More than meets the eye: A shift in the gastropod and trematode communities in Lake Kariba, Zimbabwe

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Abstract

Background: Freshwater gastropod-borne trematodes pose a great public health burden and cause major economic losses in the livestock and fish industries. Knowledge on the composition, diversity and ecology of both gastropods and trematodes communities is key to understand disease transmission dynamics and control trematodiases of economic significance. The objective of this study is to investigate the diversity and spatio-temporal ecological trends of gastropod and trematode communities on the Northern shore of Lake Kariba in Zimbabwe, the largest artificial lake in volume worldwide.

Methods: A sampling campaign was undertaken at 16 sites along the lake shoreline of Kariba town. Gastropods and water samples were collected monthly during one year. All 2477 specimens were identified, counted and subjected to shedding experiments; infection status was confirmed by the use of a Rapid Diagnostic PCR (RD-PCR). To explain spatio-temporal trends in gastropod and trematode occurrence, water samples were analysed for different physico-chemical parameters including pH, temperature, nitrates and phosphates.

Results: Gastropod species collected include *Bulinus truncatus, Bulinus forskalii, Gyraulus* sp., *Pseudosuccinea columella, Radix* sp, *Physella acuta, Bellamya* sp. and *Melanoides tuberculata. Bulinus truncatus* was found to be infected with trematode species belonging to the families Notocotylidae, Psilostomstidae, Paramphistomidae and Diplostomatidae. A species of the latter family was also found to infect B. forskalii. As previously reported, lymnaeid species *P. columella* and *Radix* sp. were both infected with a species belonging to the Fasciolidae family, while Radix sp. was also infected with amphistomes.

Conclusions: We confirm the occurrence of new species of gastropods in Lake Kariba and the absence of the previously reported *B. pfeifferi* and *B. globosus*. This explains the absence of human schistosome species, but we report the presence of other trematode families that have not been reported in Lake Kariba or Zimbabwe before. Compared to the latest study in 2001, there is a remarkable shift in the gastropod community, which is likely driven by the introduction of exotic gastropod species that are now abundant in the lake.

1. Background

Some freshwater gastropod species are of high medical and or economic importance as they act as intermediate hosts to parasitic flatworms (class: Trematoda) that infect humans and other vertebrates as final host. These gastropod-borne trematodes form a major public health burden and affect the aquaculture and livestock industry leading to billions of US dollars in economic losses worldwide (Giannelli et al., 2016). The most important trematodiases in Africa are without doubt schistosomiasis and fasciolosis, caused by the infection of trematodes of the genera Schistosoma and Fasciola respectively. Planorbid gastropod species of the Bulinus and Biomphalaria genera transmit schistosomiasis in Africa (Agatsuma et al., 2002) while fasciolosis is transmitted by species belonging to the Lymnaeidae family (Toledo et al., 2014). According to the WHO about 88% of the global mortality due to schistosomiasis occurs in Africa (World Health Organization Geneva, 2018). Fasciolosis is estimated to infect 2.4 to 17 million people in more than 60 countries, of which African countries are major endemic areas (DiNardo et al., 2014; Toledo et al., 2014). The reality however is that all gastropod-borne diseases are neglected diseases. As a result, most research on gastropod-borne trematodes is in its infancy when it comes to knowledge about their epidemiology, biology and ecology (Adema et al., 2012; Toledo et al., 2014).

Due to the high specificity of trematodes for their first intermediate host, the geographical distribution of gastropods can dictate where transmission of the associated trematodes is likely to occur (Woolhouse & Chandiwana, 1989). Therefore knowledge on gastropod abundance, distribution and ecology is essential in understanding the epidemiology of the disease. Environmental factors such as size, volume and depth of the water body, velocity, vegetation, dissolved nutrients and the presence of other gastropod species regulate gastropod distribution (Hubendick 1958;Giovanelli et al., 2005). Oloyede et al. (2016), for example, found that there was a significant relationship between water temperature and B. globosus distribution.

The creation of embankments such as dams and dikes may create new habitats and disrupt the ecological and limnological conditions of water systems, often increasing the risk of water-borne disease transmission (Araoye, 2002; Steinmann et al., 2006). Construction of dams in the tropical world has been known to intensify transmission of diseases such as schistosomiasis, malaria and filariases in endemic regions, as well as the creation of new transmission areas (Khalil, 1949; Farid, 1977;Hunter et al., 1982; Sokolow et al., 2017). The construction of the Kariba dam wall on the Zambezi River bordering Zimbabwe and Zambia is also an example of a man-made hydroelectric construction that led to increased transmission of schistosomiasis. After the dam construction and formation of the lake, studies reported increasing prevalence of human schistosomiasis, as well as Bulinus globosus and Biomphalaria pfeifferi, both intermediate gastropod hosts of Schistosoma species, on both the Zimbabwean (Machena, 1988; Chimbari & Chirundu, 2003) and the Zambian (Hira, 1969; Hira, 1970; Mungomba et al., 1993; Mungomba and Kalumba, 1995) sides of Lake Kariba. In more recent years, the prevalence has however decreased in Kariba town as a result of mollusc and schistosomiasis control strategies and improved water sanitation facilities (Chimbari et al., 2003). Because most research in Lake Kariba has focused on Schistosoma species and their intermediate hosts, little is known regarding the diversity and prevalence of other trematodes and gastropod hosts present in the lake. We adopted the same sites studied by Chimbari et al. (2003) in their investigation on the prevalence of Schistosoma spp. and their respective snail hosts, and investigate the diversity and ecology of the complete gastropod and trematode fauna of Lake Kariba. This is the first study in which molecular diagnostic tools such as DNA barcoding and Rapid Diagnostic-PCR (RD-PCR) are used alongside gastropod shedding experiments and ecological analysis to investigate the gastropod and trematode community of Lake Kariba.

2. Methods

2.1 Gastropod sampling, cercarial shedding and gastropod identification

2.1.1 Gastropod sampling and cercarial shedding

Sampling was performed monthly from May 2017 to April 2018 at 16 sites on the banks of Lake Kariba near the town of Kariba, Zimbabwe as shown in Figure 1. The months were divided according to three seasons; namely the cold season (May-August), the hot and dry season (September-December) and the hot and wet season (January-April). Gastropod samples were collected by scooping the sediment and aquatic vegetation along a 40 m stretch of lake shoreline up to 1 m deep into the lake using a scooping net. A dredge was used for sampling depths up to 4 m. Gastropods were collected by two people during approximately 30 minutes per site.

Live snails were transferred to cell culture plates (pooled per five specimens of same species and collection site) filled with aged tap water and exposed to artificial light for two hours to induce cercarial shedding. Plates were checked at 30 minute intervals using a stereomicroscope. Upon shedding, pooled gastropods were individually incubated. Infected snails were stored in 1.5 ml of 98% ethanol with a subsample of the released cercariae. Non-shedding snails were kept alive in the laboratory for a week after which they underwent another shedding experiment as described above. If negative, they were fixed in 50ml Falcon tubes in 98% ethanol, pooled per species and per site.

2.1.2 Gastropod Morphological Identification

Morphological identification was carried out by stereomicroscope analysis and following identification keys by Mandahl-Barth (1962), Brown (1994) and Frandsen & McCullough (1980), all of which use shell morphology as well as the shape of soft parts extended out of the shell; i.e., the shape of the foot and the tentacles, head and presence or absence of an operculum. High quality stacking photography was used to photograph two specimens from each morphotype before DNA extraction. Multiple pictures on a range of focal depths were taken and all pictures were stacked using Zerene Stacker® creating a high-resolution image of the gastropod along with a calibrated scale.

2.1.3. Gastropod molecular identification

For molecular identification we focused on the pulmonates as they are the main intermediate hosts of trematodes. To confirm gastropod species identity of the different morphotypes (i.e. species discriminated based on morphological identification), DNA barcoding based on the cytochrome c oxidase subunit I (*cox1*) marker was applied as described in Carolus *et al.* (2019). Gastropod DNA extraction was carried out using the E.Z.N.A.® Mollusc DNA Kit (OMEGA bio-tek, Inc.) following the manufacturer's protocol. DNA sequencing and phylogenetic analysis was performed using the protocol and primers described in Carolus *et al.* (2019).

2.2. Trematode infection prevalence in gastropods

2.2.1. DNA extraction

For the infection prevalence assessment, the Chelex® (Biorad™) DNA extraction method was used to extract gastropod and trematode DNA as described in Carolus *et al.* (2019). Gastropods were pooled per four specimens of the same morphotype, site and collection date. A maximum of 16 gastropods of the same morphotype, site and sampling date were analysed.

2.2.2. Multiplex RD-PCR

Two multiplex rapid diagnostic (RD)-PCR protocols were used as described by Carolus et al. (2019) for *Fasciola* spp. detection, and by Schols et al. (2019) for *Schistosoma* spp. detection respectively. Both multiplex RD-PCR protocols are composed of three markers: one internal positive control (i.e. a general gastropod marker that confirms a successful PCR amplification), a trematode-specific marker and a marker that targets members of a certain trematode family of interest (i.e. *Fasciola* spp. in lymnaeid gastropods and *Schistosoma* spp. in planorbid gastropods respectively).

2.3. Trematode identification

2.3.1. DNA extraction

For DNA extraction of individual cercariae, the DNeasy Blood and Tissue Kit (Qiagen™) was used. DNA was extracted from a maximum of four individual cercariae per infected gastropod. Also all snail DNA extracts that appeared positive after the Multiplex RD-PCR but not positive for the *Schistosoma* or *Fasciola* signal, were included in the simplex PCR protocol for trematode species identification.

2.3.2. Simplex PCR protocol

Primers used for the amplification of the 18S rDNA marker (1160 bp) were 18S_Digenea_F (5'- CAGCTATGGTTCCTTAGATCRTA-3') from Carolus et al. (2019) and 1270_R (5'-CCGTCAATTCCTTTAAGT-3') from Littlewood & Olson (2001) while a fragment of the gene coding for the cytochrome c oxidase subunit I (*cox1*) was targeted using primers COI1_dig_F and COI1_dig_R, and yielded a fragment of approximately 450 bp (Carolus et al. 2019); The gastropod-specific markers were targeted using primers COI_gastropod_F and COI_gastropod_R (Carolus et al. 2019). All PCR reactions were performed according to Carolus et al. (2019). PCR products were visualised by gel electrophoresis on a 2 % agarose gel with Midori Green Direct® staining and UV light. Positive PCR samples were purified using the ExoSAP (Fermentas™) PCR purification protocol and sequenced by Macrogen™ (Sanger sequencing using BigDye® chemistry).

2.3.4. Phylogenetic analysis

Consensus sequences were assembled from forward and reverse sequences using Geneious® Version 6.1.8 software; all sequences were manually trimmed and ambiguities were edited. All parts of the phylogenetic analysis described next were computed and visualized in MEGA 7 (Tamura et al., 2013). Based on the taxonomic indications from the Basic Local Alignment Search Tool (BLAST; https://blast.ncbi.nlm.nih.gov/Blast.cgi) (i.e. order, superfamily, family and genus), the NCBI GenBank (https://www.ncbi.nlm.nih.gov/) and BOLD (http://www.boldsystems.org/index.php/) databases were mined for closely related

sequences to construct a phylogenetic tree. Sequences were aligned using the MUSCLE alignment algorithm (Edgar, 2004) and trimmed homogenously for phylogenetic tree construction. The best nucleotide substitution model was calculated by Model Selection (based on the Bayesian information Criterion, BIC) in MEGA and used to compute pairwise genetic distances and construct a Maximum Likelihood (ML) tree with 500 replicates for nodal support statistics (bootstrap method). Genetic pairwise distances were calculated between *cox1* sequences with 5% as threshold for intraspecific variation (Vilas et al., 2005; Lawton et al., 2015) to discriminate between species.

2.4. Water Quality Analysis

Water temperature, pH, dissolved oxygen, oxygen saturation percentage and conductivity, were measured on site using a HACH multi-meter (HQ40D HACH, USA). A EUTECH turbidity meter (TN-100, Singapore) was used to measure water transparency. Water samples were collected in cooled polythene bottles for dissolved nutrients analysis. Chemical analysis of water was conducted in the Wet Chemistry Laboratory at the University Lake Kariba Research Station (ULKRS). Samples analysed for nitrates and phosphates were first filtered using Whatman GF/C filter papers with 47 mm diameter. The filter papers were wrapped with an aluminium foil and placed in the freezer prior to chlorophyll a analysis. Nitrates, orthophosphates, total nitrogen, total phosphorous and ammonium were analysed following guidelines by USEPA (1983) and Hach Company (2007). Chlorophyll a analysis was carried out following guidelines by Aminot & Rey (2000).

2.5. Statistical data analysis

The ecological data (physico-chemical water parameters) and gastropod count data were analysed in the statistical software RStudio® Version 3.4.2. Significance threshold for all statistical tests was $P \leq 0.05$. All R generated graphs were exported to Microsoft PowerPoint® for minor aesthetic adjustments using the *Export* package created by Wenseleers (2016). Microsoft Excel® was used to generate graphs for the visualisation of temporal trends in gastropod abundance.

2.5.1. Gastropod community composition

Two methods were used to visualize the relationships amongst sites based on their gastropod community structure: Nonmetric Multi-Dimensional Scaling (NMDS) and hierarchical cluster analysis. NMDS analysis was performed using the function metaMDS from the Vegan package (Oksanen et al., 2016). The ordination was visualized with an NMDS plot. Different distance measures, suitable for community data as described in Legendre et al. (2013), were used to create the association matrix for NMDS ordination and the distance measure resulting in the lowest stress value was used. Vegan functions goodness and stressplot were used to assess the goodness of fit (Oksanen et al., 2016).

The Unweighted average linkage agglomerative clustering (UPGMA and Ward's minimum variance method) was used as a hierarchical clustering method. The same distance measure as for NMDS analysis was used for the association matrix created by vegdist in package vegan (Oksanen et al*.,* 2016). The optimal number of clusters was determined by creating fusion level plots and calculating silhouette widths. The dendrogram was both separately plotted and projected on the NMDS plot using the ordicluster and ordiplot functions in package Vegan (Oksanen et al., 2016).

2.5.2. Physico-chemical habitat differentiation of sites

Principal component analysis (PCA) was performed on a matrix of average physico-chemical water parameters per site to visualize the differentiation amongst sites based on these parameters. PCA was done using function *prcomp* from package *stats* (R Development Core Team, 2011) on a scaled correlation matrix because of the differences in dimensions of the different variables. A scree plot was made by function fviz_eig package *factoextra* (Kassambara & Mundt, 2017). The contributions of the different variables to the principal components were visualized using *corrplot* in package *corrplot* (Wei & Simko, 2017) and functions fviz_contrib and fviz_pca_var from package *factoextra.* The biplot was plotted with *fviz_pca_biplot* in package *factoextra* (Kassambara & Mundt, 2017).

3. Results

3.1. Gastropod diversity and abundance

A total of 2,477 gastropods were collected. They were assigned to a total of 8 morphotypes including *Bulinus forskalii* (Ehrenberg, 1831), *B. truncatus* (Audouin, 1827), *B. pfeifferi*, *Pseudosuccinea columella* (Say 1817), *Radix* sp., *Physella acuta* (Draparnaud, 1805), *Melanoides tuberculata* (Müller, 1774) and *Bellamya* sp. The *cox1*-based BLAST results from morphotypes *B. forskalii*, *P. columella* and *P. acuta* were sufficiently strong (BLAST identity≥99% with query cover of 100%, sequence length of +300bp and HQ≥95%) to conclude that they represent that actual species (Supplementary Table 1; GenBank accession numbers: XXXXX). No final conclusion could be made for the *B. truncatus* morphotype as the BLAST hits were identical for *B. truncatus* and *Bulinus tropicus*. Based on pairwise genetic distances of all *cox1* sequences, all representatives of the same morphotype belonged to the same species with p-distance < 0.1% (data not shown).

A phylogeny-based barcoding approach was used to gather more taxonomic information about the morphotypes for which BLAST results were not conclusive. The Planorbidae phylogeny (Figure 2) shows that the morphotype *B. pfeifferi* does not cluster with the genus of *Biomphalaria* but is rather related to the genus *Gyraulus*. Pairwise genetic distances show that the Kariban specimen is most closely related to *Gyraulus* sp. from Turkey (KC495833.1) with a p-distance of 12.9% (data not shown). However, due to the lack of African gastropod sequences in Genbank we cannot conclude on this. The phylogenetic tree (Figure 2) confirms the identity of our *B. forskalii* morphotype. Also the *B. truncatus* morphotype clusters with the *B. truncatus* reference sequence but shows close relationship with *B. tropicus*. The identification of the *Radix* sp and *P. columella* was discussed in Carolus et al. (2019) where phylogenetic analysis identified the nearest relative to be a *Radix* sp. specimen from Vietnam.

The most abundant gastropod species collected was *P. acuta*, representing 51.91% of the total gastropods collected and being present in all sampled sites. The least abundant gastropod species was *B. forskalii* comprising 0.89% of the total gastropods collected. Gastropod diversity ranged from two to eight species per site. Table 2 shows the cumulative gastropod count per site and per species.

3.1.1. Spatial trends in gastropod community composition

Based on UPGMA hierarchical clustering, the 16 sites can be divided into six distinct groups. The sites within a group cluster together based on gastropod community data. Figure 3 shows the ordering relationship between the 16 sampled sites (displayed by numbers 1 to 16) based on their gastropod community composition in the NMDS plot (Figure 3a) as well as in the UPGMA clustered dendrogram (Figure 3b). Site 14 forms its own cluster and contained the highest abundance of *Gyraulus* sp. Sites 11 and 12 cluster together in both the dendrogram and the NMDS plot, although they have different species richness, they have similarities in abundance of *Gyraulus* sp. (Table 2). The fourth and largest cluster, represented in red, is comprised of 8 sites (Figure 3a and 2b). Sites 1 and 10 both have a species richness of seven but are situated furthest away from each other on the dendrogram. The rest of the sites in the cluster have a species richness ranging between 2 and 5. Sites 2 and 4 form a clade and are similar in their low abundance of *P. columella.* Cluster 5, represented by the colour green, comprised of sites that have very high counts in *P. columella.*

3.1.2. Temporal trends in gastropod community composition

Figure 4 shows the seasonal variation of cumulative gastropod counts per species. The highest abundance of gastropods occurred in the hot and dry season, between September and December of 2017, while the lowest occurred in the hot and wet season, between January and April of 2018 (Figure 4). *B. truncatus, B. forskalii, Gyraulus* sp.*,* and *P. acuta* followed the same trend as the cumulative abundance of gastropods (i.e. highest abundance in the hot and dry season, and lowest in the hot and wet season; Figure 4). The lymnaeids, *P. columella* and *Radix* sp., were most abundant in the hot and dry season, and least abundant in the hot and wet, and the cold season, respectively. *Melanoides tuberculata* was most abundant in the cold season (Figure 4).

3.2. Trematode infection prevalence

3.3.1 Shedding results

Shedding experiments were carried out during all twelve months of the study and they showed only 9 out of a total of 2477 gastropods to be infected (0.36 % prevalence), which shed between the months of August and November 2017. Eight of the infected gastropods were B. truncatus (from a total of 148 tested) and one was a P. columella. Six of the B. truncatus gastropods from site 16 and one from site 15 shed amphistome cercariae. One B. truncatus from site 16 shed furcocercous (fork-tailed) cercariae of the longifurcate-pharyngeate distome type (also called strigeid cercariae). The ninth shedding gastropod, a P. columella from site 15 shed gymnocephalous cercariae of which Fasciola is a type genus

3.3.2 Multiplex PCR results

The multiplex RD-PCR protocol was performed on 290 gastropods (Table 3), excluding M. tuberculata and Bellamya sp. Infection rates per species are given in Table 3 and the prevalence of infected gastropods per site per species is shown in Figure 6. Of all the gastropods tested, 25.17% were infected. B. truncatus contributed to 12.32% of all infections with an infection prevalence of 13.43%. P. columella showed to be the most infected gastropod species (overall infection prevalence 59.60%) and contributed to 80.82% of all infections (59 infections of a total of 73 infections). The data on P. columella infection was already published by Carolus et al. (2019) in a study which was part of this sampling campaign. Radix sp. comprised 15.17% of all tested gastropods, but it contributed to just 4.10% of all infections, with only 6.82% of the Radix sp. infected. There were no infections detected in Gyraulus sp. A selection of infected gastropods (as diagnosed with the multiplex RD-PCR) was used for trematode genotyping with 18S rDNA and cox1 markers.

3.3. Trematode diversity

The 18S rDNA showed high BLAST identity matches ranging from 97% to 100% while *cox1* sequences of all trematode amplicons showed lower BLAST identity matches, never exceeding 92% identity with *cox1* sequences in the GenBank or BOLD database. A total of 18 and 15 trematode sequences of the 18S and the *cox1* marker respectively had sufficient quality and length for phylogeny reconstruction and were submitted to GenBank (acc. nos. XXXXX). For the 18S rDNA phylogenetic analysis, the top five BLAST species hit results of each sequence were downloaded from GenBank, aligned with sequences and trimmed, yielding a 550bp alignment representing two orders, five super families, 11 families, 22 genera and 25 species. Phylogeny-based genotyping was used for more taxonomic resolution. For the 18S rDNA phylogeny the Kimura 2-parameter model with discrete Gamma distribution ([+G] = 0.23) and invariant sites ([+I] = 39%) was proposed for constructing the ML tree. For the *cox1*-based phylogeny, BOLD and GenBank sequences were selected from one represented genus of each family and superfamily found by 18S rDNA genotyping. However, the lack of *cox1* sequences for many families of interest (e.g. superfamily Pronocephaloidea with only 1 of 5 families represented in GenBank) constrained this effort. The trematode taxonomy of Olson et al. (2003) and additional classification of amphistomes by Laidemitt et al. (2017) were used as guideline for family selection. The General Time Reversible model with discrete Gamma distribution ([+G] = 0.60) and invariant sites ([+I] = 19.28%) was proposed for the *cox1* dataset and used for constructing the ML tree. Based on *cox1* pairwise genetic distances, six trematode species were identified (Tables 7 and 8). These trematode species (Figure 5) could however only be identified to family level: Notocotylidae and Psilostomatidae in *B. truncatus*, superfamily Paramphistomoidae in *B. truncatus* and *Radix* sp., Fasciolidae in *P. columella* and *Radix* sp. and Diplostomatidae in *B. forskalii.* There were no *Schistosoma* infections detected in the tested gastropods. Barcoding of the cercariae isolated from *B. truncatus* during the shedding experiments showed as closest BLAST hits *Alaria* sp. (80% similarity) and *Uvulifer* sp. (88% similarity), both belonging to the Diplostomidae family.

3.4. Physico-chemical habitat differentiation of sites

Figure 7 shows the PCA correlation biplot of different sites (as scores) and all physico-chemical variables included (depicted as descriptor axis). The three variables with the strongest correlation with PC1 and thus the most influence on the position of scores along this axis are: chlorophyll a, ammonia concentration and turbidity. Dissolved oxygen concentration, oxygen saturation and temperature showed the strongest correlation with PC2.

Sites 10, 11, 12, 13, 14 and 16 differentiate from the main group in the PCA. Sites 4, 7, 8 and 9 had the lowest overall gastropod counts, and additionally show no extremes in physicochemical water parameters. Sites 14 and 16 had the highest abundance of the planorbids (i.e. *B. truncatus, B. forskalii* and *Gyraulus* sp.). They also had extreme values for several physico-chemical parameters, although in different directions, with site 14 being high in nutrient concentrations while site 16 exhibited low nutrient concentrations. The lymnaeids; *P. columella* and *Radix* sp, were most abundant in sites 3 and 5, which had relatively high dissolved oxygen concentrations and oxygen saturation and a similar pH. The PCA biplot also indicates that ammonia, chlorophyll a, conductivity, phosphates and nitrates concentrations were strongly correlated to each other, while pH was strongly correlated to dissolved oxygen concentration and percentage oxygen saturation (Figure 7).

4. Discussion

4.1. Gastropod Diversity

A total of eight freshwater gastropod species were identified in this study; namely *B. forskalii, B. truncatus/tropicus, P. acuta, Bellamya* sp., *P.columella, Radix* sp*., M. tuberculata* and *Gyraulus* sp. This is slightly less compared to the study by Chimbari and Chirundu (2003) which reported the following species: *B. pfeifferi*, *B. globosus, Bulinus depressus, B. tropicus, L. natalensis, P. acuta, M. tuberculata, Bellamya capillata* (Frauenfeld, 1865) and *Cleopatra ferruginea* (Lea & Lea, 1850). The most important difference with respect to disease transmission is the absence of *B. pfeifferi* and *B. globosus* in this study. Chimbari et al. (2003) found both *B. pfeifferi* and *B. globosus* at 13 out of 16 sites, with *B. globosus* being generally more abundant than *B. pfeifferi,* although both species had a low abundance that varied between seasons. There are several factors that might explain this absence. One of these might be competitive exclusion. There are multiple invasive gastropod species present in the lake, including *Radix* sp., *P. columella* (Carolus et al., 2019), *P. acuta* and *M. tuberculata* that compete for food and space. Rondelaud et al. (2016) showed that the physid *Aplexa hypnorum* exerted competitive pressure on two lymnaied species, consequently lowering their abundance. There is also the possibility of gastropod predation by the invasive red claw crayfish, *Cherax quadricarinatus* (von Martens, 1868), which was accidentally introduced into Lake Kariba and is now at an established stage in the lake (Marufu et al., 2014; Marufu et al,. 2018). Several studies have shown that gastropods make up the diet of freshwater crayfish, such as *Procambarus clarkii* whose prey of first choice was shown to be *Biomphalaria alexandrina, B. truncatus* and *L. natalensis* (Khalil & Sleem, 2011) while it has also been reported to feed on *B. globosus* and *M. tuberculata* (Monde et al., 2017). Another factor that might play a role is the water level, which has been fluctuating greatly during the past decennia, which affects snail distribution (Chimbari and Chirundu, 2003), possibly in a species-specific manner. After the sudden rise in waterlevel between 1999 and 2001, *B. pfeifferi* and *B. globosus* were only found 200 m from the shoreline, at depths of four to six meters (Chimbari and Chirundu, 2003).

Another striking outcome is the absence of *L. natalensis*, which was the second most abundant species in 2001 (Chimbari and Chirundu, 2003). This species might have been replaced by the highly invasive *P. columella* and *Radix* sp. that currently dominate in Lake Kariba (Carolus et al., 2019). The exact species identity of the *Radix* sp. sampled is not known yet, but phylogenetic analyses point to a close relationship to *Radix* specimen from Vietnam (Carolus et al. 2019). Due to morphological similarities with *L. natalensis*, it cannot be ruled out that these two species were already present in 2001.

It is worth noting that the other two bulinid species reported by Chimbari & Chirundu (2003) in Kariba, namely *B. tropicus* and *B. depressus,* were not identified in this study. However, we cannot rule out that our *B. truncatus* morphotype is actually *B. tropicus*. Due to similarity in morphological characteristics of the shells of gastropods, especially in juveniles, distinction between shells of species belonging to the same genus, as well as those belonging to different genera, is often difficult and error-prone (Frandsen & McCullough, 1980; Brown,1994; Palasio et al., 2017). The BLAST results were not conclusive, yielding the same similarity score for *B. truncatus* and *B. tropicus* reference sequences, while phylogeny reconstruction pointed to a closer affinity with *B. truncatus.* We therefore opt to assign our morphotype to the latter species.

Three other gastropod species identified in this study were *P. acuta, Bellamya* sp. and *M. tuberculata. Physa acuta* is an invasive species native to North America, which has been reported in previous surveys in Kariba in the past (Chimbari & Chirundu, 2003). *Bellamya capillata* was previously found in the lake (Machena & Kaustky, 1988; Chimbari & Chirundu, 2003; Brodersen et al., 2010). In this study we were only able to identify our *Bellamya* specimen to genus level, but according the phylogenetic analysis it is not *B. capillata* as the BLAST ID of its *cox1* sequence showed only 91% similarity with *B. capillata.* Future molecular studies will need to establish its exact species identification. Finally, *C. ferruginea* was reported in previous studies of Kautsky & Kiibus (1997) and Chimbari & Chirundu (2003), but not identified in this study. Its absence may be attributed to the absence of the macrophyte *Vallisneria aethiopica*, to which it was reported to be restricted (Kautsky & Kiibus, 1997). In this study a few *V. aethiopica* plants were only observed at Site 5 (Gundamusaira) in the month of March 2018.

4.2. Gastropod Ecology and Temporal trends

The planorbid gastropods (i.e. *Gyraulus* sp., *B. forskalii* and *B. truncatus*) showed to be highly abundant in highly eutrophic water. These results are consistent with Watson (1958), who reported that *B. truncatus* prefers polluted waters near human habitations, which are usually characterised by high eutrophication, low pH and low oxygen levels. Site 14, which had the third highest abundance of *B. truncatus,* is highly eutrophic, as it is exposed to a wastewater stream from a nearby crocodile farm. However, *B. truncatus* was also common in other, less contaminated sites suggesting that it tolerates a larger range of water quality conditions. Lymnaeid abundance (*Radix* sp. and *P. columella),* followed an opposite trend to that of the planorbid gastropods, being more abundant in sites 5 and 3 respectively, where nutrient concentrations were low. *Radix* sp. was however observed to be present in only 7 sites, all with fairly high dissolved oxygen content while *P. columella* was present at 14 sites. This more narrow distribution of *Radix* sp. may be explained by its higher demand for oxygen compared to *P. columella* which is tolerant to lower oxygen concentrations as well as higher eutrophication levels (Grabner et al., 2014).

Two prosobranch species, *Bellamya* sp. and *M. tuberculata,* were found together at only 4 sites; that is sites 5, 11, 15 and 16, all of which had low nutrient concentration levels. Although low numbers of *Bellamya* sp. were sampled, a large number of *Bellamya* sp. empty shells were observed, though not quantified, at the lake shore of site 15, suggesting that it is or was a hotspot for this species relative to other sites. We observed that *M. tuberculata* was the second least abundant gastropod species, contrasting Kautsky & Kiibus (1997) and Chimbari & Chirundu (2003), who found it to be the most abundant gastropod in Lake Kariba. In contrast, *P. acuta* was the most abundant species, present in large numbers and at each of the 16 sites, while it was the least abundant species in the study of Chimbari and Chirundu (2003). The wide tolerance to physical and chemical gradients that *P. acuta* displays (Stoll et al., 2013), allows its successful colonisation of all sites despite their marked differences in water chemistry. Additionally, *P. acuta* lacks any association with completely submerged aquatic vegetation, but thrive on the roots of floating *E. crassipes* (De Kock & Wolmarans, 2007), as also found in this sampling campaign. Hence, the observed shift in abundance of *P. acuta* may partly be attributed to the fact that, while there is less aquatic vegetative cover in Lake Kariba than there was a decade ago (Barson, personal observation), *E. crassipes* is well established in the lake (Carolus et al., 2019). Therefore, while other gastropod species decline in abundance due to lack of habitats, *P. acuta* competes successfully against other gastropods for habitat on *E. crassipes.*

Seasonality in the overall abundance of gastropods was observed during the course of the year, with the highest overall abundance of gastropods collected in the hot-dry season, between the months of September and December 2017 and the lowest in the hot-wet season between January and April 2018. The peak abundance of all gastropods also occurred in the hot-dry season, with the exception of *P. acuta* and *M. tuberculata* which both peaked in the cold season. *Physa acuta* was the most abundant gastropod during all seasons but also present at all sites, demonstrating that the proliferation of this invasive pulmonate is widespread in Lake Kariba. Tchakonté et al. (2014) reported similar trends in the proliferation of *P. acuta* in urban and suburban streams in Cameroon.

Bulinus truncatus reached its peak abundance in October during the hot-dry season, which is contrary to the peak density reported by Chimbari et al. (2003) for *B. globosus* to have occurred in July 2001. However, the seasonal distribution of *Gyraulus* sp. which reached peak abundance in September 2017, was similar to that reported by Chimbari et al. (2003) for *B. pfeifferi* which also reached peak density in September. It was also observed that, while *P. columella* was present every month, *Radix* sp. was absent from all sites in the months of May and June 2017, as well as February and March 2018. This difference in seasonal variation between the two species was as expected, since *P. columella* is more tolerant to a wider range of abiotic conditions than *Radix* spp. and is adapted to surviving longer periods of hot and dry weather (Prepelitchi et al. 2011).

4.3. Trematode diversity

Barcoding and phylogeny reconstruction showed six different families of trematodes infecting four species of gastropods (Figure 5). The parasite fauna of both *Radix* sp. and *P. columella* in Kariba has already been described and discussed in detail by Carolus et al., (2019). *Radix* sp. was infected by an unknown amphistome, which according to pairwise genetic distances (Table 4) is related to the amphistome *Calicophoron microbothrium* (p-distance of 15.2%). Both *Radix* sp. and especially *P. columella* populations were highly infected by an unknown *Fasciola* species. Due to its close phylogenetic affinity to *F. gigantica* and *F. hepatica* (Carolus et al., 2019), it may infect similar final host species, including wild and domesticated ruminants as well as humans (Figure 5). The most common ruminants found in Kariba are hippopotami (*Hippopotamus amphibious*, Linnaeus 1758) and the African buffalo (*Syncerus caffer*, Sparrman, 1779) (Ramberg *et al.,* 2013), with hippopotami being a natural host of *F. nyanzae* and buffalo being a natural final host of *F. gigantica* Cobbold 1855 (Bindernagel 1972). Hippopotami are also susceptible to cattle-borne diseases as they can be naturally infected with amphistomes (Sey and Graber 1979). Currently, there are no documented investigations into the extent to which trematodes contribute to the mortality of hippopotami or African buffalo in Kariba.

Bulinus truncatus was shown to be infected by the most diverse trematode community, with species belonging to the Notocotylidae, Gastrothylacidae and Psilostomstidae family. This observation is in line with other studies that reported that *B. truncatus* could host up to four different trematode species, including amphistomes (Chu et al., 1962; Ahmed et al., 2006; Farahnak et al., 2008). One trematode species we found in *B. truncatus* (type I; Figure 5) belongs to the family Gastrothylacidae, one of the most speciose ruminant infecting amphistome families in Africa (Laidemitt et al., 2017). Amphistomes present a huge economic burden in the livestock industry worldwide (Toledo et al., 2014). In Zimbabwean cattle, *Carmyerius* sp. has been the only recorded Gastrothylacidae species so far. Different *Carmyerius* species also infect African wild ruminants including African buffalo and hippopotami (Sey and Graber, 1979). *Bulinus truncatus* and *B. tropicus* have been implicated as hosts of *Carmyerius microbothrium* in northern and southern Africa, respectively (Pfukenyi et al., 2005). Based on pairwise genetic distances of *cox1* (Table 4), we know the Kariba species is not *C. microbothrium.* However, distances show a divergence of 11% between the Kariban haplotype and *Carmyerius mancepatus,* sampled in Kenyan cattle (Laidemitt et al., 2017), which suggests that the species found in Kariba might be a different, potentially unknown representative of the *Carmeyerius* genus.

A second trematode species (type III; Figure 5) found in *B. truncatus* belongs to the family Notocotylidae, a family of bird and mammalian herbivore parasites closely related to the Paramphistomoidae (Olson et al., 2003; Ma et al., 2016). Notocotylids have a two - host life cycle, with adults residing in the caeca and hindgut of birds and the intestine of mammals (Kearn 1998). It could be hypothesised that bulinid gastropods can function as hosts of Notocotylidae since some bulinid species are known to be hosts of the Paramphistomoidae (Eduardo 1987; Laidemitt et al. 2017; Phiri et al. 2007). However, this hypothesis needs further investigation by experimental infection of bulinids with species of the family Notocotylidae to determine their compatibility. Both notocotylids and paramphistomids infect ruminants as their definitive host and can cause major economic losses by infecting cattle and ovine livestock. An example of a notocotylid group known to occur in Africa is the genus *Ogmocotyle*. Members of this genus infect hippopotami in Southern Africa (Junker et al., 2015). This is the first report of Notocotylidae in Lake Kariba in scientific literature.

A third trematode species found in *B. truncatus* (type IV, Figure 5) belongs to the family Psilostomatidae. This family comprises gastro-intestinal parasites of birds and mammals that closely resemble the Echinostomatidae in their morphology (Atopkin, 2011). They are known to infect amphibians, waterfowl and mammals (Wilson et al., 2005; Mazeika et al., 2009). In Zimbabwe, the only genus of Psilostomidae reported is *Ribeiroia,* of which the species *Ribeiroia congolensis* infects the great egret (*Ardrea alba* Linnaeus, 1758) and the giant kingfisher (*Megaceryle maxima* Pallas, 1769) in Kariba (Johnson et al., 2004). The life cycle of *Ribeiroia* spp. involves the infection of amphibians as their second intermediate hosts, in which it causes limb deformities that are thought to

increase the chance of predation by the definitive hosts (i.e. carnivorous birds; Johnson et al., 2004). Some members of the Psilostomidae have been implicated in human infections in Asia (Chai *et al.,* 2009). Barcoding of the cercariae isolated from *B. truncatus* also identified two species belonging to the Diplostomidae family.

Bulinus forskalii was also shown to be infected by a species of the Diplostomatidae family, which typically infect lymnaeids as their first intermediate host, and fish (Smyth, 1976) and amphibians (Horák et al., 2014) as their second intermediate host. Our barcode sequence shows the highest similarity with *Alaria mustelae* with a p-distance of 12.9% (Table 4). There is little known about alariosis and the various *Alaria* species that cause it (Wasiluk 2013). However, *Alaria* species have a wide range of hosts including fish (Locke et al. 2011), birds, amphibians, reptiles, and mammals, as well as humans (Möhl et al., 2009). Humans can act as paratenic host (which harbours the parasite without any further development before it is transmitted to its intermediate host) in the three - host life cycle of some *Alaria* species, and are infected through ingesting meat infected with *Alaria* mesocercaria (Freeman & Stuart, 1976; Möhl et al., 2009; Toledo et al., 2014). The pathogenicity of *Alaria* species in humans is an area that is still being explored, therefore further investigation is needed to confirm the presence of *Alaria* sp. in Kariba and to identify its species identity.

We did not find any sign of *Schistosoma* infection in the collected gastropod species, as confirmed by the *Schistosoma* RD-PCR (Schols et al. 2019; described below). The absence of *Schistosoma* infections may be mainly attributed to the absence of the previously reported *Schistosoma* host *B. globosus* and *B. pfeifferi* from this study. We did, however, identify *B. truncatus,* which is known to be able to host both *S. haematobium* (Mohammed et al., 2016) and *S. bovis* (Akinwale *et al*., 2011) in some parts of Africa. However, more sequencing data for this gastropod species is needed to confirm its exact species status.

Due to the lack of *cox1* reference sequences from the GenBank and BOLD database, the identification of trematodes in this study was only possible up to family level. Therefore further investigation into the trematode species sampled in Lake Kariba is required. We suggest the use of the variable internal transcribed spacer (ITS) marker that is more represented on GenBank compared to 18S rDNA or *cox1* markers, combined with extensive phylogenetic analysis (Nolan et al., 2005; Vilas et al., 2005). However, the major bottleneck is the knowledge gap of African trematodes and the associated lack of reference sequences in all major databases. Although we were only able to identify the trematode family and sometimes genus level, it is clear that Lake Kariba harbours trematodes with the potential to affect Kariba's public health and its tourism and fishing industries by causing economic losses through mortality of wild animals and fish, as well as lowering the quality of fish.

4.4. Infection prevalence

There was a sharp contrast between shedding and multiplex RD-PCR with regard to infection prevalence. The overall amount of shedding gastropods indicated an infection prevalence of 0.36% whereas the multiplex RD-PCR resulted in a prevalence of 25.17%. According to the shedding results, only eight out of 148 (5.4%) *B. truncatus* and one out of 456 (0.22%) *P. columella* were infected, whereas multiplex RD-PCR showed four species to be infected; namely *P. columella, B. truncatus, B. forskalii* and *Radix* sp., with respective infection prevalence of 59.60%, 13.43%, 16.67% and 6.82%.

It has been shown before that shedding underestimates the prevalence of trematode infections in gastropods. Born-Torrijos et al. (2014) found underestimations of up to 66% of single infections and 80% of double infections when comparing PCR-based diagnostics to classical shedding. Various explanations may be put forward like the presence of immature (pre-patent) infections and/or gastropod mortality during the shedding experiments. Also, cercarial shedding of mature infections is affected by various conditions including temperature (Rondelaud *et al.,* 2013) and light intensity (Wagenbach and Alldredge 1974), which differ according to the trematode species and may affect the success of shedding experiments.

Our overall infection prevalence based on shedding was lower but not so different from a previous study in Kariba that reported 3.7% of the planorbid snails to be infected with mammalian-type of schistosome cercariae (Chimbari et al., 2003). Figures are however much higher for Zambia (Phiri et al., 2007) and Tanzania (Loker et al., 1981), with shedding rates of 13.7% and 14.9% respectively, accounting for all trematode species from both planorbids and lymnaeids. The infection prevalence of *B. truncatus,* as revealed by shedding, was significantly lower than the 14.9% reported in *B. truncatus* by Ahmed et al. (2006).

Based on the RD-PCR results, 59.60% of all lymnaeid gastropods were infected. This is slightly higher than infection prevalence based on shedding reported by Chingwena et al. (2002) and Phiri et al. (2007): 38.2% in Zimbabwe and 42.8% in the Kafue wetlands (in the vicinity of Lake Kariba) of Zambia respectively. The infection prevalence of lymnaeid gastropods is discussed in detail by Carolus et al. (2019). Despite a report by Chimbari et al. (2003) describing 3.33% prevalence of *S. haematobium* in *B. globosus* and 4.76% prevalence of *S. mansoni* in *B. pfeifferi,* there were no *Schistosoma* species found in this study. However, a drastic decrease was already reported in the prevalence of schistosomiasis in humans (Chimbari et al., 2003; Chimbari & Chirundu, 2003) as well as in their gastropod hosts (Chimbari et al., 2003) due to improved sanitary facilities and control strategies including molluscicide and chemotherapy (Chimbari et al., 2003; Chimbari, 2012).

Conclusion

Through this study we gained new fundamental insight into the composition of the gastropod community as well as gastropod-trematode associations along the northern shore of Lake Kariba. Regardless of the differences between methods used in previous studies and this study, a remarkable shift in the gastropod communities was detected. This shift is potentially driven by the spread of exotic gastropod species, now present in high numbers in the lake. While we were unable to identify the trematodes to species level due to the lack of reference sequences in genetic databases, it is possible that they have the potential to affect Kariba's public health and wildlife, as well its fishing industry, which make up the backbone of Kariba's economic structure. One of the most remarkable findings is the absence of schistosome species in the tested planorbid population, as well as the absence of their previously reported gastropod host species

B. globosus and B. pfeifferi. The findings of this study show the need for further investigation into the complete trematode fauna of Lake Kariba as well as the implications it has on wildlife, aquaculture and the public health in surrounding communities in Kariba.

List Of Abbreviations

BLAST - Basic Local Alignment Search Tool

BOLD – Barcode of Life Data system

cox1 – Cytochrome c oxidase subunit I

ML- Maximum Likelihood

MUSCLE- Multiple Sequence Comparison by Log-Expectation.

NMDS- Nonmetric Multi-Dimensional Scaling

PCA - Principal component analysis

RD-PCR- Rapid Diagnostic PCR

UPGMA - Unweighted pair group method with arithmetic mean

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and materials

The sequences generated in this study are deposited to GenBank under accession numbers XXXX. The alignments used for the phylogenetic analysis are available from the corresponding author on reasonable request

Competing interests

The authors declare that they have no competing interests

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Authors' contributions

TH and MB conceived the study and helped with fieldwork and correcting the manuscript. KM led and conducted the year round field collection, wrote the manuscript. HC led and conducted the molecular analysis and data analysis, helped with fieldwork and wrote the manuscript. CH helped with molecular data analysis and writing. RS helped with molecular work and correcting the manuscript

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Tables

Table 1: **Information on sampling sites.** Each site has an assigned number and three-letter code as reference. Coordinates (longitude and latitude) are given per site.

Site number	Site name	Site Code	Longitude	Latitude
1	DDF Harbour	DDF	S16.53152	E028.75891
2	GDI Harbour	GDI	S16.53438	E028.76714
3	Andora Harbour	AND	S16.53229	E028.77057
4	Irvine & Johnson	InJ	S16.53050	E028.78197
5	Gundamusaira	GUN	S16.53030	E028.78521
6	Marineland	MAR	S16.53030	E028.80208
7	Breezes	BRE	S16.53605	E028.81407
8	B-Line	BLI	S16.53287	E028.81571
9	Cuttysark	CUT	S16.53468	E028.81909
10	Mopani Bay	MOP	S16.53086	E028.83055
11	UZ Harbour	UZH	S16.52934	E028.84181
12	Nyanyana	NYA	S16.52963	E028.84747
13	Lake Harvest	LHA	S16.53303	E028.85368
14	Crocodile Farm	CRO	S16.54363	E028.87697
15	Nzou Lodges	NZO	S16.56119	E028.94143
16	Charara	CHA	S16.55410	E028.95083

Table 2: **The cumulative abundance of gastropods over 12 months per site per species.** The column labelled "Species Richness" shows the species richness at each site. The column "Site" shows the specific sites of gastropod collection as numbers, the corresponding site names are shown in Table 1.

Site	*P. columella*	*Radix .*	*Gyraulus* sp.	*B. truncatus*	*B. forskalii*	*P. acuta*	*Bellamya* sp.	*M. tuberculata*	Species Richness
1	62	6	2	11	0	184	0	54	**7**
2	19	0	1	0	0	132	0	0	**3**
3	102	90	2	0	0	178	0	0	**5**
4	1	0	0	0	0	12	0	0	**2**
5	3	104	1	2	0	76	5	1	**8**
6	17	3	0	11	0	187	0	0	**5**
7	3	0	0	0	0	12	0	0	**3**
8	0	0	0	0	0	29	0	0	**2**
9	0	0	0	0	0	47	0	0	**2**
10	4	0	8	9	1	34	3	0	**7**
11	9	14	18	2	3	30	1	2	**9**
12	15	15	27	2	1	71	0	16	**8**
13	22	3	1	0	1	40	0	14	**7**
14	10	0	50	19	9	24	0	0	**6**
15	76	0	0	22	1	120	13	9	**7**
16	93	0	2	66	6	110	1	10	**7**

Table 3: Trematode infection prevalence of the gastropod specimens tested with the Multiplex RD-PCR protocol.

Species	Number of gastropods tested	Number of infected gastropods	Infection prevalence (%)
Lymnaeidae			
[a]*P. columella*	99	59	59.60
[a]*Radix* sp.	44	3	6.82
Bulinidae			
B. truncatus	67	9	13.43
B. forskalii	12	2	16.67
Planorbidae			
Gyraulus sp.	8	0	0
Physidae			
Physella acuta	60	0	0
Total	**290**	**73**	**25.17**

[a]Results regarding these species are discussed in detail by Carolus et al. (2019)

Table 4: **Pairwise genetic distance (p-distance) between trematode sequences of *cox1* (402 bp) in the *cox1* phylogeny (Figure 5)**.

Seq	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27
Calicophoron microbothrium	1																										
Carmyerius mancupatus	2	0.142																									
Cotylophoron sp.	3	0.137	0.127																								
Fasciola jacksoni	4	0.318	0.313	0.303																							
Fascioloides magna	5	0.291	0.299	0.276	0.129																						
Fischoederius elongatus	6	0.157	0.139	0.179	0.306	0.294																					
Gastrothylax crumenifer	7	0.184	0.124	0.159	0.318	0.301	0.152																				
Homalogaster paloniae	8	0.182	0.184	0.179	0.308	0.291	0.192	0.216																			
Notocotylus sp.	9	0.279	0.254	0.271	0.286	0.279	0.259	0.244	0.279																		
Ogmocotyle sikae	10	0.254	0.254	0.259	0.286	0.274	0.249	0.249	0.241	0.192																	
Paramphistomum cervi	11	0.119	0.134	0.129	0.303	0.291	0.149	0.157	0.187	0.284	0.269																
Pseudopsilostoma varium	12	0.343	0.326	0.333	0.261	0.236	0.328	0.318	0.308	0.299	0.301	0.338															
Stephanopharynx sp.	13	0.184	0.177	0.162	0.308	0.294	0.179	0.197	0.209	0.276	0.281	0.152	0.331														
Hypoderaeum conoideum	14	0.353	0.358	0.373	0.259	0.241	0.346	0.351	0.366	0.281	0.296	0.358	0.254	0.328													
Apharyngostrigea pipientis	15	0.301	0.279	0.279	0.313	0.286	0.291	0.291	0.291	0.291	0.284	0.294	0.321	0.301	0.331												
Diplostomum ardeae	16	0.296	0.291	0.306	0.279	0.269	0.289	0.291	0.311	0.301	0.296	0.296	0.351	0.294	0.323	0.197											
Fibricola sp.	17	0.281	0.269	0.279	0.276	0.256	0.279	0.274	0.286	0.276	0.286	0.279	0.326	0.276	0.323	0.187	0.137										
type II (*Radix* sp.)	18	0.152	0.157	0.157	0.306	0.286	0.174	0.172	0.189	0.249	0.231	0.159	0.331	0.197	0.331	0.286	0.291	0.264									
type III (*B. truncatus*)	19	0.266	0.254	0.251	0.286	0.279	0.259	0.249	0.266	0.229	0.201	0.234	0.336	0.266	0.308	0.289	0.303	0.286	0.291	0.259							
type V (*P. columella*)	20	0.296	0.306	0.296	0.164	0.144	0.303	0.303	0.313	0.276	0.276	0.289	0.239	0.291	0.226	0.301	0.279	0.264	0.274	0.291							
type IV (*B. truncatus*)	21	0.311	0.308	0.318	0.239	0.236	0.308	0.311	0.341	0.303	0.291	0.333	0.192	0.306	0.269	0.338	0.331	0.313	0.303	0.303	0.318	0.244					
type VI (*B. forskalii*)	22	0.296	0.276	0.291	0.291	0.299	0.308	0.291	0.313	0.294	0.303	0.281	0.328	0.301	0.338	0.184	0.134	0.114	0.281	0.281	0.259	0.343					
type I (*B. truncatus*)	23	0.179	0.112	0.157	0.308	0.299	0.142	0.139	0.224	0.256	0.261	0.172	0.328	0.211	0.331	0.294	0.301	0.291	0.184	0.269	0.321	0.311	0.303				
Alaria mustelae	24	0.316	0.308	0.316	0.281	0.284	0.318	0.316	0.321	0.316	0.311	0.301	0.343	0.311	0.323	0.199	0.137	0.134	0.321	0.301	0.281	0.356	0.129	0.313			
Notocotylidae sp.	25	0.276	0.254	0.261	0.286	0.289	0.266	0.241	0.264	0.211	0.194	0.259	0.321	0.276	0.308	0.301	0.289	0.279	0.261	0.097	0.286	0.318	0.284	0.259	0.308		
Echinostoma trivolvis	26	0.306	0.316	0.318	0.259	0.236	0.328	0.311	0.301	0.296	0.296	0.306	0.244	0.321	0.211	0.311	0.296	0.316	0.306	0.229	0.274	0.301	0.346	0.316	0.326		
Fasciola hepatica	27	0.289	0.284	0.284	0.152	0.152	0.291	0.301	0.286	0.279	0.261	0.289	0.234	0.289	0.241	0.306	0.264	0.256	0.104	0.239	0.264	0.313	0.313	0.201			
Fasciola gigantica	28	0.276	0.291	0.289	0.152	0.142	0.294	0.289	0.281	0.281	0.266	0.281	0.226	0.303	0.226	0.311	0.269	0.259	0.271	0.296	0.09	0.219	0.261	0.303	0.276	0.291	0.214	0.087

Supplementary Table 1. BLAST results for *cox1* sequences with highest quality and length for each gastropod morphotype**.** The provisionally assigned morphotype name (MT), the sequencing quality (HQ), length (len) and top 3 BLAST species hits with the respective identity value (ID). Morphotype abbreviations are BFO: *B. forskalii*, BTR: *B. truncatus*; BIO: *Biomphalaria* sp., PCO: *P. columella*, RAD: *Radix* sp., LSP: *Lymnaea* sp., PHY: *P. acuta* and BEL: *Bellamya* sp.

MT	HQ (%)	len (bp)	BLAST 1	ID (%)	BLAST 2	ID (%)	BLAST 3	ID (%)
BFO	98	399	*Bulinus forskalii*	99	*Bulinus camerunensis*	95	*Bulinus* sp.	91
BIO	87	460	*Gyraulus* sp.	88	*Biomphalaria tenagophila*	87	*Bulinus globosus*	86
BTR	97	326	*Bulinus truncatus*	99	*Bulinus tropicus*	99	*Bulinus nyassanus*	98
PCO	96	395	*Pseudosuccinea columella*	100	*Lymnaea columella*	100	/	/
RAD	92	417	*Radix* sp.	96	*Radix auricularia*	92	*Lymnaea diaphana*	85
PHY	95	483	*Physella acuta*	99	*Physa heterostropha*	98	*Radix swinhoei*	98
BEL	96	450	*Bellamya monardi*	93	*Bellamya capillata*	91	*Bellamya* sp.	92

Figures

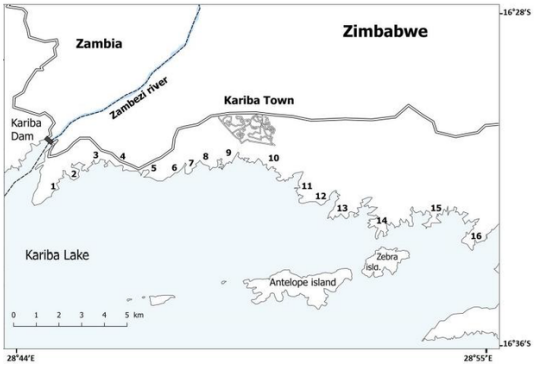

Figure 1

Map of the sampling sites along the shore of Lake Kariba.

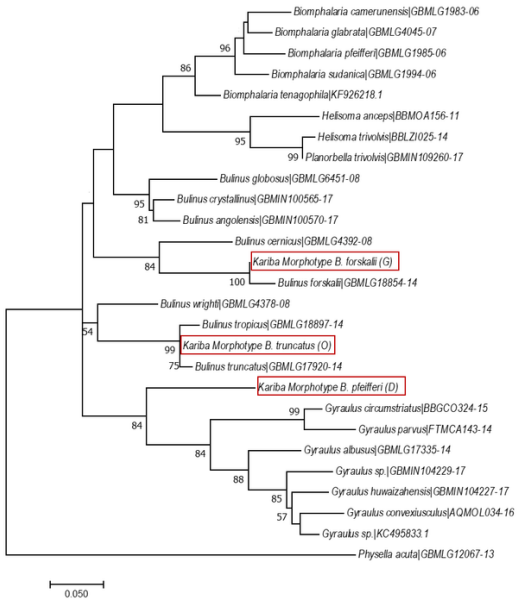

Figure 2

Maximum Likelihood phylogenetic tree for the snail species belonging to the Planorbidae family, using cox1 sequences (325bp) and the General Time Reversible model. The tree with the highest log likelihood (Ln = -2549.89) is shown. Bootstrap values (500 replicates) that are above or equal to 50 are shown next to the branches. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The BOLD or GenBank accession number of each sequence used is displayed after the | separator. Kariban morphotypes are indicated by a red box.

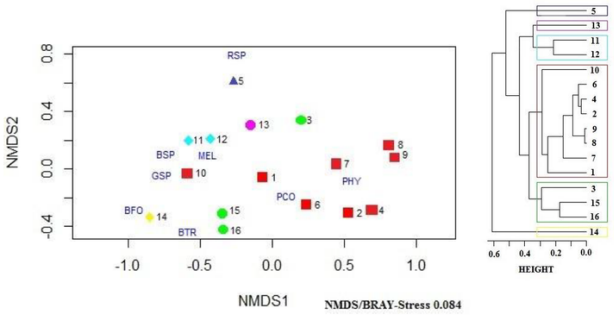

Figure 3

a) Non-metric Multidimensional Scaling plot showing relatedness of sites according to gastropod species richness. The scores represent the sites. The gastropod species are shown in blue species codes: Radix sp (RSP), Pseudosuccinea columella (PCO), Gyraulus sp. (GSP), Bulinus truncatus (BTR), Bulinus forskalii (BFO), Physella acuta (PHY), Bellamya sp. (BSP), Melanoides tuberculata (MEL) b) UPGMA clustering of sites based gastropod community data. Clustering groups are represented in different colours, also corresponding to points colours in a) .

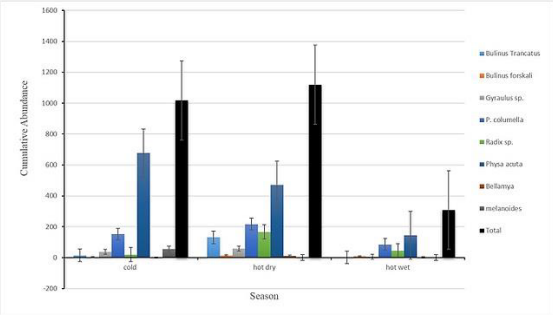

Figure 4

Cumulative abundance of gastropods per season. The different coloured bars represent cumulative abundance of gastropods per season per gastropod species; the black bar shows the cumulative abundance of all gastropods per season. The error bars represent standard error.

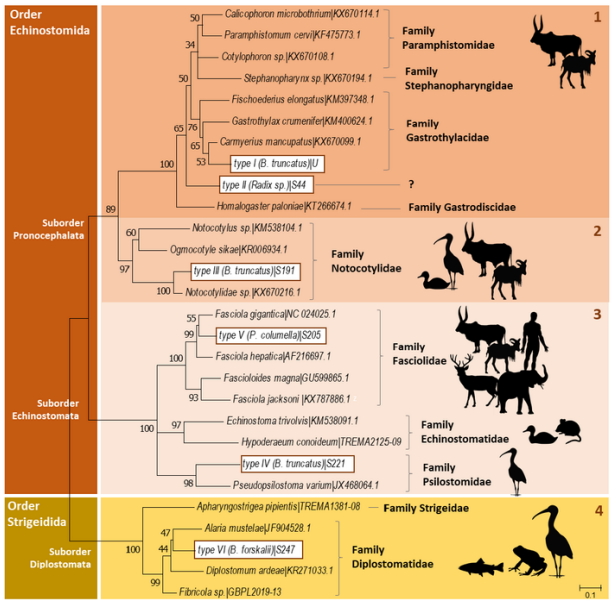

Figure 5

Phylogenetic tree showing the position of Kariba trematodes in their Orders, based on cox1 alignment of 402bp. The numbers 1 to 4 indicate the superfamilies; Paramphistomoidea (1), Pronocephaloidea (2), Echinostomoidea (3) and Diplostomoidea (4). The silhouette pictograms indicate possible final hosts of the trematode species included in the phylogeny. BOLD/GenBank accession number or sequence identifier are indicated after '|'. Each Kariba trematode type sequence, along with the host gastropod in which it was found, by a white grid.

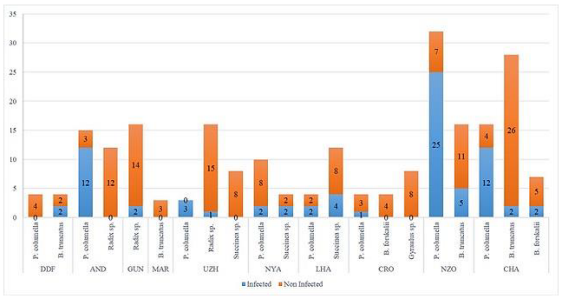

Figure 6

Multiplex RD-PCR number of infected and non-infected gastropods per site per gastropod species. The total number of gastropods sampled is represented bars. The amount of infected and non-infected gastropods is represented by the orange and blue proportions respectively. The sites are shown as three letter codes and the corresponding site names are shown in Table 1.

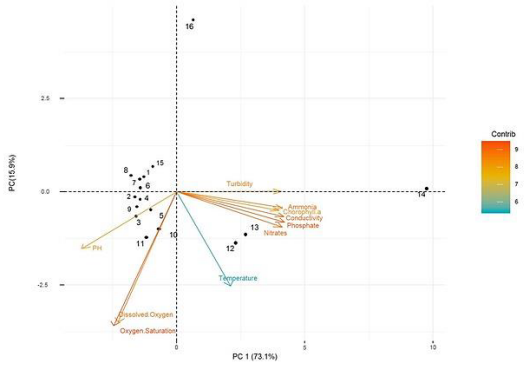

Figure 7

Principal Component Analysis plot showing relatedness of sites according to water chemistry. The scores (points) represent the sampled sites and the arrows represent the variables. The angles between the descriptor axis reflect correlations among the scores (sites), the distances between the objects do not approximate the Euclidean distance in the multidimensional space.