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Eight New Species of Microsporidia (Microspora) from Argentine Mosquitoes (Diptera: Culicidae)

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Seven new species of *Amblyospora* and one of *Parathelohania* are described from larvae of neotropical mosquitoes: *Aedes albifasciatus, Aedes crinifer, Anopheles evansae, Culex dolosus, Culex tramazayguesi, Mansonia indubitans, Psorophora ferox,* and *Uranotaenia nataliae*. This is the first formal description of microsporidia from mosquitoes in Argentina and the first report of an *Amblyospora* sp. from the genus *Uranotaenia*. New species were distinguished on the basis of meiospore morphology and presumed host specificity. The proposed new species are *Amblyospora albifasciati* n. sp., *Amblyospora criniferis* n. sp., *Amblyospora dolosi* n. sp., *Amblyospora ferocis* n. sp., *Amblyospora indubitantis* n. sp., *Amblyospora nataliae* n. sp., *Amblyospora tramazayguesi* n. sp., and *Parathelohania evansae* n. sp. A complete list of all of the described species of *Parathelohania* from mosquitoes is given. © 1994 Academic Press, Inc.

KEY WORDS: Taxonomy; ultrastructure; spore morphology.

INTRODUCTION

The first true microsporidium from mosquitoes was probably discovered in Europe by Hesse (1904) when he described *Parathelohania legeri* (Hesse, 1904) Codreanu, 1966 from larvae of *Anopheles maculipennis* Meigen. Since then, microsporidia have been reported worldwide as being among the most common parasites of mosquitoes (Hazard and Chapman, 1977; Castillo, 1980; Daoust, 1983). However, little information is available on microsporidia from neotropical Culicidae, and none from Argentina has been formally described (Camey-Pacheco, 1968; Hazard and Anthony, 1974; Hazard and Oldacre, 1975; García, 1989; García and López Lastra, 1989; García and Camino, 1990). To fill this void, a survey of mosquito larvae infected with microsporidia was conducted in three Argentina provinces from 1984 to 1991. In this paper, we present ultrastructural descriptions of meiospore morphology from microsporidia found in eight neotropical species of mosquitoes, for which we propose the creation of eight new species. In addition, information on the seasonality and natural prevalence of most of the newly described microsporidia is presented.

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MATERIALS AND METHODS

Mosquito larvae infected with microsporidia were collected in October and November 1984 during a surveillance project for larval pathogens near Formosa city [26.07 S lat.; 58.14 W long.], Formosa province near Mendoza city [32.48 S lat.; 68.52 W long.], Mendoza province, Argentina. Additional infected larvae were found during a 2-year study of the ecology of immature stages of culicids and their natural enemies at the Buenos Aires State Natural Reserve of Punta Lara (34° 54′ 53″ S, 57° 52′ 23″ W), Buenos Aires province, Argentina, from July 1989 to July 1991.

Those larvae exhibiting patent signs of infection were identified according to Lane (1953) and Darsie and Mitchell (1985). Infected larvae from Formosa and Mendoza provinces were identified by Dr. R. F. Darsie, Jr., from The International Center for Public Health Research of the University of South Carolina (McClellanville, SC).

Pieces of live infected larvae were smeared on microscope slides, air dried, fixed in methanol for 3 min and stained 10 min with 10% Giemsa-stain solution buffered at pH 7.41. Other pieces of infected larvae were fixed in 2.5% glutaraldehyde buffered in 0.1 M Na cacodylate (v/v) containing $CaCl_2$ (1 mg/ml). After 30 min, the tissues were cut into smaller pieces and fixed for an additional 2.5 hr at room temperature. Some samples were held for up to 2 weeks in buffer in the cold. The tissues were postfixed in 1% O_sO_4 (w/v) for 2 hr, dehydrated through a graded ethanol series into acetone, and embedded in Epon-Araldite. Sections were poststained in methanolic uranyl acetate followed by Reynold's lead citrate and examined and photographed with

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a Hitachi H-600 electron microscope at 75 kV. Spores (n = 32) were measured with a Vickers split image micrometer. Quantification and arrangement of polar filament coils was made from examination of a minimum of 10 meiospores of each species that were viewed in full sagittal section.

RESULTS

The isolated microsporidia were placed into either *Amblyospora* Hazard and Oldacre, 1975 or *Parathelohania* Codreanu, 1966 based on general features of the sporulation sequence in larval fat body and the ultrastructure of meiospores. The sporulation sequences were similar in each of these species and involved octosporoblastic sporogony accompanied by meiosis and ended with the production of meiospores (octospores). These spores are presumed to be infectious for an intermediate (copepod) host. Ultrastructurally, the spores shared the common features of a single nucleus; a stratified exospore which was generally 2–3 times as thick as the endospore (in the *Amblyospora* spp., the thickest part was often in the anteriolateral region) with the exception of *Parathelohania,* in which these 2 parts of the wall were of about the same thickness; endospore rather similar in all of the species of *Amblyospora* and generally unremarkable with 1 exception (Fig. 5); endospore of the single species of *Parathelohania* conspicuously ornamented at the ends in a manner characteristic of the genus; a polaroplast that was lamellate and bipartite with units of the distal part somewhat expanded; an anisofilar polar filament that constricted abruptly in the distal region; and a large posterior vacuole.

Amblyospora albifasciati n. sp. (Fig. 1, Table 1)

Amblyospora sp. García, 1989, Revta. Soc. Ent. Argent. 47(1–4), 100

Type host. *Aedes (Ochlerotatus) albifasciatus* (Macquart, 1837).

Spore. Living meiospores elongate ovoid, much attenuated anteriorly and slightly attenuated posteriorly when fresh, collapsed when stained, 7.2 ± 0.3 × 4.4 ± 0.4 μm (fresh, n = 32), 6.1 ± 0.9 × 4.2 ± 0.4 μm (fixed, n = 32). Uninucleate with a thick spore wall and large posterior vacuole. Polaroplast lamellate and more tightly arranged in the apical region. Polar filament anisofilar with 4 ½ (range = 4–5) broad proximal and 6 ½ (range = 6–8) narrow distal coils all arranged in a single row.

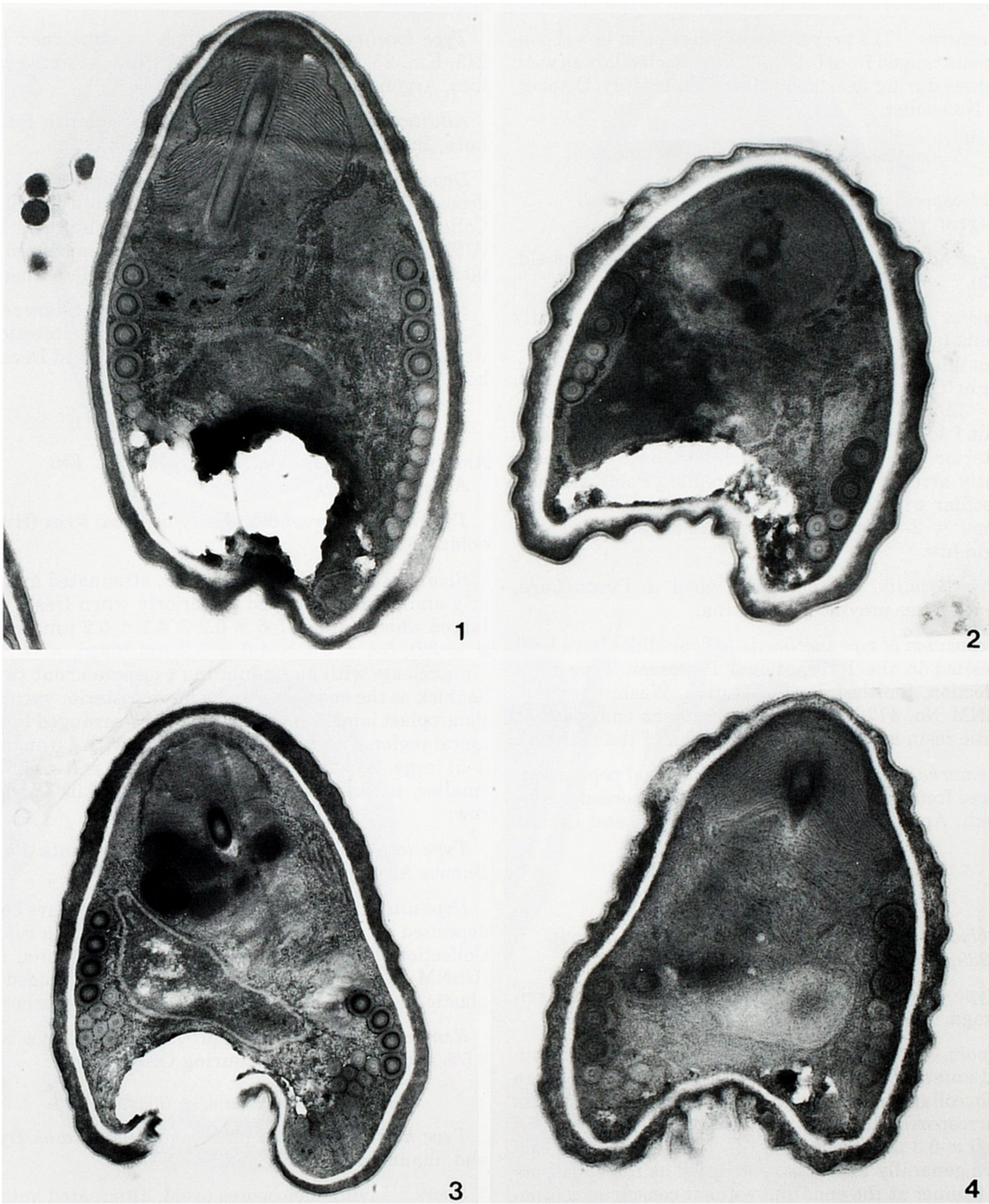
Type locality. A temporary pond in Punta Lara, Buenos Aires province, Argentina.

Deposition of type specimens. Type slides have been deposited in the International Protozoan Type Slide Collection, Smithsonian Institution, Washington, DC

TABLE 1

Comparison of Some Morphological Features for Seven Species of *Amblyospora* from Mosquitoes Collected in Argentina

Species	Mosquito host	Meiospore (μm)	Broad coils	Narrow coils	Arrangement of narrow coils
A. albifasciati	*Aedes albifasciatus*	7.2 ± 0.3 × 4.4 ± 0.4 (Fresh) 6.1 ± 0.9 × 4.2 ± 0.4 (Fixed)	4½	6½	Uniform
A. criniferis	*A. crinifer*	4.6 ± 0.2 × 3.6 ± 0.2 (Fresh) 4.2 ± 0.5 × 3.5 ± 0.2 (Fixed)	2	3	Uniform
A. dolosi	*Culex dolosus*	9.3 ± 0.3 × 5.4 ± 0.3 (Fresh) 8.8 ± 0.4 × 4.9 ± 0.3 (Fixed)	4	6	Irregular
A. ferocis	*Psorophora ferox*	5.6 ± 0.2 × 4.1 ± 0.2 (Fresh) 5.4 ± 0.4 × 4.0 ± 0.2 (Fixed)	4	4	Uniform
A. indubitantis	*Mansonia indubitans*	8.9 ± 0.3 × 7.2 ± 0.4 (Fresh) 8.1 ± 0.5 × 6.8 ± 0.4 (Fixed)	4	4½	Irregular
A. nataliae	*Uranotaenia nataliae*	4.7 ± 0.1 × 3.7 ± 0.1 (Fresh) 4.4 ± 0.4 × 3.5 ± 0.4 (Fixed)	3	4	Uniform
A. tramazayguesi	*C. tramazayguesi*	6.8 ± 0.5 × 5.7 ± 0.3 (Fixed)	3	4	Uniform



FIGS. 1–4. Meiospores of *Amblyospora* spp. from Argentine mosquitoes. (1) *Amblyospora albifasciati*. ×25,500. (2) *Amblyospora criniferis*. ×27,500. (3) *Amblyospora dolosi*. ×16,000. (4) *Amblyospora ferocis*. ×26,000.

(USNM No. 47758-59) Type specimens embedded in plastic resin are also in the collection of the authors.

Remarks. The prevalence of infection in larval populations ranged from 0.15 to 7.7%. Infected larvae were recorded during February, March, June, July, October, and November.

Amblyospora criniferis n. sp. (Fig. 2, Table 1)

Amblyospora sp. García, 1989, Revta. Soc. Ent. Argent. 47(1–4), 100

Type host. *Aedes* (*Ochlerotatus*) *crinifer* (Theobald, 1903).

Spore. Living meiospores short ovoid, only slightly attenuated anteriorly and broadly rounded posteriorly when fresh, collapsed when stained, 4.6 ± 0.2 × 3.6 ± 0.2 μm (fresh, n = 32), 4.2 ± 0.5 × 3.5 ± 0.2 μm (fixed, n = 32). Uninucleate with an undulating exospore about 1 ½ times as thick as the endospore and a large posterior vacuole. Polaroplast lamellate and more tightly arranged in the apical region. Polar filament anisofilar with 2 (range = 1–3) broad proximal and 3 (range = 2–4) narrow distal coils all arranged in a single row.

Type locality. A temporary pond in Punta Lara, Buenos Aires province, Argentina.

Deposition of type specimens. Type slides have been deposited in the International Protozoan Type Slide Collection, Smithsonian Institution, Washington, DC (USNM No. 47760-61). Type specimens embedded in plastic resin are also in the collection of the authors.

Remarks. Natural prevalence in larval populations ranged from 0.6 to 50% with infections recorded during March, April, June, September, October, and December.

Amblyospora dolosi n. sp. (Fig. 3, Table 1)

Amblyospora sp. No. 1 García and López Lastra, 1989, Neotropica 35(93), 9–14

Type host. *Culex* (*Culex*) *dolosus* (Lynch Arribálzaga, 1891).

Spore. Living meiospore ovoid, somewhat attenuated anteriorly and broadly rounded posteriorly when fresh, collapsed when stained, 9.3 ± 0.3 × 5.4 ± 0.3 μm (macrospores 11.6 × 5.8 μm) (fresh, n = 32), 8.8 ± 0.4 × 4.9 ± 0.3 μm (fixed, n = 32). Uninucleate, the exospore generally about twice as thick as the endospore but thinner at the ends and without conspicuous ornamentation and large posterior vacuole. Polaroplast lamellate and more tightly arranged in the apical region. Polar filament anisofilar with 4 (range = 4–6) broad proximal coils arranged in a single row and 6 (range = 5–7) narrow distal coils arranged in an irregular row.

Type locality. A roadside ditch on state road No. 215, Km. 25, Partido de La Plata, Buenos Aires province, Argentina.

Additional localities. A temporary pond in Punta Lara, Buenos Aires province, Argentina.

Deposition of type specimens. Type slides have been deposited in the International Protozoan Type Slide Collection, Smithsonian Institution, Washington, DC (USNM No. 47762-63). Type specimens embedded in plastic resin are also in the collection of the authors.

Remarks. The prevalence of infection in larvae of *C. dolosus* ranged from 0.5 to 17.2% and infections were recorded during October, November, and December.

Amblyospora ferocis n. sp. (Fig. 4, Table 1)

Amblyospora sp. García, 1989, Revta. Soc. Ent. Argent. 47(1–4), 100

Type host. *Psorophora* (*Janthinosoma*) *ferox* (Humboldt, 1820).

Spore. Living meiospores ovoid, attenuated anteriorly and broadly rounded posteriorly when fresh, collapsed when stained, 5.6 ± 0.2 × 4.1 ± 0.2 μm (fresh, n = 32), 5.4 ± 0.4 × 4.0 ± 0.2 μm (fixed, n = 32). Uninucleate with an undulating exospore about twice as thick as the endospore and a large posterior vacuole. Polaroplast lamellate and more tightly arranged in the apical region. Polar filament anisofilar with 4 (range = 3–5) large, broad proximal and 4 (range = 3–5) much smaller, narrow distal coils all arranged in a single row.

Type locality. A temporary pond in Punta Lara, Buenos Aires province, Argentina.

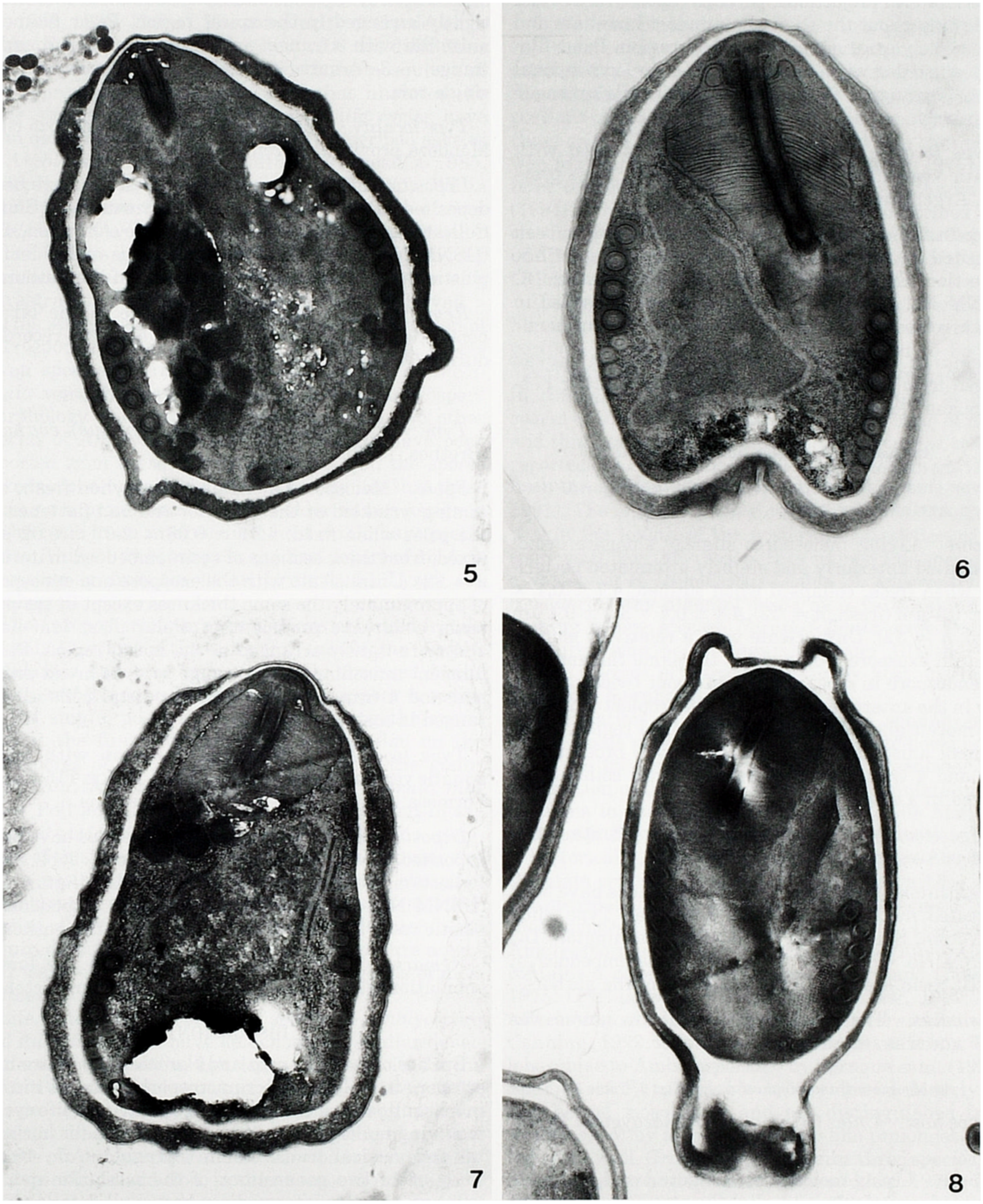
Deposition of type specimens. Type slides have been deposited in the International Protozoan Type Slides Collection, Smithsonian Institution, Washington, DC (USNM No. 47764-65). Type specimens embedded in plastic resin are also in the collection of the authors.

Remarks. The infection prevalence in larva was 4.5% and it was recorded during October.

Amblyospora indubitantis n. sp. (Fig. 5, Table 1)

Type host. *Mansonia* (*Mansonia*) *indubitans* Dyar and Shannon, 1925.

Spore. Living meiospores ovoid, attenuated anteriorly and broadly rounded posteriorly when fresh, collapsed when stained, 8.9 ± 0.3 × 7.2 ± 0.4 μm (fresh, n = 32), 8.1 ± 0.5 × 6.8 ± 0.4 μm (fixed, n = 32). Uninucleate with a slightly undulating exospore about



FIGS. 5–8. Meiospores from Argentine mosquitoes. (5) *Amblyospora indubitantis*. ×16,800. (6) *Amblyospora nataliae*. ×18,000. (7) *Amblyospora tramazayguesi*. ×16,800. (8) *Parathelohania evansae*. ×20,400.

twice as thick as the endospore and distinguished by 2 posteriolateral projections, perhaps representative of a single ring about the spore. Polaroplast lamellate and more tightly arranged in the apical region. Polar filament anisofilar with 4 (range = 3–5) broad proximal and 4 ½ (range = 3–5) narrow distal coils arranged irregularly.

Type locality. A permanent pond covered with aquatic vegetation in Punta Lara, Buenos Aires province, Argentina.

Deposition of type specimens. Type slides have been deposited in the International Protozoan Type Slide Collection, Smithsonian Institution, Washington, DC (USNM No. 47766-67). Type specimens embedded in plastic resin are also in the collection of the authors.

Remarks. The infection prevalence in larvae ranged from 1 to 2.8% and was recorded during February and April.

***Amblyospora nataliae* n. sp. (Fig. 6, Table 1)**

Type host. Uranotaenia (*Uranotaenia*) *nataliae* Lynch Arribálzaga, 1899.

Spore. Living meiospores slightly elongate ovoid, attenuated anteriorly and slightly attenuated posteriorly when fresh, collapsed when stained, $4.7 \pm 0.1 \times 3.7 \pm 0.1$ μm (fresh, $n = 32$), $4.4 \pm 0.4 \times 3.5 \pm 0.4$ μm (fixed, $n = 32$). Uninucleate with a relatively smooth and thin exospore, being about the same thickness as the endospore in most places and about twice as thick only in the anteriolateral region. Polaroplast lamellate and more tightly arranged in the apical region. Polar filament anisofilar with 3 (range = 2–4) broad proximal and 4 (range = 3–5) narrow distal coils all arranged in a single row.

Type locality. A temporary pond in Punta Lara, Buenos Aires province, Argentina.

Deposition of type specimens. Type slides have been deposited in the International Protozoan Type Slide Collection, Smithsonian Institution, Washington, DC (USNM No. 47768-69). Type specimens embedded in plastic resin are also in the collection of the authors.

Remarks. The infection prevalence in larvae was 6.6% and was recorded during October.

***Amblyospora tramazayguesi* n. sp. (Fig. 7, Table 1)**

Type host. Culex (*Allimanta*) *tramazayguesi* Duret, 1954.

Spore. Living meiospores elongate ovoid, much attenuated anteriorly and slightly attenuated posteriorly when fresh, collapsed when stained, $6.8 \pm 0.5 \times 5.7 \pm 0.3$ μm (preserved, $n = 32$). Uninucleate with an undulating exospore about twice as thick as the endospore in most places but conspicuously thicker in the anteriolateral region. Polaroplast lamellate and more tightly arranged in the apical region. Polar filament anisofilar with 3 (range = 3–4) broad proximal and 4 (range = 3–5) narrow distal coils all arranged in a single row.

Type locality. Papagayo stream near Mendoza city, Mendoza province, Argentina.

Deposition of type specimens. Type slides have been deposited in the International Protozoan Type Slides Collection, Smithsonian Institution, Washington, DC (USNM No. 47770-71). Type specimens embedded in plastic resin are also in the collection of the authors.

Remarks. The infection prevalence in the larval population was approximately 10% and was recorded during October.

***Parathelohania evansae* n. sp. (Fig. 8, Table 1)**

Type host. Anopheles (*Nyssorynchus*) *evansae* (Brethes, 1926).

Spore. Meiospores elongate, ovoid when fresh, becoming wrinkled at the posterior end and flattened at the poles when fixed, $4.51 \pm 0.05 \times 2.67 \pm 0.02$ μm (fixed, from thick sections of resin-embedded material, $n = 32$). Uninucleate with the exospore and endospore of approximately the same thickness except in the posterior collar-like prolongation. Polaroplast lamellate and more tightly arranged in the apical region. Polar filament anisofilar with 5 (range = 4–5) broad proximal and 4 (range = 3–4) narrow distal coils all arranged in a single row.

Type locality. A fresh-water lagoon with much aquatic vegetation on National Road 14, km 1158, near Formosa city, Formosa province, Argentina.

Deposition of type specimens. Type slides have been deposited in the International Protozoan Type Slide Collection, Smithsonian Institution, Washington, DC (USNM No. 47772-73). Type specimens embedded in plastic resin are also in the collection of the authors.

Remarks. The infection prevalence in the larval population was 10% and was recorded during October.

DISCUSSION

Species of *Amblyospora* and *Parathelohania* are heterosporous (polymorphic) microsporidia found mostly in mosquitoes and characterized by intricate life cycles involving multiple spore types responsible for horizontal and vertical transmission (Sprague *et al.*, 1992). They affect two generations of the mosquito and involve an obligate intermediate host. Full details of the intricate relationships between heterosporous microsporidia and their hosts have only recently been elucidated (Andreadis, 1985, 1988, 1990; Avery, 1989;

Avery and Undeen 1990a,b; Becnel, 1992; Hazard and Weiser, 1968; Sweeney and Becnel, 1991; Sweeney *et al.*, 1985, 1988, 1990a). The complexity of the development of these parasites together with the difficulties involved with the colonization of both the mosquito and the appropriate intermediate hosts has hindered life cycle studies. To date, the complete life cycles have been determined for only 5 of the 37 described species of *Amblyospora* from mosquitoes (Andreadis, 1985, 1988; Becnel, 1992; Sweeney *et al.*, 1988, 1990a; White *et al.*, 1994) and 2 of the 13 described species of *Parathelohania* (Avery, 1989; Avery and Undeen, 1990a,b). Other species have been distinguished primarily by their occurrence in a new mosquito host together with ultrastructural characters of spores found in larvae.

This study represents the first formal description of microsporidia from mosquitoes in Argentina. These seven species of *Amblyospora* and one of *Parathelohania* were distinguished on the basis of meiospore morphology and presumed host specificity. No other species of *Amblyospora* or *Parathelohania* have been reported from these mosquito species, and the spores were morphologically distinct. We recognize that these names are provisional and that older names assigned to the cycle in an intermediate host would have precedence. This study represents the first report of an *Amblyospora* sp. from the genus *Uranotaenia* Lynch Arribálzaga.

The two posteriolateral projections observed in longitudinal sections of the spore of *A. indubitans* from *M. indubitans* have not been previously observed in the genus *Amblyospora*. These projections appear to represent a single, posterior ring surrounding the spore. This is the first ultrastructural information on the meiospores of an *Amblyospora* spp. from the genus *Mansonia*. Another microsporidium, *Tricornia muhezae* Pell and Canning, 1992, has been found in the mosquito *Mansonia africana* (Theobald). The meiospore of this species is characterized by an anterior rim and a posterior knob-like projection of the exospore. Ultrastructural information on meiospores of other species of microsporidia found infecting *Mansonia* are required to determine whether these ridges are a general feature for microsporidia from this genus of mosquitoes.

Life history studies for other species of *Amblyospora* and *Parathelohania* have demonstrated similar developmental cycles and a high degree of specificity for the definitive mosquito host (Andreadis, 1988, 1989, 1990; Sweeney *et al.*, 1990b; Avery, 1989; Avery and Undeen, 1990a,b). Based on these studies, it appears likely that species of *Amblyospora* and *Parathelohania* are highly host specific. In addition, in the absence of information on the developmental sequence in adult mosquitoes and/or the intermediate host, species of *Amblyospora* can be reliably differentiated through quantitative examination of size and ultrastructural morphologies of the meiospores (Andreadis, 1994; Hazard and Oldacre, 1975; Vávra *et al.*, 1984).

Some taxonomic characters that are especially useful (because they can conveniently be quantified and tabulated) are size of the meiospore, numerical ratio of the coils formed by the broad basal and thinner distal portions of the polar filament, and the arrangement of these coils. The special usefulness of these characters was first reported by Hazard and Oldacre (1975) and later confirmed by Vávra *et al.* (1984) and Andreadis (1994). When compared with all other described species for which this information is available (in Table 2 of Andreadis, 1994), these new species of *Amblyospora* (Table 1) can also be distinguished.

Distinguishing the described species of *Parathelohania* by spore size and the ratio of the thick and thin portions of the polar filament has proved to be less discriminating (Table 2). A species of *Parathelohania* in *Anopheles messeae* Falleroni was found to have spores of the same size with a numerical ratio of thick and thin portions of the polar filament similar to that reported for *Parathelohania illinoisensis* (Kudo, 1921) from *Anopheles punctipenis* (Say) (Pankova *et al.*, 1991). The only morphological difference in the spores was in the length of the posterior constriction of the spore wall. For this reason, they named this species as a variety of *P. illinoisensis* (Table 2), apparently not considering the different hosts as a valid reason for naming a new species. *Parathelohania evansae* from Argentina can be distinguished from all other described species of *Parathelohania* by host specificity and spore morphology (Table 2).

Information on the developmental sequences in adult female mosquitoes and intermediate hosts is not available for these new species from Argentina. The recognition of these new species may stimulate investigations to characterize their complete life histories and verify the generic determinations made here.

Historically, morphology of the meiospore has been the main criterion for establishing genera within Amblyosporidae as well as distinguishing species within these genera (Hazard and Oldacre, 1975). The family Amblyosporidae Hazard and Oldacre, 1975 currently contains 4 genera: *Amblyospora* Hazard and Oldacre, 1975, *Cristulospora* Khodzhaeva and Issi, 1989, *Parathelohania* Codreanu, 1966, and *Tricornia* Pell and Canning, 1992, recently transferred from family Thelohaniidae to Amblyosporidae by Sprague *et al.* (1992). Species of *Parathelohania* are found mostly in anopheline mosquitoes and are distinguished from *Amblyospora* by a posterior collar-like prolongation of the spore wall. *Cristulospora* contains three species, all from mosquito hosts (Khodzhaeva and Issi, 1989). Two different spores were reported for the type species, *C. sherbani* Khodzhaeva and Issi, 1989: one a binucleate spore in adults and the other an "octospore" formed in larvae and distinguished by "appendages in shape of

TABLE 2

Comparison of Some Morphological Characteristics of Described *Parathelohania* Species

Species	Mosquito host	Meiospore (μm)	Broad coils	Narrow coils	Arrangement of narrow coils	References
P. africana	*Anopheles gambiae*	3.7 × 2.3 (Fresh)	2	3–4	Uniform	Hazard and Anthony (1974)
P. anomala	*A. ramsayi*	—	—	—	—	Sen (1941); Hazard and Anthony (1974)
P. anophelis	*A. quadrimaculatus*	4.0–5.5 × 2.5–3.6 (Fresh)	3½	4½	Uniform	Hazard and Weiser (1968); Hazard and Anthony (1974)
P. barra	*Aedes australis*	3.77 ± 0.34 × 2.63 ± 0.28 (Fresh)	—	—	—	Pillai (1968); Hazard and Oldacre (1975)
P. chagrasensis	*Aedeomyia squamippenis*	3.07–3.92 × 2.12–2.44 (Fresh)	2	4	Uniform	Hazard and Oldacre (1975)
P. evansae	*A. evansae*	4.51 ± 0.05 × 2.67 ± 0.02 (Fixed)	5	4	Uniform	Present paper
P. illinoisensis	*A. punctipennis*	4.8–6.0 × 3–4 (Fresh) 4.9 ± 0.05 × 3.2 ± 0.02 (Fixed)	2	4	Uniform	Kudo (1921); Anderson (1968); Hazard and Anthony (1974)
P. illinoisensis var. *messeae*	*A. messeae*	4.8 ± 0.07 × 3.4 ± 0.07	2	4	Uniform	Pankova *et al.* (1991)
P. indica	*A. hyrcanus*	4.0–5.2 × 2.4–2.8 (Preserved)	—	—	—	Kudo (1929); Hazard and Anthony (1974)
P. legeri	*A. maculipennis*	6.0–8.0 × 3.0–4.0 (Fresh)	—	—	—	Hesse (1904); Hazard and Anthony (1974)
P. obesa	*A. crucians*	3.5–5.7 × 2.8–4.2 (Fresh) 4.0–4.5 × 3.0–3.5 (Preserved)	3	4	Uniform	Kudo (1924); Hazard and Weiser (1968) Hazard and Anthony (1974)
P. obscura	*A. varuna*	4.5–5.0 × 3.0–3.5 (Preserved)	—	—	—	Kudo (1929); Hazard and Anthony (1974)
P. octolagenella	*A. pretoriensis*	6.0 × 2.6 (Fresh)	3½	5½	Uniform	Hazard and Anthony (1974)
P. periculosa	*A. franciscanus*	4.7 ± 0.06 × 2.6 ± 0.02 (Fresh) 3.8 ± 0.06 × 2.4 ± 0.04 (Fixed)	—	—	—	Kellen and Wills (1962); Hazard and Anthony (1974)

magnificent plumes on both poles." The genus was distinguished from *Amblyospora* by the presence of these appendages. *Tricornia* is monotypic with the type species, *T. muhezae,* found in the mosquito *M. africana.* Only octospores from larvae that were characterized by modification of the spore wall in the form of 3 horn-like projections, 2 anteriorly and 1 posteriorly, were reported (Pell and Canning, 1992). The authors compared this species to *Parathelohania* but created the new genus based on the morphology of the meiospore.

A discussion of Amblyosporidae would be incomplete without mentioning the recently described species, *Duboscqia dengihilli* Sweeney, Doggett and Piper, 1993 from the mosquito *Anopheles hilli* Woodhill and Lee and the copepod *Apocyclops dengizicus* (Lepeschkin). The development of this microsporidium involves vertical transmission between successive mosquito generations (via a binucleate spore) and horizontal transmission between mosquitoes and copepods (via two type of uninucleate spores) similar to *Amblyospora* and *Parathelohania* (Sweeney *et al.,* 1993). The authors distinguished this species from these two latter genera based on differences related to "sporogony in the larval fat body" which resulted in groups of 16 spores, which is characteristic for the genus *Duboscqia* Perez, 1908 in the family Duboscqiidae. The overall similarities of *D. dengihilli* to species of *Amblyospora* and *Parathelohania* may represent a closer taxonomic relationship. We speculate that the presence of 16 spores in a group may represent a case of parallel evolution in *D. legeri* and *D. dengihilli,* and the latter probably does not belong in the genus *Duboscqia* but rather in a new genus in the family Amblyosporidae.

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