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Gastropod-borne trematode communities of man-made reservoirs in Zimbabwe, with a special focus on *Fasciola* and *Schistosoma* helminth parasites

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Preface

The thesis took almost a year from preparing the field trip to completing the writing process. Many people assisted me with the data collection and the writing process of this thesis. Without them this work would not have reached the present quality and each of them deserves a personal mention of gratitude here.

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Abbreviations

Full term	Abbreviation
Biomphalaria	*Bi.*
Bulinus	*B.*
Centre for Disease Control	CDC
cytochrome oxidase 1 gene	COI
Deoxyribonucleic acid	DNA
Gastropod-Borne Trematode	GBT
Gel electrophoresis	GE
Helisoma	*H.*
Hippopotamus	*Hi.*
Human Immunodeficiency Virus	HIV
Internal transcribed spacer	ITS
International Union for the Conservation of Nature	IUCN
Mitochondrial DNA	mtDNA
Neglected Tropical Disease	NTD
Polymerase chain reaction	PCR
Royal Museum for Central Africa	RMCA
University of Zimbabwe	UZ
World Health Organization	WHO

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Summary

Gastropod-borne trematodes infect some 300 million people worldwide. An estimated 200,000 people die each year because of schistosomiasis. However, effects go beyond human mortality, and many trematodes are of significant economic and ecological importance. Identifying a parasite's complete lifecycle, including all its possible (intermediate and final) hosts is the first step in disease control and prevention of epidemics. Man-made reservoirs have heavily disturbed environments, aiding the spread of invasive species across the landscape, which in their turn potentially facilitate an *invasional cascade*. Therefore, gastropod and trematode communities of five man-made reservoirs in Zimbabwe were studied using shedding experiments and molecular tools. There, we detected 21 trematode species of seven superfamilies and 14 gastropod species. We provide the first molecular data and high-quality photographs of *Fasciola nyanzae* and report its endemicity in Zimbabwe, the spread of the gastropod *Gyraulus connollyi* north of the Limpopo River Valley, four *Schistosoma* species in Mwenje reservoir and identified *Biomphalaria pfeifferi* as a potentially new intermediate gastropod host for *S. edwardiense*. In Lake Chivero we found an invasive leech, *Helobdella europaea*, a known gastropod predator originating from South America, together with two invasive gastropods in close association with the invasive water hyacinth, *Eichhornia crassipes*, could indicate an *invasional meltdown*. Furthermore, the complete lifecycle of three hippopotamus-infecting trematode species were identified, within the *One Health* framework. This study indicates the importance of invasive gastropods in disease transmission in man-made reservoirs and will hopefully contribute to the protection of hippopotamus populations in Zimbabwe despite the crippling anthropogenic pressures.

Samenvatting

Gastropod-geassocieerde trematoden infecteren wereldwijd ongeveer 300 miljoen mensen. Elk jaar sterven naar schatting 200,000 mensen aan de gevolgen van schistosomiasis. Trematoden infecteren echter ook dieren en vele zijn van ecologisch en economisch belang. De eerste stap in het voorkomen van ziekte-uitbraken is het identificeren van alle mogelijke (finale en tussen-) gastheren. Artificiële reservoirs creëren een verstoorde omgeving die invasieve soorten helpt verspreiden en die op hun beurt kunnen leiden tot een *invasional cascade*. Daarom bestudeerden we slakken en trematoden in vijf artificiële reservoirs in Zimbabwe met behulp van *shedding* experimenten en moleculaire analyses. We vonden 21 trematode soorten van zeven superfamilies en 14 slakken soorten. DNA-sequenties en hoge kwaliteitsfoto's van *Fasciola nyanzae* werden voor het eerst gecreëerd en deze soort blijkt bovendien endemisch te zijn in Zimbabwe. Bovendien vonden we als eersten de slak *Gyraulus connollyi* ten noorden van de Limpopo River Valley, vier *Schistosoma* soorten in Mwenje reservoir en identificeerden we *Biomphalaria pfeifferi* als nieuwe tussengastheer van *Schistosoma edwardiense*. In Lake Chivero vonden we de invasieve slak-etende bloedzuiger *Helobdella europaea*, afkomstig uit Zuid-Amerika, samen met twee invasieve slakken soorten in associatie met de invasieve water hyacint, *Eichhornia crassipes*. De aanwezigheid van deze exoten duidt op een mogelijke *invasional meltdown* in dit reservoir. In het kader van de *One Health* benadering, identificeerden we de volledige levenscyclus van drie verschillende parasieten van het nijlpaard. Deze studie toont het belang van invasieve slakken in ziekte overdracht in artificiële reservoirs en zal hopelijk bijdragen tot een verdere bescherming van het nijlpaard in Zimbabwe.

Introduction

1. Role of freshwater gastropods in disease transmission

Many gastropod species serve as **obligate intermediate host** in the complex lifecycles of digenetic trematodes. Some 300 million people are affected world-wide by Gastropod-Borne Trematodes (GBTs) (WHO, 2018). Of these, 207.7 million people are affected by schistosomiasis, of which 92% live in Africa, causing an estimated 200,000 deaths each year due to direct and indirect effects of the disease. Many trematode species are of veterinary and ecological importance with the most substantial financial losses caused by schistosomes, amphistomes and fasciolids (Toledo and Fried, 2014; **Figure 1**). Many species also infect wildlife, which may also constitute a conservation threat since many wild animals (e.g. *Hippopotamus amphibious* Linnaeus, 1758) are listed as vulnerable or endangered on the IUCN Red List of Threatened Species (Stringer and Linklater, 2014; Rohr *et al.*, 2008; **Figure 1**). Adding to their ecological relevance, trematode biomass can reach high proportions in ecosystems, it can even exceed bird biomass as exemplified in three Californian estuaries (Kuris *et al.*, 2008).

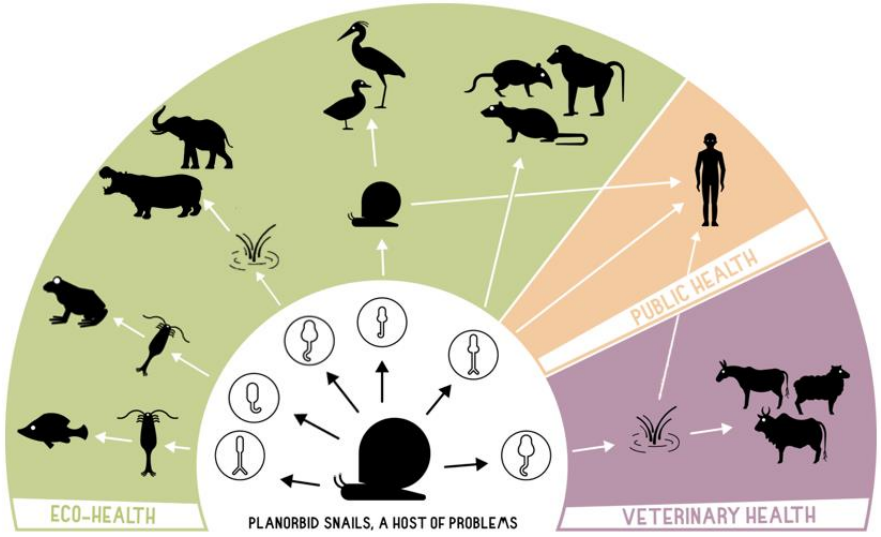


Figure 1: Planorbid gastropods, hosts of many trematodes. Planorbids are used as intermediate hosts by a wide variety of trematodes. Some species infect humans and both wild and domestic animals (e.g. *Schistosoma mattheei* Veglia & Le Roux, 1929), highlighting the importance of potential zoonoses and reservoir hosts. Image courtesy of Dr. T. Huyse (adapted).

Most GBTs occur in developing countries in the tropics and subtropics, which is why they are also referred to as 'Neglected Tropical Diseases' (NTDs). Neglected diseases are by definition understudied and many knowledge gaps still have to be filled (Toledo and Fried, 2014). Some gastropod species have been introduced to the African continent as the result of the globalized transport in agricultural and aquarium trade (Appleton and Miranda, 2015). These invasive gastropods can have serious consequences for local parasite epidemiology (Morgan *et al.*, 2001; Smith *et al.*, 2009; Grabner *et al.*, 2014; Carolus *et al.*, 2019). Man-made reservoirs are sensitive to these invasive gastropods due to their young age and ecological instability (Havel

et al., 2005). This, together with the observation that snail management is an important component of diseases control, underline the importance of malacological surveys of man-made reservoirs (Chandiwana *et al.*, 1988; Sokolow *et al.*, 2016). Our study, therefore, analyses a small subset of man-made reservoirs (Mwenje Dam, Mazowe Dam, Lake Chivero and Kariba Dam) present in Zimbabwe in an attempt to better understand invasive gastropods and local trematode epidemiology.

2. Gastropoda

1.1. Diversity

The **Class Gastropoda** (Phylum: Mollusca), often referred to as 'snails', and the Class Bivalvia have been repeatedly successful at colonizing freshwater habitats, with only the Antarctic being devoid of freshwater gastropods (Strong *et al.*, 2008). Both classes belong to the Phylum with the most described species following the Phylum Arthropoda. Continental waters host around 35 lineages of gastropods covering 4,000 described species (Strong *et al.*, 2008). Lydeard *et al.* (2004) estimated that there are still 3,000 – 10,000 undescribed freshwater species waiting to be discovered (Lydeard *et al.*, 2004). However anthropogenic threats, such as pollution and habitat degradation and loss, pose a major threat to freshwater gastropod diversity resulting in 874 species being included on the IUCN Red List of Threatened Species as vulnerable or endangered (www.iucnredlist.org).

1.2. Biology

Gastropod classification includes two main clades, the Caenogastropoda and the Pulmonata. Most Caenogastropoda are dioecious and strictly sexually reproducing with a few exceptions as for example *Melanoides* (family: Thiaridae), which have a true molluscan gill (ctenidium) and are usually habitat specialists. Pulmonates are exclusively hermaphroditic, can self-fertilize and lack a ctenidium but instead have a vascularized 'lung' that is used for gas exchange; they are mostly habitat generalists. These traits give pulmonates a high colonisation and invasion capacity (see further) (Strong *et al.*, 2008).

1.3. Classification

Morphological gastropod classifications have repeatedly been proven unsuccessful due to high intraspecies and overlapping interspecies variation in shell morphology. An example can

be found in the *Bulinus tropicus/truncatus* complex where certain individuals of the following four species, *B. tropicus* (Krauss, 1848); *B. truncatus* (Audouin, 1827); *B. depressus* (Haas, 1936) and *B. natalensis* (Küster, 1841) prove to be unclassifiable based on morphological shell characteristics (Mukaratirwa *et al.*, 1998). Mukaratirwa *et al.* (1998) were successful in using chromosome numbers, allozyme data, morphological characteristics and susceptibility to *Schistosoma haematobium* (Bilharz, 1852) to classify snail morphs from the *Bulinus tropicus/truncatus* complex found in Zimbabwe. Most classification guides use shell and soft part traits to determine snail species. However, these traits prove to be insufficient due to the correlation of shell morphology with environmental variables and the existence of species complexes based on both shell and soft part morphology (Mukaratirwa *et al.*, 1998; Pfenninger *et al.*, 2006; Correa *et al.*, 2011). Contrary to shell morphology, DNA-taxonomy provides comparable, reliable, consistent and objective species identification while additionally providing information on geographical distribution patterns when using certain markers (e.g. COI) (Pfenninger *et al.*, 2006; King and Lively, 2012).

3. Gastropod - Borne Trematodes

2.1. Lifecycles

GBTs use at least two hosts to complete their **lifecycle**, which always includes a gastropod as first intermediate host and alternations of sexual and asexual reproductive stages. The *Schistosoma* lifecycle depicted in **Figure 2** is used as an example to explain the general lifecycle of GBTs in this part. The cycle starts when fertilized eggs are produced. Embryonation occurs during the seven to ten-day migratory route to the exterior of the host (Mahmoud, 2001). This allows eggs passed in faeces, urine or other excretions of the final host, to hatch into miracidia within minutes upon contact with a suitable freshwater habitat (Mahmoud, 2001). *Schistosoma mansoni* (Sambon, 1907) eggs can survive for six to eight days on land or for as long as the faeces do not desiccate or the temperatures does not get too high (Mahmoud, 2001). Contrary to schistosome eggs that hatch immediately upon water contact, *Fasciola* eggs still need 9 - 21 days of miracidial development before hatching and can remain viable for up to a few months if hatching conditions are unfavourable (Toledo and Fried, 2014).

After a possibly long, resistant phase, miracidia hatch and **actively search** for their suitable intermediate gastropod hosts. This stage usually loses its infectivity after around 12 hours, but survival time strongly depends on abiotic conditions. Upon contact with a gastropod, penetration of the host tissue is completed in a few minutes and is facilitated with boring movements and secretions from penetration glands. The penetr-ation spot is tissue-aspecific since it can enter through any soft body part and is also not species-specific since incompatible gastropods (i.e. incompatible species or phenotypes) are also often penetrated, resulting in an aborted infection (Mitta *et al.*, 2011). Within the following 48 hours, the miracidium develops into a primary sporocyst. Within the primary sporocyst, secondary sporocysts develop within eight to ten days. Even within one species, there is a considerable variation in the number of secondary sporocysts formed as can be seen in *S. mansoni*, which creates between 40 and more than 600 secondary sporocysts (Mahmoud, 2001). These secondary sporocysts or rediae are active feeders and migrate actively or passively through the blood sinuses to their final location, the digestive glands, within the gastropod host (Mahmoud, 2001). Cercariae now develop within the secondary sporocysts. They can either burst through the sporocyst wall or through terminal birth pores and are released into the environment at certain periods in the day. This process is called 'shedding'. Many schistosome species shed during the few hours following sunrise (e.g. *S. intercalatum* (Fisher, 1943)) but many variations are known (Pages and Théron, 1990). Some species shed diurnal (e.g. *S. mattheei* (Pitchford *et al.*, 1969)) and others like the *Fasciola* sp. found in Lake Kariba and e.g. *S. rodhaini* (Brumpt, 1931) shed nocturnally (pers. observ.; Pitchford *et al.*, 1969; Carolus *et al.*, 2019). Shedding time has evolved to maximize the chance of infecting the final

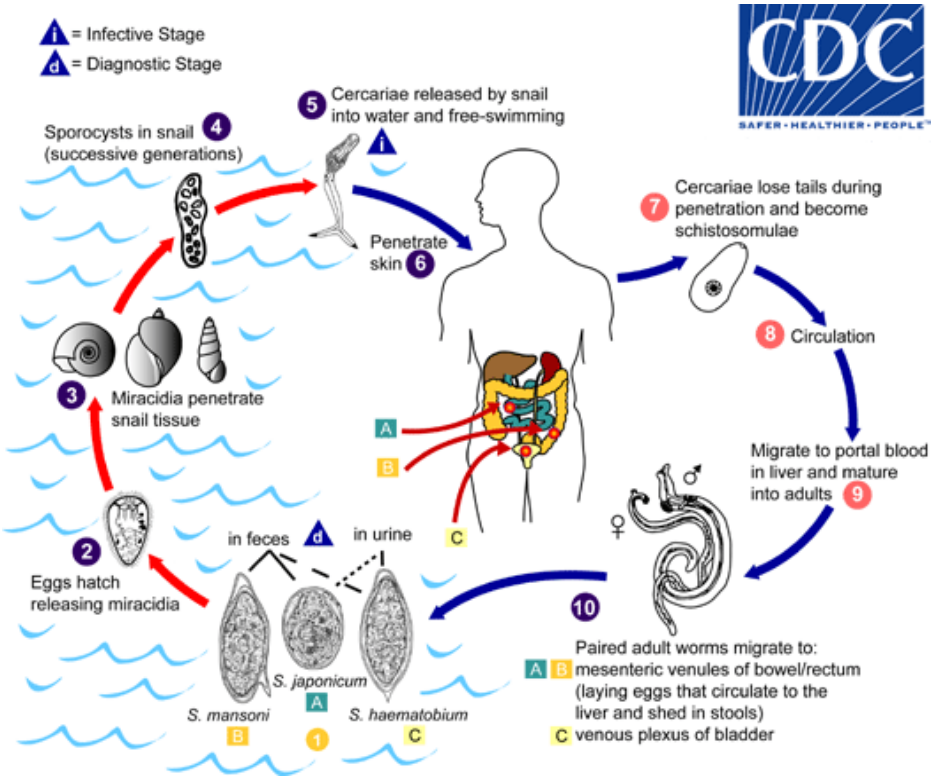


Figure 2: The *Schistosoma* lifecycle provides a generalized view on the GBTs lifecycles (source: (CDC, USA).

host and thus depends on its circadian rhythm. Cercariae, like miracidia, cannot absorb nutrients and have to find their host before their energy reserves are exhausted. This usually happens after 24 hours, but infectivity is already diminishing after six to eight hours. Schistosome cercariae, like most cercarial stages of GBTs, actively penetrate the skin of their final host. Fasciolids and amphistomes, however, form some of the exceptions where cercariae passively infect the final host through encysting and formation of metacercariae on vegetation, which is then consumed by the host. Actively infecting cercariae lose their tail upon penetration and become schistosomulae in the case of schistosomes. After invasion of the final host, migration towards the reproduction site starts. Firstly, however, these *Schistosoma* juveniles have to mature into adult flatworms. Development happens in the liver. After reaching adulthood, intersex couples form. The male now escorts the female to the reproductive site using its powerful suckers (Toledo and Fried, 2014; **Figure 3**). These are also used to anchor the pair at the final location (Toledo and Fried, 2014). This location is species-specific, an example is the venous plexus of the bladder for *S. haematobium* (Toledo and Fried, 2014). Female schistosomes need pairing with a male to complete their reproductive development (Toledo and Fried, 2014). In contrast to most trematode species that are hermaphroditic, the family Schistosomatidae is dioecious. The lifecycle is now completed when adults reproduce by means of cross-fertilization. Many other lifecycle variations exist (e.g. self-fertilization or selfing in hermaphrodite species) due to the high diversity of GBTs, but these are beyond the scope of this introduction.

Figure 3: *S. mansoni* adults laying *in copula*. The larger male holds the female within its gynecophoral canal, providing stimuli needed for reproductive and physiological development of the female (Toledo and Fried, 2014) (photo: T. Huyse).

2.2. Classification

The **class Trematoda** belongs to the phylum Platyhelminthes and constitutes of two subclasses; the extensive Digenea spanning an estimated 18,000 species and the significantly smaller Aspidogastrea containing only 100 species. The Aspidogastrea are obligate parasites of molluscs with some infecting fishes and turtles as their final hosts. Digeneans, however, are obligate parasites of, but not limited to, both molluscs and vertebrates. Classification of this subclass has been the source of many discussions since the 19th century. Many attempts to

find useful criteria have been made, for example, usage of the sucker arrangements. These proved unsatisfactory however (Gibson *et al.*, 2002). In the following decades, many more classification attempts were made based on for example secondary sporocysts or cercarial morphology but also these methods received severe criticism and conflicts. The simple classification proposed by Gibson in 1996 divides the Digenea into three orders; the Strigeida, the Echinostomida and the Plagiorchiida. This appears to be one of the most accepted classifications but only uses cercarial morphology, and thus, additional works were needed for the determination of adult stages. This was provided in *Keys to the Trematoda* by Gibson (2002) where molecular-based phylogenies were used together with morphological evidence to classify the Digenea as well as possible (Toledo and Fried, 2014).

Morphological cercarial identification is usually done based on gross morphological structures like the head, the tail and its relative dimensions, the number and position of body suckers and finally a number of specialized surface structures like a stylet if present. This usually allows for cercarial identification up to family level and sometimes even up to genus level (Frandsen and Christensen, 1984). However, similar to gastropods, cercariae are challenging to identify up to species level based on morphology alone. Hence DNA-based taxonomy is needed to provide a clear, objective and comparable result for identification. Typical markers include the nuclear ribosomal DNA regions that contain the large and small subunit of the ribosomal RNA: 18S and 28S rDNA with in between them the internal transcribed spacer regions ITS1 and ITS2. Both 18S and 28s are highly conserved regions, making them useful to determine higher taxonomic ranks. In contrast, ITS provides more resolution at the species level. In order to obtain variation at the population level, the mitochondrial cytochrome oxidase 1 gene (COI) is mainly used. Additionally, *Schistosoma* hybrids can be studied through combined analysis of both mitochondrial and nuclear genes (e.g. COI and ITS1 respectively) (See ‘5.2 Hybridization’).

4. Schistosoma

5.1. Biology and Ecology

Schistosomiasis, also known as bilharzia, is a neglected tropical disease (NTD) affecting more than 200 million people worldwide with the most substantial proportion (92%) living on the African continent (WHO, 2018). Disease symptoms are not directly caused by the adult worms but are a consequence of parasite eggs inducing lesions and inflammatory reactions (WHO, 2018). Financial losses in Africa due to disability in humans are estimated to be

nearly half a billion US$ per year (Wright, 1972). This estimation does not include any other costs associated with human or animal infections, nor does it include all schistosome species. Both *S. haematobium* and *S. mansoni* are endemic to Zimbabwe, of which the former is the most widespread human schistosome in the country (Doumenge, 1987). Adult *S. haematobium* worms live in the venous plexus around the bladder. Their eggs migrate through vein walls and ureter or bladder walls to be excreted with the urine. The process causes hematuria or bloody urine, which is a clear sign of *S. haematobium* infection. The blood loss is not very harmful to the patient. However, eggs traversing tissues cause the formation of scar tissue and around the bladder cause an increased chance of developing bladder cancer (King and Dangerfield-Cha, 2008). They also have the potential to cause urogenital complications even in children, an increased chance for HIV transmission and infertility in females (Forsyth and Macdonald, 1965; King and Dangerfield-Cha, 2008). Many other symptoms occur when eggs deviate from their normal passageway and the human immune system forms granulation tissue around them. One of them is cerebral schistosomiasis, which is capable of causing paralysis and even death.

Table 1: Other *Schistosoma* species, besides *S. haematobium* and *S. mansoni*, known to be endemic to Zimbabwe. Species are listed together with known final and intermediate hosts.

***Schistosoma* sp.**	**Gastropod host**	**True final hosts**	**Sources**
S. leiperi Le Roux, 1955	*B. globosus* and *B. africanus*	Genus *Kobus* (family: Bovidae)	(Pitchford, 1976; Christensen *et al.*, 1983)
S. mattheei	*B. globosus* and *B. africanus*	Orders Artiodactyla and Perrisodactyla	(Nelson, 1960; Pitchford, 1976)
S. rodhaini Brumpt, 1931	*Bi. pfeifferi*	Wild rodents	(Chandiwana *et al.*, 1987)

Additional *Schistosoma* species endemic to Zimbabwe or near its border are listed in **Table 1**. *S. leiperi* can sporadically infect other wildlife species and domestic animals, but these seem unable to sustain viable populations (Christensen *et al.*, 1983; Pitchford, 1976). This contrasts with the broad host range of *S. mattheei* that includes both domestic and wild animals (Nelson, 1960). *S. mattheei* is capable of infecting non-human and human primates while having true reservoir hosts in the orders Artiodactyla and Perrisodactyla, ranging, respectively, from giraffes and cattle to rhinos and zebras (Nelson, 1960). *S. mattheei* frequently infects humans, while *S. leiperi* and *S. margrebowiei* appear an insignificant

zoonotic disease (Michelson, 1989; Weyher *et al.*, 2010). Co-infections of *S. mattheei* with human schistosomes are the rule rather than the exception, with co-infections of *S. haematobium* or *S. mansoni* being reported in Zimbabwe and South Africa (Blackie, 1932; Nelson, 1960; Chandiwana *et al.*, 1987, 1988; Mahmoud, 2001). 'Pure' *S. mattheei* infections in the human host occur seldomly, are short-lived and have lower fecundity in comparison to the previously mentioned co-infections (Chandiwana *et al.*, 1987, 1988; Mahmoud, 2001). Considering that eggs traversing tissues mainly cause disease symptoms, co-infections most likely result in increased disease symptoms in humans. *S. mattheei* is not only of zoonotic importance, but it also poses a substantial economic burden on animal husbandry in Southern Africa (Vanwyk *et al.*, 1997; De Bont and Vercruysse, 1998; You *et al.*, 2018). Infection prevalence in cattle and humans can reach up to 90% and 40% respectively (Mahmoud, 2001). Worldwide 165 million cows have been estimated to have schistosomiasis with financial losses originating from decreased fertility, production loss and mortality (Vanwyk *et al.*, 1997; De Bont and Vercruysse, 1998; You *et al.*, 2018).

Schistosomes are characterised by the alternation of sexual reproduction in the final host and asexual reproduction within the intermediate gastropod host. This lifecycle allows schistosomes to experience the **benefits of both reproduction methods** (de Meeûs *et al.*, 2007). Sexual reproduction combines new alleles, which can then be tested in the local environment (de Meeûs *et al.*, 2007). The asexual or clonal reproduction phase allows the fittest genotype to reproduce clonally without dismantling its genetic set-up (de Meeûs *et al.*, 2007). Additionally, this results in much larger numbers of shedding cercariae since there are no demographic costs associated with different sexes through cloning (de Meeûs *et al.*, 2007). Cloning effectively eliminates the twofold cost of sex since all progeny can reproduce (de Meeûs *et al.*, 2007). In contrast to half the progeny when males are involved. Additionally, costs associated with meiosis and reproductive structures are avoided (de Meeûs *et al.*, 2007). *Schistosoma* species each have their own intermediate snail host species, which in turn determine their geographical distribution. The *Schistosoma* genus used to be sub-divided in groups based on egg morphology and intermediate snail host genus (Rollinson and Southgate, 1987). These groups and their intermediate snail hosts are: *S. japonicum* Katsurada, 1904, using snails from the genus *Oncomelania*; *S. indicum* Montgomery, 1906, transmitted by the snail genus *Indoplanorbis*; *S. mansoni*, using *Biomphalaria* spp. for transmission and *S. haematobium*, using the genus *Bulinus* as vectors. The two latter affect humans and a wide variety of wild and domesticated animals throughout Africa, making them of high veterinary

and medical importance. Phylogenetic studies have shown that the ancestral species infected non-human animals and that the human-infecting schistosomes most likely evolved this capacity at three separate moments in their evolution with some species; like *S. bovis* Sonsino, 1876; returning to the ancestral state (Webster *et al.*, 2006; Standley *et al.*, 2012; **Figure 4**). Known natural hosts and hybrid zones are marked in **Figure 4** because of the particular medical importance of hybridizing schistosomes (See next paragraph).

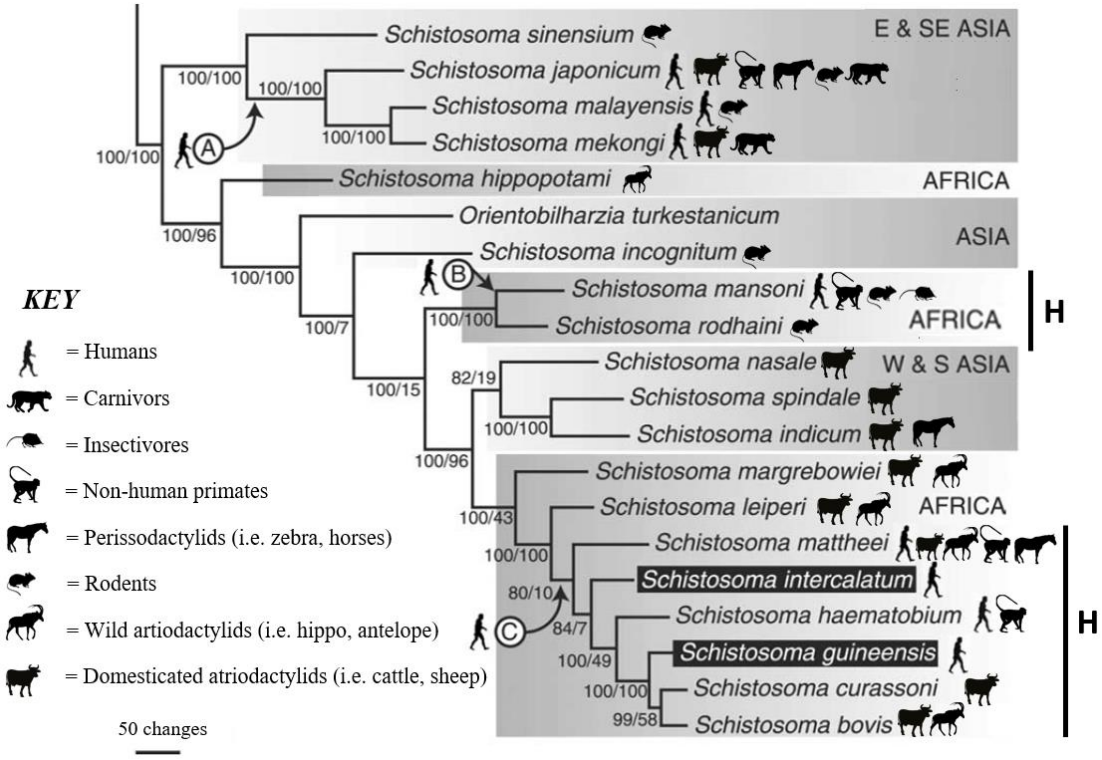


Figure 4*:* Phylogenetic tree of the *Schistosoma* genus constructed by Webster *et al.* (2006) using complete 18S, combined partial 28S and partial COI sequences. *S. kisumuensis* is not included since it was only discovered in 2009 and most likely belongs to the *S. haematobium* group (Hanelt *et al.*, 2009). *S. edwardiense* and *S. ovuncatum* are also missing in the tree for unknown reasons. The use of humans as final hosts (A, B and C) and the known hybridizing species (full line with 'H') are marked (see section 5.2). Adapted from Webster *et al.* (2006) and Standley *et al.* (2012).

5.2. Hybridization

Schistosoma **hybrids** are not isolated cases, with many studies reporting its occurrence (Théron, 1989; Webster and Southgate, 2003; Huyse *et al.*, 2009; Huyse, den Broeck, *et al.*, 2013). Hybridization is partially made possible due to the common mate finding and pairing site, of all schistosome species, in the hepatic portal system before males carry the females to their respective egg-laying sites (Armstrong, 1965). Mate pairing is a process of trial and error, resulting in same-sex and interspecific couples being temporarily formed (Armstrong,

1965). Another requirement for viable hybridization is that both mating species belong to the same *Schistosoma* clade, with viable hybrid strains being reported within the *S. haematobium* and the *S. mansoni* group respectively (Théron, 1989; Huyse *et al.*, 2009). Cross-group mating allows for the female to mature, which usually results in the female switching to parthenogenetic reproduction, rather than sexual reproduction, causing only a small proportion of eggs to be viable (Tchuente *et al.*, 1994). Notably, Huyse *et al.* (2013) detected hybrid offspring resulting from *S. mansoni* X *S. haematobium* pairing. This indicates that inter-clade hybridization can still result in viable offspring. Inter-clade hybridization could have important epidemiological implications if these hybrids are able to use both *Bulinus* and *Biomphalaria* spp. as intermediate gastropod hosts. Interspecific mating can clearly only occur when both species have the same geographical distribution, which depends on the distribution of both the intermediate gastropod host and final mammalian host. Additionally, both species should have the capacity to infect the same final host. It was suggested by Huyse *et al.* (2009) that, since *S. haematobium* is ancestral to *S. bovis* (Webster *et al.*, 2006), it is possible that *S. bovis* could have retained its ancestral capacity to infect humans allowing both species to hybridize in the Senegal River Basin. Another possible explanation given by Huyse *et al.* (2009) is that both species hybridized in rodents.

Many hybrid crosses are not viable; the ones that are viable, can pose a serious **health risk** for the local inhabitants and can potentially change local epidemiology quite drastically. An example can be found for *S. haematobium, S. intercalatum* and their hybrids in Cameroon (Southgate *et al.*, 1976). Initially the only compatible gastropod intermediate host in this region was *B. forskalii,* which transmits *S. intercalatum.* Environmental changes, however, allowed *B. truncatus* to colonize this area, providing a suitable snail host for *S. haematobium*. In the following 30 years it became clear that *S. haematobium* and recombinants became the more dominant species in the region over *S. intercalatum*. Heterosis plays an important role in the explanation for this shift. It means that, among other traits, hybrid strains can have shorter pre-patent periods, a wider snail host range and increased infectivity when compared to their pure parental species (Webster and Southgate, 2003). Male schistosomes determine the final location within the mammalian host, allowing *S. haematobium*(♂) X *S. intercalatum*(♀) hybrids to pass their eggs through urine instead of faeces, drastically changing the transmission cycle (Webster and Southgate, 2003). These improved parasitic transmission traits turn hybridization into an extremely important research subject since it can change parasite epidemiology and potentially further increase the global burden of schistosomiasis.

5. *Fasciola*

Fasciolosis is a disease caused by parasites of the genus *Fasciola*. The genus has the largest longitudinal, latitudinal and altitudinal distribution of any vector-borne parasite. The adaptive capacity of both the parasite and its lymnaeid gastropod host facilitated the wide geographical distribution (Toledo and Fried, 2014). Global estimates of human infection reach about 2.4 - 17 million, with 180 million people at risk of infection, making it an important public health problem (Hopkins, 1992; WHO). This number might increase dramatically with improved research efforts since many Asian and African countries remain understudied (Hopkins, 1992; Mas-coma, 2004). Three species of *Fasciola* are known: *Fasciola nyanzae*, Leiper, 1910; *F. hepatica* and *F. gigantica*. The latter two species infect humans on several continents, with *F. hepatica* having the largest distribution, managing to colonize and thrive on all continents excluding Antarctica. *F. hepatica* is mainly adapted to *Galba truncatula* (Muller, 1774) as intermediate gastropod host and Caprinae ruminants, such as sheep, as final hosts (Mas-Coma *et al.*, 2009). *F. gigantica* is adapted to Sub Saharan ruminants as final hosts and *Lymnaea natalensis* (Krauss, 1848) as intermediate snail host (Mas-Coma *et al.*, 2009). *L. natalensis* is known to also transmit *F. nyanzae* (Dinnik and Dinnik, 1961), which exclusively infects hippopotami (*Hi. amphibious)* (Dinnik and Dinnik, 1961; Mas-Coma *et al.*, 2009). The host range could potentially increase with further studies due to limited knowledge about this parasite and its lifecycle. *F. gigantica* has been found in hippopotami and furthermore shares many common final hosts with *F. hepatica* (Mas-Coma *et al.*, 2009). *F. gigantica* X *F. hepatica* hybrids commonly occur and are capable of infecting humans (Hoa Le *et al.*, 2008; Nguyen *et al.*, 2018). The broad host range of both *F. gigantica* and *F. hepatica* combined with the known susceptibility of *Hi. amphibious* to *F. gigantica*, suggest that hybridization with *F. nyanzae* could be possible. Resistance of *L. natalensis* to *F. hepatica* used to create a geographical barrier between *F. hepatica* and *F. nyanzae* (Mas-Coma *et al.*, 2009). However, the recent spread of the *Fasciola* susceptible invasive gastropod *Pseudosuccinea columella* (Say, 1817) across Africa might allow *F. hepatica* to colonize new regions breaking down this geographical barrier (Bargues *et al.*, 2011; Grabner *et al.*, 2014; Lounnas *et al.*, 2017; Carolus *et al.*, 2019). Ruminants can host a notable *Fasciola* sp. infection prevalence (e.g.: Sudanese cattle (91%), Ethiopian sheep (70%) and Vietnamese Buffalo (62%) (Mehmood *et al.*, 2017). Industrialized countries are not exempt from these helminth parasites. Belgian and Swiss cattle were found to be infected by *F. hepatica* at a prevalence of 37% and 18%, respectively (Rapsch *et al.*, 2006; Bennema *et al.*, 2009). The infection rates were most likely related to husbandry practices (Bennema *et al.*, 2009). Previous work in Switzerland assumed infection

rates of 10% and estimated financial losses per infected animal at € 299 (Schweizer *et al.*, 2005). The main financial burdens originate from reduced fertility and milk production (Schweizer *et al.*, 2005). Together with minor losses from growth reduction and liver condemnation result in 52 million euros of total annual losses in Switzerland (Schweizer *et al.*, 2005). As suggested by Rapsch *et al.* (2006) financial burdens are most likely higher due to the underestimated infection prevalence. Zimbabwe has approximately four times as much cattle compared to Switzerland, while having a comparable infection prevalence of 16% (Mehmood *et al.*, 2017). These observations suggest potential annual losses of around 200 million euros in the Zimbabwean cattle husbandry alone. Most *Fasciola* sp. infections in Zimbabwean cattle are caused by *F. gigantica* and a minority due to *F. hepatica* (Mucheka *et al.*, 2015). This is in contrast to South Africa, where 64% of infected cattle suffer *F. hepatica* infections (Mucheka *et al.*, 2015). World-wide financial losses most likely exceed 300 US$ billion annually as a consequence of fasciolosis (Mehmood *et al.*, 2017).

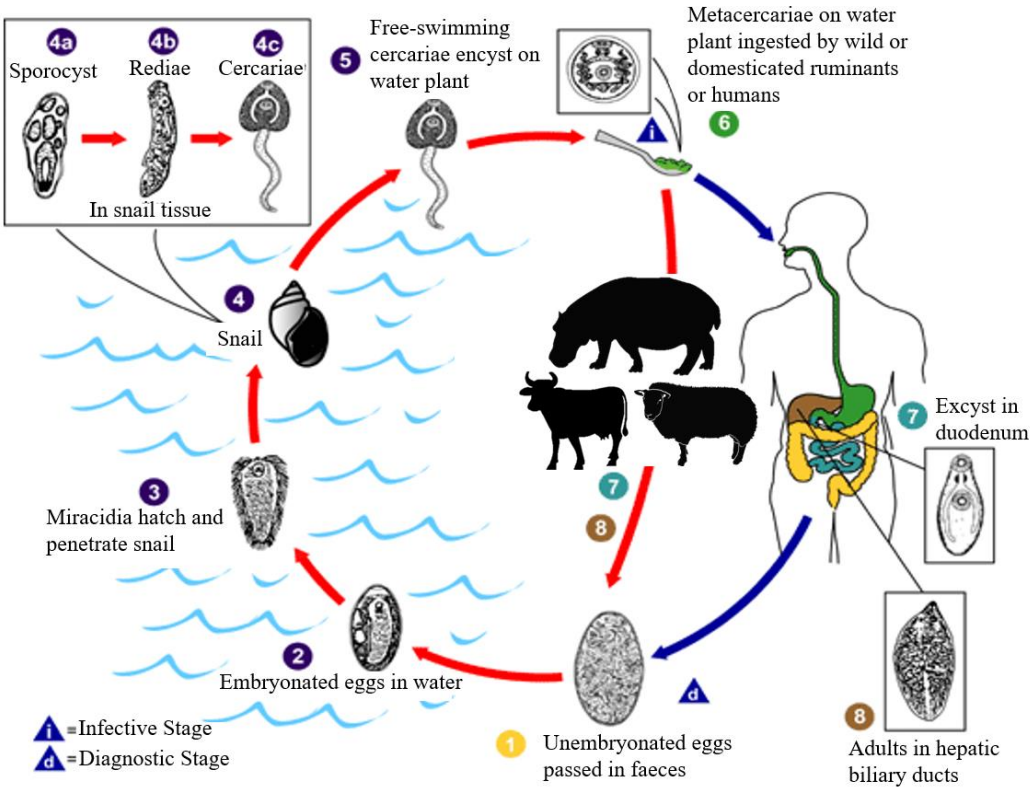


Figure 5*:* The *Fasciola* lifecycle. Notice the differences in steps one and six with the *Schistosoma* lifecycle depicted in **Figure 2**. Whereby excreted *Fasciola* eggs are unembryonated and metacercariae passively infect the final hosts (adapted from: CDC, USA).

***Fasciola* sp. infections** occur passively through ingestion of infected water vegetation, water and even terrestrial plants that have been washed with contaminated water (**Figure 5**). *F. nyanzae* is a neglected species within the *Fasciola* genus, most presumably because it is not

known to infect humans or domesticated animals. The only known final host and intermediate snail host are respectively *Hi. amphibious* and *L. natalensis* (Leiper, 1910; Dinnik and Dinnik, 1961). Thus far, only one study conducted limited experiments on the host range of this species showing that low numbers of rabbits, goats and calves failed to be experimentally infected by *F. nyanzae* (Dinnik and Dinnik, 1961). Two published papers describe the morphology of *F. nyanzae* with the most recent one being published in 1961 that allow for morphological analysis of adult *F. nyanzae.* However, no genetic data is available for this species to date. This prevents molecular identification of *F. nyanzae* from environmental samples. In this thesis we will attempt to provide genetic data on this species and screen other potential mammalian final and gastropod intermediate hosts. The data will allow future research to genetically identify *F. nyanzae* from infected samples. Describing genetic variability in fasciolids can support disease surveillance and control (Mucheka *et al.*, 2015; Khalifa *et al.*, 2016). This information could prove vital in future population management of *Hi. amphibious*, which is listed on the IUCN red list as vulnerable. Parasites have positive and negative effects on wildlife conservation efforts and are increasingly seen as biodiversity conservation targets themselves (Gómez and Nichols, 2013; Stringer and Linklater, 2014). Parasites act as evolutionary drivers behind community structures and host genetic and phenotypic diversity making them sources of biodiversity (Gómez and Nichols, 2013; Stringer and Linklater, 2014). Anthropogenic effects (e.g.: host inbreeding, climate change, habitat degradation and loss, invasive species and contact with domestic animals) could potentially increase the consequences of parasite infections due to temporary or permanent reductions in already threatened host populations (Smith *et al.*, 2009; Heard *et al.*, 2013). Large healthy wildlife populations with enough genetic diversity can co-evolve with their parasites. This not only conserves and drives both host and parasite biodiversity but also prevents the creation of naïve host populations (Smith *et al.*, 2009; Stringer and Linklater, 2014).

6. Amphistomes

Amphistomiasis or ‘stomach fluke disease’ is a neglected tropical disease infecting domesticated and wild animals world-wide and is caused by helminth parasites of the superfamily Paramphistomoidea (Fischoeder, 1901) commonly referred to as ‘amphistomes’ (Hanna *et al.*, 1988; Toledo and Fried, 2014). Nine out of twelve amphistome families occur in Africa (Laidemitt *et al.*, 2016). Three families (i.e. Paramphistomidae, Gastrodiscidae and

Gastrothylacidae) are especially speciose in Africa and contribute to over 40% of known amphistomes (Laidemitt *et al.*, 2016). Amphistomes are endoparasites in the digestive tracts of a wide variety of final vertebrate hosts, ranging from fishes and amphibians to both livestock and wild ruminant species (Toledo and Fried, 2014). Economic losses due to this disease have not been estimated to date but must be substantial since it is the most pathogenic parasitic diseases in domesticated livestock (Toledo and Fried, 2014). Amphistomiasis causes mortality, anorexia, extreme weakness, exhaustion, loss of appetite and diarrhoea. Mortality mostly occurs in young animals and is caused when high numbers of immature flukes migrate from the intestines to the rumen (Toledo and Fried, 2014). Amphistome migration is a slow and painful process that causes severe intestinal damage to the infected host (Toledo and Fried, 2014). The high mortality and morbidity in ruminant livestock make the stomach fluke disease of high veterinary importance (Hanna *et al.*, 1988; Anuracpreeda *et al.*, 2008; Toledo and Fried, 2014). Amphistomes are transmitted through Planorbidae and Lymnaeidae gastropod intermediate hosts, which transmit *Schistosoma* and *Fasciola* spp. The shared gastropod host has important implications for local disease epidemiology. Some amphistomes form rediae within the gastropod host, enabling consumption of sporocysts from other trematodes like schistosomes (Laidemitt *et al.*, 2016). The highly specific treatment only targeting fasciolosis in livestock in France might have caused the severe increase in amphistomiasis prevalence from 1990 to 1999 (5.2% to 44.7%) (Christian Mage *et al.*, 2002). The increase originates from two factors. Firstly, the reduction in *Fasciola* sp. causes reduced competition between *Fasciola* and amphistome parasites within the gastropod intermediate host (Christian Mage *et al.*, 2002). And secondly, there is no effective treatment against the stomach fluke disease (Christian Mage *et al.*, 2002). Further epidemiological complications occur due to the high percentage (77%) of amphistome species infecting both domestic and wild ruminants in Eastern and Southern Africa (Pfukenyi and Mukaratirwa, 2018). Additionally, intermediate gastropod hosts are only known for nine out of twenty-six described amphistome species in Eastern and Southern Africa (Pfukenyi and Mukaratirwa, 2018). The ratio of unknown gastropod intermediate hosts is undoubtedly higher because the review by Pfukenyi and Mukaratirwa (2018) does not include species infecting *Hi. amphibious* or *Loxodonta africana* (Blumenbach, 1797), which are barely studied. Infection rates of *Hi. amphibious* and other final and reservoir hosts, if any, could be estimated through non-invasive molecular analysis of stool samples. This method combined with studying infection prevalence in gastropod intermediate hosts and the potential effect of invasive gastropod species on disease transmission, allow for a more profound comprehension of the

parasite's lifecycle. Additionally, it allows for a preliminary understanding of potential dilution effects caused by unsuitable intermediate and final hosts (Civitello *et al.*, 2015).

7. Invasive Gastropods in Southern Africa

Increased **globalisation** has caused the spread of many invasive species. Therefore, it is important to understand their distribution pattern and population structure and the associated effects on local epidemiology. To date 14 species of non-indigenous or exotic gastropods have been documented in South Africa, while no exotic bivalve species are found (Appleton, 2003; Appleton and Miranda, 2015). Some of these gastropods became invasive: (*P. columella*, *Physa acuta* (Draparnaud, 1805) and *Tarebia granifera* (Lamarck, 1816)) or still have the potential to do so (*Gyraulus chinensis* (Dunker, 1848)). Some other species went locally extinct (*Biomphalaria angulosa* (Mandahl-Barth, 1957)) or managed to remain stable without becoming invasive (*Helisoma duryi* (Wetherby, 1879)) (Appleton and Miranda, 2015; **Table 2**).

Table 2: Adapted list from Appleton and Miranda (2015) containing **invasive or otherwise interesting exotic gastropods in South Africa.**

Species	Year of first collection	Earliest published source	Region of origin	Status in South Africa
Pseudosuccinea columella	1942	Barnard (1948)	North America	Invasive
Physa acuta	1956	Van Bruggen (1966)	North and South America	Invasive
Helisoma duryi	1966	Brown (1967)	North America	Established, not invasive
Tarebia granifera	1999	Appleton & Nadasan (2002)	Southeast Asia	Invasive
Melanoides tuberculata	2004	Genner *et al.* (2004)	Southeast Asia	Indigenous but potential replacement by an invasive morph from Asia (Genner *et al.*, 2004) or a hybrid
Radix rubiginosa	2004	Appleton and Miranda (2015)	Southeast Asia	Introduced, status unknown
Gyraulus chinensis	2006	Appleton and Miranda (2015)	Southeast Asia	Introduced, potentially invasive

Almost all exotic gastropods found in Africa have been introduced through the aquarium trade (i.e. juveniles or eggs attached to aquarium plants; Ebbs *et al.*, 2018) and show three general introduction periods (Appleton and Miranda, 2015). Between the 1940s and 1960s North American gastropods were introduced, from the 1960s to the 1980s mainly South American species were introduced and starting from the 1980s Asian species followed suit (Appleton and Miranda, 2015). The invasion capacity of these gastropods is made possible through a

wide diversity of interacting factors. The absence of native amphibious gastropods in Africa caused their associated niches to be empty and ready for invasions (Appleton, 2003). This partially explains the successful and rapid invasion of *P. columella* on the African continent (Appleton, 2003). Additionally, this gastropod is characterized by an augmented defence strategy against predatory beetle larvae compared to native snails. Rapid excretion of large quantities of slime immobilize the predator. This amount is significantly higher than the amount of mucus produced by native snails (Appleton *et al.*, 2004). *P. columella* is found strongly associated with *Eichhornia crassipes* (Solms, 1883), the invasive water hyacinth (Grabner *et al.*, 2014). This association together with its amphibious nature allow this species to quickly escape the water when threatened by either predators or molluscicides, making control of the snail almost impossible (Grabner *et al.*, 2014). The non-invasive natural establishment of *H. duryi* has been attributed to its increased need for cross-fertilisation compared to other pulmonates (Appleton, 2003). This prevents colonization of a new area by a single individual (Appleton, 2003). Contrary to *H. duryi*, most invading gastropods reproduce parthenogenetically or use cross - and self - fertilisation. The global invasion of *P. acuta* knows multiple factors that all contribute to this success. Firstly, *P. acuta* is a relatively fast moving snail that is not associated with vegetation like most gastropods, allowing it to quickly colonize disturbed habitats before plants even had the chance to grow again (Appleton, 2003). This rapid (re-) colonization capacity is made possible due to high and plastic reproduction rates, allowing for cohort production up to once every one to three months (Appleton, 2003; Ebbs *et al.*, 2018). Secondly, it has a unique musculature allowing shell flicking when a predator, as for example the leech *Helobdella europaea* (Kutschera, 1987), attempts to attack the gastropod (Appleton *et al.*, 2004). This effectively reduces the predation efficiency (Appleton *et al.*, 2004). Their defence strategy is supplemented by active avoidance of predators, which involves rapid movement away from the predator or out of the water when attacked by more rapid hunters like crayfish (Appleton *et al.*, 2004). This behaviour is not observed in native snails.

The **introduction method** of invasive gastropods usually involves eggs or young snails colonizing new areas through the aquarium trade or with biological vectors like birds (Ebbs *et al.*, 2018). Since eggs cannot be infected by trematode parasites most invasive species lose their native parasite fauna when colonizing a new geographical area (i.e. the *enemy release* hypothesis). Invasions by *P. acuta* have been found in line with this hypothesis (Ebbs *et al.*, 2018). Invasive gastropods could affect local parasite epidemiology in three distinct ways (Mitchell and Leung, 2016). Firstly, the invasive gastropods could be a compatible host for

native parasites and cause a *spillback* effect whereby they increase the number of local parasites. Secondly, the invader could be an incompatible host for some or all local parasites effectively creating a *dilution* effect due to the aspecific infection of the gastropod intermediate host by trematode miracidia (in that case they act as a "decoy" host). Finally, the invader could introduce exotic parasites into the newly colonized area (co-introduction) and cause a *spillover* effect whereby exotic parasites infect native species.

Invasive species usually have low or no **genetic variation** due to genetic bottlenecks and founder effects but still manage to adapt and thrive in new environments, leading to the 'the invasion paradox of genetic variability' (Lounnas *et al.*, 2017). The global flash invasion of a single multilocus genotype of *P. columella* is a clear example of this paradox. *Genetic purging* most likely allowed this invasion to occur since it decreases inbreeding depression (Lounnas *et al.*, 2017). This single multilocus genotype flash invasion has important medical and veterinary implications since genetically homogenous populations are more susceptible to parasites (King and Lively, 2012; Lounnas *et al.*, 2017). Additionally, this strain has been found susceptible to *Fasciola* spp. (Bargues *et al.*, 2011; Carolus *et al.*, 2019; Grabner *et al.*, 2014). Carolus *et al.* (2019) found an exceptionally high *Fasciola* sp. infection rate (65%) in *P. columella* populations in Lake Kariba, Zimbabwe. This might be a case of parasite *spillback* whereby native *Hi. amphibious* and potentially other native species suffer increased parasite burdens. The increased burden on native hosts cannot be proven at this point but due to the high infection rate in the invasive gastropod, increased transmission to native species is likely (Carolus *et al.*, 2019). Additionally, Grabner *et al.* (2014) suggest that the increased incidence of fasciolosis-cases in Egypt is due to parasite *spillback* through *P. columella.* parasite *spillback* events stress the importance of studying bioinvasions and their effect on local parasite epidemiology. The vulnerability of man-made reservoirs to invasions together with the increased human-water interactions, make artificial reservoirs interesting and important ecosystems for trematode epidemiological studies (Havel *et al.*, 2005).

8. The effect of man-made lakes on trematode epidemiology

Zimbabwe hosts about 10,000 of the estimated 12,000 man-made reservoirs in Southern Africa (excluding South Africa) while only covering 6.8% of the region (Doumenge, 1987; Sugunan, 1997). In Zimbabwe most of these man-made reservoirs were constructed during the 20th century. Their purposes range from irrigation, drinking water and fishing to the generation of hydroelectricity (Morley, 2007). Mazowe Dam, Chivero Dam, Kariba Dam and

Mwenje Dam were respectively constructed in 1920, 1952, 1959 and 1970. Kariba Dam (128 m tall) is world-wide the largest dam based on water capacity alone. Dams dramatically affect a wide variety of environmental and cultural factors (e.g. stop migration of river fish, cause flooding of vast land surfaces, force resettlement of native inhabitants and increase vector-borne disease transmission) (Lerer and Scudder, 1999; McAllister *et al.*, 2001). Dams blocking water flow inherently slow down the water velocity, shifting silt deposits from river deltas and flood plains to up-river areas (Townsend *et al.*, 2008). The sedimentation shift can have grave consequences for both human and wildlife populations (Townsend *et al.*, 2008). For example, due to the lack of sedimentation in the Nile delta, Egypt could potentially lose 19% of its habitable land, forcing almost a fifth of their population to migrate to other regions within the next 60 years (Townsend *et al.*, 2008). Dams create extensive standing waters where rivers used to flow, resulting in a shift from riverine to lake biota. Freshwater gastropods are a prime example. They are able to colonize these newly formed environments and increase in population size due to increased habitat size, refuge abundance and food availability (Havel *et al.*, 2005; Sokolow *et al.*, 2017). This results in important implications for both invasive species and gastropod-borne disease transmission (Sow *et al.*, 2002; Havel *et al.*, 2005). Firstly, invasive species can use these standing, well connected and heavily disturbed waters as stepping-stones to spread across the landscape (Havel *et al.*, 2005). Species-rich ecosystems are assumed to be more resistant to invasions under the 'biotic resistance' model compared to species-poor ones because of the more complete exploitation of resources and the presence of more potential predators and competitors of the former (Elton, 1958). In contrast to this hypothesis, the successful invasion rate increased in certain aquatic ecosystems with an increase in species richness (Ricciardi, 2001). This pattern could be explained by the 'invasional meltdown' model (Simberloff and Von Holle, 1999). Hereby established invasive species facilitate invasions by new exotic species through habitat alterations and/or disruption of local community interactions (Simberloff and Von Holle, 1999). Examples supporting the 'invasional meltdown' model have been found by Ricciardi (2001) and Carolus *et al.* (2019) in the great lakes (North-America) and lake Kariba (Zimbabwe) respectively. Carolus *et al.* (2019) found that increased densities of the invasive aquatic plant *E. crassipes* correlated with increased abundances of the invasive gastropod *P. columella*. The increase in intermediate gastropod hosts and potential gastropod community alterations can have drastic effects on parasite epidemiology. Construction of a dam on the Senegal River in 1985 provides a striking case with respect to these consequences. Prior to dam completion, local people experienced low *S. haematobium* infection rates. Contrary to

expectations, *S. mansoni* managed to colonize the area after dam construction and in the consecutive years caused serious epidemics of intestinal schistosomiasis (Morgan *et al.*, 2001). To this day the area remains one of the most intense intestinal schistosomiasis foci in the world (Morgan *et al.*, 2001). Dam completion caused marked physiological changes in the river water (e.g. reduced salinity) (Morgan *et al.*, 2001). This caused a significant increase in both *Bulinus* and *Biomphalaria* gastropods, subsequently enabling the invasion of *S. mansoni* (Morgan *et al.*, 2001). The effects of dams on schistosome transmission are not restricted to this one area in Senegal. Estimates state that dams blocking gastropod - predating river prawns (i.e. natural control agents of gastropod populations) cause an increased risk of schistosomiasis for nearly 400 million people world-wide (Sokolow *et al.*, 2017). Expanding gastropod populations will increase not only schistosomiasis but also other gastropod-borne diseases of socio-economic importance (Lerer and Scudder, 1999).

Aims

The limited literature dealing with the impact of man-made reservoirs and invasive gastropods on trematode epidemiology underline the importance of studying artificial ecosystems. Therefore, we address the following questions: 1) Do the man-made reservoirs in Zimbabwe (Mwenje dam, Mazowe dam and Lake Chivero) contain invasive gastropod species? 2) If so, do they play a role in GBT transmission? 3) Which final hosts are affected by these GBTs? To answer these questions, trematode and gastropod communities in and around the Mwenje Dam, Mazowe Dam, Lake Chivero and Kariba Dam, all found in Zimbabwe, were studied by means of morphological and molecular studies. Additionally, stool samples from wildlife and domestic ruminants were collected and studied using PCR analysis.

Materials and methods

1. Ethical statement and risk assessment

Gastropod collection was approved by the Research Ethics Committee of the University of Zimbabwe (UZ) (Harare, Zimbabwe). Collection of the adult flukes from the hippopotami were in context of the research collaboration between UZ and the Zimbabwe Parks and Wildlife Management Authority in Lake Kariba. Collection and fixation of gastropods, adult parasites and animal stool samples did not require any further certificates. Collection of the *Hi. amphibious* parasites occurred when the Zimbabwe Parks and Wildlife Authority culled a

subadult male for population control in Lake Kariba. Detailed risk assessments are given in Annex I.

2. Sampling

2.1. Sampling locations

Gastropod samples were collected (see 2.2 sampling methodology) at 12 sites spanning five man-made reservoirs and two rivers. Sampling occurred between 1 September 2018 and 16 October 2018. Sites were located in Mazowe Dam (sites 1 and 3), Henderson's reservoir (site 2), Mwenje Dam (sites 4, 5, 7, 8 and 8b), Kariba Dam (sites 3 and 16 in Kariba), Lake Chivero (site 10) and two river sites both up- and downstream of Mwenje reservoir (sites 9 and 6 resp.). All Harare region sites are shown in **Figure 6,** and all Kariban sites are shown in **Annex Figure 1**. **Table 3** provides a summary of the sampled locations, and additional information (including GPS-coordinates) is provided in **Annex Table 1**. Sites at Lake Kariba were chosen based on parasitological data collected by Hans Carolus in 2017. A selection of six sites was made based on parasite abundance found in *P. columella* snails. Only two of these locations (site 3 and 16) still harboured snails at the beginning of October and were included in this study.

Table 3: Summary of site information, including reservoir name, sampling date, observed activities and origin.

Site	Reservoir	Sampling date	Observed activities	Origin
1	Mazowe	10-9-2018	Fishing, boating, water irrigation, cattle	Man-made
2	Henderson's	12-9-2018	Fishing, water reserve fish farm	Man-made
3	Mazowe	17-9-2018	Fishing, wastewater fish farm, cattle	Man-made
4	Mwenje	20-9-2018	Swimming, humans drinking, cattle	Man-made
5	Mwenje	20-9-2018	Cattle	Man-made
6	Mwenje river downstream	20-9-2018	Washing location	Natural
7	Mwenje	27-9-2018	Cattle, fishing	Man-made
8	Mwenje	27-9-2018	Cattle, fishing	Man-made
8b	Mwenje (small puddle)	27-9-2018	Cattle	Man-made
9	Mwenje river upstream	27-9-2018	River sand mining, cattle	Natural
10	Lake Chivero	6-10-2018	Boating activity, fishing	Man-made
3	Kariba	1-10-2018	Fishing, boating, wildlife	Man-made
16	Kariba	1-10-2018	Wildlife	Man-made

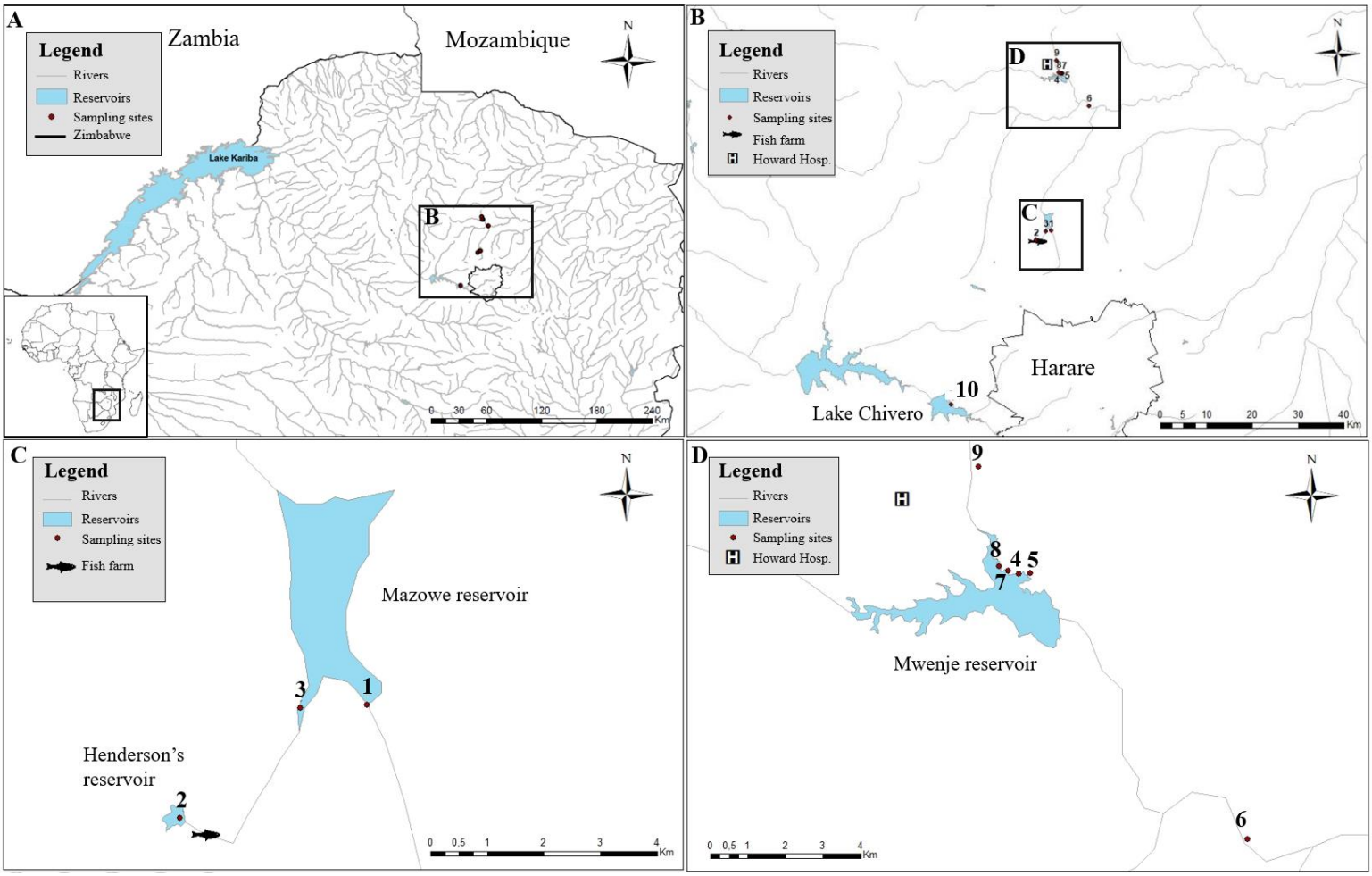


Figure 6: **Map of the sampling sites in Harare region (Zimbabwe).** Created by Birgit Vandenberghen using Arc map 10.6 ® software.

2.2. Sampling methodology

2.2.1. Gastropods

Gastropods were collected through investigation of (floating) water vegetation in trays and scooping of plants and sediment using strong nets. Sampling methods differed slightly between Lake Kariba and the other reservoirs due to the presence of dangerous wildlife and different water vegetation in Lake Kariba. Both Kariban sites were studied by two researchers collecting vegetation for 30 minutes and subsequent collection of gastropods until the whole batch of vegetation was processed. Other sites were studied for 30 minutes, including the analysis of scoop content and identification of vegetation, by two researchers. Site '8b' was exempt from this methodology because one researcher managed to collect all gastropods present in the puddle in 15 min. All gastropods collected were transported to the lab in jars containing water from the source location in coolers to prevent premature mortality. Gastropods were fixated following shedding experiments (see next paragraph) and

morphotype identification. Two identification keys were used to determine the species using only external morphological traits (Mandahl-Barth, 1962; Frandsen, 1980). Gastropod fixation was achieved through the following steps. Each gastropod was initially treated with a hot water bath (~70 °C) and subsequently submerged in 70% ethanol for a few minutes after which the final fixation in 90% ethanol followed. The hot water bath is used to relax gastropod tissue and facilitate DNA extractions. The first ethanol washing step prevents gastropod haemolymph from contaminating and degrading the final fixation ethanol. Gastropods shedding cercariae were given a code and fixated in separate tubes together with a subsample of the released cercariae. Non-infected gastropods were pooled per morphotype per site in 15 or 50 ml tubes depending on the gastropod count. Ethanol was replaced within two to three days following fixation to assure adequate fixation. Fixated gastropods were stored at 4 °C during the rest of the sampling period and sample processing in Belgium. Pictures were taken of two individuals per morphotype per site and all infected individuals prior to DNA extraction using the high-quality photography stacking apparatus at the RMCA and according to the methodology described by Brecko *et al.* (2014). Pictures taken at different focal depths were subsequently merged into one high quality stacked photograph with appropriate scaling using the Zerene Stacker® program.

2.2.2. Cercariae

Infection status of gastropods was tested using shedding experiments in Zimbabwe and PCR protocols at the RMCA (see paragraph 3.2). Experiments were conducted for all sites except for site 10 due to time restrictions. Gastropods were sorted per morphotype per site upon arrival in the field lab and individually transferred to separate wells of culture plates containing aged water from their site of origin during the whole experiment. A pooling method was used when the number of gastropods exceeded the number of separate wells. This involved selecting larger individuals that would be stored separately, while younger individuals were stored in larger pools in glasses or bottles. Such size-based selection was made because older gastropods have a higher cumulative chance of having been exposed to trematodes, and these parasites cause gastropod gigantism (Giannelli *et al.*, 2016). Gastropods were stored separately overnight to allow detection of nocturnal shedding cercariae. All gastropods underwent intense light treatment from 8 am to 12 pm to trigger parasite emergence through mimicking sunlight. The use of direct sunlight was avoided since it can cause excessive heating of the well, resulting in gastropod mortality. Few cercariae were

isolated and immobilized using a drop of ethanol prior to the microscopical determination to family or genus level using the key made by Frandsen and Christensen (1984). Multiple cercariae were compared to detect possible double infections. Of each shedding gastropod, four cercariae were stored on a Whatman FTA® card. A subsample of the remaining cercariae was stored together with the gastropod, but the majority was stored in a separate RMCA tube with a unique barcode containing 80% ethanol. Cercarial morphotypes from each gastropod were digitalized using a microscopic stacking photography set-up. The Zerene Stacker® program was again used to provide high-quality final pictures with appropriate scales.

2.2.3. Stool

Lake Kariba has a high diversity in wildlife species. Attempts were made to collect stool samples from a wide variety of wild animals in the region, which would allow preliminary data on the final host range of local trematodes. The other sampling sites had minimal wildlife activity. At these sites, stool samples from cattle were collected on the same day of gastropod sampling. Only fresh stool samples were collected because they contain viable trematode eggs and would thus provide higher quality DNA compared to older stool samples. Stools matching these criteria were fixed in high-grade ethanol (99-100%) at 0.25 g stool per ml of ethanol to allow optimal sample fixation (ten Hove *et al.*, 2008). All fixed stool samples were stored at 4 °C in the lab to further prevent DNA degradation.

2.2.4. Adult flukes

Adult parasites were collected on two distinct occasions. The first collection occurred at the abattoir 'Koala Park' close to Harare. It allowed the detection of fasciolosis and amphistomiasis in slaughtered cattle. Local veterinarians decided which livers were condemned. These selected livers were analysed for *Fasciola* sp. infections through dissection. All flukes were pooled per species and host individual. Due to time restrictions, only four livers were checked. We were not allowed to search stomach contents for amphistomes ourselves due to the abattoir's regulations. The local veterinarians luckily provided us with a small number of adult amphistomes found in the intestines of the same batch of cattle being slaughtered. The second adult fluke collection event occurred in Kariba. Local wildlife authorities culled a subadult *Hi. amphibious* male as a salary for their services to the national park and for population control. We were allowed to take a few tissue samples when about twenty soldiers were processing the kill. Sadly, the fast work of the rangers did

not allow for a detailed and complete analysis of all tissues; the liver could be completely dissection but only part of the stomach and intestines could be inspected for flukes. Helminths and nematodes were stored in separate falcon tubes containing 80% ethanol and stored at 4 °C. Amphistomes and nematodes had to be pooled respectively due to their high abundance. Each fluke morphotype was photographed and stacked using the same method as applied to gastropod samples.

2.2.5. Ecological assessments

Physicochemical parameters, depth, substrate, observed animals and activities and littoral vegetation were noted down prior to gastropod sampling. Animal presence was determined based on stool samples or direct activity. Physicochemical measurements included pH, water temperature (°C), conductivity (µS/cm), dissolved oxygen (mg/l), total dissolved solutes (mg/l), nitrates (mg/L), phosphates (mg/L) and carbonates (mg/L). Abiotic measurements are incomplete due to lack of equipment both in the field and at the University of Zimbabwe.

3. Molecular analysis

3.1. Molecular identification of gastropod and trematode species

3.1.1. DNA extraction

Tissue of the complete gastropod was extracted from its shell using a fine needle, homogenized using a scalpel and excess ethanol was eliminated using absorbent paper. Gastropods of excessive size were cut lengthwise in order to sample all organs and a tissue voucher was placed in the museum collection. All equipment was sterilized after each individual extraction using bleach and an open flame to prevent contamination between samples. The proteinase *K* - based E.Z.N.A. Mollusc DNA Kit (OMEGA bio-tek, Inc.) and the associated protocol was used to extract DNA from selected gastropod samples. The selection includes: infected individuals, morphotype representatives and gastropods collected from sites that had shown high infection prevalence based on shedding experiments. Other samples were extracted using the Chelex® (Biorad™) DNA extraction method. Following DNA extraction each sample was split in a working and a storage solution. The former was diluted 1 / 10 and served for further analysis during this study. The latter remained undiluted and served as high-quality DNA back-up and remains continuously stored at -20 °C to prevent DNA degradation. Back-up samples were only thawed in case a very light infection was detected in the infection RD-PCR to obtain PCR amplification of the parasite DNA. The E.Z.N.A. Mollusc DNA Kit eliminates inhibition by removing mucopolysaccharides more

consistently. However, this method requires significantly more financial and time resources compared to the Chelex® (Biorad™) DNA extraction method. When inhibition occurred for pooled samples, all gastropods were diluted 1:100 to prevent inhibition and tested individually tested. Samples were discarded if amplification was still unsuccessful. Both methods were combined in this study due to the previously mentioned advantages and disadvantages of each method. A maximum of 24 gastropods per site per species was extracted to reduce cost and time expenditure. Location five was exempt from this methodology since four bulinids were found shedding *Schistosoma*-type cercariae in the field. For this reason, all collected *Bi. pfeifferi* and *B. globosus* gastropods were extracted with the E.Z.N.A. method, 34 and 38, respectively.

Stool samples collected in the field were filtered by a Pitchford funnel in the lab to both eliminate ethanol residue and concentrate parasite eggs. QIAamp Fast DNA Stool Mini Kit (Qiagen™) was used to extract trematode DNA from vertebrate stool samples. Adult helminths collected from final hosts were extracted using the DNeasy Blood & Tissue Kit (Qiagen™) and associated protocol. Cercariae were extracted using a proteinase K based lysis buffer. The lysis stock buffer contained 0.90% Tween 20 (v/v), 0.90% NP40 (v/v) and 2x PCR-buffer. On the day of extraction, 12 µL of proteinase K was added to 1,000 µL of lysis buffer. 10 µL of the proteinase K lysis buffer was added to a single cercaria with 10 µL of MilliQ water. A first and second incubation step followed at 65 °C for 25 minutes and 95 °C at 10 minutes respectively.

3.1.2. Simplex PCR and Sanger sequencing

Gastropods were identified through amplification of the protein-encoding COI gene because it is the marker of choice for species identification and population studies due to its high evolutionary speed (Hebert *et al.*, 2003). Additionally, gastropod COI sequences are highly represented on both BOLD systems V4 (http://www.boldsystems.org/index.php/) and NCBI GenBank (https://www.ncbi.nlm.nih.gov/). COI1_SNAIL_F and COI1_SNAIL_R were used for gastropod COI amplification, yielding a COI fragment of approximately 500 bps. Some gastropod genera could not be amplified using these primers. Non-infected representatives were selected and subsequently analysed using the general COI primers HCO2198 and LCO1490 designed by Folmer *et al.* (1994), yielding a 710 bp COI fragment. Trematode DNA amplification was more complex and a total of twenty-two primers had to be used for sequencing during this study (**Annex Table 2**). Attempts were made to amplify the longest possible fragment. If the initial primers were not successful, other primers were used to

amplify a shorter fragment within the same marker. ITS4 / ITS5, BD1 / BD2 and Trem2F / Trem1R were used in a first step when a *Schistosoma* sp. or *Fasciola* sp. infection was detected. These primers amplify a large rDNA fragment covering 18S and 28S rDNA partially and the complete internal transcribed spacer (ITS) 1, ITS 2 and 5.8S rDNA regions. If amplification failed, Fasc-ITS1F / Fasc-ITS1R and ITS2_DIG_F / ITS2_DIG_R were used to obtain sequences of the ITS 1 region for *Fasciola* spp. and ITS 2 region for *Schistosoma* spp. respectively.

18S is the most conserved ribosomal marker and can only be used to infer higher taxonomic levels (Nolan and Cribb, 2005; Toledo and Fried, 2014). Primers 18S_DIG_F and 1270R amplified the 18S fragment for non-*Schistosoma* and non-*Fasciola* spp. infections. 18S was amplified on two distinct occasions: as an initial broad screening of trematode diversity and when comparable COI reference sequences (best BLAST < 80%) were unavailable.

Amplification success was detected through gel electrophoresis using a 1.5% agarose gel combined with the 100 bps FastGene® DNA ladder. Amplicons showing a strong signal of expected length, subsequently underwent a purification step following the ExoSAP (Fermentas™) protocol. Finally, 5 µL of the sequencing primer (5 µM) was added to 5 µL of purified PCR product before the sequencing plates were sent to Macrogen™ for Sanger sequencing using the BigDye® chemistry. Both forward and reverse primers were used to sequence all amplicons except for gastropod COI, whereby only the forward primer was used because the number of samples exceeded financial possibilities.

3.1.3. Phylogenetic trees

Sequence trimming, quality checks and alignments were made in Geneious® 6.1.8 and were subsequently exported to Mega-X version 10.1.0 (Kumar *et al.*, 2018). The invertebrate and flatworm mitochondrial genetic code were used when analysing gastropod and trematode COI sequences respectively. The standard nuclear code was used when analysing 18S amplicons and ITS regions were defined as non-coding. Phylogenetic tree layout was simplified by sequence alignment per phylum and marker. Related parasite species were bundled under one 'Type' code by using preliminary phylogenetic trees and p-distance matrices. p-distance based type-thresholds were selected at 5% for mitochondrial markers and 1% for ribosomal markers (Brant and Loker, 2009; Detwiler *et al.*, 2010). Similar tree positions of same-type original and reference sequences were used to validate the selection. A slightly different approach was used for gastropod and *Schistosoma* spp. COI sequences. Intraspecific sequences were aligned in MEGA in order to detect haplotype richness. The best quality (highest

HQ) and longest representative of each haplotype was selected to simplify tree layout. 'Find best model' was used in Mega to determine the optimal analysis model to construct the maximum likelihood (ML) tree based on AIC values. jModelTest2 was used to determine if the GTR model had a sufficiently high AIC value to perform Bayesian inference with the Markov Chain Monte Carlo (MCMC) algorithm. All ML trees were statistically tested through 1,000 bootstrap replications. The Markov Chain Monte Carlo algorithm was programmed to sample 1,000,000 times, which allows enough sampling after stasis. The p-distance model was used to construct pairwise distance matrices for each respective tree. Outgroups consisted of three species from closely related taxa outside the group of interest because it tests the monophyly of the ingroup (Barriel and Tassy, 1998). Parasite final host range, shown as silhouettes, were gathered from the host-parasite database of the Natural History Museum (London, www.nhm.ac.uk/research-curation/scientific-resources/taxonomy-systematics/host-parasites/database).

3.2. Multiplex PCR assays for detection of gastropod infection status

A pooling design of four same-species individuals per pool was used to reduce costs during the infection screening of gastropods. *Schistosoma* sp. or *Fasciola* sp. infected pools were subsequently tested individually to identify the infected gastropods from the respective pool. Three rapid diagnostic (RD, i.e. different primers are combined in one PCR reaction to obtain amplicons of different lengths) multiplex PCR protocols were used to determine infection status in gastropod samples. Both infection PCR protocols had a similar set-up and contained an internal control amplifying 18S gastropod rDNA, to avoid false negative results. Combined with an 18S rDNA trematode marker to detect a general trematode infection in the gastropod and *Schistosoma* or *Fasciola* sp. specific primers for planorbid and lymnaeid gastropods respectively. Carolus *et al.* (2019) and Schols *et al.* (under review) are referred to for the interpretation of RD-PCR output. Samples showing a schistosome infection underwent a *two-step* approach and were subsequently identified to species level based on COI amplification using the *Schistosoma* RD-PCR described in Schols *et al.* (unpublished). All primers used in the RD-PCR protocols are summarized in **Table 4**. The '*Fasciola* sp. infection PCR' protocol was performed in a 15 µL volume with 2 µL gastropod DNA using the Qiagen™ Taq DNA polymerase kit containing 1.5 mM PCR buffer (Qiagen™), 0.6 mM dNTP mix (Qiagen™), 1.5 mM $MgCl_2$, 0.45 units of Taq Polymerase (Qiagen™) and primer mix. Both *Schistosoma* RD-PCR protocols used in the two-step approach were performed in a 15 µL volume with 2 µL gastropod DNA template containing the Qiagen™ Multiplex Taq DNA polymerase kit and

the suitable primer mix. Primer concentrations in the final volume of the *Schistosoma* sp. and *Fasciola* sp. infection PCRs were: 0.2 µM '18S_Digenea_F' and '18S_Digenea_R', 0.1 µM '18S_SNAIL_F' and '18S_SNAIL_R' and 0.6 µM of 'Kaplan_F' and 'Kaplan_R' or 'ITS2_Schisto_F' and 'ITS2_Schisto_R' respectively. The *'Schistosoma* RD-PCR*'* primer concentrations in the final volume were: 0.8 µM of 'Asmit1' and 0.3 µM of each reverse primer ('Sh.R', 'Sb.R', 'Smat.R' and 'Sman.R'). The '*Schistosoma* sp. infection PCR' and the '*Schistosoma* RD-PCR' protocols consisted of the following steps: initial denaturation at 95 °C for 5 min, 40 cycles of 94 °C for 30 s, 62 °C for 45 s and 72 °C for 45 s and a final elongation step at 72 °C for 10 min in a Biometra® Tprofessional Thermal Cycler. The *Fasciola* sp. infection PCR protocol was identical except for the annealing temperature, which was lowered to 60 °C.

Table 4: Primers used in diagnostic RD-PCR protocols. The primer names, sequences and authors are given along with the targeted marker, target organism and amplicon length (in nucleotides). Fas, 18S, COI and ITS2 refer respectively to the 124 bp *Fasciola* sp. repeat, 18S rDNA, cytochrome c oxidase 1 (mtDNA) and internal transcribed spacer 2.

Primer name	Marker	Target	Length	Primer sequence	Authors
			"Infection RD-PCRs"		
18S_Digenea_F	18S	Trematoda spp.	±392	CAGCTATGGTTCCTTAGATCRTA	Carolus *et al.* (2019)
18S_Digenea_R				TATTTTTCGTCACTACCTCCCCGT	Carolus *et al.* (2019)
18S_SNAIL_F	18S	Gastropoda spp.	±500	AGTATGGTTGCAAAGCTGAAACTTA	Carolus *et al.* (2019)
18S_SNAIL_R				TACAAAGGGCAGGGACGTAAT	Carolus *et al.* (2019)
Kaplan_F	Fas	*Fasciola* spp.	124	ATTCACCCATTTCTGTTAGTCC	Krämer and Schnieder (1998)
Kaplan_R				ACTAGGCTTAAAGGCGTCC	Krämer and Schnieder (1998)
ITS2_Schisto_F	ITS2	*Schistosoma* spp.	±369	GGAAACCAATGTATGGGATTATTG	Schols *et al.* (under review)
ITS2_Schisto_R				ATTAAGCCACGACTCGAGCA	Schols *et al.* (under review)
			"Schistosoma RD-PCR"		
Asmit1	COI	*Schistosoma* spp.		TTTTTTGGTCATCCTGAGGTGTAT	Webster *et al.* (2010)
Sh.R		*S. haematobium*	543	TGATAATCAATGACCCTGCAATAA	Webster *et al.* (2010)
Sb.R		*S. bovis*	306	CACAGGATCAGACAAACGAGTACC	Webster *et al.* (2010)
Smat.R		*S. mattheei*	362	CACCAGTTACACCACCAACAGA	Schols *et al.* (under review)
Sman.R		*S. mansoni*	375	TGCAGATAAAGCCACCCCTGTG	Van den Broeck *et al.* (2011)

3.3. Haplotype and genotype mapping based on COI and ITS respectively

Both haplotypes and genotypes were defined using the DnaSP® V5 software (Librado and Rozas, 2009) and respective networks were subsequently constructed in PopART® (http://popart.otago.ac.nz) using the TCS network inference model (Clement *et al.*, 2002). Only the ITS1 genotype network could be constructed containing sequences obtained by

Mucheka *et al.* (2015) and thus covers *F. hepatica* and *F. gigantica* from Zimbabwe and South-Africa. COI sequences from Mucheka *et al.* (2015) did not overlap with our amplicons. Additionally, their primers were found amplifying gastropod mtDNA, preventing their use in this study. A COI haplotype network was also constructed for *B. globosus* and *L. natalensis* collected during this study because of their significant role as intermediate hosts in schistosomiasis and fasciolosis transfer. Haplotype networks were not made for other gastropod species, even if they were found transmitting these parasites, because of their low COI-inferred genetic diversity. No networks were constructed for *Schistosoma* spp. since intraspecific diversity was insufficient based on COI and the rDNA region.

3.4. Screening for hybrids

Potential hybrids of *Schistosoma* spp. and *Fasciola* spp. were analysed using both mitochondrial and nuclear markers, which is a common and successful practice in helminth hybrid screening (Hoa Le *et al.*, 2008; Huyse *et al.*, 2009; Huyse *et al.*, 2013; Nguyen *et al.*, 2018). The haploid inheritance of the mitochondrial COI gene allows identification of the maternal line. While nuclear markers, like ITS1 and ITS2 rRNA, allow detection of hybrids through double peaks in the chromatogram or mito-nuclear discordance following Sanger sequencing. Nuclear amplicons were aligned with sequences of potential parental species available on NCBI GenBank to detect polymorphic nucleotide sites. *Fasciola* spp. were screened using the whole ITS region or only ITS1 through a nested approach, if the initial PCR amplified gastropod instead of parasite DNA. This contrasts with gastropods infected with *Schistosoma* spp., whereby a different nested protocol resulted in ITS2 amplification. The discontinuity in hybrid screening methodology between *Fasciola* and *Schistosoma* spp. results from the limited amount of primers described in the literature. Therefore, primers had to be used that amplify markers readily available in online databases for each respective genus.

4. Analysis of gastropod communities

Ecological data was analysed using the statistical software program RStudio®. All graphs generated in R were exported to Microsoft Powerpoint® using the *export* package, designed by Wenseleers (2016) to improve figure lay-out. Most multivariate methods were unsuitable to analyse gastropod communities collected during this study for multiple reasons. The lack of equipment to measure physiological parameters resulted in the absence of data from multiple reservoirs, rendering analysis methods such as the RDA analysis insignificant. NMDS

analyses were executed on gastropod and macrophyte data of all sites. In contrast to physicochemical data for which only site 1 to 6 could be compared due to lack of data for the other locations.

Results

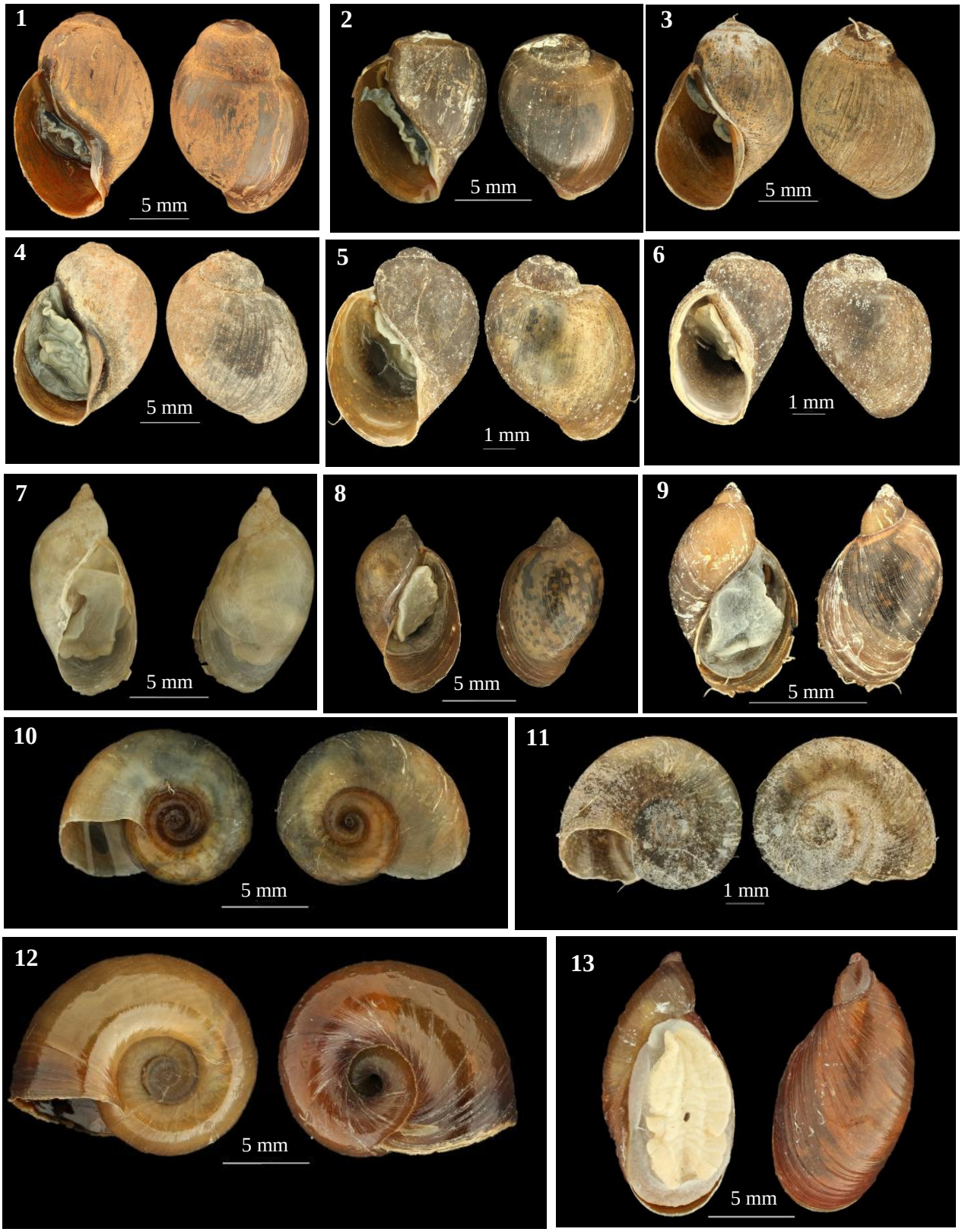
1. Gastropod and trematode diversity

1.1. Gastropod diversity

Key-based gastropod classification in Zimbabwe resulted in 13 distinct morphotypes: *B. globosus* (1 and 2), *B. truncatus* (6), *B. tropicus* (3, 4 and 5), *Radix* sp. (7), *L. natalensis* (8), *P. columella* (9), *Bi. pfeifferi* (10), *Gyraulus* sp. (11), *H. duryi* (12), *Oxyloma* sp. (13), *M. tuberculata* (14), *Bellamya* sp. (15) and *P. acuta* (16) (Mandahl-Barth, 1962; Frandsen, 1980). Each number within parenthesis links to molecularly identified specimens shown in **Figure 7**. Some of these individuals were wrongly identified based on morphology and their final identification using molecular tools is listed in the subscript of **Figure 7**. Complementary to shell-based identification, soft body parts were also used to identify snail species morphologically. These are not shown in the figures because all depicted specimens were fixated on ethanol. *Oxyloma* sp. was identified as such because it closely resembled the taxon *Oxyloma patentissima* described in this area by Brendonck *et al.* (2003). Whenever possible, shells and corresponding DNA extracts were deposited in the collection of the Royal Museum for Central Africa (Tervuren, Belgium) for future studies.

One gastropod per morphotype per site (including those shown in **Figure 7**), all shedding and all *Schistosoma* sp. and *Fasciola* sp. infected gastropods (n = 111) were identified using both BLAST and tree-based identification. COI amplifications followed by manual editing and trimming of the sequences, resulted in 111 sequences (success rate of 100%) of sufficient quality (HQ > 80% and without a single ambiguity) and length (> 400 bps), which were combined with sequences present in our molecular database from both Lake Kariba (n = 13) and Henderson's reservoir (n = 2). This resulted in the total classification of 30 unique haplotypes of 15 different species. COI1_Snail primers were sufficient for most samples except for *Bellamya*, *Helisoma*, *Oxyloma* and *Radix* spp., for which primers from Folmer *et al.* (1994) had to be used. The latter provided longer fragments (> 650 bps) of high quality for these specimens but could not be used on other samples due to aspecific amplification of trematode DNA. The best Bold Systems BLAST results (highest similarity) per haplotype are

depicted in **Table 5**. All sequence ID's compacted into one single haplotype are depicted in **Annex Table 3**, while **Annex Table 4** contains the BLAST results for all haplotypes.



(continued on next page)

Figure 7: Photographs of each gastropod species. One representative per species is shown with the exception of *B. truncatus*, *B. globosus* and *L. natalensis* for which two morphologically distinct but genetically similar individuals are shown. Pictures show the following species: *B. globosus* [BGL H1 (**1**) and BGL H3 (**2**)], *Bulinus* sp. 1 (**3**), *Bulinus* sp. 2 (**4**), *B. truncatus* [TRU H1 (**5**) and TRU H2 (**6**)], *L. natalensis* [LNA H4 (**7** and **8**)], *P. columella* (**9**), *Bi. pfeifferi* (**10**), *G. connollyi* (**11**), *H. duryi* (**12**), *Oxyloma* sp. (**13**), *M. tuberculata* (**14**), *Bellamya* sp. (**15**) and *P. acuta* (**16**). Scale bars indicate either one or five mm as indicated on each respective picture.

Table 5: Best BLAST results on BOLD systems V4 using COI. All gastropod species are provided with sequence length (bps), BLAST 1 and 2 with corresponding identification percentage and country of origin. A second BLAST result is only included of it was listed amongst the top 20 results and if species names were different. Species abbreviations are: BGL = *B. globosus*, TRU = *B. truncatus*, BPF = *Bi. pfeifferi*, GYR = *Gyraulus* sp., LNA = *L. natalensis*, COL = *P. columella*, PHY = *P. acuta*, MEL = *M. tuberculata*, HEL = *H. duryi*, RAD = *Radix* sp.

Seq.	Len. (bps)	BLAST 1	ID (%)	Country	BLAST 2	ID (%)	Country
BGL H1	479	*Bulinus globosus*	98.22	South Africa	/	/	/
TRU H2	417	*Bulinus truncatus*	99.75	Tanzania	/	/	/
***Bulinus* sp. 1**	417	*Bulinus tropicus*	99.26	Zimbabwe	*Bulinus nyassanus*	97.6	Malawi
***Bulinus* sp. 2**	417	*Bulinus tropicus*	98.02	Uganda	*Bulinus nyassanus*	97.53	Malawi
BPF H1	469	*Biomphalaria pfeifferi*	100	Zimbabwe	/	/	/
GYR H2	421	*Gyraulus connollyi*	96.3	South Africa	*Gyraulus sp.*	90.37	China
GYR H3	313	*Gyraulus sp.*	87.14	China	/	/	/
LNA H3	463	*Lymnaea natalensis*	98.7	Malawi	*Radix natalensis*	96.62	Portugal
COL H1	463	*Pseudosuccinea columella*	100	Multiple	/	/	/
PHY H4	464	*Physella acuta*	99.78	Multiple	/	/	/
MEL H1	395	*Melanoides tuberculata*	100	Malawi	/	/	/
***Bellamya* sp.**	681	*Bellamya sp.*	93.74	Namibia	*Bellamya monardi*	93.26	Malawi
Bellamya sp. Kariba	450	*Bellamya monardi*	93	Botswana	*Bellamya robertsoni*	91.86	Malawi
CHI HEL 1	678	*Planorbella duryi*	100	USA	*Planorbarius corneus*	100	China
***Oxyloma* sp.**	677	*Oxyloma verrilli*	89.09	Canada	*Succinea putris*	88.79	Russia
RAD H1	662	*Radix sp.*	97.32	China	*Radix auricularia*	95.97	Japan

No trees were constructed for *Bellamya* and *Melanoides* spp. (subclass: Caenogastropoda). The argument for doing so concerning *M. tuberculata* were BLAST identifications of such excellent quality that further investigation was not necessary. No tree was constructed for *Bellamya* spp. because they are of little importance for parasite transmission in Africa (Brown, 2008). Therefore, not all *Bellamya* samples were molecularly analysed for parasite infections. Such argument is supported through the absence of clear infections in *Bellamya* samples from both Lake Chivero through molecular analysis and Lake Kariba through shedding experiments as tested by Hans Carolus. One single specimen showed a potential trematode signal but could not be amplified for parasite identification, so could, therefore, be a false positive. No tree was constructed for the family Succineidae because they are terrestrial species and thus fall outside the scope of this study. Moreover, Hans Carolus constructed such tree in 2018 and since sequences from both studies are identical (100% identical based on a 401 bp COI sequence), the resulting phylogenetic position would be identical too.

Following alignment and trimming, 464 bp for Physidae, 394 bp for Planorbidae and 465 bp for Lymnaeidae COI sequences were used to construct phylogenetic trees. The Physidae tree was constructed using three *Bulinus* spp. sequences as outgroup. Bulinids were selected because it is a closely related genus belonging to the same Superfamily (Lymnaeoidea). The same reasoning was made in the opposite direction to select three *Physa* spp. as an outgroup for both the Lymnaeidae and Planorbidae phylogeny. The following models were selected to construct Maximum Likelihood (ML) trees: for Physidae phylogeny, the Hasegawa-Kishino-Yano model with a discrete Gamma distribution ([+G] = +0.22) was used; for Lymnaeidae and Planorbidae phylogeny, the General Time Reversible (GTR) model with a discrete Gamma distribution (for Lymnaeidae, [+G] = +0.30; for Planorbidae, [+G] = +0.48) and invariant sites (for Lymnaeidae, [+I] = 30.54%; for Planorbidae, [+I] = 27.03%) were used (Nei and Kumar, 2000). Constructed trees and pairwise genetic distance matrices are provided in **Figure 8**, **Annex Figures 4.1 and 5.1** and their corresponding **Annex Tables 5, 6 and 7** respectively for Planorbidae, Physidae and Lymnaeidae. In the Bayesian analysis the GTR model was used with a discrete gamma distribution and invariant sites (**Annex Table 8)**. Constructed trees are provided in **Annex Figure 3**, **4.2 and 5.2** respectively for Planorbidae, Physidae and Lymnaeidae.

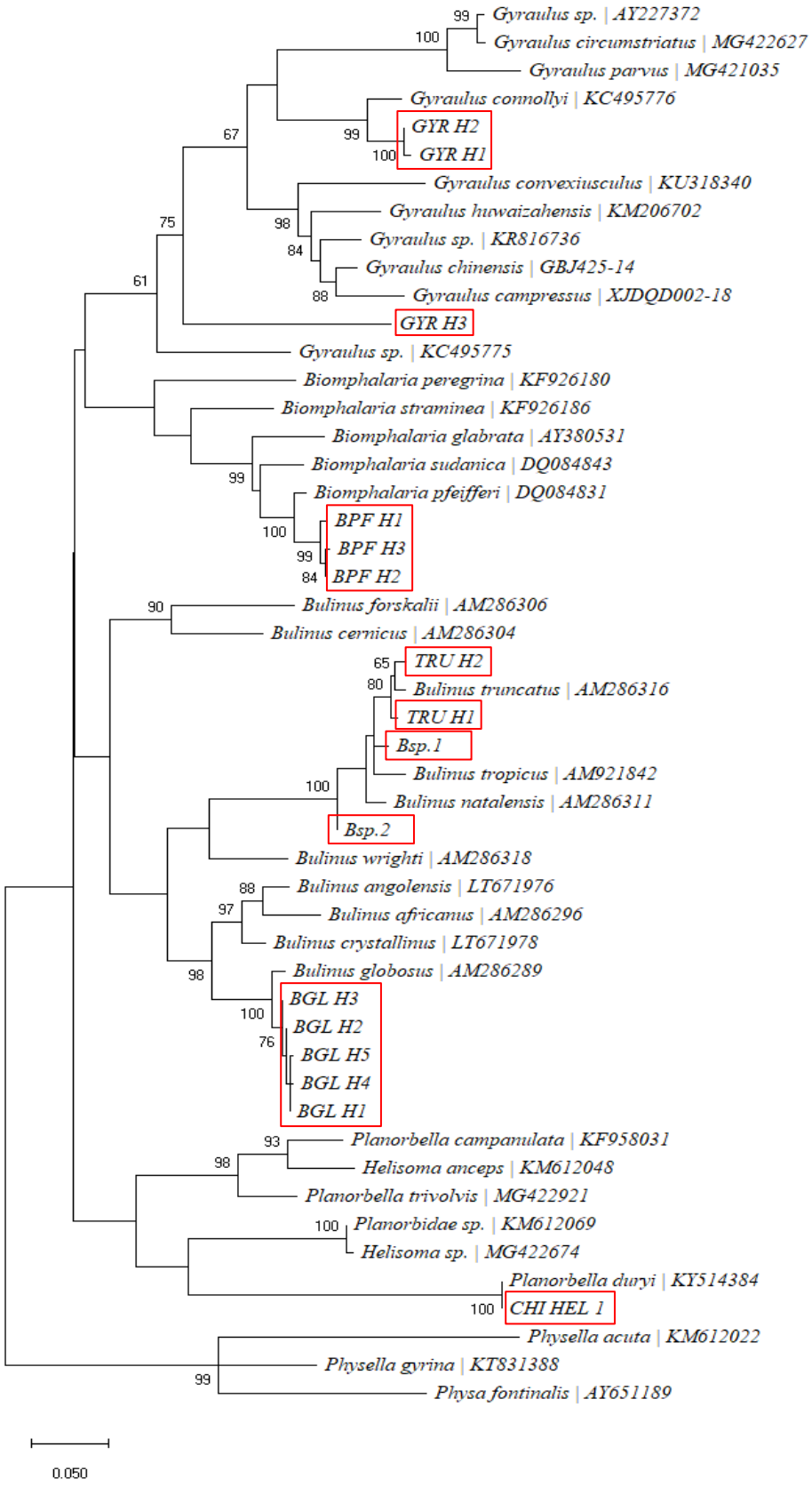


Figure 8: ML phylogenetic tree for the Planorbidae family, using a 394 bp COI alignment and the General Time Reversible (GTR) model. The tree with the highest log likelihood (-4481.98) is presented. Bootstrap values (1,000 replicates) above or equal to 60 are indicated on the nodes. The tree is drawn to scale, with branch lengths

measured in the number of substitutions per site. The analysis involved 50 different sequences. BOLD or GenBank accession number of each reference sequence is displayed after the '|' separator. Red boxes indicate sequences from Kariba and Harare.

The Physidae phylogeny confirms the identification of four *Physella* (or *Physa*) *acuta* haplotypes that form a well-supported clade. Pairwise genetic distance (p-distance) also showed close affinity to *P. virgata*. However, the p-distance to *P. acuta* is for each haplotype at least twice as small relative to *P. virgata.* Haplotype 3 (only Harare) and haplotype 4 (Harare and Kariba), show the highest resemblance with *P. acuta* (ref. from Iran), 0.2 and 0% p-distance respectively. The Lymnaeidae phylogeny confirms the morphological identification of the LNA and COL morphotype with *Radix* (or *Lymnaea*) *natalensis* (ref. from Egypt) and *P. columella* (ref. from multiple countries) respectively. P-distances were 3.0 - 4.1% for LNA and *R. natalensis* and 0% for COL and *P. columella*. The *Radix* sp. from Lake Kariba could not be brought to name as it is most closely related to an unnamed *Radix* specimen from China (3%). The Planorbidae phylogeny confirmed the identification of *B. globosus* (p-distance: 1.3 – 1.8%). Indicating on a genetic basis that our samples do not contain *B. africanus*. A p-distance for the *H. duryi* morphotype of 0% to *Planorbella* (or *Helisoma*) *duryi* confirmed BLAST results. *Planorbarius corneus* sequences were not included because some were identical to *H. duryi* and are most likely misidentifications.

Gyraulus haplotypes 1 and 2 have lowest p-distances to *G. connollyi* from South-Africa, 4.6 and 4.1% respectively and are genetically distinct species from the *Gyraulus* sp. present in Lake Kariba (p-distance 15.1%). *Bi. pfeifferi* and *B. truncatus* morphotypes are confirmed with p-distances between 2.5 and 2.8% for *B. pfeifferi* and 1.5% for *B. truncatus*. An additional alignment with reference sequences from Spain confirmed the identification of *B. truncatus* (results not shown). Both *Bulinus* sp. 1 and 2 remain unresolved, showing small p-distances to all representatives of the *truncatus/tropicus* group: *B. truncatus* (3.8 and 3.1% resp.), *B. tropicus* (3.1 and 2.8% resp.) and *B. natalensis* (3.1 and 2.8% resp.). Additionally, the clustering with either *B. tropicus* or *B. natalensis* in the ML or Bayesian inferred tree are not supported through bootstrapping or probability values. No reference sequence was available for *B. depressus*, which is part of the *truncatus/tropicus* group and present in South Africa.

Although no tree was constructed for the *Oxyloma* sp. (Family Succineidae) from lake Chivero it is notable that it is the same species as collected and described by Hans Carolus in

Lake Kariba in 2017 (100% identical on a 403 bps alignment). His results suggest a potential affinity to the genus *Oxyloma*. Therefore, we maintain the name *Oxyloma* sp. even though the p-distance is quite large.

A TCS haplotype network was built using a 403 and 474 bps COI alignment, containing all *B. globosus* (n = 23) or *L. natalensis* sequences (n = 29) respectively obtained during this study (**Figure 9.1** and **9.2** resp.; Clement *et al.*, 2002). The network indicates a star-like pattern for *B. globosus.* H1, H4 and H5 are each represented by a single sequence obtained from site 9 (H1 and H5) and site 4 (H4). Total *B. globosus* haplotype diversity (Hd) is 0.39 compared to a Hd of 0.77 for *L. natalensis*. Haplotypes of *L. natalensis* are genetically more dissimilar compared to *B. globosus* haplotypes but still belong to the same species (p-distances < 5%).

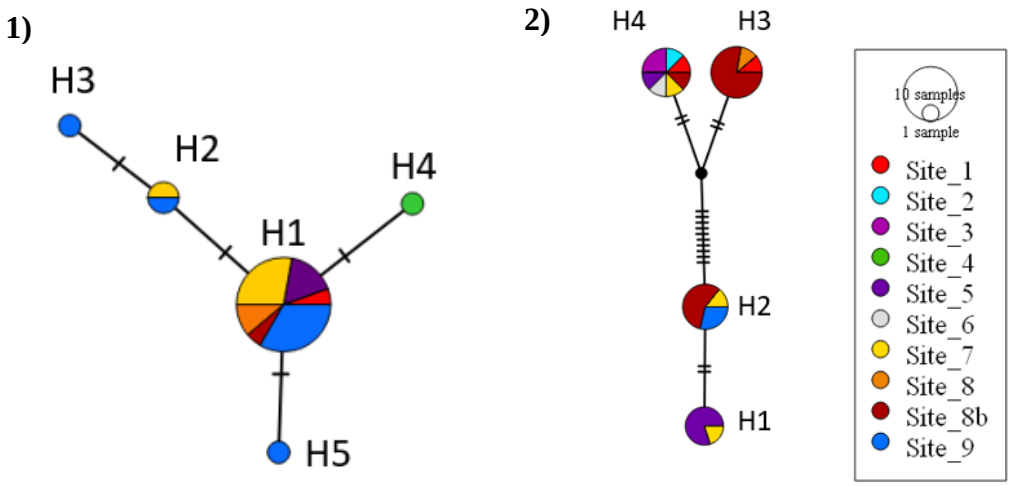


Figure 9: *B. globosus* (left, 1) and *L. natalensis* (right, 2) haplotype network. The network was built using 403 and 474 bp of the COI gene respectively. The number of mutations between each haplotype is indicated through hatch marks. Legend specifies haplotype origin and abundance through colour and size respectively. Site 10 is omitted because of the absence of the respective species.

1.3. Trematode diversity

1.3.1. Fasciola diversity

Dissection and molecular assays resulted in the detection of six adult *Fasciola* specimens and 18 *Fasciola* sp. infected gastropods (**Table 6**). Two *Fasciola* specimens were collected from *Hi. amphibious* (**Figure 10.1** and **10.2**) and four *F. gigantica* were collected in Koala Park abattoir from multiple *B. taurus* hosts (**Figure 10.5**). Sequences from metacercariae collected from *P. columella* (**Annex Figure 6.1**) and *Fasciola* sp. infections in intermediate gastropod

hosts (i. e. *L. natalensis* from Mwenje reservoir and *Radix* sp. from Lake Kariba) were also obtained.

Table 6: *Fasciola* spp. detected in Mwenje reservoir and Lake Kariba. For each species: lifecycle stage, host of origin, abundance, collection site and sequenced amplicons are listed. The whole ITS region covers ITS1, ITS2 and 5.8S rDNA.

Species	Lifecycle stage	Host	Abun-dance	Collection site	Sequenced amplicon (n=x)
'F. nyanzae'	Adult	*Hi. amphibious*	2	Kariba	18S (2), COI (2), whole ITS (2)
	Intermediate	*L. natalensis, P. columella*	16	Mwenje, Kariba	18S (1), COI (6), ITS1 (6), whole ITS (6)
F. gigantica	Adult	*B. taurus*	4	Koala Park	18S (1), COI (3), whole ITS (2)
	Intermediate	*L. natalensis*	2	Mwenje	COI (1), ITS (1), whole ITS (1)

Neither a reference sequence nor photograph of *F. nyanzae* exists online, complicating species identification. Further complications arise due to the limited morphological descriptions by Leiper (1910), Jackson (1921) and Dinnik and Dinnik (1961) combined with uncertainty in morphologically-based identification of *Fasciola* spp. due to morphological plasticity and frequent hybridisation (Itagaki *et al.*, 2005, 2009; Hoa Le *et al.*, 2008). Therefore, morphometrics are provided but molecular data and broader species traits are used to identify *F. nyanzae* and *F. gigantica*. Sequences of '*F.* nyanzae' isolated from intermediate hosts match the sequences obtained from both '*F. nyanzae'* adult flukes, metacercariae and *Fasciola* sp. sequences obtained by Carolus *et al.* (2019) for COI, 18S and the complete ITS region. Morphological measurements of adult liver flukes are provided in **Annex Table 9** and list the descriptions made by Leiper (1910) and Dinnik and Dinnik (1961) together with measurements of three potential *F. nyanzae*, three *F. gigantica* and one *F. hepatica* (**Figure 10**). One representative of each species originates from the RMCA ethanol collection for comparisons. Dinnik and Dinnik (1961) wrote that the body shape of *F. nyanzae* is very characteristic: "*Oblanceolate narrowly rounded at the posterior end and very shortly and obtusely mucronate at the anterior end. Well-developed cephalic cone and distinct shoulders.*

Greatest width between the ventral sucker and Mehlis' gland or slightly posterior to this and then gradually tapers towards the bluntly pointed posterior end." All our '*F. nyanzae*' specimens match this description while *F. hepatica* and *F. gigantica* do not have distinct shoulders or a gradual tapering towards a bluntly pointed posterior end. Leiper (1910) on the other hand mentioned that *F. nyanzae* is characterized by a seven to one body length to width ratio (*F. gigantica* 4/1 and *F. hepatica* 2/1). No measured fasciolid within this study matches this ratio. *F. nyanzae1* did, however, show a ratio of almost ten to one before fixation on ethanol. In contrast to *F. nyanzae2*, which measured a four to one ratio falling within the range provided by Jackson (1921) for *F. nyanzae*. Testis to body length ratio was significantly smaller in *F. nyanzae* compared to the two other species and fell within the range mentioned by Dinnik and Dinnik (1961, 25% - 40%). Metacercariae obtained from *P. columella* (n = 2) match the size range obtained by Dinnik and Dinnik (1961). Taking into consideration the final and intermediate hosts, the close morphological resemblance of metacercariae and especially *F. nyanzae1* to early species descriptions and the molecular proof of these morphotypes to be separate species (see below), the *Fasciola* sp. described by Carolus *et al.* (2019) is most likely *F. nyanzae*. Therefore, further mention to these samples will be made with this species name.

Figure 10: *Fasciola* spp. used for morphometrics. Pictures show the following species: *F. nyanzae* (1, 2 and 3); *F. hepatica* RMCA (4) and *F. gigantica*1 (5). Pictures are all at the same scale (5 mm).

To study the genetic diversity of *F. nyanzae,* COI-based haplotype (**Figure 11**) and ITS1-based genotype (**Annex Figure 7**) networks were constructed. The ITS1-based network also contains sequences from *F. hepatica* and *F. gigantica* from South Africa and Zimbabwe collected by Mucheka *et al.* (2015) and *F. gigantica* collected in this study. It indicates that

only one ITS1 genotype is present in Zimbabwe and South Africa for both *F. hepatica* and *F. gigantica*, in contrast to *F. nyanzae* for which three different genotypes were found in Zimbabwe. *F. nyanzae* is separated from *F. hepatica* by one mutation, and *F. hepatica* is in its turn separated from *F. gigantica* by three mutations. Based on twelve 441 bps COI sequences, ten different *F. nyanzae* haplotypes were detected, resulting in a haplotype diversity of 0.97. A COI alignment of 800 base pairs containing the three adult *F. gigantica*, collected from Koala Park abattoir, indicate the presence of two haplotypes separated by two mutations, which were not detectable in the 441 bps alignment. No hybrid species were discovered using the previously described method.

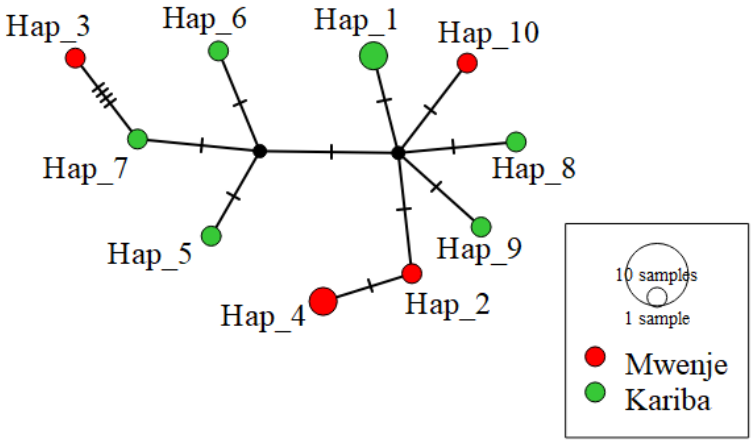


Figure 11: *F. nyanzae* COI haplotype network based on 12 sequences of 441 nucleotides, visualized using the TCS model in PopArt. Hatch marks represent mutational steps. The legend indicates the used colour-code and haplotype abundance through circle size.

1.3.2. Schistosoma diversity

COI and ITS2 were successfully amplified for 13 and 32 samples respectively. Three samples remained unamplified, of which two were identified as *S. mattheei* using the *Schistosoma* RD-PCR. The COI-based ML phylogenetic tree for species identification is shown in **Figure 12**, together with the associated p-distance matrix in **Annex Table 10**. **Annex Figure 8** shows the MCMC algorithm based Bayesian inference phylogenetic tree. Four different species have been detected: *S. haematobium* in one *B. globosus* from site 8b; *S. mansoni* in one *Bi. pfeifferi* from site 6; *S. mattheei* in one *B. truncatus* (site 2) and 12 *B. globosus* (site 4, 5, 7 and 9); *S. edwardiense* in three *Bi. pfeifferi* and six *B. globosus* from site 8b. Not any schistosome was found in Mazowe reservoir (Site 1 and 3) and Lake Chivero (site 10). No schistosome hybrids were detected when BLAST results of the same sample were compared for ITS2 and COI.

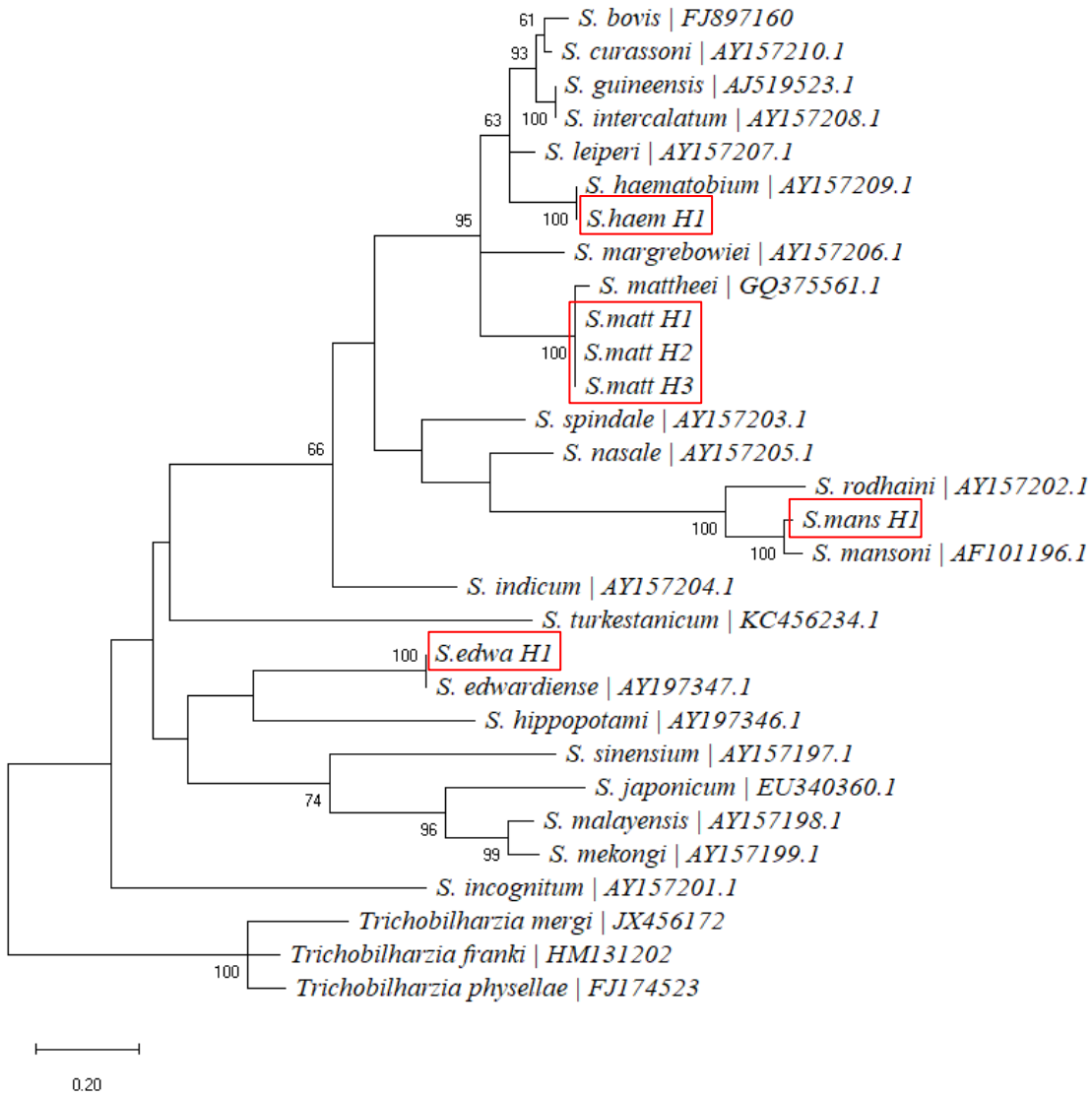


Figure 12: ML phylogenetic tree for the *Schistosoma* genus, using a 335 bp COX1 alignment and the Tamura-Nei model. The tree with the highest log likelihood (-3603.16) is shown. Bootstrap values (1,000 replicates) above or equal to 60 are indicated on the nodes. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 30 different sequences. BOLD or GenBank accession number of each reference sequence is displayed after the '|' separator. Red boxes indicate sequences obtained by this study (Country of origin: Zimbabwe).

1.3.3. General trematode diversity

Both the *Hi. amphibious* and *B. taurus* were found to host a similar fluke species diversity (n = 3). Each was found hosting two stomach fluke species (Cattleamphtype 1, n = 3 and 2, n = 10 from *B. taurus* and Hippoamphtype 1, n > 200 and 2, n = 2 from *Hi. amphibious;* **Annex Figure 6.2-5**) and one liver fluke species (*F gigantica,* n = 4, in *B. taurus* and *F. nyanzae,* n = 2, in *Hi. amphibious;* **Figure 10**).

All cercarial morphotypes (see 2.1. Shedding), adult flukes and non-schistosome and non-fasciolid infected gastropods (based on the infection RD-PCR) were used in trematode phylogeny. COI sequences were grouped under the same 'Type' followed by a roman number if pairwise genetic distance did not exceed 2% (Vilas *et al.*, 2005). This would allow species-level information to be conserved while simplifying tree layout. The 18S marker provides information for higher taxonomic levels because of its highly conserved nature (Nolan and Cribb, 2005). The pairwise genetic distance threshold to group 18S sequences under the same 'Type-code', was set for all but one type to 0.3%. This value was maximally detected by Carolus *et al.* (2019) to separate *F. gigantica, F. hepatica* and a *Fasciola* sp. from one another based on 18S and should allow clustering according to genus level. Type IV contains one additional sequence with a pairwise genetic distance of 0.8% to simplify tree layout. Best BLAST results for each type and adult fluke together with the intermediate host(s) and location(s) are listed in **Annex Table 11** for both the 18S and COI marker if obtained. COI BLAST results were highly variable, ranging from 75.4% identity to 99.8%. 18S BLASTs were higher, with all results exceeding 97%. *Schistosoma* and *Fasciola* spp. were included in a separate tree to simplify tree layout (results not shown for fasciolids). Amplification of trematode DNA from isolated cercariae went significantly better compared to trematode DNA from gastropod samples. 18S primers were capable of amplifying more samples relative to COI primers, although both had types which could not be amplified. 35 out of 42 COI sequences were successfully amplified for non-*Fasciola* and non-*Schistosoma* samples, resulting in 12 distinct types isolated from gastropod samples and four adult amphistome types (**Figure 13**). **Annex Figure 9** shows the MCMC algorithm based Bayesian inference phylogenetic tree. 18S was successfully amplified for 41 out of 46 samples, resulting in 12 types (**Figure 14**). **Annex Figure 10** shows the MCMC algorithm based Bayesian inference phylogenetic tree. Type V and VI could not be included in the 18S-based phylogeny. Although COI amplification worked, 18S failed for Type V, one representative of Type VI and amplified *Fasciola* spp. in three other representatives of Type VI. This is in contrast to COI-based phylogeny for which Types XI, XII and XIII are excluded due to unsuccessful amplification attempts. A discrepancy can be noted between the ML and Bayesian-based COI tree. The ML tree shows that the most recent common ancestor can be found with *Explanatum explanatum* although at a minimal support value (<60), while the clustering with the *Calicophoron* genus is well supported in the Bayesian tree (>0.99). Also, the suggested sister taxon for Type III is different; both clusterings are supported by strong bootstrap values. Only one discrepancy can be noted between both methods for the 18S trees, whereby *Echinostoma*

revolutum has the most recent common ancestor with Type III in the ML tree. The Bayesian-based phylogeny places *E. revolutum* with *Echinostoma caproni*. These results do not alter the tree interpretation because 18S is only used to infer information at the family level.

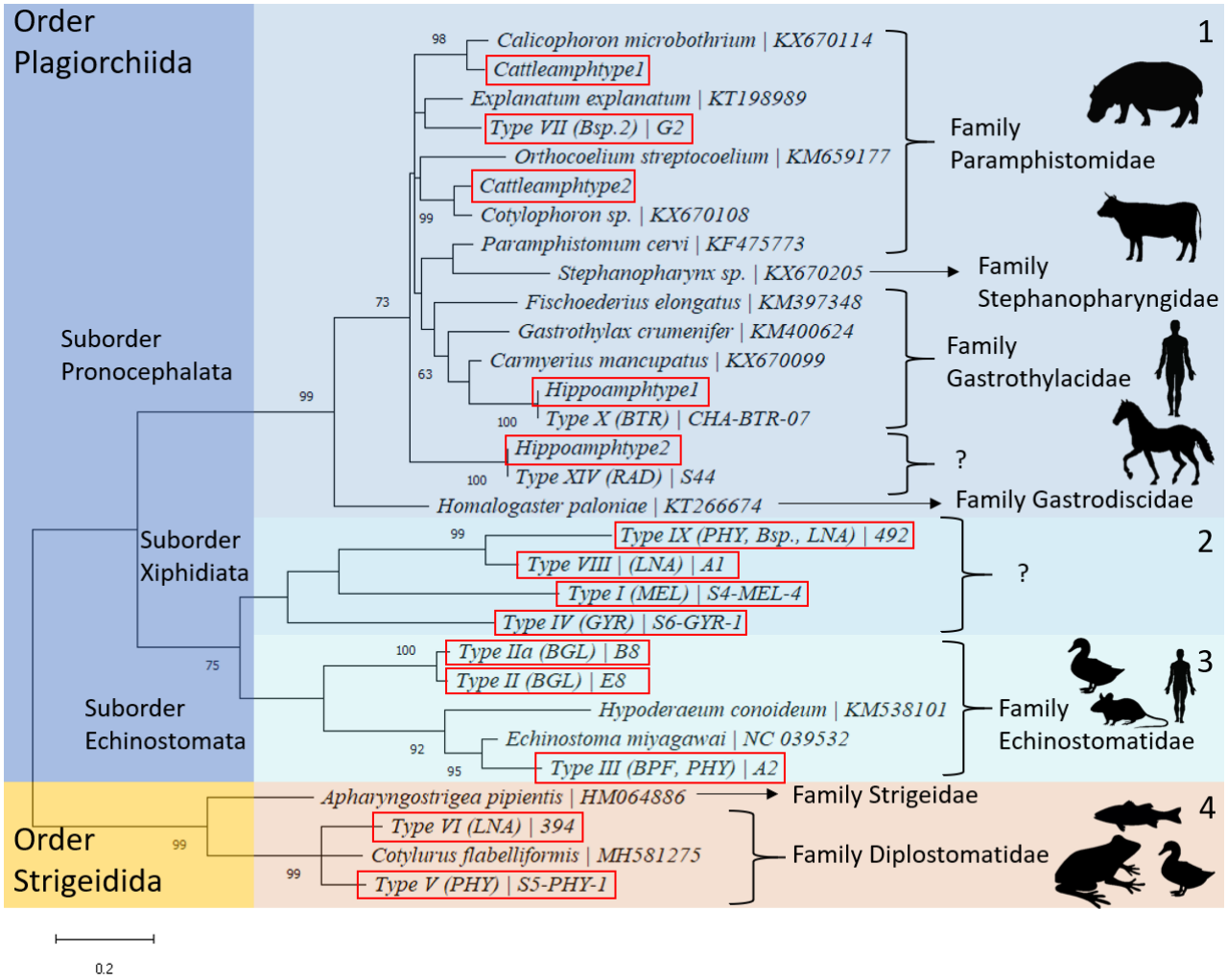


Figure 13: ML phylogenetic tree for trematodes, using a 276 bp COI alignment and the Tamura-Nei model with a discrete Gamma distribution ([+G] = +0.69) and invariant sites ([+I] = 22.11%). The tree with the highest log likelihood (-3630,93) is shown. Bootstrap values (1,000 replicates) above or equal to 60 are indicated on the nodes. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 30 different sequences. BOLD or GenBank accession number of each reference sequence is displayed after the '|' separator. The following superfamilies are indicated: Paramphistomoidea (1), Plagiorchioidea and Opisthorchioidea (2), Echinostomatoidea (3) and Diplostomoidea (4). Silhouettes indicate final hosts of GenBank represented Superfamilies. All type names were obtained in this study, except for type X and XIV (collected by Hans Carolus).

Total trematode species richness (22) found in gastropods, the hippopotami and *B. taurus* in Zimbabwe consists of two *Fasciola* spp., four *Schistosoma* spp., five amphistomes and at least 11 other trematode species. These species belong to 19 distinct genera, seven superfamilies and two different orders.

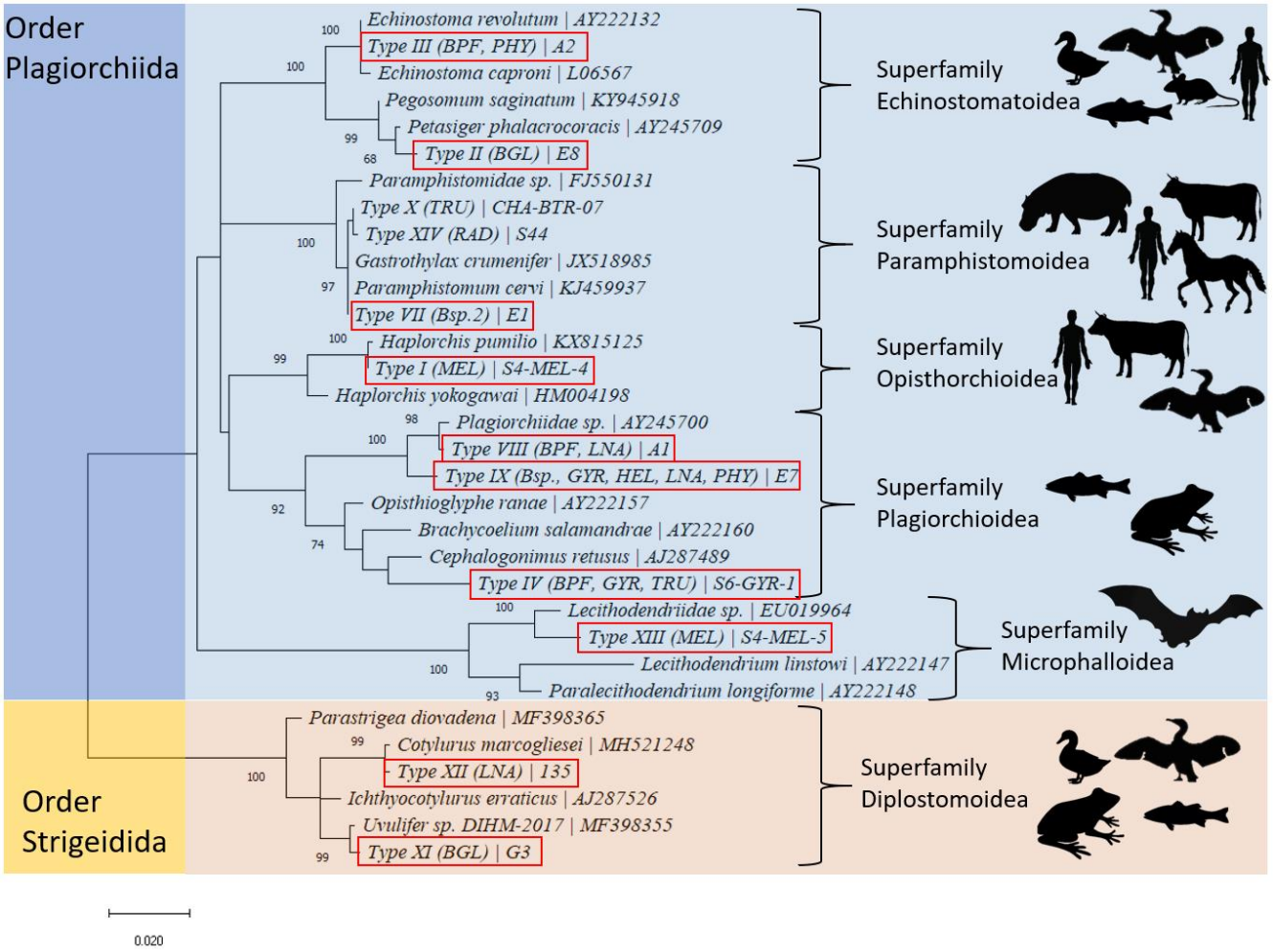


Figure 14: ML phylogenetic tree for trematodes, using a 796 bp 18S alignment, and the Kimura 2-parameter model with a discrete Gamma distribution ([+G] = +0.48) and invariant sites ([+I] = 38.76%). The tree with the highest log likelihood (-3087,25) is shown. Bootstrap values (1,000 replicates) above or equal to 60 are indicated on the nodes. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 32 different sequences. BOLD or GenBank accession number of each reference sequence is displayed after the '|' separator. Silhouettes indicate final hosts of GenBank represented species. All type names were obtained in this study, except for Type X and XIV (collected by Hans Carolus).

An overview of trematode types found in *Hi. amphibious*, *B. taurus* and per gastropod intermediate host species is given in **Table 7**. *L. natalensis* is found to transmit the highest parasite taxonomic diversity (6). This is in contrast to *P. columella* and *H. duryi*, which were found to each transmit one trematode type. Type IX is transmitted by five different gastropod species of five different genera. Natural intermediate and final hosts were identified for *F. gigantica*, *F. nyanzae*, Type X and Type XIV. The former being transmitted by the intermediate gastropod species *L. natalensis* and infecting *B. taurus* as final host. *F. nyanzae* is transmitted by both *L. natalensis* and *P. columella* gastropods and infects *Hi. amphibious* as final host. Types X and XIV are amphistomes transmitted through *B. truncatus* and *Radix* sp.,

respectively and infect *Hi. amphibious* as final mammalian host. Hippoamphtype1 (genetically identical to Type X) is a species of the *Carmyerius* genus. Only one species of this genus has been described from the hippopotamus (i.e. *Carmyerius cruciformis*, Leiper, 1910). Although the initial description by Leiper mentions a length of 0.5 to 0.8 mm, later sources corrected this to 5 - 8 mm, which matches our samples (Leiper, 1910; Sey, 2017). Additionally, Leiper mentioned the deep red colour of fresh specimens that turned brown once fixated. We noticed the same pattern in our samples. Species from this genus are transmitted through either *Ceratophallus natalensis* or *Bulinus* sp. which are all planorbids, as is our *B. truncatus*. Based on these characteristics combined with the morphological similarities we suggest our samples to belong to *C. cruciformis*. The other amphistome species retrieved from the hippopotamus, 'Hippoamphtype2', did not match any of the descriptions made by Leiper (1910) or any other record of amphistomes from the hippopotamus. Interestingly the intermediate host of this species was determined to be a *Radix* sp. most closely related to Southeast Asian sequences available on GenBank (Carolus *et al.,* 2019). The absence of records in hippopotamus of a similar species and the absence of any reference sequence of the same genus create some interesting hypotheses. It is possible that this is a newly introduced species to Zimbabwe that arrived through a vector and subsequently succeeded in infecting hippopotamus (i.e. *spillover*). This might explain the low number of flukes retrieved from the hippopotamus in view of incomplete adaptation. This is however considered unlikely because most gastropods are introduced as uninfected juveniles or eggs. Another explanation could be a recent host switch of this species from a different final host to hippopotami. Alternatively, this species was already present in Zimbabwean hippopotami but was never discovered and is now transmitted through an invasive gastropod, potentially increasing transmission rates (i.e. *spillback*). For the latter two hypotheses it is possible that this species belongs to the family Brumptiidae (Skryabin, 1949). We make this final suggestion based on the absence of reference sequences for this family on GenBank and the presence of small caudal appendages present on both of our individuals, indicating that our specimens could be immature or adapted to a different host (visible on the right individual in **Annex Figure 6.5**). *Brumptia bicaudata* (Poirier, 1909) the only described species in this genus is known to infect elephants and rhinoceros but has not been listed as a hippopotamus parasite. Again very little is known about this taxon and Willmott (1960) is the only author to provide a drawing of the species.

Table 7: Trematodes listed per host species. The host species are listed with BGL: *B. globosus*, B.spp.: *Bulinus* spp., BPF: *Bi. pfeifferi*, COL: *P. columella*, GYR: *G. connollyi*, HEL: *H. duryi*, LNA: *L. natalensis*, MEL: *M. tuberculata*, PHY: *P. acuta*, RAD: *Radix* sp., TRU: *B. truncatus* and Hippo: *Hi. amphibious*.

Host	Parasite					
COL	*F. nyanzae*					
LNA	Type VI	Type VIII	Type IX	Type XII	*F. gigantica*	*F. nyanzae*
BPF	Type III	Type IV	Type VIII	*S. edwardiense*	*S. mansoni*	
TRU	Type IV	Type X	*S. mattheei*			
B.spp.	Type VII	Type IX				
BGL	Type IIa	Type II	Type XI	*S. haematobium*	*S. mattheei*	
PHY	Type III	Type V	Type IX			
HEL	Type IX					
GYR	Type IV	Type IX				
MEL	Type I	Type XIII				
RAD	Type XIV	*F. nyanzae*				
Hippo	Type X	Type XIV	*F. nyanzae*			
Cattle	Cattleamphtype1	Cattleamphtype2	*F. gigantica*			

Stool samples were collected from cattle, zebras, crocodiles, buffalos, baboons, hippos and elephants. Isolation of trematode DNA failed for each sample. Five 18S sequences were obtained and two sequences of sufficient quality (HQ > 80%) were BLASTED. Resulting in 100% identical BLASTS with an alveolate (180 bps) in Zebra stool and one environmental eukaryote sample from the Netherlands (321 bps) from cow stool. Stool from the culled *Hi. amphibious* was collected as a positive control for DNA extraction. All amplification attempts also failed for these samples.

2. Infection prevalence

2.1. Shedding

Shedding experiments revealed 21 gastropods infected with trematodes. Of these, seven are *L. natalensis*, one *Bi. pfeifferi*, 11 *B. globosus* and three *Bulinus* sp. 2. All cercariae released by *L. natalensis* specimens were Xiphidiocercariae (site 1, 3, 5 and 7, **Figure 15.1**). Similarly, all three *Bulinus* sp. 2 shed amphistome type cercariae (site 1 and 3, **Figure 15.2**), which encysted rapidly on the side of the well to form metacercariae. *Bi. pfeifferi* shedded one echinostome cercaria (Type III, site 1, results not shown). In contrast, four different cercarial types were recovered from *B. globosus*. Seven shed furcocercous (fork-tailed) cercariae, six of which were *Schistosoma* genus type cercariae (site 5 and 7, **Figure 15.3**) and one Longifurcate-pharyngeate distome cercariae (Strigea cercariae, site 5, **Figure 15.4**). Of three

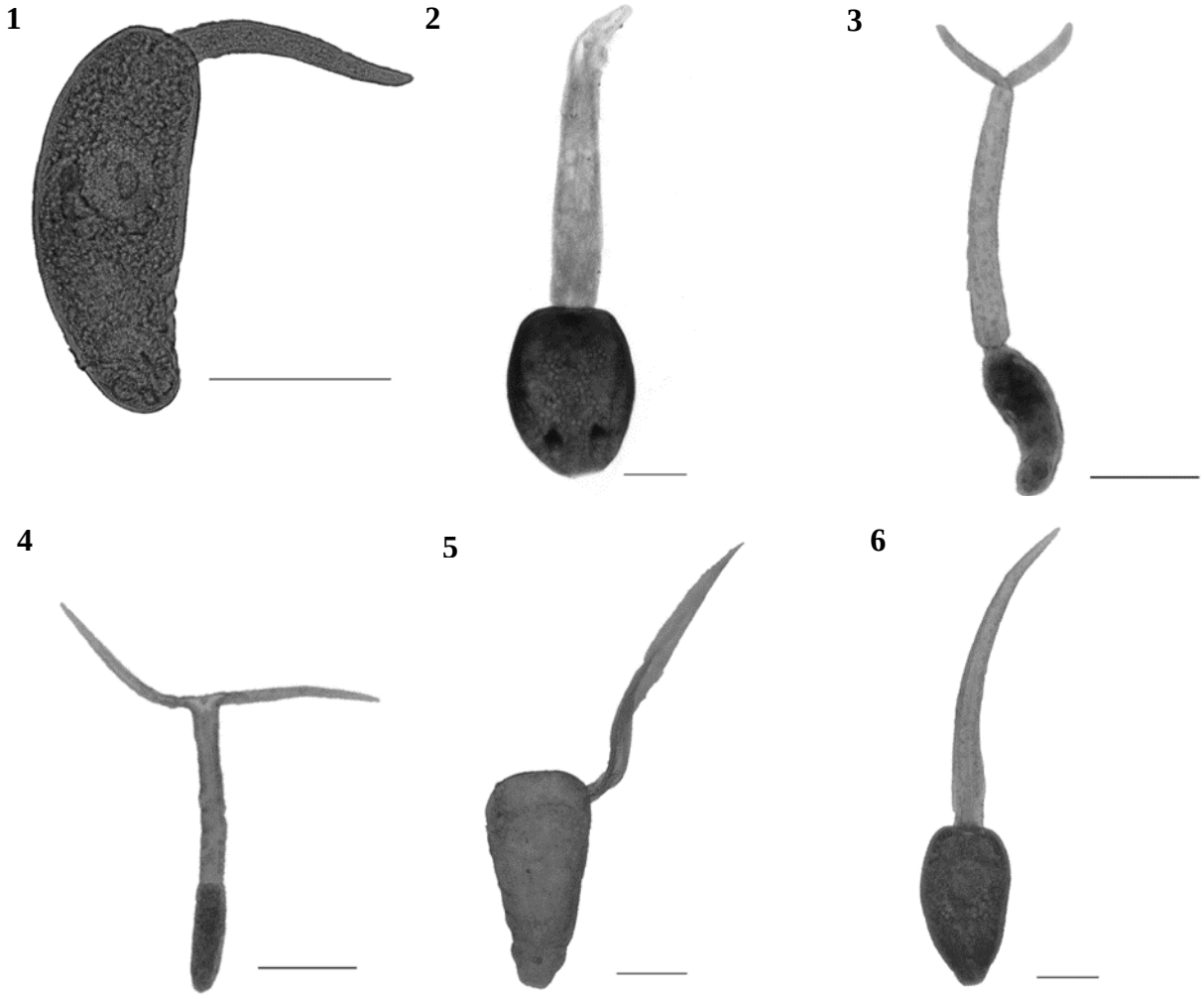


Figure 15: Cercarial morphotypes identified during shedding experiments. The following morphotypes are shown: Xiphidiocercaria (1), amphistome type cercaria (2), *Schistosoma* genus cercaria (3), Longifurcate-pharyngeate distome cercaria (4), echinostome type II cercaria (5), echinostome type IIa cercaria (6). Pictures are re-scaled for improved interpretation and are shown in greyscale. Scale bars represent 100 µm in each respective picture.

other shedding individuals in site 4, two released their own respective echinostome cercariae (Type II, **Figure 15.5** and Type IIa, **Figure 15.6**). No pictures or molecular data were obtained for the third shedding individual of site 4 because cercariae could not be retrieved from the tube. Visual inspection during the experiment had shown cercariae similar to those of Type II but this remains unresolved. With respect to total snail counts per species, seven out of 484 *L. natalensis* (1.4%), one out of 411 *Bi. pfeifferi* (0.2%), three *Bulinus* sp. 2 out of the 40 tested *Bulinus tropicus*/*truncatus* complex (7.5%) and 11 out of 233 *B. globosus* (4.7%) had trematode infections based on shedding experiments. All other gastropod species appeared either uninfected (*P. acuta, G. connollyi, Bulinus* sp. 1 and *M. tuberculata*) or were not tested (site 10: *Bellamya* sp., *Oxyloma* sp., *Helisoma duryi* and *P. columella*).

2.2. Molecular assays

The E.Z.N.A. kit caused no PCR inhibition after DNA extraction. Nine pools (8.3%) of CHELEX® - based DNA extractions failed initial PCR amplification. Following an additional 1 / 10 dilution, one (2.8%) of the individually tested samples had to be discarded since a 1 / 100 dilution still inhibited PCR amplification. In total, 61 gastropods from Lake Kariba and 735 from Harare region were tested for trematode infections. Of all tested pools, 162 (88.5%) were found to host trematode infections. Of these 61 (Kariba) and 353 (Harare region) gastropods were tested individually, resulting in trematode infection prevalences of 80.3% and 58.1% respectively. Samples not showing *Schistosoma* or *Fasciola* trematode signals in the pooled design were not individually tested. The screening set-up causes trematode infection prevalence of the remaining gastropods (n = 382) to range between 25 and 100%. This infection range is a result of the pooled design. At least one out of the four gastropods per pool hosts a trematode infection and maximally all four can host trematodes, resulting in the infection prevalence range between 1/4 (25%) and 4/4 (100%). Trematode and gastropod abundances (absolute counts) and trematode prevalences (number of hosts infected out of the tested hosts) are shown in **Annex Table 12**. Out of the 435 planorbids screened for *Schistosoma* sp. infections using the RD-PCR, 35 were infected (prevalence of 8%). 18 lymnaeids out of 195 tested samples appeared to host *Fasciola* sp. infections based on the RD-PCR (prevalence of 9.2%). This contrasts with the 80.3% *Fasciola* sp. infection prevalence found in Kariban Lymnaeids. All other species were also tested using the RD-PCR protocols, but none were found hosting *Schistosoma* or *Fasciola* sp. infections.

Double infections were found in eight different gastropods: three *L. natalensis* were infected with both *F. nyanzae* and trematode Type VI, one with *Fasciola* sp. and trematode Type VI, one with *F. nyanzae* and trematode Type IX, one double infection was detected in a *B. globosus*, hosting both *S. mattheei* and trematode Type IV and two double infections were detected in *Bulinus* sp. 1, co-infected with *S. mattheei* and trematode Type IX. Co-infected *L. natalensis* originated from site 8b, *B. globosus* from site 9 and both co-infected *Bulinus* sp. 1 were found in site 7. All but one double infection were detected by comparing sequencing results of a nested and non-nested PCR approach. The nested approach detected *Schistosoma* or *Fasciola* sp. infections, while the non-nested approach amplified other trematodes. The other double infection was detected through the infection RD-PCR combined with sequencing results, whereby a *Fasciola* sp. signal was visible in the multiplex PCR, but sequencing resulted in detection of another parasite species.

3. Gastropod ecology

3.1. Gastropod abundance

A total of 1575 gastropods were collected during this study. Between-site counts were highly variable and are shown in (**Table 8**). The highest gastropod count (n = 397) was encountered in site 3, of which 321 individuals were identified as *L. natalensis*. When comparing systems, Mazowe (site 1 and 2) and Mwenje reservoir (site 4, 5, 7, 8 and 8b) had the highest total gastropod counts (639 and 525 resp.). Despite having a lower gastropod abundance, Mwenje reservoir contained nine different species, resulting in the highest species richness amongst all sampled reservoirs.

Table 8: Total gastropod abundances per site, per reservoir and per species are provided along with species richness and site (Site prev.) and reservoir (Res. prev.) prevalence. *Bulinus* sp. 1 and 2 are listed under the common name 'Bspp.' because unclarity remains for site 1, 3 and 8 concerning the presence of one or two of these species. The presence of *Bulinus* sp. 1 and 2 is given between parentheses in each respective row, ≥1 indicates uncertainty in the presence of both species (detected through molecular analysis). Other species lister are: *B. truncatus* (TRU), *B. globosus* (BGL), *Bi. pfeifferi* (BPF), *P. acuta* (PHY), *L. natalensis* (LNA), *G. connollyi* (GYR), *M. tuberculata* (MEL), *Bellamya sp.* (BEL), *H. duryi* (HEL), *Oxyloma sp.* (OXY) and *P. columella* (COL). Site 6 and 9 are circumvented for reservoir prevalences because of their riverine origin. Sites per reservoir are listed in **Table 3**.

Site	Bspp.	TRU	BGL	BPF	PHY	LNA	MEL	GYR	BEL	HEL	OXY	COL	Total	Richness
1	5(≥1)	5	3	114	50	65	0	0	0	0	0	0	242	6
2	0	24	0	0	0	3	0	0	0	0	0	0	27	2
3	30(2)	0	8	0	38	321	0	0	0	0	0	0	397	5
4	0	2	30	6	6	2	6	0	0	0	0	1	53	6
5	0	0	50	26	46	25	0	0	0	0	0	0	147	4
6	0	0	0	3	1	1	0	7	0	0	0	0	12	4
7	2(1)	1	77	127	14	12	1	1	0	0	0	0	235	8
8	2(≥1)	0	1	2	0	28	0	0	0	0	0	0	33	4
8b	1(1)	0	9	6	24	17	0	0	0	0	0	0	57	5
9	0	1	61	127	3	10	0	0	0	0	0	0	202	5
10	0	0	0	0	1	0	0	0	5	143	6	15	170	5
Total	40	33	239	411	183	484	7	8	5	143	6	16	1575	12
Site prev.	5	5	8	8	9	10	2	2	1	1	1	2	11	
Mazowe	35	5	11	114	88	386	0	0	0	0	0	0	639	6
Henderson	0	24	0	0	0	3	0	0	0	0	0	0	27	2
Mwenje	5	3	167	167	90	84	7	1	0	0	0	1	525	9
Chivero	0	0	0	0	1	0	0	0	5	143	6	15	170	5
Res. prev.	2	3	2	2	3	3	1	1	1	1	1	2	3	

The smallest number of gastropods recovered from a single site were 12 individuals from four different taxa in site 6. Site 2 was found to be the least species diverse, solely hosting two species (*B. truncatus* and *L. natalensis*). In contrast to site 7, which hosted eight different

species and subsequently had the highest species richness. *Bi. pfeifferi* (411) and *L. natalensis* (484) showed the highest abundance of collected gastropod species. Taxa caught at lowest abundances were *G. connollyi*, *M. tuberculata*, *Bellamya* sp. and *Oxyloma* sp. (resp. 8, 7, 6 and 5). These species, together with *H. duryi* had the smallest geographical distribution and were found in maximally two different sites within a single reservoir. Exotic gastropods collected were: *P. acuta*, *H. duryi* and *P. columella*, of which *P. acuta* occurred at highest abundance. Having colonized all riverine locations and reservoirs, except for Henderson's reservoir, *P. acuta* appears to have the broadest geographical distribution. Uncertainty remains regarding the foreign or native origin of the *Oxyloma* sp.

3.2 Multivariate community analysis

the 11 sampled sites are divided into five distinct groups, based on UPGMA (Unweighted Paired Group Method with Arithmetic mean) hierarchical clustering using a Chord distance matrix. Chord distance was chosen to calculate the dissimilarity matrix because it resulted in the lowest stress value relative to the Bray-Curtis method. Additionally, it is a method often used to analyse community data. UPGMA clustering method was selected based on both the

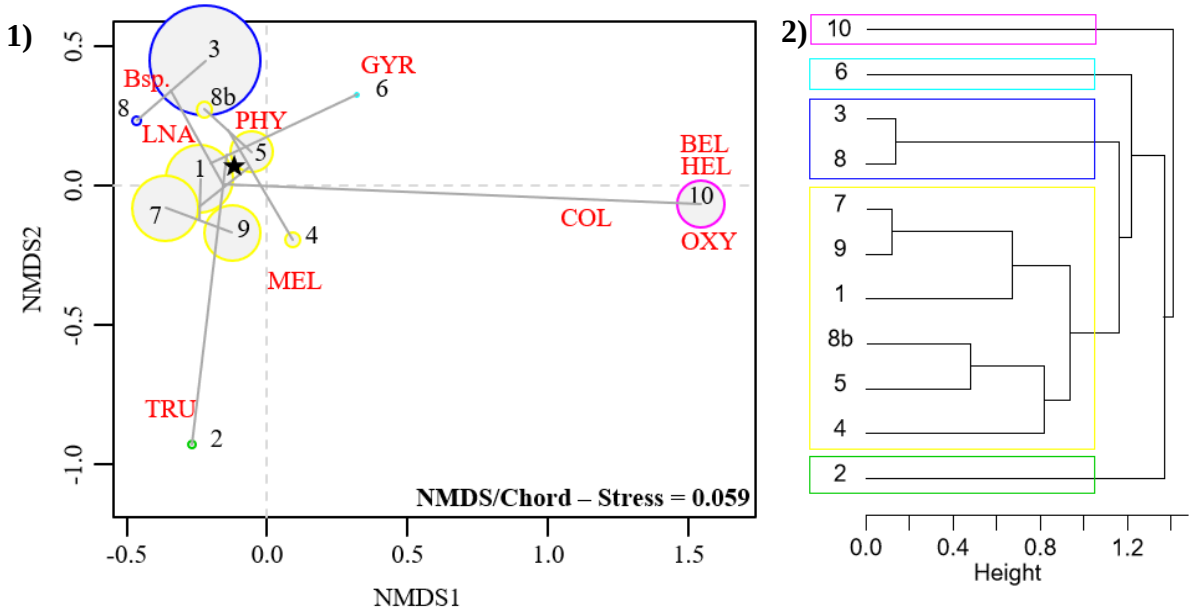


Figure 16: NMDS plot and UPGMA clustering dendrogram based on gastropod abundance per species per site. 1) NMDS plot with scores, in this case circle centres, displaying the 11 different sites. Species (variables indicated in red codes) displayed are:, *Bulinus* sp. 1 and 2 (Bsp.), *B. truncatus* (TRU), *L. natalensis* (LNA), *P. columella* (COL), *G. connollyi* (GYR), *H. duryi* (HEL), *Oxyloma* sp. (OXY), *M. tuberculata* (MEL), *Bellamya* sp. (BEL) and *P. acuta* (PHY). *B. globosus* and *Bi. pfeifferi* occupy the same coordinates and are indicated by the star symbol to improve figure readability. The clustering dendrogram is indicated by grey full lines. The size of each respective circle corresponds to the total gastropod abundance. **2)** UPGMA cluster

dendrogram for different sites based on gastropod community data. Optimal number of clusters is determined to be five and are each represented by a different colour. The same colour code is applied in 13.1.

highest cophenetic and lowest Gower distance relative to other methods. **Figure 16.1** visualizes the gastropod community per site through nonmetric multidimensional scaling (NMDS). The relative position of each score (site) to other sites and plotted variables (gastropod species) indicate their relatedness. **Figure 16.2** indicates the accompanying UPGMA-based cluster dendrogram.

Site 10 is separated from other locations and is characterized by the presence of *Oxyloma* sp., *H. duryi, Bellamya* sp. and *P. columella*. Of which, only *P. columella* was found in one other location. The three other species are thus site-specific to site 10. Site 6 forms its own cluster due to the low abundance of gastropods in this location and a relatively high count of *G. connollyi* (seven out of 12 collected gastropods). The same is true for site 2, which is characterized by the high relative abundance of *B. truncatus* (24 out of 27 gastropods). Site 3 shows the highest gastropod abundance.

The same methods as previously mentioned were used to construct UPGMA-based clustering of the 11 sampling sites based on macrophyte presence/absence data collected at each location. **Figure 17.1** and **17.2** visualize the macrophyte communities in each site through NMDS and the accompanying UPGMA-based cluster dendrogram respectively.

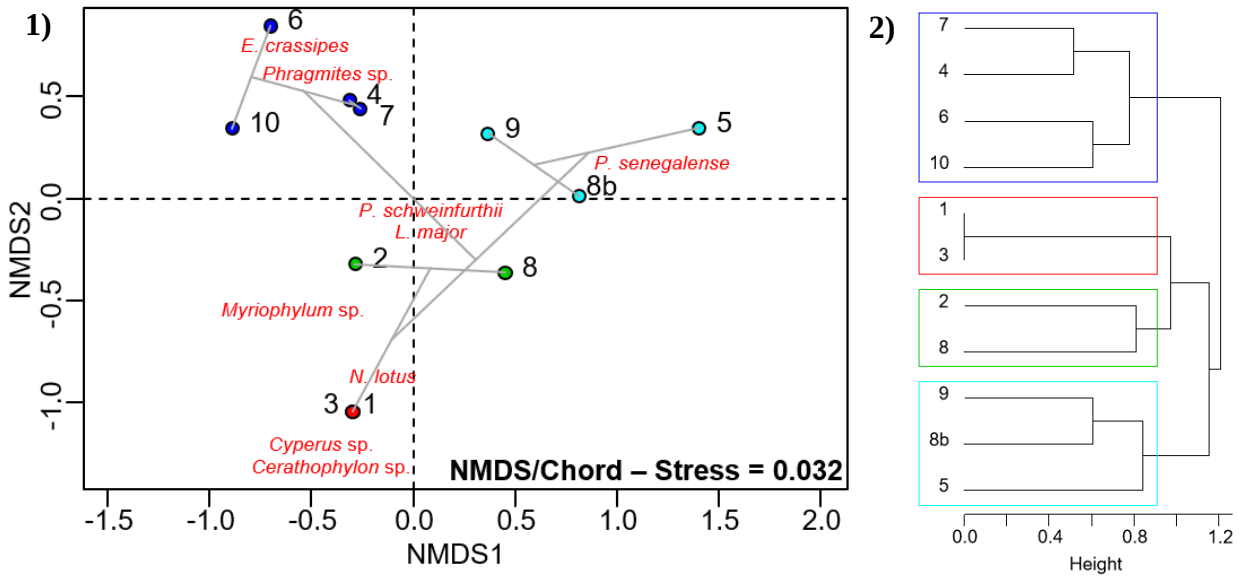


Figure 17: NMDS plot and UPGMA clustering dendrogram based on macrophyte presence or absence data per species per site. 1) NMDS plot with scores, in this case circles, displaying the 11 different sites. Macrophyte species are indicated in red. The clustering dendrogram is indicated by grey full lines. **2)** UPGMA cluster dendrogram for different sites based on macrophyte community data. Optimal number of clusters is

determined to be four and are each represented by a different colour. The same colour code is applied in 1). Site 1 and 3 have the same score but have been moved for interpretational purposes.

Site 1 and 3 form the red cluster and have the same exact score and are thus characterized by identical macrophyte communities. The light blue cluster is mainly formed through the presence of *Polygonum senegalense*. Site 4, 6, 7 and 10 mainly cluster due to the presence of *E. crassipes* since this macrophyte is absent in other locations.

The principal component analysis (PCA) explained 86.4% of the total variation in physico-chemical data across sites through the PC1 and PC2 axis. PC1 contained most of this variation (67.9%). **Figure 18** visualizes the scores for the first six sampling locations for all included variables (depicted as descriptors). Other sites were omitted due to missing data values. The complete list of abiotic measurements per site are listed in **Annex Table 13**. The large geographical variation (i.e. different reservoirs) between sites, prevented correct estimates of these missing values through inference. PC1 is strongly positively related with total dissolved solutes (TDS, in mg/l) and conductivity (µS/cm) and negatively correlated with pH. PC2 is positively correlated with dissolved oxygen (DO, in mg/l) and negatively with water temperature (T, in °C).

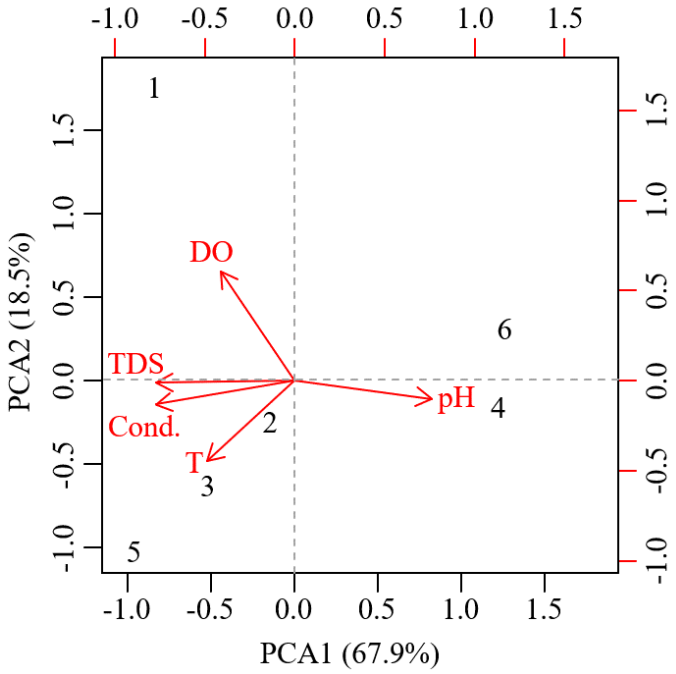


Figure 18: PCA distance biplot displaying correlations among sites according to physico-chemical water parameters. Only the six first sites could be included due to missing data from the other locations. The scores, in this case numbers, represent their corresponding site. The biplot focussed on the sites and the (dis)similarity between them in terms of the variables. Descriptors indicated through red vectors are: total dissolves solutes (TDS, in mg/l), conductivity (Cond., in µS/cm), water temperature (T, in °C), dissolved oxygen (DO, in mg/l) and pH.

Three different redundancy analysis were performed to investigate explanatory values of abiotic and biotic factors on gastropod community. Firstly, the correlation between macrophytes and gastropod communities were tested. Resulting models were all non-significant and only included one significant factor ($p < 0.05$) being *Cerathophylon* sp., *Cyperus* sp. or *Phragmites* sp (results not shown). Secondly, the variability of gastropod

communities explained by physico-chemical parameters were investigated for site 1 to 6. The resulting model was highly insignificant, and no variables proved significant during the forward stepwise model selection using permutation tests (results not shown). Finally, Both the NMDS and PCA axis from **Figure 17** and **18** respectively, were used to investigate the explanatory value of both abiotic and biotic factors on gastropod communities. The resulting models were also highly insignificant (results not shown). Insignificance remained when either abiotic or biotic factors were kept constant. Although RDA analysis did not show any correlation between gastropods and biotic and abiotic factors, two field observations are worth mentioning. We found high numbers of *B. globosus* gastropods in or adjacent to reed patches and all *P. columella* gastropods were collected from *E. crassipes*. This pattern is not seen in the RDA analysis because vegetation types were noted per site and no within site variations were collected.

Discussion

The aims of this study were: 1) Do the man-made reservoirs in Zimbabwe (Mwenje dam, Mazowe dam and Lake Chivero) contain invasive gastropod species? 2) If so, do they play a role in GBT transmission? 3) Which final hosts are affected by these GBTs? A lot of data/ a wide range of results were generated, uncovering important trends and alternations in trematode epidemiology in Zimbabwe. We will discuss a selection of the most important results below. But first we should elaborate on the limitations encountered during this study.

1. Constraints

As with any research, we experienced significant constraints. Ten locations were studied across four different reservoirs in the Zimbabwean highlands. These locations selected for a good initial overview of trematode transmission, still leave a considerable number of large reservoirs unsampled (~98%). Even the sampled reservoirs are significantly under sampled as we focussed on only a few sites per reservoir. This is important because transmission sites are heterogeneously spread across a water body (Smith, 2001). Secondly, the lack of measurement equipment on certain days impeded a standardized collection of abiotic variables. Additionally, because typically only a small percentage of a gastropod population is infected, some harbouring important infections could have been missed. Our methodology was not suitable for haplotype networks of gastropods because additional gastropods were sequenced once a *Fasciola* or *Schistosoma* sp. infection was detected. This creates a bias

towards specific locations with high infection rates. The large barcoding gap proved a major limitation for gastropod and trematode identification and is recognized as a significant issue by the IPBES report on global biodiversity (Díaz *et al.*, 2019). The reference sequences that exist might still be wrongly identified since many gastropod species show overlapping geographical distributions and similar morphologies. The knowledge gap also prevents the comparison of our sequences to exotic species, and thus the detection of potential *spillover* effects. Since we mainly relied on molecular methods to detect trematode infection (rather than classical shedding protocols), we might have some false positives. Gastropod species that are not suitable hosts will rapidly abort the infection, but the highly sensitive PCR might still detect this signal (Schols *et al.*, under review). However, we deem this chance small. Even if some detected infections are aborted, our results remain a reasonable estimate of the trematode diversity present in the environments we sampled. This is supported by shedding of some individual gastropods. The unsuccessful collaboration with Howard's hospital prevented us from analysing schistosome infection rates in the local population and the effect of the mass drug administration in the region. The failure of DNA extraction from stool samples prevented the study of host specificity of flukes in Lake Kariba and the trematode diversity infecting cattle around our sampled reservoirs. The small surface area studied of the 3 m long hippopotamus stomach (Furstenburg, 2012) and the limited samples taken from an abattoir, make it very likely that our results are a severe underestimation of the amphistome burden on ruminants in Zimbabwe. We are no specialists in amphistome or fasciolid morphological identification and a considerable knowledge gap of animal trematodiases still remain; hence, our species might be novel to science.

Possible solutions to most of these constraints are listed under perspectives.

2. Major findings

New records of trematode, gastropod and other invasive species are described. We report: the presence of water hyacinth (*Eichhornia crassipes*) upstream, downstream and in Mwenje reservoir; the gastropod *Gyraulus connollyi* North of the Limpopo River Valley and its potential role in trematode transmission; the presence of *Pseudosuccinea columella*, *Physa acuta* and *Helisoma duryi* as invasive exotic gastropods and report on trematodes they transmit in Zimbabwe; the gastropod *Bulinus truncatus* in four reservoirs and its potential function in the *Schistosoma mattheei* lifecycle; the presence of potentially two other gastropod species belonging to the *B. truncatus/ tropicus* complex; *Biomphalaria pfeifferi* as a

novel intermediate host for *Schistosoma edwardiense*; *Schistosoma mansoni* downstream of Mwenje reservoir; the presence of *S. edwardiense, S. mattheei* and *Schistosoma haematobium* in gastropods in Mwenje reservoir; the detection of seven trematode superfamilies: Diplostomoidea, Echinostomatoidea, Microphalloidea, Opisthorchioidea, Paramphistomoidea, Plagiorchiodea and Schistosomatoidea, each having zoonotic species; the liver fluke *Fasciola nyanzae* in both Lake Kariba and Mwenje reservoir and its transmission through both the native gastropod *Lymnaea natalensis* and the invasive gastropod *P. columella*; a large genetic diversity in *F. nyanzae*; *F. nyanzae* infecting hippopotami in Lake Kariba; *Fasciola gigantica* (liver fluke) infections in *L. natalensis* in Mwenje reservoir and in slaughtered cattle in Koala Park abattoir; identification of intermediate and final hosts of three hippopotamus helminth parasites in Lake Kariba and the presence of the gastropod predating leech *Helobdella europaea* in Lake Chivero.

3. Man-made reservoirs and their role in disease transmission

Man-made reservoirs create a permanent supply of water, which is used for fishing, irrigation, drinking water and hydroelectricity (Morley, 2007). Moreover, dams protect people from floods and reduce global methane emissions (Muller, 2019). However useful they might be, man-made reservoirs pose several negative pressures on the environment and many less-harmful alternatives exist (see introduction; Díaz *et al.*, 2019; Schmitt *et al.*, 2019). The most significant changes caused by man-made reservoirs with respect to this study are: a change from riverine to lake environment, providing additional breeding grounds for disease vectors; a permanent water source, providing a year-round habitat for gastropods and thus increases their population size; profoundly disturbed environments; creating a stepping-stone model for invasive species and finally bad reservoir design creates temporary puddles, increasing mosquito breeding areas and thus malaria transmission (WHO, 1982; Lerer and Scudder, 1999; McAllister *et al.*, 2001; Sow *et al.*, 2002; Havel *et al.*, 2005; Townsend *et al.*, 2008; Sokolow *et al.*, 2017). Also agrochemical use on nearby lands have a stimulating effect on gastropod populations by increasing algal growth and reducing gastropod predator populations (Halstead *et al.*, 2018). Therefore, environmental management is needed to limit the increase of vector-borne diseases. An example would be to increase the slope of reservoirs and faster drainage of puddles in the dry season to avoid temporary puddles as suggested by the WHO in 1982 to combat increasing mosquito populations. This management could also aid the control of gastropod-borne diseases. We hypothesise that temporary puddles also

increase trematode disease transmission. This could explain the increased trematode prevalence and presence of trematode species not found in other locations in site 8b. The temporary puddles formed when water levels recede, concentrate gastropods in a small area, in which a single stool dropping could infect all individuals present. Once the puddle nearly desiccates, gastropods aestivate and survive until the water levels rise again (Strong *et al.*, 2008). The increased gastropod densities and miracidial exposure in small puddles, stress the importance of gastropod diversity to provide sufficient decoy hosts to restrain trematode transmission in man-made reservoirs. Nearby reed patches, known to be associated with *B. globosus* (Cetron *et al.*, 1996), could increase transmissions further by assisting gastropod aestivation, increasing their survival rate (Cridland, 1967).

The disturbed nature of man-made reservoirs makes them especially vulnerable to invasive species. Colonisations by invasive species could trigger an *invasional cascade*, which has been reported in man-made reservoirs in Zimbabwe and the USA (Ricciardi, 2001; Carolus *et al.*, 2019). The presence of the invasive leech *H. europaea*, the invasive gastropod *P. columella* and the invasive water hyacinth, *E. crassipes*, indicate a potential *invasional meltdown* in Zimbabwe's man-made reservoirs. The leech (*H. europaea*) could assist in gastropod control. However, *P. acuta* and *P. columella* have superior anti-predation techniques when compared to endemic species (Appleton *et al.*, 2004). Therefore, we believe the invasive leech could facilitate the spread of invasive gastropods by eliminating their endemic competitors.

4. Exotic gastropods and their role in disease transmission

Three invasive gastropod species have been found in this study; namely *Helisoma duryi*, *P. acuta* and *P. columella*; some more widespread and abundant than others. *Helisoma duryi* was only found in Lake Chivero. Its susceptibility in the transmission of *Gastrodiscus aegyptiacus* might make it an important intermediate host responsible for recent outbreaks in horse and pig populations in Zimbabwe (Mukaratirwa *et al.*, 2004). The presence of this gastropod in the reservoir of a nature reserve hosting warthogs and zebras could have serious consequences for disease transmission and could threaten the conservation of these species. *P. acuta* was the most widespread invasive gastropod and was found in nearly all reservoirs. Neither of the three species was found to host unique trematodes, and therefore our results suggest the absence of a *spillover* effect. Based on shedding experiments the *enemy release* hypothesis seemed likely for *P. acuta*, although sequenced infections appear to indicate a *spillback*

effect. Both effects have previously been described for *P. acuta* populations (Mitchell and Leung, 2016; Ebbs *et al.*, 2018). Its transmission of echinostomes as a secondary intermediate host has been known for decades in Southern Africa (Appleton, 2003). This would result in human infection upon consumption of the infected gastropod. The most significant impacts of an exotic gastropod on trematode transmission have been found for *P. columella* (Bargues *et al.*, 2011; Grabner *et al.*, 2014; Lounnas *et al.*, 2017; Carolus *et al.*, 2019). Although we did not detect trematode infections in *P. columella*, the spread to at least two other reservoirs in Zimbabwe, one of which within a nature reserve, could have significant consequences for fasciolosis epidemiology.

5. Epidemiology of *Fasciola*

Even though several *Fasciola* species have been reported in Zimbabwe, little knowledge exists on gastropods transmitting *Fasciola* parasites in Zimbabwe. Pfukenyi *et al.* (2006) refer to Needham (1977), which described *L. natalensis* as the intermediate host of *F. gigantica* in Zimbabwe. Unfortunately, the article could not be validated. To the best of our knowledge, Needham (1977) is the only source reporting on *L. natalensis* transmitting *F. gigantica* in the field in Zimbabwe, prior to this study. Identifying a parasite's complete lifecycle is the first step in disease control and prevention of epidemics. Carolus *et al.* (2019) report the transmission of a *Fasciola* sp., identified as *F. nyanzae*, in Lake Kariba through the invasive gastropod *P. columella*. The broad final host range of both *F. gigantica* and *F. hepatica* combined with the known susceptibility of *Hippopotamus amphibious* to *F. gigantica* and *F. nyanzae* and the many hybridization events between *F. gigantica* and *F. hepatica* (Hoa Le *et al.*, 2008; Nguyen *et al.*, 2018) suggest that hybridization between these three species is possible. Until recently, resistance of *L. natalensis* to *F. hepatica* (Mas-Coma *et al.*, 2009) created a geographical barrier between *F. hepatica* and *F. nyanzae*. However, the recent spread of *P. columella* across Zimbabwe might allow *F. hepatica* to colonize new regions, breaking down this geographical barrier and potentially affecting disease transmission in these regions (Bargues *et al.*, 2011; Grabner *et al.*, 2014; Lounnas *et al.*, 2017; Carolus *et al.*, 2019). As discussed in the introduction (5.2 Hybridization), hybrid heterosis could play an important role in altering disease epidemiology (Webster and Southgate, 2003). Therefore, it is of utmost importance to monitor the role of *P. columella* in *Fasciola* species transmission around the world and especially in regions where multiple potentially hybridizing species occur.

6. *Schistosoma* epidemiology

Zimbabwe and neighbouring regions host six different *Schistosoma* species (see introduction). Our study managed to detect three schistosomes endemic to Zimbabwe; namely *S. mansoni, S. mattheei* and *S. haematobium*; together with a novel species, *S. edwardiense* (**Figure 12**). This latter species is known to infect *Hi. amphibious* as a final host and *Bi. sudanica* as an intermediate host. It was previously described in hippopotami in Uganda (type locality: Lake Edward) and the Kruger National Park (South Africa). Sequences of *S. edwardiense* were isolated from both *B. globosus* and *Bi. pfeifferi.* Based on the bright amplification signals of parasite DNA in *Bi. pfeifferi* and the known compatibility of this genus with *S. edwardiense* (Morgan *et al.*, 2003), we consider it highly likely that *Bi. pfeifferi* acts as a suitable intermediate host for *S. edwardiense* in Zimbabwe. The unknown compatibility of the *Bulinus* genus with *S. edwardiense* suggests *B. globosus* to be a decoy host for this parasite, although, based on PCR, twice as many were found infected compared to *Bi. pfeifferi.* However we do not exclude the potential of *B. globosus* as intermediate host because the related *S. hippopotami* is transmitted by *B. truncatus*, a representative of the same genus (Morgan *et al.*, 2003).

Zimbabwe implemented a countrywide Mass Drug Administration program (MDA) from 2012 to 2016 (Mutapi *et al.*, 2017). Praziquantel treatments, when applied according to guidelines, kills the adult worms, releasing a large amount of antigens and subsequently boosting the hosts immune response (Mutapi *et al.*, 2017). This results in increased schistosome immunity for the next five years, but it is not known whether the resistance remains or decays after that (Mutapi *et al.*, 2017). When MDAs are discontinued, models suggest that the reduced exposure to schistosomes and the removal of the antigenic stimulation can result in infection rates higher than before the MDA (Mutapi *et al.*, 2017). The same pattern was detected for malaria infections in Tanzania, whereby treated patients that became re-infected showed higher pathology compared to non-treated patients due to a more naïve immune response (Mutapi *et al.*, 2017). The presence of two human infecting schistosomes in the Mwenje catchment area, sufficient to maintain an active transmission site (Toledo and Fried, 2014), indicates that these predictions could come true. The increased infection rates could be reinforced because of drug resistance (Huyse *et al.*, 2013) and the widespread presence of *S. mattheei,* a species known to hybridize with *S. haematobium* (King *et al.*, 2015). The immune response against schistosomes is species specific, which indicates that *S. mattheei* will not reduce susceptibility to other *Schistosoma* spp. (Bickle *et al.*, 1985).

Therefore, it is vital that local inhabitants and other final and intermediate hosts are regularly screened to asses potential schistosome epidemics.

7. Trematodes in a *One Health* context

Since the land reforms in 1980, Zimbabwe has been in dire need of improved wildlife management. Hippopotamus populations have plummeted in most regions as a result of climate change (i.e. droughts), siltation of rivers, legal and illegal hunting, habitat fragmentation, destruction by expanding human populations and mercury pollution caused by illegal gold mining (van Straaten, 2000; Degeorges and Reilly, 2007; Zisadza-Gandiwa *et al.*, 2011; Utete *et al.*, 2017). The potentially increased transmission rate through invasive gastropods, the decreasing hippopotamus populations and the associated loss in genetic diversity could result in increased parasite burdens on the already declining hippopotamus populations (King and Lively, 2012). A *One Health* approach maps the trematode diversity to effectively tackle trematodiasis outbreaks in human and animal populations and assists in preventing zoonoses (Gower *et al.*, 2017). Considering the *One Health* framework, we also studied non-schistosome and non-fasciolid trematode infections in gastropods. *Melanoides tuberculata* was infected with a trematode species of the family Microphalloidea that is most closely related to the Lecithodendriidae family (Type XIII; **Figure 14**) and a species of Opisthorchioidea, closely related to the Heterophyidae family (Type I; **Figure 14**). *Bi. pfeifferi, P. acuta* (Type III; **Figure 13**) and *B. globosus* (Type II and IIa; **Figure 13**) were all found to host Echinostomatidae infections. *P. acuta* and *L. natalensis* were found infected with Diplostomatidae based on COI (Type V and VI resp., **Figure 13**). *B. globosus* and *L. natalensis* were found infected with Diplostomatidae based on 18S (Type XI and XII resp., **Figure 14**). Type IV, Type VIII and Type IX are representatives of the superfamily Plagiorchioidea and were found in a broad range of gastropod species (see **Figure 14**). Type IX was found in five gastropod species belonging to five genera and three families. The only shedding cercariae were obtained from *L. natalensis* and therefore raises the question whether all other species are compatible hosts or instead serve as decoy hosts. The latter would underline the dilution effect of high gastropod diversity in a habitat.

Four adult amphistome species were retrieved during ruminant dissections, of which two could be linked to sequences obtained from gastropod intermediate hosts. One additional species was retrieved through shedding of a '*Bulinus* sp. 2*'* (Type VII, **Figure 13**). None of the five species could be barcoded to species level, indicating the large barcoding gap for

amphistomes. More effort should be spent on mapping amphistome diversity and lifecycle traits based on their high diversity, economic burden and host pathology to both wild and domestic animals (Toledo and Fried, 2014; Laidemitt *et al.*, 2016; Pfukenyi and Mukaratirwa, 2018). The hybridizing potential of trematodes and its potential consequences for disease transmission combined with the dwindling hippopotamus populations could result in further regional declines of this large herbivore and all the ecosystem services it supports. Consequences of interactions between global change and parasites could go much further than the apparent megaherbivores. One vertebrate group, the amphibians, have been especially suffering under the spread of the invasive Asian fungus *Batrachochytrium dendrobatidis*, which causes chytridiomycosis (Skerratt *et al.*, 2007). Suspicious amounts of amphibian corpses were found in Mwenje reservoir, suggesting a potential fungal infection, which needs further investigation. Chytridiomycosis has been decimating populations around the world and has driven many species to extinction (Skerratt *et al.*, 2007). Combined pressures of environmental changes and multiple infections could prove fatal for local amphibian populations (King and Lively, 2012).

8. Perspectives

This thesis generated a lot of hypotheses, some of which that could not be addressed here. Therefore, we make some suggestions for future work. The first issue to address is species identification. Based on the BLAST outcome our bulinid specimens are *B. truncatus.* However, this species cannot survive in the tropical temperature of Lake Kariba and no records exist for other locations in Zimbabwe (Samson Mukaratirwa, pers. com.). Even though our COI sequences match with *B. truncatus* from Spain, a region were no other species of the complex occur, this should be further examined. Since *B. truncatus* is the only known tetraploid species of the *truncatus/tropicus* complex, a karyotyping experiment on live snails could provide the answer. The species hypothesis of 'Hippoamphtype1' should be confirmed through comparisons of scanning electron microscopy and transverse and longitudinal sections with Leiper (1910) and Sey (1984). To validate the hypothesis for 'Hippoamphtype2', *Brumptia bicaudata* specimens, present in the museum collection, should be sequenced and photographed. This would be an excellent example of a taxonomic effort which will help close the immense taxonomic gap.

Next Generation Sequencing (NGS) offer alternative means to uncover the trematode diversity in snail specimens. NGS allows metabarcoding and would therefore easily detect

double or triple infections, while in our design, they were usually detected by chance. Additionally, if more markers could have been sequenced, our phylogenetic trees would have an increased resolving power and the short fragment issue would have been resolved. Therefore, if sufficient resources are present, it should be the standard method when analysing trematode infections. The use of 'high throughput' genomics is justified because of the significant economic burden caused by trematode infections (see introduction). It could have also been used to analyse the collected stool samples without a PCR amplification step. The method allows detection of very minute pathogen DNA quantities. Another more basic solution would have been the use of a Pitchford funnel to concentrate parasite eggs in the field. This would have made microscopical examination of the stool samples possible and facilitate egg isolation for miracidial hatching and PCR amplification. This would allow the study of host specificity of for example *F. nyanzae*.

Echinostome infections should be sequenced with 28S and nad1 amplification to facilitate comparison with Laidemit *et al.* (2019). Due to the late publication date (May 2019) of the study, we were unable to realize it for this study. A further methodological improvement would be the addition of an amphistome-specific marker to the RD-PCR, which could provide important epidemiological data in a *One Health* context. Another interesting comparison could be made between site 1 and 3 whereby an equal number of gastropods is sequenced. This would allow the detection of a potential increase in fish-borne trematodes in Mazowe reservoir caused by the adjacent fish farm. Also, regular sampling within the fish farm is important to prevent outbreaks of for example, the 'black spot disease' or fish-eye flukes transmitted by gastropods, which pose a significant economic burden (Toledo and Fried, 2014).

Extensive contacts should be made with the staff at the Howard's hospital. This will allow the study of schistosome infections in humans around Mwenje reservoir and the potential effect the MDA has had in this region. Collaborations with Lake Kariba park rangers and the veterinary department of the University of Zimbabwe allow the study of the population size of hippopotami and other wildlife species since the construction of Kariba dam, hopefully resulting in integrated and improved wildlife management in the region.

Global change will undoubtably continue, and the impacts on nature will only increase. Many species will succumb to pathogens and anthropogenic pressures if no actions are taken. Therefore, we hope that high-quality scientific research combined with other fields and global efforts could result in the proliferation of the rich biodiversity of both animals and their parasites still present on this planet.

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Appendix

1. Risk assessment

The risk assessment is split into two parts. The first part discusses all risks associated with sampling while the second part discusses all lab hazards associated with molecular work. The sampling period in Zimbabwe resulted in significant but preventable risks. Potential hazards were: attack by wild animals, sunburn, risk of vector-borne parasite infections (e.g. schistosomiasis and malaria) and food- and water-borne diseases (e.g. through faecal contaminations such as cholera). Associated hazards went from mild discomfort (e.g. light diarrhoea or sunburns) to death by trampling of elephants or an untreated cholera infection. Sunburns and heatstroke were prevented by wearing a hat, UV-blocking shirts and sunscreen (SPF 30) and the consumption of large quantities of water. Excess water was always kept in the car in case a heatstroke would occur. Attacks of wild animals are never completely excludable but were kept to a minimum by never sampling at dusk, dawn or night time; always having at least one person watching the nearby bushes and waterline; scanning the ground for venomous snakes, rapid removal of samples and no wading in crocodile-infested waters. A car was always kept nearby, in case an attack would occur, it would provide extra protection. Additionally, a first aid kit with venom pump was always at hand in the field. Trematodiases were prevented by avoiding all contact with water. This was done by wearing waders and plastic gloves. In case water contact did occur, it is sufficient to dry the skin as soon as possible to prevent skin penetration of the parasite. Mosquito-borne diseases were prevented by closing all windows before nightfall and the rigorous application of high percentage DEET to any unprotected skin area. Additionally, malaria medication was taken one day before departure until one week after returning from Zimbabwe (seven weeks in total). Faecal borne diseases (e.g. rabies and Salmonella) potentially transmittable to humans were accounted for by avoiding any skin contact and disinfection of any contaminated equipment by bleach. Water-borne diseases were prevented by only drinking bottled or boiled water. Food-borne diseases were prevented by only eating raw vegetables if they were properly disinfected. In case sickness did occur, sufficient travel drugs and needles were present to provide sufficient care until doctor consultation occurred, which lived next-door to our residence. Additionally, all proposed vaccines were accounted for before departure through multiple consultations with a health specialist.

In the lab, DNA extractions and GE posed the most substantial health risks. An open flame and a sharp scalpel were used during the DNA extractions of gastropod tissues. Both of these objects were handled with extreme caution. Some of the buffers used during DNA extraction and GE are toxic when they come in contact with unprotected skin. To prevent such injuries, lab gloves and a lab coat were always worn when working in the lab. In case skin contact would occur excessive rinsing with water would be executed while contacting the 'anti-gif centrum'. Dangerous steps during GE include: DNA visualization with MIDORI^{Green} Direct (Nippon Genetics^{TM}) and boiling agar solution. Severe burns were prevented by wearing heat resistant rubber gloves. Chloroform:isoamyl alcohol (24:1) was the only used reagent that required additional protective measures. This was done by extreme cautionary handling and by executing all pipetting steps under the suction hood while wearing protective gear. Hazard numbers are: H351, H361d, H331, H372, H302, H319 and H315. Precautionary recommendations numbers are: P201, P281, P302, P352, P309 and P311. Additional attention was paid when visualizing GE with UV-light and double checks were executed to make sure the UV-lighting was turned off before opening the machine door. This was necessary because UV-light has mutagenic effects on both skin and eyes. The only risk associated with PCR amplification was skin burning by touching the thermocyclers plate base. This was prevented by paying attention when placing the plates in PCR machines. Lab responsibles (Kenny Meganck and Nathalie Smitz) were always nearby to provide first aid if needed. During the extended writing and sequence processing period, sufficient brakes were taken, and proper ergonomics were applied to prevent back and neck injuries.

2. Sampling sites in Lake Kariba

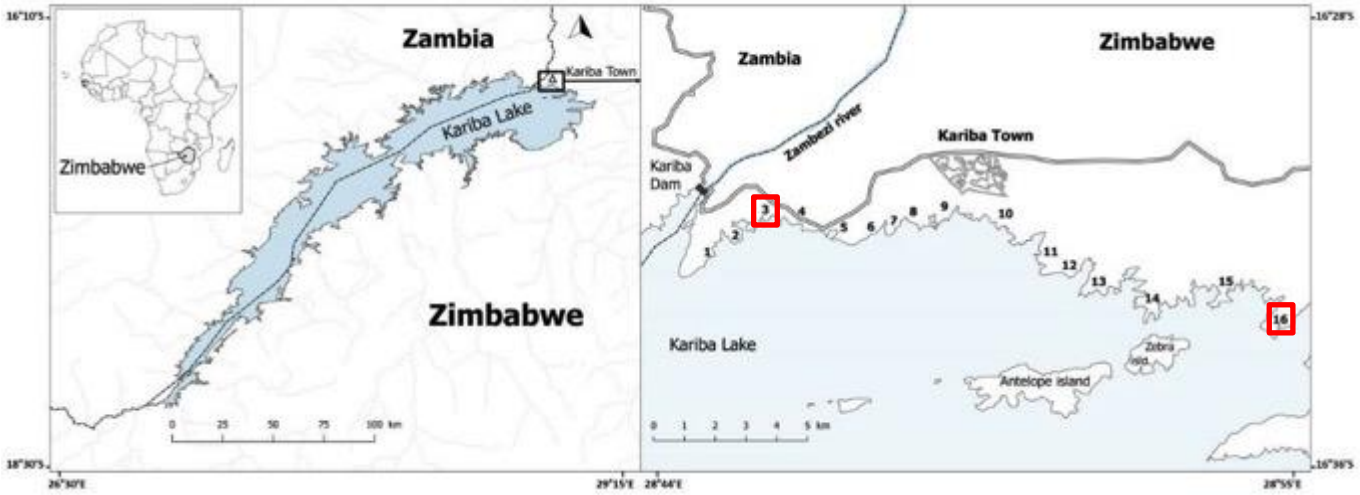

Figure 1: Sites '3' and '16' were included in our study based on both parasitological data collected by Hans Carolus and presence of gastropods. Image taken from Carolus *et al.* (2019). Maps were constructed in QGIS 2.18.28 ® software.

3. Sampling sites in Harare region

(Continued on next page)

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8b

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(Continued on next page)

Figure 2: **Photographs of all sites near Harare** region. Labels correspond to site name. Note that these pictures were taken in September and they do not represent the year-round situations because water levels fluctuate severely throughout the year.

Table 1: Harare region site information. All variables were noted in the field on the same day sampling occurred.

Site	Reservoir	Water type	Latitude (South)	Longitude (East)	Observed activities	Origin
1	Mazowe	Flowing water + standing edges	17°33'0.08"	31°00'01.6"	Fishing, boating, water irrigation, cattle	Man-made
2	Henderson's	Standing water	17°34'30.6"	30°58'26.5"	Fishing, irrigation for the fish farm	Man-made
3	Mazowe	Standing water	17°33'41.1"	30°59'31.0"	Fishing, waste water fish farm, cattle	Man-made
4	Mwenje	Standing water	17°14'54.4"	31°01'24.5"	Swimming, humans drinking water, cattle	Man-made
5	Mwenje	Standing water	17°14'51.6"	31°01'36.5"	Cattle	Man-made
6	Mwenje river	Flowing water + standing edges	17°18'42.6"	31°04'46.1"	Washing location	Natural
7	Mwenje	Standing water	17°14'53.3"	31°01'15.7"	Cattle, fishing	Man-made
8	Mwenje	Standing water	17°14'47.9"	31°01'07.7"	Cattle, fishing	Man-made
8b	Mwenje (small puddle)	Standing water	17°14'47.9"	31°01'07.7"	Cattle	Man-made
9	Mwenje river	Slow Flowing water	17°13'22.4"	31°00'51.9"	river sand mining, cattle	Natural
10	Lake Chivero	Standing water	17°53'36.4"	30°48'27.8"	Boating activity, fishing	Man-made

Site	Water vegetation	Substrate	Depth gastropods present (m)
1	*L. major, Cerathophylon sp., Cyperus sp., N. lotus, Myriophylum sp.*	Clay and Detritus	0.1 - 0.5
2	*L. major, N. lotus, Myriophylum sp., Phragmites* sp., *P. schweinfurthii*	Gravel and detritus	0.1 - 0.4
3	*L. major, Cerathophylon sp., Phragmites sp., Cyperus sp., N. lotus, Myriophylum sp.*	Detritus and silt	0.1 - 0.7
4	*L. major, E. crassipes, Phragmites* sp.	Gravel and detritus with big rocks	0.2 - 0.6
5	*P. senegalense*	Clay and detritus	0.0 - 0.3
6	*E. crassipes, Phragmites sp.*	Large rocks in fast flowing part and gravel with detritus in standing part	0.1 - 0.6
7	*L. major, E. crassipes, Phragmites sp., P. schweinfurthii*	Sand, clay and detritus	0.1 - 0.4
8	*L. major, P. senegalense, N. lotus, P. schweinfurthii*	Gravel, sand and detritus	0.1 - 0.5
8b	*L. major, P. senegalense*	sand and detritus	0.0 - 0.1
9	*Phragmites sp., L. major, P. senegalense*	Gravel, sand and small fraction detritus	0.1 - 0.4
10	*E. crassipes, Phragmites sp.*	Gravel, sand and small fraction detritus	0.0 - 0.2

4. Primers used

Table 2: All simplex primers used for Sanger sequencing during this research. Primer names are given along with the target species, primer sequence, genetic marker, annealing temperature, approximate amplicon length (bps) and the original authors that designed the primer. Amplicon length specifies the resulting fragment when the reverse primer is combined with the first abovementioned forward primer in the table, which is provided in parentheses.

Primer name	Target	Primer sequence (5'-3')	Marker	Sense	Annealing temperature	App. amplicon length (bps)	Authors
asmit1	Trematoda sp.	TTTTTTGGTCATCCTGAGGTGTAT	COI	F	/	/	Webster *et al.* (2010)
COI_schisto 3'	*Schistosoma* sp.	TAATGCATMGGAAAAAAACA	COI	R	49 °C	600 (asmit1)	Lockyer *et al.* (2003)
asmit2	Trematoda sp.	TAAAGAAAGAACATAATGAAAATG	COI	R	54 °C	423 (asmit1)	Bowles *et al.* (1992)
COI1_Dig_F	Trematoda sp.	CNATGATNTTNTTTTTTTTRATGCC	COI	F	/	/	Hammoud (unpublished)
COI1_Dig_R	Trematoda sp.	GMASWACCAAAWTTHCGATCAAA	COI	R	50 °C	450 (COI1_Dig_F)	Hammoud (unpublished)
Nasmit R	Trematoda sp.	ACATAATGAAARTCAGCNAYMACRA	COI	R	53 °C	871 (COI1_Dig_F)	Hammoud (unpublished)
ITS5	Universal	GGAAGTAAAAGTCGTAACAAG	ITS1, 5.8S, ITS2	F	/	/	White *et al.* (1990)
ITS4	Universal	TCCTCCGCTTATTGATATGC	ITS1, 5.8S, ITS2	R	50 °C	1,000 (ITS5)	White *et al.* (1990)
Trem-2F	Trematoda sp.	CAAGTCATAAGCTTGCGCTGA	ITS1, 5.8S, ITS2	F	/	/	Grabner *et al.* (2014)
Trem-1R	Trematoda sp.	ACCYAAACACCACATTGCCTA	ITS1, 5.8S, ITS2	R	55 °C	1086 (Trem-2F)	Grabner *et al.* (2014)
BD1	Trematoda sp.	GTCGTAACAAGGTTTCCGTA	ITS1, 5.8S, ITS2	F	/	/	Luton *et al.* (1992)
BD2	Trematoda sp.	TATGCT- TAAATTCAGCGGGT	ITS1, 5.8S, ITS2	R	61.6 °C	1100 (BD1)	Luton *et al.* (1992)

Fasc-ITS1 F	*Fasciola* sp.	TCTACTCTTACACAAGCGATACAC	ITS1	F	/	/	Grabner *et al.* (2014)
Fasc-ITS1 R	*Fasciola* sp.	GGCTTTCTGCCAAGACAAG	ITS1	R	55 °C	680 (Fasc-ITS1 F)	Grabner *et al.* (2014)
ITS2_DIG_F	Trematoda sp.	CAAHAAGTCGTGGMTTGG	ITS2	F	/	/	Hammoud (unpublished)
ITS2_DIG_R	Trematoda sp.	AACAACCCGACTCCAAGG	ITS2	R	50 °C	600 (ITS2_DIG_F)	Hammoud (unpublished)
123F	Paramphistomoidea	ATTCGTTTGAACTATATGGA	COI	F	/	/	Laidemit *et al.* (2016)
858R	Paramphistomoidea	CATATGATGAGCCCAAACAAC	COI	R	46 °C, 45 °C and 44 °C	690 (123F)	Laidemit *et al.* (2016)
18S_Dig_F	Trematoda sp.	CAGCTATGGTTCCTTAGATCRTA	18S	F	/	/	Carolus *et al.* (2019)
1270R	Universal	CCGTCAATTCCTTTAAGT	18S	R	50 °C	1160 (18S_DIG_F)	Littlewood and Olsen (2001)
Worm A	Universal.	GCGAATGGCTCATTAAATCAG	18S	F	/	/	Waeschenbach *et al.* (2007)
Worm B	Universal	CTTGTTACGACTTTTACTTCC	18S	R	54 °C	1940 – 2238 (Worm A)	Waeschenbach *et al.* (2007)
COI1_snail_F	Gastropoda sp.	TAATTWATTGTTACDGCWCATGC	COI	F	/	/	Hammoud (unpublished)
COI1_snail_R	Gastropoda sp.	CWCCTCCTGCWGGATCAAA	COI	R	50 °C	536 (COI1_snail_F)	Hammoud (unpublished)
LCO1490	Universal	GGTCAACAAATCATAAAGATATTGG	COI	F	/	/	Folmer *et al.* (1994)
HCO2198	Universal	TAAACTTCAGGGTGACCAAAAAATCA	COI	R	53 °C	710 (LCO1490)	Folmer *et al.* (1994)

5. Gastropod haplotype information

Table 3: All haplotypes found based on COI sequences (450 bps) per species. DNA extract codes of all identical sequences are provided and are listed under their respective haplotype code, used in tree-based species identification.

Haplotype	Matching snail	Haplotype	Matching snail	Haplotype	Matching snail	Haplotype	Matching snail	Haplotype	Matching snail
BGL_H1	S1_BGL_1	**BGL_H5**	382	**BPF_H1**	S6_BPF_1	**LNA_H1**	S9_LNA_1	**LNA_H4**	S1_RAD_1
	S4_BGL_1	**Bsp.1**	S3_BGL_2		S6_BPF_3		S9_LNA_2		S2_LNA_1
	S4_BGL_9		S7_TRO_1	**BPF_H2**	S1_BPF_1		393		S3_LNA_1
	S4_BGL_10		S7_TRO_2		S4_BPF_1		481		S3_LNA_4
	S5_BGL_1	**Bsp.2**	S1_BAF_1		S5_BPF_7		483		S6_LNA_1
	S5_BGL_2		S3_BGL_1		S7_BPF_1		487		S8b_LNA_1
	S5_BGL_3		S3_BGL_7		S8_BPF_1		489		296
	S5_BGL_4		S8b_TRO_1		S8b_BPF_3	**LNA_H2**	S5_LNA_1		394
	S5_BGL_5	**TRU_H1**	S1_TRU_1		S8b_BPF_4		S5_LNA_3	**PHY_H1**	KAR_FC_S1_3
	S7_TRU_1		S3_BGL_5		S8b_BPF_5		S5_LNA_4	**PHY_H2**	KAR_FC_S2_10
	S7b_BGL_1		S4_TRU_1		S8b_BPF_6		S7b_LNA_1	**PHY_H3**	S4_PHY_1
	S8b_BGL_7		S8_Btr_1		S9_BPF_1		295	**PHY_H4**	S5_PHY_1
	S9_BGL_1	**TRU_H2**	S2_TRU_1	**BPF_H3**	S8_BPF_2	**LNA_H3**	S1_LNA_1		S6_PHY_1
	365		S2_TRU_2	**GYR_H1**	S6_GYR_1		S8_LNA_1		S7_PHY_1
	368		S3_TRU_1	**GYR_H2**	S7_GYR_1		S8b_LNA_2		S8_PHY_1
	378		S3_BGL_4	**GYR_H3**	ZIM_Snail_D		480		AND_E2_1
	379		S3_BGL_6	**COL_H1**	S4_LNA_1		482		NZO_D7_1
	381		ZIM_Snail_S		KAR_exh_COL_1		484	**MEL_H1**	S4_MEL_1
BGL_H2	S7b_BGL_2		ZIM_Snail_T		CHI_COL_2		485		S7_MEL_1
	377		ZIM_Snail_U		32		491		S4_MEL_4
BGL_H3	S9_TRU_1		MAZ_4				492		
BGL_H4	S4_BGL_14		MAZ_5						

Table 4: Best BLAST results on BOLD systems V4. All unique COI snail haplotypes and one leech collected in Harare are provided with sequence length (bps), BLAST 1 and 2 with corresponding identification percentage and country of origin. A second BLAST result is only included of it was listed amongst the top 20 results and if species names were different. Species abbreviations are: BGL = *B. globosus*, TRU = *B. truncatus*, BPF = *Bi. pfeifferi*, GYR = *Gyraulus*, LNA = *L. natalensis*, COL = *P. columella*, PHY = *P. acuta*, MEL = *M. tuberculata*, HEL = *H. duryi*, RAD = *Radix* sp.

Seq.	Len. (bps)	BLAST 1	ID (%)	Country	BLAST 2	ID (%)	Country
BGL H1	479	*Bulinus globosus*	98.22	South Africa	/	/	/
BGL H2	417	*Bulinus globosus*	98.52	South Africa	/	/	/
BGL H3	417	*Bulinus globosus*	98.77	South Africa	/	/	/
BGL H4	413	*Bulinus globosus*	99	South Africa	/	/	/
BGL H5	424	*Bulinus globosus*	98.25	South Africa	/	/	/
TRU H1	417	*Bulinus truncatus*	99.25	Multiple	/	/	/
TRU H2	417	*Bulinus truncatus*	100	Multiple	/	/	/
***Bulinus* sp. 1**	417	*Bulinus tropicus*	99.26	Zimbabwe	*Bulinus nyassanus*	97.6	Malawi
***Bulinus* sp. 2**	417	*Bulinus tropicus*	98.02	Uganda	*Bulinus nyassanus*	97.53	Malawi
BPF H1	469	*Biomphalaria pfeifferi*	100	Zimbabwe	/	/	/
BPF H2	469	*Biomphalaria pfeifferi*	100	Zimbabwe	/	/	/
BPF H3	413	*Biomphalaria pfeifferi*	99.75	Zimbabwe	/	/	/
GYR H1	421	*Gyraulus connollyi*	95.8	South Africa	*Gyraulus sp.*	89.88	China
GYR H2	421	*Gyraulus connollyi*	96.3	South Africa	*Gyraulus sp.*	90.37	China
GYR H3	313	*Gyraulus sp.*	87.14	China	/	/	/
LNA H1	463	*Radix natalensis*	98.48	Portugal	*Lymnaea natalensis*	96.32	Malawi
LNA H2	463	*Radix natalensis*	98.05	Portugal	*Lymnaea natalensis*	95.89	Malawi
LNA H3	463	*Lymnaea natalensis*	98.7	Malawi	*Radix natalensis*	96.62	Portugal
LNA H4	463	*Lymnaea natalensis*	98.27	Malawi	*Radix natalensis*	97.4	Portugal
COL H1	463	*Pseudosuccinea columella*	100	Multiple	/	/	/
PHY H1	464	*Physella acuta*	99.56	South Africa	/	/	/
PHY H2	464	*Physella acuta*	99.13	South Africa	/	/	/
PHY H3	464	*Physella acuta*	99.56	Multiple	/	/	/
PHY H4	464	*Physella acuta*	99.78	Multiple	/	/	/
MEL H1	395	*Melanoides tuberculata*	100	Malawi	/	/	/
***Bellamya* sp.**	681	*Bellamya sp.*	93.74	Namibia	*Bellamya monardi*	93.26	Malawi
Bellamya sp. Kariba	450	*Bellamya monardi*	93	Botswana	*Bellamya robertsoni*	91.86	Malawi
CHI HEL 1	678	*Planorbella duryi*	100	USA	*Planorbarius corneus*	100	?
***Oxyloma* sp.**	677	*Oxyloma verrilli*	89.09	Canada	*Succinea putris*	88.79	Russia
RAD H1	662	*Radix sp.*	97.32	China	*Radix auricularia*	95.97	Japan
Leech	680	*Helobdella europaea*	100	Multiple	*Helobdella socimulcensis*	96.7	?

6. Gastropod phylogenetic trees and associated p-distance matrices

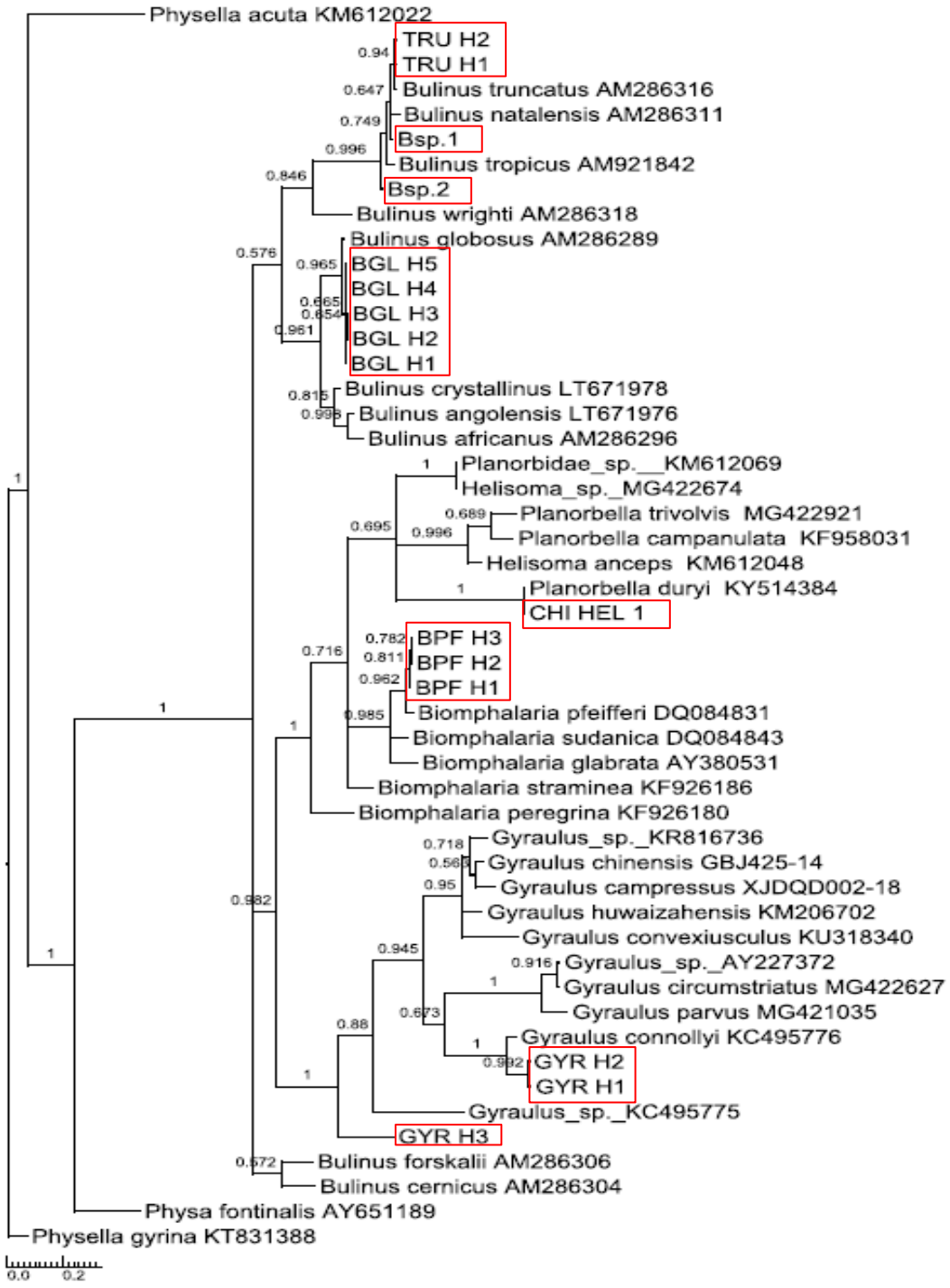

Figure 3: Bayesian inference through the MCMC algorithm phylogenetic tree for the Planorbidae family, using a 394 bps COI alignment and the General Time Reversible (GTR) model with a discrete Gamma distribution ([+G] = +0.343) and invariant sites ([+I] = 41%). Probabilities are indicated on the nodes. Ngen= 1,000,000. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 50 different sequences. BOLD or GenBank accession number of each reference sequence is displayed after the ID. Red boxes indicate sequences from Kariba and Harare.

Table 5: Pairwise genetic distance (p-distance) between Planorbidae COI sequences of 394 nucleotides.

The proportion of different nucleotides between two sequences are shown. Discussed results are highlighted.

Sequence		1	2	3	4	5	6	7	8	9	10
Planorbidae sp. | KM612069	**1**										
Planorbella trivolvis | MG422921	**2**	0.157									
Planorbella duryi | KY514384	**3**	0.168	0.185								
Planorbella campanulata | KF958031	**4**	0.150	0.086	0.185							
Helisoma sp. | MG422674	**5**	0.005	0.162	0.170	0.155						
Helisoma anceps | KM612048	**6**	0.137	0.094	0.190	0.066	0.142					
Gyraulus sp. | KR816736	**7**	0.173	0.155	0.195	0.173	0.178	0.180				
Gyraulus sp. | KC495775	**8**	0.185	0.145	0.195	0.152	0.190	0.147	0.137			
Gyraulus sp. | AY227372	**9**	0.201	0.193	0.190	0.190	0.206	0.188	0.140	0.157		
Gyraulus parvus | MG421035	**10**	0.193	0.201	0.195	0.188	0.193	0.193	0.150	0.165	0.061	
Gyraulus huwaizahensis | KM206702	**11**	0.206	0.168	0.203	0.193	0.206	0.195	0.069	0.142	0.137	0.152
Gyraulus convexiusculus | KU318340	**12**	0.180	0.180	0.218	0.193	0.185	0.203	0.096	0.152	0.142	0.147
Gyraulus connollyi | KC495776	**13**	0.185	0.178	0.188	0.193	0.190	0.188	0.114	0.137	0.119	0.135
Gyraulus circumstriatus | MG422627	**14**	0.201	0.188	0.183	0.185	0.206	0.183	0.142	0.157	0.010	0.061
Gyraulus chinensis | GBJ425-14	**15**	0.190	0.155	0.208	0.168	0.195	0.183	0.046	0.129	0.142	0.142
Gyraulus compressus | XJDQD002-18	**16**	0.206	0.185	0.221	0.188	0.211	0.195	0.066	0.152	0.137	0.155
Bulinus wrighti | AM286318	**17**	0.180	0.145	0.190	0.140	0.185	0.168	0.150	0.150	0.155	0.152
Bulinus truncatus | AM286316	**18**	0.203	0.165	0.180	0.175	0.208	0.178	0.160	0.155	0.165	0.188
Bulinus tropicus | AM921842	**19**	0.185	0.162	0.180	0.170	0.190	0.175	0.150	0.147	0.170	0.183
Bulinus natalensis | AM286311	**20**	0.180	0.145	0.183	0.170	0.185	0.162	0.145	0.147	0.168	0.183
Bulinus globosus | AM286289	**21**	0.147	0.155	0.190	0.152	0.152	0.160	0.162	0.155	0.170	0.162
Bulinus forskalii | AM286306	**22**	0.173	0.150	0.173	0.180	0.178	0.175	0.165	0.140	0.201	0.201
Bulinus crystallinus | LT671978	**23**	0.150	0.147	0.180	0.145	0.150	0.145	0.150	0.140	0.170	0.180
Bulinus cernicus | AM286304	**24**	0.173	0.140	0.190	0.162	0.178	0.170	0.152	0.147	0.180	0.193
Bulinus angolensis | LT671976	**25**	0.165	0.150	0.195	0.157	0.170	0.155	0.160	0.157	0.175	0.185
Bulinus africanus | AM286296	**26**	0.178	0.173	0.201	0.162	0.183	0.155	0.168	0.168	0.170	0.188
Biomphalaria sudanica | DQ084843	**27**	0.140	0.129	0.175	0.124	0.145	0.122	0.140	0.129	0.175	0.183
Biomphalaria straminea | KF926186	**28**	0.140	0.137	0.178	0.152	0.145	0.150	0.152	0.147	0.185	0.185
Biomphalaria pfeifferi | DQ084831	**29**	0.142	0.132	0.180	0.145	0.147	0.137	0.140	0.137	0.175	0.183
Biomphalaria peregrina | KF926180	**30**	0.168	0.157	0.188	0.168	0.173	0.162	0.155	0.152	0.195	0.193
Biomphalaria glabrata | AY380531	**31**	0.140	0.150	0.195	0.155	0.145	0.142	0.150	0.142	0.198	0.188
TRU H1	**32**	0.196	0.153	0.178	0.163	0.201	0.168	0.150	0.145	0.168	0.183
TRU H2	**33**	0.201	0.153	0.183	0.165	0.206	0.173	0.160	0.153	0.176	0.186
GYR H1	**34**	0.193	0.165	0.198	0.173	0.198	0.173	0.104	0.135	0.140	0.140
GYR H2	**35**	0.188	0.163	0.204	0.173	0.193	0.173	0.099	0.135	0.135	0.140
GYR H3	**36**	0.187	0.189	0.189	0.202	0.187	0.199	0.128	0.148	0.148	0.151
CHI HEL 1	**37**	0.168	0.185	0.000	0.185	0.170	0.190	0.195	0.195	0.190	0.195
Bsp.2	**38**	0.191	0.148	0.186	0.160	0.196	0.165	0.145	0.142	0.170	0.173
Bsp.1	**39**	0.188	0.150	0.181	0.160	0.193	0.170	0.142	0.153	0.178	0.183
BPF H1	**40**	0.150	0.140	0.183	0.150	0.155	0.140	0.148	0.135	0.176	0.186
BPF H2	**41**	0.153	0.142	0.181	0.153	0.158	0.142	0.145	0.137	0.181	0.188
BPF H3	**42**	0.155	0.142	0.183	0.155	0.160	0.145	0.150	0.142	0.183	0.190
BGL H1	**43**	0.153	0.158	0.188	0.158	0.158	0.155	0.163	0.158	0.176	0.170
BGL H2	**44**	0.150	0.155	0.186	0.155	0.155	0.153	0.160	0.155	0.173	0.168
BGL H3	**45**	0.148	0.153	0.183	0.153	0.153	0.155	0.158	0.153	0.170	0.165
BGL H4	**46**	0.153	0.155	0.188	0.155	0.158	0.155	0.160	0.158	0.176	0.170
BGL H5	**47**	0.150	0.155	0.191	0.155	0.155	0.153	0.160	0.155	0.173	0.168
Physella gyrina | KT831388	**48**	0.185	0.188	0.203	0.188	0.188	0.188	0.183	0.185	0.188	0.195
Physella acuta | KM612022	**49**	0.234	0.213	0.241	0.213	0.236	0.221	0.211	0.221	0.213	0.216
Physa fontinalis | AY651189	**50**	0.218	0.195	0.208	0.183	0.221	0.193	0.203	0.190	0.208	0.216

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	10	11	12	13	14	15	16	17	18	19	20	21	22
11	0.152												
12	0.147	0.099											
13	0.135	0.132	0.145										
14	0.061	0.140	0.145	0.127									
15	0.142	0.066	0.094	0.119	0.140								
16	0.155	0.081	0.109	0.129	0.145	0.051							
17	0.152	0.155	0.175	0.155	0.152	0.150	0.157						
18	0.188	0.173	0.178	0.157	0.165	0.178	0.190	0.117					
19	0.183	0.178	0.178	0.157	0.165	0.173	0.185	0.112	0.033				
20	0.183	0.170	0.188	0.145	0.168	0.170	0.185	0.114	0.033	0.030			
21	0.162	0.178	0.162	0.165	0.168	0.165	0.180	0.094	0.142	0.145	0.140		
22	0.201	0.180	0.173	0.168	0.193	0.173	0.190	0.142	0.145	0.152	0.155	0.135	
23	0.180	0.173	0.173	0.147	0.168	0.152	0.173	0.102	0.129	0.127	0.127	0.061	0.122
24	0.193	0.180	0.173	0.165	0.183	0.170	0.175	0.127	0.152	0.150	0.145	0.129	0.102
25	0.185	0.178	0.168	0.147	0.173	0.165	0.178	0.109	0.135	0.132	0.132	0.076	0.137
26	0.188	0.185	0.178	0.155	0.175	0.175	0.183	0.119	0.137	0.140	0.145	0.086	0.147
27	0.183	0.162	0.157	0.162	0.180	0.142	0.157	0.147	0.165	0.157	0.152	0.142	0.155
28	0.185	0.155	0.155	0.168	0.183	0.152	0.180	0.147	0.175	0.165	0.157	0.160	0.142
29	0.183	0.162	0.162	0.165	0.180	0.152	0.170	0.150	0.160	0.157	0.150	0.145	0.142
30	0.193	0.168	0.180	0.170	0.193	0.152	0.178	0.124	0.142	0.145	0.132	0.137	0.147
31	0.188	0.173	0.162	0.178	0.203	0.170	0.183	0.150	0.173	0.162	0.155	0.150	0.152
32	0.183	0.168	0.176	0.155	0.168	0.168	0.181	0.112	0.015	0.036	0.033	0.140	0.140
33	0.186	0.173	0.173	0.158	0.170	0.173	0.191	0.112	0.015	0.036	0.038	0.137	0.145
34	0.140	0.122	0.132	0.046	0.148	0.104	0.125	0.170	0.173	0.173	0.163	0.168	0.181
35	0.140	0.117	0.127	0.041	0.142	0.099	0.120	0.165	0.173	0.173	0.163	0.163	0.176
36	0.151	0.136	0.148	0.148	0.148	0.138	0.151	0.148	0.159	0.169	0.153	0.143	0.171
37	0.195	0.203	0.218	0.188	0.183	0.208	0.221	0.190	0.180	0.180	0.183	0.190	0.173
38	0.173	0.160	0.181	0.153	0.165	0.163	0.181	0.102	0.038	0.031	0.031	0.127	0.137
39	0.183	0.173	0.183	0.160	0.178	0.173	0.183	0.117	0.031	0.028	0.028	0.150	0.148
40	0.186	0.165	0.173	0.168	0.181	0.160	0.173	0.148	0.170	0.165	0.155	0.148	0.155
41	0.188	0.165	0.176	0.170	0.181	0.158	0.176	0.150	0.173	0.165	0.158	0.150	0.148
42	0.190	0.168	0.178	0.175	0.183	0.162	0.180	0.155	0.175	0.170	0.160	0.155	0.150
43	0.170	0.186	0.160	0.165	0.173	0.170	0.183	0.099	0.140	0.142	0.137	0.015	0.130
44	0.168	0.183	0.158	0.163	0.170	0.168	0.181	0.097	0.137	0.140	0.135	0.018	0.132
45	0.165	0.181	0.155	0.160	0.168	0.165	0.178	0.094	0.135	0.137	0.132	0.015	0.130
46	0.170	0.183	0.163	0.163	0.173	0.168	0.181	0.097	0.140	0.142	0.137	0.013	0.130
47	0.168	0.183	0.163	0.163	0.170	0.168	0.181	0.097	0.142	0.140	0.135	0.018	0.132
48	0.195	0.203	0.203	0.201	0.183	0.193	0.211	0.162	0.173	0.170	0.162	0.173	0.183
49	0.216	0.223	0.239	0.231	0.213	0.221	0.239	0.183	0.208	0.195	0.201	0.213	0.216
50	0.216	0.193	0.221	0.234	0.203	0.195	0.213	0.165	0.206	0.201	0.206	0.195	0.178

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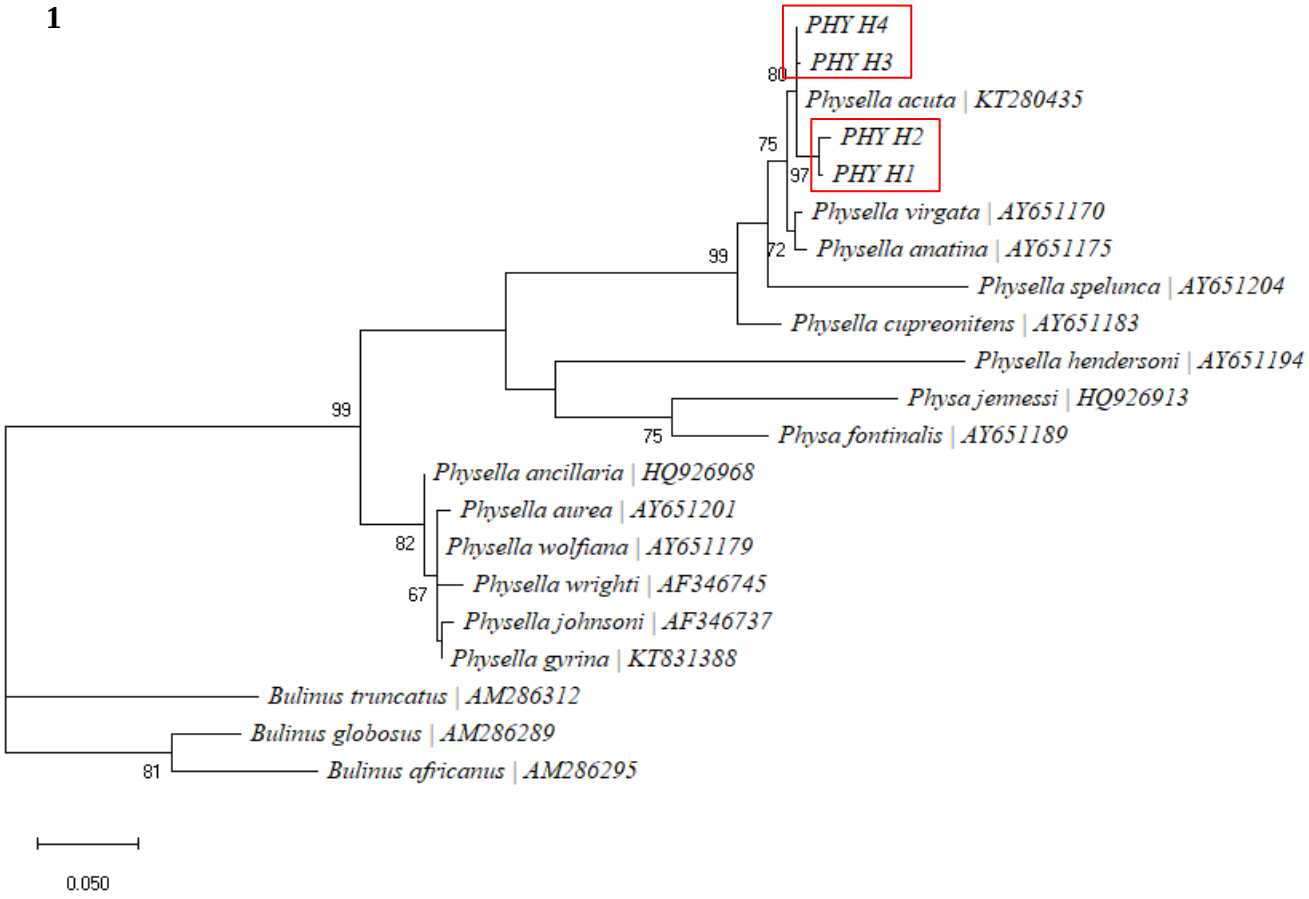
	23	24	25	26	27	28	29	30	31	32	33	34	35
24	0.129												
25	0.043	0.127											
26	0.056	0.132	0.048										
27	0.135	0.129	0.124	0.145									
28	0.155	0.132	0.162	0.175	0.096								
29	0.137	0.137	0.132	0.157	0.048	0.091							
30	0.140	0.127	0.152	0.162	0.122	0.112	0.114						
31	0.145	0.147	0.135	0.152	0.063	0.104	0.069	0.132					
32	0.125	0.148	0.130	0.140	0.155	0.168	0.148	0.137	0.160				
33	0.122	0.155	0.127	0.142	0.165	0.176	0.158	0.148	0.170	0.015			
34	0.165	0.176	0.165	0.173	0.148	0.150	0.150	0.168	0.160	0.163	0.173		
35	0.160	0.170	0.160	0.168	0.142	0.145	0.145	0.165	0.160	0.163	0.173	0.005	
36	0.136	0.156	0.153	0.161	0.151	0.164	0.148	0.146	0.174	0.156	0.169	0.156	0.151
37	0.180	0.190	0.195	0.201	0.175	0.178	0.180	0.188	0.195	0.178	0.183	0.198	0.204
38	0.112	0.148	0.125	0.140	0.158	0.165	0.153	0.140	0.153	0.033	0.033	0.165	0.165
39	0.130	0.145	0.135	0.145	0.155	0.170	0.153	0.148	0.150	0.020	0.031	0.163	0.168
40	0.140	0.145	0.137	0.158	0.053	0.104	0.028	0.125	0.064	0.160	0.170	0.155	0.150
41	0.142	0.148	0.140	0.160	0.056	0.104	0.025	0.122	0.071	0.163	0.173	0.158	0.153
42	0.147	0.150	0.145	0.165	0.058	0.107	0.028	0.124	0.071	0.163	0.173	0.160	0.155
43	0.064	0.122	0.079	0.087	0.142	0.158	0.140	0.150	0.148	0.137	0.135	0.168	0.163
44	0.066	0.120	0.076	0.084	0.140	0.155	0.137	0.148	0.145	0.135	0.132	0.165	0.160
45	0.064	0.122	0.074	0.087	0.137	0.153	0.135	0.145	0.142	0.132	0.130	0.163	0.158
46	0.061	0.120	0.076	0.084	0.142	0.158	0.140	0.148	0.148	0.137	0.135	0.165	0.160
47	0.061	0.120	0.076	0.084	0.140	0.155	0.137	0.148	0.145	0.140	0.137	0.165	0.160
48	0.162	0.178	0.168	0.185	0.188	0.203	0.195	0.203	0.208	0.173	0.168	0.219	0.214
49	0.206	0.213	0.211	0.223	0.208	0.206	0.203	0.221	0.211	0.198	0.204	0.219	0.224
50	0.180	0.193	0.175	0.193	0.175	0.198	0.180	0.203	0.190	0.201	0.206	0.224	0.219

	36	37	38	39	40	41	42	43	44	45	46	47	48	49
37	0.189													
38	0.156	0.186												
39	0.169	0.181	0.031											
40	0.149	0.183	0.158	0.160										
41	0.154	0.181	0.163	0.163	0.008									
42	0.156	0.183	0.163	0.160	0.008	0.003								
43	0.146	0.188	0.130	0.148	0.145	0.148	0.150							
44	0.144	0.186	0.127	0.145	0.142	0.145	0.148	0.003						
45	0.141	0.183	0.125	0.142	0.140	0.142	0.145	0.005	0.003					
46	0.144	0.188	0.130	0.148	0.145	0.148	0.150	0.003	0.005	0.008				
47	0.144	0.191	0.127	0.145	0.142	0.145	0.148	0.003	0.005	0.008	0.005			
48	0.210	0.203	0.165	0.176	0.196	0.198	0.203	0.176	0.173	0.170	0.176	0.178		
49	0.233	0.241	0.188	0.191	0.204	0.209	0.211	0.211	0.214	0.211	0.211	0.209	0.157	
50	0.220	0.208	0.201	0.201	0.173	0.176	0.180	0.193	0.191	0.188	0.193	0.191	0.132	0.162

1


PHY H4
PHY H3
80
Physella acuta | KT280435
75
PHY H2
97
PHY H1
Physella virgata | AY651170
99
72
Physella anatina | AY651175
Physella spelunca | AY651204
Physella cupreonitens | AY651183
Physella hendersoni | AY651194
99
Physa jennessi | HQ926913
75
Physa fontinalis | AY651189
Physella ancillaria | HQ926968
Physella aurea | AY651201
82
Physella wolfiana | AY651179
67
Physella wrighti | AF346745
Physella johnsoni | AF346737
Physella gyrina | KT831388
Bulinus truncatus | AM286312
Bulinus globosus | AM286289
81
Bulinus africanus | AM286295
0.050

2

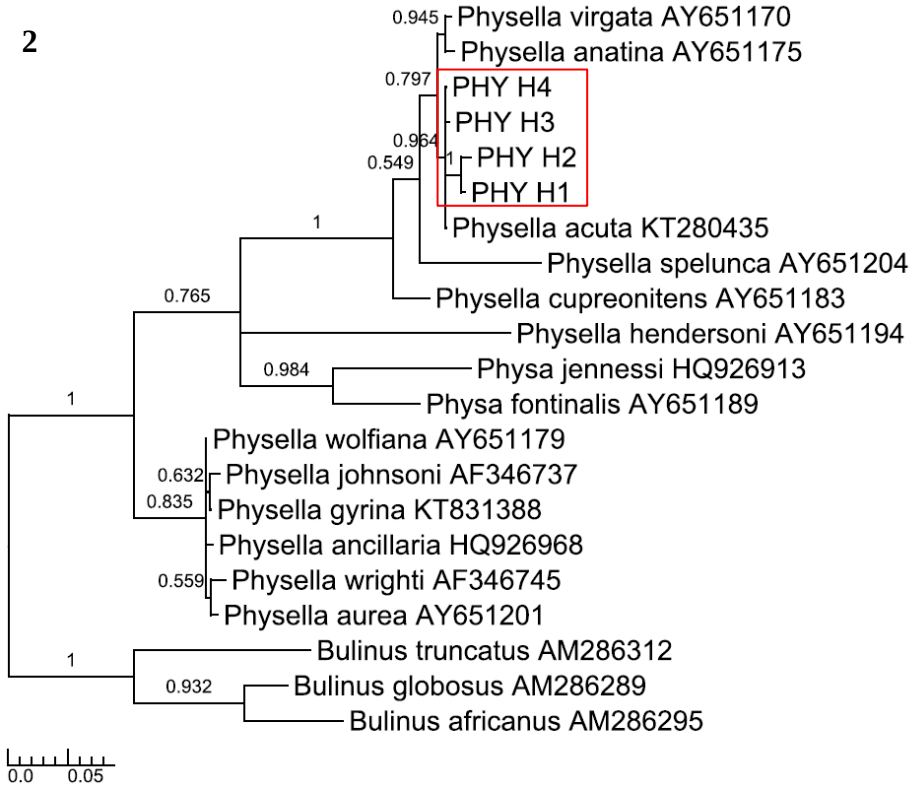

0.945
Physella virgata AY651170
Physella anatina AY651175
0.797
PHY H4
PHY H3
0.964
0.549
1
PHY H2
PHY H1
1
Physella acuta KT280435
Physella spelunca AY651204
0.765
Physella cupreonitens AY651183
Physella hendersoni AY651194
0.984
Physa jennessi HQ926913
1
Physa fontinalis AY651189
Physella wolfiana AY651179
0.632
Physella johnsoni AF346737
0.835
Physella gyrina KT831388
Physella ancillaria HQ926968
0.559
Physella wrighti AF346745
Physella aurea AY651201
1
Bulinus truncatus AM286312
0.932
Bulinus globosus AM286289
Bulinus africanus AM286295
0.0
0.05

Figure 4: Phylogenetic trees using a 464 bps COI alignment for the Physidae family. (**1**) ML phylogenetic tree using the Hasegawa-Kishino-Yano model. The tree with the highest log likelihood (-2368.12) is shown. Bootstrap values (1,000 replicates) above or equal to 60 are indicated on the nodes. (**2**) Bayesian Inference (MCMC algorithm) of the phylogenetic tree using the GTR model with a discrete Gamma distribution ([+G] = +0.204). Probabilities are indicated on the nodes. Ngen= 1,000,000. The trees are drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 21 different sequences. BOLD or GenBank accession number of each reference sequence is displayed after the sequence ID. Red boxes indicate sequences from Kariba and Harare.

Table 6: Pairwise genetic distance (p-distance) between Physidae COI sequences of 464 nucleotides. The proportion of different nucleotides between two sequences is shown. Discussed results are highlighted in yellow.

Sequence		**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Physella wrighti | AF346745	**1**								
Physella wolfiana | AY651179	**2**	0.013							
Physella virgata | AY651170	**3**	0.156	0.149						
Physella spelunca | AY651204	**4**	0.158	0.156	0.086					
Physella johnsoni | AF346737	**5**	0.017	0.009	0.158	0.156				
Physella hendersoni | AY651194	**6**	0.175	0.171	0.186	0.177	0.168			
Physella gyrina | KT831388	**7**	0.015	0.002	0.151	0.153	0.006	0.168		
Physella cupreonitens | AY651183	**8**	0.149	0.143	0.048	0.099	0.147	0.175	0.140	
Physella aurea | AY651201	**9**	0.015	0.006	0.156	0.158	0.015	0.171	0.009	0.145
Physella ancillaria | HQ926968	**10**	0.019	0.006	0.145	0.156	0.015	0.173	0.009	0.140
Physella anatina | AY651175	**11**	0.153	0.147	0.011	0.091	0.156	0.190	0.149	0.052
Physa jennessi | HQ926913	**12**	0.136	0.132	0.177	0.181	0.132	0.171	0.130	0.177
Physa fontinalis | AY651189	**13**	0.136	0.127	0.145	0.151	0.134	0.153	0.130	0.149
PHY H1	**14**	0.158	0.151	0.022	0.097	0.156	0.184	0.153	0.058
PHY H2	**15**	0.162	0.156	0.030	0.104	0.160	0.186	0.158	0.063
PHY H3	**16**	0.153	0.147	0.015	0.091	0.156	0.181	0.149	0.048
PHY H4	**17**	0.153	0.147	0.013	0.089	0.156	0.179	0.149	0.045
Physella acuta | KT280435	**18**	0.153	0.147	0.013	0.089	0.156	0.179	0.149	0.045
Bulinus globosus | AM286289	**19**	0.162	0.154	0.184	0.197	0.162	0.201	0.156	0.197
Bulinus africanus | AM286295	**20**	0.167	0.162	0.188	0.197	0.171	0.195	0.165	0.197
Bulinus truncatus | AM286312	**21**	0.158	0.154	0.180	0.203	0.162	0.225	0.156	0.197

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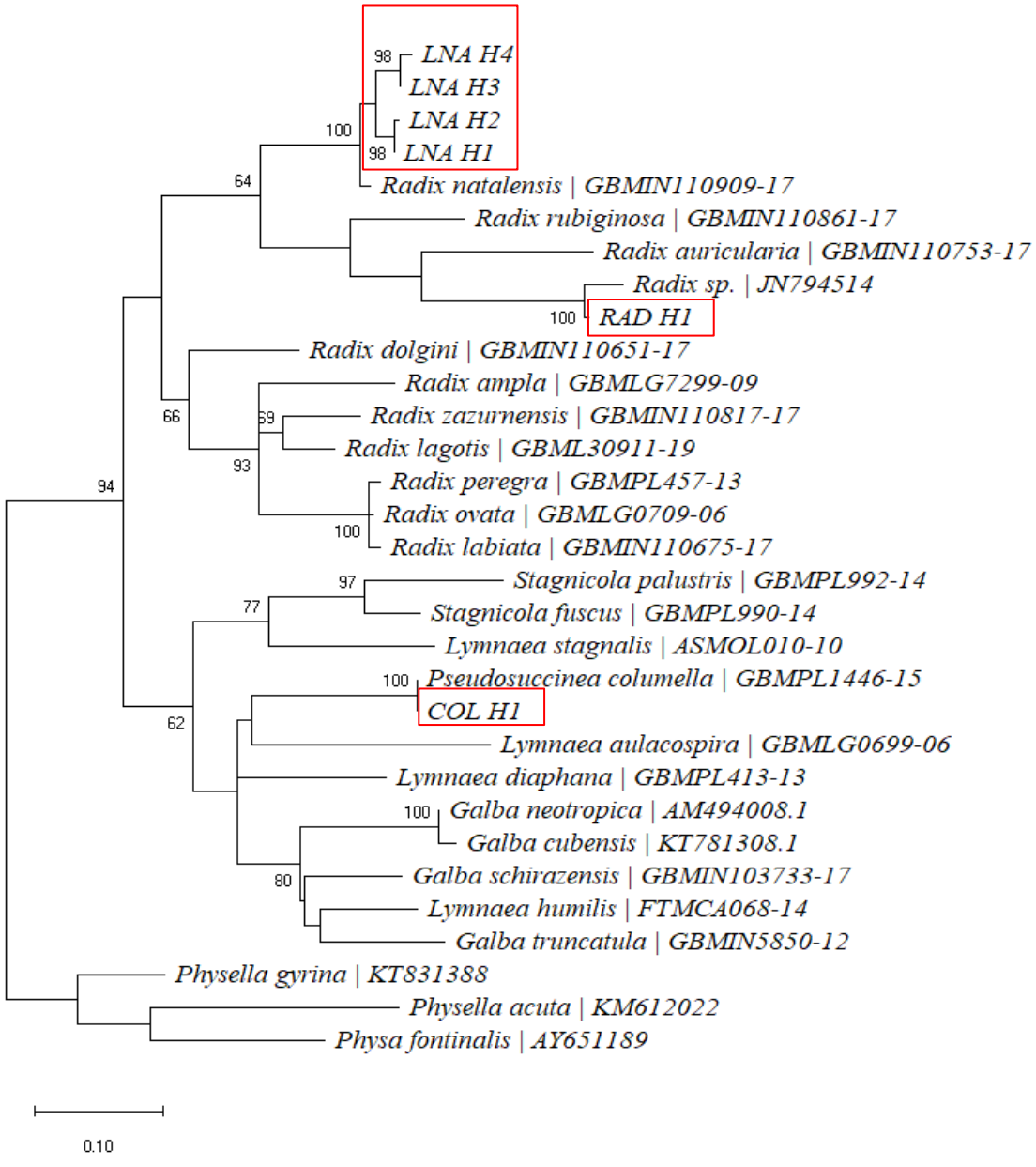
	9	10	11	12	13	14	15	16	17	18	19	20
10	0.013											
11	0.153	0.143										
12	0.134	0.134	0.175									
13	0.134	0.125	0.143	0.108								
14	0.158	0.147	0.024	0.173	0.149							
15	0.162	0.151	0.032	0.181	0.153	0.009						
16	0.153	0.143	0.013	0.173	0.138	0.015	0.019					
17	0.153	0.143	0.015	0.175	0.140	0.013	0.017	0.002				
18	0.153	0.143	0.015	0.175	0.140	0.013	0.017	0.002	0.000			
19	0.160	0.158	0.186	0.184	0.175	0.188	0.190	0.184	0.184	0.184		
20	0.160	0.162	0.195	0.171	0.175	0.199	0.201	0.195	0.195	0.195	0.080	
21	0.158	0.156	0.177	0.188	0.195	0.180	0.184	0.180	0.182	0.182	0.138	0.145



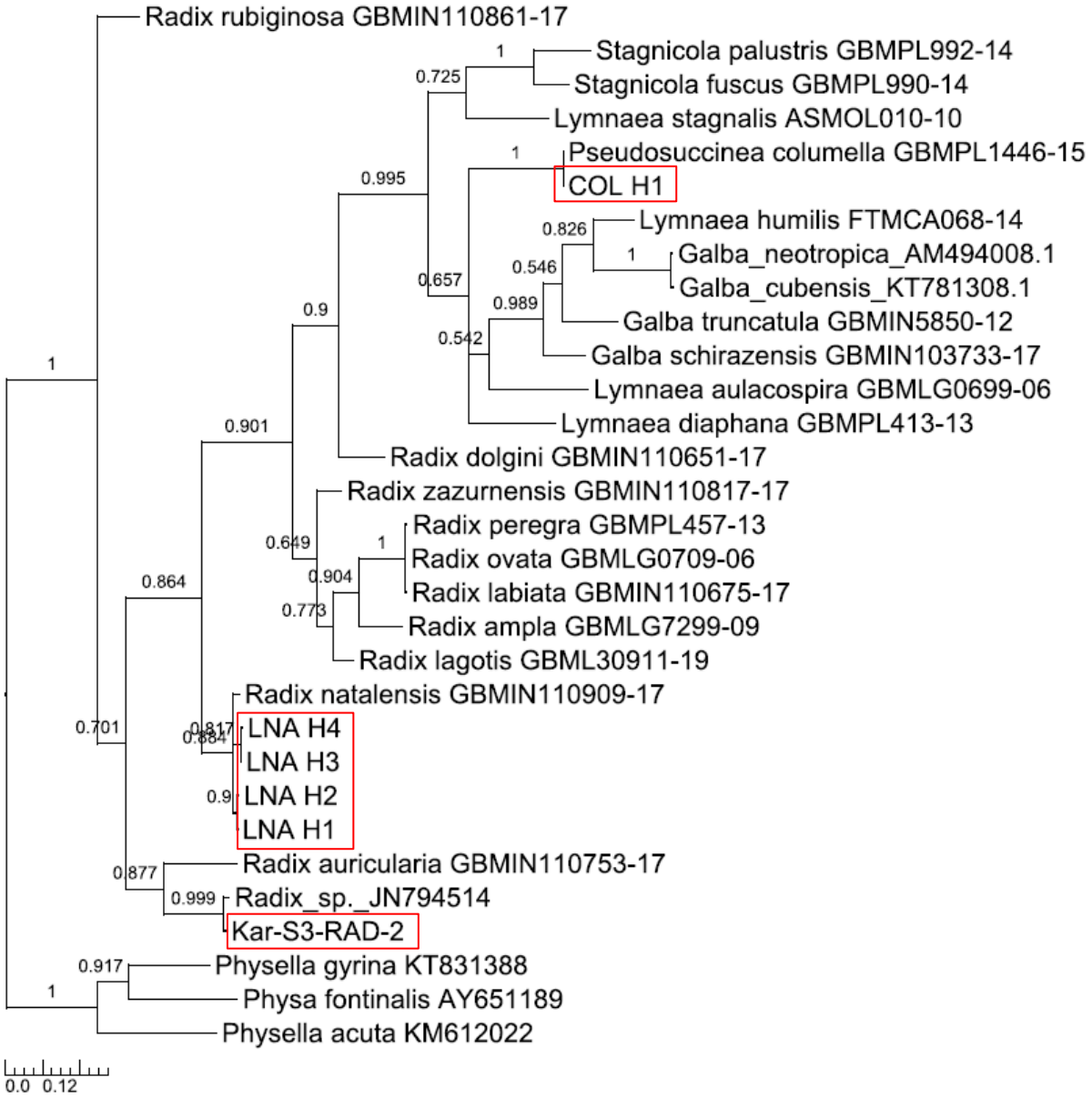


Figure 5: Phylogenetic trees using a 465 bps COI alignment for the Lymnaeidae family. (**1**) ML likelihood tree using the General Time Reversible model. The tree with the highest log likelihood (-4002.38) is shown. Bootstrap values (1,000 replicates) above or equal to 60 are indicated on the nodes. (**2**) Markov Chain Monte Carlo for Bayesian Inference of the phylogenetic tree using the GTR model with a discrete Gamma distribution ([+G] = +0.221) and invariant sites ([+I] = 43.8%). Probabilities are indicated on the nodes. Ngen= 1,000,000. The trees are drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 31 different sequences. BOLD or GenBank accession number of each reference sequence is displayed after the '|' separator. Red boxes indicate sequences from Kariba and Harare.

Table 7: Pairwise genetic distance (p-distance) between Lymnaeidae COI sequences of 465 nucleotides. The proportion of different nucleotides between two sequences is shown. Discussed results are highlighted in yellow.

		1	2	3	4	5	6	7	8
Radix sp. | JN794514	**1**								
Stagnicola palustris | GBMPL992-14	**2**	0.173							
Stagnicola fuscus | GBMPL990-14	**3**	0.186	0.099						
Radix zazurnensis | GBMIN110817-17	**4**	0.162	0.175	0.158					
Radix rubiginosa | GBMIN110861-17	**5**	0.128	0.168	0.162	0.158				
Radix peregra | GBMPL457-13	**6**	0.167	0.168	0.177	0.097	0.149			
Radix ovata | GBMLG0709-06	**7**	0.160	0.166	0.171	0.091	0.145	0.013		
Radix natalensis | GBMIN110909-17	**8**	0.145	0.186	0.166	0.134	0.125	0.140	0.134	
Radix lagotis | GBML30911-19	**9**	0.156	0.164	0.158	0.073	0.158	0.093	0.091	0.130
Radix labiata | GBMIN110675-17	**10**	0.169	0.173	0.184	0.095	0.143	0.017	0.013	0.138
Radix dolgini | GBMIN110651-17	**11**	0.156	0.156	0.145	0.106	0.138	0.119	0.117	0.123
Radix auricularia | GBMIN110753-17	**12**	0.145	0.175	0.171	0.156	0.136	0.158	0.156	0.147
Radix ampla | GBMLG7299-09	**13**	0.162	0.164	0.140	0.108	0.147	0.099	0.091	0.143
Pseudosuccinea columella | GBMPL1446-15	**14**	0.171	0.160	0.145	0.166	0.147	0.160	0.158	0.166
Lymnaea stagnalis | ASMOL010-10	**15**	0.177	0.134	0.125	0.160	0.160	0.173	0.177	0.164
Lymnaea humilis | FTMCA068-14	**16**	0.160	0.158	0.151	0.145	0.145	0.147	0.143	0.145
Lymnaea diaphana | GBMPL413-13	**17**	0.162	0.145	0.151	0.151	0.162	0.151	0.151	0.160
Lymnaea aulacospira | GBMLG0699-06	**18**	0.206	0.162	0.164	0.179	0.184	0.171	0.171	0.179
LNA H4	**19**	0.154	0.188	0.181	0.138	0.121	0.143	0.136	0.041
LNA H3	**20**	0.154	0.194	0.179	0.140	0.119	0.143	0.136	0.032
LNA H2	**21**	0.147	0.184	0.173	0.132	0.127	0.127	0.125	0.035
LNA H1	**22**	0.147	0.184	0.173	0.132	0.123	0.132	0.130	0.030
Galba truncatula | GBMIN5850-12	**23**	0.169	0.153	0.160	0.158	0.164	0.156	0.151	0.168
Galba schirazensis | GBMIN103733-17	**24**	0.169	0.166	0.145	0.145	0.156	0.145	0.143	0.158
Galba neotropica | AM494008.1	**25**	0.175	0.160	0.138	0.153	0.153	0.153	0.151	0.166
Galba cubensis | KT781308.1	**26**	0.173	0.158	0.140	0.156	0.151	0.156	0.153	0.166
COL H1	**27**	0.171	0.160	0.145	0.166	0.147	0.160	0.158	0.166
RAD H1	**28**	0.030	0.182	0.182	0.158	0.130	0.162	0.156	0.143
Physella gyrina | KT831388	**29**	0.171	0.178	0.178	0.156	0.163	0.165	0.163	0.169
Physa fontinalis | AY651189	**30**	0.167	0.195	0.182	0.184	0.158	0.174	0.174	0.178
Physella acuta | KM612022	**31**	0.184	0.208	0.191	0.165	0.178	0.191	0.195	0.189

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	9	10	11	12	13	14	15	16	17	18	19	20
10	0.099											
11	0.099	0.123										
12	0.162	0.156	0.173									
13	0.082	0.097	0.125	0.160								
14	0.147	0.166	0.143	0.171	0.153							
15	0.149	0.179	0.151	0.175	0.156	0.160						
16	0.149	0.147	0.140	0.164	0.151	0.140	0.153					
17	0.136	0.160	0.114	0.164	0.140	0.130	0.119	0.130				
18	0.175	0.175	0.153	0.188	0.168	0.151	0.173	0.147	0.143			
19	0.123	0.140	0.119	0.145	0.143	0.164	0.164	0.145	0.147	0.186		
20	0.125	0.140	0.121	0.147	0.138	0.160	0.171	0.145	0.153	0.179	0.009	
21	0.117	0.130	0.130	0.140	0.132	0.166	0.162	0.147	0.151	0.179	0.032	0.032
22	0.121	0.134	0.125	0.145	0.136	0.166	0.162	0.143	0.151	0.179	0.028	0.028
23	0.145	0.156	0.153	0.184	0.127	0.147	0.156	0.095	0.130	0.136	0.166	0.168
24	0.138	0.149	0.119	0.173	0.136	0.134	0.147	0.093	0.117	0.132	0.147	0.147
25	0.153	0.156	0.134	0.177	0.145	0.151	0.149	0.093	0.112	0.147	0.166	0.164
26	0.156	0.158	0.136	0.181	0.147	0.149	0.149	0.097	0.117	0.149	0.166	0.164
27	0.147	0.166	0.143	0.171	0.153	0.000	0.160	0.140	0.130	0.151	0.164	0.160
28	0.158	0.165	0.149	0.143	0.156	0.175	0.175	0.158	0.160	0.210	0.141	0.145
29	0.171	0.167	0.156	0.169	0.171	0.167	0.171	0.145	0.154	0.197	0.165	0.169
30	0.161	0.184	0.165	0.174	0.169	0.171	0.182	0.165	0.171	0.180	0.182	0.182
31	0.184	0.197	0.180	0.182	0.191	0.206	0.187	0.189	0.195	0.215	0.193	0.187

	21	22	23	24	25	26	27	28	29	30
22	0.004									
23	0.164	0.164								
24	0.153	0.153	0.093							
25	0.164	0.164	0.114	0.099						
26	0.164	0.164	0.112	0.099	0.013					
27	0.166	0.166	0.147	0.134	0.151	0.149				
28	0.139	0.139	0.171	0.162	0.177	0.180	0.175			
29	0.171	0.171	0.163	0.132	0.156	0.161	0.167	0.167		
30	0.167	0.171	0.163	0.161	0.169	0.176	0.171	0.171	0.126	
31	0.184	0.184	0.215	0.174	0.174	0.174	0.206	0.182	0.151	0.143

Table 8: Gamma and invariant site values for Bayesian inferred trees using the GTR model. The used marker is COI unless indicated otherwise. Absence of an invariant site value (I) indicates that is was not used in that respective model.

Taxon name	Model used	Gamma value	Invariant site value
Schistosoma	GTR + G + I	0.426	33%
Trematoda	GTR + G	0.318	/
Trematoda 18S	GTR + G + I	0.530	47.3%
Planorbidae	GTR + G + I	0.343	41%
Lymnaeidae	GTR + G + I	0.221	43.8
Physidae	GTR + G	0.204	/

7. Trematode diversity

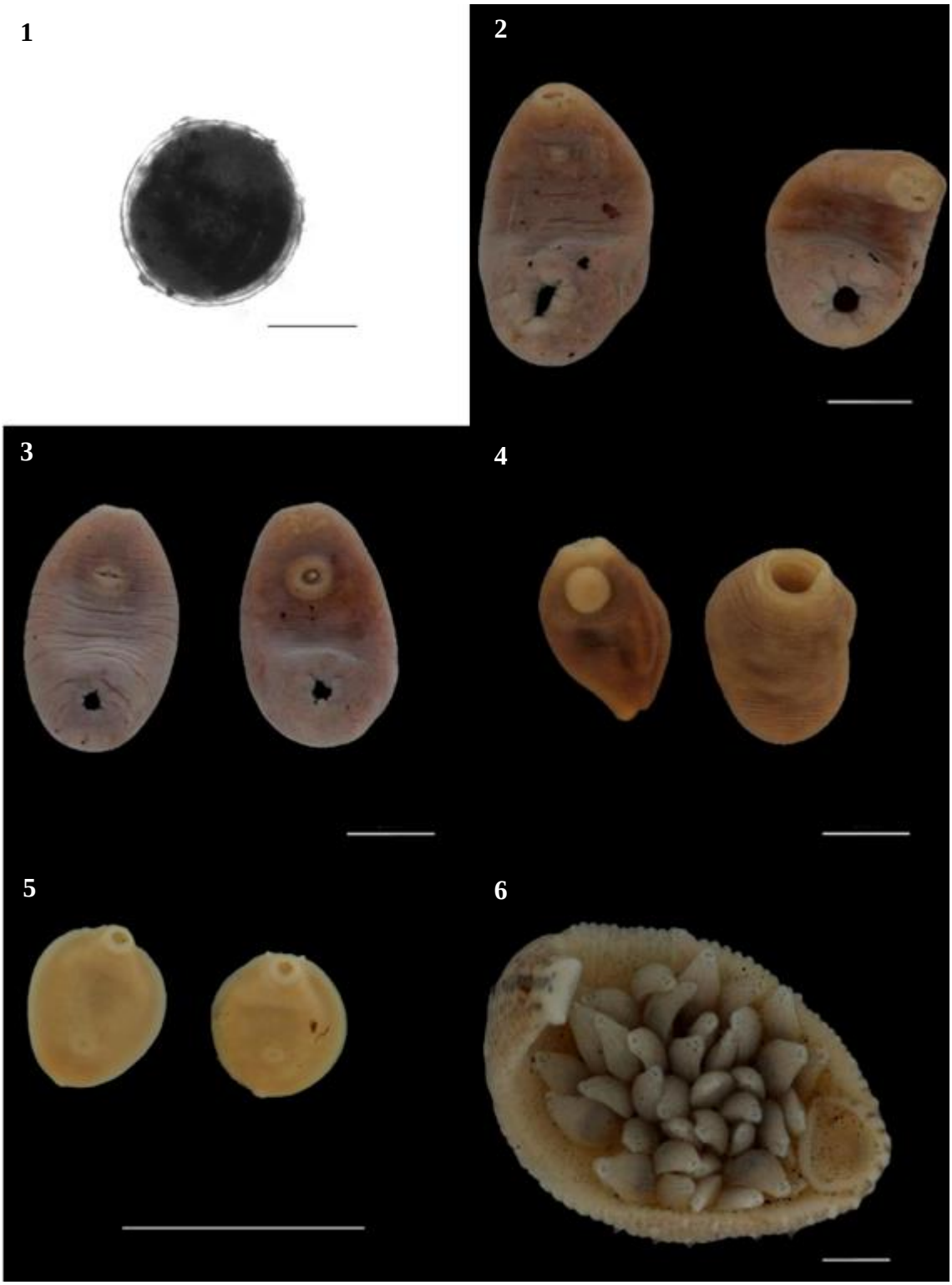

Figure 6: *F. nyanzae* metacercaria (1), Adult amphistomes (2 and 3 from *B. taurus*, 4 and 5 from *Hi. amphibious*) and *Helobdella europaea* (Phylum: Anellida, Subclass: Hirudinea) from Lake Chivero. Scale bars represent 100 µm, 5 mm and 1 mm respectively in pictures 1, 2-5 and 6.

Table 9: Morphometrics fasciolids. Morphological measurements are provided for three different *Fasciola* sp. Three reference measurements from the literature are provided for *F. nyanzae* (Leiper, 1910; Jackson, 1921; Dinnik and Dinnik, 1961). All samples are adult flukes except for 'S3_COL_1' and S3_COL_3', which are metacercariae. Sample origin, host, Length to width ratio, body width at widest point (BW), Body length (BL), Cephalic cone length until shoulders (CL), Posterior width at 2 mm from end (PW), Ventral sucker anteroposterior diameter (VS), oral sucker width (OS), testis location in body length, testis to BL ratio, vitellaria location in body length, outer diameter metacercaria (OD), inner diameter metacercaria (without fibrous layer, ID) All measurements are done in mm unless indicated otherwise. Metacercarial cyst measurements for *F. gigantica* and *F. hepatica* were taken from Alicata (1938) and Vareille-Morel *et al.* (1993) respectively.

Origin	Host	Length/width	BW	BL	CL	PW	VS	OS
Dinnik and Dinnik (1961)	*Hi. amphibious*	~7/1	~9	49 - 91	/	/	1.46 - 1.97	1.15 - 1.55
Leiper (1910)	*Hi. amphibious*	7/1	~9	69	3	3	1.25	0.9 - 0.7
Jackson (1921)	*?*	1/3.6 - 1/7.8	13.5 - 4.5	35 - 59	/	/	/	/
***F. nyanzae* 1**	*Hi. amphibious*	4.9/1	10.6	52.4	/	3.1	1.47	/
***F. nyanzae* 2**	*Hi. amphibious*	3.5/1	13.3	46.9	2.2	3.2	1.72	0.82
***F. nyanzae* RMCA**	*Hi. amphibious*	4.2/1	9.8	41.2	2.04	3	1.35	/
S3_COL_3	*P. columella*	/	/	/	/	/	/	/
S3_COL_1	*P. columella*	/	/	/	/	/	/	/
***F. gigantica* 1**	*B. taurus*	3.2/1	9.3	29.3	1.8	6.5	1.45	0.6
***F. gigantica* 2**	*B. taurus*	2.8/1	9.3	25.6	1.4	4.2	1.46	/
***F. gigantica* RMCA**	?	3.6/1	9.9	35.3	3.1	5.1	0.83	0.79
***F. hepatica* RMCA**	?	3.1/1	8.6	26.8	1.67	4.6	1.47	0.88

Origin	Testis location	testis/BL	vitellaria	OD cysts (µm)	ID cysts (µm)
Dinnik and Dinnik (1961)	anterior third	25 - 40%	/	242 - 272	212 - 228
Leiper (1910)	/	/	25	/	/
Jackson (1921)	anterior third	/	/	/	/
***F. nyanzae* 1**	anterior third	24%	34.1	/	/
***F. nyanzae* 2**	anterior third	29%	27.1	/	/
***F. nyanzae* RMCA**	anterior third	25%	25.4	/	/
S3_COL_3	/	/	/	261	221
S3_COL_1	/	/	/	259	228
***F. gigantica* 1**	anterior two third	42%	9.96	/	/
***F. gigantica* 2**	anterior two third	41%	8.2	/	/
***F. gigantica* RMCA**	anterior two third	41%	10.8	/	/
***F. gigantica* metacercariae**				238-268	180 - 206
***F. hepatica* RMCA**	anterior two third	42%	10.16	/	/
***F. hepatica* metacercariae**				230.2 +- 25.3	

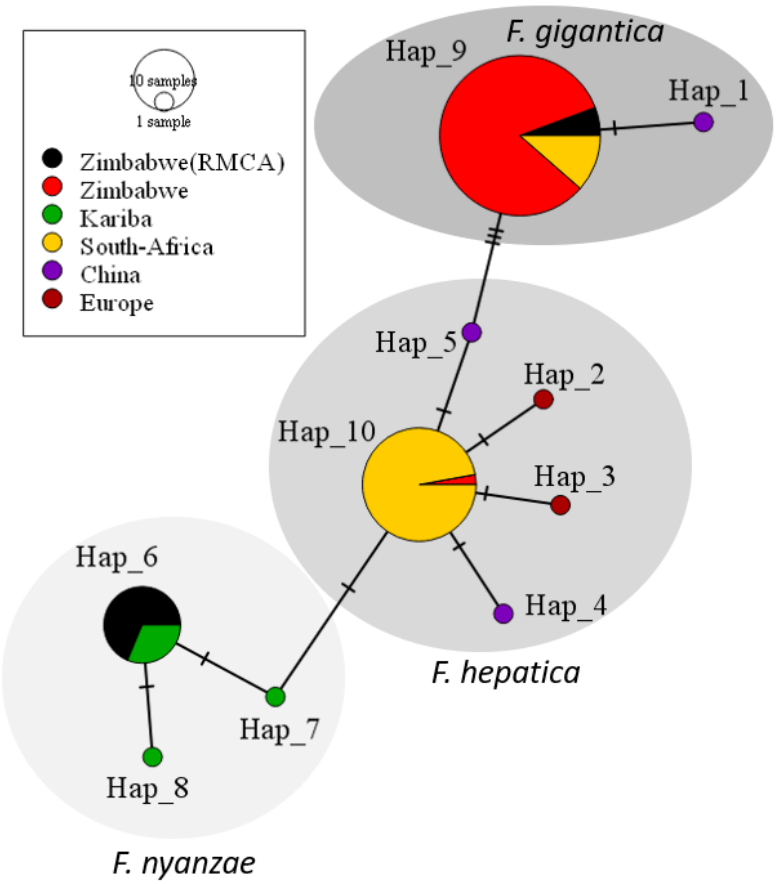

Figure 7: ITS1 genotype network based on 464 nucleotides using the TCS model. Numbers between parentheses represent mutational steps. Grey scale circles indicate same-species genotypes based on COI sequences of the same sample. Legend explains colour-code and genotype abundance. 'Zimbabwe (RMCA)' indicates samples originating from Koala Park abattoir and Mwenje reservoir. 'Zimbabwe' and 'South-Africa' shows samples collected in Zimbabwe by Mucheka *et al.* (2015). Kariba although belonging to Zimbabwe is indicated separately to better understand the geographical distribution of *F. nyanzae*.

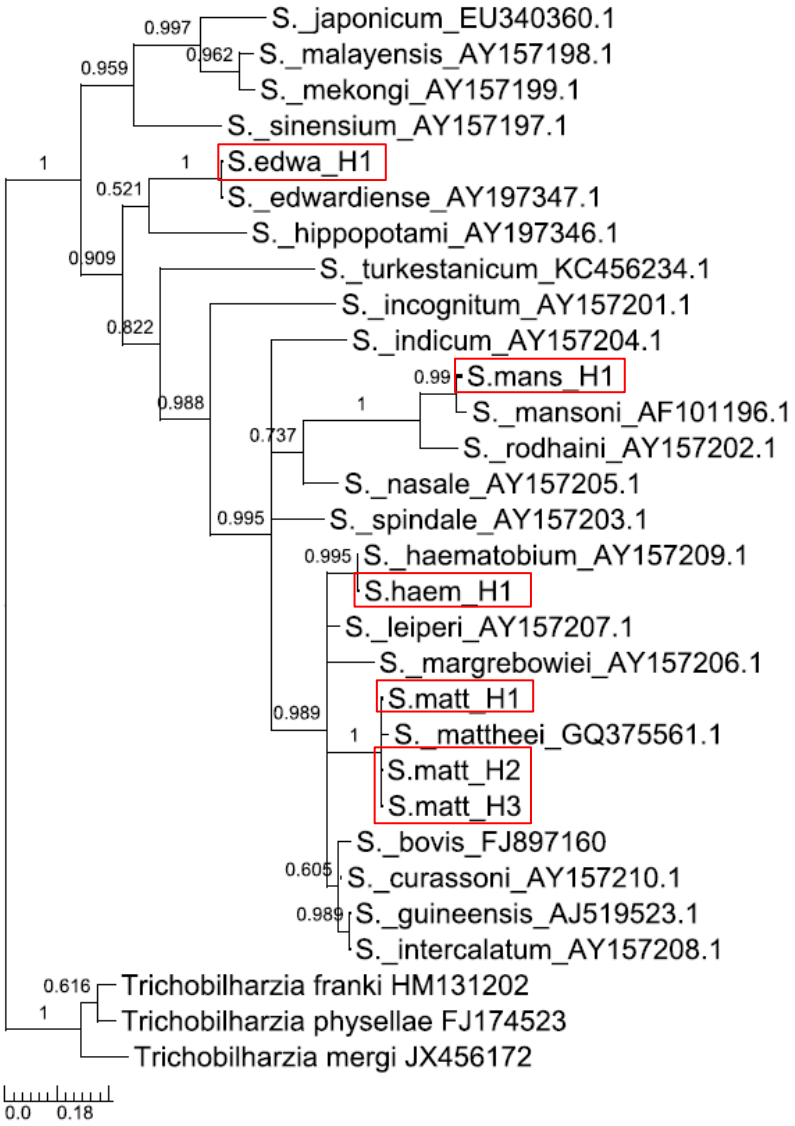

Figure 8: Bayesian inferred phylogenetic tree for the *Schistosoma* genus, using a 335 bps COI alignment and the GTR model with a discrete Gamma distribution ([+G] = +0.416) and invariant sites ([+I] = 33%). Ngen= 1,000,000 using the MCMC algorithm. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 30 different sequences. BOLD or GenBank accession number of each reference sequence is displayed after the sequence ID. Red boxes indicate sequences obtained by this study (Country of origin: Zimbabwe).

Table 10: Pairwise genetic distance (p-distance) between *Schistosoma* spp. sequences (COI) of 465 nucleotides. The proportion of nucleotide differences per site between sequences is shown. Discussed results are highlighted in yellow.

Sequence		1	2	3	4	5	6	7	8	9
S. bovis | FJ897160	**1**									
S. curassoni | AY157210.1	**2**	0.048								
S. edwardiense | AY197347.1	**3**	0.254	0.242							
S. guineensis | AJ519523.1	**4**	0.075	0.051	0.242						
S. haematobium | AY157209.1	**5**	0.113	0.101	0.257	0.116					
S. hippopotami | AY197346.1	**6**	0.254	0.254	0.218	0.251	0.245				
S. incognitum | AY157201.1	**7**	0.245	0.218	0.239	0.230	0.227	0.245			
S. indicum | AY157204.1	**8**	0.188	0.185	0.242	0.179	0.191	0.251	0.233		
S. intercalatum | AY157208.1	**9**	0.075	0.051	0.242	0.000	0.116	0.251	0.230	0.179	
S. japonicum | EU340360.1	**10**	0.272	0.263	0.260	0.263	0.275	0.251	0.269	0.224	0.263
S. leiperi | AY157207.1	**11**	0.087	0.078	0.239	0.081	0.090	0.233	0.227	0.197	0.081
S. malayensis | AY157198.1	**12**	0.233	0.224	0.239	0.242	0.260	0.257	0.236	0.218	0.242
S. mansoni | AF101196.1	**13**	0.239	0.230	0.281	0.248	0.224	0.287	0.287	0.224	0.248
S. margrebowiei | AY157206.1	**14**	0.125	0.119	0.254	0.116	0.125	0.233	0.203	0.200	0.116
S. mattheei | GQ375561.1	**15**	0.128	0.116	0.269	0.140	0.140	0.266	0.242	0.185	0.140
S. mekongi | AY157199.1	**16**	0.245	0.239	0.233	0.251	0.254	0.227	0.245	0.218	0.251
S. nasale | AY157205.1	**17**	0.194	0.182	0.266	0.200	0.170	0.284	0.227	0.203	0.200
S. rodhaini | AY157202.1	**18**	0.206	0.206	0.254	0.218	0.203	0.257	0.287	0.233	0.218
S. sinensium | AY157197.1	**19**	0.263	0.260	0.254	0.284	0.269	0.227	0.275	0.245	0.284
S. spindale | AY157203.1	**20**	0.191	0.176	0.230	0.179	0.188	0.245	0.218	0.173	0.179
S. turkestanicum | KC456234.1	**21**	0.227	0.230	0.254	0.224	0.233	0.278	0.278	0.239	0.224
S.edwa H1	**22**	0.251	0.239	0.003	0.239	0.260	0.218	0.242	0.239	0.239
S.mans H1	**23**	0.230	0.215	0.275	0.239	0.203	0.284	0.284	0.215	0.239
S.haem H1	**24**	0.116	0.104	0.254	0.119	0.003	0.242	0.224	0.188	0.119
S.matt H1	**25**	0.117	0.105	0.263	0.129	0.147	0.272	0.257	0.180	0.129
S.matt H2	**26**	0.119	0.107	0.263	0.125	0.146	0.269	0.254	0.176	0.125
S.matt H3	**27**	0.122	0.110	0.266	0.128	0.149	0.272	0.257	0.179	0.128
Trichobilharzia franki | HM131202	**28**	0.263	0.260	0.239	0.269	0.281	0.260	0.245	0.254	0.269
Trichobilharzia mergi | JX456172	**29**	0.272	0.260	0.245	0.257	0.287	0.254	0.245	0.254	0.257
Trichobilharzia physellae | FJ174523	**30**	0.269	0.257	0.266	0.266	0.260	0.236	0.236	0.245	0.266

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	10	11	12	13	14	15	16	17	18	19	20	21	22
11	0.257												
12	0.152	0.242											
13	0.278	0.236	0.284										
14	0.263	0.110	0.227	0.251									
15	0.248	0.131	0.233	0.251	0.143								
16	0.155	0.233	0.069	0.278	0.242	0.227							
17	0.287	0.176	0.257	0.206	0.185	0.200	0.254						
18	0.266	0.203	0.260	0.146	0.215	0.239	0.260	0.209					
19	0.215	0.272	0.212	0.304	0.269	0.272	0.200	0.272	0.299				
20	0.254	0.176	0.230	0.224	0.185	0.203	0.230	0.170	0.209	0.245			
21	0.301	0.215	0.269	0.319	0.251	0.260	0.266	0.266	0.281	0.293	0.245		
22	0.257	0.236	0.236	0.281	0.257	0.272	0.230	0.266	0.254	0.251	0.227	0.254	
23	0.278	0.221	0.281	0.042	0.236	0.239	0.278	0.206	0.137	0.299	0.221	0.313	0.275
24	0.272	0.093	0.257	0.221	0.128	0.143	0.251	0.167	0.200	0.266	0.185	0.236	0.257
25	0.246	0.120	0.225	0.254	0.138	0.027	0.228	0.192	0.237	0.260	0.189	0.243	0.260
26	0.245	0.116	0.221	0.254	0.137	0.024	0.224	0.191	0.236	0.260	0.185	0.248	0.260
27	0.248	0.119	0.224	0.251	0.140	0.027	0.227	0.188	0.233	0.263	0.182	0.251	0.263
28	0.251	0.254	0.233	0.290	0.257	0.269	0.236	0.278	0.284	0.260	0.269	0.284	0.242
29	0.236	0.254	0.221	0.272	0.248	0.266	0.224	0.260	0.278	0.266	0.236	0.284	0.248
30	0.257	0.263	0.242	0.278	0.236	0.281	0.227	0.257	0.266	0.254	0.251	0.269	0.269

	23	24	25	26	27	28	29
24	0.200						
25	0.237	0.150					
26	0.236	0.149	0.003				
27	0.233	0.152	0.006	0.003			
28	0.281	0.278	0.266	0.266	0.266		
29	0.269	0.284	0.266	0.263	0.263	0.113	
30	0.272	0.257	0.284	0.284	0.284	0.087	0.128

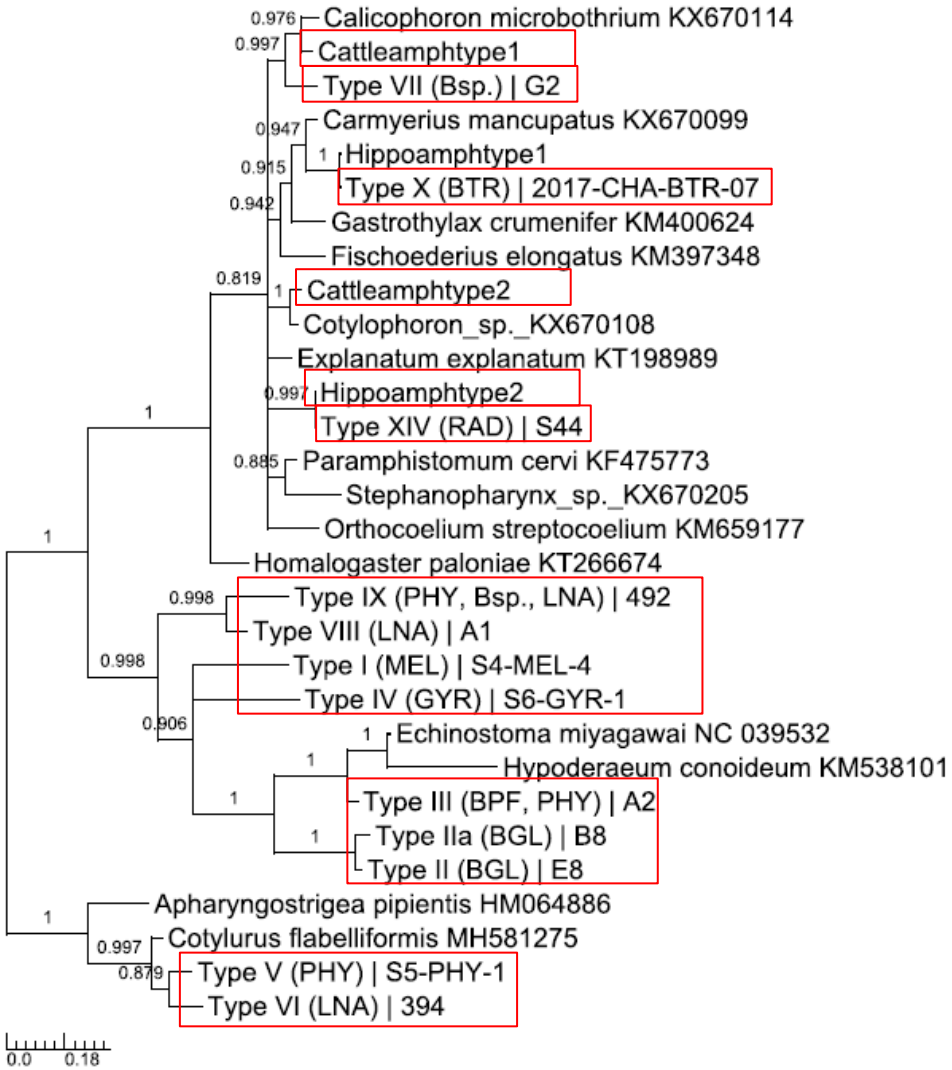

Figure 9: Bayesian inferred phylogenetic tree for trematodes, using a 276 bps COI alignment and the GTR model with a discrete Gamma distribution ([+G] = +0.318). Ngen=1,000,000 in the MCMC algorithm. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 30 different sequences. BOLD or GenBank accession number of each reference sequence is displayed after their ID. All type names were obtained in this study, except for type X and XIV (collected by Hans Carolus).

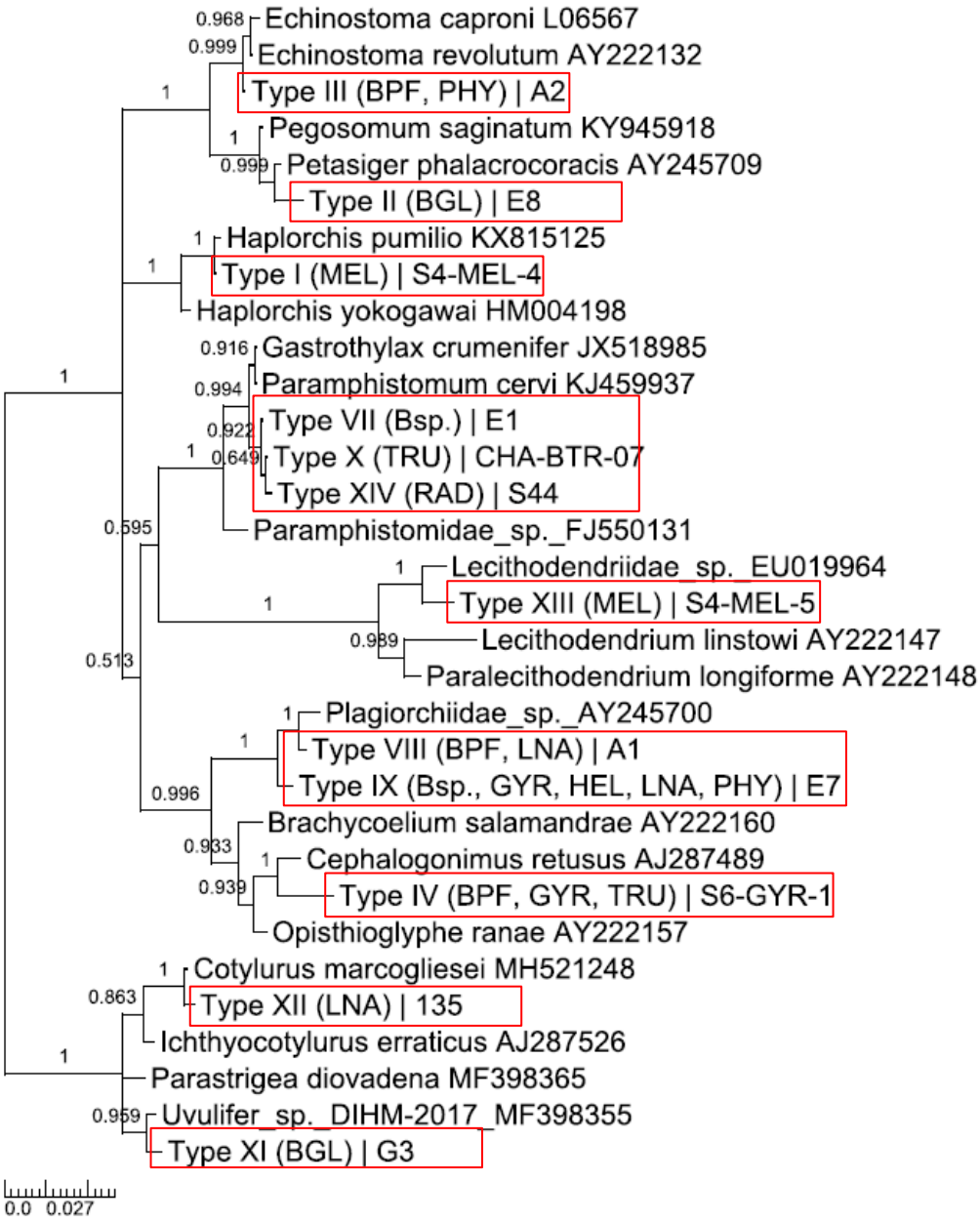

Figure 10: Bayesian inferred phylogenetic tree for trematodes, using a 796 bps 18S alignment and the GTR model with a discrete Gamma distribution ([+G] = +0.53) and invariant sites ([+I] = 41%). Ngen=1,000,000 in the MCMC algorithm. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 32 different sequences. BOLD or GenBank accession number of each reference sequence is displayed after their ID. All type names were obtained in this study, except for type X and XIV (collected by Hans Carolus).

Table 11: Summary of barcoded trematode sequences. The top two BLAST hits with their respective identity covers (ID) and length (len.) are provided for every type included in the genotype analysis. The host species are listed with BGL: *B. globosus*, *B.*sp.: *Bulinus* sp., BPF: *Bi. pfeifferi*, COL: *P. columella*, GYR: *G. connollyi*, HEL: *H. duryi*, LNA: *L. natalensis*, MEL: *M. tuberculata*, PHY: *P. acuta*, RAD: *Radix* sp. and TRU: *B. truncatus*. The site number is indicated between parentheses. Site 'Kar' indicates collection by Hans Carolus in 2017 from Lake Kariba. Host 'cow' and 'Hippo' indicate collection from slaughtered cattle or the culled *Hi. amphibious*.

Type name	Host (site)	Marker	len. (bp)	Best BLAST 1	ID (%)	Best BLAST 2	ID (%)
Type I	MEL (4)	18S	873	*Haplorchis pumilio*	99.9	*Haplorchis taichui*	99.7
		COX1	609	*Echinostoma sp.*	77.4	*Echinostoma hortense*	76.4
Type IIa	BGL (4)	18S	1051	*Petasiger phalacrocoracis*	98.9	*Pegosomum saginalum*	98.4
		COX1	645	*Acanthoparyphium sp.*	78.9	*Echinostoma miyagawai*	77.6
Type II	BGL (4)	18S	1071	*Petasiger phalacrocoracis*	98.8	*Pegosomum saginalum*	98.3
		COX1	809	*Echinostoma hortense*	81.4	*Fasciola gigantica*	81.3
Type III	BPF (1), PHY (1)	18S	1049	*Echinostoma revolutum*	100	*Echinostoma paraensei*	99.9
		COX1	738	*Echinostoma revolutum*	96.8	*Echinostoma miyagawai*	95.5
Type IV	BPF (6), GYR (6), TRU (7, 9)	18S	1048	*Cephalogonimus retusus*	97.2	*Opisthioglyphe ranae*	97.2
	GYR (6)	COX1	414	*Paruterina candelabraria*	75.4	*Haematoloechus sp.*	74.6
Type V	PHY (5)	COX1	356	*Ichthyocotylurus sp.*	86.7	*Diplostomum sp.*	86.0
Type VI	LNA (7, 8b)	COX1	437	*Cotylurus flabelliformis*	90.4	*Cotylurus marcogliesei*	89.7
Type VII	B.sp.2 (1, 3)	18S	1059	*Paramphistomum cervi*	99.9	*Gastrothylax crumenifer*	99.9
		COX1	696	*Calicophoron clavula*	91.1	*Calicophoron raja*	90.2
Type VIII	BPF (5, 9), LNA (1, 5)	18S	1055	*Plagiorchiidae sp.*	99.4	*Auridistomum chelydrae*	94.4
	LNA (1, 5)	COX1	416	*Lepotrema melichthydis*	73.2	*Neodiplostomum americanum*	72.1
Type IX	PHY (1), B.sp.? (3), LNA (3), GYR (7), HEL (10)	18S	1009	*Plagiorchiidae sp.*	98.3	*Opisthioglyphe ranae*	95.0
	PHY (3, 7, 8), B.sp? (3, 7), LNA (8b)	COX1	419	*Paragonimus mexicanus*	77.3	*Plagiorchis sp.*	77.1
Type X	TRU (Kar), Hippo	18S	1043	*Gastrothylax crumenifer*	100.0	*Paramphistomum cervi*	99.9
		COX1	376	*Carmyerius mancupatus*	89.4	*Carmyerius exoporus*	88.8
Type XI	BGL (5)	18S	1078	*Uvulifer sp.*	99.2	*Parastrigea diovadena*	97.4
Type XII	LNA (1)	18S	950	*Cotylurus marcogliesei*	99.5	*Ichthyocotylurus erraticus*	97.1
Type XIII	MEL (4)	18S	1005	*Lecithodendriidae sp.*	98.4	*Paralecithodendrium longiforme*	96.2
Type XIV	RAD (Kar), Hippo	18S	1052	*Gastrothylax crumenifer*	99.7	*Paramphistomum cervi*	99.6
		COX1	419	*Gastrothylacidae sp.*	86.4	*Calicophoron microbothrium*	85.4
Cattleamphtype2	Cattle	COX1	671	*Cotylophoron* ***sp.***	95.9	*Cotylophoron* ***sp.***	95.8
Cattleamphtype1	Cattle	COX1	692	*Calicophoron microbothrium*	92.1	*Cotylophoron sp.*	92.0
S.mans	BPF (6)	COX1	521	*Schistosoma mansoni*	99.4	*Schistosoma rodhaini*	88.3
S.haem	BGL (8b)	COX1	804	*Schistosoma haematobium*	99.8	*Schistosoma leiperi*	90.7
S.edwa	BPF (8b)	COX1	591	*Schistosoma edwardiense*	99.8	*Schistosoma sp.*	83.6
S.matt	BGL (4, 5, 7), TRU (2)	COX1	665	*Schistosoma mattheei*	99.7	*Schistosoma sp.*	89.6
F.nyanzae	LNA (7, 8b), COL (Kar), RAD (Kar)	COX1	862	*Fasciola gigantica*	93.2	*Fasciola sp.*	92.9
F.gigantica	LNA (5), cattle	COX1	885	*Fasciola gigantica*	97.9	*Fasciola sp.*	97.3

Table 12: General trematode, *Schistosoma* spp., *Fasciola* spp. infection abundances and prevalences per site.

Site	Nr. of gastropods tested	Nr. of planorbids tested	Nr. of lymnaeids tested	Nr. ind. tested	Ind. tested trematode infections	Trematode infection prevalence	Nr. of Pooled trematode infections	*Schistosoma* sp. infected gastropods	*Schistosoma* spp. prevalence	*Fasciola* sp. infected gastropods	*Fasciola* spp. prevalence
1	86	33	27	35	29	82.9%	51	0	0%	0	0%
2	27	24	3	23	5	21.7%	4	1	4%	0	0%
3	163	78	60	34	32	94.1%	129	0	0%	0	0%
4	28	20	1	20	14	70.0%	8	3	15%	0	0%
5	120	72	24	44	35	79.5%	76	5	7%	3	13%
6	13	11	1	13	6	46.2%	0	1	9%	0	0%
7	115	96	11	45	21	46.7%	70	5	5%	2	18%
8	39	5	26	35	10	28.6%	4	1	20%	0	0%
8b	36	16	17	34	30	88.2%	2	11	69%	12	71%
9	63	53	10	25	20	80.0%	38	8	15%	1	10%
10	45	27	15	45	3	6.7%	0	0	0%	0	0%
Total	735	435	195	353	205	/	382	35	/	18	/

Table 13: Abiotic measurements of all sites located near Harare (Zimbabwe). Nitrate and Phosphate absorbance values were measured at 545nm and 882nm, respectively.

Site	pH	Water temp (°C)	Conductivity (µS/cm)	Dissolved oxygen (mg/l)	Dissolved oxygen	TDS (mg/l)	Nitrates(mg/L)	Phosphates (mg/L)	Carbonates (CaCO3 mg/L)
1	6.79	21.2	268	17.2	72%	166	/	/	/
2	6.89	20.7	258	2.4	27%	156	0.096	0.712	10
3	6.99	22.5	287	6.3	85%	160	0.137	0.14	10
4	7.32	21.6	86.5	5	61%	51	0.35	0.334	5
5	6.88	23.4	326	6.7	86%	171	0.162	0.156	10
6	7.4	20	134.2	3.9	49%	82	0.108	0.324	10
7	/	23.2	86.4	/	/	49	0.022	0.404	10
8	/	26.6	86.9	/	/	47	0.03	0.353	10
9	/	26.4	194.8	/	/	106	0.051	0.3	10
10	/	/	/	/	/	/	/	/	/

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