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Trematode cercarial fauna obtained from the field-collected freshwater snails *Lymnaea natalensis* in Egypt

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Abstract

Background: *Lymnaea natalensis* is the snail intermediate host for *Fasciola* spp. parasites. The purpose of the present investigation is to detect the different trematodes' larval stages in this snail from three rural localities in Giza Governorate (ElZeidia, Oseem, and ElMansuria), Egypt as examples and detecting the percentage of infection with *Fasciola* spp. and other trematodes cercariae. The samples were collected and examined for parasitic cercariae infections by using cercarial emergence and crushing techniques.

Results: From total examined snails, it was found that the infected snails included five types of the cercarial stages beside *Fasciola* cercariae and three types of metacercariae. These cercariae were xiphidio cercariae, gymnocephalus, echinostome cercariae, virgulate cercariae, Echinochasmus pelecani, and Palaeorchis cercariae. The infection rate in snails collected from ElZeidia was the higher infection rate (84%) than others from Oseem (76%) and ElMansuria (62%).

Conclusion: These findings confirmed that *L. natalensis* snails are not only a suitable intermediate host for *Fasciola* spp. but also for several parasites and play an important role in the transfer of different parasitic diseases to animals and humans.

Keywords: Fasciolosis, *Lymnaea natalensis*, Trematodes cercariae

Background

Fasciolosis is one of the neglected tropical diseases (Beesley et al. 2017; WHO. World Health Organization 2017) and is caused by infection of trematodes belonging to the genus *Fasciola* spp. It is a serious infectious parasitic disease infecting humans and animals worldwide (Soliman 2008). *Fasciola* spp. causes disease of economic importance in sheep and cattle (Schweizer et al. 2005; Bennett and Pelaar 2005), with an estimated 250 million sheep and 350 million cattle at risk worldwide (Beesley et al. 2017; Hillyer and Apt 1997). Human fascioliasis is considered now as a zoonosis of major global and regional importance (Soliman 2008) as it is affecting nearly 50 million people worldwide (Rahman et al. 2017). Recently, the epidemiology of human fasciolosis had increased in the last two decades (Chaturvedi and Singh 2016). Individuals can also be infected by drinking water containing viable metacercariae (Sah et al. 2017), or by ingesting raw aquatic plants contaminated with the metacercariae (Lee et al. 2017). These liver flukes caused severe hepatic damage in the bile duct of the definitive hosts (Amer et al. 2016).

Freshwater snails served as intermediate hosts for a large number of trematode parasites in humans and animals (Lee et al. 2017; Chontananarth et al. 2017). *Lymnaea* snails are a group of freshwater snails distributed worldwide (Adediran and Uwalaka 2013) and *Lymnaea natalensis* snails were the main snail host for these liver flukes, *Fasciola hepatica* or *gigantica*, which were widely distributed in Africa (Moema et al. 2008). This infection had been recognized as a major constraint to animal farming, contributing to the impeded economic development in the developing countries (Amer et al. 2016).

In Egypt, liver flukes of *Fasciola* spp. negatively affected the farming industry and public health (Elshraway

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and Mahmoud 2017), and the liver fluke fragments were found in the Egyptian mummy (David 1997; Esteban et al. 2003). According to the Egyptian Academy of Scientific Research and Technology Report, losses in animal wealth due to fasciolosis in Egypt were estimated at 190 million annually. Also, human infection is a major public health problem in several areas of the world, including the Nile Delta in Egypt (Soliman 2008). Both acute and chronic fasciolosis have been found in almost all governorates and in the reclaimed desert land (Soliman 2008). Ashour et al. (2008) stated that it has a wide prevalence and distribution in the Egyptian Governorates, like Fayoum, Giza, Qaluobiya, Sharkiya, Dakahliya, Gharbiya, Kafr El-Sheikh, and Damiett Governorates and correlated the presence of these snails with the presence of the aquatic plants and the other snails. The infection rates of these snails were increased during summer months (Ahmed and Ramzy 1999; Hussein et al. 2005).

Lymnaea natalensis snails are involved in the life cycles of at least 71 trematode species belonging to 13 different families whose members use birds and both domestic and wild animals as definitive hosts (Brown 1978), and this without counting digeneans of lower vertebrates such as amphibians. These trematode species were varied according to the considered geographic region (Toledo et al. 1998) and local ecological characteristics (Esch and Fernandez n.d.). Lymnaeid species included different cercariae which were the larval form of different parasites. This field study was designed to gather information about different trematode cercariae present in *L. natalensis* snails beside *Fasciola* cercariae in three rural localities in Giza governorate, Egypt and so, can develop an initiative ways for their control.

Materials and methods

Collection of *Lymnaea natalensis* snails samples

The field groups included 3.130 *L. natalensis* snails collected during April 2016 to September 2017, from different irrigation canals belonging to three rural localities in Giza governorate namely Elmansuria, Elzeideia, and Oseem villages (Fig. 1). In each sampling site, the collection area covering 1 m^2 through the agricultural canals. Snails found under water *Lemna gibba* leaves were collected manually by a sweeping net, where snails then loosen their hold on the plants and dropped to the bottom of the net (Fig. 2). Lymnaeid snails were found in areas where water was stagnant or presented only a slow current, usually in places where livestock was present.

Snails' maintenance in lab conditions

Collected snails were transported by wet cotton to the lab at Theodor bilharz Research institute at the same day of collection. In Malacology laboratory, the snails were maintained in 60 × 30 × 30 cm plastic containers. *Lymnaea* snails were fed by lettuce 1–2 times per week and the water in the containers was changed once 24–48 h. Eggs were collected daily on transparent plastic sheets placed into the aquaria. These snails were identified morphologically as regards color, number of whorls,

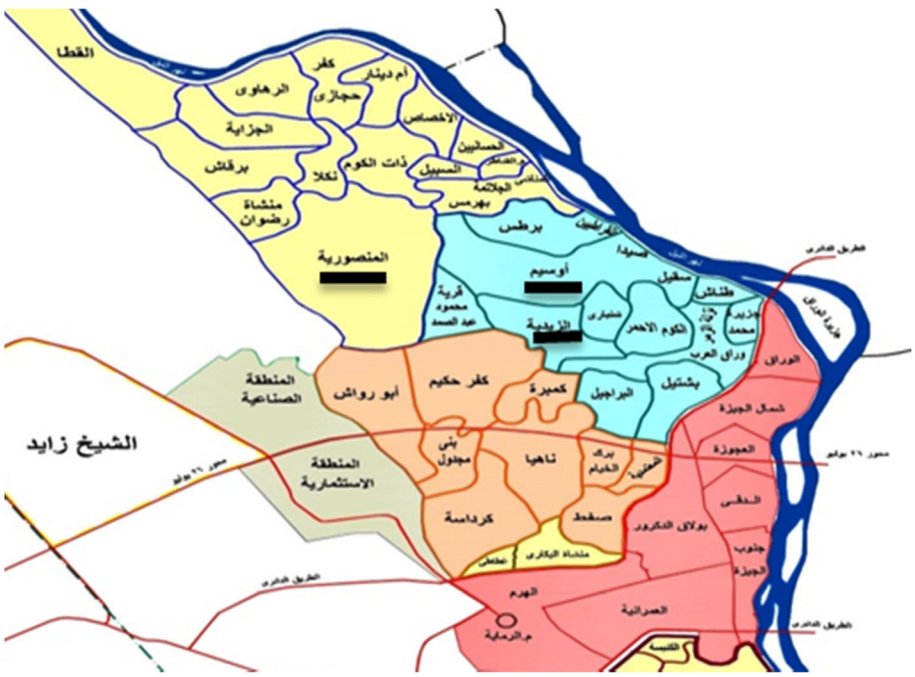

Fig. 1 Shows map of Giza; http://www.giza.gov.eg/GovMaps/Maps.aspx

Fig. 2 Shows manual collection of snails

and diameter of the shell according to keys proposed by Ibrahim et al. (1999).

Collection and study of trematode larval stages

The collected snails were examined for larval trematodes infection by two methods that were carried out either by exposure of snails to artificial light for 1 h, at 25 °C, for cercarial shedding (Liang et al. 1987) or by crushing (Chu and Dawood 1970), where snails were crushed between two slides, and were carefully investigated under the dissection microscope. The collected cercariae were identified fresh or stained samples with iodine solution using light microscope. Non-shedding snails were kept in the laboratory and re-examined weekly to verify that cercariae were not shed by snails which could have been infected only shortly before being collected. When snails proved to be negative after several examinations by the shedding method, they were gently crushed in a Petri dish containing a small amount of dechlorinated water. The fleshy part of each snail was removed and was dissected under a microscope. The number of snails shedding cercariae and those presenting infection by intramolluscan larval stages were recorded. Cercarial types were classified according to Frandsen and Christensen (1984).

Statistical analysis

Raw data were analyzed statistically using SPSS software (IBM Software Company, USA). The percentage was calculated for all data as the number of infected individuals divided by number of individuals examined and multiplied by 100.

Results

The external examination of the shell reveals that it is thin, fragile, with concavity on its side and a wide open funnel. Also, it is dextral, dark brown to black in color; the shell whorls were ranging from three to five in number and are spirally coiled (Fig. 3). As regards the size, it is about 12–16 mm (Table 1).

The present results show that the infection rate of snails infected with different trematode larvae that were collected from three rural localities in Giza governorate is higher in Elzeidia (84%) than others from Oseem (76%) and ElMansuria (62%) (Table 2).

During the present study, five species of cercariae, beside *Fasciola* cercariae, were found among the examined infected snails. The most abundant type was xiphidio cercaria, followed by gymnocephalus (faciolid) cercaria and echinostome cercariae (Fig. 4). These cercariae were divided into separate groups based on morphologically distinguishable differences in the shape and the length of the tail. All types of cercariae were single-tailed types and are classified as:

1- Xiphidio cercaria (Plagiorchidea):

The oral sucker has a stylet, and the ventral sucker located in the middle of the body with the same size of oral sucker; it develops in sporocysts (Plate 1, 1 and 2) and encystments in invertebrates, amphibians, and reptiles.

2- Gymnocephalus cercaria (Fasciolidae):

The length of its tail is almost as long as the body with dorso-ventral fin fold; the ventral sucker on mid-ventral surface of body and there is no stylet in the oral sucker. It develops in rediae and encysts on external substrates. It is produced by species of the family Fasciolidae (intestinal and liver parasites in herbivorous mammals). (Plate 1, 3 and 4).

3- Echinostome cercariae:

Its tail is stout and as long as the whole body. The ventral sucker located on mid-ventral surface of body. It develops in rediae and encysts in invertebrates, fish, and amphibians. It is produced by species of the family Echinostomatidae (intestinal parasites of birds, reptiles and mammals) (Plate 2, 5).

4- Virgulate cercaria:
It has a stout non forked tail; its length is shorter than its body and without finfolds. Ventral sucker is smaller than oral sucker, and a stylet located in the oral sucker (Plate 2, 6). It is produced by species of the family Lecithodendriidae (intestinal parasites of bats, birds, and amphibians) and by species of the genera *Lymnaea* and *Melanoides*.

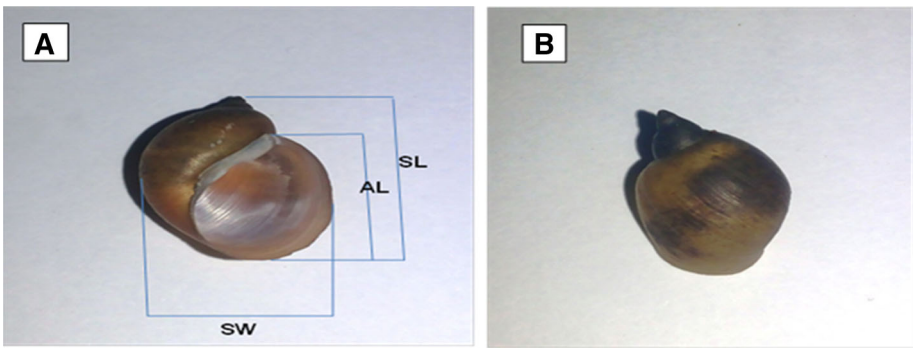

Fig. 3 Shell dimensions of *L. natalensis* snails. **a** Ventral view. **b** Dorsal view. *AL* aperture length, *SL* shell length, *SW* shell width

5- *Echinochasmus pelecani*:
Cercarial body is oval, white in color; tail is the same length as body, flexible, vacuole appearance along the tail. Cercariae were produced within rediae (Plate 2, 7). Esophagus was between pharynx and ventral sucker, ceca reaching to bladder, two main excretory tubes meet together before entering bladder. Genital primordia are two mass behind the ventral sucker.
6- Cercariaeum cercariae (*Palaeorchis* sp.):
Tail is absent, non-emerging cercariae, develop in rediae and encyst in snails. It is produced by species of the families Monorchiidae (intestinal parasites of fish) and Cyclocoelidae (parasites of the respiratory tract of birds) (Plate 2, 8). It is of no economic importance and is produced by species of the genera *Biomphalaria*, *Gabbiella*, and *Lymnaea*.

Types of metacercaria

Metacercariae had double thick cyst walls which were formed as a thin irregular wall secreted around the body of cercariae from its cystogenous glands and the tail then separated from the body (Plate 3). The present study reveals presence of:

1. *Clonorchis sinensis* metacercaria in mantle of *L. natalensis* snails. It is elliptical, has nearly equal sized two suckers and an excretory bladder found in posterior part of the body (Plate 3, 9). It also encysted in the flesh of freshwater fish.
2. *Plagiorchis elegans* metacercaria in the mantle of the snails (Plate 3, 10). Encysted inside the shell of *L. natalensis*.
3. *Fasciola* metacercaria (recently formed) shortly after emerged from snail or after crushing snails formed on slides (Plate 3, 11).

Discussion

Freshwater snails play an important role in the veterinary field and also affecting the health of the public (Chontananarth et al. 2017). Fasciolosis is a major public health problem in several areas of the world, including the Nile Delta in Egypt (Soliman 2008). *L. natalensis* is the most common species prevalent in Egypt compared to other species such as *L. columella*, *L. truncatula*, and *L. stagnalis* (*Ashour* et al. 2008).

The external examination of the shell of *L. natalensis* snails revealed that they were thin, fragile, dextral, dark brown to black in color; the shell whorls were ranging from three to five in number and are spirally coiled. These results are in accordance with (El-Dafrawy and Taha 2007), who stated that the shell height ranging between 10 and 20 mm and the shell whorls were always ranging from 3 to 5 in number in *L. natalensis* snails collected from Giza governorate.

The present study showed that the infected snails include six types of cercariae and three types of metacercariae. These cercariae included xiphidio, gymnocephalus (fasciolid), echinostome, virgulate, echinochasmus pelecani, and Palaeorchis cercariae. The highest percentage of cercariae in all snails was xiphidio cercaria followed by echinostome and gymnocephalus. This agrees with the findings of Hussein et al. (2005) and Hussein and Khalifa (2010) who stated that the infection with xiphidio cercariae was the most prevalent in

Table 1 Measurements (mm) of *Lymnaea natalensis* shells collected from three field localities. Random samples of snails were measured from each locality

Locality	Height of shell (mm)	Height of aperture (mm)	Width (mm)	Number of whorls
ElMansuria	15 ± 1.7	10 ± 1.04	9 ± 1	3–5
Oseem	13 ± 1.0	9 ± 1.25	7 ± 1.05	3–5
ElZeidia	16 ± 0.86	12 ± 1.6	10 ± 0.5	3–5

Table 2 Percentage (%) of infection in snails detected by shedding and crushing techniques in three localities in Giza governorate

Localities	Total number	Infected	% infection
ElMansuria	500	310	62
Oseem	500	380	76
ElZeidia	500	420	84

Qena Governorate, Upper Egypt, followed by echinostome cercaria. A xiphidio cercaria and an echinostome were described in Alexandria (Allam 1992). Also, the xiphidio cercaria was found to be the most abundant single-tailed cercariae in the habitats examined in the proximity of Pretoria (Moema et al. 2008). These cercariae encyst in invertebrates, amphibians, and reptiles (Frandsen and Christensen 1984). In previous studies, it has been shown that xiphidio cercaria larvae belong to Plagiorchidae trematodes (Yaraghi et al. 2011). Sharif et al. (2010) stated that *Lymnaea gedrosiana* from the Mazandaran Province were found to be infected with the Plagiorchidae, Clinostomidae, and Echinostomatidae and stated that xiphidio metacercariae were found encysted in various second intermediate hosts, i.e., in the gill chambers of freshwater shrimps and in the mantle of *L. natalensis.* Also, Faltýnková et al. (2007) stated that *L. stagnalis*, the great pond snail in Central Europe, were infected with 24 trematode species comprising 19 species of cercariae and 11 species of metacercariae (six species occurred both as cercariae and metacercariae) of eight families. The dominant cercariae were those of Opisthioglypheranae, Plagiorchis elegans, and Echinostomatidae, and 14 double infections were found.

The echinostomatid cercariae were known as avian parasites and xiphidio cercariae were parasitized avian and amphibians (Moema et al. 2008). The primary intermediate host of Plagiorchis is freshwater snail and the secondary intermediate hosts are fishes, snails, and arthropods (Rivaz et al. 2014). Palaeorchis sp. (family Monorchidae) is widely distributed fish parasites, whose larvae can use several snail species as their first intermediate host (Mastitsky 2007; Zdun 1961). The cercaria, according to the main features of morphology, was identified as echinochasmid and attributed to the genus *Echinochasmus*. The intermediate hosts of *Echinochasmus* cercariae are mainly freshwater prosobranch gastropods (Stanevičiūtė et al. 2008). The life cycles of less than half from approximately 50 known species of the genus *Echinochasmus* have been studied (Fried 2001). A wide range of fish-eating birds and mammals are known as final hosts of *Echinochasmus* spp. (Kostadinova and Gibson 2000). The fact that echinochasmid trematodes can infect man orally via the cercarial stage in contaminated water is of considerable interest to the medical community (Taylor et al. 2001). The present results showed the presence of three types of metacercariae which encysted in various second intermediate hosts, i.e., in the gill chambers of freshwater shrimps and in the mantle of *L. natalensis.* These results are in accordance with Faltýnková et al. (2007) who reported 11 species of metacercariae (six species occurred both as cercariae and metacercariae) of eight families in *L. stagnalis*, the great pond snail in Central Europe.

The present results showed that the infection rate in Elzeidia was higher than that in Oseem and ElMansuria being 84%, 76%, and 62%, respectively. This agreed with Ashour et al. (2008) who stated that the infection was present in Fayoum, Damietta, Qaluobiya, and Kafr El-Sheikh; Gharbiya, Dakahliya, Sharkiya, and Giza Governorates, which may be attributed to the presence of the aquatic plants *Lemna gibba* and *Jussiaea repens*. Also, Nagaty et al. (1959) stated that the possibility of infection of *Lymnaea* sp.

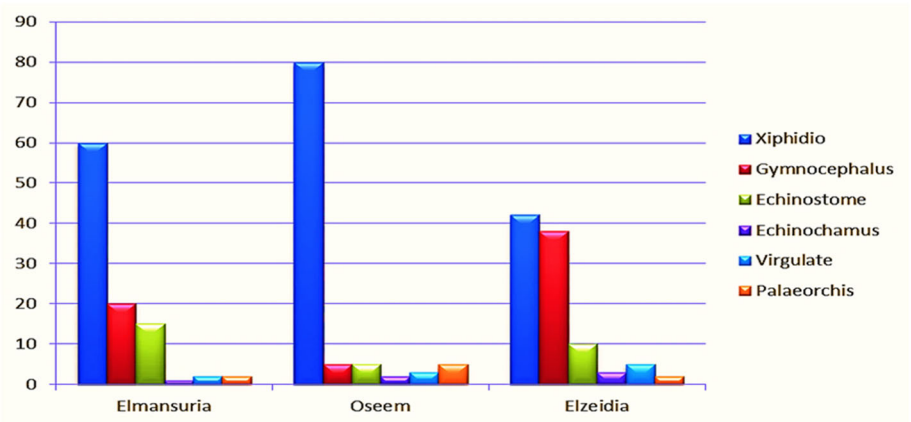

Fig. 4 Shows the percentage of each type of cercariae found in *L. natalensis* snails

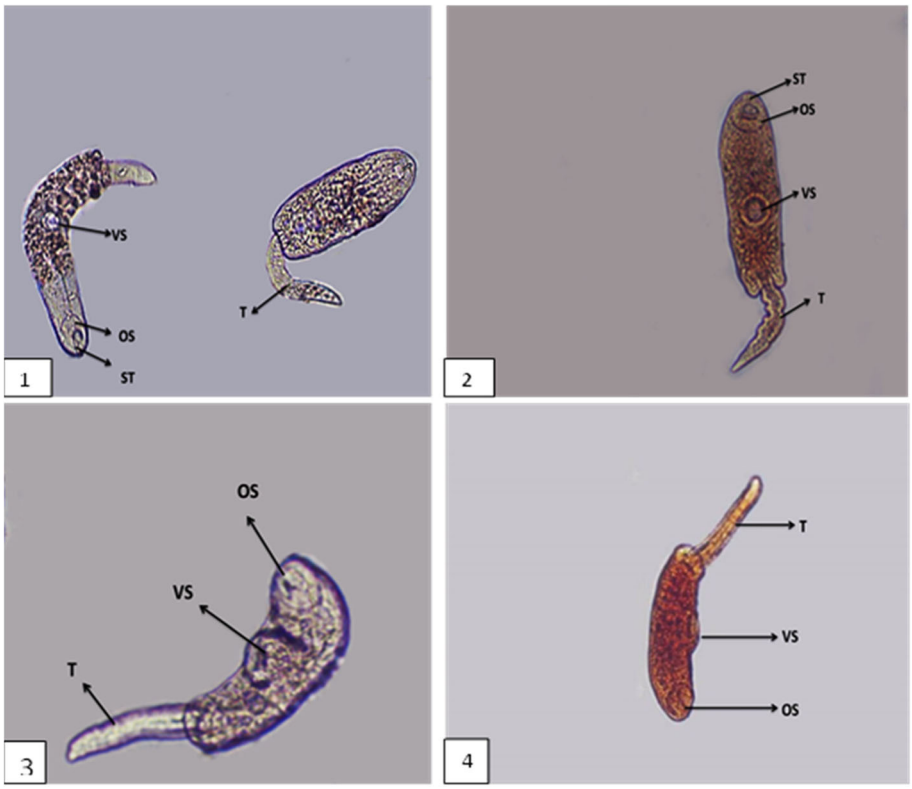

Plate 1 Light micrographs of Xiphidio cercaria (1 and 2) and gymnocephalus cercariae (3 and 4). 1 and 3 photos captured when they are alive, while 2 and 4 cercariae are stained with iodine; oral sucker (OS); ventral sucker (VS); tail (T)

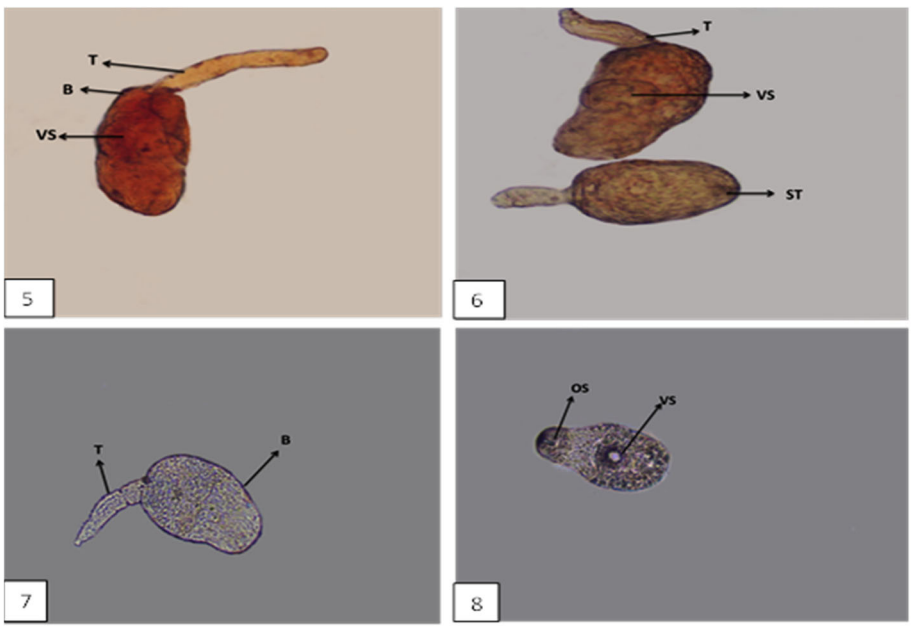

Plate 2 Light micrographs of (5) echinostome cercaria stained with iodine; (6) virgulate cercaria stained with iodine; (7) *Echinochasmus pelecani* cercaria while alive; (8) *palaeorchis* cercaria while alive; tail (T); (B) body of cercariae; (OS): oral sucker; ventral sucker (VS)

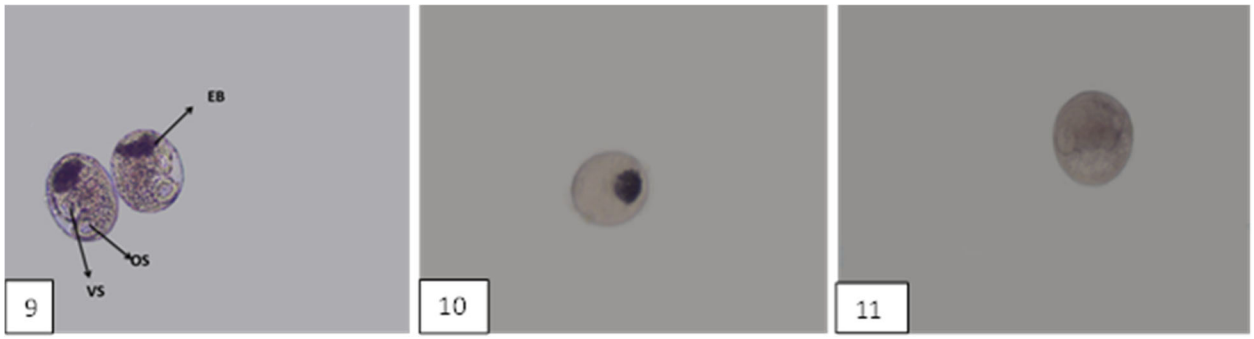

Plate 3 Light micrographs of metacercariae of (9) *Clonorchis sinensis*; (10) *Plagiorchis elegans*; (11) *Fasciola* (recently formed); a ventral sucker (VS); oral sucker (OS); excretory bladder (EB)

with *Fasciola gigantica* is higher in snails with large size.

Conclusion

The present study investigated the trematode fauna that were present in *L. natalensis* snails beside fasciolid cercariae and confirmed the presence of other cercarial species in three rural areas in Giza governorate, Egypt. These findings had served as an initial step for understanding the epidemiological situation and establishment of control programs of trematode infections in humans and animals. Thus, the complimentary studies seem necessary for survey of prevalence of trematodes in this snail all over the governorate.

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Availability of data and materials
All the data obtained during the study are presented in this manuscript. Any further enquiries for additional information are available upon request from the corresponding author.

Authors' contributions
AKA and AMI conceived and designed the study. AMI collected snails from the field, performed the experiments, and analyzed the data. AKA classified the cercarial types. AMI wrote the first draft and AKA revised and edited it. All authors read and approved the final manuscript.

Ethics approval and consent to participate
Ethical approval had been granted approval by the Ethics Committee of Theodor Bilharz Research Institute (TBRI).

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

Publisher's Note

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