# Genetic Profile of *Schistosomes* from Malian hotspot Areas with Urogenital Schistosomiasis

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# Abstract

**Background -** Although schistosomiasis is a public health issue in Mali, little is known about the genetic profile. The purpose of this study was to analyze the genetic profile of the schistosomes of *Schistosoma haematobium* group in school aged children in various sites of Mali.

**Methods** - Urine samples were collected from 07 to 21 November 2021 and subjected to a filtration method for the presence *S. haematobium* eggs. The study took place in two schistosomiasis endemic villages, qualified as hotspots according to the World Health Organization (WHO) definition Fangouné Bamanan and Diakalel in Kayes' region. Molecular biology (Cox1 and ITS2/18S) was used as eggs taxonomic parameters.

**Results** - A total of 789 miracidium were collected individually from 63 school aged children and stored on Whatman Find The Agent (FTA) cards for molecular analysis. After genotyping for species and hybrid identification using rapid diagnostic multiplex mitochondrial Cox1 Polymerase chain reaction (PCR) and Amplification-refractory mutation system -ARMS_PCR analysis of the nuclear Internal Transcribed spacer - ITS2 and 18S regions, 338 (42.8%) and 45) (57.2%) of miracidium revealed *S. bovis and S. haematobium* profiles for Cox1, respectively; 749 (94.9%) and 40 (5.1%) revealed *S. haematobium* and *S. haematobium/S. curassoni* profiles for ITS/18S genes, respectively. There was a significant difference in the Cox1 and ITS2/18S profiles distribution between the two villages ($p<0.0001$). Overall, there was 360 (45.6%) cases of hybrids of which 322 (72.0%) were from Fangouné Bamanan. Three hybrids profiles [(*Sb_ShxSc*) with 2.3%); (*Sb_ShxSh* with 40,9%) ; *(Sh_ShxSc* with 2.8%) and one pure profile [*Sh_ShxSh* with 54.5%) were identified. The hybrid *Sb_ShxSh* appeared to be more frequent in Fangouné Bamanan (68%) whereas *Sh_Sh/Sc* was lightly represented in Diakalel (5.8%).

**Conclusions** - Our findings show for the first time the presence of introgressive hybridization between *S. haematobium* and *S. bovis/S. curassoni* in humans at a large scale. More studies are needed on population genetics of schistosomes at the human and animal interface to evaluate the parasite's gene flow and its consequences on epidemiology of the disease as well as the transmission to humans.


# Introduction

Schistosomiasis is a parasitic disease of medical and veterinary importance that mainly affects tropical and subtropical areas. According to WHO(1), schistosomiasis affects almost 240 million people worldwide, and more than 700 million people live in endemic areas. From the 207 million people infected with schistosomiasis, 85% of them live in Africa. In Mali, the national prevalence of infection with *Schist. haematobium* in 2004–2006 was 38.3%, whereas for *S. mansoni (S. m.)*, prevalence in 2004–2006 was 6.7%. High prevalence of infection with *S. haematobium (S. h.)* is widespread in Mali in all the regions the country, whereas for *S. mansoni*, both surveys indicate small clusters of high infection prevalence in central Mali (Macina and Niono districts in the Office du Niger irrigation area)(2–4). In addition to humans, schistosomiasis also affects livestock. According to a study carried out in Mali(5), the authors found that 70–80% of the zebu cattle of the sahelian region of Mali were infected with the blood fluke *S. bovis*. Among the zebu cattle of the sub-desertic Saharan region, the infection rate by *S. bovis (S. b.)* was about 48%. In the native cattle of the Sudanese regions in the southern part of the country, the prevalence rate for *S. bovis* was

as row as 20%. In Sudan, all cattle examined were adults aged between 6 and 15 years; therefore, the author concluded that the low prevalence could be attributed to animal age (6). However, the findings revealed a high prevalence infection rate of up to 80% of *S. bovis* in older animals in the central delta of the Niger river in Mali (5). It seems, therefore, that the epidemiologic pattern of *S. bovis* is a reflection of local topographical conditions.

Schistosomes are parasites, with a life cycle alternating between a gastropod mollusk and vertebrate hosts. To ensure the establishment of a host-parasite system, one of the conditions is that schistosomes exhibit strong host specificity, particularly for the gastropod, the intermediate host, in which they undergo larval development(7, 8), and which also determines their geographic distribution range (9, 10). Yet, not every encounter between a schistosome and a snail species deemed compatible results in the infection of the latter(11). One of the challenges in establishing the host-parasite system is the presence/absence of the phenomenon of hybridization between schistosome species. Hybridization represents a real concern in terms of parasite transmission, epidemiology and morbidity (12). Natural hybridizations between schistosomes have already been identified between: (i) different human-specific schistosome species (S. *h. x S. m.*) (13, 14) or (*S. h x S. guineensis*)(14); (ii) different animal-specific schistosome species (*S. b. x S. curassoni.*)(15–17) ; (iii) and between human and animal-specific schistosome species (*S. h. x S. b.*)(18–23). As a consequence of this phenomenon, there could be a broadening of the intermediate host spectrum or the possibility of conferring enhanced infectivity on the hybrid parasite (*S. haematobium x S. guineensis*)(24). The study of hybridization involving human parasites has received renewed interest since the widespread use of molecular tools in parasite identification. In Mali, the only case of hybrid described between *S. haematobium* and *S. bovis* was reported in Belgian travelers who stayed at the Dogon plateau one of the most *S. haematobium* endemic area of the country (25).

To better understand the life history traits of the parasite, and the dynamics and epidemiology of the disease in the field, a genetic characterization of *S. haematobium* parasites in Kayes region was undertaken.

In Mali where several mass chemoprevention campaigns with praziquantel (PZQ) have been implemented since 2005 on the basis of the treatment of *S. mansoni* and *S. haematobium* pure strains, hybridization could lead to decrease efficacy of PZQ and require new disease control regimens. In this study, we characterized the genetic profile of *S. haematobium* and its hybrids collected from children living in a schistosomiasis endemic area subjected to several years of Mass Drug Administration (MDA) with PZQ to prevent any possible case to drug resistance.

# Methodology

# Study sites

The study was conducted in two villages endemic to *S. haematobium* in the Kayes region of western Mali. The two study villages (Fangouné Bamanan and Diakalèl), which are 300 km apart, are in Kayes region. They were chosen based on their proximity to water sources (ponds in the Diéma district, the Senegal River and its tributaries in the vicinity of the city of Kayes) (Fig. 1). The Kayes region is characterized by a northern Sudanese climate in the south and a Sahelian climate in the north with two main seasons: the rainy season

(May-June to October) marked by average annual rainfall of up to 1000 mm in the south and 600 to 800 mm in the north, and the dry season which extends from November to April-May(26). The water points created fed by rainwater (ponds and river are excellent snail beds. Agriculture and livestock are the two main economic activities of the population(26) The Sudano-Sahelian climate of the region is indeed favorable to the cultivation of dry cereals (millet, sorghum, maize) and groundnuts, and especially to extensive livestock farming where numerous herds of cattle, sheep and goats cohabit. The practice of these two activities around the same water points creates favorable conditions for the mixing of genes between animal and human schistosomes.

## Type And Period Of Study

We conducted a cross-sectional study of parasitological survey in November 2021. This is a pilot study that will be complemented by the study of the structure and diversity of schistosome populations.

## Parasitological Examination Of Urine

From 07 to 21 November 2021, urine samples were collected from 506 children aged 6–14 years. Urine samples were collected in sterile jars between 9 am and 2 pm. Each child was assigned an identification number based on the first two letters of the village name. The Whatman paper filtration technique was used to test the urine for *S. haematobium* eggs. Once the urine was homogenized in the jar, a 10 mL was taken with a syringe and filtered through a numbered Whatman filter paper (catalogue CAT No. 1001-025, 25 mm) previously placed in a filter holder. The filter was then stained with 3% ninhydrin, dried and rewetted with tap water before to be read under a microscope with an X4 or X10 objective for *S. haematobium* eggs. After reading all filters, part of the urine samples of the 63 heavily infected children (≥ 50eggs/10mL of urine)(27) was used for miracidia capture on FTA (Foam-Tipped Applicators) cards after egg hatching.

## Eggs Hatching And Isolation Of Miracidia On Fta Card For Molecular Analysis

From the selected urine samples of children heavily infected, eggs were recovered by sieving in fresh water (hypotonic solution) for 10–20 minutes to hatch for miracidia release. After eggs hatching, miracidia were collected individually using a P10 micropipette set to a volume of 3 µL and then captured on FTA card (blotting paper). A total of 30 to 35 miracidia were captured per child and deposited on FTA cards and stored at room temperature in a ziplock bag protected from moisture until used for molecular analysis.

## Molecular Analysis

# DNA extraction

Deoxyribonucleic acid (DNA) extraction from miracidia was done by the Chelex method. 3 $mm^2$ disks were cut and removed into Eppendorf tubes (1.5 mL) using a Craft Punch from the adapted FTA® classic cards

where the miracidium was captured. An initial wash step with 100 µL of Milli-Q water was performed while incubating for 10 min at room temperature then the water was removed and replaced with 60 µL of 5% Chelex® 100 molecular biology grade resin solution (Bio-Rad Laboratories, Hercules, CA, USA). The samples were then heated at 65°C for 30min at a stirring speed of 300 rpm, followed by a second heating phase at 99°C for 10 min without stirring. DNA was finally collected by centrifugation at 14,000 rpm for 2 min, and 40 µL of the supernatant (DNA) from each sample was stored at -20°C for further molecular analysis.

## Rapid Diagnostic (Rd) Multiplex Polymerase Chain Reaction

A rapid diagnostic multiplex PCR was used to target the Cox1 mitochondrial DNA (mtDNA) gene. We used the technique of Webster et *al*. (2010)(28), optimized by Van den Broeck et *al*. (2011)(29) and Angora et *al*. (2019)(30). Primers were a universal reverse primer (Shmb.R: 5'-CAA GTA TCA TGA AAY ART ATR TCT AA-3') and three species-specific direct primers for *S. haematobium* (120 bp) Sh. F: 5'-GGT CTC GTG TAT GAG ATC CTA TAG TTT G-3', for *S. bovis* (260 bp) Sb.F: 5'-GTT TAGGTA GTG TAG TTT GGG CTCAC-3' and for *S. mansoni* (215 bp) Sm.F: 5'-CTT TGA TTC GTT AAC TGG AGT G-3'). Each partial COI mtDNA PCR amplification was performed in a total volume of 10 µLl, containing 2 µL of DNA template, 2 µL of GoTaq® Flexy buffer (Promega; Madison, Wisconsin, USA), 0. 6 µL of 25 mM $MgCl_2$, 1 µL of 10× primer mix (4 µL of 100 µM universal reverse primer, 4 µL of each 100 µM forward primer, and 84 µl of milli-Q water), 0.2 µL of dNTP solutions at 10 mM each, 0.2 µL of GoTaq® G2 Hot Start Taq Polymerase (Promega), and 4 µL of milli-Q water. PCR conditions included an initial GoTaq polymerase activation phase at 95°C for 3 minutes, followed by 45 cycles amplification. Each cycle included a DNA denaturation step at 95°C for 10 s, followed by primer annealing at 52°C for 30 s, and an elongation step at 72°C for 10 s. The program ended with a final extension at 72°C for 2 min. PCR products were examined on 2.5% agarose gels at 135 V for 40 min using the 100-bp DNA size marker (Promega) for size estimation.

## Pcr Of Coi Mtdna

Partial amplification of Cox1 mtDNA was performed by PCR using the forward primer Cox1_schist F (5'-TCTTTRGATCATAA GCG-3'), and the reverse primer Cox1_schist-R (5'-TAAT GCATMGGAAAAACA-3'). The PCR conditions used were similar to those described in Savassi et *al*. (2021)(31). This one allowed the PCR products to be sent for sequencing of the Cox1 gene.

# ARMS PCR

The nuclear profile of parasites was diagnosed using ARMS_PCR. Such rapid diagnostic multiplex PCR was used to cibler the ITS/18S of nuclear DNA. We participated to perform this technic (article in submission course). Eight primers were used:

Out reverse OR18S: 5’- GCACCAGACTTGCCCTCCAATTGGTCC – 3’;

Interne Forward IF-ITS: 5’-CGCATATCAACGCGGGTTGCTGGGCA–3’;

Interne reverse IR-ITS: 5’ - TGTGGCCGGACTATTAGGACGGAGCCGTC-3’;

OR-ITS:5’-TCGTGCGTATTACACACACCATCGGTACAAACC-3’;

OF-ITS:5’-GCATGCAAATCCGCCCCGTTATTGTTCCT-3’;

IF18S: 5’ - TGGATAACTTTACTGATCGCAGTCGGACC – 3’;

IR18S: 5’- ATTTGAAAGATCCGTCGCCGACAAAA- 3’ and

OF18S: 5’ - GCATTTATTAGAACAGAACCAAYCGGGCG – 3.

For each partial PCR amplification of nuclear DNA a total volume of 25 µL, containing 3 µL of DNA matrix, 5µL of GoTaq® Flexy buffer (Promega; Madison, Wisconsin, USA), 1.5 µL of 25mM MgCl2, 0.5 µL of mixture of the 8 prediluted primers (12µM outer primers and 15 µM for the inner ones), 0.2 µL of dNTP solutions at 10 mM each, 0.5 µL of GoTaq® G2 Hot Start Taq polymerase (Promega), and 10.75 µL of milli-Q water. PCR conditions included an initial GoTaq polymerase activation phase at 94°C for 1 minute, followed by 28 cycles of amplification. Each cycle included a DNA denaturation step at 94°C for 1 minute, followed by annealing of the primers at 62°C for 45 s and an elongation step at 72°C for 40 s. The program ended with a final extension at 72°C for 6 min. PCR products were examined on 1.8% agarose gels at 135 V for 40 min using the 100-bp DNA size marker (Promega) for size estimation.

## Pcr Its And 18s For Sequencing

We performed by PCR a partial nuclear DNA amplification for ITS and 18S using the direct ITS_schist (OF-ITS: 5’ - TAACAAGGTTTCCGTAGGTGAA – 3’) primer and ITS_schist-R (OR-ITS: 5’ - TGCTTAAGTTCAGCGGGT – 3’) revert primer then the 18S_schist (OF18S: 5’- GCGAATGGCTCATTAAATCAG – 3’) direct pirmer and 18S_schist (OR18S: 5’ - TCAGGCTCCCTCTCCGGA – 3’) revert primer. The PCR conditions were similar to those of ARMS except that hybridization has been done at 40°C for ITS and 44°C for 18S(32).

## Results

Overall, 363 urine samples were examined for *S. haematobium* egg diagnostic (Table 1). The prevalence was 69.2% and it was significantly higher in Diakalèl (78.1%) than in Fangouné Bamanan (53.5%, p *< 0.0001).* From this study, 40 and 23 children were heavily infected in Diakalèl and Bambanan, respectively corresponding to a total of 63 heavily samples selected for egg hatching testing and miracidia capture of FTA cards.

Table 1
Prevalence and intensity of *S. haematobium* (light infection:1-49eggs/10mL urine; heavy infection: ≥50eggs/10mL urine) in the two study sites

| *S. haematobium*/ Sociodemographic Variables | Samples examined | Positive | Prevalence | No light infection | No heavy infection |
|---|---|---|---|---|---|
| Diakalèl | 251 | 196 | 78.1 | 148 | 40 |
| Fangoune Bamanan | 142 | 76 | 53.5 | 61 | 23 |
| Total | 393 | 272 | 69.2 | 209 | 63 |
| No.: Number | | | | | |

# Genotyping Of Schistosomes For Cox1 And Its/18s Genes

Overall, 840 miracidia (Diakalèl, n = 475 and Fangouné Bamanan, n = 365) were genotyped but only 789 miracidia (Diakalèl, n = 447 and Fangouné Bamanan, n = 342) collected from 63 children provided successfully profiles for both Cox1 and ITS/18S. Of these miracidia genotyped, 338 (42.8%) and 451 (57.7%) had *S. bovis* and *S. haematobium* profiles for Cox1, respectively. For ITS/18S genes, two profiles were identified with 749 (94.9%) for *S. haematobium* and 40 (5.1%) for *S. haematobium*/*S. curassoni*. There was a significant difference in the Cox1 et ITS2/18S profiles distribution between the two villages ($p < 0.0001$). Whereas the *S. haematobium*_cox1 frequency was higher to those *S. bovis*_cox1 in Diakalèl ($p < 0,00001$), the ITS2/18S alleles frequencies of *S. haematobium* were greater than 90% in both villages (Table 2).

Table 2
Percentage (in parenthesis) of genes identified by Rapid diagnostic (RD_ PCR) for Cox1 and ARMS_PCR for IT2/18S

| | *Haplotype Cox1* | | | *P* | *Génotype ITS/18S* | | *p* |
|---|---|---|---|---|---|---|---|
| *Village* | *Total* | *S. bovis* | *S. haematobium* | *< 0.0001* | *S. haematobium* | *S. haematobium + S. curassoni* | *< 0.0001* |
| *Diakalèl* | 447 | 18 (4.0) | 127 (85.0) | | 429 (96.0) | 18 (4.0) | |
| *Fangouné Bamanan* | 342 | 320 (93.6) | 324 (30.0) | | 320 (93.6) | 22 (6.4) | |
| *Total* | 789 | 338 (42.8) | 451 (57.2) | | 749 (94.9) | 40 (5.1) | |

Overall, 45.6% (360/789) of hybrids were recorded. The higher hybrid prevalence has been observed in Diakalèl with 72.0%. Four genetic profiles were identified after genotyping miracidium using Cox1 and ITS/18S: *S. bovis* cox1 x *S. haematobium* ITS 2/*S. curassoni*_18S (*Sb_ShxSc); S. bovis*_cox1 x *S. haematobium*_ITS 2/ *S. haematobium* _18S (*Sb_ShxSh); S. haematobium*_cox1 x *S. haematobium*_ITS 2/ *S. curassoni* _18S (*Sh_ShxSc)* and *S. haematobium*_cox1 x *S. haematobium*_ITS 2/ *S. haematobium* _18S (*Sh_ShxSh).* Three hybrid profiles *Sb_ShxSc* (2.3%); *Sb_ShxSh* (40.9%); *Sh_ShxSc* (2.8%) and one pure genetic

profile *Sh_ShxSh* (54.5%) have been described. Of the three hybrid profiles, *Sb_ShxSh* profile was highly prevalent in Diakalèl (68.0%). In contrast, the non-hybrid genetic profile *Sh_ShxSh* was more frequent in Fangouné Bamanan (88.9%) (Table 3).

Table 3
Repartition of parasites genetic profiles in the two villages.

| *Village* | *Total* | Sb_ShxSc(%) | Sb_ShxSh (%) | Sh_ShxSc (%) | Sh_ShxSh (%) | *Total Hybrid (%)* | *p* |
|---|---|---|---|---|---|---|---|
| *Diakalèl* | 447 | 16 (3.6) | 304 (68.0) | 2 (0.4) | 125 (28.0) | **322 (72.0)** | < 0.0001 |
| *Fangouné Bamanan* | 342 | 2 (0.6) | 16 (4.7) | 20 (5.8) | 304 (88.9) | 38 (11.1) | |
| *Total* | 789 | 18 (2.3) | 320 (40.9) | 22 (2.8) | 429 (54.4) | 360 (45.6) | |

# Discussion

The endemicity of *S. haematobium* in the study area had already been reported in previous studies(16). Of the 63 positive children, the Cox1 and ITS2/18S genetic profiles analysis was conducted on 789 miracidia from which, 360 (45.6%) provided three types of hybrids: *S. bovis* cox1_*S. haematobium* ITS2_*S. curassoni* 18S (*SbxShSc)*; *S. bovis* cox1_*S. haematobium* ITS2 *S. haematobium* 18S: *SbxShSh* and *S. haematobium* cox1_*S. haematobium* ITS2_ *S. curassoni* I8S: *ShxShSc*) per site. Added to this, the non-hybrid *S. haematobium* (*S. haematobium* cox1_*S. haematobium* ITS2/18S: *ShxShSh*) (54.4%) was more frequent in Fangouné Bamanan (88.9%). In Mali, Gambia, Senegal *S. bovis* and *S. curassoni* were reported in animals since 1990 (16). In this study, the authors demonstrated that *S. bovis* was the commonest schistosome with the highest prevalence (85.3%) occurred at the abattoir of Mopti in Mali. Evidence of interaction of *S. bovis* and *S. curassoni* was found in cattle from Bamako and Mopti. One of the major difficult facing the (former or previous) studies was due to the lack of performant tools for schistosomes analyzing. The eggs' morphology i.e. as diagnose criteria is sometimes limited by the presence of eggs with intermediate size and length between related species such as *S. bovis* et *S. curassoni*. Recently, ten Belgian travelers returned from Mali with a *S.haematobium– S. bovis* hybrid infection, confirmed by DNA sequencing from(16). To support the infection of the patients in this cluster of ten Belgian travelers, the authors relied on a combination of the results of 2 species-specific PCR tests on serum, the parasitological findings (morphological features and detection of eggs in both stool and urine samples), and the confirmation of hybridization between *S. haematobium* and *S. bovis* by DNA. In northern Senegal, a similar hybrid was demonstrated (17) and more recently in France (Corsica)(13, 33), although no eggs were found in stool samples in the latter outbreak and in the stool samples of the children of our study site. In the past, cases of infection with *S. haematobium* and *S. intercalatum* since 2003 recognized as *S. guineensis*(34, 35) have been reported in several clusters of travelers exposed from the Dogon Plateau. In fact, it is not excluded that some cases of these reported clusters may have been infected with this hybrid form unknown at that time. In Benin, even if the data genotyped with just two loci (Cox1 mtDNA and ITS rDNA) providing little power to detect recent hybridization,

the results showed that double *S. bovis* and *S. haematobium* ITS recombinant DNA (rDNA) profiles were not rare in Kessounou village (31) suggesting a recent hybridization, as the ITS rDNA marker can retain both parental copies for several generations before they are homogenized by concerted evolution. While our results are comparable to those obtained in Benin, particularly the presence of gene flow between *S. bovis* and *S. haematobium* in some areas as well as in Benin and Mali, they are not congruent with two recently published papers which suggest that hybridization may occur quite infrequently between *S. bovis* and *S. haematobium* (36). These authors found strong differentiation between *S. bovis* and *S. haematobium* in human populations of the Senegal River Basin (SRB), for the first and in Niger and Zanzibar for the seconds, suggesting that there is minimal gene flow between the two species. Unlike to the results in Senegal River Basin (SRB), the findings in Benin and Mali implies that parasite transmission (gene flow) between the human and animal host is extensive and that mammals are a potential reservoir for human schistosomiasis (36, 37). The study area located in the Sahelian zone is mainly characterized by low rainfall leading to early drying of water points from January to June. As a result, there is a strong pressure exerted on the same water points shared by both people and animals whose proximity is favorable to an interaction between their schistosomes, *S. haematobium* for humans and *S. bovis*/S. *curassoni* for cattle. This would have important implications for the implementation of control programs (18). Finally, whatever the presence of *S. bovis* in cattle and its hybridization with *S. haematobium* in a cluster traveler have already been described in Mali, the presence of Cox1_ITS/18S *S. bovis* haplotypes in 42.8% of the population samples confirms that introgressive hybridization between *S. bovis/* and *S. haematobium* exists on a large scale in humans in Mali. Unlike to the molecular studies undertaken so far on hybridization between schistosomes, we used three loci (Cox1, ITS/18S) to discriminate *S. bovis* from *S. curassoni* which are mostly sympatric in cattle, suggesting a higher power of detection of recent hybridization. Our results are comparable to those recorded in Ivory Cost where *S. bovis* cox1_*S. haematobium* ITS2 (42.0%) has been identified as the most commonly observed in the populations (30), *S. bovis* cox1 x *S. haematobium* ITS2/ 18S (*SbxShSh)* genotype also was the most frequently found (40.9%) among the four possible hybrid profiles observed in our study site, particularly in Fangouné Bamanan (68.0%), while the *S. haematobium* c*ox1 x S. haematobium* 1TS2_*S. curassoni* I8S (*ShxShSc*) was the main hybrid genotype described in Diakalel (5.8%). On the other hand, while Angora et *al*., 2022 (30) identified 15 miracidia (0.7%) with *S. bovis* cox1 x *S. bovis* ITS2 genotype, we did not identify any such profile in our samples.

# Conclusions

Our study shows for the first time that hybrid schistosome infections in humans may be more common than previously thought, suggesting that in the Kayes region and probably in other areas in Mali gene flow between *S. haematobium* x *S. bovis* is present. The presence of hybrids underscores the importance of the livestock reservoir of schistosomes therefore hampering schistosomiasis control and elimination. More studies are needed on population genetics of schistosomes at the human and animal interface to evaluate the parasite's gene flow and its impact on epidemiology of the disease, the transmission to humans, as well as the control of the disease.

# Abbreviations

*S*
*Schistosoma*
*h*
*haematobium*
*c*
*curassoni*
*b*
*bovis*
WHO
World Health Organization
MDA
Mass Drug Administration
FTA
Find The Agent
ITS
Internal Transcribed Spacer
ARMS
Amplification Refractory Mutation System
CAT
Catalogue
DNA
Deoxyribonucleic Acid
mt
Mitochondrial
IR
interne Reverse
IF
Interne Forward
OR
Out Reverse
OF
Out Forward
rDNA
Recombinant Deoxyribonucleic
SRB
Senegal River Basin

# Declarations

### Ethics approval and consent to participate

The study protocol was approved by the Institutional Ethics Committee of the Faculty of Medicine and Dentistry of Bamako under the reference number N° 2018/71/CE/FMPOS. School authorities, teachers, parents/guardians and children were informed about the objectives, procedures and potential risks and benefits of the study. Written informed consent was obtained from children's parents or legal guardians, while children provided oral assent. After sampling, a praziquantel treatment following WHO guidelines (40 mg/kg) was provided to children found with a *Schistosoma* infection. For data protection purposes, an identification number was assigned to each participant.

### *Consent for publication*

Not applicable

### *Availability of data and materials*

All data and materials are available in the article

### *Competing interests*

The authors declare that they have no conflict of interest. Ethical approval Ethical permission was obtained from the Ethic Committee of the « Faculté de Médecin et d'Odontostomatologie FMOS de Bamako, Mali. T.S. is an employee of Ares Trading S.A., an affiliate of Merck KGaA, Darmstadt, Germany.


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All authors read and approved the final manuscript.

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Compliance with ethical standards

***Informed cons*****ent**: The Directors of Fangouné bamanan and Diakalel school obtained informed consent from the parents of all the schoolchildren who participated to the study. Data set availability. Sequence data will be deposited in the NCBI GenBank database Cox 1 DNA.

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# Figures

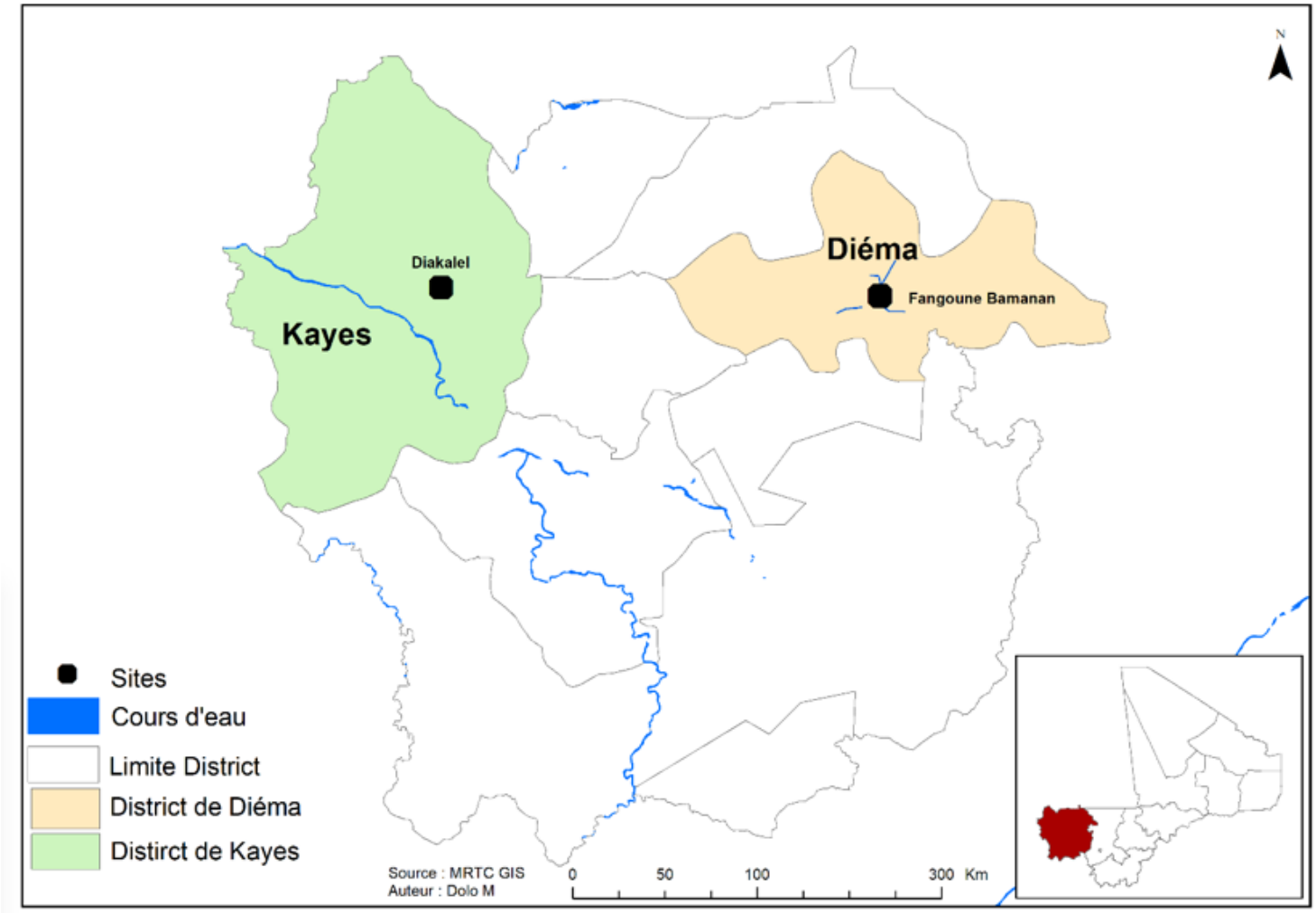
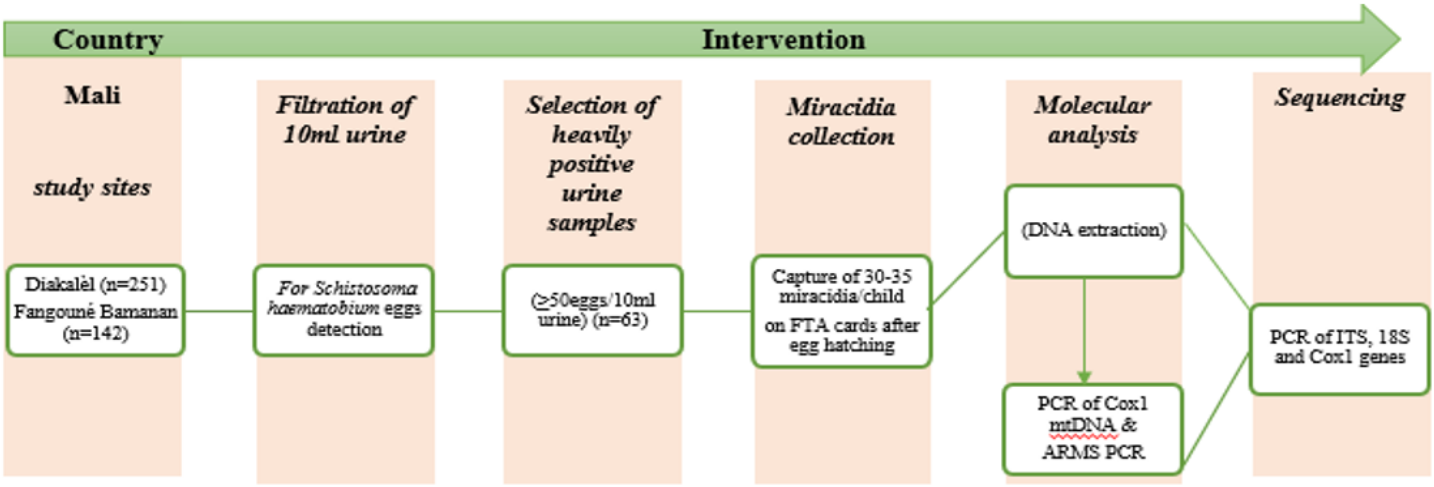



**Figure 1**

Location of the two study sites on the map of the Kayes region (Mali, West Africa).




## Figure 2

Data collection framework, November 2021 for parasitological survey and May-December for molecular analysis of *S. haematobium miracidia* samples