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Ultrastructural characterization and comparative phylogenetic analysis of new microsporidia from Siberian mosquitoes: Evidence for coevolution and host switching

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ABSTRACT

A survey of mosquito larvae infected with microsporidia was conducted from 2005 to 2008 in the Tomsk, Kemerovo and Novosibirsk regions of western Siberia, Russia. Twenty-one morphologically and genetically unique species of microsporidia were isolated from nine species of *Anopheles*, *Aedes*, *Culex* and *Ochlerotatus* mosquitoes including: (1) 14 proposed new species of *Amblyospora* (*A. bakcharia*, *A. baritia*, *A. bogashovia*, *A. chulymia*, *A. hristinia*, *A. jurginia*, *A. kazankia*, *A. mavlukevia*, *A. mocrushinia*, *A. modestium*, *A. salairia*, *A. severinia*, *A. shegaria*, and *A. timirasia*); (2) a newly proposed genus and species, *Novothelohania ovalae* and; (3) six species of *Amblyospora* (*A. flavescens*, *A. kolarovi*, *A. rugosa*), *Parathelohania* (*P. divulgata* and *P. tomski*) and *Trichoctosporea* (*T. pygopellita*) from which gene sequences had not been previously obtained. Detailed ultrastructure of meiospores revealed unique cytological features associated with the length, arrangement and ratio of broad to narrow coils of the polar filament, comparative thickness of the exospore and endospore, and overall size of each species reaffirming their value in distinguishing taxonomic relationships. SSU rDNA sequences obtained from each species of microsporidia were unique when compared with GenBank entries. Phylogenetic trees constructed by Maximum Parsimony, Maximum Likelihood and Neighbor Joining analyses yielded similar topologies with a high degree of congruence between parasite and host at the generic level. Species that parasitize *Aedes*/*Ochlerotatus* and *Culex* mosquitoes segregate into distinct monophyletic groupings mirroring their host phylogeny, while species from *Anopheles* mosquitoes group as a sister clade basal to the entire group of mosquito-parasitic microsporidia as their *Anopheles* hosts cluster as a sister clade to the entire group of culicine mosquitoes. This provides strong evidence for host-parasite coevolution by descent at the generic level and limited host lineage switching between unrelated taxa. Among parasites of *Aedes*/*Ochlerotatus* and *Anopheles* mosquitoes, we found several instances where a single mosquito species serves as a host for two or more related species of microsporidia, an observation consistent with host switching and independent parasite speciation. Among the microsporidian parasites of *Culex* mosquitoes, we found only one parasite per host indicating a higher degree of host specificity and less host switching among parasites of this genus. Findings suggest a degree of host-parasite co-speciation with host switching occurring occasionally when the "normal" host is unavailable in the aquatic ecosystem. Frequency of host switching seems to be occurring in proportion to host relatedness and does not cross generic boundaries in this system.

1. Introduction

The Amblyosporidae are common parasites of mosquitoes, and as a group, possess some of the most complex life cycles known within the phylum Microsporidia. Elements of their life cycles include asexual and sexual reproduction, the production of multiple spore types, vertical (transovarial) and horizontal transmission, and obligatory development in an intermediate host. They have successfully radiated into mosquito populations inhabiting a diverse array of aquatic habitats including temporary vernal pools, permanent freshwater swamps and bogs, open flood plains and lagoons, coastal brackish saltmarshes, and artificial and natural (tree holes, rock holes, epiphytic plants) containers. To date, more than

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150 species of microsporidia, representing 24 genera have been described from 14 different mosquito genera worldwide, and it is generally accepted that most if not all mosquitoes serve as hosts for one or more of these ubiquitous parasites (Andreadis, 2007).

Generic and species designations within the Amblyosporidae have largely been based on life history traits, developmental morphology, nuclear organization, and ultrastructural features associated with various life stages focusing most frequently on spores (see Andreadis, 2007 and references therein). More recently, small subunit ribosomal DNA (SSU rDNA) sequence data have been increasingly used to identify new genera and species, and clarify their phylogenetic relationships with existing taxa (Nilsen and Chen, 2001; Andreadis and Vossbrinck, 2002; Vossbrinck et al., 2004; Franzen et al., 2006; Simakova et al., 2008). These analyses, albeit limited and with some notable discrepancies, have thus far revealed an apparent correlation between mosquito host and parasite at the generic level, suggestive of co-speciation, and a correspondingly high level of host specificity, especially within the genus *Amblyospora* (Baker et al., 1998; Vossbrinck et al., 2004). However, due to the limited number of species that have been examined, it has not been possible to fully assess the degree of congruence between the mosquito host and their corresponding microsporidian parasite. It has further been hypothesized that while microsporidia appear to have invaded members of the Culicidae several times independently (e.g. *Anncaliia*, *Vavraia*), the "true mosquito parasites" (*Amblyospora*, *Culicospora*, *Culicosporella*, *Hazardia*, *Hyalinocysta*, *Intrapredatorous*, and *Parathlohania*) likely evolved from parasites of crustaceans, arose as a single event, and proceeded to adapt their life cycles to accommodate host ecological conditions and maximize transmission through natural selection (Vossbrinck et al., 2004; Andreadis, 2005).

In the spring and summer of 2005–2008, an extensive survey of mosquito larvae infected with microsporidia was conducted in a variety of aquatic habitats in the Tomsk, Kemerovo and Novosibirsk regions of western Siberia in Russia. During the course of this investigation, 21 distinct species of microsporidia representing three genera (*Amblyospora*, *Parathelohania*, and *Trichoctosporea*), including 14 proposed new species and one new genus (*Novothelohania* n. g.), were discovered infecting nine species of mosquitoes from four genera (*Aedes*, *Anopheles*, *Culex*, *Ochlerotatus*). In this paper, we present (1) detailed ultrastructural morphology of meiospore stages from each new microsporidian species with accompanying taxonomic descriptions, (2) SSU rDNA sequence data for all 21 species, and (3) phylogenetic reconstruction and comparative analyses of the entire clade of mosquito-parasitic microsporidia in relation to host mosquito molecular phylogeny.

2. Materials and methods

2.1. Field collection and host identification

Third and fourth instar larvae infected with microsporidia were collected from April through July of 2005 to 2008 from a variety of aquatic habitats within the Kemerovo, Novosibirsk and Tomsk regions of western Siberia, Russia. These included temporary vernal pools and swamps, permanent ponds and lakes, and pools alongside rivers. Larvae were initially identified based on morphological characters and descriptive keys of Gucevich et al. (1970).

Host identity was further confirmed by comparative analysis of the small subunit ribosomal DNA (18S rDNA) sequence with those recorded in GenBank/EMBL using amplification and sequencing primers described in Shepard et al. (2006). Genomic DNA from each mosquito host was liberated by bead-beating larval specimens for microsporidia analysis in which an aliquot was used for polymerase chain reaction (PCR) amplification of nuclear 18S rDNA. This was performed using the Taq PCR Core Kit (Qiagen Inc., Valencia, CA) according to the manufacturer's protocol with 0.6 μM of primers 28F and 16SendR (Shepard et al., 2006). PCR reactions were performed in a thermal cycler (PTC-200 DNA Engine, M.J. Research, Watertown, MA) under the following conditions: 94 °C for 3 min followed by 35 cycles of 94 °C for 45 s, 45 °C for 30 s, and 72 °C for 1 min 30 s, followed by a final extension at 72 °C for 3 min. The amplified PCR product (approximately 1862 nucleotides in length) was confirmed by standard 1% agarose gel electrophoresis, purified using QIAquick PCR Purification Kit (Qiagen Inc., Valencia, CA) according to the manufacturer's protocol, and submitted for direct nucleotide sequencing. For sequencing reactions, approximately 100 ng of purified 18S rDNA PCR product was combined with 0.6 μM of sequencing primer (Shepard et al., 2006) and sterile water. Sequencing reactions were performed at the W.M. Keck Foundation Biotechnology Resource Laboratory (Yale University, New Haven, CT).

2.2. Electron microscopy

Abdominal segments from infected larvae were fixed in a 2.5% (v/v) glutaraldehyde solution buffered in 100 mM Na cacodylate (pH 7.4) for 2.5 h at 4 °C, postfixed in aqueous 1% (w/v) OsO_4 (pH 7.4) for 2 h at room temperature, dehydrated through a graded ethanol and acetone series and embedded in Epon 812-Araldite (Fluka, Switzerland). Thin sections (60–100 nm) were stained with 2% (w/v) uranyl acetate in 50% ethanol followed by Reynold's lead citrate and examined in a JEM-100 CX II electron microscope at an accelerating voltage of 80 kV.

2.3. Molecular phylogenetic analysis of parasites

Nucleotide sequences were obtained from mature spores that were isolated from the thorax of each infected mosquito host. Whole thoracic segments initially fixed in 70% ethanol were exchanged several times with deionized water and left overnight to rehydrate. The larval tissues were then homogenized briefly in 500 μl of sterile water, filtered through a 41 μm nylon mesh into a clean 1.5 ml microcentrifuge tube, centrifuged and spun at 14,000*g* for 1 min. The supernatant was removed and 500 μl of sterile water was added. The samples were mixed and allowed to sit for 10–15 min to ensure any residual ethanol was removed from the spores. This process was repeated two to three times. Following hydration, samples were centrifuged at 14,000*g* for 2 min. The supernatant was removed, and 150 μl of STE buffer (0.1 M NaCl, 10 mM Tris–HCl, 1 mM EDTA, pH 8.0) was added to the spore pellet (Fluka, Buchs, Switzerland), resuspended and placed in a 0.5 ml microcentrifuge tube. A 10 μl aliquot of each sample was removed and examined by phase contrast microscopy (100×) for the presence of spores. One hundred fifty mg of glass beads (212–300 μm diameter) (Sigma, St. Louis, MO) were then added and the tube was shaken in a Mini-Beadbeater (Biospec Products Bartlesville, OK) for 50 s to fracture the spores. The samples were spun briefly and 10 μl was removed to verify spore disruption. The samples were incubated at 95 °C for 5 min, and then centrifuged at 14,000*g* for 5 min. The supernatant was removed to a clean 1.5 ml microcentrifuge tube and was frozen at −20 °C until use in PCR.

One to 5 μl of the STE-ruptured spore solution was used in a standard PCR reaction (94 °C for 3 min, followed by 35 cycles of 94 °C for 45 s, 45 °C for 30 s, and 72 °C for 1 min 30 s) using primers 18f and 1492r (see below). The PCR product was then purified on a Qiaquick PCR purification kit (Qiagen Company, CA) and prepared for automated sequencing at the Keck Biotechnology Resource Laboratory at Yale University with the following microsporidian primers: 18f, CACCAGGTTGATTCTGCC; SS350f, CCAAGGAYGGCAGCAG GCGCGAAA; SS350r, TTTCGCGCCTGCTGCCRTCCTTG; SS530f,

Table 1
Species list of microsporidian and mosquito host SSUrDNA sequences included in the phylogenetic analysis including GenBank accession number and geographic origin.

Microsporidium	Host mosquito	Geographic origin (M = microsporidium) (H = host mosquito)
New species		
Amblyospora bakcharia n.sp.	*Ochlerotatus excrucians*	Russia (M,H)
JF826402	JF837523	
Amblyospora baritia n.sp.	*Ochlerotatus excrucians*	Russia (M,H)
JF826403	JF837524	
Amblyospora bogashova n.sp.	*Ochlerotatus excrucians*	Russia (M,H)
JF826404	JF837525	
Amblyospora chulymia n.sp.	*Ochlerotatus caspius*	Russia (M,H)
JF826405	JF837527	
Amblyospora hristinia n.sp.	*Ochlerotatus communis*	Russia (M,H)
JF826407	JF837529	
Amblyospora jurginia n.sp.	*Ochlerotatus excrucians*	Russia (M,H)
JF826408	JF837521	
Amblyospora kazankia n.sp.	*Ochlerotatus dianaetus*	Russia (M,H)
JF826409	JF837531	
Amblyospora mavlukevia n.sp.	*Aedes cinereus*	Russia (M,H)
JF826411	JF837532	
Amblyospora mocrushinia n.sp.	*Ochlerotatus punctor*	Russia (M,H)
JF826412	JF837536	
Amblyospora modestium n.sp.	*Culex modestus*	Russia (M,H)
JF826413	JF837538	
Amblyospora salairia n.sp.	*Aedes cinereus*	Russia (M,H)
JF826415	JF837534	
Amblyospora severinia n.sp.	*Ochlerotatus excrucians*	Russia (M,H)
JF826417	JF837522	
Amblyospora shegaria n.sp	*Aedes cinereus*	Russia (M,H)
JF826416	JF837533	
Amblyospora timirasia n.sp	*Aedes cinereus*	Russia (M,H)
JF826418	JF837535	
Novothelohania ovalae n. g., n.sp	*Ochlerotatus caspius*	Russia (M,H)
JF826419	JF837528	
New isolates of described species		
Amblyospora flavescens	*Ochlerotatus dianaetus*	Russia (M,H)
JF826406	N/A	
Amblyospora kolarovi	*Ochlerotatus punctor*	Russia (M)
JF826410	U48378	United States (H)
Amblyospora rugosa	*Ochlerotatus cataphylla*	Russia (M,H)
JF826414	JF837530	
Parathelohania divulgata	*Anopheles messeae*	Russia (M,H)
JF826420	JF837537	
Parathelohania tomski	*Anopheles messeae*	Russia (M,H)
JF826421	N/A	
Trichoctosporea pygopellita	*Ochlerotatus excrucians*	Russia (M,H)
HM594267	JF837526	
Species from GenBank		
Amblyospora californica	*Culex tarsalis*	United States (M)
U68473	N/A	
Amblyospora canadensis	*Ochlerotatus canadensis*	United States (M,H)
AY090056	AY988433	
Amblyospora cinerei	*Aedes cinereus*	United States (M,H)
AY090057	AY988441	
Amblyospora connecticus	*Ochlerotatus cantator*	United States (M,H)
AF025685	AY988428	
Amblyospora criniferis	*Ochlerotatus crinifer*	Argentina (M)
AY090061	N/A	
Amblyospora excrucii	*Ochlerotatus excrucians*	United States (M,H)
AY090043	AY988430	
Amblyospora ferocis	*Psorophora ferox*	Argentina (M)
AY090062	AY988442	United States (H)
Amblyospora indicola	*Culex sitiens*	Australia (M)
AY090051	N/A	
Amblyospora khaliulini	*Ochlerotatus communis*	United States (M,H)
AY090045	AY988425	
Amblyospora opacita	*Culex territans*	United States (M,H)
AY090052	AY988450	
Amblyospora salinaria	*Culex salinarius*	United States (M,H)
U68474	AY988449	
Amblyospora stictici	*Ochlerotatus sticticus*	United States (M,H)
AY090049	AY988437	
Amblyospora stimuli	*Ochlerotatus stimulans*	United States (M,H)

(*continued on next page*)

Table 1 (*continued*)

Microsporidium	Host mosquito	Geographic origin (M = microsporidium) (H = host mosquito)
AF027685	AY988429	
Amblyospora weiseri	*Ochlerotatus cantans*	Czech Republic (M)
AY090048	N/A	
Andreanna caspii	*Ochlerotatus caspius*	Russia (M,H)
EU664450	EU700339	
Anncaliia algerae	*Anopheles stephensi*	Unknown (M)
AF069063	N/A	
Culicospora magna	*Culex restuans*	United States (M,H)
AY326269	AY988448	
Culicosporella lunata	*Culex pilosis*	United States (M)
AF027683	N/A	
Edhazardia aedis	*Aedes aegypti*	Taiwan (M)
AF027684	AY988440	United States (H)
Hazardia milleri	*Culex quinquefasiatus*	United States (M,H)
AY090067	AY988447	
Hyalinocysta chapmani	*Culiseta melanura*	United States (M,H)
AF483837	AY988453	
Intrapredatorus barri	*Culex fuscanus*	Taiwan (M)
AY013359	N/A	
Parathelohania anophelis	*Anopheles quadrimaculatus*	United States (M,H)
AF027682	AY988423	
Parathelohania obesa	*Anopheles crucians*	United States (M)
AY09006	N/A	
Senoma globulifera	*Anopheles messeae*	Russia (M,H)
DQ641245	N/A	
Vavraia culicis	*Culex pipiens*	United States (M,H)
AJ252961	AY988445	
Additional mosquito species		
	Ochlerotatus abserratus	United States (H)
	AY988426	
	Ochlerotatus aurifer	United States (H)
	AY988427	

GTGCCAGCMGCCGCGG; SS530r, CCGCGGKGCTGGCAC; 1047r, AAC GGCCATGCACCAC; 1061f, GGTGGTGCATGGCCG; 1492r, GGTTA CCTTGTTACGACTT.

Sequences were obtained from the NCBI GenBank database and were aligned using the ClustalX program (Thompson et al., 1997). Aligned sequences were analyzed by Maximum Parsimony, Maximum Likelihood and Neighbor Joining analyses using PAUP version 3.1b (Swofford, 1998). Bootstrap analysis was accomplished using 1000 Neighbor-joining replicates. Maximum Parsimony analysis was done using the heuristic search method. All characters were unordered and had equal weight, no topological constraints were enforced and 838 characters were parsimony informative. Maximum Likelihood analysis was accomplished using the heuristic search method. The substitution model was selected and the Ti/tv ration was set to two. Two microsporidian outgroups: *Anncaliia algerae* and *Vavraia culicis* were selected, both disparately related parasites of mosquitoes representing clades 3 and 5, respectively of Vossbrinck and Debrunner-Vossbrinck (2005). Additional species included in the phylogenetic analyses are shown in Table 2.

3. Results

3.1. Isolation and collection data

Twenty-one morphologically and genetically unique species of microsporidia were isolated from nine species of larval mosquitoes

Table 2
Comparison of key diagnostic morphological features among newly proposed microsporidia isolated from mosquitoes in Siberia, Russia.

Microsporidium	Mosquito host	Spore size ± SD (μm)	Spore ultrastructure				
			Polar filament		Spore wall (nm)		
			Broad coils (diameter nm)	Narrow coils (diameter nm)	Arrangement	Exospore	Endospore
Amblyospora bakcharia	*Oc. excrucians*	5.4 ± 0.6 × 3.5 ± 0.6	4½ (230)	8½ (120)	Uniform	60	140
Amblyospora baritia	*Oc. excrucians*	6.1 ± 0.6 × 4.1 ± 0.5	4½ (220)	15 (140)	Irregular	160	90
Amblyospora bogashovia	*Oc. excrucians*	7.8–6.4 × 5.4–5.5	5½ – 6 (280)	8–8½ (130)	Irregular	120	60
Amblyospora chulymia	*Oc. caspius*	5.9 ± 0.6 × 4.7 ± 0.6	4 (190)	7½ (100)	Uniform	200	150
Amblyospora hristinia	*Oc. communis*	6.8 ± 0.6 × 5.1 ± 0.5	3 (300)	13 (150)	Irregular	220	130
Amblyospora jurginia	*Oc. excrucians*	5.3 ± 0.5 × 3.8 ± 0.4	3 (290)	5 (160)	Uniform	220	60
Amblyospora kazankia	*Oc. dianaetus*	5.1 ± 0.1 × 3.5 ± 0.1	5 (200)	9 (120)	Uniform	50	110
Amblyospora mavlukevia	*Ae. cinereus*	4.4 ± 0.2 × 3.7 ± 0.2	5 (220)	14–15 (150)	Irregular	280	110
Amblyospora mocrushinia	*Oc. punctor*	4.9 ± 0.5 × 3.3 ± 0.4	4½ – 5 (240)	7–7½ (140)	Uniform	60	120
Amblyospora modestium	*Cx. modestus*	4.8 ± 0.3 × 3.6 ± 0.4	3 (300)	6 (150)	Irregular	180	150
Amblyospora salairia	*Ae. cinereus*	5.4 ± 0.6 × 3.8 ± 0.6	2½ – 3 (230)	8½ – 10½ (120)	Irregular	170	80
Amblyospora severinia	*Oc. excrucians*	5.3–5.6 × 3.9–4.7	3–4 (220)	14–15 (150)	Irregular	200	110
Amblyospora shegaria	*Ae. cinereus*	3.8 ± 0.1 × 3.4 ± 0.2	4 (250)	12 (150)	Irregular	270	60
Amblyospora timirasia	*Ae. cinereus*	4.9 ± 0.3 × 3.2 ± 0.3	3 (180)	4 (90)	Uniform	120	90
Novothelohania ovalae	*Oc. caspius*	3.4 ± 0.4 × 2.1 ± 0.3	3½ – 4 (190)	3 (130)	Uniform	160	80

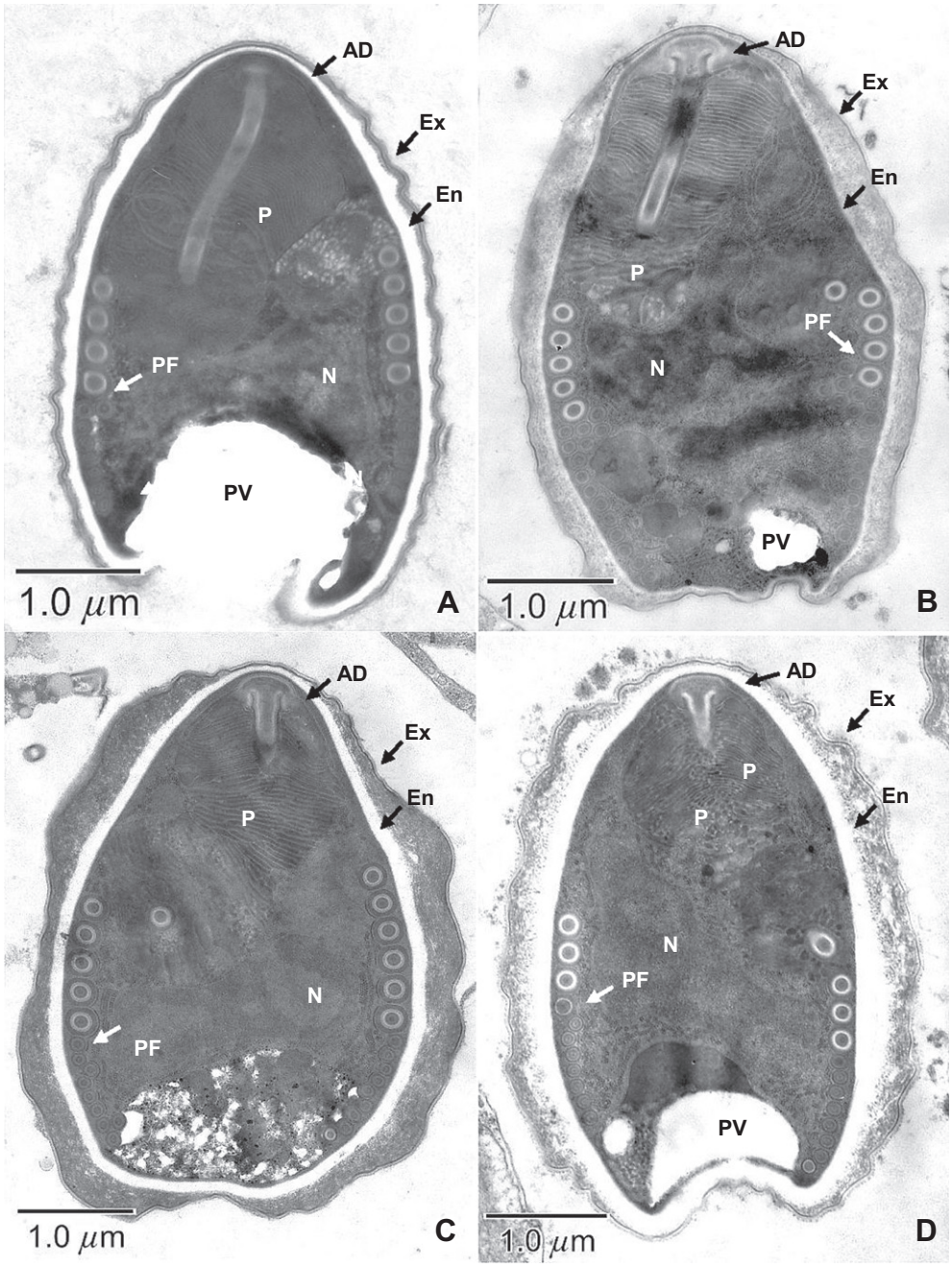

Fig. 1. Ultrastructural morphology of meiospores of *Amblyospora* species isolated from Siberian mosquitoes. (A) *Amblyospora bakcharia* from *Ochlerotatus excrucians*. (B) *Amblyospora baritia* from *Oc. excrucians*. (C) *Amblyospora bogashovia* from *Oc. excrucians*. (D) *Amblyospora chulymia* from *Ochlerotatus caspius*. AD, anchoring disc; En, endospore; Ex, exospore; N, nucleus; P, polaroplast; PF, polar filament; Pm, plasmalemma; PV, posterior vacuole.

(Table 1). These included: (1) 14 proposed new species of *Amblyospora* from *Aedes cinereus* (four species), *Culex modestus*, *Ochlerotatus caspius*, *Ochlerotatus communis*, *Ochlerotatus diantaetus*, *Ochlerotatus excrucians* (five species), and *Ochlerotatus punctor*; (2) a newly proposed genus, *Novothelohania* from *Oc. caspius* and; (3) six recently described species of *Amblyospora* (*Amblyospora flavescens* = *Oc. dianaetus*, *Amblyospora kolarovi* = *Oc. punctor*, *Amblyospora rugosa* = *Ochlerotatus cataphylla*) (Simakova and Pankova, 2005), *Parathelohania* (*Parathelohania divulgata* and *Parathelohania tomski* = *Anopheles messeae*) (Simakova and Pankova, 2004a) and *Trichoctosporea* (*Trichoctosporea pygopellita* = *Oc. excrucians*) (Simakova and Pankova, 2004b) from which gene sequences had not been previously obtained.

3.2. Host identification

Sequencing of the 18s SSU rDNA fragment from each larval mosquito host from which one or more microsporidian parasites was found generated a sequence of approximately 1862 nucleotides. GenBank assession numbers are shown in Table 1. Comparative analysis of sequences obtained from the six host mosquitoes identified as *Oc. excrucians* from Siberia, with a sequence of *Oc. excrucians* from Connecticut, USA (GenBank Accession No. AY988430) revealed no (JF837523, JF837525, JF837526), one (JF837521, JF837522), and two (JF837524), nucleotide differences. All four host mosquitoes identified as *Ae. cinereus* from Siberia (JF837532, JF837533, JF837534, JF837535) shared 100% nucleotide

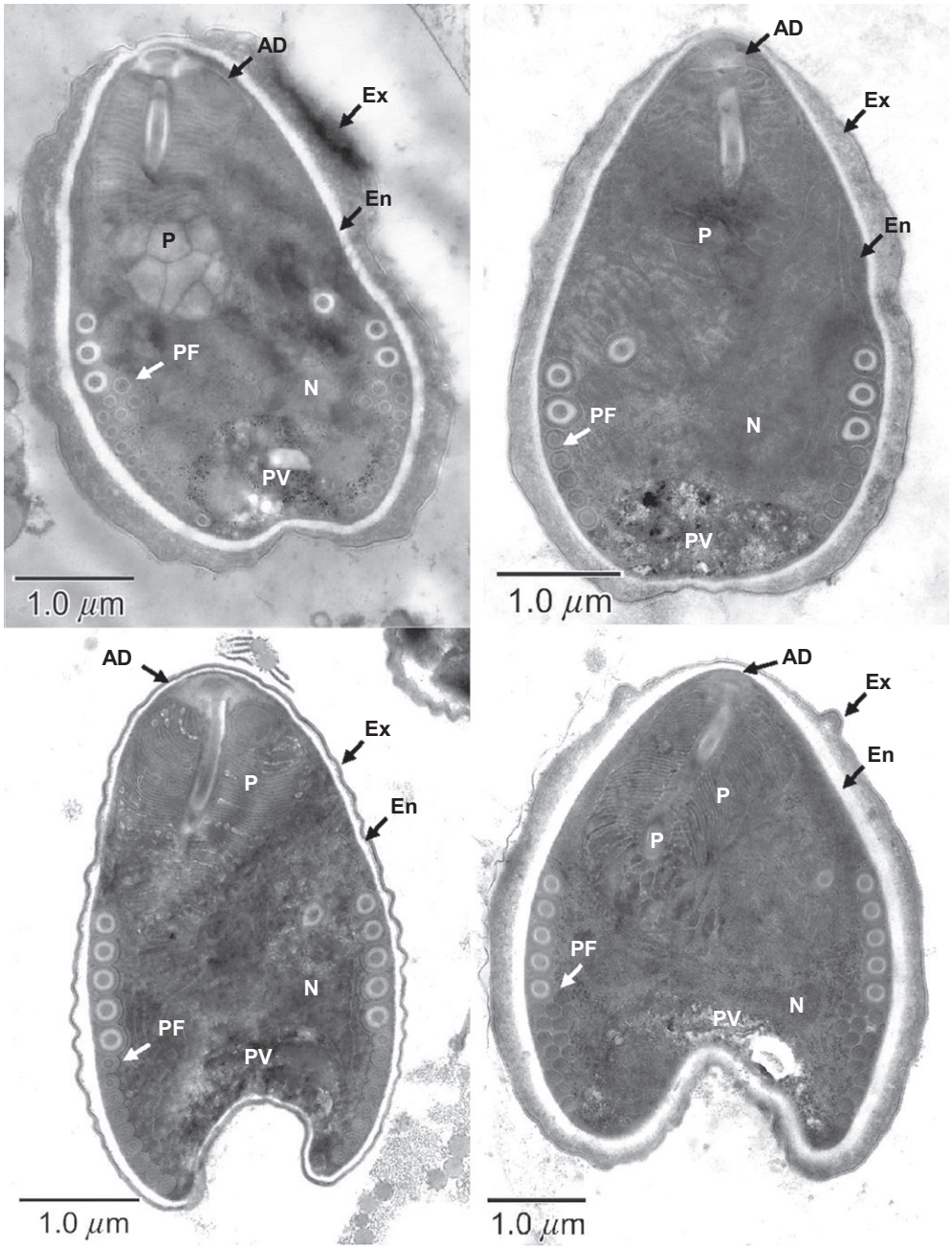

Fig. 2. Ultrastructural morphology of meiospores of *Amblyospora* species isolated from Siberian mosquitoes. (A) *Amblyospora hristinia* from *Ochlerotatus communis*. (B) *Amblyospora jurginia* from *Ochlerotatus excrucians*. (C) *Amblyospora kazankia* from *Ochlerotatus dianeatus*. (D) *Amblyospora mavlukevia* from *Aedes cinereus*. AD, anchoring disc; En, endospore; Ex, exospore; N, nucleus; P, polaroplast; PF, polar filament; Pm, plasmalemma; PV, posterior vacuole.

sequence identity with *Ae. cinereus* from Connecticut, USA (AY988441). Percent nucleotide sequence identity for the remaining mosquitoes collected from Siberia was as follows: *Oc. caspius* (JF837527, JF837528 = 99.8% with AM071383, Spain); *Oc. communis* (JF837529 = 99.9 with AY988425, USA); *Oc. punctor* (JF837536 = 99.8% with APU48378 Colorado USA). No sequences of the 18S SSU rDNA fragment were available from GenBank to compare our sequences from *An. messeae* (JF837537), *Cx. modestus* (JF837538), or *Oc. cataphylla* (JF837530).

3.3. Microsporidian ultrastructural morphology

Ultrastructural examination of the 14 proposed new species of *Amblyospora* (Fig. 1–4) revealed infections exclusively confined to larval fatbody tissue, and general morphological features diagnostic for the genus including: broadly ovoid, uninucleate meiospores formed in groups of 8 within a sporophorous vesicle, with a large posterior vacuole, well developed anchoring disc, large lamellate polaroplast, anisofilar polar filament, and stratified spore wall with exospore and endospore components. Unique quantifiable structural features associated with meiospores were observed for all species including each of the microsporidia isolated from *Ae. cinereus* ($n = 4$) (Fig. 2D, 3C and 4A and B) and *Oc. excrucians* ($n = 5$) (Fig. 1A–C, 2B and 3D). These structural features are summarized in Table 2 and included: measurable differences in overall spore size (3.4–7.8 μm × 2.1–5.5 μm), number and arrangement of the broad (3–5) and narrow coils (4–15) of the polar filament, and thickness of the exospore (60–280 nm) and endospore (60–150 nm) walls.

Spores of the newly proposed genus and species, *Novothelohania ovalae* isolated from *Oc. caspius* were similarly uninucleate

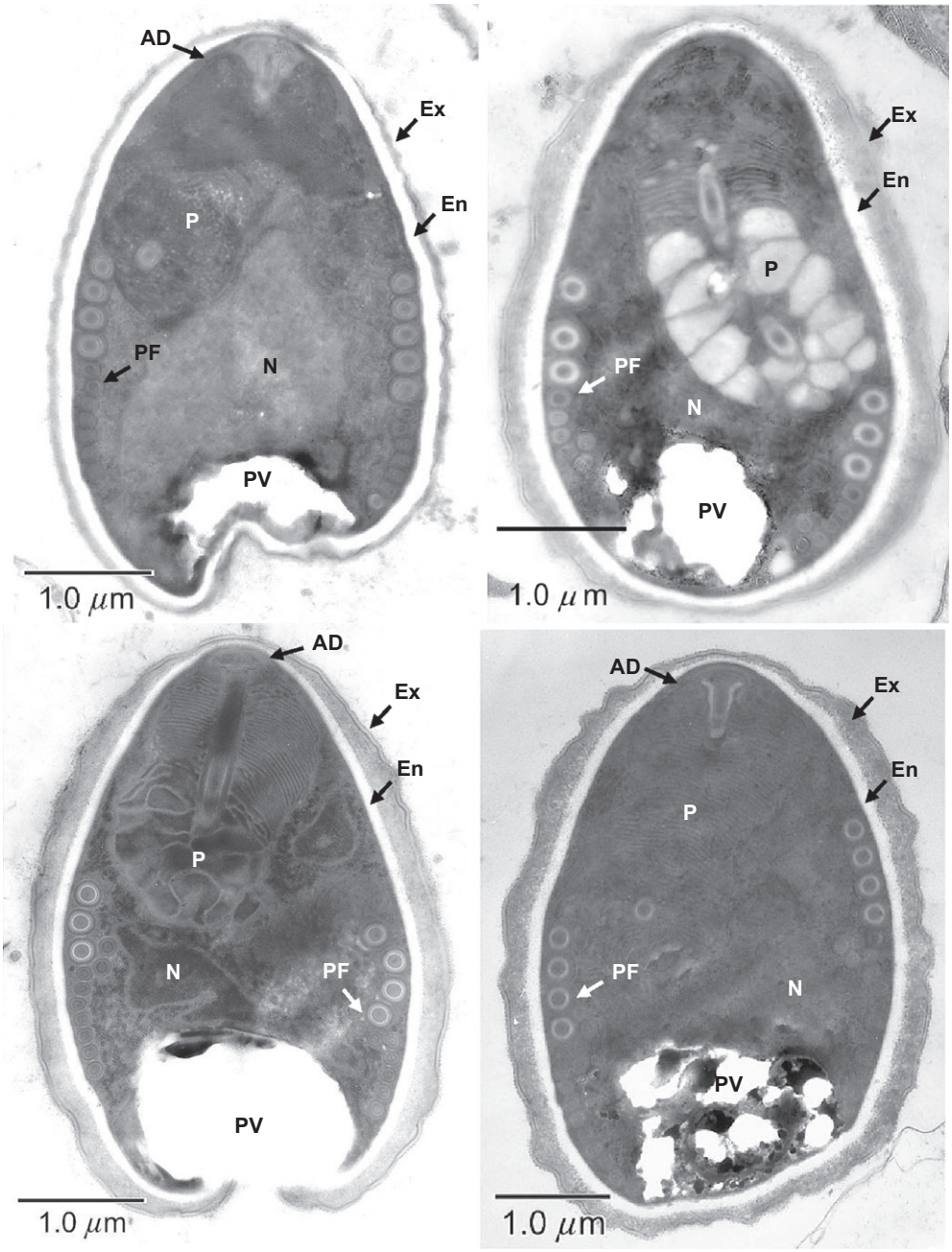

Fig. 3. Ultrastructural morphology of meiospores of *Amblyospora* species isolated from Siberian mosquitoes. (A) *Amblyospora mocrushinia* from *Ochlerotatus punctor*. (B) *Amblyospora modestium* from *Culex modestus*. C. *Amblyospora salairia* from *Aedes cinereus*. (D) *Amblyospora severinia* from *Ochlerotatus excrucians*. AD, anchoring disc; En, endospore; Ex, exospore; N, nucleus; P, polaroplast; PF, polar filament; Pm, plasmalemma; PV, posterior vacuole.

and formed in groups of 8 within a sporophorous vesicle (Fig. 5). However, they were markedly smaller in size (3.4 μm × 2.1 μm), oval in shape and possessed a large umbrella-shaped anchoring disc contiguous with a uniformly packed narrow lamellate polaroplast with no apparent vesicular components. The polar filament was anisofilar but more gradually tapered with a noticeably less severe constriction between the wide and narrow portions. Complete species descriptions are given in the taxonomic summary in Section 5.

3.4. Microsporidian molecular phylogeny

The SSU rDNA sequences (approximately 1260 bp) obtained from each of the 15 newly proposed and six previously described species of microsporidia isolated from Siberian mosquitoes were unique when compared with GenBank entries. Phylogenetic trees constructed by Maximum Parsimony (not shown), Maximum Likelihood (not shown) and Neighbor Joining (Fig. 6) analyses yielded similar topologies with a high degree of congruence between parasite and host at the generic level.

The sixteen species of *Amblyospora* and one species of *Trichoctosporea* isolated from *Aedes*/*Ochlerotatus* mosquitoes (Fig. 6) segregated with previously reported *Amblyospora* species of *Aedes*/*Ochlerotatus* mosquitoes into a monophyletic grouping containing three well-supported clades (100%, 94% and 82% bootstrap support). *Amblyospora modestium* from *Cx. modestus* similarly grouped with *Amblyospora* species from *Culex* mosquitoes as a fourth sister clade (99% bootstrap support), while *P. divulgata* and *P. tomski* from

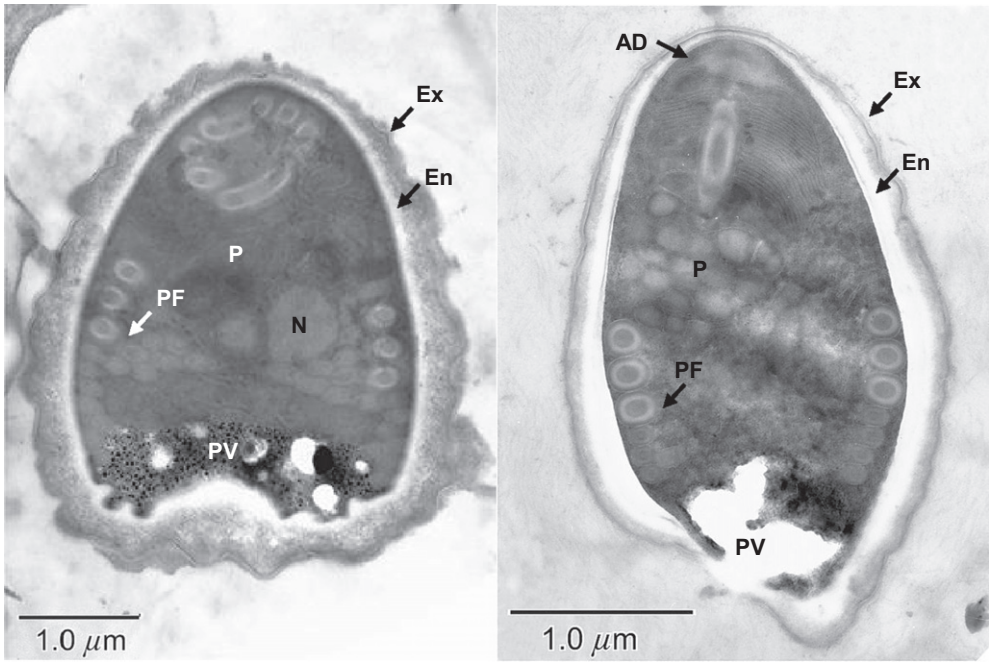

Fig. 4. Ultrastructural morphology of meiospores of *Amblyospora* species isolated from *Aedes cinereus*. (A) *Amblyospora shegaria*. (B) *Amblyospora timirasia*. AD, anchoring disc; En, endospore; Ex, exospore; N, nucleus; P, polaroplast; PF, polar filament; Pm, plasmalemma; PV, posterior vacuole.

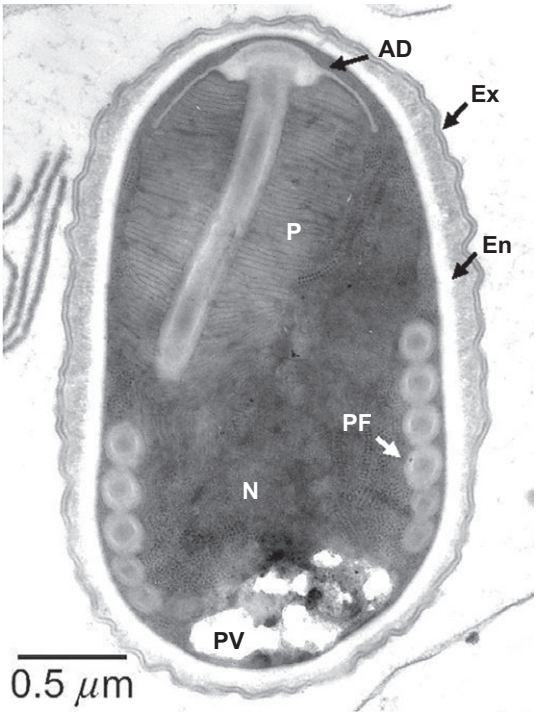

Fig. 5. Ultrastructural morphology of a meiospore of *Novothelohania ovalae* isolated from *Ochlerotatus caspius*. AD, anchoring disc; En, endospore; Ex, exospore; N, nucleus; P, polaroplast; PF, polar filament; Pm, plasmalemma; PV, posterior vacuole.

Anopheles messeae grouped with *Parathelohania* species from *Anopheles* mosquitoes in an equally well-supported clade (100% bootstrap support). The *Parathelohania* species like their *Anopheles* hosts are the sister group to their respective clades. The newly proposed microsporidium, *N. ovalae* isolated from *Oc. caspius*, was most closely related to the clade containing *Parathelohania* species (100% bootstrap support) with a sequence divergence ranging from 14% to 15% rather than the 2–7% divergence seen within the *Parathelohania*.

The five newly proposed (*Amblyospora bakcharia*, *Amblyospora baritia*, *Amblyospora bogashovia*, *Amblyospora jurginia*, and *Amblyospora severinia*) and two previously described (*Amblyospora excrucii* and *T. pygopellita*) species of microsporidia isolated from *Oc. excrucians* are genetically diverse as evidenced by their position in all three clades containing species parasitic among Aedes/Ochlerotatus mosquitoes (Fig. 7) and by their relatively low sequence similarities ranging from 83.8% to 96.1% (ave. = 88.4%) (Table 3). *A. flavescens* and *Amblyospora kazankia* isolated from *Oc. Diantaeus* (85.6% sequence identity), and *A. kolarovi* and *Amblyospora mocrushinia* isolated from Oc. punctor (87.7% sequence identity), were similarly segregated into distally related clades within the larger Aedes/Ochlerotatus/Amblyospora grouping.

The five species of *Amblyospora* (*Amblyospora mavlukevia*, *Amblyospora salairia*, *Amblyospora shegaria*, and *Amblyospora timirasia*) isolated from *Ae. cinereus*, on the other hand, grouped more closely together (Fig. 7), and with the exception of *A. timirasia*, had sequence similarities that exceeded 95% (Table 3). This clade also includes *Amblyospora cinerei*, a North American species isolated from the same mosquito host. Similarly, *Amblyospora hristinia* from Siberia, Russia and *Amblyospora khaliulini* from Connecticut, US (Fig. 7) appear to have a common origin in *Oc. communis* (100% bootstrap support, 96.8% sequence identity). *A. khaliulini* is most genetically similar to *A. kolarovi* (99.0% sequence similarity) from a different but closely related mosquito host, *O. punctor* in our analysis. Among the new species isolated from *Culex* mosquitoes, *A. modestium* was most closely aligned with *A. indicola* (94.7% sequence identity), a species of Australian origin from *Culex sitiens* (Table 4).

The SSU rDNA sequences obtained from the two *Parathelohania* species (*P. divulgata* and *P. tomski*) isolated from *An. messeae* grouped together in a clade containing *Parathlohania anophelis* and *Parathelohania obesa* with maximum bootstrap support. Sequence similarity exhibited between *P. divulgata* and *P. tomski* was 97.7%, and differed by 6.0–7.6% from *P. anophelis* and *P. obesa*

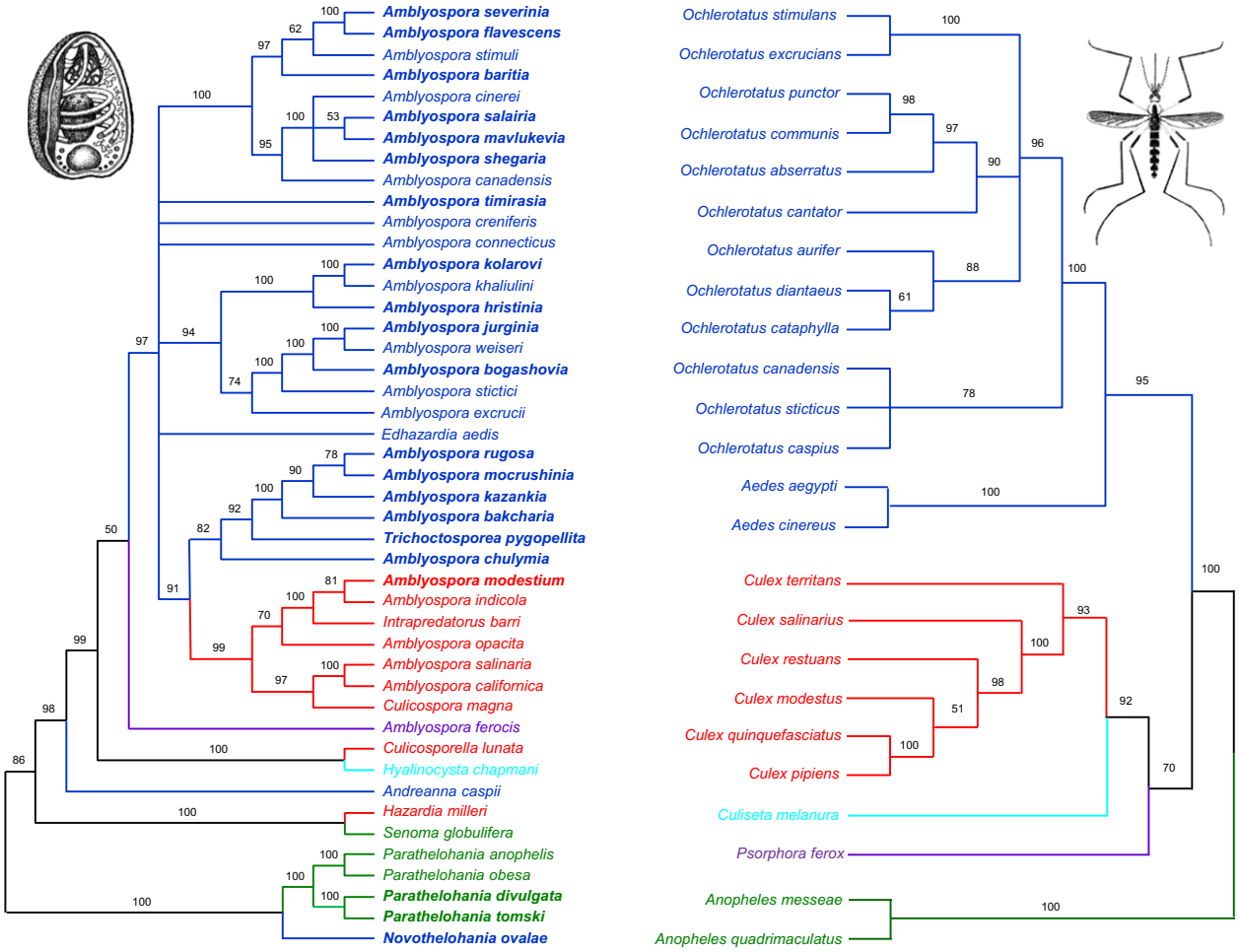

Fig. 6. Comparison of microsporidian and mosquito host phylogenetic trees. Bootstrap analysis based on 1000 replicates using Neighbor Joining analysis. Newly sequenced species of microsporidia from Siberia are in bold. Microsporidia and corresponding mosquito host genera are color-coded.

(Table 5). The sequence difference between *P. divulgata* and *P. tomski*, and *Senoma globulifera* a distally related parasite from the same host mosquito, *An. messeae* was 22.7%.

4. Discussion

4.1. Justification for creation of new Amblyospora species

The unique morphological characters observed in meiospores and correspondingly novel SSU rDNA sequences obtained from each of the 14 newly proposed species of *Amblyospora* isolated from larval mosquitoes collected in Siberia, Russia support their designation as new taxa within the *Amblyospora* clade. We found no contradictions between the morphological and molecular taxonomic data. In every instance, the combined cytological features associated with the length, arrangement and ratio of broad to narrow coils of the polar filament, comparative thickness of the exospore and endospore, and overall size of the spore were unique for each species (Table 2) reaffirming their value in distinguishing taxonomic relationships among the *Amblyospora* (Andreadis, 1994a). Among the newly proposed species, the closest affinity was seen between *A. severinia* from *Oc. excrucians* (Russia) and *A. flavescens* from *Oc. diantaeus* (Russia) which differed by ~29 nucleotides, a comparatively large number. Among all species that were sequenced within the *Amblyospora* clade, the closest affinity was seen between *A. khaliulini* from *Oc. communis* (United States) and *A. kolarovi* from *Oc. punctor* (Russia) which differed by ~12 nucleotides.

Of particular note concerning morphology, were differences in the numerical length of the polar filament between species infecting *Aedes*/*Ochlerotatus* and *Culex* mosquitoes as measured by the number of coils viewed in full sagittal section. A comparative analysis of the 17 *Amblyospora* species isolated in the present study from *Aedes*/*Ochlerotatus* and *Culex* mosquitoes collected in Russia with 26 additional species from other geographic regions (Andreadis, 1994a and references therein; Garcia and Becnel, 1994; Micieli et al., 2000a; Simakova and Pankova; 2005), revealed quantifiable differences among species parasitizing these two genera of mosquitoes (Fig. 8). Although some overlap is apparent, species parasitizing *Aedes*/*Ochlerotaus* mosquitoes as a group, possess significantly ($p = 0.005$) longer polar filaments (median no. coils = 12, range 5–19½) than species parasitizing *Culex* mosquitoes (median no. coils = 9, range 3½–16). The functional significance of this structural variation in the length of the polar filament between parasite species infecting these two host genera is not overtly apparent, but it is does concur with the molecular data where we found a very strong association between parasite lineage and mosquito host at the generic level.

The functional role of the polar filament in establishing infections in a susceptible host through penetration and inoculation of the infectious sporoplasm into a target host cell following spore

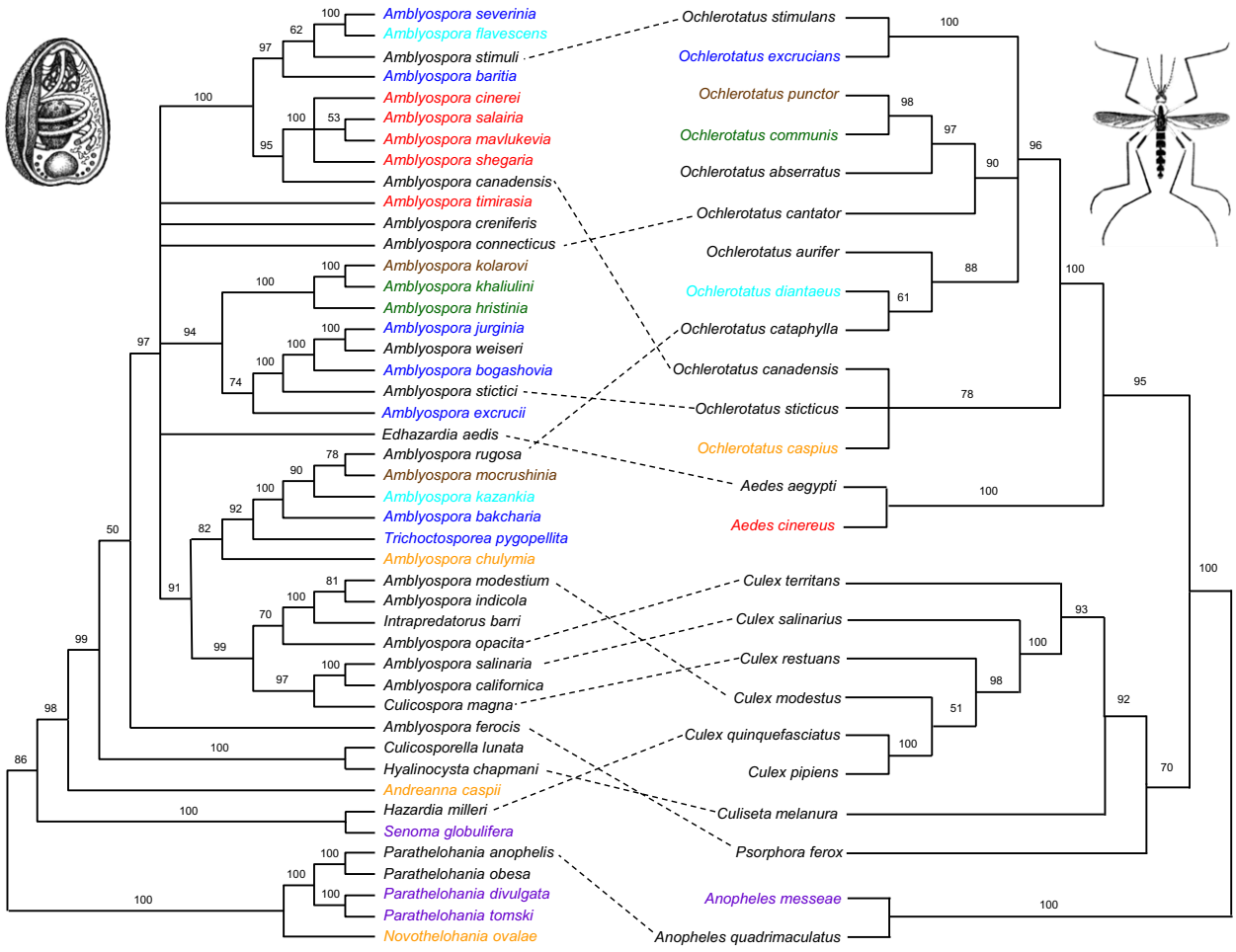

Fig. 7. Phylogenies of microsporidian parasites and their mosquito hosts. Dashed lines connecting taxa and colors indicate specific mosquito host and microsporidian parasite associations.

ingestion is well established (Keohane and Weiss, 1999), but it is unclear how the microsporidian polar filament penetrates the host cell membrane and whether the polar filament or spore itself binds to specific receptors on the host cell membrane (Franzen, 2004, 2005). It is also unclear how and to what degree the length of the polar filament helps to mediate this process. It has been suggested that polar filament "extension" and by association length, may be an adaptation of non-motile spores which allows them to infect distant target cells within the host (Franzen, 2005). In the case of the *Amblyospora*, this would include ovarian tissue in cyclopoid copepods, which thus far has been identified as the sole target tissue in all species that have been examined to date regardless of the host mosquito from which the meiospores originated (Andreadis, 1988; Becnel, 1992; Becnel and Andreadis, 1998; Micieli et al., 1998, 2000a, 2000b). Unfortunately, the limited numbers of copepod species from which observations have been made (4 from *Culex* and 2 from *Ochlerotatus* mosquito hosts) preclude any further analysis at this time.

4.2. Justification for creation of Novothelohania n. g.

Although we recognize that care should be exercised in erecting a new genus of microsporidia without including detailed morphology and development of vegetative stages, the distinctive morphological characters observed in spores and unique SSU rDNA sequence obtained from *N. ovalae* warrant the creation of a new genus and species for this microsporidium isolated from *Oc. caspius*. *N. ovalae* segregated with the *Parathelohania* clade from *Anopheles* mosquitoes (Fig. 6) but exhibited a sequence divergence of 14% (~176 nucleotides) with its nearest relative, *P. divulgata* (Table 5). Moreover, spores of this microsporidium possessed none of the diagnostic morphological features associated with *Parathelohania* meiospores, most notably, the posterior "bottleneck" extension of the spore wall and abruptly constricted anisofilar polar filament (Hazard and Anthony, 1974). The distinctive thin "umbrella-shaped" anchoring disc found in *N. ovalae* also differed markedly from the more "cap-like" structure typically seen in most species of *Parathelohania* (Hazard and Anthony, 1974; Hazard and Oldacre, 1975).

4.3. Justification for validity of P. divulgata and P. tomski

The molecular data obtained in our study support the designation of *P. divulgata* and *P. tomski* as separate valid species. The sequences obtained from these two parasites isolated from *An. messeae* differed by ~29 nucleotides, a similar magnitude found between two North American microsporidian species, *P. anophelis* and *P. obesa* from *Anopheles quadrimaculatus* and *Anopheles crucians*, respectively. The nucleotide sequence data for *P. divulgata* and *P. tomski* demonstrate greater genetic variation than might be inferred from the comparatively minor differences observed in meiospore ultrastructure. Meiospores of *P. divulgata* are slightly larger in size (*P. divulgata* = 4.6–5.3 μm × 2.4–3.2 μm; *P. tomski* = 4.4–4.9 μm × 2.1–2.5 μm) and possess marginally fewer coils of the polar filament (*P. divulgata* = 2 broad and 4.5 narrow coils; *P.*

Table 3
Percent similarity of microsporidian species isolated from *Aedes*/*Ochlerotatus* mosquitoes based on SSUrDNA sequences.[a]

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
1. *A. severinia*	-																									
2. *A. flavescens*	97.7	-																								
3. *A. stimuli*	94.6	94.3	-																							
4. *A. baritia*	94.4	94.0	96.9	-																						
5. *A. cinerei*	93.4	93.4	93.0	92.9	-																					
6. *A. salairia*	91.0	90.6	93.7	93.4	98.0	-																				
7. *A. mavlukevia*	89.9	89.8	93.1	93.3	96.2	96.4	-																			
8. *A. shegaria*	90.3	90.1	93.4	93.4	96.3	95.6	95.3	-																		
9. *A. canadensis*	90.6	89.9	93.4	93.5	94.6	92.0	91.4	91.5	-																	
10. *A. timirasia*	86.9	86.5	89.0	88.5	88.5	87.4	86.5	87.0	87.1	-																
11. *A. criniferis*	85.8	85.7	88.6	89.5	87.1	85.4	84.9	85.2	85.6	86.9	-															
12. *A. connecticus*	85.9	85.9	89.2	88.6	89.1	87.2	86.2	86.7	87.3	87.4	85.9	-														
13. *A. kolarovi*	88.8	88.1	90.8	90.8	90.2	88.7	88.1	88.0	88.5	88.7	87.6	88.8	-													
14. *A. khaliulini*	88.3	87.7	90.6	90.6	90.2	88.6	88.0	88.1	88.3	88.7	86.6	88.3	99.0	-												
15. *A. hristinia*	88.4	87.9	90.4	90.5	90.1	88.6	88.4	88.4	88.1	88.4	87.5	88.9	97.1	96.8	-											
16. *A. jurginia*	87.6	87.6	90.6	90.3	91.0	88.7	88.2	89.0	87.5	86.3	85.7	87.0	90.2	90.2	90.0	-										
17. *A. weiseri*	87.8	87.6	90.5	90.2	91.0	88.9	88.3	88.9	87.8	86.3	87.0	87.4	90.4	89.9	90.0	98.9	-									
18. *A. bogashovia*	86.2	86.0	90.7	90.0	90.4	86.6	86.8	86.8	86.2	85.6	85.8	87.2	90.0	89.8	89.9	96.1	96.3	-								
19. *A. stictici*	87.4	87.0	89.9	89.8	89.8	88.1	87.5	87.6	87.8	86.0	86.1	87.7	91.0	90.5	90.0	93.0	93.0	93.3	-							
20. *A. excrucii*	86.7	86.5	89.3	89.0	89.1	87.9	87.4	87.7	87.5	86.2	84.7	86.6	90.4	90.1	90.4	89.7	89.8	89.3	89.6	-						
21. *E. aedis*	86.1	86.2	89.4	89.8	89.8	87.9	86.8	86.5	87.4	86.6	85.3	87.4	89.2	88.9	88.1	88.1	87.8	86.5	88.1	88.0	-					
22. *A. rugosa*	85.1	85.1	88.7	89.3	88.8	87.4	86.9	87.4	86.7	86.3	84.5	86.4	87.8	87.9	87.6	86.7	86.5	86.2	86.5	86.3	87.4	-				
23. *A. mocrushinia*	85.7	85.6	88.8	89.4	88.9	87.4	87.1	87.1	87.4	86.8	84.5	86.6	87.7	87.7	87.8	86.5	86.3	86.1	86.3	85.9	87.4	95.7	-			
24. *A. kazankia*	85.7	85.6	89.0	89.2	88.7	87.0	86.6	87.1	86.1	87.1	84.6	86.3	88.4	88.6	88.3	87.0	86.9	86.4	86.4	86.4	87.4	95.2	95.4	-		
25. *A. bakcharia*	86.2	85.9	89.2	89.8	88.7	87.5	87.1	87.4	87.2	86.9	85.6	86.1	88.7	88.6	88.4	88.0	87.8	87.8	87.7	86.9	87.6	94.9	94.9	94.9	-	
26. *T. pygopellita*	84.5	84.0	89.8	89.9	88.7	85.2	85.0	85.0	84.5	84.7	84.0	84.6	88.1	85.6	87.6	85.6	86.6	84.5	83.9	83.8	85.5	88.7	89.4	89.1	89.1	-
27. *A. chulymia*	86.2	86.2	88.9	89.3	88.8	86.7	86.2	86.5	86.9	86.5	84.9	85.4	88.5	88.8	88.3	87.1	86.6	86.7	86.7	85.9	87.6	88.6	88.7	89.4	89.8	87.6

[a] Colors denote multiple microsporidian species isolated from the same mosquito host: *Ae. cinereus*, *Oc. communis*, *Oc. diantaeus*, *Oc. excrucians*, *Oc. punctor*.

tomski = 2 broad and 5 narrow coils) (Simakova and Pankova, 2004a). Both parasites were collected in late June from similar flood-plain ponds alongside rivers, but in different geographic locales in the Tomsk region (*P. divulgata* = Tom River, Kolarovo; *P. tomski* = Chulym River, Teguldet) suggesting local geographic segregation. We further note the existence of at least three other morphologically distinct species of *Parathelohania* described from *An. messeae* from this same geographic region but from which we have no equivalent molecular data: *Parathelohania formosa*, *Parathelohania sibirika* (Simakova and Pankova, 2004a), and *Parathelohania illinoisensis* var. *messeae* (Pankova et al., 1991).

4.4. Evidence for coevolution of parasite and host

The results obtained in the present study greatly expand the number of microsporidian taxa parasitic in mosquitoes from which we can more precisely evaluate phylogenetic relationships and infer coevolutionary events with their respective mosquito hosts. At the generic level, we continue to see a strong correlation between parasite lineage and host group evidenced by largely congruent phylogenies (Fig. 6). *Amblyospora* species that parasitize *Aedes*/*Ochlerotatus* and *Culex* mosquitoes segregate into distinct monophyletic groupings with high bootstrap support mirroring their host phylogeny, while *Parathelohania* species from *Anopheles* mosquitoes group as a sister clade basal to the entire group of mosquito-parasitic microsporidia as their *Anopheles* hosts similarly cluster as a sister clade to the entire group of culicine mosquitoes (Shepard et al., 2006). Although the number of species is limited, we also see the relative positions of *Amblyospora ferocis* from *Psorophora ferox* and *Hyalinocysta chapmani* from *Culiseta melanura* as distinct sister groups to the *Amblyospora* clade from *Aedes*/*Ochlerotatus* and *Culex* mosquitoes consistent with the position of these two host taxa as basal clades to the *Culex* mosquitoes. Taken in totality, these congruent phylogenies provide strong evidence for host-parasite coevolution by descent at the generic level, and with a few exceptions, indicate limited host lineage switching between unrelated taxa. The most notable exception is *Oc. caspius*, which was found to harbor three distally related microsporidia, *Amblyospora chulymia*, *Andreanna caspii*, and *N. ovalae*. With the inclusion of 29 new microsporidian taxa, our findings also provide further credibility to the hypothesis (Vossbrinck et al., 2004) that this entire group of mosquito parasitic microsporidia arose as a single event and evolved from parasites of crustaceans which serve not only as intermediate hosts for all species of *Amblyospora*, *Hyalinocysta* and *Parathelohania* that have been examined thus far but also serve as hosts to sister taxa to the Amblyosporidae (Vossbrinck and Debrunner-Vossbrinck, 2005).

4.5. Evidence for cospeciation, host switching and independent parasite speciation

The partial incongruences observed between the *Amblyospora* and *Parathelohania* tree topologies and their respective mosquito hosts (Fig. 7) indicate that pure faithful cospeciation with simulta-

Table 4
Percent similarity of microsporidian species isolated from *Culex* mosquitoes based on SSUrDNA sequences.

	1	2	3	4	5	6
1. *A. modestium*	_					
2. *A. indicola*	94.7	_				
3. *I. barri*	92.7	93.7	_			
4. *A. opacita*	90.6	90.6	89.8	_		
5. *A. salinaria*	85.5	87.0	86.3	85.5	_	
6. *A. californica*	85.2	86.3	85.7	84.8	96.6	_
7. *C. magna*	89.6	89.2	87.3	88.0	87.2	86.5

Table 5
Percent similarity of *Novothelohania ovalae* n. g., n. sp. with microsporidian species isolated from *Anopheles* mosquitoes based on SSUrDNA sequences.

	1	2	3	4	5
1. *N. ovalea*	_				
2. *P. tomski*	85.3	_			
3. *P. divulgata*	85.7	97.7	_		
4. *P. obesa*	85.0	92.5	93.7	_	
5. *P. anophelis*	84.9	92.4	94.0	97.7	_
6. *S. globulifera*	72.7	76.7	76.9	77.3	77.3

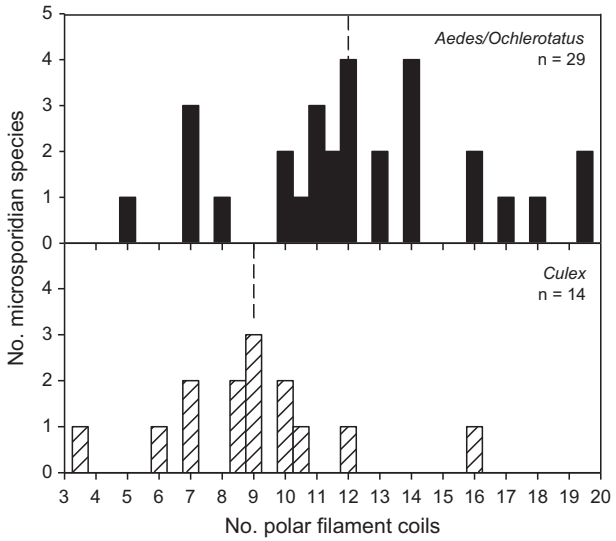

Fig. 8. Frequency distribution showing the number of polar filament coils observed in meiospores among 43 species of *Amblyospora* isolated from *Aedes*/*Ochlerotatus* and *Culex* mosquito hosts. Vertical dashed lines denote median number.

neous divergence of mosquito host and microsporidian parasite has not likely occurred within this group of parasites. Rather, the topologies of the mosquito host and microsporidian parasite phylogenies suggest mixed evolutionary events that imply various degrees of cospeciation, host switching and independent parasite speciation (Page, 2003).

Among the microsporidian parasites of *Culex* mosquitoes, we find only one parasite per host consistent with a high level of host specificity. The congruency between mosquito and microsporidian phylogenies is consistent with joint speciation between host and parasite. Among closely related parasites of *Aedes*/*Ochlerotatus* and *Anopheles* mosquitoes, on the other hand, we found several instances where a single mosquito species serves as a host for two or more related species of microsporidia, an observation more consistent with host switching and independent parasite speciation. From Figure ure7 we observe four species of *Amblyospora* (*A. cinerei*, *A. salairia*, *A. mavlukevia*, *A. shegaria*) isolated from *Ae. cinereus*, two species isolated from *Oc. communis* (*A. khaliulini* and *A. hristinia*), and two similarly related species of *Parathlohania* (*P. divulgata* and *P. tomski*) isolated from *An. messeae*. This implies that these microsporidian species evolved independently from a common ancestor within isolated populations of their respective hosts. In the absence of any phylogenetic information on the intermediate copepod hosts, we can only surmise that these microsporidia may have evolved from a closely related ancestor species found in sympatric copepod populations. It has been suggested that parasites with complex life cycles and modes of transmission such as the *Amblyospora* and *Parathelohania* may select for flexibility in their range of acceptable hosts provided these closely related hosts provide a suitable environment and opportunity for completion of the life cycle (Poulin, 2007). Our inferred interpretation for independent parasite speciation wherein the microsporidial lineage split into two or more daughter lineages associated with the same host mosquito is in agreement with large scale phylogenetic analyses that have shown strong links between parasite lineage and host groups irrespective of geography (Vossbrinck and Debrunner-Vossbrinck, 2005; Smith, 2009).

Conversely, the three widely disparate genera and species of microsporidia (i.e. *A. caspii*, *A. chulymia* and *N. ovalae*) identified from the mosquito *Oc. caspius*, appear to represent true host switching across taxonomic lines, perhaps in response to host availability. The basal phylogenetic positions of *A. caspii* and *N. ovalae* further imply early radiation of microsporidia into this mosquito species as seen with *Parathelohania* parasites in anopheline mosquitoes. All three microsporidia were collected from similar temporary vernal pool habitats in central Russia, but from different geographic locales within the region.

The wide variety of morphologically distinct *Amblyospora* species with dissimilar phylogenetic linages identified from *Oc. excrucians*, *Oc. diantaeus*, and *Oc. punctor* also seem to provide evidence for host switching but to a lesser degree. These observations suggest to us that host shifts occur more frequently in nature than had previously been recognized, and are more likely to take place between closely related hosts with ecological and/or physiological attributes more similar to the original host. This inference is supported by experimental transmission studies with *Amblyospora connecticus* from *Ochlerotatus cantator* and *Edhazardia aedis* from *Aedes aegypti*, both of which were shown to be capable of infecting closely related *Aedes* and *Ochlerotatus* species, but not distally related species in other genera including *Anopheles*, *Culex*, *Culiseta* and *Psorophora* (Hembree, 1982; Andreadis, 1989, Andreadis, 1994a,b; Becnel and Johnson, 1993). It is important to note that while these microsporidia underwent apparently normal vegetative growth and development within similar target tissues in these closely related mosquito hosts, in no instance were either species able to infect the ovaries and complete the life cycle via transovarial transmission.

4.6. Phylogenetic positions and taxonomic validity of Culicospora, Edhazardia, Intrapredatorus, and Trichoctosporea

Our expanded phylogenetic analyses continue to show that the monotypic genera *Culicospora*, *Edhazardia* and *Intrapredatorus* as well as *Trichoctosporea* fall well within the *Amblyospora* clade. *Culicospora magna* (host = *Culex restuans*) and *Intrapredatorus barri* (host = *Culex fuscanus*), segregate into a well-supported monophyletic clade with other *Amblyospora* species from *Culex* mosquitoes, while *E. aedis* (host = *Ae. aegypti*) and *T. pygopellita* (host = *Oc. excrucians*) clusters with other *Amblyospora* species from *Aedes*/*Ochlerotatus* mosquitoes.

Culicospora magna and *E. aedis* have developmental cycles and spore morphologies that largely mirror those observed in *Amblyospora* species, except neither species produces functional meiosp-

ores (*E. aedis* forms abortive meiospores), nor requires an intermediate host to complete its development. Another major difference found in *C. magna* and *E. aedis* is that functional haplosis of diplokaryotic vegetative stages is by nuclear dissociation followed by the production of uninucleate spores that are orally infectious to mosquito larvae. While in *Amblyospora* species, haplosis is by meiosis producing meiospores that are orally infectious to an intermediate copepod host where morpholocically and functionally similar uninucleate spores are subsequently formed (Becnel, 1994). Our phylogenetic analyses clearly demonstrate that these developmental and life cycle differences are not an indication of evolutionary relatedness, but more probably represent an adaptation to the ecological attributes and microhabitat of the host mosquito which likely enhance transmission (Vossbrinck et al., 2004).

The phylogenetic position of *I. barri* deep within the subclade of *Amblyospora* species isolated from *Culex* mosquitoes argues for reassignment of this monotypic species to the genus *Amblyospora.* This microsporidium has a life cycle that is nearly identical to *Amblyospora trinus*, a parasite from another predaceous mosquito host, *Culex halifaxi* (Becnel and Sweeney, 1990). It produces two uninucleate spore types in the larval mosquito host, one by meiosis forming typical meiospores, and another by dissociation forming uninucleate spores similar to those found in *E. aedis* and *C. magna* (Chen et al., 1998). Nilsen and Chen (2001) defended its establishment as a separate genus based on the large number of nucleotide differences they found in an analysis with a very limited number of other species of *Amblyospora* (129–211). However, with the addition of several more species, we found nucleotide sequence similarities ranging from 93.7% to 85.7% with other *Amblyospora* species from *Culex* mosquitoes and a difference of ~ 80 nucleotides with its nearest relative, *Amblyospora opacita.*

The genus *Trichoctosporea* was erected by Larsson (1994) to describe a microsporidium isolated from larval fat body tissue of *Ae. vexans* that had developmental and morphological features identical to *Amblyospora* but possessed distinct fibrillar "hair-like" projections from the meiospore exospore. Diagnosis of the genus was based on light microscope and ultrastructural detection of globular and fibrillar material within the sporophorous vesicle and the persistent fibril spore projections that appeared to be unique to this isolate. No DNA sequencing data were available at the time for phylogenetic comparisons. Subsequent investigations (Simakova and Pankova, 2005) and our current study have revealed similar filamentous projections on the exospore of four distally related species of *Amblyospora* (*A. bakcharia*, *A. flavescens*, *A. rugosa*, and *A. timirasia*) from four different host species that would appear to dismiss the taxonomic value of this feature. Furthermore, the phylogenetic grouping of *T. pygopellita* within a well-supported subclade of newly identified *Amblyospora* species, clearly indicate a close affinity and call into question the validity of this genus. While we are not advocating abolishment of the genus *Trichoctosporea* at this time, we suggest avoiding the establishment of monotypic genera of microsporidia until a clear connection can be made between morphological parameters and genetic relatedness.

5. Taxonomic summary and species descriptions

Phylum Microsporidia Balbiani, 1882.
Family Amblyosporidae Weiser, 1977.

5.1. Amblyospora bakcharia n. sp. (Fig. 1A)

Type host. *Oc. excrucians* (Walker) (Diptera: Culicidae)

Type locality. A temporary vernal pool near the village of Bakchar, Tomsk region, Western Siberia, Russia (57°01′28″ pr N, 82°06′02″ pr W). Collected 19 May 2007.

Site of infection. Fat body tissue of larvae.

Diagnosis. Meiospores broadly ovoid, uninucleate, with large posterior vacuole. 5.4 ± 0.6 μm × 3.5 ± 0.6 μm in size in tissues fixed for electron microscopy. Anchoring disc well developed. Polaroplast lamellar with vesicular components posteriorly. Exospore undulating, 60 μm thick, with thin filamentous tails. Endospore 140 μm thick. Polar filament anisofilar with 4½ broad (230 nm) proximal and 8½ narrow (120 nm) distal coils arranged in an irregular row.

Type material. Epon–Araldite embedded material used in the ultrastructural studies is in the collection of A. V. Simakova, Tomsk State University, Russia. Frozen DNAs of the microsporidium and the host mosquito are available from T.G. Andreadis, The Connecticut Agricultural Experiment Station, New Haven, CT.

Gene sequences. The SSU rDNA sequence of the microsporidium, *A. bakcharia* has been deposited in the GenBank/EMBL database under Accession No. JF826402. The SSU rDNA sequence of the host mosquito, *Oc. excrucians* has been deposited in the GenBank/EMBL database under Accession No. JF837523.

5.2. Amblyospora baritia n. sp (Fig. 1B)

Type host. *Oc. excrucians* (Walker) (Diptera: Culicidae).

Type locality. A temporary vernal pool near the village of Ursk, Kemerovo region, Western Siberia, Russia (54°27′36′ pr N, 85°22′05′ pr W). Collected 19 May 2007.

Site of infection. Fat body tissue of larvae.

Diagnosis. Meiospores broadly ovoid, uninucleate, with large posterior vacuole. 6.1 ± 0.6 μm × 4.1 ± 0.5 μm in size in tissues fixed for electron microscopy. Anchoring disc well developed. Polaroplast lamellar, more narrow anteriorly. Exospore undulating, 160 μm thick. Endospore 90 μm thick. Polar filament anisofilar with five broad (220 nm) proximal and 15 narrow (140 nm) distal coils arranged in an irregular row.

Type material. Epon–Araldite embedded material used in the ultrastructural studies is in the collection of A.V. Simakova, Tomsk State University, Russia. Frozen DNAs of the microsporidium and the host mosquito are available from T.G. Andreadis, The Connecticut Agricultural Experiment Station, New Haven, CT.

Gene sequences. The SSU rDNA sequence of the microsporidium, *A. baritia* has been deposited in the GenBank/EMBL database under Accession No. JF826403. The SSU rDNA sequence of the host mosquito, *Oc. cataphylla* has been deposited in the GenBank/EMBL database under Accession No. JF837524.

5.3. Amblyospora bogashovia n. sp. (Fig. 1C)

Type host. *Oc. excrucians* (Walker) (Diptera: Culicidae).

Type locality. A temporary vernal pool in Tomsk region, Western Siberia, Russia (56°27′03′ pr N, 84°59′54′ pr W). Collected 29 May 2006.

Site of infection. Fat body tissue of larvae.

Diagnosis. Meiospores broadly ovoid, uninucleate, with large posterior vacuole. 7.8–6.4 μm × 5.4–5.5 μm in size in tissues fixed for electron microscopy. Anchoring disc well developed. Polaroplast lamellar with vesicular components posteriorly. Exospore undulating, 60 μm thick. Endospore 140 μm thick. Polar filament anisofilar with 4½ broad (230 nm) proximal and 8½ narrow (120 nm) distal coils arranged in an irregular row.

Type material. Epon-Araldite embedded material used in the ultrastructural studies is in the collection of A. V. Simakova, Tomsk State University, Russia. Frozen DNAs of the microsporidium and the host mosquito are available from T. G. Andreadis, The Connecticut Agricultural Experiment Station, New Haven, CT.

Gene sequences. The SSU rDNA sequence of the microsporidium, *A. bakcharia* has been deposited in the GenBank/EMBL database

under Accession No. JF826404. The SSU rDNA sequence of the host mosquito, *Oc. excrucians* has been deposited in the GenBank/EMBL database under Accession No. JF837525.

5.4. Amblyospora chulymia n. sp. (Fig. 1D)

Type host. *Oc. caspius* (Pallas) (Diptera: Culicidae).

Type locality. A temporary vernal pool in Tomsk region, Western Siberia, Russia (56°27′03′ pr N, 84°59′54′ pr W). Collected 29 May 2006.

Site of infection. Fat body tissue of larvae.

Diagnosis. Meiospores broadly ovoid, uninucleate, with large posterior vacuole. 5.9 ± 0.6 μm × 4.7 ± 0.6 μm in size in tissues fixed for electron microscopy. Anchoring disc well developed. Polaroplast lamellar with vesicular components posteriorly. Exospore undulating, 200 μm thick. Endospore 150 μm thick. Polar filament anisofilar with 4 broad (190 nm) proximal and 7½ narrow (100 nm) distal coils arranged in a single row.

Type material. Epon-Araldite embedded material used in the ultrastructural studies is in the collection of A.V. Simakova, Tomsk State University, Russia. Frozen DNAs of the microsporidium and the host mosquito are available from T.G. Andreadis, The Connecticut Agricultural Experiment Station, New Haven, CT.

Gene sequences. The SSU rDNA sequence of the microsporidium, *A. chulymia* has been deposited in the GenBank/EMBL database under Accession No. JF826405. The SSU rDNA sequence of the host mosquito, *Oc. caspius* has been deposited in the GenBank/EMBL database under Accession No. JF837527.

5.5. Amblyospora hristinia n. sp. (Fig. 2A)

Type host. *Oc. communis* (De Geer) (Diptera: Culicidae).

Type locality. A temporary vernal pool near the village of Ursk, Komerovo region, Western Siberia, Russia (56°30′24′ pr N, 84°49′50′ pr W). Collected 19 May 2007.

Site of infection. Fat body tissue of larvae.

Diagnosis. Meiospores broadly ovoid, uninucleate, with large posterior vacuole. 6.8 ± 0.6 μm × 5.1 ± 0.5 μm in size in tissues fixed for electron microscopy. Anchoring disc well developed. Polaroplast lamellar with vesicular components posteriorly. Exospore undulating, 220 μm thick. Endospore 130 μm thick. Polar filament anisofilar with three broad (300 nm) proximal and 13 narrow (150 nm) distal coils arranged in an irregular row.

Type material. Epon–Araldite embedded material used in the ultrastructural studies is in the collection of A.V. Simakova, Tomsk State University, Russia. Frozen DNAs of the microsporidium and the host mosquito are available from T.G. Andreadis, The Connecticut Agricultural Experiment Station, New Haven, CT.

Gene sequences. The SSU rDNA sequence of the microsporidium, *A. hristinia* has been deposited in the GenBank/EMBL database under Accession No. JF826407. The SSU rDNA sequence of the host mosquito, *Oc. communis* has been deposited in the GenBank/EMBL database under Accession No. JF837529.

5.6. Amblyospora jurginia n. sp. (Fig. 2B)

Type host. *Oc. excrucians* (Walker) (Diptera: Culicidae).

Type locality. A temporary vernal pool in Tomsk region, Western Siberia, Russia (56°26′25′ pr N, 84°55′35′ pr W). Collected 22 May 2007.

Site of infection. Fat body tissue of larvae.

Diagnosis. Meiospores broadly ovoid, uninucleate, with large posterior vacuole. 5.3 ± 0.5 μm × 3.8 ± 0.4 μm in size in tissues fixed for electron microscopy. Anchoring disc well developed. Polaroplast lamellar with vesicular components posteriorly. Exospore undulating, 220 μm thick. Endospore 60 μm thick. Polar filament anisofilar with three broad (290 nm) proximal and five narrow (160 nm) distal coils arranged in a single row.

Type material. Epon–Araldite embedded material used in the ultrastructural studies is in the collection of A.V. Simakova, Tomsk State University, Russia. Frozen DNAs of the microsporidium and the host mosquito are available from T.G. Andreadis, The Connecticut Agricultural Experiment Station, New Haven, CT.

Gene sequences. The SSU rDNA sequence of the microsporidium, *A. jurginia* has been deposited in the GenBank/EMBL database under Accession No. JF826408. The SSU rDNA sequence of the host mosquito, *Oc. excrucians* has been deposited in the GenBank/EMBL database under Accession No. JF837521.

5.7. Amblyospora kazankia n. sp. (Fig. 2C)

Type host. *Ochlerotatus dianaetus* (Dyar) (Diptera: Culicidae).

Type locality. A temporary vernal pool near the village of Kolarovo, Tomsk region, Western Siberia, Russia (56°19′35′ pr N, 84°58′41′ pr W). Collected 30 May 2005.

Site of infection. Fat body tissue of larvae.

Diagnosis. Meiospores broadly ovoid, uninucleate, with large posterior vacuole. 5.1 ± 0.04 μm × 3.5 ± 0.03 μm in size in tissues fixed for electron microscopy. Anchoring disc well developed. Polaroplast lamellar, more narrow anteriorly. Exospore undulating, 50 μm thick. Endospore 110 μm thick. Polar filament anisofilar with five broad (200 nm) proximal and nine narrow (120 nm) distal coils arranged in a single row.

Type material. Epon–Araldite embedded material used in the ultrastructural studies is in the collection of A.V. Simakova, Tomsk State University, Russia. Frozen DNAs of the microsporidium and the host mosquito are available from T.G. Andreadis, The Connecticut Agricultural Experiment Station, New Haven, CT.

Gene sequences. The SSU rDNA sequence of the microsporidium, *A. kazankia* has been deposited in the GenBank/EMBL database under Accession No. JF826409. The SSU rDNA sequence of the host mosquito, *Oc. diantaeus* has been deposited in the GenBank/EMBL database under Accession No. JF837531.

5.8. Amblyospora mavlukevia n. sp. (Fig. 2D)

Type host. *Ae. cinereus* Meigen (Diptera: Culicidae).

Type locality. A permanent riverside pond (Mavlukeevskoe lake) within Tom River flood plain, Tomsk region, Western Siberia, Russia (56°27′48′ pr N, 84°56′10′ pr W). Collected 2 June 2007.

Site of infection. Fat body tissue of larvae.

Diagnosis. Meiospores broadly ovoid, uninucleate, with large posterior vacuole. 4.1 ± 0.2 μm × 3.7 ± 0.2 μm in size in tissues fixed for electron microscopy. Anchoring disc well developed. Polaroplast lamellar with vesicular components posteriorly. Exospore undulating, 280 μm thick. Endospore 110 μm thick. Polar filament anisofilar with five broad (220 nm) proximal and 14½ narrow (150 nm) distal coils arranged in an irregular row.

Type material. Epon–Araldite embedded material used in the ultrastructural studies is in the collection of A.V. Simakova, Tomsk State University, Russia. Frozen DNAs of the microsporidium and the host mosquito are available from T.G. Andreadis, The Connecticut Agricultural Experiment Station, New Haven, CT.

Gene sequences. The SSU rDNA sequence of the microsporidium, *A. mavlukevia* has been deposited in the GenBank/EMBL database under Accession No. JF826411. The SSU rDNA sequence of the host mosquito, *Ae. cinereus* has been deposited in the GenBank/EMBL database under Accession No. JF837532.

5.9. Amblyospora mocrushinia n. sp. (Fig. 3A)

Type host. *Oc. punctor* Kirby (Diptera: Culicidae).

Type locality. A temporary vernal pool in Tomsk region, Western Siberia, Russia (56°27′03′ pr N, 84°59′54′pr W). Collected 11 May 2008.

Site of infection. Fat body tissue of larvae.

Diagnosis. Meiospores broadly ovoid, uninucleate, with large posterior vacuole. 4.9 ± 0.5 μm × 3.3 ± 0.4 μm in size in tissues fixed for electron microscopy. Anchoring disc well developed. Polaroplast lamellar with vesicular components posteriorly. Exospore undulating, 60 μm thick. Endospore 120 μm thick. Polar filament anisofilar with five broad (240 nm) proximal and 7½ narrow (140 nm) distal coils arranged in a single row.

Type material. Epon–Araldite embedded material used in the ultrastructural studies is in the collection of A.V. Simakova, Tomsk State University, Russia. Frozen DNAs of the microsporidium are available from T.G. Andreadis, The Connecticut Agricultural Experiment Station, New Haven, CT.

Gene sequences. The SSU rDNA sequence of the microsporidium, *A. mocrushinia* has been deposited in the GenBank/EMBL database under Accession No. JF826412. The SSU rDNA sequence of the host mosquito, *Oc. punctor* has been deposited in the GenBank/EMBL database under Accession No. JF837536.

5.10. Amblyospora modestium n. sp. (Fig. 3B)

Type host. *Cx. modestus* Ficalbi (Diptera: Culicidae).

Type locality. A permanent pond (Krotovo Lake) located near the village of Troitskoe, Novosibirsk region, Western Siberia, Russia (53°43′40.26″ pr N, 77°53′18.52′ pr W). collected 24 August 2008.

Site of infection. Fat body tissue of larvae.

Diagnosis. Meiospores broadly ovoid, uninucleate, with large posterior vacuole. 4.8 ± 0.3 μm × 3.6 ± 0.4 μm in size in tissues fixed for electron microscopy. Anchoring disc well developed. Polaroplast lamellar with large vesicular components posteriorly. Exospore undulating, 180 μm thick. Endospore 150 μm thick. Polar filament anisofilar with three broad (300 nm) proximal and six narrow (150 nm) distal coils arranged in an irregular row.

Type material. Epon–Araldite embedded material used in the ultrastructural studies is in the collection of A.V. Simakova, Tomsk State University, Russia. Frozen DNAs of the microsporidium are available from T.G. Andreadis, The Connecticut Agricultural Experiment Station, New Haven, CT.

Gene sequences. The SSU rDNA sequence of the microsporidium, *A. modestium* has been deposited in the GenBank/EMBL database under Accession No. JF826413. The SSU rDNA sequence of the host mosquito, *Cx. modestus* has been deposited in the GenBank/EMBL database under Accession No. JF837532.

5.11. Amblyospora salairia n. sp. (Fig. 3C)

Type host. *Ae. cinereus* Meigen (Diptera: Culicidae).

Type locality. A temporary vernal pool located near the village of Timirjasevo, Tomsk region, Western Siberia, Russia (56°30′24′ pr N, 84°49′50′ pr W). Collected 6 May 2007.

Site of infection. Fat body tissue of larvae.

Diagnosis. Meiospores broadly ovoid, uninucleate, with large posterior vacuole. 5.4 ± 0.6 μm × 3.8 ± 0.6 μm in size in tissues fixed for electron microscopy. Anchoring disc well developed. Polaroplast lamellar with vesicular components posteriorly. Exospore undulating, 170 μm thick. Endospore 80 μm thick. Polar filament anisofilar with 3½ broad (230 nm) proximal and 10½ narrow (120 nm) distal coils arranged in an irregular row.

Type material. Epon–Araldite embedded material used in the ultrastructural studies is in the collection of A.V. Simakova, Tomsk State University, Russia. Frozen DNAs of the microsporidium and the host mosquito are available from T.G. Andreadis, The Connecticut Agricultural Experiment Station, New Haven, CT.

Gene sequences. The SSU rDNA sequence of the microsporidium, *A. salairia* has been deposited in the GenBank/EMBL database under Accession No. JF826415. The SSU rDNA sequence of the host mosquito, *Ae. cinereus* has been deposited in the GenBank/EMBL database under Accession No. JF837534.

5.12. Amblyospora severinia n. sp. (Fig. 3D)

Type host. *Oc. excrucians* (Walker) (Diptera: Culicidae).

Type locality. A temporary vernal pool in Tomsk region, Western Siberia, Russia (56°26′25′ pr N, 84°55′35′ pr W). Collected 22 May 2007.

Site of infection. Fat body tissue of larvae.

Diagnosis. Meiospores broadly ovoid, uninucleate, with large posterior vacuole. 5.3–5.6 μm × 3.9–4.0 μm in size in tissues fixed for electron microscopy. Anchoring disc well developed. Polaroplast lamellar, more narrow anteriorly. Exospore undulating, 200 μm thick. Endospore 110 μm thick. Polar filament anisofilar with four broad (220 nm) proximal and 14½ narrow (150 nm) distal coils arranged in an irregular row.

Type material. Epon–Araldite embedded material used in the ultrastructural studies is in the collection of A.V. Simakova, Tomsk State University, Russia. Frozen DNAs of the microsporidium and the host mosquito are available from T.G. Andreadis, The Connecticut Agricultural Experiment Station, New Haven, CT.

Gene sequences. The SSU rDNA sequence of the microsporidium, *A. severinia* has been deposited in the GenBank/EMBL database under Accession No. JF826417. The SSU rDNA sequence of the host mosquito, *Oc. excrucians* has been deposited in the GenBank/EMBL database under Accession No. JF837522.

5.13. Amblyospora shegaria n. sp. (Fig. 4A)

Type host. *Ae. cinereus* Meigen (Diptera: Culicidae).

Type locality. A temporary vernal pool located near the village of Timirjasevo, Tomsk region, Western Siberia, Russia (56°30′24′ pr N, 84°49′50′pr W). Collected 21 May 2007.

Site of infection. Fat body tissue of larvae.

Diagnosis. Meiospores broadly ovoid, uninucleate, with large posterior vacuole. 3.8 ± 0.1 μm × 3.4 ± 0.2 μm in size in tissues fixed for electron microscopy. Anchoring disc well developed. Polaroplast lamellar with vesicular components posteriorly. Exospore undulating and rugose, 270 μm thick. Endospore 60 μm thick. Polar filament anisofilar with four broad (250 nm) proximal and 12 narrow (150 nm) distal coils arranged in an irregular row.

Type material. Epon–Araldite embedded material used in the ultrastructural studies is in the collection of A.V. Simakova, Tomsk State University, Russia. Frozen DNAs of the microsporidium and the host mosquito are available from T.G. Andreadis, The Connecticut Agricultural Experiment Station, New Haven, CT.

Gene sequences. The SSU rDNA sequence of the microsporidium, *A. shegaria* has been deposited in the GenBank/EMBL database under Accession No. JF826416. The SSU rDNA sequence of the host mosquito, *Ae. cinereus* has been deposited in the GenBank/EMBL database under Accession No. JF837533.

5.14. Amblyospora timirasia n. sp. (Fig. 4B)

Type host. *Ae. cinereus* Meigen (Diptera: Culicidae).

Type locality. A temporary vernal pool located near the village of Timirjasevo, Tomsk region, Western Siberia, Russia (56°30′24′ pr N, 84°49′50′ pr W). Collected 22 May 2007.

Site of infection. Fat body tissue of larvae.

Diagnosis. Meiospores broadly ovoid, uninucleate, with large posterior vacuole. 4.9 ± 0.3 μm × 3.2 ± 0.3 μm in size in tissues fixed for electron microscopy. Anchoring disc well developed. Pola-

roplast lamellar with vesicular components posteriorly. Exospore undulating with filamentous tails, 120 μm thick, with thin filamentous tails. Endospore 90 μm thick. Polar filament anisofilar with three broad (180 nm) proximal and four narrow (90 nm) distal coils arranged in a single row.

Type material. Epon–Araldite embedded material used in the ultrastructural studies is in the collection of A.V. Simakova, Tomsk State University, Russia. Frozen DNAs of the microsporidium and the host mosquito are available from T.G. Andreadis, The Connecticut Agricultural Experiment Station, New Haven, CT.

Gene sequences. The SSU rDNA sequence of the microsporidium, *A. timirasia* has been deposited in the GenBank/EMBL database under Accession No. JF826418. The SSU rDNA sequence of the host mosquito, *Ae. cinereus* has been deposited in the GenBank/EMBL database under Accession No. JF837535.

5.15. Novothelohania n. g.

Diagnosis. Monotypic genus. Spores oval, uninucleate, formed in groups of 8 within a sporophorous vesicle. With thick undulating spore wall, large umbrella-shaped anchoring disc contiguous with a large tightly lamellate polaroplast and tapered polar filament.

5.16. Novothelohania ovalae n. sp. (Fig. 5)

Type host. *Oc. caspius* (Pallas) (Diptera: Culicidae).

Type locality. A temporary vernal pool located near the village of Troitskoe, Novosibirsk region, Western Siberia, Russia (53°43′56.8″ pr N, 77°52′04.8″ pr W). Collected 15 July 2008.

Site of infection. Fat body tissue of larvae.

Diagnosis. With characters of the genus. Spores 3.4 ± 0.4 μm × 2.1 ± 0.3 μm in size in tissues fixed for electron microscopy. Exospore 160 μm thick. Endospore 80 μm thick. Polar filament with 3½ broad (190 nm) proximal and 2 narrow (100 nm) distal coils arranged in a single row.

Type material. Epon–Araldite embedded material used in the ultrastructural studies is in the collection of A.V. Simakova, Tomsk State University, Russia. Frozen DNAs of the microsporidium and the host mosquito are available from T.G. Andreadis, The Connecticut Agricultural Experiment Station, New Haven, CT.

Gene sequences. The SSU rDNA sequence of the microsporidium, *A. chulymia* has been deposited in the GenBank/EMBL database under Accession No. JF826419. The SSU rDNA sequence of the host mosquito, *Oc. caspius* has been deposited in the GenBank/EMBL database under Accession No. JF837528.

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