BMC Infectious Diseases

RESEARCH

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Advancing schistosomiasis surveillance: standardization and application of an environmental DNA (eDNA)-based approach for detecting *Schistosoma mansoni* in Brazil

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Abstract

Background *Schistosoma* sp. transmission is linked to water bodies, poor sanitation, and the presence of intermediate hosts. Nevertheless, parasite detection in snails is hampered by challenges in snail sampling and low infection rates, mainly in moderate and low-endemic areas, as well as requiring specialized personnel and being time-consuming. Thus, there is a need to improve tools to assist schistosomiasis surveillance and an environmental DNA (eDNA) approach may help to overcome these limitations. Here, we standardized and used an eDNA-based approach to monitor *Schistosoma mansoni* occurrence in two schistosomiasis endemic areas from Minas Gerais, Brazil.

Methods The eDNA approach was standardized for local conditions by evaluating the specificity of the qPCR assay in detecting the parasite DNA. Water from snail breeding tanks containing *Biomphalaria glabrata*, either infected or not with *S. mansoni*, was used to standardize the eDNA filtration and extraction protocols. Three molecular techniques– Low-Stringency PCR (LS-PCR), Loop-mediated isothermal amplification (LAMP), and quantitative PCR (qPCR)– were applied to investigate samples from snail tanks and two field surveys. Additionally, malacological surveys and measurements of water physicochemical and microbiological parameters were conducted at the same locations to know the species of mollusks present and the ideal environmental conditions to identify hotspots.

Results The qPCR assay was specifically amplified *Schistosoma* sp. DNA without amplifying other trematodes presents in Brazil, ensuring accurate detection without cross-amplification. All three molecular assays efficiently detected *S. mansoni* DNA only from eDNA samples from tanks with infected snails. The eDNA approach, associated with LAMP and qPCR assays, successfully identified *S. mansoni* DNA at the same collection points where snails

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releasing cercariae were found and at one additional site, that was missed by traditional methods, underscoring its sensitivity.

Conclusions This study illustrates the potential of employing eDNA sampling combined with molecular techniques as an effective strategy for monitoring and identifying potential schistosomiasis transmission foci in endemic areas. This approach aligns with the WHO's roadmap for schistosomiasis elimination by 2030 and has implications for public health interventions and control measures.

Keywords Bilharziasis, *Schistosoma mansoni*, environmental surveillance, Quantitative PCR (qPCR), Loop-mediated isothermal amplification (LAMP), Low-Stringency PCR (LS-PCR)

Background

The prevalence of schistosomiasis mansoni, a neglected tropical disease that causes significant economic losses to public health in Brazil and Africa, is directly linked, among other factors, to sewage contamination in bodies of water that serve as breeding sites for *Biomphalaria* snails, intermediate hosts for *Schistosoma mansoni* parasites. Thus, there is a need for improvement in the tools for surveillance of transmission areas to achieve the goal of schistosomiasis elimination by 2030, as targeted in the WHO/NTD road map [1]. However, detecting the transmission foci is challenging due to difficulties in host and parasite species identification and in snail sampling, particularly in the dry season when the population decreases, added to low infection rates, high mortality of infected snails, and the impracticality of parasitological detection in the pre-patent stage. The scenario becomes even more complex, considering that currently, low endemicity areas prevail in Brazil. In this context, individuals often present low parasite burdens, resulting in the excretion of few eggs in the environment. Due to the limited sensitivity of diagnostic methods currently applied, the diagnosis in humans is also challenging [2]. This whole scenario makes it even more difficult to detect the parasite in snails. Consequently, in these predominantly low-endemicity areas, the likelihood of encountering infected snails is even lower, making necessary an increase in the number of specimens collected at each sampling point [3].

Environmental DNA (eDNA) analysis has revolutionized the fields of ecology and environmental science by providing an efficient and non-invasive way to study and monitor ecosystems. This approach has been effectively employed in detecting and quantifying DNA traces from parasites, including protozoans, nematodes, and trematodes, and their hosts, using water, air, or soil samples (reviewed in [4]). eDNA sampling coupled with diverse molecular techniques enables the detection of specific DNA sequences. In this context, Polymerase Chain Reaction (PCR), quantitative PCR (qPCR), and loop-mediated isothermal amplification (LAMP) are being successfully used in detecting parasites, and even parasites and their hosts when multiplexed [5–9].

The monitoring of *S. mansoni* through the detection of traces of the parasite's DNA in eDNA samples has proven to be an efficient tool in identifying active transmission sites of schistosomiasis in Africa, contributing to the monitoring of disease control programs [10–12]. However, Africa presents a whole different scenario, with high endemicity areas, the presence of three *Schistosoma* species and their hosts counterparts. In this study, we used eDNA sampling and compared three methods for detecting *S. mansoni* DNA: a Low-Stringency PCR (LS-PCR) and a LAMP assay [13] both previously employed for parasite detection during snail xenomonitoring, and a qPCR assay used for the eDNA-based monitoring of *S. mansoni* transmission focus in Madagascar [11]. For this purpose, we used water samples from snail breeding tanks containing *Biomphalaria glabrata* infected or not with *S. mansoni* and samples from five water body collections in a schistosomiasis moderate endemic area from a rural District in Northeastern Minas Gerais, Brazil (Fig. 1).

Methods

This study followed a structured approach comprising three main components: (1) standardization of Sm*coi* qPCR reactions, (2) standardization of filtering and eDNA extraction methods, and (3) field validation. An overview of the methodological workflow is provided in Fig. 2 and serves as a guide to the organization of the sections that follow.

Biological samples and DNA extraction

To evaluate the specificity of qPCR reactions, we used DNA samples extracted using the Wizard Genomic DNA Purification kit (Promega) from different species of *Schistosoma* (*Schistosoma curassoni*, *Schistosoma haematobium*, *Schistosoma intercalatum*, *Schistosoma magrebowiei*, *Schistosoma mattheei*, *Schistosoma rodhaini*), and snails (*Biomphalaria amazonica*, *Biomphalaria cousini*, *Biomphalaria glabrata*, *Biomphalaria intermedia*, *Biomphalaria kuhniana*, *Biomphalaria occidentalis*, *Biomphalaria oligoza*, *Biomphalaria peregrina*, *Biomphalaria schrammi*, *Biomphalaria straminea*, *Biomphalaria tenagophila*, *B. tenagophila guaibensis*,

Fig. 1 Map of Brazil **(A)**, Minas Gerais state **(B)**, the municipality of Joaíma **(C)**, Giru district **(D)**, and the municipality of Ribeirão das Neves **(E** and **F)**, showing collection points where malacological surveys and water samples collection were conducted. Maps were generated using the leaflet package (v. 2.2.2) in the R statistical software (v. 4.4.0) (https://www.r-project.org/)

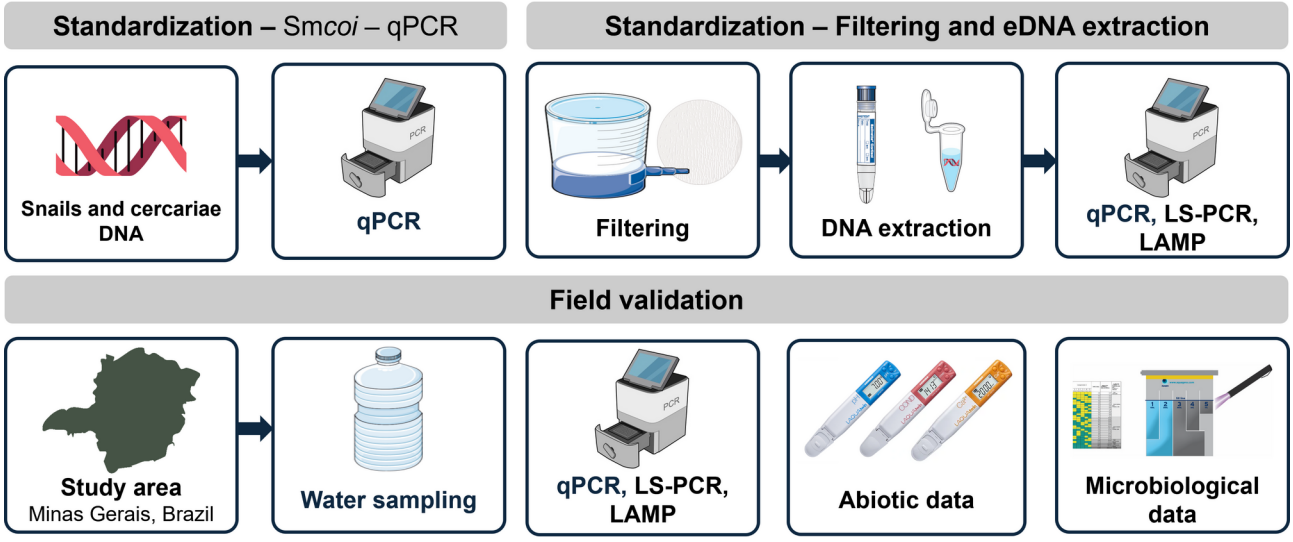

Fig. 2 Study flow diagram illustrating the three key sections of this research: standardization of *Smcoi* qPCR, standardization of filtering and eDNA extraction, and field validation. The image was created using images from Servier Medical Art (licensed under CC BY 4.0) and the NIAID NIH BIOART Source (bioart.niaid.nih.gov/bioart)

Drepanotrema anatinum, *Drepanotrema lucidum*, *Galba cubensis*, *Galba viatrix*, *Galba truncatula*, *Helisoma sp.*, *Physa marmorata*, *Pseudosuccinea columella*). These DNA samples extracted from cercariae and snails were obtained from the Medical and Malacological Collection at Fiocruz Minas (CMM-Fiocruz).

Additionally, alcohol-fixed samples of cercariae from *Clinostomum* sp., *Drepanocephalus* sp., and Spirorchiida were donated by Prof. Hudson Alvez Pinto from the Federal University of Minas Gerais (UFMG). DNA extraction from alcohol-fixed samples, after complete evaporation of alcohol, and from *Schistosoma mansoni* cercaria from the LE strain provided by the "Lobato Paraense" snail rearing facility were conducted following the QIAamp DNA Micro kit (Qiagen). DNA samples were quantified using NanoDrop One (Thermo Scientific) and then stored at -20 °C until used in quantitative PCR (qPCR) assays.

Experimental validation of species-specific primers for quantitative PCR (qPCR) assay

To verify the specificity of the qPCR assays, we used DNA from the above-mentioned trematodes species as a template. The assay amplifies a fragment of the mitochondrial gene cytochrome c oxidase (*coi*) of *S. mansoni* [11]. The reactions containing 10 µL of Taqman Universal Master Mix (Thermo Fisher Scientific), 2 µL of DNA template, 900 nM of each Sma-COI-F (5'-CAGGGGTTTCAAGTCTAATTGGAT-3') and Sma-COI-R (5'-CAAATAATAACATCGTTATTCCTCTGG-3') primers, and 125 nM of the probe Sma-COI-P (5'-FAM-TTCAAATGTTCGATAATA-NFQ-MGB-3') amplifies a 162 bp amplicon. PCR conditions consist of an initial step of 2 min at 50 °C and 10 min at 95 °C, followed by 40 cycles of 15 s at 95 °C and 60 s at 60 °C. All reactions were performed in triplicate in a final volume of 20 µL using a ViiA® 7 Real-Time PCR System or QuantStudio 12 K Flex Real-Time PCR System (Applied Biosystems). Each assay included a negative control, in which DNA/RNA-free water was used as the template. The change in fluorescence intensity (ΔRn) throughout qPCR amplification cycles was used to construct line plots using R [14].

Water sampling from tanks containing *Biomphalaria glabrata* snails

Approximately 250 *B. glabrata* snails challenged with the LE strain of *S. mansoni* were placed in 45-liter tanks at the "Lobato Paraense" snail rearing facility. The aquarium contained approximately 20 L of dechlorinated tap water and was daily subjected to partial water exchange. An air blower was used to add oxygen to the water and snails were fed with lettuce and powdered mice food added with 5% calcium carbonate *ad libitum*. Fifty days after the challenge, 500 mL of water was taken from this aquarium, and part of the water was observed under a microscope, to confirm the cercariae release. Some snails were also individually examined under artificial light for the same purpose. Additionally, we collected water (500 mL) from aquariums containing non-infected *B. glabrata* snails and from dechlorinated and filtrated water supplies to serve as negative control samples.

Filtration and eDNA extraction

Water (500 mL from each tank) was filtrated with glass microfiber filters GF/F 47 mm (Whatman) using a Nalgene® 300–4050 Reusable apparatus (Nalgene) with a vacuum pump. After filtration, approximately 5 mL of 95% ethanol was passed through filters. Filters were air-dried, individually wrapped in aluminum foil and plastic bags, and stored at -20 °C until eDNA extraction. The eDNA extraction from the filters was conducted following the protocol of the QIAamp DNA Mini Blood Kit (Qiagen), with modifications as described by Sato et al. (2018) [11]. The eDNA was quantified using NanoDrop One (Thermo Scientific) and then stored at -20 °C until used in molecular assays.

Low-stringency PCR (LS-PCR)

The PCR reactions included 2 µL of eDNA template and 200 nM of each primer ER (5'-ACCTACCGTACTATGACG-3') and EF (5'-GGTTTCTTAGTGTTATAGCC-3'), targeting adjacent in tandem minisatellite units from mitochondrial DNA. It generates a ladder pattern with fragments differing from each other by 62 bp. PCR cycling conditions consisted of an initial step of 3 min at 95 °C, followed by 35 cycles of 1 min at 40 °C, 1 min at 72 °C and 45 s at 95 °C, and a final step of 45 s at 40 °C and 5 min at 72 °C. All reactions were performed using 1.25 U GoTaq DNA Polymerase (Promega), 1X Colorless GoTaq® Reaction Buffer (Promega), and 0.2 mM dNTPs (Promega) in a final volume of 10 µL using a VeritiPro™ Thermal Cycler (Applied Biosystems) [15]. Each assay included a negative control using DNA/RNA-free water as the template and a positive control using *S. mansoni* DNA as the template. PCR amplification was checked on silver-stained 6% polyacrylamide gels. LS-PCR assay specificities were previously standardized as reported [15].

Loop-mediated isothermal amplification (LAMP)

The LAMP reactions contained 2 µL of eDNA template, 1X Isothermal Amplification Buffer (New England Biolabs), 6 mM $MgSO_4$ (New England Biolabs), 1.4 mM of each dNTP (Invitrogen), 40 nM of each inner primer (FIP– 5'-GCCAAGTAGAGACTACAAACATCTT-TGGGTAAGGTAGAAAATGTTGT-3' / BIP– 5'-AGAAGTGTTTAACTTGATGAAGGGG-AAACAAAACCGAAACCACTA-3'), 5 nM of each outer primer

(F3–5'-TTATCGTCTATAGTACGGYAGG-3' / B3–5'-ATACTTTAACCCCCACCAA), 1 M betaine (Sigma), and 8 U of *Bst* 2.0 WarmStart DNA polymerase (New England Biolabs) [16]. Outer primers target a 206 bp sequence within the mitochondrial minisatellite DNA region. Reaction conditions consisted of 1 h at 65 °C and 5 min at 80 °C [13]. All reactions were performed in a final volume of 25 µL using a VeritiPro™ Thermal Cycler (Applied Biosystems) [13]. Each assay included a negative control using DNA/RNA-free water as the template and a positive control using *S. mansoni* DNA as the template. LAMP amplification was checked on silver-stained 6% polyacrylamide gels and by color change through the addition of 2 µL of SYBR Green I ×1000 (Life Technologies) by naked eye and by exposure to ultraviolet light. LAMP assay specificities were previously standardized as described by Fernandez-Soto and collaborators [13].

Malacological surveys

Malacological surveys covered two areas in Minas Gerais, Brazil, one in the northeast and the other in the center of the state (Figs. 1A and B). Within the Giru district of Joaíma, nine collection points were covered during two surveys (totaling 10 collections, as collection point 4 was sampled in both surveys) (November 2023, May 2024) (Figs. 1C and D). Additionally, three collection points were surveyed in April 2024 in the city of Ribeirão das Neves (Figs. 1E and F).

Snails were collected using a metal scoop and stored in properly identified plastic bags. Geospatial data (latitude and longitude) was retrieved from Google Maps. Snails were then packed in damp gauze and plastic bags and sent to Fiocruz Minas, where, if alive, they were exposed to light for five weeks (one exposition per week) [17]. Dead snails and shells were stored in 75% ethanol. After ethanol evaporation, DNA extraction was performed using the Wizard Genomic DNA Purification Kit (Promega) following the manufacturer's instructions. The extracted DNA was used in assays for the molecular identification of snails of the genus *Biomphalaria* [18] as well as in the molecular examination of infectivity in snails of the genus *Biomphalaria* by LS-PCR to verify the presence of *S. mansoni* DNA [15] (Table 1). The mollusks were exposed to light to check for cercariae shed [19]. Cercariae that were morphologically compatible with *S. mansoni* were identified by LS-PCR [15], as previously described. The other morphotypes were subjected to multiplex PCR for identification at the trematode family level [20]. Multiplex PCR reactions used primers targeting the rDNA-ITS region for the four trematode families in the same reaction: Clinostomidae (115 bp) Clin-4F (5'-GGACTTGCTGGAC-3') and Clin-(5'-4R GACGCACC GTGGC-3'); Echinostomatidae (172 bp) Echi-1 F (5'-GT GCGTCAATGGC-3') and Echin-1R (5'-ATGCAGGAGA ACG-3'); Schistosomatidae (140 bp) Schis-2 F (5'-GCCA TCCCGTAGGCTTGC-3') and Schis-2R (5'-GCGTTGA GCGGTGCTTGG-3'); and Strigeidae (183 bp) Strig-5 F (5'-CCGTTGTACGACAGCTTG-3') and Strig-5R (5'-T TGCGGCTTGGCTTGTCC-3') [20]. All reactions were performed using 0.25 U GoTaq DNA Polymerase (Promega), 1X Colorless GoTaq® Reaction Buffer (Promega), 0.2 mM dNTPs (Promega) and 0.25 nM of each primer in a final volume of 20 µL using a VeritiPro™ Thermal Cycler (Applied Biosystems). PCR cycling conditions consisted of an initial step of 5 min at 94 °C, followed by 30 cycles of 30 s at 62.5 °C, 30 s at 72 °C and 30 s at 94 °C, and a final step of 7 min at 72 °C. PCR amplification was checked on 3% agarose gel stained with ethidium bromide (0.5 µg/mL).

The results of the different methodologies used were kept blind until the end of the study.

Water sampling from the environment

Field water samples were collected in triplicate 10 cm below the water surface using plastic bottles at the same locations where snail collections were conducted. Filtration was performed as described in Sect. 2.4, and the filters were sent to the Laboratory of Helminthology and Medical Malacology at Fiocruz Minas, where eDNA extraction and molecular assays were conducted as described above. A technical field negative control consisting of 500 mL of mineral water following the same procedures was carried out between each collection point, here referred to as a blank sample. LS-PCR assay was used only with the samples from the first survey in Giru. All eDNA field samples were subjected to qPCR and LAMP assays.

LS-PCR and LAMP assays were conducted as described above. For qPCR assays with field eDNA samples, the reactions containing 2 µL of DNA template, 10 µL of Taqman Environmental Master Mix 2.0 (Thermo Fisher Scientific), 0.1 µL of AmpErase Uracil N-Glycosylase (Thermo Fisher Scientific), 900 nM of each Sma-COI-F and Sma-COI-R primers, and 125 nM of the probe Sma-COI-P. PCR conditions consist of an initial step of 2 min at 50 °C and 10 min at 95 °C, followed by 55 cycles of 15 s at 95 °C and 60 s at 60 °C. All reactions were performed in triplicate in a final volume of 20 µL using a ViiA® 7 Real-Time PCR System or QuantStudio 12 K Flex Real-Time PCR System (Applied Biosystems). Each assay included a negative control using DNA/RNA-free water as the template and a positive control using *S. mansoni* DNA as the template. The change in fluorescence intensity (ΔRn) throughout qPCR amplification cycles was used to construct line plots using R [14].

Two other 500 mL samples of water were collected at the same points. One sample was used for analysis of

Table 1 Collection points for water and snail samples in the district of Giru, Joaíma, and the municipality of Ribeirão Das Neves, in Minas Gerais, Brazil, including the abiotic parameters measured, the concentration of *Escherichia coli* and total coliforms in water bodies

Collection point	Geographic coordinates		Taxonomy identification	Parasitological exam	Number of collected snails	Number of positive snails	Water temperature °C	pH	Calcium (mg/L)	Conductivity (µS/cm)	E.coli (CFU 100 mL^{-1})	Total Coliforms (CFU 100 mL^{-1})
Literature*							18 to 31†	5.6 to 9.1†	2.4 to 129†			
								Giru (First survey)				
1	-41.053185	-16.861399	Dead	-	6	0	NP	NP	NP	NP	NP	NP
2	-41.051812	-16.860166	Dead	-	2	0	NP	NP	NP	NP	NP	NP
3	-41.065900	-16.866780	*B. glabrata*	*S. mansoni*	3	1	NP	NP	NP	NP	NP	NP
4	-41.057031	-16.864323	*B. glabrata*	-	32	0	NP	NP	NP	NP	NP	NP
5	-41.044322	-16.872878	*B. glabrata*	-	3	0	NP	NP	NP	NP	NP	NP
								Giru (Second survey)				
4	-41.05715	-16.864323	*B. glabrata*	-	54	3*	31.97 ± 0.29	6.85 ± 0.07	4.33 ± 0.58	87.33 ± 1.53	$5{,}1 \times 10^2 \pm 7.50$ log	$3.6 \times 10^3 \pm 5.5$ log
6	-41.06278	-16.86698	*B. glabrata*	*S. mansoni*	322	4**	27.77 ± 0.55	7.40 ± 0.20	4.00 ± 0.00	83.67 ± 4.16	$8.2 \times 10^3 \pm 3.0$ log	$10 \times 10^3 \pm 0$ log
7	-41.07110	-16.86609	-	-	0	-	25.60 ± 0.10	7.37 ± 0.15	4.00	24.33 ± 1.53	$5.5 \times 10^2 \pm 7.1$ log	$10 \times 10^3 \pm 0$ log
8	-41.05881	-16.8603	*B. glabrata*	-	6	0	27.63 ± 0.25	6.83 ± 0.06	11.00 ± 1.00	93.33 ± 4.51	$5 \times 10^1 \pm 0.8$ log	$6.8 \times 10^3 \pm 5.4$ log
9	-41.06615	-16.86483	-	-	0	-	30.47 ± 1.20	7.60	7.13 ± 0.06	28.67 ± 2.08	$5{,}2 \times 10^3 \pm 6.7$ log	$6.8 \times 10^3 \pm 5.4$ log
								Ribeirão das Neves				
1	-44.114034	-19.770934	*B. straminea*	-	24	0	29.57 ± 1.07	6.97 ± 0.12	39.33 ± 1.53	108.00 ± 4.36	$5.4 \times 10^2 \pm 4.1$ log	$4{,}3 \times 10^2 \pm 1.1$ log
2	-44.110362	-19.786881	-	-	0	-	35.17 ± 1.23	6.83 ± 0.12	15.33 ± 0.58	62.00 ± 5.57	$4.0 \times 10^4 \pm 2.4$ log	$2.1 \times 10^4 \pm 1.0$ log
3	-44.016439	-19.813047	*B. tenagophila*	-	134	0	33.13 ± 0.85	7.47 ± 0.06	46.00 ± 1.73	437.00 ± 57,58	$2.8 \times 10^4 \pm 1.8$ log	$6.1 \times 10^4 \pm 6.5$ log

NP: Not performed

* Three snails releasing cercariae with a long, single-tailed, eyeless body and elongated shape, identified as belonging to the Echinostomatidae family by Multiplex PCR

** One snail releasing *S. mansoni* cercaria, confirmed by molecular techniques; and three snails releasing cercariae with a long, single-tailed, eyeless body and elongated shape, identified as belonging to the Echinostomatidae family by Multiplex PCR

†Ministério da Saúde, 2008 and ††Brown et al. (1994)

physicochemical parameters, while the other sample was subjected to microbiological analysis.

Water physicochemical parameters

The abiotic parameters water temperature (T), pH, conductivity (Co) and calcium (Ca) were measured to compare the presence/absence of the mollusk and the ideal conditions described in the literature [21], and also to verify if these parameters would interfere in the parasite detection.

Water samples were analyzed in situ. To define the degree of water acidity, pH was measured using LAQUAtwin pH-11 (Horiba); to measure the content of dissolved salts (conductivity) and water temperature, the LAQUAtwin EC-11 (Horiba) was used; and to measure water hardness based on calcium presence, the LAQUAtwin CA-11 (Ca^{2+}) (Horiba) was used. To obtain the values, about 0.12 mL of water was collected from the bottle with the help of a mini plastic pipette provided with the probes and deposited on the sensor to cover it completely. The analyses were carried out in triplicate, washing the sensor with distilled water after each sampling.

Water microbiological parameters

To assess whether the water collection was potentially contaminated by human and/or other mammal feces, an investigation of the abundance of *Escherichia coli* and total coliforms was carried out. These microorganisms, when in high quantities, might indicate that *S. mansoni* is present.

Microbiological analyzes were performed using Aquagenx CBT EC + TC Kit, according to the manufacturer's recommendations. The Compartment Bag Test (CBT) is a microbial water quality field test of simple design consisting of a clear plastic bag with five internal compartments of various volumes to determine a Most Probable Number (MPN) concentration of bacteria. The detection method provides quantitative next-day results for fecal bacterial concentrations between 0 and 100 *E. coli* and total coliforms per 100 mL of water.

To enumerate *E. coli* and total coliforms, each water sample was homogenized, and a 100 mL aliquot intended for analysis was serially diluted 1:10 (w/v) in sterilized water. The chromogenic substrate was added to the second dilution (10^{-2}) and after due homogenization the content was transferred to the compartmentalized bag. The samples were incubated at 37 °C for 21 ± 3 h, and the results were observed under visible light for *E. coli* and under UV light for total coliforms.

Samples in which at least one of the bag compartments showed a green color under visible light and a blue color under fluorescence were considered positive for *E. coli* and total coliforms, respectively. For greater reliability of the results, analyses were performed in triplicate and a negative control (100 mL of sterilized water) was included in each round of experiments.

Results

Species-specific qPCR assay

The specificity of the primers and probe targeting the mitochondrial gene cytochrome c oxidase (*coi*) of *S. mansoni* [11] was assessed using quantitative PCR (qPCR) assays using as a template the genomic DNA from eight trematodes and twenty snail's species. The assay demonstrated specificity to *S. mansoni*, as no amplification was observed for any of the snail or trematode species analyzed, except for *S. haematobium* and *S. intercalatum* (Fig. 3).

Schistosoma mansoni detection in experimentally infected snail aquarium water

The three molecular assays evaluated (LS-PCR, LAMP, and qPCR) proved efficient in amplifying *S. mansoni* DNA from eDNA samples collected from tanks containing infected snails. No unspecific amplifications were observed from eDNA samples from filtered or dechlorinated water supplies, nor from tanks containing non-infected *B. glabrata* (Fig. 4), in any of the molecular techniques employed.

Field trial of *Schistosoma mansoni* detection using malacological surveys

In the district of Giru, during the first survey, snails were found in all five locations, with taxonomic identification of *B. glabrata* specimens using morphology and PCR-RFLP at the collection points 3, 4, and 5 (Table 1). The snails from collection points 1 and 2 died and decomposed during the transportation to the laboratory, making it difficult the identification of the snails, as well as to detect the presence of the parasite using parasitological methods. Additionally, through parasitological examination of snails, *S. mansoni* cercaria release was observed at collection point 3 (Table 1).

During the second survey, snails were found in three locations, with taxonomic identification of *B. glabrata* specimens using morphology and PCR-RFLP at collection points 4, 6, and 8 (Table 1). Additionally, through parasitological examination of snails, *S. mansoni* cercaria was detected at collection point 6 (Table 1). Cercaria from Echinostomatidae was also identified at collection points 4 and 6 using a multiplex PCR protocol [20].

In Ribeirão das Neves, snails were found in all two locations. Using morphology and PCR-RFLP, we taxonomically identified *B. straminea* at collection point 1 and *B. tenagophila* specimens at collection point 3 (Table 1). None of the collected snails released cercaria during parasitological examinations.

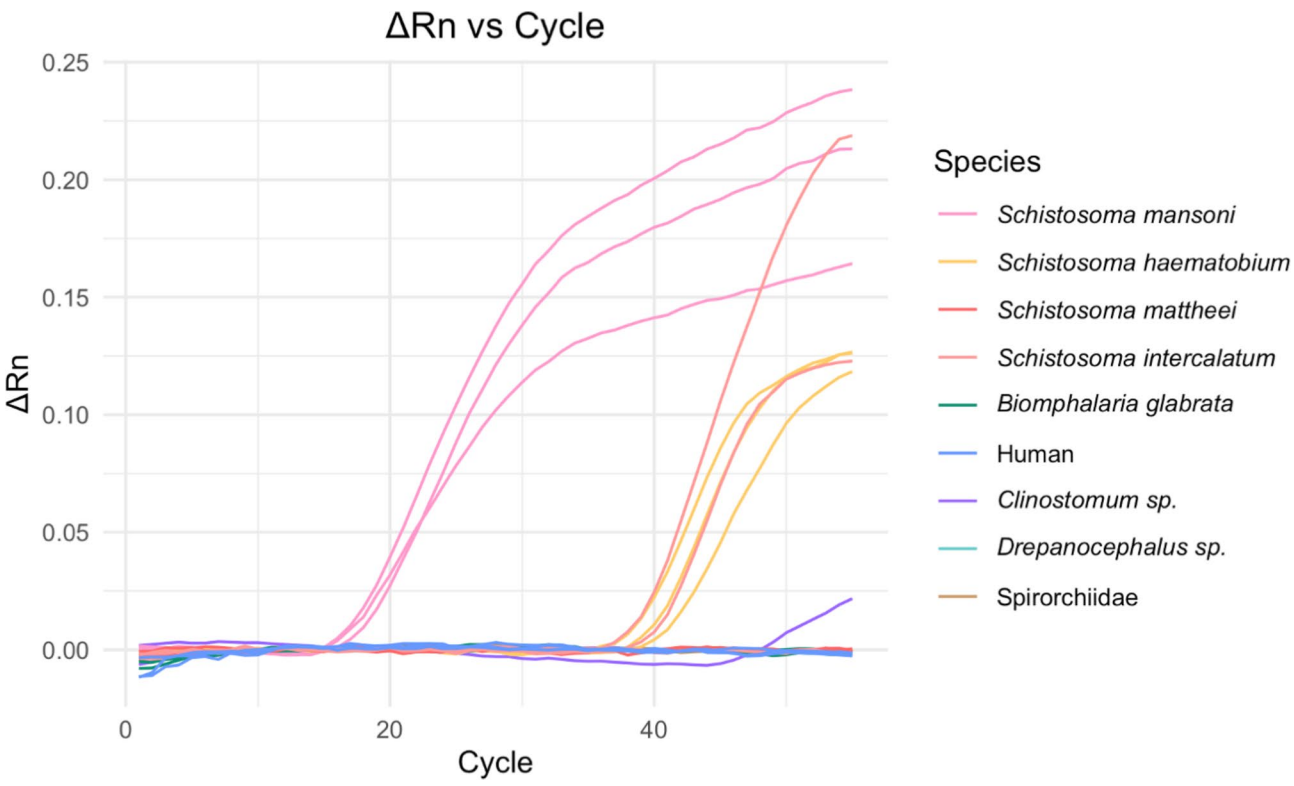

Fig. 3 Specificity of the qPCR assay targeting Sm*coi*. The graph depicts the change in fluorescence intensity (ΔRn) throughout qPCR amplification cycles for *Schistosoma mansoni* (pink), *Schistosoma haematobium* (yellow), *Schistosoma mattheei* (coral), *Schistosoma intercalatum* (salmon), *Biomphalaria glabrata* (green), human (blue), *Clinostomum* sp. (purple), *Drepanocephalus* sp. (sea green), and Spirorchiidae (sand)

Field trial of *Schistosoma mansoni* detection using eDNA samples

Simultaneously and blindly, the eDNA survey was conducted at the same collection points. The eDNA sampling at Giru district revealed the presence of *S. mansoni* DNA traces at collection points 3 and 4 during the first survey, and at collection point 6, during the second survey, as visualized by electrophoresis after LAMP assays (Fig. 5A; Table 2), and only at collection point 3 by color change with SYBR Green I by the naked eye (Fig. 5B; Table 2) or by exposure to ultraviolet light (Fig. 5C; Table 2). qPCR assays enabled parasite DNA detection at collection points 3 and 4 (Fig. 5D; Table 2). However, the LS-PCR assay did not yield any detectable amplification with eDNA samples from the first survey (Supplementary Fig. 1) and was not used in subsequent surveys due to its lower sensitivity.

Additionally, using the eDNA samples from Ribeirão das Neves, neither the LAMP nor the qPCR assays yielded any detectable amplification (Supplementary Fig. 2).

Abiotic data

Probes to measure abiotic data were acquired in 2024. Consequently, the collections carried out this year allowed us to obtain data on water temperature, pH, calcium, and conductivity from water samples collected in Ribeirão das Neves and during the second survey in Giru, Joaíma. The data are shown in Table 1.

Microbiological data

The microbiological test results indicated that all water bodies investigated in this study were positive for the presence of *E.coli* and total coliforms. The concentrations of these coliform bacteria are presented in Table 1.

Discussion

The latest recommendations from the World Health Organization (WHO) and the Oswaldo Cruz Foundation's (Fiocruz) Schistosomiasis Translational Program (FioSchisto) to reduce schistosomiasis transmission indicate the need for parasite detection using highly sensitive and specific diagnostic methods, particularly in low-endemicity areas and in cases where it is necessary to verify the elimination of transmission [1, 3]. Additionally, they recommend the development and validation of new methodologies for diagnosing infection in non-human definitive hosts. Thus, these methods are also essential for advancing schistosomiasis control and elimination.

The guidelines for elimination of schistosomiasis [1] recommend the use of molecular detection of *Schistosoma* only in communities approaching the interruption

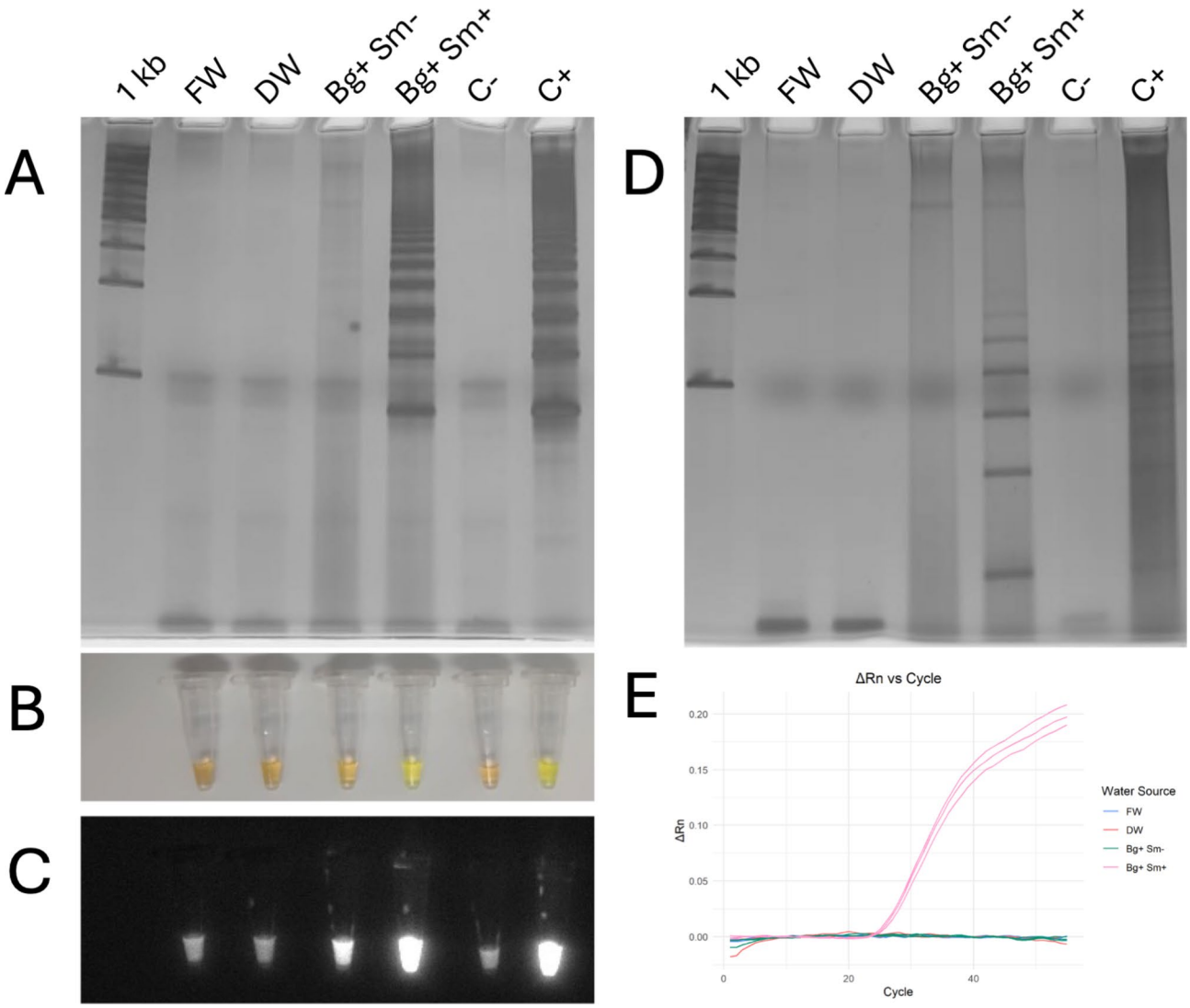

Fig. 4 Molecular assays detected *Schistosoma mansoni* infection using water sampling from aquariums containing *Biomphalaria glabrata* snails. (**A**, **B**, and **C**) Loop-mediated isothermal amplification (LAMP), (**D**) Low-Stringency PCR (LS-PCR), and (**E**) quantitative PCR (qPCR) assays. 100 pb: molecular weight (Promega); FW (blue): eDNA from filtered water; DW (red): eDNA from dechlorinated water; Bg + Sm- (green): eDNA from aquarium containing non-infected *B. glabrata*; Bg + Sm+ (pink): eDNA from aquarium containing *B. glabrata* infected with *S. mansoni*; C-: negative control; C+: positive control

of transmission, defined as no autochthonous human cases for five consecutive years. The guidelines also reinforce that more evidence is needed to support molecular-based diagnostics for detection of *Schistosoma* in snails, and that techniques such as eDNA, LAMP, and qPCR hold considerable promise for sensitivity and specificity. Thus, this work contributes to the recommendation 6, regarding the verification of interruption of transmission. FioSchisto group consider that monitoring mollusks is important for schistosomiasis control efforts [3]. However, there are limitations related to mollusks transport due to Brazilian legislation regarding biologic material. In this scenario, the eDNA is a promising monitoring technique and this work contributes to the evaluation and standardization of this technique and to improve the tools used for public health purposes in Brazil.

Parasitological methods for *S. mansoni* detection in mollusks [17] exhibits limited efficiency and precision in monitoring transmission sites, especially in low-endemicity areas [22]. Among the diagnostic problems highlighted are the difficulty of snail sampling and transportation, the difficulties in detecting the parasite in the pre-patent stage, and the time-consuming nature of these methods, which involve steps performed by specialized and well-trained professionals. Furthermore, there are limitations in parasite detection when snails release few cercariae, requiring specialists to distinguish *S. mansoni* cercariae from those of other trematodes, prolonging the entire process. In Brazil, currently, most areas present

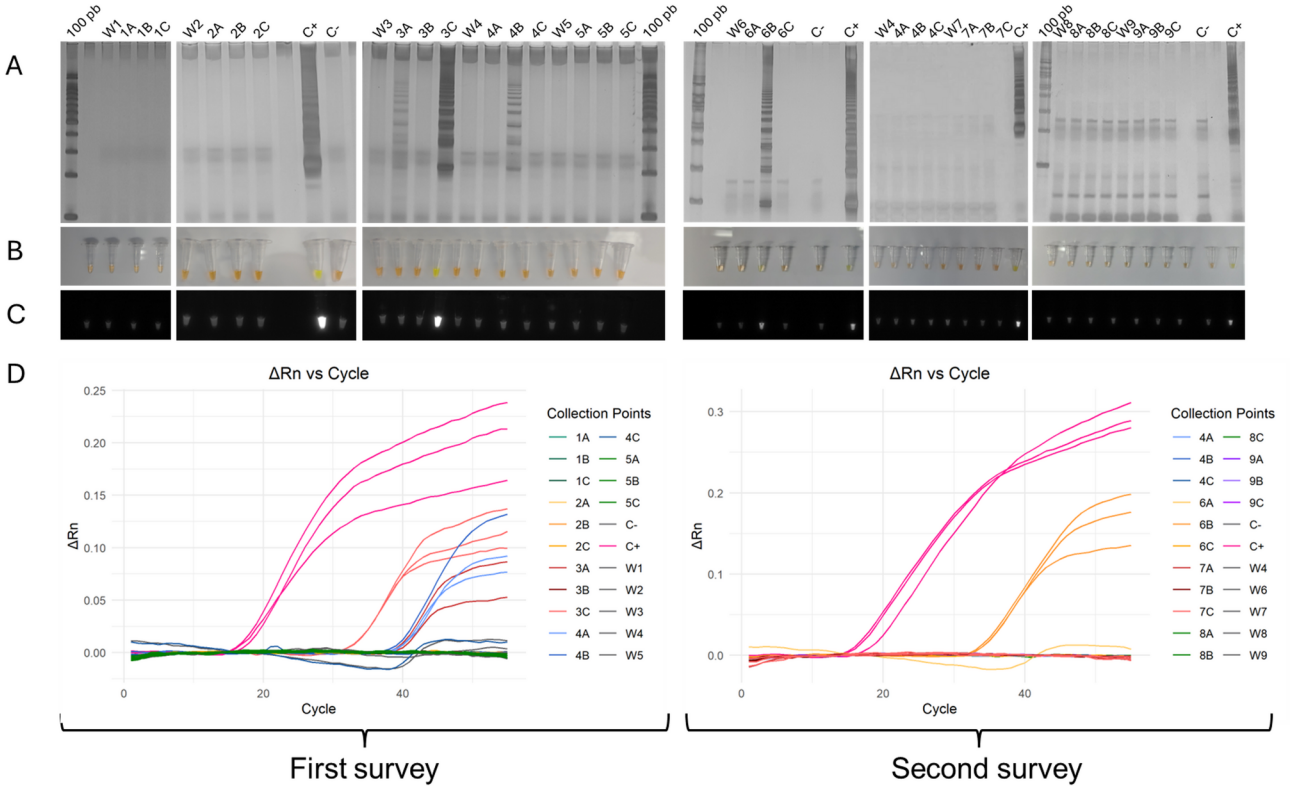

Fig. 5 Loop-mediated isothermal amplification (LAMP) and Quantitative PCR (qPCR) assays to detect *Schistosoma mansoni* detection in Giru district, Joaíma, Minas Gerais, Brazil. LAMP amplifications from collection points were visualized on silver-stained 6% polyacrylamide gels **(A)** and by color change with SYBR Green I by the naked eye **(B)** and by exposure to ultraviolet light **(C)**. 100 pb: marker (Promega); W: blank samples; A, B, C: samples from respective collection points. **(D)** The graph depicts the change in fluorescence intensity (ΔRn) throughout qPCR amplification cycles for all collection points. For all graphs, negative controls and blank samples are shown in gray, and positive controls in pink. Graphs were generated using the ggplot2 package (v. 3.5.1) in the R statistical software (v. 4.4.0) (https://www.r-project.org/)

low disease endemicity making the detection of transmission foci a technical challenge [23]. Therefore, it is necessary to develop and integrate new technologies to expand and enhance the capabilities of detecting schistosomiasis transmission.

The environmental DNA (eDNA) technique has been successfully applied in ecological surveys and served as an efficient method to detect DNA traces from diverse pathogens, thus reducing time and cost (recently reviewed [24, 25]). Xenomonitoring of *S. mansoni* through the detection of parasite DNA traces in eDNA samples has proven to be an efficient tool in identifying active sites of schistosomiasis transmission in Africa, contributing to the monitoring of disease control programs [10–12] and until now, there is no publication showing the use of this technic for schistosomiasis surveillance in Brazil. eDNA sampling offers a simpler alternative to conventional snail sampling, requiring less effort in terms of collection, processing, and transportation. This could enable the sampling of broader geographical areas. Furthermore, other studies have already concluded that eDNA surveys represent a cost reduction compared to traditional survey methods [12, 26, 27]. For Brazil, where sanitation conditions are poor, this methodology can be used to identify transmission areas and notify local health authorities.

In this study, we employed three molecular techniques (LS-PCR, LAMP, and qPCR) to detect *S. mansoni* in field eDNA samples. Primer sets that produce shorter amplicons and target high-copy number genes are advantageous for detecting specific DNA traces in environmental samples since eDNA samples are more prone to degradation caused by environmental conditions [28, 29]. While all three assays investigated in this study yield short amplicons, only LAMP and qPCR successfully detected *S. mansoni* DNA traces in field eDNA samples.

The qPCR assay showed specificity to *S. mansoni*, since, from all trematode species interrogated, only *S. haematobium* and *S. intercalatum* amplified. Although, these two species are not present in Brazil, so not interfering in *S. mansoni* detection, other primers should be considered in the African and Asian settings. Additionally, none of the snail species assayed were amplified, confirming the primers specificity.

The eDNA-based method associated with LAMP or qPCR presented higher efficacy compared to the

Table 2 Collection points for water samples in the district of Giru, Joaíma, and the municipality of Ribeirão Das Neves, in Minas Gerais, Brazil, including the results for molecular assays (LS-PCR, LAMP, and qPCR) used to detect *Schistosoma mansoni* by eDNA sampling

Collection point	Snail shedding*	LS-PCR**	LAMP**	qPCR**	eDNA in water samples***
			Giru (First survey)		
1	0/6	0/3	0/3	0/9	0/3
2	0/2	0/3	0/3	0/9	0/3
3	1/3	0/3	2/3	5/9	2/3
4	0/32	0/3	1/3	3/9	2/3
5	0/3	0/3	0/3	0/9	0/3
			Giru (Second survey)		
4	3¥/54	NP	0/3	0/9	0/3
6	4¥¥/322	NP	1/3	3/9	1/3
7	0/0	NP	0/3	0/9	0/3
8	0/6	NP	0/3	0/9	0/3
9	0/0	NP	0/3	0/9	0/3
			Ribeirão das Neves		
1	0/24	NP	0/3	0/9	0/3
2	0/0	NP	0/3	0/9	0/3
3	0/134	NP	0/3	0/9	0/3

NP: Not performed

*Number of snails shedding some cercariae / number of collected snails at each site

**Number of replicates that amplified *S. mansoni* DNA / number of LS-PCR, LAMP or qPCR reactions

***Number of positive eDNA samples / number of eDNA samples collected (An eDNA sample was considered positive for *S. mansoni* presence if one or more LAMP or qPCR technical replicates amplified *S. mansoni* DNA)

¥Three snails releasing cercariae with a long, single-tailed, eyeless body and elongated shape, identified as belonging to the Echinostomatidae family by Multiplex PCR

¥¥One snail releasing *S. mansoni* cercaria, confirmed by molecular techniques; and three snails releasing cercariae with a long, single-tailed, eyeless body and elongated shape, identified as belonging to the Echinostomatidae family by Multiplex PCR

traditional malacological survey. LAMP and qPCR presented similar sensitivity in this study being effective molecular techniques for detecting DNA traces in the environment. qPCR remains the gold standard for diagnosis purposes due to its reproducibility and quantitative capabilities, enabling precise measurement of DNA levels. It also presents higher accuracy in controlled laboratory conditions and can be multiplexed to detect multiple pathogens at the same time [30, 31]. Conversely, LAMP offers significant advantages in field settings due to its simplicity, rapidity, and minimal equipment requirements, being more cost-effective and user-friendly, particularly in resource-limited settings [32]. Moreover, LAMP has demonstrated greater tolerance to inhibitors compared to qPCR, which is especially beneficial given the often challenging nature of eDNA samples [33, 34].

Abiotic data varied between the water collections of both municipalities of Minas Gerais state, Brazil. The municipality of Ribeirão das Neves belongs to the metropolitan region of Belo Horizonte, whose biome is Cerrado and the Rio das Velhas hydrographic basin, while Joaíma belongs to the Atlantic Forest biome and the Jequitinhonha river basin. Regardless, all measured data were within the expected range in habitats frequented by *Biomphalaria* species [21, 35] showing no interference in the parasite detection in this study. A greater abundance of *Biomphalaria* was found at collection points 4 and 6 of Giru and at collection point 3 of Ribeirão das Neves, demonstrating that only the water temperature parameter seems to have an influence. This finding corroborates data recently published by Aslan et al. (2024) [36], which demonstrated that higher temperatures are more likely to increase the number of these mollusks. These authors draw attention to the impact of climate change on the geography of schistosomiasis.

Temperature is among the most important factors influencing the life-history of living organisms, and ectotherms, which depend on the environment to regulate their body temperature, are more vulnerable to fluctuations in this variable [37]. Snails and the free-living stages of *S. mansoni* are known to be sensitive to temperature. Consequently, water temperature is crucial for the abundance of *Biomphalaria*, making schistosomiasis transmission temperature-dependent [38–41]. Water temperature also impacts the snails' life cycle, including growth rates, reproduction, and resistance to pathogens.

Climate change affects the geographic distribution of these host snails, as rising temperatures may render some suitable regions inhospitable while making new areas favorable. This can lead to the expansion of schistosomiasis into previously unaffected regions, heightening outbreak risks. Additionally, changes in precipitation can impact snail habitats, with dry periods leading to mortality and reduced parasite transmission, while heavy rains create temporary habitats that promote snail proliferation. Ideal temperatures for their reproduction and survival generally range from 20 °C to 30 °C, with temperatures below 15 °C inhibiting reproduction and those above 30 °C increasing mortality, although some snails have been reported to survive in water collections with temperatures as high as 41 °C [1]. It is essential to consider temperature thresholds and seasonal variations in understanding schistosomiasis transmission. Critical thresholds, such as the inability of snails to reproduce below 15 °C or above 30 °C, limit their survival and reproductive success. Seasonal variations influence snail populations, with warmer months supporting growth and

colder or dry periods causing declines, which reduces opportunities for *S. mansoni* transmission. Rainfall also impacts water body availability, affecting *Biomphalaria* habitats. During dry spells, snails may enter estivation, and those emerging in the rainy season exhibit lower infection rates compared to snails in permanent water bodies.

The complexity of the factors related to schistosomiasis transmission makes it difficult to predict climate change effects on this parasitic disease. Examples of such complexity are the relation between strains of the parasite and different *Biomphalaria* species, the limited literature regarding the effect of temperature on free living stages of *S. mansoni*, and the dynamic interplay of environmental factors, such as air and water temperature, rain, winds, and water currents. Moreover, rising temperatures may increase human contact with water, further complicating transmission predictions. Mathematical models has been used to predict schistosomiasis thermal optimum. However, the estimated temperatures lies below those observed in endemic areas in this work and in hyperendemic regions of Africa. A recently developed mathematical model combining the effects of temperature on intermediate hosts, free living stages of *S. mansoni* and human contact rate with water, estimated that the thermal optima for *S. mansoni* transmission ranges from 23.1 to 27.3 ºC [36], which is higher than previous models that estimated transmission risk peaks at 21.7 °C [42].

The detection of coliform bacteria, including *Escherichia coli*, in water samples provides essential information regarding the sanitary conditions of water bodies. These microorganisms are directly associated to the gastrointestinal tracts of humans and warm-blooded animals, making them reliable indicators of fecal contamination [43]. The presence of coliform bacteria, as observed in this study, suggests inadequate sewage management and highlights the extent of fecal waste contamination. This pollution poses serious risks to water resources used for drinking, recreational, and other activities, as it can expose populations to a wide array of pathogenic organisms [44, 45]. In addition to immediate health concerns, fecal contamination has broader environmental and epidemiological implications, particularly in the context of neglected tropical diseases such as schistosomiasis, which is strongly linked to inadequate sanitation. The deposition of fecal waste into aquatic environments inhabited by snails of the genus *Biomphalaria*, significantly heightens the risk of disease transmission. In that regard, monitoring the concentration of coliform bacteria can aid in the evaluation of putative schistosomiasis sites and their probability of becoming the focus, especially in resource-limited settings.

The eDNA method enables the detection of the parasite's DNA at very low concentrations, making it especially effective in identifying new foci of *S. mansoni* in low-endemicity environments. By collecting water samples, this technique facilitates the mapping of the parasite's distribution and tracking its presence over time. Additionally, it can be utilized to monitor how *S. mansoni* responds to environmental changes, such as climate variations and control interventions, offering insights into the dynamics of schistosomiasis transmission. However, further standardization and additional field studies across diverse ecological settings are essential to ensure its reproducibility and reliability of this method. Ongoing research projects combining eDNA analysis with epidemiological and malacological surveys in different epidemiological settings could provide robust evidence for the utility and applicability of eDNA approaches in strengthening schistosomiasis control programs.

In conclusion, this pilot study represents a pioneering attempt in Brazil to apply field eDNA sampling associated with molecular techniques such as LAMP and qPCR as a valid strategy for monitoring schistosomiasis endemic areas in Brazil. It successfully demonstrates the effectiveness of this approach in identifying transmission foci and encourages further studies in broader areas with distinct endemicity statuses.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12879-025-11069-0.

Supplementary Material 1

Supplementary Material 2

Supplementary Material 3

Acknowledgements

The authors thank the "Lobato Paraense" snail-rearing facility of Fiocruz Minas for supplying water and parasites, the Medical and Malacological Collection (CMM) at Fiocruz Minas for providing DNA from cercariae and snails, and Prof. Hudson Alvez Pinto from the Trematodes Biology Laboratory at the Federal University of Minas Gerais (UFMG) for donating cercariae samples. The authors also thank the Program for Technological Development in Tools for Health-PDTIS/FIOCRUZ for granting access to the qPCR facility and acknowledgment is extended to Viviane Santos for her invaluable technical assistance. Our thanks to the municipal health secretariats of the municipalities of Ribeirão das Neves and Giru, district of Joaíma, State of Minas Gerais, for indicating the places frequented by the population and helping catch mollusks.

Author contributions

SGG: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Resources, Data curation, Visualization, Supervision, Project administration, Funding acquisition, Writing– original draft, Writing– review & editing; IRC: Formal analysis, Investigation, Writing– review & editing; MOS: Methodology, Writing– review & editing; MS: Methodology, Writing– review & editing; NMNF: Formal analysis, Investigation, Writing– review & editing; PMP: Resources, Funding acquisition; Writing– review & editing; AAO: Resources; SSFT: Resources; AJL: Resources; OSC: Funding acquisition; Writing– review & editing; LCM: Conceptualization, Methodology, Resources, Data curation, Supervision, Writing– review & editing; MMM: Conceptualization, Methodology, Resources, Data curation, Supervision, Writing– review & editing; RLC: Conceptualization, Methodology, Validation, Resources, Data curation, Supervision, Project administration, Funding acquisition, Writing– review & editing. All authors read and approved the submitted version.

Funding
This research was financially supported by grants from Programa Inova Fiocruz Pesquisa Clínica em Esquistossomose (09/2022–1693113295200930) to RLC and Leo Heller; Inova Fiocruz– Ideias Inovadoras (08/2022 – 1366513295200151) to RLC and PMP; Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG) - Demanda Universal 001/2022 (APQ-01533-22) to RLC and Demanda Universal 001/2024 (APQ-03078-24) to MMM; Fiocruz Minas Research and Innovation Network (001/2023) to RLC; Fiocruz Minas (Young Talents Fellowship) to SGG; and Coordination of Health Surveillance and Reference Laboratories from Fiocruz to OSC. This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior– Brasil (CAPES)– Finance Code 001. MMM is supported by a Research Productivity fellowship from Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq – 17389/2021–1).

Data availability
All data supporting the findings of this study are provided within the manuscript and its supplementary information files. Unedited full-length gel images are available in the Supplementary Material (Supplementary Figure S3).

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Clinical trial
Not applicable.

Competing interests
The authors declare no competing interests.

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Received: 6 February 2025 / Accepted: 30 April 2025
Published online: 15 May 2025

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