MICROSPORIDIAN PARASITES OF MOSQUITOES

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INTRODUCTION

The Microsporidia are a large diverse group of obligate, intracellular parasites. They are single-celled eukaryotic microorganisms that have small genomes in the size range of prokaryotic cells and are now thought to be highly evolved fungi (Keeling and Fast 2002). They are exclusive parasites of other eukaryotes and possess a unique and highly specialized mechanism for invading host cells via infectious spores. Spores are the only stage that can exist outside a living host cell and they are the primary vehicles for horizontal transmission between hosts (peroral) and vertical transmission (transovarial) within the host. Spores are diagnostic, especially at the ultrastructural level (Fig. 1), and are distinguished by their small size (2–20 μm), thick walls (consisting of an endospore and exospore), and presence of a unique set of organelles that function as an extrusion apparatus. These include a tightly coiled polar filament (tube) that is attached to an anchoring disc at the anterior pole of the spore, a membranous polaroplast, and a posterior vacuole which collectively function to explosively inoculate the spore content or "sporoplasm" through the polar filament into a host cell to initiate infection.

Microsporidia are ubiquitous in nature and exhibit a very broad host range within the animal kingdom. They have been described as parasites in all classes of vertebrates, including humans, and most invertebrates, but are particularly common to arthropods and fish.

Microsporidia represent one of the largest and most diverse groups of parasitic organisms associated with mosquito populations in nature. They have been described from 14 different genera worldwide, and it is quite likely that all mosquitoes serve as hosts for one or more of these parasites. The group currently includes a heterogeneous assemblage of over 150 described species from 23 recognized genera, 15 of which are monotypic (only one species is known) (Table 1).

Members of these genera exhibit extensive variation in their development and life cycles but generally fall into 2 broad categories. The first includes the monomorphic forms such as *Anncaliia* (formerly *Nosema* and then *Brachiola*) (Franzen et al. 2006) and *Vavraia*. These microsporidia have comparatively simple life cycles involving only one sporogonic sequence (Fig. 2). They develop asexually (merogony or schizogony) and produce a single spore type that is orally infectious to mosquito larvae. Vertical transmission may additionally occur via oral ingestion of spores on contaminated eggs (transovum), but there is no separate developmental sequence leading to ovarian infection in female hosts. These microsporidia have a very broad host range and are mildly pathogenic to mosquitoes, generally producing low larval mortality. Other genera from which only one developmental sequence has been described include *Aedispora*, *Crepidulospora*, *Polydispyrenia*, *Senoma*, *Trichotosporea*, and *Tricornia*. However, the complete life cycles and modes of transmission of members of these genera have yet to be resolved and they may be polymorphic.

The second group includes the true polymorphic forms. These parasites are more common in nature and exhibit some of the most complex life cycles yet described for any microsporidia (Fig. 2). These include elements of asexual (schizogony, merogony, and sporogony) and sexual (karyogamy, gametogenesis, and plasmogamy) reproduction; the formation of multiple spore types in various stages of the host; host sex- and tissue-dependent development; and separate developmental sequences leading to vertical (transovarial) and horizontal transmission. Many species, such as *Edhazardia aedis*, require two successive host generations to complete their life cycle, and at least 4 genera, *Amblyospora*, *Duboscqia*, *Hyalinocysta* and *Parathelohania* require obligatory development in an intermediate copepod host. These microsporidia generally exhibit higher levels of host specificity and although they do not cause any acute mortality or detectable morbidity in adult female hosts that go on to transmit infections transovarially, they have at least one phase of development that typically kills larval hosts during the last stadium. Mortality in larvae results from destruction of various host tissues and subsequent depletion of essential energy reserves necessary for pupation. The production of entomopathogenic toxins has never been documented.

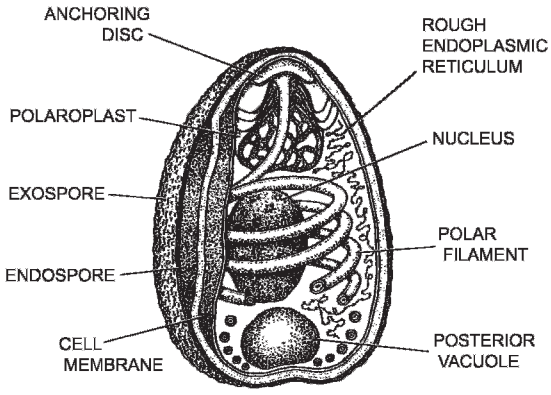

Fig. 1. Diagram of a microsporidian spore showing important structures and organelles.

In addition to the 5 aforementioned genera, other well-studied genera with polymorphic development that are not known to involve an intermediate host in the life cycle include *Culicospora*, *Culicosporella*, and the less well known *Hazardia*. Dimorphic development has also been described in species of *Cristulospora*, *Dimeiospora*, *Goldbergia*, *Intrapredatorus*, *Krishtalia*, *Merocinta*, and *Pilosporella*, but these microsporidia have life cycles and modes of transmission that are incompletely known. Details on the specific life cycles and pathologies associated with each of these genera are reviewed in the genera descriptions.

DETECTION AND DIAGNOSIS

Surveys for microsporidian parasites in mosquito populations usually involve the screening of large numbers of individuals owing to their typically low prevalence rates in nature. These are most easily conducted by examining living larvae which are the only stage that exhibit visible signs of infection. Microsporidian infections are most readily detected in late stage (4th instar) larval mosquitoes where heavy concentrations of spores, whether in the fat body, midgut or gastric caecae, can be seen through the cuticle as white or yellow masses when viewed against a black background (Figs. 3A, 3B). The use of black photographic pans with an overhead light source is ideal for screening large numbers of individuals. These gross visible signs may also be accompanied by swellings that are caused by hypertrophy of infected cells.

Alternatively, where infections are light or not overtly apparent as in adult hosts, microsporidia can be detected by microscopically examining samples of mascerated tissues or whole specimens for spores or vegetative stages. This is best achieved with the use of a compound microscope (400× or 1000×). Whole "wet mounts" examined with phase or differential (Nomarski) interference contrast microscopy, are ideal for viewing live spores. Verification that a spore is a microsporidium can further be achieved by applying light pressure to the coverslip which will cause spores

Table 1. Recognized genera of Microsporidia from mosquitoes.

	No. species	Mosquito host range	Life cycle	Transmission		Intermediate host
				Vertical	Horizontal	
Aedispora	2	*Oc.*	monomorphic	unknown	unknown	unknown
Amblyospora	> 100	*Ad.*, *Ae.*, *An.*, *Cq.*, *Cs.*, *Cx.*, *Ms.*, *Oc.*, *Ps.*	polymorphic	transovarial	oral	yes
Anncaliia	1	*Ae.*, *An.*, *Ar.*, *Cx.*, *Wy.*	monomorphic	transovum	oral	no
Crepidulospora	1	*An.*	monomorphic	unknown	unknown	unknown
Cristulospora	3	*Cx.*, *Oc.*	dimorphic	unknown	unknown	unknown
Culicospora	1	*An.*, *Cx.*, *Oc.*	dimorphic	transovarial	oral	no
Culicosporella	1	*Cx.*	polymorphic	transovarial	oral	no
Dimeiospora	1	*Oc.*	dimorphic	unknown	unknown	unknown
Duboscqia	2	*An.*, *Oc.*	polymorphic	transovarial	oral	yes
Edhazardia	1	*Ae.*	polymorphic	transovarial	oral	no
Goldbergia	1	*Cx.*	dimorphic	no	oral	no
Hazardia	1	*An. Cx.*	polymorphic	unknown	oral	unknown
Hyalinocysta	1	*Cs.*	dimorphic	no	oral	yes
Intrapredatorus	1	*Cx.*	dimorphic	unknown	unknown	unknown
Krishtalia	1	*Cx.*	dimorphic	likely	unknown	unknown
Merocinta	1	*Ms.*	dimorphic	transovarial	unknown	unknown
Parathelohania	22	*Ad.*, *An.*, *Oc.*	polymorphic	transovarial	oral	yes
Pilosporella	2	*Oc.*, *Wy.*	dimorphic	transovarial	unknown	unknown
Polydispyrenia	2	*Cs.*, *Cx.*, *Oc.*	monomorphic	transovarial	unknown	unknown
Senoma	1	*An.*	monomorphic	unknown	likely	unknown
Trichotosporea	2	*Ae. Oc.*	monomorphic	likely	unknown	unknown
Tricornia	1	*Ms.*	monmorphic	unknown	unknown	unknown
Vavraia	1	*Ae.*, *An.*, *Cs.*, *Cx.*, *Oc.*, *Or.*	monomorphic	transovum	oral	no

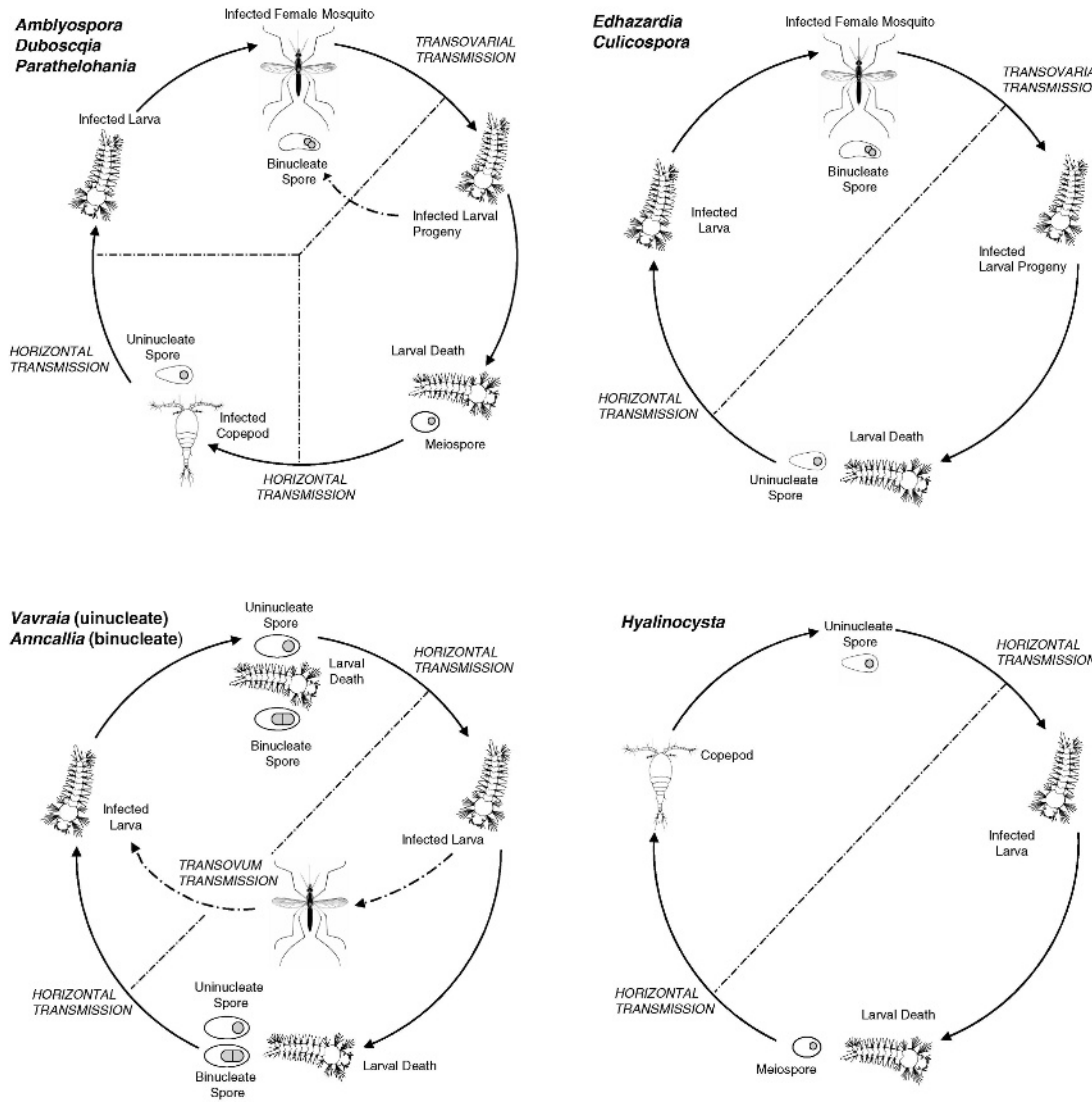

Fig. 2. Life cycle drawings of representative genera of microsporidia showing major transmission pathways.

to appear to germinate and evert the diagnostic polar filament (Fig. 4B). A clean pencil eraser is a useful tool for applying pressure to the coverslip. Vegetative or developmental stages by contrast are more readily observed through examination of Giemsa-stained smears of infected tissues with bright field optics (1000×). Best results are obtained with infected tissues from live hosts that are air-dried and fixed with 100% methanol. The Giemsa stain of choice is a modified Romanowsky's containing Azur II, Azure, glycerin, and methanol in phosphate (pH 7.4) buffer. The cytoplasm of meronts, sporonts and other vegetative stages will stain blue while the nulclei stain red. The presence of stages with nuclei in the diplokaryotic arrangement (paired nuclei) is almost always indicative of a microsporidian infection. Mature spores stain blue or purple but the nuclei are not usually visible.

The identification of infected tissues is best determined through microscopic (bright-field) examination of paraffin-embedded histological sections fixed in Carnoy's solution and stained with Heidenhain's haematoxylin and Eosin. This classic procedure stains nuclei and mature spores deep blue to black while vegetative cells appear red to brown. Recipes for preparation of stains and specific protocols for their use with microsporidia can be found in Hazard et al. (1981), Becnel (1997) and Undeen and Vavra (1997). While these procedures and techniques will effectively identify and diagnose most microsporidian infections in mosquitoes, it is important to recognize that in almost all instances, definitive

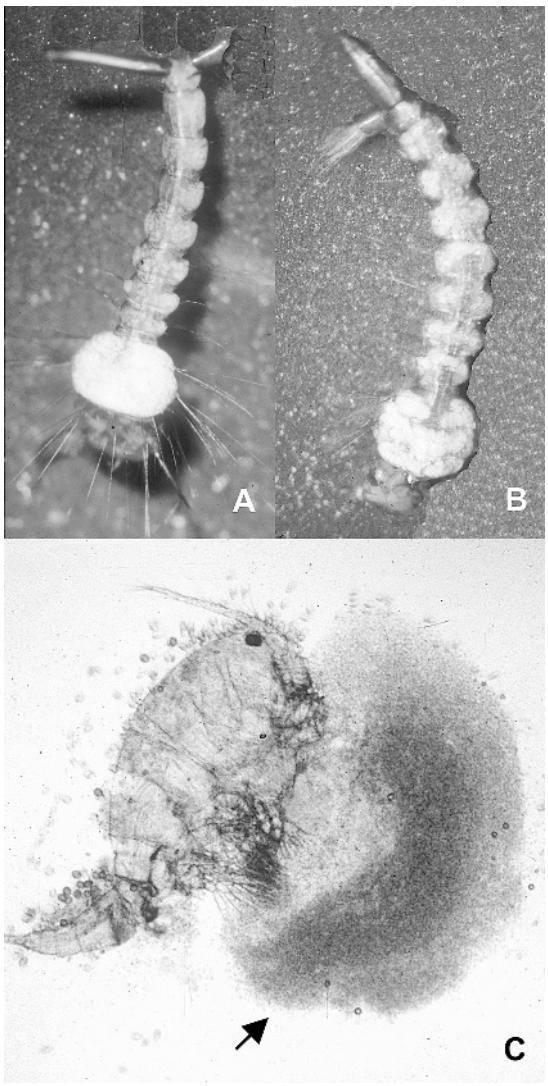

Fig. 3. (A) A 4th instar *Culiseta melanura* infected with *Hyalinocysta chapmani*. (B) A 4th instar *Ochlerotatus stimulans* infected with *Amblyospora stimuli*. (C). A female copepod, *Orthocyclops modestus* infected with spores (arrow) of *Hyalinocysta chapmani*.

identification of a particular isolate to the species level will require an examination of detailed ultrastructure of life stages, especially the spore (Fig. 1) (see Vavra and Larsson 1999), and comparative analysis rRNA sequence data (see Vossbrinck et al 2004, Vossbrinck and DeBrunner-Vossbrinck 2005).

ECOLOGY AND EPIZOOTIOLOGY

Much progress has been made in the study of mosquito-parasitic microsporidia over the last 20 years. This has led to an elucidation of the life cycles and modes of transmission with accompanying pathology, ultrastructure, and host specificity of a number of genera including *Amblyospora*, *Anncaliia*, *Culicospora*, *Culicosporella*, *Duboscqia*, *Edhazardia*, *Hyalinocysta*, *Parathelohania*, and *Vavraia*. Many new species also have been discovered, and several new genera, most represented by one or two species, have been created. Unfortunately, the majority of these have been based solely on the ultrastructural morphology of the spore with little or no knowledge of their developmental cycles and modes of transmission. These include *Aedispora*, *Crepidulospora*, *Cristulospora*, *Dimeiospora*, *Intrpredatorous*, *Krishtalia*, *Merocinta*, *Senoma*, *Trichotosporea*, and *Tricornia*.

Despite these advances, detailed life history and epizootiological studies have been conducted on only a handful of species, and less progress has been made in understanding the ecology and population dynamics of these mosquito-parasitic microsporidia in natural host populations. Most reports on prevalence rates have been based on a few isolated observations and with a few exceptions, most species have been found to occur at very low levels, typically no more than 1 or 2%. However, detailed studies on 2 species *Amblyospora connecticus* (Andreadis 1990) and *Hyalinocysta chapmani* (mosquito host = *Culiseta melanura*, copepod host = *Orthocyclops modestus*) (Andreadis 2002), have shown that these microsporidia are important natural enemies that consistently cause seasonal epizootics in larval populations, with prevalence rates ranging from 60% to near 100% in their respective mosquito hosts. Furthermore, these studies have revealed a remarkable variety of unique and highly specialized adaptations particular to each parasite species that directly facilitate transmission and are intimately interwoven into the biological attributes of their hosts and the varied aquatic environments they inhabit. It is quite likely that other microsporidian species exhibit similar regulatory affects on their mosquito hosts but these remain to be discovered. We are in critical need of more basic long-term epizootiological investigations. There is an urgent need for more quantitative field studies that carefully assess the contribution of major and minor routes of transmission (horizontal and vertical) to the initiation and development of both enzootic and epizootic infections in mosquito populations. These efforts will allow us to identify specific factors (abiotic and biotic) that directly impact host-parasite population dynamics in the field. This is essential if we hope to exploit the control potential of these naturally occurring ubiquitous parasites.

MOLECULAR PHYLOGENY

Small subunit ribosomal (ssrDNA) sequence data are available for 11 of the 23 microsporidian genera recognized here from mosquitoes. These include *Amblyospora*, *Anncaliia*, *Culicospora*, *Culicosporella*, *Edhazardia*, *Hazardia*, *Hyalinocysta*, *Intrapredatorus*, *Parathelohania*, *Senoma* and *Vavraia*. The largest number of species that have been examined thus far is included within the genus *Amblyospora*. Figure 5 is a phylogenetic tree adapted from Vossbrinck et al. (2004) of 28 microsporidian taxa from mosquitoes and copepods based on partial ssrDNA sequences using maximum parsimony analysis. Mosquito host associations and the involvement of an intermediate host in the life cycle are also indicated. With the exception of *Anncalina* and *Vavraia* that exhibit very broad host ranges across a variety of mosquito genera, the analysis shows a high degree of correlation between the mosquito host and the microsporidian parasite at the generic level. Species of *Amblyospora*, *Culicospora*, and *Intrapredatorus* that parasitize *Culex* mosquitoes form a distinct group, as do species of *Amblyospora* and *Edhazardia* that parasitize *Aedes* and *Ochlerotatus* mosquitoes. *Amblyospora ferocious* further appears as a distinct sister taxon and is confined to *Psorophora* mosquitoes. The analysis also clearly demonstrates that 3 monotypic genera *Culicospora*, *Edhazardia*, and *Intrapredatorus* cluster well within the *Amblyospora* clade. This makes *Amblyospora* a paraphyletic taxon and supports defining *Amblyospora* as a much broader group. It has been proposed (Vossbrinck et al. 2004) that if further sequence analysis of other genes supports these findings, strong consideration should be given to reassigning these 3 monotypic genera to the genus *Amblyospora*. The analysis further demonstrates a high level of host specificity by species of *Amblyospora* for their definitive mosquito hosts.

Hyalinocysta and *Culicosporella* are sister taxa to the *Amblyospora* and are also monotypic but are sufficiently different based on evolutionary relatedness to designate separate generic status (Andreadis and Vossbrinck 2002). *Hyalinocysta* is known from *Culiseta* mosquitoes only while *Culicosporella* infects *Culex* (*Cx. pilosis*). *Hyalinocysta* is distinguished from the *Amblyospora* by the diplokaryotic meronts formed by karyokinesis rather than by plasmogamy, and by the absence of a developmental sequence leading to the production of binucleate spores and transovarial transmission, a universal trait in *Amblyospora* (Andreadis and Vossbrinck 2002). *Culicosporella* is distinguished from *Amblyospora* by its production of binucleate-lanceolate spores rather than uninucleate-lanceolate spores for the oral infection of the mosquito host (Becnel and Fukuda 1991).

Species of *Parathelohania*, *Hazardia* and *Senoma* appear as a sister group to the aforementioned mentioned taxa. With the exception of *H. milleri* which infects *Culex*, these microsporidia are restricted to *Anopheles* mosquitoes. It is not known how closely the mosquito and microsporidian phylogenies parallel each other. However, the microsporidian phylogeny is consistent with the conventional classification of their mosquito hosts, where *Anopheles* mosquitoes (Subfamily Anophelinae) are thought to be the sister group to the culicines (Subfamily Culicinae), as the *Parathelohania*, *Hazardia* and *Senoma* appear to be the sister group of the *Amblyospora*.

Examination of the phylogenetic relationships among these microsporidian taxa from mosquitoes and copepods also provides insight into the origin and evolution of both the intermediate host and transovarial transmission. The analysis suggests that microsporidian parasites of anopheline and culicine mosquitoes evolved from parasites of crustaceans, and that parasitism of mosquitoes by all 8 of the true mosquito-parasitic genera (*Amblyospora*, *Culicosporella*, *Culicospora*, *Edhazardia*, *Hazardia*, *Intrapredatorus*, *Parathelohania* and *Senoma*) likely arose from a single event (Vossbrinck et al. 2004). However, the position of *Anncaliia* and *Vavraia* indicates that microsporidia have invaded members of the Culicidae several times independently.

Aside from the shape of the meiospore found in infected mosquito larvae, the life cycles, pathology and developmental morphologies of *Parathelohania* and *Amblyospora* are virtually identical; both genera have copepod intermediate hosts and are transovarially transmitted (Fig. 2). *Culicospora* and *Edhazardia* also have morphologies and life cycles similar to the *Amblyospora* including transovarial transmission, but lack functional meiospores and the requirement for an intermediate copepod host (Becnel et al. 1987, 1989, Becnel 1994). *Culicosporella* is transovarially transmitted but similarly lacks functional meiospores and an intermediate copepod host (Hazard et al. 1984, Becnel and Fukuda 1991). Thus, the absence of an intermediate host in the life cycles of these 3 genera (*Culicospora*, *Culicosporella*, and *Edhazardia*) most likely reflects an ecological adaptation to the aquatic habitat of the larval host and is not a reflection of evolutionary relatedness. Analysis of the ssrDNA data further suggests that transovarial transmission and the developmental sequence leading to ovarian infection have been secondarily lost in *Hyalinocysta* as they occur in all other closely related genera (*Amblyospora*, *Edhazardia*, *Culicosporella* and *Culicospora*) including the likely sister group *Parathelohania*. These observations collectively suggest that 1) mosquito-parasitic microsporidia are adjusting their life cycles to accommodate host ecological conditions; 2) the ancestral state

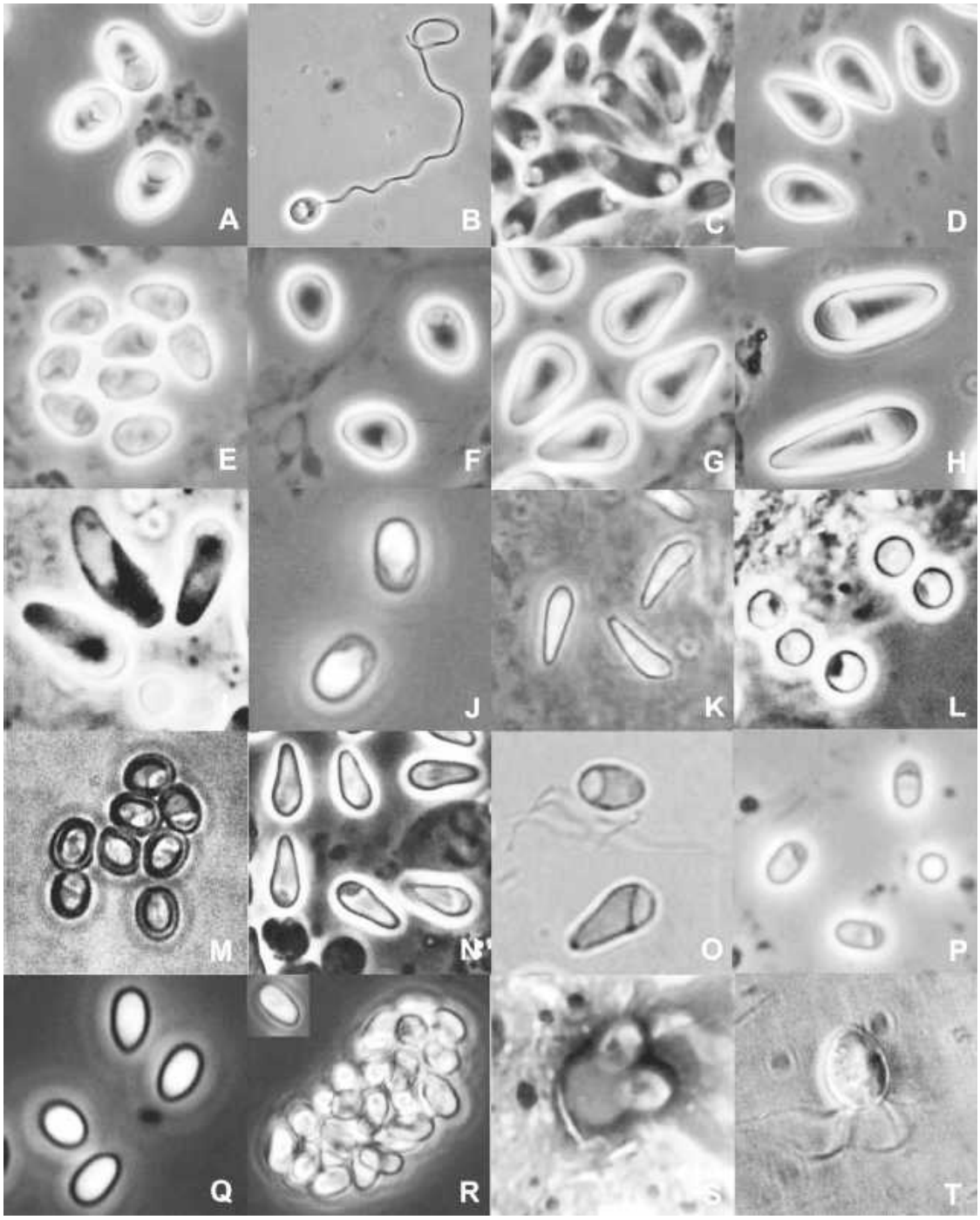

Fig. 4. (A) Ovoid uninucleated meiospores of *Amblyospora stimuli* from a larval *Ochlerotatus stimulans*. (B) Germinated meiospore of *Amblyospora stimuli* with extruded polar filament. (C) Elongated binucleated spores of *Amblyospora connecticus* from the ovaries of an adult female *Ochlerotatus cantator*. (D) Lanceolate uninucleated spores of *Amblyospora connecticus* from the intermediate copepod host, *Acanthocyclops vernalis*. (E) Ovoid uninucleated meiospores of *Hyalinocysta chapmani* from a larval *Culiseta melanura*. (F) Lanceolate uninucleated spores of *H. chapmani* from the intermediate copepod host, *Orthocyclops modestus*. (G) Lanceolate uninucleated spores of *Edhazardia aedis* from larval *Aedes aegypti*. (H). Lanceolate uninucleated spores of *Culicospora magna* from larval *Culex restuans*. (I) Lanceolate binucleated spores of *Culicosporella lunata* from larval *Culex pilosis*. (J) Ovoid meiospores of *Parathlohania obesa* from larval *Anopheles quadrimaculatus*. (K) Lanceolate uninucleated

included a complex life cycle involving transovarial transmission as well as an intermediate host; and 3) that parts of the life cycle can be gained and lost relatively rapidly over evolutionary time (Baker et al. 1997, 1998, Vossbrinck et al. 2004).

MICROSPORIDIA AS BIOLOGICAL CONTROL AGENTS

Interest in using microsporidia for control of mosquitoes has existed for several decades. Early efforts focused on 2 monomorphic species, *Anncaliia algerae* (syn. *Nosema algerae* Vavra and Undeen 1970; syn. *Brachiola algerae* Lowman, Takvorian, and Cali 2000) and *Vavraia culicis* because they were the only microsporidians that could be readily transmitted to mosquito larvae by feeding, and they had comparatively simple life cycles. These investigations (Reynolds 1972, Anthony et al. 1978b) demonstrated that while both species had a broad host range and were capable of significantly reducing adult longevity and fecundity, they did not appear to have great potential for long-term control of mosquitoes in the field because they did not cause high mortality in larval hosts and they did not persist or recycle in the environment at significantly high enough levels to adversely affect the population.

The recent discovery of *A. algerae* as the cause of a fatal myositis (inflammation and damage to muscle fibers) in a human patient (Coyle et al. 2004), and the recognition that this microsporidian parasite represents a threat to public health (Visvesvara et al. 2005), especially among immunodeficient humans, will undoubtedly preclude any further development of *A. algerae* as a biological agent for mosquito control. A similar fate will likely rest with *V. culicis*, as this microsporidium is very closely related to *Trachipleistophora hominis* (Cheney et al. 2000), another opportunistic myositic parasite of AIDS patients which readily infects larval stages of *Anopheles quadrimaculatus* and *Culex quinquefasiatus* via oral ingestion of spores, and can be passively transferred from infected adults during feeding (Weidner et al. 1999).

More recently, research efforts to evaluate microsporidia as applied biological controls for mosquitoes have focused on those species with polymorphic development, most notably *Amblyospora* spp. and *Edhazardia aedis*. These microsporidia are distally related to *Anncaliia* and *Vavraia* based on their molecular phylogeny (Vossbrinck and DeBrunner-Vossbrinck 2005), and generally exhibit much higher levels of host specificity that are confined to mosquitoes and their intermediate crustacean hosts in the case of *Amblyospora* (Andreadis 1989b, 1994b; Becnel 1992b, Becnel and Johnson 1993). Thus, they pose little or no risk to humans or other nontarget animals. Furthermore, despite their complex life cycles, they have much greater potential than the monomorphic forms because they are ubiquitous among mosquitoes in nature, infect a wide variety of species, are more virulent to larval hosts, are efficiently transmitted both vertically and horizontally, and have the capacity for long-term persistence. Their evaluation as practical mosquito control agents was facilitated by the discovery of the role of the intermediate copepod host in the life cycle of *Amblyospora* spp. (Andreadis 1985, Sweeney et al. 1985), and an elucidation of the complete life cycle and methods of transmission of *E. aedis*, that, while restricted to *Ae. aegypti*, was found to be readily transmissible and did not require an intermediate host (Becnel et al. 1989). Subsequent field trials with *Amblyospora connecticus* (mosquito host = *Ochlerotatus cantator*, copepod host = *Acanthocyclops vernalis*) (Andreadis 1989a) and *E. aedis* (Becnel and Johnson 2000) demonstrated that both microsporidia could be successfully introduced into a larval mosquito population via release of infected copepods (*A. connecticus*) or infected larvae (*E. aedis*), and produce moderately high infection rates, resulting, in the case of *E. aedis*, in vertical transmission to the filial generation and elimination of a semi-natural population of *Ae. aegypti* in 11 wk (see narrative in genera descriptions for additional details). To date, these are the only published field trials that have been conducted with polymorphic microsporidia.

In assessing the regulatory potential and strategies for use of microsporidia for mosquito control, it is essential to consider their inherent characteristics. They possess a number

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spores of *P. anophelis* from the intermediate copepod host *Microcyclops varicans*. (L) Oval uninucleated spores of *Pilosporella chapmani* from larval *Ochlerotatus triseriatus*. (M) Ellipsoid uninucleated meiospores of *Tricornia muhezae* from larval *Mansonia africana*. (N) Pyriform spores of *Hazardia milleri* from larval *Culex pipiens quinquefasciatus*. (O) Oval uninucleated meiospore (top) and uninucleate lanceolate spore (bottom) of *Intrapredatorus barri* from larval *Culex fuscanus*. (P) Ovoid spores of *Polydisprenyia caecorum* from larval *Ochlerotatus cantator*. (Q) Ellipsoidal spores of *Anncaliia algerae* from larval *An. quadrimaculatus*. (R) Ovoid spores of *Vavraia culicis* from *Culex pipiens quinquifasciatus*. (S) Egg-shaped binucleated spore matrix of *Senoma globulifera* from larval *Anopheles messae*. (T) Ovoid spores with fibrous extensions of *Trichotosporea pygopellita* from larval *Aedes vexans*.

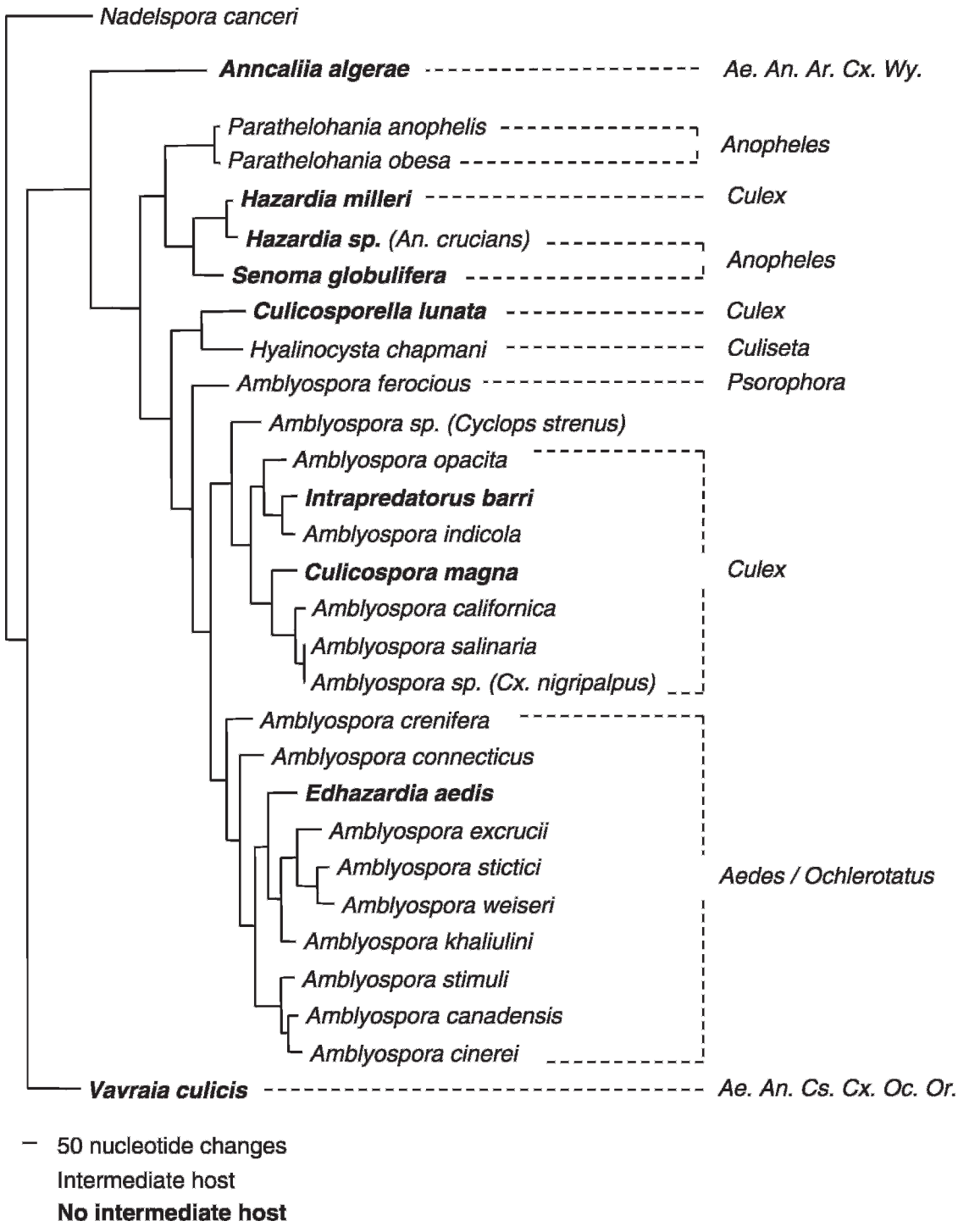

Fig. 5. Phylogenetic tree of microsporidia from mosquitoes based on partial sequence data of the small subunit ribosomal RNA gene using maximum parsimony analysis.

of attributes that would appear to make them amenable for development as biological control agents consistent with conventional criteria for the selection of candidate agents that will persist within a host population and help regulate host abundance to a steady state (Anderson and May 1981, Anderson 1982, Service 1985). These include 1) moderate pathogenicity and the ability to

kill the host after density dependant mortalities have acted; 2) high transmission efficiency with vertical and horizontal components; 3) well synchronized development with the host(s); 4) low rate of recovery from infection; 5) high rates of direct reproduction of transmissible stages (spores); and 6) the capacity to recycle and thereby persist in the biotic environment.

Conversely, they also possess a number of disadvantageous attributes that would likely limit their ability to regulate mosquito populations and thus the way in which they might be utilized. These include 1) reliance on the host for survival and dispersal (spores do not tolerate drying or freezing); 2) the production of short-lived infective stages; 3) long incubation period before producing transmissible stages (e.g., spores are not released into the environment until death of the host); 4) high host specificity; 5) lack of *in vitro* mass culture techniques; and in some instances, 6) the involvement of an intermediate host in the life cycle.

Considering these traits, it is clear that few if any microsporidian parasites offer much promise as strict microbial insecticides. Their greatest potential lies as classical biological control agents through the intentional creation of epizootics or the utilization of naturally occurring epizootics as outlined by Harper (1987). This would include 1) inoculative or inundative introductions into ecosystems where they do not occur or where they are present but not functional. Inoculative releases with the expectation of permanent long-term establishment and re-cycling are more likely to be effective in more stable environments (i.e., permanent swamps and bogs) that do not dry out and thus allow for continuous host(s)-parasite interaction. Inundative releases of spores or infected hosts to achieve more rapid suppression of mosquito populations, on the other hand, could be an effective strategy for control of stenogamous mosquitoes such as *Aedes aegypti* or *Culex pipiens* form *molestus* that breed in confined areas and have no history of infection with the particular microsporidium in that habitat but are susceptible hosts. 2) Augmentation via release of additional parasite units (i.e., spores or infected primary or intermediate hosts) into the environment at critical times in the life cycle to increase disease prevalence. This approach could be useful in those host-parasite systems where the prevalence of the microsporidium is too low to adversely affect the population. The objective would be to increase the prevalence of infection in the host population by helping to overcome ''weak links'' in the transmission cycle(s). 3) Conservation to promote transmission and the likelihood of epizootics through selective use of insecticidal controls so as not to interfere with natural transmission cycles. The objective of this approach is to recognize the contributions of natural mortality factors and take advantage of them. This approach could also include environmental manipulation to increase habitat stability and therein create conditions to enhance transmissibility of the microsporidum.

GENERA DESCRIPTIONS OF MOSQUITO-PARASITIC MICROSPORIDIA

Aedispora Kilochitskii, 1997

Type species: *Aedispora dorsalis* Kilochitskii, 1997.

Type host: *Ochlerotatus caspius dorsalis* (Meigen).

Mosquito host range: *Ochlerotatus*.

Number species from mosquitoes: 2 - *Aedispora dorsalis* (host = *Oc. caspius dorsalis*), *Aedispora tuzetae* (Tour, Rioux and Croset 1971) Kilochitskii, 2002 (host = *Oc. detritus*); (one additional undescribed species from *Oc. caspius caspius*).

Distribution: Europe (France, Ukraine).

Life cycle and transmission: Only one sporulation sequence is known. Mature spores are uninucleated and formed in groups of 8 within a non-persistent sporophorous vesicle. They are elongated and pyriform (10.0–14.5 μm × 3.5–4.4 μm). The mechanisms and pathways of transmission are unknown.

Site of infection and pathology: Infections occur in fat body tissue of larvae. Heavily infected larvae appear opaque white and typically die during the 4th stadium prior to pupation.

Host specificity: Unknown.

Epizootiology and field prevalence: *Aedispora dorsalis* has been found in larval populations of *Oc. caspius dorsalis* inhabiting open ephemeral and semi-ephemeral pools with prevalence rates in 4th instars ranging from 6–15%.

NCBI GenBank® nucleotide accession numbers: None.

References: Kilochitskii (1997, 2002).

Amblyospora Hazard and Oldacre, 1975 (Figs. 2, 3B, 4A–4D)

Type species: *Amblyospora californica* (Kellen and Lipa, 1960) Hazard and Oldacre, 1975.

Type host: *Culex tarsalis* Coquillett.

Mosquito host range: *Aedeomyia*, *Aedes*, *Anopheles*, *Coquillettidia*, *Culex*, *Culiseta*, *Mansonia*, *Ochlerotatus*, *Psorophora*, *Uranotaenia*.

Number species from mosquitoes: >100.

Natural geographical distribution: Africa, Asia, Australia, Europe, North America, South America.

Life cycle and transmission: All species studied thus far have been shown to exhibit obligatory development in an intermediate (cyclopoid)

copepod host and polymorphic development with the formation of 3 different spore types (Fig. 2): a lanceolate, uninucleated spore (8.0–13.2 μm × 3.8–6.0 μm) in the copepod (Fig. 4D); an elongated, binucleated spore (8.0–9.0 μm × 3.0–3.2 μm) in adult female mosquitoes (Fig. 4C); and a broadly oval, uninucleated ''meiospore'' (4.2–9.3 μm × 3.5–5.1 μm) formed in groups of 8 (in a sporophorous vesicle) in larval mosquitoes (Fig. 4A,B). Transovarial transmission of infection to larval progeny is universal and takes place via binucleated spores that are formed in adult female mosquitoes. In some species, sporogenesis and subsequent ovarian infection are dependent on host blood feeding and may be controlled by the secretion of host reproductive hormones (20-hydroxyecdysone). Parasite development in larval progeny is dimorphic and in many species is dependent on the host's sex; progressive in males leading to death and benign in females leading to ovarian infection and transovarial transmission. Horizontal transmission of infection from mosquito larvae to copepods occurs via oral ingestion of ''meiospores'' that are liberated from larval cadavers. Horizontal transmission of infection from copepods to larval mosquitoes is similarly facilitated via oral ingestion of extra-cellular uninucleated spores that are released into the aquatic habitat with the death of infected copepods.

Site of infection and pathology: These microsporidia exhibit high tissue specificity, low virulence and delayed pathogenicity that is synchronized with host development. In the female copepod host, infections are confined to the median ovary and paired lateral oviducts. This prevents egg development and eventually results in death. Heavily infected copepods appear orange to amber in color when viewed against a white background. In larval mosquitoes with orally acquired infections, the microsporidium initially invades the gastric caeca and then spreads to the oenocytes, muscle and ovarian tissue in adult female stages (via transstadial transmission) with no apparent acute pathology. A reduction in hatchability of transovarially infected eggs has been noted in some species. In larval hosts with transovarial infections, infections are localized in fat body tissue. Parasite development is progressive, depleting the host of essential reserves and typically results in death just prior to pupation. Heavily infected 4th instars appear opaque white when viewed against a black background (Fig. 3B).

Host specificity: Species are highly host specific for mosquitoes but copepods may serve as intermediate hosts for more than one species. Experimental infections have been achieved with *Amblyospora connecticus* (host = *Ochlerotatus cantator*) in alternate mosquito hosts of the same genera (i.e., *Oc. atropalpus*, *Oc. epactius*, *Oc. sierrensis*, and *Oc. triseriatus*) in the laboratory following oral ingestion of spores from the copepod (*Acanthocyclops vernalis*), but the microsporidium is unable to infect the ovaries and complete its life cycle via transovarial transmission.

Epizootiology and field prevalence: Detailed studies are available for only a handful of species. Species exhibit well defined seasonal transmission cycles that are intimately linked to the ecology of each host and the aquatic environments in which they inhabit. Epizootics of lethal meiospore infections in larval mosquitoes have been reported to be as high as 80–90%. Prevalence rates of horizontally acquired infections in copepods range from 40–80% and up to 60% in larval mosquitoes. In northern climates, overwintering occurs in copepods and diapausing mosquito eggs.

Field introductions: *Amblyospora connecticus* was successfully introduced into a larval field population of *Ae. cantator* via the release of live infected *A. vernalis* copepods. The tests were conducted in steel drums that were placed within a saltmarsh pool that supported breeding populations of both hosts in coastal Connecticut. The majority of infections were acquired by 2nd and 3rd instars during the 1st 3-wk of exposure, and maximum infection rates ranging from 16–24% were obtained by the time of pupation (6 wk post introduction).

NCBI GenBank® nucleotide accession numbers: *Amblyospora californica* (*Culex tarsalis*) – U68473; *Amblyospora canadensis* (*Ochlerotatus canadensis*) – AY090056; *Amblyospora cinerei* – AY090057 (*Ochlerotatus cinereus*), AY090058, AY090059 (*Acanthocyclops vernalis*), AY090060 (*Cyclops venustoides*); *Amblyospora connecticus* (*Ochlerotatus cantator*, *Acanthocyclops vernalis*) – AF025685; *Amblyospora crenifera* (*Ochlerotatus crenifera*) – AY090061: *Amblyospora excrucii* – AY090043 (*Ochlerotatus excrucians*), AY090044 (*Acanthocyclops vernalis*); *Amblyospora ferocious* (*Psorophora ferox*) – AY090062; *Amblyospora indicola* (*Culex sitiens*) – AY090051; *Amblyospora khaliulini* – AY090045 (*Ochlerotatus communis*), AY090046, AY090047 (*Acanthocyclops vernalis*); *Amblyospora opacita* (*Culex territans*) – AY090052; *Amblyospora salinaria* (*Culex salinarius*) – AY326270, U68474; *Amblyospora sticticus* (*Ochlerotatus sticticus*) – AY090049; *Amblyospora stimuli* – AF027685 (*Ochlerotatus stimulans*), AY090050 (*Diacyclops bicupidatus*); *Amblyospora weiseri* (*Ochlerotatus cantans*) – AY090048; *Amblyospora* sp. (*Culex nigripalpus*) – AY090053; *Amblyospora* sp. (*Cyclops strenus*) – AY090055.

References: Kudo (1922), Kellen and Lipa (1960), Welch (1960), Franz and Hagman (1962), Kellen (1962), Kellen and Wills (1962a, 1962b); Kudo and Daniels (1963), Kellen et al. (1965, 1966a, 1966b, 1967); Wills and Beaudoin

(1965), Chapman et al. (1966, 1967, 1969, 1973); Bailey et al. (1967), Anderson (1968), Tsai et al. (1969), Hazard and Oldacre (1975), Andreadis and Hall (1979a, 1979b); Hazard et al. (1979), Lipa and Bartkowski (1981), Lord et al. (1981), Weiser and Prasertphon (1981), Andreadis (1983a, 1983b, 1985a, 1985b, 1988a, 1988b, 1989a, 1989b, 1990, 1991, 1993, 1994a, 1999, 2005); Lord and Hall (1983a, 1984); Hazard and Brookbank (1984), Sabwa et al. (1984), Vavra et al. (1984), Hall (1985, 1990); Larsson (1985), Sweeney et al. (1985, 1988, 1989a, 1989b, 1990a, 1990b); Toguebaye and Marchand (1985, 1986a, 1986b); Hall and Washino (1986), Goettel (1987), Becnel and Sweeney (1990), Dickson and Barr (1990), Lukes and Vavra (1990), Darwish and Canning (1991), Becnel (1992b, 1994); Diarra and Toguebaye (1992, 1994, 1997), Kilochitskii (1992a, 1992b, 1995, 1996), Garcia and Becnel (1994), White et al. (1994), Chen and Barr (1995), Flegel and Pasharawipas (1995), Larkin et al. (1995), Baker et al. (1997, 1998); Becnel and Andreadis (1998), Micieli et al. (1998, 2000a, 2000b, 2001, 2003), Vossbrinck et al. (1998, 2004); Pankova et al. (2000), Simakova and Pankova (2005).

Anncaliia Issi, Krylova and Nicolaeva 1993 (Figs. 2, 4Q)

Type species: *Anncaliia meligethi* (Issi and Radishcheva 1979) Issi, Krylova and Nicolaeva 1993.

Type host: *Megligethes aeneus* (Coleoptera, Nitidulidae).

Mosquito host range: *Aedes*, *Anopheles*, *Armigeres*, *Culex*, *Wyeomyia*.

Number species from mosquitoes: 2 – *Anncaliia algerae* (syn. *Nosema algerae* Vavra and Undeen, 1970; syn. *Brachiola algerae* Lowman, Takvorian, and Cali, 2000) Franzen, Nassonova, Scholmerich and Issi, 2006 (original host = *An. stephensi*); *Anncaliia gambiae* (syn. *Nosema stegomyiae* Fox and Weiser, 1959; syn. *Brachiola stegomyiae* Weiser and Zizka, 2004) Franzen, Nassonova, Scholmerich and Issi, 2006 (original host = *An. gambiae*).

Natural geographical distribution: Africa (Liberia, Nigeria), Asia (India, Pakistan), Australia (Cairns), North America (El Salvador).

Life cycle and transmission: These microsporidia are monomorphic, have a comparatively simple developmental cycle and produce a single spore type (Fig. 2). Spores are typically ellipsoidal with rounded poles (3.0–4.3 μm × 1.8–2.5 μm) or oval (2.5–3.0 μm × 1.5–2.0 μm) (Fig. 4Q). Horizontal transmission occurs via oral ingestion of spores. Vertical transmission may also occur via oral ingestion of spore contaminated eggs (transovum) but there is no separate developmental cycle leading to ovarian infection in the female host. Transmission in insectaries is common; spores are released by adults in fecal pellets and saliva during feeding and transmission typically occurs when adults feed on contaminated sugar solutions.

Site of infection and pathology: *Anncaliia algerae* infects a variety of tissues of both larvae and adults and is usually pathogenic for its host. Tissues invaded vary with the host. In general almost all tissues (fat body, gut, Malpighian tubules, muscle, nerve, salivary glands) are attacked in *Anopheles* and *Culex*, but only the nerve tissues in *Ae. aegypti*. Heavily infected anopheline larvae can be recognized by opaque white areas visible through the cuticle. Lightly infected larvae pupate normally, while heavily infected larvae usually die before, during, or after pupation. There is a sharp reduction in the longevity and fecundity of infected adult survivors and infected anophelines have a reduced capacity to transmit malaria. *Anncaliia gambiae* infects most tissues of adults, destroying the connective tissue, fat body, hypoderm, midgut, and Malpighian tubules.

Host specificity: *Anncaliia algerae* has a very broad host range that in addition to mosquitoes includes Coleoptera, Hemiptera, Lepidoptera, and Digena (oral); Decapoda, Megaloptera, Odonata, Orthoptera, and Rodentia (injection). It has also been isolated from humans where it is capable of causing fatal disseminated disease.

Epizootiology and field prevalence: *Anncaliia algerae* has mostly been found in laboratory colonies of anopheline mosquitoes. However, it has been recorded at very low incidences in *An. gambiae* from Liberia and Nigeria, *An. stephensi* from India, *An. albimanus* from El Salvador, and *Cx. sitiens* from Australia.

Field introductions: Field trials have been conducted with *A. algerae* against *An. albimanus* in Panama via release of spores (2.15×10^7 to 2.15×10^9 spores per m^2) in natural breeding areas. Infection rates in larvae were dose dependant and ranged from 16% to a high of 86% (after 2 wk) in a site treated 4 times at the highest dosage rate. A similar study was conducted in pools seeded with *An. stephensi* larvae in Pakistan. Slight reductions were seen in the number of early instars with infection rates ranging from 31–50%. However, no long-term reductions in larval populations were obtained. In both studies the loss in spore activity was generally attributed to rapid settling of spores to the bottom of the treated pools.

NCBI GenBank® nucleotide accession numbers: *Ancaliia algerae* (*Anopheles stephensi*) – AF069063, AY963290.

References: Fox and Weiser (1959), Alger and Undeen (1970), Canning and Hulls (1970), Vavra and Undeen (1970), Hazard and Lofgren (1971), Hulls (1971), Reynolds (1971), Savage et al.

(1971), Anthony et al. (1972, 1978a, 1987b); Ward and Savage (1972), Canning and Sinden (1973), Undeen and Alger (1975), Van Essen and Anthony (1976), Bai et al. (1979), Gajanana et al (1979), Haq et al (1981), Avery and Anthony (1983), Fournie et al. (1990), Henn et al. (1998), Weiser and Zizka (2004), Franzen et al. (2006).

***Crepidulospora* (Simakova, Pankova and Issi, 2003) Simakova, Pankova and Issi, 2004**

Type species: *Crepidulospora beklemishevi* (Simakova, Pankova and Issi, 2003) Simakova, Pankova and Issi, 2004.

Type host: *Anopheles beklemishevi* Stegnii and Kabanova.

Mosquito host range: Unknown.

Number species from mosquitoes: 1 – monotypic.

Natural geographical distribution: Asia (Russia – West Siberia).

Life cycle and transmission: Spores found in larvae are formed in groups of 8 in a non-persistent sporophorous vesicle and are held together by micro fibular structures. They are ''sandals-like'' and broadly oval (4.2 μm × 2.0 μm) with a bottleneck constriction posteriorly, similar to *Parathelohania*. The mechanisms and pathways of transmission are unknown but infections in larvae are likely to result from transovarial transmission.

Site of infection and pathology: Infections have been described from the fat body tissue of larvae only, presumably resulting in death during the 4th stadium.

Host specificity: Unknown.

Epizootiology and field prevalence: This microsporidium has been sporadically found in larval *An. beklemishevi* inhabiting permanent riparian pools in West Siberia with very low prevalence rates.

NCBI GenBank® nucleotide accession numbers: None.

References: Simakova et al. (2003, 2004).

***Cristulospora* Khodzhaeva and Issi, 1989**

Type species: *Cristulospora sherbani* Khodzhaeva and Issi, 1989.

Type host: *Culex modestus* Ficalbi.

Mosquito host range: *Culex, Ochlerotatus*.

Number species from mosquitoes: 3 – *Cristulospora aedis* Khodzhaeva and Issi, 1989 (host = *Oc. caspius*); *Cristulospora cadyrovi* Khodzhaeva and Issi, 1989 (host = *Cx. pipiens*); *C. sherbani* (host = *Cx. modestus*).

Natural geographical distribution: Asia (Uzbekistan).

Life cycle and transmission: The species is reported to undergo dimorphic development similar to *Amblyospora*. Two sporulation sequences are known; one in adult females producing single, binucleated, oval-cylindrical spores (6.3–11.8 μm × 2.5–5.0 μm); and a second in larvae producing uninucleated spores (5.6–6.8 μm × 3.7–5.0 μm) with distinct ''plume-like appendages'' on both poles that are formed in groups of 8 within a sporophorous vesicle. The methods of transmission are unknown but transovarial transmission is likely.

Site of infection and pathology: Infections are localized within the genital ducts of adult females and presumably in the fat body tissue of larvae. The pathology has not been established but is likely to result in death in larvae.

Host specificity: Unknown.

Epizootiology and field prevalence: Natural prevalence rates of 16% have been observed in *Cx. modestus* larvae and 13.8% in adult females infected with *C. sherbani* from permanent pools in Uzbekistan. A prevalence rate of 12.5% has been similarly observed in *Cx. pipiens* infected with *C. aedis*.

NCBI GenBank® nucleotide accession numbers: None.

References: Khodzhaeva and Issi (1989).

***Culicospora* Weiser, 1977 (Figs. 2, 4H)**

Type species: *Culicospora magna* (Kudo, 1920) Weiser, 1977.

Type host: *Culex pipiens* L. (likely misidentified *Culex restuans* Theobald).

Mosquito host range: *Anopheles*, *Culex*, *Ochlerotatus*.

Number species from mosquitoes: 1 (one additional species from a blackfly, Simulidae).

Natural geographical distribution: North America (USA).

Life cycle and transmission: This microsporidium exhibits dimorphic development (Fig. 2) producing single, oblong-ovate, slightly bent binucleated spores (11.0 μm × 4.4 μm) in adult females; and lanceolate or elongate-pyriform, uninucleated spores (12.0–16.5 μm × 3.3–4.6 μm) in larvae (Fig. 4H). Transovarial transmission via the binucleated spores from adult females to larval progeny is well established and results in the formation of the lanceolate spores in the filial generation. These spores are orally infectious to other larvae and are involved in direct horizontal transmission resulting in the formation of binucleated spores in adult females to complete the life cycle.

Site of infection and pathology: Infections occur in the fat body tissue, oenocytes and ovaries of adult females producing no demonstrable pathology. Infections in larvae of the filial generation are mainly in the fat body but can also be found in the esophageal valve and hind gut. The microsporidium kills slowly and infected larvae usually succumb during the 4th stadium.

Host specificity: Naturally acquired infections have been reported from *An. stephensi*, *Oc. sierrensis* and 3 species of *Culex* (*Cx. pipiens*, *Cx. restuans*, and *Cx. territans*). Infections have been orally transmitted to *Cx. pipiens*, *Cx. restuans* and *Cx. territans* larvae exposed to spores in the laboratory but no infections have been achieved in similar feeding trials with *Ae. aegypti*, *An. quadrimaculatus*, *Cx. salinarius*, *Cx. quinquefasciatus*, *Culiseta inornata*, *Oc. sierrensis*, or *Oc. triseriatus*.

Epizootiology and field prevalence: Natural infections ranging from 1–45% have been recorded in field collected *Cx. restuans* larvae, 26% in *Oc. sierrensis* adults, 80% in larvae of *Cx. pipiens*/*Cx. restuans* reared from a single egg raft.

NCBI GenBank® nucleotide accession numbers: *Culicospora magna* (*Culex restuans*) – AY326269, AY090054.

References: Kudo (1921, 1925, 1962); Wills and Beaudoin (1965), Bailey et al. (1967), Clark and Fukuda (1967), Anderson (1968), Weiser (1977), Hazard et al. (1985), Becnel et al. (1987, 1994).

***Culicosporella* Weiser, 1977 (Fig. 4I)**

Type species: *Culicosporella lunata* (Hazard and Savage, 1970) Weiser, 1977.

Type host: *Culex pilosis* (Dyar and Knab).

Mosquito host range: Unknown.

Number species from mosquitoes: 1 – monotypic.

Natural geographical distribution: North America (USA – Florida).

Life cycle and transmission: The microsporidium is polymorphic and 3 sporulation sequences are involved in the life cycle. A small oblong-ovoid binucleated spore (2.4 μm × 1.4 μm) is formed in adult females and is presumed to be responsible for transovarial transmission of infection to larval progeny. Two sporulation sequences occur in larvae; one resulting in large, lanceolate, binucleated spores (7.1 μm × 3.8 μm) (Fig. 4I) and a second that usually aborts but may produce uninucleated "meiospores" (3.9 μm × 4.5 μm) enclosed in a sporophorous vesicle (usually fewer than 8). The binucleated lanceolate spores are involved in horizontal transmission and are orally infectious to mosquito larvae.

Site of infection and pathology: Infections occur in hemocytes and fat body tissue of larvae usually resulting in death during the 4th stadium. Surviving females harbor infections in the ovaries.

Host specificity: Unknown.

Epizootiology and field prevalence: A prevalence rate of 23% was documented in larvae of *Cx. pilosis* collected from a roadside ditch near Gainesville, FL in the original collection.

NCBI GenBank® nucleotide accession numbers: *Culicosporella lunata* (*Culex pilosus*) – AF027683.

References: Hazard and Savage (1970), Weiser (1977), Hazard et al. (1984), Becnel and Fukuda (1991).

***Dimeiospora* Simakova, Pankova and Issi, 2003**

Type species: *Dimeiospora palustris* Simakova, Pankova and Issi, 2003.

Type host: *Ochlerotatus punctor* Kirby.

Mosquito host range: Unknown.

Number species from mosquitoes: 1 – monotypic.

Natural geographical distribution: Asia (Russia – West Siberia).

Life cycle and transmission: The species is dimorphic producing 2 morphologically different spore types in larval hosts; an oviform uninucleated spore (6.1 μm × 4.9 μm) and a broadly-ovate uninucleated spore (4.6 μm × 3.7 μm) both formed in groups of 8 and enclosed in a sporophorous vesicle. Ultrastructural differences in the spore wall, polaroplast, and polar tube of these 2 spores have also been documented. The mechanisms and pathways of transmission are unknown.

Site of infection and pathology: Infections occur in the fat body tissue of larvae and kill the host during the 4th stadium.

Host specificity: Unknown.

Epizootiology and field prevalence: This microsporidium has been sporadically found in larval *Oc. punctor* populations inhabiting sphagnum bogs in West Siberia. Prevalence rates are reported to be very low.

NCBI GenBank® nucleotide accession numbers: None.

References: Simakova et al. (2003).

***Duboscqia* Perez, 1908 (Fig. 2)**

Type species: *Duboscqia legeri* Perez, 1908.

Type host: *Reticulotermes lucifugus* Rossi (Isoptera: Rhinotermitidae).

Mosquito host range: *Anopheles*, *Ochlerotatus*.

Number species from mosquitoes: 2 – *Duboscqia aediphaga* Kettle and Piper, 1988 (host = *Oc. vigilax*); *Duboscqia dengihilli* Sweeney, Doggett and Piper, 1993 (host = *An. hilli*).

Natural geographical distribution: Australia.

Life cycle and transmission: Members of this genus, which infect mosquitoes, have a life cycle that is very similar to *Amblyospora* and *Parathelohania*. They undergo obligatory development in an intermediate (cyclopoid) copepod host and exhibit polymorphic development with the formation of 3 different spore types: a clavate, slightly curved, uninucleated spore (8.8 μm × 3.0 μm) in the copepod; a broadly rounded, binucleated spore (7.4 μm × 4.6 μm) in adult (male and female) mosquitoes; and a broadly oval to ellipsoid, uninucleated "meiospore" (5.0 μm × 2.8 μm), formed in groups of 16 (in a sporophorous vesicle)

in larval mosquitoes. Transovarial transmission of infection to larval progeny takes place via binucleated spores formed in adult female mosquitoes. Parasite development in larval progeny is mostly progressive in both males and females usually leading to death during the 4th instar. Horizontal transmission of infection from mosquito larvae to copepods occurs via oral ingestion of ''meiospores'' that are liberated from larval cadavers. Horizontal transmission of infection from copepods to larval mosquitoes is similarly facilitated via oral ingestion of extracellular uninucleated spores that are released into the aquatic habitat with the death of infected copepods.

Site of infection and pathology: Infections occur in the oenocytes and ovaries of adult female mosquitoes producing no demonstrable pathology. Infections in larval mosquitoes of the filial generation are localized in the fat body. Infected larvae of *Oc. vigilax* develop a marked reddish coloration in the thorax and/or opaque white patches. The microsporidium kills slowly and infected larvae die in the 4th instar or on pupation, or alternatively give rise to abnormal pupae in which the abdomen is greatly swollen and the respiratory horn poorly developed. The site of infection and pathology in copepods has not been reported.

Host specificity: Unknown.

Epizootiology and field prevalence: A prevalence rate of less than 1% was estimated for *D. aediphaga* infection in *Oc. vigilax* larvae collected from temporary brackish pools in coastal marshes.

NCBI GenBank® nucleotide accession numbers: None.

References: Kettle and Piper (1988), Sweeney et al. (1993).

***Edhazardia* Becnel, Sprague and Fukuda 1989 (Figs. 2, 4G)**

Type species: *Edhazardia aedis* (Kudo, 1930) Becnel, Sprague and Fukuda 1989.

Type host: *Aedes aegypti* L.

Mosquito host range: *Ae. aegypti*.

Number species from mosquitoes: 1 – monotypic.

Natural geographical distribution: Asia (Thailand), North America (Puerto Rico).

Life cycle and transmission: This microsporidium has a characteristic *Amblyospora*-like life cycle but an intermediate host is not involved (Fig. 2). It exhibits polymorphic development and produces 4 different spore types: an ovate, ''early,'' binucleated spore (6.7 μm × 4.5 μm) that is formed in the gastric caeca of larval stages and is responsible for autoinfection; a second larger, oblong-ovate, slightly bent, binucleated spore (9.1 μm × 3.4 μm) that develops in the oenocytes and oocytes of adult females and is responsible for transovarial transmission; a pyriform-lanceolate, uninucleated spore (8.3 μm × 4.5 μm) that is formed in the fat body of larval stages, is orally infectious to other mosquito larvae and responsible for horizontal transmission (Fig. 4G); and a broadly oval ''meiospore'' (7.6 μm × 6.0 μm) that is usually abortive.

Site of infection and pathology: Infections are initially established in the gastric caeca of larvae following oral ingestion of the uninucleated pyriform spore. This results in the formation of early binucleated spores that are responsible for dispersal of the microsporidium to the oenocytes, and transstadial transmission to adult stages where the second binucleated spore is formed in the ovaries. The effects of these infections are sub-lethal. Infected adults have significantly smaller body sizes and females exhibit reduced fecundity and egg hatch when compared to uninfected controls. Transovarially transmitted infections that result in the production of the orally infectious pyriform spores are localized within the fat body of larval progeny. These infections kill slowly and most larvae typically die during the 4th stadium.

Host specificity: This microsporidium appears to be specific for *Ae. aegypti*. Experimental infections have been achieved in several alternate mosquito hosts following exposure to uninucleated spores. These include: *Ae. albopictus*, *Ae. vexans*, *Anopheles quadrimaculatus*, *Ochlerotatus atropalpus*, *Oc. taeniorychus*, *Oc. triseriatus*, *Orthopodomyia signifera*, and *Toxorrhynchites rutilus rutilus*. However, the normal developmental sequence of ovarian infection and transovarial transmission does not occur and the entire life cycle cannot be completed.

Epizootiology and field prevalence: Although this microsporidium has been well studied in the laboratory, no data are available on its prevalence in natural field populations of *Ae. aegypti*.

Field introductions: The effectiveness of *E. aedis* to control a semi-natural population of *Ae. aegypti* in a large screened enclosure was evaluated over a 2-year period in Florida. In the 1st year, inoculative release of the microsporidium via infected pupae that were allowed to emerge as adults resulted in dispersal of the parasite via transovarial transmission to all larval breeding containers over a 20-wk period. However, *E. aedis* did not survive the winter in sufficient numbers to become reestablished. In the 2nd year, an inundative release of *E. aedis* via infected larvae (containing uninucleated spores) produced high larval (46%) and adult infections, with a 76% vertical infection rate in progeny, and successfully eliminated the mosquito population within 11 wk.

NCBI GenBank® nucleotide accession numbers: *Edhazardia aedis* (*Aedes aegypti*) – AF027684.

References: Kudo (1930), Hembree (1979, 1982); Hembree and Ryan (1982), Becnel et al. (1989, 1995); Becnel (1992b, 1994); Becnel and Undeen (1992), Nasci et al (1992), Becnel and Johnson (1993, 2000); Undeen et al (1993), Andreadis (1994b), Agnew and Koella (1997, 1999); Johnson et al. (1997), Koella and Agnew (1997, 1999); Koella and Offenberg (1999).

***Golbergia* Weiser, 1977**

Type species: *Golbergia spinosa* (Golberg, 1971) Weiser, 1977.

Type host: *Culex pipiens* L.

Mosquito host range: Unknown.

Number species from mosquitoes: 1 – monotypic.

Natural geographical distribution: Europe (Russia).

Life cycle and transmission: This microsporidium undergoes dimorphic development producing 2 morphologically distinct spore types: an elongated, ''pear-like'' (pyriform), uninucleated spore (5.4–6.6 μm × 2.4–2.7 μm) that is produced in groups of 4, 8, 12 and 16 within a sporophorous vesicle; and a binucleated spore (approx. same size and also produced in groups of 4–16) that is flattened at the narrow end and ornamented with ridges and nail-like protrusions at the broad end. The microsporidium is orally infectious to larvae, but it is unclear which of the 2 spores is involved.

Site of infection and pathology: Infections occur in the fat body and salivary glands of larvae, pupae and rarely adults. The pathology associated with infection has not been reported.

Host specificity: Unknown.

Epizootiology and field prevalence: The microsporidium was isolated from a collection of *Cx. pipiens* larvae found in the suburbs of Moscow, but prevalence rates were not reported. Development of the binucleated spore is apparently seasonal and is reported to occur in the host during the autumn.

NCBI GenBank® nucleotide accession numbers: None.

References: Golberg (1971), Weiser (1977).

***Hazardia* Weiser, 1977 (Fig. 4N)**

Type species: *Hazardia milleri* (Hazard and Fukuda, 1974) Weiser, 1977.

Type host: *Culex pipiens quinquefasciatus* Say.

Mosquito host range: *Culex*, *Anopheles*.

Number species from mosquitoes: 1 (an additional undescribed but molecularly distinct species has been isolated from *An. crucians*).

Natural geographical distribution: North America (USA – Florida, Louisiana, Texas), Asia (Thailand).

Life cycle and transmission: This microsporidium is polymorphic and produces 3 different spore types in the larval mosquito host: A small, oval, thin-walled, binucleated spore (3.5 μm × 2.0 μm) that is rarely seen; a lanceolate, binucleated spore with a rugose surface (4.9 μm × 2.5 μm); and a predominant lanceolate to elongate-pyriform, thin-walled, uninucleated spore (4.9 μm × 2.5 μm) that is formed in groups of 2–16 (usually 8) (Fig. 4N). Horizontal transmission occurs via oral ingestion of uninucleated spores.

Site of infection and pathology: Infections initially occur in the hemocytes of larvae and then spread to fat body tissue throughout the thorax and abdomen. Infections appear as grayish-white cysts under the cuticle and the microsporidium kills slowly. Lightly infected larvae often pupate and survive to emerge as adults.

Host specificity: Susceptibility of *H. milleri* appears to be limited to *Culex* spp. It has been experimentally transmitted to *Cx. pipiens pipiens*, *Cx. salinarius*, *Cx. territans*, and *Cx. tarsalis* following exposure to spores in the laboratory. No infections have been achieved with any species of *Aedes*, *Anopheles*, *Culiseta*, *Ochlerotatus*, *Psorpohora* or *Uranotaenia*. An undescribed but molecularly distinct species has been isolated from *An. crucians*.

Epizootiology and field prevalence: Natural infections have been detected in larval *Cx. p. quinquefasiatus* collected in Texas (in the fall) and Louisiana, and from *Cx. p. fatigans* collected in Bangkok, Thailand but prevalence rates were not reported.

Field introductions: *Hazardia milleri* was established for a short period of time (58 days) in a natural population of *Cx. p. quinquefasciatus* following the release of naturally infected larvae into a site in Texas that was fed by sewage effluent, but did not survive drying. The parasite survived over 2 months in the same host mosquito following a similar introduction into a artificial container habitat.

NCBI GenBank® nucleotide accession numbers: *Hazardia milleri* (*Culex pipiens quinquefasciatus*) – AY090067; *Hazardia* sp. (*An. crucians*) – AY090066.

References: Hazard and Fukuda (1974), Miller and Scanlon (1976), Weiser (1977), Hazard et al. (1985).

***Hyalinocysta* Hazard and Oldacre, 1975 (Figs. 2, 3A, 3C, 4E, 4F)**

Type species: *Hyalinocysta chapmani* Hazard and Oldacre, 1975.

Type host: *Culiseta melanura* Coquillett.

Mosquito host range: *Culiseta*.

Number species from mosquitoes: 1 (one additional species from a blackfly, *Simulium ornatum* – *Hyalinocysta expilatoria*).

Natural geographical distribution: North America (USA – Connecticut and Louisiana).

Life cycle and transmission: This microsporidium undergoes obligatory development in an intermediate (cyclopoid) copepod host, has dimorphic development, and produces a different spore type in each host (Fig. 2): an ovoid, uninucleated "meiospore" (4.5 μm × 2.8 μm) that is formed in groups of 8 in a sporophorous vesicle in the mosquito host (Fig. 4E); and a larger ovoid, uninucleated spore (5.3 μm × 3.5 μm) that is formed in the copepod host (Fig. 4F). Horizontal transmission of infection from copepod to mosquito and visa-versa occurs via oral ingestion of spores formed in each respective host. There is no developmental sequence leading to ovarian infection in the mosquito host and transovarial transmission does not occur.

Site of infection and pathology: This microsporidium exhibits high tissue specificity and delayed pathogenicity that is synchronized with development of each host. Infections are confined to fat body tissue in the larval mosquito host. Parasite multiplication proceeds slowly as the host larva develops and mortality generally takes place just prior to pupation. Heavily infected 4th instars have a typical opaque white color when viewed against a black background (Fig. 3A). Parasite development and reproduction in the copepod host proceed more rapidly in accordance with copepod development and are restricted to the ovaries and oviducts of female stages which are prevented from forming eggs and eventually die. Heavily infected copepods appear orange when viewed against a white background (Fig. 3C).

Host specificity: Unknown.

Epizootiology and field prevalence: The microsporidium is maintained in a continuous cycle of horizontal transmission between each host that occurs in subterranean habitats and extends from April to November in the northeastern USA The microsporidium overwinters in diapausing *Cs. melanura* larvae (prevalence rate 10%) and horizontal transmission of infection to copepods (*Orthocyclops modestus*) is initiated in the spring. Subsequent transmission to mosquito larvae ensues with peak infection rates of 48–60%.

NCBI GenBank® nucleotide accession numbers: *Hyalinocysta chapmani* – AF483837 (*Culiseta melanura*), AF483838 (*Orthocyclops modustus*).

References: Hazard and Oldacre (1985), Andreadis (2002, 2005); Andreadis and Vossbrinck (2002).

Intrapredatorus Chen, Kuo and Wu 1998 (Fig. 4O)

Type species: *Intrapredatorus barri* Chen, Kuo and Wu 1998.

Type host: *Culex fuscanus* Wiedemann.

Mosquito host range: Unknown.

Number species from mosquitoes: 1 – monotypic.

Natural geographical distribution: Asia (Taiwan).

Life cycle and transmission: This microsporidium undergoes concurrent dimorphic development in the larval mosquito host producing 2 different spore types: an oval, uninucleated, "meiospore" (7.4 μm × 4.6 μm) that is formed in groups of 8 in a sporophorous vesicle (predominant) (Fig. 4O, top); and a lanceolate, uninucleated spore (scant) (8.1 μm × 4.4 μm) (Fig. 4O, bottom). The method(s) of transmission are unknown.

Site of infection and pathology: All development takes place in the fat body of larvae presumably resulting in death.

Host specificity: Unknown.

Epizootiology and field prevalence: This microsporidium was originally described from *Cx. fuscanus* larvae collected from an artificial tank in Liu-Chui Islet, Ping-Tung, Taiwan but no prevalence rates of infection were given.

NCBI GenBank® nucleotide accession numbers: *Intrapredatorus barri* (*Culex fuscanus*) – AY013359.

References: Chen (1998), Chen et al. (1998), Nilsen and Chen (2001).

Krishtalia Kilochitskii, 1997

Type species: *Krishtalia pipiens* Kilochitskii, 1997.

Type host: *Culex pipiens pipiens* L.

Mosquito host range: *Cx. p. pipiens*, *Cx. pipiens* form *molestus*, *Cx. theileri*.

Number species from mosquitoes: 1– monotypic.

Distribution: Europe (Ukraine).

Life cycle and transmission: This microsporidium is reported to undergo dimorphic development producing 2 different spore types in larvae, pupae and adults; uninucleated thin walled pyriform spores (4.0–6.3 μm × 1.9–2.5 μm); and binucleated thick walled oval spores (4.4–6.5 μm × 2.3–2.5 μm) that are joined together by "mucose strands on spurs" on the posterior ends. Horizontal transmission to *Cx. pipiens* form *molestus* larvae has been achieved in the laboratory (up to 60%) but it is not know which spore type is responsible. Transovarial transmission has not been demonstrated but is presumed to occur.

Site of infection and pathology: Infections occur in the fat body tissue of larvae that characteristically appear opaque white. Infections in adults (both sexes) are found throughout the body cavity. They develop swollen abdomens and exhibit reduced activity.

Host specificity: Unknown.

Epizootiology and field prevalence: Infected *Cx. p. pipiens* larvae have been found in artificial container habitats with heavily polluted water in

the Ukraine. Natural prevalence rate ranging from 5–10% are reported from 4th instars.

NCBI GenBank® nucleotide accession numbers: None.

References: Kilochitskii (1997, 2002).

***Merocinta* Pell and Canning, 1993**

Type species: *Merocinta davidii* Pell and Canning, 1993.

Type host: *Mansonia africana* (Theobald).

Mosquito host range: Unknown.

Number species from mosquitoes: 1 – monotypic.

Natural geographical distribution: Africa (Tanzania).

Life cycle and transmission: This microsporidium is dimorphic and produces 2 different spore types: a small oval, slightly flattened, uninucleated spore (2.5 μm × 1.5 μm) that is formed in groups of 40–60 within a sporophorous vesicle in larval progeny following transovarial transmission; and an oval, binucleated spore (3.4 μm × 1.8 μm) that is formed in the ovaries of adult females and is responsible for transovarial transmission. Horizontal transmission is unknown.

Site of infection and pathology: Infections in host larvae are concentrated around the midgut and these individuals show no overt symptoms even when heavily infected. Infections also occur in the ovaries of adult females.

Host specificity: Unknown

Epizootiology and field prevalence: Natural prevalence rates of infection ranging from 2.5–5.7% have been reported in larval populations of *Ms. africana* from 3 field sites in Tanzania. A prevalence rate of 10.9% has been additionally observed in field-caught adult females from the same locale, and 11.3% of progeny reared from these infected females develop infections within the midgut as a result of transovarial transmission.

NCBI GenBank® nucleotide accession numbers: None.

References: Pell and Canning (1993).

***Parathelohania* Codreanu, 1966 (Figs. 2, 4J,K)**

Type species: *Parathelohania legeri* (Hesse, 1904) Codreanu, 1966.

Type host: *Anopheles maculipennis* Meigen.

Mosquito host range: *Aedeomyia*, *Anopheles*, *Ochlerotatus*.

Number species from mosquitoes: 22.

Natural geographical distribution: Africa, Asia, Europe, New Zealand, North America, South America.

Life cycle and transmission: These microsporidia have a life cycle that is very similar to *Amblyospora* (Fig. 2). It involves an intermediate (cyclopoid) copepod host and polymorphic development with the formation of 4 different spore types: an elongated, lanceolate, uninucleated spore (11.3 μm × 2.8 μm) in the copepod (Fig. 4K); an ovoid, uninucleated ''meiospore'' (3.1–8.0 μm × 2.1–4.2 μm) that possesses a ''bottleneck-like'' posterior extension of the spore wall, that is formed in groups of 8 (in a sporophorous vesicle) in larval mosquitoes (Fig. 4J); and a cylindrical and oval binucleated spore (3.0–6.1 μm × 1.5–3.2 μm) in adult female mosquitoes. Transovarial transmission of infection to larval progeny appears to be universal and takes place via binucleated spores formed in adult female mosquitoes. Parasite development in larval progeny is typically dimorphic and in many species is dependent on the host sex: progressive in males leading to death, and benign in females leading to ovarian infection and transovarial transmission. Horizontal transmission of infection from mosquito larvae to copepods occurs via oral ingestion of ''meiospores'' that are liberated from larval cadavers. Horizontal transmission of infection from copepods to larval mosquitoes is similarly facilitated via oral ingestion of extra-cellular uninucleated spores that are released into the aquatic habitat with the death of infected copepods.

Site of infection and pathology: Infected copepods are identifiable by a dorsal opaque white band in the metasome that is visible against a black background. Infections appear systemic but the specific tissue where development occurs has not been acsertained. Parasite development in male larval mosquito hosts with transovarial infections leading to meiospores occurs within in the oenocytes and fat body tissue. This typically results in death just prior to pupation. Heavily infected larvae appear opaque white when viewed against a black background. Infections in females leading to binucleated spores and transovarial transmission are confined to the oenocytes and ovaries in the adult stage with no discernable pathology.

Host specificity: Unknown.

Epizootiology and field prevalence: Natural prevalence rates ranging from 3.7–23.5% have been reported from field populations of larval *An. quadrimaculatus* infected with *P. anophelis* in rice fields in Louisiana, USA; 3.5% in *Oc. australis* infected with *Parathelohania barra* in brackish water pools in New Zealand; 10% in *An. evansae* infected with *Parathelohania evansae* from a freshwater lagoon in Argentina during October; and 2.0% and 2.6% in *An. messae* and *An. beklemishevi* infected with *Parathelohania divulgata* in Russia and Kazakhstan.

NCBI GenBank® nucleotide accession numbers: *Parathelohania anophelis* (*Anopheles quadrimaculats*) – AF027682, L28969; *Parathelohania obesa* (*Anopheles crucians*) – AY090065.

References: Hesse (1904), Kudo (1924, 1929); Missiroli (1929), Sen (1941), Kellen and Wills

(1962), Wills and Beaudoin (1965), Anderson (1968), Hazard and Weiser (1968), Pillai (1968), Hazard and Anthony (1974), Simmers (1974), Hazard and Oldacre (1975), Hazard et al. (1979), Weiser and Prasertphon (1981), McLaughlin et al. (1988), Avery (1989), Avery and Undeen (1990), Pankova et al. (1991), Garcia and Becnel (1994), Garcia et al. (1993), Kilochitskii (1998), Osborne (2002), Simakova and Pankova (2004a).

***Pilosporella* Hazard and Oldacre, 1975 (Fig. 4L)**

Type species: *Pilosporella fishi* Hazard and Oldacre, 1975.

Type host: *Wyeomyia vanduzeei* Dyar and Knab.

Mosquito host range: *Ochlerotatus*, *Wyeomyia*.

Number species from mosquitoes: 2 – *Pilosporella chapmani* Hazard and Oldacre, 1975 (host = *Ochlerotatus triseriatus*), *P. fishi* (host = *Wy. vanduzeei*).

Natural geographical distribution: North America (USA – Connecticut, Florida, and Louisiana).

Life cycle and transmission: This microsporidium is dimorphic and produces 2 different spore types: a subspherical uninucleated spore (2.3–3.1 μm) that is formed in groups of 8 within a sporophorous vesicle in larval hosts (Fig. 4L); and a slender elongated binucleated spore (3.2–5.1 μm × 1.4–1.7 μm) that is formed in adults and is responsible for transovarial transmission to progeny. Horizontal transmission is unknown.

Site of infection and pathology: Infections in larvae are confined to fat body tissue. Pathology is variable depending on the host. *Pilosporella chapmani* infection in *Oc. triseriatus* appears as small, distended opaque whitish patches in the 6th abdominal segment (rarely in the head or 2nd abdominal segment) and is not usually lethal to the host. *Pilosporella fishi* by contrast, causes more extensive pathology in *Wy. vanduzzeei* that often results in death during late larval instars and during pupation. *Pilosporella fishi* also causes greater mortality of female than of male immature *Wy. vandusseei*. Some infected individuals survive to become adults where infections leading to the production of the binucleated spores are found in the oenocytes.

Host specificity: Unkown.

Epizootiology and field prevalence: Infected larvae of *Oc. triseriatus* have been found in flower urns (LA) and used tire casings (CT, LA) in July, but prevalence rates have not been reported for this species. A natural prevalence rate of 0.14% was reported for larvae of *Wy. vanduzzeei* infected with *P. fishi* in leaf axils of bromeliad plants (FL) with no obvious seasonality of occurrence.

NCBI GenBank® nucleotide accession numbers: None.

References: Hazard and Oldacre (1975), Frank and Curtis (1977), Becnel et al. (1986).

***Polydispyrenia* Canning and Hazard, 1982 (Fig. 4P)**

Type species: *Polydispyrenia simulii* Lutz and Spendor, 1908) Canning and Hazard, 1982.

Type host: *Simulium venustum* Say = *Simulium pertinax* Kollar (Diptera: Simuliidae).

Mosquito host range: *Culex*, *Culiseta*, *Ochlerotatus*.

Number species from mosquitoes: 2 – *Polydispyrenia caecorum* (Chapman and Kellen 1967) Canning and Hazard, 1982 (host = *Cs. inornata*), *Polydispyrenia chapmani* (Clark and Fukuda 1971) Canning and Hazard, 1982 (host = *Cx. territans*). An additional undescribed species has been reported from *Oc. sierrensis*.

Natural geographical distribution: North America (USA – California, Louisiana).

Life cycle and transmission: Only one sporulation sequence is known. Spores are uninucleated and are produced in multiples of 8 within a persistent and sometimes thick-walled vesicle or cyst ranging in size from 10–40 μm in diameter. The number of spores within the vesicle is variable ranging from 24 to several hundred. Individual spores are small (2.2–4.0 μm × 1.4–1.7 μm) and subspherical (Fig. 4P). *Polydispyrenia caecorum* is transovarially transmitted in *Cs. inornata*. All attempts to transmit these microsporidia to healthy larvae via exposure to spores from infected tissues or to contaminated water have been unsuccessful.

Site of infection and pathology: *Polydispyrenia caecorum* infections in *Cs. inornata* are confined to the larval gastric caeca, which are difficult to detect in the field, but appear white in late 3rd and 4th instars when examined with a bright light against a black background in the laboratory. Infections are not lethal to any stage of the mosquito host and this microsporidium does not adversely affect the fecundity or life span of adults. *Polydispyrenia chapmani* infections in *Cx. territans* occur in the larval midgut and gastric caeca. The linings of these cells become completely destroyed causing the thorax and abdomen to appear swollen and lighter in color. No infections have been detected in adults. Infections in *Oc. sierrensis* are restricted to posterior portions of the midgut and heavily infected larvae usually die when infected in the 1st or 2nd instar.

Host specificity: Unknown.

Epizootiology and field prevalence: *Polydispyrenia caecorum* has been found in *Cs. inornata* larvae inhabiting salt marsh pools in Louisiana; *P. chapmani* has been found in *Cx. territans* larvae inhabiting cypress swamp in Louisiana; and infected larvae of *Oc. sierrensis* have collected

from natural tree holes in California. Prevalence rates have not been reported.

NCBI GenBank® nucleotide accession numbers: None.

References: Chapman and Clark (1967), Clark and Fukuda (1971), Sanders and Poinar (1976), Canning and Hazard (1982).

***Senoma* Simakova, Pankova, Tokarev and Issi 2005 (Fig. 4S)**

Type species: *Senoma gloulifera* (Issi and Pankova, 1983) Simakova, Pankova, Tokarev and Issi 2005.

Type host: *Anopheles messeae* Fall.

Mosquito host range: Unknown.

Number species from mosquitoes: 1 – monotypic.

Natural geographical distribution: Asia (Russia – Siberia).

Life cycle and transmission: This microsporidium produces ''egg-shaped'' binucleated spores (3.3–4.4 μm × 2.2–2.8 μm) that are formed in groups of 2 and are connected to each other by a homogeneous ''globular'' matrix (Fig. 4S). The method(s) of transmission are unknown but oral transmission is likely owing to the site of infection within the midgut epithelium.

Site of infection and pathology: Infections appear to be confined to the midgut epithelium of larval and pupal stages of the host. Decreased ''locomotory activity'' has been noted in infected larvae. There are no overt symptoms of infection.

Host specificity: The host range of this microsporidium is unknown. It was erroneously reported from *An. maculipennis*.

Epizootiology and field prevalence: This microsporidium is reported to be routinely recovered but at low prevalence rates (0.5%) from larvae and pupae of *An. messeae* developing in flood plain ponds along rivers in Western Siberia.

NCBI GenBank® nucleotide accession numbers: *Senoma gloulifera* (*Anopheles messeae*) – DQ641245.

References: Issia and Pankova (1983), Simakova et al. (2005).

***Trichoctosporea* Larsson, 1994 (Fig. 4T)**

Type species: *Trichoctosporea pygopellita* Larsson, 1994.

Type host: *Aedes vexans* (Meigen).

Mosquito host range: *Aedes*, *Ochlerotatus*.

Number species from mosquitoes: 2 – *Trichoctosporea colorata* (Pankova, 1988) Simakova and Pankova, 2004 (hosts = *Ae. cinereus Oc. eudes*, *Oc. punctor*), *T. pygopellita* (hosts = *Ae. vexans*, *Oc. flavescens*).

Natural geographical distribution: Europe (Sweden), Asia (Russia-Siberia).

Life cycle and transmission: Only one sporulation sequence is known. Mature spores (6.3–7.0 μm × 4.2–4.6 μm) are uninucleated and formed in groups of 8 within a sporophorous vesicle as in *Amblyospora* and *Parathelohania*. They are oval, with a pointed anterior pole, and with as many as 5 fibrous extensions on the spore wall (Fig. 4T). No data are available on transmission but transovarial transmission is likely.

Site of infection and pathology: Infections occur in the fat body tissue of larvae. Infected larvae appear grayish white especially in the thorax region and typically die during the 4th stadium.

Host specificity: Unknown.

Epizootiology and field prevalence: Larvae of *Ae. vexans* infected with *T. pygopellita* have been collected in April and May (but not March, September or October) from small temporary pools in flooded meadows in Scania in the south of Sweden. Prevalence rates are reported to be low with only a few visually infected larvae in a sample of a hundred or more. *T. colorata* and *T. pygopellita* have similarly been found in univoltine *Aedes* and *Ochlerotatus* larvae inhabiting permanent and ephemeral pools in Siberia.

NCBI GenBank® nucleotide accession numbers: None.

References: Larsson (1994), Simakova and Pankova (2004b).

***Tricornia* Pell and Canning, 1992 (Fig. 4M)**

Type species: *Tricornia muhezae* Pell and Canning, 1992.

Type host: *Mansonia africana* (Theobald).

Mosquito host range: Unknown.

Number species from mosquitoes: 1 – monomorphic.

Natural geographical distribution: Africa (Tanzania).

Life cycle and transmission: Only one sporulation sequence is known. Spores are formed in groups of 8 within a sporophorous vesicle as in *Amblyospora* and *Parathelohania*. They are broadly ellipsoid and uninucleated with one end flattened (3.0 μm × 2.2 μm) (Fig. 4M). The spore wall is uniquely ornamented with 3 knob-like projections (one posterior and two anterior) that are only visible at the ultrastructural level. The methods of transmission are unknown.

Site of infection and pathology: Infections are found in the fat body of larval hosts. Overtly infected larvae appear swollen with opaque white thoracic segments.

Host specificity: Unknown.

Epizootiology and field prevalence: Natural prevalence rates ranging from 21.5–6.9% have been reported from larva collected from several pond sites in Tanzania.

NCBI GenBank® nucleotide accession numbers: None.

References: Pell and Canning (1992).

***Vavraia* Weiser, 1977 (Figs. 2, 4R)**

Type species: *Vavraia culicis* (Weiser, 1947) Weiser, 1977.

Type host: *Culex pipiens* L.

Mosquito host range: *Aedes*, *Anopheles*, *Culex*, *Culiseta*, *Ochlerotatus*, *Orthopodomyia*.

Number species from mosquitoes: 1 (a second isolate showing 99% similarity in the 16S rRNA sequence has been identified from a laboratory colony of *An. stephensi*; 6 other species have been described from the following: a brine shrimp, *Artemia* sp. – *Vavraia anostraca*; a caddisfly, *Holocentropus dubius* – *Vavraia holocentropi*; a sandfly, *Lutzomyia longipalpus* – *Vavraia lutzomyiae*; a shrimp, *Crangon crangon* – *Vavraia mediterranica*; a crayfish, *Cherax tenuimanus* – *Vavraia parastacida*; and several Coleoptera and Lepidoptera – *Vavraia oncoperae*).

Natural geographical distribution: Africa, Europe, North America (USA – Florida; Polynesia.

Life cycle and transmission: These microsporidia are monomorphic and only one sporulation sequence is known (Fig. 2). Spores (3.7–7.9 μm × 2.2–6.2 μm) are uninucleated and ovoid and are produced in multiple groups of 8, 16, 32 commonly and 64 rarely within a thick persistent sporophorous vesicle (Fig. 4R). Horizontal transmission occurs readily in larval hosts via oral ingestion of spores. Vertical (transovum) transmission has been demonstrated in the laboratory via ingestion of spores adhering to the outside of the eggshell picked up from ovarian connective tissue.

Site of infection and pathology: Infections mainly occur in the Malpighian tubules, fat body midgut and muscles of the larval host. The microsporidium is not very pathogenic and larval mortality is low. Infected tissues typically appear as white opaque patches in the fat body of 4th and 5th abdominal segments. Infected *Ae. aegypti* larvae exhibit longer developmental times and emerge as smaller and lighter adults with reduced longevity. Infected female *Cx. pipiens* pupate earlier than uninfected females and also tend to emerge as smaller adults. They exhibit reduced fecundity and longevity. The life history traits of infected male *Cx. pipiens* are not altered.

Host specificity: *Vavraia culicis* is orally infectious for a wide range of mosquitoes. Infections have been recorded from wild-caught *Aedes*, *Anopheles*, *Culex*, *Culiseta*, *Ochlerotatus*, and *Orthopodomyia* species. Experimental laboratory feeding trials have shown higher susceptibility and more severe infections in *Anopheles* and *Culex* species rather than in *Aedes* species. This microsporidium is also a common parasite of mosquito colonies around the world, and has been found infecting colonies of *An. gambiae* and *An. stephensi*.

Epizootiology and field prevalence: Natural prevalence rates of infection ranging from less than 1% to as high as 53% have been recorded for *V. culicis* from the following species: *Ae. albopictus* (0.3–53.8%), *Oc. triseriatus* (6.3%), and *Or. signifera* (2.7–4.6%) from artificial and natural container habitats in Florida; *Ae. polynesienis* (1.9–46.2%) from the Tokelau Islands in Polynesia; and *An. gambiae* (6.6%) from Senegal, West Africa.

Field introductions: An attempt was made to introduce and establish *V. culicis* in a wild population of *Cx. pipiens fatigans* via the release of spores in several sites (wells, cisterns, metal drums) on the Pacific island of Naru. Two years after the introduction, the parasite was still present in the wild population, but the infection rate (2%) was similar to that found in naturally occurring infections in other mosquitoes, and thus did not appear to be high enough to adversely affect the population.

NCBI GenBank® nucleotide accession numbers: *Vavraia culicis* (*Aedes albopictus*) – AJ252961, AJ278956.

References: Weiser (1947, 1977, 1978); Canning (1957), Bano (1958), Weiser and Coluzzi (1964, 1972); Reynolds (1966, 1970, 1972); Kelly et al. (1981), Canning and Hazard (1982), Laird (1982), Wang (1982), Undeen and Dame (1987), Diarra and Toguebaye (1990, 1991); Fukuda et al. (1997); Agnew et al. (1999, 2004); Cheney et al. (2000); Bedhomme et al. (2004), Biron et al. (2005); Lobo et al. (2006).

MICROSPORIDIA OF DOUBTFUL TAXONOMIC DESIGNATION REQUIRING REEXAMINATION

Microsporidium fibriatum

Host: *Ochlerotatus taeniorhynchus*
Reference: Lord and Hall (1983)

Pleistophora milesi

Host: *Maorigoeldia argyropus*
Reference: Pillai (1973)

***Toxoglugea* sp.**

Host: *Anopheles culicifacies*
Reference: Sharma et al. (1979)

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