A FAUNISTIC SURVEY OF DIGENEAN LARVAE INFECTING FRESHWATER SNAILS *BIOMPHALARIA*, *RADIX* AND *BULINUS* SPECIES IN THE LAKE VICTORIA AND MINDU DAM, MOROGORO IN TANZANIA

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ABSTRACT

Biomphalaria and Bulinus spp have been reported in Tanzania as vectors of Schistosoma mansoni and Schistosoma haematobium respectively. Thus most schistosomiasis control efforts have focussed on elimination of these molluscs in freshwater systems or deworming infected persons. In addition almost there is limited information of larval trematodes infecting freshwater snails in Tanzania and whether the trematode antagonism found elsewhere could be used for control of schistosomiasis. We report and describe larval digeneans infecting Biomphalaria pfeifferi, Bulinus spp and Radix natalensis, snails collected at Mindu dam, Morogoro and Mwanza gulf of the Lake Victoria from January 2011 to January 2012. Out of a total 9194 snails, belonging to Biomphalaria pfeifferi, Bulinus tropicus and Radix natalensis, collected from Mindu dam and the Mwanza gulf, Lake Victoria, 678 (7.34%) were infected with 14 larval trematode species: 3 xiphidiocercariae, 6 furcocercariae, l gymnocephalous cercariae, 1 amphistome, 2 echinostome and 1 undescribed metacercaria. From 386 infected B. pfeifferi 67 (1.69%) were infected with S. mansoni, the causative agent of intestinal schistosomiasis. These findings show that despite the increased effort to control schistosomiasis and other neglected tropical diseases in Tanzania, the diseases are still highly prevalent in freshwater systems. However, the absence of trematode antagonism or low multiple infections mean redia cannot coexist with sporocysts, hence can be used for control of schistosomiasis.

Keywords: *Bulinus*, *Biomphalaria*, *Radix*, Digenean larvae, neglected tropical diseases

INTRODUCTION

The trematodes, along with the cestodes and monogeneans constitute the subphylum Neodermata within the phylum Platyhelminthes (Cribb et al. 2003); have a cosmopolitan distribution and are implicated in some of the most important parasitic diseases of man and animals. The classification of trematode groups is largely based on their habitat in the host (liver, lung, intestine) (Chai et al. 2005). The most common ones, in the tropics and subtropics in particular, are the blood flukes of the genus *Schistosoma* the agents of schistosomiasis (or bilharziasis) (Chitsulo et al. 2000). Other trematodes such as liver flukes *Fasciola*, *Clonorchis*, *Opisthorchis* and lung flukes *Paragonimus* species are obtained from the consumption of raw or undercooked freshwater plants, crabs, fish or shellfish of many species with infective metacercariae (Abdussalam et al. 1995; Sun et al. 2011). These parasites cause devastating impacts on human, veterinary and aquaculture health resulting in slower economic growth.

It is worth noting that freshwater snails play a key role in the transmission of these eye-catching digenetic trematodes. The snails provide the parasites with not only the nutrients and space for reproduction but also transport by which the parasites can proceed to infect the next host generation (Lockyer et al. 2004). The peculiarity of the snail-trematode guild comes from the fact that the majority of the known digenetic trematode species utilize snails as the mandatory first intermediate host (Littlewood and Bray 2001). Snails become infected after eating eggs that are associated with the droppings of the definitive host, or after the penetration by the free-swimming miracidia hatched from eggs (Cribb et al. 2003). In the snail hosts, trematodes multiply asexually to produce large quantities of morphologically distinctive larvae (sporocysts and/or rediae), which give rise to cercariae that escape the snail hosts and disperse in the external environment actively locating and penetrate the next host or encyst on vegetation. Thus examination of naturally infected freshwater snails increases the possibility of understanding (i) the sources of infection of the second and definitive hosts (Faltýnková et al. 2007, Żbikowska and Nowak 2009), (ii) the larvae trematodes that may act as regulators of snail populations in high infections (Loker et al. 1981, Brown et al. 2005), (iii) the useful trematodes for biological control of snail transmitted diseases (Davis 1998) and (iv) the bio-indication of environmental quality (Kuris and Lafferty 1994, Keas and Blankespoor 1997).

Several studies of larval trematodes have been carried out in Europe for over a hundred years (Faltýnková et al. 2007, 2008, Żbikowska and Nowak 2009). On the contrary, in Africa these studies are few in number and most of them have been restricted mainly to schistosomiasis (Kasuku et al. 1988) and fasciolisis (Ogambo-Ongoma 1971), while there is relatively little information on the abundance, morphology, taxonomy and even host relationships of other trematode species responsible for foodborne zoonotic trematodiases, and those of less medical or veterinary importance. The only all-inclusive studies known on larval digenean are those carried out by Fain (1953), Vercammen – Grandjean (1960), Loker et al. (1981), King and Van As (2000) Chingwena et al. (2002), Nkwengulila and Kigadye (2005) and Moema et al. (2008). However, these studies covered only small geographical areas thus leaving out the majority of freshwater snails unattended. In addition, Loker et al. (1981) and Nkwengulila and Kigadye (2005) who carried out their studies in Tanzania, did not identify all the cercariae recovered from the freshwater snails.

In the present study we provide data on the abundance, morphology and taxonomy of larval trematodes occurring in three freshwater snails (*Bulinus* spp, *Biomphalaria pfeifferi* and *Radix natalensis*) collected from Mwanza Gulf, Mwanza and Mindu dam, Morogoro. This is a preliminary step to defining the snails that harbour digenetic trematodes of medical, veterinary or economic importance. It was also expected that the study would identify the unidentified trematodes species reported by Loker et al. (1981) and Nkwengulila and Kigadye (2005) as well as highlighting the status of schistosomiasis and possible use of other digeneans (see Davis, 1998) to its control in Tanzania and thus provide a useful baseline information for future studies.

MATERIAL AND METHODS

Snail collection

Field work involved the collection of three most common genera of snails *Biomphalaria*, *Radix* and *Bulinus*, of which two, *Biomphalaria* and *Bulinus*, have been implicated in the transmission of schistosomiasis (Brooker 2002). The specimen of the snails, *Biomphalaria* and *Radix* spp. were collected from the Mwanza Gulf of Lake Victoria (situated at 2º 41′ S and 32º 51′ E) and *Bulinus* spp. were collected from Mindu Dam, Morogoro (37º 36′ E and 6º 50′ S) in Tanzania (Fig. 1), from January 2011 to January 2012. The snails were mostly found in places where water was stagnant or slowly moving, and were collected using a strainer or located visually and handpicked from underneath of waterweeds on the shores (Faltýnková 2005). Snails collected from each site were placed in separate labelled plastic containers with water from the site and lettuce or Chinese cabbage; and transported to the laboratory where they were placed in small aquaria containing dechlorinated water and fed with lettuce or Chinese cabbage. The snails collected from each site were then experimentally subjected to cercarial emergence by placing them separately in small vials of 12.5 ml or 50 ml under a strong artificial light and heat (60W) for a period of 6 to 48 hours. Both cercariae emergence and snails with no emergence were crushed between two glass slides and examined for intramolluscan stages. At Mindu Dam *Biomphalaria* and *Lymnaea* could not be found, thus only *Bulinus* spp. were collected. Because of some difficulty in separating *Bulinus* species (see Mukaratiwa et al. 1998, Brown 2005), bulinid species were not classified to species level.

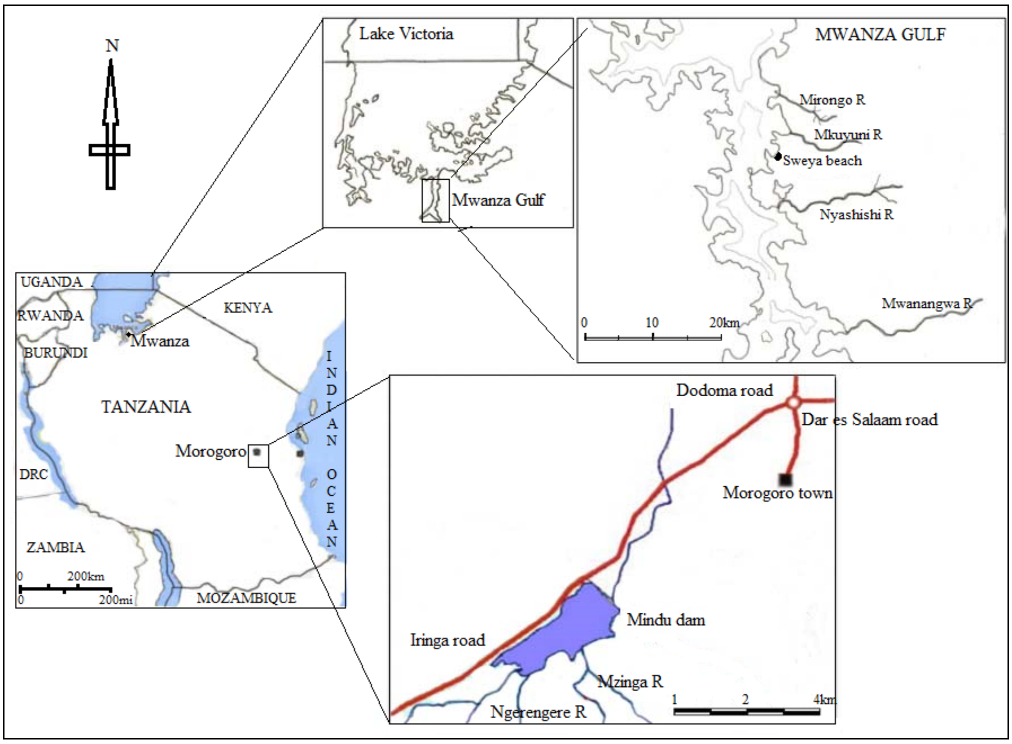

Figure 1: Map of Tanzania showing study sites (Mindu dam and the Mwanza gulf, Lake Victoria).

Morphological studies and identification of snails and digenean larvae

The snails were identified based on field guides proposed by the Danish Bilharziasis Laboratory (1987) and Brown (2005) for Africa freshwater snails. The cercarial morphology was studied from live specimen emerging from the snails using neutral red and Nile blue solution as intravital stains and observed under light microscope. Measurements were taken from specimen placed in a drop of water and killed by heat (Niewiadomska and Kiseline 1994). The digenean larvae were identified based on keys by Miller (1926), Nasir and Erasmus (1964), Vercammen and Grand-jean (1960), Dawes (1966), Combes (1980) and Frandsen and Christensen (1984) and descriptions in the literature.

RESULTS

Out of a total 9194 snails, belonging to *Biomphalaria pfeifferi*, *Bulinus* spp and *Radix natalensis*, collected from Mindu dam and the Mwanza gulf, Lake Victoria, 678 (7.34%) were infected with 14 trematode species consisting of 13 species recovered in cercarial form and one as a metacercaria. Gymnocephalous and amphistome cercarial forms had the lowest prevalence across the two localities while xiphidiocercariae had the highest prevalence as (Table 1).

In the Mwanza gulf *Bulinus* species were not abundant, and when they were found they had no infection. On the other hand, *Bulinus* species were abundant at the Mindu dam and about 7.65 % of the collected samples were infected either with furcocercariae (1.32%) or echinostomes (6.33%). However, from the recovered furcocercarial forms *Schistosoma* species were absent. On the contrary, *Radix* and *Biomphalaria* species were absent at Mindu dam, but were abundant at the Mwanza gulf. Out of 2,341 *R. natalensis* collected in Mwanza gulf 263 (11.23%) were infected, mostly with xiphidiocercaria, a *Plagiorchis* sp (10.81%). From 3,966 *B. pfeifferi* examined, 357 (9%) were infected with 8 trematode species and *Oligolecithus alianae* (Plagiorchiidae) was the most prevalent (6.78%). *O. alianae* and *Schistosoma mansoni* were the most abundant species found in *Biomphalaria* (Table 2.1). The figures of all larval trematodes we recovered the three freshwater genera are presented in figures 2 - 6.

Table 1: Occurrence of digenean larvae (cercariae and metacercariae) in snails in their natural localities

Cercaria No.	Morphological types	Locality	Snail Host	Identification	Examined	Infected	Prevalence %
I	Xiphidiocercaria	Mwanza gulf	*R. natalensis*	*Plagiorchis* sp	2341	253	10.81
II		Mwanza gulf	*B. pfeifferi*	*Oligolecithus alianae*	3966	269	6.78
III		Mwanza gulf	*B. pfeifferi*	Undescribed	3966	30	0.76
IV	Furcocercaria	Mwanza gulf	*B. pfeifferi*	*Schistosoma mansoni*	3966	67	1.69
V		Mwanza gulf	*B. pfeifferi*	*Trichobilharzia* sp	3966	1	0.03
VI		Mindu dam	*Bulinus* spp	Dipostomid sp 1	758	9	1.19
VII		Mwanza gulf	*B. pfeifferi*	Dipostomid sp 2	3966	1	0.03
VIII		Mindu dam	*Bulinus* spp	*Spirorchis* sp	758	1	0.13
IX		Mwanza gulf	*R. natalensis*	*Clinostomum* sp	2341	2	0.09
X	Gymnocephalous	Mwanza gulf	*R. natalensis*	*Fasciola gigantica*	2341	8	0.34
XI	Amphistome	Mwanza gulf	*B. pfeifferi*	*Paramphistomum* sp	3966	2	0.05
XII	Echinostome	Mwanza gulf	*B. pfeifferi*	*Paryphostomum* sp 1	3966	2	0.05
XIII		Mindu dam	*Bulinus* spp	*Paryphostomum* sp 2	758	48	6.33
XIV	Metacercaria	Mwanza gulf	*B. pfeifferi*	Undescribed	3966	2	0.05

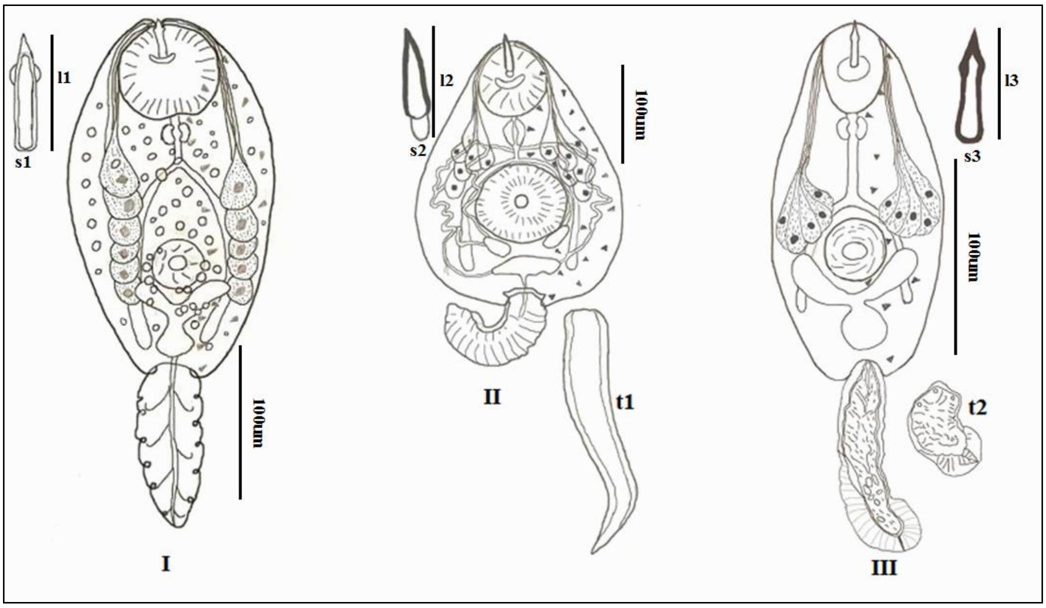

Figure 2: Xiphidiocercariae: I, *Plagiorchis* sp from *Radix natalensis*; II, *Oligolecithus elianae* and III, *Haematoloechus* sp from *Biomphalaria pfeifferi* at Mwana gulf, Lake Victoria (t1 = unfolded tail of *O. elianae* and t2 = folded tail of *Haematoloechus* sp; l1.

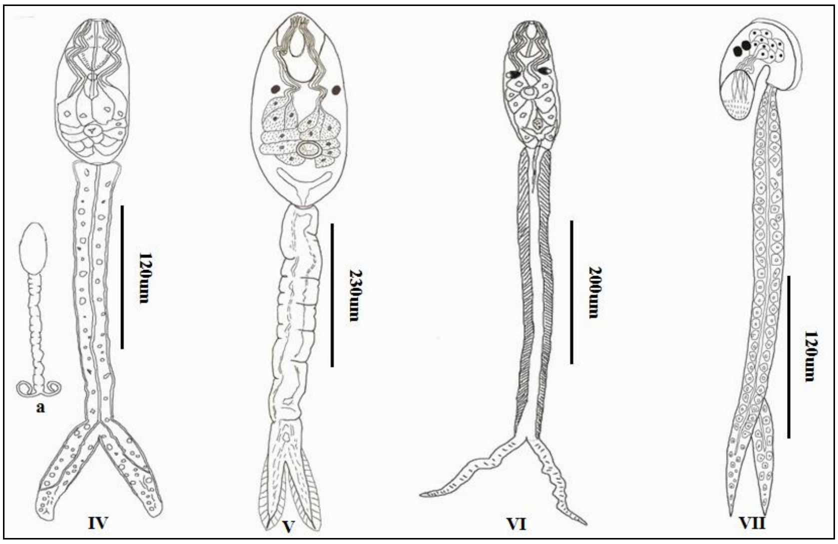

Figure 3: Furcocercariae: IV, *Schistosoma mansoni* and V, *Trichobilharzia* sp from *Biomphalaria pfeifferi* at Mwanza gulf, Lake Victoria; VI, *Spirorchis* sp from *Bulinus* spp at Mindu dam and VII, *Clinostomum* sp from *Radix natalensis* at Mwanza gulf, Lake Victoria.

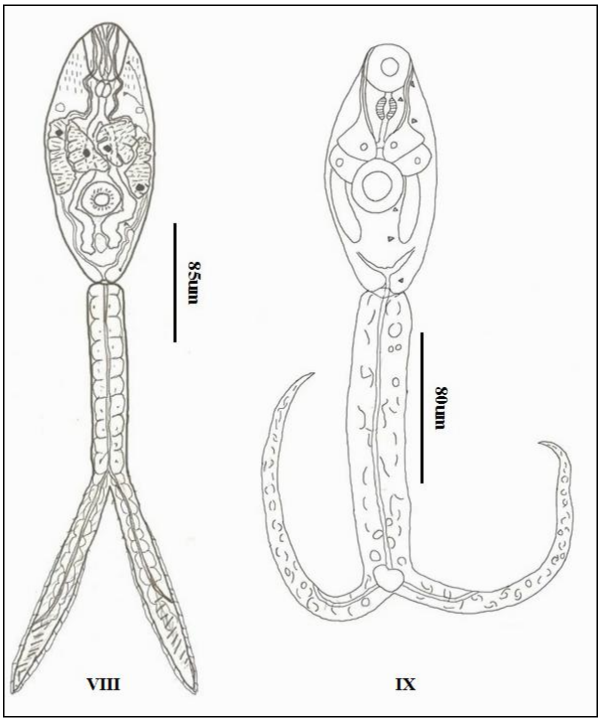

Figure 4: Diplstomid cercariae; XIII from *Bulinus* spp (Mindu dam) and IX, from *Biomphalaria pfeifferi* (Mwanza gulf).

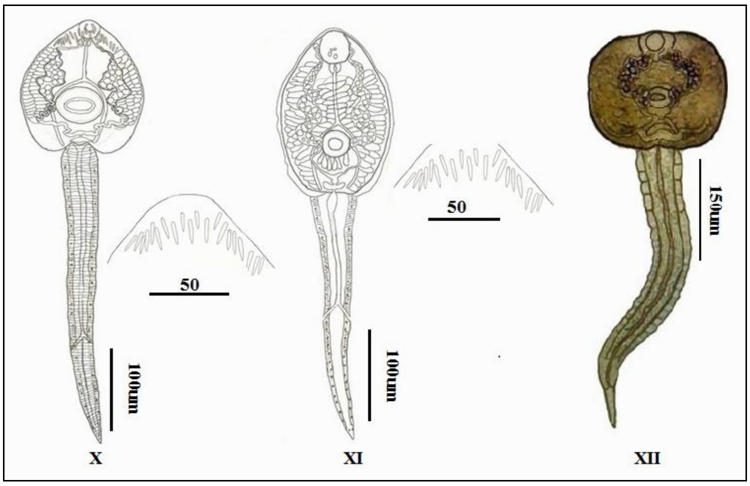

Figure 5: Echinostome cercaria; X and XI, *Paryphostomum* spp from *Bulinus* spp (Mindu dam) and *Biomphalaria pfeifferi* (Mwanza gulf), respectively; XII, Gymnocephalous cercaria (*Fasciola* sp) from *Radix natalensis* (Mwanza gulf).

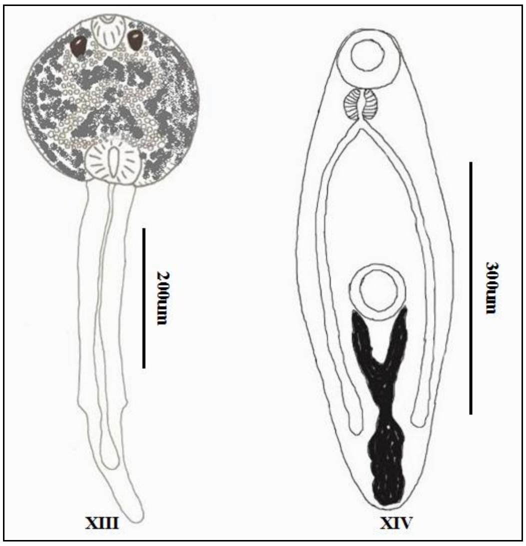

Figure 6: Amphistome cercaria X (*Paramphistomum* sp) and undescribed metacercaria from *Biomphalaria pfeifferi* (Mwanza gulf).

Multiple infections were, as usual, not common in the present study. Only 4 (0.1%) individual snails were infected with two trematode larvae, and all the four snails had similar pairs i.e. *Oligolecithus alianae* (xiphidiocercariae) and *Schistosoma mansoni* (furcocercariae). However, individual snail double infection was only recorded among *Biomphalaria pfeifferi*, despite the fact that multiple infection across the populations are not uncommon. In Mwanza gulf *B. pfeifferi* and *R. natalensis* populations were parasitized with 8 and 4 species, respectively while *Bulinus* spp population at Mindu dam were three trematode species.

DISCUSSION

Out of a total 9194 snails, belonging to *Biomphalaria pfeifferi*, *Bulinus* spp and *Radix natalensis*, collected from Mindu dam and Mwanza gulf in the present study, only 678 (7.34%) were infected with digenean larvae. This rate of infection (7.34%) is relatively lower than 14.9% reported by Loker et al. (1981) and twice as much as 3.5% documented by Nkwengulila and Kigadye (2005). The low prevalence of larval trematodes in mollusc intermediate hosts is not uncommon, it has also been reported in previous studies by Huspeni and Lafferty (2004), Dechruksa et al (2007), Sharif et al. (2010) and Bdir and Adwan (2011), who attributed several factors to low infection in snail hosts. Firstly, host mortality induced by a castrating parasite may wipe out a large proportion of the infected population, especially when starved or in poor condition, leading to low infection in the following generation (Anderson and May 1979; Jokela et al. 1999; Krist et al. 2004). Secondly, hosts with short life span do not have enough time to allow high prevalence of parasites (Esch and Fernandez 1993). And lastly, in the course of host-parasite co-evolution, hosts might have acquired immune system that would counteract infection in most of the potential hosts (Begon et al. 1990; Poulin 1997). Obviously, the results of the present study contrast reports by Loy and Haas (2001), Faltýnková (2005) and Soldánová and Kostadinova (2011) to mention a few, which showed higher prevalence. This could be explained by the fact that most of these studies were conducted in small manmade fishponds (relative to Mindu dam and Lake Victoria), which besides having a broad spectrum of migrating birds and other vertebrates, that serve as the main sources of infection for the snails, have an influence on the density or dissemination of eggs and hence maximize infection rates in snails.

The 11 larval trematode species recorded in Mwanza gulf in this study constitute only about 28.95% of the 38 species reported by Loker et al. (1981). Although this survey did not exactly use the same localities reported by Loker et al. (1981), the discrepancy might be explained by recent ecological changes in the framework of aquatic food–web alterations, catchment disturbance and natural ecosystem variability as a result of anthropogenic effects in the shape of human population increase and agricultural activities in the Lake Victoria drainage basin (Verschuren et al. 2002). The present study found most localities surveyed for snails by Loker *et al*. (1981), are either dry or no longer habitable by snails and birds. It is, therefore, logical to incriminate the decreased bird diversity in a diminished diversity of digenean species recorded by this study in Mwanza gulf.

A study by Loker et al. (1981) recovered *Schistosoma mansoni* from *B. sudanica* (22 out of 2393 infected) and *B. pfeifferi* (79 out of 1913 infected). Since specimen under the genera *Bulinus* and *Biomphalaria* are difficult to distinguish to species due to overlapping of their anatomical and morphological characters (Brown 2005), *Biomphalaria* species from the Mwanza gulf, in the present study, were treated as *B. pfeifferi* (Loker et al. 1981) and *Bulinus* species as *Bulinus* spp. Therefore the pooled prevalence in *B. pfeifferi*, according to Loker et al. (1981), was 2.35% which is more than 1.69% found in this study. However, our findings in relation to control of schistosomiasis in Tanzania are, still overwhelming due to the collective effort invested therein. We recommend more research to be conducted in order to unveil novel feasible control strategies or improve the current ones so that schistosomiasis could be eliminated.

Bulinus species were scarce in the vicinity of Lake Victoria; and when found at Sweya beach and Mirongo drainage systems, they were not infected. Both of the two places were severely impacted by human activities. At Sweya beach, the beach was cleared to make Galati beach resort for recreation purposes. These findings contrast those of Loker et al. (1981) that reported *S. haematobium* from *Bulinus nasutus* (3.32) and *B. africanus* (3.22%). When a habitat like that is destroyed either by natural catastrophes or human activities, it requires several years for both snail and trematode populations to recover (Aguirre-Macedo et al., 2011). In as much as habitat destruction becomes a major threat to bird hosts in the tropics, it also turns out to be a threat to their parasite diversity (Dobson et al. 2008).

The most dominant cercarial form recovered in all localities was xiphidiocercaria, i.e. *Plargiorchis* sp, *Oligolecuthus alianae* and *Haematoloechus* sp, which contributed 81.78% of all larval digeneans recorded. *Plargiorchis* sp and *O. alianae* in particular were found almost in all sites where their respective hosts occurred. The dominance of xiphidiocercariae in planorbids was also noted by Loker et al. (1981) and De Moraes et al. (2009). De Moraes et al. (2009) associated the commonness of xiphidiocercariae with the abundance of frogs of the genus *Leptodactylus* that act as their definitive hosts. On the other hand, Loker et al. (1981) related the high prevalence of xiphidiocercariae with intramolluscan antagonism between *C. guttera* and *F. gigantica*, which counter-effected each other consequently lowering their infection rates. However, the present study corroborates De Moraes et al. (2009) that the abundance of vertebrates (amphibians, reptiles, birds and mammals) definitive hosts for Plagiorchiids in and around Mwanza gulf waters, influenced xiphidiocercariae abundance.

Conclusively, the present study described 14 digenean species infecting *Bulinus* species from Mindu dam, Morogoro, and *Biomphalaria pfeifferi* and *Radix natalensis* from the Mwanza Gulf, Lake Victoria. The trematodes were identified using the extant keys and other literature available, which included Porter (1938), Vercammen and Grandjean (1960), Nasir and Erasmus (1964), Dawes (1966), Combes (1980), Frandsen and Christensen (1984) and Faltýnková et al. (2007, 2008). Most of these literatures like Faltýnková (2007, 2008) were not very useful to African material as they typically cover European species. The lack of specific keys for identification of Tanzanian larval trematodes coupled with inconsistent descriptions in the literature, precluded identification to species level. In addition, in the present study measurements were taken from heat killed specimens placed in a drop of water according to Brooks (1943) and Niewiadomska and Kiseline (1994), meaning that identification based on morphometrics would be tentative due to the lack of standardized fixation and staining methods across the literature (see Brooks 1943; Krailas et al. 2003). However, we have managed to provide baseline information on what can be expected when dealing with African snails.

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