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Larval stages of digenetic trematodes in *Melanopsis praemorsa* snails from freshwater bodies in Palestine

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ABSTRACT

Objective: To detect the species of larval trematodes (cercariae) in *Melanopsis praemorsa* snails from 5 different fresh water bodies in Palestine. **Methods:** A total of 1 880 *Melanopsis praemorsa* snails were collected from different fresh water bodies in Palestine from October, 2008 to November, 2010. Cercariae in *Melanopsis praemorsa* snails were obtained by lighting and crushing methods. The behavior of cercariae was observed using a dissecting microscope. **Results:** Three different species of larval trematodes were identified from *Melanopsis praemorsa* snails collected only from Al-Bathan fresh water body, while snails from other water bodies were not infected. These species were microcercous cercaria, xiphidiocercaria and brevifurcate lophocercous cercaria. These cercariae called *Cercaria melanopsi palestina I*, *Cercaria melanopsi palestina II* and *Cercaria melanopsi palestina III* have not been described before from this snail in Palestine. The infection rate of *Melanopsis praemorsa* collected from Al-Bathan fresh water body was 5.7%, while the overall infection rate of snails collected from all fresh water bodies was 4.3%. Details are presented on the morphology and behavior of the cercariae as well as their development within the snail. **Conclusions:** These results have been recorded for the first time and these cercariae may be of medical and veterinary importance.

1. Introduction

Melanopsis praemorsa (L. 1758, Buccinum) (*M. praemorsa*) is a gastropod snail which belongs to the prosobranchia to the superfamily *Cerithioidea* and to the family *Melanopsidae*. This specie is highly polymorphic; within the same population, conically elongated, dark-brown to black prosobranch snail which may exceed 2 cm in length. The sculpture of the shell is highly varied, being either smooth or ribbed to a greater or lesser extent. The growth and the phenotypic enzyme profile varied as well. *M. praemorsa* is widely prevalent in freshwater bodies of the Mediterranean region^[1,2]. In Palestine, it is also one of the most abundant snails present in fresh water bodies. Therefore, it is expected that it may act as an intermediate host for many digenetic trematodes of various vertebrates in the area.

Most freshwater snails can become intermediate hosts for trematode cercariae which may be transmitted to people and animals^[3–5]. Trematodes have a complex life cycle involving one or two (rarely three) intermediate hosts prior to infecting the definitive host. Snail species are the first intermediate hosts for the majority group of digenetic trematode. Snail infection occurs through ingestion of the eggs or penetration by free-swimming miracidia. Inside the first intermediate host, the process of polyembryony occurs and several different intramolluscan larval stages (*i.e.*, sporocyst, redia, and cercaria) are formed by asexual reproduction. After emerging from the snail tissue, the larvae of trematodes (cercariae) may find the suitable secondary intermediate host or definitive host by means of passive transmission (metacercaria) or active penetration, respectively.

Mollusks are regularly found harbouring larval stages of parasitic trematodes or digenea^[6]. Various studies have been done on fauna of cercariae from *Melanopsis* snails ^[4,7–12]. Following the discovery of human philophthalmiasis cases in Israel, it showed that *Philophthalmus* cercariae were collected from *M. praemorsa*^[8,9,13]. In Palestine, the

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knowledge on freshwater larval trematodes is poor. The purpose of the present study is to investigate the infection rate of trematodes in *M. praemorsa* and the type of cercariae released from *M. praemorsa* collected from different fresh water bodies in Palestine.

2. Material and methods

A total of 1 880 *M. praemorsa* snails were collected from different freshwater bodies in Palestine from October, 2008 to November, 2010. These water bodies included Al–Bathan, Al–Nassareya, Al–Oja, Al–Jeftlek and Wadi Qana. The number of snails collected from these water bodies were 1400, 150, 150, 100 and 80, respectively. These water bodies were used for bathing, drinking and washing by the people. The collected snails were kept in a glass aquaria containing water and thin layer of sediment from the same habitat of snails. Aquaria were continuously aerated using air pumps.

Examination of snails for larval trematodes was carried out as described previously [4,14]. Snails were examined for larval trematodes within 2 days after collection. Cercariae in *M. praemorsa* snails were obtained by lighting and crushing methods. In the lighting method snails were put in the dishes containing distilled water and illuminated for 12 hours in the room. In the crushing method, snails were broken with tweezers and the soft tissues were placed between 2 slides and squashed. Recovered larval trematodes were studied alive, unstained or vitally stained 0.5% neutral red or 0.5% brilliant cresyl blue. They were fixed in acetic acid–formalin–alcohol (AFA) solution and subsequently stained in acetocarmine. Measurements were taken on a minimum of 10 specimens of live and fixed larval trematodes. Figures were drawn with freehand, from preparations examined under a light microscope. The behavior of cercariae was observed using a dissecting microscope.

Table 1

Measurements (μm) of the various structures of *Cercaria melanopsi palestina I, II and III* encountered in *M. praemorsa* snails collected from Al–Bathan freshwater bodies, Palestine.

Structure	Type of <i>Cercaria melanopsi</i>		
	I	II	III
Body length ^a	90–130 100–120	75–110 110–136	250–310 230–280
Body width ^a	57–70 60–68	20–34 30–35	60–120 70–85
Tail length ^a	40–120 45–90	220–300 ^b 230–280	50–80 45–65
Tail width ^a	8–15 9–16	15–20 17–19	30–60 32–45
Oral sucker (diam.)	30–35	–	42–47
Ventral sucker (diam.)	21–23	–	40–45
Pharynx	7×7	–	18×14
Flame cell formula	2[(2)+(2)+(3)+(2)]=18	2[(1)+(1)+(1)+(1)]=8	2[(2)+(3)+(1)+(1)]=14

^a Measurements are given for live (upper values) and fixed (lower values) specimens.

^b This measurement includes the length of tail rami which are 50–75 by 5–10 μm (live) and 40–50 by 7–12 μm (fixed).

3. Results

Snails collected from water bodies of Al–Nassareya, Al–Oja, Al–Jeftlek and Wadi Qana were not infected. The overall infection rate of snails collected from all fresh water bodies was 4.3%. However, from the total of 1400 *M. praemorsa* collected from Al–Bathan fresh water body, 80 (5.4%) were infected with various larval trematodes including *Cercaria melanopsi palestina I* ($n=56$, 4%), *Cercaria melanopsi palestina II* ($n=14$, 1%) and *Cercaria palestina III* ($n=10$, 0.7%). Three different species of larval trematodes were recorded, which were xiphidiocercaria, a brevifurcate lophocercous cercaria and a microcercous cercaria. These cercariae called *Cercaria melanopsi palestina I*, *Cercaria melanopsi palestina II* and *Cercaria melanopsi palestina III* have not been described before from this snail in Palestine. Details of measurements of the various structures and the flame cell formula of each type of cercariae are presented in Table 1.

3.1. *Cercaria melanopsi palestina I* (Figure 1 A, B ; Table 1)

It has a variable size and shape depending on the contraction and extension movements of the body, with a simple tail not quite as long as the body. The body is covered with minute delicate spines, with a 2 μm thick tegument and has an oval and elongated shape. The anterior end of the body is armed with a stylet situated on the oral sucker which was 14 μm long and 3 μm wide at the round part in the posterior end. The stylet lacking conspicuous lateral thickening. The oral sucker is larger than the ventral one, while the later is located in the final third of the body. The genital premordium cell mass lies in the posterior of the ventral sucker. Alimentary canal is composed of a mouth and a pharynx which is located just behind the oral sucker. The excretory system consists of a V-shaped excretory

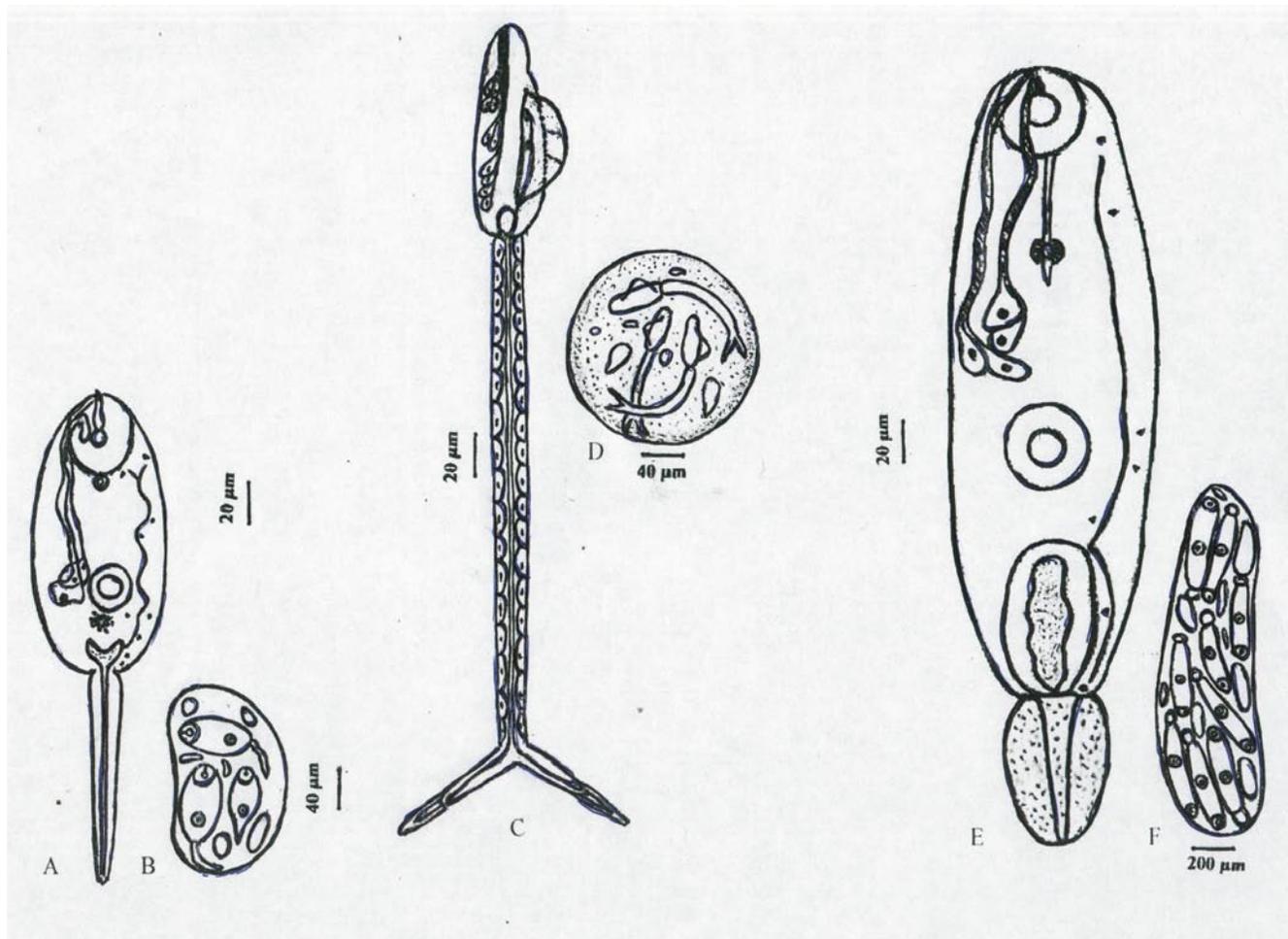


Figure 1. *Cercaria melanopsi palestina I, II and III* encountered in *M. praemorsa* snails from Al-Bathan. *C. melanopsi palestina I* and its sporocyst (A and B), *C. melanopsi palestina II* and its sporocyst (C and D) and *C. melanopsi palestina III* and its sporocyst (E and F).

vesicle measuring $10\ \mu\text{m}$ to $18\ \mu\text{m}$, opening into the posterior part of body through a canal extending to the tail. It also has two main lateral ducts, which run anteriorly to the oral sucker. Flame cells are arranged symmetrically in the body with a flame cell formula $2[(2) + (2) + (3) + (2)] = 18$, but they are missed in the tail. *Cercaria melanopsi palestina I* has two pairs of well developed penetration glands arranged symmetrically on both sides of the ventral sucker. One pair of these glands opens into two small pores located lateral to the stylet, while the other pair opens into two sacs located on both sides of the mouth. When the cercaria swims, the body is strongly contracted and the tail lashes in all directions, rarely pelagic or with powers of floatation. Once the cercaria comes to the surface, it pauses and starts sinking slowly, but often resumes swimming before hitting the bottom and after a few seconds of resting period. *Cercaria melanopsi palestina I* develops within ovoid sporocysts measuring $110\text{--}190\ \mu\text{m}$ long and $80\text{--}130\ \mu\text{m}$ wide. Sporocysts are filled with one or two fully developed cercariae and germ balls.

3.2. *Cercaria melanopsi palestina II* (Figure 1C, D; Table 2)

It is furcocercous cercaria and neither sucker nor alimentary

canal was seen. It has a long tail which is contractile and bifurcated into two rami. The length of the rami are less than that of half of the tail stem. The cercaria is equipped with an anterior protrusible organ, delimited from the body by a conspicuous constriction at about $18\text{--}20\ \mu\text{m}$ from the anterior edge. The typical median dorsal fin-fold extended from the posterior end of the body nearly to the region of the anterior protrusible organ, which is $40\text{--}60\ \mu\text{m}$ long and $13\text{--}17\ \mu\text{m}$ at its maximum width. The last third of each ramus is surrounded by a thin fin-fold. The cercaria body is covered with minute spines, with a $2\ \mu\text{m}$ thick tegument. The glandular system consists of two groups of glands. The anterior glands, four on each side of the body, are located behind the anterior protrusible organ. The posterior-lateral glands, six on each side, lead into two glandular bundles. Ducts of these glands open at the anterior edge of the protrusible organ. The excretory vesicle is small measuring $8\text{--}10\ \mu\text{m}$ by $4\text{--}6\ \mu\text{m}$. It has two main lateral ducts which run anteriorly, while other two canals run posteriorly extending into the tail with each one entering into the excretory pores at about $18\ \mu\text{m}$ from the branch tip. Flame cells are organized symmetrically in the body with a flame cell formula $2[(1) + (1) + (1) + (1)] = 8$, but they are missed in the

tail stem. *Cercaria melanopsi palestina II* swims actively by the action of its tail which shows great power of contractility. This cercaria develops within a spherical sporocyst and measures 130–200 μm in diameter. Birth pore is not discernible. Sporocysts contain 3–6 developed cercariae, several developing cercariae and germ balls.

3.3. *Cercaria melanopsi palestina III* (Figure 1E, F; Table 2)

This cercaria has an elongate cylindrical body with stumpy knob-like tail which may developed as a sucker. The body is covered with a smooth tegument (2 μm thick). Both ventral and oral suckers are round and nearly equal in size with ventral one near the middle region of the body. The oral sucker has a stylet in the anterior margin measuring 10 μm long and 4 μm wide. The alimentary canal consists of a mouth, a pre-pharynx and a pharynx. The excretory vesicle is relatively large, located in the space between the posterior end of the body and ventral sucker. It measures 60–75 μm long and 34–50 μm wide and is characterized by having a thick wall. It has two main lateral ducts which run anteriorly. Other two canals run posteriorly to the end of the body. Flame cells are arranged symmetrically in the body with a flame cell formula $2[(2) + (3) + (1) + (1)] = 14$, but they are missed in the tail. There are four pairs of penetration glands located above the ventral sucker. Gland ducts, on each side, form two bundles. Each of the outer and the inner bundle consists of two ducts. All bundles run anteriorly to open on both sides of the stylet. This specie of cercariae shows no swimming activity because it has a stumpy knob-like tail. This cercaria develops from the elongate sporocyst varying greatly in size. The sporocyst is 500–2000 μm long and 130–580 μm wide and has a birth pore at its anterior end. It is filled with many developed cercariae, developing cercariae and germ balls.

4. Discussion

Freshwater gastropods of the genus *Melanopsis* snails are apparently obligate intermediate hosts for many species of trematode parasites that may exist in several vertebrate hosts [2,4,7–9,13,15,16]. A detailed description of the various types of cercariae has been reported previously [17–21]. Some of these cercariae are classified according to the position and number of body suckers. Some are categorized according to the shape and relative size of their tails, while others are categorized morphologically by specialized body structures like the xiphidiocercariae. In the present study, three types of cercariae have been described. These are xiphidiocercaria, brevifurcate lophocercous and microcercous. The characteristics for xiphidiocercariae differentiation have been reviewed previously [22]. These characteristics include flame cells, shape of stylet, presence or absence of fin-folds, number and location of penetration

glands and others. However, the author concluded that none of these characteristics have significant taxonomic value and the xiphidiocercariae group should be left without subdivisions. There are several families of digenetic trematodes have xiphidiocercariae as their larval forms, for example, *Plagiorchidae*, *Telrchiidae*, *Ochetosomatidae*, and *Lecithdendriidae*. The presence of well developed stylet in *Cercaria melanopsi palestina I* is identified as xiphidiocercaria. *Cercaria melanopsi palestina I* belongs to microcotylae sub-group of the xiphidiocercariae due to the absence of the tail fin-fold, the small size of the body, and the location and size of the ventral sucker. Moreover, it belongs to "Pusilla" sub-type due to the possession of two pairs of penetration glands, undeveloped gut, and development within small ovoid sporocyst, except that the number of flame cells is greater (18 vs 12) [19]. *Cercaria melanopsi palestina I* is closely related to *Cercaria melanopsi I* described previously from *M. praemorsa* snails collected from Yarmouk River, Jordan [15], except that the number of pairs of penetration glands (2 vs 3) and the number of flame cells (18 vs 20) are different.

Cercaria melanopsi palestina II is an *Furcocercous cercaria*, apharyngeal, brevifurcate monostome cercaria. It belongs to the "Lophocerca" group which is characterized by the presence of a dorsal median fin-fold on the body, lacking ventral sucker, having excretory system with two canals in the tail and absence of flame cells in the tail stem. It resembles *Cercaria cristata* which develops in *Lymnaea stagnalis* snails. However, *Cercaria melanopsi II* is different from *Cercaria cristata* because it lacks gut, has eight flame cells instead of six, 10 pairs of penetration glands instead of one pair and develops within a small spherical rather than in a thread-like sporocyst [19]. Also *Cercaria melanopsi palestina II* is different from *Cercaria brevifurca* which develops in *Planorbis trivolvis* as well as *Cercaria cristata* except that *Cercaria brevifurca* has four pairs of glands ventral to cecum and rami are not bearing fin-folds [23]. *Cercaria melanopsi palestina II* resembles that of *Cercaria melanopsi IV* described previously from *Melanopsis praemorsa* snails collected from Yarmouk River, Jordan [15], except that the number of pairs of the posterior-lateral glands (6 vs 7) are different. It resembles *Sanguinicola* cercaria which develops in *Potamopyrgus antipodarum* snails and was recovered in France [24]. *Sanguinicola* cercaria is characterized by a body and furca fin-fold, suckerless, the absence of a pharynx and development in spherical sporocyst, but the flame cells are not discernible and the number of penetration glands and other characteristics were not mentioned. *Sanguinicola sp.* is a digenetic blood fluke of brackish or fresh water fishes.

Cercaria melanopsi palestina III belongs to a group of cercariae called "Microcercous" cercariae, whose tail is short and stumpy. This cercaria resembles that of *Cercaria melanopsi V* described previously from *Melanopsis praemorsa* snails collected from Yarmouk River, Jordan [15],

except that the number of pairs of penetration glands (4 vs 5) and the number of flame cells (14 vs 16) are different. It also resembles that of *Sphaerostoma bramae* which develops in a sporocyst in *Bithynia tentaculata*^[19]. However, *Cercaria melanopsi palestina III* differs from that of *Sphaerostoma bramae* in that the former has two suckers of equal size and its gut consists of only a pharynx and a short oesophagus. The *Microcercous cercariae* may develop into trematodes of several families, such as, the *Allocreadiidae* and *Gorgoderidae*, which are reported from a freshwater fish and frog, respectively^[19].

In summary, due to the presence of various cercariae in *M. praemorsa* snails and their potential for establishment of zoonotic diseases in animals and may be in humans, these findings may be concerned by Health Ministry Officials for surveillance of fresh water snails in this fresh water body as well as others.

Conflict of interest statement

We declare that we have no conflict of interest.

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