Light and TEM studies on the gills of Tuna infecting with *Allodidymozoon pharyngi* (Didymozoid trematodes) from Libya

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Key Words : TEM, Gills,Tuna, *Allodidymozoon*, Didymozoid, trematoda

Abstract

Allodidymozoon pharyngi Abul-Salam and Sreelatha,1995 (Didymozoid trematodes) belonging to the subfamily Didymozoinae (Ishii,1935), genus *Allodidymozoon* Yamaguti,1959 was recorded from the marine fish *Thunnus albacarcs* (locally called Tuna) collected from Misurata fish market in Libya. The worms found encysted on the gill arch and gill filaments of fish. There are an encysted sinuous strings of eggs masses resembling the encysted worms .The worms as well as the eggs masses of the worm were sectioning, for light and TEM.The cyst wall is mainly a reaction product of host`s tissues and consists of two layers .. The eggs are non-operculate instead of operculate in the original description,it possesses two projections in their posterior and lateral sides . The egg shell is composed of three layers, outer delicate vitelline layer , thick chitinous layer and inner narrow lipid layer. The eggs contains ovum, yolk particles, polyphenol granules ,fibrous materials, mitochondria, endoplasmic reticulum ,cortical cytoplasm and refringent granules. The external features agreed fully with the original description, but the present description added more details about the internal structure of the encysted worms and ultrastructure of eggs .The present work represent a new host and locality records.

Introduction

Didymozoid trematodes are known to occur encysted on the gills, gill-covers or the bones of the head of marine fishes. The first record of Didymozoid trematodes in freshwater fishes was in (1955) during a survey of the Nile fish carried out by McClelland in Sudan. He described *Nematobothrium labeonis* free in the orbit of *Labeo niloticus.* Williams (1957) described the anatomy of *Kollikeria filicollis* (Rudolphi,1819) Cobbold, 1860 from the Bloch *Brama raii* .Cable and Nahhas (1962) reported some crustacean serve as the second intermediate hosts of

Didymozoid trematodes . Madhavi (1968) reported a Didymozoid metacercaria from the copepod *Paracalanus aculeatus* from Bay of Bengal. Lengy and Fishelson (1972) reported immature Didymozoid larvae from the dorsal muscles and swim bladder of the coral-reef fish *Anthias squamipinnis* from Israel. Noble (1975)described *Nematobibothrioides histoidii* from the body wall of the sunfish *Mola mola* from California. Fischthal and Kuntz (1964) described immature didymozoid larvae from *Euthynnus yaito* from Palawan Islands in Philippines. No didymozoid life cycle has been worked out experimentally , but larval forms have been found in small fishes and planktonic invertebrates (Nikolaeva ,1965; Koie and Lester,1985) . The larvae are probably eaten by the definitive hosts while consuming prey, and from the digestive tract they migrate to their normal site of maturation (Cable and Nahhas,1962 ;Lester,1980) . Several species of didymozoid have been recorded in barracudas in the Indo-West Pacific region (Yamaguti,1959 ; Job,1961a ,1961b,1962,1964,1966 ; Ku and Shen, 1965 ; Madhavi,1982 ; Shen,1984,1989 ,1990 ; Hussain et al.,1985a 1985b ; Mordvinova and Nikolaeva,1990).The affinity of barracudas for didymozoid infections is probably related to their diet , which consists predominantly of small fishes, potential intermediate,or paratenic hosts (Abdul-Salam and Sreelatha,1995). Abdul-Salam and Sreelatha (1993) reported 8 species of didymozoids in the barracuda *Sphyraena obtusata* Cuvier in Kuwait Bay.They also in (1995) described *Allodidymozoon pharyngi* and *A. trilobata* from cysts in the pharyngeal muscles and the muscles on the inner surface of the operculum respectively from the same fish and the same locality.

During the present investigation *Allodidymozoon pharyngi* Abul-Salam and Sreelatha,1995 redescribed from *Thunnus albacarcs* from Misurata fish market in Libya . The present work represent a new host and locality records and the first study illustrate the egg of this species by TEM in Egypt.

Material and methods

Numerous specimens of parasites were collected from the gills of the marine fish *Thunnus albacarcs* (locally called Tuna) from Misurata fish market in Libya.The worms were found capsuolated on the gill-arch and gills fillaments of fish.The worms excysted then relaxed and fixed in 10% formaldehyde, then washed and stained by using carmine stain. Drawing were made to the scale of Camera lucida. For transmission electron

microscopy, the specimens were transferred from formalin, via a series of Sorensen`s phosphate buffers (pH 7.3) and graded alcohols, back to Sorensen`s buffer, and then osmicated in 1% osmium tetroxide in Sorensen s buffer. The specimen were embedded in Spurr`s embedding resin, and sectioned. The sections were stained with lead citrate and examined in a Zeiss 10 CA transmission electron microscope in Ruhr-Universitat, Bochum, Germany .All measurements are in millimeter unless otherwise stated .

Description

In the first observation the writer showed a large orange masses on the gill arch and gill filament of fishes (Figs. 2,3).He think that a fungus infection, but the studies revealed that the materials are eggs of didymozoid trematodes.

The worm enclosed in globular cyst on the gill-arch and gill filament of fish (Fig. 2).There are a large sinus of eggs masses encysted on the gill arch alternating with the encysted worms (Fig.3). These sinus of eggs masses are larger than the encysted worms ,it measures 3.9-5 long and 0.11-1.7 wide. Hindbody subcylindrical,3.5-4.5 long and 0.9- 1.4 wide, flat ventrally,covex dorsally. The posterior end slightly broader than anterior. Forebody flattened 0.51-0.60 long and 0.06-0.1 wide attached to hindbody on its flat side (Fig.1). Oral sucker pyriform, terminal,0.064-0.084 in length . Prepharanx absent . Pharynx round ,muscular 0.034-0.046 in diameter .Oesophagus tubular surrounded by gland cells,bifurcating into ceca (Fig. 1). Ceca tubular,running to near posterior extremity . Testes paired , elongate,tubular each 1.36-2.30 in length .Vas deferens running forward in forebody . Genital pore ventrolateral to oral sucker. Ovary tubular divided into 2 winding branches reaching to near opposite extremities. Vitellarium tubular and branched ,extending throughout hindbody. Receptaculum seminis and Mehlis gland not seen . Uterine coils convoluted,occupying all available space in hindbody(Fig. 1). The transverse sections revealed that the cyst wall which rests on the host tissues is mainly a reaction product of the host, it consists of two layer, thick outer layer and thin inner layer (Fig. 5) . The body cavity contains spherical ovary, testes, branches of intestine , vas deference and uterus (Fig.4). Dorso-ventral muscles fibres occur very spasmodically throughout the body(Fig.4) . Immediately beneath the musculature of the cyst there is a single layer of regularly spaced subcuticular gland cells (Fig.5). The parenchymatous cells are present throughout the body (Fig. 4).The eggs are typically boat shaped and produced in vast number and entirely fill the

uterus in mature specimens. They are oval in outline and non-operculate, measuring 0.031-0.036 long and 0.020-0.025 wide. The thickness of the egg-shell being 0.8-1µm . In the longitudinsl sections, the egg shell extend posteriorly to form prominent process in each side (Figs.8,9). It have a flat ventral surface , convex dorsal surface and the lateral surface provided with prominent process in each side (Fig.7). The body cavity supported by connective tissues septa (Fig.4) . The eggs sinus also supported by connective tissues (Fig.5), they contains a large numbers of different sized eggs (Fig.5). In the TEM sections, the eggs contains semispherical ovum, large yolk particles, fibrous materials, pigment cells and ciusters of polyphenol granules inside and outside the yolk particles (Figs 7,8,9) . A fully formed egg shell consists of three layers, an sticky delicate outer vitelline layer, thin inner lipid layer and thick chitinous layer in between (Fig.7).

Discussion

Yamaguti (1970,1971,1975) in his studies on Didymozoids speculated that the larvae of Didymozoids are usually referred to under the groups names Torticaecum and Monilicaecum . He also outlined that three larval stages occur in between the cercarial and adult stage, the pre-monlicaecum and pre-torticaecum stages in crustaceans, the torticaecum and monilicaecum stages in fishes which serve as paratenic hosts and post-torticaecum and post-monilicaecum stages which undertake extensive migration in the definitive host before becoming adults. Williams (1959) outlined that the cyst of *Kollikeria filicolis* (didymozoid trematodes) is mainly a reaction product of the host tissue against secretions from some of the subcuticular gland cells of the female worm . He slso stated that the sexes are not entirely separate and the eggs are operculated. In agreement with Williams,1959 that the cyst of *Allodidymozoon pharyngi* is mainly a reaction product of the host tissue and the sexes are not entirely separate . McClleland (1955) outlined that the eggs of *Nematobothrium labeonis* (didymozoid trematodes) are non-operculated . The present work agree with McClleland,1959 that the eggs of *Allodidymozoon pharyngi* are non-operculated as like *Nematobothrium labeonis* .The complete life cycle of didymozoid trematodes is unknown . Associated with *Nematobibothrioides histoidii* were small masses of eggs lying in host connective tissue . Occasionally these masses occurred in sinuous strings resembling a worm, as through the parent trematode snaked its way through tissues leaving eggs behind. How the eggs get out of the host is

unknown. Possibly they are released into water only when fish are attacked by their natural enemies and their tissues are ruptured (Noble,1975). The present work agree with the findings of Noble (1975) that the occurrence of sinus of eggs masses of *Allodidymozoon pharyngi* on the gills of fish originates from the parent trematodes.

A fully formed egg shell in most trematodes consists of three layers : 1- an outer vitelline layer, often not detectable by light microscopy, 2- a chitinous layer , 3- an innermost lipid layer. Immediately after sperm penetration , a new plasma membrane forms beneath the original ; the old plasma membrane becomes the vitelline layer and separates from peripheral cytoplasm, then the cytoplasm shrinks back leaving a clear space within which the chitinous layer forms. Refringent bodies , previously dispersed throughout the cytoplasm migrate to the periphery and expel their contents, the fusion of which forms the lipid layer (Roberts et-al., (2005). In agreement with Roberts et-al., (2005) the egg shell consists of three layers, outer vitelline layer ,chitinous layer and inner lipid layer. The main structure of protein in the egg-shell are sclerotin , o-quinone and o-phenol (Smyth, 1954) . Stephenson (1947) and others stated that the egg shell of trematodes, and in particular that of *Fasciola hepatica,* originates from granules present in the vitelline cells and not from a secretion of Mehlis s gland, this secretion being reinforced by the granules of the vitelline cells. The present work agreed with Nigrelli,1939 and Stephenson ,1947 that the polyphenol granules which occur inside and outside the yolk particles play a main role in the formation of the egg shell.

Abdul-Salam and Sreelatha (1995) described *Allodidymozoon pharyngi* from the barracuda *Sphyraena obtusata* Cuvier in Kuwait Bay. The present work agreed fully with Abdul-Salam and Sreelatha (1995) description in the external features, but there are certain minor differences in the body size and the egg-shaped . The present work added more details about the internal structures of the encysted worms and ultrastructures of the eggs. The present work represent a new host and locality record.

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Explanation of figures

Fig. 1 : Camera lucida drawing of the entire worm , h-hindbody ; f-forebody ; o-ovary ; t-testis ; v-vitellarium ; u-uterus (S.bar 500µm).

Fig. 2 : Light microscopy micrograph showing the encysted worms on the gill arch of the infected fish (S. bar 900µm).

Fig. 3 : Light microscopy micrograph showing sinuous strings of eggs masses resembling the encysted worm on the gill arch , ga-gill arch (S. bar 800 µm).

Fig. 4 : Light microscopy micrograph showing part of transverse section of the worm, t-testis ; u-uterus ; e-egg ; ca-intestinal cecum ; vd-vas deferens ; ct-connective tissues ; pc-parenchymatous cell ; o-ovary (S.bar 19 µm).

Fig.5 : Light microscopy micrograph showing part of transverse section of the cyst of eggs masses , scg-subcuticular gland cell ; ow-outer cyst wall ; iw-inner cyst wall; ct-connective tissues e-egg ; ht-host tissues (S.bar 19 µm).

Fig.6 : Light microscopy micrograph showing the eggs (S. bar 17 µm).

Fig. 7 : TEM micrograph of transverse section of the egg, fr-fibrous materials; y-yolk particles; cc-cortical cytoplasm; d-dorsal surface of the egg; m-mitochondria; vl-outer vitelline layer of egg shell ; cl-chitinous layer of egg shell ; ll-inner lipid layer of egg shell ; lp-lateral projection of the egg shell ; ve- ventral surface of the egg(S. bar 3µm)

Fig. 8 : TEM micrograph of longitudinal section showing the lateral view of egg, r-endoplasmic reticulum ; sw-sticky surface of the egg ; ov-oocyte ; ph-polyphenol granules ; y-yolk particles ; fr-fibrous material ; p-posterior projection of the egg shell; cc-cortical cytoplasm (S. bar 4 µm).

Fig. 9 : TEM micrograph of longitudinal section showing the ventral view of the egg, y-yolk particles; g-pigment materials; p-posterior projection of the egg shell; m-mitochondria ; rg-refringent granules (S.bar 3µm)

الملخص العربى

دراسة بالميكروسكوب الضوئى والالكترونى القاطع لخياشيم اسماك التونا المصابة بدودة *الوديديميزويد فارينجى* من ليبيا

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تم فى هذة البحث دراسة خياشيم اسماك التونا من نوع *تونس الباكارس* المجمعة من سوق الاسماك بمدينة مصراتة بليبيا وقد اظهرت الدراسة وجود ديدان من نوع *الوديديميزويد فارينجى* متحوصلة على خياشيم اسماك التونا وكذلك وجود اكياس بيض اسطوانية الشكل وكبيرة الحجم على الخيوط الخيشومية لنفس الاسماك ومحتوية على كمية كبيرة من البيض وقد لوحظ انة عند تشريح تلك الاكياس انتثرت البيوض بمجرد لمس الاكياس بمقص التشريح بما يشبة الجراثيم وقد تم عمل قطاعات فى الديدان واكياس البيض ووجد فى القطاعات ان جدار الحوصلة مكون من طبقتين طبقة خارجية واخرى داخلية وتجويف جسم الدودة مقسم بحواجز من نسيج ضام بها قطاعات عرضية للمبيض والخصية والوعاء الناقل وتفرعات القناة الهضمية والرحم الملوء بالبيض ولوحظ وجود خلايا بارنشيمية عديدة ووجد ايضا الياف عضلية دائرية واخرى طولية ولقد تم عمل قطاعات فى البيوض بالمجهر الالكترونى القاطع ووجد ان البيوض لها سطح بطنى مستوى وظهرى محدب وقد وجد ان البيض له شكل القارب ولة حافتان جانبيتان ممتدتان بطول البيضة وبارزتان من الناحية البطنية و الخلفية ووجد ان تلك الزائدتان خاليتان من الداخل من اى انسجة داخلية ووجد ان البيضة لها جدارمكون من ثلاث طبقات الخارجية رقيقة والوسطى كيتينية سميكة والداخلية دهنية رقيقة ويحتوى البيض على خلية بيضية كروية الشكل والعديد من كريات المح المحتويى على حبيبات البوليفينول التى توجد داخل وخارج خلايا المح هذا بالاضافة لوجود لييفات عضلية دقيقة ومواد صبغية قريبة من السطح وليس للبيض غطاء كما كان يعتقد وهذا الجدار علية مادة خارجية لزجة ومتعرجة قليلا ولقد اضافت الدراسة معلومات عن شكل البيض والتشريح الداخلى للدودة بالاضافة الى لوجود هذة الدودة لاول مرة من اسماك التونا ومن ليبيا

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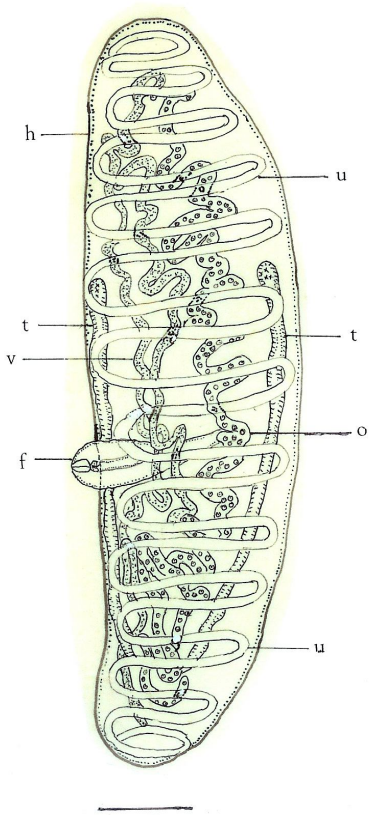


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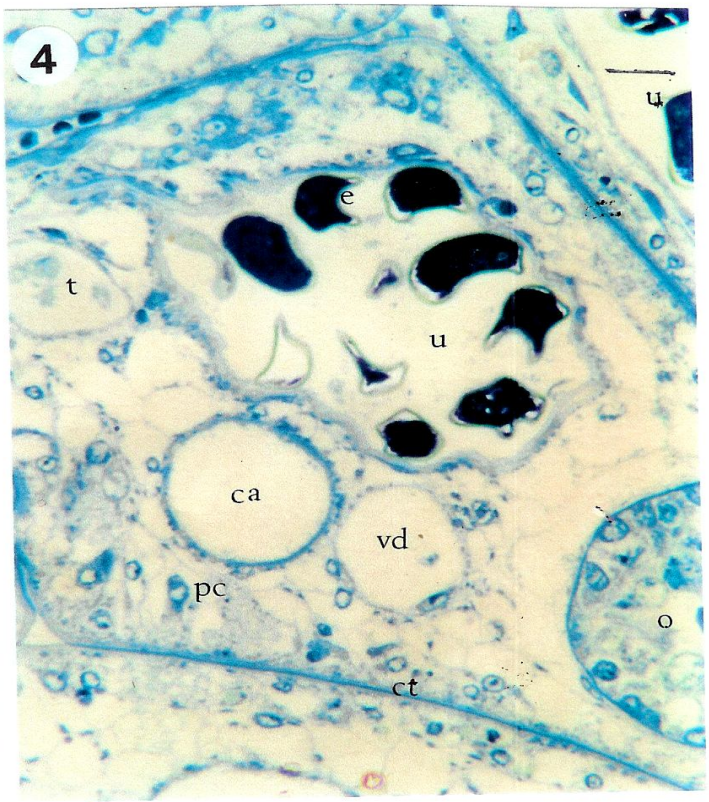
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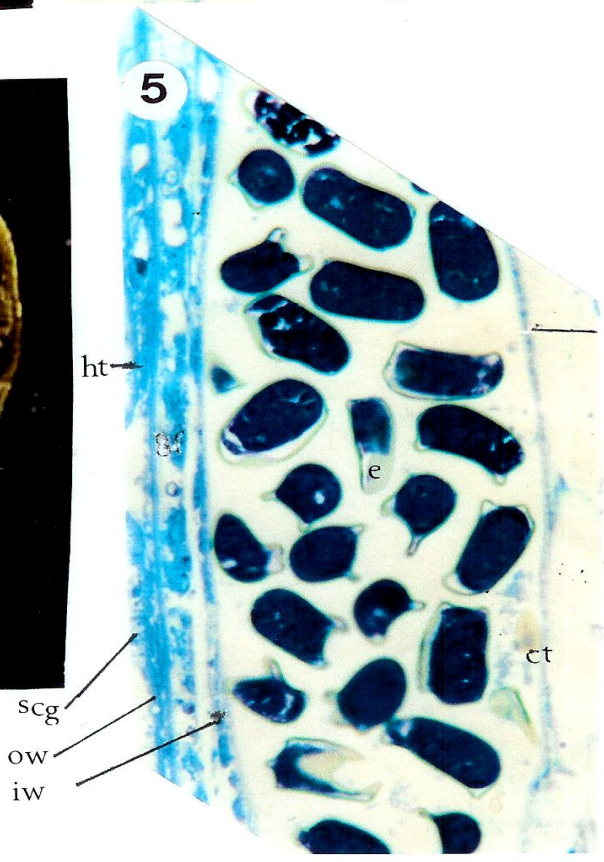
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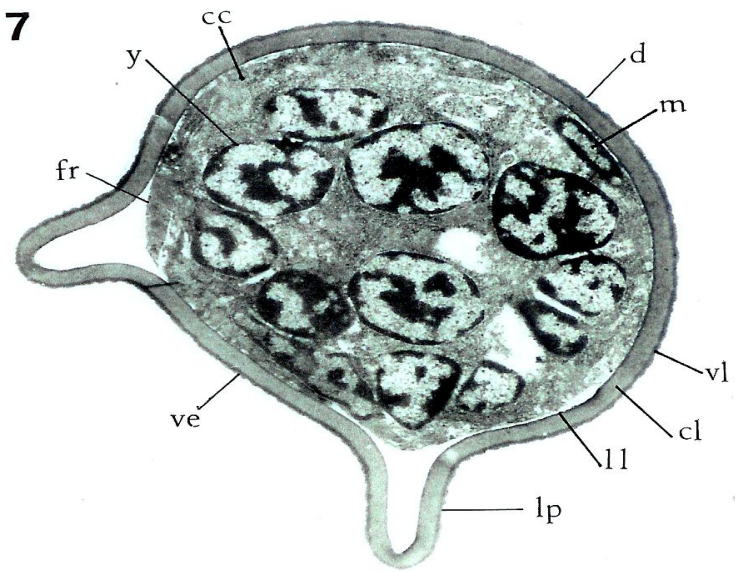
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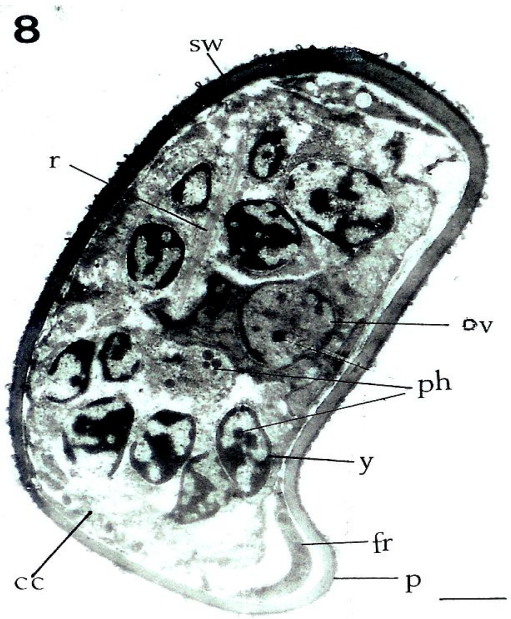
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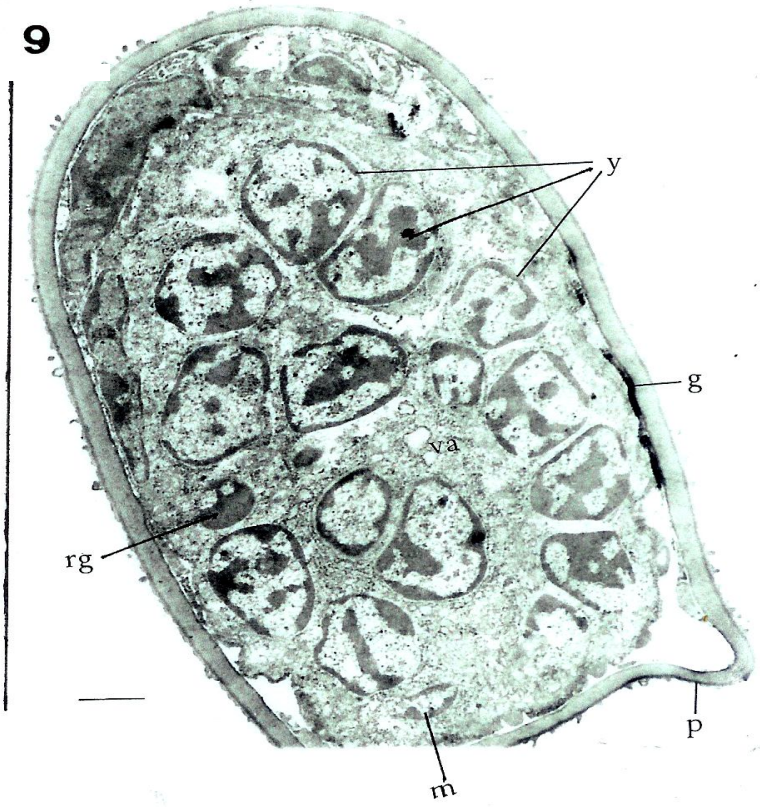
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