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MÉMOIRES ORIGINAUX

Microsporidian infection in the cyst wall of Trematode metacercariae encysted in fish

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Résumé.

Infection microsporidienne de la paroi kystique de métacercaires de Trématodes enkystées chez des poissons.

L'infection microsporidienne de *Liza ramada* (Risso), Mugilidae, pêché dans la lagune « Bardawill » sur la côte méditerranéenne du Sinaï, se trouve située dans les fibroblastes de la capsule métacercarienne d'*Heterophyes heterophyes* (Siebold). L'infection du kyste métacercarien a pour résultat l'hypertrophie de la paroi du kyste et la dégénérescence ou éventuellement la mort de la métacercaire enkystée.

Summary.

Microsporidian infection is reported in *Liza ramada* (Risso), Mugilidae, from Bardawil Lagoon, Mediterranean coast of Sinai, in the fibroblasts of the metacercarial capsule of *Heterophyes heterophyes* (Siebold). Infection of the metacercarial cyst resulted in an hypertrophy of the cyst wall and degeneration and eventual death of the encapsulated metacercaria.

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Introduction

Grey mullets (Mugilidae) in the hypersaline Bardawil Lagoon on the mediterranean coast of the Sinai Peninsula were found to be heavily infected with heterophyiid metacercariae, predominantly *Heterophyes heterophyes* (Siebold). In these fish, infection prevalence is maintained at a 90-100 % level with an infection load as high as 200-6000 metacercariae per 1 gram of muscle (Paperna, 1975). The present communication reports the findings of microsporidian infection in the metacercarial cyst of *H. heterophyes* recovered from muscles of *Liza ramada* (Risso), from Bardawil Lagoon.

Materials and methods

Only post mortem material was available for histology and electron microscopy, fish were obtained from landings of commercial fishing. Cysts and microsporidian spores were studied by light microscope from preparations of fresh material in saline and of material fixed in 4 % formalin and then embedded in glycerine gelatine. Muscles containing metacercariae were fixed in buffered neutral formol for histology. Sections of paraffin embeded tissue (6-7 μm thick) were stained in Harris' hematoxylin eosin and Mallory's method for collagen. Isolated cysts were fixed in 2 % glutaraldehyde in cacodylate or phosphate buffer, 0.1M, pH 7.4 for twelve hours at room temperature for electron microscopy. Following extensive buffer washing, post fixation was in 1 % OsO_4 for an hour at room temperature. After enbloc staining in aqueous uranil acetate and dehydration in ascending alcohols, tissue was embeded in Spurr's Epon. Thin sections were cut on glass with an LKB ultratome, stained on grid with lead citrate and examined in an AEI 6B electron microscope. Sections from Spurr's Epon stained in methylene blue were used for light microscopy.

PLANCHE I

FIG. 1. — Infected (M) and uninfected (N) metacercariae of *H. heterophyes* in fresh muscle impression, × 25.

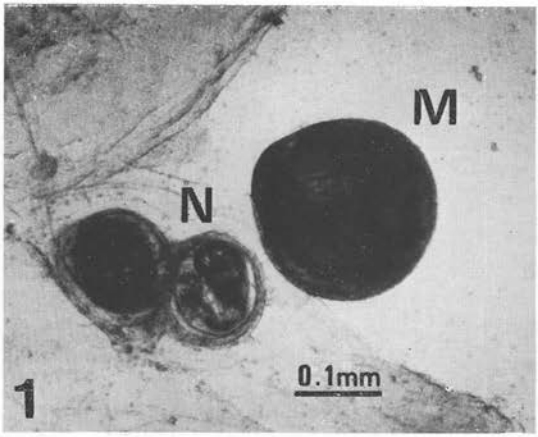
FIG 2. — Cross sections of two infected metacercaria. A. Earlier stage of infection with the trematode (T) still alife. B. Final stage when microsporidian spore fill the entire volume of the cyst. 1 μm section from cyst embedded in Spurr's Epon, methylene blue stain, × 160.

FIG. 3. — Infected metacercaria in freh muscle impression: M. Zone containing the microsporidian spores, T. Active larval trematode. × 160.

FIG. 4. — Uninfected metacercaria in fresh muscle impression. × 160 (same scale as fig. 3).

FIG. 5. — Microsporidian spores isolated in saline, × 1000.

FIG. 6. — Spores with extruded polar filament, after treatment with 1 % KOH, × 1000.


M
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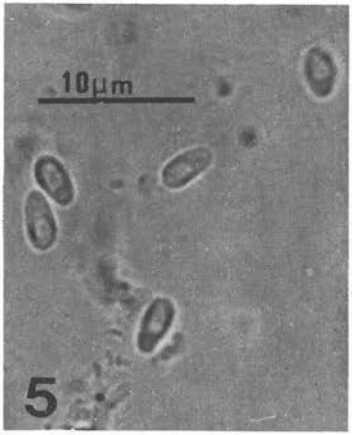
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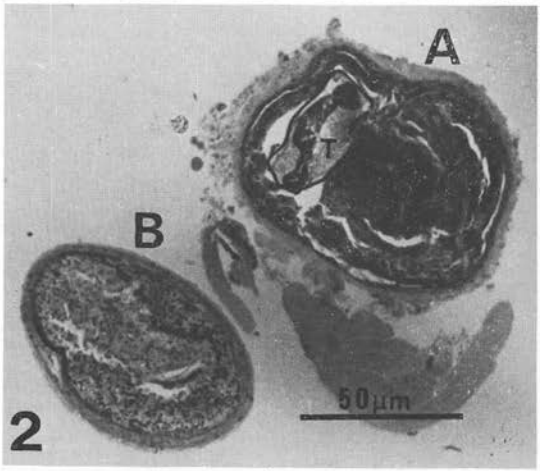
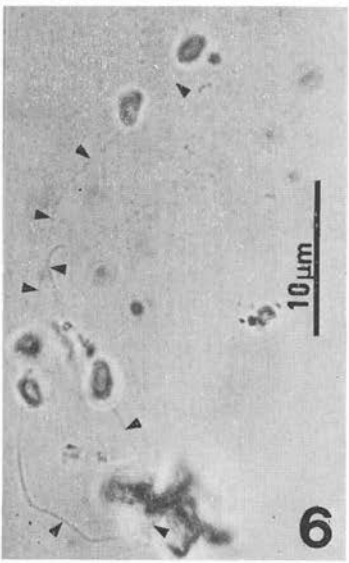

A
T
B
50µm
2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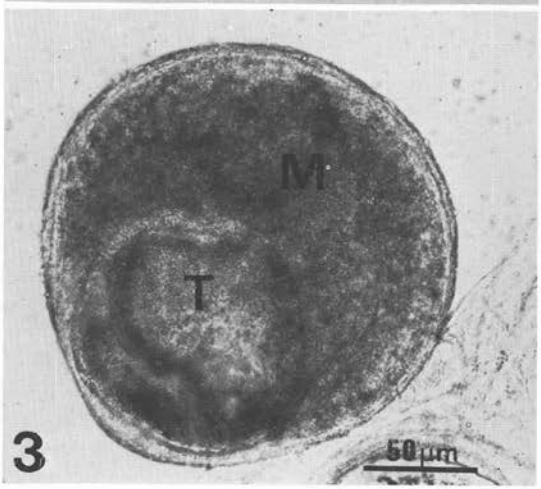
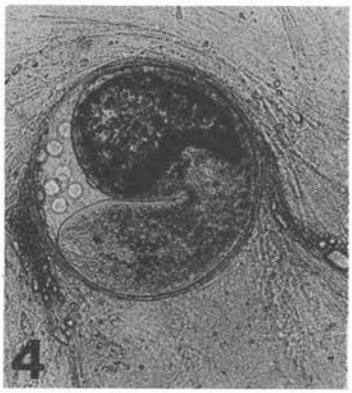
6


M
T
50µm
3


4

Results

The infected metacercaria.

Infected cysts of *H. heterophyes* were larger, with diameter of 0.16-0.22 mm ($\bar{x}$ = 0.19, SD = 0.02, n = 10), than uninfected cysts, whose diameter ranged from 0.10-0.15 mm ($\bar{x}$ = 0.13, SD = 0.04, n = 10), (*fig. 1*). While the active cyst was transparent, the infected cyst appeard heavily granulated (*fig. 3, 4*). The host cells for the microsporidians were the fibroblasts of the cyst enclosing the metacercaria, which become infected while the encysted worm is alive (*fig. 2, A*). As infection in the cyst wall develops, the cellular layer undergoes hypertrophic changes, as a result, the cyst gradually increase in size. The process continues until the volume of the cyst is filled with microsporidian infected cells, while the metacercaria dies and disintegrates (*fig.* 2, *B*). No microsporidian spores were observed within the tissues of the encysted trematode (*fig. 2, A; 10*).

The microsporidian spore.

LIGHT MICROSCOPY : The microsporidian spores were pyriform circular in cross section, refractile in an unstained state (*fig. 5*). The spore's mean lengh was 3.7 µm (SD = 0.39, n = 15) and width 1.70 µm (SD = 0.13, n = 10). The posterior vacuole was distinct, 1.99 µm (SD = 0.58, n = 5) in length ; anterior vacuole was not always distinct. The site of the polar filament coiles may be seen as a distinct dark spot on both sides of the spore margin at the mid posterior vacuole level. The coiles of the polar filament however, could not be differentiated. The extruded polar filament reach the length of 250 µm (*fig.. 6*).

ELECTRON MICROSCOPY : Preparation of isolated cysts for electron microscopy studies yielded unsatisfactory results, due to the inadequate penetration of the fixatives through the fibrous layers of he infected metacercaria. Attemts to obtain new material for ultrastructure studies have been unsuccessful. Therefore the available photomicrographs could not yield all the necessary ultrastructural details for a complete description of the spore (*fig. 11*).

PLANCHE II

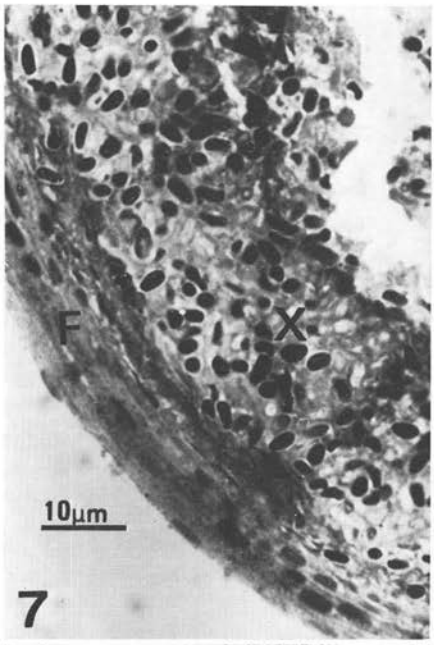
FIG. 7. — Section through the cyst wall of infected metacercaria, 1 µm section from Spurr's Epon embeded cyst. F. Uninfected external fibrous layer. ×. Infected inner layer. Methylene blue stain, × 1000.

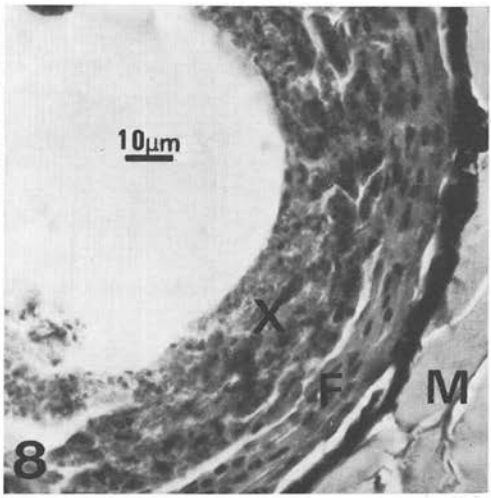
FIG. 8. — Section through the cyst wall of infected metacercaria, 6 µm section from paraffin embedded tissue. M. Host fish muscle. Harris' Hematoxylin eosin, × 1000.

FIG. 9. — Section through the cyst wall of uninfected metacercaria, 6 µm section from paraffin embedded tissue, B. Fibroblast envelope. Harris' hematoxylin eosin. × 1000.

FIG. 10. — Cross section through infected metacercariae still containing live trematode (T). L. A-cellular inner envelope. 1 µm sections from Spurr's Epon block. Methylene blue stain, × 450.

FIG. 11. — EM photomicrograph of the microsporidian spore: e-endospore, f-polar filament. m-plasmalemma. n-nuclear zone. p-polar cap. pa-polaroplast. pp-polaroplast-like structure accompaning the polar filament coils. ×-exospore. × 7500.


F
X
10µm
7


10µm
X
F
M
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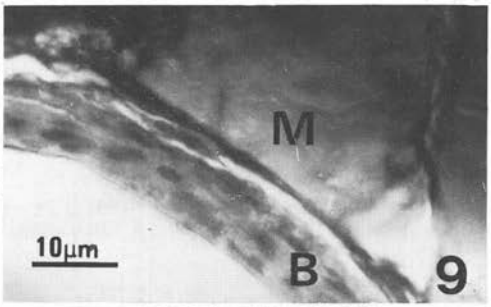
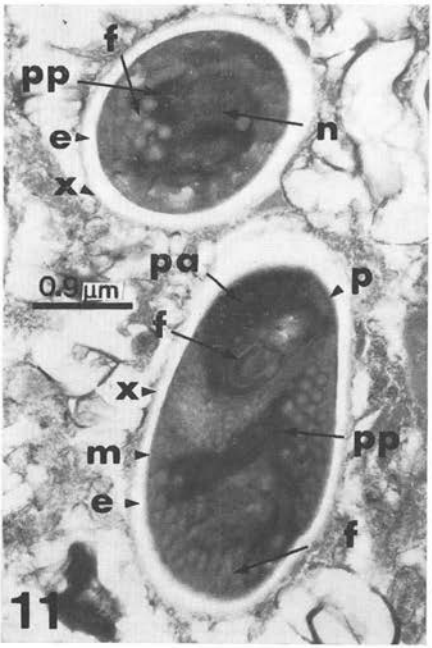
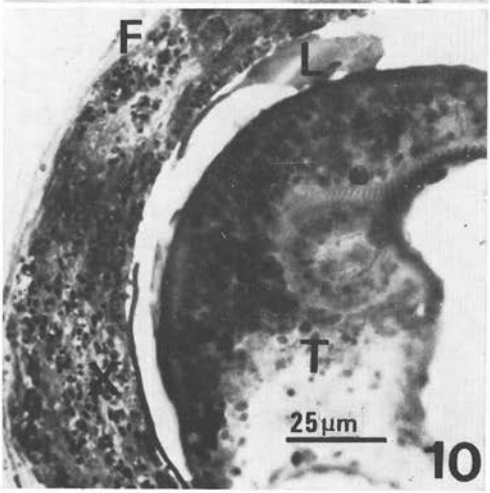

M
10µm
B
9


f
pp
e
n
x
pa
p
0,9µm
f
x
m
pp
e
f
11


F
L
T
25µm
10

The spore coat composed of thin, laminated electron dense exospore, less than 0.05 µm thick and an electron lucent endospore, 0.12-0.17 µm thick. The electron dense inner layer as described by Sinden and Canning (1974), if present was indistinguishable from the plasmalemma. Coiles of the polar filament numbered seventeen to nineteen. A polar cap was observed at the apical end of the polar filament. The apical part of the polar filament as well as the coiles were surrounded by a large electron dense zone of fine laminated configuration similar to that of the polaroplast complex described in other forms. This laminated structure which accompanies the coiles of the polar filament could not be identified with any of the structures previously described from other microsporidians to occupy a similar location in the spore. In this are included the « Cavum » described by Sprague *et al.* (1968) and the « filament forming substance » described by Canning and Sinden (1973). A nuclear zone was observed between the coiles of the polar filament, however, structural details were indifferentiable. Outlines of structures comparable to endoplasmatic reticulum were observed in sections of some spores. However, the above discussed laminated structure might well be part of the endoplasmatic reticulum complex.

TAXONOMY : The taxonomic position of this microsporidian is inconclusive as our material was inadequate for detailed ultrastructural study of the spores. Moreover only mature microsporidian spores were seen in the material studied. The microsporidian cysts within the fibroblasts contained over 20 spores, without any evidence of internal limiting membranes between the spores. We propose therefore to tentatively include these microsporidians (according to Putz, *et al.*, 1965, taxonomic system) in the genus *Pleistopora* Gurley, 1893.

Histological changes in the infected cyst wall.

The cellular capsule of uninfected metacercaria in cross section was less than 10 µm in thickness, consisting predominantly of fibroblasts with rounded or oval nuclei and a few flat elongated fibrocytes with elongated nuclei (*fig. 9*). This capsule stained red to orange with residual blue in mallory's method (e.g. contained only residual amount of collagen). The inner a-cellular envelop of the cyst stained blue in Mallory's stain.

In the infected cysts the cellular capsule may reach a thickness of 50 µm with the metacercaria present and will eventually fill the entire volume of the cyst (*fig. 2, 8, 10*). Only the cells of the inner layer of the cellular capsule were infected with microsporidians while the cells of the outer layer remained intact. This layer, 10-15 µm thick, consisted of a dense envelope of concentricly arranged fibrocysts with compressed nuclei, and stained blue in Mallory's stain, e.g. rich in collagen. The inner layer was loose and consisted entirely of infected fibroblasts containing the parasite spores (*fig. 7, 8*). This layer of infected cells stained red to orange in Mallory's stain. The metacercarial innermost a-cellular envelop remained intact while the metacercaria existed (*fig. 10*), but disappeared as the metacercaria was destroyed.

Occurrence and prevalence of infection.

Throughout the years 1973-1977, grey mullets, *Mugil cephalus, L. ramada* and *L. aurata* as well as the sea basses *Dicentrarchus labrax* and *D. punctatus* were routinely examined bi-tri monthly for heterophyasis. Though *H. heterophyes* infection was very high throughout the year in all fish, cysts infected with microsporidia were found only in *L. ramada* and only in fish caught during December, 1975 and February, 1976. Microsporidian spores in these fish were found only within the metacercarial cysts and never in any other tissue in the fish. Thus far, it has been observed in metacercarial cysts of no other trematode but *H. heterophyes.*

In February samples, infected cysts were found in four out of seventeen examined fish, the ratio of infected to non infected cysts ranged in these fish from one to eight per 100. Prevalence of infection was much lower in December, being limited to one fish out of 13 examined, with ratio of infected to non infected cysts of one to 300.

Discussion

The presently described microsporidian infection demonstrates a high degree of host cell specifity, as it is exclusive to the cells of the metacercarial cyst. Though the fish is the host of this parasite, it is rather the encysted trematode in the infected cyst which is implicated in the consequences of this infection. This microsporidian infection results in the eventual elimination of the encysted metacercaria without it being directly involved in the infection process. The possible cause for the degeneration of the infected metacercaria is the apparent pressure effect of the hypertrophing infected cells of the cyst wall. Furthermore the formation of a thick fibrous layer on the outer surface of the metacercarial cyst could reduce the metabolic exchange or oxygen uptake (Stunkard, 1930, Hunter and Verenberg, 1955) of the encysted trematode.

This host-parasite system differs entierly from previously described microsporidian or haplosporidian infections of metacercariae where the infection is established in the trematode rather than in the fish host (Martin, 1936, Cort *et al.* 1960, Stanier *et al.* 1968 and Perkins, 1971). In spite of the biological implication of this interesting host-parasite interaction, we doubt if this microsporidian infection has any significant role in the population dynamics of *H. heterophyes.* In the presently studied system the pressure of heterophyiid infection on the fish population is very high, while the prevalence of the microsporidian infection is too low to have any significant effet on the survival rate of the encysted metacercariae.

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