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A cascade of biological invasions and parasite spillback in man-made Lake Kariba

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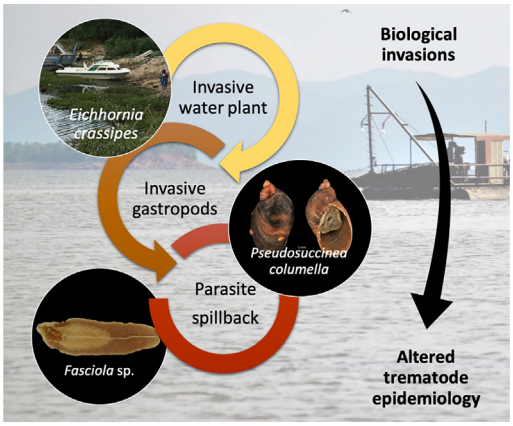
HIGHLIGHTS

- Discovery of two abundant non-indigenous lymnaeid gastropod species in Lake Kariba.
- Exceptionally high rate of infection with a hitherto unknown *Fasciola* species.
- A link between invasive water hyacinth and invasive gastropod species abundance.
- Design of a multiplex Rapid Diagnostic PCR for the detection of trematode and *Fasciola* sp. infections.

GRAPHICAL ABSTRACT



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ABSTRACT

Parasite spillback, the infection of a non-indigenous organism by a native parasite, is a highly important although understudied component of ecological invasion dynamics. Here, through the first analysis of the parasite fauna of lymnaeid gastropods of Lake Kariba (Zimbabwe). We illustrate how the creation of an artificial lake may lead to a cascade of biological invasions in which an invasive aquatic plant promotes the proliferation of invasive gastropods, which in turn alters the epidemiology of trematodiases of potential medical and veterinary importance. Using a new multiplex Rapid Diagnostic PCR assay, we assessed the prevalence of *Fasciola* sp. infections in the gastropod populations. Both gastropod hosts and trematode parasites were identified using DNA barcoding. We provide the first record of the invasive North-American gastropod *Pseudosuccinea columella* in Lake Kariba. This species was found at 14 out of 16 sampled sites and its abundance was strongly positively correlated with the abundance of the invasive South-American water hyacinth (*Eichhornia crassipes*). About 65% of the *P. columella* specimens analysed were infected with a hitherto unknown *Fasciola* species. Phylogenetic analyses indicate close affinity to *Fasciola hepatica* and *F. gigantica*, which cause fasciolosis, an important liver disease affecting both ruminants and humans. In addition, another non-native Lymnaeid species was found: a *Radix* sp. that clustered closely with a Vietnamese Radix species. *Radix* sp. hosted both amphistome and *Fasciola*

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trematodes. By linking an invasion cascade and parasite spillback, this study shows how both processes can act in combination to lead to potentially important epidemiological changes.

1. Introduction

Artificial water bodies are particularly sensitive to the introduction of non-indigenous species, due to their young age and increased niche availability (Havel et al., 2005; Johnson et al., 2008). Impoundments are usually larger, less isolated and more disturbed compared to natural water systems, which promote species introduction and random effects in species arrival and establishment. Artificial water reservoirs are therefore more likely to harbour (multiple) invasive species compared to their natural counterparts (Johnson et al., 2008). Another trend that has been associated with dam construction and irrigation schemes is the invasion of parasites (Morley, 2007) and subsequent changes in the epidemiology of parasitic water-related diseases such as malaria, trypanosomiasis and schistosomiasis (Stanley and Alpers, 1975; Lerer and Scudder, 1999; Sow et al., 2002). Novel water bodies function as new breeding grounds for vectors or intermediate hosts of parasites, such as gastropods in the case of trematode parasites. An infamous example is the construction of two dams on the Senegal River and its tributary, leading to one of the largest epidemics of schistosomiasis worldwide (Picquet et al., 1996). Trematode flatworms can infect humans and other vertebrates as definitive hosts, while gastropods serve as the intermediate hosts. Gastropod-borne trematodes form a major public health burden and have a great impact on the aquaculture and livestock industry, leading to billions of US$ in economic losses worldwide (Giannelli et al., 2016).

Lake Kariba forms the Zimbabwe - Zambia border and is the largest man-made water reservoir in the world by volume. The lake was formed following the impoundment of the Zambezi River by the Kariba hydro-electric dam wall, completed in 1959 (Magadza, 2006). An assessment of medical risks in the 1950s mentioned no risk of spreading gastropod-borne trematodiases near the dam wall construction area, because of its rocky soil and absence of freshwater gastropod habitat (Webster, 1960). Later however, schistosomiasis was detected in colonizing gastropods and resident villagers of the two major towns near the dam wall: Siavonga (Zambia) and Kariba (Zimbabwe) (Chimbari and Chirundu, 2003; Chimbari et al., 2003; Hira, 1969; Mungomba et al., 1993). *Schistosoma mansoni* (Sambon, 1907) and *S. haematobium* (Bilharz, 1852) are the only two trematode species ever identified in Lake Kariba. Another gastropod-borne trematodiasis of major veterinary and medical importance but never investigated in Lake Kariba, is fasciolosis, caused by liver flukes of the *Fasciola* genus (Toledo and Fried, 2014). There are four species of *Fasciola* described, of which two species are known to infect livestock and humans: *F. hepatica* (Linnaeus, 1758) and *F. gigantica* (Cobbold, 1855). *F. hepatica* has the broadest geographic range of all trematodes that infect humans, which is due to the generalist nature of both the trematode and its lymnaeid host (DiNardo, 2015). *F. gigantica* is prevalent in Sub-Saharan Africa, Hawaii and parts of Asia (Toledo and Fried, 2014). Acute fasciolosis results in abdominal pain, loss of appetite and fever. Chronic infections may lead to hepatomegaly, portal liver cirrhosis and infections of the pancreas and gallbladder (Toledo and Fried, 2014). Global prevalence estimates of human fasciolosis vary from 2.4 (WHO, 2017) to 17 million people (Hopkins, 1992) infected and 180 million people at risk of infection in >60 countries. The global economic losses due to *Fasciola* infections in livestock are estimated to be over 3.2 billion US$ per year. In poor, highly endemic African countries the effect on the economy is thought to be underestimated (Mehmood et al., 2017).

The endemic gastropod hosts for *Fasciola* species in Africa are the lymnaeids *Galba truncatula* (Muller, 1774) and *Radix natalensis* (Krauss, 1848) (Lotfy et al., 2008). The invasive lymnaeid *Pseudosuccinea columella* (Say, 1817) has also been found to transmit both *F. hepatica* and *F. gigantica* in Egypt (Grabner et al., 2014). This gastropod species is endemic to North and Central America but it was introduced with agricultural aquatic crops to South Africa in the 1950s where it has become the most successful invasive freshwater gastropod species (De Kock et al., 1989; Appleton, 2003). Gastropod snails are very responsive to environmental and anthropogenic change as their capacity for self-fertilization and rapid development can result in explosive population growth after introduction in new habitats. As such, they can be strongly invasive and some species are listed as a potential major pest (Cowie et al., 2009; Pointier et al., 2005). In addition to these biological traits, gastropod species can easily be transported over large distances through passive dispersal, for example via international trade in (aquatic) plants or attached to migrating, aquatic birds (Patoka et al., 2016; Lounnas et al., 2017).

Another non-indigenous species that can become highly invasive in artificial and natural water systems is the water hyacinth *Eichhornia crassipes* (Mart. Solms, 1883). This floating water plant has been associated with thriving gastropod populations hosting *Schistosoma* species in East Africa (Plummer, 2005; Güereña et al., 2015) and with invasion of *P. columella* and the subsequent spread of *Fasciola* species in Egypt (Grabner et al., 2014). *E. crassipes* originated from South America and invaded all continents, except Antarctica, via the ornamental plant trade. By the end of the 19th century it became highly prevalent in African waters (Edwards and Musil, 1975). Since then, it has destabilized many aquatic ecosystems by forming vast mats that block sunlight and outcompete local water plants (Adams et al., 2002). The mats provide habitat and shelter for many aquatic organisms, including freshwater gastropods (Coles and Kabatereine, 2008).

The trematode fauna of the lymnaeid gastropod species in Lake Kariba has never been studied despite the importance of lymnaeid gastropods in invasion biology (Lounnas et al., 2017), the possible impact of parasite spillback on disease epidemiology (Grabner et al., 2014; Lounnas et al., 2017), and the fact that Lake Kariba is one of the largest artificial lakes worldwide. Therefore we address the following questions: 1) which lymnaeid species occur in Lake Kariba, 2) what is their role in trematode transmission, with a focus on *Fasciola* species, and 3) is there an association between the occurrence of lymnaeid species and the introduced water hyacinth *E. crassipes*? To this end, we developed a multiplex Rapid Diagnostic PCR to diagnose trematode infections in gastropod tissue and identify infections of *Fasciola* sp. in particular. The use of the Chelex®-resin based DNA extraction method and a pooled screening design allows for a cost- and time-efficient diagnosis.

2. Materials and methods

2.1. Sampling

Sampling was performed monthly from June 2018 until December 2018 at 16 sites on the banks of Lake Kariba near the rural village of Kariba, Zimbabwe (see Fig. 1). The sampling sites were adopted from Chimbari et al. (2003) for later data comparison. The study is part of a larger project that aims at documenting the spatio-temporal changes of the gastropod communities involved in trematode transmission. Gastropod species were collected by examination of sediment and water plant vegetation along a 40 m stretch of lake bank using a scooping net (sieve mounted on a stick) up to 1 m into the lake taking one scoop per meter and using a dredge (sieve attached to a rope) up to 4 m into the lake. Gastropods were collected by two people during approximately 30 min per site. All lymnaeid specimens collected were

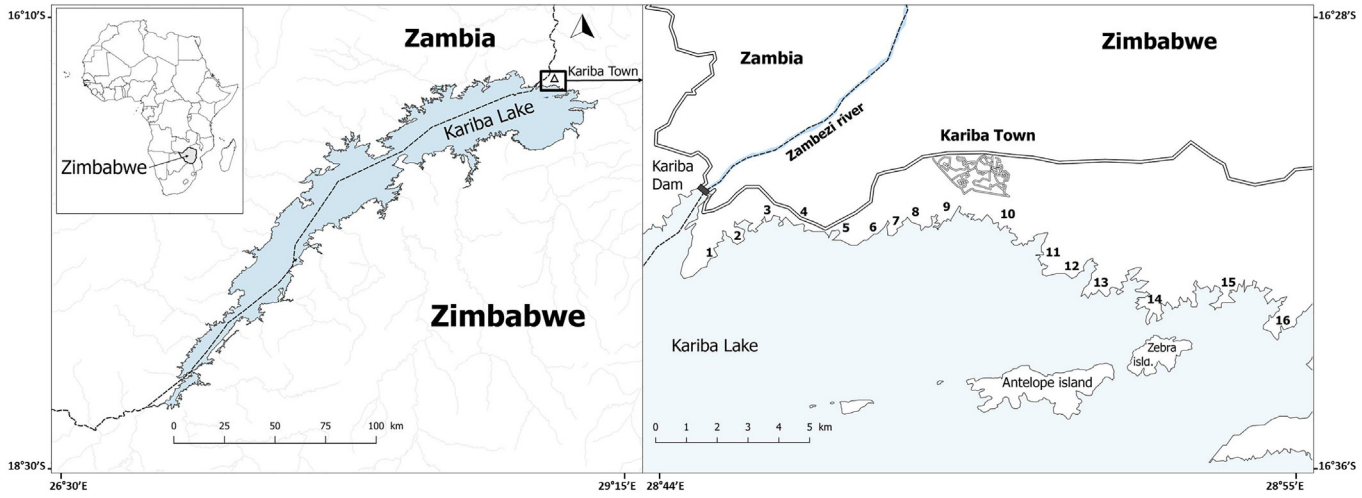

Fig. 1. Map of the sampling sites along the shore of Lake Kariba
Sites adapted from Chimbari et al. (2003). Map data © 2018 Google.

pooled according to species morphotype (based on Brown (1994) and Mandahl-Barth (1962)) and fixated in pure ethanol. The overall density of *Eichhornia crassipes* was estimated and categorized at three levels for each site. 'High' when at least 50% of a 250 m^2 quadrant was covered with floating *E. crassipes*, 'low' with less than 50% coverage and 'none' when *E. crassipes* was absent. Gastropods were left overnight, individually placed in cell culture plates the next morning, and exposed to artificial light for 2 h to induce shedding.

2.2. DNA extraction

The soft tissue of the preserved gastropod specimens was removed from the shell and dried on sterile absorbent paper. Tissue was homogenized with a scalpel on a glass plate. Cross sample contamination was prevented by sterilizing scalpel and forceps with ethanol and flame. Glass plate and gloves were rinsed with DNA AWAY™ (Thermofisher™) between sample homogenization. The Chelex® (Biorad™) DNA extraction method was used to reduce cost and process time (Caron et al., 2011). The tissue homogenate was incubated for 1 h at 56 °C and 30 min at 95 °C in a 100 μL 5% Chelex solution in a spinning Thermocycler™. Samples were centrifuged for 7 min at 13,000 ×*g* and the supernatant was stored at −20 °C. Pure DNA extract was diluted 1:10 to avoid PCR inhibition. Cercariae isolated from shedding gastropods were extracted with the DNeasy Blood and Tissue kit (Qiagen™) according to the manufacturer's protocol.

2.3. Multiplex rapid diagnostic PCR

Trematode infection prevalence in lymnaeid gastropods was assessed by multiplex RD-PCR (i.e. diagnosis based on amplicons of differential length using multiple primers in a single PCR reaction). Three different markers were targeted: an internal control (i.e. a general gastropod marker that indicates successful PCR amplification), a general trematode-specific marker and a marker that targets *Fasciola* sp. specifically (see Caron et al., 2011 for a similar approach). Primers for the internal control and general trematode markers were designed by identifying conserved regions in alignments including all African and European gastropod and trematode genera available from NCBI GenBank (https://www.ncbi.nlm.nih.gov/) in the Geneious® software. To identify *Fasciola* sp. infections, a 124 bp *Fasciola* sp. repeat was targeted using the primers designed by Krämer and Schnieder (1998) based on findings of Kaplan et al. (1995). Primer sequences and fragment length are summarized in Table 1. All PCR reactions were performed in a 15 μL volume with 1.5 μL of gastropod DNA template 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. Primer concentrations in the final volume were: 0.2 μM 18S_Digenea_F and 18S_Digenea_R, 0.1 μM FW_SNAIL2 and REV_SNAIL2 and 0.6 μM of Kaplan_F and Kaplan_R. The PCR reaction was performed as follows: initial denaturation at 94 °C for 15 min, 39 cycles of 94 °C for 30 s, 60 °C for 1 min 30 s and 72 °C for 1 min and a final elongation step at 72 °C for 10 min in a Tprofessional Thermocycler (Biometra™). Analysis of the PCR products was carried out by gel electrophoresis (GE) on a 3% agarose gel with Midori Green direct® staining method and UV light. To separate fragments, the GE run was performed on 120 V for 1 h 45 min. To reduce cost and increase sample capacity, gastropod DNA extracts were pooled as recommended by Caron et al. (2011). Four specimens of the same species and site were pooled for multiplex RD-PCR diagnosis. Sites with less than 4 specimens per lymnaeid species successfully fixated were excluded from the analysis. If no internal control amplicon could be obtained (a sign of PCR inhibition), the DNA sample was further diluted (1:100) and PCR was repeated. For each infected pool, all four samples were individually tested using the same multiplex PCR.

2.4. DNA sequencing and phylogenetic analysis

Gastropods that appeared infected based on the multiplex RD-PCR were used for sequencing to identify both the gastropod and the

Table 1
Primers used in the multiplex RD-PCR assay to diagnose trematode and *Fasciola* infections in gastropod tissue.

Primer name/ref.	Marker	Target	Length	Primer sequence (5′-3′)
18S_Digenea_F	18S	Trematoda sp.	±392	CAGCTATGGTTCCTTAGATCRT
18S_Digenea_R				TATTTTTCGTCACTACCTCCCCGT
FW_SNAIL2	18S	Gastropoda sp.	±500	AGTATGGTTGCAAAGCTGAAACTTA
REV_SNAIL2				TACAAAGGGCAGGGACGTAAT
(Krämer and Schnieder, 1998)	Nuclear repeat	*Fasciola* sp.	124	ATTCACCCATTTCTGTTAGTCC
				ACTAGGCTTAAAGGCGTCC

trematode species. For the identification of trematodes, the cytochrome *c* oxidase subunit I (COI), 18S rDNA and the ribosomal internal transcribed spacer 1 and 2 (ITS) markers were targeted. Primers used for the amplification of a trematode-specific 18S rDNA marker of approximately 1160 bp were 18S_Digenea_F (5′-CAGCTATGGTTCCTTAGAT CRTA-3′) and 1270_F (5′-ACTTAAAGGAATTGACGG-3′) while COI was targeted using primers COI1_dig_F (5′-CNATGATNTTNTTTTTTTT RATGCC-3′) and COI1_dig_R (5′-GMASWACCAAAWTTHCGATCAAA-3′) yielding a fragment of 450 bp. The primer pair Trem2F (5′-CAAGTC ATAAGCTTGCGCTGA-3′) and Trem1R (5′-ACCYAAACACCACATTGC CTA-3′) was used to amplify regio of the trematode ITS gene of approximately 1000 bp (Grabner et al., 2014). For gastropod genotyping, only the COI marker was targeted using newly designed primers: COI_gastropod_F (5′-TAATGTWATTGTTACAGCACATGC-3′) and COI_gastropod_R (5′-GTTGRTATAAAATAGGATCACCWCC-3′) for a 536 bp COI fragment. All PCR reactions were performed in a 15 μL volume with 1.5 μL DNA (1:10 diluted) using the Qiagen™ Taq DNA polymerase kit with 1.5 mM PCR buffer (Qiagen™), 0.6 mM dNTP mix (Qiagen™), 1.5 mM $MgCl_2$, 1.1 μM of each primer and 0.45 units of *Taq* Polymerase (Qiagen™). The PCR was performed by initial denaturation at 94 °C for 5 min, followed by 39 cycles of 94 °C for 30 s, 45 °C for 30 s and 72 °C for 45 s and a final elongation step at 72 °C for 5 min in a Biometra® Tprofessional Thermal Cycler. PCR products were visualised by gel electrophoresis on a 2% agarose gel with Midori Green Direct® staining and UV light. Samples that showed a clear amplified PCR product of the expected length were purified using the ExoSAP (Fermentas™) PCR purification protocol. All successful PCR products were sequenced by Macrogen™ (Sanger sequencing using BigDye® chemistry). Sequences of sufficient quality (HQ > 50%) were processed using the Geneious® software version 6.1.8. All sequences were manually trimmed and ambiguities were edited; consensus sequences were assembled from forward and reverse sequences (if both available). All unique sequences used in our phylogenetic analyses are available on GenBank (accession numbers: MK330622-MK330630, MK333465 and MK333466). The Basic Local Alignment Search Tool (BLAST; Altschul et al., 1990) was used for species identification followed by phylogenetic analyses using MEGA X (Kumar et al., 2018). We included all available species from the Fasciolidae family from NCBI GenBank (https://www.ncbi.nlm.nih.gov/) and BOLD (http://www.barcodinglife.org) databases. All sequences were aligned with the MUSCLE alignment algorithm (Edgar, 2004) and trimmed for phylogenetic tree building. Model selection (based on the Bayesian information Criterion, BIC) was performed in MEGA X to select the best model of sequence evolution in order to compute pairwise genetic distances to perform a Maximum Likelihood (ML) phylogenetic analysis. The robustness of the inferred tree was tested using 1000 bootstrap replicates. For species discrimination, genetic pairwise *p*-distances were calculated between the COI sequences with up to 5% divergence regarded as intraspecific variation (Vilas et al., 2005; Lawton et al., 2015).

2.5. Ecological data analysis

Lymnaeid gastropod species abundance and the discrete ecological variable 'abundance of *E. crassipes*' from all sites were analysed with the statistical software program RStudio®. Generalized Linear Models (GLM) were fitted using *glm* from package *stats* (R Development Core Team, 2011). Akaike Information Criterion (AIC) based model selection was performed using the function *stepAIC* in package *MASS* (Venables and Ripley, 2002). Overdispersion was assessed by changing the distribution to 'quasipoisson' (instead of poisson) and checking the dispersion parameter. Systematic deviations of observed values compared to model predictions were checked by using *residualPlots* from package *car* (Fox and Weisberg, 2011). If the gastropod species abundance showed to be significantly influenced by the abundance of *E. crassipes* according to likelihood ratio tests (type III Anova with Chi square test statistics), the effect was plotted with the function *ggplot* from the *ggplot2* package (Wilkinson, 2011). Significance threshold for all statistical tests was $P \leq 0.05$.

3. Results

3.1. Lymnaeid diversity

Pseudosuccinea columella and *Radix* sp. (Fig. 2) were the only lymnaeid gastropod species found in Lake Kariba. COI barcoding was used to confirm the species identity of the two species. The General Time Reversible (GTR) model with Gamma distribution ([+G] = 0.90) and invariant sites ([+I] = 51.18%) was used for distance calculation (Nei and Kumar, 2000). An alignment was built with a selection of *Radix* species sequences downloaded from GenBank that produced an overlap of at least 349 bp. with our sequenced COI marker. The lowest pairwise genetic distance between the *Radix* sp. from Kariba and *Radix* species mined from GenBank was 3.7%, with a *Radix* sp. specimen from Vietnam (Table 2). Pairwise genetic distances between the 349 bp COI sequences of *P. columella* from Kariba and from different geographical regions retrieved from BOLD (http://www.barcodinglife.org; Ratnasingham and Hebert, 2007) (i.e. Egypt [GBMIN110283-17], Australia [GBMLG0711-06], Colombia [GBMPL238-13], Spain [GBMPL1442-15] and USA [GBMPL484-13]), showed that sequences were 100% identical. No mutations were found between sequenced specimens of *P. columella* (520 bp) collected in Lake Kariba in this study.

Fig. 2. Photographs of the Lymnaeid species collected in Lake Kariba: *Pseudosuccinea columella* (left) and *Radix* sp. (right). Scale bars are provided.

Table 2
Pairwise genetic distances (*p*-distance) between *Radix* sequences of COI (349 bp). The lymnaeid genotypes from Kariba are indicated by '(Kariba)'. GenBank accession number is given after the '|' separator.

Species	1	2	3	4	5	6	7	8	9	10	11	12	13
1. *P. columella*|LC015520													
2. *Radix ampla*|EU818804	0.138												
3. *Radix auricularia*|KT867312	0.178	0.163											
4. *Radix balthica*|KX832493	0.169	0.092	0.163										
5. *Radix dolgini*|KT030067	0.149	0.115	0.181	0.126									
6. *Radix labiata*|EU818832	0.163	0.109	0.175	0.129	0.097								
7. *P. columella* (Kariba)|MK333465	0	0.138	0.178	0.169	0.149	0.163							
8. *Radix* sp. (Kariba)|MK333466	0.178	0.16	0.158	0.175	0.158	0.169	0.178						
9. *Radix lagotis*|LT623601	0.152	0.072	0.169	0.1	0.103	0.097	0.152	0.166					
10. *Radix natalensis*|LC015518	0.175	0.143	0.16	0.135	0.126	0.152	0.175	0.166	0.138				
11. *Radix ovata*|AY227364	0.169	0.077	0.169	0.014	0.123	0.126	0.169	0.169	0.092	0.135			
12. *Radix rubiginosa*|KU318330	0.169	0.132	0.129	0.158	0.14	0.152	0.169	0.158	0.152	0.143	0.155		
13. *Radix* sp.|JN794514.1	0.169	0.166	0.158	0.178	0.163	0.169	0.169	0.037	0.16	0.166	0.172	0.146	
14. *Radix zazurnensis*|JN794510	0.178	0.097	0.172	0.109	0.112	0.12	0.178	0.175	0.077	0.14	0.1	0.166	0.178

3.2. Fasciola sp. infection prevalence

The multiplex RD - PCR protocol was performed on a total of 44 *Radix* sp. and 92 *P. columella* specimens. Three samples of *P. columella* remained unamplified and were discarded. A total of 58 *P. columella* specimens (65.17%) tested positive for trematode infection, all displaying the *Fasciola* sp. signal. We also obtained cercariae from a single *P. columella* gastropod that was shedding (see Supplementary Fig. 1). *Radix* sp. only showed three infected specimens (6.8%) of which two tested positive for *Fasciola* sp. (4.5%). The number of gastropods tested for infection per species and per site (Fig. 3) was highly dependent on the presence and abundance of the different species at different sites (see Supplementary Table 1). Unsuccessful DNA extraction represented another limitation to testing equal numbers of gastropod specimens for infection per site and per species.

3.3. Trematode identification

Both multiplex RD-PCR and BLAST analysis showed that all successfully sequenced (18S rDNA, ITS and COI markers) trematode infections of *P. columella* (seven specimens), one cercaria and two of the three infections of *Radix* sp. were *Fasciola* infections. For the third infection of *Radix* sp., only 18S rDNA was successfully sequenced and the top BLAST hits showed affinity to the Paramphistomoidea superfamily. Four unique *Fasciola* haplotypes (from four sites) were found based on COI sequences (417 bp): S9, S84, S201 and S151 (Table 3) and three genotypes based on ITS (899 bp; Table 4). No variation was observed at the 18S rDNA (586 bp) marker between specimens. Sequences from all available representative species of the Fasciolidae family were mined from GenBank for phylogeny reconstruction. We tried to include as many African sequences as possible, and to select strains for which both ITS and COI sequences were available. We also included a sequence of *F. gigantica* and of *F. hepatica* from areas where both species do not occur in sympatry, being Niger and Spain respectively, in order to include 'pure' sequences instead of putative hybrids (Mas-Coma et al., 2009). All these requirements led to an overlapping fragment of 351 bp for the COI phylogeny (Fig. 4A) and 442 bp of the ITS phylogeny (Fig. 4B). The same species as in Lotfy et al. (2008) were included for the ITS tree to facilitate comparison and to interpret the position of the Kariba sequence within the Fasciolidae. Pairwise genetic distances between COI, ITS and 18S rDNA sequences are shown in Tables 3, 4 and Supplementary Table 2 respectively. The 18S rDNA marker showed 99.98% homology with *F. gigantica* and *F. hepatica* (1 point-mutation in 586 bp) (see Supplementary Table 2). Pairwise genetic distances using the COI and ITS alignments of the four Kariba genotypes confirmed that they all belong to the same species (intraspecific variation less than 5% and 1% for COI and ITS respectively), but none belonged to any of the species available in the NCBI GenBank database.

3.4. Abundance of Eichhornia crassipes and gastropods

Generalized Linear Models were built using Poisson distributed gastropod counts per species in relation to the abundance of *E. crassipes* (on three levels: none, low and high) for all sites. A significantly positive correlation ($Chi^2 = 6.32$; $p = 0.04239$) between the coverage of *E. crassipes* and counts of *P. columella* at the 16 sites was found (Fig. 5). The abundance of *E. crassipes* is given in Supplementary Table 3.

4. Discussion

Malacological surveys have been carried out before in Lake Kariba, but were mainly focused on planorbid gastropods that transmit schistosome parasites (Hira, 1969; Mungomba et al., 1993; Mubila and Rollinson, 2002; Chimbari and Chirundu, 2003; Chimbari et al., 2003). Here we focused for the first time on the trematode fauna of lymnaeid gastropod species. The outcome is relevant from both the parasitology

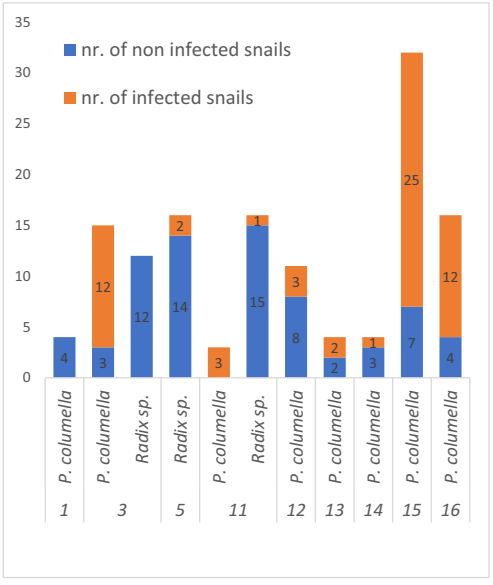

Fig. 3. Number of lymnaeid gastropod specimens tested for infection with trematode parasites using the multiplex RD-PCR. The total number infected (orange) versus non-infected specimens (blue) is given. Species and sites are indicated on the x-axis. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 3
Pairwise genetic distances (*p*-distance) between *Fasciola* sequences of the COI (351 bp) marker used in the phylogenetic analysis (Fig. 4A). All Kariba haplotypes (S201, S84, and S151) were obtained from *P. columella*. The haplotype found in *Radix* sp. was identical to S201.

Sequences	1	2	3	4	5	6	7	8	9	10
1. *Fasciola gigantica* (Zimbabwe)										
2. *Fasciola gigantica* (South Africa)	0									
3. *Fasciola gigantica* (Niger)	0.014	0.014								
4. *Fasciola hepatica* (Spain)	0.057	0.057	0.051							
5. *Fasciola hepatica* (Zimbabwe)	0.054	0.054	0.054	0.011						
6. *Fasciola hepatica* (South Africa)	0.057	0.057	0.057	0.006	0.006					
7. *Fascioloides magna*	0.114	0.114	0.108	0.088	0.085	0.091				
8. *Fasciola jacksoni*	0.117	0.117	0.108	0.125	0.125	0.128	0.111			
9. *Fasciola* sp. (Kariba)|S84	0.077	0.077	0.074	0.068	0.071	0.071	0.103	0.125		
10. *Fasciola* sp. (Kariba)|S151	0.074	0.074	0.071	0.066	0.068	0.068	0.103	0.123	0.003	
11. *Fasciola* sp. (Kariba)|S201	0.074	0.074	0.071	0.074	0.071	0.077	0.1	0.123	0.011	0.009

and invasion ecology perspective. The most important results obtained through our study are: 1) the discovery of two abundant non-indigenous lymnaeid species in Lake Kariba, 2) the exceptionally high rate of infection with a hitherto unknown *Fasciola* species and 3) the link between the invasive water hyacinth and the invasive gastropod species abundance. From these findings we deduce that this man-made lake has set the stage for a cascade of biological invasions, resulting in an increased transmission of an indigenous fasciolid through parasite spillback. In the following paragraphs, we discuss the arguments corroborating this scenario.

4.1. Invasive gastropod species at Lake Kariba

We provide the first report of the lymnaeid *P. columella* in Lake Kariba. This species, native to North America, has been reported as invasive species in South Africa since the 1940s or 1950s (Appleton, 2003) but records in Zambia, Mozambique, Zimbabwe and Kenya also exist (Brown, 1994). It was the second most abundant species in the lake, and it was found in 14 out of 16 sites. The haplotype of *P. columella* showed a 100% sequence similarity with isolates from Australia, Egypt, Colombia, Spain and the USA, suggesting a recent introduction in Zimbabwe. Even though the comparison was based on a relatively short COI fragment, it corresponds with the 'flash invasion' scenario described for this species by Lounnas et al. (2017). These authors found one single multilocus genotype of *P. columella* distributed throughout the world. *P. columella* represents a striking example of 'the invasion paradox of genetic variability'. One of the reasons might be the fact that they can reproduce parthenogenetically. A meta-analysis by Roman and Darling (2007) showed >60% of the aquatic invasions with loss of genetic diversity were species that could reproduce without sexual recombination. Similar to this is the observation that some invertebrate species switch to clonality in the invasive range, which has also been observed for the invasive water hyacinth (Caron et al., 2014). These findings could indicate that this mode of reproduction can help to overcome the negative consequences of low diversity. Also other biological characteristics may explain the invasion success of *P. columella*: it is a eurythermal and amphibious species and it is capable of colonizing a range of habitats, being far less sensitive to climatic variations compared to most African freshwater gastropods (Brown, 1994). An example is the capability of surviving submerged in mud when water levels retract. This specific niche is not occupied by many other African molluscs in areas where *P. columella* is found (van Eeden and Brown, 1966). In South Africa, *P. columella* commonly co-occurs with *Radix* (*Lymnaea*) *natalensis* in slow flowing rivers or stagnant lakes with dense vegetation and muddy substrate. The superior reproduction of *P. columella* capacity and its semi-amphibious way of foraging, generate putative competitive advantages over *R. natalensis* (Brown, 1994). Competitive exclusion might therefore explain the absence of local *R. natalensis* in our study, although sampling bias or seasonal variation in *R. natalensis* distribution cannot be excluded. Another possibility is that *P. columella* was misidentified as *R. natalensis* in previous studies of Lake Kariba and Zimbabwe. The account of Lake Kariba was based on morphology only (Hira, 1969); due to similarities in shape misidentification is possible (Brown, 1994).

For the second lymnaeid gastropod species sampled, our genotyping analysis suggests a close affinity to the *Radix* genus. The highest relatedness was found to a *Radix* species from Vietnam (von Oheimb et al., 2011) with a genetic *p*-distance of 3.7% between both COI sequences (Table 2). This suggests that our unidentified *Radix* species could be relatively recently introduced form Southeast Asia but a lack of reference sequences in the GenBank database precludes any conclusion. It has indeed been demonstrated that the *Radix* clade originated in the Indo-

Table 4
Pairwise genetic distances (*p*-distance) between *Fasciola* sequences of the ITS rDNA (442 bp) marker used in the phylogenetic analysis (Fig. 4B). The three unique genotypes (S201, S84 and S151) were obtained from *P. columella*. No ITS sequence could be obtained from *Radix* sp. infections.

	Sequences	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	*Fasciola gigantica* (Burkina Faso)																
2	*Fasciola gigantica* (Niger)	0,000															
3	*Fasciola gigantica* (Zimbabwe)	0,002	0,002														
4	*Fasciola hepatica* (Peru)	0,012	0,012	0,014													
5	*Fasciola hepatica* (Spain)	0,012	0,012	0,014	0,000												
6	*Fasciola* sp. (Kariba)|S151	0,007	0,007	0,009	0,005	0,005											
7	*Fasciola* sp. (Kariba)|S84	0,009	0,009	0,012	0,002	0,002	0,002										
8	*Fasciola* sp. (Kariba)|S201	0,009	0,009	0,012	0,007	0,007	0,002	0,005									
9	*Fasciola jacksoni*	0,042	0,042	0,045	0,035	0,035	0,040	0,038	0,042								
10	*Fasciola jacksoni*	0,042	0,042	0,045	0,035	0,035	0,040	0,038	0,042	0,000							
11	*Fasciolopsis buski*	0,110	0,110	0,110	0,114	0,114	0,110	0,112	0,112	0,098	0,098						
12	*Fasciolopsis buski*	0,100	0,100	0,100	0,110	0,110	0,105	0,107	0,107	0,093	0,093	0,026					
13	*Fascioloides magna*	0,061	0,061	0,064	0,057	0,057	0,061	0,059	0,064	0,059	0,059	0,132	0,125				
14	*Fascioloides magna*	0,064	0,064	0,066	0,059	0,059	0,064	0,061	0,066	0,061	0,061	0,135	0,127	0,002			
15	*Protofasciola robusta*	0,194	0,194	0,196	0,196	0,196	0,191	0,194	0,194	0,199	0,199	0,203	0,203	0,208	0,210		
16	*Parafasciolopsis fasciolaemorpha*	0,092	0,092	0,094	0,089	0,089	0,089	0,092	0,092	0,089	0,089	0,129	0,129	0,121	0,123	0,172	
17	*Echinostoma caproni*	0,167	0,167	0,169	0,169	0,169	0,167	0,169	0,169	0,163	0,163	0,167	0,170	0,173	0,176	0,174	0,167

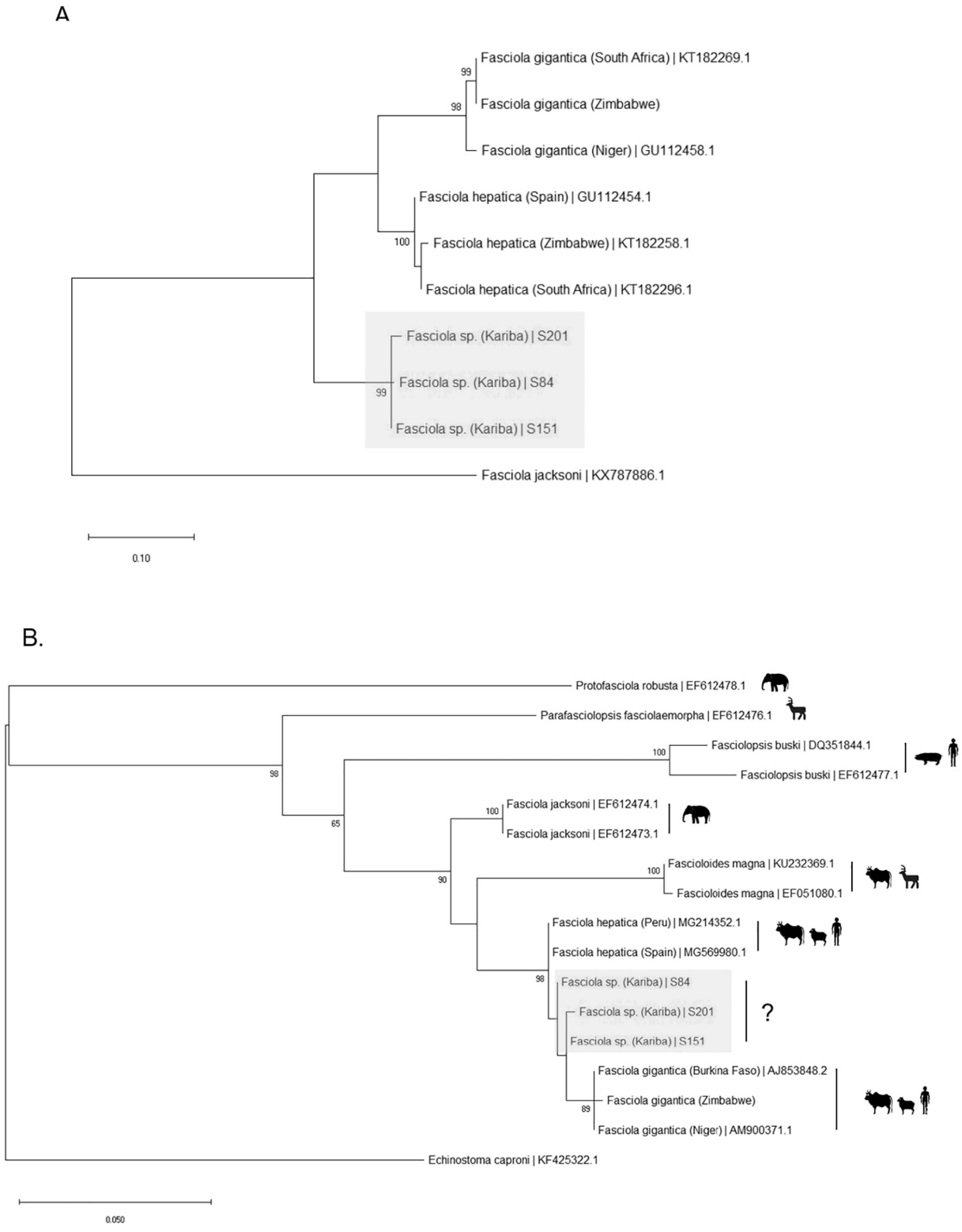

Fig. 4. The Maximum Likelihood phylogenetic tree of Fasciolidae constructed using COI (351 bp) sequences (A) and ITS (442 bp) sequences (B). Hasegawa-Kishino-Yano model (Hasegawa et al., 1985) with discrete Gamma distribution ([+G] = 0.67) and invariant sites ([+I] = 69%) was selected for the COI dataset while the General Time Reversible model (Nei and Kumar, 2000) with discrete Gamma distribution ([+G] = 0.42) was selected for the ITS dataset. Bootstrap values (1000 replicates) that are above 60 are shown next to the branches. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The GenBank accession number of each sequence used is displayed after the '|' separator.

Pacific region, followed by an expansion towards Eurasia and Africa (Correa et al., 2010). Also recent introductions from members of this clade have been identified like *Radix rubiginosa* (Michelin, 1831) in South Africa, originating from Southeast Asia (Appleton and Miranda, 2015).

4.2. High prevalence of unidentified Fasciola sp.

Based on the multiplex RD-PCR results of approximately 20% of all lymnaeid gastropods sampled, 45.86% was infected. This number is similar to infection rate estimates based on shedding of lymnaeid

Fig. 5. The total count of *P. columella* specimens in respect to the abundance of the water plant *Eichhornia crassipes*. A generalized linear model showed that the amount of gastropods significantly correlates with the abundance of *E. crassipes*.

gastropods throughout Zimbabwe (38.2% infected, Chingwena et al., 2002) and the Kafue wetlands (in the vicinity of Lake Kariba) of Zambia (42.8% infected, Phiri et al., 2007). Most remarkable however is the exceptionally high prevalence (65.17%) of *Fasciola* sp. in *P. columella*. This high figure, together with the high abundance of *P. columella* across all sites, suggests very intensive transmission in the lake near Kariba town. As suggested by Lounnas et al. (2017), these high rates might be linked with the low genetic diversity of the invasive *P. columella* populations, which could increase their susceptibility.

P. columella is a well-known host of *F. hepatica* and *F. gigantica* and has been shown to be susceptible to *Fasciola* infections upon introduction (Grabner et al., 2014). However, being one of the most successful invasive gastropod species in South Africa, no *Fasciola* infections have ever been found in previous studies of South African molluscs and no record exists of this particular host - parasite association in Sub-Saharan Africa (Toledo and Fried, 2014). One of the reasons might be that previous studies did not use PCR based diagnosis of gastropod infections. Also species of the *Radix* genus have been identified as intermediate hosts of *Fasciola hepatica* (Lawton et al., 2015; Mas-Coma et al., 2009). In Vietnam and other parts of South East Asia, fasciolosis is thought to be transmitted through the lymnaeids *Radix auricularia* and *Radix rubiginosa* (Bui et al., 2016) and in Africa, *Radix natalensis* has been found to transmit both *F. gigantica* (Grabner et al., 2014) and the endemic African *Fasciola nyanzae* (Leiper, 1910) (Dinnik and Dinnik, 1961). Also in this study we found *Radix* sp. to be infected with *Fasciola* sp., but at a much lower rate of 4.5%. Our sequence analysis suggests it is the same species as the one that infects *P. columella* as the COI sequence of *Radix* sp. was identical to the one of haplotype S201 from *P. columella* (Table 3).

Our data strongly suggest that the fasciolid found in lake Kariba is closely related to *F. gigantica* and *F. hepatica* (Fig. 4, Tables 3, 4 and Supplementary Table 2). The 18S rDNA marker (Supplementary Table 2) shows only 0.2% of sequence heterogeneity with both *F. hepatica* and *F. gigantica* while the interspecific variation between the latter two species amounts to 0.3%. The 18S rDNA region is however too conserved to draw phylogenetic conclusions at the species level (Nolan and Cribb, 2005). Also the ITS marker clearly underlines a close affinity of the Kariba *Fasciola* species with *F. hepatica* and *F. gigantica*, (Fig. 4B, Table 4). Especially the distances between genotype S84 and *F. hepatica* from Spain and Peru is very small (p-distance = 0.4% for the entire ITS region and 0.2% for the 442 bp fragment used for phylogeny reconstruction). The interspecific variation between *F. hepatica* and *F. gigantica* is 1.05% in the entire ITS region while intraspecific variation is very low in *F. hepatica* and non-existent in *F. gigantica* (Mas-Coma et al., 2009). Therefore we think that the low genetic distance we find between our Kariba strain and *F. hepatica*, is still indicative of distinct species status. This is corroborated by the COI phylogeny and the distance table that both suggest that the Kariba sequences are neither *F. hepatica* nor *F. gigantica*. The lowest p-distance between the Kariba sequences and either of the two *Fasciola* species in the COI alignment is 6.6% (Table 3). This is higher than the intraspecific variation usually found within the COI barcoding region, ranging between 0.3 and 2.2% (max. 5%) (Vilas et al., 2005). Species identification and delineation is however complicated by frequent hybridisation between *F. gigantica* and *F. hepatica* (Agatsuma et al., 2000; Itagaki et al., 2005; Amer et al., 2011). The close affinity of the *Fasciola* species from Kariba with *F. hepatica* based on the nuclear ITS marker, but the considerable difference at the mitochondrial COI level, could suggest a hybrid origin through a cross between a male *F. hepatica* and a species that has not been included in our analysis. The family Fasciolidae contains five described genera and nine species, of which only five species are available as COI reference sequences in the GenBank database. No reference sequence exists for example from *Fasciola nyanzae*, which infects hippopotami and is endemic to Southern Africa.

Although our analysis didn't allow exact species identification, we expect the *Fasciola* species to be native rather than the result of an introduction with the invasive gastropod species. Indeed, invasive gastropods are generally transported passively as eggs or juveniles on traded plants or sticking to feet of migrating birds. This means that they are still free from parasites since eggs cannot become infected yet (Ebbs et al., 2018). Also, *Fasciola* parasites are mainly spread and introduced to new areas by means of their final ruminant hosts that can be traded over large distances (Mas-Coma et al., 2009). Finally, the fact that we found different *Fasciola* haplotypes (four different COI haplotypes out of eight sequences), also suggests that they are not the result of a recent introduction with *P. columella*, as this would have created a founder effect in the parasite population (reduced genetic diversity). Taken all results and literature together, we therefore consider the *Fasciola* species as local.

The close relatedness of the *Fasciola* sp. to *F. gigantica* and *F. hepatica*, compared to other fasciolid species, suggests that it probably shares similar final hosts, namely wild or domesticated ruminants and humans (see Fig. 4B). We therefore expect that the very high prevalence of this parasite within a widespread and abundant invasive intermediate host might have important ecological, economic and/or medical consequences (Grabner et al., 2014). Hippopotami are very abundant in and around Lake Kariba, as is the African buffalo. The latter has been found to be naturally infected with *F. gigantica* with prevalences up to 50% in Uganda (Bindernagel, 1972). Screening of faeces from wildlife in and around Kariba would help to identify which final hosts are involved in the transmission.

4.3. Biological invasions and parasite spillback

The water hyacinth abundance correlated significantly with the *P. columella* count (Fig. 5). Water plants offer freshwater gastropods protection from the sun and from visual predators such as fish and birds (Plummer, 2005), they provide oxygen to the water, act as substrate for egg mass deposition and function as food source. Vegetation mats also reduce water velocity and tidal wave action, which benefits freshwater gastropods (Plummer, 2005). *E. crassipes* is a problematic water plant in Lake Kariba because of the vast mats they form, thereby impeding boat traffic and fishing, and outcompeting local water flora (Adams et al., 2002). Even though we cannot prove a causal relationship, we suggest that, in addition to the aforementioned detrimental effects, *E. crassipes* boosts the infection dynamics of endemic fasciolosis in Lake Kariba by supporting the invasion of a highly competent intermediate gastropod host species. Because the encountered *Fasciola* species is most probably native, our study illustrates how an invasive gastropod species devoid of its native parasites can become the host of a local parasite, boosting transmission of this parasite due to its high abundance and exceptional competence as intermediate host – a phenomenon referred to as 'parasite spillback' (Kelly et al., 2009). To be more precise, at least the first step of parasite spillback is evidenced, as increased infection in the native final host cannot be proven at this stage, because we

do not know the final host yet. However, the unusually high infection rate in *P. columella* implicates high exposure of the final hosts, which most likely leads to high infection levels in the final hosts.

The success of the native parasite at exploiting its new host is potentially explained by multiple factors evocated earlier; the competence of the host, the very low genetic diversity of the established population (Lounnas et al., 2017) and the absence of competition with its natural parasites. Experimental infection of *P. columella* collected in France showed that all 26 gastropods developed infection after exposure to *F. hepatica* miracidia (Pointier et al., 2007), demonstrating it is a highly competent host. The current scenario was actually already 'predicted' by Lounnas et al. (2017), who warned for the serious risk of fasciolosis outbreaks around the globe as a consequence of the rapid worldwide invasion of a single *P. columella* strain, being susceptible to *Fasciola* infections. A similar case of parasite spillback has also been reported from Egypt where the invasive *P. columella* hosted endemic *F. gigantica* infections leading to an increased infection prevalence of fasciolosis in humans and livestock (Grabner et al., 2014). Also in the latter case, high abundance of water hyacinth was reported.

The putative cascade of biological invasions followed by parasite spillback is likely to have highly important ecological and economic consequences by dramatically increasing the prevalence of *Fasciola* sp. infections in wild and domesticated mammals around Lake Kariba. It might therefore be useful to consider the control of water hyacinth in order to control (invasive) gastropods and their trematodes. Since *E. crassipes* causes many other problems besides supporting trematodiases such as hampering boat transport and fishing, its control can have multiple beneficial outcomes.

5. Conclusions

This study corroborates the idea that man-made water reservoirs are sensitive to cascades of biological invasions, which can have far-reaching consequences when combined with parasite spillback. These invasions probably happened in a cascade fashion: the introduction and spread of the invasive water hyacinth promoted the establishment of large populations of invasive *P. columella* and *Radix* sp. of which the *P. columella* population appeared to be particularly suitable to transmit a (probably) native *Fasciola* species (spillback hypothesis). The exceptionally high proportion of infected *P. columella* populations suggests that the transmission of this *Fasciola* species dramatically intensified. However, various aspects remain unclear and require further investigation. More work is needed to morphologically and molecularly describe the respective *Fasciola* species and to identify its final host(s) in order to assess the risk it constitutes. Investigating nearby natural systems might also shed light on the provenance and on the 'endemic' intermediate host of the parasite. Very little literature exists regarding the distribution of *P. columella* and its infection status, despite the possible economical and epidemiological consequences. Given our findings and the previous warnings of Lounnas et al. (2017) regarding the potential of *P. columella* to trigger fasciolosis epidemics, it is crucial to investigate the distribution of this invasive gastropod in Zimbabwe and neighbouring countries.

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References

Adams, C.S., Boar, R.R., Hubble, D.S., Gikungu, M., Harper, D.M., Hickley, P., Tarras-Wahlberg, N., 2002. The dynamics and ecology of exotic tropical species in floating plant mats: Lake Naivasha, Kenya. Hydrobiologia 488, 115–122. https://doi.org/10.1023/A:1023322430005.

Agatsuma, T., Arakawa, Y., Iwagami, M., Honzako, Y., Cahyaningsih, U., Kang, S.Y., Hong, S.J., 2000. Molecular evidence of natural hybridization between *Fasciola hepatica* and *F. gigantica*. Parasitol. Int. 49 (3), 231–238. https://doi.org/10.1016/S1383-5769(00)00051-9.

Alicata, J.E., 1938. Observations on the Life History of *Fasciola gigantica*, the Common Liver Fluke of Cattle in Hawaii, and the Intermediate Host, *Fossaria ollula*. Bulletin no 80. University of Hawaii Press. https://doi.org/10.5962/bhl.title.87642.

Altschul, S.F., Gish, W., Miller, W., Myers, E.W., Lipman, D.J., 1990. Basic local alignment search tool. J. Mol. Biol. 215 (3), 403–410. https://doi.org/10.1016/S0022-2836(05)80360-2.

Amer, S., Dar, Y., Ichikawa, M., Fukuda, Y., Tada, C., Itagaki, T., Nakai, Y., 2011. Identification of *Fasciola* species isolated from Egypt based on sequence analysis of genomic (ITS and ITS2) and mitochondrial (NDI and COI) gene markers. Parasitol. Int. 60 (1), 5–12. https://doi.org/10.1016/j.parint.2010.09.003.

Appleton, C.C., 2003. Alien and invasive freshwater Gastropoda in South Africa. Afr. J. Aquat. Sci. 28 (1), 69–81. https://doi.org/10.2989/16085914.2003.9626602.

Appleton, C.C., Miranda, N.A.F., 2015. Two Asian freshwater snails newly introduced into South Africa and an analysis of alien species reported to date. Afr. Invertebr. 56 (1), 1–17. https://doi.org/10.5733/afin.056.0102.

Bindernagel, J.A., 1972. Liver fluke *Fasciola Gigantica* in African buffalo and antelopes in Uganda, East Africa. J. Wildl. Dis. 8 (4), 315–317. https://doi.org/10.7589/0090-3558-8.4.315.

Brown, D.S., 1994. Freshwater Snails of Africa and Their Medical Importance. 2nd ed. vol. 75. UK Taylor & Francis Ltd. https://doi.org/10.1016/0035-9203(81)90097-3.

Bui, T.D., Doanh, P.N., Saegerman, C., Losson, B., 2016. Current status of *Fasciolosis* in Vietnam: an update and perspectives. J. Helminthol. 90 (5), 511–522. https://doi.org/10.1017/S0022149X15000929.

Caron, Y., Righi, S., Lempereur, L., Saegerman, C., Losson, B., 2011. An optimized DNA extraction and multiplex PCR for the detection of *Fasciola* sp. in Lymnaeid snails. Vet. Parasitol. 178 (1–2), 93–99. https://doi.org/10.1016/j.vetpar.2010.12.020.

Caron, V., Ede, F.J., Sunnucks, P., 2014. Unraveling the paradox of loss of genetic variation during invasion: superclones may explain the success of a clonal invader. PLoS One 9 (6), 97744. https://doi.org/10.1371/journal.pone.0097744.

Chimbari, M.J., Chirundu, D., 2003. Prevalence and intensity of the schistosomiasis situation along the Zimbabwean urban and Peri-urban shoreline of Lake Kariba. Cent. Afr. J. Med. 49, 8–12.

Chimbari, M.J., Dhlomo, E., Mwadiwa, E., Mubila, L., 2003. Transmission of schistosomiasis in Kariba, Zimbabwe, and a cross-sectional comparison of schistosomiasis Prevalences and intensities in the town with those in Siavonga in Zambia. Ann. Trop. Med. Parasitol. 97 (6), 605–616. https://doi.org/10.1179/000349803225001508.

Chingwena, G., Mukaratirwa, S., Kristensen, T.K., Chimbari, M., 2002. Larval trematode infections in freshwater snails from the Highveld and Lowveld areas of Zimbabwe. J. Helminthol. 76 (4), 283–293. https://doi.org/10.1079/JOH2002132.

Coles, G.C., Kabatereine, N.B., 2008. Water hyacinth and the transmission of schistosomiasis. Trans. R. Soc. Trop. Med. Hyg. 102 (6), 619–620. https://doi.org/10.1016/j.trstmh.2008.01.009.

Correa, A.C., Escobar, J.S., Durand, P., Renaud, F., David, P., Jarne, P., Pointier, J., Hurtez-Boussès, S., 2010. Bridging gaps in the molecular phylogeny of the Lymnaeidae (Gastropoda: Pulmonata), vectors of fascioliasis. BMC Evol. Biol. 10, 381. https://doi.org/10.1186/1471-2148-10-381.

Cowie, R.H., Dillon, R.T., Robinson, D.G., Smith, J.W., 2009. Alien non-marine snails and slugs of priority quarantine importance in the United States: a preliminary risk assessment. Am. Malacol. Bull. 27, 113–132. https://doi.org/10.4003/006.027.0210.

De Kock, K.N., Joubert, P.H., Pretorius, S.J., 1989. Geographical distribution and habitat preferences of the invader freshwater snail species *Lymnaea columella* (Mollusca: Gastropoda) in South Africa. Onderstepoort J. Vet. Res. 56 (4), 271–275. http://www.ncbi.nlm.nih.gov/pubmed/2626264.

DiNardo, A., 2015. Helminth infections and their impact on global public health. Clin. Infect. Dis. 60. https://doi.org/10.1093/cid/ciu891.

Dinnik, J.A., Dinnik, N.N., 1961. On the morphology and life history of *Fasciola nyanzae* Leiper, 1910 from the hippopotamus. J. Helminthol. 35 (1), 53–62. https://doi.org/10.1017/S0022149X00017570.

Ebbs, E.T., Loker, E.S., Brant, S.V., 2018. Phylogeography and genetics of the globally invasive snail *Physa acuta* Draparnaud 1805, and its potential to serve as an intermediate host to larval digenetic trematodes. BMC Evol. Biol. 18, 103. https://doi.org/10.1186/s12862-018-1208-z.

Edgar, R.C., 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res. 32 (5), 1792–1797. https://doi.org/10.1093/nar/gkh340.

Edwards, D., Musil, C.J., 1975. *Eichhornia crassipes* in South Africa - a general review. J. Limnol. Soc. South. Afr. 1 (1), 23–27. https://doi.org/10.1080/03779688.1975.9632904.

van Eeden, J.A., Brown, D.S., 1966. Colonization of fresh waters in the Republic of South Africa by *Lymnaea columnella* Say (Mollusca: Gastropoda). Nature 209, 694–696. https://doi.org/10.1038/2101172a0.
Fox, J., Weisberg, S., 2011. An R Companion to Applied Regression. 2nd ed. Sage, Thousand Oaks CA.
Giannelli, A., Cantacessi, C., Colella, V., Dantas-Torres, F., Otranto, D., 2016. Gastropod-borne helminths: a look at the snail-parasite interplay. Trends Parasitol. 32 (3), 255–264. https://doi.org/10.1016/j.pt.2015.12.002.
Grabner, D.S., Mohamed, F.A.M.M., Nachev, M., Méabed, E.M.H., Sabry, A.H.A., Sures, B., 2014. Invasion biology meets parasitology: a case study of parasite spillback with Egyptian *Fasciola gigantica* in the invasive snail *Pseudosuccinea columella*. PLoS One 9 (2), 1–7. https://doi.org/10.1371/journal.pone.0088537.
Güereña, D., Neufeldt, H., Berazneva, J., Duby, S., 2015. Water hyacinth control in Lake Victoria: transforming an ecological catastrophe into economic, social, and environmental benefits. Sustain. Prod. Consum. 3, 59–69. https://doi.org/10.1016/j.spc.2015.06.003.
Hasegawa, M., Kishino, H., Yano, T., 1985. Dating of the human-ape splitting by a molecular clock of mitochondrial DNA. J. Mol. Evol. 22 (2), 160–174. https://doi.org/10.1007/BF02101694.
Havel, J.E., Lee, C.E., Vander Zanden, M.J., 2005. Do reservoirs facilitate invasions into landscapes? Bioscience 55 (6), 518. https://doi.org/10.1641/0006-3568(2005)055[0518:DRFIIL]2.0.CO;2.
Hira, P.R., 1969. Transmission of schistosomiasis in Lake Kariba, Zambia. Nature 224, 670–672. https://doi.org/10.1038/224488a0.
Hopkins, D.R., 1992. Homing in on helminths. Am. J. Trop. Med. Hyg. 46 (6), 626–634. https://doi.org/10.4269/ajtmh.1992.46.626.
Itagaki, T., Kikawa, M., Sakaguchi, K., Shimo, J., Terasaki, K., Shibahara, T., Fukuda, K., 2005. Genetic characterization of parthenogenic *Fasciola* sp. in Japan on the basis of the sequences of ribosomal and mitochondrial DNA. Parasitology 131 (5), 679–685. https://doi.org/10.1017/S0031182005008292.
Johnson, P.T.J., Olden, J.D., Vander Zanden, M.J., 2008. Dam invaders: impoundments facilitate biological invasions into freshwaters. Front. Ecol. Environ. 6 (7), 357–363. https://doi.org/10.1890/070156.
Kaplan, R.M., Dame, J.B., Reddy, G.R., Courtney, C.H., 1995. A repetitive DNA probe for the sensitive detection of *Fasciola hepatica* infected snails. Int. J. Parasitol. 25 (5), 601–610. https://doi.org/10.1016/0020-7519(94)00159-L.
Kelly, D.W., Paterson, R.A., Townsend, C.R., Poulin, R., Tompkins, D.M., 2009. Parasite spillback: a neglected concept in INvasion ecology? Ecol. Soc. Am. 90 (8). https://doi.org/10.1890/08-1085.1.
Køie, M., Nansen, P., Christensen, N.Ø.Z.F., 1977. Stereoscan studies of rediae, cercariae, cysts, excysted metacercariae and migratory stages of *Fasciola hepatica*. Parasite 54, 289. https://doi.org/10.1007/BF00390120.
Krämer, F., Schnieder, T., 1998. Sequence heterogeneity in a repetitive DNA element of *Fasciola*. Int. J. Parasitol. 28 (12), 1923–1929. https://doi.org/10.1016/S0020-7519(98)00162-3.
Kumar, S., Stecher, G., Li, M., Knyaz, C., Tamura, K., 2018. MEGA X: Molecular Evolutionary Genetics Analysis across Computing Platforms. Mol. Biol. Evol. 35 (6), 1547–1549. https://doi.org/10.1093/molbev/msy096.
Lawton, S.P., Lim, R.M., Dukes, J.P., Kett, S.M., Cook, R.T., Walker, A.J., Kirk, R.S., 2015. Unravelling the riddle of *Radix*: DNA barcoding for species identification of freshwater snail intermediate hosts of zoonotic digeneans and estimating their inter-population evolutionary relationships. Infect. Genet. Evol. 35, 63–74. https://doi.org/10.1016/j.meegid.2015.07.021.
Lerer, L.B., Scudder, T., 1999. Health impacts of large dams. Environ. Impact Assess. Rev. 19 (2), 113–123. https://doi.org/10.1016/S0195-9255(98)00041-9.
Lotfy, W.M., Brant, S.V., DeJong, R.J., Le, T.H., Demiaszkiewicz, A., Rajapakse, R.P., Perera, V.B., Laursen, J.R., Loker, E.S., 2008. Evolutionary origins, diversification, and biogeography of liver flukes (Digenea, Fasciolidae). Am. J. Trop. Med. Hyg. 79 (2), 248–255. https://doi.org/10.4269/ajtmh.2008.79.248.
Lounnas, M., Correa, A.C., Vázquez, A.A., Dia, A., Escobar, J.S., Nicot, A., Arenas, J., et al., 2017. Self-fertilization, long-distance flash invasion and biogeography shape the population structure of *Pseudosuccinea columella* at the worldwide scale. Mol. Ecol. 26 (3), 887–903. https://doi.org/10.1111/mec.13984.
Magadza, C.H.D., 2006. Kariba reservoir: experience and lessons learned. Lakes Reserv. Res. Manag. 11 (4), 271–286. https://doi.org/10.1111/j.1440-1770.2006.00308.x.
Mandahl-Barth, G., 1962. Key to the identification of east and central African freshwater snails of medical and veterinary importance. Bull. World Health Organ. 27 (1), 135–150.
Mas-Coma, S., Valero, M.V., Bargues, M.D., 2009. Chapter 2. *Fasciola*, lymnaeids and human fascioliasis, with a global overview on disease transmission, epidemiology, evolutionary genetics, molecular epidemiology and control. Adv. Parasitol. 69, 41–146. https://doi.org/10.1016/S0065-308X(09)69002-3.
Mehmood, K., Zhang, H., Sabir, A.J., Abbas, R.Z., Ijaz, M., Durrani, A.Z., Saleem, M.H., et al., 2017. A review on epidemiology, global prevalence and economical losses of fasciolosis in ruminants. Microb. Pathog. 109, 253–262. https://doi.org/10.1016/j.micpath.2017.06.006.
Morley, N.J., 2007. Anthropogenic effects of reservoir construction on the parasite fauna of aquatic wildlife. EcoHealth 4 (4), 374–383. https://doi.org/10.1007/s10393-007-0130-4.
Mubila, L., Rollinson, D., 2002. Snail-parasite compatibility and prevalence of *Schistosoma haematobium* on the shores of Lake Kariba, Zambia. Ann. Trop. Med. Parasitol. 96 (2), 165–173. https://doi.org/10.1179/000349802125000592.
Mungomba, L.M., Chandiwana, S.K., Madesen, H., 1993. Schistosomiasis around Siavonga, on the shores of Lake Kariba, Zambia. Ann. Trop. Med. Parasitol. 87 (4), 365–371. https://doi.org/10.1080/00034983.1993.11812780.
Nei, M., Kumar, S., 2000. Molecular Evolution and Phylogenetics. Oxford University Press.
Nolan, M.J., Cribb, T.H., 2005. The use and implications of ribosomal DNA sequencing for the discrimination of *Digenean* species. Adv. Parasitol. 60, 101–163. https://doi.org/10.1016/S0065-308X(05)60002-4.
von Oheimb, P.V., Albrecht, C., Riedel, F., Du, L., Yang, J., Aldridge, D.C., Bößneck, U., Zhang, H., Wilke, T., 2011. Freshwater biogeography and limnological evolution of the Tibetan Plateau - insights from a plateau-wide distributed gastropod taxon (*Radix* spp.). PLoS One 6 (10). https://doi.org/10.1371/journal.pone.0026307.
Patoka, J., Bláha, M., Kalous, L., Vrabec, V., Buřič, M., Kouba, A., 2016. Potential pest transfer mediated by international ornamental plant trade. Sci. Rep. 6, 25896. https://doi.org/10.1038/srep25896.
Phiri, A.M., Phiri, I.K., Chota, A., Monrad, J., 2007. Trematode infections in freshwater snails and cattle from the Kafue wetlands of Zambia during a period of highest cattle–water contact. J. Helminthol. 81 (1), 85–92. https://doi.org/10.1017/S0022149X07387786.
Picquet, M., Ernould, J.C., Vercruysse, J., Southgate, V.R., Mbaye, A., Sambou, B., Niang, M., Rollinson, D., 1996. The epidemiology of human schistosomiasis in the Senegal river basin. Trans. R. Soc. Trop. Med. Hyg. 90 (4), 340–346. https://doi.org/10.1016/s0035-9203(96)90501-5.
Plummer, M.L., 2005. Impact of invasive water hyacinth (*Eichhornia crassipes*) on snail hosts of schistosomiasis in Lake Victoria, East Africa. EcoHealth 2 (1), 81–86. https://doi.org/10.1007/s10393-004-0104-8.
Pointier, J.P., David, P., Jarne, P., 2005. Biological invasions: the case of planorbid snails. J. Helminthol. 79 (3), 249–256. https://doi.org/10.1079/JOH2005292.
Pointier, J.P., Coustau, C., Rondelaud, D., Theron, A., 2007. *Pseudosuccinea columella* (Say 1817) (Gastropoda, Lymnaeidae), snail host of *Fasciola hepatica*: first record for France in the wild. Parasitol. Res. 101 (5), 1389–1392. https://doi.org/10.1007/s00436-007-0656-y.
R Development Core Team, R, 2011. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing. vol. 1. The R Foundation for Statistical Computing, Vienna, Austria.
Ratnasingham, S., Hebert, P.D.N., 2007. Bold: the barcode of life data system (http://www.barcodinglife.org). Mol. Ecol. Notes 7 (3), 355–364. https://doi.org/10.1111/j.1471-8286.2007.01678.x.
Roman, J., Darling, J.A., 2007. Paradox lost: genetic diversity and the success of aquatic invasions. Trends Ecol. Evol. 22 (9), 454–464. https://doi.org/10.1016/j.tree.2007.07.002.
Sow, S., de Vlas, S.J., Engels, D., Gryseels, B., 2002. Water-related disease patterns before and after the construction of the Diama dam in northern Senegal. Ann. Trop. Med. Parasitol. 96 (6), 575–586. https://doi.org/10.1179/000349802125001636.
Stanley, N.F., Alpers, M.P., 1975. Man-made Lakes and Human Health. Academic Press Inc.
Toledo, R., Fried, B., 2014. Digenetic trematodes. Advances in Experimental Medicine and Biology. Springer, NY, p. 766. https://doi.org/10.1007/978-1-4939-0915-5.
Venables, W.N., Ripley, B.D., 2002. MASS: Modern Applied Statistics With S. 4th ed. Springer. https://doi.org/10.1198/tech.2003.s33.
Vilas, R., Criscione, C.D., Blouin, M.S., 2005. A comparison between mitochondrial DNA and the ribosomal internal transcribed regions in prospecting for cryptic species of platyhelminth parasites. Parasitology 131 (6), 839–846. https://doi.org/10.1017/S0031182005008437.
Webster, M.H., 1960. The medical aspects of the Kariba hydro-electric scheme. Cent. Afr. J. Med. 10, 3–36.
WHO, 2017. Foodborne trematode infections: fascioliasis. http://www.who.int/foodborne_trematode_infections/fascioliasis/en/, Accessed date: 15 September 2018.
Wilkinson, L., 2011. Ggplot2: elegant graphics for data analysis by WICKHAM, H. Biometrics 67 (2), 678–679. https://doi.org/10.1111/j.1541-0420.2011.01616.x.