IDENTIFICATION OF SOME TREMATODE CERCARIAE COLLECTED FROM SIX MARINE SNAIL SPECIES OF THE GENUS NERITA.

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Abstract
Transmission of trematode cercariae depends mainly on the presence of marine snails as an intermediate host for zoonotic diseases. Parasitic infections in marine snails are poorly discussed. The current study was aimed to investigate the collected snails and determine the prevalence of larval trematode infections as well as identifying the cercarial types from January to December 2016. A total of 549 marine snails belonging to the genus, Nerita were randomly collected from Obhor bay, Red Sea at Jeddah city, Saudi Arabia. To harvest cercariae, snails were exposed to artificial light and the non-shedding snails were crushed. Eight distinct cercariae were identified as well as their sporocysts. Seven of them were found belonging to non-virgulate xiphidiocercaria: Ascorhysis charadriformis, cercariae, Haematoloechus similis, Litorina saxatilis V, Litorina saxatilis VII, Maritrema lingulla, Microphallus similis, Microphallaceae, and one ocellate furcocercous. The most prevalent type of cercariae was Maritrema lingulla (16.96%). The described cercariae are part of a diverse group of about eight trematode species inhabiting marine Nerita snails as first intermediate hosts. Such studies can assist in collecting data on disease distribution in the sympatric fish and the configuration of trematodes transmission by snails and eventually, in the avoidance and control of the subsequent fish and human diseases.

Key words: Marine snails, Nerita, Sporocyst, Cercariae, Trematoda, Red Sea

Introduction
Most snails are intermediate hosts for trematode cercariae infecting humans and animals. The life cycle of trematodes is very complex, as they require an intermediate snail or fish for the maturation to the infective stage. Cercariae are adapted for dispersal outside snail and exhibit a large variety in morphology to penetrate the second intermediate host (Graczyk and Fried, 1999).

Trematode groups often consist of different species inhabiting the same host population, with two or more trematodes sometimes sharing the same host (Born-Torrijos et al., 2014). The detection of infection in snails depends on; stimulation of cercarial shedding by exposing snails to light, crushing of snails, followed by microscopic examination and finally rearing of snails in the laboratory occurs until they shed cercariae (Hanelt et al., 1997).

Taxonomic position of numerous trematode cercariae often cannot be established based on their morphology alone due to the shortage of consistent taxonomic distinguishing features at this stage of trematodes development. Cercariae can be identified to the family or superfamly level. Laboratory experiments on life cycles using natural definitive hosts are not always possible (Bar-toli et al., 2000; Brant et al., 2006; Pina et al., 2007; Jensen and Bullard, 2010; Locke et al., 2011). Little studies were carried out on diversity and abundance of cercariae in the Red Sea snails. Such studies gave data on environmental factors influencing emergence of cercariae in marine system (Koprininakar and Poulin, 2009).

The current study was aimed to investigate the collected marine snails, Nerita spp., to determine prevalence of larval trematode infections and to identify the cercariae infecting them.

Materials and methods
Snails were gathered from Obhor Bay located about 30 km north of Jeddah City on
east coast of the Red Sea, and were transferred to the Parasitology laboratory, Science college, King Abdulaziz University. Collection was carried out from January to December 2016. Snails were identified using standers keys (Knaap and Loker, 1990; Rusmore-Villaume, 2008). They belong to genus Nerita, comprises six species: Nerita albicilla, N. grayana, N. histrio, N. polita, N. orbignyana and N. quadricolor.

Shedding and harvesting of cercariae: Snails were cleaned, individually separated in small glass beakers containing seawater and were kept in the laboratory at 23°C. They were exposed to artificial light for two hours. Under a dissecting microscope, water drops from each beaker was investigated at short intervals searching for emerging cercariae. Non-shedding snails were crushed and investigated using the dissecting microscope searching for early larval trematode stages. Living cercariae were stained with diluted vital stain methylene blue. The stained cercariae were characterized and photographed. Cercariae were identified according to their morphological features using taxonomic keys (Hassan et al, 2017).

Results

Eight distinguishable types of cercariae were collected from Nerita spp, seven of them were found belonging to xiphidiocercaria and one ocellate furcocercus. Infection prevalence for each cercarial type was recorded; cercaria of Ascorhytis charadriformis found 1.7%. Cercaria of Haematoloechus similis in 5.94%, cercaria of Litorina saxatilis V in 11.87%, cercaria of Litorina saxatilis VII in 7.64%, cercaria of Maritrema lingula in 16.96%, cercaria of Microphallus similis in 3.40% and cercaria of Microphalliciae in 16.96% and ocellate furcocercous cercaria in 1.7%. All cercariae developed in sporocysts. Measurements were given (Tab. 1). Cercaria of Ascorhytis charadriformis (Young, 1949) Ching, 1965: Cercaria was found in snail, Nerita orbignyana and N. histrio. Body is oval to elongate, creamy color. Tail is simple and longer than body. Oral sucker is terminal, rounded in shape without virgula organ. A well-developed stilet arises from oral sucker with an arrow like terminal end with sharp tip and broad base. Ventral sucker not detected. Pharynx detected and digestive system remainder could be detected. Four pairs of penetration glands on ventral sucker sides. An excretory bladder is circular found in body posterior end (Fig. 1A, B).

Cercaria of Haematoloechus similis: Cercaria was found in snail, Nerita orbignyana and N. histrio. Body is oval, creamy color. Tail is simple and shorter than body. Oral sucker is terminal, rounded freed from virgula organ. A well-developed stilet arises from oral sucker with an arrow like terminal end. Ventral sucker is rounded with six pairs of penetration glands on either side. Excretory bladder is Y shape in body posterior end (Fig. 1, C, D).

Cercaria of Litorina saxatilis V Popiel, 1976: Cercaria was found in snail, Nerita albicilla and N. grayana. Body is oval, creamy color. Tail is simple and shorter than body. Oral sucker is sub terminal, rounded in shape without virgula organ. A well-developed stilet arises from oral sucker with an arrow like terminal end and broad base. Ventral sucker not detected. Esophagus bifurcates into ventral sucker anterior part. Two pairs of penetration gland on ventral sucker each side. Excretory bladder not identified (Fig. 2A, B).

Cercaria of Litorina saxatilis VII Cercaria Newell, 1986: Cercaria was found in snail, Nerita grayana. Body is elongate, creamy color. Tail is simple and nearly same as body length. Oral sucker is terminal, rounded in shape without virgule organ. A well-developed stilet arises from the oral sucker and has an arrow like terminal end with sharp tip with broad base. Four pairs of penetration glands, two lying on either sides of oral sucker, and two at genital premordium level, open on each side of stilet tip. Excretory bladder is irregular in shape in body posterior end. Genital primordium found in
between excretory bladder and posterior penetration glands (Fig. 2C, D).

Cercaria of *Maritrema lingua* ll Jagerskio ld, 1908: Cercaria was found in *Nerita albicylla*, *N. quadricolor* and *N. orbignyana*. Body is transparent and elongate in shape. Tail is simple and shorter than body. Oral sucker is subterminal, rounded in shape without virgula organ. A well-developed stylet arises from oral sucker with an arrow like terminal end with sharp tip. Ventral sucker undetected. Four pairs of penetration glands on each side of ventral sucker. Excretory bladder is circular in shape, found in body posterior end (Fig. 3A, B).

Cercaria of *Microphallus similis* Jagerskio ld, 1900: Cercaria was found in snail, *Nerita orbignyana*. Body is oval to elongate with creamy color. Tail is simple and longer than body. Oral sucker is subterminal, rounded in shape freed from virgula organ. A well-developed stylet arises from oral sucker with an arrow like terminal end and broad base. Ventral sucker is absent. Four pairs of penetration glands. Excretory bladder is circular in shape found in body posterior end (Fig. 4C, D).

Cercaria of *Microphallidae spp*. Travassos, 1920: These cercariae were obtained from

![Table 1: Measurements of cercariae collected from Nerita snails (All measurements in μm)](image)

**Discussion**
In the present work, eight types of cercariae were identified from *Nerita* spp, seven of which belong to non-virgulate xiphidiocercaria; *Ascorhytis charadriformis*, cercariae, *Haematoloechus similis*, *Litorina saxatilis V*, *Litorina saxatilis VII*, *Maritrema lingulla*, *Microphallus similis*. Micro-phalliae and one ocellate furcocercous cercaria.

*Ascorhytis charadriformis* was collected from the brackish water snail, *Littorinopsis intermedia* in the central and east coast of the gulf in Thailand by Namchote et al. (2015). They found the adult in large intestine of dunlin, *Calidris alpina* and was originally described from charadriform birds in California (Young, 1949). Gulls are other avian hosts in the life cycle which involves littorine snails and grapsid crab intermediate hosts (Cheng, 1963). The present record of *Ascorhytis charadriformis* cercaria in the marine snails, *Nerita orbignyana* and *N. histrio* is the first in Saudi Arabia.

In the present work, *H. similis* cercariae

253
were collected from marine snails, *Nerita orbignyana* and *Nerita histrio*. This cercaria was reported only from planorbid snails in central Europe by Faltynkova et al. (2007). The life cycle of *Haematoloechus similis* was traced experimentally. The first intermediate host was *Planorbis planorbis*. The metacercariae are found in the larvae of several species of *Insecta*. Adult flukes were obtained from *Rana esculenta*. The cercariae of *H. similis* resemble those of the group "Prima" of the "Cercariae ornatae" in size and body proportions but also resemble those of the "Cercariae armatae" group in the nature of the excretory system and in the absence of a caudal finfold; the cercariae of *H. similis* differ from those of both groups by possessing prominent penetration glands and by the absence of cystogenous glands (Grabda et al., 1960).

Irwin et al. (1983) recorded that *littorinae saxatilis* V had been previously described by Popiel (1976) on the eastern Atlantic seaboard. Irwin et al., 1990 pointed in his study that cercariae of *M. arenaria* are the previously known as cercaria *littorinae saxatilis* V. The current study recorded *l. saxatilis* V from snails, *Nerita albicilla* and *Nerita grayana* for the first time in Saudi Arabia. *littorina saxatilis* VII cercariae were originally described by Newell (1986), these samples were collected from *Littorina saxatilis* snails in the Scilly Isles. The morphology and morphometry discussed by Newell (1986) agreed with that recorded in Iceland. Evidences from the previous work suggest that *Onoba aculeus* is the main first intermediate host for *littorina saxatilis* VII cercariae. *littorina saxatilis* VII cercariae entered and encysted in the isopod, *Jaera alibifrons* (Leach) which mean that waders inhabiting the intertidal coast may represent the final hosts for this cercaria. In the present study, these cercariae were recorded from the marine snails, *Nerita grayana* for the first time.

Cercariae of *Maritrema linguilla* were collected from marine snails, *Nerita albicilla*, *N. quadricolor* and *N. orbignyana* in the present study. Newell (1986) documented *M. linguilla* infection from *L. saxatilis* in the studied coastal regions. *M. linguilla* cercariae were recorded also from *L. saxatilis* snails found on French coast (Richard, 1976), this can clarified by the specific environmental conditions in the island as intertidal zone. *M. linguilla* was not detected in *Littorina* spp. in Barents Sea coast or the Norwegian Sea (Chubrik, 1966; Podlipaev, 1979; Galaktionov and Bunnes, 1999).

Adult cercariae of *M. murmanica* were defined by (Deblock and Capron, 1960; Newell, 1986; Benjamin and James, 1987). The second intermediate host for *M. linguilla* is the, *Ligia oceanica* (L.) host belonging to Crustacea. The present record of this cercaria is the first in Saudi Arabia.

Stunkard (1957) recorded the life-history of *Microphallus similis* by experimental infection of both intermediate and final hosts. The cercariae are minute, stylet-bearing monostomes. *Microphallus* species are mainly intestinal parasites of birds and mammals, and its metacercariae have been revealed in the crab (Guk et al., 2008). *Microphallus similis* invests numerous hosts to complete its life cycle, starting with two *Littorina* snails (*L. saxatilis* and *L. obtusata*), where the trematode reproduces asexually to give many cercariae. These cercariae leave the snail into water searching for the second intermediate host, the green crab, *Carcinus maenas*, to encyst within it to metacercariae. The crab host must be eaten by a definitive host, often a *Larus gull* species, where the trematode reproduces sexually, and their eggs are then deposited into the surrounding marine environment within the feces of the birds.

Kudlai et al. (2016) described the life cycles of Microphallids. They involve gastropods, mostly marine and brackish water, as first intermediate hosts and crustaceans as second intermediate hosts. Martorelli et al. (2006) found cercaria in *Zeacumantus subcarinatus* in New Zealand that agreed with Microphallid cercariae. Also, two pairs of
anterior cephalic glands behind oral sucker Illus Travassos 1920 and Megalophallus Cable, Connor & Balling, 1960 and in accordance with Microphallid cercarial type in the present study. Galaktionov et al. (2012) reported that Microphallus species' life cycle has two hosts; metacercariae develop (intertidal and subtidal gastropods, mostly of genus Littorina) and infective to marine birds (ducks, gulls and waders). Faltynkova et al. (2007) reported eight species of furcocercous cercariae of families (Strigeidae, Diplostomidae, Schistosomatidae and Sanguinicolidae), from Lake Konnevesi in Finland in (Valvata macrostoma, Lymnaea stagnalis, Bathymophalus contortus and Planorbarius corneus). Snail hosts of furcocercous cercaria were B. siamensis, F. polygramma and M. tuberculata. Chontananarth and Chai (2013) in Thailand collected this cercaria from M. tuerculata and Tarebia scabra. In the current study, this cercaria from Nerita polita, which life cycle must be studied?

Conclusion

The described cercariae are part of a diverse group of about eight trematode species inhabiting marine Nerita snails. Such studies can assist in collecting data on disease distribution in the sympatric fish and the configuration of trematodes transmission by snails and eventually, in avoidance and control of subsequent fish and human diseases.

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**Explanation of figures**

Fig.1: Photomicrograph showing A, B. *Ascorhytis charadriformis* A, sprocyst and B, cercaria, (EB) excretory bladder, (OS) oral sucker, (PG) penetration glands, (S) stylet, (TA) small tail

Fig. 2: Photomicrograph showing A, B. *litonina saxatilis* V, A, sprocyst and B, cercaria; C, D: *litonina saxatilis* VII, C, sporocyst and D, cercaria. (EB) excretory bladder, (OS) oral sucker, (PG) penetration glands, (S) stylet, (TA) small tail.

Fig. 3: Photomicrograph showing A, B. *Maritrema linguilla* A, sprocyst and B, cercaria, C, D: *Microphallus similis*. C, sporocyst and D, cercaria. (EB) excretory bladder, (OS) oral sucker, (PG) penetration glands, (S) stylet, (TA) small tail.

Fig. 4: Photomicrograph showing A, B Microphallidae. A, sprocyst and B, cercaria, C, D: Ocellate furcocercus. C, sporocyst and D, cercaria. (EB) excretory bladder, (OS) oral sucker, (PG) penetration glands, (S) stylet, (TA) small tail.