



Research



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One Health insights into local transmission of zoonotic *Schistosoma mattheei* in southern Malawi

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Schistosoma mattheei is a zoonotic schistosome species in central and southern Africa and is of increasing public health concern in southern Malawi. To gain insight into its local transmission, we investigated the biology of *Schistosoma mattheei* in southern Malawi, integrating epidemiological, environmental and genetic data within a One Health framework. Cattle, goats, humans and snails were surveyed, with DNA barcoding revealing nine mitochondrial *S. mattheei* haplotypes. Two haplotypes were shared across species, indicating cross-host transmission. Infected snails were detected year-round, with seasonal variation linked to vegetation cover (Normalized Difference Vegetation Index (NDVI)). Praziquantel (40 mg kg⁻¹) treatment in selected cattle herds reduced infection prevalence over 12 weeks. These findings highlight the zoonotic potential of *S. mattheei* and the need for integrated control strategies.

This article is part of the Science+ meeting issue 'Parasite evolution and impact in action: exploring the importance and control of hybrid schistosomes'.

1. Introduction

Schistosomiasis is a waterborne neglected tropical disease present throughout much of sub-Saharan Africa [1]. Urogenital schistosomiasis (UGS), a manifestation of the disease, is predominantly caused by *Schistosoma haematobium*, which utilizes several species within the genus *Bulinus* as intermediate freshwater snail hosts [2,3]. This species of schistosome is of particular research interest of late in being able to form various viable hybrids with other schistosomes that are livestock-associated, such as *Schistosoma bovis* and *Schistosoma mattheei*, with the potential to establish novel zoonotic transmission cycles [1–8]. Despite its known presence in livestock, the latter species has historically been overlooked in public health strategies, especially in central Africa [9], even though it has now been firmly implicated in both female and male genital schistosomiasis in Malawi [9,10].

Hybrid schistosomes are increasingly recognized as a challenge for schistosomiasis control and future efforts in elimination of public health problems (EHPH). These hybrids may exhibit altered host specificity,

increased infectivity and variations in drug sensitivity [11–13]. Recent evidence highlights the risk of hybrid vigour, or heterosis, which may enhance parasite fecundity, expand geographical ranges and complicate control efforts in endemic and non-endemic areas alike [9,12]. The hybridization of *S. haematobium* with livestock-associated species, such as *S. mattheei*, is a critical aspect that requires further investigation, especially in relation to its impact on the genetic diversity of schistosome populations alongside developing any inheritable adaptations that diminish the effectiveness of current tools used in parasite control, e.g. preventive chemotherapy. Adopting One Health approaches that recognize the role of animal reservoirs in sustaining transmission [13] is important, and molecular surveillance of livestock is essential to better understand the zoonotic potentials and to improve future integrated control strategies [14].

With a focus here on *S. mattheei*, a long-known important veterinary parasite [15,16], there is mounting contemporary evidence demonstrating its natural hybridization with *S. haematobium*, with such variants being found in humans [9]. Despite early descriptions in the 1970s and experimental confirmation of its zoonotic potential, *S. mattheei* has long remained underappreciated. The Hybridization in Urogenital Schistosomiasis (HUGS) project recently confirmed its involvement in human genital infections and co-infections, using real-time polymerase chain reaction (PCR) diagnostics in both male and female patients in Malawi [9]. These findings have significant implications for zoonotic spillover and necessitate a broader epidemiological perspective on schistosomiasis epidemiology.

Until recently, formal surveillance of livestock schistosomiasis in Malawi has been limited. However, emerging research has highlighted a significant burden of bovine and caprine schistosomiasis, particularly in southern districts [7,8]. Despite this and hitherto, there has been no molecular population genetic analysis of *S. mattheei* populations although a molecular epidemiological survey conducted in 2022 in Mangochi District revealed a high prevalence of *S. mattheei* infections in cattle, including the first formal molecular identification of *S. haematobium* × *S. mattheei* hybrids and pure *S. haematobium* in faecal samples from several animals [7]. These seminal findings confirmed the zoonotic potential of *S. mattheei* and *S. haematobium* in Malawian cattle. A parallel study of goats from the same region uncovered *S. mattheei* and a pure *S. haematobium* infection in goats' faecal samples, further augmenting the potential for zoonotic transmission [8]. Concurrently, targeted snail surveys have shown that *Bulinus africanus*, in addition to *Bulinus globosus*, can shed *S. mattheei* and its hybrids, underscoring the likely role of intermediate host compatibility in transmission dynamics [8,9,17].

In this study, we adopt the WHO/World Organization for Animal Health (OIE) definition of a One Health approach as 'integrated, unifying efforts to sustainably balance and optimize the health of people, animals and ecosystems'. This perspective frames our investigation into zoonotic schistosomiasis transmission. This study focuses on Chimbende village, a previous sampling site in Malawi, where hybrid schistosomes were found in cattle. We aimed to resample these animals to assess potential seasonal variation in hybrid infections. We also evaluated the feasibility of praziquantel treatment. Building on prior mitochondrial DNA barcoding studies of *Schistosoma mansoni* and *S. haematobium* [18,19], our study applies a similar mitochondrial (mt) cytochrome oxidase subunit I (*cox1*) gene-based barcoding or haplotyping approach to *S. mattheei*. By integrating host species, geographical origin and *cox1* haplotype information into a visual haplotype network, we aim to identify potential zoonotic transmission links.

2. Material and methods

(a) Study design, area and population

The first identification of hybrid schistosomes and *S. haematobium* in local cattle in Chimbende, in Mangochi District, occurred in 2022, prompting further investigation into the region's schistosomiasis burden [7]. The follow-up survey was conducted in June 2024 in Chimbende when a total of 15 herds (labelled 1–15) were examined, and two herds (1 and 10) were selected for praziquantel (40 mg kg⁻¹) treatment based upon initial infection rates (see electronic supplementary material). All 15 herds consisted solely of cattle (*Bos taurus*), owned by separate households. Herd sizes ranged from 4 to 17 animals. Cattle were grazed communally on open pasture and watered at shared streams during the dry season. A single follow-up assessment was conducted in September 2024 to reassess the infection status of animals (figure 1A–G).

To complement disease surveillance in cattle, we monitored intermediate host snail populations at the Mangochi 7 (M7) (S–14.44386°, E35.30623°) sampling site: a large natural stream crossing under a road located near Chimbende village, with a large pool on the northern side. As part of the HUGS project, we conducted systematic surveys of intermediate host snails in the Chimbende region, with quarterly sampling over a 3 year period (2022–2024) (12 inspections in total), to assess both snail abundance and the presence of infected individuals (figure 2). On each visit, two collectors searched for 15 min along a 20 m transect using hand scoops and visual inspection. Collected snails were screened for cercarial shedding within 24 h and then preserved in ethanol. Of note, snails collected in Chimbende were identified as *Bulinus globosus* using molecular DNA barcoding.

(b) Parasitological surveys and praziquantel treatment assessment

Except for herd 1 (*n* = 17), which was larger, all other herds contained 4–9 cattle and were owned by independent livestock keepers from nearby households. In herds 1 and 10, all animals were individually identified using plastic ear tags, and rectal faecal samples were collected from each tagged animal (*n* = 23). This allowed accurate determination of individual infection status and dosing for treatment, monitoring over time. In all other herds (*n* = 14), five faecal samples per herd (total *n* = 75) were randomly collected from fresh deposits on the ground within the holding kraal (fenced sleeping area). As these samples

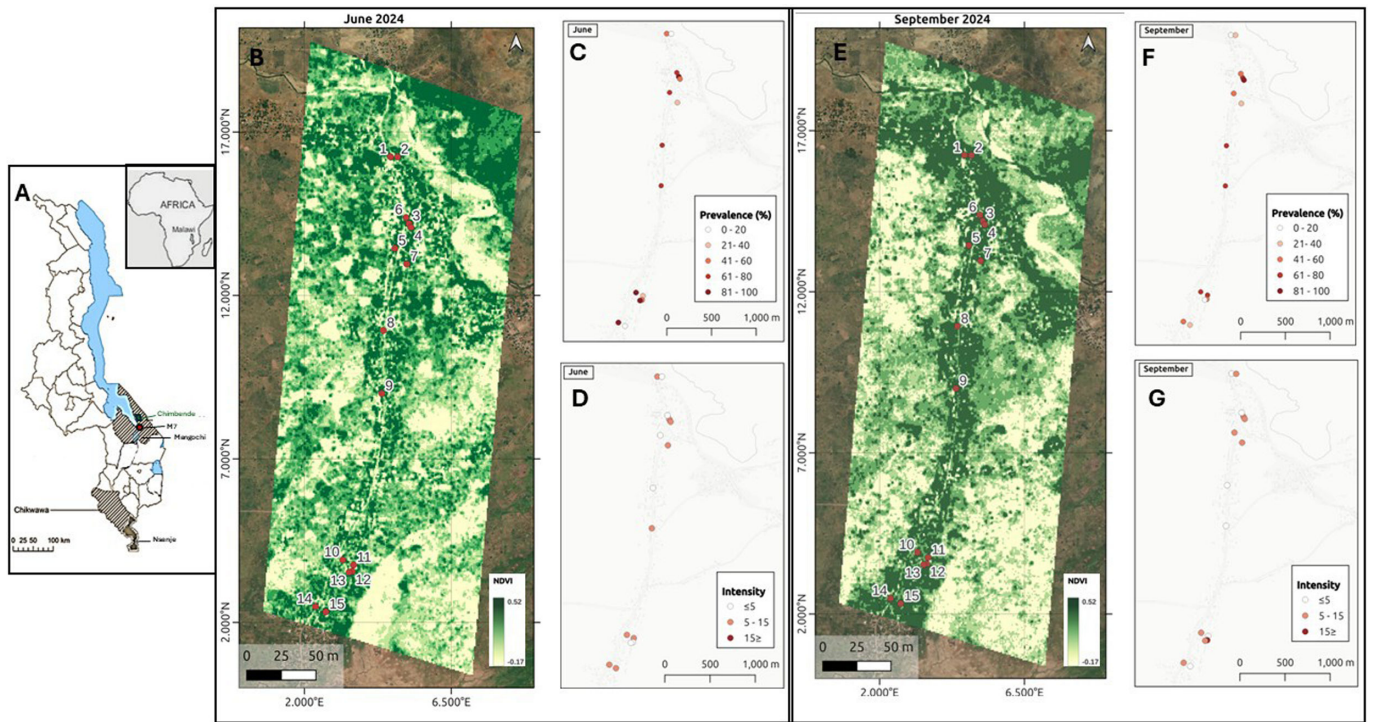


Figure 1. (A) Sampling area. (B) Map of Malawi. (B, E) Maps illustrate vegetation changes in the study area over time using the Normalized Difference Vegetation Index (NDVI). It compares NDVI values from June and September, highlighting variations in vegetation density and health. Higher NDVI values (dark green) indicate denser and healthier vegetation, while lower values (lighter shades) represent sparse or stressed vegetation, or areas with little to no vegetation. This change is attributed to weather conditions rather than human activity. (C, D, F, G) Infection status—prevalence and intensity—defined as the number of hatched miracidia, over 12 weeks in 15 cow herds (1–15) naturally infected with schistosomes.

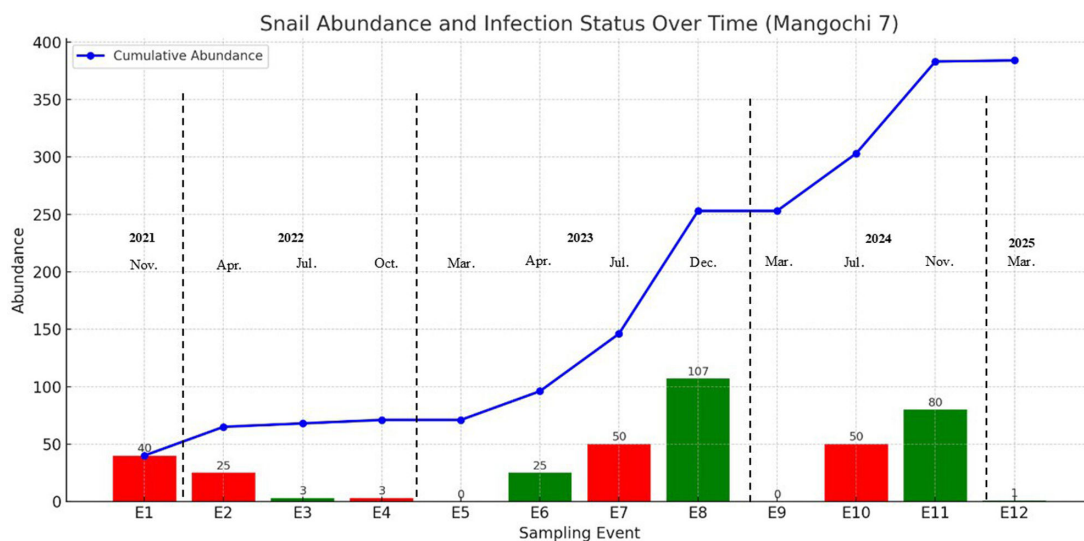


Figure 2. Snail abundance and infection status across 12 surveys at Chimbende. Bars represent the number of snails collected during each sampling event (E1–E12). Bar colour indicates infection status: red bars show the presence of snails infected with *Schistosoma* species, while green bars indicate uninfected snails. Numerical labels above each bar show the total number of snails collected during that event. The blue line represents the cumulative snail abundance over time, illustrating the gradual increase in potential exposure risk and highlighting periods when infected snails were present in the environment.

were not linked to individual animals, repeat sampling from the same animal could not be excluded; this is acknowledged as a limitation in interpreting prevalence. All samples were transported to the laboratory for parasitological and genetic analysis.

To enable individual-level monitoring of treatment efficacy, all tagged animals from Herd 1 ($n = 17$) and three animals from Herd 10 were treated with a single oral dose of praziquantel at 40 mg kg^{-1} body weight (600 mg Biltricide®; IDA Pharmacy). This higher-than-standard dose was selected based on evidence from Juhász *et al.* [8] indicating improved efficacy in bovine schistosomiasis and aligns with veterinary recommendations.

Faecal samples were analysed using a modified miracidium hatching test (MHT) to assess the prevalence and intensity of infection, as described previously [7]. Faecal samples (approx. 15 g) were homogenized in dechlorinated water and filtered sequentially through a metal sieve, a $212 \mu\text{m}$ mesh, and two fine nylon sieves ($65 \mu\text{m}$, $32 \mu\text{m}$). Filtrates were washed into Petri dishes, debris removed and incubated under natural light at approximately 35°C . The presence of miracidia was assessed after 15 min, and the sample was considered positive if miracidia were observed within a 4 min observation period. Infection

intensity was estimated by individually harvesting each observed miracidium using a micropipette and transferring it into 2.5 µl of water on Whatman FTA® cards (GE Healthcare Life Sciences, Amersham, UK), allowing for enumeration.

(c) DNA preparation and molecular analysis

To gain additional insight into schistosome species genetic diversity, real-time PCR high-resolution melting (rtPCR-HRM) assays were performed on all miracidia and cercariae from humans, cows, goats and snails extracted from FTA cards using alkaline DNA extraction. To validate the HRM results, amplification of the mt *cox1* gene was also attempted from the same samples [7,20]. Sanger DNA sequencing was used on a subset of samples identified using the real-time PCR as being samples of interest. These methods have been previously described by Huyse *et al.* [21]; however, in brief, primers targeting a 583 bp region of the *cox1* (Asmit1F: TTTTGGTGCATCCTGAGGTGTAT & SCHR: TAATGCATMGGAAAAAACA) genes of *S. mattheei* were used to generate sequences for further phylogenetic analysis. This analysis formed part of the HUGS project, a 4 year multidisciplinary longitudinal population study seeking to reveal the transmission biology, epidemiological impact and clinical importance of *S. haematobium* hybrids in Malawi. Samples were collected from livestock (cattle and goats), humans and intermediate host snails across three southern districts of Malawi—Mangochi, Chikwawa and Nsanje. The present study compares these archived sequences with newly generated sequences from cattle sampled in Chimbende, Chikwawa and Samama, from goats in Chikwawa and Nsanje, and from snails from Sunbird, Montfort, Chikwawa, Nsanje, Chimbende and Samama, allowing for a broader phylogeographical assessment of haplotype distribution and host association across southern Malawi.

(d) DNA sequencing, *cox1* data and population genetic analysis

Sequences were aligned using MAFFT (Multiple Alignment using Fast Fourier Transform) in Geneious [22]. Trimming was performed conservatively to ensure maximum coverage with minimal sequence loss, ensuring uniform start and end positions across samples. The final alignment was imported into PopART [23] for haplotype network construction. *Cox1* haplotype maps were constructed with TCS v. 1.13 [24]. Two network visualizations were created to explore the relationships among sequences: (i) a host-based network to visualize cross-species sharing, and (ii) a geography-based network to investigate haplotype distribution across different regions.

Prevalence and intensity data were analysed using statistical resampling methods to identify patterns and deviations from expected distributions in Chimbende's herds. Spearman's rank correlation was used to compare prevalence and intensity rankings. To assess vegetation cover and potential habitat suitability for schistosomiasis intermediate host snails, we incorporated the NDVI as an environmental variable. NDVI values were derived from satellite imagery to identify areas with higher vegetation density, which may indicate the presence of water bodies and favourable conditions for snail populations. The NDVI data were processed and analysed using Google Earth Engine [25] using Sentinel image collection (June and September 2024) with values categorized in QGIS 3.3.4 (<https://qgis.org/>) to examine their spatial correlation with schistosomiasis prevalence.

3. Results

(a) Prevalence and intensity of schistosomiasis

The intensity of schistosome infection in cattle varied between the two time points. The data show that the intensity values measured in June (2024) were generally lower, while in some herds ($n = 8/15$) an increase was observed during the second follow-up (in September 2024; figure 1).

The prevalence trends also varied among the herds. During the baseline survey, the distribution of infection showed greater variability, whereas by September, prevalence values appeared to stabilize. The prevalence of infection varied markedly among the herds at baseline, ranging from 20% (herds 2 and 15) to 100% (herds 3, 10, 13, 14). By the 12th week, values appeared to stabilize, with most herds showing intermediate prevalence levels. By contrast, several herds (herds 7, 8, 9, 12) maintained stable prevalence over time. Mean prevalence decreased from 68.0% (95% CI: 56.2–77.9) in June to 57.7% (95% CI: 46.2–68.6) in September, with prevalence distribution exhibiting spatial heterogeneity. The 2nd herd displayed significantly lower prevalence (20%) compared with its adjacent 13th. Herds 3, 10, 13, 14 exhibited absolute prevalence, although intensity levels varied, suggesting differences in exposure or transmission dynamics. Notably, the 1st herd showed a prevalence of 60% but an intensity of only 7.6, indicating a mismatch between prevalence and intensity (figure 1, table 1). Notable deviations included an extreme peak in the 11th herd during the 12 week follow-up, while the herds 13 and 14 maintained consistently higher prevalence levels. Overall, while baseline data demonstrated structured increases in intensity and prevalence, follow-up assessments suggested stabilization, with some locations exhibiting significant deviations indicative of variable disease trajectories (figure 1, table 1).

The comparison reveals a noticeable decline in NDVI values in September, suggesting a reduction in vegetation cover or plant health (figure 1E). This change is attributed to seasonal weather conditions rather than human activity. The NDVI results indicate a potential link between vegetation cover and schistosomiasis prevalence. Areas with higher NDVI values may correspond to the presence of water bodies and dense vegetation, which create suitable habitats for snail populations that serve as intermediate hosts for the parasite. This environmental condition could increase the risk of disease transmission in these regions.

Table 1. Prevalence (%) and intensity of *Schistosoma mattheei* in faecal samples of cows across 15 herds in June and in September 2024.

herd no.	coordinates		cattle		<i>Schistosoma mattheei</i>				
	latitude (S)	longitude (E)	NMHT*	no. positive (June)	intensity (June)	prevalence June (%) [95% CI]	no. positive (Sept)	intensity (Sept)	prevalence Sept (%) [95% CI]
1 ^a	14.40629°	35.31257°	17	3	7.7	60.0 (25.7–86.3)	0	0.0	0.0 (0–26.5)
2	14.40630°	35.31305°	5	1	3.0	20.0 (3.6–62.4)	2	9.5	40.0 (12.2–73.8)
3	14.41077°	35.31382°	5	5	6.0	100.0 (56.6–100.0)	4	9.8	80.0 (38.6–96.4)
4	14.41101°	35.31395°	5	3	9.0	60.0 (25.7–86.3)	5	10.0	100.0 (56.6–100.0)
5	14.41242°	35.31287°	5	4	5.0	80.0 (38.6–96.4)	3	5.3	60.0 (25.7–86.3)
6	14.41035°	35.31362°	5	3	3.0	60.0 (25.7–86.3)	3	3.7	60.0 (25.7–86.3)
7	14.41347°	35.31367°	5	2	4.5	40.0 (12.2–73.8)	2	10.5	40.0 (12.2–73.8)
8	14.41791°	35.31210°	5	4	3.75	80.0 (38.6–96.4)	4	4.3	80.0 (38.6–96.4)
9	14.42213°	35.31199°	5	4	6.5	80.0 (38.6–96.4)	4	4.3	80.0 (38.6–96.4)
10 ^a	14.43326°	35.30938°	5	5	14.2	100.0 (56.6–100.0)	2	14.0	66.7 (20.8–93.9)
11	14.43360°	35.31010°	5	3	8.67	60.0 (25.7–86.3)	4	2.3	80.0 (38.6–96.4)
12	14.43403°	35.31001°	5	3	5.0	60.0 (25.7–86.3)	3	24.0	60.0 (25.7–86.3)
13	14.43409°	35.30980°	5	5	4.0	100.0 (56.6–100.0)	1	13.0	20.0 (3.6–62.4)
14	14.43638°	35.30755°	5	5	9.6	100.0 (56.6–100.0)	3	6.3	60.0 (25.7–86.3)
15	14.43674°	35.30824°	5	1	10.0	20.0 (3.6–62.4)	2	3.0	40.0 (12.2–73.8)
Total			75	51	6.4	68.0 (56.2–77.9)	44	7.2	57.7 (46.2–68.6)

^aHerds receiving praziquantel treatment (herds 1 and 10). *Number of cattle sampled by miracidial hatching technique.

Treatment was administered to the herds 1 and 10, selected based on alignment with overall prevalence trends. Statistical resampling confirmed focal transmission patterns, with certain sites consistently deviating from expected distributions. Intensity appeared to correlate with exposure, although variations warrant further investigation. Analysis of the MHT data revealed a marked reduction in both prevalence and intensity in treated herds 1 and 10. In contrast, untreated herds exhibited variable levels of persistent infection, suggesting ongoing transmission (figure 1, table 1).

Snail monitoring in Chimbende revealed variable abundance and infection status across sampling periods, with cumulative abundance increasing markedly over time (figure 2). Cumulative abundance integrates data from all sampling periods, allowing a clearer assessment of long-term trends and overall population changes, rather than short-term fluctuations that may be influenced by seasonal or stochastic factors.

(b) *Schistosoma mattheei* population genetics and phylogenetical structuring

Melting temperature profile clustering confirmed the presence of *S. mattheei* across sampled populations. Barcoding analyses did not detect hybrid infections, although epidemiological patterns were assessed. Genetic relationships among the samples were investigated using haplotype network analysis, which identified a total of nine haplotypes (C34, C38, H1–H4, M1, S17 and U1). The H5 sample represented a *S. haematobium* reference sequence [GU257335]. Four haplotypes (H1, H2, H3, H4) contained samples from multiple host species, suggesting potential cross-host transmission. For instance, haplotypes H2 and H3 included samples derived from humans, cattle, goats and snails, while H1 primarily comprised samples from cattle, humans and snails. Haplotype H5 included only the *S. haematobium* reference sequence and was genetically distant from the other haplotypes, as indicated by the multiple hatch marks along the branch in the network.

Goat-derived haplotypes were also observed, though they were more focal and less diverse, probably owing to the lower number of samples and limited geographic sampling. In addition, sequences from Zambia available in GenBank (derived from *S. mattheei* obtained from a baboon [GQ375561], a mouse [AY157211] and an unknown host, presumed to be a cow [AJ519518]) were included as reference data and these formed a distinct cluster (figure 3). The geographically colour-coded network further illustrates the spatial distribution of genetic diversity. Samples from Chikwawa, Chimbende and Samama were found across several haplotypes (H1–H4), while samples from Nsanje were mostly associated with haplotypes H2 and H4 (figure 4). These findings indicate substantial genetic diversity within *S. mattheei* populations and suggest that certain haplotypes may be capable of infecting multiple host species. Additionally, the observed geographical patterns point to both local and regional transmission dynamics.

The geographically stratified haplotype network (figure 4) revealed distinct spatial structuring of *S. mattheei* genetic diversity across the surveyed regions in southern Malawi. Multiple haplotypes (H1–H4) were observed among samples collected from

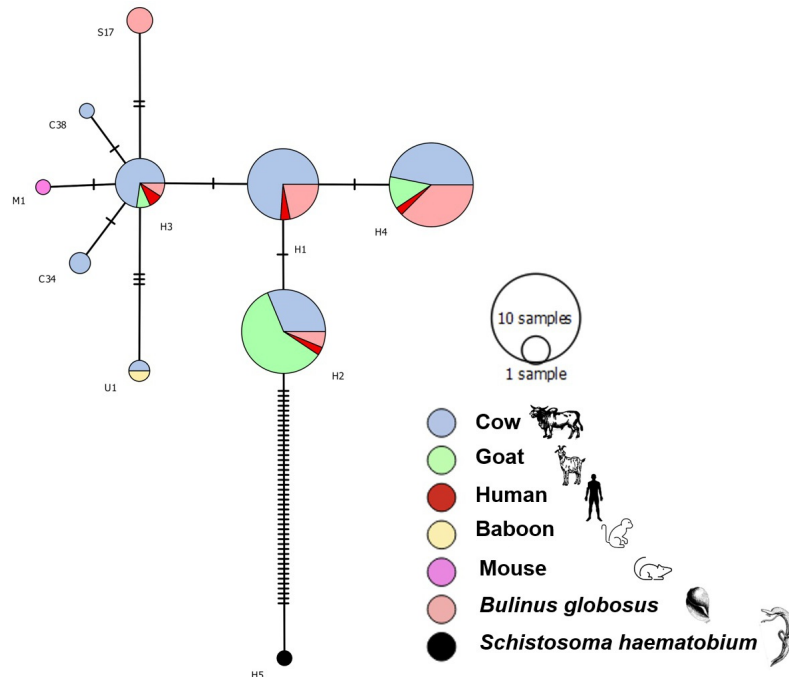


Figure 3. Haplotype network analysis. Median-joining network based on a fragment of the mitochondrial *cox1* gene. Black lines on the branches indicate the number of mutational changes between two different haplotypes. Haplotype colours correspond to the host species, as annotated in the legend, illustrating cross-species sharing of some haplotypes. Haplotype H5 samples from *Schistosoma haematobium* are included as a reference point.

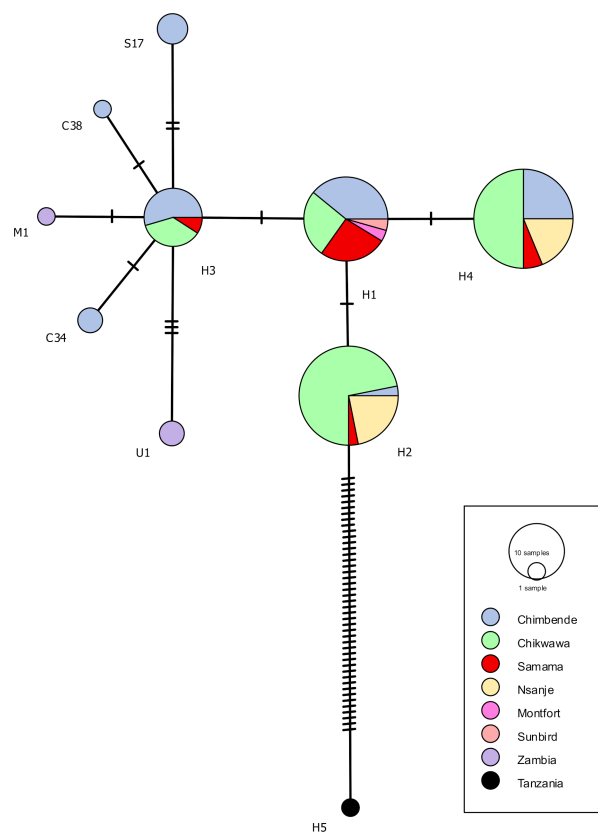


Figure 4. Haplotype *cox1* network showing relationships among *S. mattheei* samples from different geographical locations. The median-joining network represents genetic relationships among mitochondrial haplotypes detected in the study populations. Circle sizes are proportional to the number of samples sharing each haplotype, while colours within circles indicate the sampling locations (see key). Each line between haplotypes represents a mutational step, with black lines on the branches denoting the number of mutations separating them. Haplotype H5, which is genetically distinct and represented solely by samples from *S. haematobium* in Tanzania, is included as a reference point.

Chikwawa, Chikwawa and Samama, indicating substantial intra- and inter-regional genetic heterogeneity. By contrast, samples from Nsanje exhibited a narrower haplotypic range, primarily clustering within haplotypes H2 and H4. These patterns suggest that certain haplotypes are more broadly distributed, while others remain geographically restricted. The broad range of haplotypes found in cattle would also suggest that bovines are the primary host of *S. mattheei* and that other animals become infected through transmission sites associated with cattle.

4. Discussion

This study reinforces the high burden of *S. mattheei* in cattle from Chimbende and its potential role in zoonotic transmission. The results indicate that schistosome infection intensity and prevalence followed different patterns over time. The observed genetic variation indicates dynamic schistosome populations, possibly maintained by multiple transmission sources. DNA 'barcoding' approaches to access levels of population structure and diversity within species are now more frequently being used. The most commonly studied *Schistosoma* spp., *S. mansoni*, has shown high levels of mtDNA diversity within and between populations from endemic areas [26,27]. Variation of the *cox1* mtDNA of schistosome populations is probably not that surprising given that cross-species comparison of mtDNA has shown that the *cox1* gene accumulates a considerable number of nucleotide substitutions [28]. However, Zarowiecki *et al.* [28] concluded that the *cox1* marker is not actually ideal for species identification or population studies, and they propose the *mt* gene's cytochrome oxidase subunit 3 (*cox3*) and NADH dehydrogenase (*nad5*) as better markers for these studies, although accumulation of data across these regions will require future sampling.

(a) Complex *Schistosoma mattheei* dynamics

Our *cox1* haplotype analysis revealed eight haplotypes across samples, with four (H1, H2, H3, H4) shared across multiple host species, including humans, cattle and goats. This strongly supports the hypothesis of ongoing cross-host transmission and the zoonotic potential of *S. mattheei*. In particular, the detection of identical haplotypes in humans and livestock highlights overlapping transmission cycles and challenges the assumption that *S. haematobium* is the sole driver of human UGS in this region. In light of recent clinical findings in Malawi, where *S. mattheei* was identified in both female and male genital schistosomiasis cases, its role in human pathology must be reconsidered and more directly addressed in future diagnostic and surveillance frameworks [9].

The spatial distribution of haplotypes highlights the presence of both localized and regional transmission dynamics within *S. mattheei* populations in southern Malawi. Interestingly, geographical structuring of haplotypes was also evident, with samples from Chikwawa, Chimbende and Samama dispersed across several haplotypes, while Nsanje samples were more spatially restricted. The occurrence of shared haplotypes across districts supports the possibility of parasite movement facilitated by livestock mobility, human water contact behaviour or shared intermediate host habitats [29,30]. In particular, the presence of identical haplotypes (H2, H4) in multiple host species and geographical areas underscores the zoonotic potential of *S. mattheei* and the role of interconnected ecological systems in sustaining transmission.

These findings reinforce the need for regionally coordinated control strategies that go beyond administrative boundaries. A One Health framework—integrating veterinary, environmental and human health perspectives—is essential to address the complexities introduced by cross-host and cross-region parasite gene flow [31]. Furthermore, the observed geographical structuring may reflect differential selective pressures or habitat-specific intermediate snail host compatibility, warranting further investigation.

Although hybrid infections were not identified in this dataset, the inclusion of the *S. haematobium* reference haplotype (H5) underscores the importance of continued molecular surveillance, especially considering prior evidence of hybridization in Malawi [8,32]. Considering the potential for heterosis in hybrids, including increased egg output and pathogenicity, future work should closely examine their fitness and epidemiological impact [9]. Moreover, the co-circulation of multiple haplotypes within single herds or communities may facilitate genetic recombination or introgression in the future [33,34].

The effectiveness of praziquantel treatment was confirmed, as treated herds showed only two residual or reinfections in the investigated three month period. However, untreated herds exhibited persistent infections, emphasizing the need for sustained treatment programmes and improved environmental management strategies [7,8,35]. Nevertheless, recent evaluations have indicated that reinfection can occur rapidly post-treatment, and current strategies remain short-term solutions unless complemented by snail control and environmental management [9]. Our findings underscore the importance of intermediate snail host surveillance, as the cumulative snail abundance and detection of infected snails across multiple sampling events indicate sustained transmission potential in the Chimbende region. Moreover, previous molecular xenomonitoring has confirmed that *B. africanus*, previously unappreciated in Lake Malawi, is shedding *S. mattheei* and hybrid forms, raising questions about expanded compatibility ranges and possibly underrecognized transmission hotspots [7,17].

An environmental assessment by remote sensing, inspecting NDVI signatures, demonstrated seasonal variation probably influencing local cattle grazing patterns with altered spatial needs for animal watering, concurrent with fluctuating (infected) snail population densities. These results highlight the importance of incorporating ecological factors into disease risk assessment models and suggest that NDVI monitoring could aid in identifying high-risk areas for targeted control strategies [36–38].

Several studies have investigated the systematics, genetic diversity and structure of *Schistosoma* species by analysis of the *cox1* gene. Globally, partial *cox1* analysis of *S. mansoni* isolates has revealed a geographical separation into five main groups, with the highest diversity observed in East Africa [5,9,26,27,39–41]. By contrast, partial *cox1* data for *S. haematobium* suggest extremely low levels of intra- and interpopulation diversity, with two major groups [26,40,41]. High genetic diversity in a parasite population may contribute to the development of drug-resistant genotypes [42]. As praziquantel remains the cornerstone of schistosomiasis control in endemic areas [43–45], there is a growing need to understand how parasite genetic variation might influence treatment outcomes and resistance development. While *S. mattheei* is known as a zoonotic causative agent, its genetic diversity is poorly characterized, with limited data available in public databases such as GenBank.

Our findings highlight the need for integrated control strategies that involve routine and targeted praziquantel administration at shorter intervals to ensure effective control of schistosomiasis in cattle. Additionally, improved water management

to reduce snail populations is crucial to limit transmission. Enhanced molecular surveillance is also essential to monitor the emergence of potential hybrid schistosomes, which may pose new challenges to control efforts. The high genetic diversity observed among *S. mattheei* isolates aligns with previous studies on schistosome populations [18]. This variation may have important implications for vaccine and drug development, as genetic differences can influence both antigenicity and treatment response [46,47]. Furthermore, the presence of multiple haplotypes suggests that cattle are exposed to diverse parasite populations, potentially owing to multiple transmission sources, including contaminated water bodies. Further genomic studies may help identify specific markers associated with hybrids and potential zoonotic transmission pathways to humans. Only through comprehensive One Health approaches, integrating human, veterinary and ecological data, can schistosomiasis elimination targets be realistically achieved.

5. Conclusion

This study provides new insights into the genetic diversity of *S. mattheei* and demonstrates the short-term effectiveness of treatment in reducing parasite burden. The identification of multiple unique haplotypes suggests significant geographical structuring of parasite populations, which may influence transmission dynamics and host specificity. Our findings reinforce the emerging understanding that *S. mattheei* is not only a veterinary concern but also a relevant human pathogen with demonstrable involvement in genital schistosomiasis. Future research should explore long-term treatment outcomes and transmission dynamics in endemic regions.

Ethics. This study received ethical approvals from The College of Medicine Research Ethics Committee, Malawi (approval no. P.08/21/3381) and the Liverpool School of Tropical Medicine Research Ethics Committee, United Kingdom (approval no. 22-028).

Data accessibility. The datasets supporting this article have been uploaded as part of the Supplementary Material.

Supplementary material is available online [48].

Declaration of AI use. We have not used AI-assisted technologies in creating this article.

Authors' contributions. A.J.: data curation, formal analysis, investigation, methodology, writing—original draft, writing—review and editing; P.M.: formal analysis, investigation, methodology, writing—original draft, writing—review and editing; L.C.: formal analysis, investigation, methodology, software, visualization, writing—original draft, writing—review and editing; C.N.: formal analysis, methodology, software, visualization, writing—review and editing; J.A.: formal analysis, investigation, methodology, writing—original draft, writing—review and editing; S.J.: data curation, formal analysis, investigation, methodology, writing—original draft, writing—review and editing; G.N.: investigation, methodology, writing—original draft, writing—review and editing; P.C.: investigation, methodology, writing—original draft, writing—review and editing; B.M.: investigation, methodology, writing—original draft, writing—review and editing; D.L.: data curation, investigation, methodology, writing—original draft, writing—review and editing; S.A.K.: data curation, writing—review and editing; J.LaC.: writing—original draft, writing—review and editing; A.M.O.: data curation, investigation, writing—original draft, writing—review and editing; J.M.: conceptualization, formal analysis, funding acquisition, investigation, methodology, project administration, supervision, validation, writing—original draft, writing—review and editing; R.S.: conceptualization, data curation, formal analysis, funding acquisition, investigation, methodology, project administration, supervision, visualization, writing—original draft, writing—review and editing.

All authors gave final approval for publication and agreed to be held accountable for the work performed therein.

Conflict of interest declaration. We declare we have no competing interests.

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References

- World Health Organization. 2020 *Ending the neglect to attain the Sustainable Development Goals: a road map for neglected tropical diseases 2021–2030*. Geneva, Switzerland: World Health Organization.
- Rollinson D *et al.* 2013 Time to set the agenda for schistosomiasis elimination. *Acta Trop.* **128**, 423–440. (doi:10.1016/j.actatropica.2012.04.013)
- Buonfrate D, Ferrari TCA, Adegnik AA, Stothard JR, Gobbi FG. 2025 Human schistosomiasis. *Lancet* **405**, 658–670. (doi:10.1016/S0140-6736(24)02814-9)
- Huyse T, Van den Broeck F, Hellemans B, Volckaert FAM, Polman K. 2009 Hybridisation between the two major African schistosome species of humans. *Int. J. Parasitol.* **39**, 1501–1508. (doi:10.1016/j.ijpara.2013.04.001)
- Webster BL, Diaw OT, Seye MM, Webster JP, Rollinson D. 2013 Introgressive hybridization of *Schistosoma haematobium* group species in Senegal: species barrier break down between ruminant and human schistosomes. *PLoS Neglected Trop. Dis.* **7**, e2110. (doi:10.1371/journal.pntd.0002110)
- Leger E, Webster JP. 2017 Hybridizations within the genus *Schistosoma*: implications for evolution, epidemiology and control. *Parasitology* **144**, 65–80. (doi:10.1017/S0033182016001190)
- Juhász A *et al.* 2024 Revealing caprine schistosomiasis and its One Health importance in Malawi: a molecular epidemiological investigation augmented with a praziquantel treatment and GPS animal tracking pilot sub-study. *One Health* **19**, 100918. (doi:10.1016/j.onehlt.2024.100918)
- Juhász A *et al.* 2024 Revealing bovine schistosomiasis in Malawi: connecting human and hybrid schistosomes within cattle. *One Health* **19**, 100761. (doi:10.1016/j.onehlt.2024.100761)
- Stothard JR, Juhász A, Musaya J. 2025 *Schistosoma mattheei* and zoonotic schistosomiasis. *Trends Parasitol.* **41**, 87–90. (doi:10.1016/j.pt.2024.12.008)
- Kayuni SA, Musaya J, Stothard JR. 2024 Highlighting male genital schistosomiasis in Malawi. *Trends Parasitol.* **40**, 546–548. (doi:10.1016/j.pt.2024.05.003)
- Steinauer ML *et al.* 2008 Introgressive hybridization of human and rodent schistosome parasites in western Kenya. *Mol. Ecol.* **17**, 5062–5074. (doi:10.1111/j.1365-294x.2008.03957.x)

12. Mathieu-Bagné E, Kincaid-Smith J, Chaparro C, Allienne JF, Rey O, Boissier J, Toulza E. 2024 *Schistosoma haematobium* and *Schistosoma bovis* first generation hybrids undergo gene expressions changes consistent with species compatibility and heterosis. *PLoS Neglected Trop. Dis.* **18**, e0012267. (doi:10.1371/journal.pntd.0012267)
13. O'Ferrall AM, Musaya J, Stothard JR, Roberts AP. 2024 Aligning antimicrobial resistance surveillance with schistosomiasis research: an interlinked One Health approach. *Trans. R. Soc. Trop. Med. Hyg.* **118**, 498–504. (doi:10.1093/trstmh/trae035)
14. Boissier J *et al.* 2016 Outbreak of urogenital schistosomiasis in Corsica (France): an epidemiological case study. *Lancet Infect. Dis.* **16**, 971–979. (doi:10.1016/S1473-3099(16)00175-4)
15. Pitchford RJ. 1965 Differences in the egg morphology and certain biological characteristics of some African and Middle Eastern schistosomes, genus *Schistosoma*, with terminal-spined eggs. *Bull. World Health Organ.* **32**, 105–120.
16. Pfukenyi DM, Mukaratirwa S. 2004 A retrospective study of the prevalence and seasonal variation of *Fasciola gigantica* in cattle slaughtered in the major abattoirs of Zimbabwe between 1990 and 1999. *Onderstepoort J. Vet. Res.* **71**, 181–187. (doi:10.4102/ojvr.v71i3.258)
17. Alharbi MH, Irvogga C, Kayuni SA, Cunningham L, LaCourse EJ, Makaula P, Stothard JR. 2022 First molecular identification of *Bulinus africanus* in Lake Malawi implicated in transmitting *Schistosoma* parasites. *Trop. Med. Infect. Dis.* **7**, 195. (doi:10.3390/tropicalmed7080195)
18. Webster BL *et al.* 2013 DNA 'barcoding' of *Schistosoma mansoni* across sub-Saharan Africa supports substantial within locality diversity and geographical separation of genotypes. *Acta Trop.* **128**, 250–260. (doi:10.1016/j.actatropica.2012.08.009)
19. Archer J *et al.* 2024 Molecular epidemiology and population genetics of *Schistosoma mansoni* infecting school-aged children situated along the southern shoreline of Lake Malawi, Malawi. *PLoS Neglected Trop. Dis.* **18**, e0012504. (doi:10.1371/journal.pntd.0012504)
20. Cunningham LJ *et al.* 2024 A rapid DNA screening method using high-resolution melt analysis to detect putative *Schistosoma haematobium* and *Schistosoma mattheei* hybrids alongside other introgressing schistosomes. *Front. Trop. Dis.* **5**, 1350680. (doi:10.3389/ftd.2024.1350680)
21. Huyse T, Webster BL, Geldof S, Stothard JR, Diaw OT, Polman K, Rollinson D. 2009 Bidirectional introgressive hybridization between a cattle and human schistosome species. *PLoS Pathog.* **5**, e1000571. (doi:10.1371/journal.ppat.1000571)
22. Kears M *et al.* 2012 Geneious Basic: An integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics* **28**, 1647–1649. (doi:10.1093/bioinformatics/bts199)
23. Leigh JW, Bryant D. 2015 popart: full-feature software for haplotype network construction. *Methods Ecol. Evol.* **6**, 1110–1116. (doi:10.1111/2041-210X.12410)
24. Clement M, Posada D, Crandall KA. 2000 TCS: a computer program to estimate gene genealogies. *Mol. Ecol.* **9**, 1657–1659. (doi:10.1046/j.1365-294x.2000.01020.x)
25. Gorelick N, Hancher M, Dixon M, Ilyushchenko S, Thau D, Moore R. 2017 Google Earth Engine: Planetary-scale geospatial analysis for everyone. *Remote Sens. Environ.* **202**, 18–27. (doi:10.1016/j.rse.2017.06.031)
26. Morgan JAT *et al.* 2005 Origin and diversification of the human parasite *Schistosoma mansoni*. *Mol. Ecol.* **14**, 3889–3902. (doi:10.1111/j.1365-294X.2005.02709.x)
27. Standley CJ, Kabatereine NB, Lange CN, Lwambo NJS, Stothard JR. 2010. Molecular epidemiology and phylogeography of *Schistosoma mansoni* around Lake Victoria. *Parasitology* **137**, 1937–1949. (doi:10.1017/S0031182010000788)
28. Zarowiecki MZ, Huyse T, Littlewood DTJ. 2007 Making the most of mitochondrial genomes – markers for phylogeny, molecular ecology and barcodes in *Schistosoma* (Platyhelminthes: Digenea). *Int. J. Parasitol.* **37**, 1401–1418. (doi:10.1016/j.ijpara.2007.04.014)
29. Platt RN *et al.* 2022 Genomic analysis of a parasite invasion: colonization of the Americas by the blood fluke *Schistosoma mansoni*. *Mol. Ecol.* **31**, 2242–2263. (doi:10.1111/mec.16395)
30. Reed AT *et al.* 2024 A geospatial analysis of local intermediate snail host distributions provides insight into schistosomiasis risk within under-sampled areas of southern Lake Malawi. *Parasit. Vectors* **17**, 272. (doi:10.21203/rs.3.rs-3949127/v2)
31. Díaz AV, Walker M, Webster JP. 2023 Reaching the World Health Organization elimination targets for schistosomiasis: the importance of a One Health perspective. *Phil. Trans. R. Soc. B* **378**, 20220274. (doi:10.1098/rstb.2022.0274)
32. Lubanga AF, Bwanali AN, Munthali LE, Mphemo M, Chumbi GD, Kangoma M, Matola Y, Kaonga B, Moyo CS. 2024 Exploring the role of community involvement in reducing the burden of schistosomiasis and other neglected tropical diseases in Malawi: where are we in the fight against neglected tropical diseases? *Res. Rep. Trop. Med.* **15**, 51–58. (doi:10.2147/rrtm.s448425)
33. Rey O *et al.* 2021 Diverging patterns of introgression from *Schistosoma bovis* across *S. haematobium* African lineages. *PLoS Pathog.* **17**, e1009313. (doi:10.1371/journal.ppat.1009313)
34. Landeryou T, Rabone M, Allan F, Maddren R, Rollinson D, Webster BL, Tchuem-Tchuente LA, Anderson RM, Emery AM. 2022 Genome-wide insights into adaptive hybridisation across the *Schistosoma haematobium* group in West and Central Africa. *PLoS Neglected Trop. Dis.* **16**, e0010088. (doi:10.1371/journal.pntd.0010088)
35. Gower CM, Vince L, Webster JP. 2017 Should we be treating animal schistosomiasis in Africa? The need for a One Health economic evaluation of schistosomiasis control in people and their livestock. *Trans. R. Soc. Trop. Med. Hyg.* **111**, 244–247. (doi:10.1093/trstmh/txr047)
36. Abdel-Rahman MS, El-Bahy MM, Malone JB, Thompson RA, El Bahy NM. 2001 Geographic information systems as a tool for control program management for schistosomiasis in Egypt. *Acta Trop.* **79**, 49–57. (doi:10.1016/S0001-706X(01)00102-4)
37. Simoonga C, Utzinger J, Brooker S, Vounatsou P, Appleton CC, Stensgaard AS, Olsen A, Kristensen TK. 2009 Remote sensing, geographical information system and spatial analysis for schistosomiasis epidemiology and ecology in Africa. *Parasitology* **136**, 1683–1693. (doi:10.1017/S0031182009006222)
38. Chimfwembe K, Mutesu LM. 2024 Predicting *Schistosoma haematobium* risk distribution in Zambia using geographic information system (GIS) and remote sensing (RS) technologies. *Asian J. Health Res.* **3**, 233–243. (doi:10.55561/ajhr.v3i3.198)
39. Rollinson D, Webster JP, Webster B, Nyakaana S, Jørgensen A, Stothard JR. 2009 Genetic diversity of schistosomes and snails: implications for control. *Parasitology* **136**, 1801–1811. (doi:10.1017/S0031182009990412)
40. Webster BL *et al.* 2012 Genetic diversity within *Schistosoma haematobium*: DNA barcoding reveals two distinct groups. *PLoS Neglected Trop. Dis.* **6**, e1882. (doi:10.1371/journal.pntd.0001882)
41. Betson M, Sousa-Figueiredo JC, Kabatereine NB, Stothard JR. 2013 New insights into the molecular epidemiology and population genetics of *Schistosoma mansoni* in Ugandan pre-school children and mothers. *PLoS Neglected Trop. Dis.* **7**, e2561. (doi:10.1371/journal.pntd.0002561)
42. Webster JP, Gower CM, Norton AJ. 2008 Evolutionary concepts in predicting and evaluating the impact of mass chemotherapy schistosomiasis control programmes on parasites and their hosts. *Evol. Appl.* **1**, 66–83. (doi:10.1111/j.1752-4571.2007.00012.x)
43. World Health Organization. 1993 *The control of schistosomiasis: second report of the WHO expert committee*. Geneva, Switzerland: WHO.
44. World Health Organization. 2013 Schistosomiasis: number of people treated in 2011. *Wkly. Epidemiol. Rec.* **88**, 81–88.

45. Sesay S, Paye J, Bah MS, McCarthy FM, Conteh A, Sonnie M, Hodges MH, Zhang Y. 2014 *Schistosoma mansoni* infection after three years of mass drug administration in Sierra Leone. *Parasites Vectors* **7**, 14. (doi:10.1186/1756-3305-7-14)
46. Parsons DAJ, Walker AJ, Emery AM, Allan F, Lu DB, Webster JP, Lawton SP. 2025 Evolution of antigenic diversity in the zoonotic multi-host parasite *Schistosoma japonicum*: implications for vaccine design. *Int. J. Parasitol.* **55**, 447–460. (doi:10.1016/j.ijpara.2025.04.004)
47. Lund AJ *et al.* 2022 Integrating genomic and epidemiologic data to accelerate progress toward schistosomiasis elimination. *eLife* **11**, e79320. (doi:10.7554/eLife.79320)
48. Juhasz A, Makaula P, Cunningham L, Nkolokosa C, Archer J, Jones S *et al.* 2025 Supplementary material from: One Health insights into local transmission of zoonotic *Schistosoma mattheei* in southern Malawi. Figshare. (doi:10.6084/m9.figshare.c.8186557)