Epizootiological studies of *Amblyospora camposi* (Microsporidia: Amblyosporidae) in *Culex renatoi* (Diptera: Culicidae) and *Paracyclops fimbriatus fimbriatus* (Copepoda: Cyclopidae) in a bromeliad habitat

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Abstract

The epizootiology of *Amblyospora camposi* was studied in a natural population of *Culex renatoi*, a bromeliad-inhabiting mosquito, and its intermediate host, *Paracyclops fimbriatus fimbriatus*, over a 2-year period. Twenty *Eryngium cabrerae* plants were sampled monthly from January 2003 to January 2005 and the prevalence of *A. camposi* in *P.f. fimbriatus* and *Cx. renatoi* populations was determined. The monthly prevalence rates of meiospore infections in *Cx. renatoi* larvae never exceeded 5.5% and was detected in 50% of the monthly samples. Meiospores were available in plants over the course of the study at a mean concentration of $2 \times 10^4$ meiospores/ml. Within each plant the parasite was maintained by horizontal transmission. *P.f. fimbriatus* with vegetative stages and mature spores were found regularly in bromeliads suggesting efficient meiospore infectivity to field copepod populations. The mean concentration of spores from copepods found in plants was $8 \times 10^2$ spores/ml. Infections in copepods were detected in 54% of the monthly samples with a prevalence rate ranging from 0.55 to 17.4% and an overall average of 5.1%. Vegetative stages in fourth instar mosquito larvae (probably derived from the horizontal pathway via spores formed in copepods) were detected in 12.5% of the monthly samples with an overall prevalence rate of 1.1%. Infections in female and male adults were detected in 20.8% of the monthly samples with an overall average of 4.1% and 6.8%, respectively.

Keywords: *Amblyospora camposi*; Microsporidia; *Culex renatoi*; *Paracyclops fimbriatus fimbriatus*; Epizootiology

1. Introduction

The genus *Amblyospora* (Hazard and Oldacre) represents the largest group of microsporidia that infect natural populations of mosquitoes (Hazard and Oldacre, 1975; Hazard and Chapman, 1977; Castillo, 1980). Several *Amblyospora* species have been reported infecting mosquitoes from Argentina (García, 1989; García and Becnel, 1994; Micieli and García, 1997; Micieli et al., 2000). Thus far, epizootiological studies have been conducted for two of these species; *A. albifasciati* (García and Becnel) in *Ochlerotatus albifasciatus* (Macquart), a floodwater mosquito (Micieli et al., 2001) and *A. feroxis* (García and Becnel) a parasite of *Psorophora ferox* (Humbold), a forest mosquito that develops in temporary ponds in Argentina (Micieli et al., 2003).

The life cycle of *Amblyospora camposi* Micieli et al., 2000 was described from the mosquito *Culex renatoi* Lane and Ramalho and the copepod *Paracyclops fimbriatus fimbriatus* (Fischer) collected in the leaf axils of the plant *Eryngium cabrerae* (Umbelliferae) in Argentina (Micieli et al., 2000). This microsporidium has an intricate life cycle with multiple types of spores responsible for horizontal and vertical transmission and therefore, is comparable to life cycles described for other species of *Amblyospora* (Andreadis, 1985a,b, 1988; Sweeney et al., 1985, 1988; Becnel, 1992).
However, in the original description of *A. camposi* it was not possible to verify vertical transmission because *Cx. renatoi* has not been colonized. Vertical transmission was implicated by the detection of binucleate spores observed in field collected adult mosquitoes. Meiospores from infected mosquito larvae (presumably derived from infected adults) are infectious to the copepod *P.f. fimbriatus*. Spores formed in the ovaries of copepods are released upon death and responsible for horizontal transmission of *A. camposi* to larvae of *Cx. renatoi*. In these horizontally infected mosquito larvae, vegetative stages (uninucleate stages, gametes and diplokarya) were observed in laboratory transmission experiments but it was not possible to obtain progeny from the infected adults to verify vertical transmission to the next generation.

We have examined the epizootiology of *A. camposi* in field populations of its primary mosquito host *Cx. renatoi* and its intermediate host *P.f. fimbriatus* over a 2-year period to elucidate the transmission and survival strategies used by this microsporidium in this specialized habitat.

2. Materials and methods

2.1. Host biology

*Culex renatoi* is unique in that it is strictly a bromeliad-inhabiting mosquito. It is host-plant specific occurring only in the phytotelmata of *E. cabrerae* (Campos and Lounibos, 1999). *Paracyclops f. fimbriatus* is a small copepod species found in a wide range of freshwater habitats with worldwide distribution (Reid, 1985). It is commonly found associated with *Cx. renatoi* larvae in the phytotelmata of *E. cabrerae* (Micieli et al., 2000).

2.2. Study site and host collections

The study site was located in Punta Lara (34° 51’53”S, 57° 52’23”W) near the Rio de La Plata river in Buenos Aires Province, Argentina. The site consists of an open field close to forest habitats that border the river. Ten *E. cabrerae* plants located in this site were randomly selected on each sample day, twice a month from January 2003 to 2005. The water-holding leaf axils of the plants, containing mosquitoes and associated microcrustacea, were extracted with an aspirator and water volume measured as described in Micieli et al. (2000). The samples were placed into labeled containers and transported to the laboratory. The larvae were identified using the diagnostic key of Darsie and Mitchell (1985) and the number of immature stages of mosquitoes from each plant recorded.

2.3. Processing of mosquitoes and copepods

*Culex renatoi* larvae were examined for infections with *A. camposi* using stereoscopic and light microscopes. The overall prevalence of each type of infection was recorded as well as the relative abundance of each larval instar and pupa. Meiospore infections in larvae of *Cx. renatoi* were determined based on the chalky white appearance of the fat body and confirmed with Giemsa-stained smears (10%, pH 7.4). Mosquito larvae without gross lesions were individually smeared, stained with Giemsa and examined for the presence of vegetative stages (i.e., uninucleate stages, gametes, diplokarya) of *A. camposi* as evidence of horizontal transmission (Micieli et al., 2000). The number of larvae examined was variable depending on the weekly collection. Field collected pupae from each plant were placed into a cage for adult emergence. All adults were sexed, individually smeared, stained with Giemsa as above and examined for the presence of vegetative stages and/or spores of *A. camposi*.

Copepods were identified using the keys of Reid (1985) and Ringuellet (1958). Presence and relative abundance of female and male adults of *P.f. fimbriatus* were recorded. The prevalence of *A. camposi* in a *P.f. fimbriatus* population was determined for each sample by microscopic examination (1000 £) of Giemsa-stained smears for a variable number of copepod female adults. This method allows for distinguishing early infections (vegetative stages) from late infections (spores). Juvenile stages of the copepods (nauplii and copepodids) and male adults were not examined.

The distribution of larval mosquitoes infected with meiospores of *A. camposi* and the infected intermediate host was determined monthly. To obtain the total number of meiospores, patently infected larvae were triturated in a glass tissue grinder and the large matter was removed by passing it through cotton in a syringe. Meiospore counts were made with a hemacytometer from each infected individual and the total number was determined monthly. To determine the total number of uninucleate spores for a given month, 10 infected copepods were triturated in a glass tissue grinder and counted to obtain the mean number of spores per copepod. The monthly number of uninucleate spores was expressed as the mean number of spores per copepod times the number of infected copepods for each sample.

The mean concentration of meiospores and uninucleate spores in plant axils was calculated based on the volume of water extracted from each plant where infected individuals (mosquito larvae and copepods, respectively) were collected. The total number of spores from both types of infection was obtained as above and the concentration was expressed as the number of spores per milliliter per plant.

3. Results

3.1. Host populations

*Paracyclops fimbriatus fimbriatus* and immature stages of *Cx. renatoi* were consistently recovered from *E. cabrerae* during the two-year sampling period. Mean numbers of copepods ranged from 13 (July) to 53 (November–December) per plant during the first year and from 7 (October) to 69 (November) in the second year (Fig. 1). A high percentage...
of plants were found to contain *P. f. fimбриatus* during the course of this study and varied from 75 to 100%. Kruskal-Wallis ANOVA for the mean number of copepods per plant was not significantly different between seasons (*H* = 8.96, *n* = 12, *P* = 0.06 in 2003; *H* = 3.2, *n* = 12, *P* = 0.36 in 2004). Three other copepod species, *Acanthocyclops robustus* (Sars) (*n* = 12), *Ectocyclops* sp. (Brady) (*n* = 1762) and *Microcyclops* sp. (Claus) (*n* = 8) were found living sympatrically with *P. f. fimбриatus*, in the water-holding leaf axils of *E. cabrerae*. *Amblyospora* spp. infections were not present in any of these three associated copepod species. For the first year of the study, the mean number of mosquito larvae per plant ranged from 1.65 in February to 39 in November. Larval populations declined in the second year ranging from 17.7 larvae/plant in January to 4.8% in November 2004 (Fig. 1). The proportion of infected fourth instar larvae was always low, ranging from 0.4% (*n* = 225) in January to 4.8% (*n* = 168) in August (Fig. 2). Early instar larvae showing fat body infections were found in most of the samples. Patently infected fourth instar larvae were detected in January, August, and November (Table 1). In 2004 the prevalence of *A. camposi* ranged from 1% (*n* = 193) in November to 5.4% (*n* = 37) in March (Fig. 2). Infected early instar larvae were found in February, March, April, and June. Patently infected fourth instar larvae were detected in November. The overall average of infected larvae with meiospores was 1.4% (*n* = 2500) during the 2-year study. Based on the volume of water extracted from each plant where infected instar larvae were found, we calculated a mean concentration of $2 \times 10^4$ meiospores/ml (*n* = 25 plants).

### 3.2. *Amblyospora camposi*

#### 3.2.1. Meiospore infections

Patently infected larvae were detected in seven months during the first year of the study (Table 1). The prevalence of meiospore infections in the larval population was always low, ranging from 0.4% (*n* = 225) in January to 4.8% (*n* = 12) in August (Fig. 2). Early instar larvae showing fat body infections were found in most of the samples. Patently infected fourth instar larvae were detected in January, August, and November (Table 1). In 2004 the prevalence of *A. camposi* ranged from 1% (*n* = 193) in November to 5.4% (*n* = 37) in March (Fig. 2). Infected early instar larvae were found in February, March, April, and June. Patently infected fourth instar larvae were detected in November. The overall average of infected larvae with meiospores was 1.4% (*n* = 2500) during the 2-year study. Based on the volume of water extracted from each plant where infected instar larvae were found, we calculated a mean concentration of $2 \times 10^4$ meiospores/ml (*n* = 25 plants).

### 3.2.2. Infections in copepods

Infected copepods were present in 8 of the 12 months sampled in 2003 with the highest prevalence in December (4.4%, *n* = 614). In 2004 infected *P. f. fimбриatus* were found in five of the months sampled with the highest prevalence (4.4%, *n* = 614). Three other copepod species, *Acanthocyclops robustus* (Sars) (*n* = 12), *Ectocyclops* sp. (Brady) (*n* = 1762) and *Microcyclops* sp. (Claus) (*n* = 8) were found living sympatrically with *P. f. fimбриatus*, in the water-holding leaf axils of *E. cabrerae*. *Amblyospora* spp. infections were not present in any of these three associated copepod species. For the first year of the study, the mean number of mosquito larvae per plant ranged from 1.65 in February to 39 in November. Larval populations declined in the second year ranging from 17.7 larvae/plant in January to 4.8% in November 2004 (Fig. 1). The proportion of infected fourth instar larvae was always low, ranging from 0.4% (*n* = 225) in January to 4.8% (*n* = 168) in August (Fig. 2). Early instar larvae showing fat body infections were found in most of the samples. Patently infected fourth instar larvae were detected in January, August, and November (Table 1). In 2004 the prevalence of *A. camposi* ranged from 1% (*n* = 193) in November to 5.4% (*n* = 37) in March (Fig. 2). Infected early instar larvae were found in February, March, April, and June. Patently infected fourth instar larvae were detected in November. The overall average of infected larvae with meiospores was 1.4% (*n* = 2500) during the 2-year study. Based on the volume of water extracted from each plant where infected instar larvae were found, we calculated a mean concentration of $2 \times 10^4$ meiospores/ml (*n* = 25 plants).

### Table 1: Distribution of *Amblyospora camposi* in *Culex renatoi* larvae during the two year study

<table>
<thead>
<tr>
<th>Month</th>
<th>Number of instars and number of infected larvae per plant</th>
<th>Number of meiospore/ month$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>January</td>
<td>1L, 3L2, 1L2, 1L2</td>
<td>$2 \times 10^5$</td>
</tr>
<tr>
<td>May</td>
<td>1L2, 1L3, 1L3, 2L3</td>
<td>$5 \times 10^5$</td>
</tr>
<tr>
<td>June</td>
<td>1L2, 1L3, 2L3, 1L3, 3L3, 1L3</td>
<td>$2 \times 10^6$</td>
</tr>
<tr>
<td>July</td>
<td>2L3, 1L3, 1L3</td>
<td>$8 \times 10^5$</td>
</tr>
<tr>
<td>August</td>
<td>1L4, 1L3, 2L3, 1L3, 3L3, 1L4</td>
<td>$5 \times 10^6$</td>
</tr>
<tr>
<td>October</td>
<td>1L2, 1L3</td>
<td>$3 \times 10^5$</td>
</tr>
<tr>
<td>November</td>
<td>1L2, 4L4, 1L2</td>
<td>$1 \times 10^6$</td>
</tr>
<tr>
<td>February</td>
<td>1L2, 1 L3</td>
<td>$4 \times 10^5$</td>
</tr>
<tr>
<td>March</td>
<td>1L2, 1 L3</td>
<td>$2.5 \times 10^5$</td>
</tr>
<tr>
<td>April</td>
<td>1L2, 2 L2</td>
<td>$3 \times 10^5$</td>
</tr>
<tr>
<td>June</td>
<td>1L3</td>
<td>$3 \times 10^5$</td>
</tr>
<tr>
<td>November</td>
<td>2L4</td>
<td>$8 \times 10^5$</td>
</tr>
</tbody>
</table>

$^a$ Total number of meiospore per month was calculated based on the number of infected larvae present (10 plants sampled twice a month).

Fig. 1. Abundance of *Culex renatoi* larvae and pupae and *Paracyclops fimбриatus fimбриatus* copepods from monthly samples of 20 *Eryngium cabrerae*. 
recorded in January (17.4%, n = 275) (Fig. 2). The majority of A. camposi infections detected in copepods were in the form of vegetative stages with an average infection rate over the course of the entire study of 5.1% (n = 4281). The highest numbers of infected female copepods with mature spores were found in January (n = 30) and February (n = 35) of 2004 (Fig. 3). Based on the volume of water extracted from each plant where infected copepods were collected, we calculated a mean concentration of uninucleate spores of $8 \times 10^2$ spores/ml (n = 44 plants).

3.2.3. Non-patent infections in Cx. renatoi larvae

Larvae infected with vegetative stages of A. camposi were detected in Giemsa-stained smears. Larvae infected with uninucleate stages and gametes (indicative of horizontal transmission from spores formed in copepods) were found in July (18%, n = 22) and August (4.76%, n = 21). In November of 2003, 1.9% of infected larvae (n = 52) had uninucleate stages, gametes, diplokarya and binucleate spores (Fig. 2). For 2004, no larvae were found infected. The overall average of larvae infected via this pathway was 1.1% (n = 610) for the two years of the study.

3.2.4. Adult mosquito infections

Adult females of Cx. renatoi infected with binucleate spores of A. camposi were detected in November (3.4%, n = 29) and December (33.3%, n = 6) of 2003 (Fig. 2) and in 20% of the adults collected in August, 2004 (n = 5). Male adults were found infected in August (30%, n = 10), September (14.2%, n = 7), November (7.14%, n = 14) and December (16.6%, n = 12) of 2003 (Fig. 2). In August, gametes, diplokarya and binucleate spores were found in two infected adult males while in the third infected male both binucleate spores and meiospores were detected. In September and November, infected males contained only binucleate spores. In December, one of the infected males

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Fig. 2. Prevalence of Amblyospora camposi in field populations of female Paracyclops fimбриatus fimбриatus; horizontally transmitted infections and meiospore infections in larval Culex renatoi; and infections in the adult male and female mosquitoes.

Fig. 3. Number of Paracyclops fimбриatus fimбриatus infected with Amblyospora camposi. Monthly comparison of presence of mature spores and vegetative stages in Paracyclops fimбриatus fimбриatus.
had both binucleate spores and meiospores while the other had only diplokaryotic stages. Infected male adults were not found in 2004. The overall average infection rates for female and male mosquito adults was 4.1% \((n = 96)\) and 6.8% \((n = 102)\), respectively. Adult infections were recorded from only six plants out of the total number of bromeliads sampled during both years of the study \((n = 511)\).

3.3. Distribution of infection with *A. camposi*

The distribution of larval mosquitoes infected with meiospores of *Amblyospora camposi* in the bromeliads varied by month within each sample year. This ranged from one plant \((n = 20)\) in January and October of 2003, and June and November of 2003 to 5 plants \((n = 20)\) in August of 2003. The total number of meiospores available for each month of the year varied between \(2 \times 10^4\) and \(5 \times 10^6\) meiospores/month (Table 1). Meiospore infections were detected in 4.9% of the total number of bromeliads examined (25/511).

The mean number of uninucleate spores was 6000 spores/copepod \((n = 10)\). The monthly number of uninucleate spores was calculated from the total number of infected copepods collected (10 plants sampled twice a month) and ranged from \(2 \times 10^4\) to \(2 \times 10^5\) spores/month. The number of plants with infected copepods by month varied from 1 to 7, with variable numbers of copepods per plant (range 1–17). Most (60%) of the bromeliads positive for infected copepods contained only one or two infected individuals (Table 2). Copepods infected with *A. camposi* were observed in 8.6% of the bromeliads examined (44/511).

4. Discussion

*Amblyospora camposi* was found to be enzootic over a two year period within this population of *Cx. renatoi*, a multivoltine bromeliad-inhabiting mosquito from the Neotropical region of Argentina. Prevalence levels were low but *A. camposi* was consistently present in the mosquito host population. Seasonal epizootics were not observed in either the copepod or mosquito hosts as has been described for *A. connecticus*, a parasite of the univoltine mosquito, *Ochlerotatus cantator* (Andreadis, 1990) and in the larval stages of *Amblyospora* spp. parasitizing multivoltine mosquitoes (Andreadis, 1993). Other *Amblyospora* spp. in univoltine mosquitoes have been reported in previous studies to only occur at enzootic levels (Anderson, 1968; Andreadis, 1994, 1999). *Amblyospora albifasciata*, a parasite of the multivoltine freshwater mosquito *Ochlerotatus albifasciatus* was enzootic in its mosquito host but reached epizootic levels in the copepod intermediate host population (Micieli et al., 2001).

*Cx. renatoi* was present throughout the year as immature stages with overlapping generations in bromeliads. Larvae infected with meiospores were found in 50% of the samples over the course of the 2-year study. Patent infections were detected in early instar mosquito larvae in most of the samples but fourth instar larvae infected with meiospores were observed for only three months each year. We speculate that the presence of early instar mosquito larvae with mature meiospores could be a consequence of delayed physiological development as a result of infection by *A. camposi* in a habitat with limited resources.

The monthly prevalence of larvae infected with meiospores of *A. camposi* never exceeded 5.5%. Low rates of infected larvae have previously been reported for several *Amblyospora* spp. (Anderson, 1968; Andreadis, 1999; Micieli et al., 2001). Although monthly prevalence rates were low, meiospores were available in many plants at a mean concentration of \(2 \times 10^4\) meiospores/ml. Micieli et al. (2000) determined in laboratory transmission test that a concentration of \(1 \times 10^3\) meiospores/ml produced between 33% and 93% infection rates in copepods. The field concentrations of meiospores determined in this study was sufficient for successful transmission of *A. camposi* to the copepod population which is reflected by the data presented in this study (Fig. 2). Furthermore, the distribution of infected larvae in the bromeliads and the total number of meiospores available for several months of the year with levels reached \(10^5–10^6\) meiospores/month suggests a widespread distribution of the parasite within the host populations.

Transovarial transmission is an important strategy for the dispersal of *Amblyospora* spp. to new habitats (Lucchini and Andreadis, 1995). In a specialized habitat such as phytotelmata, vertical transmission could be expected to be the main mechanism for parasite dissemination to each plant where the hosts are present. However, the production of infected adults was restricted to only 1% of the bromeliads sampled. It appears that the dispersal of this microsporidium via infected adults is limited to the time periods of November–December and August. Based on previous life cycle studies for *Amblyospora*, it is likely that the binucleates spores in female adults are responsible for transovarial transmission to the next generation. This restricted production of infected adults would seem to limit the occurrence

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### Table 2

<table>
<thead>
<tr>
<th>Month</th>
<th>Number of plants with infected copepod</th>
<th>No. of infected copepod per plant</th>
<th>Number of spores/montha</th>
</tr>
</thead>
<tbody>
<tr>
<td>January</td>
<td>3</td>
<td>2–8–2</td>
<td>7 (\times 10^4)</td>
</tr>
<tr>
<td>April</td>
<td>2</td>
<td>2–6</td>
<td>5 (\times 10^4)</td>
</tr>
<tr>
<td>July</td>
<td>2</td>
<td>2–1</td>
<td>2 (\times 10^4)</td>
</tr>
<tr>
<td>August</td>
<td>1</td>
<td>3</td>
<td>2 (\times 10^4)</td>
</tr>
<tr>
<td>September</td>
<td>2</td>
<td>5–1</td>
<td>3 (\times 10^4)</td>
</tr>
<tr>
<td>October</td>
<td>3</td>
<td>1–17–2</td>
<td>1 (\times 10^5)</td>
</tr>
<tr>
<td>November</td>
<td>5</td>
<td>2–1–2–2–6</td>
<td>8 (\times 10^4)</td>
</tr>
<tr>
<td>December</td>
<td>3</td>
<td>1–4–14</td>
<td>1 (\times 10^5)</td>
</tr>
<tr>
<td>January</td>
<td>5</td>
<td>5–8–11–4–1</td>
<td>2 (\times 10^5)</td>
</tr>
<tr>
<td>February</td>
<td>7</td>
<td>2–10–2–4–7–4–2</td>
<td>2 (\times 10^5)</td>
</tr>
<tr>
<td>March</td>
<td>6</td>
<td>3–1–1–1–1–1</td>
<td>5 (\times 10^4)</td>
</tr>
<tr>
<td>May</td>
<td>6</td>
<td>1</td>
<td>6 (\times 10^3)</td>
</tr>
<tr>
<td>June</td>
<td>4</td>
<td>1–3–1</td>
<td>4 (\times 10^3)</td>
</tr>
</tbody>
</table>

*a Monthly number of uninucleate spores was calculated from the total number of infected copepods collected (10 plants sampled twice a month).*
of vertically infected larvae with meiospores to these discrete periods. Since larvae infected with meiospores were detected regularly during the year, pathways other than vertical transmission may be responsible for this observation. One such pathway has been identified for *Hyalinocysta chapmani* Hazard and Oldacre (1975) in *Culiseta melanura* where spores produced in a copepod intermediate host infect mosquito larvae with the direct production of meiospores (Andreadis, 2005). This possibility would provide for long term maintenance of *A. camposi* within each plant via repeated horizontal transmission events where infectious uninucleate spores are produced in copepods and infectious meiospores are produced in larvae. In this scenario, the primary role of vertical transmission in *A. camposi* would be to spread the infection to new phytophagous and not crucial for long term maintenance within each plant. Spread of infection could possibly be enhanced by the unique undulating and climbing movement of these mosquito larvae and copepods observed by the authors under laboratory condition. This behavior could permit them to colonize neighboring plants and disperse *A. camposi*. The fate of meiospores and uninucleate spores found in male adults is unclear.

Copepod populations were abundant and omnipresent in *E. cabrerae, P.f. fimbriatus* infected with mature spores and vegetative stages was found on a consistent basis. In laboratory bioassays, Micieli et al. (2000) reported that 10–12 days were required for the production of uninucleate spores in copepods exposed to meiospores under laboratory conditions. The presence of copepods with *A. camposi* vegetative stages was indicative of a recently acquired infection, suggesting that meiospores were ingested 1–9 days before sampling. However, despite the availability of uninucleate spores in the breeding sites, infections via the horizontal pathway to mosquito larvae were detected from only three samples and at a very low level. Previous studies with *A. albifasciati* reported low levels of horizontal transmission, which was apparently due to the low infectivity of copepod spores to mosquito larvae (Micieli et al., 2001). For *A. stimuli*, a low copepod population was believed to be responsible for the low rates of infection and considered to be the principal limiting factor for microsporidian proliferation (Andreadis, 1999). Transmission experiments to first instar mosquito larvae with *A. camposi* spores formed in copepods (concentration of approximately $1 \times 10^3$ spores/ml) produced 6% infection levels in pupa (Micieli et al., 2000). In this study we observed $10^3$ spores/ml per plant in 34% of the bromeliads with infected copepods, while the remaining 66% of plants had $10^2$ spores/ml per plant. Another factor could be the low density of susceptible larval instars at the appropriate time, when spores from copepods were mature and infectious *per os*. Further studies are required to establish if the horizontal pathway from copepods to mosquito larvae will always develop into uninucleate spores in adults and subsequent vertical transmission as other *Amblyospora* species (Andreadis and Hall, 1979; Andreadis, 1985a; Sweeney et al., 1988, 1990; Becnel, 1992) or under some circumstances undergo meiosis and form meiospores (Andreadis, 2005). It could explain the distinct periods of horizontal transmission in copepods and mosquito host observed in this study. In the *A. camposi* system, horizontal transmission may be the predominant maintenance mechanism with vertical transmission via adults occurring at crucial times to distribute the parasite to new plant habitats. The host–parasite relationship of *A. camposi* could be yet another example of how a microsporidium has adapted to the ecological parameters of its hosts and the specialized habitat where they are found in nature to ensure long term survival.

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**References**


