



## RESEARCH PAPER

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## Diversity and prevalence of digenean trematode larvae in five freshwater Prosobranch snails from Burkina Faso

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Article published on December 06, 2022

**Key words:** Parasite, Cercariae, Gastropods, Reservoir of Loumbila, Burina Faso

### Abstract

Many trematode parasites use molluscs as intermediate host to ensure their life cycle. In Burkina Faso, few studies have been carried out on diversity of trematode larvae on snails. For that reason, the present study aims to determine the diversity and the prevalence of trematodes larvae in snails in the reservoir of Loumbila for better safeguarding of biodiversity and effective control of parasitosis transmitted by snails. This study was conducted between September 2019 and March 2020. During this period, 479 gastropods were collected using three complementary methods; a kick net was used to collect molluscs in the littoral and pelagic areas, a grab Eckman for deep benthic areas and hand picking for shoreline molluscs. The cercariae larvae stage of trematode were investigated using the shedding crushing method (by using cercarial emergence and crushing snails). The infection rate was found to be 3.55%, i.e. 17 animals infected in a total of 479. Twelve digeneans species cercariae were recorded from five species of Prosobranch snail, *Cleopatra bulimoides*, *Cleopatra* sp, *Lanistes lybicus*, *Lanistes ovum*, *Bellamyia unicolor*. These cercariae were divided into four major groups including *Cercariaeum cercariae* (Two species), *Xiphidocercariae* (Three species), *Furcocercariae* (Six species) and cercaria type 2. *Cleopatra* sp showed the highest diversity of trematode with nine groups of larvae among the gastropod species collected. The genus *Cleopatra* was the most parasitized host with the highest prevalence and *Lecithodendrium* sp was the most abundant parasite species during this study.

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## Introduction

Gastropods are intermediate hosts of several species of Trematodes. Many species of Trematodes of biomedical and veterinary importance such as schistosomes and flukes also use these freshwater gastropods to ensure their life cycle (Martin and Cabrera, 2018). The freshwater snails are involved in the transmission of trematode species belonging to the superfamilies Schistosomatoidea, Fascioloidea, Clinostomoidea, Paramphistomoidea, Ehinostomatoidea, Diplostomoidea and Pronocephaloidea (Islam *et al.*, 2013), which cause disease when transmitted to humans and animals. Larval Trematodes which frequently parasitize freshwater gastropods constitute a biotic stress factor which reduces the physical condition of their host (Esch and Fernandez, 1994); increases mortality and reduces animal health in multiple ways; may also reduce resistance or tolerance to other infections (Morley, 2010).

Studies of gastropods parasitic fauna have multiplied in recent years in certain regions of Africa. Indeed, the work of Mohammed *et al.* (2016) had shown cases of Xiphidiocercariae trematodes infection in *Cleopatra bulimoides* and *Biomphalaria pfeifferi* in Sudan in the East Nile locality. Parasitological studies conducted by Yousif *et al.* (2010) and Lotfy *et al.* (2017) in Egypt had also reported the presence of eleven groups of morphologically different Trematodes in *Melanoides tuberculata*.

In the West African sub-region, some cases of gastropod parasitic infections have been observed, particularly in Nigeria, with the studies of Luka and Mbaya (2015) who reported the presence of *Schistosoma haematobium* and *Fasciola* sp in *Bulinus globosus* and *Lymnea natalensis*. Seven morphologically different Trematode species in *Melanoides tuberculata*, *Cleopatra bulimoides*, *Bellamyia unicolor* and *Lanites Varicus* were discovered by Abdulkadir *et al.* (2018).

Despite their abundance, freshwater molluscs are rarely studied in Burkina Faso. Local studies have been limited to their biogeographical distribution,

biodiversity, inventory (Ouedraogo *et al.*, 2018), and the gastropods responsible for the transmission of schistosomiasis such as *Bulinus* spp, *Melanoides tuberculata*, *Cleopatra bulimoides*, *Bellamyia unicolor* and *Biomphalaria* spp (Poda *et al.*, 2006; Zongo *et al.*, 2012; Bagayan *et al.*, 2016, Kpoda *et al.*, 2022). According to Morley and Lewis (2006) infection by Trematode larvae influenced the physiology of gastropods and led to host castration (Sorensen and Minchella, 2017). Moreover, trematodes are indicators of ecological changes such as the decrease in free life, biodiversity of aquatic ecosystems over time (Morley and Lewis, 2006).

This study aims to discover the diversity and the prevalence of trematode larvae into the prosobranch snails.

## Material and methods

### Study area

This study was carried in the reservoir of Loubila located in 12°29' N, 01°24' W, on the river of Nacambe. The Loubila's reservoir (12°29' N 01°24' W), built in 1947, has a capacity of 42.2 million cubic meters and covers an area of about 16.80 km<sup>2</sup>. Fishing and agriculture activities have developed around the reservoir. It is impacted, mainly by activities such as agriculture especially gardening. During the dry season, the bed of this reservoir is used for crops and often more than chemicals are used.

### Host sampling

Sampling was performed from September 2019 to March 2020. Snails were collected using a kick net composed of a 500µm mesh net for the littoral and pelagic zone; an Eckman grab was used for deep benthic areas. Manual collection was also done for shoreline gastropods by handpicking. The snails were harvested randomly using the "sight" search method (Cucherat and Demuyneck, 2008). Sampling was carried out in 15 minutes for each zone, according to the microhabitats found. Gastropods are sought in all favorable environments and were picked up by hand with a glove. The collected gastropods were kept alive in wide-mouthed, ventilated jars containing site water.

*Measurements and Identification of Gastropods*

Morphometric parameters of each individual gastropod (height for conical shells and shell diameter for discoid shells) were measured using a caliper nearest 0.1mm. For identification, all the gastropods were identified in the laboratory down to the species level according to the keys provided by Lévêque (1980) and Brown (1994).

*Collection and count of cercariae*

The snails were examined for cercarial infection using the shedding and crushing methods. The specimens were placed individually in a shedding tube and submerged with dechlorinated tap water and checked for the shedding of cercariae by exposing them to artificial light for about 3 hours. Emerged cercariae were examined under the stereoscopic microscope and observed for their swimming behavior. The cercariae were transferred to a glass slide and covered with a coverslip for morphological observation under a Zeiss microscope.

Then, the snails were examined to observe young larvae remaining in the snails using the crushing method according to the method of Curtis and Hubbard (1990). This method makes it possible not only to determine the distribution of the parasites obtained and their abundance according to the sites of infection in the host but also to observe the other stages of development of trematodes. Dissection was performed with two forceps by placing the snail in a dissecting dish after crushing the shell with shears (Chu and Dawood, 1970). The different organs, the hepatopancreas, the muscle tissue, the gonads and the digestive tract were examined under a Leica MZ8 stereomicroscope.

*Cercariae Identification*

Identification is made by observing anatomical and morphological characteristics. Thus, cercariae were stained with a drop of 1% iodine and different measurements are taken on the cercariae to allow their identification. The Measurements were made with a calibrated ocular micrometer. The length of the body, esophagus, tail, furcates and the diameter of the

suckers were measured as described by (Smyth and Wakelin, 1994). Then, they were identified using the identification keys of Frandsen and Christensen (1984); Hechinger (2012). The prevalence, abundance and mean intensity were calculated according to the terminology of Bush *et al.* (1997).

**Results**

*Prevalence and abundance*

For this study, a total of 479 gastropods representing 3 families of Prosobranch (Thiaridae, Ampullariidae and Viviparidae), 3 genera and 5 species including *Cleopatra bulimoides*, *Cleopatra* sp (Thiaridae), *Lanistes lybicus*, *Lanistes ovum* (Ampullariidae), *Bellamyia unicolor* (Viviparidae) were collected on the reservoir of Loumbila. 3.55% (17/479) of snail were infected with digenean larvae. Digeneans were observed in the five snail species: *Cleopatra bulimoides*, *Cleopatra* sp, *Lanistes lybicus*, *Lanistes ovum*, *Bellamyia unicolor* (Table 1). Among the snail's species, *Cleopatra* sp was the most prevalent (45%) and *Cleopatra bulimoides* the lowest prevalence (Table 1). However, the snail *L. ovum* contains the most abundant trematodes (Table 1). Nine types of cercariae infected *Cleopatra* sp and one type in *Cleopatra bulimoides* two for *Lanistes* species and three for *Bellamyia unicolor* (table 2).

**Table 1.** Prevalence of snail's species infected by trematod.

Host species	Examined number	Infected number	Prevalence (%)	Abondance	Mean intensity
<i>Cleopatra bulimoides</i>	129	1	0.78	1	1
<i>Cleopatra</i> sp	20	9	45.00	186	20.67
<i>Lanistes lybicus</i>	73	2	2.74	3	1.50
<i>Lanistes ovum</i>	170	2	1.18	354	177
<i>Bellamyia unicolor</i>	87	3	3.55	24	8

*Diversity of Trematoda*

The collected Trematodes were composed of cercariae and rediae classified into four major groups of cercariae. Group1: Cercariaeum cercariae, group 2: Xiphidiocercous cercariae, group 3: Furcocercous cercariae and group 4: cercariae type 2. Twelve species of digenean trematodes belonging to different

families were found parasitizing the snails' species. Two species of the group Cercariaeum cercariae, three

species for Xiphidocercariae, six for furcocercariae and one species of cercaria type 2.

**Table 2.** Prevalence (%) of trematode larvae recorded in snail species.

Host species	<i>Cleopatra bulimoides</i>	<i>Cleopatra sp</i>	<i>Lanistes lybicus</i>	<i>Lanistes ovum</i>	<i>Bellamya unicolor</i>	Global prevalence
Examined number	129	20	73	170	87	
<i>Lecithodendrium</i> sp	-	-	1.37	1.76	-	0.83
<i>Haematoloechus</i> sp	-	-	-	0.59	-	0.21
<i>Apatemon</i> sp1	-	5	-	-	-	0.21
<i>Apatemon</i> sp2	0.78	5	-	-	1.15	0.63
<i>Apatemon</i> sp3	-	5	-	-	1.15	0.42
<i>Xiphidocercaria</i> sp1	-	5	-	-	-	0.21
<i>Xiphidocercaria</i> sp2	-	5	-	-	-	0.21
<i>Aporocotyld</i> sp1	-	5	-	-	-	0.21
<i>Aporocotyld</i> sp2	-	5	-	-	-	0.21
<i>Plagiorchioid</i> sp	-	-	1.37	-	1.15	0.42
Furcocercariae1	-	5	-	-	-	0.21
Cercariae 1	-	5	-	-	-	0.21
Total						3.55

Two types of cercariae have not been previously described in Africa. There are furcocercaria type1 and cercaria type 2. The furcocercous cercariae are divided into four subgroups: Brevifurcate-apharyngeate distome cercariae, Brevifurcate-apharyngeate monostome cercariae, Longifurcate-apharyngeate distome cercariae (Strigca cercariae, three type) and furcocercariae type 1. The two species of Cercariaeum cercariae corresponding to *Acaudate Xiphidocercarie* sp1 and sp2 and the three species of Longifurcate-pharyngeate distome cercariae to genus

*Apatomon*. Xiphidicercariae were divided into three subgroups such as Plagiorchiid cercariae, *Lecithodendrium* cercariae and *Haematoloechus* cercariae. So, a total of 12 morphologically distinct trematode species have been observed. All parasite species and their prevalence are listed in table 2 and their abundance in table 3. The cercariae *Lecithodendrium* sp was the most abundant and the most prevalent. Fig. 1 show the mean intensity of trematode larvae. All of the cercaria types were described as follows:

**Table 3.** Abundance of trematode larvae in snail.

Host species	<i>Cleopatra bulimoides</i>	<i>Cleopatra sp</i>	<i>Lanistes lybicus</i>	<i>Lanistes ovum</i>	<i>Bellamya unicolor</i>	Total abundance
<i>Lecithodendrium</i> sp	-	-	2	342	-	344
<i>Haematoloechus</i> sp	-	-	-	12	-	12
<i>Apatemon</i> sp1	-	10	-	-	-	10
<i>Apatemon</i> sp2	1	111	-	-	2	114
<i>Apatemon</i> sp3	-	6	-	-	2	8
<i>Xiphidocercaria</i> sp1	-	20	-	-	-	20
<i>Xiphidocercaria</i> sp2	-	7	-	-	-	7
<i>Aporocotyld</i> sp1	-	25	-	-	-	25
<i>Aporocotyld</i> sp2	-	3	-	-	-	3
<i>Plagiorchioid</i> sp	-	-	1	-	20	21
Furcocercaria1	-	2	-	-	-	2
Cercaria1	-	2	-	-	-	2
Total	1	186	3	354	24	568

*Cercariaeum cercariae* group

*Acaudate xiphidocercaria* sp 1: Larvae were recovered from *Cleopatra* sp in cercariae stages with a prevalence of 5%. A morphological analysis of the cercariae showed there were cercariae with stylet and

without tail. Live cercariae were mobile. Body was elongate, oval, piriform, and measured 370µm long x 175µm width. Oral sucker was round and measured 60µm, with a vertical stylet 50µm long. A prepharynx was present and conspicuous; the protruding

pharynx, globular measured 25µm; the esophagus long, measured 60µm.

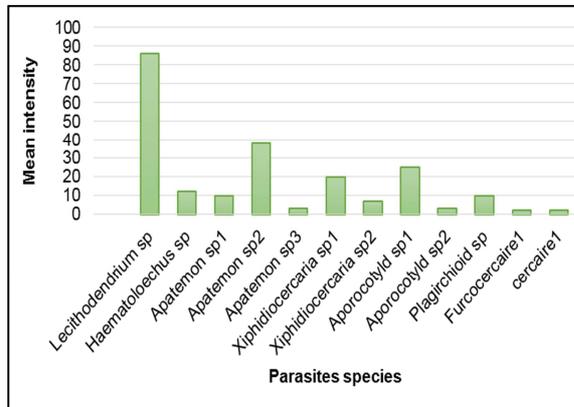


Fig. 1. Mean intensity of snail parasites.

The ventral sucker was found at the posterior mid of the body and was almost half the size of the oral sucker. Ventral sucker measured 28.5µm. The excretory bladder is Y-shaped. The surface of the body was covered with tiny spines. The intestinal caeca reached the posterior end of the body (Fig. 2). The posterior end of the body was broad and rounded.

*Acaudate xiphidiocercaria* sp2 : Trematodes were recovered from *Cleopatra* sp (5%) in rediae and cercariae stages. Rediae (Fig. 3A) in live were animated by slow movement, average length 1187.5 x 75µm. The number of developing cercariae was around 50, these cercariae are folded into a relatively small space.

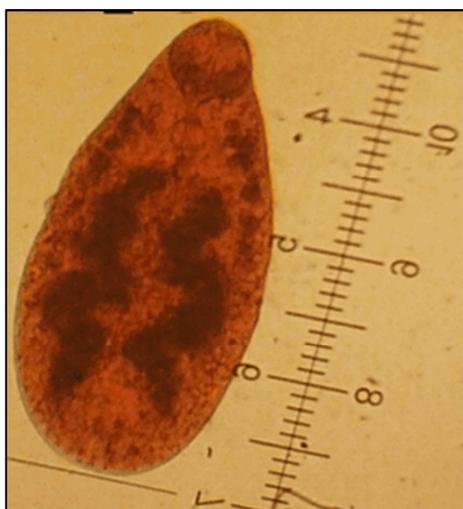


Fig. 2. Acaudate xiphidiocercaria sp1, scale bar: 100µm.

Cercariae were non-oculate without a tail, with an oral sucker but lacking a veritable characteristic ventral sucker. They had a simple elliptical stylet, body length measured on average 231.5 x 96.87µm. Oral sucker measured 25µm. Presence of penetration glands in the half of body (Fig. 3B).

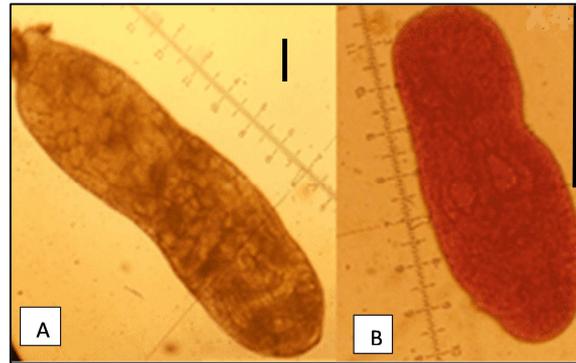
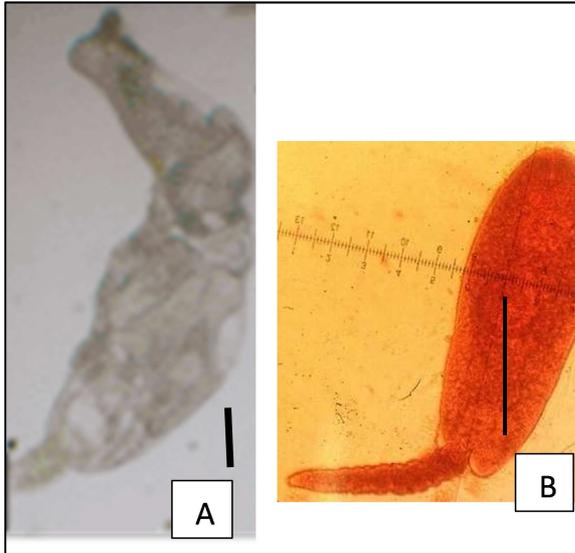


Fig. 3. Acaudate xiphidiocercaria sp 2. A. redia, B. cercaria. Scale bar, 100µm.

*Xiphidiocercous cercariae* group

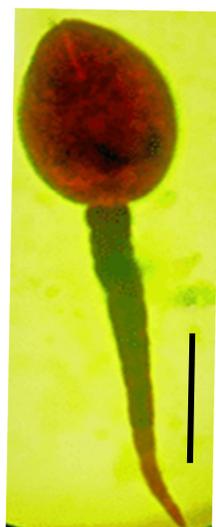
*Lecithodendrium* cercariae: The stages observed were rediae and cercariae from *Lanistes lybicus* (1.37%) and *L. ovum* (1.76%). The observed rediae in the host specimens were rare and measured in size 800 to 1000µm. These rediae were brown sometimes whitish in live with very slow movements; they often show constrictions which give them a moniliform appearance (Fig. 4A). Each redia can contain 20 to 30 developing cercariae.

The observed cercariae had tail and body with stylet. The body was longer than the tail (Fig. 4B). The body was small, oval- elongate shaped, measuring 250-257.5µm x 92.5µm. The tail, measuring 100-137µm in length, was simple with indented margins, without a finfold. The oral and ventral suckers are almost equal; we note the presence of a vertical stylet 20 – 22.5µm long in the oral sucker. The oral sucker was sub-terminal, round and muscular 38-40µm. The ventral sucker was round, measured 36-42.5µm in diameter, and is located at mid-length of the body. The excretory bladder is Y-shaped. The surface of the body is covered with spines. A long esophagus of 35-37.5µm was observed and the intestinal caeca reached the posterior end of the body.



**Fig. 4.** *Lecithodendrium* sp. A. redia, B. cercaria. Scale bar, 100µm.

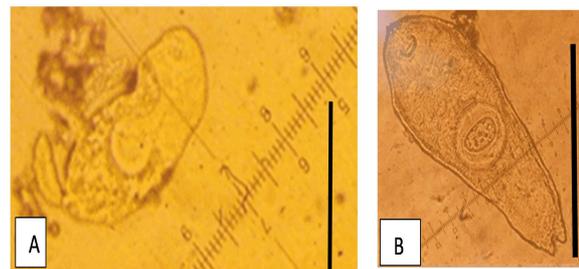
*Haematoloechus* cercariae: These cercariae were emitted naturally by *Lanistes ovum* with a prevalence of 0.59%. Sporocyst and radiae no observed. The cercaria was a single-tailed xiphidiocercaria. Tail was long twice that of the body. virgula organ absent (Fig. 5). The length of tail was 300µm, and the body 150µm. Cercaria had a stylet on oral sucker. Oral sucker was about four times larger than the ventral sucker. The length of oral sucker was 52.5µm and ventral sucker 12.5µm. Stylet length was 30µm.



**Fig. 5.** *Haematoloechus* cercariae. Scale bar, 100µm.

Plagiorchioid cercariae: cercariae were recorded from *Lanistes lybicus* (1.37%) and *Bellamyia unicolor*

(1.15%). The cercariae were non-oculate, with oral and ventral suckers; the excretory bladder is Y-shaped, and a very short simple tail with an indented edge (Fig. 6A). The ventral sucker was larger than the oral sucker (Fig. 6B). virgula organ absent. The body length measured in average 350 x 80µm; tail length 100µm. The buccal stylet was ball-shaped with length 30µm, located in the roof of the oral sucker. The ventral sucker measured 75µm and the oral sucker 25µm; the body was covered with spines.



**Fig. 6.** *Plagiorchioid* cercariae. A. whole cercaria; B. body of cercaria, scale bar 200µm.

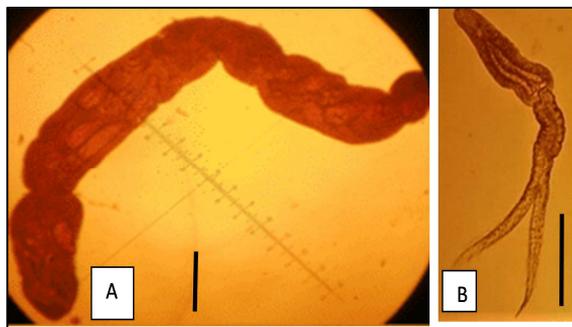
#### *Furcocercous* cercariae group

1.67% of snail were infected by furcocercariae. They had the high prevalence among all the cercariae. *Apatemon* sp.1 (Strigea cercariae): only cercariae were recorded from *Cleopatra* sp. with a prevalence of 5%. The cercariae were longifurcate-pharyngeate distome, non-oculate with oral and ventral sucker (Fig. 7). length of forcae (305µm) was greater than half of stem (365µm). Pharynx present, the length of the body was 205x100µm. The ventral sucker was just post-equatorial, smaller 10µm than the oral sucker 30µm.



**Fig. 7.** *Apatemon* sp1., whole cercaria. Scale bar, 100µm.

*Apatemon* sp2 (Strigca cercariae) : rediae and cercariae were observed from *Cleopatra bulimoides* (0.78%), *Cleopatra* sp (5%) and *Bellamyia unicolor* (1.15%). The rediae were great 800- 1650µm and had an average of 30 cercariae (Fig. 8A). The cercariae were longifurcate-pharyngeate distome whose body, tail stem and forcae length were approximately equal (Fig. 8B). Body length 230-250x67.5-75µm. Tail stem 250µm and forcae length 210µm. Furcae without finfold, presence of an oral and ventral sucker and a stylet in the roof of the oral sucker; stylet length, 23.75µm. Oral sucker 40µm greater than ventral sucker 10µm. Intestinal caeca reached to the posterior end of the body (Fig. 8B).

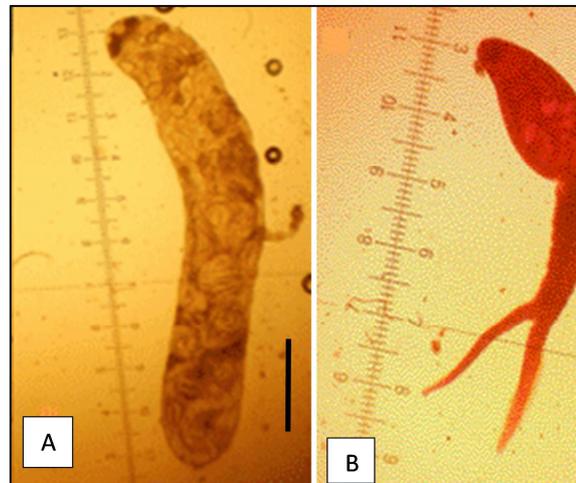


**Fig. 8.** *Apatemon* sp2. A. redia, B. cercaria, scale bar 200µm.

*Apatemon* sp3 (Strigca cercariae) : observed stages were rediae and cercariae in *Cleopatra* sp (5%) and *Bellamyia unicolor* (1.15%). Rediae measured 1130-1600µm and each redia contained 30 to 40 cercariae (Fig. 9A). Cercariae were longifurcate pharyngeate distome, furcae with finfold (Fig. 9B). They are similar to the previous *Apatemon* sp2 but differs from it by the furcae size, the presence of finfold on tail fork and variable morphology of the redia. Body length measured 200-225 x 105- 125µm; tail stem 250µm; furcae 175-200µm. Oral sucker measuring 30µm was larger than ventral sucker 10µm. Intestinal caeca reached to the posterior end of body.

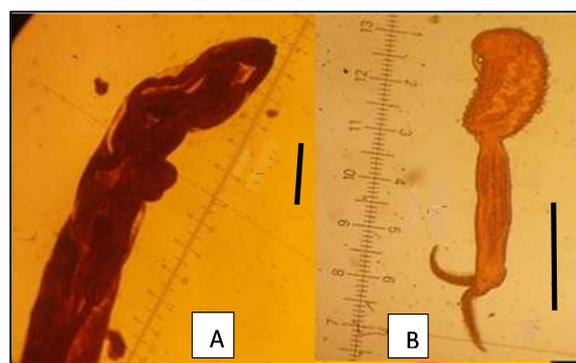
*Aporocotylid* sp1 cercariae: the Trematodes observed were rediae and cercariae stages from *Cleopatra* sp with a prevalence of 5%. The rediae were large (Fig. 10A) and measured in average 1500µm. The number of developing cercariae can reach up to more than 30.

Cercariae were large Furcocercariae, measuring in average 1000µm.



**Fig. 9.** *Apatemon* sp3. A. redia, B. cercaria, scale bar 200µm.

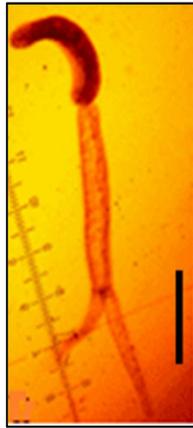
They were brevifurcate -apharyngeate cercariae (the length of the caudal fork was less than half of the caudal stalk) (Fig. 10B). They lacked a pharynx, with a protrusible anterior organ modified into an oral sucker, and without a ventral sucker (Fig. 10B). They had dorso-ventral furcal finfold. The body length was 250 x 190µm. The length of the tail stem was 450µm, furcae 140µm and stylet length 75µm, oral sucker measured 30µm, ventral sucker was not observed.



**Fig. 10.** *Aporocotylid* sp1. A. A. redia, B. cercaria. Scale bar, 200µm.

*Aporocotylid* sp2 cercariae: The Trematodes were cercariae collected from *Cleopatra* sp. (5%). Cercariae were characterized by the presence of a crown of thorns around the oral sucker. These were brevifurcate-apharyngeate monostomes with finfold

on furcae. Body length measured 200- 250µm; tail stem length 400-430µm, furcae length 250µm. Oral sucker measured 35x30µm. Stylet was present with length 25µm. Pharynx and ventral sucker were absent (Fig. 11).



**Fig. 11.** Aporocotylid sp2. whole cercaria. Scale bar, 200µm.

Furcocercariae type 1: the Trematodes observed were cercariae recorded from *Cleopatra* sp (5%). They were characterized by the presence of an elongate-oval body with a bifurcation nearly the base of the body. Body length 325 x 50µm; tail with furcate, tail stem almost absent  $\square$ 20µm in the form of a belt at the level of which the bifurcation originates (Fig. 12). Furcae measured 275µm long. Presence of fine spots in furcae.



**Fig. 12.** Furcocercous type 1 : whole cercaria. Scale bar, 200µm.

Cercariae type 2: Only cercaria stage was observed from *Cleopatra* sp with a prevalence of 5%. The body and the tail were almost equal in length (Fig. 13); length of the body was 270 x 60µm; the tail length was 260µm. The body was covered with an

undulating membrane. Ventral sucker and oral sucker not observed. Stylet absent. The tail had fine internal granulations.



**Fig. 13.** Cercaria type 2 : whole cercaria, 200µm.

## Discussion

### Prevalence

The infection prevalence of our study was low, but higher than those of Ibrahim and Ahmed (2019) in Nageria and Dunghungzin *et al.* (2017) in Thailand who found respectively a prevalence of 1.51% and 1.99% in their study. According to Dronen (1978), the low prevalence can cause by a number of limiting factors *such as*: infertility of some eggs when passed; the final host may defecate in deep water or on land where the parasite's eggs are not available to snails. The eggs may be digested or hatch in a non-susceptible host and thus be effectively removed from the ecosystem, or eggs may be produced at a time of year when proper environmental conditions, snail host species or suitable age groups of snails are not available (Dronen, 1978). The most prevalent and the high number of larvae species of *Cleopatra* sp. showed that the specimens of *Cleopatra* sp. were more receptive to cercariae than *C. bulimoides* and the two species of *Lanistes* in the reservoir of Loumbila. Our results were similar to those reported by Mohammed *et al.* (2016) who found two types of cercariae in *Cleopatra bulimoides*.

### Diversity

Nails of the reservoir of Loumbila contains a large number of trematode larvae. Twelve species grouped

in four major groups were recorded. There are *Cercariaeum cercariae*, *Xiphidocercous cercariae*, *Furcocercous cercariae* and cercariae type 2 (Frandsen and Christensen, 1984). Xiphidocercariae and furcocercariae were regularly observed by many authors (Krailas *et al.*, 2014; Lotfy *et al.*, 2017; Anucherngchai *et al.*, 2017; Martin and Cabrera, 2018; Enabulele *et al.*, 2018; Doanh *et al.*, 2019; Schwelm *et al.*, 2020). Chontanarith and Wongsawad found 9 groups of cercariae in Thailand in 2013. Many previous studies found these parasites in more different species of snail but for furcocercaria type 1 and cercaria type 2, we hadn't found document describing this form. Trematode larvae have final host like vertebrates. According to Sorensen and Minchella (2017) the degree of damage that trematodes cause their hosts, ultimately depends upon the relative success cercariae have in finding and infecting their next host and the extent to which this damage jeopardizes host survival. All the digenians species observed infested a single genus of snail except Plagiorchid sp and *Apatomon* sp2 and *Apatomon* sp3 who infected two or three different species of snail. According to Sorensen and Minchella (2017), the populations where prevalence is higher, trematode infections will alter host life history more so than in the same parasite–host combination at lower prevalence. The transmission of many digeneans depends on the ability of the cercariae to invade specific hosts (Kiatsopit *et al.*, 2014). Larval trematodes are the main parasites of snails, and they play a crucial role because they usually castrate their snail hosts and can thus alter their population and community dynamics (Gilardoni *et al.*, 2019). Some hosts may alter either their reproductive activities or growth, presumably to enhance survival (Sorensen and Minchella, 2017).

#### *Cercariaeum cercariae*

Two species namely *Acaudate Xiphidocercariae* sp1 and *Acaudate Xiphidocercariae* sp 2 of the type cercarium were recorded. These Cercariae were characterised by lacking tail, with oral stylet (Hechinger, 2012). Niewiadomska and Valtonen (2007) redescribed *Cercariaeum crassum* at the

cercariaeum stage and the daughter-rediae from *Pisidium amnicum* in Finland. They confirmed, basing on the developmental stages observed in daughter-redia, the cercariaeum stage (a type of cercaria without a tail) developed directly from germ balls. So, *Cercariaeum cercariae* are not some immature cercaria but the mature cercaria because Niewiadomska and Valtonen (2007) showed before cercariaeum stage, developing cercariae have sometimes tail. Many trematodes, in their life cycle include this stage of development to ensure their cycle. Indeed, Petkevičiūtė *et al.* (2020) described this type of cercaria in the development of *Palaeorchis incognitus*. They showed the live developmental stages of *Palaeorchis incognitus* describing their cercariae (type cercariaeum) and the adult. The specimens of *Acaudate Xiphidocercariae* sp 2 resemble to *Acaudate xiphidocercaria 1* recorded from *Potamopyrgus antipodarum* by Hechinger (2012).

#### *Xiphidocercous cercariae* group

Three species of this type of cercaria were recorded. There is *Lecithodendrium* sp., *Haematoloechus* sp. and Plagiorchid sp. Xiphidocercous cercariae had an unforked tail with ventral sucker on mild-ventral surface of body and a stylet in oral sucker (Frandsen and Christensen, 1984). According tomc Dowell (1953), the xiphidocercariae or stylet forms are characterized by the presence of a stylet on the anterior margin in the oral sucker near the mouth. The prevalence of infection in xiphidocercaria was 1.46% (7/479). They were recorded from *L. lybicus*, *L. ovum* and *B. unicolor*.

The *Lecithodendrium* cercariae identified were an armatae Xiphidocercariae (Frandsen and Christensen, 1984). This cercaria was observed in snail of the genus *Lanistes* (*L. Lybicus*, *L. ovum*). The cercariae resemble to those observed by Lotfy *et al.* (2017) and (Enabulele *et al.*, 2018). They resembled an armatae cercaria in which the tail is without dorso-ventral finfold shorter than body, the virgula organ is absent, and the oral and ventral suckers are of equal size. The cercaria form, the position of suckers and stylet with the penetration gland allow to say the

cercaria is into the genus of *Lecithodendrium*. The final host of *Lecithodendrium* cercaria is bats (Matskási, 1971). Many studies were carried on *Lecithodendrium* cercariae and adulte (Enabulele *et al.*, 2018; Anucherngchai *et al.*, 2017; Doanh *et al.*, 2019; Schwelm *et al.*, 2020; Shchenkov *et al.*, 2020). The molecular and phylogenetic analyses show that the xiphidiocercariae observed from *Radix balthica* in the UK (Enabulele *et al.*, 2018) and from *Bithynia tentaculata* in Central Europe (Schwelm *et al.*, 2020) was *Lecithodendrium linstowi*. Anucherngchai *et al.* (2017) observed it from *Melanoides tuberculata* (15%) *Tarebia granifera* (51.16%) in Thailand. The prevalence of cercaria we recorded was slow (1.37% in *L. lybicus* and 1.76% in *L. ovum*) to compare to those of Anucherngchai *et al.* (2017). *Lecithodendrium spathulatum* from *Melanoides tuberculata* was reported by Doanh *et al.* (2019). Shchenkov *et al.* (2020) described by molecular and morphological analyses 4 cercaria of Lecithodendriidae from *Bythinia tentaculata* and *Viviparus viviparus*. Our specimens were different from them described by Enabulele *et al.*, 2018; Schwelm *et al.* (2020) and Shchenkov *et al.* (2020) in size.

*Haemaloechus* cercariae were recorded in *L. ovum*, but studies proved Planorbis snail were the primary intermediate host (Dronen (1975). The cercaria of *Haematoloechus similis* was collected from *M. tuberculata* (Krailas *et al.*, 2014) with an infection rate of 1.46%. The body of this cercaria was longer than tail. Whereas, the cercaria we found had a body shorter than tail (body, 1/2 of tail). Our cercariae resemble those recorded by Outa *et al.* (2020) from *Pila ovata*. Not only are the characteristics of the individuals almost the same (the tail twice the length of the body, the oral sucker about four times that of the ventral sucker) but also the hosts of these cercariae (*P. ovata* and *L. ovum*) are of the same family (Ampullariidae). The second intermediate host of *Haematoloechus* is an arthropod and the definitive host is a vertebrate (Snyder and Janovy, 1996).

#### *Furcocercous cercariae group*

Furcocercous or forked-tailed cercariae are those that have a tail which consists of a stem and two distal

furcae or appendages (McDowell, 1953). 1.67% of snail were infected by furcocercariae. They had the high prevalence among all the cercariae. We found furcocercous from the genus *Cleopatra* and *Bellamyia*. Some studies showed them in the same genus (Lotfy *et al.*, 2017). Almost of research found them from *M. tuberculata* and *T. scabra* (Chontanarith and Wongsawad, 2013; Krailas *et al.*, 2014), in Lymnaeid Snails (Martin and Cabrera, 2018). Abdulkadir *et al.* (2018) recorded furcocercariae longifurcate from *Bilinus unicolor*, *Biomphalaria*, *Cleopatra bulimoides*.

According to Lotfy *et al.* (2017), this type of cercariae is mainly characterised by a forked tail into which the body is not retractable. The forked-tail may be either brevifurcate or longifurcate. Brevifurcate forked-tail is that in which the length of each of the furcal ramus is less than half the length of the main tail stem; Longifurcate forked tail is that in which the furcal ramus is more than half the length of the main tail stem (McDowell, 1953; Frandsen and Christensen, 1984). Furcocercariae found named *Apatomon* sp 1, *Apatomon* sp 2, *Apatomon* sp 3, *Aporocotylid* sp1, *Aporocotylid* sp2 and furcocercaria no previously described. Krailas *et al.* (2014) described *Apatemon gracilis* with an infection rate of 0.17% from *M. tuberculata* and Krailas *et al.* (2014) found furcocercaria in *M. tuberculata* namely *Alaria mustelae* with 0.15% of infection rate.

#### *Cercariae type 2*

The characteristics of this cercaria resemble to those of the cercariae type Megalurous. But the absence of the oral and ventral suckers does not allow us to say that it is Megalurous cercariae. More study classed this cercaria in Megarulous cercaria and others researches put him in *Gymnosphalus* type (Ayoub *et al.*, 2020; Chontanarith *et al.*, 2017; Chontanarith (2015) identified the Megarulous cercaria from *Melanoides tuberculata*, *Tarebia granifera* and *Thiara scabra* as *Philophthalmus* sp. Krailas *et al.* (2014) found Megalurous cercariae in *M. tuberculata* and identified molecular analyses, two species *Cloacitrema philippinum* and *Philophthalmus* sp.

Jabal *et al.* (2022) reported the presence of Megarulous cercariae and redia from three freshwater Prosobranch snail (Ampullariidae: *Pila* sp., *Pomacea* sp. and *Viviparidea: Bellamya* sp.) in Indonesia. The specimen found in our study was recorded also in prosobranch snail, *Cleopatra* sp with prevalence 5%. Anucherngchai *et al.* (2017) observed Megarulous cercariae of *Melanoides tuberculata* (2.33%) and *Tarebia granifera* (20%) in Thailand. Ayoub *et al.* (2020) described that this eye fluke as *Philophthalmus palpebrarum* and the *Melanoides tuberculata* snails emphasized as its intermediate host in natural and experimental infection in Egypt.

### Conclusion

The snails of Loumbila reservoir harbor many digeneans larvae with low prevalence. The snail who presents the more diversity of trematode and the more prevalent was *Cleopatra* sp. and the more harbored trematode was *Lanistes ovum* in this study. Cercaria no presenting the adult characteristics, molecular analyses are necessary to determine species of these trematode.

### Conflict of interest statement

We declare that we have no conflict of interest.

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