# Population genetic structure and hybridization of schistosomes in schoolchildren in Côte d'Ivoire

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# Summary

**Background:** Schistosomiasis is a water-based chronic parasitic disease caused by trematode worms of the genus *Schistosoma*. Considered as a neglected tropical disease by the World Health Organization (WHO), it affects more than 250 million people, primarily in sub-Saharan Africa. In Côte d'Ivoire, both *Schistosoma haematobium* (causing urogenital schistosomiasis) and *Schistosoma mansoni* (causing intestinal schistosomiasis) co-exist. The chronic disease is characterized by anaemia, abdominal pain, bloody stool or urine, portal hypertension, swelling of the liver, enlarged spleen, and degradation of intestinal or urogenital organs. The main strategy for control endorsed by WHO is preventive chemotherapy using praziquantel. Despite mass drug administration campaigns, the prevalence of schistosomiasis remains high in many parts of Côte d'Ivoire. Investigating the population genetics of schistosomes, using molecular markers, may help elucidate its dynamic epidemiology and provide better insight into parasite gene flow.

**Objectives:** This Ph.D. thesis had two major aims. The first was to expand our understanding of the parasite's transmission dynamics to update epidemiological data on schistosomiasis by determining the prevalence and associated risk factors of the disease. The second aim was to better understand the genetic structure and diversity of *S. haematobium* in schoolchildren.

**Methods:** Urine samples were examined from schoolchildren in four sampling sites (Adzopé, Agboville, Duekoué and Sikensi). A filtration method was used to identify *S. haematobium* eggs, while stool samples were subjected to duplicate Kato-Katz thick smears to identify and quantify eggs of *S. mansoni* and soil-transmitted helminths. Data on sociodemographic, socioeconomic and environmental factors were obtained using a pretested questionnaire. Eggs were hatched and miracidia were individually collected and stored on Whatman® FTA cards for molecular analysis. Individual miracidia were molecularly characterized by analysis of mitochondrial cox1 gene and nuclear internal transcribed spacer 2 (ITS 2) DNA region. The cox1 and ITS 2 amplicons were Sanger sequenced from 40 randomly selected miracidia to confirm species and hybrids status. To achieve our second aim on the genetic distribution of *S. haematobium*, we systematically first reviewed the scientific literature of studies focused on methods used for population genetic structuring and diversity of *S. haematobium* and we genotyped and analysed miracidia using a set of 18 microsatellite markers.

**Results:** Of 1,187 schoolchildren, the overall prevalence was 14.0% for *S. haematobium* and 6.1% for *S. mansoni.* In the southern part of Côte d'Ivoire, the prevalence of *S. haematobium* was 16.1% with a particularly high prevalence in Sikensi (35.6%), while *S. mansoni* was most prevalent in Agboville (11.2%). We found that fishing and washing clothes in open freshwater bodies and swimming were positively associated with schistosomiasis. 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 ITS2 sequences analysed, 77.5% were assigned to pure *S. haematobium*, 10.0% to pure *S. bovis* and 12.5% to *S. haematobium* x *S. bovis* hybrids. The literature review elucidated that few methods are available for the population genetics of *S. haematobium*. Variable genetic diversity and population structure were observed within and across countries. We found that microsatellite markers are commonly used for *Schistosoma* population genetics research and they remain the best tool for population genetic analysis. Of 2,164 miracidia successfully amplified in both cox1 and ITS2, 1,966 (90.9%) yielded reliable peak calls for at least ten microsatellite loci and were retained for population genetic analysis. Significant differences were found between sampling sites in all genetic diversity indices and a genetic structure was observed. The analysis of cox1-ITS profile showed that no significant genetic clustering was observed between *S. haematobium* and hybrids *S. haematobium* x *S. bovis*. We also observed a clear separation between "pure" *S. bovis* (Sb_SbSb) and the other cox1-ITS profiles.

**Conclusion:** Our study confirms that schistosomiasis remains prevalent in the southern and western parts of Côte d'Ivoire, and we reported that *S. haematobium* x *S. bovis* hybrids are common in Côte d'Ivoire. The current results provide novel insights about the population genetics of *S. haematobium* in Côte d'Ivoire and reiterate the utility of microsatellite markers designed for this parasite. A genetic diversity and population structure were observed and "pure" *S. bovis*, previously thought to infect bovines, was identified in children samples. Hence, intense investigation in domestic and wild animals is warranted to determine whether zoonotic transmission occurs. These findings could serve as a benchmark to enhance schistosomiasis control and surveillance to measure the effect of treatment on parasite population genetics in Côte d'Ivoire. New research is needed on population genetics in human and animal schistosomes to evaluate the parasite's gene flow.

# Résumé

**Contexte et Justification :** La schistosomiase est une maladie parasitaire chronique causée par des vers trématodes du genre *Schistosoma*. Considérée comme une maladie tropicale négligée par l'Organisation Mondiale de la Santé (OMS), cette parasitose touche plus de 250 millions de personnes, principalement en Afrique subsaharienne. En Côte d'Ivoire, *Schistosoma haematobium* (agent de la schistosomiase urogénitale) et *Schistosoma mansoni* (agent de la schistosomiase intestinale) coexistent. La chronicité de cette affection se caractérise par une anémie, des douleurs abdominales, des selles ou des urines sanglantes, une hypertension portale, une hépatomégalie, une splénomégalie et une atteinte des organes intestinaux ou urogénitaux. La principale stratégie de lutte approuvée par l'OMS est la chimiothérapie préventive à base de praziquantel. Malgré les campagnes de traitement de masse, la prévalence de la schistosomiase reste élevée dans de nombreuses régions de la Côte d'Ivoire. L'étude de la génétique des populations de schistosomes, à l'aide de marqueurs moléculaires, permettra à élucider sa dynamique de transmission et à mieux comprendre le flux de gènes du parasite.

**Objectifs :** Cette thèse de doctorat avait deux buts principaux. Le premier était d'élargir notre connaissance de la dynamique de transmission du parasite afin d'actualiser les données épidémiologiques sur la schistosomiase en déterminant la prévalence et les facteurs de risque associés à la maladie. Le second but était d'étudier la structuration et la diversité génétique de *S. haematobium* chez les écoliers.

**Matériel et méthodes :** Des échantillons d'urines ont été collectés chez les écoliers dans quatre sites d'étude : Adzopé, Agboville, Duekoué et Sikensi. La méthode de filtration des urines a été utilisée pour identifier les œufs de *S. haematobium*, tandis que les selles ont été soumises à la méthode de Kato-Katz afin d'identifier et quantifier les œufs de *S. mansoni* et des géohelminthes. Les données sur les paramètres sociodémographiques, socio-économiques et environnementaux ont été obtenues à l'aide d'un questionnaire pré-testé. Après éclosion des œufs, les miracidia ont été collectés et stockés individuellement sur des cartes Whatman® FTA pour une analyse moléculaire. Les miracidia ont été individuellement caractérisés par analyse moléculaire du gène mitochondrial (cox1) et de la région interne transcrit 2 (ITS 2) de l'ADN nucléaire. Les amplicons de cox1 et ITS 2 ont été séquencés par la méthode Sanger à partir de 40 miracidia choisis au hasard pour déterminer le profil génétique. Pour atteindre notre second but, nous avons effectué une revue de la littérature axée sur les méthodes utilisées dans la génétique des populations de *S. haematobium*. Les miracidia ont été génotypés en utilisant un ensemble de 18 marqueurs microsatellites afin d'analyser la structuration et la diversité génétique.

**Résultats :** Sur 1187 écoliers, la prévalence globale était de 14,0% pour *S. haematobium* et de 6,1% pour *S. mansoni*. Au sud de la Côte d'Ivoire, la prévalence de *S. haematobium* était de 16,1 %, avec une fréquence particulièrement élevé à Sikensi (35,6 %), tandis que *S. mansoni* était plus répandu à Agboville (11,2 %). La pêche, le lavage des vêtements dans des plans d'eau douce et la baignade ont été identifiés comme facteurs de risque de la schistosomiase. Parmi les 33 séquences de Cox1 analysées, 15 de *S. haematobium* (45,5%) appartenant à sept haplotypes et 18 de *S. bovis* (54,5%) appartenant à 12 haplotypes. Sur les 40 séquences de ITS2 analysées, 77,5% étaient des *S. haematobium* purs, 10,0% à des *S. bovis* purs et 12,5% étaient des hybrides *S. haematobium* x *S. bovis*. La revue de la littérature a permis de constater que peu de méthodes sont disponibles pour l'étude de la génétique des populations de *S. haematobium*. Une diversité et une structuration génétique variables ont été observées à l'intérieur et entre les pays. Il ressort que les marqueurs microsatellites étaient les plus utilisés pour l'analyse génétique des schistosomes. Sur les 2164 miracidia amplifiées avec succès par la PCR de cox1 et ITS2, 1966 (90,9%) avaient des pics fiables pour au moins dix marqueurs microsatellites et ont été retenus pour l'analyse génétique des populations. Des différences significatives ont été obtenues entre les sites d'étude pour tous les indices de diversité génétique et une structure génétique a été observée. L'analyse des génotypes de parasites n'a montré aucun regroupement génétique significatif entre *S. haematobium* et les hybrides *S. haematobium* x *S. bovis*. Par contre, une structuration génétique nette entre *S. bovis* pur (Sb_SbSb) et les autres profils génétiques a été observée.

**Conclusion :** Notre étude confirme que la schistosomiase est toujours endémique au sud et à l'ouest de la Côte d'Ivoire avec une fréquence élevée d'hybrides entre *S. haematobium* x *S. bovis*. Ces résultats fournissent de indications sur la génétique des populations de *S. haematobium* en Côte d'Ivoire et réitèrent l'utilité des marqueurs microsatellites conçus pour ce parasite. Une diversité et une structure génétique ont été observées et *S. bovis* pur, espèce animale, a été identifiée chez les écoliers. Il est donc opportun de mener d'autres études chez les animaux domestiques et sauvages afin de rechercher un réservoir animal. Ces résultats pourraient servir de référence pour améliorer le contrôle et la surveillance de la schistosomiase afin de mesurer l'effet du traitement sur la diversité génétique des schistosomes en Côte d'Ivoire. De nouvelles recherches sont également nécessaires chez les schistosomes humains et animaux afin d'évaluer le flux génétique du parasite.

# List of Abbreviations

| | |
|---|---|
| **ATP** | Adenosine triphosphate |
| **Cox** | Cytochrome oxidase |
| **DALYs** | Disability-adjusted life years |
| **DNA** | Deoxyribonucleic acid |
| **eDNA** | Environmental deoxyribonucleic acid |
| **ELISA** | Enzyme-linked immunosorbent assay |
| **ETS** | External transcribed sequence |
| **GBD** | Global Burden of Disease |
| **HALE** | Healthy life expectancy |
| **HIV** | Human immunodeficiency virus |
| **IFAT** | Immunofluorescent-antibody test |
| **IGS** | Intergenic spacer |
| **IHA** | Indirect haemagglutination assay |
| **ITS** | Internal transcribed spacer |
| **LAMP** | Loop-mediated isothermal amplification |
| **LTR** | Long terminal repeat |
| **MDA** | Mass drug administration |
| **MEG** | Micro exon genes |
| **NAD** | Nicotinamide adenine dinucleotide |
| **NTS** | Non-transcribed spacer |
| **PCA** | Principal component analysis |
| **PCR** | Polymerase chain reaction |
| **PNLMTN-CP** | Programme National de Lutte contre les Maladies Tropicales Negligées à Chimioprophylaxie Préventive |
| **RAPD** | Randomly amplified polymorphic DNA |
| **RFLP** | Restriction fragment length polymorphism |
| **RNA** | Ribonucleic acid |
| **RPA** | Recombinase polymerase amplification |
| **rRNA** | Ribosomal ribonucleic acid |
| **rrnL** | Large rRNA subunit |
| **rrnS** | Small rRNA subunit |

| | |
|---|---|
| ***Sm*PoMuc** | *Schistosoma mansoni* mucin-like proteins |
| ***Sm*SULT-OR** | *Schistosoma mansoni*-specific sulfotransferase |
| **SNP** | Single nucleotide polymorphism |
| **STHs** | Soil-transmitted helminths |
| **tRNA** | Transfer ribonucleic acid |
| **VNTR** | Variable number of tandem repeats |
| **WHO** | World Health Organisation |

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# Chapter 1:

# Introduction

## 1.1 Epidemiology and global burden of schistosomiasis

Schistosomiasis is a chronic neglected tropical disease caused by the genus *Schistosoma*, which is widespread in sub-Saharan Africa (Lai et al., 2015). It is a parasitic disease of humans and animals and causes serious public health and veterinary concerns worldwide, particularly in tropical and subtropical areas. Schistosomiasis ranks second to malaria in terms of morbidity and mortality in humans (King, 2010). The World Health Organization (WHO) estimates that the disease is transmitted in over 78 countries, throughout a wide belt of the tropics and subtropics (WHO, 2018) **(Figure 1.1)**. The number of schistosomiasis cases worldwide is considered to be greater than 250 million, mostly in Africa (Hotez et al., 2014). The disability-adjusted life years (DALYs) index of schistosomiasis was estimated at 1.4 million in 2017 (GBD 2017 DALYs and HALE Collaborators, 2018). Typically, the infection occurs in childhood with the highest prevalence and intensity in young adolescents and generally decreasing in adulthood. Moreover, people are infected by contact with contaminated water during daily activities such as laundry, bathing, fishing or playing. In endemic areas, serological surveys showed that almost every long-term resident becomes infected with schistosomes at some point in their life ( Steinmann et al., 2006; Colley et al., 2014). Six species of schistosomes can infect humans, such as *Schistosoma mansoni*, *S. haematobium*, *S. intercalatum* and *S. guineensis* in Africa, and *S. mekongi* and *S. japonicum* in Asia (Rollinson et al., 1997). Each species has a specific range of suitable snail hosts, so the distribution is defined by host snail habitat range **(Table 1.1)**. Environmental and climate changes can either increase or decrease the transmission of schistosomiasis (Steinmann et al., 2006; Wang et al., 2009). Change in snail habitat and predators are crucial determinants of transmission, and prepatent periods can affect the efficacy of treatment regimens (Utzinger et al., 2007).

In endemic regions of schistosomiasis, the most prevalent form of the disease is chronic schistosomiasis, resulting from repeated exposure to infectious cercariae. In such settings, a child's initial infection often occurs by age 2 years with the burden of infection increasing in intensity during the next 10 years as new worms colonize child's body (Colley et al., 2014).

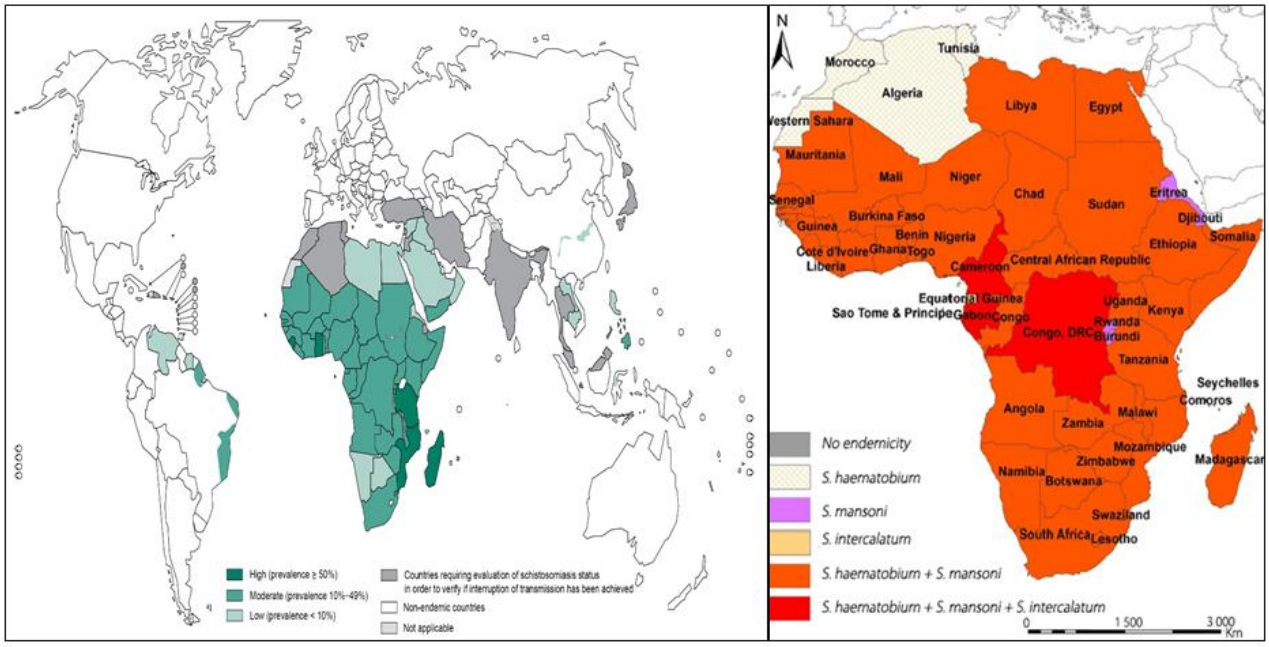


**Figure 1.1: Worldwide distribution of schistosomiasis** (Abdullahi Hudu and Umar, 2019; Colley et al., 2014; WHO, 2018).

**Table 1.1: Parasite species and geographical distribution of human schistosomiasis**.
Adapted from: (Manson et al., 1987; WHO, 2018)

| | Species | Intermediate host | Endemic area |
|---|---|---|---|
| **Intestinal schistosomiasis** | *Schistosoma guineensis* | *Bulinus forskalii* | West Africa |
| | *Schistosoma intercalatum* | *Bulinus spp.* | Africa |
| | *Schistosoma japonicum* | *Oncomelania spp.* | China, East Asia, Philippines |
| | *Schistosoma mansoni* | *Biomphalaria spp.* | Africa, the Middle East, the Caribbean, South America |
| | *Schistosoma mekongi* | *Neottricula aperta* | Several districts of Cambodia and the Lao People's Democratic Republic, Southeast Asia |
| **Urogenital schistosomiasis** | *Schistosoma haematobium* | *Bulinus spp.* | Africa, Middle East, Corsica (France) |

## 1.2. Biology and life cycle of the parasite

*Schistosoma* adult worms are digenetic trematodes with two separated sexes. They have an oral and ventral sucker and live within the blood vessel lumen in contact with the vascular endothelium; their longevity is great and can reach 20 years (Olson et al., 2003). The different species have a similar morphology and the sexual dimorphism is very evident. The male is more robust than the female, measuring 10 to 15 mm with a longitudinal gutter, the gynecophoral canal, in which the female resides during mating (Humans, 2012). Females are longer and thinner, measuring 15 to 20 mm with a tegumental texture like an elephant trunk. Females remain in a male's gynecophoral canal in a monogamous state of copulation for many years inside the host, where they produce tens to thousands of eggs daily (Cheever et al., 1994). Eggs released by females are passed out of the body in faeces (intestinal schistosomiasis) or urine (urogenital schistosomiasis) to continue the life cycle or remain lodged in body tissues and organs such as the liver. They contain a mobile miracidium 70 to 200 µm long according to species. The miracidium as the first larval form is about 100 µm long. Cercariae are the second larval form and measure about 500 µm. They are viable for one to three days and in this period, they pursue their specific host using prototaxis and chemotaxis (McKerrow and Salter, 2002). The head of the cercariae is connected to a forked tail, which has earned its name furcocercariae.

Humans are infected during contact with freshwater bodies contaminated by cercariae. The cercariae penetrate through host skin using the propulsive force of their tail combined with secreting serine proteases from the acetabular gland that digest skin proteins. The tail breaks off during the penetration process that takes 24 hours (Salter et al., 2000). Young parasites (schistosomula) migrate with the bloodstream via the lungs to the liver, where they mature into adult worms in the portal vein and subsequently mate. The paired worms migrate against the bloodstream and relocate to the perivesicular veins of mesenteries, or in the case of urogenital schistosomiasis, vesicular veins of the bladder or urogenital organs (Gryseels, 2012). At around four to six weeks after infection, they start producing eggs, which continues throughout their adult life. The lifespan of an adult worm averages 3-5 years, but can be longer than 30 years, and they employ many immune evasion mechanisms to protect themselves from the humoral environment of the host (Gryseels et al., 2006; Pearce and MacDonald, 2002). An infected person probably harbours an average of hundreds of worms. Some animal schistosomes can have the same intermediate host as human ones; e.g *S. bovis* shares *Bulinus* as an intermediate host with *S. haematobium* **(Figure 1.2)**.

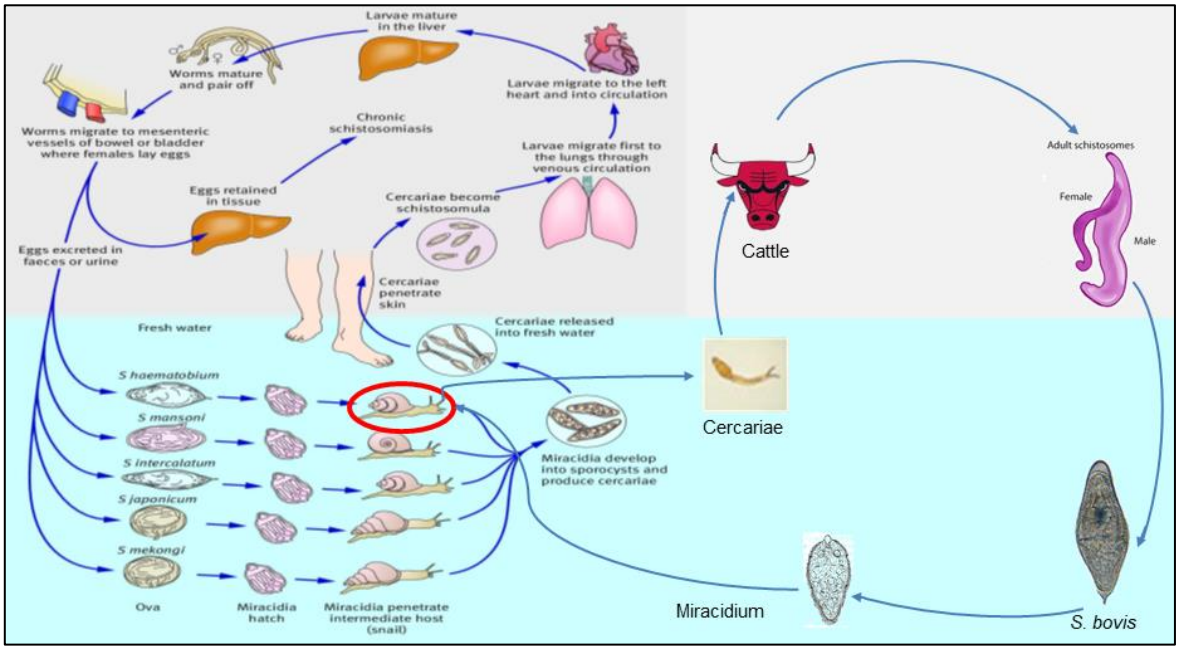


**Figure 1.2: The life cycle of *Schistosoma spp***. Adapted from: (Gray et al., 2011; Moné et al., 2015; Boon et al., 2018)

## 1.3. Pathogenesis and morbidity

Schistosome eggs, and not adult worms, are known to induce the morbidity caused by schistosome infections (Burke et al., 2009). Many eggs are not excreted and become permanently lodged in the intestines or liver (intestinal schistosomiasis) or the bladder and urogenital system (urogenital schistosomiasis). Within 12 hours of infection, an individual may complain of a tingling sensation or papular rash, commonly referred to as "swimmer's itch", due to irritation at the point of penetration of cercariae through the skin (James et al., 2005). Symptoms usually appear 4–6 weeks from the time of infection. Eggs induce a granulomatous host immune response largely characterized by lymphocytes (which mainly produce T-helpers cytokines: interleukins 4, 5 and 13), eosinophils and, alternatively, activated macrophages (Pearce and MacDonald, 2002; Fairfax et al., 2012). These granulomas contain egg proteolytic enzymes to prevent tissue necrosis, but the process of granuloma formation induces chronic inflammation that leads to clinical symptoms of schistosomiasis (Peterson and Von Lichtenberg, 1965).

Schistosomiasis begins with an acute phase usually called Katayama syndrome. Symptoms include fever, myalgia, eruption of pale temporary bumps associated with severe itching (urticarial) rash, headache, eosinophilia, fatigue, coughing and abdominal pain (Ross et al., 2007). This acute schistosomiasis (Katayama fever) may occur weeks or months after the initial infection as a systemic reaction against migrating schistosomula (Gryseels et al., 2006). Similar to swimmer's itch, Katayama fever is more commonly seen in people with a first infection such as migrants or tourists, and both are absent in endemic populations. However, it is seen in native residents infected with *S. japonicum* (Ross et al., 2001; Zhou et al., 2005). Because it can appear from several weeks to months with an unspecific presentation, the acute schistosomiasis phase is most likely to be misdiagnosed by travel physicians (Ross et al., 2007).

Most of the schistosomiasis morbidity is due to the chronic form in which adult worms lay eggs that can cause inflammatory reactions (Murray et al., 2012). If untreated, adult worms continue to shed eggs each day and the faeces or urine only expels a few. The majority of eggs secrete proteolytic enzymes that help them to migrate and become lodged in proximal organs (Colley et al., 2014). The enzymes also cause an eosinophilic inflammatory reaction when eggs are trapped in tissues or embolize to the liver, spleen, lungs or brain (Gryseels et al., 2006). The long-term manifestations are dependent to the species of schistosome, as the adult worms of different species migrate to different areas (Mandell et al., 2010). Many infections are symptomatic, with anaemia and malnutrition being common in endemic areas. Thus, the

severity of the disease is highly correlated with the intensity of infection (Pearce and MacDonald, 2002; Russell et al., 2015).

In gastrointestinal schistosomiasis, adult worms migrate to the veins of the intestinal tract and liver. Eggs in the gut wall can lead to pain, bloody stools, and diarrhoea, especially in children (Ross et al., 2002). Severe disease can lead to narrowing of the colon or rectum and rectal stenosis (Gray et al., 2011). Eggs also migrate to the liver leading to fibrosis in 4% to 8% of people with chronic infection, and mainly those with long-term heavy infection experience hepatomegaly and liver fibrosis (Ross et al., 2002; Barsoum et al., 2013). *Schistosoma mansoni* infection epidemiologically overlaps with high HIV prevalence in sub-Saharan Africa, where gastrointestinal schistosomiasis has been linked to increased HIV transmission (Yegorov et al., 2019).

In the case of urogenital schistosomiasis, *S. haematobium* worms migrate to the veins around the bladder and ureters (Mandell et al., 2010). This can lead to blood in the urine (haematuria) 10 to 12 weeks after infection. Over time, fibrosis can lead to obstruction of the urinary tract, hydronephrosis and kidney failure (Gryseels et al., 2006; Gray et al., 2011). Bladder cancer diagnosis and mortality are generally elevated in affected areas; efforts to control schistosomiasis in Egypt for instance led to a decreased bladder cancer rate (Mostafa et al., 1999; Gray et al., 2011). In women, genitourinary schistosomiasis is due to eggs trapped in various organs of the genital tract, also causing cancer and often neglected consequences for female reproductive health (Kjetland et al., 2012). This form can also include genital lesions that may lead to increased rates of HIV transmission (Feldmeier et al., 1995; Gray et al., 2011; Yegorov et al., 2019). In men, urogenital schistosomiasis can cause haematospermia, orchitis, prostatitis, dyspareunia and oligospermia. Usually, these symptoms disappear after antischistosomal treatment (Leutscher et al., 2000, 2009). *Schistosoma mansoni* and *S. japonicum* rarely affect the genital tract (Kjetland et al., 2012).

Ectopic localization of *Schistosoma* eggs is possible and can lead to unexpected morbidities. The most common is cerebral schistosomiasis in which adult worms or eggs distribute in the central nervous system and provoke spinal compression or encephalopathy. Moreover, it occurs during infection with *S. japonicum*. Clinical signs include symptoms of meningoencephalitis and altered sensorium or Jacksonian epilepsy (Ross et al., 2012).

Similarly, cerebral granulomatous lesions can involve *S. mansoni* and *S. haematobium* eggs in the spinal cord and lead to transverse myelitis with flaccid paraplegia (Freitas et al., 2010). Eggs are thought to travel to the central nervous system via embolization (Ross et al., 2002). In long term infection, all *Schistosoma* species cause systemic morbidities including anaemia,

malnutrition and impaired childhood development (King and Dangerfield-Cha, 2008). The consequence is the effect of continued inflammation on normal growth, iron metabolism, physical fitness and cognitive function in infected people (Friedman et al., 2005; Bustinduy et al., 2011). Mostly, the anaemia of inflammation is caused by the reduction of iron rate during trapping within the body mediated by the hepatic hormone hepcidin (Nemeth et al., 2004). The downstream consequence of chronic anaemia is the dramatic effects on physical work output in endemic regions for schistosomes (Ndamba et al., 1993; Bustinduy et al., 2011).

## 1.4. Diagnosis

The diagnosis of schistosomiasis involves a medical history and clinical symptoms combined with confirmation using laboratory tests. The diagnostic standard for active schistosomiasis is detecting viable eggs in urine for urogenital schistosomiasis, faeces for intestinal schistosomiasis, or tissue biopsies by using microscopic examination to identify species-specific eggs (Gray et al., 2011). The direct thick smear with the formalin and ether concentration method is also used to diagnose *S. mansoni* eggs (Utzinger et al., 2010). Because of the low sensitivity of the standard method for urine and stool examination, epidemiological surveys for schistosomiasis diagnosis involve more quantitative methods. The diagnosis of intestinal schistosomiasis is confirmed by identification of eggs in the stool. Eggs of *S. mansoni* are about 140 µm by 60 µm in size and have a lateral spine. The most widely used diagnosis is the Kato-Katz thick smear method that allows quantifying eggs per gram of stool (Katz et al., 1972). Because eggs may be expelled in faeces intermittently or in small numbers, their detection is enhanced by repeated examinations or concentration procedures. Simultaneously, helminth infections can also be detected by this method. For urogenital schistosomiasis, syringe filtration of urine and microscopic examination of polycarbonate filters allows quantification of eggs per 10 ml of urine (Mott et al., 1982). Microhaematuria reagent strips can also be performed on urine samples for light intensity of *S. haematobium* infection (Knopp et al., 2018). The sedimentation technique is an important substitute method for filtration methods. However, in light intensity infection the sensitivity and specificity of the sedimentation method are low, compared to the filtration of 10 ml of urine. The epithelial cells, for example, in urine samples might disguise eggs of *S. haematobium* in sediment (Gray et al., 2011). Rectal biopsy for all species of schistosomes may demonstrate eggs when stool or urine samples are negative (Gray et al., 2011).

Furthermore, the FLOTAC and its subsequent Mini-FLOTAC are new sensitive methods for the diagnosis of schistosomiasis using the egg-flotation technique for multiple sampling settings and large quantification of stool samples. They should therefore considerably improve the sensitivity of the aforementioned diagnostic tools but detection is limited to 10 eggs per gram of stool (Glinz et al., 2010; Barda et al., 2013; Catalano et al., 2019). However, they require centrifugation, and hence might be out of reach in resource-constrained settings (Cringoli et al., 2017).

The detection of schistosomal antigens in blood, urine, stool or sputum is possible with a highly effective method of diagnosis called point-of-care circulating cathodic antigen (POC-CCA) assay (Utzinger et al., 2015). This method has superior sensitivity over the Kato-Katz thick smears (Coulibaly et al., 2011; van Dam et al., 2015; Danso-Appiah et al., 2016) for mapping of *S mansoni*-endemic regions (Colley et al., 2013) and detection of *S. japonicum* and *S. mekongi* infections (Stothard et al., 2006).

Serologic testing for the antischistosomal antibody is indicated for the diagnosis of travellers or immigrants from endemic areas who have not previously been treated appropriately for schistosomiasis. Commonly used serologic tests detect antibodies to the adult worm (Tsang and Wilkins, 1997). However, test sensitivity and specificity can vary widely and are dependent on both the type of antigen preparations used (crude, purified, adult worm, egg and cercariae) and the test procedure (Doenhoff et al., 2004; Gray et al., 2011). Thus, these serological tests include enzyme-linked immunosorbent assay (ELISA), indirect haemagglutination assay (IHA) (Angora et al., 2015) and immunofluorescent-antibody test (IFAT) (Kinkel et al., 2012). They cannot be used for a population in endemic areas, as they do not discriminate active infection from previous exposure in patients who have been repeatedly infected and treated in the past because specific antibodies can persist despite cure (Doenhoff et al., 2004). For new infections, the serum sample tested should be collected at least 6 to 8 weeks after likely infection, to allow for the full development of the parasite and antibody to the adult stage (Gray et al., 2011).

Recently, the detection of schistosome DNA or RNA by conventional or more advanced PCR-based techniques (e.g. real-time quantitative PCR or multiplex PCR) is an important advance which can be combined with parasitological and serological diagnostic tests for accurate schistosomiasis diagnosis (Weerakoon et al., 2015, 2018; Guegan et al., 2019). These molecular approaches for detecting schistosomiasis have been developed to detect *Schistosoma* DNA in clinical human samples such as faeces, sera (Pontes et al., 2002) and urine (Sandoval et al., 2006) and confirm the existence of parasite DNA in the host.

Mitochondrial and nuclear genes are used in this molecular analysis. Although, several attempts have been made to simplify the isolation of eggs on the filter paper since it does not require laboratory facilities, DNA can still be successfully extracted at the latter stage (Reinstrup et al., 2012). The mitochondrial genes are very useful to demonstrate the relationship between different parasite species. Polymerase chain reaction (PCR) - based testing is accurate and rapid. However, they are not frequently used in countries where the disease is common due to the cost of the equipment and the technical experience required to run them (Utzinger et al., 2015).
Loop-mediated isothermal amplification (LAMP) is being studied at a lower cost but is not yet commercially available (Utzinger et al., 2015; Nigo et al., 2019). It is a relatively simple, cost-effective and rapid DNA detection compared to the commonly used PCR-based assays. This method does not require thermocycler, electrophoresis gel and is applicable in poor settings (Tomita et al., 2008). Moreover, the assay is highly specific to the intended target sequence and more sensitive, because it uses specific inner and outer primer sets (Notomi et al., 2000; Xu et al., 2010). Recombinase polymerase amplification (RPA) is another isothermal amplification technique that uses lower temperatures (around 40°C). DNA sequences are amplified using DNA polymerase and oligonucleotide primers (Piepenburg et al., 2006; Poulton and Webster, 2018). Similar to LAMP, the RPA technique is useful in resource-poor areas as it has now been integrated with a chip and lateral flow devices making it a convenient portable application as a point of care diagnostic tool (Piepenburg et al., 2006; Zanoli and Spoto, 2012). The RPA has been applied in the diagnosis of both intestinal and urinary schistosomiasis, and its detection time and diagnostic sensitivity are superior to microscopic examination and serological tests (Rosser et al., 2015; Xing et al., 2017; Poulton and Webster, 2018; Rostron et al., 2019). Another important public health aspect is the detection of schistosome infections in snail intermediate hosts and freshwater. This allows for identification of environmental contamination (Allan et al., 2013). Recently, environmental DNA (eDNA) of schistosomes has been detected in freshwater using a xenomonitoring method (Alzaylaee et al., 2020).

## 1.5. Strategies of control and treatment

The control of schistosomiasis is based on the large-scale treatment of population at-risk groups, access to safe water, improved sanitation, hygiene education, and snail control. Praziquantel is the main treatment recommended by WHO against all forms of schistosomiasis. This drug is effective, safe, and low-cost. Even though re-infection may occur after treatment, the risk of developing severe disease is diminished and reversed when treatment is initiated and repeated in childhood. Because of its excellent tolerability and ability to effectively cure or diminish worm burden (70-90%), praziquantel can be distributed annually (or every other year) by moderately trained teachers or community health workers during mass drug administration (MDA) campaigns (Fenwick et al., 2003; Utzinger and Keiser, 2004). This provides sufficient coverage to control morbidity in children, even despite the possibility of reinfection, with prevention of serious complications (Savioli et al., 1990; WHO, 2006). Despite several studies, the mechanism of action of praziquantel is not exactly known (Wu et al., 2011) and for full efficacy, it needs an effective host antibody response (Doenhoff et al., 2008). Praziquantel acts against adult schistosome worms but has poor activity against immature schistosome larvae. It increases the permeability of schistosome membranes cells towards calcium ions. The drug induces contraction of the parasite, resulting in paralysis in the contracted state (Greenberg, 2005; Angelucci et al., 2007). Another hypothesis concerning the mechanism of action of praziquantel is its binding to beta subunits of voltage-gated calcium ions channels as target (Kohn et al., 2003; Pica-Mattoccia et al., 2007). Besides, the drug seems to interfere with adenosine and glutathione S-transferase receptors. This effect may have therapeutic relevance given that a schistosome is unable to synthesize purines such as adenosine *de novo* (Angelucci et al., 2007; Tallima and El Ridi, 2007). The single dose of 40 mg/kg of praziquantel is effective for the treatment of *S. haematobium* and *S. mansoni*. In contrast, for *S. japonicum* and *S. mekongi*, the recommended dose is 60 mg/kg (Montresor et al., 2001; Kovač et al., 2018). For preschool children (generally, younger than age 5 years), a new dose pole extends below 94 cm (Stothard et al., 2013). Praziquantel tablets are large and taste bitter and no readily available paediatric formulation exists (Stothard et al., 2013; Coulibaly et al., 2017). Therefore, the treatment of young children involves crushing tablets in carriers such as orange juice. The common adverse events of praziquantel include abdominal pain, headache and dizziness.

Oxamniquine is another drug active against schistosomes, but it is effective only against *S. mansoni* and is no longer readily available (Katz et al., 1991). Its mode of action is well documented, unlike that of praziquantel. This prodrug binds particularly the *S. mansoni*-specific

sulfotransferase (*Sm*SULT-OR) encoded on chromosome 6 and creates a conjugation with the 3'phosphoadenosine 5'phosphosulfate to produce an unstable substance which can degrade DNA and proteins (Pica-Mattoccia et al., 2006; Valentim et al., 2013). Molecular markers of oxamniquine resistance allow efficient monitoring of the distribution of resistant alleles in schistosome populations. A resistance mutation, p. E142del has been reported and could impact the disease control (Chevalier et al., 2019).

In addition to preventive chemotherapy, additional control measures should be integrated into national and regional programme to effectively control schistosomiasis in areas at risk (Rollinson et al., 2013). These include the control of freshwater snails, the main intermediate hosts of the parasite. Although some chemicals, environmental management, mollusk predators and biological competitors are used to reduce snail populations, current efforts focus mainly on niclosamide, which is an effective molluscicide useful against snails at low concentrations and not toxic to humans. However, it is toxic to some freshwater fishes (Oliveira-Filho and Paumgartten, 2000; Dai et al., 2008).

Communication for behaviour change in the population is essential but difficult in the control of the environmental disease. However, adequate community participation is necessary in reducing both human exposure to schistosome-contaminated water and the contamination of snail habitat through human excrement containing schistosome eggs. This change in behaviour includes improving living conditions of the population and supplies of drinking water, avoidance of bathing in and contact with suspect waters and environmental sanitation as the main means of choice for effective elimination of schistosomiasis (Ross et al., 2014; Qian et al., 2018). The improvement to water supply focuses on preventing consumption of contaminated water. However, since schistosomes infect people by passing through intact skin, the success of water supply improvements in preventing schistosome infection also depends on prevention of water contact.

Furthermore, the role of sanitation in schistosomiasis control is to prevent contamination of freshwater with excreta, rather than to prevent the ingestion of faecal pathogens. Since the parasite stages in the excreta cannot directly infect people, hand washing following defecation or urination will not affect schistosome transmission during contact with freshwater, but could reduce the infectivity of miracidia, and reduce infected snail numbers (Grimes et al., 2015). Widespread elimination will almost certainly need integrated use of many or all the applicable methods: preventive chemotherapy, snail control, behavioural modification, sanitation and water supply improvements, and possibly a prophylactic vaccine could break the transmission chain.

## 1.6. Genomes of schistosomes

Schistosome genomes are rich in repeated sequences and turn out to be large (363 Mb for *S. mansoni*) (Berriman et al., 2009). Approximately, 40% of the genome involves repeated sequences, including retrotransposons, (endogenous DNA sequences capable of moving and especially multiplying in the host genome giving rise to dispersed repeated sequences), long terminal repeat (LTR) and non-long terminal repeat (Le Paslier et al., 2000). Despite the high proportion of repeated sequences, 50% of the genome is assembled (> 824 kb) and 43% is located on chromosomes (7 pairs of autosomes and sex-determining Z and W chromosomes). The originality of genes concerns introns. Introns in 5' of the genes are globally smaller than those in 3', suggesting an atypical mode of transcriptional control (Le Paslier et al., 2000). **Figure 1.3** shows the retrotransposons, terminal repeats, and chromosomes.

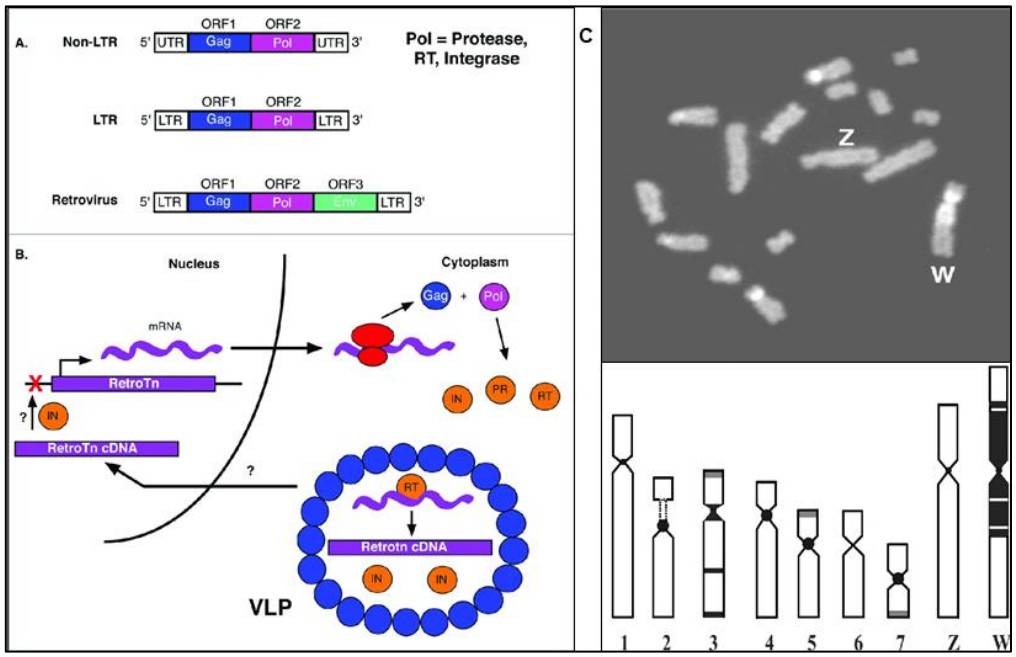


**Figure 1.3: The genomes of schistosomes including (A) long terminal repeat (LTR) and non-long terminal repeat (non-LTR), (B) Retrotransposons, (C) Chromosomes.** Adapted from: (Le Paslier et al., 2000).

*Schistosoma* genes are characterized by several micro-exons named micro exon genes (MEG). In *S. mansoni*, a specific group of genes *Sm*PoMuc codes for mucin-like proteins, specifically expressed in the parasite stages that interact with *Biomphalaria glabrata* as intermediate host (Oliveira et al., 2004). The genomes of schistosomes are expressed in both nucleus and mitochondrion.

Recently, a high quality of *S. haematobium* genome has been achieved using single-molecule and long-range sequencing that showed an improvement in terms of accuracy and contiguity of genome assembly compare to schistosome species (Stroehlein et al., 2019).

The nuclear genome mostly used in schistosomes is the internal transcribed spacer (ITS**) (Figure 1.4)**. These genes coding for ribosomal RNA and spacers are in the form of tandem repeats several thousand copies long, each separated by regions of non-transcribed DNA called intergenic spacer (IGS) or non-transcribed spacer (NTS). Each ribosome group contains the 5' external transcribed sequence (5' ETS), 18S rRNA gene, ITS1, 5.8S rRNA gene, ITS2, 26S or 28S rRNA gene, and the 3' external transcribed sequence (3'ETS). During maturation of the rRNA, some portions are excised. As non-functional by-products of this maturation, they are rapidly degraded (Choudhary et al., 2015).

The mitochondrial genome of schistosomes is usually used for phylogenetic analysis. It contains 36 genes, including 12 genes coding for proteins (cox13, nad16, nad4L, atp6); 2 rRNAs (rrnL: large rRNA subunit and rrnS: small rRNA subunit); and 22 transfer RNA (tRNA) genes **(Figure 1.4)**. This might be vital because it allows the exploration of genetic heterogeneity of parasites among a sample (Morgan et al., 2003a; Webster et al., 2006; Kosakyan et al., 2012).

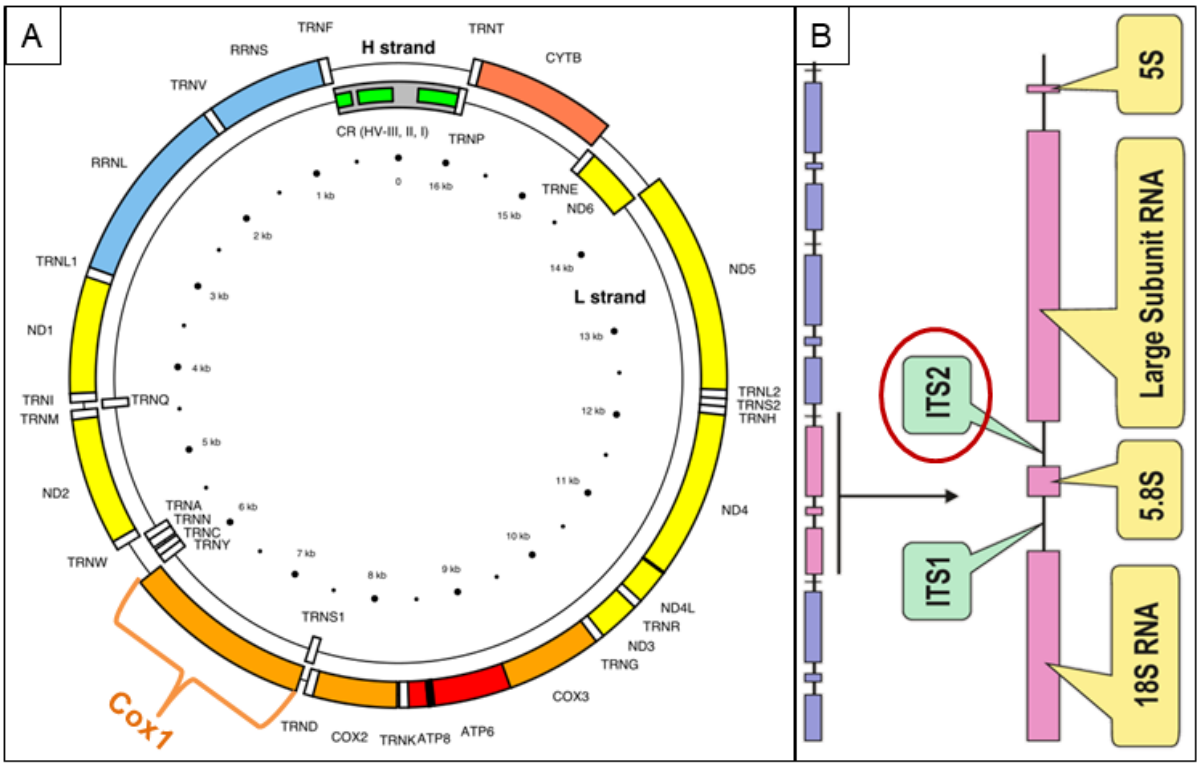


**Figure 1.4: Location of (A) Cox1 gene in the mitochondrial genome (B) the internal transcribed spacer region**. Adapted from: (Kosakyan et al., 2012; Choudhary et al., 2015).

The phylogeny and speciation of schistosomes have been assessed using DNA sequencing, molecular tools also that have promoted greater records and exploration of genetic diversity of schistosome species and their hosts (Webster et al., 2006). Variations in the sequences of mitochondrial cytochrome oxidase subunit 1 (Cox1) gene are very useful to identify the evolutionary differences, and possible similarities (Johnston et al., 1993; Kane et al., 2003). Also, the genus *Schistosoma* includes six clades that correlate to different geographical distributions of the parasites (Webster et al., 2006). African schistosomes form two distinct clades, the *S. mansoni* and the *S. haematobium* clade **(Figure 1.5)**.

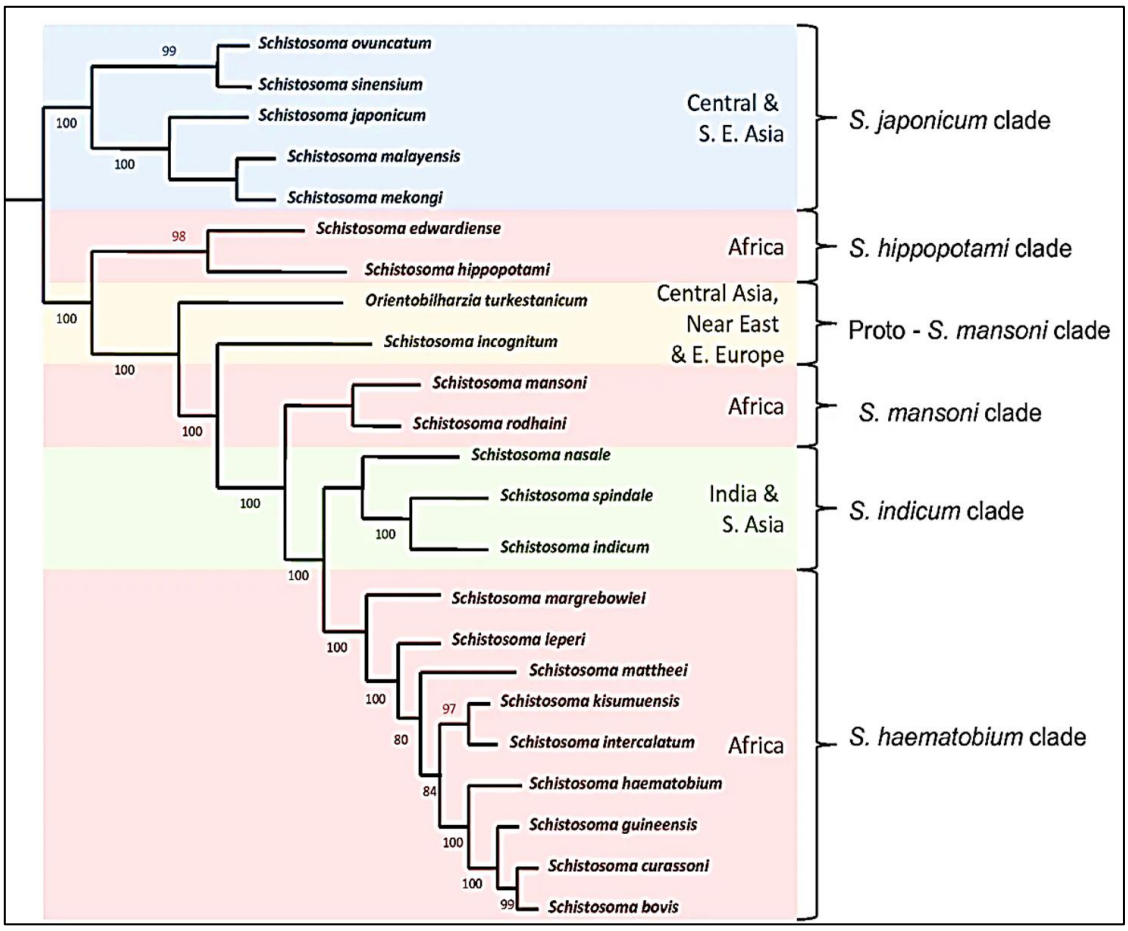


**Figure 1.5: Schematic phylogeny of the interrelationships of members of the species within the genus *Schistosoma*.** Adapted from: (Abdullahi Hudu and Umar, 2019)

## 1.7. Hybridization

Crossing between different species to produce hybrids by introgression (gene transfer) remains a relevant phenomenon in schistosome species. Two molecular markers are involved in this event, a nuclear marker: ribosomal internal transcribed spacer 2 (ITS2) with two-parent heritability, and a mitochondrial marker: cytochrome oxidase subunit I (Cox1) only passed on by the mother **(Figure 1.6)**. These markers are classically used for the identification of schistosome species (Huyse et al., 2009; Boissier et al., 2016). Hybridization between two *Schistosoma* species can complicate diagnosis and expand the host spectrum (Webster and Southgate, 2003). Adult worms of *S. haematobium* live in the capillaries of the periventricular venous plexus, with eggs being evacuated through urine. In contrast, *S. bovis* is found in the mesenteric veins and the eggs are released through the faeces (Webster et al., 2013). The question remains: where are the eggs of the hybrid form between species of parasite evacuated? In schistosomes, male and female meet at the portal vein. The two worms mate and then move towards the egg-laying site, with the male carrying the female in his gynecophoral canal. It can, therefore be assumed that in a first-generation cross, the male of one species leads the female of the other species to her breeding site. For subsequent generations, the choice of the egg-laying site could depend on the level of genetic introgression.

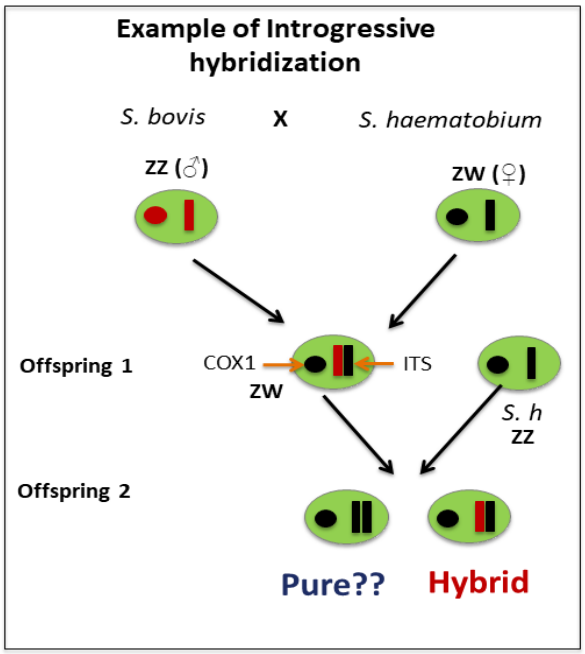


**Figure 1.6: Example of introgressive hybridization between *S. haematobium* and *S. bovis***

All phenotypic changes induced by hybridization can lead to serious implications in terms of human health and disease control. They can affect both parasitological diagnosis and treatment of population. Moreover, hybridization can modify the morphology of eggs produced by the parasites and also mode of excretion leading to a discrepancy between symptomatology and parasitological diagnosis. In the case of hybrids between *S. haematobium* and *S. bovis*, eggs have been identified in both the faeces and urine of patients (Huyse et al., 2009). Therefore, either specific or combined excretion of eggs from hybrid parasites in the faeces and/or urine should be considered in endemic countries to improve the diagnosis of the disease.

From an epidemiological perspective, hybridization is common in schistosomes from several African countries. Hybridization of *S. haematobium-S. guineenis* was observed in Cameroon in 1996. *Schistosoma. haematobium* could establish itself only after deforestation of the tropical rainforest in Loum next to the endemic *S. guineensis*; hybridization led to the competitive exclusion of *S. guineensis* (Tchuenté et al., 1997). In 2003, *S. mansoni-S. rodhaini* hybrids were found in snails in western Kenya (Morgan et al., 2003b). In 2009, *S. haematobium–S. bovis* hybrids were described in northern Senegalese children. The Senegal River Basin changed very much since the 1980s after the Diama Dam in Senegal and the Manantali Dam in Mali were built. The Diama dam prevented ocean water from entering and allowed new forms of agriculture. Human migration, an increasing number of livestock and sites where humans and cattle both contaminate the water facilitated mixing between the different schistosomes in Nder (Huyse et al., 2009). The same hybrid profile was identified during the 2015 investigation of a schistosomiasis outbreak in humans on the Cavu river of Corsica, France (Boissier et al., 2016) and also in snails in Côte d'Ivoire (Tian-Bi et al., 2019). Recently, hybrid *S. haematobium -S. mansoni* were described in Côte d'Ivoire (Depaquit et al., 2019; Le Govic et al., 2019).

## 1.8. Microsatellites

Microsatellites are tandems of repetitive DNA in which DNA sequences (ranging in length from one to six or more base pairs) are repeated, typically 5- 50 times, and they occur at thousands of locations within an organism's genome with highest mutation rate (Brinkmann et al., 1998; Richard et al., 2008; Gulcher, 2012). Unlike point mutations, which affect only a single nucleotide in the DNA sequence, microsatellite mutations may be responsible for the gain or loss of one or more entire repeat units. Thus, the mutation rate at microsatellite loci could probably be different from other mutation rates such as base substitution (Tautz and Schlötterer, 1994). Microsatellites and their longer counterparts, minisatellites, are classified together as a variable number of tandem repeats DNA (VNTR) and many microsatellites are located in non-coding DNA, others in regulatory or even coding DNA. Thus, microsatellite mutations in such positions can lead to phenotypic changes (Gymrek et al., 2016).

Variation at a single microsatellite locus can be measured in much the same way as variation at a single nucleotide site (SNP: single nucleotide polymorphism), though there are usually many more than two alleles (often more than 10 alleles). These alleles can also differ quite a lot in terms of repeat lengths depending on the structure of the microsatellite (Valdes et al., 1993). Moreover, microsatellite loci are highly variable DNA markers in widespread use within the schistosomiasis research community as they enable population-level analysis (Glenn et al., 2013). The advantage of microsatellite markers resides in their use for mapping locations within the genome to identify a gene or a mutation responsible for a given disease. As a special case of mapping, they can be used to study population genetics and in species conservation (Pemberton et al., 2013).

Despite the rise of single nucleotide polymorphism (SNP) platforms, microsatellites remain the most powerful genetic markers for schistosome genome analysis in genomic variation for linkage and association (Webster et al., 2015). Their continuing advantage lies in their greater allelic diversity than SNPs, such that microsatellites can be used to differentiate alleles within a block of binding disequilibrium defined by the SNP of interest (Gulcher, 2012; Ott et al., 2015).

## 1.9. Aim and objectives

The transmission dynamics of schistosomiasis are determined by the complex life cycle of the parasitic worm (schistosome), environmental factors (suitable water bodies), and the social-ecological context. The dynamics are further complicated by uncontrolled and changing environmental, climatic and socio-economic factors, which generate many challenges for its control. Environmental changes due to natural phenomena or human activities affect the epidemiology and distribution of schistosomiasis and can facilitate interspecies genome modification events.
Despite mass drug administration campaigns, water supply, sanitation and hygiene improvement and behavioural interventions, the prevalence of schistosomiasis remains high in many parts of Côte d'Ivoire. Investigating the population genetics of schistosomes, using molecular markers, may help elucidate its dynamic epidemiology.

In this light, this Ph.D. project had two main aims. The first was to expand our understanding of the parasite's transmission dynamics to update epidemiological data on schistosomiasis by determining the prevalence and associated factors of the disease. The second aim was to better understand the genetic structure and diversity of *S. haematobium* in schoolchildren.

To achieve these aims, the following specific objectives were assigned:

1. To determine the prevalence of schistosomiasis in the southern and western parts of Côte d'Ivoire.
2. To molecularly characterise hybrids between *S. haematobium* and *S. bovis* among schoolchildren in Côte d'Ivoire.
3. To review the current knowledge on genetic structure and diversity of *S. haematobium* in Africa.
4. To analyse genetic differentiation of *S. haematobium* in schoolchildren in Côte d'Ivoire.

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# Chapter 2:

# Prevalence of schistosomiasis

# Prevalence and risk factors for schistosomiasis among schoolchildren in two settings of Côte d'Ivoire

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# Prevalence and Risk Factors for Schistosomiasis among Schoolchildren in two Settings of Côte d'Ivoire

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**Abstract:** Schistosomiasis is a parasitic disease affecting more than 250 million people, primarily in sub-Saharan Africa. In Côte d'Ivoire both *Schistosoma haematobium* (causing urogenital schistosomiasis) and *Schistosoma mansoni* (causing intestinal schistosomiasis) co-exist. This study aimed to determine the prevalence of *S. haematobium* and *S. mansoni* and to identify risk factors among schoolchildren in the western and southern parts of Côte d'Ivoire. From January to April 2018, a cross-sectional study was carried out including 1187 schoolchildren aged 5–14 years. Urine samples were examined by a filtration method to identify and count *S. haematobium* eggs, while stool samples were subjected to duplicate Kato-Katz thick smears to quantify eggs of *S. mansoni* and soil-transmitted helminths. Data on sociodemographic, socioeconomic, and environmental factors were obtained using a pretested questionnaire. Multivariate logistic regression was employed to test for associations between variables. We found a prevalence of *S. haematobium* of 14.0% (166 of 1187 schoolchildren infected) and a prevalence of *S. mansoni* of 6.1% (66 of 1089 schoolchildren infected). In the southern part of Côte d'Ivoire, the prevalence of *S. haematobium* was 16.1% with a particularly high prevalence observed in Sikensi (35.6%), while *S. mansoni* was most prevalent in Agboville (11.2%). Swimming in open freshwater bodies was the main risk factor for *S. haematobium* infection (adjusted odds ratio (AOR) = 127.0, 95% confidence interval (CI): 25.0–634.0, $p < 0.001$). Fishing and washing clothes in open freshwater bodies were positively associated with *S. haematobium* and *S. mansoni* infection, respectively. Preventive chemotherapy using praziquantel should be combined with setting-specific information, education, and communication strategies in order to change children's behavior, thus avoiding contact with unprotected open freshwater.

**Keywords:** Côte d'Ivoire; prevalence; risk factors; *Schistosoma haematobium*; *Schistosoma mansoni*; schistosomiasis

## 1. Introduction

Schistosomiasis is a water-based chronic parasitic disease caused by trematode worms of the genus *Schistosoma*. Considered as a neglected tropical disease by the World Health Organization (WHO), schistosomiasis affects more than 250 million people worldwide with an estimated global burden of 1.4 million disability-adjusted life years (DALYs) in 2017 [1–3]. Schistosomiasis remains a public health problem in countries of the tropics and subtropics with approximately 90% of cases concentrated in Africa [3,4]. Humans are the definitive host for adult parasites, while specific freshwater snails act as intermediate hosts [3,4]. Hence, the transmission of schistosomiasis is governed by social-ecological systems (e.g., conditions of poverty and living near open freshwater bodies) [5]. Schistosome eggs are excreted by humans with feces or urine. After hatching, miracidia infect specific snails to produce cercariae. Schistosome cercariae penetrate the unbroken skin of humans during domestic (e.g., washing clothes or dishes) and recreational activities (e.g., bathing and swimming in unprotected open freshwater bodies). Various factors have been shown to facilitate transmission of schistosomiasis in Africa, such as living in close proximity to freshwater bodies (e.g., rivers, small dams, irrigation schemes, and lakes), socioeconomic factors which influence occupational activities (e.g., poor people without running water at home are likely to contact freshwater bodies) and climate change [6–8]. The lack of access to improved sanitation contributes to open defecation, which results in environmental contamination that enhances the transmission of schistosomiasis [9].

In Côte d'Ivoire, snails of the genera *Biomphalaria* and *Bulinus* are the intermediate hosts for *Schistosoma mansoni* and *Schistosoma haematobium*, respectively [10]. While both *S. mansoni* and *S. haematobium* are endemic in Côte d'Ivoire [11], the former species is predominantly found in the western part of the country [12,13] and *S. haematobium* is mostly present in the central and southern parts [9,14]. In northern Côte d'Ivoire, a recent study reported low prevalence rates of 1.9% and 3.5% for *S. haematobium* and *S. mansoni* among school-aged children, respectively [15]. To enhance control efforts and shift the focus from morbidity control toward interruption of transmission, fine-grained information on the distribution of the disease is important, including underlying risk factors.

The purpose of this study was to determine the prevalence of schistosomiasis and risk factors among schoolchildren in the western and southern parts of Côte d'Ivoire where the disease is most prevalent. The results will assist public health authorities of Côte d'Ivoire to refine control measures and complement preventive chemotherapy with specific information about infection prevalence and intensity, and to enhance education and communication approaches that are readily tailored to specific social-ecological contexts.

## 2. Materials and Methods

### *2.1. Study Settings and Population*

The study was carried out in two settings of Côte d'Ivoire, including four health districts: (i) Agboville (geographical coordinates: 5° 55′ 41″ N latitude, 4° 13′ 01″ W longitude); (ii) Adzopé (6° 06′ 25″ N, 3° 51′ 36″ W); and (iii) Sikensi (5° 40′ 34″ N, 4° 34′ 33″ W), all located in the southern part of Côte d'Ivoire; and (iv) Duekoué (6° 44′ 00″ N, 7° 21′ 00″ W) in the western part (Figure 1). We included one health district from the western part of Côte d'Ivoire in order to contrast with the three health districts in the South, thus enriching ecological features (the western part of Côte d'Ivoire is hilly as opposed to mainly flat terrain in the South) and biological characteristics (setting-specific parasite-intermediate host systems). In view of limited financial and human resources, we were unable to include the same amount of health districts in the western compared to the southern setting of Côte d'Ivoire.

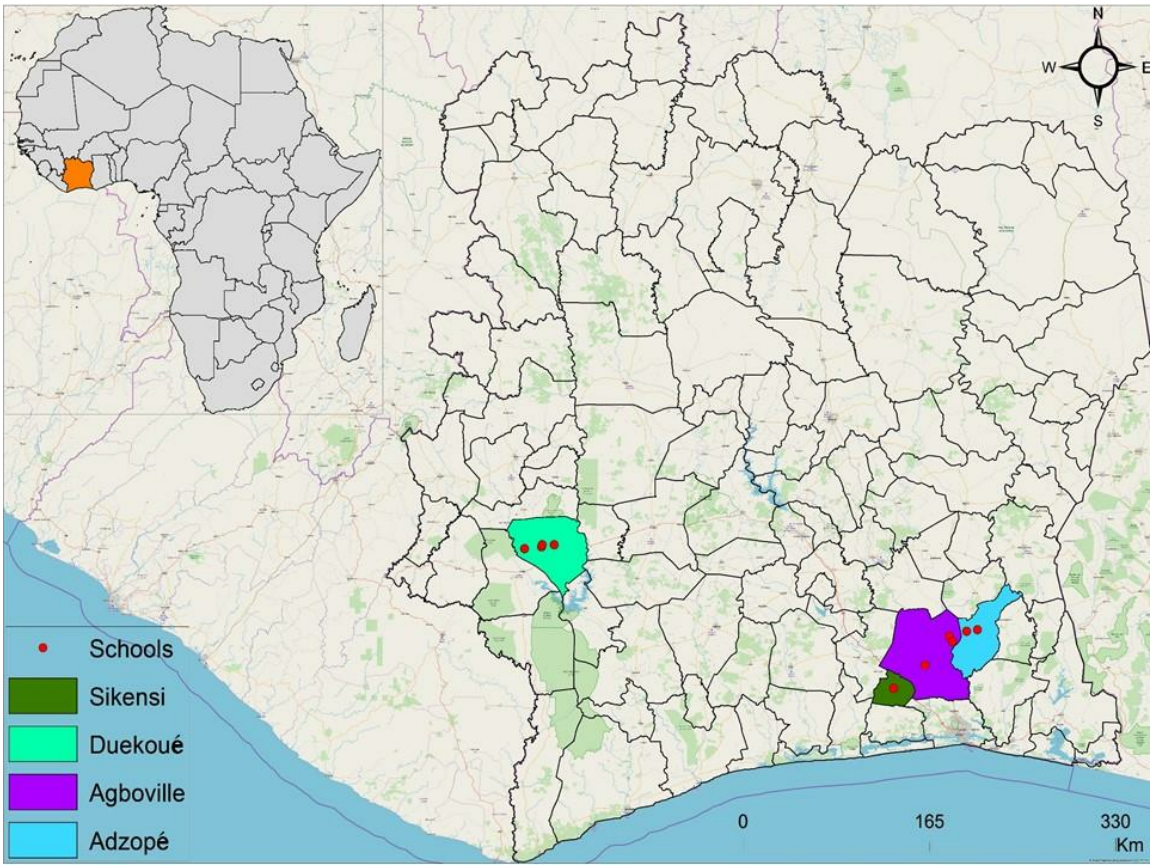


**Figure 1.** Collection of maps displaying the study area in Côte d'Ivoire, West Africa: Adzopé, Agboville, and Sikensi in the southern setting; Duekoué in the western setting of Côte d'Ivoire. Primary schools (red dots) were selected in each health district on the basis of close proximity to open freshwater bodies (distance: < 10 km).

Subsistence farming is the main economic activity in all study settings, which are well known for their endemicity of *S. haematobium* [16] and *S. mansoni* [12,17]. In each of the health districts, there are rivers which act as main transmission sites for schistosomiasis [18]. Of note, Duekoué is additionally appreciated as tourist destination. The Guemon River is the predominant river running right through the city. Sikensi has several rivers that discharge in the Agnéby River. Water flowing through Agboville also discharges in the Agnéby River [19]. Adzopé shares rivers from different catchment areas [18].

The study population consisted of schoolchildren aged 5–14 years. Schools were chosen on the basis of their proximity to a stream, river, lake, or backwater used by children at a distance of less than 10 km.

### 2.2. Design and Sample Size

A cross-sectional study was carried out in the two study settings from January to April 2018. The sample size ($n$) was adjusted to 1187 children based on the following formula: $n = (Z^2 \times p\,(1-p) \times C) / i^2$, where $Z = 1.96$, $p = 40\%$ is the prevalence expected based on a previous study [9], $i$ is the precision or margin of the error (5%), and $C$ is the correction coefficient ($C = 2$).

### 2.3. Data and Sample Collection

Only schoolchildren aged 5–14 years who had lived in the study area for at least one year prior to the survey were included. The number of children per school was proportionally allocated according to population size in each school. Children were randomly selected using readily available school lists and identified by unique codes. A questionnaire was administered to collect data about each

child's habits and behaviors, such as swimming/bathing in open freshwater bodies, washing clothes in rivers, and fishing. In total, 1187 urine and 1089 stool samples were collected from children in plastic containers and transferred to nearest health centres for parasitological examination. Urine samples were collected between 10 a.m. and 12 a.m. [20].

### *2.4. Parasitological Examination*

A urine filtration method was employed to identify *S. haematobium* eggs [21]. In brief, 10 mL of urine was vigorously shaken and filtered through a Nytrel filter with a 40 µm mesh size and examined microscopically for the presence of *S. haematobium* eggs that were counted by experienced laboratory technicians. Stool samples were subjected to the Kato-Katz technique [22]. Two thick smears from each stool sample were microscopically examined to identify and quantify eggs of *S. mansoni* and soil-transmitted helminths.

After examination, all schoolchildren were treated with a single 40 mg/kg oral dose of praziquantel (600 mg; Biltricide, Bayer, Leverkusen, Germany) through the "Programme National de Lutte contre les Maladies Tropicales Negligées à Chimioprophylaxie Préventive" (PNLMTN-CP) of Côte d'Ivoire. Children with soil-transmitted helminths were treated with albendazole (400 mg).

### *2.5. Statistical Analysis*

Statistical analyses were performed with STATA version 15.0 (Stata Corporation; College Station, TX, USA). Univariate analysis ($\chi^2$ and Fisher's exact test, as appropriate) was used for comparison between groups. Children were stratified into three age groups (5–8, 9–11, and 12–14 years). Parasitic infections were defined as positive for *S. haematobium* or *S. mansoni* when at least one egg was identified in a urine or stool sample, respectively. Associations between parasitic infections and sociodemographic, socioeconomic, or environmental factors were assessed by mixed multivariable logistic regression models with random intercepts for schools and for classes nested within schools. The study area was used as a fixed factor. The risk factors investigated were occupation and educational attainment of parents/legal guardians, and swimming, fishing, and playing in freshwater by children. Associations and differences with a *p*-value below 0.05 were considered statistically significant.

### *2.6. Ethical Consideration*

Ethical clearance 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, parents/guardians, and participants were informed about the objectives, procedures, and potential risks and benefits of the study. Written informed consent was obtained from children's parents or legal guardians. Oral assent was obtained from children.

## 3. Results

### *3.1. Sociodemographic Characteristics of the Population*

A total of 1187 schoolchildren were included in the study. There were considerably more boys than girls (61.2% vs. 38.8%) and the highest proportion of children was included in Agboville. The mean age was 9.9 years (standard deviation (SD) = 2.4 years) with a median age of 10 years. Children aged 9–11 years were the most common age class in Adzopé (43.8%), Duekoué (40.3%), and Sikensi (54.1%) but the least common age class in Agboville (31.6%). Table 1 shows the sociodemographic characteristics of the study population, stratified by setting.

**Table 1.** Sociodemographic characteristics of the study population subjected to schistosomiasis diagnosis in different settings of Côte d'Ivoire in early 2018.

| Variable | Western Setting | Southern Setting | | | Total (N = 1187) |
|---|---|---|---|---|---|
| | Duekoué (*n* = 372) | Adzopé (*n* = 208) | Agboville (*n* = 402) | Sikensi (*n* = 205) | |
| **Sex** | | | | | |
| Girl (%) | 132 (35.5) | 83 (39.9) | 182 (45.3) | 63 (30.7) | 460 (38.8) |
| Boy (%) | 240 (64.5) | 125 (60.1) | 220 (54.7) | 142 (69.3) | 727 (61.2) |
| **Age (years)** | | | | | |
| 5–8 (%) | 109 (29.3) | 55 (26.4) | 135 (33.6) | 58 (28.3) | 357 (30.1) |
| 9–11 (%) | 150 (40.3) | 91 (43.8) | 127 (31.6) | 111 (54.1) | 479 (40.5) |
| 12–14 (%) | 113 (30.4) | 62 (29.8) | 140 (34.8) | 36 (17.6) | 351 (29.4) |

*n*: number of children included in each study site; N: number of children included overall; %: percentage in each category.

### *3.2. Urine and Stool Examination*

#### 3.2.1. Infection with *S. haematobium*

All 1187 schoolchildren included in the study provided a single urine sample (100%). *S. haematobium* eggs were found in 166 of the children, owing to an overall prevalence of 14.0% (95% confidence interval (CI): 12.1%–16.1%). The prevalence of *S. haematobium* was considerably higher in the three school locations of the southern compared with the western Côte d'Ivoire (16.1% vs. 9.4%). The highest prevalence was found in Sikensi (35.6%). Boys and girls showed similar *S. haematobium* prevalence (14.2% vs. 13.7%; $p = 0.781$). No statistically significant difference was observed in the prevalence between age groups ($p = 0.337$).

Two cases of co-infection with *S. haematobium* and *S. mansoni* were found in Agboville. In two children from Duekoué, eggs identified in urine samples were morphologically determined as *S. mansoni* (Table 2).

**Table 2.** Prevalence rate of *Schistosoma haematobium* and *Schistosoma mansoni* infection, stratified by study settings, sex, and age group among schoolchildren from Côte d'Ivoire in early 2018.

| Characteristic | *S. haematobium* | | *S. mansoni* | |
|---|---|---|---|---|
| | Total | Positive *n* (%) | Total | Positive *n* (%) |
| **Western setting** | | | | |
| Duekoué | 372 | 35 (9.4)[2] | 274 | 20 (7.3) |
| **Southern setting** | | | | |
| Adzopé | 208 | 22 (10.6) | 208 | 1 (0.9) |
| Agboville | 402 | 36 (9.0)[1] | 402 | 45 (11.2)[1] |
| Sikensi | 205 | 73 (35.6) | 205 | 0 |
| *p*-value | | < 0.001 | | < 0.001 |
| **Sex** | | | | |
| Boy | 727 | 103 (14.2) | 408 | 27 (6.6) |
| Girl | 460 | 63 (13.7) | 681 | 39 (5.7) |
| *p*-value | | 0.781 | | 0.551 |
| **Age (years)** | | | | |
| 5–8 | 357 | 60 (16.8) | 307 | 15 (4.9) |
| 9–11 | 479 | 65 (13.6) | 443 | 24 (4.4) |
| 12–14 | 351 | 41 (11.7) | 339 | 27 (8.0) |
| *p*-value | | 0.337 | | 0.199 |

[1]: Two children were co-infected with *S. haematobium* and *S. mansoni.* [2]: Two of these 35 positive cases were identified microscopically as *S. mansoni* eggs.

*n*: number of positive children after microscopic examination

#### 3.2.2. Infection with *S. mansoni*

Stool samples were obtained from 1089 children (91.7%). The overall prevalence of *S. mansoni* infection was 6.1% (95% CI: 4.8%–7.6%). *S. mansoni* was most commonly found in Agboville (11.2%), while no infections were found in Sikensi. Age and sex were not associated with *S. mansoni* infection ($p > 0.05$) (Table 2). The arithmetic mean of *S. mansoni* eggs per gram of stool (EPG), including standard error (SE) from positive samples, was 91.1 EPG (SE: 11.2 EPG; 95% CI: 68.7–113.4 EPG) with a minimum and maximum of 20 and 400 EPG, respectively. The geometric mean of *S. mansoni* eggs from positive stool samples was 4.1 (SD: 0.9).

#### 3.2.3. Other Helminths and Co-Infection

Three species of soil-transmitted helminths were identified in stool samples at very low rates: *Trichuris trichiura* (2.3%), *Ascaris lumbricoides* (1.7%), and hookworm (0.2%). In two school locations, children with concurrent *Schistosoma* and soil-transmitted helminth infections were identified; in Sikensi (*S. haematobium-T. trichiura* and *S. haematobium*-hookworm) and in Agboville (*S. mansoni-A. lumbricoides*, *S. mansoni-T. trichiura,* and triple species infection with *S. mansoni*, *A. lumbricoides,* and *T. trichiura*).

#### 3.2.4. Multivariate Logistic Regression Models

Table 3 shows the association between *Schistosoma* infection and sociodemographic factors, socioeconomic status, and environmental factors. The key risk factors for *S. haematobium* were swimming (adjusted odds ratio (AOR): 127.0; 95% CI: 25.0–634.0) and playing in water (AOR: 74.0; 95% CI: 3.8–144.3). For *S. mansoni*, children who lacked tap water at home (AOR: 2.7; 95% CI: 1.2–5.8) and who washed their clothes in open freshwater bodies (AOR: 5.3; 95% CI: 2.3–12.1) were the most infected.

The educational status as illiterate, of fathers (crude odds radio (COR): 3.3; 95% CI: 1.0–10.9) and mothers (COR: 11.5; 95% CI: 1.6–84.0) were also significantly associated with *S. mansoni* infection.

**Table 3.** Multivariate logistic regression model analysis of variables associated with *S. haematobium* and *S. mansoni* infection among schoolchildren non-adjusted and adjusted for sociodemographic factors, socioeconomic status, and environmental factors.

| Characteristics | *S. haematobium* | | | | *S. mansoni* | | | |
|---|---|---|---|---|---|---|---|---|
| | Total | Positive | Crude OR (95% CI) | Adjusted OR (95% CI) | Total | Positive | Crude OR (95% CI) | Adjusted OR (95% CI) |
| **Sociodemographic factors** | | | | | | | | |
| *School locations* | | | | | | | | |
| Agboville | 402 | 36 | 1.00 | 1.00 | 402 | 45 | 0.04 (0.01–0.28) | 1.88 (0.02–2.20) |
| Adzopé | 208 | 22 | 1.20 (0.69–2.10) | 1.72 (0.25–11.61) | 208 | 1 | 1.00 | 1.00 |
| Duekoué | 372 | 35 | 1.06 (0.65–1.72) | 0.67 (0.10–4.37) | 274 | 20 | 0.62 (0.36–1.08) | 1.66 (0.54–5.10) |
| Sikensi | 205 | 73 | 5.62 (3.60–8.78)* | 4.43 (0.10–198.43) | 205 | 0 | – | – |
| ***Sex*** | | | | | | | | |
| Girl | 460 | 63 | 1.00 | 1.00 | 408 | 27 | 1.00 | 1.00 |
| Boy | 727 | 103 | 1.04 (0.74–1.46) | 0.92 (0.32–2.68) | 681 | 39 | 0.86 (0.52–1.42) | 0.98 (0.52–1.82) |
| *Age (years)* | | | | | | | | |
| 5–8 | 357 | 60 | 1.00 | 1.00 | 307 | 15 | 1.00 | 1.00 |
| 9–11 | 479 | 65 | 0.78 (0.53–1.14) | 0.15 (0.02–0.99) | 443 | 24 | 1.11 (0.58–2.16) | 0.37 (0.10–1.35) |
| 12–14 | 351 | 41 | 0.65 (0.42–1.00) | 0.28 (0.03–2.80) | 339 | 27 | 1.68 (0.88–3.23) | 0.23 (0.05–1.09) |
| **Socioeconomic factors** | | | | | | | | |
| *Father's education* | | | | | | | | |
| Illiterate | 710 | 104 | 1.30 (0.69–2.46) | 1.20 (0.05–28.41) | 620 | 57 | 3.34 (1.03–10.88)* | – |
| Primary school | 101 | 12 | 1.02 (0.44–2.40) | 1.15 (0.01–25.70) | 101 | 0 | – | – |
| Secondary school | 104 | 12 | 0.99 (0.42–2.32) | 0.45 (0.01–6.46) | 104 | 1 | 0.32 (0.03–3.13) | – |
| Expert level | 103 | 12 | 1.00 | 1.00 | 102 | 3 | 1.00 | – |
| *Mother's education* | | | | | | | | |
| Illiterate | 856 | 139 | 3.39 (0.46–26.35) | 3.18 (0.03–26.50) | 767 | 58 | 11.54 (1.58–83.96)* | – |
| Primary school | 142 | 17 | 2.45 (0.31–19.53) | 3.98 (0.04–38.00) | 142 | 1 | 0.42 (0.10–4.54) | – |
| Secondary school | 51 | 5 | 1.96 (0.21–17.93) | 0.34 (0.04–28.20) | 53 | 0 | – | – |
| Expert level | 19 | 1 | 1.00 | 1.00 | 18 | 1 | 1.00 | – |
| *Father's occupation* | | | | | | | | |
| Farmer | 735 | 123 | 1.00 | 1.00 | 694 | 48 | 1.00 | 1.00 |
| Fisherman | 152 | 20 | 0.75 (0.45–1.25) | 2.26 (0.57–9.00) | 105 | 6 | 0.82 (0.34–1.96) | 0.72 (0.17–3.07) |
| Official | 269 | 21 | 0.42 (0.26–0.68) | 0.23 (0.01–3.56) | 265 | 10 | 0.53 (0.26–1.06) | 1.08 (0.33–3.51) |

**Table 3.** *Cont.*

| Characteristics | *S. haematobium* | | | | *S. mansoni* | | | |
|---|---|---|---|---|---|---|---|---|
| | **Total** | **Positive** | **Crude OR (95% CI)** | **Adjusted OR (95% CI)** | **Total** | **Positive** | **Crude OR (95% CI)** | **Adjusted OR (95% CI)** |
| *Mother's occupation* | | | | | | | | |
| Farmer | 617 | 115 | 1.00 | 1.00 | 551 | 42 | 1.00 | 1.00 |
| Householder | 520 | 49 | 0.45 (0.32–0.65) | 1.13 (0.32–4.05) | 495 | 23 | 0.59 (0.35–1.00) | 1.13 (0.42–3.01) |
| Official | 22 | 1 | 0.21 (0.03–1.56) | 0.63 (0.10–9.90) | 21 | 0 | – | – |
| **Environmental factors** | | | | | | | | |
| *Using tap water* | | | | | | | | |
| Yes | 539 | 74 | 1.00 | 1.00 | 447 | 17 | 1.00 | 1.00 |
| No | 618 | 91 | 1.08 (0.78–1.51) | 0.55 (0.14–2.17) | 616 | 49 | 2.19 (1.24–3.85) | 2.65 (1.22–5.79)* |
| *Swimming* | | | | | | | | |
| No | 922 | 1 | 1.00 | 1.00 | 879 | 63 | 1.00 | 1.00 |
| Yes | 265 | 165 | 152 (21–1097) | 127 (25–634)* | 210 | 3 | 0.18 (0.06–0.60) | 0.35 (0.09–1.40) |
| *Washing clothes* | | | | | | | | |
| No | 118 | 4 | 1.00 | 1.00 | 917 | 38 | 1.00 | 1.00 |
| Yes | 1069 | 162 | 0.20 (0.07–0.54) | 0.70 (0.10–82.0) | 118 | 28 | 7.64 (4.48–13.02) | 5.26 (2.28–12.10)* |
| *Fishing* | | | | | | | | |
| No | 386 | 40 | 1.00 | 1.00 | 338 | 9 | 1.00 | 1.00 |
| Yes | 801 | 126 | 1.61 (1.11–2.36) | 74.0 (3.8–144.3)* | 751 | 57 | 3.00 (1.47–6.14) | 1.88 (0.34–10.33) |
| *Playing* | | | | | | | | |
| No | 493 | 55 | 1.00 | 1.00 | 432 | 12 | 1.00 | 1.00 |
| Yes | 694 | 111 | 1.52 (1.07–2.14) | 1.98 (0.09–45.32) | 657 | 54 | 3.13 (1.66–5.93) | 0.61 (0.11–3.25) |

* $p$-value < 0.05, $p$-value obtained from a mixed logistic regression model with fixed effects for the prevalence of *S. haematobium* or *S. mansoni* and each variable in the table.

## 4. Discussion

This study was designed to determine the prevalence of the two known human *Schistosoma* species and to identify risk factors associated with infection among 5–14 year-old schoolchildren in southern and western parts of Côte d'Ivoire. We employed widely used diagnostic methods; namely a filtration method for detection and quantification of *S. haematobium* eggs in urine samples and the Kato-Katz technique for detection and quantification of *S. mansoni* (and soil-transmitted helminth) eggs in fecal samples. We found an overall prevalence of 14.0% for *S. haematobium* and 6.1% for *S. mansoni*, which classify our study settings as moderate and low endemic areas, respectively, for urogenital schistosomiasis and intestinal schistosomiasis, according to WHO guidelines [23]. The arithmetic mean of *S. mansoni* egg counts (91.9 EPG) recorded was low and would be classified as a light infection.

As expected, the current study reports a low prevalence of *S. haematobium* in Duekoué in the western part of Côte d'Ivoire, corroborating results from previous studies [11,17]. The highest prevalence of *S. mansoni* was found in Agboville, in one of the three school locations included in the southern part of Côte d'Ivoire. Similar studies showed the prevalence to be lower for *S. haematobium* (0.9%–4.4%), and higher for *S. mansoni* (17.5%–61.3%) in western Côte d'Ivoire [24]. Another study reported a high prevalence of *S. mansoni* (58.7%–68.4%) and low prevalence for *S. haematobium* (10.9%–18.4%) in southern Côte d'Ivoire [9]. The difference between the prevalence of the two schistosome species could be explained by the variation in ecological factors that influence the transmission dynamics. The low prevalence rate of *S. haematobium* and *S. mansoni* infections reported in our study is most likely the result of preventive chemotherapy campaigns pursued on an annual basis since several years by the PNLMTN-CP in Côte d'Ivoire[25].

We found similar prevalence rates for boys and girls, corroborating results from previous studies in Côte d'Ivoire [9]. However, it must be noted that the number of boys in our final study sample was considerably higher than that of girls (727 vs. 460), which was particularly pronounced in Sikensi in the southern part (142 vs. 63) and Duekoué in the western part of Côte d'Ivoire (240 vs. 132). This observation is in line with a large epidemiological study conducted in the late 1990s in the Man region of western Côte d'Ivoire. Among 12,227 children interviewed from 121 schools, there were 7489 boys and 4738 girls, pointing to a gender-bias in terms of school enrolment that tended to increase with age of children [26]. Interestingly, prior and current observations from Côte d'Ivoire are in contrast to a study from Senegal, where boys showed a higher prevalence of *Schistosoma* infection [27]. Results from other studies showed that when children are in contact with freshwater bodies for longer periods of time, they are more likely to be infected [9,28]. The risk of disease occurrence can increase because children are more often involved in recreational activities; hence, they are exposed to unprotected open surface freshwater for longer periods [29].

*S. mansoni* eggs, unmistakendly characterised by a lateral spine, were identified in two urine samples obtained from schoolchildren in Duekoué. The appearance of *S. mansoni* eggs in urine is unusual and has not been studied extensively [30]. In our investigation, *Schistosoma* species were determined by widely used methods based on egg morphology and light microscopy. Future studies should employ concurrent molecular approaches to improve diagnostic sensitivity.

In the current study, no differences in the prevalence of *Schistosoma* infection were observed among the three investigated age groups, which is in line with several other studies [20,31,32]. However, there are also studies reporting an increase in the prevalence with age of children [33,34]. Indeed, children aged 10–14 years can become more vulnerable for schistosomiasis during recreational activities, i.e., swimming and playing in water, or while fetching water for household use, or agriculture activities [35,36]. Most of the children in our study who did not have tap water at home were infected by *S. mansoni*, contrary to results reported from South Africa [34].

Socioeconomic factors were significantly associated with the occurrence of schistosomiasis. In particular, a significant relationship between illiteracy of the parents/guardians and *S. mansoni* infection was reported. Similar results were found in a previous study in Nigeria, which showed that

better educated parents can understand the preventive campaigns more deeply and thus better explain them to their children [35].

The association between the occurrence of schistosomiasis and contact with freshwater bodies is well documented. In our study, swimming and fishing in freshwater by schoolchildren was strongly associated with infection with *S. haematobium*. This might be explained by the fact that, while swimming, infected children emit urine with *S. haematobium* eggs. The eggs hatch, infecting snails which produce furcocercariae that penetrate the skin of children exposed to the contaminated water. This corroborates the findings of other studies that reported high infection rates among children who swim in rivers [25,37]. Other researchers did not report a significant correlation between swimming and occurrence of schistosomiasis [20,38]. Our finding that washing clothes in water and lack of tap water at home were associated with the occurrence of *S. mansoni* infection is in line with results from other studies [9,34].

The low prevalence of any of the three common soil-transmitted helminths in our study confirms observations from previous surveys [39,40]. This observation is likely attributed to large-scale preventive chemotherapy campaigns by the PNLMTN-CP, coupled with systematic sensitization and deworming carried out by non-governmental organizations (NGOs) and improvements in sanitation in face of social and economic development.

The current study has several limitations, and hence, the findings should be interpreted with care. First, stool and urine samples were collected only on a single day, though duplicate Kato-Katz thick smears were examined from each stool sample to enhance diagnostic sensitivity. It is conceivable that a number of infections, particularly those of light intensity were missed, and hence, the overall prevalence of both *S. haematobium* and *S. mansoni* might be somewhat higher than reported here [41]. Second, data about sociodemographic, socioeconomic, and environmental factors were collected through a pretested questionnaire administered to children. There might be some kind of reporting bias. Third, no specific information was collected on water, sanitation, and hygiene behavior, although these are known risk factors for schistosomiasis [42,43].

## 5. Conclusions

Our study confirms that schistosomiasis remains prevalent in the southern and western parts of Côte d'Ivoire, although the overall prevalence in school-aged children was much lower than reported a few years earlier. Swimming, washing clothes, playing in unprotected open freshwater bodies, and low educational attainment of parents/guardians were identified as key risk factors of schistosomiasis in schoolchildren. Hence, preventive chemotherapy using praziquantel—which is the current mainstay of the national schistosomiasis control program—should be combined with targeted information and education campaigns to change children's behaviors, with the goal of reducing the frequency of contact of children with open freshwater bodies.

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# Chapter 3:

# Identification of schistosome hybrids in humans

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

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

## Research Article

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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 $\mu$m 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 $\mu$L 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 $\mu$L of double-distilled water for 10 min. Water was removed and the disc incubated in 80 $\mu$L 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 $\mu$L 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 $\mu$L, comprising 2 $\mu$L of the DNA extract, 2 $\mu$L of Green GoTaq Flexi Buffer 5X (Promega; Madison, Wisconsin, USA), 0.6 $\mu$L of 25 mM $MgCl_2$ (Promega), 0.2 $\mu$L of 10 mM dNTP mix (Promega), 1 $\mu$L of 10 × primer mix (4 $\mu$L of 100 $\mu$M reverse primer, 4 $\mu$L of each 100 $\mu$M forward primer and 84 $\mu$L 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 $\mu$L, comprising 4 $\mu$L of DNA template, 5 $\mu$L of 5X Colorless GoTaq® Flexi Buffer (Promega), 1.5 $\mu$L of $MgCl_2$ (25 mM), 0.5 $\mu$L of dNTP (10 mM), 0.8 $\mu$L of each 10 $\mu$M primer and 0.2 $\mu$L 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


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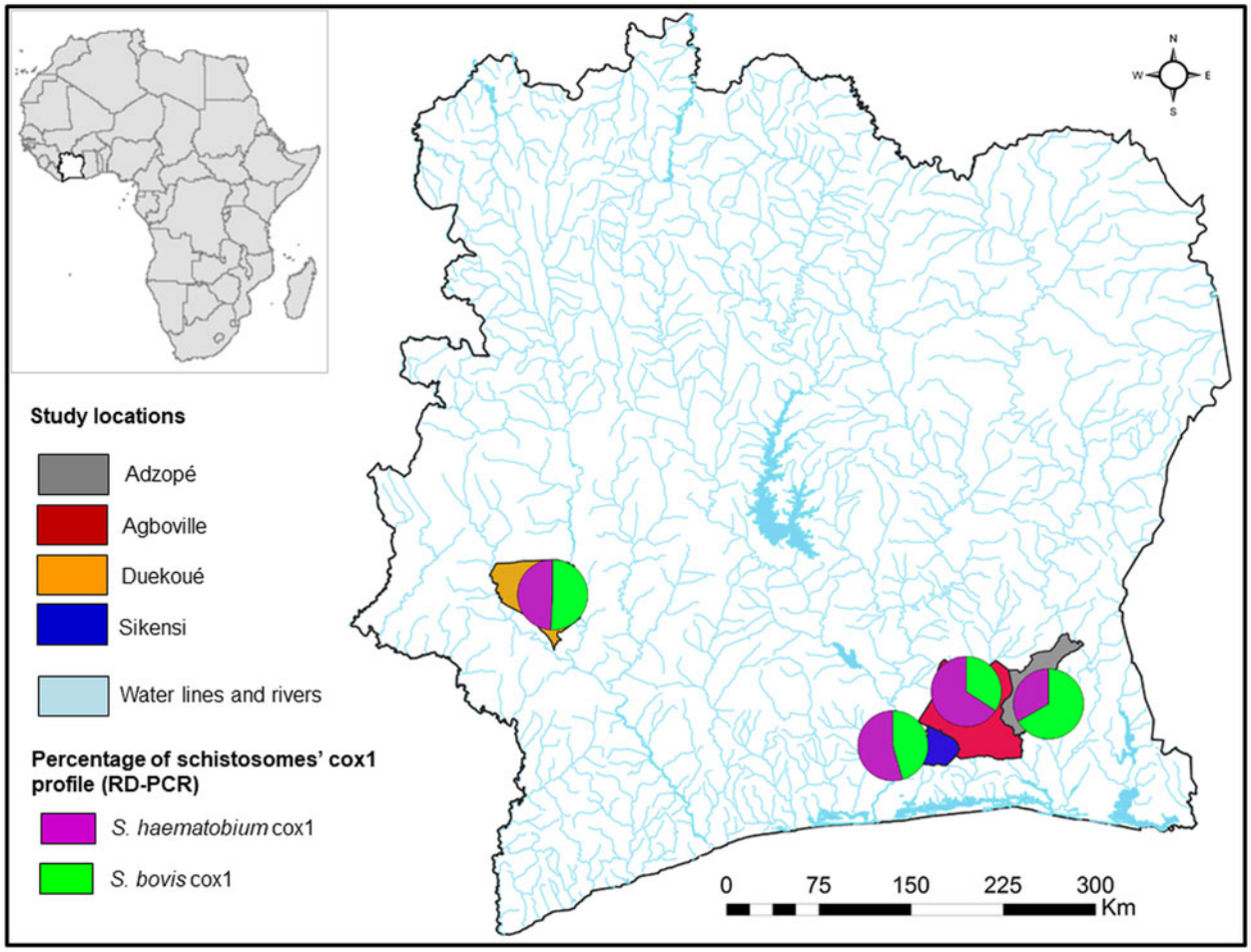


**Fig. 1.** Map of the four study locations in Côte d'Ivoire (Adzopé, Agboville, Duekoué and Sikensi) showing the distribution and the proportion of *Schistosoma haematobium* cox1 or a *Schistosoma bovis* cox1 genetic profile. The cox1 profile of each miracidium was identified using a rapid diagnostic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

mitochondrial cox1 and nuclear ITS2 PCR products (4 $\mu$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 [±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


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**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

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

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Sikensi | SI028 | 30 | 18 (60.0) | 12 (40.0) | 2 | 1 | *S. bovis* Sb3 | *S. haematobium* | Hybrid |
| | | | | | | 1 | *S. bovis* Sb4 | *S. haematobium* | Hybrid |
| | SI052 | 26 | 12 (46.2) | 14 (53.8) | 2 | 1 | *S. haematobium* Sh7 | *S. haematobium* | *S. haematobium* |
| | | | | | | 1 | *S. haematobium* undet[a] | *S. haematobium* | *S. haematobium* |
| | SI109 | 22 | 9 (40.9) | 13 (59.1) | 1 | 1 | *S. haematobium* undet[a] | *S. haematobium* | *S. haematobium* |
| | SI114 | 19 | 11 (57.9) | 8 (42.1) | 1 | 1 | *S. bovis* Sb3 | *S. haematobium* | Hybrid |
| | SI122 | 19 | 11 (57.9) | 8 (42.1) | 2 | 1 | *S. bovis* Sb2 | *S. haematobium* + *S. bovis* | Hybrid |
| | | | | | | 1 | *S. bovis* Sb1 | *S. haematobium* | Hybrid |
| | SI136 | 23 | 15 (65.2) | 8 (34.8) | 2 | 1 | *S. haematobium* Sh1 | *S. haematobium* | *S. haematobium* |
| | | | | | | 1 | *S. haematobium* undet[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.
[a]undet., 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 (±S.D.) was $0.922 \pm 0.051$ and $0.724 \pm 0.121$ for *S. bovis* and *S. haematobium*, respectively. Nucleotide diversity (±S.D.) was $0.0094 \pm 0.0013$ and $0.0011 \pm 0.0003$ for *S. bovis* and *S. haematobium*, respectively. Among the ITS2 sequences, 31 gave an *S. haematobium* profile and four an *S. bovis* profile. Five miracidia gave double chromatogram peaks at the polymorphic positions between *S. haematobium* and *S. bovis*, suggesting heterozygosity (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 × *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


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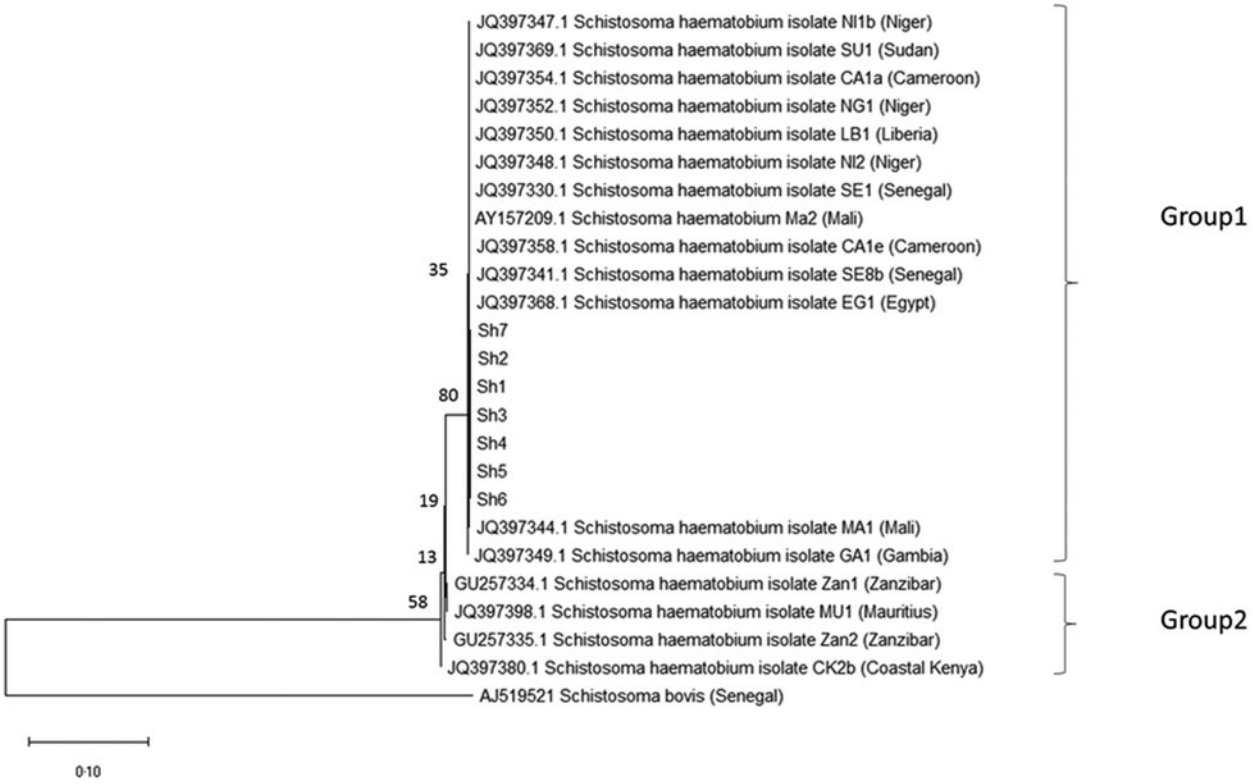


**Fig. 2.** Majority rule consensus tree from maximum likelihood analysis of the mitochondrial cox1 sequences for *Schistosoma haematobium* haplotypes Sh1–Sh7, including data from Genbank. Groups 1 and 2 indicate the major clades defined by Webster *et al.* (2012). Clade support values for each node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

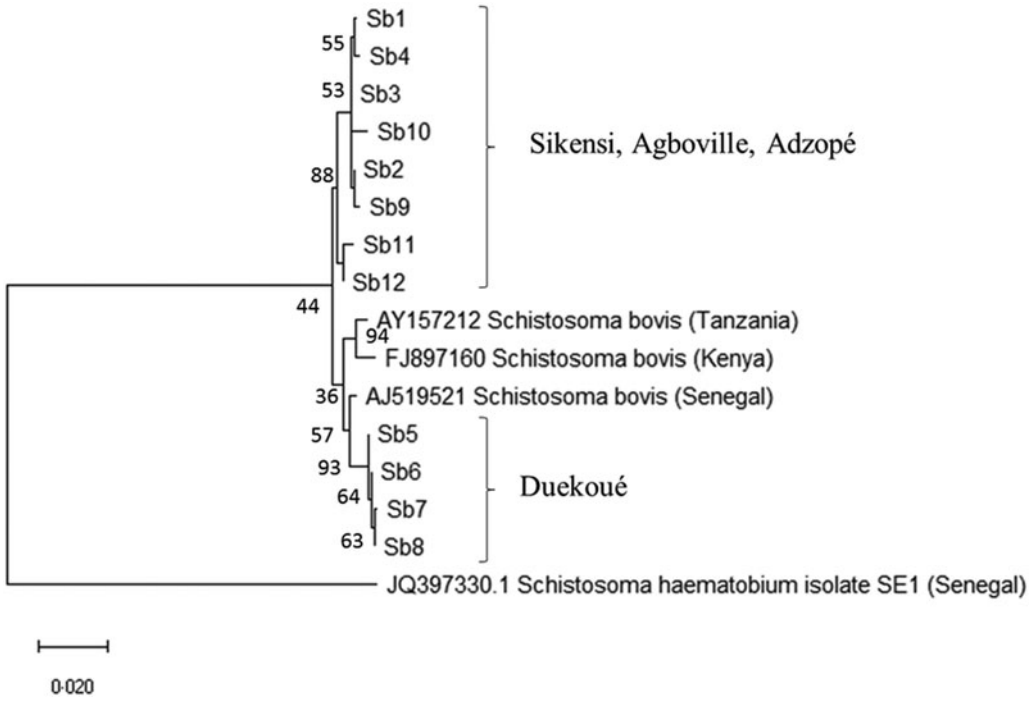


**Fig. 3.** Majority rule consensus tree from maximum likelihood analysis the mitochondrial DNA cox1 sequences for *Schistosoma bovis* haplotypes Sb1–Sb12 and data from GenBank. There are two clusters of *S. bovis* haplotypes: Duekoué (Sb5–Sb8) and the three other study sites (Sb1–Sb4 and Sb9–Sb12). 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


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*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.

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**Conflict of interest.** None.

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

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# Chapter 4:

# Review of molecular methods in population genetics of *Schistosoma haematobium*

Chapter 4

# A review of molecular methods for genetic structure and diversity of *Schistosoma haematobium* in Africa

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Chapter 4

# A review of molecular methods for genetic structure and diversity of *Schistosoma haematobium* in Africa

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**Abstract**

Highly sensitive PCR-based assays have been developed for the detection of schistosome DNA to confirm infection, infecting species and to genetically characterize schistosome populations. Despite the enormous number of people infected with *Schistosoma haematobium*, the vast majority of population genetics studies of *S. haematobium* are from Asia, with only a few from Africa. A review of the methods used and genetic patterns found in populations of *S. haematobium* is needed. We systematically reviewed the scientific literature pertaining to the methods used to study population genetic structuring and diversity of *S. haematobium* across Africa. Available electronic databases (i.e. MEDLINE/PubMed, Science Direct, Web of Science and Google Scholar) were searched and published articles from 1997 to 2020 of any language, which focused on population genetics of *S. haematobium* were included. The titles and abstracts of studies were screened for eligibility and relevant articles were read in full for inclusion in the review. Seventeen peer-reviewed publications were considered for the final analysis. These papers reported four methods used for determining population genetic structuring and diversity of *S. haematobium*, these include restriction fragment length polymorphism (RFLP), randomly amplified polymorphic DNA (RAPD), DNA barcoding and microsatellites genotyping. Variable genetic diversity and population structure were observed within and across countries.

The current research suggests that *S. haematobium* has variable genetic diversity and structure within and across countries. Microsatellite analysis seems to be the suitable approach to assess population genetics of *S. haematobium*.

**Keywords:** Africa, Genetic structure, Diversity, Molecular methods, *S. haematobium*

## Introduction

Helminth infections caused by nematodes, cestodes and trematodes are a significant contributor to the global burden of disease, particularly in populations from the tropics and subtropics (Hotez et al., 2008). Schistosomiasis, a neglected tropical disease according to World Health Organization, is a water-associated, chronic parasitic infection caused by a trematode worm of *Schistosoma* genus. *Schistosoma* species infect humans, wildlife and livestock (definitive hosts) as well as snails (intermediate hosts). Schistosomiasis affects more than 250 million people in the world and is estimated to cause at least 11,000 deaths per year (Knowles et al., 2017). The disease remains a great public health and socioeconomic problem in several tropical and subtropical countries (Gryseels et al., 2006; Jordan, 2000; Southgate et al., 2005). According to the Global Burden of Disease (GBD) study, 1.4 million Disability-Adjusted Life Years (DALYs) were lost due to schistosomiasis in 2017 and the number of cases in the world is considered to be greater than 250 million, of which most occur in Africa (GBD 2017 DALYs and HALE Collaborators, 2018; Hotez et al., 2014). Six species of schistosomes are known to infect humans: *Schistosoma mansoni*, *S. haematobium*, *S. intercalatum* and *S. guineensis* in Africa, and *S. mekongi*, as well as *S. japonicum* in Asia (Colley et al., 2014; Rollinson et al., 1997). *Schistosoma haematobium* causes the urogenital form of the disease and is endemic in sub-Saharan Africa and the Middle East. It is the most prevalent species in sub-Saharan Africa. *Schistosoma* eggs that become lodged in the tissues cause granulomatous inflammation, ulceration and squamous-cell carcinoma of the bladder, or pseudopolyposis of the vesical and uterine walls. The common early signs include dysuria, proteinuria, and especially haematuria. Chronic lesions can evolve to fibrosis or calcification of the bladder and lower ureters (Akinwale et al., 2008; Gray et al., 2011; Gryseels et al., 2006; IARC, 2012; van der Werf et al., 2003).

Laboratory methods are necessary for diagnosis and treatment of schistosomiasis and are commonly used to identify eggs in faeces or urine, depending on the infecting species and where the parasite resides. In the case of urogenital schistosomiasis, microscopic examination of urine samples by filtration remains the gold standard for the diagnosis (Colley et al., 2014). Molecular characterization using specific and highly sensitive PCR-based assays have been developed for the detection of schistosome DNA to confirm infection (Alzaylaee et al., 2020; Gomes et al., 2010; Mulero et al., 2019; Oliveira et al., 2010; Wichmann et al., 2009). This approach has the potential to diagnose schistosomiasis at all phases of clinical disease, including the acute (Katayama syndrome) and chronic stages of disease, and for the evaluation of treatment (Gray et al., 2011).

Two molecular markers are frequently used to identify parasites species: a nuclear marker (*ITS2*, ribosomal internal transcribed spacer 2), which is diploid, and a mitochondrial marker (*COI* or *Cox1*, cytochrome oxidase subunit I) which is haploid and is maternally inherited (Boissier et al., 2016; Huyse et al., 2009). Mitochondrial markers have a higher mutation rate than nuclear markers, and are often used for phylogenetic analyses within species. These methods and markers are now frequently used to genetically characterization parasites (Huyse et al., 2009; Littlewood et al., 2006). Molecular methods are also commonly used to identify the *Schistosoma* species of adult worms, cercariae, eggs and miracidia (Barber et al., 2000; Boissier et al., 2016; Huyse et al., 2013). Maternal and nuclear markers are used in combination for this purpose. Prior to the advent of molecular methods, *Schistosoma* species could only be determined by egg morphology and unusual egg phenotypes led researchers to suspected that hybrids between schistosome species existed as far back the 1940s (Leger and Webster, 2017). The use of molecular markers has confirmed these suspicions, with genetically verified schistosome hybrids found across sub-Saharan Africa (Leger and Webster, 2017; Boon et al., 2018; Webster et al., 2019).

Despite the considerable burden of *S. haematobium*, research on the genetic diversity of this schistosome species is sparse, particularly in Africa (Gower et al. 2011). There is a need to review the methods used and genetic patterns found in populations of *S. haematobium*. Therefore, a literature review was conducted to summarize the methods used to assess genetic structure and diversity of *S. haematobium* and patterns found in Africa.

## Methods

### *Search strategy*

This systematic literature review was performed to identify all scientific studies investigating genetic structure and diversity of *S. haematobium* in Africa. The research was conducted and reported according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (Moher et al., 2009).

The following electronic databases were systematically searched: MEDLINE/PubMed, Science Direct, Web of Science and Google Scholar. The search strategy included the keywords related to schistosomiasis, genetics, variability, molecular methods and Africa. The full search strategy outlined used a combination of terms including at least the concept "schistosome" **(Table 1)** and the PRISMA checklist is provided in **Additional file 1**.

Publications were screened first by title and then by relevance of the abstracts. All manuscripts that met the selection criteria were included for analysis. A subsequent snowball search of the reference lists of all full text articles that met inclusion criteria was conducted to find further relevant sources. All articles published from 1997 to 2020 were eligible for inclusion without language restrictions. Conference abstracts or book chapters detected through these databases were also considered. Studies that were conducted outside Africa, but with African samples were included, however publications focusing on non-African samples or other schistosome species were excluded. Laboratory studies without field samples were excluded, as well.

Data from the full text articles were extracted using a standardized spreadsheet. Items obtained included study date, country, type of samples, method of determining genetic structure or diversity for *S. haematobium* and major findings of genetic analyses.

## Results

### *Search results*

The literature search yielded 392 published studies, with an additional four articles from other sources (snowballing) for a set of 396 articles **(Fig. 1)**. After removing 12 duplicates, 384 articles were evaluated in more detail and from that, 337 studies were further excluded based on the relevance of the titles and abstracts. We performed the analysis of full text on the remaining 47 studies and 30 articles were excluded because their scope did not include population genetics of *S. haematobium* in Africa, leaving 17 articles that were included in the review **(Table 2)**.

### *Samples and study sites*

The selected publications investigated samples from many African countries **(Fig. 2)** and employed different methods to collect samples. Six studies collected *Schistosoma* eggs (Afifi et al. 2016; Ezeh et al. 2015; Gasmelseed et al. 2014; Quan et al. 2015) or miracidia (Angora et al. 2020; Gower et al. 2013) from urine samples, depositing and storing them on Whatman-FTA® cards (GE Healthcare Life Sciences; Amersham, UK). Another six publications collected cercariae from snails that were infected with miracidia in the laboratory (Shiff et al. 2000; Brouwer et al. 2001, 2003; Gower et al. 2011; Webster et al. 2012; Glenn et al. 2013; Boon et al. 2019). Some studies performed genetic analyses directly on the cercariae (Shiff et al. 2000; Brouwer et al. 2001; Tian-Bi et al. 2019), while others used the cercariae to infect laboratory hamsters. Adult schistosome worms were used for genetic analysis after being collected from the mesenteric vein system of the hamsters by means of perfusion and dissection (Dabo et al. 1997; Gower et al. 2011; Webster et al. 2012, 2013; Boon et al. 2019).

### *Molecular methods used*

Four molecular methods were used to assess the genetic diversity and population structure of *S. haematobium* in the studies included in our review: microsatellite genotyping, randomly amplified polymorphic DNA (RAPD), restriction fragment length polymorphism (RFLP) and DNA barcoding (**Table 2)**.

Microsatellite analyses used sets of four to eighteen markers to assess genetic diversity and population structure of *S. haematobium* within Senegal, Zanzibar and Mali (Boon et al. 2019; Webster et al. 2015; Gower et al. 2011) and across Kenya, Tanzania, Uganda, Cameroon, Niger, Mali and Nigeria (Ezeh et al. 2015; Gower et al. 2013). This method showed high resolution in terms of genetic analysis and fingerprints of genetic patterns in the genome. A

variable level of genetic diversity and population structure of *S. haematobium* were reported within and across countries.

Randomly amplified polymorphic DNA (RAPD) was the most commonly used method to analyze the genetic diversity of *S. haematobium* within Sudan, Zimbabwe, Mali (Gasmelseed et al. 2014; Brouwer et al. 2003, 2001; Shiff et al. 2000; Dabo et al. 1997) and across Egypt, Nigeria, Senegal, Zimbabwe, South Africa, Malawi and Zanzibar (Afifi et al. 2016; Glenn et al. 2013). The number of primers used for the PCR varied from four to 32 and produced at least 15 high-quality unambiguous loci. A variable genetic diversity was observed with a mean number of 32.7 genotypes. In the study of Glenn et al. (2013) , 15 high quality, unambiguous microsatellite loci were identified. High genetic diversity and population genetic structure were observed across countries using this method.

Restriction fragment length polymorphism (RFLP) was employed to characterize the genetic diversity of *S. haematobium* among human hosts in Sudan. To select samples for the RFLP analysis, a multiplex PCR was performed using genomic DNA isolated from patients with microscopically confirmed urinary schistosomiasis using primers specific for two microsatellite markers of *S. haematobium* (Quan et al. 2015). In this study, RFLP used the digestion enzyme Sau3A1 that cut the internal transcribed spacer 2 region (ITS2) of *S. haematobium* in three places, producing fragments at 237, 98, 83, and 50 bp. No genetic difference was observed in *S. haematobium* isolated from Sudan compared to those collected in Kenya.

DNA barcoding was employed in four studies within Côte d'Ivoire and Zanzibar (Angora et al. 2020; Tian-Bi et al. 2019; Webster et al. 2013), and across several African countries (Webster et al. 2012). The mitochondrial cytochrome oxidase subunit I (*Cox1*), was the most commonly used marker, however the partial region of the mitochondrial NADH-dehydrogenase subunit 1 (nad1) gene was also utilized occasionally (Webster et al. 2012). In one study, molecular analysis was performed using cercariae collected from naturally infected *Bulinus* snails (Tian-Bi et al. 2019). Ninety-six *S. haematobium Cox1* haplotypes were identified in the four studies. In several countries, more than one molecular method was employed to analysis genetic patterns of *S. haematobium*. **Fig. 2** shows sampling areas and molecular methods used for analysis.

## Discussion

This review aimed to provide an overview of the methods used for analysis of genetic variability and population structure of *S. haematobium* in humans across Africa and of the patterns emerging from these studies. To our knowledge, this is the first systematic literature review on this topic. Four molecular methods were used (DNA barcoding, RAPD, RFLP and microsatellite genotyping). Taken together, the available studies suggest that *S. haematobium* is genetically diverse within and across countries. A population genetic structure was consistently observed in the studies investigating patterns using microsatellite genotyping.

### *Microsatellite genotyping*

Microsatellites or short tandem repeats (STRs) are repetitive DNA motifs ranging in length from two to six base pairs, that are tandemly repeated 5-50 times and occur at thousands of locations within the genome (Gulcher, 2012; Richard et al., 2008). They have a higher mutation rate than most other genome regions, leading to high genetic diversity (Brinkmann et al., 1998). They are found in prokaryotes and eukaryotes and are used for a variety of purposes, including forensics, paternity testing, population genetics, genetic mapping, and phylogeography (Vieira et al., 2016). Five studies included in this review have employed microsatellite genotyping to assess the genetic variation and structure of *S. haematobium*, using between four to eighteen loci (Nele A. M. Boon et al., 2019; Ezeh et al., 2015; Webster et al., 2015; Gower et al., 2013, 2011). These markers have also been used to differentiate *S. haematobium* from *S. bovis* (Djuikwo-Teukeng et al., 2019).

Variable genetic diversity and population genetic structure were observed within and across countries. Boon et al. (2019) reported significant genetic diversity indices between *S. haematobium* from humans and *S. bovis* from cattle, suggesting species boundary between human and cattle schistosomes, despite the evidence of hybridization.

Similarly, significant genetic diversity of *S. haematobium* was found between parasite populations (Ezeh et al. 2015; Webster et al. 2015; Gower et al. 2011). The population structure analysis showed clustering of parasite populations within countries (Gower et al. 2013). All these findings revealed that microsatellite analyses provide opportunities to investigate many important topics. The most relevant being the relationships between parasite genetics and disease burden and consequences of various control activities on parasite populations (Norton et al., 2010). Furthermore, the potential for the circulation of strains adapted to human and reservoir hosts (Lu et al., 2010) and the likely evolution and spread of drug resistance (Webster et al., 2008) could be investigated with this method. Although the development of high-

throughput single nucleotide polymorphism (SNP) platforms for genome analysis is accelerating, microsatellite markers remain highly informative measures of genomic variation for linkage and association assessment (Ott et al., 2015). Up until now, SNPs have not been developed for schistosomes, but would be a very helpful tool. They would likely also reveal further markers to distinguish between species like the known polymorphisms in the nuclear *ITS2* region that differentiate between *S. haematobium* and *S. bovis* (Angora et al. 2020; Webster et al. 2013; Huyse et al. 2009). Microsatellite genotyping remains the principal method for population genetics related to high information content, low cost, high throughput and sufficient resolution for population structure.

*Randomly amplified polymorphic DNA (RAPD)*

The genetic diversity analyses using this method are related to the number of unambiguous loci recovered after PCR. This method allows differentiation of both inter- and intraspecific relationships. This method requires a low amount of DNA, which is a critical issue when analysing schistosome miracidia or cercariae (Brouwer et al. 2001; Shiff et al. 2000). It require neither a DNA probe nor information about the genome of the organism. However, the main drawback is the segregation of dominant markers with heterozygous and homozygous dominant individuals having the same banding pattern at a locus while homozygous recessive individuals having no band at that locus (Williams et al., 1990). Limitations of the RAPD method due to a notorious inconsistency of patterns have been improved by running duplicate reactions, increasing the quantity of DNA and using high quality reagents (Shiff et al. 2000). To enhance genotype banding patterns produced by RAPD-PCR, Brouwer et al. (2003) have added an additional procedure to estimate the related groupings of the population. This provides a picture of the genetic relationships among individuals. In five studies, the extent of genetic diversity of *S. haematobium* infections was assessed by using RAPD on parasite samples to produce unambiguous loci (Afifi et al. 2016; Gasmelseed et al. 2014; Brouwer et al. 2003, 2001; Dabo et al. 1997). Glenn et al. (2013) used this method to produce microsatellite markers and compare results between populations in Senegal, Zanzibar, Malawi, Mauritius, Nigeria and South Africa. The microsatellite analysis in this study showed strong significant structuring between all sites ($F_{ST}$ = 0.16 – 0.54, p=0.001), but especially Senegal and South Africa ($F_{ST}$ = 0.65, p=0.001). There were no significant deviations from Hardy-Weinberg Equilibrium or significant signs of inbreeding, although samples sizes were small (between 10 and 12 parasites). Expected heterozygosity was quite high for all sites but South Africa (0.51-0.70 and 0.06, relatively). From this RAPD analyses, a variable genetic diversity of *S. haematobium* populations was

reported with levels ranging from low (Dabo et al., 1997) to high (Afifi et al. 2016; Glenn et al. 2013; Brouwer et al. 2001; Shiff et al. 2000). Randomly amplified polymorphic DNA has also been used to assess the association between genetic diversity of *S. haematobium* and the severity of the disease in which no correlation was observed (Gasmelseed et al. 2014; Brouwer et al. 2003).

*Restriction fragment length polymorphism (RFLP)*

This method uses differences in the length of a fragment of DNA between restrictions sites to quantify genetic diversity in populations. The polymorphisms in homologous DNA causes the difference in fragment length and are used to distinguish individuals, populations, or species (Barber et al., 2000). Quan et al. (2015), used this method in combination to characterize the genetic diversity of *S. haematobium* in Sudan using the *ITS2* region. The multiplex PCR results showed that most of the samples did not produce the expected PCR band patterns for *S. haematobium* (Quan et al. 2015). The genetic patterns of *S. haematobium* were observed in Sudan similar to those from Kenya (Quan et al. 2015). This could be explained by the possible inflow of a Kenyan strain into Sudan in spite of the geographical distance and likely the lack of sensitivity of this method.

*DNA barcoding*

DNA barcoding is a method that identifies organisms by matching a standardized genomic DNA fragments from a reference sequence library (Kress et al., 2015). Four publications in our review used this approach to document the genetic variation of *S. haematobium* from several geographical areas using historical collections from across Africa (Angora et al. 2020; Tian-Bi et al. 2019; Webster et al. 2012) and from Zanzibar Island (Webster et al. 2013). Different gene regions are used to identify the different species. The most commonly used barcode regions are the cytochrome C oxidase subunit 1 (abbreviated *COI* or *Cox1*) and the NADH-dehydrogenase subunit 1 (*Nad1*), both found in mitochondrial DNA (Standley et al., 2010). The nuclear internal transcribed spacer (*ITS*) is another gene region suitable for DNA barcoding (Kress et al., 2015). The haplotype diversity analysis showed that *S. haematobium* was less polymorphic than *S. bovis* (Angora et al. 2020; Webster et al. 2013). This variation in mitochondrial DNA (mtDNA) is commonly used in systematics to infer species phylogenies and sub-species ancestry (Webster et al., 2006). The DNA barcoding approach is useful to assess haplotype diversity and to infer species identification and phylogeny. However, the strong limitations of this method reside particularly in defining species boundaries (Witt et al., 2006).

## Conclusions

The current systematic review elucidated that *S. haematobium* has variable genetic diversity and population structure within and across African countries using molecular analyses. Microsatellite analysis seems to be the suitable approach to assess population genetics of *S. haematobium*. From the perspective of schistosomiasis elimination in Africa, it is important to better identify and understand the challenges that actually affect control interventions. However, the deployment of any intervention must be accompanied by diagnostic activities involving the use of molecular methods to monitor the success of the intervention.

## Abbreviations

DALYs: disability-adjusted life years; GBD: Global Burden of Disease; PCR: Polymerase chain reaction; RAPD: Randomly amplified polymorphism DNA; RFLP: Restriction fragment length polymorphism; SNP: Single nucleotide polymorphism.

## Declarations

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Authors' contributions: EKA conducted the systematic review of the literature, extracted and analysed data, and drafted the first version of the manuscript. JGE, HM, JB, JU and OB revised the manuscript. All authors read and approved the final manuscript.

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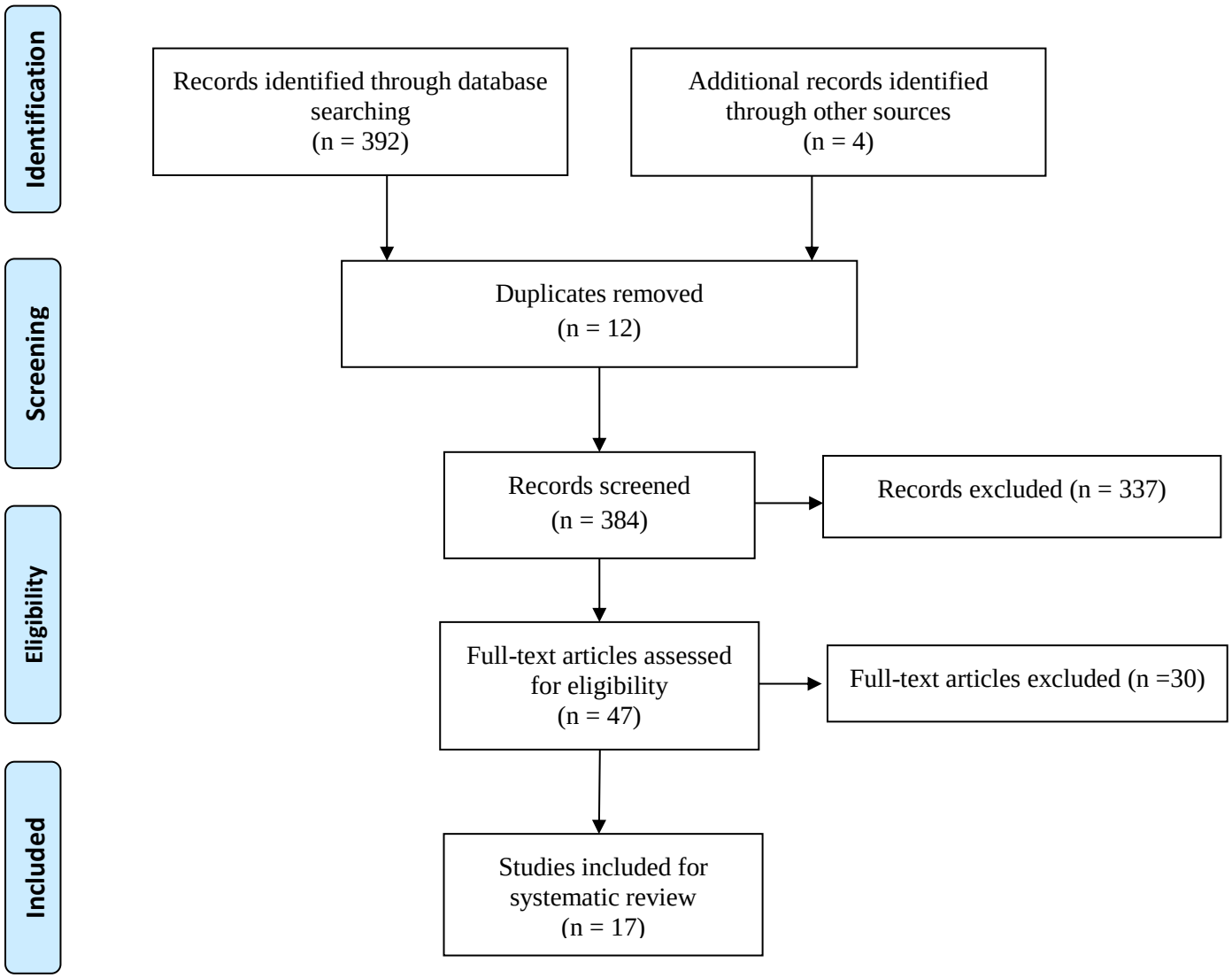


**Fig. 1** PRISMA Flow Diagram of the studies reviewed.

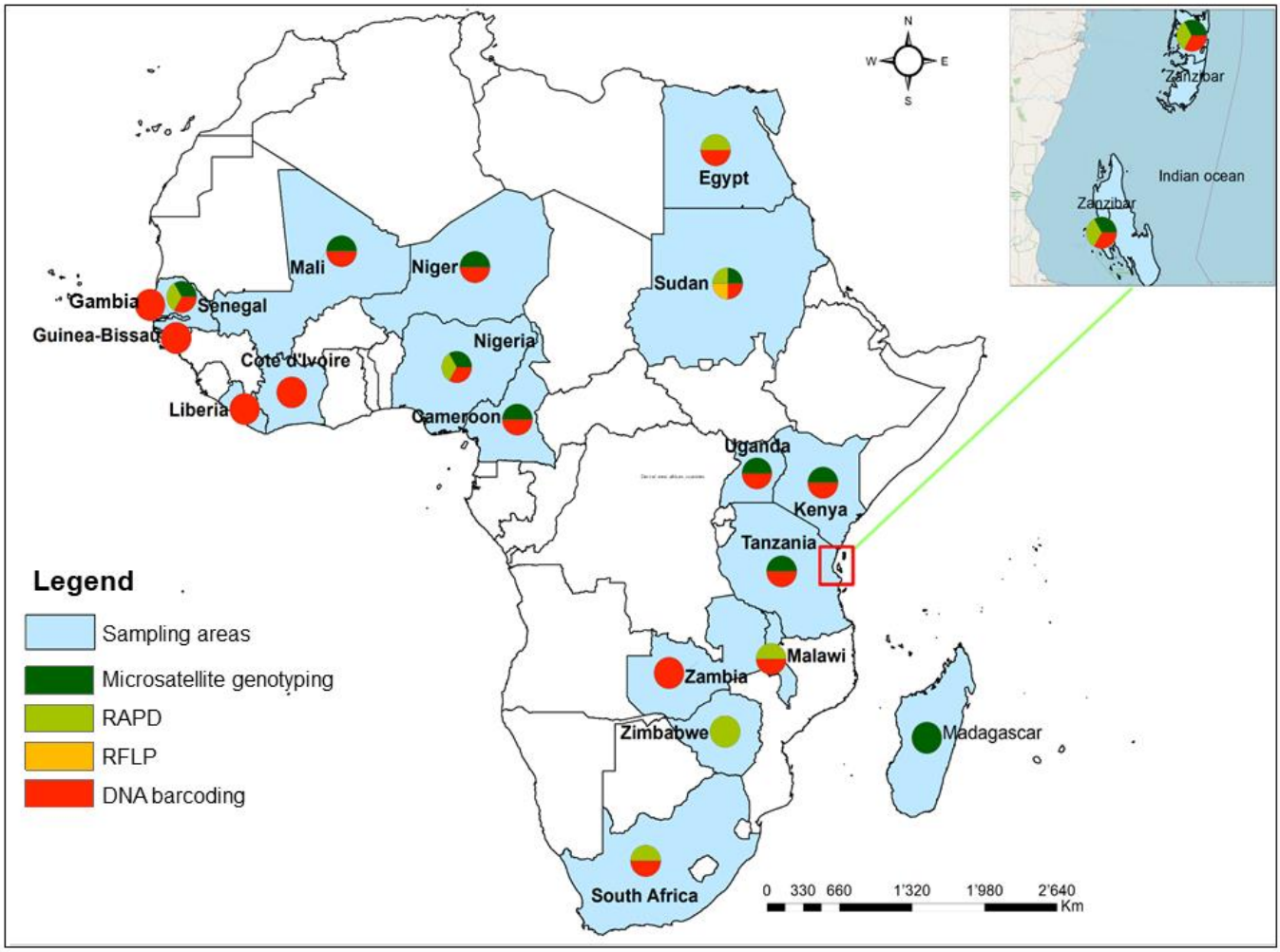


**Fig. 2** Map of sampling areas of the publication included in the review and molecular methods applied. RAPD, randomly amplified polymorphic DNA; RFLP, restriction fragment length polymorphism.

**Table 1:** Concepts, search terms and combinations of key words used in search strategy.

| # | Concepts | Search strategy |
|---|---|---|
| 1 | Schistosome | Schistosom* OR Bilharz* |
| 2 | Genetic | Gen* OR Genom* OR Single nucleo* |
| 3 | Diversity | Divers* OR Varia* OR Polymorph* OR Distrib* |
| 4 | Technique | Techni* OR Method* OR Diagnos* OR Molecular* OR Labo* |
| 5 | Africa | Afri* |
| | | **Combinations** |
| | | 1+5; 1+2+3; 1+2+5; 1+3+5; 1+2+4; 1+3+4 |

**Table 2:** Study locations, type and sample size, and main findings of 17 studies investigating genetic variability and population structure of *Schistosoma haematobium* across Africa, sorted by molecular methods.

| Molecular methods | Reference | Country | Samples | Sample size | Main findings |
|---|---|---|---|---|---|
| Microsatellite genotyping | Boon et al. (2019) | Senegal | Miracidia, cercariae, adult worms | 606 samples (529 miracidia, 13 cercariae; 64 adult worms) | *S. haematobium* was spatially more differentiated than *S. mansoni*. Strong species boundary between human and cattle schistosome was observed, despite the opportunity for hybridization. |
| | Ezeh et al. (2015) | Nigeria, Mali | Eggs | 245 | High level of genetic variability of *S. haematobium* was found between Mali and Nigeria suggesting that geographical population differentiation may occur in the regions. |
| | Webster et al. (2015) | Zanzibar | Miracidia and cercariae | 26 samples (10 miracidia and 16 cercariae) | Higher genetic diversity was observed in Zanzibar compared to that from Niger. |
| | Gower et al. (2013) | Cameroun, Kenya, Niger, Mali, Tanzania, Uganda | Miracidia | 2737 | High level of genetic diversity of *S. haematobium* populations was found. |
| | Gower et al. (2011) | Mali | Miracidia, adult worms | 884 samples (862 miracidia and 22 adult worms) | High genetic diversity of parasite populations was found within schools. Low genetic structure was observed. |
| Randomly amplified polymorphic DNA (RAPD) | Afifi et al. (2016) | Egypt, Zimbabwe and South Africa | Eggs | Eggs from three urine samples | *S. haematobium* from the three locations are genetically distinct with the possible existence of two phylogenetic groups. |
| | Gasmelseed et al. (2014) | Sudan | Eggs | 83 urine samples | No association between genetic diversity of *S. haematobium* and the severity of the disease. |
| | Glenn et al. (2013) | Malawi, Nigeria, Senegal, Zanzíbar | Cercariae | 3703 cercariae of which 69 individual were genotyped | High genetic diversity was observed and variation between countries. Strong |

| | | | | | |
|---|---|---|---|---|---|
| | | | | using 15 microsatellites identified after sequencing | population genetic structure between countries was found. |
| | Brouwer et al. (2003) | Zimbabwe | Cercariae | 133 | Genetic diversity of *S. haematobium* does not influence the severity of urinary schistosomiasis in children |
| | Brouwer et al. (2001) | Zimbabwe | Cercariae | 133 | Genetic differentiation of *S. haematobium* was observed between children. |
| | Shiff et al. (2000) | Zimbabwe | Cercariae | 1000 | High degree of genetic diversity was found in *S. haematobium* from Zimbabwe |
| | Dabo et al. (1997) | Mali | Adult worms | 414 | Low genetic variation among populations compared to within populations. |
| Restriction fragment length polymorphism (RFLP) | Quan et al. (2015) | Sudan | Eggs | 13 | No genetic diversity was observed in *S. haematobium* isolated from Sudan compare to those collected in Kenya. |
| DNA barcoding | Angora et al. (2020) | Côte d'Ivoire | Miracidia | 549 | Low genetic variation of *S. haematobium* compared to *S. bovis* using *Cox1* gene |
| | Tian-Bi et al. (2019) | Côte d'Ivoire | Cercariae | 75 | Molecular data from schistosome cercariae clearly show the sympatric distribution of *S. haematobium* and *S. bovis*. |
| | Webster et al. (2013) | Zanzibar | Adult worms | 214 | High levels of parasite diversity were found across Zanzibar |
| | Webster et al. (2012) | East, central and west Africa | Miracidia, cercariae, adult worms | 1978 samples (1869 miracidia, 46 cercariae and 241 adult worms) | Low level of diversity within *S. haematobium* across most of mainland Africa |

**Supplementary file 1**: Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) checklist.

| Section/topic | # | Checklist item | Reported on page # |
|---|---|---|---|
| **TITLE** | | | |
| Title | 1 | Identify the report as a systematic review, meta-analysis, or both. | 1 |
| **ABSTRACT** | | | |
| Structured summary | 2 | Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number. | 2 |
| **INTRODUCTION** | | | |
| Rationale | 3 | Describe the rationale for the review in the context of what is already known. | 3, 4 |
| Objectives | 4 | Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS). | 4 |
| **METHODS** | | | |
| Protocol and registration | 5 | Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number. | N/A |
| Eligibility criteria | 6 | Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale. | 5 |
| Information sources | 7 | Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched. | 5 |
| Search | 8 | Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated. | 5 |
| Study selection | 9 | State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis). | 5 |
| Data collection process | 10 | Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators. | 5 |
| Data items | 11 | List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made. | 5 |
| Risk of bias in individual studies | 12 | Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis. | N/A |
| Summary measures | 13 | State the principal summary measures (e.g., risk ratio, difference in means). | N/A |
| Synthesis of results | 14 | Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., $I^2$) for each meta-analysis. | 5 |

| Section/topic | # | Checklist item | Reported on page # |
|---|---|---|---|
| Risk of bias across studies | 15 | Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies). | N/A |
| Additional analyses | 16 | Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified. | N/A |
| **RESULTS** | | | |
| Study selection | 17 | Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram. | 6 |
| Study characteristics | 18 | For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations. | 7 |
| Risk of bias within studies | 19 | Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12). | N/A |
| Results of individual studies | 20 | For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot. | N/A |
| Synthesis of results | 21 | Present results of each meta-analysis done, including confidence intervals and measures of consistency. | N/A |
| Risk of bias across studies | 22 | Present results of any assessment of risk of bias across studies (see Item 15). | N/A |
| Additional analysis | 23 | Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]). | N/A |
| **DISCUSSION** | | | |
| Summary of evidence | 24 | Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers). | 8, 9, 10 |
| Limitations | 25 | Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias). | 8, 9 |
| Conclusions | 26 | Provide a general interpretation of the results in the context of other evidence, and implications for future research. | 13 |
| **FUNDING** | | | |
| Funding | 27 | Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review. | N/A |

# Chapter 5:
# Genetic diversity and differentiation of *Schistosoma haematobium*

# Population genetic structure of *Schistosoma haematobium* in schoolchildren in Côte d'Ivoire

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# Population genetic structure of *Schistosoma haematobium* in schoolchildren in Côte d'Ivoire

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**Abstract-** Schistosomiasis is a neglected parasitic disease that affects both humans and animals worldwide and particularly in sub-Saharan Africa. Population genetics of *Schistosoma haematobium* have been investigated in West Africa. However, no data are available from Côte d'Ivoire. This study aimed to analyze genetic variability among *S. haematobium* and quantify *S. haematobium* x *S. bovis* hybrids in schoolchildren in Côte d'Ivoire. Urine samples were collected and examined by filtration method to identity *Schistosoma* eggs in four sampling sites across western and southern Côte d'Ivoire. A total of 2692 miracidia were individually collected and stored on Whatman® FTA cards and 2561 were genotyped using a set of 18 microsatellite markers. Hybrids were identified using both a multiplex PCR for the mitochondrial cox1 gene and restriction fragment length polymorphism (RFLP) analysis for the nuclear ITS2 region. Of 2164 miracidia successfully amplified in both cox1 and ITS2, 1966 (90.85%) yielded reliable peak calls for at least ten microsatellite loci and were retained for population genetic analysis. Significant differences were found between sampling sites in all genetic diversity indices and strong genotypic differentiation was observed between Duekoué and the other sites. The analysis at the infrapopulation level revealed that almost each parasites from each child belonged to one cluster, particularly in Duekoué and Sikensi. Of the six parasite genotypes, *S. bovis* cox1 x *S. haematobium* ITS2 (41.96%) were the most commonly observed in the population. Fifteen cases (0.70%) of "pure" *S. bovis* (*S. bovis* cox1 x *S. bovis* ITS2) were identified. Our study reported a genetic diversity and a population structuring of parasites between populations. These results could serve as a baseline to evaluate the effects of selective pressure due to massive drug administration on the selection of schistosome genetic diversity and enhance schistosomiasis.

**Keywords:** *Schistosoma haematobium*, Côte d'Ivoire, Microsatellites, Population genetics.

## Introduction

Schistosomiasis is a chronic neglected tropical disease caused by parasites of the *Schistosoma* genus (Colley *et al.*, 2014; Lai *et al.*, 2015). This parasitic disease infects humans and animals and is of great public health and veterinary concern worldwide and particularly in tropical and subtropical zones. The disease ranks second to malaria in terms of morbidity and mortality in humans (King, 2010). World Health Organization (WHO) estimates that it is transmitted in over 78 countries, throughout a wide belt of the tropics and subtropics (World Health Organization, 2018). The number of worldwide schistosomiasis cases is considered to be greater than 250 million, mostly in Africa, and the "Disability-Adjusted Life Years" (DALYs) index was estimated at 1.4 million in 2017 (Hotez *et al.*, 2014; GBD 2017 DALYs and HALE Collaborators, 2018). Six species of schistosomes are known to infect human: *Schistosoma mansoni*, *S. haematobium*, *S. intercalatum* and *S. guineensis* in Africa, and *S. mekongi* and *S. japonicum* in Asia (Rollinson *et al.*, 1997). *Schistosoma haematobium* causes the urogenital form of the disease and is endemic in Africa and the Middle East. Classified as a group I carcinogen, urogenital schistosomiasis can lead to squamous-cell carcinoma of the bladder (IARC, 2012). In most countries, while human schistosomiasis is well documented, little is known about the prevalence and transmission dynamics of animal schistosomiasis.

In Côte d'Ivoire, both *S. mansoni* and *S. haematobium* are endemic (Chammartin *et al.*, 2014). The former is predominant in western (Utzinger *et al.*, 2000; Assaré *et al.*, 2015) and the latter in the central and southern parts (Coulibaly *et al.*, 2013; Soumahoro *et al.*, 2014). We recently reported high prevalence of *S. haematobium* among schoolchildren in southern Côte d'Ivoire (Angora *et al.*, 2019). Limited data are available on animal-infecting schistosomes such as *S. bovis*, a parasite of livestock and rodents. A previous study on post-mortem examinations of cattle in Côte d'Ivoire reported a prevalence of 35% (Achi *et al.*, 2003).

Climate change can influence the epidemiology and distribution of schistosomiasis and enhance the occurrence of interspecies hybridization between human and animal schistosomes (King *et al.*, 2015). *Schistosoma haematobium* x *S. bovis* hybrids are well documented in West Africa, particularly in Benin, Mali, Niger and Senegal (Huyse *et al.*, 2013; Leger and Webster, 2017); in Malawi (Webster *et al.*, 2019) and Corsica, France, as well (Berry *et al.*, 2016; Boissier *et al.*, 2016). Recently, hybrids have been identified in Côte d'Ivoire between *S. haematobium* x *S. mansoni* in humans (Depaquit *et al.*, 2019; Le Govic *et al.*, 2019), and between *S. haematobium* x *S. bovis* in *Bulinus* snails (Tian-Bi *et al.*, 2019), and schoolchildren (Angora *et al.*, 2020).

Population genetic structure and genetic diversity varies by *Schistosoma* species concerned. Among African schistosome species, *S. mansoni* is the most studied species. Several studies have shown important genetic diversity and strong genetic structure in several countries including Kenya, Uganda, Ethiopia and Senegal (Agola *et al.*, 2006; Stothard *et al.*, 2009; Van den Broeck *et al.*, 2015; Aemero *et al.*, 2015). In contrast, *S. haematobium* is less studied and only weak population genetic structure was observed in Mali (Gower *et al.*, 2011). This difference in genetic structuration between *S. mansoni* and *S. haematobium* is also visible at the continental scale (Webster *et al.*, 2012, 2013b; Gower *et al.*, 2013). Data on *S. bovis* are even more sparse. Only one study has investigated population genetic patterns of *S. bovis*, which revealed an intermediate pattern with high genetic diversity (i.e. like in *S. mansoni*) and but no genetic structuration (i.e. like in *S. haematobium*) across Cameroon (Djuikwo-Teukeng *et al.*, 2019). Importantly, this last work studied parasites collected from cows and failed to detect hybrids. Recently, a study analyzed the genetic patterns of parasites collected from humans and animals in the North Senegal (Boon *et al.*, 2019) where *S. haematobium* x *S. bovis* hybrids were first discovered (Huyse *et al.*, 2009). The authors demonstrated (i) a strong boundary between parasites recovered in animal compare to those from human, (ii) no genetic structuration between hybrids and pure parasites from human hosts, and (iii) significant genetic differentiation between different villages of the Senegal River Basin. This study reported no barrier breakdown between *S. haematobium* and *S. bovis*. Therefore, animals do not represent a real reservoir for human schistosomiasis.

This study aimed to analyze the genetic pattern among *S. haematobium* and *S. haematobium* x *S. bovis* hybrids in four sampling sites of Côte d'Ivoire. First, we characterized the hybrid status of parasites using both mitochondrial and nuclear markers and a novel restriction fragment length polymorphism (RFLP) approach for species determination. Second, we genotyped parasites using a set of microsatellite markers to analyze the genetic structuring and diversity.

## Materials and methods

*Study areas and miracidia collection*

This study was carried out in four sampling sites of Côte d'Ivoire: (i) Agboville (5° 55′ 41″ N, 4° 13′ 01″ W) and (ii) Adzopé (6° 06′ 25″ N, 3° 51′ 36″ W) in the south-eastern part; (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 (**Fig. 1**). The study was integrated into a cross-sectional survey of the prevalence of schistosomiasis among schoolchildren from January to April 2018 (Angora *et al.*, 2019). *Schistosoma* miracidia from schoolchildren aged 5 to 14 years were collected after egg hatching and stored on Whatman® FTA (GE Healthcare Life Sciences, Amersham, UK) as previously described (Boissier *et al.*, 2016). FTA cards were dried for 1 hour at room temperature before being stored in a sealed plastic bag and then transferred to the "Interactions Hôtes-Pathogènes- Environnements" (IHPE) laboratory in Perpignan, France for molecular analysis.

*Molecular methods*

Genomic DNA from individual miracidia was extracted using Chelex® (Bio-Rad; Hercules, USA) beads, and a multiplex PCR was performed to identify species-specific variants of the mitochondrial cox1 gene, as described before (Angora *et al.*, 2020). For the nuclear internal transcribed spacer 2 (ITS 2) region, a new restriction fragment length polymorphism (RFLP) method was developed using forward primer Sc_ITS_F: 5'-GGC TGC AGC GTT AAC CAT TA -3' and reverse primer Sc_ITS_R: 5'-ACA CAC ACC ATC GGT AC AAA-3', which produces sequence of 505 bp. We performed PCR in a total reaction volume of 25 µL, comprising 2 µL of DNA, 5 µL of Green GoTaq flexi buffer 5X (Promega; Madison, WI, USA), 1.5 µL of 25 mM $MgCl_2$ (Promega; Madison, WI, USA), 0.5 µL of 10 mM dNTP mix (Promega; Madison, WI, USA), 1 µL of each primer 10 µM and 1 U of GoTaq Hot Start Polymerase (Promega; Madison, WI, USA). The reaction conditions included an activation step of 95°C for 3 min, followed by 45 cycles of 95°C for 40 sec, 58°C for 40 sec and 72°C for 40 sec, and a final extension at 72°C for 6 min. In the subsequent step, PCR products were digested using restriction enzyme MboI (NEB, UK) which targets ↓GATC or CTAG↑ in CutSmart Buffer for 15 min incubation and inactivation at 60°C for 20 min. This enzymatic digestion produces banding patterns on stained agarose gels that distinguish *S. haematobium* (281, 145, 78 bp bands) from *S. bovis* (426 and 78 bp bands) (**Supplementary Fig. S1**).

All digestion products were visualized using two percent agarose electrophoresis gels stained with GelRed™ (Biotium Inc, CA, USA). The combination of the nuclear ITS2 and mitochondrial cox1 markers was assessed to classify into six possible cox1-ITS profiles (cox1 haplotype x ITS2 alleles, Sb: *S. bovis*, Sh: *S. haematobium*): Sb x SbSb, Sb x ShSb, Sb x ShSh, Sh x SbSb, Sh x ShSb and Sh x ShSh.

*Microsatellite genotyping*

Individual miracidia were further genotyped using a set of 18 microsatellite markers divided in two panels developed by Webster et *al.* ( 2015). We used the Microsatellite PCR Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. The forward primers were fluorescently labelled with 6-FAM, VIC, NED and PET dyes (Applied Biosystems, Foster City, USA) as previously described (Webster *et al.*, 2015). Microsatellite PCR was performed in a final volume of 10 µL including 4 µL of DNA template, 5 µL of 2X microsatellite PCR Buffer Kit (Qiagen, Hilden, Germany) and 1 µL of 10X microsatellite primer mix. Thermal cycling was performed with an initial hot-start activation of 15 min at 95 °C, followed by 45 cycles of 94 °C for 30 s, 56 °C for 90 s and 72 °C for 60 s, with a final extension at 60 °C for 30 min. The microsatellite reaction products were sent to Genoscreen (Lille, France) for genotyping. All microsatellite loci were visually peak called using GS500Liz size standard (Applied Biosystems), and GeneMarker Software. Only 16 loci were used for analysis because two (C131 and Sh8) amplified in less than 20% of the samples. Errors due to large allele dropout or stutter bands and evidence for the presence of null alleles at each locus were checked using Micro-checker 2.2.3 (Oosterhout *et al.*, 2004).

*Genetic diversity*

Genetic diversity was measured between sampling sites and between parasite genotypes. Expected heterozygosity (He), number of alleles (A), allelic richness (Ar) and the inbreeding coefficient ($F_{IS}$) of each microsatellite locus were computed per sampling site using FSTAT v.2.9.4 (Goudet *et al.*, 2002). Tests for deviation from Hardy-Weinberg equilibrium per locus and study site were carried out using Genepop 4.0 (Rousset, 2008). The genotypic disequilibrium test for pairs of loci overall and the adjusted p-value for 5% nominal level was performed using FSTAT v.2.9.4.

The number and percentages of cox1-ITS profiles were calculated for each sampling site. The equipartition of these profiles among sampling sites was tested using Chi-Square test. We also calculated the number of *S. bovis* and *S. haematobium* alleles for each molecular marker among sampling sites.

The mitochondrial Cox1 marker presents an unique allele while the ITS is scored one for heterozygous and two for homozygous. The equipartition of *S. bovis* vs *S. haematobium* alleles was tested using a Binomial test. For these profiles, we calculated the expected heterozygosity (He), allelic richness (Ar) and inbreeding coefficient ($F_{IS}$) per sampling site using FSTAT v.2.9.4, as well.

*Population structure*

Population differentiation between sampling sites was assessed using pairwise genetic estimators, Principal Component Analysis (PCA) and Bayesian approach. Pairwise $F_{ST}$ values (Weir and Cockerham, 1984) were performed using FSTAT v.2.9.4. Significance was obtained after 300 permutations and indicative adjusted nominal level (5%) for multiple comparisons values was computed. Principal Component Analysis was performed using GENETIX software. The uppermost level of genetic structure for all individuals was determined by Bayesian clustering approach using Markov Chain Monte Carlo (MCMC) permutation analysis implemented in STRUCTURE 2.3 (Falush *et al.*, 2003). The length of Burn-in Period was 250,000 with the number of MCMC replicates after Burn-in at 1,000,000 and K from 1 to 8 using an admixture model. The log likelihood for each K was averaged over 3 runs with the corrsieve package in R and the delta K-values were then computed to determine the most likely K number among the K tested (Evanno *et al.*, 2005). For the most likely number of genetic clusters, an additional 10 runs were computed with the same initial parameters as those described. The probability of each miracidium to belong to the four clusters was averaged over the 10 runs and graphically represented using CLUMPP version 1.1.2 (Francis, 2017) and DISTRUCT version 1.1 (Rosenberg, 2004).

Analysis of molecular variance (AMOVA) was performed to evaluate the partitioning of the overall genetic variance to the five hierarchical levels “within miracidia”, “among miracidia within child”, “between children within sampling sites” and “between sampling sites” using ARLEQUIN version 3.5 (Excoffier and Lischer, 2010).

Population genetic structure between parasite infrapopulation was assessed independently for each sampling site using STRUCTURE 2.3 with the same procedure as previously described, but the first run used from K=24 to K=28 according sampling site.

*Ethical consideration*

Ethical clearance 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, parents/guardians, and participants were informed about the objectives, procedures, and potential risks and benefits of the study. Written informed consent was obtained from children's parents or legal guardians. Oral assent was obtained from children. Following sampling, a praziquantel treatment (40 mg/kg) was offered to infected children.

## Results

*Schistosoma haematobium and hybrid genotypes identification*

Of 2692 miracidia collected in urine samples from 91 *Schistosoma* egg-positive schoolchildren in four sampling sites, 2561 were analyzed. Therefore, 2164 were positive with both Cox1-PCR and ITS2-RFLP methods (495 for Adzopé; 501 for Agboville; 610 for Sikensi and 558 for Duekoué) **(Fig. 1)**. Of these positive schoolchildren, 89 (97.8%) harbored hybrid parasites. Among all possible hybrid profiles, *S. bovis* cox1 x *S. haematobium* ITS 2 (Sb x ShSh) was the most found in Adzopé (64.6%), Duekoué (56.3%) and Sikensi (30.2%) while *S. haematobium* cox1 x *S. haematobium* ITS 2_*S. bovis* ITS 2 (Sh x ShSb) was the most common hybrid genotype in Agboville (27.7%). We identified 15 cases (0.7%) of "pure" *S. bovis* (*S. bovis* cox1 x *S. bovis* ITS2: Sb x SbSb) from 7 children and 791 cases (36.6%) of "pure" *S. haematobium* (*S. haematobium* cox1 x *S. haematobium* ITS2: Sh x ShSh) in the overall study. The Chi-square test showed a significant difference in the distribution of cox1-ITS profiles across sampling sites (**Table 1)**. The frequency of *S. bovis* cox1 (50.7%) was similar to that of *S. haematobium* cox1 (49.3%) while *S. haematobium* ITS2 allele frequency was much higher at 87.9% than *S. bovis* ITS allele frequency (12.1%). A high significant difference was found in the number of *S. bovis* vs *S. haematobium* ITS2 alleles ($p<0.00001$) but not for the cox1 haplotypes ($p= 0.27$) (**Table 1)**. Analysis of the multiplicity of infection showed that 52 schoolchildren (71.1%) were infected by parasites exhibiting three different genotypes of *Schistosoma*. One child (Id: AG122) from Agboville was infected by parasites exhibiting all the six possible cox1-ITS profiles **(Supplementary Tab. S1).**

*Genetic diversity*

Among the 2164 miracidia that yielded bands for both Cox1-PCR and ITS2-RFLP methods, 1966 (90.8%) produced reliable peak calls of allele for at least 10 microsatellite loci and were used in subsequent analyses. All loci were highly polymorphic and the number of alleles and allelic richness ranged from 7 to 17 and 7.0 to 15.8, respectively per locus in the whole dataset. High level of genetic diversity of *S. haematobium* populations was found in Duekoué with individual microsatellite loci detecting a mean of 10.50 alleles and 10.22 allelic richness per locus compared to the other sampling sites. Genetic diversity indices (He, A, Ar and $F_{IS}$) and the probability of deviation from Hardy-Weinberg equilibrium ($P_{HWE}$) for each microsatellite locus by sampling site are shown in **Table 2**. The Friedman test (p=0.0001) showed a significant difference in the mean of all the genetic diversity indices between the four sampling sites with parasites from Duekoué more diverse. Virtually, all individual loci significantly deviated from Hardy-Weinberg equilibrium (without sequential Bonferroni adjustment) across sampling sites. We found a significant genotypic disequilibrium (adjusted p-value at 5% level =0.00014) for 120 pairwise locus combinations across the whole dataset.

The comparison of genetic diversity indices between the six possible cox1-ITS profiles in each sampling site did not show a significant difference in mean (averaged over sampling sites) expected heterozygosity (p=0.079) and number of alleles (p=0.274) while the mean allelic richness (p=0.039) and the mean inbreeding coefficient (p=0.030) significantly differed between cox1-ITS profiles **(Table 3)**.

*Population genetic structure*

The analysis of pairwise $F_{ST}$ values showed a weak but statistically significant differentiation between sampling sites with Duekoué consistently most differentiated **(Table 4)**. The $F_{ST}$ values between parasite genotypes within each sampling sites did not show any genetic differentiation **(Table 5)**. The principal component analysis (PCA) revealed only weak structuration among miracidia from Agboville and Sikensi while those from Adzopé and Duekoué were well separated **(Fig. 2)**. Analysis of the different parasite genotypes by PCA showed a clear separation in two distinct genetic clusters **(Fig. 3)**. The cluster of "pure" *S. bovis* cox1-ITS profile (Sb_SbSb) and that of the five other parasite genotypes which includes pure *S. haematobium* and hybrid parasites.

The population genetic structure was assessed both at population level (across sampling sites) and at infrapopulation level (within each sampling site). K = 4 displayed maximal delta K at population level suggesting that all miracidia were grouped into 4 clusters. The analysis using STRUCTURE showed that genetic structure was relatively strong between sampling sites despite considerable gene flow **(Fig. 4)**. The analysis at infrapopulation level revealed 19 and 22 clusters in Duekoué and Sikensi, respectively that reflected the individual children sampled. However, the clusters were not uniformly distributed among children and some children belonged to many clusters **(Supplementary Fig. S2)**.

At population level, an average of 51% of the parasites' genomes were assigned to the respective dominant cluster in Adzopé, 63% in Agboville, 69% in Sikensi, and 83% in Duekoué. The analysis of molecular variance (AMOVA) showed that apart from the obvious predominant contribution of the within-miracidia level (i.e. variance introduced by the variability of the microsatellite loci), most of the genetic variance was found among children within sampling sites (7.5% of the total or 57.8% of the residual variance). Low genetic variance was observed among miracidia within children (1.35%; or 10.4% of the residual variance) **(Table 6)**.

**Discussion**

The current study is the first one to investigate the population genetic structure and identify hybrid status of *S. haematobium* in humans in Côte d'Ivoire. Our study showed 62.7% of *S. haematobium* x *S. bovis* hybrids, and among 91 schoolchildren, 97.8% emitted hybrid parasites. Similar results have been shown in a large-scale study in Senegal (Webster *et al.*, 2013a). The authors reported that most of children (88%) surveyed in areas of suspected species overlap excreted hybrid miracidia.

We have classified hybrids in four possible profiles, and we observed that hybrids with genotype *S. bovis* cox1 x *S. haematobium* ITS2 (i.e. homozygous ITS2) were the most commonly identified at 41.9%. Such hybrids have recently been documented in Senegal and ascribed to bidirectional introgressive hybridization (Huyse *et al.*, 2009; Webster *et al.*, 2013a). Beside this general pattern, significant variation was observed between sampling sites and most of hybrids were identified in Adzopé (79.0%). Interestingly, the high frequency of hybrids is due to a higher frequency of *S. bovis* Cox1 mitochondrial haplotype than that of *S. bovis* ITS2 nuclear allele. Globally, the frequency of *S. bovis* or *S. haematobium* ITS alleles was more stable than the mitochondrial haplotypes. This difference could be explained by the concerted evolution of ITS region (Ganley and Kobayashi, 2007).

One interesting finding in this study was the identification of parasites with a pure genetic signature of *S. bovis* (*S. bovis* cox1 x *S. bovis* ITS2) in 15 cases from seven children. This *S. bovis* genotype has equally been reported in eggs collected from humans in Corsica (Boissier *et al.*, 2016). *Schistosoma bovis* is supposed to infect only livestock and rodents, but its presence in humans reported here implies a zoonotic transmission of this parasite. The occurrence of such parasite could also due to the result from backcrosses between hybrids. Furthermore, we only used one nuclear marker that can mask some *S. haematobium* part of the genome of these supposed "pure" *S. bovis*. This interesting aspect is also related to the fact that these parasites are the only ones standing out in the PCA analysis. This clear separation between "pure" *S. bovis* (Sb_SbSb) and the other profiles could be interpreted by the fact that these parasites come from ruminants and has not been introgressed by either pure or *S. bovis* x *S. haematobium* hybrids. To our knowledge, no studies on human schistosomes have yet shown these results. Moreover, no hybrid has been identified in animals whatever in Senegal (Webster *et al.*, 2013a), Cameroon (Djuikwo-Teukeng *et al.*, 2019) and Corsica (Oleaga *et al.*, 2019. More thorough analyses using single nucleotide polymorphisms (SNPs) and studies on animal should be conducted to elucidate the frequency of zoonotic transmissions.

Genetic diversity indices (Ar and He) obtained here are similar to those from previous studies (Gower *et al.*, 2013; Ezeh *et al.*, 2015; Webster *et al.*, 2015). We found only significant differences in allelic richness of parasites between populations. Parasites from Duekoué presented a high genetic diversity compared to those of the three other sampling sites. The highest genetic diversity seems to be independent of hybrids frequency. This genetic diversity could be attributed to the substantial gene flow in parasite populations due to the high frequency of alleles (Lu *et al.*, 2010).

Data on the genetic structuring of *S. haematobium* populations are sparse (Gower *et al.*, 2011, 2013). Previous findings based on a phylogeographic approach at the African continental scale, suggested that *S. haematobium* is less structured than *S. mansoni* (Webster *et al.*, 2013b). In contrast, the significant differentiation between sampling sites and even within individual hosts reported here, demonstrates a genetic structure of *S. haematobium* at small geographical scales. The large geographical distance separating Duekoué in the western part of Côte d'Ivoire to the three other sampling sites is likely to limit contacts between populations, and can lead to population structure. More surprising was the genetic structure observed at the patient level. We reported that in the vast majority each child hosted an infrapopulation different from the other children. This result does not validate the 'genetic mixing bowl' (Curtis and Minchella, 2000) hypotheses which proposed that definitive host owns a variety of profiles from a snail intermediate host, and lead to the homogeneity of infrapopulation. The pattern found in our study could be likely explained by a recent treatment of children and the 'bowl' did not have time to occur. Therefore, children could be infected with one or a few pairs of parasites.

No genetic structuring of parasites was observed between *S. haematobium* versus hybrids *S. haematobium* x *S. bovis*. This result suggests that there are no genetic mating restrictions upon the population and therefore, all recombination are possible (Gorton *et al.*, 2012; Boon *et al.*, 2019). Similar results have recently been reported in Senegal (Boon *et al.*, 2019). These authors considered only the cox1 haplotype and not the nuclear gene; therefore, they could not observe the "pure" *S. bovis* cluster identified in the present study.

## Conclusions

Our study reported a genetic diversity and a population structuring of parasites at population level. Each child harbored a genetic cluster of parasites at infrapopulation level and could lead to putative resistant parasites. Hybrid parasites identified can lead to difficulties in accurately diagnosing schistosome species by conventional techniques using microscopic examination. The "pure" *S. bovis* profiles observed in children suggest a zoonotic transmission of this parasite. Our results could serve as a baseline to evaluate the effects of selective pressure due to massive drug administration on the selection of schistosome genetic diversity and enhance schistosomiasis control and surveillance.

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

**Conflict of interest.** None.

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**Legends of tables**

**Table 1:** Total number of miracidia analyzed (n), mean number (± SE) of miracidia analyzed per child ($\overline{x}$), and number and percentage (in parentheses) of the six possible cox1-ITS profiles identified using the haploid mitochondrial cox1 gene (first two letters) and the diploid nuclear ITS2 region (last four letter), i.e. "pure" *Schistosoma haematobium* (*S. haematobium* cox1 x *S. haematobium* ITS2: Sh x ShSh), "pure" *S. bovis* (*S. bovis* cox1 x *S. bovis* ITS2: Sb x SbSb) and four types of hybrid (*S. bovis* cox1 x *S. haematobium* ITS2_*S. bovis* ITS2: Sb x ShSb; *S. bovis* cox1 x *S. haematobium* ITS2: Sb x ShSh; *S. haematobium* cox1 x *S. bovis* ITS2: Sh x SbSb; *S. haematobium* cox1 x *S. bovis* ITS2_ *S. haematobium* ITS2: Sh x ShSb) per sampling site 2164 miracidia analyzed.. Total number of *S. haematobium* (Sh) and *S. bovis* (Sb) cox1 haplotype and ITS2 alleles (percentage in parentheses) per sampling site.

**Table 2:** Genetic diversity indices. Mean expected heterozygosity (He), total number of alleles detected (A), Allelic richness for 362 diploid individuals (Ar), mean inbreeding coefficient ($F_{IS}$) and the probability of deviation from Hardy-Weinberg Equilibrium ($P_{HWE}$) for each microsatellite locus per study location. n, number of miracidia genotyped per study location.

**Table 3:** Genetic diversity indices: heterozygosity (He), number of alleles (A), allelic richness for 12 diploid individuals (Ar) and inbreeding coefficient ($F_{IS}$) for each parasite genotype per sampling site. The number of each parasite genotype (n) and the average of each index per sampling site for the 16 microsatellites loci together are given.

**Table 4**: Pairwise estimates of $F_{ST}$ (below the diagonal) and significance (above diagonal) between parasite populations of the four sampling sites based on 16 microsatellite loci.

**Table 5:** Pairwise estimates of $F_{ST}$ (below the diagonal) and significance (above diagonal) between parasite genotypes of the four sampling sites based on 16 microsatellite loci.

**Table 6:** Analysis of molecular variance (AMOVA) partitioning the total variance observed at 16 microsatellite loci in 1966 miracidia between four hierarchical levels.

## Legends of figures

**Fig. 1:** Sampling sites in southern and western Côte d'Ivoire and number (percentage of total sample size) of miracidia collected and genotyped from *Schistosoma*-infected children.

**Fig. 2:** Principal components analysis (PCA) plot. Each miracidium is represented by a dot and the color label corresponds to its population origin. The first two axes of the PCA explain 41.14% and 33.75% of total inertia of the data set, respectively.

**Fig. 3:** Principal components analysis (PCA) plot. Each miracidium is represented by a dot and the color label corresponds each cox1-ITS profile. The first two principal components (PC) of the PCA explain 61.6% and 21.0% of total inertia of the data set, respectively.

**Fig. 4:** Bar plots depicting the genetic population structure of 1966 *Schistosoma* miracidia from the four sampling sites produced by STRUCTURE for K = 4. Each column represents one miracidium with colors indicating the proportional contribution of each of the four identified clusters.

**Legends of supplementary files**

**Supplementary Tab. S1:** Results of cox1- PCR and ITS2- RFLP analysis of miracidia collected per child. The total number and percentage of each cox1-ITS profile.

**Supplementary Fig. S1:** Banding patterns of restriction fragment length polymorphism (RFLP) analysis of the nuclear ITS2 region of *Schistosoma*. *S. haematobium* exhibits three bands at 281, 145 and 78 bp (C). *S. bovis* exhibits two bands at 426 and 78 bp (A, D) Hybrid (first generation) between *S. haematobium* and *S. bovis* exhibits bands at 426, 281 and 78 bp (B).

**Supplementary Fig. S2**: Bar plots depicting the percentage for each miracidium to belong to each cluster at infrapopulation in Duekoué and Sikensi, and significance for Chi-Square test.

**Table 1:** Total number (n) and mean number ($\bar{x}$) of miracidia genotyped per child using microsatellite markers. Number and percentage (in parentheses) of the six possible cox1-ITS profiles identified using the haploid mitochondrial cox1 gene (first two letters) and the diploid nuclear ITS2 region (last four letter), i.e. "pure" *Schistosoma haematobium* (*S. haematobium* cox1 x *S. haematobium* ITS2: Sh x ShSh), "pure" *S. bovis* (*S. bovis* cox1 x *S. bovis* ITS2: Sb x SbSb) and four types of hybrid (*S. bovis* cox1 x *S. haematobium* ITS2_*S. bovis* ITS2: Sb x ShSb; *S. bovis* cox1 x *S. haematobium* ITS2: Sb x ShSh; *S. haematobium* cox1 x *S. bovis* ITS2: Sh x SbSb; *S. haematobium* cox1 x *S. bovis* ITS2_ *S. haematobium* ITS2: Sh x ShSb) per sampling site. Total number of *S. haematobium* (Sh) and *S. bovis* (Sb) cox1 haplotype and ITS2 alleles (percentage in parentheses) per sampling site. The two largest values per sampling site are set in bold face.

| Sampling site | Sb x SbSb | | Sh x ShSh | | Sb x ShSb | | Sb x ShSh | | Sh x SbSb | | Sh x ShSb | | Total hybrids | All total | Cox1 haplotypes | | ITS2 alleles | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | n (%) | $\bar{x}$ (SE) | n (%) | $\bar{x}$ (SE) | n (%) | $\bar{x}$ (SE) | n (%) | $\bar{x}$ (SE) | n (%) | $\bar{x}$ (SE) | n (%) | $\bar{x}$ (SE) | n (%) | | **Sb** | **Sh** | **Sb** | **Sh** |
| Adzopé | 3 (0.6) | 1.5 (0.7) | 101 (20.4) | 5.1 (3.1) | 47 (9.5) | 3.4 (2.8) | 320 (64.7) | 15.2 (8.9) | 4 (0.8) | 2.0 (1.4) | 20 (4.0) | 2.9 (2.0) | 391 (79.0) | 495 | 370 (74.7) | 125 (25.3) | 81 (8.2) | 909 (91.8) |
| Agboville | 9 (1.8) | 2.3(1.3) | 170 (33.9) | 7.7 (5.4) | 59 (11.8) | 4.5 (4.1) | 90 (18.0) | 6.4 (4.3) | 34 (6.8) | 4.9 (4.3) | 139 (27.7) | 6.6 (4.3) | 322 (64.3) | 501 | 158 (31.5) | 343 (68.5) | 284 (28.4) | 718 (71.6) |
| Sikensi | 3 (0.49) | 1.5 (0.7) | 311 (51.0) | 12.0 (6.2) | 49 (8.0) | 2.9 (2.2) | 184 (30.2) | 7.7 (4.6) | 2 (0.33) | 1.0 (0) | 61 (10.0) | 3.4(2.5) | 296 (48.5) | 610 | 236 (46.3) | 374 (53.7) | 120 (9.8) | 1100 (90.2) |
| Duekoué | 0 | - | 209 (37.5) | 11.2 (8.6) | 19 (3.4) | 2.8 (1.8) | 314 (56.3) | 15.6 (6.8) | 3 (0.54) | 1.6 (0.9) | 13 (2.33) | 8.9 (10.8) | 349 (62.5) | 558 | 333 (59.7) | 225 (40.3) | 38 (3.4) | 1078 (96.6) |
| **Total** | **15 (0.7)** | **1.8 (0.9)** | **791 (36.6)** | **9.0 (5.8)** | **174 (8.0)** | **3.4 (2.7)** | **908 (42.0)** | **11.2 (6.2)** | **43 (2.0)** | **2.4 (1.7)** | **233 (10.8)** | **5.4 (4.9)** | **1358 (62.7)** | **2 164** | **1097 (50.7)** | **1067 (49.3)** | **523 (12.1)** | **3805 (87.9)** |

*SE*: Standard error, Chi-square test=555.9; degrees of freedom =15; p<0.0001.
Binomial test of *S. bovis* vs *S. haematobium* allele's equipartition for Cox1 (p=0.27) and ITS2 (p<0.00001).

**Table 2:** Genetic diversity indices. Mean expected heterozygosity (He), total number of alleles detected (A), Allelic richness (Ar) rarefied to 362 diploid individuals per population. Mean inbreeding coefficient ($F_{IS}$) and the probability of deviation from Hardy-Weinberg Equilibrium ($P_{HWE}$) for each microsatellite locus per study location. n, number of miracidia genotyped per study location.

| Locus | Adzopé (n=440) | | | | | Agboville (n=465) | | | | | Duekoué (n=527) | | | | | Sikensi (n=534) | | | | | Total (n=1966) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **He** | **A** | **Ar** | **$F_{IS}$** | **$P_{HWE}$** | **He** | **A** | **Ar** | **$F_{IS}$** | **$P_{HWE}$** | **He** | **A** | **Ar** | **$F_{IS}$** | **$P_{HWE}$** | **He** | **A** | **Ar** | **$F_{IS}$** | **$P_{HWE}$** | **He** | **A** | **Ar** |
| **Sh9** | 0.72 | 9 | 8.86 | 0.37 | <0.001 | 0.58 | 11 | 10.49 | 0.41 | <0.001 | 0.78 | 12 | 11.67 | 0.50 | <0.001 | 0.54 | 10 | 8.68 | 0.33 | <0.001 | 0.66 | 14 | 10.98 |
| **Sh3** | 0.83 | 14 | 13.96 | 0.20 | <0.001 | 0.78 | 15 | 14.60 | 0.11 | <0.001 | 0.88 | 16 | 15.53 | 0.08 | <0.001 | 0.83 | 14 | 13.47 | 0.01 | 0.836 | 0.83 | 16 | 15.00 |
| **C102** | 0.44 | 4 | 3.84 | 0.10 | 0.220 | 0.46 | 6 | 5.76 | -0.06 | <0.001 | 0.43 | 7 | 6.70 | 0.08 | 0.819 | 0.50 | 5 | 4.66 | -0.06 | <0.001 | 0.46 | 7 | 6.05 |
| **Sh1** | 0.77 | 9 | 8.88 | 0.01 | <0.001 | 0.68 | 9 | 8.55 | -0.01 | <0.001 | 0.75 | 12 | 11.97 | 0.03 | <0.001 | 0.74 | 8 | 7.99 | -0.03 | <0.001 | 0.74 | 13 | 11.85 |
| **Sh14** | 0.81 | 14 | 14.00 | 0.04 | <0.001 | 0.83 | 14 | 13.84 | 0.03 | <0.001 | 0.83 | 15 | 14.63 | 0.00 | <0.05 | 0.82 | 16 | 15.56 | -0.01 | <0.001 | 0.82 | 17 | 15.78 |
| **Sh6** | 0.53 | 7 | 6.98 | 0.24 | <0.001 | 0.58 | 7 | 6.99 | 0.07 | <0.001 | 0.48 | 7 | 7.00 | -0.01 | <0.001 | 0.60 | 7 | 6.99 | -0.11 | <0.001 | 0.55 | 7 | 7.00 |
| **C111** | 0.55 | 4 | 4.00 | 0.18 | <0.001 | 0.44 | 5 | 5.00 | 0.11 | <0.001 | 0.65 | 8 | 7.59 | 0.03 | 0.276 | 0.56 | 8 | 7.44 | 0.01 | 0.688 | 0.55 | 9 | 7.12 |
| **Sh7** | 0.68 | 7 | 6.83 | 0.26 | <0.001 | 0.74 | 5 | 5.00 | 0.32 | <0.001 | 0.64 | 7 | 6.99 | 0.31 | <0.001 | 0.64 | 5 | 4.74 | 0.32 | <0.001 | 0.68 | 7 | 6.69 |
| **Sh13** | 0.66 | 15 | 14.67 | 0.01 | <0.001 | 0.51 | 14 | 13.37 | 0.01 | <0.001 | 0.69 | 15 | 14.52 | -0.02 | <0.001 | 0.71 | 14 | 13.64 | -0.03 | 0.408 | 0.64 | 17 | 15.68 |
| **Sh4** | 0.74 | 9 | 9.00 | 0.05 | <0.01 | 0.73 | 9 | 8.92 | 0.08 | <0.001 | 0.80 | 11 | 10.82 | 0.04 | <0.001 | 0.85 | 9 | 9.00 | 0.07 | <0.001 | 0.78 | 11 | 10.22 |
| **Sh11** | 0.51 | 7 | 6.62 | -0.01 | <0.001 | 0.68 | 6 | 5.81 | 0.12 | <0.001 | 0.46 | 8 | 7.54 | 0.13 | <0.001 | 0.70 | 8 | 7.66 | 0.09 | <0.001 | 0.59 | 10 | 7.80 |
| **Sh15** | 0.49 | 7 | 7.00 | 0.16 | <0.001 | 0.54 | 6 | 6.00 | 0.17 | <0.001 | 0.50 | 6 | 5.97 | 0.13 | <0.001 | 0.46 | 6 | 6.00 | -0.02 | <0.001 | 0.50 | 7 | 7.00 |
| **Sh2** | 0.78 | 13 | 13.00 | 0.35 | <0.001 | 0.80 | 12 | 12.00 | 0.32 | <0.001 | 0.89 | 14 | 13.96 | 0.45 | <0.001 | 0.86 | 12 | 12.00 | 0.35 | <0.001 | 0.83 | 15 | 14.64 |
| **Sh5** | 0.73 | 10 | 9.90 | 0.44 | <0.001 | 0.47 | 13 | 12.34 | 0.26 | <0.001 | 0.80 | 15 | 14.70 | 0.10 | <0.001 | 0.64 | 9 | 8.73 | 0.16 | <0.001 | 0.66 | 16 | 13.95 |
| **Sh10** | 0.48 | 8 | 7.85 | 0.51 | <0.001 | 0.53 | 6 | 5.79 | 0.35 | <0.001 | 0.48 | 8 | 7.88 | 0.31 | <0.001 | 0.33 | 7 | 6.64 | 0.10 | <0.001 | 0.46 | 8 | 7.56 |
| **Sh12** | 0.10 | 4 | 3.83 | -0.04 | 0.972 | 0.29 | 6 | 5.95 | 0.03 | <0.001 | 0.07 | 7 | 6.06 | -0.03 | 1.0 | 0.23 | 8 | 7.58 | -0.06 | <0.001 | 0.17 | 10 | 7.68 |
| ***Mean*** | *0.61* | *8.81* | *8.70* | *0.18* | - | *0.60* | *9.00* | *8.78* | *0.15* | - | *0.63* | *10.50* | *10.22* | *0.13* | - | *0.63* | *9.13* | *8.80* | *0.07* | - | *0.62* | *11.50* | *10.31* |
| ***SE*** | *0.19* | *3.60* | *3.61* | *0.17* | - | *0.15* | *3.61* | *3.47* | *0.14* | - | *0.22* | *3.61* | *3.59* | *0.17* | - | *0.18* | *3.28* | *3.22* | *0.15* | - | *0.18* | *3.88* | *3.65* |

*SE*: Standard error, Ar: (Friedman test= 53.59, p=0.0001); A: (Friedman test=52.83, p=0.0001); He: (Friedman test: F=50.59, p=0.0001); $F_{IS}$ (Friedman test: F=48.75, p=0.0001)

**Table 3:** Mean value of genetic diversity indices: heterozygosity (He), number of alleles (A), allelic richness (Ar) (for one diploid individual in Adzopé, eight in Agboville, one in Sikensi and one for Duekoué) and inbreeding coefficient ($F_{IS}$) for each cox1-ITS profile per sampling site. The number of each parasite genotype (n) and the average of each index per sampling site for the 16 microsatellites loci together are given.

| **Sampling Sites** | **Sb x SbSb (n=15)** | | | | **Sh x ShSh (n= 692)** | | | | **Sb x ShSb (n= 164)** | | | | **Sb x ShSh (n= 839)** | | | | **Sh x SbSb (n=42)** | | | | **Sh x ShSb (n=214)** | | | | **All (n=1966)** | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **He** | **A** | **Ar** | **$F_{IS}$** | **He** | **A** | **Ar** | **$F_{IS}$** | **He** | **A** | **Ar** | **$F_{IS}$** | **He** | **A** | **Ar** | **$F_{IS}$** | **He** | **A** | **Ar** | **$F_{IS}$** | **He** | **A** | **Ar** | **$F_{IS}$** | **He** | **A** | **Ar** | **$F_{IS}$** |
| Adzopé | 0.52 | 2.38 | 1.55 | -0.10 | 0.58 | 6.75 | 1.58 | 0.23 | 0.61 | 5.63 | 1.61 | 0.11 | 0.61 | 8.38 | 1.61 | 0.16 | 0.56 | 2.44 | 1.52 | 0.32 | 0.61 | 4.81 | 1.60 | 0.29 | 0.58 | 8.81 | 1.61 | 0.18 |
| Agboville | 0.60 | 4.0 | 3.96 | 0.10 | 0.60 | 7.06 | 4.05 | 0.15 | 0.59 | 6.50 | 4.03 | 0.04 | 0.60 | 7.31 | 4.05 | 0.13 | 0.56 | 4.38 | 3.36 | 0.18 | 0.61 | 7.31 | 4.07 | 0.16 | 0.59 | 9.0 | 4.09 | 0.13 |
| Sikensi | 0.61 | 2.75 | 1.61 | -0.02 | 0.63 | 8.75 | 1.63 | 0.11 | 0.61 | 6.19 | 1.61 | 0.03 | 0.62 | 7.50 | 1.62 | 0.02 | 0.60 | 2.25 | 1.58 | 0.15 | 0.61 | 6.19 | 1.61 | 0.04 | 0.61 | 9.13 | 1.63 | 0.05 |
| Duekoué | - | - | - | - | 0.62 | 9.31 | 1.62 | 0.13 | 0.62 | 5.63 | 1.62 | 0.11 | 0.64 | 9.69 | 1.64 | 0.13 | 0.66 | 2.69 | 1.57 | 0.25 | 0.62 | 4.44 | 1.61 | 0.12 | 0.63 | 10.50 | 1.63 | 0.15 |
| ***Mean*** | *0.58* | *3.* | *2.37* | *-0.01* | *0.61* | *8.80* | *2.22* | *0.16* | *0.61* | *6.25* | *2.22* | *0.07* | *0.62* | *8.25* | *2.23* | *0.11* | *0.60* | *2.75* | *2.01* | *0.23* | *0.61* | *5.50* | *2.22* | *0.15* | *0.60* | *8.50* | *2.24* | *0.13* |
| ***SE*** | *0.05* | *1.0* | *1.37* | *0.10* | *0.02* | *1.15* | *1.22* | *0.05* | *0.01* | *0.50* | *1.21* | *0.04* | *0.02* | *1.26* | *1.21* | *0.06* | *0.05* | *0.96* | *0.90* | *0.08* | *0.01* | *1.29* | *1.23* | *0.10* | *0.02* | *1.29* | *1.23* | *0.06* |

*SE*: Standard error He: (Friedman test: F=5.08 p=0.079); A: (Friedman test: F=2.58 p=0.275); Ar: (Friedman test= 11.08, p=0.039); $F_{IS}$: (Friedman test=7.0, p=0.030)

**Table 4:** Pairwise estimates of $F_{ST}$ (below the diagonal) and significance (above diagonal) between parasite populations of the four sampling sites based on 16 microsatellite loci.

| Sampling site | Adzopé | Agboville | Duekoué | Sikensi |
|---|---|---|---|---|
| Adzopé | - | S | S | S |
| Agboville | 0.049 | - | S | S |
| Duekoué | 0.069 | 0.065 | - | S |
| Sikensi | 0.047 | 0.041 | 0.056 | - |

S, significant values at $p<0.01$

**Table 5:** Pairwise estimates of $F_{ST}$ (below the diagonal) and significance (above diagonal) between cox1-ITS profiles of the four sampling sites based on 16 microsatellite loci.

| Genotype | Sb_SbSb | Sb_ShSb | Sb_ShSh | Sh_SbSb | Sh_ShSb | Sh_ShSh |
|---|---|---|---|---|---|---|
| Sb_SbSb | - | NS | S | S | NS | NS |
| Sb_ShSb | 0.003 | - | S | S | S | S |
| Sb_ShSh | 0.011 | 0.0071 | - | S | S | S |
| Sh_SbSb | 0.015 | 0.023 | 0.027 | - | S | S |
| Sh_ShSb | 0.006 | 0.005 | 0.014 | 0.009 | - | S |
| Sh_ShSh | 0.004 | 0.003 | 0.006 | 0.021 | 0.008 | - |

NS: no statistically significant; S: statistically significant at the $p < 0.05$ level

**Table 6:** Analysis of molecular variance (AMOVA) partitioning the total variance observed at 16 microsatellite loci in 1966 miracidia between four hierarchical levels.

| Hierarchical level | df | Sum of squares | Variance component | Percentage of variation |
|---|---|---|---|---|
| Among sampling sites | 3 | 25659.07 | 7.83 | 4.11 |
| Among children within sampling site | 87 | 68119.04 | 14.26 | 7.48 |
| Among miracidia within child | 1875 | 320706.33 | 2.78 | 1.35 |
| Within miracidia | 1966 | 326144 | 165.89 | 87.06 |
| Total | 3931 | 740628.44 | 190.55 | |

*df, degrees of freedom*

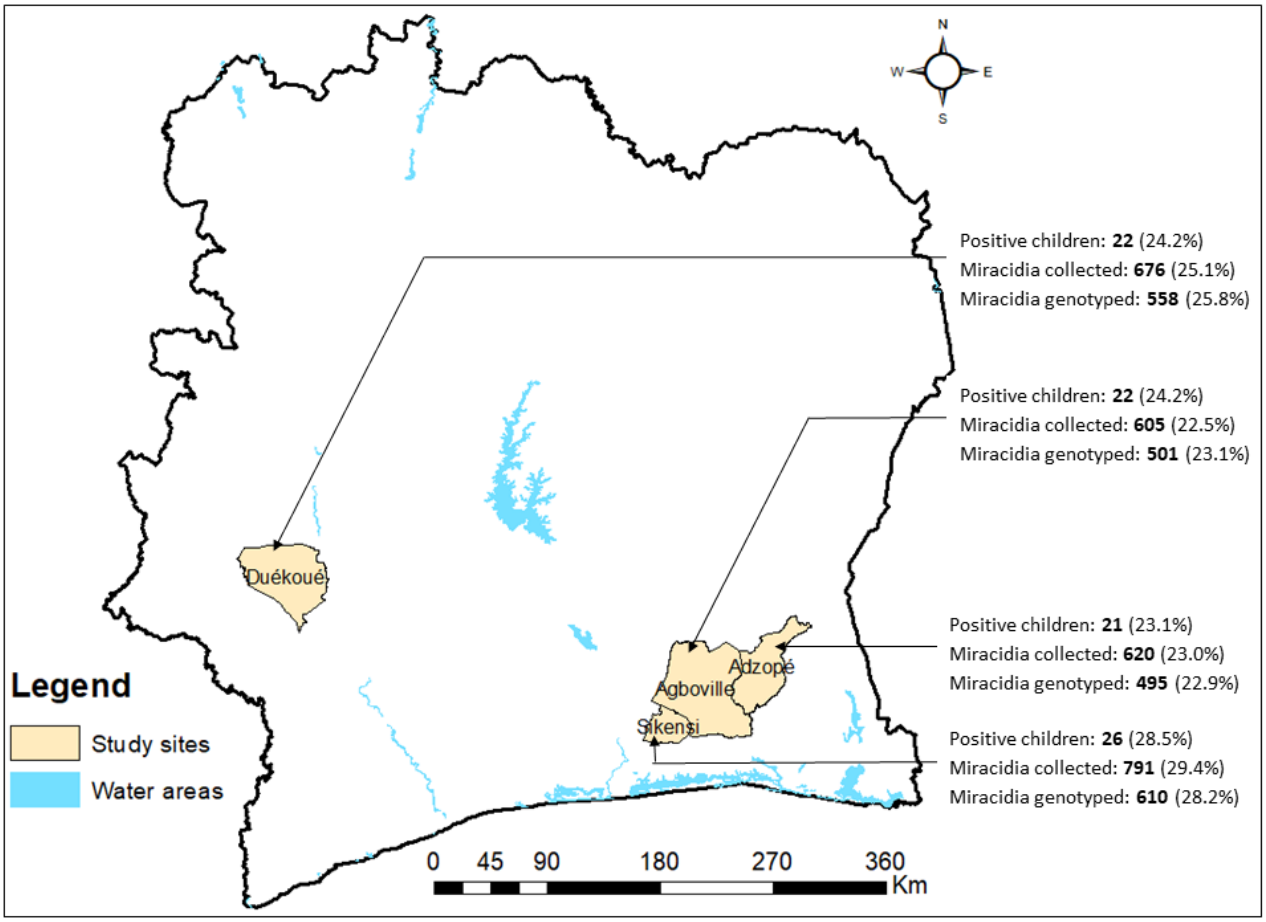



**Fig. 1:** Sampling sites in southern and western Côte d'Ivoire and number (percentage of total sample size) of miracidia collected and genotyped from *Schistosoma*-infected children.

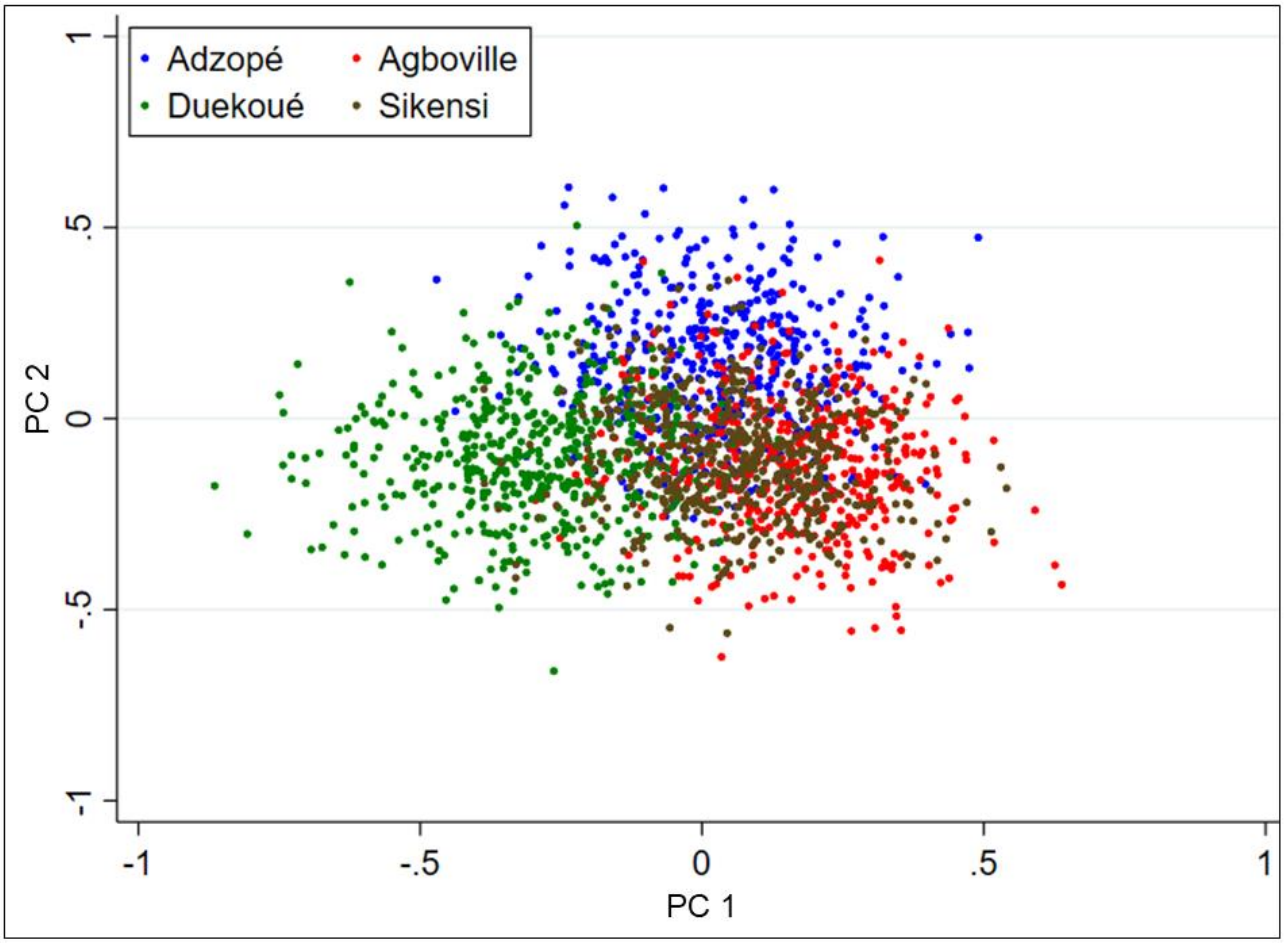



**Fig. 2:** Principal components analysis (PCA) plot. Each miracidium is represented by a dot and the color label corresponds to its population origin. The first two principal components (PC) of the PCA explain 41.1% and 33.7% of total inertia of the data set, respectively.

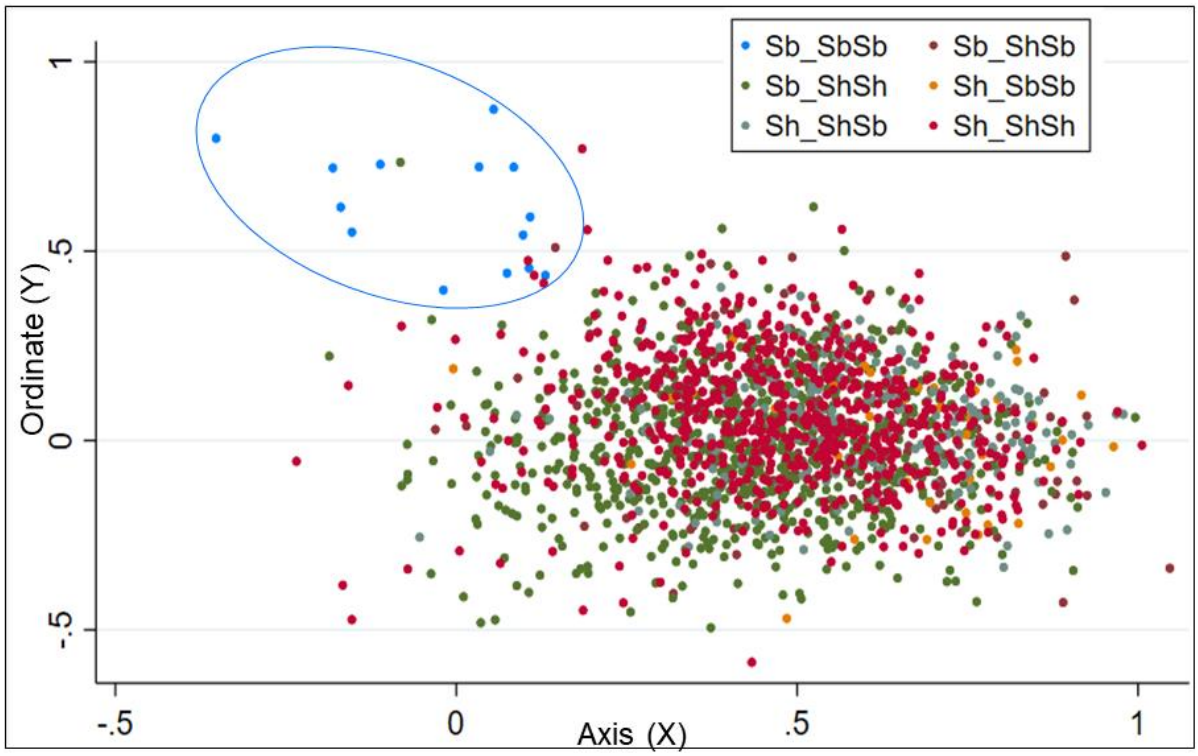



**Fig. 3:** Principal components analysis (PCA) plot. Each miracidium is represented by a dot and the color label corresponds each cox1-ITS profile. The first two principal components (PC) of the PCA explain 61.6% and 21.0% of total inertia of the data set, respectively.

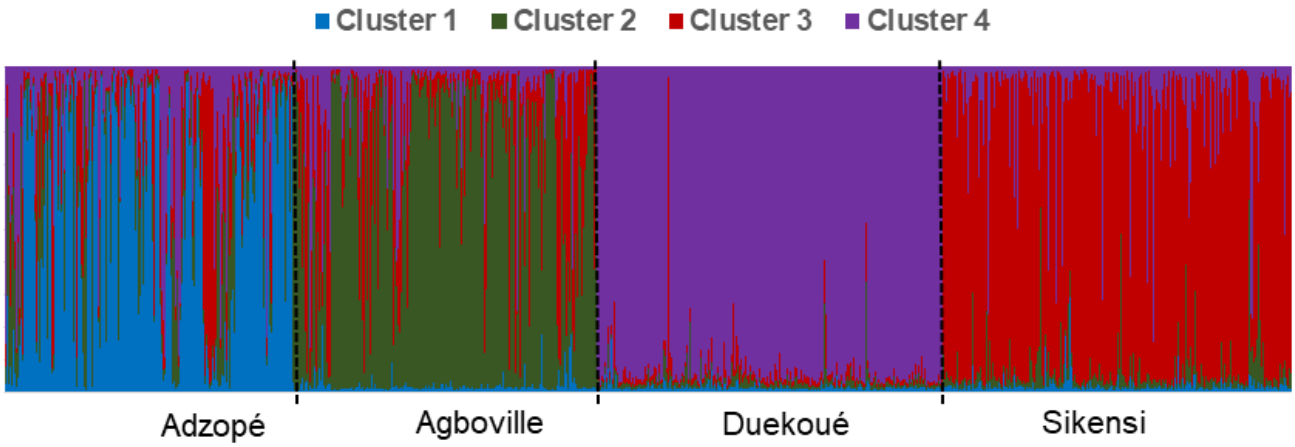



**Fig. 4:** Bar plots depicting the genetic population structure of 1966 *Schistosoma* miracidia from the four sampling sites produced by STRUCTURE for K = 4. Each column represents one miracidium with colors indicating the proportional contribution of each of the four identified clusters.

**Supplementary Tab. S1:** Total number of miracidia analyzed (n) and number (percentage) of miracidia assigned to the six possible cox1-ITS profiles defined by cox1- PCR and ITS2- RFLP analysis per child and sampling site.

| Sampling site | Child Id | Sb x SbSb | Sb x ShSb | Sb x ShSh | Sh x SbSb | Sh x ShSb | Sh x ShSh | Total |
|---|---|---|---|---|---|---|---|---|
| | | n (%) | n (%) | n (%) | n (%) | n (% | n (%) | |
| Adzopé | AD078 | 0 | 3 (10.3) | 25 (68.2) | 0 | 0 | 1 (3.4) | 29 |
| | AD088 | 0 | 0 | 1 (12.5) | 3 (37.5) | 4(50.0) | 0 | 8 |
| | AD091 | 0 | 0 | 1 (14.3) | 0 | 4 (57.1) | 2 (28.6) | 7 |
| | AD094 | 0 | 0 | 1 (12.5) | 0 | 3 (37.5) | 4 (50.0) | 8 |
| | AD122 | 0 | 8 (28.6) | 18 (64.3) | 0 | 0 | 2 (7.1) | 28 |
| | AD127 | 0 | 1 (4.2) | 15 (62.5) | 0 | 0 | 8 (33.3) | 24 |
| | AD128 | 0 | 1 (6.2) | 9 (56.2) | 0 | 0 | 6 (37.5) | 16 |
| | AD138 | 0 | 1 (2.9) | 27 (79.4) | 0 | 0 | 6 (17.6) | 34 |
| | AD140 | 0 | 0 | 23 (82.1) | 0 | 0 | 5 (17.9) | 28 |
| | AD142 | 0 | 0 | 22 (73.3) | 0 | 0 | 8 (26.7) | 30 |
| | AD145 | 1 (3.4) | 3 (10.3) | 20 (69.0) | 0 | 0 | 5 (17.2) | 29 |
| | AD155 | 0 | 5 (17.9) | 17 (60.7) | 0 | 0 | 6 (21.4) | 28 |
| | AD156 | 2 (10.0) | 4 (20.0) | 6 (30.0) | 0 | 1 (5.0) | 7 (35.0) | 20 |
| | AD158 | 0 | 0 | 1 (7.1) | 0 | 6 (42.9) | 7 (50.0) | 14 |
| | AD160 | 0 | 1 (4.5) | 17 (77.3) | 0 | 0 | 4 (18.2) | 22 |
| | AD161 | 0 | 10 (29.4) | 9 (26.5) | 0 | 1 (2.9) | 14 (41.2) | 34 |
| | AD165 | 0 | 4 (14.3) | 21 (75.0) | 0 | 1 (3.6) | 2 (7.1) | 28 |
| | AD179 | 0 | 1 (3.6) | 23 (82.1) | 0 | 0 | 4 (14.3) | 28 |
| | AD180 | 0 | 0 | 23 (88.5) | 0 | 0 | 3 (11.5) | 26 |
| | AD182 | 0 | 2 (8.0) | 16 (64.0) | 1 (4.0) | 0 | 6 (24.0) | 25 |
| | AD197 | 0 | 3 (10.3) | 25 (86.2) | 0 | 0 | 1 (3.4) | 29 |
| | **Total** | **3 (0.6)** | **47 (9.5)** | **320 (64.6)** | **4 (0.8)** | **20(4.0)** | **101 (20.4)** | **495** |

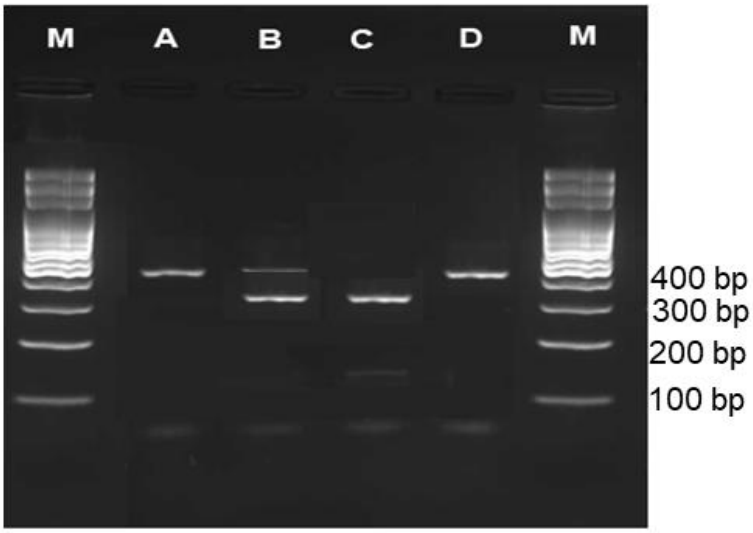
| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Agboville | AG008 | 1 (4.5) | 2 (9.1) | 11 (50.0) | 0 | 3 (13.6) | 5 (22.7) | 22 |
| | AG035 | 0 | 6 (24.0) | 5 (20.0) | 0 | 7 (28.0) | 7 (28.0) | 25 |
| | AG044 | 4 (23.5) | 3 (17.6) | 4 (23.5) | 0 | 2 (11.7) | 4 (23.5) | 17 |
| | AG045 | 0 | 0 | 0 | 1 (5.9) | 7 (41.2) | 9 (52.9) | 17 |
| | AG047 | 0 | 0 | 0 | 4 (15.4) | 15 (57.7) | 7 (26.9) | 26 |
| | AG057 | 0 | 0 | 2 (7.1) | 0 | 15 (53.6) | 11 (39.3) | 28 |
| | AG058 | 0 | 5 (20.0) | 13 (52.0) | 0 | 6 (24.0) | 1 (40.0) | 25 |
| | AG062 | 0 | 2 (10.5) | 4 (21.0) | 0 | 7 (36.8) | 6 (31.6) | 19 |
| | AG066 | 0 | 0 | 0 | 0 | 11 (73.3) | 4 (26.7) | 15 |
| | AG067 | 0 | 0 | 1 (50.0) | 0 | 0 | 1 (50.0) | 2 |
| | AG068 | 0 | 0 | 0 | 14 (56.0) | 3 (12.0) | 8 (32.0) | 25 |
| | AG073 | 0 | 1 (3.6) | 0 | 5 (17.9) | 9 (32.1) | 13 (46.4) | 28 |
| | AG077 | 2 (8.3) | 10 (41.7) | 9 (37.5) | 0 | 1 (4.2) | 2 (8.3) | 24 |
| | AG086 | 0 | 0 | 0 | 0 | 3 (25) | 9 (75.0) | 12 |
| | AG107 | 0 | 0 | 0 | 0 | 6 (33.3) | 12 (66.7) | 18 |
| | AG108 | 0 | 0 | 4 (24.7) | 3 (20.0) | 2 (13.3) | 6 (40.0) | 15 |
| | AG115 | 0 | 10 (37.0) | 12 (44.4) | 0 | 2 (7.4) | 3 (11.1) | 27 |
| | AG122* | 2 (4.0) | 13 (26.0) | 8 (16.0) | 2 (4.0) | 7 (14.0) | 18 (36.0) | 50 |
| | AG144 | 0 | 1 (5.3) | 12 (63.2) | 0 | 2 (10.5) | 4 (21.0) | 19 |
| | AG149 | 0 | 1 (4.8) | 0 | 0 | 11 (52.4) | 9 (42.9) | 21 |
| | AG219 | 0 | 4 (12.1) | 4 (12.1) | 5 (15.1) | 12 (36.4) | 8 (24.2) | 33 |
| | AG250 | 0 | 1 (3.0) | 1 (3.0) | 0 | 8 (24.2) | 23 (69.7) | 33 |
| | **Total** | **9 (1.8)** | **59 (11.8)** | **90 (18.0)** | **34 (6.8)** | **139 (27.7)** | **170 (33.9)** | **501** |
| Sikensi | SI028 | 1 (2.9) | 3 (8.8) | 9 (26.5) | 0 | 4 (11.8) | 17 (50.0) | 34 |
| | SI032 | 0 | 0 | 5 (17.9) | 0 | 7 (25.0) | 16 (57.1) | 28 |
| | SI036 | 0 | 4 (14.3) | 4 (14.3) | 0 | 9 (32.1) | 11 (39.3) | 28 |
| | SI044 | 0 | 0 | 15 (57.7) | 0 | 0 | 11 (42.31) | 26 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | SI047 | 0 | 1 (3.1) | 11 (34.4) | 0 | 0 | 20 (62.5) | 32 |
| | SI052 | 0 | 9 (31.0) | 5 (17.2) | 0 | 4 (13.8) | 11 (37.9) | 29 |
| | SI059 | 0 | 0 | 3 (13.0) | 0 | 0 | 20 (87.0) | 23 |
| | SI064 | 0 | 0 | 9 (37.5) | 0 | 8 (33.3) | 7 (27.2) | 24 |
| | SI070 | 0 | 0 | 3 (12.0) | 1 (4.0) | 0 | 21 (84.0) | 25 |
| | SI075 | 0 | 1 (4.8) | 4 (19.0) | 0 | 3 (14.3) | 13(61.9) | 21 |
| | SI078 | 0 | 3 (42.9) | 3 (42.9) | 0 | 0 | 1 (14.3) | 7 |
| | SI091 | 0 | 0 | 2 (15.4) | 0 | 1 (7.7) | 10 (76.9) | 13 |
| | SI092 | 0 | 0 | 4 (22.2) | 0 | 4 (22.2) | 10 (55.6) | 18 |
| | SI098 | 0 | 2 (9.5) | 4 (19.0) | 1 (4.8) | 2 (9.5) | 12 (57.1) | 21 |
| | SI101 | 0 | 1 (4.5) | 12 (54.5) | 0 | 2 (9.1) | 7 (31.8) | 22 |
| | SI104 | 0 | 2 (13.3) | 6 (40.0) | 0 | 1 (6.7) | 6 (40.0) | 15 |
| | SI109 | 0 | 2 (8.3) | 11 (45.8) | 0 | 1 (4.2) | 10 (41.7) | 24 |
| | SI110 | 0 | 0 | 0 | 0 | 2 (10.5) | 17 (89.5) | 19 |
| | SI112 | 0 | 1 (4.2) | 8 (33.3) | 0 | 3 (12.5) | 12 (50.0) | 24 |
| | SI114 | 0 | 2 (8.0) | 10 (40.0) | 0 | 0 | 13 (52.0) | 25 |
| | SI115 | 0 | 2 (6.9) | 11 (37.9) | 0 | 1 (3.4) | 15 (51.7) | 29 |
| | SI122 | 0 | 3 (14.3) | 6 (28.6) | 0 | 5(23.8) | 7 (33.3) | 21 |
| | SI125 | 0 | 0 | 0 | 0 | 1 (3.6) | 27 (96.4) | 28 |
| | SI133 | 0 | 7 (30.4) | 14 (60.9) | 0 | 0 | 2 (8.7) | 23 |
| | SI134 | 0 | 4 (15.4) | 20 (76.2) | 0 | 0 | 2 (7.7) | 26 |
| | SI136 | 2 (8.0) | 2 (8.0) | 5 (20.0) | 0 | 3(12.0) | 13 (52.0) | 25 |
| | **Total** | **3 (0.5)** | **49 (8.0)** | **184 (30.2)** | **2 (0.3)** | **61 (10.0)** | **311 (50.9)** | **610** |
| Duekoué | DU053 | 0 | 0 | 13 (100.0) | 0 | 0 | 0 | 13 |
| | DU114 | 0 | 5 (29.4) | 11(64.7) | 0 | 0 | 1 (5.9) | 17 |
| | DU197 | 0 | 0 | 0 | 1 (25.0) | 3 (75.0) | 0 | 4 |
| | DU309 | 0 | 0 | 11 (34.4) | 1 (3.1) | 2 (6.2) | 18 (56.2) | 32 |

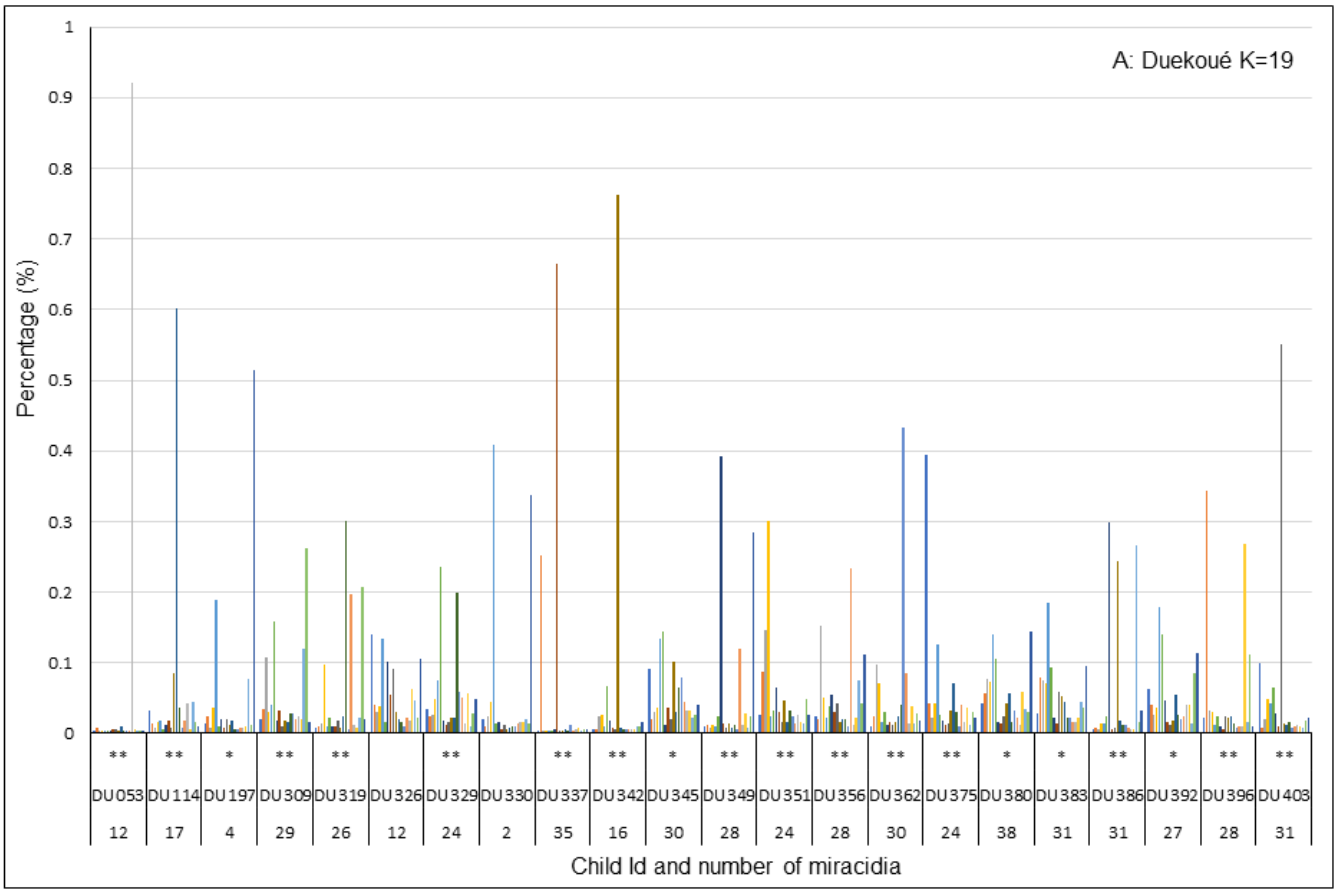
| | | | | | | | |
|---|---|---|---|---|---|---|---|
| DU319 | 0 | 0 | 6 (21.4) | 0 | 2 (7.1) | 20 (71.4) | 28 |
| DU326 | 0 | 0 | 10 (76.9) | 0 | 0 | 3 (23.1) | 13 |
| DU329 | 0 | 3 (10.3) | 8 (27.6) | 0 | 2 (6.9) | 16 (55.2) | 29 |
| DU330** | 0 | 0 | 0 | 0 | 0 | 2 (100.0) | 2 |
| DU337** | 0 | 0 | 0 | 0 | 0 | 35 (100.0) | 35 |
| DU342 | 0 | 0 | 15 (78.5) | 0 | 0 | 4 (21.0) | 19 |
| DU345 | 0 | 0 | 19 (59.4) | 0 | 0 | 13 (40.6) | 32 |
| DU349 | 0 | 0 | 17 (56.7) | 0 | 0 | 13 (43.3) | 30 |
| DU351 | 0 | 0 | 12 (48.0) | 0 | 0 | 13 (52.0) | 25 |
| DU356 | 0 | 2 (7.1) | 6 (21.4) | 0 | 4 (14.3) | 16 (57.1) | 28 |
| DU362 | 0 | 0 | 20 (62.5) | 0 | 0 | 12 (37.5) | 32 |
| DU375 | 0 | 1 (4.0) | 21 (84.0) | 0 | 0 | 3 (12.0) | 25 |
| DU380 | 0 | 2 (5.3) | 23 (60.5) | 0 | 0 | 13 (34.2) | 38 |
| DU383 | 0 | 1 (3.2) | 18 (58.1) | 0 | 0 | 12 (38.7) | 31 |
| DU386 | 0 | 0 | 28 (87.5) | 0 | 0 | 4 812.5) | 32 |
| DU392 | 0 | 2 (6.4) | 0 | 2 (6.4) | 27 (87.1) | 0 | 31 |
| DU396 | 0 | 6 (13.4) | 0 | 3 (9.7) | 22 (77.0) | 0 | 31 |
| DU403 | 0 | 0 | 27 (87.1) | 1 (3.2) | 0 | 3 (9.7) | 31 |
| **Total** | **0** | **19 (3.4)** | **314 (56.3)** | **3 (0.5)** | **13 (2.3)** | **209 (37.5)** | **558** |
| **All total** | **15 (0.7)** | **174 (8.0)** | **908 (42.0)** | **43 (2.0)** | **233 (10.8)** | **791** | **2 164** |

*: Infected by parasites of all the six possible cox1-ITS profiles. **: Infected by only pure parasite genotype.

**Supplementary Fig. S1:** Banding patterns of restriction fragment length polymorphism (RFLP) analysis of the nuclear ITS2 region of *Schistosoma*. *S. haematobium* exhibits three bands at 281, 145 and 78 bp (C). *S. bovis* exhibits two bands at 426 and 78 bp (A, D). Hybrids between *S. haematobium* and *S. bovis* exhibit bands at 426, 281 and 78 bp (B). M, 100 bp molecular ladder.

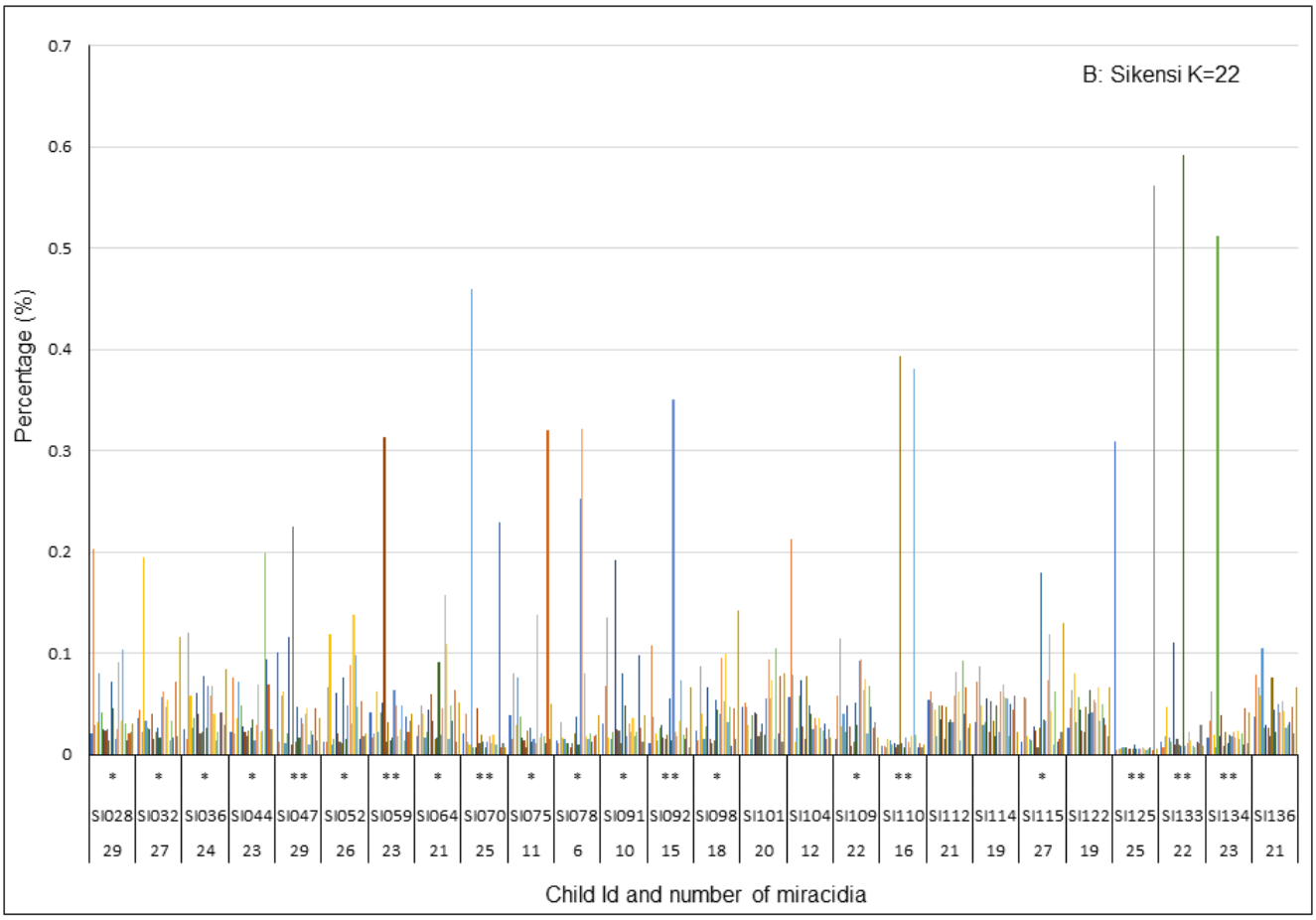


**Supplementary Fig. S2:** Bar plots depicting the percentage for each miracidium to belong to each cluster at infrapopulation in Duekoué and Sikensi, and significance for Chi-Square test.




Significance of Chi-Square test: * p<0.05, ** p<0.01

**Fig. S2 A:** Miracidia belong to 19 clusters in Duekoué, each color of bar represents one cluster. Child "DU053" belongs preferentially to one cluster.




Significance of Chi-Square test: * p<0.05, ** p<0.01

**Fig. S2 B:** Miracidia belong to 22 clusters in Sikensi, each color of bar represents one cluster.

# Chapter 6:

# Discussion

Worldwide, schistosomiasis remains significant in terms of prevalence and morbidity (Murray et al., 2012; Vos et al., 2016). This chronic parasitic disease is prevalent in tropical and subtropical areas, especially in poor and rural communities without access to safe drinking water and adequate sanitation (Colley et al., 2014). The best method for diagnosis is detecting parasite eggs in urine or stool samples (Utzinger et al., 2010; Gray et al., 2011). Control or elimination of schistosomiasis includes improving access to clean water, sanitation and hygiene improvements, behavioural interventions, reducing the number of snails and particularly, increasing treatment coverage with praziquantel (Barry et al., 2013; Webster et al., 2014). Water supplies and access to and use of adequate sanitation play important roles in reducing *Schistosoma* eggs and preventing miracidia from infecting intermediate host snails (Grimes et al., 2015). However, mass drug administration (MDA) increases drug pressure and therefore positions parasite populations increasingly to selection pressure. This can lead to a possible reduction of drug effectiveness, thus genetic monitoring and examination of long-lived multi-cellular parasites are needed (Norton et al., 2010). Population genetics allows understanding factors capable of changing the distribution and frequency of alleles within *Schistosoma* populations. It also reveals the importance of genetic monitoring and examination of long-lived parasites under novel or increased chemotherapeutic selective pressures.

Species have commonly been considered as groups of natural populations that interbreed to produce viable and fertile offspring. However, this biological species concept is often challenged (De Queiroz, 2007; Aldhebiani, 2018) by evidence of gene flow between species. Hybridization has long been considered as a rare event related to an abnormality in reproductive system of species (Mallet, 2008). Reproduction between hybrids or between parents and hybrids can lead to introgression. This corresponds to the transfer of a gene from one species to others. Thus, the offspring includes a complex mixture of genes from these species and may eventually lead progressively to genetic invasion (Mallet, 2005).

Genetic diversity describes variety of genes within species, which are often subdivided into populations with differing allelic and genotypic frequencies (Davies et al., 1999). The purpose of population genetics is the measurement of this variation and its origin, but also the dynamics of diversity, to understand the evolution mechanisms, in particular using theoretical approaches based on mathematical models. Methods for schistosomes' genotyping are diverse. They include the abundance and degree of polymorphism, simple genotyping and data analysis.

Restriction fragment length polymorphism (RFLP) (McCutchan et al., 1984), randomly amplified polymorphic DNA (RAPD) (Neto et al., 1997), DNA barcoding using mitochondrial markers (Le et al., 2000) and microsatellite genotyping (Webster et al., 2015) are commonly employed to assess intraspecific geographical distribution. For population genetics, mitochondrial markers have a limited number of possible alleles that can influence the interpretation of data related to the potential bi-parental inheritance in schistosomes (Jannotti-Passos et al., 2001).

In this thesis, the two main aims were to expand understanding to update data on schistosomiasis and to assess the population genetics of *Schistosoma* populations. In the framework of the first aim, I determined the prevalence and associated factors of the disease. The second aim was addressed by analysing the genetic diversity and population structure of *S. haematobium* in schoolchildren by investigating the gene flow of parasites between different geographical locations.

Throughout this thesis, many of the findings were discussed in detail in the included manuscripts. Thus, in this discussion part, I would like to point out the relevant lessons learned from the entire study.

**Table 6.1: Main contribution of individual chapters to the three areas of innovation, validation and application**

| | Innovation | Validation | Application |
|---|---|---|---|
| **Chapter 2** | | Microscopic examination for schistosomes identification in urine and stool samples | Update of schistosomiasis prevalence |
| **Chapter 3** | Identification of hybrids *S. haematobium* x *S. bovis* in humans | Use of PCR methods and sequencing for molecular analysis | |
| **Chapter 4** | | Utilisation of microsatellite analysis in population genetics of *S. haematobium* | Basis for future population genetic studies |
| **Chapter 5** | - Pure *S. bovis* identified in humans<br>- Genetic clustering of *S. haematobium* within children | Use of microsatellite genotyping for genetic diversity of *S. haematobium* | Novel insights informing future sampling designs |

## 6.1. Prevalence of schistosomiasis in schoolchildren

Schistosomiasis is a human parasitic disease widely distributed throughout tropical and subtropical areas. Many factors are associated with the persistent transmission of schistosomiasis in sub-Saharan Africa (Jordan, 2000). The risk of infection has increased over the years and this has been attributed to environmental, socio-economic and demographic factors (Kalinda et al., 2018). Moreover, evidence proved that the target population of high transmission includes children, as many were found to harbor infections of varying intensity (Coulibaly et al., 2013; Stothard et al., 2013; Hotez et al., 2014; Diakité et al., 2017; Gbalégba et al., 2017). There is evidence that from a young age, childhood development is the most affected by this chronic parasitic disease, which can promote inflammatory homeostasis by triggering an immune response (Mishra et al., 2014), as is likely the case for other helminth infections (Kvalsvig and Albonico, 2013). However, schistosomes also infect adult people during water-contact (Bakuza et al., 2017; Toor et al., 2018). The main epidemiological feature of schistosomiasis is the focal distribution, which involves the interplay of humans, intermediate host snails and human-water contact patterns (McManus et al., 2018).

The current study showed that schistosomiasis remains prevalent in children in Côte d'Ivoire although the prevalence was low **(Chapter 2)**. Diagnostic methods employed were those commonly used in epidemiological surveys (Katz et al., 1972; Mott et al., 1982). We presented evidence of the effectiveness of preventive chemotherapy campaigns using praziquantel pursued on an annual basis for several years by the "Programme National de Lutte contre les Maladies Tropicales Negligées à Chimioprophylaxie Préventive" (PNLMTN-CP) in Côte d'Ivoire (Tian-Bi et al., 2018). We reported that swimming, washing clothes, playing in unprotected open freshwater bodies, and low educational attainment of parents/guardians were identified as key risk factors of schistosomiasis in schoolchildren. The lack of access to clean water, poor sanitation and hygiene and activities involving contact with water, including domestic, recreational (playing and swimming in lakes or rivers) and professional (car washing and collection), put the population at risk of schistosome infections when exposed to contaminated water bodies (Grimes et al., 2014; Tian-Bi et al., 2018). Despite schistosome infections, soil-transmitted helminth (STH) infections were identified at a low prevalence rate. Association of parasite infections is frequently found in the human body including schistosomiasis and STH infections (Coulibaly et al., 2013). The low prevalence of these STH infections in our study could in part be explained by prior interventions, in the frame of research and control strategies targeting neglected tropical diseases.

This includes annual treatment with albendazole plus ivermectin for the control/elimination of lymphatic filariasis (Fürst et al., 2012; Lo et al., 2017; Hürlimann et al., 2018). Finally, the main effective action to prevent this parasitic disease is combining preventive chemotherapy using praziquantel, the current mainstay of the national schistosomiasis control program, with targeted information and education campaigns to change people's behaviors, and improved environmental sanitation by creating and using latrines.

## 6.2. Identification of schistosome hybrids in humans

Hybrid schistosomes expansion can decrease in the sensitivity of parasites to praziquantel used in massive drug administration chemotherapy (Webster et al., 2014). Hence, identification of such hybrids is important for future control strategies.

The most adaptive capacity of hybrids compared to parental species is the "hybrid vigour". This is the best performance of hybrids to become virulent and invasive, and to expand the host spectrum (Bar-Zvi et al., 2017; Kincaid-Smith et al., 2019). Hybridization can also lead to rapid changes in genome structure and function to produce new offspring (Baack et al., 2005; Ungerer et al., 2006). It potentially increases the risk of zoonotic transmission of the disease, as well. *Schistosoma haematobium* group is frequently involved in this hybridization (Leger and Webster, 2017). We reported a high prevalence of *S. haematobium* x *S. bovis* hybrids in children and the results are discussed in depth in **Chapter 3.** Here, I would like to present key findings from the characterization that used molecular markers representative of the mitochondrial (Cox1: cytochrome oxidase subunit I) and nuclear (ITS2: transcribed internal spacer 2) genomes of the parasite. Most of the hybrid profiles found were *S. bovis* cox1 x *S. haematobium* ITS2 (i.e. homozygous ITS2). Hybrids occurred at similar frequencies in all age classes of the children included. The cox1 haplotype analysis showed that *S. bovis* was more polymorphic than *S. haematobium*. Some miracidia gave double chromatogram peaks at the polymorphic positions between *S. haematobium* and *S. bovis* **(Figure 6.1)**, previously described (Huyse et al., 2009; Webster et al., 2013b), indicating hybridization between the two species.

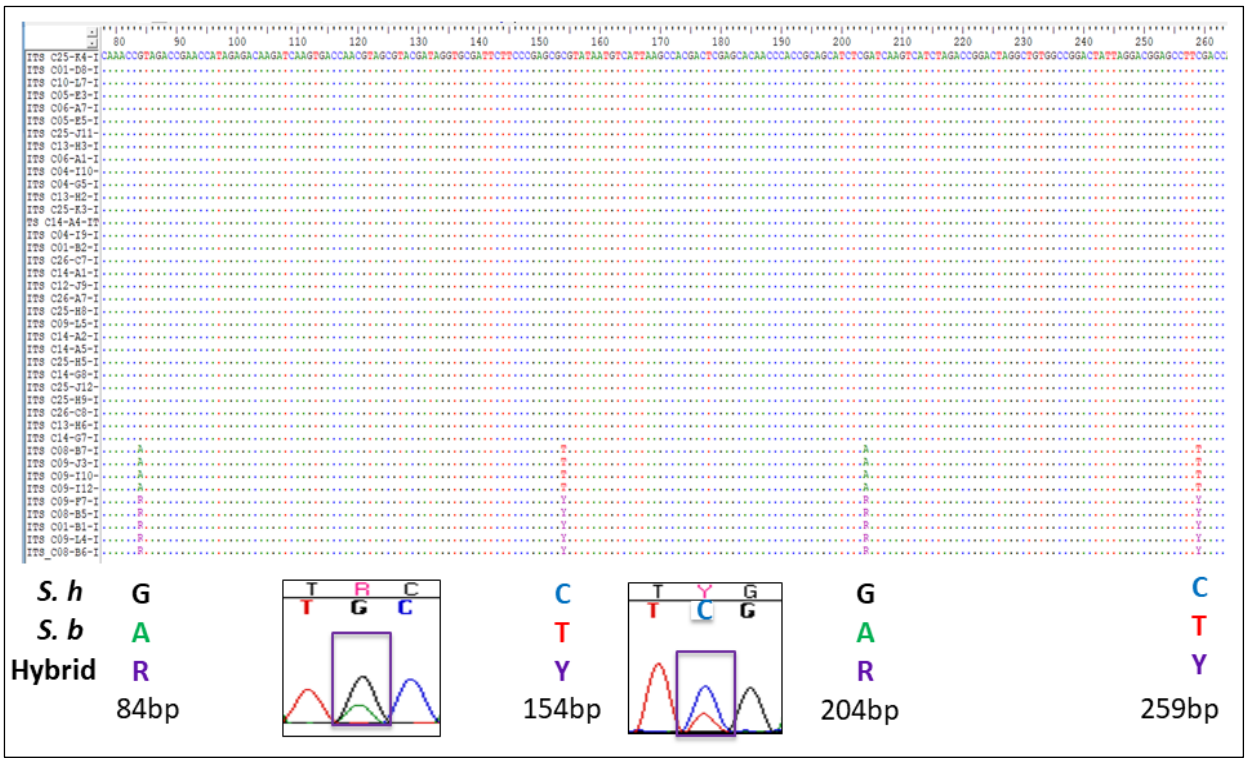



**Figure 6.1: *Schistosoma haematobium* (*S. h*) ITS and *S. bovis* (*S. b*) ITS sequences alignment; double chromatogram peaks at the polymorphic positions indicate hybrids between the two species.**

For *S. haematobium* x *S. bovis* hybrids, there is heterogeneity in the distribution of haplotypes across the country, with haplotypes from Duekoué in the western part differentiated from those from the southern part of Côte d'Ivoire. These hybrids are well documented in West Africa, particularly in Benin, Mali, Niger, and Senegal (Huyse et al., 2013; Leger and Webster, 2017). As expected, *S. haematobium* haplotypes from our study cluster with "group 1", as already defined (Webster et al., 2012).

The most common method to characterize individuals as "pure" or "hybrid" parasites is to amplify both the mitochondrial (haploid) cox1 gene and the nuclear (diploid) ITS2 region and check if the cox1 haplotype and the ITS2 alleles are all from the same species **(Table 6.2)**. However, this method does not detect all hybrids, because recombination and successive breeding events between hybrids and pure individuals can lead to the partial congruence between these two genomes. Therefore, whole-genome sequencing of adult parasites is employed to characterize the genomic composition of hybrids (Kincaid-Smith et al., 2019). This hybridization phenomenous between human and animal schistosomes is related to livestock breeding. However, Côte d'Ivoire had no pastoral tradition before independence and since 1974, livestock exporting turned to a priority sector. Therefore, cattle was exported from

the coastal countries (Mali, Burkina Faso, and Niger) and breeds, well adapted to local tropical climate, are also resistant to many parasites and are trypanotolerant. Farming area including our sampling sites heavily infested by tsetse flies leaded to significant decline in livestock productivity, especially for farmers with small units (Sokouri et al., 2007).

**Table 6.2:** Designation of parasites as "pure" or "hybrid" based on the six possible cox1-ITS profiles at one mitochondrial and one nuclear marker, as used in this thesis.

| | | **Nuclear marker (ITS)** | | |
|---|---|---|---|---|
| | | **Species 1** | **Species 1 and 2** | **Species 2** |
| **Mitochondrial marker (Cox1)** | **Species 1** | Pure parasite | Hybrid parasite | Hybrid parasite |
| | **Species 2** | Hybrid parasite | Hybrid parasite | Pure parasite |

## 6.3. Genetic diversity and differentiation of *Schistosoma haematobium*

Currently, the most widely used genetic markers for schistosome population genetics are microsatellite markers. They have high information content, since each locus can have great polymorphism in a population (Sachidanandam et al., 2001). In **Chapter 4**, we highlighted the most important findings on methods used for *S. haematobium* population genetics analysis. We reported that few methods are available for the genetic structure and diversity of *S. haematobium*. Variable genetic diversity and population structure were observed within and across countries. We presented the evidence of using microsatellite genotyping including use within the schistosomiasis research community to enable population-level analysis (Glenn et al., 2013). Single nucleotide polymorphisms (SNPs) designed to identify genes associated with traits and diseases produce finer even resolution than microsatellite markers (Sachidanandam et al., 2001). Single nucleotide polymorphisms have also been used to differentiate various levels of hybridisation between schistosome species (Webster et al., 2013b; Boissier et al., 2016) but have not yet been applied foo population genetic analyses. For the being time, microsatellite genotyping remains the principal method for population genetics due to its high information content, low cost, high throughput and sufficient resolution for population structure. I used two panels of microsatellites with 18 loci **(Figure 6.2)**.

| Panel | Locus | Dye | Size (bp) |
|---|---|---|---|
| Panel 1 | C102 | VIC | 184-199 |
| | Sh1 | VIC | 245-284 |
| | Sh14 | NED | 184-240 |
| | C131 | NED | 253-265 |
| | Sh6 | NED | 309-321 |
| | Sh9 | 6-FAM | 197-227 |
| | Sh3 | 6-FAM | 270-366 |
| | C111 | PET | 201-225 |
| | Sh7 | PET | 293-311 |

| Panel | Locus | Dye | Size (bp) |
|---|---|---|---|
| Panel 2 | Sh2 | NED | 155-218 |
| | Sh5 | NED | 263-314 |
| | Sh13 | 6-FAM | 163-211 |
| | Sh4 | 6-FAM | 268-313 |
| | Sh10 | PET | 183-207 |
| | Sh12 | PET | 245-278 |
| | Sh11 | VIC | 183-231 |
| | Sh8 | PET | 282-321 |
| | Sh15 | VIC | 274-301 |

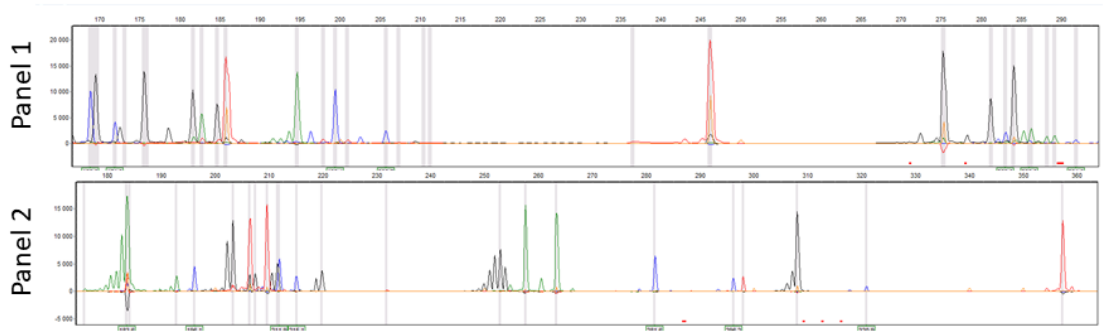



**Figure 6.2: Microsatellite set for parasite genotyping and fluorescent dyes. The lower panels show typical electrophoregrams for each of the two microsatellite panels.**

Our findings in **Chapter 5** revealed genetic structuring of parasites between populations. The geographical distance separating Duekoué in the western part of Côte d'Ivoire and the three other sampling sites (about 400 km) can lead to limited contacts between populations and parasite distribution across sampling sites. The lack of association between genetic differentiation and distance could lead to isolation by distance, since the areas are located in geographically distant sites, and local adaptations or variations in environmental conditions. Another explanation may be that the number of sampling sites was too small to observe a significant statistical link between genetic and geographic distances (Aemero et al., 2015). More intensive sampling sites could help to test for a pattern of isolation by distance across southern and western parts of the country.

We found no genetic structuring separating pure *S. haematobium* from *S. haematobium* x *S. bovis* hybrids. This suggests that within infected children, there are no genetic mating restrictions upon the population and therefore all recombination are possible (Boon et al., 2019). From the epidemiological perspective, hybrids may have the capacity to take over the geographical range of pure species through genetic extinction (Tchuenté et al., 1997). "Pure" *S. bovis* (Sb_SbSb) identified in the human body suggests an animal reservoir leading to possible zoonotic transmission of this parasite. Mitochondrial cox1 and nuclear ITS2 markers were employed to identify hybrid profiles, thus the nuclear one can be affected and cause a silent genetic signal from one of the parental species (Webster et al., 2013a; Boissier et al., 2016). These "pure" *S. bovis* were clearly differentiated from the other parasite genotypes. It could be interpreted by the fact that these parasites come from ruminants and have not been introgressed by either pure *S. haematobium* or by *S. bovis* x *S. haematobium* hybrids. To our knowledge, no studies on human schistosomes have yet shown these results. Our findings strongly advocate for the use of single nucleotide polymorphisms (SNPs) analysis to elucidate this possible zoonotic transmission in more depth.

Moreover, we noticed during analysis a strong genetic clustering of miracidia within many children. However, the clusters were not uniformly distributed among children and some children carried miracidia belonging to many genetic clusters **(Figure 6.3)**. This means that there is another level of finer structuring observed in infrapopulation with a weak gene flow between children. This pattern could arise through various mechanisms, which I cannot distinguish with my data. It could mean that many children only carry one couple of parasites excreting egg or that only one couple was excreting at the moment of sampling. It could be related to effects of host immune system in the selection of schistosome genetic diversity due to the protective immunity during infection (Beltran et al., 2011). Another explanation could be the fact of a recent treatment of children that exposes the body to more antigens from the parasite population. Therefore, during a new infection, new parasites genetically different from the first ones are selected by the immune system. This pattern fit with field observations after treatment using praziquantel (Norton et al., 2010). Whatever, the reason for its existence, this clustering of miracidia at children level could be problematic for the common molecular methods used because it violates the common assumption of independence of multiple miracidia sampled from the same patient. Together with the thesis advisors Jérôme Boissier and Oliver Balmer, it was decided to investigate this clustering and it effects in detail and include this analysis in the population genetic manuscript when it is submitted for publication. However, it is not included in the thesis document.

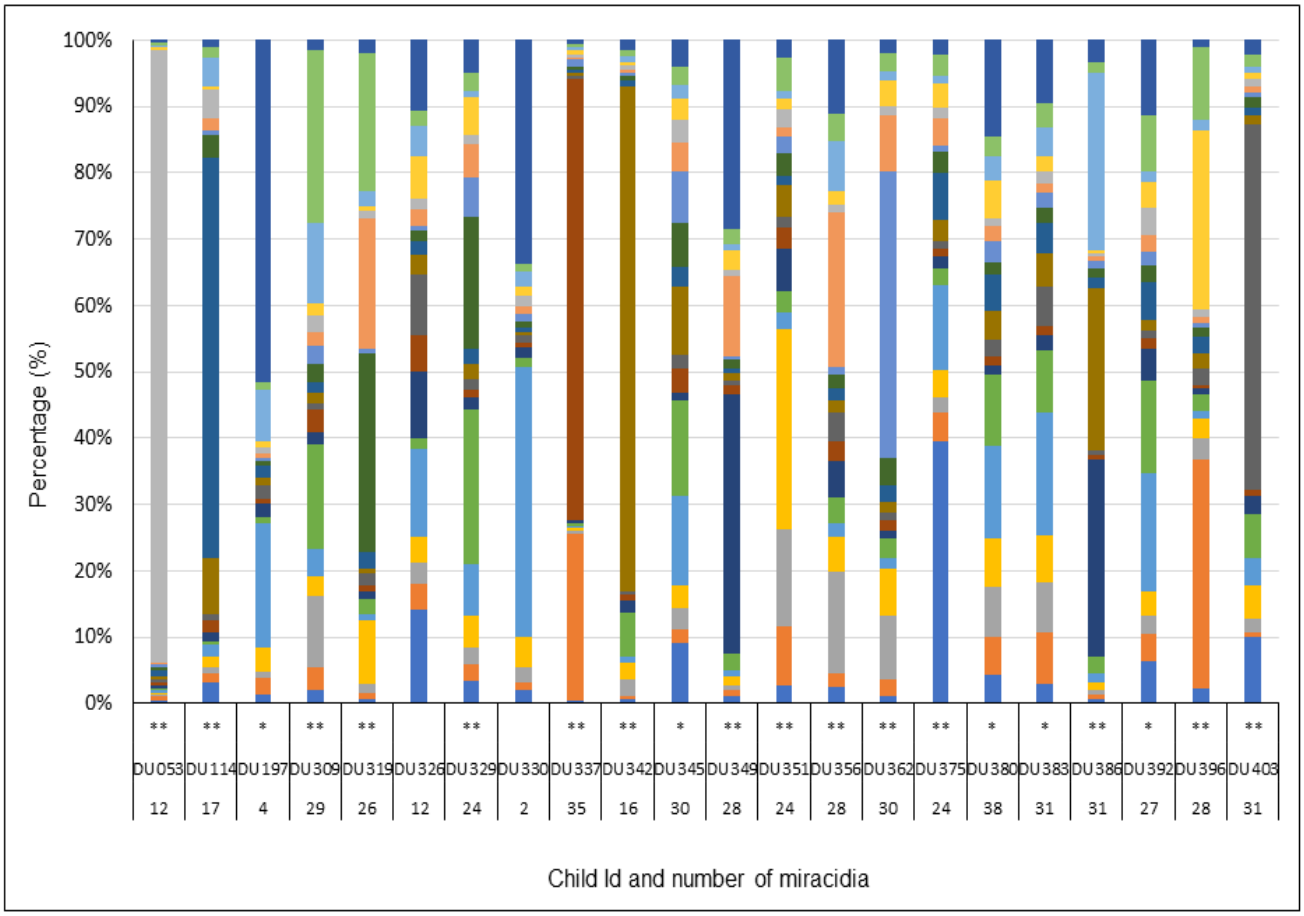



**Figure 6.3: *Schistosoma haematobium* genetic clustering within children in the example of Duekoué. Each bar corresponds to a child and each color to the percentage assignment of all miracidia of that child to the 19 predefined genetic clusters (K = 19). Children with one predominant color (e.g. DU053, DU114 and DU337) excrete miracidia, with close to identical genetic profile, i.e. very limited genetic variability. Stars indicate significant deviation from random expectation in the distribution of genetic cluster. Number below the child ID indicate the number of miracidia per sampled per genotype.**

We did not collect snails to observe cercariae shedding. However, human populations in each sampling site live in close contact with their livestock which could lead to the risk of infection with animal schistosome species, creating the opportunity for interspecies interactions and the occurrence of first-generation hybrids (Webster et al., 2013b; Catalano et al., 2018).

# Chapter 7:

# Conclusion and Outlook

The present work emphasized that schistosomiasis remains prevalent in the southern and western parts of Côte d'Ivoire, although the overall prevalence in school-aged children was much lower than reported a few years earlier. This is likely due to better sanitation and behaviour changes of the population, with the goal of reducing the frequency of contact of children with open freshwater bodies, and the preventive chemotherapy campaigns in the whole country. *Schistosoma haematobium* x *S. bovis* hybrids are common in *Schistosoma* egg-positive children in Côte d'Ivoire. Changes in life-history traits of these hybrids can lead to a high prevalence of the disease in areas where hybridization occurs. It could also be related to the high ability of these parasites to infect intermediate hosts of both parental species.

"Pure" *S. bovis* supposed to infect bovines was found in children. The methods used for hybrid identification, based on the analysis of two main markers such as mitochondrial (cox1) and nuclear (ITS), do not allow determining the degree of introgression and can underestimate hybrid frequency. An alternative technique would be to use a greater number of discriminating markers between species, including single nucleotide polymorphism (SNP) markers representative of many genomic regions. However, these markers are not yet available. The presence in many areas of intermediate hosts involved in the transmission of the parasite could affect control strategies. Therefore, one of the priorities remains to determine the sensitivity of hybrids to schistosomiasis treatment.

Lastly, this study reported a genetic diversity and a population structuring of parasites at population level. Genetic clustering of parasites was also observed at infrapopulation level and could lead to putative resistant parasites. These results could serve as a baseline to enhance schistosomiasis control and surveillance to measure the effect of treatment on parasite population genetics in Côte d'Ivoire. More studies are needed on population genetics in human and animal schistosomes to evaluate the parasite's gene flow across the whole country.

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# Curriculum Vitae

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## PERSONAL INFORMATIONS

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| Date of birth | 14.08.1982 |
| Place of birth | Yézimala, Côte d'Ivoire |
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## OCCUPATION

**Assistant Professor**, Department of Parasitology-Mycology, Zoology, School of Pharmacy, Felix Houphouët-Boigny University (Abidjan, Côte d'Ivoire)

**Pharmacist Biologist**, Head of Parasitology Mycology Unit, Service of Medical Biology, University Hospital of Angré, Abidjan, Côte d'Ivoire

## DIPLOMAS

**Sept. 2017- May 2020** — **PhD in Epidemiology**, Swiss Tropical and Public Health Institute, and University of Basel, Switzerland
Thesis tile: **Population genetic structure and hybridization of schistosomes in schoolchildren in Côte d'Ivoire**
Supervision: Pr. Jûrg Utzinger/Dr. Oliver Balmer and Pr. Jérôme Boissier

**2013-2014** — **Master in Functional and Molecular Biology**, Felix Houphouët-Boigny University, Abidjan, Côte d'Ivoire
Thesis title: **Mise en place d'une technique d'extraction de l'ADN génomique de *Schistosoma haematobium* à partir des urines prélevées chez des sujets infectés**
Supervision : Pr. Joseph Djaman

**2014** — **Certificate of Special Studies (CES) in Immunology**, Felix Houphouët-Boigny University, Abidjan, Côte d'Ivoire

**2013** — **Certificate of Special Studies (CES) in Medical and Technical Parasitology**, Felix Houphouët-Boigny University, Abidjan, Côte d'Ivoire
**Certificate of Special Studies (CES) in Hematology-Biology**, Felix Houphouët-Boigny University, Abidjan, Côte d'Ivoire

**2012** — **Master in Public Health**, University of Cocody, Abidjan, Côte d'Ivoire

**2007-2012** — **Hospital Internship**, Pasteur Institute of Côte d'Ivoire and National and Public Health Institute of Adjamé, Abidjan, Côte d'Ivoire

**2001-2008** — **Pharmacy Doctor Degree**, University of Cocody, Abidjan, Côte d'Ivoire

**2000** — **High School Leaving Certificate**, Mathematic and Physical Sciences, Côte d'Ivoire

## ADDITIONNAL TRAINING

| | |
|---|---|
| **Mar 2020** | **Advanced technical presentation techniques**, University of Lausanne, Switzerland |
| **Feb 2020** | **Learning Package**, Centre Suisse de Recherches Scientifiques, Abidjan, Côte d'Ivoire |
| **Oct 2018** | **Hands on Data Analysis for genetic profiling of schistosomes**, Interaction Host-pathogen and Environment (IHPE) laboratory, Perpignan, France |
| **2015** | **Bioinformatics Training**: Phylogenetic Analysis of Sequence Data, Abidjan |
| **2012** | **Workshop** in molecular biology, Pasteur Institute of Côte d'Ivoire |

## TEACHING EXPERIENCE

| | |
|---|---|
| **Jan -Feb 2020** | **Instructor, Master of Human and Tropical Biology**, Faculty of Pharmacy, Felix Houphouët-Boigny University, Abidjan, Côte d'Ivoire<br>**Instructor, Practical work of "Diplôme d'Etude Spéciale de Biologie (D.E.S Bio), Option : Parasitologie- Mycologie",** Felix Houphouët-Boigny University, Abidjan, Côte d'Ivoire |
| **2013- 2020** | **Teaching Assistant in Parasitology and Mycology**, Faculty of Pharmacy, Felix Houphouët-Boigny University, Abidjan, Côte d'Ivoire |
| **2012** | **Instructor of health workers in health districts** on the use of rapid diagnostic tests for malaria in Aboisso, Côte d'Ivoire |

## RESEARCH EXPERIENCE

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| **Sept 2017- May 2020** | **PhD Thesis, Swiss Tropical and Public Health Institute**<br>Population genetic structure and hybridization of schistosomes in schoolchildren in Côte d'Ivoire |
| **Oct 2019** | **Lab help, Human and Animal Health Unit, Swiss Tropical and Public Health Institute**<br>Molecular characterization of schistosomes |
| **2015** | **Co-investigator of clinical trial**, Efficacy and tolerance of the combination artesunate- amodiaquine versus artemether- lumefantrine in the treatment of uncomplicated malaria in Abidjan (Côte d'Ivoire). |

## PUBLICATIONS

**Etienne K. Angora**, Allienne Jean-François, Olivier, Hervé Menan, André O. Touré, Jean T. Coulibaly, Giovanna Raso, William Yavo, Eliézer K. N'Goran, Jürg Utzinger, Oliver Balmer, Jérôme Boissier. High prevalence of *Schistosoma haematobium* x *Schistosoma bovis* hybrids in schoolchildren in Côte d'Ivoire. Parasitology 2020, 147: 287–294, doi: 10.1017/S0031182019001549

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Konaté A, Kiki-Barro PCM, Kassi KF, Djohan V, **Angora KE**, Bosson-Vanga H, Doukouré CY, Yavo W and Menan EIH. Assessment of diagnostic performances of parasitological stool examinations performed in public sector medical laboratories of Abidjan (Côte d'Ivoire). *African Journal of Parasitology Research* 2017, 4 (6): 227-233

**Angora KE**, Offianan AT, Yavo W, Djohan V, Vanga-Bosson AH, Ira-Bonouman A, Konaté A, Kassi KF, Dou GS, Kiki-Barro PC and Menan EIH. Seroepidemiology and serodiagnosis of schistosomiasis at Pasteur Institute of Côte d'Ivoire from 2006 to 2014: A retrospective case study. *International Journal of Parasitology Research* 2015, 7(2) : 164-167. ISSN: 0975-3702&E-ISSN: 0975-9182. Article Id : BIA0002631. Impact factor: 4.242

MK Soumahoro, AH Bosson-Vanga, KJ Coulibaly, S Sidibé, **E Angora**, K Kouadio, A Kakou-N'Douba, D Sissoko, M Dosso. Investigation d'un foyer épidémique de bilharziose urinaire dans l'école primaire du village de Guébo 2, Abidjan, Côte d'Ivoire. *Bull. Soc. Pathol. Exot.* 2014 107:185-187. ISSN 2343-6549.

## CONFERENCES: PRESENTATIONS AND POSTERS

**Population genetic structure and hybridization of schistosomes in Côte d'Ivoire.**
Oral presentation at Swiss Tropical and Public Health Institute, Student meeting, 20 May 2019, Basel, Switzerland

**Population genetic structure and hybridization of schistosomes among schoolchildren in Côte d'Ivoire.**
Oral presentation at Interaction Host-pathogen and Environment (IHPE) laboratory, 13 November 2019, Perpignan, France

**Prevalence and associated factors of schistosomiasis among schoolchildren in western and southern Côte d'Ivoire.**
Oral presentation at 9th Congress of African Society of Parasitology, 5 to 7 December 2018, Cotonou, Benin.

**Investigation of hybridization between *Schistosoma haematobium* and *Schistosoma bovis* among schoolchildren in Côte d'Ivoire.**
Oral presentation at 9th Congress of African Society of Parasitology, 5 to 7 December 2018, Cotonou, Benin.

**Séro-épidémiologie et sérodiagnostic de la schistosomiase au laboratoire de parasitologie de l'Institut Pasteur de Côte d'Ivoire de 2006 à 2014.**
Oral presentation at 8th Congress of African Society of Parasitology, 5 to 7 December 2016, Bamako, Mali.

**Recherche des hémoparasites chez les donneurs de sang au Centre National de Transfusion Sanguine (CNTS) d'Abidjan, Côte d'Ivoire.**
Oral presentation at 8th Congress of African Society of Parasitology, 5 to 7 December 2016, Bamako, Mali.

**Connaissances, attitudes et pratiques des prescripteurs sur la prise en charge du paludisme à l'Hôpital Général de Toumodi, Côte d'Ivoire.**
Oral presentation at 8th Congress of African Society of Parasitology, 5 to 7 December 2016, Bamako, Mali.

**Helminthiases intestinales au laboratoire de parasitologie de l'Institut Pasteur de Côte d'Ivoire : étude rétrospective de 2011 à 2015.**
Poster presentation at 8th Congress of African Society of Parasitology, 5 to 7 December 2016, Bamako, Mali.

**Profil de sensibilité aux antifongiques des souches de *Candida* isolées dans les vulvo-vaginites récidivantes à Abidjan, Côte d'Ivoire.**
Oral presentation at 2nd Congress of Ivoirian Society of Parasitology and Mycology, 9 to 10 March 2016, Abidjan, Côte d'Ivoire.

## MEMBERSHIP

Ivorian Society of Parasitology and Mycology
Ivorian Society of Venimology
African Society of Parasitology
American Society of Parasitologists

## AWARDS

**Prize for Young Researcher in Parasitology, PASRES**, May 2017
Organized by the Ivorian Society of Parasitology and Mycology, Abidjan, Côte d'Ivoire

**Best Poster Award, December 2016**
Poster Title: **Helminthiases intestinales au laboratoire de parasitologie de l'Institut Pasteur de Côte d'Ivoire : étude rétrospective de 2011 à 2015.**
During the Congress of the African Society of Parasitology (SOAP), Bamako, Mali

## REFERENCES

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