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CROCODILES AS A SOURCE OF NEMATODE LARVAL INFESTATION AMONG FISH SPECIES IN LAKE NASER, EGYPT

(With 9 Figures)

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التماسيح كمصدر ليرقات الديدان الأسطوانية التى تصيب الأسماك في بحيرة ناصر

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تم فحص ٣ تماسيح من بحيرة ناصر لمعرفة أنواع الديدان الأسطوانية التى قد تنتقل إليها من خلال الأسماك كعائل وسيط. بالفحص تبين إصابة إحدهم بذكور و إناث دودة الدوجارديني دوجارديني و الأخر بالطور اليرقى لنوع الأمبليسيكم أما الثالث فكان خاليا من الإصابة. أجريت دراسات تجريبية على إمكانية انتقال الدوجارديني دوجارديني إلى الأسماك كعائل وسيط باستخدام أو بدون استخدام القشريات كعائل له دور و قد تبين أن للأسماك دور العائل الوسيط أما القشريات كانت كعائل انتقالي. تم أيضا وصف شامل لكل الديدان و اليرقات تحت الدراسة مع عمل الرسم و التصوير.

SUMMARY

Three *Crocodilus niloticus* were collected from Lake Naser and examined for the presence of nematode parasites that were transmitted via fish intermediate host. The examination revealed the detection of adult males and females *Dujardinia dujardini* (Travassos, 1920) in one of them as a first report in Egypt. The second crocodile was infested with *Amplicaecum* species larvae, while the third one was found free. Experimentally, the patterns of transmission of *Dujardinia dujardini* to fish as intermediate host with and without using invertebrate host (*Cyclops*) was done revealing the role of such crustacean as paratenic host. Morphological description of the adult *Dujardinia* species,

Amplicaecum species larvae as well as the obtained stages during the experimental transmission was described and microphotographed.

Keywords: *Crocodile, Dujardinia, Amplicaecum, Fish.*

INTRODUCTION

Despite many years of Parasitological research, there is as yet little available information on the parasites of Egyptian reptiles, with only sporadic works had been done (Linstow 1894 &1899; Belle, 1957; Yamaguti, 1958 & 1961; Myers et al., 1962; Moravec et al., 1987; Daszak and Ball, 1991; Ashour et al., 1994; Al-Bassel, 1994; Alaa Al-Deen et al., 1995 and Mazen et al., 1996).

Crocodiles are particularly incriminated in being the source of many nematode species (Paperna; 1980) that mostly developed via fish intermediate hosts, which form part of the food chain of the final host. The presence of such nematode larval stages in fish is considered actually a problem of great economic importance. Mass communication in Egypt has recently erected this problem which propagated and still being uncontrolled among fishes in Lake Naser. Mahmoud et al (1989), reported an infestation rate of 52.38% of nematode larvae isolated from *Tilapia species* in Lake Naser and was identified as *Amplicaecum* type larvae.

The present article is devoted to investigate the nematode parasites of crocodiles in order to identify their role as a source of nematode larvae infesting fish species in Southernmost part of Egypt; Lake Naser .

MATERIAL and METHODS

I. Parasitological examination of crocodiles:

Three Nile crocodiles (*Crocodilus niloticus)* of body length, 130, 86 and 63 cm were captured from Lake Naser at Aswan Governorate, during spring season, 1998 and examined for the presence of nematode parasites according to *Reichenbach-Klinke and Elkan (1965)*. After removing the skin, the abdomen was longitudinally opened to explore the visceral cavity. The muscular tissues are macroscopically investigated in addition to microscopic examination of random samples

of muscles using digestion technique mentioned by Meyer and Olsen (1971). The oral cavity was inspected, stomach and intestinal canal were split along their whole length and their wall as well as contents were examined. The detected worms were fixed and preserved in glycerin alcohol, cleared in lactophenol and mounted in gelatin (Soulsby, 1982) and then examined, measured and microphotographed.

II. Collection and preparation of Nematode eggs:

Eggs were obtained from the detected adult females, which were freshly collected from the infested crocodiles as well as from crocodile's gut contents. The number of eggs/one gram content was calculated according to Soulsby (1982). After several washes and sedimentation in normal saline, eggs were incubated in 1% formol saline in petri dishes at 23-25°C (Anderson, 1988) and they were daily aerated and microscopically observed for further development.

As the 2nd larval stages (L_2) developed and hatched, a small drop containing 2nd stage larvae was put on a slide, fixed in diluted Lugol's Iodine, covered then microscopically examined, counted and microphotographed. Furthermore, groups of these 2nd stage larvae were washed and kept in petri dishes containing normal saline and their number/1 ml of saline was calculated.

III. Experimental infection of fish:

1) **Infection of invertebrate host**: In a large petri dish, group of the 2nd larvae were exposed to parasite free fresh water *Cyclops sp* (laboratory breed). Samples of *Cyclops* were examined microscopically immediately and every 6 hours to observe that they have already ingested the 2nd larvae.
2) **Fish inoculation**: Two groups of nematode-free *Tilapia species* fish (9-11 cm body length); obtained from Beni Suef hatcheries; were kept in the laboratory in a clean aerated large aquarium and fed on dry meal. Each group consisted of 15 fish (12 experimental and 3-control non-infected; marked by cutting part of the tail). In group I, each experimental fish was orally dosed with about 100 motile 2nd larvae by using capillary Pasteur pipettes. In-group II, each experimental fish was orally infected with 15 *cyclops* containing 2nd larvae. Two fish from each group were sacrificed and examined for the presence of larvae after 12 hours, 7 days, 15 days then every 2 weeks up to 60

days post-infection. The detected larvae were measured and microphotographed.

RESULTS

I. Parasitological examination of crocodiles:

Examination of three Nile crocodiles showed that one of them (130 cm length) was infested with one nematode species identified as *Dujardinia dujardini (Travassos, 1920); Syn. Dujardinascaris helicina, Molin, 1860* represented by 4 females and 3 males. The second crocodile (86 cm length) was found infested with three larvae of *Amplicaecum sp. (Baylis; 1920)*, while the third crocodile was found free of nematode parasites.The number of *D. dujardini* eggs/gm of gut content of the infested crocodile was 800-1400.

II. Morphological description:

1. **Adult** *Dujardinia dujardini: (Order: Ascarididea; Family: Heterocheilidae,* Railliet & Henry, 1915; *Subfamily: Filocapsulariinaesyn. Anisakinae, Genus Dujardinia,* Gedoelst, 1916- *syn. Dujardinascaris,* Baylis, 1947. Adult male and female were detected from the pyloric region of the stomach, Fig. (1); both were dark creamy in colour with spiral brown band around the body which appears more clear in females. The body of the worm tends to form several coils. They measure 13.35-15.4 (mean 14.38) mm in length and 0.53-0.65 (mean 0.59) mm as maximum breadth as well as 27.2-35.6 (mean 31.4) mm in length and 0.93-1.08 (mean 1.01) mm as maximum breadth in male and female respectively. The body cuticle is smooth and transversely striated. The anterior end is provided with three large lips (one dorsal and two sub-ventral) with small interlabia. Lips are without dentigerous ridges, the cuticle of their internal surface produced into large tooth-like structures, which are carried by three main cuticular lobes on the anterior border of each lip. The oesophagus is formed of anterior muscular portion and a small posterior bulb, its whole length is 2.26-2.49 (mean 2.45) mm and 3.21-3.70 (mean 3.45) mm in male and female respectively, Fig. (2 A & C). There is an anterior coecum springing from the intestine and extends forward alongside the oesophagus to the length of 1.66-1.73 (mean 1.71) mm in male and 2.10-2.33 (mean 2.22) mm in

female. The excretory pore opens 0.65-0.69 (mean 0.67) mm from the anterior end of the worm.

Male posterior end, Fig. (2 D) is curved and provided with two equal spicules measuring 2.9-3.04 (mean 2.97) mm long. The gubernaculum is large, with an expanded and solid head while tapered and hallows posteriorly. The caudal papillae are five in number. Small caudal alae are present in the cloacal region.

In female, valva is situated in the anterior half of the body and opens into a muscular sucker like atrium which lies 2.59-3.31 (mean 2.95) mm and 1.26-1.35 (mean 1.31) mm from the anterior end and posterior oesophago-intestinal junction respectively. The female posterior end Fig. (2 B) is pointed sharply ended and the tail region is 0.56-0.61 (mean 0.59) mm long. The gravid females contain unembryonated eggs in the proximal part of the uterus. The collected eggs from female worms and gut contents of crocodiles are unembryonated, subglobular in shape, with transparent, finely pitted shell, measuring0. 053-0.071 X0. 084-0.092 (mean 0.060 X0. 088) mm; Fig. (3 A).

2. ***Amplicaecum sp.*** **larvae**: Three larvae were detected from the muscular tissue of the crocodile abdomen. They are cylindrical in shape, with 2.22-3.65 cm body length. About the anterior third of the larval body was found embedded in the abdominal muscles. The oral opening is surrounded by three small lips with dentigerous ridges and small interlabia. The oesophagus is muscular and measures 3.19-4.30 (mean 4.11) m.m in length with the oesophageo-intestinal valve posteriorly that connect it to the intestine. The nerve ring is noticed around the oesophagus about 0.11-0.14 (mean 0.13) mm from the anterior body end. The intestine opens into the anal opening, which lies on the ventral aspect of the body. The posterior end is straight and is 0.20-0.23 (mean 0.22) mm in length with a gently pointed end(Fig.4).

III. Results of *D.dujardini* egg embryonation:

The incubated eggs were embryonated to the 1st larvae within 3-5 days at 23-25° C, Fig. (3 A), which developed further and moult in the eggs into the 2nd larvae after 6-8 days Fig. (3 B). They retain the cuticle of the 1st larvae and hatched spontaneously within 2-3 days at the same temperature, Fig. (5 A). The sheathed larvae were actively motile in the petri dishes and after 1-2 days, some larvae

retained the 1st stage cuticle, Fig. (5 D) while others shed it, Fig. (5 B).

Morphologically these 2nd stage larvae have curved body with a total length of 0.392-0.462 (mean 0.427) mm and a maximum breadth of 0.012-0.015 (mean 0.135) mm. The cuticle is smooth with transverse striations along the whole length. The cephalic end has four small elevations and the posterior end terminates by a sharp point. The oesophagus is elongated and composed of two parts separated with a narrow constriction into an anterior cylindrical part and a posterior pyriform (rhabditiform oesophagus). The total length of the oesophagus is 0.083-0.141 (mean 0.112) mm. The nerve ring surrounds the oesophagus at a distance of 0.051-0.112 (mean 0.081) mm from the anterior tip of the worm. Numerous oesophageal glands extended along the whole length of the oesophagus. The intestine appeared packed with refractive granules and is connected to the anal opening with the rectum (measures 0.021-0.032 mean 0.026 mm in length) which was surrounded with 4-7 relatively large glands. The tail measures 0.046-0.051 (mean 0.048) mm in length Fig. (5 C).

IV. Result of experimental infection:

2-5 larvae were observed alive in the haemocoel of the infected *Cyclops* 6-12 hours Fig. (6) after putting the active free 2nd larvae with the mentioned crustacean.

The experimentally infected fish with free L_2 and those with *Cyclops* containing L_2 as well as the control group were examined and revealed the following: -

1) Presence of living L_2 in the gut of the examined fish in both groups 12 hours post infection.
2) Detection of the exsheathed larvae (0.8-1.2 mm in length) in the intestinal mucosa of the examined fish in both groups one week post infection.
3) The early developed 3rd larvae (L_3) which measured 3.63-5.42 (mean 5.27) mm in length and 0.161-0.190 (mean 0.175) mm in breadth were detected in the abdominal cavity of fish in both groups 15 and 30 days post infection. They are cylindrical in shape having an oral opening surrounded with rudimentary lips and a tooth like structure present ventro-lateral to the oral opening. The oesophagus is divided into an anterior tubular and posterior widened parts with well

distinct sphincter, its whole length is 1.12-1.46 (mean 1.3) mm, the posterior end measures 0.085-0.13 mm long, Fig. (7 A, B &C).

4) At days 45 and 60 post infection, the detected L_3 in the abdominal cavity of the examined fish were more developed and increase in size; the length ranged from 8.13-10.91 (mean 9.61) mm and the breadth was 0.24-0.35 (mean 0.29) mm. The oesophagus appears more developed with a whole length of 1.83-2.06 (mean 1.94) mm and provided with a prominent posterior ventriculus that measuring 0.34-0.55 (mean 0.45) mm (Fig. 8).
5) The intensity of infection with the 3rd stage larvae in the abdominal cavity of the infested fish was 4-9/fish in the 1st group that administrated free L_2 while it was 3-5/fish in the group administrated *Cyclops* containing L_2 .Fig.(9) shows 3rd stage larvae in the abdominal cavity of infested *Tilapia sp.* fish.
6) Control non-infected fish of both groups were found free from any nematode larvae till the end of the trial (60 days).

DISCUSSION

The present work was carried out on three *Crocodilus niloticus* captured from Lake Naser, Egypt and revealed the detection of males and females *Dujardinia dujardini* nematode species. Morphologically, the detected worms came in agreement with the descriptions mentioned by York and Maplestone (1926), Yamaguti (1961), Hartwish (1974) and Sprent (1977). The presence of this nematode in the stomach of crocodiles supported the findings of Paperna (1980) who denoted that, crocodiles are definitive hosts for the heterocheilid *Dujardinia species.*

Experimentally, *Dujardinia dujardini* could be transmitted to fish intermediate host (*Tilapia species)* whether the second stage larvae passed through invertebrate host "*Cyclops sp.*" or not. This finding concluded that, the invertebrate host might act as a paratenic not as an intermediate host for this nematode species. Also, Kahl (1936) believed that, the second stage larvae could directly infect fish intermediate host. Moreover, Anderson (1992) mentioned that, the use of vertebrates as intermediate host and invertebrate as paratenic host in order to place the second stage larvae in the food chain of the vertebrate intermediate host is a basic feature of the transmission of the ascaridoides in aquatic habitats. On the other hand, Paperna (1980) considered *Crustacea* as

first intermediate host for heterocheilid nematodes. The worker concluded here that, compating crustaceans (paratenic host) could be valuable in controlling the spreading of these nematode parasites.

Concerning the detected *Amplicaecum sp.* larvae in one crocodile, they were morphologically identical to those described by Mahmoud et al (1989) who collected them from the abdominal cavity and sinus venosus of *Tilapia sp.* in Lake Naser. The presence of such larvae in crocodiles might support the report of Paperna (1980) in that crocodiles are final hosts for *Amplicaecum sp.* and / or their presence might be resulted from eating fish containing such nematode larvae, a point that needs more research in the near future.

Although, the report of *Dujardinia sp.* larvae from fish in Lake Tanganyika, Congo, it was worthy to mention that, the present work is considered as the first study dealting with the patterns of transmission of *Dujardinia species* from crocodiles as final host to the fish intermediate host. In addition, no reports were recorded on larvae as natural infestation among Egyptian fish species, particularly in Lake Naser.

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Fig. (1): *Dujardinia dujardini* adult in the stomach of crocodile.

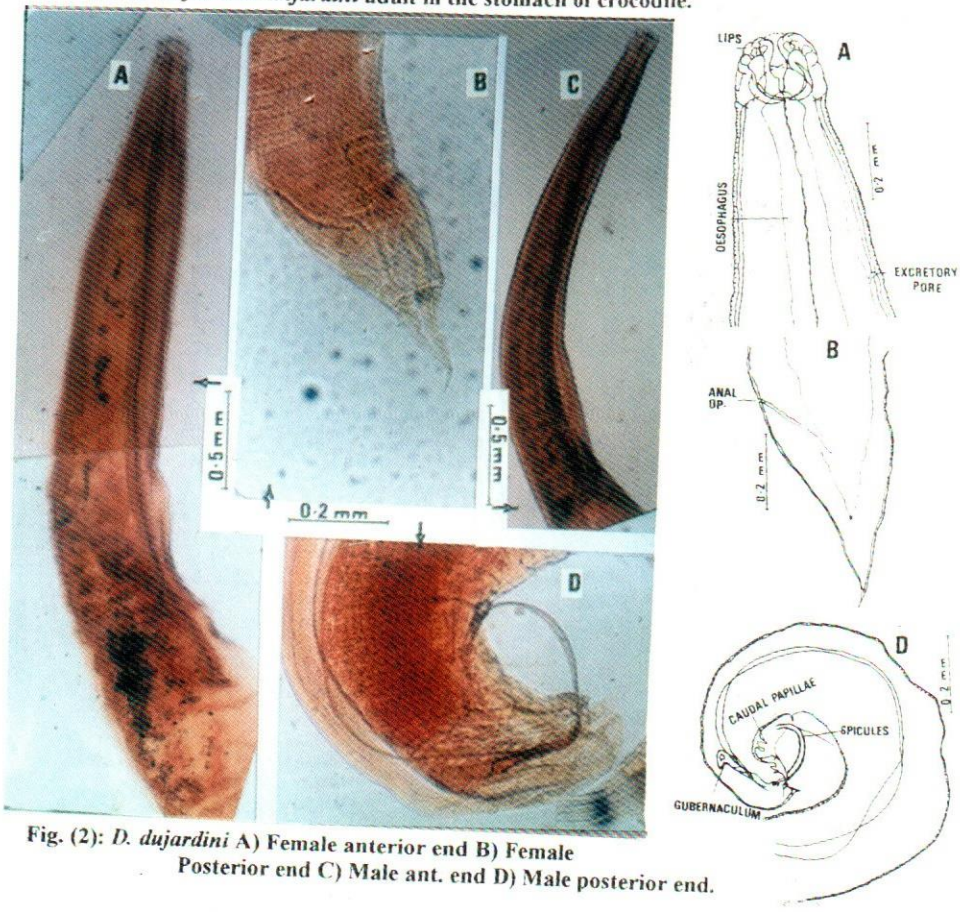


Fig. (2): *D. dujardini* A) Female anterior end B) Female Posterior end C) Male ant. end D) Male posterior end.

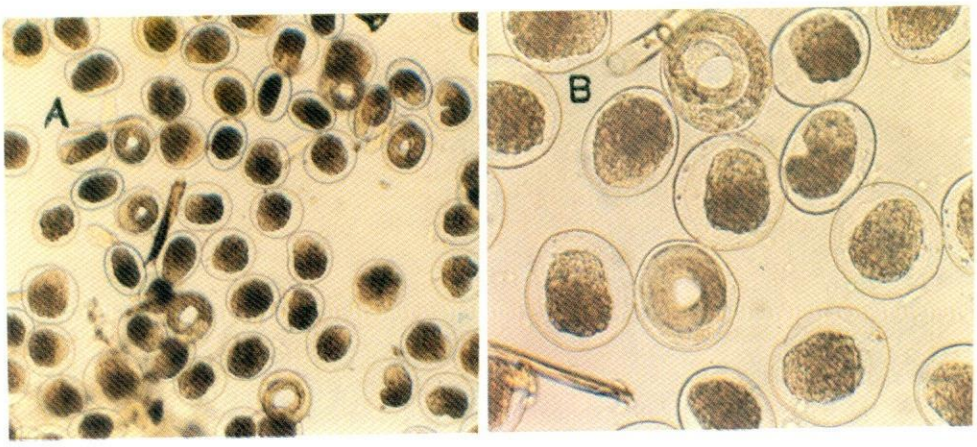


Fig. (3): *D. dujardini* eggs A) Unembryonated and containing L_1 B) Eggs containing L_2.

Fig. (4): *Amplicaecum sp.* larvae.

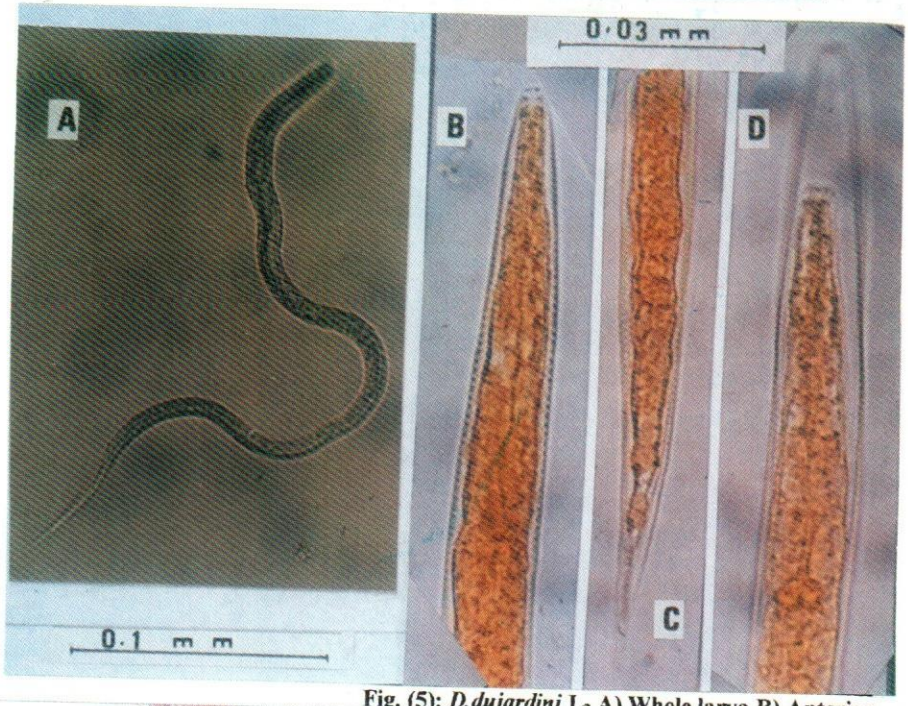
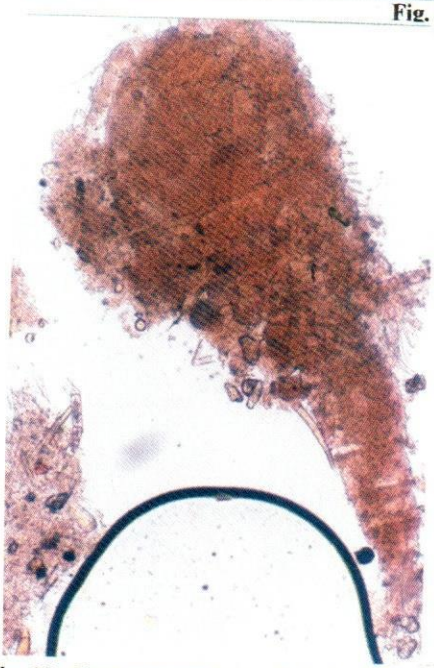


Fig. (5): *D.dujardini* L_2 A) Whole larva B) Anterior end C) posterior end D) Ant. end sheathed.



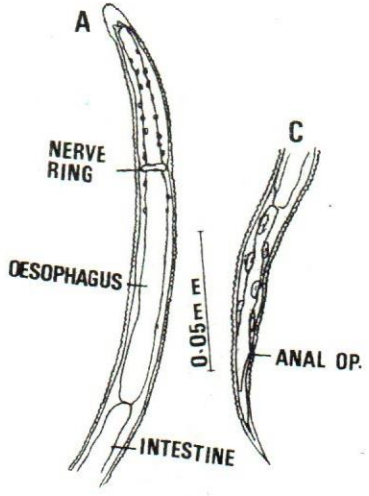


Fig. (6): Cyclops containing *D. dujardini* L_2.

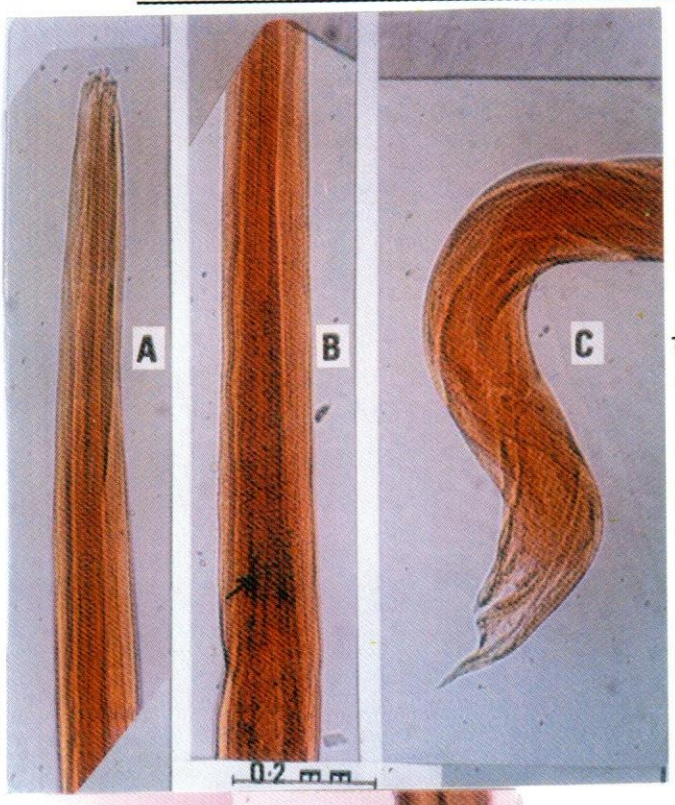


Fig. (7): *D. dujardini* early 3rd larva A) ant. end
B) Oesophogeal region C) posterior end.

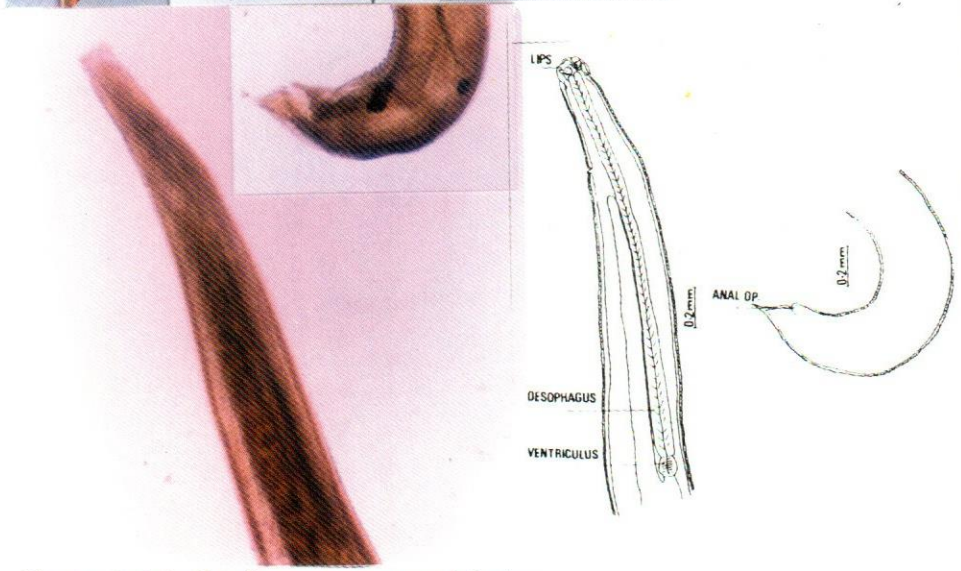


Fig. (8): *D. dujardini* 3rd larva 60 days post infection.

Fig. (9): *Tilapia sp.* fish with *D. dujardini* L_3.