

## *Amblyospora connecticus* sp. nov. (Microsporida: Amblyosporidae): Horizontal Transmission Studies in the Mosquito *Aedes cantator* and Formal Description

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The morphology, development, and ultrastructure of an *Amblyospora* (Microsporida) species parasitic in the salt marsh mosquito, *Aedes cantator*, were investigated following horizontal transmission of infection from the intermediate copepod host, *Acanthocyclops vernalis*. Infections were readily transmitted to *A. cantator* in the laboratory when larvae were allowed to feed on spores harvested from *A. vernalis*. The microsporidium was observed to initially invade the midgut and gastric caeca of host larvae and then spread to the muscles, oenocytes, and Malpighian tubules. It divides by binary fission within these host tissues and produces uninucleated gametes that undergo plasmogamy to form diplokaryotic stages in adult hosts. Diplokaryotic sporonts sporulate in adult female hosts only when a blood meal is taken and this results in the formation of binucleated spores that are responsible for transovarial transmission to the next host mosquito generation. Quantitative studies show that male mosquito hosts are more susceptible and develop more progressive infections than females. However, the microsporidium never sporulates in males and the fate and function of these infections in male hosts remain unknown. A new species, *Amblyospora connecticus*, is proposed for this isolate and a formal taxonomic description is given based on its entire life cycle in both hosts. © 1988 Academic Press, Inc.

**KEY WORDS:** *Amblyospora connecticus* sp. nov.; Microsporida; *Aedes cantator*; mosquito; *Acanthocyclops vernalis*; copepod; horizontal transmission; life cycle; ultrastructure; description.

### INTRODUCTION

The genus *Amblyospora* (Microsporida: Amblyosporidae) is composed of a large number of closely related species that infect a variety of aquatic arthropods. They are particularly common to mosquitoes and have been isolated from nearly 70 different host species in eight genera (Hazard and Oldacre, 1975; Hazard and Chapman, 1977; Castillo, 1980).

All species of *Amblyospora* are polymorphic, have two sporulation sequences in the primary mosquito host, and are transovarially transmitted via ovarian infection of the adult female (Hazard and Oldacre, 1975; Andreadis and Hall, 1979). Recent studies (Andreadis, 1985a; 1986; Sweeney et al., 1985, 1988; Becnel, 1986) have established that in at least three, and undoubtedly other, mosquito hosts, these microsporidian parasites can also be transmitted horizontally (per os) following obligatory devel-

opment in an intermediate copepod host. The discovery of this "missing link" in the life cycle of *Amblyospora* has now made it possible to fully categorize a particular isolate and provide a formal taxonomic description based on its entire life cycle.

In this paper, I present previously unknown information on the morphology and development of an *Amblyospora* species parasitic in the mosquito, *Aedes cantator*, following horizontal transmission of infection from the intermediate copepod host, *Acanthocyclops vernalis*. A formal taxonomic description is also given based on the complete life cycle in both hosts and a new species, *Amblyospora connecticus*, is proposed. Parasite development in the copepod host has been described in a previous report (Andreadis, 1985a), as has each developmental cycle resulting from transovarial transmission in the primary mosquito host (Andreadis, 1983a).

## MATERIALS AND METHODS

**Infection of copepods.** The *A. vernalis* copepods used in all transmission studies were initially collected from two coastal salt marsh pools in Guilford, Connecticut, from October 1985 through January 1986. Copepods were infected by placing 50 to 100 adult females in 100 × 80-mm culture dishes to which 250 ml of a balanced salt solution (Trager, 1935) and five to ten moribund or dead 4th-instar, field-collected larvae of *A. cantator* that were heavily infected with meiospores had been added. Copepods were maintained in these dishes at room temperature (20°–22°C) under a natural photoperiod, and within 2 to 3 weeks they were infected with large numbers of mature spores. This procedure would routinely result in the infection of 60–70% of the copepods and these spores were used to infect mosquito larvae.

**Infection of mosquitoes.** Horizontal transmission of the parasite from *A. vernalis* to *A. cantator* was achieved by allowing *A. cantator* larvae to feed on spores obtained from *A. vernalis* as described above. Since *A. cantator* is not readily colonized (i.e., will not free mate in the laboratory), all larvae used in these tests were hatched from eggs obtained from wild-caught females collected during the summer (June–August) of 1985 from a coastal salt marsh in Milford, Connecticut. The procedures for collecting adults, obtaining individual oviposition in the laboratory, and conditioning and hatching of eggs have been described previously (Andreadis, 1983a, 1985b).

To infect *A. cantator* larvae, twenty-five 2-day-old 2nd instars were initially placed in 60 × 15-mm Petri dishes containing 20 ml of Trager's salt solution. Three macerated *A. vernalis*, heavily infected with mature spores, were added to each dish along with a small quantity of an aqueous suspension of dried liver powder and brewer's yeast (3:2 mixture). Larvae were maintained in these dishes for 2 days at 22°C under a 16:8 LD photoperiod and were then collectively

transferred to 100 × 80-mm culture dishes for further rearing to adulthood or until they were to be examined for infection. There was a control for each group of exposed individuals consisting of sibling larvae hatched from the same egg batch. These larvae were reared identically except that they were not exposed to infected copepods.

Characterization of pathogen development in the mosquito host was made from Giemsa-stained smears, histological section, and ultrastructural examination of larvae and adults of both sexes at various time intervals following exposure to spores. In the first trial, larvae were sacrificed every 2 days, smeared on slides, stained with Giemsa, and examined microscopically. Other individuals in this initial feeding trial were allowed to develop to adulthood. They were individually isolated in small cages and supplied with a 10% (w/v) sucrose solution before being examined in a similar manner. A few females were additionally allowed to blood feed on restrained guinea pigs and were then examined 2–3 days later for pathogen sporulation. The prevalence of infection and various developmental stages observed in each stage and sex of the host mosquito were recorded, and every exposed and control individual was examined for infection.

In the second trial, emphasis was placed on determining the site(s) of infection within each developmental stage of the mosquito host. This was accomplished through histological examination of paraffin-embedded 3rd- and 4th-instar larvae and adults of both sexes that had been exposed to spores from copepods as in the previous trial. The various tissues infected in each individual were quantified and the sex of the infected host larva was recorded so that statistical comparisons could be made with the prevalence of infection in those individuals that were allowed to develop to adulthood. As before, every exposed and control individual was examined either by histological section or by Giemsa-stained

smear and the prevalence of infection was quantified for each sex. Paraffin sections were cut at 6  $\mu\text{m}$  and stained with iron hematoxylin and eosin Y.

**Ultrastructure.** Pathogen development was also characterized ultrastructurally by the examination of infected tissues in both larvae and adults. Individuals used for these studies were fixed overnight at 4°C in 2.5% (v/v) glutaraldehyde/2% (w/v) paraformaldehyde that contained 0.1% (w/v)  $\text{CaCl}_2$ , and 1% (w/v) sucrose and was buffered with 0.1 M sodium cacodylate, pH 7.4. Specimens were post-fixed for 2 hr at room temperature in 1% (w/v)  $\text{OsO}_4$ , stained en bloc with 0.5% (w/v) uranyl acetate in 70% (v/v) ethanol, dehydrated through an ethanol and acetone series, and embedded in an LX-112/Araldite mixture. Sections were post-stained with 5% (w/v) methanolic uranyl acetate, followed by Reynold's lead citrate, and examined in a Zeiss EM 10C electron microscope at an accelerating voltage of 80 kV.

## RESULTS

### Quantitative Studies

*A. connecticus* was readily transmitted to *A. cantator* in the laboratory when larvae were allowed to feed on spores harvested from the intermediate copepod host, *A. vernalis*. In the first trial (Table 1), a total of

51% ( $n = 155$ ) of the exposed individuals developed benign infections that could be detected in Giemsa-stained smears. Examinations of exposed *A. cantator* that were allowed to develop to adulthood also revealed a significantly ( $p > 0.01$ ) higher prevalence rate of infection among male as compared to female hosts.

A similar trend in the comparative susceptibility of each host sex to infection was observed in the second feeding trial as well. Although higher prevalence rates (overall = 74.5%,  $n = 98$ ) were achieved with both sexes in this test, males once again were found to be more susceptible to infection. Histological examination of 3rd- and 4th-instar larvae further demonstrated that this phenomenon was true in larvae as well as in adults. Infection rates in larvae of both sexes were nearly identical to that seen in individuals that were not examined until they reached adulthood.

There was no differential mortality associated with infection in either feeding trial and no infections of any type were detected in unexposed sibling larvae reared from the same egg batches as exposed larvae.

### Life Cycle

Examinations of Giemsa-stained smears of 2nd-instar larvae of *A. cantator*, 4 days after exposure to spores from *A. vernalis*, revealed the presence of a few small, oval,

TABLE 1

PREVALENCE OF *Amblyospora connecticus* IN LARVAL AND ADULT *Aedes cantator* FOLLOWING EXPOSURE TO SPORES FROM *Acanthocyclops vernalis*<sup>a</sup>

Developmental stage examined	Host sex					
	Undetermined		Male		Female	
	No.	% Infected	No.	% Infected	No.	% Infected
<b>Trial 1</b>						
Larva	25	44.0	—	—	—	—
Adult	—	—	75	72.0a	55	25.4b
<b>Trial 2</b>						
Larva	—	—	32	93.8a	20	40.0b
Adult	—	—	24	95.8a	22	54.5b

<sup>a</sup> Prevalence rates (% infection) within each trial that are followed by a common letter are not significantly different by  $\chi^2$  contingency table analysis ( $p < 0.05$ ).

uninucleated meronts (Fig. 1). These meronts were seen to divide by binary fission (Figs. 2–4) and form additional stages that were also uninucleate but were typically fusiform to pyriform in shape with the nucleus at one pole (Fig. 5). Similar developmental stages were seen in 3rd- and 4th-instar larvae and occasionally some fusiform-shaped meronts were observed in union with one another (Figs. 6–7). These stages, believed to be gametes, were always found in pairs and they appeared to be undergoing plasmogamy to form diplokarya.

Examinations of infected individuals that were allowed to develop to adulthood, usually revealed large numbers of diplokaryotic stages (Fig. 8). It was also not uncommon to find a few uninucleated stages as seen in larvae. Similar developmental stages were observed in both host sexes but infections were typically more pronounced in males. Diplokaryotic meronts appeared to divide repeatedly by multiple fission with synchronous division of each member of the diplokaryon (Fig. 9). No sporulation was ever detected in male hosts that were maintained for up to 14 days. Sporulation was routinely induced in female hosts that were provided with a blood meal, however, and resulted in the formation of large num-

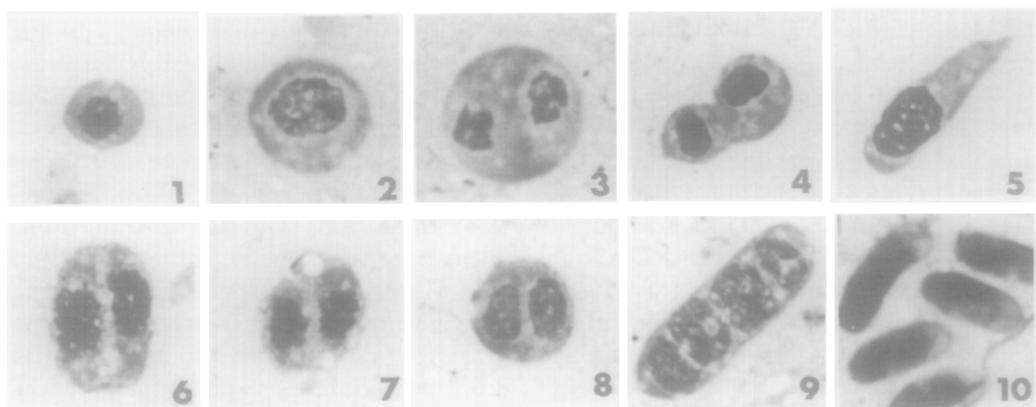
bers of binucleated spores (Fig. 10) previously shown to be responsible for transovarial transmission.

#### Histology

Histological examination of infected larvae of *A. cantator* showed that in both host sexes, the primary sites of infection were the muscles and midgut epithelial cells (Table 2, Figs. 11, 12). Also infected, but to a lesser degree, were the gastric caeca (Fig. 13), oenocytes (Fig. 14), and Malpighian tubules (Fig. 15). Pathogen development in adults was limited to the oenocytes and muscle tissue and no infections were ever detected in any portion of the alimentary tract. Infected oenocytes (Fig. 16) were found scattered throughout thorax and abdomen of the host and were seen as grossly hypertrophied cells, especially in males which were usually more heavily infected.

#### Ultrastructure

Early meronts (Fig. 17) were seen as simple cells with single nuclei enclosed by a simple plasmalemma. Their cytoplasm characteristically contained numerous free ribosomes, cisternae of endoplasmic reticulum, and one or more well-developed zones of Golgi apparatus. Spindle plaques,



Figs. 1–10. Developmental stages of *Amblyospora connecticus* from larval (1–7) and adult (8–10) *Aedes cantator* following exposure to spores from *Acanthocyclops vernalis*. All are Giemsa-stained. (1) Uninucleated meront. (2–4) Dividing meronts. (5) Gamete. (6, 7) Gametes undergoing plasmogamy. (8) Diplokaryotic stage. (9) Dividing diplokaryon. (10) Binucleate spores. ( $\times 1700$ )

TABLE 2

PREVALENCE OF *Amblyospora connecticus* IN VARIOUS TISSUES OF *Aedes cantator* LARVAE FOLLOWING EXPOSURE TO SPORES FROM *Acanthocyclops vernalis*

Host sex	No. examined	Percentage with infected tissue			
		Muscle	Midgut	Gastric caecum	Oenocyte
Male	32	59.4	40.6	34.4	18.8
Female	20	40.0	40.0	20.0	0

spindle microtubules, and polar vesicles were often seen in association with the nuclei of dividing meronts (Figs. 18, 19) but no synaptonemal complexes were ever detected, thus indicating that the divisions were mitotic.

Developmental stages believed to be gametes were also observed in late stage larvae and newly emerged adults. These stages (Fig. 20) were pyriform in shape and had an irregular but uniformly thick plasmalemma, a very dense cytoplasm and a single large nucleus located at one pole. Paired gametes were occasionally observed in which the nuclei of the two cells were closely appressed (Fig. 21). These were presumably undergoing plasmogamy, as was similarly observed in the light microscopical examinations of Giemsa-stained smears (Figs. 6, 7). These stages were often intermingled with other developmental stages that had nuclei in the diplokaryotic arrangement (Fig. 22) and could be distinguished by their unikaryotic condition and electron-dense cytoplasm. These observations suggested that plasmogamy was responsible for diplokaryotic formation.

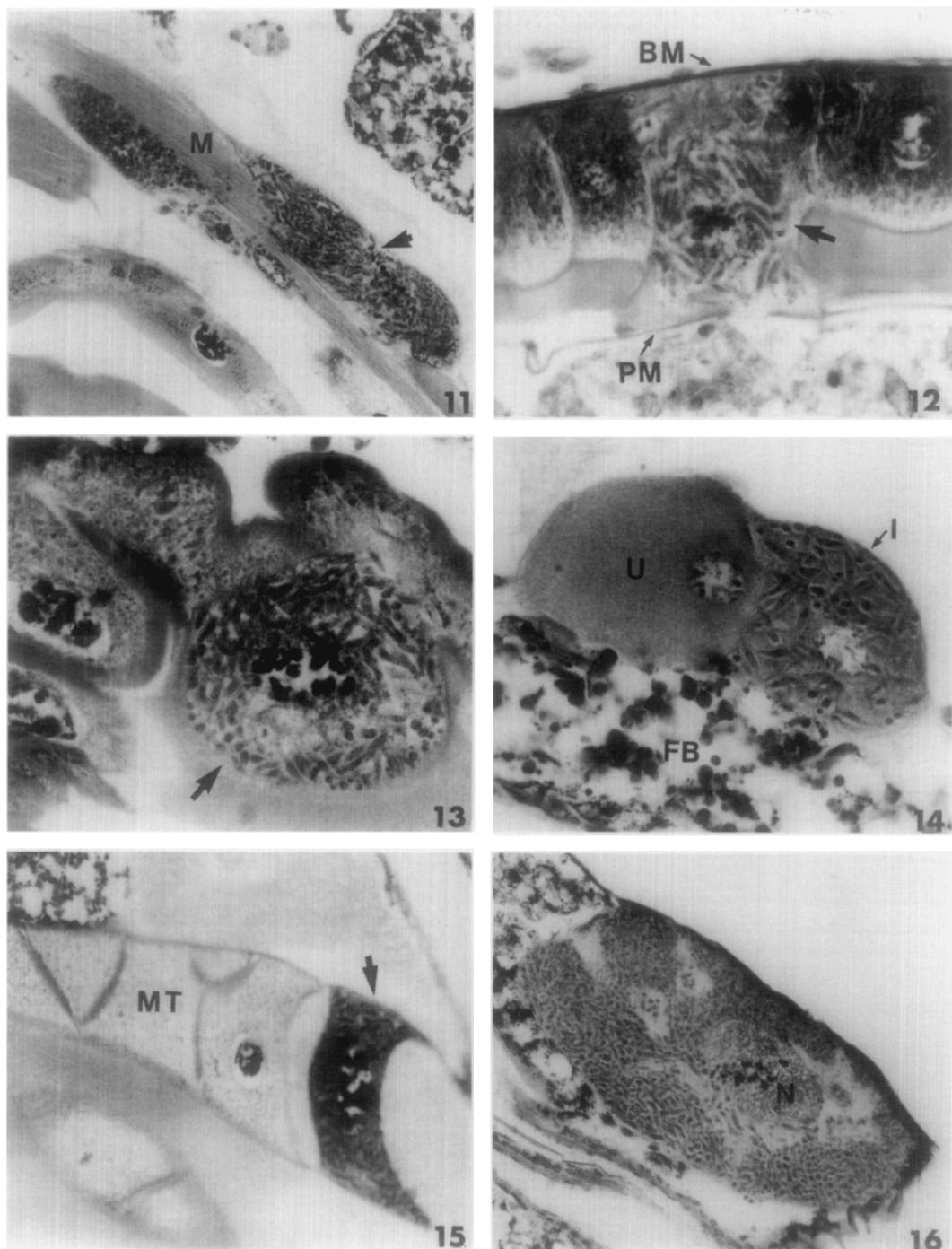
Diplokaryotic stages (Fig. 23) predominated in adult hosts. They were typically fusiform in shape and possessed a ribosome-rich cytoplasm and numerous cisternae of rough endoplasmic reticulum. Diplokarya were also seen to undergo synchronous nuclear division of each member of the diplokaryon to form additional vegetative stages (Fig. 24). No synaptonemal complexes were found within the nuclei of these cells.

## DISCUSSION

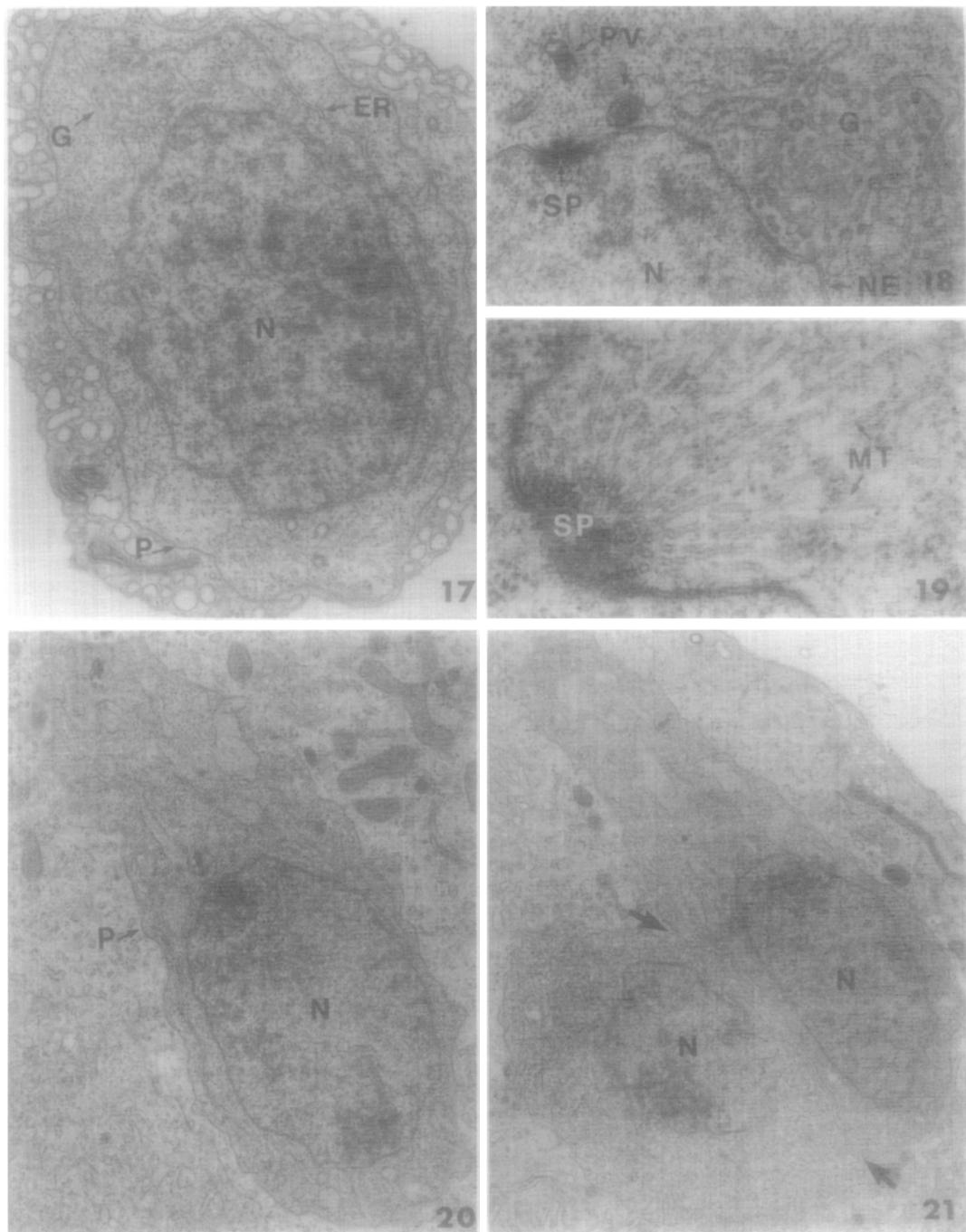
With this study, the complete life cycle of *A. connecticus* has now been elucidated. Unikaryotic spores, produced in the intermediate copepod host, via prior infection with larval meiospores, are directly infectious to *A. cantator* larvae by feeding. This mechanism of horizontal transmission provides the necessary pathway through which *A. connecticus* can be introduced to new mosquito populations, and thereby be maintained.

Infections appear to initially arise within epithelial cells of the midgut and gastric caeca of young larvae shortly following the ingestion of spores. Here, the microsporidium divides repeatedly by binary fission, causing cells to swell and eventually rupture. This results in the release of many meronts that apparently spread to the muscles, oenocytes, and occasionally the Malpighian tubules where they undergo further multiplication and plasmogamy to form diplokaryotic stages and restore the diploid condition in adult hosts. Sporulation occurs in female hosts only and this results in the formation of binucleate spores that are responsible for transovarial transmission of *A. connecticus* and the production of meiospores in progeny of the next generation. The pattern of parasite development in oenocytes of adult female hosts is identical to that observed in transovarially transmitted infections (Andreadis, 1983a).

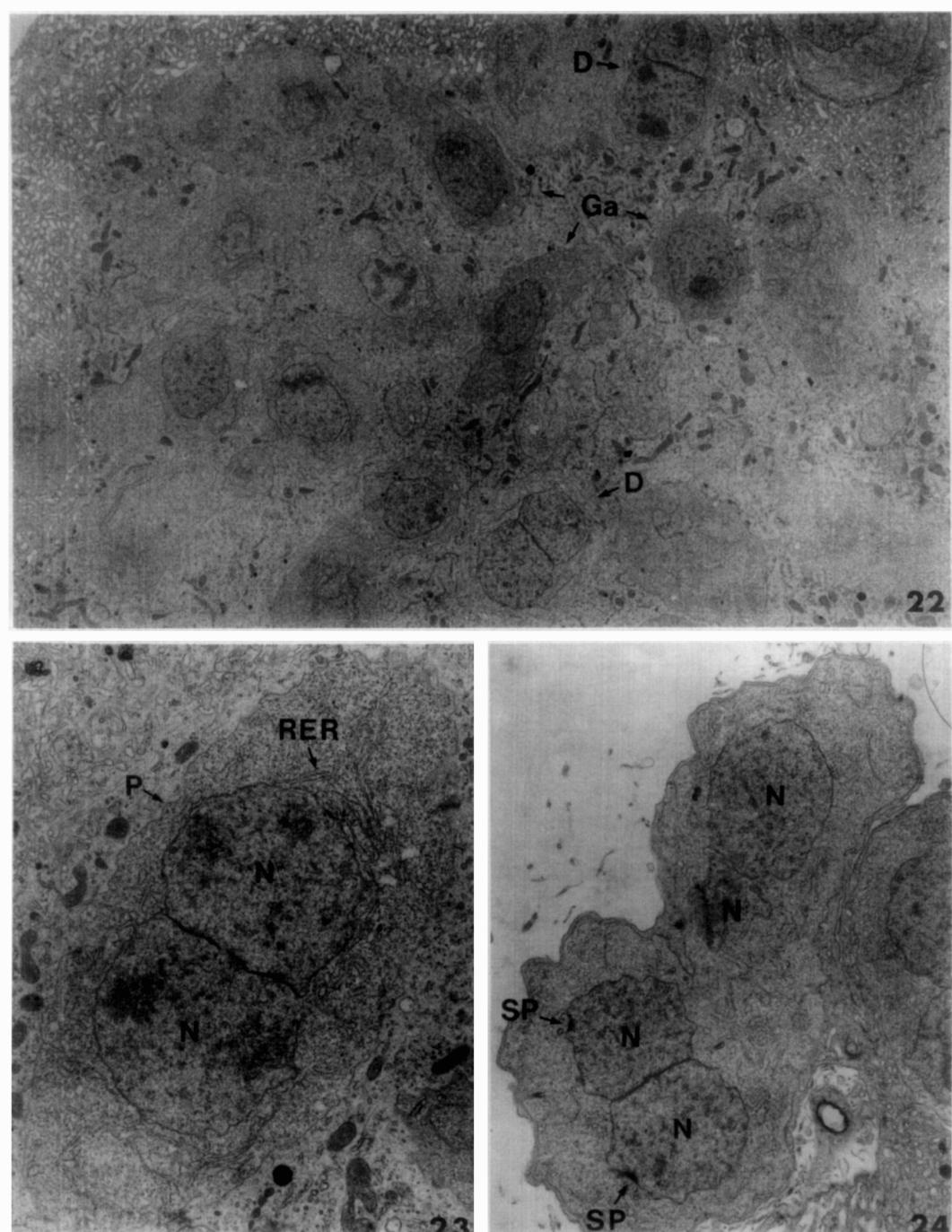
The fate and function of horizontally acquired infections in male hosts are not known since the microsporidium never sporulates in males, regardless of their age.



Figs. 11-16. Histological sections of tissues infected with *Amblyospora connecticus* from larval (11-15) and adult (16) *Aedes cantator*. (11) Intersegmental muscle ( $\times 240$ ). (12) Midgut epithelium ( $\times 490$ ). (13) Gastric caecum ( $\times 450$ ). (14) Larval oenocytes ( $\times 530$ ). (15) Malpighian tubule (MT) ( $\times 260$ ). (16) Adult oenocyte ( $\times 260$ ). Large arrows indicate infection; BM, basement membrane; FB, fat body; I, infected cell; N, host cell nucleus; PM, peritrophic membrane; U, uninfected cell.



FIGS. 17-21. Electron micrographs of *Amblyospora connecticus* from larval (17-19) and adult (20, 21) *Aedes cantator*. (17) Uninucleated meront ( $\times 21,400$ ). (18, 19) Detailed view of structures associated with nuclear division (18,  $\times 33,300$ ; 19,  $\times 57,600$ ). (20) Gamete ( $\times 12,500$ ). (21) Gametes prior to plasmogamy, arrows indicate appressed region ( $\times 10,700$ ). ER, endoplasmic reticulum; G, Golgi apparatus; MT, spindle microtubules; N, nucleus; NE, nuclear envelope; P, plasmalemma; PV, polar vesicles; SP, spindle plaque.



FIGS. 22-24. Electron micrographs of *Amblyospora connecticus* from adult *Aedes cantator*. (22) Section from an infected oenocyte showing gametes (Ga) and diplokaryotic stages (D) ( $\times 3300$ ). (23) Diplokaryotic stage ( $\times 8700$ ). (24) Dividing diplokaryon ( $\times 6800$ ). N, nucleus; P, plasmalemma; RER, rough endoplasmic reticulum; SP, spindle plaque.

There is also no evidence to suggest venereal transmission of the microsporidian from infected males to females or direct paternal infection of ova via infected sperm, as no infections were ever detected in male testes.

The morphologies of the various developmental stages and basic sequence of development of *A. connecticus* in *A. cantator* following horizontal transmission of infection show many similarities with *Amblyospora dyxenoides* from *Culex annulirostris* (see Sweeney et al., 1988) and an undescribed *Amblyospora* sp. from *Aedes stimulans* (see Andreadis, 1985b), the only other mosquito hosts from which the details of this process have been reported. However, some notable differences do exist. In *C. annulirostris* and *A. stimulans*, for example, sporulation of diplokaryotic stages occurs in both host sexes, but in *A. cantator* is clearly restricted to female hosts only. Moreover, in female *C. annulirostris*, sporulation occurs independent of the host blood meal and spore formation does not necessarily coincide with the development and maturation of the host oocytes as it does in *A. cantator*.

The pathway of infection in *A. cantator*, involving initial invasion of the midgut and subsequent spread to other host tissues, has similarly been observed in *A. stimulans* (see Andreadis, 1985b). However, it is not presently known whether this same sequence of events takes place in *C. annulirostris*, where infections have only been observed in host oenocytes (Sweeney et al., 1988).

In *A. stimulans*, infected cells of the gastric caeca are often sloughed off with the onset of pupation before vegetative stages can successfully spread into the hemocoel and infect the oenocytes (Andreadis, 1985b). This greatly reduces the prevalence of infection in adult hosts, especially females, that were infected as larvae. In *A. cantator*, however, the microsporidium appears to be much more efficient in achieving this transition, as nearly identical levels

of infection were observed in larvae and adults (Table 1). This might suggest a longer standing relationship between *A. connecticus* and its host, *A. cantator*, and help explain why transovarially induced epizootics have been observed in field populations of this mosquito host (Andreadis, 1983b) and not others.

I have no explanation as to why male larvae of *A. cantator* are more susceptible to infection than are females and it is not presently known whether this same phenomenon occurs in other host mosquitoes infected horizontally with *Amblyospora*. In *A. stimulans*, the higher prevalence of infection observed in adult males appears more to be due to the ability of the microsporidium to successfully spread from gastric caecal cells to oenocytes in male hosts (Andreadis, 1985b) rather than any differential susceptibility at the time of exposure, as may be the case with *A. cantator*. It is also not known why infections are more progressive in male hosts but this could certainly be due to some yet to be discovered host-mediated humoral factor that may similarly be involved in the dimorphic expression of transovarially transmitted infections in each host sex (Andreadis, 1983a).

My observations of gametogenesis and plasmogamy in *A. connecticus*, which restore the diploid condition following haploid development in the copepod host (Andreadis, 1985a), are entirely consistent with observations of these processes in *A. dyxenoides* (see Sweeney et al., 1988) and other horizontally transmitted, polymorphic microsporidia of mosquitoes (i.e., *Culicospora magna* in *Culex restuans*, *Hazardia milleri* in *Culex quinquefasciatus*, and an undescribed *Microsporidium* in *Aedes aegypti*) (Hazard et al., 1985; Becnel et al., 1987). Vegetative development of gametes and the events taking place during plasmogamy (i.e., cytoplasmic fusion) appear to be nearly identical for all five species. However, in *C. magna*, *H. milleri*, and the *Microsporidium* sp., the earliest

stages of gametogenesis occur in mosquito blood cells, rather than in the midgut, and plasmogamy takes place in the host hemocoel rather than in the oenocytes or muscle tissue, as apparently occurs with *A. connecticus* in *A. cantator*. Ultrastructurally, the gamonts and gametes show many affinities except that I could find no evidence of any "nipple-like" structure on the gamete plasmalemma of *A. connecticus* that distinguished gametes of *C. magna* and the *Micromsporidium* sp.

Plasmogamy and karyogamy have also recently been reported in *Amblyospora culicis*, a parasite of *Culex quinquefasciatus* (see Toguebaye and Marchand, 1986). However, according to the authors, these events take place in larval fat body tissue prior to merogony and are initiated by gametes that are presumed to arise directly from haploid meiospores produced in the same mosquito host. Gametes emerge from meiospores and first undergo cytoplasmic and then nuclear fusion to form a diploid uninucleate zygote. The nucleus of this zygote subsequently divides, forming a diplokaryotic meront that will undergo merogony, meiosis, and a prolonged sporulation sequence to form eight meiospores. This appears to be highly unusual for an *Amblyospora*, as this sequence of development leading to meiospores has always been shown (Hazard and Oldacre, 1975; Andreadis and Hall, 1979; Lord et al., 1981; Andreadis, 1983a, 1985b; Sweeney et al., 1988) to occur via transovarial transmission and to be initiated by binucleate spores produced in adult female hosts. It is also inconsistent with my findings and directly contradicts what has been documented for other *Amblyospora* spp. whose meiospores have never been shown to be infectious to mosquito larvae (Andreadis and Hall, 1979; Hazard et al., 1979; Andreadis, 1983a, 1985a, b; Hazard and Brookbank, 1984; Sweeney et al., 1985, 1988; Becnel, 1986). Moreover, it should be noted that the authors have based their conclusions on ob-

servations of field-collected mosquito larvae with infections of unspecified origin. However, if their observations are accurate, then this would be a clear departure from other *Amblyospora* spp. and represent the only instance where meiospores are directly reinfectious to the same mosquito host.

## SYSTEMATICS

### *Amblyospora connecticus* sp. nov.

*Amblyospora* sp. Andreadis, 1983a, *J. Protozool.* 30, 509; Andreadis, 1983b, *J. Invertebr. Pathol.* 42, 427; Andreadis, 1985, *Proc. Natl. Acad. Sci. USA* 82, 5574.

*Thelohania* near *opacita*. Anderson, 1968, *J. Invertebr. Pathol.* 11, 440.

*Hosts.* The brown salt marsh mosquito, *Aedes cantator* (Coquillett) and the copepod, *Acanthocyclops vernalis* (Fisher, 1853) Kiefer, 1927.

*Type locality.* Several salt marshes in Guilford and Milford, Connecticut.

*Site of infection.* *Aedes cantator*—Transovarially transmitted infections in fat body, oenocytes, and ovaries; horizontally transmitted infections in the gastric caeca, midgut, muscles, oenocytes, and Malpighian tubules. *Acanthocyclops vernalis*—in ovarian tissue.

*Diagnosis.* *Aedes cantator*—Transovarially transmitted infections produce two sporulation sequences; one in oenocytes and ovaries of most female progeny that results in the production of binucleate spores that are responsible for transovarial transmission and the other in larval fat body tissue of all male and some female progeny that results in the production of haploid meiospores that are orally infectious to copepods. Meronts in both sequences have diplokaryotic nuclei and divide repeatedly by binary and multiple fission, forming merogonial plasmodia with up to four diplokarya. In female hosts with oenocytic infections, diplokaryotic stages develop di-

rectly into nonmembrane bound binucleate spores when a blood meal is taken. In larval hosts with fat body infections, diplokaryotic stages secrete a sporophorous vesicle, undergo meiosis, and develop into sporogonial plasmodia that produce eight sporoblasts and eight haploid meiospores enclosed in a persistent sporophorous vesicle. Quadrinucleate and octonucleate sporonts possess prominent tubular extensions on the plasmalemma and secrete numerous metabolic products that accumulate within the sporophorous vesicle cavity.

Horizontally transmitted infections arise within the midgut and gastric caeca of host larvae and spread to the muscles, oenocytes and Malpighian tubules. Young meronts are uninucleate and divide by binary fission to produce gametes that have a single nucleus at one pole. Gametes also divide by binary fission and undergo plasmogamy to form diplokaryotic stages that sporulate in adult female hosts to produce binucleate spores that are responsible for transovarial transmission.

*Acanthocyclops vernalis*—Development is unikaryotic. Young meronts are uninucleate and undergo repeated nuclear divisions, producing multinucleated plasmodia with up to 12 nuclei. Sporonts are ovoid, possess a single nucleus at one pole, and form rosette-shaped plasmodia. Following cytoplasmic cleavage, sporoblasts secrete a nonpersistent sporophorous vesicle and develop into uninucleated spores that are orally infectious to mosquito larvae.

**Spore morphology.** Meiospores in larval *Aedes cantator*—uninucleate and broadly oval with a lamellar polaroplast and thick spore wall; polar tube anisofilar with 4 anterior and 7–8 posterior coils;  $7-7.2 \times 4.8-5 \mu\text{m}$  (fresh).

**Spores in adult *Aedes cantator***—binucleate and elongate with a vesicular polaroplast and thin spore wall; polar tube isofilar with 9–10 coils;  $8-9 \times 3-3.2 \mu\text{m}$  (fixed).

**Spores in *Acanthocyclops vernalis***—

uninucleate and pyriform to lanceolate with a large vesicular polaroplast and thin spore wall; polar tube isofilar with 11–12 coils;  $8-10 \times 5-6 \mu\text{m}$  (fresh).

**Type slides.** Type slides are in the collection of the author and will be deposited with the International Protozoan Type Slide Collection, Smithsonian Institution, Washington, DC.

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