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Amblyospora salinaria n. sp. (Microsporidia: Amblyosporidae), Parasite of *Culex salinarius* (Diptera: Culicidae): Its Life Cycle Stages in an Intermediate Host

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Horizontal transmission testing with an *Amblyospora* species from the mosquito *Culex salinarius* has documented the involvement of a copepod intermediate host. Meiospores (one type of uninucleate spore) of the *Amblyospora* sp. were infectious *per os* to female *Macrocyclops albidus* adults. All developmental stages in the copepod had unpaired nuclei (were haplophasic), starting with the sporoplasms from the meiospore, continuing as a succession of schizonts undergoing binary division and ending with sporulation, producing a second type of uninucleate spore. These spores, formed in the ovaries of *M. albidus,* were lanceolate, slightly curved and measured 13.23 × 3.85 μm. They infected *C. salinarius* larvae, both male and female, when ingested. In addition, cross-infectivity testing was conducted and demonstrated that while *A. californica* from *C. tarsalis* will infect *C. salinarius,* it does not complete its life cycle in this host. Based on these findings, we conclude that *Amblyospora* sp. from *Culex salinarius* is a distinct species and assign it the name *Amblyospora salinaria* n. sp.

***Key Words:Amblyospora salinaria* n. sp.; Microsporidia; *Culex salinarius*; mosquito; *Macrocyclops albidus*; copepoda; taxonomy; ultrastructure; host specificity.**

INTRODUCTION

Andreadis and Hall (1979a) provided a detailed description of the development, ultrastructure, and mechanism of transovarial transmission of an undescribed species of *Amblyospora* from the mosquito, *Culex salinarius* Coquillett, 1904. At that time, the mode of horizontal transmission of this and other *Amblyospora* species was unknown (Andreadis and Hall, 1979b). Because of the close affinities of the isolate from *C. salinarius* with the type species, *Amblyospora californica* Hazard and Oldacre, 1975, a parasite of *Culex tarsalis* Coquillett, 1896, the authors deferred the assignment of a new species to the former until complete life cycle studies and horizontal transmission testing could be completed with the latter.

In 1985, the involvement of a copepod intermediate host in the life cycle of *Amblyospora* was discovered (Andreadis, 1985; Sweeney *et al.*, 1985). This was followed by a series of host range studies (Andreadis, 1989; Sweeney *et al.*, 1990) in which a high level of specificity for the definitive mosquito host was demonstrated for several *Amblyospora* spp., thereby suggesting the likely existence of a distinct species for each mosquito.

The intermediate host for *A. californica* was recently discovered by Becnel (1992) who provided detailed accounts on horizontal transmission and subsequent development of this microsporidium in both hosts (copepod and mosquito). Herein, we now present definitive evidence that *Amblyospora* sp. from *C. salinarius* is also horizontally transmitted via a copepod intermediate host and in addition, describe results of cross infectivity testing with *A. californica* from *C. tarsalis*. Based on these findings, we conclude that *Amblyospora* sp. from *C. salinarius* is a distinct species and assign it the name *Amblyospora salinaria* n. sp.

MATERIAL AND METHODS

Larvae of *C. salinarius* were obtained from a healthy colony maintained at the USDA/ARS/CMAVE laboratory in Gainesville, FL. *Culex salinarius* larvae infected with *A. salinaria* were collected in the vicinity of Gainesville. A healthy colony of *C. tarsalis* and one infected with *A. californica* were obtained and maintained as previously described (Becnel, 1992). A laboratory colony of *Macrocyclops albidus* (Jurine, 1820) was established from copepods isolated in the vicinity of Gainesville. Specimens for electron microscopy were prepared as previously described (Becnel, 1992).

Groups of approximately 100 adult *M. albidus* (primarily females) were counted into small containers

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(80 × 45 mm) in 100 ml deionized water. These were exposed to meiospores of *A. salinaria* at a concentration of 1×10^4 spores/ml at room temperature. Control groups were handled in a similar manner but without the addition of meiospores.

Spores obtained from *M. albidus*, infected as described above, were fed to *C. salinarius* larvae as follows: Three groups of fifty 1st instar *C. salinarius* larvae (24 h old) were exposed in small petri dishes (60 × 15 mm) containing 10 ml of filtered field water (0.22 μm) at a concentration of 1×10^4 spores/ml. A small amount of a 5% aqueous infusion of 3:2 (liver: yeast) was added to each group. Exposed groups were held for 24 h at room temperature and then transferred to 18 × 29 × 4.5-cm enamel pans with 500 ml deionized water and reared to adults according to normal protocol. A control group was handled in a similar manner but without the addition of spores. Adults from the exposed groups were combined and held in small screened cages (21 cm^3), provided a 10% sugar solution, and held for 72 h. Females were then offered a blood meal (chicken) and egg rafts were collected 72 h later. The control group was handled in a similar manner. Rafts were hatched and the larvae were reared individually according to normal rearing protocols and examined for patent fat body infections as 4th instar larvae. All remaining adults were smeared, stained with Giemsa, and examined for infection.

The specificity of *A. californica* was tested in a separate set of experiments. Spores of *A. californica* from *M. albidus* were obtained as described previously (Becnel, 1992). Groups of 100 1st instar *C. tarsalis* and *C. salinarius* were counted separately into small petri dishes (60 × 15 mm) containing 10 ml of deionized water. Spores of *A. californica* from *M. albidus* were added to groups of each species to give a final concentration of 1×10^5 spores/ml. There were two exposed groups for each species. One additional group for each species without the addition of spores served as a control. From this point on, the protocol given above for the *A. salinaria* exposures was followed except that only a sample of the adults was examined for infection. The test was repeated one time.

An effort has been made in this contribution to use the terminology for developmental stages suggested by Sprague *et al.* (1992). Specifically, we reserve the term "meront" for diplokaryotic cells and "schizont" for cells with unpaired nuclei.

RESULTS

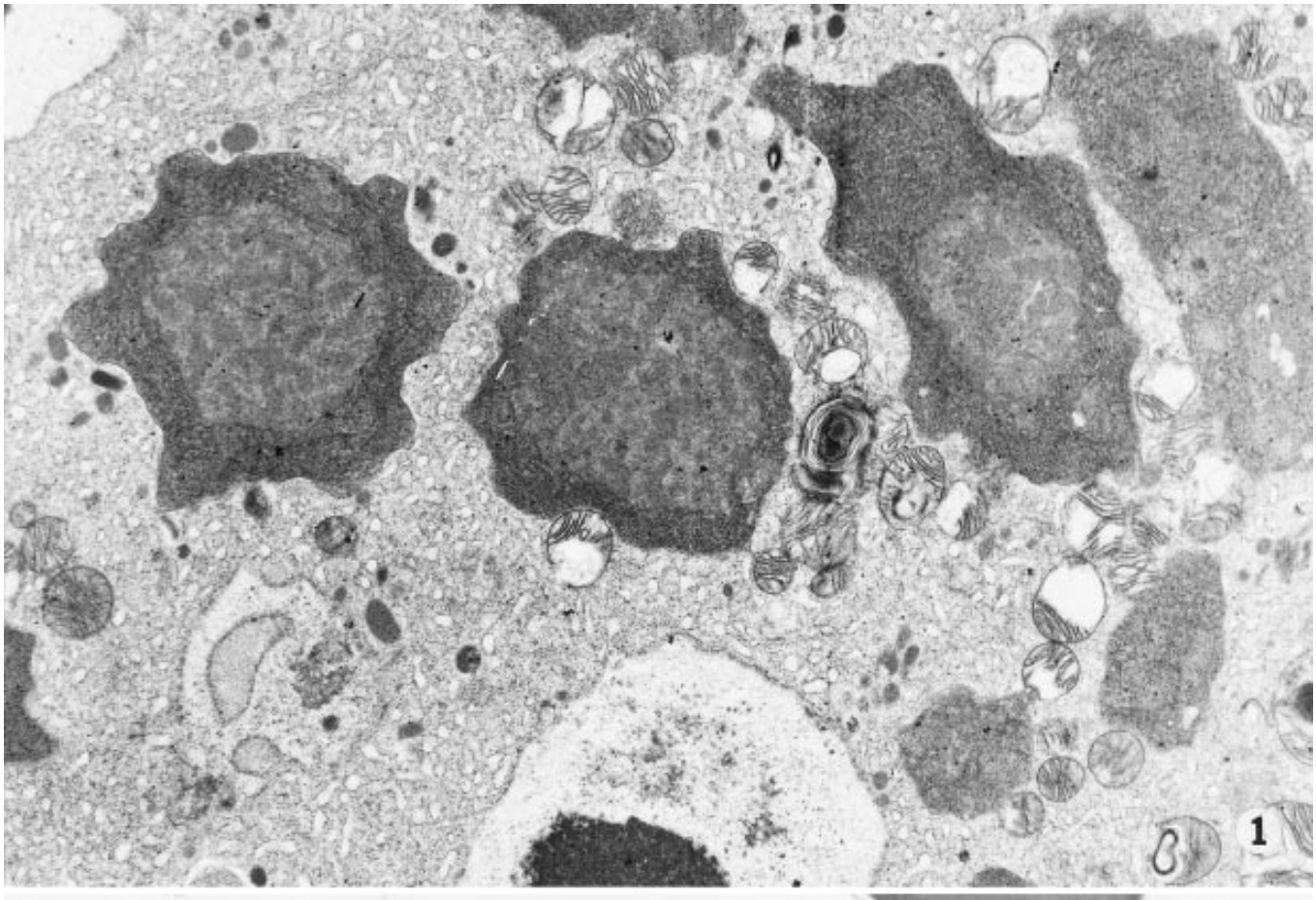
After 11 days, 95% of *M. albidus* exposed to *A. salinaria* meiospores had acute infections restricted to the ovaries; no infections were found in the control group. All developmental stages in the copepod had unpaired nuclei (Fig. 1.) with multiplication proceeding as a succession of schizonts undergoing binary division. Sprogonial plasmodia with up to four nuclei undergo multiple fission producing a second type of uninucleate spore. Spores in the ovaries were uninucleate, lanceolate, slightly curved, and measured 13.23 ± 1.37 × 3.85 ± 0.62 μm (fresh, $n = 32$) (Fig. 2). Each spore was individually contained within a sporontogenic sporophorous vesicle and characterized by an extensive, compartmentalized polaroplast with an isofilar polar filament with 12–14 turns in the posterior end. The spore wall was very thin with the endospore about twice as thick as the unlayered exospore.

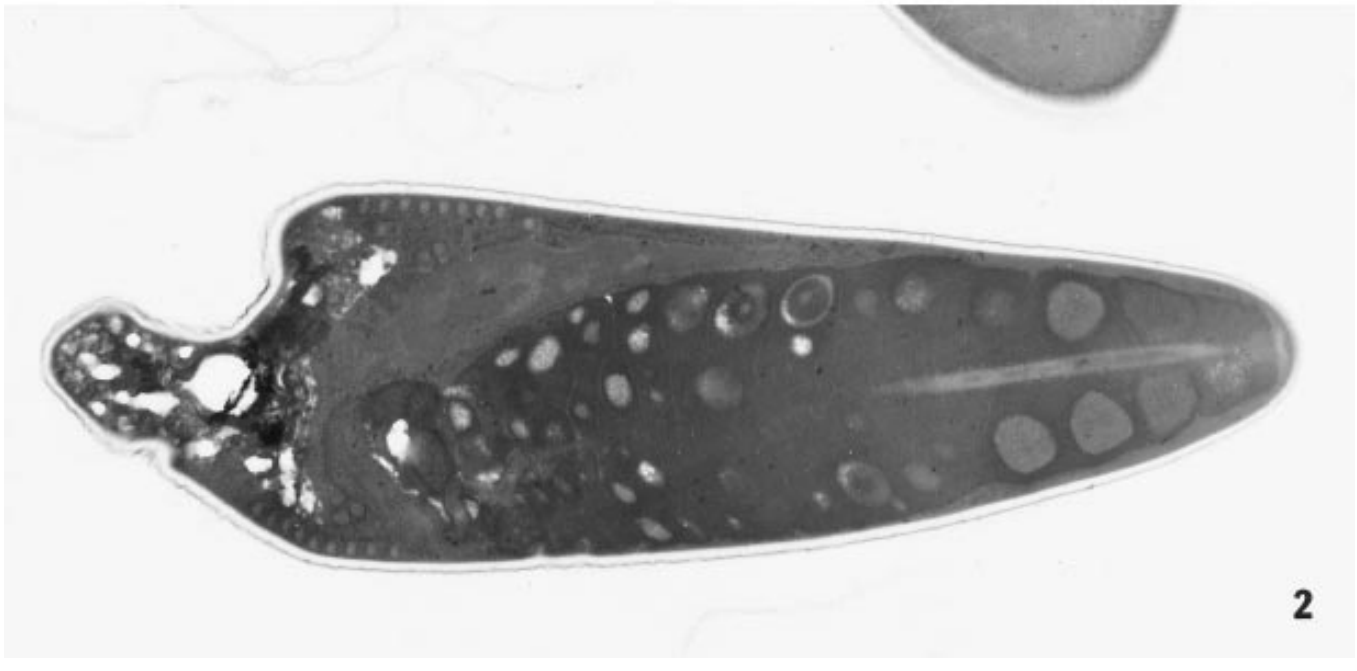
In *C. salinarius* adults exposed to spores from *M. albidus*, 31% of males (15/49) and 26% females (12/46) were infected. Only five egg rafts were obtained from the exposed adult females but two of these rafts had male larvae with fat body infections containing meiospores. These meiospores measured 8.42 ± 0.07 × 5.77 ± 0.07 μm (fresh, $n = 32$). This is consistent with the spore measurements originally reported for this species of 8.68 × 5.87 μm (Chapman *et al.*, 1966). No infections were found in the control adults or progeny.

Data from the two replicates testing the specificity of *A. californica* were combined and the results were as follows. No infections were found in the control adults or progeny of either species. In *C. tarsalis* adults exposed to *A. californica*, 100% of females (14/14) and 94% of males (31/33) were infected. The progeny of the families from *C. tarsalis* females were examined with 83% (15/18) infected with *A. californica*. In *C. salinarius* adults exposed to *A. californica*, 50% of females (8/16) and 42% of males (18/43) were infected. Infections in *C. salinarius* were generally light with mostly uninucleate stages observed. The progeny from 104 families of *C. salinarius* were examined but were not infected.

DISCUSSION

The life cycle of *A. salinaria* is essentially identical to that described for the type species *A. californica* (Becnel, 1992, Fig. 29) and is viewed as an alternation of haploid (haplophasic) and diploid (dihaplophasic) generations requiring two host species. The new data presented in this study has documented a haplophasic developmental sequence in the copepod *M. albidus* initiated by the meiospores produced in male mosquito larvae. This sequence results in a sporulation sequence forming the second type of uninucleate spore that is infectious orally to mosquito larvae continuing the haplophasic developmental sequence that presumably involves schizogony and gametogony and ends with plasmogamy. Nuclear association results in diplokaryotic meronts to begin the dihaplophase and supplements the previous report of Andreadis and Hall (1979a) on the life cycle stages of *A. salinaria* that occurs in *C. salinarius* (definitive host).





FIGS. 1 and 2. Stages of *Amblyospora salinaria* in the ovarian tissue of the copepod *Macrocyclops albidus*. (Fig. 1) Group of uninucleate stages early in the infection process. ×11,450. (Fig. 2) Mature uninucleate spore within a sporophorous vesicle. ×20,300.

We have demonstrated here that *A. salinaria* utilizes one (*M. albidus*) of two copepod hosts that are competent to serve as intermediate hosts for *A. californica*. The other host for *A. californica* is *Mesocyclops leuckarti* (Claus, 1857) as reported by Becnel (1992). The present study, like previous ones, provides evidence that species of *Amblyospora* lack the high degree of specificity for the intermediate hosts that they have for the definitive hosts. Thus, it may be that both *A. californica* and *A. salinaria* use any number of copepod species as intermediate hosts but little or nothing is known about the preferences in nature.

There are clear differences in size and morphology of the meiospores of *A. californica* and *A. salinaria* (Andreadis, 1994) and recent examination of the small subunit rRNA genes of the 2 species indicate that they are closely related but distinct (Baker *et al.*, 1997). In addition, the cross-infectivity data reported here demonstrates that while *A. californica* will infect *C. salinarius* it does not complete its life cycle in this host. This is consistent with the results of host specificity testing previously conducted with other species of *Amblyospora* (Andreadis, 1989, Sweeney *et al.*, 1990). Therefore, we conclude from the data presented in this study together with previously reported information, that the *Amblyospora* sp. from *C. salinarius* is a distinct species.

TAXONOMIC SUMMARY

Amblyospora salinaria n. sp.

Thelohania sp. Chapman, Woodard, Kellen, and Clark, 1966, *J. Invertebr. Pathol.* **8,** 452; Kellen, Chapman, Clark, and Lindegren, 1966, *J. Invertebr. Pathol.* **8,** 355; Chapman, Woodard, and Peterson, 1967, *Proc. N. J. Mosq. Exterm. Assoc.* **54,** 54; Chapman, Clark, Peterson, and Woodard, 1969, *Proc. N. J. Mosq. Exterm. Assoc.* **56,** 203.

Amblyospora sp. Hazard and Oldacre, 1975, *U. S. Dept. Agric. Tech. Bull. No.* **1530**, 44; Andreadis and Hall, 1979a, *J. Protozool.* **26,** 444; Andreadis and Hall, 1979b, *J. Invertebr. Pathol.* **34,** 152; Hazard, Andreadis, Joslyn, and Ellis, 1979, *J. Parasitol.* **65,** 117; Greenstone, 1983, *J. Invertebr. Pathol.* **41,** 250; Lord and Hall, 1983, *Parasitol.* **87,** 377; Hazard and Brookbank, 1984, *J. Invertebr. Pathol.* **44,** 3; Lord and Hall, 1984, *J. Invertebr. Pathol.* **43,** 276; Hall, 1985, *J. Am. Mosq. Control Assoc.* **1,** 514; Greenstone, 1986, *J. Kan. Entomol. Soc.* **59,** 658; Hall, 1990, *J. Invertebr. Pathol.* **55,** 291; Andreadis, 1994, *J. Euk. Microbiol.* **41,** 147; Flegel and Pasharawipas, 1995, *Can. J. Microbiol.* **41,** 1; Baker, Vossbrinck, Becnel, and Maddox, 1997, *J. Euk. Microbiol.* **44,** 220.

Amblyospora near *californica* Jahn, Hall, and Zam, 1986, *J. Florida Anti-Mosq. Assoc.* **57,** 24.

Type definitive host. *Culex salinarius* Coquillett, 1904 (Diptera: Culicidae).

Type intermediate host (laboratory). *Macrocyclops albidus* (Jurine,1820) (Copepoda: Cyclopidae).

Type locality. Calcasieu Parish, Louisiana.

Additional localities. Alachua County, FL.

Transmission. *Per os* to *C. salinarius* larvae via uninucleate spores from *M. albidus*. Transovarially to successive generations of *C. salinarius* larvae via binucleate spore in female. *Per os* to adult female *M. albidus* via meiospores from male *C. salinarius* larvae.

Site of infection. Oenocytes and ovary of *C. salinarius* female. Oenocytes and adipose tissue of male *C. salinarius* larva. Ovary of *M. albidus*.

Interface. Interfacial envelope produced by sporont (sporontogenic) in the sporulation sequence involving meiosis in the mosquito host. Sporulation stages in the copepod host within a sporontogenic interfacial envelope. All other parasite stages in direct contact with the host cell cytoplasm.

Development. (1) Stages in *C. salinarius* of a first generation: Uninucleate spores from copepod ingested by mosquito larvae of both sexes. Sporoplasm injected into oenocytes, initiates schizogonic series that ends with gametes (postulated). Gametes unite in pairs (gametogony) and nuclei associate as diplokarya, producing the first meronts (a first meront being the first stage in the dihaplophase). Meronts undergo a series of binary divisions (merogony), finally initiating a sporulation sequence. Sporulation in the female ends with binucleate spores that transovarially infect filial generations (see below). Sporulation in males preceded by karyogamy, accompanied by meiosis, and ends with sporoblasts transforming into meiospores, the first individuals in the haplophase. (2) Stages in *M. albidus*, entirely haplophasic: Meiospore ingested by copepod ejects sporoplasm which initiates a schizogonic sequence leading to sporulation and production of a second type of uninucleate spore. (3) Stages in *C. salinarius*, filial generation: *Females*: Merogony by binary division of meronts in oenocytes. Sporulation initiated after host blood feeding, proceeds directly from diplokaryotic cells to form binucleate spores responsible for transmitting the parasite from the female to the developing oocytes. *Males*: Merogony as above in oenocytes and adipose tissue, followed by karyogamy and restoration of the diplokaryon. Sporogony in adipose tissue, octosporoblastic and accompanied by meiosis, producing uninucleate meiospores within a sporophorous vesicle.

Spores. *Meiospore*: Oval with posterior end more broadly rounded than anterior, measuring 8.3–10.0 ×

5.8–6.7 µm fresh. Uninucleate with a thick undulating spore wall and large posterior vacuole. Polaroplast lamellar and more tightly arranged in the anterior end. Polar filament, anisofilar with 5–6 broad proximal and 10–11 narrow distal coils all arranged in a single row. *Binucleate spore:* Elongate, measuring 14.2–15 × 4.2–5.0 µm fresh. Binucleate with a thin spore wall and large posterior vacuole. Polaroplast lamellar. Polar filament isofilar with eight coils arranged in an irregular row. *Spore formed in copepod:* Lanceolate and slightly curved, measuring 11.9–14.6 × 3.2–4.5 µm fresh. Uninucleate with a thin spore wall and large posterior vacuole. Polaroplast vesiculate. Polar filament isofilar with 12–14 coils arranged in a single row.

Deposition of type materials. Hapantotype slides from each host (mosquito and copepod) have been deposited in the International Protozoan Type Slide Collection, Smithsonian Institute, Washington, DC (USNM Nos. 47908, 47909, 47910). Additional hapantotypes and type specimens embedded in plastic resin are also in the collection of each author.

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