

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

Research Article

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Cite this article: Angora EK *et al* (2020). High prevalence of *Schistosoma haematobium* × *Schistosoma bovis* hybrids in schoolchildren in Côte d'Ivoire. *Parasitology* **147**, 287–294. <https://doi.org/10.1017/S0031182019001549>

Received: 31 May 2019

Revised: 5 September 2019

Accepted: 10 October 2019


First published online: 21 November 2019

Key words:

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

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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 prospection of domestic and wild animals is warranted to determine whether zoonotic transmission occurs.

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 (Huyse *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 Nytrel 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 MgCl₂ (Promega), 0.2 μL of 10 mM dNTP mix (Promega), 1 μL of 10 \times 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 MgCl₂ (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

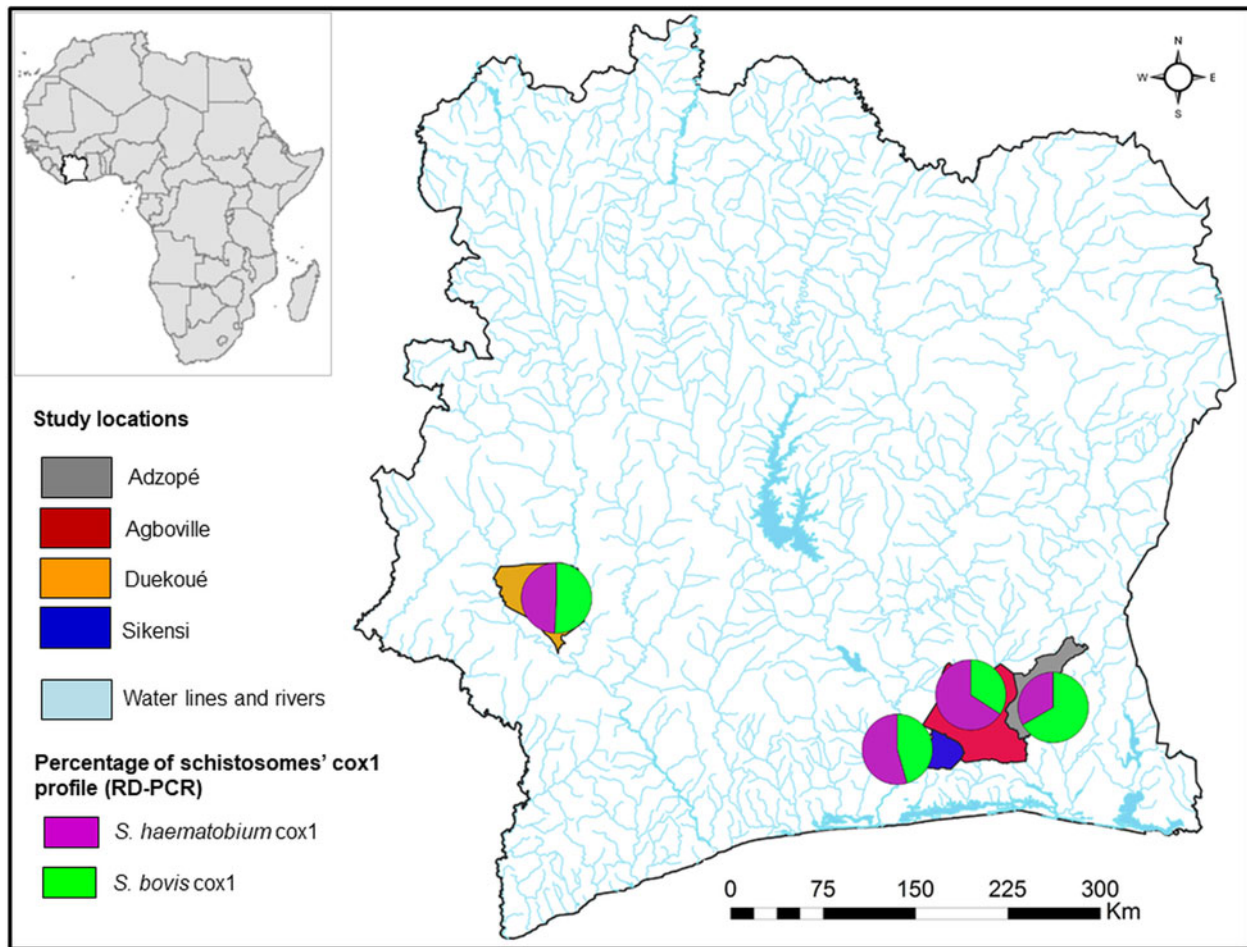


Fig. 1. Map of the four study locations in Côte d'Ivoire (Adzopé, Agboville, Duekoué and Sikensi) showing the distribution and the proportion of *Schistosoma haematobium* cox1 or a *Schistosoma bovis* cox1 genetic profile. The cox1 profile of each miracidium was identified using a rapid diagnostic (RD) multiplex PCR.

mitochondrial cox1 and nuclear ITS2 PCR products (4 μ L) 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

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 (<i>n</i> = 459)				Sequence analysis (<i>n</i> = 40)								
		<i>n</i>	<i>S. haematobium</i> cox1 (%)	<i>S. bovis</i> cox1 (%)	<i>P</i> value	<i>n</i>	No. of times observed	cox1 haplotypes	ITS2 alleles	Classification				
Adzopé	AD138	31	3 (9.7)	28 (90.3)	0.0001	5	1	<i>S. bovis</i> Sb3	<i>S. haematobium</i>	Hybrid				
							1	<i>S. bovis</i> Sb12	<i>S. haematobium</i>	Hybrid				
							1	<i>S. bovis</i> undet ^a	<i>S. haematobium</i>	Hybrid				
							2	<i>S. haematobium</i> Sh1	<i>S. haematobium</i>	<i>S. haematobium</i>				
	AD140	27	5 (18.5)	22 (81.5)	0.0001	3	1	<i>S. haematobium</i> Sh1	<i>S. haematobium</i>	<i>S. haematobium</i>				
							1	<i>S. haematobium</i> Sh2	<i>S. haematobium</i>	<i>S. haematobium</i>				
							1	<i>S. haematobium</i> undet ^a	<i>S. haematobium</i>	<i>S. haematobium</i>				
	AD145	23	19 (82.6)	4 (17.4)	0.0001	2	1	<i>S. bovis</i> Sb11	<i>S. haematobium</i>	Hybrid				
						1	<i>S. bovis</i> Sb9	<i>S. haematobium</i>	Hybrid					
	Total	81	27 (33.3)	54 (66.7)	0.0001									
Agboville	AG062	19	13 (68.4)	6 (31.6)	0.0001	1	1	<i>S. bovis</i> Sb10	<i>S. haematobium</i> + <i>S. bovis</i>	Hybrid				
							3	2	<i>S. haematobium</i> Sh3	<i>S. bovis</i>	Hybrid			
	AG068	23	23 (100)	0	0.0001		1	<i>S. haematobium</i> Sh4	<i>S. bovis</i>	Hybrid				
						AG077	24	3 (12.5)	21 (87.5)	3	1	<i>S. bovis</i> Sb3	<i>S. haematobium</i> + <i>S. bovis</i>	Hybrid
												1	<i>S. bovis</i> undet ^a	<i>S. haematobium</i> + <i>S. bovis</i>
	AG108	13	9 (69.2)	4 (30.8)	0.0001	2	1	<i>S. bovis</i> Sb9	<i>S. bovis</i>	<i>S. bovis</i>				
								1	<i>S. haematobium</i> Sh6	<i>S. haematobium</i> + <i>S. bovis</i>	Hybrid			
								1	<i>S. haematobium</i> Sh5	<i>S. haematobium</i>	<i>S. haematobium</i>			
AG219	32	25 (78.1)	7 (21.9)	0.0001	1	1	<i>S. bovis</i> Sb3	<i>S. haematobium</i>	Hybrid					
	Total	111	73 (65.8)	38 (34.2)	0.0001									
Duekoué	DU330	2	2 (100)	0	0.721	2	1	<i>S. haematobium</i> Sh1	<i>S. haematobium</i>	<i>S. haematobium</i>				
							1	<i>S. haematobium</i> undet ^a	<i>S. haematobium</i>	<i>S. haematobium</i>				
	DU337	35	35 (100)	0	0.721	2	2	<i>S. haematobium</i> Sh1	<i>S. haematobium</i>	<i>S. haematobium</i>				
	DU345	30	11 (36.7)	19 (63.3)	0.721	3	2	<i>S. bovis</i> Sb7	<i>S. haematobium</i>	Hybrid				
								1	<i>S. bovis</i> Sb8	<i>S. haematobium</i>	Hybrid			
	DU362	30	12 (40.0)	18 (60.0)	0.721	2	1	<i>S. bovis</i> Sb5	<i>S. haematobium</i>	Hybrid				
								1	<i>S. bovis</i> Sb6	<i>S. haematobium</i>	Hybrid			
	DU386	31	3 (9.7)	28 (90.3)	0.721	1	1	<i>S. haematobium</i> Sh1	<i>S. haematobium</i>	<i>S. haematobium</i>				
	Total	128	63 (49.2)	65 (50.8)	0.721									

Sikensi	S1028	30	18 (60.0)	12 (40.0)	2	1	S. bovis Sb3	S. haematobium	Hybrid
						1	S. bovis Sb4	S. haematobium	Hybrid
	S1052	26	12 (46.2)	14 (53.8)	2	1	S. haematobium Sh7	S. haematobium	S. haematobium
						1	S. haematobium undet ^a	S. haematobium	S. haematobium
	S1109	22	9 (40.9)	13 (59.1)	1	1	S. haematobium undet ^a	S. haematobium	S. haematobium
	S1114	19	11 (57.9)	8 (42.1)	1	1	S. bovis Sb3	S. haematobium	Hybrid
	S1122	19	11 (57.9)	8 (42.1)	2	1	S. bovis Sb2	S. haematobium + S. bovis	Hybrid
						1	S. bovis Sb1	S. haematobium	Hybrid
	S1136	23	15 (65.2)	8 (34.8)	2	1	S. haematobium Sh1	S. haematobium	S. haematobium
						1	S. haematobium undet ^a	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 area 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.

^aundet., sequences for which the exact haplotype could not be determined due to sequence quality.

P. value < 0.05 was considered significant.

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 ± 0.051 and 0.724 ± 0.121 for *S. bovis* and *S. haematobium*, respectively. Nucleotide diversity (\pm s.d.) was 0.0094 ± 0.0013 and 0.0011 ± 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 (Huyse *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-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

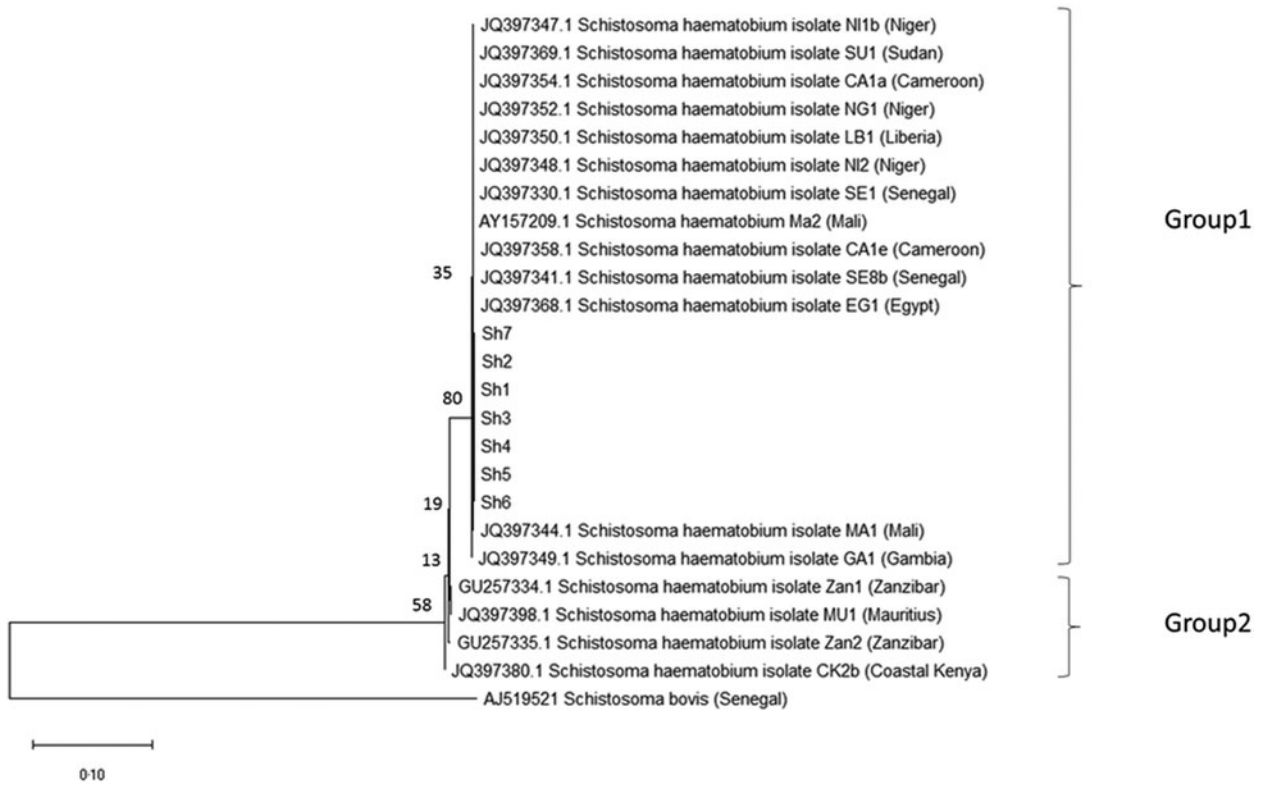


Fig. 2. Majority rule consensus tree from maximum likelihood analysis of the mitochondrial *cox1* sequences for *Schistosoma haematobium* haplotypes Sh1–Sh7, including data from GenBank. Groups 1 and 2 indicate the major clades defined by Webster *et al.* (2012). Clade support values for each node are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

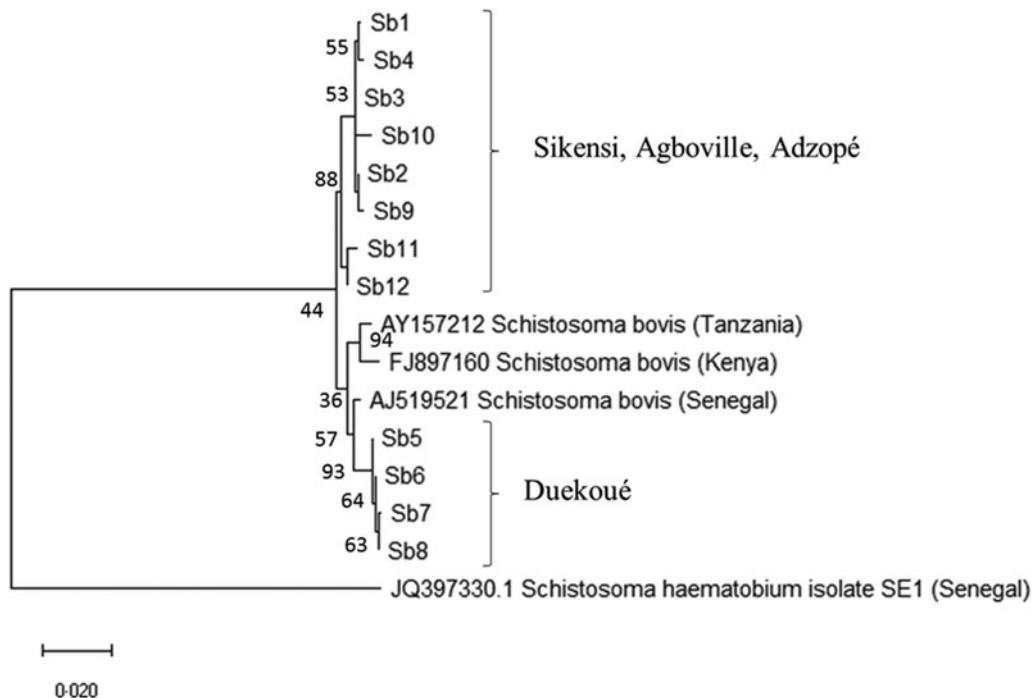


Fig. 3. Majority rule consensus tree from maximum likelihood analysis the mitochondrial DNA *cox1* sequences for *Schistosoma bovis* haplotypes Sb1–Sb12 and data from GenBank. There are two clusters of *S. bovis* haplotypes: Duekoué (Sb5–Sb8) and the three other study sites (Sb1–Sb4 and Sb9–Sb12). Clade support values for each node are maximum parsimony bootstrap percentages. *Schistosoma bovis* reference sequences are from schistosomes collected from bovines.

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-bovis* hybrids occurred in schoolchildren from each of the four study locations.

No *S. haematobium-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-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-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-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-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.

Supplementary material. The supplementary material for this article can be found at <https://doi.org/10.1017/S0031182019001549>.

Acknowledgements. We are grateful to the district health and village authorities of the four study areas for their support and facilitating the implementation of this study. We also thank the participants (children and their parents/guardians) for their commitment throughout the study.

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.

Financial Support. This work was financially supported by the Agence Nationale de Recherche (ANR), which supports the Hybrid Swarm project (HySWARM) in Perpignan that is set within the frame of the 'Laboratoire d'Excellence (LABEX) TULIP (ANR-10-LABX-41). Additional financial support was obtained by the Swiss National Science Foundation for EKA, JU and OB (grant no. 31003A_170113). EKA is a recipient of a Swiss Government Excellence Scholarship (ESKAS, grant no. 2017-0746) for which he is deeply grateful.

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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