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## High prevalence of *Schistosoma haematobium* × *Schistosoma bovis* hybrids in schoolchildren in Côte d'Ivoire

Etienne K. Angora<sup>1,2,3</sup>, Jean-François Allienne<sup>4</sup>, Olivier Rey<sup>4</sup>, Hervé Menan<sup>3</sup>,  
André O. Touré<sup>5</sup>, Jean T. Coulibaly<sup>1,2,6,7</sup>, Giovanna Raso<sup>1,2</sup>, William Yavo<sup>3</sup>,  
Eliézer K. N'Goran<sup>6,7</sup>, Jürg Utzinger<sup>1,2</sup>, Oliver Balmer<sup>1,2,\*</sup> and Jérôme Boissier<sup>4,\*</sup>

1 Swiss Tropical and Public Health Institute, Basel, Switzerland;

2 University of Basel, Basel, Switzerland;

3 Unité de Formation et de Recherche Sciences Pharmaceutiques et Biologiques, Université Félix Houphouët-Boigny, Abidjan, Côte d'Ivoire;

4 IHPE, Univ. Montpellier, CNRS, Ifremer, Univ. Perpignan Via Domitia, Perpignan, France;

5 Institut Pasteur de Côte d'Ivoire, Abidjan, Côte d'Ivoire;

6 Unité de Formation et de Recherche Biosciences, Université Félix Houphouët-Boigny, Abidjan, Côte d'Ivoire

7 Centre Suisse de Recherches Scientifiques en Côte d'Ivoire, Abidjan, Côte d'Ivoire

Author for correspondance : Jérôme Boissier, E-mail: [boissier@univ-perp.fr](mailto:boissier@univ-perp.fr)

### Abstract

Schistosomiasis is a neglected tropical disease, though it is highly prevalent in many parts of sub-Saharan Africa. While *Schistosoma haematobium*-*bovis* hybrids have been reported in West Africa, no data about *Schistosoma* hybrids in humans are available from Côte d'Ivoire. This study aimed to identify and quantify *S. haematobium*-*bovis* hybrids among schoolchildren in four localities of Côte d'Ivoire. Urine samples were collected and examined by filtration to detect *Schistosoma* eggs. Eggs were hatched and 503 miracidia were individually collected and stored on Whatman® FTA cards for molecular analysis. Individual miracidia were molecularly characterized by analysis of mitochondrial *cox1* and nuclear internal transcribed spacer 2 (ITS 2) DNA regions. A mitochondrial *cox1*-based diagnostic polymerase chain reaction was performed on 459 miracidia, with 239 (52.1%) exhibiting the typical band for *S. haematobium* and 220 (47.9%) the *S. bovis* band. The *cox1* and ITS 2 amplicons were Sanger sequenced from 40 randomly selected miracidia to confirm species and hybrids status. Among the 33 *cox1* sequences analysed, we identified 15 *S. haematobium* sequences (45.5%) belonging to seven haplotypes and 18 *S. bovis* sequences (54.5%) belonging to 12 haplotypes. Of 40 ITS 2 sequences analysed, 31 (77.5%) were assigned to pure *S. haematobium*, four (10.0%) to pure *S. bovis* and five (12.5%) to *S. haematobium*-*bovis* hybrids. Our findings suggest that *S. haematobium*-*bovis* hybrids are common in Côte d'Ivoire. Hence, intense prospecting of domestic and wild animals is warranted to determine whether zoonotic transmission occurs.

### Keywords

Côte d'Ivoire; hybrids; molecular analysis; *Schistosoma bovis*; *Schistosoma haematobium*

## **Introduction**

Schistosomiasis is a parasitic disease caused by trematodes of the genus *Schistosoma*. It is widespread in sub-Saharan Africa; yet, a key epidemiological feature of schistosomiasis is its focal distribution (Colley et al., 2014; Lai et al., 2015). The disease affects more than 250 million people and it is estimated to have caused 1.4 million disability-adjusted life years in 2017 (Hotez et al., 2014; GBD 2017 DALYs and HALE Collaborators, 2018). In Côte d'Ivoire, urogenital and intestinal schistosomiasis, caused by *Schistosoma haematobium* and *Schistosoma mansoni*, respectively, are endemic in humans and crossing of open water sources is a key risk factor for transmission of schistosomiasis (Chammartin et al., 2014; Krauth et al., 2015). While *S. mansoni* is widespread in the western part of the country (Assaré et al., 2015), *S. haematobium* is mostly present in the central and southern parts of Côte d'Ivoire (Coulibaly et al., 2013). In the northern part of Côte d'Ivoire, a recent study found a low prevalence among school-aged children for both *S. haematobium* (1.9%) and *S. mansoni* (3.5%) (M'Bra et al., 2018). *Schistosoma bovis*, a parasite of domestic animals, has also been reported in Côte d'Ivoire, but there is a paucity of recent data. In 1997, post-mortem examinations of cattle in the savannah area of Côte d'Ivoire revealed a prevalence of 35% (Achi et al., 2003).

*Schistosoma haematobium* and *S. bovis* are phylogenetically closely related and freshwater snails of the genus *Bulinus* act as intermediate hosts for both species (Cook and Zumla, 2009). The close phylogenetic association between these two species enables inter-species mating and can result in hybridization between the two species, which might influence disease transmission and alter phenotypic characteristics of parasites in both human and animal (Huysse et al., 2009; Boissier et al., 2016). Of particular concern, hybridization could enhance transmission and expand the distribution of these species. For instance, laboratory hybrids exhibit particularly enhanced life-history traits, including increased virulence, expanded snail host spectrum, maturation and egg production (Leger and Webster, 2017). There is a need to study *S. haematobium-bovis* hybrids and determine how such hybrids might influence the epidemiology and control of schistosomiasis in terms of virulence, zoonotic potential and resistance to treatment. Previous work focused on Benin, Mali, Niger and Senegal (Leger and Webster, 2017). Recently, *S. haematobium-bovis* hybrids have been found in Malawi (Webster et al., 2019) and hybrids have been involved in infection in Corsica, France (Boissier et al., 2016), which demonstrates the capacity for long-range dispersion. A high prevalence and focal transmission of *S. haematobium-bovis* hybrids in *Bulinus* snails have been shown in Côte d'Ivoire (Tian-Bi et al., 2019), but the occurrence of such hybrids in humans has not yet been investigated.

This study molecularly characterized schistosome miracidia collected from schoolchildren in four locations in Côte d'Ivoire to investigate the presence and extent of *S. haematobium-bovis* hybrids.

## **Materials and methods**

### ***Ethical consideration***

Ethical clearance for this study was obtained from the Ministère de la Santé et de l'Hygiène Publique de Côte d'Ivoire (reference no. 003-18/MSHP/CNER-kp). School authorities, teachers, participating children and their parents/guardians were informed about the objectives, procedures, and potential risks and benefits of the study. Written informed consent was obtained from children's parents/guardians, while children provided oral assent.

### ***Study area***

The study was carried out in four locations of Côte d'Ivoire: (i) Agboville (5°55' 41"N latitude, 4°13' 01"W longitude) and (ii) Adzopé (6°06' 25"N, 3°51' 36"W) in the south-eastern part of the

country; (iii) Sikensi (5°40' 34''N, 4°34' 33''W) in the south-central part; and (iv) Duekoué (6°44' 00''N, 7°21' 00''W) in the western part. The study was integrated into a cross-sectional survey determining the prevalence of *Schistosoma* infection among school-aged children (Angora et al., 2019). The four locations are well known for their high endemicity of *S. haematobium* (N'Guessan et al., 2007) and *S. mansoni* (Raso et al., 2005). Figure 1 shows the study area.

### ***Collection of miracidia***

From January to April 2018, a total of 1187 children aged 5–14 years from the four locations (Agboville, n = 402; Adzopé, n = 208; Sikensi, n = 205; and Duekoué, n = 372) were invited to provide a mid-day urine sample. Urine samples were transferred to the nearby health centres for parasitological examination. *Schistosoma haematobium* infection was identified by urine filtration (Mott et al., 1982). Ten millilitres of vigorously shaken urine was filtered through a Nylrel filter with a 40 µm mesh size and examined under a microscope by experienced laboratory technicians for *S. haematobium* egg detection. The presence of eggs was recorded, but infection intensities were not determined.

Urine samples from 19 randomly selected infected children were chosen for further analysis in the four locations: Sikensi (n = 6), Agboville (n = 5), Duekoué (n = 5) and Adzopé (n = 3) (Table 1). Under a dissecting microscope, eggs were removed from each urine sample with an elongated Pasteur pipette and placed in a petri dish filled with tap water to facilitate miracidial hatching. Miracidia were collected individually in 3 µL of water using a micropipette and preserved on Whatman-FTA® cards (GE Healthcare Life Sciences; Amersham, UK), as described previously (Webster et al., 2012; Boissier et al., 2016). All samples were transferred to the University of Perpignan in France for molecular analysis.

### ***Genomic DNA extraction***

Genomic DNA was extracted individually from 503 miracidia. A 2.0 mm disc containing the sample was removed from the FTA card with a Harris-Micro-Punch (VWR; London, UK) and incubated in 50 µL of double-distilled water for 10 min. Water was removed and the disc incubated in 80 µL of 5% Chelex® (Bio-Rad; Hercules, California, USA) solution successively at 65 °C for 30 min and then 99 °C for 8 min. Finally, 60 µL of the supernatant was stored at –20 °C for subsequent molecular analysis.

### ***Mitochondrial cox1 profiling***

DNA from each miracidia was analysed using a new rapid diagnostic mitochondrial cox1 rapid diagnostic polymerase chain reaction (RD-PCR) in order to infer mitochondrial species designation. We used species-specific primers to amplify a specific cox1 DNA region (differing in length) for *S. bovis* (260 bp), *S. mansoni* (215 bp) and *S. haematobium* (120 bp). Primers employed were a universal reverse (Shmb.R: 5' -CAA GTA TCA TGA AAY ART ATR TCT AA -3' ) and three species-specific forward primers (Sb.F: 5' -GTT TAG GTA GTG TAG TTT GGG CTC AC-3' ; Sm.F: 5' -CTT TGA TTC GTT AAC TGG AGT G-3' ; and Sh.F: 5' -GGT CTC GTG TAT GAG ATC CTA TAG TTT G-3' ). Each PCR was performed in a total reaction volume of 10 µL, comprising 2 µL of the DNA extract, 2 µL of Green GoTaq Flexi Buffer 5X (Promega; Madison, Wisconsin, USA), 0.6 µL of 25 mM MgCl<sub>2</sub> (Promega), 0.2 µL of 10 mM dNTP mix (Promega), 1 µL of 10 × primer mix (4 µL of 100 µM reverse primer, 4 µL of each 100 µM forward primer and 84 µL of distilled water) and 1 U of GoTaq Hot Start Polymerase (Promega). The reaction conditions included an activation step of 95 °C for 3 min, followed by 45 cycles of 95 °C for 10 s, 52 °C for 30 s and 72 °C for 10 s, and a final extension at 72 °C for 2 min. The cox1-PCR products were visualized for electrophoresis on a 3% agarose gel stained with ethidium bromide (see Supplementary File 1: Fig. S1).

The prevalence of schistosomes with each mitochondrial *cox1* signature was computed and stratified by study location using Epi Info version 7 (Centers for Disease Control and Prevention; Georgia, Atlanta, USA). Fisher's exact test was used and a P value of 0.05 was considered statistically significant.

### ***Cox1 and internal transcribed spacer 2 (ITS2) analysis***

Based on the RD-PCR results, we randomly selected a subsample of 10 miracidia (five *S. haematobium* *cox1* and five *S. bovis* *cox1*) per study location. We performed PCR on 40 miracidia and products were sequenced on both mitochondrial *cox1* and nuclear ITS2 gene (Table 1), using the following primers: *Cox1*.R: 5' -TAA TGC ATM GGA AAA AAA CA-3' and *Cox1*.F: 5' -TCT TTR GAT CAT AAG CG-3' for *cox1* (Lockyer et al., 2003) and ITS 5.R: 5' -GGA AGT AAA AGT CGT AAC AAG G-3' and ITS4.F: 5' -TCC TCC GCT TAT TGA TAT GC-3' for ITS2 (Barber et al., 2000). The PCRs were performed in a final reaction volume of 25 µL, comprising 4 µL of DNA template, 5 µL of 5X Colorless GoTaq® Flexi Buffer (Promega), 1.5 µL of MgCl<sub>2</sub> (25 mM), 0.5 µL of dNTP (10 mM), 0.8 µL of each 10 µM primer and 0.2 µL of Go Taq®G2 Hot Start Polymerase (Promega). The PCR conditions were the same for both markers: 3 min at 95 °C, followed by 45 cycles at 95 °C for 40 s, 48 °C for 40 s and 72 °C for 70 s, followed by a final extension of 2 min at 72 °C. The mitochondrial *cox1* and nuclear ITS2 PCR products (4 µL) were visualized on 1.5% agarose gels stained with ethidium bromide to verify band size (expected size 1200 bp) and quality of the amplicons. All successfully amplified PCR products were purified and sequenced with the *Cox1*.R: 5' -TAA TGC ATM GGA AAA AAA CA-3' or the ITS4.F: 5' -TCC TCC GCT TAT TGA TAT GC-3' primers, respectively, on an Applied Biosystems Genetic Analyser at Genoscreen (Lille, France).

### ***Sequences analysis***

The partial *cox1* and ITS2 sequences were assembled separately and edited using Sequencher version 4.5 (Gene Codes Corporation; Ann Arbor, Michigan, USA). All sequences were aligned using BioEdit version 7.0.9 (Ibis Therapeutic; Carlsbad, California, USA) and compared to sequences deposited in the GenBank Nucleotide Database (<https://www.ncbi.nlm.nih.gov/nucleotide/>). The nuclear-ITS2 region differs at five polymorphic sites between *S. haematobium* and *S. bovis*, and hence, the sequence chromatograms were checked at these mutation points to identify possible heterogeneity, as previously described (Webster et al., 2013). The mitochondrial-*cox1* haplotype and nucleotide diversity [ $\pm$ standard deviation (S.D.)] were calculated using DnaSP version 6.0 (Rozas et al., 2017). Phylogenetic trees were constructed separately for *S. haematobium* and *S. bovis* *cox1* haplotypes using MEGA version 6.0.6 (Penn State University; Philadelphia, Pennsylvania, USA) and employing a maximum likelihood and the HKY + G nucleotide substitution model, which was determined by MEGA version 6.0.6 as the model best describing the data. The support for tree nodes was calculated with 1000 bootstrap iterations. The phylogenies include all the haplotypes identified in this study plus reference haplotypes obtained from GenBank Nucleotide Database. The phylogeny of the *S. bovis* *cox1* data was rooted with an *S. haematobium* haplotype (JQ397330.1) and the *S. haematobium* *cox1* data with an *S. bovis* haplotype (AJ519521.1). All *cox1* sequences were uploaded onto the GenBank Nucleotide Database (GenBank accession nos. MK757162–MK757168 for *S. haematobium* and MK757170–MK757181 for *S. bovis*).

## **Results**

### ***Cox1 rapid diagnostic PCR***

Of the 1187 urine samples examined, 166 (14.0%) were found positive for *Schistosoma* eggs, as described elsewhere (Angora et al., 2019). Overall, 503 miracidia were collected from 19 *Schistosoma*-

infected children and stored on Whatman® FTA cards and the *cox1* RD-PCR was successful for 459 miracidia. Of these, 239 miracidia (52.1%) gave an *S. haematobium* *cox1* profile and 220 (47.9%) an *S. bovis* *cox1* profile, with no statistically significant difference between the two proportions ( $P = 0.081$ ). No miracidia gave an *S. mansoni* *cox1* profile. The *S. haematobium* *cox1*:*S. bovis* *cox1* ratio varied according to study area (76:63 for Sikensi; 73:38 for Agboville; 63:65 for Duekoué; and 27:54 for Adzopé). The proportion of *S. haematobium* *cox1* was higher than that of *S. bovis* *cox1* in Sikensi ( $P = 0.026$ ) and Agboville ( $P < 0.001$ ), whereas *S. bovis* *cox1* was the predominant species found in Adzopé ( $P < 0.001$ ). In Duekoué, *S. haematobium* *cox1* and *S. bovis* *cox1* were equally distributed (Fig. 1). Table 1 shows for each parasite infra-population, the proportion of *S. haematobium* *cox1* and *S. bovis* *cox1* from the 459 *cox1* RD-PCR miracidia.

### ***Cox1 and ITS2 sequence analysis***

The *cox1* and ITS amplicons of a total of 40 miracidia were sequenced. Good quality ITS2 sequences were obtained for all samples while, for *cox1*, only 33 samples yielded good sequences. Among the 33 *cox1* sequences, we identified 15 *S. haematobium* sequences (Table 1) belonging to seven distinct haplotypes with a very low diversity showing few single nucleotide polymorphisms (Supplementary File 2: Table S2) and 18 *S. bovis* sequences belonging to 12 distinct haplotypes (Supplementary File 3: Table S3). Haplotype diversity ( $\pm$ S.D.) was  $0.922 \pm 0.051$  and  $0.724 \pm 0.121$  for *S. bovis* and *S. haematobium*, respectively. Nucleotide diversity ( $\pm$ S.D.) was  $0.0094 \pm 0.0013$  and  $0.0011 \pm 0.0003$  for *S. bovis* and *S. haematobium*, respectively. Among the ITS2 sequences, 31 gave an *S. haematobium* profile and four an *S. bovis* profile. Five miracidia gave double chromatogram peaks at the polymorphic positions between *S. haematobium* and *S. bovis*, suggesting heterozygosity (Huysse et al., 2009; Webster et al., 2013).

### ***Miracidia identified as hybrids and non-hybrids***

Discordance in the *cox1* and ITS2 profiles showed that *S. haematobium*-*bovis* miracidia were present in all the four study locations and were excreted by 12 children. Among these 12 children, nine excreted *S. haematobium*-*bovis* hybrids and seven excreted pure *S. haematobium*. Two children excreted both *S. haematobium*-*bovis* hybrids and pure *S. haematobium* miracidia, while one child excreted both *S. haematobium*-*bovis* hybrids and pure *S. bovis* miracidia (Table 1). From all the schistosome miracidia sequenced, 16 were *S. haematobium*, one was *S. bovis* and 23 were hybrids (57.5%). The different hybrid profiles according to the discordance in the *cox1* (*S. haematobium* or *S. bovis*) and ITS2 (*S. haematobium* or *S. bovis*) profiles are presented in Table 1. Most of the hybrid profiles found were *S. bovis* *cox1*  $\times$  *S. haematobium* ITS2. Hybrids occurred at similar frequencies in all age classes of the children included in the study.

### ***Cox1 phylogenies***

Figures 2 and 3 show phylogenies of all *S. haematobium* and *S. bovis* *cox1* haplotypes, respectively. Note that *S. bovis* reference sequences used were obtained from animals. All *S. haematobium* *cox1* haplotypes from Côte d'Ivoire cluster with group 1, as defined by Webster et al. (2012). *Cox1* *S. bovis* haplotypes were split into two clusters separating those from Duekoué (Sb5–8), from those from the three remaining locations (Sb1–4 and Sb9–12).

## **Discussion**

Hybridization of certain parasites is an emerging public health concern at the interface of infectious disease biology and evolution (King et al., 2015). From a population of 459 miracidia obtained from schoolchildren in Côte d'Ivoire, we have identified 47.9% and 57.5% of *S. haematobium*-*S. bovis* hybrids using the diagnostic mitochondrial *cox1* analysis and by sequencing of the *cox1* and ITS regions, respectively. The analysis of partial mitochondrial *cox1* regions showed seven haplotypes for *S. haematobium* and 12 for *S. bovis*, which demonstrates the existence of a mitochondrial introgressive hybridization of *S. haematobium* *cox1* by *S. bovis*. Similar results have been reported in Corsica (Moné et al., 2015; Boissier et al., 2016). Our findings were corroborated by analysis of the nuclear ITS2 region.

The polymorphism analysis of the *cox1* gene shows that *S. bovis* is more polymorphic than *S. haematobium*. This result is consistent with a recent microsatellite-based population genetic study in Cameroon, which reported higher gene diversity and higher allelic diversity for *S. bovis* compared to *S. haematobium* (Djuikwo-Teukeng et al., 2019). Of note, the cited Cameroon study compared the diversity of *S. bovis* to previous data for *S. haematobium* obtained from Niger and Zanzibar (Webster et al., 2015). The low polymorphism of *S. haematobium* *cox1* is in line with previous studies (Webster et al., 2012, 2013; Gower et al., 2013). Results obtained at a regional scale (i.e. in different countries) corroborate our results from a finer spatial scale (i.e. four sites within a single country).

*Schistosoma haematobium* is known to be weakly structured (Webster et al., 2012). It has been shown that two groups can be identified across the parasite's range in sub-Saharan Africa: 'group 1' clusters parasites from mainland Africa, while 'group 2' clusters parasites exclusively from the Indian Ocean islands and the neighbouring African coastal regions (Webster et al., 2012). As expected, *S. haematobium* haplotypes from our study cluster with 'group 1'. Our study also shows that for *S. bovis*, there is heterogeneity in the distribution of haplotypes across the country with the haplotypes from Duekoué in the western part differentiated from those from the southern part of Côte d'Ivoire. Furthermore, the current study shows that *S. haematobium*-*S. bovis* hybrids occurred in schoolchildren from each of the four study locations.

No *S. haematobium*-*S. bovis* hybrids were identified, even though such hybrids have been shown in a migrant boy from Côte d'Ivoire upon examination in France (Le Govic et al., 2019). Recently, it has been shown that *Bulinus* snails from the northern and central parts of Côte d'Ivoire were infected with *S. bovis*, *S. haematobium* and/or *S. haematobium*-*S. bovis* hybrids (Tian-Bi et al., 2019). The authors showed that *S. bovis* was particularly prevalent in *Bulinus truncatus*, *S. haematobium* was most prevalent in *B. globosus* and *S. haematobium*-*S. bovis* hybrids infected the two *Bulinus* species similarly. *Schistosoma bovis*-infected *Bulinus* were predominantly found in the northern part, while *S. haematobium* and hybrid-infected snails were mainly found in the central part of Côte d'Ivoire. These results show the importance of snail's involvement in the transmission of *S. haematobium*-*S. bovis* hybrids.

Most of the hybrids in our study showed *cox1* sequences from *S. bovis* and nuclear ITS2 sequences from *S. haematobium*. This type of hybrid is the most common hybrid reported, including cercariae collected from infected snails in Côte d'Ivoire (Tian-Bi et al., 2019), miracidia collected from infected patients in Senegal (Huyse et al., 2009) and miracidia collected during a recent schistosomiasis outbreak on Corsica (Boissier et al., 2016).

The current study found that some children excreted both pure *S. haematobium* and *S. haematobium*-*S. bovis* hybrids, which is in line with observations from Senegal (Huyse et al., 2009; Webster et al., 2013). Interestingly, we have also observed a single miracidium with a 'pure' *S. bovis* signature (*S. bovis* ITS2 and *S. bovis* *cox1*) in one child, suggesting that this patient may be infected with *S. bovis*, which is traditionally considered a parasite of bovines. Such a 'pure' *S. bovis* has been

reported in eggs recovered from humans in Corsica (Boissier et al., 2016). These accounts suggest that zoonotic transmission might occur. However, additional research is needed to confirm this speculation. We assume that the genome of *S. bovis* is strongly introgressed, and hence, it is plausible that we may have missed signatures of *S. haematobium* ancestry due to the standard analyses performed (Webster et al., 2013). A broader coverage of the genome would no doubt identify even more hybrids and would allow a clearer distinction between 'pure' parasites of each species and different levels of introgression.

### **Conclusion**

Our study has shown that *S. haematobium-bovis* hybrids are common in *Schistosoma* egg-positive children in Côte d'Ivoire. Our observations are relevant because hybrid parasites could affect transmission dynamics, treatment efficacy and morbidity, which might jeopardize control of, and progress towards, elimination of schistosomiasis. Our findings are relevant as the presence of hybrids calls into question our present understanding of parasite transmission and host ranges, which in turn may affect the effectiveness of current control strategies. Intensive prospection of domestic and wild animals is warranted to determine whether real zoonotic transmission occurs.

### **Acknowledgements**

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### **Author contributions**

EKA, HM, OB and JB conceived and designated the study. EKA, OR and JB wrote the first draft of the manuscript. EKA and JFA performed the molecular analyses. EKA and JB performed statistical analysis. OR, AOT, JTC, GR, WY, EKN, JU and OB revised the manuscript. All authors read and approved the final manuscript prior to submission.

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### **Conflict of interest**

None.

### **Ethical standards**

Ethical clearance for this study was obtained from the Ministère de la Santé et de l'Hygiène Publique de Côte d'Ivoire (reference no. 003-18/MSHP/CNER-kp). Written informed consent was obtained from the children's parents or legal guardians. Oral assent was obtained from children.



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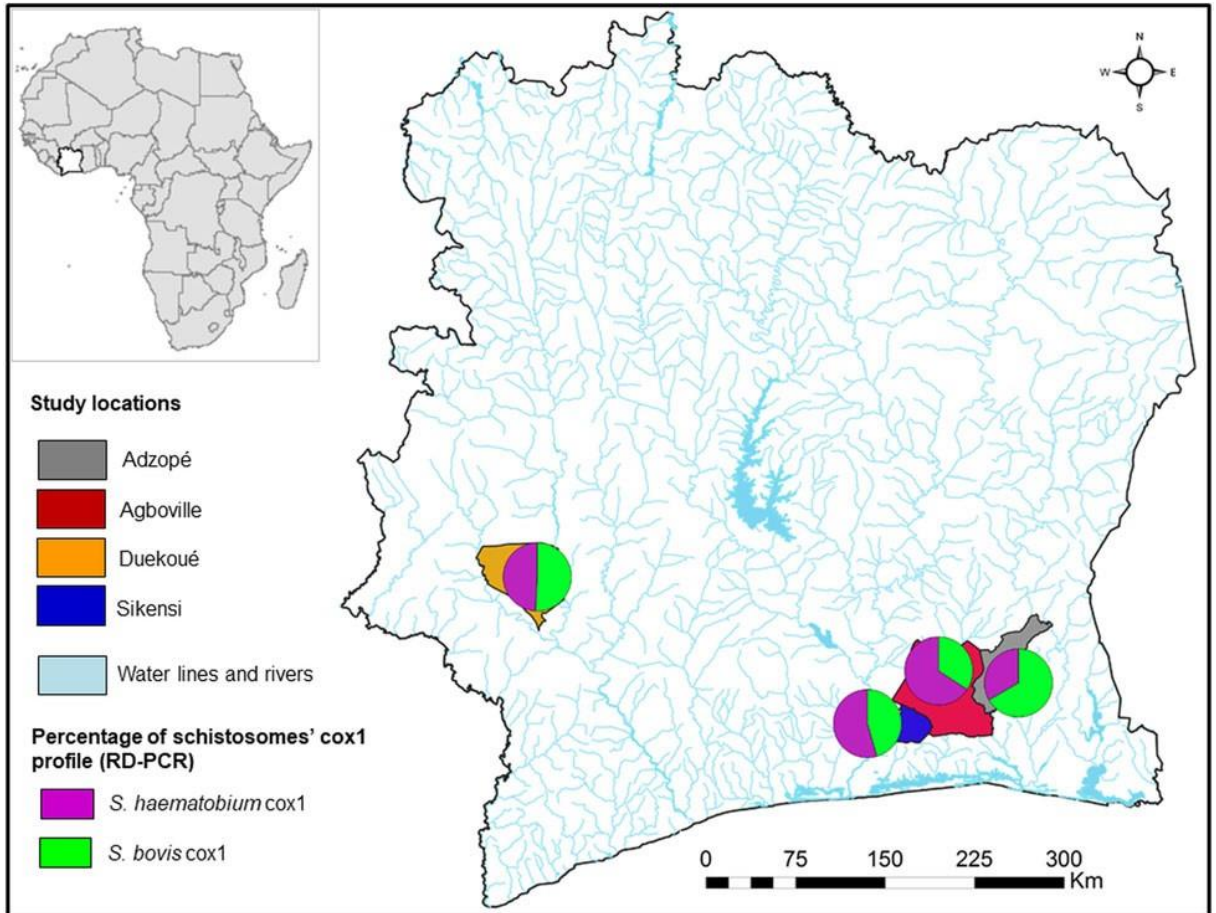
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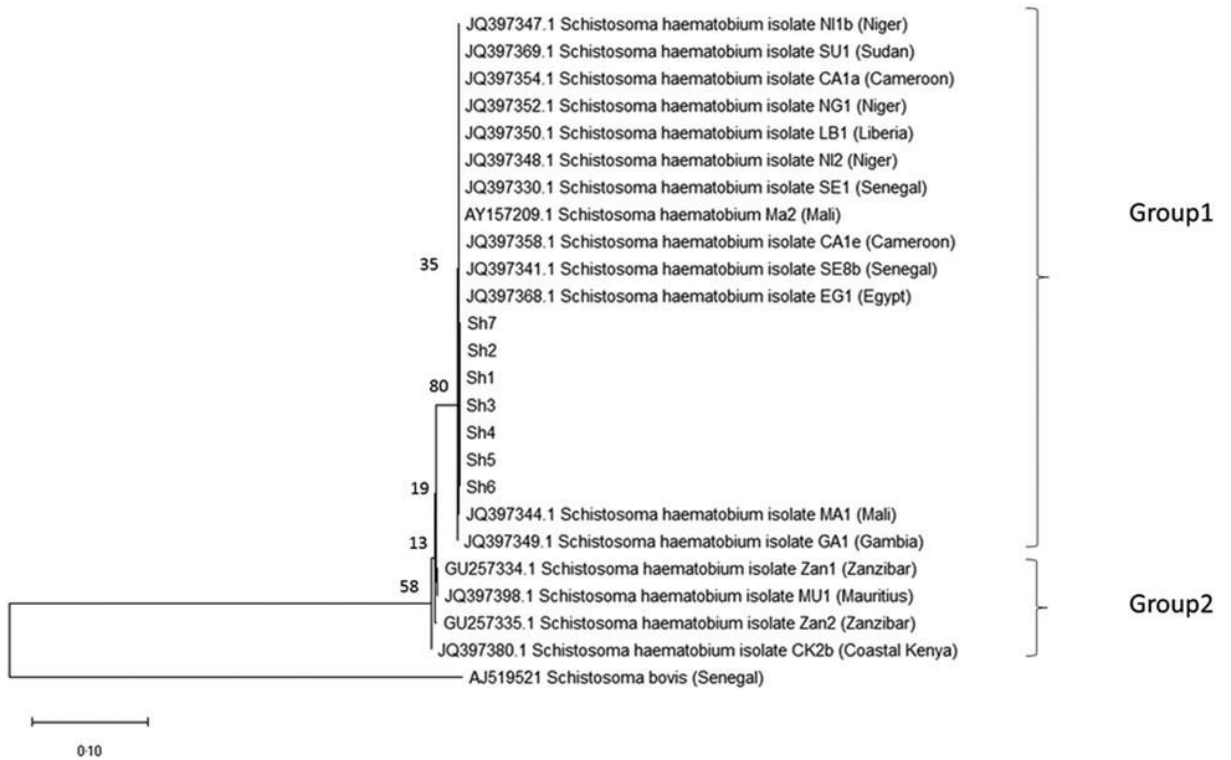
**Figure 1**

Map of the four study locations in Côte d'Ivoire (Adzopé, Agboville, Duekoué and Sikensi) showing the distribution and the proportion of *Schistosoma haematobium* cox1 or a *Schistosoma bovis* cox1 genetic profile. The cox1 profile of each miracidium was identified using a rapid diagnostic (RD) multiplex PCR.



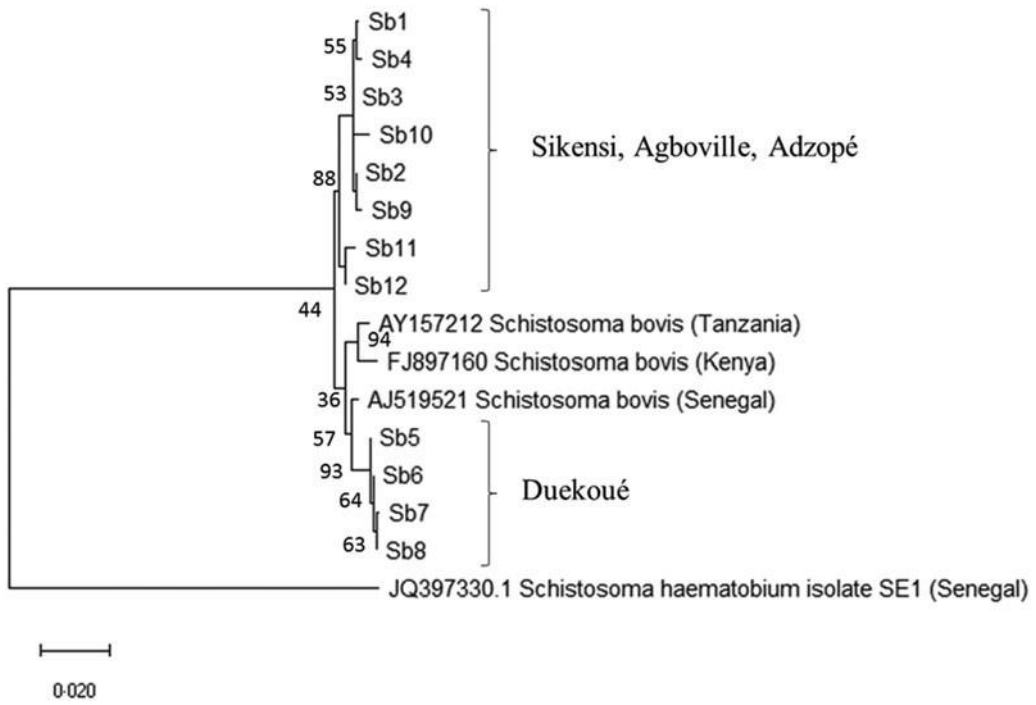
**Figure 2**

Majority rule consensus tree from maximum likelihood analysis of the mitochondrial *cox1* sequences for *Schistosoma haematobium* haplotypes Sh1–Sh7, including data from Genbank. Groups 1 and 2 indicate the major clades defined by Webster et al. (2012). Clade support values for each node are maximum parsimony bootstrap percentages



**Figure 3**

Majority rule consensus tree from maximum likelihood analysis the mitochondrial DNA *cox1* sequences for *Schistosoma bovis* haplotypes Sb1–Sb12 and data from GenBank. There are two clusters of *S. bovis* haplotypes: Duekoué (Sb5–Sb8) and the three other study sites (Sb1–Sb4 and Sb9–Sb12). Clade support values for each node are maximum parsimony bootstrap percentages. *Schistosoma bovis* reference sequences are from schistosomes collected from bovines.



**Table 1**

Results of cox1-based rapid diagnostic (RD) PCR analysis of all miracidia collected per patient and of subsequent sequence analysis of a subsample

Study location	Patient	RD-PCR analysis (n = 459)				Sequence analysis (n = 40)				
		n	S. haematobium cox1 (%)	S. bovis cox1 (%)	P value	n	No. of times observed	cox1 haplotypes	ITS2 alleles	Classification
Adzopé	AD138	31	3 (9.7)	28 (90.3)		5	1	S. bovis Sb3	S. haematobium	Hybrid
							1	S. bovis Sb12	S. haematobium	Hybrid
							1	S. bovis undet <sup>a</sup>	S. haematobium	Hybrid
							2	S. haematobium Sh1	S. haematobium	S. haematobium
							3	1	S. haematobium Sh1	S. haematobium
	AD140	27	5 (18.5)	22 (81.5)		1	S. haematobium Sh2	S. haematobium	S. haematobium	
						1	S. haematobium undet <sup>a</sup>	S. haematobium	S. haematobium	
	AD145	23	19 (82.6)	4 (17.4)		2	1	S. bovis Sb11	S. haematobium	Hybrid
						1	S. bovis Sb9	S. haematobium	Hybrid	
	Total	81	27 (33.3)	54 (66.7)	0.0001					
Agboville	AG062	19	13 (68.4)	6 (31.6)		1	1	S. bovis Sb10	S. haematobium + S. bovis	Hybrid
	AG068	23	23 (100)	0		3	2	S. haematobium Sh3	S. bovis	Hybrid
							1	S. haematobium Sh4	S. bovis	Hybrid

	AG077	24	3 (12.5)	21 (87.5)	3	1	S. bovis Sb3	S. haematobium + S. bovis	Hybrid
						1	S. bovis undet <sup>a</sup>	S. haematobium + S. bovis	Hybrid
						1	S. bovis Sb9	S. bovis	S. bovis
	AG108	13	9 (69.2)	4 (30.8)	2	1	S. haematobium Sh6	S. haematobium + S bovis	Hybrid
						1	S. haematobium Sh5	S. haematobium	S. haematobium
	AG219	32	25 (78.1)	7 (21.9)	1	1	S. bovis Sb3	S. haematobium	Hybrid
	Total	111	73 (65.8)	38 (34.2)					0.0001
Duekoué	DU330	2	2 (100)	0	2	1	S. haematobium Sh1	S. haematobium	S. haematobium
						1	S. haematobium undet <sup>a</sup>	S. haematobium	S. haematobium
	DU337	35	35 (100)	0	2	2	S. haematobium Sh1	S. haematobium	S. haematobium
	DU345	30	11 (36.7)	19 (63.3)	3	2	S. bovis Sb7	S. haematobium	Hybrid
						1	S. bovis Sb8	S. haematobium	Hybrid
	DU362	30	12 (40.0)	18 (60.0)	2	1	S. bovis Sb5	S. haematobium	Hybrid
						1	S. bovis Sb6	S. haematobium	Hybrid
	DU386	31	3 (9.7)	28 (90.3)	1	1	S. haematobium Sh1	S. haematobium	S. haematobium
	Total	128	63 (49.2)	65 (50.8)					0.721



Sikensis	SI028	30	18 (60.0)	12 (40.0)	2	1	S. bovis Sb3	S. haematobium	Hybrid
						1	S. bovis Sb4	S. haematobium	Hybrid
	SI052	26	12 (46.2)	14 (53.8)	2	1	S. haematobium Sh7	S. haematobium	S. haematobium
						1	S. haematobium undet <sup>a</sup>	S. haematobium	S. haematobium
	SI019	22	9 (40.9)	13 (59.1)	1	1	S. haematobium undet <sup>a</sup>	S. haematobium	S. haematobium
	SI114	19	11 (57.9)	8 (42.1)	1	1	S. bovis Sb3	S. haematobium	Hybrid
	SI122	19	11 (57.9)	8 (42.1)	2	1	S. bovis Sb2	S. haematobium + S.bovis	Hybrid
						1	S. bovis Sb1	S. haematobium	Hybrid
	SI136	23	15 (65.2)	8 (34.8)	2	1	S. haematobium Sh1	S. haematobium	S. haematobium
						1	S. haematobium undet <sup>a</sup>	S. haematobium	S. haematobium
	Total	139	76 (54.7)	63 (45.3)		0.026			

For RD-PCR analysis, the total number of miracidia, the number (and percentage) determined as *Schistosoma haematobium* cox1 and *S. bovis* cox1 and the level of significance of differences between parasites per study are using Fisher's exact test are shown.

For sequencing, 10 miracidia were randomly selected (five *S. haematobium* and five *S. bovis*) per study area, based on the mitochondrial-cox1 RD-PCR results. For sequence analysis, number analysed (n) and the times different combinations of cox1 haplotype and ITS2 alleles found are given together with the resulting species classification.

Cox1, cytochrome oxidase subunit I gene ; ITS, internal transcribed spacer region.

<sup>a</sup>undet, sequences for which the exact haplotype could not be determined due to sequence quality.

P value <0.05 was considered significant.