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Review Article

African *Diplostomum* (*sensu* Dubois 1961): Minireview on taxonomy and biology

Abstract

Freshwater fisheries has a significant contribution to development as an important source of human proteins as well as in sport fishing and aquarium. Despite their importance, both wild and aquaculture fish suffer from a problem of parasitism, notably *Diplostomum* species, causing fish mortalities particularly in fingerlings. Although a considerable effort in understanding *Diplostomum* species taxonomy, biology and control of fish parasites has been well developed in the northern hemisphere, the knowledge of these aspects in Africa is not clearly known. Therefore the present work reviews the aspects of biology and taxonomy of African *Diplostomum*. The traditional approach to uncover these aspects would be to complete the life cycles in the laboratory, which would lead to morphological characterisation of all life cycle stages. However, establishing and maintaining life cycles of *Diplostomum* species is difficult, laborious and expensive. Although, molecular methods have been proven to provide an alternative solution, are not common in Africa due to lack of equipment and expertise. However, improvement of some weaknesses for some studies like providing pictures or diagrams of the *Diplostomum* species found is recommended. In addition, for laboratories that have the capacity to do molecular analysis, the use of a familiar molecular marker like a barcode region could be a prospective development in future.

Introduction

Freshwater fisheries has a significant contribution to development as an important source of proteins. More than 90% of all freshwater fisheries, i.e. wild capture and aquaculture, occurs in developing countries [1]. Besides providing food and a livelihood for millions of the world's poorest people, freshwater fisheries contributes to the overall economic income by means of export commodity trade, tourism and recreation [2]. As a consequence, freshwater fisheries has become an important economic activity for both rural and urban populations in Africa and globally. The widening gap between supply and demand for fish products, has further made capture fisheries to be the largest extractive use of wildlife worldwide. Increases in human population, rising incomes and increasing urbanisation coupled with stagnation or decline of supplementary proteins, further exacerbates the situation. As a result most communities globally have responded by venturing into aquaculture to supplement capture fisheries. Despite its progress, African aquaculture needs to address a cascade of challenges including lack of national policies to guide aquaculture development, unfavourable investment policies, the absence of linkages between farmers, lack of research/technology development and extension, and unfavourable investment climates, inadequate quality seed and feed, and above all infectious and parasitic diseases [3,4].

Diplostomum species, especially larval stages namely cercariae and metacercariae are among the main agents of important diseases in fish and aquaculture systems. *Diplostomum* species are strigeoid digeneans of the family Diplostomidae [5,6]. The family Diplostomidae has four subfamilies: Diplostominae, Crassiphialinae, Alariinae and Codonocephalinae, which are classified on the basis of host specificity and metacercariae types [7]. However, the present review focuses on members of the compound genus *Diplostomum* [8], that includes three subgenera, often elevated to genera level [9]. They are *Diplostomum*, *Tylodelphys* and *Dolichorchis*. These parasites are ubiquitous in freshwater systems, but the most frequently encountered stages are the metacercariae that reside unencysted in the eyes, cranial and brain cavities of freshwater fishes. *Diplostomum* can be highly pathogenic to fish and thus threaten the natural and aquaculture practices globally. Fingerlings in particular may experience high rates of mass mortalities when heavily infected with metacercariae, or as a result of massive cercarial penetration [5]. The metacercariae can also impair the fish escape response, diminish fish crypsis and thus may increase their vulnerability to predation [10]. In addition, cercarial penetration can increase the chances of bacterial infection in fish [11] and productivity of farms could be severely compromised.

Many studies on *Diplostomum* species have been reported in the Northern Hemisphere where their life cycles and

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general biology have been well investigated. In Africa, on the other hand, a few studies are known and most of them have been restricted to reporting occurrence [12-15]. In general the taxonomy, biology and life cycles of *Diplostomum* species in Africa remains incomplete. With this regard, the present review focuses on *Diplostomum* species occurring in the African continent on the aspects of biology, taxonomy and life cycles based on the available literature. I highlighted areas where there is a dearth of information that require further research. This approach is a reflection of my personal view and not an attempt to be exhaustive.

Life cycle and host specificity

Diplostomum species, like other digenean trematodes, have attracted attention of countless studies due to their complex life-cycles, which involve a series of larval stages coupled with sexual and asexual modes of reproduction [16]. In the process they incorporate parasitic stages (sporocyst, metacercaria and adult) and free-living stages (egg, miracidium and cercaria). For a successful completion, diplostomid life cycle requires three hosts, namely, a mollusc intermediate host, a wide variety of fishes and amphibians as second intermediate host and a bird definitive host [6,10].

Sexually matured adults of *Diplostomum* species are found in the alimentary tract of bird host where they produce eggs that reach the external environment with the host's faeces. Eggs hatch to release a ciliated free-swimming miracidium that seeks out a molluscan intermediate host. Snail hosts becomes infected after actively being penetrated by the miracidium. Once in the molluscan tissue, the miracidium transforms into a mother sporocyst, which produce asexually embryos that develop into daughter sporocysts generation. Moreover, the second intra-molluscan generations, daughter sporocysts, produce cercariae after another rounds of asexual reproduction [16]. Cercariae are well equipped with features and behaviours geared towards fish-finding, recognition and penetration [6]. After emergence, the cercariae actively infect a wide variety of fishes to become metacercariae. In the host, the metacercaria migrate along specific neural tracts to access sites with relatively minimal immune resistance like visceral organs, optic lobes and the brain so that they remain viable for successful transmission to the next host [16]. Bird hosts normally get infected upon consuming metacercariae in fish hosts [17].

Nonetheless, *Diplostomum* species life cycles have been well documented in the Palaearctic region, but not fully understood in Africa. Usually, lymnaeid snails (*Lymnaea* and *Radix* spp) and planorbid snails (*Planorbarius corneus*) are the main intermediate hosts [6,18], and in South America the snails of genus *Biomphalaria* such as *B. prona*, *B. straminea* and *B. glabrata* have been reported as the potential first intermediate [19]. In Africa, on the other hand, the range of snail hosts are far from completely understood, despite several experimental attempts undertaken [13,20]. The only known life cycle is that of *Tylodelphys xenopi*, in which the freshwater snail *Bulinus tropicus* is the first intermediate host [21]. Beverley-Burton (1963) also tried to infect *Radix natalensis* (Lymnaeidae) with *Diplostomum* (*Tylodelphys*) *mashonense*, but could not obtain cercariae. Even so, strigeoid cercariae purported to belong to the genus *Diplostomum* have been reported from *Biomphalaria* species from three fish farms in Kibos area within Kisumu, Kenya [22]. Similarly, at Mindu dam in Tanzania, *Diplostomum*-like furcocercariae had been reported once from the snail *Biomphalaria pfeifferi* [23,24], but since then *B. pfeifferi* have not been spotted in the dame. Thus the consistently high prevalence of the metacercariae of *T. mashonense* in *C. gariepinus* at Mindu Dam [23,25], brought a suspicion that non-lymnaeid snail species could serve as snail hosts, particularly because lymnaeid snails have not been recorded within or around the dam. As such it was hypothesised that other snails besides lymnaeids and *Biomphalaria*, could be responsible for the transmission and *Bulinus* spp were shown to support that hypothesis [25], as shown in figure 1.

In *Diplostomum* species, host specificity is mostly restricted in the first intermediate host while less specific in the second intermediate host and final hosts. For instance in Europe and North America, metacercariae of *Diplostomum* species have been recorded from over 150 species of fish from families Percidae, Salmonidae, Coregonidae, Clupeidae, Gobiidae, to name a few [26,27] and a broad range of piscivorous bird species serving as definitive hosts [10]. Similarly in Africa, *Diplostomum* species have been found in almost every fish family i.e. Characidae, Centrarchidae, Cichlidae, Claridae, Cyprinidae, Hepsetidae, Salmonidae, Schilbeidae to mention but a few [28,29]. However, the catfish *Clarias gariepinus* (family Claridae) is the most examined fish [29] and references therein. Nonetheless the range of fish hosts in Africa is not well known as the level of *Diplostomum* studies in Africa is still at an infant stage.

As far as definitive hosts are concerned, in Africa adult *Diplostomum* have been reported from the Egyptian kite *Milvus migrans aegypticus*, the Egyptian moorhen *Gallinula chloropus chloropus* and the giant heron *Ardea goliath* in Egypt [20,30,31], Pel's fishing owl *Scotopelia peli* in Ivory Coast [32], the grey heron *Ardea cinerea* in Zimbabwe and Tanzania [13,23,33] the

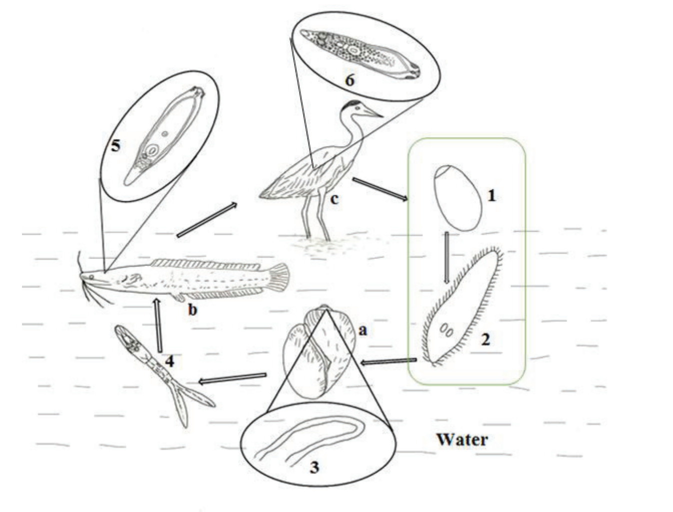

Figure 1: Life cycle of *Tylodelphys mashonense*; stages 1 and 2 have been elucidated from experimental establishment (Musiba, unpublished); stages 3, 4, 5 and 6 identified by molecular methods [25].

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African darter, *Anhinga rufa rufa* in Ghana [34] and the great white egret *Ardea alba* in Tanzania [23,33]. Generally, studies of *Diplostomum* species in bird definitive hosts in the African continent are scarce and limited. This is attributed to (i) low sampling efforts in the tropical countries due to inadequate expertise and resources (ii) difficulty in getting study permits to sacrifice some birds as they are either found in the protected areas (national parks and game reserves) or lack of interests in fish parasitology.

Taxonomy of *diplostomum* (Sensu Dubois, 1970) species in africa

Precise identification of members of the genus *Diplostomum* is usually difficult because of remarkable morphological similarity within and among species at almost every developmental stage [6]. Also, lack of a well-defined criterion further exacerbates the delineation difficulty within the *Diplostomum* group [6,35]. Furthermore, the taxonomic problem is aggravated by deformation of the body in the course of fixation, staining and mounting of permanent preparations [6]. In addition, many species have been described on the basis of one or two life cycle stages as a result different stages of the same species have been given different names or different species are known by the same name [6].

The taxonomy of *Diplostomum* species in Africa in general remains not well understood because the full range of species that may occur in Africa is far from completely known. As a consequence the biology of all reported species such as the range of hosts and life cycle stages (eggs, miracidia, intramolluscan stages, cercariae) is poorly understood. So far in the whole of Africa the adults of only five *Diplostomum* species have been described; they are *D. tregenna* [12], *D. marahoueense* [32], *D. magnicaudum* [30], *D. ghanense* [34] and *D. ardeae* [31]. On the other hand, only four *Tylodelphys* species adults are known i.e. *T. mashonense* [13], *T. aegypteus* [31], *T. clavata* [36] and *T. xenopi* [21]. Nevertheless, more than 15 *Diplostomum* species have been reported as metacercaria in different fish hosts species [29,37,38]. Although some authors tried to name the metacercarial stages to species level, for example *D. garrae*, *D. longicollum*, *D. montanum* and *D. tilapiae* described by Zhokhov [38], have further exacerbated the taxonomic problem. Moreover the taxonomic status of some *Diplostomum* species that have been recorded from some fish hosts is confusing. For instance, when the genus *Clarias* was reviewed by [39], several widespread species i.e *Clarias ngamensis*, *C. melandi* and *C. capensis* of southern Africa, *C. mossambicus* of central Africa and *C. lazera* of west and north Africa were synonymized under the name *Clarias gariepinus*. With this regard, the taxonomic status of *Diplostomum* (*Dolichorchis*) *tregenna* recovered from *C. lazera* and *Tylodelphys mashonense* found in *C. gariepinus* is questionable.

In addition Niewiadomska [9] moved *D. marahoueense* Baer 1957 to the genus *Dolichorchis* due to the presence of a genital cone and asymmetrical anterior testes in the adult form. This taxonomic re-evaluation of *D. marahoueense* implies that other African *Diplostomum* species with similar features may follow a similar fate. This idea was supported by Zhokhov et al. [40] and Zhokhov [38] who considered *Diplostomum tregenna* as *Dolichorchis tregenna*. At the metacercarial stage the fundamental features that enable distinguishing genera or species are the shape of fore- and hindbodies, presence or lack of additional organs of attachment like pseudosuckers, the structure of the holdfast organ, structure of the reserve bladder, the shape and spread of the calcareous bodies [35]. However these features could only be used on *T. mashonense*, *D. tregenna* and other known diplostomid metacercariae, but not on the metacercaria of *D. marahoueense*, which is hitherto unknown. As a result the metacercarial status of *D. marahoueense* is not understood.

Various literatures regarding *Diplostomum* species described from Africa reveal a complicated taxonomic situation. In particular, views over the taxonomic position of the named *Diplostomum* species differ. Dubois [8] considered the genus *Diplostomum* to be under three subgenera, namely, *Diplostomum*, *Tylodelphys* and *Dolichorchis* (Figure 2). The idea which was supported by Niewiadomska [9], but elevated them to genera. Accordingly, the classification of the three forms was based on the anatomical features of the adults, i.e. the asymmetrical nature of the anterior testis (as in *Diplostomum* spp.) and the presence of a genital cone (as in *Tylodelphys* spp.). The genus *Dolichorchis* is given to materials exhibiting an intermediate position between *Diplostomum* and *Tylodelphys*, i.e. asymmetrical anterior testis and the presence of a genital cone. As a result, African material *Diplostomum tregenna*, *D. marahoueense* and *D. mashonense* have been assigned within the genus *Dolichorchis* [9], although they originally belonged to the genus *Diplostomum*. However, phylogenetic analysis of *Diplostomum mashonense* by Chibwana et al. [41] suggested a re-allocation to *Tylodelphys* as previously viewed by Sudarikov [42].

Furthermore, records of *Tylodelphys* species in Africa are generally scarce. The only descriptions of adults *Tylodelphys* species are those of immature *T. clavata* from the intestine of a jackal, buzzard *Buteo rufofuscus* in the Democratic Republic

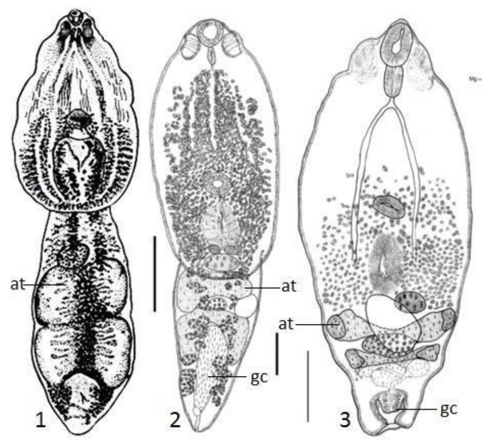

Figure 2: *Diplostomum* [8]: 1 – 374 *Diplostomum* [51], 2- *Dolichorchis* (Lunaschi and Drago, 2006) & 3 – *Tylodelphys* (Drago and Lunaschi, 2008) with their taxonomical reproductive structures (at = anterior testis; gc = genital cone)

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of Congo (DRC) [36] and *T. xenopi* from an experimental host, the African darter *Anhinga melanogaster* in South Africa [21] and *T. mashonense* from *Ardea cinerea* and *A. alba* [13,33]. Other *Tylodelphys* species have only been described at the metacercarial stages. For instance *Tylodelphylus* (*Diplostomulum*) *victorianus* was described by Vercammen-Grandjean [43] from the pericardial cavity of *Xenopus laevis victorianus* in Nyakabere River in DRC. *T. grandis* was described from *C. gariepinus* by Zhokov et al. [40] from Lake Tana in Ethiopia. Four other *Tylodelphys* species, namely, *T. claviformis* (from *Barbus*, *Labeobarbus*, *Garra*), *T. mutica* (from *Barbus*), *T. fusiliformis* (from *Oreochromis niloticus*) and *T. clariae* (from *C. gariepinus*) were also described by Zhokov [44] from Lake Tana in Ethiopia. However, there are several metacercariae of *Tylodelphys* species from numerous fishes in Africa (Table 1), which have not been described. In addition, the adults of these metacercariae and their life cycles are hitherto unknown, and their classifications only end at the genus level.

Table 1: A list of *Diplostomum* species (*sensu* Dubois, 1961) occurring in Africa with their hosts, developmental stage, site of occurrence and freshwater systems they were recovered.

Parasite	Hosts	Stage	Site	Locality/Country	Reference
Diplostomum/Dolichorchis tregenna	Milvus migrans aegypticus	Adult	Small intestine		Nazmi, 1932; Dubois, & Pearson, 1963
Diplostomum/Dolichorchis tregenna	Clarias lazera/gariepinus Channa obscurra	Metacercaria	Brain	Nile River, Sudan; Osse River, Benin Lk Tana, Ethiopia	Khalil, 1963; Okaka & Akhigbe, 1999 Zhokov et al., 2010
Diplostomum spathaceum	Clarias gariepinus	?	Intestine/stomach	Lamingo Dam, Nigeria	Goselle et al., 2008
Diplostomulium tregenna	Tilapia zilli	?	Intestine	Lamingo Dam, Nigeria	Goselle et al., 2008
Diplostomum marahoueense	Scotopelia peli	Adult	Small intestine	Cote d'Ivoire	Baer 1957
Diplostomum magnicaudum	Gallinula chloropus	Adult	Small intestine	Egypt	El-Naffar, 1979
Dipostomum ghanense	Anhinga rufa rufa	Adult	Small intestine	Ghana	Ukoli, 1968
Diplostomum ardeae	Ardea goliath	Adult	Small intestine	Egypt	El-Naffar et el., 1980
Diplostomum commutatum	Cynoglossus senegalensis	Adult	Small intestine	Cross River, Nigeria	Abraham et al., 2004
Diplostomum sp	Barbus poechii	Metacercaria	Eye	Lake Naivasha, Kenya	Otachi et al., 2015
Diplostomum sp	Brycinus lateralis	Metacercaria	Eye/brain	Okavango RS, Botswana	Grobbelaar et al., 2014
Diplostomum sp	Hepsetus odoe	Metacercaria	Eye	Okavango RS, Botswana	Grobbelaar et al., 2014
Diplostomum sp	Schilbe intermedius	Metacercaria	Brain	Okavango RS, Botswana	Grobbelaar et al., 2014
Diplostomum sp	Oreochromis andersonii	Metacercaria	Eye/brain	Okavango RS, Botswana	Grobbelaar et al., 2014
Diplostomum sp	Oreochromis macrochir	Metacercaria	Eye	Okavango RS, Botswana	Grobbelaar et al., 2014
Diplostomum sp	Sargochromis greenwoodi	Metacercaria	Eye/brain	Okavango RS, Botswana	Grobbelaar et al., 2014
Diplostomum sp	Serranochromis angusticeps	Metacercaria	Eye	Okavango RS, Botswana	Grobbelaar et al., 2014
Diplostomum sp	Coptodon rendalli	Metacercaria	Eye/brain	Okavango RS, Botswana	Grobbelaar et al., 2014
Diplostomum sp	Tilapia sparrmanii	Metacercaria	Eye/brain	Okavango RS, Botswana	Grobbelaar et al., 2014
Diplostomum sp	Labeo umbratus	Metacercaria	Eye	Okavango RS, South Africa	Grobbelaar et al., 2014
Diplostomum sp	Labeo capensis	Metacercaria	Eye	Okavango RS, South Africa	Grobbelaar et al., 2014
Diplostomum sp	Cyprinus carpio	Metacercaria	Eye	Okavango RS, South Africa	Grobbelaar et al., 2014
Diplostomum sp	Marcusenius macrolepidotus	Metacercaria	Eye/brain	Okavango RS, Botswana	Grobbelaar et al., 2014
Diplostomum sp	Pollimyrus castelnaui	Metacercaria	Eye/brain	Okavango RS, Botswana	Grobbelaar et al., 2014
Diplostomum sp	Petrocephalus catostoma	Metacercaria	Eye/brain	Okavango RS, Botswana	Grobbelaar et al., 2014
Diplostomum sp	Labeobarbus marequensis Barbus trimaculatus	Metacercaria	Eyes Eyes	Nwanedi-Luphephe dams, South Africa	Mbokane et al. 2015
Diplostomum garrae	Garra dembecha	Metacercaria	Eye	Lake Tana, Ethiopia	Zhokhov, 2014
Diplostomum longicollum	Barbus humilis	Metacercaria	Eye	Lake Tana, Ethiopia	Zhokhov, 2014
Diplostomum montanum	Barbus humilis	Metacercaria	Eye	Lake Tana, Ethiopia	Zhokhov, 2014
Diplostomum tilapiae	Oreochromis niloticus	Metacercaria	Eye	Lake Tana, Ethiopia	Zhokhov, 2014
Diplostomum sp	Tilapia sparrmanii, Pseudocrenilabrus philander	Metacercaria	Eye	Supersand Dam, South Africa	Moema et al., 2013
Diplostomum sp	Barbus intermedius Clarias gariepinus	Metacercaria Metacercaria	Eye Cranial cavity	Koka reservoir, Ethiopia	
Diplostomum sp	Oreochromis niloticus	Metacercaria	Eye	Dams & farms, Eldoret, Kenya	Migiro et al., 2012
Diplostomum spathaceum	Clarias gariepinus		Intestine/stomach	Lamingo Dam, Nigeria	Goselle et al., 2008

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Diplostomum commutatum	Pseudotolithus elongatus		Intestine/stomach	Cross River, Nigeria	Abraham et al., 2005
Diplostomum sp	Clarias gariepinus	Metacercaria	Cranial cavity/eye	NL Dams, SA	Madanire-Moyo et al., 2010
Diplostomum sp	Oreochromis niloticus	Metacercaria	Eye	Kisumu, Kenya	Ndeda et al., 2013
	Clarias gariepinus	Metacercaria	Brain	Koka Res., Ethiopia	Gulelat et al., 2013
Diplostomum/Tylodelphys mashonense	Clarias gariepinus	Metacercaria	Cranial cavity	RR, KR, LV, MD- Tanzania	Musiba & Nkwengulila, 2006; Chibwana & Nkwengulila, 2010
Diplostomum/Tylodelphys mashonense	Aredea cinerea, Albas alba	Adult	Intestine	Lake Victoria, Tanzania	Chibwana et al 2015
Diplostomum/Tylodelphys mashonense	Clarias gariepinus	Metacercaria	Cranial cavity	Lebowa, South Africa	Mashego & Saayman, 1989
Diplostomum sp	Schilbe intermedius	Metacercaria	Eye	N-L Dams, South Africa	Smit & Luus-Powell, 2011
Diplostomum sp	Oreochromis niloticus	Metacercaria	Eye	Kibos Area, Kenya	Ndeda et al., 2013
Diplostomum sp	Synodontis nigrita	Metacercaria	Eye	Nigeria	Chibwana et al 2013
Tylodelphys sp	Oreochromis niloticus	Metacercaria	Eye	Ngeki reservoir, Kenya	Otachi et al., 2011
Tylodelphys claviformis	Barbus, Labeobarbus, Garra	Metacercaria			Morozova, 2011
Tylodephys grandis	Clarias gariepinus,	Metacercaria	Cranial cavity	Lake Tana, Ethiopia	Zhokhov et al., 2010
T. mutica	Barbus	Metacercaria		Lake Tana, Ethiopia	Morozova, 2011
T. fusiliformis	Oreochromis niloticus	Metacercaria		Lake Tana, Ethiopia	Morozova, 2011
T. clariae	Clariasgariepinus	Metacercaria	Cranial cavity	Lake Tana, Ethiopia	Morozova, 2011

Techniques and Methods

In order to have a reliable research output, researchers need a good criterion to filter from diversely available techniques and methods. For instance Chappell [45], reported that methods for identification of *Diplostomum* species depend upon: (i) site of infection in a host; (ii) metacercarial morphology; (iii) infected host species; (iv) chaetotaxy of cercariae (not reliable unless supported by evidence obtained from experimental infections); (v) experimental establishment of the life cycle and recovery of the adult worm. Of the five methods above only three i.e. site of infection, infected host species and metacercarial morphology are the most common in African studies. In other words most studies have been dealing with occurrences. *Diplostomum* metacercariae have been reported in the cranial cavity [13,14,46,47], eyes [29,48] and abdominal cavities [21]. Although other *Diplostomum* species recorded by some studies occur in unusual sites in fish host or unusual host, they are neither accompanied with morphometrics nor figures. For example metacercariae and adults of *Diplostomum commutatum* were isolated from the stomachs and intestines of fishes *Pseudotolithus elongatus* and *Cynoglossus senegalensis*, respectively [49]. Generally most of studies on *Diplostomum* species in Africa are not accompanied with the known taxonomic methods i.e. morphometrics and illustrations. With this regard it is difficult for other researchers to support or refute conclusions.

According to Niewiadomska [7], a precise identification of *Diplostomum* species requires the a *priori* knowledge of all the features in the developmental cycle i.e. adult, cercaria and metacercaria. However, a correct description of cercariae and metacercariae relies on traditional and modern methods of morphological research like numerical taxonomy, as well as verification of the correctly identified adult forms obtained experimentally. So far, in Africa, identification of *Diplostomum* species across the life cycle has not been done due to incomplete information on the cercarial stage [13,20]. In addition, maintaining life cycles using *in vivo* systems is difficult, laborious and expensive and *in vitro* cultivation is almost impossible [6]. All these considerations indicate that both the identification of the larval stages and uncovering the diversity of *Diplostomum* species in Africa is challenging. Furthermore, experimental approaches cannot be applied to a large-scale screening of natural infections in intermediate hosts (fish or snails) due to the impossibility of linking each larval stage with its corresponding adult stage [25]. Although, the application of molecular techniques on *Diplostomum* has advanced significantly elsewhere [9,50-54], only a few researches in Africa have ventured into these techniques.

As polymerase chain reaction (PCR)-based methods for molecular analysis have started to advance in Africa, and a variety of molecular markers have been applied in different studies. Some of the gene regions that have been used to assess genetic diversity and variability among species and linking of life cycle stages are partial 18S rDNA sequences [23,55], 28S recombinant DNA [47], ITS rDNA (ITS1-5.8S-ITS2 [25,41.48] and the DNA barcode region of cytochrome c oxidase I (COI) [25,56]. *T. mashonense* is the most analysed diplostomid species in Africa, and almost every molecular marker mentioned above has been tested on it (see above citations). However, studies based on molecular methods on African *Diplostomum* are few in number, and the majority of them have used different markers, thus making it difficult to compare the findings taxonomically. For instance, it is not clearly understood if the *T. mashonense* studied by Chibwana et al. [25,41], is similar to that dealt by Moema et al. [47]. In other words, although DNA sequences of those workers' materials have been deposited in the public nucleotide databases (like GenBank), they cannot be aligned together for identification purposes. As a result, more and more of the so-called *Diplostomum* species are discovered

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spatially and temporally, but their sequences cannot be used to harmonise their identities. Increased synonyms in *Diplostomum* species as shown by Niewiadomska [6] could be the ultimate outcome.

Future prospects

Occurrence of *Diplostomum* species in their hosts across the life cycle, i.e. in snails, fish or birds, is one of the first steps that need to be performed for the subsequent analyses. However, a correct identification of hosts is one of the most challenging endeavour in Africa, which requires an involvement of experts in specific fields viz. ornithologist, malacologist and fish biologist. Understanding the biology of *Diplostomum* species and their hosts would help fisheries managers and extension workers to deal with the threat of the disease that might be posed by these parasites. As a consequence, it will raise the economic returns of fish farms as correct identification of species is critical in making correct decisions for disease control. Such information would also be invaluable for planning, implementation and assessment of control strategies for these parasites in fish farms. An experimental establishment of the life cycle of diplostomid species is needed in order to study the biology of the partially studied developmental stages like eggs, miracidia, intramolluscan stages, cercariae and metacercariae. In other words, studies on infectivity, pathology and migration through the fish host of the parasite would only be possible if a life cycle is maintained in the laboratory. Since *Diplostomun* species can easily be cultivated in the laboratory [6], identification and naming of species should be based on adult stages.

An alternative to completing the life cycle experimentally, which is tedious and laborious, would be DNA sequencing. Unfortunately, in Africa, DNA sequencing has rarely been employed not only in the assessment of *Diplostomum*, but also other animal groups. In cases where DNA sequencing was applied, there has been little coordination (the sequences of various studies cannot be compared because they targeted exclusive DNA regions) of the four genes (i.e. 18S, 28S, ITS and mtDNA) frequently used with great success in these trematode studies. Consequently, the many sequences available on public databases such as GenBank could not be assembled and analysed together because they do not represent homologous gene regions. To alleviate this difficulty, in future studies, sequencing of all four genes would be an ideal solution in a quest to identify more species. Alternatively, DNA barcoding efforts for trematode species across Africa would be recommented.

DNA barcoding, the use of single locus cytochrome c oxidase subunit I (COI) of mitochondrial DNA, has already been popular in revealing illegal imports of bushmeats (Eaton et al., 2009), labelling requirements violations in marketed fish (Wong and Hanner, 2008; Lowenstein et al., 2009) and accurate identification of potentially toxic tuna (Lowenstein et al., 2010). The uniqueness of the locus COI across species is purported to allow rapid and accurate identification of almost all animal species (Hebert et al., 2003). Although this technique has been widely adopted by other biological fields, the parasitological community has barely used it. In parasitology and *Diplostomum* in particular, DNA barcoding has been able to reveal diversity and specificity of metacercariae in hosts [52], disentangle cryptic species [53] and linking life cycle developmental stages [25]. As already shown by Besansky et al. (2003), DNA barcoding is potentially a great tool to improve the rate of discovery of parasites' species diversity and life cycles. The author of this review, therefore recommends the increased use of this genetic region in African diplostomids in order to fill the taxonomic gaps prevailing in *Diplostomum* species emanating from various morphological challenges. It will also enable quick identification of diplostomid species irrespective of their developmental stage once their sequences are deposited in the public nucleotide databases.

Conclusion

Since the first *Diplostomum* species in Africa was described in the intestines of Egyptian kite *Milvus migrans aegypticus* in Sudan by Nazmi (1932), many other *Diplostomum* have been found in other countries and hosts albeit most studies have been conducted in the sub-Saharan Africa. However, a majority of studies have been reporting their occurrences in fish either in natural waters or fish farms. The influence of most *Diplostomum* in the fish populations is not well understood. Although, interest on *Diplostomum* studies has increased, most aspects like their biology, epidemiology, taxonomy and immunology are well known. The traditional approach to uncover these aspects would be to complete the life cycles in the laboratory, which would lead to morphological characterisation of all life cycle stages. However, establishing and maintaining the life cycles of these *Diplostomum* species using in vivo systems is difficult, laborious and expensive. Although, molecular methods have been proven to provide an alternative solution, are uncommon in African studies due to lack of equipment, resources and expertise. However, improvement of some weaknesses for some studies like providing pictures or diagrams of the *Diplostomum* species found. For laboratories or researchers that have the capacity to do molecular analysis, the use of a molecular marker that is commonly used like barcode region CO1 and ITS could be a prospective development in future.

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