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Phylogeny of *Amblyospora* (Microsporida: Amblyosporidae) and Related Genera Based on Small Subunit Ribosomal DNA Data: A Possible Example of Host Parasite Cospeciation

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Small subunit ribosomal RNA (SSU rRNA) gene sequences were analyzed for six species and four genera of microsporidia from mosquito hosts; *Amblyospora stimuli* (*Aedes stimulans*), *Amblyospora californica* (*Culex tarsalis*), *Amblyospora* sp. (*Culex salinarius*), *Edhazardia aedis* (*Aedes aegypti*), *Culicosporella lunata* (*Culex pilosus*), and *Parathelohania anophelis* (*Anopheles quadrimaculatus*). Comparison of these sequences to those of other microsporidia show that these sequences are longer with the SSU rRNA gene of *E. aedis* being the longest microsporidia sequenced to date (1447 base pairs). Parsimony, maximum likelihood, and distance methods produced identical trees, suggesting that the above microsporidian taxa, contrary to current classification schemes, form a monophyletic group. Relationships within this group are further supported by high bootstrap and decay analysis values. Based on the molecular analysis, *P. anophelis* is the most divergent species in this group of mosquito parasites. *Amblyospora* is paraphyletic with *A. californica* and *Amblyospora* sp., forming a sister taxon to a clade composed of *E. aedis* and *A. stimuli*. *Culicosporella lunata* comprises a sister taxon to the *Amblyospora/Edhazardia* clade. The pattern of host relationships on the tree provides preliminary evidence that the branching pattern seen here may indicate that host–parasite cospeciation is an important mechanism of evolution in this group.

Key Words: phylogeny; ribosomal DNA; comparative sequence analysis; Culicidae; mosquito; Amblyospora stimuli; Amblyospora californica; Amblyospora sp.; Edhazardia aedis; Culicosporella lunata; Parathelohania anophelis; Vavraia oncoperae; Endoreticulatus schubergi; Encephalitozoon cuniculi; Nosema bombycis; Aedes stimulans; Aedes aegypti; Culex tarsalis; Culex salinarius; Culex pilosus; Culex territans; Anopheles quadrimaculatus.

INTRODUCTION

Weiser (1977) erected the family Amblyosporidae (Order Pleistophoridida) to include those genera whose species underwent two different developmental cycles, each producing a different type of spore; one in the adult hosts and the other in larval hosts. In the larva, groups of eight, thick-walled, uninucleate spores with anisofilar polar filaments were produced within a pansporoblastic membrane (sporophorous vesicle). The other sequence, that found in the adults, produced single, thin-walled, diplokaryotic spores with isofilar polar filaments. The majority of the species placed in the family Amblyosporidae were described from mosquitoes. Included within the Amblyosporidae were the genera *Amblyospora* Hazard & Oldacre and *Parathelohania* Codreanu. In the same classification scheme, Weiser erected the family Culicosporidae which he believed to be closely related to the Amblyosporidae. Within the Culicosporidae were placed genera whose species produced sporophorous vesicles consisting of 4, 8, or 16 pyriform spores with helicoidal polaroplasts and had either one or two sporulation sequences. This included the monotypic genus *Culicosporella* Weiser (type species, *Culicosporella lunata* (Hazard & Savage); type host, *Culex pilosus* (Dyar & Knab)).

Sprague (1982) removed *Culicosporella* to the family Caudosporidae in the order Apansporoblastina. At that time, only one sporulation sequence for *Culicosporella* was known, which resulted in single diplokaryotic spores via rosette formation in the sporoblast stage. This corresponded well with the definition of the family. The above generic relationships proposed by Weiser

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(1977) remained essentially the same. Larsson (1986) created an intuitive character state tree of the microsporidia. In it, *Amblyospora* and *Parathelohania* were represented as sister taxa with *Culicosporella* included in a lineage branching just prior to the *Amblyospora/Parathelohania* lineage.

In 1989, Becnel *et al.* redescribed a microsporidium, *Nosema aedis* Kudo, from *Aedes aegypti* (L.), renaming it *Edhazardia aedis* (Kudo). *Edhazardia aedis* has three sporulation sequences: a diplokaryotic cycle in the adult female responsible for transovarial transmission; a uninucleate cycle in the larva which results from nuclear dissociation of transovarial transmitted forms; and an abortive meiotic sequence in the transovarially infected larva, resulting in groups of meiospores enclosed in a sporophorous vesicle. The uninucleate spores produced in the larval cycle are responsible for horizontally infecting other larvae and continuing the infection in the adult. The abortive meiospores appear to be nonfunctional since there is no alternate host. Becnel *et al.* (1989) and Becnel (1994) suggested affinities between *E. aedis* and the genus *Amblyospora* based on life cycle similarities. Both species are vertically and horizontally transmitted and involve two successive generations of the mosquito host. Horizontal transmission to larval hosts occurs via oral ingestion of a uninucleate spore. This is followed by identical developmental sequences in larvae that involve gametogony followed by plasmogamy and nuclear association to form diplokarya. These diplokaryotic stages then form binucleate spores that are responsible for transovarial transmission to the filial generation. The most significant difference is that in *E. aedis* haplosis of diplokaryotic sporonts in larval progeny occurs by nuclear dissociation. This results in the production of uninucleate spores that are orally infectious to the next generation of larval mosquitoes. In *Amblyospora*, haplosis of diplokaryotic sporonts ocurrs by meiotic division. This results in the production of functional meiospores that are orally infectious for an intermediate copepod host, wherein similar uninucleate spores infectious for larval mosquitoes are formed (Andreadis, 1985a; Sweeney *et al.*, 1985). Because of these similarities, *E. aedis* was assigned to the family Amblyosporidae.

Becnel and Fukuda (1991) described the ultrastructural cytology of and reported on new developmental information for *C. lunata*. They found that *C. lunata* had three developmental cycles similar to those found in *E. aedis*. The *C. lunata* developmental cycle in mosquito larvae did not, however, undergo nuclear dissociation. The spores resulting from this sequence were diplokaryotic. Although, similarities existed between *Culicosporella*, *Amblyospora*, and *Edhazardia*, Becnel and Fukuda (1991) chose to create a new family Culicosporellidae, for *C. lunata*.

Sprague *et al.* (1992) proposed the most recent hypothesis on the systematics of the microsporidia. Basing their main divisions on cell cycle characteristics, they developed an interpretation of the above genera that was different from those of previous workers. As previously hypothesized, they assigned *Amblyospora* and *Parathelohania* to the family Amblyosporidae (Superfamily Amblyosporoidea; Order Meiodihaplophasida). The remaining aforementioned genera were placed in the Order Dissociodihaplophasida within which the superfamily Culicosporoidea housed *Edhazardia* (Culicosporidae) and *Culicosporella* (Culicosporellidae).

Recent work in our lab, using molecular data, has suggested that the genera *Amblyospora*, *Parathelohania*, *Culicosporella*, and *Edhazardia* are all closely related, relative to the other 20 or so genera that we have analyzed. These four genera probably represent a single family. In this paper we discuss the relationships between these genera based on molecular data, relative to the evolution of various morphological characters.

MATERIALS AND METHODS

Table 1 lists the species of microsporidia used in this study and the hosts and sources from which they were obtained. Spore purification, nucleic acid preparation, PCR amplification, template purification, and sequencing were performed as previously described (Baker *et al.*, 1995). For *C. lunata* and *P. anophelis* (Kudo), the primers 18f and 1537r were used for amplification, while 18f and 1492r were used to amplify *A. stimuli* Andreadis and *E. aedis*.

Sequences were aligned visually on a PC using the

TABLE 1

Microsporidia Used in Analysis, Hosts from Which They Were Obtained and Genbank Accession Numbers

Microsporidia	Mosquito host	Genbank accession no.	Source
Parathelohania anophelis (Kudo)	*Anopheles quadrimaculatus* (Insecta: Diptera)	AF027682	Lab colony, A. H. Undeen ID No. 93-056, (11-29-93)
Culicosporella lunata (Hazard & Savage)	*Culex pilosus* (Insecta: Diptera)	AF027683	Field collected, Gainesville, FL
Edhazardia aedis (Kudo)	*Aedes aegypti* (Insecta: Diptera)	AF027684	Lab colony, source: Thailand
Amblyospora stimuli Andreadis	*Aedes stimulans* (Insecta: Diptera)	AF027685	Field collected, Mt. Carmel, CT (5-4-93)
Amblyospora californica (Kellen & Lipa)	*Culex tarsalis* (Insecta: Diptera)	U68473	Lab colony, source: California
Amblyospora sp. Hazard & Oldacre, 1975	*Culex salinarius* (Insecta: Diptera)	U68474	Lab colony, source: Lake Charles, LA

Sage Professional Editor program (Sage Software, Beaverton, OR). Only those portions of the sequence which could be unambiguously aligned (approximately 1120 base pairs) were used in the analyses (alignments may be obtained from the author). Once an acceptable alignment was obtained the sequences were transferred to a Power Macintosh computer where they were analyzed using parsimony [PAUP-Phylogenetic Analysis Using Parsimony, Version 3.1.1 (Swofford, 1993)], the various distance methods (Neighbor Joining, UPGMA, and Fitch-Margoliash) found in PHYLIP v3.5c (Felsenstein, 1993), and FastDNAML (Olsen *et al.*, 1994). The most parsimonious tree was found using the branch and bound option of PAUP. Bootstrap and decay analyses were performed, to test the robustness of the data, using the heuristic search option with random addition (five replications) and tree bisection and reconnection method of branch swapping. For distance-based methods (NJ, UPGMA, and Fitch-Margoliash), sequences were converted to distances using the Jukes-Cantor, Kimura 2-parameter, and the maximum likelihood models of base substitution. Additionally, bootstrap analyses were performed with trees generated using the neighbor joining algorithm.

RESULTS

Sequence inspection revealed that the lengths of the SSU rRNA genes for this group of microsporidia were mostly on the high side of the range of known microsporidian SSU rRNAs (Table 2). *Parathelohania anophelis* seemed to be the exception at 1287 base pairs. *Edhazardia aedis* represents the longest microsporidian SSU rRNA gene (1447 base pairs) known to date. Most of this variation in length can be accounted for in region V4 (135 base pairs in *E. aedis*) and region V9/helix 47 (157 base pairs in *E. aedis*). The length of the *E. aedis* V4 region was more than twice that of all other known microsporidia while the *E. aedis* V9 region was three or more times longer than all other non-*Amblyospora* microsporidia. Smaller size differences of the V9 region can be seen in the three *Amblyospora* species. However, excluding *E. aedis*, the V4 region sizes within the *Amblyospora* group do not show any significant size difference (Table 2). Region V2 is lacking for the species used in this analysis. Regions are comparable to those proposed by Neefs *et al.* (1991). GC content for these species is fairly typical for most microsporidia with *P. anophelis* having the highest content at 55% and *E. aedis* the lowest at 47% (Table 2).

TABLE 2
Lengths of SSU rRNA Gene and V4 and V9 Regions; and GC Content of *Amblyospora* Group Species and Selected Outgroups

Species	SSU rRNA	V4 region	V9 region	% GC
Nosema bombycis	1234	3	59	34
Encephalitozoon cuniculi	1298	54	58	52
Endoreticulatus schubergi	1252	40	58	51
Vavraia oncoperae	1322	62	57	56
Parathelohania anophelis	1287	59	60	55
Culicosporella lunata	1343	64	65	44
Amblyospora californica	1359[a]	60	106	49
Amblyospora sp.	1358[a]	60	114	49
Amblyospora stimuli	1360[a]	60	126	48
Edhazardia aedis	1447[a]	135	157	47

Note. Lengths are in base pairs.
[a] Represents incomplete sequence lengths (see text).

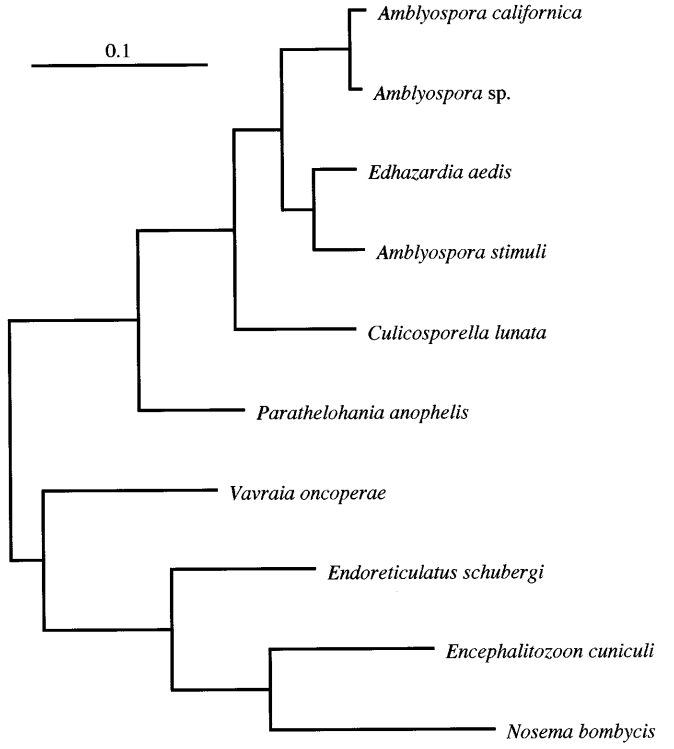

FIG. 1. Most parsimonious tree (1369 steps) found using the branch and bound option of PAUP. Consistency index (CI) = 0.748, CI (excluding uninformative characters) = 0.683, Retention index (RI) = 0.589. Identical topology also generated using neighbor joining and maximum likelihood analyses. *V. oncoperae, E. schubergi, E. cuniculi,* and *N. bombycis* rare used as outgroups.

Figure 1 shows the most parsimonious tree generated by PAUP when *Vavraia oncoperae* (Milner & Beaton), *Endoreticulatus schubergi* (Zwolfer), *Encephalitozoon cuniculi* Levaditi, Nicolau & Schoen, and *Nosema bombycis* Naegeli are used as outgroups. Each of these outgroup species are representative of a monophyletic group of microsporidia (Baker *et al.*, 1995), which when combined form a sister taxon to the *Amblyospora* group. The topology of the parsimony tree was identical to that of the neighbor joining, UPGMA, Fitch-Margoliash, and maximum likelihood trees. The relationships suggested by the tree in Fig. 1 support a paraphyletic *Amblyospora* with *A. stimuli* being more

TABLE 3
Pairwise Distances between Taxa

		1	2	3	4	5	6	7	8	9	10
1	*Amblyospora californica*	—	0.019	0.213	0.182	0.108	0.120	0.302	0.312	0.316	0.344
2	*Amblyospora* sp.	20	—	0.205	0.173	0.099	0.111	0.290	0.306	0.306	0.338
3	*Parathelohania anophelis*	226	216	—	0.226	0.198	0.202	0.282	0.313	0.319	0.359
4	*Culicosporella lunata*	195	184	243	—	0.163	0.164	0.304	0.315	0.317	0.325
5	*Edhazardia aedis*	116	105	210	174	—	0.071	0.302	0.322	0.318	0.337
6	*Amblyospora stimuli*	128	118	215	175	76	—	0.297	0.328	0.318	0.336
7	*Vavraia oncoperae*	320	304	300	326	320	314	—	0.291	0.281	0.331
8	*Endoreticulatus schubergi*	330	322	333	337	341	347	311	—	0.267	0.311
9	*Encephalitozoon cuniculi*	334	321	339	339	336	336	299	286	—	0.296
10	*Nosema bombycis*	365	356	385	349	358	357	354	334	317	—

Note. Numbers above diagonal, mean distances; numbers below diagonal, absolute distances.

closely related to *E. aedis* than it is to the other two *Amblyospora* species. The relationship between *E. aedis* and *A. stimuli* is not, as suggested by branch lengths (Fig. 1) and mean distances (0.071 versus 0.019) (Table 3), nearly as close as the relationship between *Amblyospora* sp. and *Amblyospora californica* (Kellen & Lipa). *Culicosporella lunata* forms a sister taxon to the *Amblyospora/Edhazardia* clade with distances between these two groups ranging from 0.163 to 0.182. Finally, *P. anophelis* represents the most divergent species of the *Amblyospora* group with distances at or above 20% (Table 3).

The topology in Fig. 1 appears to be very well supported based on bootstrap and decay analysis values (Fig. 2). In both bootstrap analyses all branches within the *Amblyospora* group are supported in 99 or 100% of the bootstrap replicates. Similarly, relatively high decay analysis values (13 additional steps to collapse the first branch) were calculated for all branches within this group. Two clades in particular were very well supported, the *Amblyospora* group as a whole and the group composed of *Culex* infecting *Amblyospora* (*Amblyospora* sp. and *A. californica*). The branches leading to these two groups collapsed at 46 and 38 steps, respectively, longer than the most parsimonious tree.

DISCUSSION

The similarities in topology generated between the various analyses and the bootstrap and decay analyses values suggest a high degree of support for the relationships between species within this group of microsporidia. There is considerable incongruence between this tree and some of the current systematic hypotheses for this group of microsporidia. Recent classification schemes (Issi, 1986; Larsson, 1986; and Sprague *et al.*, 1992) failed to show affinities among all of these genera, although similar morphological characteristics indicate that they are related (Becnel *et al.*, 1989; Becnel, 1994). At least two developmental cycles appear to represent apomorphic characters supporting a monophyletic *Amblyospora* group (Fig. 1). They are (1) the *Nosema*-like cycle in adult mosquitoes resulting in diplokaryotic cylindrical spores required for transovarial transmission and (2) the *Thelohania*-like cycle initiated by the transovarially transmitted spores which, through meiosis, produce groups of eight meiospores

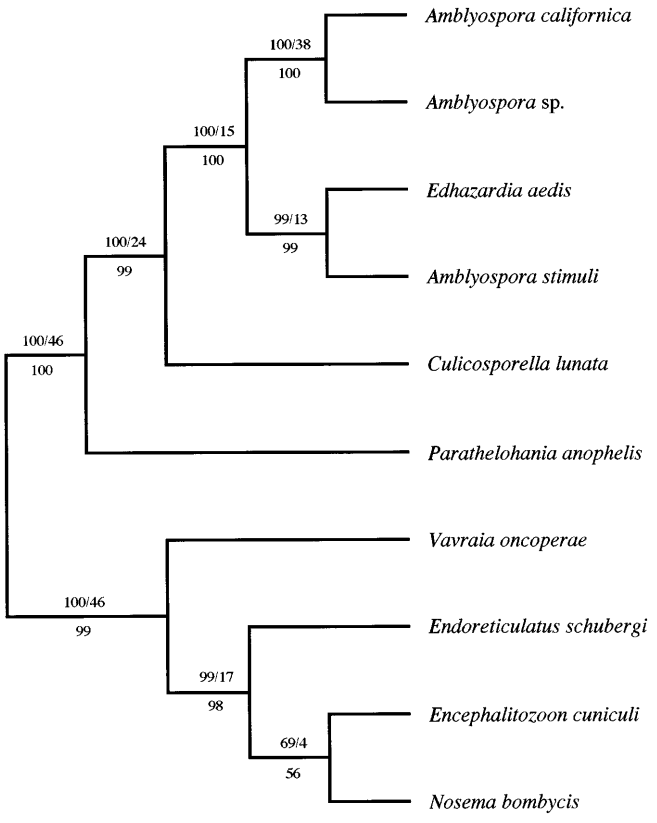

FIG. 2. Bootstrap and Decay analysis support for tree in Fig. 1. Numbers above branch represent bootstrap (1000 replicates) values (left) and decay analysis values (right) calculated using parsimony. Numbers below branch represent bootstrap (400 replicates) values calculated using neighbor joining analysis. *V. oncoperae, E. schubergi, E. cuniculi,* and *N. bombycis* rare used as outgroups.

enclosed in a sporophorous vesicle. These two cycles were used by Weiser (1977) as characters to define the family Amblyosporidae. Unfortunately, not enough was known about *Culicosporella* or *Edhazardia* for placement in Amblyosporidae at that time.

The above apomorphies may be pleisiomorphic to the microsporidia, however, and therefore not unique to the *Amblyospora* group. A third possible apomorphic life cycle sequence for this group is the unikaryotic cycle producing lanceolate spores that are responsible for horizontal transmission to the definitive host. For *Amblyospora* and *Parathelohania,* this developmental sequence follows meiotic division of diplokaryotic stages to produce meiospores and proceeds within an intermediate host (Andreadis, 1985a; Becnel, 1992; Avery and Undeen, 1990). In *Edhazardia* this cycle is initiated by a diplokaryotic stage that undergoes dissociation rather than meiosis (Hazard *et al.,* 1984; Becnel *et al.,* 1989; Becnel and Fukuda, 1991). Because of the differences in timing and origination of this portion of the life cycle, it has been overlooked as an apomorphic character uniting these species. Furthermore, changes in the timing of the lanceolate spore cycle (without regard to nucleation) may have reduced the selective pressure for the production of the meiospores, resulting in the abortive meiosis and nonfunctional meiospores seen in *Edhazardia* and *Culicosporella.* Because of the abortive meiosis, the haplophase of these species is not initiated through meiosis. Therefore, Sprague *et al.* (1992) did not recognize the evolutionary relationships of these genera and chose to place *Edhazardia* and *Culicosporella* in a different order from *Amblyospora* and *Parathelohania.*

By mapping the morphological characters onto the tree generated by molecular data, we can make inferences as to the evolutionary history of these characters. Since Weiser (1977) erected the family Amblyosporidae to accomodate *Amblyospora* and *Parathelohania,* these two genera have always been closely united. Larsson (1986) treated these two genera as sister taxa, as did Sprague *et al.* (1992), who included them in the same family. *Amblyospora* and *Parathelohania* have very similar life cycles (Avery and Undeen, 1990; Becnel, 1992). The only significant difference appears to be in the morphology of the meiospores which in *Parathelohania* possess ridges on their exterior surface. One species, *P. anophelis* has recently been found to possesses an additional sporulation sequence in the adult female definitive host resulting in oval diplokaryotic spores (Garcia *et al.,* 1993). Whether this is present in all *Parathelohania* species, representing an additional difference between these two genera, is not known. Analysis of the molecular data (Fig. 1) show that while *Parathelohania* and *Amblyospora* are relatively close, they do not show the direct sister group relationship that has been suggested previously. Considering the *Parathelohania/Amblyospora* type life cycle as ancestral (see below) negates the usefulness of this character for defining relationships within this group.

Because *P. anophelis* is the basal branch of the *Amblyospora* group, it appears that some sort of complex life cycle, which employs an intermediate host, is the pleisiomorphic state for this group of microsporidia. We feel that the genus *Parathelohania* is a well-defined taxon based on the ornamentation of the meiospore and the host preference of *Parathelohania* for *Anopheles* mosquitoes rather than *Culex* and *Aedes* species (Fig. 3). Therefore, it seems more likely that the *Amblyospora*-type life cycle is more representative of the ancestral condition of this group. This would help to explain the paraphyletic nature of the *Amblyospora.* If this is so, then the change in timing of the lanceolate spore cycle would have had to occur at least twice in the evolutionary history of this group, once in the *Culicosporella* lineage and again in the *Edhazardia* lineage (Fig. 3). Independent switching of this cycle in these two lineages can therefore be treated as an apomorphic character to define both. Furthermore, independence of this switching event could explain differences in this cycle in *Culicosporella* (diplokaryotic) and *Edhazardia* (uninucleate). Two other species, *Amblyospora trinus* Becnel & Sweeney and *Culicospora magna* (Kudo) possess life cycles which are identical to that of *E. aedis* with the exception of the meiospore stages (Becnel *et al.,* 1987; Becnel and Sweeney, 1990; Becnel, 1994). No meiospores are produced in *C. magna,* while in *A. trinus* meiospores appear fully functional. This may represent a transformation series with the following polarity: typical *Amblyospora* → *A. trinus* → *E. aedis* → *C. magna.* Sequence data is needed for *C. magna* to confirm this relationship.

Synapomorphic characters defining *E. aedis* and *A. stimuli* as a monophyletic unit are difficult, if not impossible, to determine based on the literature. Possibilities include lack of an intermediate host and characteristics of the life cycle in horizontally infected larvae. Andreadis (1985b) provided evidence for horizontal transmission of *A. stimuli* to *Aedes stimulans* (Walker) larvae, but the source of infection could not be determined. The possibility that *A. stimuli* lacks an intermediate host still remains but seems unlikely since larvae are not orally susceptible to meiospores. Andreadis (1985b) also noticed in these horizontal transfers that an initial infection originates in the gastric caeca of the host which culminates in the production of a small number of spores. Early developmental cycles such as this, which are thought to disseminate the infection within the host, have been reported in some species of microsporidia (Iwano and Ishihara, 1991; J. J. Becnel and J. V. Maddox, personal communication). The uninucleate larval cycle in *Edhazardia,* responsible for horizontal transmission of the parasite, also initiates

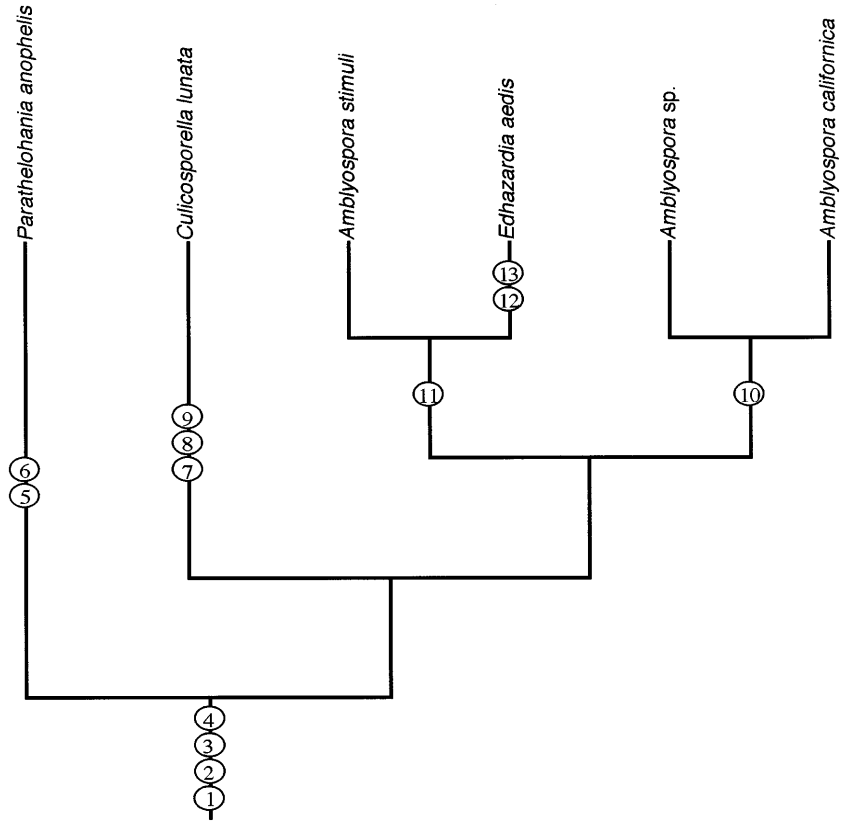

FIG. 3. Possible apomorphic states for the *Amblyospora* group species. (1) dipolkaryotic sporulation sequence in adult fematle host responsible for transovarial transmission; (2) developmental cycle ending in the production of meiospores; (3) developmental cycle in intermediate host ending in the production of lanceolate spores with vesiculate polaroplast, each of which is surrounded by sporophorous vesicle; (4) parasites of Culicidae; (5) meiospore with ornamentation; (6) second sporulation sequence in adult resulting in binucleate oval spores; (7) reduction of meiospore cycle; (8) lanceolate spore cycle diplokaryotic; (9) lanceolate spore cycle shift to mosquito host; (10) parasites of *Culex;* (11) parasites of *Aedes;* (12) lanceolate spore cycle shift to mosquito host; (13) lanceolate spore cycle uninucleate via nuclear dissociation.

an early infection in the gastric caeca (Becnel *et al.*, 1989). Early development has yet to be explored in other *Amblyospora* species primarily because no one knew what to look for. We suspect that it is just a matter of time before it is demonstrated in other members of this group.

The host may represent a synapomorphic character for *E. aedis* and *A. stimuli* and may prove to be very important for understanding the evolution of this group as a whole. Baker *et al.* (1995) have demonstrated that the host is an important character in other microsporidian groups. In the *Amblyospora* group the character of host species seems to be much more critical than in most groups. First, almost all of the *Amblyospora* group species infect Culicidae; *E. aedis* and *A. stimuli* are *Aedes* parasites; *Amblyospora* sp., *A. calfornica,* and *C. lunata* are parasites of *Culex;* and *P. anophelis,* like most species in the genus *Parathelohania,* infects *Anopheles* (Table 1). Second, these relationships somewhat follow the systematics of the host, in that the monophyletic group composed of *Aedes* (Culicinae) parasites are more closely related to the *Culex* (Culicinae) parasites than either is to the *Anopheles* (Anophelinae) parasite, *P. anophelis.* This initial study suggests that host–parasite cospeciation may be responsible for this pattern of *Amblyospora* group species diversity. Therefore, the *Amblyospora* group may represent another example of a taxon which shows evolutionary patterns consistent with Fahrenholz's rule (*i.e.,* that host phylogeny mirrors parasite phylogeny) (Glen and Brooks, 1985; Bandoni and Brooks, 1987; Deets, 1987; Hafner *et al.,* 1994). Obviously, more *Amblyospora* group species must be examined along with the accompanying phylogeny of the hosts before adherence to Fahrenholz's rule can be confirmed.

Lyal (1985) suggested that, for taxa complying to Fahrenholz's rule, one would predict the following: (1) the number of parasite species equals the number of host species; (2) the number of parasite species associated with any one host species can be no greater that one; (3) no parasite species will be found in more that one host species; and (4) host specificity is high, decreas-

ing the amount of colonization of new hosts. Discussion of how these predictions describe the *Amblyospora* group will be limited to the genus *Amblyospora* as it represents the vast majority of this group. Although *Amblyospora* species have not been described from all species of Culicidae, it cannot yet be ruled out. As far as multiple parasites described from single hosts only 6 of the 89 host species have been determined to possess more than one parasite species (Andreadis, 1994). Only 3 of the 31 described species of *Amblyospora* have been described from multiple hosts with the extreme being 15 host species for *A. opacita*. However, many of these are based on gross morphology of the spore as observed under the light microscope. White *et al.* (1994) have shown that, at least for an *Amblyospora opacita* (Kudo) isolate infecting the type host, *Culex territans* Walker, there is a high degree of host specificity, suggesting that the *A. opacita* isolates from other mosquito species may not be conspecific. Furthermore, there exists a high degree of host specificity in all of the *Amblyospora* species thus far examined (Andreadis, 1989; Sweeney *et al.*, 1990a,b; White *et al.*, 1994), suggesting that each species described from a different host may represent a separate species. The genus *Amblyospora* has been described primarily from *Aedes* and *Culex* hosts; however, it does have a wider distribution across several genera of mosquitoes (for host list see Andreadis, 1994; Garcia and Becnel, 1994). *Amblyospora* have also been described in the Simuliidae. It will be interesting to see if these relationships hold up as we sequence more *Amblyospora* and *Parathelohania* species from other genera of Culicidae, especially *Anopheles* and the Simuliidae.

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