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Light and Electron Microscopic Examination of *Pleistophora Cepedianae* Putz, Hoffman, and Dunbar (Microsporida: Pleistophoridae) from Gizzard Shad, *Dorosoma Cepedianum* LeSueur

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ABSTRACT

Developmental and mature stages of *Pleistophora cepedianae* from gizzard shad, *Dorosoma cepedianum*, were examined by light and electron microscopy. Cysts were found in connective tissue associated with all portions of the digestive tract as well as in the caudal musculature. Schizonts are initially amorphous multinucleate bodies which elongate resulting in nuclei aligning in a straight row. Ultrastructural characteristics of developmental stages differ from those published for *Pleistophora* in lacking membrane lined channels in the amorphous coat of schizonts and formation of sporoblasts from rosette shaped sporogonial plasmodia. Mature spores average 5.0 μm in length and 2.4 μm in width. Polar filaments have 15 to 17 coils aligned in a single row. Both vesicular and lamellar polaroplast regions are present.

INTRODUCTION

The importance of microsporidians as obligate intracellular parasites has been recognized for many years as evidenced by monographs such as Kudo's (1924) Biologic and Taxonomic Study of the Microsporidia and numerous papers dated

before 1900. Unfortunately, the status of many taxa within the group is unclear as evidenced by the shifting of genera and families in the various taxonomic schemes which have been suggested (Kudo, 1924; Sprague, 1977).

Sprague (1977) characterised the genus *Pleistophora* as an inadequately defined genus that will eventually need to be broken up. He also suggested that since the type species is in fish it will probably remain a genus primarily of fish parasites, while many of the species in invertebrates will eventually go into new genera. Canning and Hazard (1982) subsequently examined several species of *Pleistophora* by electron microscopy and concluded the genus consisted of an assemblage of at least three genera, suggesting further ultrastructural examination of species in vertebrates would result in these remaining in *Pleistophora*, while species in invertebrates may need to be shifted to other genera.

Developmental and mature stages of *Pleistophora cepedianae* were examined by light, and transmission and scanning electron, microscopy. The only previous reports of *P. cepedianae* have been by Putz et al. (1965) who described developmental and mature stages by light microscopy and Price (1982) who discussed prevalence of the parasite in gizzard shad of Carlyle Lake, Illinois.

METHODS AND MATERIALS

Gizzard shad were collected by seine from Carlyle Lake, Clinton County, Illinois, from July to September, 1982 and during July, 1983. Fish were pithed and immediately examined for opaque white cysts characteristic of microsporidian infections. Fresh spores were prepared by the paraffin oil method of Vavra (1964) for measurement of spore size.

Cysts prepared for paraffin embedding were preserved in 10% phosphate buffered formalin, washed in distilled water, dehydrated through a graded ethanol series and embedded in paraffin. Eight micrometer sections were stained with either Harris' hematoxylin or Mallory's modified aniline blue which is specific for collagenic connective tissue (Preece, 1965).

Cysts prepared for transmission electron microscopy (TEM) were preserved in 3% glutaraldehyde prepared with 0.1M cacodylate buffer (pH 7.2), post fixed in 2% aqueous osmium tetroxide, washed in distilled water, and dehydrated through a graded ethanol series. Infiltration and embedding were performed in vacuo with either Spurr's low viscosity embedding media (Spurr, 1969) or the medium formula of LR White embedding media by the method of Murphy and Price (1983).

Two micrometer sections of cysts prepared in Spurr's and LR White were obtained on glass knives and stained with 0.1% toluidine blue prepared with 1.0% borax. Sections for TEM were obtained on diamond knives on a Reichert OM-U2 ultramicrotome and stained with 2% aqueous uranyl acetate and Reynold's lead citrate (Reynolds, 1963). Examination was in the Hitachi H500H at 75 kV.

Cysts prepared for scanning electron microscopy (SEM) were preserved as for TEM, critical point dried in a Samdri 790, mounted on aluminum stubs using Scotch 666 double sticky tape and silver conducting paint, and coated with gold palladium in a Hummer V sputter coater. Examination was in a Cambridge Mark IIA at 10 and 20 kV.

Measurement of polar filament angle was performed by the method of Burges et al. (1974) using median longitudinal sections of mature spores.

RESULTS

Cysts were found in connective tissue associated with all portions of the digestive tract as well as the caudal musculature (Fig. 1). By light microscopy spores appeared either slightly tapered at the anterior end or oval. Fresh spores averaged 5.0 μm in length and 2.4 μm in width. Little information concerning internal organelles was visible in fresh and stained smears due to small spore size and the chitinous endospore. Pansporablasts contain more than 16, and usually more than 30, developing spores. Pansporoblast size varied with number of spores developing within.

Two micrometer sections of young cysts show a thin cyst wall with a developmental zone of schizonts, sporonts, and pansporoblasts immediately interior (Fig. 2). Sporoblasts separate from a rosette shaped sporogonial plasmodium within a pansporoblast membrane (Fig. 3). Greater than 20 spores were frequently seen in a 2 μm section of a pansporoblast (Fig. 4). Width of the developmental zone decreases as cysts matured until finally spores lined the cyst wall (Fig. 5). Host fibroblasts line the thin cyst wall in young cysts (Fig. 2) and are replaced by a wide zone of collagen fibers in mature cysts (Fig. 5).

Transmission electron micrographs through the wall of a young cyst shows a narrow zone of host cell organelles with numerous mitochondria and fragments of host nuclei. Inside the zone of host organelles are developmental stages of the parasite (Fig. 6).

Schizonts are first seen as amorphous multinucleate bodies. Mitotic spindles in dividing nuclei were occasionally seen. Zones of endoplasmic reticulum were usually adjacent to nuclei (Fig. 7). As schizonts elongate nuclei arrange in a straight row and the limiting membrane becomes thicker in preparation of pansporoblast membrane formation (Fig. 8). As many as twelve nuclei were observed in a thin section.

As the schizont wall thickens vesicles form (Fig. 9) which continue expanding to form the pansporoblast membrane (Fig. 10). As the pansporoblast membrane develops schizonts divide to form sporonts. Sporogonial plasmodia form rosette shaped structures (Fig. 11) from which sporoblasts divide. Sporoblasts retained within a pansporoblast membrane elongate and nuclei migrate to one end. Endoplasmic reticulum orients along the length of the sporoblast (Fig. 12).

Polar filament formation is closely associated with structures Vavra (1965) identifies as "primitive" Golgi apparati (Fig. 13). The filament is formed in several segments which coalesce and align along the inside periphery of the late sporoblast. The endospore and exospore are both well formed before the filament completes alignment and polaroplast areas are differentiated (Fig. 14).

Mature spores have 15 to 17 coils in the polar filament which are aligned in a single straight row along the inside periphery of the spore (Fig. 15). Angle of anterior coils averages 80° while that of posterior coils averages 73.5°. The manubrium of the polar filament courses through the vesicular and lamellar polaroplast ending in a terminal or slightly subterminal anchoring disc (Fig. 16). Higher magnifications of filament coils show they consist of six to seven concentric electron dense and electron transparent rings (Fig. 17). The posterior vacuole occupies up to one half of the posterior spore and contains dense flocculent material. Spore walls consist of an exospore 32.4A in thickness and endospore 48.6A thick except over the anchoring disc where it thins to 14A (Fig. 15).

Spores remain encased in pansporoblast membranes until mature. Spore surfaces are smooth with no apparent ornamentation (Fig. 18).

DISCUSSION

Importance of electron microscopy in the taxonomy of the genus *Pleistophora* has taken on added significance with the attempt by Canning and Hazard (1982) to split the genus into at least three genera, *Pleistophora*, *Vavraia*, and *Polydispyrenia*. *Polydispyrenia* can easily be distinguished from *Pleistophora* and *Vavraia* by diplokarya in young sporonts and formation of the sporophorous vesicle wall (pansporoblast membrane) at the onset of sporogony. Sporophorous vesicles in both *Pleistophora* and *Vavraia* begin formation during schizogony and are derived from amorphous secretions laid down external to the plasmalemma. In *Pleistophora* the amorphous material is permeated by membrane lined channels which are absent in *Vavraia*.

Sporulation in *Pleistophora* is polysporous with stepwise division through multinucleate segments into many uninucleate sporoblasts. Both microspores and macrospores are found, although macrospores are rare. Sporulation in *Vavraia* is by multiple fission of a rosette formation resulting in uninucleate sporoblasts. Only microspores are found.

Lack of membrane lined channels in the amorphous coat of schizonts, and formation of sporoblasts by multiple fission of a rosette, may indicate *P. cepedianae* should be shifted to the genus *Vavraia*. However, *Pleistophora* sp. from the stoneroller, *Campostoma anomalum*, also lacks membrane lined channels in the amorphous coat of schizonts, but does form sporoblasts by stepwise division through multinucleate segments (Price, 1983). I hesitate to transfer *P. cepedianae* to *Vavraia* since it does occur in a vertebrate host. After ultrastructural examination and interpretation of developmental stages from several more species of *Pleistophora* in both vertebrates and invertebrates a clearer distinction of genera may be possible.

LITERATURE CITED

Burges, H.D., E.U. Canning, and I.K. Hulls. 1974. Ultrastructure of *Nosema oryzaephili* and the taxonomic value of the polar filament. J. Invert. Pathol. 23:135-139.

Canning, E.U., and E.I. Hazard. 1982. Genus *Pleistophora* Gurley, 1893: An assemblage of at least three genera. J. Protozool. 29:39-49.

Kudo, R.R. 1924. A Biologic and Taxonomic Study of the Microsporidia. Ill. Biol. Monogr. 9:1-268.

Murphy, J.A., and R. L. Price. 1983. Use of LR White embedding medium for routine transmission electron microscopy. 41st Ann. Proc. EMSA G.W. Bailey (ed). San Francisco Press, Inc. San Francisco, CA. p. 624-625.

Preece, A. 1965. A Manual for Histological Technicians. Little Brown and Company. Boston. 287 p.

Price, R.L. 1982. Incidence of *Pleistophora cepedianae* (Microsporida) in gizzard shad (*Dorosoma cepedianum*) of Carlyle Lake, Illinois. J. Parasitol. 68:1167-1168.

Price, R.L. 1983. Light and electron microscopic examination of *Pleistophora cepedianae* and *Pleistophora campostomae* n. sp. (Microsporida: Pleistophoridae) from fishes of southern Illinois. Ph.D. Dissertation, Southern Illinois University at Carbondale, Carbondale, Illinois. 107 p.

Putz, R.E., G.L. Hoffman, and C.E. Dunbar. 1965. Two new species of *Pleistophora* (Microsporidea) from North American fish with a synopsis of Microsporidea of freshwater and euryhaline fishes. J. Protozool. 12:228-236.

Reynolds, E.S. 1963. The use of lead citrate at high pH as an electron opaque stain in electron microscopy. J. Cell Biol. 17:208-212.

Sprague, V. 1977. Annotated list of species of Microsporidia. In: Comparative Pathobiology Volume 2. Systematics of the Microsporidia. Bulla, L.A. and T.C. Cheng (eds). Plenum Press. New York. 501 p.

Spurr, A.R. 1969. A low-viscosity epoxy resin embedding medium for electron microscopy. J. Ultrastr. Res. 26:31-43.

Vavra, J. 1964. Recording microsporidian spores. J. Insect Pathol. 6:258-260.
Vavra, J. 1965. Étude au microscope electronique de la morphologie du developpement de quelques Microsporidies. C.R. Acad. Sci. 261:3467-3470.

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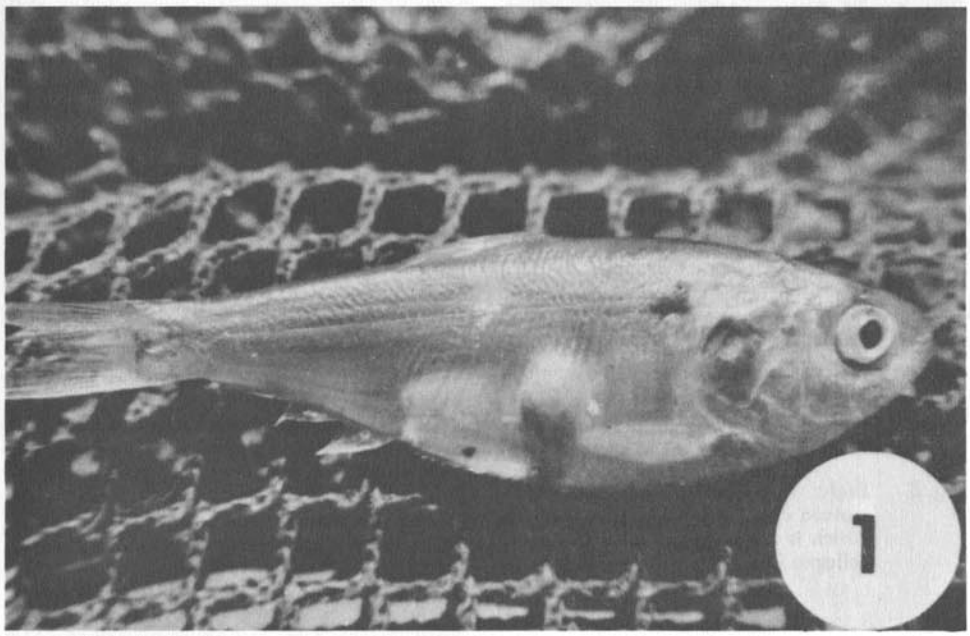
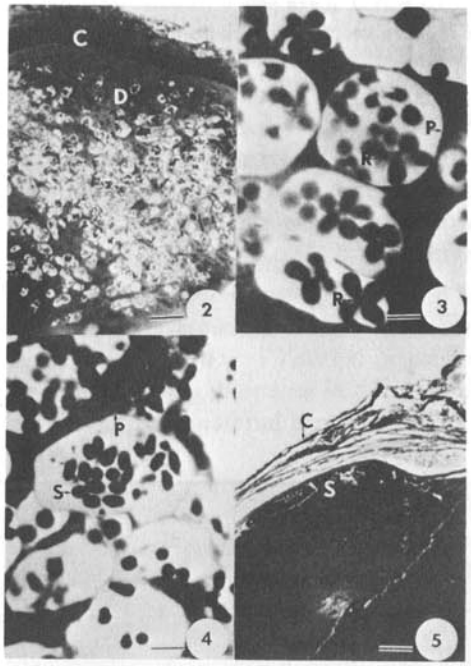


Fig. 1. Gizzard shad, *Dorosoma cepedianum*, with large *Pleistophora cepedianae* cyst protruding from the body cavity.



Figs. 2-4. Two micrometer sections through developing *Pleistophora cepedianae* cyst. 2. Section through thin cyst wall (C) and zone of developing parasites (D). Scale = 50 μm. 3. Pansporoblasts (P) containing rosettes (R) of developing sporonts. Scale = 8 μm. 4. Pansporoblast (P) with at least 20 spores. Scale = 8 μm.

Fig. 5. Eight micrometer section through mature cyst. Spores (S) line cyst wall which is composed of a thick zone of collagen fibers (C). Scale = 50 μm.

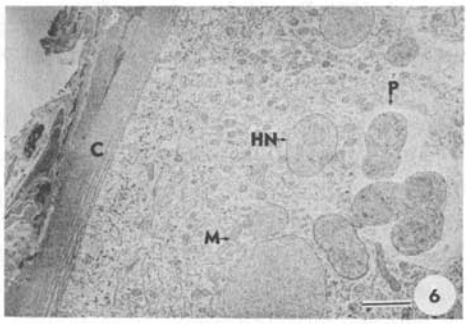
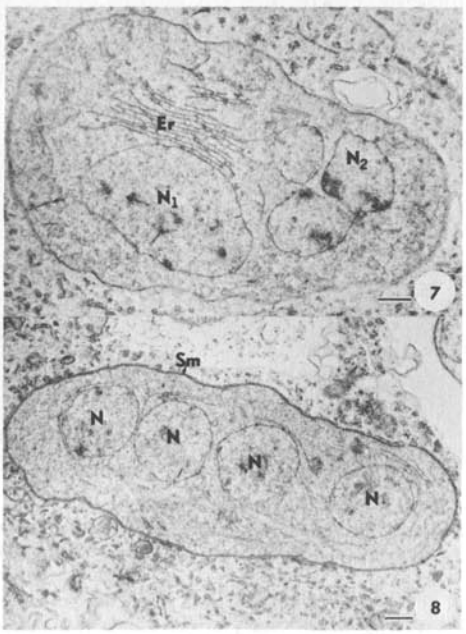


Fig. 6. Transmission electron micrograph through cyst wall of developing *Pleistophora cepedianae* cyst. Inside cyst wall (C) is zone of host cell organelles, including numerous mitochondria (M) and nuclei (HN). Early developmental stages of the parasite (P) are inside the zone of host organelles. Scale = 5 μm.



Figs. 7-8. Transmission electron micrographs of *Pleistophora cepedianae* schizonts. 7. Amorphous multinucleate schizont with dividing nucleus (N_2) and endoplasmic reticulum (Er) closely associated with nuclei. Scale = 1 μm. 8. Elongate schizont with nuclei (N) in a straight row. The schizont membrane (Sm) is thicker than that seen in Figure 7 in preparation of pansporoblast membrane formation. Scale = 1 μm.

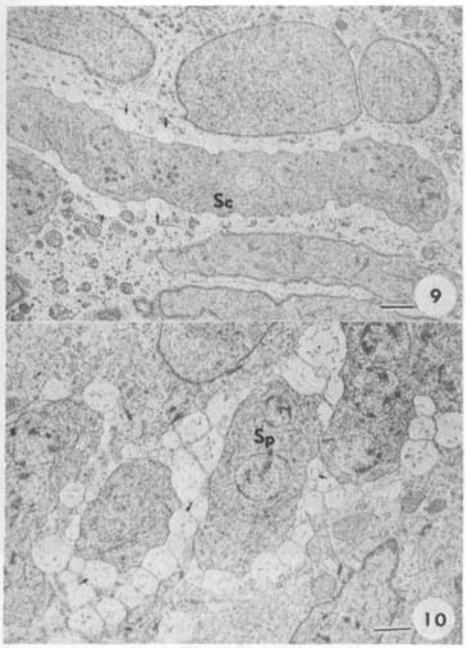


Fig. 9. Wall of schizont (Sc) forming vesicles (arrows) initiating pansporoblast membrane formation. Scale = 3 μm.

Fig. 10. Vesicles (arrows) have expanded and schizont has divided to form sporonts (Sp). Scale = 3 μm.

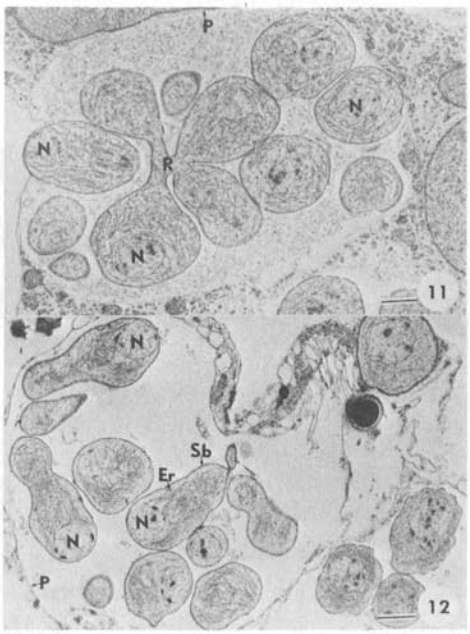
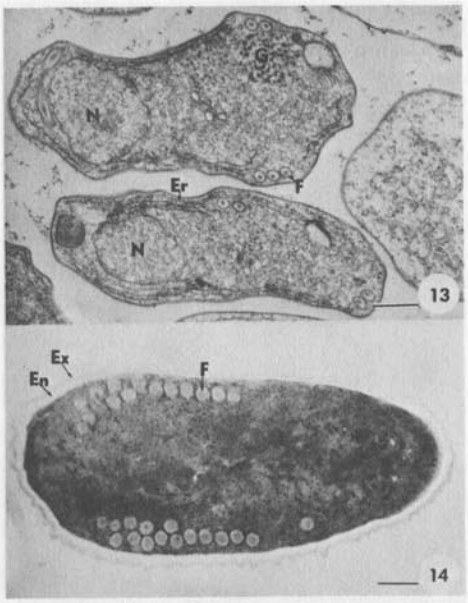
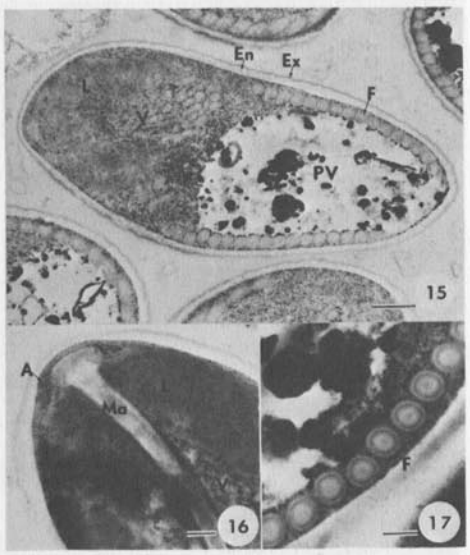


Fig. 11. Rosette shaped sporogonial plasmodium (R) dividing to form unincleate (N) sporoblasts within pansporoblast membrane (P). Scale = 3 μm.

Fig. 12. Elongate sporoblasts (Sb) with nuclei (N) at one end and endoplasmic reticulum oriented along length. Scale = 3 μm.



Figs. 13-14. Late sporoblasts of *Pleistophora cepedianae*. 13. Development of polar filament in close association with "primitive" Golgi apparatus (G). Scale = 1 μm. 14. Late sporoblast with polar filament (F) aligning around periphery as endospore (En) and exospore (Ex) develop. Scale = 0.5 μm.



Figs. 15-17. Mature *Pleistophora cepedianae* spores. 15. Section through spore showing arrangement of lamellar (L) and vesicular (V) polaroplast regions, posterior vacuole (PV), polar filament (F), and spore wall (Ex, En). Scale = 0.5 μm. 16. Anterior end of spore showing arrangement of anchoring disc (A), manubrium of polar filament (Ma), and lamellar and vesicular polaroplast regions. Scale = 0.05 μm. 17. High magnification illustrating 6 to 7 concentric rings of polar filament. Scale = 0.05 μm.

Fig. 18. Scanning electron micrograph of mature *Pleistophora cepedianae* spores encased in pansporoblast membranes. Scale = 5 μm.