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Population genetic structure and hybridization of urogenital schistosomiasis among primary school-age pupils in Nigeria

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THÈSE

Pour obtenir le grade de
Docteur

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UNIVERSITE DE PERPIGNAN VIA DOMITIA

Préparée au sein de l'école doctorale
Énergie Environnement ED305

Et de l'unité de recherche
**UMR5244 - Interactions Hôtes-Pathogènes-Environnements
(IHPE)**

Spécialité : **Biologie**

Présentée par
Amos ONYEKWERE Mathias

**Population genetic structure and hybridization of
urogenital schistosomiasis among primary school-age
pupils in Nigeria.**

Soutenue le 21 Avril 2022 devant le jury composé de

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CHAPTER I: INTRODUCTION

A. *Schistosomiasis, a Neglected Tropical Disease*

1. Epidemiology of schistosomiasis.

Schistosomiasis is a waterborne parasitic disease caused by digenean trematodes of the genus *Schistosoma* which causes serious public health and veterinary concern. The disease is considered as a neglected tropical disease (NTD) and affects more than 250 million people worldwide (fig 1) in about 76 countries (GBD, 2017), with the areas of high endemicity being the sub-Saharan Africa (SSA) and some parts of Asia (Edungbola, et al., 1998). With global burden of about 1.4 million Disability-Adjusted Life Years (DALYs) in 2017, the disease is ranked second after malaria based on morbidity (Hotez, et al., 2012; Hotez, et al., 2014). Typically, the disease occurs in school-age children between the age of 5-15 years' old which constitute the highest risk group for the infection with highest intensities in endemic areas (WHO, 2018b). The distribution of this disease is focal and being a major poverty-related disease among the rural populace, infection with this disease is mainly by contact with water infested cercariae. People are infected during daily activities like fetching water for domestic use, bathing, recreation, washing and fishing. Also, people may be infected when they wade through the streams while going to and from school, farm or bath in them early in the morning before the day's activities (Nwosu, et al., 2006). This parasitic infection shows wide definitive hosts spectrum that ranges from humans to domestic and wild animals. The genus *Schistosoma* has 23 recognized species with no fewer than 19 species that infect livestock and wild animals. Although only 5 species manifest infection severity in these domestic animals while about 6 species infect humans (Leger, & Webster, 2016). The human schistosomes are mainly; *Schistosoma haematobium* (the causative agent of urogenital schistosomiasis), *S. mansoni* and *S. japonicum* (the causative agents of intestinal schistosomiasis). *S. haematobium* and *S. mansoni* occur in Africa and the Middle East, while only *S. mansoni* that is found in the Americas. *Schistosoma japonicum* is present in the Asian countries like in the Philippines, Indonesia and China. The disease has since been eradicated in Japan from where it originated (Tanaka, & Tsuji, 1997). There are three locally distributed Schistosome species that cause human schistosomiasis. These are *Schistosoma mekongi*, *S. guineensis* and *S. intercalatum*. *S. mekongi* occur along the

Mekong River basin in Laos and Cambodia while the two other *species*; *S. intercalatum* and *S. guineensis* are found in some rainforest of west and central Africa, (Colley et al., 2014). Humans could be infected with one or more of the six human infecting species; five of which are intestinal parasite while one resides at the urogenital region, and this may lead to combined disease symptoms and co-morbidities. Except *S. guineensis*, all schistosome species are known to infect domestic or wild mammalian animal hosts (Pitchford, 1977; Panzner, & Boissier, 2021).

The *schistosoma* species that infect definitive mammalian animal hosts are *S. bovis*, *S. intercalatum*, *S. matthei*, *S. rodhaini*, *S. nasale* and *S. japonicum*, out of which the most important schistosomiasis causing agents in ruminant animals are *S. bovis* and *S. matthei*, (Zamdayu et al., 2019). The disease is less recognized in the veterinary sector including livestock and wild animals (Leger, & Webster, 2017). In Africa, three species of schistosomes are concerned with livestock infection: *S. bovis* and *S. matthei* infect members of the orders of Cetartiodactyla (mainly Bovidae), Rodentia, Primates and Perissodactyla and *S. curassoni* infects members of the family of Bovidae (Panzner, & Boissier, 2021). There is little or no documentation available on the prevalence, intensity, repartition, transmission dynamics and phylogeography of these livestock schistosomes (Mone, et al., 1999; Djuikwo-Teukeng et al., 2019). In addition to their veterinarian importance the animal infecting schistosomes have recently received peculiar interest due to their potential zoonotic importance through hybridization with human infecting parasites. Schistosomiasis is characterized by poverty, lack of access to good water and influenced by environmental factors like development of dams and the migration of people, (Otineme, et al., 2014). Humans and animals move from one place to the other. These movements, in combination with the global climate change bring about the transport of disease agents from their places of origin to new areas where such disease agents may not have existed, (Oleaga et al., 2019). Some infectious diseases infect humans as well as animals. The interbreeding between the human and the animal species of these parasites might result to hybrids. This heterospecific crosses can occur naturally and can lead to genetic diversity and hybrid vigor which will result to a greater threat to drug treatment and global control of the infection (Boon et al., 2019).

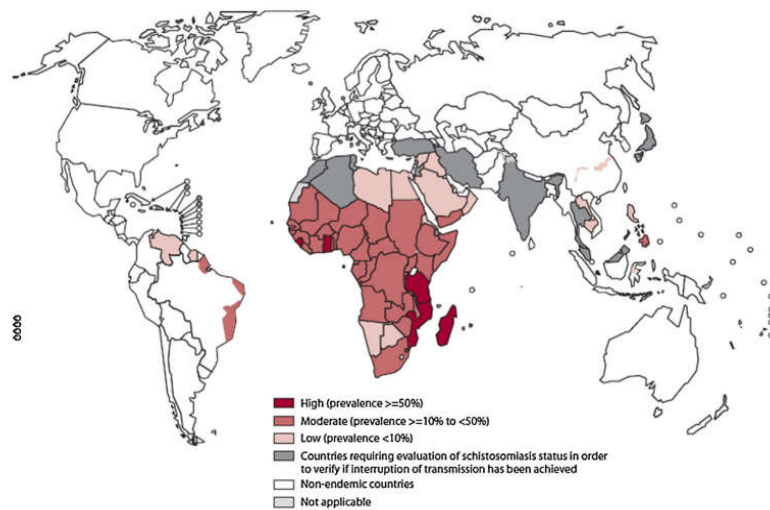


Fig 1. Global distribution of schistosomiasis showing the most at-risk populations within Africa. Highly endemic countries marked dark-red are proposed to be the focus of future integrated control efforts (Gryseels, et al., 2006; Ross, et al., 2016).

There is a specific association between larval schistosomes and their aquatic molluscan intermediate hosts suitable for its distribution (Table 1). This close-host relationship enhances a population of schistosomes to become better adapted to its local race of snail than it is to other races of the same snail species (Rollinson, & Southgate, 1985; Morand, et al., 1996). The intermediate hosts for *S. haematobium* are the *Bulinus* species freshwater snails while the *Biomphalaria* species freshwater snails are the intermediate hosts for *S. mansoni*. Amphibious freshwater *Oncomelania* species serves as the snail intermediate hosts for the dissemination of *S. japonicum*, (Colley et al., 2014).

Therefore, the distribution of schistosomes species is largely influenced by the distribution of their specific intermediate snail hosts, whose distribution in turn depends on environmental and ecological factors (Ratard, et al., 1990).

Table 1. Global distribution of human *Schistosoma* species and their molluscan intermediate hosts. Adapted from: (Manson, et al., 1987; WHO, 2018).

<i>Schistosoma</i> species	Site of infection	Snail intermediate host	Endemic area(s)
<i>S. mansoni</i>	Intestine	<i>Biomphalaria</i> species	Africa, middle east, the Caribbean, South America
<i>S. intercalatum</i>	Intestine	<i>Bulinus</i> species	Africa
<i>S. guineensis</i>	Intestine	<i>Bulinus forskalii</i>	West Africa
<i>S. japonicum</i>	Intestine	<i>Oncomelania</i> species	China, Indonesia, the Philippines
<i>S. mekongi</i>	Intestine	<i>Neotricula aperta</i>	Several districts of Cambodia and the Lao People’s Democratic Republic
<i>S. malayensis</i>	Intestine	<i>Robertsiella</i> species	Southeast Asia
<i>S. haematonium</i>	Bladder	<i>Bulinus</i> species	Africa, Middle east, Corsica (France)

2. Global distribution and prevalence of schistosomiasis

Globally, the disease can be found in about 76 countries with about 700 million people at risk of infection with almost 200 million people infected (Figure 1). It is estimated that about 300,000 die of the diseases annually (Dawet, et al., 2012; Dawaki, et al., 2016; Nwachukwu et al., 2018) while an estimated 10 million pregnant women and lactating mothers are infected. The SSA and Asia accounts for about 90% of cases of this disease worldwide (Hotez, & Kamath, 2009). Nigeria has the largest number of about 70 million cases of the infection globally (Hotez, et al., 2012). Other countries that have large number of cases of the disease after Nigeria are Ethiopia, DR Congo, and Kenya. The countries that have the highest prevalence of the disease are Angola and Gabon, (Herrick et al., 2017). Concerning *S. mansoni*, the most common of the schistosome species (Table 1), the parasite could be found in Angola, Gabon, Eritrea, Madagascar, Somalia, Mozambique,

Kenya, Benin, DR Congo, Nigeria, Tanzania, and Cote d'Ivoire. The infection is also present in other African countries like Egypt, Libya, and Sudan with the African continent accounting for about 90% of the parasite world-wide, (Ossai et al., 2014; Onile et al., 2016). Other countries where *S. mansoni* can be found include Arab Peninsula, Brazil, Venezuela, and some Caribbean Islands. *S. japonicum* is another intestinal schistosomiasis of health importance. This parasite is prevalent in the Asian countries like China, Indonesia and the Philippines where more than one million people are currently infected, and more than 50 million people are currently at risk of the infection. *S. japonicum* as the name implies, originated from Japan, but the infection has been eradicated from the area since 1977 (Tanaka, & Tsuji, 1997; Salem et al., 2016). *S. haematobium* causes urogenital schistosomiasis. The parasite has less geographical coverage compared to *S. mansoni*, yet endemic in about 53 Africa countries and also present in the Middle East where more than 100 million people are infected.

Most parts of the world have almost eliminated the disease, while there is an ongoing large-scale control measures in northern parts of Africa. In Egypt the prevalence of urogenital schistosomiasis has reduced and ranges from 0%-13.9% due to the implementation of control measures which includes mass drug administration (MDA) of praziquantel (PZQ), (El-Kady et al., 2020). Conversely, there is little or no significant control measures on the disease in the sub-Saharan Africa. Out of about 290 million people infected world-wide in 2013, only about 30% change has occurred since 1990. (Herrick et al., 2017).

3. Epidemiology of Schistosomiasis in Nigeria

Nigeria is one of the most impacted SSA country by both the urinary and intestinal schistosomiasis with about and accounts for almost 14% of the disease worldwide, (Hotez, et al., 2012; Herrick, et al., 2017). Nigeria also has the highest number of people that are in need of intervention for this disease, (Uchendu et al., 2017; Ajakaye et al., 2017). *Schistosoma haematobium* (Figure 2) and *S. mansoni* (Figure 3) has been reported to be endemic in Nigeria, but the latter is less prevalent with lower geographical coverage (Garba, et al., 2004). A parasitological survey carried out in 2015 in 19 states of Nigeria showed the proportion of urogenital to intestinal schistosomiasis to be 80%-20% (Global Network, 2015) respectively. *S. bovis*, an animal infecting parasite has also been detected in some parts of the country (Ndifon, et al., 1988; Zamdayu, et al., 2019). The overlapping distribution of these *Schistosoma* species could enhance the chance of co-infection and the

potential severe effect on morbidity. The first case of *Schistosoma* infection reported in Nigeria was in 1908, though the disease was discovered by Theodor Bilharz in 1851 (Bishop, 2017). The information about the epidemiology and prevalence of this disease in Nigeria is so scanty and maybe conflicting, (Emmanuel et al., 2017). No reliable systematic report has been given on the epidemiological status of this disease in many parts of Nigeria, (Houmsou et al., 2011). The Federal Ministry of Health (FMOH), Nigeria, for the first time on June 4th, 2015

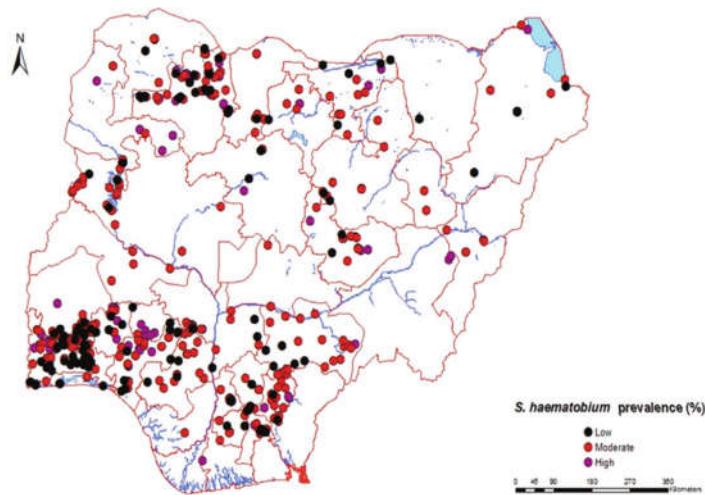


Fig 2. Prevalence of *S. haematobium*, adapted from the atlas of the global distribution of schistosomiasis in Nigeria, 1987.

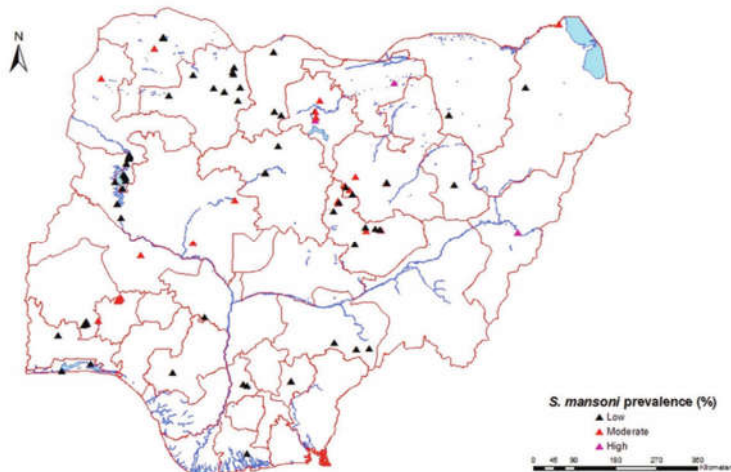


Fig 3. Prevalence of *S. mansoni*, adapted from the atlas of the global distribution of schistosomiasis in Nigeria, 1987.

officially released the epidemiological data on the prevalence of this disease in Nigeria (Global Network, 2015). The data showed that about 24 million of the population are at the risk of infection with a national prevalence of almost 9.5%, (Bishop, 2017). The control program is mainly by chemotherapy; giving PZQ to infected individuals or those at the risk of the infection, (Ejike et al., 2017). The control programs are initiated by the FMOH, individual State Ministry of Health, while Research Institutions, Non-Government Organizations (NGOs) and Faith Based Organizations (FBOs), have only had a very minimal contribution to the control or elimination of this disease (Ejike et al., 2017). This is because there are several hurdles that are encountered in the process, such as hard to reach areas, ethnic violence, religious crisis, insurgency as well as the marshy stream or river systems that is favorable for the multiplication of the molluscan intermediate hosts (Ejike, et al., 2017; Yauba et al., 2018). It is being suggested that integrating school-based health education with preventive chemotherapy will bring the prevalence rate of the disease to the barest minimum, by overcoming the hurdle of re-infection, resurgence in the prevalence rate after treatment and resistance to drugs from the parasites (Ajakaye, et al., 2017).

B. Life cycle of *Schistosoma* species (Figure 4)

The schistosome adult worms pair at the hepatic portal vein from where the male worm carry the female to the egg laying site (Armstrong, 1965). The adult male and female worms live in *copula* in the capillary plexuses of urinary bladder or mesenteric veins of humans (depending on the species) for a long time, with an average of 3-5 years, but may even live for as much as 30-40 years. The female worm is long and thin and is carried by the short, stout male, in the latter's gynaecophoric canal where the pair mate and the female produce fertilized eggs, (Colley et al., 2014). Some of the eggs laid enter the lumen of the blood vessels from where they transverse the various tissues of the host, while the remaining eggs find their way through the intestinal wall or bladder and are discharged into the surrounding environment through urine (*S. haematobium*), or feces (*S. mansoni*, *S. japonicum*, *S. mekongi*, *S. intercalatum* or *S. guineensis*) (Mawa, et al., 2021). Eggs hatch into motile miracidia upon contact with fresh water. The miracidia will find and penetrate a specific freshwater snail intermediate host suitable for the *Schistosoma* species within 24 hours. *Schistosoma haematobium*, *S. intercalatum* and *S. guineensis* infect *Bulinus* species while *S. mansoni*, *S. japonicum*, *S. mekongi* are compatible with *Biomphalaria* species,

Oncomelania species and *Neotricula aperta*, respectively (WHO, 2018; Molehin, 2020) (Table 1).

The larvae undergo series of asexual reproduction within the snail intermediate host which result to the development of sporocyst. The sporocysts multiply and grow into cercariae. When these freshwater snails are exposed to light, motile cercariae the etiological agent of this disease are released from the sporocysts to penetrate and infect suitable mammalian definitive hosts. When the cercaria penetrate the human or animal host, it sheds the tail and becomes a migrating larva

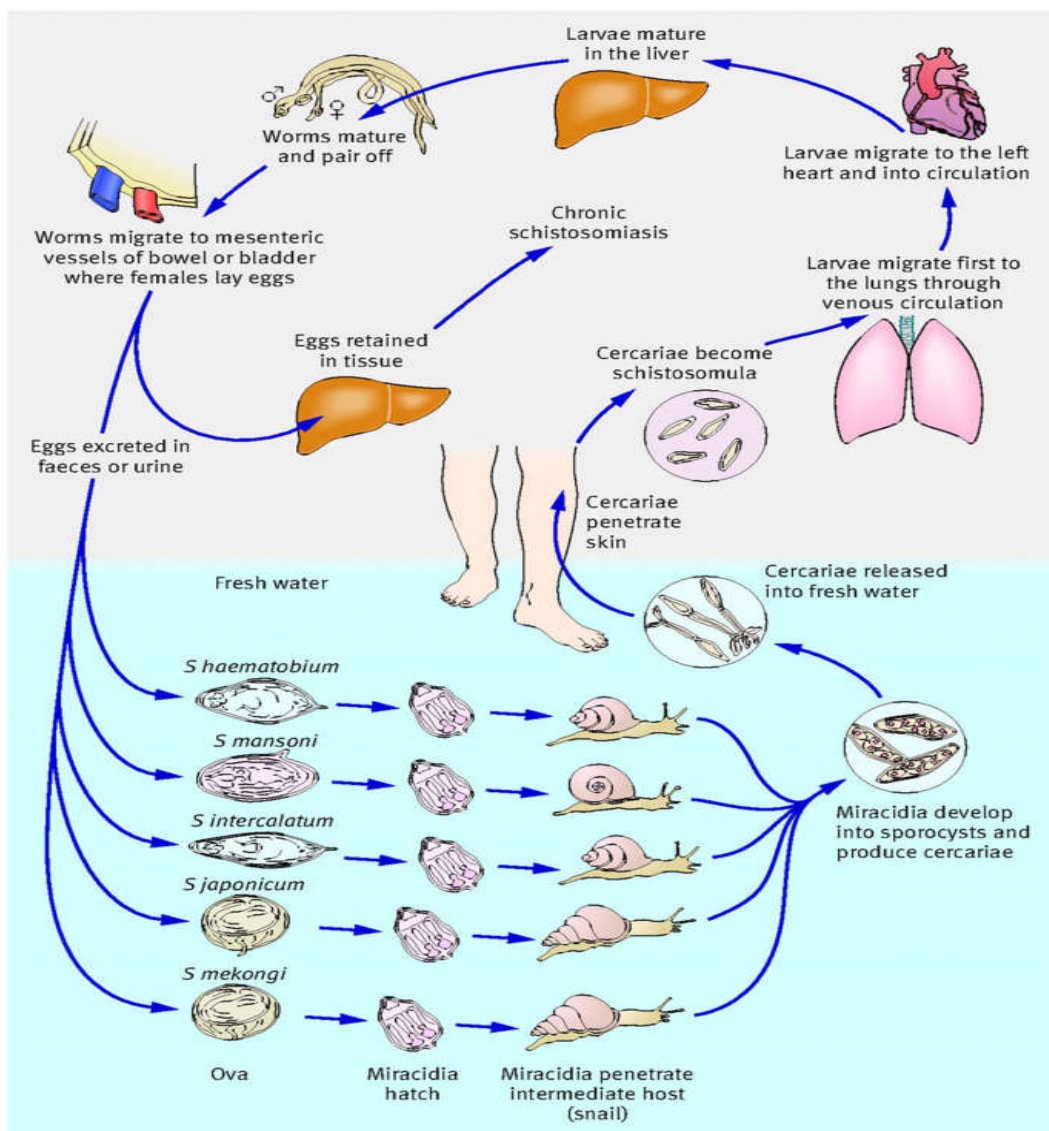


Fig 4. Life cycle of five human *Schistosoma* species adapted from Gray et al, 2011.

known as schistosomula and enters blood circulation, where it transverse the different organs of the host: lungs, heart, liver and the mesenteric vessels of bowel or bladder. The worms leave the lungs after many days and move to the veins of the portal system where they form male and female pairs upon maturity. Depending on the species, the worm's pairs move to either the vesical plexus and veins draining the ureters (*S. haematobium*), or the superior mesenteric veins (*S. mansoni*), or the inferior mesenteric and superior hemorrhoidal veins (*S. japonicum*), (Molehin, 2020). The parasite next mature to adults after 4-6 weeks of penetrating their definitive human or animal host and sexual reproduction begins on maturity and continues in the definitive human or animal host throughout the lifespan of the worm (Houmsou, et al., 2018). This disease is acquired when the unprotected skin of the host (human or animal) is penetrated by the free-swimming larvae called cercariae which is released by the different snail intermediate host, when the definitive host is in contact with freshwater infested cercariae. The release of arginine from the human skin attracts the cercariae to them which pierces the human skin with the aid of the lytic enzyme secreted from the postacetabular glands (Ajakaye et al., 2017).

C. *Symptoms of Schistosomiasis*

Both humans and animals display one or more forms of symptoms when infected with this disease. In many infections, anaemia, malnutrition and impaired childhood development are common symptoms in endemic areas and the severity of the disease depend on age and intensity of infection (Pearce, & MacDonald, 2002; Russell, et al., 2015). In addition, there may be loss of man hours in physical work output in endemic regions (Ndamba, et al., 1993). The symptoms associated with schistosomiasis in humans within 12 hours of infection is irritation at the point of the cercariae penetration. This is referred as papular rash or “swimmer’s itch” (James, et al., 2005). Katayama syndrome is an acute phase of the infection with symptoms like fever, severe itching (urticarial), eosinophilia, rash, headache, fatigue, coughing and abdominal pain. This may occur from several weeks to months with no specific presentation, resulting to misdiagnosis (Ross, et al., 2007). Most cases of morbidity are due to inflammation caused by eggs released from adult worms (Murray, et al., 2012). Adults continue to shed eggs in untreated cases with only few eggs release through urine or feces and majority of eggs being lodged in the tissues of liver, spleen lungs or brain (Gryseels, et al., 2006). Intestinal schistosomiasis may cause abdominal pain, bloody stool and diarrhea

(Ross, et al., 2002). Chronic form of anaemia, haematuria, frequent urination, burning micturition are not uncommon. *Schistosoma haematobium* can affect both the male and female reproductive systems, where it can cause infertility. Female genital schistosomiasis (FGS) can increase the risk of ectopic pregnancy or abortion in females (Kjetland, et al., 2012). The eggs of the parasite that are not removed by urination are embedded in the walls of the urinary bladder, these may cause fibrosis, glomerulonephritis and even cancer (Ibironke, et al., 2011; Colley, et al., 2014). Some of these eggs may move to the reproductive tract where they cause inflammation of both the lower genital tract (vulva, vagina) and the upper genital tract (cervix, uterus, fallopian tube, ovaries), (Herrick et al., 2017).

Male genital schistosomiasis (MGS) or testicular schistosomiasis is very rare, though *S. haematobium* and *S. mansoni* have been isolated from the testes, in seminal fluid. This could be because of the link between the veins of the internal spermatic cord and the mesenteric veins. Testicular schistosomiasis have been reported in the cases of a 13 and 16 years old patients mimicking testicular cancer (Badmus, 2012; Ekenze et al., 2015). The disease may be difficult to diagnose because it may not present any index for suspecting schistosomiasis. The presentations of testicular schistosomiasis may include testicular mass with no pain, haematuria, heamospermia and infertility.

Other symptoms reported in ultrasonography examination of the urinary tract of patients infected with urinary schistosomiasis include irregular bladder wall, thickening of bladder wall, mass formation on the bladder wall, bladder and kidney lesions (Ekwunife et al., 2009). The World Health Organization (WHO) through the International Agency for Research on Cancer (IARC) placed *S. haematobium* as group 1 carcinogen due to the granulomatous lesions caused by the eggs (Awosolu, et al., 2020).

D. *Diagnosis of schistosomiasis*

The first step in the diagnosis of *Schistosoma* infection is based on medical history and clinical symptoms. This is confirmed by microscopic examination which is the gold standard (Colley, et al., 2014) laboratory diagnosis by detection of viable eggs with terminal spine in urine (urogenital), lateral spine in stool (intestinal), or microscopic examination of tissue biopsy to identify schistosome eggs (Gray, et al., 2011). In the diagnosis of urogenital schistosomiasis, the use of

centrifugation to achieve sedimentation is commonly used when the filtration method is not available. However, the sensitivity of the former technique is low in areas of light intensity infection. Improved diagnosis is achieved with the use of Syringe filtration of urine and microscopic examination of polycarbonate filters to quantify the number of eggs in 10ml urine (Mott, et al., 1982). Microhaematuria reagent strips can also be performed on urine specimens for light intensity of urogenital schistosomiasis infection (Knopp, et al., 2018). Direct thick smear of formalin-ether concentration technique is use for diagnosis of *S. mansoni* eggs (Utzinger, et al., 2010). The modified Kato-Katz smear technique is of higher sensitivity and has been used to improve the detection and quantification of *S. mansoni* eggs and other helminths in stool (Katz, et al., 1972). Rectal biopsy can be used for all species of schistosomes to demonstrate eggs when stool or urine specimens are negative (Gray, et al., 2011). In addition, the FLOTAC and mini-FLOTAC are new sensitive techniques for diagnosis of schistosomiasis using the egg-floatation technique for multiple sampling settings and large quantification of stool specimens with detection limited to 10 eggs per gram of stool (Glinz, et al., 2010; Catalano, et al., 2019). This technique requires improvement and may be out of reach in resource-constrained areas due to the need for centrifugation (Cringoli, et al., 2017). The availability of reliable high-definition techniques that quantify the schistosome circulating cathodic antigens (CCA) and anodic antigens (CAA) in serum and urine has recently replaced these microscopy methods (Bergquist, 2013). Testing for CCA and CAA, both in urine and serum in Lao PDR and Cambodia in addition to stool examination, showed that detection of circulating schistosome antigens is on average about eight times more sensitive than egg detection (Peters, et al., 1976).

The quantification of *Schistosoma* species antigens in blood, urine, stool and sputum is possible with the extremely efficient testing technique called point-of-care circulating cathodic antigen assay: POC-CCA (Utzinger, et al., 2015). This technique is of higher sensitivity when compared to the Kato-Katz method (Coulibaly, et al., 2011) and has been used for mapping *S. mansoni* endemic areas (Colley, et al., 2013) and detection of *S. japonicum* and *S. mekongi* infections (Stothard, et al., 2006). Commonly used serologic test detect antibodies to adult worms. The test sensitivity and precision vary widely and may depend on both the type of antigen preparation used and test procedure (Gray, et al., 2011). However, serological tests like enzyme-linked immunosorbent assay (ELISA), indirect haemagglutination assay (IHA), Western Blot (WB) assay and immunofluorescent-antibody test (IFAT) (Kinkel, et al., 2012) can not be used in a population

in endemic regions. This is because antibodies can persist in circulation despite cure (Doenhoff, et al., 2004) and active infection can not be discriminated from previous exposure in patients who might have been repeatedly infected and treated in the past. With recent development, schistosome mitochondrial DNA gene (Cox1 mtDNA) and nuclear gene (rDNA ITS2) are detected with rapid diagnostic multiplex polymerase chain reaction (RD-PCR) and can be integrated with serological and parasitological techniques for accurate diagnosis (Guegan, et al., 2019). These molecular tools have been developed to detect *Schistosoma* DNA in clinical samples like stool, blood (Pontes, et al., 2002) and urine (Sandoval, et al., 2006) and confirm the existence of parasite DNA in the host. Mitochondrial genes have been used to differentiate parasite species in rapid diagnostic multiples PCR. However, this method is not commonly used in regions where the infection is endemic due to high cost of equipment and lack of trained personnel to run them (Utzing, et al., 2015). Loop-mediated isothermal amplification (LAMP) is being studied as an alternative due to low cost, relatively simple and rapid DNA detection but is not yet commercially available (Utzing, et al., 2015). The LAMP assay uses specific inner and outer primer sets which enhances its sensitivity and makes it highly specific to target sequence (Notomi, et al., 2000). This assay does not require thermocycler, electrophoresis gel and can be used in poor settings (Tomita, et al., 2008). Recombinase polymerase amplification (RPA) is a technique similar to LAMP. It uses lower temperatures of about 40°C to amplify DNA sequences using DNA polymerase and oligonucleotide primers. The technique is useful in poor-resource areas as it has now been integrated with chip and lateral flow devices and a convenient portable device as a point of care diagnostic tool (Piepenburg, et al., 2006). This device has been applied in the diagnosis of urogenital and intestinal schistosomiasis and its detection time and precision level are higher than microscopic examination and serological test (Rosser, et al., 2015).

E. *Control and treatment*

1. Global situation

The cornerstone of control to date has been mass drug administration (MDA) of PZQ, though there are problems with this approach. PZQ is the drug of choice recommended by WHO for all forms of schistosomiasis. The drug is effective, tolerant, safe and cheap, with little side effects like headache, abdominal pain and dizziness. Due to its excellent tolerability and effectivity, it can be

distributed annually or biennial by trained personnel or primary health workers during MDA campaigns (Utzinger, & Keiser, 2004). WHO's latest schistosomiasis fact sheets emphasize the need to attain the goal of regular treatment with PZQ of not less than 75% of children by 2020 (WHO, 2015), in addition to provision of essential medicines, water, sanitation and hygiene (WASH) (WHO, 2012). Also of paramount importance is the need for new complementary drugs, local guidance for snail control, surveillance of hotspots and a shift to highly sensitive diagnostics. This provides sufficient coverage to control morbidity and complications associated with the disease among the age group at risk, despite the possibility of reinfection after medication due to unavoidable contacts of humans to hotspots of transmission (WHO, 2006). The mechanism of action of PZQ is not yet known (Wu, et al., 2011) and for full efficacy, it requires an effective host antibody response (Doenhoff, et al., 2008). The drug is thought to act against adult worms by inducing contraction which results to paralysis of the parasite (Greenberg, 2005) but has little efficacy on immature larvae. In addition, the drug appears to interfere with adenosine and glutathione S-transferase receptors. This may have therapeutic relevance since schistosomes are unable to synthesize purines such as adenosine (Angelucci, et al., 2007). The effective treatment of *S. mansoni* and *S. haematobium* is single oral dose of 40mg/kg body weight of PZQ, unlike *S. japonicum* and *S. mekongi* that require higher dose of 60mg/kg (Montresor, et al., 2001). Concerning treatment of young children, there is no readily available pediatric formulation (Stothard, et al., 2013). Therefore to treat preschool children, the tablet is crushed in carriers such as orange juice. Oxamniquine has been used against *S. mansoni* but it is no longer readily available (Katz, et al., 1991). This drug mode of action is well documented. The drug is effective against *Schistosomes* invasive stages and adult worms. Metabolism of the drug is by oxidation and excretion is through urine. This prodrug binds the *S. mansoni*-specific sulfotransferase (*SmSULT-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-Mattocchia, et al., 2006). Molecular markers of oxamniquine resistance is use for monitoring distribution of resistant alleles in schistosome populations. A resistant mutation p. E142del was observed and could impact on disease control (Chevalier, et al., 2019). Artemisinin, an isolated compound of *Artemisia annua* L. Compositae is alternative group of drugs being considered (Araujo, et al., 1991). The artemisinin derivatives: artesunate and artemether are known antimalaria drugs but observed as possible antischistosomal drugs in the 1980's, with early studies focusing

on *S. japonicum* (Utzinger, et al., 2000). Artemisinin are more effective on young stages of the parasite than adults (Utzinger, et al., 2000; Obonyo, et al., 2010). In addition to preventive treatment, control measures like Integrated control, targeting the parasite life cycle, is the only approach that will lead to sustainability and future elimination. Habitat modification, snail predators and biological competitors of freshwater snail intermediate host should be integrated into the national and regional control programs. The use of chemical molluscicides against snails are considered at concentrations not toxic to humans and other aquatic organisms (Oliveira-Filho, & Paumgarten, 2000). Community enlightenment on behaviour change towards contact with freshwater infested cercariae and contamination of snail habitat with human excreta is essential to control this disease. Moreso, improving the living conditions of the community by environmental sanitation, health education, clean water supply and adequate disposal of human excreta is essential for elimination of the disease (Inobaya, et al., 2014). Although the methods deployed in schistosomiasis control programs have had considerable success in reducing the burden of infection, these methods have limitations. MDA which is the main strategy for control does not prevent reinfection. Therefore, the search for integrated control measures with a more lasting effect must continue. Human and bovine vaccines are in various stages of development. A few human schistosomiasis vaccine candidates have reached the stage of clinical trials (Bergquist, & McManus, 2016), while a transmission blocking vaccine has already yielded empirical results in buffaloes and cattle in People's Republic (PR) of China (Gray, et al., 2014). It is therefore likely that the use of transmission-blocking vaccine may soon be part of an integrated approach for the prevention, control and elimination of schistosomiasis in areas of zoonotic transmission. Sm 14 kDa is a fatty acid binding protein of *S. mansoni* (Bethony, et al., 2011) and is already in phase I clinical trials as a candidate vaccine (Beaumer, et al., 2013). Sm-TSP-2, a member of the tetraspanin family of proteins, is undergoing toxicology testing. Sh28GST (Bhilvax) is the vaccine candidate in the most advanced stage of development, it is already in phase II clinical trials (Beaumer, et al., 2013). However, approval of human schistosomiasis vaccine may take longer time and the costs of development may be exorbitant.

2. Progress in schistosomiasis control in Nigeria

A model has shown that annual 75% MDA coverage especially within the age group 5-15 years which constitute the highest risk group would reduce schistosomiasis burden significantly by 2020

(Anderson, et al., 2015). The Nigeria schistosomiasis MDA coverage index is currently at 74% (Oyeyemi, et al., 2020) which is a significant increase from 57% recorded in 2016. This may not be suitable for many endemic areas that require much higher benchmark coverage index. Even though the Nigerian government in recent time has scaled up efforts to combat the infection, funds allocated to this disease are not adequate (FMoH, 2013). Several international organizations (Bill and Melinda Gates, the Carter Centre), global partners (WHO/Merck KGaA, Germany) and NGOs (Izumi Foundation, Envision, Sight Savers, DFID and Children's Investment Fund Foundation (Oyeyemi, et al., 2020), have come to the rescue to eliminate the disease. The Carter Centre in partnership with WHO/Merck KGaA (Germany) received a donation of 1.5 million PZQ tablets in 2009 which greatly expanded the Centre's outreach. In the northeast region of Nigeria, the Federal Capital Territory, the CBM in partnership with the NGO Health and Development Support (HANDS) has successfully distributed MDA to almost 2 million people. In early 2020, Merck KGaA, Germany donated one billion PZQ tablets to WHO, of which Nigeria benefitted a delivery of almost 37 million tablets. However, the implementation of schistosomiasis and other NTDs management policy suffers significantly from bureaucratic setbacks in the process of policy making, under funding, inadequate motivation of workers, poor data collection and lack of systemic coordination of the program at national and regional levels (Omole, et al., 2015). A national assessment from ten states on the impact of PZQ received from Merck KGaA, Germany in 2016 showed an overall prevalence of 10.4% (Enabulele, et al., 2021) which is slightly above the national average of 9.5% (Nduka, et al., 2019). The report revealed high intensity of infections (≥ 50 eggs/10ml urine) in most participants from all infected population studied. Before this study in 2018, 93.3% of the communities studied except for the population from Kano, have at least received a single dose of treatment with PZQ since 2016. This report suggests that despite the ongoing MDA in Nigeria, transmission of urogenital schistosomiasis is still common with high intensity of infection and that the infected patients reported here, may have been reinfected after treatment (Enabulele, et al., 2021).

A survey study, an integral part of this Ph.D thesis, was carried out in order to provide adequate and new data on the disease based on wider geographical coverage in comparison with previous studies. Such information is critical to draw a current epidemiological status of the infection and the impact of control measures rolled out to combat the disease in the past years.

F. *Schistosoma* population genetics

Molecular epidemiological studies provide useful insights into population genetics of *Schistosoma* species in time and space. Population genetics is a molecular characterization using highly sensitive PCR-based assay developed to detect schistosome DNA, confirm the species and to genetically characterize schistosome populations (Wichmann, et al., 2009). Understanding the genetic structure of *S. haematobium* will lead to a better knowledge of the variation in natural populations and the transmission dynamics of schistosomes between hosts and across geographical origins. This is relevant for both epidemiological and evolutionary perspectives (King, et al., 2015). The methods used for determining genetic structure and diversity of *S. haematobium* are randomly amplified polymorphic DNA (RAPD), restriction fragment length polymorphism (RFLP) and microsatellite genotyping. We used microsatellite analysis to assess population genetic diversity and structuration of *S. haematobium* because it appears to be the most suitable tool. Two molecular markers are used in combination to identify and assign parasite species. First, the mitochondrial marker (cytochrome C oxidase subunit I mtDNA, Cox1 or COI1), which is haploid and is inherited from the mother. Second, the nuclear marker (ribosomal internal transcribed spacer II, rDNA, ITS2), which is diploid and bi-parentally inherited (Huyse, et al., 2009; Boissier, et al., 2016). Investigations into schistosome population genetic structure, diversity and evolution has been an area of important research. *Schistosoma* population genetic structure and diversity are important characteristics that may reflect variation and selection pressure caused by snail and mammalian hosts, environment, habitat change, movement, treatment and control programs. The sexual reproduction in these parasites ensures genetic mixing and the emergence of new combinations of genes in offspring. All these shape speciation and evolution and allows schistosome populations to potentially adapt quickly to new environments and challenges to ensure survival and overcome global efforts for control, elimination and eventual eradication. Host specificity will also affect population genetics and increase genetic diversity where multiple hosts are involved versus reduced diversity where parasites are more specific.

I have participated to a review on Population genetics of African *Schistosoma* species. This review synthesizes 135 relevant studies on the subject. **The full publication is presented in Annex 1. Below a detailed abstract of the paper.**

Rey, O., Webster, B.L., Huyse, T., Rollinson, D., Van den Broeck, F., Kincaid-Smith, J., **Onyekwere, A.**, Boissier, J., 2021. Population genetics of African *Schistosoma* species. *Infect. Genet. Evol.* 89, 104727.

1. Genetic structuration

For *Schistosoma* species, different levels of nested populations can be defined. First, all parasites from an individual host (infra-population), Second, all parasites from a community (transmission sites) and third, all the villages from a region and fourth, all regions of the area of repartition of a given species. The extent of gene flow between these populations will determine the degree of population structure at each level. The “local scale” is the first level which is made up of subpopulations between hosts (spatial populations) with a distance coverage of 100’s metres to 10’s of kilometers (km). Second, is the “regional scale” in which analysis of parasites subpopulations far from each other by 10’s-100’s km. The third level is the “phylogeography” which analyses parasites populations far apart (within or between countries) by more than 100’s of km and provides insight to the evolutionary history of the parasites.

a. *Local scale*

The first empirical study aimed at analyzing infra-population on genetic structuration of schistosome species was on *S. mansoni* adult worms recovered from naturally infected *Rattus rattus* on the island of Guadeloupe (Sire, et al., 2001). The study revealed that *S. mansoni* exhibited high genetic diversity and this diversity occurs on average more within than between hosts (Sire, et al., 2001). The first study that investigated *S. mansoni* from humans also used adult worms obtained from successive passage of miracidia excreted by Brazilian patients through experimental snail and rodent hosts (Thiele, et al., 2008). The study revealed that the genetic diversity and variation within the host accounted for the majority of the variance observed (Thiele, et al., 2008). The advent of microsatellite genotyping has advanced our knowledge of population genetics.

These methods collect, store and amplify multiple (>10) microsatellite markers from a single miracidium (Gower, et al., 2007). Microsatellite analysis of individually genotyped miracidia of *S. mansoni* from infected children in Kenya at a local scale between villages (<7 km) showed significant pairwise genetic estimates (F_{ST} values) between patients (0.44%-3.98%) and between schools (0.16%-3.92%) (Agola, et al., 2009). The genetic structure was more similar between patients than between sites. This showed high levels of gene flow between infrapopulations. Similar results were obtained from Uganda and Senegal from analysis of mitochondrial cytochrome c oxidase (Cox1) or nuclear microsatellite DNA regions (Betson, et al., 2013; Van den Broeck, et al., 2014). No genetic variation was observed between infra-populations in one village on the shore of Lake Albert, Uganda while a very weak differences (about 3.5% variation) were observed within another village on the shore of Lake Victoria, Uganda (Betson, et al., 2013). This difference can be due to the fact that people living in village associated with Lake Victoria might have acquired the infections from various micro-foci hosting different parasite infrapopulation (Betson, et al., 2013). In Senegal, the genetic structure did not show any spatial-temporal structure in *S. mansoni* from three communities located on the west shore of Lake Guiers (Van den Broeck, et al., 2014). Even though significant parasite genetic variation was found to be low between the three communities that are 4-22 km apart (F_{ST} , 0.003) and between infrapopulations within each community (F_{ST} , 0.004) (Van den Broeck, et al., 2014). This suggests that the three communities were highly connected. Few studies on population genetic structure on *S. haematobium* at a local scale revealed similar results observed for *S. mansoni*. Gower, et al., (2011) noticed low genetic variation between (F_{ST} , 0.004) two schools about 7 km apart and among (F_{ST} , 0.001) children within the school and this accounted for only 0.35% and 0.1% variations respectively, while the remaining variation was attributed to variation within each child. The genetic structure of *S. haematobium* from 10 villages north Senegal showed weak genetic structuration from 7 villages clustering within 45 km, while isolation by distance (IBD) was observed for two villages away from the cluster. Therefore, these studies relating to human host suggest few barriers to gene flow between populations exist at the local scale. Also, similar results were obtained in studies on *S. bovis* on cows. The study revealed that most variation is noticed within individual host (98.09% and 88.83%) for microsatellites and Cox1 markers respectively) rather than between hosts (Djuikwo-Teukeng et al., 2019).

b. *Regional scale*

At this scale, the population structure depends on migration of the definitive hosts between the different transmission sites. It is expected that little gene flow exists and genetic differentiation is determined by the effective population size rather than gene flow between sites. The restriction in gene flow is supported by the fact that the infection is mainly transmitted by children who do not move as much as adults. Furthermore, the snail-miracidium compatibility also limit the gene flow between different sites (Mitta, et al., 2017). Population structure of *S. mansoni* at regional scale analysis of worms obtained from laboratory mice exposed to cercariae from naturally infected snails (Agola, et al., 2006) showed maximum F_{ST} of 0.273 between two villages that are 175 km apart. In Uganda, the same sampling approach was used to genotype adult worms after passage through a laboratory hosts. This study also revealed strong genetic structure between Lakes Victoria and Albert (Stothard, et al., 2009). In Ethiopia, analysis of miracidia collected from infected patients showed both low and high F_{ST} values (Aemero, et al., 2015). In 2015 Van den Broeck and collaborators carried out parasites sampling for a period of 14 years in 7 villages located within 4-65 km range. Their findings revealed that most parasites belong to the same genetic population, with comparatively low genetic variation between most villages suggesting relatively high gene flow in the region (Van den Broeck, et al., 2015). Considering all the studies reviewed, the inter-population indices revealed that the populations do not follow a pattern of IBD, but instead show an island model of gene flow. This may be attributed to random and/or restricted gene flow between sites, sib-transmission or local adaptations.

c. *Phylogeography*

About 13 *Schistosoma* species have been identified in Africa, of which four (*S. mansoni*, *S. haematobium*, *S. intercalatum* and *S. guineensis*) infect humans (Webster, et al., 2006), with the others infecting mainly animals. The *S. mansoni* and *S. haematobium* are the two most common species with major impacts on human health and thus has received research attention. However, our knowledge on the distribution and evolutionary history of the sister species *S. intercalatum* and *S. guineensis* seems so limited (Tchuem Tchuente, et al., 2003; Webster, et al., 2006) with no respective current population genetic studies. Similarly, *Schistosoma* species that infect animals have received little research attention and their phylogeography is under documented. However,

some animal *Schistosoma* species, such as *S. bovis* have recently attracted research attention principally at the local scale due to its ability to hybridize with human associated species which can lead to zoonotic transmission (Leger, & Webster, 2017; Catalano, S., et al., 2018). Presently, our global vision on the distribution and phylogeography of most *Schistosoma* species at the continental level is still scanty. For now, *S. mansoni* is the most studied species at local, regional and global scales, with the parasite showing high level of genetic diversity at the mitochondrial and nuclear DNA, which at global scale is geographically well structured (Morgan, et al., 2003; Gower, et al., 2013; Webster, et al., 2013a; Webster, et al., 2013b; Van den Broeck, et al., 2015). Although *S. mansoni* shows compatibility in several *Biomphalaria* species and a wide spectrum of vertebrate definitive hosts; humans, rodents and non-human primates, but no genetic structure has been associated with host use (Lawton, et al., 2011; Webster, et al., 2013a; Webster, et al., 2013b). First, this lack of structure by host could be due to ongoing gene flow between parasites using different hosts. Second, populations of *S. mansoni* from East Africa are more diverse than populations from West Africa (Morgan, et al., 2003; Gower, et al., 2013; Webster, et al., 2013a; Webster, et al., 2013b), this pattern has led to several school of thoughts that the parasite originated from Eastern Africa from where it expanded to Western part (Morgan, et al., 2003; Lawton, et al., 2011; Webster, et al., 2013a; Webster, et al., 2013b). It further added that this evolutionary scenario may explain global geographical patterns of genetic structure and the gradual reduction of genetic diversity from Eastern to Western Africa, with only one exception from Coastal Kenya and Zambia ((Webster, et al., 2013a; Webster, et al., 2013b).

Concerning *S. haematobium*, molecular studies are few at global scale, and mainly based on analysis of mitochondrial DNA regions, few nuclear DNA (ITS) regions and more recently microsatellites (Webster et al., 2015) and SNPS at the genome scale (Rey, et al., 2021a; Landeryou, et al., 2022). Despite the scarce molecular studies coupled with poor resolution of routinely used genetic markers, common patterns emerged concerning genetic diversity, genetic structure and genome-wide evolutionary history of *S. haematobium* populations. *S. haematobium* has been particularly characterized by very low genetic diversity compared to *S. mansoni* (Webster, et al., 2012; Gower, et al., 2013). In spite of the low genetic variability, two major clades: East Africa and West Africa (Webster, et al., 2012) have been identified based on their mtDNA. Populations established in some Indian Ocean islands: Madagascar and Mauritius have been incorporated into the Eastern clade. Interestingly, unlike *S. mansoni*, no differences in genetic

variability has been evidenced between Eastern and Western populations, even though Gower, et al., (2013) found important genetic differentiation among populations based on IBD patterns although such patterns have been called to question (Glenn, et al., 2013). Even though there is no genetic data for other *Schistosoma* species at the continental scale efforts have been made to draw general phylogeographic patterns of some schistosomes based on their geographical distribution, evolutionary history and compatibility with their respective freshwater snail host species.

2. Factors affecting schistosome genetic diversity

Schistosoma species genetic diversity within the definitive host: genetic mixing bowl (Curtis, & Minchella, 2000) is increased by the movement and long life span of the definitive host as well as the genotype dependent host re-infection. Also, the quick turnover of infected intermediate snail hosts, the snail-parasite compatibility and PZQ treatment increase genetic diversity (Beltran, et al., 2011). However, the parasite genetic diversity could be regulated by some intrinsic (sex and species of parasite) and extrinsic (sex, age, infection status or PZQ intake of patient) factors detailed in the publication.

G. *Hybridization in schistosomes*

Hybridization is a common phenomenon in *Schistosoma* species. There is increasing evidence for natural hybridization between species possibly enhanced by increased environmental changes which may be natural or man made. Naturally, host specificity and distribution may have limited the potential for hybridization between *Schistosoma* species. In addition, natural and anthropogenic environmental changes, accompanied by hosts movement, introduction or radiation can all contribute to alter the distribution of schistosome species. This can result to new interactions between schistosome species and hosts and also between different schistosomes infecting the same hosts. For example, dam construction and irrigation may give rise to modifications in farming practice and create novel habitats for intermediate snail hosts bringing humans into even closer contact with their livestock and also parasites of these animals, certain species that can successfully hybridize with human schistosomes (Webster, et al., 2013b). Species interaction and interbreeding has led to increased number of parasites species with hybrid progenies been more harmful than the parent species, show host range expansion, host

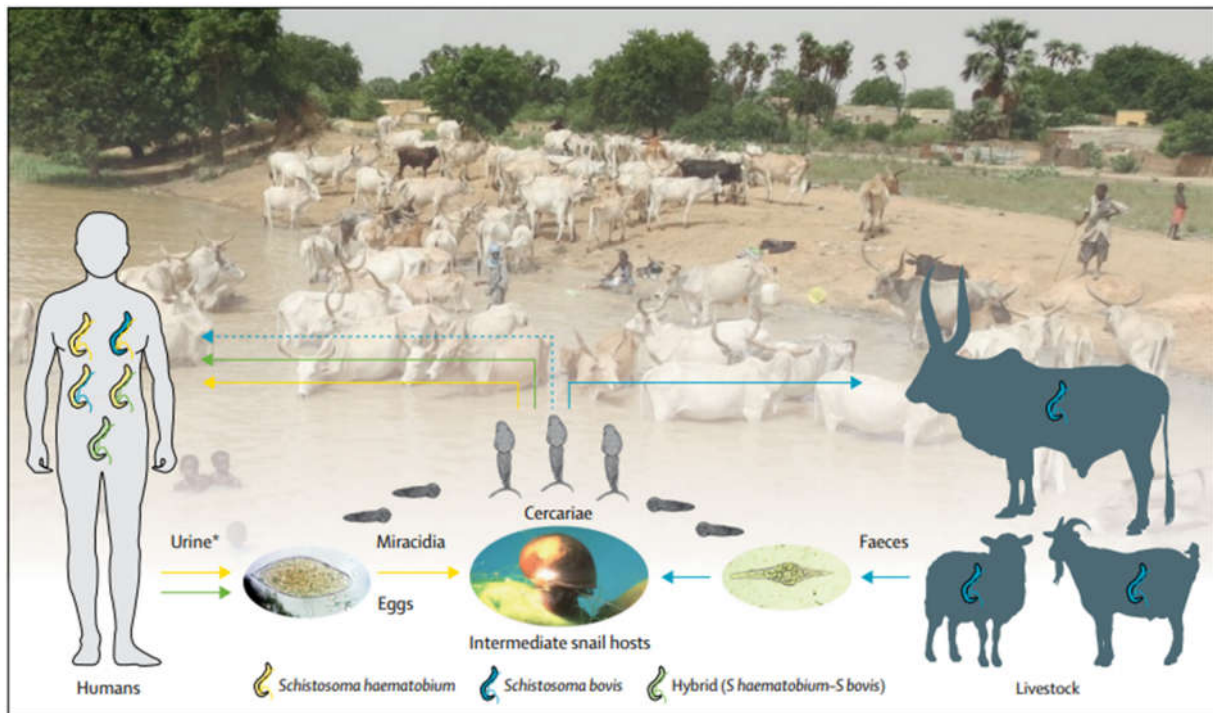


Fig 5. Proposed life cycle of *S. haematobium* group in hybrid zones in West Africa: multi-hosts and multi-parasites transmission cycle. *S. bovis* (blue) is present in livestock: cattle, sheep and goats. Transmission is maintained within these species by emission of eggs, miracidia and cercariae of *S. bovis* (blue arrows). Evidence shows that *S. bovis* cercariae can infect humans (dashed blue arrow) but unable to complete their life cycle in humans unless paired with the human schistosome, *S. haematobium* (yellow arrows). This cross-species pairing leads to viable hybrid eggs and miracidia (green arrows) which are compatible with snail intermediate hosts and re-infect human hosts. In humans, repeated back crosses and introgression lead to complex range of miracidia genotypes shed in hybrid zones. *Faecal transmission of *S. haematobium* x *S. bovis* hybrids is also possible. Adapted from Léger, et al., 2020

morbidity as well as complicate parasite diagnosis (Webster, & Southgate, 2003; Kincaid-Smith, et al., 2021). In addition, the relationship of changes in disease transmission and hosts health with inter-specific interactions, the potential for novel reservoirs and zoonotic transmission are relevant in the disease control and elimination.

Depending on the phylogenetic distance of the species, the heterologous sexual interactions in schistosome species will lead to either hybrid (introgression) or parthenogenetic offspring (parthenogenesis) (Taylor, 1970; Jourdane, & Southgate, 1992). Schistosome species are commonly identified using two molecular markers: mitochondrial DNA marker; cytochrome

oxidase c subunit I (Cox1) acquired from the maternal gene and nuclear rDNA marker; ribosomal internal transcribed spacer II (ITS2) which is bi-parentally inherited (Huysse, et al., 2009; Boissier, et al., 2016). Hybrid parasites are incriminated when there is a clear discordance between mitochondrial and nuclear signatures. Several studies have shown that there are no physiological barriers preventing encounters and mating of schistosome individuals of different species, even belonging to a different genus, in the definitive host (Armstrong, 1965; Rollinson, et al., 1990; Tchuem Tchuente, et al., 1993). So, in endemic areas where several species exist which are capable of co-infecting the same definitive host, there will be frequent opportunities for interspecific interactions and hybridization in human or non-human hosts. The hybridization of phylogenetically closely related schistosomes are usual and have been severally observed both in nature (Cunin, et al., 2003), and in the laboratory (Mansour, et al., 1984). For the first generation of hybrids, the breeding site is determined by the muscular male which holds the thin female within its gynaecophoric canal where the pair mate and the female begin to produce fertilized eggs (Colley, et al., 2014). However, for subsequent generations, the anatomical site of infection could depend on the extent of genetic introgression. Hybridization can modify the morphology and mode of egg excretion and this can lead to variation between clinical symptoms and laboratory diagnosis. Eggs of hybrids between *S. haematobium* and *S. bovis* have been identified in both urine and faeces of infected people (Huysse, et al., 2009). Therefore, mixed excretion of eggs from hybrid parasites in excreta will not be uncommon in endemic areas. Moreover, natural hybrids of schistosome have been identified in several African countries: *S. haematobium* x *S. guineensis* hybrids were noticed in Cameroon (Wright, et al., 1974; Webster & Southgate, 2003; Southgate, et al., 1976; Ratard, et al., 1990; Ratard, & Greer, 1991). The natural occurrence of *S. haematobium* x *S. guineensis* hybrids have also been evidenced in Gabon and Benin (Zwingenberger, et al., 1990; Moné, et al., 2012), *S. mansoni* x *S. rodhaini* hybrids were identified in sympatric East Africa zones (Morgan, et al., 2003; Steinauer, et al., 2008b), with natural hybrids noticed in Tanzania and Kenya (Morgan, et al., 2003; Steinauer, et al., 2008b). Concerning *S. haematobium* x *S. mattheei*, (De Bont, et al., 1994) found evidence for suspecting these hybrids in cattle, while *S. haematobium* x *S. mattheei* mix/hybrid infections were also identified in tourists infected in north east of South Africa (Cnops, et al., 2021). Natural *S. haematobium* x *S. bovis*, have shown high numbers of *hybrids* excreted by humans in West African countries with the first observation in Niger (Brémond, et al., 1993). High abundance and wide distribution of *S. haematobium* x *S. bovis* hybrids were evidenced in humans

in Senegal and none in livestock (Huyse, et al., 2009; Webster, et al., 2013a; Webster, et al., 2013b). Also, *S. haematobium* x *S. bovis* hybrids which is thought to have been imported from West Africa were noticed in infected tourists in Corsica, France (Moné, et al., 2015; Boissier, et al., 2016). *Schistosoma haematobium* and *S. mansoni* are two co-endemic parasites that are highly prevalent in humans, but are of high phylogenetic distance. Though there have been reports of lateral-spine eggs (typical of *S. mansoni*) been observed in urine samples and terminal-spine eggs (typical of *S. haematobium*) been observed in stool samples in several co-endemic areas: Kenya, Egypt, Senegal, Malawi, Mali and Cote D'Ivoire. However, these atypical egg excretion in connection to heterologous interaction is not common in all endemic areas and in all patients. Also, a case of *S. haematobium* x *S. mansoni* hybrids molecularly identified from a migrant boy from Cote D'Ivoire to France, but again the viability of the offspring was not able to be tested (Le Govic, et al., 2019). Even though such hybrids may not be viable, impact on human health are likely to cause increased pathology due to ectopic egg deposition in organs and tissues.

Aim and objectives of this Study.

The transmission dynamics of *Schistosoma* species are determined by the complex life cycle of the parasite, environmental factors, the molluscan intermediate and mammalian definitive hosts. Environmental changes due to human activities influence the epidemiology and distribution of the parasite and can enhance interspecies genome modification. In spite of MDA campaigns, safe water supply, improved environmental sanitation, community enlightenment, urogenital schistosomiasis is still prevalent with heavy intensity of infection in some parts of Nigeria. In addition, hybrids of human urogenital schistosome and bovine intestinal schistosome have been reported in humans in several of Nigeria's neighboring West African countries. Also, there have been a number of studies concerning hybridization of *Schistosoma* species experimentally in the laboratory and reports have shown that there are no physiological barriers preventing encounters and mating of schistosome individuals of different species, even belonging to a different genus, in the definitive host. Therefore, investigating the presence and prevalence of hybrids and population genetic structure of this infection using molecular tools in addition to experimental approach may help elucidate its transmission dynamics. Based on this, this Ph. D thesis is aimed at the study of genetic diversity of *Schistosoma haematobium* among primary school-age pupils in Nigeria and the mating interactions between a major human intestinal *Schistosoma* species in Africa (*S. mansoni*) and bovine intestinal schistosome (*S. bovis*). In order to accomplish these goals, this research will:

- i. determine the current prevalence of the infection among primary school-age children in Nigeria and the risk factors associated with the disease transmission.
- ii. determine the presence and prevalence of *S. haematobium* x *S. bovis* hybrids in different sampling sites.
- iii. determine the population genetic diversity and structuration of *S. haematobium* eggs obtained from positive patients.
- iv. study the mating interactions between two intestinal parasites: *S. mansoni* (human) and *S. bovis* (bovine).

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CHAPTER II, Epidemiology, Prevalence and Risk Factors Associated with Urogenital Schistosomiasis among Primary School Pupils in Nigeria

Nigeria is one of the most impacted sub-Saharan African (SSA) country by both urogenital and intestinal schistosomiasis and accounts for almost 14% of the disease globally. My study started by a large survey in the south of my country in order to assess recent data on prevalence and associated risk factors. I have also collected samples for the subsequent molecular analysis. A parasitological survey was done on 5,514 primary school-age pupils in 12 sites in the south of Nigeria. My results ranged from 4.6% (east) to 15.9% (west) Nigeria with an overall prevalence of 7.1% which is below the national prevalence of 9.5%. The significant risk factors associated with the infection are frequent contact with freshwater bodies infested cercariae among others. This survey outcome showed a fall in prevalence in most of the studied areas when compared with results of the past years. This suggests the efforts rolled out in the past several years to fight the disease seem to bring positive results. Our result outcome showed similarities to the report from the assessment of preventive chemotherapy on schistosomiasis prevalence in 44 SSA countries which showed global reduction, comparing 2000-10, with 2011-14 and 2015-19 periods during which preventive chemotherapy was scaled-up. This three-time periods assessment also showed that complementary interventions such as access to clean water, improved sanitation, snail control and behaviour change, will all lower the risk for infection and stop the parasite transmission.

Epidemiology, Prevalence and Risk Factors Associated with Urogenital Schistosomiasis among Primary School Pupils in Nigeria.

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SUBMITTED ON JULY 2021 TO PARASITE EPIDEMIOLOGY AND CONTROL: MAJOR REVISION

Abstract: Urogenital Schistosomiasis is a Neglected Tropical Disease endemic in Nigeria and continues to pose public health problem especially among school-age children in rural communities. This study was carried out in remote areas where most of the people depend on natural water bodies and rainwater for their daily water need. The research aims at studying the prevalence of urogenital schistosomiasis and the significant risk factors associated with the infection among primary school pupils in Nigeria. A total of 5,514 primary school-age pupils from August 2019 to December 2019 from twelve sites were diagnosed from the presence of *Schistosoma haematobium* eggs in their urine. We also collected socio-demographic,

sociocultural, socioeconomic indices and data on behaviors (e.g contact frequency to freshwater bodies) for each diagnosed individual using pretested questionnaire. Associations between each of these variables and disease infection were tested using multivariate logistic regression. A total of 392 of the 5514-urine samples were positive for the infection, the overall prevalence reached 7.1% and ranged from 4.6% (East Nigeria) to 15,9% (West Nigeria). Multivariate logistic regression analyses showed that the significant risk factors associated with *S. haematobium* infection are frequent contact with freshwater bodies (rivers/streams) adjusted odds ratio (AOR): 4.92; 3.34-7.24, washing/swimming AOR: 46.49; 27.64-78.19, and fishing, AOR: 11.57; 8.74-15.32. For socioeconomic factor, primary education of fathers AOR: 1.63; 1.01-2.45 was significantly associated with the infection. The socio-demographic factor age group 12-14 years AOR: 1.68; 1.21-2.33) was also significantly associated with the disease. Nigeria remains endemic for urogenital schistosomiasis as indicated by the data obtained at all studied sites, hence the need to upgrade control measures.

Keywords: Nigeria; schistosomiasis; *Schistosoma haematobium*; prevalence; risk factors

1. Introduction

Schistosomiasis is a Neglected Tropical Disease (NTD) known world-wide, which is caused by dioecious blood fluke (digenetic trematodes), of the genus *Schistosoma* (Atalabi, et al., 2018). It is ranked as the second parasitic disease after malaria based on socio-economic and public health importance. It is the most prevalent water-borne disease that occurs among the rural populace. The parasite is transmitted by specific freshwater intermediate molluscan host, while humans of all age groups act as the definitive host, (Uchendu, et al., 2017). It is estimated that about 700 million people live in areas where the disease is endemic. About 200 million people are infected in about 76 countries of the world (Ossai, et al., 2014), with the areas of high endemicity being in the sub-Saharan Africa and some parts of Asia. The sub-Saharan Africa has about 90% of the infected cases, and it is estimated that about 11,700 people die of this disease annually in this region, (Dawet, et al., 2012).

Of the six *Schistosoma* species that infect humans (*S. haematobium*, *S. mansoni*, *S. japonicum*, *S. intercalatum*, *S. mekongi* and *S. malayensis*) (Manson, et al., 1987), three species (*S. haematobium*,

S. mansoni and *S. intercalatum*) are present in Nigeria. The urogenital schistosomiasis caused by *S. haematobium* occur at a higher frequency in remote areas of the country. Nigeria is one of the most impacted Sub-Saharan country by both the urinary and intestinal schistosomiasis with about and accounts for almost 14% of the disease worldwide, (Hotez, et al., 2012; Herrick, et al., 2017). Nigeria thus also requires the greatest intervention for the disease (Uchendu, et al., 2017). The first case of this disease in Nigeria was in 1908, Bishop, (2017) and numerous studies are available on the epidemiology, prevalence and risk factors associated with this disease. Although, sometimes, these reports maybe conflicting (Emmanuel, et al., 2017). No reliable systematic report has been given on the epidemiological status of this disease in many parts of Nigeria (Houmsou, et al., 2011). It was just on June 4th, 2015, that Nigeria's Federal Ministry of Health (FMOH), officially released the first epidemiological data on the prevalence of this disease (Global Network Neglected Tropical Diseases, 2015). The data showed that about 24 million of the population are at the risk of infection and a prevalence of almost 9.5% (Bishop, 2017). The control programs initiated by the FMOH, State Ministry of Health, and other institutions is mainly chemotherapy, by mass drug administration (MDA), giving praziquantel to infected individuals or those at the risk of the infection (Ejike, et al., 2017). Despite Government efforts in addition to the contributions of Research Institutions, Non-Government Organizations (NGOs) and Faith Based Organizations (FBOs), the disease remains unabated (Ejike, et al., 2017). This maybe as a result of several hurdles encountered in the process. These includes, hard to reach areas, , ethnic violence, religious crisis as well as the marshy stream or river systems that are favorable for the multiplication of the snail intermediate host (Yauba, et al., 2018). It has been suggested that integrating school-based health education with preventive chemotherapy will bring the prevalence of the disease to the barest minimum. This will overcome the hurdle of re-infection, resurgence in the frequency of infection after treatment and resistance to drugs from the parasites (Ajakaye, et al., 2017). Previous researchers have provided information on the endemicity of this disease in one or few communities within the same geographical coverage (Opara, et al., 2021). However, the data on this subject are based on either results collated from studies carried out not only at different times but by different researchers (Abdulkadir, et al., 2017).

In this survey, we used a simple and rapid method that yields results within five minutes to screen the patients. Furthermore, we used questionnaires in addition to conducting oral interviews with each subject to scrutinize and draw a tentative conclusion on the prevalence and risk factors

associated with the disease. This study aims at providing ample and new information on the disease based on wider geographical coverage in comparison with previous studies. Such data is crucial to draw a current epidemiological status of the disease. This will unravel the efforts made by national control programs (The Carter Center, Bill and Melinda Gates Foundation) and several internationally funded partners (Merck Group) in the several years of praziquantel treatments. This work will serve as robust evidence of the impact of control measures rolled out on the disease in the past years.

2. Materials and Methods

2.1. Study Area and Study Population

This study was carried out in 12 sites in 7 states in South Nigeria, West Africa to give a wide geographical coverage. No study was carried out in north due to security challenges. At each site, we investigated the urine samples of the local population. Our target was primary school-age children within the age group 5-14 years which constitute the highest risk group for the infection with highest intensities in endemic areas. We chose primary schools, based on information obtained from previous surveys as well as where we can easily get the required number of samples. The study was approved by the research and ethical committee of the Alex Ekwueme Federal University, Ndufu – Alike Ikwo, Ebonyi State, department of Biology/Microbiology/Biotechnology. A letter of ethical clearance was obtained from each of the States’ “Universal Basic Education Board”. Parents Teachers Association (PTA) of schools were mobilized and educated and each gave approval. Informed consents of parents and pupils were obtained before samples were collected from participants. The whole school was invited to the screening. After consultations with the PTA, the head teacher directed each class teacher to organize their respective pupils for the screening. For the youngest children the teacher assisted the pupils to fill the questionnaire.

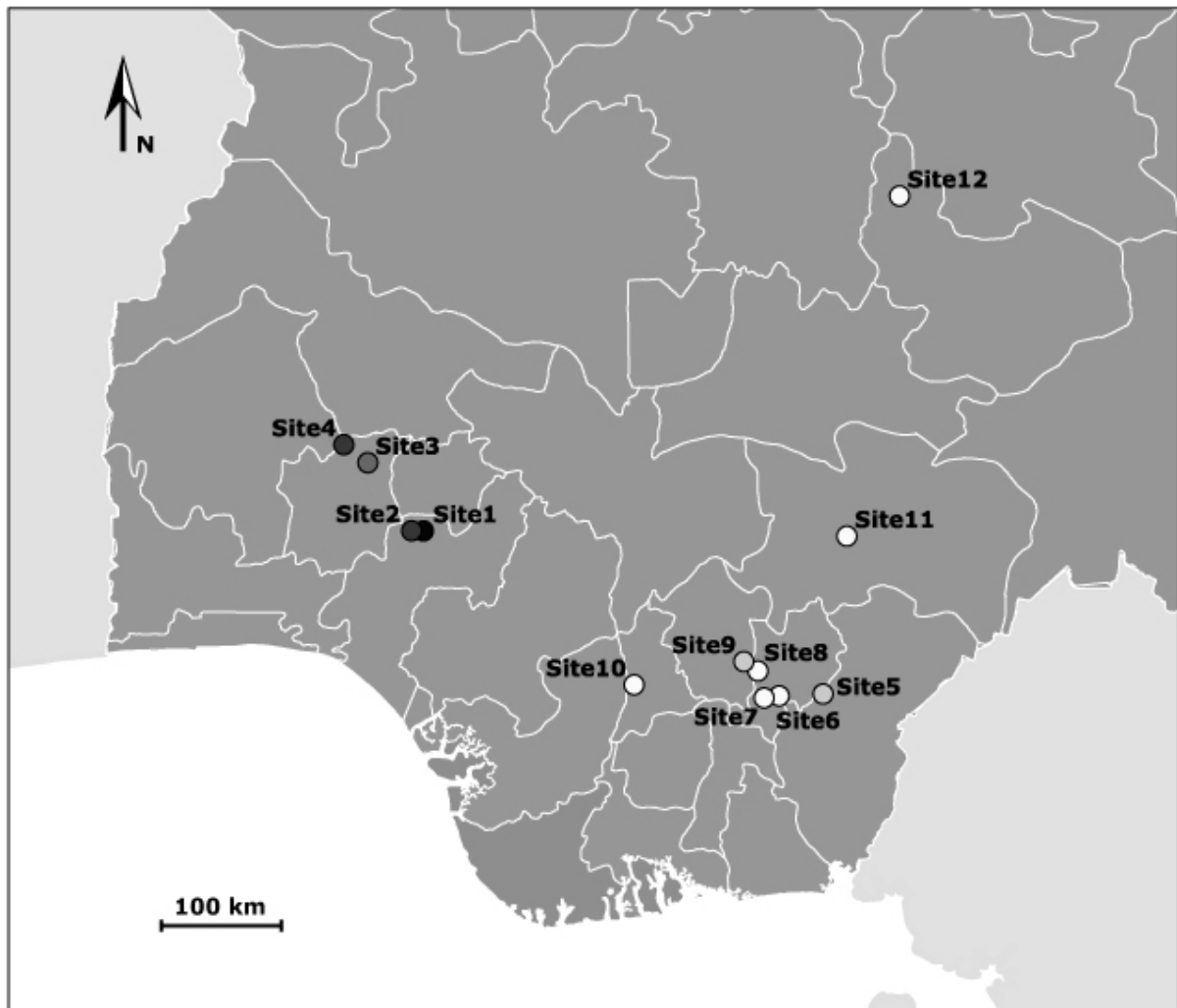


Fig 1. Map of Nigeria (West Africa), showing the sampling sites. Sampling sites were represented according to the prevalence of *S. haematobium* measured among primary school pupils (see results). Lighter to Darker colors correspond to lower to higher prevalence observed in the studied sites.

2.2 Sample Collection

Urine samples were collected between 10.00 hr and 14.00 hr when the excretion of *S. haematobium* is expected to be very high. The excretion peak generally considered as being at 12.00hr mid-day (Singh, et al., 2016). Only primary school pupils who might have lived in the study area for at least one year, whose parents or legal guardians gave oral consent were selected. Each patient was given an ‘identification code’ for anonymity, and a clean labelled, plastic container of 20 ml for urine collection. We used a questionnaire to collect data on each child’s sociodemographic,

behaviors, such as source of drinking water, bathing, washing, farming, and fishing. Most participants visit the streams about 3-5 times per week especially in the dry season to fetch water for domestic use. This visit reduces to once or twice in the rainy season when rainwater becomes an alternative source of water. Fishing activity is predominantly carried out by males. Nonetheless, males and females still wade through the streams while going to and from school, farm or bath in them early in the morning before the day's activities. Other information collected include symptoms like dysuria (painful urination) and haematuria (bloody urine) and socioeconomic status of the parents or guardian. This does not seem to influence the frequency at which participants have contact with water bodies, but to enable us to identify the potential risk factors associated with the disease. A total of 5,514 urine samples were collected for urogenital schistosomiasis screening.

2.3 Parasitological Examination

On the spot examination of each urine sample was performed using a rapid diagnostic technique which is made up of a field microscope and Nytrell® filter at 40µm mesh size. Each urine sample of about 20 ml was homogenized by shaking vigorously and filtered through a Nytrell® filter and the residue released into a small Petri dish using wash bottle filled commercial spring water (Mott, et al., 1982; Colley, et al., 2014). The resulting residue was examined under of a binocular microscope at 20X or 40X magnifications, for the presence of a characteristic 'terminal spine' *Schistosoma haematobium* egg or of hatched miracidia. After the urine examination, each child infected with *Schistosoma haematobium* was treated with a single oral dose of 40mg/kg body weight of praziquantel (600mg, Biltricide, Bayer, Leverkusen, Germany), through their Primary Health Centre (PHC).

2.4 Statistical Analysis

Statistical analysis was carried out on twelve parameters using the STATA version 15.0 (Stata cooperation, college station, Texas, USA). Prevalence comparisons between groups (Site, Sex or Age) were tested using univariate analysis (χ^2 or Fisher's exact test). Patients were said to be positive for the infection when one or more eggs/miracidia of schistosome was seen during the microscopy examination. Participants were divided into three age groups (5-8, 9-11 and 12-14 years) for each sex. We performed statistical analysis to assess the relationship between parasitic

infections and demographic, sociocultural and socioeconomic factors using multivariate logistic regression with random intercepts for sites and groups nested within sites. The risk factors investigated were domestic water source, washing, swimming, fishing, parents' occupation and parents' educational status. Associations and differences with a p-value < 0.05 was considered as statistically significant.

3. Results

3.1 Socio-demographic Study (Population parameter)

A total of 5,514 primary school children were tested and more males 3,190 (57.85%) participated than the females 2,324 (42.15%). The minimum, mean and maximum age was 5 years, 9.02 years and 14 years respectively, with a standard deviation of 2.84 years. Table 1 shows the demographic analysis of pupils.

Table 1: Socio-demographic characteristics of study population (n=5,514).

Variable	Number of children	Percentage of the total sample (%)
Study site/state		
1 Ipogun (Ondo)	308	5.59
2 Ilara-Mokin (Ondo)	302	5.48
3 Alie Ilie (Osun)	300	5.44
4 Lie Twon (Osun)	301	5.46
5 Ikwo (Ebonyi)	536	9.72
6 Ohaozara (Ebonyi)	548	9.94
7 Onicha (Ebonyi)	537	9.74
8 Ishielu (Ebonyi)	548	9.94
9 Nkanu east (Enugu)	572	10.37
10 Anambra west (Anambra)	539	9.78

11 Gwer east (Benue)	567	10.28
12 Jos north (Plateau)	456	8.27
Gender		
Female	2 324	42.15
Male	3 190	57.85
Age (years)		
5-8	2 579	46.77
9-11	1 596	28.95
12-14	1 339	24.28

3.2 Prevalence of *Schistosoma haematobium*

A total of 392 (7.1%) of the 5,514 urine samples were found positive for *S. haematobium*. The prevalence ranged from 4.6% in East Nigeria to 15.9% in West Nigeria. The highest prevalences were recorded in West Nigeria (sites 1-4), while the lowest were in East or central part of the country (sites 5-12). There was a statistically significant difference among the study sites, $p \leq 0.001$. Prevalences for the sites 1-4 seems comparable; similarly, that for site 5-12. Moreover, 244 (7.6%) males were positive, while 148 (6.4%) females were positive but no statistically significant difference in prevalence was found between the two genders ($p = 0.068$). There was a statistically significant difference in the prevalence among the age groups ($p \leq 0.001$), with the prevalence of the infection increasing with increase in the age of the patients.

Table 2: Infection status stratified by study settings, sex and age group.

Characteristic	Infection status		p-value
	Total	Positive n (%)	
Study site/state			<0.001
1 Ipogun(Ondo)	(308)	49 (15.91)	
2 Ilara-Mokin (Ondo)	302	37 (12.27)	
3 Alie Ilie (Osun)	300	32 (10.67)	

4 Lie Twon (Osun)	301	38 (12.62)	
5 Ikwo (Ebonyi)	536	36 (6.72)	
6 Ohaozara (Ebonyi)	548	28 (5.11)	
7 Onicha (Ebonyi)	537	27 (5.03)	
8 Ishielu (Ebonyi)	548	27 (4.93)	
9 Nkanu east (Enugu)	572	35 (6.12)	
10 Anambra west (Anambra)	539	25 (4.64)	
11 Gwer east (Benue)	567	32 (5.64)	
12 Jos north (Plateau)	456	26 (5.70)	
Gender			0.068
Female	2 324	148 (6.37)	
Male	3 190	244 (7.65)	
Age (years)			<0.001
05- 8	2 579	149 (5.78)	
09- 11	1 596	121 (7.58)	
12-14	1 339	122 (9.11)	

Table 3: Multivariate logistic regression analysis of variables associated with the infection non-adjusted and adjusted for socio-demographic factors, socio-economic status and environmental factors. OR: Odd ratio; CI: confidence interval

Variable	Total	Positive	Crude OR (95% CI)	Adjusted OR (95% CI)
Socio-demographic factors				
<i>Age (years)</i>				
05- 8	2 579	149	1.00	1.00
09- 11	1 596	121	1.34 (1.04-1.72)	1.30 (0.94-1.81)
12-14	1 339	122	1.63 (1.27-2.10)	1.68 (1.21-2.33) *

Gender

Female	2 324	148	1.0	1.00
Male	3 190	244	1.23 (0.98-1.50)	1.14 (0.86-1.50)

Socio-economic factors*Father's educational status*

Illiterate	843	45	1.00	1.00
Primary school	3 346	251	1.44 (1.04-1.99)	1.63 (1.01-2.45)*
Secondary school	1 169	88	1.44 (1.00-2.09)	1.28 (0.80-2.05)
Tertiary school	156	8	0.96 (0.44-2.07)	0.95 (0.36-2.47)

Mother's educational status

Illiterate	538	34	1.00	1.00
Primary school	2 887	190	1.04 (0.72-1.52)	1.14 (0.71-1.85)
Secondary school	2 039	162	1.23 (0.87-1.87)	1.39 (0.85-2.27)
Tertiary school	50	6	2.02 (0.80-5.08)	2.01 (0.63-6.45)

Father's occupation

Peasant farmer	3 925	272	1.00	1.00
Petty trader	991	74	1.08 (0.83-1.41)	1.24 (0.87-1.76)
Fisherman	214	18	1.23 (0.75-2.03)	1.14 (0.60-2.19)
Civil service	383	28	1.06 (0.71-1.59)	1.13 (0.67-1.93)

Mother's occupation

Peasant farmer	3 643	257	1.00	1.00
Petty trader	1 647	114	0.98 (0.78-1.23)	0.77 (0.73-1.04)
Housewife	92	7	1.09 (0.50-2.34)	1.10 (0.38-3.10)
Civil service	132	14	1.56 (0.88-2.76)	1.52 (0.74-3.12)

Sociocultural factors*Using borehole water*

No	3 003	237	1.00	1.00
Yes	2 511	115	0.77 (0.62-0.95)	0.80 (0.61-1.05)

Using river/stream water

No	1 917	46	1.00	1.00
Yes	3 597	346	4.33 (3.17-5.92)	4.92 (3.34-7.24) *

Washing/ swimming in river/stream water

No	3 500	21	1.00	1.00
Yes	2 014	371	37.41 (24.0-58.29)	46.49 (27.64-78.19) *

Fishing in freshwater

No	4 776	113	1.00	1.00
Yes	738	219	11.23 (9.02-13.98)	11.57 (8.74-15.32) *

* *P-value*<0.05, *p-value* obtained from mixed logistic regression with fixed effects for infection status and each variable in the table.

The multivariate logistic regression analysis of variables on table 3 shows the relationship between urogenital schistosomiasis and sociodemographic, sociocultural and socioeconomic factors. The significant risk factors associated with *S. haematobium* infection are frequent contact with freshwater bodies (rivers/streams) AOR: 4.92; 3.34-7.24), washing/swimming AOR: 46.49; 27.64-78.19, and fishing AOR: 11.57; 8.74-15.32. For socioeconomic factor, primary education of fathers AOR: 1.63; 1.01-2.45 was significantly associated with the disease. The sociodemographic factor (age group 12-14 years) AOR: 1.68; 1.21-2.33 was also significantly associated with the infection.

4. Discussion

Infection to urogenital bilharziasis was found at all studied sites although the prevalence is heterogeneous over the country. The East and central parts of the country fall below the national average prevalence of 9.5%, while all the sites in the West are slightly above the national average prevalence. A systemic review from 1994-2015, using a unified pooled population, revealed a pooled prevalence range from 31.0% to 38.50% for all regions (Abdulkadir, et al., 2017). A 50-

year review by Ezech, et al., (2019), ranked most of our study sites (Benue, Ebonyi, Enugu, Osun and Plateau) as hyper endemic zones (>50% prevalence), while a few (Anambra and Ondo) were in moderately endemic zones (10-50% prevalence). Results of this survey showed a fall in prevalence in most of the studied areas when compared with previous results highlighted. This suggests the efforts rolled out in the past years to fight the disease seem to bring positive results. Nigeria has increased control efforts, with global partners renewing their support to eliminate the infection. Yet, risk of reinfection and transmission of the parasite is still active in many areas in Nigeria. MDA has helped to reduce morbidity, but it is not sufficient to stop transmission in many parts of the country. Long-time well-structured control approach could bring a more sustainable result like was achieved in China, Japan and Brazil (Bergquist, et al., 2017).

Table 4: Comparative analysis of our results with previous results

Present Survey				Previous Survey		References
S/No	State	Sample site	Prevalence (%)	Date	Prevalence (%)	
1.	Ondo	Ifedore	15.91	Oct 2002- Oct 2003 ¹	59.0 ¹	(Oniya ¹ , & Odaibo, 2006)
2.	Ondo	Ifedore	12.27			
3.	Osun	Irepodun	10.67	Nov 2006- Jun 2007 ¹ , Feb-July 2012 ²	62.0 ¹ , 55.8 ² , 51.10 ³	(Ugbomoiko ¹ , et al., 2010), (Awosolu ² , 2016), (Bishop ³ , 2017)
4.	Osun	Irepodun	12.62	2013- 2015 ²	30.10 ¹ , 5.30 ²	(Igbeneghu, et al., 2018), (Amuga, et al., 2020)

5.	Ebonyi	Ikwo	6.72	July-Dec 2016 ²	17.50 ¹ , 10.0 ²	(Nwosu ¹ , et al., 2015), (Umoh ² , et al., 2020),
6.	Ebonyi	Ohaozara	5.11	2012- 2013 ¹ , Feb 2016-Jan 2017 ³	15.3 ¹ , 6.30 ² & 79.40, 3.90 ³	(Ivoke ¹ , et al., 2014), (Bishop, 2017), (Nwachukwu, et al., 2018)
7.	Ebonyi	Onicha	5.03	Aug 2005- July 2006 ¹	26.8 ¹ , 27.0 ²	(Uneke ¹ , et al., 2006), (Elom ² , et al., 2017)
8.	Ebonyi	Ishielu	4.93	Jan-March 2009 ¹	46.18 ¹ , 22.7 ²	(Ozowara, et al., 2011), (Onwe, et al., 2016)
9.	Enugu	Nkanu East	6.12	---	34.1 ¹ , 13.60 ²	(Bishop ¹ , 2017), (Aribodor ² et al., 2019)
10.	Anambra	Anambra west	4.64	Oct 2007- Sept 2008 ¹	12.8-19.8 ¹ , 2.9 ²	(Ugochukwu ¹ , et al., 2013), (Ndukwe, et al., 2019)
11.	Benue	Gwer east	5.64	Nov 2008- Sept 2009 ¹ , Sept, 2012 ² , 2013- 2015 ⁵	38.6 ¹ , 36.0- 64.0 ² , 41.50 ³ , 20.70 ⁴ , 13.10 ⁵	(Houmsou ¹ , et al., 2012), (Amuta ² , & Houmsou 2014), (Bishop ³ , 2017), (Emmanuel ⁴ , et al., 2017), (Amuga ⁵ et al., 2020)

12.	Plateau	Jos north	5.70	---	6.40 ¹	(Dawet, et al., 2012)
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An overall prevalence of 7.1% was obtained from a total of 5,514 urine samples observed. The male patients were infected more than their female counterparts as other findings have revealed (Otineme, et al., 2014, Gbonhinbor & Abah., 2019), although this was not statistically significant. This could be as a result of lack of differences in sociocultural behaviors between the two genders. Generally, in most parts of south and central Nigeria, there is no gender based cultural restrictions to water bodies. Even though Ezech, et al., (2019), reported that the females have higher prevalence compared with their male counterparts. Our study also revealed that there is increase in prevalence with increase in the age group among the primary school pupils (age group 12-14 years). This is consistent with the findings of Gbonhinbor & Abah., (2019), Amuga, et al., (2020),), and maybe attributed to the frequent water contact activities exhibited by pupils within this age group. Although Angora et al., (2020) did not observe any difference in prevalence among the three age groups investigated in a study conducted in Ivory Coast.

There was no significant association between parents' educational status (except fathers' primary education) and occupation and the infection status. This could be attributed to the fact that fetching water from the stream may not be related to one's parent's profession or societal status, but a form of cultural activity in some rural areas. Even though the findings of Ugbomoiko et al., (2010) showed that parents with better education can understand the preventive strategies and explain them to their children. Nonetheless, Geleta, et al., (2015), Angora et al., (2019),) showed that 'farmer' as parents' occupation was statistically significant to the frequency of the disease.

The relationship between the prevalence of schistosomiasis and contact with freshwater bodies infested with cercariae is well established (Ugbomoiko, et al., 2010, Gbonhinbor & Abah., 2019). In fact, and as expected, we here found that frequent contact with freshwater bodies infested with cercariae, like contact when fetching water for domestic use, recreation, swimming, washing and fishing was associated with urogenital schistosomiasis. This is to some extent logical since bilharziasis is directly linked to the exposition of schistosomiasis contaminated freshwater.

S. haematobium and *S. mansoni* have been reported to be endemic in Nigeria, but the latter is less prevalent and widespread (Garba, et al., 2004). A survey carried out in 2015 in 19 states of Nigeria showed the proportion of *S. haematobium* and *S. mansoni* as 82% to 18% respectively. The overlapping distribution of the two species could increase the chance of co-infection and the potential severe effect on morbidity. Also worrisome is the possible ongoing control challenges in these areas.

5. Conclusions

The result obtained in this survey shows that the disease is still endemic in the studied areas despite significant increase in praziquatel treatment coverage index. This imposes a health hazards among the infected children who could play a leading role in the spread of the parasite. Recent studies have identified in particular hybrids of *S. haematobium* x *S. bovis* in some West African countries Webster, et al., (2013) which may naturally infect rodents and cattle Savassi, et al., (2020). These animal reservoir hosts can pose potential zoonotic implications. We advocate research priority in this area of hybridization events in parasites in Nigeria. The role of animal reservoirs in the parasite transmission can pose serious challenges to prevention, control and treatment of the disease.

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CHAPTER III, Population Genetic Structure and Hybridization of *Schistosoma haematobium* in Nigeria

S. haematobium and *S. mansoni* have been evidenced to be endemic in Nigeria, but the latter is less prevalent with lower geographical coverage. *S. bovis*, an animal infecting parasite has also been detected in some parts of the country. The overlapping distribution of these *Schistosoma* species could enhance the chance of co-infection and the potential of hybridization. Indeed, there is increasing evidence for natural hybridization between schistosomes possibly due to increased environmental changes. *S. haematobium* x *S. bovis* hybrids have been reported in humans in several of Nigeria's neighboring West African countries in addition to the population genetic structure of the parasite, but such study has not been evidenced in Nigeria. I have done molecular genotyping using both Cox1 gene and microsatellite markers on 1,364 parasites recovered during the survey. Of the 1,364 miracidia, 1,212 (89%) and 152 (11%) showed a *S. bovis* Cox1 profile and *S. haematobium* Cox1 profile respectively. All the study sites except two showed 100% of *S. bovis* Cox1 haplotypes. The population genetic analyses also showed two main clusters: one each at the east and west of Nigeria. This study provide evidence that *S. haematobium* x *S. bovis* hybrids are common in Nigeria. We advocate research priority in domestic and wild animals to study the role of zoonotic transmission.

Population Genetic Structure and hybridization of *Schistosoma haematobium* in Nigeria.

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Abstract: Schistosomiasis is a major poverty-related disease caused by dioecious parasitic flatworms of the genus *Schistosoma* with health impact on both humans and animals. Hybrids of human urogenital schistosome and bovine intestinal schistosome have been reported in humans in several of Nigeria's neighboring West African countries. No empirical studies have been carried out on the genomic diversity of *Schistosoma haematobium* in Nigeria. Here we present novel data on the presence and prevalence of hybrids and population genetic structure of *S. haematobium*. 165 *Schistosoma* positive urine samples were obtained from 12 sampling sites in Nigeria. *Schistosoma haematobium* eggs from each sample were hatched and each individual miracidium picked and preserved in Whatman® FTA cards for genomic analysis. 1,364 parasites were molecularly characterized by rapid diagnostic multiplex polymerase chain reaction (RD-PCR) for mitochondrial DNA gene (Cox1 mtDNA) and a subset of 1,136 miracidia were genotyped using a panel of 18 microsatellite markers. No significance difference was observed in population genetic diversity ($P>0.05$), though significant difference was observed in the allelic richness of the sites

except sites 7, 8 and 9 ($P < 0.05$). Also, we observed two clusters of populations: west (populations 1-4) and east (populations 7-12). Of the 1,364 miracidia genotyped, 1,212 (89%) showed a *S. bovis* cox1 profile and 152 (11%) showed a *S. haematobium* cox1 profile. All parasites showed a *S. bovis* Cox1 profile except for some at sites 3 and 4. *Schistosoma* miracidia full genotyping showed 59.3% of *S. bovis* ITS2 allele. This study provides novel insight into hybridization and population genetic structure of *S. haematobium* in Nigeria. Our findings suggest that *S. haematobium* x *S. bovis* hybrids are common in Nigeria. More genomic studies on both Human and animal infecting parasites are needed to ascertain the role of animal in schistosome transmission.

Keywords: Nigeria, hybrids, genomic analysis, *Schistosoma haematobium*, *Schistosoma bovis*

1. Introduction

Schistosomiasis is one of the major neglected tropical diseases with public and veterinary health concern and endemic in tropical and subtropical regions. With a global burden of about 1.4 million disability-adjusted life years (DALYs), the disease is ranked second after malaria based on morbidity (Hotez, et al., 2014). *Schistosoma* genus show wide definitive host spectrum that ranges from humans to domestic and wild animals. Humans could be infected with one or more of the six human infecting Schistosome species, and this may lead to combined disease symptoms and comorbidities. Four of human infecting parasites (*Schistosoma haematobium*, *S. mansoni*, *S. intercalatum*, and *S. guineensis*) are common in Africa, while two (*S. japonicum* and *S. mekongi*) are prevalent in Asia (Boissier, et al., 2019). Except *S. intercalatum*, all schistosome species are known to infect domestic or wild mammalian animal hosts (Panzner, & Boissier, 2021). The disease is less recognized in the veterinary sector including livestock and wild animals (Leger, & Webster, 2017).

In Africa, three species of schistosomes are concerned with livestock infection: *S. bovis* and *S. mattheei* infect members of the orders of Cetartiodactyla (mainly Bovidae), Rodentia, Primates and Perissodactyla and *S. curassoni* infects members of the family of Bovidae (Panzner, & Boissier, 2021). There is little or no documentation available on the prevalence, intensity, repartition, transmission dynamics and phylogeography of these livestock schistosomes (Mone, et al., 1999, Djuikwo-Teukeng et al., 2019). In addition to their veterinarian importance the animal infecting schistosomes have recently received peculiar interest due to their potential zoonotic

importance through hybridization with human infecting parasites. In Africa, hybrids between *S. haematobium* and the 3 livestock schistosomes has been evidenced in humans: *S. haematobium* x *S. bovis* (Huyse, et al., et al., 2009; Sene-Wade, et al., 2018; Angora et al., 2020), *S. haematobium* x *S. mattheei* (Wright, & Ross, 1980; Kruger, & Evans, 1990) and *S. haematobium* x *S. curassoni* (Webster, et al., 2013b).

The hybrid of human uro-genital schistosome (*S. haematobium*) with bovine intestinal schistosome (*S. bovis*) is certainly the most studied hybrid form. *S. haematobium* x *S. bovis* hybrids have been reported in infected humans in several West African countries like Benin, Cote d'Ivoire, Mali, Niger and Senegal (Leger, & Webster, 2017, Angora et al., 2020). No empirical studies have been carried out in Nigeria to identify presence of hybrids as has been done in some of its neighboring countries. Hybrids are commonly identified based on a single nuclear and single mitochondrial marker. A discordance in species assignation on these two markers or the presence of animal parasite allele or haplotype in human are thus generally considered as a hybrid parasite. Based on such approach, studies have shown hybridization between: *S. haematobium* and *S. mansoni* (Huyse, et al., 2013; Le Govic, et al., 2019; Depaquit, et al., 2019), *S. haematobium* and *S. bovis*, (Brémond, et al., 1993; Huyse, et al., 2009 ; Webster, et al., 2013b ; Boissier, et al., 2015; Moné, et al., 2015; Catalano, et al., 2018 ; Angora et al., 2020 ; Savassi, et al., 2020 ; Savassi, et al., 2021). *S. haematobium* and *S. guineensis*, (Rollinson, & Southgate, 1985 ; Vercruysse, et al., 1994; Webster, et al., 2005; Moné, et al., 2012), *S. haematobium* and *S. curassoni* (Webster, et al., 2013b), or *S. haematobium* and *S. mattheei*, (Wright, & Ross, 1980; Kruger, & Evans, 1990; De Bont, et al., 1994).

If the presence/absence of *S. haematobium* hybrids has been assessed in some west African countries the population genetic structure and diversity of this parasite has received less attention. Nevertheless, genomic introgression through hybridization is expected to influence both the genetic diversity and the population structure. Most population genetic studies have concerned *S. mansoni* and very few *S. haematobium*. Some common feature can be observed whatever the *Schistosoma* species such as few barriers to gene flow at a local scale or the most important genetic variation within than between definitive host (Rey et al 2021). However, *S. haematobium* is particular from the other species because its present less genetic diversity and less genetic structuration compared to *S. mansoni* or *S. bovis* (Rey et al 2021). To date, a single study has

analyzed both the hybrid status of the parasite using the nuclear/mitochondrial marker discordance approach and population genetic structure based on microsatellite markers (Boon et al. 2019). This last study showed no clustering when parasites are grouped according to their hybrid vs pure status.

Our study aims to complete our knowledge on *S. haematobium* in the identification of the presence and prevalence of *S. haematobium* verses *S. bovis* hybrids in Nigeria at a large geographical scale as well as microsatellites genotyping to analyze the population genetic structure and diversity.

2. Materials and methods

2.1.1. Study area and study population

This study was carried out in twelve sites in Nigeria, West Africa (Fig 1). This study was integrated into a survey carried out on epidemiology, prevalence and risk factors associated with urinary schistosomiasis among primary school age pupils in Nigeria (Onyekwere et al. Submitted).

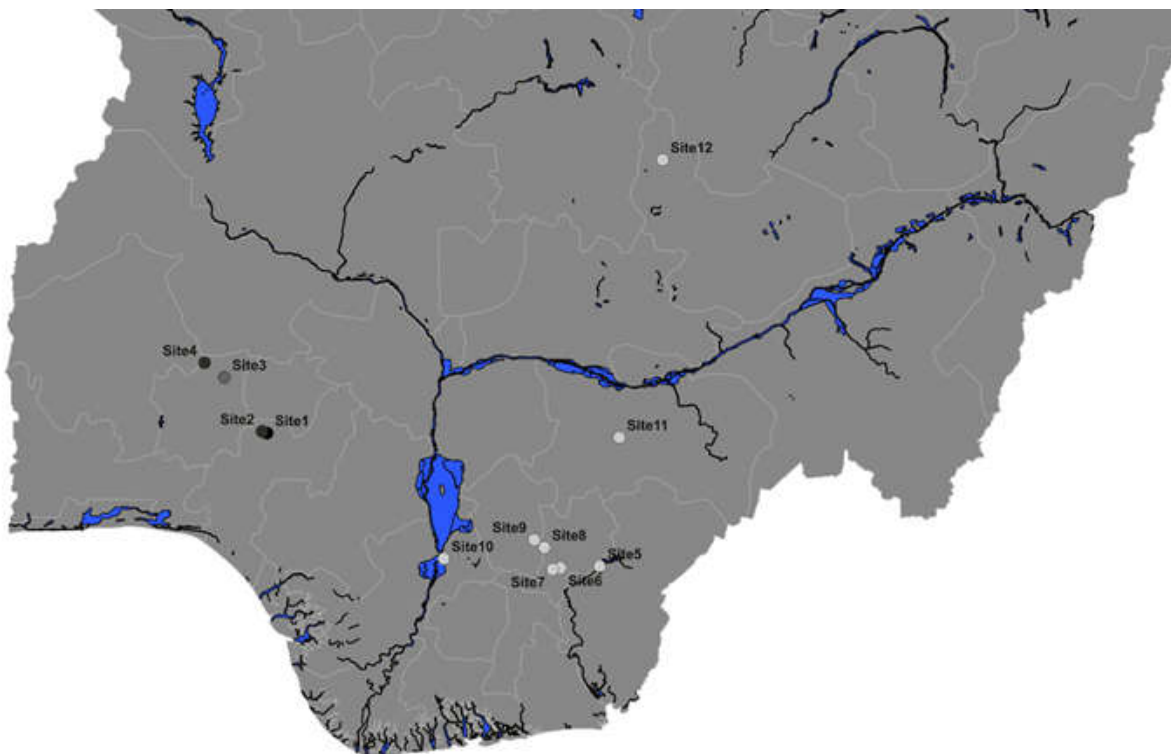


Figure 1. Map showing sampling sites 1-12 in the survey study carried to determine the prevalence of *S. haematobium* infection among primary school-age pupils across Nigeria (Onyekwere, et al.

Submitted). Sampling sites were represented according to infection status of the disease among the participants. Darker to lighter colors correspond to higher to lower infection status observed at the studied sites.

2.1.2. *Urine sample collection*

A labelled clean and sterile plastic container with “identification code” for anonymity, of 20ml was given to each patient whose parents or legal guardians gave oral consent. Each participant whose urine sample was positive for the parasite was treated with a single oral dose of 40mg/kg body weight of praziquantel (600mg, Biltricide, Bayer, Leverkusen, Germany) through their Primary Health Centre (PHC).

2.1.3. *Miracidia sampling (Table 1)*

Individual miracidium was harvested using P10 Gilson micropipette in 3 μ L of water under 20X or 40X magnification binocular microscope. About 20-25 miracidia were individually captured for each participant with each miracidium being checked in the pipette tip before placing on the Whatman FTA® cards (GE Healthcare Life Sciences; Amersham, UK). Each FTA® card filled with miracidia was stored at room temperature while on the field and transferred to “Laboratoire Interactions Hotes-Pathogenes-Environnements” (IHPE), France for genetic analysis.

2.2. Genomic analysis

2.2.1. *DNA extraction*

Genomic DNA from *Schistosoma* eggs were individually extracted from FTA® cards using Chelex method (Beltran et al., 2008). Harris-Micro-Punch (VWR; London, UK) was used to perforate a 2mm disc at the centre where the sample was placed. The disc was washed in 50 μ L ultra-pure water for 10 minutes, the water discarded, and the disc was incubated in 80 μ L of 5% Chelex® solution (Bio-Rad; Hercules, California, USA) at 65°C for 30 minutes with agitation. This was incubated again at 99°C for 8 minutes without agitation. The solution was centrifuged at 14,000 rpm for 2 minutes and 60 μ L of the supernatant transferred into a micro-plate of 96 wells and stored at -20°C for genomic analysis.

Table 1. Number of miracidia collected from participants and genotyped with Cox1 and microsatellites for each of the sampling site.

Site No	Sampling site	Number of children	Number of miracidia collected	Number of miracidia genotyped with Cox1	Number of miracidia genotyped with microsatellites
1	Ipogun	10	268	95	74
2	Ilara-Mokin	20	560	156	206
3	Alie Ilie	18	405	152	219
4	Lie Twon	11	279	74	76
5	Ikwo	3	82	30	0
6	Ohaozara	12	279	66	0
7	Onicha	12	277	84	77
8	Ishielu	12	278	103	90
9	Nkanu east	17	418	164	87
10	Anambra west	12	279	90	77
11	Gwer east	20	465	193	61
12	Jos north	18	417	162	169
	Total	165	4,007	1,364	1,136

2.2.2. Estimation of hybrid prevalence by mitochondrial DNA identification

Hybrid schistosomes are generally characterised by the combination of the maternal DNA (mt-DNA) from Cox1 and the nuclear DNA (rDNA) from ITS2 (Huyse, et al., 2009, Angora et al., 2020). The results will be used to assign each parasite a genetic signature based on the haplotype-alleles combinations: *Sb x SbSb*, *Sb x ShSh*, *Sb x SbSh*, *Sh x ShSh*, *Sh x SbSb* or *Sh x SbSh*. We have obtained this full genotyping only for a subsample of miracidia (see below). However, a basic estimation of the hybrid frequency could be assessed only by the Cox1 gene characterization considering that a human infected by an animal parasite (i.e *S. bovis*) gene is an hybrid parasite (Boon, et al., 2018). Hence, the frequency of hybrids is a synonym for the frequency of miracidia with a *S. bovis* Cox1 profile. This method has for consequence to underestimate the frequency of hybrids because *Sh x SbSb* or *Sh x SbSh* genotypes are considered as pure *S. haematobium* instead

of hybrids. For this purpose, each miracidium was molecularly characterized by rapid diagnostic multiplex PCR (RD-PCR) on Cox1 (Webster et al., 2010). The number of miracidia tested by site is presented in table 1. We used species specific primers to amplify region to discriminate each *Schistosoma* species fragment: *S. haematobium* 120 bp, *S. mansoni* 215 bp and *S. bovis* 260 bp (Webster et al., 2010). The primers we used were a single universal reverse primer; (Shmb.R, 5'-CAA GTA TCA TGA AAY ART ATR TCT AA-3') and three species-specific forward primers; (Sh. F, 5'-GGT CTC GTG TAT GAG ATC CTA TAG TTT G-3') for *S. haematobium*, (Sm. F, 5'-CTT TGA TTC GTT AAC TGG AGT G-3') for *S. mansoni* and (Sb. F, 5'-GTT TAG GTA GTG TAG TTT GGG CTC AC-3') for *S. bovis*. Each PCR is made up of 1.2µL of ultra-pure water, 2µL of buffer (Green GoTaq Flexi buffer, 5X; Promega; Madison, Wisconsin, USA), 1.2µL of 25 mM MgCl₂ (Promega), 0.4µL of 10 mM dNTPs mix (Promega), 1µL of 10X primer mix (4µL of 100µM reverse primer, 4µL of each 100µM forward primer and 84µL of ultra-pure water), 0.2µL of 5U/µL of GoTaq G2 Hot Start Polymerase (Promega), and 4µL of DNA extract, making a total volume of 10µL for the PCR mix. Thermal cycling was performed in (plate thermal cycler) a PerkinElmer 9600 Thermal Cycler (PerkinElmer, Waltham, Massachusetts, USA) and the PCR conditions used were: pre-denaturing at 95°C for 3 minutes; 45 cycles of 10 seconds at 95°C (denaturing), 30 seconds at 52°C (annealing), and 10 seconds at 72°C (extending). This was followed by a final extending period of 2 minutes at 72°C. The PCR product was stored in the refrigerator at 4°C until used. The PCR products (Cox1) was visualized on 2% agarose gel stained with 8µl Midori dye. 9µL of the PCR product was loaded into each well using multi-channel micro-pipette (including wells for positive controls; *S. haematobium*, *S. mansoni*, *S. bovis* and water for negative control) and 4µL for size standard 100 bp (base-pair) ladder. The PCR products in the gel was analyzed by electrophoresis at 135 V for 30-35 minutes and transferred to the UV trans-illuminator where the pictures in the gel were taken.

2.2.3. Mitochondrial DNA (Cox1) and nuclear internal transcribed spacer II (ITS2) sequencing

Full genotyping on a sub-sample was assessed by sanger sequencing of the two genes (Cox1 and ITS2). Cox1 and ITS2 gene of six to seven miracidia harboring *S. bovis* Cox1 RD-PCR profile were sequenced on all sites. Seven more miracidia were sequenced for site 3 and 4, the only sites harboring *S. haematobium* Cox1 RD-PCR profile (see results). *S. haematobium* Cox1 PCR mix

was performed in 96 wells with a single forward primer COI1_F: 5'-GGGGGTTTTATTGGTTTAGGTT-3' and a single reverse primer COI1_R: 5'-CCAATTATAAAAGGCCATCACC-3', while *S. bovis* COI1 PCR mix was performed in 96 wells with a single forward primer COI1_F: 5'-GAGGTGGTTTTATTGGTCTTGG-3' and a single reverse primer COI1_R: 5'-GGCCACCATCATACCAACAT. Schistosome ITS2 PCR mix was performed with a single forward primer ITS4_F: 5'-TAACAAGGTTTCCGTAGGTGAA-3' and a single reverse primer ITS5_R: 5'-TGCTTAAGTTCAGCGGGT-3' (Kane, & Rollinson, 1994). The PCR mix was made up of 17.35µL of ultra-pure water, 6µL of buffer (colorless GoTag Flexi buffer 5X; Promega; Madison, Wisconsin, USA) 1.8µL of 25mM MgCl₂ (Promega), 0.6µL of 10mM dNTPs mix (Promega), 1µL of each 10µM primer, 0.25µL GoTaq G2 Hot Start polymerase (Promega) and 2µL of DNA extract, making a total volume of 30µL for each PCR mix. The PCR thermal cycling conditions used was the same for all markers and was performed in (plate thermal cycler) a PerkinElmer 9600 Thermal Cycler (PerkinElmer, Waltham, Massachusetts, USA): pre-denaturing at 95°C for 3 minutes, 45 cycles of 30 seconds at 95°C (denaturing), 40 seconds at 56°C (annealing), and 80 seconds at 72°C (extending). This was followed by a final extending period of 2 minutes at 72°C. The PCR product was stored in the refrigerator at 4°C until used. 4.5µL of the product was mixed with 1.5µL of a green loading dye to make 6µL which was loaded into each well of 1% agarose gel with 8µL Midori dye using multi-channel micro-pipette and 5µL for size standard 100 bp ladder. This was analyzed by electrophoresis at 135 V for 30 minutes and transferred to the UV trans-illuminator where the picture in the gel was taken. The expected band size was 1000-1100 bp. Fifty-nine (59) samples were selected based on the quality of the amplicons. This successfully amplified PCR products were purified and sequenced on an Applied Biosystem Genetic Analyzer at Genoscreen, Lille, France.

2.2.4. Sequence analysis

The Cox1 and ITS2 sequences were assembled separately and edited with 4.5 sequencer version: (Gene Codes Corporation; Ann Arbor, Michigan, USA). The sequences were aligned using BioEdit Version 7.0.9 and ClustalW software (Ibis Therapeutic, Carlsbad, California, USA). The aligned sequences were compared with the sequences in the GenBank Nucleotide Database for species designation: (<https://www.ncbi.nlm.nih.gov/nucleotide/>). The nuclear ITS2 region

between *S. haematobium* and *S. bovis* differs at five polymorphic sites, hence the sequence chromatograms were checked at these SNPs to identify any possible heterozygosity. We constructed a Cox1 gene phylogenetic tree only using *S. bovis* sequences because Cox1 *S. haematobium* gene is known to be poorly variable and phylogenetic study reveals only two clusters on all the area of repartition of the parasite (Webster, et al., 2012). Phylogenetic tree was constructed using MEGA version 6.0.6 (Pennsylvania State University, Philadelphia, Pennsylvania, USA) using a HKY+G nucleotide substitution model identified as the best model describing data. The support for tree nodes was calculated with 1000 bootstrap iterations. The phylogenetic analysis includes *S. bovis* sequences from various African countries retrieved from GenBank databases with a minimum length of 778 bp (See Supp Table 1). The tree was rooted with the *S. haematobium* haplotypes of the present study. All sequences were uploaded onto GenBank database (OL840258-OL840278).

2.2.5. Microsatellite genotyping

Microsatellite genotyping was performed on parasites from all sites except 5 and 6. A total of 1,136 samples (Table 1) were individually genotyped with 18 microsatellite markers divided into two panels of 9 loci (Webster et al., 2015). The multiplex PCR mix for each panel in two tubes was performed using Qiagen® multiplex PCR kit (Qiagen, Hilden, Germany) according to manufacturer's standard amplification protocol. The forward primers were fluorescently labeled using 6-FAM, VIC, NED and PET dyes (Applied Biosystems, Foster City, USA). The PCR mix consist of 5µL Qiagen MM 2X, 1µl of 10X microsatellite primer mix and 4µl DNA extract making a final volume of 10µl. The thermal cycling was performed in a plate thermocycler, PerkinElmer 9600 Thermal Cycler (PerkinElmer, Waltham, Massachusetts, USA): pre-denaturing at 95°C for 15 minutes, 40 cycles of 30 seconds at 94°C (denaturing), 90 seconds at 56°C (annealing), and 60 seconds at 72°C (extending). This was followed by a final extending period of 30 seconds at 60°C (Webster et al., 2015). The microsatellite PCR products were sent to Genoscreen, Lille, France for genotyping. Each microsatellite locus was visibly peak called with GS500Liz size standard (Applied Biosystem) and GeneMarker software. 80% of our samples were successfully amplified by 14 loci and were used for result analysis while 4 markers (C131, Sh4, Sh8 and Sh15) which amplified less than 20% of the samples were excluded.

2.2.6. Population genetic structure

Linkage disequilibria and departures from Hardy-Weinberg expectations were tested using exact tests (1200 permutations) adjusted for multiple tests using Bonferroni's correction as implemented in the FSTAT software 2.9.3.2 (Goudet, 2001). We analyzed the genetic variability of schistosomes from each study site by computing the expected heterozygosity (H_e), number of alleles (A), allelic richness (A_r) and the inbreeding coefficient (F_{is}) in each microsatellite's locus with FSTAT v.2.9.3.2 (Goudet, et al., 2002). Heterozygosity (H_e) and allelic richness (A_r) between the populations were compared using the pairwise Friedman rank test followed by Nemenyi post-hoc test.

Genetic structuration was first assessed calculating pairwise F_{ST} values between sites according to (Weir, & Cockerham, 1984) using FSTAT version 2.9.3.2. Second, we used the principal components analysis (PCA) implemented in Genetix (Belkhir, et al., 2004). Third, we used the Bayesian clustering approach implemented in the Structure software to determine the uppermost level of genetic structure (Pritchard, et al., 2000). We tested the number of clusters from $K=1$ to $K=12$, by computing three runs for each cluster which is made up of 10^6 iterations after a "burn-in" period of 250,000 iterations with other parameters set by default and admixture model. The mean logarithm probability for each cluster (K) was taken for the three runs with the *corrsieve* package in R. The ΔK -values were then computed in R to determine the probable cluster number from the total clusters (ΔK) tested according to Evanno, et al., (2005), from which we identified $K=2$ as the most probable genetic clusters. Lastly, an additional 10 runs were computed for $K=2$ using 10^6 iterations and setting the same parameters as earlier described. The mean probability for a miracidium to belong to each cluster over the 10 runs was taken as Q-values, and we used Clumpp version 1.1.2 according to Francis, (2017), and Distruct version 1.1 according to Rosenberg, (2004).

3. Results

3.1. Schistosome genotyping using *Cox1* and *ITS2*

We obtained a total of 4,007 miracidia from 165 *Schistosoma* positive urine samples and analyzed 1,364 successful miracidia. Of the 1,364 miracidia, 1212 (89%) and 152(11%) showed a *S. bovis*

Cox1 profile and *S. haematobium* Cox1 profile respectively (table 2). All the study sites except sites 3 and 4 showed 100% of Cox1 haplotypes. No miracidium was identified as a *S. mansoni* Cox1 profile. Full genotyping (i.e Cox1 and ITS2 sequencing) was obtained from a sub-sample. The *Schistosoma* miracidia full genotyping showed 6 (5.1%) for nuclear gene of *S. haematobium* (*ShxShSh*), 46 (39.0%) alleles were assigned to *S. bovis* (*SbxSbSb*) and 66 (55.9%) alleles were assigned to *S. bovis* x *S. haematobium* hybrids (*SbxShSh*, *SbxSbSh* and *ShxShSb*) while no hybrid parasite was identified as *ShxSbSb* genotype (table 3).

Table 2. Number of miracidia collected from participants and analyzed by Cox1 marker rapid diagnostic (RD) PCR to show the minimum percentage of *S. haematobium* x *S. bovis* hybrids.

Sampling site	No of children tested	No miracidia genotyped	No of miracidia with Cox1 <i>S. bovis</i>	No of miracidia with Cox1 <i>S. haematobium</i>	Min % of hybrids (<i>S. bovis</i> Cox1)
1	10	90	90	0	100%
2	20	156	156	0	100%
3	18	152	28	124	18%
4	11	74	46	28	62%
5	3	30	30	0	100%
6	12	66	66	0	100%
7	12	84	84	0	100%
8	12	103	103	0	100%
9	17	164	164	0	100%
10	12	90	90	0	100%
11	20	193	193	0	100%
12	18	162	162	0	100%
Total	165	1364	1212	152	89%

Table 3. Prevalence of *S. haematobium*, *S. bovis* and *S. haematobium* x *S. bovis* hybrids for each sampling site based on Cox1 x ITS2 combinations of full genotyped 59 sub-samples.

Test	Site	1	2	3	4	5	6	7	8	9	10	11	12	Total alleles (%)		
														<i>Sb</i> ITS2	<i>Sh</i> ITS2	
COX1	ITS2															
<i>Sb</i>	<i>SbSb</i>	3	1	0	0	0	0	2	0	4	4	5	4	23(39.0)	46(39.0)	0(0)
<i>Sb</i>	<i>ShSh</i>	2	0	4	2	0	0	0	0	0	1	0	0	9(15.3)	0(0)	18(15.3)
<i>Sb</i>	<i>SbSh</i>	2	6	2	1	0	2	0	1	3	2	1	2	22(37.2)	22(18.6)	22(18.6)
<i>Sh</i>	<i>SbSb</i>	0	0	0	0	0	0	0	0	0	0	0	0	0(0)	0(0)	0(0)
<i>Sh</i>	<i>ShSh</i>	0	0	2	1	0	0	0	0	0	0	0	0	3(5.1)	0(0)	6(5.1)
<i>Sh</i>	<i>SbSh</i>	0	0	2	0	0	0	0	0	0	0	0	0	2(3.4)	2(1.7)	2(1.7)
Total		7	7	10	4	0	2	2	1	7	7	6	6	59(100)	70(59.3)	48(40.7)

3.2. *Cox1* phylogenetic trees

On the 59 *Cox1* sequences we have sequenced 21 different haplotypes: 2 *S. haematobium* and 19 *S. bovis* haplotypes. *S. bovis* *Cox1* phylogenetic tree performed with the haplotypes recovered from Nigeria and several other haplotypes from Cameroon, Benin, Senegal, Ivory Coast, Kenya and Tanzania do not reveal any spatial structuration (See supplementary Figure 1).

3.3. Microsatellite analysis

No significant deviation from HW equilibrium or linkage disequilibrium was observed across loci. The genetic variability indices (He, A, Ar and Fis) are shown in table 4 from the 14 microsatellite loci. Mean heterozygosity across population range from 0.527-0.598 but we did not observe any significant difference ($P>0.05$). For allelic richness (Ar), the mean values range from 4.718-6.929. Significant differences were observed between site 9 and sites 2 to 4 ($p<0.05$).

3.4. Population genetic structure

The pairwise genetic differentiation estimates (F_{ST}), between most of the sampling sites are statistically significant after Bonferroni correction, except for between sites 1 and 2, 8 and 9, 8 and 10, 9 and 10, 7 and 11, and 7 and 12 (Table 5). The PCA (fig 2) showed a structuration among populations with the population from the east (1-4) separated from the population in the west (sites 7-12). This genetic structuration was confirmed using Structure software that revealed a highest probability for two clusters ($K=2$) (fig 3).

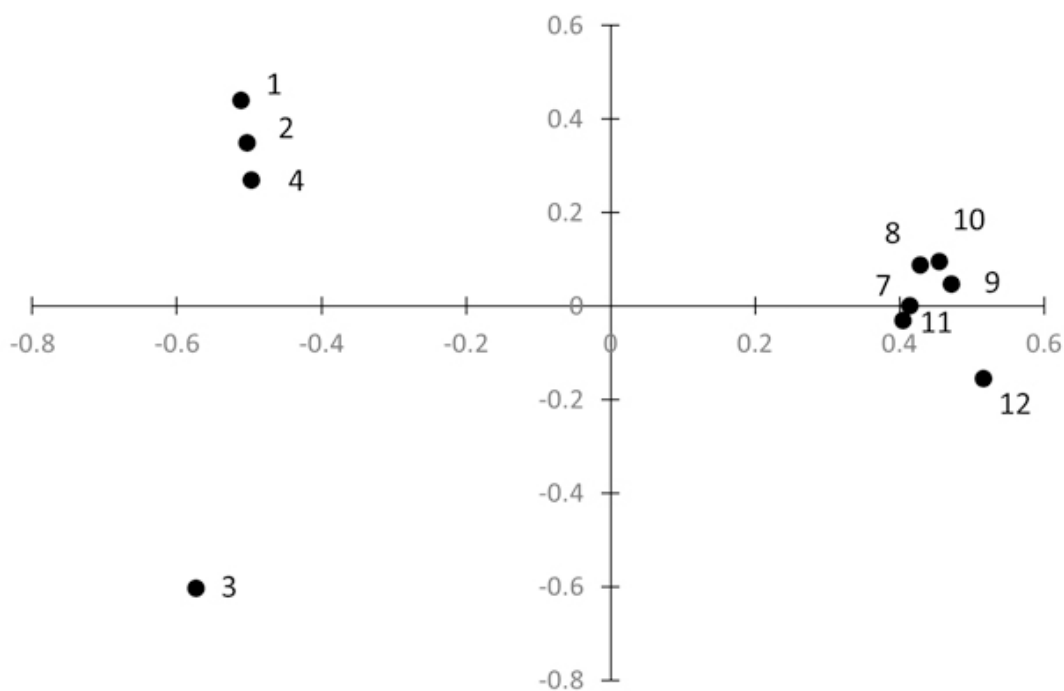


Fig 2. Population genetic structure graph assessed by principal component analysis of 1,136 *S. haematobium* parasites collected in Nigeria revealed by graph (PCA). Each sampling site is

represented by a dot. The first and second axis of the PCA represent 43.8% and 22.9% respectively of the total variation in allele frequency.

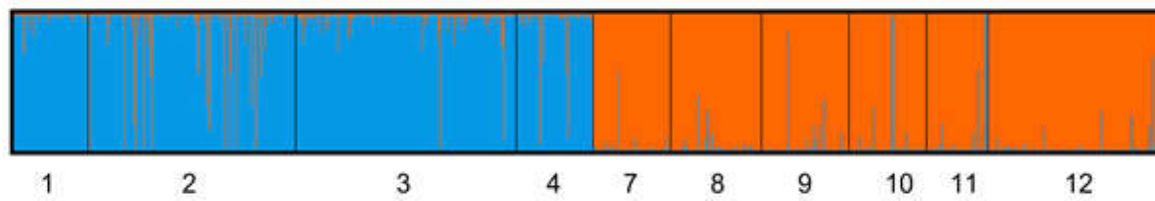


Fig 3. Bar plot showing the population genetic structure using Structure software of 1,136 *S. haematobium* miracidia collected in Nigeria. Each column represents one miracidium. The colors show the proportion of contribution of each cluster to each genotype. The cluster structure K=2, produced by structure software for 10 sampling sites.

Table 4. Population genetic diversity indices per study and per locus. Mean and Standard error (SE) of expected heterozygosity (He), number of alleles detected (A), allelic richness (Ar), mean inbreeding coefficient (Fis).

Locus	Sh9	Sh3	C102	Sh1	Sh14	Sh6	C111	Sh7	Sh13	Sh11	Sh2	Sh5	Sh10	Sh12	Mean	SE
Site 1 n=74																
He	0.633	0.824	0.000	0.704	0.875	0.365	0.608	0.560	0.696	0.454	0.877	0.841	0.536	0.335	0.593	0.247
A	5	10	1	6	11	5	5	4	8	5	10	9	7	4	6.429	2.848
Ar	4.590	9.603	1.000	5.550	10.934	4.947	4.645	3.636	7.330	4.980	9.574	8.710	6.014	3.956	6.105	2.774
Fis	0.569	0.241	NA	0.177	0.082	0.273	0.088	0.377	-0.026	0.416	0.411	0.415	0.501	0.048	0.275	0.196
Site 2 n=206																
He	0.624	0.860	0.025	0.659	0.883	0.335	0.636	0.677	0.710	0.480	0.821	0.821	0.365	0.473	0.598	0.241
A	11	11	2	6	12	6	6	4	10	5	12	13	8	5	7.929	3.518
Ar	7.111	9.906	1.694	5.502	11.469	4.337	4.960	4.000	7.839	4.465	10.851	10.045	5.225	4.212	6.544	3.017
Fis	0.414	0.116	-0.010	0.013	0.063	0.090	0.039	0.468	-0.010	0.411	0.264	0.423	0.219	-0.086	0.172	0.191
Site 3 n=219																
He	0.698	0.801	0.331	0.624	0.767	0.360	0.630	0.656	0.656	0.231	0.833	0.875	0.326	0.136	0.566	0.241

A	9	14	7	12	10	5	6	5	11	5	12	13	6	5	8.571	3.368
Ar	6.925	10.399	5.671	8.790	8.091	3.698	5.003	4.623	9.302	3.715	10.252	12.187	4.802	3.541	6.929	2.890
Fis	0.363	0.107	0.130	-0.006	0.151	0.344	0.108	0.094	0.103	0.562	0.335	0.202	0.399	0.158	0.218	0.157

Site 4 n=76

He	0.583	0.885	0.039	0.675	0.865	0.419	0.602	0.579	0.652	0.580	0.796	0.821	0.308	0.409	0.587	0.235
A	5	11	3	6	12	5	6	4	8	5	11	9	5	4	6.714	2.946
Ar	4.683	10.865	2.354	5.831	11.624	4.757	5.418	3.863	7.602	4.675	10.836	8.517	4.322	3.934	6.377	2.986
Fis	0.216	0.135	-0.009	-0.015	0.183	0.040	0.145	0.330	-0.062	0.655	0.392	0.513	0.333	-0.033	0.202	0.219

Site 7 n=77

He	0.585	0.781	0.208	0.569	0.731	0.000	0.659	0.534	0.822	0.194	0.750	0.758	0.666	0.703	0.569	0.253
A	8	9	2	5	7	1	6	3	11	2	7	7	7	4	5.643	2.925
Ar	6.624	8.526	2.000	4.714	6.506	1.000	5.133	2.998	9.906	2.000	5.969	6.680	6.897	4.000	5.211	2.591
Fis	0.399	0.179	0.126	0.214	0.088	NA	0.262	1.000	0.083	0.525	-0.167	0.330	0.675	0.196	0.301	0.298

Site8 n=90

He	0.732	0.802	0.163	0.525	0.673	0.000	0.629	0.509	0.736	0.229	0.711	0.671	0.677	0.664	0.552	0.245
A	9	8	2	5	7	1	4	4	5	4	7	8	9	7	5.714	2.525
Ar	8.112	7.737	2.000	4.448	6.387	1.000	3.859	3.669	4.999	3.424	6.643	7.285	7.628	6.179	5.241	2.242
Fis	0.700	0.213	-0.092	0.102	-0.008	NA	0.043	0.690	0.233	0.630	-0.102	0.109	0.636	0.089	0.249	0.300

Site 9 n=87

He	0.755	0.778	0.269	0.457	0.702	0.000	0.578	0.400	0.771	0.052	0.737	0.714	0.494	0.667	0.527	0.263
A	8	8	2	5	6	1	4	2	6	2	7	8	7	6	5.143	2.507
Ar	7.445	7.121	2.000	3.973	5.971	1.000	3.488	2.000	5.518	1.983	6.718	6.826	6.302	5.709	4.718	2.245
Fis	0.470	0.044	0.081	-0.080	-0.043	NA	0.376	0.826	0.408	-0.018	-0.192	0.159	0.349	0.104	0.191	0.275

Site 10 n=77

He	0.761	0.762	0.232	0.534	0.677	0.000	0.567	0.490	0.766	0.262	0.787	0.727	0.702	0.704	0.569	0.244
A	8	8	3	3	8	1	5	2	7	3	8	6	8	6	5.429	2.563
Ar	7.334	7.561	2.553	3.000	7.071	1.000	4.120	2.000	6.151	2.667	7.614	5.856	7.568	5.791	5.020	2.379

Fis	0.371	0.075	0.092	0.241	-0.113	NA	0.201	0.819	0.231	0.697	-0.059	0.089	0.719	0.105	0.267	0.298
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Site 11 n=61

He	0.747	0.768	0.242	0.692	0.677	0.075	0.557	0.551	0.763	0.406	0.776	0.723	0.593	0.696	0.590	0.213
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A	9	9	2	4	6	4	4	3	9	2	8	9	8	5	5.857	2.742
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Ar	8.542	8.910	2.000	4.000	5.962	3.543	3.700	3.000	10.162	2.000	7.439	8.736	7.466	4.999	5.747	2.779
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Fis	0.596	0.030	0.256	0.261	0.091	0.494	0.281	0.637	-0.063	0.707	-0.013	0.202	0.603	0.116	0.300	0.262
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Site 12 n=169

He	0.760	0.752	0.200	0.619	0.725	0.045	0.608	0.420	0.816	0.123	0.738	0.712	0.468	0.728	0.551	0.259
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A	10	11	4	6	7	2	5	4	11	3	8	9	8	7	6.786	2.914
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Ar	7.487	8.692	3.555	4.973	6.448	1.895	4.220	3.188	9.908	2.340	6.648	7.411	6.542	4.797	5.579	2.401
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Fis	0.513	0.167	0.038	0.134	-0.025	0.855	0.147	0.836	0.020	0.471	-0.034	0.174	0.665	-0.000	0.283	0.321
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Total (n=1,136)

Table 5. Pairwise genetic differentiation estimate (F_{ST} - above the diagonal) and the Euclidian geographic distances (Km – below the diagonal) between the sampling sites. Most F_{ST} values are statistically significant (in bold) with the level of significance adjusted with Bonferroni correction ($p < 0.0011$)

Population number	1	2	3	4	7	8	9	10	11	12
1	--	0.0104	0.0681	0.0441	0.1493	0.1454	0.1544	0.1295	0.1424	0.1579
2	5.6	--	0.0546	0.0187	0.1206	0.1286	0.1323	0.1157	0.1188	0.1348
3	72.7	68.2	--	0.0445	0.1195	0.1497	0.1387	0.1346	0.1179	0.1332
4	97.6	92.8	25.5	--	0.1274	0.1683	0.1688	0.1521	0.1181	0.1487
7	372.3	377.9	436.3	461.5	--	0.0358	0.0371	0.0318	0.0112	0.0209
8	311.7	317.3	376.4	401.6	24.3	--	0.0122	0.0052	0.0356	0.0241
9	298.9	304.5	363.3	388.5	36.1	13.2	--	0.0194	0.0485	0.0261
10	225.1	230.5	295.4	320.9	113.8	105.4	96.2	--	0.0244	0.0286
11	365.8	371.2	414.7	437.4	153.3	138.0	138.0	221.5	--	0.0220
12	497.9	501.3	506.5	518.5	439.3	419.8	415.4	471.3	291.0	--

4. Discussion

We report for the first time the population genetic structure and hybridization of *S. haematobium* in Nigeria. Based on Cox1 profile, our study revealed a country-wide minimum proportion of 89% prevalence of *S. bovis* x *S. haematobium* hybrids and almost equal repartition among the study sites. Most studied sites revealed a hybrid prevalence of 100% except for sites 3 and 4. *S. bovis* x *S. haematobium* hybrid prevalence obtained from other West African countries: Cote d'Ivoire 57.5% (Angora et al., 2020) and Senegal 9%-72% (Huyse, et al., et al., 2009; Webster, et al., 2013b; Sene-Wade, et al., 2018; Boon, et al., 2018; Boon et al., 2019; Léger, et al., 2020) revealed

that Nigeria has the highest prevalence of hybrids. Important variation in hybrid frequency, ranging from 2% to 26%, between different villages has been evidenced in Senegal (Boon, et al., 2018). The authors have positively associated this variation with the prevalence of *S. mansoni*. They hypothesize that a first schistosome infection would favour ongoing infections and subsequently hybridizations. Because hybrid prevalence is 100% in the majority of the sites we have sampled we cannot test for an eventual link with proximal factors such as prevalence and socio-demographic factors we have measured (Onyekwere et al....).

We have obtained full genotypes (both Cox1 and ITS2) for a sub-sample of 59 parasites. Interestingly, we found a high percentage (39%) of *Sb x SbSb* genotypes. This genotype has not been found in Senegal (Boon, et al., 2018; Boon et al., 2019 ; Sene-Wade, et al., 2018 ; Webster, et al., 2013b), and in very low percentage in Cote d'Ivoire (Angora et al., 2020), or in Corsica (Boissier, et al., 2016). The high percentage of *Sb x SbSb* genotype we found is associated to a preponderance of both *S. bovis* Cox1 haplotype and *S. bovis* ITS2 alleles compare to *S. haematobium*. Concerning the ITS2, in Cote d'Ivoire and in Senegal the frequency of *S. haematobium* allele is 87% and more than 88%, respectively (Angora et al., 2020 ; Boon, et al., 2018 ; Boon et al., 2019 ; Sene-Wade, et al., 2018 ; Webster, et al., 2013b). We found only 40% of *S. haematobium* ITS2 alleles in Nigeria. When population is at equilibrium the ITS is expected to harbor a single allele from one of the parent resulting of the concerted evolution (Sang, et al., 1995). This suppose that in Nigeria, contrary to other countries the population of hybrid schistosomes is not stabilized.

Concerning the Cox 1, in Cote d'Ivoire and in Senegal, the frequency of *S. haematobium* haplotype is 46% and more than 77%, respectively (Angora et al., 2020 ; Boon, et al., 2018 ; Boon et al., 2019 ; Sene-Wade, et al., 2018 ; Webster, et al., 2013b). We found only 11% of *S. haematobium* haplotype in Nigeria, and these haplotypes were restricted to two sites. As previously proposed by Boon, et al., (2018), two main factors could explain a variation in Cox1 haplotype frequency: genetic drift and/or selection. Because the mitochondrial genes are only maternally inherited, they are more prone to genetic drift compare to bi-parentally (i.e. nuclear) inherited marker. Mate choice or mate competition could select for a given mitochondria species. Recently, it has been shown random mating between *S. haematobium* and *S. bovis* excluding selection of mitochondria through sexual selection (Kincaid-Smith, et al., 2021). Boon, et al., (2018), proposed that the

environment could select for different mitochondrial haplotypes. For instance, these authors hypothesized that the snail strain host could select for hybrid parasites in a given area. This interesting snail driver selection hypothesis needs to be tested.

Concerning Nigeria, the high frequency of *S. bovis* genes could be explained by active zoonotic transmission and ongoing gene flow between animal (i.e. *S. bovis*) and human parasites. Recent genomic studies have shown that *S. haematobium* x *S. bovis* hybrid is certainly the result of an ancient introgression event (Platt, et al., 2019; Rey, et al., 2021a). The age of the hybridization does not exclude ongoing zoonotic transmission. This zoonotic transmission has been evidenced in Benin with cows and rodent (Savassi, et al., 2020; Savassi, et al., 2021) and only with rodent in Senegal (Catalano, et al., 2018). *S. haematobium* x *S. bovis* hybrids have not been evidenced in cows in Cameroon (Djuikwo-Teukeng et al., 2019). Considering the high prevalence of *S. bovis* genes in parasite infecting human in Nigeria, looking for the presence of hybrid schistosomes in animal (rodents or cows) seems necessary.

To determine the genetic structure among the populations, we measured the pairwise genetic estimates (F_{ST} values), for all pairs. Generally, values <0.05 , $0.05-0.15$, $0.15-0.25$ and >0.25 indicate low, moderate, high and very high genetic differentiations respectively (Djuikwo-Teukeng et al., 2019). Our study revealed F_{ST} values of $0.0104-0.1688$ which is an indication of low to very high genetic differentiation among the populations. Few population genetic studies concerned *S. haematobium* compared to *S. mansoni* Rey et al., (2021), and the studies have concerned local scales, between 8 and 45 km distances between sites for (Gower, et al., 2011), and (Boon et al., 2019), respectively. Our study proposed a wider range from local (10's of kilometers) to regional (10's to 100's of kilometers) scale. When populations are separated by few kilometers paired F_{ST} values are in agreement with previous studies and ranged from 0.01 to 0.04 (Gower, et al., 2011). At regional scale the F_{ST} values for *S. haematobium* are similar than for *S. mansoni* (Agola, et al., 2006, Aemero, et al., 2015).

Whatever the method we used (PCA or Bayesian analyses using Structure software), we showed a clear clustering into two groups of populations: one from the west (populations 1-4) and one from the east (populations 7-12). *S. haematobium* populations are usually not well structured compared to *S. mansoni* (Rey et al., 2021). These two parasite species possess a similar transmission dynamic than could influence on parasite genetic structuration. For instance, for both

species, the transmission is focused to water bodies, the intermediate and definitive hosts have similar mobility, and the number of intermediate host species is restricted. It is well established that *S. haematobium* have less genetic diversity than *S. bovis* or *S. mansoni* (Djuikwo et al 2019, Rey, et al., 2021a). This low genetic diversity reduces the power of determination of structuring units. Our study shows that no structuring unit are detectable under around 250 km distances between populations. Fst values are also lower among populations 1-4 or among populations 5-10 than between the two clusters of populations. In comparison, it has been shown clear population structures between *S. mansoni* populations separated by 127 km distance in Ethiopia (Aemero, et al., 2015).

Various factors including hybridization can favor genetic structuration (Rey et al., 2021). Introgression through hybridization can influence the genetic structuration by adding new alleles in a given area and in term favorize population clustering. We have obtained 100% of hybrids in the majority of sites we have sampled. Nowadays this does not exclude an influence of hybridization in genetic structuring. Indeed, the molecular barcoding method we have used only infer the presence/absence of hybrids and not the genomic introgression level. Molecular markers such SNP are needed to infer the role of hybridization in genetic structuring.

5. Conclusions

This study revealed that *S. haematobium-bovis* hybrids are predominant in the *Schistosoma* eggs isolated in the urine samples of primary school-age pupils in Nigeria. Our findings provide evidence that *S. haematobium* x *S. bovis* hybrids are common in Nigeria. Based on the high prevalence of *S. haematobium* x *S. bovis* hybrids, we advocate research priority in domestic and wild animals to investigate the role of zoonotic transmission.

Contributions of authors: AOM, MNC, MA and CUU collected the field data. AOM, OR and JB conceived and updated the methodology of the study. AOM, J-FA and JB performed the genomic analysis. AOM and JB performed the computer analysis. AOM drafted the manuscript. JB and OR review the manuscript for submission.

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CHAPTER IV, Mating Interactions between *Schistosoma bovis* and *S. mansoni* and compatibility of their F1 hybrids with *Biomphalaria glabrata* and *Bulinus truncatus*

Species interaction and inter-breeding has led to increased number of parasites species with hybrid progenies been more harmful than the parent species, show host range expansion, host morbidity as well as complicate parasite diagnosis. Depending on the evolutionary lineage of the species, the interspecific sexual interactions in *Schistosoma* species will lead to the production of either hybrid or parthenogenetic offspring. Crossing outcome between two phylogenetically distant species have been reported to result in the production of non-viable or apparently parthenogenetic eggs: *S. mansoni* x *S. haematobium*, *S. guineensis*, *S. mattheei* or *S. japonicum* resulted in non-viable or parthenogenetic eggs. However, several evidence have confirmed that certain closely related schistosomes (*S. haematobium* x *S. guineensis*, *S. mattheei*, *S. bovis* and also in *S. mansoni* x *S. rodhaini*) can successfully pair and hybridize both experimentally and in the wild for several generations. Results of my experiments on mating interactions between *S. mansoni* males' x *S. bovis* females and vice versa showed that the two parasite species can cross and produce viable progeny in experimentally infected mice. Mate preferences exist for *S. mansoni* but not for *S. bovis*. The F1 progeny can only infect the snail corresponding to the mother species: female *S. bovis* x male *S. mansoni* F1 cross can only infect *Bulinus truncatus* snail while male *S. bovis* x female *S. mansoni* F1 cross can only infect *Biomphalaria glabrata* snail. The loss of compatibility is explained by the fact that the offspring exhibited uniparental maternal origin.

Mating interactions between *Schistosoma bovis* and *S. mansoni* and compatibility of their F1 hybrids with *Biomphalaria glabrata* and *Bulinus truncatus*

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Abstract

In contrary to the majority of Trematoda *Schistosoma* species are gonochoric. So, in endemic areas where several schistosome species exist which are capable of co-infecting the same definitive host, there will be frequent opportunities for interspecific pairing. Our experimental study provides novel insight on pairing behavior between *Schistosoma bovis* and *S. mansoni* in mixed infections in mice. We used six mate choice experiments to demonstrate mating interactions between the two schistosome species. We show that mating between the two *Schistosoma* species is not random and that *S. mansoni* exhibits greater mate recognition compared to *S. bovis*. We also produced

reciprocal hybrids (male *S. mansoni* x female *S. bovis*) and (female *S. mansoni* x male *S. bovis*). These two last crosses produce active swimming miracidia. These miracidia were genotyped by ITS2 sequencing and proposed for mollusc infection. Molecular analyses show that all the miracidia are parthenogenetically produced (i.e. they harbor the mother ITS2 genotype) and as a consequence can only infect the mollusc of the mother. Male *S. mansoni* x female *S. bovis* can only infect *Bulinus truncatus* and female *S. mansoni* x male *S. bovis* can only infect *Biomphalaria glabrata* snails. We conclude that this cross is certainly a dead end for the parasite.

Keywords: *Schistosoma bovis*, *Schistosoma mansoni*, mating interactions, F1 hybrids, compatibility, *Bulinus truncatus*, *Biomphalaria glabrata*

Introduction

Trematoda is a diversified phylum of worms characterized by their parasitic way of life. Trematoda are usually hermaphroditic; However, the hundred species of the family of Schistosomatidae are exceptional because they are gonochoric (Loker, & Brant, 2006). This family contains some species of considerable medical and veterinary importance (Lockyer, et al., 2003). Among them the *Schistosoma* genus is the most important in terms of human health impact. Human schistosomiasis affects about 200 million people in about 76 countries worldwide, with the largest disease burden throughout the sub-Saharan Africa (Huyse, et al., 2009; Colley, et al., 2014). The *Schistosoma* genus has about 23 recognized species with no fewer than 19 species that infect livestock and wild animals (Panzner, & Boissier, 2021). Although only 5 species manifest infection severity in these domestic animals while about 6 species infect humans (Panzner, & Boissier, 2021). Because several of these species are co-endemic and can share the same definitive host, interspecific crossing opportunities can occur. Several studies, both in the field and in the lab have addressed the potentiality of different *Schistosoma* species to meet, to mate and to interbreed.

In the field, numerous studies have reported of potential interspecific crosses between different species of schistosomes (Leger, & Webster, 2016). Early reports were mainly based on the physical appearance of the eggs (Alves, 1948). However, the viability of these eggs were not evaluated and these early physical observations have often been considered as misdiagnosis (Teesdale, 1976; Kinoti, & Mumo, 1988). The use of molecular techniques confirmed hetero-specific pairing or

resulting hybrid progeny of different schistosome species crosses, when adult worms or miracidia were genotyped, respectively (Leger, & Webster, 2016). Interspecific natural hybridization occurs between human *Schistosoma* species (*S. haematobium* x *S. guineensis* or *S. mansoni*), animal schistosomes (*S. bovis* x *S. curasonni*) or between human and animal schistosomes (*S. haematobium* x *S. bovis* or *mattheei* or *curasonni* or *S. mansoni* x *S. rodhaini*). Interspecific pairing can reveal some important outcomes in the field. For example, in Cameroon, hybridization between *S. haematobium* and *S. guineensis* has led to the disappearance of the latter species and the establishments of the former and the hybrids offspring of the two parasites (Tchuem Tchuente, et al., 2003; Webster, et al., 2005). Other important outcome is the zoonotic potential of some crosses as it has been observed in *S. haematobium* x *S. bovis* crosses (Savassi, et al., 2020; Savassi, et al., 2021).

In parallel to field identification, experimental approaches allowed to analyze interspecific interactions and to infer some biological aspects, such mate choice, mate competition or genetic outcome of the crosses, that cannot be address easily in the field. Several interbreeding experiments in the laboratory have confirmed that *Schistosoma* species can successfully hybridize for several generations (Southgate, et al., 1998). These experimental studies have evidenced either random mating or a preponderance of homo-specific pairing according to the phylogenetic distance of the interacting species. When closely related species interact such *S. haematobium* x *S. bovis* (Kincaid-Smith, et al., 2021a), *S. bovis* x *S. curassoni* (Rollinson, et al., 1990a), *S. haematobium* x *S. intercalatum* (Southgate, et al., 1982) or *S. intercalatum* x *S. guineensis* (Pagès, et al., 2001) the pairing is random. All these latter species are included in the same monophyletic *S. haematobium* group (Lockyer, et al., 2003). At the opposite when the species belong to two different evolutionary lineages mate preference is observed as for *S. mansoni* x *S. intercalatum* crosses (Tchuem Tchuente, et al., 1993). The genetic background of the resulting progeny also depends on the phylogenetic distance between the interacting species from parthenogenetic individuals (Jourdane, et al., 1995) to complete genomic introgression (Platt, et al., 2019; Rey, et al., 2021a; Kincaid-Smith, et al., 2021b). One visible consequence of these last differences is the mollusc compatibility. When the progeny is parthenogenetically produced the mollusc host spectrum is limited to the host spectrum of the mother (Jourdane, et al., 1995). At the opposite, for closely related species, hybridization can enlarge the host spectrum like that it has been evidenced in *S. haematobium* x *S. intercalatum* crosses (Webster, & Southgate, 2003a).

The present study addresses inter-specific interactions between *S. mansoni* and *S. bovis* in the laboratory. These species belong to two different evolutionary lineages of schistosomes; the *S. mansoni* and the *S. haematobium* groups. *S. mansoni* is known to infect Human, Non-Human Primate or rodents while *S. bovis* are known to infect livestock and rodents (Panzner, & Boissier, 2021). In the field, both parasite species can share the same rodent host as it has been found in *Mastomys huberti* and *Arvicanthis niloticus* in Senegal (Catalano, et al., 2018). This last study also evidenced interspecific pairs between *S. mansoni* and *S. haematobium* x *S. bovis* hybrids in *Mastomys huberti* (Catalano, et al., 2018). No experimental data are available on this cross. In the context of potential zoonotic transmission, this study proposes to experimentally analyse i) mate interactions thanks to mate choice experiment ii) After forced hybridization experiment, the compatibility of the progeny for the parental mollusc species (*Biomphalaria glabrata* and *Bulinus truncatus*) and iii) the nuclear genetic background of the progeny.

Materials and methods

Ethics statement

This research was carried out according to national ethical standards established in the write of February 1st 2013 (NOR: AGRG1238753A), setting the conditions for approval, planning and operation of establishments, breeders and suppliers of animals used for scientific purposes and controls. The experiments carried out for this study was approved and provided permit A66040 for animal experimentation by the French Ministry of Agriculture and Fishery (Ministere de l'Agriculture et de la Peche), and the French Ministry for Higher Education, Research and Technology (Ministere de l'Education Nationale de la Recherché et de la Technologie). The investigator has the official certificate for animal experimentation obtained from both ministries (Decret n° 87-848 du 19 octobre 1987; number of authorization 007083).

Origin and maintenance of schistosome strains

Schistosoma bovis and *S. mansoni* were maintained in the laboratory using *Bulinus truncatus* (Spain strains) and *Biomphalaria glabrata* (Brazilian strain) respectively. The definitive hosts used were Swiss OF1 mice (Charles River Laboratories L'abresle, France). The parasite strains *S. bovis* and *S. mansoni* originated from Yegua-Salamanca (Spain) and Recife (Brazil), respectively. The

S. bovis strain isolated in the early 80's Silva, et al., (1977) originates from Villar de la Yegua-Salamanca, was provided by Ana Oleaga from the Spanish laboratory of parasitology of the Institute of Natural Resources and Agrobiology in Salamanca. The *S. mansoni* strain isolated in 1975 originated from Recife (Brazil), was provide by Pr. Y. Golvan from the Faculty of Medicine St Antoine, Paris (France).

Experimental protocol

Snail infection and obtention of unisexual clonal population of schistosome

Methods for miracidium recovery follow previously published procedure (Boissier, & Mone, 2000; Boissier, et al., 2003). 72 *Biomphalaria glabrata* snails were exposed individually overnight to single miracidium of *S. mansoni* each and 96 *Bulinus truncatus* snails were individually exposed overnight to single miracidium of *S. bovis* each so that each infected snail would produce single-sex cercarial population. The molluscs were separated into two breeding tanks according to species and fed *ad libitum* for a duration of 35 days for miracidium to develop to cercariae. Molluscs in 24 well-plates according to species were stimulated under light to emit cercariae. After 2-3 h, the 24 well-plates were examined under a binocular microscope for the presence of cercariae and any snail found to emit cercariae was assigned with an identity number. Three cercariae from each infected snail was individually captured for molecular sexing and each snail was separated into a plastic cup and fed *ad libitum*. Molecular sexing of *S. mansoni* and *S. bovis* cercaria was performed according to Portela, et al., (2010) and Kincaid-Smith, et al., (2016) respectively (see Supplementary File for detailed molecular biology protocols). The snails were finally separated into four distinct tanks according to sex and species of cercariae.

Mice infection, parasite recovery and species identification

Methods for mice infection follow previously published procedure (Boissier, & Mone, 2000; Boissier, et al., 2003). The sex, species and number of cercariae combination used for each mouse exposure are shown in table 1.

Table 1. Number of cercariae used for each experiment according to sex and species of parasite

Experiment Number	<i>S. bovis</i>		<i>S. mansoni</i>		Number of mice
	Males	Females	Males	Females	
Limited choice experiment					
1. limiting sex: female <i>S. bovis</i>	60	60	60	--	8
2. limiting sex: female <i>S. mansoni</i>	60	--	60	60	8
3. limiting sex: male <i>S. bovis</i>	60	60	--	60	8
4. limiting sex: male <i>S. mansoni</i>	--	60	60	60	8
Full choice experiment					
5. no limiting sex	45	45	45	45	8
Forced choice experiment					
6. F1 hybrid	--	100	100	--	4
6. F1' hybrid	100	--	--	100	4

The experimental design to quantify the frequency of homo- and hetero-specific coupling between *S. bovis* and *S. mansoni* is made of five experiments (n°1-5 - table 1). Experiments n° 1-4 aimed to test the individual choice of each species and sex. In experiments 1 and 2, we tested the female choice for *S. mansoni* and *S. bovis*. In experiments 3 and 4, we tested the male choice for *S. mansoni* and *S. bovis*. Experiments 1-4 served as a restricted choice of mate where excess of one sex of the two species compete for pairing, while ensuring that all individuals of the other sex that

had the choice for homo- or hetero-specific mating will be paired. Experiment n° 5 served as full choice of mate. Mice were infected with the same number of cercariae of both sexes and species so that we can evaluate all combination of pairing simultaneously. Experiments 6. F1 and F1' hybrids are forced hybridization experiments. These last crosses were designed to obtain a first generation of hybrid miracidia in order to know whether these hybrids are compatible with the snail intermediate host.

After cercarial exposure, the mice were euthanized at two months and adult worms recovered by hepatic perfusion. We used magnifier lens and a small paintbrush to separate the worms according to their sex (male or female). Each worm whatever mated or unmated was placed in a 1.5 micro tube and labeled appropriately. Tubes containing worms were stored in the freezer at -20°C for genomic analysis. The species of all worms (mated and unmated) were identified using DNA extraction and amplification methods (see Supplementary File for detailed molecular biology protocols)

Mollusc exposition with F1 and F1' hybrid miracidia

Albino mice infected with male *S. mansoni* x female *S. bovis* cercariae and vice versa (F1 & F1'), were euthanized at two months and eggs from the livers were hatched to produce hybrid miracidia (Boissier, & Mone, 2000; Boissier, et al., 2003). 40 miracidia of each cross were store on FTA card (Kebede, et al., 2020). From these miracidia, DNA were extracted and a part of the ITS2 gene was sequenced for 66 specimens (see molecular biology methods supplementary file for details). The sequence has been compared to reference sequences from Genbank database (AF531314.1 for *S. mansoni* and FJ588862.1 for *S. bovis*). We used 24-wells plate to expose 48 *Biomphalaria glabrata* snails each of Brazil and Guadeloupe strains individually overnight with 10-12 F1 or F1' hybrid miracidia. The same procedure was used to expose *Bulinus truncatus* (Morocco and Spain strains). The protocol for molluscs infection with F1 and F1' hybrid miracidia is shown on table 2 below. The molluscs were separated into eight breeding tanks according to their infected hybrid miracidia (F1 or F1'), snail species (*B. glabrata* or *B. truncatus*) and snail strain (Brazil, Guadeloupe, Morocco or Spain). Snails were fed *ad libitum* for a duration of 60 days for miracidia to develop to cercariae. Molluscs in 24 well-plates according to group were stimulated under light to emit cercariae.

Table 2. Snail infection with F1/F1' miracidia for compatibility. Sb: *S. bovis*, Sm: *S. mansoni*

Exp	Intermediate snail host strain	Number of snails exposed	Hybrid miracidia
A	<i>Biomphalaria glabrata</i> (Brazil)	48	♂Sb x ♀Sm (F1')
B	<i>Biomphalaria glabrata</i> (Guadeloupe)	48	♂Sb x ♀Sm (F1')
C	<i>Biomphalaria glabrata</i> (Brazil)	48	♀Sb x ♂Sm (F1)
D	<i>Biomphalaria glabrata</i> (Guadeloupe)	48	♀Sb x ♂Sm (F1)
E	<i>Bulinus truncatus</i> (Morocco)	48	♂Sb x ♀Sm (F1')
F	<i>Bulinus truncatus</i> (Spain)	48	♂Sb x ♀Sm (F1')
G	<i>Bulinus truncatus</i> (Morocco)	48	♀Sb x ♂Sm ♂ (F1)
H	<i>Bulinus truncatus</i> (Spain)	48	♀Sb x ♂Sm ♂ (F1)

Statistical analysis

The total worm number of adult worms recovered for each schistosome species was counted, e.g., homo-specific pairs, hetero-specific pairs and single worms. We used the null hypothesis of random pairing to calculate the expected number of single and paired worms. For e.g., in experiment 1 the expected number of homo-specific paired *S. bovis* females equals the total number of *S. bovis* females, times the total number of *S. bovis* males over the total number of males. We used the Chi-square tests with Yates correction for continuity to compare the expected and observed numbers of homo- and hetero-specific pairs. The p-value was adjusted for multiple comparisons using Benjamini & Hochberg method. Statistical analyze was done using R Studio v1.4.1106.

Results

Table 3. Summarized information (limited choice experiments: exp1-4), to show number of paired (homo- and hetero-specific) and unpaired worms recovered from mice exposed to a limited

combination of cercariae. Expected number of pairs under random mating is shown in brackets.

Sb: *S. bovis*, Sm: *S. mansoni*

Exp	Host	Choosing partner	Homo-specific pairs	Hetero-specific pairs	Single worms	
1.	Mouse	♀Sb	♀Sb x ♂Sb	♀Sb x ♂Sm	♂Sm	♂Sb
	1		2	0	5	4
	2		8	4	7	4
	3		1	0	5	6
	4		5	3	5	3
	5		6	6	5	5
	6		4	3	4	5
	7		1	12	0	1
	8		1	1	1	0
Total			28 (30)	29 (27)	32	24
2.		♀Sm	♀Sm x ♂Sm	♀Sm x ♂Sb	♂Sm	♂Sb
	1		5	1	7	8
	2		13	4	0	1
	3		8	1	6	4
	4		12	0	6	13
	5		4	3	0	0
	6		8	2	0	3
	7		9	1	0	1
	8		7	6	0	0
Total			66 (54)	18 (30)	19	30
3.		♂Sb	♂Sb x ♀Sb	♂Sb x ♀Sm	♀Sm	♀Sb

1		4	2	2	3
2		1	3	6	4
3		1	7	2	2
4		3	6	4	8
5		0	5	4	4
6		0	3	2	1
7		0	1	7	4
8		2	2	2	4
Total		11 (16)	29 (24)	29	30
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4.	♂Sm	♂Sm x ♀Sm	♂Sm x ♀Sb	♀Sm	♀Sb
1		15	1	11	0
2		8	0	1	4
3		4	0	5	4
4		5	0	0	0
5		9	1	0	2
6		8	2	0	9
7		6	2	0	1
8		12	3	0	3
Total		67 (55)	9 (21)	17	23
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Table 4. Summarized information of homo-specific pairs, hetero-specific pairs and unpaired worms recovered from mice exposed in simultaneous infections to full combination of cercariae. Expected number of pairs under random mating is shown in brackets. Sb: *S. bovis*, Sm: *S. mansoni*

Exp 5.	Homo-specific	Homo-specific	Hetero-specific	Hetero-specific	Single worms			
Mouse	♂Sm x ♀Sm	♂Sb x ♀Sb	♂Sm x ♀Sb	♂Sb x ♀Sm	♂Sm	♂Sb	♀Sm	♀Sb
1	6	3	1	0	3	4	0	0
2	4	2	3	0	1	1	0	0
3	5	3	6	0	3	3	0	1
4	5	5	3	0	0	1	0	0
5	9	4	4	0	4	2	0	1
6	6	2	2	0	1	2	0	0
7	5	3	5	0	0	0	0	2
8	6	5	1	0	0	1	0	0
Total	46 (30)	27 (18)	25 (36)	0 (15)	12	14	0	4

For each experiment, Table 2 shows the sex and species of the choosing partner e.g., in experiments 1 and 2, male competition for limited female partners, and in experiments 3 and 4, male preference for homo- or hetero-specific mating. Random pairing is observed when *S. bovis* is species who choose whatever male ($\chi^2=2.73$; $p=0.8$ after Benjamini & Hochberg correction) or female ($\chi^2=0.29$; $p=1.00$ after Benjamini & Hochberg correction). Homo-specific are more numerous than heterospecific pairs when *S. mansoni* is the species who choose whatever male ($\chi^2=9.85$; $p=0.016$ after Benjamini & Hochberg correction) or female ($\chi^2=7.66$; $p=0.048$ after Benjamini & Hochberg correction). Table 3 shows the number of homo-, hetero-specific pairs and single worms when

mice are exposed to equal number of cercariae whatever their sex and their species. The number homo-specific pairs are more numerous than expected under the hypothesis of random association ($\chi^2=31.86$; $p<0.001$ after Benjamini & Hochberg correction). No ♂Sb x ♀Sm pairs are observed because all *S. mansoni* female has been monopolized by male *S. mansoni*.

Table 5. Compatibility of F1 hybrids of *S. bovis* (male) x *S. mansoni* (female) and *S. mansoni* (male) x *S. bovis* (female) in *Biomphalaria glabrata* and *Bulinus truncatus*. Each snail was exposed to 12 miracidia. Sb: *S. bovis*, Sm: *S. mansoni*

Exp	Snail species	Hybrid miracidia	Snails exposed	Snails surviving	Snails infected	% of snails infected
A	<i>B. glabrata</i> (Brazil)	♂Sb x ♀Sm (F1')	48	46	23	50
B	<i>B. glabrata</i> (Guadeloupe)	♂Sb x ♀Sm (F1')	48	48	10	20.8
C	<i>B. glabrata</i> (Brazil)	♀Sb x ♂Sm (F1)	48	48	0	0
D	<i>B. glabrata</i> (Guadeloupe)	♀Sb x ♂Sm (F1)	48	48	0	0
E	<i>B. truncatus</i> (Morocco)	♂Sb x ♀Sm (F1')	48	29	0	0
F	<i>B. truncatus</i> (Spain)	♂Sb x ♀Sm (F1')	48	45	0	0
G	<i>B. truncatus</i> (Morocco)	♀Sb x ♂Sm ♂ (F1)	48	30	1	3.3
H	<i>B. truncatus</i> (Spain)	♀Sb x ♂Sm ♂ (F1)	48	47	5	10.6

Table 5 shows compatibility of F1 (female *S. bovis* x male *S. mansoni*) and F'1 (male *S. bovis* x female *S. mansoni*) miracidia with the intermediate snail hosts. F1' hybrid miracidia readily infect *B. glabrata* from Brazil (50%) and from Guadeloupe (20.8%). F1 hybrid miracidia readily infect in *B. truncatus* from Spain (10.6%) and from Morocco (3.3%). Loss of compatibility was noted in F1 miracidia for *Biomphalaria* and in F1' miracidia for *Bulinus* snail. 505 base pairs of the ITS2 nuclear gene has been sequence for 66 miracidia. On the 33 sequences of the F1 miracidia all

exhibit a *S. bovis* gene profile (no heterozygous profile) and on the 33 sequences of the F1' miracidia all exhibit a *S. mansoni* gene profile (no heterozygous profile).

Discussion

Several studies have shown that in experimental mixed infections, there are no physiological barriers preventing encounters and mating of schistosomes of different species, even species belonging to a different genus in the definitive host (Jourdane, et al., 1995). Our overall findings on the experimental combinations between *S. mansoni* males' x *S. bovis* females and vice versa demonstrated that i) mating between the two *Schistosoma* species is not random ii) *S. mansoni* exhibits greater mate recognition compared to *S. bovis* iii) the progeny is parthenogenetic and as a consequence iv) the mollusc host spectrum of the F1 progeny is limited to the mother host spectrum.

Random pairings have been observed in crosses between *S. haematobium* x *S. bovis* (Kincaid-Smith, et al., 2021a), *S. bovis* x *S. curassoni* (Rollinson, et al., 1990a), *S. haematobium* x *S. intercalatum* (Southgate, et al., 1982) and *S. intercalatum* x *S. guineensis* (Pagès, et al., 2001). Our study outcome shows mate choice recognition, and is in tandem with results obtained in mixed infections of *S. haematobium* x *S. mattheei*, (Southgate, et al., 1995) or *S. mansoni* x *S. intercalatum*. Mate recognition seems to be dependent on the genetic proximity of the interacting species: *S. bovis*, *S. curassoni*, *S. intercalatum*, *S. haematobium* and *S. guineensis* are more related among each other compared to *S. haematobium* and *S. mattheei* and even worse between *S. mansoni* and *S. bovis* or *S. intercalatum*.

Our study also shows that *S. mansoni* exhibited greater specific mate preference than *S. bovis* and this indicates that intra-*S. mansoni* recognition is stronger than intra-*S. bovis* attraction, itself stronger than inter-specific attraction and this attest to differences between intra- and inter-specific chemo-attractants (Fried, & Haseeb, 1990). Evidence of *S. mansoni* exhibiting greater mate recognition than *S. intercalatum* (Tchuem Tchuente, et al., 1993) and *S. haematobium* exhibiting greater mate recognition than *S. mattheei* (Southgate, et al., 1995) have been reported and our results further buttress the existence of mechanisms in *Schistosoma* species favoring the pairing of homo-specific partners (Tchuem Tchuente, et al., 1993). In addition, it has been reported that *S. mansoni* males show a stronger competitiveness at coupling with females of either genotype than *S. intercalatum* males. It has been demonstrated that the former will change partner to mate with

conspecific females in preference to hetero-specific females whenever the opportunity arise (Southgate, et al., 1998). Also, it has been evidenced that in the absence of *S. mansoni* female worms, unpaired *S. mansoni* male worms that arrive in a pre-established *S. intercalatum* infection could pull away female *S. intercalatum* from male *S. intercalatum* (Southgate, et al., 1998).

Depending on the evolutionary lineage of the species, the interspecies sexual interactions in schistosomes will lead to either hybrids offspring or parthenogenetic offspring (Taylor, 1970; Jourdane, & Southgate, 1992). Our experimental design on mating interactions between *S. mansoni* males' x *S. bovis* females (two species of schistosomes that belong to two different evolutionary lineages) and vice versa shows that the F1 progenies are parthenogenetically produced. Within the family of schistosomatidae, parthenogenesis has been reported in some species (Vogel, 1941; Short, 1952; Taylor, et al., 1969; Taylor, 1970; Taylor, 1971; Basch, & Basch, 1984). Apart from female *Schistosomatium douthitti* which regularly mature and produce numerous viable eggs in unisexual infections (Short, 1952), the induction of parthenogenesis in other female schistosomes has been evidenced in crosses with males from a different species group. Experimental studies have shown that incomplete parthenogenesis, with the production of non-viable eggs occurs in female *S. mattheei* (Taylor, et al., 1969; Taylor, 1971) and in female *S. haematobium* (Sahba, & Malek, 1977) in single sex infections. However, in an induced parthenogenesis in crosses between *S. mansoni* males and *S. mattheei* females (Armstrong, 1965; Taylor, 1970) *S. mattheei* females which are not well developed in unisexual infections reached complete maturity and produced viable eggs typical of the maternal species when they were paired with *S. mansoni* males. Basch, & Basch, (1984) have shown parthenogenesis in *S. mansoni* females paired with *S. douthitti* males. The paired females reached sexual maturity and produced viable eggs without having been inseminated. Such eggs produced miracidia compatible only with the snail corresponding to the maternal species (*B. glabrata*). Sporocysts arising from this miracidia were haploid and produced cercariae infective to mice. Both haploid male and female cercariae were obtained. The uniparental maternal origin of offspring originating from each kind of pair has been confirmed using species-specific marker. Our result is in tandem with findings evidenced in several parthenogenesis models in *Schistosoma* species: the insemination of female worms by non-conspecific males has been clearly demonstrated (Imbert-Establet, et al., 1994; Tchuente, et

al., 1994). However, as pointed out by Whitfield, & Evans, (1983), this sexual interaction suggests the possibility of parthenogenesis with pseudogamy (joining of sperm with the egg cell, but without contribution of sperm to the egg nucleus), as happens in some organisms with parthenogenetic reproduction in which the male is always required (Bogart, 1980).

The present data reflects significant outcome of the infectivity of the *S. mansoni* males' x *S. bovis* females and vice versa F1 hybrid larvae with their two parental intermediate snail hosts. Analysis of the data obtained suggests that there are disparities between the infectivity of *S. mansoni* males' x *S. bovis* females and vice versa F1 hybrid larvae in the intermediate snail hosts *B. glabrata* and *B. truncatus* (table 5). We observed that the F1 progeny can only infect the snail corresponding to the mother species. Loss of compatibility was noticed in F1 miracidia for *B. glabrata* and in F1' miracidia for *B. truncatus*. This results outcome was not in tandem with the results obtained in *S. haematobium* x *S. intercalatum* hybrid larvae infectivity with *B. truncatus* and *B. forskalii* snail intermediate hosts (Webster, & Southgate, 2003a) and *S. mansoni* males x *S. japonicum* females and vice versa F1 hybrid miracidia compatibility with *Oncomalania h. chiui* and *B. glabrata* intermediate snail hosts (Fan, & Lin, 2005).

Conclusion

The production of hybrids offspring in laboratory experiments is a useful approach to determine levels of reproductive compatibility or incompatibility in schistosome species. This will also predict if this hybrid offspring could be evidenced in the field. Our study demonstrated that pairing is possible between *S. mansoni* and *S. bovis* with the production of viable parthenogenetic offspring. These new hybrids can develop into new emerging pathogen, necessitating new control strategies in zones where both parental species overlap (Huyse, et al., et al., 2009).

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CHAPTER V, Discussion

Schistosomiasis is a debilitating chronic disease affecting both humans' and livestock. It is considered as one of the neglected tropical diseases (NTDs) by the World Health Organization (WHO) and ranks second only to malaria in terms of public health importance. The disease affects more than 200 million people worldwide (Hotez, et al., 2009; Colley, et al., 2014) in low and middle-income countries in the sub-Saharan African (SSA) regions and accounts for almost 200,000 deaths annually (King, 2010), with mainly school-age children and young adults that constitute the highest population at risk for the disease. Extreme poverty, lack of knowledge of the risks, and inadequate or total lack of public health facilities along with the unsanitary conditions in which millions of people live their daily lives especially in the rural areas of developing countries, contribute to the risk of the disease (WHO, 2002).

Nigeria is one of the most impacted SSA country by both urinary and intestinal schistosomiasis and accounts for about 14% of the disease worldwide (Hotez, et al., 2012; Herrick, et al., 2017). Nigeria also has the highest number of people that are in need of preventive chemotherapy for this disease (Uchendu, et al., 2017). *S. haematobium* and *S. mansoni* have been evidenced to be endemic in Nigeria, but the latter is less prevalent with lower geographical coverage (Garba, et al., 2004). *S. bovis*, an animal infecting parasite has also been reported in some parts of the country (Ndifon, et al., 1988; Zamdayu, et al., 2019). The overlapping distribution of these *Schistosoma* species could enhance the chance of co-infection and the potential severe effect on morbidity. My PhD started with a large parasitological survey on 5,514 primary school-age pupils in 12 sites in the south of Nigeria that revealed an overall prevalence of 7.1%. The prevalence ranged from 4.6% in east Nigeria to 15.9% in west Nigeria. The highest prevalence was recorded in west Nigeria, while the lowest were in east part of the country. Thanks to a questionnaire I have evaluated the risk factors associated to schistosome infection. The significant risk factors associated with *S. haematobium* infection are frequent contact with freshwater bodies during washing, swimming and fishing. For socioeconomic factor, primary education of fathers was significantly associated with the disease and the sociodemographic factor age group 12-14 years was significantly associated with the infection. This survey outcome showed a fall in prevalence in most of the studied areas when compared with results of the past years (ranging from 4.9% to 15.9). This

suggests the efforts rolled out in the past several years to fight the disease seem to bring positive results. Our result outcome showed similarities to the report from the assessment of preventive chemotherapy on schistosomiasis prevalence in 44 SSA countries which showed global reduction, comparing 2000-10, with 2011-14 and 2015-19 periods (Kokaliaris, et al., 2022). Assessment of changes in *Schistosoma* species prevalence were estimated by a categorical variable with values corresponding to the three-time periods 2000-10, 2011-14 and 2015-19 during which preventive chemotherapy was scaled-up. Before the early 2000s, mass preventive chemotherapy using praziquantel (PZQ) was uncommon in SSA. However, from 2005 when 250 million doses of PZQ were provided every year by Merck KGaA (Germany), WHO report showed that by 2018, about 76.2 million (61.2%) school-age children and 19.1 million (18.2%) adults were treated with PZQ (WHO, 2018). By contrast, only 7 million people received PZQ treatment in 2006 and this emphasize why all countries had lower prevalence than 2000-10 period. The results outcome of these assessment showed that the prevalence of schistosomiasis among school-age children in 44 SSA countries decreased from 23.3% in 2000-10 to 9.6% in 2015-19; indicating an overall reduction of 58.3%. The reduction of 67.9% (*S. haematobium*) and 53.6% (*S. mansoni*) was noticed when comparing 2000-10 with 2015-19 periods. This three-time periods assessment also showed that complementary interventions such as access to clean water, improved sanitation (Grimes, et al., 2014; Lo, et al., 2017) snail control and behaviour change (King, et al., 2015; Sokolow, et al., 2016) will all lower the risk for infection and stop the parasite transmission. These interventions have not been applied widely in SSA countries due to insufficient human and financial resources and challenges identifying water bodies harbouring intermediate-host snails (Tchuem Tchuente, et al., 2017). Also, the association between closeness to freshwater bodies with higher risk of the parasite and the majority of high risk settings found in the vicinity of large rivers (Niger River) or lakes (Lake Victoria) is in tandem with our findings.

There is increasing evidence for natural hybridization between *Schistosoma* species possibly enhanced by increased environmental changes. Species interaction and inter-breeding has led to increased number of parasites species with hybrid progenies been more harmful than the parent species, show host range expansion, host morbidity as well as complicate parasite diagnosis. Depending on the evolutionary lineage of the species, the heterologous sexual interactions in schistosome species will lead to either hybrid (introgression) or parthenogenetic offspring (parthenogenesis) (Jourdane, et al., 1995; Southgate, et al., 1998). During my PhD, I have analyzed

hybrid Schistosomes using both field and experimental approaches. Regarding the field, I focused my study on *S. haematobium* x *S. bovis* cross, a hybrid form with a broad repartition in west Africa. Regarding the experimental approach, I have studied the interaction between *S. mansoni* and *S. bovis*.

Table 1 summarizes all observations of potential natural hybridization among *Schistosoma* species. Although, early suspicion of human infections with pure animal *Schistosoma* species such as *S. bovis*, *S. curassoni* or *S. mattheei* (Raper, 1951; Grétilat, 1962; Albaret, et al., 1985; Chunge, et al., 1986; Mouchet, et al., 1988) based on egg morphologies that were dismissed as misdiagnosis (Capron, et al., 1965; Vercruyssen, et al., 1984; Rollinson, et al., 1987; Kruger, & Evans, 1990; Brémond, et al., 1993) have been confirmed with molecular markers. Molecular tools have revealed new hybridization between different species and some individuals previously considered to be *S. haematobium* based on egg morphology and sequence data alone, have been confirmed to be hybrids by use of high-resolution analysis.

The most widely distributed and studied hybrid is *S. haematobium* x *S. bovis* (Table 1). This hybrid was first identified in Senegal (Huyse, et al., 2009). Since 2009 it has been identified in several west African countries like Benin, Cote d'Ivoire, Mali, Cameroon and Niger (Huyse, et al., 2013; Leger, & Webster, 2016; Angora et al., 2020). I have assessed the percentage of these latter hybrid in my country using molecular genotyping using Cox1 gene. Of the 1,364 miracidia, I have showed a *S. bovis* Cox1 1,212 (89%) profile and *S. haematobium* Cox1 152 (11%) profile. All studied sites except two showed 100% of *S. bovis* Cox1 haplotypes. This study revealed for the first time that *S. haematobium-bovis* hybrids are predominant in the *Schistosoma* eggs isolated in the urine samples of primary school-age pupils in Nigeria. Our findings provide evidence that *S. haematobium* x *S. bovis* hybrids are common in Nigeria

Table 1. List of various combinations of *Schistosoma* species hybrids documented within snails, livestock, wildlife and humans

<i>Schistosoma</i> species combination and original host	Host of hybrid offspring	Countries	Reference
<i>S. haematobium</i> (H) x <i>S. mattheei</i> (L)	Human, cattle*	Zimbabwe, S/Africa, Zambia & Mali	Alves, 1948; Le Roux, 1954b; Pitchford, 1959; Pitchford, 1961; Wright, & Ross, 1980, Kruger, et al., 1986a; Kruger, et al., 1986b; Kruger, 1987, 1988; Kruger, 1990; Kruger, & Hamilton-Attwell, 1988; Kruger, & Evans, 1990; De Bont, et al., 1994*; Vercruyssen, et al., 1994, 1994*;
<i>S. haematobium</i> (H) x <i>S. mattheei</i> (L)	Multimammate mouse	S/Africa	Kruger, et al., 1986a, 1986b; Kruger, 1987, 1988, Kruger, 1990; Kruger, & Hamilton-Attwell, 1988; Kruger, & Evans, 1990.
<i>S. haematobium</i> (H) x <i>S. guineensis</i> (H)	Human	Cameroon, Gabon & Benin	Wright, et al., 1974; Southgate, et al., 1976; Burchard, & Kern, 1985; Rollinson, & Southgate, 1985; Zwingenberger, et al., 1990; Ratard, et al., 1990; Ratard, & Greer, 1991. Vercruyssen, et al., 1994; Tchuem Tchuenté, et al., 1997b; Webster, et al., 2003; Webster, et al., 2005; Moné, et al., 2012.

<i>S. haematobium</i> (H) x <i>S. guineensis</i> (H)	<i>Bulinus forskalii</i>	Cameroon	Rollinson, & Southgate, 1985
<i>S. haematobium</i> (H) x <i>S. guineensis</i> (H)	<i>Bulinus truncatus</i> , <i>B. cameruneesis</i>	Cameronn Cameroon	Webster, et al., 2003, 2005.
<i>S. haematobium</i> (H) x <i>S. mansoni</i> (H)	Human	Cameroon, Mali, Senegal & Kenya	Cunin, et al., 2003; Koukounari, et al., 2010; Huyse, et al., 2013; Gouvras, et al., 2013; Le Govic, et al., 2019; Depaquit, et al., 2019; Brémond, et al., 1993;
<i>S. haematobium</i> (H) x <i>S. bovis</i> (L)	Human	Niger, Senegal, France, Benin, Cote d'Ivoire & Cameroon	Huyse, et al., 2009; Webster, et al., 2013b; Boissier, et al., 2015; Moné, et al., 2015; Sene- Wade, et al., 2018; Angora et al., 2020; Djuikwo-Teukeng et al, 2022
<i>S. haematobium</i> (H) x <i>S. bovis</i> (L)	Multimammate mouse	Benin	Savassi, et al., 2020, 2021.
<i>S. haematobium</i> (H) x <i>S. bovis</i> (L)	<i>B. truncatus</i> <i>B. globosus</i>	Senegal Senegal	Huyse, et al., 2009.
<i>S. haematobium</i> (H) x <i>S. intercalatum</i> (H)	Human	E/Africa	Añé, et al., 1997.
<i>S. haematobium</i> (H) x <i>S. curassoni</i> (L)	Human	Senegal	Webster, et al., 2013b.

<i>S. bovis</i> (L) x <i>S. curassoni</i> (L)	Sheep, cattle*	Senegal, Niger Senegal*, Niger*, Mali*	Southgate, et al., 1985; Rollinson, et al., 1987*; Brémond, 1990, 1990*; Brémond, et al., 1990, 1990*; Rollinson, et al., 1990a*; Webster, et al., 2013b*.
<i>S. bovis</i> (L) x <i>S. curassoni</i> (L)	Goats	Niger	Brémond, 1990; Brémond, et al., 1990.
<i>S. mansoni</i> (H) x <i>S. rodhaini</i> (W)	<i>Biomphalaria</i> <i>sudanica</i> ,	Kenya	Morgan, et al., 2003; Steinauer, et al., 2008, 2008*.
	<i>B. pfeifferi</i> *	Kenya	
<i>S. mattheei</i> (L) x <i>S. leiperi</i> (L)	Cattle	Zambia	De Bont, et al., 1994; Vercruyssen, et al., 1994.

H: human; **L:** livestock; **W:** wildlife.

We used mitochondrial and nuclear genes in the molecular analysis of the samples. These markers are traditionally used for the identification of *Schistosoma* species. We performed full genotypes (both Cox1 and ITS2) on a sub-sample of 59 parasites. A discordance in the Cox1 (*S. haematobium* or *S. bovis*) and ITS2 (*S. haematobium* or *S. bovis*) profiles shows *S. haematobium* x *S. bovis* hybrids miracidia to be present. This molecular barcoding method does not deduce the genomic introgression level. A broader coverage of the parasite whole genome sequencing would no doubt identify even more hybrids and would allowed a clearer distinction between pure parasites of each species and different levels of introgression of *S. bovis* genome tracts into *S. haematobium* lineages (Platt, et al., 2019). Importantly however, individuals harboring both a nuclear and a mitochondrial genetic signature of *S. bovis* are seldom detected in humans' (Boissier, et al., 2016; Angora et al., 2020). More recently, individuals biologically characterized as *S. haematobium* (according to egg morphology and chronobiology) and genetically identified as *S. haematobium-bovis* hybrids were detected in cattle (Savassi, et al., 2020) and rodents (Savassi, et al., 2021) for the first time in Benin. Together these results suggest that current simultaneous infection of animals (cows, rodents) by *S. haematobium* and of humans by *S. bovis* may occur at some level but it is not common. However, the genomic composition of those individuals from Benin has not been characterized to ascertain the possibility of introgression. More thorough analysis using single nucleotide polymorphisms (SNPs) and studies on animals (rodents, cows) need to be conducted to elucidate the frequency of zoonotic transmission. There is need to conduct a genomic comparative study to investigate the genomic relationship between the *S. haematobium*, *S. bovis* and their hybrids (Rey, et al., 2021a). New research is needed on population genetics of schistosomes at the human-animal interface to evaluate the parasites' gene flow.

The main concern with *S. haematobium* x *S. bovis* hybrid is certainly it's zoonotic capacity. Two recent genome-wide study has suggested that hybridization between *S. bovis* and *S. haematobium* would certainly be an ancient event (Platt, et al., 2019; Rey, et al., 2021a). An ancient origin does not mean that the genetic mixture between *S. haematobium* and *S. bovis* would not always be ongoing. In a study performed in Cameroon the authors showed a positive link between the percentage of hybrids and the genetic diversity measured by both allelic richness and heterozygosity (Djuikwo-Teukeng, et al., 2022). I could not test this link in my study because contrary to Cameroon where the percentage of hybrid ranges from 5% to 20%, there was little or no variations in the percentage of hybrids in the different populations I have sampled in Nigeria.

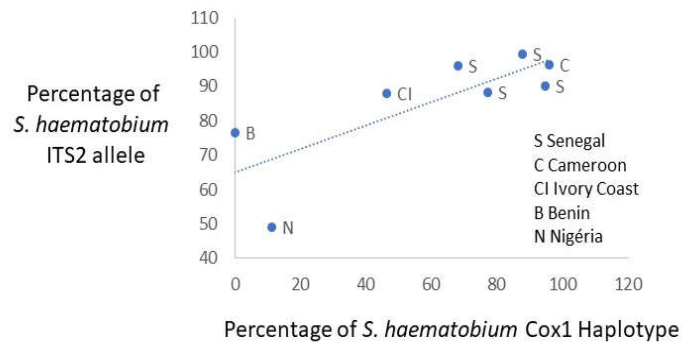
This link between hybrid percentage and genetic diversity highlights the fact that hybridization is a major biological process that shapes the genetic diversity of *S. haematobium*. In table 2, I have referenced the study using the same method (Cox1/ITS2 genotyping) to identify *S. haematobium* x *S. bovis* hybrids in different countries. This table shows contrasted situation among the different countries with a percentage of hybrid ranging from 11.3% in Cameroon to 89% in Nigeria. We can also observe consistent results when several studies are performed in the same country (i.e. Senegal).

Table 2: Studies genotyping *S. haematobium* x *S. bovis* hybrids in West Africa (sample size in parenthesis)

Percentage of hybrid parasites (number of miracidium)	Patients infected by at least one hybrid parasite (number of patient)		ITS2. <i>S. haematobium</i> allele (number of miracidium x2)		Country	Reference
	Cox1 <i>S. haematobium</i> haplotype (number of miracidium)	Heterozygous ITS2 (number of miracidium)				
11.3% (1,327)	81.8 (55)	95.8% (1,327)	96.2% (2,654)	7.5% (1,327)	Cameroon	Djuikwo-Teukeng et al., 2019
21% (823)	88% (52)	94.7% (823)	90.1% (1,646)	9% (823)	Senegal	Webster, et al., 2013b
22.6% (164)	NA	77.2% (570)	88.2% (328)	0.6% (164)	Senegal	Boon et al., 2019
NA	NA	87.7% (1,200)	99.3% (492)	1.2% (246)	Senegal	Boon, et al., 2018
37% (81)	63% (11)	68% (81)	96.0% (162)	4.3% (81)	Senegal	Sene-Wade, et al., 2018
29.8% (47)	100% (5)	0% (47)	76.6% (94)	29.8% (47)	Benin Cote	Savassi, et al., 2020, 2021
62.7% (2,164)	97.8% (91)	46.3% (2,164)	87.9% (4,328)	18.8% (2,164)	d'Ivoire	Angora et al., 2020
89% (1,364)	100%	11.1% (1,364)	49.1% (118)	41.3% (59)	Nigeria	My study

Interestingly, we also observe a positive link between the percentage of Cox1 haplotype and ITS2 *S. haematobium* allele (n=8, r²=74%, p<0.05) (Figure 1).

Figure 1. Relationship between the percentage Cox1 haplotype and ITS2 *S. haematobium* allele in some West African countries.



This link may seem obvious, but it shows that the process of genetic introgression is not the same in all countries and that there is certainly an ongoing genetic mixing between species. This genetic mix can exist if an animal is implicated in the transmission (i.e. zoonotic transmission). Despite the dramatic consequences of zoonotic transmission, the research on *S. haematobium* x *S. bovis* in animal have concerned few studies. No hybrids have been found in cows in Cameroon (Djuikwo-Teukeng et al., 2019) or in Senegal (Boon, et al., 2018; Sene-Wade, et al., 2018; Boon et al., 2019; Webster, et al., 2013b) but they have been found in both cows and rodent in Benin (Savassi, et al., 2020; Savassi, et al., 2021) and in rodent in Senegal (Catalano, et al., 2018). The research on *S. haematobium* x *S. bovis* hybrids in animals in Nigeria need to be done.

An alternative way to study hybrid schistosome in the field is to analyze mating interactions and resulting progeny in the lab. Table 3 shows several experimental infections and crossing outcomes.

Crossing outcome between two phylogenetically distant species have been reported to result in the production of non-viable or apparently parthenogenetic eggs: *S. mansoni* x *S. haematobium*, *S. guineensis*, *S. mattheei*, *S. japonicum* or *S. bovis* resulted in non-viable or parthenogenetic eggs (Taylor, et al., 1969; Taylor, 1970; Tchuente, et al., 1994; Khalil, & Mansour, 1995; Webster, et al., 1999). However, several evidence have confirmed that certain closely related schistosomes (*S. haematobium* x *S. guineensis*; F7, *S. mattheei*; F4, *S. bovis*; F3, and also in *S. mansoni* x *S. rodhaini*; F4) can successfully pair and hybridize both experimentally and in the wild for several generations (Taylor, 1970; Morgan, et al., 2003; Webster, & Southgate, 2003b; Webster, et al., 2013b). Our result outcome on the experimental combinations between *S. mansoni* males' x *S. bovis* females and vice versa show that pairing between the two *Schistosoma* species is possible

but not random. *S. mansoni* shows greater mate recognition compared to *S. bovis* and the sibling species exhibited uniparental maternal origin. Random pairings have been observed in crosses between *S. haematobium* x *S. bovis* (Kincaid-Smith, et al., 2021a) *S. bovis* x *S. curassoni* (Rollinson, et al., 1990a), *S. haematobium* x *S. intercalatum* (Southgate, et al., 1982) and *S. intercalatum* x *S. guineensis* (Pagès, et al., 2001). Our study outcome shows mate choice recognition, and is in agreement with results obtained in mixed infections of *S. haematobium* x *S. mattheei*, (Southgate, et al., 1995) or *S. mansoni* x *S. intercalatum* (Tchuem Tchuente, et al., 1993). Mate recognition seems to be dependent on the genetic closeness of the interacting species: *S. bovis*, *S. curassoni*, *S. intercalatum*, *S. haematobium* and *S. guineensis* are more related among each other compared to *S. haematobium* and *S. mattheei* and even more distant between *S. mansoni* and *S. bovis* or *S. intercalatum* (Lawton, et al., 2011). Our results are in agreement with the few result outcomes of (Taylor, 1970) in the pairing between male *S. mansoni* x female *S. bovis* which resulted to the production of numerous eggs typical of *S. bovis* phenotype, but only few contained viable miracidia which failed to infect snails on several attempts.

Table 3. List of various documented experimental hybridizations.

<i>Schistosoma</i> species crossing	Crossing outcomes	Reference year
<i>S. mansoni</i> x <i>S. haematobium</i>	Low viable parthenogenetic offspring	Vogel, 1941, 1942; Basch, & Basch, 1984; Khalil, & Mansour, 1995; Webster, et al., 1999.
<i>S. mansoni</i> x <i>S. japonicum</i>	Viable parthenogenetic offspring	Vogel, 1941; Vogel, 1942; Imbert-Establet, et al., 1994; Fan, & Lin, 2005.
<i>S. mansoni</i> x <i>S. rodhaini</i>	Viable offspring up to F4	Le Roux, 1954a; Taylor, M.G., 1970; Taylor, & Andrews, 1973; Taylor, et al., 1973; Bremond, et al., 1989; Théron, 1989; Norton, et al., 2008b.
<i>S. mansoni</i> x <i>S. mattheei</i>	Parthenogenetic offspring viable up to F3	Taylor, et al., 1969; Taylor, 1970; Taylor, & Andrews, 1973; Taylor, et al., 1973.
<i>S. mansoni</i> x <i>S. bovis</i>	Non-viable offspring	Taylor, 1970; Taylor, & Andrews, 1973; Taylor, et al., 1973.
<i>S. mansoni</i> x <i>S. guineensis</i>	Low viable parthenogenetic offspring	Tchuem Tchuente, et al., 1993 ; Tchuem Tchuente, et al., 1994 ; Tchuem Tchuente, et al., 1995 ; Tchuem Tchuente, et al., 1996.
<i>S. mansoni</i> x <i>S. intercalatum</i>	Unknown	Cosgrove, & Southgate, 2003b.

<i>S. mansoni</i> x <i>S. margrebowiei</i>	Non-viable offspring	Cosgrove, & Southgate, 2002.
<i>S. haematobium</i> x <i>S. mattheei</i>	Viable offspring up to F4	Taylor, 1970; Taylor, & Andrews, 1973; Taylor, et al., 1973; Wright, & Ross, 1980; Kruger, & Evans, 1990. Rollinson, et al., 1990b; Southgate, et al., 1995; Tchuem Tchuente, et al., 1997a.
<i>S. haematobium</i> x <i>S. guineensis</i>	Viable offspring up to F7	Wright, et al., 1974; Wright, & Southgate, 1976; Southgate, et al., 1976; Southgate, et al., 1982; Mutani, et al., 1985 Rollinson, & Southgate, 1985); Pages, & Theron, 1990; Rollinson, et al., 1990b, Cosgrove, & Southgate, 2003a. Webster, & Southgate, 2003a; Webster, & Southgate, 2003b; Webster, et al., 2003; Webster, et al., 2005; Webster, et al., 2007.
<i>S. haematobium</i> x <i>S. bovis</i>	Viable offspring up to F3	Taylor, 1970; Taylor, & Andrews, 1973; Taylor, et al., 1973; Pages, & Theron, 1990; Brémond, et al., 1993; Webster, et al., 2013b.
<i>S. haematobium</i> x <i>S. curassoni</i>	Viable offspring up to F2	Webster, et al., 2013b.
<i>S. bovis</i> x <i>S. mattheei</i>	Very low viable offspring up to F3	Taylor, 1970; Taylor, & Andrews, 1973; Taylor, et al., 1973; Rollinson, et al., 1990b.

<i>S. bovis</i> x <i>S. curassoni</i>	Viable offspring up to F4	Rollinson, et al., 1990a; Brémond, et al., 1993; Webster, et al., 2013b.
<i>S. bovis</i> x <i>S. guineensis</i>	Viable offspring up to F1	Pages, & Theron, 1990.
<i>S. guineensis</i> x <i>S. mattheei</i>	Viable offspring up to F4	Wright, 1974.
<i>S. guineensis</i> x <i>S. intercalatum</i>	Viable offspring up to F4	Frandsen, 1978; Bjørneboe, & Frandsen, 1979; Pagès, et al., 2001; Pages, et al., 2002.
<i>S. japonicum</i> x <i>S. mekongi</i>	Viable offspring up to F1	Kruatrachue, et al., 1987.

The results outcome of the mating interactions between *S. mansoni* males' x *S. bovis* females and vice versa showed that the F1 sibling species can only infect the snail corresponding to the mother species: female *S. bovis* x male *S. mansoni* F1 siblings can only infect *Bulinus truncatus* snail while male *S. bovis* x female *S. mansoni* F1 siblings can only infect *Biomphalaria glabrata* snail. The loss of dual compatibility is explained by the fact that the offspring have uniparental maternal origin and as a consequence, compatibility with intermediate snail host is limited to the mother host spectrum. This result outcome is not in agreement with the reports of (Pages, et al., 2002; Webster, & Southgate, 2003a and Fan, & Lin, 2005).

Pages, et al., (2002) showed that hybrid miracidia produced from the pairing interactions between male *S. intercalatum* (CAM) and female *S. intercalatum* (ZAI) and vice versa, exhibited dual infectivity for each of the parental snail species: *Bulinus forskalii* and *B. globosus* for several generations. Also, Webster, & Southgate, (2003a) showed that in the pairing interactions between *S. haematobium* males' x *S. intercalatum* females, both hybrid crosses developed in both parental intermediate snail hosts, *B. truncatus* and *B. forskalii*, though infectivity was more with the former than the latter. Finally, Fan, & Lin, (2005), showed that sibling species resulting from pairing between two human *Schistosoma* species: *S. mansoni* males x *S. japonicum* females infected both *Biomphalaria glabrata* and *Oncomelania h. chiui* intermediate snail hosts, whereas sibling species from reciprocal crossing could only infect *B. glabrata*. The reports of Pages, et al., (2002) and Webster, & Southgate, (2003a) could be due to the fact that the two parasites involved in each of the experiments are phylogenetically closed and could share the same intermediate snail hosts. Whereas in Fan, & Lin, (2005), the two parasites involved are of high phylogenetic distance; but sometimes successful pairing could vary in part with geographical origin as well as the strain of the parasites (Taylor, 1970) and this may be responsible for the compatibility of the sibling species with parent intermediate snail hosts.

My PhD analyzes for the first-time mating interactions between *S. mansoni* males' x *S. bovis* females and vice versa in mixed infections.

Here, two co-endemic *Schistosoma* species, one is human intestinal parasite (*S. mansoni*) and the other is traditionally considered an intestinal parasite (*S. bovis*) of bovines, which are transmitted by specific intermediate freshwater snails, *Biomphalaria* species for the former and *Bulinus* species for the latter. On a biological point of view, these two species use different definitive hosts,

the former being associated with humans and the latter livestock. Though within their respective host, they do not differ in their tropisms: both parasites utilize the mesenteric system. It is generally accepted that the two *Schistosoma* species are not able to hybridize, due to the high phylogenetic distance between them. These three specific biological characteristics (different definitive and intermediate hosts and distant evolutionary lineage) greatly limit possible interspecific reproduction events between the two parasites (Rey, et al., 2021a). Crossing outcome between two phylogenetically distant species have been reported to result in the production of non-viable or apparently parthenogenetic eggs: *S. mansoni* x *S. haematobium*, *S. guineensis*, *S. mattheei*, *S. japonicum* or *S. bovis* resulted in non-viable or parthenogenetic eggs (Taylor, et al., 1969; Taylor, 1970; Tchuente, et al., 1994; Khalil, & Mansour, 1995; Webster, et al., 1999).

Our result show that the two parasite species can pair and produce viable parthenogenetic progenies in experimentally infected mice. We observed that mate preferences exist for *S. mansoni* but not for *S. bovis* and that the F1 progenies can only infect the snail corresponding to the mother species: female *S. bovis* x male *S. mansoni* F1 cross can only infect *Bulinus truncatus* snail while male *S. bovis* x female *S. mansoni* F1 cross can only infect *Biomphalaria glabrata* snail. The loss of compatibility is explained by the fact that the progeny exhibited uniparental maternal origin. Our findings on *S. mansoni* x *S. bovis* mating interactions and compatibility of F1 hybrids with the parental snail intermediate hosts is an addendum to several reports (Pages, et al., 2002; Webster, & Southgate, 2003a) on experimental hybridizations and compatibility of hybrids with parent intermediate snail hosts.

In conclusion, first, this study showed that schistosomiasis is prevalent in some parts of Nigeria, although, the overall prevalence is lower than reported in the past several years. This suggests the efforts rolled out in the past several years to combat the infection seem to yield positive results. Second, this finding revealed that *S. haematobium-bovis* hybrids are predominant in the *Schistosoma* eggs isolated in the urine samples of primary school children in Nigeria and provide evidence that *S. haematobium* x *S. bovis* hybrids are common in Nigeria. The population genetic analyses showed two main clusters; one each on the east and west part of the country. Third, this work showed that *S. mansoni* males' x *S. bovis* females and vice versa can pair and produce viable parthenogenetic siblings in experimentally infected mice. *S. mansoni* exhibited mate preference, but *S. bovis* did not and the F1 progenies can only infect the snail corresponding to the mother

species due to their uniparental maternal origin. Finally, I suggest research priority in domestic and wild animals to investigate the role of zoonotic transmission of this infection in combination with the reports of this study to better our understanding on schistosomiasis control and elimination activities.

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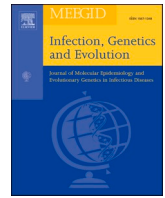
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Annex

Rey, O., Webster, B.L., Huyse, T., Rollinson, D., Van den Broeck, F., Kincaid-Smith, J., **Onyekwere**, A., Boissier, J., 2021. Population genetics of African *Schistosoma* species. *Infect Genet Evol* 89, 104727.



Review

Population genetics of African *Schistosoma* speciesO. Rey^{a,1}, B.L. Webster^{b,c,1}, T. Huyse^{d,e}, D. Rollinson^{b,c}, F. Van den Broeck^{f,g},
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ABSTRACT

Blood flukes within the genus *Schistosoma* (schistosomes) are responsible for the major disease, schistosomiasis, in tropical and sub-tropical areas. This disease is predominantly present on the African continent with more than 85% of the human cases. Schistosomes are also parasites of veterinary importance infecting livestock and wildlife. *Schistosoma* population genetic structure and diversity are important characteristics that may reflect variations in selection pressures such as those induced by host (mammalian and snail) environments, habitat change, migration and also treatment/control interventions, all of which also shape speciation and evolution of the whole *Schistosoma* genus. Investigations into schistosome population genetic structure, diversity and evolution has been an area of important debate and research. Supported by advances in molecular techniques with capabilities for multi-locus genetic analyses for single larvae schistosome genetic investigations have greatly progressed in the last decade. This paper aims to review the genetic studies of both animal and human infecting schistosome. Population genetic structures are reviewed at different spatial scales: local, regional or continental (i.e. phylogeography). Within species genetic diversities are discussed compared and the compounding factors discussed, including the effect of mass drug administration. Finally, the ability for intra-species hybridisation questions species integrities and poses many questions in relation to the natural epidemiology of co-endemic species. Here we review molecularly confirmed hybridisation events (in relation to human disease) and discuss the possible impact for ongoing and future control and elimination.

1. Introduction

Schistosomes are parasitic trematodes of mammals that are transmitted by freshwater intermediate molluscan hosts. A total of 23 described *Schistosoma* species belong to the *Schistosoma* genus (Boissier et al., 2019) (Fig. 1). Among trematodes, this genus is particularly well studied because six species are of medical importance. Schistosomes cause schistosomiasis or bilharziasis, a chronic neglected tropical disease (NTD). It is the most prevalent NTD in sub-Saharan Africa (Colley et al., 2014; Lai et al., 2015; McManus et al., 2018) and a major public health problem in several tropical and subtropical countries. The

number of schistosomiasis cases in the world is considered to be greater than 250 million, mostly in sub Saharan Africa and the DALYs index (“Disability-Adjusted Life Years”) was estimated at 1.4 (Hotez et al., 2014).

Schistosoma species have a two host life-cycle including a mammalian definitive host and a snail intermediate host. A unique feature of schistosomes is that they have separate sexes (i.e. gonochoric) whereas trematodes are typically hermaphroditic (Beltran and Boissier, 2008, 2010; Mone and Boissier, 2004). Within the mammalian hosts, highly sexually dimorphic male and female adult worms pair in the veins of either the peri-vesical vascular system (*S. haematobium*) or in the

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mesenteric system (most other species). This sexual reproduction ensures genetic mixing and the emergence of novel combinations of genes in each offspring. This allows schistosome populations to potentially adapt to selection pressures, both natural and anthropogenic, with natural selection leading to highly successful and diverse populations. The mammalian host spectrum varies greatly according to the species concerned. For instance, some species only infect humans (e.g. *S. guineensis*), others have a narrow host spectrum (e.g. *S. mansoni* infects humans, rodents and non-human primates) while *S. japonicum* is able to infect many mammalian species from various groups including Cetartiodactyla, primates, Rodentia, Carnivora, Insectivora or Lagomorpha (Pitchford, 1977), and is considered to be highly zoonotic. Multi host versus restricted host specificities appears to be evolutionary, with the multi host species being more ancestral. Host specificity will also affect population genetics, increasing diversity where multiple hosts are involved versus reduced diversity where schistosomes are more specific.

Eggs from adult worms are released via the urine (*S. haematobium*) or the faeces (other species) and hatch in freshwater systems, releasing the highly motile larva: the miracidium. This larva actively searches for a specific/compatible planorbid host. As with all trematodes the intermediate host spectrum is often narrow with clear species-specific compatibilities, which governs geographical species distributions. Within the snail, asexual reproduction occurs with the clonal production of the free swimming larvae: the cercariae which once released from the snail actively search for their mammal definitive hosts, penetrating the skin and infecting their host. This clonal replication is highly productive with a single miracidia resulting in many thousands of cercariae produced

over many weeks. The tight compatibilities between individual snails and their schistosomes leads to high selection pressures and reduced population variation, however the prolific cercarial production ensures ongoing transmission. It is clear that schistosomes have evolved unique life-history traits to maintain high-levels of transmission whilst maintaining populations diversity through alternate asexual and sexual life-cycle stages.

Molecular epidemiological studies provide valuable insights into the genetic variation of *Schistosoma* species and populations in time and space. This may reflect variations in selection pressures such as host (mammalian and snail) environments, habitat change, emergence in new areas and also treatment/control interventions, all of which will also shape speciation and evolution. Several population genetic studies have shown their importance in the identification of reservoir hosts, the potentiality/likelihood of drug resistance development and spread, the understanding of the recruitment pattern in each host and the genetic structure and gene flow in the context of disease control. This review aims to review several of these studies, highlights how dynamic both human and animal *Schistosoma* species are in terms of their ability to adapt and evolve. Certainly, evidence shows that *Schistosoma* species adapt quickly to new environments and challenges to ensure their survival, a trait that should not be underestimated in global efforts for control, elimination and eventual eradication.

2. Nested Russian doll genetic structuration

For *Schistosoma* spp., different levels of nested populations can be

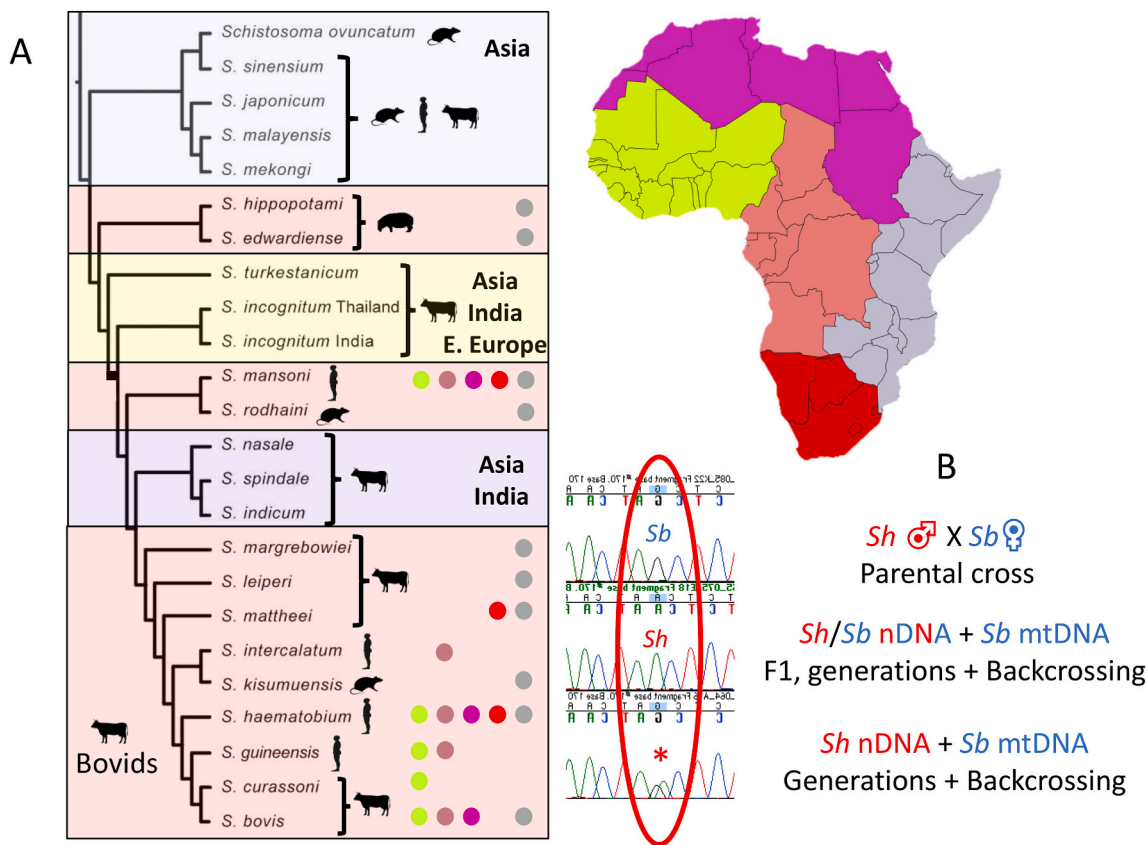


Fig. 1. A) Showing the interrelationships of the 23 *Schistosoma* species (adapted from Webster and Littlewood, 2012), the main definitive hosts and the geographical distribution. The African clades are shown in the red boxes and their fine scale African distribution shown by the coloured dots that correspond to the map, highlighting areas of sympatry and opportunities for inter-species interactions. B) Demonstrates the basic molecular profiling of *Schistosoma* hybrids. This shows maternal inheritance of the mitochondrial DNA from the maternal line and the mixed inheritance of the nuclear DNA, with the difficulty encountered with deciphering the hybrid generation or backcrossing. The sequence chromatograms show the pure and mixed signal in the nuclear data with * showing the double sequence chromatogram showing bi-parental inheritance of the nuclear DNA. Species specific SNP's within the nuclear ITS and 18S rDNA regions are used to identify hybrids, together with the maternal mitochondrial DNA.

defined as i) all the parasites (worms, sporocysts, excreted miracidia or emitted cercariae) from an individual host (i.e. infrapopulation) ii) all the parasites from a community iii) all the villages from a region and iv) all the regions of the area of repartition of a given species. Since schistosome transmission is restricted to fresh water bodies, the 'community' scale could be equivalent to a small number of transmission sites. The extent of gene flow between these populations will determine the degree of population structure at each level. Here we define three levels of spatial scales. The first level, usually referred to as the 'local scale', includes the study of subpopulations between hosts or spatial populations distanced from each other by 100's of metres to 10's of kilometres (Table 1). The second level, usually referred to as the 'regional scale', includes analyses of parasite subpopulations far from each other by 10's to 100's of kilometres (Table 1). The differentiation between these two first scales can be blurry. The third level, usually referred to as 'phylogeography', analyses parasites subpopulations far from each other by more than 100's of kilometres (i.e. between and within countries) and give insights on the evolutionary history of the species.

2.1. Local scale

For schistosomes or trematodes in general, an infrapopulation (i.e. all individuals from a same host individual) cannot be considered a single population because it depends on a continuous recruitment of new individuals during re-infection and not through intra-host natality. As for any population genetic study on schistosomes, the miracidial populations are used as a proxy of genetic diversity of parental populations locked inside the mammal host. Moreover, the fact that the parasite asexually reproduces inside the snail, creating clonal populations, means that different definitive hosts may be infected by genetically identical schistosomes. Theoretically, the structuration of the infrapopulation and the differentiation between infrapopulations is a balance between factors having tendencies to homogenize the population, and factors having tendencies to differentiate the parasite infrapopulations (Theron et al., 2004). At a local scale, it seems that the majority of these factors are expected to favour the homogenization of the population; (i) snails are not highly mobile within and between sites supporting infections of different definitive hosts with the same clonal schistosomes, (ii) definitive hosts are infected through a repeated light exposure mechanism (i.e. trickle infection process), (iii) mammalian hosts are mobile and long lived and (iv) genetic exchange due to sexual reproduction of adult worms, with the ability to change partners. Opposite to this (i) the rapid turn-over of infected snails and (iii) the acquired immunity of the definitive host, could lead to infrapopulation discriminations. Finally, an expected consequence for parasites in general is a deviation from Hardy-Weinberg equilibrium at the global scale but not within each host. This could be explained by a Wahlund effect with a non-random mating of parasites between hosts and random mating within each host.

The first empirical study aiming to analyse the infrapopulation

genetic structure of schistosomes concern adult *Schistosoma mansoni* worms recovered from naturally infected *Rattus rattus* on the island of Guadeloupe (Sire et al., 2001). At that time no method was available for the molecular analysis of individual larval stages (samples were subject to passage through laboratory rodents) and the genetic marker used was non-allelic (e.g. Random Polymorphic DNA marker). The main finding was that *S. mansoni* exhibited high genetic diversity and this diversity occurs on average more within than between hosts (Sire et al., 2001). Despite this, a significant degree of differentiation was also observed between the schistosome infrapopulations (Sire et al., 2001). The first study that investigated *S. mansoni* from humans also used adult worms resulting from successive passage through experimental snail and rodent hosts using miracidia excreted by Brazilian patients (Thiele et al., 2008). This passage system presents obvious sampling bias, bottlenecks and/or selection pressures (Gower et al., 2007). Despite this, the study showed both an important genetic diversity within the host and that the genetic variation within the infrapopulations accounted for the majority of the variance observed (Thiele et al., 2008). Later, the development of methods to collect, store and to amplify multiple (>10) microsatellite markers from a single larval stage has greatly advanced our knowledge of schistosome population genetics (Gower et al., 2007). The microsatellite analysis of individual miracidia collected from infected children in Kenya showed significant F_{ST} values both between patients (ranging from 0.44% to 3.98%) and between schools (ranging from 0.16% to 3.92%) (Agola et al., 2009). At this scale (<7 km between villages), the genetic structure was more similar between patients than between sites suggesting high levels of gene flow between infrapopulations. Similar results were obtained from Uganda and Senegal, from analysis of both mitochondrial cytochrome *c* oxidase (*cox1*) or nuclear microsatellite DNA regions, respectively (Betson et al., 2013; Van den Broeck et al., 2014). In one village located on the shore of Lake Albert (Uganda), no genetic differentiation was observed between infrapopulations and very weak differences (accounting for only 3.5% of variation) were observed within another village on the shore of Lake Victoria (Uganda) (Betson et al., 2013). The authors explained this difference by the fact that the shore of Lake Victoria is more convoluted than that of Lake Albert, providing micro-environments for transmission. People living in the village associated with Lake Victoria appeared to acquire their infections from different micro-foci, hosting different parasite infrapopulation (Betson et al., 2013). In Senegal, hierarchical analyses of genetic structure revealed no spatial-temporal structure in *S. mansoni* from three communities located on the Western shores of Lake Guiers (Van den Broeck et al., 2014). Although significant, parasite genetic differentiation was found to be low between the three communities located 4 to 22 km from each other ($F_{ST} = 0.003$), and between infrapopulations within each community ($F_{ST} = 0.004$) (Van den Broeck et al., 2014). Genetic differentiation between infrapopulations from all three communities ranged between -0.027 and 0.067 (Table 1), and the large majority of F_{ST} -values were not significant after Bonferroni correction, suggesting relatively high connectivity among the three communities (Van den

Table 1

F_{ST} values at local or regional scales for schistosome populations analysed using microsatellite markers.

F_{ST} (range)	Species	Distances (Km) between site	Number of sites	Number of microsatellite markers used	IBD	Reference
Local scale						
0.0016–0.0392	Sm	0.8–8	4	9	NS	(Agola et al., 2009)
0.01–0.056	Sm	7–45	4	20	NS	(Steinauer et al., 2009)
0.0011–0.05	Sm	1–3	7	7	ND	(Curtis et al., 2002)
0.010–0.0564	Sm	7–40	4	20	NS	(Steinauer et al., 2009)
–0.027–0.067	Sm	4–22	3	9	ND	(Van den Broeck et al., 2014)
0.004	Sh	8	2	8	ND	(Gower et al., 2011)
Regional scale						
0.036–0.273	Sm	25–490	7	5	NS	(Agola et al., 2006)
0.0357–0.136	Sm	127–553	4	11	NS	(Aemero et al., 2015)
0.014–0.069	Sm	600–1200	10	9	ND	Van den Broeck et al., 2015
0.0002–0.0048	Sb	175–672	4	14	NS	(Djuikwo-Teukeng et al., 2019)

NS: Not significant; ND: Not Done; IBD: Isolation By Distance. Sm = *Schistosoma mansoni*; Sb = *S. bovis*; Sh = *S. haematobium*.

Broeck et al., 2014). Most studies on population structure have focused on *S. mansoni* while only two studies have focused on *S. haematobium* at a local scale (Boon et al., 2019; Gower et al., 2011). These studies are in accordance with results already observed for *S. mansoni*. Gower et al. (2011) show low genetic differentiation between two schools separated by 7 km ($F_{ST} = 0.004$) and among children within the schools ($F_{ST} = 0.001$) and these variations accounted for only 0.35% and 0.1%, respectively. The 99.5% remaining variation was attributable to the variation within each child. Boon et al. (2019) analysed the genetic structure of *S. haematobium* from 10 villages in the North of Senegal. Weak genetic structuration was observed from 7 villages clustering in a 45 km range. A tendency for isolation by distance was noticed for two other villages further away from this cluster. To conclude all these studies, related to human hosts, suggest that few barriers to gene flow between populations exist at the local scale. Finally, similar results have been obtained in the single study on *Schistosoma bovis* (Djuikwo-Teukeng et al., 2019). This study showed that most variation is observed within individual animals (98.09% and 88.83% for microsatellites and *cox1* markers, respectively) rather than between animals.

2.2. Regional scale

At a regional scale the population structure mainly depends on the movement of human or animal hosts between different suitable transmission sites. At this scale a strong genetic population structure is expected. It is expected that at this scale little gene flow exists and that the genetic variation is more determined by the effective population size, rather than by gene flow between sites. This restriction in gene flow is reinforced by the fact that schistosomiasis is primarily transmitted by children who do not move much between regions compared to adults. The snail-miracidium compatibility could also restrict the gene flow between different sites (Mitta et al., 2017), due to fine scale snail-schistosome strain compatibilities.

The first empirical study on *S. mansoni* at the regional scale, analysed worms recovered from infected laboratory mice after exposure to cercariae from naturally infected molluscs (Agola et al., 2006). This approach will reduce the number of genotypes represented within the given population and in turn artificially increase the differentiation between the populations. The maximum F_{ST} was 0.273 for two villages distanced by 175 km. Using the same sampling approach (i.e. genotyping adult worms after passage through a laboratory host), similar strong genetic structure was been observed in Uganda between lake Victoria and lake Albert (Stothard et al., 2009). This latter studies used a DNA barcoding approach and showed two distinct lineages corresponding to the two lakes (Stothard et al., 2009). Using miracidia collected from infected patients both low and high F_{ST} values were also obtained in Ethiopia (see Table 1) (Aemero et al., 2015). In 2015, Van den Broeck and colleagues reconstructed the disease outbreak of intestinal schistosomiasis in Northwest Senegal (Van den Broeck et al., 2015). Parasites were sampled over a period of 14 years in seven villages located 4 to 65 km from each other. They found that most parasites belonged to the same genetic population, with relatively low genetic differentiation between most villages, suggesting relatively high gene flow in the region (Table 4 in Van den Broeck et al., 2015). An interesting observation was that *S. mansoni* parasites from Mbodjène in the Lampsar region were significantly differentiated from most other samples in the vicinity of Lake Guiers and the Senegal River. Likewise, the *B. pfeifferi* population sampled close to this locality was genetically different from the other two populations near the Lampsar River and the populations around Lake Guiers (Campbell et al., 2010). This correspondence between host and parasite geographic structure suggests that the genetic composition of the intermediate snail hosts could be an important factor determining establishment success of *S. mansoni* in a certain region (Van den Broeck et al., 2015). At the molecular level, this could comply with the hypothesis of a matching phenotype model where the interactions between parasite antigens and host immune receptors

during the early stages of the infection determine the success or failure of the infection (Mitta et al., 2012). Van den Broeck et al. (2015) also estimated genetic differentiation between the eight villages in North-West Senegal, one village in South-East Senegal and one village in Western Mali. At this scale, almost all pairwise F_{ST} estimates were significant after Bonferroni correction ($p < 0.001$). F_{ST} estimates between samples from the same year (2007) ranged between 0.032 and 0.066 when compared between sites from North-West Senegal and Western Mali (located 1200 km from each other), and between 0.014 and 0.069 between sites from North-West Senegal and South-East Senegal (located 600 km from each other) (Table 1). One noticeable exception of this general pattern was observed for *S. bovis* (Djuikwo-Teukeng et al., 2019). Indeed, at very large distances (175–672 km between sampled sites) no evidence for inter- or intrapopulation structuring was observed. Because the parasites were recovered from naturally infected cows, there was no possible bias due to selection within experimental snail or rodent hosts. The absence of genetic structure for *S. bovis* at a country scale (i.e. Cameroon) was explained by a large effective population size and/or movement of the definitive hosts within the country (Djuikwo-Teukeng et al., 2019). More studies on genetic diversity and population genetic structuring of animal schistosomes are needed to infer the role of the definitive hosts on parasite gene flow.

Within all the studies reviewed the interpopulation similarity indices showed that the populations do not follow a pattern of isolation by distance (Table 1) but instead they exhibit an island model of gene flow. This could be due to several factors such as restricted gene flow between sites, sib-transmission or due to local adaptations.

2.3. Phylogeography

A total of 13 *Schistosoma* species are described in Africa, four of which are associated with humans: *S. mansoni*, *S. haematobium*, *S. guineensis* and *S. intercalatum* (Webster et al., 2006) with the others being pathogens primarily of other animals. *Schistosoma mansoni* and *S. haematobium* are the two most prevalent species in Africa with important impacts on human health and de facto they have received considerable research interest. Conversely, our knowledge on the distribution and the evolutionary history of the other human-associated *Schistosoma* species (*S. intercalatum* and *S. guineensis*) is very limited (e.g. Webster et al., 2006; Tchuem Tchuente et al., 2003) and there are currently no genetic population studies on these species. Animal *Schistosoma* species also receive very little attention and their phylogeography is still under documented. Yet, some animal *Schistosoma* species such as *S. bovis* have been recently studied, mainly at a local scale, mainly because they have been shown to interbreed with human associated species raising the possibility for the emergence of zoonoses (Leger and Webster, 2017; Catalano et al., 2018; see sections below). However, we still lack a global vision on the distribution and the phylogeography of most *Schistosoma* species at the continental scale. Below we review our knowledge on the phylogeography of the two best studied species, *S. mansoni* and *S. haematobium* and also the evolutionary history of *S. bovis*.

Schistosoma mansoni is by far the best studied species at various geographical scales, from local to global (even intercontinental) scales. Globally, *S. mansoni* displays high levels of genetic diversity at both the mitochondrial and nuclear level, which at a global scale is geographically well structured (Van den Broeck et al., 2015; Gower et al., 2013; Morgan et al., 2003; Webster et al., 2013a, 2013b); but see Crellen et al., 2016). Conversely, although *S. mansoni* can infect several molluscs including, among others, *Biomphalaria pfeifferi*, *B. sudanica* and *B. choanonphala* and a range of vertebrate definitive hosts; humans, rodents and non-human primates, no genetic signature is associated with host use (Lawton et al., 2011; Webster et al., 2013a, 2013b). Lawton and collaborators argue that this lack of structure by host results from continuous gene flow between parasites using different hosts hence arguing the need to consider a 'One Health' approach for *S. mansoni*

control. Second, populations from Eastern Africa are far more diverse than Western populations (Gower et al., 2013; Morgan et al., 2003; Webster et al., 2013a, 2013b). This pattern has led several authors to hypothesise that *S. mansoni* has emerged in the former region and migrated toward Western Africa (Lawton et al., 2011; Morgan et al., 2003; Webster et al., 2013a, 2013b). More recent genomic evidence suggests that *S. mansoni* emerged in Eastern Africa around 126,5 KYA and that populations were confined to this area over a long period until around 7 KYA (Crellen et al., 2016). Populations have then expanded and further divergence events occurred giving rise to populations now present in Cameroon six thousand years ago (KYA) and more recently in Senegal 1.5 KYA (Crellen et al., 2016). If confirmed, this evolutionary scenario might at least partly explain global geographical patterns of genetic structure and the gradual decrease of genetic diversity from Eastern to Western Africa. One exception concerning the globally observed geographical structure of *S. mansoni* populations concern those established in Coastal Kenya and Zambia (Webster et al., 2013a, 2013b). These two closely related populations genetically cluster together and display high genetic divergence compared to other West-African populations (Webster et al., 2013a, 2013b). Based on this former study and based on further genomic evidence that Coastal Kenya is genetically distinct from other East African populations, Crellen et al. (2016) argue that Zambia and Coastal Kenya share a common evolutionary history probably due to important human movements between these two countries during the 19th century. These results suggest that some cryptic diverging lineages of *S. mansoni* that emerged under the influence of human history could occur in other regions of Africa and that the overall genetic diversity of this species might therefore still be underestimated.

Molecular studies on *S. haematobium* are scarce at the global scale and mainly based on the analysis of mt DNA regions, few nuclear DNA regions (e.g. *ITS*) and more recently microsatellites (Webster et al., 2015). Although useful to identify potential hybrids, the rDNA *ITS* region has been found to be monomorphic and cannot be used for reconstructing genetic relationships between populations at any scale (Webster et al., 2012). Despite these few studies and the poor resolution of the routinely used genetic markers, some common patterns emerged regarding the genetic diversity, genetic structure and more recently the genome-wide evolutionary history of *S. haematobium* populations. The first major characteristic of *S. haematobium* is that this species displays very low genetic diversity compared to *S. mansoni* (Gower et al., 2013; Webster et al., 2012) but see Glenn et al. (2013). Despite such low genetic diversity, two major clades have been identified based on their mitochondrial haplotypes including populations from Eastern Africa on one hand and Western Africa on the other hand (Webster et al., 2012). The Eastern clade also includes populations established in some Indian Ocean islands (e.g. Madagascar, Mauritius). Interestingly, one mitotype (H1) is overrepresented at a global scale (Webster et al., 2012). This pattern has been interpreted as the result of a recent and important population bottleneck before recent expansion of this parasite species across Africa (Webster et al., 2012). However, contrary to *S. mansoni*, Webster et al. (2012) did not find the differences in genetic diversity between Eastern and Western populations. Gower et al. (2013) found important genetic differentiation among populations according to an Isolation-by-distance pattern although such patterns have been called into question (Glenn et al., 2013). Results from the latter study might at least partly be explained by the sampling of a highly diverging population in South Africa which increases genetic differentiation indexes computed between geographically close populations.

Although no genetic data exists at a global continental scale for the other *Schistosoma* species, some authors attempted to draw general phylogeographic patterns of some species based on their geographical distribution, the evolutionary history and the compatibility to their respective host snails. For instance, based on the comparison of the geographical distribution of *S. bovis* and that of their potential snail host species, combined with experimental assessments of compatibility

between different *S. bovis* populations and different snail species, authors found important diversity among *S. bovis* populations and clear differences between populations from southern Mediterranean zones and southern Saharan zones (another distinct population being identified in southern Europe) (Mone et al., 1999). Based on these results the authors proposed a three-step evolutionary scenario for *S. bovis*. First this species emerged from (Western) South Sahara and extended its distribution to the South, East and West of Africa. Second and more recently this species reached the Mediterranean zone together with an important reduction of host spectrum. Finally, *S. bovis* colonised the Iberian Peninsula most likely through dispersion events over the Arabian Peninsula first and next around the Mediterranean basin. So far, no genetic data exists to confirm this scenario. We expect that with the recent interest for hybridization events between *S. bovis* with other *Schistosoma* species, together with the development of genomic resources for this species in particular, we will soon better understand the evolutionary history of this broadly distributed parasite species across Africa (and southern Europe).

3. Factors affecting schistosome genetic diversity

Parasite genetic diversity is expected to be balanced by the clonality within the mollusc host and by the fact that snails are usually infected by only a few genotypes (Theron et al., 2004). Opposite to this, the definitive host mobility, the rapid turnover of infected snails, the snail-parasite compatibility, the long lifespan of the definitive host and the genotype dependent host re-infection (Beltran et al., 2011), should all increase the parasite genetic diversity within the definitive host. In this context, some authors have proposed that the definitive host is a 'genetic mixing bowl' for the parasite (Curtis and Minchella, 2000). The 'genetic mixing bowl' hypothesis was validated by several studies. The classical indexes used to infer population diversity such as heterozygosity or allelic richness are always very high for *S. mansoni* or *S. haematobium* infrapopulations (Agola et al., 2009; Gower et al., 2011; Sire et al., 2001; Steinauer et al., 2009; Thiele et al., 2008; Van den Broeck et al., 2014). Using relatedness indices Steinauer et al. (2009) showed that 94.44% of the schistosomes coming from a single patient were unrelated (Steinauer et al., 2009). Similarly, Aemero et al. (2015) using sibship analysis showed that the estimated number of unique *S. mansoni* worm pairs within a single human host was between 66% and 92% of the entire worm infrapopulation (Aemero et al., 2015). This parasite genetic diversity could also be modulated by intrinsic factors such as sex of the parasite or the species concerned, or by extrinsic factors such as the sex of the host, the age of the host, the infection intensity or the drug treatment.

3.1. A sex-specific population genetic structure

Sexual dimorphism is a remarkable trait in schistosomes (Mone and Boissier, 2004). The muscular male holds the thin female within its gynaeophoric canal. Considering this sexual dimorphism it has been shown that the male elicits a stronger immune response compared to females (Boissier et al., 2003). After repeated light exposure in experimentally infected mice it has been shown that male parasites induced a genotype-dependant negative effect, via the host immune system, on subsequent male infections (Dumont et al., 2007). Females alone seem unable to induce this protective effect. The same authors have demonstrated that the protective effect is genotype dependent and correlates with genetic dissimilarity between the male schistosomes (i.e. the smaller the genetic distance between the initial and secondary infecting schistosomes, the lower the infectivity rate of secondary infecting males) (Beltran et al., 2011). Comparative 2D proteomic analysis on different male clones revealed differences in antigens known to be involved in the induction of the immune system (Beltran et al., 2011). This gender difference in host-parasite interaction may be at the origin of a sex-specific population structure found in naturally infected rats

(Prugnolle et al., 2002). These last authors have shown that the female parasites were more genetically similar than the males in infected rats (Prugnolle et al., 2002). This pattern could be the result of the gender differences previously demonstrated in experimental approaches and the successive trickle infection process that will in term lead to more dissimilar males compared to females in a single host. However, this sex-specific pattern has never been investigated for parasites recovered from humans.

3.2. Differences of genetic diversity between species

Important differences in genetic diversity indices have been observed according to the schistosome species. Several studies have shown that *S. mansoni* is much more diverse than *S. haematobium* (Gower et al., 2013; Sady et al., 2015; Webster et al., 2013a; Webster et al., 2012). At the African continental scale, the haplotype diversity (h) and the nucleotide diversity (Π) of the *cox1* gene were 0.94 ± 0.0067 and 0.02553 (Webster et al., 2013a) and 0.36 ± 0.014 and 0.00434 (Webster et al., 2012) for *S. mansoni* and *S. haematobium*, respectively. The haplotype diversity is also higher for *S. bovis* (0.961 ± 0.012), even at a small population size than for *S. haematobium* (Djuikwo-Teukeng et al., 2019). The higher haplotype diversity of *S. bovis* compared to *S. haematobium* were confirmed by comparing microsatellite markers common to these two sister species (Djuikwo-Teukeng et al., 2019). However, if *S. bovis* is similar to *S. mansoni* concerning its haplotype diversity it differs concerning its nucleotide diversity ($\Pi = 0.00602$), which is more similar to *S. haematobium*. As a consequence, the Tajima's D^* index did not differ from zero for *S. mansoni* or *S. haematobium* (Sady et al., 2015) but it is significantly negative for *S. bovis*, which could constitute a signature of population demographic expansion (Djuikwo-Teukeng et al., 2019). This last study hypothesises that this difference in both nuclear and mitochondrial diversity could be the consequence of the absence of praziquantel treatment of infected animals compared to humans, imposing different bottlenecks/and selective pressures within these hosts accordingly.

3.3. The effect of the host age on parasite genetic diversity

The trickle infection model is expected to lead to more diverse parasite populations in older patients because they should be exposed to a wider variety of parasites (mixing bowl hypothesis). This expectation has been confirmed for *S. mansoni* populations in Senegal (Van den Broeck et al., 2014). The study showed significant correlations between inbreeding (negative correlation) or heterozygosity (positive correlation) and the age of the patients: the children being more infected by related parasites than adults. To date, this study is the only one showing this pattern. The explanation could be that the authors have sampled a variety of ages (between 4 and 50 years) and not only children, as in the majority of other studies (Agola et al., 2009; Gower et al., 2011). A more recent study failed to find a difference in parasite genetic diversity between mothers and young children (Betson et al., 2013). These authors proposed that the trickle dynamics is rapidly saturated and that two year old children could host the same parasite genetic diversity as their mothers (Betson et al., 2013). The different findings in these two studies could also relate to the difference in the genetic markers used. The first study used a more polymorphic and biparentally inherited genetic marker (microsatellite) compared to the second one that looked at haplotypic diversity of the maternal mitochondrial marker. It could also relate to the exposure and mobility of the hosts, the mothers (Betson et al., 2013) might be less mobile than males (Van den Broeck et al., 2014).

3.4. The effect of host sex on parasite genetic diversity

Because of behavioural or immunological differences, the sex of the host could also influence the parasite infrapopulation. For instance, it is

commonly observed that boys are more heavily infected than girls due to difference in sexual roles in work, play and ultimately water contact (Angora et al., 2019; Kebede et al., 2018; Mohamed-Ali et al., 1999). This gender bias is also observed in traveller infections (Nicolls et al., 2008). It has also been observed in mouse experimental models that females were more sensitive to schistosome infection than males due to physiological differences between sexes (Boissier et al., 2003; Nakazawa et al., 1997). These host gender differences in either behaviour and/or physiology are expected to shape the parasite genetic diversity. In natural *S. mansoni* infected rats, no difference was observed between male and females in the genetic composition of the parasite infrapopulation (Prugnolle et al., 2002). The same tendency has been observed for *S. mansoni* and *S. haematobium* from infected humans (Gower et al., 2011; Thiele et al., 2008; Van den Broeck et al., 2014). However, two recent studies showed that males have a higher schistosome genetic diversity than females (Faust et al., 2019; Kebede, 2020). This could reflect the differences in behaviour with young males having higher mobility and greater water contact compared to young females. One interesting outcome that differs between these two studies is that parasite genetic diversity increases only with male or female age (Kebede (2020) or Faust et al. (2019), respectively). This confirms that human behaviour can shape the parasite genetic composition but also, that acquired immunity does not seem to limit this diversity because no decline in genetic diversity was observed (Faust et al., 2019).

3.5. Infection intensity and schistosome genetic diversity

Schistosomiasis infection intensity is estimated by the number of eggs per gram of faeces for *S. mansoni* or per 10 mls of urine for *S. haematobium*. This intensity is dependent on several factors including the sex, age or the occupation of the host, the history of treatment, the transmission foci, the virulence of the parasite etc.... Intuitively, we would expect a positive link between infection intensity and parasite genetic diversity. Using mitochondrial *cox1* analysis, no correlation between *S. mansoni* infection intensity and either haplotype or nucleotide diversity was observed (Betson et al., 2013). Similar results were obtained for *S. haematobium* (Gower et al., 2011). Based on microsatellite markers the authors showed that the allelic richness is the same whatever the intensity of infection, from low to very high (Gower et al., 2011). In this same study, the link between sibship analysis (measured as the number of unique parental genotypes) and infection intensity did not give consistent results. First, when comparing two villages, the higher the intensity of infection the less diverse the schistosome infrapopulations appear to be. Second, no difference in infection intensity was observed between girls and boys but the number of unique parental genotypes was lower in girls than in boys. Third, counter-intuitively children with light infections had more diverse parasites than those with high infections. Finally, after controlling for several demographic parameters (village, gender and age) and co-infection status with *S. haematobium*, no link was observed between *S. mansoni* infection intensity and heterozygosity or allelic richness in Senegalese patients (Van den Broeck et al., 2014).

3.6. Effect of praziquantel treatment

Praziquantel (PZQ) is the drug of choice to treat schistosomiasis because of the absence of toxicity (Cioli and Pica-Mattocchia, 2003; Thetiot-Laurent et al., 2013), low cost (Fenwick, 2006) and the effectiveness against all *Schistosoma* species (Kumar and Gryseels, 1994). In 2002, the "Schistosomiasis Control Initiative" was founded with the aim for broad-scale elimination of schistosomiasis by regular (mass) treatment of school-aged children. This could exert an enormous selection pressure on parasite infrapopulations, which might, among others, increase the risk of drug resistance development (Doenhoff et al., 2002; Fenwick and Webster, 2006), although there is currently no conclusive evidence for PZQ-induced adaptive evolution.

Here, we consider the neutral evolution of *Schistosoma* populations in response to Mass Drug Administration (MDA) treatment programs. To this end, we explore the genetic concepts and tools that have enabled investigations into the possible impact of PZQ on the population genetic structure of schistosomes, and demographic changes over time to verify whether current programs actually decrease parasite transmission (and could ultimately lead to their elimination).

A theoretical framework to assess the impact of PZQ treatment on schistosome population genetics was outlined in (Huyse et al., 2013b). Within this framework it is assumed that praziquantel randomly kills worms in the treated individuals, leaving a random subset of worms from the infrapopulation after treatment. This will decrease the allelic richness and gene diversity of the treated infrapopulation, while observed heterozygosity is less sensitive to changes in the effective population size. Low effective population size will furthermore lead to a random loss and fixation of alleles (genetic drift) and thus an increase in genetic differentiation between the treated infrapopulations, and between the pre- and post-treatment infrapopulations. In addition, pre- and post-treatment infrapopulations could become genetically different due to re-infection of parasites from genetically differentiated source populations. In conclusion, the following population statistics are important to assess the impact of PZQ treatment on schistosome populations: allelic richness (AR), gene diversity (D), inbreeding coefficient (F_{IS}) and genetic differentiation of infrapopulations (F_{ST}). The inbreeding coefficient is included as it is also sensitive to changes in effective population size (see Huyse et al., 2013b).

Norton and colleagues were the first to investigate the impact of treatment on the population biology of schistosomes (Norton et al., 2010). Using seven microsatellite markers, they compared the genetic diversity and differentiation of *S. mansoni* populations in Tanzanian school aged children before (in April 2005) and twelve months after (in April 2006) the first MDA. The authors found a small but significant difference in AR and D as estimated per infrapopulation in 2005 and 2006, suggesting that parasite infrapopulations may have experienced genetic bottlenecks in response to PZQ treatment. However, this conclusion should be nuanced as the authors also reported a decline in AR in a control group of children that had not been previously treated as they were not yet of school age during baseline sampling (Norton et al., 2010), suggesting that factors other than preventive chemotherapy may have caused the decline in infrapopulation genetic diversity. This hypothesis is supported by the study of (Gower et al., 2017) that revisited the two Tanzanian schools in 2010 and genotyped miracidia using the same microsatellite markers as Norton et al. (2010). Children had received two (in 2005 and 2007) or four (in 2005, 2006, 2007 and 2009) rounds of treatment depending on the school. The authors observed a significant increase in infrapopulation AR and D in 2010 compared to baseline estimates in April 2005. The initial decline in 2006 (Norton et al., 2010) and then increase in 2010 (Gower et al., 2017) may reflect temporal changes in parasite transmission dynamics, rather than being a direct consequence of PZQ treatment. Gower et al. (2017) also found a significant decline in worm burdens within an individual host as estimated with parentage analyses. Although the authors did not correct their estimates for family structure or the number of sampled miracidia (Steinauer et al., 2013), their observations could suggest that PZQ treatment reduced infrapopulation sizes, but not to an extent that it resulted in concomitant genetic bottlenecks of the entire parasite population. Both Norton et al. (2010) and Gower et al. (2017) found higher F_{ST} between years (within schools) compared to between schools (within years), an observation that may suggest high re-infection levels in the Tanzanian villages.

These reports of a long-term decrease in genetic diversity (Norton et al., 2010) and number of adult worms per infrapopulation (Gower et al., 2017) in response to PZQ treatment could not be reproduced by other studies. In Senegal, 12 Senegalese children were followed over two years and samples were collected prior to treatment: once in April 2007 before the first double treatment (i.e. two PZQ treatments 3 weeks

apart), once six months after the first double treatment and two years after baseline surveys with a total of five treatments (Huyse et al., 2013b). Although miracidial genotypes (based on nine microsatellite markers) could not be generated for all children at all timepoints, the authors found no change in AR, D , F_{IS} or F_{ST} . More-over, K -means clustering revealed 11 distinct genetic clusters, most of which were present in all children at all timepoints, suggesting that the genetic composition of parasite infrapopulations remained largely stable for two years despite a relatively large number of PZQ treatments (see Fig. 1 in Huyse et al., 2013a, 2013b). This observation, together with the sustained high egg counts and high genetic diversity after several treatments is indicative of a surviving parasite population, that is tolerant to the administered PZQ dose. In Kenya, Lelo et al. (2014) followed parasite genetic diversity for four years (2008–2012) in 15 children that received four treatments during a MDA program, whereby samples were taken once a year prior to treatment (Lelo et al., 2014). One of the major strengths of this study is that it is the only African-based study that followed a relatively large cohort of children for four years (but see also (Blanton et al., 2011) for an example in Brazil), allowing comparable genetic statistics of the same infrapopulations before and after PZQ treatments (see Table 2 in Lelo et al., 2014). In addition, the authors performed a relatively deep miracidial sampling per child (average of 82 miracidia per child), allowing robust parentage analyses and reducing the amount of bias introduced due to family structure (Steinauer et al., 2013). Contrary to expectations, Lelo et al. (2014) found a small but significant increase in AR and D after four years of MDA, but the number of full sibling families and effective number of breeders per infrapopulation did not change over time. In Uganda, Betson et al. (Betson et al., 2013) used mt *cox1* data to compare the genetic diversity of *S. mansoni* before and 6 months after PZQ treatment of cohorts of pre-school aged children and mothers. Note that all participants were treated at baseline with PZQ, albendazole and artemether-lumefantrine, and that samples were taken from the same individual or from the same family. The authors found no decline in haplotype or nucleotide diversity. Finally, also in Uganda, Faust et al. (2019) performed one of the most extensively designed sampling studies (Faust et al., 2019). Children aged 6–12 years from three primary schools were sampled at 11 timepoints over two years, including one week, four weeks and six or 12 months after PZQ treatment. Most children received a total of four treatments. The major strength of this study is that the sampling scheme allowed examination of the parasite genetic diversity over short follow-ups after treatment (but see also (Blanton et al., 2011) for an example in Brazil). One of the main insights from this study is that AR only decreased in samples taken four weeks after treatment, suggesting that PZQ induced a genetic bottleneck, but this then recovered six months after treatment (Faust et al., 2019). The authors argued that high rates of gene flow in combination with refugia in snails and untreated individuals ensure rapid recovery of parasite genetic diversity. Sibship analysis furthermore demonstrated that adult worms can survive treatment but high gene flow and refugia prevent fixation of resistant/tolerant genotypes (Faust et al., 2019). All these observations together made the authors conclude that MDA alone is not sufficient for schistosomiasis elimination (Faust et al., 2019). Also, Lelo et al. (2014) concluded that MDA programs targeting school-aged children only may have limited impact on parasite transmission at the community level. This was also the main finding of a simulation study that used an island model to investigate the impact of MDA on long-term parasite population dynamics (Van den Broeck et al., 2020). The study revealed that repeated treatments induced strong and lasting declines in parasite infrapopulation sizes, resulting in concomitant genetic bottlenecks. However, parasite genetic diversity recovered quickly in a few generations due to re-infection, and there was little or no impact of treatment on the genetic diversity of the component population when treatment coverage was 95% or lower (Van den Broeck et al., 2020). The authors concluded that community-wide distribution of praziquantel is essential to induce long-term effects on parasite population dynamics (Van den Broeck et al., 2020).

Table 2
 Details of the detected natural hybridisation between different *Schistosoma* species combinations in Africa. Accounts shown in (O) are based on atypical/morphological observations and biochemical markers, whereas the other cases (not in (O)) have been confirmed by molecular data. The years are the first and last year of the reported hybridisation. The main host is the typical definitive host. The detected host is the definitive or intermediate host that the hybrids/introgressed forms were recovered from.

Years	Country	Main Definitive Host	<i>Schistosoma</i> species	Detected Host	Reference
2003–2008	Tanzania, Kenya	Humans Rodents	<i>S. mansoni</i> <i>S. rodhaini</i>	Snails	(Morgan et al., 2003; Steinauer et al., 2008a; Steinauer et al., 2008b)
1974–2012	Cameroon; (Gabon); Benin; (Mali)	Humans	<i>S. haematobium</i> <i>S. guineensis</i>	Humans	(Burchard and Kern, 1985; Mone et al., 2012; Raitard and Greer, 1991; Raitard et al., 1990; Southgate et al., 1976; Tchuem Tchuente et al., 1997; Verccruysee et al., 1994; Webster et al., 2003; Webster et al., 2005; Wright et al., 1974; Zwingenberger et al., 1990)
1948–2019	(Zimbabwe), (South Africa), (Zambia), Malawi	Humans Livestock	<i>S. haematobium</i> <i>S. mattheei</i>	Humans & Livestock	(Alves, 1948; De Bont et al., 1994; Kruger, 1987, 1990; Kruger and Evans, 1990; Kruger and Hamilton-Attwell, 1988; Kruger et al., 1986; Le Roux, 1954; Pennance et al., 2020; Pitchford, 1959, 1961; Platt et al., 2019; Verccruysee et al., 1994; Webster et al., 2019; Wright and Ross, 1980)
1993–2020	Senegal, France, Niger, Mali, Benin, Cote D'Ivoire, Malawi	Humans Livestock	<i>S. haematobium</i> <i>S. bovis</i>	Humans & Snails (Livestock [*])	(Boissier et al., 2016; Boon et al., 2017; Boon et al., 2018; Brémond et al., 2019; Boon et al., 2018; Brémond et al., 1993; Catalano et al., 2018; Huysse et al., 2009; Leger et al., 2020; Mone et al., 2015; Ramalli et al., 2018; Rothe et al., 2021; Savassi et al., 2020; Soentjens et al., 2016; Tian-Bi et al., 2019; Webster et al., 2013b)
1993–2012	Senegal, (Niger)	Humans Livestock	<i>S. haematobium</i> <i>S. curassoni</i>	Humans	(Brémond et al., 1993; Webster et al., 2013b)
2010–2020	Senegal, (Mali), (Kenya), Cote D'Ivoire	Humans	<i>S. haematobium</i> <i>S. mansoni</i>	Humans	(Gouvras et al., 2013; Huysse et al., 2013a; Koukounari et al., 2010; Le Govic et al., 2019)

* This is the suspected occurrence of *S. haematobium-bovis* hybrids in cattle based on cercarial circadium rhythms (Savassi et al., 2020).

However, as also concluded in Barbosa et al. (2016), more studies are needed to cover the enormous heterogeneity in transmission contexts in Africa before any firm conclusions can be made (Barbosa et al., 2016). But as it stands now, comparative analyses are complicated due to differences in sampling design, molecular markers and type of population genetic analysis used in the studies. Adopting standardized procedures is therefore key. Future studies may also need to distinguish intrapopulation from component population genetics. At the intrapopulation level, parentage analyses using genome-scale polymorphism data (e.g. (Shortt et al., 2020)) of large miracidial samples per child may become a powerful tool to assess the strength of the genetic bottleneck and dissect re-infection from persistent infections following treatment. At the component population level, genomic data of a few miracidial samples from a large number of hosts will provide major insights into the long-term regional effects of treatment programs, ultimately allowing the identification of the main factors/interventions that could help schistosomiasis elimination.

4. Schistosome genetic variation and disease outcome

Several experiments have been performed to study the influence of the parasite and/or hosts genetic background on the host/parasite association. The work of André Theron's group is an example of how the genetic background of both the parasite and its mollusc host is important for successful infection (e.g. a phenomenon called "compatibility") (Mitta et al., 2017; Theron et al., 2014). Interestingly, experimental comparative studies have shown adaptative trade-offs according to the parasite strain (Davies et al., 2001; Le Clec'h et al., 2019). However, the influence of parasite genetic variations on virulence in terms of human disease outcome is certainly an understudied aspect. Brouwer et al. (2013) was first to find genetic differences, measured by randomly amplified polymorphic (RAPD) DNA regions, associated with pathology observed within *S. haematobium* infected children (Brouwer et al., 2003). No link was observed between heterozygosity and the severity of infection, however they found a positive association between genetically related clusters and pathology, measured as bladder or kidney pathology and infection intensity (Brouwer et al., 2003). In comparison this link was not observed in a study that investigated *S. haematobium* infections in Sudanese patients (Gasmelseed et al., 2014). However, this study used only one RAPD marker showing only 3 distinct genotypes clearly lacking the power to conclude there is any link between genetic diversity and pathology. There is only one study that has used microsatellite markers to investigate any links between genetic diversity, prevalence and intensity of infection (Aemero et al., 2015). However, with only three studied sites no statistical tests could be validated. Only one study has clearly shown an association between genetic variation, in terms of microsatellite allelic variation, and human disease phenotype for *S. mansoni* (Huysse et al., 2018). The study showed a positive association between a specific microsatellite allele and infection intensity, while a negative association for another allele of the same locus was also associated (Huysse et al., 2018). Twenty one percent of the variation at this locus was explained by infection intensity, host age and bladder morbidity (Huysse et al., 2018). This microsatellite allele is located near the cGMP-dependent protein kinase gene that is expressed in reproductive organs of adult schistosome worms and appears to be linked to egg production (Leutner et al., 2011; Leutner et al., 2013).

5. Hybridization

5.1. *Schistosoma* inter-species interactions and hybridization

It is clear that *Schistosoma* species are diverse and have evolved several genetically well-defined species that have distinct phenotypic traits. In Africa both biological (host specificities/preferences, relatedness and anatomical site of infection, inter-species dominance/competition) and ecological barriers (geographical distribution, intermediate

snail host habitats, transmission site ecology) have certainly driven speciation within the genus and also prevent inter-species interactions and hybridisation. However, it is well known that given the opportunity species can interact and also inter-breed, facilitated by the dioecious sexual stage in the definitive host, and this has been clearly demonstrated through multiple experimental hybridization studies involving different *Schistosoma* species (reviewed in (Leger and Webster, 2017)). This means that the presence or absence of reproductive isolating mechanisms cannot be used as a criterion for defining the different species. The success of inter-species interactions and the production of viable hybrid offspring is highly dependent of the species and also geographical strains involved, and there are also several biological features observed within different combinations. These include non-reciprocal crosses, species competition (particularly male worm competition) and dominance, parthenogenetic reproduction, hybrid vigour and also hybrid instability. These features are not uniform, are highly dynamic and probably play a crucial role in preventing prolific admixing in nature, particularly for species that co-infect the same definitive host. Relatedness also plays a key role in preventing genetic exchange and hybrid speciation (Fig. 1, Table 2), with interactions between more distantly related species resulting in parthenogenetic reproduction or no viable hybrid offspring, compared to viable hybridisation between more closely related species. It does appear that under natural conditions interspecies genetic exchanges are limited by the existence of geographic separation and/or the existence of pre- or postzygotic reproductive isolating barriers. However, there is increasing evidence for natural hybridization between species possibly influenced by increased environmental changes, both manmade and natural, together with the movement of parasites within their definitive hosts into new areas enabling inter-species interactions. Additionally, our improved ability to characterise schistosome populations and identify hybrids, with the utility of molecular and genomic analyses, may also be uncovering hybridization and genetic introgression that have been occurring as part of the natural epidemiology of these species, but until recently remained undetected. The existence of genetic exchanges between species of schistosomes and the formation of natural hybrids is indeed a topical subject under investigation in several African foci. The relationship of changes in disease transmission and human/animal health with inter-species interactions, together with the potential for novel reservoirs of infection and zoonotic transmission are clearly important in terms of disease control and elimination. In the next sections we review and discuss the genetic evidence for some inter-species interactions between African *Schistosoma* species in their natural settings with a focus on those involving human species.

5.2. Molecular identification of hybrids

Modern genetic methods allow for a more accurate assessment of infections and also allow for the identification and deciphering of inter-species hybridization. Certain DNA regions are now routinely used for species and hybrid identification. These include a mitochondrial (mtDNA) DNA region (routinely *cox 1*, although other gene regions have been used) to identify the maternal line. Species specific SNP's within nuclear ribosomal DNA regions (nrDNA) (routinely the ITS1 and ITS2 rDNA regions are used however other markers are needed for certain species combinations) are used to provide the nuclear genetic profile of the species involved (Huysse et al., 2009; Pennance et al., 2020; Webster et al., 2019; Webster et al., 2012) (Fig. 1). The occurrence of hybridisation events are often identified by the unexpected mitochondrial signatures presented by samples that do not relate to the infected hosts that are being sampled e.g. *S. bovis* mt signatures for samples retrieved from human hosts. This then leads to more scrutiny of the nuclear profile of the samples with clear discordance between mt and nuclear signatures incriminating hybrid forms (Fig. 1). It is acknowledged that this basic genetic profiling does not enable the identification of the hybrid generation or parental backcrosses however (Platt et al., 2019), it infers

that inter-species genetic exchange has occurred successfully with the generation of hybrid offspring. Mixed nuclear profiles (copies from both parental species) do suggest earlier generation hybrids, and possibly F1 generations however, it still remains unknown as to the generational speed of genetic homogenization, and loss of signal from one of the parental species preventing accurate hybrid identification (Leger et al., 2020). This may also mean that hybrids are often missed by this form of identification and that they may be more prevalent than the data suggests. Whole genome studies are providing a more recent and valuable opportunity for investigating and understanding these hybridization events, and their outcomes, and should form an integral component of natural hybridization investigations going forward (Kincaid-Smith et al., 2018; Platt et al., 2019). These enable the assessment of the genetic composition of hybrids across the whole genome allowing us to decipher different generations of hybrids and backcrossed/introgressed forms by the assessment of the different genomic levels from the two parental forms. From this we can determine if hybridization is active, rare and/or ancient and can also identify directional hybridization/introgression (Platt et al., 2019). This relates directly to any pre- and post-reproductive isolating barriers that may be in place that help maintain species integrities and prevent hybrid speciation (Kincaid-Smith et al., 2018; Oey et al., 2019; Platt et al., 2019).

5.3. Natural *S. mansoni* group species hybridisation (*S. mansoni* X *S. rodhaini*)

As described previously, *S. mansoni* and *S. rodhaini* are closely related sister species and both have the ability to infect rodents. This provides the opportunity for these two species to encounter each other. *S. rodhaini* is highly specific to rodents and has also developed phenotypic traits supporting its transmission via nocturnal emergence patterns. *S. mansoni* is a human pathogen that has important zoonotic reservoirs including non-human primates and rodents (Standley et al., 2012b; Standley et al., 2012c).

Natural hybrids between *S. mansoni* and *S. rodhaini* have been identified in sympatric East African zones (Morgan et al., 2003; Steinauer et al., 2008b) (Table 2). *S. rodhaini* has a more focal distribution compared to *S. mansoni* and is currently only found transmitted in Rwanda, Kenya, Tanzania, Burundi, Uganda, Zaire and also there is a report from Nigeria. *S. mansoni* overlaps with *S. rodhaini* in several of these countries and natural hybrids have been identified in Tanzania and Kenya (Morgan et al., 2003; Steinauer et al., 2008b). Hybrids have only ever been found emitted from *Biomphalaria* snails with the molecular data concluded that no F1 hybrids were present with all appearing as introgressed forms. The data suggested a relatively low hybridisation rate and that hybrid backcrossing was rapidly occurring. True hybridisation appeared episodic and strongly influenced by the transmission dynamics of the different species, particularly related to *S. rodhaini* with transmission and the opportunity to hybridise being highly seasonal. There also appeared to be strong directional introgression of genetic material from *S. rodhaini* into *S. mansoni*.

5.4. Natural *S. haematobium* group hybridisation

The *S. haematobium* group is the most diverse *Schistosoma* species group with nine species several of which are sympatric (Fig. 1). Three closely related species are human pathogens providing opportunities for interactions and hybridisation. Several of the others also have opportunities to interact within their non-human hosts particularly bovids. Interestingly hybridisation within this group centres around the most widespread, prevalent and medically important species *S. haematobium*. Particularly its hybridisation with bovid species is attracting attention due to the zoonotic components that may be facilitating this or the potential risk of the emergence of zoonotic strains and the creation of zoonotic reservoirs (Leger et al., 2020).

5.4.1. *S. haematobium* and *S. guineensis*

Until 2003 *S. guineensis* and *S. intercalatum* were considered one species (*S. intercalatum*) but were split based on their mitochondrial divergence (Pagès et al., 2003; Webster et al., 2006). When *S. guineensis* and *S. intercalatum* were split into their separate species, *S. intercalatum* was recognised as being restricted to more central African regions, namely the Democratic Republic of Congo (DRC) whereas *S. guineensis* is found on South West coastal countries and Sao Tome (Fig. 1). In the literature many of the earlier reports related to *S. guineensis* will refer to *S. intercalatum* and so for ease of understanding going forward, this will be described as *S. guineensis* (*intercalatum*).

It is apparent that *S. haematobium* and *S. guineensis* readily hybridise in nature. This is probably not surprising given the fact that they are sister species and human pathogens, however they do utilize different anatomical sites of infection (urogenital and intestinal respectively) probably reducing interactions. Evidence of hybridisation between *S. haematobium* and *S. guineensis* (*intercalatum*), has been reported in several areas of Cameroon (Loum, Kinding Ndjabi, Kumba, Barombi Kotto (Ratard and Greer, 1991; Ratard et al., 1990; Southgate et al., 1976; Webster et al., 2003; Wright et al., 1974) predominately based on polymorphic eggs excreted in the urine of patients and this was later backed up with molecular data obtained from preserved isolates (Webster et al., 2003, 2007). Molecular analyses, using mt *cox1* and nrDNA ITS2 + IGS genetic markers, of samples collected from various time periods during the fluctuation of *S. haematobium* and *S. guineensis* (*intercalatum*) in Cameroon clearly showed the presence of *S. haematobium* X *S. guineensis* (*intercalatum*) hybrids, with a predominance of hybrids probably resulting from interactions between *S. haematobium* ♂'s with *S. guineensis* (*intercalatum*) ♀'s (Webster and Southgate, 2003; Webster et al., 2005; Webster et al., 2007). The data also clearly showed a decline in numbers of hybrids over time suggesting a replacement of *S. guineensis* (*intercalatum*) by *S. haematobium* through introgressive hybridization (Tchuem Tchuente et al., 2003; Webster et al., 2005).

The natural occurrence of *S. haematobium* X *S. guineensis* (*intercalatum*) hybridisation has also been reported in Gabon and also Benin (Mone et al., 2012; Zwingenberger et al., 1990). The Gabon account was based on egg morphology and remains unconfirmed. Mone et al. (2012) molecularly identified *S. haematobium* X *S. guineensis* hybrid miracidia collected from human urine samples in Benin. All samples showed maternal mtDNA *cox1* *S. haematobium* genetic profiles suggesting that these were not early generation hybrids but probably introgressed forms. Current foci for *S. guineensis* remain severely underreported due to their scarcity and lack of knowledge related to this species. However, foci do still exist in Central African/South Western regions and are likely in some areas to overlap with *S. haematobium*.

5.4.2. *S. haematobium* and *S. matthei*

Many sympatric zones exist for these species however, compared to *S. haematobium* *S. matthei* has a more restricted distribution to East African regions and typically below a latitude of 10° South (Fig. 1). *S. matthei* is considered to be a potential pathogen of humans although it is primarily a pathogen of bovids (Standley et al., 2012a). It still needs to be elucidated if *S. matthei* actually infects humans or is only found in humans as part of mixed infections and/or in a hybrid form.

Many historical observations exist for *S. matthei* shaped eggs being excreted by humans in South and South Eastern regions of Africa, where *S. haematobium* and *S. matthei* are sympatric (reviewed in (Leger and Webster, 2017) (Table 2)). At the time molecular methods could not be used and only observational data are available from these earlier studies. Wright and Ross (1980), followed up this earlier work and confirmed natural hybrids between *S. haematobium* and *S. matthei* using species-specific biochemical markers, with field isolates showing the same enzymatic patterns as laboratory produced hybrid offspring (Wright and Ross, 1980).

In the late 1980's Kruger (Kruger, 1987, 1988, 1990; Kruger and

Evans, 1990; Kruger and Hamilton-Attwell, 1988; Kruger et al., 1986) and colleagues further investigated *S. matthei* X *S. haematobium* interactions, using both morphological and biochemical methods, identifying *S. matthei* X *S. haematobium* hybrids from a patient from Nelspruit, in the north east of South Africa. Phenotypic differences suggested that the *S. matthei* populations that were sympatric with *S. haematobium*, may have been infiltrated by genetic introgression from *S. haematobium* in these areas. However, it was evident that the proportion of hybrid forms was low and that parental backcrossing with introgression of *S. matthei* genetic material into the *S. haematobium* populations was probable.

In 1994, De Bont and colleagues (De Bont et al., 1994), found evidence, based on egg morphology and biochemical markers, for suspected *S. haematobium*-*matthei* hybrids in cattle however, they could not conclude if there were either *S. matthei* x *S. haematobium* or *S. matthei* x *S. leiperi* hybrids. Since then, very few studies have followed up on the occurrence of the natural hybridisation between *S. haematobium* and *S. matthei*, but very recently molecular data (mt *cox1*, ITS and 18S nrDNA) has provided indisputable evidence for the occurrence of *S. haematobium*-*matthei* in Malawi (Webster et al., 2019). The study was conducted due to the observations of atypical eggs (resembling *S. matthei* and also *S. bovis*) excreted in the urine of children at specific *S. haematobium* foci in Malawi. Additionally, very recently *S. haematobium*-*matthei* mix/hybrid infections were also identified, via the molecular analysis of serum, in a group of tourists who became infected in the north east of South Africa in 2016/2017 (Cnops et al., 2020). As with the more historical accounts these more recent findings still point to asymmetric interactions highlighting the extreme biases observed in these hybridisation systems with pre- and postzygotic reproductive barriers in place to reduce prolific admixing.

5.4.3. *S. haematobium* and *S. bovis*

These two species are widespread across Africa and are sympatric in many countries. *S. bovis* has a slightly more restricted distribution primarily above a latitude of 10° South below which it is replaced by *S. matthei*. Natural hybridisation between *S. haematobium* X *S. bovis* has become the most recent subject of several research groups mainly due to the identification of high numbers of hybrids excreted by humans in West African countries and the possible implications for schistosomiasis control, in relation to zoonotic transmission (Table 2).

The first observations of natural hybridisation were made by Brémond et al. in 1993 who identified schistosomes excreted by humans in Niger, with allozyme profiles that were intermediate between *S. bovis* and *S. haematobium* or *S. curassoni*, but more sensitive markers were needed to discriminate *S. haematobium* from *S. curassoni* (Brémond et al., 1993). However, these findings did indicate that some sort of interspecies interaction had or was taking place. No further studies investigated these hybridisations in nature occurred until 2009, where Huyse et al. (2009) using molecular methods (mt *cox1* and nuclear ITS analysis) identified the high abundance and wide distribution of *S. haematobium* X *S. bovis* hybrids along the Senegal River Basin. These hybrids were found in multiple sites and were collected from both humans and snail hosts (*B. truncatus* and *B. globosus*). An expanded study was conducted in Senegal (both in the North and South of Senegal) in 2009–2010 (Webster et al., 2013a, 2013b), that also examined infections in domestic livestock and humans. It was found that high levels of hybrids occurred in the human population in areas of sympatry but no hybrids or *S. haematobium* were found in livestock. This clearly showed the biases within the hybrid system, with humans being the primary host for the hybrids with strong correlation with urinary excretion of the hybrids. Further studies by (Boon et al., 2017; Boon et al., 2018) showed the heterogeneity of these hybrids and also the close association with coinfections with *S. mansoni*. In 2013, a surprise came with the outbreak of urogenital schistosomiasis on the Mediterranean Island of Corsica (France) (Boissier et al., 2015), with hybrids molecularly identified within the schistosome populations excreted from several infected tourists

(Boissier et al., 2016; Mone et al., 2015). It was also concluded, via molecular phylogenetic analyses that the parasites had been imported from West Africa and in particular Senegal, where there are high levels of migration between these French colonies (Boissier et al., 2016). Interestingly, a single egg excreted from one of the infected tourists showed a genetic profile suggesting a pure *S. bovis* origin (both nuclear ITS and mt *cox1* DNA giving a *S. bovis* profile) presenting some evidence for potential *S. bovis* zoonotic infection however, more in-depth genomic analysis would be needed to determine the genetic make-up of this egg. Sporadic eggs like this have also been found in studies in Senegal and also suggest a pure *S. bovis* origin however, they are very rare and their viability needs to be determined. Whole genome analyses of miracidia from Senegal and Niger failed to identify F1 hybrids within the samples analysed and suggested that the hybrid populations are highly introgressed forms, with hybridisation being ancient and not active. Additionally, genetic microsatellite analyses of hybrids in Senegal and Niger have shown clear population differentiation between the schistosomes infecting humans (*S. haematobium* and the *S. haematobium* - *bovis* hybrids versus *S. bovis*) suggesting no or very limited active interactions/hybridisation and gene flow between the populations (Boon et al., 2019; Pennance et al., 2020). Further areas of hybridisation between *S. haematobium* and *S. bovis* have now been molecularly identified, including areas in Niger, Benin, Mali, Cote D'Ivoire and also Malawi (Leger et al., 2020; Pennance et al., 2020; Savassi et al., 2020; Soentjens et al., 2016; Tian-Bi et al., 2019). The Benin study (Savassi et al., 2020) indicated the possible occurrence of *S. haematobium-bovis* hybrids in cattle, identified by the shift in cercarial shedding patterns of the resultant progeny isolated from infected cattle together with atypical genetic profiles of the adult worms. It is clear from the data from multiple studies that this hybridisation is common but it is not random and there are specific biological and genetic influences taking place, which may also differ between foci. This raises several questions with regard to the impact of this hybridisation which, only further detailed epidemiological and in depth genetic/genomic studies can help unravel.

5.4.4. *S. haematobium* and *S. curassoni*

Interactions between these two species are rarer, mainly due to the more restricted distribution of *S. curassoni* to specific foci in a few West African countries with most transmission in Senegal (Leger et al., 2020; Leger and Webster, 2017; Webster et al., 2013b) (Table 2). In the early 1990's suspected hybrids between *S. haematobium* and *S. curassoni*, based on egg morphology and biochemical markers, were observed excreted by humans in Niger but the species involved could not decisively be determined as either *S. bovis* or *S. curassoni* (Brémond et al., 1993). More recent work in Senegal has provided evidence of *S. haematobium-curassoni* hybrids excreted via the urine of patients in sympatric areas of Senegal, although more in-depth genetic analysis would support our understanding of these interactions in these endemic zones (Webster et al., 2013b) (Table 2). Particularly the distinction between *S. bovis* and *S. curassoni* using traditional nuclear markers is not always robust with a single species-specific SNP in the ITS1 nrDNA that allows distinction between the two species (Pennance et al., 2020; Webster et al., 2013b), with the use of other nrDNA regions such as the 18S needed for further clarification. A very recent report by (Leger et al., 2020) showed no *S. haematobium* and *S. curassoni* interactions in sympatric zones in Senegal suggesting that interactions between these species are rare and unstable. More detailed studies into the dynamics of these interactions and hybridisation events in different settings is certainly warranted as it is likely that differences occur at both micro and macro geographical scales (Stothard et al., 2020).

5.5. *S. haematobium* and *S. mansoni*

S. haematobium and *S. mansoni* represent two co-endemic pathogens that are both highly prevalent in humans but highly divergent from each other, a key postzygotic reproductive isolating barrier retaining these

species integrities. There have been many reports of the possible interactions between these species in their human hosts based on atypical egg excretion, ectopic egg deposition and host morbidity assessments and competitive exclusion of *S. mansoni* by *S. haematobium* in some areas (Gouvras et al., 2013; Koukounari et al., 2010; Stothard et al., 2020) (Table 2). Lateral-spined eggs, with a typical *S. mansoni* morphology, have often been observed in urine samples and terminal-spined *S. haematobium* shaped eggs have been observed in stool samples in several co-endemic areas including Kenya, Egypt, Senegal, Malawi, Mali and Cote D'Ivoire. However, the occurrences of these atypical egg excretions in relation to heterospecific interactions are dynamic and are not observed in all co-endemic areas and in all patients. Huysse et al. (2013a) conducted the first molecular analyses of eggs/miracidia using nrDNAITS and mt DNA *cox 1* markers and showed that the offspring contained genetic material from both parental species. This disputes parthenogenesis and suggests hybridisation has occurred between these two distantly related species however, the viability of the offspring to infect snails remains unknown. Additionally, a case of *S. haematobium* - *mansoni* hybrids were molecularly identified from atypical eggs excreted from a migrant boy who had migrated from Cote D'Ivoire, via West Africa to France, but again the viability of the offspring was not able to be tested (Le Govic et al., 2019). The impact of such inter-species interactions, even if they are not viable, on human health should not be dismissed as they are likely to cause increased pathology due to ectopic egg deposition and a higher abundance of eggs being deposited in organs and tissues.

6. Conclusion

Molecular epidemiological studies on the diverse range of *Schistosoma* species have provided valuable insights into transmission dynamics, population and species level diversity over time and space, and how selective pressures such as drug treatment may shape and change the schistosome populations. Results obtained in the last decade clearly show, and in particular for *S. mansoni* and *S. haematobium*, that whatever the schistosome species involved only few barriers to gene flow exist at a local and regional scale. However, despite this, species have maintained their integrity with natural selection processes making them highly adapted to their environments with successful and ongoing transmission. It is acknowledged that our knowledge on the genetic distribution and the evolutionary history of other human-associated and all animal-associated African *Schistosoma* species is currently very limited if not completely absent at a large geographical scale. It is thus crucial to gather more information on the population genetic structuring of these schistosomes including the recently identified hybrids in order to; i) infer the role of the definitive hosts on the parasite gene flow and ii) to better understand their relative importance in the epidemiology and evolution of African *Schistosoma* species, with parasite genetic variation known to be an important factor in explaining the variation in host disease phenotype.

Another important aspect in studying animal infecting schistosomes, potential zoonotic hybrids and animal reservoirs of infection is the role they may play in spreading resistance or virulence alleles among and between populations. Indeed, despite several years of vast MDA coverage in Africa and extensive treatment campaigns, many areas characterized as "persistent hotspots" fail to substantially decrease transmission, with on-going high levels of prevalence, intensity and associated human morbidity.

Studies focusing on the genetic structure of *Schistosoma* populations and their response to MDA suggest that rapid re-infection of parasites from genetically differentiated source populations occurs and that MDA alone is not sufficient to control the disease transmission (Van den Broeck et al., 2020). High levels of gene flow between parasite populations before and after treatment together with refugia may facilitate a rapid recovery of population genetic diversity with treatment having only a limited impact on parasite transmission at the community level.

In addition, whether drug resistance or an increased genetic diversity brought about by inter-species/population genetic exchange, maybe influencing reduced drug efficacy in some foci needs to be investigated.

Broader and more fine-scale molecular epidemiological analyses of schistosome populations are warranted both to understand transmission and infection dynamics, but also in relation to treatment responses. Powerful genetic analysis tools, such as Whole-Genome Single Nucleotide Polymorphisms (SNP), now exist and are quickly being optimised to study natural *Schistosoma* populations and will provide greater insights into many aspects of schistosome biology, evolution, genetics, transmission dynamics, inter-species interactions and resistance/versus reinfection dynamics, all of which will support control and elimination of this debilitating disease.

Declaration of Competing Interest

Authors of the manuscript “Genetics and evolution of African schistosomes” declare that they have no competing interests.

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