PRELIMINARY REPORT OF MICROSPORIDIA IN SIMULIIDAE LARVAE FROM ARGENTINA

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ABSTRACT. *Amblyospora bracteata, Polydispyrenia simulii,* and 3 other undescribed microsporidia are reported from 11 species of neotropical blackfly larvae collected from streams in Buenos Aires (eastern) and Neuquen (southwestern) provinces of Argentina. Preliminary light and electron microscopy studies indicate that 2 of the undescribed species belong in the family Thelohaniidae and the third species is placed in the family Caudosporidae. Ten species of Argentine blackflies are recorded as new host records for *A. bracteata* and *P. simulii.*

INTRODUCTION

Microsporidia have been recorded as common parasites of blackfly larvae worldwide (Jenkins 1964, Roberts and Strand 1977, Roberts et al. 1981). However, little information is available on species infecting blackfly larvae from Argentina. Marino et al. (1979) found 5 blackfly species from Argentina infected with a microsporidium originally described as *Pleistophora simulii* (Lutz and Splendore 1904) which was subsequently transferred by Canning and Hazard (1982) to their newly created genus *Polydispyrenia.* In the present study we report the presence of *Polydispyrenia simulii* and *Amblyospora bracteata* in several species of blackfly larvae from Argentina and also 3 other microsporidia of uncertain taxonomic status. Two appear to belong in the family Thelohaniidae and the third in the family Caudosporidae. A brief description of the microsporidia found in Argentine blackfly larvae, as well as host identity and localities, is offered in this paper.

MATERIALS AND METHODS

During the early spring of 1981, blackfly larvae were collected in 7 streams from Buenos Aires Province located in eastern Argentina and in 10 mountainous streams from Neuquen Province in southwestern Argentina. Larvae were collected from submerged plants or stones, brought to the laboratory in plastic bags and then processed immediately. Smears of infected larvae on microscope slides were air dried, fixed in 95% methanol and stained with Giemsa's stain buffered at pH 7.41. Wet smears were fixed in aqueous Bouin's fluid and stained with Heidenhain's iron hematoxylin. Giemsa- and Heidenhain-stained spores were measured with a Vicker's image-splitting micrometer at a magnification of 1,000×. Lacto-aceto-orcein (LAO)-stained preparations were used to observe meiotic stages according to the technique of Hazard et al. (1979). For electron microscopy, small pieces of infected tissue were fixed in 2.5% glutaraldehyde buffered in 0.1 M sodium cacodylate for 4 hr at 4°C. After fixation, the tissue was washed twice in 0.1 M sodium cacodylate buffer and stored in Histocon® at 4°C until field work concluded. After one month, the tissue was postfixed in 1% osmium tetroxide for 2 hr, dehydrated through a graded ethanol series into acetone and embedded in Epon-Araldite (Mollenhauer, 1964). Sections were poststained in methanolic uranyl acetate followed by lead citrate (Reynolds 1963). The sections were examined with a Hitachi HS-8 electron microscope at 50 kV.

The heads of infected larvae were dissected and kept in 70% ethanol for host identification at the Museo de Ciencias Naturales, La Plata, Argentina.

RESULTS

Five different microsporidian species were found infecting 11 species of neotropical simuliids from Buenos Aires and Neuquen Provinces. Hosts and localities are listed in Table 1.

Thelohaniidae Species No. 1.: This microsporidium (Fig. 1) was found in larvae of *Gigantodax chilense* and *G. rufidulum* from San Martin de Los Andes, Neuquen Province. Infected larvae were identified by the milky white appearance of the infected adipose tissue.

Small diplokaryotic meronts with deeply stained nuclei were observed in LAO- and Giemsa-stained preparations. Binary division of meronts increased their number; chains with 4 diplokaryotic nuclei were also observed when cytokinesis was delayed. Early diplokaryotic

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Table 1. Summary of microsporidia found infecting Argentine blackflies of Buenos Aires and Neuquen provinces during 1981.

Microsporidium	Host	Locality
Thelohaniidae		
Species no. 1	*Gigantodax chilense*	Neuquen
	G. rufidulum	Neuquen
Species no. 2	*Cnesia dissimilis*	Neuquen
Caudosporidae		
	G. antarcticum	Neuquen
	G. chilense	Neuquen
	G. fulvescens	Neuquen
	G. rufescens	Neuquen
	G. rufidulum	Neuquen
Amblyospora bracteata	*G. bonorinorum*	Neuquen
	G. chilense	Neuquen
	G. fulvescens	Neuquen
	G. rufescens	Neuquen
	G. rufidulum	Neuquen
	Simulium bonaerense	Buenos Aires
	S. rubiginosum	Buenos Aires
	S. wolffhuegeli	Buenos Aires
Polydispyrenia simulii	*S. bonaerense*	Buenos Aires
	S. limay	Neuquen
	S. rubiginosum	Buenos Aires

sporonts were oval with lightly stained nuclei. These sporonts underwent meiosis and then mitosis to produce 8 and often 16 uninucleate spores within a sporophorous vesicle. Ultrastructurally, tubular secretions were observed inside the vesicle. The spore had a thin wall with a rugose exospore surface and contained a polar tube that appeared to be isofilar and uncoiled (Fig. 9). Mature spores fixed and stained with Heidenhain's were spherical, measuring 1.55–1.81 μm.

Thelohaniidae Species No. 2.: This microsporidium (Fig. 2) was observed from one larvae of *Cnesia dissimilis*, collected in San Martin de Los Andes, Neuquen Province. This larva was milky-white in hue due to the heavily infected adipose tissue.

Merogony was not observed because of the advanced state of the infection. Diplokaryotic sporonts underwent a meiosis-like division which was observed in LAO-stained preparations. Electron microscopy revealed bi-, tetra- and octonucleated sporonts within a sporophorous vesicle. Cytokinesis of octonucleate sporonts resulted in the formation of 8 uninucleate sporoblasts within the sporophorous vesicle. Tubular secretions were observed inside the vesicle during early sporogony; however, these secretions gradually disappeared, resulting in a sporophorous vesicle with mature spores almost void of tubular secretions (Fig. 10). Spores had a thin spore wall with a flattened posterior area; the polar tube was isofilar and uncoiled (Fig. 11). Mature fixed and Heidenhain-stained spores were rod-shaped, measuring 3.28–4.22 × 1.12–1.47 μm.

Caudosporidae: This microsporidium (Fig. 3) was found infecting adipose tissue in larvae of 5 species of blackflies collected from San Martin de Los Andes, Neuquen Province (Table 1).

The earliest stages observed were spherical diplokaryotic meronts (Fig. 4), which divided by binary fission (Fig. 5). Round plasmodia with 4 and 8 diplokaryotic nuclei were observed later in sporogony as the result of 3 divisions of the diplokaryotic nuclei. Cytokinesis occurred, forming rosettes containing 8 binucleate sporoblasts. Ultrastructurally, abundant filamentous appendages were observed to be attached to the sporoblasts as well as to the exospore surface of spores. The resulting binucleate spores had a thick endospore and a thin exospore. The polar tube was isofilar, with 16 or 17 coils (Fig. 12). No vesicle was observed around spores. Fixed Heidenhain-stained spores were oval without caudal appendages and measured 8.44–9.22 × 4.13–4.82 μm.

Amblyospora bracteata (Strickland 1913): This species (Fig. 6 and 7) was the most frequently found microsporidium in the survey (Table 1). Sporogonial stages were the most frequently observed because of the advanced state of the infection in larvae. Diplokaryotic sporonts underwent meiosis followed by mitotic division to produce 8 uninucleate spores within a sporophorous vesicle. Fresh spores were oval and had a thick spore wall. Spores stained with Heidenhain's iron hematoxylin were truncate and somewhat invaginated at their posterior end. Preserved spore measurements of *A. bracteata* were host variable and are summarized in Table 2.

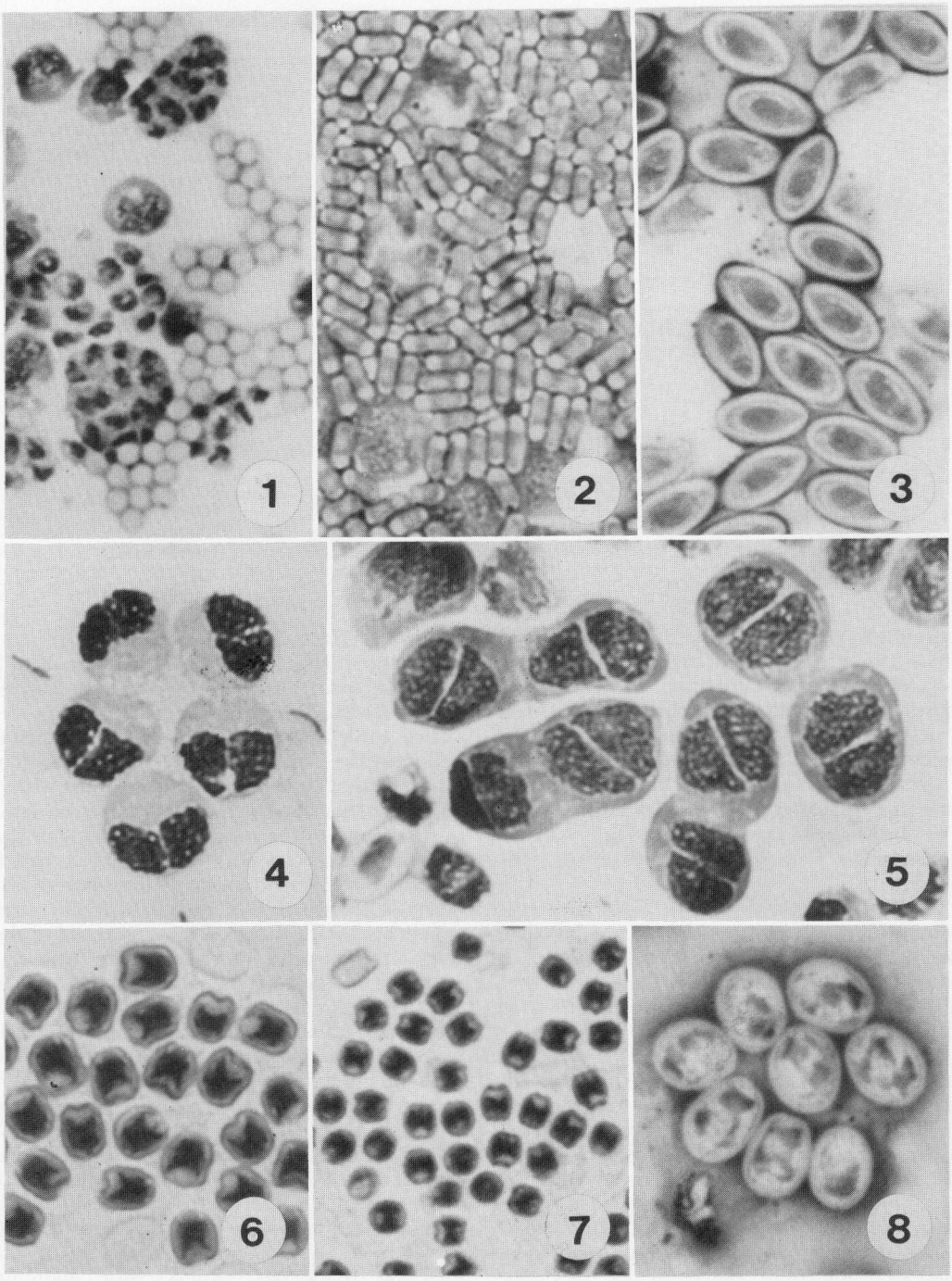

Fig. 1–8. Light micrographs of microsporidia found infecting Argentine blackfly larvae collected during 1981. 2,000×. 1) Sporonts and spores of Thelohaniidae species no. 1 infections in *Gigantodax chilense* and *G. rufidulum*. Giemsa. 2) Spores of Thelohaniidae species no. 2 infection in *Cnesia dissimilis*. Giemsa. 3) Spores of the Caudosporidae microsporidium infection in *Gigantodax fulvescens*. Heidenhain's hematoxylin. 4) Meronts of the Caudosporidae microsporidium. Giemsa. 5) Dividing meronts of the Caudosporidiae microsporidium. Giemsa. 6) Spores of *Amblyospora bracteata* infections in *Simulium rubiginosum*. Heidenhain's hematoxylin. 7) Spores of *Amblyospora bracteata* infections in *Simulium bonaerense*. Heidenhain's hematoxylin. 8) Spores of *Polydispyrenia simulii* in *Simulium limay*. Giemsa.

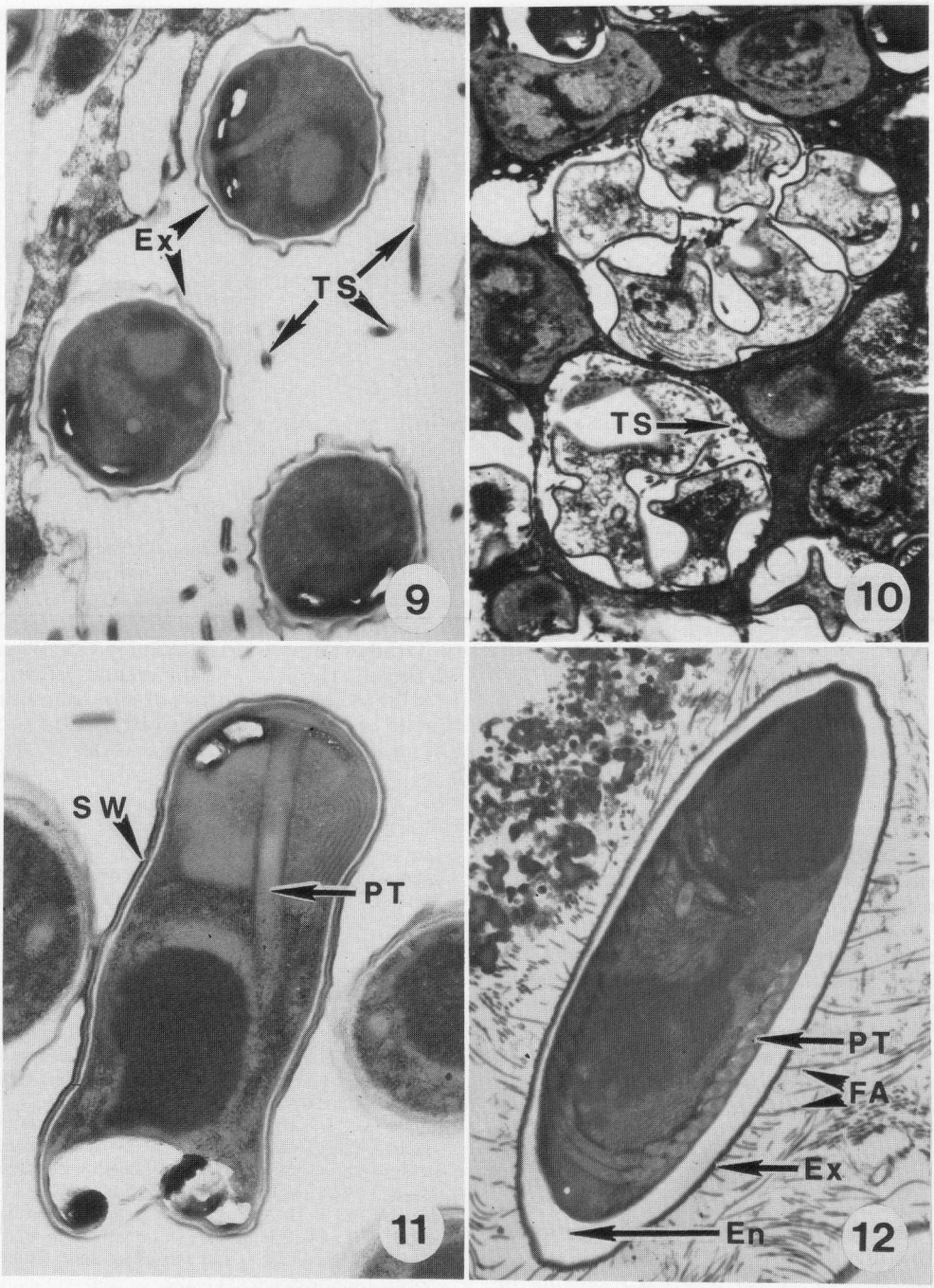

Fig. 9–12. Electron micrographs of microsporidia found infecting Argentine blackfly larvae collected during 1981. 9) Sporophorous vesicle containing spores of Thelohaniidae species no. 1. 20,000×. EX, exospore; TS, tubular secretions. 10) Sporonts of Thelohaniidae species no. 2. 8,500×. TS, tubular secretions. 11) Spore of Thelohaniidae species no. 2. 32,000×. PT, polar tube; SW, spore wall. 12) Spore of the Caudosporidae microsporidium. 15,000×. En, endospore; Ex, exospore; FA, filamentous appendages; PT, polar tube.

Polydispyrenia simulii (Lutz and Splendore 1904): This species was frequently found infecting *Simulium rubiginosum* and *S. bonaerense* larvae in the streams of Buenos Aires Province and larvae of *S. limay* collected in San Martin de Los Andes, Neuquen Province. In LAO-, Giemsa- and Heidenhain-stained preparations, plasmodia were observed with diplokaryotic nu-

Table 2. Measurements (μm)[a] of *Amblyospora bracteata* spores from different species of infected Argentine blackfly larvae.

Host Species	Length		Width	
	Average ± SD	Range	Average ± SD	Range
Gigantodax bonorinorum	3.0 ± 0.1	2.9–3.2	2.7 ± 0.1	2.7–2.8
G. chilense	3.2 ± 0.1	3.1–3.4	2.8 ± 0.1	2.7–2.9
G. fulvescens	3.1 ± 0.1	3.0–3.2	2.8 ± 0.1	2.7–2.9
G. rufescens	2.9 ± 0.1	2.9–3.0	2.7 ± 0.1	2.6–2.8
G. rufidulum	3.4 ± 0.1	3.3–3.5	3.0 ± 0.1	2.8–3.1
Simulium bonaerense	3.1 ± 0.5	2.7–4.2	2.7 ± 0.3	2.4–3.4
S. rubiginosum	4.5 ± 0.5	4.0–5.8	3.8 ± 0.5	3.3–5.2
S. wolffhuegeli	4.1 ± 0.2	3.6–4.4	3.4 ± 0.1	3.1–3.5

[a] n = 10, Heidenhain-stained.

clei which after division produced 64 or 128 uninucleate spores within a sporophorous vesicle. These spores were oval and varied in size according to the host. Giemsa-stained spores from larvae of *S. rubiginosum* and *S. bonaerense* measured 4.25 × 3.31 μm. Giemsa-stained spores from *S. limay* measured 6.46 × 5.16 μm (Fig. 8). The fat body of the larvae was the site of infection for this microsporidium.

DISCUSSION

We placed microsporidian species no. 1 and no. 2 in the family Thelohaniidae because meiosis and 8 uninucleate spores in a sporocyst were observed in their life cycle. The third microsporidium was placed in the family Caudosporidae since the division of diplokaryotic meronts by binary fission resulted in plasmodia with up to 8 diplokaryotic nuclei. Rosette stages were also found in its developmental cycle. These sequences were described in *Caudospora* and *Weiseria* by Doby and Saguez (1964a, 1964b), Doby et al. (1965), Weiser (1947), Jamnback (1970) and Maurand (1975). The binucleate spore was large, oval and did not have caudal appendages or lateral alae as in *Caudospora* species; however, it also did not have ridges subtending a membrane or the bell shape of *Weiseria* species. Since it has not yet been possible to study all stages of these 3 microsporidia, elucidation of host-parasite and taxonomic relationships at the species level is a matter for future studies.

Marino et al. (1979) reported *P. simulii* infecting larvae of *S. perflavum*, *S. romanai*, *S. rubiginosum*, *S. wolffhuegeli* and *Simulium* sp. from Argentina and larvae of *S. annulatum* from Chile. They also mentioned a microsporidium similar to *P. simulii* found in larvae of *Gigantodax* sp. and *S. pertinax* that they did not include under this species because the spores were larger (5.3–2.8 μm) than those mentioned above (4.3–4.8 × 2.0–2.5 μm). Nevertheless, the present findings constitute new host records of *P. simulii* and *A. bracteata* in 10 species of neotropical blackflies from Argentina.

ACKNOWLEDGMENTS

The authors wish to thank Ms. Susan W. Avery for her suggestions and aid in the preparation of this manuscript and Mr. James J. Becnel for his advice and aid with the ultrastructure studies. We are also grateful to Drs. Wayne M. Brooks, Harold C. Chapman and Jeffrey C. Lord for their critical reviews of this manuscript. We are especially indebted to Dr. Sixto Coscarón for identifying the host blackflies.

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