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Larval stages of trematodes isolated from the *Lymnaea auricularia* (freshwater snail) in Basra.

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Abstract:

Freshwater snail *Lymnaea auricularia* that is known as the intermediate host for Trematode larval stages were studied by using cercarial emergence and crushing. 659 samples were collected from August 2018 to July 2019 from five stations represented by Karmet Ali, Musab, Qurna, Shafi and El Deer. It was found the infection rate was 27.01%. Five species belong to three different groups including two species were recorded for the first time in Basra. *Echinostoma cercariae* B, *Armata xiphidiocercous cercariae* B and three species were recorded previously: *Virgulate xiphidiocercous*, *Echinostoma cercariae* A and *Armata xiphidiocercous*. The aim of the study is to determine the effect of the environmental agents in Basra Province on the cercaria diversity and spread in *Lymnaea auricularia*.

Key words: Trematode, Cercariae, Metacercariae, *Echinostoma*, *Xiphidiocercous*, *Virgular*, *Armata*.

Introduction:

The fresh water gastropods have a tremendous effect on human and veterinary health since they are intermediate hosts for the human and animal's infections including schistosomiasis, fascioliasis and other trematode infections. Few studies were made on trematodes larval stages. Wajdi *et al*, (1979) examined the susceptibility of Iraqi fresh water snails to infection with *Schistosoma haematobium* and *Schistosoma mansoni*. Egyptian strains at the seventies decade of the 20th century characterized with coming of millions of Egyptian workers in Iraq. Jaffer (1980). commented on larval trematodes in Iraqi snails giving numbers descriptions and measurements. Yacoub (1985) studied the epidemiology of *Schistosoma haematobium* infection in Basra, southern Iraq. Al- Mayah (1990) pointed out his search for helminthes in some aquatic birds, wrote some notes about swimmer itch in Basra. southern Iraq. Al-Hussein (2000). made a taxonomic study of several races of the trematode described their inferiority under the family in Basra, southern Iraq. Al-Ali (2002); Al-Mayah *et al* (2005) and Al-Mayah and Awad (2005) showed the favorite growth and development of *Fasciola gigantica* in the snail intermediate host *Lymnaea auricularia*.

MATERIAL AND METHODS:

About 659 snails of *Lymnaea auricularia* were collected from five stations including: Karmt Ali, Masehab, Qurna, Shafi and El Deer during the period from August 2018 to July 2019. The samples were either collected with a scoop or picked up directly by hand, maintained in suitable size plastic container filled with freshwater from the irrigation channel and brought to the lab as soon as possible. The snails were placed in a Petri dish with a little sterile water after taking the length measurement with the Fern instrument. The snail breaks down to release the cercariae under dissecting microscope. Then the cercariae were examined under the light microscope and Photomicrographs by (Leica ICC50 HO) camera.

Results:

It was found that 178 out of the 659, (27.01%) samples were infected with the trematodes. The average length of snails was {10.73-18.58 (av.14.46)} millimeter (Table 1).

During the whole study period in August 2018 to July 2019 snail samples were obtained except 8-11 months.

The infection of the snails with *Echinostoma* cercariae occurred during months January, February, March and May.

While the infection with *Armatae xiphidiocercous* cercariae observed in the period from December, Feb, Mar, May, Jun and Jul. The infection with *Virgulate Xiphidiocercous* cercariae was recorded in Feb, April and June.

Table1: the number of examined snails and the rate infected fact shows the percentage of infection

Mont hs	Tested snails	Average lengths of snails infected	Numb er of snails infect ed	Injury rate	Types cercaiea
Aug	0	0	—	—	—
Sep	0	0	—	—	—
Oct	0	0	—	—	—
Nov	0	0	—	—	—
Dec	45	13.09-18.24	8	17.39%	<i>Armatae xiphidiocercous</i> cercariae A
Jan	113	11.33-17.35	21	18.58%	<i>Echinostomae</i> cercariae B and <i>Armatae xiphidiocercous</i> cercariae B
Fab	29	10.73-16.36	3	10.34 %	<i>Armatae xiphidiocercous</i> cercariae A and <i>Virgulate</i> <i>xiphidiocercous</i> cercariae
Mar	169	11.25-17.22	30	17.75%	<i>Echinostomae</i> cercariae A and <i>Armata xiphidiocercous</i> cercariae A

Apr	125	12.22-18.89	42	33.6%	Virgulate xiphidiocercous cercariae
May	43	10.34-16.32	16	37.2%	Echinostomae cercariae A ,B and Armata xiphidiocercous B
Jun	57	10.34-16.32	21	36.84%	virgulate xiphidiocercous cercariae and Armata xiphidiocercous A
Jul	78	10.13-18.32	37	47.43%	Armatae xiphidiocercous cercariae B and Echinostoma B
Sum	659		178	27.01%	

In the present study, three types of cercariae were observed represented by Echinostoma cercariae, Virgulate xiphidiocercous and Armata xiphidiocercous. Five species were diagnosed for the three types; two species were recorded for the first time in Iraq.

Type 1: Echinostome cercariae

Echinostoma cercariae A

The cercariae appeared in the study during varying periods in different collection areas (plate 1).

Description: Large Cercaria body with oval shape. It contains two types of suckers: the muscular circular, the anterior oral sucker which is surrounded by a row of spines. And the ventral sucker that is located in the center of the body

The esophagus was between pharynx and venter sucker, ceca reaching to bladder meet together before entering bladder. The penetration glands not present. The body has numerous number of cystogenous glands which in result gave it the dark color, flame cells pattern not determine. Tail is longer then the body. Cercariae were produced within redia which have one the birth opening (table 2).

Movement behavior: The cercariae move in or on the water surface by rolling up and springing back for about 7-10 seconds in speed. They remain active for 3-5 hours after emergence in the water.

Echinostoma cercariae B:

Description: (plate 2) The cercariae characteristic by a big spherical distinctive. They have two suckers the anterior oral sucker is located at front the body and surrounded by about 27 collar spines. Venter sucker located in the center of the body. The penetration glands not present. The dark body color due to the Large number for cystogenous glands they contain, sack quickly on snail sensors, Pharynx was lager muscular and two main excretory tubes meet before entering. They a very long tail with a distinctive extrusion at its end {95x35} mm. Cercariae were produced within redia is have two the birth opening (table 2). Echinostome cercariae B was recorded for the first time in Iraq.

Movement behavior: The cercariae actively move, by swimming in rotational form or by striking in all directions in a speed ranging between 5-10 seconds. They stay active for about 4-5 hours after emergence in the water.

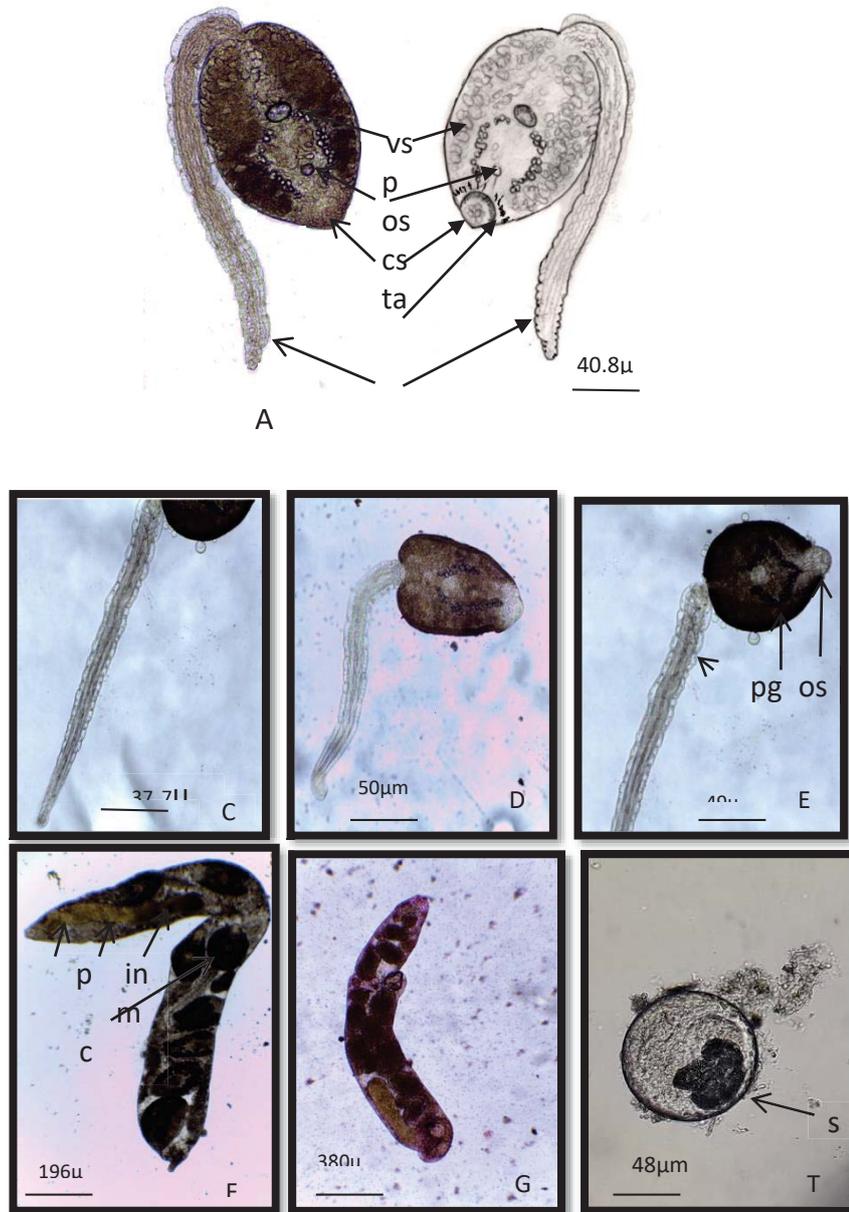


Plate (1). Image *Echinostoma cercariae* A; A. Cercaria without staining. B. Drawing of cercariae structure. (C, D, E). Cercaria without staining. (G, F). Redia without staining. T. Metacercaria without staining. Abbreviations : os -oral sucker, vs- venter sucker , p-pharynx , ta-tail , in-intetine , mo-mouth , sc- spine collar.

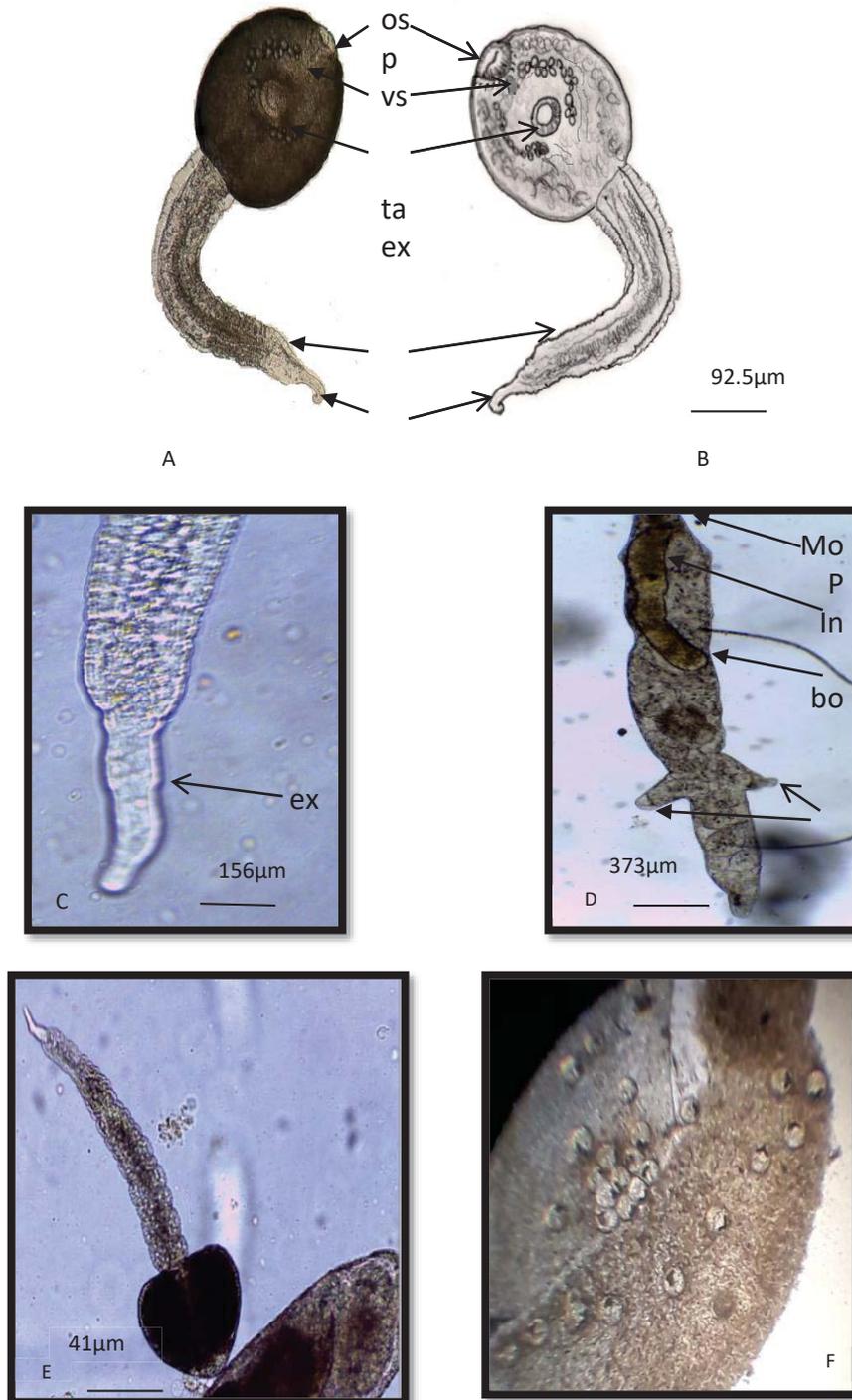


Plate 2. Image Echinostoma cercariae B; A. Cercaria without staining. B. Drawing of cercariae structure. C- extrusion. (E). Cercaria without staining. (D). Redia without staining. (F). Metacercaria without on snail sensors. Abbreviations: os -oral sucker, vs- venter sucker, p-pharynx, ta-tail, in-intestine, mo-mouth, ex-extrusion, bo- birth open.

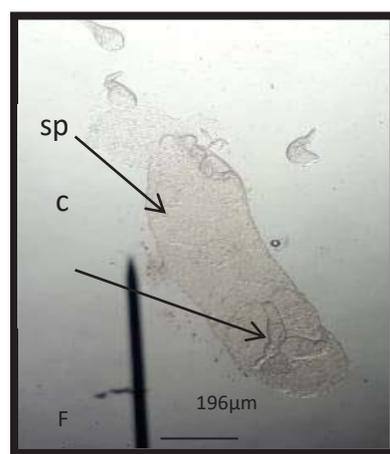
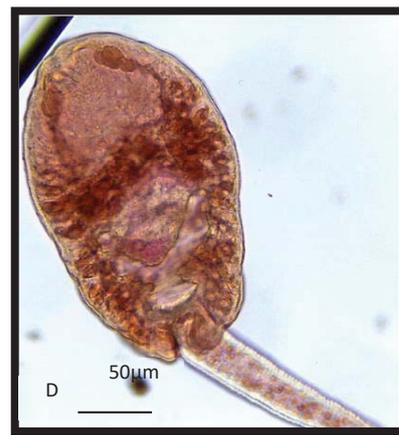
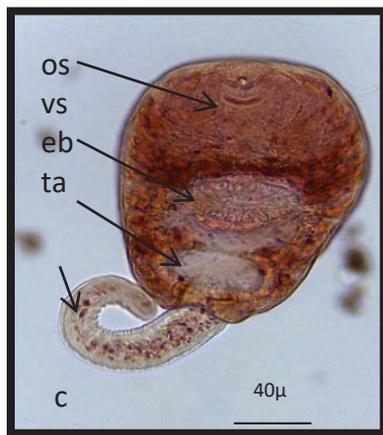
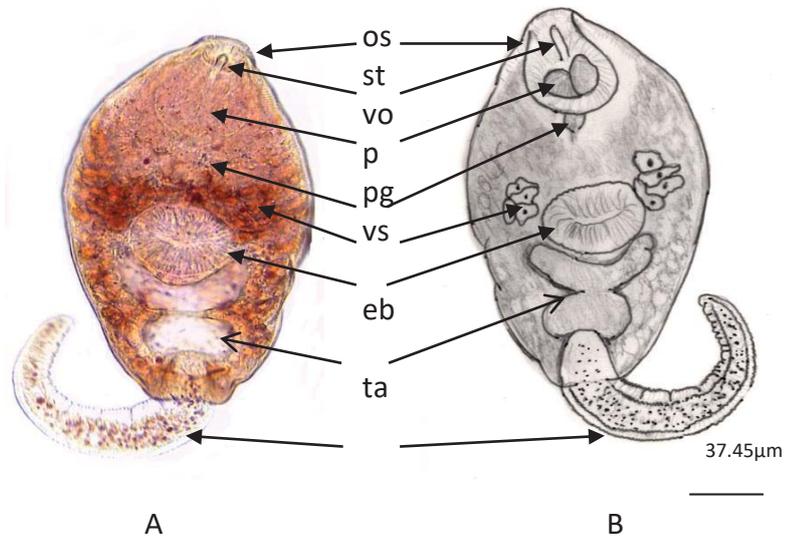


Plate 3. Image virgulate xiphidiocercariae; A. Cercaria with staining. B. Drawing of cercariae structure. (D, C). Cercaria with staining. F. sporocyst without staining. E. cercaria without staining. Abbreviations: os -oral sucker, vs- venter sucker, st-stylet, ta-tail, ed-excretory bladder, pg-penetration glands, sp-sporocyst, P-pharynx, c-cercariae.

Type 2: Virgulate xiphidiocercous cercariae.

Description: (plate 3) The body is oval-shape is covered with small spines that are denser in the oral sucker, which is circular in front of the middle is a small installation is the stylet. A virgular organ is located in the region of the oral sucker. The venter sucker is circular smaller than oral sucker. The pharynxes round and short. the excretory bladder is Y-shaped and located at the end of the body. Four pairs penetration glands exist, two anterior pairs and a posterior pair, the penetration glands had granules and ducts, the ducts opened near the tip of the stylet. The tail is very short and looks like spring (table 2). Cercariae were produced within the sporocyst.

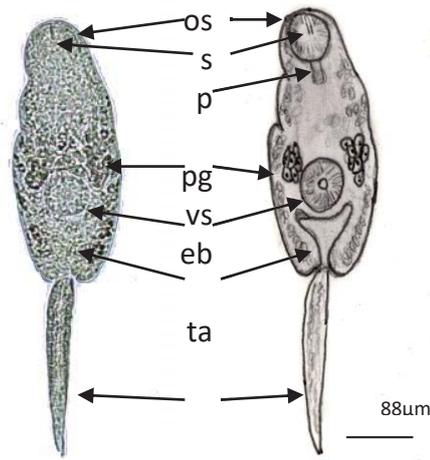
Movement behavior: The cercariae moved in or on water surface. The body dive lower than tail. The cercariae moved for about 30-45 seconds, and rested for about 5-10 seconds. They live for 4-5 hours in the water before they die.

Type 3: Armata xiphidiocercous cercariae

Armata xiphidiocercariae A

Description: (plate 4) The body of cercariae is elongated oval –shape. The y contains large circular oral sucker which houses a stylet. Without of virgular organ and finfold. The venter sucker is equal in size to oral sucker. Seven pairs of granular and nucleated penetration glands located laterally to esophagus. four of them are located on each side are irregularly shape and coarsely granulated. Excretory bladder is Y-shaped with thick, granulate walls. Tail short is without of spines and dorso -venter finfold (table 2). The cercariae develop in a sac-like sporocyst.

Movement behavior: cercariae swims randomly using their tail. Finally, it dives upside down motionless with its tail. Once the cercariae touches the bottom it moves on it. They stay for 2-3 hours in the water after emergence.



A

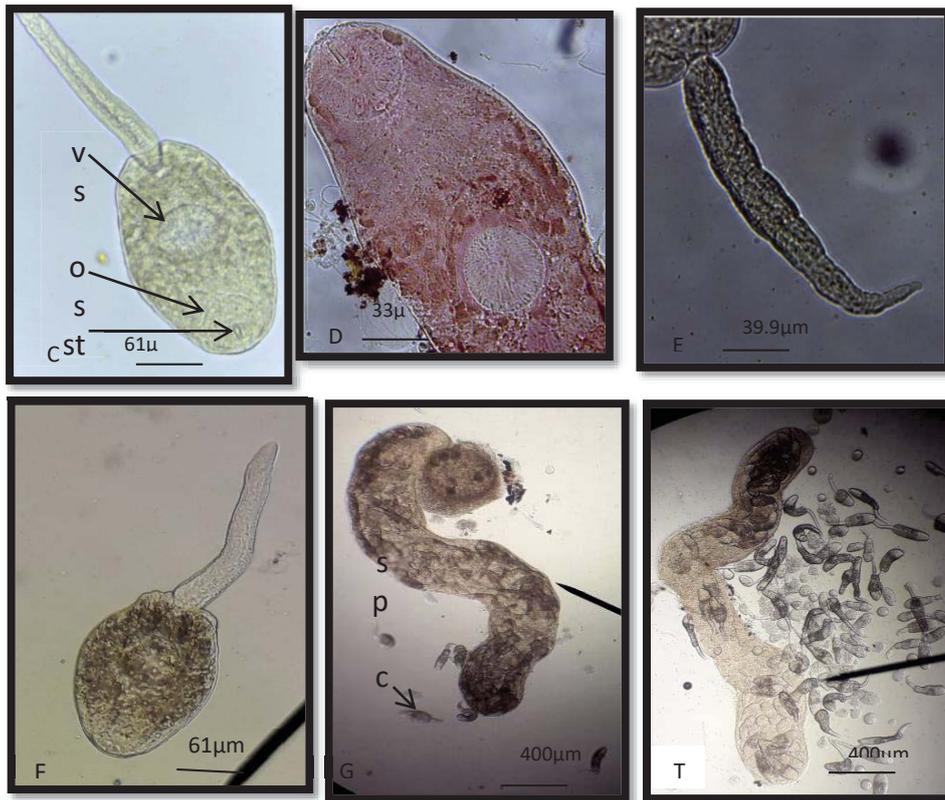


Plate 4. Image Armata xiphidiocercariae A; A. Cercaria without staining. B. Drawing of cercariae structure. (D, C). Cercaria with staining. E. Tail cercariae (G, T). sporocyst without staining. F. cercaria without staining. Abbreviations: os -oral sucker, vs- venter sucker, st-stylet, p-pharynx, ta-tail, eb-excretory bladder, pg-penetration glands, sp-sporocyst, s-spine.

Armata xiphidiocercariae B:

Description: (plate 5) The cercariae body is oval-shape, and surface is covered with spines, oral sucker large circular located in the middle of the mouth protrudes from it stylet. Venter sucker smaller than oral sucker. Six pairs of penetration glands with large nuclei and fine granules, this ducts are bundled one on each side, opening near of the stylet. Pharynx poorly differentiated esophagus, ceca and ducts are not developed, the excretory bladder is Y-shaped, its walls very thin. Tail is a slim with a certain length, without finfold. The cercariae develops in a branched sporocyst, infecting the digestive gland of the snail host. This cercariae was first recorded in Basrah (table 2).

Movement behavior: This cercariae swim through the water by its whipping tail, almost constantly, near the surface of the water, resting periodically. It stays for about 2 hours in the water after emergence. Many of these cercariae were encysted within few minutes after emergence on plastic Petri dish wall.

Table 2: Size rang and average size (in micrometers, calculated from 15-25 cercariae):

Cercariae	Echinostoma A	EchinostomaB	Virguate xiphidi	Armata xiphidioA	Armata xiphidio B
Body Lx W mm mean	110-95x78-68 (av.106-72)	256-221x270-210 (av.232-247.7)	202-173x132-119 (av.184-126)	270-220x140-110 (av.243-123)	230-170x145-100 (av.202.5-121)
Oral sucker mm mean	24-19x24-16 (av.21-18.5)	105-94x74-63 (av.99.5-66.7)	62-54x62-46 (av.57.5-53)	63.4-45x61-51 (av.58-56)	48.8-39x46.4-36.6 (av.43-41.5)
Venter sucker mm mean	22-14x22-16 (av.18-19)	211-200x221-147 (av.207-185.7)	62-51x46-3 (av.57-40)	53.7-48.8x46.4-45 (av.51-45)	29.3-26.8x29.2-26.8 (av.28)
Tail l xw mm mean	259-189x24-16 (av.209-20.7)	716-589x105-94 (av.207-185)	200-132x32-30 (av.168-31)	160-150x190-90 (av.155-140)	310-290x40-30 (av.300-36.7)
Redia or sporocyst Lxw mm mean	Redia 1641-1368x308-158 (av.1504.5-240)	Redia 2410-2230x270-210 (av.2350-247.5)	Sporocyst 718x410	Sporocyst 2925-2350x600-350 (av.2662-442)	Sporocyst 1330-900x320-170 (av.1170-265)

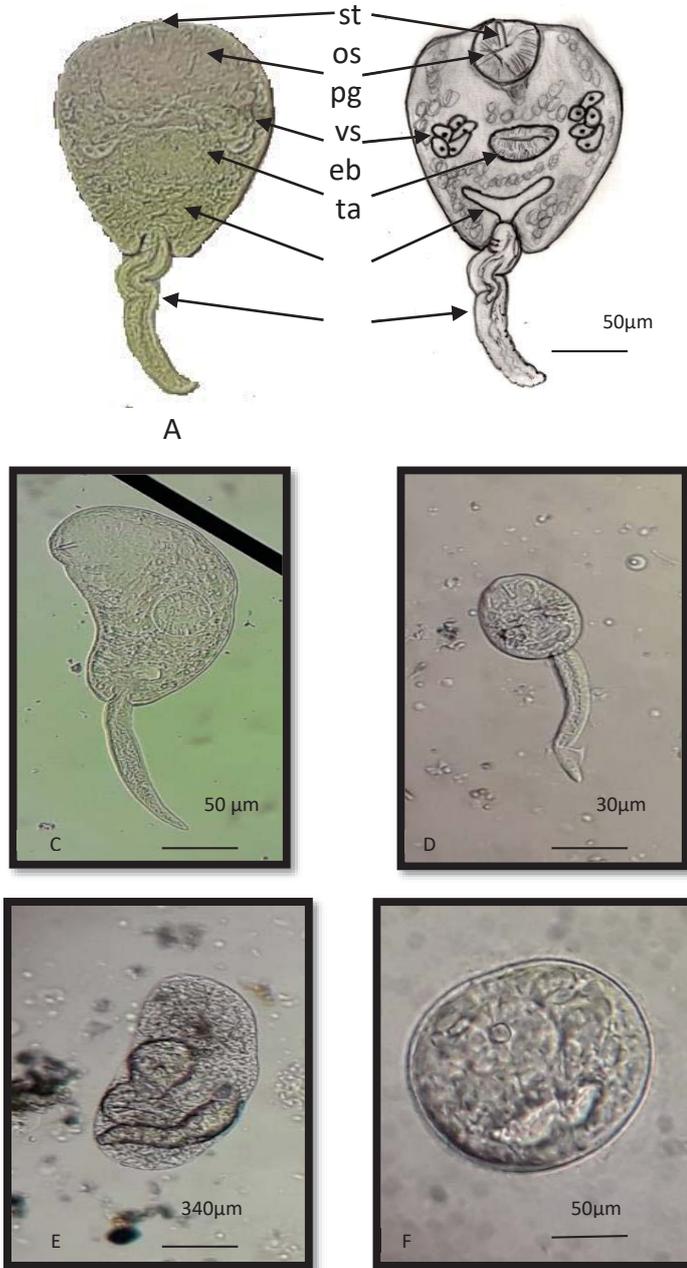


Plate 5. Image Armata xiphiocercariae B; A. Cercaria without staining. B. Drawing of cercariae structure. (D, C). Cercariae without staining. (E). sporocyst without staining. (F). metacercariae without staining. Abbreviations: os -oral sucker, vs- venter sucker, st-stylet, ta-tail, eb-excretory bladder, pg-penetration glands.

Discussion:

The present study showed low prevalence of *Lymnaea auricularia* in the province of Basrah. No samples were obtained during August to November that might be ascribed to the high temperature in the area, drought and high salinity, Asadi (2011) that the concentration salinity and high temperature adversely affects the growth of the *L. auricularia*. Abdul-Sahib (1986) referred to the disappearance of the snails during the summer time in Basrah. On the contrary the snail's numbers increased in winter and spring

(Al-Jibouri *et al* ,2011) who ascribed that to climatic changes and rainfall. The diversity of trematode in *L. auricularia* as recall (Zhang *et al* ,2005) due to the snail fragility and high food content. Two species were recorded during the present study in Basrah Governorates, Echinostome cercariae B according to (Georgieva *et al* ,2013.; Chantima *et al* ,2013). And Armata xiphidiocercariae B according (Flowers &Miller ,1995).

Five species of cercariae were recorded during the study which categorized into three groups: Echinostoma cercariae, Virgulate xiphidiocercous cercariae and Armata xiphidiocercous cercariae. Echinostoma cercariae group are characterized by a row of spines around oral sucker, the Echinostoma cercariae A measurements were similar to that found in a study made by Al- Hussein, (2000) but they have birth opening in radia and no penetration glands. while Echinostoma cercariae B was recorded for the first time in Iraq, has distinctive appearance. Large body size, long tail a terminal extrusion. While radia contains two birth openings. That group have a large number of Tissue glands effect on the dark color. gave it cystogenous glands which and also we have, cercariae were encysted on the plastic Petri dish meta cercariae on the snail sensors. The dark color characteristic cercariae). *Fasciola* parasite (Al-Jibouri *et al* 2011).

While Virgulate xiphidiocercariae cercariae have a kidney shaped virgule organ present in the region of the oral sucker, ventral sucker is smaller than oral sucker. They belong to the family Lecithodendriidae, which infected bats, birds and amphibians. (Frandsen &Christensen,1984).

While Armata xiphidiocercariae group have tail without a finfold and virgule organ. Armata xiphidiocercariae A have oral sucker and venter sucker of equal sizes. Seven pairs of penetration glands are also found, excretory bladder is I-shaped (Al-Hussein, (2000).

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