

## **Experimental infection of fresh water snail *Lymnaea auricularia* with *Clinostomum complanatum* (Trematoda: Clinostomidae)**

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### **Abstract:**

The digenetic trematode *Clinostomum complanatum* is naturally parasitizes the throat and esophagus of piscivorous birds, used aquatic snail and fishes (rarely frogs and toads) as first and the second intermediate hosts respectively.

The aim of this study was to investigate the possibility of infection of fresh water snail *Lymnaea auricularia* with miracidium of *Clinostomum* because lacking the study on the life cycle of this parasite in Iraq.

Snails were collected from vegetation and debris in shallow water of Euphrates river and identified as *Lymnaea auricularia*, their eggs were isolated for production of new generation which used for the infection with *Clinostomum* miracidium.

Eggs of mature trematodes *Clinostomum complanatum* were isolated from the buccal cavity and esophagus of *Bubulicus ibis* and hatched to miracidium after 21 days of incubation, The fresh water snails *Lymnaea auricularia* were experimentally infected with miracidium of *C. complanatum*. The miracidium were ciliated with eye spot in the anterior third of body, the infected snails with these miracidium released cercariae after 30 days post infection. The cercariae of *C. complanatum* were fork tailed with two eye spots in the anterior part of middle third of the body.

**Keywords:** experimental infection, *Lymnaea auricularia*, *Clinostomum complanatum*.

## إصابة تجريبية لقواقع المياه العذبة *Lymnaea auricularia* بمتقوية *Clinostomum complanatum* (Trematoda: Clinostomidae)

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### الخلاصة:

تتطفل المتقوية ثنائية المضيف *Clinostomum complanatum* في بلعوم ومريء الطيور التي تقتات على الاسماك وتستخدم القواقع المائية والاسماك (احيانا الضفادع والعلاجيم) كمضيف وسطي اول ومضيف وسطي ثاني على التوالي. هدفت هذه الدراسة للبحث عن امكانية اصابة قواقع المياه العذبة *Lymnaea auricularia* بمهدبات *Clinostomum* بسبب عدم وجود اي دراسة حول دورة حياة هذا الطفيلي في العراق. عزلت البيوض من الديدان البالغة لمتقوية *Clinostomum complanatum* المتواجدة في التجويف الفمي والمريء لطيور أبي قردان *Bubulicus ibis* وبعد مرور 21 يوما من الحضانة فقسّت إلى مهدبات، أصيبت قواقع المياه العذبة *Lymnaea auricularia* بمهدبات طفيلي *C. complanatum* مختبريا. اتصفت المهدبات بكونها مغطاة بالأهداب وباحنواؤها على زوج من البقع العينية الواقعة في مقدمة الثلث الأمامي من الجسم ، انطلقت المذنبات من القواقع المصابة بهذه المهدبات بعد مرور ثلاثين يوما على الإصابة. أن مذنبات طفيلي *C. complanatum* هي من المذنبات مشطورة الذنب والمزودة بزوج من البقع العينية الواقعة في مقدمة الثلث الوسطي من الجسم.

## 1- Introduction:

The digenetic trematode *Clinostomum complanatum* is naturally parasitizes the throat and esophagus of piscivorous birds (Yamaguti, 1958), used aquatic snail and fishes (rarely frogs and toads) as first and the second intermediate hosts respectively (Lo *et al.*, 1982).

Hunter and Hunter (1934) described the life cycle of *Clinostomum spp.* in which the ciliated larva miracidium hatches from the *egg* in water, enters a snail (first intermediate host) and, shedding its cilia to changed into a sporocyst, rediae and cercariae. Cercariae leave the snail and come into contact with and penetrate fishes (second intermediate hosts). On entering the fish, cercariae shed their tails and secrete cysts, becoming metacercariae (yellow grub). These become adults when the fish ingested by the definitive host (a bird, usually a heron). The metacercariae migrate to the esophagus where the worms attach to the inner lining of the buccal cavity, upper esophagus, or trachea and become sexually mature adults in four to six hours. As the heron feeds, eggs are expelled into the water and the cycle is completed.

The whole or part of the life cycle of this species has been studied by several authors, who demonstrated that it includes a mollusc as first intermediate host, a fish as second intermediate host, and a fish-eating bird as the final host (Liao, 1993 ; Chung *et al.* 1998).

Metacercariae and adult stages of *Clinostomum* have been reported from freshwater fishes and water birds of Iraq respectively (Al-Salim and Ali, 2010), but there is no study on experimental infection of intermediate host in Iraq.

The aim of this study was to investigate the possibility of infection of fresh water snail *Lymnaea auricularia* with miracidium of *Clinostomum*.

## 2- Materials and Methods:

Snails were collected by hand-picking them from vegetation and debris in shallow water of Euphrates river in Thi-Qar governorate / south of Iraq . The snails were brought into a laboratory of parasites/Biology Department/College of Education for Pure Science/Basrah university in March 2017. The snails were identified as *Lymnaea auricularia* (Din Kakar *et al.*, 2017), their eggs were isolated in jars and incubated in laboratory for production of new generation which used for the infection with *Clinostomum* miracidium Fig. (1).



Fig.(1): *Lymnaea auricularia* (1 month age)

Adult *Clinostomum* worms Fig. (2) were isolated from buccal cavity and esophagus of cattle egret *Bubulicus ibis* (final host of parasites) in normal saline 0.85% and the eggs were isolated by fine needles from uterus of adult worms and transferred by fine glass pipette to small Petri dishes with enough a mounts of distal water.

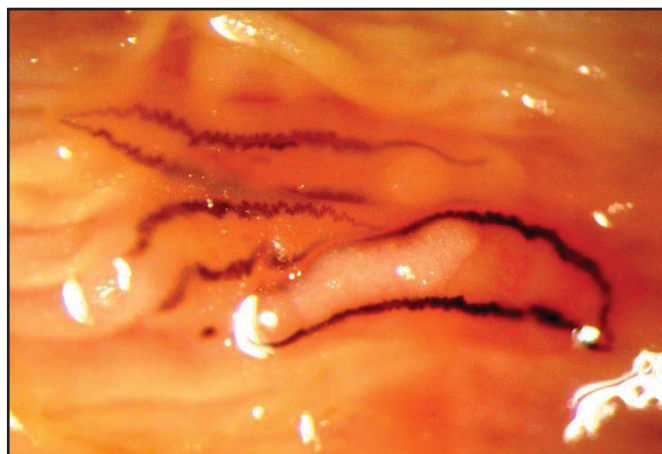


Fig. (2): Adults of *C. compalاناتum* attached to the esophagus of *B. ibis*

The eggs were incubated for 22 days on 25-30°C under constant illumination, the distal water in dishes were changed every day during the incubation period. Some eggs were examined daily under the light microscope to follow the embryonic development steps until the miracidium hatched from the eggs.

Five miracidiae were added for each snail kept in incubation chamber and leaved for 24 hours then returned to jars and incubated for 30 days, the snails were exposed to artificial light to verify the release of cercariae then isolated and preserved in formalin 10%, all the measurements were done in mm and the parasites painted by camera lucida and photographed by digital camera.

### 3- Results and discussion:

#### The Adult:

The adult worms in the current study were obtained from buccal cavity and esophagus of *B. ibis*. Adult stage of parasite has been reported from *Nycticorax nycticorax*, *Ardea purpurea*, *Egretta garzetta* and *Egretta alba* in Iran (Shamsi *et al.*, 2013).

Chung *et al.* (1995) revealed that the *C. complanatum* accidentally infected human and caused pharyngitis, many of fresh water fishes play a role as the source of human infection throughout the world and they described and measured the adult specimens of *C. complanatum* recovered from the buccal cavity of experimentally infected chicks after four days

The adult worms appeared with longitudinal shape and were 4.466-7.076 (6.857) mm length by 1.832-2.394 (1.857) mm width, posterior end more rounded than the anterior end, oral sucker was transverse 0.470-0.580 (0.535) mm length by 0.514-0.928 (0.857) mm width, pharynx and esophagus were unclear, ventral sucker was elongated 0.725-0.899 (0.857) mm length by 0.714-0.841(0.821) mm width and located in the anterior third of the body, ceca were branched behind the oral sucker and stained in dark color, oval ovary was located between the testes and measured 0.348-0.435(0.428) mm length by 0.696-0.761(0.714) width, anterior testis 0.551-0.761(0.714) mm length by 0.609-0.725(0.642) mm width and the posterior one 0.435-0.609(0.571) mm length by 0.638-0.891(0.891) mm width, uterus located between the ventral sucker and the posterior testis and contain many oval shaped eggs, cirrus pouch obscure and the follicular vitellaria were distributed along the lateral margin of the body between the posterior part of ventral sucker to the posterior end of the body. Fig.3. (A, B).

Al-Salim and Ali (2010) described that adult stage of *C. complanatum* isolated from buccal cavity of *Ardea cinerea* and pharynx, tongue of *Ardeola ralloides* This was the first record and description of parasite. Both *A. cinerea* and *A. ralloides* regarded as new hosts record in Iraq, they also isolated *C. dasi* from *Botaurus stellaris* and *Clinostomum* sp. from each of *A. cinerea* and *A. ralloides* in the east Al-Hammar marsh.

Al- Mayah (1994) recorded that immature specimens of *C. phalacrocoracis* from proventriculus of pygmy cormorant in Al-Sweeb Marsh north-east of Basrash province.

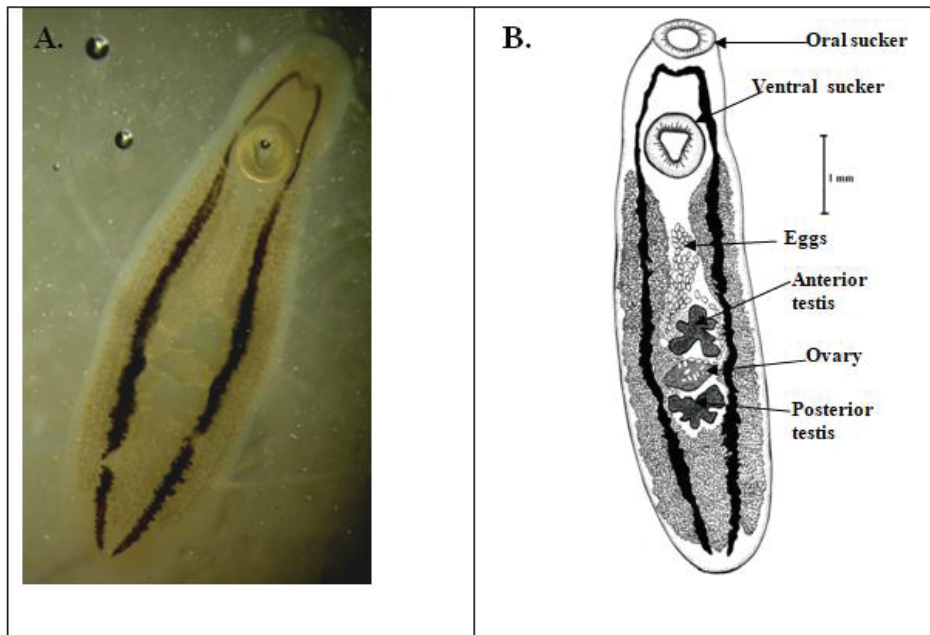


Fig.(3): A. Adult of *C. complanatum*. B. Lucida painting.

#### The eggs:

The eggs were oval in shape 0.107-0.124(0.116) mm length by 0.074-0.066 (0.073) mm width, and with operculum in one side of egg. Fig. 4. (A, B). The eggs measurement and shape of the present study agreed with previous measurements and shape of matured eggs of *C. complanatum* (Chung *et al.*, 1995 ; Al-Salim and Ali, 2010).

After seven days of incubation under constant condition on (25-30)<sup>o</sup>C with illumination, the egg contain many cleaved cells looked very crowded in the center of egg Fig. 5 (A, B). The development of embryo inside the egg continued and after sixteen days the miracidum looked very clear with two eye spots inside egg shell, Fig. 6. (A, B). Then egg was hatched and free swimming miracidium was release after twenty two days of incubation.

Miracidiae in current study were penetrate the bodies of the *L. auricularia* snails and developed to larval stages and finally released cercariae after 30 days of infection with miracidiae.

Several mollusc species have been described as natural first intermediate hosts for *C. complanatum*: *Radix auricularia coreana* in Korea (Chung *et al.*, 1998), *Radix auricularia swinhoei* in Taiwan (Lo *et al.*, 1981) and *Helisoma antrosum* and *Helisoma campanulatum* in the USA (Hunter and Hunter, 1934). Three species of fresh water snails, *Lymnaea auricularia*, *L. stagnalis* and *Radix ovate*, have been reported as the first intermediate hosts of *C. complanatum* in Poland (Grabda-Kazubaska, 1974) and Taiwan (Lo *et al.*, 1982), while *L. japonica* and *L. ollula* were reported as the experimental snail hosts in Japan (Aohagi *et al.*, 1993).

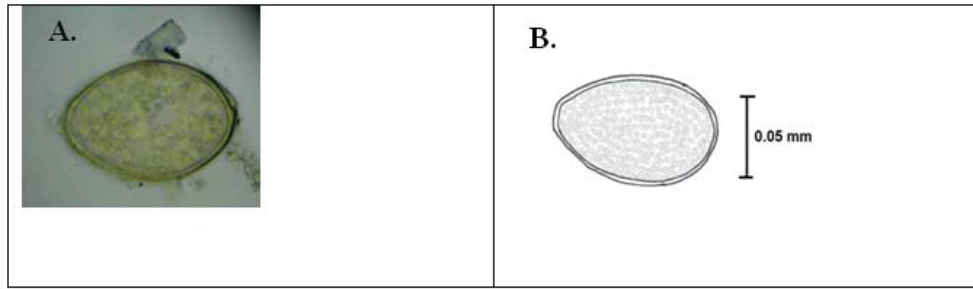


Fig. (4): A. Egg before incubation 40X. B. Lucida painting.

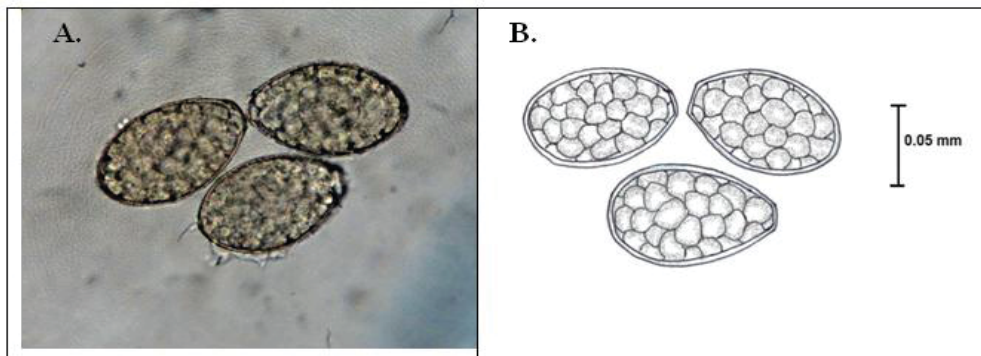


Fig.(5):A. Egg after 7 days 40X. B. Lucida painting.

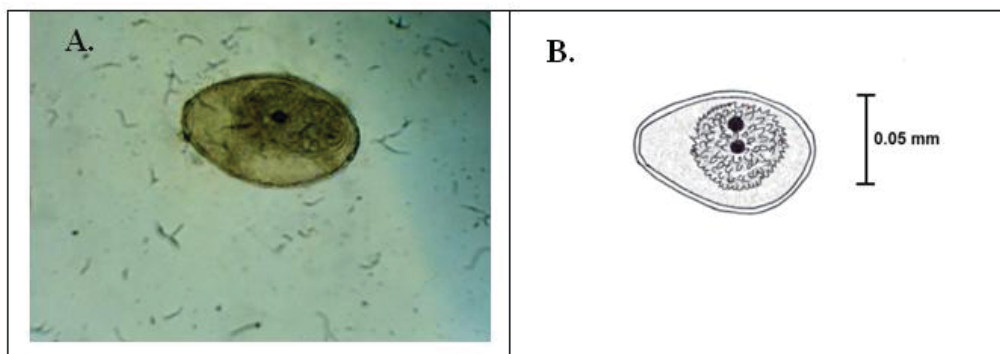


Fig.(6): A. Egg contain miracidium after 16 days 40X. B. Lucida painting.

### **The miracidium description:**

The miracidium, whose length 0.104-0.076 (0.091) mm is greater than its width 0.061-0.053 (0.057) mm, is characterized by the movement of cilia (hair-like structures) on the body surface with eye spot in the anterior third part of the body, Fig. 7. (A,B). It was characterized with speed swimming movement and they were die after few hours from hatching.

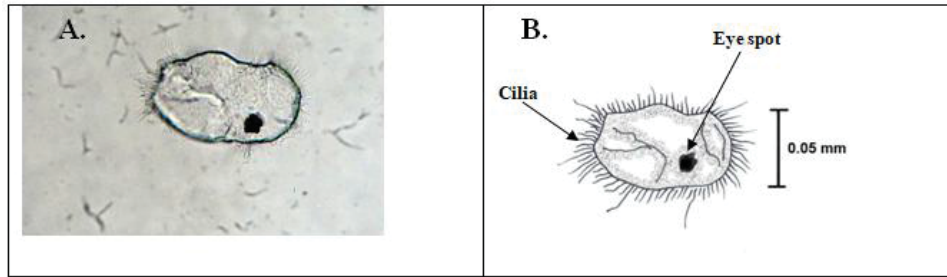


Fig.(7): A. Miracidium hatched after 22 days 40X. B. Lucida painting.

**The cercariae description:**

The cercaria of *C. complantum* was fork tailed with two eye spots in the anterior part of middle third of the body, Fig. 8. (A, B). The cercaria characterized with floating movement in the middle level of water during the resting and speed spiral turnover toward the light source, total length 0.292-0.395 (0.350) mm, the body measured 0.105-0.143 (0.120) mm length by 0.036-0.040 (0.038) mm width, the tail stem 0.210-0.350 (0.250) mm length by 0.022-0.027 (0.025) mm width, furca 0.048-0.090 (0.064) mm length by 0.01-0.024 (0.017) width and eye spot diameter 0.0045-0.0061 (0.0052) mm.

The measurements of cercariae of *C. complantum* which released from experimentally infected *L. auricularia* in current study were compared with previous studies in table 1.

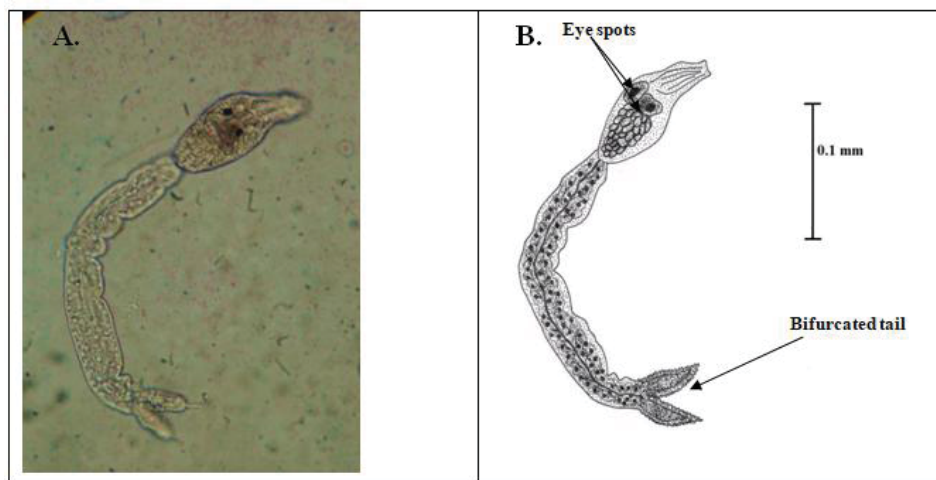


Fig.(8): A. Dorsal view of *C. complantum* cercaria. B. Lucida painting.

Table 1. Measurements of *C. complantum* cercariae (in mm)

compared with other studies.

The studies	Current study	Chun <i>et al.</i> (1998)	Aohagi <i>et al.</i> (1993)	Aohagi <i>et al.</i> (1993)	Lo <i>et al.</i> (1982)
Snail host	<i>L. auricularia</i>	<i>Radix auricularia coreana</i>	<i>L. japonica</i>	<i>L. ollula</i>	<i>R. ovata</i>



Total length	0.292-0.395 (0.350)	0.492-0.542 (0.521)	0.497-0.549 (0.516)	0.479-0.546 (0.510)	0.528
Body length	0.105-0.143 (0.120)	0.119-0.147 (0.127)	0.104-0.124 (0.114)	0.110-0.122 (0.116)	0.130
width	0.036-0.040 (0.038)	0.033-0.036 (0.034)	0.035-0.040 (0.036)	0.032-0.038 (0.034)	0.033
Tail stem length	0.210-0.350 (0.250)	0.275-0.370 (0.320)	0.294-0.319 (0.304)	0.277-0.319 (0.298)	0.195- 0.360
width	0.022-0.027 (0.025)	0.019-0.026 (0.023)	0.021-0.027 (0.024)	0.022-0.027 (0.024)	0.013- 0.020
Furca length	0.048-0.090 (0.064)	0.072-0.104 (0.085)	0.076-0.107 (0.093)	0.076-0.107 (0.094)	0.060- 0.100
width	0.01-0.024 (0.017)				
Eye spot	0.0045-0.0061 (0.0052)	0.0044-0.0062 (0.0051)	0.0045-0.0063 (0.0053)	0.0045-0.0063 (0.0053)	0.0051

Sharif *et al.* (2010) recorded 0.2% of snails *L. gedrosiana* were infected with clinostomidae furcocercariae in Iran.

Cercariae of *C. complanatum* were swam in aquatic environment until find the second intermediate host (Fishes) and then penetrate fish body to develop to the metacercarial stage in different fish tissue sites. The importance of fishes for the life cycle of the *C. complanatum* was related to the probability of being preyed on by the definitive avian hosts (Dias *et al.*, 2003).

In Iraq Bashe and Abdullah (2010) have isolated *C. complanatum* metacercariae from branchial cavity of spiny eel *astacembelus mastacembelus* from Greater Zab river, north of Iraq.

Abdullah and Mhaisen (2011) have isolated *C. complanatum* metacercariae from branchial cavity of *Barbus luteus*, *Cyprinion macrostomum*, *Leusiscus lepidus* and *Varicorhinus umbla* fishes in Greater Zab river, north of Iraq with percentage 37.5%, 13.3%, 13.7% and 14.2% respectively.

*C. complanatum* are widely distributed parasites in Iraq with high infection rates in final and intermediate hosts, there for these parasites need more studies about their stages of life cycle and pathological effects in these hosts

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