Molecular Biology Reports
https://doi.org/10.1007/s11033-020-05541-0

ORIGINAL ARTICLE

Genetic diversity of *Biomphalaria pfeifferi*, the intermediate host of *Schistosoma mansoni* in Shamva district, Zimbabwe: role on intestinal schistosomiasis transmission

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Received: 23 July 2019 / Accepted: 23 May 2020

Abstract

The fresh water snail *Biomphalaria pfeifferi* is the intermediate host for *Schistosoma mansoni,* which causes human intestinal schistosomiasis in Zimbabwe. Despite the medical importance of this intermediate host, there are no current data on its molecular characterization in Zimbabwe. In 2016, human water contact sites were identified in four communities in Madziwa area, Shamva district, Zimbabwe. The survey sites were recorded and mapped using a global positioning system. A 655 bp region of the mitochondrial cytochrome oxidase subunit I gene was amplified in 70 *B. pfeifferi* snails. The sequence data were analysed to determine the relationships between the individual snails, their inter, intra population diversity and structure. Overall, four unique *cox*1 haplotypes, with a haplotype diversity of 0.608, were identified in the snails. One haplotype spanned across most of the sites. There was no clear geographical clustering of haplotypes. The mean diversity among the haplotypes was very low (0.009), while the net divergence among the collection sites ranged from 0.000 to 0.026. The diversity within and between the sites was 0.017 and 0.012 respectively. This data advances our knowledge of the understanding of the population structure of *B. pfeifferi* in Madziwa area, Zimbabwe, with the high occurrence of one haplotype indicating the possibility of a recent bottleneck followed by population expansion. The population genetic structure of *B. pfeifferi* snails described here has provided an opportunity to investigate the contribution of snail genetics to variation in disease burden; and development of control strategies that exploit genetic differences in susceptibility to parasites.

Keywords *Biomphalaria pfeifferi* · Zimbabwe · Phylogenetics · *Cox*1 · Schistosomiasis

Abbreviation

*Cox*1 Cytochrome oxidase subunit 1

Introduction

The hermaphroditic *Biomphalaria* (Preston 1910) freshwater snails of the family Planorbidae are the intermediate host snails of the human intestinal parasitic trematode, *Schistosoma mansoni* [1]. The genus is widely distributed, both in Africa and in the Neotropics, with molecular evidence suggesting that it originated in South America and colonised Africa, facilitating the wide geographical distribution of intestinal schistosomiasis [2, 3]. However, there is a high degree of variation in compatibility between *S. mansoni* and the different *Biomphalaria* species [4, 5]. The difference in compatibility is driven by both biotic and abiotic factors resulting in localised differences in disease epidemiology/transmission [1, 4, 5].

Amongst the *Biomphalaria* species, *B. pfeifferi* (Krauss 1848) is the most common intermediate host snail for *S. mansoni* in Sub-Saharan Africa [5] including Zimbabwe [6, 7]. While a number of subspecies of *B. pfeifferi* have been described [5], there is no clear geographical pattern explaining their variation [8]. Nevertheless, the existence of genetic variation among *B. pfeifferi* populations is speculated to be due to flooding and drought, which are the major ecological forces leading to bottlenecks and/or recolonization [9] and possibly extinction. However, parasitic infection and other

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s11033-020-05541-0) contains supplementary material, which is available to authorized users.

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Published online: 12 June 2020

environmental factors such as habitat differences cannot be ruled out [10].

A notable feature for *B. pfeifferi* is that it is a selfing species, with few copulations between different snails [11, 12]. Selfing and bottlenecks result in limited genetic diversity within a population but large genetic differentiation between populations [13]. Thus, the understanding of the evolutionary history affecting snail genetic diversity, consequences of environmental factors, host-parasite compatibility, bottlenecks and mating patterns of snail populations within a certain locality is imperative [9–11, 14]. This knowledge will assist in interpreting current transmission heterogeneity within endemic communities as large-scale control and elimination programmes are implemented [15].

With the advent of molecular genetics, several molecular marker based methods for elucidating the genetic structure and phylogeny of snail species have been developed. The molecular markers include both mitochondrial (mt) [2, 4, 15–17] and nuclear DNA [14, 15, 18–22]. More recently, sequence analysis of the internal transcribed spacer (ITS1) of ribosomal RNA operon [4], the mitochondrial cytochrome oxidase subunit 1 (*cox*1) [2, 4, 16, 23–25] and complete mitochondrial and nuclear genomes [26] are being used for the characterisation of *B. pfeifferi* and to investigate their evolutionary history. The mitochondrial *cox*1 marker has been recognized by the International Barcode of Life as an official marker for animal identification [27, 28]. The 'universal' *cox*1 marker for invertebrates developed by Folmer and others [29] has generated informative sequence data for the identification and phylogenetic analysis of snail hosts in Africa and beyond [2, 4, 16, 17, 23–25]. Such information is vital for identifying areas that are at risk of schistosomiasis transmission [30].

A previous study [17] describing the molecular phylogeny of *B. pfeifferi* in Zimbabwe, using *cox*1 data was based on a single snail sampled from Chiweshe. The study used basic phylogenetic analysis showing close clustering of the Zimbabwe *B. pfeifferi* with West African *B. pfeifferi*; however, no haplotype or diversity estimates were made. In this study, we have explored the *cox*1 diversity of *B. pfeifferi* populations in Madziwa area, Shamva district, Zimbabwe in addition to describing their phylogeny. The data are discussed in relation to local environmental characteristics and patterns of intestinal schistosomiasis transmission.

Materials and methods

Study area

Natural populations of *B. pfeifferi* were collected from freshwater habitats in four rural communities in Mashonaland Central province, Shamva district, Zimbabwe. Although both urogenital and intestinal schistosomiasis is endemic in the area, Shamva district was chosen based on its high endemicity of urogenital schistosomiasis [31]. The main study to which the current study is a sub-study was focusing on monitoring prevalence, reinfections and new infections of urogenital schistosomiasis in preschool aged children [32] and the risk factors of infection [33]. The main study also monitored the spatial and seasonal distribution of intermediate host snails and their infection status throughout the one-year study period. The study area covering approximately 13 villages with human settlements has one major perennial river, Mupfure. While there is no corresponding data on *S. mansoni* infection in local communities near the sampling sites, Midzi et al. [31] reported a prevalence of > 10% for *S. mansoni* in Shamva district in 2010. The villages in the local communities are characterised as semi-arid areas subject to high levels of poverty, and rely on rivers and streams for most household activities including bathing, fishing, swimming, washing, gardening and subsistence farming. Despite more than 75% of the residents in the communities having toilets at home, most individuals use river water for the majority of household activities, thus potentially exposing themselves to schistosomiasis infection [33]. There are no major water development projects in the area. The communities rely on a few boreholes available in the area for safe water sources, which they mostly use for drinking purposes. The study area has distinct seasonality with the rainfall period starting from late October and ending in April.

Human water contact site selection and snail collection

Selection of the snail survey sites was based on asking for water contact points frequently used by the local people for water collection, gardening, bathing, washing, swimming, or playing. Within the sampling time, all intermediate host snails were collected regardless of size. Twenty-seven water contact sites were identified in three main river systems namely Mupfure, Nyarukunda and Nyamaruru and two streams (Zvisokwe and Kamoyo). Of these, 20 had *B. pfeifferi* snails during the one year study period. Most communities rely on the perennial Mupfure River that crosses through approximately 80% of the villages in the area. To assess the spatial distribution of the snail populations, all the surveyed sites were mapped using a global positioning system (GPS) (Trimble Navigation Ltd, California, USA). The snail collection points are shown in Fig. 1 and Table 1 below. The snails were collected at three months intervals corresponding to different seasons over one year (February 2016 to February 2017 (Table 1). Snail sampling at all sites was performed using a metal scoop or handpicking for 30 min from rocky sites and from the edges and main parts of the water body, where they

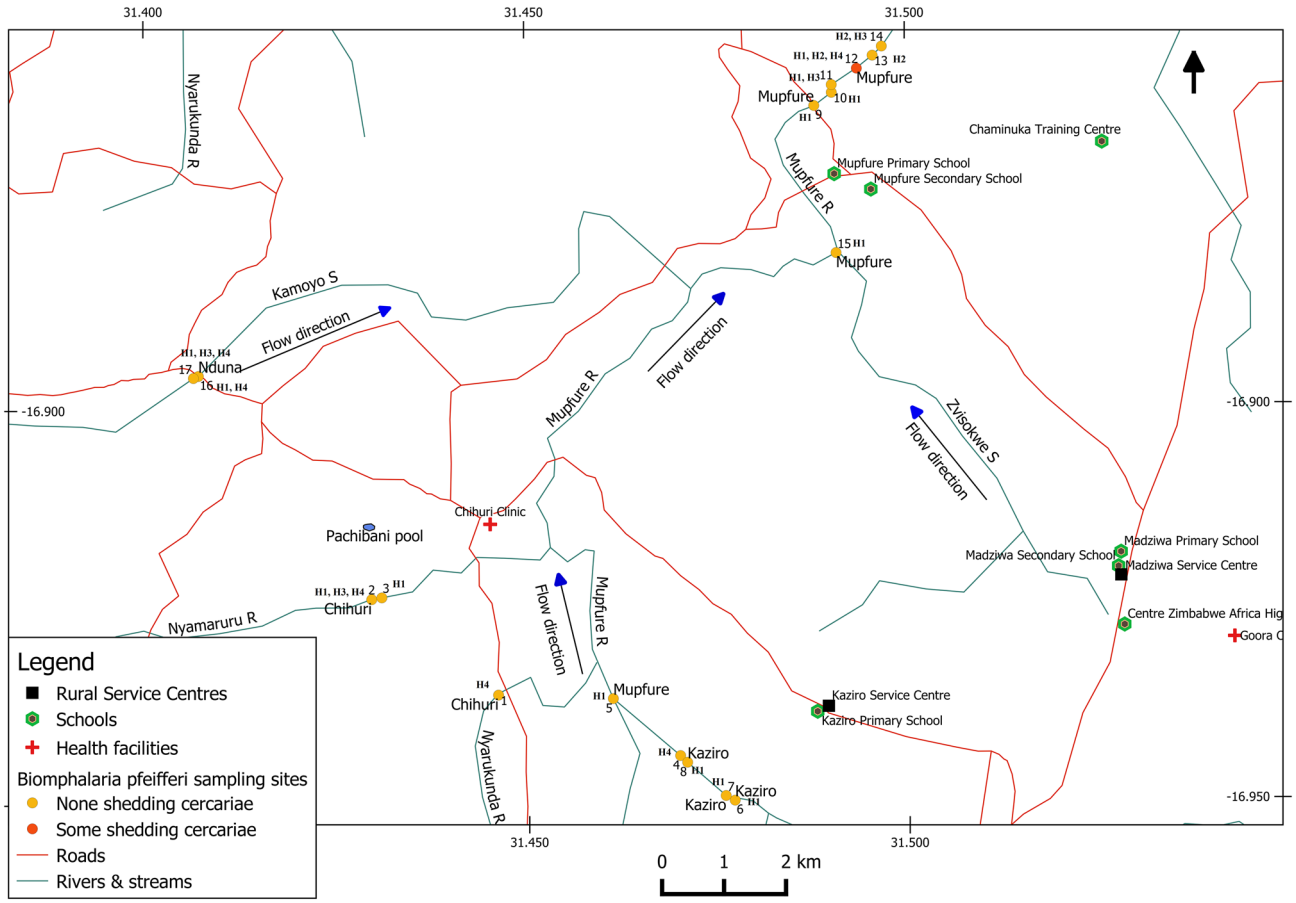

Fig. 1 Location of the water contact sites where *B. pfeifferi* snails were collected and their *cox1* gene sequenced in Madziwa area, Shamva district. *Legend* The coordinates of these water contact sites are detailed in Table 1.The locations shown are for those sites where *B. pfeifferi* snails were collected for sequencing and also indicated sites which had snails shedding cercariae

were removed from the underside of aquatic vegetation. All the snails collected were placed in labelled plastic containers with cotton wool soaked in water from the collection site and transported to the field laboratory. Each snail was identified to the species level by shell morphology as described by Brown in [34]. *Biomphalaria pfeifferi* were identified by its planular, disc shaped shell, flattened spire when raised above succeeding whorls. They were then checked for patent schistosome infections by cercarial shedding before being preserved in absolute ethanol. The snails were placed individually in flat-bottomed glass vials containing 2.5 ml of untreated borehole water and exposed to artificial light for a maximum duration of four hrs [35]. Cercarial identification was based on morphology using a binocular microscope as described by Frandsen and Christensen [36]. Bifurcate cercariae, swimming to the surface, resting momentarily before sinking back into the container either partially or to the bottom before they resumed swimming again were considered to be *S. mansoni*. No further tests were carried out to distinguish infections of different *Schistosoma* species.

DNA extraction

Due to limited resources and depending on the number of adult *B. pfeifferi* snails available per site, at least 5% of the snails per site were selected for molecular analysis. In total, 70 adult snails were randomly selected and shipped to the Key Schistosomiasis Laboratory, National Institute of Parasitic Diseases, Shanghai, China for molecular characterisation. Individual snails were soaked in TE buffer (10 mM Tris, 0.1 mM EDTA) pH 7.4 for 1 h. After removing the shells, genomic DNA was extracted from the head and foot part of the soft tissue using the DNeasy Blood and Tissue kit (Qiagen, Crawley, UK) following the manufacturer's instructions. Double lysis buffer and proteinase K was used and the extracted DNA was eluted into 200 µl AE buffer. A Nanodrop ND-1000 spectrophotometer (Nanodrop Technologies Inc., Thermo Fisher Scientific, Wilmington, DE, USA) was used for quantification and analysis of DNA purity. DNA was extracted from 70 snails only. The snails analysed were from 17 of the 20 human water contact sites that had *B. pfeifferi* in the area (Table 1; Fig. 1).

Table 1 Collection information for *B. pfeifferi* snails analysed

Collection site	Name of water body	Latitude	Longitude	Number of *B. pfeifferi* collected	Number of *B. pfeifferi* infected	Number of *B. pfeifferi* molecularly analysed	Period of collection
1	Nyarukunda R	16° 56.185′ S	031° 26.762′ E	7	0	1	February 2016
2	Nyamaruru R	16° 55.452′ S	031° 25.768′ E	18	0	4	February 2016
3	Nyamaruru R	16° 55.439′ S	031° 25.848′ E	18	0	1	February 2016
4	Mupfure R	16° 56.655′ S	031° 28.194′ E	6	0	6	February, June 2016
5	Mupfure R	16° 56.218′ S	031° 27.667′ E	183	0	9	February, June 2016
6	Mupfure R	16° 56.997′ S	031° 28.622′ E	42	0	4	February 2016
7	Mupfure R	16° 56.961′ S	031° 28.551′ E	28	0	7	February, September 2016
8	Mupfure R	16° 56.706′ S	031° 28.249′ E	15	0	1	September 2016
9	Mupfure R	16° 51.723′ S	031° 29.283′ E	4	0	4	February, September 2016
10	Mupfure R	16° 51.624′ S	031° 29.422′ E	9	0	1	February 2016
11	Mupfure R	16°51.565′S	031° 29.422′ E	33	0	2	September 2016
12	Mupfure R	16° 51.442′ S	031° 29.621′ E	121	4	12	February, June, September 2016
13	Mupfure R	16° 51.344′ S	031° 29.746′ E	8	0	2	September 2016
14	Mupfure R	16° 51.275′ S	031° 29.819′ E	4	0	4	February, September 2016
15	Mupfure R	16° 52.842′ S	031° 29.450′ E	6	0	1	June 2016
16	Kamoyo S	16° 53.746′ S	031° 24.415′ E	23	0	4	February 2016
17	Kamoyo S	16° 53.761′ S	031° 24.376′ E	17	0	7	February 2016, February 2017
Total				542	4	70	

Mitochondrial DNA amplification and sequencing

A fragment of the cytochrome c oxidase subunit 1 gene (*cox*1) approximately 655 bp in size was amplified using the Folmer 'universal' primers [29] in a C1000TM thermal cycler (BIO-RAD, UK). The *cox*1 PCR amplification was carried out in a 25 µl reaction containing a premixed mastermix [2.5 µl of 20 mM MgCL2, 2.5 µl of 20 mM 5 × buffer, 2.5 µl dNTPs, one unit of Taq DNA polymerase], 10 pmol of each of the forward and reverse primers and 1 µl (10–100 ng) of the DNA template. A no-template negative control was used with each set of reactions run. The PCR amplification conditions consisted of an initial denaturing step at 95 °C for 5 min followed by 45 cycles of denaturation at 95 °C for 3 min, annealing at 45 °C for 30 s, and extension at 72 °C for 1 min and a final extension step at 72 °C for 10 min. The PCR amplicons were analysed using a 1% gel electrophoresis (electrophoresis power supply E455) and the gel was visualised using a Molecular Imager, Gel DocTM XRt Imaging system (BIO-RAD, UK). Positive PCR amplicons were purified and sequenced in both forward and reverse directions using an Applied biosystems 3730XL analyser (Life Technologies, Northumberland, UK) at Sangon Biotech, Shanghai, China.

Data analysis

The number of infected *B. pfeifferi* snails per every visit and overall were recorded and expressed as a percentage. The nucleotide sequences were manually edited using Sequencher v5.1 (https://www.Genecodes) to remove any ambiguities between forward and reverse strands and to trim the ends / remove primer sequence. The consensus sequences of each forward and reverse sequence for each samples was created and aligned in Sequencher. Polymorphic positions observed between individual consensus sequences were confirmed by visualisation of the chromatograms of the original sequence. A BLAST [37] (https://www.ncbi.nlm.nih.gov/) was performed for all sequences to confirm that the species of the snail was *B. pfeifferi.*

Haplotype analysis

The consensus sequences from each sample were grouped and aligned in MacClade 4.05 and then collapsed together

using Collapse V1.2 (https://www.darwin. uvigo.es/software/collapse.html) to identify samples with identical sequences. Within each collection site, mitochondrial haplotypes were collapsed to a set of unique haplotypes, such that any repeated haplotypes were represented only once in the final site-specific data set. The consensus sequence for the unique haplotype were created and given a site-haplotype identifier code, consisting of a site number, letter H to represent the word haplotype and a number to identify the different haplotypes, in each locality. The number of individual sequences presenting each haplotype was also recorded. The identified unique sequences (haplotypes) were submitted to the GenBank and have accession numbers MN397781-MN397784.

Phylogenetic analysis

The consensus sequences were aligned using the Clustal W algorithm [38] using Geneious v10.2. For phylogenetic reconstruction, Neighbor-Joining (NJ), Maximum Likelihood (ML) and Minimum Evolution (ME) methods were used. The analysis was run using the Tamura-Nei nucleotide substitution model which was the best fit model for the data, inferred using the model test function in MEGA 6 (Molecular Evolutionary Genetic Analysis) software [39, 40]. A total of 1000 bootstrap replicates were used to test the reliability of phylogenetic relationships inferred in the tree methods [41]. *B. glabrata* from Egypt was used as the outgroup. Additional published *Biomphalaria pfeifferi* sequence data from Senegal, Mali, Cameroon, Ethiopia, Sudan, Uganda, Kenya, Madagascar and Zimbabwe available from the GenBank were also included in the analysis (Supplementary Table S1).

Population genetic analysis

The aligned sequences were exported into MEGA 6. The interpopulation and intrapopulation diversity was assessed together with the demography among the *B. pfeifferi* populations using pairwise distance and diversity measures. The level of sequence diversity including the number of haplotypes (H), haplotype diversity (h), nucleotide diversity (π), was analysed in DNaSP V6 using the Juke-Cantor corrrections [42]. Additionally, the data was analysed to produce a haptoype network in PopArt to investigate the relationships between the haplotypes [43].

Test for selection

Tajima's test of neutrality [44] was conducted in MEGA 6 to detect violations of mutation-drift equilibrium caused by selection or changes in population size.

Results

In total, 70 *B. pfeifferi* snails were analysed from 17 water contact sites. After alignment and editing, the query length of the *cox*1 sequence was 526 bp.

Haplotype analysis

Overall, there were four haplotypes with one main haplotype (H1) found across most localities and it appeared most frequently in localities in the Mupfure river representing 41 (58.6%) out of the overall 70 samples analysed. Haplotype 2 (H2) representing 9 (12.9%) of the overall number of samples, was observed only in Mupfure river in three sites within very close proximity. Haplotype 3 (H3) and haplotype 4 (H4) were scattered in all the river systems but being more dominant in Kamoyo river which passes through Nduna community. Although there were four haplotypes, all the phylogenetic tree topologies were consistently showing two well supported main clusters of *B. pfeifferi* from the study site (Figs. 2, 3, 4). One cluster contained three out of the four haplotypes (H1, H2 and H3) clustering closely with the published haplotype, DQ084829, from Chiweshe, Zimbabwe. Although not supported, H3 and DQ084829 branched off from H2, with DQ084829 branching off earlier than H3. H1 was significantly similar to MG780150, a haplotype from Sudan (bootstrap value of 93%). The first cluster also contained subclades of other published data from Senegal, Mali, Cameroon, Ethiopia and Madagascar. These branched off earlier than the Zimbabwe haplotypes. The second cluster contained haplotype 4 (H4) and other published data from Sudan, Uganda, and Kenya. Similar to the pattern in the first cluster, the haplotypes from Sudan in the second cluster (MG780167 and MG780152) were significantly similar to the Zimbabwe haplotype (H4) (bootstrap value of 96%). Within the same cluster there is good support for divergence between H4 and the haplotype from Kenya. The relationships among the haplotypes are also supported by the haplotype network analysis showing closer interrelationships between the main clusters and a more divergent cluster (Fig. 5).

Haplotype diversity and population genetics

The diversity among haplotypes was 0.608 with a variance of 0.003. The overall genetic divergence between the haplotypes was 0.028. The haplotypes were not highly divergent, with 0.004–0.025 pairwise distances (Table 2). The highest divergence was between haplotype 1 and 3. In sites

Fig. 2 Cladogram resulting from the Maximum likelihood analysis of the *cox*1 of *B. pfeifferi* from Madziwa, Zimbabwe in relation to other *B.pfeifferi* data available. *Legend*: The bootstrap values (1000 replicates) are shown next to the branches as a percentage

that had more than one haplotype, the diversity within and between sites was 0.017 and 0.012 respectively. Between the sites, the average evolutionary distance in haplotypes ranged from 0.005 to 0.014 and was 0.004–0.026 within the sites.

The number of samples genotyped for some sites was very low, which would have affected the diversity analysis so we pooled the samples by population. Measures of overall haplotype and nucleotide diversity together with within communities are presented in Table 3. The overall mean genetic diversity within the whole populations was 0.009 (genetic distance ranging from 0.000 to 0.015) while the average diversity among the populations was 0.000 (genetic distance ranging from 0.005 to 0.014). While H1 spread throughout the area, H2 was locally restricted in only three geographically close sites in Mupfure community. Kaziro community had complete homogeneity. Although the sampling was biased towards Mupfure River, which had the highest number of water contact sites in the area, the river also had the highest number of unique haplotypes compared to the other river systems. However, the highest diversity among the haplotypes was recorded in Chihuri.

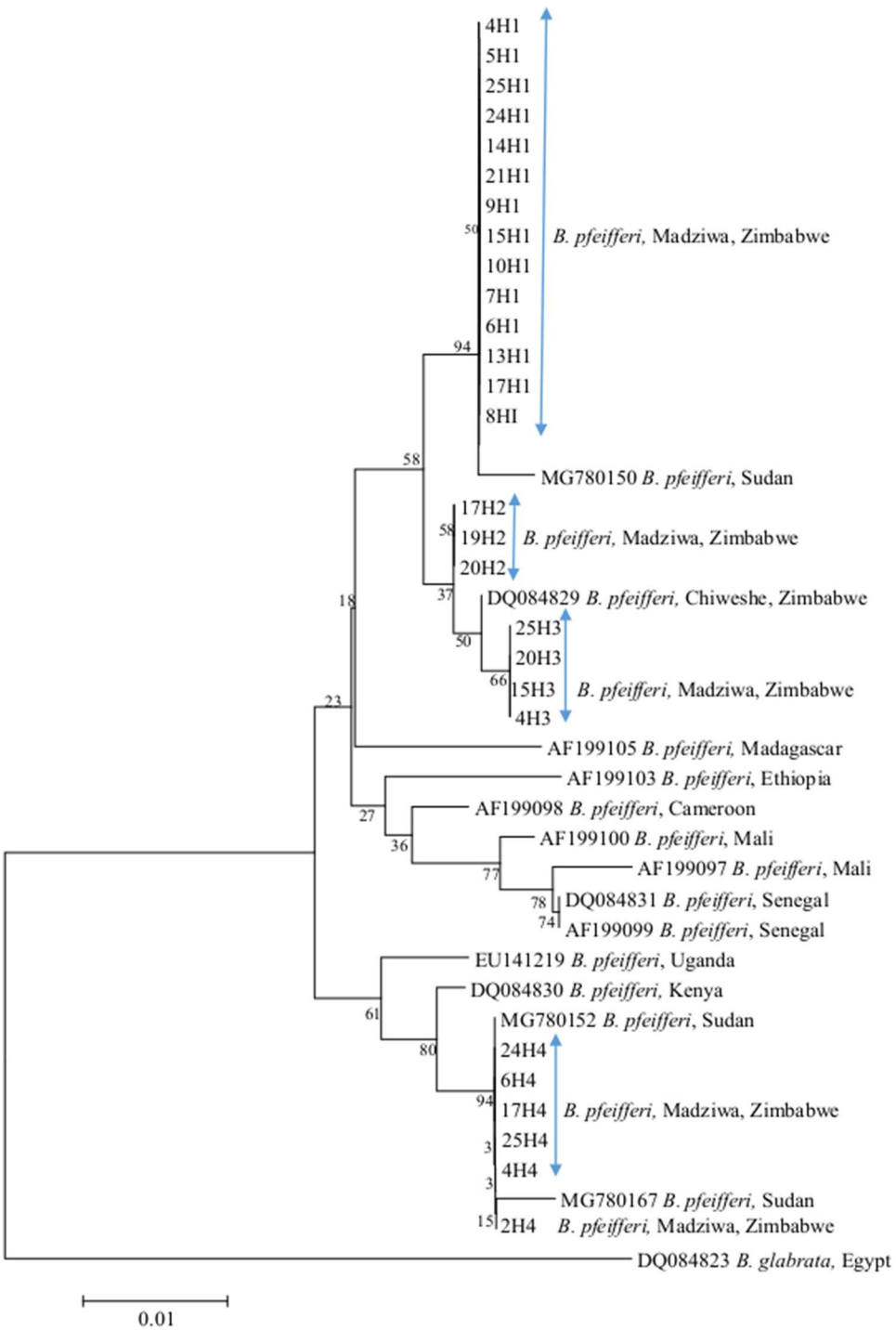

Fig. 3 Cladogram resulting from the Neighbor-Joining analysis of the *cox*1 of *B. pfeifferi* from Madziwa, Zimbabwe in relation to other *B.pfeifferi* data available. *Legend*: The bootstrap values (1000 replicates) are shown next to the branches as a percentage

Neutrality test

Tajima's D and Fu's F statistic showed that there was no strong selection occurring in *B. pfeifferi* ($P > 0.10$) overall and within populations.

Genetic divergence and genetic differentiation

The total number of polymorphic sites was 14. Table 4 describes genetic differentiation among populations. Pairwise F_{st} ranged from -0.130 to 0.540. Average number

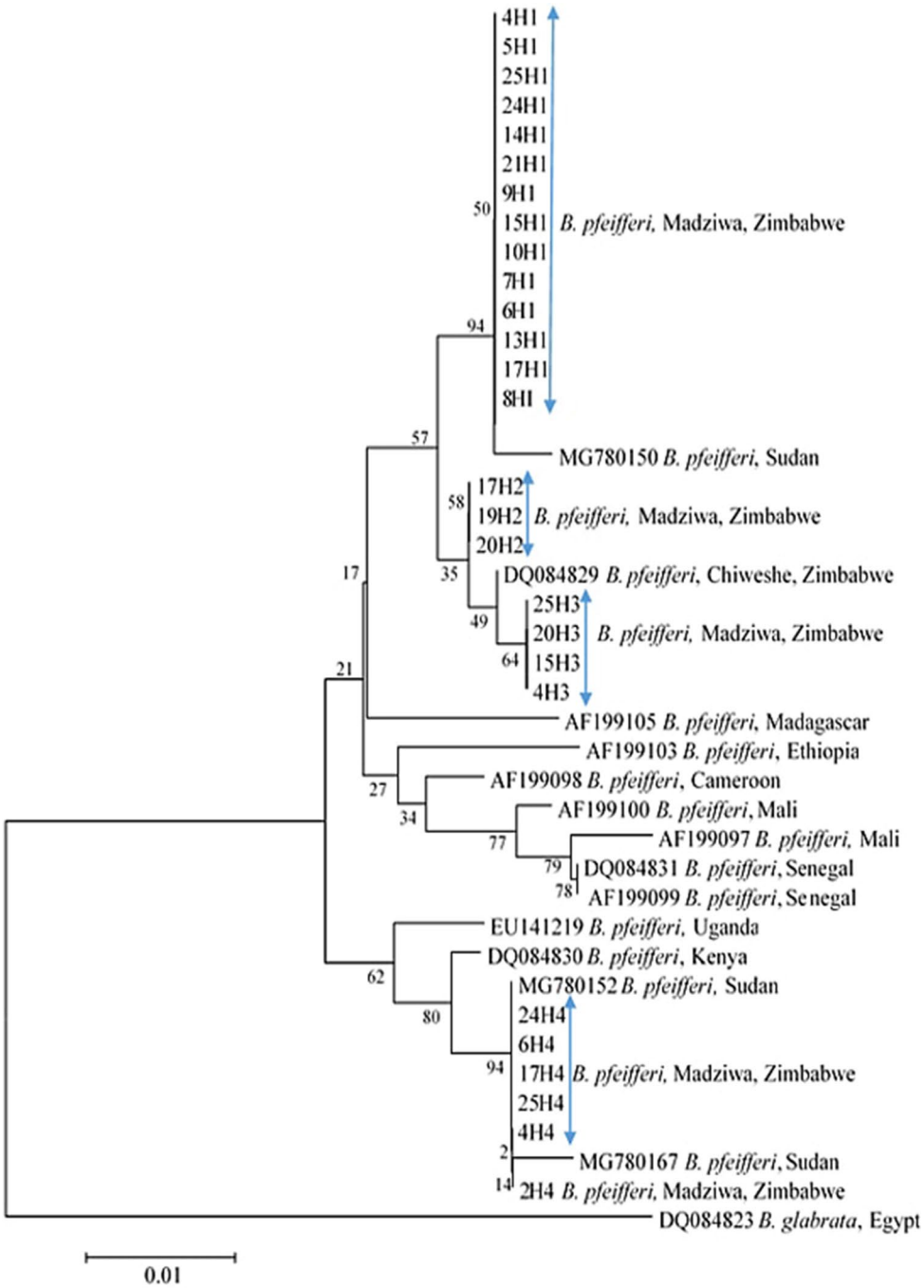

Fig. 4 Cladogram resulting from the Minimum Evolution analysis of the *cox*1 of *B. pfeifferi* from Madziwa, Zimbabwe in relation to other *B.pfeifferi* data available

of pairwise nucleotide differences was 4.537. Nucleotide divergence between populations were as follows; Nduna and Chihuri = 0.012, Nduna and Kaziro = 0.013, Nduna and Mupfure = 0.012, Chihuri and Kaziro = 0.011, Chihuri and Mupfure = 0.011, Mupfure and Kaziro = 0.005.

The genetic distance among the samples within a single collection site was low ranging from 0.000 to 0.014 while in some sites it could not be calculated. While the net divergence between the populations among the collection sites ranged from 0.000 to 0.026, interpopulation diversity among the sites was 2.147 with a coefficient of evolutionary differentiation of 2.427. There was generally low genetic distance among the samples within the Mupfure River collection sites.

Infection status of *B. pfeifferi species*

Among the *B. pfeifferi* collected and tested for patent infections, 4/542 (0.7%), were shedding fork-tailed schistosome cercariae (Table 1). The infected snails were only

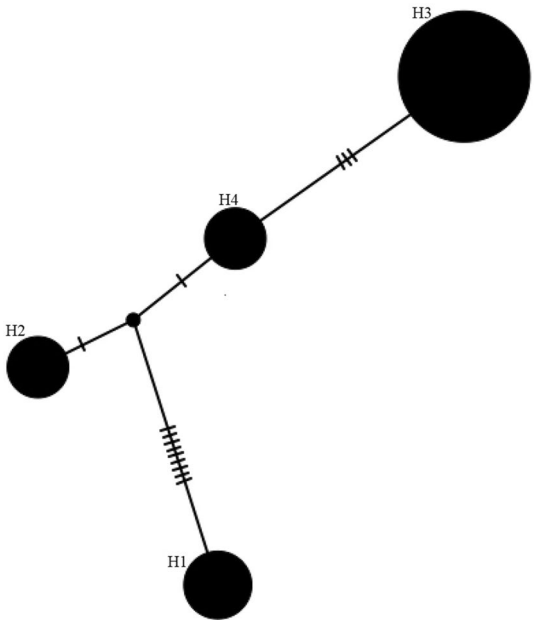

Fig. 5 Minimum spanning network inferred from the *B. pfeifferi* mitochondrial cox1 from Madziwa, Zimbabwe

Table 2 Mean Pair wise distance analysis among the haplotypes

Haplotype ID	1	2	3	4
1	–			
2	0.020			
3	0.025	0.009		
4	0.020	0.004	0.006	–

observed at 6 months follow up (September 2018-summer). None of the *B. pfeifferi* were shedding cercariae at baseline (February-rainy season), three (June-winter season), nine (November-early rainy season), or 12 (February-rainy season) months follow-up. The infected snails were observed at only one site. All the infected snails observed belonged to one haplotype (H2).

Discussion

From the seventy *B. pfeifferi* samples analysed, four haplotypes were found adding to previous studies where only one haplotype was found [17].

Our findings support the divergence between the Zimbabwean *B. pfeifferi* with those from Kenya. Although not supported by significant bootstrap values, the results also show divergence between Zimbabwean *B. pfeifferi* with the same species from Mali, Cameroon, Ethiopia, Uganda Senegal and Madagascar *B. pfeifferi* and other *B. pfeifferi* in Africa. The variation of the observed haplotypes in the current study to the data documented by Jørgensen and others [17] and the variation observed among the *B. pfeifferi* in the African region suggests that there may be more diversity still to be described in Zimbabwe or sub-Saharan Africa [5]. Such diversity should be investigated in relation to intestinal schistosomiasis transmission. Accordingly, malacological surveys in at-risk populations should be combined with genetic diversity studies to identify intraspecific differences in intermediate host snails and relate the differences to transmission patterns in the locality.

Despite that the main objective for intermediate host snail collection was to determine their spatial and temporal distribution in the study area (data to be published elsewhere);

Table 3 Estimation of diversity and summary statistics of *B. pfeifferi* identified using the *cox*1 marker among communities and water bodies

Collection area	Collection sites	N	H	Hd	± Hd SD	π	± π SD	Θ	Tajima's D	Fu's Fs statistic
Overall	All	70	4	0.608	0.053	0.009	0.001	0.006	1.615	9.869
Community										
Chihuri	1, 2, 3	6	3	0.800	0.122	0.014	0.003	0.012	1.337	3.996
Nduna	16 and 17	11	3	0.691	0.086	0.012	0.002	0.009	1.527	6.396
Mupfure	4, 5, 9–15	41	4	0.577	0.070	0.007	0.002	0.006	0.530	6.475
Kaziro	6–8	12	1	–	–	0.000	–	0.000	n/c	–
Water bodies										
Mupfure River	5–14	52	4	0.489	0.073	0.006	0.001	0.006	0.110	5.967
Nyarukunda River	1	1	1	–	–	–	–	–	–	–
Nyamaruru River	2 and 3	5	3	0.800	0.164	0.013	0.004	0.013	− 0.130	2.879
Kamoyo River	16 and 17	11	3	0.691	0.086	0.012	0.002	0.009	1.527	6.396
Zvisokwe stream	15	1	1		–	–	–	–	–	–

N number of sequences, *H* number of haplotypes, *Hd* haplotype diversity, *π* nucleotide diversity, *Θ* theta per site, ± *Hd SD* ± haplotype diversity standard deviation, ± *π SD* ± nucleotide diversity standard deviation

Table 4 Estimation of fixation index (F_{st}) pairwise population comparisons of genetic differentiation among communities

Community	Mupfure	Chihuri	Kaziro
Chihuri	0.049		
Kaziro	0.231	0.378	
Nduna	0.205	− 0.130	0.540

an opportunity was taken to molecularly characterize 70 of the 542 *B. pfeifferi* snails collected. Besides that the sample size characterised was limited, the low level of diversity observed is expected, given that the mitochondrial *cox*1 genetic marker often shows low intraspecific variation over a small geographical area as covered in this study [45]. On the other hand, regardless of the fact that analysis of few samples per site might distort genetic diversity [46], some studies have generated informative data by analysing two to six snail samples per site [47]. It is recommended to include samples from a wider geographic areas and other genetic markers such as the internal transcribed spacer sequences of the ribosomal DNA [4, 17], mitochondrial 16S [4] that have previously been used for investigating *B. pfeifferi* population genetics. The recent publication of the complete mitochondrial and nuclear genomes of *Biomphalaria* [26] could also facilitate the development of more informative markers for population genetic analysis allowing the interpretation of heterogeneous patterns of schistosomiasis transmission in endemic foci.

At one site there were four snails shedding fork-tailed cercariae. The cercariae had characteristic features similar to that of *S. mansoni*. In the absence of molecular test to confirm the parasite, this is not sufficient evidence of human schistosomiasis transmission. Nevertheless, the infected snails were collected together with other snails not shedding cercarie. The infected snails belonged to one haplotype (H2). This may largely be attributed to the highly focal nature of schistosome transmission, the low vagility of the snail host, and the very limited window of opportunity for detecting patent infection in the current study. From the same site, there were two other different haplotypes, which were not shedding cercarie. Despite that the haplotype was also present in two other close by sites; the presence of one infected haplotype might suggests that there may be a specific haplotype that is more susceptible to infection. Although among the haplotypes observed at the site those infected were the only ones which belonged to this haplotype, it is also highly probable that other snails collected at the site, but were not molecularly characterised belong to the same haplotype. Depending on the ability of the haplotype to survive in the environment for a longer period which will allow development of the parasite to cercarial stage, this may pose a potential risk of high snail host-parasite adaptation that will result in proliferation of *S. mansoni* transmission in the area resulting in an unexpected increase in prevalence of intestinal schistosomiasis. Future studies should test host snail-parasite compatibility to identify the haplotypes with an active role in *S. mansoni* transmission.

Mupfure River had the highest number of haplotypes but the highest diversity was recorded in Chihuri. The samples from Chihuri maybe from different waterways upstream of the sites. Although the waterways upstream are small, they are perhaps serving as stable sources of different populations. However, overall, the highest values of intraspecific divergence within these populations appear to be a consequence of different river systems in the different communities. Expectedly for such a small geographic area as covered in this study, there was generally a low interpopulation diversity among the sites in the Mupfure river system.

The topologies of the tree based methods and the haplotype network analysis showed partitioning of the *B. pfeifferi* samples into four distinct groups with genetic divergence ranging from 0.004–0.025. The low nucleotide diversity and proliferation of H1 as observed here is an indication of a recent bottleneck followed by population expansion.

It should be noted that the species identity of these snail sample has been based on the closest genetic match in relation to previously published data. More sophisticated statistical analysis of these haplotypes should be performed to examine the levels of divergence within the species.

Overall, the haplotypes had no clear geographical clustering except for H2 that was localised in three sites in the Mupfure River. The fact that H2 was found in very few sites in the Mupfure River might be attributed to a bottleneck where only a few individual snails survive and reproduce in a locality. It may also reflect the dispersal of snails by passive migration along the river system, in most cases due to flooding although a systematic variation in environmental conditions cannot be ruled out [48]. The genetic divergence among the populations in two very close sites in the Nduna community, in the Kamoyo stream was relatively high. The sites lie within 100 m from one another. While it is acknowleged that the sample size and geographic area covered is too small to make conclusive remarks, this may also suggests that a local phenomenon, such as human activity affecting habitat suitability, may drive genetic differentiation in these *B. pfeifferi* populations as they try to adapt.

Understanding the intraspecific genetic diversity within schistosomiasis intermediate host snail populations may help us understand schistosome transmission dynamics, predicting epidemiological risk and snail-schistosome relationships [2, 21]. Malacological surveys in at risk populations should be combined with genetic diversity studies of both the intermediate host and the schistosomes to identify intraspecific differences, which may have a bearing on disease transmission in the area.

Conclusion

The cox1 mitochondrial genetic diversity investigated here has enabled us to study the micro-biodiversity of these biomedically important snail hosts. The high occurrence of one haplotype indicates the possibility of a recent bottleneck followed by population expansion. The diversity of the intermediate host snails may help in our understanding of the transmission patterns of human intestinal schistosomiasis in the local area. In light of the presence of susceptible snails in the area, it is also important for the public health managers to advocate for human behaviour change such that their actions are not inadvertently increasing snail population sizes.

Acknowledgements We are grateful to the Madziwa area community leaders, the village heads and the community residents for allowing us to work in the area. We are also grateful to the village health workers from the communities under study for their assistance in data collection. Acknowledgement also goes to Professor Jinhu Chen and Dr Kassergene D. Vera for their support during laboratory analysis of samples.

Author contributions MJM NM and XNZ conceived the study. MJM, NM and CT undertook fieldwork. MJM, NM, and EA performed the laboratory analysis. MJM, BW, JM, TM, FA and NM analysed the data. MJM wrote the initial draft of the manuscript. NM, BW, XNZ, NC, EA, FA, TM and CT reviewed the manuscript. All authors read and approved the final version of the manuscript.

Funding This study was partly funded by the Thrasher Research Fund (12440).

Data availability The datasets analysed during the current study are available from the corresponding author on reasonable request.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interests.

Ethical approval Prior to the commencement of the study, approval was obtained from the community leaders in the area. Ethical clearance of the study was sought from the Joint Research Ethics Committee (JREC) for the University of Zimbabwe, College Of Health Sciences, and the Parirenyatwa Group of Hospitals (JREC/251/16). Further approval was sought from the national ethical review board, the Medical Research Council of Zimbabwe (MRCZ) (MRCZ/A/2149). The communities were sensitized about the nature of the study and study objectives in local language (Shona). All applicable international, national and institutional guidelines for the care and use of animals were followed.

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