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Horizontal Transmission of *Amblyospora dolosi* (Microsporidia: Amblyosporidae) to the Copepod *Metacyclops mendocinus* (Wierzejski, 1892)

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This study documents the involvement of a copepod intermediate host in the life cycle of *Amblyospora dolosi* from the neotropical mosquito *Culex dolosus* in Argentina. Meiospores of *A. dolosi* from the mosquito host were infectious *per os* to female adults of the copepod *Metacyclops mendocinus.* All developmental stages in the copepod were haplophasic (unpaired nuclei), with sporulation producing a second type of uninucleate spore. These spores, formed in the ovaries of *M. mendocinus,* were lanceolate, curved, and measured 14.3 × 3.8 μm. This study supports previous life cycle studies which demonstrate that most if not all *Amblyospora* spp. in mosquitoes require an obligate intermediate copepod host to complete the life cycle.

Key Words: **Amblyospora dolosi; Microsporidia; *Culex dolosus;* mosquito; *Metacyclops mendocinus;* copepod; taxonomy; ultrastructure; host specificity.**

INTRODUCTION

The microsporidium *Amblyospora dolosi* Garcia and Becnel, 1994, was described from the neotropical mosquito *Culex dolosus* (Lynch Arribálzaga, 1891) collected in Argentina. This species was distinguished from other described species of *Amblyospora* based on ultrastructure of the meiospore and presumed host specificity; no other information on the life cycle was available at that time (Garcia and Becnel, 1994). This report identifies a copepod, *Metacyclops mendocinus* (Wierzejski, 1892), as the intermediate host for *A. dolosi* and presents developmental and ultrastructural information on this new sporulation sequence. Data are also presented on host specificity and the natural prevalence of *A. dolosi* in both the copepod and mosquito hosts.

MATERIALS AND METHODS

Field studies. The collection site was a semipermanent pond (approximately 50 × 20 m in size and 0.30 m in depth) located near La Plata, Buenos Aires Province, Argentina. Collections were made daily for a 2-week period in early November 1995 and for a 2-week period in early February 1996. The collection site was without water from the end of November to the beginning of January. Mosquito larvae and copepods were collected from the pond on each sampling date by taking 100 random samples with a 300-ml mosquito dipper. The samples were immediately returned to the laboratory for examination. Copepods were identified according to Dussart (1969), Reid (1985), and Ringuelet (1958). The species of adult female copepods and their relative abundance were recorded. The prevalence of microsporidia within the copepod populations was determined by daily microscopic examination of whole wet mount preparations of individual copepods for the presence of spores. All individuals were examined if the sample size was small or 10% of the total sample if the population was large. Larvae of *C. dolosus* were sorted by instar and counted and the number of fourth instars with patent fat body infections of *A. dolosi* was determined by examination under a dissecting microscope. The natural prevalences of *A. dolosi* infections in *C. dolosus* and *M. mendocinus* were determined daily for each 2-week sampling period by the examination of individual copepods for the presence of spores.

Copepod infection experiments. Meiospores of *A. dolosi* for testing the susceptibility of copepods were isolated from infected *C. dolosus* obtained in the field. Examination of the field-collected copepods revealed that four cyclopoid species were commonly associated with *C. dolosus; Metacyclops mendocincus, Eucyclops* sp., *Paracyclops fimbriatus fimbriatus* (Fischer), and *Tropocyclops prasinus* (Fischer). A colony of each spe-

0022-2011/98

cies was established by isolating and rearing families from adult females. Each species was maintained in 500-ml trays held at 26°C. Groups of 25 adult copepods from each species were exposed to meiospores from one dead or moribund *C. dolosus* larvae infected with *A. dolosi*. Infected larvae were cut into small pieces that were readily fed upon by the copepods. Samples of copepods were removed from the test groups 3–13 days postexposure. Individual copepods were smeared, stained with Giemsa (Hazard and Oldacre, 1975), and examined for the presence of developmental stages and/or spores. Groups of copepods not exposed to meiospores were included in each experiment as controls and handled in a similar manner.

Development and ultrastructure of A. dolosi in the copepod host. Giemsa-stained smears of infected individuals were examined 3–13 days postexposure. Specimens for ultrastructural examination were fixed in 2.5% glutaraldehyde, postfixed in 1% OsO_4, dehydrated in an ethanol series to absolute acetone, and embeded in Epon Araldite. Thin sections were stained with uranyl acetate followed by lead citrate and examined with a Hitachi H-600 at 75 kV.

RESULTS

Natural prevalence. For the 13 sampling days in November, 1995, a total of 20,900 larvae of *C. dolosus* ($\bar{x}$ = 16 larvae/300 ml) and 852 female adult *M. mendocinus* ($\bar{x}$ = 0.6 females/300 ml) were collected. Fourth instar *C. dolosus* larvae infected with *A. dolosi* were found on 7 of 13 sampling days, with prevalences ranging from 0.4 to 3%. Based on the results of the copepod specificity experiments (presented below), data on field infections in the copepod population were restricted to *M. mendocinus*. Infected adult female *M. mendocinus* were found on 6 of 13 sampling days, with infection rates ranging from 0.8 to 25%.

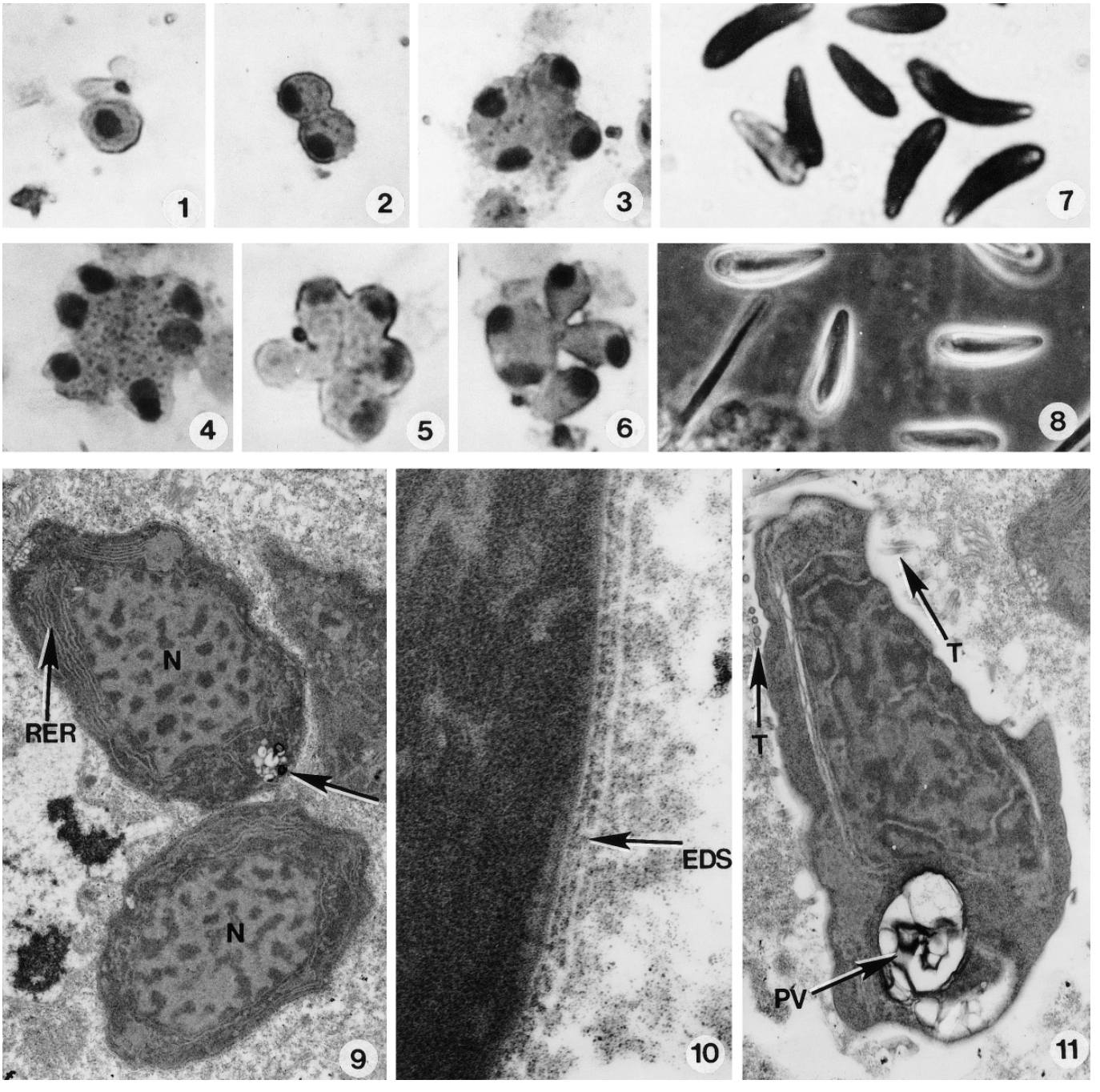
Results from the February sampling period showed that, unlike in the November sampling period, the mosquito populations were greatly reduced, while the copepod populations had greatly increased. For the 15 sampling days in February 1996, a total of 1306 larvae of *C. dolosi* ($\bar{x}$ = 0.8 larvae/300 ml) and 14,030 female adult *M. mendocinus* ($\bar{x}$ = 9 females/300 ml) were collected. Fourth instar *C. dolosus* larvae infected with *A. dolosi* were found on 2 of 15 sampling days, with infection rates of 4.9 to 7.7%. Infected female adult *M. mendocinus*, however, were found on each of the 15 sampling days, with infection rates ranging from 0.4 to 7.7%.

Copepod specificity experiments. Meiospores of *A. dolosi* infected *M. mendocinus* but none of the other copepod species tested. The infection was restricted to ovaries of females and no egg sacks were produced. Males were never infected. Mature spores of *A. dolosi* were first detected in infected females 6–8 days postexposure. Infection rates ranged from 70 to 100% in 17 separate cohorts of copepods exposed to *A. dolosi*. None of the controls examined from these experiments developed microsporidian infections. The laboratory infections of *M. mendocinus* were identical in tissue specificity, development, and morphology to the infected individuals collected from the field.

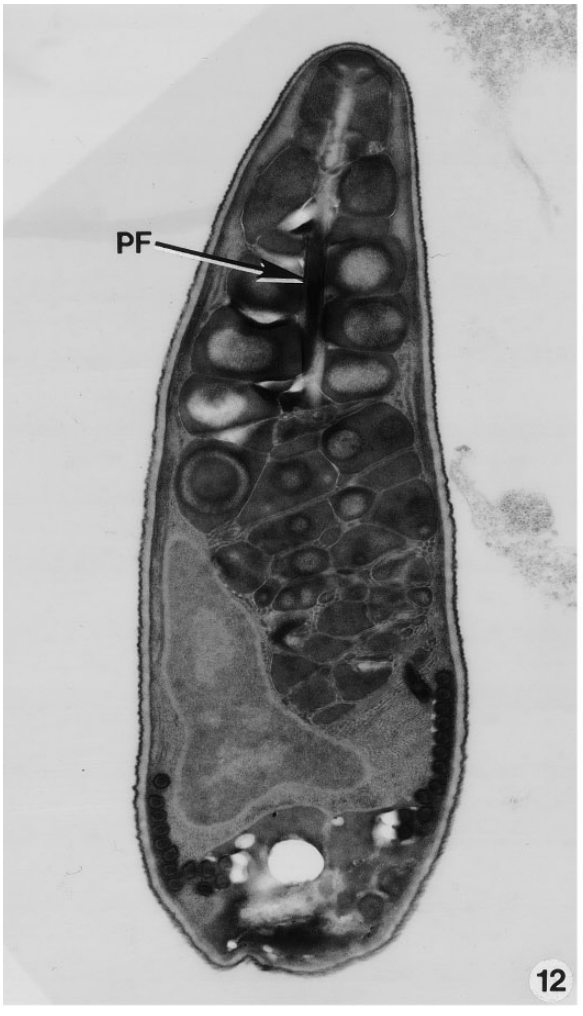
Light microscopy. Development of *A. dolosi* in *M. mendocinus* was completely haplophasic (unpaired nuclei). The stages observed probably represented cells in various stages of the sporulation process. Uninucleate (transitory) cells (Fig. 1) multiplied either by binary divisions (Fig. 2) or by multiple fission of plasmodia (Figs. 3–6). Plasmodia, interpreted to be sporogonial, typically contained four nuclei (Fig. 3) but occasionally six nuclei were found (Fig. 4). Multiple fission of the plasmodia, often in the shape of a rosette (Figs. 5 and 6), resulted in the production of uninucleate sporoblasts. These developed into large, lanceolate, uninucleate spores (Figs. 7 and 8) that measured 12.2 ± 0.5 × 3.3 ± 0.5 μm (mean ± SE, n = 15, fixed, Giemsa-stained) and 14.3 ± 0.6 × 3.8 ± 0.2 μm (mean ± SE, n = 15, fresh).

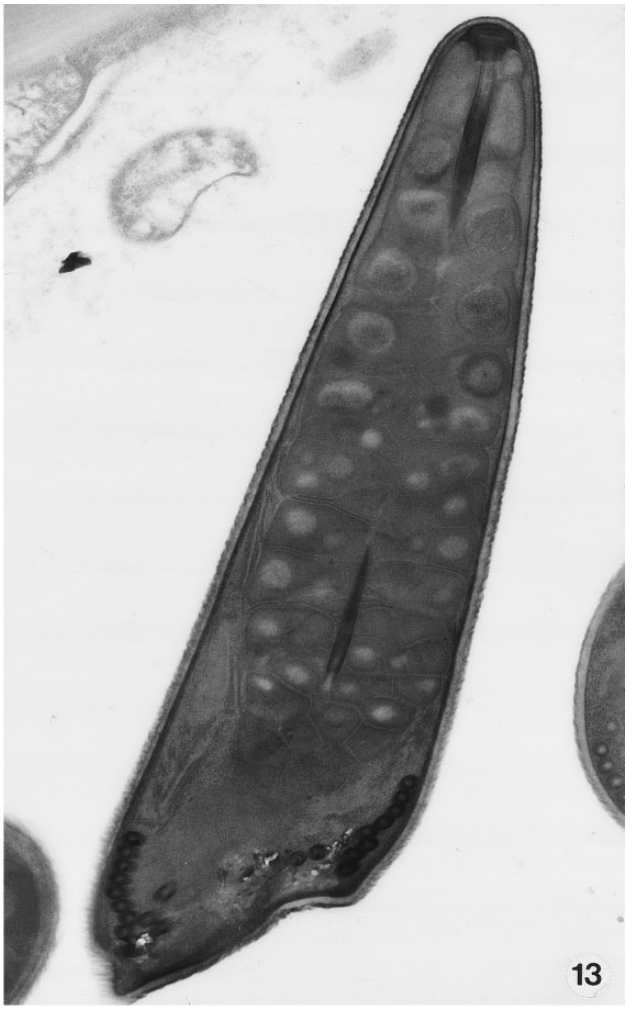
Ultrastructure. The earliest vegetative stages observed were late stage sporonts in the process of transforming into sporoblasts. These stages were electron dense, with the nucleus occupying a large portion of the cell (Fig. 9). The cytoplasm was dominated by arrays of rough endoplasmic reticulum (RER) and free ribosomes. A specialization of the host–parasite interface was found on these cells at this point in development. This interface was composed of small regularly spaced electron-dense structures with the general appearance of RER (Fig. 10). It was unclear whether this was a parasite- or host-derived structure or a combination of both. The first indications of transformation into sporoblasts was the elongation of the cell and the formation of the posterior vacuole at one pole (Fig. 11). The cell continued to elongate, accompanied by the presence of transitory tubules that were associated with the early sporoblast (Fig. 11). There was no indication of a parasite-derived interfacial envelope, although sporoblasts and spores appeared to be individually isolated from the host cytoplasm (Fig. 14).

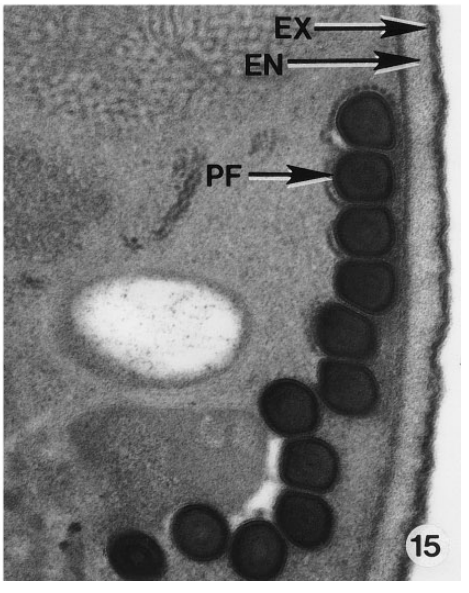
The spore was dominated by an extensive compartmentalized and vacuolated polaroplast that occupied three-fourths of the spore (Figs. 12, 13). The isofilar polar filament made 12–13 coils in the posterior region



FIGS. 1–6. Giemsa-stained developmental stages of *Amblyospora dolosi* from the ovarian tissue of the copepod *Metacyclops mendocinus*. ×2000. (1) Uninucleate stage. (2) Binary fission of uninucleate stage. (3) Quadrinucleate sporogonial plasmodium. (4) Sporogonial plasmodium with six nuclei. (5–6) Multiple fission of sporogonial plasmodium. Figs. 7–8. Mature spores of *A. dolosi* from *M. mendocinus*. ×2000. (7) Giemsa-stained. (8) Fresh, phase contrast. Figs. 9–11. Stages of *A. dolosi* during sporulation in the ovarian tissue of the copepod *M. mendocinus*. (9) Sporonts transforming into sporoblasts. Note the formation of the posterior vacuole (arrow). *N*, nucleus; RER, rough endoplasmic reticulum. ×9600. (10) Parasite–host interface of stages in Fig. 9. EDS, electron-dense structures. ×12,400. (11) Early sporoblast. Note the tubules in cross and longitudinal sections (T). PV, posterior vacuole. ×12,400.







FIGS. 12–15. Spores of *A. dolosi*. (12) Early spore. PF, polar filament. ×20,800. (13) Mature spore. ×18,400. (14) Group of mature spores in the ovarian tissues of *M. mendocinus*. ×3900. (15) Details of the spore wall (EN, endospore; EX, exospore) and polar filament (PF). ×66,500.

of the spore (Figs. 12, 13, and 15). The spore wall was thin with the endospore approximately four times the thickness of the unlayered exospore (Fig. 15).

DISCUSSION

This study has documented, for the first time, the involvement of an intermediate host in the life cycle of an *Amblyospora* species from a neotropical mosquito. The sporulation sequence of *A. dolosi* in the copepod *M. mendocinus* is essentially the same for the type host *A. californica* (Kellen and Lipa, 1960) as described by Becnel (1992). All developmental stages in the copepod were haplophasic, with sporulation involving the production of multinucleate plasmodia to produce a second type of uninucleate spore. The spore was characteristic of those previously described for other *Amblyospora* spp., with the most notable feature being the large, vacuolated polaroplast that occupies the majority of the spore (Sweeney *et al.*, 1985, 1988, 1990a; Andreadis, 1985, 1988; Becnel, 1992; White *et al.*, 1994).

Recently, the genus *Trichotuzetia* Vavra, Larsson, and Baker, 1997, has been created for a new microsporidium *T. guttata* (Vavra *et al.*, 1997) described from the copepod *Cyclops vicinus* Uljanin. *T. guttata* is cytologically different from the spores of *Amblyospora* and *Parathelohania* spp. formed in the copepod intermediate host in shape, construction of the exospore, polaroplast, and sporophorous vesicle. This is only notable here because molecular analysis of the small subunit rDNA shows that *T. guttata* is most closely related to a group of microsporidia including *A. californica* (see Vavra *et al.*, 1997). The authors speculated that *T. guttata* may have a more complex life cycle involving a second host because of unsuccessful infection experiments and the results of the molecular analysis.

A. dolosi is only the second species of *Amblyospora* that does not form an interfacial envelope during sporulation and therefore does not have a sporophorous vesicle. Sporulation of *A. opacita* (Kudo, 1922) in the copepod host also does not involve the formation of a parasite-derived interfacial envelope (White *et al.*, 1994). These authors suggested that since the interfacial envelope is generally a higher order character, its absence is unimportant in the delineation of similar species. Our observations support the conclusion that the presence or the absence of an interfacial envelope during sporulation of *Amblyospora* spp. in the copepod host probably represents a species character.

Field and laboratory data have confirmed that of the four cyclopoid species commonly associated with *C. dolosus*, only *M. mendocinus* was susceptible to *A. dolosi*. This high degree of specificity for a single copepod intermediate host has also been found by Sweeney *et al.* (1990b) in *A. indicola* Vavra, Bai, and Panicker, 1984, and *A. dyxenoides* Sweeney, Graham, and Hazard, 1988, and by Andreadis (1988) in *A. connecticus* Andreadis, 1988. *Amblyospora californica* and *A. opacita*, however, have been shown to have two copepod species that can serve as intermediate hosts (Becnel, 1992; White *et al.*, 1994). It might be expected that a low degree of specificity for copepod intermediate hosts would have greater survival value for the parasite. However, it is apparently not the specificity for *M. mendocinus* that assures survival of *A. dolosi* but rather unknown features of the ecological linkage of the two hosts that are responsible for continued survival.

C. dolosus populations are reported to peak in Argentina during spring (October–November) and fall (March–April) and are reduced during the months with extreme temperatures (winter and summer) (Macia *et al.*, 1997). This is consistent with our field observations of *C. dolosus* populations. *M. mendocinus* populations, however, peaked in the summer (February) and were reduced in the spring (November). Infections of *A. dolosi* in *M. mendocinus* were more common during the February sampling period, even though *C. dolosus* populations declined and infections in fourth instar larvae were low. The presence of a continuous source of *A. dolosi* in copepods could serve to maintain the parasite until the fall population peak of *C. dolosus*. Additional epizootiological studies over a longer period of time are needed to determine the dynamics involved in maintenance of *A. dolosi* in the populations of this two-host system.

C. dolosus has not been colonized and therefore infectivity of the spore formed in *M. mendocinus* for *C. dolosus* has not been verified. Based on previous studies (Sweeney *et al.*, 1985, 1988, 1990a, b; Andreadis, 1985, 1988; Becnel, 1992; White *et al.*, 1994), we believe that the function of the uninucleate spore of *A. dolosi* formed in copepods is to infect the larval stages of *C. dolosus*.

The involvement of intermediate copepod hosts for *Amblyospora* spp. has now been verified from three widely separated geographic regions; Australia (Sweeney *et al.*, 1985), North America (Andreadis, 1985) and, in this report, from South America. Studies in other geographic regions, specifically Africa, Europe, and Asia, are needed to determine if a two-host system is a general features of *Amblyospora* life cycles or if other variations occur in microsporidia with complex life cycles.

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