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Digenetic trematodes dependent on fish of Burkina Faso

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Abstract

Fish in Burkina Faso are parasitized by many types of parasites. In these study, digenetic trematodes of fishes were investigated. The aim is to inventory the digenetic trematodes dependent of commonly consumed fish in Burkina Faso. The sampling of the fish was carried out in February 2015, in March 2016 and from February 2017 to December 2017. Sampled fish were consisted of *Oreochromis niloticus*, *Coptodon zillii*, *Sarotherodon galilaeus*, *Gymnarchus niloticus*, *Clarias anguillaris*, and *Synodontis schall* in the stations of Kompienga, Bagré, Lemourdougou, Ziga and, Loumbila dams; and Tingréla, Di (Sourou), and Diarbakoko (Comoé) Lakes. The fish were dissected under the binocular microscope and their organs and abdominal cavity were carefully examined for digeneans. Digeneans were identified with the keys of references of Trematoda. A total of 806 fish were examined. Analysis of this ichthyofauna made it possible to find digenetic trematodes of the genus *Gymnatrema* (16.6%), *Clinostomum* (51.32%), *Basidiodiscus* (18.18%), *Glossidium* (0.74%), and metacercariae that we have been able to identify as *Tetracotyle* sp, *Ornithodiplostomum* sp, *Tylodelphys* sp, *Diplostomum* sp1, and *Diplostomum* sp 2. The present study provides knowledge on the fauna of digenetic trematodes encountered in fish of Burkina Faso.

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Introduction

One of the biggest problems today in the world is the lack of food (Abdel-Ghaffar *et al.*, 2015). Lack of protein in food is a major challenge in Third World countries in general and specifically in Burkina Faso, in the South of the Sahara. Fish is an important source of animal protein in worldwide. In Burkina Faso, freshwater fish generate significant financial resources. According to Kabré *et al.* (2013) the apparent annual consumption went from 1.5 kg to 3.5 kg per inhabitant between 2003 and 2009, either a doubling in 5 years. According to these same authors, fishing has become an economic activity which occupies nearly 10,000 fishermen with national production in 2009 estimated at 18 billion CFA francs. Despite its economic and nutritional importance, fish constitutes a very favorable biotope to the development of a large number of parasites such as the digenetic trematodes. Freshwater fish are intermediate or final hosts of several parasitic helminths. Parasites affect the health, growth and survival of fish (Moyo *et al.,* 2009). The impacts exerted by parasites on their hosts can be mechanical, chemical or physical (Adeyemo and Agbede, 2008). The metacercariae of *Clinostomum marginatum* trematodes are known to be responsible for considerable damage in the viscera and in the musculature of several species of fish in the USA (Hoffman and Meyer, 1974). Parasitic diseases of fish are very common worldwide and are specific in certain tropics (Schimidt *et al.,* 2000; Moyo *et al.,* 2009). The helminthology of fish in Africa is not widely studied like the other aspects of aquatic parasitology and fish biology (Abo Esa, 2008; Abdel – Gaber *et al.*, 2016). The digenetic trematodes of fish in Burkina Faso are not widely studied like other fish parasites (Sinaré, 2009). Very few studies have been devoted to parasitic fish trematodes from Burkina Faso (Coulibaly, 1995; Kabré, 1997; Boungou, 1998; Sinaré, 2009, 2017).

It was with a view to compensating for this insufficiency of data on digeneans, that this study was initiated. The objective of this study is to identify the digenetic trematodes dependent of commonly consumed fish in Burkina Faso.

Materials and methods

Sampling area

The choice of sites was made so that the samples were as representative as possible of the Burkinabe ichthyofauna and therefore likely to best translate the Burkinabe ichtyoparasito fauna relating to the digenetic trematodes.

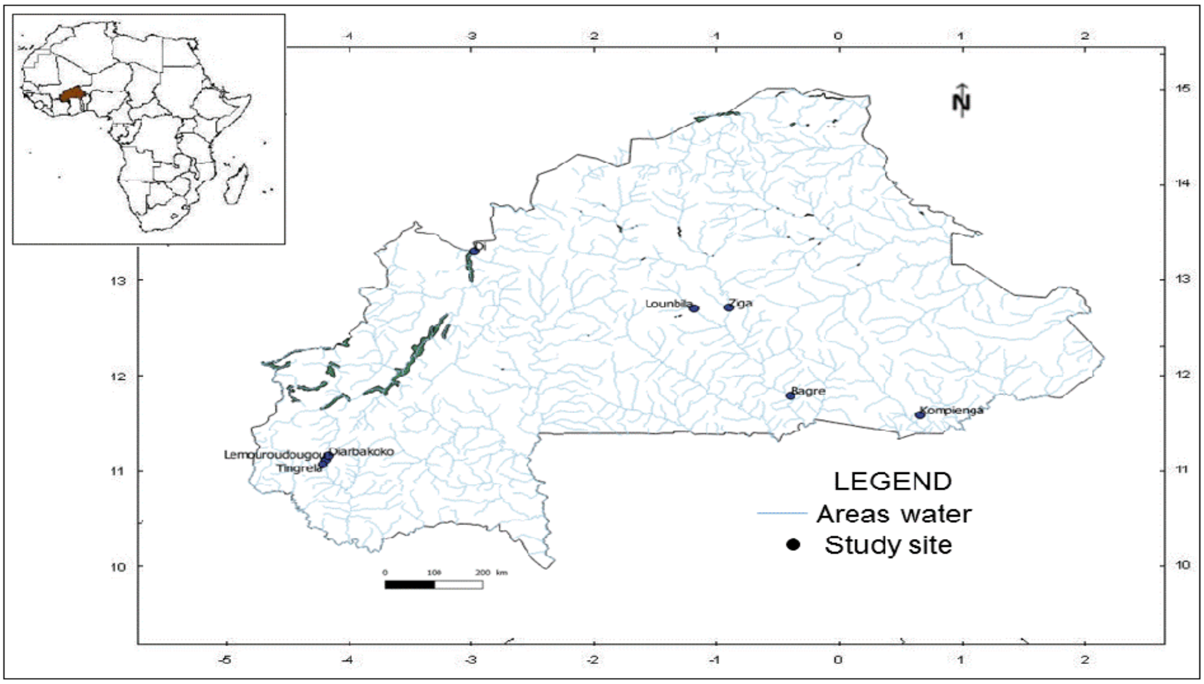

Fig. 1. Fish harvest sites.

The stations located on the Kompienga, Bagré, Lemourdougou, Ziga, and Loumbila dams; and Lakes Tingréla, Di (Sourou) and Diarbakoko (Comoé) were surveyed (Fig. 1).

Sampling period

The sampling of the fish took place in February 2015, March 2016, and February 2017 to December 2017.

Biological material

It consisted of *Oreochromis niloticus* Linnaeus, 1758, *Coptodon zillii*, Gervais, 1848, *Sarotherodon galilaeus* (Linnaeus, 1758), *Gymnarchus niloticus* Cuvier, 1829, *Clarias anguillaris* Linné, 1762, and *Synodontis schall* (Bloch et Schneider, 1801). A total of 806 fish were purchased at the various study sites from professional fishermen. Some fish were analyzed on the spot and others were brought back to the laboratory in coolers containing ice. The fish were identified according to Paugy *et al.* (2004).

Identification of parasites

The measurements of each fish collected were carried out. After measuring the standard size, the fish were observed under a binocular magnifier for the search of ectoparasites. Then, they were dissected under the binocular microscope and the digestive tract, muscles, liver, gills and, abdominal cavity were carefully examined for digeneans. The Trematodes encountered were removed with flexible forceps and placed in clean salt shakers containing 2% isotonic saline solution.

Digeneans were rinsed in distilled water after a stay in 70° alcohol. They were stained with Boracic Carmine, then passed for a few seconds in 1% hydrochloric alcohol. They were then dehydrated in a series of increasing degrees of ethyl alcohol (70 to 100). After clarification in the clove essence, the specimens were mounted between blades and strips, without pressure in Canada balsam. Observations and diagrams of digeneans were made using a Leitz Orthoplan and Wild M20 microscope equipped with a drawing tube. Some observations were made with a JEOL 6300F scanning electron microscope. For the identification of digeneans, we used the references of Gibson *et al.* (2002) and Jones *et al.* (2005).

Results and discussion

Gymnatrema gymnarchi Dollfus 1950 (Fig 2)
Family of Cryptogonimidae
Subfamily: Acanthostominae
Genre Gymnatrema Morosov, 1955
Host: Gymnarchus niloticus
Location at the host: Rectum
Sampling site: Di (Sourou)
Prevalence: 16.6%

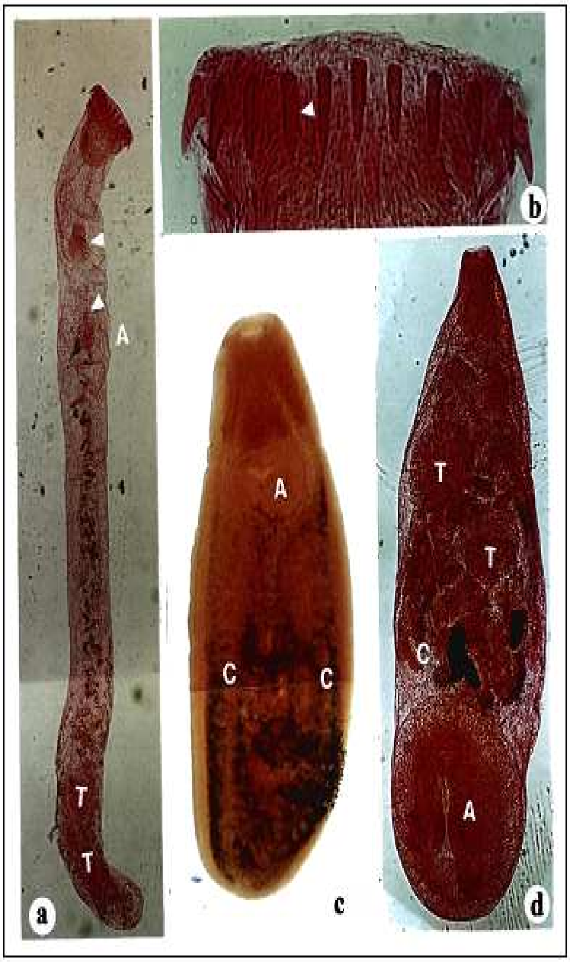

Fig. 2. *Gymnatrema gymnarchi*, *Clinostomum* sp and *Basidiodiscus ectorchis*: a: *Gymnatrema gymnarchi* in toto seen in light microscopy (x10). b: Anterior region in scanning electron microscopy, showing the hooks (arrowhead) surrounding the mouth (x20) c: *Clinostomum* sp. in toto seen in light microscopy (x10). d: *Basidiodiscus ectorchis* in toto seen in light microscopy. Note the two testicles (T), the postero-ventral acetabulum, and caeca (x10).
Abbreviations: A: Acetabulum, C: Caeca, T: Testicles

Gymnatrema gymnarchi is a long, thread-like worm with cuticular spines and especially circumoral hooks (Fig.2b, arrowhead). It has a funnel-shaped oral sucker marked with a crown of 21 large thorns that can be inserted into a skin fold. The surface of his body is covered with small integumentary spines. Its ventral acetabulum is smaller than its oral sucker and it is located at the level of the anterior 1: 4 of the body (Fig. 2a). It has a digestive tract starting at the bottom of the oral sucker and comprising 3 parts: a long pre pharynx, a small globular pharynx and a short esophagus. Its intestine is separated into two uneven lateral branches ending near the posterior end of the body. We did not observe the opening in the excretory vesicle. The two ovoid testicles are placed in tandem in the posterior quarter of the body (Fig. 2a). From each testicle leaves a long efferent canal which rises forward to unite with its symmetrical at the base of a long seminal vesicle. A vesicle ending in an ejaculatory duct and the uterus opens into the genital atrium. The terminal part of the uterus surrounded by a muscle mass, the gonostyle, and median can protrude outside and cover the anterior part of the acetabulum.

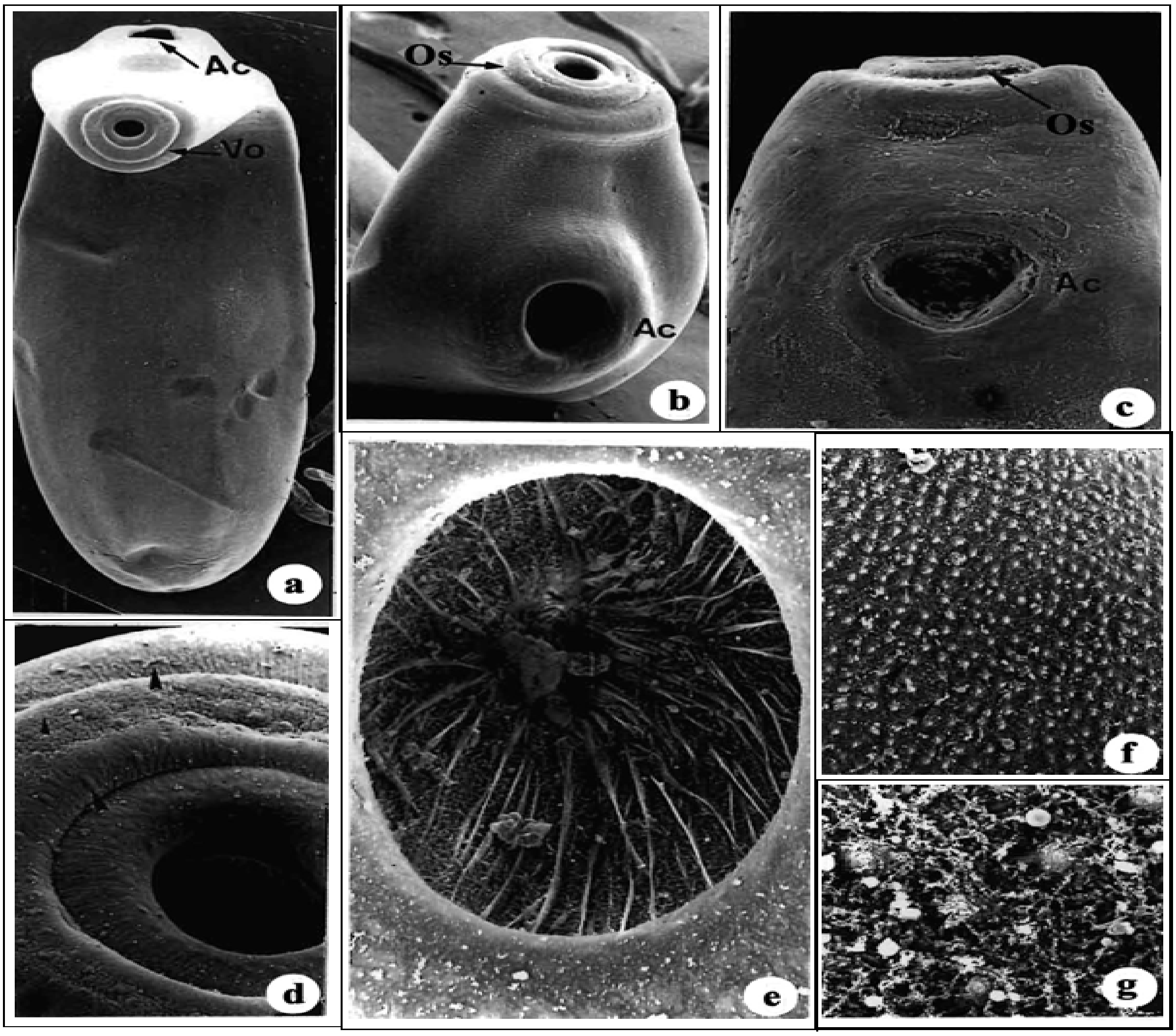

Fig. 3. *Clinostomum* sp. a: Parasite in toto seen in scanning electron microscopy (x25); b: Anterior region in scanning electron microscopy, showing the mouth surrounded by the oral sucker (Os) with a serpentine structure and the acetabulum (Ac) located on the ventral side (x 50); c: Detail of the anterior region seen in scanning electron microscopy. It shows details of the acetabulum and the buccal region viewed in profile (x 30); d: Detail of the anterior region in scanning electron microscopy (x250); e: Detail of the anterior region in scanning electron microscopy showing details of the back of the mouth (x 600); f: Cuticular detail (x 600); g: Cuticular detail at higher magnification (X 2000).

It has a spherical, ventral ovary located in the inter caecal space immediately in front of the most anterior testicle. Its oviduct goes to the genital crossroads where the laurer canal, the seminal receptacle canal, and the odd median vitelloduct terminate. The oviduct then goes up along the left side of the seminal receptacle and gives the ootype. The laurer's canal opens outwards in the middle of the dorsal surface at the level of the ovary.

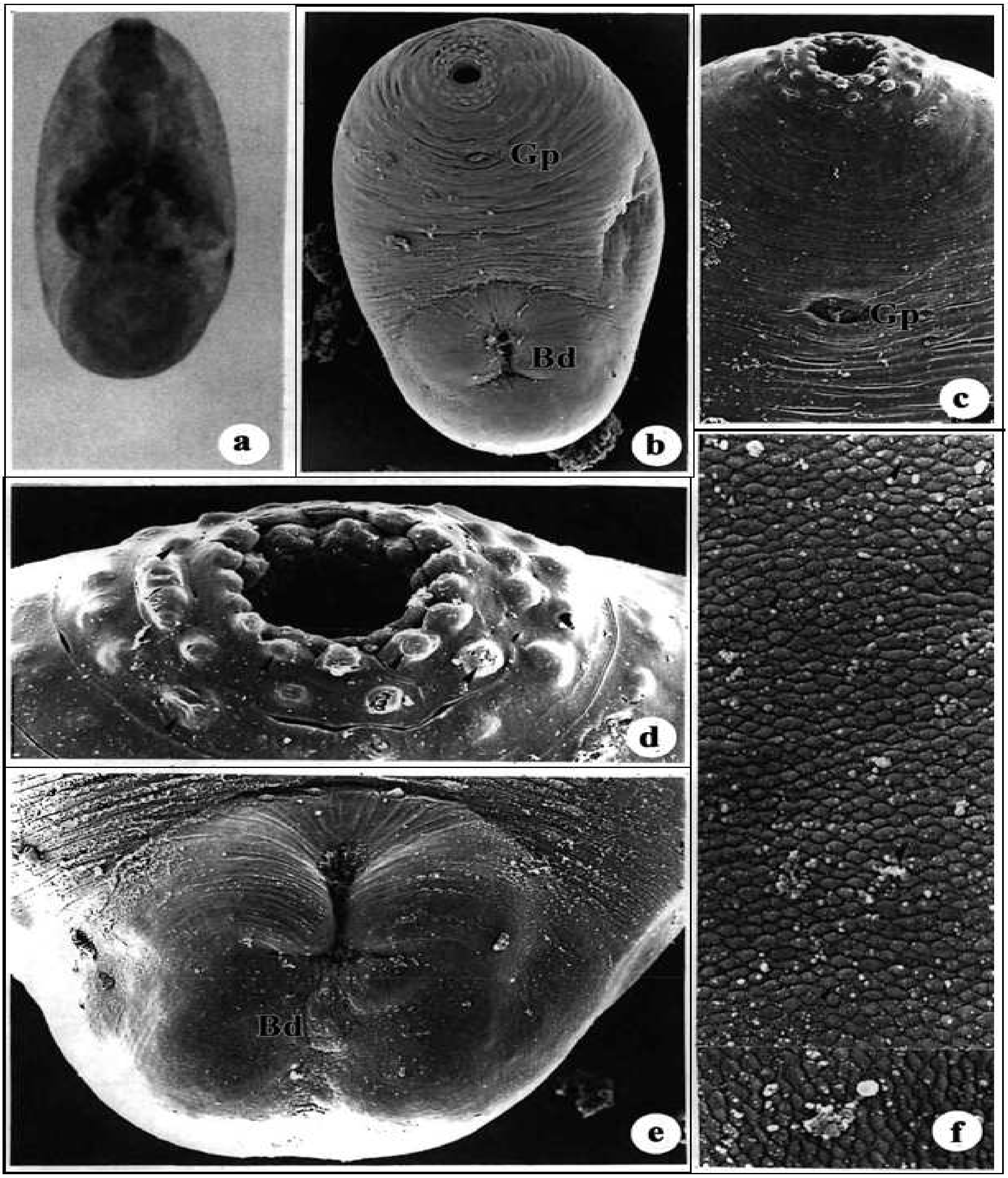

Fig. 4. *Badiodiscus ectorchis*: a: Parasite seen in toto in light microscopy (x 5) ; b: parasite seen in toto by scanning electron microscopy (x1 00); c: Anterior region in scanning electron microscopy (x 200); d: Detail of the anterior region seen in scanning electron microscopy (x 600); e: Detail of the posterior region in scanning electron microscopy (x 200); f: Cuticular detail (x 1000).

Abbreviations: A: Acetabulum, Pg: Glandular papillae, Db: Basal disc.

The yolk follicles spread laterally and dorsally and extend from the anterior testicle halfway up the body. The ventral uterus, directed backwards before going up forward to lead into the genital atrium. "Y" shaped excretory apparatus. We encountered small, sealed eggs. By its anatomo-morphological characteristics (elongated body, large circumoral spines, developed oral suction cup, pharynx present and acetabulum of small size), this trematode belongs to the genus *Gymnatrema* Morosov, 1950. Few *Gymnatrema* have to date been the object of description in Africa. The only data concern *Gymnatrema gymnarchi* (Dollfus, 1950) Syn.:*Acanthochasmus gymnarchi* Dollfus, 1950, observed in *Gymnarchus niloticus* in Sudan (Dollfus, 1950); Khalil, 1969). The specimen we are describing can be compared to *Gymnatrema gymnarchi* (Fig. 2 a) which has 21 circumoral spines, which has a comparable size (length between 2.5 and 3.5 mm) and an identical host. The presence of *Gymnatrema gymnarchi* in Burkina Faso widens the range of this trematode.

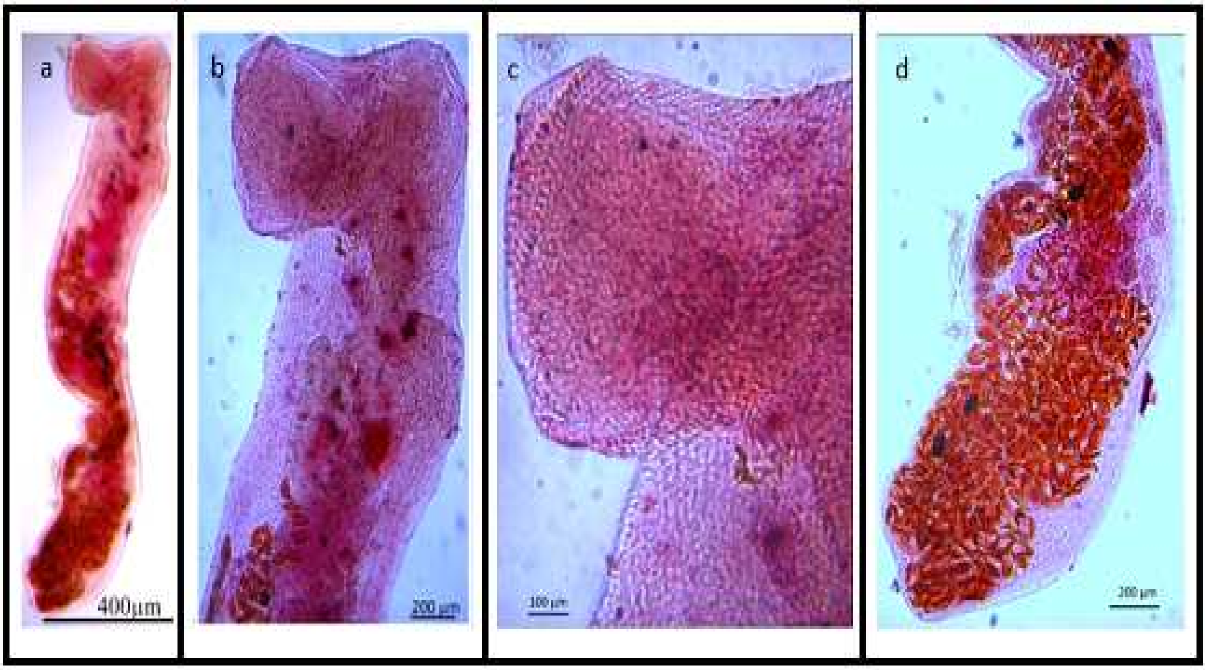

Fig. 5. Photograph of *Glossidium* sp, a: whole worm, b: anterior part, c: anterior part of the worm showing the presence of body hair and the posterior part.

Clinostomum sp. (Fig. 3)

Family of Clinostomidae Lühe, 1901
Genus: *Clinostomum* Leidy, 1856
Hosts: *Coptodon zillii*, *Oreochromis niloticus*,

Sarotherodon galilaeus
Location in the host: Gills, pharynx, Intestine
Sampling sites: Kompienga, Bagré, Lemourdougou, Di (Sourou), Diarbakoko (Comoé), Ziga and Loumbila dams.

Prevalences: the overall prevalence was 51.32% (428 parasitized fish / 834 fish examined. According to the hosts, it was 62.16%; 45.30% and 49.36% respectively in *Coptodon zillii* (115 parasites out of 185 fish examined), *Oreochromis niloticus* (82 parasites out of 181 fish examined) and *Sarotherodon galilaeus* (231 parasites out of 468 fish examined).

Clinostomum sp. has a robust, elongated body with oral sucker surrounded by a kind of falling collar when they retract (Fig. 3a). The ventral acetabulum with a diameter much larger than the oral sucker is located at the level of the anterior 1/3 of the body (Fig. 3b). *Clinostomum* sp. has a typical pharynx, a swollen, bulbous esophagus at the posterior end. At the back of the mouth, we note the presence of species of partitions (Fig. 3e). It has intestinal caeca with a sinuous wall, opening into the excretory vesicle by narrow passage. The testicles are located in the middle of the body or near the posterior end. The cirri pocket, anterior to the ovary or anterior testicle, contains a coiled seminal vesicle and an ejaculatory duct. The prostate complex is apparent or absent.

The genital atrium opens on the right side or in front of the anterior testicle and its ovary on the right side of the median plane between the two testicles. It has a uterus that can reach the acetabulum. The excretory vesicle is small and "V" shaped with a dorso-ventral terminal pore. It is a worm with a slightly flattened dorso-ventrally body (Fig. 3c) with a circular oral sucker (Fig. 3b). It has small projections in papillae arranged irregularly on the edges of this sucker and on the anterior part of the body pursued over the whole body (Fig. 3d). The surface of which appears rough (Fig. 3f-g).

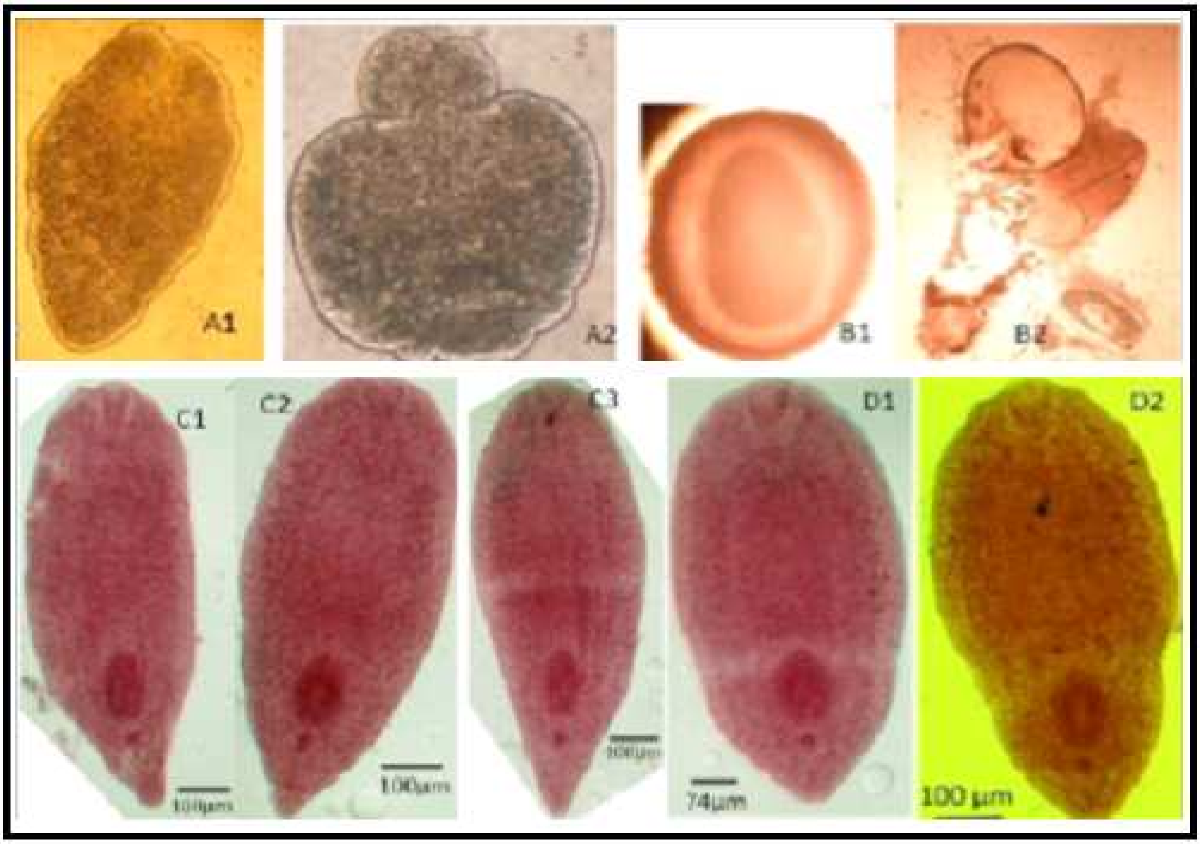

Fig. 6. Photograph of diplostomatids observed in *C. anguillaris*. A Metacercaria 1 (*Ornithodiplostomum* sp.), B Metacercaria 2 (*Tetracotyle* sp.), C-D2 Metacercaria 3 (C1-C2 *Diplostomum* sp.1, C3 *Tylodelphys*, D *Diplostomum* sp.2).

Clinostomum sp. has always been found only in Cichlidae. According to Coulibaly (1995), Cichlidae are more receptive to the larvae of *Clinostomum*. They constitute the second dominant species of parasite observed in Cichlidae during these studies. Digeneans (*Clinostomum* sp) have complex life cycles involving three hosts (Bonett *et al.,* 2011), snail, fish or amphibians and birds. The snail is considered the first intermediate host, with fish acting as the second intermediate host, and the water birds as the final hosts. Cichlidae feed mainly on benthic materials, including detritus, by picking up the larval stages of parasites.

Maguza-Tembo and Mfitilodze (2008) reported that *Oreochromis shiranus* (Cichlidae) was likely to harbor the Trematode (*Clinostomum*). Moema *et al.* (2019) found a species of the genus *Clinostomum* in Tilapia sparrmanii. Clinostomum metacercariae are known to have damaged the muscles of Kompienga fish and made them disgusting and non-commercial (Coulibaly, 1995).

Basidiodiscus ectorchis Fischthal and Kuntz, 1959 (Fig. 4).

Genus: *Basidiodiscus* Fischthal and Kuntz, 1959
Host: *Synodontis schall*
Location at the host: Intestine
Sampling site: Tingrela Lake
Prevalence: 18.18%

This *Basidiociscus ectorchis*, with a conical body, has a circular anterior oral opening (Fig. 4a). We note the presence of papillae arranged in 5 circles of 15 papillae around the mouth opening (Fig.4b-c). The papillae tight near the oral opening, interannular space become more and more important as one moves away from this opening (Fig. 4d). The cuticular surface is not smooth, but rather rough (Fig. 4f). The genital pore is located at the level of the anterior 1/3 of the animal's body. The ventro-terminal acetabulum is larger than the body and does not has papillae.

The pharynx has two saccular diverticula. There is a short esophagus, with a posterior bulb. The intestine is divided into two lateral sinuous ceca ending near the posterior region of the body. Two globular testicles, ventral and located in the intercaecal space. Uterus hardly observable in the specimen here in description. The intercaecal uterine loops can extend from the ovary to the end of the cecum. The posterior region is made of a very muscular disc (Fig. 4e).

Basidiociscus ectorchis was first described in *Synodontis schall*. According to Khalil *et al.* (1969) this species of digeneans can be found in several species of fish of the genus *Synodontis*. In the present study, *B. ectorchis* (Fig. 3) was encountered in Synodontis. Our results confirm the hypothesis of Ashour *et al.* (2006) that *B. ectorchis* is subservient to species of the genus *Synodontis*. Indeed, all hosts except for *Synodontis schall* which were autopsied did not harbor any specimen of *B. ectorchis*. This same result was found by Kabré in 1997.

Glossidium sp. (Fig. 5).

Family of Macroderoididae McMullen, 1937
Genus: *Glossidium* Looss, 1899
Host: *Clarias anguillaris*
Location: Loumbila reservoir
Infection site: intestine
Prevalence: 0.74%

The genus *Glossidium* has been encountered by several authors in fish of the genus *Clarias*. According to Matla (2012) *Glossidium pedatum* is specific to fish from the Clariidae and Bagrididae family. Effect, among the species of the genus *Glossidium*, G. pedatum is most encountered in this host. Indeed, it was found in *Clarias gariepinus* by Saayman *et al.* (1991), Moyo *et al.* (2009) in Zimbabwe. In the present study, Glossidium sp. (Fig. 5) was found in the intestines of *Clarias anguillaris*. This localization of this trematode is the same in *Clarias batrachus*. Indeed, Nimbalkar *et al.* (2010) found *G. pedatum* in the intestines of *Clarias batrachus* in India. According to these authors, the genus *Clarias* is more exposed to digeneans because of their habitats characterized by an environment that is covered with vegetation and significant turbidity.

Family of Diplostomatidae Poirier, 1886 (Fig.6). The Diplostomatidae family particularly contains metacercariae. Metacercariae differ in the shape of their cysts and their size. On the basis of the encystment and the type of cyst, the three types of metacercariae collected during the present study were named as metacercaria 1, metacercaria 2 and metacercaria 3. Trematodes of the genus *Diplostomum*, *Ornithodiplostomum*, *Tylodelphys* are known to parasites *Clarias* fish (Barson and Avenant-Oldewage, 2006b; Barson *et al.*, 2008; Mwita, 2014). The first form (Metacercaria 1) is encysted in the cavity on the mesenteries and on the liver. It is more abundant in Ziga than in Loumbila. Metacercaria 1 is surrounded by a single, less hard envelope (Fig. 6 A). The larva out of its envelope is shown in Fig. 6 A1. It does not have pseudo suckers and it seems to be divided in two. The second form (Metacercaria 2) encysted in the cavity and on the mesenteries, on the gonads, in the muscles, on the intestine and the liver. It has 3 envelopes: a hard external, another internal which surrounds the larva, and another in the middle between the 2 envelopes. Fig. 6B shows a metacercaria 2 burst after pressure and released the larva by exposing the 3 envelopes. It has suckers and pseudo suckers. Metacercaria 1 and 2 correspond respectively to *Tetracotyle* sp. and *Ornithodiplostomum* sp. The metacercaria 1 by its morphology, resembles the specimens of *Ornithodiplostomum* sp. The Metacercaria 2 is surrounded by three cystic membranes and has pseudo suckers and suckers. It is comparable to *Tetracotyle*-type metacercaria de *Clarias gariepinus* (Barson *et al.*, 2008). Metacercaria 2 also resembles the genus *Ornithodiplostomum* encountered in the ventro-lateral muscle of Clarias gariepinus (Barsonand Avenant-Oldewage, 2006b; Barson, 1998). According to Gibson (1996), *Tetracotyle* is encysted in tissues especially on membranes such as pericardium, the general cavity of teleost freshwater fish. It has also been found in muscle, in the abdominal cavity, on the mesenteries, gonads, liver and in the outer wall of the intestine of *Clarias anguillaris*. But the fact that the metacercaria has pseudo suckers, leads us to think that metacercaria 2 is of the *Tetracotyle* type.

The third form (Metacercaria 3) is encountered freely and only in the abdominal cavity. This metacercaria is somewhat advanced and is not in an envelope. The Metacercaria 3 is without encystment membrane and bathes in the cavity. Elongated in shape, it looks like an adult with two prominent suckers (apical sucker and acetabulum). We have three categories of metacercaria 3 (Fig. 6 C-E), one is more or less short and has two pseudo suckers in addition to the oral sucker and the acetabulum and an anal opening; It is presented in Figure 6 C1-C2. The second category is elongated with an oral sucker, a clearly visible pharynx, a digestive tract divided in two, an acetabulum and an anal opening (Fig. 6 C3). The third category in addition to these characters presents a sort of tail (Fig. 6D). These three categories correspond to three types of species which are: *Diplostomum* sp1, *Tylodelphys* sp, and *Diplostomum* sp2. The three categories can represent 3 species or subspecies of Diplostomum. Paradoxically, it is possible to think that these three types of metacercariae belong to a single species and the different metacercariae correspond to the different stages of development of the species in the organism of the host. This is because studies have emphasized the complex nature of migration and the growth of metacercariae of the genus *Ornithodiplostomum* and have shown that their growth and encystment phases occur in different habitats inside their intermediate hosts (Matisz and Goater, 2010). These authors showed that in four weeks, all the metacercariae were encysted, the integumentary extensions had disappeared, and several metacercariae were free in the visceral cavity. The morphology of the specimens that Matisz and Goater (2010) observed resembles our metacercaria 3. Raissy *et al.* (2011) observed metacercariae of *Ornithodiplostomum* in the eyes, gills, on the wall of the esophagus and intestine, in the abdominal cavity, muscles and gonads of a Cyprinodontidae; the encysted forms resemble our metacercaria 1 and the free forms dispersed in the abdominal cavity are comparable to metacercaria 3.

In sum, we can think that the different types of metacercariae that we encountered during our investigations in *C. anguillaris* belong to the genus *Ornithodiplostomum*; but the different forms (rounded, elongated, body divided in two or not) and characters (presence of sucker and pseudo sucker), allow us to conclude that these metacercariae correspond to different species that we have tried to identify based on Gibson's key (1996). However, additional and further studies will be needed to clearly identify these metacercariae.

Conclusion

The analysis of the fish consumed in Burkina Faso allowed us to find *Gymnatrema gymnarchi* in *Gymnatrema niloticus*, *Basidiodischus ectorchis* in *Synodontis schall*, *Clinostomum* sp. in three species of fish, *Coptodon zillii*, *Oreochromis niloticus*, and

Sarotherodon galilaeus, *Glossidium* sp in *Clarias anguillaris*, and metacercariae that we have been able to identify as *Tetracotyle* sp, *Ornithodiplostomum* sp, *Tylodelphys* sp, *Diplostomum* sp1 and *Diplostomum* sp2. The present study provides knowledge on the fauna of digenetic trematodes encountered in fish of Burkina Faso. However, an additional study is important and necessary to understand the development of the metacercariae.

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