

## Original Article

# Larval stages of digenetic trematode parasitizing freshwater snails in the water bodies of Maysan Governorate, Iraq

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**Abstract:** A survey was conducted on the larval stages of digenetic trematodes parasitizing freshwater snails in the water bodies of the Degla River passing through Maysan Governorate, southern Iraq, from March 2022 to February 28, 2023. The rates of snail infection with cercariae varied. Examination of 5,109 snails revealed four types infected with cercariae, namely: *R. auricularia*, *P. acuta*, *M. tuberculata*, and *Melanopsis* spp., with infection rates of 44.5, 16, 26.5, and 13%, respectively. The researcher identified 21 species of cercariae belonging to 12 families: Plagiorchiidae, Echinostomatidae, Microphallidae, Fasciolidae, Gymnophallidae, Telorchidae, Lecithodendriidae, Haematoloechidae, Heterophyidae, Haploporidae, Transversotrematidae, and Cyathocotylidae. Thirteen species were recorded for the first time in Iraq: Xiphidiocercariae II, Monostomatous I, and Gymnocephalous II from *R. auricularia* snails, Xiphidiocercariae IV from *P. acuta* snails, Gymnocephalous III, Parapleurolophocercous I, Pleurolophocerca I, Monostomatous II, Pleurolophocerca II, and Gymnocephalous IV from *Melanopsis* spp. snails, and Furcocercous I from *M. tuberculata* snails, as well as Macrocerous I and Macrocerous II.

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## Introduction

Digenetic trematodes pass through at least two hosts: the final host, which houses the adult stages for sexual reproduction and is usually a vertebrate, and the intermediate host, which houses the immature stages for asexual reproduction and is typically an invertebrate, especially snails, that serve as food for many vertebrates such as fish, waterfowl, and mammals, including humans (Cribb et al., 2014). Trematodes undergo two reproductive cycles, the sexual and asexual cycles, culminating in the intermediate invertebrate host with the formation of cercariae (Veeravechskij et al., 2018). The phenotypic classification of these cercariae depends on host specificity, the presence of oral and ventral suckers, the presence of the stylet, the number of penetration glands, the presence and type of the tail, whether the tail is split, the presence of the fin fold, and the presence of spines on the tail (Krailas et al., 2020). Cercariae are typically classified at the family

level and, in rare cases, at the genus level. Classification at lower levels is challenging due to significant variations in phenotypic and behavioral characteristics (Al-Mayah, 1998); classification at lower levels necessitates completing life cycles, obtaining adult worms or their eggs, or using polymerase chain reaction (PCR).

The study of digenetic trematodes in freshwater snails in Iraq was conducted by Al-Mayah (1998), Al-Hussein (2000), and Al-Katea (2020) in Basra Governorate, and by Al-Waeli (2014) in central and southern Iraq. The relationship of snails with the larval stages of the digenetic trematodes they harbor in Iraq has not received as much attention as in other regions. This is evident from the lack of ongoing studies on the subject, in Iraq in general or Misan Governorate in particular. Therefore, the current work aims to detect the infection of snails in the water bodies of Misan Governorate by the larval stages of digenetic trematodes and to attempt to classify them.

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Table 1. Percentage of infection of four studied snail species.

| Name of the snails                           | No. of infected snails No. (%) | Family               | Name of cercaria           |
|--|--------------------------------|----------------------|----------------------------|
| <i>R. auricularia</i><br>44.5%               | 5 (1.1%)                       | Plagiorchiidae       | Xiphidiocercariae I        |
|  | 19 (5.6%)                      | Echinostomatidae     | Xiphidiocercariae II*      |
|  | 44 (12.9%)                     | Echinostomatidae     | Xiphidiocercariae III      |
|  | 12 (3.5%)                      | Microrphallidae      | Monostomatous I*           |
|  | 45 (13.5%)                     | Fasciolidae          | Gymnocephalous I           |
| <i>P. acuta</i> 16%                          | 27 (7.9%)                      | Gymnophallidae       | Gymnocephalous II*         |
|  | 19 (16%)                       | Telorchidae          | Xiphidiocercariae IV*      |
| <i>M. tuberculata</i><br>26.5%               | 41 (2.0%)                      | Echinostomatidae     | Gymnocephalous III*        |
|  | 35 (3.7%)                      | Haematolichidae      | Xiphidiocercariae V        |
|  | 44 (2.1%)                      | Heterophyidae        | Parapleurolophocercous I*  |
|  | 33 (1.8%)                      | Heterophyidae        | Pleurolophocerca I*        |
|  | 39 (1.9%)                      | Heterophyidae        | Monostomatous II*          |
|  | 47 (2.9%)                      | Heterophyidae        | Pleurolophocerca II*       |
|  | 36 (1.7%)                      | Haploporidae         | Gymnocephalous IV*         |
|  | 38 (1.9%)                      | Transversotrematidae | Furcocercous I*            |
| <i>M. tuberculata</i><br>+ <i>Melanopsis</i> | 262 (6.2%)                     | Heterophyidae        | Parapleurolophocercous II  |
|  | 98 (2.3%)                      | Heterophyidae        | Parapleurolophocercous III |
| <i>Melanopsis</i> (13%)                      | 21 (0.2%)                      | Lecithodendriidae    | Macrocerous I*             |
|  | 5 (0.1%)                       | Lecithodendriidae    | Macrocerous II*            |
|  | 97 (2.0%)                      | Cyathocotylidae      | Furcocercous II            |
|  | 94 (2.0%)                      | Cyathocotylidae      | Furcocercous III           |

## Materials and Methods

Snail samples were randomly collected from the Degla River's water bodies passing through Misan Governorate. The snails were transported to the laboratory in plastic boxes containing water from the sampling sites.

In the laboratory, the snails were examined to confirm their infection with cercariae using two methods, namely shedding and crushing methods (Arouna and Remy, 2005). The cercariae were examined while alive, without staining, by preparing glass slides based on Rankin (1939). Additionally, they were examined after staining while alive using Vital stain, which consists of Neutral red and Methylene blue, to observe important structures for classification, such as spines and fin folds that are only visible in living specimens and disappear when cercariae stabilize or die (Al-Mayah, 1998). This staining method also allowed for observation of behavioral characteristics of cercariae, such as swimming and resting in the water column, which are crucial for diagnosis and remain unaffected by these vital dyes (Blair, 1977).

The method used for stimulating cercariae to

emerge was artificial (electric) light to obtain fully developed cercariae for diagnosis. This approach was chosen because the immature cercariae cannot be accurately diagnosed when breaking the snail's shell due to their varying sizes, and the absence of important structures and characteristics necessary for classification. Additionally, the confined space between the snail shell and its membrane may contain numerous protozoa and small crabs that can be challenging to differentiate from cercariae (Al-Mayah, 1998). The method of breaking the shell and dissecting the snail tissues was employed to determine the stages from which the cercariae emerged, distinguishing between sporocysts and rediae. Sporocysts typically possess a mouth, pharynx, and saccular intestines, while rediae lack these structures (Fried and Graczyk, 1997).

## Results and Discussions

This study aimed to detect the larvae of digenetic trematodes, which utilize freshwater snails of the gastropod class as intermediate hosts to complete their life cycle. An examination of 5,109 shells collected from the study stations revealed that 992 shells were

Table 2. The morphological characteristics of the cercariae examined in the present study.

| Name of Cercariae          | Oral sucker | Style | Ventral sucker | Eye spots | Spiny collar | Phoryhx | Lateral finfold | Tail type |          | Develop in |          |
|----------------------------|-------------|-------|----------------|-----------|--------------|---------|-----------------|-----------|----------|------------|----------|
|                            |             |       |                |           |              |         |                 | forked    | unforked | Redia      | Sporcyst |
| Xiphidiocercariae I        | +           | +     | +              | -         | -            | +       | -               | -         | +        | -          | +        |
| Xiphidiocercariae II*      | +           | -     | +              | -         | +            | +       | +               | -         | +        | +          | -        |
| Xiphidiocercariae III      | +           | -     | +              | -         | +            | +       | +               | -         | +        | -          | +        |
| Monostomatous I*           | +           | +     | -              | -         | -            | -       | -               | -         | +        | -          | +        |
| Gymnocephalous I           | +           | -     | +              | -         | -            | +       | -               | -         | +        | +          | -        |
| Gymnocephalous II*         | +           | -     | -              | -         | -            | -       | -               | +         | -        | -          | +        |
| Xiphidiocercariae IV*      | +           | +     | +              | -         | -            | +       | -               | -         | +        | -          | +        |
| Gymnocephalous III*        | +           | -     | +              | -         | -            | +       | -               | -         | +        | +          | -        |
| Xiphidiocercariae V        | +           | +     | +              | -         | -            | +       | -               | -         | +        | -          | +        |
| Parapleurolophocercous I*  | +           | -     | +              | +         | -            | +       | +               | -         | +        | +          | -        |
| Pleurolophocerca I*        | +           | -     | +              | +         | -            | +       | +               | -         | +        | +          | -        |
| Monostomatous II*          | +           | -     | -              | +         | -            | +       | +               | -         | +        | +          | -        |
| Pleurolophocerca II*       | +           | -     | -              | +         | -            | +       | +               | -         | +        | +          | -        |
| Gymnocephalous IV*         | +           | -     | -              | +         | -            | +       | -               | -         | +        | +          | -        |
| Furcocercous I*            | -           | -     | +              | +         | -            | +       | +               | +         | -        | +          | -        |
| Parapleurolophocercous II  | +           | -     | -              | +         | -            | -       | +               | -         | +        | +          | -        |
| Parapleurolophocercous III | +           | -     | -              | +         | -            | -       | +               | -         | +        | +          | -        |
| Macrocerous I*             | +           | -     | -              | -         | -            | -       | -               | -         | +        | -          | +        |
| Macrocerous II*            | +           | +     | +              | -         | -            | +       | -               | -         | +        | -          | +        |
| Furcocercous II            | +           | -     | -              | -         | -            | +       | +               | +         | -        | -          | +        |
| Furcocercous III           | +           | -     | -              | -         | -            | +       | -               | +         | -        | -          | +        |

+ present; - absent

infected with 21 species of cercariae (Table 1), distributed among the snail species *R. auricularia*, *P. acuta*, *M. tuberculata*, and *Melanopsis*, with infection rates of 44.5, 16, 26.5, and 13%, respectively. To avoid repetition, only their main phenotypic characteristics will be mentioned (Table 2).

**Family Plagiorchiidae:** This family comprises a large number of digeneans that parasitize the liver, lungs, bile duct, and kidneys of amphibians, reptiles, and birds (Tkach, 2008). The characteristics of the cercariae Xiphidiocercariae I (Table 2, Fig. 1A) isolated from the shell of *R. auricularia* in the current study as well as with cercariae previously associated with the genus *Plagiorchis* (Dempster, 1988), with molecular confirmation of its classification using PCR by Faltynkova (2005) in the Czech Republic and Barton et al. (2022) in Australia. Al-Hadithi et al. (1989) documented two parasitic species in toads and frogs, and Mhaisen et al. (1990) recorded *Plagiorch elegans* parasitizing the intestines of waterfowl in Basra. Al-Hussein (2000) described one of the

species, characterized by the absence of the caudate fold and virgula organ, with the two cups being equal, placing the caudate below the armata group, distinguishing it from the type described in the current study.

**Echinostomatidae:** Members of the Echinostomatidae family are characterized by a spiny collar around the mouth consisting of two rows of spines, with the size, number, and arrangement of these spines serving as taxonomically valuable characteristics to differentiate between genera (Tkach et al., 2016). Georgiev et al. (2013) described two types of cercariae isolated from *Radix* snails, and they were placed in the genus *Echinostoma*, with their specialization in infecting snails of the genus *Radix*. The characteristics mentioned by the researchers apply to the two models, Xiphidiocercariae II (Table 2, Fig. 1B) and Xiphidiocercariae III (Fig. 1C), which were isolated from the shell of *R. auricularia* in the current study. Based on their specialization in infecting snails of the genus *Radix*, they belong to the genus *Echinostoma*.

Al-Hussein (2000) showed a type of cercariae

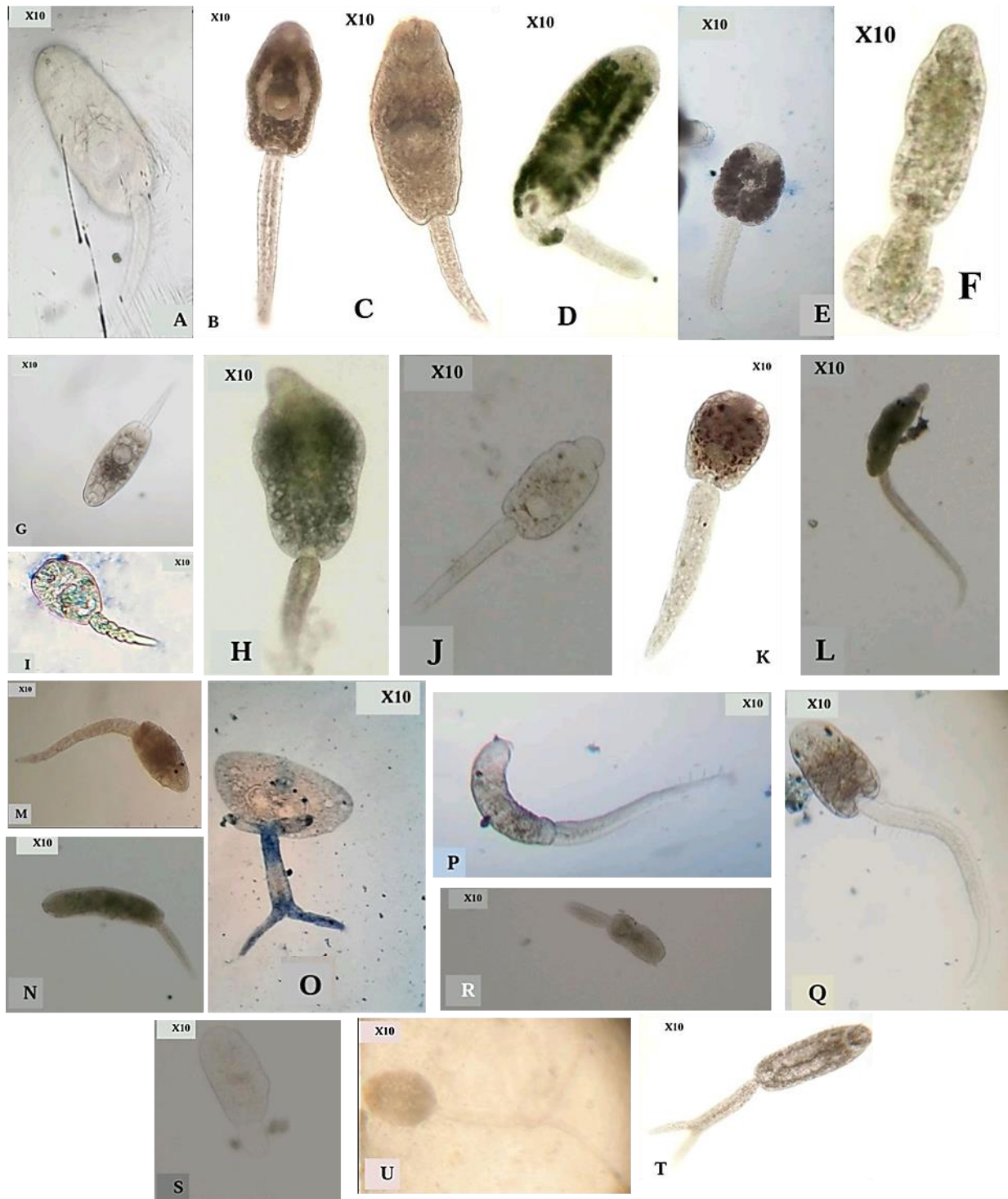


Figure 1. Images of cercariae were recorded in the current study. A- Xiphidiocercariae I, B- Xiphidiocercariae II, C- Xiphidiocercariae III, D- Monostomatous I, E- Gymnocephalous I, F- Gymnocephalous II, G- Xiphidiocercariae IV, H- Gymnocephalous III, I- Xiphidiocercariae V, J- Parapleurolophocercous I, K- Pleurolophocerca I, L- Monostomatous II, M- Pleurolophocerca II, N- Gymnocephalous IV, O- Furcocercous I, P- Parapleurolophocercous II, Q- Parapleurolophocercous III, R- Macrocerous I, S- Macrocerous II, and T- Fucocercous II.

matching Xiphidiocercariae III in all its phenotypic characteristics and attributed the model recorded to the Macroderoididae family due to its caudal fold that does not cover the entire tail. However, Al-Waeli

(2014) recorded the same model and placed it within the Echinostomatidae family. Xiphidiocercariae II was not previously recorded in Iraq, making it a potential new species in the current study. Regarding

Gymnocephalous III (Table 2, Fig. 1H), isolated from *M. tuberculata*, it belongs to the same family and it is characterized by a dark body and contracted tail with a division of the esophagus in front of the abdominal retractor. It may be related to the genus *Echinochasmus*, whose adult species have been found parasitizing waterfowl in Iraq. The general characteristics of this model agree with one described by Grabda-Kazubaska (1991) in Lithuania. It differs in its host shell, being isolated from the shell of *Bithynia tentaculata*. No previous studies in Iraq have mentioned a description similar to the model in the current study, suggesting it may be a new record for Iraq. Abdullah (1988) and Al-Mayah (1990) recorded several species of adult trematodes of the genera *Echinostoma* and *Echinochasmus* parasitizing migratory waterbirds in Basra Governorate. These larval stages may be related to them.

**Microrophallidae:** The most significant taxonomic characteristic of the cercariae in this family is their monostome nature, indicating they possess only an anterior chamber with a stylet penetrating organ and develop within egg-shaped sporocysts (Galaktionov and Skirnisson, 2007).

*Spelotrema pygmaeum*, a genus within this family, was documented parasitizing the intestines of water chickens on a fish farm in Babylon Governorate (Mhasien and Abul-Eis, 1992). Al-Husseini (2000) described a member of this family without specifying the genus, attributing it to the subgroup Microcotylae due to its small size, presence of a fin fold, three penetrating glands, and an underdeveloped intestine, suggesting it belongs to the Pusilla group. Young (1938) described adults of the trematode *Levinseniella* from shorebirds and their cercariae from the snails *Olivella biplicata*, noting a high infection rate in snails but a very low rate in birds. Nicol et al. (1985) detailed the life cycle of the trematode *Levinseniella* in California, completing it in the laboratory using frogs and snakes as definitive hosts and leeches as a second intermediate host.

The phenotypic characteristics of the model Monostomatous I (Table 2, Fig. 1D) from the current study, isolated from the shell of *R. auricularia*, align

with those described by the researchers. These include a longer esophagus than the pharynx, the absence of a ventral retractor, and a short, V-shaped excretory bladder. This finding is reported for the first time in Iraq.

**Fasciolidae:** Adults of this family reside in the livers of sheep, buffalo, goats, and pigs, causing fasciolosis, with rare cases of accidental human infections reported in Japan, northern Iran, and Thailand (Inoue et al., 2007). Snails of the Lymnaeidae family serve as intermediate hosts for the most significant species, *F. hepatica* and *F. gigantica*, as studied by Phalee et al. (2015) in an experimental study tracking the life cycle of *F. gigantica* cercariae from egg to adult. The researcher utilized *L. auricularia* shells, employing a medium of water and parsley leaves to encyst the cercariae. These encysted leaves were fed to mice, and the adults were isolated from their bile ducts 3-4 days post-infection. The cercariae were described as having a discoid body with two chambers, oral and ventral, saccular and pharyngeal glands, and a long tail. These characteristics align with Gymnocephalous I from the current study (Table 2, Fig. 1E), isolated from *R. auricularia*, potentially belonging to the same species. Al-Waeli (2014) employed molecular methods, specifically the PCR method, to diagnose *F. gigantica* without detailing its phenotypic characteristics.

**Gymnophallidae:** The Gymnophallidae family comprises seven genera: *Meiogymnophallus*, *Gymnophallinae*, *Gymnophallus*, *Paragymnophallus*, *Pseudogymnophallus*, *Gymnophalloides*, and *Bartolius*. These names are inspired by the names of sports games (Cremonte, 2001). In an experimental study conducted by Galaktionov (2006), the type of intermediate host for the genera *Gymnophallids* was investigated, focusing on snails or oysters. Snails were infected with miracidia, leading to the production of sporangia and cercariae within the shell. Cercariae were found in the tissues of oysters and in the adults of seagulls used in the experiment. The characteristics of the cercariae of the studied type resemble the model in the current study, Gymnocephalous II (Table 2, Fig. 1F), with a small trunk of the tail compared to the body

of the caudate and the absence of a ventral retractor. This species has not been previously documented in Iraq, making this its first recording in the country.

**Telorchiiidae:** Members of the family Telorchiiidae are known as intestinal parasites that utilize snails as the first intermediate host, frogs as the second intermediate host, and snakes as the final host (Yousesfi et al., 2013). Xiphidiocercariae IV (Table 2, Fig. 1G), isolated from the shell of *P. acuta* in the current study, shares similarities with the species described by Flatyknova (2005), belonging to the genus *Opisthioglyhe*. This species is characterized by a notch in the middle of the oral receptacle, a larger ventral receptacle compared to the oral receptacle, and a tail surrounded by terminal spines. The model observed in the current study represents a new finding in Iraq.

**Haematoloechidae:** The adult parasites of this family reside in the lungs, digestive tract, bladder, and blood vessels of their vertebrate hosts, with the most significant genera being *Haematoloechis*, *Megalodiscus*, and *Ozolaimus*. The genus *Haematoloechis* has been documented as a parasite in frogs and snakes (Snyder and Tkach, 2001). Shinad et al. (2023) reported two species of the genus *Haematoloechus*, namely *H. almorai* and *H. variegatus*, found in the lungs of frogs for the first time in India. The characteristics of Xiphidiocercariae V (Table 2, Fig. 1I), isolated from the snail *M. tuberculata*, align with the cercarial characteristics of the species *H. similis* described by Krailas et al. (2014) from the snail, placing it in the Xiphidiocercariae. Key characteristics include an oval body covered with spines, the presence of a stylet facilitating the oral sucker, six irregularly shaped penetrating glands, a short pharynx, an undeveloped esophagus, and a Y-shaped excretory sac. The cercariae are produced from a sporocyst. Al-Hussein (2000) documented a model similar to the one observed in the current study, categorizing it as a saber cercariae with a member of *Virgula*, placing it within the family Lecithodendriidae.

**Heterophyidae:** This family comprises approximately 50 genera, with the genus *Heterophyes* being the most significant, as some of its species infect

humans, leading to intestinal ulcers. Sukontason et al. (2005) documented three cases of ulceration, bleeding of the mucous layer, chronic inflammation, and fibrosis of the submucosal layer. Farahnak et al. (2004) identified flukes as the causative agent of heterophyiasis in birds that consume fish in Iran.

The characteristics of Parapleurolophocercous I (Table 2, Fig. 1J), isolated from the shell of *M. tuberculata*, resemble those of cercariae from the genus *Pygidiopsis* described by Simoes et al. (2009) in Brazil, where the adults parasitize bats. The researchers achieved the genus by experimentally infecting hamsters with teleosts isolated from fish. The cercariae were described as having a body shorter than the tail, a short pharynx, rectangular eye spots at the beginning of the body, and a tail that extends deep into the body. This species has not been previously documented in Iraq, making this study the first to record it.

The model Pleurolophocerca I (Table 2, Fig. 1K), isolated from the snail *M. tuberculata*, is classified within the Pleurolophocerca group due to the presence of a lateral fold along the tail. This model shares similarities with cercariae of the genus *Centrocestus* isolated from the same host and described previously by Krailas et al. (2014) and Dechruksa et al. (2017) characterized by a pair of black eye spots, a strong connection of the tail to the body, a short and unclear pharynx, an excretory bladder shaped like the letter V, and the production of cercariae from rudiments. This species has not been previously documented in Iraq, marking its first recording in the country.

The descriptions of *Stictodora tridactyla* cercariae provided by Ukong et al. (2007) and Veeravechskij et al. (2018) align with the characteristics of the Monostomatous II model (Table 2, Fig. 1L), isolated from the shell of *M. tuberculata*. This model features an oval body with a yellowish-brown color, an oral sucker with three rows of spines, a pair of eyespots, and seven pairs of penetrating glands between the pharynx and the V-shaped excretory bladder with a thick wall. The tail is longer than the body and has dorsal and ventral lateral folds, suggesting it may be a similar species. This cercariae has not been previously

documented in Iraq, making this study the first to report it.

Pleurolophocerca II (Table 2, Fig. 1M), isolated from the host *M. tuberculata*, exhibits some similarities to the life cycle described by Dutt and Srivastava (1966) for the cercariae of the genus *Gastrodisus*. These similarities include the shape of the pharynx, the presence of eyespots in front of the pharynx, and the excretory bladder at the posterior end. The body of Pleurolophocerca II is closely connected to the tail. Researchers have observed that some cercariae of this genus utilize the shell *Helicorbis coenous* as their first intermediate host. After leaving the snail host, these cercariae swim in the aquatic environment, attaching to surrounding plants and eventually forming black cercariae. These black cercariae then infect their final hosts, which include horses and buffalo. Notably, this particular cercariae species has not been previously recorded in Iraq, making its description in the current study the first such record for the country.

Additionally, Parapleurolophocercous II (Table 2, Fig. 1P) and Parapleurolophocercous III (Table 2, Fig. 1Q) were isolated from snails of both *M. tuberculata* and *Melanopsis*. These species exhibit two folds—one lateral and one dorsal-ventral—and can be classified as part of the Parapleurolophocerca group (Chontanarith and Wongsawad, 2017). The characteristics described by Hechinger (2019) for cercariae of the genera *Acanthotrema* and *Euhaplorchis* align with the features observed in these species. These cercariae are produced from transparent, gray, or light-yellow sausage-shaped rudiments, with a semi-transparent body. They possess an oral sucker but lack a ventral sucker (or it may be vestigial). The body length is approximately one and a half times shorter than the tail, and they have seven penetrating glands. The lateral fin fold begins near the body and extends to the middle of the tail's last third. The ventral and dorsal fins span from the middle third of the tail to the last third. These cercariae exhibit intermittent swimming patterns with rest periods.

Interestingly, Parapleurolophocercous II and

Parapleurolophocercous III were recorded in Iraq for the first time in this study. Al-Hussein (2000) documented their isolation from *Melanoides* and *Melanopsis* snails, respectively, and provided detailed characteristics and measurements consistent with the current study. Al-Waeli (2014) also described Parapleurolophocercous II and included it in the family *Heterophyidae*. Other adult species from this family have been reported in Iraq, including five species parasitizing waterfowl in Basra. Additionally, Al-Ali (1998) recorded the species *Ascocotyle coleostoma*, which belongs to the same family as *M. tuberculata* snails, parasitizing the gills of redbfish, sea bream, and carp in Basra. Al-Mayah (1998) documented an *Ascocotyle coleostoma* species within the same family.

**Haploporidae:** The final host for members of this family is often a species of marine fish, particularly mullet. However, it has also been found parasitizing a small number of freshwater fish in Korea, Japan, Brazil, and Argentina. Manter (1963) explained this occurrence as a consequence of mullet fish transitioning to freshwater environments. In such cases, invertebrate hosts cannot transfer the adult parasites directly. Instead, transferring the final host to a new environment necessitates either the first intermediate host adapting to the new environment or changing its intermediate hosts to sustain the parasite's life cycle.

The characteristics of the genus *Saccocoelioides*, as described by Szidat (1954), align with the specimen studied in the current research—Gymnocephalous IV (Table 2, Fig. 1N). This specimen was isolated from the shell of *M. tuberculata*. Notably, Gymnocephalous IV exhibits a curved body on the dorsal side, and its oral sucker is larger than the vestigial ventral sucker. Additionally, eye spots are present in the cercariae. Martin (1973) previously described cercariae of the *S. pearson* type, which closely resembles the model studied in our current research. The primary difference lies in the first intermediate host: whereas our study identifies the *Posticobia brazier* shell as the intermediate host, Martin's work involved a different host. The specimen described in our study represents

the first recorded instance of this species in Iraq.

**Transversotrematidae:** This family is highly specialized, with the first intermediate host being snails of the genus *Melanoides*. Its final host consists of fish inhabiting the Indian Ocean and tropical regions (Hunter et al., 2012). Krailas et al. (2014) introduced the cercaria *Transversotrema laruei*, which exhibits several distinctive features: bowl-like body shape, light brown color, numerous spines on the body surface, resembling fish scales, prominent eye spots, presence of a pair of testicles, oral sucker disappearing, longer tail compared to the body, adhesive pad in the cercaria produced by redia. All these characteristics align with the cercariae observed in the current study, specifically Furcocercous I (Table 2, Fig. 1O). This particular specimen was isolated from the snail *M. tuberculata* and may belong to the same species. Interestingly, while a similar model of this cercaria was previously isolated in India from the snail *M. tuberculata*, it was not officially classified as a distinct species. Therefore, the current study represents the first recorded instance of this cercaria in Iraq.

**Lecithodendridae:** Members of this family are globally distributed and primarily parasitize Chiroptera bats. Rarely, they infect mammals, birds, and other reptiles as definitive hosts. According to the latest survey by Tkach et al. (2000), the present study recorded two specimens belonging to the family Macrocerous I (Table 2, Fig. 1R). These specimens were isolated from the intermediate host *Melanopsis* spp. Notably, they are characterized by their small tail size, placing them within the *Macrocerous* group. Due to the absence of a ventral sucker, they are classified as monostomes.

The most distinguishing feature of these cercariae is the presence of a rhombic or triangular mass in front of the excretory bladder, which may be considered the primary reproductive cells. Interestingly, this species has not been previously recorded in studies conducted in Iraq. Therefore, its description in the current study represents the country's first documented record of this species.

Moving on to the model Macrocerous II (Table 2,

Fig. 1S), which was isolated from the intermediate host *Melanopsis* sp., it exhibits characteristics reminiscent of the cercariae genus *Ochoterenatrema*, as described by Fernandes et al. (2022). Specifically, the caudate contains a pseudogonotyl consisting of thick crowns on the left side of the ventral sucker. When inverted, this structure appears swollen and takes on the shape of a bag. Interestingly, Tkach et al. (2018) previously misidentified this structure as an opening for the reproductive system. Although the function of the pseudogonotyl remains unknown, it holds taxonomic significance for distinguishing between genera within the family.

The *Ochoterenatrema* genus comprises six species, and the characteristics observed in the current study's model consistently align with cercariae of this genus. As a result, they can be classified as cercariae of one of its types, thereby establishing a new record for this species in Iraq.

**Cyathocotylidae:** The distinctive feature of this family is the wide spectrum of parasitism (reptiles, birds, and mammals), and the characteristics of the cercariae are more stable, unlike the stages of metacercariae and adults, which show great morphological diversity (Martin, 1961). Isolation of two types of cercariae of this family in the current study from the snails of *Melanopsis* spp.

The first type, Furcocercous II (Table 2, Fig. 1T), is characterized by the length of the two halves of the tail, the presence of a pharynx, and the absence of a ventral retractor. Al-Mayah (1998) and Al-Hussein (2000) isolated this type of cercaria from snails in Basra Governorate. It falls under the Longifurcate group – Pharyngeate – Monostome, sometimes called Vivax sub-group. Al-Waeli (2014) and Al-Katea (2020) have confirmed their presence in Iraqi freshwater snails of the genus *Melanopsis*.

The second type, Furcocercous III (Table 2, Fig. 1U), is similar in description to cercariae belonging to the genus *Mesostephanus*, as detailed by Myer (1958). Myer stated that their first intermediate host is freshwater snails, and their second intermediate host is frogs and fish. The same researcher conducted an experiment infecting birds and snakes with distant



cercariae. The result showed that snakes were more affected than birds.

Krailas et al. (2014) described the cercariae of the species *M. appendiculatus*, which utilizes *Melanoides* snails as their intermediate host. These cercariae are characterized by an oval body containing spines with coarse granules inside. The pharynx is small, and the esophagus is long, divided into two ridges that end at a small excretory bladder. They lack an abdominal volume. The tail is covered with many spines, and its stem is longer than its two parts. The caudate is produced from a transparent, rectangular sporangium, as explained by Hechinger (2019). The presence of the caudate is concentrated in the rectum of the snail. These transparent-bodied cercariae swim in a column, with intermittent rest periods and responsiveness to vibrations. Al-Katea (2020) recorded them for the first time in Iraq, making the current study the second documented instance.

The appearance of previously unrecorded cercariae, along with those infecting snails in coastal waters, may result from bird migration between countries or the trade of infected animals from other regions, introducing new trematode species.

## Conclusions

Misan Governorate has a somewhat high biodiversity of cercariae, which indicates a decline in water quality and safety. Some cercariae may be important for public health. The absence and non-recording of cercariae belonging to the family Diaphoresis, especially those causing human diarrhea. There is a high prevalence of the Heterophyidae family, indicating its spread in the final hosts.

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