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Three new species of cercariae from *Melanopsis praemorsa* (L. 1758, Buccinum) snails in Al-Bathan fresh water body, Palestine

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ABSTRACT

Objective: To investigate other new species of cercariae encountered in *Melanopsis praemorsa* (*M. praemorsa*) snails collected from Palestine. **Methods:** A total of 1 100 *M. praemorsa* were collected from Al-Bathan water body, Palestine, from November, 2010 to November, 2011. Cercariae in *M. praemorsa* were obtained by emerging and crushing methods. **Results:** Other three new different species of cercariae have been identified from this snail. These species were Xiphidiocercaria (*Cercaria melanopsi palestinia* IV), Microcercous (*Cercaria melanopsi palestinia* V) and Longifurcate cercaria (*Cercaria melanopsi palestinia* VI). The infection rate of *M. praemorsa* with these three different cercariae was (42.2%). Coinfection with Xiphidiocercariae and Longifurcate cercariae or Xiphidiocercariae and Microcercous cercariae has been noted and coinfection rate was 1.23% among the infected snails. The highest cercarial infection rate was in June (64.3%). No infected snails were found in September. It was also noted that infected snails attained a larger size than uninfected ones and all infected snails had a size between 17-22 mm (average 20 mm). **Conclusions:** Our studies imply that there are potentially more new species of trematodes in this area than were found until now. Due to the presence of infected *M. praemorsa* and may be species of other snails, water resources could be contaminated by the emerging new cercariae, consequently attack the local people directly via the skin or are transferred to them by metacercariae ingestion. More surveys are needed to identify the real prevalence of the trematodes both in human and animal hosts, and also to determine the range of snail hosts of the parasite in enzootic areas of the disease, as well as the life cycle and biology of trematodes and its effects on man should be elucidated.

1. Introduction

Freshwater snails play a vital role in the life cycle of digenetic trematodes. These snails provide these parasites with resources for development, reproduction and also constitute a means of transport by which trematodes can reach their next host. Most authors agree that trematodes were associated with molluscs before they adapted to other hosts[1]. Trematodes are a diverse group of endoparasite requiring intermediate host (molluscan and vertebrate) and definitive host to complete their life cycle. The distribution of fresh water mollusks varies with biological, physical and chemical characteristics[2]. *Melanopsis praemorsa* (*M. praemorsa*) (L. 1758, Buccinum) is widely prevalent in freshwater bodies of the Mediterranean region. In Palestine, it is considered one of the most abundant snails present in fresh water bodies especially in Al-Bathan freshwater body[3].

Three larval stages of digenetic trematodes were reported from freshwater *M. praemorsa* in Palestine. These larvae were Xiphidiocercaria (*Cercaria melanopsi palestinia* I), Brevifurcate lophocercous (*Cercaria melanopsi palestinia* II) and Microcercous (*Cercaria melanopsi palestinia* III) [3]. Description new types of Cercariae from *M. praemorsa* and other snails from other countries has been reported in the literature. Therefore, it is expected that *M. praemorsa* may act as an intermediate host for many digenetic trematodes of various vertebrates in Palestine. Seven types of cercariae have been found in *M. praemorsa* collected from Yrmouk River, Jordan[4]. These included three types of Xiphidiocercariae, one type of a Brevifurcate lophocercous cercaria, one type of a Microcercous Cercaria, and two types of Pleurolophocereous Cercariae. A total of four cercarial families and one cercarial group were

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identified from *Melanopsis* spp as follows: Heterophyidae: *Haplorchis pumilio, H. taithui, Stellantchasmus falcatus* and *Centrocestus formosanus*; *Echinostomatidae: Echinochasmus milvi; Cyathocotylidae, Philophthalmidae* and *Monostome group cercariae* (probably Notocotylidae)[5]. Recently, a total of eight different cercarial types have been reported in freshwater snail *M. praemorsa* from the Kura River in Azerbaijan[6-9]. These cercraiae included: three types of stylet cercaria, four types of virgulate cercaria and one type of lecithodendroid cercaria. Eleven types of cercariae were detected from the freshwater prosobranch snail *Melanoides tuberculata* collected from the Nile and irrigation system in several localities of Egypt[10]. These cercariae belong to four main type groups: two types xiphidiocercariae, two types furcocercous cercariae, four types pleurolophocercous and three types gymnocephalous cercariae. In addition to these, twenty four trematode species comprising 19 species of cercariae and 11 species of metacercariae (six species occurred both as cercariae and metacercariae) were identified in Central Europe from Lymnaea stagnalis snails[11].

In Palestine, our knowledge on freshwater larval trematodes is still poor. The present study describes three new species of cercariae encountered in specimens of *M. praemorsa* collected from Al-Bathan freshwater body, Palestine.

2. Material and methods

A total of 1 110 *M. praemorsa* snails were collected from Al-Bathan freshwater body in Palestine from November, 2010 to November, 2011. Examination of snails were carried out as described previously[3]. The collected snails were kept in a glass aquarium containing water and thin layer of sediment from the same habitat of snails. Aquaria were continuously aerated using air pumps. Snails were examined for larval trematodes within 2-3 d after collection. Cercariae were obtained from snails by emerging or crushing methods. In the emerging method snails were placed in the dishes containing distilled water and then, they were exposed to artificial illumination for 12 h in the lab. 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% brilliant cresyl blue or 0.5% neutral red) specimens. Some specimens 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. The behavior of cercariae was observed using a dissecting microscope.

3. Results

From the total of 1 100 *M. praemorsa*, 464 (42.2%) snails were infected with three different cercariae. These new cercariae were Xiphidiocercaria (*Cercaria melanopsi palestinia* IV), Microcercous cercaria (*Cercaria melanopsi palestinia* V) and Longifurcate cercaria (*Cercaria melanopsi palestinia* VI). The infection rates were 31.4%, 7.0%, and 3.8% for Xiphidiocercaria, Microcercous cercaria and Longifurcate cercaria, respectively. These cercariae have not been described before from this snail. Coinfected snails with Xiphidiocercariae and Longifurcate crcariae or Xiphidiocercariae and Microcercous cercariae has been noted but rare, and the coinfection rate was 1.23% among the infected snails. The highest cercarial infection rate was in June (64.3%). No infected snails were found in September. It was also noted that infected snails attained a larger size than uninfected ones and all infected snails had a size between 17-22 mm (average 20 mm). Details of measurements of the various structures of each type of cercariae are presented in Table 1.

Table 1.
Measurements (μm) of the various structures of *Cercaria melanopsi palestinia IV,V* and *VI* encountered in *M. praemorsa* snails collected from Al-Bathan freshwater body, Palestine.

Structure	Type of *Cercaria melanopsi palestinia*		
	IV	VI	VI
Body length[a]	65-120	270-330	400-420
	60-110		380-410
Body width[a]	40-60	70-130	210-220
	35-50		200-210
Tail length[a]	47-75	70-90	440-460[b]
	40-70		420-440
Tail width[a]	8-14	50-70	100-110
Oral sucker (diam.)	25-30	55×58	80-90×82-93
Ventral sucker (diam.)	20-25	50×53	28×28
Pharynx	6×12	23×30	8-12
Excretery vesicle (diam)	16×22	(50-70) ×(40×56)	30×60
Flame cell formula	2[(3) + (5) + (2) + (3)]=26	[(2+2+2)+(2)]=16	2[(1+1+1+1+1+1+1+1)+ (1+1+1+1+1+1+1+1) +[(1+1+1+1)]]=36

[a]Measurements are given for live (upper values) and fixed (lower values) specimens. [b]This measurement includes the length of tail rami which are 213-222 by 44-46 μm (live) and 200-205 by 40-44 μm (fixed).

3.1. Cercaria melanopsi Palestinia IV (Figure 1 A, B; Table 1)

This cercaria has a small, oval shaped and transparent body with fine spined, 1-2 μm thick tegument. The ventral sucker which is located in posterior half of the body, is smaller than oral sucker. This type of cercaria has an oral sucker, of which possesses not only the stylet but also a peculiar structure, called "virgula organ". In Cercaria melanopsi Palestinia IV, the dagger-shaped stylet measuring 14 μm × 3 μm and a bilobed virgula that covers more than the half of the oral sucker area, which contains non-granulated material. In addition, it has two pairs of penetration gland cells containing coarse granular matter, located antero-laterally to the ventral sucker, open on both sides of the stylet. The digestive system consists only of a mouth and a pharynx, but oesophagus and caeca are apparently absent. The excretory system consists of a shaped like kidney excretory vesicle measuring 20-24 μm × 15-17 μm, located at the posterior end of the body. There are two main excretory ducts, one on each side of the body. These ducts extend posteriorly, to join antero-laterally the excretory vesicle. The excretory vesicle is opening into posterior body extremity through a caudal canal extending posteriorly through the length of the tail to open at its tip. Flame cells are arranged symmetrically in the body and has a flame cell formula 2[(3+5+2+3)]=26, but are missed in the tail. The anlagen of reproductive organs are found posterior and around the ventral sucker.

These cercariae actively swim using their tail near the water surface, while in the bottom start wiggling rather than creeping. *C. melanopsi* palestinia IV develops within ovoid sporocysts measuring 87-180 μm long by 84-160 μm wide. It is covered with a 1-2 μm thick tegument and filled with 1-4 fully developed cercariae, 1-2 developing cercariae, and several germ cells.

3.2. Cercaria melanopsi Palestinia V (Figure 1 C; Table 1)

The body of this cercaia is covered with a smooth 3 μm thick tegument and longer than tail stem. However, several rows of fine spines are found at the inner edges of both oral and ventral suckers. The body of this cercaria is filled with refractile granules which appear to be cystogenous material. The tail is short, stumpy, muscular, and sometime may be used for attachment. Both suckers are round, ventral sucker is a little bit smaller than oral sucker and located centrally near the middle region of the body. The stylet is in the anterior edge of the oral sucker measuring 12 by 5 μm. The digestive system consists of a mouth that leads into a thin pre-pharynx which connects with an oval pharynx, while oesophagous is rudimentary. This type of cercaria has

Figure 1. *Cercaria melanopsi palestinia IV*, and *V* encountered in *M. praemorsa* snails from Al-Bathan. *C. melanopsi palestinia IV* and its sporocyst (A and B), *C. melanopsi palestinia V* (C) and its sporocyst is not shown.

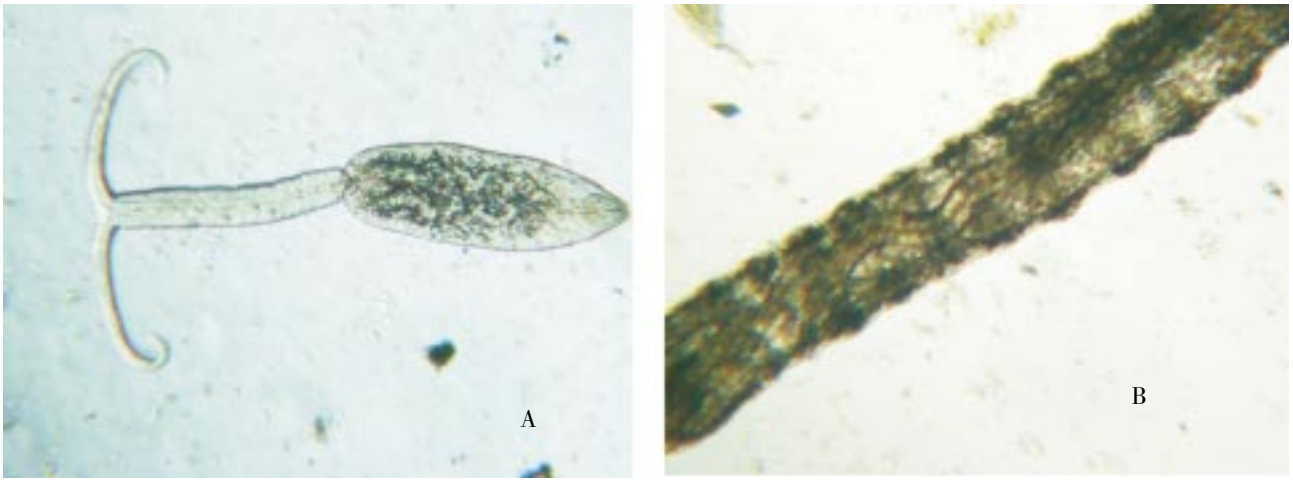

Figure 2. *Cercaria melanopsi palestinia* VI and its sporocyst (A and B) encountered in *M. praemorsa* snails from Al-Bathan.

three pairs of penetration glands located above the ventral sucker. Gland ducts, on each side, form two bundles. The outer bundle consists of two ducts, while the inner consists of one duct. All bundle run anteriorly to open on both sides of the stylet. The excretory vesicle is relatively large, filling major portion of the space between the ventral sucker and the posterior end of the body. It measures 70-85 μm by 40-56 μm and is characterized by having a 11-14 μm thick wall. Two main lateral ducts run anteriorly, while others 2 short ducts run to posterior end of the body. Flame cells are arranged symmetrically in the body but are absent in the tail. Flame cells formula is 2[(2+2+2) +(2)]=16.

This type of cercariae shows no swimming activity, because it has short tail, which may developed as a sucker for attachement. It was clear that it used the tail to attach itself to the bottom of the water container. When detached from the bottom the cercaria creeps, for a short period of time, in a worm- like manner using both sucker for positioning. The cercaria casts off its tail and dies without encystment after several hour of emergence from the snail. The cercaria develops in a sausage- shaped sporocysts varying greatly in size (800-2 300 μm × 380-610 μm). The sporpcyst has a birth pore at its anterior end and is filled with many developed cercariae, developing cercariae, and germ balls.

3.3. Cercaria melanopsi Palestinia VI (Figure 2 A, B ; Table 1)

This cercaria comprises an oval body, longifurcate, non-oculate, pharyngeate. The length of the tail stem (226-237 μm) is approximately the same as well as the length furcal ramus (213-222 μm), no furcal finfolds. It is covered with 2-4 μm thick tegument which is provided with fine spines usually more concentrated at the anterior part of the body. A total of 7 pairs of sensory cilia found along the tail stem measuring 14 μm long, another four pairs of sensory cilia of 4 μm long are found at each tail ramus. It has a rudimentary ventral sucker measuring 28 μm × 28 μm, located post-equatorial. Cercaria melanopsi Palestinia V has an oral sucker which is modified to relatively large penetration organ measuring 80-90 μm × 82-93 μm. The digestive system consists of a mouth that leading posteriorly into a very short distinct pre-pharynx (6-7 μm), followed by a muscular spherical pharynx (8-12 μm), then a short oesophagus, as long as pharynx approximately (14 μm), the oesophagus then leading into two long intestinal caeca (17-20 μm wide) that extend to posterior extremity of the body terminating close to anlagen of reproductive organs. The excretory system consists of spherical excretory vesicle with a diameter (30-60 μm). From the excretory vesicle a caudal excretory duct extends posteriorly along the tail stem that terminates in an opening on the tip of each caudal ramus. Two pairs of main primary excretory ducts run anteriorly, the outer pair runs to about the level of penetration glands, while the inner pair runs to about the level rudimentary ventral sucker. A total of 36 flame cells have been detected in Cercaria melanopsi Palestinia V, seven on each of outer and inner duct, and 4 pairs along the caudal duct, flame cells are missed in rami.

The possible flame cell formula is 2[(1+1+1+1+1+1+1)+(1+1+1+1+1+1+1)+[(1+1+1+1)]]=36. The genital primordium consists of relatively large cells found immediately above the excretory vesicle. The glandular system composed of 5 pairs of granular cells arranged symmetrically inside the penetration organ, and open at the anterior margin, as well as, 10 pairs of granular cells are found outside the penetration organ.

This cercaria is a rapid swimmer but its movements are interrupted by frequent resting pauses. As typical for furcocercous cercaria, the swimming is tail directed, and when inactive, the larva sinks slowly to the bottom. This cercaria develops within unbranched thread-like spotrocysts located in the heptopancreas of the snails. The sporocysts are extremely elongated (2 700-6 300 μm × 240-380 μm), annulated and have one pointed end to form a birth pore. Sporocysts usually contain 8-14 developed cercariae, 4-12 developing cercariae, and germ balls.

4. Discussion

This study adds more information to the trematode fauna in Palestine and represents the first step to understand the host-parasite relationship including the completion of their life cycles as well as defining their final hosts. High prevalence of infection in June, this might be due to low levels of water, slow-flowing in the stream during hot non-rainy season, also it could be due to high contamination by the faecal matter of livestock, birds and other animals. It is probably that snail abundance is low in the summer and miracidia enter most available snails. It is noted that more than 93% of infected snails were could not creep to the wall of aquarium, this may be due to pathological effect of these cercariae on the host.

M. praemorsa snail is apparently intermediate host for many digenetic trematodes of various vertebrates living in this area. Three types of cercariae have been described previously and were encountered in this snail in Al-Bathan water body[3]. Our studies revealed that this snail could carry at least six different types of cercariae in Palestine. Xiphidiocercaria was coined for the group of non-oculate distome cercariae in which the tail is slender and a stylet is present at the round anterior end[12]. Development of these cecariae occurs in sporocysts and encystment takes place in intermediate hosts. The main features used to distinguish between Xiphidiocercariae are the size of the suckers, position of ventral sucker, nature of the gut, number and position of the penetration glands, and presence or absence of a fin-fold along the margins of the tail. The presence of well developed stylet and "virgula" organ in *C. melanopsi* palestinia IV identifies this type as

Xiphidiocercaria and belongs to "Cercaria Virgulae" subgroup[13]. "Cercaria Virgulae" subgroup erected for spinous Xiphidiocercaria in which the ventral sucker is smaller than the oral, tail lacks a fin-fold, excretory vesicle almost V-shaped, and a highly characteristic 'virgula' organ present, consisting of two pyriform sacs which are fused in the median line and have their pointed ends directed forward and situated near the oral sucker[13]. The virgula organs are residual ducts of the mucoid body gland-cells and remain after the gland cell bodies degenerate. The ducts are markedly enlarged and appear as paired comma-shaped reservoir, open to the exterior and, during penetration of the second intermediate host, release materials that forms a pseudocyst around the cercarial body to attach it to the host as penetration occurs[14]. *C. melanopsi* palestinia IV closely related to *C. melanopsi III* described previously from *M. praemorsa* snails collected from Yarmouk River, Jordan[4], except that the latter having three pairs of penetration glands instead of two and having 30 flame cells instead of 26 in *C. melanopsi* Palestinia IV. On the other hand, *C. melanopsi* palestinia IV is different in may aspects from *C. melanopsi* II[4]. *C. melanopsi* II having larger penetration glands; the "Vurgula" organ is unilobed; and the ventral sucker is located in the middle. Also *C. melanopsi* Palestinia IV is different from *C. melanopsi* palestinia I which was described previously [3], the later differs in being the "Vurgula" organ is absent and having 18 flame cells. *C. melanopsi* Palestinia IV is different from Virgulate xiphidiocercariae called *Cercaria* sp. IX Malabar n.sp and *Cercaria* sp. X Malabar n.sp, which are described recently from *Thiara tuberculata* snails collected from freshwater bodies in the Palakkad district of Kerala[15]. *C. melanopsi* Palestinia IV is distinct from these in size of body and stylet, shape of virgula organ, arrangement and number of penetration glands and flame cells, absence of intestine and also in the intermediate host. Rrecently, two types Virgulate xiphidiocercariae were described[10], these are collected from the freshwater prosobranch snail *Melanoides tuberculata* from the Nile and irrigation system in several localities of Egypt. These Virgulate xiphidiocercariae are distinct from *C. melanopsi* Palestinia IV in may aspects such as shape of stylet, virgula, genital primordium, shape of excretory vesicle, number of penetration glands and intermediate host. Number and flame cells formulae for these Virgulate xiphidiocercariae are not mentioned.

Microcercous cercariae is one of the lesser groups of cercariae, though, in recent times, a number of both marine and fresh-water species have been described[13]. Cercaria melanopsi Palestinia V belongs to a group designated as a "Microcercous". Cercaria melanopsi Palestinia V is closely related to *C. melanopsi* V which was described previously from *M. praemorsa* snails collected from Yarmouk River, Jordan[4], except that number of pairs of penetration glands (3 vs the 5) and ventral sucker is a little smaller than oral sucker. *C. melanopsi* Palestinia V resembles that of larva of Sphaerostoma bramae (*Cercaria micrura*) which develops in a sporocyst in *Bithynia tentaculata*[13]. However, *C. melanopsi* Palestinia V differs from *Cercaria micrura* in which the later has ventral sucker larger than oral sucker; its gut consists of a pre-pharynx, oesophagus and caeca relatively long; has four penetration glands and the intermediate host is *B. tentaculata*. *C. melanopsi* palestinia III has been recently described from *M. praemorsa* in Palestine[3]. *C. melanopsi* Palestinia V differes from *C. melanopsi* palestinia III in number of penetration glands and flame cells. Another related microcercous cercaria, C. theodoxi II has been described from *Theodoxus jordani* snails in Yarmouk River[16]. *C. melanopsi Palestinia V* differs from *Cercaria theodoxi* II in many aspects such as the arrangement of penetration glands, flame cells formula and number, the larger size of the tail, the absence of long spines at the periphery of the anterior end and the intermediate host.

The presence of forked tail in *C. melanopsi* Palestinia VI identifies it as a fucocercous cercariae. There are two forms of fucocercous cercariae; the brevifurcate fucocercous form, the length of each of the furcal ramus is less than the half of the length of the main tail stem and in the longifurcate fucocercous form, each of the fucal ramus is more than half of the length of the main tail stem[17]. *C. melanopsi* Palestinia VI is belonged to a group longifurcate fucocercous, due to the length of each rami is more than half of the length of the main tail stem. The presence of a pharynx, absence of eyespots, distome, this cercaria belongs to pharyngeal longifurcate distome cercaria, which belongs to the "Vvivax" group[13]. *C. melanopsi* palestinia VI closely related to *C. melanopsi* XV described previously from *M. praemorsa* snails collected from Jarash Falls, Jordan [18]. In *C. melanopsi* XV, the length of the tail of the ramus is about two thirds of the tail stem, while in *C. melanopsi* palestinia VI the tail ramus has a length of about the same as the tail stem. *C. melanopsi* XV has 16 pairs of flame cells in the body and 5 pairs of flame cells in the tail stem, while 14 pairs of flame cells in body and 4 pairs of flame cells in tail stem of *C. melanopsi* palestinia VI. *C. melanopsi* XV has 11 pairs of granular glands are found outside the penetration organ, while *C. melanopsi* palestinia VI has 5 pairs inside and 10 pairs of granular glands outside the penetration organ. Moreover, *C. melanopsi* XV has finfolds covering most of the tail rami, while this is absent in *C. melanopsi*

palestinia VI.

Our findings in this study and the previous one[3], imply that there are potentially more species of trematodes in Al-Bathan area than were found until now. Due to the presence of infected *M. praemorsa* and species of other snails, water resources could be contaminated by the emerging cercariae and consequently cercariae attack the local people directly via the skin or are transferred to them by metacercariae ingestion. Our studies provide preliminary information about the distribution and prevalence of trematode species based on identification of larvae. Although identification of cercariae to species level using specific characteristics such as, nervous system elements, sensory apparatus, body papillae such cheatotaxy is possible but it is very laborious and subjective[19]. Therefore DNA analysis is a better reliable method. Determination the host-parasite relationship in enzootic areas of the disease, as well as the life cycle and biology of trematodes and its effects on man should be elucidated.

Conflict of interest statement

We declare that we have no conflict of interest.

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