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).

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

This study molecularly characterised schistosome miracidia collected from schoolchildren in four locations in Côte d'Ivoire to investigate the presence and extent of *S. haematobium* x *S. 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 1,187 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 nearby health centres for parasitological examination. *S. 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. 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 on a petri dish filled with fresh 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 a laboratory in Perpignan, France pending molecular analysis.

Diagnostic by polymerase chain reaction (PCR)

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, 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.

Extracted DNAs were analysed using a rapid diagnostic mitochondrial *cox1* RD-PCR in order to infer mitochondrial species designation. We employed 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 DNA extract, 2 µl of Green GoTaq flexi buffer 5X (Promega; Madison, USA), 0.6 µl of 25 mM MgCl₂ (Promega; Madison, USA), 0.2 µl of 10 mM dNTP mix (Promega; Madison, USA), 1 µl of 10x primer mix (4 µl of 100 µM reverse primer, 4 µl of each 100 µM forward primer and 84 µl of double distilled water) and 1 U of GoTaq Hot Start Polymerase (Promega; Madison, USA). The reaction conditions included an activation step of 95°C for 3 min, followed by 45 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 for 2 min. The *cox1*-PCR products were visualized on 3% percent agarose gels stained with ethidium bromide. The prevalence of schistosomes with each mitochondrial *cox1* signature was computed and stratified by study location using EpiInfo version 7 (Centers for Disease Control and Prevention; Atlanta, USA). Fisher's exact test was used and a p-value of 0.05 was considered significant.

Cox1 and ITS2 sequencing

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. Overall, 40 miracidia were sequenced on both mitochondrial cox1 and nuclear ITS2 gene 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; Madison, USA), 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; Madison, USA). The PCR conditions were the same for both markers: 3 min at 95°C, followed by 45 cycles at 95°C for 40 sec, 48°C for 40 sec and 72°C for 70 sec, 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% percent agarose gels stained with ethidium bromide to verify band size (expected size 1,200 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, USA). All sequences were aligned using BioEdit version 7.0.9 (Ibis Therapeutic; Carlsbad, USA) and compared to sequences deposited in the GenBank Nucleotide Database. 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 diversities ($\pm$ standard deviation [SD]) 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; Pennsylvania, USA) and employing a maximum likelihood and the HKY + G nucleotide substitution model, which was determined by MEGA version 6.0.6 (Penn State University; Pennsylvania, USA) as the model best describing the data. The support for tree nodes was calculated with 1,000 bootstrap iterations. The phylogenies include all the haplotypes identified in this study plus some reference haplotypes obtained from GenBank Nucleotide Database. The phylogeny of the *S. bovis* cox1 data was rooted with a *S. haematobium* haplotype (JQ397330.1) and the *S. haematobium* cox1 data with a *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 1,187 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 hatched eggs of 19 *Schistosoma*-infected children on Whatman® FTA cards and the cox1 RD-PCR was successful for 459 miracidia. Of these, 239 miracidia (52.1%) gave a *S. haematobium* cox1 profile and 220 (47.9%) gave a *S. bovis* cox1 profile, with no statistically significant difference between the two proportions ($p=0.081$). No miracidia gave a *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

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

($p < 0.001$), 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 (Figure 1). Table 1 shows the proportion of *S. haematobium* **cox1** and *S. bovis* **cox1** from the 459 **cox1** RD-PCR **miracidia**, stratified by study location at the unit of the child.

Cox1 and ITS2 sequence analysis

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 (SNPs) (Supplementary file 1: Table S1) and 18 *S. bovis* sequences belonging to 12 distinct haplotypes (Supplementary file 2: Table S2). Haplotype diversity ($\pm$ SD) was 0.922 ± 0.051 and 0.724 ± 0.121 for *S. bovis* and *S. haematobium*, respectively. Nucleotide diversity ($\pm$ SD) was 0.0094 ± 0.0013 and 0.0011 ± 0.0003 for *S. bovis* and *S. haematobium*, respectively. Among the ITS2 sequences, 31 gave a *S. haematobium* profile and four a *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

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

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

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). We have identified 47.9% of *S. bovis* *cox1* profile by RD-PCR and 57.5% of *S. haematobium* x *S. bovis* hybrids using both *cox1* and ITS2 sequences of miracidia collected in urine samples obtained from schoolchildren in Côte d'Ivoire. The analysis of mitochondrial *cox1* gene sequences 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.

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

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* x *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 in, 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* x *S. bovis* hybrids, which is in line with observations in 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* x *S. 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

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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318
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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 children’s parents or legal guardians. Oral assent was obtained from children.

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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>cox1</i> (%)	<i>S. bovis</i> <i>cox1</i> (%)	<i>p-value</i>	n	No. of times observed	<i>cox1</i> haplotypes	ITS2 alleles	Classification
Adzopé	AD138	31	3 (9.7)	28 (90.3)	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*	<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)		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*	<i>S. haematobium</i>	<i>S. haematobium</i>
	AD145	23	19 (82.6)	4 (17.4)		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)	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
<i>Sh1</i>	8	T	A	T	G	T
<i>Sh2</i>	1	.	.	.	.	A
<i>Sh3</i>	2	G	.	C	.	.
<i>Sh4</i>	1	G	T	C	.	.
<i>Sh5</i>	1	.	.	C	.	.
<i>Sh6</i>	1	G	.	.	.	.
<i>Sh7</i>	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
<i>Sb1</i>	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	G	A
<i>Sb2</i>	1	.	C	.	.	.	T	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
<i>Sb3</i>	5	.	C	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
<i>Sb4</i>	1	.	C	A	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
<i>Sb5</i>	1	.	C	.	.	A	.	G	G	T	T	G	.	T	T	A	T	C	A	A	.	G	A	T	.	.	.	.
<i>Sb6</i>	1	.	.	.	.	A	.	G	G	T	T	G	.	T	T	A	T	C	A	A	.	G	A	T	.	.	.	.
<i>Sb7</i>	2	C	.	.	.	A	T	G	G	T	T	G	.	T	T	A	T	C	A	A	.	G	A	T	.	.	.	.
<i>Sb8</i>	1	.	.	.	.	A	T	G	G	T	T	G	.	T	T	A	T	C	A	A	.	G	A	T	.	.	.	.
<i>Sb9</i>	2	C	.	.	.	.	T	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
<i>Sb10</i>	1	.	C	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	A	C	C	C
<i>Sb11</i>	1	C	.	.	.	.	T	G	.	.	.	.	C	.	T	.	T	.	A	A	T	.	.	.	.	.	.	.
<i>Sb12</i>	1	.	C	.	.	.	.	G	.	.	.	.	C	.	T	.	T	.	A	A	T	.	.	.	.	.	.	.