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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: *Ascorhytis charadriformis*, cercariae, *Haematoloechus similis*, *Litorina saxatilis* V, *Litorina saxatilis* VII, *Maritrema lingulla*, *Microphallus similis*, Microphalliae 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 superfamily level. Laboratory experiments on life cycles using natural definitive hosts are not always possible (Bartoli *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 (Koprivinikar 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 identifythe 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 lingulla* in 16.96%, cercaria of *Microphallus similis* in 3.40% and cercaria of Microphalliae 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 orignyana* 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-deve-loped stylet 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).

Cercaiae 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 stylet 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 *littorina 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 stylet 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 glan-ds 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 stylet 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 stylet 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 linguilla* Jagerskiold, 1908*:* Cercaria was found in *Nerita albicilla, 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* Jagerskiold, 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. 3C, D).

Cercaria of Microphallidae spp. Travassos, 1920: These cercariae were obtained from snail, *Nerita histrio.* Body is oval to elongate in shape and creamy in color. Tail is simple and smaller than body. Oral sucker is sub terminal, rounded in shape free from virgula organ. A well-developed stylet arises from oral sucker with an arrow like terminal end and broad base. Digestive system is not developed. Penetration glands arranged in two pairs, located in third quarter of body. Posterior pairs of penetration glands not observed. Ducts open ventrolaterally to stylet. Genital primordium forms an oval mass in body posterior third. Excretory bladder is Y-shaped (Fig. 4A, B).

Ocellate furcocercous cercaria Faust, 1918: This cercaria was collected from snail, *Nerita polita.* Body is long, flat-like and oval shaped, creamy in color. Tail is longer than body and divided into two furcae measuring about 45µm in length. Pharynx is quite small with two pairs of large penetration glands. Two eyespots are globularly shaped located in anterior position near pharynx. Penetration glands two pairs in body third quarter. Posterior pairs of penetration glands not observed. Genital primordium is an oval mass in body posterior third. Excretory bladder is Y-shaped (Fig. 4C, D).

Type of cerceriae	Body	Tail	Oral sucker	Stylet	Ventral sucker	Excretory bladder	Sporocyst
Ascorhytis charadriformis	25×66.67	58.33	12.5	5.83	absent	15	37.5×75
Haematoloechus similis	18. 50×18.33	15	8.33	10.83	4.16	10	75×21.67
Iitorina saxatilis V	12.5×50.17	21.67	8.3	4.17	absent	Unidentified	23.33×75
Iitorina saxatilis VII	54.17×12.5	29.17	12	6.67	absent	13	91.6×50
Maritrema linguilla	50×16.67	29.17	8.33	10	absent	9	45.83× 20
Microphallus similis	33.33×12.5	40.5	8.33	4.17	absent	7	65× 17.5
Microphallidae spp	47.5×19.17	29.17	12.5	4.17	absent	10	70.83× 20
Ocellate furcocercous	20.83×0.42	45	absent	absent	absent	absent	100× 18.33

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

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 charadriiform 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 orignyana* and *N. histrio* is the first in Saudi Arabia.

In the present work, *H. similis* cercariae 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 *Iittorinae 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 albifrons* (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 Bustnes, 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) recoded 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 *llus* 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, Schistosomati dae* and *Sanguinicolidae*), from Lake Konnevesi in Finland in (*Valvata macrostoma, Lymnaea stagnalis, Bathyomphalus 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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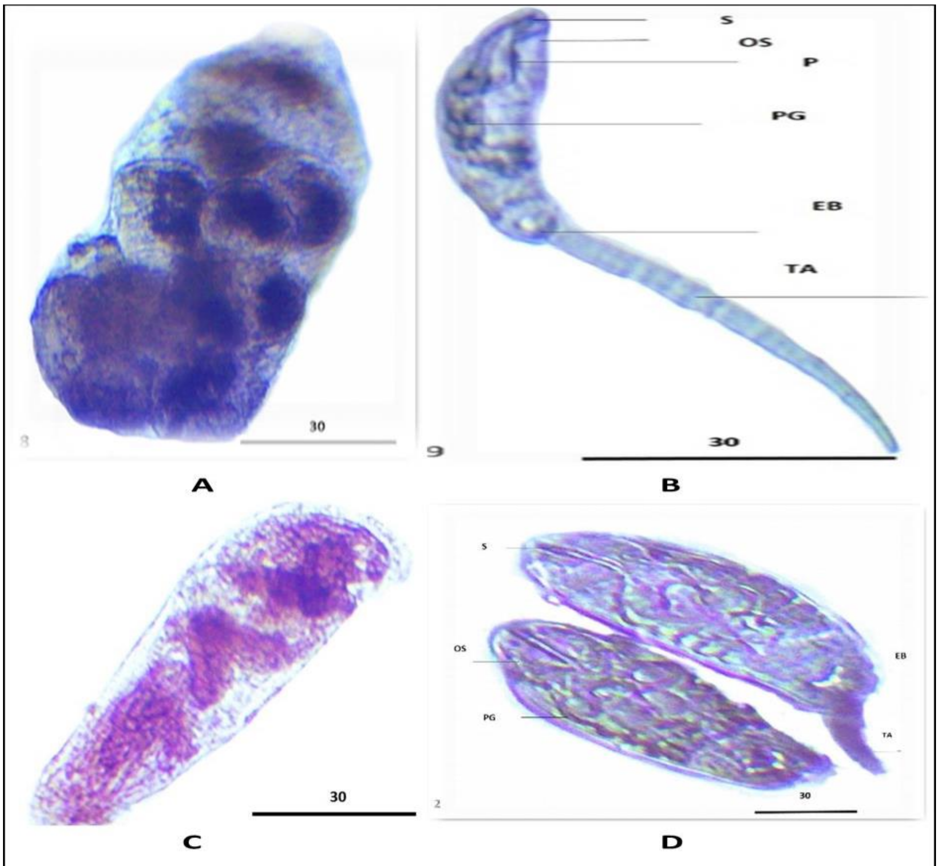
Explanation of figures

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

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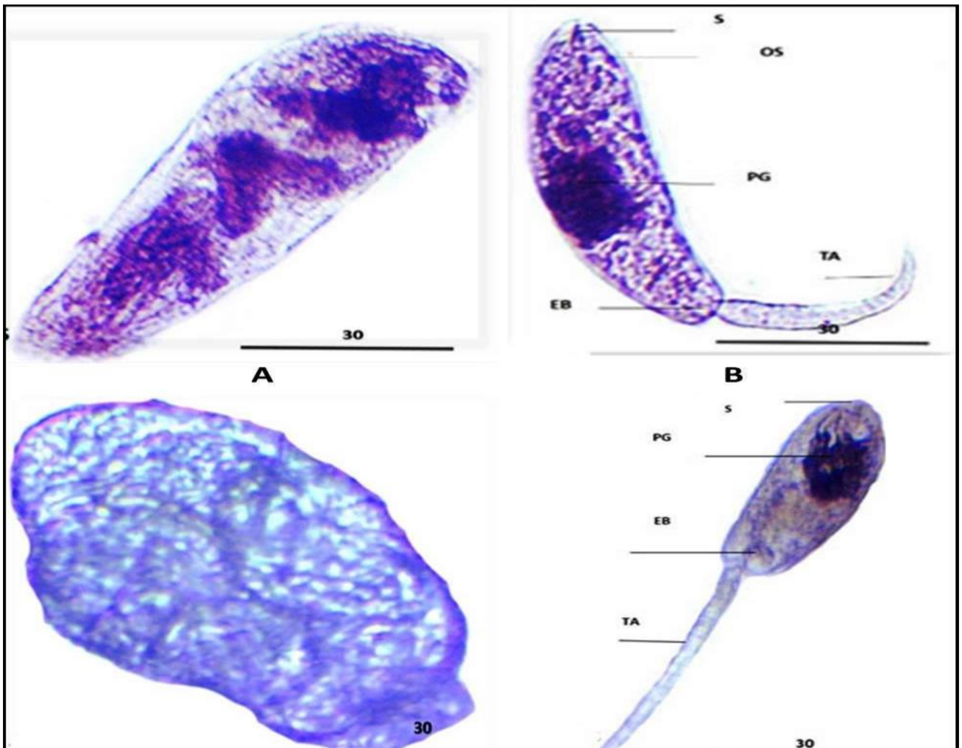
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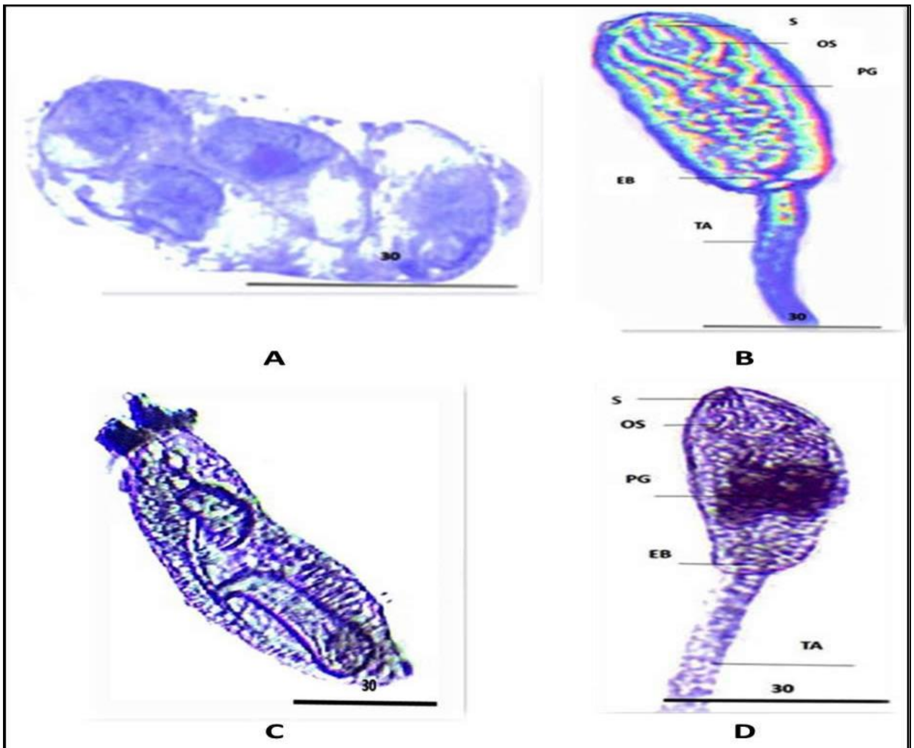
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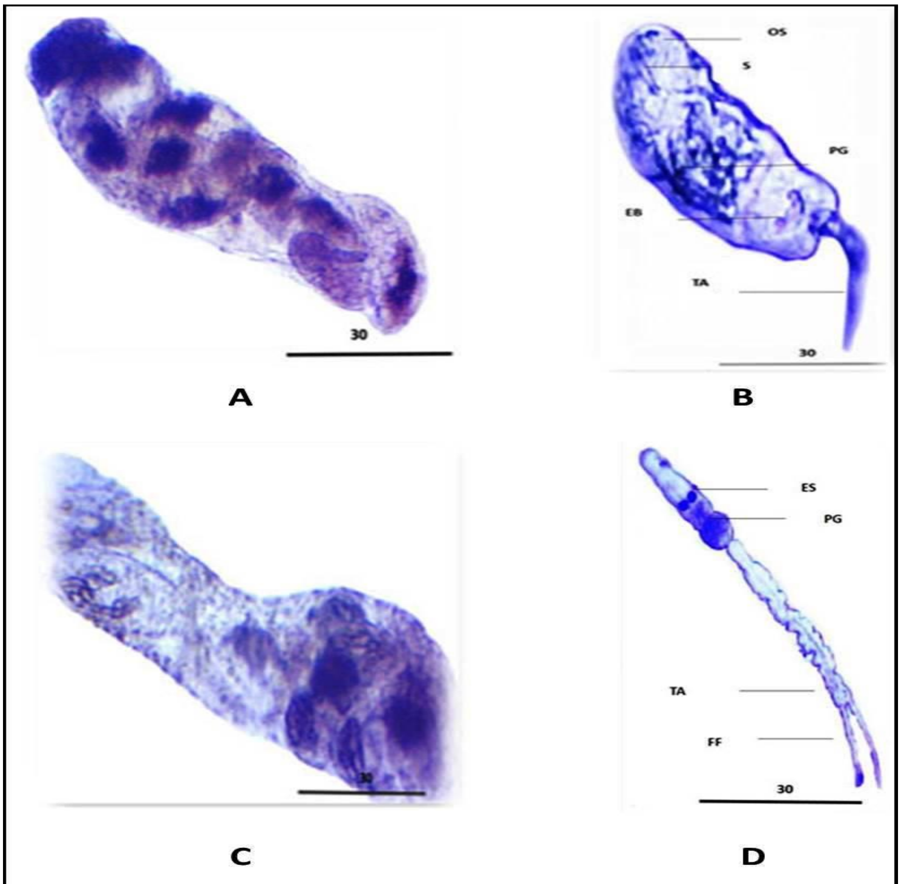
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