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Light Microscope and Ultrastructural Observations of a Microsporidian Parasite of *Mesocyclops rarus* (Copepoda: Cyclopoida) in Tanzania

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A dimorphic microsporidian infection was found in *Mesocyclops rarus* copepods collected at three sites near Muheza in NE Tanzania. The mean prevalence of infection varied between 0 and 24.3% at the three sites. Both spore types were pyriform and varied in size but not ultrastructure. The smaller ones measured 8.9 × 3.9 μm and the larger 15.0 × 6.3 μm. They were always found together in the same host individual and therefore assumed to be the same species. Ultrastructurally both types showed marked similarities to those of the copepod phase of *Amblyospora* spp. which cause polymorphic infections in copepods and mosquitoes. Although it was not demonstrated in transmission experiments, the infection in *M. rarus* is possibly conspecific with one of two dimorphic infections in *Mansonia africana* mosquitoes (either *Tricornia muhezae* or *Merocinta davidii*) recently described from the same sites.

KEY WORDS: Microsporidium; copepod; ultrastructure.

INTRODUCTION

Dimorphism in the genera *Amblyosopora* and *Parathelohania* (Family: *Thelohaniidae*) has long been known to involve two different developmental sequences, the first resulting in free binucleate spores, which germinate and inoculate their sporoplasms into developing eggs of the host. The larvae that hatch usually develop fatal infections with a second spore type (octospores) by the fourth instar. These spores do not infect healthy larvae (Hazard and Oldacre, 1976).

It was not until the work of Sweeney *et al.* (1985) and Andreadis (1985) that the remainder of the life cycle of at least two *Amblyospora* species from mosquitoes was elucidated. They discovered an additional spore type which developed in an intermediate copepod host. Octospores were found to be infective to copepods where a third developmental sequence occurred, producing the third spore type and resulting in infections. The unikaryotic spores produced in the copepod were directly infective *per os* to mosquito larvae. These larvae developed benign diplokaryotic infections and emerged as adults in which free binucleate spores formed, fatally infecting the progeny.

At three sites in NE Tanzania (5° 11′, 38° 5′ E), Kisiwani Pond, Muheza Sisal Estate Swamp and Mamakingi Pond two species of dimorphic microsporidia were found in *Mansonia africana* mosquitoes. These were *Tricornia muhezae* (Family: *Thelohaniidae*) (Pell and Canning, 1992) which is closely related to *Amblyospora* spp. and *Parathelohania* spp. and *Merocinta davidii* new gen. new sp. (Pell and Canning, 1993). This second species was shown to have free binucleate spores in adults, giving rise to multisporous infections in progeny. In an attempt to discover intermediate hosts of these organisms, therefore, copepods and other invertebrates were collected from the three sites and examined for microsporidia. A dimorphic microsporidian was discovered in the copepod *Mesocyclops rarus*. This article describes its morphology and discusses its possible links with *T. muhezae* and *M. davidii*.

MATERIALS AND METHODS

Copepods were collected with a fine mesh pond net from the three sites. The mesh of the net was fine enough to enable both adult copepods and juvenile stages (copepodids) of all species to be collected. A universal tube was attached to the net and all organisms not passing through the mesh accumulated in it, in a small volume of water. Other organisms trapped in the net were removed and retained separately to prevent predation on the copepods. Water was sampled from light and dark, open and edge, deep and shallow areas, to ensure that representative numbers of all species were collected. All collecting pots were kept shaded during collection before being transported to the laboratory. The net and pots were cleaned with boiling water and alcohol after each collection to prevent transfer of infections between sites.

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TABLE 1

Mesocyclops rarus Infection at Muheza Sisal Estate Swamp and Mamakingi Pond

Date	Site	No. collected	No. infected with developmental stages and spores (%)	No. infected with developmental stages only (%)
25.7.87	Muheza Sisal Estate	35	8 (22.0)	1 (2.8)
20.9.87	Muheza Sisal Estate	72	14 (19.4)	3 (4.2)
27.7.87	Mamakingi Pond	37	3 (8.1)	1 (2.7)
8.9.87	Mamakingi Pond	59	8 (13.6)	1 (1.7)

For light microscopy adult copepods of both sexes (females were distinguished by the presence of egg sacs) were smeared, and the smears air dried, fixed in methanol, stained for 30 min in 10% Giemsa's stain (Gurr's improved R66) at pH 7.2, and examined for microsporidian infection. A proportion of copepods was preserved in 70% alcohol for later identification and some were fixed for electron microscopy. Care was taken, before fixation for electron microscopy, to rupture the body of each copepod to enhance the penetration by fixative. Copepods were transferred first to Karnovsky's fixative for 10 min at ambient temperature and then into fresh fixative for an hour at 4°C. After washing twice in 0.12 M sodium cacodylate buffer, the material was not processed further until return to the U.K. 1–3 months later. It was then postfixed in 2.5% 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.

RESULTS

Mesocyclops rarus was the commonest copepod species, and the only one occurring in abundance at all three sites. Occasional *Ectocyclops hirsutus, Afrocyclops gibsoni,* and *Cryptocyclops linjanti* were found but only *M. rarus* was infected with microsporidia and only at Mamakingi Pond and Muheza Sisal Estate Swamp (Table 1). Although copepods (126 *M. rarus*) were collected at Kisiwani Pond on three occasions, none were infected. Infections occurred in adult females which were recognized by their paired egg sacs, and in those individuals of similar size or slightly smaller than females, but without egg sacs. The latter were thought to be males of the same species or females temporarily without egg sacs. Other common invertebrates including species of Notonectidae in these ponds were not infected with microsporidia.

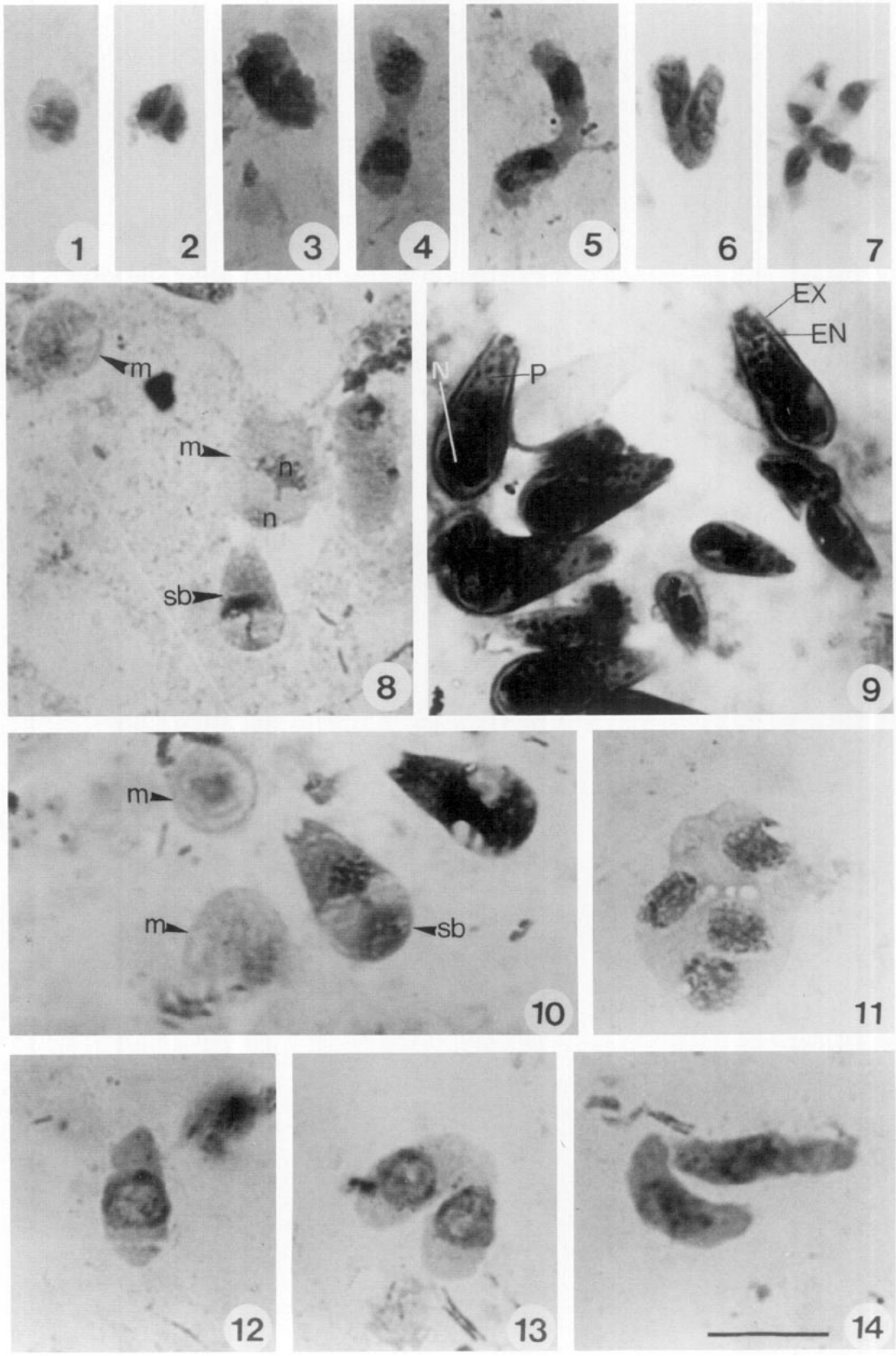
Morphology of Infection

Light microscopic observations. What appeared to be two separate developmental sequences were detected. These sequences were similar, differing only in the size of the parasite stages and resultant spores, and were always found together entirely packing the body cavity of the copepod. Macroscopically, copepods did not appear infected.

In one sequence the earliest stages seen were small rounded uninucleate and binucleate meronts (Figs. 1 and 2). Early sporogonic stages were also uninucleate and binucleate but more oval in shape (Fig. 3). After division of the nucleus, the two resulting nuclei moved toward the poles of the cytoplasm (Fig. 4) before separating into two sporoblasts (Figs. 5 and 6). Tetranucleate sporonts were also present; these became stellate during division (Fig. 7). The sporoblasts became pyriform (Fig. 8) and matured into small pyriform spores measuring $8.9 \pm 0.5 \times 3.9 \pm 0.3$ μm ($n = 9$) (Fig. 9).

In the other sequences the earliest stages were also

FIGS. 1–14. Photomicrographs of a microsporidian parasite in *Mesocyclops rarus.* All are Giemsa-stained smears. Scale Bar = 10 μm.
FIG. 1. Small uninucleate meront.
FIG. 2. Small binucleate meront.
FIG. 3. Early uninucleate sporont of the small staged developmental sequence.
FIG. 4. Small binucleate sporont. The nuclei have migrated to the poles.
FIG. 5. Small sporont. A U-shape forms before division.
FIG. 6. Early sporoblasts of the small developmental sequence.
FIG. 7. Tetranucleate stellate sporont of the small developmental sequence.
FIG. 8. Late pyriform sporoblast (sb) of the small developmental sequence and uninucleate and binucleate meronts (m) of the larger developmental sequence. n, nucleus.
FIG. 9. Pyriform spores. The spores are of two sizes belonging to both developmental sequences, but, within each exospore (EX), endospore (EN), polaroplast (P), and nucleus (N) are clearly visible.
FIG. 10. Large uninucleate meronts (m) and large mature pyriform sporoblast (sb).
FIG. 11. Large tetranucleate meront.
FIG. 12. Large uninucleate sporont.
FIG. 13. Large binucleate sporont. A U-shape forms before division.
FIG. 14. Early crescentic sporoblasts of the larger developmental sequence.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rounded uninucleate and binucleate meronts, although rather larger (Figs. 8 and 10). Occasional tetranucleate meronts were also seen (Fig. 11). As with the first sequence uninucleate sporonts (Fig. 12) became binucleate and aligned themselves into a U-shape (Fig. 13). Division produced crescentic sporoblasts (Fig. 14) which became pyriform (Fig. 10) and matured into large pyriform spores measuring 15.0 ± 0.3 × 6.3 ± 0.1 μm (n = 11) (Fig. 9). Tetranucleate stellate forms were never seen. Even at the light microscope level, the polaroplast, nucleus, endospore, and exospore of the spore wall were clearly visible in both large and small spores.

Ultrastructural observations. As in the light microscope study, the earliest stages seen were meronts which were rounded cells limited by a fine unit membrane and surrounded by host mitochondria (Fig. 15). Sporogonic stages were more elongate than merogonic stages, and electron dense material was laid down patchily on the plasma membrane. At this stage large blisters were formed by the sporophorous vesicle membrane as it separated from the parasite surface. Within sporonts membranous whorls were commonly found, possibly formed from endoplasmic reticulum during polaroplast development (Fig. 16). Within the copepod there were distinct regions in which different developmental stages were found. From the cuticle inwards there was first striated muscle which was uninfected and allowed the host to remain mobile. Within this, developmental stages occurred, which were both merogonic and sporogonic. The spores were located toward the center of the copepod (Fig. 17).

Sporoblasts, the stage before mature spores, were not seen. Pyriform spores of two sizes lay singly within large, empty sporophorous vesicles (Fig. 17). The small spores measured 5.6 ± 0.05 × 1.9 ± 0.1 μm (n = 3). Due to the great size of the larger spore, there were no entire cross sections. Ultrastructurally there was little difference between the two types of spores. The polar filament was isofilar and less than 20 coils were observed (Fig. 18).

The straight part of the polar filament ran through the polaroplast blocks to terminate beneath the polar cap. In cross section five layers could be distinguished in each polar filament. At the center was an electron lucent core surrounded by a layer of moderate electron density, then a lucent ring, another moderately dense ring, and an outer sleeve of two distinct membranes (Fig. 19). The polaroplast consisted of a series of blocks separated by membranes, the blocks becoming less electron dense toward the posteriad. All spores had a prominent posterior vacuole, the contents of which were disrupted during fixation (Fig. 18).

DISCUSSION

The dimorphic microsporidian infection of *M. rarus* produced spores which differed in size but not in ultrastructure. From developmental stages seen using light and electron microscopy, it was assumed that the two sequences culminating in two spore types were distinct and that small sporogonic stages developed from small merogonic stages and large sporogonic stages from large merogonic stages. However, as the size of meronts within a single species can be highly variable (due to fixation) it is possible and perhaps more probable that only one merogonic developmental sequence occurs with stages of variable size until the onset of sporogony when development follows two paths culminating in the production of spores of two sizes. However, no stages possessed diplokarya and each spore was uninucleate within a vesicle. As all developmental stages and both spore types were always found together in a single host, they were presumed to belong to the same species.

Using the annotated list of Sprague (1977) of microsporidia reported from the Copepoda, none corresponded with the species in *M. rarus*. Species of *Pyrotheca, Gurleya, Pleistophora,* and *Thelohania* which have been reported from copepods produce more than one spore per sporophorous vesicle (Maurand *et al.*, 1972; Leblanc, 1930; Moniez, 1887; Lemmerman, 1990; Cépède, 1911; Weiser, 1945; cited in Sprague, 1977). *Tuzetia* spp. also reported from copepods (Maurand *et al.*, 1972; Kudo, 1921; cited in Sprague, 1977) produce single spores in sporophorous vesicles but form plasmodia with many nuclei. Also the vesicles have electron dense trilaminate membranes and divide with the sporont (Larsson, 1983). *Mrazekia cyclopis* (Vávra, 1962; cited in Sprague, 1977) produces binucleate spores and the *Duboscqia* sp. described from several copepod species (Lom and Vávra, 1963; cited in Sprague, 1977) have spores covered in mucus layers. *Courgourdella magna* and *Courgourdella pusilla* from copepods (Hesse, 1935; cited in Sprague, 1977) both

FIGS. 15–19. Transmission electron micrographs of a microsporidian parasite in *Mesocyclops rarus*. Scale bars = 1 μm.

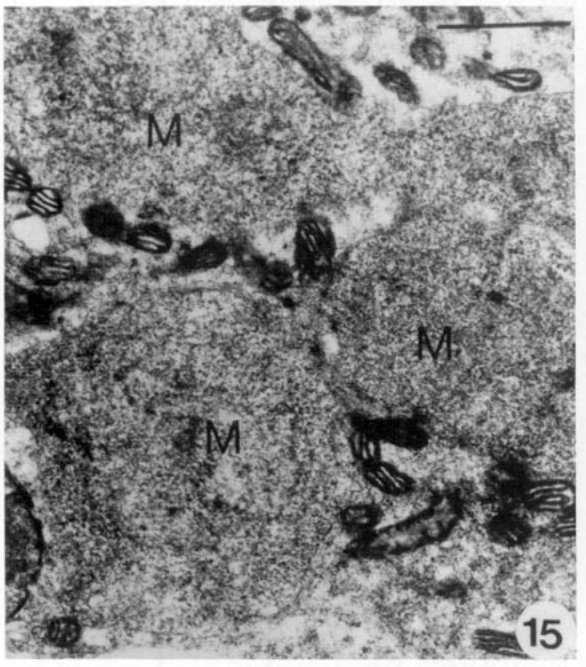
FIG. 15. Uninucleate meronts (M). Rounded cells limited by single-unit membranes and surrounded by host mitochondria.

FIG. 16. Sporont. Electron dense material is laid down patchily on the plasma membrane (indicated by double arrows) and large blisters are formed by the sporophorous vesicle as it lifts away from the parasite surface (indicated by a single arrow). Membranous whorls form.

FIG. 17. Relative position of the developmental stages of the parasite within the copepod. Spores are found centrally within the copepod, surrounded by earlier developmental stages and finally host muscle.

FIG. 18. Mature spore of the small type. The polar filament is isofilar with a maximum of 20 coils. The polaroplast consists of a series of blocks becoming less electron dense toward the posteriad.

FIG. 19. Inset. Coils of the polar filament. The filament has five layers in cross section.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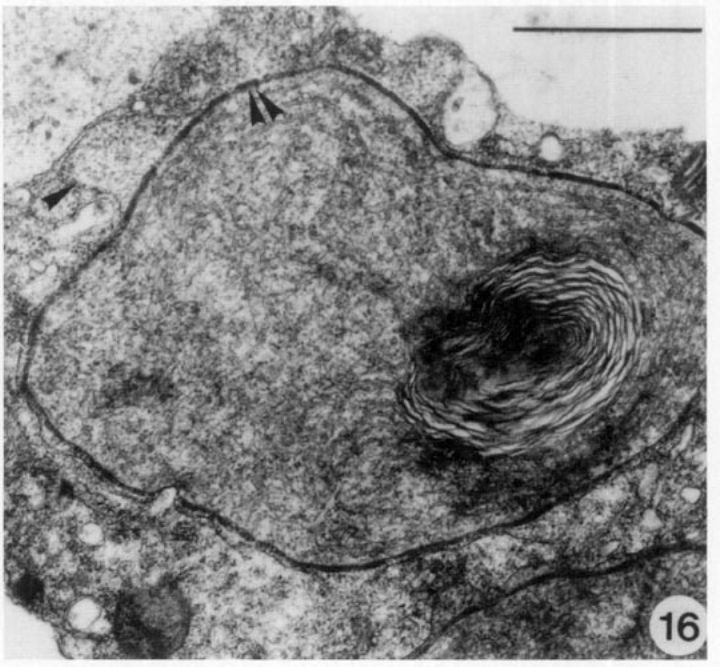
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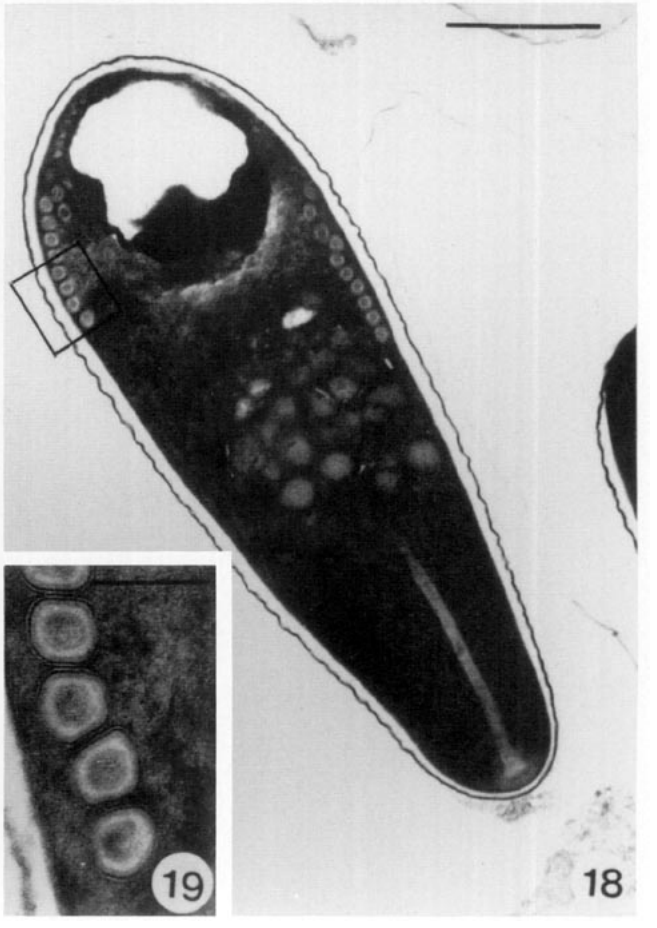
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have paired sporoblasts and lageniform spores of a different structure to the spores in *M. rarus*.

The only other species described from copepods are *Amblyospora dyxenoides* and *Amblyospora connecticus* in *Mesocyclops albicans* and *Acanthocyclops vernalis*, respectively (Andreadis, 1985, 1988; Sweeney *et al.*, 1985, 1988), and they bear most resemblance to the species from *M. rarus* in structure and development. In *A. dyxenoides* the sporonts divide after polarization of the nuclei and formation of the sporont into a U-shape. Stellate forms also occurred, although more rarely. Spores were formed singly in sporophorous vesicles, and the polaroplast had the same series of blocks separated by membranes, the blocks becoming less electron dense toward the posteriad (visible by light and electron microscopy). These features were also seen in the species from *M. rarus* but two spore types were not described for *A. dyxenoides* or *A. connecticus*. However, in *Amblyospora cambelli* from *Culiseta incidens* (Dickson, 1988), two different spore types were seen in the copepod alternate host, *Diaptomus* sp. One spore type was pyriform, very like those previously described for the copepod stages of *Amblyospora* spp. and the other type was derived from an octosporous sporogony. The pyriform spores were responsible for transmission to mosquito larvae and the octospores for transmission to other copepods.

As the species *T. muhezae* (possibly dimorphic and related to *Amblyospora* spp.) and *M. davidii* (a dimorphic species) were found in the mosquito *M. africana* in the same sites, it is possible that the infection in *M. rarus* is part of the complex polymorphic life cycles of one of these species (Pell and Canning, 1992; Pell and Canning, 1993). Transmission experiments are required to prove this hypothesis.

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