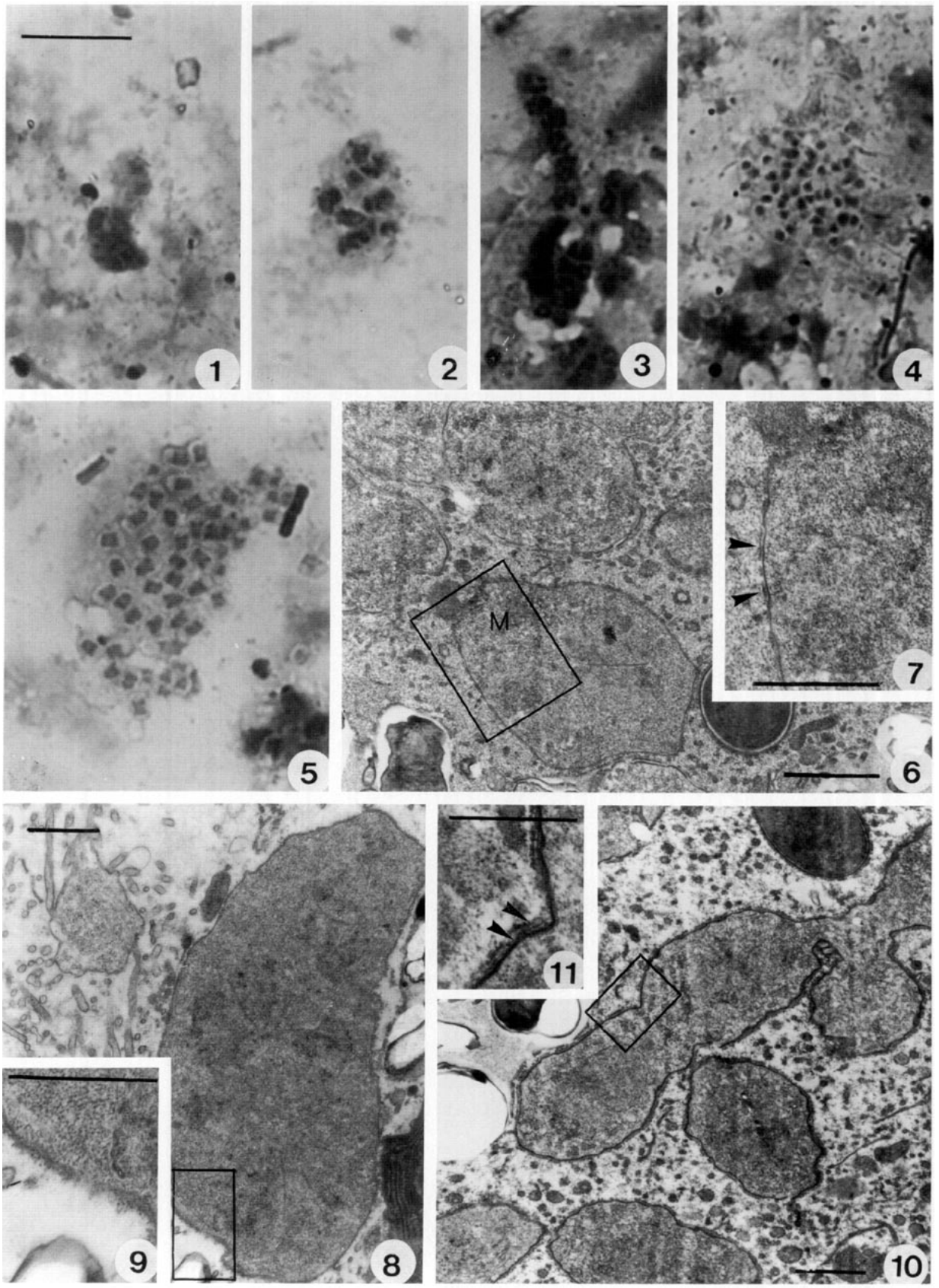
FIG. 7. Inset: Detail of area indicated in Fig. 6 showing strings of ribosomes close to the parasite surface (indicated by arrows).

FIG. 8. Multinucleate merogonic plasmodium.

FIG. 9. Inset: Detail of the area indicated in Fig. 8. The plasmodia is almost entirely surrounded by two other membranes (thought to be host endoplasmic reticulum), the outer of which is studded with ribosomes.

FIG. 10. Ribbon-like meront with thickened surface coat prior to division. The host endoplasmic reticulum encasing the meronts divides with the parasite.

FIG. 11. Inset: Detail of the area indicated in Fig. 10. Ribosomes are present on both the outer and the inner membranes of the endoplasmic reticulum (ribosomes indicated by an arrow).


1
2
3
4
5
M
6
7
8
9
10
11

diplokaryotic meronts (Fig. 6), limited by a single, somewhat thickened, unit membrane. Short strings of ribosomes were seen close to the meront surface (Fig. 7, inset). Repeated division of the diplokaryotic nuclei produced multinucleate plasmodia (Fig. 8). The plasma membrane of the larger plasmodia was almost entirely surrounded by two other membranes, the outer of which was abundantly studded with ribosomes (Fig. 9, inset). These membranes were interpreted as host endoplasmic reticulum closely applied to the parasites' surface. The inner two membranes were so close together that in many regions they were difficult to resolve.

The surface coat of the multinucleate meronts increased in thickness and the meronts became ribbon-like prior to division (Fig. 10). Division was by constriction of the cytoplasm around each diplokaryon and the encasing cisterna of host endoplasmic reticulum divided with the parasite. As a result, the daughter meronts were also encased in host endoplasmic reticulum. At this stage it could be seen that sparsely distributed ribosomes were also present on the inner membrane of the endoplasmic reticulum (Fig. 11, inset).

Sporogony was signaled in multinucleate sporonts (sporogonial plasmodia) by two events: the separation of the two nuclei of each diplokaryon and sloughing of the electron-dense coat into the space between its surface and the cisterna of endoplasmic reticulum so that the parasite surface again became a simple unit membrane (Fig. 12). The effect of sloughing of the surface coat was to increase the space around the developing sporogonial plasmodium (Fig. 13) which proceeded to divide sequentially into segments with fewer and fewer nuclei and eventually to produce uninucleate sporoblasts. During this process an electron-dense coat was secreted again onto the surface of the segments and the cavity bounded by the endoplasmic reticulum became full of granular metabolic products (Fig. 13). The electron-dense coat was secreted in patches onto the sporogonial plasmodia with unthickened regions between (Figs. 13, 14). The result, after fixation, was to cause blistering at the surface of the plasmodia (Fig. 14). As the thickening became more extensive the blistering diminished until regularly shaped sporont products were formed (Fig. 15).

The final cytoplasmic divisions gave uninucleate sporoblasts (Fig. 15): by this stage development of the surface coat had been completed and the surface had become smoother, having lost the blistered appearance. Late sporoblasts, however, were crenated and showed the developing polar filaments and polaroplast (Fig. 16). The surface coat was 0.045 nm thick. Within the vesicle the granular homogeneous material remained, but in addition tubular profiles were present. These remained in the vesicles containing maturing spores, but disappeared as the spores matured. Although spores were not well preserved, signs of endospore formation under the exospore were present. The polaroplast was not clear but endoplasmic reticulum, a single nucleus, and one coil of the polar filament were visible (Fig. 17). No more than one coil of the polar filament was observed in any spore, suggesting that mature spores have a single coil (Fig. 17). Spores were oval and measured 2.1 ± 0.1 μm × 1 ± 0.1 μm (n = 5).

Adult Infection

Two types of infection were found in adult females, one with small diplokaryotic developmental stages (designated Type A) infecting 10.9% of female *M. africana* (n = 174) and the other with much larger diplokaryotic developmental stages (designated Type B) infecting 5.8% of female *M. africana* (n = 174). Type B microsporidian infections were also found in 5.6% (n = 72) of females identified as *M. uniformis* and 3.1% of females that could not be identified to species level and were so recorded as *Mansonia* spp. Mixed infections were not seen. Before mosquitoes were identified they had undergone much handling, resulting sometimes in loss of scales. The differentiation between *M. africana* and *M. uniformis* depends mainly on the strength of the banding colors on the legs, which are directly attributable to the presence of certain scales. Because of scale loss some specimens could not be assigned to one or other species and were thus assigned to the group *Mansonia* sp. It is also possible that some *M. africana* were misidentified as *M. uniformis* due to loss of leg and wing scales. The infections were therefore not just predominantly, but possibly exclusively, in *M. africana*.

FIGS. 12–17. Transmission electron micrographs of *Merocinta davidii* from *Mansonia africana* larva. Scale bars = 1 μm.

FIG. 12. Early multinucleate sporont (sporogonial plasmodium). The nuclei (n) of the diplokarya are separated and the electron-dense coat present in meronts is sloughed into the space between the sporont surface and the cisterna of host endoplasmic reticulum.

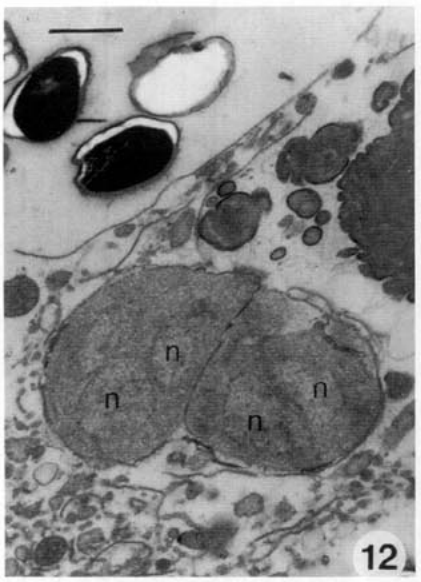
FIG. 13. Sporont. The sporogonial plasmodium divides sequentially. Electron-dense material continues to be sloughed off into the vesicle cavity (indicated by a single arrow), but in addition another electron-dense coat is in the process of being secreted in patches (indicated by a double arrow). The contents of the vesicle are granular.

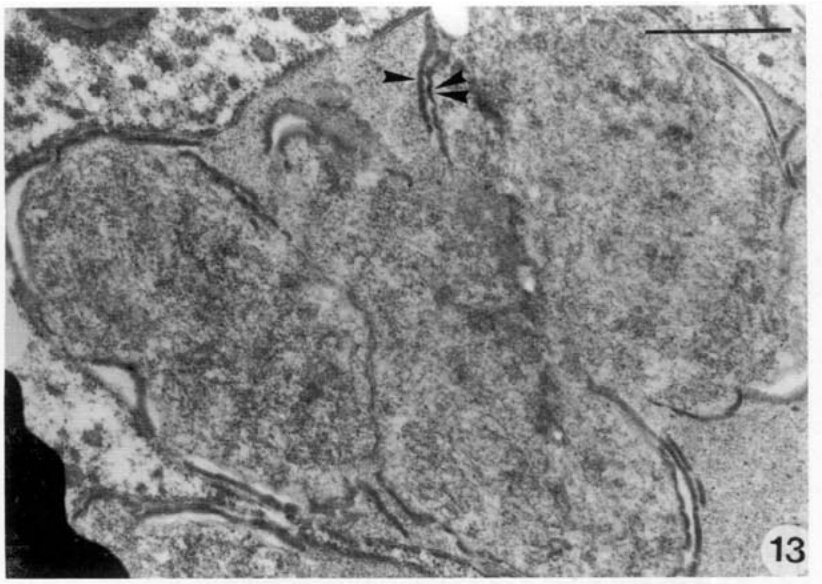
FIG. 14. Sporont products. The surface coat appears blistered as an artifact of fixation.

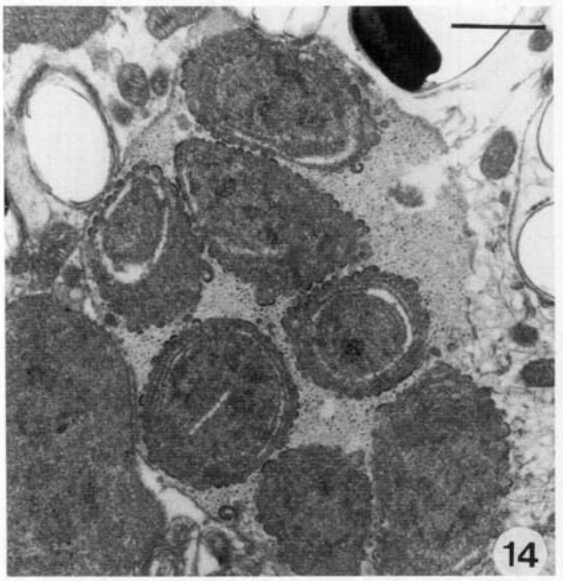
FIG. 15. Uninucleate sporoblasts.

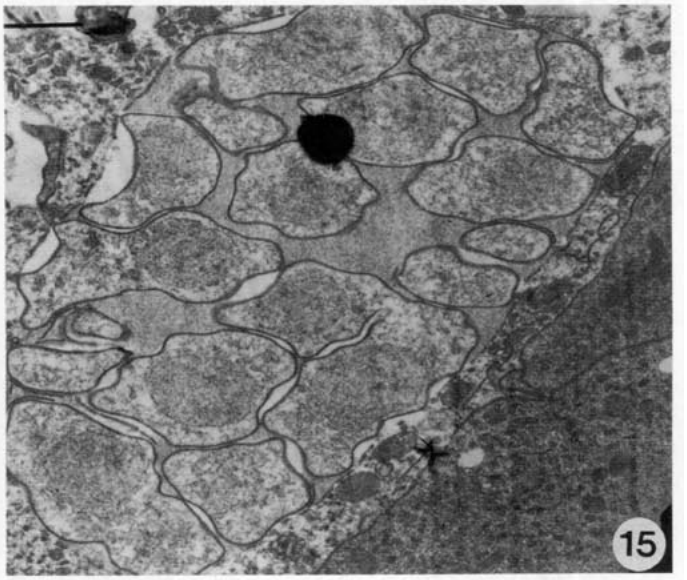
FIG. 16. Late sporoblasts. These are crenated in shape and show the developing polar filaments and polarplast (PP).

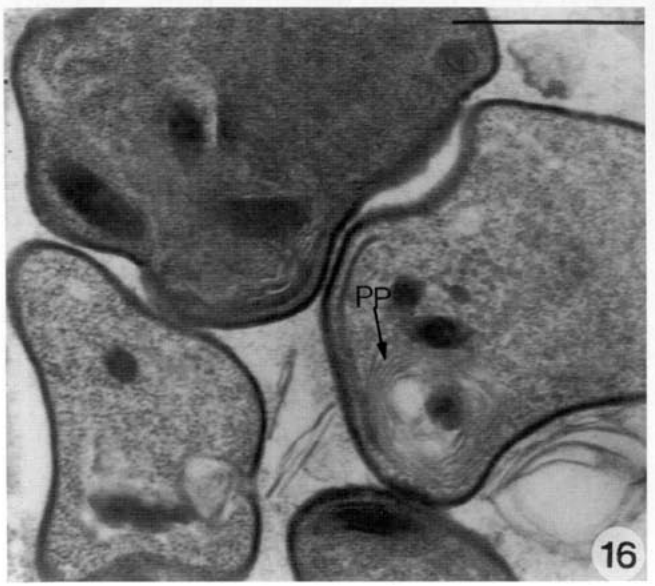
FIG. 17. Maturing spore. Signs of endospore (EN) formation below the exospore (EX) are present. Spores have a single nucleus and only one coil of the polar filament.

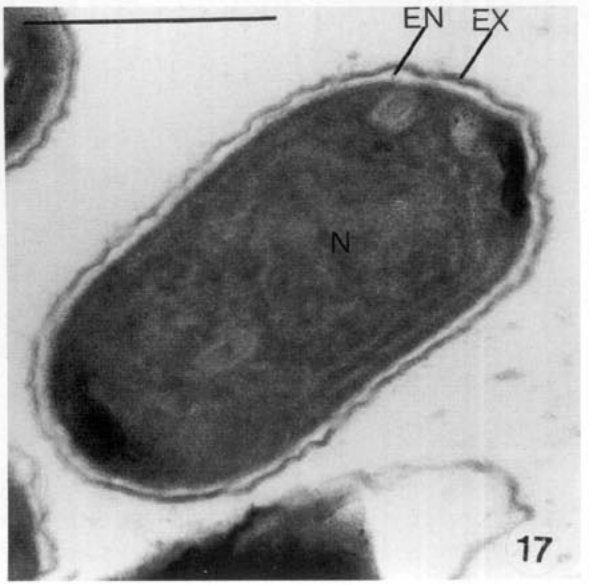

n
n
n
n
12


13


14


15


PP
16


EN
EX
N
17

Light Microscopical Appearance of Type A and Type B

Type A

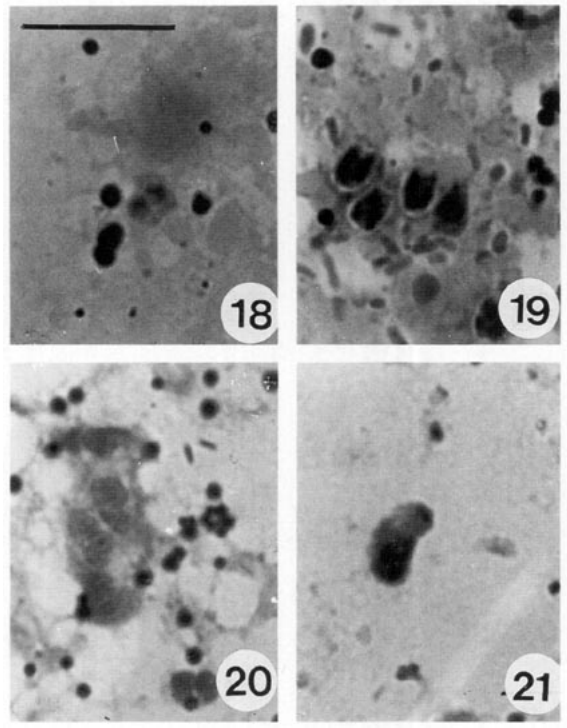
The earliest stages seen were diplokaryotic meronts or early sporonts (Fig. 18) the diplokaryon of which measured 2.6 ± 0.3 μm × 1.1 ± 0.3 μm (n = 7). All subsequent stages were binucleate and never tetranucleate. Their development resulted in binucleate spores (Fig. 19), measuring 3.4 ± 0.3 μm × 1.8 ± 0.3 μm (n = 6).

Type B

The earliest stages presumed to be merogonic were diplokaryotic with 1–4 diplokarya (Fig. 20). They measured from 4.5 ± 0.5 μm × 2.2 ± 0.3 μm to 2.6 ± 0.5 μm × 1.1 ± 0.13 μm (n = 10). Of the sporogonic stages only binucleate sporoblasts and spores were seen (Fig. 21). Spores measured 6.6 ± 0.3 μm × 2.9 ± 0.3 μm (n = 10) and were slightly curved into a characteristic sausage shape.

Transovarial Transmission Trial

Three larval bowls were prepared. The first bowl contained four egg batches laid by *M. africana* females infected with the Type A microsporidium. The second bowl contained five egg batches, two from *M. africana* females infected with Type A and three from *M. africana* infected with Type B microsporidia. The third bowl contained progeny from three uninfected female *M. africana* and served as the control. None of the 80 control larvae were infected. In the second bowl only 4 larvae survived, none of which showed any signs of infection. However, of the 62 larvae in the first bowl, 7 had multisporous infections (11.3%) indistinguishable from the multisporous infection found in field collected larvae.



FIGS. 18–21. Photomicrographs of Type A (*Merocinta davidii*) and Type B (unidentified microsporidium) developmental sequences in *Mansonia africana* adults. Figs. 18 and 19: Giemsa-stained smears of Type A. Figs. 20 and 21: Giemsa-stained smears of Type B. Scale bar = 10 μm.

FIG. 18. Rounded diplokaryotic meront.

FIG. 19. Binucleate spore.

FIG. 20. Merogonic plasmodium with two pairs of diplokaryotic nuclei.

FIG. 21. Binucleate spore.

DISCUSSION

Apart from two species in the collective genus *Microsporidium,* microsporidia which undergo multisporous sporogony are included within the *Pleistophora* complex. In this complex there are 10 genera, *Pleistophora* Gurley 1893, *Glugea* Thelohan 1891, *Pseudopleistophora* Sprague 1977, *Vavraia* Weiser 1977, *Baculea* Loubès and Akbarieh 1978, *Polydispyrenia* Canning and Hazard 1982, *Cystosporogenes* Canning *et al.* 1985, *Ovavesicula* Andreadis and Hanula 1987, *Endoreticulatus* Brooks *et al.* 1988, and *Flabelliforma* Canning *et al.* 1991.

In the species from *M. africana,* meronts were diplokaryotic, often forming multinucleate plasmodia. At the surface was a plasma membrane which was thickened during development by an electron-dense coat. Host ribosomes could be seen close to the parasite's surface and the larger meronts were surrounded by two additional membranes; the outer abundantly studded and the inner sparsely studded with ribosomes. This membrane, which we have interpreted as a cisterna of host endoplasmic reticulum, divided with the meronts. A similar arrangement at the surface of meronts occurs in *Glugea anomala* (Canning *et al.,* 1982). However, in this species, the endoplasmic reticulum and the electron-dense coat on the meront plasma membrane disperse at the onset of sporogony and the developing sporonts secrete a sporophorous vesicle by pushing out a new series of blisters from the surface of the sporogonial plasmodium. In the species in *M. africana* the outer layer of the endoplasmic reticulum cisterna persisted to form the vesicle (not a sporophorous vesicle) in which the sporoblasts and spores were formed. Further, *Glugea* has isolated nuclei throughout development and stimulates the host to form a xenoma.

The meronts of *Polydispyrenia* are diplokaryotic, finally producing uninucleate spores but the sporophorous vesicle in the meronts is fine, delicate, and of parasite rather than host origin (Canning and Hazard, 1982). *Pleistophora* and *Vavraia* have a thick amorphous layer around meronts and sporonts, quite unlike

the surface coat in the *M. africana* parasite (Canning and Nicholas 1980; Weiser, 1977), and together with *Cystosporogenes* and *Flabelliforma* (Canning *et al.*, 1985, 1991) they are uninucleate throughout development. *Pseudopleistophora,* on the other hand, has diplokaryotic nuclei in all stages (Sprague, 1977). The sporophorous vesicle in *Ovavesicula* is of parasite origin, is extremely thick and electron dense, has a constant shape, and always contained 32 spores (Andreadis and Hanula, 1987). The spores of *Baculea* are uniquely elongate (Loubès and Akbarieh, 1978). The parasite of *M. africana* cannot therefore be attributed to either *Ovavesicula* or *Baculea.* The sporophorous vesicle of *Endoreticulatus* is formed from host endoplasmic reticulum and the meronts form ribbon-like strings during division just as in the species in *M. africana* but all developmental stages of *Endoreticulatus* are uninucleate (Brooks *et al.*, 1988).

In addition, there are three enigmatic species within the *Pleistophora* complex that have not yet been assigned to a genus. None of these showed great similarities to the parasite of *M. africana.* The first was from *Aedes sierrensis* (Sanders and Poinar, 1976). Early sporogonic stages retained the diplokaryotic nuclear arrangement as in the species from *M. africana* but there were no ribbon-like forms and the sporophorous vesicle was of parasite not host origin. The second, from *Pristophora erichsonii* (Percy *et al.*, 1982) had diplokaryotic meronts and uninucleate sporonts and the sporophorous vesicle was of host origin but meronts were never ribbon-like and never had a thickened plasma membrane. The third, from *Spodoptera depravata* (Iwano and Ishihara, 1984), was uninucleate throughout its development.

Finally there are also two species in the collective genus *Microsporidium* with multisporous sporogony. These are *M. itiiti* from the Argentine stem weevil *Listronotus bonariensis* (Malone, 1985) and *M. novacastriensis* (Jones and Selman, 1985) from the slug *Deroceras reticulatum.* These have ribbon-like stages, but these stages are sporogonic not merogonic. Spores form within a membrane (formed initially around meronts) derived from host endoplasmic reticulum, as in the parasite from *M. africana;* however, stages are uninucleate throughout development.

For the reasons discussed the multisporous species from *M. africana,* although it has some characteristics in common with *Endoreticulatus, Polydispyrenia,* and the collective genus *Microsporidium,* cannot be assigned to an existing genus.

The transovarial transmission trial showed that the parasite from *M. africana* is dimorphic, because the small binucleate spores (Type A) detected in the ovaries of field collected adults are part of the life cycle of the microsporidium that forms multisporous sporophorous vesicles with a different spore type in larvae. This is important taxonomically, as it suggests links between this parasite and *Polydispyrenia,* the only other genus of the *Pleistophora* complex for which there is some evidence of dimorphism (Canning and Hazard, 1982).

It is also possible that like *Polydispyrenia simulii* the parasite from *M. africana* undergoes a sexual cycle with meiosis. Meiosis can be recognized by the presence of synaptonemal complexes in the two nuclei of the diplokarya, as they separate in early sporonts (Loubès, 1979). Such complexes were not seen in the multisporous material from *M. africana,* probably because the material had been unavoidably kept in buffer solution for several weeks after fixation, before final processing for electron microscopy. However, the separation of the two nuclei of the diplokaryon and subsequent development, producing uninucleate spores, corresponded to the sequence in *P. simulii,* for which meiosis has been demonstrated (Canning and Hazard, 1982).

Due to its unique morphology and dimorphic life cycle (multisporous sporogony in larvae and production of free binucleate spores in adults) this parasite from *M. africana* is ascribed to a new genus for which the name *Merocinta* is suggested. The generic name is *Mero,* from merogony, and *cinta,* from the Spanish for ribbon, and refers to the ribbon-like merogonic stages. The species name is *davidii* after the senior author's late father Mr. David Pell.

In addition to the Type A diplokaryotic spores in adults there were other larger spores of Type B and there remains the possibility that these larger spores represent part of the life cycle of *Tricornia muhezae* which was also found in *Mansonia africana* larvae (Pell and Canning, 1992). The close morphological similarity of *T. muhezae* to the dimorphic genera *Amblyospora* and *Parathelohania* supports this hypothesis.

Taxonomic Summary[2]

Merocinta n.g.

Larval infection. Meronts were diplokaryotic with a thickened unit membrane. Repeated division of the diplokarya produced multinucleate plasmodia, the plasma membranes of which were entirely surrounded by two other membranes formed from host endoplasmic reticulum. Meronts became ribbon-like before division, the host endoplasmic reticulum dividing with the parasite. During early sporogony the nuclei of the diplokaryon separated and the electron-dense coating on the plasma membrane was sloughed off. Nuclear and cytoplasmic division gave rise to uninucleate sporoblasts within a vesicle of host endoplasmic reticulum. Electron-dense material was again secreted

[2] Type material is deposited at the Natural History Museum, Zoology Department, Cromwell Rd., London SW7 5BD, UK, under Accession No. 1991:11:7:1.

around each sporoblast (the future exospore) which ultimately matured into oval spores.

Adult infection. Meronts and sporonts were diplokaryotic ultimately producing binucleate spores. These spores were transmitted transovarially to progeny.

Merocinta davidii n.sp. (type species)

Uninucleate spores in larvae measured 2.5 × 1.5 μm and binucleate spores in the adult measured 3.4 × 1.8 μm. Spores from larvae had a single coil to the polar filament.

Type host: Mansonia africana. Infection was concentrated around the midgut in larvae and ovaries in adults.

Type locality. Locality was NE Muheza and its environs, Tanzania.

ACKNOWLEDGMENTS

Financial support for this study was provided by the Natural Environment Research Council. We thank Mr. J-P. Nicholas and Mr. R. Hartley for technical assistance with electron microscopy and photography and Dr. N. Wilding for useful comments on the manuscript. We are grateful to the Medical Research Council of Tanzania for permission to collect material, and thank Dr. C. F. Curtis, Dr. J. D. Lines, Mr. T. J. Wilkes, and all the staff at the Ubwari Field Station, Muheza, Tanzania, particularly Mr. C. Chambika, for help.

REFERENCES

Andreadis, T. G., and Hanula, J. L. 1987. Ultrastructural study and description of *Ovavesicula popilliae* ng. n.sp., (Microsporida: Pleistophoridae) from the Japanese beetle, *Popillia japonica* (Coleoptera: Scarabaeidae) *J. Protozool.* 34, 15–21.

Brooks, W. M., Becnel, J. J., and Kennedy, G. G. 1988. Establishment of *Endoreticulatus* N.G. for *Pleistophora fidelis* (Hostounsky and Weiser, 1975) (Microsporida: Pleistophoridae) based on the ultrastructure of a microsporidium in the Colorado Potato Beetle, *Leptinotarsa decemlineata* (Say) (Coleoptera: Chrysomelidae). *J. Protozool.* 35, 481–488.

Canning, E. U., Barker, R., Nicholas, J. P., and Page, A. M. 1985. The ultrastructure of three microsporidia from winter moth, *Operophtera brumata* (L.), and the establishment of a new genus *Cystosporogenes* Canning, Lom and Nicholas, n.g. for *Pleistophora operopterae* (Canning, 1960). *Systematic Parasitol.* 7, 213–225.

Canning, E. U., and Hazard, E. I. 1982. Genus *Pleistophora* Gurley, 1893: An assemblage of at least three genera. *J. Protozool.* 29, 39–49.

Canning, E. U., Killick-Kendrick, R., and Killick-Kendrick, M. 1991. A new microsporidian parasite, *Flabelliforma montana* n.g., n.sp., infecting *Phlebotomus ariasi* (Diptera, Psychodidae) in France. *J. Invertebr. Pathol.* 57, 71–81.

Canning, E. U., Lom, J., and Nicholas, J. P. 1982. Genus *Glugea* Thelohan 1891 (Phylum Microspora): Redescription of the type sp. *Glugea anomala* (Moniez 1887) and recognition of its sporogonic development within sporophorous vesicles (pansporoblastic membrane). *Protistologica* 18, 193–210.

Canning, E. U., and Nicholas, J. P. 1980. Genus *Pleistophora* (Phylum Microspora): Redescription of the type species *Pleistophora typicalis* Gurley, 1893 and ultrastructural characterisation of the genus. *J. Fish. Diseases* 3, 317–338.

Iwano, H., and Ishihara, R. 1984. A Microsporidian, *Pleistophora* sp. isolated from the lawn grass cutworm *Spodoptera depravata* Butler (Lepidoptera: Noctuidae). *Bull. Coll. Agr. Vet. Med., Nihon University* 41, 25–33.

Jones, A. A., and Selman, B. J. 1985. *Microsporidium novacastriensis* n.sp., a microsporidian parasite of the grey field slug *Deroceras reticulatum*. *J. Protozool.* 32, 581–586.

Laurence, B. R. 1960. The biology of 2 species of mosquito, *Mansonia africana* (Theobald) and *Mansonia uniformis* (Theobald) belonging to the subgenus Mansonioides (Diptera: Culicidae). *Bull. Entomol. Res.* 51, 491–517.

Laurence, B. R., Page, R., and Smith, S. A. 1962. Laboratory colonisation of *Mansonia* mosquitoes. *Bull. Entomol. Res.* 53, 515–519.

Loubès, C. 1979. Recherches sur la méiose chez les microsporidies: Conséquences sur les cycles biologiques. *J. Protozool.* 26, 200–208.

Loubès, C., and Akbarieh, M. 1978. Etude ultrastructurale de la microsporidie *Baculea daphniae* n.g. n.sp. Parasite de l'epithelium intestinal de *Daphnia pulex* Leydig 1860 (Crustace: Caldocere). *Protistologica* 14, 23–38.

Malone, L. A. 1985. A new pathogen *Microsporidium itiiti* from the Argentine stem weevil. *J. Protozool.* 32, 535–541.

Pell, J. K., and Canning, E. U. 1992. Ultrastructure of *Tricornia muhezae* gen. et.sp. nov. (*Microspora*, Thelohaniidae) a parasite of *Mansonia africana* (Diptera: Culicidae) from Tanzania. *J. Protozool.* 39, 242–247.

Percy, J., Wilson, G., and Burke, J. 1982. Development and ultrastructure of a microsporidian parasite in midgut cells of the larch sawfly *Pristiphora erichsonii* (Hymenoptera: Tenthredinidae). *J. Invertebr. Pathol.* 39, 49–59.

Sanders, R. D., and Poinar, G. O. 1976. Development and fine structure of *Pleistophora* sp. (Cnidospora: Microsporida) in the mosquito *Aedes sierrensis*. *J. Invertebr. Pathol.* 28, 109–119.

Service, M. W. 1976. "Mosquito Ecology: Field Sampling Methods." Applied Science Publishers Ltd, London.

Sprague, V. 1977. Systematics of the microsporidia. *In* "Comparative Pathobiology" (L. A. Bulla, and T. C. Cheng, Eds.), Vol 2. Plenum, New York/London.

Weiser, J. 1977. Contribution to the classification of microsporidia. *Vest. Cesk. Spol. Zool.* 61, 308–320.