

## **High prevalence of *Schistosoma haematobium* x *Schistosoma bovis* hybrids in schoolchildren in Cote d'Ivoire**

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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 Cote d'Ivoire. This study aimed to identify and quantify *S. haematobium*-*bovis* hybrids among schoolchildren in four localities of Cote 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(R) 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 Cote d'Ivoire. Hence, intense prospection of domestic and wild animals is warranted to determine whether zoonotic transmission occurs.

**Keywords :** Cote d'Ivoire, hybrids, molecular analysis, *Schistosoma bovis*, *Schistosoma haematobium*

48 **Introduction**

49 Schistosomiasis is a parasitic disease caused by trematodes of the genus *Schistosoma*. It is  
50 widespread in sub-Saharan Africa; yet, a key epidemiological feature of schistosomiasis is its  
51 **focal distribution** (Colley *et al.*, 2014; Lai *et al.*, 2015). The disease affects more than 250  
52 million people and caused an estimated 1.4 million disability-adjusted life years (DALYs) in  
53 2017 (Hotez *et al.*, 2014; GBD 2017 DALYs and Hale Collaborators, 2018).

54 In Côte d'Ivoire, urogenital and intestinal schistosomiasis, caused by *Schistosoma*  
55 *haematobium* and *Schistosoma mansoni*, respectively, are endemic in humans (Chammartin *et*  
56 *al.*, 2014). While *S. mansoni* is widespread in the western part of the country (Assaré *et al.*,  
57 2015), *S. haematobium* is mostly present in the central and southern parts of Côte d'Ivoire  
58 (Coulibaly *et al.*, 2013). In the northern part of Côte d'Ivoire, a recent study found a low  
59 prevalence among school-aged children for both *S. haematobium* (1.9%) and *S. mansoni* (3.5%)  
60 (M'Bra *et al.*, 2018). *Schistosoma bovis*, a parasite of domestic animals, has also been reported  
61 in Côte d'Ivoire, but there is a paucity of recent data. In 1997, post-mortem examinations of  
62 cattle in the savannah area of Côte d'Ivoire revealed a prevalence of 35% (Achi *et al.*, 2003).

63 *Schistosoma haematobium* and *S. bovis* are phylogenetically closely related and freshwater  
64 snails of the genus *Bulinus* act as intermediate hosts for both species (Cook and Zumla, 2009).  
65 The close phylogenetic association enables inter-species mating and can result in hybridization  
66 between the two species, which might influence disease transmission and alter phenotypic  
67 characteristics of parasites in both human and animal (Boissier *et al.*, 2016; Huyse *et al.*, 2009).

68 Of particular concern, hybridization might enhance transmission and expand the distribution of  
69 schistosomiasis. For instance, laboratory hybrids exhibit particular life-history traits, including  
70 increased virulence, expansion of host spectrum, maturation and egg production (Leger and  
71 Webster, 2017). There is a need to study *S. haematobium* x *S. bovis* hybrids and determine how  
72 such hybrids might influence the epidemiology and control of schistosomiasis. Previous work  
73 focused on Benin, Mali, Niger and Senegal (Huyse *et al.*, 2013; Leger and Webster, 2017).  
74 Recently, hybrids have also been involved in infections in Corsica, France (Boissier *et al.*,  
75 2016). While *S. haematobium* x *S. bovis* hybrids have been observed in *Bulinus* snails (Tian-Bi  
76 *et al.*, 2019) in Côte d'Ivoire, no data are currently available for humans.

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

80

## 81 Materials and methods

### 82 Ethical consideration

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

88

### 89 Study area

90 The study was carried out in four locations of Côte d'Ivoire: (i) Agboville ( $5^{\circ} 55' 41''$  N latitude,  
91  $4^{\circ} 13' 01''$  W longitude) and (ii) Adzopé ( $6^{\circ} 06' 25''$  N,  $3^{\circ} 51' 36''$  W) in the south-eastern part  
92 of the country; (iii) Sikensi ( $5^{\circ} 40' 34''$  N,  $4^{\circ} 34' 33''$  W) in the south-central part; and (iv)  
93 Duekoué ( $6^{\circ} 44' 00''$  N,  $7^{\circ} 21' 00''$  W) in the western part. The study was integrated into a cross-

94 sectional survey determining the prevalence of *Schistosoma* infection among school-aged  
95 children (Angora *et al.*, 2019). The four locations are well known for their high endemicity of  
96 *S. haematobium* (N'Guessan *et al.*, 2007) and *S. mansoni* (Raso *et al.*, 2005). Figure 1 shows  
97 the study area.

98

#### 99 Collection of miracidia

100 From January to April 2018, a total of 1,187 children aged 5–14 years from the four  
101 locations (Agboville, n=402; Adzopé, n=208; Sikensi, n=205; and Duekoué, n=372) were  
102 invited to provide a mid-day urine sample. Urine samples were transferred to nearby health  
103 centres for parasitological examination. *S. haematobium* infection was identified by urine  
104 filtration (Mott *et al.*, 1982). Ten millilitres of vigorously shaken urine was filtered through a  
105 Nytrel filter with a 40 µm mesh size and examined under a microscope by experienced  
106 laboratory technicians for *S. haematobium* egg detection. Infection intensities were not  
107 determined.

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

116

#### 117 Diagnostic by polymerase chain reaction (PCR)

118 Genomic DNA was extracted individually from 503 miracidia. A 2.0 mm disc containing  
119 the sample was removed from the FTA card with a Harris-Micro-Punch (VWR; London, UK)

120 and incubated in 50 µl of double distilled water for 10 min. Water was removed and the disc  
121 incubated in 80 µl of 5% Chelex® (Bio-Rad; Hercules, USA) solution successively at 65°C for  
122 30 min and then 99°C for 8 min. Finally, 60 µl of the supernatant was stored at -20°C for  
123 subsequent molecular analysis.

124 Extracted DNAs were analysed using a rapid diagnostic mitochondrial cox1 RD-PCR in  
125 order to infer mitochondrial species designation. We employed species-specific primers to  
126 amplify a specific cox1 DNA region (differing in length) for *S. bovis* (260 bp), *S. mansoni* (215  
127 bp) and *S. haematobium* (120 bp). Primers employed were a universal reverse (Shmb.R: 5'-  
128 CAA GTA TCA TGA AAY ART ATR TCT AA -3') and three species-specific forward  
129 primers (Sb.F: 5'-GTT TAG GTA GTG TAG TTT GGG CTC AC -3'; Sm.F: 5'-CTT TGA  
130 TTC GTT AAC TGG AGT G -3'; and Sh.F: 5'-GGT CTC GTG TAT GAG ATC CTA TAG  
131 TTT G -3'). Each PCR was performed in a total reaction volume of 10 µl, comprising 2 µl of  
132 DNA extract, 2 µl of Green GoTaq flexi buffer 5X (Promega; Madison, USA), 0.6 µl of 25 mM  
133 MgCl<sub>2</sub> (Promega; Madison, USA), 0.2 µl of 10 mM dNTP mix (Promega; Madison, USA), 1  
134 µl of 10x primer mix (4 µl of 100 µM reverse primer, 4 µl of each 100 µM forward primer and  
135 84 µl of doubled distilled water) and 1 U of GoTaq Hot Start Polymerase (Promega; Madison,  
136 USA). The reaction conditions included an activation step of 95°C for 3 min, followed by 45  
137 cycles of 95°C for 10 sec, 52°C for 30 sec and 72°C for 10 sec, and a final extension at 72°C  
138 for 2 min. The cox1-PCR products were visualized on 3% percent agarose gels stained with  
139 ethidium bromide. The prevalence of schistosomes with each mitochondrial cox1 signature was  
140 computed and stratified by study location using EpiInfo version 7 (Centers for Disease Control  
141 and Prevention; Atlanta, USA). Fisher's exact test was used and a p-value of 0.05 was  
142 considered significant.

143 *Cox1 and ITS2 sequencing*

144 Based on the RD-PCR results, we randomly selected a subsample of 10 miracidia (five  
145 *S. haematobium* cox1 and five *S. bovis* cox1) per study location. Overall, 40 miracidia were  
146 sequenced on both mitochondrial cox1 and nuclear ITS2 gene using the following primers:  
147 Cox1.R: 5'-TAA TGC ATM GGA AAA AAA CA -3' and Cox1.F: 5'-TCT TTR GAT CAT  
148 AAG CG -3' for cox1 (Lockyer *et al.*, 2003) and ITS 5.R: 5'-GGA AGT AAA AGT CGT AAC  
149 AAG G -3' and ITS4.F: 5'-TCC TCC GCT TAT TGA TAT GC -3' for ITS2 (Barber *et al.*,  
150 2000). The PCRs were performed in a final reaction volume of 25 µl, comprising 4 µl of DNA  
151 template, 5 µl of 5X Colorless GoTaq® Flexi Buffer (Promega; Madison, USA), 1.5 µl of  
152 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  
153 Hot Start Polymerase (Promega; Madison, USA). The PCR conditions were the same for both  
154 markers: 3 min at 95°C, followed by 45 cycles at 95°C for 40 sec, 48°C for 40 sec and 72°C  
155 for 70 sec, followed by a final extension of 2 min at 72°C. The mitochondrial cox1 and nuclear  
156 ITS2 PCR products (4 µl) were visualized on 1.5% percent agarose gels stained with ethidium  
157 bromide to verify band size (expected size 1,200 bp) and quality of the amplicons. All  
158 successfully amplified PCR products were purified and sequenced with the Cox1.R: 5'-TAA  
159 TGC ATM GGA AAA AAA CA -3' or the ITS4.F: 5'-TCC TCC GCT TAT TGA TAT GC -  
160 3' primers, respectively, on an Applied Biosystems Genetic Analyser at Genoscreen (Lille,  
161 France).

162

163 *Sequences analysis*

164 The partial cox1 and ITS2 sequences were assembled separately and edited using  
165 Sequencher version 4.5 (Gene Codes Corporation; Ann Arbor, USA). All sequences were  
166 aligned using BioEdit version 7.0.9 (Ibis Therapeutic; Carlsbad, USA) and compared to  
167 sequences deposited in the GenBank Nucleotide Database. The nuclear-ITS2 region differs at  
168 five polymorphic sites between *S. haematobium* and *S. bovis*, and hence, the sequence

169 chromatograms were checked at these mutation points to identify possible heterogeneity, as  
170 previously described (Webster *et al.*, 2013). The mitochondrial-cox1 haplotype and nucleotide  
171 diversities ( $\pm$ standard deviation [SD]) were calculated using DnaSP version 6.0 (Rozas *et al.*,  
172 2017). Phylogenetic trees were constructed **separately** for *S. haematobium* and *S. bovis* cox1  
173 haplotypes **using** MEGA version 6.0.6 (Penn State University; Pennsylvania, USA) and  
174 employing a maximum likelihood and the HKY + G nucleotide substitution model, which was  
175 determined by MEGA version 6.0.6 (Penn State University; Pennsylvania, USA) as the model  
176 best describing the data. The support for tree nodes was calculated with 1,000 bootstrap  
177 iterations. The phylogenies include all the haplotypes identified in this study plus some  
178 reference haplotypes **obtained** from GenBank Nucleotide Database. The phylogeny of **the**  
179 *S. bovis* cox1 data was rooted with **a** *S. haematobium* haplotype (JQ397330.1) and **the**  
180 *S. haematobium* cox1 data with **a** *S. bovis* haplotype (AJ519521.1). All **cox1** sequences were  
181 uploaded onto the GenBank Nucleotide Database (GenBank accession nos. MK757162-  
182 MK757168 for *S. haematobium* and MK757170- MK757181 for *S. bovis*).

183

## 184 Results

### 185 Cox1 rapid diagnostic PCR

186 Of **the** 1,187 urine samples examined, 166 (14.0%) were found positive for *Schistosoma*  
187 eggs, **as described elsewhere** (Angora *et al.*, 2019). Overall, 503 miracidia were collected from  
188 hatched eggs of 19 *Schistosoma*-infected children on Whatman® FTA cards and the cox1 RD-  
189 PCR **was** successful for 459 miracidia. Of these, 239 miracidia (52.1%) **gave a** *S. haematobium*  
190 cox1 profile and 220 (47.9%) **gave a** *S. bovis* cox1 profile, with no statistically significant  
191 difference **between the two proportions** ( $p=0.081$ ). No miracidia **gave a** *S. mansoni* cox1 profile.  
192 The *S. haematobium* cox1:*S. bovis* cox1 ratio varied according to study area (76:63 for Sikensi;  
193 73:38 for Agboville; 63:65 for Duekoué; and 27:54 for Adzopé). The proportion of  
194 *S. haematobium* cox1 was higher than that of *S. bovis* cox1 in Sikensi ( $p=0.026$ ) and Agboville

**Commented [KEA1]:** As the RD-PCR of cox1 gene is routinely used to identify mitochondrial cox1 signature in schistosomes, we did not display images.

195 (p<0.001), whereas *S. bovis* cox1 was the predominant species found in Adzopé (p<0.001). In  
196 Duekoué, *S. haematobium* cox1 and *S. bovis* cox1 were equally distributed (Figure 1). Table 1  
197 shows the proportion of *S. haematobium* cox1 and *S. bovis* cox1 from the 459 cox1 RD-PCR  
198 miracidia, stratified by study location at the unit of the child.

199

200 *Cox1 and ITS2 sequence analysis*

201 A total of 40 miracidia were sequenced. Good quality ITS2 sequences were obtained for all  
202 samples while, for cox1, only 33 samples yielded good sequences. Among the 33 cox1  
203 sequences, we identified 15 *S. haematobium* sequences (Table 1) belonging to seven distinct  
204 haplotypes with a very low diversity showing few single nucleotide polymorphisms (SNPs)  
205 (Supplementary file 1: Table S1) and 18 *S. bovis* sequences belonging to 12 distinct haplotypes  
206 (Supplementary file 2: Table S2). Haplotype diversity ( $\pm$ SD) was  $0.922 \pm 0.051$  and  $0.724 \pm 0.121$   
207 for *S. bovis* and *S. haematobium*, respectively. Nucleotide diversity ( $\pm$ SD) was  $0.0094 \pm 0.0013$   
208 and  $0.0011 \pm 0.0003$  for *S. bovis* and *S. haematobium*, respectively. Among the ITS2 sequences,  
209 31 gave a *S. haematobium* profile and four a *S. bovis* profile. Five miracidia gave double  
210 chromatogram peaks at the polymorphic positions between *S. haematobium* and *S. bovis*,  
211 suggesting heterozygosity (Huyse *et al.*, 2009; Webster *et al.*, 2013).

212

213 *Miracidia identified as hybrids and non-hybrids*

214 Analysis of both cox1 and ITS2 sequences together showed that *S. haematobium* x *S. bovis*  
215 miracidia were present in all the four study locations and were excreted by 12 children. Among  
216 these 12 children, nine excreted *S. haematobium* x *S. bovis* hybrids and seven excreted pure  
217 *S. haematobium*. Two children excreted both *S. haematobium* x *S. bovis* hybrids and pure  
218 *S. haematobium* miracidia, while one child excreted both *S. haematobium* x *S. bovis* hybrids  
219 and pure *S. bovis* miracidium (Table 1). Seventeen parasites were pure (16 *S. haematobium* and  
220 one *S. bovis*) and 23 were hybrids (57.5%). Among the six *S. haematobium* x *S. bovis*

221 combinations possible with our two markers (two possibilities in the haploid cox1  
222 [*S. haematobium* cox1; *S. bovis* cox1] and three possibilities in the diploid ITS2  
223 [*S. haematobium* ITS2; mixed *S. haematobium* ITS2-*S. bovis* ITS2; *S. bovis* ITS2]), four  
224 combinations indicate hybrids [*S. bovis* cox1 x *S. haematobium* ITS2; *S. bovis* cox1 x  
225 *S. haematobium* ITS2-*S. bovis* ITS2; *S. haematobium* cox1 x *S. haematobium* ITS2-*S. bovis*  
226 ITS2; *S. haematobium* cox1 x *S. bovis* ITS2]. Most of the hybrid profiles found were *S. bovis*  
227 cox1 x *S. haematobium* ITS2. Hybrids occurred at similar frequencies in all age classes.  
228

#### 229 *Cox1 phylogenies*

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

235

#### 236 **Discussion**

237 Hybridization of certain parasites is an emerging public health concern at the interface of  
238 infectious disease biology and evolution (King *et al.*, 2015). We have identified 47.9% of  
239 *S. bovis* cox1 profile by RD-PCR and 57.5% of *S. haematobium* x *S. bovis* hybrids using both  
240 cox1 and ITS2 sequences of miracidia collected in urine samples obtained from schoolchildren  
241 in Côte d'Ivoire. The analysis of mitochondrial cox1 gene sequences showed seven haplotypes  
242 for *S. haematobium* and 12 for *S. bovis*, which demonstrates the existence of a mitochondrial  
243 introgressive hybridization of *S. haematobium* cox1 by *S. bovis*. Similar results have been  
244 reported in Corsica (Moné *et al.*, 2015; Boissier *et al.*, 2016). Our findings were corroborated  
245 by analysis of the nuclear ITS2 region.

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

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

264 No *S. haematobium* x *S. mansoni* hybrids were identified, even though such hybrids have  
265 been shown in a migrant boy from Côte d'Ivoire upon examination in France (Le Govic *et al.*,  
266 2019). Recently, it has been shown that *Bulinus* snails from the northern and central parts of  
267 Côte d'Ivoire were infected with *S. bovis*, *S. haematobium* and/or *S. bovis* x *S. haematobium*  
268 hybrids (Tian-Bi *et al.*, 2019). The authors showed that *S. bovis* was particularly prevalent in  
269 *Bulinus truncatus*, *S. haematobium* was most prevalent in *B. globosus* and *S. haematobium* x  
270 *S. bovis* hybrids infected the two *Bulinus* species similarly. *S. bovis*-infected *Bulinus* were  
271 predominantly found in the northern part, while *S. haematobium* and hybrid-infected snails

272 were mainly found in the central part of Côte d'Ivoire. These results show the importance of  
273 snail's involvement in the transmission of *S. haematobium* x *S. bovis* hybrids.

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

279 The current study found that some children excreted both pure *S. haematobium* and  
280 *S. haematobium* x *S. bovis* hybrids, which is in line with observations in Senegal (Huyse *et al.*,  
281 2009; Webster *et al.*, 2013). Interestingly, we have also observed a single miracidium with a  
282 "pure" *S. bovis* signature (*S. bovis* ITS2 and *S. bovis* cox1) in one child, suggesting that this  
283 patient may be infected with *S. bovis*, which is traditionally considered a parasite of bovines.  
284 Such a "pure" *S. bovis* has been reported in eggs recovered from humans in Corsica (Boissier  
285 *et al.*, 2016). These accounts suggest that zoonotic transmission might occur. However,  
286 additional research is needed to confirm this speculation. We assume that the genome of  
287 *S. bovis* is strongly introgressed, and hence, it is plausible that we may have missed signatures  
288 of *S. haematobium* ancestry due to the standard analyses performed (Webster *et al.*, 2013). A  
289 broader coverage of the genome would no doubt identify even more hybrids and would allow  
290 a clearer distinction between "pure" parasites of each species and different levels of  
291 introgression.

292

### 293 Conclusion

294 Our study has shown that *S. haematobium* x *S. bovis* hybrids are common in *Schistosoma*  
295 egg-positive children in Côte d'Ivoire. Our observations are relevant because hybrid parasites  
296 could affect transmission dynamics, treatment efficacy and morbidity, which might jeopardize  
297 control of, and progress towards, elimination of schistosomiasis. Our findings are relevant as

298 the presence of hybrids calls into question our present understanding of parasite transmission  
299 and host ranges, which in turn may affect the effectiveness of current control strategies.  
300 Intensive prospection of domestic and wild animals is warranted to determine whether real  
301 zoonotic transmission occurs.

302  
303 **Supplementary files**

304 *Supplementary file 1:* Table S1: Nucleotide sequence at the five polymorphic sites within the 1  
305 200 bp cox1 sequences of the different *S. haematobium* haplotypes found using haplotype Sh1  
306 as reference. n, number of miracidia with the respective haplotypes. (DOCX 15 KB)

307  
308 *Supplementary file 2:* Table S2: Nucleotide sequence at the 27 polymorphic sites within the 1  
309 200 bp cox1 sequences of the different *S. bovis* haplotypes found, using haplotype Sb1 as  
310 reference. n, number of miracidia with the respective haplotype. (DOCX 21 KB)

311  
312 **Contributions of authors.** EKA, HM, OB and JB conceived and designated the study. EKA,  
313 OR and JB wrote the first draft of the manuscript. EKA and JFA performed the molecular  
314 analyses. EKA and JB performed statistical analysis. OB, AOT, JTC, WY, JU and OB revised  
315 the manuscript. All authors read and approved the final manuscript prior to submission.

316  
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328

329 **Ethical standards.** Ethical clearance for this study was obtained from the Ministère de la Santé  
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331 informed consent was obtained from children’s parents or legal guardians. Oral assent was  
332 obtained from children.

333

334 **References**

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- 458

459 **Figure legends**

460

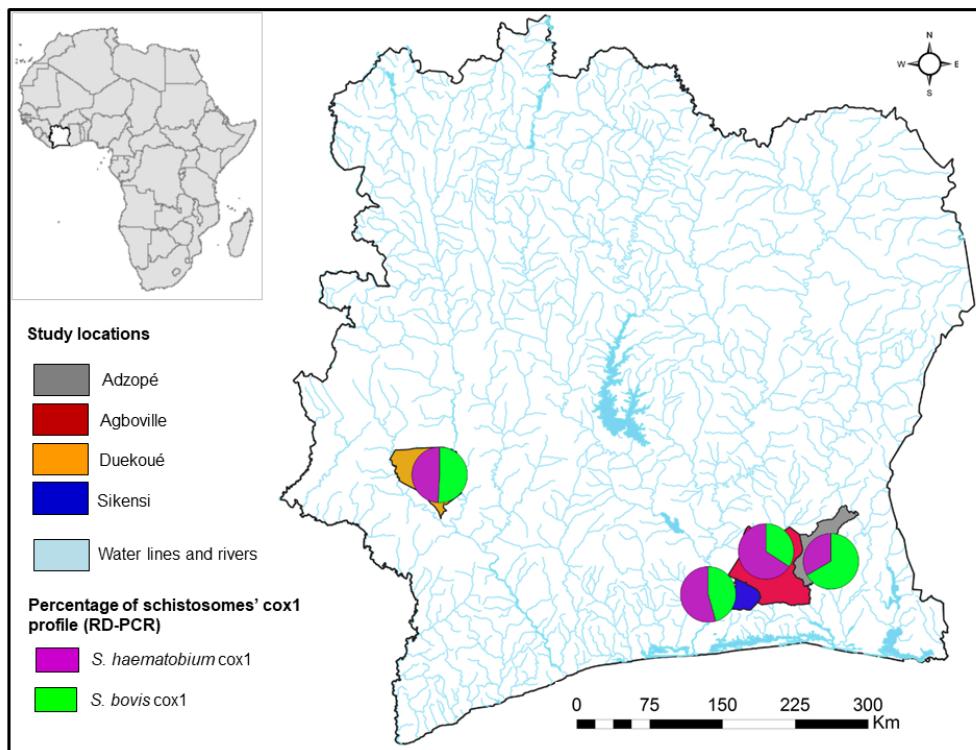
461 **Fig. 1.** Map of the four study locations in Côte d'Ivoire showing the distribution and the  
462 proportion of *Schistosoma haematobium* cox1 and *Schistosoma bovis* cox1 identified by RD-  
463 PCR.

464

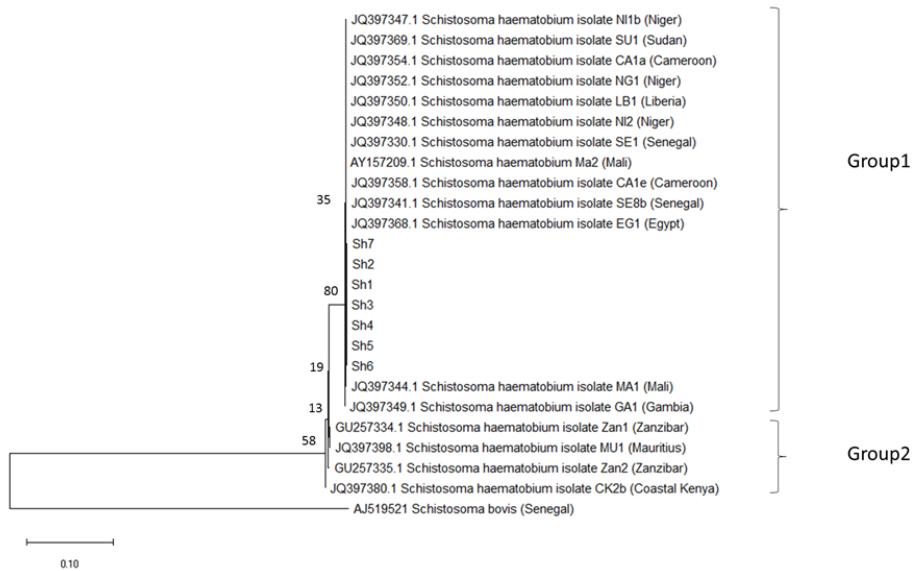
465 **Fig. 2.** Majority rule consensus tree from maximum likelihood analysis of 1,200 bp  
466 mitochondrial cox1 sequences for *Schistosoma haematobium* haplotypes Sh1-Sh7 and  
467 sequences downloaded from GenBank. Groups 1 and 2 indicate the major clades defined by  
468 Webster *et al.* (2012). Clade support values for each node are maximum parsimony bootstrap  
469 percentages.

470

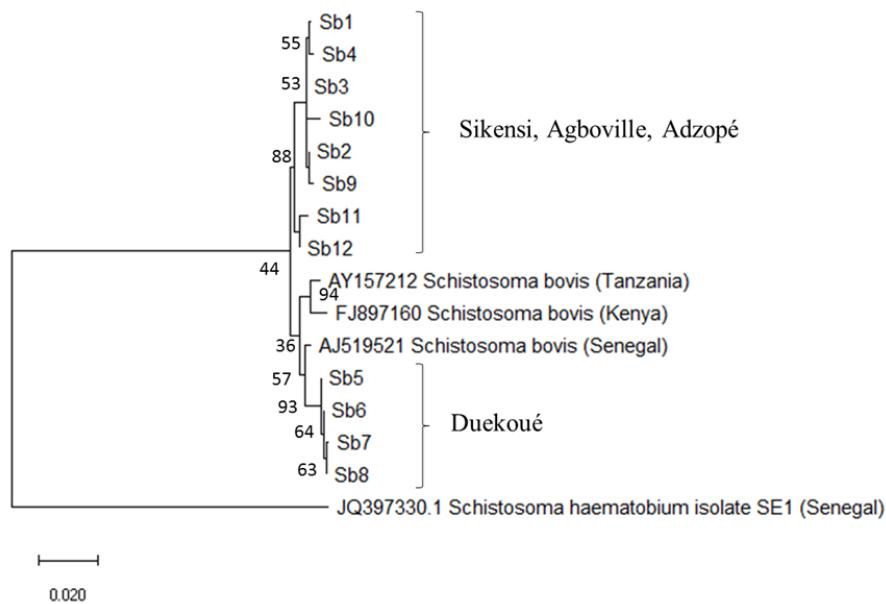
471 **Fig. 3.** Majority rule consensus tree from maximum likelihood analysis of 1,200 bp  
472 mitochondrial DNA cox1 sequences for *Schistosoma bovis* haplotypes Sb1-Sb12 and sequences  
473 downloaded from GenBank. There are two clusters of *S. bovis* haplotypes: Duekoué (Sb5-Sb8)  
474 and the three other study sites (Sb1-Sb4 and Sb9-Sb12). Clade support values for each node are  
475 maximum parsimony bootstrap percentages. *S. bovis* reference sequences come from parasites  
476 collected from animals.



254x190mm (96 x 96 DPI)



254x190mm (96 x 96 DPI)



254x190mm (96 x 96 DPI)

**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. 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.

Study location	Patient	RD-PCR analysis (n = 459)				Sequence analysis (n = 40)				
		n	<i>S. haematobium</i>	<i>S. bovis</i> cox1	<i>p</i> -value	n	No. of times observed	cox1 haplotypes	ITS2 alleles	
			cox1 (%)	(%)						
Adzopé	AD138	31	3 (9.7)	28 (90.3)	0.0001	5	1	<i>S. bovis</i> Sb3	<i>S. haematobium</i>	Hybrid
						1	1	<i>S. bovis</i> Sb12	<i>S. haematobium</i>	Hybrid
						1	1	<i>S. bovis</i> undet*	<i>S. haematobium</i>	Hybrid
						2	2	<i>S. haematobium</i> Sh1	<i>S. haematobium</i>	<i>S. haematobium</i>
	AD140	27	5 (18.5)	22 (81.5)		3	1	<i>S. haematobium</i> Sh1	<i>S. haematobium</i>	<i>S. haematobium</i>
						1	1	<i>S. haematobium</i> Sh2	<i>S. haematobium</i>	<i>S. haematobium</i>
						1	1	<i>S. haematobium</i> undet*	<i>S. haematobium</i>	<i>S. haematobium</i>
AD145	23	19 (82.6)	4 (17.4)	0.0001	2	1	1	<i>S. bovis</i> Sb11	<i>S. haematobium</i>	Hybrid
						1	1	<i>S. bovis</i> Sb9	<i>S. haematobium</i>	Hybrid
Total	81	27 (33.3)	54 (66.7)							

Agboville	AG062	19	13 (68.4)	6 (31.6)	1	1	<i>S. bovis</i> Sb10	<i>S. haematobium</i> + <i>S. bovis</i>	Hybrid
	AG068	23	23 (100)	0	3	2	<i>S. haematobium</i> Sh3	<i>S. bovis</i>	Hybrid
						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*	<i>S. haematobium</i> + <i>S. bovis</i>	Hybrid
						1	<i>S. bovis</i> Sb9	<i>S. bovis</i>	<i>S. bovis</i>
	AG108	13	9 (69.2)	4 (30.8)	2	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)	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	2	1	<i>S. haematobium</i> Sh1	<i>S. haematobium</i>	<i>S. haematobium</i>
						1	<i>S. haematobium</i> undet*	<i>S. haematobium</i>	<i>S. haematobium</i>
	DU337	35	35 (100)	0	2	2	<i>S. haematobium</i> Sh1	<i>S. haematobium</i>	<i>S. haematobium</i>
	DU345	30	11 (36.7)	19 (63.3)	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)	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)	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	SI028	30	18 (60.0)	12 (40.0)	2	1	<i>S. bovis</i> Sb3	<i>S. haematobium</i>	Hybrid
						1	<i>S. bovis</i> Sb4	<i>S. haematobium</i>	Hybrid
	SI052	26	12 (46.2)	14 (53.8)	2	1	<i>S. haematobium</i> Sh7	<i>S. haematobium</i>	<i>S. haematobium</i>
						1	<i>S. haematobium</i> undet*	<i>S. haematobium</i>	<i>S. haematobium</i>
	SI109	22	9 (40.9)	13 (59.1)	1	1	<i>S. haematobium</i> undet*	<i>S. haematobium</i>	<i>S. haematobium</i>
	SI114	19	11 (57.9)	8 (42.1)	1	1	<i>S. bovis</i> Sb3	<i>S. haematobium</i>	Hybrid
SI122		19	11 (57.9)	8 (42.1)	2	1	<i>S. bovis</i> Sb2	<i>S. haematobium</i> + <i>S. bovis</i>	Hybrid
						1	<i>S. bovis</i> Sb1	<i>S. haematobium</i>	Hybrid
	SI136	23	15 (65.2)	8 (34.8)	2	1	<i>S. haematobium</i> Sh1	<i>S. haematobium</i>	<i>S. haematobium</i>
						1	<i>S. haematobium</i> undet*	<i>S. haematobium</i>	<i>S. haematobium</i>
Total	139	76 (54.7)	63 (45.3)	0.026					

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

p-value<0.05 was considered significant

\* undet., sequences for which the exact haplotype could not be determined due to sequence quality

**Supplementary file 1**

**Table S1.** Nucleotide sequence at the five polymorphic sites within the 1200 bp cox1 sequences of the different *S. haematobium* haplotypes found, using haplotype Sh1 as reference. n, number of miracidia with the respective haplotypes.

Haplotype	n	Position of polymorphic site				
		13	18	66	635	998
Sh1	8	T	A	T	G	T
Sh2	1	.	.	.	.	A
Sh3	2	G	.	C	.	.
Sh4	1	G	T	C	.	.
Sh5	1	.	.	C	.	.
Sh6	1	G	.	.	.	.
Sh7	1	.	.	.	A	.

**Supplementary file 2**

**Table S2.** Nucleotide sequence at the 27 polymorphic sites within the 1200 bp cox1 sequences of the different *S. bovis* haplotypes found, using haplotype Sb1 as reference. n, number of miracidia with the respective haplotype.

Haplotype	n	Position of polymorphic site																										
		106	108	132	133	136	192	293	295	319	353	355	616	666	685	712	763	829	949	973	976	980	982	994	996	1000	1001	1008
Sb1	1	T	T	G	T	G	A	A	A	C	C	T	T	C	C	G	C	T	G	C	C	A	G	C	T	G	A	
Sb2	1	.	C	.	.	.	T	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
Sb3	5	.	C	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
Sb4	1	.	C	A	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
Sb5	1	.	C	.	.	A	.	G	G	T	T	G	.	T	T	A	T	C	A	A	.	G	A	T	.	.		
Sb6	1	.	.	.	.	A	.	G	G	T	T	G	.	T	T	A	T	C	A	A	.	G	A	T	.	.	.	
Sb7	2	C	.	.	.	A	T	G	G	T	T	G	.	T	T	A	T	C	A	A	.	G	A	T	.	.	.	
Sb8	1	.	.	.	.	A	T	G	G	T	T	G	.	T	T	A	T	C	A	A	.	G	A	T	.	.	.	
Sb9	2	C	.	.	.	.	T	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Sb10	1	.	C	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	A	C	C	C	C
Sb11	1	C	.	.	.	.	T	G	.	.	.	C	.	T	.	T	.	A	A	T	.	.	.	.	.	.	.	
Sb12	1	.	C	.	.	.	.	G	.	.	.	C	.	T	.	T	.	A	A	T	.	.	.	.	.	.	.	