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NOSEMA AEGYPTI N. SP. (MICROSPORIDA) HYPERPARASITE OF *PROCAMALLANUS ELATENSIS*, AN INTESTINAL NEMATODE OF SIGANID FISHES FROM THE RED SEA, EGYPT.

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ABSTRACT: During a survey of parasites of fishes from the Red Sea, Egypt, *Nosema aegypti* (Microsporida) n. sp. was observed for the first time in the coelomic cavity and musculature of *Procamallanus elatensis*. *Procamallanus elatensis* is a common camallanid nematode infecting the digestive tract of siganid fishes in the Red Sea. During a recent survey (1993-1994) of parasites of fishes from the Red Sea, 69% of *Siganus luridus* (9/13) and 49% of *Siganus rivulatus* (38/78) were infected with *P. elatensis*. *Nosema aegypti* was noted in most of the host tissues of one Nematode in different stages of development. Spores (1.5 to 2.5 µm long) were observed beneath the cuticle and in the musculature of the nematode. Both the polaroplast and diplokaryon spores are small for microsporidans. The schizonts are small, irregularly shaped cells with electron dense grains in their cytoplasm. Sporonts contain two elongated nuclei and mature spores are characterized by 9 to 10 coils of the polar filament. Only one nematode was infected with *N. aegypti*. This is the first record of *Nosema* in nematodes from siganid fishes.

Keywords: *Nosema Aegypti* n.s.p. hyperparasite, siganid fishes, Red Sea.

INTRODUCTION

The Phylum Microspora represents a group of intracellular parasites of most eucaryotic organisms including fish (Canning and Lom 1986, Lom and Dykova 1992). In fish, the infection with microspora can be widespread and diffuse throughout tissues of the host and often localize in encapsulated cysts (Canning and Lom 1986). The first record of a microsporidan infection for a marine nematode was reported by Hopper et al (1970). He studied the incidence of infection of *Pleistophora* in the coelomic cavity of the nematode, *Metoncholaimus scissus* in Florida (USA). In 1908, Lutz and Splendore described *Nosema mystacis* from two infected females of *Toxocara mystax* from the gut of a cat. Other species of microsporidans

have been recorded in the coelomic cavity and viscera of nematodes (Poinar and Jansson 1988, Uzhakhav and Poltavtseva 1983, Veremtchuk and Issi 1980). Canning (1975) reported on the hyperparasitism of trematodes by microsporidans. It is not uncommon for parasites to become hosts to other parasites. Palmieri et al (1976) were successful in using *Nosema strigeoideae* as a biological control of the eye fluke, *Diplostomum spathaceum*, in fish. Canning and Lom (1986) listed the names of vertebrate hosts and the names of genera and species of microsporida infecting those hosts. Azevedo and Canning (1987) and Canning et al 1979 reported positive results for the hyperparasitism of larval and adult trematodes through the use of microsporidans. Other parasites have been hosts to microsporida such as *Nosema ceratomyxae* infecting the myxosporidan *Ceratomyxa* sp. (Diamant and Paperna 1985). *Ceratomyxa* sp. had been observed in the gall bladder of Red Sea rabbitfish (Siganidae) from Israel. The potential to control parasites with parasites (biological control) is feasible and may be an alternative to the use of chemicals (Canning 1974).

During a survey of parasites and diseases of siganid (rabbitfish) fishes from the Red Sea, microsporidans were found in the coelomic cavity of one of the intestinal nematodes, *Procamallanus elatensis*. *Procamallanus elatensis* is a common camallanid nematode infecting the intestinal tract of rabbitfish from the Red Sea, Egypt.

MATERIALS AND METHODS

Nematodes, *Procamallanus elatensis* (Camallanidae), were collected from the intestine of *Siganus luridus* and *Siganus rivulatus* (Siganidae, rabbitfish) from Hurghada, Egypt during 1993-1994. The nematodes were washed with tap water and further prepared for observation as follows:

Scanning Electron Microscopy:

Whole specimens of *P. elatensis* with potential infections of microsporidans were cut into 3-4 parts to allow fixation of the internal viscera. Sections were fixed for 2 hr in 2% buffered glutaraldahyde and then washed several times in 0.1 M sodium cacodylate buffer for ten minutes. The samples were then fixed in OsO_4 for 12 hr, washed in buffer and immersed into 2% tannic acid for 8 hr. After the fixation steps the sample was washed in buffer six times each for 10 min, dehydrated in an ascending series of ethanol and critical point dried. Each sample was coated with gold using a CSA Mini-Coater Sputter apparatus and then examined with a scanning electron microscope.

Transmission Electron Microscopy:
Sections of nematodes were fixed in 2% buffered glutraldehyde and then washed six times in an 0.1 M sodium cacodylate buffer ten minutes each. Dehydration was accomplished with a graded series of ethanol followed by 3 changes of acetone each for 10 min. Before embedding in 100% Spurr resin, the samples were immersed in 25% resin and 75% acetone for 1hr followed by 75% resin and 25% acetone for another 1hr and finally embedded in 100% resin overnight. The tissue was sectioned with an ultramicrotome, placed on the grids and stained with lead citrate and 1% uranyl acetate. Each grid was viewed with a high resolution transmission electron microscope.

RESULTS

Microsporida were observed in one of the nematodes removed from the intestine of siganid fishes from the Red Sea. Small rounded egg-like spores were observed in the coelomic cavity of *Procamallanus elatensis*. At first it was assumed that these rounded spheres were nematode eggs (Figure 1).

Scanning Electron Microscopy: SEM
Several sections of the nematode *P. elatensis* were infected with spores of *Nosema aegypti* n.sp. Throughout the coelomic cavity and within the organs of the nematode thousands of microsporidan spores were observed (Figures 2A, 2B). Only one nematode was infected. The spores were oval to spherical in shape and measured 1.5 to 1.6 µm in width. The outer surface of the spores appears to be corrugated with some extruding their polar filament. The corrugations are prominent with sections used for transmission electron microscopy.

Transmission Electron Microscopy: TEM
The microsporidan spores of *N. aegypti* n. sp. are widely distributed throughout the coelomic cavity and underneath the cuticle of *P. elatensis* (Figures 3A, 3B). The spores were observed among both epithelial and muscle cells of the host and free in the coelomic cavity. Areas of the nematode with high number of spores displayed cellular necrosis and lysis (Figures 4 and 5).
The schizonts (merogony phase) of *N. aegypti* n. sp. are rounded to irregularly in shape with a small, compact nucleus. A thin double membrane surrounds the schizont with electron dense granules throughout the cytoplasm. It was observed that often 4 schizonts appeared to be attached together (Figure 6).
For the formation of spores, the sporont is irregular in shape with a multi layered electron dense cell membrane. Each spore is a diplokaryon with two elongated nuclei, well developed rough endoplasmic reticulum and cisternae (Figures 7A,

7B). Sporoblasts at various stages of development were observed (Figures 8A, 8B). In the early development, the sporoblast is rounded to oval with an outer electron dense layer followed by a transparent layer. The nucleus is small, located at a lateral position in the sporoblast with a dense, fine-grained nucleoplasm. Ribosomes are represented by dark granules within the sporoblast (Figure 9). In subsequent stages of development, multiple nuclei appear in the sporoblast.

For the polar filament, the developing spore first developes one coil and then 9 to 10 coils, which is typical for mature spores, are formed (Figure 10). The ribosomes in the spore are often concentrated in the anterior half in ribbon-like arrangements. The polarplast is prominent (Figure 9) with a manubrium situated at the anterior pole of the spore with the polar filament extending from this structure. There are typically 9 to 10 coils for the polar filament. The initial part of the polar filament is surrounded by the lamellar-like polarplast. Transverse sections of the coils, displays two alternating dark and light areas for the structure (Figure 11). The outer electron dense layer of the spore membrane is folded or corrugated.

DISCUSSION

Previous records of microsporidans as hyperparasites in nematodes are limited. Poinar and Jansson (1988) reviewed diseases and parasites of nematodes and included microsporida as an organism which infects the coelomic cavity of roundworms. The earliest reports describe a possible microsporidan from the ascarid nematode, *Toxocara mystax*, a parasite of the intestine of cats (Poinar and Jansson 1988, Lutz and Splendore 1908). Micoletsky (1925) observed some internal microsporidans and microparasites of freshwater nematodes.

Other early records of nematodes infected with microsporidan organisms include; *Thelohania reniformis* infecting the gut epithelial cells of *Protospirara muris* a nematode found in the stomach of a house mouse (Kudo and Hetherington 1922). Poinar and Hess (1986) observed *Microsporidium rhabdophilum* infecting *Rhabditis myriophila* a nematode found in the garden millipede, *Oxidis gracilis*. Hopper et al (1970) described a species of *Pleistophora* found in the tissues of a marine nematode *Metoncholaimus scissus*. This is the first recorded data of *Nosema* in a nematode host. The species name of *Nosema* (*N. aegypti*) is based on the location of the host.

In addition to Nematodes, the genus *Nosema* has been found in other parasitic organisms. Palmieri et al (1976) reported on the use of *Nosema strigeoideae* as a biological control of the eye fluke, *Diplostomum spathaceum*. *Nosema ceratomyxae* has been found in the myxosporidan parasite *Ceratomyxa* sp. This myxosporidan

is a parasite of the gall bladder of three species of rabbitfish (siganids) from the Red Sea (Daimant and Paperna 1985).

In the present study, *Nosema aegypti* n. sp. was recorded for the first time in the coelomic cavity and tissues of a common nematode parasite of rabbitfishes in the Red Sea, Egypt. *Nosema* is characterized by small diplokaryan spores in mainly invertebrate hosts (Canning and Lom 1986), with some vertebrates infected with the genus (Lom and Dykova 1992). The diplokaryotic characteristic is consistent through most of the life cycle of *Nosema*. Both merogony and sporogony are in direct contact with the cytoplasm of the host cell. The merogoney phase is disporoblastic (Canning and Lom 1986). *Nosema aegypti* n. sp. differs from *N. ceratomyxae* in the absence of a posterior vacuole and the number of coils for the polar filament in the mature spores (*N. aegypti* 9-10 coils and *N. ceratomyxae* 11 coils). The polaroplast differed for the two species in which in which *N. ceratomyxae* is characterized by both lamellar and vesicular processes within the polaroplast while *N. aegypti* had only lamellar structures. The spores of *N. ceratomyxae* are larger (4.2 to 5.2 um long and 1.5 to 1.6um wide) than *N. aegypti* (1.5 to 2.5um long and 1.5 to 1.6um wide). This is the first known record of *Nosema* inhabiting the internal visceria and coelomic cavity of a nematode from Red Sea fishes.

Taxonomic Summary: (Hyperparasite)

Parasite:	*Nosema aegypti* n. sp.
Type Host:	*Procamallanus elatensis* (Nematode) found in intestine of rabbitfish (Siganids).
Type Locality:	Coelomic cavity and viscera of *P. elatensis*
Site:	Red Sea, Egypt
Prevalence:	One of thirty nematodes (*Procamallanus elatensis*)
Abundance:	Numerous in the host
Nematode Host:	Siganid (rabbitfish) fishes; *Siganus rivulatus* and *Siganus luridus* from Red Sea, Egypt
Etymology:	Named due to location of host fish
Specimens:	Nematode, *P. elatensis* infected with *N. aegypti* mounted on glass slides and ultrathin sections mounted on grids deposited at the University of Nebraska State Museum, Number HWML 38929. Both authors have additional samples in their collections.

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CAPTIONS FOR FIGURES

Figure 1: Micrograph of *Procamallanus elatensis* with internal structure (E) that appear to be eggs of the femal Nematode. C equals outer cuticle of the round-worm.

Figure 2A: Scanning Electron Micrograph (SEM) of a specimen of *Procamallanus elatensis* with egg-like structures which were identified as Nosema aegypti spores (S).

Figure 2B: Higher magnification of the Nosema spores (S). Note corrugated outer cell wall of the spores. Bare equals 1um.

Figure 3A: Transmission Electron Micrograph (TEM) of *P. elatensis* with spores of *N. aegypti* immediately under the cuticle (C) and next to a muscle (M) Layer of the nematode. Bar equals 1.0um.

Figure 3B: TEM of P. elatensis showing the invasion of *Nosema* spores (S) in between the cuticle (C) and muscle (M) of the nematode. Bar equals i.0um.

Figure 4: TEM of P. elatensis invaded by *N. aegypti* (S) causing necrosis (N) of host tissue. Note mature spores (S) and developing spores (arrowheads). Bar equals 1.0um.

Figure 5: Necrosis of striated muscle (M) in *P. elatensis* due to invasion of *N. aegypti* (S). Bar equals 0.1um.

Figure 6: Developing spores (S) of *N. aegypti* with four schizonts (SC) attached together. The ribosomes (R) are visible within the cells. Bar equals 1um.

Figure 7A: Mature spore with a corrugated outer membrane (arrows) with wraps of the polar filanment (arrowheads), polaroplast (P) and manubrium (M). Numerous ribosomes (R) are arranged in a lamellar fashion within spore. Bar equals 0.5um.

Figure 7B: Mature spore (arrow) showing the diplokaryon (n) nucleus which is characteristic of *Nosema*. Arrowheads represents the polar filament and (R) ribosomes. Bar equals 0.5um.

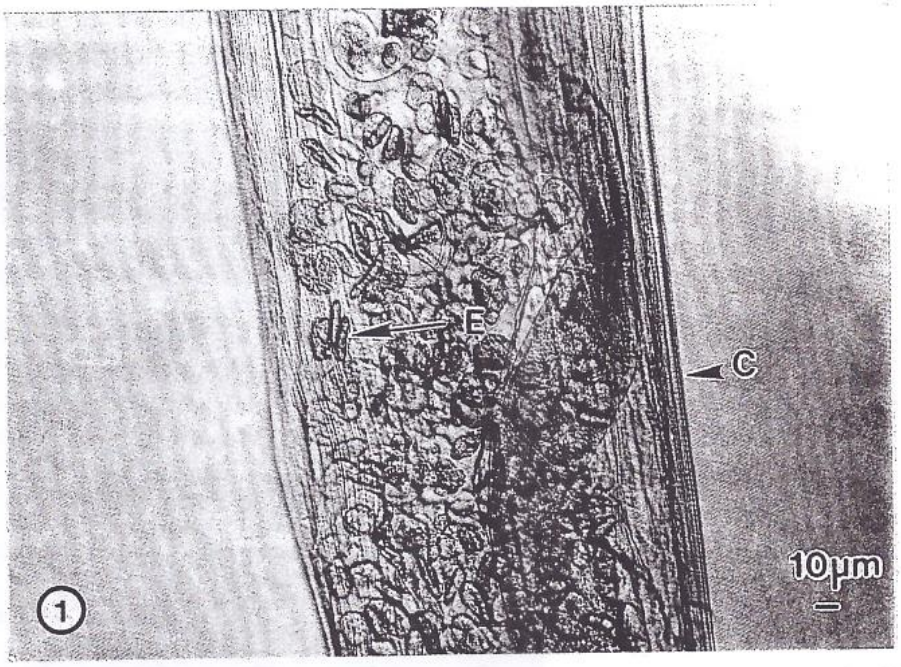
Figure 8A: Developing sporoblasts (S) of *N. aegypti* (arrows) with ribisomes (R) and developing out membrane (Arrow). Bar equals 0.5um.

Figure 8B: Maturing spore for *N. aegypti* (arrows) with two nuclei (N), diplokaryon and ribosomes (R). The polar filament (arrowheads) has formed. Bar equals 0.5um.

Figure 9: Mature spore (arrow) of *N. aegypti* with well developed ribosomes (R) represented by dark dots within spore. Manubrium (M) and polarplast (P) are visible. Bar equals 0.5um

Figure 10: Mature spores (arrow) of *N. aegypti* displaying the typical number of wraps for the poalr filament (P). Note corrugated out membrane and ribosomes (R). Bar equals 1.0um.

Figure 11: Higher magnification of polar filament (arrows). Note light and dark (electron dense) areas of filament. Ribosomes (R) and part of the diplokaryon nucleus (N) are visible. Bar equals 0.1um.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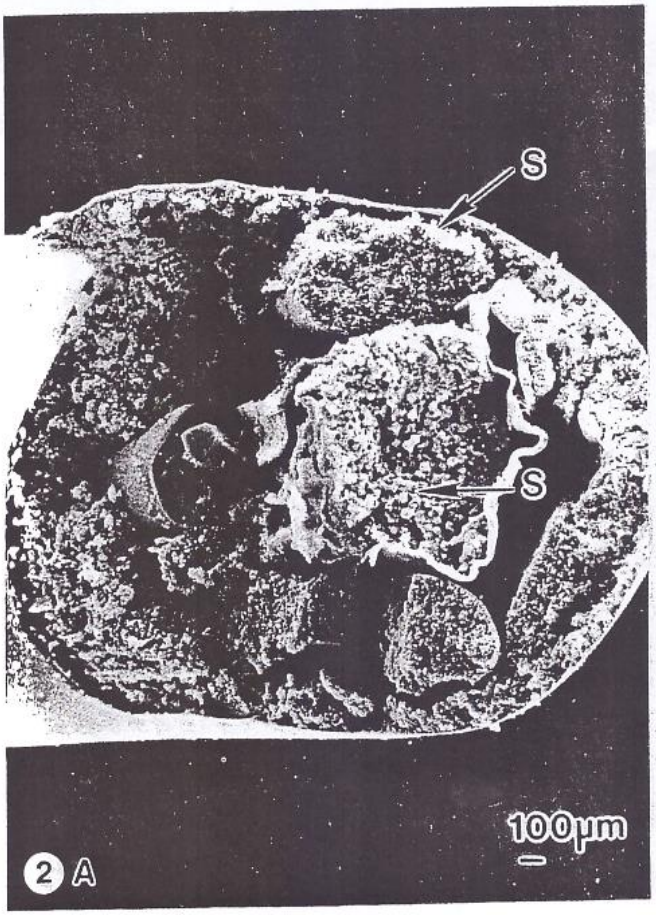
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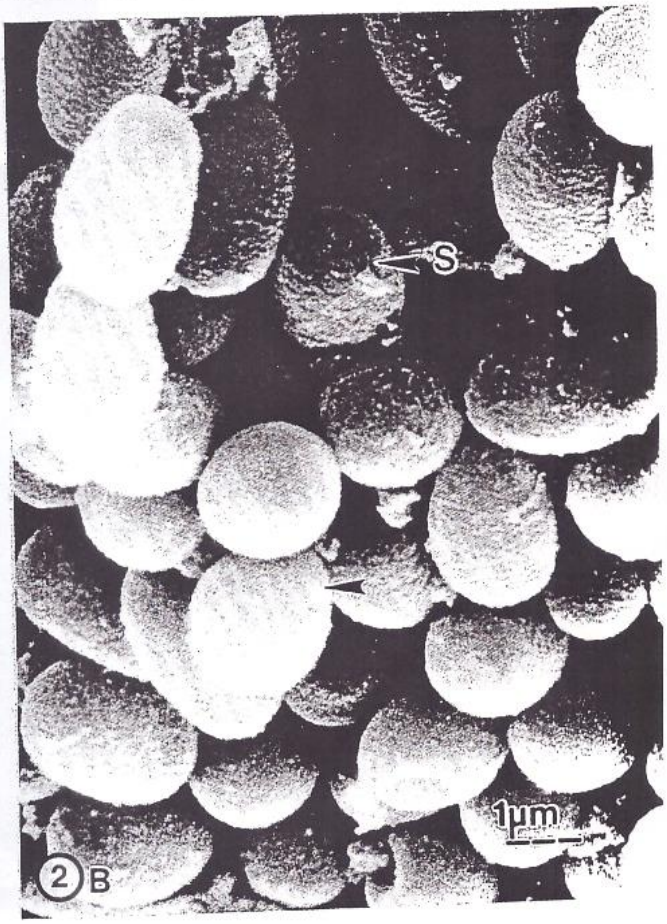
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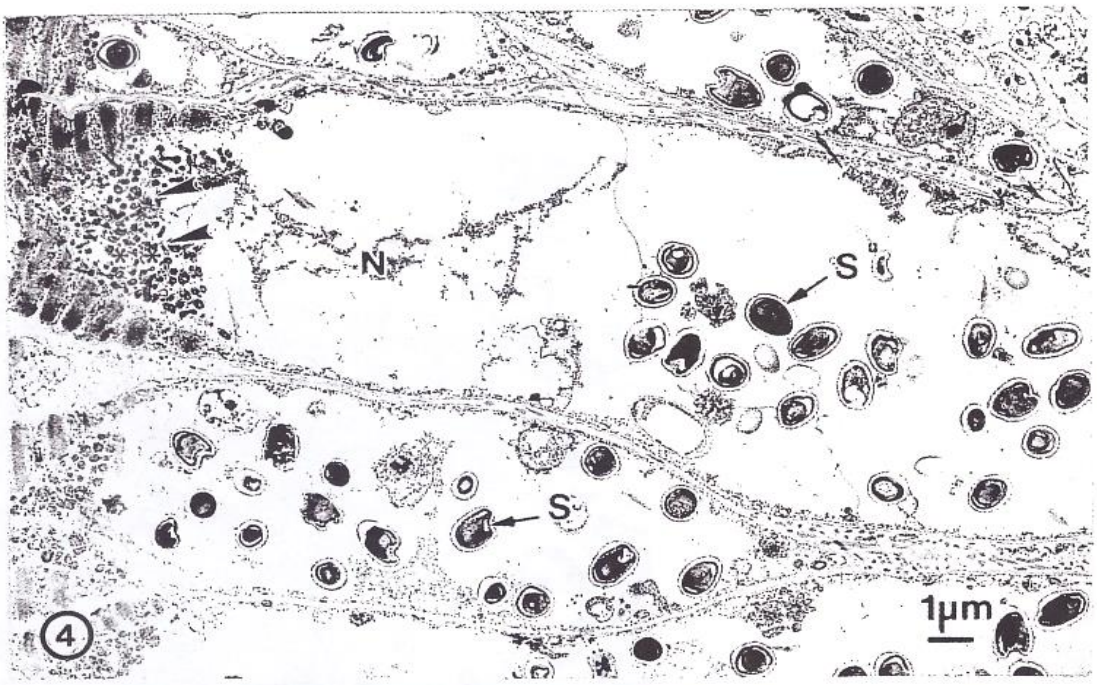
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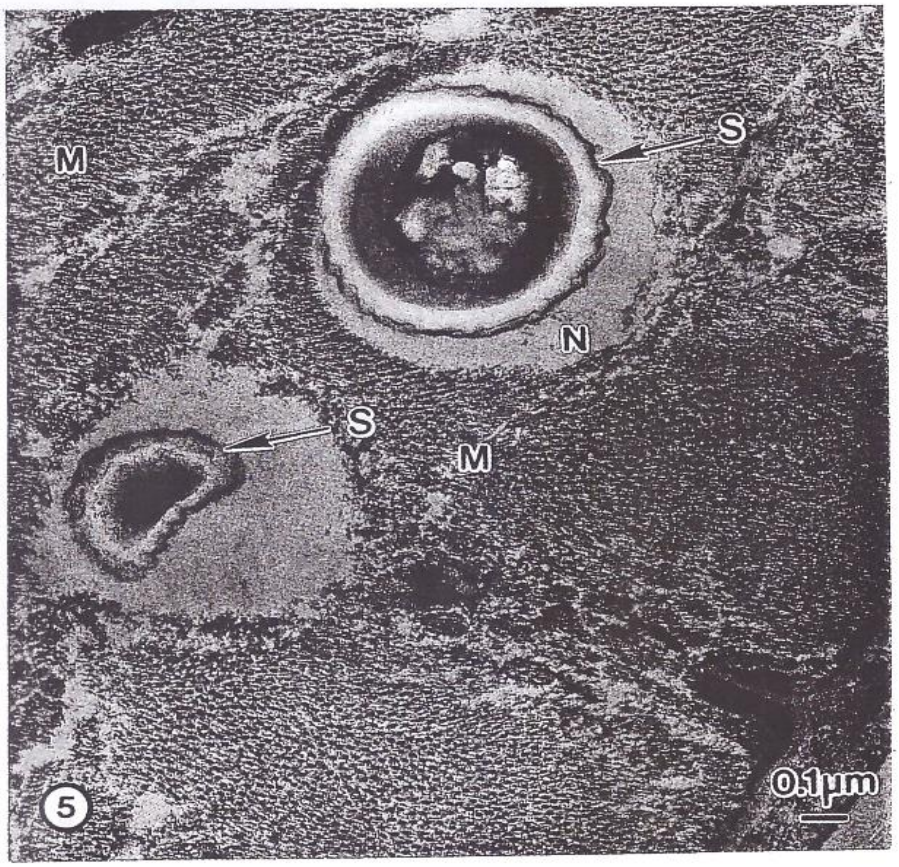
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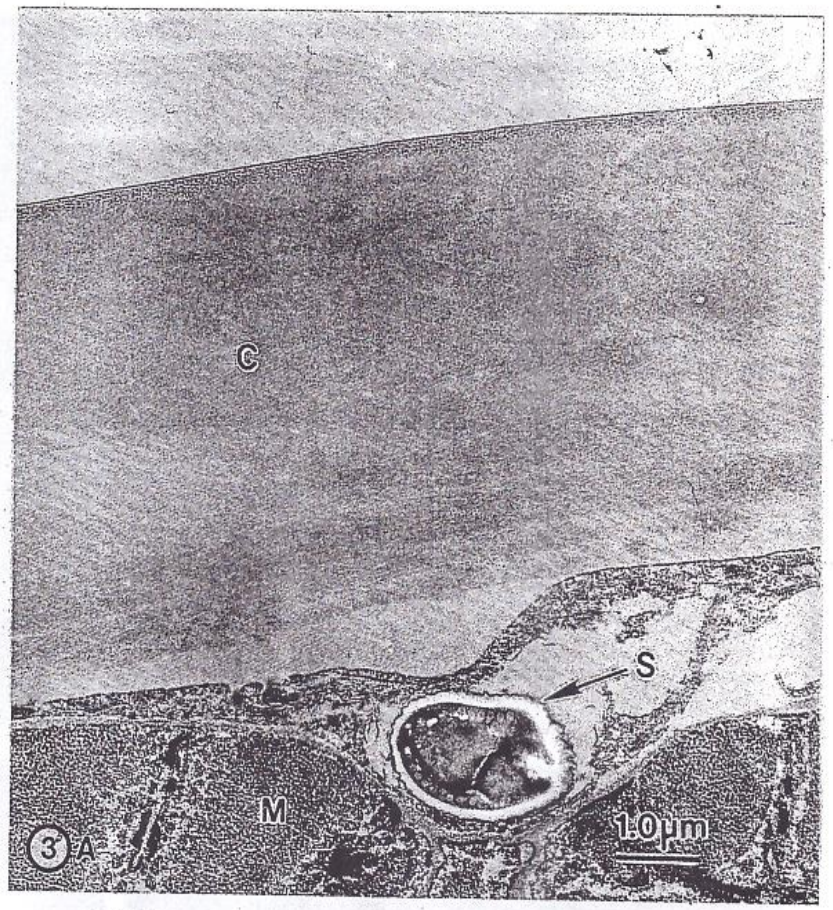
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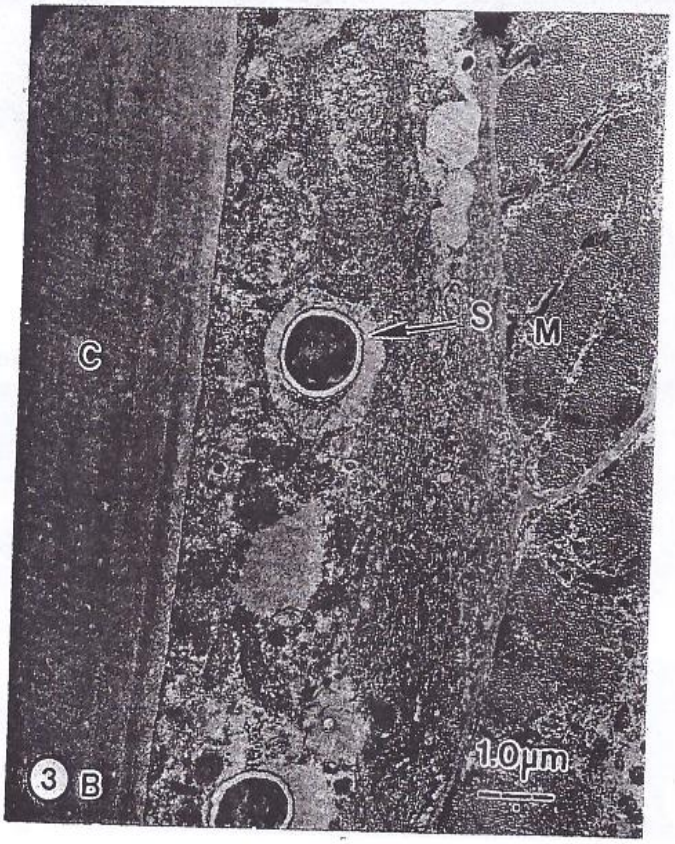
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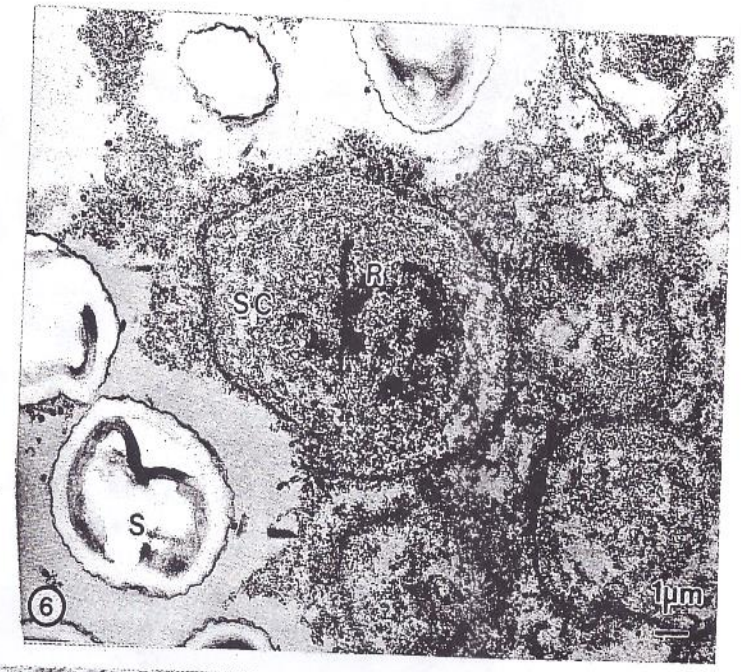

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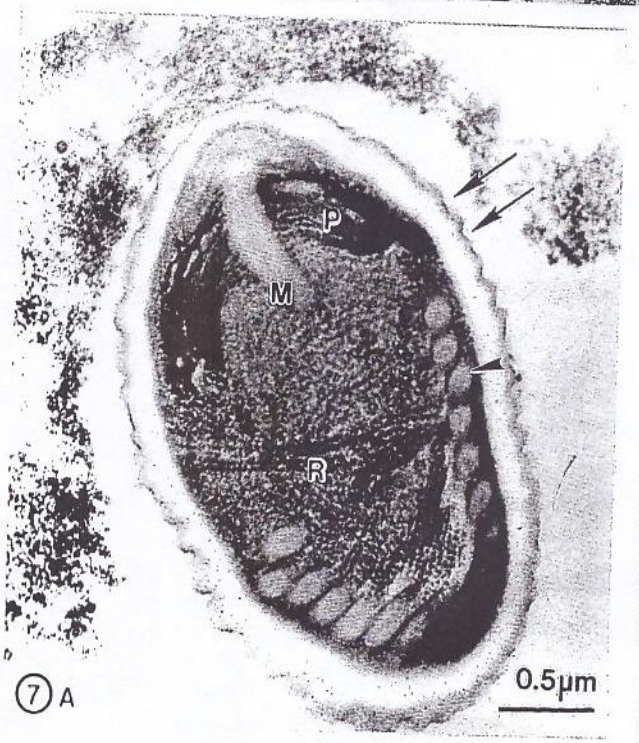

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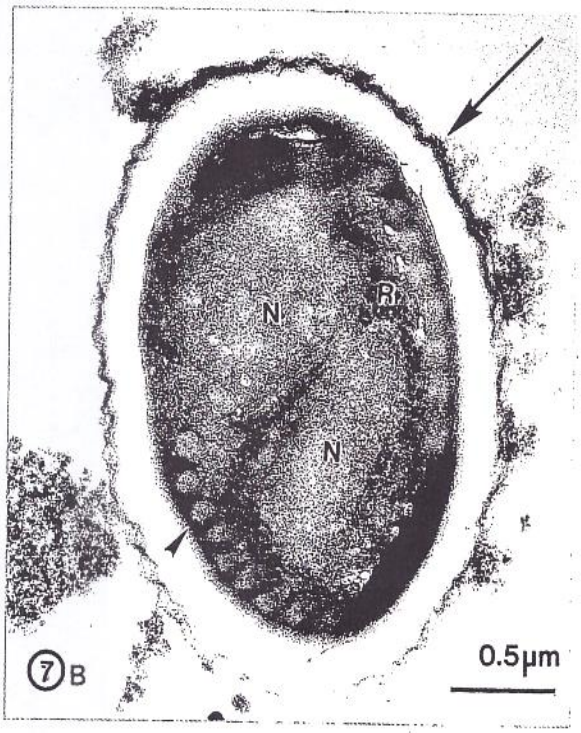

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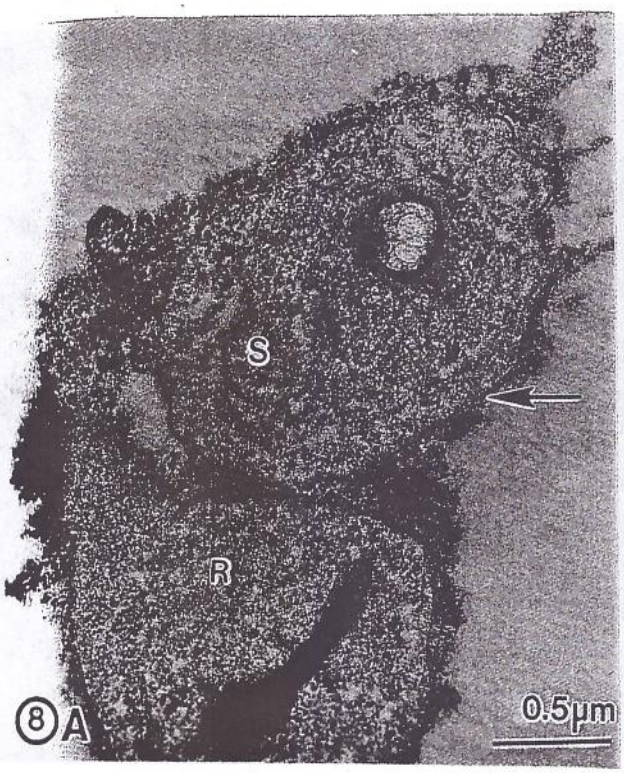

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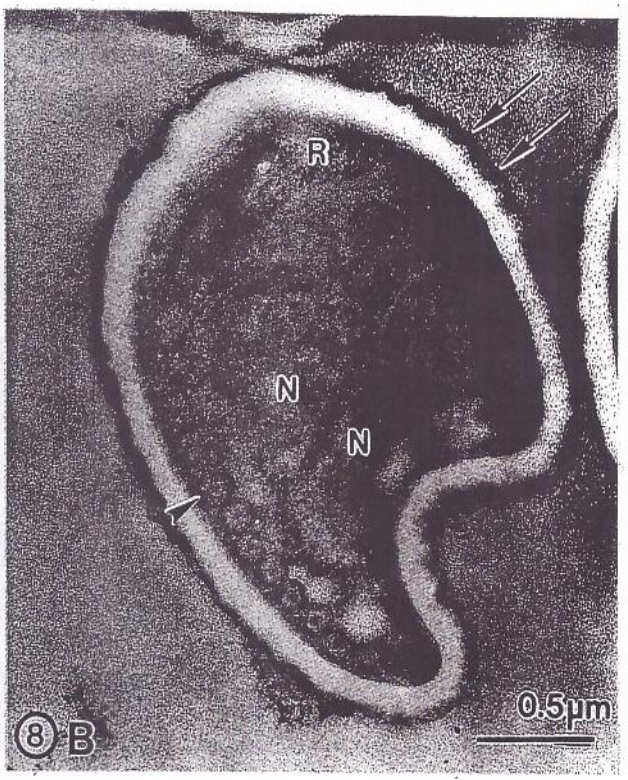

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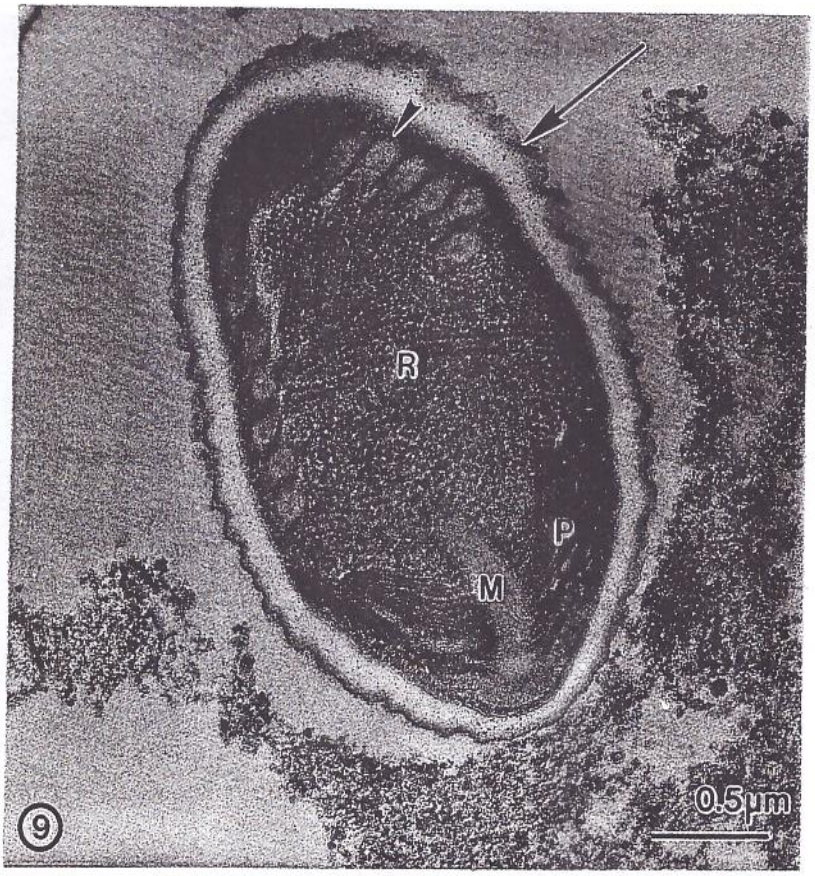

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