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Ultrastructural Characterization of Meiospores of Six New Species of *Amblyospora* (Microsporida: Amblyosporidae) from Northern *Aedes* (Diptera: Culicidae) Mosquitoes

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ABSTRACT. The ultrastructure of meiospores of six different species of *Amblyospora* found infecting larval mosquitoes of *Aedes abserratus, Aedes aurifer, Aedes cinereus, Aedes excrucians, Aedes sticticus,* and *Aedes stimulans* are described. Meiospores of all species exhibited characteristics typical for the genus *Amblyospora* including: a single nucleus, a large posterior vacuole, a thick undulating exospore and thinner endospore, a bipartite and lamellate polaroplast with more tightly stacked membranes in the proximal region, and an anisofilar polar filament with distal coils reduced in thickness. Distinct differences were found in the arrangement and number/ratio of coils formed by the broad basal and narrow distal portions of the polar filament. These differences, when quantified and averaged, were unique from all other mosquito-parasitic species that have been examined ultrastructurally. Information on parasite development, natural field prevalence and transmission to suspected intermediate copepod hosts is presented. The creation of six new species, *Amblyospora abserrati, Amblyospora auriferi, Amblyospora cinerei, Amblyospora excrucii, Amblyospora stictici,* and *Amblyospora stimuli* is proposed. Synonymies and complete host lists of all forms and species of *Amblyospora* described from mosquitoes are given.

Supplementary key words. Microsporidia, taxonomy, spore morphology.

MICROSPORIDIA belonging to the genus *Amblyospora* Hazard and Oldacre, 1975 (Microsporida: Amblyosporidae) are among the most common and widespread parasites associated with mosquito populations in nature. To date, at least 31 described species and 49 undescribed species or forms have been reported from 71 different host mosquitoes representing eight genera worldwide (see Table 1).

The genus is heterosporous and possesses a complex life cycle that requires obligatory development in an intermediate copepod host and two mosquito generations to complete [7, 33, 34]. All species have similar gross morphologies and developmental pathways but exhibit a high level of specificity for the definitive mosquito host [6, 36] (J. Becnel, pers. commun.). This specificity is accompanied by demonstrable differences in each of three morphologically distinct spores. These include: 1) an elongate, diploid, "binucleate spore," which is formed in adult female mosquitoes and is responsible for transovarial transmission of the parasite to larval progeny; 2) an oval, haploid "meiospore," which develops in transovarially infected larval mosquitoes and is orally infectious to the intermediate copepod host; and 3) a pyriform to lanceolate, haploid "uninucleate spore," which is formed in the copepod host and is orally reinfectious to mosquito larvae.

Because of the complexity of the life cycle and extreme difficulty in finding the appropriate intermediate host, complete life histories have been documented for only four of the 31 described species of *Amblyospora* [3, 5, 10, 35, 37]. Other species have generally been distinguished by ultrastructural examination of meiospores found in larval mosquitoes, the most frequently encountered host stage. Distinguishing characters have specifically included the number and arrangement of coils in the polar filament and the ratio of coils formed by the broad basal portion of the filament to its narrower distal portion, which thus far have proven to be unique for each species [27] therein having important taxonomic value.

For the last decade I have been investigating the host parasite relationships of a number of different undescribed species of *Amblyospora* parasitizing northern *Aedes* Meigen mosquitoes, which are the most common but least understood of all hosts. Unfortunately, these microsporidia and their mosquito hosts have not been amenable to laboratory colonization (i.e. most are univoltine) and this has precluded attempts to elucidate their complete life histories. However, essential information on meiospore development and ultrastructure, natural field prevalence rates, and laboratory transmission to suspected copepod hosts has been obtained. This is, accordingly, documented herein and the creation of six new species of *Amblyospora* is proposed. Complete synonymies and type localities are also given for each species.

MATERIALS AND METHODS

Field collections and transmission studies. Larval mosquitoes infected with different species of *Amblyospora* were collected from April 20, 1979 through May 4, 1989 during a surveillance program of larval woodland habitats in Connecticut. Field-collected larvae were initially screened in black photographic pans. Those exhibiting patent symptoms of infection (milky white opaque coloration) were identified according to Darsie and Ward [18] and Means [30] and immediately processed for electron microscopy.

All cyclopoid copepods found inhabiting pools where *Amblyospora*-infected mosquito larvae were detected were collected and identified according to Pennak [32]. Some copepod specimens were additionally submitted to Dr. Janet Reid, National Museum of Natural History, Smithsonian Institution for species confirmation. Copepods were screened for microsporidian infections via Giemsa-stained smears. In those instances in which copepods were found to be infected with *Amblyospora*-like spores, horizontal transmission studies were conducted with field-collected mosquito larvae as described previously [6]. Adult female copepods were also exposed to meiospores of various isolates of *Amblyospora* obtained from infected mosquito larvae in laboratory bioassays [5] to assess their role as a potential intermediate host.

Electron microscopy. Whole live infected mosquito larvae were dissected in 2.5% (v/v) glutaraldehyde or a 2.5% (v/v) glutaraldehyde/2% (v/v) paraformaldehyde solution containing 0.1% (w/v) $CaCl_2$ and 1% (w/v) sucrose that was buffered with 0.1 M Na cacodylate (pH 7.4) and then fixed for one–two days at 4° C. Specimens were post-fixed at room temperature in 1% (w/v) OsO_4 in the same buffer, stained *en bloc* overnight with 0.5% (w/v) uranyl acetate in 70% (v/v) ethanol and dehydrated through an ethanol and acetone series. Tissue blocks (1 mm^3) were sequentially (1:1, 2:1, 3:1, resin : acetone) infiltrated over 24 h, subjected to a minimum of three changes in pure resin over another 24 h, and embedded in Epon/Araldite or LX-112/Araldite. Thin sections showing gold interference color (80–140 nm) were post-stained with 5% (w/v) uranyl acetate in 50% (v/v) methanol followed by Reynold's lead citrate, and examined in a Zeiss EM 10C electron microscope operating at 80 kV.

Quantification of ultrastructural features (i.e. number and arrangement of polar filament coils) was made from examination

Table 1. Host list of *Amblyospora* species reported from mosquitoes in nature.

Mosquito host	Species	Reference
Adeomyia squamipennis (Lynch Arribalzaga)	*Amblyospora keenani* Hazard & Oldacre, 1975	[27]
Aedes abserratus (Felt & Young)	*Amblyospora abserrati* Andreadis, 1994	Present paper
Aedes annulipes (Meigen)	*Amblyospora* sp. Hazard & Oldacre, 1975	[27]
Aedes aurifer (Coquillett)	*Amblyospora auriferi* Andreadis, 1994	Present paper
Aedes behningi Martini	*Amblyospora opacita* sensu Kilochitskii & Sheremet, 1978	[19]
Aedes canadensis (Theobald)	*Amblyospora canadensis* (Wills & Beaudoin, 1965) Hazard & Oldacre, 1975	[27]
Aedes cantans Meigen	*Amblyospora weiseri* Lukes & Vavra, 1990	[29]
Aedes cantator (Coquillett)	*Amblyospora connecticus* Andreadis, 1988	[5]
Aedes caspius (Pallas)	*Amblyospora* sp. Hazard & Oldacre, 1975	[27]
Aedes cataphylla Dyar	*Amblyospora* sp. Hazard & Oldacre, 1975	[27]
Aedes cinereus Meigen	*Amblyospora cinerei* Andreadis, 1994	Present paper
Aedes communis (De Geer)	*Amblyospora khaliulini* Hazard & Oldacre, 1975	[27]
Aedes cyprius Ludlow	*Amblyospora minuta* sensu Khaliulin, 1973	[12]
	Amblyospora opacita sensu Khaliulin, 1973	[12]
Aedes detritus (Haliday)	*Amblyospora* sp. Hazard & Oldacre, 1975	[27]
Aedes diantaeus Howard, Dyar & Knab	*Amblyospora minuta* sensu Khaliulin, 1973	[12]
	Amblyospora opacita sensu Khaliulin, 1973	[12]
Aedes dorsalis (Meigen)	*Amblyospora* sp. Hazard & Oldacre, 1975	[27]
Aedes excrucians (Walker)	*Amblyospora excrucii* Andreadis, 1994	Present paper
Aedes fitchii (Felt & Young)	*Amblyospora* sp. Hazard & Oldacre, 1975	[27]
Aedes flavescens (Muller)	*Amblyospora khaliulini* sensu Khaliulin, 1973	[12]
	Amblyospora minuta sensu Khaliulin, 1973	[12]
	Amblyospora opacita sensu Levchenko & Issi, 1973	[12]
Aedes grossbecki Dyar & Knab	*Amblyospora* sp. Hazard & Oldacre, 1975	[27]
Aedes hexodontus Dyar	*Amblyospora* sp. Hazard & Oldacre, 1975	[27]
Aedes increpitus Dyar	*Amblyospora* sp. Hazard & Oldacre, 1975	[27]
Aedes intrudens Dyar	*Amblyospora minuta* sensu Khaliulin, 1973	[12]
	Amblyospora opacita sensu Khaliulin, 1973	[12]
Aedes kasachstanicus Gutsevich	*Amblyospora opacita* sensu Levchenko & Issi,1973	[12]
Aedes melanimon Dyar	*Amblyospora unica* (Kellen & Wills, 1962) Hazard & Oldacre, 1975	[27]
Aedes montchadskyi Dubitsky	*Amblyospora opacita* sensu Levchenko & Issi, 1973	[12]
Aedes nigrinus (Eckstein)	*Amblyospora opacita* sensu Levchenko, 1975	[12]
Aedes pionips Dyar	*Amblyospora opacita* sensu Chadzieva & Gulii, 1977	[19]
Aedes pullatus (Coquillett)	*Amblyospora* sp. Hazard & Oldacre, 1975	[27]
Aedes punctor (Kirby)	*Amblyospora* sp. Hazard & Oldacre, 1975	[27]
Aedes riparius Dyar & Knab	*Amblyospora* sp. Hazard & Oldacre, 1975	[27]
Aedes rossicus Dolbeskin, Gorickaja & Mitrofanova	*Amblyospora khaliulini* sensu Khaliulin, 1973	[12]
	Amblyospora minuta sensu Khaliulin, 1973	[12]
Aedes simanini Gutsevich	*Amblyospora opacita* sensu Levchenko & Issi, 1973	[12]
Aedes sollicitans (Walker)	*Amblyospora* sp. Hazard & Oldacre, 1975	[27]
Aedes squamiger (Coquillett)	*Amblyospora bolinasae* (Kellen & Wills,1962) Hazard & Oldacre, 1975	[27]
Aedes sticticus (Meigen)	*Amblyospora stictici* Andreadis, 1994	Present paper
Aedes stimulans (Walker)	*Amblyospora stimuli* Andreadis, 1994	Present paper
Aedes stramineus Dubitsky	*Amblyospora opacita* sensu Levchenko & Issi, 1973	[12]
Aedes taeniorhynchus (Weidmann)	*Amblyospora polykarya* Lord, Hall & Ellis, 1981	[28]
Aedes thibaulti Dyar & Knab	*Amblyospora* sp. Hazard & Chapman, 1977	[26]
Aedes ventrovittis Dyar	*Amblyospora* sp. Hazard & Oldacre, 1975	[27]
Aedes vexans (Meigen)	*Amblyospora* sp. Hazard & Oldacre, 1975	[27]
Anopheles eiseni Coquillett	*Amblyospora mojingensis* Hazard & Oldacre, 1975	[27]
Anopheles gambiae Giles	*Amblyospora coluzzii* Weiser & Prasertphon, 1981	[42]
Anopheles maculipennis Meigen	*Amblyospora opacita* sensu Levchenko, 1974	[12]
Coquillettidia perturbans (Walker)	*Amblyospora* sp. Hazard & Oldacre, 1975	[27]
Culex annulirostris Skuse	*Amblyospora dyxenoides* Sweeney, Graham & Hazard, 1988	[38]
Culex apicalis Adams	*Amblyospora benigna* (Kellen & Wills, 1962) Hazard & Oldacre, 1975	[27]
Culex erraticus (Dyar & Knab)	*Amblyospora minuta* (Kudo, 1924) Hazard & Oldacre, 1975	[27]
Culex erythrothorax Dyar	*Amblyospora gigantea* (Kellen & Wills, 1962) Hazard & Oldacre, 1975	[27]
Culex halifaxi Theobald	*Amblyospora trinus* Becnel & Sweeney, 1990	[11]
Culex modestus Ficalbi	*Amblyospora opacita* sensu Levchenko & Issi, 1973	[12]
Culex peccator Dyar & Knab	*Amblyospora* sp. Hazard & Oldacre, 1975	[27]
Culex peus Speiser	*Amblyospora* sp. Hazard & Oldacre, 1975	[27]
Culex pipiens Linnaeus	*Amblyospora* sp. Hazard & Chapman, 1977	[26]
	Amblyospora sp. Darwish & Canning, 1991	[20]

Table 1. Continued.

Mosquito host	Species	Reference
Culex quinquefasciatus Say	*Amblyospora culicis* Toguebaye & Marchand, 1985	[39]
	Amblyospora kadunae Weiser & Prasertphon, 1981	[42]
	Amblyospora nigeriana Weiser & Prasertphon, 1981	[42]
Culex salinarius Coquillett	*Amblyospora* sp. Hazard & Oldacre, 1975	[27]
Culex sitiens Wiedemann	*Amblyospora indicola* Vavra, Bai & Panicker, 1984	[40]
Culex tarsalis Coquillett	*Amblyospora californica* (Kellen & Lipa, 1960) Hazard & Oldacre, 1975	[27]
Culex territans Walker	*Amblyospora opacita* (Kudo, 1922) Hazard & Oldacre, 1975	[27]
Culex theileri Theobald	*Amblyospora opacita* sensu Alikhanov, 1978	[19]
Culex thriambus Dyar	*Amblyospora noxia* (Kellen & Wills, 1962) Hazard & Oldacre, 1975	[27]
Culex tritaeniorhynchus Giles	*Amblyospora tritaeniorhynchi* Diarra & Toguebaye, 1992	[21]
Culiseta annulata (Schrank)	*Amblyospora* sp. Hazard & Oldacre, 1975	[27]
Culiseta impatiens (Walker)	*Amblyospora* sp. Hazard & Oldacre, 1975	[27]
Culiseta incidens (Thomson)	*Amblyospora campbelli* (Kellen & Wills, 1962) Hazard & Oldacre, 1975	[27]
Culiseta inornata (Williston)	*Amblyospora inimica* (Kellen & Wills, 1962) Hazard & Oldacre, 1975	[27]
Culiseta particeps (Adams)	*Amblyospora* sp. Hazard & Oldacre, 1975	[27]
Mansonia dyari Belkin, Heinemann & Page	*Amblyospora* sp. Hazard & Oldacre, 1975	[27]
Mansonia leberi Boreham	*Amblyospora* sp. Hazard & Oldacre, 1975	[27]
Psorphora columbiae (Dyar & Knab)	*Amblyospora* sp. Hazard & Oldacre, 1975	[27]

of a minimum 30 meiospores of each species that were viewed in full sagittal section. Meiospore sizes for species that had not been previously documented were determined from measurements on 50 spores observed in 3-μm sections of embedded material with an ocular micrometer at 1,000×.

Ultrastructural observations were also made on various stages of parasite development, including merogony and sporogony, in each respective larval mosquito host. No distinguishing features of taxonomic value could be found among any stages, however, and since these events have been documented repeatedly for numerous other species [2, 9, 11, 20–22, 28, 29, 38–40], no individual micrographs were prepared and the information was presented in text only.

RESULTS

Amblyospora abserrati n. sp.
(Fig. 1, Table 1, 2)

Thelohania near *opacita* parasitizing *Aedes abserratus*, Anderson, 1968 [1, Fig. 1, 11–16].

Amblyospora sp. Hazard & Oldacre, 1975 [27].

Type definitive host. *Aedes abserratus* (Felt & Young) (Diptera: Culicidae).

Type intermediate host. *Amblyospora abserrati* has been experimentally transmitted to *Acanthocyclops vernalis* (Fischer) (Copepoda: Cyclopidae) in the laboratory but reinfection of mosquito larvae has not been achieved. Other associated cyclopoids shown to be non-susceptible to meiospores in laboratory bioassays include *Macrocyclops albidus* (Jurine).

Type locality. A permanent pond in Cornwall, Connecticut, USA.

Additional localities. Hamden and Granby, Connecticut, USA [1].

Site of infection. Fat body tissue of male and female larvae [1].

Meiospore size. Living meiospores measure 7.0 ± 0.4 × 5.1 ± 0.2 μm [1]. Preserved meiospores measure 6.2 ± 0.6 × 4.9 ± 0.6 μm [1].

Meiospore morphology (Fig. 1). Meiospores cordiform in sagittal section with posterior end more broadly rounded than anterior. Uninucleate with a thick undulating spore wall and large posterior vacuole. Polaroplast lamellar and more tightly arranged in the apical region. Polar filament anisofilar with 3½ (range = 3–3½) broad proximal and 7½ (range = 7–9) narrow distal coils all arranged in a single row.

Prevalence of field infection. The prevalence of infection in larval populations of this univoltine mosquito has been less than 1% at all locales.

Deposition of type specimens. Syntype slides have been deposited in the International Protozoan Type Slide Collection, Smithsonian Institution, Washington, DC, USA (USNM No. 43209). Type specimens embedded in plastic resin are also in the collection of the author.

Amblyospora auriferi n. sp.
(Fig. 2, Table 1, 2)

Type definitive host. *Aedes aurifer* (Coquillett) (Diptera: Culicidae).

Type intermediate host. No data.

Type locality. A temporary vernal pool in Cornwall, Connecticut, USA.

Site of infection. Larval fat body tissue.

Meiospore size. Preserved meiospores measure 4.5 ± 0.4 × 3.1 ± 0.2 μm.

Meiospore morphology (Fig. 2). Meiospores cordiform in sagittal section with posterior end more broadly rounded than anterior. Uninucleate with a thick undulating spore wall and large posterior vacuole. Polaroplast lamellar and more tightly arranged in the apical region. Polar filament anisofilar with 3 (range = 2½–3½) broad proximal and 9 (range = 7½–9½) narrow distal coils all arranged in a single row.

Prevalence of field infection. The prevalence of infection in larval populations of this univoltine mosquito is less than 1%.

Deposition of type specimens. Syntype slides have been deposited in the International Protozoan Type Slide Collection, Smithsonian Institution, Washington, DC, USA (USNM No. 43210). Type specimens embedded in plastic resin are also in the collection of the author.

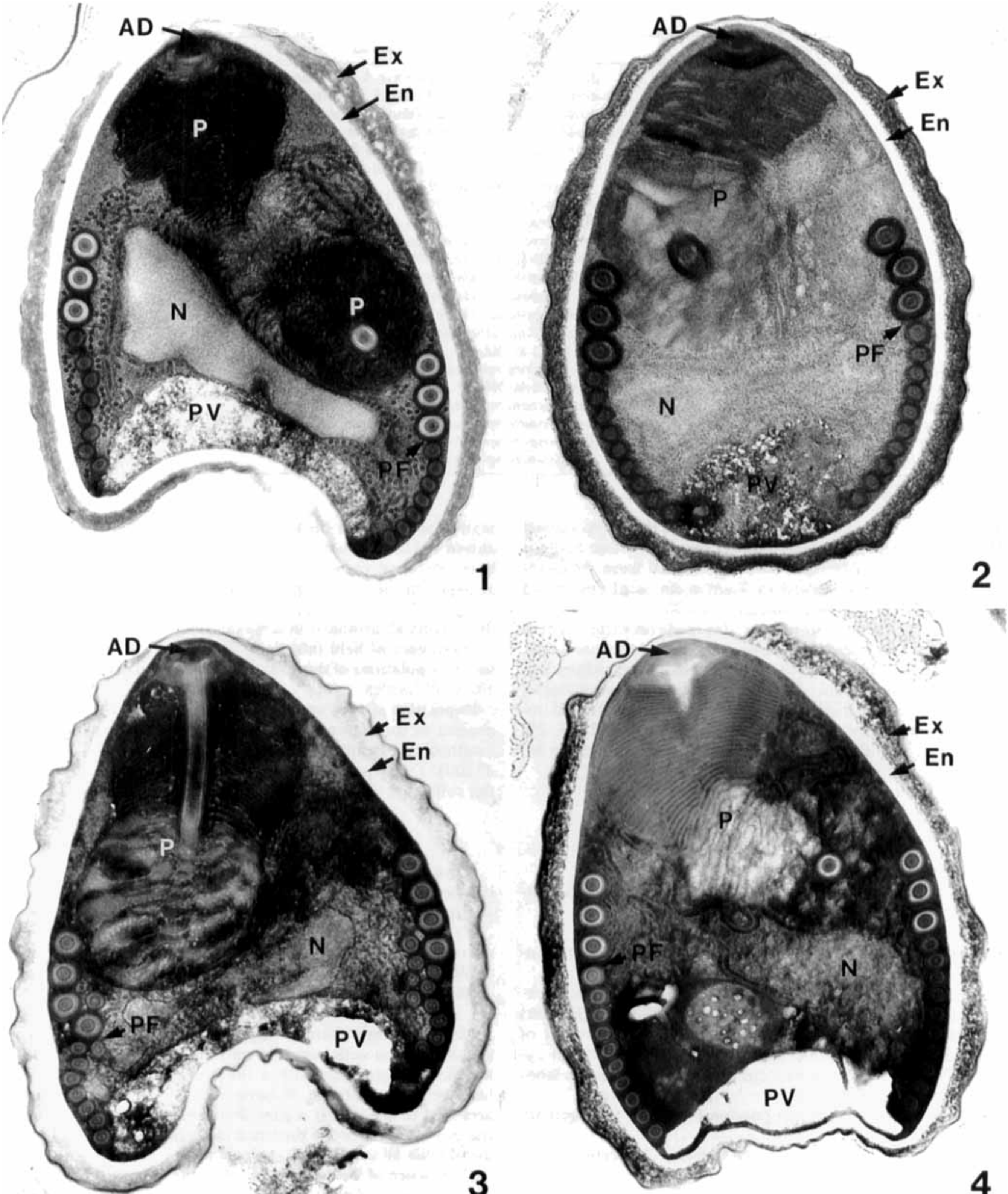

Fig. 1–4. Ultrastructural morphology of meiospores of *Amblyospora* spp. from mosquitoes. **1**. *Amblyospora abserrati.* ×22,600 **2**. *Amblyospora auriferi.* ×23,900. **3**. *Amblyospora cinerei.* ×23,700. **4**. *Amblyospora excrucii.* ×23,400. AD, anchoring disc; En, endospore; Ex, exospore; N, nucleus; P, polaroplast; PF, polar filament; PV, posterior vacuole.

Amblyospora cinerei n. sp.
(Fig. 3, Table 1, 2)

Thelohania sp. Chapman, 1966 [13].

Thelohania near *opacita* parasitizing *Aedes cinereus*, Anderson, 1968 [1, Fig. 35–40].

Amblyospora sp. Hazard & Oldacre, 1975 [27], Andreadis, 1993 [8].

Type definitive host. *Aedes cinereus* Meigen (Diptera: Culicidae).

Type intermediate host. *Amblyospora cinerei* has been experimentally transmitted to *A. vernalis* in the laboratory but reinfection of mosquito larvae has not been achieved. Other associated cyclopoids shown to be non-susceptible to meiospores in laboratory bioassays include *Eucyclops agilis* (Koch), *M. albidus* and *Orthocyclops modestus* (Herrick).

Type locality. A temporary vernal pool in Cornwall, Connecticut, USA.

Additional localities. Hamden, Granby and Voluntown, Connecticut [1, 8] and Nevada, USA [13].

Site of infection. Fat body tissue of male and female larvae [1].

Meiospore size. Living meiospores measure 6.8 ± 0.6 × 4.9 ± 0.4 μm [1]. Preserved meiospores measure 5.9 ± 0.6 × 4.4 ± 0.7 μm [1].

Meiospore morphology (Fig. 3). Meiospores cordiform in sagittal section with posterior end more broadly rounded than anterior. Uninucleate with a thick undulating spore wall and large posterior vacuole. Polaroplast lamellar and more tightly arranged in the apical region. Polar filament anisofilar with 4 (range = 3–4½) broad proximal coils arranged in a single row and 10 (range = 9½–11) narrow distal coils arranged in an irregular row.

Prevalence of field infection. The prevalence of infection in first generation (spring brood) larvae is typically less than 1% at all locales. An epizootic has been reported [8] in second generation (fall brood) larvae with a prevalence rate of 13%.

Deposition of type specimens. Syntype slides have been deposited in the International Protozoan Type Slide Collection, Smithsonian Institution, Washington, DC, USA (USNM No. 43211). Type specimens embedded in plastic resin are also in the collection of the author.

Amblyospora excrucii n. sp.
(Fig. 4, Table 1, 2)

Thelohania near *opacita* parasitizing *Aedes excrucians*, Anderson, 1968 [1, Fig. 23–28].

Thelohania sp. Chapman et al., 1973 [15].

Amblyospora sp. Hazard & Oldacre, 1975 [27].

Type definitive host. *Aedes excrucians* (Walker) (Diptera: Culicidae).

Type intermediate host. No data.

Type locality. A temporary vernal pool in Hamden, Connecticut, USA.

Additional localities. Granby, Connecticut [1] and Alaska, USA [15].

Site of infection. Fat body tissue of mostly male larvae [1].

Meiospore size. Living meiospores measure 6.8 ± 0.3 × 4.8 ± 0.2 μm [1]. Preserved meiospores measure 6.3 ± 0.8 × 5.2 ± 1.0 μm [1].

Meiospore morphology (Fig. 4). Meiospores cordiform in sagittal section with posterior end more broadly rounded than anterior. Uninucleate with a thick undulating spore wall and large posterior vacuole. Polaroplast lamellar and more tightly arranged in the apical region. Polar filament anisofilar with 3 (range = 3–3½) broad proximal and 10 (range = 8½–11) narrow distal coils all arranged in a single row.

Prevalence of field infection. The prevalence of infection in larval populations of this univoltine mosquito has been less than 1% at all locales.

Deposition of type specimens. Syntype slides have been deposited in the International Protozoan Type Slide Collection, Smithsonian Institution, Washington, DC, USA (USNM No. 43212). Type specimens embedded in plastic resin are also in the collection of the author.

Amblyospora stictici n. sp.
(Fig. 5, Table 1, 2)

Thelohania sp. Chapman et al., 1966 [16], 1967 [17], 1969 [14]. *Amblyospora* sp. Hazard & Oldacre, 1975 [27].

Type definitive host. *Aedes sticticus* (Meigen) (Diptera: Culicidae).

Type intermediate host. No data.

Type locality. A temporary pool in Sharon, Connecticut, USA.

Additional localities. Louisiana, USA [14, 16, 17].

Site of infection. Fat body tissue and oenocytes of male larvae [16].

Meiospore size. Living meiospores measure 7.0 ± 0.5 × 4.7 ± 0.3 μm [16]. Preserved meiospores measure 5.2 ± 0.5 × 3.3 ± 0.4 μm.

Meiospore morphology (Fig. 5). Meiospores cordiform in sagittal section with posterior end more broadly rounded than anterior. Uninucleate with a thick undulating spore wall and large posterior vacuole. Polaroplast lamellar and more tightly arranged in the apical region. Polar filament anisofilar with 4 (range = 3½–4½) broad proximal and 6 (range = 6–7½) narrow distal coils all arranged in a single row.

Prevalence of field infection. The prevalence of infection in larval populations of this multivoltine flood-water mosquito is typically less than 1% in Connecticut but has been reported to be as high as 50% at some locales in Louisiana [14].

Deposition of type specimens. Syntype slides have been deposited in the International Protozoan Type Slide Collection, Smithsonian Institution, Washington, DC, USA (USNM No. 43213). Type specimens embedded in plastic resin are also in the collection of the author.

Amblyospora stimuli n. sp.
(Fig. 6, Table 1, 2)

Thelohania sp. Franz & Hagman, 1962 [25].

Thelohania near *opacita* parasitizing *Aedes stimulans*, Anderson, 1968 [1, Fig. 4, 29–34].

Amblyospora sp. Hazard & Oldacre, 1975 [27], Morrison & Andreadis, 1992 [31].

Amblyospora Andreadis, 1985 [4].

Type definitive host. *Aedes stimulans* (Walker) (Diptera: Culicidae).

Type intermediate host. Infections have been experimentally transmitted to *Ae. stimulans* larvae following exposure to spores obtained from the indigenous copepod *Diacyclops bicuspidatus thomasi* (Forbes) in the laboratory but reinfection of *D. b. thomasi* with meiospores from *Ae. stimulans* larvae has not been achieved. Other associated cyclopoids shown to be non-susceptible to meiospores in laboratory bioassays include *A. vernalis* and *M. albidus*.

Type locality. A temporary vernal pool in Hamden, Connecticut, USA.

Additional localities. New Jersey, USA [23].

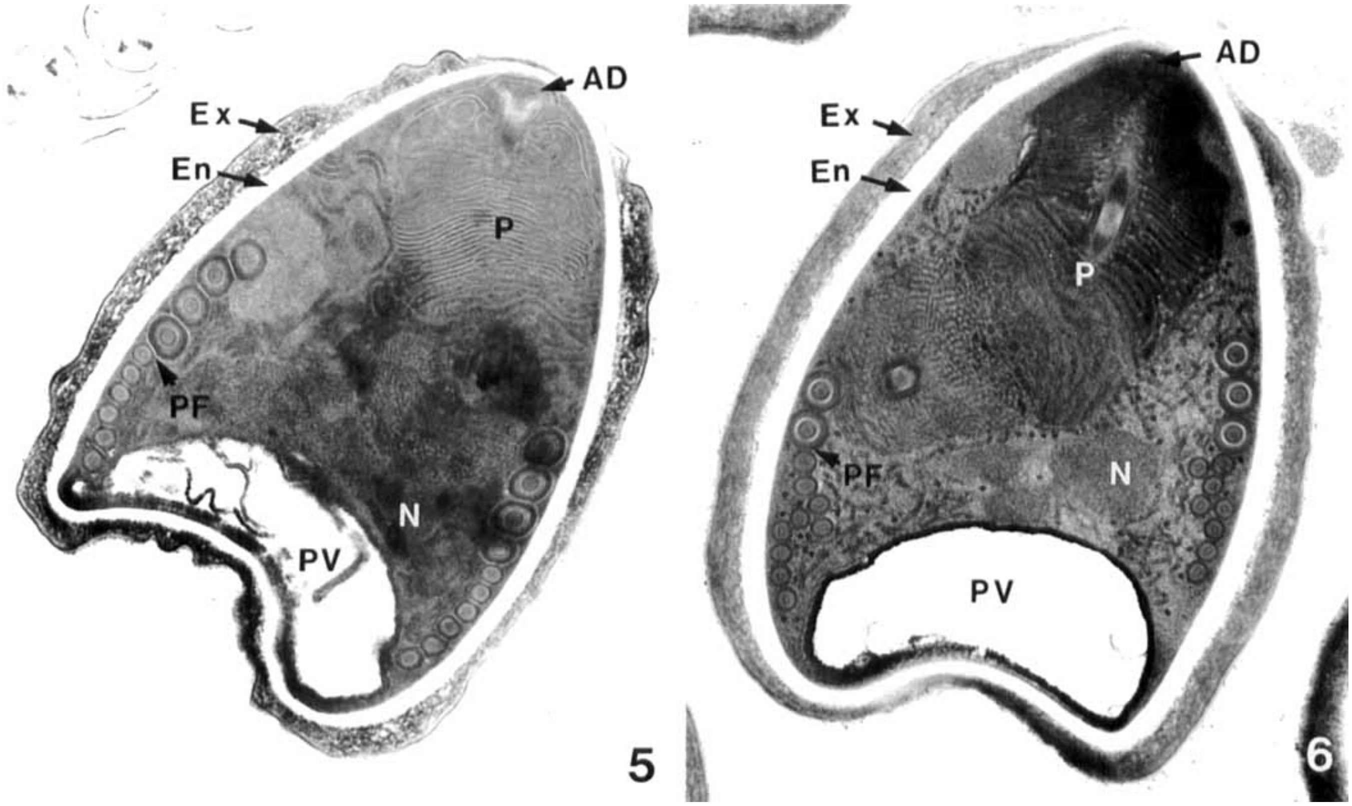

Fig. 5, 6. Ultrastructural morphology of meiospores of *Amblyospora* spp. from mosquitoes. **5.** *Amblyospora stictici.* ×20,100. **6.** *Amblyospora stimuli.* ×20,900. AD, anchoring disc; En, endospore; Ex, exospore; N, nucleus; P, polaroplast; PF, polar filament; PV, posterior vacuole.

Site of infection. Fat body tissue of male and female larvae.

Meiospore size. Living meiospores measure 7.2 ± 0.3 × 3.9 ± 0.2 μm [1]. Preserved meiospores measure 5.9 ± 1.0 × 4.6 ± 0.2 μm [1].

Meiospore morphology (Fig. 6). Meiospores cordiform in sagittal section with posterior end more broadly rounded than anterior. Uninucleate with a thick undulating spore wall and large posterior vacuole. Polaroplast lamellar and more tightly arranged in the apical region. Polar filament anisofilar with 3 (range = 2–3½) broad proximal coils arranged in a single row and 9 (range = 7–10½) narrow distal coils arranged in an irregular row.

Prevalence of field infection. The prevalence of meiospore infections in larval populations of this univoltine mosquito have ranged from less than 1% to 6% [1, 4].

Deposition of type specimens. Type slides have been deposited in the International Protozoan Type Slide Collection, Smithsonian Institution, Washington, DC, USA (USNM No. 43214). Type specimens embedded in plastic resin are also in the collection of the author.

DISCUSSION

The ultrastructures of the meiospores of the six species described herein were characteristic of the genus *Amblyospora* [27, 33]. These included: a single nucleus, a large posterior vacuole, a thick undulating exospore and thinner endospore, a bipartite and lamellate polaroplast with more tightly stacked membranes in the proximal region, and an anisofilar polar filament with distal coils reduced in thickness. Parasite development, including merogony, sporogony, and sporogenesis, was also virtually identical for each species. Infections were generally restricted to larval fat body tissue. All meronts had diplokaryotic nuclei and divided by binary fission to form merogonial plasmodia with up to eight diplokarya. Early sporonts were diplokaryotic. During sporogony they secreted a sporophorous vesicle, underwent meiosis, and developed into octonucleated plasmodia that underwent cytokinesis to form eight haploid sporoblasts. No distinguishing features were found in meront or sporont stages from any of the species and the only differences noted were the host sex in which these transovarially transmitted infections were expressed.

Despite these similarities, measurable differences of taxonomic value were found in the arrangement and numerical ratio of coils formed by the broad basal and narrow distal portions of the polar filament. These differences, when quantified and averaged, proved to be distinct from published accounts of all other mosquito-parasitic species that have been examined ultrastructurally (Table 2). Even in nearly identical species such as *A. auriferi* and *A. stimuli,* which had the same ratios, a distinction could be made in the arrangement of the narrow coils, which were irregular in *A. stimuli* and uniform in *A. auriferi. Amblyospora cinerei* and *A. excrucii* were similarly differentiated. The significance of the irregular arrangement of the narrow coils of the polar filament is unknown. It does not appear to be related to the overall filament length or size of the spore, and may simply represent a species-specific trait that has no apparent ecological or physiological significance. It has been observed in three different host genera (*Aedes, Anopheles* Meigen and *Culex* L.), but is considerably less common than the singly coiled uniform arrangement.

Some variability and overlap in the number of polar filament coils were observed among individual meiospores of each iso-

Table 2. Comparison of the arrangement and average number of broad and narrow coils of polar filaments in published accounts of ultrastructurally examined species of *Amblyospora* from mosquito hosts.

Species	Mosquito host	Broad coils	Narrow coils	Arrangement of narrow coils	Reference
A. abserrati	*Ae. abserratus*	3½	7½	Uniform	Present paper
A. auriferi	*Ae. aurifer*	3	9	Uniform	Present paper
A. californica	*Cx. tarsalis*	5	7	Uniform	[27]
A. campbelli	*Cs. incidens*	3½	6	Uniform	[22]
A. canadensis	*Ae. canadensis*	3	8½	Uniform	[27]
A. cinerei	*Ae. cinereus*	4	10	Irregular	Present paper
A. connecticus	*Ae. cantator*	4	7	Uniform	[2]
A. culicis	*Cx. quinquifasciatus*	5	5½	Uniform	[39]
A. dyxenoides	*Cx. annulirostris*	3½	5	Uniform	[38]
A. excrucii	*Ae. excrucians*	3	10	Uniform	Present paper
A. indicola	*Cx. sitiens*	3	3	Uniform	[40]
A. inimica	*Cs. inornata*	5	5	Uniform	[27]
A. keenani	*Ad. squamipennis*	6	2	Uniform	[27]
A. khaliulini	*Ae. communis*	3½	10½	Irregular	[27]
A. minuta	*Cx. erraticus*	2½	1	Uniform	[27]
A. mojingensis	*An. eiseni*	4	3	Irregular	[27]
A. opacita	*Cx. territans*	3½	3½	Uniform	[27]
A. polykarya	*Ae. taeniorhynchus*	4	3	Uniform	[27]
A. stictici	*Ae. sticticus*	4	6	Uniform	Present paper
A. stimuli	*Ae. stimulans*	3	9	Irregular	Present paper
A. tritaeniorhynchi	*Cx. tritaeniorhynchus*	5	4	Irregular	[21]
A. trinus	*Cx. halifaxi*	4	5	Uniform	[11]
A. weiseri	*Ae. cantans*	2½	4½	Uniform	[29]
Amblyospora sp.	*Cx. pipiens*	5½	4½	Irregular	[20]
Amblyospora sp.	*Cx. salinarius*	5½	10½	Uniform	[9]

late. Thus, it is important to emphasize that assessment of this characteristic for taxonomic purposes should be made from examination of a number of meiospores viewed in full sagittal section. It should also be noted that the values presented in Table 2 for previously described species are based on published micrographs and the author's description, which may not necessarily represent true averages. Nevertheless, each species does appear to have a distinct ratio. It would seem to be inevitable that as more forms are discovered from different hosts, more overlap will be found and distinctions based upon this characteristic will prove less reliable in determining taxonomic relationships. In these instances, ultrastructural examination of spores formed in adult female mosquito and copepod hosts should reveal further morphological differences unique for each particular species. The use of biochemical and immunological techniques (i.e. SDS-PAGE, Western blot) and/or molecular analysis of ribosomal DNA sequences, as has recently been done to separate two morphologically similar human microsporidia, *Encephalitizoon cuniculi* Levaditi, Nicolau & Schoen, 1923 and *Encephalitizoon hellem* Didier et al., 1991 [23, 24, 41], may also help to demonstrate differences when no morphological markers are apparent.

The data obtained on the intermediary role of various cyclopoid copepods that were found in habitats with *Amblyospora*-infected mosquito larvae were inconclusive. The most abundant and frequently encountered copepod was *A. vernalis*, the recognized intermediate host of another closely related microsporidium, *Amblyospora connecticus* Andreadis, 1988, whose definitive host is the salt marsh mosquito *Aedes cantator* (Coquillett) [3, 5]. Female *A. vernalis* were shown to be susceptible to meiospores of two species, *A. abserrati* and *A. cinerei*, in laboratory bioassays. However, in no instance could reinfection of mosquito larvae be achieved when larvae were exposed to spores formed in *A. vernalis*. In contrast to this, larvae of *Ae. stimulans* were readily infected with *Amblyospora*-like spores found in the copepod *D. b. thomasi*, but I could not reinfect copepodid or adult female stages of *D. b. thomasi* with meiospores from *Ae. stimulans*. Similar difficulties in demonstrating the infectivity of meiospores of other *Amblyospora* spp. to copepods that occur regularly in habitats with infected mosquito larvae have been reported by others [21, 29]. These observations suggest that other, extrinsic environmental factors may be involved in oral transmission of these microsporidia from one host to another.

In conclusion, it is hoped that the documentation presented herein on meiospore ultrastructure and infectivity to suspected intermediate hosts will provide a basis from which future investigations will be conducted in an attempt to elucidate the complete life histories of these complex and enigmatic microsporidian parasites.

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