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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 palestinia* I, *Cercaria melanopsi palestinia* II and *Cercaria melanopsi palestinia* 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.

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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 miriacidia. 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

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.

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 palestinia* I (n=56, 4%), *Cercaria melanopsi palestinia* II (n=14, 1%) and *Cercaria palestinia* 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 palestinia* I, *Cercaria melanopsi palestinia* II and *Cercaria melanopsi palestinia* 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 palestinia 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

Table 1
Measurements (μm) of the various structures of *Cercaria melanopsi palestinia* I, II and III encountered in *M. praemorsa* snails collected from Al-Bathan freshwater bodies, Palestine.

Structure	Type of *Cercaria melanopsi*		
	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 (diameter)	30-35	_	42-47
Ventral sucker (diameter)	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).